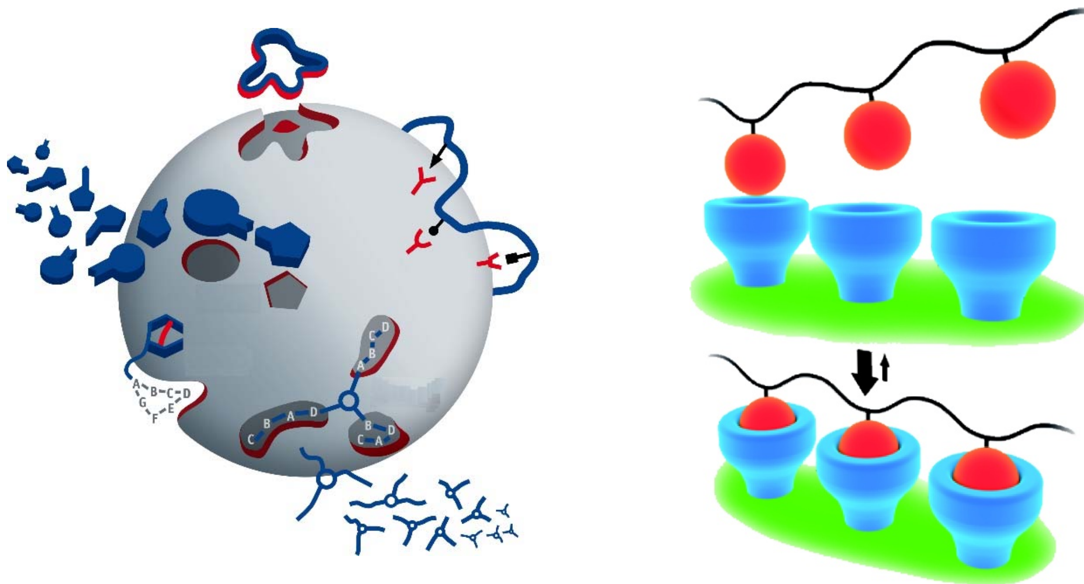
UNIVERSITÄT DUISBURG ESSEN

Offen im Denken

Freie Universität Berlin

CRC 1093 and CRC 765 Graduate Student Symposium



Protein-Ligand Interactions

31st August – 2nd September 2016; Hannover

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Program of the Graduate Student Symposium 2016 – Day 1

Wednesday 31st of August 2016	
11 – 13:00	Check-in GHotel Hannover
13:15	**Dr. Lydia Didt-Koziel**, *UDE* Welcome remarks
13:30	**Prof. Dr. Carsten Schmuck,** *UDE* Introduction into the research network of the CRC1093
13:45	**Graduate Student Speakers,** *Free University of Berlin* Introduction into the research network of the CRC765
14:00	**Small Snack and Coffee Break**
Session 1 *Chemical Toolbox*	
15:00	**Prof. Dr. Carsten Schmuck,** *UDE* “Combinatorial chemistry”
15:45	**Andrea Sowislok,** *UDE* “Novel diphosphate tweezers for regioselective protein surface recognition.”
16:00	**Florian Schulz,** *UDE* “Synthesis of selected AQUA peptides and method development for absolute quantification of proteins in biological matrices by high resolution mass spectrometry."
16:15	**Jean-Noël Grad,** *UDE* “Computational investigation of multivalent peptidic dendrimers.”
16:30	**Sumit Mittal,** *UDE* “Inhibition of Huntingtin exon-1 aggregation by a molecular tweezer.”
17:00	**Coffee Break**
17:30	EVENING LECTURE **Dr. Peter Nussbaumer,** *Lead Discovery Center & Translation, Dortmund* „Professional translational research: A new efficient drug discovery paradigm by merging the strengths of academia and industry.”
19:00	**Symposium Dinner**

Program of the Graduate Student Symposium 2016 – Day 2

Thursday 1st of September 2016	
Session 2 *Inhibitor screening*	
09:00	**Prof. Dr. Mark Brönstrup,** *University of Hannover* “Chemical biology directed to anti-infective drug discovery.”
09:45	**Miriam Bertazzon,** *Free University of Berlin* “Profiling and inhibition of multivalent WW domain interaction.”
10:00	**Christina Fischer,** *Free University of Berlin* “Multivalent peptide-polymer conjugates as inhibitors for protein-protein interactions.”
10:15	**Johannes von Sass** *Free University of Berlin* “Production of multivalent (oNB)DOPA-containing flagella forest.”
10:30	**Coffee Break**
Session 3 *Think Big – DNA Cages and Molecular Machines*	
11:00	**Dr. Barbara Saccà,** *UDE* “Tailored protein encapsulation into a DNA origami host through geometrically organized supramolecular interactions”.
11:45	**Wolfgang Pfeifer,** *UDE* “Molecular recognition in the assembly of hierarchical DNA superstructures.”
12:00	**Kai Walstein,** *Max-Planck Institute, Dortmund* “Investigation of CENP-A loading on centromeric chromatin”
12:30	**Lunch**
Session 4 *Structure Based Drug Design*	
13:30	**Prof. Dr. Teresa Carlomagno,** *University of Hannover* “Structural mechanisms of drugs studied by INPHARMA-NMR: methodology and applications.”
14:15	**Gunnar Bachem,** *Humboldt University of Berlin* “DNA-Programmed Spatial Screening of Multivalent Carbohydrate-Protein Interaction.”
14:30	**Saba Nojoumi,** *Free University of Berlin* “Next level drug design - Proteins on the rise”
15:00	**Poster Session**
17:30	**Symposium Dinner**
19:30	**Social Event “Altstadtrundgang Hannover”**

Program of the Graduate Student Symposium 2016 – Day 3

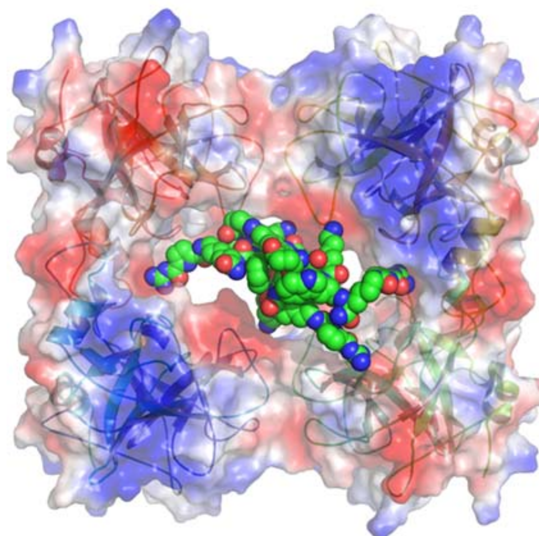
Friday 2nd of September 2016	
Session 5 *Protein-Ligand Interactions in Cells*	
09:00	**Prof. Dr. Matthias Epple,** *UDE* “Nanoparticle-mediated transport of proteins and other biomolecules into cells.”
09:45	**Sascha Büscher,** *UDE* „Synthesis, purification and characterization of calcium phosphate nanoparticles as carriers for biomolecules“
10:00	**Cecilia Vallet ,** *UDE* „Molecular impact of Survivin acetylation on its biological function”.
10:15	**Lisa Brencher,** *University Hospital Essen* “Attenuation of intestinal I/R-injury by β-alanine: A glycine- receptor mediated effect?”
10:30	**Coffee Break**
Session 6 *Functionalized Scaffolds / Interaction Dynamics*	
11:00	**Prof. Dr. Beate Paulus,** *Free University of Berlin* “Theoretical investigations for host-guest interactions -- how we can link results from the computer to results in the lab.”
11:45	**Susanne Liese,** *Free University of Berlin* “Influence of length and flexibility of spacers on the binding affinity of divalent ligands**.”**
12:00	**Andreas Achazi,** *Free University of Berlin* “Cooperativity effects in multivalent systems − A case study.”
12:15	**Anirudh Gupta,** *Free University of Berlin* “Calculating hydration interaction between hydrophobic and hydrophilic surfaces from computer simulations.”
12:30	***Poster Prizes and Closing of the Symposium***
13:00	**Lunch** **End of the Symposium**

Session 1 Chemical Tools

Recognition of biological targets with supramolecular ligands

Prof. Dr. Carsten Schmuck

Molecular Recognition of biomolecules such as proteins or DNA is of utmost importance for numerous biological processes. Supramolecular ligands which specifically bind to such targets can help to better understand the fundamentals of these recognition events. Furthermore, these ligands might be able to modulate the biological function of the target. Often molecular recognition of biological targets is based on ion-pair formation in combination with hydrophobic interactions. We use small, but tailor-made combinatorial libraries which are specifically designed and hence biased for a given biological target, to identify supramolecular ligands which can bind to charged hot spots on a protein surface or polyanions such as DNA or certain polysaccharides (e.g. heparin). Part of our ligand design involves also the use of a tailor-made anion binding site, the guanidiniocarbonyl pyrrole cation, which we developed as a superior binding site for oxoanions such as carboxylates or phosphates. We have successfully used this approach to identify supramolecular ligands which modulate protein-protein-interactions, which act as efficient gene transfection vectors or which can be used to monitor nucleic acids, proteins or polysaccharides even within cells.



References:

Schmuck et al. *Chemical Science* **2012**, *3*, 996; *Chem. Sci.*, **2015**, *6*, 1792; *Angew. Chem. Int. Ed.* **2013**, *52*,14016; *Angew. Chem. Int. Ed.* **2015**, 54, 2941

CURRICULUM VITAE

Prof. Dr. Carsten Schmuck

Date of birth 20th of February 1968
in Oberhausen (Germany)

Education and Career

Since June 2008	W3-Professor of Organic Chemistry (Chair) at the Institute of Organic Chemistry of the University Duisburg-Essen/Germany
March 2002 – May 2008	C3-Professor of Organic Chemistry at the Institute of Organic Chemistry of the University Würzburg/Germany
Sep. 1997 – March 2001	Assistant professor at the University of Cologne, Department of Organic Chemistry, Habilitation Thesis (2001)
April 1995 – June 1997	Postdoctoral fellow at Columbia University, New York City, USA with Prof. Ronald Breslow
July 1992 – Nov. 1994	Doctoral research work at the Institute of Organic Chemistry of The Ruhr-University in Bochum (Germany) with Prof. Dr. W. R. Roth
Oct. 1987 – May 1992	Study of Chemistry at the Ruhr-University Bochum (Diploma)

Selected Awards and Fellowships

- Fritz-Winter-Award of the Bavarian Academy of Science (2005)
- Dozentenstipendium of the Fonds der Chemischen Industrie (2002)
- Karl-Arnold-Award of the Academy of Science of the State of Nordrhein-Westfalen (2002)
- Heisenberg-Fellowship of the German Science Foundation (2002)
- Habilitation-Award of the Albertus-Magnus-University of Cologne (2001)
- Fellowship of the Kurt-Alder Foundation Cologne (2001)
- Award of the Hellmut-Bredereck-Foundation (2001)
- Otto Röhm Memorial Award (2001)
- German Science Foundation Habilitanden Fellowship (1999-2001)
- SYNTHESIS–SYNLETT Journals Award (1999)
- Liebig Fellowship of the Fonds der Chemischen Industrie (1997-1999)
- Feodor Lynen Fellowship of the Alexander von Humboldt Foundation (1995-1997)
- Doctoral Fellowship of the Studienstiftung des Dt. Volkes (1992-1994)
- Award for the best diploma thesis, Ruhruniversity Bochum (1993)
- Student-Felowship of the Studienstiftung des Dt. Volkes (1987-1992)
- Silver-medalist at the International Chemistry Olympiad in Vesprem/Hungary (1987)

Memberships and Functions

Since 10/2011	Dean of the faculty of chemistry
Since 02/2012	Member of the Scientific Advisory Board of the journal “ChemKon”, Wiley-VCH
Since 2011	vice head of the local GDCh-section “Essen-Duisburg”
2011	Organizer and chair of the first German national meeting on supramolecular chemistry (held from 24th – 25th of February 2011 in Essen)
Since 2009	Member of the Scientific Advisory Board of the European Journal of Organic Chemistry (*EuJOC*), Wiley-VCH
Since 2009	Member of the Centre for molecular biotechnology (ZMB), University Duisburg-

	Essen
Since 2009	Member of the Centre for Nanointegration University Duisburg-Essen (CeNIDE), University Duisburg-Essen
2012-15	Member of the Board of Executives of the Liebig-Organization for Organic Chemistry, German Society of Chemistry
2008-10	Member of the Senate Committee “Entwicklungsplanung und Finanzen” (KEF), University Duisburg-Essen
2007-08	Dean of studies, Julius-Maximilians-University Würzburg, faculty of chemistry
2007-08	Member of the Board of the faculty of chemistry, Julius-Maximilians-University Würzburg
2006-08	Head of the local GDCh-section “Unterfranken”
2006	National Chairman of the Transatlantic Frontiers of Chemistry Conference (coorganized by the GDCh, ACS, RCS)
2003-09	Member of the Collaborative Research Centre 630 “Recognition, Preparation and Functional Analysis of Agents against Infectious Diseases” Julius-Maximilians-University Würzburg
Since 2005	served as Member of the selection Committee of the Studienstiftung des deutschen Volkes for fellowships for advanced students on several occasions

Project relevant Selected Publications (from recent years)

1. M. Li, M. Ehlers, S. Schlesiger, E. Zellermann, S. Knauer, C. Schmuck,* Incorporation of a non-natural arginine analogue into a cyclic peptide leads to formation of positively charged nanotubes capable of gene transfectio, *Angew. Chem*. In press, DOI: anie.201508714
2. K. Samanta, C. Schmuck*, Two-component self-assembly of a Tetra-Guanidiniocarbonyl Pyrrole Cation and Na_4EDTA: Formation of pH switchable supramolecular networks, *Chem. Commun.* **2015**, *51*, 16065-16067; DOI: 10.1039/c5cc06392b.
3. M. Li, S. Schlesiger, S. K. Knauer, C. Schmuck;* A tailor made specific anion binding motif in the side chain transforms a tetrapeptide into an efficient gene delivery vector. *Angew. Chem.* **2015**, *127*, 2984-2987; *Angew. Chem. Int. Ed.* **2015**, *54*, 2941-2944; DOI: 10.1002/ange.201410429 bzw. anie.201410429.
4. M. Fleischer, C. Schmuck;* Transforming polyethylenimine into a pH-switchable hydrogel by additional supramolecular interactions, *Chem. Commun.* **2014**, *50*, 10464-10467; DOI: 10.1039/c4cc03281k.
5. L. Chen, J. Wu,* C. Schmuck, H. Tian;* A Switchable Peptide Sensor for Real-time Lysosomal Tracking, *Chem.Commun.* **2014**, *50*, 6443-6446; DOI: 10.1039/C4CC00670D.
6. Q. Wang, X. Shi, X. Zhu, M. Ehlers, J. Wu,* C. Schmuck;* A fluorescent light up probe as an inhibitor for intracellular □-tryptase. *Chem. Commun.* **2014**, *50*, 6120-6122; DOI:10.1039/C4CC02208D.
7. H. Y. Kuchelmeister, S. Karczewski, A. Gutschmidt, S. Knauer, C. Schmuck;* Utilizing Combinatorial Chemistry and Rational Design: Peptidic Tweezers with Nanomolar Affinity to DNA can be transformed into Efficient Vectors for Gene Delivery by Addition of a Lipophilic Tail. *Angew. Chem.* **2013**, *125*, 14266-14270, *Angew. Chem. Int. Ed.* **2013**, *52*, 14016-14020; DOI: 10.1002/anie.201306929 bzw. 10.1002/ange.201306929.
8. H. Y. Kuchelmeister, A. Gutschmidt, S. Knauer, C. Schmuck*; Efficient Gene Delivery into Cells by a Surprisingly Small Three-Armed Peptide Ligand. *Chem. Sci.* **2012**, *3*, 996-1002 (back cover); DOI:10.1039/C2SC01002J.
9. J. Wu, Y. Zou, W. Sicking, I. Piantanida, T. Yi*, C. Schmuck*; A Molecular Peptide Beacon for the Ratiometric Sensing of Nucleic Acids. *J. Am. Chem. Soc.* **2012**, *134*, 1958-1961; DOI:10.1021/ja2103845.

Session 2 Inhibitor Screening

Chemical biology directed to anti-infective drug discovery

Prof. Dr. Mark Brönstrup

Our efforts to generate novel antibacterial and antiviral lead substances through chemical biology methods will be highlighted through two projects.

Infections caused by pathogenic bacteria represent a major health threat that is expected to rise further in the future. The need for novel antibiotics is currently not met by R&D efforts, in particular in the area of infections caused by Gram-negative bacteria. A main scientific hurdle is the lack of understanding how to assure a sufficient translocation of bioactive molecules across the Gram-negative cell wall. In the talk, our efforts to induce an active transport of small molecules into Gram negative bacteria and methods to quantify such uptake will be presented. We report a series of theranostics agents based on 1,4,7,10-tetraazacyclododecane-1,4,7,10- tetraacetic acid amide (DOTAM) derivatives comprising siderophores that actively target bacteria, inhibit bacterial growth and demonstrate efficacy to visualize bacterial infections in mice by optical imaging *in vivo*. In addition, two orthogonal approaches to quantify the intracellular accumulation of such conjugates will be presented.

In the second part of the talk, antiviral natural products with broad-spectrum action against multiple human pathogenic viruses will be presented with a focus on labyrinthopeptins. Labyrinthopeptins are post-translationally modified peptides with lanthionine and labionine bridges that confer a largely enhanced stability in plasma compared to unmodified peptides. Following a first report on the broad anti-HIV and anti-HSV activities of LabyA1 (Férir et al. 2013), we have extended our studies to a variety of enveloped but evolutionary unrelated viruses such as Herpesviridae (HSV, CMV, KSHV), Retroviridae (HIV, RSV) or the Dengue virus (DENV). Mechanistic studies demonstrated that Labyrinthopeptins interact with lipid components of the viral surface, thereby inhibiting virus entry into target cells.

CURRICULUM VITAE

Prof. Dr. Mark Brönstrup

Education and Career

Mark Brönstrup studied Chemistry at the Philipps-Universität Marburg and at the Imperial College in London.

In 1999 he received his doctorate from the Technical University Berlin in Organic Chemistry. After his graduation, he worked from 2000 to 2004 as a laboratory head for Mass Spectrometry at Aventis, complemented by a research sabbatical in 2003 at Harvard Medical School.

From 2005 to 2010 he led the Natural Product Sciences section at Sanofi-Aventis in Frankfurt with the goal of discovering leads from natural sources and optimising them to clinical candidates. He dealt with translational research projects from 2010 to 2013 as a section head for Biomarkers & Diagnostics in the Diabetes Division, and a domain head for Biomarkers, Bioimaging & Biological Assays at Sanofi.

Since December 2013, he heads the department Chemical Biology at the Helmholtz Centre for Infection Research; additionally, he holds a Professorship (W3) at the Leibniz Universität Hannover.

Selected Publications

1. Hoffmann, H., Kogler, H., Heyse, W., Matter, H., Caspers, M., Schummer, D., Klemke-Jahn, C., Bauer, A., Penarier, G., Debussche, L. & Bronstrup, M**.** Discovery, Structure Elucidation, and Biological Characterization of Nannocystin A, a Macrocyclic Myxobacterial Metabolite with Potent Antiproliferative Properties. *Angew Chem Int Ed Engl* (2015)
2. Kling, A., Lukat, P., Almeida, D.V., Bauer, A., Fontaine, E., Sordello, S., Zaburannyi, N., Herrmann, J., Wenzel, S.C., Konig, C., Ammerman, N.C., Barrio, M.B., Borchers, K., Bordon-Pallier, F., Bronstrup, M**.**, Courtemanche, G., Gerlitz, M., Geslin, M., Hammann, P., Heinz, D.W., Hoffmann, H., Klieber, S., Kohlmann, M., Kurz, M., Lair, C., Matter, H., Nuermberger, E., Tyagi, S., Fraisse, L., Grosset, J.H., Lagrange, S. & Muller, R. Antibiotics. Targeting DnaN for *tuberculosis* therapy using novel griselimycins.*Science* 348, 1106-12 (2015)
3. Martinez, J.P., Sasse, F., Bronstrup, M., Diez, J. & Meyerhans, A. Antiviral drug discovery: broad-spectrum drugs from nature. *Nat Prod Rep* 32, 29-48 (2015)
4. Ferir G., Petrova, M.I., Andrei, G., Huskens, D., Hoorelbeke, B., Snoeck, R., Vanderleyden, J., Balzarini, J., Bartoschek, S., Bronstrup, M**.,** Sussmuth, R.D. & Schols, D. The lantibiotic *peptide* labyrinthopeptin A1 demonstrates broad anti-HIV and anti-HSV activity with potential for microbicidal applications. *PLoS One* 8, e64010 (2013)
5. Krawczyk J.M., Voller, G.H., Krawczyk, B., Kretz, J., Bronstrup, M. & Sussmuth, R.D. Heterologous expression and engineering studies of labyrinthopeptins, class III lantibiotics from Actinomadura namibiensis. *Chem Biol* 20, 111-22 (2013)

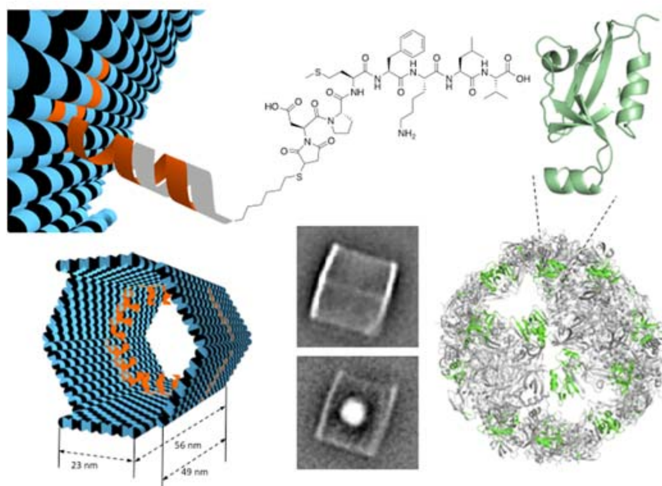
Session 3 Think Big – DNA Cages and Molecular Machines

Tailored protein encapsulation into a DNA origami host through geometrically organized supramolecular interactions

A. Sprengel,[1] P. Lill,[2] P. Stegemann,[1] K. Bravo Rodriguez,[3] E.-C. Schöneweiß,[1] M. Merdanovic,[1] D. Gudnason,[4] M. Aznauryan,[4] L. Gamrad,[1] S. Barcikowski,[1] E. Sancez Garcia,[3] V. Birkedal,[4] C. Gatsogiannis,[2] M. Ehrmann[1] and B. Saccà[1]

[1]ZMB and CENIDE, University of Duisburg-Essen, Universitätstr. 2, 45117 Essen (Germany)
[2]MPI of Molecular Physiology, Otto-Hahn-Straße 11, 44227 Dortmund (Germany)
[3]MPI für Kohlenforschung, Kaiser-Wilhelm-Platz 1, 45470 Mülheim an der Ruhr (Germany)
[4]Interdisciplinary Nanoscience center (iNANO), Aarhus University, Gustav Wieds Vej 148000 (Denmark)

Confining a single protein molecule in a chemically engineered environment may be used to modulate its properties,[1] allowing for example to enhance its stability, alter its function, induce conformational changes and facilitate protein structural elucidation by single-molecule methods. DNA origami structures have recently emerged as ideal protein cages[2, 3] and their effect on the activity and stability of the internalized cargo discloses a primordial chaperone-like role of DNA that promises exciting future applications. Nevertheless, full exploitation of this property is still limited by the chemical strategies used for DNA-protein conjugation,[4] with low control over the stoichiometry and regioselectivity of the modification, leading to increase in sample heterogeneity and possible alteration of protein function. Here, we describe the use of hollow DNA nanostructures, whose internal cavity has been functionalized with a distinct number of peptide ligands, targeting specific domains on the surface of the oligomeric DegP protein.[5] In this way, selective protein encapsulation within the DNA channel is achieved through geometrically constrained supramolecular interactions, i.e. non-covalent and coordinated action of many ligands converging to a common target. This would therefore allow to employ virtually any kind of protein in its native state as a payload of synthetic DNA hosts.



Selective encapsulation of the oligomeric DegP protein within a DNA origami nanocontainer through multivalent supramolecular binding of pre-oriented peptide ligands.

References:

D. Fujita et al. 2012. Protein encapsulation within synthetic molecular hosts. **Nat Commun** 3: 1093.
S. M. Douglas et al. 2012. A logic-gated nanorobot for targeted transport of molecular payloads. **Science** 335: 831-834.
Z. Zhao et al. 2016. Nanocaged enzymes with enhanced catalytic activity and increased stability against protease digestion. **Nat Commun** 7: doi: 10.1038/ncomms10619.
B. Sacca et al. 2011. Functionalization of DNA nanostructures with proteins. **Chem Soc Rev** 40: 5910-5921.
M. Merdanovic et al. 2010. Determinants of structural and functional plasticity of a widely conserved protease chaperone complex. **Nat Struct Mol Biol** 17: 837-843.

CURRICULUM VITAE

Dr. Barbara Saccà

born 25.06.1971 (w) in Messina (Italy)

Academic training and professional career

1990-1996	Undergraduate studies in Chemistry (Dipl.), University of Padova (Italy)
1997-1998	Post-Laurea fellowship, University of Padova
1999-2002	Ph.D. at Max-Planck Institute of Biochemistry, Martinsried (Prof. Dr. L. Moroder)
2003-2004	Postdoc with Dr. J.L. Mergny, Museum National d´Histoire Naturelle, Paris.
2004-2005	Postdoc with Dr. A. Bensimon, Institut Pasteur, Paris.
2006-2009	Postdoc with Prof. Dr. C.M. Niemeyer, Technical University Dortmund.
2009-2012	Principal Investigator, Technical University Dortmund.
2012- today	Group leader, Faculty of Biology, University of Duisburg-Essen, Germany

Functions and honours

1997	Student scholarship from the University of Padova, Italy
1998	Debiopharm S.A. Fellowship from the University of Padova, Italy
2002	PhD awarded with *Summa cum Laude*.
2003	Yvette-Mayent grant from the Institut Curie, France
2004	Post-doctoral fellowship from the ARC foundation for cancer research
2005	Roux fellowship from the Institut Pasteur
2009	DFG Fellowship for a temporary position as Principal Investigator
2012-today	Member of CENIDE, University Duisburg-Essen
2012-today	Member of the ZMB research program, University Duisburg-Essen
2011-2012	Member of the Global Young Faculty, Mercator Stiftung

Career breaks

01.06.2005 – 14.02.2006	Parental leave for the birth of the first child (8.5 months)
07.09.2007 – 31.03.2008	Parental leave for the birth of the second child (6.5 months)

Most relevant publications

W. Pfeifer and B. Saccà.* ***ChemBioChem*** (2016), *in press*.

R. Meyer, B. Saccà and C.M. Niemeyer. ***Angew Chem Int Ed Engl.*** (2015) *54*:12039-12043.

B. Saccà,* Y. Ishitsuka, R. Meyer, A. Sprengel, E.C. Schöneweiß, G.U. Nienhaus* and C.M. Niemeyer.* ***Angew Chem Int Ed Engl.*** (2015) *54*:3592-3597. *Flagged as "hot" paper*

B. Saccà,* B. Siebers, R. Meyer, M. Bayer and C.M. Niemeyer. ***Small*** (2012) *8*:3000-3008.

B. Saccà* and C.M. Niemeyer*. ***Angew. Chem. Int. Ed.*** (2011) *51*:58-66.

B. Saccà, R. Meyer, M. Erkelenz, K.S. Rabe, K. Kiko, A. Arndt, and C.M. Niemeyer. ***Angew. Chem. Int.*** **Ed. *Engl.*** (2010) *49*:9378-9383. *Flagged as "hot" paper*

B. Saccà,* R. Meyer and C.M. Niemeyer.* ***Nat. Protoc.*** (2009) *4*:271-285.

B. Saccà,* R. Meyer and C.M. Niemeyer.* ***ChemPhysChem*** (2009) *10*:3239-3248.

B. Saccà,* R. Meyer, U. Feldkamp, H. Schroeder and C.M. Niemeyer*. ***Angew. Chem. Int. Ed. Engl.*** (2008) *47*:2135-2137.

B. Saccà, C. Conti, J. Herrick, C. Lalou, Y. Pommier and A. Bensimon. ***Mol. Biol. Cell*** (2007) *18*:3059-3067.

(** corresponding authors)*

Session 4 Structure Based Drug Design

Structural mechanisms of drugs studied by INPHARMA-NMR: methodology and applications

Prof. Dr. Teresa Carlomagno

Small molecules play a fundamental role in the regulation of the function of proteins, nucleic acids and molecular machines. The development of specific binders that selectively alter the function of only one or a few cellular targets relies on the availability of structural information for the target active site and its mode of interaction with low affinity ligands, identified for example in screening experiments. When this structural information is not available, the rationale design of a selective drug is impossible and the process of drug development has to rely on the screening of large libraries of molecular fragments accompanied by many, lengthy, parallel routes of chemical synthesis.

Our lab developed an NMR-based methodology, INPHARMA (*1,2*), which provides access to the relative binding mode of low-affinity ligands to a common target. The method is based on the observation of interligand, spin diffusion mediated, transferred-NOE data, between two ligands A and B, binding competitively and weakly, to a macromolecular receptor T. In accordance with existing SBDD workflows, the experimental information derived from the INPHARMA NOEs is used to select the correct binding mode among many possible binding orientations obtained by molecular docking (*3*). The method requires a small amount of non-isotope-labelled target and is widely applicable to different receptor classes.

In this lecture, I will explain the principle of the method, its validation in standard drug design projects, its application to the "induced-fit" case (*4*) and its expansion to INPHARMA-STRING. In addition, I will demonstrate its usefulness on the basis of several applications (*5*).

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3. J. Orts et al., **Angew. Chem**. 47, 7736 (2008).

4. L. Skjærven et al., **J. Am. Chem. Soc**. 135, 5819 (2013)

5. J. Sikorska et al., **Med. Chem. Comm.** 6, 1501 (2015)

CURRICULUM VITAE

Prof. Dr. Teresa Carlomagno

Education and Career

Teresa Carlomagno started her scientific career at the University of Naples, Italy. During her Ph.D. studies and her following post-doctoral work at the University of Frankfurt, Germany, she developed NMR methods to study the structure and the dynamics of proteins, nucleic acids and small molecules.

During a second post-doctoral period at The Scripps Research Institute, La Jolla, USA, she matured a strong interest in the mechanisms of intermolecular recognition and function in ribonucleoprotein complexes.

In 2001, she joined the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, and started her own research group.

In 2007, she moved to the EMBL in Heidelberg as a group leader in biomolecular NMR spectroscopy in the Structural and Computational Biology Unit. Her research program focuses on two areas: half of the laboratory studies the structural basis for the function of ribonucleoprotein (RNP) complexes with catalytic activity in RNA metabolism and *gene* regulation; the other half of the laboratory studies the interaction of small molecules with macromolecular receptors and develops NMR-based methodologies for drug design. Interdisciplinarity and advanced technology are key elements to her vision for *structural biology*.

In 2015 she will move to the Leibniz University of Hannover, as a W3 professor in Structural Chemistry. She is a joined group leader at the HZI in Braunschweig.

Selected Publications

1. AsamiS, Rakwalska-Bange M, Carlomagno T, Reif B. *Protein*-RNA interfaces probed by 1H-detected MAS solid-state NMR spectroscopy. **Angew. Chem. Int. Ed. Engl**. 52(8):2345-2349.
2. Lapinaite A, Simon B, Skjaerven L, Rakwalska-Bange M, Gabel F, Carlomagno T. The structure of the box C/D enzyme reveals regulation of RNA methylation. **Nature** 502(7472):519-523
3. Skjærven L, Codutti L, Angelini A, Grimaldi M, Latek D, Monecke P, Dreyer MK, Carlomagno T.Accounting for conformational variability in protein-ligand docking with NMR-guided rescoring.**J. Am. Chem. Soc.** 135(15):5819-5827
4. Latek D, Pasznik P, Carlomagno T, Filipek S. Towards improved quality of GPCR models by usage of multiple templates and profile-profile comparison. **PLoS ONE** 8(2):e56742
5. Ballare C, Lange M, Lapinaite A, Martin GM, Morey L, Pascual G, Liefke R, Simon B, Shi Y, Gozani O, Carlomagno T, Benitah SA, Di Croce L. Phf19 links methylated Lys36 of histone H3 to regulation of Polycomb activity. **Nat. Struct. Mol. Biol.** 19(12):1257-1265

Session 5 Protein-Ligand Interactions in Cells

Nanoparticle-mediated transport of proteins and other biomolecules into cells

Prof. Dr. Matthias Epple

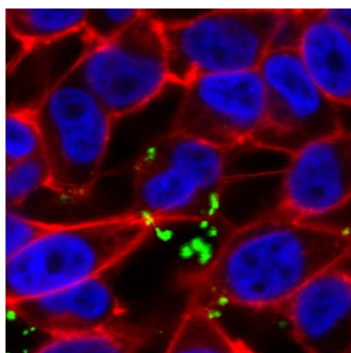
The cell membrane is an efficient barrier against the intrusion of external molecules. Biomolecules and synthetic molecules are often not able to penetrate the cell membrane on their own. This applies to nucleic acids (e.g. DNA, siRNA) and many proteins as well as to synthetic compounds like drugs.

On the other hand, nanoparticles are actively taken up by almost all cell types, typically via endocytosis or related processes. If the nanoparticles are loaded with (bio-) molecules, they can carry this cargo across the cell membrane and deliver it into the cell. Thereby, a desired biological effect can be triggered, e.g. transfection, gene silencing, or stimulation of the immune system. The latter is efficient for prophylactic or therapeutic vaccination against viral infections.

The synthesis of multi-shell nanoparticles allows to incorporate more than one biomolecule into one nanoparticles, allowing to induce synergetic effects, and also protects them against enzymatic attack, e.g. by nucleases or proteases. The attachment of targeting moieties like antibodies or peptides permits a cell-specific targeting, also *in vivo*. After the attachment of fluorescent labels, tracking of nanoparticles and their cargo during uptake by a cell and also after *in vivo* administration are possible.

The uptake of nanoparticles occurs via endocytosis/micropinocytosis, directing the particles into early endosomes and finally lysosomes. In the lysosome, the particles are dissolved and their cargo is delivered into the cytoplasm.

The synthesis and application of nanoparticles of calcium phosphate nanoparticles (100 nm), of calcium phosphate/polylactide nanoparticles (100 nm) and of ultrasmall gold nanoparticles (1-3 nm) will be presented.



Laser confocal microscopy on cells, incubated with nanoparticles (green). Nucleus: blue, cell membrane: red.

References:

V. Sokolova, M. Epple. **Angew. Chem**. 120 (2008) 1402-1416

O. Rotan, V. Sokolova, P. Gilles, W. Hu, S. Dutt, T. Schrader, M. Epple. **Mater.-Wiss. Werkstofftechn.** 44 (2013) 176-182.

V. Sokolova, D. Kozlova, T. Knuschke, J. Buer, A.M. Westendorf, M. Epple, **Acta Biomater.** 9 (2013) 7527-7535.

G. Doerdelmann, D. Kozlova, S. Karczewski, R. Lizio, S. Knauer, M. Epple, **J. Mater. Chem.** B 2 (2014) 7250-7259.

CURRICULUM VITAE

Prof. Dr. Matthias Epple

Date of Birth:	02.01.1966
Location:	Reutlingen/Germany
Nationality:	German
Family:	Married, 4 sons (Felix 1991, Tim 1994, Paul 1999, Jakob 2003)

1984-1989	Study of Chemistry at the Technical University of Braunschweig
1989-1992	PhD (TU Braunschweig; Advisor: Prof. Dr. H.K. Cammenga)
1993	Postdoctoral Studies at the Department of Chemical Engineering, University of Washington, Seattle, USA, with Prof. Dr. J. C. Berg
1994-1997	Postdoctoral Studies (Habilitation) at the Department of Inorganic and Applied Chemistry, University of Hamburg, with Prof. Dr. A. Reller
1995, 1996	Postdoctoral Studies at the Royal Institution, London, and at the synchrotron source at Daresbury with Prof. Dr. Sir J. M. Thomas
08.12.1997	Habilitation in Solid State Chemistry at the University of Hamburg
10.06.1998	Privatdozent (Lecturer) for Inorganic Chemistry
May to December 1998	Guest Professor for Solid State Chemistry at the University of Augsburg
01.04.2000-30.09.2003	Associate Professor (C3) of Inorganic Chemistry at the Ruhr-University of Bochum
since 01.10.2003	Full Professor (C4; Chair of Inorganic Chemistry) at the University of Duisburg-Essen

Main Current Research Interests:

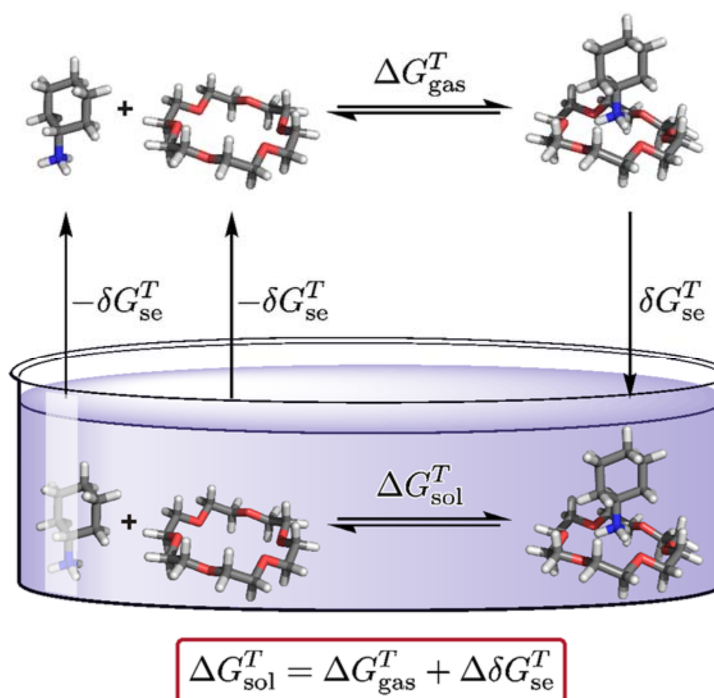
- Synthesis and functionalization of inorganic nanoparticles, e.g. for gene and drug delivery and immunization
- Development and application of biomaterials: polymers, ceramics, metals, composites; in close collaboration with physicians
- Study of biominerals with high-end solid-state chemical methods
- Biomimetic crystallization of inorganic materials, pathological crystallization phenomena
- Reactivity of solids

Session 6: Theoretical investigations of Multivalent Interaction

Theoretical investigations for host-guest interactions -- how we can link results from the computer to results in the lab

Prof. Dr. Beate Paulus

Very different contributions to the binding occur in host-guest interactions. To achieve a reasonable agreement of value determined with theoretical methods with the measured quantities in experiment there are many steps to climb. In a first step the quantum chemical contributions describing the forming and breaking of chemical bonds has to be described properly. Many so-called electronic structure methods are available, the most used ones based on density functional theory (DFT). But experiments take place at finite temperatures, therefore vibrational and rotational contributions, both for energy and entropy have to be taken into account. Only very seldomly gas phase reactions take place, therefore the influence of solvents and if applicable counter ions has to be regarded.



As example of the application of the above mentioned methods the investigation for the interaction of primary alkyl ammonium ions and crown ethers will be presented. Measurements are performed by isothermal titration calorimetry and calculations are with density functional theory including Grimme's dispersion correction D3(BJ). The translational, rotational, and vibrational contributions to the Gibbs energy of association are taken into account by a rigid-rotor-harmonic-oscillator approximation with a free-rotor approximation for low lying vibrational modes. Solvation effects are taken into account by applying the continuum solvation model COSMO-RS. A good agreement of theory and experiment is only achieved, when solvation and the effects of the counterions are explicitly taken into account.

Reference:

A. J. Achazi, L. K. S. von Krbek, C. A. Schalley, B. Paulus: Theoretical and experimental investigation of crown/ammonium complexes in solution, J. Comp. Chem, 37, 18-24 (2016).DOI: 10.1002/jcc.23914

CURRICULUM VITAE

Prof. Dr. Beate Paulus

Date of Birth:	15.04.1967
Location:	Freie Universität Berlin, Institut für Chemie und Biochemie Theoretische Chemie Takustr. 3, 14195 Berlin

Scientic education:

Physics (10/1987-03/1993), Universität Regensburg, diploma thesis with Joachim Keller

Degrees:

2005 Habilitation in Physics; Universtität Regensburg and MPI für Physik komplexer Systeme,Dresden;
1995 Dr. ret. nat., Universität Regensburg and MPI für Physik komplexer Systeme, Dresden; Mentor: Prof. Peter Fulde

Scientific Vita:

07/2011 - today	W3 professorship for Theoretical Chemistry, Freie Universität Berlin
06/2011- 10/2007	W2 professorship for Theoretical Chemistry (5 years), Freie Universität Berlin
09/2007- 01/1996	Scientific coworker at the MPI for the Physics of Complexe Systems, Dresden, with Prof. Peter Fulde

Editorships:

Reviewer for grant proposals of the DFG, several European science foundations, AvH-foundation, ERC grants
Panel Reviewer for the annual romanian (2012) and finnish (2010) science foundation
Reviewer for more than 15 different journals

Scienctific administration:

Scientific board of the HLRN (north-german computer alliance) (since 07/13)
Head of the Dahlem Research School Molecular Science (since 2011)
Head of the graduate school of the CRC 765 (since 2013)
Head of the "Institut für Chemie und Biochemie“ (since 04/15)

Organizational activities:

Workshop on Local correlation methods: From Molecules to Crystals (Dresden 2007)
Workshop on Entanglement Based Approaches in Quantum Chemistry (Dresden 2012)
Workshop on Systematically Improvable Electronic Structure Methods (Ax les Thermes, France, 2013)
CECAM TCCM Winter School on Large Systems (Saint-Lary-Soulan, France, 2014)

Honours and awards:

Price for Excellent Teaching of the Institute of Chemistry (2011)
Guest professorship of the University Paul Sabatier, Toulouse (2011)

POSTER ABSTRACTS

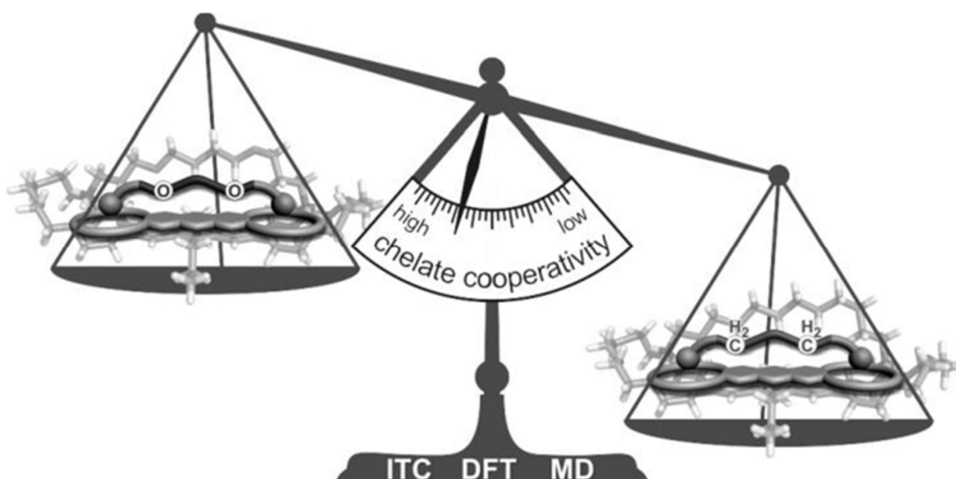
P1 – Achazi: Cooperativity effects in multivalent systems − A case study

Andreas J. Achazi[1], Larissa K. S. von Krbek[1], Marthe Solleder[2], Marcus Weber[2], Beate Paulus[1], Christoph A. Schalley[1]

1) Institute of Chemistry and Biochemistry, Freie Universität Berlin

2) Zuse Institute Berlin (ZIB), Germany (Takustr. 3, 14195 Berlin)

The particularly strong as well as reversible interaction in multivalent systems arises increasing interest, especially as it is progressively used in the fields of supramolecular and medicinal chemistry. To understand and quantify the unique enhancement of the binding constant K in multivalent systems, different cooperativity effects have to be considered individually. Besides, various possible unbound, bound and partly bound states occur during the association process of a multivalent guest to a multivalent host. The number of these states influences the value of the over-all binding constant K of the multivalent system by statistical factors. Hence, these statistical factors have to be quantified prior to any experimental analysis of the association process. Subsequently, a double mutant cycle analysis [1] may be used to quantify all cooperativity effects in the multivalent system.



We use this approach to study the cooperativity effects of divalent crown-ammonium-complexes in different solvent mixtures. Isothermal titration calorimetry is the only tool to directly and simultaneously determine the binding constant K as well as the binding enthalpy ΔH and thus derive the Gibbs energy ΔG and the entropy ΔS. Furthermore, we compare our experimental data with DFT and molecular-dynamics calculations which give further insight into the processes occurring upon multivalent binding in solution. [2,3]

References:

1. W. Jiang, K. Nowosinski, N. L. Löw, E. V. Dzyuba, F. Klautzsch, A. Schäfer, J. Huuskonen, K. Rissanen, C. A. Schalley, ***J. Am. Chem. Soc.*** **2012**, *134*, 1860 – 1868.
2. A. J. Achazi, L. K. S. von Krbek, C. A. Schalley, B. Paulus, ***J. Comput. Chem.*** **2016**, *37 (1)*, 18–24.
3. L. K. S. von Krbek, A. J. Achazi, M. Solleder, M. Weber, B. Paulus, C. A. Schalley, **2016**, submitted.

P2 – Bachem: DNA-Programmed Spatial Screening of Multivalent Carbohydrate-Protein Interaction

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a) Department of Chemistry, Humboldt-University Berlin, Germany
b) Structural Glycobiology, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Carbohydrate-protein interactions play an important role in many biological recognition processes. While single carbohydrate-protein interactions are typically weak, multivalent interactions between multiple carbohydrates (as found on viruses, bacteria, parasites etc.) and receptor clusters are responsible for much higher and biologically relevant binding affinities and selectivities. [1]

Langerin is a mannose binding C-type lectin involved in immune cell response and currently believed to be a natural barrier to HIV infection. It exists as a stable trimer with primary sugar-binding sites separated by a distance of 42 Å (*Figure 1a*). [2]

We have recently developed a new ligand for Langerin, Glc-2NHTs, with a 10 fold increase in affinity to mannose, its natural binder. Our further efforts have focused on the multivalent presentation of Glc-2NHTs with the help of peptide nucleic acid (PNA)-DNA duplexes.[3,4] Thiol-modified PNA was synthesized by SPPS and labelled with maleimide-functionalized Glc-2NHTs (figure 1b). The hybridization of the Glc-2NHTs-PNA conjugates to complementary DNA strands allowed the modular approach to a series of bivalent PNA:DNA complexes with Ångström-scale precise positioning of Glc- 2NHTs-ligands as shown in figure 1c. The spatial screening led to bivalent Glc-2NHTs-PNA-DNA complexes with much higher affinity for Langerin than the monovalent ligand.

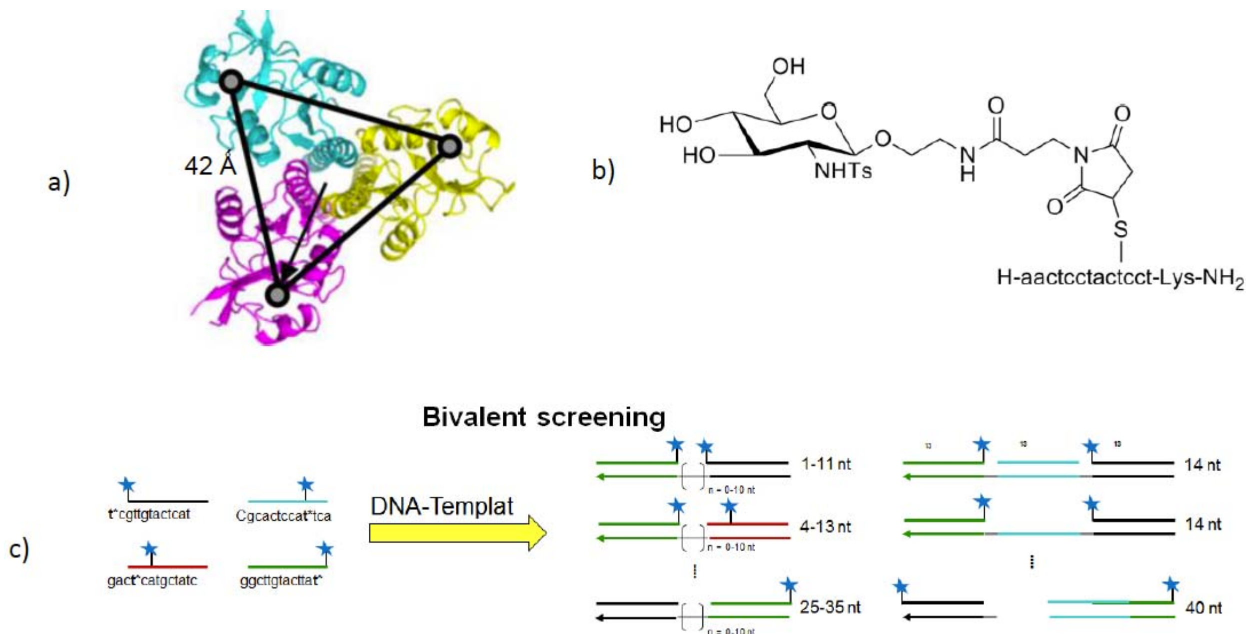

Figure 1: a) Crystal structure of Langerin b) Example of Glc-2NHTs-PNA conjugate c) Assembly of bivalent ligand-PNA-DNA complexes

References:
1. V. Wittmann et al., *Chem. Soc. Rev*. **2013**, 42, 4492
2. H. Feinberg et al., *J. Biol. Chem.* **2010**, 285, 13285
3. C. Scheibe, et al. *ChemBioChem* **2013**, 14, 236
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P3 – Bäcker: 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.

References:

S. K. Knauer, C. Bier, N. Habtemichael, R. H. Stauber: The survivin-Crm1 interaction is essential for chromosomal passenger complex localization and function. ***EMBO Rep.*** **2006**, *7*, 1259-1265.

S. K. Knauer, O. H. Kramer, T. Knosel, K. Engels, F. Rodel, A. F. Kovacs, W. Dietmaier, L. Klein-Hitpass, N. Habtemichael, A. Schweitzer, J. Brieger, C. Rodel, W. Mann, I. Petersen, T. Heinzel, R. H. Stauber: Nuclear export is essential for the tumor-promoting activity of survivin. ***FASEB J.*** **2007**, *21*, 207-216.

B. Unruhe, E. Schröder, D. Wünsch, S. K. Knauer: An Old Flame Never Dies: Survivin in Cancer and Cellular Senescence. ***Gerontology*, 2015**.

P4 – Bertazzon: Profiling and inhibition of multivalent WW domain interactions

Bertazzon M.1, Henning L.1, Fischer C.2, Bhatia S.1, Kühne R.3, Rademann J.2, Haag R.1, Freund C.1

[1]Institut für Chemie und Biochemie der Freie Universität Berlin; [2]Institut für Pharmazie, Freie Universität Berlin; [3]Leibniz-Institut für molekulare Pharmakologie, Berlin

In the spliceosome are involved several low affinities interactions which modulate splicing events, one of these appears from WW domain and proline-rich sequences (PRS). The WW domain has multivalent effect property that allows enhancing the affinity binding with the target during splicing. Within the spliceosomal protein, we focus on FBP21 because contains two WW domains separated by a short and flexible linker and seems to be involved in the alternative splicing.

Analyzing the interaction between the tandem WW domains (tWW) and ligands containing one or more PRS we could show that the tandem arrangement of WW domains and the valency of the proline-rich ligand both contribute to an apparent affinity enhancement (Fig.1) [1].

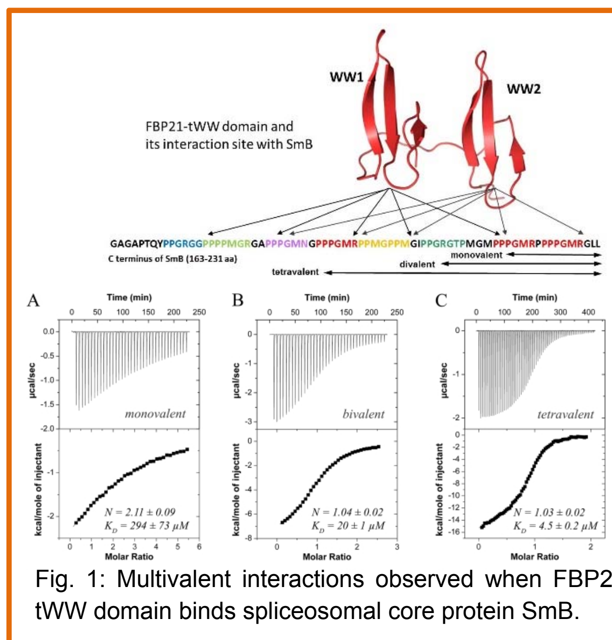


Fig. 1: Multivalent interactions observed when FBP21-tWW domain binds spliceosomal core protein SmB.

Huang and co-workers have demonstrated that FBP21-tWW activates pre-mRNA splicing and localizes to nuclear speckles in which splicing takes place [2]. However, molecular details how FBP21 is involved in splicing remain elusive. In order to understand and alter FBP21's cellular function, we aim at creating an inhibitor against FBP21-tWW.

We are following several ideas for inhibitor design. On the one hand, we are trying to increase binding affinity of the monovalent WW ligand. To achieve this, we optimized a peptide ligand by screening a nonapeptide phage display library for an optimal binding partner and analyzed the binding affinities of the top hits by ITC. The best binding candidate was WPPPPRVPR (Kd of 90 µM and 150 µM respectively for WW1 and WW2):

On the other hand, we are presenting the binding peptide on polymeric scaffolds, to gain avidity through multivalent ligand presentation. This is done in collaboration with AG Haag, using their dendrimeric polymer (Fig. 2) [3,4], and in collaboration with AG Rademann, in which a multivalent dextran is employed to display the peptide ligand.

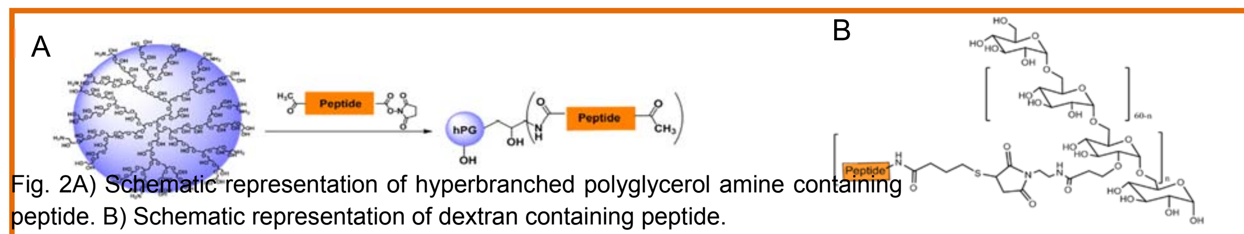


Fig. 2A) Schematic representation of hyperbranched polyglycerol amine containing peptide. B) Schematic representation of dextran containing peptide.

References:

1. Klippel S., Wieczorek M., Freund C. et al. (**2011**) ***J. Biol. Chem.***, 286: 38478-38487.
2. Huang X., Beullens M., Bollen M., Shi Y.et al. (**2009**) ***J. Biol. Chem.***, 284: 25375-25387.
3. Sisson A.L., Haag R. et al. (**2009*) Angew. Chem. Int. Ed.***, 48: 7540-7545.
4. Henning L.M., Bhatia S., Bertazzon M., Haag R. and Freund C.,et al.(**2015**) **Beilstein *J.Org. Chem.***, 11: 701–706.

P5 – Bharate: Self-Assembling Carbohydrate-functionalized Graphene for Capturing and Killing *E. coli* Bacteria

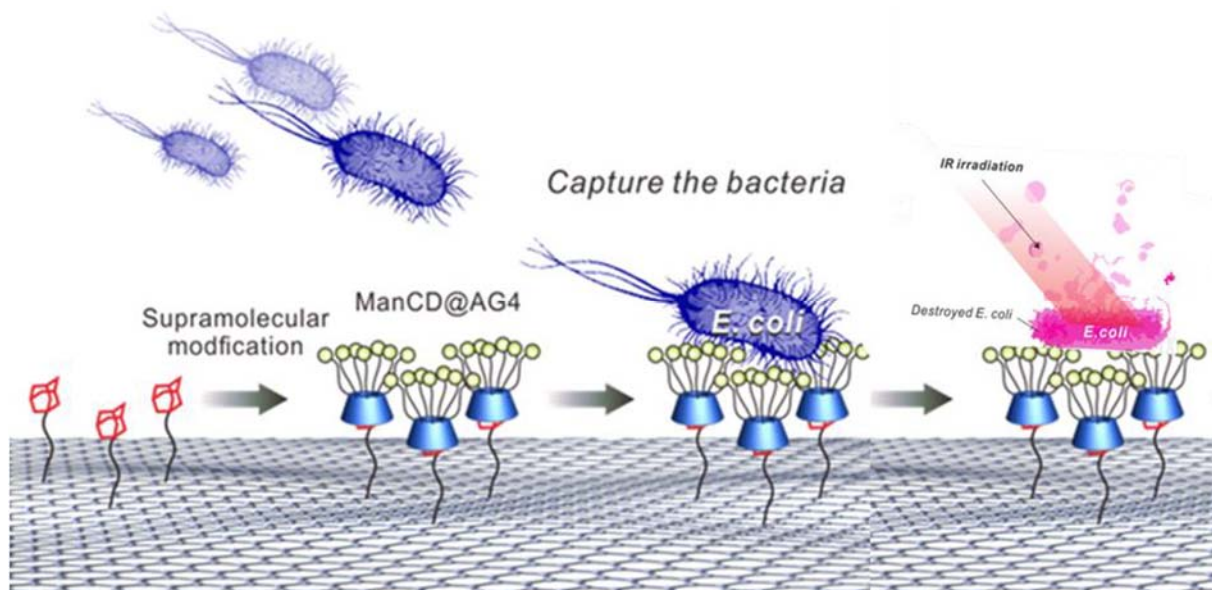
Priya Bharate, Peter H. Seeberger

Max-Planck-Instut für Kolloid-und Grenzflächenforschung, Germany

Carbohydrate-protein interactions (CPIs) play a crucial role in several biological processes including cell-cell interaction, proliferation, bacterial and viral infections.[1] However, serious problems arise while attempting to employ these specific interactions for constructing an artificial recognition interface due to the weak binding constants for mono- and oligosaccharides to most lectins (*K*a between 102 and 105 M-1).[2] The use of multivalent scaffolds provides a strategy to increase those interactions.[3]

Herein, we designed and synthesized a supramolecular carbohydrate-functionalized two-dimensional (2D) surface using a host-guest method. Adamantyl functionalized graphene (AG4) is decorated with mannose-β-cyclodextrin (ManCD) and used for bacterial binding assay exploiting CPIs. The developed self-assembled sensor system (ManCD@AG4) can bind the bacteria due to the specific interaction of mannose to *E.Coli* strain ORN178 in reversible way. Exploiting the unique thermal IR-absorption properties of graphene derivatives, [4] IR-laser irradiation of the ManCD@AG4-*E.coli* complex provides a facile method to kill the captured bacteria (99% elimination).[5]

Figure:



References:

1. D. C. Kennedy, D. Grunstein, C. H. Lai and P. H. Seeberger, *Chemistry*, 2013, 19, 3794 3800.
2. Y. Sato, K. Yoshioka, T. Murakami, S. Yoshimoto and O. Niwa, *Langmuir: the ACS journal of surfaces and colloids*, 2012, 28, 1846-1851.
3. D. Grunstein, M. Maglinao, R. Kikkeri, M. Collot, K. Barylyuk, B. Lepenies, F. Kamena, R. Zenobi and P. H. Seeberger, *J Am Chem Soc*, 2011, 133, 13957-13966.
4. M. Acik, G. Lee, C. Mattevi, M. Chhowalla, K. Cho, Y. J. Chabal, *Nat. Mater*. 2010, 9, 840-845.
5. Z. Qi,+ P. Bharate,+ C. Lai, P. H. Seeberger,*R. Haag* et al. *Nano Lett*., 2015, 15 (9), pp 6051–6057.

P6 – Blümke: Role of HTRA1 in the prion-like transmission of Tau aggregates in neurodegenerative diseases

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The abnormal aggregation of the microtubule-associated protein Tau into fibrillar deposits is a hallmark of neurodegenerative tauopathies, such as Alzheimer's disease. Tau pathology spreads along distinct neural networks through the intercellular transfer of *low-order* Tau oligomers, or seeds (1-2). In a prion-like manner, Tau seeds are secreted into the extracellular space and are subsequently endocytosed by neighboring cells (2). Upon internalization, seeds nucleate the fibrillization of endogenous Tau via direct protein-protein contact (2). The highly conserved serine protease HTRA1 degrades Tau fibrils by disintegrating their core structure and proteolyzing the remaining polypeptides (3). The PDZ domain of HTRA1 provides additional binding sites for Tau und thus enhances the destabilization of fibrils (3). HTRA1 is largely localized in the extracellular space, however, its spontaneous uptake in various cell lines was demonstrated (3). In a cellular model of Tau aggregation, internalized HTRA1 was shown to co-localize with Tau fibrils and degrade them, indicating an intercellular spread of HTRA1 that counteracts the propagation of Tau (3).

Here, the antagonistic spreading of Tau seeds and HTRA1 will be investigated by applying an indirect co-culture system for HTRA1 overexpressing HEK293T cells and SH-SY5Y neuroblastoma cells that contain induced Tau aggregates. Media exchange between both cell lines occurs via a microporous membrane that hinders cell migration. Further, the effect of HTRA1-activating peptides on the Tau fibril proteolysis will be analyzed. However, HTRA1-mediated fibril disintegration might be impaired, since peptides bind to the PDZ domain and occupy binding sites for Tau interaction. Hence, a substoichiometric peptide concentration needs to be established that enables allosteric activation of HTRA1 and concomitantly allows PDZ binding to Tau fibrils.

References:

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Kfoury, N., Holmes, B. B., Jiang, H., Holtzman, D. M., Diamond, M. I.: Trans-cellular propagation of Tau aggregation by fibrillar species, *J. Biol. Chem.*, 2012, 287: 19440-19451

Poepsel, S., Sprengel, A., Sacca, B., Kaschani, F., Kaiser, M., Gatsogiannis, C., Raunser, S., Clausen, T., Ehrmann, M.: Determinants of amyloid fibril degradation by the PDZ protease HTRA1, *Nat. Chem. Biol., 2015,* 11: 862-869

P7 – Brencher: Attenuation of intestinal I/R-injury by β-alanine: A glycine- receptor mediated effect?

Lisa Brencher, Rabea Verhaegh, Michael Kirsch

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Background Acute mesenteric ischemia is often caused by embolization of the mesenteric arterial circulation [1]. Coherent intestinal injury due to ischemia and following reperfusion get visible on macroscopic and histological level. In previous studies, application of glycine caused an ameliorated intestinal damage after ischemia-reperfusion in rats [2]. Since we speculated that glycine acted here as a signal molecule, we investigated whether the glycine-receptor agonist β-alanine evokes the same beneficial effect in intestinal ischemia-reperfusion [3].

Material and Methods β-alanine (10, 30 and 100 mg/kg) was administered *intravenously*. Ischemia/reperfusion of the small intestine was induced by occluding and re-opening the superior mesenteric artery in rats. After 90 min of ischemia and 120 min of reperfusion, the intestine was analyzed with regard to macroscopic and histological tissue damage, activity of the saccharase, and accumulation of macrophages. Additionally, systemic parameters and blood gas analysis (e.g. acid base balance, electrolytes, blood glucosis) were measured at certain points in time.

Results All three dosages of β-alanine did not change systemic parameters but prevent from hyponatremia during period of reperfusion. Most importantly, application of 100 mg β-alanine clearly diminished intestinal tissue damage, getting visible on macroscopic and histological level. In addition, IR-induced decrease of activity of saccharase as well as accumulation of macrophages in the small intestine was ameliorated.

Conclusion The present study demonstrated that β-alanine was a potent agent to ameliorate I/R-induced injury of the small intestine. Due to its diminishing effect on the accumulation of macrophages β-alanine is strongly expected to mediate its beneficial effect *via* glycine-receptors.

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P8 – Büscher: Purification of functionalized calcium phosphate nanoparticles by asymmetric flow field-flow fractionation

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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 have used FFF as a method develop a new route of purification for functionalized 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 FFF as a more gentle method for purification.

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P9 – Dubel: Exploring the range of multivalency by DNA-based spatial screening

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Multivalency is used by nature to strengthen weak receptor-ligand interactions.[1, 2] By now the limits and range of multivalency are not explored. An example is the adapter protein 2 (AP2), which is a central interaction hub in the clathrin mediated endocytosis. The two ear domains of AP2 are connected to the core via long and flexible linkers. Because of this, it is not clear whether they are interacting in synergy.[3] DNA-based model systems offer a unique set of properties which are useful for this study. DNA-double strands can be seen as molecular rulers on which the spatial arrangement and the orientation of the ligands can be precisely controlled. Cucurbit[7]Uril (CB[7]) is a molecular cage which is able to build strong guest-host-complexes with several molecules and dyes, like adamantane and Pyronine B.[4, 5] By interacting with CB[7] the fluorescence of Pyronine B increases.[5] With a spatial screening the limit of bivalency can be examined. Therefore we constructed a bivalent system by attaching CB[7] and a suitable host on the 5'-end of the DNA (Fig. A). At larger distances of the guest-host-complexes the binding affinity lowers. When the bivalent affinity is as low as the monovalent binding affinity the limit of bivalency is reached. We already showed that a guest-host-complex is build between Pyronine B and a CB[7]-modified DNA. Furthermore an adamantane, which is connected to a DNA, is able to replace the Pyronine B in the CB[7] (Fig. B).

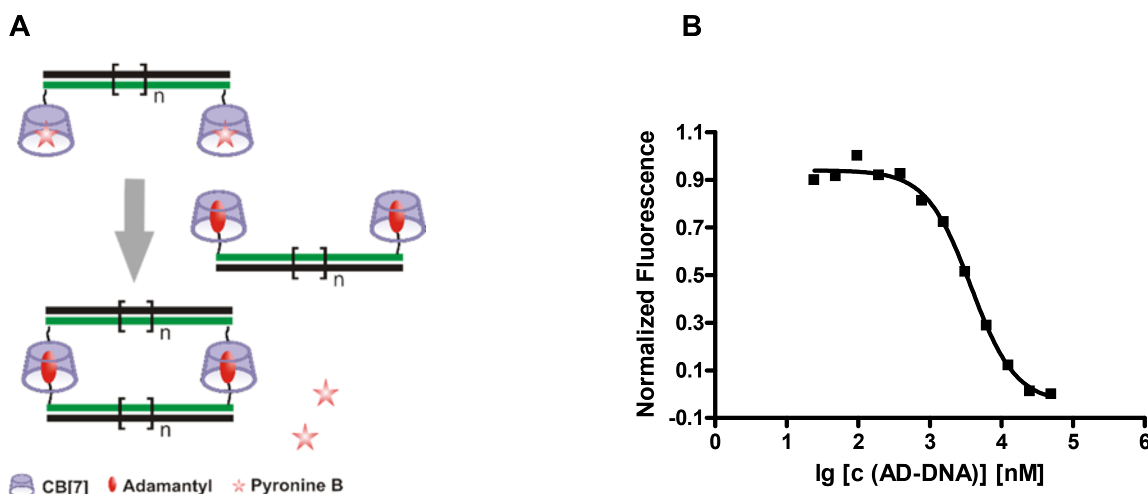

Fig. A: Concept of the competition experiment. Fig. B: Fluorescence intensity upon Pyronine B replacement.

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P10 – Fischer: Multivalent peptide-polymer conjugates as inhibitors for protein-protein interactions

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Recently, we have employed peptide-polymer conjugates as ligands for multivalent protein targets to inhibit intracellular protein-protein interactions.[1] It was demonstrated that the multivalent presentation of ligands leads to the enhancement of binding affinities and therefore to an increase of the inhibitory effect of the ligands.[2] However, an exponential increase in binding affinities can only be expected for well-fitted, rigid systems of multivalent receptors and ligands (Fig 1a). In Nature, though, many multivalent receptors are characterized by the flexible arrangement of binding sites (Fig 1b). It is therefore harder to estimate the magnitude of the enhancement of binding affinities due to multivalent effects for these flexible ligand-receptor systems. Thus, it was our goal to further investigate structure-activity relationships (SAR) of peptide-polymer conjugates to specific protein targets in order to design and synthesize potent, flexible and multivalent inhibitors.

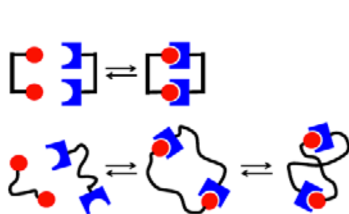

Figure 1 a: rigid ligand-receptor system b: flexible ligand-receptor arrangement

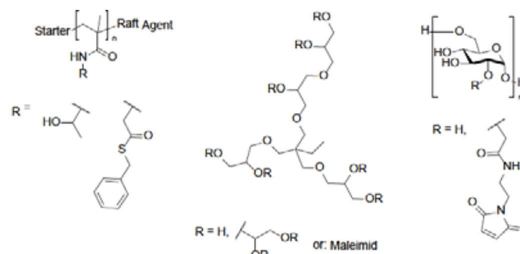

Figure 2: different scaffold systems (poly HMPA, hyperbranched polyglycerol and dextran)

The protein target in this work is the tandem-WW domain of FBP21, which plays an important role in the m-RNA splicing process. It has been shown that monovalent peptides with proline-rich sequences possess moderate binding affinities, which can be enhanced through a multivalent presentation of the peptides.[3] In previous works, the SAR of various polymeric carriers (dextran, hyperbranched polyglycerol and poly HPMA) with different structure, flexibility and morphology (Fig 2) were tested.[4] Thermodynamics of binding and stoichiometry of the different peptide-polymers to the bivalent receptor protein were investigated by ITC, showing dextran-based conjugates as the most promising carrier systems ($K_{D,\ mono}$ >1000µM, $K_{D,\ 10\ peptides\text{-}conjugate}$ = 1.2 µM). This led to the utilisation of optimized peptide-dextran conjugates, which used an improved, bivalent peptide sequence as a ligand ($K_{D,\ 4\ peptides\text{-}conjugate}$ = 0.84 µM). Subsequent confocal imaging showed cellular uptake of the conjugates into endosomes (using nona-arginine) or the cytoplasm (using TAT as a cell-penetrating peptide). Further studies of the functional inhibition of the conjugates on the tandem-WW domains of FBP21will include splicing assays in cell lysates and *in vivo*.

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P11 – Grad: Computational investigation of multivalent peptidic dendrimers

Jean-Noël Grad

Project A7, AG Daniel Hoffmann (Centre for Medical Biotechnology, Universitätsstraße, 45117 Essen, jean-noel.grad@uni-due.de)

14-3-3 proteins play a key role in cell proliferation, differentiation and survival.[1] In particular, they regulate the interaction between C-Raf and Ras.GTP through selective binding to C-Raf. Evidence for stabilization of C-Raf/14-3-3 interactions by supramolecular ligands featuring guanidiniocarbonyl pyrrole (GCP) cations was recently obtained *in vitro*, however elucidation of the binding mechanism at a molecular level remains challenging due to ligand flexibility.[2]

In the absence of X-ray structure of the complex, we used molecular modelling to gain insight into the interactions between 14-3-3/C-Raf and a 2.5 kDa dendrimer with 3 GCPs (QQJ-096). Molecular Dynamics (MD) simulations revealed highly flexible binding poses involving specific regions of 14-3-3 (Figure 1.a and 1.b) that could be critical to the stabilizing effect of QQJ-096. A similar observation was made in Epitopsy (Figure 1.b), a fast alternative to MD currently under development.

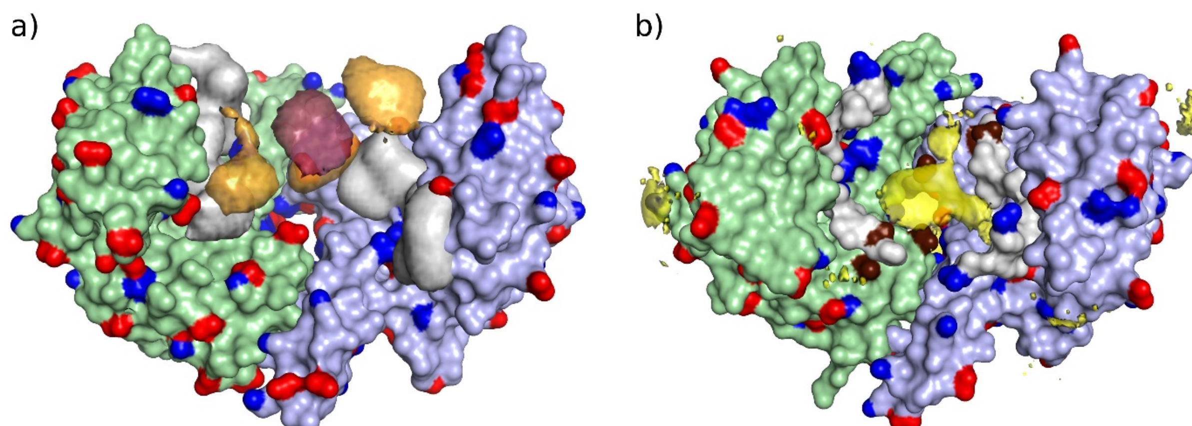

Figure 1: 14-3-3ζ dimer in pale green/blue, ASP/GLU colored in red and ARG/LYS in blue. a) Result of a 50 ns MD simulation; the volumes represent the space occupied by two C-Raf fragments (white), the QQJ-096 central hydrazone scaffold (raspberry) and its 3 GCP arms (orange) for at least 25% of the simulation time. b) Residues frequently involved in salt bridges with QQJ-096 in 6×50 ns MDs are depicted in dark red. The yellow isosurface drawn at – 1 k_BT was calculated in Epitopsy with a single GCP cation as molecular probe.

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P12 – Gupta: Calculating hydration interaction between hydrobhobic and hydrophilic surfaces from computer simulations

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Using all-atom molecular dynamics (MD) simulations at constant water chemical potential in combination with basic theoretical arguments, we study hydration-induced interactions between two overall charge neutral yet polar planar surfaces. Hydration repulsion dominates the interaction between polar surfaces in water at nanometer separations and ultimately prevents the sticking together of biological matter. Although confirmed by a multitude of experimental methods for various systems, its mechanism remained unclear. A simulation technique (thermodynamic extrapolation) is introduced that yields accurate pressures between solvated surfaces at prescribed water chemical potential. In a separate study the effect of co-solutes on hydration repulsion is also discussed.

P13 – Heid: New Approaches to Symmetric and 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] Their unique binding mode counteracts pathologic protein aggregation with potential applications in the treatment of Alzheimer's disease[2] and HIV infection.[3] However, molecular recognition of lysines and arginines on protein surfaces is not selective. We now developed a new method for direct esterification of monoaryl phosphates. This method allows the convenient introduction of one or two additional linker arms for subsequent click reactions with modified peptides. These peptides act as additional recognition sites and direct the tweezers to a selected lysine residue on the protein surface. Herein we present the synthesis of these new molecular tweezers, including binding experiments.

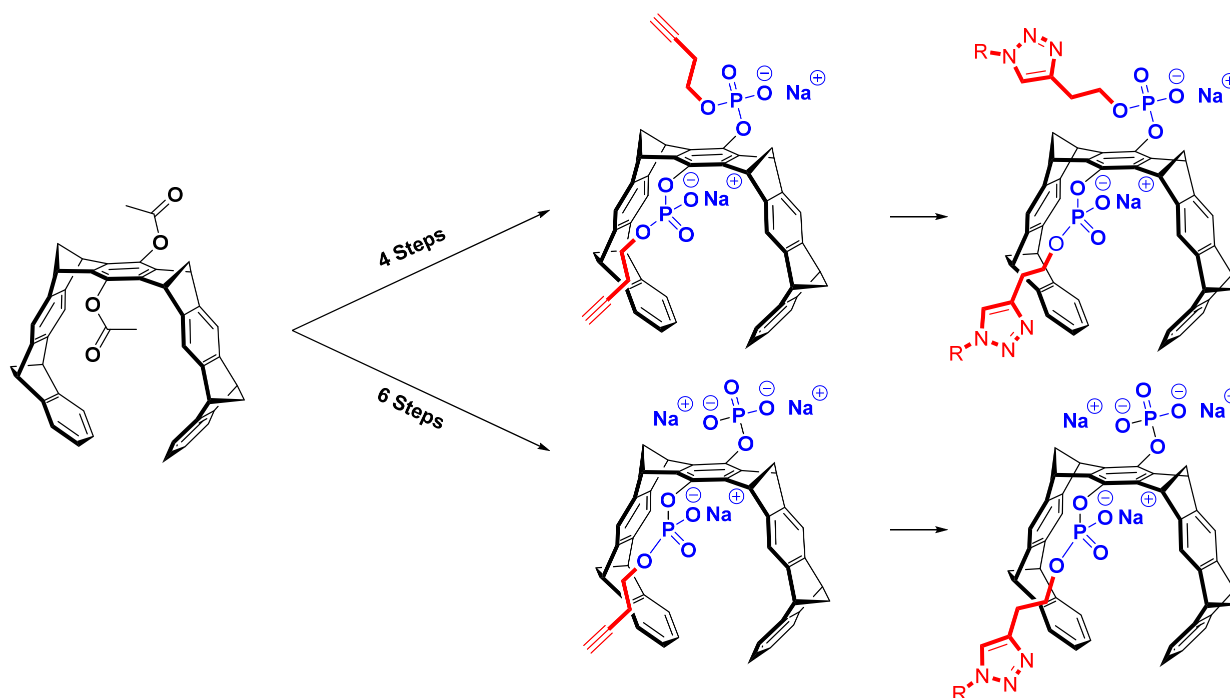

Figure 1. Synthesis of new molecular diphosphate tweezers with additional recognition unit.

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P14 – Homa: The role of unit geometry in the self-assembly of hierarchical DNA superstructures

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The complexity of natural system is often achieved through the step-wise assembly of small units into larger architectures of high hierarchical order.[1] To reach this goal, a subtle balance of intermolecular recognition forces and geometrical factors is necessary and should be taken into consideration when trying to mimic natural strategies of self-assembly.[2, 3] In this work, we apply the DNA origami method for the construction of planar superstructures starting from small building blocks of rectangular shape (Figure 1). Besides canonical base hybridization, design approaches are investigated, which allow to control inter-unit association through base stacking of their edges. Our preliminary results demonstrate that the efficiency of superlattice formation as well as the resulting ultrastructure are strongly affected by the geometric properties of the interacting tiles, such as the crossover pattern at their edges, their global twisting and relative curvature orientation. Full control of force- and shape-driven inter-unit interactions is therefore critical for programmable hierarchical self-assembly and will enable to extend our capability to control matter distribution and manipulation to macroscopic scales.

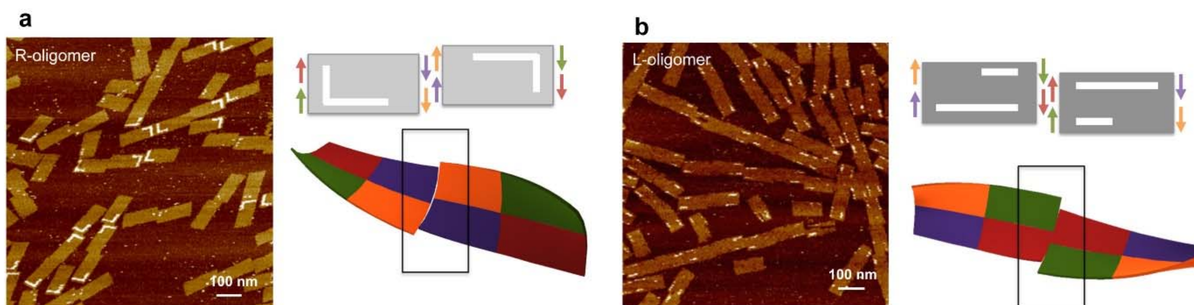

Figure 1. The global twist of the DNA origami tiles, imposed by specific crossover pattern, is responsible for their regioselective base stacking at the edges. Both R-twist (**a**) and L-twist (**b**) rectangular shapes give rise to oligomers with a regular orientation of the component units. The mechanisms regulating the hierarchical assembly process are still under investigations.

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P15 – Kiran: Multivalent Macromolecules as Inhibitors of Influenza Virus

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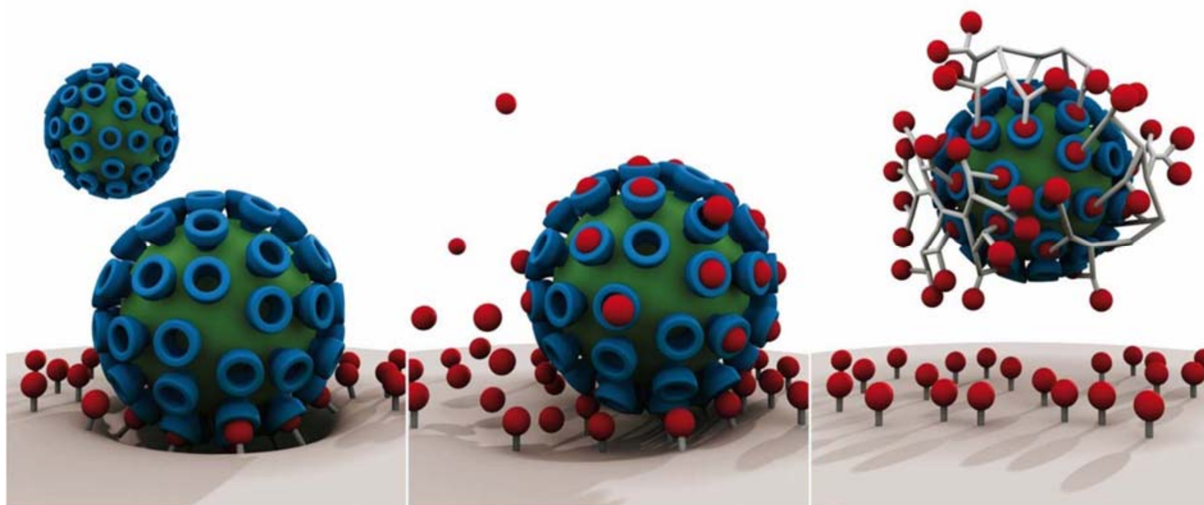
Multivalency is a ubiquitous phenomenon in nature involving complex binding mechanisms for achieving non-covalent strong yet reversible interactions. Interfacial multivalent interactions at pathogen-cell interfaces can be competitively inhibited by multivalent scaffolds that prevent pathogen adhesion to the cells during the initial stages of infection. The lack in understanding of complex biological systems makes the design of an efficient multivalent inhibitor a toilsome task and is the reason why as of yet no multivalent anti-infective (apart from antibodies) has emerged on the market until now.



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P16 – Kumari: Synthesis of Multivalent Polymer Architecture Targeting Inflammation

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Atherosclerosis is an inflammatory disease, characterized by the uptake of low density lipoprotein (LDL) particles into macrophages and inflamed vascular endothelial cells [1]. Native LDL is modified by reactive oxygen species (ROS) generated by vascular tissues and yields oxidized LDL (oxLDL) that triggers the expression of the scavenger receptor LOX-1 on different cell types-macrophages, vascular endothelia, smooth muscle cell and platelets. LOX-1 is a type II membrane protein and consists of four domains:N-terminal cytoplasmic domain, transmembrane domain, extracellular neck domain and a C-terminal lectin-like domain (CTLD). For efficient oxLDL uptake, LOX-1 requires at least homodimerization [2,3].

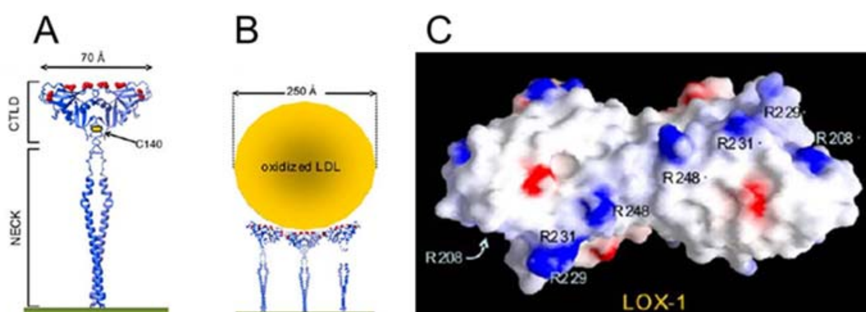

Figure 1, LOX-1 Structures. A, extracellular domains of homodimer; B, oxLDL particle bound to surface assembled LOX-1; C, CTLD surface [3]

Apart from oxLDL binding, LOX-1 also mediates bacterial adhesion to vascular endothelia and lung epithelial cells and contributes to effective colonization [3].

In this project we are interested in exploiting the dynamic nature of synthetic sulphated multivalent star like and dendritic polymer architecture in a therapeutic approach to antagonize oxLDL uptake that might be beneficial to treat atherosclerosis. The specific targets is to synthesis and characterization of sulphated star like multivalent architecture and PEG conjugated sulphated dendritic architecture. Further SPR and other biological assay will be done to show the interaction of the molecules with the receptor.

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P17 – Köcher: 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.

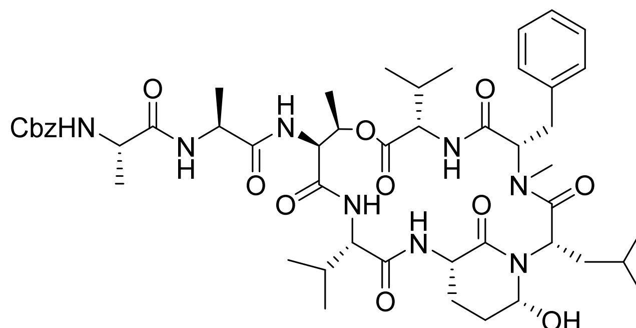

Fig. 1: Chemical structure of an Ahp-Cyclodepsipeptide

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P18 – Kujawski: Tailor-made imprinted polymers for protein recognition

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Protein recognition is a challenging but very promising research field. Many diseases are caused by protein-misfolding, assembly or controlled by specific proteins and their interactions with other proteins. Therefore it is important to find selective and high specific chemical tools to interact with these proteins and e.g. block their function. Addressing those targets with crosslinked polymers, containing tailor-made monomers, is an up-and-coming goal for us in supramolecular chemistry. For many amino acids *Schrader et al*. have functional addressing monomer-units that can be added to the polymerization mixture.[1] This mixture for imprinted polymers (MIP) contains beside the functional monomer/-s (FM), cross linker (CL), radical initiator and buffer, the target molecule (template). This template will be imprinted by the polymer-network and the FM as a kind of anchor. After the polymerization step it is necessary to remove the template, in our case the protein. This is possible with enzymes that cuts the proteins out or with a washing procedure (dialysis). In the end rebinding studies show the effect and efficiency of imprinting.[2,3]

Our task will be first synthesizing non-imprinted (NIP) crosslinked polymer-networks, evaluate them with binding and affinity studies, and later involve the imprinting-effect by repeating the most promising NIP procedures in presence of the target protein and create the desired MIPs.

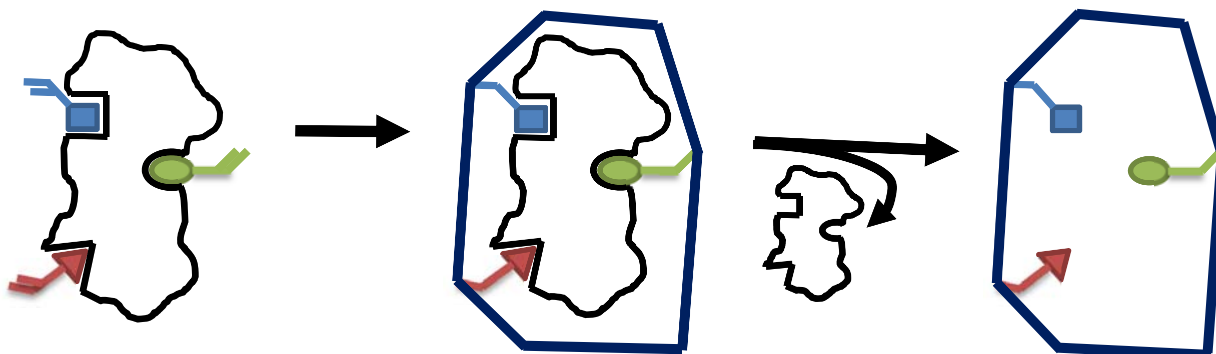

Figure 1: Principle of molecular imprinting - 1. Preparation of covalent conjugate or non-covalent adduct between a functional monomer and a template molecule; 2. Polymerization of this monomer-template conjugate (or adduct); 3. Removal of the template from the polymer

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P19 – Mertel: Development and investigation of GCP-derived small molecules targeting the central pore of 14-3-3 Proteins

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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 molecules will be used in order to establish a structure activity relationship (SAR) with regard to their binding properties to the different 14-3-3 isoforms. Furthermore we want to develop an anchor molecule that binds inside the central pore and which can be connected to other moieties like fluorophores, for potential biosensing applications, as well as to known inhibitors or stabilizers to benefit from cooperative binding effects. These composite molecules could provide fundamental knowledge about the biological behavior of 14-3-3 proteins and could lead to medicinal applications in drug development and anti-cancer therapy.

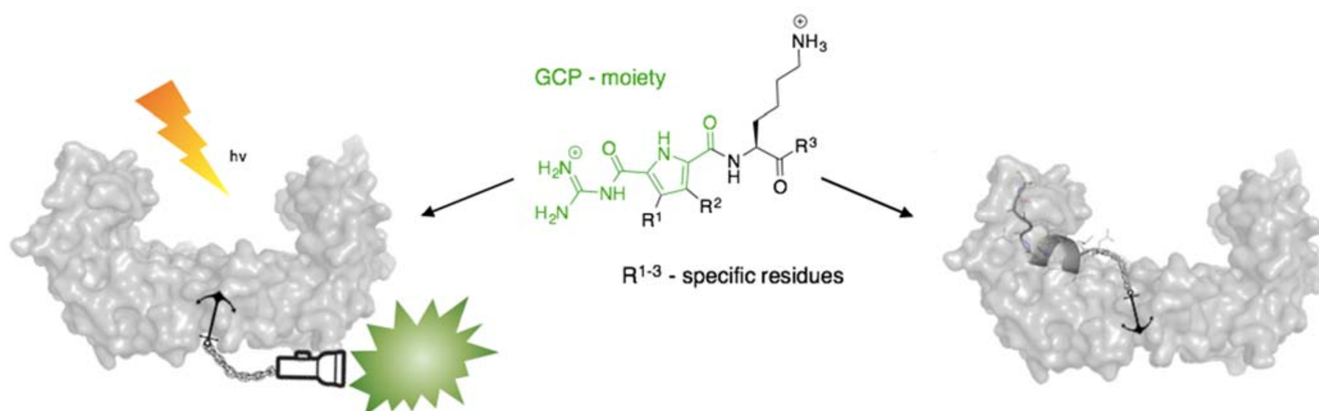

Figure 1: Schematic depiction of the substituted anchor moiety binding to the central pore of a 14-3-3 protein

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P20 – Mittal: Inhibition of Huntingtin exon-1 aggregation by a molecular tweezer

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Huntington's disease (HD) is associated with the pathogenic expansion of the polyglutamine (polyQ) stretch of the huntingtin (htt) protein[1]. The 17-residue N-terminal fragment of the exon 1 (N17) has been suggested to play a crucial role in modulating aggregation propensity and toxicity [2].

The molecular tweezer CLR01 selectively binds to lysine and, to a lesser extent, arginine residues. CLR01 has been reported to inhibit toxic aggregation in amyloidogenic proteins like amyloid polypeptide (hIAPP), α-synuclein and amyloid beta (Aβ) without toxic side effects[3]. Since the N17 region of htt contains three lysine residues, which could be important to the function of the huntingtin protein, we studied the possible effect of the binding of CLR01 molecules on a htt protein with a pathogenic polyQ tract. For this purpose, we used classical and enhanced sampling molecular dynamics simulations to show that the binding of CLR01 induces structural rearrangements within the crucial N17 region of htt exon 1 monomer. QM/MM calculations allowed us to propose the preferred lysine for the tweezer. We also studied the interaction of another small molecule, CLR03, as a control system that does not form inclusion complexes with lysine and arginine residues. Our predictions were experimentally corroborated by circular dichroism spectroscopy and other biochemical techniques.

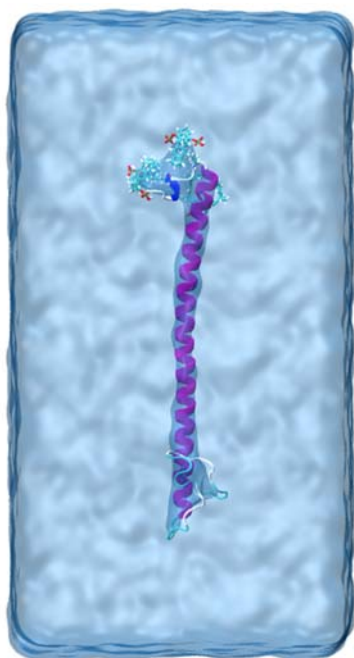

Figure. Htt model with molecular tweezers in explicit solvent

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P21 – Nitschke: Calcium phosphate nanoparticles as delivery and surface presentation system

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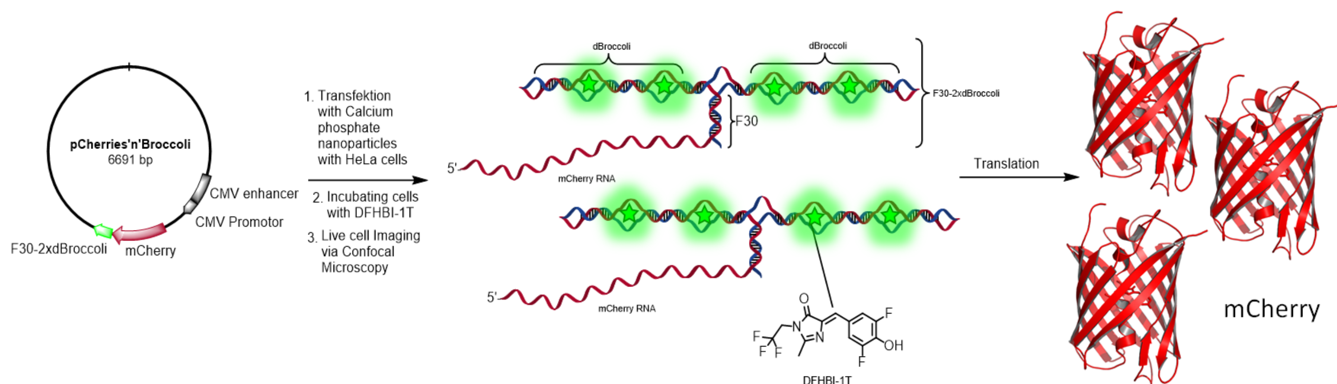
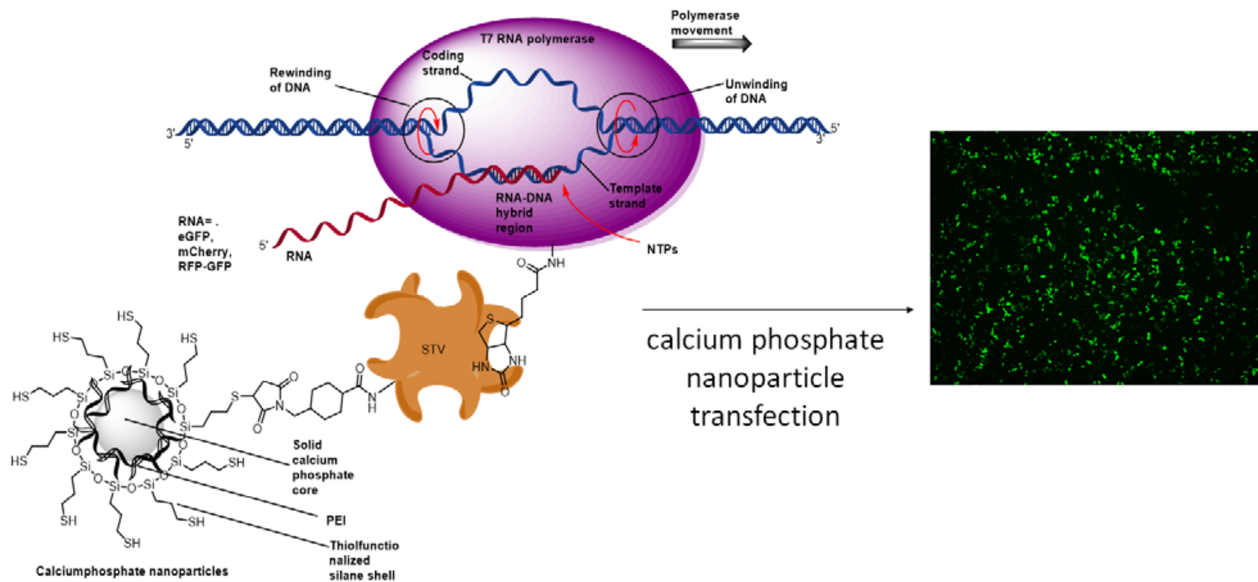
The transport of bioactive and biomedically relevant proteins across the cell membrane into cells is still a challenge in current research. Calcium phosphate nanoparticles are good carriers due to their high biocompatibility and biodegradability to transport (bio-)molecules across the cell membrane.[1] In particular, calcium phosphate nanoparticles have been used to transport cDNA of fluorescent proteins into different cell lines.[2] To elucidate the pathway from cDNA to mRNA, mRNA can be labeled and visualized with a self-folding scaffold originated from phage f29 bearing two copies of the DHFBI-1T binding RNA aptamer dBroccoli[3]. The formed G-quadruplex of the RNA aptamer around the DHFBI-1T is fluorescent. We describe some projects in which we are trying to use this RNA aptamer to visualize RNA (e.g. mRNA, RNA-Aptamers).

Project A: Transfection of mRNA with calcium phosphate nanoparticles.

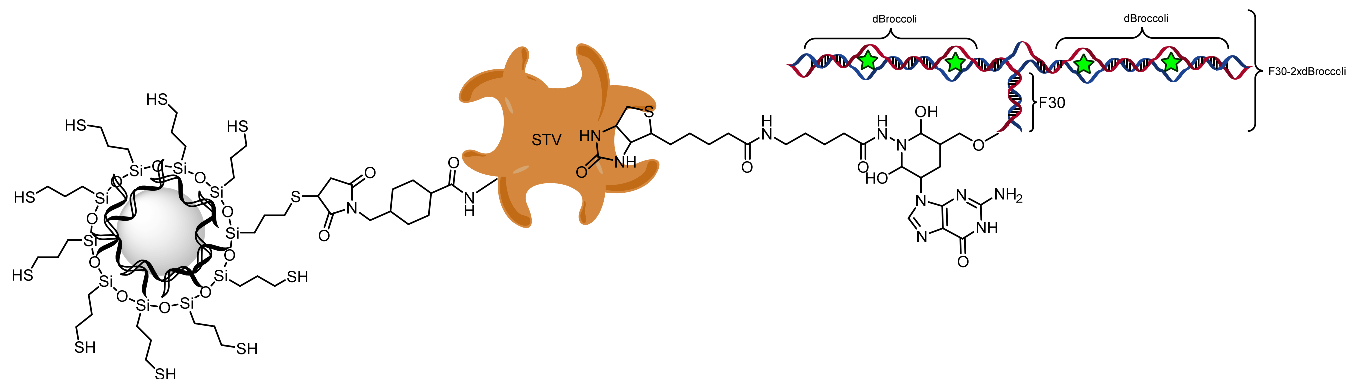


Beside of this possible approach, calcium phosphate nanoparticles have been used for the immobilization of biomolecules (e.g. avidin, antibodies) on its surface[4]. Here we describe the idea of a nanoparticle-based surface presentation system by the immobilization of the T7 RNA polymerase and the viral RNA self-folding scaffold that is bearing two copies of DHFBI-1T binding RNA aptamer dBroccoli, F30-2xdBroccoli. These approaches will help to elucidate the uptake pathway of calcium phosphate nanoparticles and give insight into the processing of mRNA that has introduced by nanoparticle transfection. Furthermore, it would answer the question whether our nanoparticles can be used as a nanoparticle based surface presentation system for enzymes and nucleic acids, serving as template display system for supramolecular drugs in future.

Project B: Immobilization of T7 RNA Polymerase.



Project C: **Immobilization of the viral RNA scaffold F30-2xdBroccoli.**



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P22 – Nojoumi: Next level Drug design - Proteins on the rise

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4) Institut für Chemie und Biochemie der Freien Universität Berlin

The novel tools of Synthetic Biology have broadened the possibilities to utilize proteins for various applications. Since antiinfectives are gaining more importance with increasing infection types, addressing these has become the main challenge to date. Chemical remedies for fighting viruses and other infectious diseases are by far not specific enough to avoid unwanted side effects. At the same time multivalent ligand–receptor binding events are known to be crucial in nature and responsible for mediating biological interactions. Therefore, here we are reporting novel antiinfectives based on biological systems as multivalent scaffold proteins to address the pathogenic influenza A virus. Viral infection occurs by the recognition and binding of sialic acid terminated cell-surface glycans on the viral membrane protein hemagglutinin (HA). The inhibition of this binding event by a suitable HA inhibitor is regarded as a very promising strategy to develop novel antiviral drugs. The Qβ virus like particle (VLP) is a self-assembling icosahedral biological structure tolerant to Temperature, pH and modification in protein sequence being a monodisperse multivalent system, in which the valency, spatial arrangement and ligand identity can be tuned as desired. The unnatural amino acid HPG bearing alkyne functionality was used as a single mutation in the sequence of the capsid protein (cp) exposed on the surface and assembled to the VLP. The modified VLPs were conjugated with sialic acid bearing azide functionality in a biorthogonal copper mediated azide-alkyne cycloaddition (CuAAC) to expose 180 sialic acid residues per VLP. Fully functionalized and intact Qβ particles, analyzed by mass spectrometry and cryo transmission electron microscopy were obtained. Hemagglutination and in vivo inhibition assays revealed the sialic acid decorated Qβ VLP variant as a highly potent virus inhibitor proven to be effective as an antiinfective validated in cryo electron microscopic measurements. Next, an alternative multivalent scaffold based on self- assembled nanotubes like bacterial flagella is investigated for pathogen inhibition.

P23 – Ohl: Modeling Copolymer-Protein Interactions

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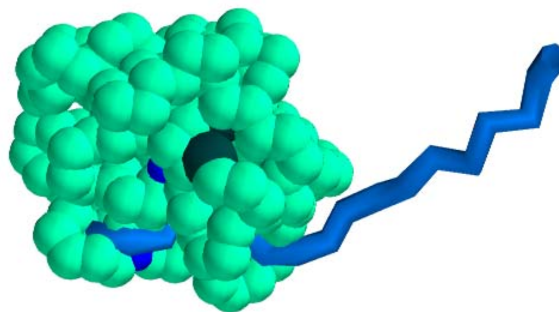
University Duisburg-Essen/ZMB/Bioinformatics

Interactions of copolymers like glycosaminoglycans and proteins in the extracellular matrix play a vital role in biological organisms and yet many aspects still remain poorly understood. Many of these complexes formed by proteins and copolymers are facilitated by a multitude of weak binding spots that lead to strong avidity which makes them distinct from the small organic molecules usually used for strong binding.

Experiments have revealed interesting binding-properties like slow on-set binding and sub-stoichiometric inhibition that hint at more complex interactions.

We have developed a coarse-grained molecular model that will help to investigate the nature of copolymer-protein interactions. Preliminary results show encouraging agreement with experimentally measured inhibitions[1], but our model still needs refinement. In the next steps we will simulate larger and more complex copolymer-protein systems which will help in the understanding of sub-stoichiometric inhibition.



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P24 – Pfeifer: Hierarchical assembly of modular and programmable polymer-like DNA materials with tailored properties

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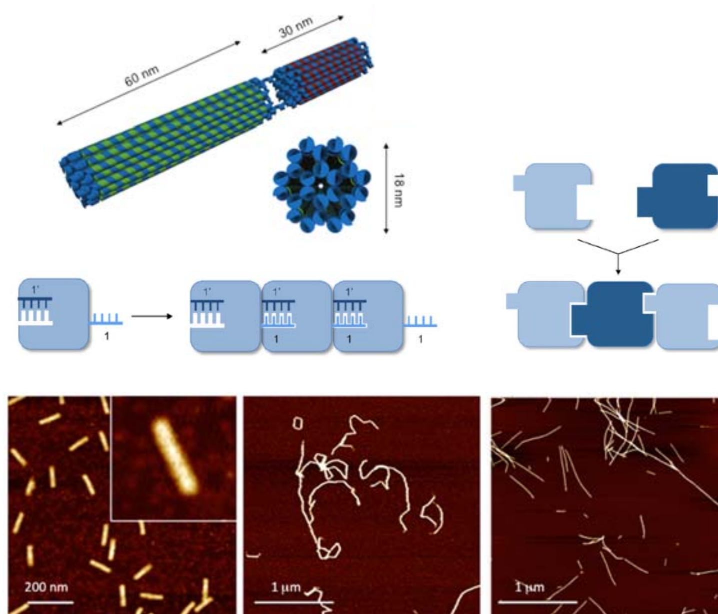
Structural DNA nanotechnology allows for the programmable construction of sophisticated nanoscale objects using Watson-Crick base-pairing rules. Recent developments, particularly in the field of DNA origami, have demonstrated that other forces, such as base stacking interactions and shape complementarity, may be advantageously exploited to drive the assembly of DNA units into hierarchical structures of micrometer dimensions, thus contributing to bridge the nano- to the macroscopic world in a predictable fashion.[1, 3] Applying strict design rules, such as staple crossover spacing, nick-positioning and staple strand length, we recently developed a robust 3D origami structure, composed of two quasi-independent domains connected by a variable module.[2] Whereas the length and flexibility of the intra-domains region can be regulated by switchable DNA motifs, the inter-domain interfaces feature self-complementary shapes, which allow for their connection into oligomeric structures of distinct periodical pattern. Thus, combining hierarchical strategies of DNA self-assembly[3] with dynamic control of structural reconfiguration, we aim at the realization of artificial polymer-like materials with tailored properties.



A 24-helix bundle composed of two quasi-independent domains connected by a variable region has been realized observing strict design rules. Combining base hybridization, stacking interactions and shape complementarity with the modularity of the intra-domains region, polymer-like filaments of variable flexibility and periodical pattern can be obtained.

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P25 – Pöhler: 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 alternative complexes with different adaptors, p97 is involved in many different cellular pathways. For example, p97 represents a central component of the ubiquitin-proteasome system and targets ubiquitinated proteins for degradation in signaling. Its role in protein homeostasis qualifies it as a potential cancer drug target.

The underlying mechanisms governing the functions of p97 are still poorly understood. Hence, 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 called I8. In vitro assays showed that I8 inhibits p97 in the micromolar range by an allosteric mechanism. In addition, cell based 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 TNFα-induced degradation of IκBα in HeLa cells is delayed by treatment with I8.

P26 – Rey: Allosteric regulation of HTRA1 activity and its implications in MAPK signaling

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Serine proteases of the high temperature requirement A (HtrA) family are involved in protein quality control and cellular stress signaling (1). The human family member HTRA1 was described as a tumour suppressor (2) and was shown to be downregulated in various types of cancer. In contrast to classic serine proteases, HTRA activity can be reversibly switched on and off involving allosteric mechanisms. For HTRA1 the C terminal PDZ domain was so far thought to be redundant for activity regulation and substrate processing.

Prior to this work a large-scale peptide screen was performed in cooperation with the HU Berlin to identify new potential HTRA1 interactors. In this screen the C termini of all human proteins were tested for binding to HTRA1. Various binding peptides were chosen afterwards to be analysed for their potential to activate this enzyme. Furthermore RAS-related and RAF proteins were identified as candidate ligands, implicating HTRA1 in MAPK signalling. This pathway, which involves the GTPase RAS and subsequent signalling via a phosphorylation cascade through RAF, MEK and ERK, is important for regulation of cell proliferation, migration and survival.

Here we investigate the role of HTRA1 on MAPK pathway members and the allosteric regulation of HTRA1 via peptidic PDZ ligands. To address these questions various biochemical approaches such as ITC, activity assays and crosslinking experiments are performed.

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P27 – von Sass: Production of a multivalent (oNB)DOPA-containing flagella forest

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Bacterial flagella are shown to be a promising bio-template to obtain a wide three-dimensional surface and a highly variable and flexible nano scaffold.[1] The bacterial flagellum macromolecule should act as flexible carrier bearing different chemical functionalities. A single flagellum filament is composed by tens of thousands of flagellin monomers and growth by self-assembly.[2]

The non-canonical amino acid dihydroxyphenylalanine (DOPA) is found in mussel proteins where the DOPA side chains are responsible for the sticky behavior of the mussels even in aqueous solutions.[3] These properties are of a huge interest for industrial and medical applications. By the incorporation of DOPA on exposed positions of the flagellin, a highly functionalized flagellum can be created. The side specific incorporation of DOPA will occur via stop codon suppression at in frame stop codon positions in the flagellin gene. Therefore our working group recently found an orthogonal aminoacyl-tRNA-synthetase/tRNA-pair which is capable to activate this non-canonical amino acid of interest. By using DOPA variants with UV-cleavable protecting groups, the sticky behavior can be switched on controlled via light radiation.

Additionally the incorporation of homopropargylglycine (HPG) via selective pressure incorporation (SPI) method at the N-terminus of the protein allows the immobilization of the flagellin on an azide-containing surface via click chemistry.[4] Summarized, the *in vitro* synthesis of a flagella forest as a polyfunctional scaffold for different applications and investigations shall be realized and established.

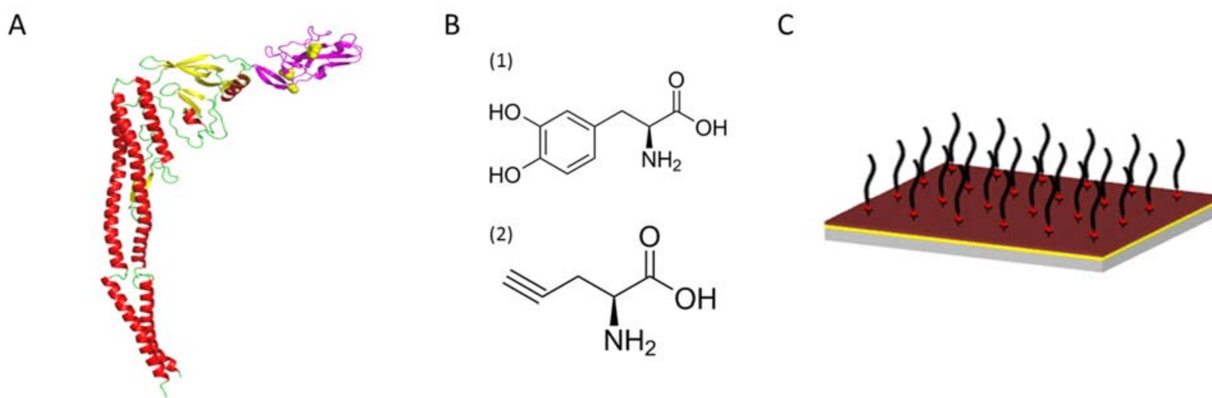

Figure 1: Crystal structure of a flagellin monomer. B) Non-canonical amino acids, which shall be introduced into the flagellin monomer: (1) dihydrooxyphenylalanine (DOPA), (2) homopropargylglycine (HPG). C) Schematic representation of an immobilized flagella forest.[1]

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P28 – Scheer: Survivin Expression Pattern in the Intestine of Normoxic and Ischemic Rats

Alexandra Scheer, Rabea Verhaegh, Shirley Knauer, Herbert de Groot*

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Survivin acts as an apoptosis-inhibiting protein. Besides, it regulates mitosis and chromosome segregation. The expression of survivin proceeds during the embryonic phase. In the small intestine and colon of adult rats survivin expression has already been shown[1]. The very weak expression of survivin is mentioned only as a side note without detailed information[1]. A detailed evaluation of survivin expression in the individual sections of the small intestine or within the individual cell layer/cell types is missing. In order to determine the expression pattern of survivin in the small intestine, we used an immunhistochemical approach in normal adult small intestinal tissue and also investigated its expression in mesenteric ischemia-reperfusion (I/R) injury. Mesenteric ischemia-reperfusion injury was induced in male Wistar rats by occlusion of the superior mesenteric artery for 90 min and subsequent reperfusion for 120 min. Paraffin sections of untreated or ischemically treated tissue were assessed immunhistochemically by survivin and Ki-67 staining. Survivin could be detected in the small intestine and colon of the normoxia group. It was expressed mainly in the epithelial cells of the crypts and only slightly in the villi. Likewise, the expression of survivin was detected in the ischemically damaged small intestine and ascending colon. The expression pattern corresponded to the normoxic animals, as far as verifiable due to the existing tissue damage. Its expression was located in cells with a high proliferation rate and regenerative capacity. This further supports the decisive role of survivin in cell division. Surprisingly, the ischemically damaged small intestinal and colonic tissue showed a comparably high expression level. These results suggest that there is already a maximal survivin expression under normal conditions. However, the intestine is able to maintain the regenerative capacity even in spite of an ischemic injury.

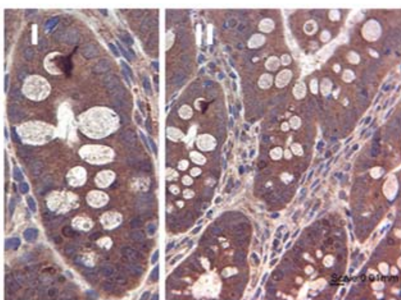

Figure 1: Survivin staining within the crypts of intestinal segment III in the normoxia group (representative figure). *Survivin* positive cells are immunohistochemically labeled in brown. I: single crypt; II: small intestine (*jejunum*). Nuclei are stained blue by counterstaining with hematoxylin. Magnification: 400x.

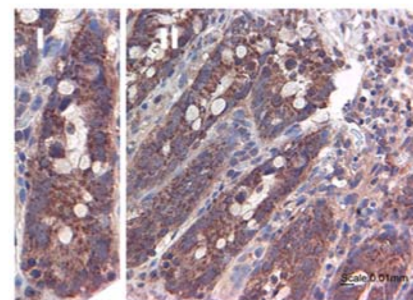

Figure 2: Survivin staining within the crypts of intestinal segment III in the ischemia group (representative figure). Survivin positive cells are immunohistochemically labeled in brown. I: single crypt; II: small intestine (*jejunum*). Nuclei are stained blue by counterstaining with hematoxylin. Magnification: 400x.

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* Deceased 10.05.2016

P29 – Schöneweiß: Reversible reconfiguration of DNA origami nanochambers

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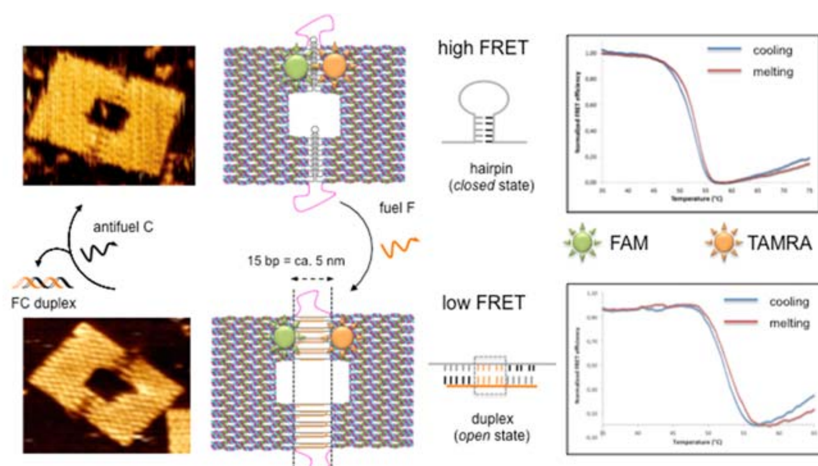
Through the physical separation of biological reactions into specialized compartments, nature achieves control over matter distribution, both in space and time.[1] In the past few years, many brilliant examples of DNA-based nanocages have been reported for the spatial confinement of molecular cargos.[2-3] 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 and reversible transformations in response to external events. 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 a single protein copy and the effect of DNA caging on the enzymatic activity. 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. The energetics associated to device functioning is currently investigated by thermal-dependent FRET spectroscopy.



Structural reconfiguration of 2D DNA origami compartments is achieved through insertion of switchable DNA motifs in the central seam. The energetics of the device can be investigated by thermal-dependent FRET spectroscopy.

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P30 – Schulz: Synthesis of selected peptides and method development for absolute quantification of proteins in biological matrices by high resolution mass spectrometry

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In the analysis of biological systems, it is of interest to identify the components of the system and to monitor their changes in abundance under different conditions. The AQUA (for 'absolute quantification') method allows sensitive and specific targeted quantification of protein and posttranslational modifications in complex protein mixtures using stable isotope–labeled peptides as internal standards. Each AQUA experiment is composed of two stages: method development and application to a biological scenario. In the method development stage, peptides from the protein of interest are chosen and then synthesized with stable isotopes such as 13C, 2H or 15N. The abundance of these internal standards and their endogenous counterparts can be measured by mass spectrometry with selected reaction monitoring or selected ion monitoring methods. Once an AQUA method is established, it can be rapidly applied to a wide range of biological samples, from tissue culture cells to human plasma and tissue. Here we selected and synthesized AQUA peptides to quantify the human serine protease HTRA1 in blood serums. Preliminary calibration experiments reveal for single pure AQUA peptides a linearity down to the lower amol range. This work has a strong focus on developing mass spectrometry methods for quantification on MS1 level on Orbitrap instruments. It is desired to spread the scope of the AQUA method on additional proteins of interest to obtain a valuable tool for our proteomic toolbox.

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P31 – Smolin: Tailor-made copolymers as selective enzyme inhibitors

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A major complication in pancreatitis, ischemia and cardiac surgery is the enzymatic self-digestion of the gastrointestinal walls. Most digestive enzymes are serine proteases and can hence be generally blocked by unspecific inhibitors such as aprotinin. However, stoichiometric amounts are required and other enzymes may be also affected (e.g. in blood clotting).

Our motivation is the development of selective serine protease inhibitors based on a polymeric backbone. In recent years we have developed a library of amino acid-selective monomers which can be subjected to free radical copolymerization and yield linear multivalent copolymers [1-3]. These affinity polymers recognize the surface of a given enzyme and in some cases work in a highly substoichiometric manner. The mechanism of efficient enzyme shut down operates either by steric blocking of the active site or by irreversible enzyme denaturation.

In order to confine polydispersity and to explore the lower limits of polymer size for efficient inhibition, we now pursue the controlled synthesis of multivalent copolymers via ARGET ATRP and metal free ATRP protocols. Here we present our new polymeric materials and their application in trypsin inhibition.

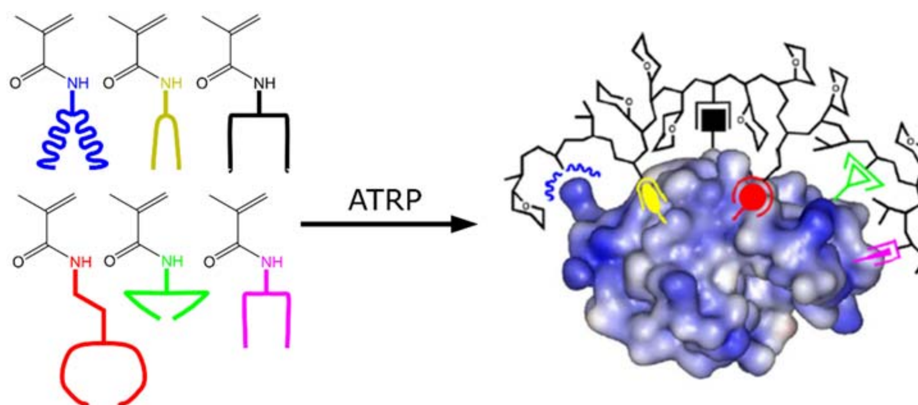

Figure 1. Amino acid-specific binding monomers are copolymerized via ARGET ATRP or metal free ATRP. The resulting multivalent linear affinity polymer binds to a complementary protein surface which leads to enzyme-specific shut down.

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P32 – Sowislok: Synthesis of Non-symmetric, Clickable Diphosphate Tweezers for Regioselective Protein Surface Recognition

A. Sowislok, T. Schrader*

Prof. Dr. Thomas Schrader, Universität Duisburg-Essen, Universitätsstr. 7, 45117 Essen

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. The introduction of the phosphate moiety containing the alkyne unit follows a classic phosphoramidite approach.

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.

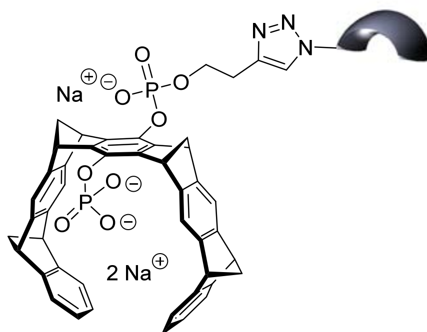

Figure 1. Molecular diphosphate tweezer for the binding of lysine and arginine with attached peptide ligand unit.

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P33 – Tötsch: Carbenes as Reactive Intermediates: Computational Methods for S-T gaps and Intersystem Crossings

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The reactivities of carbenes are multiplicity-dependent. Thus, it is crucial to predict whether the ground state is a singlet or triplet. 38 density functionals are benchmarked for a test set of carbenes with a focus on predicting the S-T gap $\Delta E_{S\text{-}T}$. Special interest lies in double hybrids (DHYBs) as these have been very successfully applied to other systems.

Some computationally inexpensive functionals lead to reasonable results although the bulk of them does not yield the desired accuracy. The more sophisticated DHYBs perform more consistently. Furthermore, the benchmark covers different types and sizes of basis sets. Of those tested, aug-cc-pVTZ yields the most accurate results.

Moreover, we are interested in how triplet carbenes convert to singlets. Costa et al. have demonstrated conversion of triplet bis(p-methoxyphenyl)carbene (DMeOPC) upon annealing (>10 K).[1] For diphenylcarbene (DPC), this is not the case. We aim to

1. Develop and implement a novel approach to determine intersystem crossings based on molecular dynamics simulations and additional spin-orbit coupling calculations. We are particularly interested in studying solvated carbenes.
2. Use our approach to provide insights into the conversion mechanism of DMeOPC and discover why it does not apply to DPC. Once the governing factors are understood and the molecular flexibility required for a transition is known, solvent and temperature can be selected to enable or disable conversion.

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P34 – Vallet: 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 established 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.

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P35 – Walstein: Reconstitution of polynucleosome arrays as a template for *in vitro* CENP-A loading studies

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In the eukaryotic cell cycle the DNA of a cell is first duplicated and subsequently equally parted to the nascent daughter cells. During mitosis, the sister chromatids in each pair are attached to opposite spindle poles. These dynamic attachments to spindle microtubules are mediated by kinetochores. Centromeres are the chromosomal loci at which kinetochores assemble. The centromere region is epigenetically defined by an enrichment of the histone H3 variant centromere protein A (CENP-A)[1]. In contrast to other histones, CENP-A is not replenished during S-phase but in early G1-phase. CENP-A replenishment is accomplished by an evolutionarily conserved loading machinery[2]. To understand the molecular mechanism of CENP-A loading on centromere chromatin, we generated polynucleosome arrays that can be used as a template for *in vitro* CENP-A loading studies in a cell-free environment. For the reconstitution of polynucleosomes we assembled purified histones on linearized plasmid DNA harbouring 20 tandem repeats of a nucleosome assembly sequence. We further included 6 repeats of a symmetric lac operator sequence within the non-repetitive part of the plasmid DNA to couple the chromatin arrays to lac repressor coated magnetic beads. Furthermore, using the SpyTag reaction we purified full-length centromere protein C (CENP-C), which has been reported to recruit the CENP-A loading machinery to the centromere[3] and therefore is a crucial component for future *in vitro* CENP-A loading studies.

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P36 – Yilmaz: 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. They are included in numerous cell biological processes like signal transduction, apoptosis or cell cycle regulation. Consequently, they are also involved in the development of diseases that are triggered for example by a malfunction of the respective protein. Therefore it is an attractive aim to generate inhibitors that are able to bind specifically to 14-3-3 proteins to prevent diseases. The idea is to synthesize bivalent inhibitors to increase the effect of inhibition. Virtual screening approaches have already identified phosphophenyl ethers as a novel class of 14-3-3 inhibitors. To prove the efficiency of bivalent inhibitors the phosphophenyl ethers are connected to each other with a linker. Due to their high polarity it is advisable to use PEG molecules as linkers, as they can increase the solubility of the inhibitor in aqueous media.

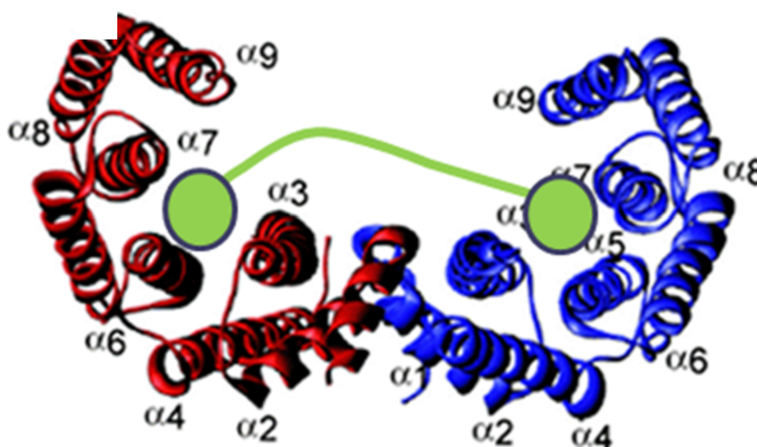

Figure 1: 14-3-3 protein with a bivalent inhibitor (green).

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P37 – Zakeri: Label-free Raman spectroscopy of a new generation of unsymmetrical molecular tweezers

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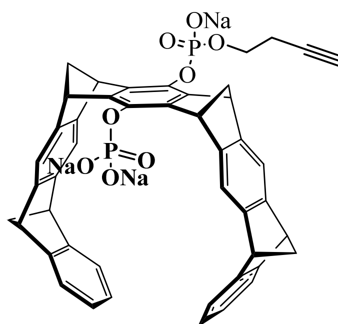
Quite often protein function and signaling is localized on hot spots which involve only a few amino acids or sequences of amino acids. Therefore, artificial binders specific for single amino acids, represent a promising tool for protein targeting with potential applications in mechanistic elucidation, diagnostics and disease-modifying therapy.

Molecular tweezers for basic amino acid chains, have a high potential to interfere with biologically relevant peptides or proteins in which lysine or arginine residues play crucial roles. Very recently, unsymmetrical tweezers carrying additional recognition sites were introduced recently for evaluating the effects of their individual linker groups in complex formation. The binding properties were characterized by techniques such as NMR spectroscopy, ITC, and molecular modeling [1].

Laser Raman spectroscopy is a promising optical technique for label-free monitoring of peptide recognition of supramolecular ligands with peptide and proteins targets in aqueous solution under physiologically relevant conditions. Advantages of this approach are high molecular specificity and selectivity, in particular when combined with resonant excitation (resonance Raman scattering) [2].

Here we present the characterization of an unsymmetrical molecular tweezer (Figure) and its binding to peptide sequences by label-free Raman spectroscopy.

Financial support from the German Research Foundation is gratefully acknowledged.



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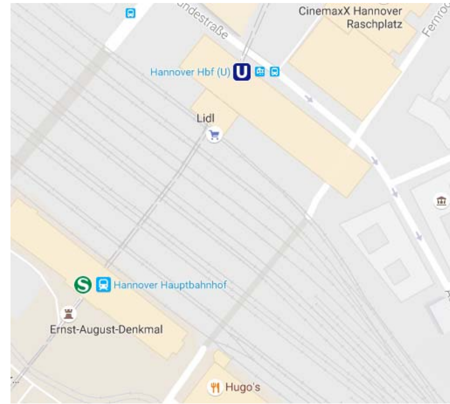
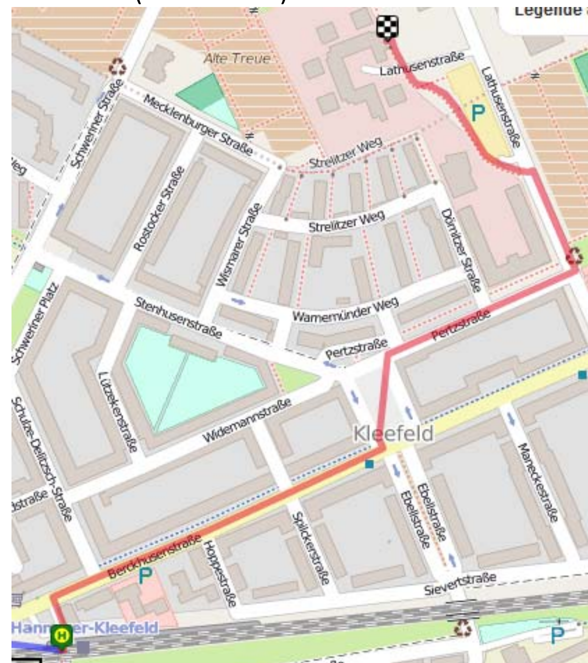


Option 1: S-Bahn (20 min, fastest connection)

Transport	Station/Direction	Platform
10:04	Hannover main train station	14
S 7 (dur. 3 min)	**Direction**: Celle Next alternative “S-Bahn” (train) every 3-4 minutes: S 3 at 10:34, S 7 at 11:04, S 3 at 11:34, S 7 at 12:04, S 3 at 12:34 and so on (a.s.o.)	
10:07	Hannover-Kleefeld	1
On foot (dur. 17 min) **A**	**Distance**: 0.7 km	
10:24	Hannover Lathusenstraße 15 (GHotel)	

A On foot (dur. 17 min)

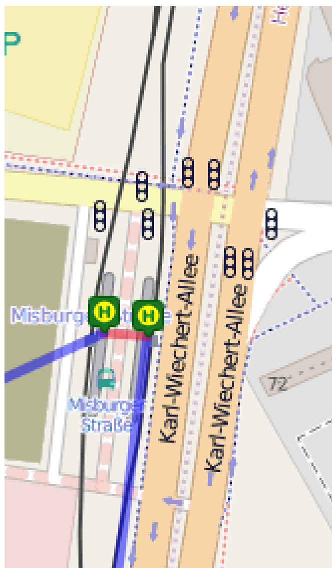


Option 2: Tram & Bus (29 min)

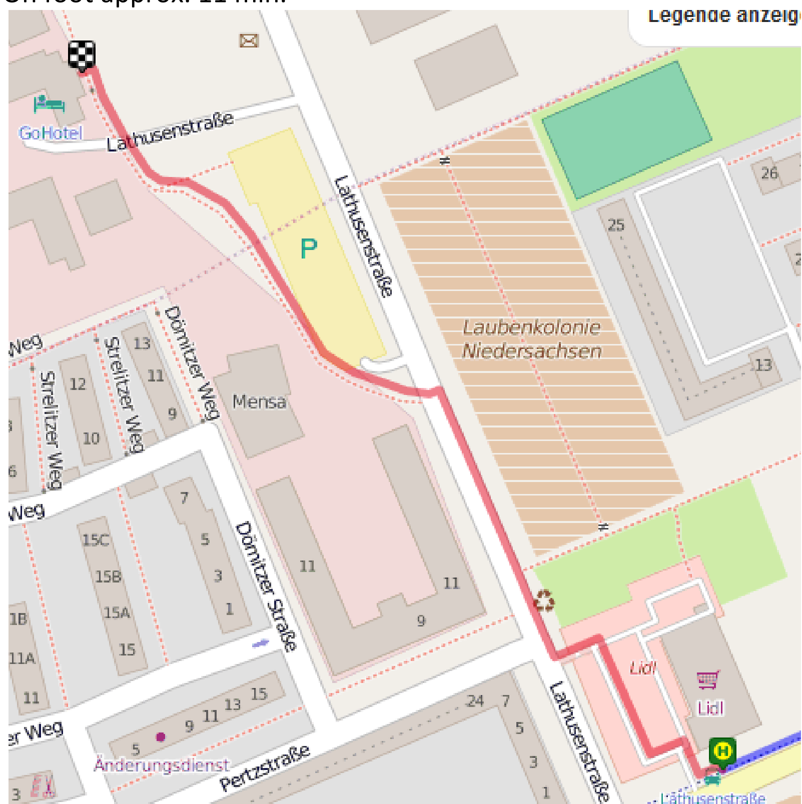
From “Hannover Hauptbahnhof“(underground station)

Transport	Station/Direction
10:05	Hannover main train station (U)
U Stb 1 (dur. 3 min)	**Direction**: Sarstedt (Endpunkt GVH) Next alternative Stb every 3-4 min: Stb 2 at 10:09, Stb 8 at 10:12, Stb 1 at 10:15, Stb 2 at 10:19, Stb 8 at 10:22 a.s.o.
10:08	Hannover Aegidientorplatz (U) Change to Stb 4
10:08	Hannover Aegidientorplatz (U)
Stb 4 (dur. 12 min)	**Direction**: Roderbruch next alternative Stb every 10 min: Stb 4 at 10:18, Stb 4 at 10:28, Stb 4 at 10:38, Stb 4 at 10:48, Stb 4 at 10:58 a.s.o.
10:20	Hannover Misburger Straße
On foot (dur. 1 min) **A**	Right across the street change to bus line 127
10:21	Hannover Misburger Straße
Bus 127 (dur. 2)	**Direction**: Pferdeturm next alternative Bus every 10 min: Bus 137 at 10:31, Bus 127 at 10:41, Bus 137 at 10:51, Bus 127 at 11:01, Bus 137 at 11:11
10:23	Hannover Lathusenstraße
On foot (dur. 11 min)	**Distance**: approx. 0.4 km
10:34	Hannover Lathusenstraße 15 (GHotel)

A On foot approx. 1 min.



B On foot approx. 11 min.



Option 3: Tram & Bus (30 min)

From "Hannover Hauptbahnhof" (underground station)

Transport	Station/Direction
10:09	Hannover main train station (U)
Stb 2 (dur. 3 min)	**Direction**: Döhren/Bf Next alternative every 3-4 minutes: Stb 2 at 10:12, Stb 1 at 10:15, Stb 2 at 10:19 Stb 8 at 10:22, Stb 1 at 10:25 a.s.o.
10:12	Hannover Aegidientorplatz (U) Change to Stb 5
10:12	Hannover Aegidientorplatz (U)
Stb 5 (dur. 7 min)	**Direction**: Anderten Linie der üstra - Hannoversche Verkehrsbetriebe AG, Info: 0511 1668-0 next alternative every 4-6 min) : Stb 4 at 10:18, Stb 5 at 10:22, Stb 4 at 10:28, Stb 5 at 10:32, Stb 4 at 10:38 a.s.o.
10:19	Hannover Uhlhornstraße
On foot (dur. 20 min) A	**Distance**: approx. 0.9 km
10:39	Hannover Lathusenstraße 15 (GHotel)

On foot (dur. 20 min) A

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