Peptide Inhibitor of RED-SMU Interaction as a new anti-infectious agent

This project lies at the interface between chemistry and biology and involves a chemistry lab of Sorbonne University (LBM, UMR 7203) that gathers bioorganic chemists (Roba Moumné, Nicolas Pietrancosta, Lou Rocard, Emmanuelle Sachon, Ludovic Carlier) specialists of synthesis, characterization, computer modelisation and structural investigation of biomolecules and especially peptides (= Partner 1), and a group of virologists of Institut Pasteur (Nadia Naffakh, Catherine Isel) expert in influenza viruses and with a long-standing interest in the development of novel anti-influenza therapies (= Partner 2).

The increasing incidence of drug-resistant pathogens calls for the development of novel therapeutic strategies. In recent years, the concept of host-directed therapies, which target host determinants essential for the infectious life cycle and/or pathogenesis rather than pathogen components, has been rapidly expanding.[1] They show clinical safety while providing the advantage of broad-spectrum efficacy and reduced antiviral resistance. Following this approach, Naffakh *et al* (Partner 2) have recently characterized the structure of a human splicing factor, RED-SMU1, which is essential for influenza A virus (IAV) life cycle, and investigated this cellular factor as a potential target for IAV therapy.[2] They have shown that disrupting this protein-protein complex allow decreasing the level of endogenous RED-SMU-1 levels and inhibiting viral mRNA splicing and viral multiplication while preserving cell viability. Interfering with this complex represents thus a potential strategy for the development of new antiviral therapy. However, because of their extensive, shallow, and highly hydrophobic complementary interfaces, the design of compounds that specifically disrupt protein-protein interactions is quite challenging. Mimicking proteins with small "drug-like" compounds is rather tricky and many interactions involving proteins are described as "un-druggable".[3] Peptides on the other side could play a key role in this context: their intermediate molecular size between that of traditional drug-like compounds and much larger biologics allows them to cover large surface areas on their binding partner leading to high affinity and selectivity, while maintaining pharmacokinetic properties that are much more favorable than biologics.[4] Proteins interact most often with their partner *via* an array of side chains that are displayed by highly ordered segments, such as helices or strands.[5] This sequence can in principle be directly extracted and used as mimetic of the whole protein, incarnating thus the simplest functional protein mimetic, but most often when removed from their native proteic context, peptide segments fail to adopt the bioactive conformation leading to entropic penalty and loss of affinity. Methods for pre-organizing peptide 3D structure have been developed over the last century, involving the use of constrained residues, head to tail or side-chain to side-chain macrocyclization, or projection of crucial side chains on 3D scaffolds.[6] These methods allow the access to shorter Protein Domain Mimetic (PDM) that retains the activity of the whole protein and are particularly useful for targeting interactions that have revealed intractable with small molecules. **In this project we wish to downsize the RED protein into a short helical peptide corresponding to RED amino acids 211-222, that retains the target affinity and activity of the whole protein and is embedded with pharmacological properties favorable for its *in vivo* use, particularly cell membrane permeability and stability toward proteolytic degradation.** For this purpose, different strategies combining rational design and libraries screening will be explored. The X-ray structure of a minimal RED-SMU1 complex has been solved at 3.0 Å and reveals two interacting domains at the interface.[2] One of them involves an α-helix of RED that project 4 hydrophobic side-chains residues located at one face of the helix into a hydrophobic groove of SMU1. This helical domain could represent a suitable sequence for the development of PDM.

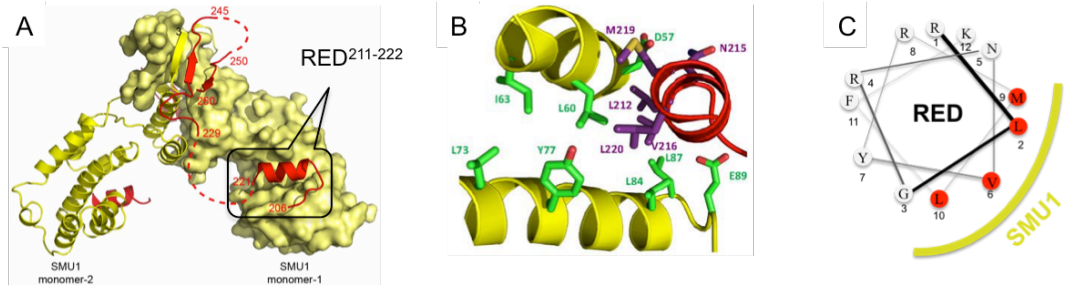
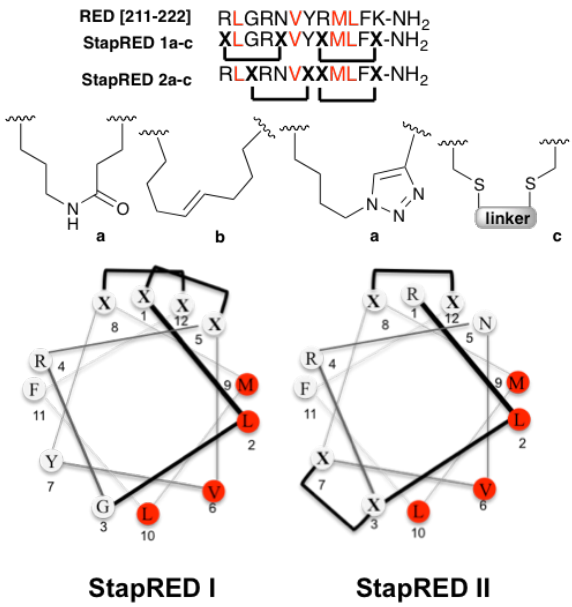


Figure 1. (A) Crystal structure of the recombinant human RED–SMU1Nter complex (RED and SMU1Nter proteins are colored red and yellow, respectively) (B) Location of SMU1 and RED residues (colored green and purple, respectively) involved in interactions (C) Schematic representation of the interface using a wheel diagram of RED. Adapted from ref [2]

Rational Design of a stapled RED peptide (StapRED) as a first generation RED-SMU1 inhibitor

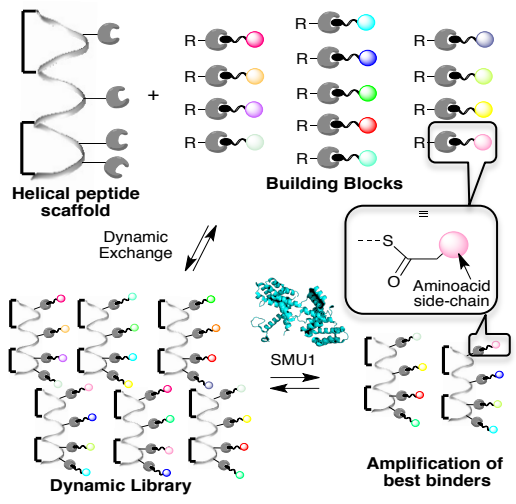
First a rational design will be performed to introduce covalent constraints into the RED[211–222] sequence, in order to stabilize its overall 3D structure. Due to the helix periodicity, the amino acid residues found at positions *i*, *i+4*, *i+7* and *i+11* of an α-helix point towards the same direction and constitute an opportunity for introducing a cross-link between their side-chains, therefore locking the conformation of the peptide: the so-called peptide stapling.[7] This method allows to increase protein binding affinity by reducing the entropic cost of binding and could also provide extra contact between the staple linkers and target proteins. Moreover, constrained peptides often exhibit improved proteolytic stability and membrane permeability compared to their linear counterpart.

Starting from the X-ray structure of RED in complex with SMU1,[2] we have identified different combinations of *i* and *i+4* residues on the solvent exposed face of the helix that do not interfere with its recognition by SMU1 and could thus be exploited for staple introduction (**StapRED I** & **II** figure on the right side). Two covalent bridges will be introduced in these positions in order to lock the helical conformation. Because the nature of the linkages obtained for stapled peptide can dramatically impact the overall physicochemical and biological properties of the obtained compound, different chemical strategies that have been successfully applied to other bioactive peptides will be considered: lactam, hydrocarbon or triazole bridge. Alternatively, a two-components stapling strategy in which different linkers react simultaneously with two thiol groups located at *i*/*i+4* position will be envisaged.[8] The main advantage here is that several cross-linkers can be easily screened from a single linear peptide. Docking studies will be performed on the different designed compounds to help the selection of the best strategy. The best candidates will be prepared by SPPS. Structural investigations will be performed (NMR and circular dichroism) in order to evaluate the helix stabilization in each sequence. The binding affinities of the compounds will be measured by bio-layer Interferometry (collaboration D. Hart, IBS, Université Grenoble-Alpes). For this purpose the SMU1 protein will be produced in *E. Coli* using a well-established and highly efficient procedure[2] (collaboration T. Crépin, IBS, Université Grenoble-Alpes). The stability against proteolytic enzymes and intrinsic ability to penetrate cell membranes will be evaluated (Partner 1). This work will deliver a first-generation short RED[211-222] mimetic that will be then evaluated for its ability to disrupt the RED-SMU1 complex and to inhibit IAV replication, using well-established cell-based assays [2] (Partner 2).



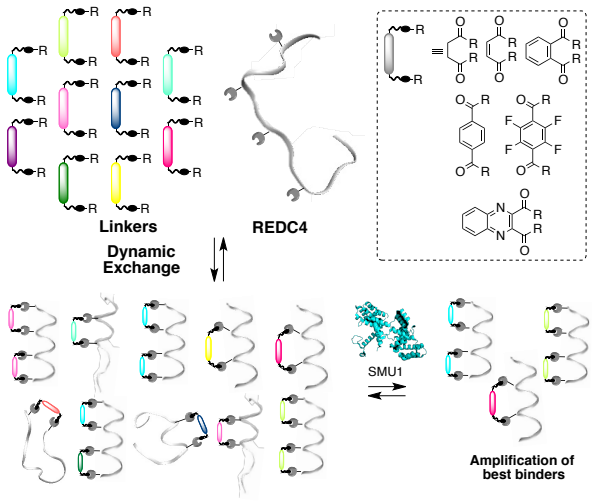
Optimization of the recognition element of StapRED by dynamic libraries screening

Having validated the use of a stapling strategy to improve the conformational stability of RED peptide, the next step will be to optimize the sequence. In general optimization of peptides sequence is performed by synthesis and screening of libraries of peptides and is often the most challenging and time-consuming step in the discovery of bioactive peptides. Partner 1 has recently designed a new strategy that exploits Dynamic Combinatorial Chemistry (DCC)[9] as a tool to functionalize peptide backbone.[10] This method (schematized on the right) allows generating dynamic libraries of peptide with defined 3D structure that bears virtually all side-chains combination on their surface. This strategy will be used to prepare a library of α-helical peptides with a wide variety of side-chains on one of the



helical face. For this purpose, an α-helical scaffold based on the structure of the developed RED mimetic will be prepared, in which residues that are crucial for SMU1 recognition are replaced by cysteine residues that can participate in reversible thiol to thioester exchange in the DCC process. This peptide will be mixed with a series of building blocks (BB) bearing natural and non-natural amino acids side-chains *via* a thioester linkage, in physiological conditions, leading to a dynamic chemical library of well-ordered peptides with multiple side-chains combinations. Upon addition of SMU1, the best ligands will bind the target and shift the equilibrium to favor their own formation at the expense of the other compounds. Changes in library composition upon introduction of the target will be thus exploited to probe favorable interactions. After identification of amplified sequences (LC-MS, CE/MS & MS-MS, collaboration MS platform SU),[11] the best combinations will be individually re-synthesized by replacing the thioester linkage with a more robust amide function in order to transpose the hit into a stable peptide. Structural investigation and binding studies will be then performed to confirm the conformation of the ligand and its affinity to the target. This second part will deliver a new RED domain mimetic with high affinity to SMU1, that will be then submitted to biological evaluation as described above (Partner 2). Notably, it would represent the first proof of concept of the exploitation of this DCC strategy for the discovery of PPI inhibitors.

Methodological development: peptide stapling by DCC



A new approach for the dynamic anchoring of a staple into a peptide sequence will be developed using RED as a model helical peptide, in order to address the synthetic challenge of efficiently screening different staple component for a given peptide. (Partner 1) For this purpose, a new reversible stapling reaction will be established using thiol/thioester exchange reaction on peptide surface. Very few examples of dynamic stapling of peptide are reported in the literature but in these studies no combinatorial aspect are considered. Starting from a peptide REDC4 derived from RED[211-222] bearing two pairs of cysteine residues in relative positions *i* to *i*+4, a peptide library will be generated using a series of linkers of different chemical nature, length and geometry, functionalized at each ends with thioester moiety (Several BB of this type have already been prepared). Thiol to thioester exchange will occur, leading to all possible peptide/linker combinations that are in thermodynamic equilibrium. Upon addition of the biological partner SMU1, the combination that possesses the most suitable conformation to interact with the target should be amplified. This new methodology would allow an easy and rapid identification of the best stapling strategy for a given peptide.

This project will deliver new compounds for innovative anti-influenza therapies. They would also have the potential of becoming inhibitors of other viruses that rely on the RED–SMU1 complex, or anti-tumor drugs.

This interdisciplinary doctoral project will leverage theoretical and practical knowledge both in chemistry and biology. It will rely on a diverse range of techniques: organic and peptide synthesis, separation technics, mass spectrometry MALDI-TOF and LC-ESI MS/MS, NMR, biophysical analysis and cellular assays.

[1] Kaufmann S.H.E.; Dorhoi A.; Hotchkiss R.S.; Bartenschlager R. *Nat Rev Drug Discov*. **2018**, *17*, 35–56. [2] Ashraf, U.; Tengo, L.; Le Corre, L.; Fournier, G.; McCarthy, A. A.; Rameix-Welti, M.-A.; Gravier-Pelletier, C.; Ruigrok, R. W.; Jacob, Y.; Vidalain, P.-O.; Pietrancosta, N.; Crepin, T.; Naffakh, N. *PNAS* **2019**, *116*, 10968–10977. [3] Milroy, L.-G.; Grossmann, T. N.; Hennig, S.; Brunsveld, L.; Ottmann, C. *Chem. Rev.* **2014**, *114* (9), 4695–4748. [4] M. Muttenthaler, G. F. King, D. J. Adams, P. F. Alewood *Nat. Rev. Drug Discov.* **2021**; *20*(4), 309. [5] Sawyer, N.; Watkins, A. M.; Arora, P. S. *Acc. Chem. Res.* **2017**, 50, 1313. [6] Lenci, E.; Trabocchi, A. *Chem. Soc. Rev.* **2020**, *49*, 3262. [7] Li, X.; Chen, S.; Zhang, W.-D.; Hu, H.-G. *Chem. Rev.* **2020** *120* (18), 10079–10144. [8] Lau, Y. H.; de Andrade, P.; Wu, Y.; Spring, D. R. *Chem. Soc. Rev.* **2015**, *44* (1), 91–102. [9] Frei, P.; Hevey, R.; Ernst, B. *Chem. Eur. J.* **2019**, *25* (1), 60. [10] Zagiel, B.; Peker, T.; Marquant, R. Webb, G.; Cazals, G. Miclet, E. Bich, C., Sachon, E.; Moumné, R. 10.26434/chemrxiv-2022-thdpk. [11] Peker, T.; Zagiel, B.; Cazals, G.; Bich, C.; Sachon, E.; Moumné, R. Mass Spectrometry Analysis of Cyclic Peptides obtained by Dynamic Combinatorial Chemistry, *in preparation.* [12] Haney, C. M.; Horne, W. S. *Org. Biomol. Chem.* **2015**, *13* (14), 4183–4189. [13] Shi, X.; Zhao, R.; Jiang, Y.; Zhao, H.; Tian, Y.; Jiang, Y.; Li, J.; Qin, W.; Yin, F.; Li, Z. *Chem. Science* **2018**, *9* (12), 3227–3232.