using DTT. Going further, we tested them preparing several short peptides containing a Cys residue and Vasopressin, a therapeutic nonapeptide containing a disulfide bridge. The removal of the Cys protecting groups and subsequent cyclization in vasopressin were done in solid phase. All peptides were obtained in good yield and high purity.

Later, we have also explored the chemoselective disulfide formation by thiol-disulfide interchange in SIT-protected cysteinyl peptides. Being less sterically hindered than groups like StBu, SIT facilitated the exchange under mild basic conditions (pH≤8). To demonstrate, we synthesized therapeutic peptides like oxytocin and somatostatin containing one disulfide bond. In both cases, SIT directed the disulfide based cyclization in a shorter time without the need of any oxidizing reagent or reprotection. The quality of the final products demonstrated the total applicability of this new building block as a convenient tool for disulfide formation in peptides.

In conclusion, we have developed two promising disulfide Cys side chain protecting groups with additional activating/directing properties (SIT) which could replace the ones commercially available nowadays.

O23 | An amyloidogenic fragment of human alpha hemoglobin with a combined antibacterial and antiviral activity

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Antimicrobial peptides (AMPs) are essential components of the immune system, defending the host against invading pathogens. Research of the past years showed that a common feature of many AMPs is to self-assemble into amyloid-like structures to exert an antimicrobial activity. In this study, we aim to identify and characterize novel endogenous AMPs with amyloidogenic properties. To this end, we extracted all peptides from human spleen by chromatographic means, and screened the resulting peptide library for amyloid-containing fractions by Thioflavin T (ThT) assay. A ThT-positive fraction mainly contained a C-terminal 32-mer fragment of human alpha hemoglobin, termed HBA. We show that the synthetic peptide forms positively charged fibrils by incubation at 37°C. In-vitro antimicrobial activity was analyzed in bacterial growth and survival assays, and viral infection assays. The freshly dissolved peptide displayed antibacterial activity against Gram-negative (P. aeruginosa, A. baumannii) and Gram-positive (L. monocytogenes, E. faecium) bacteria. In contrast, HBA fibrils, but not the freshly dissolved peptide, inhibited Herpes Simplex 1 (HSV-1), Herpes Simplex-2 (HSV-2), Human cytomegalovirus (HCMV) and Measles virus (MeV) infections in a dose-dependent manner, with a mean IC50 of ~100 μg/ml, while having no effect on Zika Virus (ZIKV), Influenza virus (IAV) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Peptide generation from the full-length precursor was analyzed by an in vitro digestion of hemoglobin with different proteases. Our data indicates that the peptide is generated upon digesting full-length hemoglobin with aspartic proteases Pepsin and Napsin A. Of note, concentrations necessary to fully block microbial infections can be achieved by proteolytic degradation of 5 % of plasma hemoglobin suggesting a relevant role in vivo. Our study shows that proteolytic processing of highly abundant hemoglobin results in the generation of an amyloidogenic hemoglobin alpha fragment with combined antiviral and antibacterial activity.

O24 | Peptide/ionic liquid conjugates to tackle complicated skin infections: antimicrobial, antibiofilm and collagen-boosting effects

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Complicated skin and soft tissue infections (cSSTI) like, e.g., diabetic foot ulcers (DFU), are severe cases of cutaneous and deeper soft tissue infections.[1] Their symptoms are consistent with local polymicrobial biofilms, which are difficult to eliminate and delay the healing process. The standard-of-care for cSSTI requires oral antibiotics and other measures, often complex and distressing (e.g., amputations). Due to widespread multidrug resistant (MDR) microbes, efficient treatments for cSSTI are being exhausted. These should promote both antimicrobial protection and fast tissue regeneration, to atone the inefficient healing in elderly people afflicted with, e.g., diabetes or venous/arterial insufficiency.[2]

We advance peptide/ionic liquid conjugates as potential active pharmaceutical ingredients for topical formulations to tackle cSSTI. Such conjugates are anticipated to concomitantly display antimicrobial and anti-biofilm action along with fast healing through, e.g., neocollagenesis-inducing effects. Promising results were obtained with chimeric peptides combining a host-defense sequence[3] with a collagenesis-inducing peptide widely used in cosmetics.[4] The best constructs exhibited: (i) antibacterial and anti-biofilm activity against Gram-positive and Gram-negative bacteria, including MDR clinical

isolates; (iii) improved action against S. aureus (prevalent pathogen in chronically-infected DFU) in simulated wound fluid; and (v) antifungal activity.5 Relevantly, their ionic liquid-modified conjugates were proven to display equally potent antimicrobial and anti-biofilm activities, and retain or enhance the collagenesis inducing action of the cosmeceutical parent peptide. These findings will be herein communicated.

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O25 | Designed conformationally constrained peptides as potent inhibitors of amyloid self-assembly and reciprocal cross-seeding of IAPP and Aβ42

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Amyloid self-assembly is linked to the pathogenesis of more than 40 devastating cell- and neurodegenerative diseases including Alzheimer's disease (AD) and type 2 diabetes (T2D). Epidemiological and pathophysiological evidence suggest that AD and T2D are linked to each other. Cross-seeding interactions between Aβ and IAPP, the key amyloid polypeptides of AD and T2D, dramatically accelerate amyloid self-assembly of both polypeptides and might be possible molecular links between the two diseases (O'Nuallain et al. (2004) & Moreno-Gonzalez et al. (2017)). Molecules targeting amyloid self-assembly and cross-seeding interactions of Aβ and IAPP could thus be valuable leads for anti-amyloid drugs for both AD and T2D. In fact, except for a recently approved and controversially discussed anti-Aβ amyloid antibody, treatments targeting AD or T2D amyloid do not yet exist.
Previous work of our group has shown that IAPP/Aβ cross-amyloid interactions can be used for designing potent inhibitors of amyloid self-assembly of both polypeptides (Armiento et al. (2020)). Here we will present design, synthesis, and biophysical/biochemical studies of a novel class of conformationally constrained peptides designed to mimic Aβ/IAPP cross-interaction surfaces, which are nanomolar inhibitors of amyloid self-assembly of both IAPP and Aβ42, effectively suppress their cross-seeding effects, and function via a novel and unexpected mechanism. Their favorable features make this kind of peptides to promising leads for the development of anti-amyloid drugs for both AD and T2D.

O26 | Design of short peptides and peptide assemblies aided by machine learning

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The discovery of new peptides (i.e., antimicrobial, antiviral, catalytic) is challenging, as they are part of a very large search space and the principles responsible for the desired activities at the sequence level are not yet fully understood. To avoid expensive and time-consuming guesswork and experimental failure, our strategy is to apply soft computing techniques to accelerate peptide discovery. Search-based algorithms allow for a faster exploration of peptide permutation space which grows exponentially with peptide length and whose amount and dimensionality is too overwhelming to rationally comprehend. Machine learning can find patterns or regularities in data, build mathematical models based on the theory of statistics and make up for the lack of knowledge. To date, both strategies have been applied to a variety of chemical problems to maximize the chance of successful and rapid solving of complex issues.
Our team has already reported on a multi-objective evolutionary approach for the exploration of mass and sequence diversity-oriented random peptide libraries[1] and on the bottom-up approach for their design[2], which combines physico-chemical properties obtained experimentally and theoretically to cover larger parts of the peptide chemical space. Our current activities involve the application of machine learning to find peptides with catalytic activity, to predict their predisposition towards self-assembly[3] and to estimate the antiviral or antimicrobial activities (fig 1). For this purpose, we developed a new sequential properties representation scheme that combines physico-chemical properties with the amino acid order within the sequence. Moreover, we applied the generative adversarial network that enables the design of de-novo sequences.

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