Sorbonne Université/China Scholarship Council program 2021

Thesis proposal

Title of the research project: Peptides Stapling by Dynamic Combinatorial Chemistry

Keywords: Peptide Chemistry – Dynamic Combinatorial Chemistry – Protein Domain Mimics- Inhibition of Protein Protein Interaction

Joint supervision: no

Joint PhD (cotutelle): no

Thesis supervisor: Roba Moumné

Email address of the thesis supervisor: roba.moumne@sorbonne-universite.fr

Institution: Sorbonne Université/ENS

Doctoral school (N°+name): ED406 Chimie Moléculaire Paris Centre

Research laboratory: Laboratoire des Biomolécules

Address of the laboratory:
Campus Pierre et Marie Curie
Tour 23-33 5ème étage
4 place Jussieu
75005 PARIS

Name of the laboratory director: Olivier Lequin

Email address of the laboratory director: mailto:olivier.lequin@sorbonne-universite.fr

SORBONNE UNIVERSITÉ

Subject description (2 pages max):

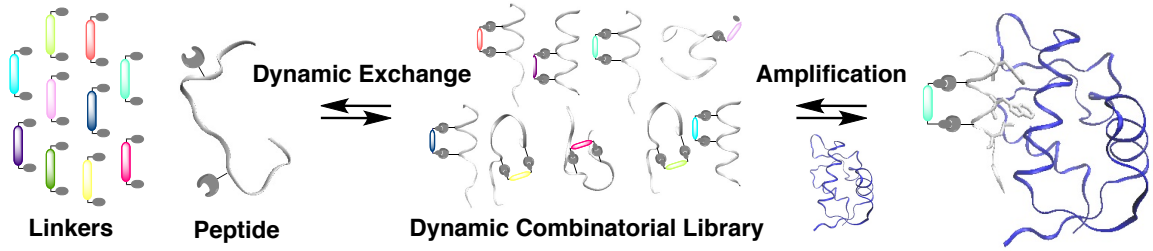
1) Study context

The project aims at developing a new strategy for the auto-assembly of bioactive peptides, driven by their molecular recognition by a biological target. Small peptides are attractive and underexploited biomolecules that occupy an intermediate molecular space (1-2 kDa) between that of traditional drug-like compounds and much larger "biologics". Because their sequence can be directly derived from proteins, they can closely reproduce their specific side-chains arrangement and should thus incarnate the simplest protein mimetics. Particularly, proteins interact most often with their partner *via* an array of side chains that are displayed in defined orientation by highly ordered segments, such as helices or strand.[1] The importance of these 3D structures led to the assumption that this segments could be used as **Protein Domain Mimics** (PDB) to imitate the whole protein structure. However, when removed from their native context, peptide segments usually fail to adopt the bioactive conformation, leading to entropic penalty upon binding that affects their affinity. To circumvent this, two tactics have been suggested. One consists in introducing covalent conformational constraints into the isolated peptide sequence.[1] The second one often called "grafting strategy" consists in virtually "dissecting" the set of residues that make crucial contribution in a molecular recognition event, the so-called "hotspots", and "grafting" them on a stable 3D scaffold that can approximate their spatial arrangement.[2] The limit of both these strategies is that structural data on the protein to mimic have to be available. In addition, they usually deliver a first low affinity hit, which is in turn optimized through cycles of design and screening. In order to bring a new alternative, our group wish to combine these strategies based on rational design with a combinatorial method allowing to explore more quickly and efficiently a wide array of combinations for a given target. For this purpose we propose to use **Dynamic Combinatorial Chemistry (DCC)**.[3] We have recently establish a new strategy in which a small peptide of defined 3D structure is used as a scaffold to graft amino acid side-chains in a dynamic reaction.[4] In this project, we wish to develop a similar strategy in which conformational constrains are dynamically introduced on a flexible peptide that already contains this time the protein epitope, in order to select the conformation that fit the best to the target. These new strategy will be used in the highly challenging field of **Protein-Protein Interactions** (PPI).

2) Details of the proposal

α-helix is one of the most common structural elements found at proteins interfaces. Due to the helix periodicity, the amino acid residues at positions *i*, *i+4*, *i+7* and *i+11* point toward the same direction and constitute therefore an opportunity to lock the conformation by introducing a cross-link between their side-chains: the so-called peptide stapling.[5] An expanding repertoire of macrocyclization chemistry has been developed for this purpose. Depending on the chosen reaction and staple position, the obtained linkage differs completely and this can dramatically impact the overall structural, physicochemical and biological properties of the staple peptide. Consequently there is no single optimal stapling strategy for all peptides and for each target, the staple must be carefully designed and screening of libraries of peptides is most often required. We propose here to develop a dynamic combinatorial stapling method, in which the staple is reversibly auto-assembled on a peptide sequence, in the presence of a biological partner. In this

way, the target chooses the most suitable staple by amplification of the staple/peptide combination that possesses the best affinity. The use of reversible chemistry in the presence of a target would allow an easy and rapid identification of the best stapling strategy for a given peptide; this has never been reported so far.[6] Starting from a linear peptide derived from a biologically relevant protein sequence, cysteine residues will be introduced in relative positions *i* to *i*+4. These peptides will be reacted with a library of linkers of different chemical nature, length and geometry, functionalized at each ends with thioester moiety, allowing their exchange with the peptide, leading to a library of stapled peptide that differ only from the nature of the staple. The generated libraries will be screened against the biological partner, in order to select the best staple/peptide combination. Analysis of the library content will be performed by LC and CE and identification of the peptide structure will be performed by MS and/or MS-MS. The most promising compounds will be then transposed into stable peptides through replacement of the labile thioester moiety by a robust amide bond. Structural investigation (NMR, Molecular modelling) as well as biophysical studies with conventional methods available in LBM (FP, SPR, ITC) will be then performed to confirm the conformation of the ligand and its affinity to the target and validate the method as a new tool for the development of PPI inhibitors.



3) References

[1] Sawyer, N.; Watkins, A. M.; Arora, S., *Acc. Chem. Res.* **2017**, *50*, 1313; [2] Baker, E. G.; Bartlett, G. J.; Porter Goff, K. L.; Woolfson, D. N. *Acc. Chem. Res.* **2017**, *50*, 2085; [3] Liu, Y.; Lehn, J.-M.; Hirsch, A. K. H. *Acc. Chem. Res.* **2017**, *50* (2), 376; [4] Zagiel, B.; Peker, T.; Webb, G.; Miclet, E.; Sachon, E.; Moumné, R. *manuscrit en preparation;* [5] Lau, Y. H.; de Andrade, P.; Wu, Y.; Spring, D. R. *Chem Soc Rev* **2015**, *44*, 91; [6] Only one example of reversible reaction for stapling of peptides is reported in the literature, using oxime side-chain cross-links; however, no combinatorial aspect is considered in this study: Haney, C. M.; Loch, M. T.; Horne, W. S. *Chem. Comm.* **2011**, *47* (39), 10915.

4°) Profile of the Applicant (skills/diploma…)

Candidates should have a Master Degree in organic or biological chemistry and an advanced training in organic synthesis and analytical chemistry (NMR, Liquid Chromatography (LC), Mass Spectrometry (MS)). A background and/or practice in peptide chemistry would be highly appreciated. The successful candidate is expected to be highly motivated, have scientific independence, excellent English speaking and writing skills.

Contacts: Thesis supervisor

Roba Moumné

Email address of the thesis supervisor: roba.moumne@sorbonne-universite.fr