Peptide Materials

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# A Decade of Peptide Materials

October 26-28, 2023

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**ABSTRACT BOOK**

**Organized by:**
Research Center on Bioactive Peptides (CIRPeB) – University of Naples "Federico II"
Dept. of Chemical Science and Technologies University of Rome Tor Vergata

**Under the auspicies of:**
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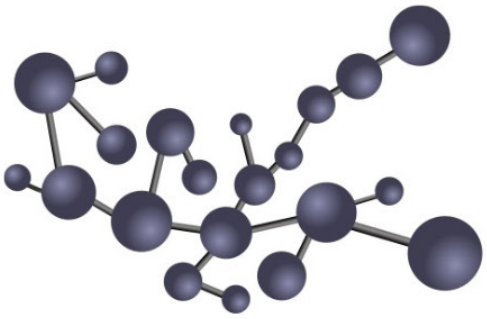

Peptide Materials

# Plenary Lectures

PL 1

# Engineering Biofunctional Metal Phenolic Materials via Supramolecular Assembly

F. Caruso

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The development of rapid and versatile coating strategies for interface and materials engineering is of widespread interest. This presentation will focus on our studies on the formation of a versatile class of metal–organic materials, metal–phenolic networks (MPNs), which can be formed on various substrates by coordinating polyphenols and metal ions through self-assembly[1,2]. This robust and modular assembly strategy is substrate independent (covering organic, inorganic, and biological substrates) and has been used for the preparation of various materials, including thin films, particles, superstructures and macroscopic assemblies[3]. It will be shown that a range of polyphenols and a library of metal ions are suitable for forming MPNs and that by altering the type of metal ions, different functions can be incorporated in the MPN materials, ranging from fluorescence to MRI and catalytic capabilities. Furthermore, the use of polypeptides[4] and proteins[5–7] to form engineered films and particles for a range of biological applications will be highlighted. The ease and scalability of the assembly process, combined with the tuneable properties of MPNs, provide a new avenue for functional interface engineering and make MPNs potential candidates for biomedical, environmental, and advanced materials applications.

*References*

1. Ejima, J. J. Richardson, K. Liang, J. P. Best, M. P. van Koeverden, G. K. Such, J. Cui, F. Caruso Science (2013), 341, 154.
2. J. Guo, Y. Ping, H. Ejima, K. Alt, M. Meissner, J. J. Richardson, Y. Yan, K. Peter, D. v. Elverfeldt, C. E. Hagemeyer, F. Caruso Angew. Chem. Int. Ed. (2014), 126, 5546.
3. J. Guo, B. L. Tardy, A. J. Christofferson, Y. Dai, J. J. Richardson, W. Zhu, M. Hu, Y. Ju, J. Cui, R. R. Dagastine, I. Yarovsky, F. Caruso Nat. Nanotechnol. (2016), 11, 1105.
4. Y. Han, R. P. M. Lafleur, J. Zhou, W. Xu, Z. Lin, J. J. Richardson, F. Caruso J. Am. Chem. Soc. (2022), 144, 12510.
5. Y. Han, Z. Lin, J. Zhou, G. Yun, R. Guo, J. J. Richardson, F. Caruso Angew. Chem. Int. Ed. (2020), 59, 15618.
6. Y. Han, J. Zhou, Y. Hu, Z. Lin, Y. Ma, J. J. Richardson, F. Caruso ACS Nano (2020), 14, 12972.
7. J. Chen, S. Pan, J. Zhou, Z. Lin, Y. Qu, A. Glab, Y. Han, J. J. Richardson, F. Caruso Adv. Mater. (2022), 34, 2108624.

PL 2

# Peptide-Based Supramolecular Systems

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We study how peptides can be used as building blocks to create functional materials and adaptive systems using bottom-up approaches. Instead of copying biological designs, we use integrated computational and experimental approaches to search and map the peptide sequence space and create guiding principles for the formation of peptide materials. The talk will include our latest research on three ongoing projects: (i) Design of peptide modalities that give rise to formation of liquid condensates; (ii) Mechano-responsive peptide crystals; (iii) Drug-matched peptide nanoparticles; (iv) Experimental learning and memory using sequence-adaptive peptide mixtures. Overall, the research demonstrates that peptides, and dynamically exchanging mixtures of peptides, show significant potential as designable and tunable nanomaterials for a variety of applications in biomedicine and green nanotechnology.

PL 3

# Peptide-Based Electronic Materials

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The emerging need for bioelectronic devices that interface with biological substances is accompanied by a growing need for biocompatible, biodegradable, conducting materials. However, the inherently sustainable biomaterials commonly are commonly poor conductors. In order to overcome this challenge, our group is developing bio-inspired materials based on de-novo designed peptides. These materials capture many of the features of proteins on one hand, and are optimized for electronic and/ or protonic conduction applications on the other hand. In this talk, I will discuss the guiding lines for the design of peptides as building blocks of conductive materials. Specifically, I will show that the sequence of the peptide can greatly influence the conductivity with sensitivity to even single sequence mutation. These effects are shown to originate both from the differences in the intrinsic ability of a specific amino acid to participate in the charge transport process, and also from their effects on the assembly of the structure, which in return affect the conductivity. Design concepts for improving either electron[1] or proton[2-4] conductivity, or both,[5-6] will be presented. These outlined structure function relationships can be used for the design of novel biocompatible organic bio-electronic devices.

*References*

1. D. Ivnitski, M. Amit, O. Silberbush, Y. Atsmon-Raz, J. Nanda, R. Cohen-Luria, Y. Miller, G. Ashkenasy, and N. Ashkenasy, Angewandte Chemie Int. Ed., (2016) 55, 9988.
2. O. Silberbush, M. Amit, S. Roy and N. Ashkenasy Adv. Func. Mat. (2017) 27, 1616.
3. O. Silberbush, M. Engel, I. Sivron, S. Roy, N. Ashkenasy, J. Phys. Chem. B (2019) 123, 9882.
4. S. Roy, L. Zheng, O. Silberbush, M. Engel, Y. Atsmon-Raz, Y.Miller, A. Migliore, D. N. Beratan, and N. Ashkenasy, (2021) J. Phys. Chem. B, 125, 12741.
5. M. Amit, S. Appel, R. Cohen, G. Cheng, I. W. Hamley, and N. Ashkenasy, Adv. Func. Mat. (2014) 24, 5873.
6. S. M. M. Reddy, E. Raßlenberg, S. Sloan-Dennison, T. Hesketh, O. Silberbush, T. Tuttle, E. Smith, D. Graham, K. Faulds, R. V. Ulijn, N. Ashkenasy, and A. Lampel Adv. Mat. (2020) 32, 2003511.

Topic:

Peptide Materials for Opto/Electronic Applications

PL 4

# Controlled therapeutic peptide release using nanoparticles and hydrogels

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Therapeutic peptides are bioactive agents with a well-defined primary structure and usually small in size. Although the field of therapeutic peptides started with natural human hormones, currently it is well accepted that naturally occurring peptides are often not directly transferable to therapeutics on account of their own limitations (i.e. low chemical and physical stability). However, two strategies can be combined to overcome such drawbacks. Firstly, the chemical and physical stability of therapeutic natural peptides can be improved through rational design, thus replacing specific amino acids by synthetic amino acids. Through efficient substitutions, this approach produces peptide analogues with resistance against enzymatic degradation, enhanced stability of the bioactive conformation, and reduced tendency towards aggregation. Secondly, specific carriers for therapeutic peptides can be developed to improve the bioavailability and medicinal effect of those compounds. In this communication, we review how, after overcoming the intrinsic limitations of anticancer peptides by designing stable analogues, controlled release can be achieved through the application of external stimuli if appropriate responsive carriers are used. For this purpose, we have focused on CR(NMe)EKA (Cys-Arg-N-methyl-Glu-Lys- Ala) as a benchmark case.

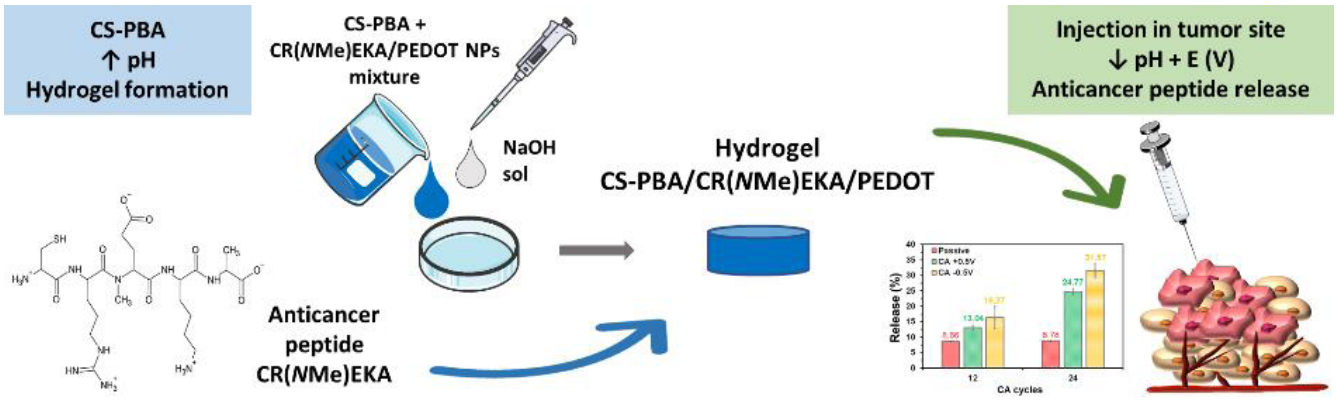


Figure 1. Sketch showing the development of a doubly-responsive carrier to release therapeutic peptides.

PL 5

# β-sheet forming peptides: from molecular self-assembly to biomedical materials design

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2. Manchester Institute of Biotechnology, The University of Manchester, UK

3. Department of Chemical Engineering, School of Engineering, Faculty of Science and Engineering, The University of Manchester, UK

The use of non-covalent self-assembly has become a prominent strategy in material science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from electronic to biotechnology. A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is de-novo designed peptides. Our group work focusses on the development of a technological platform for the design of novel biofunctional hydrogels exploiting the self-assembly of so-called b-sheet forming peptides. These hydrogels can be easily functionalised using specific biological signals and can also be made responsive through the use of enzymatic catalysis and/or conjugation with responsive polymers. Through the fundamental understanding of the self-assembly and gelation processes of these peptides across length scales we have been able to design hydrogels with tailored properties for a range of applications from tissue engineering, cell culture and drug delivery to 3D bioprinting and biosensing.[1]

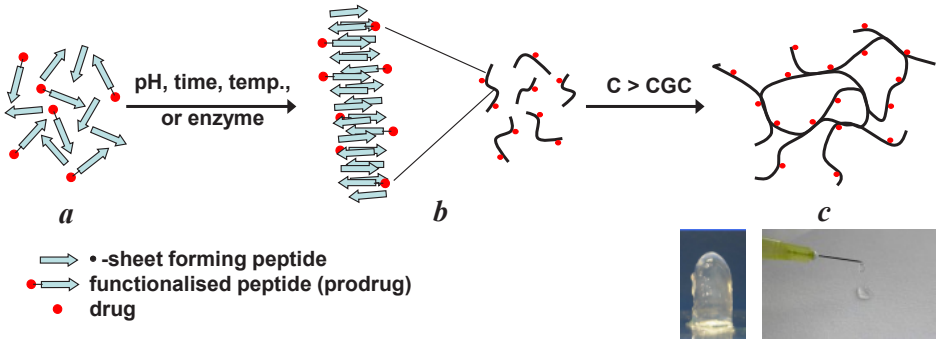


Figure 1. Schematic representation of the self-assembly and gelation pathway of b-sheet forming peptides

Here I will discuss our group's journey from biomolecular design and materials formulation to their potential use in the biomedical sector. I will in particular focus on the challenges encounter along the translational pathway of a work stemmed from basic scientific interest.[1]

*Reference*

1. www.polymersandpeptides.co.uk

PL 6

# Peptide Self-Assembly to Control Intracellular Activities for Biomedical Applications

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3 Department of Biochemistry, Weill Medical College of Cornell University, 413 East 69th Street, New York, NY 10021 (USA)

In this presentation, I will cover how both self-assembly of peptides as well as disassembly of peptide have important medical applications. The intracellular peptide self-assembly can be applied as a stimulus to intracellular activities and signal pathways. Among such interests in mechano-stimulation and mechano-transduction, we currently focus on the large-scale engineering of therapeutic nanoparticles of exosomes. By designing the self-assembly in specific areas in cells, triggered cellular stress could influence intracellular trafficking, multivesicular body formation (MVB), and genetic packaging, aiming to large-scale generation of exosomes with improved therapeutic functionalities for immunotherapy and cancer-targeting vehicles.[1] These aims will be discussed with nanoparticle tracking analysis (NTA), proteomics, and cancer biology. Such biotechnology in combination with peptide self-assembly could help overcome the main hurdle of clinical translation of exosome therapeutics. On the other hand, peptide self-assembly could have negative impact in health. We are using magnetic Brownian motion of iron oxide nanocages[2] to disassemble the peptide aggregates in an on-and-off manner, which will improve the efficacy of immunotherapy for treating cancers and metastasis. The mechanism of such magnetic mechano-stimulation and the influence in intracellular structures will be discussed.

*References*

1. H. Matsui, M.A. Kang, K. Shiba. U.S. Provisional Patent Application (2021), 17/366,950.
2. M.A. Kang, J. Fang, A. Paragodaarachich, D. Yakobashvili, K. Kodama, Y. Ichiyanagi, H. Matsui. Nano Lett. (2022), 22, 8852 (2022).

PL 7

# Bioactive Self-Assembling Peptides and Lipopeptides

Ian W Hamley and Valeria Castelletto

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I will review selected examples of recent work in my group that exploits the self-assembly propensity of designed peptides and lipopeptides to control their diverse structures and bioactivities in aqueous solutions and gels. I will discuss highlights of recent research on arginine-containing surfactant-like peptides which show diverse nanostructures including a new type of a-helical nanotube,[1-2] and hydrogel and emulsion formation along with remarkable bioactivity such as selective antimicrobial activity.[3-5] I will also briefly outline selected discoveries on bioactive self-assembling lipopeptides including those with anti-cancer,[6] and organocatalytic properties,[7-8] and those with applications in tissue engineering and the production of cultured meat.[9] I will also discuss our recent work on a conserved coronavirus spike protein peptide that forms amyloid structures, differing from the native helical conformation and not predicted by amyloid aggregation algorithms.[10] We examined the conformation and aggregation of peptide RSAIEDLLFDKV, a sequence common to many animal and human coronavirus spike proteins. This sequence is part of a native α-helical spike glycoprotein domain, close to and partly spanning the fusion sequence, and it is not predicted to form amyloid by aggregation propensity algorithms. However, we found that this peptide aggregates into β-sheet amyloid nanotape structures close to the calculated pI =4.2, but forms disordered monomers at high and low pH. We also uncovered conditions for hydrogelation, relevant to potential future applications.

*References*

1. Castelletto, V.; Seitsonen, J.; Ruokolainen, J.; Hamley, I. W., Soft Matter 2020, 17, 3096.
2. Castelletto, V.; Seitsonen, J.; Ruokolainen, J.; Piras, C.; Cramer, R.; Edwards-Gayle, C. J. C.; Hamley, I. W., ChemComm 2020, 56, 11977.
3. Castelletto, V.; Barnes, R. H.; Karatsas, K.-A.; Edwards-Gayle, C. J. C.; Greco, F.; Hamley, I. W.; Seitsonen, J.; Ruokolainen, J., Langmuir 2019, 35, 1302.
4. Castelletto, V.; Barnes, R. H.; Karatzas, K. A.; Edwards-Gayle, C. J. C.; Greco, F.; Hamley, I. W.; Rambo, R.; Seitsonen, J.; Ruokolainen, J., Biomacromolecules 2018, 19, 2782.
5. Castelletto, V.; Edwards-Gayle, C. J. C.; Hamley, I. W.; Pelin, J. N. B. D.; Alves, W. A.; Alguilar, A. M.; Seitsonen, J.; Ruokolainen, J., ACS Applied Bio Materials 2019, 2, 3639.
6. Castelletto, V.; Edwards-Gayle, C. J. C.; Greco, F.; Hamley, I. W.; Seitsonen, J.; Ruokolainen, J., ACS Appl. Mater. Interfaces 2019, 11, 33573.
7. Pelin, J. N. B. D.; Edwards-Gayle, C. J. C.; Aguilar, A. M.; Kaur, A.; Hamley, I. W.; Alves, W. A., Soft Matter 2020, 16, 4615.
8. Pelin, J. N. B. D.; Edwards-Gayle, C. J. C.; Castelletto, V.; Aguilar, A. M.; Alves, W. A.; Seitsonen, J.; Ruokolainen, J.; Hamley, I. W., ACS Appl. Mater. Interfaces 2020, 12, 13671.
9. Rosa, E.; Mello, L. R.; Castelletto, V.; Dallas, M. L.; Accardo, A.; Hamley, I. W., Biomacromolecules 2023, 24, 213.
10. Castelletto, V.; Hamley, I. W., ACS Nano 2022, 16, 1857.

PL 8

# Peptide-Based Functional Coatings and Assemblies

Meital Reches

Institute of Chemistry and the Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem, Israel

I will present bio-inspired functional coatings that are spontaneously formed by extremely short peptides. These peptide-based coatings self-assemble on metals, oxides, and polymers under mild conditions without any need for a curing step. The coating can serve many functions. One application is preventing biofouling - the undesirable adhesion of biomolecules and organisms to surfaces. This process leads to numerous adverse effects including hospital-acquired infection, blockage of water desalination facilities, and food contamination. We showed that this coating prevents the first step of biofouling, which involves the adsorption of bioorganic molecules to the substrate. [1] Moreover, the coating significantly reduces the attachment of various organisms, such as bacteria, viruses and fungi, to surfaces. [2,3] Another function that these peptide-based coatings can mediate is the adhesion of mammalian cells to implants. [4] This function is important for the integration of implants into the human body. Moreover, these peptides self-assemble in solution into particles that adsorb and release active compounds that synergistically reduce the number of bacteria and viruses on the surface. They can also be integrated into polymeric films by a simple co-extrusion protocol. [5]

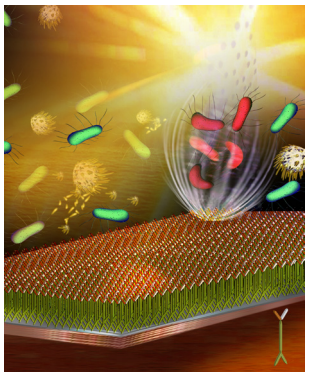
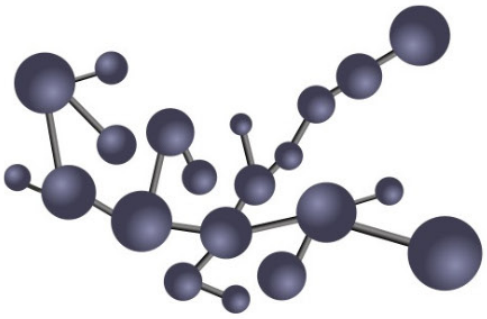


Figure 1. Peptide-based coating that prevents bacterial adhesion

*References*

1. S. Maity, S. Nir, T. Zada, M. Reches Chem. Commun. (2014), 50,11154.
2. T. Hu, O. Agazani, S. Nir, M. Cohen, S. Pan, M. Reches ACS Appl. Mater. Interfaces (2021), 13, 48469.
3. T. Hu, M. Kaganovich, Z. Shpilt, A. Pramanik, O. Agazani, S. Pan, E. Tshuva, M. Reches Adv. Mater. Interfaces (2023), 10, 2202161.
4. S. Yuran, A. Dolid, M. Reches, ACS Biomater. Eng. (2018), 4, 4051.
5. M. Kaganovich, C. Shlosman, E. Goldman, M. Benchis, T. Eitan, R. Shemesh, A. Gamliel, M. Reches Chem. Commun (2022) 58, 9357.



Peptide Materials

# Oral

O 1

# Peptide hydrogels as a long-acting injectable implant for combined HIV/AIDS-contraceptive drug delivery

G. Laverty[1], S. Coulter[1], S. Pentlavalli[1], Y. An[1], H. Sun[1], J. Moore[1], E. Cross[1], M. Zbiri [2], R. Schweins [2]

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**Introduction:** There is a need for a convenient and effective long-acting formulation to deliver a combination of HIV/AIDS and contraceptive drugs. Women in the developing world represent are the most significant group for new HIV/AIDS infections.[1] Our aim was to develop an injectable *in situ* forming hydrogel implant for the delivery of antiretroviral (zidovudine, MIV-150, cabotegravir) and contraceptive drugs (etonogestrel) for ≥28 days using peptides that form hydrogels in response to phosphatase enzymes present in the subcutaneous space. The formulation is a D or L-α peptide hydrogelator composed of phosphorylated (naphthalene-2-ly)-acetyl-diphenylalanine-lysine-tyrosine-OH (NapFFKY[p]-OH) to which drugs are conjugated covalently forming an injectable solution.
**Methods:** Drug-peptides synthesised using solid-phase synthesis. Mechanical properties characterized using oscillatory rheology. The structure of hydrogel fibre networks studied using small angle neutron scattering (SANS). Cell toxicity assessed using MTS, Live/Dead and LDH assays. Biostability studied using protease Proteinase K. Drug release from hydrogels assessed for 28 days in PBS (pH7.4) and drug plasma concentrations over 5 weeks in Sprague Dawley rats.
**Results:** Rheology showed peptides rapidly formed hydrogels within minutes in the presence of phosphatase enzyme. SANS demonstrated peptide gels closely fit model data for flexible cylinder elliptical model. D-peptides were promising as a long-acting drug delivery platform, displaying resistance to protease degradation for 28 days. Burst release from physically encapsulated MIV-150 and etonogestrel combinations was reduced by >40% via chemical conjugation of drugs to the same D-peptide gelator (NapffkYG-OH). This system was also able to deliver clinically relevant plasma concentrations of drugs within their IC50 range in rats for 35 days.
**Conclusions:** This work is a proof-of-concept for the development of a long-acting combined injectable *in situ* forming implant using a peptide hydrogel formulation strategy. Our discrete technology can provide extended contraceptive cover and HIV/AIDS prevention within one product, empowering women to take control of their sexual health and improving adherence to medication.[2]

**Acknowledgements:** Funding provided by EPSRC, MRC, Wellcome Trust, Innovate UK and Invest NI. SANS at ILL experiment number 9-13-972 (DOI:10.5291/ILL-DATA.9-13-972).

*References*

*1.* Karim,S. et al. Lancet Global Health.( 2019), 7, e1470-e1471.
2. UNAIDS.Fact sheet-Global HIV & AIDS statistics 2022.

O 2

# Self-assembled peptide-based photothermal hydrogels: cancer theranostic combining thermo-chemotherapy and MRI

Teresa Pereira[1], Juan Gallo[2], Manuel Bañobre-López[2], Elisabete Coutinho[3], Loic Hilliou[4], Paula M.T. Ferreira[1], and José A. Martins[1]

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4 Institute for Polymers and Composites, Department of Polymer Engineering, University of Minho, Campus de Azurém,4800-058 Guimarães, Portugal.

Peptide hydrogels are the current paradigm soft materials for in vivo applications owing to high water content, biocompatibility and degradability and responsiveness to environmental stimuli. A new family of succinic acid N-capped dehydropeptide hydrogelators was developed recently by our research group (Figure 1).1 Rheological studies showed that the succinic acid N-capped dehydropeptide hydrogels display high elasticity, thermal and mechanical stability, injectable and self- healing properties. Moreover, composite hydrogels - with incorporated tannic acid-based Fe nanoparticles, retain the rheological properties of the pristine hydrogels. The composite hydrogels were characterised as theragnosticplatforms. Phantoms and T1,2 relaxation maps showed that despite a slight decrease of relaxivity (r1, mM-1s-1), comparing to the nanoparticles in aqueous solution, the composite hydrogels retain MRI reporting properties. The heating capacity of the nanoparticles and of the composite hydrogels, revealed suitable for cancer photohyperthermia - hydrogels reached temperatures above 40 oC upon laser irradiation (15 minutes, 808 nm, 1W/cm2). The composite hydrogels were tested also as agents for photothermia-triggered drug delivery, potentially allying thermo-chemotherapeutic capabilities. Injectable and self-healing properties allied to photothermia and MRI reporting properties make these composite hydrogels promising theranostic cancer platforms[1].

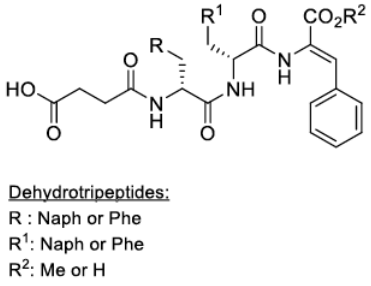


Figure 1. General chemical structure of the dehydrotripeptides

*Reference*

1. Teresa Pereira, 2021, Master's thesis in Medicinal Chemistry, University of Minho, Portugal.

O 3

# Construction of a medical device for blood purification based on biocompatible materials conjugated with antimicrobial peptides

Alessandro Pini

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Sepsis is a complex life-threatening clinical condition associated with significant morbidity and mortality. It is due to organ dysfunction caused by a dysregulated host response to infection.
A novel medical device for the purification of blood of sepsis patients is under development in collaboration among University of Siena, SetLance srl and Medica Spa, two private companies involved in peptide science and biomaterial-based devices, respectively. The device is based on biocompatible matrices derivatized with the antimicrobial peptide SET-M33[1-3], already reported in the past for its strong activity for binding LPS and LTA and killing bacteria. The device is thought to be applied in an extracorporeal circulation system (Fig.1) where the peptide-based cartridge is used as sorbent decoy to eliminate toxins and bacteria from the blood of sepsis patients in Intensive Care Units.

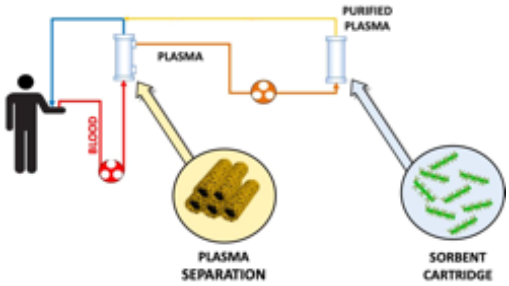


Figure 1. Schematic representation of an extracorporeal circulation system where a sorbent cartridge containing peptides able to retain toxin and bacteria is used for blood purification.

The peculiar features of the peptide SET-M33 renders this device the first tool in development able to remove, at the same moment, bacterial toxins such as LPS and LTA, the major triggers of sepsis onset, and living Gram-negative and Gram-positive bacteria.
Description of the conjugation of biocompatible materials with the peptide and the efficacy of the prototype device, along with the onset of preclinical development phase will be reported.

*References*

1. A. Pini et al. FASEB J. (2010), 24, 1015.
2. J. Brunetti et al. Sci Reports. (2016), 6, 26077.
3. L. Cresti et al., Sci Reports (2022), 12, 19294.

O 4

# Designing a Peptide Hydrogel for Cancer Early Detection

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3. School of Pharmacy, University of Manchester, Stopford Building, Oxford Road, Manchester, M13 9PT, UK

Cancer early detection (CED) is pivotal to patient survival. The small non-coding nucleic acid sequences, microRNA (miRNA), are a captivating molecular target for cancer early detection. miRNA is dysregulated during the early stages of cancer[1], and is found in stable amounts in blood plasma and serum. Therefore, a minimally invasive liquid biopsy-screening device would allow for point-of-care diagnostics. Current miRNA detection methods are cumbersome and lack reproducibility. To overcome these challenges, we aim to develop a new diagnostic platform using a functional 3D peptide hydrogel for sequence-specific[2], PCR-free, fluorescent detection of miRNAs in a "one-pot" assay.

Using a split probe FRET pair in an anti-parallel β-sheet peptide hydrogel, we can formulate a system that allows complementary strands of cancer miRNA biomarkers to be identified via fluorescence. Diffusion characteristics were evaluated via plate reader and Fluoroblok well insert. Cell culture of Panc-1 and MIA PaCa-2 in 2D and 3D forms was undertaken to validate the sensor.

Four de novo designed self-assemble peptide hydrogels (SAPH) were tested to understand the diffusion characteristics of miRNA and select a system that allows fast trapping and detection of miRNA. It was found that positively charged hydrogels promoted miRNA trapping. The mesh size of the hydrogel used (<40nm) provided a filter to avoid interference with cell debris. Consistent high quenching was observed in negative hydrogels, achieving a low limit of detection that is on par with other nucleic acid detection methods. The biocompatibility of the SAPH provides a 3D platform for cancer cell culture. The SAPH are an extremely versatile material, with the potential to harbor fluorescent properties, for biosensor application in CED.

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*References*

1. Kosaka et al. "Circulating microRNA in body fluid..." Cancer science 101.10 (2010): 2087-2092.
2. Yousaf, et al. "Sequence-Specific Detection of Unlabeled Nucleic Acid Biomarkers Using a "One-Pot" 3D Molecular Sensor." Analytical chemistry 91.15 (2019): 10016-10025.

O 5

# Supramolecular assemblies of histidine-rich designer peptides with multiple biological functions

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A family of designed histidine-rich peptides will be presented which can assemble in a number of different aggregation states. Interestingly, whereas sequences that occur in soluble form exhibit considerable antimicrobial activities, nucleic acid transfection is best by peptides that form large complexes of tuneable size with nucleic acids (DNA, siRNA, mRNA…) and these also exhibit strong cell penetrating activities for large proteins, peptide vaccines, adeno associated viruses and nanodots. Due to the presence of four histidines their membrane interactions are strongly pH dependent. The delivery of cargo by these peptides is complex, involving many steps, which we investigated on a structural and biophysical level.

More recently, vectofusin-1, a member of the same family of LAH4 peptides has been shown to spontaneously self-assemble into helical oligomers, spherical aggregates, that further assemble into annular and extended nanofibrils and hydrogels as a function of phosphate concentration and in a pH-dependent manner. This bears considerable interest for the design of biomaterials.

Importantly, the peptide has a strong capacity to enhance the gene transfer by lentiviral vectors into the cell interior. Thereby, the fibres formed by this relatively short sequence have therapeutic applications ranging from monogenic and infectious diseases to cancer, by enhancing transduction levels of target cells and reducing the amount of lentivirus for greater safety and reduced costs. Vectofusin-1 associates with viral particles and promotes the entry of several retroviral pseudotypes into target cells when added to the culture medium, without cytotoxicity. The vectofusin-1 fibrils have a unique coiled-coil α-helical structure whereas most other viral transduction enhancers form β-amyloid fibrils and are investigated by solid-state NMR and other biophysical approaches. Our observations define vectofusin-1 as a member of a new class of α-helical lentiviral transduction enhancers. Its coiled-coil fibril formation is reversible which bears considerable advantages in handling the peptide in conditions of gene therapy protocols.

*References*

1. Lointier, M., Dussouillez, C., Glattard, E., Kichler, A., and Bechinger, B., Toxins, 13, 363 (2021)
2. Lointier, M., Aisenbrey, C., Marquette, A., Kichler, A., Bechinger, B., BBA 1862(8):183212 (2020)
3. Aisenbrey, C., Douat, C., Kichler, A., Guichard, G., Bechinger, B., J. Phys Chem B 124:4476 (2020)
4. Vermeer, L.S., et al. Acta Biomoaterialia 64, 259 (2017)
5. Moulay, G., et al. J. Pep. Scie. 23, 320 (2017)

O 6

# Cysteinyldopa as a key connectivity for polymerizing peptides to artificial mussel glue proteins

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Bio-inspiration has emerged as one of the key strategies for the design of advanced materials.1 For instance, the field of underwater adhesive engineering was inspired by marine mussels, resulting in a rich platform of mussel-glue inspired polymers.2 Meanwhile, L-dihydroxyphenylalanine (L-Dopa) functionalities can be enzymatically generated on demand,3 enzymatically activatable adhesives can be specifically selected by combinatorial means to bind to surfaces of interest3,4 and the binding secrets of these mussel-glue-mimetic peptides were revealed in divergent-convergent analysis efforts.5

Here, we summarize work going beyond these adhesives, by exploiting one of the follow-up chemical pathways of Dopa moieties to realize a generic polyaddition route. The thiol-quinone Michael-addition, enabled polymerization of peptides or fully synthetic analogs and proved to lead to artificial mussel- glue polymers, which exhibited thiol-catechol-connectivities (TCCs).6 The route was capable to polymerize minimal peptides7 as well as peptide sequences integrating complex functions, such as activatable β-sheet domains.8 Thus, the adhesion properties of TTC functionalities were combined with mechanisms to activate cohesion by pH-changes or presence of Zn-ions to highlight the potentials for next generation under water glues.8-9

Ultimately, a generic platform of TCC-polymers was established by exploiting on the one hand commodity monomers to address ease of scale up.10-11 On the other hand, the robust chemistry proved to allow the use of various bio-based multi-phenols as well as sustainable waste materials to synthesize adhesives for underwater applications.

*References*

1. Dujardin E., Mann S. Adv. Mater. 2002,14,775.
2. Lee H., Messersmith P.B., et al. Nature 2007,448,338.
3. Wilke P., Börner H. G., et al. J. Am. Chem. Soc. 2014,136,12667.
4. Juds C., Börner H. G., et al. J. Am. Chem. Soc. 2020,142,10624.
5. Venkatareddy N. L., Börner H. G., et al. Adv. Mater. Interfaces 2019,6,1900501.
6. Horsch J., Börner H. G., et al. Angew. Chem. 2018,57,15728.
7. Kohn J. M., Börner H. G., et al. Macromol. Rapid Commun. 2020,41,1900431.
8. Arias S., Börner H. G., et al. Angew. Chem. 2020,59,18495.
9. Arias S., Börner H. G., et al. Soft Matter 2021,17,2028.
10. KrügerJ.M.,BörnerH.G.Angew.Chem.2021,60,6408.
11. KrügerJ.M.,BörnerH.G.;etal.,Macromolecules2022,55,989.

O 7

# Mineral: Peptide interactions: using our understanding of molecular interactions to develop a time efficient biopanning approach

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Keywords: phage display, biopanning, peptide, silica

Minerals and biomolecules can interact via a range of binding modalities from electrostatic interactions, though hydrogen bonding to hydrophobic interactions and van der Waals interactions. These binding possibilities lead to a range of biomolecule sequences that can interact with a particular mineral. From our understanding of peptide-mineral interactions gained by extensive, detailed experimental and computational studies we have developed an optimised biopanning approach, utilising multiple chemical eluants that allows the identification and recovery of a wide mineral binder pool in just one or two biopanning rounds. In this presentation I will describe our current understanding of peptide silica interactions for silica and show how this led to the development of the optimised biopanning approach.

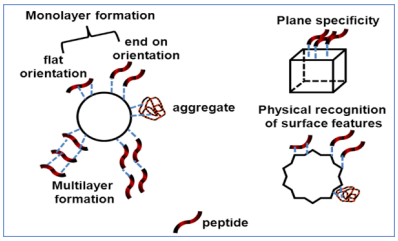


Figure 1. possible binding modes for biomolecule-material interactions

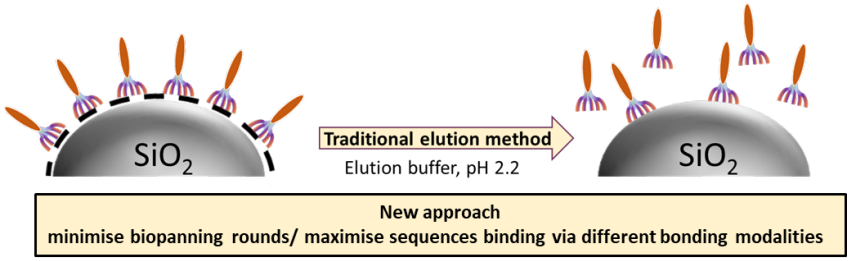


Figure 2. Traditional vs. new biopanning/ elution approach

O 8

# Biomolecules for non-biological things: Peptide 'Bundlemer' design for model colloidal particle creation and hierarchical solution assembly or polymerization

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A new solution assembled system comprised of theoretically designed coiled coil bundle motifs, also known as 'bundlemers', will be introduced as model colloidal particle systems[1] as well as super monomers for covalent assembly into polymers[2]. The molecules and nanostructures are not natural sequences and provide opportunity for controlled solution behavior and arbitrary nanostructure creation with peptides. With control of the display of all amino acid side chains (both natural and non-natural) throughout the peptide bundles, desired physical and covalent (through appropriate 'click' chemistry) interactions have been designed to control interparticle interactions in solution of both individual bundlemer particles as well as polymers of connected bundlemers.[2] One-dimensional polymer nanostructures span exotically rigid rod polymers that produce liquid crystal phases to semi-flexible chains, the flexibility of which are controlled by the interbundle linking chemistry. The bundlemer particles can be responsive to temperature since the individual bundle building blocks are physically stabilized coiled coil bundles that can be melted and reformed with temperature. They are also responsive to salt and pH since computational design is used to design bundlemers with different net charged character in order to manipulate their interactions in solution.[3] Finally, other patches of interaction (e.g., hydrophobic) can be designed on the surface of the particles to dictate their interaction in solution and the control of particle assembly (e.g., crystalline lattice vs. amorphous aggregate). Polymers made from bundlemers can be used in the creation of fiber materials.[4] Included in the discussion will be molecule design, hierarchical assembly pathway design and control, and the characterization of solution nanostructure via electron microscopy, neutron and x-ray scattering, and rheological measurements.

*References*

1. N. Sinha, R. Guo, R. Misra, J. Fagan, A. Faraone, C.J. Kloxin, J.G. Saven, G.V. Jensen, D.J. Pochan. J. Coll. Int. Sci. (2022), 606, 1974.
2. D.D. Wu, N. Sinha, J.Y. Lee, Y. Tian, H. Zhang, B. Sutherland, C.J. Kloxin, J.G. Saven, D.J. Pochan. Nature (2019), 574, 658.
3. R. Guo, N. Sinha, , R. Misra, Y. Tang, M. Langenstein, K. Kim, J. Fagan, C.J. Kloxin, D.J. Pochan, J.G. Saven, J. Coll. Int. Sci. (2022), 606, 1974.
4. K. Kim, C.J. Kloxin, J.G. Saven, D.J. Pochan. ACS Applied Materials and Interfaces, (2021), 13, 26339.

O 9

# Peptides and polysaccharides: towards an antibacterial and collagen regenerative textile for wound treatment

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In recent years, the need to create textile materials with distinctive properties to achieve protection in the prevention from pathogens has grown significantly. In this regard, endowment of a textile material with biologically active compounds (antibacterial, antiviral…) would be useful for many applications. Our work presents a study on the modification of cotton with peptides. To this end, enzymatic oxidation of cellulose was successfully applied using a method that allowed reuse of the oxidation solution multiple times. Then, model peptides have been conjugated to cotton via either thiazolidine or oxime chemoselective ligations and the stability of the two chemical bonds has been compared [1].

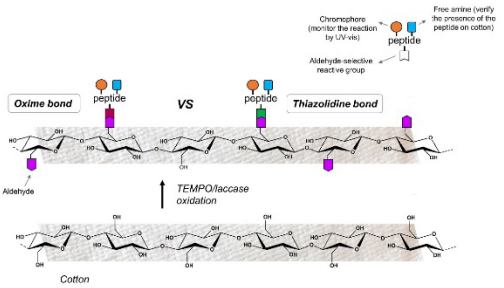


Figure1. Cellulose functionalization

This approach has been extended to other natural polysaccharides, which are characterised by low cost, non-toxicity, biocompatibility, and biodegradability, which make them candidates for the production of sustainable materials. Peptides capable of fighting bacterial infections or repairing skin lesions were combined with carbohydrates, without affecting the biological properties of the peptides [2].
In addition, we applied the electrospinning technique that allows the tissues obtained to be characterised by good haemostasis, absorbability, and oxygen permeability [3], useful for the preparation of plasters or gauze. Special attention was paid to the use of water and green solvents in the electrospinning process, in order to make the method of general interest for the modern biomedical industry.

*References*

1. Albini, F., et al., Cellulose (2023), 30, 5573.
2. Biondi, B., et al. (2023) Submitted
3. Teixeira, M.A., et al., Nanomaterials (2020), 10, 557.

O 10

# Peptide Nanophotonics

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Fluorescence (FL) is a basic optical phenomenon that is widely used inmolecular spectroscopy, high-resolution microscopy, and bioimaging. It has exciting and emission spectra defined by an electronic structure of particular fluorochrome molecules. This FL phenomenon is observed both in biological amyloid and bioinspired amyloidogenic assemblies, folded into specific biomolecular β-sheet secondary structure. A fine mechanism of this fold-sensitive FL effect, arising at the earliest stages of seeding and nucleation of β-sheet nanofibers, and its growth under thermally activated conformational refolding of helical to β-sheet state in bioinspired ultrashort peptide structures are studied. This FL effect demonstrates different properties compared to the classical FL from non-folded biomolecules or molecular dyes. Its excitation and emission spectra are similar in any β-sheet peptide/protein assemblies irrespective of their biochemical composition, primary structure, and origin. It is shown that this fold-sensitive FL effect exhibits a wideband spectrum, covering the entire visible region (400–650 nm), high quantum yield, and tunable FL wavelength. In this work, a new generation of bioinspired optical fibers is proposed. Developed amyloidogenic peptide fibrillary structures with tailored β-sheet conformation exhibit unique optical properties of full overlapping of broadband visible fluorescence (FL) and optical absorption spectra. This study reports on unexpected lossless propagation of the FL light along 100 μm length β-sheet microfibers. It is shown that the found FL long-distance lossless radiative energy transport occurs due to highly effective FL photon recycling phenomenon supported by FL zero Stokes shift and high quantum yield. This new non-Beer–Lambert FL lossless propagation mechanism is observed in very thin ≈1 μm diameter fibers, providing a single/few-mode waveguiding regime. The developed model and computer simulations, based on the finite difference time domain method, are consistent with the experimental results. Fabricated peptide FL fiber probes permit delivering intensity-modulated FL signal with selected wavelength over the whole visible spectrum in wide modulation frequencies range.

*Reference*

1. B.Apter, I.Lapsker, A. Inberg, G.Rosenman, Adv. Optical Mater. 2102342 (2021)

O 11

# Exploring Properties of Helical Oligourea Foldamers

S. Sęk[1], K. Pułka-Ziach[2], J. Juhaniewicz-Dębińska[1], P. Bachurska[1], A. K. Puszko[2] and D. Dziubak[1]

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Helical N,N-linked oligourea foldamers are a versatile class of compounds mimicking peptides. The synthesis of oligoureas with urea-based monomers allows for the incorporation of side chains similar to natural amino acids, resulting in remarkable robustness and tunability of these compounds. Unlike polypeptides, the folding process of oligoureas is independent of the side chain composition, making them highly adaptable. The sequence of just four residues is sufficient for complete helical turn formation in oligoureas. These foldamers adopt a 2.5-helix structure stabilized by 3-centered hydrogen bonding. These exceptional characteristics make oligoureas highly attractive for the design of functional materials. Our research group has focused on investigating the properties of molecular films composed of oligourea foldamers. We observed that the conductance of these films is thickness-dependent and obeys either a tunneling or hopping mechanism depending on the length of the molecules forming the films. Additionally, we discovered an intriguing property of oligoureas—the directional dependence of electron transport—which results in current rectification in the current-voltage characteristic. The rectification ratio exceeded 12, surpassing that of helical peptides of similar length by a factor of three. This effect arises from the alignment of a large dipole moment along the molecular axis and from the stability of the helical structure.

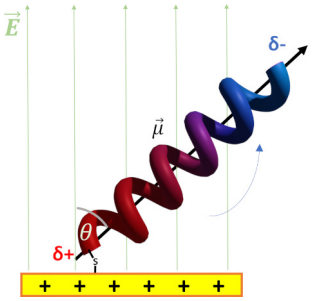


Figure 1. Helical oligourea foldamers, when adsorbed onto conductive surfaces, exhibit a responsive behavior to applied electric fields by altering their orientation.

Furthermore, we demonstrated that the direction of the dipole moment significantly influences the electron transfer behavior of oligourea monolayers deposited on gold electrodes. The dynamics of the adsorbed molecules on the electrode surface are governed by the charge distribution along the molecule, which leads to electric field-induced effects. The extent of molecular motion affects the kinetics of electron transfer between monolayer-confined ferrocene redox sites and the electrode. These findings emphasize the crucial role of molecular dynamics in long-range electron transport facilitated by molecular bridges. Our results indicate also that molecular layers of oligourea helical foldamers have the potential to be employed as materials with stimuli-responsive properties. For example, they can act as molecular lifters triggered by an electric field. These characteristics open up possibilities for applications in molecular actuation and switching systems. Moreover, appropriately modified oligoureas also exhibit significant antibacterial activity, thereby expanding the potential utility of oligoureas in various fields.

*References*

1. K. Pułka-Ziach, A.K. Puszko, J. Juhaniewicz-Dębińska, S. Sęk, J. Phys. Chem. C (2019), 123, 1136.
2. D. Dziubak, K. Pułka-Ziach, S. Sęk, J. Phys. Chem. C (2020), 124, 17916.
3. D. Dziubak, A.K. Puszko, P. Bachurska, K. Pułka-Ziach, S. Sęk Electrochim. Acta (2022), 403, 139634.

O 12

# Peptaibol-decorated gold nanoparticles self-assembly guides their cell internalization

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Trichogin is a naturally-occurring, cytotoxic peptaibol produced by a fungus of the genus Trichoderma. Several trichogin analogs are endowed with antitumor activity. A few years ago, we identified two trichogin analogs displaying membrane activity devoid of cytotoxicity [1]. Here, we describe the results of our study on their potential as targeting agents in cancer therapy. Circular dichroism (CD) analysis carried out in the presence of breast cells showed the presence of the peptide helical structure, thus proving the stability of the two analogs in the biological environment. Gold nanoparticles (GNPs) decorated with the thiol-containing versions of those analogs were produced and characterized. By a combination of microscopy investigations and intracellular uptake assays both in cancer and in the corresponding normal cell lines, we could draw a correlation between peptide-GNPs propensity to self-assembly and their internalization, finding that peptide decoration can increase GNP uptake by cancer cells while at the same time limiting it by normal cells [2].

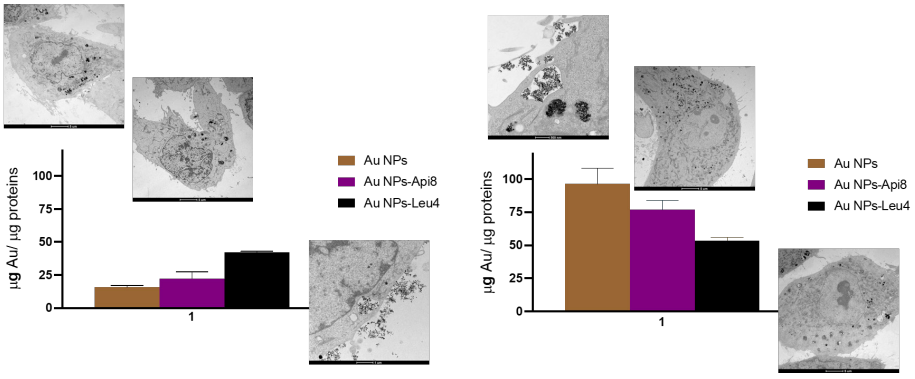


Figure 1. Uptake of GNPs conjugated with peptaibols in mammary cells. Left, in a cancer cell line. Right, in the corresponding normal cell line. Atomic Absorption spectroscopy, 24 h of incubation.

*References*

1. A. Dalzini, C. Bergamini, B. Biondi, M. De Zotti, G. Panighel, R. Fato, C. Peggion, M. Bortolus, A.L. Maniero Sci. Rep. (2016), 6, 24000.
2. F. Moret, L. Menilli, C. Milani, G. Di Cintio, C. Dalla Torre, V. Amendola, M. De Zotti, Int. J. Mol. Sci. (2023), 24, 5537.

O 13

# Zinc Oxide-Based Electrochemical Immunosensor for Evaluating Antibody-Mediated Immunity to Wild-Type and Gamma SARS-CoV-2 Strains Induced by Vaccination

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The evaluation of serological responses to COVID-19 is crucial for population-level surveillance, the development of new vaccines, and the assessment of different immunization programs. The research and development of advanced point-of-care testing technologies are essential for improving immunity assessment, particularly for SARS-CoV-2 variants that partially evade vaccine-induced immune responses. In this study, we employed an impedimetric biosensor based on the immobilization of the recombinant trimeric wild-type Spike Protein (S-protein) on zinc oxide nanorods (ZnONRs) for serological evaluation.[1] We successfully demonstrated its applicability using serum samples from individuals vaccinated with Spike-based COVID-19 vaccines, namely ChAdOx1-S (Oxford-AstraZeneca) and BNT162b2 (Pfizer-BioNTech). Overall, the ZnONRs/Spike-modified electrode exhibited high sensitivity for both vaccines, showcasing its excellent potential as a tool for assessing and monitoring seroprevalence in the population. A further advancement of this technology was achieved by functionalizing the ZnO immunosensor with the S-protein from the Gamma variant (P.1 lineage).[2] We obtained robust serological responses against samples from vaccinated individuals, demonstrating outstanding performance. The data from the ZnONRs/Spike immunosensor revealed that individuals vaccinated with Oxford-AstraZeneca exhibited significantly lower antibody-mediated immunity against the Gamma variant compared to those who received the Pfizer-BioNTech vaccine, highlighting the tremendous potential of this point-of-care technology in evaluating vaccine-induced humoral immunity against emerging SARS-CoV-2 strains.

*References*

1. F.A. Nunez, A.C.H. Castro, V.L. de Oliveira, A.C. Lima, J.R. Oliveira, G.X. de Medeiros, G.L. Sasahara, K.S. Santos, A.J.C. Lanfredi, W.A. Alves. ACS Biomater. Sci. Eng. 2023, 9, 458.
2. F.A. Nunez, A.C.H. Castro, I.P. Daher, E. Cunha-Neto, J. Kalil, S.B. Boscardin, A.J.C. Lanfredi, V.L. de Oliveira, W.A. Alves. Biosensors 2023, 13, 371.

O 14

# Hierarchical peptide materials from agricultural resources

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Protein-derived materials could play important roles in the development towards a more sustainable society, as proteins are renewable resources and biodegradable. Moreover, protein materials can provide unique functionality in e.g. water remediation or bioelectronics devices and constitute a fundamental building block in food. Natural occurring protein materials, such as silk, have served as inspiration for manmade structures for long time but the challenge to produce synthetic materials with comparable properties from biobased resources remains. The key to achieve this is to gain control over the assembly of hierarchical structures from the protein building blocks.[1] Amyloid-like protein nanofibrils (PNFs) have emerged as a promising foundation for the synthesis of novel bio-based materials for a variety of applications. Such nanofibrils are formed through self-assembly and have mechanical properties comparable to silk. We have demonstrated the formation of PNFs from a range of agricultural resources[2] and how these fibrils can be assembled into ordered, hierarchical structures.[3-5] A key step in the assembly of large and complex proteins into PNFs is the hydrolytic cleavage of the polypeptide chain into smaller peptides, which occur spontaneously at low pH and high temperature. Our work suggests that this process also dictates the morphology of the PNFs that are formed and thereby an opportunity to control the material properties.[6] Access to morphological distinct fibrils allows us to explore the relationships between nanoscale- and macroscale structures in PNF-based fibres, films and foams. We have also developed an approach to create cross-linked protein materials with very good thermal- and chemical stability without any non-protein additives.[7]

*References*

1. Lendel, C. & Solin, N. RSC Adv. (2021), 11, 39188.
2. Herneke, A., Lendel, C., Johansson, D., Newson, W., Hedenqvist, M. S., Karkehabadi, S., Jonsson, D. & Langton, M. ACS Food Sci. & Technol. (2021), 1, 854.
3. Kamada, A., Mittal, N., Söderberg, L. D., Ingverud, T., Ohm, W., Roth, S. V. Lundell, F. & Lendel, C. Proc. Natl. Acad. Sci. USA. (2017), 114 1232.
4. Kamada, A., Herneke, A., Lopez-Sanchez, P., Harder, C., Ornithopoulou, E., Wu, Q., Wei, X., Schwartzkopf, M., Müller-Buschbaum, P., Roth, S. V., Hedenqvist, M., S., Langton, M. & Lendel, C. Nanoscale (2022), 14, 2502.
5. Ye, X., Capezza, A. J., Davoodi, S., Wei, X.-F., Andersson, R. L., Chumakov, A., Roth, S. V., Langton, M., Lundell, F., Hedenqvist, M. S. & Lendel, C. ACS Nano (2022), 16, 12471.
6. Ye, X., Hedenqvist, M. S., Langton, M. & Lendel, C. RSC Adv. (2018), 8: 6915.
7. Ye, X., Capezza, A., Gowda, V., Olsson, R. T., Lendel, C. & Hedenqvist, M. S. Adv. Sustain. Syst. (2021), 2100063.

O 15

# Biological Refolding and Fold-Sensitive Physical Properties

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Peptide and protein biomolecules, folded into two fundamentally different either a-helical or b-sheet conformations, carry out dissimilar biological functions. In living organisms a-helical secondary structure is adopted by different types of proteins such as myoglobin, keratin, collagen and more. They can be found in diverse biological tissues of muscle, bone, cartilage and more. Biological functions of b-sheet peptide/protein structures are different and associated with a wide range of human mental amyloid diseases such as Alzheimer, Parkinson, and more. The fundamental basis of these diseases is refolding of natively soluble a-helical amyloid proteins into solid-state b-sheet fibrillary structures.

In this Lecture we show that these two structural conformations, native (a-helix) and b-sheet, exhibit exclusive and different sets of fold-sensitive physical properties which are surprisingly similar in both biological and bioinspired materials. Native (helix-like) self-assembled fold having asymmetric structure, demonstrates ferroelectric-like pyroelectric, piezoelectric, nonlinear optical and electrooptical effects. b-sheet peptides/proteins structures acquire unique visible fluorescence (FL), and reveal a new property of lossless FL photonic transport followed by a long-range FL waveguiding in amyloidogenic fibers. Applied thermally-mediated refolding native-to-b-sheet allows to observe in details adoption, disappearance, and switching of the revealed physical properties in each fold and study dynamics of all critical stages of refolding from the metastable (native) helix-like conformation via intermediate disordered state to stable b-sheet fibrillary ordering. In the intermediate state appearance of the visible FL provides imaging, monitoring and direct observation of the early stages of seeding and nucleation of b-sheet fibrils. Found diverse fold-sensitive physical properties give a new insight in biological refolding processes and paves the way for development of advanced physical methods of folds recognition, bio-nano-imaging, peptide/protein nanophotonics towards new diagnostic methods of neurodegenerative diseases.

O 16

# The peptide is the key: Mussel-inspired pressure-sensitive adhesives (mPSA)

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Pressure-sensitive adhesives (PSAs) are a key technology platform with application in modern electronic devices like smartphones or electric cars. However, many polymeric adhesives are only available from petrochemical feedstocks and lack in aspects like sustainability, biodegradability, and universal adhesion. Marine mussels have often been cited as an inspiration for alternative adhesives. In their mussel foot proteins (MFPs), the amino acid L-3,4-dihydroxyphenyl-alanine (DOPA) has been identified as one of the key residues responsible for the strong adhesion to a variety of surfaces exhibited by the mussel even under seawater conditions.[1] Therefore, mussel-inspired polymers are being explored as biomimetic adhesives with the potential for adaptive adhesiveness.

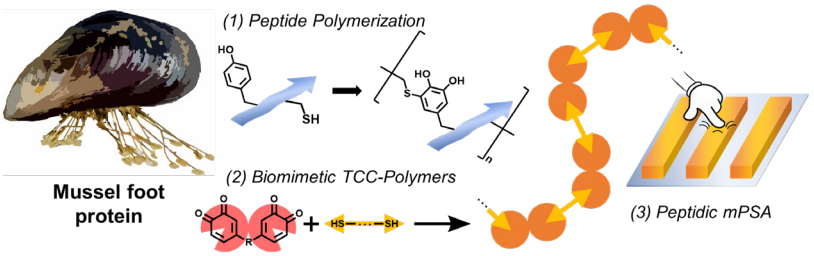


Figure 1. mPSAs are developed by abstraction of peptide sequences and chemical motifs from mussel foot proteins.

This concept was demonstrated b y polymerizing a decapeptide abstracted from MFP-1, which could be activated by tyrosinase and polymerized via cysteinyl-DOPA linkages.[2] Such polymers showed adhesion energies exceeding those of their MFP-1 role model. The cysteinyl-DOPA linkages were abstracted to create biomimetic thiol-catechol-connectivities (TCCs) to obtain TCC-polymers, advancing such adhesives toward an industrially relevant platform.[3] Recently, both approaches have been combined by incorporating a tailored peptide as a building block in TCC-polymer networks. These show permanent tack and act as mPSA with promising adhesive properties.

*References*

1. J. H. Waiter, Ann. N. Y. Acad. Sci. (1999) 875, 301.
2. J. Horsch, H. G. Börner et al., Angew. Chem. Int. Ed. (2018) 57,15728.
3. J. M. Krüger, H. G. Börner, Angew. Chem. Int. Ed. (2021) 60, 6408.

O 17

# Entropically-Driven Co-assembly of L-Histidine and L-Phenylalanine to Form Supramolecular Materials

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Molecular self- and co-assembly allow the formation of diverse and well-defined supramolecular structures with notable physical properties.[1,2] Among the associating molecules, amino acids are especially attractive due to their inherent biocompatibility and simplicity.[1,2] The biologically active enantiomer of L-histidine (L-His) plays structural and functional roles in proteins but does not self-assemble to form discrete nanostructures. In order to expand the structural space to include L-His-containing materials, we explored the co-assembly of L-His with all aromatic amino acids, including phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp), all in both enantiomeric forms. [3] In contrast to pristine L-His, the combination of this building block with all aromatic amino acids resulted in distinct morphologies including fibers, rods, and flake-like structures. Electrospray ionization mass spectrometry (ESI-MS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and isothermal titration calorimetry (ITC) suggested the highest affinity between L-His and L-Phe where the formation of co-assembled structures was driven by entropy. In accordance, among all the combinations, the co-assembly of L-His and L-Phe produced single crystals. The structure revealed the formation of a 3D network with nanocavities stabilized by hydrogen bonding interactions between the two amino acids, further confirming the co-assembly. In the present study, using the co-assembly approach we expanded the field of amino acid nanomaterials and showed the ability to obtain discrete supramolecular nanostructures containing L-His based on its specific interactions with L-Phe, useful in the various technological applications.

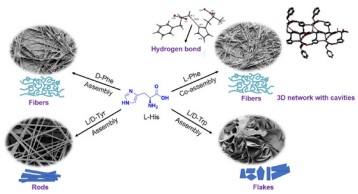


Figure 1. Co-assembly of L-His with all the coded aromatic amino acids in both enantiomeric forms.

*References*

1. L. Adler-Abramovich, L. Vaks, O. Carny, D. Trudler, A. Magno, A. Caflisch, D. Frenkel, E. Gazit, Nat. Chem. Biol. (2012), 8, 701.

2. O. S. Tiwari, S. Rencus-Lazar, E. Gazit, ChemNanoMat (2022), 8, e202200055.

3. O. S. Tiwari, R. Aizen, M. Meli, G. Colombo, L. J. W. Shimon, N. Tal, E. Gazit, ACS Nano (2023) 17, 4, 3506.

O 18

# Peptide-Hyaluranon Bioconjugates designed to improve bioactivity of electrospun scaffolds for tissue engineering

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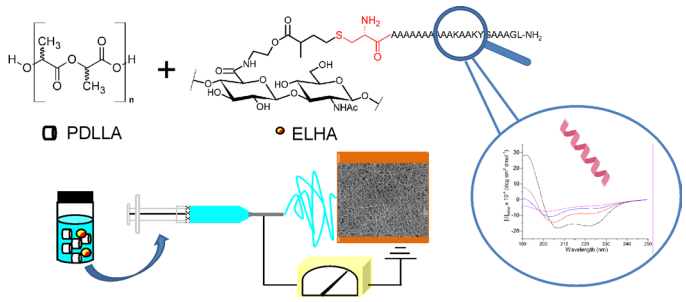
Hyaluronic acid or hyaluronan (HA) and elastin-inspired peptides (EL) have been widely recognized as bioinspired materials useful in biomedical applications. The present work aims to produce electrospun scaffolds as wound dressing materials which would benefit from the synergic action of the bioactivity of elastin peptides and the regenerative properties of hyaluronic acid. Taking advantage of thiol-ene chemistry, bioactive elastin peptides can be successfully conjugated to methacrylated hyaluronic acid and electrospun together with poly-D,L-lactide (PDLLA)[1].
The conformational studies carried out by Circular Dichroism (CD) on the bioconjugated derivatives confirmed the preservation of the peptide secondary structure after conjugation while Scanning Electron Microscopy (SEM) revealed the supramolecular structure of the electrospun scaffolds.
Moreover, no cytotoxic activity on dermal fibroblast cells was observed as preliminary evaluated.




Overall, the study demonstrates that the bioconjugation of hyaluronic acid with the elastin peptide improved the electrospinning processability with improved characteristics in terms of morphology of the final scaffolds and biocompatibility.

*Reference*

1. A. Laezza, A. Pepe, B. Bochicchio. Chem. Eur. J. (2022), e202201959.

O 19

# New synthetic peptides with potential applications in medicine and biotechnology

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Due to the modern way of living and increased life expectancy, cancer, neurological diseases, bacterial and viral infections, among others, must be treated [1,2]. Antimicrobial peptides (AMPs) are peptides that are capable of destroying microorganisms. They can be used to treat cancer, genetic disorders, cardiovascular diseases, infectious diseases, and inflammatory diseases.

Using solid-phase peptide synthesis (SPPS) and the labile fluorenylmethyloxycarbonyl group (Fmoc chemistry) strategy, six newly designed buforine derivatives were synthesized. These were analyzed using MALDI-TOF-MS, and their purity was evaluated using analytical HPLC. By calculating the minimum inhibitory concentration (MIC), the antimicrobial activity of the six newly-obtained peptides was determined. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of the peptides in two bacteria (Staphylococcus aureus and Klebsiella pneumoniae) according to Wiegand et al.'s (2008) protocol [3].The peptides were effective against the microbes tested. Microscopy and Alamar Blue assays were used to measure cytotoxicity, cell proliferation, and cellular metabolic activity of the peptides in MDA-MB-231 and MCF-7 breast cancer cells, and in mesenchymal stem cells.

To expedite the development of peptide-based drug systems, it is essential to design peptides that can, among other things, traverse cell membranes or biological barriers, remain in circulation for longer time, and are neither toxic nor immunogenic to humans.

*Acknowledgements*

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*References*

1. D.M.Copolovici, K. Langel, E. Eriste, Ü. Langel. (2014). ACS Nano, 8(3): 1972.
2. D.M. Copolovici, A.I. Lupitu (2018) Peptide-based systems for drug delivery in Peptide Applications in Biomedicine, Biotechnology and Bioengineering, Edited by Sotirios Koutsopoulos, Woodhead Publishing Series in Biomaterials, An imprint of Elsevier, ISBN: 978-0-08-100-742-6 (online), 409.
3. I. Wiegand, K. Hilpert, R.E.W. Hancock, Nature Protocols, (2008), 3, 163.

O 20

# Fibers formation upon self-assembly of PNA-peptide conjugates

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Self-assembly of biomolecules such as peptides or nucleic acids is dictated by interactions between the backbone and side chains of peptides or nucleobases in nucleic acids. Hybrid molecules containing both peptides and nucleic acids could exploit multiple hydrogen bond donor/acceptors and hydrophobic moieties on the peptide or nucleic acid to assemble. Indeed, not all the mentioned interactions contribute equally to promote assembly and the steric hindrance of amino-acids and nucleobases affects the packing ability of these molecules. In order to unveil the general guidelines to produce homogeneous ordered self-assembled structures we and others have investigated the self-assembly of a number of Peptide Nucleic Acid (PNA)-peptide conjugates, containing PNA monomers or dimers conjugated to short peptides. [1-3] The peptide moiety seems to drive aggregation; the structure and morphology of aggregates change depending on the composition of the hybrid. In this work we report our recent studies on conjugates of PNA dimers to hydrophobic peptides (Figure 1). Spectroscopic studies aimed at deriving information on the molecular structure and fluorescence properties of the assembled systems are described. Atomic force microscopy studies, demonstrating formation of fibers are reported. Finally, a structural model showing the arrangement of the building blocks in the fibers is described.

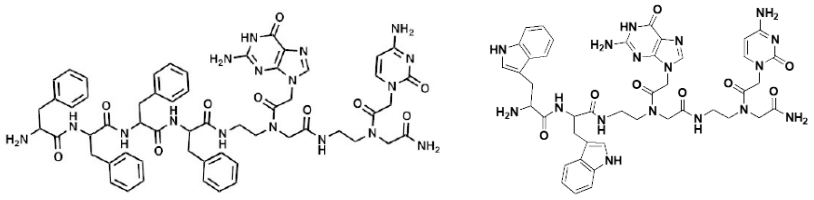

Figure 1. Chemical structure of the self-assembling PNA-peptide conjugates

*References*

1. C. Avitabile et al. Chemistry (2019) 25, 14850.
2. T. Giraud et al. Nanoscale (2921) 13, 10566.
3. C. Diaferia et al. Chemistry (2021) 27, 14307.

O 21

# Peptide nanotubes self-assembled from leucine-rich alpha helical surfactant-like peptides

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The designed arginine-rich surfactant-like peptide $R_3L_{12}$ (arginine$_3$– leucine$_{12}$) is shown to form a remarkable diversity of self-assembled nanostructures in aqueous solution, depending on pH, including nanotubes, mesh-like tubular networks in three-dimensions and square planar arrays in two-dimensions.[1] These structures are built from a-helical antiparallel coiled–coil peptide dimers arranged perpendicular to the nanotube axis, in a ‘‘cross-a’’ nanotube structure. The aggregation behaviour is rationalized based on the effects of dimensionality, and the balance of hydrophobic and electrostatic interactions. The nanotube and nanomesh structures display arginine at high density on their surfaces, which may be valuable for future applications.

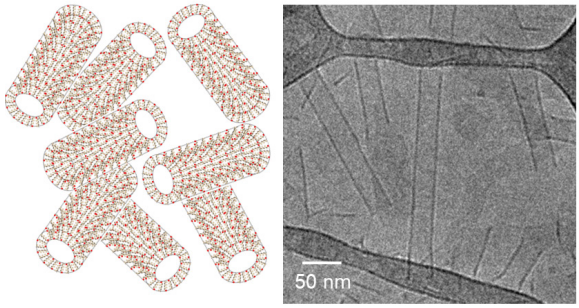


Figure 1. Cross-a $R_3L_{12}$ peptide nanotubes.

*Reference*

1. V. Castelletto, J. Seistonen, J. Ruokolainen et al Chem. Commun. (2020), 56, 11977.

O 22

# Self-assembled short peptides and pseudopeptides: balance between aromatic and ionizable residues

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Non-covalent interactions are key players in protein structure and become determinant for fundamental processes such as folding and aggregation. These interactions are mainly manifested by the amino acid side chains which respond to environmental stimuli such as pH, temperature, or salinity. Those interactions similarly affect to the self-assembly of short peptides as they behave as minimalistic protein-like materials.[1]
Here we present two families of dipeptides and pseudopeptides bearing non-polar (F) and ionizable (K, E) residues and study their aggregation behaviour under the influence of external stimuli. [2]

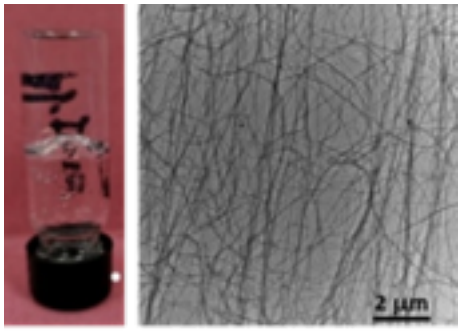


Figure 1. Macroscopic (left) and microscopic (right) appearance of a pseudopeptide-based hydrogel.

*References*

1. N. Singh, M. Kumar, J.F. Miravet, R.V. Ulijn, B. Escuder Chem. Eur. J. (2017), 23, 981.
2. R. Martí-Centelles, B. Escuder ChemNanoMat (2018), 4, 796.

O 23

# Self-assembling peptide hydrogels; the journey to commercialisation

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Over the past few decades, self-assembling peptide materials have attracted considerable attention due to their ability to form an array of nanostructures with programmable structure, function and properties. In particular, peptide fibrillar hydrogels have captured the imagination of the biomedical and clinical communities as these resulting structures mimic the cell micro-environment and they have tuneable properties to simulate the natural environment of a wide range of human tissues. Such hydrogel materials can be specifically designed and tailored to overcome the key limitations with current 3D biomaterial scaffolds for regenerative medical applications. They are 100% ethical, animal free and chemically defined, and are prepared with no batch-to-batch variability. This gives end users the confidence to achieve reproducible and reliable results every time, within the growing fields of 3D cell culture, 3D bioprinting, organoid growth, tissue regeneration and drug discovery. Such hydrogel global markets have an estimated CAGR of ~20% and are predicted to be worth ~£25bn by 2030.

Here, I will outline how we exploited a material platform technology we developed over 20 years and established the start-up company, Manchester BIOGEL Ltd, in 2013. I will highlight the key drivers for founding the company and talk through the highs, lows and key learnings made as we transitioned from academic research to a commercial venture, as well as personally pivoting from an academic to CEO mindset. Key inflection points of raising investment funds from Venture Capital, Private and Catapult Venture Funds, building the team, scaling both the technology production and commercial operation will be discussed, along with winning R&D funds to generate IP and feed the product pipeline. Throughout our journey we increased our staff count, our company footprint, regulatory compliance and revenue generation; all of which came with their own challenges. Our ten year journey culminated with the company being named as one of the Top 10 BioTech Start-Ups in Europe by Start-Up City in 2021 and winning the Most Impactful Emerging Technology Award at Lab Innovations 2021 before the technology was taken up by a third party. With current research into the application of peptide-based platforms to meet unmet clinical needs growing, the need to share learnings from commercial ventures is ever increasing. Here I hope to share such learnings with the aim to accelerate future knowledge exchange and generation of tangible commercial impact.

To achieve successful commercialization, the spin-out must address key challenges, including scalability, regulatory compliance, and market penetration. Scalability will be crucial to meet the demands of the biomedical research and pharmaceutical industries. Developing efficient and cost-effective synthesis methods, as well as establishing reliable manufacturing processes, will be paramount. Ensuring regulatory compliance is essential to navigate the complex landscape of biomedical product development. The spin-out will undertake comprehensive preclinical and clinical studies to evaluate safety, efficacy, and product performance. Collaboration with regulatory authorities from an early stage will help streamline the approval process and expedite market entry. Market penetration will require strategic partnerships with academic institutions, research organizations, and pharmaceutical companies. These collaborations can facilitate technology transfer, provide access to funding and resources, and aid in the validation of peptide hydrogels for specific applications. By establishing a university spin-out dedicated to commercializing peptide hydrogels for 3D cell culture, the research and development efforts in this field can be translated into tangible products with widespread impact. The spin-out's innovative solutions have the potential to revolutionize drug discovery, disease modeling, and tissue engineering, ultimately contributing to advancements in healthcare and regenerative medicine.

*References*

1. P. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.
2. P. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.

O 24

# Stimuli-responsive self-assemblies of synthetic and recombinant polypeptides

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Our group "Polymer Self-Assembly and Life Sciences" at LCPO focuses on the design and synthesis of amphiphilic (block) copolymers able to self-assemble in a controlled and predictable manner into functional nano- or micron-sized particles with specific morphologies (e.g., core-shell particles, worm-like micelles, polymersomes) able to mimic or interact with living systems for diverse applications (e.g., biomimicry, nanomedicine, tissue engineering). Towards this goal, our favorite polymers are those based on amino acid residues, namely protein-like polymers obtained by ring opening polymerization, or genetically engineered and produced by bacterial fermentation, two alternative approaches that can also be combined to access biocompatible, biodegradable and stimuli-responsive polymers.[1] A particular focus is dedicated to the development of synthetic methodologies to access bioactive or biomimetic self-assemblies.

In this presentation, I will specifically highlight our most recent works exploring a dual biotechnological and chemical approach, combining recombinant technologies and biosynthesis of elastin-like polypeptides (ELPs) with orthogonal bioconjugation techniques to enlarge the diversity of relevant ELP-based macromolecules for subsequent self-assembly and biological applications. (Figure 1A) Chemoselective modifications at the N-terminal chain and of ELPs or at the side chain of specific residues have in particular been explored to access stimuli-responsive glycoconjugates and multivalent nanoparticles thereof,[2] (Figure 1B) and amphiphilic lipopolypeptides self-assembling into thermally-responsive lipo-proteinosomes.[3] (Figure 1C)

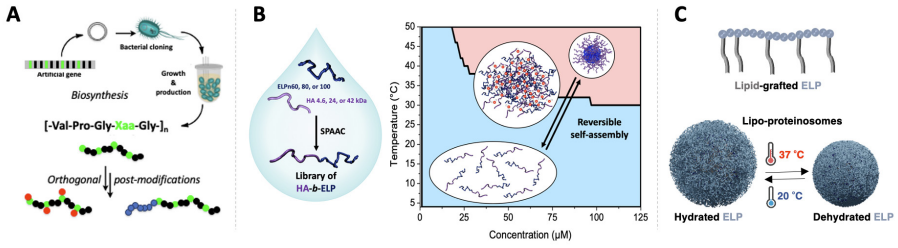


Figure 1. Orthogonal modifications of recombinant ELPs to access multivalent nanoparticles and thermo-responsive vesicles.

*References*

1. E. Garanger, S. Lecommandoux Adv. Drug Deliv. Rev. (2022), 191, 114589.
2. M. Levêque et al. Biomater. Sci. (2022), 10, 6365.
3. V. Ibrahimova et al. Angew. Chem. Int. Ed. Engl. (2021), 60, 15036.

O 25

# Enzymatically Triggered Lipid Conjugation of Membrane Active Peptides

Alexandra Iversen[1], Johanna Utterström[1], Robert Selegård[1], Daniel Aili[1]

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Liposomal drug delivery systems are widely used to improve drug pharmacokinetics, but often suffer from slow and non-targeted release of the active pharmaceutical ingredient (API). Release kinetics can be modulated using membrane-active peptides, but controlling the interactions between the peptides and lipid membranes is challenging. Here we show a novel enzyme-mediated strategy for the conjugation of a de novo designed membrane-active peptide to vesicles. The peptide is a lysine-rich 29 amino acid helical peptide that triggers lipid membrane destabilization when conjugated to vesicles as a result of peptide folding and membrane partitioning. An N-terminal cysteine enables peptide conjugation to maleimide-functionalized vesicles via Michael addition reaction. Incorporation of a cysteine-protection group, Phacm, on the thiol-moiety prevents undesired thiol-oxidation prior to conjugation. The Phacm group is removed by Penicillin G Acylase (PGA), generating a free thiol that can then react with the maleimide lipids, resulting in a peptide concentration dependent release of encapsulated cargo. The possibility to optimize peptide-lipid conjugation provides better means to tune the release process. Additionally, Phacm prevents the issue of thiol-oxidation, allowing for better means of controlling peptide surface concentration, which facilitates the development of bioresponsive liposome-based drug delivery systems.

O 26

# Design and Self-Assembly of Symmetrical Bolaamphiphiles Derived from Dehydrodipeptides: A Pathway to Tailored Nanostructures

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Bolaamphiphiles based on small peptides have received significant attention in recent years due to their unique structure and functional properties.[1] Bolaamphiphiles molecules are composed of two hydrophilic groups linked by a hydrophobic residue. Due to their amphiphilic nature, these compounds can spontaneously form various supramolecular structures, including micelles, vesicles, nanotubes, and gels. Self-assembly can be modulated by altering the length and nature of the hydrophobic and/or hydrophilic moieties, as well as the pH, temperature, and concentration of the compound.

In this work we describe the synthesis, characterization and self-assembly of several symmetrical bolaamphiphiles based on dehydrodipeptides (Figure 1). Depending on their structural characteristics, some compounds exhibited the ability to self-assemble into hydrogels while others formed different types of nanostructures. By studying the properties and behaviour of these compounds, we were able to gain insights into the structural requirements necessary to obtain specific types of nanostructures. This knowledge is crucial for designing and developing new materials with tailored properties and functions.

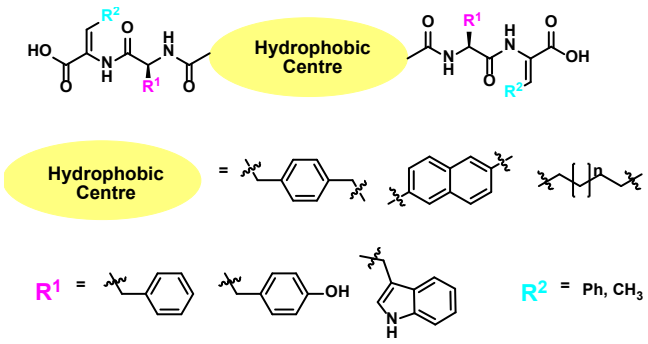


Figure 1. General structure of the symmetrical bolaamphiphiles prepared.

*Reference*

1. C. Amorim, S.R.V. Veloso, E.M.S. Castanheira, L. Hilliou, R.B. Pereira, D.M. Pereira, J.A. Martins, P.J. Jervis, P.M.T. Fereira. Gels. (2021), 7, 52.

O 27

# Functionalizations with BMP2-derived peptide for overcoming Polyetheretherketone bioinertness

L. Cassari[1], A. Zamuner[2], G.M.L. Messina[3], M. Marsotto[4], H.C. Chang[5], T. Coward[5], C. Battocchio[4], G. Iucci[4], G. Marletta[3], L. Di Silvio[5], and M. Dettin[1]

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2 University of Padova, Department of Civil, Environmental, and Architectural Engineering, 35129 - Padova;
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Polyetheretherketone (PEEK) is a substitute material for bone tissue engineering (BTE) thanks to its biocompatibility and mechanical properties.[1] However, its bio-inertness does not induce bone integration once the material is implanted. In this work, we proposed to add bioactive properties to the surfaces of 3D-printed PEEK disks by simply grafting the fragment (48-69) of BMP2 protein (GBMP1α) proved to promote osteoblast migration, proliferation, and differentiation.[2] The peptide was designed in order to explore and compare two covalent functionalization methods. In the first method, the ketones on the PEEK surface react with an amino-oxy group (Aoa) specifically introduced on the N-terminus of GBMP1α (named Aoa-x- GBMP1α, where x is a spacer).[3] The second method uses an azido group ($N_3$), introduced at peptide N-terminus (named $N_3$-x-GBMP1α), which was photoactivated to produce a nitrene through photoactivation at 254 nm.[4] The peptides were synthesized through Solid Phase Peptide Synthesis, purified and characterized with Reverse Phase-High Performance Liquid Chromatography, and identified through MALDI-TOF mass spectroscopy. X-ray photoelectron spectroscopy (XPS) measurements of functionalized surfaces confirmed the presence of nitrogen. The N/C ratio of the peptide-grafted surfaces resulted in about 5 times higher than peptide physio-adsorbed ones. Force spectroscopy showed that functionalization with Aoa-x-GBMP1α increased superficial Young's modulus while $N_3$-x-GBMP1α did not, and enhanced adhesion tip-sample forces were detected in functionalized PEEK samples versus non-functionalized samples. The functionalization enhances the biological properties of PEEK. In fact, Live and Dead™ assay showed that human osteoblasts (HOB) colonized the functionalized samples with no cytotoxicity. Moreover, AlamarBlue™ and Alizarin Red S assays showed significant increases in proliferation and calcium deposition after 1 and 21 days in culture, respectively.

*References*

1. D.F. Williams, A. McNamara, R.M. Turner. Journal of Materials Science Letters (1987), 6, 188–190.
2. E. Rampazzo et al. BBA – General Subjects (2017), 1861, 9.
3. L. Cassari et al. Biomolecules (2023), 13, 246.
4. M. Dettin et al. Journal of Peptide Science (2015), 21, 10.

O 28

# Novel Peptide as a Recognition Element for C-Reactive Protein Detection – Characterization and Application in Electrochemical Sequential Microfluidic Device

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We investigate the interactions between C-reactive protein (CRP) and new CRP binding peptide materials using experimental (biological and physicochemical) methods with the support of theoretical simulations (computational modelling analysis). Three specific CRP-binding peptides (P2, P3, P9) derived from an M13 bacteriophage have been identified using phage-display technology. The binding efficiency of the peptides exposed on phages towards the CRP protein was demonstrated via biological methods. Fibres of the selected phages/peptides interact differently due to different compositions of amino acid sequences on the exposed peptides, which was confirmed by transmission electron microscopy. Numerical and experimental studies consistently showed that the P3 peptide is the best CRP binder. A combination of theoretical and experimental methods demonstrates that identifying the best binder can be performed simply, cheaply, and fast. Such an approach has not been reported previously for peptide screening and demonstrates a new trend in science, where calculations can replace or support laborious experimental techniques. Finally, the best CRP binder – the P3 peptide – was used for CRP recognition on silicate-modified indium tin oxide-coated glass electrodes. The obtained electrodes exhibit a wide range of operation (1.0-100 μg $mL^{-1}$) with a detection limit (LOD= 3σ/S) of 0.34 μg $mL^{-1}$. The dissociation constant Kd of 35 ± 1.2 nM was evaluated from the change in the current. The selectivity of the obtained electrode was demonstrated in the presence of three interfering proteins. Moreover, the affinity of the P3 peptide towards CRP in a real setting was demonstrated. The P3 peptide was used as a recognition element in a point-of-care testing sequential microfluidic device. The device was tested with serum, plasma, and whole blood samples to validate its applicability, yielding satisfactory results and a very low limit of detection compared to an antibody-based device on the same platform. These results prove that the presented P3 peptide is a potential candidate as a receptor for CRP, which can replace specific antibodies.

O 29

# An engineered nanosugar enables rapid and sustained glucose-responsive insulin delivery in diabetic mice

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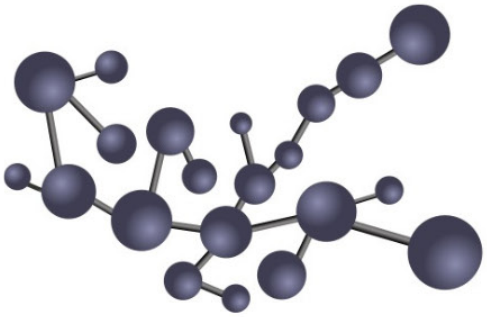
Glucose-responsive insulin delivery platforms that are sensitive to dynamic glucose concentration fluctuations and provide both rapid and prolonged insulin release have great potential to control hyperglycemia and avoid hypoglycemia diabetes. Here, we engineered biodegradable and charge-switchable phytoglycogen nanoparticles capable of glucose-stimulated insulin release. The nanoparticles are "nanosugars" bearing glucose sensitive phenylboronic acid groups and amine moieties that allow effective complexation with insulin (95% loading capacity) to form nanocomplexes. A single subcutaneous injection of nanocomplexes showed a rapid and efficient response to a glucose challenge in two distinct diabetic mouse models, resulting in optimal blood glucose levels (below 200 mg/dL) for up to 13 h. The morphology of the nanocomplexes was found to be key to controlling rapid and extended glucose-regulated insulin delivery *in vivo*. Our studies revealed that the injected nanocomplexes enabled efficient insulin release in the mouse, with optimal bioavailability, pharmacokinetics, and safety profiles. These results highlight a promising strategy for the development of a glucose-responsive insulin delivery system based on a natural and biodegradable nanosugar.

*Reference*

1. Rong Xu, Sukhvir Kaur Bhangu, Karly C Sourris, Domitilla Vanni, Marc-Antoine Sani, John A Karas, Karen Alt, Be'eri Niego, Anukreity Ale, Quinn A Besford, Brendan Dyett, Joshua Patrick, Irena Carmichael, Jonathan E Shaw, Frank Caruso, Mark E Cooper, Christoph E Hagemeyer, Francesca Cavalieri. *Advanced Materials,* **2023**, 35, 2210392



Peptide Materials

# Short

SC 1

# Peptide-based materials as Magnetic Resonance Imaging (MRI) supramolecular probes.

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Magnetic Resonance Imaging (MRI) is one of the most important diagnostic techniques. Gadolinium Gd(III) complexes are used as $T_1$ contrast agents (CAs) to improve the quality of the images. However, their clinical use has been questioned because of the evidence of ions accumulation in body districts of patients receiving multiple doses.[1] Additionally, even if highly accurate, MRI is a low sensitive technique. For all these reasons, peptide-based materials, including hydrogels, nanogels and fibers, have been proposed and scrutinized as possible supramolecular tools to improve the CAs safety profile and sensitivity. Encapsulating or chemically derivatized with Gd (III) complexes, peptide materials may protect metal complexes from transmetallation phenomena occurring in vivo and being responsible of Gd (III) release and, in turn, of their toxicity.[2] Moreover, the generation of supramolecular CAs can allow to increase the efficiency of the contrast, in terms of relaxivity value. Here we describe the synthesis, and the formulation of some examples of peptide-based materials, nanofibers, hydrogels and nanogels encapsulating or supporting Gd complexes CAs (Figure 1).

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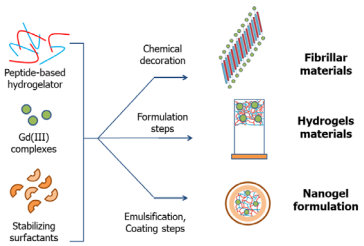


Figure 1. Proposed peptide-based materials as platforms for MRI applications.

*References*

1. T. Kanda, et al. Radiology 2014, 270(3), 834−841
2. E. Rosa, et al. Pharmaceuticals 2022, 15(12), 1572

SC 2

# Effect of Serine Residue in Lipopeptide Sequences on the Interaction of Glyphosate Pesticide in Colorimetric Analysis

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In a previous study,[1] we proposed a lipopeptide sequence consisting of a hydrophilic region composed of the amino acids L-proline (P), L-arginine (R), L-tryptophan (W), and L-glycine (G), and a hydrophobic region containing either one (C1) or two (C2) hydrocarbon chains (C18). These novel molecules, PRWG(C1) and PRWG(C2) have demonstrated their potential as biomimetic microenvironments for AChE and exhibit excellent capabilities for detecting organophosphate pesticides. Serine (S) has been identified in the literature as a crucial amino acid for the interaction between AChE protein and pesticides due to its side chain containing an OH group, which enhances the hydrolysis process between the protein and the pesticide. To investigate this further, we introduced the serine residue into the molecules as mentioned above, resulting in SPRWG(C1) and SPRWG(C2). For the first time, we conducted a systematic study varying the pH and assessing its influence on the supramolecular lipopeptide structures in solution to identify the most stable system for developing a colorimetric biosensor. In the case of SPRWG(C2), we observed minimal structural differences in the self-assembly of the solution in the presence of serine, as the compact planar structures remained unchanged.[1] Interestingly, this molecule exhibited lower pesticide detection efficiency than its serine-free counterpart. In contrast, several structural changes were observed when considering SPRWG(C1). The system became more hydrophilic, and the interaction between OH groups and water molecules favored the formation of alpha-helix secondary structures, as evidenced by circular dichroism (CD) assays. Small-angle X-ray scattering (SAXS) revealed morphological changes from spherical to cylindrical micelles as the pH was raised, further confirmed by cryogenic transmission electron microscopy (Cryo-TEM) images. Additionally, titration of the N-(phosphonomethyl) glycine into SPRWG(C1) resulted in an exothermic-endothermic transition, suggesting modifications in the supramolecular structure of the compound in the presence of the pesticide. UV-Vis kinetic experiments demonstrated that SPRWG(C1) exhibited a high catalytic effect and great sensitivity, outperforming its serine-free version. Overall, these results suggest that SPRWG(C1) holds promise as a biomimetic lipopeptide sequence for AChE and represents an excellent candidate for detecting organophosphate pesticides.

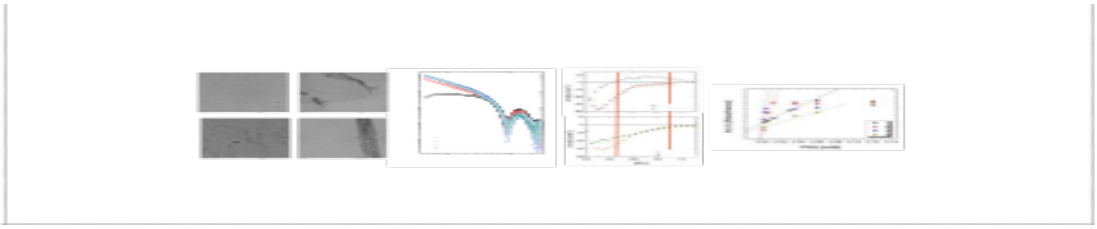

Figure 1. Characterization results for the SPRWG(C2) molecule: (a)-(d) Cryo-TEM images of SPRWG(C2), and (e) SAXS d ata at different pH values. Comparison of results for all four molecules using (f) ITC and (g) UV-Vis kinetic experiments.

*Reference*

1. B. B. Gerbelli, L. O. Filho, , I. W. Hamley, J. Seitsonen and W. A. Alves, Nanoscale Adv. 2022, 4, 3592.

SC 3

# Valorisation of Rapeseed oil by product for the development of protein/peptide based biomaterial

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Petrol-based polymers are widely used in the production of both common and high added value products, due to their exceptional properties, versatility, and low price. However, their uncontrolled disposal and extremely low degradability have resulted in one of the most serious environmental problems in the last two centuries. Therefore, in recent years there has been a rising interest in the development of polymeric materials based on natural biopolymers as a renewable alternative to petrol-based plastics[1]. The availability of proteins from agricultural by-products and their favourable properties fostered a renewed interest in protein-based materials, fuelling research in innovative technologies for the preparation of bioplastics[2]. The presented study is the first step toward the development of a protein-based material for multiple applications. Proteins from rapeseed meal have been chosen as first candidates for our bioplastics main ingredient. Rapeseed meal samples were collected as a by-product from the crude oil production after the oil pressing and hexane extraction process[3]. The proposed protein extraction process is eco-friendly, easy to scale up, and lead to the production of two protein isolates with good recovery yield. Further, the rapeseed meal was processed by pression moulding and chemical reactions have been performed to form cross-links between protein chains in order to modulate the material thermo-mechanical properties. In addition, the enzymatic hydrolysis of rapeseed meal with selected proteases lead to hydrolysate mixtures. Those are currently tested as additives with the goal to obtain a fully sustainable protein-rich material. Purification and peptidomic study of the hydrosylates will allow us to characterise the number and sequence of the peptides obtained[4] with the final aim to identify their behaviour in the protein based material. The achieved results will contribute to the green transition achieving the goals of the European Green Deal.

*References*

1. Hatti-Kaul, R. et al. Trends Biotechnol 2020, 38 (1), 50–67.
2. DeFrates, K. G. et al. Nanomaterials 2018, 8 (7), 457.
3. Newson, W. R. Industrial Oil Crops 2012, 59
4. Hellinger, R., et al. Nat Rev Methods Primers 3, 25 (2023).

*Topic*: Peptide Biomaterials & Tissue Regeneration

SC 4

# Supramolecular hydrogels for the controlled release of active compounds and fragrances

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Low-molecular-weight gelators (LMWGs) are small compounds that self-assemble thanks to weak interactions.[1] Peptide derivatives are ideal candidates as LMWGs because of their biocompatibility, biodegradability and efficient synthesis. The tunability of gelators and of the conditions for gel formation allows the preparation of materials with different features and applications.

We recently reported that Boc-L-DOPA(Bn)$_2$-OH is a robust gelator, able to form gels with different triggers and with the addition of many fillers. The gels obtained from ethanol/water mixture were used to study the release of odorant molecules from two classes of profragrances, responding to pH or light triggers.[2,3] The release of fragrances resulted more controlled from gels then solutions.

The formation of hydrogels was also evaluated, using a second gelator as trigger, Boc-AUV-OH.[4] These multicomponent gels have rheological properties and pH that can be tuned by the ratio between the two gelators. The gels were used to study the permeation of two bioactive tripeptides with antiaging activity, TFA-L-Val-L-Tyr-L-Val-OH and Pal-L-Lys-L-Val-L-Lys-OH, through pig ear skin using Franz cells. The multicomponent gels constitute a valid and biocompatible formulation for both drug delivery and cosmetic applications.

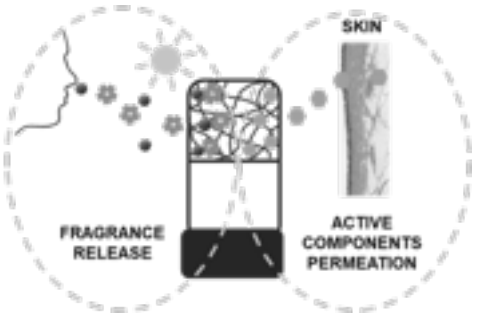


Figure 1. Example of release from self-assembled gels: fragrances (left) and active compounds (right).

*References*

1. E. Draper, D. J. Adams Chem. (2017), 3, 390.
2. G. Nicastro, L. M. Black, P. Ravarino, S. D'Agostino, D. Faccio, C.Tomasini, D.Giuri, Int. J. Mol. Sci (2023), 23, 3105.
3. F. Cenciarelli, G. Falini, D. Giuri, C. Tomasini, Gels (2023), 9, 350.
4. S. R. Shariati Pour, S. Oddis, M. Barbalinardo, P. Ravarino, M. Cavallini, J. Fiori, D. Giuri, C. Tomasini, Molecules (2023), 28, 2528.

SC 5

# Bioinspired short peptide hydrogels: chemical modifications and functional applications

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Bioactive peptides, deriving from natural protein fragments, capable of self-assembly into ordered supramolecular structures are ideal building blocks to form hydrogels.

Self-assembling peptide (SAP)-based hydrogels show unique characteristics: biocompatibility, biodegradability, tunable mechanical stability and low production costs. Thus bio-based hydrogels are promising in many biomedical applications: drug delivery, diagnostic, tissue engineering and regenerative medicine.[1]

To modulate the mechanical properties of self-assembling peptides the insertion of chemical modifications as the change of the chirality of single amino acids is a successful tool. A short stretch spanning residues 268-273, $^{268}$FINYVK$^{273}$, in the C-terminal domain of Nucleophosmin 1 showed amyloid-aggregation features.[2] Recently, the conformational investigations of peptides deriving from a D-scan of this sequence, demonstrated the common ability to self-assemble to form hydrogels endowed with different intermediates related to the nature of introduced chirality.[3] Since the presence of net charges at the extremities of sequences can heavily influence peptide-hydrogels, we recently focused on the self-assembly processes of the pentapeptide covering residues 269-273, $^{268}$INYVK$^{273}$, in four different forms: i) free N- and C-term, ii) free N-term and amidated C-term, iii) acetylated N-term and free C-term and iv) acetylated N-term and amidated C-term. Interestingly, our results highlighted that $^{268}$INYVK$^{273}$ self-assembly process and consequent hydrogel formation is strictly dependent on the presence of charges: indeed, only the protect sequence (acetylated /amidated) was able to form a stable hydrogel while the free N- and C-term version, did not show any type of aggregation.[4]

*References*

1. S. La Manna, C. Di Natale, V. Onesto, D. Marasco. Int. J. Mol. Sci. (2021), 22, 23.
2. C. Di Natale, P.L. Scognamiglio, R. Cascella, C. Cecchi, A. Russo, M. Leone, A. Penco, A. Relini, L. Federici, A. Di Matteo, F. Chiti, L. Vitagliano, D. Marasco. FASEB J. (2015), 29, 9.
3. D. Florio, C. Di Natale, P.L. Scognamiglio, M. Leone, S. La Manna, S. Di Somma, P.A. Netti, A.M. Malfitano, Marasco, D. Bioorg Chem. (2021), 114, 105047.
4. S. La Manna, D. Florio, V. Panzetta, V. Roviello, P.A. Netti, C. Di Natale, D. Marasco. Soft Matter. (2022), 18, 44.

SC 6

# Antibody conjugated with gold nanoparticle for construction of a biosensor for COVID-19

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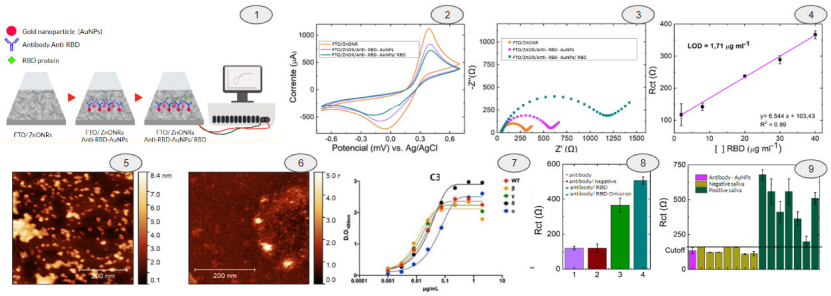
In this study, we have developed an electrochemical biosensor to diagnose COVID-19. The biosensor utilizes gold nanoparticles conjugated with antibodies that specifically target the RBD domain of the Spike protein from SARS-CoV-2. We modified FTO electrodes with zinc oxide nanorods to create the impedimetric biosensor.[1] The immobilized AuNPs-anti-RBD were characterized by observing a decrease in the oxidation peak potential of the redox couple and an increase in the diameter of the semicircle in the Nyquist diagrams. We obtained a calibration curve with a detection limit (LOD) of 1.7 µg $mL^{-1}$. Furthermore, we performed an AFM analysis, which revealed distinct behavior between the pure antibody and the antibody conjugated with the AuNPs. Using ELISA, we also evaluated the antibody's performance in detecting the RBD recombinant protein from five Variants of Concern (VoC) of SARS-CoV-2. The electrochemical tests showed similar effectiveness in detecting the Omicron variant, and we achieved excellent results using real samples, specifically saliva. The antibody demonstrated a strong detection-response performance, indicating its potential for recognizing the viral proteins of interest. These findings highlight the significant prospects for developing a biosensor using this antibody.




Figures: (1) Schematic representation of the biosensor's working principle; (2) Cyclic voltammogram from the electrochemical test; (3) Nyquist diagram illustrating the electrode surface of the biosensor; (4) Calibration curve showing the relationship between analyte concentration and biosensor response; (5) Atomic Force Microscopy (AFM) image of the Antibodies; (6) AFM image of the Antibodies conjugated with AuNPs; (7) ELISA test results using the anti-RBD antibody and the Alpha, Beta, Gamma, Delta, and Omicron variants of the SARS-CoV-2 virus; (8) Bar graph comparing the Rct values obtained for RBD, RBD Omicron, and negative protein; (9) Bar graph comparing the Rct values obtained from patients' SARS-CoV-2 positive and negative saliva samples.

*Reference*

1. F.A. Nunez, A.C.H. Castro, V.L. de Oliveira, A.C. Lima, J.R. Oliveira, G.X. de Medeiros, G.L. Sasahara, K.S. Santos, A.J.C. Lanfredi, W.A. Alves. ACS Biomater. Sci. Eng. 2023, 9, 458.

SC 7

# Diving into the blue: Synthetic methodologies toward aggregation induced fluorescent peptide materials

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Fluorescence spectroscopy proved to be a powerful tool to investigate complex biological processes, such as the interactions of a protein with other proteins as well as with nucleic acids. However, fluorescent techniques show weak or quenched emission in the aggregate stage or in highly concentrated solutions due to the aggregation-caused quenching effect (ACQ). Unlike to ACQ fluorophores, aggregation induced emissive (AIE) luminogens show bright emission in the aggregate state. AIE is a photophysical phenomenon in which molecular aggregate exhibits stronger emission than a singlet molecule. Thus, continuous efforts are made for the design and synthesis of unnatural amino acids with various aggregation induced emissive moiety fluorophores that could easily and successfully incorporate into peptide sequences. In the present work, L-phenylalanine was used as a chiral template for the preparation either in liquid or on solid phase of unnatural amino acids, that will bear a tetraphenylethene (TPE) moiety on their side chain. TPE is a classic AIE molecule widely used as a fluorescent probe[1] and could easily build on L-phenylalanine via Suzuki-Miyaura cross coupling reactions. The morphology of the new fluorescent phenylalanine analogues was characterized by SEM microscopy and DLS analysis, whereas fluorescence spectroscopy assays were performed to investigate the fluorescence properties of aggregates in solid state and in solution.

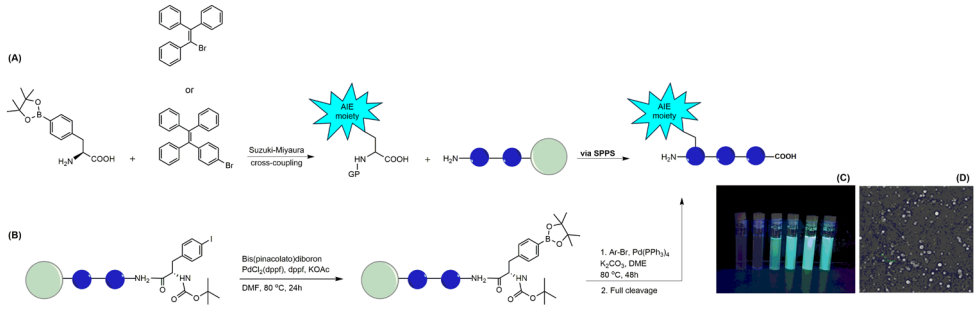


Figure 1. Synthesis of TPE-based AIEgens (A) in liquid phase and (B) on solid phase;
(C) Example of aggregates in different ratios of organic solvent/$H_2O$ (water is increasing from left to right); (D) SEM analysis.

*Reference*

1. W. Chen, C. Zhang, X. Han, S. H. Liu, Y. Tan and J. Yin J. Org. Chem. (2019), 84, 14498.

SC 8

# Self-assembled peptides as smart materials

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Inspired by self-assembly in nature, scientists have explored the self-assembly process of short peptides, used as building blocks. Their synthesis and chemical modifications are facile and can assemble with remarkable efficiency into biocompatible and controllably biodegradable materials.
As proof of concept, we designed a fiber based on three amphiphilic peptides labelled P1, P2 and P3. P1 and P2 consist of alkyl tail (C19, made of 19 carbon atoms), hydrophobic segment (made of 6 alanine), and charged segment. In particular, negative charges are present in P1 and positive charge in P2, respectively. P3 consists of the same sequence of P2 but differ at the N terminal side covalently linked to the cell-penetrating peptide (CPP) gH625, which is known to favor the uptake of the nanofiber into cell. [1,2]
Nanofiber presents a diameter of ca. 12 ± 2 nm and a length of ca. 150 ± 50 nm, was characterized with several technique such as fluorescence, transmission electronic microscopy, dynamic light scattering, atomic force microscopy, circular dichroism, and Raman spectroscopy.

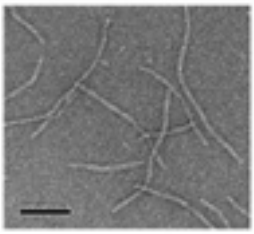

Figure 1. TEM image of fiber composed by P1 + P2 + P3 mixture

This represent a starting point that can be easily update with peptides opportunely designed to obtain a valid weapon for a wide range of applications, as antimicrobial [3] and/or anticancer disease [4].

*References*

1. D. Guarnieri, et al. Small (2013), 9, 6.
2. A. Falanga, et al. Scientific Reports (2018), 8, 1.
3. L. Lombardi, et al. Biomacromolecules 20.3 (2019), 20, 3.
4. V. Del Genio, et al. Pharmaceutics (2022), 14, 8.

SC 9

# Chitosan functionalized with bioactive peptides for bone tissue engineering

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The increase in bone pathologies due to the ongoing of the population pushes research to formulate increasingly sophisticated and performing biomaterials. Chitosan is a biocompatible, biodegradable, and antibacterial biomaterial employed for various biomedical applications. In this work, two synthetic peptides were covalently grafted to chitosan in order to improve polymer bioactivity. The first sequence, the nonapeptide (351-359) of human vitronectin (HVP), was demonstrated to enhance osteoblast adhesion and migration.[1] The second peptide, a 22mer sequence reproducing the fragment (48-69) of BMP-2, was able to increase the osteogenic differentiation of mesenchymal stem cells.[2] Both peptides were synthesized using solid phase peptide synthesis and Fmoc chemistry. A Serin was added at the N-terminal of both sequences to produce an alpha-oxo-aldehyde group after oxidation. The anchoring chemistry consists of Schiff base formation, successively reduced, between chitosan amino groups and the aldehyde group at peptide N-terminus. This method allows a selective anchoring of a bioactive sequence in a single-step reaction carried out in aqueous solution and mild conditions. Seven scaffolds were created to evaluate the biological relevance of different concentrations of anchored peptides mixing pure chitosan with functionalized chitosans: one of pure chitosan (control), five mixed matrices, and a scaffold of chitosan functionalized simultaneously with both peptides. Sponges of different chitosan blends were prepared by freeze drying. Matrices were characterized through biomechanical tests, scanning electron microscope (SEM), x-ray photoelectron spectroscopy (XPS), and biological assays with human osteoblasts. Almost all functionalized scaffolds significantly increased osteoblast adhesion, with chitosan-HVP as the best-performing scaffold. Functionalized scaffolds significantly promoted cellular proliferation, especially chitosan-GBMP1α. Furthermore, all matrices induced an up-regulation in the expression of vitronectin, osteopontin, and RUNX-2 genes compared to the control. Finally, scaffolds were implanted in vivo in the ectopic site of a murine model, to assess the cytotoxicity and osteoinduction capacity of the functionalized chitosan.

*References*

1. A.Zamuner et al., Biomed. Mater., (2021), vol. 16, no. 5.
2. E.Rampazzo et al., Biochim. Biophys., (2017), vol. 1861, no. 9.

SC 10

# Versatile magneto-plasmonic composites based on dehydropeptide hydrogels for multimodal cancer therapy

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Supramolecular peptide hydrogels present multiple advantages for biomedical applications. In the context of cancer therapy, they can entrap and release drugs in a fashion that can be tuned by changing the composition of the network [1]. Recently, efforts have been made to combine these materials with inorganic nanoparticles to make the former responsive to stimuli [2]. For example, the inclusion of magneto-plasmonic liposomes in the matrix of peptide-based hydrogels enables their targeting to the disease area by magnetic forces, and both optical and magnetic hyperthermia. Furthermore, these organic-inorganic nanohybrids allow the encapsulation of a wide range of drugs (hydrophobic and hydrophilic) and their compartmentalisation. In this communication, we report novel supramolecular hydrogels based on lysine dehydrodipeptides and address their functionalization with gold-manganese ferrite plasmonic magnetoliposomes (figure 1).

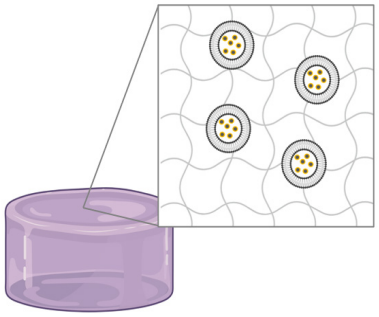
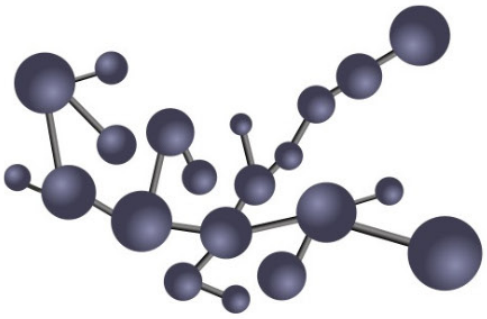


Figure 1. Dehydropeptide-based hydrogels containing magneto-plasmonic liposomes.

The (micro/nano)structure of the composite gels was evaluated by STEM, Raman and fluorescence spectroscopies and the respective viscoelastic properties were studied by rheology. Ultimately, their relevance as drug delivery platforms and hyperthermia agents was tested.

*References*

1. S. Veloso, P. Ferreira, J. Martins, P. Coutinho, E. Castanheira. Pharmaceutics. (2018), 10(3), 145.
2. V. Gomes, S.Veloso, M. A. Correa-Duarte, P. M .T. Ferreira, E. M. S. Castanheira. Int. J. Mol. Sci. (2022), 24(1), 186.



Peptide Materials

# Sponsor

# Why using microfluidics for peptide-guided lipid nanoparticles synthesis offers you so many advantages

Roberto Santoliquido

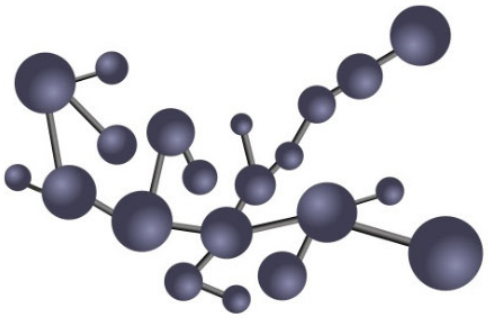
Alfatest S.r.l., Roma, Italy

The Nanoparticles are a proven delivery method for nanomedicine (vaccines, drugs, gene therapies), as they offer a range of key benefits over traditional approaches. For

instance, a significant part of the medical industry's research and development focuses on Liposomes and Lipid Nanoparticles (LNPs) because they allow enhanced efficacy and efficiency of APIs, bio-targeting with peptides, stability and controlled release.

However, there is still a key challenge surrounding some of the traditional methodology used. How to ensure that each and every produced particle is consistent in terms of composition, size and payload?

Microfluidics for nanoparticle synthesis offers so many advantages, it enables control, consistency and precision in the created nanoparticles: excellent monodispersity, consistent (targeted) particle size, high encapsulation efficiency, low sample volume, very low waste, high particle integrity, high reproducibility, high-throughput, linear scalable manufacturing, consistent process/API loading, quick and readily optimizable protocols, preserved particle integrity, encapsulation of hydrophobic or hydrophilic cargo.



Peptide Materials

# Poster

P 1

# Capacitive Peptide Interfaces for Electroanalytical Applications

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Electrochemical capacitance methodologies are promising tools for biological recognition, with reducing time and cost. Self-assembled monolayers peptides have been used due their versatility and capacity to form stable interfaces. The advantages of peptides include design flexibility, easy synthesis, strong thiol gold interactions (via cysteine residues). The sequence Fc-Glu-Ala-Ala-Cys-$NH_2$)[1,2] has been used in a matrix coupled with a DNA aptamer as a bioreceptor to detect the C- reactive protein (CRP, a biomarker of inflammation. This carboxamide peptide has a C-terminal cysteine, used for bound in the gold electrode surface. Two alanine residues were used to promote film crystallinity and an N-terminal glutamic acid containing one ferrocene redox probe at the N-terminus and have the carboxyl side-chain unmodified to enable subsequent bioconjugation of the aptamer/antibody/protein. In the present work, different ferrocene-tagged peptides with a structure of Fc-Glu-XX-XX-Cys-$NH_2$ (XX = Serine, Phenylalanine, Glycine) were used to form self-assembled monolayers on gold. The applicability of these sequences was verified by detecting the NS1 DENV (Dengue Virus) biomarker. The peptide was obtained by Solid Phase Peptide Synthesis (SPFS) and the electrochemical properties were evaluated by impedance-derived electrochemical capacitance spectroscopy and cyclic voltammetry. The NS1 protein (non-structural protein 1), a biomarker for dengue diagnosis, was coupled to the carboxyl group of the peptide monolayer after activating using EDC/NHS.

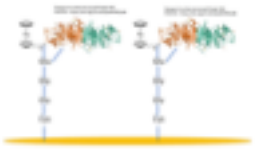

Figure 1. Schematic representation of electroactive biossensor obtained by the assembly of Fc-Glu(NS1)-Ala-Ala-Cys-NH2 on gold electrode

The biossensor containing these three peptides surfaces present different NS1 detection. The Gly peptide SAM exhibited the highest normalized electronic density of states (DOS) and electrochemical capacitance and was more sensitive for NS1 detection. The biossensor with Phe-peptide SAM showed poor analytical performance, with R2 < 84%, relatively low sensitivities (<1.2% per decade), and higher Limit Of Detection (LOD) and Limit Of Quantification (LOQ) than the Phe peptide. The Ser-peptide did not showed response for the NS1 biomarker and the sensibility, LOD, and LOQ were not determinate. These data indicate that small amino acids sequence form the best self-assembled monolayers for biosensing devices. In addition, our data indicated that optimized analytical parameters is important to obtain sensitive interfaces with highest detection and quantification limits.

*References*

1. Picooli, J.P. et al. Anal. Chem. (2018) 90, 3005.
2. Piccoli, J.P; Soares, A.C; Oliveira Jr, O.N,; Cilli, E.M. Bioelectrochemistry (2021) 138, 107692.
3. P. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.

P 2

# Next-generation silk-based materials for tissue regeneration: a peptide functionalization of degummed silk fibroin fibers

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Tissue engineering helps regenerating compromised tissues by promoting adhesion and survival of selected cell involved in tissue injury as an alternative to tissue transplant [1]. Among the various classes of materials (e.g., metals, polymers, ceramics, etc. [2]), natural materials are the most promising ones, as they are not toxic, abundant, they have customizable mechanical properties, and good biocompatibility for cell activity [3][4]. We focused on degummed silk fibroin fibers (DSFFs), derived from Bombyx Mori cocoons. The use of silk in the biomedical field can only take place after the removal of the external sericin (degumming process), due to its hypersensitivity and immunogenicity effects. DSFFs were used as a scaffold to link a newly designed peptide containing the arginine-glycine-aspartate motif (named MO-07), with anti-inflammatory and cell adhesion properties [5]. The peptide was synthetized via induction assisted peptide synthesis, purified by flash chromatography, and then linked to the DSFFs where -COOH groups on the backbone were previously activated [6]. In the resulted regenerated silk films, different mechanical properties were found, due to peptide functionalization [7]. Peptide-functionalized silk fibroin was also used as a bio-ink for 3D printing of grids used to promote wound healing which were shown to be efficient in promoting cell proliferation and migration [8].

*References*

1. Langer, R. Acc. Chem. Res. (2000) 33, 2.
2. Liu, C.; Xia, Z.; Czernuszka, J. T. Chem. Eng. Res. Des. (2007) 85, 7.
3. Tandon, S.; Kandasubramanian, B.; Ibrahim, S. M. Ind. Eng. Chem. Res. (2020) 59, 40.
4. Su, C.; Chen, Y.; Tian, S.; Lu, C.; Lv, Q. Gels (2022) 8, 11.
5. Bellis, S. L. Biomaterials (2011) 32, 18.
6. Manchineella, S.; Thrivikraman, G.; Basu, B.; Govindaraju, T. ACS Appl. Mater. Interfaces (2016) 8, 35.
7. Valentini, L.; Pacini, L.; Errante, F.; Morchio, C.; Sanna, B.; Rovero, P.; Morabito, A. Molecules (2022) 27, 14.
8. Ceccarini, M. R.; Palazzi, V.; Salvati, R.; Chiesa, I.; De Maria, C.; Bonafoni, S.; Mezzanotte, P.; Codini, M.; Pacini, L.; Errante, F.; Rovero, P.; Morabito, A.; Beccari, T.; Roselli, L.; Valentini, L. Int. J. Mol. Sci. (2023) 24, 2.

*Topic*: Peptide Biomaterials & Tissue Regeneration

P 3

# Self-assembly as a molecular strategy of improve adsorbents of pollutants in water

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Although a lot of progress have been made in studying the molecules mechanism of peptide assembly, it remains a challenge to accurately regulate the assembly structure of peptides to achieve pre-designed structure and function. Chirality, an inherent property of peptides, has been recognized as a vital factor that can exert essential impacts on peptide assembly structures. In the development of peptide biomaterial, the chirality of peptide and amino acid residues is an important factor that has been taken into consideration by researchers. Numerous helical or twisted nanostructures and ordered ensembles have been successfully produced by molecular self-assembly from either single or multiple molecular components but it still remains a challenge to construct chiral nanostructures with desirable conformation (i.e., right-handed, P; left-handed, M) from specific chiral building blocks at will.
Alternating D,L-oligopeptides are able to assume specific conformations including, among others, various kinds of single and double stranded β-helical structures. In previous study the conformational characteristics of Boc-(L-Val-D-Val)$_4$-OMe in solution have been investigated by using NMR techniques. Different species that interconvert slowly compared with the proton spin time scale occur in chloroform solution and two of them are left-handed, double-stranded helical species of the type ↓↑β5.6 with 14 interstrand hydrogen bonds (Figure1).

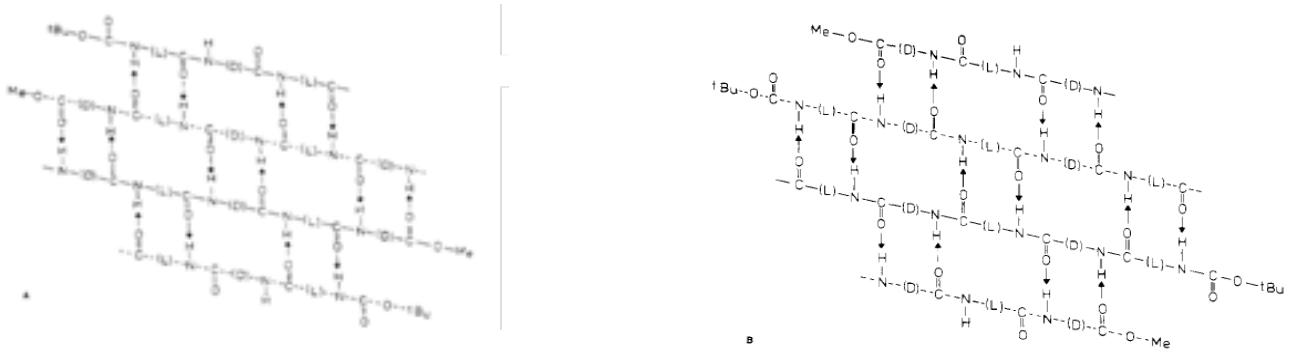

Figure 1. Hydrogen-bonding pattern of the two left-handed ↓↑ $\beta^{5.6}$-helical structures of Boc-(L-Val-D-Val)$_4$-OMe with 14 interturn hydrogen bonds.

As illustrated in Figure 1 in the helix A the hydrogen bonds connect three pairs of D residues and four pairs of L residues, in the helix B the hydrogen bonds connect four pairs of D residues and three pairs of L residues. Note that the helix A can be converted into helix B (or vice versa) simply by transposing one strand by two residues with respect to the other. Here we report about synthesis and conformational behavior of a peptidic system that can be used for the removal of a wide variety of micropollutants of different size from natural and engineered water systems.

*References*

1. D.U. Römer, E. Fenude-Schoch, G.P. Lorenzi, Helvetica Chimica Acta (1993) 76, 451-458
2. G.P. Lorenzi, H. Jackle, L. Tomasic, V. Rizzo, C. Pedone, J. Am. Chem. Soc. (1985) 104, 1728-1733

P 4

# Exploring protein regions prone to amyloid aggregation: a key pathway for biomaterial development

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Getting inspiration from nature and further developing functional architectures provides an effective way to design innovative materials and systems. The exploitation of protein regions prone to amyloid aggregation and the morphological characterization of deriving amyloid fibers offer promising avenues for the development of novel self-assembling biomaterials1. Amyloid fibrils are characterized by a cross-β-sheet structure, providing exceptional mechanical stability and resistance to proteolytic degradation. This unique property prompted the investigation of the potential of amyloid fibrils as versatile building blocks for the development of biomaterials with diverse applications, ranging from tissue engineering scaffolds to drug delivery systems2.

Nucleophosmin 1 (NPM1) is a nucleolar phosphoprotein that has garnered our attention for the conformational consequences of its point mutations in acute myeloid leukemia (AML), they induce a clear self-assembly propensity of specific protein regions3-4. The deep investigation of structural determinants causing amyloidogenicity enables rational design and engineering of biomaterials with improved properties and customised functionality5. The manipulation of self-assembled architectures is always a critical prerequisite to achieve desired functionalities.

Here we present our investigative path from NPM1 entire protein toward the functional self-assembly of small hexa-penta-peptides that demonstrated biocompatible6-7.

*References*

1. Chu, S.; Wang, A. L.; Bhattacharya, A.; Montclare, J. K., Protein based biomaterials for therapeutic and diagnostic applications. Progress in Biomedical Engineering 2021, 4 (1), 012003.
2. Das, S.; Jacob, R. S.; Patel, K.; Singh, N.; Maji, S. K., Amyloid Fibrils: Versatile Biomaterials for Cell Adhesion and Tissue Engineering Applications. Biomacromolecules 2018, 19 (6), 1826-1839.
3. Zarka, J.; Short, N. J.; Kanagal-Shamanna, R.; Issa, G. C., Nucleophosmin 1 Mutations in Acute Myeloid Leukemia. Genes (Basel) 2020, 11 (6).
4. La Manna, S.; Florio, D.; Di Natale, C.; Napolitano, F.; Malfitano, A. M.; Netti, P. A.; De Benedictis, I.; Marasco, D., Conformational consequences of NPM1 rare mutations: An aggregation perspective in Acute Myeloid Leukemia. Bioorganic Chemistry 2021, 113, 104997.
5. Zhang, J.; Wang, Y.; Rodriguez, B. J.; Yang, R.; Yu, B.; Mei, D.; Li, J.; Tao, K.; Gazit, E., Microfabrication of peptide self-assemblies: inspired by nature towards applications. Chemical Society Reviews 2022.
6. Florio, D.; Di Natale, C.; Scognamiglio, P. L.; Leone, M.; La Manna, S.; Di Somma, S.; Netti, P. A.; Malfitano, A. M.; Marasco, D.. Bioorganic Chemistry 2021, 114, 105047.
7. La Manna, S.; Florio, D.; Panzetta, V.; Roviello, V.; Netti, P. A.; Di Natale, C.; Marasco, D.,. Soft Matter 2022, 18 (44), 8418-8426.

P 5

# Peptide-based hydrogels and nanogels for delivery of Dexamethasone

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In the last years, innovative strategies based on the use of nanomedicine, have been proposed for the solving of problems related with drug administration.[1] Recently, novel classes of soft biomaterials such as nanofibers, hydrogels (HGs) and nanogels (NGs) have emerged as alternative drug delivery vehicles to conventional supramolecular structures.[2] In this context, we developed HGs and NGs, based on the self-assembling of the well know peptide hydrogelator Fmoc-FF.[3] These supramolecular structures allow to efficiently encapsulate large quantities of Dexamethasone (DEX), a glucocorticoid medication used to treat many inflammatory and autoimmune disorders and as a direct chemotherapeutic agent in certain haematological malignancies, such as acute lymphoblastic leukemia (ALL). The biomaterials were characterized from the structural, mechanical and morphological point of view. Moreover, we evaluated the DEX loading capability of supramolecular structures and their drug release properties over time. Finally, we also assessed the NGs stability in human serum, the haemotoxicity and the selective internalization in leukemic cells.

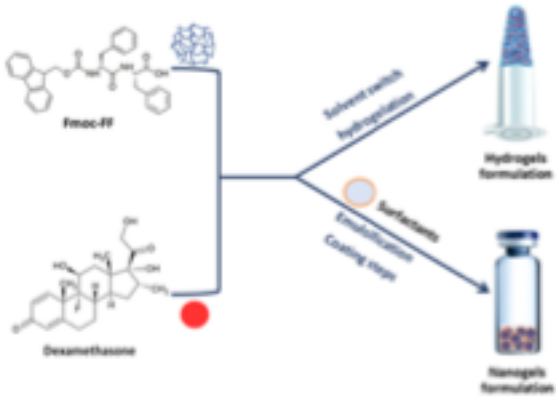


Figure 1. Schematic representation of components and methodologies for the formulation of DEX filled HGs and NGs.

*References*

1. C. Diaferia Sci. Rep. (2017), 7, 307.
2. E. Gallo Int. J. of Nanomed (2021), 16, 1617.
3. C. Diaferia Pharmaceuticals (2022), 15, 1048.

P 6

# Novel biocompatible alloys via peptides surface engineering

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Improving cell-material interactions is a major goal in the field of biomaterials. Current functionalizing strategies, like native full-length proteins, are associated with drawbacks, thus the need to develop novel methodologies. In this regard, the use of synthetic peptides encompassing specific bioactive regions of proteins represents a promising option. Furthermore, the combination of peptide sequences with complementary or synergistic effects makes it possible to address more than one biological target at the biomaterial surface.

To this aim, peptide sequences from the Bone Morphogenetic Protein 2[1,2] (BMP2 like peptides) have been thus used to coat the surface of the selected metal alloys (high entropy alloys, HEAs)[3] to develop a new generation of medical metal implant, with improved osseointegration properties compared to already used ones in clinic. In fact, to date,[4] the main challenge in the biomedical field is to find a biomaterial that meets all the imposed requirements for biomedical applications such as consisting of biocompatible elements, low elastic modulus to prevent the stress-shielding effect on bone fixation, high wear corrosion resistance, etc.

The physicochemical and biochemical characterization of the engineered alloys has been carried out, focusing on how they will interact with the biological system. The characterizations were conducted on different substrates functionalized through various pathways, including covalent grafting and electrodeposition. Besides coating characterization by physicochemical techniques, we also investigated the coatings' biological performances in vitro. To this aim, we studied the biocompatibility of the coatings and their interaction with cells.

*References*

1. K. Schmidt-Bleek, et al Factor Rev.( 2016), 27, 141.
2.L. Weng, et al, Adv. Healthcare Mater. (2018), 7, 1701415.
3. M. Gueye, et al, Surface and Coatings Technology, (2020), 385, 125374.
4. M. Dinu, S. Franchi, V. pruna et al., "in: Titanium in medical and dental applications" Edited by F. H.Froes and M. Qian, Woodhead Piblishing series in Biomaterials, ELSEVIER, 2018, section 2.4, p. 175

P 7

# Bioadhesive and biocompatible polysaccharide-peptide hybrid IPN as a promising scaffold for biomedical application

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The use of tissue engineering in regenerative medicine opened the route to the possibility of restoring normal biological function of damage tissues through the combination of cells, scaffolds, and growth factors.[1] Polysaccharide-based hydrogels have been widely used as scaffold by virtue of their biocompatibility and biodegradability, however, since most of them lack of suitable mechanical properties and bioadhesive characteristics, further modifications are required[2].

In this work, dextran (Dex) and gellan gum (Ge) have been selected as the starting material for the preparation of semi-IPN systems potentially useful for tissue engineering purposes. Dex was modified by introduction of methacrylic groups, which, when exposed to the UV light, allows the formation of a chemically crosslinked matrix. On the other hand, Ge was derivatized with an RGD tripeptide, a sequence that is recognized by cell proteins and allows them to adhere on the scaffold surface.

The study of the rheological and dynamo-mechanical properties of the matrices, together with the 3D printing of the scaffold and cell adhesion experiments, have proved the capability of the system to support cell proliferation and reconstitution of the damage area.

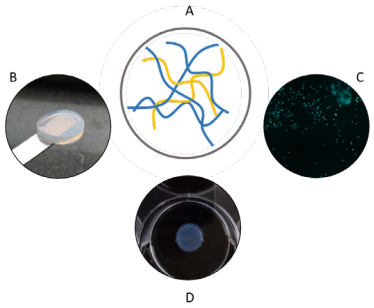


Figure 1. A) Dex-methacrylate and Ge-RGD solution; B) semi-IPN after UV crosslinking; C) Fluorescent image of cell adhesion; D) 3D-printed IPN film.

*References*

1. A. Khademhosseini, R. Langer Nat. Protoc. (2016), 11, 1775–178.

2. M. Jin, J. Shi, W. Zhu, H. Yao, D.-A. Wang Tissue Engineering Part B: Reviews (2021), 27:6, 604-626.

P 8

# Synthesis of peptidomimetics using Diaryl-β amino acids for the preparation of smart materials

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α-Amino acids are at the basis of the living world, being the building blocks of peptides and proteins. The advantages of α-peptides, i.e. versatility, biocompatibility and ease of preparation, make them the perfect candidates in different applications, for example biology and pharmaceutical science. However, they show some drawbacks mainly due to their protease sensitivity. To overcome this limitation, non-standard amino acids such as β-amino acids are inserted in the peptide sequences to produce peptidomimetics, molecules with the same biological activity of the natural peptides but with enhanced proteolytic and conformational stability.[1] In addition, the ability of peptides or amino acids alone to self-assemble and self-organize, allows their use in wide and variable applications, from nanomedicine to electrochemistry and catalysis, as well as bioelectronic materials.[2]

Here we present an unnatural fluorine-substituted $\beta^{2,3}$-diarylamino acid, synthesized for the first time in our laboratory, by a stereoselective Mannich-like reaction.[3] This β-amino acid triggers the formation of different bioinspired architectures, depending on the peptide sequence in which it is inserted. So far, different nanomaterials such as nanotubes,[4] supramolecular conductive ropes,[5] and cationic nanospheres,[6] have been obtained and characterized.

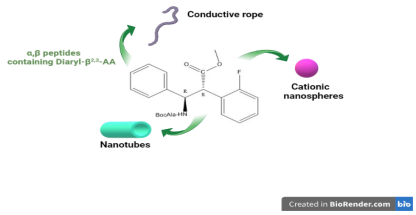


Figure 1. Diaryl-$\beta^{2,3}$-amino acid structure and nanomaterials obtained by peptidomimetics self-assembly

*References*

1. R. Bucci, F. Foschi, C. Loro, E. Erba, M.L. Gelmi, S. Pellegrino, Eur. J.O.C. (2021), 20, 2887-2900.
2. F. Clerici, E. Erba, M.L. Gelmi, S. Pellegrino,Tetrahedron Lett. (2016), 57, 5540-5550.
3. A. Bonetti, F. Foschi, D. Nava, S. Pellegrino, M. Penso, R. Soave, M.L. Gelmi, Eur. J.O.C. (2014), 15, 3203-3209.
4. A. Bonetti, S. Pellegrino, P. Das, S. Yuran, R. Bucci, N. Ferri, F. Meneghetti, C. Castellano, M. Reches, M. L. Gelmi, Org. Lett. (2015), 17, 4468-4471.
5. N. Forlano, R. Bucci, A. Contini, M. Venanzi, E. Placidi, M.L. Gelmi, R. Lettieri, E. Gatto, Nanomaterials. (2023), 13, a.n. 333.
6. R. Bucci, P. Das, F. Iannuzzi, M. Feligioni, R. Gandolfi, Gelmi M. L., Reches M., Pellegrino S. Org. Biomol. Chem. (2017), 15, 6773-6779.

P 9

# Sanidrink: an innovative strategy toward the development of antimicrobial surfaces

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Antimicrobial peptides (AMPs) are a promising class of non-toxic natural and synthetic molecules representing a valid alternative to antibiotics being multifunctional therapeutic agents, able to fight a huge repertoire of microorganisms and the spread of several diseases.[1] Remarkably, AMPs act as suitable building blocks for the development of active coatings on surfaces of different materials. As a matter of fact, the incorporation of AMPS onto/into metal, polymeric or silicon surfaces, either by adsorption or through covalent coupling, allows the preparation of hybrid tools with potential use in the prevention of implant-associated infections, gene delivery or the treatment of microbial infections in the mouth.[2] For instance, bottled water, microbiologically pure, is usually used to prevent water-borne diseases, especially for hospitalized patients with a compromised immune system. Nonetheless, water can be easily contaminated by bacteria of the oral flora or the external environment, which can be harmful to health. In addition to that, it is worth remembering the environmental pollution caused by the massive use of plastic containers, which drove the social push for the adoption of environmentally friendly reusable water bottles.[3]
In this scenario, Sanidrink, an innovative start-up, provides a sustainable technology based on the functionalization of several materials (e.g., silicone, PET) with patented AMPs, which paves the way for the development of reusable containers. This technology has a wide field of application, spanning from food packaging to food&beverage, ensuring, for example, a higher healthiness of water stored for a long time in bottles, offering a strategy against the spread of pathogens.

Figure 1. Reusable water bottles proposed by Sanidrink.

*References*

1. Y. Huan, Q. Kong, H. Mou, H. Yi Front. Microbiol. (2020), 11, 1.
2. R. Akhilesh, R. Ferrao, P. Palma, T. Patricio, P. Perreira, E. Anes, C. Tonda-Turo, M. C. L. Martins, N. Alves and L. Ferreira J. Mater. Chem. B (2022), 10, 2384.
3. H. Liu, Q. Liu Ann Civil Environ Eng (2017), 1, 055.

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# Antimicrobial peptides with applications in medicine

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Antimicrobial peptides, also known as AMPs, have been shown to be effective against a wide variety of microbes, including bacteria, fungi, viruses, and parasites. In addition, antimicrobial peptides have been linked to immunomodulatory and anticancer effects.[1,2,3] The vast majority of AMPs feature amphiphilic architectures and net positive charges. Cationic AMPs are able to kill bacteria by selectively damaging their membranes, but cationic cell-penetrating peptides (CPPs) are able to infiltrate bacterial cells without disrupting the cell membrane and then assault intracellular targets, proving that they have antimicrobial activity. AMPs are able to kill bacteria through the process of selective membrane destruction. We will exhibit the mechanisms of cell-penetrating of AMPs with dual effects that may be used either on their own or as part of combination therapy (for example, with antibiotics). These AMPs represent an alternative platform of therapeutic agents that can be used to treat intracellular infections.[1,2,3,4,5]

Acknowledgements

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*References*

1. I.P. Encalada, L.M.C. Cocom, N.D.Q. Bojórquez, M.R.S. Campos, Int. J. Pept. Res. Therapeut., (2023), 29:62.
2. D.M.Copolovici, K. Langel, E. Eriste, Ü. Langel. ACS Nano, (2014), 8, 1972.
3. L.Diao, M. Liu, Adv. Sci. (2023), 2300121.
4. J. Xie, Y. Bi , H. Zhang, S. Dong, L. Teng, R.J. Lee, Z. Yang. Front. Pharmacol. (2020) 11, 697.
5. D.F. Buccini, M.H. Cardoso, O.L. Franco, Front. Cell. Infect. Microbiol. (2021) 10, 612931.
6. H.K. Kang, J. Park, Chan, C.H. Seo, Y. Park, ACS Infectious Diseases, (2021), 7, 2620.

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# Molecular basis for the changes in the assembly structures of bioactive peptides

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Peptide supramolecular assemblies can compete with designed proteins in their capacity to offer useful biological functions and structural diversity to synthetic soft matter. Besides the extensive use of peptidic sequences to mimic the functional domains of large proteins, the ability to program a vast array of structures through changes in sequences via straightforward solid-phase synthesis has led to the use of peptides as self-assembling building blocks. In particular, the combination of bioactive and self-assembling epitopes generates customized nanomaterials for various biomedical applications. This potential is particularly interesting given the possibility of integrating multiple biological functionalities into a supramolecular scaffold of peptides. From structural perspective peptide assemblies can generate filaments, 2D-sheets, spheres, networks, helices and more complex shapes that will no doubt be discovered in the future as we learn to master morphogenesis of peptide assemblies. Here we focus on a peptidic system composed composed by a short multifunctional peptide linked to a hydrophobic peptide sequence.

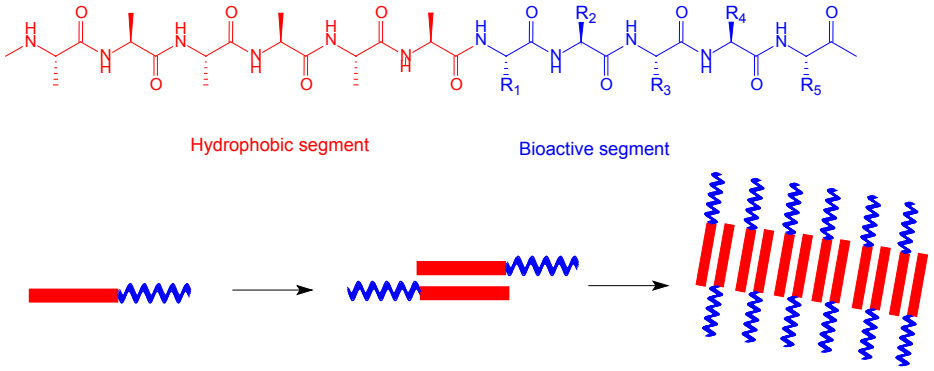


Fig.1 Types of peptide-based self-assemblies:

The hydrophobic sequence can be designed to form β-sheets among the hydrophobic side chains, while the residue farthest from the tail are the multifunctional sequence and in some cases promote solubility. In water β-sheet formation and collapse of the hydrophobic sequence induce assembly of the molecules into supramolecular, one-dimensional nanostructures. These nanostructures hold significant promise for biomedical functions due to their ability to display a high density of biological signals on their surface for targeting or to activate pathways, as well as for biocompatibility and biodegradable nature. Recent studies show that selectivity and potency of self-assembled bioactive peptides are effected in relation to transport system used. The future success of bioactive self-assembling nanostructures in biomedical applications strongly depends on the ability to fine-tune the density and display of bioactive epitopes- creating more complex heterovalent structures- while nor interfering whit the self-assembly process. Therefore, the development of bioactive self assembling structures is strongly coupled to a detailed understanding of the self-assembling properties of the systems, its dynamic and its susceptibility to change. This work aims to highlight the diversity of self-assembled nanostructures constructed from mono-dispersed synthetic building blocks, with a particular focus on their design, self-assembly, functionalization with bioactive ligands and effects thereof on the self-assembly, and possible applications.

*References*

1. E. Fenude-Schoch, D.U. Römer, G.P. Lorenzi, Int. J. Peptide Protein Res. (1994) 44, 10-18
2. G.P. Lorenzi, C. Gerber, H. Jackle, Macromolecules (1985) 18, 154-159

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# Study of lipopeptide doping in DPPC monolayer for PNG detection in water

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In recent years, the utilization of pesticides in agriculture has become more prevalent in managing agricultural pests. Given this scenario, developing new analytical tools capable of detecting these substances in food and water is crucial. In this study, we use lipopeptides as functional mimics of the enzyme acetylcholinesterase (AChE) in monolayers of phosphatidylcholine (PC) to detect the presence of glyphosate and N-(phosphonomethyl)glycine (PNG) in the environment. The lipopeptides contain hydrophilic amino acids, L-serine (S), L-proline (P), L-arginine (R), L-tryptophan (W), and L-glycine (G), covalently linked to a long aliphatic chain (18-carbons).[1] We used different molar ratios between the lipopeptides (LP) and PC ([LP/PC]). For each monolayer composition, we varied the concentration of PNG from 1 to 15 µmol L-1. We used the Langmuir-Blodgett technique to investigate the influence of the LP on the lipid monolayer, and we observed changes in the adsorption isotherm with increasing amounts of LP, such as a decrease in the mean molecular area and a decrease in the maximum pressure. For [LP/PC] = 0.5, increasing the concentration of PNG did not modify the collapse pressure, but for [LP/PC] = 1.0, the maximum pressure decreased until it reached a value of around 23 mN/m. The Nyquist diagram shows an increase in resistance to charge transfer due to the modification of ITO in the presence of the monolayer and then adding the pesticide, indicating that the monolayer adheres to the ITO surface and the pesticide interacts with the monolayer. A calibration curve was established by adjusting the concentration of PNG, resulting in a detection limit (LOD)of 48 nmol L-1. This results is very promissor once using the same lipopeptide in water solution as colorimetric biosensor we obtained a LOD around 0.3umolL-1, showing us a high senbility of this electrochemical biosensor.

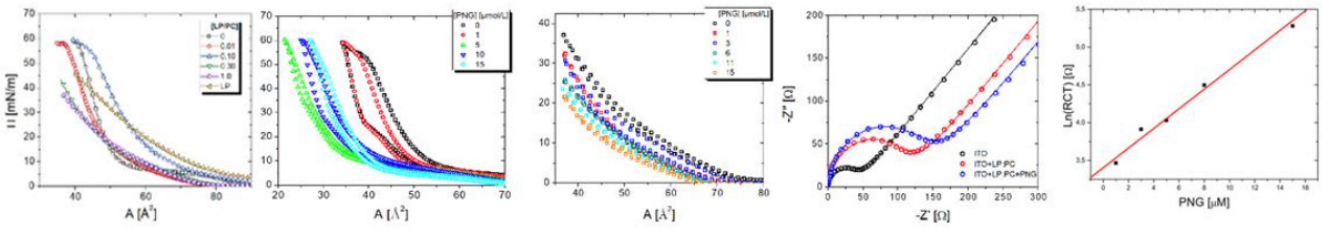


Figure 1. Adsorption isotherm: (A) Various molar ratios of LP and PC (0.05≥[LP/PC]≥1), (B) Different molar concentrations of PNG at [LP/PC]=0.3, and (C) [LP/PC]=1. (D) Nyquist diagram illustrating EIS measurements in the presence and absence of pesticides. (E) Calibration curve depicting the variation in PNG concentration.

*References*

1. Gerbelli, B.B.; Oliveira-Filho, P.L.; Cortez, B.; Sodre, P.T.; Coutinho-Neto, M.D.; Hamley, I.W.; Seitsonen, J.; Alves, W.A. Nanoscale Adv. 2022, 4, 3592.

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# Tailored Graphene Nanoparticles-Peptide conjugates for Drug-Delivery

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The chemical modification of graphene nanoparticles (GNPs) can allow their internalization by targeting cells and their use as platforms for achieving the selective delivery of drugs. We developed an effective protocol for preparing GNPs from pure graphite by ball-milling and liquid-phase exfoliation methods. The goal requires a careful control over GNPs size and defects and a suitable functionalization of the particles. A thorough characterisation showed that we can obtain GNPs with small size (average lateral sizes <L> in the range 100 – 200 nm, formed by few, <N> = 1-10 nm stacked layers), carrying carboxyl functional groups on the edges [1]. These GNPs form stable water dispersions without the need of any surfactant, and are suitable for a further chemical functionalization, specifically devoted to increase the biocompatibility and/or to impart targeting properties. Among the highly cationic peptides that can improve cellular uptake, we selected the cell permeable peptide (CPP) poly-arginine-11 (R11) as a potential delivery system for bladder cancer cell lines [2]. With this aim, the Peptide R11 was covalently grafted onto GNPs via a well-known EDC/NHS coupling reaction where the activated carboxyl groups of graphene surface react with the N-terminal amino group of R11.

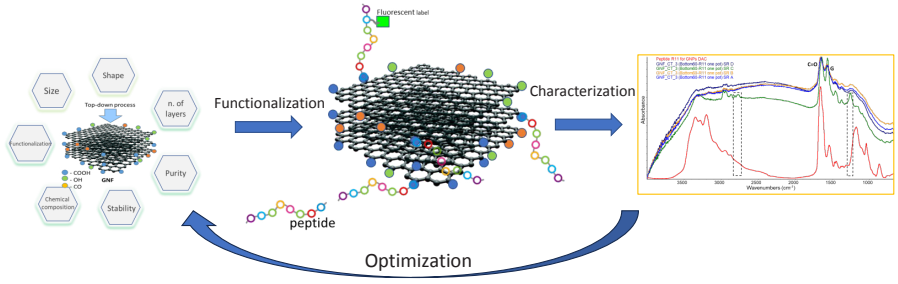


Figure 1. Schematic diagram of controlled, tailored and functionalized GNPs.

We present several investigations, aiming to the structural characterization of the bare GNPs and to assess the functionalisation of GNPs with R11, which is proven by means of IR and Raman spectroscopies.

*References*

1. K. Hu, L. Brambilla, P. Sartori, C. Moscheni, C. Perrotta, L. Zema, C. Bertarelli, C. Castiglioni,. Molecules, 2023, 28, 565.
2. Oncotarget, 2017, Vol. 8, (n. 3), pp: 4718-4729

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# Design and engineering of sweet proteins for biotechnological applications

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Protein amyloid aggregation processes play an important role in some neurodegenerative disorders, such as Alzheimer and Parkinson diseases. At the same time, the capability of some small globular proteins to form ordered aggregates can be exploited to obtain nanostructured materials. In this respect, the sweet protein MNEI has a particular appeal. This protein has been deeply studied to be used as a potential sweetener. However, an important feature of MNEI is related to its ability to form fibrils under different environmental conditions.[1] In particular, the mechanism by which MNEI forms ordered, or amorphous aggregates is strongly influenced by different physico-chemical parameters affecting the protein structure. Indeed, it is necessary an accurate balance of temperature, pH, and ionic strength to control the morphology of the aggregates. To further improve MNEI stability and sweetening properties, different mutants have been designed using point mutations experiments, as well as circular permutation. Here, we report recent studies indicating that MNEI can form ordered fibrillar aggregates at 65°C, a temperature far below from its melting temperature, at pH 2.5 and in the presence of NaCl.[2] Spectroscopic and structural techniques, like fluorescence and TEM, have been chosen to follow the kinetic of the aggregation process step by step for MNEI and some selected mutants. This kind of studies support the increasing evidence that MNEI and its variants are suitable model to module the structural features of protein aggregates that can be used as building blocks for the construction of nanomaterials for various technological and biological applications, such as drug delivery, in cancer therapy, or as environmental biosensors. [3]

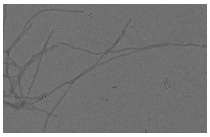

Figure 1. TEM image of MNEI incubated in 20 mM sodium phosphate buffer at pH 2.5 and 65 °C in the presence of 150 mM NaCl.

*References*

1. Donnarumma F, Leone S, Delfi M, et al. Probing structural changes during amyloid aggregation of the sweet protein MNEI. FEBS J. 2020;287(13):2808-2822.
2. Pica A, Leone S, Di Girolamo R, et al. pH driven fibrillar aggregation of the super-sweet protein Y65R-MNEI: A step-by-step structural analysis. Biochim Biophys Acta Gen Subj. 2018;1862(4):808-815.
3. Wei G, Su Z, Reynolds NP, et al. Self-assembling peptide and protein amyloids: from structure to tailored function in nanotechnology. Chem Soc Rev. 2017;46(15):4661-4708.

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# Functional cryogles for ab detection

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Early diagnosis of Alzheimer's Disease (AD) is essential for the development of preventive strategies.[1] Unfortunately, AD's initial symptoms are unspecific, and its diagnosis is often delayed to a time when the pathology is widespread.[2] Therefore, the identification or reliable signatures of specific biomarkers at the preclinical stage is of great interest.[3] The original design is the development of cryogel (Cyg) materials, functionalized with a peptide sequence able to identify β-amyloid protein. Such a system might represent a suitable non-invasive approach in the early diagnosis of Alzheimer's disease. The peptide GPGKLVFF (KL), which is deputed to the specific recognition of the Ab peptides, was grafted to the cryogel material according to different chemical protocols described in this communication. The success of the functionalization of Cygs was assessed by RAMAN and IR spectroscopies, while the morphology of the materials was observed by SEM imaging.

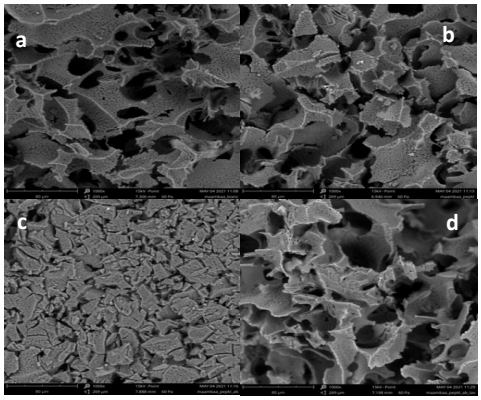


Figure 1. a: pristine Cyg; b: Cyg-KL; c: Cyg-KL_Ab loaded; d: Cyg-KL_Ab released.

The functional Cygs were also preliminary tested for their ability to "fish out" the Aβ protein from aqueous solution. These experiments were accomplished by means of MS analyses. The results presented in this communication might represent an encouraging starting point for the development of medical devices able to identify Aβ protein from biological fluids.

*References*

1. B. Veerabhadrappa, et al., Crit. Rev. Clin. Lab. Sci. (2019), doi: 10.1080/10408363.2019.1678011.
2. R.J.Bateman, et al., New Eng. J. Med., (2012), 367, 795-804.
3. Y. Zhou, et al., Chem. Asian J., (2016), 11, 805-817.

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# Lipopeptides-based nanoformulations for tissue engineering applications

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Lipopeptides represent peptide analogues in which the primary sequence is linked to one or more alkyl chains conferring amphiphilic properties and a general aggregative behavior, often resulting in extended fibril structures. [1] Lipidation is also a widely used strategy to improve the stability of peptide therapy in vivo, since lipopeptide derivatives confer more stability to peptidase activity. [2] All these interesting features make lipopeptides the starting point for the development of many bio-inspired/bio-derived materials for applications in nanotechnology, nanobiotechnology and nanomedicine, with involvement also of the tissue engineering field. Several lipopeptides were designed, synthesized and the effect of both the length of the alkyl chain and the peptide primary sequence on the self-assembling behavior was analyzed. [3,4] Their critical aggregation concentration (CAC) was determined through several comparative methods using circular dichroism (CD) and fluorescence; the secondary organization was assessed using spectroscopic methods (CD and FTIR) and the morphology of the aggregates was studied through cryogenic transmission electron microscopy (cryo-TEM) and Small-angle X-ray Scattering (SAXS). Lipopeptides capability to generate macroscopic hydrogel was tested as well. Cell viability assays were performed using both L929 fibroblasts and C2C12 myoblasts to examine the potential uses of the lipopeptides in tissue engineering, with a specific focus on application to cultured (lab-grown) meat based on myoblast cytocompatibility.

*References*

1. I.W. Hamley Soft Matter (2011), 7, 4122−4138.
2. I.W. Hamley Introduction to Peptide Science. Wiley: Chichester (2020).
3. E. Rosa et al. Biomacromolecules (2023), 24, 213−224.
4. E. Rosa et al. Soft Matter (2023), 19, 4686-4696.

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# Supramolecular Peptide-Folding Mediated Interactions for Tuning Lipid Vesicle Permeability

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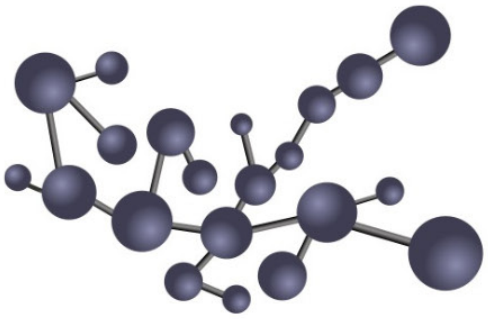
Membrane active peptides that offer tuneable interactions with lipid membranes are of large interest for development of novel therapeutics and drug delivery systems. The coiled coil peptide $KV_4C$ was originally de novo designed for self-assembly of nanomaterials[1] but was also found to be highly membrane active when conjugated to lipids.[2] Conjugation to lipids in vesicles results in rapid release of encapsulated compounds. Introducing the complementary peptide EI results in heterodimerization and folding into parallel coiled coils. At high stoichiometric excess of EI, the $KV_4C$-lipid membrane interactions are inhibited as a result of competing peptide-peptide interactions preventing vesicle cargo release.[3] Here we explore the lipid membrane activity of a large synthetic peptide (JUM1) comprising both $KV_4C$ and EI, linked via a flexible peptide spacer. JUM1 displays high helicity in both absence and presence of lipid vesicles indicating fully folded a-helical peptides in both cases. In the absence of vesicles, dynamic light scattering show formation of peptide complexes in the size range of 4-8 nm and formation of a small number of larger structures, indicating assembly of supramolecular peptide structures formed as a result of intra- and intermolecular oligomerization. Interestingly, in the presence of vesicles with maleimide headgroup functionalized lipids, JUM1 triggers rapid and extensive release of encapsulated carboxy fluoresceine (CF). No aggregation or degradation of the vesicles was observed. The helicity after conjugation was not significantly changed indicating that the folded supramolecular structures are interacting with the vesicles, resulting in disruption of lipid membrane integrity. These observations highlight the complexity of peptide-lipid interactions and demonstrate a novel supramolecular peptide complex for tuning vesicle release.

*References*

1. C. Aronsson, S. Dånmark,F. Zhou et al. Sci Rep 5 (2015), 14063.
2. C. Skyttner, R. Selegård, J. Larsson, C. Aronsson, K. Enander, D. Aili, Biochimica et Biophysica Acta (BBA) – Biomembranes (2019), 1861, 2.
3. C. Skyttner, K. Enander, C. Aronsson, D. Aili, Langmuir (2018), 34, 22.



Peptide Materials

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