Constrained peptides targeting protein–protein interfaces

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Constrained peptides are useful tools to mimic structural and functional motifs of peptides and proteins: indeed, while remaining very similar to their natural source, they may be superior for binding affinity and specificity towards the respective biological targets, as well as for metabolic stability. Different chemical approaches are exploited to develop constrained peptides reproducing native β-turns, β-hairpins, two or more helical turns, and extended motifs. Some of these approaches are based on (i) cyclization of the backbone, (ii) linkage between two side chains or between a side chain and the backbone, and (iii) replacement of α-amino acids with Cα-alkylated α-amino acids, β- or γ-amino acids. We have used side-chain–to–side-chain cyclization to develop constrained peptides mimicking helical motifs involved in protein–protein[1,2] and hormone–receptor[3] interactions, and cyclic β-amino acids to gain receptor-subtype ligand specificity.[4,5] Examples of biological applications of such constrained peptides will be given.

References

Ab initio design of foldamer tertiary structures

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Over the last decades, numerous non-natural foldamer backbones have been developed for the construction of original, predictable, and well defined molecular architectures.\textsuperscript{[1]} The research was mainly focused on the design of structures analogous to secondary motifs of biopolymers, such as helices, sheets and turns.\textsuperscript{[2]} However, most of the functions of biopolymers, especially of proteins, emerge at the level of their tertiary structures and would not be achieved by an isolated α-helix or β-sheet. Thus, the design of artificial tertiary folds that comprise several secondary structural elements represents a major challenge. Tertiary folds based on non-natural monomers remain an unexplored area and may give access to shapes and functions different to those of peptides and nucleotides. Due to the stability and the predictability of their folded secondary structures, aromatic oligoamide foldamers possess a high potential in the field of peptidomimetics.\textsuperscript{[3]} Therefore, the high stability of aromatic amide helices can be exploited to create complex artificial tertiary structures. In this context, we focused our attention into the design, the synthesis and the characterization of oligoamide-quinolines foldamers that have side chains designed to interact with each other to form helix-turn-helix motifs.\textsuperscript{[4]}

References

Figure 1. Design of helix-turn-helix motif from primary sequence to tertiary structure
Design, Synthesis, Structural and Biochemical characterization of AIF(370-394) constrained peptide.

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Apoptosis inducing factor (AIF) is a bifunctional mitochondrial flavoprotein, critical for energy metabolism and induction of caspase-independent apoptosis in neurons.[1] Upon apoptotic stimuli, the proteolytic form of AIF, AIF(Δ1–121), moves to nuclei, where it triggers chromatin condensation, large-scale DNA fragmentation and cell death, by its direct interaction with the cytoplasmic protein cyclophilin A (CypA).[2] In a previous study, we showed that the β-hairpin region of AIF, spanning residues 370-394, mediates the protein complex between AIF(Δ1–121) and CypA.[3] A peptide mimicking this region, hereafter AIF(370-394), inhibits the formation of the complex in vitro and provides neuroprotection if transfected into neuronal cells, by competing with AIF for the same interaction site on CypA.[4,5] Data obtained demonstrated that the peptide is a good model for studying the complex AIF(Δ1–121)/CypA and for developing new inhibitors with potential therapeutic value. On the basis of these data, to improve the biological activity of the AIF(370-394) peptide, we have designed, synthesized and structurally characterized AIF peptide analogues, containing structural constraints to limit the molecule conformational freedom and to induce a more native-like conformation.

In particular, we have investigated the conformational features of a series of highly constrained bi- and mono- cyclic analogues containing both disulphide and 1,4-disubstituted 1,2,3-triazole bridges.[6] Comparative biochemical assays have also been carried out to evaluate the ability of the new analogues to bind the target protein CypA[3,5] and to obtain structure-activity relationship insights.

References
Conformationally Constrained Analogues of Amyloidogenic Segments of Islet Amyloid Polypeptide

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Pancreatic islet amyloid is a characteristic feature of type 2 diabetes and islet amyloid polypeptide (IAPP) is its major protein component. As the conformational transition of α-helical IAPP conformations to β-sheet ones underlies IAPP amyloidogenesis$^{1,2}$, we designed a series of peptides in which we attempted to stabilize the α-helical structure in the amyloidogenic IAPP segments IAPP(8-18) and IAPP(8-28) via i-to-i+4 side chain-to-side chain cyclization. Analogues B1cyclo and B2cyclo exhibited higher α-helical propensities than their linear precursors while the low solubility of B3cyclo and B4cyclo prohibited their study. Importantly, significant α-helix stabilization was observed in B5cyclo; by contrast, B6cyclo had a high β-sheet forming propensity and lower α-helical content than the linear precursor. Most importantly, B6cyclo but not B5cyclo was found to be able to suppress IAPP amyloid formation and cytotoxicity. Thus, B6cyclo is a promising candidate for the development of inhibitors of IAPP amyloidogenesis and related cell-damage. Limited proteolysis experiments using insulin-degrading enzyme (IDE) and/or trypsin to hydrolyse IAPP either in the absence or in the presence of B6cyclo were then performed. IDE is a zinc metalloprotease able to degrade several different substrates including insulin, β-amylloid peptides (Aβ) and IAPP. These experiments$^3$ coupled with LC-MS analysis allow for identification of the IAPP region involved in the interaction with the B6cyclo peptide.

Analogues (8-18)

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<th>Ac-cyclo(DTQRK)ANFLVH-NH$_2$</th>
<th>Ac-ATQRLAcyclo(DFLVK)-NH$_2$</th>
<th>Ac-Cyclo[Nle(ε-N3)-TQR-Pra]ANFLVH-NH$_2$</th>
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Analogues (8-28)

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<th>Ac-ATQRLAcyclo(DFLVK)SSNNFGAILS-NH$_2$</th>
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<td>B6cyclo</td>
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References


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Design and Analysis of Peptide Inhibitors of the Interaction between SAM Domains of Ship2 and the EphA2 Receptor

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Ship2 is a lipid phosphatase involved in different diseases including cancer [1]. It contains at the C-terminus a sterile alpha motif domain (Ship2-Sam) that associates with the Sam domain from the EphA2 receptor (EphA2-Sam) [2]. This interaction is expected to have pro-oncogenic effects in cancer cells [3] thus, compound inhibitors of the Ship2-Sam/EphA2-Sam complex may represent innovative tools in anti-cancer drug discovery.

We designed several peptide sequences encompassing the interacting region of EphA2-Sam for Ship2-Sam, and performed conformational analyses and interaction assays through a variety of techniques (CD, NMR, SPR and MST). These studies led to identification of a peptide -named (KRI)₃- in which a positively charged penta-amino acid motif belonging to EphA2-Sam is repeated thrice in tandem. (KRI)₃ gives appreciable binding to Ship2-Sam [4] and NMR experiments indicate that it mainly targets the negatively charged binding site of Ship2-Sam for EphA2-Sam [4]. The peptide is also able to induce necrosis of the PC-3 prostate cancer cell line and is more cytotoxic to cancer cells with respect to normal dermal fibroblasts, as indicated by preliminary in vitro cell-based assays [4].

References
Phage-encoded bicyclic peptide inhibitors of a tumor-associated serine protease

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Bicyclic peptides are small (<2 kDa) and highly constrained molecules that combine multiple favourable properties such as good binding affinity, exquisite selectivity, high proteolytic stability and low toxicity that make them an attractive format for the development of therapeutics1. Combinatorial libraries of billion of different bicyclic peptide binders can be generated and screened by using a phage display-based methodology2. By applying this approach, we identified bicyclic peptide inhibitors of human urokinase-type plasminogen activator (uPA), a serine protease implicated in tumor growth and invasion3. Selected bicyclic peptides appear to have properties typical of proteins explaining their good binding affinity and exquisite specificity4. Their binding affinity and specificity can be further tuned by varying i) the length of the peptide loops and ii) the nature and the size of the chemical linker connecting them5,6. Importantly, their half-life could be extended to several days by fusing them to an antibody Fc domain or non-covalently appended it to serum albumin7,8. In vivo studies revealed that long-lived bicyclic peptides can stay fully intact and functional for >24 hrs in mice, overcoming a limitation faced by many peptide leads8,9. Finally, when tested in tumor-bearing mice, bicyclic peptides diffused deeply into tissues reaching high nanomolar concentrations in wide areas of solid tumors8,9. Given these encouraging qualities, in vitro evolved bicyclic peptides offer a promising format for the development of next generation peptide therapeutics.

References
G Protein-Coupled Receptors (GPCRs) family are well-established drug targets within pharmaceutical intervention, and to date about 50% of the marketed drugs exert their activity by modulating distinct members of this class of transmembrane signal pathway. Among GPCRs, peptides-binding receptors play a crucial role in many pathological and physiological pathways.\textsuperscript{1,2} The assessment of the receptor-bound conformation of a peptidic ligand within a membrane receptor such as a GPCR is of great impact for a rational drug design of more potent analogues. In this work, we applied ligand-based NMR methods to study the interaction of heptapeptides, derived from the C-X-C Motif Chemokine 12 (CXCL12), and the C-X-C Chemokine Receptor Type 4 (CXCR4) located on the cytosolic membrane of CCRF-CEM cells. This study represents the first structural investigation on the binding mode of a peptide to a GPCR directly on a living cell. As model for our investigation, we selected CXCR4 since it represents an important potential therapeutic target for various severe diseases involving immune system, including cancer.\textsuperscript{2,3} The results obtained in the field of CXCL12/CXCR4 are proofs of concept, although important information for researchers dealing with the CXCR4 field arise. General application of the presented NMR methodologies is possible and surely may help to boost the development of new therapeutic agents targeting GPCRs.

\textbf{References}
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Peptide Decorated Gold Nanostructures for high effective SERRS Detection of Colorectal Cancer Cells


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Due to their optical properties, facile surface chemistry and biocompatibility, Gold Nanoparticles are increasingly utilized for biotechnological applications including imaging, diagnostics and therapy. In order to fully exploit their potentialities, they need to be directed to the target using biomolecular targeting agents such as antibodies, peptides or other small molecules. Recently, the dodecapeptide YHWYGYPQNV (GE11) was identified as a specific ligand for the Epidermal Growth Factor Receptor (EGFR), frequently overexpressed in many types of cancer (1). According to this knowledge, the present study aims to demonstrate that Gold Nanostructures properly decorated with the EGFR binding peptide GE11 (2) can effectively target and detect different types of colorectal tumour cells overexpressing EGFR.

Gold Nanostructures were prepared from naked gold nanoparticles obtained by laser ablation in water and encoded with a SERRS reporter (3) to achieve very intense Surface-Enhanced Raman Resonance Scattering (SERRS) signals. Nanostructures were then functionalized with the targeting peptide and PEG polymers, as antifouling agents, in different arrangements to investigate how they affect the receptor recognition. Peptides were directly linked to gold nanoparticles, exploiting an extra cysteine residue, or through a thiolated PEG chain. The Gold Nanostructures targeting activity on colorectal cancer cells, expressing or not EGFR, was quantified by recording the SERRS signals cell by cell. Nanoaggregates covered with PEG alone or with Cetuximab, an anti-EGFR antibody already used in the clinical settings, were used as negative and positive control, respectively. (2) The overall results show that the presentation of the targeting peptide on the nanostructures surface plays the pivotal role for the high selective and sensitive association with the EGFR Receptor.

References

Peptidomimetics of suppressor of cytokine signaling 1 and 3: new therapeutics in cancer-inflammation diseases

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The suppressor of cytokine signaling (SOCS) proteins are negative regulators of the JAK/STAT pathway [1]. Many studies outline that they can play crucial roles in a variety of diseases including cancers with acute inflammation [2]. Only two members of this family, SOCS1 and SOCS3, contain a unique small kinase inhibitory region (KIR) that is directly involved in inhibition of JAKs. We investigated the abilities of the peptidomimetic called PSS [3] to mimic SOCS1 biological functions in cellular vascular smooth muscle cells and to test new compounds following medicinal chemistry rules to improve the affinity and cellular stability of PSS a new binding assay based on a Surface Plasmon Resonance (SPR) experiment was set up. Several analogues of PSS were analyzed through SPR, Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopies [4].

Furthermore, recent studies showed that recombinant SOCS3 was able to prevent triple negative breast cancer (TNBC) growth and metastasis by suppressing inflammatory cytokines [5]. To develop new potential therapeutics in TNBC, we designed and characterized, by CD and SPR spectroscopies, several SOCS3’mimetics derived from the N-terminal region of SOCS3 encompasses KIR and extended SH2 domain (ESS) domain that interface the complex with JAK2. The activity of the most promising sequence, KIRESS, was further investigated in vivo in mouse xenografts of MDA-MB-231-luci tumours as model of human TNBC subtype. This peptide demonstrated capable to eliminate pulmonary metastasis and showed a significant reduction of primary tumour growth [6].

Overall data could be considered as a promising scaffold to develop higher-affinity, -stability compounds active in cancer-inflammation diseases.

References
Functional characterization of a selective αvβ5 antagonist

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Integrins are cell surface receptors involved in cell-cell and cell-extracellular matrix adhesion, recognizing the RGD triad found in many extracellular matrix proteins. Their overexpression is associated with different pathologies such as inflammation, psoriasis, wound-healing and, in particular, cancer and tumor angiogenesis thus contributing to drug resistance and tumor recurrence1. For these reasons, integrins are considered interesting targets in the development of new anticancer and anti-inflammatory molecules. We reported the design and characterization of RGDechi, a chimeric peptide containing a cyclic RGD motif linked to an echistatin C-terminal fragment, able to recognize selectively αvβ3 integrin both in vitro and in vivo 2. A computational analysis, combined with the NMR data, provided a three-dimensional model of the RGDechi-αvβ3 complex elucidating the molecular requirements for the peptide specific recognition of the receptor3. On the basis of the identified molecular determinants, a RGDechi mutant was designed to be selective for αvβ5 integrin, a widely studied member of integrin receptor family involved in the process of liver metastasis and epithelial-metastatic transition 4. In this work we performed a deeply functional characterization of the new synthetized peptide on a human hepatocarcinoma cell line (HepG2) overexpressing αvβ5 integrin, investigating its effect on cell adhesion, proliferation and invasion.

References
The mechanism of action of the GKY20 antimicrobial peptide

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Antimicrobial peptides (AMPs) are membrane-active peptides with a broad spectrum of activity against different pathogenic organisms (e.g. bacteria, viruses, cancer cells).1 The emergence of resistance to antibiotics in bacteria has catalyzed the attention of the scientific community on AMPs as drug of the future. In fact, it is believed that AMPs, interacting in a non-specific way with the lipid matrix of the membrane, could circumvent the problem of resistance.2 For their application as drugs, it is mandatory to reveal their mechanism of action. Some models have been proposed which may, in part, explain the mechanism of action.1 Unfortunately, despite extensive studies, most details are still unknown. As complicating factors, the mechanism strongly depends on the lipid composition and molecular properties of both lipids and peptide.3

In this study, we report a comprehensive physico-chemical study of the interaction of a human thrombin derived peptide, GKY20,4 with liposomes composed by POPC and POPC/POPG (8/2 mol/mol) as models of an eukaryotic and bacterial membrane, respectively. The reported fluorescence and circular dichroism data suggest that the peptide is able to interact with both model membranes with high affinity, adopting a helical structure upon binding. The percentage of α-helices, obtained from the deconvolution of the CD spectra, is significant less with POPC (13%) compared to POPC/POPG (46%). Upon the interaction with the bacterial model membrane, atomic force microscopy (AFM) and differential scanning calorimetry (DSC) experiments revealed the formation of lipid domains enriched in POPG and peptide molecules. Further, the AFM data showed that beyond a certain threshold concentration, complete disruption of the membrane takes place through lipid extraction via formation of micelles. Overall, the data presented support a description based on a carpet-like mechanism of action for the GKY20 peptide.

References

1. Role of lipids in the interaction of antimicrobial peptides with membranes. V. Teixeira et al., Prog. Lipids Res. 51, 149-177 (2012).
Mild reactions for peptide-cotton bond formation: preparation of biocompatible, antimicrobial fabrics

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The need to develop new biocompatible fabrics for a variety of applications is greatly promoting academic and industrial research. In this connection, we prepared cotton-based, antibacterial textiles characterized by the covalent attachment of short peptides. Peptide covalent grafting on the cotton surface was achieved in different ways, but always exploiting the naturally occurring hydroxyl groups of cellulose. In search for green and mild reactions, we exploited also chemoselective ligations through an oxime or a thioazolidine ring in aqueous solution. With this last approach we were able to link an octapeptide, derived from the N-terminal domain of a dermaseptin 1S mutant, known for its antimicrobial properties. In this example, we used the chemoenzymatic, TEMPO-mediated oxidation of the hydroxyl groups into aldehydes by means of laccase in mild acidic aqueous conditions. The subsequent reaction with the β-aminothiol of a Cys-peptide gave a stable covalent bond (Figure 1).

![Figure 1. Laccase mediated cotton oxidation and chemoselective reaction with a Cy-peptide](image)

We characterized our cotton-peptide samples by means of FT-IR, UV-Vis and XPS and determined their antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Interestingly, some of the materials gave promising results against the Gram positive strain, responsible for most hospital-acquired infections.

References

II° Convegno Società Italiana Peptidi

Derivatives of the frog skin peptide esculentin-1a kill Pseudomonas aeruginosa biofilm on soft contact lenses and preserve their bactericidal activity upon conjugation to the lens surface

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Antimicrobial peptides (AMPs) represent a class of molecules with interesting biological properties, including a broad spectrum of action ranging from fungi to Gram-positive and Gram-negative bacteria. Among the latter, one of the most dangerous microorganisms is Pseudomonas aeruginosa, a motile bacterium capable of colonizing biological and abiotic surfaces, such as contact lenses (CLs), forming biofilm communities that are difficult to eradicate. This increases the risks of CL wearers of developing microbial keratitis. Therefore, novel strategies and compounds to reduce the onset of CL-associated ocular infections are needed. It has been demonstrated that the frog-skin AMP Esc(1-21) and its diastereomer Esc(1-21)-1c have potent anti-Pseudomonal activity against the free and biofilm form of this pathogen. Here we first evaluated the ability of both peptides to kill P. aeruginosa biofilm formed on soft CLs by microbiological assays and scanning electron microscopy analysis. It turned out that both peptides have the ability to eradicate Pseudomonas biofilms with the diastereomer showing a stronger potency (up to 85% killing vs no killing of the all-L peptide at 4 μM for some bacterial strains). Furthermore, we covalently immobilized both peptides to soft CLs. The peptides-immobilized CLs were found to cause more than four log reduction in the number of bacterial cells within 20 minutes and to reduce bacterial adhesion to the CL surface (77%–97% reduction) in 24 hours without any harmful effect on mammalian cells. In addition peptides immobilization to CLs did not affect the lens properties. These promising results suggest the potential use of peptides tethered to medical devices for the prevention and treatment of CL-associated P. aeruginosa keratitis [1].

References

Novel peptide-based nanomaterials with antimicrobial activity

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The extensive use of antibiotics in both human and animal healthcare has resulted in the development of bacterial resistance towards these drugs. Great attention has been recently devoted to the possibility to build self-assembling peptide nanostructures with intrinsic antibacterial activity.¹,²

Here we present a versatile self-assembling nanostructures with antimicrobial activity due to the presence of antimicrobial peptides (AMPs) on the surface. For this purpose, we synthetized two different peptide sequences: one shorter, which aids in the self-assembling process, and a longer one containing two moieties (the one serving for the assembly and the one serving as antimicrobial agent). As a proof of concept, an active analogue of the antimicrobial peptide myxinidin³ was developed.

The self-assembled nanostructures were characterized by fluorescence spectroscopy to determine the critical aggregation concentration, circular dichroism, NMR, DLS and transmission electron microscopy for a structural study, biological activity tests to evaluate the eukaryotic cell toxicity and the capability to inhibit the growth or promote the eradication of bacterial and fungal biofilms. This study showed that our peptide nanostructures are promising tools against biofilm formation at low concentration especially against fungal biofilm.

References

From peptides to peptidomimetics: spectroscopic studies of potential antimicrobial agents

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Bacteria are showing increasing resistance against available antibiotics. Naturally occurring antimicrobial peptides (AMPs) are promising molecules to fight multi-resistant microbes. Notwithstanding the potential of AMPs in the fight against drug resistance, their therapeutic application presents some limitations, such as their susceptibility to proteolytic degradation and high manufacturing cost. To address these issues, recently several research efforts were devoted to the development of synthetic peptidomimetics of AMPs. However, the rational design of protease-resistant peptidomimetic molecules with the same properties of AMPs, is complicated by the fact that multiple equilibria (self-assembly, water-membrane partition, insertion in the bilayer, pore formation, etc.) influence the membrane-perturbing activity, and any modification in molecular properties perturbs all of them to different extents. Spectroscopic approaches, in particular fluorescence methods, are extremely powerful in the characterization of the behavior of AMPs or peptidomimetics in interaction with lipid bilayers [1]. By using these techniques, together with molecular dynamics simulations, we studied the mechanism of action of an antimicrobial norspermidine-based peptidomimetic (Nor Trp) characterized by a cationic charge and an aliphatic tail, and centered on norspermidine, an easily available and inexpensive compound that allows easy functionalization. This peptidomimetic exhibited a strong activity and very low toxicity [2]. We characterized aggregation, membrane binding, insertion in the membrane, pore formation and bilayer perturbation. Overall, our findings provide a clear picture of the interaction of Nor Trp with lipid bilayers, and of its mechanism of membrane perturbation.

References
Synthesis and characterization of Glucosylated Peptides: toward selective plasmapheresis-based treatment of Multiple Sclerosis

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Multiple Sclerosis (MS) is a neurodegenerative disease presumably involving in some forms an antibody-mediated mechanism in the damage of myelin sheath surrounding the axons in the central nervous system. Since the aetiology is still unknown and the therapies are feeble, isolating the specific autoantibodies is a major goal to understand and treat this complex pathology. Previous studies already assessed that the glucosylation of the H. Influenzae adhesin protein HMW1 on asparagine residues in consensus sequons (N-X-T/S) is necessary for protein secretion and efficient adherence to host cells, hence vital to assure infectivity of the bacterium.1 Recently, the presence of N-Glucosyl epitopes in H. influenzae C-terminal adhesin fragment HMW1ct (1205-1526) was proven to be essential for the identification of the highest affinity antibodies in MS, showing a very specific recognition. Hence, hyperglucosylated HMW1ct is the first example of an N-glucosylated antigen that can be considered a relevant candidate for triggering pathogenic antibodies in MS.2

From a fundamental point of view, finding the minimal epitope recognized by antibodies and understanding the impact of glucosylation on adhesin sequons is a crucial task. To do so, a collection of adhesin-derived sequences was synthesized through solid-phase peptide synthesis and analysed by CD and NMR techniques. These peptides contain differently glucosylated consensus sequences that were able to recognize antibodies in patients’ sera. Moreover, these putative antigens were loaded onto polymeric scaffolds with the aim to obtain multivalent macromolecules ideally able to snare selectively circulating antibodies. Indeed, the development of a tentacle-like, antigen-decorated polymer is a promising stratagem for the isolation and characterization of reactive antibodies found in the sera of patients suffering from autoimmune diseases. For this scope, the achievement of synthetically accessible, peptide-based microarchitectures may provide a great step forward both in the treatment and in the comprehension of MS.

References
Patients with cystic fibrosis require pharmacological treatment against chronic lung infections due to antibiotic resistant pathogens. Antimicrobial peptides are among the leading compound for developing new drugs because of the broad spectrum of activity and the slow rate in acquire resistance. In order to obtain a peptide stable in the pulmonary environment we truncated and modified the natural antimicrobial peptide BMAP27, obtaining the all-D peptide D-BMAP18 [1]. It is an α-helical peptide which has a MIC_{90} = 16 μg/ml against Pseudomonas aeruginosa CF-isolates [2] and has a good activity against their biofilm. We tested with success the bactericidal activity of D-BMAP18 toward P. aeruginosa in CF-sputum by co-treatments with DNase and NaCl. We also tested its in vitro and in vivo toxicity observing a non-negligible toxicity when it was intratracheally administrated to mice [3], a side effect that may be linked to this route of administration. Preliminary test on a systemic infection model in Galleria mellonella (wax moth) are in progress to verify this hypothesis. In order to improve its efficacy and to minimize undesired side effects, we are also trying new routes of administration in mice and the design of a modified in situ activable D-BMAP18 prodrug. The final aim is to obtain a safe drug for the treatment of pulmonary infections in cystic fibrosis.

References

Use of peptide mimetics of proteins to characterise immune response in different pathologies: a powerful approach

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Peptides are a powerful tool for the identification of antibodies and for the study of chemical and biological mechanisms involved in several disorders. We present the synthesis and the screening of different libraries of peptides related to some specific diseases for in-depth studies of these pathological conditions. In particular:

**Type 1 Diabetes**: our data show a cross-reactivity of T1D and LADA autoantibodies in patient sera against two hGAD-derived peptides and one Coxsackievirus-derived peptide¹. These results suggest a possible correlation between viral infections and the onset of T1D.

**Psoriasis**: we preliminarily highlighted a specific interaction between autoantibodies in psoriatic arthritis and rheumatoid arthritis patient sera and the endogenous antimicrobial peptide LL37².

**Fabry disease**: through affinity measures we successfully identified a promising alpha-galactosidase epitope involved in the autoimmune mechanism which lead to the Fabry disease³.

**Rett syndrome**: our preliminary data show a correlation between hyperglucosylated adhesin and Rett syndrome, suggesting a plausible involvement of *Haemophilus influenzae* in its pathogenesis⁴.

**References**

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Photocurrent Generation in Supramolecular DNA-Inspired Nanoarchitectures on Gold Surface: from 2D to 3D

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Fabrication of ordered three-dimensional nanostructures on surface is an ongoing challenge in the field of materials science. In particular, molecular order and perfect positioning of different redox centers are of fundamental importance in building-up artificial photosynthetic systems, to control the direction of the electronic flow. Different systems with all subunits covalently linked have been reported in the literature, but the synthetic effort to obtain these multicomponent molecules is considerable. In Nature all supramolecular architectures are built up by non-covalent interactions, between them hydrogen bonds. In particular, DNA molecules are engineered using pair up of nucleobases. In this communication, we describe preliminary studies on photocurrent generating supramolecular components, built with an unprecedented approach: we have used the thymine-adenine DNA base pairs approach to construct supramolecular films in 3D, composed of different 2D layers. To this end, we have engineered two types of photocurrent generating films on gold surfaces. Film 1 and 2 consists of multilayered systems where the light absorbing group (ZnTPP chromophore) is noncovalently linked to a gold surface through thymine-adenine hydrogen bond. These films are assembled by consecutive deposition of each layer. In film 1, two components are used: adenine linked to a lipoic acid molecule (Lipo-A) to covalently bind the gold surface, and ZnTPP linked to a thymine molecule (T-ZnTPP). Film 2 has an additional noncovalently linked layer: an undecapeptide analogue of the Thricogin GA IV peptide, in which the two extremities were functionalized with thymine and adenine, for binding of, respectively, Lipo-A and T-ZnTPP. In this work the photocurrent generation properties of film 1 and 2 are studied by electrochemical and spectroscopic techniques. The importance of molecular organization for the optimization of the photoconversion efficiency will be emphasized.

References

Structural metal ion recruitment influences folding mechanism and self-association propensity of high homologous proteins

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Using three isostructural proteins of the prokaryotic zinc finger family as model systems (M1452-151 lacking zinc binding and M1153-149 and Ros87 that bind a structural metal ion), we propose the study of the structural metal ion influence on proteins structure and function, folding mechanism and self-association propensities1,2. The prokaryotic zinc finger domain3 shows a ββα motif similar to the eukaryotic domain, is stabilized by an extensive hydrophobic core of 15 amino acids and uses different combinations of amino acids to coordinate the structural metal ion when present4. We will discuss how the recruitment of the structural metal can modify the folding pathway of these relatively small domains, control conformational accessibility to aggregation-prone states and change aggregation kinetics. While these model domains have little direct disease-relevance, our findings should be of broad general interest as many disease-relevant proteins bind metal ions, which could similarly influence their structures, folding pathways and aggregation.

References