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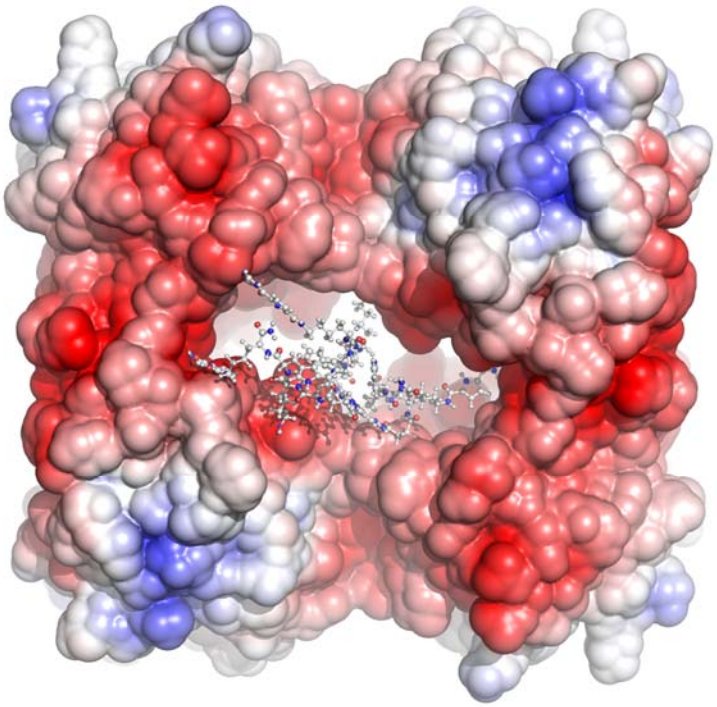

Supramolecular Chemistry on Proteins

29 – 30 September 2015; University of Duisburg-Essen, Essen, Germany

Poster Abstracts

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Poster category: A

(1) Dissection and Modulation of (patho)biological Survivin Functions by supramolecular Ligands

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Survivin plays an important role in cancer. While it is mostly absent in normal resting adult tissues, it is highly up-regulated in almost all cancer types. Its overexpression is associated with a resistance of tumors against chemo-and radiotherapy, making Survivin an attractive target for novel therapeutic strategies[3].

As a member of the IAP family, Survivin plays a role in the inhibition of cell death, but as part of the CPC it is also crucially involved in mitotic regulation. In both cases, an interaction with the nuclear export receptor Crm1 mediated by Survivin's nuclear export signal (NES) is necessary for executing its function[1-3]. Thus, an inhibition of the Survivin/Crm1 interaction will most likely lead to a loss of Survivin's anti-apoptotic and mitotic function and therefore to an inhibition of cancer cell proliferation.

Our project aims to establish novel *in vitro* and *in vivo* assays to quantitatively assess the Survivin/Crm1 interaction and to analyze the biological effects of supramolecular binders targeting Survivin's NES.

Following verification of cellular co-localization of Survivin and Crm1 by IF staining, pulldown assays biochemically demonstrated a Survivin/Crm1 interaction and an inhibiting effect of the molecular tweezer. Additionally, we are now able to recombinantly express and purify not only Survivin, but also the high molecular weight export receptor Crm1 and its cofactor Ran in order to employ the highly pure proteins in ongoing *in vitro* assays and protein structure analyses.

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Poster category: A

(2) Stabilizing Protein-Protein-Interactions with Multivalent Ligands

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In all living organisms protein-protein-interactions (PPIs) mediate a huge variety of biologically relevant processes. To garner insights into these processes it is of great importance to address these PPIs specifically. At the same time the modulation of PPIs is not fully understood, as they depend on molecular recognition processes on the proteins surface, and hence a challenging task.

A promising target is the class of 14-3-3-proteins and their interactions with several binding partners. As a member of the adapter protein family the highly conserved 14-3-3-proteins are involved in the regulation of other proteins, e. g. p53 or RAF[1] (Figure 1a) which are also of therapeutic significance.

We present here the synthesis of multivalent peptide ligands (Figure 1b) featuring an artificial carboxylate binding motif **1** developed by us[2] (Figure 1c) as well as the first results of their stabilizing effect on the 14-3-3/RAF-interaction.

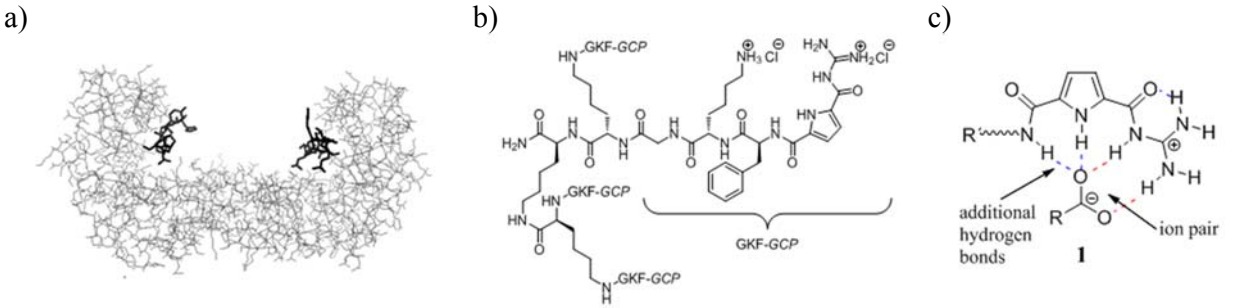

Figure 1: *a) Representation of 14-3-3ζ dimer (light grey) in complex with C-RAFpS233pS259 (black); pdb* ***4FJ3*** *b) multivalent ligand featuring different amino acids/artificial binding motif c) 2-(guanidiniocarbonyl)-pyrrol-5-carboxylate (GCP)* ***1****.[2]*

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Poster category: A

(3) *Intravenous* Application of the Glycine Receptor Agonist β-Alanine in Acute Mesenteric Ischemia

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Introduction: In previous studies, glycine showed beneficial effects in acute mesenteric ischemia (AMI) *in vivo* [1]. It clearly diminished hypoxia-induced damage and showed a modulatory effect on macrophages and leucocytes *in vitro* [2, 3]. To analyze whether glycine mediates its protective effects *in vivo* via the glycine receptor (GlyR), we investigated the GlyR agonist β-alanine, whose metabolism is completely different from glycine, in regard to comparable properties in the AMI.

Methods: In male Wistar rats the *arteria mesenterica superior* was occluded (t = 0 min) that way inducing AMI. Subsequent to the ischemic period reperfusion was started (t = 90 min). Finally (t = 210 min) animals were sacrificed by resection of the small intestine, kidney and liver. To evaluate the injury induced by ischemia-reperfusion, the small intestine was analyzed macroscopically and histologically. Besides, the number of macrophages in liver and small intestine were determined. At predefined points of time, blood samples were taken for blood gas analysis and the measurement of the activity of alanine and aspartate aminotransferases (ALAT, ASAT).

Results: β-alanine caused a significantly reduced injury of the small intestine and led to a diminished number of macrophages in liver and small intestine. In addition, a beneficial effect on the mean arterial blood pressure and the acid-base balance was observed.

Conclusion: These results indicate a receptor-mediated effect of glycine in AMI.

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Poster category: A

(4) Supramolecular Modulation of 14-3-3 Protein-Protein Interactions

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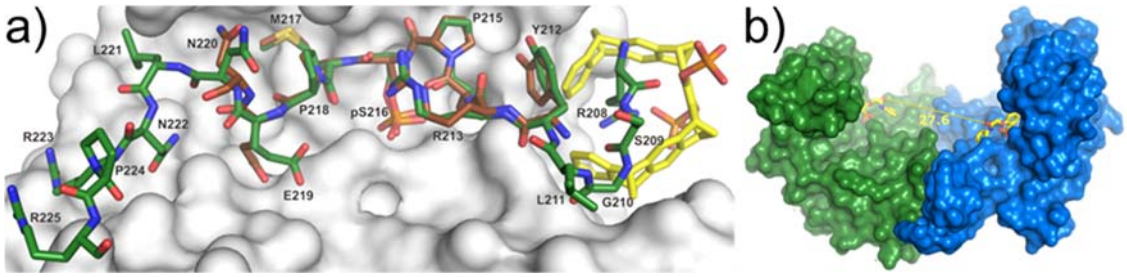
The adaptor protein 14-3-3 is found in a diverse range of pathologically relevant protein-protein interactions (PPIs). As 14-3-3 is a hub protein with very diverse interactions, it is able to influence the intracellular localization of their binding partners (BPs) and regulate signal transduction processes as well as cell cycle functions[1]. These numerous interaction pathways render 14-3-3 PPIs an attractive novel drug target.

One of these targets is the supramolecular tweezer molecule (CLR01), which was found to modulate 14-3-3 PPIs[2]. By binding to a surface exposed lysine located near the amphipatic groove of 14-3-3, the affinity of a multitude of BPs was greatly reduced. In contrast to these findings, the binding of one peptide mimic of BP Cdc25C was found to be enhanced. Biophysical and structural analysis showed that CLR01 stabilizes the interaction between Cdc25C and 14-3-3 (Figure 1 a).

An alternative strategy, making use of the dimeric structure of 14-3-3, novel small-molecule inhibitors were tethered to exploit the bivalent effect. Potent derivatives of a phenylphosphonic inhibitor scaffold[3] were bridged by linkers of varying lengths, thereby facilitating the compound to reach both binding sites of the 14-3-3 dimer (Figure 1 b). Biophysical evaluation revealed an 8-fold increase of the inhibitory effect for the bivalent phenylphosphonate towards 14-3-3 and its BP.



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Poster category: A

(5) Separation of biomolecule-loaded nanoparticles by asymmetric flow field flow fractionation (AF4)

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Since its development in 1966 by John Calvin Giddings, field-flow fractionation (FFF) has become a growing field of interest.[1] Being capable of separating nanoscopic substances like nanoparticles, polymers or peptides, FFF represents an effective analytical method in terms of selectivity, applicability and soft practice conditions.[2] Therefore, we chose asymmetric flow field-flow fractionation (AF4) from available FFF-techniques as a method to develop a new route of purification for biomolecule-loaded calcium phosphate nanoparticles. This type of particle is well known as efficient delivery agent for molecules, proteins or peptides into living cells.[3] Unfortunately, the traditional way of purification, i.e. centrifugation followed by ultrasonication, is rather rough and in case of proteins or peptides may lead to the destruction or denaturation of the biomolecules. To overcome these negative aspects, we have established AF4 as a more gentle method for purification.

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Poster category: A

(6) Amine immobilization on self-assembled monolayers via triazol formation employing microcontact printing.

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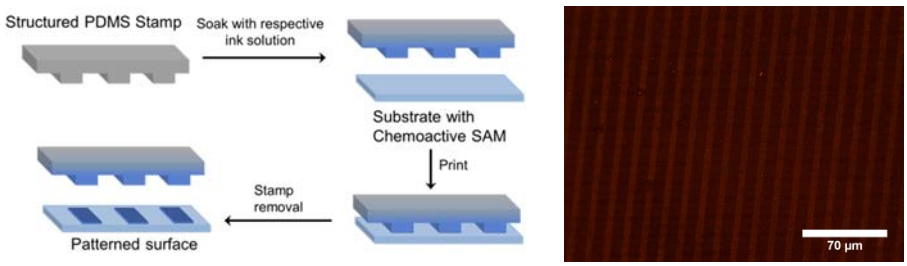
In many fields of materials sciences, such as industrial or medical applications, the surface properties of the employed materials play a key role for the feasibility and efficiency of the respective functions. Most of the common materials do not meet the demands of their specific assignment due to the unsuitable combination of their bulk and surface properties. In these fields surface modifications have gained a huge interest due to the possibility of selectively modifying materials to generate surfaces with the desired properties. The spatiotemporal covalent immobilization of molecules on surfaces is another field of interest due to the possibility of generation of microarrays and electronic storage devices. Microcontact printing is suitable for these areas of research due to its easy and fast performance and set up. It is possible to immobilize a small amount of substance on a large area in patterns that show a high resolution. In recent studies it was possible to immobilize a wide variety of molecules and proteins on surfaces employing click reactions[1,2]. In this study we show the generation of amine patterns *via* tosylhydrazone based triazol formation by microcontact printing. This reaction is based on the condensation of a primary amine with α, α –dichlorotosylhydrazone. The latter was bound to a substrate to form a chemoactive surface on which patterns of amines were formed. The success of the coupling was verified via XPS, condensation experiments and fluorescence microscopy. With this chemoselective reaction it is possible to immobilize primary amines at ambient conditions. This method opens a window to a wide variety of substances like basic molecules as well as bio molecules such as proteins.



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Poster category: A

(7) Multi-Component Assembly of an 14-3-3/Cucurbit[8]uril Supramolecular Protein Complex

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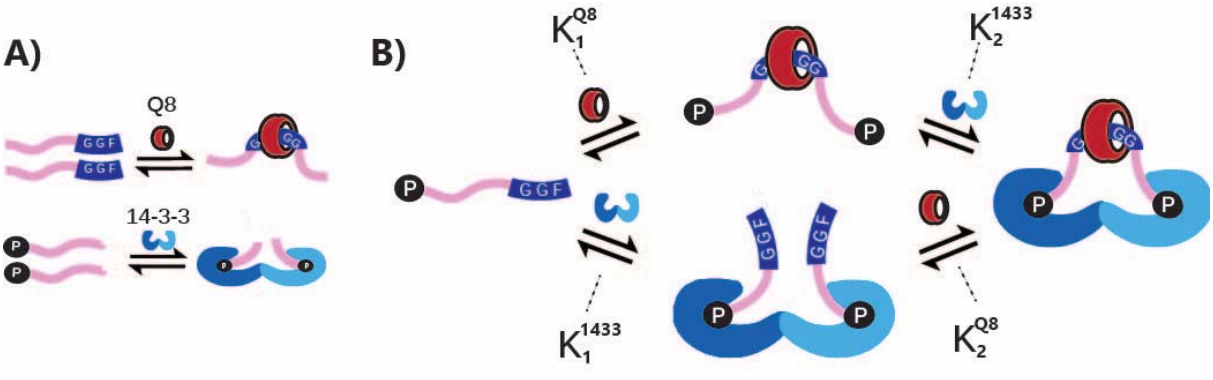
Cucurbit[8]uril (Q8) is a supramolecular host molecule able to induce dimerization of proteins carrying a FGG-tag (Phenylalanine-Glycine-Glycine)[1]. On the other hand, 14-3-3 proteins are natural scaffolds that can bring two partner proteins into mutual proximity and are vital to many cellular processes[2].

Herein, a hybrid supramolecular/protein scaffold approach is used to create a multicomponent supramolecular protein complex, involving both Q8 and 14-3-3. By combining multiple interactions, enhanced affinity and specificity are achieved.

A library was made of peptides having both a 14-3-3 binding motif and a FGG-tag, allowing dimerization involving either 14-3-3 or Q8 (see Figure 1A). When 14-3-3 and Q8 are offered both, a stable multi-component complex is formed (see Figure 1B).

Combining multiple interactions is a general strategy to increase affinity and specificity of binding[3]. The various binding steps were studied by different techniques. By comparing peptide binding to Q8 in presence and in absence of 14-3-3, an enhancement factor β of >100 is observed, caused by induced bivalency.

Due to the inherent symmetry, templating by Q8 and 14-3-3 lead to the same complex. Because of this, their enhancement effects are equal ($\beta = K_2^{Q8}/K_1^{Q8} = K_2^{1433}/K_1^{1433}$). This dual perspective allows for a scheme of internal validation between experiments, which makes these complexes interesting as a platform to study multivalency.



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Poster category: A

(8) Investigating multiarmed peptidic dendrimers using MD simulations

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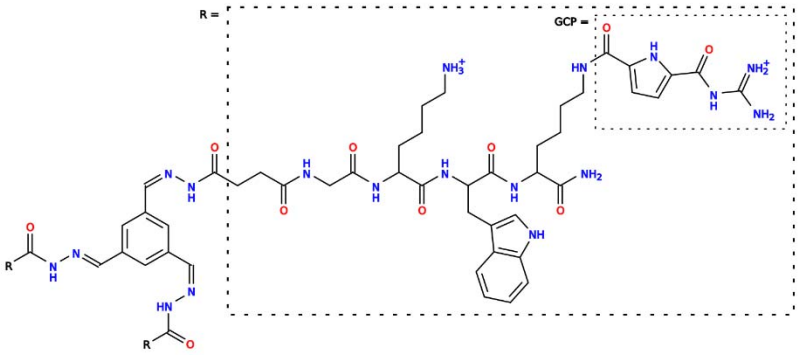
14-3-3 proteins play a critical role in the mitogen-activated protein kinase signaling cascade responsible for cell proliferation, differentiation and survival[1]. They are known to promote or prevent the recruitment of C-RAF by the Ras.GTP protein depending on its phosphorylation state[2], a behavior that could be altered using supramolecular ligands as binding partners.

Using Molecular Dynamics, ligand QQJ082 developed by Qian-Qian Jiang and coworkers[3] (Project A1, molecule depicted below) was investigated at a molecular level to reveal critical interactions with 14-3-3 These results will be used to facilitate future *in vitro* experiments with 14-3-3 and provide a basis for rational ligand design.

A recent X-ray structure of the dipeptide GCP-LYS-methylester (GCP = guanidiniocarbonyl pyrrole moiety) bound to 14-3-3 revealed the presence of a binding site into the 14-3-3 pore. Using Propka for pK_a prediction and Epitopsy for electrostatic calculations, we were able to detect this binding site and reproduce *in silico* the unusual protonation state of the dipeptide in the bound state, where the LYS side chain undergoes a significant pK_a shift and loses its positive charge due to the proximity of R18 and R55.



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Poster category: A

(9) Synergistic cooperation of supramolecular aminopyrazole-based Tau ligands and the human serine protease HTRA1 in the degradation of Tau fibrils

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One of the hallmarks of Alzheimer's disease is the occurrence of Tau-based neurofibrillary tangles. To approach the issue of Tau fibril formation various pharmacological strategies have evolved. Most of these strategies aim at the inhibition of aggregate formation by interrupting a single step in the process of fibril formation (i.e. Tau-phosphorylation, fibril elongation etc.).

In this study a series of supramolecular aminopyrazole derivatives previously shown to hinder the aggregation of Amyloid-β was investigated for its effects on Tau aggregation. Several of the tested compounds were found to have restricting effects on Tau aggregation, while one was shown to prevent aggregate formation entirely under the conditions tested.

Future work will investigate the compounds' effects on Tau fibrils in the presence of the widely conserved serine protease HTRA1, which was recently shown to disintegrate the tightly packed core structure of Tau-fibrils thus allowing for their efficient degradation. The aim of this study is to combine the effects of HTRA1 and the aminopyrazole derivatives thereby allowing for a more efficient disintegration and clearance of Tau-aggregates due to the concerted action of a supramolecular Tau-binder and a human protein.

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Poster category: A

(10) Design and synthesis of “Molecular Glue” for the human serine protease HtrA1

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The field of research of protein-protein-interactions (PPIs) is an intersection of biochemistry and supramolecular chemistry. These PPIs, which are not fully understood yet, form a whole class of essential reactions in living organisms like enzyme-substrate-interactions or other protein-surface-interactions. Through analysis and specific design in previous works of our group, we were capable of the synthesis of ligands which inhibit or stabilize proteins via surface-interactions [1]. Thereby the Human High Temperature Requirement Serine Protease A1 (HtrA1) is of particular interest. The native form of HtrA1 is predominantly a homo-trimer [2]. As decomposed monomer, for example though natural mutation of the amino acid sequence at the monomer contact area, the protein loses its biological activity. This biological activity shall be restored through custom-built ligands. For this purpose, the ligands shall lay between the contact areas of the monomers like „molecular glue“. Thereby the monomers should be able to reassemble to the biological active trimer.

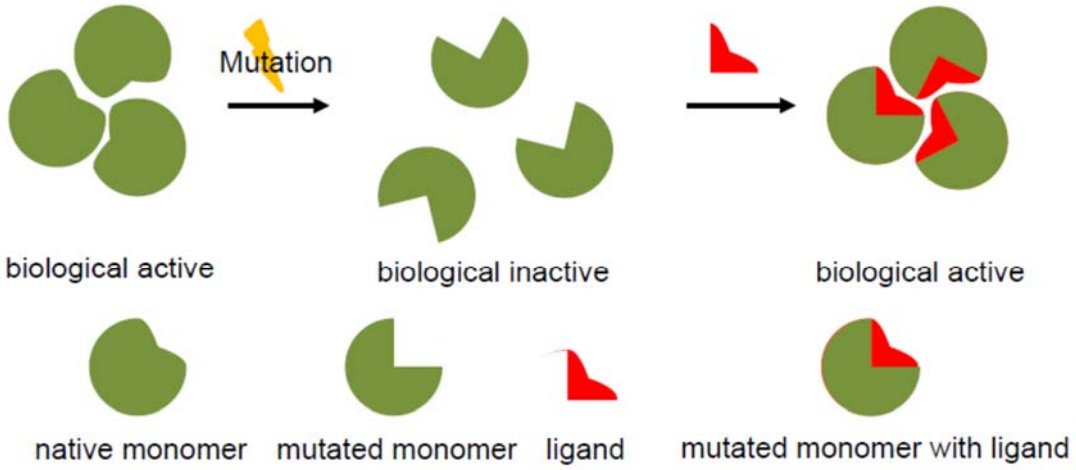

Figure 1: Exemplary sketch of the functionality of the “molecular glue”.

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Poster category: A

(11) Evaluation of the OPEP force field for protein-protein docking

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Knowing the binding site of protein-protein complexes leads to an understanding of their function and shows possible regulation sites. We refine and rescore rigid body protein-protein predictions. The predictions are generated by ZDOCK, a rigid body global search algorithm. For rescoring and refining we use the coarse-grained force field OPEP. We show the predictive power of the coarse-grained force field for protein-protein docking. The use of a force field, instead of a scoring function, gives one the possibility to introduce more flexibility later. By ZDOCK produced predictions were shortly energy minimized. Afterwards, they were ranked by a soft rescoring function based on OPEP. This rescoring function was further trained for different complex classes, as defined by Chen et al. We showed on a test of 46 complexes, which were not contained in the training data set, that the new rescoring functions perform better than the general OPEP rescoring. In the beginning, each prediction has secondary structures defined by the crystal structures of the binding partners. The energy minimization changes the secondary structures depending on the prediction. Near native predictions contain less amount of coils than incorrectly docked predictions after minimization. This observation can be used as a filter for ranking the predictions.

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Poster category: A

(12) Modulation of 14-3-3 dimers by GCP-derived small molecules targeting the dimerization interface

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14-3-3 proteins play a key role in controlling cell survival and proliferation. Thus, it is to assume that their dysfunction is related to a huge range of severe diseases such as cancer. It has been shown that specific human tumor tissues expose an enhanced expression of certain 14-3-3 isoforms and that a down-regulation of the 14-3-3ζ isoform in lung and neck cancer cells leads to a higher sensitivity for chemotherapy, which substantiates the hypothesis of an involvement of 14-3-3 proteins in cancer associated pathophysiological pathways. In this context the 14-3-3 protein family represents a highly interesting target for the modulation of protein-protein interactions (PPIs), since up to 500 interaction partners are known. Based on a guanidiniocarbonyl-pyrrole(GCP)-derived lead structure, which was developed in our working group and binds to the dimerization interface of 14-3-3ζ homo dimers with high affinity (as proven by X-ray crystallography, MST and UV/Vis-titration), we designed a library of GCP-substituted small molecules. These will be used in order to establish a structure activity relationship (SAR) with regard to the dimerization behavior of the 14-3-3 isoforms and the impact on biological systems, respectively. As the functions of 14-3-3 proteins are strongly dependent on an effective dimerization of two defined subunits, a specific modulation of these interactions could provide fundamental knowledge about their biological relevance and could lead to medicinal applications in drug development and anti-cancer therapy.

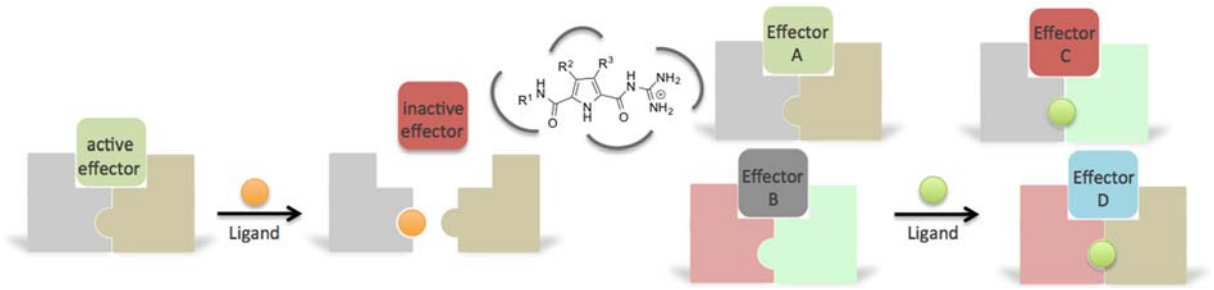

Figure 1: Schematic depiction of potential ligand-induced effects on the dimerization behavior of 14-3-3 monomers

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Poster category: A

(13) Alternative splicing of PFKFB4 and PFKFB2 as an important factor of the regulation of glycolysis and cell surviving

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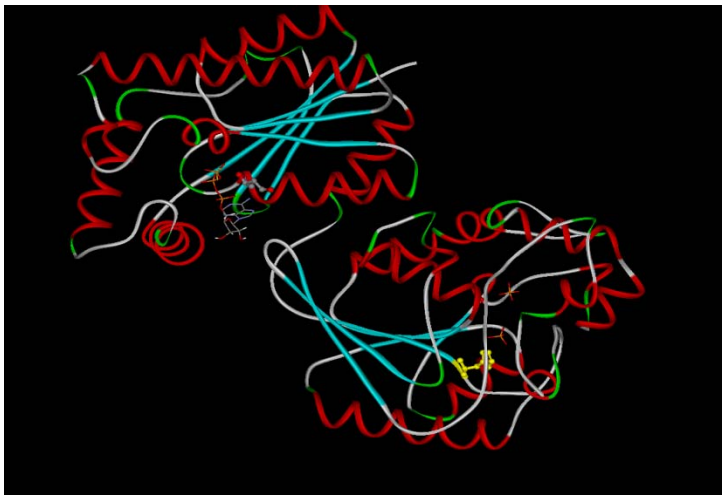
The 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB) is a key enzyme in the regulation of both glycolysis and cell surviving and proliferation. Four genes located in separate chromosomes encoded different variants of this enzyme with unique properties. Moreover, each pre-mRNA can create several alternative splice variants. The PFKFB is a bifunctional enzyme with 6-phosphofructo-2-kinase and fructose-2,6-bisphosphatase activities. The PFKFB4 enzyme is strongly upregulated in various cancers and is very important for cancer cell survival. We have shown that PFKFB4 and PFKFB2 have alternative splice variants with deletions or inserts in both catalytic domains or in N-terminal regulatory region. As a result of an alternative splicing the PFKFB4 and PFKFB2 variants can have only one enzymatic activity: 6-phosphofructo-2-kinase or fructose-2,6-bisphosphatase. Alternative splice variants PFKFB4 and PFKFB2 were observed in both human and animal cell lines and tissues. They are similar in different organisms with variable length of deletions and inserts. Moreover, in mouse tissues were identified two unique alternative splice variants of PFKFB4. One of them can produce two separate enzymes: 6-phosphofructo-2-kinase and fructose-2,6-bisphosphatase. Other one is very short and contains only N-terminal and C-terminal regulatory regions without catalytic domains. It is interesting to note that alternative splicing of PFKFB4 is sensitive to toxicants, particularly to MTBE.



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Poster category: A

(14) Computational modeling of supramolecular binding

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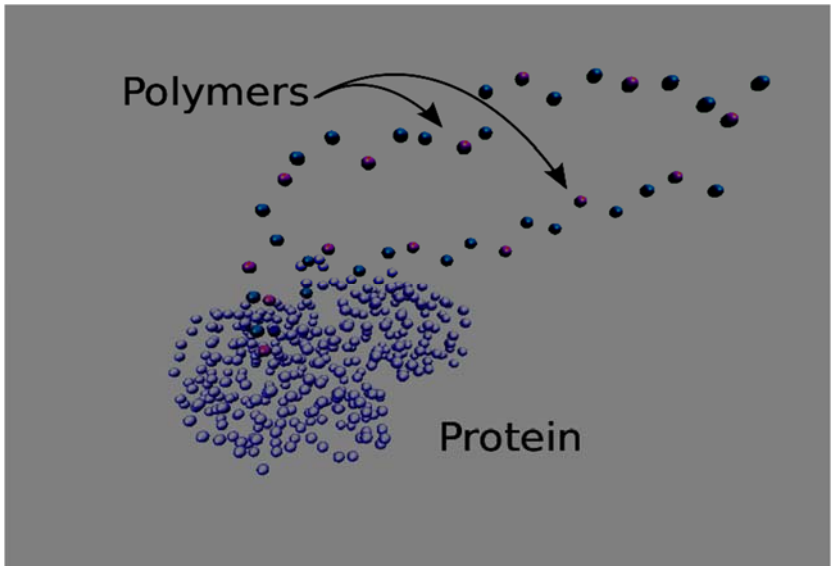
While the interaction of copolymers, e.g. glycosaminoglycan, and proteins plays a vital role in the biological organisms, many aspects of the interaction still remain unclear. These interactions occur throughout the extracellular matrix and are of significant importance for processes like intracellular signal transduction, cell migration and viral invasion of cells and angiogenesis. Many of these complexes formed by proteins and copolymers are facilitated by a multitude of weak binding spots that cumulate in total to strong avidity which makes them distinctly different from the small organic molecules usually used for strong binding. We are interested in the conformation of these complexes, as well as the makeup of the copolymers forming them. Additionally, the inhibitory activity of the copolymers towards specific proteins is of great importance [2]. For this, we conduct a series of coarse grained molecular dynamics simulations [1]. Results from preceding docking experiments between the copolymers' monomers and the target protein are used to implement topological constraints on the protein's surface. From the results of these simulations, we want to find out if we can predict the inhibitory activity of copolymer by optimizing composition and length of the copolymers. Furthermore, we are interested in optimizing the specificity of the copolymers towards targeted proteins.



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Poster category: A

(15) The flexibility of His516 negatively impacts the catalytic activity of glucose oxidase.

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The catalytic ability of an enzyme originates from the stabilization of a transition state geometry for a ligand conversion.[1] Catalytic groups, in complex with a ligand, lower the activation energy for a chemical transformation. The preorganization of the active site is among the key elements for enhanced catalysis.[2] The relative residue rigidity is often higher for the catalytic than for the non-catalytic amino acids.[3] We examined two new crystal structures of the highly active glucose oxidase (GOx) mutants in order to identify the cause of the increased catalytic potency. The His516 residue in the wt-GOx active site has a very flexible side chain. Its flexibility negatively impacts the catalytic activity by making the active site geometrically less suitable for the concerted proton and hydride transfer. The molecular dynamics analysis supplements the crystallographic data, showing that His516 stays more rigid in the mutants. The comparative free energy surface analysis as a function of the His516 side chain dihedral angles χ_1 and χ_2 shows the presence of one free energy minimum in the mutant GOx. However, the wild-type enzyme is characterized by two distinct minima. The mutant possesses the stiffest active site with the optimal geometry for glucose interconversion to gluconolactone. We conclude that the antiperiplanar arrangement of His516 is catalytically active while the synclinal conformation is catalytically inactive, or it contributes to catalysis only negligibly.

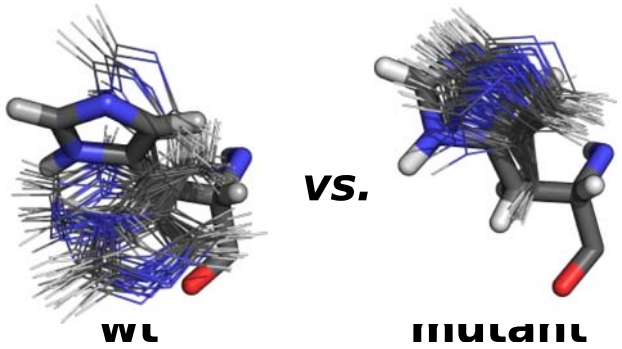

Figure 1. *His516 conformational sampling in the wt and mutant GOx.*

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Poster category: A

(16) Novel small molecule inhibitor of VCP/p97

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VCP/p97 is an abundant and essential AAA+ ATPase. The hexamer consists of two stacked rings of ATPase domains and an outer ring comprised of the adaptor binding N-domains. By forming alternate complexes with different cofactors, p97 is involved in many different cellular pathways. For example, p97 represents a central component of the ubiquitin-proteasome system, targeting ubiquitinated proteins for degradation in signaling and is an important part of protein quality control pathways. Its role in protein homeostasis qualifies it as a potential cancer drug target. However, the underlying mechanisms governing the functions of p97 are still poorly understood. Inhibitors of p97 provide a valuable tool that will facilitate further functional studies and possibly enable identification of pathway specific p97 cofactors. Here, we study a new small organic inhibitor I8 in *in vitro* as well as *in vivo* assays. *In vitro* assays showed that I8 inhibits p97 in the micromolar range by an allosteric mechanism. *In vivo* assays were used to assess cell viability and the effect of I8 on impairing degradation of proteins. While degradation of a p97-dependent substrate is inhibited, a p97-independent substrate of the ubiquitin-proteasome system is degraded, thus confirming specificity of the new inhibitor. Regarding the role of p97 in signaling, we show that the TNF-induced degradation of IκBα in HeLa cells is delayed by treatment with I8.

Poster category: A

(17) Expression Pattern of Survivin in the Small Intestine of Normoxic and Ischemic Rats

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Survivin acts as an apoptosis-inhibiting protein. Besides, it regulates mitosis and chromosomal segregation. The expression of survivin proceeds during the embryonic and fetal phase. In adult rats intestine survivin expression has already been shown[1]. However, a detailed evaluation of survivin expression in the different tissue layers and sections of the small intestine, as well as in ischemically damaged intestinal tissue is missing. In order to determine the expression pattern of survivin in the intestine of normoxic and ischemic adult rats, we induced mesenteric ischemia-reperfusion (I/R) injury by occlusion of the superior mesenteric artery (90 min) and subsequent reperfusion (120 min). Afterwards, immunohistochemical staining of paraffin sections of untreated or ischemically treated tissue was performed. In the small intestine of normoxic rats, survivin was expressed mainly in the epithelial cells of the crypts and only slightly in the villi. Likewise, the expression of survivin could be detected in the ischemically damaged intestine. The expression pattern corresponded to the normoxic animals, as far as verifiable due to the existing tissue damage. In addition, the expression pattern of survivin matched with that of the proliferation marker Ki-67. These results suggest a high regenerative capacity of the unimpaired small intestine. In spite of the ischemic damage, the small intestine still shows a similar high regenerative capacity.

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Poster category: A

(18) Ultrasmall gold nanoparticles for selective protein interaction

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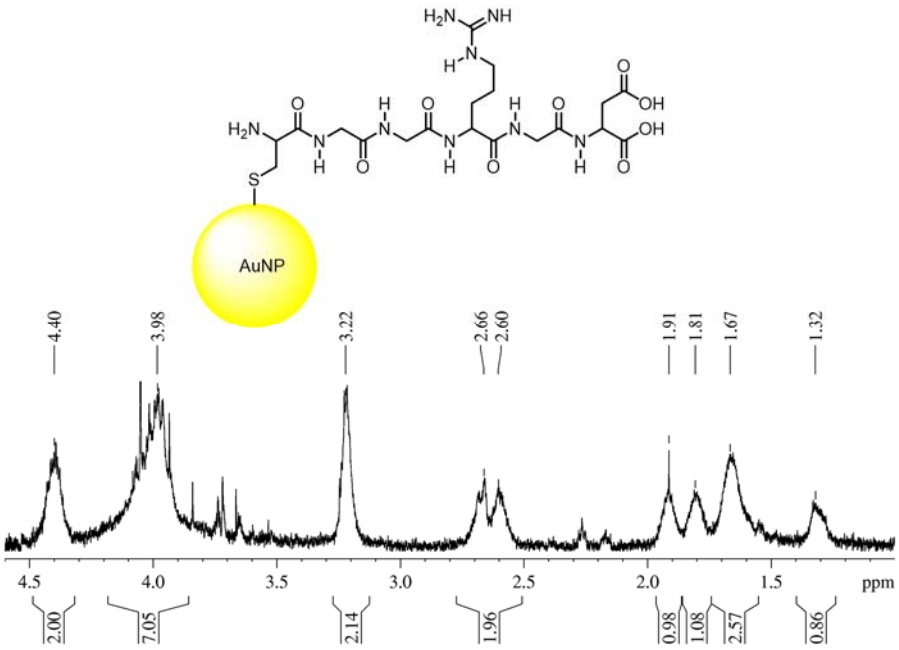
Gold nanoparticles functionalized by different aliphatic and aromatic thiol-functionalized carboxylic acids or with two small peptides (CG and CGGRGD) were synthesized by reduction of $HAuCl_4$ with $NaBH_4$ in the present of one of the above ligands. After purification by centrifugation or filtration and redispersion, the dispersed nanoparticles were analyzed by differential centrifugal sedimentation (DCS) and a variety of NMR techniques: ^{1}H-NMR, ^{1}H,^{1}H-COSY and ^{1}H-DOSY. The hydrodynamic diameter of the particles was between 1.8 and 4.4 nm. Diffusion ordered spectroscopy (DOSY) proved to be a valuable and non-destructive tool to determine the hydrodynamic diameter of dispersed nanoparticles and to control the purity of the finished particles. The coordination of the organic molecules to the gold nanoparticles resulted in distinct changes in the ^{1}H-NMR spectra which are only partially explainable but are clearly caused by the vicinity of the molecules and the gold nanoparticle.



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Poster category: A

(19) Tailor-made copolymers for protein-selective enzyme inhibition

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Linear multivalent polymers which are generated by free radical and controlled radical (ATRP) copolymerization of several amino acid-selective monomers can be used as enzyme-specific receptors. They are able to shut down enzyme activity in a reversible and irreversible manner, either by sterically blocking the active site or by enzyme denaturation after binding.[1-3]

We recently found substoichiometric inhibition of trypsin by the polymers. Here we present our results and mechanistic studies for the unique supramolecular inhibition process of trypsin.

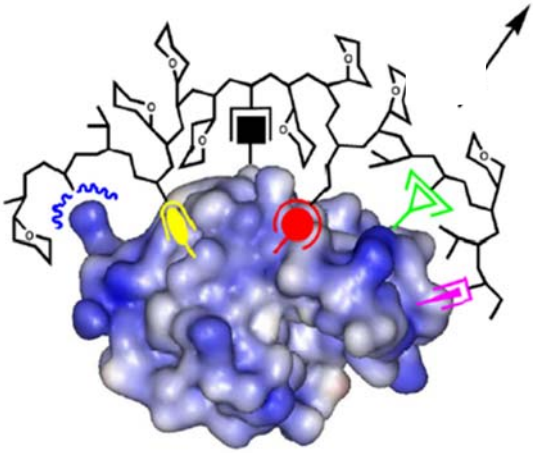

Figure 1: Amino acid-selective copolymer binds to the protein surface.

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Poster category: A

(20) Hybrid Quantum Mechanics/Molecular Mechanics/Coarse Grained Modeling: A Triple-Resolution Approach for Biomolecular Systems

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We present a hybrid quantum mechanics/molecular mechanics/coarse-grained (QM/MM/CG) multiresolution approach for solvated biomolecular systems. The chemically important active-site region is treated at the QM level. The biomolecular environment is described by an atomistic MM force field, and the solvent is modeled with the CG Martini force field using standard or polarizable (pol-CG) water. Interactions within the QM, MM, and CG regions, and between the QM and MM regions, are treated in the usual manner, whereas the CG–MM and CG–QM interactions are evaluated using the virtual sites approach. The accuracy and efficiency of our implementation is tested for two enzymes, chorismate mutase (CM) and *p*-hydroxybenzoate hydroxylase (PHBH). In CM, the QM/MM/CG potential energy scans along the reaction coordinate yield reaction energies that are too large, both for the standard and polarizable Martini CG water models, which can be attributed to adverse effects of using large CG water beads. The inclusion of an atomistic MM water layer (10 Å for uncharged CG water and 5 Å for polarizable CG water) around the QM region improves the energy profiles compared to the reference QM/MM calculations. In analogous QM/MM/CG calculations on PHBH, the use of the pol-CG description for the outer water does not affect the stabilization of the highly charged FADHOOH-pOHB transition state compared to the fully atomistic QM/MM calculations. Detailed performance analysis in a glycine–water model system indicates that computation times for QM energy and gradient evaluations at the density functional level are typically reduced by 40–70% for QM/MM/CG relative to fully atomistic QM/MM calculations. This approach is particularly useful to accurately model small molecule interactions occurring in very large molecular systems.

Poster category: A

(21) Synthesis of novel asymmetric, clickable diphosphate tweezers for regioselective protein surface recognition

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Molecular diphosphate tweezers are able to bind to lysine and arginine residues on protein surfaces and are used as new enzyme inhibitors and blockers of pathological protein aggregation.[1,2] In a first attempt to enhance selectivity, we replaced one phosphate group by a neutral linker unit for the introduction of additional recognition elements.[3] Since the neutral linkers generally lowered tweezer affinities towards basic amino acids and peptides, we now pursue a new strategy which keeps both phosphate anions and allows introduction of one recognition unit by “click” chemistry.

This poster presents synthetic methodology towards novel clickable asymmetric diphosphate tweezers and binding studies. These results will be used in the future to generate more potent tweezers species with higher specificity for their target proteins.

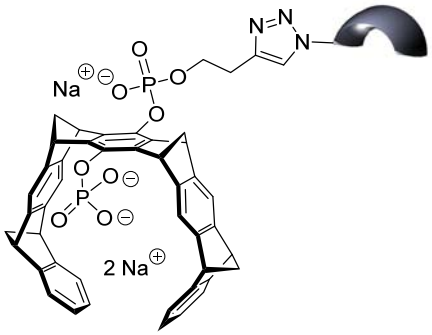

Figure 1: Molecular diphosphate tweezer for the binding of lysine and arginine with attached peptide ligand unit.

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Poster category: A

(22) Molecular impact of Survivin acetylation on its biological function

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Survivin was demonstrated to conduct an at least dual biological role. As a member of the IAP family, it exhibits anti-apoptotic functions, but is also necessary for proper chromosome segregation during mitosis[1]. Survivin was found to be upregulated in virtually all types of human cancers and is associated with resistance against chemo- and radiotherapy, making it a promising target for cancer therapy.

Survivin is regulated by multiple means of dynamic cellular localization, protein interactions and posttranslational modifications, including acetylation at K129. It has been postulated that this acetylation event might regulate not only Survivin´s mitotic activity but also its dimerization behavior, which in turn affects its Crm1-mediated nuclear export[2, 3].

Our project aims to further investigate the mechanism by which acetylation on K129 affects Survivin regarding its dimerization, dynamic localization and anti-apoptotic and mitotic functions. Using size-exclusion chromatography, we were able to demonstrate that mimicking acetylation on K129 does not seem to influence its dimerization *in vitro*.

To investigate dimerization in a cell-based system, we currently establish a FRET assay and a proximity ligation assay. In addition, we are setting up an apoptosis assay to analyze the influence of acetylation on Survivin´s anti-apoptotic functions. Effects on mitotic localization and function will be quantitatively assessed by the high-content screening (HCS-A) module of our Leica SP5 confocal microscopy system to automatically detect rare events like cell divisions in a statistically robust manner.

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Poster category: A

(23) Impact of Tranexamic Acid on Intestinal Ischemic Injury and Shock

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Tranexamic acid (TXA) inhibits the fibrinolytic activity of plasmin. It was brought into clinical use a few decades ago to promote hemostasis. Based on the latest clinical trials it is used in trauma surgery in severely injured patients at high risk of bleeding to prevent or stop the bleeding[1,2]. The intestine is attributed to a central (enhancing) role in the pathogenesis of shock. However, it is unknown how the intestine mediates this aggravating role. Mediators caused by the injury are assumed to provoke distant site injury and initiate the shock when released (most likely via the intestinal lymphatic vessels) into the systemic circulation. Previous studies suggest that the mediators of intestinal shock are the result of a secondary process triggered by the intestinal injury with a decisive participation of plasmin. Here, we studied the protective effect of intravenous infusion of tranexamic acid in a rat model of severe intestinal ischemia-reperfusion (I/R) injury and shock. Mesenteric I/R was induced by occlusion of the superior mesenteric artery (90 min) and subsequent reperfusion (120 min)[3]. TXA attenuated injury of the small intestine as indicated by less histopathological alterations and prevented shock as indicated by improved blood pressure. These results suggest that TXA possess a protective effect, which is independent of its antifibrinolytic effect and likely to be mediated via the participation of plasmin in pathophysiological/proinflammatory processes.

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Poster category: B

(24) Stimuli-responsive supramolecular nanoparticles

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Supramolecular nanoparticles (NPs) open new opportunities for biomedical applications. When the control over a stimuli-responsive host-guest complex is incorporated in nanoparticles, this can be used for encapsulation and targeted release of growth factors and other agents. The cavity of cucurbit[8]uril (CB[8]) handcuffs the redox-responsive methylviologen and the light-responsive trans-azobenzene in a stable 1:1:1 ternary complex.[1] This complex becomes an amphiphile owing to the fact that these guests are modified respectively with a hydrophobic and a hydrophilic tail. Moreover this supramolecular amphiphile self-assembles in orthogonally switchable NPs. Finally their void can be loaded with a cargo and their external leaflet readily modified via host-guest chemistry to target cells.

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Poster category: B

(25) A powerful stereoselective entry to 4,5-disubstituted prolines: Application in the synthesis of trandolapril

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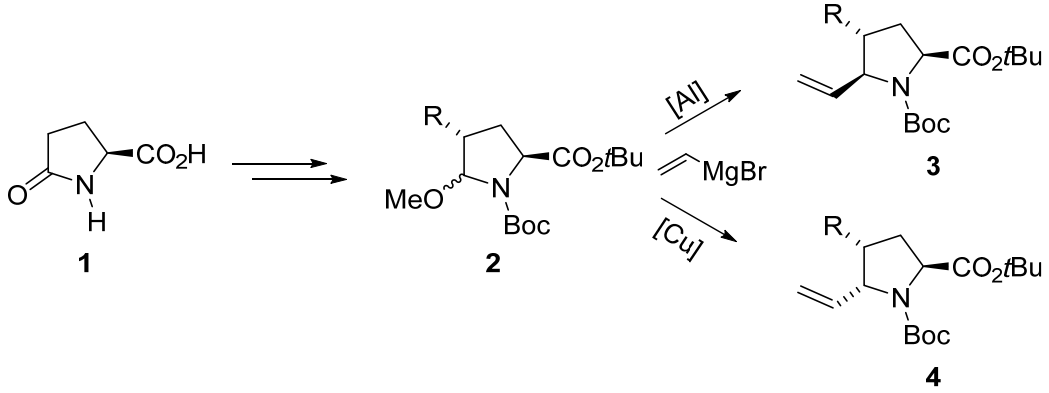
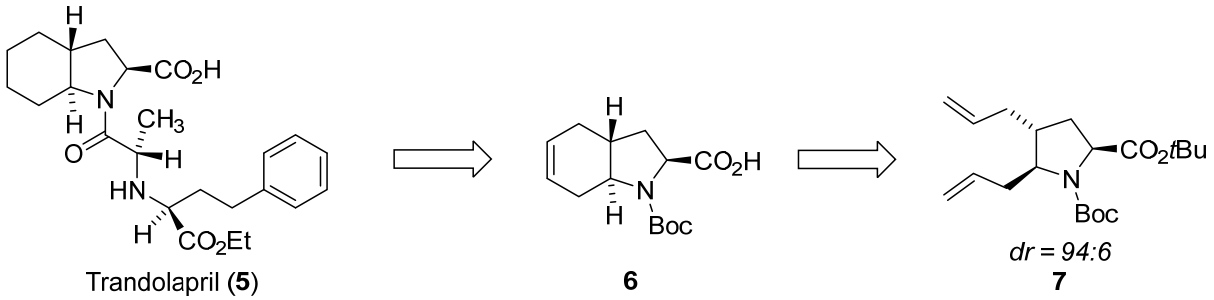
In the course of our studies towards the synthesis of proline-based dipeptide mimetics with defined conformation,[1] we have developed a methodology for the stereoselective preparation of proline derivatives starting from pyroglutamic acid (**1**).



This method led to a variety of *trans*-4-substituted prolines (**2**) and allowed the subsequent introduction of a second side-chain, which could be installed with high diastereoselectivity, to afford either the *cis*-(**3**) or the *trans*-isomer (**4**) depending on the metal additive used.[2] The power of the method was demonstrated in an efficient synthesis of the ACE-inhibitor trandolapril (**5**),[3] a synthetic drug used for the treatment of high blood pressure.



Key steps of this synthesis include the formation of a diallylated proline derivative **7** and a Ruthenium-catalyzed ring closing metathesis to the hexahydroindole species **6**.

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[2] (a) I. Collado, J. Ezquerra, C. Pedregal, *J. Org. Chem*. **1995**, *60*, 5011-5015; (b) A. Soicke, *Dissertation* **2014**, University of Cologne.

[3] G. Kretzschmar, K. Rossen, *WO2014202659,* **2014**.

Poster category: B

(26) Delivering Proteins and DNA to Cells using Photo-sensitive Supramolecular Complexes

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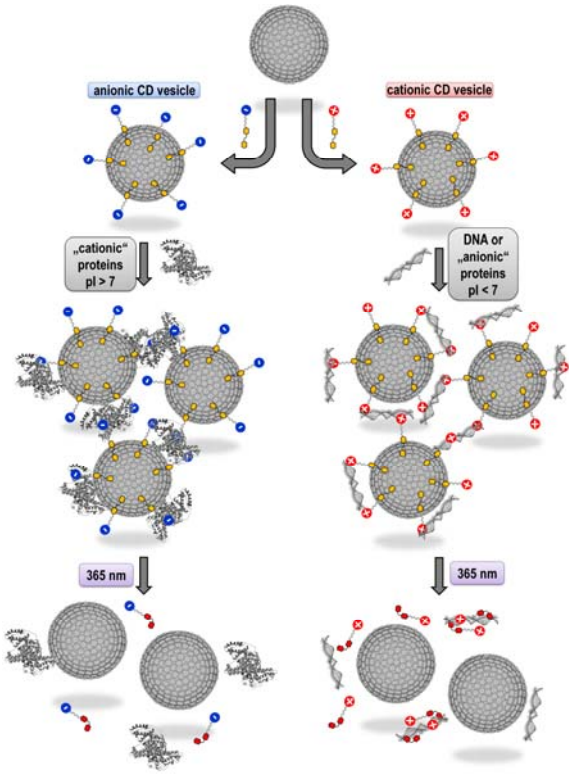
Light-induced capture and release of proteins and DNA has been realized, combining host-guest chemistry and electrostatic attraction.[1] In aqueous solution, spontaneous self-assembly of a supramolecular complex containing vesicles of amphiphilic cyclodextrin (CDV), a bifunctional linker molecule and target biomolecules is observed. The linker molecules contain an azobenzene functionality, forming photo-switchable host-guest inclusion complexes with CDV, and a charged moiety, thus inducing orthogonal aggregation of either positively or negatively charged proteins or DNA. It was demonstrated that irradiation with ultraviolet light causes complex dissociation, based on the switching between a modus of multivalent electrostatic interactions and a low-affinity monovalent state.



Multivalent capture and photo-induced release of target biomolecules.[1]

Interestingly, the threshold linker concentration needed for agglutination was found to be independent on the amount of vesicles and protein. These results imply that the protein clusters the linker molecules on the CDV, similar to recognition processes *in vivo*, where the target molecule assembles the corresponding receptor units in the cell membrane.

Currently we are investigating this systems capacity to serve as a transporter for biomolecules. The cell uptake and biocompatibility of supramolecular complexes containing DNA which encodes for a green fluorescent protein was confirmed by fluorescence microscopy. Future focus will lie on studying the release of the DNA inside the cells.

References:

1. J. Moratz, A. Samanta, J. Voskuhl, S. K. M. Nalluri, B. J. Ravoo, *Chem. Eur. J.* **2015**, *8*, 3271–3277.

Poster category: B

(27) Synthesis of novel [2]catenanes based on 1,1´-binaphthyl-phosphoric acids and -guanidines

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Mechanically interlocked molecules (MIMs), such as rotaxanes and catenanes, have fascinated chemists for decades. Viable synthetic routes for these topologically interesting species have been developed, which nowadays allows for their synthesis in useful quantities.[1] While rotaxanes have found various applications, for example as molecular switches and even as molecular machines, the application of catenanes has found limited attention so far.[2] However, their specific topology makes them highly interesting candidates for a use in sensing, catalysis and other fields.

We aim for the synthesis of chiral, bifunctional [2]catenanes based on 1,1´-binaphthyl-phosphoric acids and 1,1´-binapthyl-guanidines. On one hand we would like to generate the corresponding bis-phosphoric acid homocatenanes, while on the other hand we envisage the synthesis of the corresponding heterocatenanes based on the phosphoric acid - guanidine pair (see figure 1).

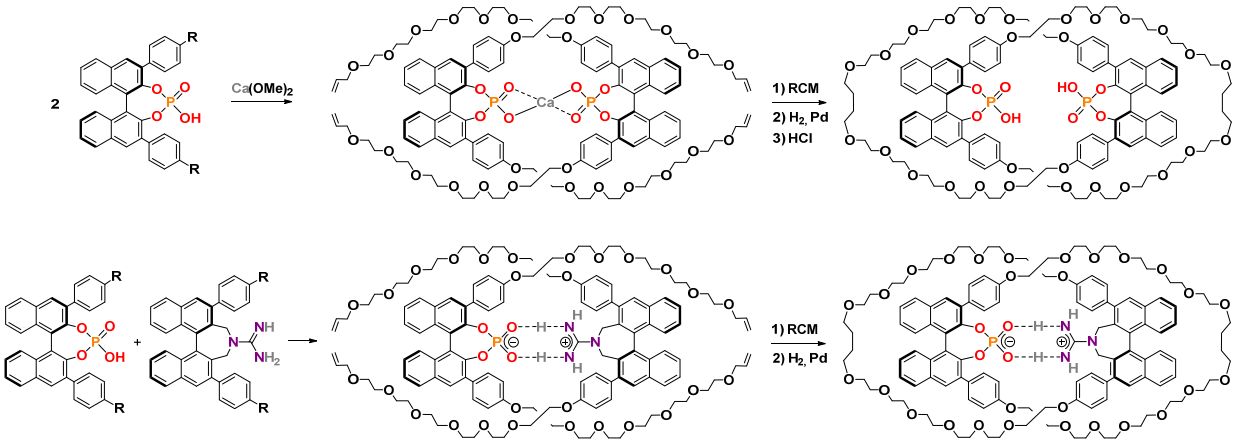

Figure 1: Envisaged synthesis of homo-[2]catenanes (top) and hetero-[2]catenanes (bottom) based on 1,1´-binapthyl-based phosphoric acids and guanidines

The resulting structures feature two Brønsted-acidic groups or one acidic and one basic group, respectively, combined in a [2]catenane. The functional groups are attached to a pseudo-monomolecular framework, while the mechanical bond between the macrocycles allows for their flexible arrangement in space. This concept, which is strongly inspired by the induced-fit principle found in enyzme-catalysis, might be a particular advantage for a use of these systems in catalytic applications.

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Poster category: B

(28) Phenylboronic Acid based Multivalent Receptors

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Multivalence plays an important role in biochemistry, in particular in carbohydrate recognition by proteins at interfaces and in solutions. These processes are often poorly understood. To get a better understanding of these processes we designed a supramolecular system based on vesicles of amphililic cyclodextrins (CDV), a functional guest and multivalent receptors with a defined amount of binding sites.
In the past we established vesicles of amphilic cyclodextrins as a versatile tool to study recognition processes on the membrane surface.[1,2] We now decorated these vesicles with a bifunctional linker molecule, which contains a adamantyl and a catechol moiety, and investigated the spontaneous self-assembly of supramolecular complexes by adding phenylboronic acid based multivalent receptors (Figure 1). Future focus will lie on exploring the kinetics of these recognition processes.

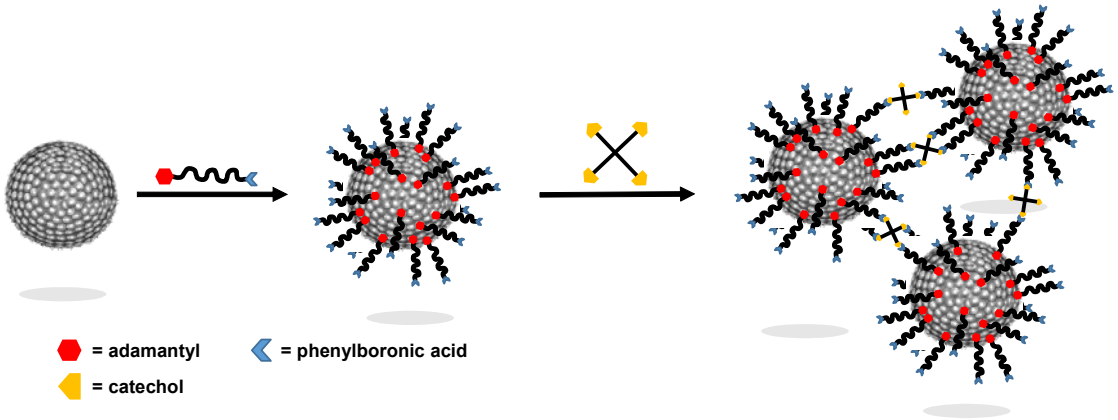

Figure 1: Multivalent complexation of CDV, linker molecules and phenylboronic acid based multivalent receptors.

References:

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2. *Moratz, J.; Samanta, A.; Voskuhl, J.; Nalluri, S.K.M.; Ravoo, B.J.: Chem. Eur. J.* **2015**, *8, 3271-3277.*

Poster category: B

(29) Switchable Bioactive Ligands for Supramolecular Surfaces

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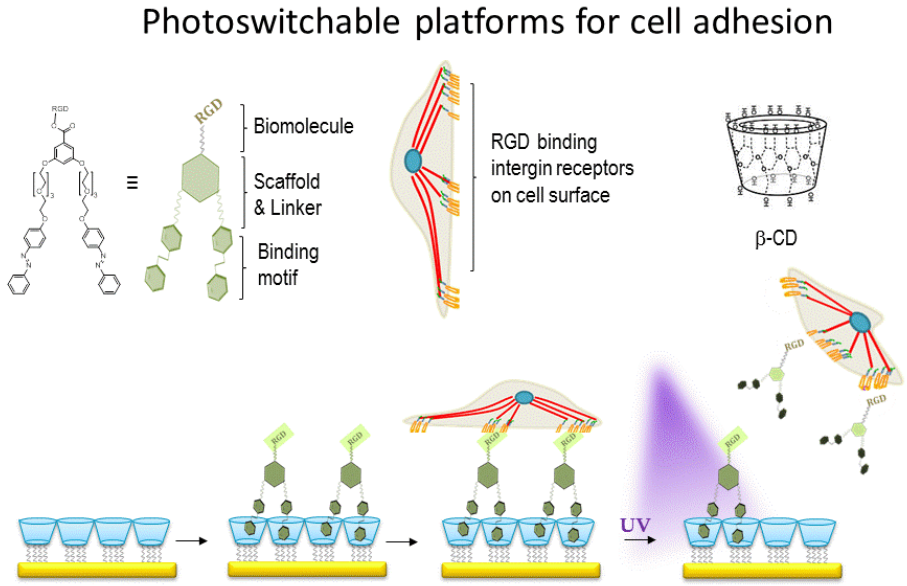
Multivalent interactions are the biological answer to many cellular processes such as immune response and molecular recognition.[1] Bioactive multivalent ligands have recently attracted much interest since they allows the immobilization of cells and bacteria to supramolecular surfaces. Host-guest chemistry is therefore a valuable tool because the concept is inspired by nature. The interactions are non-covalent, reversible and are fulfilling a number of biological requirements.[2]

We present here a number of multivalent ligands which are able to undergo these host-guest interactions and are simultaneously able to interact with cells. This approach allows for temporal and spatial control over studying cell adhesion and spreading. For host-guest chemistry we selected β-cyclodextrin (β-CD) as host molecule, which can be covalently bound to a surface and an azobenzene binding moiety as guest molecule. The adhesion and release of the cells can be controlled by applying UV-light as an external trigger.



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Poster category: C

(30) Pinpointing supramolecular tweezer binding to Lysine and Arginine residues using Lys- & Arg-specific NMR experiments

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Supramolecular phosphate tweezers[1] can bind Lysine (Lys) and Arginine (Arg) within their aromatic cavities. These residues can also be bound when presented on protein surfaces, modulating protein-protein interactions like reversal of amyloid fibril formation. Interestingly, not all Lys or Arg residues on a protein surface are bound equally, and few is known what local properties determine the binding specificity.

NMR spectroscopy is well suited to monitor ligand binding to proteins. If the backbone assignments of the protein are known, shifting or intensity changes of the amide signals in a $^{1}H,^{15}N$-HSQC spectrum are mapped onto the protein sequence, and, if available, protein structure. The tweezer affects NMR signals over a large distance due to its multiple aromatic moieties[2]. Therefore, often 2 or 3 possible binding sites lie within the affected area, and the binding site cannot be determined unambiguously by relying on amide signals only.

We employed Lys- and Arg-specific NMR experiments[3] to directly observe the atoms inserted into the tweezer cavity, for which the most drastic changes in the NMR signals are expected. In the $^{1}H,^{15}N$-HSQC of the hPin1-WW domain several signals shift but don’t allow to distinguish between two residues as preferred site. The Lys and Arg-specific experiments clearly pinpoint the order of tweezer binding to all of the two Lys and four Arg residues, furthermore revealing the first example of a protein where binding of tweezers to Arg is preferred.

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Poster category: C

(31) Toward Molecular Recognition: Three Point Halogen Bonding in the Solid State and in Solution[1]

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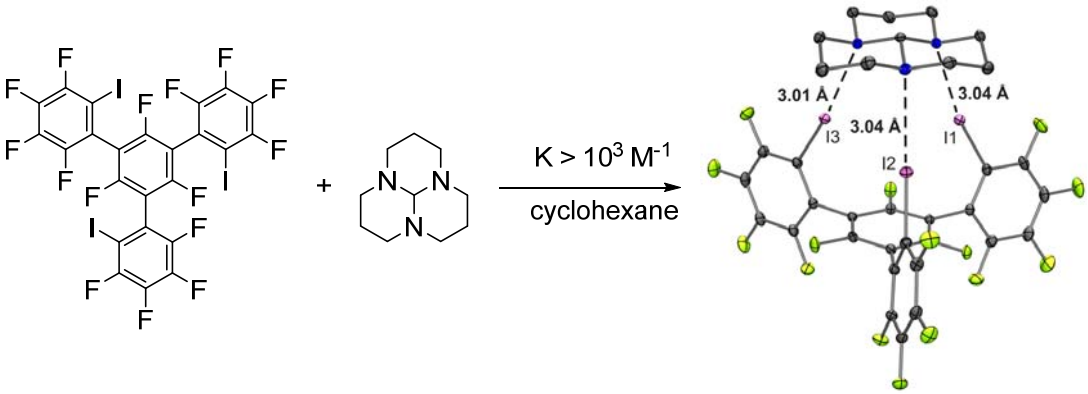
Ruhr-Universität Bochum, Universitätsstr. 150, 44801 Bochum, NC4/171, Stefan.M.Huber@rub.de

The non-covalent interaction between electrophilic halogen substituents and Lewis bases is called halogen bonding.[2] It was formerly mostly studied in the solid state (crystal engineering) but has recently also found applications in solution, e.g. in catalysis.

Molecular recognition is based on multipoint interactions between donor and acceptor species. Herein, we present a tridentate halogen bond donor which binds in solution and solid state to an orthoamide as a representative trifold Lewis base. Titration experiments of the halogen bond donor shown below with the triamine acceptor show binding constants which are three orders of magnitude higher than those for comparable monodentate amines. The crystal structure shows the perfect fit between donor and acceptor. Related multipoint interactions based on halogen bonding are envisioned as the basis for future biochemical applications.



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Poster category: C

(32) Synthetic Supramolecular Modulators of 14-3-3 Protein Interaction

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[2] Department of Biomedical Engineering, University of Technology. Postbus 513, 5600-MB-Eindhoven (The Nederlands)

The adapter proteins 14-3-3 are an attractive therapeutic target as several of their partner proteins are involved in many human diseases.[1],[2] In this regard, the design of supramolecular ligands which could recognize specific parts of the 14-3-3 protein surface will open the possibility of modulating those physiological functions in which 14-3-3 is involved throughout protein-protein interactions (PPI). In this work, we have focused on several surface-exposed aspartic and glutamic acids in the vicinity of the central binding channel of 14-3-3 where effectors like c-Raf or ExoS and Cotylenin-A (natural product that stabilize14-3-3/c-Raf interaction) bind (Figure 1).[3] Binding of a positively charged ligand to these negative residues should lead to the modulation (inhibition or stabilization) of 14-3-3 interactions with such partner proteins.

Fortunately a screening of several cationic ligands from our home-made library showed that compounds labelled **qq82** and **qq96** stabilized c-Raf peptide phosphorylated at Ser259 in its binding to 14-3-3ζ with EC_{50} values in the micromolar range (Figure 2). Therefore, and based on these validated hits, we have performed a screening of a new family of compounds built by means of a combinatorial dynamic library strategy as modulators of 14-3-3ζ protein with different effectors. Preliminary results with this novel series confirm that these cationic supramolecular ligands act as potential stabilizers.

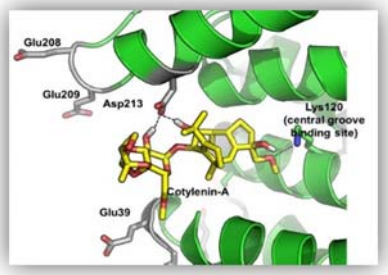

Figure 1

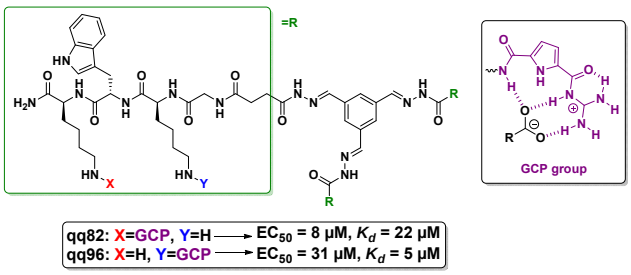

Figure 2

References:

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Poster category: C

(33) Synthesis of Ahp-Cyclodepsipeptides as Serine Protease Inhibitors

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S1 serine proteases are by far the largest and most diverse family of proteases encoded in the human genome and are involved in many important physiological processes like protein turnover, digestion or blood coagulation. Consequently, uncontrolled or undesired proteolysis can induce many diseases, for example stroke, viral infections or inflammation.
In the present project, we aim to explore if Ahp-cyclodepsipeptides can be developed into suitable chemical tools for serine protease research. This family of natural products features a unique chemical scaffold structure including a denoting 3-amino-6-hydroxy-2-piperidone (Ahp) residue (Fig. 1). Ahp-cyclodepsipeptides bind simultaneously non-covalently to the S- and S'-subsites of S1 serine proteases. So far, we investigated diverse solid phase-based chemical approaches to Ahp-cyclodepsipeptides and found that a synthetic route consisting of i) anchoring of a Fmoc-(5-OH)-Nva-OAll building block via its side-chain hydroxyl group to a solid phase resin, ii) solid phase peptide synthesis to incorporate all amino acids, iii) on-bead cyclization to generate the cyclic structure and iv) oxidation with Dess-Martin reagent to install the Ahp residue can be used to generate Ahp-cyclodepsipeptides. We currently explore the scope of this synthetic procedure to generate customized Ahp-cyclodepsipeptides as a prerequisite for developing tailored chemical tools.

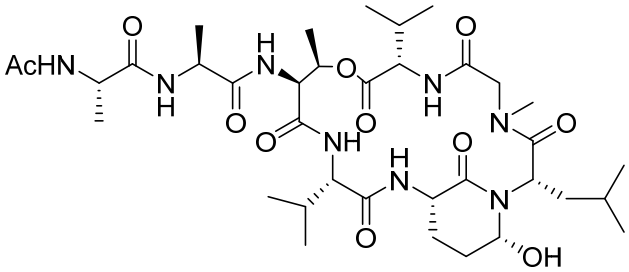

Fig. 1: Chemical structure of an Ahp-Cyclodepsipeptide

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Poster category: C

(34) Influence of Molecular Environment on Protein Interactions

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Molecular environment of a biomolecule can affect its structure and function to different extents. We aim to study such influences for the following systems:

1. Human Islet amyloid polypeptide (hIAPP) is a 37-residue peptide which forms amyloid deposits associated with type-II diabetes mellitus. It is still unclear what triggers the conversion of soluble monomeric hIAPP into insoluble amyloid fibrils. We used replica exchange molecular dynamics (REMD) to sample the conformational space of the hIAPP dimer in explicit water, thus our work provides insights into the structural properties relevant to amyloid formation.

2. Huntington’s disease (HD) is associated with the expansion of polyglutamine (polyQ) stretch of the huntingtin (htt) protein. Above a threshold of 37 glutamines huntingtin exon 1 starts to aggregate in a nucleation dependent manner. A 17-residue N-terminal fragment of exon 1 (N17) was shown to play a crucial role in modulating aggregation propensity and toxicity of htt exon 1. We used molecular dynamics simulations to show that binding of CLR01 induces structural rearrangements within the N17 region of htt exon 1 monomer that leads to change in aggregation pathway of htt.

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Poster category: C

(35) Harnessing allostery in a photo-responsive promiscuous protein

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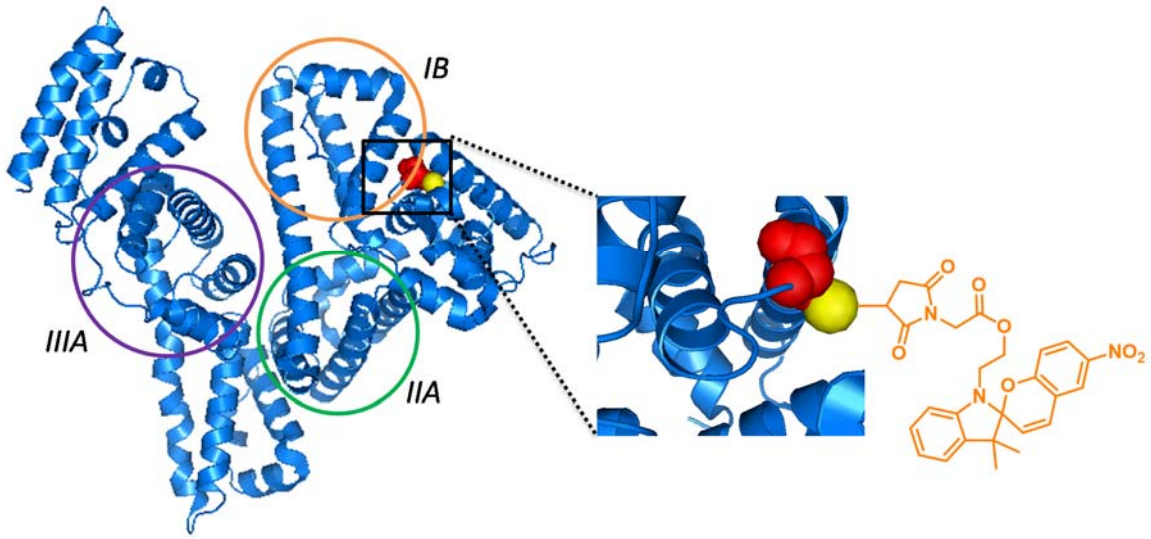
Allostery is the process by which distinct binding sites of a protein actively influence each other and is known as the second secret of life. With allostery, Nature regulates protein activity through mechanisms that remain largely unknown. Controlling allosteric mechanisms would consequently constitute a radically new approach towards regulating vital cellular processes. Here, we perform the site-specific covalent incorporation of a light-responsive molecular switch in the human serum albumin. In this re-engineered protein, light-induced conformational modifications occur at one binding site specifically and result in a significant decrease of the binding affinity for ligands of that site. Importantly, we also show that the light-responsive binding site transmits information to other binding sites by allosteric communication, which allows multiple ligands to be released when only one switch is activated. This approach provides a powerful tool to gain insight into the mechanisms of allostery and to amplify molecular switching in bio-molecular systems.



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Poster category: C

(36) Highly fluorescent water soluble dye and its interactions with proteins

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The research on new fluorescent markers for proteins and organelles is still a challenging field, even though some high luminescent compounds are already commercially available.[1] Spirobifluorene is a well-known building block for fluorescent molecules with an application in many technological fields like molecular electronics and solar cells.[2] However, its use in biological environment is limited due to the low solubility in water.

Here we present a novel water soluble highly emitting spirobifluorene-based dye and its photophysical properties in presence of bovine serum albumin and protamine. Studies about the behaviour of the dye in living cells are also reported.

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Poster category: C

(37) Effect of the covalent closure on the activity of cyclic peptides: Hydrocarbon- *vs.* lactam-bridged derivatives as dimerization inhibitors of *Leishmania infantum* trypanothione reductase

Pedro A. Sánchez-Murcia,[1,2] Marta Ruiz-Santaquiteria,[2] Miguel A. Toro,[3] Héctor de Lucio,[3] María Ángeles Jiménez,[4] Antonio Jiménez-Ruiz,[3] María-José Camarasa,[2] Federico Gago,[1] and Sonsoles Velázquez[2]

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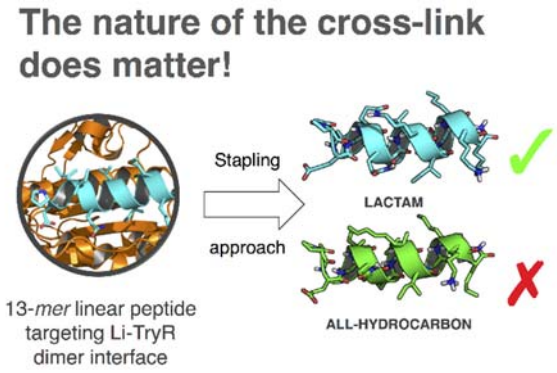
All-hydrocarbon and lactam-bridged staples linking amino acid side-chains have been used to stabilize the α-helical motif in short 13-mer peptides that target critical protein–protein interactions at the dimerization interface of *Leishmania infantum* trypanothione reductase (Li-TryR). The design of the best positions for covalent hydrocarbon closure relied on a theoretical prediction of the degree of helicity of the corresponding cyclic peptides in water. Selected (i, i + 4) and (i, i + 7) hydrocarbon-stapled peptides were prepared by using solid-phase synthesis protocols and optimized ring-closing metathesis reactions under microwave conditions. Structural analysis by NMR spectroscopy confirmed high helical contents in aqueous TFE solutions for both types of helix-constrained cyclic peptides. Remarkably, the ability to prevent Li-TryR dimerization was reduced in both (i, i + 4) and (i, i + 7) hydrocarbon stapled peptides but was retained in the corresponding (i, i + 4) Glu–Lys lactam-bridged analogue, which also showed a higher resistance to proteolytic degradation by proteinase K relative to the linear peptide prototype. *In silico* studies indicated that the introduction of a hydrocarbon staple vs. a lactam bridge likely perturbs critical interactions required for proper binding of the peptide to the Li-TryR monomer.



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Poster category: C

(38) 6×His-tagged chemical probes for quantitative affinity enrichment mass spectrometry

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In chemical proteomics, small molecule probes are utilized to profile proteins based on their functional properties. One of these functional technologies is activity-based protein profiling (ABPP). This technique enables direct monitoring of the availability of enzyme active sites. An extension of the ABPP approach is Proteome Reactivity Profiling (PRP) where probes with a broad specificity are used to profile reactivity patterns in whole proteomes.
Here we report the synthesis and evaluation of two such broad band probes based on α-chloroacetamide (CA) and α-iodoacetamide (IA) reactive moiety. Both probes are directed against with the thiol group in cysteine residues. To facilitate the enrichment of target proteins, the reactive groups are linked via an alkyl chain to a hexahistidine-tag for simplified affinity enrichment (e.g. on Ni-IDA IMAC resin). The probes were evaluated by labelling *E. coli* lysates followed by affinity enrichment on Ni-IDA resin. Incorporation of the probe was verified by Western blotting with a specific 6×His antibody and parallel quantitative label-free mass spectrometry. The whole protocol was adapted to a 96-well format and allows parallel screening of several hundred samples. First experiments in *E. coli* indicate cysteine containing thioredoxin and glutharedoxins as probe binding partners identified by LC-MS/MS.
All in all we show that our chemical probes are suitable for Nickel affinity enrichment and that the described protocol is a valuable addition to the proteomics tool box.

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Poster category: C

(39) Supramolecular ligands for 14-3-3 proteins

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14-3-3 Proteins are a highly conserved and abundant protein family found in all plants, vertebrates and higher eukaryotes in which they play prominent roles in signal transduction, apoptosis or cell cycle regulation. While their relevance for intracellular cell regulation is nowadays well established, it was recently found that 14-3-3 proteins are also found in the extracellular environment where they seem to trigger secretion of matrix metalloproteases (MMPs). In order to provide a chemical tool for studying the function of extracellular 14-3-3 proteins, we therefore set out to develop potent and selective cell-impermeable 14-3-3 protein inhibitors. To this end, we generated a set of bivalent PEG-linked phosphophenyl ethers and tested them for their biochemical inhibitory properties. We are currently exploring their bioactivity on a cellular level.

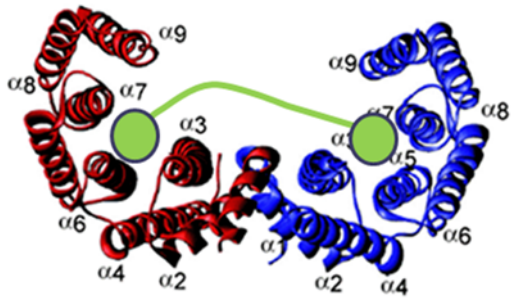

Fig.1: Schematic view of a 14-3-3 protein with a designed bivalent inhibitor (green).

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Poster category: C

(40) Label-Free Monitoring of Peptide Recognition by Supramolecular Ligands with UV Resonance Raman Spectroscopy and Binding studies

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Molecular recognition by biological or artificial receptors is of great importance for investigating the principles of protein-protein interactions. Among the different label-free techniques for monitoring, the site-specificity and high sensitivity make UVRR spectroscopy an ideal tool for probing these binding processes. This site specificity derives from the selective enhancement of vibrations that are coupled to the electronic transition upon resonant optical excitation [1].

In this work, the label-free and site-specific detection of some supramolecular ligands in solution is achieved by UVRR. The carboxylate binding site (CBS)-based artificial receptors developed by Schmuck and co-workers comprise two parts: a guanidiniocarbonyl pyrrole that serves as carboxylate binding site and a peptide part (e. g. tripeptide, Figure) that controls the selectivity of the receptors [2]. The results from binding studies of the complex with these ligands are analysed by non-negative Matrix Factorization (NMF).

We apply a 266 nm UV laser beam to excite the sample in different concentrations in a custom-made rotating quartz cuvette to minimize photochemical degradation. The scattered light is collected at 90° and focused on the entrance slit of a monochromator (50 cm focal length, 2400 grooves mm holographic grating). A liquid-nitrogen cooled CCD is used for detection. First results from UVRR binding studies are presented.

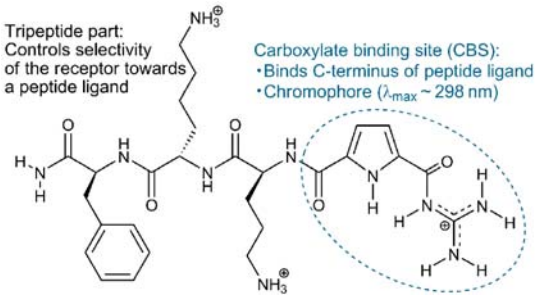

Figure: CBS-based peptide receptor

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Poster category: D

(41) Calcium phosphate nanoparticles as drug carriers across the intestinal barrier

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During acute mesenteric ischemia the blood circulation in the intestine is insufficient over a period of time. Even after or because of reperfusion the damaging of the tissue continues, which ultimately leads to death in 59 – 93 % of the incidences.

Among other cytokines, TNF-α seems to play a role in the onset of the intestinal ischemia/reperfusion injury.[1] Functionalized calcium phosphate nanoparticles have been used to inhibit the expression of the cytokine in intestinal epithelial cells by gene silencing.[2] Besides the transport of siRNA, calcium phosphate nanoparticles have been used to carry different biomolecules across the cellular membrane *in vitro*. Thus, calcium phosphate nanoparticles can be useful in the treatment of ischemia/reperfusion injury.

In order to investigate the feasibility of calcium phosphate nanoparticles as carriers for therapeutic molecules across the intestinal barrier, we studied their distribution in the small intestine of rats *in vivo*. Calcium phosphate nanoparticles were functionalized with a fluorescent oligonucleotide to visualize them in fluorescence microscopy. By intravital microscopy and microscopy of intestinal cross sections the distribution in the intestinal lumen and transfer through the intestinal mucosa of positively and negatively charged calcium phosphate nanoparticles was analyzed.

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Poster category: D

(42) Impact of strict design-rules on DNA origami self-assembly

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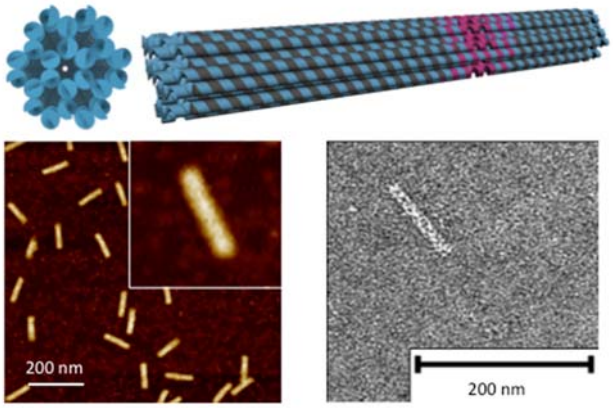
Molecular self-assembly using DNA as building block allows for the programmable construction of sophisticated nanoscale objects on the basis of simple base-pairing rules. Especially since the upcoming of DNA origami in 2006,[1] a growing amount of different structures with ever increasing complexity and difficulty have been produced. Recently, alternative design strategies have been developed for the modular assembly of DNA bricks into complex shapes[2] and the construction of mesh–like polygonal objects.[3] Finally, exploiting Watson-Crick rules in combination with base stacking interactions and single-strand displacement, hierarchical assemblies of higher order and dynamic behavior were produced.[4] DNA design is therefore a field in continuous evolution and although general self-assembly strategies were reported,[5] a common underlying concept is still desirable. Here we show how the application of strict design rules allows for the successful realization of a robust, yet complex 3D-DNA origami, composed of two quasi-independent domains connected by a variable module. Besides staple crossover spacing, other parameters such as nick-positioning, staple strand length and symmetry were taken into consideration, leading to high-yield formation of the target shape even at non-standard conditions.



A 24-helix bundle composed of two quasi-independent domains connected by a variable module (in magenta) has been realized observing strict design rules.

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[1] P. W. Rothemund, *Nature* **2006**, *440*, 297-302.
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Poster category: D

(43) G-quartet spacer controlled FRET study within G-quadruplex DNA using ^{Py}A/^{Per}A FRET pair

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We have studied the G-quartet spacer-controlled fluorescence resonance energy transfer (FRET) within G-quadruplex DNA using nucleoside-tethered FRET pair embedded in the human telomeric G-quadruplex forming sequence 5'-A GGG TTA GGG TTA GGG TTA GGG-3'. After careful investigation of the 3D structure of this quadruplex (PDB ID: 143D), we chose the A7 and A13 positions to place a polycyclic aromatic fluorophore almost parallel to G-quartet plane (Figure 1). We have used fluorescently-labelled 8-substituted 2'-deoxyadenosine units containing the well-known FRET pair comprising of pyrene (donor) and perylene (acceptor) chromophoric units (Figure 1c). Formation of tetraplex/G-quadruplex structure needs at least two G-quartet units. Thus, we can control the distance of the FRET donor/acceptor partners by varying the G-quartet spacer. In our preliminary study we have focused on G-quartet spacers of 2, 3, and 4 units between the covalently attached FRET donor/acceptor partners.

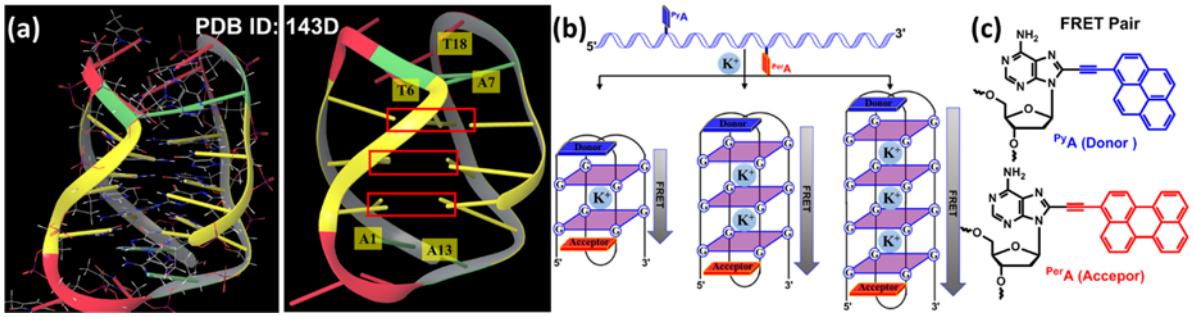

Figure 1. (a) 3D structure of G-quadruplex, (b) schematic presentation of FRET, and (c) FRET pair.

References:

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Poster category: D

(44) Transport of the proteins HTRA1 and HTRA2 across the cell membrane with the help of calcium phosphate nanoparticles

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The successful intracellular delivery of many proteins into living cell still remains an obstacle for biomedicine. Many proteins and drugs are not able to cross the cell membrane alone which is a problem if an intracellular action is desired. Calcium phosphate nanoparticles can serve as carriers for small and large biomolecules and for synthetic compounds.[1, 2]

Calcium phosphate nanoparticles were synthesized and colloidally stabilized with either polyethyleneimine (PEI) or carboxymethyl cellulose (CMC) and loaded with the fluorescently labelled proteins HTRA1 and HTRA2.[3] The nanoparticles were purified by ultracentrifugation and characterized by dynamic light scattering and scanning electron microscopy. The concentration of proteins on the nanoparticles was determined by UV-Vis spectroscopy.

HeLa, MG-63, THP-1 and hMSC cell lines were incubated with HTRA1- and HTRA2-loaded nanoparticles respectively, for 3h up to 48h. The cellular uptake was followed by light and fluorescence microcopy as well as by confocal laser scanning microscopy (CLSM). In addition, the uptake mechanism was examined by selective blockage of particular endocytotic pathways.

Both proteins were transported by calcium phosphate nanoparticles into the cells, but some cell lines were also able to take up HTRA1 protein without nanoparticles. The selective inhibition of endocytotic pathways into MG-63 cells revealed the difference in the uptake mechanisms for nanoparticles and soluble HTRA1.

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Poster category: D

(45) Reversible reconfiguration of DNA origami nanochambers

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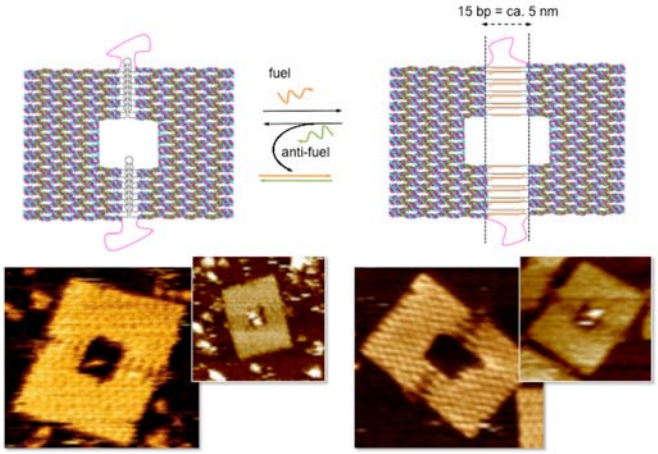
Through the physical separation of biological reactions into specialized compartments, nature achieves control over matter distribution, both in space and time.[1] In the past few years, many brilliant examples of DNA-based nanocages have been reported for the spatial confinement of molecular cargos.[2] However, many of them are static and mostly serve as structural frameworks for the precise positioning of molecules. In this sense, they lack one of the essential properties of their natural counterparts: that is the capability to undergo dynamic transformations in response to external events. Dynamic DNA nanotechnology offers this possibility.[3] Combining the spatial addressability of DNA origami structures with the switchable movement of simple DNA motives, we recently realized quasi-planar reconfigurable DNA origami nanochambers, whose inner cavity can be reversibly extended or contracted in response to external actuator sequences.[4] We are currently investigating the feasibility of such systems for the selective and reversible encapsulation/release of proteins with variable size and symmetry. At this purpose, different ligands are tethered to the inner cavity of the nanochambers and orthogonal sets of switchable motifs are employed to trigger reconfiguration of the structures in a modular fashion. Finally, thermal-dependent fluorescent studies are in course to gain a deeper insight into the device functioning.



Structural reconfiguration of 2D DNA origami compartments is achieved through insertion of switchable DNA motifs in the central seam. Modification of the inner cavity with suitable ligands allows for the selective tethering of target proteins.

References:

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Poster category: D

(46) Calcium phosphate nanoparticles as a delivery system for supramolecular drugs and biomolecules

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The successful transport of supramolecular drugs and biomolecule into living cells is highly important in biomedicine and pharmaceutics. Molecules alone usually cannot penetrate the cell membrane, therefore, an efficient carrier is needed. Calcium phosphate nanoparticles are well suited as a carrier for such molecules due to their high biodegradability and the absence of toxicity for cells if applied as a colloidal dispersion. Calcium phosphate nanoparticles, were loaded either with different artificial protein- and DNA-binders (polyfunctional anionic polymers, cationic calixarene dimers or amphiphilic molecular tweezers) or with fluorescently-labeled small and large biomolecules (nucleic acids, proteins or peptides). They were efficiently taken up by HeLa cells *in vitro*. Calcium phosphate nanoparticles represent a versatile carrier for all kinds of molecules across the cell membrane.

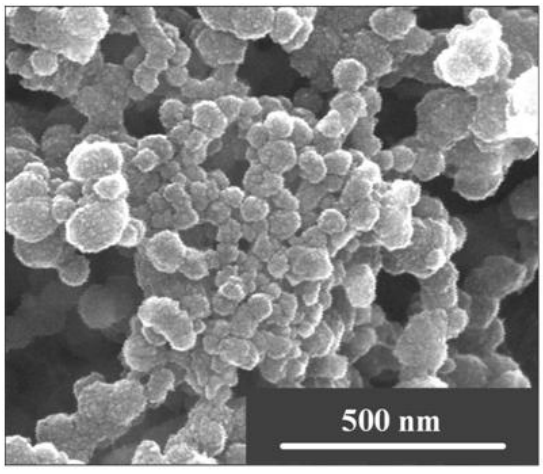

Figure 1: Scanning electron micrograph of calcium phosphate nanoparticles, functionalized with oligonucleotides.

References:

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Poster category: D

(47) DNA-based nanocontainers for predesigned spatial confinement of proteins

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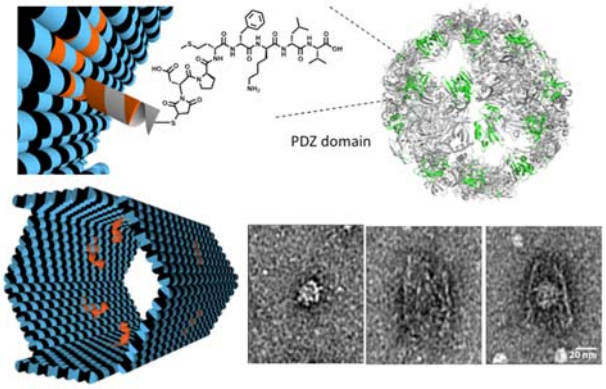
Structural DNA nanotechnology uses the unique recognition properties of the DNA molecule to realize two- and three-dimensional finite-sized objects of desired shape and full molecular addressability.[1] Control over the spatial arrangement of proteins anchored to predesigned DNA scaffolds has been successfully used to investigate biochemical processes at the single-molecule level.[2] However, current methods for functionalization of DNA-nanostructures with proteins normally lack in the regioselective modification of protein sites, require extensive protein engineering and manipulation or are limited to DNA-binding proteins.[3] Here, we describe the use of hollow DNA nanostructures, whose internal cavity has been functionalized with a distinct number of DPMFKLV peptide ligands, targeting the PDZ domains on the surface of the oligomeric DegP protein.[4] In this way, selective protein encapsulation within the DNA nanocontainer is achieved through multivalent and non-covalent ligand binding. The potential role of the DNA envelope in preventing protein degradation and enhancing its structural and functional lifetime is currently under investigation. We envisage that further modification of the external origami surface will allow to add desired properties to the system, such as specific recognition capability, physical/thermal stability or traceable optical activity, which can be advantageously employed for targeting and delivery applications.



Selective encapsulation of the oligomeric DegP protein within a DNA origami nanocontainer through multivalent and non-covalent binding of preoriented peptide ligands.

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Poster category: A

(48) New approaches to non-symmetric molecular tweezers for protein surface recognition

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Molecular tweezers are excellent binders for lysine and arginine by non-covalent interactions.[1] The application of the established CLR01-tweezer reaches from curing Alzheimer[2] disease up to HIV[3], as shortly reported in the literature. However, binding of lysines and arginines on protein surfaces is not selective. Therefore, we try to introduce a new direct method for the synthesis of unsymmetrical molecular phosphate tweezers. To these unsymmetrical tweezers, we want to attach a small peptide by click reaction. The peptide moiety acts as an additional recognition unit. Test reactions recently showed very promising results. In this poster we present these new approaches on the way to a new generation of molecular tweezers.

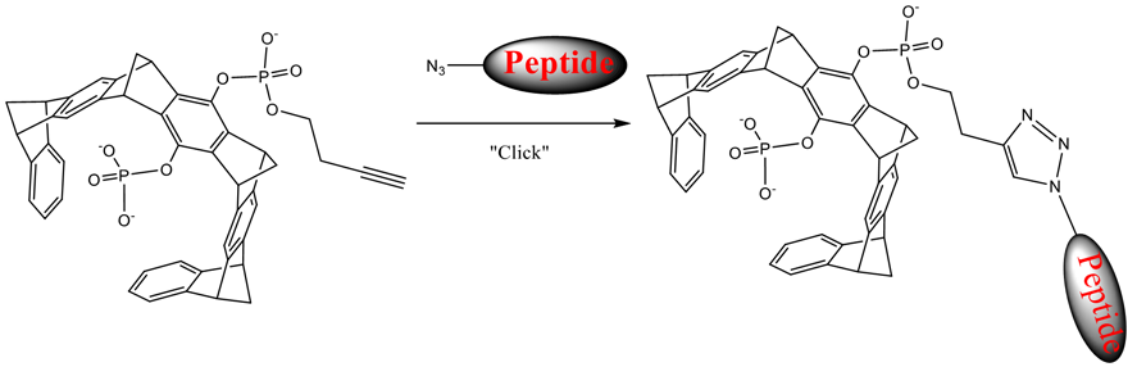

Figure 1: Molecular diphosphate tweezer for the binding of lysine and arginine with attached peptide ligand unit.

References:

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Poster category: A

(49) Mutant Huntingtin Aggregation Inhibition by Lysine Specific Molecular Tweezer

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Abstract

Huntington's disorder (HD) is one of the neurodegenerative disorders in which the polyglutamine (polyQ) tract of the protein huntingtin (htt) is found to be expanded. First exon of this mutant htt protein is found in the inclusions in the brain of the patient suffering from HD, which is the pathological hallmark of the disease[1]. Exon-1 of htt comprises of 17 amino acid long N-terminal helix (Nt17), polyQ tract in between and polyP-rich C-terminal. Nt17 has been reported to self-associate and enhance the rate of mutant htt aggregation which leads to protein aggregation resulting in the inclusion formation. The lysine residues K6 and K15 in Nt17 are thought to be involved in inter helical association and are proposed to form the template for initial oligomerization[2]. To prevent these specific residues from interacting, we have utilized a lysine specific molecular tweezer (MT) [3]. Here, we have studied the effect of MT on the structure and oligomerization process of the mutant htt. By MD simulations and CD spectroscopy we are able to show the disruption of the helical structure. A FRET based aggregation assay showed that the MT is effective in inhibiting mutant htt aggregation.

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