Editor: Malgorzata M. Jaworska, Faculty of Chemical and Process Eng., Warsaw University of Technology, ul. Warynskiego 1, 00-645 Warsaw (POLAND).
E-mail: jaworska@ichip.pw.edu.pl

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Dear Society members,
I am pleased to send you the copy of the new issue of the European Chitin Society bulletin. I must say that it was an honor for me to serve you as a Secretary of EUCHIS and editor of the Chitin NewsLetter for last two years. I did my best to carry out the duties as well as possible. During the General Assembly I was elected as a vice-President of EUCHIS and Prof. Jacques Desbieres (France) was elected as a new Secretary of EUCHIS. Farwell to the position of an editor of ChitinNewsLetters I would like to thank Prof. George Roberts, Honorary Secretary of the Society for his priceless help.

This year we could attend the 11th International Conference of European Chitin Society that was held on May 05-08th in Porto (Portugal); you will find my short report later in this issue. During the conference the winner of the Bracconot Prize was announced. This year it was Dr Luca Luca Casettari who worked on application of chitosan for drug delivery. Please find the abstract of his thesis in this issue. There were also three poster awards. Jury took under account the scientific level and presentation of the subject. Abstracts of the awarded posters are also presented.

Malgorzata M. Jaworska
Dear EUCHIS colleagues:

From these pages of our journal I want to send my greetings to all of you. First of all it is my duty but also my pleasure and my sincere wish to thank the former Board members for their dedication and for the work done and very particularly Sevda Senel who conducted the Society with honesty, intelligence and remarkable skill. The former EUCHIS presidents deserve also our gratitude as they devoted generous efforts and enthusiasm to our common interests.

Now at the beginning of 2014, after the successful Oporto Meeting we should proceed ahead, working together in Science and Technology just to make chitosan more known to companies, to Academia and to people in general. In this time of economic crisis with widespread diminishing research funds EUCHIS has the duty of keeping alive the flame of our pet research field which we should move forward to produce new knowledge and new applications.

For this purposes you can count on me as President of EUCHIS

With my best wishes,

Angeles Heras
Dear Chitosan Family,

It is my pleasure to inform you that we have completed another very successful meeting (EUCHIS’15) in Porto, Portugal. I want to thank to the organisers Prof. Dr. Bruno Sarmento and Prof. Dr. Manuela Pintado for their great efforts for such an excellent organisation. It was a very successful meeting both scientifically and socially. The detailed information about the meeting will be given in the coming issue of EUCHIS Newsletter.

As the President of EUCHIS, as you know it was my second and last term, which ended by May, 2013. Bylaw, my term as the board member has also ended. It was indeed a great pleasure and honor for me to serve for the European Chitin Society since 2004. I will certainly continue supporting our society as a member.

I am passing the torch to Prof. Angeles Heras from Spain, who was elected as the President of the EUCHIS at the board meeting held in Porto. I am sure she will continue this mission with great enthusiasm.

With my kindest regards
Sevda Senel
General Assembly of the European Chitin Society

A General Assembly of the members of European Chitin Society was held on 7th May 2013 at the Alfândega Congress Centre (Porto, Portugal).

President's Report:
Prof. Sevda Senel, the President of European Chitin Society, presented information on the Society and mentioned that at the present moment there were 134 active members.

The jury, with Professor Senel as Chairperson and Prof. Carla Carmella, Prof. Malgorzata Jaworska, Prof. Jacques Desbrieres, Prof. Francisco Goycoolea, Prof. Verena Seidl-Seiboth as members, had selected the winner of the Braconnot Prize who was awarded during the closing ceremony.
Prof. Senel also announced that there will be also three Poster Awards. The jury (Dr Svetlana Bratskaya, Prof. George Roberts, Prof. Francisco Goycoolea) after careful selection chose the winners who were announced during the closing ceremony.

Treasurer's Report
The treasurer, Prof Francisco Goycoolea, presented the financial report to the General Assembly. The Financial report was accepted by the General Assembly with no abstentions or abjections.

Election of the new Board of Directions
According to the statute of the European Chitin Society, Section III, part of the members of the Board of Directions must be elected every four-year period. This year the following members stood down from service: Prof. Sevda Senel, Prof. Bruno Moerschbacher, Prof. Massimiliano Fenice and Prof. Henryk Pospieszny
On their place the new members were proposed: Prof. Francesca Cabrera Esconbano (Spain), Prof. Jiri Simunek (Czech), Prof. Hakan Erpelu (Turkey), Prof. Manuela Pintado (Portugal).
The General Assembly chose new members to sit on the Board of Directors.
Prof. Bruno Moerschbacher was elected as ad-hoc member of the Board of Directors
The new Board held its first meeting the day after the General Assembly.

EUCHIS'15 Conferences:
The President informed the meeting that Prof. Bruno Moerschbacher officially proposed organisation of the next EUCHIS conference in Munster (Germany) and this subject will be discussed at the meeting of the Board of Directors.
Meeting of the Board of Directors of European Chitin Society

The meeting of the Board of Directors was held on 8th of May 2013. In the first step the Committee of the Board was elected:

President: Angeles Heras (Spain)
Vice-President: Malgorzata Jaworska (Poland)
Vice-President: Manuela Pintado (Portugal)
Secretary: Jacques Desbrieres (France)
Assistant Secretary: Svetlana Bratskaya (Russia)
Treasurer: Francisco Goycoolea (Germany)
Assistant Treasurer: Laurent David (France)
Members: Carla Caramella (Italy), Hacan Ereglu (Turkey), Francesca Cabrera Escribano (Spain), Katja Heppe-Richter (Germany), Verena Seidl-Seiboth (Austria), Jiri Simunek (Czech), Valery Varlamov (Russia), Suzana Vilchez (Spain)
Ad-hoc member: Bruno Moerschbacher

The Committee discussed the problem of the next EUCHIS conference and decided to accept the proposal of Prof. Bruno Moerschbacher. The EUCHIS’15 will be held in Munster (Germany).
The 11th International Conference of the European Chitin Society was held on 5-8 May 2013 in Porto (Portugal) (http://www.skyros-congressos.pt/euchis2013/index.html). The conference started with the Welcome Party. After the official part of the evening, the banquet started. We were able to try excellent Portuguese food as well as taste Porto. This party gave us an opportunity to meet friends and discuss not only chitin/chitosan matters.

The scientific program of the Conference was divided into 6 parts:

A. Production and modification of chitin and chitosan via chemical reaction (6 oral presentations + 6 posters)
B. Characterization of chitin and chitosan (6 oral presentations + 12 posters)
C. Enzymatic synthesis, modification and degradation of chitin and chitosan (9 oral presentations + 10 posters)
D. Medical applications (11 oral presentations + 54 posters)
E. Agro-Food and other applications (10 oral presentations + 22 posters)
F. Industrial application and regulatory issues (5 oral presentations + 4 posters).

We were able to listen to 6 keynote lectures and 3 invited plenary lectures given by:

1. Prof. Jacques Desbrieres (France): “Re-invent chitosan for the future”
2. Prof. Joao F. Mano (Portugal): “Functional and instructive chitosan based devices for biomedical application”
3. Dr Michael Dornish (Norway): “Specifications of chitosans for industrial application”
All of them were very interesting, showing successes and problems met in chitin/chitosan investigation and applications.

Except the scientific program, the organizers offered us Douro river cruiser and excursion to Porto wine cellars where we had Conference dinner.

To end, I would like to thank Prof. Bruno Sarmento and Prof. Manuela Pintado and their team for excellent organization of the Conference and nice atmosphere during our stay in Porto.
We are pleased to announce that the winner of the Braconnot Prize 2013 is Dr Luca Casettari

**Nationality:** Italian  
**Date of birth:** 15.02.1981

**Email:** luca.casettari@uniurb.it  
**Web site:** www.uniurb.it/freerad

**EDUCATION**

**PhD Degree:**  
*Analyses of the chitosan and PEGylated chitosan properties. Formulation of mucoadhesive microparticles produced in scCO2 and nanoparticles for the release of DNA/siRNA*  
University of Urbino “Carlo Bo”, (Italy)

**Bachelor’s Degree:**  
*Studies of drugs release from traditional and innovative matrix*  
University of Urbino “Carlo Bo”, (Italy)

**Publications on international peer-reviewed journals**

1. **Rheological characterization of polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®) water dispersions**  
   Cespi M., Casettari L., Palmieri G.F, Perinelli D. R., Bonacucina G.  
   Submitted – 2013

2. **Evaluation of PEG-PLA di-block copolymers as additive in hypromellose film coating**  
   Cespi M., Casettari L., Bonacucina G., Giorgioni G., Perinelli D.R., Palmieri G.F.  
   Submitted – 2013

3. **Folic acid conjugated chitosan nanoparticles for tumor targeting of therapeutic and imaging agents**  
   Vllasaliu D., Casettari L.*, Bonacucina G., Cespi M., Palmieri G.F, Illum L.
4. Effect of phosphate buffer on the micellization process of poloxamer 407: microcalorimetry, acoustic spectroscopy and dynamic light scattering studies
Perinelli D.R., Cespi M., Pucciarelli S., Casettari L., Palmieri G.F, Bonacucina G.
Colloids and Surfaces A: Physicochemical and Engineering Aspects - Volume 436, September 2013, Pages 123-129

5. Effect of temperature increase during the tableting of pharmaceutical materials
Cespi M, Bonacucina G, Casettari L, Ronchi S, Palmieri GF.
doi: 10.1016/j.ijpharm.2013.03.014

6. Rheological and thermo-mechanical properties of Sepifilm-Sepisperse water dispersion and films
Marco Cespi, Giulia Bonacucina, Luca Casettari, Giovanni Filippo Palmieri.
Thermochimica Acta - Volume 557, April 2013, pages 7-12. - IF 1.805
doi: 10.1016/j.tca.2013.01.020

7. The use of acoustic spectroscopy in the characterisation of ternary phase diagrams
Giulia Bonacucina, Marco Cespi, Giovanna Mencarelli, Luca Casettari, Giovanni Filippo Palmieri.
doi:10.1016/j.ijpharm.2012.10.037

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Luca Casettari, Giulia Bonacucina, Marco Cespi, Giovanni Filippo Palmieri.
Carbohydrate Polymers - Volume 91, Issue 2, January 2013, Pages 597-602. - IF 3.628
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9. Biomedical applications of amino acid-modified chitosans: A review
Luca Casettari, Driton Vllasaliu, Jenny Ka-Wing Lam, Mahmoud E. Soliman, Lisbeth Illum.
Biomaterials – Accepted 2012 - IF 7.404
doi:10.1016/j.biomaterials.2012.06.104

10. Absorption-promoting effects of chitosan in airway and intestinal cell lines: a comparative study
Driton Vllasaliu, Luca Casettari, Robyn Fowler, Ruth Exposito-Harris, Martin Garnett, Lisbeth Illum, Snow Stolnik

Luca Casettari, Marco Cespi, Enzo Castagnino
Drug Development and Industrial Pharmacy – Volume 38, Issue 8, August 2012, Pages 979-984 - IF 1.494

12. PEGylated chitosan derivatives: Synthesis, characterisations and pharmaceutical applications.
Luca Casettari, Driton Vllasaliu, Enzo Castagnino, Snjezana Stolnik, Steven Howdle, Lisbeth Illum
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15. Thermo-dynamic-mechanical characterization of hypromellose 2910 free films.
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18. Tight junction modulation by chitosan nanoparticles: Comparison with chitosan solution.
   International Journal of Pharmaceutics, Volume 400, Issue 1-2, November 2010, Pages 183-193 -
   IF 3.607  doi:10.1016/j.ijpharm.2010.08.020

   Carbohydrate Polymers, Volume 74, Issue 3, 4 November 2008, Pages 640-647 - IF 3.628
Summary of research work carried out by Luca Casettari during his PhD and three years of PostDoc (2008 to 2012)

1. Chitosan and PEGylated chitosan as solution or nanoparticles for enhancement of drug delivery

Effect of PEGylation on the Toxicity and Permeability Enhancement of Chitosan.

The studies showed that PEGylation of chitosan, led to a reduction of chitosan's toxicity towards the nasal mucosa while maintaining its ability to open the cellular tight junctions and, consequently, producing an enhancement of macromolecular permeability as compared to chitosan.

In these studies, different graft copolymers of methoxy-polyethylenglycol and chitosan were synthesized, resulting in an increase in their solubility in aqueous solutions in the 2.0-9.6 pH range. Moreover there were considerable decrease in cytotoxicity of PEGylated chitosan as compared to chitosan, with toxicity of the conjugates found to be dependent on the degree of PEGylation. TEER and permeability experiments showed that the synthesized copolymers exhibited strong tight junction opening effects, though a slightly acidic pH was required, suggesting the necessity for chitosan to be in its protonated form for an effect on epithelial tight junctions, as suggested previously by other authors.

Remarkably, the permeability experiments found that mPEG-g-chitosan conjugates produced a notably larger permeability enhancing effect relative to unmodified chitosan. Our results therefore suggest that mPEG-g-chitosan conjugates may find applications as absorption promoters in macromolecular therapeutics formulations designed for nasal administration [1].

Tight junction modulation by chitosan nanoparticles: Comparison with chitosan solution.

Comparing chitosan nanoparticles with chitosan solution at equivalent chitosan concentrations, the data demonstrated that chitosan nanoparticles (formulated by the ionic gelation technique) produced a sharp and reversible decrease in TEER and increased the permeability of two model permeants, FITC-dextrans of 4 and 10 kDa in Calu-3 cell monolayers, to a similar magnitude to chitosan solution. Chitosan nanoparticles produced changes in ZO-1 (tight junction protein) distribution similar to chitosan solution, indicating a tight junction effect. While chitosan nanoparticles did not demonstrate an improvement in permeability, compared to the solution, nanoparticles afford the potential for drug incorporation and therefore controlled drug release and protection from enzymatic degradation at mucosal surface [2].

Absorption-promoting effects of chitosan in airway and intestinal cell lines: A comparative study.

The work investigated the ability of chitosan solution to modulate the tight junctions and consequently promoting drugs absorption enhancement across monolayers of different cell lines, the mucus producing cell line (Calu-3) in comparison with no-mucus producing one (Caco-2). The work demonstrated that Calu-3 and Caco-2 cultures displayed a significantly different sensitivity towards chitosan. However, chitosan produced a powerful effect on the tight junctions of both cell lines, despite the cytotoxicity-determined dosage adjustment for each cell line. The interaction of chitosan with the cells and its absorption-promoting capacity are strongly dictated by the presence of mucus on the epithelial surface, with data suggesting that mucus-rich epithelial surfaces are more sensitive to the tight junction opening effects of chitosan. The application of chitosan as an excipient intended to improve the absorption of therapeutic macromolecules across the mucosal surfaces may therefore require dosage optimisation (for optimal absorption enhancement without toxic effects) depending on the nature (including the extent of mucus presence) of the mucosal surface [3].

2. Chitosan and some of its derivatives as excipients for different drug delivery formulation such as microparticles, tablets and thermogels.

Surface Characterisation of Bioadhesive PLGA/Chitosan Microparticles Produced by Supercritical Fluid Technology.
Chitosan was formulated with biodegradable PLGA microparticles and using different analytical surface techniques, it was shown that the presence of chitosan on the surface of the particles could increase the mucoadhesion and consequently potentially the residence time when administered via the oral route [4]. Biodegradable PLGA microparticles formulated with the mucoadhesive biopolymer chitosan were produced by a specific PGSS technique that uses supercritical carbon dioxide to liquify the polymer. This innovative process allows several drawbacks of conventional techniques for the production of microparticles to be overcome, such as the use of organic solvents, surfactants and high temperatures. Such standard techniques require subsequent treatments in terms of washing processes to reduce solvent residue below safety limits, risk of denaturation of protein drugs and of the loss of a high amount of the encapsulated drug. These studies have shown that scCO2 does not dissolve in (liquefy) chitosan; hence, chitosan remains in the solid state during processing and is distributed in the matrix of the PLGA polymer, and dependent on the composition of the polymer matrix also on the surface of the microparticles. Characterization of the microparticles was carried out by laser diffraction, SEM and innovative surface analyses techniques, such as XPS and ToF-SIMS. The latter two techniques determined the presence of chitosan on the surface of the particles, which were consequently tested for their mucoadhesiveness properties by mucin assay. An in vitro assay measuring mucoadhesiveness highlighted the strong capacities of the particles to interact with a mucin solution. The mucin was found to adsorb on the surface of the chitosan-PLGA microparticles. It is the first time that such bioadhesive microparticles have been produced by means of the PGSS technique in a one-step process. These microparticles could potentially find use as oral gastroretentive and controlled release carriers for drugs, especially for proteins and peptides, because the method of preparation avoids the use of organic solvents, surfactants and cross-linkers, use mild temperature and pressure conditions and hence is able to preserve the biological activity of the molecule.

**Evaluation of dibutyrylchitin as new excipient for sustained drug release.**

As excipient for tablets, **dibutyrylchitin**, a lipophilic derivative of chitin, has been synthesized and used as a carrier for sustained release of drug. Dibutyrylchitin provided satisfactory results thanks to its poor susceptibility to enzymatic hydrolysis in the short term, to the poor propensity to dissolve in physiological media and to its high compressibility and good mechanical properties. In practice, dibutyrylchitin tablets loaded with metformin were able to release this drug at such a rate that could assure more effective therapeutic results with respect to the analogous commercially available products. Thus dibutyrylchitin should be considered a suitable compound for pharmaceutical applications in the area of sustained drug release [5].

**Characterisation of the interaction between chitosan and inorganic sodium phosphates by means of rheological and optical microscopy studies.**

More recently the ability of chitosan to form thermogel trough the interaction of inorganic sodium phosphates was evaluated. This work confirmed a clear inter-dependence of temperature, pH, and phosphate concentration on the gelling properties of the chitosan dispersion. Particularly, it highlighted how previous works have in fact overlooked some key issues in analyzing the role of the type of salt used as well as the fundamental importance of pH and chitosan/salt ratio, giving rise to often questionable results. The final pH value of the prepared chitosan/inorganic phosphate systems strongly affected the behavior of rheological samples, conferring pseudo thermogelling properties. Thus, in line with the literature, our data confirm that a pH value close to 7.0 is of primary importance. Moreover, the ratio between the chitosan and the salts, more than the type of inorganic phosphate, is key in determining the equilibrium state between the global positive charge of the polysaccharide and the negative charge of the salt [6].

### 3. Radical scavenging activity of chitosan and its derivatives.

**ORAC of chitosan and its derivatives.**
The antioxidant capacity of chitosan and its derivatives have been evaluated. Particularly in two different papers, it has been highlighted how through EPR technique and ORAC assay it is possible to discriminate the antioxidant capacity of these different derivatives.

The short communications presents the suitability of the ORAC assay for ranking the peroxyl radical scavenging ability of chitosan derivatives in slightly acid aqueous media. In this paper the N-Acetyl Cysteine-g-chitosan and the gallic acid-g-chitosan showed an enhanced ability to scavenge peroxyl radicals compared to the unmodified chitosan, with a stronger activity of NAC-g-chitosan respect to gallic acid-g-chitosan. On the contrary a lower antioxidant capacity was found for p-hydroxybenzoic acid-g-chitosan, whereas the antioxidant capacity found for p-methoxybenzoic-g-chitosan was near to that of the ungrafted chitosan [7].

Radical scavenging activity of 5-methylpyrrolidinone chitosan and dibutyryl chitin.

In a second paper, chitin and chitosan derivatives were found to be able to annihilate radicals with a mechanism that not always finds them chemically involved with structural modifications and with radical cascade propagation. The application of reactivity of PTOC esters described in this work as radical affording substances onto the chito-polysaccharides, demonstrated that the radical scavenging properties of such polymers not only depend on the presence of free alcohol and amino groups as hydrogen atom donors but also on the ability of these matrices to work as radical cages, entrapping and constraining free radicals to undergo copulation reactions. The copulation of radicals is the most effective process leading to the quenching of a radical chain reaction. However, this is a rare event when radicals are generated in vivo where they can freely interact with the biochemical environment affording irreversible damages. It is of interest to protect this delicate biochemical environments against radicals with the aid of modified chitosans. This study demonstrated that modified polysaccharides, MPC and DBC in this case, behaved as effective radical scavengers since they were able to prevent the propagation of chain reaction onto polymeric framework when free radical species were generated inside the matrix. Such behaviour, that can be related to the electronic character of the radical species (nucleophilic or electrophilic) and to the degree of substitution of the polysaccharides, became even more interesting when one considers that a couple of radicals usually arises when a chemical bond is cleaved as a consequence of photolytic events. In this case, the presence of modified polysaccharides such as DBC, would force radicals to copulate on themselves, preventing the damage induced by their propagation. It is deemed that the present information is useful for further developing functional biomaterials [8].

4. During this year two full reviews have been published in *Progress in Polymer Science* and *Biomaterials*, respectively.

**PEGylated chitosan derivatives: Synthesis, characterizations and pharmaceutical applications.**

This first review highlighted the advantages provided by the chemical modification of chitosan by PEGylation with respect to its potential biomedical applications such as absorption enhancement for mucosal delivery of biomolecular drugs. Different approaches used to PEGylate chitosan were discussed, together with their advantages and limitations. The reported improvements in numerous physicochemical and biological properties of chitosan following conjugation of PEG were extensively discussed. In summary, grafting PEG to the backbone of chitosan improves the aqueous solubility of the polysaccharide, and importantly allows solubility at basic pH values. This is highly desirable considering the limited aqueous solubility of chitosan above pH 6,3, which is one of the most important obstacles for its use in a wide range of pharmaceutical applications. The reduction in cytotoxicity of chitosan observed with PEGylation, as documented in some studies, allows a further improvement in the well know biocompatibility of chitosan. There is some evidence that PEGylation further promotes chitosan's well-documented absorption-enhancing properties. This is particularly interesting considering the associated benefits of an improved toxicity profile with PEGylation. In terms of the potential toxicity of chitosan following its derivatization, it is of fundamental importance that any unreacted reagents are completely removed to prevent cytotoxicity and to ensure product homogeneity. The modified product should
then be thoroughly tested for its potential as a safe material for medical applications. Examining the literature on PEGylated chitosan and other chitosan derivatives (as well as chitosan itself), it is apparent that a large number of techniques are currently used to characterize these compounds and these were also discussed in detail. It is therefore evident that a more adequate harmonization is required, particularly regarding the determination of its molecular weight and the degree of substitution. Novel PEGylated chitosan copolymers are continuously synthesized by many groups using various chemistries; studies with some of these novel copolymers have demonstrated potential for a wide range of interesting pharmaceutical applications, from drug and gene delivery to tissue engineering. Overall, PEGylated chitosan copolymers are promising candidate compounds with the potential for use in a wide range of biomedical applications [9].

Biomedical applications of amino acid-modified chitosans.

In the second review, the conjugation of chitosan with amino acid moieties was highlighted. The feasibility of preparation of some of these derivatives together with their enhanced biomedical properties, useful especially in the areas of drug delivery and tissue engineering, is an exciting prospect. One research area that may particularly benefit from the use of amino acid modified chitosans is nucleic acid (DNA and siRNA) delivery, where some of these systems have demonstrated great promise (e.g. reduction in the toxicity associated with some amino acids used as gene carriers such as poly-lysine and poly-arginine and increase in efficiency).

An improved mucoadhesive property resulting following the thiolation of chitosan has also attracted a great deal of interest. This strategy has been shown to produce benefits in the area of mucosal delivery of poorly absorbed drugs, especially peptides and proteins, whereby prolonging drug residence time at the mucosal surface is beneficial.

Modification of chitosan with poly-glutamic acid and cell-binding peptide sequences, such as RGD, opens the way to a plethora of possibilities to improve both drug and gene delivery systems and those supporting cell growth, tissue repair and regeneration in the area of tissue engineering. It must be noted, however, that even though a tremendous effort is being made to produce novel chitosan derivatives, harmonisation of their characterisations remains a challenge. Finally, the toxicity profile of new amino acid chitosan derivatives must be thoroughly evaluated in vivo to ensure that the well-established biocompatibility profile of chitosan is not compromised following its derivatisation [10].

REFERENCES


Three Poster Awards were given during the Conference of EUCHIS’13. The winners are:

1\textsuperscript{st} Prize: F.Costa, S.Maia, P.Gomes, M.C.L.Martins “Enhancement of chitosan antibacterial properties by antimicrobial peptide grafting”

2\textsuperscript{nd} Prize: H.Demey, T.Vincent, M.Ruiz, A.M.Sastre, E.Guibal “Removal and recovery of boron by using a novel composite of chitosan nad Ni(OH)$_2$”

3\textsuperscript{rd} Prize: Rebecca Melcher “A medium throughput protocol for screening plant resistance inducing activities of chitosan”