consequences for millions of men, women and children. Despite the high prevalence of parasitic diseases worldwide, in most cases their treatment is inadequate, generating an urgent demand for new antiparasitic drugs. However, in addition to the traditional challenges involved in the complex process of drug discovery and development, there is the hurdle of the lack of investments in this field. This situation is especially problematic in de novo drug discovery, regarded as a high risk and costly process. Therefore, strategies that allow high quality hit identification rate as well as reduction in drug discovery costs are extremely useful in this field. The biology of parasitic organisms has been continuously studied in detail, providing a solid base for the selection of relevant molecular targets for drug discovery. Virtual screening strategies, including the use of both ligand- and structure-based methods, have been employed in the search for new inhibitors of relevant therapeutic targets related to parasitic diseases. More recently, the combination of computational and experimental techniques has been explored as a useful approach for the identification of high quality hits. This talk outlines the progresses and applications of the combined approach of protein structure determination followed by *in vitro* and *in silico* screening strategies for the discovery of innovative chemotherapy agents for a variety of neglected diseases of poverty, highlighting the challenges, limitations, and future perspectives in medicinal chemistry. We will conclude by reviewing our work on Chagas disease, with both ligand- and structure based drug design strategies, resulting in promising new leads and drug candidates.

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**Keywords: trypanosome cruzi, schistosoma mansoni, drug candidates**

## MS.16.4

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### X-ray microdiffraction reveals structural features of amyloid deposits *in situ*

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Amyloidoses, including Alzheimer's disease, belong to emerging public health problems in Western countries. All amyloidoses share the same morphologic, structural, and tinctorial properties consisting of insoluble fibrils stained by specific dyes, a fibrillar aspect in electron microscopy and a typical cross-$\beta$ folding in X-ray diffraction pattern.

Most studies aiming at deciphering the amyloid structure rely on fibres generated *in vitro,* or extracted from tissues using protocols, which may modify their intrinsic structure. Therefore, *in situ* fine architecture of the deposits remains unknown.

Here, we present the first study, carried out on paraffin-embedded and frozen human renal tissue sections, aiming at revealing the structural features of amyloid fibrils in deposit without any possible structure modification induced by extraction [1]. To this end, we used synchrotron-based microdiffraction, which is the unique technique that gives access to micron-sized spatial resolution. This allowed detecting variations at the micron-scale in the fibres molecular structure. Hence, fibres orientation could be followed along a glomerulus and a variation inside and around the whole glomerulus unit could be monitored.

We further show that the *in situ* fibres in amyloid deposits are partly folded into cross-$\beta$ sheets exhibiting a diffraction feature at 4.7 Å as *in vitro*-formed fibres. This validates the structural data from extracted fibres and strengthens the use of this diffraction signal as a main criterion of fibrils formation *in vitro*. We provide the experimental conditions for detecting the characteristic 4.7Å reflexion inside tissue cuts. Working on the tissue gives access to intrinsic information about the molecules, we thereby show a correlation between the position inside the glomerulus and the amyloid fibres partial orientation.

*Materials and Methods.* Experiments were performed at ESRF beamline ID13 using a 2 µm beam diameter. The samples consisted in 40 µm thick tissue slices.

These results are of highest importance to understand amyloid deposit formation and spark interest to investigate tissues. Given the possibility to access to the intrinsic structural parameters such as the fibre orientation or fibre size, relevant information about *in situ* mechanisms and conditions of deposits formation will become available with potential diagnostic and therapeutic consequences.

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**Keywords: in situ X-ray structure imaging, cross-β folding, amyloidosis, fiber diffraction**

## MS.16.5

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### Insights into the Food-Poisoning toxin, *Clostridium perfringens* enterotoxin

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*Clostridium perfringens* enterotoxin (CPE) is a major cause of antibiotic-associated and sporadic diarrhoea, it is also a major cause of food-poisoning [1]. The toxin is the second most common cause of hospital-acquired enteric disease, after *Clostridium difficile*. CPE is a 319-residue protein produced by type A or C isolates of *C. perfringens* and can be chromosome or plasmid located [2]. CPE is a pore-forming toxin that acts by forming pores in intestinal epithelial cells [3]. The toxin has been shown to form a number of complexes with claudin, and perhaps occludin, at the tight junctions of CaCo-2 cell cultures [4]. The exact composition of these complexes continues to be a matter for discussion. However, it appears CPE forms a small complex (of around 90 kDa on initial binding to membrane), followed by a larger pre-pore complex and finally the largest complex, the active pore form.

We present the X-ray crystallographic structure of CPE determined to 2.7 Å resolution. The fold of the protein reveals an expected homology to the HA-3 haemagluttinin from *Clostridium botulinum* and unexpected homology to the Aerolysin-like family of beta-pore-forming proteins. We have shown CPE to be a trimer in several different crystal forms, by Electron Microscopy and also in solution. The trimer has the characteristics of a biologically-significant oligomer, but does not form a pore, and the previously identified claudin-binding cleft [5] is unoccluded and oriented in a single plane on one side of the molecule. Residues previously associated with forming a membrane-inserted beta-hairpin [6] are folded in a small helix at the centre of the complex. However, Aerolysin-like pore-forming toxins are normally hexameric or heptameric and there is evidence to support a hexameric pore for CPE.

In this presentation we will discuss the homology between CPE

and other beta-pore-forming toxins. This homology and its trimeric form will allow us to make some conclusions regarding the formation of complexes with Claudin at tight junctions.

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**Keywords: toxin, food, hairpin**

## MS.17.1

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**Designer enzymes**

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Protein design is a challenging problem. We do not fully understand the rules of protein folding, and our knowledge of structure-function relationships in these macromolecules is at best incomplete. Nature has solved the problem of protein design through the mechanism of Darwinian evolution. From primitive precursors, recursive cycles of mutation, selection and amplification of molecules with favorable traits have given rise to all of the many thousands of gene products in every one of our cells. An analogous process of natural selection can be profitably exploited in silico and in the laboratory on a human time scale to create, characterize and optimize artificial catalysts for tasks unimagined by Nature. Recent progress in combining computational and evolutionary approaches for enzyme design will be discussed, together with insights into enzyme function gained from studies of the engineered catalysts.

**Keywords: enzyme, computational design, evolution**

## MS.17.2

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**Molecular probes as starting point for structure-based lead development**

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In addition to high-throughput screening and structure-based drug design, fragment- based approaches have recently become increasingly popular for lead development in pharmaceutical drug research. Here, a small but well selected library of low molecular weight compounds (< 300 Da) is screened by biophysical methods such as surface plasmon resonance (SPR), nuclear magnetic resonance (NMR) or X-ray crystallography.

In this study, we started with even smaller, highly-soluble probe molecules, such as aniline, urea, N-methylurea, propanediol, bromophenol and phenol. These probe molecules were selected to experimentally map out protein binding pockets by detecting hot spots of binding with respect to hydrophobic and hydrophilic properties. Furthermore, they should be applicable to a wide range of target proteins. As model protein the zinc protease thermolysin was selected. Subsequently, our studies were extended to additional proteins such as protein kinase A (PKA), D-xylose isomerase (DXI), 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (IspD) and the aspartyl protease endothiapepsin (ETP). The obtained crystal structures clearly show that the probe molecules could be located in these protein binding pockets. These probe molecules form similar interactions as larger ligands containing analogical chemical features and therefore are deemed suitable for hotspot detection.

Next, the structure of PKA in complex with phenol was used as template for docking of a virtual in-house fragment library of about 4000 entries. With one promising candidate a crystal structure was subsequently determined. Using the structural information and the experimental hot spot analysis, a putative lead skeleton was obtained that was translated into a synthetically accessible compound class. Of the synthesized compound series, one first representative showed an affinity of 70 μM. Based on this complex structure further lead optimization is in progress.

**Keywords: probe molecules, fragments, drug design**

MS

## MS.17.3

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**Supramolecular synthons in crystal engineering**

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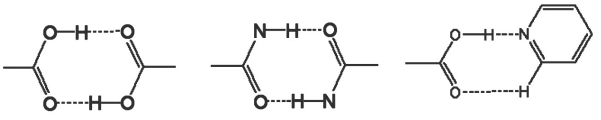
Crystal engineering deals with creation of novel materials using controlled arrangements of molecules in the crystal lattice using intermolecular interactions.[1], [2] Various common functional groups such as carboxylic acids, amides, phenols and other weak hydrogen bonding functional groups known to form certain recognition motifs in a repetitive manner (Scheme 1). These motifs were termed as supramolecular synthons signifying the importance of such motifs in the aggregation of molecules in the crystal lattice. Supramolecular synthons are defined by Desiraju as "structural units within supermolecules which can be formed and/or assembled by known or conceivable synthetic operations involving intermolecular interaction" [1]. In this talk the utility of synthons and robustness and interference effects of popular synthons will be discussed in detail by using some of our recent results [3-11]. Further the discovery of new synthons and transfer of supramoleuclar synthons observed in organic molecules into coordination polymers will also be discussed.



Scheme 1: Popular strong hydrogen bonding synthons

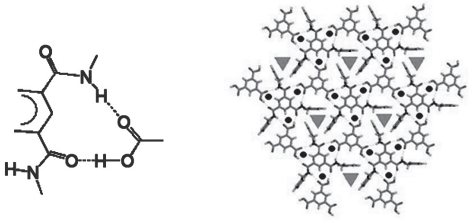


Figure 1: A new robust synthon in the generation of two-dimensional layer between two chemical components.

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