PhD Report

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Biochemical and environmental sensing with cavitands

Research activity:

Post-translational modification (PTMs) such as methylation is known for being related to particular neurodegenerative diseases. Because of its implications in epigenetics, the study of the methylated lysine residues on histones is crucial for understanding the causes of activation or repression of genes during transcription and developing efficient therapies for diseases caused by this epimutation. To circumvent this issue, water soluble synthetic receptor called tetraphosphonate cavitand (Tiiii) bearing four pyridinium moieties at the lower rim was therefore designed and its molecular recognition properties towards mono-methylated lysine residues were verified. The objective of this thesis was to continue the investigations of this synthetic receptor on proteins employing them as a model system in reductive methylation of lysines and N-termini carried out following an established protocol. To prevent per-methylation of the lysines during the reaction, Tiiii was used as a stopper. Then, because the presence of the cavitand was demonstrated to be irrelevant for the overcome of the reaction, new multifunctional organic-inorganic FeNPs were designed. Decorating the FeNPs surface with tetraphosphonate cavitands, the multivalency features of the system was exploited by performing mono-methylation reactions on lysine residues on proteins. To achieve this, water soluble Tiiii cavitand was synthetized bearing four carboxylic acid moieties at the lower rim that provide the grafting onto the FeNPs surface. Reductive amination was performed using a stoichiometry amount between formaldehyde, cavitand and the reactive amines present on the protein (e.g. formaldehyde 6 eq, cavitand 6 eq).
Since methylation can occurred also on arginine residues and is still related to genes expression, a comprehensive study on diversely methylated arginine residues recognition by Tiiii cavitands was performed both in organic and water media *via* NMR and ITC.

Despite few examples, sensors for aromatic and essential aminoacids (AAs) such as phenylalanine and tryptophan are very little investigated even if an overabundance of these can cause Phenylketonuria or Hartnup disorder. A considerable challenge is the discrimination among similar PTMs, in particular changes in the position of single modification and the detection of different changes in a single protein. In this context, synthetic receptors provide an appealing solution, since they are effective, easily synthesized and efficient in biding selectively different residues on protein scaffolds. To demonstrate the potential of molecular recognitions properties of deep cavitands towards aromatic AAs, receptors (QxCav and BzPyCav) possessing a deep hydrophobic cavity suitable for aromatic guests complexation were synthetized and their recognition properties tested in biological media inducing binding *via* hydrophobic effect. In particular, the recognition of aromatic AAs, namely phenylalanine and tryptophan, were performed *via* NMR titrations, ITC experiments and, in solid state, *via* co-crystallization methods. QxCav were also tested at the solid/liquid interphase by grafting the receptors onto the surface of gold nanoparticles for the recognition of catecholamine metabolites *via* NOE pumping and STD experiments.

Highly selective carcinogenic benzene detection is socially relevant and technologically challenging, due to the concurring requirements of high selectivity and extreme sensitivity. Nowadays, real-time measurements systems based on the sensors designed to transform chemical

information into a useful signal are performed by bulky, expensive high-end systems based on advanced laboratory equipment, which require continuous service to operate. Stand-alone sensors, where the quinoxaline cavitand (EtQxBox) receptor acts as selective preconcentrator and GC-like separation device, allow for ppb level detection of benzene in air represent a key element in current strategies against pollution. However, discrimination between similar aromatic volatile organic compounds (VOCs), in particular benzene, was not achieved yet. By reducing the dimension of the entrance of the cavity we are aiming to reverse the complexation trend of EtQxBox in favour of benzene over the TEX. To achieve this, synthetic studies were performed. New dioxolane- and dioxepane- quinoxaline cavitands were synthetized. Furthermore, a new Qx-based cavitand (EtQxAC), in which only two quinoxaline walls are linked together at the upper rim to boost the binding of benzene with respect to TEX by reducing the dimensions of the entrance of the cavity, was designed and synthetized. Using this approach we have obtained two different isomers: one has the linker on the same side of the scaffold while the other has the linker that crosses the cavity.

In closing, different water soluble macrocyclic receptors were synthetized and their recognitions abilities towards aminoacids were tested in biological media. The synergic effect of at least three molecules of Tiiii involved in the binding event in the Tiiii@FeNPs appears to be the more appealing demonstrating the possibility to reach an acceptable grade of mono-methylated lysine residues on native proteins. Whereas with methylated arginines, a decrease in the selectivity of the receptor was observed.
Five water soluble deep cavitand were synthetized for the recognition of aromatic amino acids. Unfortunately, the capability of this class of molecules to switch from a so called vase conformation to a kite one causes the opening of the cavitand and the formation of a dimer when dissolved in water. Thus, the lack of a suitable cavity for guest complexation makes their recognition impossible.
The narrowed dimension of the cavity in EtQxAC eliminates the problem of strong retention of BTEX by EtQxBox, prolonging the lifetime of the device since BTEX have not to be exhaustively desorbed at temperatures higher than 250 °C. Moreover, these studies open the new possibilities of quinoxaline walls' functionalization with linker of different length and in different positions.

Scientific activities:

Overview on taken conferences:

Date (duration)	**Name**
12-14/07/2017	NanoDays
22/11/2018	Molecular machines and motors
17/12/2018	Giornata della Chimica - SCI

Overview on taken schools:

Date (duration)	**Name**
29-01 + 03-02/2017	Wispoc
28-29/09/2017	Suprabarrier
29-30/11/2018	Suprabarrier
12-14/12/2018	Supralyon

Overview on taken courses:

Date (duration)	**Name**
1° semester (A.A. 2016-2017)	Materiali Nanostrutturati a base di Carbonio
2° semester (A.A. 2016-2017)	Advace organic chemistry
1° semester (A.A. 2017-2018)	Metodi fisici in chimica organica e laboratorio
19/06/2017	Jeol NMR ECZ600R
18/07/2017	MestReNova
16/02/2018	Solid state NMR
SOFT SKILLS	
29/09/2017	Notte dei ricercatori
28/09/2018	Notte dei ricercatori
27/09/2019	Notte dei ricercatori
17/05/2017	CV day
04/05/2018	Public speaking
21-22/02/2019	Project design and writing
06/2017	PLS
06/2018	PLS

Secondment:

Date: 11/03/2019 - 09/08/2019
Location: NUI Galway – Ireland
Subject: Monomethylation studies of lysines present in model proteins (i.e. lysozyme); Co-crystallization studies of water-soluble synthetic receptors (previously synthesized in the University of Parma) with aromatic amino acids (phenylalanine, tryptophan and tyrosine), with proteins synthesized in the laboratory of Professor Crowley (RSL) and with the above mentioned monomethylated lysine residues in proteins deriving from previous methylation studies.
Crystallization tests were carried out also with readily available myoglobin and DNAse I, both with the previuosly synthesized receptors as well as with commercially available calixarenes.