Peptides in Biology and Materials

Bridging Simulation and Experimental Data

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In memoriam Lorenzo Stella

Book of Abstracts

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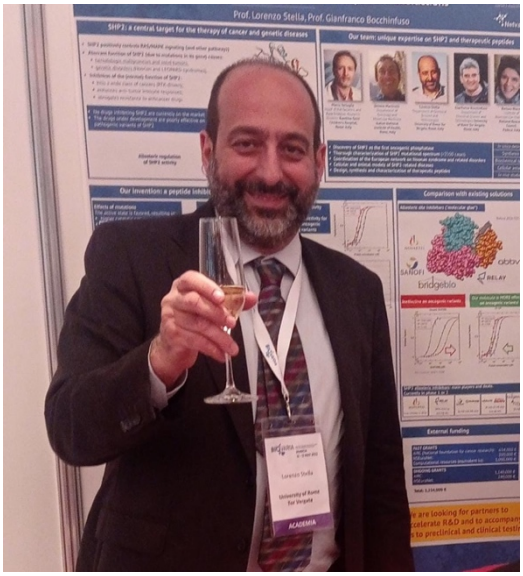
SOFIA UNIVERSITY St. KLIMENT OHRIDSKI

In memoriam Lorenzo Stella



The first edition of this workshop, held in Florence in 2024, was strongly promoted by Lorenzo Stella. Sadly, Lorenzo was unable to attend that workshop, as a severe illness had already left him greatly disabled. That same illness took him away on August 24, 2025.

Lorenzo Stella, born in Rome in 1968, was a Full Professor of Physical Chemistry at the Department of Chemical Science and Technologies, Tor Vergata University of Rome, which he joined in 1997 after graduating in Physics at the La Sapienza University of Rome (advisor Prof. G. Careri) and gaining international research experience at the University of Illinois at Urbana-Champaign, U.S.A. (advisor Prof. E. Gratton) and in the Laboratorium für Biochemie of the ETH Zürich.

Lorenzo was firmly convinced that collaboration and dialogue among researchers are the key to the advancement of science and he really loved taking part in and promoting conferences and workshops. He was also an active member of several scientific societies, including the Biophysical Society, being part of the Editorial Board of the Biophysical Journal (in the "Membranes" section), the Italian Peptide Society, which he co-founded and was also Secretary of, and the Italian Society for Pure and Applied Biophysics (SIBPA) as a member of the steering committee.

His choice to promote this workshop reflected the very essence of his scientific career, in which he combined spectroscopic techniques and computational methods with rare mastery, particularly fluorescence spectroscopy and molecular dynamics simulations. In the later stages of his career, he focused increasingly on peptides with therapeutic potential, including antimicrobial peptides to counter drug resistance and peptides interacting with proteins involved in carcinogenesis.

His absence leaves an immense void for all who knew him, both professionally and personally. We all owe him gratitude for his profound moral integrity, intellectual depth, and creativity. His legacy, rooted in the fundamental importance of bridging the experimental and computational worlds, will endure through all future editions of this workshop.

Oral Communications

Multiscale Simulations of Molecular Binding Reveal Mechanisms of Drug Action

Vittorio Limongelli

Faculty of Biomedical Sciences, Euler Institute, University of Lugano (USI), Switzerland
e-mail: vittoriolimongelli@gmail.com

Molecular binding events, including drug-protein and protein-protein interactions, underpin essential biological processes and represent a central focus of modern drug discovery. Yet their structural, thermodynamic, and kinetic characterization remains challenging because of the wide range of timescales and length scales involved. Here, we show how advanced molecular simulation methods can overcome these limitations by combining funnel metadynamics [1] with coarse-grained molecular dynamics [2] to capture binding processes under near-physiological conditions. This multiscale strategy enables quantitative prediction of binding thermodynamics and kinetics, offering mechanistic insight into molecular recognition and drug action.

We illustrate this framework in two relevant systems. First, we investigate G protein-coupled receptors (GPCRs), targets of about 30% of marketed drugs, probing both ligand binding and receptor organization into functional membrane dimers through minute-timescale simulations [3-5]. Second, we study Cy-9B, a rationally engineered stapled peptidomimetic that binds eIF4E and disrupts its interaction with eIF4G, thereby suppressing cap-dependent translation. Free-energy calculations reveal a non-canonical binding mode that helps explain its biological activity, including inhibition of lung cancer progression and rescue of disease-related phenotypes in neurodevelopmental models. These results demonstrate the growing potential of molecular binding simulations to drive next-generation drug design.

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HIV-1 Vpu-host calmodulin complexes: Mechanisms and inhibition

Elka R. Georgieva,[1] Adeyemi Ogunbowale,[1] Elaheh Hadadianpour,[1] Olamide Ishola,[1] Peter P. Borbat,[2] Md Majharul Islam,[1] Arvin Saffarian Delkhosh,[1] Natalie Ramos[1]

[1]Department of Chemistry and Biochemistry, Texas Tech University, TX, USA
[2]Department of Chemistry and Chemical Biology, Cornell University, NY, USA
e-mail: elgeorgi@ttu.edu

My lab is interested in understanding how HIV-1 proteins interact with host calmodulin (CaM) and design drugs to inhibit these interactions. Most HIV-1 proteins have unique, non-canonical CaM-binding motifs. These interactions support HIV-1 protein trafficking, membrane insertion, apoptosis regulation, and contribute to CD4+ lymphocyte loss. Higher CaM levels in HIV-1 infected cells highlight its importance in the viral life cycle. While HIV-1 efficiently exploits CaM, the precise mechanisms remain unclear.

We study how HIV-1 Vpu protein interacts with Ca^{2+}-CaM. We recently discovered that this membrane protein can exist in a soluble oligomeric form, which may serve as a protein storage or condensate with unknown physiological function. We further demonstrated for the first time that the soluble Vpu forms a complex with Ca^{2+}-CaM, suggesting this interaction may help Vpu shuttle within the cell.

Here, we utilized ensemble FRET, EPR spectroscopy and mutational analysis to quantify and characterize in detail the Vpu-Ca^{2+}-CaM association. We found that the wild-type full-length (FL) Vpu binds Ca^{2+}-CaM with energy of about -10 kcal/mol, thus forming a stable complex. Reduction of about 1-1.3 kcal/mol in the binding energy was observed for a fragment of only the Vpu's C-terminal region, which does not contain the cluster of hydrophobic residues in the CaM-binding motif. Further, the Vpu-Ca^{2+}-CaM complex was destabilized yielding a reduction of 2-2.5 kcal/mol in the binding energy when we used FL Vpu mutants in which either four positively charged residues in the soluble region or hydrophobic residues in the TM helix 1 of the CaM-binding motif were substituted for different amino acids. Pulse EPR (DEER) studies on spin-labeled Vpu and CaM are ongoing to capture the conformational differences in the heterocomplex caused by these mutations. Thus, our results suggest that both hydrophobic and electrostatic forces contribute to the Vpu-Ca^{2+}-CaM complex stabilization. We further used AI-based software to rationally design short peptides, which interact with the CaM-binding motif of Vpu, and experiments are ongoing to verify whether these peptides can prevent Vpu-CaM association. If successful, we may develop a mean to inhibit Vpu delivery to the membrane through the CaM-shuttle pathway, thus abolishing key HIV-1 mechanisms in the cell.

Phosphates Drive the Assembly of Fibers Made from the α-Helical Lentiviral Transduction Enhancer Vectofusin

B. Bechinger,[1,2] A. Saad[1], J. Jiang[1], M. Lointier[1], J. Raya[1]

[1]University of Strasbourg / CNRS, Chemistry Institute UMR7177, F-67000 Strasbourg
[2]Institut Universitaire de France, F-75005 Paris

e-mail: bechinge@unistra.fr

Vectofusin-1, a member of the 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 fibers formed by this relatively short sequence have potent therapeutic potential. The vectofusin-1 fibrils have a unique structure being assembled from short α-helices in the presence of multivalent anions whereas most other viral transduction enhancers form β-amyloid fibrils. We will present a detailed structural analysis using solid-state NMR, EM and biochemical approaches. Our observations define vectofusin-1 as a member of a new class of α-helical lentiviral transduction enhancers. Its fibril formation in the presence of phosphates is reversible which bears considerable advantages in handling the peptide in conditions of gene therapy protocols. Furthermore, similar interactions involving a diffuse electrostatic cloud should also happen when polypeptides interact with phospholipids, nucleic acids and metabolites which makes the study of general significance.

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Molecular Dynamics and Experimental Synergy to Understand Biomolecular Behavior

Alessandra Villa

Royal Institute of Technology, Center for High Performance Computing, Stockholm, Sweden
e-mail: avilla@kth.se

In this talk, I will discuss how molecular dynamics simulations can be integrated with experimental approaches and other modeling techniques to provide a comprehensive view of biomolecular behavior in biological contexts. Peptide based molecules play essential roles across biology, medicine, and materials science. Understanding the relationship between biomolecular sequence, environment, and molecular function remains challenging and requires a combination of approaches that can connect atomistic mechanisms with experimentally observable behavior.

I will present several use cases and highlight case studies in which molecular dynamics simulations of peptides, proteins, and cell membranes provide detailed insight into the structural and dynamical features of biological systems and their interactions with the environment (e.g., solvents, membranes, and surfaces), while experimental data supply critical validation and constraints.

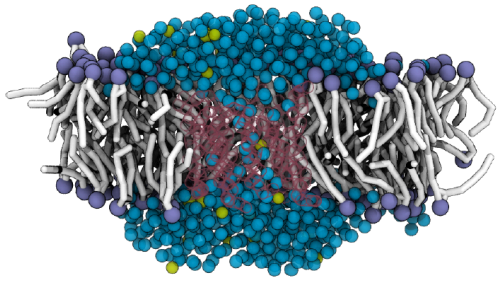
Alamethicin pore formation in Martini 3 lipid membranes

Ana C. Borges-Araújo, Manuel N. Melo

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal

e-mail: *ana.araujo@itqb.unl.pt*

Transmembrane pores are responsible for regulating ion flux, which is essential for the maintenance of cell life. Antimicrobial peptides (AMPs) are known for forming pores in pathogen membranes that disrupt this metabolite concentration control, leading to cell death. Alamethicin (Alm) is an AMP produced by the fungus *Trichoderma viride*, known for its action against bacteria and fungi. As its mechanism of action, Alm aggregates to form ion-conducting channels according to the barrel-stave model — in which the peptides' hydrophilic group is aligned towards the centre of the pore. Previous all-atom molecular dynamics studies have tried to simulate Alm's action mechanism, but computational power is a limitation. Coarse-grain (CG) modeling is an appealing approach, as it can cover the time and size scales of peptide association and membrane pore formation. However, the widely used Martini 2 CG force field could not accurately simulate Alm's pore formation behavior. In our work, we employ the newly improved Martini 3 model and show that pore formation by Alm can now be observed. We also simulated systems using the recently released GōMartini 3 model for proteins, which significantly improved pore conductivity. Alm conductance was optimal when peptides were in parallel conformation. Additionally, with the GōMartini model, we are now closer to replicating the discrete levels of conductance that are seen experimentally. Overall, our results suggest that a more flexible Martini 3 protein model may improve pore formation modelling and provide us with more accurate insight.



Top view of an Alm cluster (peptide backbone represented in see-through). Lipid heads are colored in lilac, lipid tails in white, the alamethicin backbone in red, water molecules in blue and ions in yellow.

Acknowledgements

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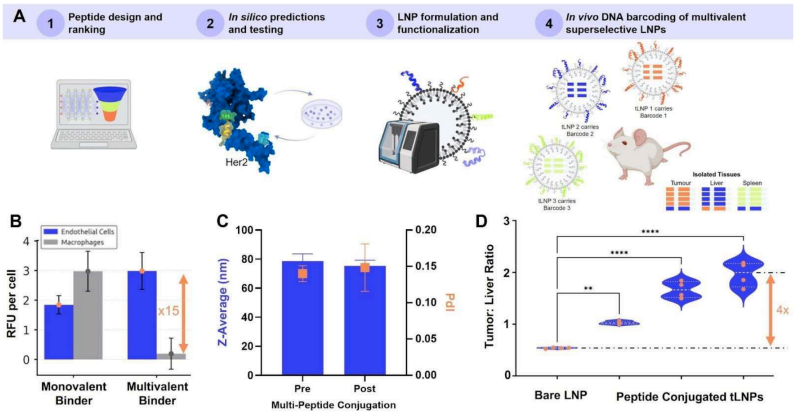
Multi-peptide targeted lipid nanoparticles (tLNPs) for cell-specific delivery

Sara Carvalho[1,†], Daniele Visco[1,†], Shanil Panara[1], Christopher Lau[1], Debesh Mandal[1], Stefano Angioletti-Uberti[1,2,*]

[1]Nanograb, Ltd, London, UK.
[2]Department of Materials, Imperial College London, London, UK.
[†]Shared contribution; [*]Corresponding author

e-mail: sara.carvalho@nanograb.com

Breast cancer, the world's most prevalent malignancy, relies on HER2 as a critical therapeutic target. However, treating it is hampered by baseline HER2 expression in healthy tissues, causing severe on-target, off-tumor toxicity. Current therapies fail to differentiate tumor overexpression from normal receptor levels. While targeted lipid nanoparticles (tLNPs) offer a scalable in vivo delivery solution, precise tumor specificity remains difficult. To solve this, we developed a multivalent peptide tLNP. Leveraging multivalent peptide binder combinations to multiple HER2 epitopes, our design achieves "super-selectivity," preferentially targeting cells with high-density HER2 overexpression. Peptide design was achieved via a bespoke generative pipeline: large peptide libraries created via ColabDesign and ProteinMPNN underwent structural filtering (AlphaFold2-Multimer) and 1 µs molecular dynamics simulations in explicit water to investigate binding stability. A proprietary algorithm then identified optimal binder combinations maximizing LNP selectivity and binding strength. To facilitate targeting, LNPs were functionalized to longer PEG chains via click-chemistry and barcoded with unique DNA sequences. These formulations consistently demonstrated excellent physicochemical properties and colloidal stability. LNPs were multiplexed and screened against untargeted benchmarks in NSG mice bearing BT474 xenografts. DNA barcode distribution in tumor, liver and spleen was quantified via NGS. Analysis of barcoded DNA targeting efficiency revealed a 4-fold improvement in tumor accumulation for the lead tLNP formulation compared to the untargeted benchmark at 24 hours post-administration. This platform successfully enhanced solid tumor targeting through unique peptide combinations selected via a proprietary algorithm rooted in the theory of multivalent super-selectivity.



*Integrated Generative Pipeline and In Vivo Validation of Super-selective tLNPs. (A) Rational design and testing of multivalent superselective tLNPs. (B) In vitro selective cellular uptake of fluorescently labelled polymersomes showing tunable selectivity via multivalent binder combinations (adapted from Tian et al. 2020). (C) Size and polydispersity characterization of proprietary LNP formulation pre and post functionalization with HER2 specific peptide binders. (D) Ratio of DNA barcodes measured in the tumor versus the liver of bare LNP and lead tLNP formulations showing a 4x increase in tumor targeting. **: p<0.01, ****: p < 0.0001.*

SARS-CoV-2 NSP8-derived peptides can inhibit NSP13

Peicho Petkov[a], **Elena Lilkova**[b], Nevena Ilieva[b], Shina Pashova[c], Elena Krachmarova[d], Anastas Pashov[e], Miroslav Rangelov[f], and Leandar Litov[a]

[a]Sofia University "St. Kl. Ohridsky", Physics Faculty
[b]Institute of Information and Communication Technologies, Bulgarian Academy of Sciences
[c]Institute of Biology and Immunology of Reproduction "Acad. K. Bratanov", Bulgarian Academy of Sciences
[d]Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences
[e]"Stephan Angeloff" Institute of Microbiology, Bulgarian Academy of Sciences
[f]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences
e-mail: elena.lilkova@gmail.com

This work explores a novel antiviral approach against SARS-CoV-2 by targeting the helicase NSP13, a key enzyme involved in viral RNA replication and modulation of host innate immunity. In addition to its role within the replication–transcription complex (RTC), NSP13 suppresses type I interferon (IFN-I) responses through interaction with the host kinase TBK1, making its interaction interfaces attractive targets for intervention.

We investigated whether peptides derived from the viral cofactor NSP8 can disrupt NSP13 function through competitive binding. Combining molecular dynamics simulations, structural interface analysis, and neural-network-based binding free energy estimation, we identified the N-terminal fragment of NSP8 (residues 1–87) as a potent NSP13 binder. Structural comparisons indicate that the NSP8 N-terminal peptide overlaps with the TBK1-binding surface, suggesting direct competition, and calculated binding energies show stronger association than native NSP8–NSP13 or NSP13–TBK1 interactions.

Cell-based assays in poly(I:C)-stimulated A549 cells confirm these findings: NSP13 reduces IFN-β expression, whereas co-expression with full-length NSP8 or its N-terminal domain restores interferon signaling. In contrast, the C-terminal region of NSP8 does not reverse this suppression.

Overall, the results support a dual mechanism in which the NSP8 N-terminal segment sequesters NSP13, blocking both its participation in viral replication and its inhibition of host immune signaling, representing a promising template for peptide-based inhibitor design.

Acknowledgements

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Development of a broad-spectrum peptide inhibitor of flaviviruses assembling and replication

Eva Dias[a], Nelly Silva[a,b], Ivo C. Martins[a,b], Gonçalo Seixas[a], **Nuno C. Santos**[a,b]

[a] GIMM – Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal
[b] Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

e-mail: nsantos@medicina.ulisboa.pt

Dengue (DENV) and Zika (ZIKV) viruses are closely related flaviviruses that have become a major global threat due to climate change and expansion of their mosquito vectors[1]. Their virion includes an outer lipid bilayer containing two viral proteins and, within, a nucleocapsid core with the viral RNA complexed with multiple copies of the capsid (C) protein[1-6]. This crucial structural protein is involved in viral assembly and encapsidation. As previously studied by us[2-6], DENV replication requires its C protein binding to host lipid systems, namely lipid droplets (LDs) and very low density lipoproteins (VLDL). Those studies allowed us to develop pep14-23[2,3], a peptide inhibitor of the binding to host lipid systems, based on a flavivirus C protein conserved segment among related viruses. Following, we identified the targets of C protein within each host lipid system: apolipoprotein E (ApoE) and perilipin 3, structurally analogous proteins found at the surface of, respectively, VLDL and LDs[4,5]. Afterwards, we established that West Nile virus (WNV)[7] and ZIKV[8] rely on the same interactions with host lipid systems.

Recently, we developed a new peptide as a potential broad-spectrum antiviral against related falviviruses, mostly based on an ApoE domain, but also with structural similarities with a perilipin 3 domain. Vero 81 cells were infected with ZIKV or DENV, and treated with pep14-23 or this new peptide. Viral replication was monitored using plaque assays for ZIKV and indirect immunofluorescence for DENV. In ZIKV-infected cells, pep14-23 caused only a modest decrease in viral replication, whereas the new peptide exhibited strong inhibition both at 12 and 24 h post-treatment. In DENV-infected cells, pep14-23 showed no significant effect, while the new molecule achieved complete suppression of viral replication, with no detectable DENV from 3 to 24 h. This strong antiviral activity (without associated cytotoxicity) was totally absent on a scrambled version of the new peptide, demonstrating sequence specificity. These results highlight the translational potential of the new peptide, opening new avenues for its development as antiviral.

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Translocation of Amphiphilic Peptides Across Cell Membranes

Robert Vacha

CEITEC and Faculty of Science, Masaryk University, Brno, Czech Republic
e-mail: robert.vacha@muni.cz

Cell membranes act as semi-permeable barriers that restrict the entry of large or hydrophilic molecules into cells, limiting the development of new drugs. Peptides with the right properties can bypass the cellular machinery and spontaneously translocate across membranes. However, it remains elusive what these "right" peptide properties are. We developed a new collective variable describing the translocation process, which allowed us to calculate the translocation free energy profiles of various peptides and demonstrate that amphiphilic peptides can spontaneously translocate across the membrane. We have shown that membrane asymmetry could reduce the translocation barrier. However, even in these cases, the translocation free energy barrier remained significant. We therefore investigated whether the barrier could be reduced by transmembrane proteins. Using two very different models, we consistently show that transmembrane proteins with hydrophilic residues in the hydrophobic core of the membrane can enhance peptide translocation. We have shown that there are natural membrane proteins with such properties, scramblase or insertase. These proteins facilitate the translocation of lipids or the insertion of proteins into the membrane, and we have shown that they share the common mechanism of local membrane deformation, which also reduces the translocation barrier of amphiphilic peptides. Therefore, scramblases and insertases could be exploited for more efficient drug delivery systems, especially those involving the translocation of amphiphilic peptides into the cell.

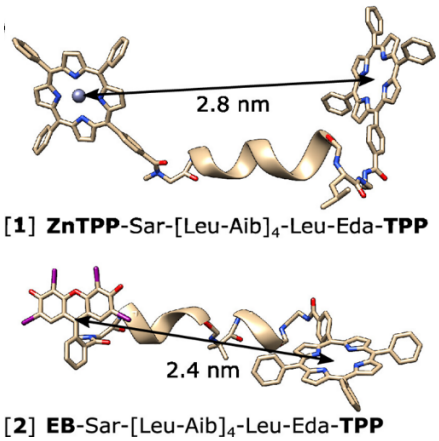
Bis-labeled helical Aib-peptides as reliable building blocks for spectroscopic and photoelectrochemical applications

M. De Zotti,[a] E. Gatto,[b] A. Bertran,[c] A. M. Bowen,[c]

[a]Department of Chemistry, University of Padova, Padova, Italy
[b]Department of Chemical Science and Technologies, University of Rome, Tor Vergata, Rome, Italy
[c]University of Manchester, Manchester, UK

e-mail: marta.dezotti@unipd.it

Peptides based on Aib (α-aminoisobutyric acid) residues are able to adopt, even when short, well-defined helical conformations, endowed with great stability outside the physiological environment. For this reason, they have been used as reliable rulers for the development and validation of new spectroscopies, such as 2D-Infrared spectroscopy [1]. In this presentation, we will show several applications of the peculiar conformational properties of Aib-containing peptides. We will describe how bis-labeled Aib-peptides containing orthogonal chromophore pairs (*e.g.*, Figure 1) enabled the first parallel distance measurements by electron spin resonance (ESR) and Förster resonance energy transfer (FRET), allowing for direct comparison [2]. Besides, we will see how the rigid 3_{10}-/α-helical structure of Aib-containing peptides can efficiently mediate electron transfer between a porphyrin dye and a gold electrode and how it might be possible to exploit them as self-assembling biomolecular wires.



Examples of bis-labeled Aib-peptides, with the chromophore center-to-center distances determined by in vacuo DFT optimization. Eda, ethylenediamine linker.

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Self-Association and Cooperative Assembly in Antimicrobial Peptide Solutions

Peicho Petkov[a], Rossitsa Marinova[a], Lyudmila Velkova[b], Aleksandar Dolashki[b], Petar Petrov[b], Leandar Litov[a], Elena Lilkova[c], Petya Sirakova[a], Kamelia Kamburova[e], Elena Krachmarova[f], Stefka Taneva[g], Frédéric Allemand[h], Assia Mouhand[h], Aurélien Thureau[i], Nathalie Sibille[h], Pavlina Dolashka[b], Nevena Ilieva[c,d]

[a]*Faculty of Physics, Sofia University "St. Kliment Ohridski"*
[b]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences*
[c]*Institute of Information and Communication Technologies, Bulgarian Academy of Sciences*
[e]*Institute of Physical Chemistry, Bulgarian Academy of Sciences*
[f]*Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences*
[g]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
[h]*Centre de Biologie Structurale, CNRS – Université de Montpellier – INSERM*
[i]*HélioBio Section, Synchrotron SOLEIL*

e-mail: peicho@phys.uni-sofia.bg

Antimicrobial peptides (AMPs) are promising alternatives to conventional antibiotics, but their mechanisms in solution remain unclear. Recent studies combining computational, physicochemical, and biological approaches highlight peptide self-association and cluster formation as key determinants of antimicrobial activity. Simulations of peptides derived from *Cornu aspersum* mucus show spontaneous aggregation into dynamic nanoscale oligomers driven mainly by hydrophobic interactions, with reduced solvent exposure and burial of aromatic residues such as tryptophan.

Spectroscopic analyses confirm concentration-dependent oligomerization, with more hydrophobic peptides forming larger, more stable clusters at lower concentrations. In mixed systems, co-aggregation produces distinct structural organization, suggesting functional specialization. Biological assays link aggregation to antimicrobial efficacy: larger clusters show stronger activity, and mixtures exhibit synergistic effects. Overall, AMP self-assembly into transient nanoscale clusters appears central to their function, connecting physicochemical properties, structural dynamics, and biological activity, and informing peptide-based therapeutic design.

Acknowledgements

The computational resources for this study were provided by the Discoverer supercomputer, the HPC cluster BioSim at Sofia University, CI TASK – Gdansk (Poland). We thank the synchrotron facility SOLEIL for allocating regular beam time and the DISCO beamline for the acquisition of CD data on their in-house time. We thank the Biophysical Characterization facility of the Biocampus PIBBPS platform.
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Liquid-liquid phase separation-derived peptide coacervates as catalytic microenvironments

Ana S. Pina

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal

e-mail: ana.pina@itqb.unl.pt

Nature has optimized enzymes that exploit liquid–liquid phase separation (LLPS) to enhance catalytic efficiency in key metabolic pathways, by using dynamic, membrane-less compartments where flexibility and disorder are harnessed for biochemical control[1]. Although catalysis is often linked to rigid macromolecular scaffolds, flexible dynamic domains and their local environments are now recognized to play a key role in enzyme function[2]. By contrast, peptide catalysts in water remain challenging because this same conformational plasticity is typically viewed as a liability. Using LLPS to sculpt peptide conformational landscapes and microenvironments offers a direct strategy to convert flexibility into a design feature and to probe how sequence-encoded dynamics give rise to catalytic function in biological and synthetic systems.

Starting from a phosphotyrosine-binding hairpin peptide (P7) discovered by supramolecular targets- guided phage display[3], we show that a single catalytic pptide sequence can drive LLPS, which constrains conformational heterogeneity into structured β-hairpin domains and boosts phosphatase-like activity by ~15 000-fold relative to the soluble state[4]. These peptide-based coacervates function as programmable microreactors that concentrate guests and selectively recruit phosphorylated protein assemblies, demonstrating that molecular recognition, compartmentalization and catalysis can be encoded within one low-complexity sequence. Complementary bioinformatics on droplet-promoting regions from 178 phase-separating proteins identify 129 enriched short motifs, dominated by Gly/Pro-rich segments interspersed with aromatic, charged and polar residues, and reveal family-specific biases. Motif co-occurrence analysis then guided us towards the design of minimal (10–14-mer) LLPS-prone peptides that form liquid-like coacervates under physiological conditions, with tunable internal dynamics and encapsulation properties validated experimentally[5]. Together, our results bridge computational motif discovery and experimental peptide engineering to deliver sequence-centric design rules for synthetic peptide coacervates that couple LLPS, molecular recognition and catalysis for sustainable catalysis and cell-free synthetic biology.

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Characterizations of amyloid-like assemblies: the cases of transthyretin and low complexity sequences

Luigi Vitagliano

Institute of Biostructures and Bioimaging, CNR, Naples, Italy
e-mail: luigi.vitagliano@cnr.it

Proteins and peptides can form supramolecular assemblies through self-association, which often possess unique structural, functional, and spectroscopic properties. In the last two decades, to gain a deep understanding of the unique properties of these assemblies, we have used a combination of experimental and computational techniques. Starting from model systems potentially involved in diseases, our focus has progressively shifted toward self-assembling peptides of biotechnological interest[1-6]. Very recently, our activities have focused on exploiting the growing structural information in the Protein Data Bank to design new self-assembling peptides. In particular, using transthyretin as a model system, we have demonstrated that the PDB constitutes a treasure trove for developing new, attractive peptide-based materials[7]. A similar experimental/computational procedure is currently being used to investigate the structural features of peptides with low-complexity sequences. Within this framework, we are developing stimuli-responsive hydrogels from liquid-liquid phase separations of FUS-derived peptides8 and structurally characterizing self-assembling fragments of intrinsically disordered proteins (manuscript in preparation).

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Aggregation of transthyretin in the presence of chitosan-based nanogels loaded with quercetin

V. Milkova[a,b], A. Guyrova[a], D. Arabadzhieva[a], I. Minkov[a]

[a]Rostislaw Kaischew Institute of Physical Chemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl.11, Sofia, Bulgaria
[b]National Centre of Excellence Mechatronics and Clean Technologies, 8 bul. Kliment Ohridski, P.C – 1756, Sofia, Bulgaria

e-mail: viktoria.milkova44@gmail.com

Human transthyretin (TTR) is a globular protein with a natural tetrameric structure involved in the transport of the thyroid hormone thyroxine and retinol. As a result of mutations, TTR may undergo misfolding and subsequent formation of fibril aggregates (amyloidosis), which can cause several pathological conditions, such as familial amyloid polyneuropathy, familial amyloid cardiomyopathy, senile systemic amyloidosis, and can also accumulate deposits in the eye, contributing to the rise in the ocular pressure (glaucoma). Recently, the potential of natural, non-toxic compounds, in particular polyphenols, has been explored for their ability to interact with amyloidogenic proteins, preventing fibril formation by stabilising their initial structure.

In this study, the aggregation kinetics of TTR were studied by monitoring the variation of the size distribution of the mixed dispersion of the peptide and nanoformulations. Nanogels from chitosan with different molecular weights were produced using a well-established procedure. The developed quercetin-loaded and unloaded nanostructures had a highly negative electrokinetic potential and remarkable stability.

The appearance and evolution of the aggregation of TTR were studied using variations in a few parameters: the quartiles (D10, D50, and D90) in the particle size distribution of the dispersion, the average diffusion coefficient of the samples, the Congo red assay, and microscopy.

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Antimicrobial peptides interaction with live cells: quantitative insights and future challenges

Daniela Roversi

Department of Chemical Science and Technologies, Tor Vergata University of Rome, 00133 Rome, Italy.

e-mail: daniela.roversi@uniroma2.it

Antimicrobial peptides (AMPs) are promising agents against drug-resistant microbes due to their ability to kill bacteria by disrupting their cellular membranes. While extensively studied in model systems, quantitative data in living cells remain limited. We developed spectroscopic assays to characterize both the thermodynamic and kinetic aspects of AMP interactions with live bacterial cells, bridging the gap between biophysical studies and microbiological assays. Our results showed that millions of molecules accumulate on each cell to cause bacterial death.[1,2] In addition to causing pore formation, we demonstrated through fluorescence measurements and molecular dynamics simulations that this high coverage of the membrane surface leads to significant perturbation of the lipid dynamics, which might contribute to the bactericidal mechanism.[3]

To gain further insight into the sequence of events involved in bacterial killing, we carried out kinetic characterization of AMPs-bacteria interaction. By combining stopped-flow measurements with fluorescence-based kinetic analyses, we examined these interactions over a wide range of timescales, from milliseconds to minutes. Our findings indicate that AMP associates with bacterial membranes in under a second, perturbs them causing bacterial death before any perturbation of the inner membrane occurs, and ultimately accumulates within the cells.

AMP activity and selectivity were found to depend strongly on the concentrations of both target and host cells, highlighting limitations of standard activity assays performed at fixed, standardized cell densities.[4] Extending these quantitative analyses to eukaryotic cells, we evaluated peptide selectivity toward pathogens versus host cells.

Finally, we demonstrated that the peptide–cell binding equilibrium also plays a key role in the "mutant selection window," the antimicrobial concentration range in which some bacteria are killed while others survive, allowing resistance to develop.[5]

Overall, our findings demonstrate that quantitative data can be obtained directly from live cells, shedding light on key aspects of AMP behavior while opening up new, largely unexplored directions for future research, including machine learning approaches to design more efficient molecules, and raising important questions for further investigations.

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The IgOme as a Universal Biosensor: Peptide Mimotopes of the Public IgM Repertoire Across Diseases

Shina Pashova-Dimova[a], Dilyan Ferdinandov[b], Lubomir Balabanski[c], Gabriel Elmadjian[a], Elena Keskinova[d], Sena Karachanak-Yankova[e], Yavor Zhelev[f], Shima Mehrabian[f], Draga Toncheva[e], Lachezar Traykov[f], **Anastas Pashov**[d]

[a] Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia
[b] Clinic of Neurosurgery, St. Ivan Rilski University Hospital, Sofia
[c] Genomics Laboratory, Hospital "Malinov", Sofia
[d] Institute of Microbiology, Bulgarian Academy of Sciences, Sofia
[e] Department of Medical Genetics, Medical University, Sofia
[f] Clinic of Neurology, UH "Alexandrovska", Medical University, Sofia

e-mail: a_pashov@microbio.bas.bg

We have developed and validated a scalable platform for monitoring changes in the IgM antibody repertoire as a constitutive sensor of the internal environment. It is based on the concept of the IgOme: a mimotope set consisting of a 7-mer random peptide library panned on IgM pooled from ~10,000 healthy donors. The IgOme library of over 200,000 mimotopes is clustered into 790 groups, yielding a representative set of ~700 mimotopes that samples this sequence space symmetrically and encodes more than 10^{70} potential reactivity profiles when tested in a peptide microarray.

We demonstrate the broad applicability of IgOme across distinct disease contexts. In neurodegenerative diseases, Alzheimer's disease and frontotemporal dementia were distinguished by the loss of public IgM reactivities that cross-react with other antibodies. In antiphospholipid syndrome (APS), profiling of the IgM and IgG IgGomes yielded 55 distinct patterns of differential representation of sequence motifs across 4 libraries (IgM and IgG in controls and APS). Antibody/antibody connectivity was quantified by cross-matching against a library of 6×10^6 naive IgH CDR3 sequences, revealing that antibody/antibody reactivity was high in IgM and low in APS IgG. Many IgM reactivities were lost in IgM APS. This coupling between the repertoire-wide IgM changes and antibody/antibody interactions reveals changes in APS beyond the pathogenic IgG auto-reactivities. At the same time, among various motifs lost in the APS IgM repertoire, a small number matched the known anti-β2GPI epitope.

The IgOme/reactivity graph framework thus provides a disease-agnostic and accessible window into the global immune state. It is a broadly applicable peptide-based platform for diagnostics, monitoring, and the mechanistic dissection of repertoire-level immunopathology.

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Function-driven design of protein assemblies

Alena Khmelinskaia

Ludwig-Maximilians-Universität München; Butenandtstr. 11, Haus B, B2.025; 81377 München
e-mail: akhmelin@cup.lmu.de

Recent computational methods have been developed for designing novel protein assemblies with atomic-level accuracy. Yet, when compared to their natural counterparts, the structural and functional space covered by de novo designed assemblies remains limited. I will share with you our ongoing efforts in diversifying the structural repertoire of protein assemblies and developing strategies to dynamically control protein assembly state. First, I will describe our approaches to the diversify assembly geometries beyond simple polyhedral geometries, such as linked architectures assembled from rigid building blocks following quasi-equivalence principles. Then, I will present our generalizable interface-seeded design approach for the generation of environemnt responsive oligomers driven by ion-mediate, small molecule-dependent orphosphorylation-triggered protein-protein interfaces. By leveraging novel architectures and a diversity of endogenous and exogenous signals, we aim to generate orthogonal, programmable control elements for synthetic biology.

Peptides in viral pandemics: From entry mechanisms to therapeutic strategies

Carolina C. Buga[a,b], Diogo H. Silva[a], Mariana Valério[a], Pedro Moreira[a,c], Luís B. Araújo[1], Carlota Conceição[a], Mariana Parada[a], Marta Miranda[b,d], Christine Cruz-Oliveira[b], Manuel N. Melo[a], César Santiago[e], José M. Valpuesta[e], M. Alenquer[d], M. J. Amorim[d], Ana S. Veiga[b,f], Miguel A. R. B. Castanho[b,f], João B. Vicente[a], Cláudio M. Soares[a] and **Diana Lousa**[a]

[a]Instituto de Tecnologia Química e Biológica António Xavier – ITQB NOVA, Oeiras, Portugal
[b]Gulbenkian Institute for Molecular Medicine, Lisboa, Portugal
[c]CEB, Universidade do Minho
[d]Catolica Biomedical Research Centre, Faculdade de Medicina, Universidade Católica Portuguesa
[e]Spanish National Research Council (CSIC), Madrid, Spain
[f]Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

e-mail: dlousa@itqb.unl.pt

The COVID-19 pandemic, responsible for over seven million deaths worldwide, exposed the urgent need to better understand viral entry mechanisms and to develop novel antiviral strategies. Peptides are central to both challenges, since they not only play key roles in viral infection but also represent powerful tools for therapeutic intervention. Within the international projects EvaMobs, BioPlaTTAR, and SHIELD, we are addressing these challenges comprehensively.

Using SARS-CoV-2 as a model, we investigated the mechanisms of two critical players in viral entry: the receptor-binding domain (RBD) and the fusion peptide. Through combined computational and experimental analyses, we discovered that SARS-CoV-2 employs a bipartite fusion module formed by two noncontiguous segments of the spike protein that act synergistically. One region initiates membrane contact, while the other penetrates more deeply, promoting membrane perturbations required for fusion. This module strongly impacts membrane structure, inducing pore formation, lipid tail protrusion, and local disorder, collectively lowering the energy barrier for membrane fusion. In parallel, we characterized the RBD structural dynamics, revealing high conformational flexibility and variant-dependent structural adaptation.

Building on these insights, we designed potent peptide-based antivirals against SARS-CoV-2 using a protocol that integrates AI-driven and computational biophysical methods, including molecular dynamics simulations. We achieved a success rate over 50%, as measured by the ability of designed binders to attach to their targets. Remarkably, the top candidates displayed neutralization IC_{50} values below 100 pM, without requiring any experimental optimization. Together, these findings demonstrate that peptides are not only crucial players in viral infection mechanisms but also promising and highly effective components of next-generation antiviral strategies.

Poster Communications

Assessing Peptide Dissociation Kinetics in MHC Class II Allotypes via τRAMD Simulations

Vinicius Ávila Cabral,[a,c] Hugo Verli,[a,b] Rebecca C. Wade [c]

[a] Federal University of Rio Grande do Sul (UFRGS)
[b] Brazilian Center for Research in Energy and Materials (CNPEM)
[c] Heidelberg Institute for Theoretical Studies (HITS)
e-mail: vinicius.cabral@h-its.org

Peptide binding and dissociation from major histocompatibility complex class II (MHC-II) molecules are central to antigen presentation and immune recognition. MHC-II is a heterodimer composed of α and β chains, with a highly polymorphic β chain, making atomistic characterization of peptide residence times and dissociation mechanisms across allotypes challenging[1]. Here, we apply τ-Random Acceleration Molecular Dynamics (τRAMD)[2] to investigate peptide unbinding across the twelve most prevalent human MHC-II allotypes, focusing on HLA-DR isotype in the presence and absence of the peptide exchange catalyst HLA-DM.

τRAMD simulations on DR and DR–DM complexes bound to the placeholder peptide $CLIP_{102-120}$[3] and the influenza hemagglutinin peptide $HA_{306-318}$[4] using the CHARMM36m force field. For HLA-DR–peptide complexes, we observe a better correlation of computed residence times with experimental data for high-affinity peptides such as HA than for CLIP, likely due to CLIP's greater length and flexibility. We further assessed different force field (AMBER14SB, AMBER19SB, and AMBER99SB-disp) for three allotypes, obtaining results consistent with CHARMM36m. In contrast, no clear correlation of computed and experimental residence times was observed for HLA-DR–DM complexes bound to CLIP, suggesting additional complexity in DM-mediated peptide exchange.

Applying the dissociation force to the four N-terminal residues, rather than the peptide center of mass, better captures the "unzipping" mechanism of peptide release and reduces variance. MD-IFP analyses reveal two distinct CLIP egress pathways from the N- and C-termini.

Overall, this work establishes τRAMD as a robust framework for probing peptide–MHC-II dissociation kinetics, with ongoing efforts to refine the approach and better capture complex cases such as DM-mediated peptide exchange.

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Analysis of reactivity repertoires of monoclonal antibodies using random peptide libraries

Elena Keskinova, Anastas Pashov

Department of Immunology, Institute of Microbiology, BAS, Sofia, Bulgaria

e-mail: Elena.keskinova@microbio.bas.bg

Antibodies exhibit vast diversity exceeding 10^{15} clonotypes, enabling recognition of nearly any antigen. Selection from phage-display random peptide libraries followed by next-generation sequencing (IgOme analysis) provides high-throughput mapping of the overlapping antibody reactivities in the repertoire. The interpretation of the repertoire representation should start with the understanding of single antibody IgOme profiles.

Here, IgOme data from four monoclonal antibodies (Herceptin, 21c, 17b, and b12) was analyzed[1]. Unique mimotopes (n=35601) were extracted and used to construct graphs from 5-mer common subsequences, which were clustered using the Leiden algorithm. The motifs of the clusters were compared to the sequence of the known conformational epitopes. Chosen representative mimotopes were docked with HADDOCK to analyze the common contacts of mimotopes and epitope with the paratope amino acid residues.

The Leiden algorithm generated from 110 to 262 clusters per antibody, from which only up to 7 corresponded to epitope-resembling motifs. The lack of similarity to the epitope could be partially due to the underrepresentation of some motifs in the library. The motif analysis showed a large variety of potential antibody ligands, suggesting that the otherwise observed antibody specificity is relative to the antigenic landscape. Molecular docking experiments underlined the need for developing a more appropriate algorithm for determining correspondence between mimotopes and epitopes.

Thus, the presented approach provides a framework for dissecting the complete range of antibody specificities of single clones, helping to understand how they shape the human antibody repertoire.

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Development of Enzymatic Methods for C-terminal Amidation of Peptides

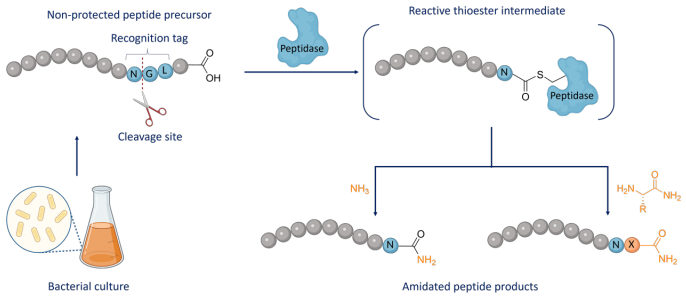
K. A. Kostadinova,[1] L. Jodaitis,[2] A. Fountain,[2,3] T. E. Nielsen,[4] W. F. J. Hogendorf,[4] M. Hyvönen,[2] D. R. Spring[1]

[1]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom.
[2]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom.
[3]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.
[4]Research Chemistry, Global Research Technologies, Novo Nordisk A/S, Maaloev, Denmark

e-mail: email: kak48@cam.ac.uk

A vast number of naturally occurring peptide hormones possess a C-terminal amide, which is vital for their structure and biological activity. This is evident from the hundred- or even thousandfold increase in potency, compared to their free-carboxylate counterparts.[1] As a result, this modification is also present in many biotherapeutics. With the growing demand for peptide drugs, there has been an increased interest in affordable and scalable methods for amidated peptide production to provide a viable alternative to solid-phase peptide synthesis.[2] However, the utility of existing chemical and enzymatic amidation methods is limited. Chemical methods are often not very selective or high yielding and require the use of protecting groups.[3] On the other hand, amidation using the native PAM enzyme is generally very efficient but is held back by production of the enzyme in mammalian cells, followed by tedious purification.[4]

This work explores the production of C-terminally amidated peptides from unprotected carboxylate precursors using a plant ligase enzyme[5]. The C-terminal ligation of each of the 20 natural amino acids was explored, evaluating the behaviour of the different side chains in the context of the reaction. After reaction time optimisation, model amidated peptides with different C-termini were produced via the addition of ammonia or the amides of the relevant amino acids. The project is currently moving towards application of the method for the synthesis of biologically relevant peptides. We envision that the method will become a useful tool in the field of protein engineering for generating peptides and proteins bearing post-translational modifications or unnatural amino acids. Additionally, it may enable the amidation of precursors produced in bacteria, providing an efficient green alternative to solid-phase peptide synthesis, thus making it of relevance both to academic and industrial settings.



C-terminal amidation of unprotected peptides - workflow

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Atomistic model of Carnauba wax solid lipid nanoparticle

Valentina Mileva, Boyana Atanasova, Mariana Vasilaki, Jasmina Petkova, Galia Madjarova, Anela Ivanova, Hristo Tzachev

Faculty of Chemistry and Pharmacy, University of Sofia, Bulgaria.

e-mail: fhgm@chem.uni-sofia.bg

Carnauba wax solid lipid nanoparticles are colloidal drug delivery systems with lipid matrix remaining in the solid state at room and body temperatures. One or more surfactants are typically used for their physical stabilization. The lipid matrix allows for the incorporation of both hydrophilic and hydrophobic active substances, ensuring their protection from chemical and biological degradation.

The complex nature of carnauba wax and the setup of a model system for atomistic MD simulations is a challenging task that involves complex considerations regarding the choice of particle composition with conservation of the nanoscale size of the system.

The study introduces the molecules that compose carnauba wax in an explicit aqueous environment and a fully atomistic model of a solid lipid nanoparticle obtained thereof. The analysis of the results of the MD molecular modeling enables the determination of the shape of the resulting particle and the arrangement of molecules therein.

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Surfactant counterion effects on salmon calcitonin hydrophobic ion pairs in aqueous media studied by molecular dynamics simulations

Passant M. Al-Maghrabi[a], **Fatmegyul Mustan**[b], Zahari Vinarov[b], Anette Müllertz[a,c,*], Thomas Rades[a]

[a]Department of Pharmacy, University of Copenhagen, Universitetsparken 2, Copenhagen 2100, Denmark.
[b]Department of Chemical and Pharmaceutical Engineering, Faculty of Chemistry and Pharmacy, University of Sofia, Bulgaria.
[c]Bioneer A/S, Kogle Allé 2, Hørsholm, 2970, Denmark
e-mail: email: fm@lcpe.uni-sofia.bg

Hydrophobic ion pairs (HIPs) increase peptide lipophilicity through complexation with oppositely charged surfactants and facilitate incorporation into lipid-based carriers such as self-nanoemulsifying drug delivery systems (SNEDDS). In an experimental study the effect of surfactant type on salmon calcitonin (sCT) HIPs, comparing sodium oleate (OLA; fatty acid) and sodium deoxycholate (DCH; bile salt) with respect to peptide–surfactant interactions within the HIP, proteolytic stability, and pharmacodynamic response have been evaluated, both as HIPs and after incorporation of each HIP into SNEDDS. It has been found that the complexation efficiency exceeded 95 % at a 1:4 molar ratio for both surfactants but upon *in vitro* proteolysis, higher protection with sCT:OLA compared to sCT:DCH has been shown (unpublished data).

In the present study, all-atom molecular dynamics simulations of HIPs in aqueous solution were conducted to investigate peptide–surfactant association. The systems were prepared at sCT:surfactant molar ratios from 1:1 to 1:8, comprising two sCT molecules and the corresponding number of surfactant molecules. Simulations for 400 ns were performed and the last 200 ns were subjected to further analysis. Visualizations of the systems explored, indicated the formation of much more compact well-arranged molecular aggregates for sCT:OLA than for sCT:DCH complexes. Structural and energetic analyses indicate that sCT:OLA exhibits a higher number of peptide–surfactant contacts than sCT:DCH, especially at shorter distances between OLA carboxylate headgroups and basic residues of sCT, leading to higher interaction energies as determined by MM-PBSA.

The computational findings demonstrate that surfactant counterion type influences sCT–surfactant association and experimental work confirmed higher proteolytic stability. Incorporation of the HIPs into SNEDDS enabled a significant oral pharmacodynamic response.

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The effect of stimulation by Gram-negative bacteria on the composition and biological activity of *Lucilia sericata* larval secretions

Kubasik Izabela,[a] Jaspreet Kaur Jandoo,[a] Alicja Carrara,[a] Marlena Golec,[b] Ryszard Smolarczyk,[b] Tomasz Skalski,[a] Artur Góra[a], **Katarzyna Papaj**[a]

[a] Tunneling Group, Biotechnology Centre, Silesian University of Technology, Akademicka 2A, 44-100 Gliwice, Poland
[b] Centre for Translational Research and Molecular Biology of Cancer Maria Sklodowska-Curie, National Research Institute of Oncology, Gliwice Branch, Wybrzeże Armii Krajowej 15, 44-102, Gliwice, Poland
e-mail: izabela.kubasik@polsl.pl, katarzyna.papaj@polsl.pl

Lucilia sericata (green bottle fly) is a species whose larvae are used in the treatment of hard-to-heal wounds in a process known as larval therapy. This therapy works on four levels: (I) removing dead tissue, (II) eliminating infecting microorganisms, (III) promoting cell growth, and (IV) providing the proper environment inside the wound. The anti-pathogenic effect arises, among other things, from the larvae's immune system. Within the humoral response, specific reactions to recognized groups of pathogens activate relevant genes and induce the production of targeted antimicrobial peptides.[1]

The aim of this study was to determine whether the presence of Gram-negative bacteria (Pseudomonas aeruginosa) in the larval environment influences the composition and activity of secretions produced by *Lucilia sericata* larvae. In this study, low-molecular-weight peptides (<3 kDa) were isolated from larvae previously exposed to bacteria. Fractions from larval secretions, obtained following separation by size-exclusion chromatography, were assessed for antimicrobial activity against reference strains of Gram-positive and Gram-negative bacteria using both an agar-based radial diffusion assay and a broth-based microtiter cell viability assay. Additionally, their effect on cell proliferation was evaluated on an endothelial cell line using the MTS assay.

Our findings demonstrate that larval secretions exhibit diverse effects following induction. Some peptide fractions were active against both Gram-positive and Gram-negative bacteria while also significantly stimulating cell proliferation. Fractions with a more selective antibacterial effect were also identified. These results indicate the dual antibacterial and pro-proliferative properties of larval secretions following immunization, highlighting their potential as a source of compounds with broad applications.

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Impact of Allelic Polymorphism on the Structural Stability and Binding Dynamics of MICA-NKG2D Complexes

Jasmina Petkova[a], Galia_Madjarova[a], Velizar Shivarov[b,c], Milena Ivanova[d]

[a]Department of Physical Chemistry, Faculty of Chemistry and Pharmacy, University of Sofia, Bulgaria.
[b]Department of Experimental Research, Medical University Pleven, Pleven, Bulgaria
[c]Department of Clinical Hematology, St. Sophia General Hospital, Sofia, Bulgaria
[d]Department of Clinical Immunology, Alexandrovska University Hospital, Medical University Sofia, Sofia, Bulgaria

e-mail: fhjp@chem.uni-sofia.bg

MICA (MHC class I polypeptide-related sequence A) is a highly polymorphic stress-induced ligand that is expressed on the cell surface in different types of cancer, including myeloid malignancies. It plays a critical role in immune surveillance by binding to the NKG2D receptor, thereby triggering the activation of Natural Killer (NK) cells and cytotoxic T cells. MICA exhibits extensive allelic polymorphism, with large number of identified variants that lead to functional diversity in immune recognition.

Atomistic molecular dynamics simulations were performed to evaluate the structural stability and binding ability of three specific MICA alleles each in complex with the NKG2D homodimer. MD simulations reveal distinct conformational behavior of the studied complexes. Mutations of amino acids makes the structure more flexible presented by two different highly populated clusters of structures. The loss of original contacts is effectively balanced by the development of alternative electrostatic and polar bonds which may influence the overall stability and remodeling of the protein-receptor interface.

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A Systematic Workflow for Peptide-Based Inhibition of SHP2 Protein–Protein Interactions

Daniela Roversi,[1] Chiara Innamorati,[1] Alessandro Strofaldi,[1] Luca Mignini,[1] Claudia Storti,[2] Luca Pannone,[3] Simone Martinelli,[3] Paolo Calligari,[1] Barbara Biondi,[4] Cristina Peggion,[2] Fernando Formaggio,[2] Marco Tartaglia,[5] Gianfranco Bocchinfuso,[1] Lorenzo Stella[1]

[1]Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Rome, 00133, Italy
[2]Department of Chemical Sciences, University of Padua, Padua, 35131, Italy
[3]Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, 00161, Rome, Italy
[4]Institute of Biomolecular Chemistry, Padua Unit, CNR, 35131 Padua, Italy
[5]Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesu , IRCCS, Rome 00146, Italy

e-mail: daniela.roversi@uniroma2.it

Src homology 2 (SH2) domain-containing phosphatase 2 (SHP2) is a non-receptor protein tyrosine phosphatase that plays a central role in multiple signalling pathways. Mutations in its encoding gene, PTPN11, are implicated in various cancers, including juvenile hematologic malignancies, and developmental disorders such as Noonan syndrome. The protein is composed of two regulatory SH2 domains (N-SH2 and C-SH2) which mediate interactions with phosphotyrosine-containing binding partners and regulate its enzymatic activity.

Often SHP2 variants destabilize its autoinhibited conformation, leading to increased basal phosphatase activity and enhanced binding affinity: a key determinant of pathogenicity. Current therapeutic strategies, including allosteric inhibitors, often fail against these mutants, highlighting the need for alternative approaches. Experimental evidence suggests that inhibiting the interaction between SHP2 and its binding partners acting through SH2 domains is the most effective way to counteract pathogenic mutations.

Clinical and research applications of SH2 ligands have been limited due to low affinity and selectivity. Here, we develop a systematic framework to generate peptide inhibitors of SHP2 protein–protein interactions (PPIs) by targeting its SH2 domains. By integrating data from natural ligands and peptide libraries with molecular dynamics simulations, we identified the structural determinants required for high affinity and selectivity. Several peptide sequences were then evaluated through diverse enhanced sampling methods to predict binding affinity, synthesized based on these predictions, and experimentally tested using fluorescence anisotropy to determine dissociation constant values. Iterative optimizations were performed until single-digit nanomolar affinities were achieved. Further optimization with unnatural amino acids enhanced peptide stability for intracellular and in vivo applications. Conjugation with cell-penetrating peptides enabled cytosolic delivery. Using this approach, we developed and patented a peptide targeting N-SH2, and we are extending the strategy to the C-SH2 domain. Overall, this integrated approach provides a method for developing PPI inhibitors targeting SH2 domains.

MD structural analysis of folate-based peptide conjugates for active targeting of folate receptor-α

Marianna Vasilaki, Nina Ilieva, Gergana Gocheva, Stoyan Iliev, Anela Ivanova

Laboratory of Quantum and Computational Chemistry, Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", 1 James Bourchier blvd., 1164 Sofia, Bulgaria

e-mail: fhmv@chem.uni-sofia.bg

Neoplasia represents a major global health threat that leads to mortality worldwide, underscoring the crucial need for pioneer strategies that can overcome the limitations and constraints of conventional chemotherapy. Targeted drug delivery is a rapidly evolving approach that seeks to optimize therapeutic efficacy, while mitigating unwanted side effects, stemming from non-specific delivery of chemotherapeutics. The design of drug delivery systems (DDSs), implemented within an active targeting context, utilizes vector-drug conjugates that possess the ability to specifically bind to cancer cell hallmarks, recognizing their cellular components.[1] Folate receptor-α (FRα) is a membrane-anchored protein with high affinity for folate, which mediates the endocytic internalization, critical for DNA synthesis and cell proliferation. Its expression is typically limited in normal tissues but the receptor is overexpressed on various types of neoplastic cells, making it a suitable target for folate-based drug conjugates.[2]

In this study, a series of folate-based[3] peptide-spaced conjugates are simulated at physiological conditions by two-step atomistic molecular dynamics to assess their potential as targeting constructs for receptor-specific delivery of the chemotherapeutic doxorubicin. The models scale from a single conjugate in saline up to the conjugate in the presence of FRα in a salinized lipid bilayer mimicking a neoplastic cell membrane.[4] An optimum spacer and linker modification is sought to enhance the conjugate-receptor interactions. Spontaneous binding is observed across all studied conjugate-receptor-membrane systems but the time, position and persistence depend strongly on the targeting ligand. Analysis of the key conjugate-receptor interactions reveals that vector-drug π-stacking is the primary factor preventing specific binding of the conjugates to the FRα active site. Two of the studied conjugates are outlined as the most prospective candidates for FRα-positive cells as potential targeting drug delivery systems.

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