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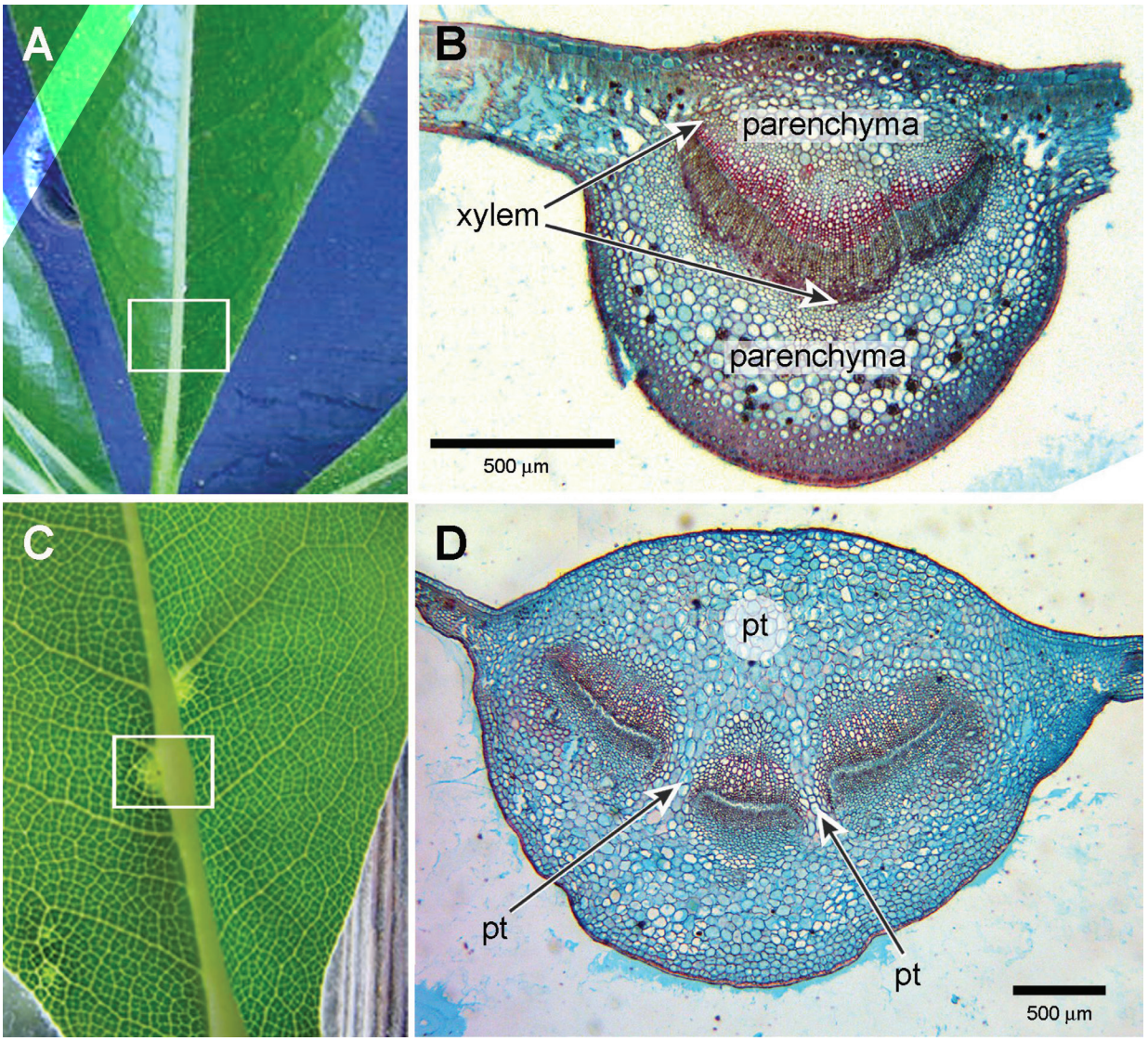

Malta Chamber of Scientists

www.xjenza.com

The Journal of the Malta Chamber of Scientists

Editor-in-Chief: Giuseppe Di Giovanni

Xjenza Online - Journal of Malta Chamber of Scientists
http://www.mcs.org.mt/

Editorial Board

Scope of Journal

Xjenza is the Journal of the Malta Chamber of Scientists and is published by the Chamber in in electronic format. Xjenza is a peer-reviewed, open access international journal. The scope of the journal encompasses research articles, original research reports, reviews, short communications and scientific commentaries in the fields of mathematics, statistics, geology, engineering, computer science, social sciences, natural and earth sciences, technological sciences, linguistics, industrial, nanotechnology, biology, chemistry, physics, zoology, medical studies, electronics and all other applied and theoretical aspect of sciences.

The first issue of the journal was published in 1996 and the last volume (No. 12) was published in 2007. We are now planning to restart publishing this journal regularly and will have a new number in March 2013. The new editorial board has been formed with internationally recognised scientists and we will produce 2 issues per year. One of the aims of Xjenza, besides highlighting the exciting research being done nationally and internationally by Maltese scholars, is to provide training for undergraduate and graduate students and young researchers in the art of scientific publishing in a peer-reviewed environment. Xjenza, therefore, might become an essential tool in the scientific development of early-stage researchers, and represent a learning platform where they can learn not only how to write a scientific paper, but also how to get it published. Initially Xjenza was mainly open to Maltese researchers.

Instructions for Authors

Xjenza is the journal of the Malta Chamber of Scientists and is published by the Chamber in electronic format on the website *http* : *//www.xjenza.com*. Xjenza will consider for publication manuscripts in the following categories:

- (01) Communications
- (02) Research Articles
- (03) Research Reports
- (04) Reviews
- (05) Notes
- (06) News
- (07) Errata

Communications are short peer-reviewed research articles (limited to three journal pages) describing new important results meriting urgent publication. These are often followed by a full Research Article.

Research Articles form the main category of scientific papers submitted to Xjenza. The same standards of scientific content and quality that applies to Communications also applies to Research Articles.

Research Reports are extended reports describing research work carried out in Malta or by Maltese researchers of interest to a wide scientific audience characteristic of Xjenza. Please contact the editor to discuss the suitability of topics for Research Reports.

Reviews articles describe work of interest to the wide readership characteristic of Xjenza. They should provide an in-depth understanding of significant topics in the sciences and provide a critical discussion of the existing state of knowledge of the subject matter with current primary literature from the previous 5-10 years. Review Articles should be written in a way that the length is kept to a minimum consistent with comprehensibility (normally should not exceed 6000 words). Authors are strongly advised to contact the Editorial Board before writing a Review.

Notes are fully referenced peer-reviewed short articles limited to three journal pages describing new theories, concepts and developments made by the authors in any branch of science and technology. Notes need not contain results from experimental or simulation work.

News: The News section provides a space for articles up to three pages in length describing leading developments in any field of science and technology or for reporting items such as conference reports. The Editor reserves the right to modify or reject articles for consideration as 'news items'.

Errata: Xjenza also publishes errata, in which authors correct significant errors of substance in their published manuscripts. The title should read: Erratum: "Original title" by XXX, Xjenza, vol. XXX (year). Errata should be as short as consistent with clarity.

Invited Articles and Special Issues: Xjenza regularly publishes Invited Articles and Special Issues that is/are, articles written on invitation by the Editor or member of the editorial board.

Submission of Manuscripts

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Department of Physiology and Biochemistry
Faculty of Medicine and Surgery (On Campus)
University of Malta
Msida MSD 06
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Tel: (+356) 2340 2776
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All manuscripts submitted to Xjenza are peer reviewed. Authors are requested to submit with the manuscript, the names and addresses of three referees, preferably from overseas. All effort will be made to use the recommended reviewers; however the editor reserves the right to also consult other competent reviewers.

Conflict of Interest

Authors are expected to disclose any commercial or other associations that might pose a conflict of interest in connection with the submitted article. All funding sources supporting the work, and institutional or corporate affiliations of the authors, should be acknowledged on the title page or at the end of the article.

Policy and ethics

The work described in your article must have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans *http* : *//www.wma.net/en/*30*publications/*10*policies/b*3*/index.html*; EU Directive 2010/63/EU for animal experiments *http* : *//ec.europa.eu/environment/chemicals/lab_animals/ legislation_en.htm*; Uniform Requirements for manuscripts

submitted to Biomedical journals http://www.icmje.org. This must be stated at an appropriate point in the article.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that the manuscript has been approved for publication by all authors, and tacitly or explicitly, by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

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Preparation of Manuscripts

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc.

Article Structure

An article for publication in Xjenza will ordinarily concise of in the following order: Title page with contact information, Abstract, Highlights, Keywords, Abbreviations, Introduction, Materials and Methods, Results, Discussion, Conclusions and Appendices. The article will be divided into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'.

Title page

• The title should be concise yet informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• **Author names and affiliations.** Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• **Corresponding author.** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.

• **Present/permanent address.** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required up to about 250 words. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references should be avoided. Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Highlights

Highlights are mandatory for Xjenza. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Keywords

Immediately after the abstract, provide a maximum of 10 keywords. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and Methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results

Results should be clear and concise. Discussion This should explore the significance of the results of the work, yet not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.

• Save text in illustrations as 'graphics' or enclose the font.

• Only use the following fonts in your illustrations: Arial, Courier, Times, Symbol.

• Number the illustrations according to their sequence in the text.

• Use a logical naming convention for your artwork files.

• Provide captions to illustrations separately.

• Produce images near to the desired size of the printed version.

• Submit each figure as a separate file. A detailed guide on electronic artwork is available on our website: http://www.xjenza/authorguidelines

Formats

Regardless of the application used, when your electronic artwork is finalised, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS: Vector drawings. Embed the font or save the text as 'graphics'.

TIFF: Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF: Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF: Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

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Colour artwork

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Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

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Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference style

Text: All citations in the text should refer to:

1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication;
2. Two authors: both authors' names and the year of publication;
3. Three or more authors: first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically. Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Borg J., Papadopoulos P., Georgitsi M., Gutiérrez L., Grech G., Fanis P., Phylactides M., Verkerk A.J., van der Spek P.J., Scerri C.A., Cassar W., Galdies R., van Ijcken W., Ozgür Z., Gillemans N., Hou J., Bugeja M., Grosveld F.G., von Lindern M., Felice A.E., Patrinos G.P., Philipsen S. (2010) Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. *Nat. Genet.* 42(9), 801-805.

Cope D.W., Di Giovanni G., Orban G., Fyson S.J., Errington A.C., Lorincz M.L., Gould T.M., Carter D.A., Crunelli V. (2009) Enhanced tonic GABAA inhibition is required in typical absence epilepsy. *Nat. Med.* 15(12), 1392-1398.

Reference to a book: Di Giovanni G. (2012) Nicotine Addiction: Prevention, Health Effects and Treatment Options. Nova Publishers, New York.

Reference to a chapter in an edited book:

Di Giovanni G., Pierucci M., Di Matteo V. (2011). Monitoring Dopamine in the mesocorticolimbic and nigrostriatal systems by microdialysis: relevance for mood disorders and Parkinson's disease. In: Applications of Microdialysis in Pharmaceutical Science. Ed: Tsai T-H. John Wiley and Sons, Inc., Hoboken, NJ, USA.

Journal abbreviations source

Journal names should be abbreviated according to

Index Medicus journal abbreviations: http://www.nlm.nih.gov/tsd/serials/lji.html;

List of title word abbreviations: http://www.issn.org/2-22661-LTWA-online.php;

CAS (Chemical Abstracts Service): http://www.cas.org/sent.html.

Video data

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Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

• One author has been designated as the corresponding author with contact details:

• E-mail address

• Full postal address

• Telephone and fax numbers All necessary files have been sent, and contain:

• Keywords

• All figure captions

• All tables (including title, description, footnotes)

Further considerations

• Manuscript has been 'spell-checked' and 'grammar-checked'

• References are in the correct format for this journal

• All references mentioned in the Reference list are cited in the text, and vice versa

• Permission has been obtained for use of copyrighted material from other sources (including the Web)

After Acceptance

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

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Table of Contents

Xjenza Online Volume 1 - Issue 1 - (March 2013)
Formerly *Xjenza*

Xjenza Online - Journal of Malta Chamber of Scientists
http://www.mcs.org.mt/

Editorial: The new Xjenza Online

Giuseppe Di Giovanni
Editor-in-Chief of Xjenza online

Last June I was offered the position of Editor-in-Chief of Xjenza, the official Journal of the Malta Chamber of Scientists (ChoSci) by the acting President, Prof Alex Felice. The journal's production has been interrupted since 2007 and now the Chamber, revitalised by a new spring, wanted to publish it again and cover the gap left in the scientific Maltese community, this time as on online only journal. I was excited by this challenge and very grateful for the confidence expressed in me by the ChoSci council in recognition of my extensive international editorial experience. I would like to take this opportunity to share with the membership my general vision and plans for the journal as we move forward in this new beginning and publish the first issue.

First, I would thank the previous editorial team, who, we believe, did an excellent job in advancing the breadth and appeal of Xjenza. Under the leadership of Prof Giuseppe Grima and Prof Richard Muscat, the journal continues as the premier outlet for work carried out in Malta. Further, their efforts to increase the readability of the journal are highly admirable and I plan to continue, and hope to expand upon, these efforts. Indeed, I will build upon their efforts in the hope of making a great journal even better.

Generally, I will continue the vision for the journal as established by the founding Editor Prof Angela Xuereb and pursued by my predecessors, but we aim higher by taking it to an international level. I will strive to publish national and international scholarly research that is of exceptional merit, that focuses on important issues from different branches of science, technology and the humanities, is of general interest, and understandable to as many scholars as possible. As with the ChoSci I am also intent on NOT creating a journal which is a collection of niches: my intent is to create synergies, not fiefdoms. More specifically, together with my new appointed editorial team I plan to pursue four major goals regarding the new Xjenza, to:

1. Increase the diversity and quality of submissions and open the journal to international authors;
2. Encourage and publish student papers (both reviews and research articles)
3. Advertise local and international opportunities for research collaborations, grants, meetings and successful stories
4. Improve the production process and decrease the time required for reviewing manuscripts.

Our aim to increase the diversity and quality of submissions via broad and systematic outreach can only be addressed by a more deliberate and systematic outreach plan, and by improving communication with the membership of different professions. Although we certainly applaud previous efforts to specifically invite high quality submissions, we believe that much more can be done. Significant and important high standard work, for instance, is being presented at the national and international conferences of many area studies associations by Maltese colleagues. Reaching out to these scholars, and others employing underrepresented approaches, should stimulate more submissions to the journal. We believe that it is important to broaden the appeal of Xjenza by engaging well recognised scientists. We believe that the problem of submissions to Xjenza is rooted in a, perception held by many in the profession that Xjenza is only "for local related issues." We do not intend to dissociate the journal from the Maltese reality, which would be a great loss. Instead, continuing the tradition there will be a session of Xjenza dedicated to News and Notes where information on grant opportunities (locally and internationally) on related to Maltese achievements and recognition can be found. We hope that Xjenza Online will take back its role of forum for academic discussion. At the same time we want to start the internationalisation of this journal, opening it up to researchers worldwide. Our goal is to take Xjenza among the other international journals, we hope with the help of affirmed publishing houses, amongst whom we have already started preliminary contacts.

We have already engaged in a systematic outreach effort which involved, first, communication with the organized sections of the ChoSci to ask for input on suggested names for the Xjenza's editorial board (and we intend to continue to ask for input and advice); and second, to attend, in person, as many national and international conferences as possible to speak to scientists about our journal.

The new and important aim of Xjenza Online, besides highlighting the exciting research being done nationally and internationally, is to offer training in the art of scientific publishing in a peer-reviewed environment for graduate students and young researchers. Indeed, publication of research work is essential in order to advance science. It is also essential for people pursuing a scientific career as their recognition as researchers depends on their publications and contributions to scientific progress. Scientists live in a culture of "publish or perish". Xjenza Online, therefore, can become an essential tool in the scientific development of early-stage researchers, and represent a learning platform where they can learn not only how to write a scientific paper, but also how to get it published. In this important task we will be assisted by the students' supervisors that will guide them in the writing process. In this first number of Xjenza Online there is a section dedicated to student papers. Mr Cassar, under the guidance of Prof Hunter, produced an interesting review on Serpines and Luana Chetcuti Zammit, supervised by Dr Kenneth, presented a research article on computational models to study air Pollution in Malta. We hope to see more and more papers signed by students.

To improve the production process as in the first issue, we are committed to the practice of collective decision making, where the members of the editorial team consult with one another before the final acceptance of manuscripts. The Xjenza's team is made up of six editors so far, a small team, in alphabetical order, Sebastiano D'Amico (Geoscieces), Sandro Lanfranco (Life Science), David Magri (Physics and Chemical Science), Kenneth Scerri (Mathematics, Communications and Informatics Science), Victoria Sultana (Human Interest Science), Mario Valentino (Biomedical Sciences). In addition there is the Copy Editor Jackson Levi Said as well as a web administrator John Gabarretta, and we aim to involve in the next future Post Doctoral Assistants and graduate student Editorial Assistants.

We have started the practice of having multiple editors with varied perspectives and methodological skills. In addition, I plan to include an Advisory Board made of international scholars.

As far as the editorial process is concerned, as lead editor, I will scan each new submission carefully but quickly and will direct it to the most appropriate Associate Editor. I believe the process is most efficient where one person (preferably with expertise in the subfield) is responsible for overseeing the review process of a manuscript. The responsible associate editor will then assign the referees, consulting with the lead editor or members of the Editorial Board in cases that are difficult and/or outside the editor's area of expertise. If a submission falls into an area which we do not feel competent to handle, we will seek the advice of an appropriate member of the Board for recommendations for referees. Indeed, we will regard it as one of the most important functions of the Advisory Board to lend their expertise in areas where we lack intimate knowledge. The result, we hope, will be a fair hearing for every manuscript from every area of science, technology and the humanities.

We believe that we will be able to maintain a high degree of continuity in our team. All members of the team are established and committed senior faculty in our University. Finally, we are keenly aware of the enormous responsibility we are taking on, and have no illusions about the amount of hard work required. I am, however, eager to meet this challenge, and am extremely grateful for the support and confidence expressed in our editorial team by the ChoSci Council on behalf of its members. We will do our utmost to live up to this responsibility. We welcome any comments or suggestions by our colleagues from the different disciplines-we are very open to any input and advice as to how to make Xjenza online better.

Thank you all for this opportunity to serve the Chamber, colleagues and our wonderful Island. We will not let you down.

Xjenza Online - Journal of Malta Chamber of Scientists
http://www.mcs.org.mt/
Doi: http://dx.medra.org/10.7423/XJENZA.2013.1.01

Research Article

EARTHQUAKE GROUND-MOTION SIMULATIONS FOR THE MALTESE ARCHIPELAGO

Sebastiano D'Amico[1] **and Pauline Galea**[1]
[1]Department of Physics, University of Malta, Msida, Malta

Abstract. The main goal of this paper is to provide earthquake ground motion simulations for particular earthquake scenarios, in terms of ground motion parameters for the Maltese islands. We used a stochastic approach to simulate high-frequency strong-ground motions, using an extended-source model code. This code was developed for earthquake simulations using stochastic finite-fault modelling and a dynamic corner frequency approach. The extended-source model code is a reliable and practical method to simulate ground motion records of moderate and large earthquakes especially in regions where structural damage is expected, but sparse ground motion recordings are available. In this paper, we show that in the Maltese archipelago, the ground motion from the repeat occurrence of historically recorded earthquakes, or from other potential sources, coupled with existing geological conditions and building typologies has the potential to cause significant structural damage in the area.

Keywords Ground motion; Numerical Simulations, Malta, Central Mediterranean

EXSIM: Extended Finite Fault Simulations

NEHRP: National Earthquake Hazards Reduction Program

Correspondence to: S. D'Amico (sebdamico@gmail.com)

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1 Introduction

Large and moderate earthquakes that have occurred in recent years in densely populated areas of the world dramatically highlight the inadequacy of a massive portion of the buildings erected in and around the epicentral areas (e.g.: Izmit, Turkey, 17th August 1999; Duzce, Turkey, 12th November 1999; Chi-Chi, Taiwan 20th September 1999, Bhuj, India, 26th January 2001; Sumatra, Indonesia 26th December 2004; Wenchuan, China, May 12th, 2008; L'Aquila, Italy, April 6th, 2009; Haiti, January 2010; Emilia, Italy, May 2012). It has been observed that many houses, industrial complexes and cultural heritage sites were unable to withstand the ground shaking. In this context, earthquake ground motion scenarios, combined with a probabilistic seismic hazard analysis and proper source characterizations can be used to better understand the expected earthquake impact, and help plan for the future (D'Amico et al. 2010a, b, D'Amico et al. 2012a, b; Ugurhan et al. 2012; Secomandi et al. 2013). In particular, they could help decision makers to better visualize specific problems that are based on scientific and engineering knowledge. Furthermore, a scenario improves awareness of what an earthquake can do to a community as a whole.

Malta is a zone of low-to-moderate seismic hazard, and earthquake awareness is culturally not strong. However the Maltese islands have been affected by a number of earthquakes in the historical past, the epicentre of these earthquakes being in Eastern Sicily, Sicily Channel or as far away as the Hellenic arc. Some of these earthquakes produced considerable damage to buildings (Galea 2007). The fact that the last damaging earthquake occurred around a hundred years ago probably explains the general complacency that exists. Consequently, general seismic preventive and preparedness plans have not been properly developed.

The main goal of this paper is to provide, for the first time, earthquake ground motion simulations for the Maltese archipelago in order to generate earthquake scenarios mainly based on the ground motion parameters. Malta represents a site of particular historical interest, and it has an important role in the tourism industry. In general, buildings are located in a diversity of topographical and geological settings, and a variety of building types and ages can be identified. Therefore, although this paper deals mainly with ground motion parameters, the area provides a suitable setting for the subsequent evaluation of a number of other factors that contribute to the damage potential, and hence the holistic assessment of seismic risk, which has not been adequately tackled so far.

Modern broadband seismic recording has been available on Malta only since 1995, and therefore no instrumental data for strong events is available. It is therefore necessary to adopt the approach of artificial earthquake simulation using numerical methods and realistic earthquake scenarios. Such methods have been used with success in numerous other regions (e.g. Taiwan, D'Amico et al. 2012a; Western Anatolia, Akinci et al. 2013; Central Italy, Ugurhan et al. 2012; Southern Italy, D'Amico 2012; D'Amico et al. 2011)

2 Methodology

The estimation of ground motion for a particular region and also site-specific investigation is essential for the design of engineered structures. Estimates of expected ground motion at a given distance from an earthquake of a given magnitude are fundamental inputs to earthquake hazard assessments. It has been proven that it is possible to make numerical predictions of ground motion parameters for regions where strong-motion data are lacking or where even data for moderate and large earthquakes are not available (e.g. D'Amico et al. 2012a, b). In order to predict the expected ground motion parameters, for example peak ground acceleration (PGA) peak ground velocity (PGV), and Spectral Acceleration (SA), as a function of distance and magnitude we used the latest version of the EXSIM program (Boore 2010; Motezedian et al. 2005).

2.1 THE EXSIM Procedure

The EXSIM code is based on Boore's stochastic method for simulating high frequency ground motion using a finite fault geometry (Boore 1983). The Fourier acceleration amplitude spectrum of ground motion at a distance r from a source of seismic moment Mo, may, in a general way, be written as the product of three factors: the source spectrum, S(f, M), the propagation term, G(r,f), and the site amplification term, Site(f):

$$A\left(f, r, M\right) = S\left(f, M\right) \cdot G\left(r, f\right) \cdot Site\left(f\right) \qquad (1)$$

where f,r, and M represent frequency, distance and magnitude respectively.

In the finite fault simulation, the source is represented by a rectangular plane fault, whose dimensions are proportional to the moment magnitude M_W. The fault is discretized into a grid of rectangular sub-faults, and the rupture is considered to begin at the centre of one of the sub-faults (in this case randomly chosen), and spread with a rupture velocity 0.8 times the shear wave velocity at the source. The acceleration time series from each sub-fault is derived by inverse Fourier transform, and the time history at the site is obtained by summation of the individual time series, with appropriate time delays. It has been shown that only the gross features of slip distribution on a fault plane that does not diverge significantly from the average value of slip may be reliable; all other complexities could be extremely uncertain (Berensev et al. 2002). We thus find it reasonable to assume a random slip distribution, since we have no constraint on the particular faults that we shall be modelling. For each sub-fault, the source spectrum follows Brune's ω^2 source model

$$\frac{CM_0\left(2\pi f\right)^2}{1+\left(f/f_c\right)^2} \qquad (2)$$

where C is a scaling factor (Boore 2003), M_o is the subfault seismic moment (proportional to the stress drop $\Delta\sigma$, and f_c is the corner frequency). In this application, a dynamic corner frequency approach is adopted, whereby the corner frequency changes with the subfaults being activated in order to reflect the decrease in frequency content during the rupture history (Motazedian and Atkinson, 2005).

The propagation term contains the geometrical spreading term $g(r) = r^n$ where n is a function of distance, the inelastic attenuation term given by $e^{-\pi fr/Q(f)\beta}$, where Q is the average, frequency-dependent quality factor for the whole path ($Q(f) = Q_o f^n$), and the upper crustal attenuation factor $e^{-\pi\varkappa f}$ where $\varkappa$governs the high frequency decay of the spectrum at the site.

In this simulation, we argue that the Maltese islands and Malta Channel (separating the islands from Sicily) belong to the same geological domain as the southeastern tip of Sicily (Malta-Hyblean plateau) and it is justified to use the crustal propagation parameters that were derived for SE Sicily by Scognamiglio et al (2005). These parameters were derived following a regression procedure on local earthquake waveform data that defined the excitation, propagation and site terms. The best-fit values yielded by the above regression, and adopted in this study, are summarised in Table 1.

Table 1. Source, path and site parameters used for the EXSIM simulations.	
Parameter identification	*Parameter value*
Dimension of the faults	According to Wells and Coppersmith (1994)
Pulsing area	50%
Slip distribution	Random
Crustal shear wave velocity	3.5 km/s
Density (crustal)	2.8 g/cm^3
Rupture velocity	0.8×shear wave velocity
Anelastic attenuation, Q(f) and	$Q_o = 400$, $\eta = 0.26$ (Scognamiglio et al. 2005)
Geometrical spreading	$g(r) = r^{-1} r < 40$ km $g(r) = (1/40)(40r)^{-0.4} r > 40$ km (Scognamiglio et al., 2005)
Kappa (sec)	0.035
Windowing function	Saragoni-Hart
Stress drop ($\Delta\sigma$)	For Mw = 5.0 $\Delta\sigma = 210$ bar For Mw = 7.6 $\Delta\sigma = 280$ bar
Site Geology/ NEHERP Amplification factors/ Number of sites	LC/Site Class 4A/74 GLOB/Site Class B/160 UC/Site Class C/74 BC/Site Class D/101

In addition, in our simulation we considered also the potential seismic effect due to the local geology (Vella et al. 2013) which will permit to create reliable earthquake scenarios. Site effects at a specific station are very important and may be used for engineering purposes to define the regional predictive law and the seismic hazard. A generalized site response concept is useful to create a detailed shaking map for a region where the different outcropping lithologies are known. The generic site response represents the average response expected for a site with specific superficial geologic characteristics. In this study in order to consider different site conditions, we will refer to the NEHRP classification (BSSC 1994; Boore et al. 1997).

2.2 Source Models

We selected two potential faults: the first located on the northernmost segment of the Hyblean-Malta Escarpment offshore eastern Sicily, and the second at about 20 km south of Malta (Fig. 1).

On the first fault we simulated a magnitude $M_W = 7.6$ event, intended to replicate the 11 January 1693 earthquake that caused the highest impact on the Maltese islands in historical times. A similar event had also occurred in 1169 on the same fault (Azzaro and Barbano, 2000). On the second fault we modelled a magnitude 5.0 event motivated by the occurrence of a band of seismicity located instrumentally during the last decades (Fig. 1). This source region appears to lie on the Malta graben which passes very close to the south of Malta, and is seismically active. Although no event of magnitude 5.0 has been recorded in this region in recent times, a magnitude 5.0 earthquake on the faults bounding other parts of the Sicily Channel rift is likely to have occurred at least once (Galea 2007). The dimensions of the faults were derived in the EXSIM code using the Wells and Coppersmith relations (Wells and Coppersmith, 1994). Another important parameter is the stress drop, which may cause differences in the ground motion levels at short distances. In order to properly represent the source characteristics we adopted a stress drop value of 210 bar for the $M5$ earthquake as suggested by Di Bona et al. (1995) and a value of 280 bar (Malagnini 2012, personal communication) for the $M7.6$ earthquake located on the Malta escarpment. This is reasonable, and in fact, Mayeda and Malagnini (2009) hypothesized a step-like change in the stress parameter around M_w 5.5 using different data sets from Hector Mine (Mayeda et al. 2007), Wells (USA; Mayeda et al. 2010), San Giuliano (Malagnini et al. 2008). The necessary parameters for ground motion simulations used in this study are given in Table 1.

3 Results and Discussion

The Maltese Islands are made up of a geological sequence of sedimentary rocks, mainly limestones and clays. The sequence is made up of distinct layers of varying hardness and resistance to erosion. In the order of deposition, the main layers in the sequence are the Lower Coralline Limestone (LC), Globigerina Limestone (GL), Blue Clay (BC), and Upper Coralline Limestone (UC) (Pedley et al. 2002). The simulations were run at a total of 409 points defined over the whole of the archipelago in such a way that all types of outcropping geology were represented. Preliminary investigations of

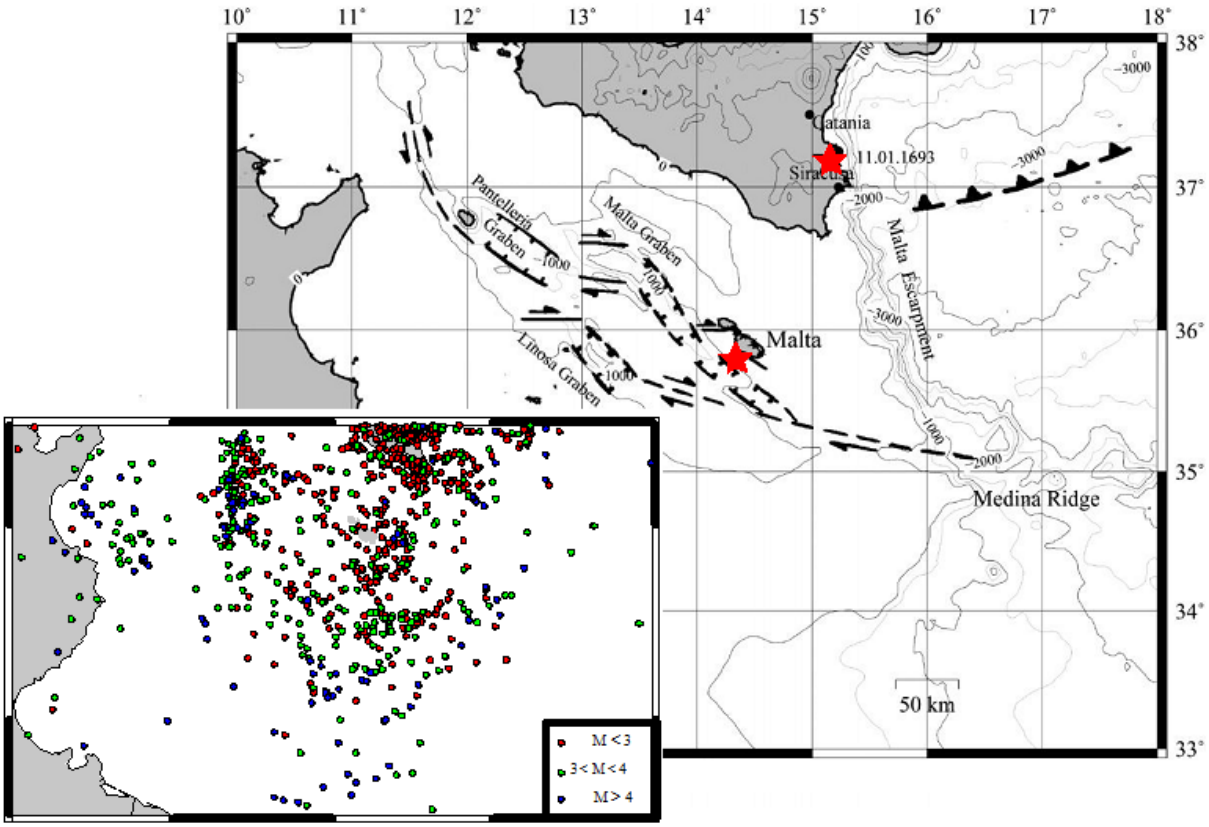

Figure 1: Bathymetry of the Sicily Channel and main tectonic features of the Sicily Channel Rift Zone-bounding normal faults and strike-slip lineaments. Also shown are the Calabrian Arc subduction zone and epicentre of the 11/01/1693 earthquake (Galea 2007), as well as the location of the second simulated earthquake (red stars). The inset (same lat/long range of the main figure) shows seismicity around the Maltese islands in the last 10 years (data are from INGV catalogue http://iside.rm.ingv.it/ and the catalogue of the Seismic Monitoring Research Unit, University of Malta http://seismic.research.um.edu.mt/)

shallow crustal properties on the Maltese islands indicate a high variability of outcrop categories (Panzera et al. 2012; 2013). In this simulation, the average shear wave velocities that characterise the uppermost layers were used to assign site classes to each point. As a preliminary attempt, NEHRP site class A, B, C, D were assigned to the LC, GL, UCL and BC respectively, and the generic site response applied for each class (Fig. 2). The assignment of site class C to sites of outcropping UCL (mainly in the west of the archipelago) is justified by recent results showing that seismic site response at these sites exhibits considerable amplification owing to the presence of underlying clays (Vella et al. 2013). However, further studies will be necessary in order to properly characterize the soil classification for the Maltese Islands including parameters such as the soil fundamental frequency. In fact, it has been recently shown (e.g Luzi et al. 2011) that the use of average shear wave velocity of the uppermost layers to discriminate soil categories is not the best tool to use in building-code or preparation of seismic hazard maps, although it is a parameter internationally used.

The ground motion simulation was run at each grid point, outputting the peak ground acceleration (PGA), peak ground velocity (PGV) and spectral acceleration (SA) at each of four chosen frequencies - 0.33 Hz, 1.0 Hz, 3.0 Hz and 5.0 Hz. This range of frequencies is considered adequate for engineering purposes. The results of the simulations for each hypothetical source are shown in Figures 3 and 4.

For the eastern Sicily earthquake, about 150 km away, (Fig. 3), the effect of geology is the most conspicuous. The distinction between the eastern and western sides of the archipelago is immediately clear, since only the western side is characterised by the presence of Blue Clay and therefore contains mostly C and D sites. Peak ground accelerations reach their maximum value (approximately 0.2 g) at sites where the BC is directly exposed. However, PGA values exceeding 0.1 g are seen to be common in almost all areas of the archipelago, including the urbanised areas in the eastern half of the island, and in particular also the high elevation areas comprising Rabat, Mdina and Mellieha, as well as the whole of Gozo. Spectral effects show that the maximum expected ground motions occur around frequencies of 1 Hz

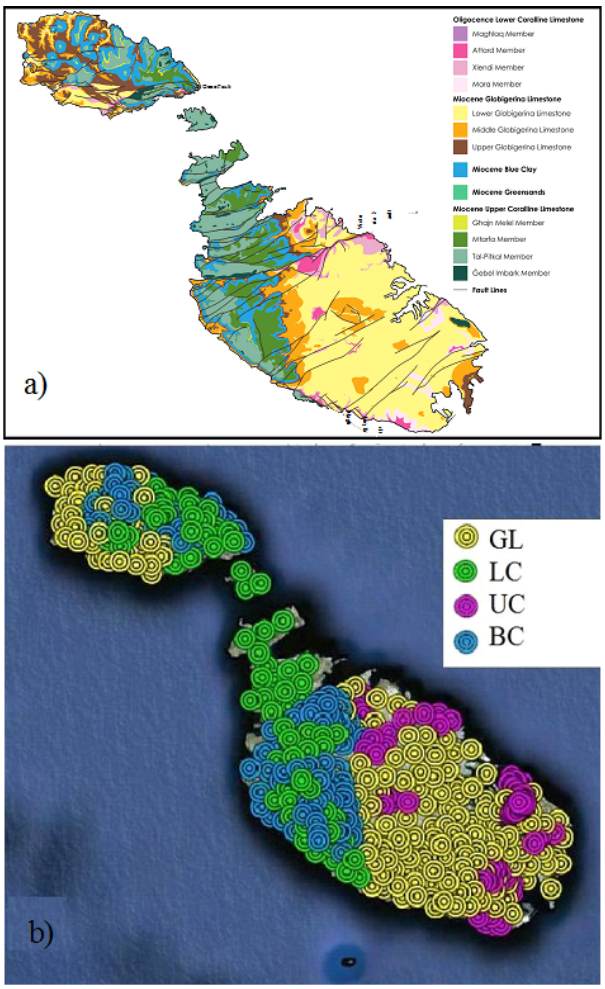

Figure 2: Location of the points used for the stochastic simulation; each point is colour coded according to the local geology (see text for details).

On the other hand, the magnitude 5.0 event produces effects that are predominantly linked with distance, since the epicentre is only about 20 km away (Fig. 4). In fact maximum ground motion is observed towards the south of the island, although the added effects of the lithology in that area cannot be excluded. Even in this case, however, peak ground accelerations exceeding 0.2 g are observed on the island of Malta, whereas on Gozo, PGA values are limited to below 0.1 g. The frequency content of the ground shaking is also different to the M 7.6 event, being shifted more towards higher frequencies.

These results are highly significant with respect to the evaluation of seismic risk on the Maltese islands. Admittedly they represent scenarios of rare events, however such simulations pave the way towards a combined methodology that will take into account both the seismic hazard evaluation (which yields the probability that such an event will occur in a given time period) as well as the deterministic prediction of the effects of any given earthquake source. Moreover they constitute a required input to the civil engineering community which is responsible for evaluating the interaction of the predicted ground motion with local building stock. Because of the inherent brittleness, lack of ductility and lack of tensile strength of unreinforced masonry buildings (URM), it is expected that even moderate ground accelerations could cause significant damage in these buildings (Hess 2008).

Finally, in order to double-check the performance of our simulations we converted the predicted PGA and PGV into seismic intensity and compared with the observed one. For the conversion from PGA to seismic intensity we used the formula implemented within the ShakeMap® packages (Wald et al. 1999). This relationship is valid in the range of V-VIII of Modified Mercalli intensities. Our estimates for the simulation related to the 1693 event yield a maximum intensity of VII-VIII and are therefore in complete agreement with the observed seismic intensity data reported by Galea (2007). Seismic intensity is an important parameter that has been traditionally used worldwide as a method for quantifying the shaking pattern and the extent of damage for earthquakes, especially for past earthquake for which there is no instrumental record. However, even if nowadays large instrumental recordings are available, it still provides a useful means of describing information contained in these recordings (e.g. Secomandi et al. 2013).

4 Concluding Remarks

These scenarios are for only two particular earthquake events. Events on other sections of the rift system (eg the western extremity of the Malta and Pantelleria grabens, or indeed a large magnitude event on the Hellenic arc) will have different effects in terms of both spectral response as well as geographical distribution. This deterministic approach will need to be combined with a probabilistic hazard assessment in order to provide a better picture of the risk faced by the islands.

Despite some uncertainties mostly due to source complexity, stochastic finite-fault modeling based on a dynamic corner frequency approach appears to be a reliable and practical method to simulate ground motion records of moderate and large earthquakes especially in regions where structural damage is expected, but only sparse ground motion recordings are available. In this paper, we have shown that in the Maltese archipelago, the ground motion from the repeat occurrence of historically recorded earthquakes, coupled with existing geological conditions and building typologies has the potential to cause significant structural damage on the islands. These preliminary results motivate us to carry out more detailed studies, in particular a comprehensive microzoning exercise with respect to shallow structure and ground response, and the formulation of a framework

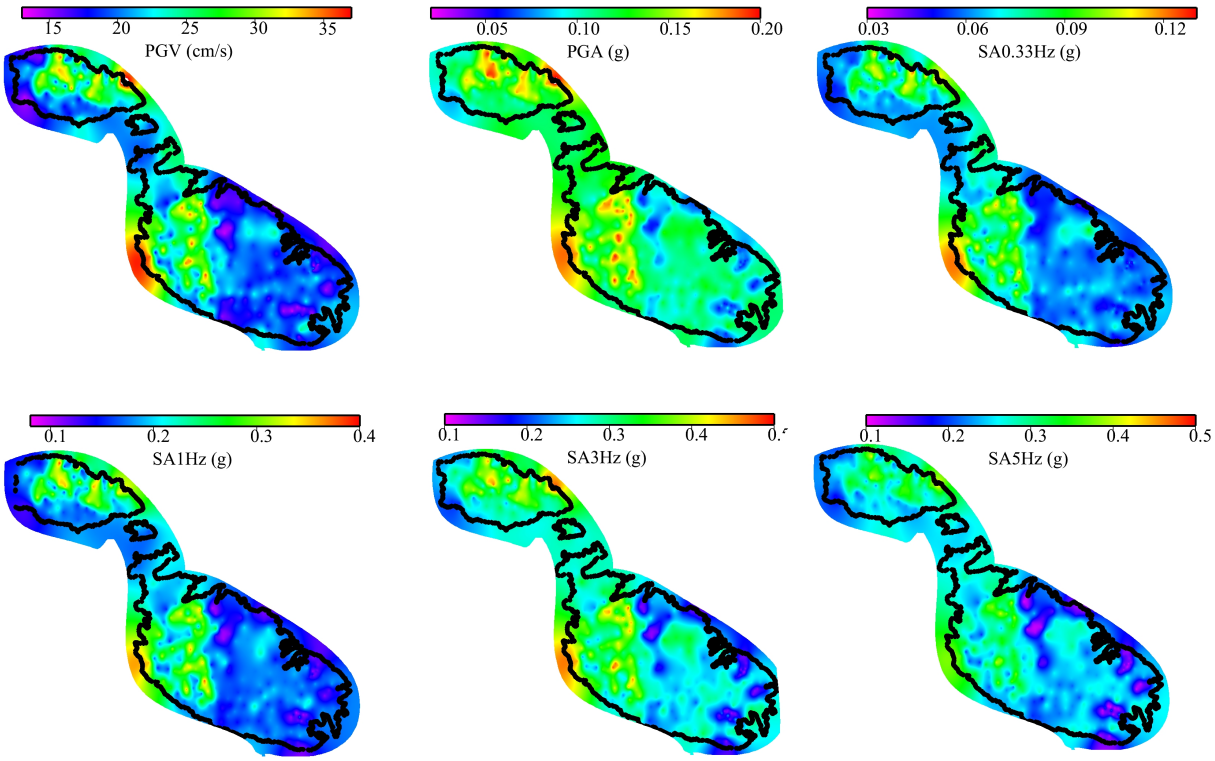

Figure 3: Ground motion scenarios for earthquake of Mw=7.6 located on the Hyblean-Maltese escarpment.

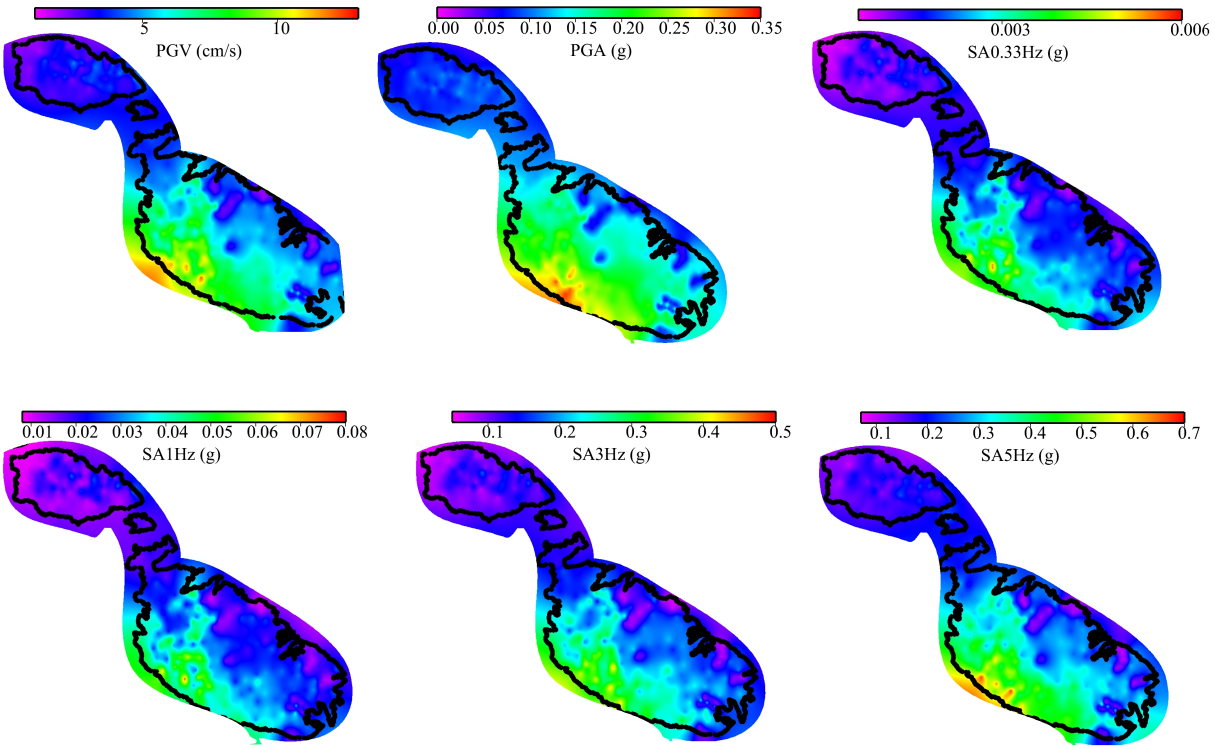

Figure 4: Ground motion scenarios for earthquake of Mw = 5.0 located about 20k south of Malta.

for the functional seismic vulnerability assessment.

We can conclude by saying that a well-crafted scenario provides a powerful tool for decision makers, emergency planners, private industry, and the general public to begin to draft mitigation policies and programs. It will help the community weigh various risks associated with the earthquake and begin to set priorities that will systematically reduce the impact of a likely future event.

5 Acknowledgments

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Research Article

PITTOSPORUM PIT SCALE, *PLANCHONIA ARABIDIS* (HEMIPTERA: ASTEROLECANIIDAE) AND ITS LEAF GALLS INDUCED ON PITTOSPORUM TOBIRA IN SOUTHERN ITALY

Alessio Vovlas[1], Chris Malumphy[2] and David Mifsud[3]
[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università Degli Studi di Torino, Via Accademia Albertina, 13, Italy
[2]The Food and Environment Research Agency, Sand Hutton, York, United Kingdom
[3]Department of Biology, Junior College, University of Malta, Msida, Malta

Abstract. The morphology of the adult female pittosporum pit scale, *Planchonia arabidis*, a commonly encountered sap-feeding insect in Europe, is described and illustrated, based on material collected from southern Italy on Pittosporum tobira. Histopathological observations are made for the first time on the aforementioned host-plant on which typical pit galls are induced by *P. arabidis*. Distribution and host-plant data is also provided for this species at a global level.

Keywords Pit scales, Mediterranean, Histopathology

1 Introduction

The family Asterolecaniidae constitutes a well defined group of 23 genera of scale insects (Hemiptera: Coccoidea), commonly referred to as pit scales, and currently accommodates 229 species worldwide (Ben-Dov 2006). They are highly specialised plant-sap feeders and many species produce deep circular pits that disfigure the host-plant, hence their common name. All of the European species are rather similar in appearance, with the adult female being circular or oval in shape, and between $0.5-5.0$ mm in length. General body colouration can vary from pale yellow or green, to dark brown. Each female is enclosed in a glassy wax test with a marginal fringe of wax filaments. The pit scale genus *Planchonia* contains ten species worldwide, of which six are established in Europe and the Mediterranean. A further species, Euphorbia pit scale *P. stentae* (Brain), has been found in the UK on *Hoodia gordonii* (Masson) Sweet ex Decne (Euphorbiaceae) imported from Namibia, but has not established (Malumphy 2009). *Planchonia arabidis* Signoret is the most widespread and abundant species of *Planchonia* in Europe. It is a serious pest of some ornamental plants in the USA, causing distortion and death of the growing tips (Gill 1993), and on one occasion damaged a sugar beet (*Beta vulgaris* L.) crop in Brownstown, Washington State, USA, resulting in about 50% yield reduction (Landis 1968). It has also been recorded causing severe damage to Crimean ivy (*Hedera taurica* Carr.) in the Crimea, Ukraine (Vasil'eva 1986), a plant widely used for vertical landscaping and ground cover. Feeding by *P. arabidis* usually induces a deep 'pit gall' on the surface of the host plant, whereas most *Planchonia* spp. only induce a shallow depression in the host, or have no visible effect on the surface of the plant, apart from localised chlorosis. Pit gall development varies with host-plant species and feeding location, the pits are usually more pronounced when the scales feed near the growing tips.

The purpose of this study is to present a detailed morphological description and illustration of the adult female *Planchonia arabidis*, review its global distribution and host-plant data, and to provide detailed histopathological observations on the leaf galls induced by the in-

Correspondence to: D. Mifsud (david.a.mifsud@um.edu.mt)
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sect on Pittosporum tobira.

2 Material and methods

Specimens of *Planchonia arabidis* used in the present study were collected in May 2011 from a private garden in Specchiolla, Brindisi province, Southern Italy (geographical coordinates 40° 44′ 84”N; 17° 44′ 21”E). The *P. arabidis* were preserved in 75% alcohol, slide-mounted in Canada balsam according to published methods (Malumphy 2002), and studied with a Zeiss compound microscope (Axioscope 2 plus). For the histopathological observations, galled and ungalled midrib portions of the host-plant (Pittosporum tobira) were fixed in FAA (formaldehyde - Acetic solution), dehydrated in a tetriary butyl alcohol series, and embedded in paraffin ($58°C$ melting point). Embedded material was sectioned at 15 micrometers thick sections, and stained with safranin and fast green and mounted permanently for microscopic examination (Johansen 1940).

Figure 1: Adult female pittosporum pit scales, *Planchonia arabidis* on Pittosporum ©US National Collection of Scale Insects Photographs Archive, USDA Agricultural Research Service, Bugwood.org

3 Results

Planchonia arabidis Signoret, 1877 - Pittosporum pit scale (Figs. 1 and 2)
Planchonia arabidis Signoret, 1877: 608.
Planchonia hederae Lichtenstein, 1880: xlv. Synonymy by Russell, 1941: 44.
Planchonia valloti Lichtenstein, 1882: lxxv. Synonymy by Fernald, 1903: 51.
Asterolecanium massalongianum Targioni Tozzetti, 1893: 295. Synonymy by Fernald, 1903: 51.
Pollinia thesii Douglas, 1893: 55. Synonymy by Russell, 1941: 44.
Asterolecanium arabidis; Cockerell, 1896: 327. Change of combination.
Planchonia arabidis; Kozár and Drozdják, 1998: 30. Revived combination

3.1 Description

Adult female. *Habitus.* Test varies from oval to broadly pyriform, dorsum strongly convex, whitish to pale brown, translucent to opaque, with a wax fringe around the margin and along the mid-dorsum (these are often missing as they are easily rubbed off), usually about 2.0−3.5 mm long and 1.5−2.5 mm wide, occasionally up to 5.0 mm long and 3.5 mm wide. *Slide mounted.* Oval to broadly pyriform, posterior end tapering, apex of abdomen usually slightly concave, 1.7 − 3.2 mm long and 1.2−2.2 mm wide. Antennal tubercles circular, with 2 large thick and 2 − 7 short setae. Labium with 2 pairs of setae. Leg vestiges entirely absent. Anal lobes undeveloped, with 6 pairs of short setae, and a pair of long (100 − 128 microns) apical setae. Anal-ring well developed with about 46 pores and 6 setae (80 − 104 microns), situated in anal tube. Body margin with 2 − 3 rows of large 8-shaped pores, usually becoming a single row posteriorly; these joined sub-marginally by 2 − 3 parallel rows of quinquelocular pores, becoming a single row anteriorly and posteriorly. Spiracles with broad bar, heavily sclerotised, peritreme with quinquelocular pores, and each with a band of quinquelocular extending along the stigmatic furrow to the body margin. Ventral surface with small 8-shaped pores scattered, but also forming a submarginal band anteriorly. Multilocular disc pores with 6−11 loculi (most with 10 loculi), in 3 or 4 complete transverse bands, and 2 − 4 interrupted rows. Dorsal surface with numerous large 8-shaped pores, these often form 5 − 7 transverse bands in median area. Small 8-shaped pores scattered between large 8-shaped pores and margin, occasionally scarce or absent. Simple disc pores numerous. Tubular ducts distributed evenly, ca 40 microns long. The above description is adapted from Russell (1941), Kosztarab et al. (1988), Gill (1993), Kosztarab (1996) and from the material examined during the present study. *Planchonia arabidis* is morphologically highly variable, particularly regarding the number and distribution of large and small 8-shaped pores, quinquelocular pores, and multilocular pores. Descriptions and illustrations of the adult female are provided by Russell (1941), Borchsenius (1950), Ferris (1955), Kosztarab et al. (1988), Gill (1993), Kosztarab (1996) and Stumpf and Lambdin (2006). Russell (1941) provides a key for the identification of *Asterolecanium* of the world, which includes all six species now assigned to *Planchonia*, established in Europe and the Mediterranean.

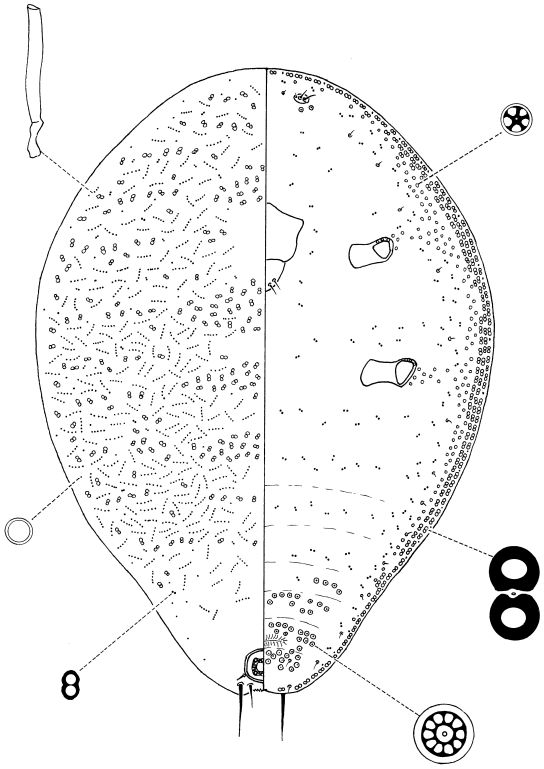

Figure 2: *Planchonia arabidis* Signoret; dorsum on the left and venter on the right

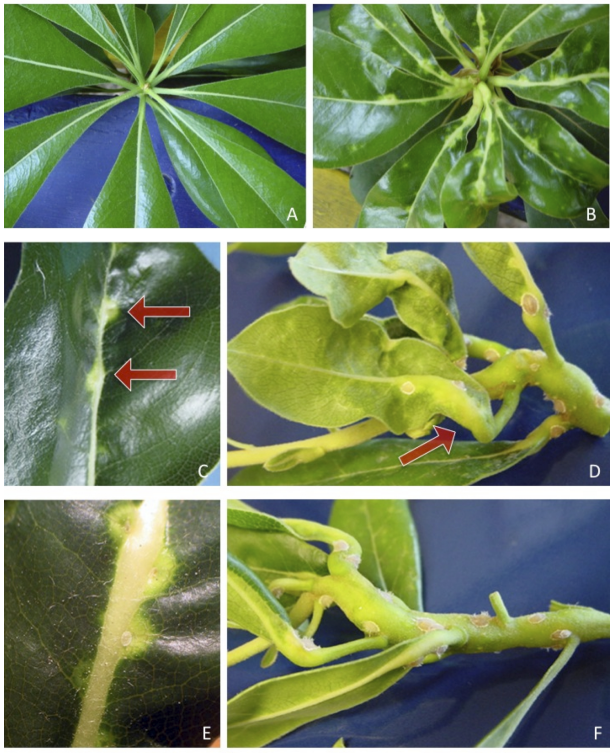

Figure 3: Leaf and stem galls of Pittosporum tobira plants induced by *Planchonia arabidis* (Italian population); A. Healthy apical foliage for comparison; B. Heavy infestation of apical foliage, with numerous galls prevalently located on midribs; C and E. Close up illustration of midrib galls (arrows), growth at scale's feeding sites; D and F. Stem and leaf infestation, showing deformed leaves (arrow), caused by the disorder of xylematic structures induced during the neoplatic expansion of the gall.

3.2 Geographical distribution

Planchonia arabidis occurs throughout Europe, from the Mediterranean in the south to Sweden in the north, and from Portugal in the west to the Ukraine in the east. It also occurs in the Near East, and has been introduced to Madeira, North America and the Caribbean (Ben-Dov 2012).

3.3 Host plants

Planchonia arabidis is polyphagous, recorded feeding on 27 plant genera belonging to 23 plant families, including many ornamental species (Ben-Dov 2012). In Europe it is most frequently found on common ivy (*Hedera helix* L.), and in the USA on *Ceanothus* and Pittosporum.

3.4 Histopathology of induced galls on Pittosporum

Induced galls by *Planchonia arabidis* on Pittosporum tobira are mainly found on or near the midrib of leaves (Figs. 3B, C, and E) but can be occasionally observed on the stem (Fig. 3 D and F). Gall induction starts only after insertion of the insect stylets, and is frequently associated with various degrees of deformation of infected leaves. The gall morphogenesis is characterised by large hyperplasia around the feeding points where radial proliferation of parenchyma cells (more than $12-15$ cell layers) causes strong disorganisation of the vascular elements (Fig. 4D). The midribs in un-infected leaves are iso-diametric in size, and their vascular system in cross section shows xylem and phloem in compact and regular arrangement (Figs. 4 A and B). The main changes in relation to the galled midribs are exclusively observed in the vascular system. The xylematic elements are disorganized and divided in three separated bundles to the hyperplasia of the associated parenchyma (Fig. 4D). In mature midrib galls, the gall diameter is about $1.5-2$ times the size of healthy midribs. The stimulus of this gall induction start from the saliva (soon after the host-parasite relationship is well established), but the precise mechanism of induction and subsequent growth, is unknown. Additional observations are needed in the next growth season.

The process of gall induction by *P. arabidis* and development on Pittosporum (although a wide host-range of the species is known worldwide) is unusual and unique for this cosmopolitan scale and is reported here for the first time. The gall-making ability of *P. arabidis* enlarges the list of Asterolecanidae gall inducers. Although galls and leave deformations can be conspicuous

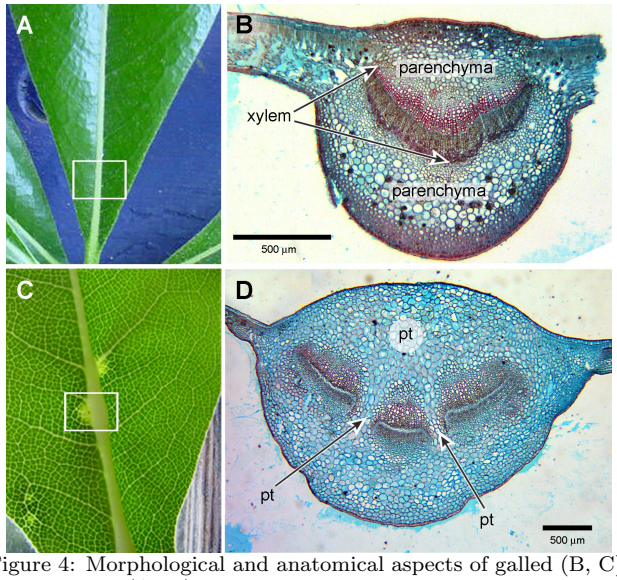

Figure 4: Morphological and anatomical aspects of galled (B, C), and ungalled (A, B) midrib portions of Pittosporum tobira; B. Cross section of ungalled midrib portion, showing entire structure of xylem and parenchyma tissues; C. Midrib *Planchonia* - feeding point with evident abaxial and adaxial expansion; D. Cross section of mature midrib gall showing hyperplasia (parenchyma cell proliferation) disorganising the vascular structure. Note the evident interruptions (arrowed) by the neoplastic parenchymatic tissues (pt).

mainly at the apical foliage (Fig. 3B), they rarely do any real agronomic damage to plants. It's only in cases of heavy infestations that occur repeatedly over several seasons which may slow the growth of the plant or make the appearance unattractive.

Our preliminary results presented here suggest that future research is needed. This pest species has a potential to cause serious problems on ornamental plants, and could be a potential threat within Mediterranean regions.

4 Acknowledgments

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Review Article

THE SPECTRUM OF ISCHEMIA-INDUCED WHITE MATTER INJURY VARIES WITH AGE

Christian Zammit[1], Richard Muscat[2] and Mario Valentino[2]
[1]Department of Anatomy, University of Malta, Malta
[2]Department of Physiology and Biochemistry, University of Malta, Malta

Abstract. Stroke is a neurological condition that targets the whole range of the human population, from the pre-term infant to the elderly and is a major cause of death worldwide (Ingall 2004). During its lifespan, the brain's vulnerability to hypoxia-ischemia varies. Term infants who suffer this insult usually exhibit widespread neuronal injury in the cerebral cortex with a stroke-like distribution of damage (Deng 2008), whereas in pre-term infants immature oligodendrocytes and subplate neurons below the neocortex are most vulnerable and result in Periventricular Leukomalacia (PVL) (Back et al. 2007; McQuillen et al. 2005). The incidence of stroke decreases in young adulthood, but peaks again in the elderly. Moreover, the underlying pathological mechanisms that occur following ischemia are different at each stage.

Experimental stroke research on stroke has traditionally focused on grey matter injury, but recent evidence indicates that white matter injury is a critical part of its pathophysiology. In this debilitating condition the mechanisms of ischemia-induced damage differ with age and all cellular components of white matter (axons, oligodendrocytes and astrocytes) are affected.

This review paper focuses on the relative vulnerability to ischemia of white matter during the course of development and on our recent findings of how individual cellular components are affected during each stage.

Keywords White Matter Development; Axon; Oligodendrocyte; Astrocyte; Optic Nerve; White Matter Injury; Ageing; Ischemia; Periventricular Leukomalacia.

Correspondence to: M. Valentino (mario.valentino@um.edu.mt)

1 Introduction

There are currently more than 250 identified neurological diseases. These constitute a worldwide problem that affects over 2 million people in Britain alone (Brain Research Trust, 2003). Fifteen million people suffer from stroke worldwide yearly (World Health Organisation 2002), and in those who survive the initial insult, mortality during the first year is about 20% (Dewar et al., 1999). The disease affects not only the patients, but also causes considerable burden on family members and on society in general. Taking into consideration inpatient rehabilitation and follow-up care, the estimated direct and indirect costs of stroke for 2009 were 68.9 billion in the U.S.A. and 32.3 billion in the countries of the European Union (Annunziato 2009).

There is substantial evidence that with age, ischemia-induced brain injury is more pronounced (Ay et al. 2005; Davis et al. 1994; Duverger et al. 1988; Kharlamov et el. 2000; Shapira et al. 2002; Sutherland et al. 1996). Thus, middle cerebral artery occlusion in 30-month-old mice resulted in a significantly larger volume of infarction, than in $<$ 17-month-old mice (Davis et al. 1994) and there was a 23% increase in infarct volume in 20 to 24-month-old rats compared to those 4-months old (Kharlamov et al. 2000). A study on sixty patients with an acute ischemic stroke reported an age-dependent increase in conversion of ischemic tissue into infarcted tissue (Ay et al. 2005).

Age has been regarded as the most significant risk factor for stroke for several decades and to result from a combination of atherosclerosis in the cerebral arteries

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associated with age-related co-morbidities such as cardiac dysfunction, hypertension, diabetes mellitus and hypercholesterolemia (De Grey, 2005). However, ageing also alters the relationship between myelin and axons and changes the relative densities of the brain's cellular constituents (Hinman et al. 2006; Hinman et al. 2007; Peters et al. 2002; Sandell et al. 2002). Therefore, an understanding is required of whether the ageing process underlies the susceptibility of white matter to ischemic injury and therefore whether this heightened vulnerability is associated with a change in the course of the underlying mechanisms that contribute to this selective type of injury.

The magnitude of the problem posed by ischemic injury on pre-term infants is very significant. In the U.S.A. approximately $50,000$ low birth-weight infants ($< 1500\,g$) are born every year. Advances in medical treatment have led to survival of almost 90% of such infants (Deng 2008). About 10% of the survivors later develop spastic motor deficits (Doyle 2010), and about $20-25\%$ later exhibit cognitive, attentional, behavioural, and/or socialisation defects that significantly impair their quality of life (Msall 2010; Johnson 2009). PVL is the predominant form of brain injury that underlies mortality and morbidity in pre-term and term infants who suffer from a hypoxic-ischemic insult and is the leading cause of cerebral palsy in premature infants (Deng 2008).

Since the establishment of the central role of the excitotoxic cascade in the neurochemical pathological process that occurs during ischemia, numerous clinical trials have been performed, but virtually every drug that conferred protection to neurons in experimental models failed in those trials (Del Zoppo 1998, Del Zoppo 1995; Dirnagl 2006; O'Collins et al. 2006). A primary reason for this was the failure of the drugs used to protect white matter. Most experimental work on stroke was performed on rodent brains, but white matter constitutes only 13% of their brain (Zhang et al. 2000) whereas it accounts for 50% of the volume of the human brain (Zhang et al. 2000). Besides, the metabolic rate of white matter is only modestly reduced in comparison to that of grey matter (Nishizaki 1988). Moreover, ischemic injury is never limited to grey matter alone, and white matter injury contributes significantly to the clinical deficits that lead to mortality and morbidity.

The mechanisms that underlie white matter injury are unique and very complex (Agrawal et al. 1997; Fern et al. 1997; Follett et al. 2000; McDonald et al. 1998; Sanchez-Gomez et al. 1999; Stys 2004; Tekkök et al. 2001; Tekkök et al. 2007; Wrathall et al. 1992). Two distinct mechanisms seem to operate sequentially or simultaneously following energy depletion and can be traced to intra-axonal ionic distribution and excitotoxicity with over-activation of AMPA and kainate receptors (Stys 2004; Tekkök et al. 2007). However, most of the data is derived from experimental work carried out in young adult animals (Tekkök et al. 2008). This review highlights the main difference in the mechanisms of ischemic white matter injury during different developmental stages. A preliminary account of part of our work mentioned here has been published elsewhere (Zammit et al. 2011, Alix et al. 2012).

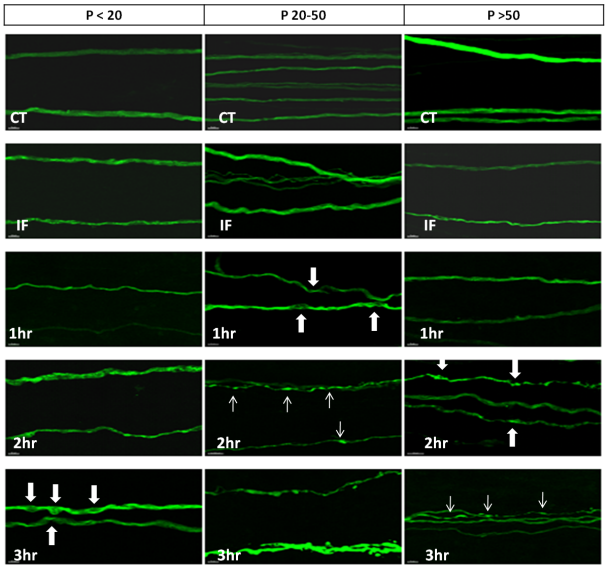

Figure 1: Progress of axonal injury following 30 mins of OGD There is progression of injury in all age groups, but features of axonal damage are first evident in $P20-50$ mice, followed by $P > 50$ mice, and finally $P < 20$ mice. Thick arrows mark axonal swelling; thin arrows mark beading formation. CT - controls; IF - immediately fixed after OGD; 1hr - 1 hour reperfusion; 2hr - 2 hours reperfusion; 3hr - 3 hours reperfusion (Zammit et al. 2011). (Magnification X60 lens - X400 digital zoom)

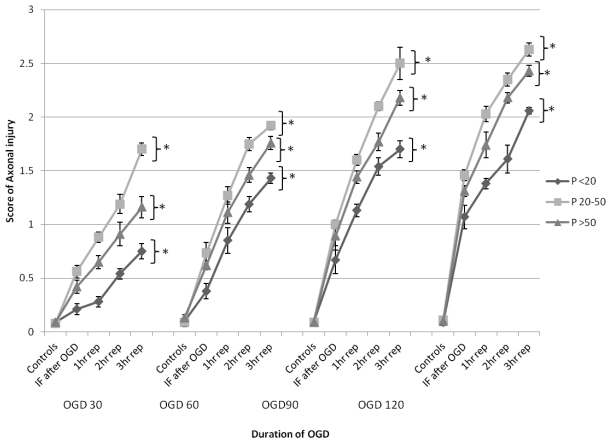

Figure 2: Axons from $P20-P50$ mice are extremely vulnerable to ischaemia Comparing axonal injury following ischemia between different age groups. There was a statistically significant difference ($*p \leq 0.001$) in axonal injury score between the different age groups after each duration of OGD. $P20-50$ mice (box) were the most vulnerable to injury, and $P < 20$ mice (diamond) were the most resistant (Zammit et al. 2011).

2 Vulnerability of axons to ischemia and age

The central nervous system of the neonatal mammal was formerly regarded to be more resistant to ischemia than that of the adult (Duffy et al. 1975). This was often interpreted to be a safety mechanism of the neonate brain which is more prone than that of the young adult to suffer from periods of hypo-perfusion during its pre- and post-natal phases (Vannucci 1990; Volpe 1992).

Fern et al. (1998) showed that neonatal white matter to be very resistant to ischemia. Using optic nerves from neonatal mice at P2 (postnatal day 2) they showed that axons at this age group were more tolerant than older aged to anoxia, aglycaemia or their combination in maintaining evoked compound action potential (CAP), and in recovery of function during reperfusion (Fern et al. 1998). This result was supplemented by an imaging study that tested the observed preservation of CAP with maintenance of axon structural integrity (Zammit et al. 2011) during variable degrees of an ischemic insult. In this study, optic nerves from Thy-1/GFP-M mice from three different age groups ($<$ P20, P20$-$P50 and $>$ P50) were exposed to variable durations of oxygen-glucose deprivation (OGD) at 30, 60, 90 and 120 mins respectively. Quantitative scoring of axon injury revealed that the degree of structural damage in axons from neonatal mice (¡ P20) was significantly smaller than that in older mice (Figure 1).

The sensitivity of rat grey matter to anoxia and aglycaemia increases progressively from birth to adulthood, consistent with the rise in metabolic rate of this tissue (Cherubini et al. 1989; Crépel et al. 1992). White matter does not follow a similar pattern. Fern et al. (1998) showed that white matter in mice between P20 and P50 is most vulnerable to an ischemic insult in terms of decrease in CAP during OGD and of the rate of recovery from the insult. This vulnerability starts to decrease at P50 which is in agreement with our published observations (Zammit et al., 2011). In that study, the degree of axonal injury in P20 - P50 mice was significantly higher than in any other age group, and this sensitivity stabilised in mice older than P50 (Figure 2).

In view of the above findings the following questions arise: Why is there such a difference in vulnerability? What types of axons are present at each developmental stage? Do the underlying ischemia-induced pathological mechanisms vary at different developmental stages?

3 Mechanisms of ischemia-induced injury in axons and development

In the mouse optic nerve, myelination starts at about P7, with few axons having only one whorl of myelin at this age (Foster et al. 1982). The rate of myelin deposition thereafter peaks at P21 $-$ P28, and from this point onward, the process of myelination is at its highest (Skoff et al. 1976). The increased tolerance to OGD-induced damage is dominated by unmyelinated axons and may be attributed to the lower metabolic rate of neonatal white matter (Duffy et al. 1975; Hansen 1985). At this developmental stage, there is also increased glycogen deposition in astrocytes (Kohle et al. 1977), and, but only transiently, in immature axons (Bruckner et al. 1981).

The mechanism of ischemia-induced injury in these axons differs from that at other stages of development. In young adult white matter, ischemic injury is mediated by AMPA/Kainate receptors (Tekkök et al. 2001; Baltan et al. 2008). However, McCarren et al. (2007) showed that ionotropic glutamate receptor agonists did not damage rodent white matter axons at P3 and damaged them only minimally at P7. Since unlike myelinated white matter, premyelinated axons do not express functional glutamate receptors on their axolemma, it was suggested that there could be a distinct mechanism of injury at this developmental stage, coupled to ionic imbalances culminating in deleterious intra-axonal Ca^{2+} overload (McCarran et al. 2007).

The period of low tolerance to ischemia (between P20 and P50) coincides with the process of myelination, and the increase in sensitivity to ischemia could be attributed to the onset of the associated heightened metabolic activity (Azzarelli et al. 1980; Davison et al. 1966; Wiggins 1982). Fowler and colleagues (2003) also proposed that myelination may increase axonal vulnerability to oligodendrocyte-induced damage, as perturbation of the oligodendrocyte-myelin-axon interaction in myelinated white matter decreased axonal damage after AMPA injection. Myelination is not the only contributor as Na^{+}-channel density in optic nerve axons also varies with age, starting from $< 2\,\mu m^2$ in the neonate (Waxman et al. 1989), increasing up to the age of about P25, and declining in adulthood (Xia et al. 1994). During myelination, the Na^{+} channels aggregate at the nodes of Ranvier and the change in their density results in a persistent non-inactivating Na+ current, which exacerbates white matter injury after anoxia (Alzheimer et al. 1993). Ca^{2+} may also have a role at this stage since Ca^{2+} currents were observed to increase in magnitude in the postnatal period (Lorenzon et al. 1995), and have been shown to contribute directly to anoxic injury in white matter (Fern et al. 1995).

We have recently reported that large ($> 0.4\,\mu$m in diameter) pre-myelinating axons are more sensitive to OGD than smaller pre-myelinated and myelinating axons. This heightened sensitivity could not be attributed to the myelination process per se, and blockage of intracellular Ca^{2+} was not protective during a $60-$ min period of OGD (Alix et al., 2012). Blockade of NMDA and non-NMDA glutamate receptors (GluRs) alone provided only partial protection to P10 axons in rat optic nerves following 60 mins of OGD plus 60 mins of recovery and addition of L-type and P/Q-type voltage-gated calcium channel (VGCC) blockers to those GluRs blockers produced complete recovery of CAP following the same ischemic insult (Alix et al. 2009). Comparison of OGD-induced damage to small ($< 0.4\mu$m) and to large ($> 0.4\mu$m) premyelinating axons showed that the former were protected by GluR blockers alone, whilst the latter needed addition of VGCC-blockers to confer protection (Alix et al. 2012). That study gave direct evidence of the importance of VGCC in this age group and provided new insight on the pathophysiological mechanism of injury during ischemia in these very sensitive axons.

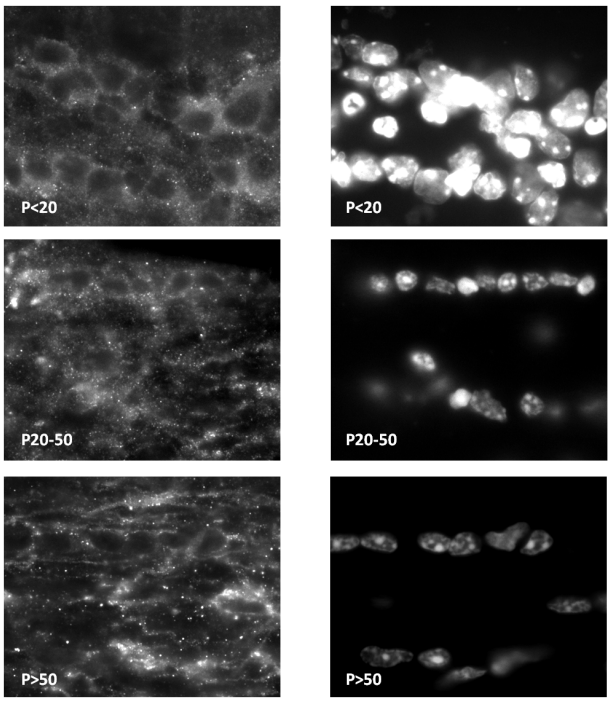

Figure 3: APC +ve oligodendrocytes in mouse optic nerve after 60 mins OGD Cropped sections from high power micrographs (X60) of optic nerve sections from 3 different age groups ($P < 20$, $P20-50$, and $P > 50$) stained with APC (left) and Hoechst stain (right) after 60 mins OGD. Optic nerves from $P < 20$ mice had a greater number of pyknotic nuclei when compared to older age groups (Zammit et al., 2011).

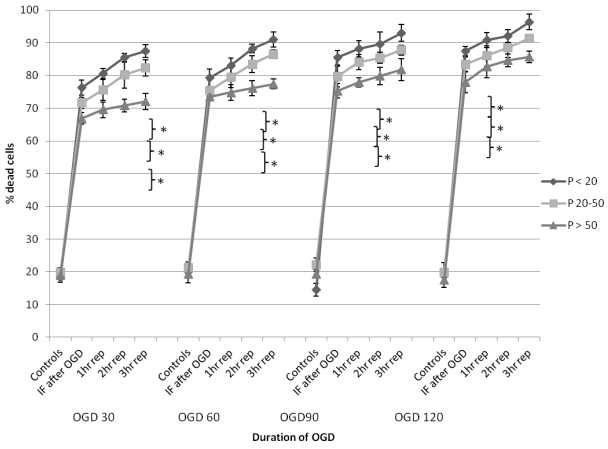

Figure 4: Oligodendrocytes vulnerability to ischaemia decreases with age Comparing percentage dead oligodendrocytes following ischemia between different age groups. There was a statistically significant difference ($*p \leq 0.05$) in percentage dead oligodendrocytes between the different age groups after each duration of OGD. APC +ve oligodendrocytes at $P < 20$ were the most vulnerable to injury, and tolerance to ischemia increased with age (Zammit et al., 2011).

4 Axonal injury and the effect of age

In young adults and in ageing white matter, glutamate excitotoxicity plays a central role in ischemia-induced injury. It is therefore not surprising that AMPA/Kainate receptors have been found to mediate excitotoxic injury in ageing white matter tracts (Tekkök et al. 2008). These authors studied ischemia-induced injury in the optic nerve of $1-, 6-, 12-, 18-$ and 24-month-old mice. Excitotoxic events occurred more quickly and more vigorously in ageing white matter, but were not mediated by Ca^{2+} influx (Tekkök et al. 2008). In contrast, in young adults, ischemia-induced injury could be almost entirely prevented if the OGD was performed in a Ca^{2+} -free medium (Fern et al. 1995; Tekkök et al. 2001; Tekkök et al. 2007). In this context, accumulation of Na+ that leads to lethal cellular swelling and reversal of Na^{+}-dependent glutamate transporter function with further efflux of glutamate (Baltan et al. 2008) and release of intracellular Ca^{2+} (Ouardouz et al. 2003), could be the underlying mechanisms of ischemia-induced white-matter injury in the ageing brain. Of note is the study by Baltan et al. 2008, that showed a significant and selective up-regulation of GLT1 in older rodents.

Besides enhanced glutamate excitotoxicity, other factors predispose to the vulnerability in ischemic injury to white matter elements as the brain ages beyond maturity. Na^{+}/K^{+} ATPase activity decreases with age. This leads to inability to maintain an appropriate transmembrane ion gradient, which results in slower restoration of normal ion gradients in ageing tissue following energy deprivation. Consequently, pathological pro-

cesses initiated by ion dysregulation would last longer, and produce more damage (Scavone et al. 2005). Besides, decline in mitochondrial function in brain cells (Toescu 2005) and increase in free radical generation (Droge et al. 2007) also occur with increasing age.

5 Early oligodendrocyte progenitor cells and Periventricular Leukomalacia

Periventricular Leukomalacia (PVL) is the most common cause of brain injury in premature infants (Back et al. 2004) and results from an ischemic insult through the high-risk developmental period of 23 and 32 weeks gestation (Alix 2006). The pathogenesis of PVL comprises three major interacting factors: cerebral ischemia, systemic infection and inflammation, and maturation-dependent intrinsic vulnerability of premyelinating oligodendrocytes (Volpe et al. 2011).

Oligodendrocyte injury has long been regarded as the hallmark of PVL. Oligodendrocyte development occurs in four stages: early oligodendrocyte progenitor cell (OPC), late OPC (also called premyelinating oligodendrocytes), immature myelinating oligodendrocyte, and mature myelinating oligodendrocyte (Back 2006). Back and Volpe used brain slices containing corpus callosum from *P*2 mice to demonstrate the relative susceptibility of early OPC, late OPC, and immature oligodendrocytes. After induction of OGD, early OPC were significantly more resistant to ischemia than late OPC (Back et al. 2002). This maturation-sensitivity of late OPC leads to preferential white matter injury in the neonate (Volpe et al. 2011) and coincides with the high-risk period for PVL in humans (Craig et al. 2003).

The major factors that underlie the maturation-dependent susceptibility of late OPC are: abundant production of reactive oxygen and nitrogen species during PVL, delayed development of glutathione antioxidant defences, acquisition of Fe^{2+}, and exuberant expression of the major glutamate transporter, of AMPA receptors deficient in the GluR2 subunit (and therefore Ca^{2+}-permeable), and of NMDA receptors (also Ca^{2+}-permeable) (Volpe et al. 2011).

6 Vulnerability of immature and mature myelinating oligodendrocytes to ischemia

As indicated above, late OPCs are important contributors to PVL. Therefore several questions immediately come to mind. What role do myelinating oligodendrocytes feature in ischemic injury? Do immature and mature myelinating oligodendrocytes vary in their vulnerability to ischemic damage? Do they contribute to the continuum of white matter susceptibility during ischemia?

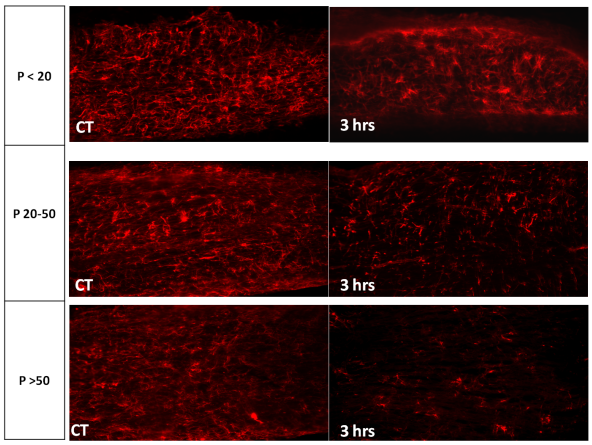

Figure 5: GFAP-stained astrocytes in mouse optic nerve after 60 mins OGD Low power micrographs (X20) showing GFAP staining in optic nerve from mice (P < 20, P20 – 50, and P > 50) after 60 mins OGD. Images on the left (CT) shows control images of each age group; images on the right (3 hrs) shows images taken after OGD 60 min + 3 hrs reperfusion. There is a decrease in GFAP intensity between controls and injured nerves and this is more marked in older mice (Zammit et al. 2011).

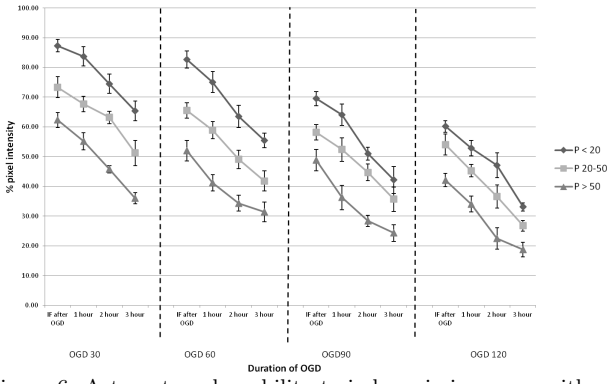

Figure 6: Astrocyte vulnerability to ischaemia increases with age Comparing percentage decrease in GFAP-pixel intensity following ischemia between different age groups. Astrocytes from mice P < 20 (triangle) retained a higher percentage of pixel intensity than other age groups, which suggests to a higher tolerance to ischemia-induced injury (Zammit et al. 2011).

To gain further insight into these questions, the effect of different durations of OGD (0, 60, 90, 120 mins) on the viability of myelinating oligodendrocytes in mouse optic nerves from three different age groups (< P20, P20 – P50 and > P50), were studied by Zammit et al. 2011 and Alix et al., 2012. In these studies, the authors used anti-APC antibody in combination with Hoechst 33342 to determine the percentage number of dead oligodendrocytes in each stage. (Figure 3). Data from these experiments clearly show that 30 mins of OGD was sufficient to kill almost 70% of oligodendrocytes in all age groups, with the number of dead oligodendrocytes in neonatal mice (P < 20) being significantly higher ($p > 0.05$) than that in the older age groups (Figure 4).

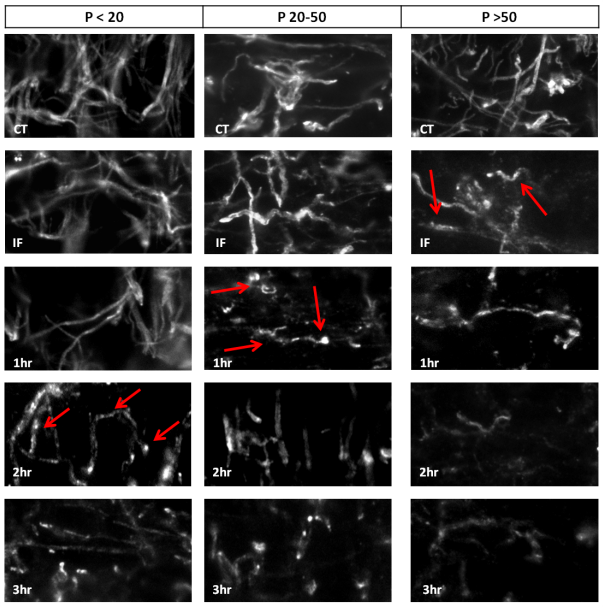

Figure 7: Astrocyte process damage is more pronounced and occurs earlier in older mice Cropped sections from high power micrographs (X60) of optic nerve sections from 3 different age groups ($P < 20$, $P20-50$, and $P > 50$) stained with GFAP, showing detail of astrocyte processes. Astrocyte processes damage (red arrows) is visible after 2 hour of reperfusion in $P < 20$ mice, after 1 hour reperfusion in $P\ 20-50$ mice, and in immediately fixed slices after OGD in $P > 50$ mice (Zammit et al. 2011).

In mouse optic nerve, immature myelinating oligodendrocytes predominate at around P7 to P10, and mature myelinating oligodendrocytes appear at around P14 and increase in number from P20 onwards (Craig et al. 2003). Therefore, our results showed that immature myelinating oligodendrocyte (which predominates in P ¡20 mice) are more vulnerable than mature myelinating oligodendrocytes (which predominate in older age groups). In cell cultures, mature oligodendrocytes ($A2B5^-$ /GC^+) were more resistant to ischemia than immature ones (O4+/GC-) which led Fern et al. 2000 to propose that rapid ischemic cell death of the immature oligodendrocytes was mediated by Ca^{2+} influx via non-NMDA glutamate receptors, and exacerbated by significant autologous feedback of glutamate from cells on their own receptors (Fern et al. 2000).

7 Astrocytes are vulnerable to ischemia

Astrocytes have long been thought to be very resistant to ischemia, probably because most of the early studies were performed in dissociated cultures (Goldberg et al. 1993). However, studies performed by Fern 2001 show that neonatal white matter astrocytes are more vulnerable to ischemic injury than axons at the same developmental stage.

The mechanism of ischemia-induced astrocyte injury varies with age. In P2 mice, significant astrocyte death was apparent just after $10-20$ min of ischemia, with death in approximately 50% after 80 min of OGD. This high sensitivity results from Ca^{2+} influx through T-type channels (Fern 1998). In older mice (P10), induced Na^+-K^+,-Cl^- and HCO_3^- channels contribute to osmoregulatory challenge (cell swelling), and are considered to be the main determinant of cell death (Thomas et al. 2004).

Live imaging of $P7-P14$ GFP-GFAP mouse optic nerves showed approximately 50% decrease of astrocyte cell bodies and 40% decrease of astrocyte processes after 20 mins of OGD and 1 hour of reperfusion (Shannon et al. 2007). This led to the proposal that astrocytes of actively myelinating white matter have a heightened sensitivity to ischemic-type injury, especially during the period of reperfusion.

8 Astrocyte vulnerability and age

In an immunocytochemical study, Zammit et al. 2011, and Alix et al. 2012, reported a predisposition of varying vulnerability to ischemia of successive developmental stages in astrocytes from mouse optic nerve exposed to different durations of OGD. In these studies, OGD resulted in a gradual decrease in pixel intensity in all age groups, and reperfusion further exacerbated the injury induced by the initial insult. The decline in GFAP staining following ischemia in $P < 20$ mice was less than in $P20-P50$ or in $>$ P50 mice (Figure 5 and 6). Also, the same duration of OGD resulted in loss of structural integrity of astrocyte processes at an earlier stage in $P20-50$ and $P > 50$ mice than in $P < 20$ mice (Figure 7). These findings support the hypothesis of Shannon et al., 2007 that astrocytes in myelinated white matter are more vulnerable than those in unmyelinated white matter. The mechanism behind this difference and the reason why astrocytes from older age groups were even more vulnerable is still unclear. However, caution must be exercised in the interpretation of these results since GFAP-staining intensity as a measure of astrocyte viability is not optimal (Shannon et al. 2007) and is highly variable although widely used as an assessment tool of viability by several groups (Chen et al. 1993; Davies et al. 1998; Fern 2001; Garcia et al. 1993; Petito et al. 1993; Schmist-Kastener et al. 1993).

9 Conclusion

There is convincing evidence that the vulnerability of white matter elements is dependent on their developmental stage.

Unmyelinated axons are very resistant to ischemia; injury to these axons is mediated by ionic imbalances with intra-axonal Ca^{2+} overload and not via

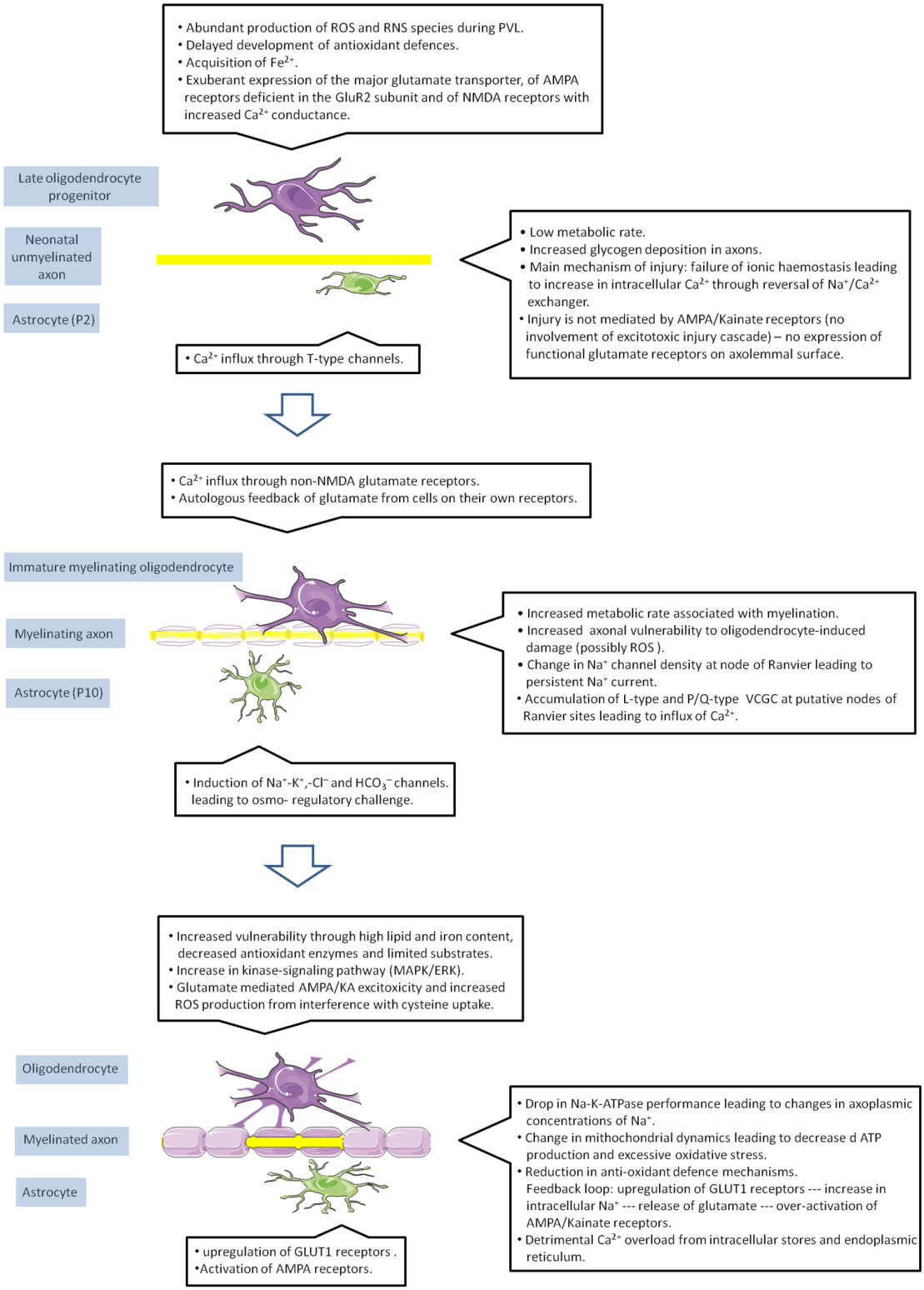

Figure 8: Cellular mechanisms of injury in white matter ischemia changes from development through ageing. Cartoon depicts the sequential and stage-specific mechanisms that are thought to contribute to white matter injury at P2, P10 and mature (> 1 year) white matter. The cellular targets are oligodendrocytes and axons in all age groups. This emphasizes the need for therapeutic approaches in white matter ischaemia to be more selective and cell-specific when considering age-related differences in neurobiology and pathophysiology.

AMPA/Kainate-receptor activation, which is followed by a period of heightened sensitivity to ischemia (Figure 8).

Large premyelinated axons ($> 0.4\,\mu$m in diameter) are extremely vulnerable and their injury is mediated by VGCC. Axons going through the process of myelination are also very sensitive to ischemia and their increased vulnerability is thought to coincide with the increased metabolic demand needed for myelination through the redistribution of Na^+ channels and an increase in Ca^{2+} currents. At this stage they have just initiated diameter expansion and express clusters of functional VGCC at future nodes of Ranvier.

The vulnerability of late OPCs can no longer be regarded as the sole contributor to immature white matter ischemic injury, and the central role of these axons must be appreciated. Myelination of the CNS is a timely and systematic process that occurs in an orderly spatial and temporal sequence. All CNS neurons are formed before birth, while white matter begins to develop and expand in the third trimester of gestation. White matter development is still incomplete at birth, and only 90% complete by 2 years of age. Before the onset of myelination when late OPCs and large calibre pre-myelinating axons co-exist and contribute to white matter injury, the susceptibility of a particular white matter region to ischemic injury will depend on axonal and oligodendrocyte maturation at that site. In young adult white matter, once axons are fully myelinated, their vulnerability to ischemia decreases, and ischemic injury is mediated by AMPA/Kainate receptor activation and calcium overload. With increasing age, white matter suffers another period of increased risk in vulnerability to ischemia. In the ageing brain, excitotoxic events occur earlier and more vigorously and are not mediated by Ca^{2+} influx. Instead, there is an accumulation of intracellular Na^+ leading to lethal swelling and reversal of Na+-dependent glutamate transporter (which increases in expression) and release of intracellular Ca^{2+}.

PVL is the most common cause of brain injury in the premature infant with the stage specific and most sensitive developmental stage of oligodendrocytes identified as the late OPC. This maturation-dependent intrinsic vulnerability plays a vital role in the pathophysiology of PVL since the late OPC are vulnerable to free radical attack and are very sensitive to excitotoxicity. Our recent findings suggest that immature myelinating oligodendrocytes are more vulnerable than the mature myelinating ones. The immature myelinating oligodendrocytes co-exist with axons undergoing myelination, and their vulnerability might contribute to the increased sensitivity to OGD of myelinating axons. Ischemia-induced mechanisms of injury in these cells has been postulated to be mediated by Ca^{2+} influx via non-NMDA glutamate receptors, and it is exacerbated by a significant element of autologous feedback of glutamate from cells onto their own receptors.

Astrocytes are also equally vulnerable to ischemia. Ischemic injury in immature astrocytes (P2 mice) is mediated by Ca^{2+} influx via T-type channels, whilst that in more mature astrocytes (P10 mice) is mediated by Na^+-K^+-Cl^- and HCO_3^- channel activation. Our recent study found that astrocytes present in younger mice ($P < 20$) are more resistant to ischemia than those present in older age groups.

White matter injury during ischemia plays a central role in the pathophysiology of stroke in all human age groups. Future therapeutic strategies should take into consideration selective white matter protection and recognize that the mechanisms that lead to this type of injury are variable with age. Extrapolation of findings and results from one age group to another may contribute to strategy failure. A better understanding of these differences, might give new insights on developing new therapeutic modalities for such a challenging disease.

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Review Article

Germination responses in *Callitriche truncata* Gussone

Jilly Camilleri[1] **and Sandro Lanfranco**[1]
[1]Department of Biology, University of Malta, Msida, Malta.

Abstract. This study investigated the germination responses of seeds of *Callitriche truncata*, an obligate hydrophyte that colonises temporary ponds in the Mediterranean, when subjected to different depths of burial and to varying patterns of initial flooding, and to examine the effect of flooding date on the growth and reproduction effort of this plant. All investigations were carried out at two different seed densities in order to investigate whether this factor would exert any effect on germination success and on accumulation of biomass. Seeds germinated from the 'no burial' treatment and from burial under 1 cm of sterile sediment with the rates of germination success declining rapidly with depth of burial. No germination was recorded from seeds buried deeper than 1 cm. The density of seeds per pot did not influence the results significantly. There was no significant difference in germination success of seeds subjected to 'Autumn flooding' and 'Winter flooding' treatments or across seed densities. Plants grown during the 'Winter flooding' treatment produced less total biomass and a lower proportion of reproductive biomass at the end of the experiment than seeds grown during the 'Autumn flooding' treatment. Although characterised by lower reproductive success, later flooding still permitted completion of life cycles and restocking of the seed bank. These findings are generally consistent with the results of previous studies in other temporary waters of the Mediterranean.

Keywords *Callitriche truncata*, Maltese Islands, germination, burial, biomass, flooding

Correspondence to: S. Lanfranco (sandro.lanfranco@um.edu.mt)

1 Introduction

The Southern Water Starwort, *Callitriche truncata* Gussone, is a hydrophyte or amphiphyte (Lansdown 2006) that colonises freshwater habitats in the Mediterranean and Western Europe. It is a frequent colonist of ephemeral freshwater habitats that alternate an aquatic phase with a terrestrial phase (Grillas et al. 2004). In the Maltese islands, this species colonises temporary freshwater rockpools that are inundated by rainfall at the start of the wet season, in September/October, and that subsequently experience one or more hydroperiods before undergoing desiccation at the start of the dry season in March/April (Lanfranco 2004). Nonetheless, in the Maltese Islands, *C.truncata* has only been observed in deeper pools characterised by a single, uninterrupted hydroperiod (Lanfranco and Sammut, in prep.). The life cycle of this species in such pools starts with germination shortly following the first flooding of the pools, growth and production of seeds during the aquatic phase, and survival in the seed stage on or in the bottom sediment of the pool throughout the dry season. Seeds are not actively dispersed but are deposited on the surface of the bottom sediment in the immediate vicinity of the parent plant and may subsequently undergo burial in the sediment (Camilleri 2012). The cyclic nature of the habitat, alternating a dry phase with an aquatic phase, imposes an annual life cycle on the species, necessitating re-establishment of populations every autumn. As such, the germination success of the seeds in the seed bank at the start of the wet season is a proximal initial determinant of the establishment of this species in the pool macrophyte community, implying that response to environmental cues for germination is a key factor in the persistence of this plant across years. Previous studies

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have indicated that germination responses of *C.truncata* seeds from temporary marshes in the Camargue are sensitive to depth of burial (Bonis et al. 1994) and seed density (Bonis et al. 1996) whilst the date of initial flooding has been found to lead to higher biomass and seed production of macrophytes in the temporary marshes of the Camargue (Grillas et al. 1998).

This study aimed to investigate the germination responses of seeds of *Callitriche truncata* when subjected to different depths of burial and to varying patterns of initial flooding, and to examine the effect of flooding date on the growth and reproduction effort of this plant. A negative relationship between density of seeds and rate of germination of *C.truncata* was observed by Bonis et al (1996). As such, all investigations in this study were carried out at two different seed densities in order to test whether this factor would exert any effect on germination success and on accumulation of biomass in local samples.

The following hypotheses were addressed:

Hypothesis 1: Germination success is reduced with greater depth of seed burial

Previous work, including Bonis et al. (1994) and Jurik et al. (1994) has shown that germination of seeds and emergence of seedlings from wetland seed banks is known to depend on the depth of burial of seeds in the sediment. Bonis et al. (1994) showed that most seeds, including those of *Callitriche truncata*, that germinated from seed banks of temporary marshes, did so from the seed reserve in the top 2 cm of sediment. Jurik et al. (1994) indicated that overlying sediment of depth as low as 0.25 cm significantly reduced the number of species and the total number of individuals recruited from wetland seed banks. It is hypothesised that, in accordance with the findings of Bonis et al. (1994), germination of *Callitriche truncata* seeds in freshwater rockpool seed banks of the Maltese Islands is sensitive to depth of seed burial, with higher germination success expected from seeds at the surface of the sediment layer.

Hypothesis 2: Germination success is higher at the start of the wet season

Later emergence from the seed bank would be expected to reduce the probability of macrophytes surviving to reproduction, since germination in periods closer to the end of the wet-season may not provide sufficient time for completion of the life-cycle. It is hypothesised that the germination success of *Callitriche truncata* seeds is higher in autumn, at the start of the wet-season, than it would be in winter.

Hypothesis 3: Production of biomass and reproductive success are dependent on flooding date

Early flooding has been found to increase the biomass and seed production of macrophytes in the temporary marshes of the Camargue (Grillas et al. 1998). It is hypothesised that a similar pattern occurs in *Callitriche truncata* populations in Maltese pools, where earlier flooding dates would lead to the formation of more vegetative and reproductive biomass than that produced in response to later flooding.

2 Material and methods

All seeds used in this study were collected from a single rock pool, situated at San Pawl tat-Targa, Malta, which was known to be colonised by *C.truncata*. Samples of dry sediment, which were assumed to contain recent seeds deposited at the end of the previous wet season, were collected from the surface of the sediment layer of the basin in August 2011. The sediment was stored in paper bags and subsequently stored in closed containers, in dark and dry conditions before undergoing sorting and analysis. Isolation of seeds from the sediment was started in mid-September 2011. During this process, the sediment was sieved under low-intensity jets of water through different mesh-sizes using the method described by Grillas et al. (1993) and Greenwood et al. (2005). The seeds and other debris collected by each sieve were filtered, and the residue left to air dry for 24 hours. The residue was subsequently observed through a stereomicroscope and seeds of *C. truncata* were removed using fine forceps. The seeds were subsequently stored in closed containers, in dark and dry conditions until required for the germination experiments.

The number of seeds recovered from the sediment samples was small compared to that utilised in studies with comparable objectives, such as Bonis et al. (1993). This was a consequence of the restricted volume of the sediment layer (and hence, of the seed bank) of the rockpools in the area of study. As such, the range of experimental treatments and number of replicates was constrained by the small number of seeds available. In all treatments, the criterion for germination was the emergence of the first plant structure (radicle or shoot) above the sediment layer. Use of this criterion therefore classified seeds that would have germinated but whose structures did not actually emerge above the sediment surface as 'ungerminated'. This limitation was considered unavoidable. During the course of the experiments, the pots were checked for germinated seeds at intervals ranging from three days to nine days, well within the monitoring ranges suggested by Baskin et al. (2001). Germlings were not removed from the pots so as to simulate natural intraspecific competitive effects.

Experiment 1: Effect of depth of burial on germination of seeds

The effect of depth of burial on germination responses was investigated using opaque, circular plastic containers (henceforth referred to as 'pots') of diameter 6.5 cm and height 8.3 cm in which a thin, level bed of sterile

sediment was placed. Ten or twenty seeds, depending on the treatment, were placed in each pot using fine tweezers and distributed on the sediment surface along a uniform grid. The seeds were subsequently buried under different depths of sterile sediment. Five different treatments were used: no burial, burial under 1 cm, 2 cm, 3 cm and 4 cm of sterile sediment. The upper limit of burial was based on the conclusions of Bonis et al. (1994), who, investigating a number of species from temporary marshes, indicated that only Charophyte oospores germinated from burial under more than 4 cm of sediment. Each treatment was repeated with ten seeds per pot and with twenty seeds per pot in order to investigate any possible effects of density-dependence on germination rates. Four replicates were prepared for each treatment and these were arranged in a grid pattern in a small ventilated greenhouse and completely filled with water obtained from a domestic reverse-osmosis apparatus. Filling of the pots was carried out at the beginning of October 2011 and these were subsequently examined regularly for a period of five months during which water levels were replenished when necessary. The positions of the pots were rotated weekly in order to eliminate any effects that may have been attributable to the position of specific pots. Germination rates were calculated using Maguire's equation (M) as described by Ranal et al. (2006) and as applied by Greenwood et al. (2005):

$$M = \sum \frac{n_i}{t_i}$$

where n_i is the cumulative number of seeds that would have germinated at time t_i (days).

The duration of this experiment is much longer than the two-week to four-week duration for germination experiments suggested by Baskin et al. (2001). The long duration of this experiment was intended to mimic the natural situation, where seeds in rockpools are inundated for several months. As such, the length of this experiment would have detected any 'late' germination that may have occurred.

Experiment 2: Effect of flooding date on germination success

The effect of different flooding dates on germination success were investigated using pots identical to the ones utilised for the 'burial' experiment described previously, with a number of uniformly-distributed seeds being placed on the surface of a level bed of sterile sediment of approximate thickness 2 *cm* in each pot. Two treatments were designed, each simulating a different flooding period:

(1): 'Autumn flooding'. Pots were inundated on 15th October 2011 in order to simulate flooding in the warmer part of the wet season. The experiment proceeded for 61 days.

(2): 'Winter flooding'. Pots were inundated on 15th December 2011 in order to simulate a late wet-season. The experiment proceeded for 63 days.

Each treatment was repeated with ten seeds per pot and twenty seeds per pot with four replicates being prepared for each treatment. Flooded pots were maintained in a small ventilated greenhouse, exposed to diurnal cycles of ambient light and temperatures and their positions rotated weekly in order to eliminate any effects that may have been a consequence of the position of specific pots. The pots were examined regularly until the end of the experiments and the number of germinated seedlings noted.

Experiment 3: Effect of flooding date on accumulation of biomass

The effect of flooding date on accumulation of biomass was investigated by subjecting seeds to two treatments simulating different dates of flooding followed by a continuous hydroperiod and subsequent desiccation. This experiment was carried out using plastic pots measuring 17 cm × 9.5 cm × 7 cm (depth) with a number of seeds (either 25 or 50, depending on the treatment) being placed on the surface of a level bed of sterile sediment of approximate thickness 2 cm in each pot. The same treatments and number of replicates described for Experiment 2 were used. The 'Autumn flooding' treatment was started at the end of October 2011 and the 'Winter flooding' treatment at the end of December 2011. The 'Autumn flooding' and 'Winter flooding' treatments proceeded for 135 and 73 days respectively, ending in mid-March 2012. During this period, the pots were examined regularly and flowers and fruits were noted and counted and left on the parent plants. At the end of the experiment, the plants in each pot were harvested, rinsed carefully to remove any sediment, and dried for three hours at 45°C − 65°C. The total dry weight of plant material in each pot, including below-ground biomass, was subsequently measured. An estimate for reproductive effort was calculated by comparing the biomass of fruit (representing reproductive structures) with biomass of vegetative material. Seven randomly-selected plants from each pot utilised for this purpose. The fruits were detached and weighed separately and fruit biomass was expressed as a proportion of total biomass.

3 Analysis of data

Proportion data was subject to an arcsine square-root transformation in order to normalise the distribution of the data (Legendre et al. 2012). Student's t-test was used when comparison of two datasets (after controlling for normality and equal variance) was required. The Shapiro-Wilk test was used to test for normality of the data whilst an Equal Variance test was utilised to com-

pare variances in the groups being tested. When the assumptions of normality or equal variance were not satisfied, the groups were compared using a Mann-Whitney Rank Sum Test. Comparisons involving more than two groups were carried out using a One-way ANOVA , Two-Way ANOVA or a Kruskal-Wallis One Way Analysis of Variance on Ranks after controlling for distribution and variance. All tests were carried out using SigmaPlot for Windows version 11.0 (Systat Software 2008).

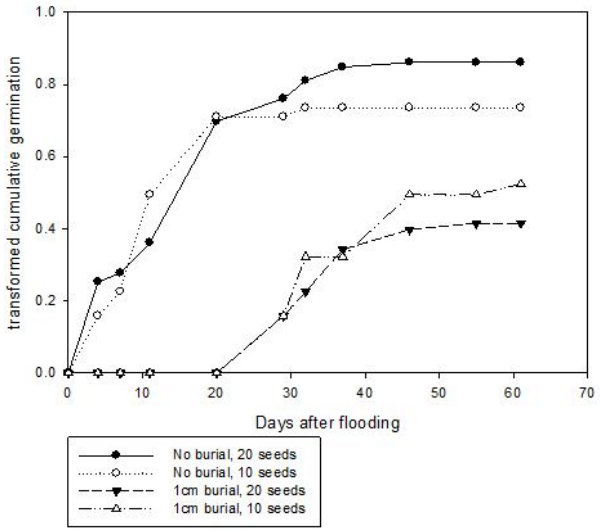

Figure 1: Cumulative germination success of seeds placed on the sediment surface and seeds buried under 1 *cm* of sterile sediment at two different seed densities (20 seeds per pot and 10 seeds per pot). Proportions of germinated seeds were arcsine square-root transformed.

4 Results

Effect of depth of burial on germination success
The effects of the depth of burial on seed germination are summarised in, Figure 1, and in Table 1. Seeds that were buried under 2 *cm*, 3 *cm* and 4 cm of sediment did not germinate and the results of these treatments were therefore omitted from subsequent analyses. Cumulative germination at the end of the experiment was highest in seeds that were not subject to burial ($58\% \pm 6\%$ with 20 seeds per pot; $45\% \pm 24\%$ with 10 seeds per pot) and declined considerably in seeds that were buried under 1 cm of sediment ($16\% \pm 14\%$ with 20 seeds per pot; $25\% \pm 17\%$ with 10 seeds per pot). The difference in cumulative germination between the seeds placed on the surface and those buried under 1 cm of sediment was tested using Student's t-test and was statistically-significant ($t = 3.738$; $P = 0.002$; $\pi = 0.931$). Seeds buried under 1 cm of sediment also germinated much later than seeds that had not been buried (Figure 1), although that may partly be a consequence of the time required for emergence of the shoot from deeper layers of sediment. First and last germination events in unburied seeds were noted 5.8 ± 3.5 days and 37.3 ± 6.9 days after flooding respectively whilst the analogous events in buried seeds were recorded 32.7 ± 4.0 days and 45.5 ± 13.1 days after flooding respectively. Peak germination rates were generally attained between $20-46$ days after initial flooding of the pots in all treatments. The interaction of seed density and depth of burial was examined using a Two-Way ANOVA. The results obtained indicated that the interaction between depth of burial and seed density was not significant in its effect on rates of germination ($F = 1.645$; $P = 0.224$; $\pi = 0.109$).

Table 1. Rates of germination success; mean number of days after flooding on which first germination events were recorded and mean number of days after flooding on which last germination events were recorded. Results are given for each treatment (different depths of burial of seeds) and for different seed densities per pot (20 seeds per pot and 10 seeds per pot). No germination was recorded from seeds buried under 2 cm, 3 cm and 4 cm of sediment.

	Germination success		First germination		Last germination	
Depth of burial	20 seeds	10 seeds	20 seeds	10 seeds	20 seeds	10 seeds
0 *cm*	$58\% \pm 6\%$	$45\% \pm 24\%$	5.8 ± 3.5	9.3 ± 3.5	37.3 ± 6.9	23.0 ± 6.0
1 *cm*	$16\% \pm 14\%$	$25\% \pm 17\%$	32.7 ± 4.0	38.5 ± 15.1	38.7 ± 14.2	45.5 ± 13.1
2 *cm*	0%	0%	-	-	-	-
2 *cm*	0%	0%	-	-	-	-
2 *cm*	0%	0%	-	-	-	-

Effect of flooding date on germination responses
The effects of early-onset and late-onset flooding on germination responses are summarised in Figure 2 and in Table 2. Mean germination rates ranged from $70\% \pm 8\%$ of seeds in the 'Autumn Flooding' treatment to $55\% \pm 38\%$ of seeds in the 'Winter Flooding' treatment. A One-Way ANOVA did not indicate any overall significant difference in germination rates ($F = 0.309; P = 0.819; \pi = 0.05$) across flooding treatments ($t = 0.533; P = 0.602; \pi = 0.05$) and across density of seeds per pot ($t = -0.425; P = 0.677; \pi = 0.05$). First germination in all treatments was recorded within

5.3±6.7 to 10.0±6.0 days of flooding whilst latest germination was recorded between 13.3±9.6 to 56.0±0.0 days after flooding. Peak germination rates were attained within 3 to 15 days after initial flooding (Figure 2) with no consistent difference in germination rate being noted across flooding treatments and across different seed densities. A Two-Way ANOVA indicated that differences in germination success across seed densities per pot were not statistically significant ($F = 0.4405$; $P = 0.537$). No significant interaction between seed density and flooding date, in relation to germination success, was detected ($F = 0.351$; $P = 0.565$).

Table 2. Rates of germination success; mean number of days after flooding on which first germination events were recorded and mean number of days after flooding on which last germination events were recorded. Results are given for each treatment (different flooding dates) and for different seed densities per pot (20 seeds per pot and 10 seeds per pot).

	Germination success		First germination		Last germination	
Treatment	20 seeds	10 seeds	20 seeds	10 seeds	20 seeds	10 seeds
'Autumn'	$56\% \pm 18\%$	$70\% \pm 8\%$	9.5 ± 2.9	10.0 ± 6.0	33.0 ± 12.7	27.0 ± 9.2
'Winter'	$59\% \pm 24\%$	$55\% \pm 38\%$	10.0 ± 3.5	5.3 ± 6.7	56.0 ± 0.0	13.3 ± 9.6

Effect of flooding date on accumulation of biomass
The effects of flooding date on accumulation of biomass and reproductive biomass are summarised in Figure 3, Figure 4, and in Table 3. Plants that germinated during the 'Autumn Flooding' treatment accumulated greater total biomass (after standardisation to account for the initial number of seeds per pot) at the end of the experiment than plants that germinated during the 'Winter Flooding' treatment with ranges of standardised total biomass of $0.94 \pm 0.58\,g$ to $1.16 \pm 0.40\,g$ per pot for the 'Autumn Flooding' treatment and from $0.13 \pm 0.05\,g$ to $0.14 \pm 0.08g$ per pot in the 'Winter Flooding' treatment. The difference in production of biomass between the two flooding treatments was statistically significant ($T = 100.00; P < 0.001$). The plants in the 'Winter Flooding' treatment also produced significantly less reproductive biomass than those in the 'Autumn Flooding' treatment ($t = 3.378; P = 0.005; \pi = 0.864$). Seed density did not exert significant effects on production of biomass in either treatment ($t = -1.314; P = 0.237; \pi = 0.108$ for 'Autumn Flooding' and $t = -0.318; P = 0.761; \pi = 0.05$ for 'Winter Flooding'). A Two-Way ANOVA did not detect any significant interaction between flooding date and seed density in relation to accumulation of reproductive biomass ($F = 0.272; P = 0.612$).

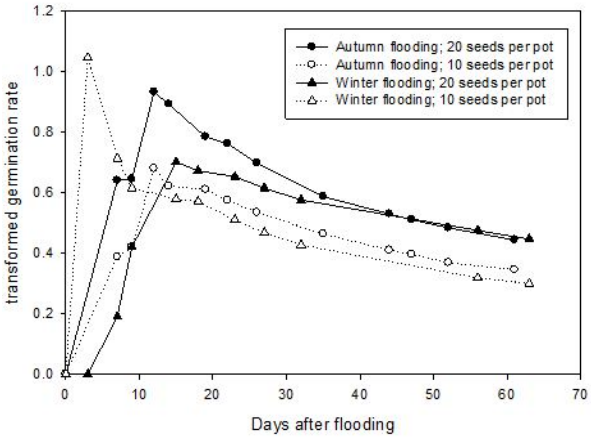

Figure 2: Germination rate over time for seeds subjected to 'Autumn flooding' and 'Winter flooding' treatments at two different seed densities (20 seeds per pot and 10 seeds per pot). Germination rates were arcsine square-root transformed.

5 Discussion

Effect of burial on germination success
Germination success was highest in seeds situated on the surface of the sediment and declined sharply with depth, with no germination recorded from seeds buried under 2 cm of sediment or more. These results corresponded with those of other studies (Galinato et al. 1986; Bonis et al. 1994; Jurik et al. 1994, Rhazi et al. 2007), all of which indicated that burial was associated with a sharp decrease in germination capacity of seeds. Germination success for seeds of *C.truncata* placed on the surface of the sediment was comparable to that recorded for seeds of the same species from the surface layers of the Cerisière marsh by Bonis et al. (1994). These authors recorded a germination rate of 57.1% when these seeds were placed on the surface of a layer of sterile sediment, and no germination for buried seeds.

In a natural setting, seeds are deposited on the surface of the sediment layer and may subsequently percolate into deeper layers of sediment following transport by wind during the dry season (Espinar et al. 2007). Burial of seeds would attenuate the influence of environmental cues, including light (Bonis et al. 1994) and temperature fluctuations (Thompson et al. 1983), which would be important for the germination process. Inhibition of germination of buried seeds is adaptive for relatively small-seeded species such as *Callitriche truncata* since these are constrained by limited endosperm reserves that may not be sufficient to enable seedlings emerging from buried seeds to reach the surface of the sediment and

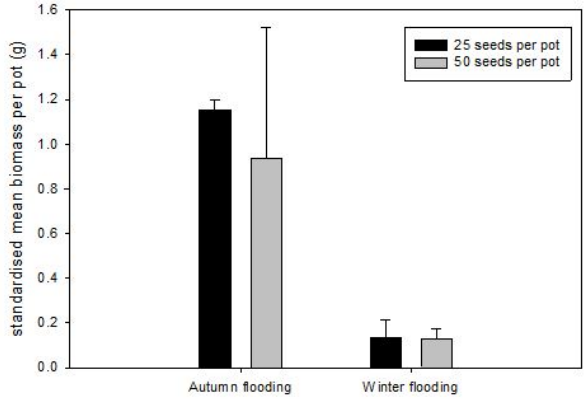

Figure 3: Standardised mean total biomass, expressed in grams per pot, produced by plants germinating under two different flooding treatments: 'Autumn flooding' and 'Winter flooding'. Each treatment was repeated with two different seed densities: 50 seeds and 25 seeds per pot. Biomass measurements were standardised to account for the number of seeds per pot in order to facilitate comparisons.

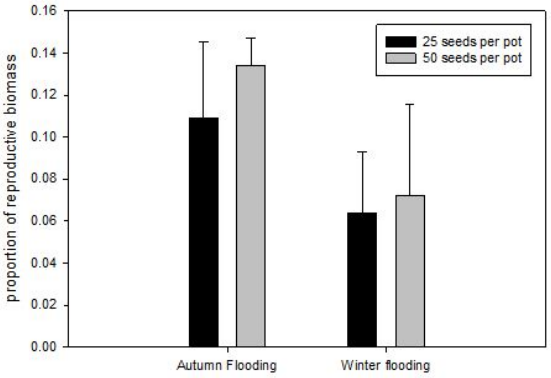

Figure 4: Proportion of reproductive biomass relative to total biomass produced for plants germinating under two different flooding treatments: 'Autumn flooding' and 'Winter flooding'. Each treatment was repeated with two different seed densities: 50 seeds and 25 seeds per pot.

start photosynthesising. Buried seeds may act as perennial repositories, giving rise to a 'storage effect' (Chesson 1985) by magnifying the effect of 'favourable' years and diluting the effect of 'unfavourable' years, ensuring the survival and re-establishment of the plant throughout several years.

Effect of flooding date

Rates of germination success of seeds subjected to the 'Autumn flooding' and 'Winter flooding' treatments were comparable and not significantly different. Nonetheless, plants that emerged in winter accumulated significantly less total biomass and reproductive biomass than plants that emerged in autumn. This decrease in total biomass as a consequence of later flooding is analogous to that reported by Grillas et al. (1998) for marshland plants in the Camargue, where a three-week delay in flooding led to a reduction in biomass produced at the end of the growing season. This reduction in production of biomass was attributed to a decrease in the time available for plant growth before the onset of environmental conditions (colder temperatures and shorter day length) that limited photosynthesis and consequently limited growth (Grillas et al. 1998). The results obtained in Experiment 3 of this study suggest that delayed flooding in a natural setting, as may occur during a wet season in which the hydroperiod is fragmented, would limit plant growth but would still permit successful completion of life cycles and sufficient fruit production for restocking of the seed bank. Such compressed life cycles are characteristic of macrophytes of temporary ponds in general.

Density-dependent effects

The density of seeds in each pot did not exert significant effects on any of the experimental results. This is in contrast to the results of Bonis et al (1996), in which higher densities of seeds were associated with a statistically-significant reduction in germination rates. In a natural setting, seeds are deposited directly on the sediment surface in the vicinity of the parent plant and such localised patches may be characterised by relatively high densities of seeds. The results obtained do not suggest that clustering of seeds leads to any density-dependent inhibition of germination, a physiological response that would be adaptive as it would maximise replenishment of the seed bank.

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Review Article

Medical Diagnostics using Designed Molecules with Sense and Logic

Maria Cardona[1], **Kristina Farrugia**[1] **and David C. Magri**[1]
[1]Department of Chemistry, University of Malta, Msida, Malta.

Abstract. Luminescent molecules responsive to cations, anions and even small molecules can be designed with the appropriate selectivity and sensitivity for monitoring physiological and pathological levels of analytes. We highlight some recent examples of designed molecules that can sense for a specific analyte or a combination of analytes in blood and in living cells. Furthermore, we demonstrate how molecules can be designed with built-in algorithms according to principles of Boolean logic to perform information processing. The potential future application of molecular systems able to perform multi-analyte sensing as 'lab-on-a-molecule' systems for medical and environmental diagnostics is also presented.

Keywords Metal ions; Chemosensors; Photoinduced electron transfer; Molecular logic gates; Lab-on-a-molecule; Biomedical diagnostics

1 Introduction

Fluorescent chemosensors are molecules that selectively bind an analyte resulting in an alteration in the photophysical properties of the system via an optical signal (Valeur et al. 2012). These types of sensors are advantageous as they can be designed with good selectivity and sensitivity besides having a rapid response. Furthermore, they require only simple instrumentation for their use: a hand-held UV lamp is enough for qualitative studies. For quantitative studies, the traditional tools are absorption and fluorescence spectrometers.

Correspondence to: D. C. Magri (david.magri@um.edu.mt)
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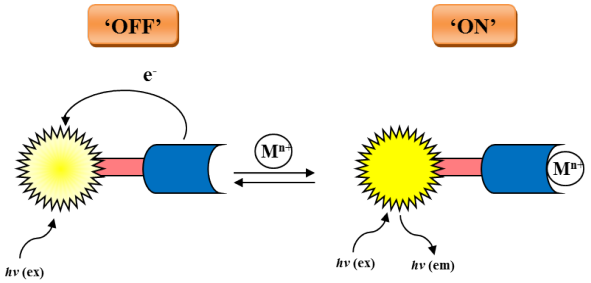

Figure 1: Diagram showing the design format (fluorophore (yellow), spacer (pink), and receptor (blue)) and 'off' and 'on' states of a PET chemosensor.

For real-life applications, fluorescent chemosensors must meet several requirements (Lakowicz 2009). Foremost, they must be selective for a specific targeted metal ion even in the presence of other metals ions found at higher concentration levels and should also be sensitive to the pathological concentration range of the analyte (Burtis et al. 2001). The rational design of such selective chemosensors owes a lot to the principles governing guest-host and coordination chemistry (Cotton 1999). The sensors must also be compatible with the biological matrix and generally water-soluble (Domaille et al. 2008). Typically, a 'turn-on' emission response or a wavelength shift is preferred over a 'turn-off' emission quenching response for maximizing spatial resolution, notably with a light microscope. In cell biology studies, a higher fluorescence brightness is advantageous as less of the intrusive indicator is needed, thus minimizing the possibility of toxicity and altering the cellular environment (Que, 2008). Many applications use fluorophores that emit in the visible region ($400 - 650\,\text{nm}$); however, those emitting at even longer wavelengths in the near infra-red region ($650 - 900\,\text{nm}$) are needed for biomedical uses (Johnson et al. 2010). This range is useful in penetrating deep within the tissue without causing photo-damage to samples, and without interference from background autofluorescence of cellular com-

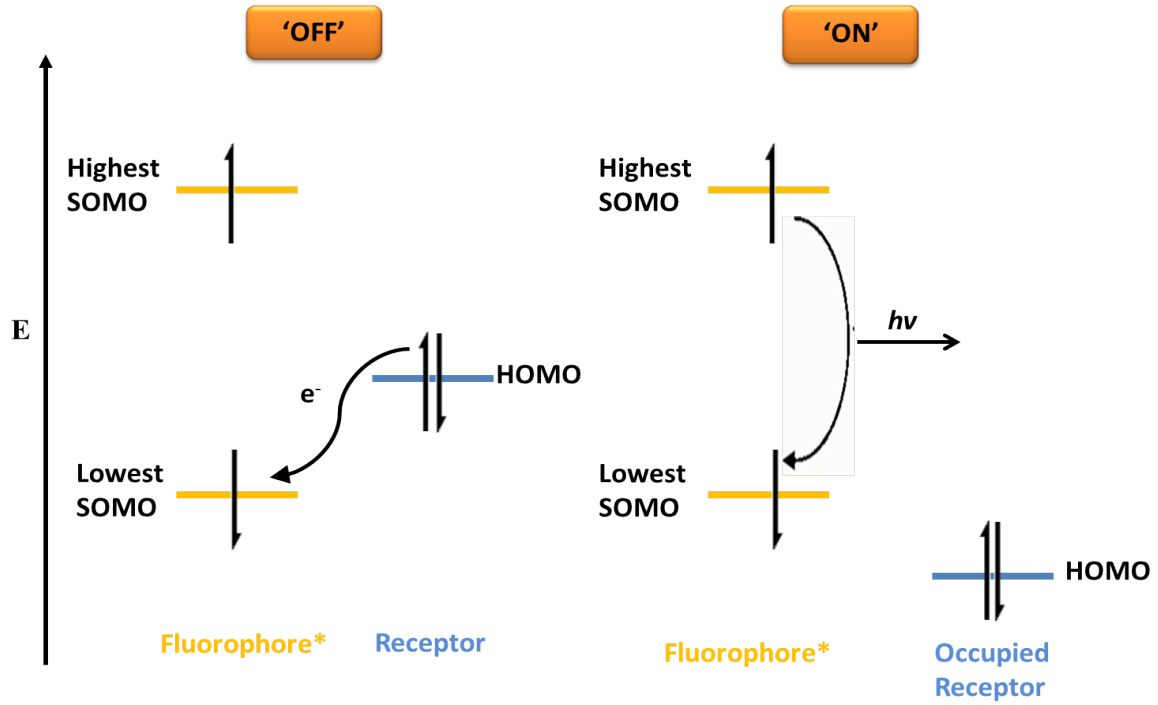

Figure 2: Molecular orbital energy diagrams showing the relative energy of the frontier orbitals of receptor and fluorophore in the "off" and "on" states during the PET process.

ponents. This problem can also be compensated for by using chemosensors with a high extinction coefficient and high fluorescence quantum yield.

This review highlights some examples of fluorescent molecules that have been used for sensing and diagnosing purposes. Some notable examples have been selected with proven application in clinical chemistry or cell imaging. We have included representative examples of molecules able to sense for various species including protons, metal cations and anions, and examples of molecular logic gates that can detect for a combination of analytes (Callan et al. 2005; Magri et al. 2007; de Silva et al. 1997; Prodi et al. 2000; Formica et al. 2012 and Jeong et al. 2012).

2 Photoinduced Electron Transfer (PET)

The basic design of an 'off-on' PET sensor involves a 'fluorophore-spacer-receptor' format as illustrated in Figure 1 (de Silva 2011). They operate based on the competition between photoinduced electron transfer and fluorescence. Such chemosensors have three components: (i) a luminescent component (fluorophore), (ii) a receptor for binding the analyte and (iii) a spacer connecting (i) and (ii). In the 'off' state, upon irradiation of the fluorophore, electron transfer occurs from the unbound receptor to the fluorophore resulting in essentially no fluorescence. However, in the 'on' state, the analyte is bound by the receptor preventing electron transfer and resulting in fluorescence. The ideal PET probe causes an 'off-on' switching of the fluorescence intensity only with no change in the wavelength.

Figure 2 illustrates simplified molecular orbital diagrams for the 'off' and 'on' states. Upon excitation of the fluorophore, an electron from the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO) of the fluorophore, which become singly occupied molecular orbitals (lowest SOMO). When the lone electron pair in the HOMO of the unbound receptor has a slightly higher energy than the SOMO of the fluorophore, a fast intramolecular PET occurs resulting in quenching of the fluorescence (Kavamos 1993). However, when the receptor binds to the analyte, the oxidation potential of the donor is increased so that the HOMO of the bound receptor becomes lower in energy than the lowest SOMO of the fluorophore. The intramolecular PET process is now not feasible and quenching is suppressed, representing the 'on' stage of the fluorescent sensor. It is the competition between electron transfer and fluorescence that is the basis of PET chemosensors (de Silva 2008).

Having a design criterion is common in engineering, but rare in chemistry (Bissell 1992). Hence, the design of fluorescent PET sensors is a rare example of molecular engineering (de Silva 2009). The key parameter in the overall design is the binding constant β (which is the inverse of the dissociation constant, K_d), which relates

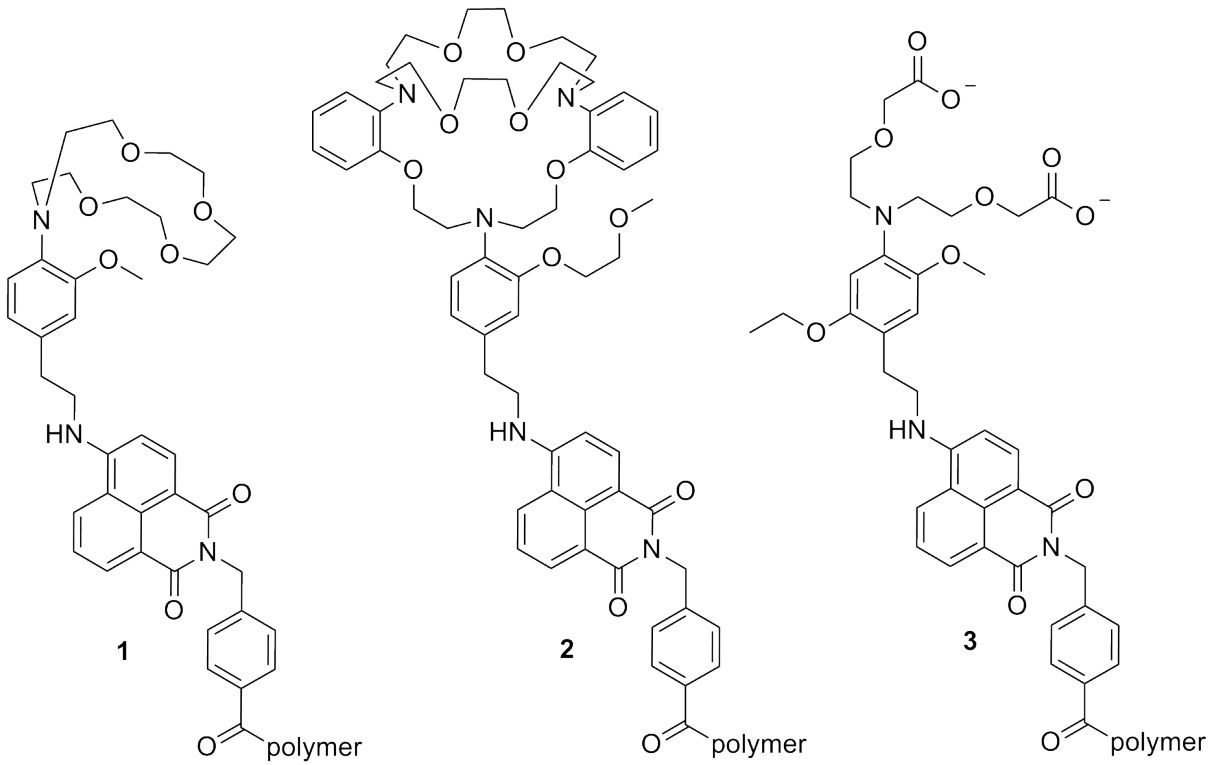

Figure 3: The chemosensor for Na^+ (1), K^+ (2) and Ca^{2+} (3) ions used in the OPTI disposable cassettes.

the fluorescence signal to the ion concentration. The reciprocal of the binding constant for the receptor-analyte interaction determines the average analyte concentration to be sensed. As mentioned, at the design stage the selectivity of the receptor towards the targeted analyte in the presence of elevated levels of other analytes is an important consideration.

3 The State-of-the-Art in Point-of-Care Technology

The Osmetech OPTI® system, consisting of a disposable cassette with incorporated molecular chemosensors, and the critical care analyzer, measure the analyte concentration of a patient from a tiny sample of blood. The device can simultaneously measure six critical care analytes at a time on a 120 µL sample of whole blood in about 2 minutes. There are five types of OPTI disposable cassettes available: a standard model measures pH, pCO_2 and tHb, Na^+, K^+, Ca^{2+} and Cl^- levels (Tusa et al. 2005). The pH and CO2/tHb sensor exploits the physicochemical properties of pyranine (HPTS), a water soluble pyrene-based indicator (Han et al. 2010). The measuring of Cl^- concentration is based on a collisional quenching mechanism (Bissell et al. 1992). The sensors for Na^+, K^+ and Ca^{2+} on the disposable cassette, as shown in Figure 3, are based on the design principle of photoinduced electron transfer (PET) (He et al., 2003a). Selective recognition of Na^+, K^+ and Ca^{2+} is achieved using carefully designed chemosensors consisting of a naphthalimide fluorophore attached to an aza-crown ether for Na^+, a cryptand for K^+ and a chelator for Ca^{2+} attached at one end of the fluorophore and to the other end to a polymer support (He et al. 2003b).

The chemosensors on the OPTI cassette are designed with a linker for immobilization to a hydrophilic polymer (Tusa et al. 2005). In water or blood serum, 1 with an aza-15-crown-5 ether reversibly binds Na^+ with a dissociation constant of 119 mM or a log Na^+ of 0.92 at a near-neutral of pH 7.4, physiological temperature of 37°C and ionic strength of 160 mM. Similarly, 2 reversibly binds K^+ at concentrations about 17 mM and 3 binds Ca^{2+} at concentrations about 1.1 mM. Excitation with a blue LED causes a green fluorescence signal from the 4-aminonaphthalimide fluorophore in all three cases. The modified iminodiacetic moiety in the Ca^{2+} sensor is reminiscent of the BAPTA receptor popularized in cell biology studies (Tsien, 1980). The OPTI device, shown in Figure 4, is the first commercial success to exploit the switching phenomena between PET and fluorescence for medical diagnostic applications.

Figure 4: A disposable cassette (left) and OPTI critical care analyser (right). The black spots (left picture) are organic polymer fibre mats attached to the appropriate sensor molecules for: Na^+, K^+, Ca^{2+}, pH and CO_2. The orange spot is the sensor for O_2. Adapted from reference 20 and reproduced with permission of the Royal Society of Chemistry (RSC).

4 Alkali and Alkaline Earth Cation Chemosensors

The monitoring of monovalent and divalent metal ions, notably Na^+, K^+, Mg^{2+} and Ca^{2+}, in blood and urine are of major medical diagnostic importance (Burtis et al. 2001). Table 1 lists typically concentration levels for various metal cations in blood including upper and lower limits, known as the critical values, which are indicative of a potential life-threatening condition. Sodium is the most abundant cation in whole blood at levels of about 140 mM. The normal concentration range of these analytes in various biological fluids can be significantly different, particularly for potassium, which has a concentration of 4 mM in blood and 70 mM in urine. Monitoring of sodium and potassium blood serum levels is routinely done for patients with high blood pressure; monitoring of sodium and potassium in urine is generally a concern for kidney problems.

Table 1. Typical concentration levels of metal cations in blood.

Cation	Blood Serum[a]/mM	Lower Limit/mM	Upper Limit/mM
Na^+	140	120	160
K^+	4.0	2.8	6.2
Ca^{2+}	1.2	1.0	3.2
Mg^{2+}	0.8	0.4	1.9
Fe^{3+}	19	9.0	31
Zn^{2+}	15	11	19
Cu^{2+}	16	11	22

[a]At physiological pH between 7.35 – 7.42.

5 Heavy Metal Cation Chemosensors

Many heavy metal ions are essential to the composition of the human body and in living organisms. The most abundant biologically important heavy metals are iron, copper and zinc. Their average concentration and critical values in blood are included in Table 1. Iron (Fe^2+ and Fe^{3+}) is ubiquitous in cells and plays a key role in oxygen transport (Sahoo 2012). Zinc (Zn^{2+}) is an integral part of hundreds of enzymes for the synthesis of genetic material and proteins and for growth and reproduction (Xu and Yoon, 2010). Copper (Cu^{2+} or Cu^+), mainly associated with many metalloproteins, is required for proper iron metabolism (Lippard and Berg, 1994). Other metals ions, including vanadium, chromium, manganese, cobalt, nickel and molybdenum are currently thought to be required in trace amounts for normal health (Burtis and Ashwood, 2001). Many diseases, such as Alzheimer's disease and Parkinson's disease, have been linked to an improper balance of metal ions in the body. As the most abundant heavy metal ions in living beings, Fe^{+3}, Zn^{2+} and Cu^+ are of interest in blood medical diagnostics. Many other metal ions are not involved in essential biological reactions; however, some of these (arsenic, aluminum, lead, mercury, and tin) are toxic. Consequently, from a medical diagnostics point-of-view, early detection of these species is highly desirable. Hence, there is considerable motivation for developing novel fluorescent chemosensors for trace-level environmental and clinical detection (Dutta et al. 2012; Kaur et al. 2012).

A number of reviews specifically on fluorescent chemosensors for heavy metals ions have recently appeared in the literature (Formica et al. 2012; Jeong et al. 2012; Kim et al. 2012): we highlight a few interesting examples in Figure 5. The pyrazoline-based probe **4** with an azatetrathiacrown receptor was used for visualizing labile copper Cu^+ in cellular systems (Thomas Morgan 2011). The four hydroxymethyl groups and the sulfonated triarylpyrazoline fluorophore provide water solubility (de Silva et al. 1993). On capturing Cu^+, **4** displays a 65-fold fluorescence enhancement at 508 nm with an observed quantum yield of 0.083 and a negligible background fluorescence of 0.002. A rare example of a Pb^{2+} chemosensor used for living cell imaging is 5 (He et al. 2006). The xanthenone-based probe has a pseudo crown-ether with two different carboxylate ligands. In the absence of Pb^{2+}, **5** has a background fluorescence with a negligible quantum yield of 0.001. Under tested physiological conditions, an 18-fold fluorescence enhancement is observed. The probe responds to changes of Pb^{2+} in the cytosol of living mammalian cells in a similar fashion. The chemosensor **6** for Zn^{2+} is comprised of a 4-amino-1,8-naphthalimide fluorophore and a phenyl iminodiacetate ligand as the binding site (Parkesh et al. 2007). The compound shows an absorption peak at 450 nm and emits a green fluorescence at 550 nm on binding Zn^{2+}. The low pK_a value of 3.2 is advantageous for monitoring Zn^{2+} in the physiological pH range of 7.4. Sensor **6** has a fluorescence quantum yield of 0.004 in water in the absence of Zn^{2+}, which increases to 0.21 in the presence of 5 µM Zn^{2+} with a 56-fold fluorescence enhancement.

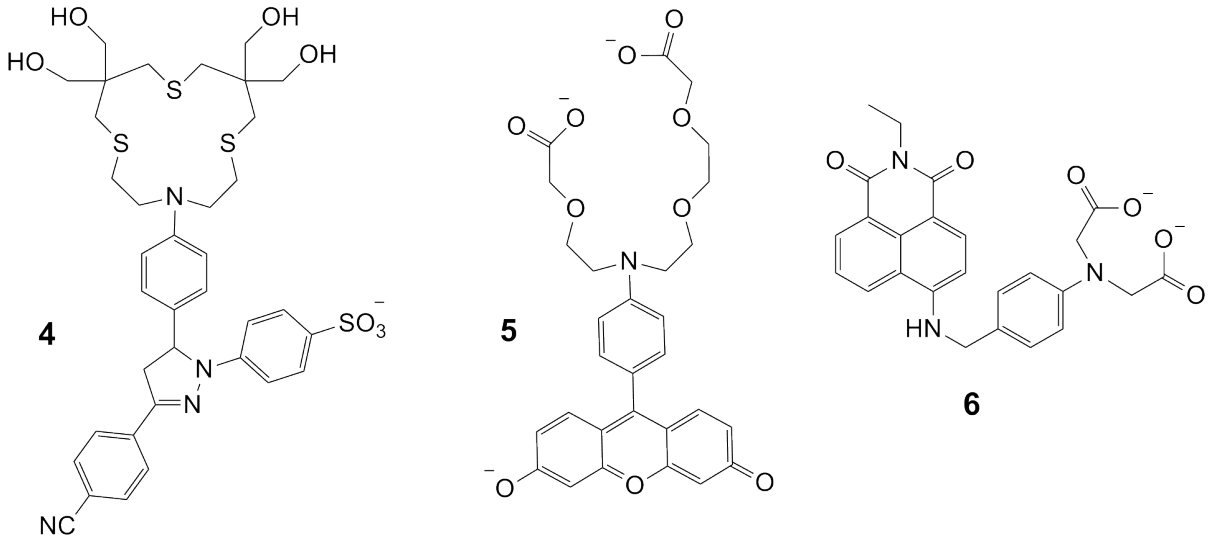

Figure 5: Representative examples of water-soluble chemosensors for Cu^{+} (4), Pb^{2+} (5) and Zn^{2+} (6).

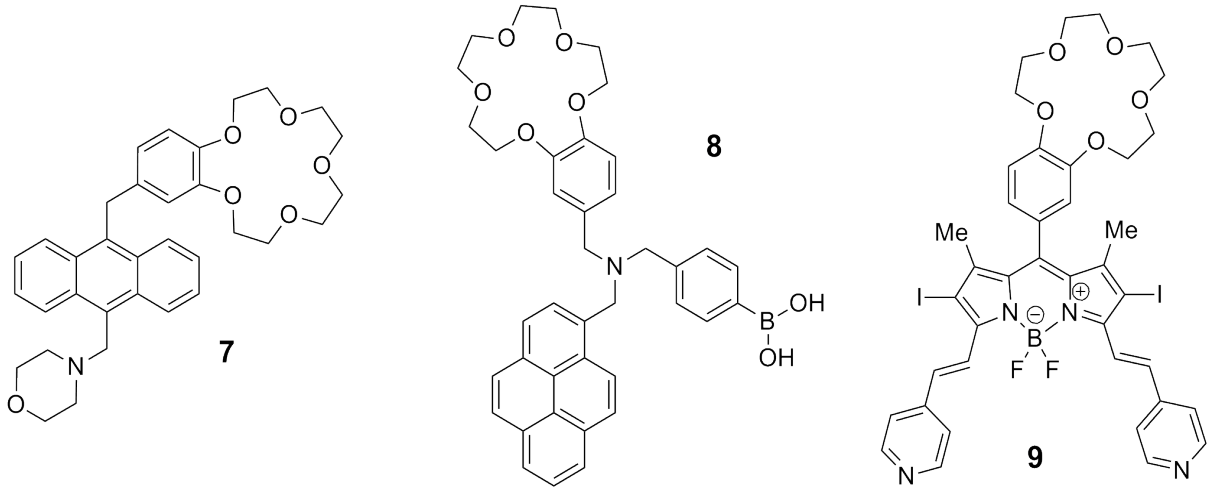

Figure 6: Representative examples of two-input AND logic gates.

6 Two-Input AND Logic Gates

Sensing for more than one analyte within a single molecule has been around for two decades with the demonstration of the first two-input molecular AND logic gate (de Silva et al. 1993). Since then many two-input logic gates (de Silva et al. 2004; de Silva et al. 2007; Szacilowski 2008) and many three and four-input logic gate arrays (de Silva, 2011) have been demonstrated to perform information processing at the molecular level (Magri 2012).

One of the earliest examples of a PET fluorescent sensor with two receptors 7, shown in Figure 6, consists of an anthracene fluorophore, a tertiary amine for binding protons and a benzo-15-crown-5 ether for complexing Na^{+} (de Silva et al. 1997). The molecule responds to H^{+} and Na^{+} inputs and fluorescence as the output according to an AND Boolean algorithm. When one or both of these analytes are in low concentration, essentially no fluorescence is observed. However, on addition of 10 mM Na^{+} and 1 mM acid, which correspond to the high input levels, 7 shows a high fluorescence output with a quantum yield of fluorescence of 0.24 as tabulated in Table 2.

Another example **8**, consisting of a pyrene fluorophore with a tertiary amine, a crown ether and a boronic acid group, is an example of a molecule that can detect for an ion pair (Koskela et al. 2005). A fluorescent enhancement was observed on addition of 6 mM potassium fluoride to the solution. In comparison, no enhancement was observed on the addition of potassium chloride or potassium bromide.

The chemosensor **9** is another AND logic gate with H^{+} and Na^{+} as the inputs, but with the generation of singlet oxygen as the output (Ozlem et al. 2009). Unlike **7** and **8**, where the fluorescence signal is the output, **9** harvests the fluorescence output signal to convert triplet oxygen (the form readily available in the atmosphere) to

singlet oxygen, which is used as a cytotoxic agent against cancer cells in photodynamic therapy (PDT). A current issue during PDT therapy is that many healthy cells are killed by singlet oxygen along with cancerous cells. However, it is known that certain types of cancer cells have higher levels of H^+ and Na^+ ion levels in the lysosomes compared to those of healthy normal cells (Iessi et al. 2008). By designing molecules, like **9**, it may be possible to have chemosensors that only generate single oxygen on excitation with a laser on detecting high levels of both H^+ (pH below 4) and Na^+ ions in specific cells according to an AND logic algorithm. The implications could be improved recovery times for patients after PDT treatment.

Table 2 Truth table for two-input AND logic gate 10.

$Input_1$	$Input_2$	Output
Na^+ [a]	H^+ [b]	Fluorescence [c]
0 (low)	0 (low)	0 (low, 0.003)
0 (low)	1 (high)	0 (low, 0.006)
1 (high)	0 (low)	0 (low, 0.005)
1 (high)	1 (high)	1 (low, 0.24)

[a]High input level $10^{-2.0}$ M sodium methanesulfonate. Low input level maintained with no added sodium salt. [b]High and low input levels correspond to $10^{-3.0}$ M and no added methanesulfonic acid. [c]Fluorescence quantum yields in methanol.

7 Three-Input AND Logic Gates: 'Lab-on-a-Molecule' Systems

The first reported illustration of the potential cross-fertilization between Boolean algebra and biomedical sensing was reported for a 'lab-on-a-molecule' - a three-input AND logic gate **10** based on a competition between PET and fluorescence (Magri et al. 2006). Three receptors are incorporated within a single molecule: a benzo-15-crown-5 ether for Na^+, a tertiary amine for H^+, and a phenyliminodiacetate for Zn^{2+}. In the absence of one, or two or all three analytes, the fluorescence emission in water of **10** is low due to PET from a vacant receptor to the excited state fluorophore. However, when all three analytes are bound in excess threshold concentrations, a high fluorescent signal is observed. The modular arrangement of the receptors, spacers and fluorophore facilitates a cooperative sensing algorithm as seen from Table 3 (Magri and de Silva 2010).

Another 'lab-on-a-molecule' **11** is based on the highly fluorescent boron-dipyrromethene dye (Bozdemir et al 2010). Combining both internal charge transfer and PET processes **11** incorporates three selective receptors that simultaneous detect Ca^{2+}, Hg^{2+} and Zn^{2+}. Advantageously, **11** uses a visible wavelength for excitation at 620 nm, with a fluorescence emission maximum at 656 nm and a significant fluorescence quantum yield of 0.266 when all three analytes are present at elevated concentrations.

Table 3 Truth table for three-input AND logic gate 11.

$Input_1$	$Input_2$	$Input_3$	Output
Na^+ [a]	H^+ [b]	Zn^{2+} [c]	Fluorescence [d]
0 (low)	0 (low)	0 (low)	0 (low, 0.001)
0 (low)	1 (high)	0 (low)	0 (low, 0.001)
0 (low)	0 (low)	1 (high)	0 (low, 0.002)
0 (low)	1 (high)	1 (high)	0 (low, 0.003)
1 (high)	0 (low)	0 (low)	0 (low, 0.006)
1 (high)	0 (low)	1 (high)	0 (low, 0.006)
1 (high)	1 (high)	0 (low)	0 (low, 0.007)
1 (high)	1 (high)	1 (high)	1 (high, 0.020)

[a]High input level 5 M sodium methanesulfonate. Low input level maintained with no added sodium salt. [b]High and low input levels correspond to $10^{-6.0}$ M and $10^{-9.5}$ M protons adjusted with methanesulfonic acid and tetramethylammonium hydroxide. [c]High input level corresponds to pZn = 3.1 at pH 6.0 and pZn = 4.8 at pH8.0. [d]Fluorescence quantum yields in water.

8 Conclusions

The current paradigm is to measure the blood ions such as Na^+, K^+ and Ca^{2+} using a specific molecule for a single analyte on a one-for-one basis. In the future, we envision the development of engineered molecules that could detect for many analytes simultaneously. The targeted analytes could also include anions and neutral molecules in addition to the above mentioned cations. These 'lab-on-a-molecule' systems with built-in algorithms would be able to sense for specific disease conditions by testing for numerous medically relevant parameters simultaneously, and make an intelligent diagnosis autonomously (Konry et al. 2009). They could even communicate a 'yes' or 'no' decision on a disease condition. Such technology could increase productivity at hospitals and clinical laboratories by saving time, especially in emergency point-of-care situations where every second is precious.

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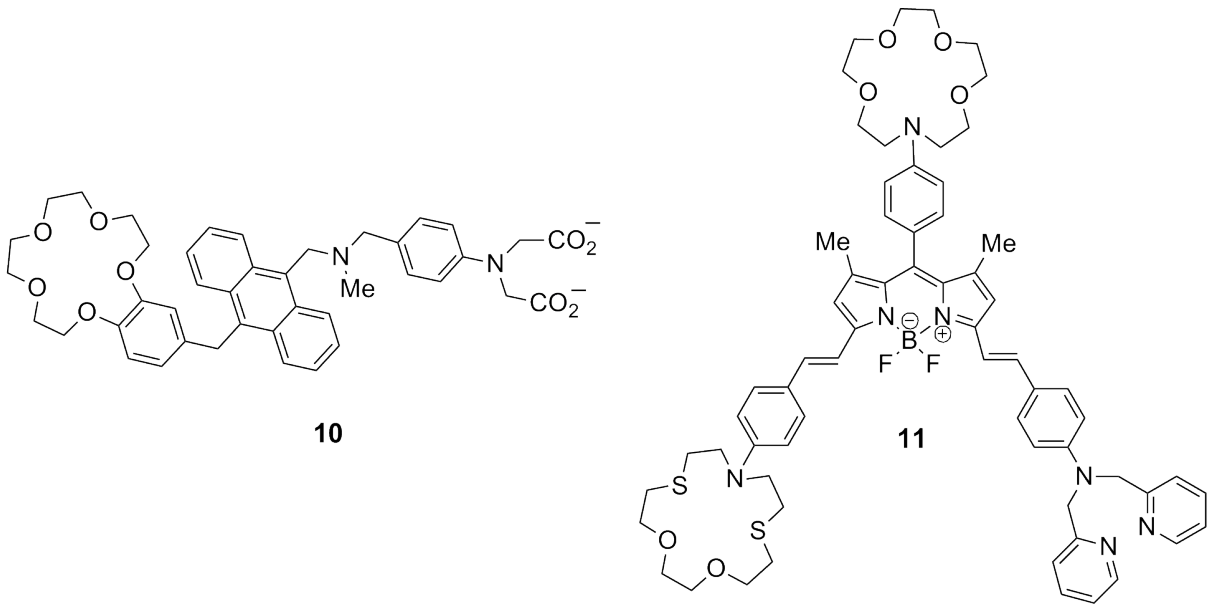

Figure 7: Examples of three-input AND logic gates.

Abela and Ray Scicluna.

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Student Research Article

COMPUTATIONALLY EFFICIENT ESTIMATION OF HIGH-DIMENSION AUTOREGRESSIVE MODELS - WITH APPLICATION TO AIR POLLUTION IN MALTA

Luana Chetcuti Zammit[1], Kenneth Scerri[1], Maria Attard[2] and Thérèse Bajada[2]
[1]Department of System and Control Engineering, University of Malta, Msida, Malta
[2]Institute for Sustainable Development, University of Malta, Msida, Malta

Abstract. The modelling and analysis of spatio-temporal behaviour is receiving wide-spread attention due to its applicability to various scientific fields such as the mapping of the electrical activity in the human brain, the spatial spread of pandemics and the diffusion of hazardous pollutants. Nevertheless, due to the complexity of the dynamics describing these systems and the vast datasets of the measurements involved, efficient computational methods are required to obtain representative mathematical descriptions of such behaviour. In this work, a computationally efficient method for the estimation of heterogeneous spatio-temporal autoregressive models is proposed and tested on a dataset of air pollutants measured over the Maltese islands. Results will highlight the computation advantages of the proposed methodology and the accuracy of the predictions obtained through the estimated model.

Keywords Data-driven modelling; Spatio-temporal autoregressive (STAR) models; Sparse datasets

1 Introduction

Mathematical modelling and analysis is an indispensable tool in the study of both natural and anthropogenic phenomena ranging from the spread of social media to the analysis of the human brain. Due to complexity of the interactions involved, mathematical modelling through the use of known physical, biological, chemical or economic laws is often unfeasible. Nevertheless, such systems often provide large datasets of measurements that indirectly describe the relationships involved. Thus, in a data-driven modelling framework, models are extracted directly from the data through a process of successive estimation and validation until a required level of predictive accuracy is obtained. Such strategies have proved useful in various applications including biology (Shen et al. 2006, Shen et al. 2008); ecology (Ikegami and Kaneko, 1992; Nikolus and Gonzalez, 2002); meteorology (Amani and Lebel, 1997; Berliner et al. 2000); physics (Guo et al. 2006; Kessler et al. 1990); econometrics (Cliff et al. 1974; Giacinto et al. 2006) and chemistry (Shibata et al. 2002; Reiter, 2005).

One of the most widely accepted mathematical descriptions for data-driven modelling is the family of time-series models (Cliff et al. 1974; Martin et al. 1975). In their simplest form, Auto-Regressive (AR) models aim to capture the temporal relationships between successive measurements allowing for the description to consider data as far back in time as deemed fit for each application. Both stochastic and deterministic variables contributing to the measurements can be included through the use of Auto-Regressive Moving-Average (ARMA) or Auto-Regressive with eXogenous input (ARX) models, respectively. Although most applications consider only the linear relationships among the dataset, nonlinear variants such as the Nonlinear AR (NAR), Nonlinear ARMA (NARMA) and the Nonlinear

Correspondence to: L. Chetcuti Zammit (lche0003@um.edu.mt)
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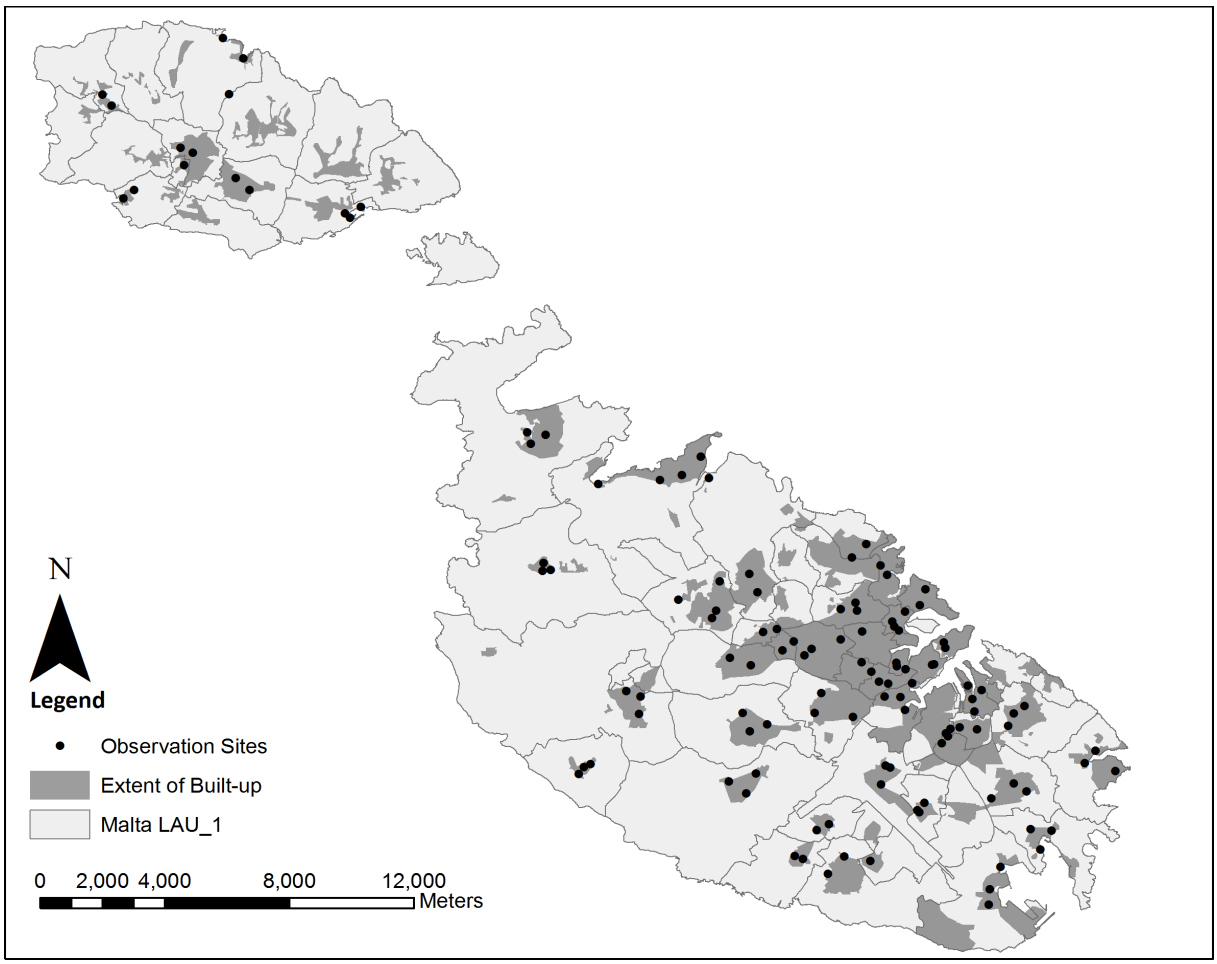

Figure 1: The Distribution of Passive Diffusion Tubes in Malta.

ARX (NARX) models have been proposed (Leontaritis et al. 1985a, Leontaritis et al. 1985*b*).

Multivariate AR (MAR) models are also widely used in applications where multiple measurements related to the same behaviour are being gathered. Such models are widely applicable to the emerging field of spatio-temporal modelling and analysis (Cressie et al. 2011), where data related to the same behaviour is gathered from various spatial locations. In such applications, modelling for both analysis and prediction will surely benefit from the inclusion of spatial interactions together with temporal dynamics. This observation contributed to the development of the Spatio-Temporal AR (STAR) models first proposed in (Pfeifer et al. 1980a, Pfeifer et al. 1980b) as one of the first tools to capture spatio-temporal behaviour from data.

Any data-driven modelling procedure follows a three step-strategy; starting with pre-analysis, filtering and model structure choices, prior to estimation of the model parameters and finally validation of the results obtained. Each step is well documented in literature for both temporal (Ljung et al. 1999; Chatfield et al. 2004) and spatial processes (Cressie et al. 1993) separately, but far less literature is available on methods for spatio-temporal modelling. This can be mostly attributed to the vast datasets usually associated with spatio-temporal studies resulting in significant computational challenges in the data-driven modelling procedure. Most significantly, methods widely used for estimation such as the least square criterion, require the repeated evaluation of a matrix inversion of the dimension of the spatial domain (Lutkepohl et al. 2005; Peña et al. 2001) which is intractable in higher-dimensional problems.

Thus in this work we present a method first proposed in (de Luna et al. 2005) in a MVAR setting for the efficient estimation of AR model parameters for high-dimensional problems. This method was set in a spatio-temporal setting in (Chetcuti Zammit et al. 2011) where, the spatial dependency is usually significant only among measurement sites located in close vicinity. Here this work is generalized to consider any STAR model and tested on a dataset of air pollution measurements taken over the Maltese Islands. Due to the sparsity of the multidimensional parameters in such applications, the

proposed method simultaneously estimates the model parameters together with the number of parameters required to capture the significant spatial relationships in the data. This reduction in the number of model parameters to be estimated allows for improvements not only in the computational demands but also in the parameter accuracy.

The remainder of this paper is structured as follows. First, a theoretical overview of spatio-temporal autoregressive models is given, followed by the proposed methodology. This methodology is validated on a dataset of air pollutant concentrations gather in Malta over the period 2004 to 2010. Some remarks on the spatio-temporal behaviour of these pollutants are then presented and finally conclusions are drawn on the applicability of the proposed methodology to such applications. Finally, various other possible future additions are identified and briefly discussed.

2 Spatio-Temporal Auto-Regressive (STAR) Models

A STAR model of order (p, q) is given by:

$$\begin{aligned}\mathbf{z}(\mathbf{s},t) = \mathbf{A}_1\mathbf{z}(\mathbf{s},t-1) + \mathbf{A}_2\mathbf{z}(\mathbf{s},t-2) + K \\ + \mathbf{A}_p\mathbf{z}(\mathbf{s},t-p) + \mathbf{e}_t \end{aligned} \quad (1)$$

where, $\mathbf{z}(\mathbf{s},\mathrm{t}) \in \Re$ represents the spatio-temporal process of interest as a stationary temporal series (Chatfield 2004), p denotes the temporal order, q denotes the full spatial order, $\mathrm{A_i} \in \Re^{\mathrm{q}\times\mathrm{q}}$ are the autoregressive parameters, and $\mathbf{e}_\mathrm{t}$ denotes white noise with the expectations $E[\mathbf{e}_\mathrm{t}] = \mathbf{0}$, $E[\mathbf{e}_\mathrm{t}\mathbf{e}'_\mathrm{t}] = \Sigma$ and $E[\mathbf{e}_\mathrm{t}\mathbf{e}'_\mathrm{t}] = \mathbf{0}$ for $u \neq t$.

Classical methods for the estimation of the model (1), require the inference from data of the temporal order p and the $(q^2 \times p)$ model parameters $\{\mathbf{A}_\mathrm{i},\, \mathrm{i} = 1, \ldots \mathrm{p}\}$. In a frequentist statistical setting, the model parameters are usually estimated by the maximum likelihood or the least squares criteria. For the linear model (1), it is well known that both these criteria give equal estimates (Ljung, 1999). Nevertheless, both methods suffer severely from the curse of dimensionality, with the number of scalar parameters increasing quadratically with the number of spatial locations.

An adequate temporal model order is usually identified by the use of various model selection criteria such as the Akaike Information Criterion (AIC) (Akaike 1974) or the Bayesian Information Criterion (BIC) also called the Schwarz Criterion (Schwarz 1978). Both these criteria aim to identify the temporal order that best satisfy the principle of parsimony (Chatfield 2004), that is, the temporal order which best balances the model demands for generality and prediction accuracy. Nevertheless, this modelling strategy requires continuous user intervention with the user ultimately deciding on the preferred model order after fitting models of various dimensions.

3 The Proposed Methodology

The methodology being proposed in this work aims to mitigate the two limitations highlighted above by: limiting the number of model parameters to be estimated based on the known independence of non-neighbouring measurements and provide a single algorithm to identify both the models order and the system's parameters without any user intervention. This method was first proposed in (de Luna et al. 2005) and is here being modified, to make use of the natural spatial ordering of measurements based on their vicinity as highlighted in Algorithm 1.

Notes:

1. The spatial nature of the spatio-temporal phenomena being considered provide a natural ordering for the sites based on their spatial vicinity. Although such an interpretation is advantageous in various applications with diffusive behaviour (such as the pollution application being considered in this study), it does not allow for the identification of long distance interactions present in some biological applications such as the spatio-temporal modelling of the electrical activity inside the human brain.
2. The comparative measures used in this work are the AIC and BIC, although the methodology can easily accommodate any other measure (such as the modified AIC (McCullagh et al. 1989) or partial correlation measures (Peng et al. 2009)); as deemed appropriate for the particular application.
3. The user is only required to the make choices for the maximum temporal and spatial orders to be considered, that is pmax and qmax, respectively. If the final model choice is given by these maximum values, the user should consider increasing these values to ensure finding the true global minimum for each site.
4. Although the algorithm first loops in time and then in space, this ordering can be reversed without any effect on the results obtained.
5. Since all sites are allowed to take a different number of neighbours, the heterogeneity of the solution depends exclusively on the measured data and thus no homogeneous or heterogeneous assumptions are taken by the user. This has the benefit of both limiting the user intervention and also allowing the data to identify the model best suited for the each application.
6. A significant computational advantage is obtained if the spatial dependence is limited to a number of sites smaller then the total number of measure-

Algorithm 1 Iterative Model Building

for $i = 1, 2, ...q$ **do**
  Order all sites in ascending order of distance relative to site s_i
  for $n = 1, 2, ...p_{max}$ **do**
    for $k = 1, 2, ...q_{max}$ **do**
      Estimate the first k elements of the i^{th} row of $A_1, A_2, ...A_n$, setting all other elements of the row to zero.
      Calculate the comparative predictive measure.
      Set the optimal temporal and spatial orders of s_i equal to the values of n and k giving the best comparative measure.
    end for
  end for
end for

ments being considered. Such an condition is common of many spatial studies as the pollution study being considered in this work.

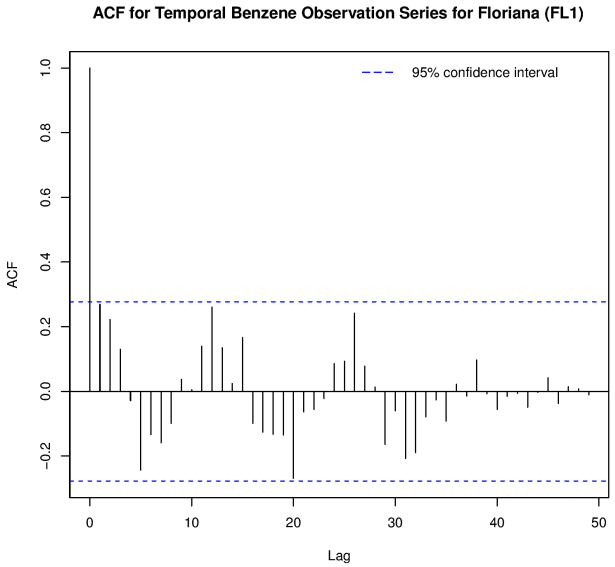

Figure 2: Autocorrelation Plot for the Benzene Observation Series at Floriana ('FL1').

4 An Example - Data-Driven Modelling of Air Pollution in Malta

To test the proposed methodology on a high-dimensional spatio-temporal application, a data-set of air pollution measurements gathered over the Maltese Islands for the period 2004 to 2010 will be used. This data is collected by the Malta Environment and Planning Authority (MEPA) using 123 passive diffusion tubes spread across the island, as shown in Figure 1. Three tubes are usually installed in each locality in sites categorized as near-road, intermediate or urban background. These tubes gather pollution levels for nitrogen dioxide, sulphur dioxide, ozone, benzene, toluene, xylene, ethyl benzene and o-xylene on a monthly basis. In this study, the pollutants usually associated with traffic, that is, nitrogen dioxide (NO_2) and benzene will be considered.

An initial analysis of the data indicated the presence of outliers and missing values. As typical of such studies (Barnett et al. 1994), outliers with measurements above $1.5 \times$ *Interquartile* Range+Upper Quartile or below 1.5×Interquartile Range-Lower Quartile were replaced by linear interpolations in time (Chatfield 2004). Missing values were also replaced by temporal linear interpolations. Note that such measures account only for 2% of the full dataset with respect to outliers and 4% with respect to missing values.

A typical temporal autocorrelation plot based on the pre-processed dataset is shown Figure 2. The low correlation values at each time delay indicate a short temporal dependence and thus point towards the choice of models with low temporal order. Similar plots for spatial correlations also reveal short distance spatial interactions and thus highlight the short range spatial dependency in the data. Partial correlation plots (rather then the full correlation plot of Figure 2), give similar indications. These chararteristics, common to various spatio-temporal applications, justify the need for methods that can efficiently deal with the spatial sparsity in the STAR model parameters.

The estimated models given by the methdology summarised in Algorithm 1 confirm these low-order dimensions for both the spatial and temporal domains. Specifically, for both benzene and nitrogen dioxide, a mean spatial order of 1.4 is obtained per site, for a total of approximately 172 parameters. Such results highlight a significant numerical advantage of approximately 99% in the number of estimated model parameters when comapared to the full model with (123 123) parameters for each temporal order. Figures 3 and 4 show AIC and BIC values for one of the sites situated in St Anne Street Floriana ('FL1') for benzene and nitrogen dioxide, re-

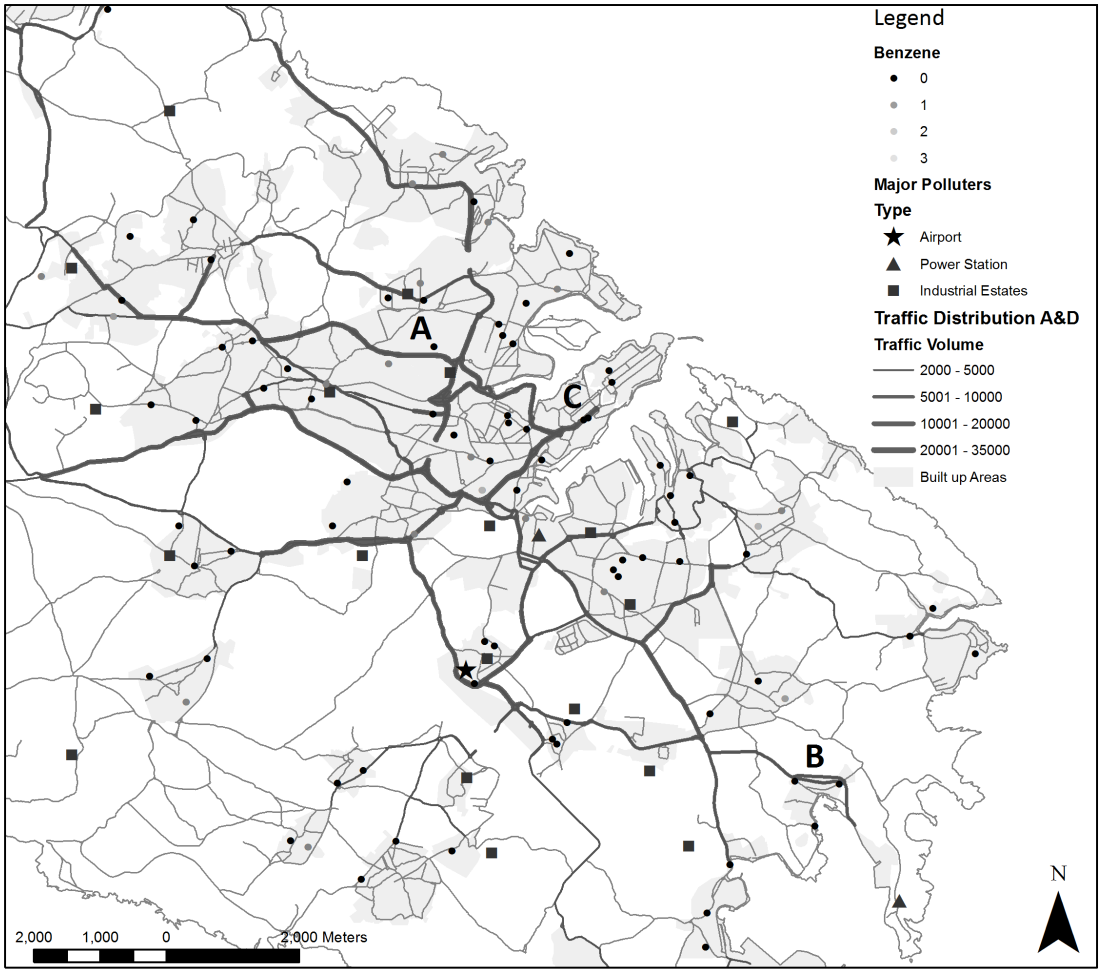

Figure 3: Number of Dependent Sites for Benzene.

spectively. Figure 3 indicates that benzene datapoints at Floriana can be described with a model of spatial order 1 and a temporal order 2, while Figure 4 indicates that nitrogen dioxide at the same site can be described using a model of a spatial and temporal order 1.

One step-ahead prediction estimates on a validation dataset (not used for estimation) of 12 months were used for model validation. For benzene datapoints the the Root Mean Squared Error (RMSE) for each monthly prediction has a mean of $1.661\,\mu\text{gm}^{-3}$ with a standard deviation of $0.664\,\mu\text{gm}^{-3}$, while for nitrogen dioxide datapoints the RMSE is $12.749\,\mu\text{gm}^{-3}$ with a standard deviation of $5.904\,\mu\text{gm}^{-3}$. These value represent 20% and 28% of the mean measurement respectively, and thus provide an acceptable predictor for the monthly pollutant concentrations. Moreover, the residues are spatio-temporally white up to a mean confidence interval of 98.7% for benzene and 95.4% for nitrogen dioxide, thus further confirming the validity of the predictions obtained.

5 Analysis of the Pollution Models

Air quality is of a major environmental concern in Malta as documented in several policy documents published over the past years (Government of Malta, 2002; Office of the Prime Minister, 2010). This concern, along with Malta's membership to the European Union in 2004, pose new obligation on the authorities to monitor the air quality. The main contributors to air pollution in Malta are the high demands for energy generation and the growth in private car use (NSO, 2010). The Maltese Islands were home to 229,016 private vehicles in 2009 (NSO, 2009), one of the highest car ownership rates in the world with approximately 669 cars per 1000 inhabitants. Such high vehicle ownership rates therefore highlight the need for continuous monitoring and analysis of the air pollutants mostly associated with traffic. The models obtained using the proposed methodology can thus be used to analyse local air pollution data and also

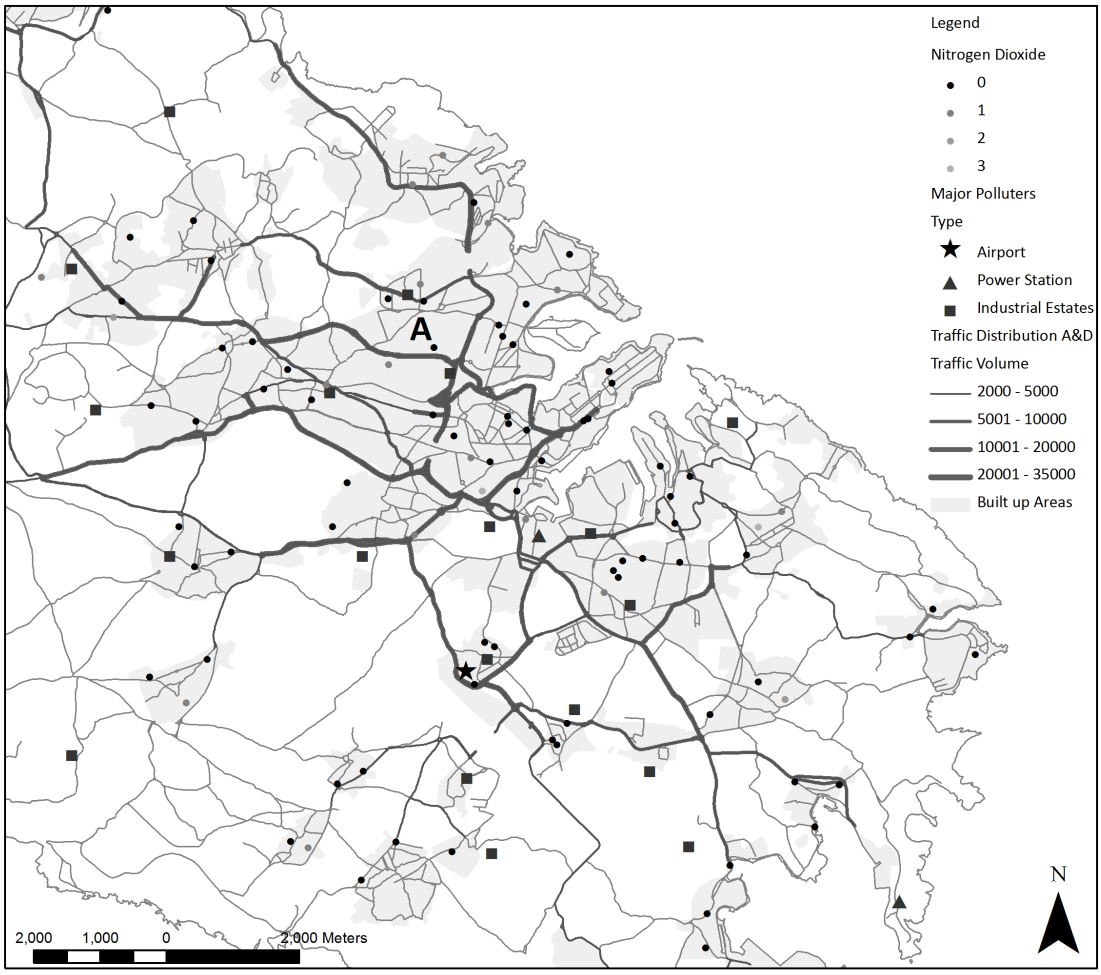

Figure 4: Number of Dependent Sites for $N0_2$.

evaluate the impact of future transport measures and possibly aid in the assesment of the health risks posed by air pollution.

Based on the models obtained, Figures 5 and 6 show the spatial model orders obtained for all observation sites for benzene and nitrogen dioxide, respectively. Note that, a value of 0 indicates that the reading at that particular site is only dependent on previous readings at the same site and a value of 1 indicates that the measurements are dependent on the site itself and its first closest neighbour, and so on for the other values. The low spatial orders observed in Figures 5 and 6 show that most sites are independent even though most of the modelled datapoints are located relatively close to each other and to pollution sources. Thus, the assumption that the dispersal of pollutants is equidistant and therefore one source of pollution in one area has an effect on the neighbouring areas is not supported by this data. This implies that the local pollution sources, rather than diffusion, have a predominant effect on a particular site. This is further highlighted by the inclusion in Figures 5 and 6 of potential sources of pollution in the main island such as traffic density, industrial estates, power stations and the airport.

The overall spatially independent behaviour of these pollutants would suggest that there are other, more local factors that are affecting air pollution. Some possible interpretations follow.

1. Since there is input from a stable source (such as traffic), similar temporal patterns can be observed. However, at different locations the source input levels may change (due to different traffic patterns) and therefore the behaviour of that point, even though it is relatively close, is independent. This is most evident in the area northwest of the Grand Harbour (marked A in Figures 5 and 6). This is reasonable since in the Maltese urban environment, the urban density, urban fabric and traffic, change considerably over a relatively short distance.
2. A few points experience higher spatial dependencies. These are marked with the letters B and C in Figure 5. In these cases we note that (i) the

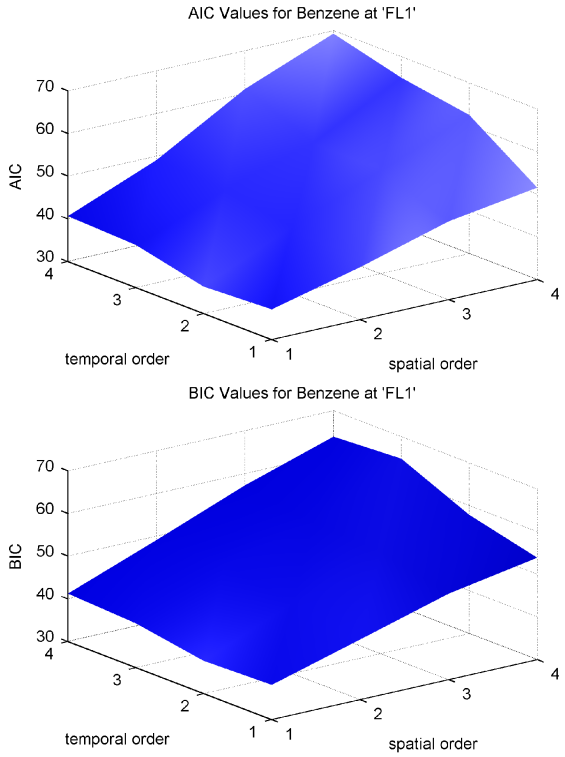

Figure 5: AIC and BIC Values for Benzene Datapoints at Floriana ('FL1').

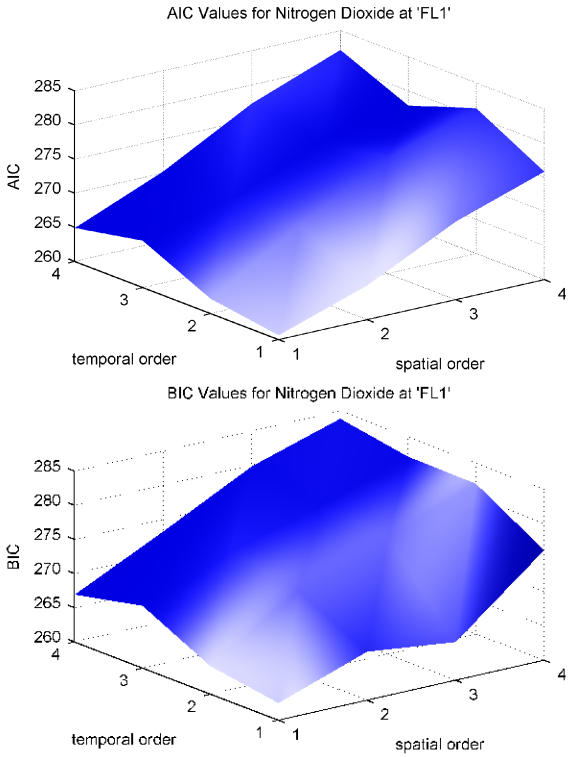

Figure 6: AIC and BIC Values for Nitrogen Dioxide Datapoints at Floriana ('FL1').

pollution values at some of these locations are relatively low, thus affecting the accuracy of the modelling procedure (area marked B) (ii) there are very similar environmental conditions (traffic and urban density) affecting these sites (area marked C).

3. These results are reasonable due to MEPA's approach in the identification of measurement sites. MEPA selects two to three sites per locality, one of which is a traffic site and the others are background sites without traffic. Thus, although geographically close, sites may exhibit significantly different traffic patterns and therefore different pollution measurements.

6 Conclusions

In this paper, a computationally efficient method for modelling heterogeneous spatio-temporal behaviour from large datasets was presented. This significant computational improvement was achieved through the use of the sparse spatial dependencies in the data. For the pollution measurement considered, a 99% reduction in the number of model parameters is obtained, resulting in a significant computational gain over classical estimation methods. In addition, one-step ahead predictions for air pollution concentrations performed on a validation dataset indicate estimation compatibilities comparable with classical methods.

Future work will focus on introducing measured pollution sources to the mathematical model to further examine the dependencies of the pollution readings on these sources. Alternative estimation techniques, such as the orthogonal least squares (Chen et al. 1989) and Expectation-Maximization (EM) algorithms (McLachlan et al. 2008), could also be used with the benefit of dealing with the estimation of the missing values in a more rigorous manner. In addition, one limitation to the available dataset is that only temporal dependencies over a monthly period can be captured due to the data's temporal resolution and thus shorter term dependencies cannot be ruled out. However the model and method presented can be readily applied to daily measurements when available, without any modifications. Such a finer temporal resolution has the added advantage of allowing for the introduction of exogenous inputs, such as wind and therefore avoiding the need to generalise such characteristics to monthly averages.

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Student Review Article

SERPINS: FORM, FUNCTION, AND DYSFUNCTION

Nicholas J. Cassar[1] **and Gary J. Hunter**[1]
[1]Department of Physiology and Biochemistry, University of Malta, Msida, Malta

Abstract. The serpin superfamily of serine protease inhibitors is one of the most ubiquitous and successful classes of inhibitors in the living world. Their unique mechanism of suicide inhibition has led to much research and several important discoveries. They function via rapid incorporation of a reactive centre loop (RCL) within a β-sheet following the former's proteolysis by the target protease: the serpin thus achieves a conformation which is more stable than the native form. Through this conformational change, the target protease structure is distorted and its function disrupted. Alpha-1-antitrypsin (AAT) has often been studied as an archetype for the serpin superfamily, and is discussed in more detail in this review. Of particular interest are the mutant variants of AAT, which have a tendency to polymerise, and thus offer insights into some mechanisms of serpin polymerisation.

Keywords Serpin, RCL, glycosaminoglycan, AAT, loop-sheet polymerisation, serpinopathy

1 The Serpin Superfamily

1.1 Introduction

Serpins are a diverse superfamily of proteins, most of which are serine protease inhibitors - hence their name (Huntington 2011; Khan et al. 2011). The size and ubiquity of this superfamily is testament to the evolutionary success of the serpin structure and function. There are more than 1500 serpin-like genes identified in a wide spectrum of organisms (Law et al. 2006). While the distribution may be vast, it is not even: all multicellular eukaryotes possess serpins (Law et al. 2006), whereas they are found only infrequently in prokaryotes (Irving et al. 2002b). Similarly, serpin structure differs between kingdoms of life. In fact, 'classical' serpins are found in higher eukaryotes and viruses, but not in prokaryotes (Irving et al. 2002b).

Some serpins not only inhibit protease inhibitors, but also cysteine proteases (Irving et al. 2002a) such as the caspases (Lockett et al. 2012), cathepsins (Fluhr et al. 2011; Higgins et al. 2010), and calpains (Luke et al. 2007). Still other serpins have no inhibitory activity, such as chicken ovalbumin (Huntington 2011), and corticosteroid binding globulin (CBG) and thyroxine binding globulin (TBG) in humans (Carrell et al. 2011). HSP47 is another non-inhibitory human serpin, which serves as a collagen-specific molecular chaperone (Nagata, 2003), and has potential as a target for Alzheimer's disease therapy (Bianchi et al. 2011).

So far, 36 serpins have been identified in humans, 27 of which are inhibitory (Law et al. 2006) as shown in Table 1. They serve functions including regulation of inflammation (Horn et al. 2012; Huntington 2011; Khan et al. 2011; Law et al. 2006), coagulation (Huntington 2011; Khan et al. 2011; Law et al. 2006), fibrinolysis (Huntington 2011; Khan et al. 2011), complement system (Khan et al. 2011), apoptosis (Law et al. 2006), and blood pressure (Ricagno et al. 2010). In the clinic, serpins could also serve as biomarkers in the diagnosis and therapy of cancer (Ghazy et al. 2011; Lim et al. 2012). These potential markers include SERPINB11 (Lim et al. 2012) and maspin - a non-inhibitory serpin which is involved in apoptosis and reduces risk of metastasis (Ghazy et al. 2011). This diverse array of functions is

RCL: reactive centre loop

AAT: alpha-1-antitrypsin

Correspondence to: N. J. Cassar (ncas0016@um.edu.mt)

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down to the unique biochemistry of serpins.

Table 1. The Human Serpins

Serpin name[a]	Target protease (if inhibitory) Function (if non-inhibitory)	Official gene symbol	Chromosomal location	Source
Alpha-1-antitrypsin	Elastase, plasmin, thrombin, trypsin, chymotrypsin, and plasminogen activator	SERPINA1	14q32.1	NCBI, Gene ID: 5265 (2013)
Alpha-1-antitrypsin-like	Pseudogene	SERPINA2	14q32.1	Seiksas et al. (2006)
Alpha-1-antichymotrypsin	Chymotrypsin	SERPINA3	14q32.1	NCBI, Gene ID: 12 (2013); Rubin et al. (1990)
Kallistatin	Kallikrein	SERPINA4	14q32.13	Chai et al. (1993); NCBI, Gene ID: 5267 (2013)
Protein C inhibitor	Protein C, kallikreins, various plasminogen activators	SERPINA5	14q32.1	NCBI, Gene ID: 5104 (2013)
Corticosteroid binding globulin	Binds corticosteroid hormones	SERPINA6	14q32.1	NCBI, Gene ID: 866 (2013)
Thyroxine-binding globulin	Binds thyroxine	SERPINA7	Xq22.2	NCBI, Gene ID: 6906 (2013)
Angiotensinogen	Precursor of angiotensin I	SERPINA8	1q42.2	NCBI, Gene ID: 183 (2013)
Germinal center B-cell expressed transcript-1	Trypsin, thrombin, plasmin	SERPINA9	14q32.13	NCBI, Gene ID: 327657 (2013); Paterson et al. (2007)
Protein-Z related protease inhibitor	Factor Xa, Factor XIa	SERPINA10	14q32.13	NCBI, Gene ID: 51156 (2013)
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 11	Serine-type endopeptidases	SERPINA11	14q32.13	NCBI, Gene ID: 256394 (2013); Nextprot BETA (2013)
Vaspin	Insulin-sensitising adipocytokine	SERPINA12	14q32.13	Hida et al. (2005); NCBI, Gene ID: 145264 (2013)
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 13	Pseudogene	SERPINA13	14q32.13	NCBI, Gene ID: 388007 (2013)
Monocyte neutrophil elastase inhibitor	Neutrophil elastase, cathepsin G, and proteinase-3	SERPINB1	6p5	NCBI, Gene ID: 1992 (2013)
Plasminogen activator inhibitor-2	Urinary plasminogen activator, tissue-type plasminogen activator	SERPINB2	18q21.3	Harrop et al. (1999); NCBI, Gene ID: 5055 (2013)

Squamous cell carcinoma antigen-1	Cathepsin K, cathepsin L, cathepsin S	SERPINB3	18q21.3	NCBI, Gene ID: 6317 (2013); Schick et al. (1998)
Squamous cell carcinoma antigen-2	Cathepin G, mast cell chymase	SERPINB4	18q21.3	NCBI, Gene ID: 6318 (2013); Schick et al. (1998)
Maspin	Tissue-type plasminogen activator	SERPINB5	18q21.33	NCBI, Gene ID: 5268 (2013); Sheng et al. (1998)
PI6	Cathepsin G	SERPINB6	6p25	NCBI, Gene ID: 5269 (2013); Scott et al. (1999);
Megsin	Plasmin, matrix metalloproteinases	SERPINB7	18q21.33	NCBI, Gene ID: 8710 (2013); Ohtomo et al. (2008);
PI8	Furin	SERPINB8	18q21.1	Leblond et al. (2006); NCBI, Gene ID: 5271 (2013)
PI9	Granzyme B	SERPINB9	6p25	NCBI, Gene ID: 5272 (2013)
Bomapin	Thrombin, trypsin	SERPINB10	18q21.3	NCBI, Gene ID: 5273 (2013); Riewald and Schleef (1995)
Serpin peptidase inhibitor, clade B (ovalbumin), member 11	Unclear function in host-pathogen interactions	SERPINB11	18q21 cluster	NCBI, Gene ID: 89778 (2013); Seixas et al. (2012)
Yukopin	Trypsin, plasmin	SERPINB12	18q21 cluster	Askew et al. (2001); NCBI, Gene ID: 89777 (2013)
Headpin	Cathepsin L, cathepsin V	SERPINB13	18q21.33	NCBI, Gene ID: 5275 (2013); Welss et al. (2003)
Antithrombin	Thrombin, factor Xa, chymotrypsin	SERPINC1	1q25.1	NCBI, Gene ID: 462 (2013); Yang et al. (2010)
Heparin cofactor II	Thrombin	SERPIND1	22q11.21	NCBI, Gene ID: 3053 (2013)
Plasminogen activator inhibitor type 1	Urinary plasminogen activator, tissue-type plasminogen activator	SERPINE1	7q22.1	NCBI, Gene ID: 5054 (2013)
Protease nexin-1	Thrombin	SERPINE2	2q36.1	Li et al. (2012); NCBI, Gene ID: 5270 (2013)
Pigment epithelium derived factor	Neurotrophic factor	SERPINF1	17p13.3	NCBI, Gene ID: 5176 (2013)
Alpha-2 antiplasmin	Plasmin	SERPINF2	17p13	NCBI, Gene ID: 5345 (2013)

Complement-1 inhibitor	Activated C1r, activated C1s	SERPING1	11q12.1	NCBI, Gene ID: 710 (2013)
Heat shock protein 47	Chaperone protein	SERPINH1	11q13.5	NCBI, Gene ID: 871 (2013)
Neuroserpin	Tissue-type plasminogen activator	SERPINI1	3q26.1	NCBI, Gene ID: 5274 (2013)
Pancpin	Tissue-type plasminogen activator	SERPINI2	3q26.1	NCBI, Gene ID: 5276 (2013); Silverman et al. (2001)

[a]Serpin name is shaded if it is a known protease inhibitor

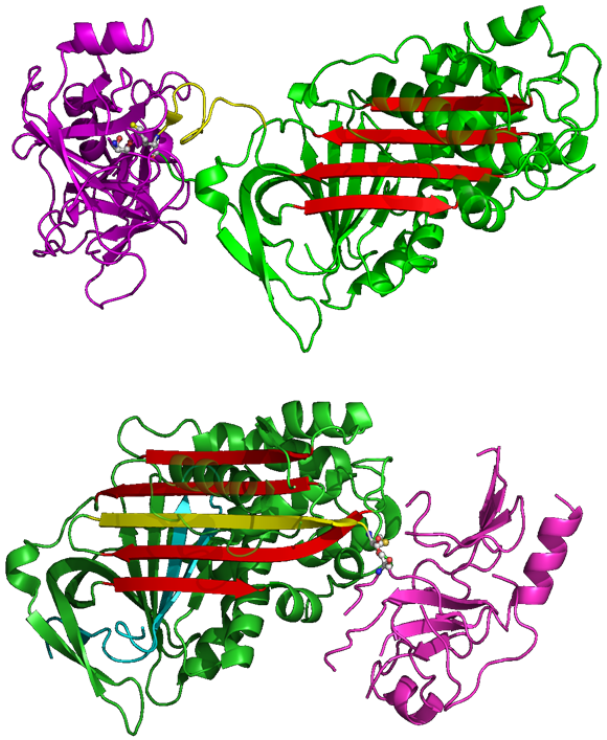

Figure 1: Serpin (AAT) and serine protease (trypsin) interaction
The trap is set (top)
The serpin (green) presents the RCL (yellow) containing the P1 residue (white) for its serine protease target (magenta). The active site serine is white. The 4-stranded β-sheet A in red is a metastable 2° structure.
The serine protease takes the bait (bottom)
After cleavage of the RCL the protease is covalently bound via its reactive serine residue to the serpin (white). The RCL (yellow) now inserts into β-sheet A to form a highly stable conformation. The serine protease is irreversibly inhibited. The cleaved serpin has a new chain (cyan). Note that the serine protease has been dragged over $70, \mathring{A}$ from the original position of the bait residue to the serpin's distal end.
Source: Pymol rendering using PDB entry 1K9O from Ye et al., (2001) (top) and PDB entry 1EZX from Huntington (2000) (bottom)

1.2 Serpin Structure and Conformational Changes

Serpins are approximately $350 - 400$ amino acids long (Patson 2000) and with a relative molecular weight roughly $40 - 60$ kDa (Gettins, 2002). They are globular (Ricagno et al. 2010) glycoproteins in their native conformation (Hopkins et al. 1997). Their secondary structure consists of helical (N-terminal) and β-barrel (C-terminal) domains (Huntington 2011). There are a total of nine α-helices (Patschull et al. 2011) as well as three β-sheets (Patschull et al. 2011).

Serpins also possess a reactive centre loop or RCL (Huntington 2011), exposed for the initial interaction with the protease to be inhibited (Lawrence et al. 1994). This peptide chain can be between $20 - 24$ residues in length (Huntington 2011). In the case of inhibitory serpins, the RCL is characterised by an electrically neutral residue at position P14, separated from the N-terminal end of the 'bait' amino acid (P1) by 12 residues (Lawrence et al. 1994). The specificity of the serpin-protease reaction is heavily dependent on the RCL , making RCL sequences a target for molecular engineering (Bottomley and Stone, 1998) and a source of serpin mutational dysfunctions (Yamasaki et al. 2010).

The mechanism of inhibitory serpins involves a significant change in their structure (Huntington 2000) as well as that of the target protease (Huntington 2011). This change from native to inhibiting state is associated with an increase in the stability of the serpin structure (Singh and Jairajpuri, 2011). In the native metastable state, the serpin has a 5 stranded β-sheet A and an exposed RCL; in the hyperstable state (following serpin-protease interaction), the RCL has been cleaved and becomes inserted in the β-sheet A as the new fourth strand (Figure 1).

Serpins can also take on the hyperstable inactive conformation without protease interaction. This mechanism involves incorporating the RCL into β-sheet A of the same serpin molecule following disruption of the intermolecular bonds between the first chain in β-sheet C and the rest of the sheet (Na and Im, 2007). The likelihood of taking this 'latent' form affects the serpin's half-life (Thompson et al. 2011).

Research by Seo et al. (2000) showed that the strain associated with the metastable structure is not localised to one structure of the serpin, but is more likely to

be diffuse. They identified several surface hydrophobic regions on α-1-antitrypsin (AAT) as contributors to this global tension. Other reasons include overclustered side-chains, thermodynamically unfavourable polar-nonpolar non-covalent bonds, and surface cavities (Im et al. 1999). However, Seo et al. (2000) point out that there is little, if any, effect on serpin inhibitory activity following stabilising mutations in regions of tension which are not vital to serpin mechanism and which are not involved in conformational change. One residue which seems central to conformational change in AAT and other serpins is lysine 335 (Im and Yu, 2000), which interacts with helix F (hF) and the loop between hF and β-sheet A's third strand (thFs3A) (Seo et al. 2000).

A study by Baek et al. (2007) introduced disulfide bonds into AAT in order to investigate the conformational changes occurring in serpins during inhibition. Their study showed that in order for RCL incorporation as the fourth strand of β-sheet A, hF and thFs3A must be able to move away from the fifth strand. Krishnan et al. (2011) also studied AAT and confirmed that there is dissociation of hF from β-sheet A. They identified other conformational changes in the serpin preceding RCL incorporation, such as dissociation of strands 5 and 6 from the rest of β-sheet A, and disruption of the intermolecular bonds between RCL and β-sheets B and C.

1.3 Protease Conformational Change

Serpins have a profound effect on target serine proteases, not only by inhibiting the active site but also by distorting the structure of the protease (Huntington 2000).

Serpins and their targets interact in a 1 to 1 ratio, forming an SDS-stable complex (Egelund et al. 1998). The resistance of this complex to SDS is explained by the covalent bond formed between serpin and protease (O'Malley et al. 1997). This, coupled with the ratio of interaction, has led to serpins being called 'suicide' inhibitors (Lawrence et al. 1995).

The P1 − P1′ bond of the serpin RCL serves as 'bait' for the attacking protease, P1′ being the amino acid closer to the C-terminal end (O'Malley et al. 1997). The protease cleaves the bond to form an acyl-intermediate with the serpin (Lawrence et al. 1995). Normally, protease action is completed by hydrolysis of the acyl-intermediate to form a tetrahedral intermediate which then disintegrates, resulting in separation of the protease-product complex (Hedstrom 2002). However, the formation of the acyl-intermediate is followed by rapid burying of the RCL into β-sheet A as the new fourth strand (Lawrence et al. 1995). This prevents hydrolysis either by preventing entry of water into the active site (Lawrence et al. 1995) or by disturbing the conformation of the active site (Dementiev et al. 2006; Huntington 2011), or both (O'Malley et al. 1997). Thus, serpin-protease complexes are trapped in an acyl-intermediate stage (Egelund et al. 1998).

The incorporation of the RCL into β-sheet A also drags the attached protease over 70$\mathring{A}$ from the original position of the bait residue to the distal end of the serpin and results in close approximation between serpin and protease (Huntington 2000) as shown in Figure 1. This could explain the disturbance of active site geometry (Stratikos et al. 1999).

The active site is not the only portion of the protease which is affected. Another study on AAT-trypsin complex (Huntington 2000) showed that approximately 37% of the protease becomes disordered as a result of the dragging force exerted on Ser195 (in the active site) as the RCL is incorporated into β-sheet A. This action also destroys a salt-bridge between Ile16 and Asp194 of the protease, formed during zymogen activation (Huntington 2000). The distortion of serine protease structure has been proposed as another facet to the serpin-inhibition mechanism (Huntington 2000), in view of the fact that such disordered proteases are more prone to proteolytic attack (Egelund et al. 2001) and decreased stability (Kaslik et al. 1997).

The degree of serine protease distortion varies from one serpin-protease complex to another, possibly dependent on factors including RCL length, the presence of protease ligands such as Ca^{2+}, and particular characteristics of serpin and protease loops such as location, length, and sequence (Huntington 2011).

1.4 Serpin Modulation

The glycosaminoglycans heparin and heparin sulphate are modulators of many serpins' function, activating most of the serpins involved in haemostasis (Huntington 2003). However, heparin can also inhibit serpins, such as kallistatin (Chen et al. 2001).

The majority of glycosaminoglycan-modulated serpins possess a sequence neighbouring or involving helix D for interaction with the glycosaminoglycan; the same is true of helix H for protein C inhibitor (PCI) (Rein et al. 2011). The mechanism of activation often involves heparin binding to the serpin and the target protease, bringing them closer and facilitating serpin-protease interaction. Complexes falling under this category include those between PCI and thrombin or activated protein C (Li et al. 2008); antithrombin and thrombin, fIXa or fXa (Olson et al. 2010); glia-derived nexin and thrombin (Baker et al. 1980); heparin cofactor II (HCII) and thrombin (Verhamme, 2012); as well as protein Z inhibitor (PZI) and fXa or fXIa (Huang et al. 2011). The bridging effect of heparin on PZI enables it to inhibit free factors Xa and XIa, whereas other activating cofac-

tors such as lipid, Ca^{2+}, and protein Z promote PZI's inactivation of membrane-bound factor Xa (Huang et al. 2011).

Interestingly, the 'bridging effect' that heparin has on serpins could have therapeutic relevance in oncology. A study by Higgins et al. (2010) showed that heparin improves the inhibition of the papain-like cathepsin L by squamous cell carcinoma antigen-1 and -2 (SCCA-1, SCCA-2), both of which are serpins. This find is interesting in that not only could it explain heparin's anti-metastatic properties, but it is also the first report of heparin promoting serpin inhibition of cysteine protease (Higgins et al. 2010).

Glycosaminoglycans can also enhance serpin function through allosteric alterations (Rein et al. 2011), such as with antithrombin and HCII. Heparin and heparan sulphate cause allosteric activating changes in antithrombin mostly through a mutual pentasaccharide sequence (Olson et al. 2010). This allosteric change does not increase antithrombin's inhibition of thrombin but of factors IXa and Xa (Olson et al. 2010) as well as plasma kallikrein (Olson and Björk, 1991). Ca^{2+} also increases antithrombin's inhibition of factor IXa by allosterically activating the latter (Bedsted et al. 2003).

HCII's inhibition of thrombin is also enhanced by heparin through allosteric modifications (Baglin et al. 2002). Similarly to heparin, dermatan sulphate is another glycosaminoglycan that activates HCII through bridging and allosteric activation mechanisms (Verhamme et al. 2004).

Alternatively, allosteric modification of the target protease can enhance serpin action. For example, interaction of thrombomodulin with thrombin causes the protease configuration to alter, providing a binding-site for the serpin PCI (Yange et al. 2003).

Other modulators of serpin function include vitronectin and cations. The serpin plasminogen activator inhibitor-1 (PAI-1) inhibits β-trypsin, tissue-type (tPA) and urokinase-type (uPA) plasminogen activator (Komissarov et al. 2007) hence playing a crucial role in the regulation of fibrinolysis. The half-life of PAI-1 is normally $1-2$ hours, however this can be altered by modulating factors like vitronectin and cations (Thompson et al. 2011). Vitronectin alone can prolong PAI-1 half-life by approximately 1.5 times (Thompson et al. 2011), through binding of vitronectin's somatomedin B domain to α-helix F of PAI-1 (Komissarov et al. 2007). Cations such as Mg^{2+} and Ca^{2+} prolong half-life slightly, whereas Cu^{2+} and Co^{2+} without vitronectin reduce half-life significantly but prolong it in the presence of vitronectin (Thompson et al. 2011). Calcium can also inhibit the activity of AAT [discussed below].

Serpin modulation can also occur at the level of the nucleus. Serpinin, released from neuroendocrine cells during exocytosis of dense core granules (DCGs), interacts with extracellular receptors to increase transcription of protease nexin-1 (PN-1) (Koshimizu et al. 2010). PN-1 is a serpin which prevents the proteolysis of DCG proteins in the Golgi complex, thus favouring the replacement of the exocytosed DCGs (Kim et al. 2006). On the other hand, PAI-1 transcription is increased by transforming growth factor (TGF)-β, a mechanism which has been implicated in vascular disease associated with non-insulin dependent diabetes (Nakayama et al. 2011). PAI-2 transcription is inhibited by heparin (Pepe et al. 1997), which contrasts with the activation of PAI-1 by heparin. Maspin can also be regulated through the rate of its transcription, either inhibited, such as by protease activated receptor-1 (Villares et al. 2011), or activated, such as by nitric oxide (Khalkhali-Ellis et al. 2003).

2 ALPHA-1-ANTITRYPSIN (AAT)

2.1 The relevance of AAT

AAT has often been studied as an archetype for the structure, function, and dysfunction of the serpin superfamily (Baek et al. 2007; Ekeowa et al. 2010; Huntington 2000; Krishnan et al. 2011; Mushero et al. 2011; Sengupta et al. 2009; Seo et al. 2000), thus emphasising its importance in understanding serpins.

2.2 Structure and function

Plasma AAT is 394 residues long (Janciauskiene et al. 1998), with a relative molecular weight of 52 kDa (Dickens et al. 2011).

AAT has three glycans and eight alpha-helices, the latter of which consist mostly of the most N-terminal 150 residues (Loebermann et al. 1984). AAT inhibits neutrophil elastase, and is produced mainly in the liver at a rate of 2 g/day (Greene and McElvaney, 2010) but also in other sites (Dickens and Lomas 2011), particularly the lungs (van't Wout et al. 2011). In the lungs, AAT production is higher in pro-inflammatory macrophages than in anti-inflammatory macrophages and immature dendritic cells: in all cases, lipopolysaccharide stimulates an increase in AAT release (van't Wout et al. 2011). The main role of AAT is to limit the damage inflicted by neutrophil elastase on tissues at sites of inflammation (Dickens and Lomas, 2011).

The 'bait' residue for elastase is methionine 358: in fact, oxidation of methionine at this position can block inhibitory function (Taggart et al. 2000). Replacement of this residue with arginine can result in AAT inhibiting thrombin, causing heparin-independent anticoagulant activity and a subsequent bleeding disorder (Owen et al. 1983).

AAT not only prevents lung tissue damage by inhibiting neutrophil elastase, but also by inhibiting lung endothelial cell apoptosis (Petrarche et al. 2006). This is an example of cross-class inhibition, since RCL-intact AAT is internalised by endothelial cells to directly inhibit caspase-3, a cysteine protease involved in apoptosis (Petrarche et al. 2006). The Z-variant of AAT [discussed below] also has direct caspase-3 inhibitory activity (Greene et al. 2010).

Another serine protease inhibited by AAT is matriptase, an enzyme which spans the plasma membrane and whose catalytic activity is extracellular (Janciauskiene et al. 2008). Given the role of matriptase in activating prostatin which then modulates epithelial sodium channels, inhibition of matriptase by AAT offers therapeutic potential for patients with cystic fibrosis (characterised by a defect in sodium absorption) (Janciauskiene et al. 2008).

2.3 AAT fragments

Elastase cleaves AAT to release a C-terminal product of 4kDA (Schulze et al. 1992), corresponding to residues $358-394$ (Janciauskiene et al. 1998). Tryspin gives the same product: AAT-trypsin complex is composed of two peptides which can be separated by SDS-page, showing that the C-terminal product is bound to the complex by non-covalent forces (Boswell et al. 1983).

The cleaved form of AAT has been shown to increase LDL capture, internalisation, and breakdown in HepG2 cells (Janciauskiene et al. 1997). The cellular response is likely initiated through binding of the C-terminal fragment of cleaved AAT (Janciauskiene et al. 1998). Since AAT is an acute-phase protein, this observation could provide an explanation for hypocholesterolemia succeeding inflammation (Janciauskiene et al. 1997).

The 36 residue C-terminal segment of cleaved AAT also confers chemoattractant properties to elastase-AAT complex, and thus mediates inflammation in the absence of bacteria or complement activation (Banda et al. 1988).

Another truncated form of AAT is SPAAT. SPAAT (short peptide from AAT) is composed of the 44 most C-terminal residues of AAT and can be found bound to the extracellular matrix in humans (Niemann et al. 1997*a*). Here, it could serve a protective role from excess tissue degradation since it is a competitive reversible inhibitor of neutrophil elastase (Niemann et al. 1997b). This contrasts with the irreversible inhibition of elastase by full-length AAT. SPAAT can also be cleaved to release an octapeptide sequence (Wright et al. 2000).

This octapeptide (MFLEAIPM), formed from residues P8-P1 of AAT RCL, was shown to inhibit elastase in a study by Wright et al. 2000. Further kinetic analysis showed that this was uncompetitive inhibition through non-covalent interactions, mostly attributable to the four most N-terminal residues. The study showed that the octapeptide can also form an acyl-enzyme intermediate with elastase, and the uncompetitive inhibition is possibly through stabilisation of this intermediate. Taken together, SPAAT and MFLEAIPM present a possible 'cascade' of protease inhibition by AAT (Wright et al. 2000).

2.4 Regulation of AAT

In the lungs, the activity of AAT can be regulated by surfactant A, a normal component of lung secretions (Sarker et al. 2011). Surfactant A has been shown to bind to AAT to limit its inhibition of elastase in a calcium-dependent manner, involving the carbohydrate side-chains of one or both of the glycoproteins (Gorrini et al. 2005).

Elastase directly promotes transcription of AAT mRNA in monocytes and bronchoalveolar macrophages (Perlmutter et al. 1988). This is the case even in individuals homozygous for the Z variant of the AAT gene; elastase has no effect on AAT secretion however, resulting in intracellular accumulation of AAT in these patients (Perlmutter et al. 1988).

Cations can also modulate the inhibition of trypsin. Inactive trypsin (in an AAT-trypsin complex) exists in equilibrium with the active form: the equilibrium can be shifted towards formation of the latter by stabilising it with Ca^{2+} ions (Calugaru et al. 2001).

2.5 AAT in immunity and inflammation

As an acute-phase protein, AAT serum levels can be used as a marker of inflammatory response (Ziakas et al. 2011). Elastase-AAT complex also corresponds with inflammatory activity: neutrophil degranulation releases neutrophil elastase, which is then inhibited by AAT, forming the complex. For this reason, elastase-AAT can be used as an indirect indication of reperfusion injury following kidney transplant (Zynek-Litwin et al. 2010) or of decreased survival chances in cystic fibrosis patients colonised with Burkholderia cenocepacia (Downey et al. 2007).

AAT dampens inflammation in islet cells and other tissues (Kalis et al. 2010), possibly due to impairment of nuclear factor-kappaB (NFκB) function (Churg et al. 2001; Kalis et al. 2010). The mechanism of AAT's interference with NFκB is unclear: however, it is associated with increased levels of inhibitor of NF-κB (IκB), not due to AAT's protease inhibitory function (Churg et al. 2001).

Interestingly, AAT has shown promise as an adjunct to immunosuppressive therapy to prolong graft viability in insulin-dependent diabetic patients who have received an islet transplant (Lewis et al. 2005). AAT directly

inhibits a mediator of β-cell apoptosis, the cysteine protease caspase-3 (Zhang et al. 2007). AAT can also prevent TNF-αmediated apoptosis in islet β-cells (Zhang et al. 2007), as well as inhibiting other pro-inflammatory cytokines (Pott et al. 2009); the mechanisms remain elusive.

2.6 AAT in vascular disease

The lungs are not the only sites susceptible to damage by imbalance between elastase and AAT levels. Patients with ruptured and unruptured cerebral aneurysms have been shown to have a serum elastase to AAT ratio almost double that of controls, implicating skewed elastase:AAT as a cause of vessel wall damage (Baker et al. 1995).

AAT can protect against vascular disease (through its elastase inhibition function) when associated with HDL (Ortiz-Muñoz et al. 2009). AAT complexed with LDLs (AAT-LDL) could also protect against vascular disease, in women without metabolic syndrome (Kotani et al. 2010). However, oxidative stress due to smoking increases AAT-LDL levels, suggesting that AAT-LDL might have a role to play in cardiovascular disease associated with smoking (Wada et al. 2012).

2.7 AAT variants

Over 95% of AAT deficient individuals are homozygous or heterozygous for the Z-allele (Greene and McElvaney, 2010) on chromosome 14 (Elzouki 1999). The Z-variant of AAT (ZAAT) is characterised by a replacement of Glu342 with Lys (Lomas et al. 1995). ZAAT is not secreted efficiently, hence the AAT deficiency: homozygotes (PiZZ) for the mutant allele have $15 - 20\%$ of normal circulating AAT levels (Elzouki, 1999). Thus, PiZZ is characterised by emphysema (due to circulating AAT deficiency) and chronic liver disease (due to inclusion of ZAAT in hepatocyte endoplasmic reticulum) (Elzouki 1999). The hepatic inclusion bodies are periodic acid-Schiff diastase (PASD)-resistant positive (Francalanci et al. 2009) since ZAAT is a glycoprotein. The buildup of ZAAT in endoplasmic reticulum (ER) causes ER stress and deranged function (Greene et al. 2010). The accumulation of ZAAT is associated with its ability to form polymers: in fact, reduced polymerisation results in increased circulating ZAAT (Parfrey et al. 2003).

ZAAT polymerises by a mechanism known as loop-A-sheet polymerisation (refer to Figure 2), whereby the RCL of one ZAAT molecule is inserted into the β-sheet A of another ZAAT (Wilczynska et al. 2003). ZAAT loop-sheet polymerisation is due to abnormal opening of β-sheet A: a mutation of phenylalanine (position 51, within the hydrophobic core) to leucine was shown to inhibit β-sheet A opening, thus interfering with formation of ZAAT aggregates (Kim et al. 1995).

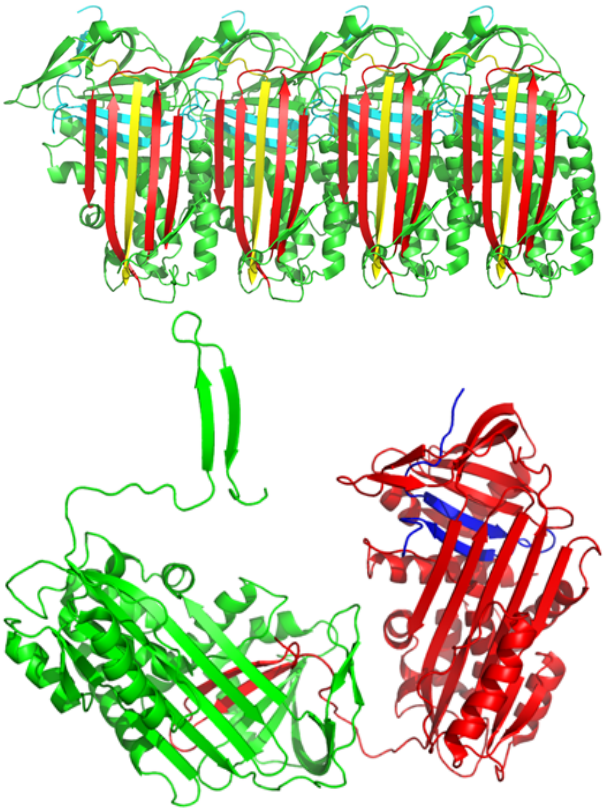

Figure 2: Hypothetical serpin polymerisation schemes
Top: Illustrates loop-A-sheet serpin (AAT) polymerisation. One way that serpin subunits might form a polymeric structure is by inserting the RCL as a fifth β-strand into a neighbouring subunit's β-sheet, in a loop-A-sheet polymerisation mechanism. The distances and links between subunits is exaggerated for clarity. It is envisaged that the close proximity of the β sheets in a serpin polymer would encourage the formation of amyloid-like interactions between subunits.
Bottom: Recent discoveries of dimers and trimers of the serpin antithrombin III suggest a novel interaction through domain swapping, whereby two β-strands are contributed by one subunit to a neighbouring subunit's β-sheet. Two complete subunits are shown (green and red) while only the β-strand contribution is shown of a third (blue). Continuation of the polymer would involve more subunits using similar interactions.
Source: Pymol rendering using PDB entry 1EZX from Huntington (2000) (top) and PDB entry 3T1P, Yamasaki et al. unpublished.

As mentioned previously, there are many sites on a serpin which reduce the stability of its native state. One AAT residue which marks such a site (a hydrophobic surface cavity) is glycine 117 (Lee et al. 2000). This pocket - formed by helix D, helix E, and β-sheet A strand 2 - is obliterated in polymerisation of AAT (Elliott et al. 2000). However, it can be 'filled' by replacing glycine 117 with phenylalanine (bulky side-chain) to increase the stability of native AAT and limit polymerisation without eliminating AAT's inhibitory action (Parfrey et al. 2003). Similar results were observed in ZAAT, where filling the pocket by replacing threonine 114 with phenylalanine resulted in decreased polymerisation and increased ZAAT extracellular release (Parfrey et al. 2003).

Elliott et al. (2000) also studied the Gly117 cavity as well as another four cavities, and compared the sizes of these cavities between four serpins (AAT, alpha-1-antichymotrypsin, PAI-1, and antithrombin). The design of small drugs which can occupy pockets such as these without inhibiting serpin function can have an important role in limiting the pathological conditions associated with intracellular accumulation of polymerised AAT and other serpins (Patschull et al. 2011; Elliott et al. 2000).

Another variant of AAT which exhibits loop-A-sheet polymerisation and accumulation in the endoplasmic reticulum is Siiyama (S53F) (Lomas et al. 1995). The Siiyama variant of AAT (SAAT) is prone to polymerisation due to a propensity for opening of β-sheet A, and impedance of its polymerisation results in increased secretion (Sidhar et al. 1995). Also similar to ZAAT, this variant results in hepatic disease and deficient serum AAT (Janciauskiene et al. 2004).

Siiyama and ZAAT are the most frequent mutant forms of AAT, and result in AAT deficiency in individuals homozygous for the alleles (PiSS and PiZZ genotypes respectively) or possessing both alleles (PiSZ genotype) (Ringenbach et al. 2011). Other variants exist though, such as Mmalton, in which there is deletion of Phe52 (Curiel et al. 1989). Its frequency even exceeds that of SAAT and ZAAT alleles in parts of the Southern Mediterranean (Denden et al. 2010). Like SAAT and ZAAT, Mmalton results in AAT deficiency and polymerises to form hepatic inclusions (Francalanci et al. 2009). However, plasma short-chain polymers of Mmalton were found to be formed by insertion of RCL of one Mmalton molecule into the β-sheet C of another (Lomas et al. 1995). The exposed C-termini of these polymers are more likely to attack by proteases, possibly explaining why Mmalton extracted from blood contains RCL-cleaved AAT (Yamasaki et al. 2011)

3 Serpin polymerisation

The loop-C-sheet polymerisation described above for Mmalton can also be observed in C1 inhibitor (Eldering et al. 1995) and antithrombin dimers (Carrell et al. 1994). In the case of antithrombin dimers, one molecule (in the latent form) has the first strand of β-sheet C separated from the rest of the sheet to permit insertion of the other molecule's RCL (Devlin and Bottomley, 2005). Loop-C-sheet polymers have also been observed in in vitro studies on typical AAT and antithrombin when heated with citrate (Devlin and Bottomley, 2005). Zhang et al. (2008) propose that loop-C-sheet interactions could also account for the polymerisation of the latent forms of some serpins. Their crystallography study of the latent form of tengpinDelta42 (a bacterial serpin) showed hyperinsertion of the RCL into β-sheet A, causing full exposure of β-sheet C. This then allows for hydrogen-bonding between the exposed part of the RCL of one latent serpin molecule with the second strand of β-sheet C of another (Zhang et al. 2008).

Loop-A-sheet polymerisation occurs in AAT, neuroserpins (Santangelo et al. 2012), and α-1-antichymotrypsin (Crowther et al. 2003). Tsutsui et al. (2008) - using wild-type AAT as a paradigm for other serpins - proposed that the mechanism of loop-A-sheet polymerisation begins by disruption of β-sheet C. This then leads to movement of the first strand from the rest of the sheet via serpin-serpin interaction, causing conformational changes. One such change is the opening of β-sheet A, which allows insertion of another serpin molecule's RCL into the sheet for polymerisation to occur (Tsutsui et al. 2008). Krishnan and Gierasch (2011) point out that even under normal conditions, an equilibrium exists between native serpin and an intermediate with an open β-sheet A. Although normally low in concentration, this intermediate's formation is increased in certain AAT variants (e.g. ZAAT) due to a lower thermodynamic barrier, explaining ZAAT's tendency to polymerise after release from hepatocytes (Krishnan et al. 2011). However, the polymerisation of ZAAT and other AAT mutants within hepatocytes is mostly due to delayed folding to the native serpin state, giving intermediates more opportunity chance to polymerise (Yu et al. 1995).

'S7A' polymerisation can be considered another loop-sheet mechanism. The RCL of one molecule forms hydrogen-bonds with the sixth strand of another molecule's β-sheet A, acting as a seventh strand (S7A) (McGowan et al. 2006). Serpins which exhibit such polymerisation include myeloid and erythroid nuclear termination stage-specific protein (MENT) (McGowan et al. 2006) and PAI-1 (Sharp et al. 1999). A mechanism of 'S5A' polymerisation was also proposed by Yamasaki et al. (2008), wherein both the RCL and the fifth strand of the β-sheet A of one molecule are inserted into the β-sheet A of the other. This mechanism might explain the highly chemically stable polymer which human neuroserpin forms when incubated at 85°C (Ricagno et al. 2010).

Yamasaki et al. (2011) propose that polymerisation via RCL insertion occurs via an intermediate which can return to native state or form a polymer. If it is more likely that the RCL is inserted into another molecule, a polymer forms this intermediate state. However, RCL insertion competes with the inclusion of the C-terminus in the folded serpin, in which case the intermediate form returns to native state. In fact, if the RCL insertion process is slowed down, there is reduced polymerisation and increased functional secretion in ZAAT (Yamasaki et al. 2010).

The loop-sheet mechanisms are the best described for serpin polymerisation, but they are not exclusive. Marszal et al. (2003) described the polymerisation of disulfide-linked dimers of wild-type AAT. The dimers were obtained in vitro, using a mild denaturing buffer without reducing agents, and polymerised through intermolecular interactions on the surface with β-sheet A. The relevance of this find is unclear; however, the similarity in structure (under the electron microscope) of dimer polymers to loop-sheet polymers suggests that the latter may involve disulfide bonds (Marszal et al. 2003).

Not all serpin multimers are pathological. For example, S7a polymerisation of MENT could actually participate in normal chromatin condensation (McGowan et al. 2006). However, the vast majority of serpin polymers are linked to disease states, such as those described above for AAT variants.

Diseases may be due to deficiency of the serpin, which is not secreted but is trapped as polymers in the endoplasmic reticulum (ER) of the secretory cell. This is true for individuals homozygous for the mutant alleles of AAT (as described above). Mutations of antithrombin, α1-antichymotrypsin, and C1-inhibitor can also result in intrahepatocyte polymer formation and subsequent deficiency disease (Belorgey et al. 2007). Deficiency disease can also occur with spontaneous polymerisation following secretion, hence limiting the amount of available serpin: for example, for individuals heterozygous and homozygous for the F229L mutant allele of antithrombin (Picard et al. 2003). Serpins need not necessarily be mutant to polymerise and cause deficiency: wild-type PAI-2 can undergo loop-sheet polymerisation within the cytosol to eventually limit its own secretion (Mikus et al. 1996).

Gain-of-function toxicity is another cause of disease. One such case is that of mutant neuroserpin polymers within ER, resulting in familial encephalopathy with neuroserpin inclusion bodies (FENIB) (Miranda et al. 2008). One possible mediator of this disease is nuclear factor kappa B (NF-ϰB), which is activated by the intraendoplasmic accumulation of neuroserpin polymers (Davies et al. 2009). The pro-inflammatory mediator NF-κB is also elevated with intraendoplasmic deposition of ZAAT polymers: inhibiting NF-ϰB's actions (and subsequent inflammation) may prove to be a line of therapy for this genetic disease (Lawless et al. 2004).

Toxicity can also be a result of serpin oligomers, rather than polymers. Carrell et al. (2008) used AAT and antithrombin to demonstrate that in the initial stages of serpin oligomer formation, the opening of the A-sheet creates a β-acceptor site which can potentially bind to physiologically significant peptides such as neurotransmitters, resulting in toxicity. Hence, extension of the oligomer to form a serpin auto-polymer is actually protective in that auto-polymerisation sequesters otherwise toxic oligomers.

4 Conclusion

To conclude, although serpins' roles in physiology and disease are varied, they share a common structure which allows great versatility and has proven to be an evolutionary success. Understanding serpin structure and their mechanism of inhibition is crucial to developing treatments for their dysfunctions.

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Note Articles

THE UNIVERSITY OF MALTA (SEISMIC MONITORING AND RESEARCH UNIT), UNIVERSITY OF BASILICATA AND IMAA-CNR (ITALY) OPERATIONS DURING THE 2012 EMILIA SEISMIC SEQUENCE

Sebastiano D'Amico[1], **Maria Rosaria Gallipoli**[2], **Marco Mucciarelli**[3,4] **and Luigi Vignola**[5]
[1]Department of Physics, University of Malta, Msida, Malta
[2]Istituto di Metodologie per l'Analisi Ambientale, Consiglio Nazionale delle Ricerche, Tito Scalo (PZ), Italy
[3]Dipartimento di Strutture, Geotecnica, Geologia Applicata all'Ingegneria, Universita' della Basilicata, Potenza, Italy
[4]Istituto Nazionale di Oceanografia e Geofisica Sperimentale, Trieste, Italy
[5]Mallet SRL, Marsicovetere (PZ)

Abstract. On 20th May 2012 (02:03 UTC), and on 29th May 2012 (07.00 UTC) two major earthquakes occurred in Northern Italy. The two earthquakes caused 27 people to be killed (7 on 20th May and 20 on 29th May), at least 400 injured, and up to 45,000 homeless in total, with initial estimates placing the total economic loss at several billion Euros. The main goal of this communication is to describe the operations and efforts of several researchers and Institutions during the seismic crises of the Emilia sequence. The acquired data can provide tools to reduce the impact of future earthquakes on the local communities.

Keywords Seismic crises, HVSR, Data Acquisition during emergency

1 Introduction

On 20th May 2012 (02 : 03 UTC) May 2012, and on 29th May 2012 (07.00 UTC) two major earthquakes occurred in Northern Italy (Anzidei et al. 2012). They were felt throughout northern Italy, 27 people were killed (7 on 20th May and 20 on 29th May), at least 400 injured, and up to 45,000 homeless in total, with initial estimates placing the total economic loss at several billion Euros. The two earthquakes struck in the Emilia-Romagna region, about 40 kilometres north of the city of Bologna.

The epicentre (depth of 9 km) of the first one was located between Finale Emilia and San Felice sul Panaro; while the second one was in Medolla at a depth of about 10 kilometres. Both earthquakes were followed by thousands of aftershocks, which were detected and located by the Istituto Nazionale di Geofisica e Vulcanologia (INGV) using a portable network of seismometers installed a few hours after the earthquake, which detected even the smallest events. The Po Plain is part of the active front of the Northern Apennines fold and thrust belt. The area is characterized by a series of active thrust faults and related folds. These faults are roughly WNW-ESE trending, parallel to the mountain front, and dip shallowly towards the south-southwest (Toscani et al. 2008). Several damaging historical earthquakes, such as the 1570 Ferrara earthquake (Boschi et al. 2000), have occurred in the area of the 2012 seismic sequence.

Both the 20th May and 29th May events caused extensive damage. After the two large events of the seismic sequence, inspections were underway to determine

Correspondence to: S. D'Amico (sebastiano.damico@um.edu.mt)

which buildings were safe to re-enter. Total and partial collapses were observed in the old and monumental buildings in the historical centres (Fig. 1). At least 100 structures of historical significance were damaged or destroyed. Many churches and towers in towns around the epicentre suffered damage. Half of a clock tower in one of the small villages in the epicentral area dating from the 13th century (known as the torre dei modenesi) fell down in the mainshock and the remaining part collapsed completely during an aftershock later that day. The 15th-century cathedral of Mirandola, already damaged on 20th May, collapsed after the 29th May shock. Heavy damage to industrial bases and farmhouses, and also the collapse of numerous barns, was observed through the whole epicentral area. There was also significant damage to factories and agricultural land in the region. Some of the deaths were caused by the collapse of recently constructed factory buildings. Old traditional brick houses suffered widespread cracks in the walls, detachment of tiles, and chimney falls. In general, it has been observed that reinforced-concrete buildings suffered minor damage. However, a few reinforced-concrete buildings collapsed or suffered a partial collapse (for instance in the village of Cavezzo and Rovereto sulla Secchia). In some cases, the damage was increased due to the huge liquefaction phenomenon that affected the deposits below the buildings (Galli et al. 2012; Tertulliani et al. 2012).

Right after the first shock the Italian Civil Protection Department arranged 35 reception camps set up in Emilia-Romagna and 10 in Lombardia. Other structures able to host people affected by the quake were also made available (tensioned structures, train carriages, covered structures and hotels). One of the reception camps in Mirandola (Fig. 1f) was able to host the group of scientists conducting the field work briefly presented in this note.

2 Instrumentation Installed, Data Acquired, Processing, and Preliminary Results

The main goal of this communication is to describe the operations and efforts of several researchers and Institutions during the seismic crises of the Emilia sequence. The acquired data can provide tools to reduce the impact of future earthquakes on the local communities as well as to contribute to the installation of seismic networks In fact, rapid-response seismic networks are an important element in the response to seismic crises. They temporarily improve the detection performance of permanent monitoring systems during seismic sequences. The improvement in earthquake detection and location capabilities can be important for decision makers to assess the current situation, and can provide invaluable data for scientific studies related to hazard, tectonics and earthquake physics (Moretti et al. 2012). The day after the mainshock, the Istituto Nazionale di Geofisica e Vulcanologia (INGV) rapid response network for site effects, called EMERSITO, deployed three linear arrays with a total of 22 sites instrumented, 16 of them equipped with both velocimeters and accelerometers (Bordoni et al. 2012)

Our team of scientists installed 3 accelerometers (ETNA-Kinemetrics) in the hospital of Mirandola and in the urban area of the same village (Fig. 1g: Fig. 2). They were placed in order to evaluate the variability of the seismic site response and they have been continuously functioning from May 23rd to June 7th recording about 700 earthquakes including the magnitude 5.8 which occurred on May 29th. Some examples of earthquake recordings are shown in Figure 2.

During the campaign we also recorded ambient noise at 57 sites (Fig. 1e-g; Fig. 2) using a 3- component portable seismometer (Tromino, www.tromino.eu). Time series of ambient noise, having a length of $10 - 20$ min, were recorded following the guidelines suggested by the SESAME project (2004) in order to compute the Horizontal to Vertical Spectral Ratio (HVSR) which will contribute to investigate the shallow geological structures in the study area. A direct estimate of the polarization angle (Fig. 2) at all the recording sites was also achieved by using the Time- Frequency (TF) polarization method based on the combination of complex polarization analysis (Vidale 1986) and the continuous wavelet transform (CWT) (Burjánek et al. 2010; Burjánek et al. 2012).

The passive array data was collected using a 24 vertical 4.5 Hz Geospace geophones, arranged in an L-shaped array with an inter-distance ranging from 1 to 60 meters and the longest arm 250 meters long. The data were collected using a 24 bit A/D digitiser (Geode Geometrics) with a $125\,\mu$s sampling rate. In order to process and interpret the seismic array results we used the ESAC (Extended Spatial Autocorrelation) procedure (Okada, 2003; Ohori et al. 2002; Parolai et al. 2006).

HVSR analyses, at the investigated sites, show a resonance peak frequency at about 0.9 Hz, while results using earthquake recordings show a much more complex behaviour that that one observed by ambient noise. The array measurements show a quite uniform Vs profile (Fig. 2). At a first instance, the differences observed between the HVSR obtained by using noise and earthquake data may be due to source effects or to complex propagation at depths larger than the one presently investigated (see e.g. Malagnini et al. 2012 for the possible role of crustal propagation). However, on-going studies will try to shade light on this.

Figure 1: Panels a to e show some examples of damage caused by the strongest earthquakes of the seismic sequence. Panel f shows one of the Mirandola camps set by the Italian Civil Protection Department, Panel g reports an example of the installation of the temporary seismic instruments. Panel e shows also the installation of the Tromino® during the measurements in the vicinity of one building severely damaged during the maninshock. The children drawing presented in panel h has the aim to illustrate the effects of earthquakes on society (the word "PRIMA" stands for before while the word "DOPO" stands for after). The drawing has been realized by one the children hosted in the Mirandola camp in which also the research team has stayed.

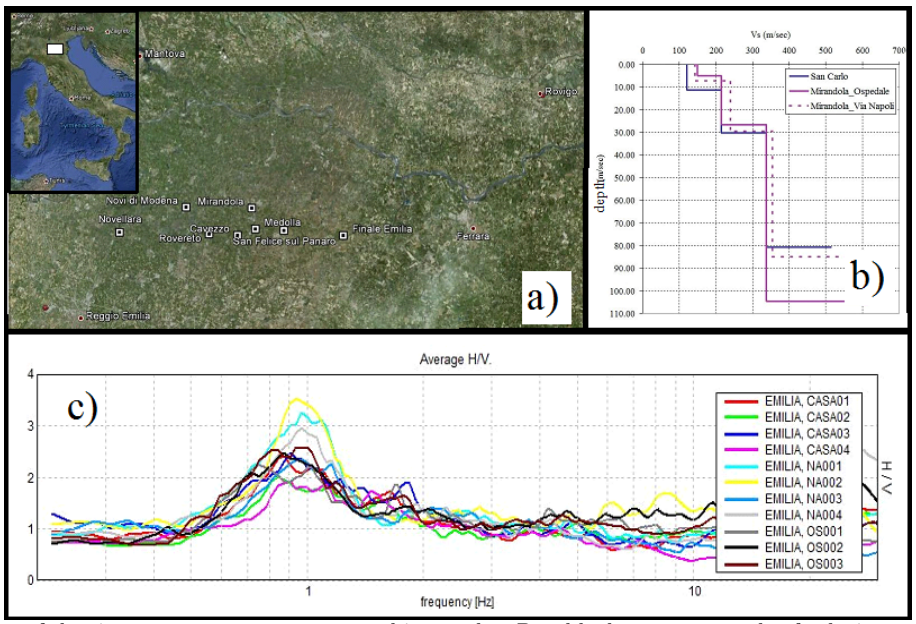

Figure 2: The location of the site measurements are reported in panel a. Panel b shows an example of velocity profile derived by using the ESAC technique. Panel c reports some example of HVSR analysis.

3 Concluding Remarks

Rapid earthquake response by scientists is very challenging and requires a high degree of preparedness.

In recent years the acquisition, transmission, exchanges and archiving of data have succeeded in vastly improving real-time monitoring systems and are widely available to the scientific community. Many improvements have been achieved through several international projects (e.g. Network of European Research Infrastructures for Earthquake Risk Assessment and Mitigation). Such kind of approach represents an important first step towards being prepared. The data collected during emergency response consist of a remarkable quantity of high-quality data that spans the entire range of ground motion and is usually recorded under near-field conditions. However, several further improvements are foreseen and needed. For example, in this context, the organization of regular seismic-risk emergency simulation can play a key role, and surely would help and contribute to improve technical and scientific exchange among the different research groups, institutions, and governments.

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News Article

COST: Matchmaking for Researchers

Janet Mifsud[1,2] and Claire Bellia[3]
[1]COST Malta Country National Contact, Malta Representative on COST Committee of Scientific Officers (CNC CSO)
[2]Department of Clinical Pharmacology and Therapeutics, University of Malta, Msida, Malta
[3]International Relations and Policy Executive, Malta Council for Science and Technology, Villa Bighi, Kalkara, Malta

1 Introduction: What is COST?

As many researchers are aware, while the EU offers a huge variety of funding possibilities, many of the funding mechanisms are tied to projects which require consortia made up of partners from different countries. Many researchers based in Malta, especially younger researchers, query on how one gets invited to participate in such consortia. In addition, the complex funding and administrative procedures involved in many of these EU projects, may put off several local scientists and researchers from applying for such funds.

COST, Cooperation in Science and Technology, offers a facilitated mechanism which tackles and overcomes these hurdles. Since the early 1970s, COST, European COoperation in Science and Technology, has brought researchers from various countries together to promote transnational coordination of nationally-funded research. This is a unique approach which enables the sharing of research interests across thirty-five European countries, and has even attracted the participation of many non EU countries such as Australia and Canada. COST not only supports the organization of the networking meetings, but also other activities as described later on. It attracts scientists and experts working not only within academia and industry, but also from NGOs and the public sector. Moreover, COST involves very simple administrative and funding procedures.

COST is indeed a unique research tool since it connects and acts as a 'matchmaker' for scientists across Europe and internationally, and enables the creation of communities of research in various fields. The COST networking platform allows researchers to meet and build consortia which can lead to the submission of transnational project proposals for future funding such as the EU's Framework Programme, now Horizon 2020. COST also funds publications and promotes the dissemination of information collected from these networking events. This increases the impact of the outcomes of COST Actions on policy-makers and other decision-making bodies.

2 How does COST work?

COST, *www.cost.eu*, is an intergovernmental framework based at European Council, which allows the coordination of nationally-funded research on a European level. As already explained, COST contributes to reducing and opening the European Research Area to cooperation worldwide and thus makes it possible for the various national facilities, institutes, universities, public sector and private industry to work jointly on a wide range of activities. COST is based around the funding of so called COST Actions. COST is unique in that the objectives of these COST Actions are the result of a bottom-up approach to research in the ten key domains: Biomedicine and Molecular Biosciences (BMBS); Chemistry and Molecular Sciences and Technologies (CMST); Earth System Science and Environmental Management (ESSEM); Food and Agriculture (FA); Forests, their Products and Services (FPS); Individuals, Societies, Cultures and Health (ISCH); Information and Communication Technologies (ICT); Materials, Physical and Nanosciences (MPNS); Transport and Urban Development (TUD) and also Transdomain Proposals (TDP-SAB).

Correspondence to: J. Mifsud (janet.mifsud@um.edu.mt)

Each COST Action lasts for four years and requires a minimum participation of five COST Member countries. Proposals for new COST Actions can be submitted by any type of institution, through a twice-yearly open call for proposals with deadlines usually in March and September. These proposals are evaluated by the relevant Domain Committee, through a number of steps, including peer-review of full proposals and organisation of oral presentations. The Committee for Scientific Officers (CSO), which consists of high level officials from each member state, then takes the final decision as to which COST Actions are funded. The whole process takes around eight months from the proposal-collection date.

Following approval by the CSO, the COST Action usually starts within three months from the date of approval. The activities of each COST Action are setup and organised by the Management Committee (MC) which is appointed once the Action is launched. Presently the COST Office and European Science Foundation (ESF) support services are responsible for both the administration and scientific coordination of the COST Actions and handling the reimbursement procedures.

3 How can a researcher based in Malta apply to participate in COST?

What is so attractive about COST is that researchers from different countries, who were not involved in the original consortium which submitted the original proposal, can also apply to join the COST Action, once it has been approved by CSO. This is done on a national level, whereby researchers submit an application to their COST National Contact (CNC). Indeed, in Malta, over ninety percent of the participations by researchers and scientists in COST Actions is the result of an application to an already approved COST action, and the researchers did not form part of the consortium who submitted the application.

In Malta, applications from local researchers and scientists to participate in COST Actions, require very minimal administration. The best way to get involved in COST is to undertake an online search, using such tools as the search engine on the COST website *http : //www.cost.eu/domains_Actions/all_Actions*, in order to find ongoing or new COST Actions that match one's expertise and research interests. The researcher is then required to fill in a simple application form available on the MCST website *http : //www.mcst.gov.mt/networking/cost* and submit it as a soft copy to the Malta COST National Contact (CNC) as indicated on the application form.

These applications are then evaluated by the Malta CNC and the relevant Malta Domain Committee (DC) Representative to ensure that the researcher's expertise 'matches' the aims of the COST action. If positively evaluated, the Malta CNC and Malta DC forward their recommendation to the Executive Board of the Malta Council for Science and Technology (MCST), which manages COST in Malta. The whole process is usually complete in about two weeks from submission of the application.

Once the researcher's application is approved by MCST, the CNC then submits a request to the COST office based at the European Council, in order that Malta formally accepts the Memorandum of Understanding of the COST Action. This is done since, it is to be highlighted that through participation in COST Actions, the researcher is representing their country (and not themselves). The participant's details are then uploaded on the e-COST website by the CNC, and the participant is then formally nominated as a COST Management Committee (MC) member. S/he is then able to participate fully in the COST action and its working groups, will be informed of all meetings, and will also be reimbursed for travel and subsistence expenses related to the attendance of COST meetings.

Thus, in COST, there are no funds for research as such, but participants are reimbursed for expenses in conjunction with flights and accommodation to COST meetings. It leads to excellent contacts (meetings are generally held 2-3 times per year) and thus one can establish excellent relations over the four years of the COST action.

What makes COST even more attractive in the local Maltese context is that, for once, our small size is an added advantage. Indeed, irrespective of the country size, every COST country is allowed to nominate two members on the Management Committee of each COST action, together with a number of substitute MC members. This gives Malta an added advantage, since it puts Malta COST MC members en par with the MC members from much larger countries.

In the last three years, the number of Maltese participants taking part in COST Actions has taken an exponential leap: from six participants in 2009, to over 130 Malta COST MC members at the end of 2012. Table 1 lists just a few of the COST Actions, researchers based in Malta are participating in. While some COST Actions look at very basic research such as those on quantum physics and black holes MP0905 Black Holes in a Violent Universe, others have a more practical basis such as Action IS0804 Language Impairment in a Multilingual Society: Linguistic Patterns and the Road to Assessment. As can be seen from Table 1, a whole variety of areas are investigated, attracting participants

from outside the traditional academic research community. These include COST Actions such as IS1005 Medieval Europe, FA1004 Conservation Physiology of Marine Fishes and IS1102 Social Services, Welfare State And Places. The restructuring of social services in Europe and its impacts on social and territorial cohesion and governance to mention just a few.

Table 1: Selection of 2012 approved COST Actions, which include researchers based in Malta.	
BM 1105 GnRH deficiency: Elucidation Of The Neuroendocrine Control Of Human Reproduction:	Prof Josanne Vassallo, Dr Robert Formosa, Faculty of Medicine and Surgery, University of Malta
BM 1106 The Genes in Irritable Bowel Syndrome Research Network Europe (GENIEUR):	Dr Mario Vassallo, Department of Medicine, Mater Dei Hospital
BM 1201 Developmental Origins of Chronic Lung Disease:	Dr Cynthia Jones, Dr Brendan Caruana Montalto, Department of Medicine, Mater Dei Hospital
FA 1105 Towards a Sustainable and Productive EU Organic Greenhouse Horticulture:	Mr Mario V. Balzan, Institute of Applied Science, Malta College of Arts, Science and Technology
FA 1202 A European Network For Mitigating Bacterial Colonisation and Persistence On Foods and Food Processing Environments:	Dr Vasilis Valdramidis, Department of Food Studies and Environmental Health, University of Malta
FA 1208 Pathogen-Informed Strategies for Sustainable Broad-Spectrum Crop Resistance:	Mr Mark Causon, Ms Katarina Kohutova, Europe Direct Malta
FP 1204 Green Infrastructure Approach: Linking Environmental with Social Aspects in Studying and Managing Urban Forests:	Dr Joseph Buhagiar, Department of Biology, University of Malta
ES 1104 Arid Lands Restoration and Combat of Desertification: Setting Up a Drylands and Desert Restoration Hub:	Mr Daniel Sultana, Malta Environment and Planning Authority
ES 0905 Basic Concepts for Convection Parameterization in Weather Forecast and Climate Models:	Dr Charles Galdies, Institute of Earth Systems, University of Malta
ES 1106 Assessment of EUROpean AGRIculture WATer Use And Trade Under Climate Change (EURO-AGRIWAT):	Dr. Eman Calleja, Institute of Applied Sciences, Malta College of Arts Sciencce and Technology,
ES 1206 Advanced Global Navigation Satellite Systems Tropospheric Products For Monitoring Severe Weather Events And Climate (GNSS4SWEC)	Dr Charles Galdies, Institute of Earth Systems, University of Malta
MP 1204 TERA-MIR Radiation: Materials, Generation, Detection and Applications:	Dr. Louis Zammit Mangion, Prof. Charles Sammut, Department of Physics, University of Malta
MP 1209 Thermodynamics in the Quantum Regime:	Dr André Xuereb, Faculty of Science, University of Malta
MP 1210 The String Theory Universe	Dr Ivan Debono, International Research Fellow, European Space Agency
IS 1105 NETwork of experts on the legal aspects of MARitime SAFEty and security (MARSAFENET):	Capt Anand Dayal, Maritime Institute, Malta College of Arts, Science and Technology
IS 1106 Offender Supervision in Europe	Dr Sandra Scicluna, Department of Criminology, University of Malta; Ms Mariella Camilleri, Department of Probation and Parole, Ministry of Justice, Dialogue and the Family
IS 0907 Childbirth Cultures, Concerns, and Consequences: Creating a Dynamic EU Framework For Optimal Maternity Care:	Ms Marika Connor, Department of Primary Health, Ministry for Health, the Elderly and Community Care; Dr Rita Borg Xuereb, Department of Midwifery, University of Malta
IS 1104 The EU in the New Complex Geography of Economic Systems: Models, Tools and Policy Evaluation:	Prof Joseph Falzon, Dr Frank Bezzina, Faculty of Economics, Management and Accountancy, University of Malta.

Figure 1: Group Conference on Joint-Conference, COST Quantum Malta 2012: Fundamental Problems in Quantum Physics.

IS 1202 Dynamics of Virtual Work:	Dr Mark Micallef Department of Computer Science, University of Malta, Dr Anna Borg, Centre for Labour Studies, University of Malta; Dr Patrick Camilleri, Mathematics, Science and Technical Education, University of Malta
IS 1203 Transcultural Migration:	Dr Simon Mercieca, Mediterranean Institute, University of Malta
IS 1205 Social Psychological Dynamics Of Historical Representations In The Enlarged European Union	Dr Emmanuel Buttigieg, Department of History, University of Malta; Dr Gordon Sammut, Department of Psychology, University of Malta
IS 1208 Collaboration of Aphasia Trialists (CATs)	Dr ienne Grima, Department of Communication Therapy, University of Malta
IS 1209 Comparing European Prostitution Policies: Understanding Scales and Cultures of Governance (ProsPol)	Mr. Trevor Calafato, Department of Criminology, University of Malta
IS 1201 Disaster Bioethics: Addressing Ethical Issues Triggered by Disasters	Prof Pierre Mallia, Department of Family Medicine, University of Malta; Dr Ray Zammit, Department of Moral Theology, University of Malta
IC 1203 European Network Exploring Research into Geospatial Information Crowdsourcing: software and methodologies for harnessing geographic information from the crowd (ENERGIC)	Dr Maria Attard, Institute of Sustainable Development, Dr Matthew Montebello, Department of Intelligent Computer Systems, University of Malta

IC 1201 Behavioural Types for Reliable Large-Scale Software Systems (BETTY):	Dr Adrian Francalanza, Department of Computer Science, University of Malta
IC 1205 Computational Social Choice:	Dr Patrick J Camilleri, Faculty of Education, University of Malta; Dr Ernest Cachia, Department of Computer Information Systems, University of Malta
IC 1206 De-identification For Privacy Protection In Multimedia Content:	Prof Joseph Cannataci, Department of Information Policy and Governance, University of Malta
IC 1207 PARSEME: PARSing and Multi-word Expressions. Towards Linguistic Precision And Computational Efficiency In Natural Language Processing:	Ms Claudia Borg, Institute of Linguistics, University of Malta; Dr Mike Rosner, Department of Intelligent Computer Systems, University of Malta
TU 1104 Smart Energy Regions:	Perit Reuben P. Borg, Construction and Management Unit, University of Malta
TU 1102 Towards Autonomic Road Transport Support Systems:	Dr. Kenneth Scerri, Department of Systems and Control Engineering, University of Malta
TU 1209 Transport Equity Analysis: Assessment and Integration of Equity Criteria in Transportation Planning (TEA):	Ms Deborah Mifsud, Institute of Sustainable Development, University of Malta
TD 1201 Colour and Space in Cultural Heritage (COSCH):	Dr Claire Baluci, Conservation Division, Heritage Malta: Ms Jacqueline Micallef Grimaud, Institute of Business and Commerce, Malta College of Arts, Science and Technology, Mr Herman Bonnici, International Institute for Baroque Studies, University of Malta
TD 1203 Food Waste Valorisation For Sustainable Chemicals, Materials	Fuels (EUBis) and Dr Everaldo Attard, Mr Adrian Bugeja Douglas, Institute of Earth Systems, University of Malta
TD 1202 Mapping and the Citizen Sensor,	Dr Matthew Montebello, Department of Intelligent Computer Systems, University of Malta
TD 1206 Development and Implementation of European Standards on Prevention of Occupational Skin Diseases (StanDerm):	Prof Joseph Pace, Founder President Maltese Dermatology Association
TD 1209 European Information System for Alien Species:	Prof Patrick Schembri, Department of Biology, University of Malta
TA 1201 Gender, Science, Technology and Environment (genderSTE)	Prof Marie Therese Podesta Camilleri , Department of Anatomy University of Malta; Dr Marion Zammit Mangion, Department of Physiology and Biochemistry, University of Malta
IS 1103 Adapting European health systems to diversity (ADAPT),	Dr Sandra Buttigeg, Faculty of Health Sciences, University of Malta; Dr Claire Bellia; Dr Maria Pisani, Integra Foundation (newly appointed in 2012)
IC 1106 Integrating Biometrics and Forensics for the Digital age:	Mr Ramon Cassar, Forensic Lab Malta Police ; Ms Joanna Vella, Lab of Molecular Genetics, University of Malta

Table 2: COST Malta National Contacts and and Malta COST DC members.	
Malta COST CNC and CSO	Dr Janet Mifsud
MCST International Relations and Policy Executive	Dr Claire Bellia
Malta DC Biomedicine and Molecular Biosciences (BMBS)	Prof Giuseppe de Giovanni
Malta DC Chemistry and Molecular Sciences and Technologies (CMST)	Prof Joe Grima
Malta DC Earth System Science and Environmental Management (ESSEM)	Prof Ray Ellul
Malta DC Food and Agriculture (FA)	Dr Anna McElhatton

Malta DC Forests, their Products and Services (FPS)	Mr Larry Shoemake
Malta DC Individuals, Societies, Cultures and Health (ISCH)	Prof Helen Grech
Malta DC Information and Communication Technologies (ICT)	Eng Saviour Zammit
Malta DC Materials, Physical and Nanosciences (MPNS)	Prof Luciano Mule Stagno
Malta DC Transport and Urban Development (TUD)	Dr Maria Attard
Malta DC Transdomain Proposals Standing Assessment Body (TDP-SAB)	Prof Vincent Buhagiar

4 What types of activities are funded by COST?

There are four main Action activities which are funded by COST:

• Meetings: The Management Committee can organise meetings in any of the COST countries participating in the Action. These include Management Committee meetings, Working Group meetings, Workshops, and Conferences. Indeed to date an average of 4 such meetings have been held every year, in Malta, since 2011.

• Short-term Scientific Missions (STSMs): Scientists participating in a COST Action have the opportunity to participate in missions or exchange visits to institutions or laboratories in another COST country. These allow participants to build collaborations, learn new techniques, or access instruments or methods not available in their country. These STSMs are particularly targeted to early stage researchers.

• Training Schools: These activities allow training or re-training for researchers, particularly those at an early stage of their career, in a new emerging subject relevant to the Action. These are usually hosted by institutions or laboratories which have the equipment or know-how to support the training.

• Dissemination, Publications: COST also disseminates outcomes of COST Actions through publications, electronic media, news, events, success story releases, and e-mail notifications. The dissemination of information on Action activities is key to ensure awareness of these activities by international scientific communities and to reach policy-makers and decision-making bodies.

5 The COST Organization in Malta

In Malta, MCST is the managing authority for COST. The author of this paper was appointed as COST CNC and CSO by MCST in 2010. In the last year, the co-author was asked to help with the increased workload due to the huge interest COST is having among the local research community. MCST has also appointed Domain Committee (DC) representatives following an open call for applications in 2010. These represent Malta in the various Domains (see Table 2).

While in 2010, Malta was participating in only 6 COST Actions, in 2011 the number increased to 19 COST Actions with the participation with 30 researchers. In 2012, Malta began to participate in 38 new COST Actions, with the new involvement of a further 56 researchers, bringing a total of nearly sixty new Malta participations in COST Actions since 2010.

Researchers from Malta have also contributed to COST publications and organization of training schools and conferences. Malta was the host of three COST events in 2012.

The SPLASHCOS Training School, held in Malta in November 2012, was organised as part of the SPLASHCOS COST Action TD0902 by Dr. Timothy Gambin, Department of Classics and Archaeology, University of Malta. The goal of this COST action is to promote the coordination of the work of archaeologists, marine geoscientists and heritage managers.

Two COST Actions were brought together during 'Quantum Malta 2012' in 24-27th April 2012. This joint conference saw the convergence of around 200 of the world's leading theoretical physicists to our Island to discuss foundational problems with our understanding in the nature of reality. The event was organized by two COST actions: MP1006 - Fundamental Problems in Quantum Physics and MP0905 - Black Holes in a Violent Universe. The organisers included Mr. Jackson Said, Department of Physics, Malta (see Photo 1).

In April 2012, the Department of Civil and Structural Engineering at the University of Malta hosted an International Conference on Fire Engineering, followed by a PhD Training School as part of International Fire Engineering and Response COST Action TU0904. This

included a European research group made up of experts from 23 countries, including academics and representatives of fire brigades from different European countries. The conference and the PhD Training School were coordinated by Perit Ruben Paul Borg of the Department of Civil and Structural Engineering of the Faculty for the Built Environment, University of Malta.

6 Conclusion

Deciding to participate in COST may seem a small step to some, an added administrative burden to others, and yet more travel commitments to others. Indeed many researchers in Malta hesitate and require persistant prodding, reminders and one to one meetings in order to persuade them of the unique advantages COST offers. Yet once a researcher takes this decision, the unique opportunities which open up to them can really lead to a huge cascade effect and open wide new avenues of opportunities and networking. The internationalization of research based in Malta is essential if Malta is to achieve the critical mass needed for excellent research. COST offers the response to the conundrum of how to overcome our physical (and perhaps in some instances also mental) insularity. You should not let this opportunity pass...

Note:
COST in Malta is managed by the Malta Council for Science and Technology. For more information see http://www.mcst.gov.mt/networking/cost or email the National Contact Point Dr Janet Mifsud janet.mifsud@um.edu.mt or cost.mcst@gov.mt

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News Article

NATIONAL STRATEGY FOR HEALTH RESEARCH AND INNOVATION

Alec Lapira[1], Nicholas Sammut[2], Christian Scerri[3], Gordon Cordina[4], Janet Mifsud[5], Jennifer Cassingena Harper[2], Ramona Saliba Scerri[2] and Nadine Castillo[2]
[1]Institute of Health Care, University of Malta, Msida, Malta
[2]Malta Council for Science and Technology, Malta
[3]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
[4]E-Cubed Consultants, Malta
[5]Clinical Pharmacology and Therapeutics, University of Malta, Msida, Malta

1 Malta Council for Science and Technology (MCST)

In 2011, the Malta Council for Science and Technology (MCST) commissioned the Development of a dedicated strategy for health research and innovation in line with its mandate from Government to identify areas of national priority and design and to also implement strategic approaches to enhance economic competitiveness and quality of life.

The Strategy was drawn up by a steering group which also included people from outside the health sector, to ensure that it also keeps note of the economic side of things.

2 Mission and Vision

The Health Research and Innovation Strategy for Malta aims to develop an enabling health research and innovation ecosystem as a springboard for securing sustainable health care. This can be done through the identification of areas and opportunities for undertaking health research in processes, diagnoses, treatments and delivery of health care services.

The objective is to improve the effectiveness and efficiency in these areas, attracting investment and achieving long-term sustainability.

Correspondence to: N. Sammut (*MCST – ceo.mcst@gov.mt*)

3 Enhancing the efficiency and effectiveness of health care

Health research and innovation generate a range of direct and indirect benefits leading to the enhancement of the efficiency and effectiveness of healthcare.

Health research and innovation enhances the quality of health services through the development of new and innovative healthcare systems. Research leads to the implementation of evidence-based measures and policies, which are aimed at enhancing the quality of life of society in general. Improvement in health system is however a partial although direct impact of health research.

Environmental, social and economic developments are considered as indirect impacts which contribute to advancements in the welfare system. Health Research should cover a wide spectrum of academic fields in order to be effective. Fields include social sciences, engineering, agriculture and food technology among others.

4 Economic growth through health research

Health research in Malta promotes economic growth in the long-term, particularly because it tackles demographic challenges. Despite the smallness and inherent disadvantages faced by the Maltese economy, the development of health research in Malta is deemed to be economically viable especially when considering the challenges brought about by demographic development.

Smallness may actually be advantageous for local researchers, if they specialize on specific niches which are

not easily accessible in larger countries. Furthermore, health research is expected to enhance the potential of the Maltese economy, diversifying local economic activity. Research improved productivity within the economy while reducing healthcare costs and increasing healthcare effectiveness.

Policy-makers in Malta should seek to encourage the uptake of promising markets, particularly those related to innovation, which open up new opportunities for the local economy. The aim of the Strategy is to ensure that health research activity and innovation in health care is promoted and furthered in Malta. In addition, the Strategy promoted measures that help in bridging the gap between research and policy implementation.

The implementation of this Strategy involves a number of economic benefits, which are identified and monitored through various indicators. Such benefits include prevention through increased awareness of diseases, enhanced efficiency within the healthcare system, and increased profits for the manufacturing sector.

In addition, capacity building and knowledge benefits are improved through research results and the more research capacity available (it increases as more institutions involve themselves in research projects). Health benefits include the improvement of the healthcare system as well as the well-being of the citizens.

Health research is a financially worthwhile investment as it will attract more local and international firms to the industry. Indicators are also useful to identify whether the resources involved are being used optimally.

5 Promoting Long-Term Sustainability

The Strategy identifies the most prominent areas and opportunities for health research and innovation, and the investments required to develop a vibrant health research and innovation ecosystem. This will provide the springboard for improvements within the local healthcare system, taking into account the structures that are already in place including the relevant infrastructure, policies and initiatives.

Long-term sustainability in the health sector is an important target for this Strategy and the main recommendation is the setting up of a National Research and Innovation Centre. Sustainability is expected to be attained through a range of capacity-building measures implemented in the short, medium and long-term.

Research will focus on enhancing the efficiency and effectiveness of processes, diagnosis, treatments and the delivery of healthcare services. This way, the maximum social and economic benefits from the strategy can be attained.

6 Funding Opportunities

Future economic growth depends on reforms in various economic sectors including health. Research is an essential component for change. Given the limited availability of local funding, the Health Research and Innovation Strategy recommends a strong internationalization drive, based mainly on proactive participation in the relevant EU funding programmes and related international activities. This would enable local organizations and individuals to increase and achieve their potential.

The success of the health research and innovation strategy depends primarily on the amount of funds allocated by the government and the private sector, together with the availability of additional funding provided at an EU Level. This funding (at EU Level) includes the FP7 programme (to be followed by Horizon 2020), the European Social Fund (ESF), European Regional Development Fund (ERDF) and related programmes including the Interreg and the MED Programmes.

Hence, research funds have to be secured and allocated accountably. Funds are also needed to generate the required skills for any potential future growth of health research industry in Malta. It is therefore important to build, strengthen and sustain human and physical capacity to conduct, absorb and utilize health research.

7 Four main goals of the Strategy

The Strategy sets out four goals which, if implemented within the specified timeframe, will result in an innovative, efficient and effective health research strategy in Malta.

These goals are:

i. Developing a vibrant and sustainable health research and innovation ecosystem

ii. Building the necessary capacity and competence for high quality research to improve well-being

iii. Supporting evidence-based policy-making in human health and ensuring outreach and take-up

iv. Leveraging internationalisation opportunities for economic growth and innovation in the health sector

8 Ten Key Recommendations to achieve these goals:

For Goal 1 - Towards a vibrant and sustainable health research and innovation ecosystem

(i) **Set up a National Governance Framework for Health Research and Innovation**: to oversee the development of an enabling ecosystem for health research and innovation.

(ii) **Increase Funding for Health Research and Innovation**: to drive capacity-building of the sector which supports an appropriate balance of specialization

and interdisciplinarity and coves transnational research in a variety of disciplines in meeting societal challenges.
(iii) **Ensure enhanced access to health research facilities**: and also enable researchers to network between each other as well as join international research networks)
(iv) **Enhance use of public procurement to stimulate Research and Innovation**:
a. Encourage proactive use of EU public procurement directives as a means for stimulating public and private sector investments in health research and innovation.
b. Enhance the role of the Public Service (both as a purchaser and regulator) as early user of health innovative products by developing capacities for implementing public procurement for research and innovation.
c. Support the Public Service to act as a catalyst in private procurement, through the establishment of credit guarantees for innovative health services, training in innovative procurement techniques and intellectual property protection, and the purchase for private use of innovative services and products.

For Goal 2: Building the necessary capacity and competence for high quality research to improve well-being
(v) **Attract high quality researchers**:
a. Provide a set of attractive conditions to increase the number and profile of local researchers engaged in medical and health research and to attract high quality researchers from overseas, particularly, in areas of national priority.
b. Develop an enabling environment conducive to research, through the establishment of an excellent health research management system
(vi) **Support Capacity-building and forward planning**
a. Map current research capacity and competence by area in order to project current research strengths, locally and abroad, and as a means to define better future needs for capacity-building.
b. Facilitate forward planning by tertiary institutions at post-graduate, doctoral and post-doctoral level to build critical mass and develop defined and structured research units.
c. Encourage postgraduate and doctoral level studies and research in health through well-designed programmes and incentives.
(vii) **Build critical mass and enhancing potential of researchers**:
a. Set up incentives to encourage clustering of researchers from various fields including ICT, medical and engineering.
b. Encourage the setup of Knowledge Transfer Partnerships (KTP) between academic institutions and industrial partners (both local and foreign) to encourage rewarding collaborations with innovative businesses as well as gain ideas for further research and development of projects.

For Goal 3: Supporting evidence-based policy making in human health: outreach and take-up
(viii) **Ensure dissemination and take-up of results**:
a. Increase the publication and dissemination of research findings in peer-reviewed and non-peer-reviewed journals
b. Build the competence for communication and exploitation of research results to develop new tools and research applications to improve the health of the population.
c. Utilise results as an educational tool to change the habits, behaviour and opinion of the general public on health issues.
(ix) **Enable access to research results and new knowledge**:
a. Enable access to research results and the transfer of knowledge needs to be improved to ensure that research evidence is transformed into practice
b. Integrate and validate data obtained from routine clinical examinations and investigations.
c. Set up an online portal to disseminate information and research evidence.
d. Introduce compulsory training in entrepreneurship.

For Goal 4: Leveraging internationalisation opportunities for economic growth and innovation in the health sector
(x) **Invest in competitiveness and job creation**:
a. Study and design a competitive package of policy measurements and incentives to target local and foreign investment in particular niche areas.
b. Review the full range of internationalisation opportunities instrumental in addressing the Strategy's objectives.
c. Ensure a strong national drive to coordinate health, biotech and life science initiatives which exploit Malta's competitive advantage.
d. Increase private sector awareness of the value-added gained from investments in research and innovation to enhance competitiveness.
e. Introduce compulsory training in entrepreneurship and related hands-on experience.

9 The Importance of Good Governance

In order to ensure the set-up and implementation of the Strategy, good governance and stewardship are necessary. These are important not only to establish a vision

for national health research, but also to identify the appropriate health research priorities and research partnerships with the relevant international organisations, especially those established in the EU. Good governance is ensured if ethical standards for health research are established and monitored.

10 Concluding Remarks

The National Strategy for Health Research and Innovation will only succeed if there is enhanced communication among all the main stakeholders. There is an urgent need for a more proactive and dynamic approach in health research and innovation.

Many clinicians and researchers feel the need to own research-it has to be a bottom up approach. The enabling research and innovation ecosystem needs to be put in place as the basis for implementing the Strategy. This ecosystem will facilitate the transformation of research outcomes in innovative products and services on the local as well as international market.

Malta needs to identify its Unique Selling Propositions-Malta is ideal as a centre for pilot projects in health care, such as bio banking, testing new drugs, servicing clinical trials and health tourism.

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Table of Contents