WG1 Workshop (Virtual Meeting 1)
COST ACTION 18132
11 January 2021

Working group 1: Innovative glyconanomaterials for biomedical prognostic and diagnostic devices; Synthesis and application

9:50−10:00: Opening**: Priyanka Sahariah** (WG1 Leader)

Session 1: *Chair* ***Cristina Nativi***
Plenary talk
10:00−10:30: *Glycosylated Bioactive Peptide Nanomaterials*. **Mustafa O. Guler**, The University of Chicago, USA.

Oral Presentations
10:30−10:50: *Carbohydrate synthesis for solving biological problems*. **M. Rita Ventura***,* Universidade Nova de Lisboa, Portugal
10:50−11:10: *Synthesis of different multivalent glyconanosystems for bioapplications*, **Cristian Rosales-Barrios**, CSIC-University of Seville, Spain
11:30−11:50: *Multifunctional hydrogels for stiffness-modulated cell behavior*. **Seda Kizilel**, Koç University, Turkey
11:50−12:10: *Stereoselective Catalytic Synthesis of 2-Deoxytrehalose Derivatives*, **Robin A. Jeanneret**, University of Bristol, United Kingdom.

Session 2: **Flash Presentations,** *Chair* ***Priyanka Sahariah***
12:10−12:15: *Structure-based design and stereoselective synthesis of human α-galactosidase A (GALA) activity regulators*, **Manuel González-Cuesta**, University of Seville, Spain.
12:15−12:20: *The Potential of Neo-Glycoproteins in Biomedical Diagnosis,* **Viktoria Heine**, Institute for Biotechnology and Helmholtz-Institute for Biomedical Engineering, Germany.
12:20−12:25: *Photoactive molecules bearing uracil-alditols: synthesis and biological assessment*. **Cristina Dias**, University of Aveiro, Portugal
12:25−12:30: *Antibacterial activity of chitosan derivatives*, **Georgia-Ioanna Kontogianni**, University of Crete, Greece

12:30−13:30: **Break**

Session 3: **Oral Presentations,** *Chair* ***Priyanka Sahariah***
13:30−13:50: *Highly functional, hybrid coatings based on chitosan, combining biocompatibility and a strong and renewable antimicrobial action*, **Evangelia Vasilaki**, University of Crete, Greece
13:50−14:10: *Grafting Strategies to Prepare Porphyrin-Cellulose Materials for Antimicrobial Photodynamic Therapy (aPDT)*. **Carlos J. P. Monteiro**, University of Aveiro, Portugal
14:10−14:30: *A tweezer-shaped diaminocabazolic receptor for the selective recognition of N,N'-diacetylchitobiose in water*, **Francesco Milanesi**, University of Florence, Italy
14:30−14:50: *Starch-based films as glycocarriers of porphyrinoid photosensitizers*. **Idalina Gonçalves,** CICECO, University of Aveiro, Portugal.
14:50−15:10: *Controlled spatiotemporal hyperthermia in magnetic chitosan films*, **Ana Barra**, University of Aveiro, Portugal

Session 4: **Flash Presentations,** *Chair* ***Filipa Marcelo***
15:10−15:15: *Glycosidic bond formation in liquid SO2*. **Krista Gulbe**, Riga Technical University, Latvia
15:15−15:20: *Development of crosslinked chitosan/gelatin/k-carrageenan hydrogel scaffolds for bone tissue engineering*. **Konstantinos Loukelis**, University of Crete, Greece.
15:20−15:25: *Antibacterial properties of photoactive starch/porphyrin-based materials.* **Ana Joaquinito,** University of Aveiro, Portugal.
15:25−15:30: *Deciphering the structural features of Siglecs recognition using NMR spectroscopy*. **Helena Coelho**, Universidade Nova de Lisboa, Portugal.

15:30: Closing Remarks: **Carmen Galan** (Action Chair)

WG1 Workshop (Virtual Meeting 1)
COST ACTION 18132
11 January 2021

Organizers: Priyanka Sahariah

Cristina Nativi

Abstracts

Glycosylated Bioactive Peptide Nanomaterials

Mustafa O. Guler[1]

The Pritzker School of Molecular Engineering, The University of Chicago, 60637, Chicago, IL, USA

Email address: mguler@uchicago.edu

Natural glycosylated biomacromolecules play essential roles in important biological functions such as extracellular and intracellular signaling, formation of defined cell structures, cellular differentiation, cancer and cell death. Protein-carbohydrate interactions are central to cell adhesion and they find applications in tissue engineering through selective differentiation of stem cells. The diverse chemical functional groups on carbohydrates are important for developing new materials with controlled and precise chemical, physical and biological properties. Materials for targeted and sustained delivery of drugs are developed to allow efficient dosing of therapeutics. More effective and safer vaccines are also possible through engineered glycomaterials.

This talk will illustrate concepts of development of glycosylated self-assembled peptide nanomaterials, which mimic the structure and function of the biological materials such as glycosaminoglycans and proteoglycans. Programmed self-assembly of small molecules and their applications in protein binding, targeted drug delivery, bioimaging, regenerative medicine and functional materials will be discussed. The supramolecular nanostructures are formed through noncovalent interactions such as hydrogen bonds, electrostatic and hydrophobic effect and diverse carbohydrate units were incorporated into the peptides for providing additional bioactive properties. These glyconanomaterials were observed to be efficient in protein binding, differentiation and proliferation of stem cells for tissue engineering, and targeted delivery of bioactive therapeutics.

References
1. C. Eren Cimenci, et. al., *Bioconjugate Chemistry,* **2019**, *30*, 2417-2426.
2. E. Arslan et. al., *Acta Biomaterialia*, **2018**, *73*, 263-274.
3. O. Yasa, *Journal of Materials Chemistry B*, **2017**, *5*, 4890-4900.
4. B. Senturk, *Biomaterials Science*, **2017**, *5*, 1293-1303.
5. G. Gunay, *ACS Applied Materials and Interfaces*, **2017**, *9*, 16035-16042.
6. F. Yergoz, *Biomaterials*, **2017**, *134*, 117-127.
7. O.S. Caliskan, *Bioconjugate Chemistry,* **2017**, *28*, 740-750.
8. M. Sever, *Acta Biomaterialia*, **2016**, *46*, 79-90.
9. B. Mammadov, *RSC Advances*, **2016**, *6*, 110535-110547.
10. G. Uzunalli, *ACS Biomaterials Science and Engineering*, **2017**, *3*, 1296-1303.
11. B. Senturk, *ACS Biomaterials Science and Engineering*, **2016**, *2*, 7 1180-1189.
12. D. Mumcuoglu, *ACS Applied Materials and Interfaces*, **2016**, *8*, 11280-11287.
13. S. U. Yaylaci, *Biomacromolecules*, **2016**, *17*, 679-689.
14. G. Uzunalli, *Acta Biomaterialia*, **2015**, *22*, 8-18.
15. R. Mammadov, *Biomacromolecules*, **2012**, *13*, 3311-3319.
16. R. Mammadov, *Biomacromolecules*, **2011**, *12*, 3508-3519.

Carbohydrate synthesis for solving biological problems

M. Rita Ventura

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal, rventura@itqb.unl.pt

rventura@itqb.unl.pt

Carbohydrates are still a relatively unexplored source of new drugs even though they are involved in many life processes which are fundamental for the well function and the survival of organisms. As such, they have a huge potential for therapeutic applications. However, the synthesis of carbohydrates, from simpler structures to more complex oligosaccharides is still challenging, especially the stereoselective construction of glycosidic bonds.

Some projects ongoing in our lab based on carbohydrate synthesis will be presented (Figure 1), such as: 1) the structure elucidation of methylglucose lipopolysaccharide (MGLP) and the synthesis of intermediate saccharides for characterisation of new enzymes involved in the construction of rare intracellular polymethylated polysacharides in Micobacteria[1]; 2) the design and synthesis of new chemical probes for the identification of specific glycosyltransferases from *Trypanosoma brucei*, as new drug targets for the treatment of African trypanosomiasis[2]; 3) the structure elucidation and the synthesis of (poly)phenol metabolites for pharmacological studies[3]; 4) the syntheses of 2-*O*-(indole-3-acetyl)-myo-inositol[4].

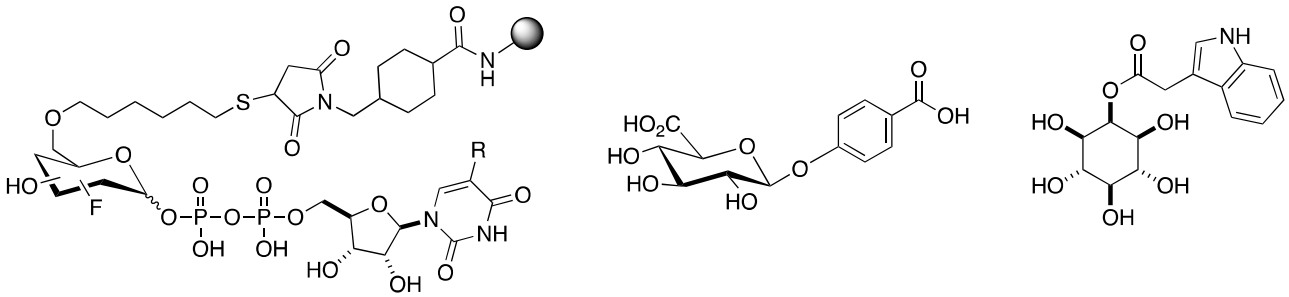


Figure 1.

Acknowledgements: This work is the result of very fruitful collaborations and we acknowledge all collaborators and students involved in the projects. GlycoPar (GA. 608295) - EU FP7 Marie Curie Initial Training Network, FCT projects PTDC/BIA-BQM/4056/2020, PTDC/BIA-BQM/30421/2017, PTDC/BBB-BSS/0827/2014. We thank MostMicro Research Unit (financially supported by LISBOA-01 0145-FEDER-007660 funded by FEDER funds through COMPETE2020 (POCI) and by national funds through FCT). The NMR data was acquired at CERMAX, ITQB-NOVA, Oeiras, Portugal with equipmentfunded by FCT, project AAC 01/SAICT/2016.

References

1. Ripoll-Rozada, J.; Costa, M.; Manso, J. A.; Maranha, A.; Miranda, V.; Sequeira, A.; Ventura, M. R.; Macedo-Ribeiro, S.; Pereira, P. J. B.; Empadinhas, N. *PNAS* **2019**, *116*, 835.
2. J. G. M. Bevan, E. C. Lourenço, M. C. Ferreira, J. A. Rodrigues, M. R. Ventura, *Eur. J. Org. Chem.* **2018**, 908.
3. Almeida, F. A.; Santos, C. N.; Ventura, M. R. *J. Agric. Food Chem.* **2017**, *65*, 6460.
4. Silva, S.; Ascenso, O. S.; Lourenço, E. C.; Archer, M.; Maycock, C. D.; Ventura, M. R. *Org. Biomol. Chem.* **2018**, *16*, 6860.

Synthesis of different multivalent glyconanosystems for bioapplications

Cristian Rosales-Barrios, Elena Romero-Ben, Mª Carmen Castillejos, Noureddine Khiar

Chemical Research Institute. CSIC-University of Seville. Calle Américo Vespucio nº49, 41092, Sevilla (Spain)

cristian.rosales@iiq.csic.es

Interactions between carbohydrate ligands and carbohydrate binding proteins (lectins) are at the origin of many important processes like fertilization, immune response, cell adhesion, infections by pathogens and toxins or tumor cell metastasis. However, these interactions are characterized by low affinity, high affinity interactions in nature being achieve by organizing carbohydrates as multivalent structures or glycoclusters. Therefore, synthetic multivalent surrogates for the adequate recognition of carbohydrate receptors based on the so called "cluster glycoside effect" would be a major advance in the development of glycodevices for biomedical applications. Using synthetic designs based on supramolecular self-assembly mediated by non-covalent forces, such as hydrogen bonding, π-π stacking, electrostatic and charge-transfer interactions, is the most appropriate approximation to induce self-assembling glyco-monomers into multivalent systems with diverse topology, composition and assembly dynamics. The drawback of this approach is the low stability of these aggregates. Diacetylenic amphiphiles are well suited to overcome this issue as they can undergo, upon UV-irradiation, a clean photo-polymerization *via* 1,4-addition reaction, affording functional polydiacetylene (PDA)-nanomaterials with enhanced stability and interesting chromatic properties [1].

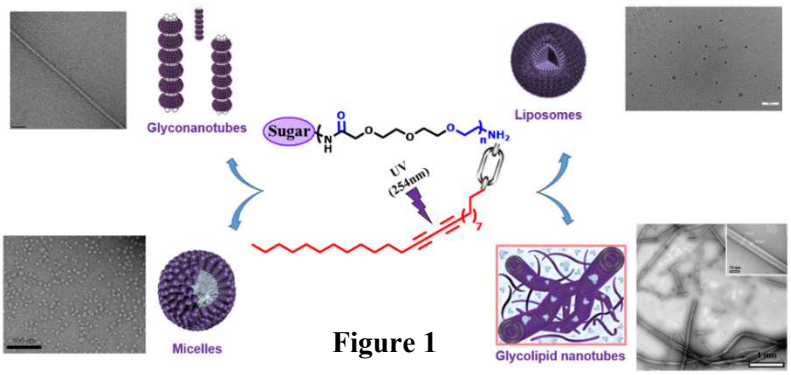


Figure 1

Based on these premises, we report here the design and synthesis through a 'bottom-up' approach of complex PDA-derived glyconanosystems, which allow us the facile comparison of nanovectors with different glycoligands, hydrophilic-lipophilic balance, sizes and topologies (figure 1), capable of recognize a plethora of lectins for their use in diagnostic and treatment applications. First, we synthesized mannose-coated micelles differing in the length of PEG chains and the oxidation state of the anomeric sulphur atom, to analyse the influence of these factors in the carbohydrate-lectin interaction [2]. These micelles can be loaded with hydrophobic imaging and cytotoxic agents. Due to our versatile design, we could compare the active targeting of sorafenib to hepatocellular carcinoma (HCC) by galactose- and mannose-coated micelles, directed to asialoglycoprotein and mannose receptors, respectively. To date, it was thought that asialoglycoprotein receptor was the ideal target for HCC, but it was shown that mannose micelles directed to over-expressed mannose receptor showed an increased endosomal incorporation with increased intracellular sorafenib concentration that induced apoptosis and reduced cell proliferation. Finally, we functionalised SWCNTs with these glycomonomers and analysed the influence of shape and size in selective bacterial cell agglutination, showing that 1-D structures (glyconanotubes or glycolipid nanotubes) regulate the agglutination and proliferation of bacterial cells more efficiently than 3D-structures (micelles or liposomes). [1,3]

Acknowledgements. We thank the MINECO (CTQ2013-49066-C2-1-R and CTQ2016-78580-C2-1-R) for financial support.

References

1. J.J. Cid Martín, M. Assali, E. Fernández-García, V. Valdivia, E.M. Sánchez-Fernández, J.M. García Fernández, R.E. Wellinger, I. Fernández, N. Khiar. *J. Mater. Chem. B.*, **2016**, 4, 2028.
2. E. Romero-Ben, T.Mena Barragán, E. García de Dionisio, E.M. Sánchez-Fernández, J.M. García Fernández, E. Guillén-Mancina, M. López-Lázaro, N. Khiar. *J. Mater. Chem. B,* **2019**, *7*, 5930.
3. M. Pernía Leal, M. Assali, J.J. Cid, V. Valdivia, J.M. Franco, I. Fernández, D. Pozo, N. Khiar. *Nanoscale*, **2015**, *7*, 19259.

Multifunctional hydrogels for stiffness-modulated cell behavior

Syeda Rubab Batool,[1] Muhammad Anwaar Nazeer,[2,3] Erdost Yilidiz,[1] Afsun Sahin,[3,4]

Seda Kizilel [1,2,3]*

[1]Biomedical Sciences and Engineering, Koç University, Sariyer 34450, Istanbul, Turkey
[2]Chemical and Biological Engineering, Koç University, Sariyer 34450, Istanbul, Turkey
[3]Reserach Center for Translational Medicine, Koç University, Sariyer 34450, Istanbul, Turkey
[4]Ophthalmology Department, Koç University Hospital, Zeytinburnu 34010, Istanbul, Turkey

skizilel@ku.edu.tr

In this study, we report the synthesis of single and dual crosslinked anthracene-functional chitosan-based hydrogels in the absence of toxic initiators. Single crosslinking was achieved through dimerization of anthracene, where dual crosslinked hydrogel was formed through dimerization of anthracene and free radical photopolymerization of methacrylated-chitosan in the presence of non-toxic initiator riboflavin, a well-known vitamin B2 as shown in Figure 1. We characterized the synthesized materials and hydrogels using Fourier transform infrared (FTIR) spectroscopy, UV-Vis spectroscopy, 2, 4, 6-Trinitrobenzenesulfonic acid (TNBS) assay, and scanning electron microscopy (SEM). Both single and dual crosslinked hydrogels were found to be elastic, as was determined through rheological analysis. We observed that the dual crosslinked hydrogels exhibited higher Young's modulus than the single crosslinked hydrogels, where the modulus for dual and single crosslinked hydrogels were measured as 26 ±2.8 kPa and 14 ±0.6 kPa, respectively. Furthermore, we investigated cytotoxicity of both hydrogels towards 3T3-J2 fibroblast cells was determined through CellTiter-Glo assay. Finally, immunofluorescence staining was carried out to evaluate the impact of hydrogel modulus on cell morphology. This study comprehensively presents functionalization of chitosan with anthracene, uses nontoxic initiator riboflavin, and modulates the degree of crosslinking through dimerization of anthracene and free radical photopolymerization, and further modulates cell behavior through the alterations of hydrogel properties on surfaces coated with these engineered networks.

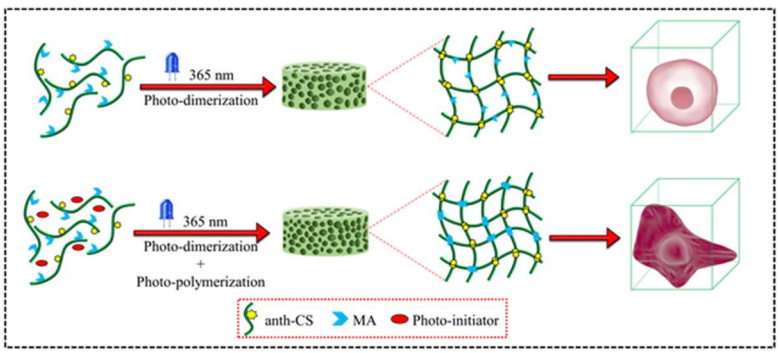


Figure1. Single and dual crosslinked hydrogel formation through photopolymerization and photodimerization for cell behavior studies

Acknowledgements: This study was supported by Koç University Seed Fund SF.0028. The authors gratefully acknowledge the use of facilities of Koç University Surface Science and Technology Center (KUYTAM), Koç University Tüpraş Energy Center (KUTEM) and Koç University Research Center for Translational Medicine (KUTTAM), funded by the Presidency of Turkey, Presidency of Strategy and Budget. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Presidency of Strategy and Budget.

Stereoselective Catalytic Synthesis of 2-Deoxytrehalose Derivatives

Robin A. Jeanneret, M. Carmen Galan

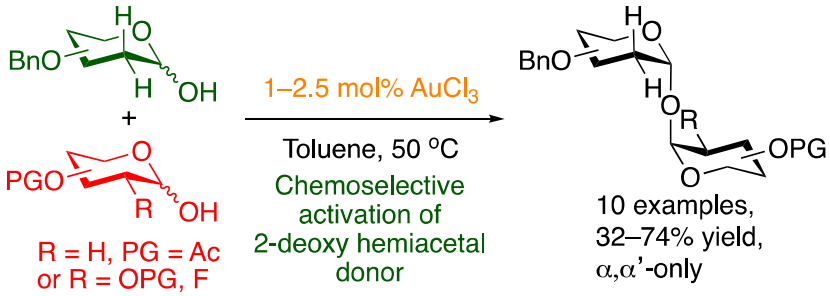
School of Chemistry, Cantock's Close, University of Bristol, BS8 1TS, Bristol, United Kingdom.

Email address – rj18703@bristol.ac.uk.

One of the key features of the bacterium *Mycobacterium Tuberculosis (Mtb)*, the main cause of tuberculosis (TB) a leading cause of death worldwide[1], is a highly complex cell wall that acts as an extremely effective barrier to the penetration of many antibiotics. This features an outer 'mycomembrane', predominantly consisting of mycolic acids linked to trehalose, a disaccharide composed of two 1,1-α,α-linked glucose units. These glycolipids have been shown to play essential roles in *Mtb* cell wall biosynthesis and in the viability and virulence of the pathogen.[2]

The incorporation of various unnatural, functionalised trehalose derivatives into the mycomembrane of live mycobacteria has been reported, exploiting the broad substrate specificity of the trehalose mycolyltransferase enzymes.[3] Accessing structurally defined trehalose derivatives via chemical synthesis is complicated by the difficulty in performing chemo- and stereoselective glycosylation to construct the 1,1-α,α-glycosidic bond due to the formation of unwanted symmetrical derivatives or difficult to separate anomeric mixtures. Alternative methods involve lengthy, often low-yielding, desymmetrisation of trehalose. Enzymatic methods have been successfully used for the efficient synthesis of trehalose derivatives but can be limited by the substrate specificity of the enzymes.[4]

Synthetic efforts within the Galan group focus on developing mild, catalytic methods for the stereoselective synthesis of deoxy glycosides.[5] Herein we present our recent developments on the application of gold catalysis towards the synthesis of 2-deoxy trehalose derivatives. We have developed a method for the catalytic stereoselective synthesis of unsymmetrical 2-deoxy trehalose derivatives via chemoselective activation of 2-deoxy hemiacetal donors by $AuCl_3$ in the presence of less reactive hemiacetal acceptors. Varying the protecting group patterns on the donor and/or acceptor and performing post-glycosylation functionalisation allows access to functionalised 2-deoxy trehalose analogues.



Acknowledgements: We would like to thank the European Research Council (ERCCOG: 648239) for funding.

References

1. World Health Organization, *Global tuberculosis report* **2019**.
2. P. J. Brennan, *Tuberculosis* **2003**, *83*, 91−97.
3. a) K. M. Backus, H. L. Boshoff, C. S. Barry, O. Boutureira, M. K. Patel, F. D'Hooge, S. S. Lee, L. E. Via, K. Tahlan, C. E. Barry and B. G. Davis, *Nature Chemical Biology*, **2011**, *7*, 228–235, b) B. M. Swarts, C. M. Holsclaw, J. C. Jewett, M. Alber, D. M. Fox, M. S. Siegrist, J. A. Leary, R. Kalscheuer and C. R. Bertozzi, *Journal of the American Chemical Society*, **2012**, *134*, 16123–16126.
4.a) S. Jana, S. S. Kulkani, *Org. Bionol. Chem.,* **2020**, *11*, 2013–2037, b) K. Kalera, A. I. Stothard, P. J. Woodruff, B. M. Swarts, *Chem. Commun,* **2020**, *56*, 11528–11547.
5. C. S. Bennett, M. C. Galan, *Chem. Rev.,* **2018**, *118*, 7931–7985.

Highly functional, hybrid coatings based on chitosan, combining biocompatibility and a strong and renewable antimicrobial action

Evangelia Vasilaki,[1,2] *Theodore Manouras,*[1,2] *Nikos Konios,*[2] *Eleftherios Koufakis,*[1,2] *Maria Kaliva,*[1,2] *Ioanna Peraki,*[3] *Maria Vamvakaki*[1,2]

[1]Institute of Electronic Structure and Laser, Foundation for Research and Technology, 700 13 Heraklion, Crete, Greece
[2]Department of Materials Science and Technology, University of Crete, 700 13 Heraklion, Crete, Greece
[3]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, 700 13 Heraklion, Crete, Greece

evasilaki@iesl.forth.gr

In this work, organic and hybrid (organic-inorganic) coatings, exhibiting an effective antimicrobial action and stimuli-renewable properties are presented. Chitosan was first modified to introduce permanent biocidal quaternary ammonium salt (QAS) groups along the polymer backbone. Next the polymer was cross-linked with bifunctional molecules that act as cross-linkers to form a stable polymer coating on glass and flexible polymer substrates. The use of a novel, acid-degradable acetal-based cross-linker enabled the pH-responsive polishing behaviour of the polymer coatings. TiO_2 nanoparticles, modified with reduced graphene oxide (rGO) sheets, to narrow their band gap energy value and shift their photocatalytic activity in the visible light regime, were introduced within the polymer film to enhance its antibacterial activity under visible light irradiation [1]. The coatings exhibited an effective biocidal activity in the dark for both Gram-negative and Gram-positive bacteria, when only the QAS sites interacted with the bacteria membrane, and an excellent biocidal action upon visible light irradiation, due to the synergistic antimicrobial effect of the QAS moieties and the rGO modified TiO_2 nanoparticles. The stimuli-renewal behaviour of the pH-polishable coatings was verified by the gradual decrease in the film thickness upon immersion in acidic media, whereas their effective antimicrobial action was still retained (Figure 1). In addition, the biocompatibility of the films was verified in human cell cultures studies. The proposed approach enables the facile development of highly functional coatings, combining biocompatibility and long-lasting bactericidal action for different applications.

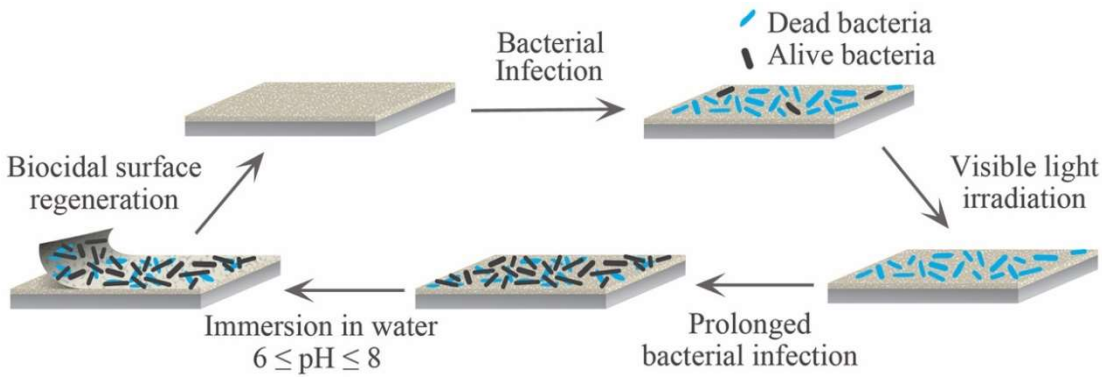


Figure 1: Schematic representation of the biocidal action and stimuli-renewable behaviour of the hybrid coatings.

Acknowledgements: This research has been financed by the Operational Program "Human Resources Development, Education and Lifelong Learning" co-financed by the European Union (European Social Fund) and Greek national funds (program MIS 5006044), and by the "European Union and Greek national funds through the Operational Program Competitiveness, Enterpreneurship, and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code: T1EDK - 04052)."

1. H.L. Poh, F. Sanek, A. Ambrosi, G.J. Zhao, Z.; Sofer, M. Pumera, *Nanoscale,* **2012**, *4*, 3515-3522.

Grafting Strategies to Prepare Porphyrin-Cellulose Materials for Antimicrobial Photodynamic Therapy (aPDT)

Carlos J. P. Monteiro,[1] *Maria G. P. M. S. Neves,*[1] *Adelaide Almeida*[2]*, M. Amparo F. Faustino*[1]

[1]LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[2]CESAM, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

cmonteiro@ua.pt

The advent of drug-resistance among pathogenic microbes is a major concern and recent strategies have focused on the development of novel treatment options and alternative antimicrobial therapies. Antimicrobial Photodynamic Therapy (aPDT) involves the combination of photoactive dyes and harmless visible light to produce reactive oxygen species that can selectively kill microbial cells and is being recognized as an effective method to inactivate a broad spectrum of microorganisms, including those resistant to conventional antimicrobials/biocides [1]. In the last few years, the development and biological assessment of new photosensitizers for aPDT were accompanied by their immobilization in different supports having in mind the extension of the photodynamic principle to new applications, such as wound disinfection, sterilization of medical materials and surfaces in different contexts (industrial, household and hospital) or prevention of microbial contamination in packaged food. In the field of medical sciences, functionalized cellulose-based materials have attracted much attention [2,3]. Cellulose is the most abundant natural biopolymer, considered a great starting material for developing new and more sustainable materials from renewable resources. As cellulose owns a carbohydrate nature, it has inherent compatibility with biological tissues and, consequently, possesses unique utility with respect to their bioavailability, biocompatibility and biodegradability considerations [4]. Photosensitizers for aPDT can become more efficacious when adsorbed, entrapped, or linked to cellulose-like supports. In addition, these cellulose-based systems provide an increase in the surface area, are able to achieve the targeted drug delivery, as well as used for the preparation of packaging materials with improved mechanical strength, barrier, and antimicrobial properties. In this communication, we intended to summarize recent work considering a diversity of photosensitizers supported in cellulose or cellulose derivative materials to achieve an effective photoinactivation with particular attention to the synthetic strategies behind the preparation of the photosensitizers-cellulose functional materials.

Acknowledgements: The authors thank University of Aveiro and FCT/MCT for the financial support provided to the Associate Laboratory LAQV-REQUIMTE (UIDB/50006/2020), CESAM (UUID/AMB/50017 — POCI-01- 0145-FEDER-007638) and to Project PREVINE - FCT-PTDC/ASP-PES/29576/2017), through national funds (OE) and where applicable co-financed by the FEDER-Operational Thematic Program for Competitiveness and Internationalization-COMPETE 2020, within the PT2020 Partnership Agreement. Thanks are also due to the Portuguese NMR and Mass Networks. Thanks are also due to GLYCOnanoPROBES COST ACTION CA18132.

References

[1] Yin, R.; Agrawal, T.; Khan, U.; Gupta, G. K.; Rai, V.; Huang, Y. Y.; Hamblin, M. R., *Nanomedicine* 2015, 10 (15), 2379-2404.
[2] Mesquita, M. Q.; Dias, C. J.; Neves, M. G. P. M. S.; Almeida, A.; Faustino, M. A. F. *Molecules* 2018, 23, 2424.
[3] Alves, E.; Faustino, M. A. F.; Neves, M.; Cunha, A.; Nadais, H.; Almeida, A., *J. Photochem. Photobiol. C-Photochem. Rev.* 2015, 22, 34-57.
[4] Moon, R. J.; Martini, A.; Nairn, J.; Simonsen, J.; Youngblood, J., *Chem. Soc. Rev.* 2011, 40 (7), 3941-3994.

A tweezer-shaped diaminocabazolic receptor for the selective recognition of *N,N'*-diacetylchitobiose in water.

Francesco Milanesi,[1,2] Cristina Nativi,[1] Stefano Roelens,[1] Oscar Francesconi [1]

[1] Department of Chemistry ''Ugo Schiff'' and INSTM
University of Florence, Polo Scientifico e Tecnologico, I-50019, Sesto Fiorentino, Firenze (Italy)
[2] Magnetic Resonance Center CERM
University of Florence, Via L. Sacconi 6, I-50019, Sesto Fiorentino, Firenze (Italy)

francesco.milanesi@unifi.it

Carbohydrates recognition on cells surfaces play a key role in many physiological and pathological processes.[1] An effective tool to interfere with these processes are biomimetic receptors, synthetic small molecules that are able to recognize carbohydrates in water using the same non-covalent interactions used by many carbohydrate-binding proteins, such as lectins.[2] Generally, biomimetic receptors are structured on an aromatic scaffold, that interacts with the aliphatic backbone of the carbohydrate through hydrophobic and CH-π interactions, endowed with one or more hydrogen bonding units deputed to interact with the hydroxylic groups of the saccharide. Development of biomimetic receptors for monosaccharides is a widespread research topic, instead, biomimetic receptors for oligosaccharides are less common in literature. This is due to the complexity of the guest that requires more complicated receptors to be opportunely bound.[3] This issue has frequently been addressed by developing macrocyclic architectures which, however, often require long synthesis with low overall yields. Our research group has a strong background on the development of biomimetic receptors for carbohydrates. Recently, we demonstrated that diaminocarbazole is an effective hydrogen bonding motif for carbohydrates' recognition in water,[4] and, we developed an effective diaminocarbazole-based receptor that shown high affinities for some biologically relevant monosaccharides.[5] Starting from the diaminocarbazole as elective binding motif, in this study, we demonstrate that a simply synthesizable tweezers-shaped structured receptor (**Figure 1**) could be effective in recognizing all equatorial disaccharides in water and at physiological pH.

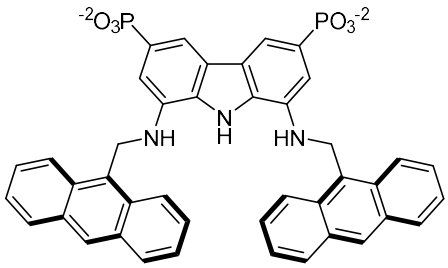


Figure 1: Tweezers-shaped structured receptor.

Such receptor selectively recognizes *N,N*'-diacitylchitobiose among other structurally related 1,4-disaccchrides with a marked affinity, that is unprecedent in the literature, and exceeds that of some lectin-like protein, such as hevein, from *Hevea brasiliensis*.

References
1. B. Ernst, W. Hart, P. Sinay, *Carbohydrates in Chemistry and Biology*, **2000**, *Wiley-VCH,* Weinheim
2. O. Francesconi, S. Roelens, *ChemBioChem*, **2019**, *20*, 1329-1346
3. T.Mooibroek, J. Casas-Solvas, R. Harniman *et al.*, *Nature Chem*, **2016**, *8*, 69-74
4. O. Francesconi, F. Cicero, C. Nativi, S. Roelens, *ChemPhysChem*, **2020**, *21*, 257-262
5. O. Francesconi, M. Martinucci, L. Badii, C. Nativi,S. Roelens, *Chem. Eur. J.*, **2018**, *24*, 6828-6836

Starch-based films as glycocarriers of porphyrinoid photosensitizers

Paloma Lopes,[1,2] Nuno M. M. Moura, [2] Ana T. P. Gomes,[3] M. Amparo F. Faustino,[2] Adelaide Almeida,[3] Paula Ferreira,[4] Manuel A. Coimbra,[2] M. Graça P. M. S. Neves,[2] Idalina Gonçalves[4]

[1] CICECO & [2] LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193, Aveiro, Portugal
[3] CESAM, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal.
[4] CICECO, Department of Materials Engineering and Ceramics, University of Aveiro, 3810-193 Aveiro, Portugal

idalina@ua.pt

In times of growing antibiotics resistance, porphyrinoid photosensitizers, due to their ability of generating reactive oxygen species, have been used in anti-infective strategies such as the photodynamic antimicrobial therapy. Aiming to extend their application range, porphyrinoid photosensitizers have been combined with polymer matrices that work as carriers and/or immobilization supports. In this work, owing to develop a photosensitive glycocarrier, potato starch-based films doped with the cationic 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin tetraiodide (TMPyP) were developed. The influence of TMPyP concentration on optical, photophysical, physicochemical, mechanical, and biological properties of starch-based films was evaluated. TMPyP conferred a reddish coloration to starch-based films. It also increased the films' hydrophobicity and elasticity and decreased their traction resistance and stretchability. Moreover, starch/TMPyP-based films were able to photoinactive Gram-negative *Escherichia coli* bacterium. Therefore, the incorporation of TMPyP into starch-based formulations revealed to be a suitable strategy to develop newly photosensitive glycocarriers with improved mechanical and water tolerance performance.

Acknowledgements: Thanks are due to the University of Aveiro and FCT/MCTES for the financial support to the Associate Laboratory *CICECO-Aveiro Institute of Materials (FCT Ref. UID/CTM/50011/2019)* LAQV-REQUIMTE (UIDB/50006/2020) and CESAM (UUID/AMB/50017 — POCI-01- 0145-FEDER-007638), CESAM (*UID/AMB/50017/2019*) *through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. The authors acknowledge to POTATOPLASTIC project (POCI-01-0247-FEDER-017938), financed by FEDER through POCI, to "Isolago – Indústria de Plásticos, S. A.", the project leader, and to "A Saloinha, Lda." for providing the starch-rich potato washing slurries. FCT is also thanked for the Investigator FCT program (PF) and the Scientific Employment Stimulus program (IG and NMMM). NMM Moura thanks his research contract (CDL-CTTRI-88-89-97-ARH/2018) which is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in numbers 4, 5 and 6 of the article 23, of the Law Decree 57/2016, of August 29, changed by Law 57/2017, of July 19. Thanks are also due to GLYCOnanoPROBES COST ACTION CA18132.*

Controlled spatiotemporal hyperthermia in magnetic chitosan films

Ana Barra,[1,2] Jacek K. Wychowaniec,[3] Liliana P. Ferreira,[4,5] Margarida M. Cruz,[5] Cláudia Nunes,[2] Dermot F. Brougham,[3] Paula Ferreira[1]

[1] Department of Materials and Ceramic Engineering, CICECO – Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal
[2] Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal
[3] School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland
[4] Physics Department, University of Coimbra, 3004-516 Coimbra, Portugal
[5] Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

abarra@ua.pt

Magnetic fluid hyperthermia is a promising therapy for cancer, that relies on the heating of the unhealthy tissue by localized magnetic nanoparticles (MNPs) exposed to an external alternating magnetic field (AMF). Temperature increase up to 40–45 °C were shown to be sufficient to trigger tumour cells death.[1] Nevertheless nanoparticle aggregation and unsuccessful targeting of nanoparticles to the tumour cells may produce hot spots and damage healthy tissue.[2] Stabilization of MNPs in composite materials prevents their agglomeration, restricts the generated heat to the localized tumour area and can enable precise spatiotemporal heat dosing. Herein, magnetic bionanocomposite films were prepared by dispersing magnetite nanoparticles (MNPs) into a chitosan biopolymer solution. MNPs were synthesized by a simple co-precipitation method,[3] and the flexible films were prepared by solvent casting.[4] The spatiotemporal heat dissipation of the bionanocomposites was measured using a live-cell alternating magnetic field (frequency of 664.2 Hz for 15 minutes) exposure system (LC-AMF),[5] both in dry and cellular medium conditions. Careful selection of the MNP concentration, the amount of glycerol used as the plasticizer, and the thickness of films on heat dissipation allowed tuning the generated heat response. The magnetic properties of the bionanocomposites were also evaluated by SQUID magnetometry. The film containing 75 w/v% magnetite and 30 w/v% of glycerol, a thickness of 78.0 µm and a magnetization saturation of 41.33 emu/g, achieved the highest local temperature increase (from room temperature) of up to 97 ºC when exposed to the AMF in dry conditions, or of up to 49 ºC when submerged in 2 mL of cell culture medium. The presented results indicate that these films are a promising platform for magnetic hyperthermia therapies.

Acknowledgements: This work was developed within the scope of the projects CICECO - Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020 and M-ERA-NET2/0021/2016 – BIOFOODPACK – Biocomposite Packaging for Active Preservation of Food*. AB and PF are thankful to FCT for grant SFRH/BD/148856/2019 and the Investigator FCT, respectively. CN is grateful to Portuguese national funds (OE), through FCT, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. COST action 18132, Grant No. 46265 is also acknowledged. J.K.W. and D.B. acknowledge support from Science Foundation Ireland (16/IA/4584).*

References

[1] V. F. Cardoso et. al., Adv. Healthc. Mater. **2017**, 1700845, 1-35.
[2] Y-J Kim et. al., Adv. Funct. Mater. **2013**, 23, 5753-5761.
[3] A. F. Alves et. al., J Nanopart Res **2016**, 18, 1-13.
[4] A. Barra et. al., J Mater Chem B **2020**, 8, 1256-1265.
[5] P. Monks et. al., Small, **2020**, 2004452.

Structure-based design and stereoselective sythesis of human α-galactosidase A (GALA) activity regulators

M. González Cuesta, R.A. Ashmus, Y. Wang, D.T. King, B. Tiet, P.A. Gilormini, J.M. García Fernández, C. Ortiz Mellet, R. Britton, D.J. Vocadlo

[1] Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada
[2] Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada
[3] Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Sevilla 41012, Spain
[4] Instituto de Investigaciones Químicas (IIQ), CSIC-Universidad de Sevilla, Sevilla 41092, Spain

mgonzalez78@us.es

The lysosomal GH27 α-galactosidase A (GALA) is responsible for cleaving terminal α-galactose residues from various glycoconjugates including, most notably, the glycolipid globotriaosylceramide (Gb3). Loss-of-function mutations in the gene *GLA*, which encodes GALA, are at the origin of Fabry disease (FD), a rare congenital lysosomal storage disorder. These mutations lead to a reduction in GALA activity and the accumulation of the substrate Gb3. The iminosugar 1-deoxy-galactonojirimycin (DGJ, Galafold ®) was recently approved for clinical use as pharmacological chaperone for the treatment of FD. DGJ enhances the activity of the endogenous mutant enzyme in the patients, thereby reversing the toxic accumulation of Gb3, but bears several shortcomings. [1] Different groups have synthesized DGJ derivatives in an effort to get better candidates. [2] Here we detail a rational structure-based approach to the design and synthesis of simple derivatives of DGJ through asymmetric synthesis [3] that retain potency, yet offering higher selectivity for GALA over *N*-acetyl-α-galactosaminidase (NAGAL), a structurally similar GH27 homologue, and other functionally related GHs. We further demonstrate that DGJ-C2Pr is cell active and drives reductions of over 50% in the cellular levels of Gb3 within FD patient fibroblast.

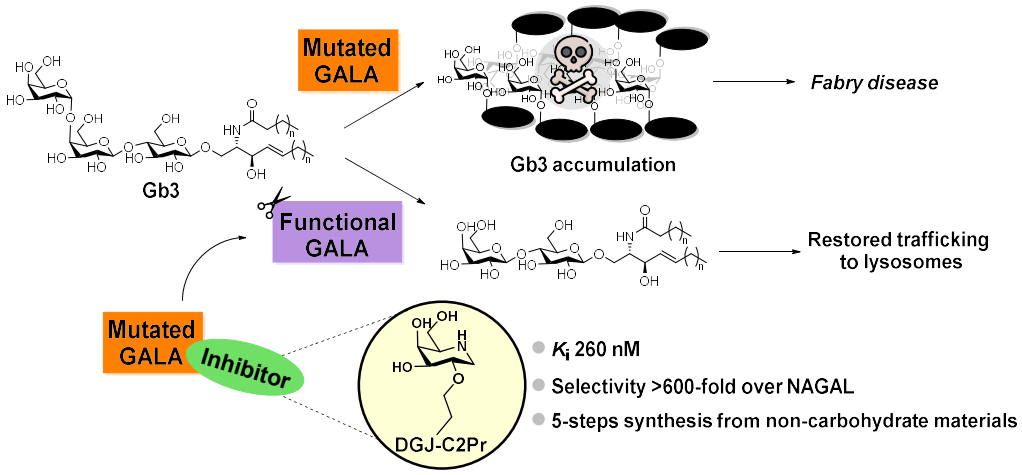


Figura 1. Squematic representation of the GALA function and structure of the best DGJ analogue prepared in this work for pharmacological chaperone use.

Acknowledgements: thanks to MICIU, AEI, FEDER, UE (RTI2018-097609-B-C21) and MINECO, FEDER, UE (PID2019-105858RB-I00) for providing financial support

References

1. Germain, D. P.; Hughes, D. A.; Nicholls, K.; Bichet, D. G.; Giugliani, R.; Wilcox, W. R.; Feliciani, C.; Shankar, S. P.; Ezgu, F.; Amartino, H.; et al. *N. Engl. J. Med.* **2016**, *375*, 545-555.
2. Aguilar-Moncayo, M.; Takai, T.; Higaki, K.; Mena Barragán, T.; Hirano, Y.; Yura, K.; Li, L.; Yu, Y.; Ninomiya, H.; García Moreno, M. I.; Ortiz Mellet, C. García Fernández, J.M. *Chem. Commun*. **2012**, *48*, 6513-6514.
3. Yu, Y.; Mena Barragán, T.; Higaki, K.; Johnson, J. L.; Drury, J. E.; Lieberman, R. L.; Nakasone, N.; Ninomiya, H.; Tsukimura, T.; Sakuraba, H.; Ortiz Mellet, C. García Fernández, J.M. *ACS Chem. Biol*. **2014**, *9*, 1460-1469.
4. Meanwell, M.; Sutherland, M.; Britton, R. J. *Can. J. Chem*. **2018**, *96*, 144-147.

The Potential of Neo-Glycoproteins in Biomedical Diagnosis

Viktoria Heine,[1,2] Tom Kremers,[3] Sarah Boesveld,[4] Nora Menzel,[1,3] Pavel Strnad,[4] Christian Trautwein,[4] Helena Pelantová,[2] Vladimír Křen[2], Uwe Schnakenberg,[3] Lothar Elling[1]

[1] Laboratory for Biomaterials, Institute for Biotechnology and Helmholtz-Institute for Biomedical Engineering RWTH Aachen University Pauwelsstraße 20, D-52074 Aachen, Germany
[2] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, CZ-14220, Prague 4, Czech Republic
[3] Chair of Micro- and Nanosystems and Institute of Materials in Electrical Engineering 1, RWTH Aachen University, Sommerfeldstraße 24, D-52074 Aachen, Germany
[4] Department of Internal Medicine III, University Hospital, RWTH Aachen University, Pauwelsstrasse 30, D-52074 Aachen, Germany

v.heine@biotec.rwth-aachen.de; l.elling@biotec.rwth-aachen.de

Fast and feasible screening methods for pathogenic biomolecules as toxins, viruses, and proteins are highly desired to make diagnosis of infections and diseases more convenient. Since toxins as the *Clostridium difficile* toxin A (TcdA) comprise repetitive carbohydrate recognition domains that bind multiple ligands per molecule [1, 2], multivalent ligands are highly promising means for scavenging of pathogens. We established a method to assemble a multivalent glycan ligand library in microtiter plate scale and transferred this system to biosensor gold chips for electrochemical impedance spectroscopy (EIS). This method enables sensitive real-time monitoring [3] of binding events between ligand and protein [4].
In detail, we produced standard neo-glycoproteins, carrying *N*-acetyllactosamine tetrasaccharides and attached them to microtiter plates. We then elongated them with galactosyltransferases, *N*-acetylglucosaminyltransferases, *N*-acetylgalactosaminyltransferases, fucosyltransferases, and sialyltransferases. Successful glycan transfer was monitored by specific lectins. The resulting glycan library was then tested with the receptor domain of TcdA. From the first screening, nine glycan structures were screened for binding of the holotoxin TcdA resulting in Lewis^{y}-Lewis^{x}-BSA as effective scavenger for TcdA in subsequent cell assays [5]. To elevate neo-glycoproteins to a diagnostic level, *N*-acetyllactosamine-carrying neo-glycoproteins were immobilized on EIS sensor chips to monitor the binding and catalysis of a bacterial fucosyltransferase in real-time [4]. Subsequent incubation with the fucose-specific *Aleuria aurantia* lectin (AAL) confirmed the fucosylation of the neo-glycoprotein's glycans. The EIS measurements enabled visualization of the processes and determination of rate constants.
The use of a sensor chips makes the assembly and analysis of ligand library even more sensitive compared to microtiter plates or micro arrays. The EIS sensor makes glycosyltransferase reactions visible, even without the use of lectins; the application of lectins is optional. Further, the sensor chips can be re-used by thorough removal of the immobilized layers [6]. Monitoring glycosyltransferases in real-time shall be exploited for the substrate screening on multivalent (neo-)glycoproteins. In summary, the developed EIS biosensors are potential diagnostic and detection tools for pathogen-related lectins, toxins, and glycosyltransferases.

References

1. K. Aktories, C. Schwan, T. Jank, *Annu. Rev. Microbiol.*, **2017**, *71*, 281-307.
2. D. Laaf, P. Bojarová, L. Elling, V. Křen, *Trends Biotechnol.*, **2019**, *37*, 402-415.
3. T. Kremers, M. Tintelott, V. Pachauri, X. T. Vu, S. Ingebrandt, U. Schnakenberg, *Electroanalysis*, **2020**, *32*, 1-12.
4. V. Heine*, T. Kremers*, N. Menzel, U. Schnakenberg, L. Elling, *submitted*.
5. V. Heine, S. Boesveld, H. Pelantová, V. Křen, C. Trautwein, P. Strnad, L. Elling, *Bioconjug. Chem.*, **2019**, *30*, 2373-2383.
6. D. J. Kim, R. Pitchimani, D. E. Snow, L. J. Hope-Weeks, *Scanning*, **2008**, *30*, 118-122.

Photoactive molecules bearing uracil-alditols: synthesis and biological assessment

Dias CJ,[1,2] Moura NMM,[1] Neves MGPMS,[1] Fardilha M,[2] Almeida A,[3] Faustino MAF[1]

[1]QOPNA & LAVQV REQUIMTE and Department of Chemistry, University of Aveiro, 3810-193, Aveiro, Portugal
[2]Laboratory of Signal Transduction, IBIMED, Medical Sciences Department, University of Aveiro, 3810-193, Aveiro, Portugal
[3]Microbiology Group, CESAM and Department of Biology, University of Aveiro, 3810-193, Aveiro, Portugal

cristina.jesus.dias@ua.pt

Photodynamic therapy (PDT) is a modality of treatment of various oncological and non-oncological diseases such as bacterial infections [1-4]. This therapy requires the presence of a photoactive molecule, named as photosensitizer (PS), visible light and molecular oxygen [1,2]. The conjugation of these three elements is responsible for the formation of reactive oxygen species (ROS) which will induce tissue damage leading to cell death [1,2]. Several molecules have been studied as PSs in PDT, but porphyrins derivatives and analogues are of particular interest due to their distinct features, such as good absorption in the red region of the electromagnetic spectrum and high generation of singlet oxygen [2]. However, in some cases these PSs presents low solubility in physiological medium and low selectivity to neoplastic tissues [2]. The possibility to obtain better porphyrinic systems for a specific application through the adequate functionalization of the macrocycle core has been an important field of research [2,5-8]. In this sense, the conjugation of the PSs with specific molecules with recognized biological functions, such as carbohydrates, is being considered a good approach, once it can bring a positive impact on their selectivity, solubility and photosensitizing properties [6-8].
In this communication it will be reported an efficient access to new photoactive molecules functionalized with different uracil-alditols moieties and will be discussed their photodynamic efficiency.

Acknowledgements: Thanks are due to the University of Aveiro and FCT/MCT for the financial support for the QOPNA (FCT UID/QUI/00062/2019), CESAM (UIDB/50017/2020+UIDP/50017/2020), and Institute for Biomedicine – iBiMED (UID/BIM/04501/2013 and to project PREVINE (FCT-PTDC/ASP-PES/29576/2017) through national funds (OE) and where applicable co-financed by the FEDER-Operational Thematic Program for Competitiveness and Internationalization-COMPETE 2020, within the PT2020 Partnership Agreement. Thanks are also due to the Portuguese NMR and Mass Networks. C. J. Dias also thanks FCT for her research grant (SFRH/BD/150676/2020) and N.M.M. Moura his research contract respectively (REF.-048-88-ARH/2018) supported by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia. Thanks are also due to GLYCOnanoPROBES COST ACTION CA18132.

References

1. E. Alves, M. A. F. Faustino, M. G. P. M. S. Neves, Â. Cunha, H. Nadais, A. Almeida, *J Photochem Photobiol C Photochem Rev*, **2015**, 22, 34.
2. H. Abrahamse, M. Hamblin, *Biochem J*, **2016**, 473, 347.
3. M. Q. Mesquita, C. J. Dias, S. Gamelas, M. Fardilha, M. G. P. M. S. Neves, M. A. F. Faustino, *Ann Brazilian Acad Sci*, **2018**, 90, 1101.
4. M. Q. Mesquita, C. J. Dias, M. G. P. M. S. Neves, A. Almeida, M. A. F. Faustino, *Molecules*, **2018**, 23, 2424.
5. A. Pałasz, D. Ciez, *Eur. J. Med. Chem*, **2015**, 97, 582.
6. C. J. Dias, I. Sardo, N. M. M. Moura, J. Felgueiras, M. G. P. M. S. Neves, M. Fardilha, M. A. F. Faustino, *Dyes Pigm,* **2020**, 173, 107996.
7. V. V. Serra, A. Zamarrón, M. A. F. Faustino, M. C. I. D. Cruz, A. Blázquez, J. M. M. Rodrigues, M. G. P. M. S. Neves, J. A. S. Cavaleiro, A. Juarranz, F. Sanz-Rodríguez, *Bioorganic Med Chem*, **2010,** 18, 6170.
8. A. T. P. C. Gomes, M. A. F. Faustino, M. G. P. M. S. Neves, V. F. Ferreira, A. Juarranz, J. A. S. Cavaleiro, F. Sanz-Rodríguez, *RSC Adv*, **2015**, 5, 33496.

Antibacterial activity of chitosan derivatives

Georgia-Ioanna Kontogianni[1], Priyanka Sahariah[2], Maria Chatzinikolaidou[1,3]

[1] Department of Materials Science and Technology, University of Crete, GR-70013, Heraklion, Greece
[2] Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Hofsvallagata 53, IS-107 Reykavik, Iceland
[3] Foundation for Research and Technology Hellas (FO.R.T.H)-IESL, GR-70013, Heraklion, Greece

gi.kontogianni@gmail.com

Chitosan-based biomaterials have attracted much interest from the scientific community for their antibacterial properties against gram-positive and gram-negative bacteria [1-3]. Several studies reported the chemical modification of chitosan to enhance its antimicrobial property and increase its application[4]. In this study, we selectively modified the amino group of the biopolymer using cationic and lipophilic moieties with varying chain lengths. We enhanced the lipophilicity of chitosan by N-acylating the amino group using acetyl, hexyl and lauryl chains and imparted quaternization by trimethylation. Utilizing the series of four water soluble chitosan derivatives we evaluated the antibacterial activity against the gram-negative bacterium, *Escherichia coli* (*E. coli*). The tested concentrations ranged from 4.096 µg/ml to 16 µg/ml. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values revealed that N,N,N-trimethyl chitosan exhibits the highest antibacterial activity against *E.coli* at all tested concentrations. The MIC of the other three derivatives ranged between 16 µg/ml and 128 µg/ml. To confirm the conclusions from the MIC of the derivatives, we evaluated the MLC which showed that N,N,N-trimethyl chitosan is bactericidal at 64 µg/ml, N-(acetyl-trimethyl) chitosan at 1.024 µg/ml, N-(hexanoyl-trimethyl) chitosan at 256 µg/ml and N-(lauroyl-trimethyl) chitosan at 64 µg/ml respectively. Scanning electron microscopy (SEM) images taken after 3, 6 and 24 hours of incubation of *E.coli* with the highest concentrations of the derivatives showed an alteration of the physiological rod-shaped bacteria after the initial time point of 3 hours. Longer interaction times for 6 and 24 hours indicated a more pronounced change in bacterial shape resulting in smaller and round morphologies with damaged membranes.

References

1. Sahariah, P., et al., *The effect of substituent, degree of acetylation and positioning of the cationic charge on the antibacterial activity of quaternary chitosan derivatives.* Mar Drugs, 2014. **12**(8): p. 4635-58.
2. Mellegard, H., et al., *Antibacterial activity of chemically defined chitosans: influence of molecular weight, degree of acetylation and test organism.* Int J Food Microbiol, 2011. **148**(1): p. 48-54.
3. No, H.K., et al., *Antibacterial activity of chitosans and chitosan oligomers with different molecular weights.* Int J Food Microbiol, 2002. **74**(1-2): p. 65-72.
4. Sahariah, P. and M. Másson, *Antimicrobial Chitosan and Chitosan Derivatives: A Review of the Structure–Activity Relationship.* Biomacromolecules, 2017.

Glycosidic bond formation in liquid SO_2

Krista Gulbe and Māris Turks

Faculty of Materials Science and Applied Chemistry, Riga Technical University,
P. Valdena Str. 3, Riga, LV-1048, Latvia

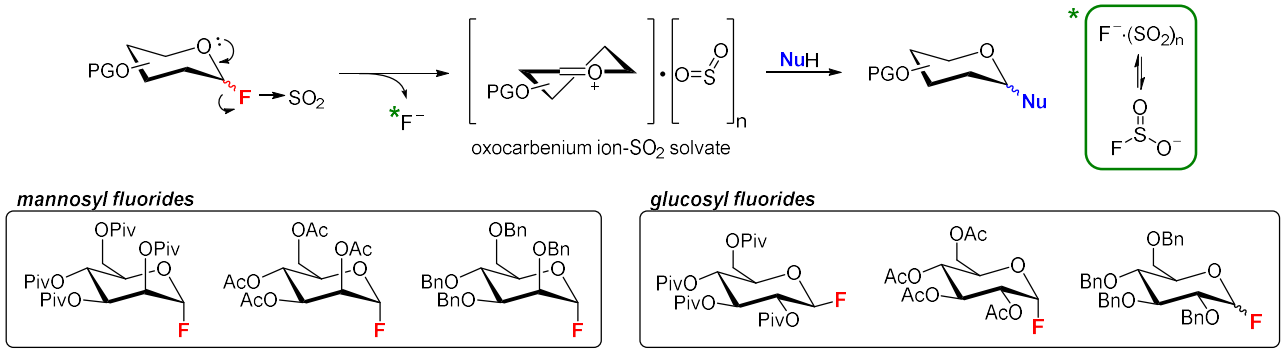
E-mail: maris.turks@rtu.lv

Glycosylation is still one of the most important and basic synthetic strategies in a carbohydrate chemistry that provides an access to the various types of glycoconjugates [1]. To ensure high yielding and regio- and stereoselective glycosidic bond formation, proper combination of glycosyl donor and acceptor, protecting and leaving groups, promoter, solvent and temperature has to be applied.

During the last few decades glycosyl fluorides have become one of the most widely used glycosyl donors in carbohydrate chemistry [2]. Comparing to other glycosyl halides, glycosyl fluorides possess considerably higher thermal and chemical stability. Since the first report in 1981, a series of fluorophilic promoting systems for glycosyl fluorides have been developed. Among them, Sn(II) species, group IVB metallocenes, $BF_3{\cdot}OEt_2$ and protic acids are the most frequently used.

Liquid SO_2 is known as a one of the few polar solvents that possess Lewis acid properties. It has been applied for a variety of Lewis acid-mediated chemical transformations, especially for those with carbenium ion intermediates [3-5]. Additionally, it has been reported that SO_2 can covalently bind fluoride ion to form fluorosulfite anion that is relatively stable even in highly polar media [6]. Thus, we proposed that plausible formation of fluorosulfite species and stabilization of oxocarbenium intermediate could facilitate glycosylation with glycosyl fluorides as glycosyl donors in liquid SO_2 without an external promoter.

Herein we report a novel glycosylation procedure in liquid SO_2 by employing differentially protected mannosyl and glucosyl fluorides. Depending on the reactivity of glycosyl donor various temperature regimes were applied and various types of *O*-, *S*- and *C*-glycosides were isolated in yields up to 95%. Similar results were obtained when saturated solution of SO_2 in dichloromethane was used. Thus, specific equipment is not required and reaction can be carried out in a glass pressure tubes. Finally, mechanistic studies by ^{19}F NMR spectroscopy proved formation of fluorosulfite species.



Acknowledgements: This work was supported by the Latvian Council of Science Grant No. LZP-2018/1-0315.

References

1. B. O. Fraser-Reid, K. Tatsuta, J. Thiem, Eds., *Glycoscience: Chemistry and Chemical Biology*, Springer: Berlin, 2008.
2. K. Toshima, *Carbohydr. Res.*, **2000**, *327*, 15-26.
3. D. Posevins, K. Suta, M. Turks, *Eur. J. Org. Chem.*, **2016**, *2016*, 1414-1419.
4. K. Suta, M. Turks, *ACS Omega*, **2018**, *3*, 18065-18077.
5. K. Leškovskis, K. Gulbe, A. Mishnev, M. Turks, *Tetrahedron Lett.*, **2020**, *61*, 152528.
6. W. Eisfeld, M. Regitz, *J. Am. Chem. Soc.*, **1996**, *118*, 11918-11926.

Development of crosslinked chitosan/gelatin/k-carrageenan hydrogel scaffolds for bone tissue engineering

Konstantinos Loukelis[1], Maria Chatzinikolaidou[1,2]

[1] Department of Materials Science and Technology, University of Crete, 70013 Heraklion, Greece
[2] Foundation for Research and Technology Hellas (FO.R.T.H)-IESL, 70013 Heraklion, Greece

Email: loukelisk@yahoo.com, mchatzin@materials.uoc.gr

Chitosan and gelatin are two of the most prominent and widely studied natural biomaterials. Although they do not present great mechanical strength, their biocompatibility, biodegradation and contribution to the formation of extracellular matrix have been considered remarkable for the development of biomaterials [1]. K-carrageenan is another natural biopolymer extracted from red edible algae that has been examined for its biological properties [2,3]. Its many hydroxyl groups and its one sulfonic group per unit seem to be its main attributes when it comes to biochemical interactions. In this study, we aim to integrate k-carrageenan into a 2.5 w/v% chitosan/5 w/v% gelatin blend in order to understand how k-carrageenan can affect the biocompatibility, integrity, porosity and mechanical stiffness of hydrogels composed of k-carrageenan/chitosan/gelatin when compared to chitosan/gelatin scaffolds. Strong polyelectrolyte attraction occurs among the NH_3^+ group of chitosan and OSO_3^- of k-carrageenan, leading to gelation at room temperature [3]. Exploiting this property while also using 0.25 v/v% glutaraldehyde as crosslinker, we currently synthesize 2.5 w/v% chitosan/5 w/v% gelatin scaffolds with different k-carrageenan concentrations of 0.5 w/v%, 0.75 w/v% and 1 w/v% by means of freeze drying and assess their physicochemical and biological characteristics. A mechanical analysis of the blend scaffolds in compression mode with a load cell of 50 N at 1 mm/s indicated increasing Young modulus with increasing concentrations of k-carrageenan obtaining values of 66 kPa, 109 kPa and 130 kPa for 0.5%, 0.75% and 1% content of k-carrageenan in chitosan/gelatin blends, respectively. Water uptake expressed as ratio of wet to dry weight of blend scaffolds was found to be approximately 30-fold for all three blends.

References

[1] A. Georgopoulou, F. Papadogiannis, A. Batsali, J. Marakis, K. Alpantaki, A.G. Eliopoulos, C. Pontikoglou, M. Chatzinikolaidou, Chitosan/gelatin scaffolds support bone regeneration, J Mater Sci-Mater M 29(5) (2018).

[2] J. Nourmohammadia, F. Roshanfara, M. Farokhib, M.H. Nazarpak, Silk fibroin/kappa-carrageenan composite scaffolds with enhanced biomimetic mineralization for bone regeneration applications, Materials Science and Engineering: C, (2017)

[3] A. Pourjavadia, M. Doroudiana, A. Ahadpoura, S. Azarib, Injectable chitosan/κ-carrageenan hydrogel designed with au nanoparticles: A conductive scaffold for tissue engineering demands, International Journal of Biological Macromolecules (2018)

Antibacterial properties of photoactive starch/porphyrin-based materials

Ana Joaquinito[1], Paulo Brites[2], Mariana Vallejo[1], Carlos Monteiro[1], Maria Bartolomeu[3], Isaac Benito González[4], Nuno M. M. Moura[1], Ana T. P. Gomes[3], Marta Martínez Sanz[4], M. Amparo López Rubio[4], Artur Ribeiro[5], Adelaide Almeida[3], Paula Ferreira[2], Manuel A. Coimbra[1], M. Graça P. M. S. Neves[1], Idalina Gonçalves[2], M. Amparo F. Faustino[1]

[1] LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[2] CICECO - Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, 3810-193 Aveiro, Portugal
[3] Department of Biology & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[4] Food Safety and Preservation Department, IATA-CSIC, Avda. Agustín Escardino 7, 46980 Paterna, Valencia, Spain
[6] Centre of Biological Engineering, University of Minho, Campus De Gualtar, 4710-057, Braga, Portugal

a.joaquinito@ua.pt

Photodynamic therapy (or PDT) has been used with success for many years as a cancer therapy but more recently has gained popularity as an alternative to the conventional antimicrobial approaches. Microorganisms have the ability to evolve and select resistant strains to antimicrobials [1,2]. In fact, it is estimated that by 2050, deaths due to antimicrobial resistance will exponentially rise above 10 million deaths per year globally, surpassing the current number of 700,000 deaths per year [1]. Therefore, other methodologies and treatments against resistant microorganisms are needed and antimicrobial photodynamic therapy (or aPDT) may prove to be a solution. Porphyrin derivatives when combined simultaneously with light and dioxygen, have been identified as good antimicrobial agents through a photodynamic action on the microbial structures. However, considering some clinical applications, like wound treatments, for instance, the delivery in a solution may not be practical. The evidence that the incorporation of porphyrinic sensitizers (PS) into solid supports maintain the photodynamic activity lead us to use glycomaterials, namely potato starch to develop antimicrobial photoactive materials. In this work the sensitizer 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin tetra-iodide (TMPyP) was prepared and further incorporated through melt-mixing into starch-based formulations. The biological properties of starch/TMPyP-based material was assessed. In this communication, it will be discussed the synthesis of TMPyP, its immobilization on the support and *in vitro* and *ex vivo* (porcine skin) antimicrobial properties of the developed photoactive starch/TMPyP-based material against methicillin-resistant *Staphylococcus aureus* (MRSA), a strain responsible for many hospital acquired infections, and with increasing prevalence [3].

Acknowledgements: Thanks are due to the University of Aveiro and FCT/MCTES for the financial support to the Associate Laboratory *CICECO-Aveiro Institute of Materials (FCT Ref. UID/CTM/50011/2019)* LAQV-REQUIMTE (UIDB/50006/2020) and CESAM (UUID/AMB/50017 — POCI-01- 0145-FEDER-007638, UID/AMB/50017/2019)) *through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. The authors acknowledge to POTATOPLASTIC project (POCI-01-0247-FEDER-017938), financed by FEDER through POCI, to "Isolago – Indústria de Plásticos, S. A.", the project leader, and to "A Saloinha, Lda." for providing the starch-rich potato washing slurries. FCT is also thanked for the Investigator FCT program (PF) and the Scientific Employment Stimulus program (IG and NMMM). NMM Moura thanks his research contract (CDL-CTTRI-88-89-97-ARH/2018) which is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in numbers 4, 5 and 6 of the article 23, of the Law Decree 57/2016, of August 29, changed by Law 57/2017, of July 19. Thanks are also due to GLYCOnanoPROBES COST ACTION CA18132.*

References.

1. M. J. Renwick, V. Simpkin, E. Mossialos, *Targeting innovation in antibiotic drug discovery and development, 2016;*
2. F. Cieplik, D. Deng, W. Crielaard, W. Buchalla, A. Al-ahmad, T Maisch, *Crit. Rev. Microbiol,* 2018, *vol. 44*, pp 571-589;
3. G. Ippolito, S. Leone, F. N. Lauria, E. Nicastri, R. P. Wenze, *Int. J. Infect. Dis.,* 2010, *vol. 14*, pp 7-11.

Deciphering the structural features of Siglecs recognition using NMR spectroscopy.

Coelho H[1], Soares C[1], Lenza MP[2], Oyenart I[2], Jiménez-Barbero J[2], Orbea J[2], Marcelo F[1],

[1]UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516Caparica, Portugal
[2]CIC bioGUNE Bizkaia, 48160 Derio, Spain

h.coelho@campus.fct.unl.pt

Glycans play a central role on the regulation of innate and adaptive immune responses. Through the interaction with glycan-binding proteins (lectins), present on the surface of immune cells, glycans are relevant in pathogen recognition, autoimmune diseases, and cancer [1]. Several studies demonstrated that specific lectins such as sialic acid-binding immunoglobulin-like lectins (siglecs), expressed by immune cells, mediate immune suppression by interaction with tumour-associated glycans [2].
Herein, we will report a multidisciplinary approach that span from molecular biology, to improve Siglecs expression and purification, to advance NMR binding methods (STD-NMR, transferred-NOESY) to characterize the molecular determinants that govern glycan-siglecs interactions. Key information on the epitope mapping and conformation of sialic-containing glycans, including the tumour-associated STn epitope, in the bound state to Siglec-7 and -15, will be presented. Our results provide new structural insights to understand the specificity of siglecs and potential contribute to the design of novel glycomimetics able to modulate the immunological activity of Siglec-7/-15.

Acknowledgements:
Helena Coelho thanks to the FCT-Portugal for the Projects IF/00780/2015, PTDC/BIA-MIB/31028/2017 and UIBD/Multi/04378/2020 and to the European commission for the COST Action 18132 - GLYCONANOPROBES. The NMR spectrometers are part of the National NMR Network (PTNMR) and are partially supported by Infrastructure Project No 22161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

References

1. J. Lübbers, E. Rodríguez, and Y. van Kooyk, *Front Immunol.* **2018**; 9: 2807.
2. M. S. Macauley, P.R. Crocker, J.C. Paulson *Nat Rev Immunol.* **2014,** *14*, 653.