

**Dynamic Host–Guest Complexes as Key Elements of
Supramolecular Sensors, Transporters,
and Stimuli-Responsive Materials**

by

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by Dr. Andreas Hennig

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*“Technology always develops from the primitive
to the complicated and then to the simple”*

(Antoine de Saint-Exupéry)

Statutory Declaration

I, **DR. ANDREAS HENNIG**, HEREBY DECLARE THAT I HAVE WRITTEN THIS THESIS INDEPENDENTLY, UNLESS WHERE CLEARLY STATED OTHERWISE. I HAVE ONLY USED THE SOURCES, THE DATA, AND THE SUPPORT THAT I HAVE CLEARLY MENTIONED. THIS THESIS HAS NOT BEEN SUBMITTED ELSEWHERE AND NO APPLICATIONS FOR A HABILITATION HAS BEEN MADE PREVIOUSLY ELSEWHERE.

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Preface

Driven by an early interest in “molecules, which can do something”, I started my scientific career with the synthesis of fluorescent molecules with peculiar photophysical properties (e.g. extremely long fluorescence lifetimes of up to 1 μ s) and their application in enzyme assays.ⁱ The same class of fluorescent molecules was at the same time used by the research group to investigate supramolecular recognition principles, which inspired us to develop the first fluorescent, label-free enzyme assay using water-soluble macrocyclic host molecules.ⁱⁱ With the goal to use supramolecular chemistry to address fundamental questions in biochemical research, I subsequently joined the only other research group worldwide, which had used supramolecular recognition to monitor enzymatic reactions. Besides following the line of supramolecular enzyme assays,ⁱⁱⁱ I soon became interested in how to control transport of molecules across lipid bilayer membranes,^{iv} and how to follow membrane transport processes.^v

Having developed analytical methods from the viewpoint of fundamental research in a physical-organic and a bioorganic chemistry lab, I subsequently joined the Department of Analytical Chemistry at the BAM Federal Institute for Materials Research and Testing with the goal to broaden my knowledge in analytical chemistry applications. The major focus was to compare analytical methods for the quantification of surface functional groups, which included – as a first piece of independent research – also the development of novel, innovative surface quantification methods based on supramolecular chemistry.^{vi} In addition, I was given the opportunity to develop enzyme-activatable probes for molecular optical and magnetic resonance imaging applications.^{vii}

After this round trip to physical-organic, bioorganic, and analytical chemistry, including a short excursion into quality management, I have returned to Jacobs University, where I previously obtained my PhD, with the goal to pursue my habilitation. During the last years, we have intensified our efforts to understand and apply the supramolecular recognition principles of host-guest complexes in more and more complex and competitive environments such as in biofluids, at biomembrane interfaces and on particle surfaces. This cumulative habilitation thesis introduces and summarizes the scientific work of the habilitation and serves as a framework for the already published papers. It also contains some unpublished results and an outlook.

ⁱ *ChemBioChem* **2006**, 7, 733; *J. Am. Chem. Soc.* **2007**, 129, 15927.

ⁱⁱ *Nat. Methods* **2007**, 4, 629; *J. Am. Chem. Soc.* **2009**, 131, 11558; *Chem. Eur. J.* **2012**, 18, 3444.

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^v *Chirality* **2008**, 20, 932; *Org. Biomol. Chem.* **2009**, 7, 1784.

^{vi} *Anal. Chem.* **2011**, 83, 4970; *Chem. Commun.* **2011**, 47, 7842; *J. Am. Chem. Soc.* **2012**, 134, 8268; *J. Phys. Chem. C* **2014**, 118, 20393; *Analyst* **2015**, 140, 1804.

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Introduction

After the serendipitous discovery of the crown ethers in 1960, the synthesis, design, and physicochemical investigation of host-guest complexes has largely driven the development of the field of supramolecular chemistry.¹⁻³ This has led to a more thorough understanding of the weak, noncovalent forces that govern supramolecular host-guest interactions, which are also responsible for the selective association of other molecules. Electrostatic interactions, hydrogen bonds, the hydrophobic effect, and van der Waals interactions also play key roles in the highly specific molecular recognition processes that occur in biology, and they largely control the properties of polymers, liquid crystals, surfactants, and other materials. Supramolecular chemistry thus naturally intersected with other research areas for decades and it is nowadays a highly interdisciplinary and largely diversified field involving biologists, environmental scientists, engineers, physicists, mathematicians, and others.⁴

Although the complexity and functionality of biological systems has always been a major source of inspiration for supramolecular chemists, current applications of supramolecular chemistry are mainly in the area of materials sciences.⁵ Materials are in the solid state and they are often processed with organic solvents, whereas biological applications require water, which represents a more challenging environment for supramolecular recognition.⁶⁻⁸ Several physicochemical characteristics of water are not well understood, and the reason why two molecules associate more or less strongly in water is much more complex than in organic solvents. Even less is known about aqueous interfaces such as the water/biomembrane or solid-state interface.⁹ For example, it has only recently been reported that the Na⁺ ion concentration near the surface of anionic micelles was up to 160times higher than that in bulk water.¹⁰ It is thus a persistent challenge to apply supramolecular systems, in particular artificial host molecules, in highly competitive environments such as bulk water, aqueous interfaces, or even inside cells.⁷ This is, however, a particularly fruitful exercise, because the combination of supramolecular systems with biology will ultimately allow us to better understand and control major processes in nature, and thus contribute to medicine or address environmental issues.^{6,11}

Within this habilitation thesis, I am going to summarize my efforts to explore, understand, and control supramolecular host-guest systems, which are not only functional in complex heterogeneous (micro)environments, but which also have high potential to pave the way for genuinely useful applications. In the following, I will briefly introduce the supramolecular structures and principles that we relied on, and will then describe in the subsequent chapters our efforts to dynamically follow concentration changes of bioanalytes, to transport peptides, and to characterize the surface of micro- and nanoparticles with supramolecular host-guest systems.

Supramolecular Structures and Principles

The research described in this thesis focused on water-soluble, supramolecular, macrocyclic host molecules and their interaction with various guest molecules. Among the most well-known water-soluble host molecules are cyclodextrins,¹²⁻¹⁴ substituted calixarenes,^{15,16} and cucurbiturils (Figure 1).¹⁷⁻¹⁹ These molecules are characterized by a hydrophilic surface and a hydrophobic cavity into which guest molecules can bind. The apparent similarity to protein-ligand complexes prompted their use as models for studies in molecular recognition. Various techniques such as nuclear magnetic resonance (NMR) spectroscopy, isothermal titration calorimetry (ITC), absorption, circular dichroism (CD), as well as steady-state and time-resolved fluorescence spectroscopy have been applied to elucidate the dependence of thermodynamic parameters (binding constant, enthalpy, entropy) and kinetic parameters on size, charge, and spatial arrangement of host and guest. These studies are fundamental for the rational choice of host and guest in the development of advanced applications of supramolecular host-guest systems.

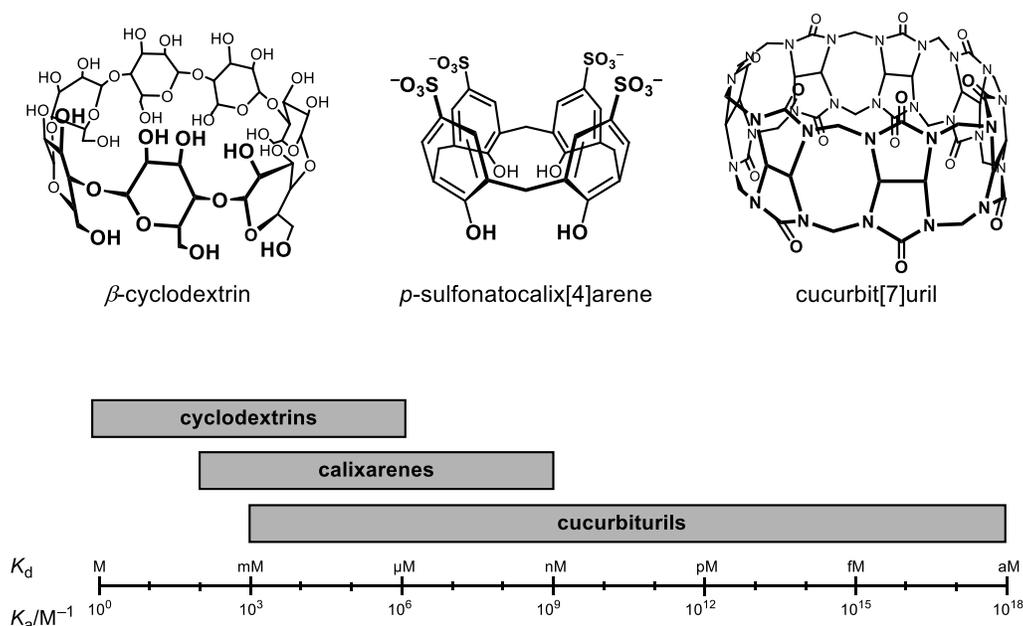


Figure 1. Prototypical examples of water-soluble macrocyclic host molecules and range of binding constants reported for the different classes.

Cyclodextrins are among the most studied water-soluble hosts, but their binding constants are generally low. Mono-, di- and persubstitution at the C2, C3, and C6 hydroxy groups of cyclodextrins with permanently charged cations and anions has been shown to significantly

increase binding affinities in water.²⁰ A notable example for a cyclodextrin host is sugammadex, a γ -cyclodextrin persubstituted at C6 with negatively charged carboxylic acid groups, which has been tailored to bind positively charged neuromuscular blocking agents in order to reverse their anesthetic effects.^{21,22} However, the affinity does not exceed 10^6 M^{-1} .

Calixarenes, substituted with ionizable groups, comprise another class of water-soluble macrocyclic hosts, which have been studied as receptors for biologically relevant analytes, as phase transfer catalysts, and as sensors for cations and anions.^{15,16} Interesting differences in the ability to bind guest molecules were found between calixarenes and cyclodextrins, such that both classes complement each other with respect to applications. Utilizing ion-ion interactions between persubstituted calixarenes and highly charged guests, affinities of the respective host-guest complexes can exceed 10^9 M^{-1} in selected cases.²³⁻²⁵

The newest class of water-soluble macrocyclic hosts are cucurbit[n]urils (CBn) composed of n glycoluril units.¹⁷⁻¹⁹ An outstanding feature of CBs is their propensity to bind hydrophobic and cationic species with unprecedentedly high binding constants exceeding even those of the streptavidin-biotin system ($>10^{15} \text{ M}^{-1}$).²⁶⁻²⁹ Recent results suggest that this is mainly due to high-energy water in the inner cavity, which is frustrated due to the lack of the possibility to form a sufficient number of hydrogen bonds to other water molecules.³⁰⁻³³ The main driving force is thus the energy gained from the release of this water upon guest binding. The binding is further reinforced by ion-dipole interactions with their carbonyl-fringed portal. CBs are as versatile as cyclodextrins and calixarenes with respect to applications, including, e.g., fluorescent sensors,³⁴⁻³⁶ peptide recognition,³⁷⁻⁴⁰ and drug delivery systems.^{41,42}

Despite their apparent similarity to proteins, macrocyclic hosts show interesting differences in their molecular recognition properties compared to, e.g., antibody-antigen binding. This includes their commonly much faster exchange kinetics compared to proteins, which is an important aspect for real-time monitoring, in which the sensor response needs to be faster than the changes in the concentration of the analyte.⁴³ Furthermore, supramolecular hosts are generally less selective than antibodies. Although this may appear at first sight as a disadvantage, a relative selectivity among various different analytes in conjunction with a thoughtful experimental design often suffices.⁴⁴ For example, a promiscuous binding of supramolecular hosts allows their use in various related applications with respect to closely related analytes without the necessity to design and synthesize a new host for each analyte. Another advantage of synthetic hosts is the principal possibility for large-scale production. This is well established for cyclodextrins, which are available on a multigram- to ton-scale at fair prices, and there is no apparent constraints for the large-scale production of calixarenes and cucurbiturils, although the synthesis of substituted cucurbiturils still presents a major challenge.

Biosensing

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The molecular recognition principles of supramolecular macrocyclic host molecules can be advantageously used for the design of new or refined biosensors and bioassays and thus to determine the presence, concentration, or functional activity of a (bio)analyte.⁴⁵⁻⁴⁸ Compared to established assays and sensors, which most often rely on biomolecular recognition principles, synthetic receptors offer several advantages. This includes, for example, the possibility to synthesize host molecules at competitive prices compared to, e.g., antibodies, or the possibility to target low molecular-weight analytes.

The most established method to convert a supramolecular host molecule into a sensor applies the indicator displacement assay (IDA) principle (Figure 2).^{49,50} Therein, a guest molecule (yellow), which changes its spectroscopic signal output upon host-guest complexation (red), is used as a reporter to form a self-assembled sensor system, which restores the spectroscopic response upon addition of a competing analyte (green). Spectroscopic methods, that have been applied within the framework of IDAs include, e.g., absorption, fluorescence, and CD spectroscopy spectroscopy, NMR spectroscopy, electrochemical methods, electron spin resonance (ESR) spectroscopy, and mass spectrometry (MS).⁴⁶ Alternative to IDAs, which were established more recently, include associative binding assays (ABAs), in which the heteroternary complex formation between a reporter, an analyte, and a sufficiently large macrocyclic host molecule are used, as well as hosts covalently modified with reporter dyes.⁵¹

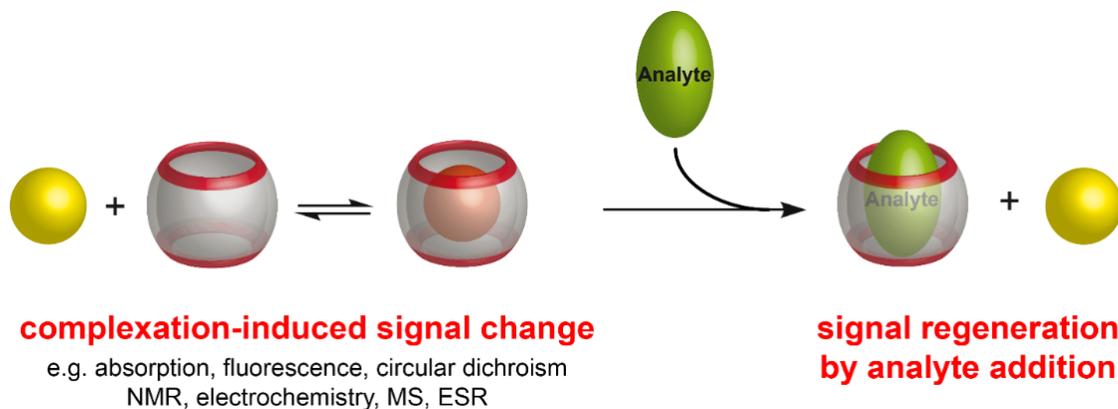


Figure 2. Indicator displacement assay (IDA) principle. Host-guest complexation of a spectroscopically active guest molecule affords a supramolecular reporter pair. Addition of a more strongly binding analyte displaces the guest leading to a spectroscopic response.

A particular advancement of the IDA principle are supramolecular tandem assays, in which the analyte is either generated or consumed by a chemical reaction.⁵² This allows following the concentration changes of the analyte in real time and was first demonstrated with enzymatically catalyzed reactions by me within my PhD studies.^{43,53} Efforts in the design and application of supramolecular tandem enzyme assays were recently reviewed including the work that was subsequently continued by Nau and co-workers.^{54,55}

One limitation for IDAs as well as for tandem enzyme assays is the promiscuous binding of supramolecular host molecules, which renders the detection of an analyte difficult when other interfering binders are present. However, this potential shortcoming can often be overcome by consciously controlling the experimental conditions as well as by a careful choice of reporter guest molecules. For example, in the seminal contribution on tandem enzyme assays for amino acid decarboxylases, a sodium-containing buffer needed to be avoided, because the presence of sodium cations severely reduced the fluorescence response of the host-guest reporter pair composed of CB7 and the dapoxyl dye.⁵³

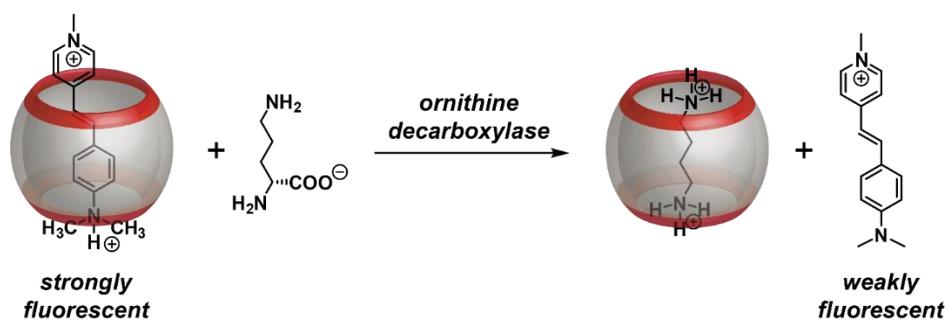


Figure 3. Refined fluorescence-based supramolecular tandem enzyme assay for ornithine decarboxylase (ODC) using CB6 and *trans*-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (DSMI) as reporter pair. Taken from ref. ⁵⁶.

A significantly enhanced version of the ornithine decarboxylase (ODC) assay was developed during this habilitation by using CB6 and *trans*-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (DSMI) as reporter pair (Figure 3).^{56,57} The CB6/DSMI reporter pair is not only insensitive towards the presence of sodium cations, but also affords a larger binding affinity, which allows to reduce the concentration of the reporter pair significantly. Since putrescine, the product of ornithine decarboxylation, has a very high affinity to CB6, a very sensitive ODC assay could be developed enabling the detection of <5 pmol putrescine in a 384-well microplate format. This was the second lowest limit of detection reported and was only excelled by an LC/MS-based method, which is much more time-consuming and thus not compatible with a high-throughput screening (HTS) of enzyme activity as it would be desired for the pharmaceutical industry. Noteworthy, also the determination of the inhibition modes of enzyme inhibitors, such as suicide inhibition, could be demonstrated for the first time with this supramolecular tandem enzyme assay.

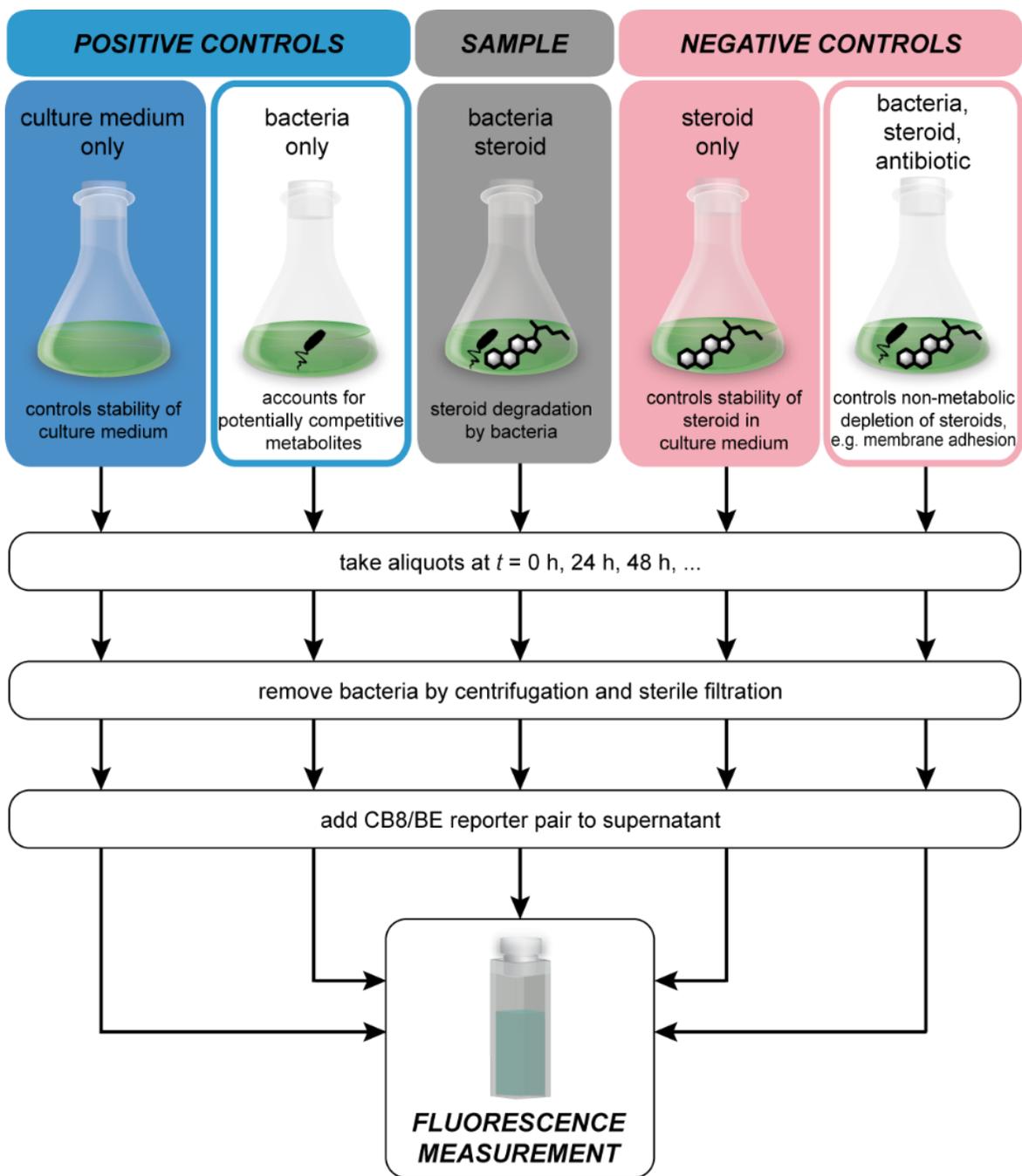


Figure 4. Screening of steroid-degrading bacteria by a supramolecular tandem enzyme assay. The experimental design included the tested bacterial culture (gray) as well as positive (blue) and negative (red) controls, in which either steroids (“bacteria only”), bacteria and steroids (“culture medium only”), or bacteria (“steroid only”) were omitted or in which bacteria were killed by an antibiotic (“bacteria, steroid, antibiotic”). Taken from ref. ⁵⁸.

In highly competitive environments, e.g. in bacterial culture broths, a further refinement of the supramolecular tandem assay procedures may be required, such as in the development of a tandem enzyme assay for the detection of steroid-degrading bacteria.^{22,58} The use of CB8/(BE)₂ as a reporter pair enabled the detection of testosterone at low micromolar concentrations directly in the bacterial culture broth medium, but batch-to-batch variations afforded sometimes inconclusive results. By considering appropriate controls (Figure 4) that could account for potential instabilities, competitive metabolites, and non-metabolic steroid depletion, the assay was much more reliable as indicated by a good Z'-factor of >0.5.

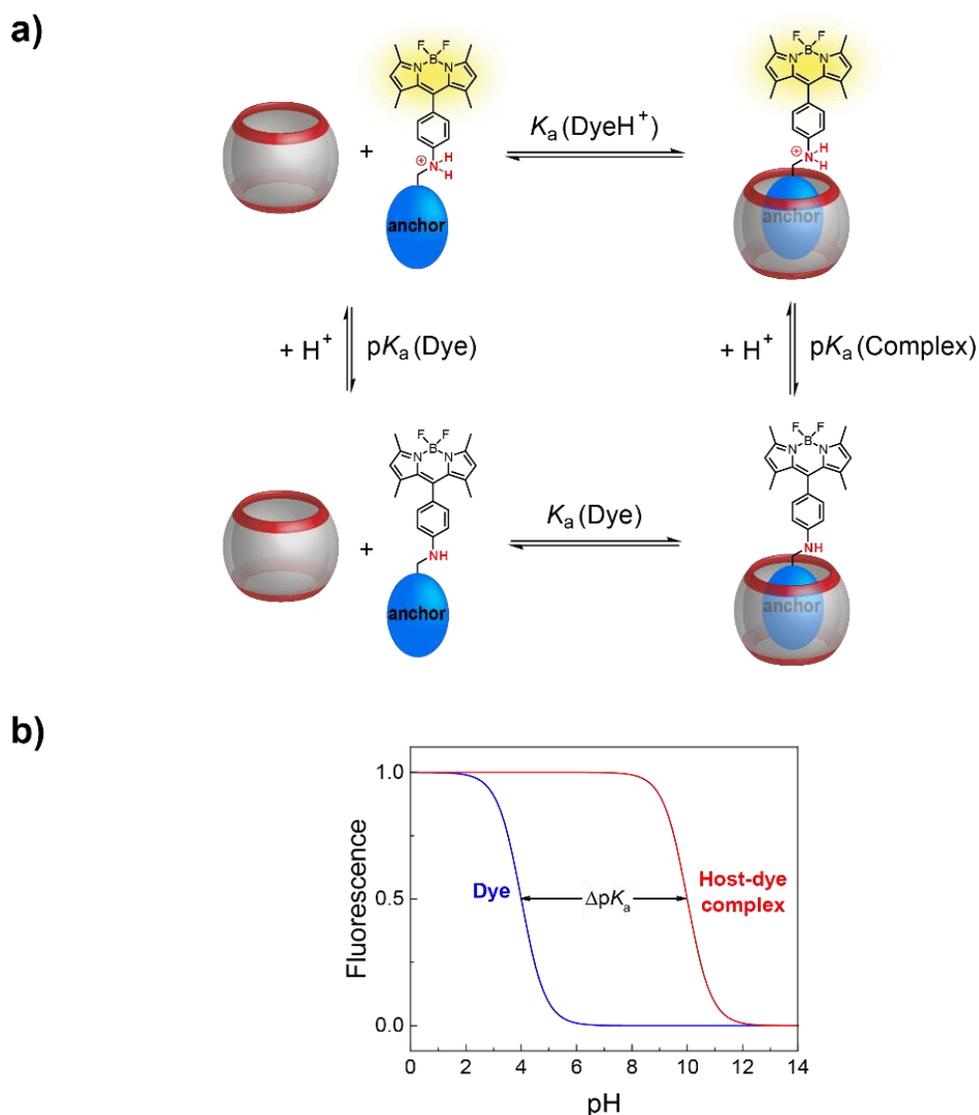


Figure 5. Anchor group strategy with boron-dipyrrromethene (BODIPY) dyes as an approach to tailored reporter dyes for cucurbiturils. a) Thermodynamic cycle of the involved binding equilibria. b) Complexation-induced pKa shifts and the resulting range of suitable pH values for sensing. Taken from ref.⁵⁹.

Considering the established supramolecular host-guest reporter pairs for fluorescence assay and sensor development, several desirable properties are so far not available, for example, absorption maxima of the reporter pairs are typically <450 nm, binding constants are often <10⁶ M⁻¹, and the maximal change in fluorescence may be too low.⁶⁰ A rational design strategy is therefore attractive to tailor the spectroscopic properties as well as the affinity of fluorescent dyes for host-guest reporter pairs. Therefore, the so-called anchor group strategy has been established (Figure 5), which exploits the host-assisted protonation of a cavity-binding functional moiety (the “anchor group”) and a suitably attached protonation-sensitive fluorescent dye.^{59,61-64}

This strategy was recently explored with boron-dipyrromethene (BODIPY) dyes,⁵⁹ which are established dyes for applications as fluorescent labels in biology, biochemistry, medical imaging, and related life science applications.^{65,66} BODIPY dyes with an aniline group in the meso position were known to be efficiently quenched by photoinduced electron transfer (PET) in their unprotonated forms, whereas the protonated BODIPY dyes were highly fluorescent.⁶⁷ The idea was thus to attach the anchor group to the nitrogen atom of the aniline group in order to position the nitrogen atom close to the carbonyl rim of cucurbiturils in the supramolecular complex. The possibility to tune binding affinities and p*K*_a values was demonstrated by introducing benzylamine, cyclohexylmethylamine, putrescine, and adamantylmethylamine as anchor groups, which afforded BODIPY reporter dyes with affinities from 1.5 × 10⁴ M⁻¹ to >10⁹ M⁻¹ and p*K*_a values of 5.0 to 8.2. This afforded host-dye reporter pairs with favourable absorption and emission wavelengths in the visible spectral region, which were subsequently applied in indicator displacement assays, fluorescence correlation spectroscopy, and noncovalent surface functionalization with fluorophores.

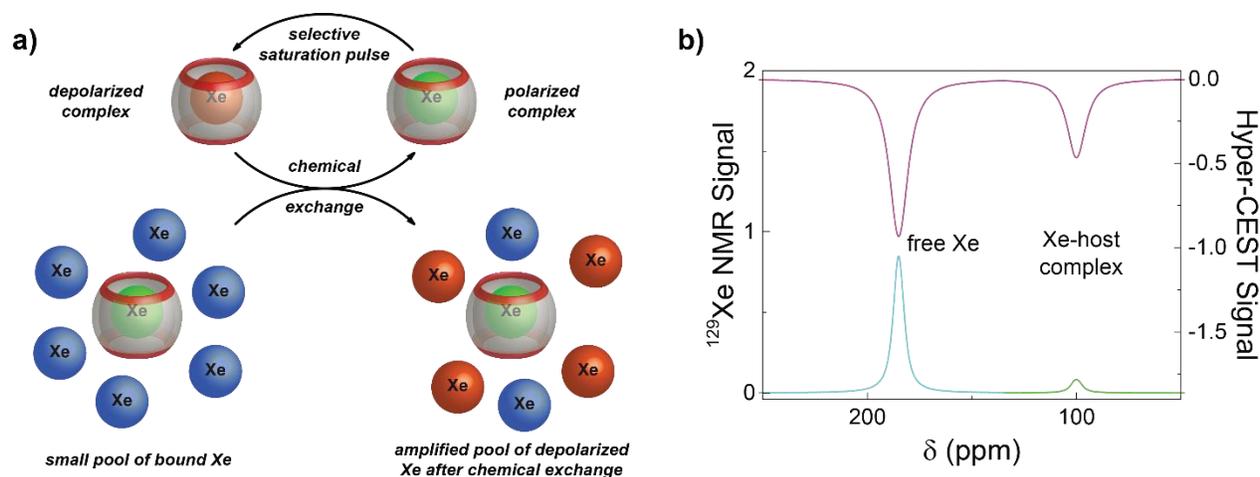


Figure 6. a) Principle of chemical exchange saturation transfer for signal amplification in Xe-based biosensors and b) conventional ^{129}Xe NMR spectra of the mixture (blue and green) as well as resulting hyper-CEST spectrum (red) with enhanced signal from the Xe-host complex. Adapted from ref. ⁶⁸.

Another advancement of supramolecular tandem enzyme assays that could be achieved was their application in magnetic resonance imaging (MRI). Xe was early known to bind to the macrocycle CB6 and was thus proposed as a potential contrast agent for Xe-based MRI, but the sensitivity was considered too low for practical applications.⁶⁹⁻⁷¹ However, the interest in CB6 as a contrast agent was revived by the development of Xe biosensors using ^{129}Xe hyperpolarized chemical exchange saturation transfer (^{129}Xe hyper-CEST).⁶⁸ Therein, hyperpolarized Xe, which is per se more sensitive than unpolarized Xe, is combined with chemical exchange saturation transfer (Figure 6). In this method, a saturation or an inverse pulse is applied at the resonance frequency of the biosensor to selectively depolarize the pool of bound Xe, which leads to an accumulation of depolarized Xe in the pool of free Xe by a continuous and rapid exchange with free Xe. Overall, an amplified signal can be detected enabling detection of CB6 down to picomolar concentrations in phosphate-buffered saline and in blood serum.^{72,73} Since even in vivo detection of CB6 has been claimed,⁷⁴ this method has the potential to become a clinically relevant molecular imaging modality.

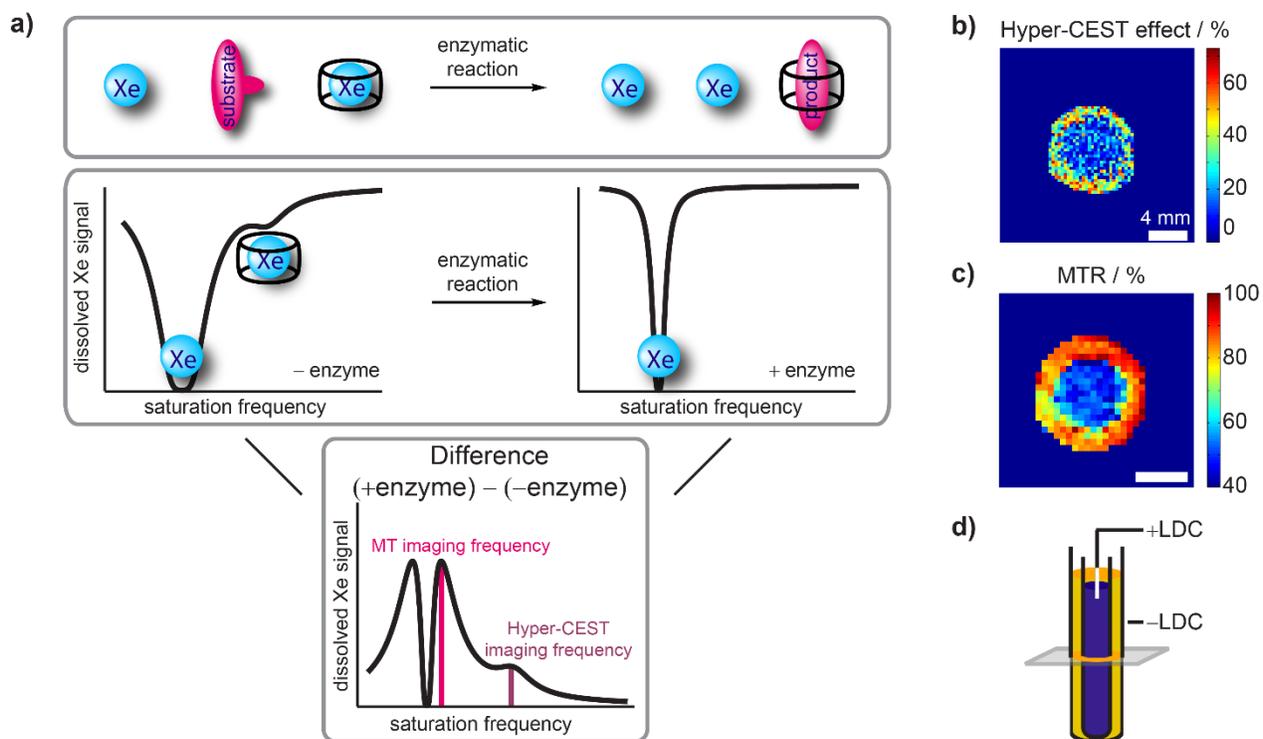


Figure 7. a) Application of the supramolecular tandem enzyme assay principle to Xe magnetic resonance imaging (MRI). b) Hyper-CEST MRI image with CB6 in buffer. c) MT MRI image with CB7 in cellular lysates. d) Experimental setup using two nested NMR tubes with lysine decarboxylase (LDC) in the inner tube. Taken from ref. ⁶².

The combination of hyper-CEST with the tandem enzyme assay principle was therefore considered to develop the first Xe enzyme biosensor for MRI.^{62,75} As a model enzyme, lysine decarboxylase (LDC) was chosen, which converts lysine into the biogenic amine cadaverine. The latter is strongly binding to CB6 and thus occupies the cavity. As a consequence, the signal at the resonance frequency of the CB6/Xe complex disappears in presence of LDC. Unfortunately, significant line broadening was observed with CB6 in cell lysates, which rendered the Xe enzyme activity determination impractical with CB6. As a remedy, magnetization transfer (MT), which is an established ¹H MRI method that applies a saturation pulse slightly off-resonant from the exchange-broadened bulk signal, was considered, and with the idea that further line broadening could be achieved, CB6 was replaced by CB7, which enabled for the first time the unambiguous detection of enzyme activity by Xe MRI in cell lysates (Figure 7).

Membrane Transport

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The transport of molecules across phospholipid bilayer membranes is a quintessential function for all living organisms exerted by wide variety of structures in live cells including peptides, proteins, as well as low-molecular-weight, natural products. This has stimulated supramolecular chemists to create structures with similar functions in order to ultimately provide biorthogonal control over membrane transport, which could pave the way for real-world applications in drug delivery, sensing, and antibiotic development.⁷⁶⁻⁸⁷

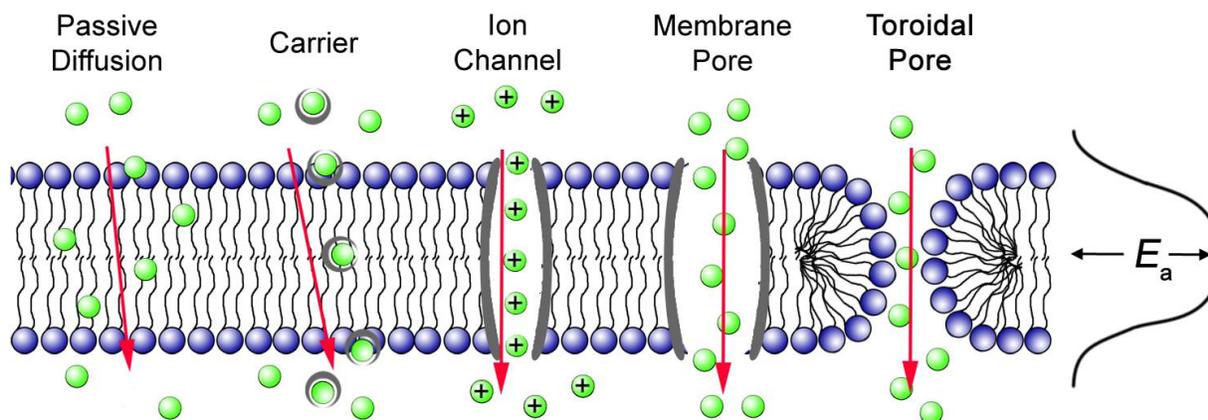


Figure 8. Selected mechanisms of membrane transport.

In addition to the challenge to create synthetic membrane transporters, it also of interest to better understand the transport mechanisms involved in natural and synthetic membrane transporters (Figure 8). It may involve, for example, passive membrane diffusion (permeation), carriers, ion channels, or pores, but due to a limited availability of characterization methods, the transport mechanisms often remain ill-characterized. Moreover, transport mechanism can also vary with different experimental conditions.⁸³ For example, typical ion carriers such as

valinomycin can act as ion channel under appropriate conditions, detergents like triton X-100 can exhibit single-channel currents, and most carriers, ion channels and pores destroy bilayer membranes in a detergent-like manner at high-enough concentrations. It has now been shown during this habilitation work, how supramolecular host molecules can contribute to the design and application of synthetic membrane transport systems as well as to the mechanistic investigation of membrane transporters.

Inspired by counterion-activated transport of cell-penetrating peptides (CPPs), amphiphilic *p*-sulfonatocalix[4]arenes with alkyl chains at the lower rim were tested as counterion activators (Figure 9).²⁵ This revealed that the calixarene amphiphiles are far more active than other established counterion activators and that only nanomolar concentrations are required to activate membrane transport of model CPPs in liposomes. As a consequence of this high capability to affect membrane transport of established CPPs, it was also explored whether the amphiphilic calixarenes could also transport other peptides. Therefore, enzyme substrates for protein kinase A (PKA) and protein kinase C (PKC), i.e. P1 and P2 (see Figure 9) were selected, which were also efficiently transported by the calixarenes.

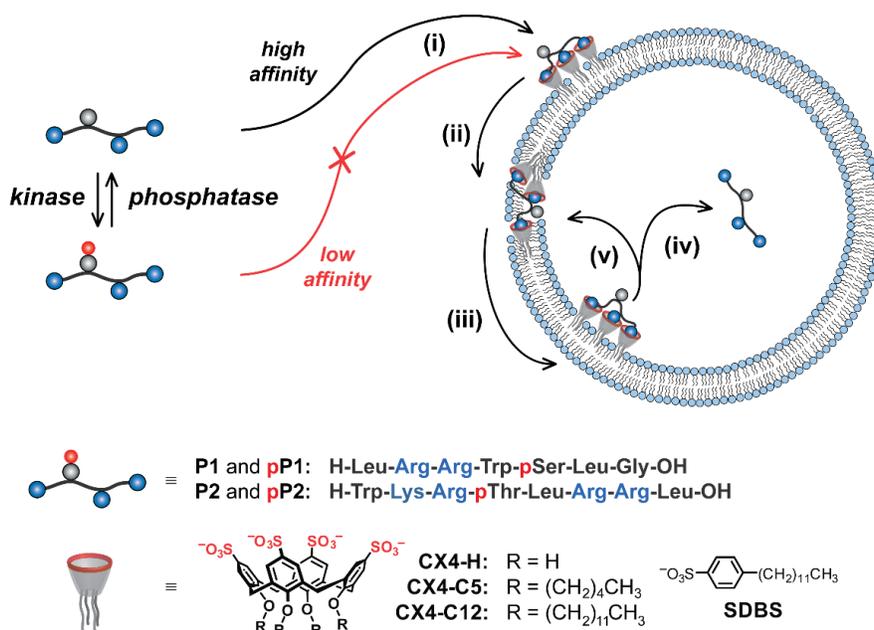


Figure 9. Principle of counterion-activated membrane transport by amphiphilic calixarenes and its inhibition by peptide phosphorylation. Kinases and phosphatases control the affinity of the peptides for membrane-bound calixarenes (i), and only the non-phosphorylated peptides have sufficient affinity to allow charge neutralization and membrane partitioning (ii). After membrane translocation (iii), either the peptides are released into the vesicle lumen (iv) or shuttle back into the membrane (v). Taken from ref. ²⁵.

This afforded the possibility to design the first artificial enzyme-responsive membrane transport system for peptides. It was shown that the introduction of a negative charge to the peptides serine and threonine residues of the peptides P1 and P2 decreased their binding affinity towards the negatively charged amphiphilic calixarenes and, as a consequence, their transport activity could be significantly down-regulated by phosphorylation by a kinase (Figure 10).

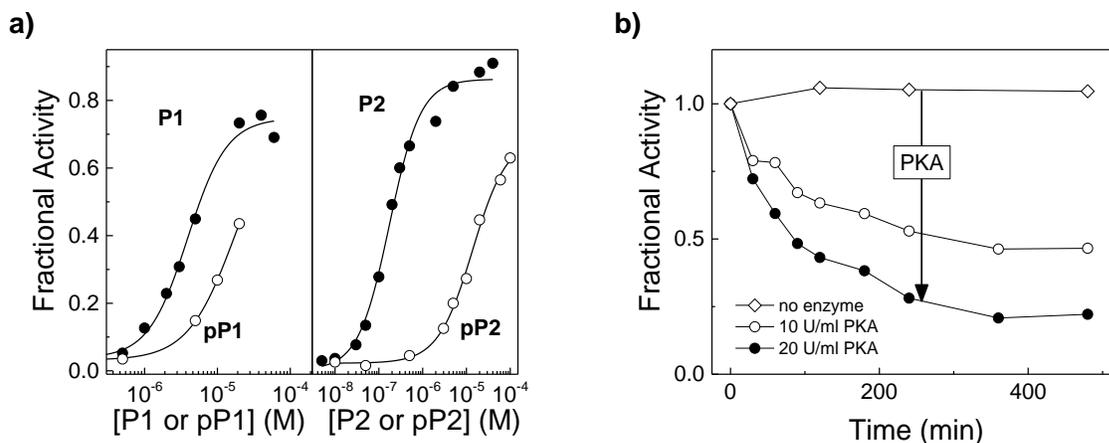


Figure 10. a) Hill plots obtained with the CF assay illustrating the concentration-dependent transport activity of the kinase substrates P1 and P2 (filled circles) and the phosphorylated products pP1 and pP2 (open circles) in presence of 0.8 μ M CX4-C5. b) PKA-dependent stalling of membrane transport activity followed with the CF assay. P1 was incubated with different concentrations of PKA and aliquots were added after varying incubation times to EYPC \supset CF liposomes. Taken from ref. ²⁵.

Subsequently, the series of amphiphilic calixarenes was extended to CX4-C_n with n = 3-8, and 12 and investigated for their ability to act as counterion activators in liposomes and cells. This indicated a clear dependence of the transport activity on the length of the alkyl tails in liposomes and corroborated that the calixarenes are superior counterion activators compared to the established pyrenebutyrate.^{88,89}

The results from the cell experiments were, however, initially disappointing showing an unexpectedly low intracellular intensity in fluorescence microscopy imaging. However, we found out that this is not due to an inefficient delivery of the peptides into cells, but due to the formation of calixarene-peptide complexes, in which the fluorescence of the dye labels is quenched. Addition of cetyltrimethylammonium bromide (CTAB) to calixarene-peptide complexes as well as to cell lysates led to a recovery of the fluorescence. Presumably, the quaternary trimethylalkylammonium group, which is a known binding motif for *p*-sulfonatocalixarenes, acts

as a competitor leading to a dissociation of the calixarene-peptide complexes. The stable fluorescence intensity in cell lysates enabled a determination of the intracellular peptide concentration, which confirmed a superior performance of calixarenes compared to pyrenebutyrate, which was subsequently also shown in fluorescence microscopy by addition of CTAB as a final step after fixing the cells (Figure 11). Interestingly, the dependence on the alkyl chain length was inverted compared to the liposome experiments, i.e., CX4-C5 was most active in liposomes and least active in cells. The origin of this behavior remains so far unclear and further experiments are currently ongoing.

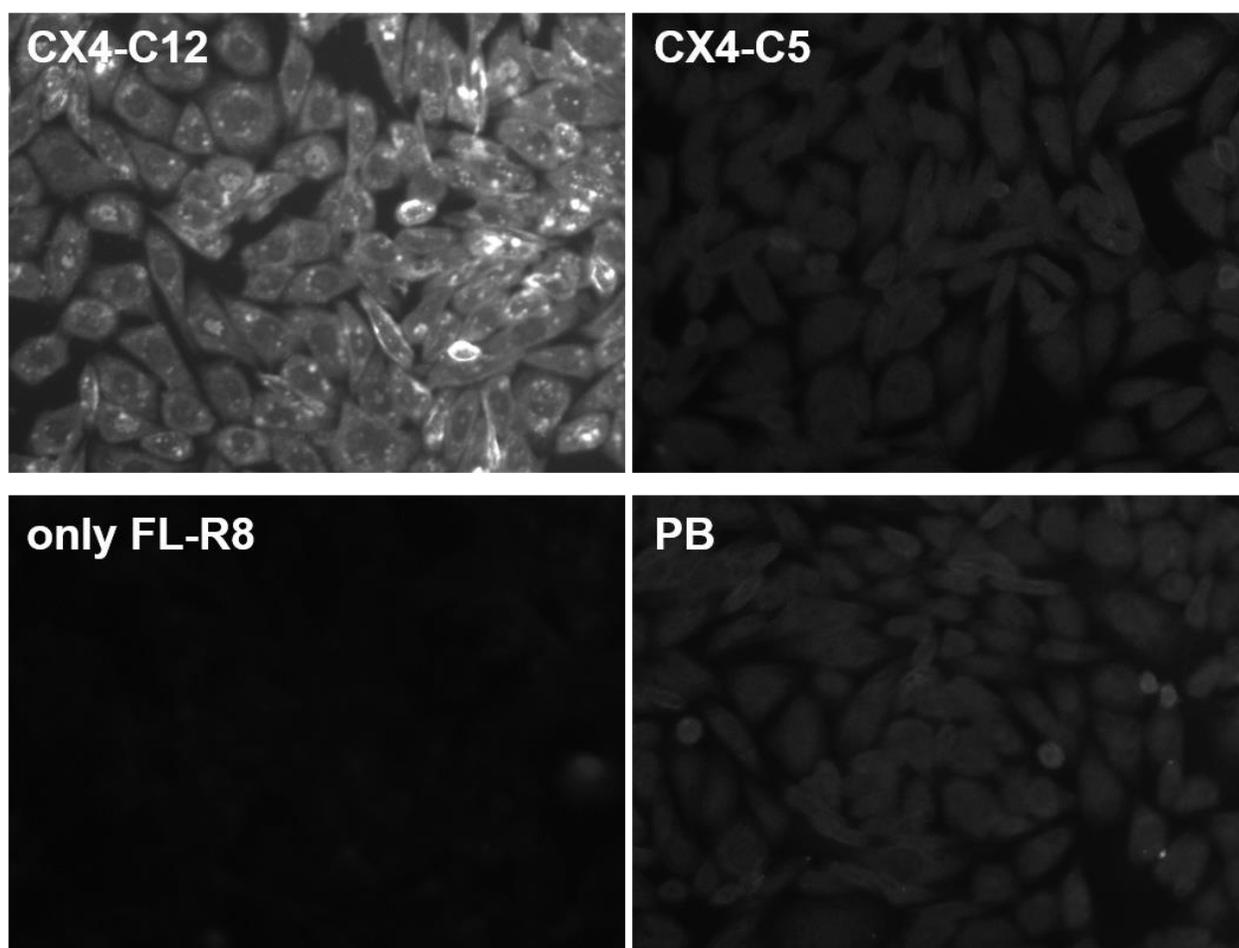


Figure 11. Cellular imaging of fixed CHO-K1 cells. The cells were incubated with fluorescein-labelled octaarginine (FL-R8) and counterion activator (calixarenes or pyrenebutyrate), membrane-bound peptide was removed by washing with hyaluronic acid, and 1% CTAB was added.

A persistent problem in characterizing membrane transporters involves, however, the frequent use of dye efflux assays (Figure 12). Therein, fluorescent dyes are encapsulated in

As a remedy for this uncertainty, liposome-encapsulated supramolecular host/dye reporter pairs were considered, which afford a direct and sensitive fluorescence-based method to monitor membrane transport of unlabeled molecules.²⁴ This has been previously demonstrated with membrane pores and for passively permeating, small molecules, and was now extended to CPPs and counterion activation. The molecular recognition between the CPP and the encapsulated host molecules affords a method to unambiguously establish that the CPPs peptide are transported and released into the vesicle lumen.

The supramolecular tandem membrane assay was first evaluated with the silently translocating peptide TP2 as well as with counterion activation of heptaarginine by perfluorinated fatty acids. In both cases, membrane transport could be reliably monitored with encapsulated host/dye pairs, whereas dye efflux assays gave false negative results. Moreover, the supramolecular tandem membrane assay principle enabled a detailed mechanistic investigation of our calixarene-based counterion activators, in which we showed that the peptides are actually transported and that the supramolecular tandem membrane assays afford reliable transport activities regardless of the encapsulated host/dye reporter pairs. This was particularly established by consistent transport efficiencies, i.e. EC_{50} values, obtained with three different reporter pairs (Table 1). For example, an influence of the binding affinity of the encapsulated host could be ruled out by comparing the results from the CX4/LCG assay and the CB7/BE assay with the peptide H-WKRTLRRRL-OH and its phosphorylated counterpart. While the phosphorylated peptide binds less efficiently to CX4 because of a reduced electrostatic attraction, both peptides bind with identical affinity to CB7 via its *N*-terminal tryptophan residue, and despite the different affinity, both assays gave comparable EC_{50} values for their transport.

Table 1. Dissociation constants, K_d , and effective concentrations to afford 50% transport obtained with different host/dye combinations in supramolecular membrane transport assays.^a

Peptide	CX4/LCG		CB7/BE		CB8/MDAP	
	K_d (μ M)	EC_{50} (μ M)	K_d (μ M)	EC_{50} (μ M)	K_d (μ M)	EC_{50} (μ M)
H-WKRTLRRRL-OH	0.85 \pm 0.09	0.021 \pm 0.002	24 \pm 2	0.050 \pm 0.008	2.3 \pm 0.3	0.046 \pm 0.002
H-WKRpTLRRRL-OH	24 \pm 4	3.9 \pm 0.6	25 \pm 2	13.8 \pm 5.2	2.5 \pm 0.4	16.5 \pm 0.8
H-FRRRRRRR-OH	0.23 \pm 0.09	0.59 \pm 0.05	0.034 \pm 0.011	0.05 \pm 0.03	0.5 \pm 0.1	0.025 \pm 0.003

^a All experiments were performed with the amphiphilic counterion activator CX4-C5 ([CX4-C5] = 0.8 μ M).

Surfaces

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The use of micro- and nanoparticles currently emerges in a wide range of applications for example, in DNA sequencing, fluorescence-based assays, as sensors, for in vivo imaging, drug delivery, and in optoelectronic devices.^{90,91} The key to successfully apply these particles lies in the possibility to control their surface chemistry, which largely determines the material's properties and the interaction of the particles with other molecules. In addition, it is essential to precisely know the number, chemical nature, and spatial distribution of functional groups on surfaces. As a consequence, numerous analytical methods have been devised and applied to quantify surface functional groups, for example, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), solid-state NMR (ssNMR), isothermal titration calorimetry, potentiometry and conductometry, as well as colorimetric or fluorometric assays.⁹²⁻⁹⁸

My interest in the surface chemistry of micro- and nanoparticles started with the development of a supramolecular host/guest-based method to determine the number of accessible (or available) functional groups.⁹⁹ This number is different from the total number of functional groups and represents the number of application-relevant molecules that can be principally conjugated to a particle surface. Established methods rely on specific detection labels, for example fluorescent probes, radiotracers or heteroatom-containing XPS labels, which can be subsequently read out with the respective analytical technique. As an alternative, an adamantylmethylamine derivative (AMADA-Put) was synthesized, which can be reacted to carboxy-modified particle surfaces and has a high affinity to CB7. After reacting the particles with AMADA-Put, CB7 is added to the suspension, which binds to the adamantylmethylamine part of

AMADA-Put. Centrifugation then affords the supernatant with excess CB7 in solution, which can be quantified by the change in fluorescence after addition of a suitable fluorescent dye (Figure 13). This method offers several advantages compared to established methods: It does not require radioactive material, it can be performed with a standard fluorometer, and it avoids typical artifacts in conventional fluorescence labeling, like fluorescence quenching originating from high local concentrations at the particle surface, an uncertainty about the micropolarity near the surface, and stray light from larger particles.^{100,101}

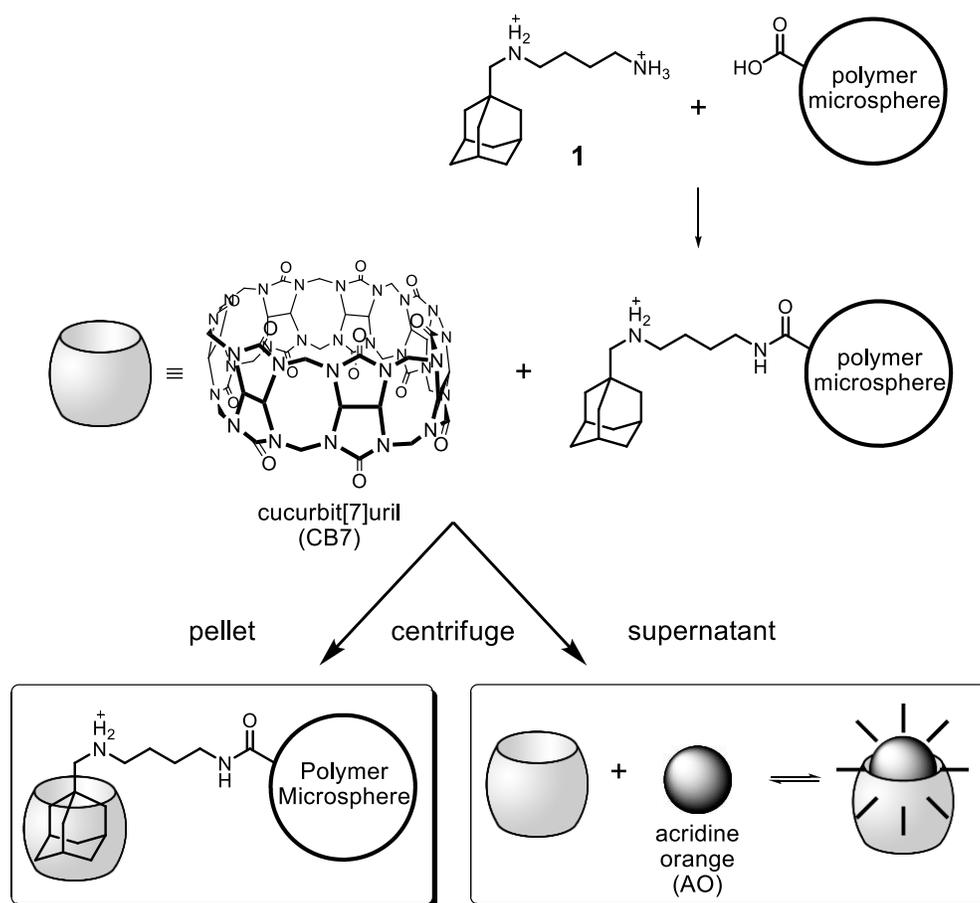


Figure 13. Quantification of accessible surface functional groups using host-guest binding between CB7 and an adamantylmethylamine derivative reacted with surface carboxylic acid groups on polymer microspheres. After centrifugation, remaining CB7 in solution is quantified by addition of the fluorescent dye acridine orange (AO). The difference between added and remaining CB7 in solution affords the number of CB7 molecules bound to the microsphere surface. Taken from ref. ⁹⁹.

This method was more recently also applied to the characterization of gold nanoparticles (AuNPs).¹⁰² The AuNPs were different from the initial model particles bearing a poly(acrylic acid) layer grafted from a poly(methyl methacrylate) core and carried mixed monolayers of surface carboxylic acid groups and unreactive surface ligands. Specifically, AuNPs with mixtures of sodium 3-mercaptopropanoic acid (MPA) and 3-mercapto-1-propanesulfonate (MPS) as well as mixtures of 11-mercaptoundecanoic acid (MUA) and triethyleneglycol mono-11-mercaptoundecyl ether (TEG) were prepared to afford MPA/MPS-AuNPs or MUA/TEG-AuNPs, respectively, with varying ligand densities of surface carboxylic acid groups. The method was thoroughly validated by comparison with quantitative NMR and a colorimetric assay, which indicated an excellent accuracy and a good precision of the method.

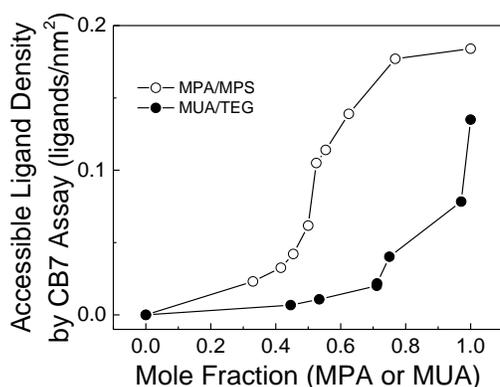


Figure 14. Dependence of the accessible ligand density on the mole fraction of reactive surface carboxylate groups in mixed ligand-protected MPA/MPS-AuNPs (open circles) and MUA/TEG-AuNPs (filled circles). Taken from ref. ¹⁰².

Most interestingly, the results suggested that the CB7-based assay can also be used to obtain structural information on the surface functional groups in addition to the quantitative information. This already transpired from the initial results with the polymer particles, in which the surface concentration of CB7 was higher than the maximum surface concentration of a closely packed monolayer of CB7 molecules. This indicated that the poly(acrylic acid) surface is not a densely packed shell, but a rather relatively rough structure, eventually with flexible “pores” to accommodate the CB7 complex with AMADA-Put. Even more intriguing were the results with the AuNPs, which showed a striking difference when the dependence of the accessible ligand density on the molar fraction of the carboxylic acid surface groups was compared for the mixed MPA/MPS and MUA/TEG monolayers (Figure 14).

This indicated that the amount of the surface-coupled AMADA-Put/CB7 complex decreased significantly with only small amounts of surface-TEG, whereas the shape of the curve for the MPA/MPS-AuNPs was clearly sigmoidal requiring a mole fraction of 0.5 of unreactive MPS to afford a reduction of the surface ligand density of AMADA-Put/CB7 complex by 50%. A potential reason for this difference in the resulting surface ligand density may be the formation of (nano)domains on the surface of the mixed-ligand shell protected AuNPs. It is conceivable that at comparable molar fractions of reactive ligands, a random distribution of the ligands on the surface would sufficiently separate the reactive ligands to accommodate a significant amount of the sterically demanding AMADA-Put/CB7 complex on the surface, whereas a confinement of the reactive ligand to small domains would prevent CB7 binding. Similar conclusions were independently reached for self-assembled monolayer on planar gold surfaces, which were probed with a ferrocene/CB7 complex.¹⁰³

CB derivatives in conjunction with ultrahigh-affinity guests have been suggested to complement the use of streptavidin/biotin in various applications.¹⁰⁴⁻¹⁰⁶ They were used, e.g., for immobilization of proteins and cells on planar surfaces,¹⁰⁷⁻¹⁰⁹ for affinity-based enrichment and isolation of proteins,¹¹⁰⁻¹¹² and for supramolecular PEGylation of biopharmaceuticals.¹¹³ Supramolecular host-guest complexes were also explored in conjunction with nanoparticles;^{114,115} for example, CBs were adsorptively attached to metal surfaces such as planar^{108,109} and spherical^{116,117} gold surfaces, to iron oxide nanoparticles through multidentate binding of their carbonyl-fringed portals,^{118,119} interfaced with quantum dots to afford CB polymer capsules with encapsulated quantum dots,¹²⁰ and used for the creation of hot spots in surface enhanced Raman scattering (SERS).^{116,117,121,122}

In order to explore CBs for nanobioconjugation, we have synthesized particles with surface-bound CB7, which could then be functionalized with model compounds by simply mixing the particles with a solution containing the compound that should be immobilized and a brief period of incubation.¹²³ To demonstrate this, a propargyl derivative of CB7 was synthesized and coupled to azide surface groups by copper-catalyzed click chemistry (Figure 15). The amount of surface-bound CB7 was then subsequently quantified by incubation with adamantylmethylamine (AMADA) as a strong binder, centrifugation, and quantifying the remaining amount of AMADA in solution by addition of CB7 and acridine orange to the supernatant. In accordance with the previous results in which AMADA-Put was reacted with the surface, the resulting CB7 surface coverage densities (ca. 0.3 nmol cm⁻²) were significantly higher than the value for a CB7 monolayer on planar gold surfaces (ca. 0.08 nmol cm⁻²), which is due to the grafted layer of poly(acrylic acid).

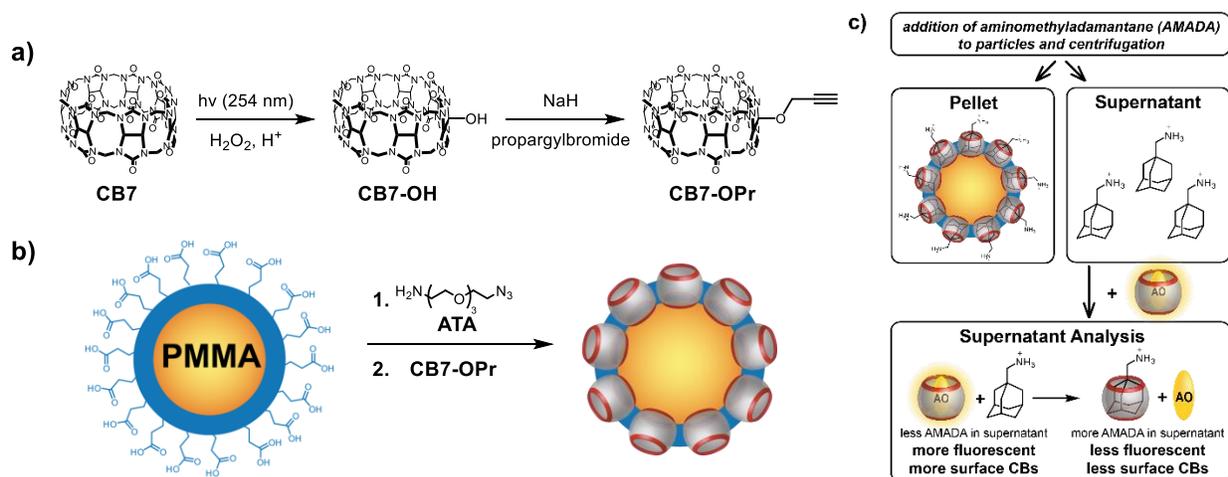


Figure 15. a) Synthesis of monofunctionalized hydroxyl-CB7 (CB7-OH) and conversion into propargyl-CB7 (CB7-OPr) by nucleophilic substitution. b) Surface-functionalization with CB7 of poly(methyl methacrylate) (PMMA) particles with a grafted layer of poly(acrylic acid) by introducing azide groups and subsequent click chemistry. c) Quantification of surface-bound CB7 by extraction of AMADA and quantification of remaining AMADA in the supernatant using acridine orange and CB7. Taken from ref. ¹²³.

Subsequently, the possibility to immobilize application-relevant molecules on the CB7-functionalized particles was demonstrated with an aminoadamantyl-labeled rhodamine (Ada-Rho), which was achieved by addition of CB7-functionalized particles to a buffered aqueous solution containing Ada-Rho. Strong, quantitative binding between Ada-Rho and the CB7-functionalized particles was inferred from a linear decrease of the absorbance and fluorescence of the supernatant with increasing amounts of CB7-functionalized particles, which also established that the maximal amount of Ada-Rho on the surface was significantly lower ($3.0 \mu\text{mol g}^{-1}$) than the amount of surface-bound CB7 ($5.7 \mu\text{mol g}^{-1}$).

In view of the different surface coverage densities of Ada-Rho and CB7, it came as a surprise that incubation of the CB7-functionalized particles with mixtures of AMADA and Ada-Rho gave surface coverage densities of Ada-Rho, which depended linearly on the molar fraction of AMADA and Ada-Rho (Figure 16). In addition, the resulting surface coverage densities of two competitors should, in a thermodynamically equilibrated mixture, also depend on their binding affinities, and the simple linear relationship suggested a kinetically controlled occupation of the CB7 binding cavities, which is presumably limited by diffusion of AMADA and Ada-Rho into the poly(acrylic acid) layer. It was further shown that addition of high concentrations of AMADA to particles surface-functionalized with Ada-Rho caused a slow dissociation of Ada-Rho from the particle surface demonstrating the principal reversibility of the host-guest interaction. Overall,

the present work paves the way for on-demand configurable and stimuli-responsive nanobioconjugates using CB7 host-guest interactions.

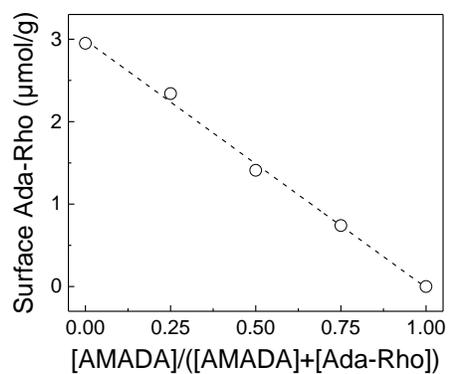


Figure 16. Dependence of resulting Ada-Rho surface coverage densities on the mole fraction of the competitor AMADA in mixtures of AMADA and Ada-Rho. Taken from ref. ¹²³.

Outlook

Since its infancy, supramolecular chemistry has become a widely interdisciplinary research field. The reason is that the fundamental concepts of supramolecular chemistry apply as well in biological processes and determine the key properties of materials. This enables the design and synthesis of artificial structures that report or interact with biological systems and it allows implementing new functions into advanced materials. As summarized in this habilitation thesis, my contribution to these research activities included the development, investigation, and application of supramolecular host-guest systems as chemosensors, membrane transporters, and for the controlled functionalization of surfaces. Future endeavors include the optimization of the amphiphilic calixarenes to enhance the cellular uptake of cell-penetrating peptides and the development of supramolecular chemosensors that work reliably in cells.

In order to rationally design these applications of host-guest systems, it is essential to know the binding affinity as well as the exchange kinetics of the various combinations of hosts and guests, particularly in the complex environments, in which the applications are being pursued, i.e. biological buffers of various composition, in cells, and at interfaces. Noteworthy, these parameters, in particular the exchange kinetics, and how different environments have an influence on these parameters is largely unknown. For example, host-guest binding affinities of cucurbiturils in water have been reported to cover a wide range from millimolar to attomolar affinities (see Figure 17 for an example overview for CB7).¹⁸ This principally enables their use as anchor groups (cf. Figure 5), but which affinity is required to tightly hold host and guest together in a competitive cellular environment is hitherto unknown.

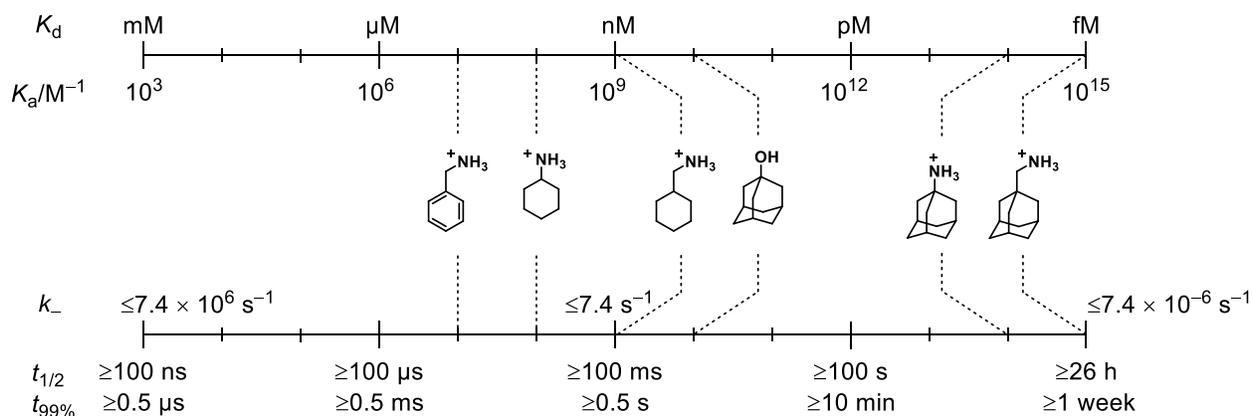


Figure 17. Approximate binding affinities and minimal exchange kinetics (assuming diffusion-limited association) of selected guests for CB7.

In addition, it is worthwhile to consider that two strongly binding guests (working concentrations above $1/K_a$), which compete for a limiting amount of host, will establish equilibrium concentrations according to:

$$\frac{[HG_1]}{[HG_2]} = \frac{K_{a,1}[G_1]_{\text{tot}}}{K_{a,2}[G_2]_{\text{tot}}}$$

Therefore, it can be deduced that exchange will be quantitative at equimolar concentrations of the two guests, if the binding constants differ by a factor of >100 ($>99\%$ exchange). Conversely, another guest of the same affinity can quantitatively replace a first guest, when the competitor concentration is 100times larger than the concentration of the first guest. This enables a controlled, quantitative guest exchange regardless of the competitive environment.

It is tempting to rely on host-guest pairs with an ultrahigh affinity to afford stable complexes in competitive environment,^{105,106} but this sacrifices the possibility to dynamically exchange the guests and therefore the possibility of a response towards an external stimulus. To demonstrate the limits of dynamic exchange, it worthwhile to consider that the maximal dissociation rate constant, k_- , is given by binding affinity, K_a , and the maximal, diffusion-limited association rate constant, $k_+ = 7.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$:

$$K_a = \frac{k_+}{k_-} \Leftrightarrow k_- < \frac{7.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}}{K_a}$$

The integrated form of the first-order reaction rate law then gives the time, t , required for dissociation of the host-guest complex as well as the time required to exchange 99% of the guest, $t_{99\%}$:

$$t = \frac{1}{k_-} \ln \frac{[HG]_0}{[HG]} \quad \text{and} \quad t_{99\%} = \frac{\ln 99}{k_-}$$

The resulting times and rates have been included in Figure 17 to enable a direct comparison between the binding affinity and the exchange rates. This clearly demonstrates that certain assumptions previously made for the design of enzyme and membrane transport assays (dye exchange much faster than the enzymatic reaction or the transport) break down and need to be reconsidered for high-affinity guests in more competitive environments. In particular, this calls for an established series of anchor groups with known affinities and exchange rates in various environments, which would form the basis for a rational choice of host-guest pairs to enable future applications of supramolecular host-guest chemistry in complex environments.

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Teaching Concept

This teaching concept has evolved from the conceptualization, preparation and the feedback and experience from the courses that I have taught at Jacobs University Bremen. The courses include: Supramolecular Chemistry lecture (5.0 ECTS) and lab course (2.5 ECTS), and the courses Scientific and Experimental Skills (2.5 ECTS) and Enzyme and Binding Assays (2.5 ECTS). Various course materials have been prepared for the courses, for example, handouts and lab manuals, which are available upon request.

The goal of my teaching is to convey to students how the knowledge that has been acquired through research efforts can be applied by them in their future lives. This includes teaching factual knowledge to students as well as promoting their self-development and supporting them to acquire essential soft skills, such as prudence, care, diligence, frustration tolerance, and an attentiveness to details.

In order to convey factual knowledge, I summarize the educational objective at the beginning of each session, then explain the fundamental principles, and finally use selected examples to further illustrate new concepts. For revision and consolidation of the study content, I refer to the contents of textbooks as well as to the primary scientific literature. I regularly offer small tests to enable students to assess and monitor whether they have successfully acquired the learning targets. This also requires a continuous participation and an independent practice of the subject matter by the students.

The soft skills of the students are systematically promoted by me using the different education formats: lecture, seminar, tutorial, and lab. For example, the experiments in the lab are conducted in small groups to promote the students' ability to cooperate and work in teams, or we discuss in seminars different approaches to solve a specific problem. Sometimes I conceptualize arithmetic problems in such a way that only the correct answer instead of the algorithm is being graded, which requires due diligence from the students. Scientific writing is practiced by increasing the standards in lab protocols with every semester, and during lab courses, I am conducting colloquia in order to promote communication skills and the ability to respond positively to criticism. The core competence of any university graduate is the ability for independent and self-motivated learning. Therefore, I offer recommended readings in addition to the compulsory reading material. This piques the curiosity of the students for more advanced topics.

The students consider my courses to be demanding, but they also appreciate that I try to convey my enthusiasm for science. This is usually achieved by referring to current developments in science or to challenges of human society. Furthermore, a close personal contact to students is important to me. In small lectures or seminars, I ask questions and strongly encourage students to do so as well. This gives me direct feedback on their current state of knowledge, and it conveys that the individual learning success of every student is important to me. For courses involving larger numbers of students, I offer open office hours as well as the possibility to make individual appointments. When I realize that students have particular difficulties, I proactively initiate a personal conversation.

As time went by, I have learned to adapt my teaching style more individually to the different courses and students, because I have experienced that what has worked well with one batch of students may fail with a different batch. What I, however, always demand is a continuous and active participation, in which the students independently practice in order to give their best.

Curriculum Vitae

Dr. Andreas Hennig

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Professional Experience

10/2014 – present	<i>Habilitation</i>	Jacobs University Bremen, Bremen (D) Department of Life Sciences and Chemistry
05/2014 – 09/2014	<i>Customer Relations Manager</i>	Deutsche Akkreditierungsstelle GmbH, Berlin (D) Division 4: Chemistry and Environment
11/2009 – 04/2014	<i>Research Scientist</i>	Federal Institute for Materials Research and Testing, Berlin (D) Department 1: Analytical Chemistry; Reference Materials
08/2007 – 10/2009	<i>Postdoc</i>	L'Université de Genève, Genève (CH) Département de Chimie Organique

Education

03/2004 – 07/2007	<i>Doctor of Philosophy</i>	Jacobs University Bremen, Bremen (D) School of Engineering and Science
10/1998 – 02/2004	<i>Diploma in Chemistry</i>	TU Braunschweig, Braunschweig (D)
10/1997 – 10/1998	<i>Civilian Service</i>	Alten- und Pflegeheim des Landkreises Peine, Peine (D)
until 06/1997	<i>High School</i>	Gymnasium Groß Ilsede, Groß Ilsede (D)

Selected Awards

2011	Start-up grant of the “DFG-Excellence Academy for Medical Engineering”
2004 – 2007	PhD fellowship: F. Hoffmann-La Roche AG
2001 – 2004	Member of “Studienstiftung des deutschen Volkes”
2001	Award of “Freunde des Instituts für Organische Chemie” (for the best pre-diploma)
1993	Dr.-Mya-Tha Memorial Award (for excellence in natural sciences)

Funding

DFG HE 5967/4-1:	“Supramolekulare Tandem-Membran-Assays”	219.750 EUR
DAAD 57190254:	“Quantifizierung von chemisch zugänglichen Oberflächengruppen” im Förderprogramm “Projektbezogener Personenaustausch mit Ägypten (GE-SEED) 2015”	4.886 EUR
DFG HE-5967/2-1:	“Effizient gelöschte zellgängige NIR Sonden für die molekulare in vivo Bildgebung”	50.000 EUR

Teaching

Lectures and Tutorials

Spring 2019

02/2019 – 05/2019	Enzyme and Binding Assays (13 x 75 min, 2.5 ECTS Points)
03/2019 – 04/2019	Supramolecular Chemistry Lab (6 x 255 min, 2.5 ECTS Points)

Fall 2018

09/2018 – 12/2018	Supramolecular Chemistry (28 x 75 min, 5.0 ECTS)
09/2018 – 10/2018	Scientific and Experimental Skills (18 x 75 min, 2.5 ECTS)

Spring 2018

02/2018 – 05/2018	Advanced Scientific and Experimental Skills (13 x 75 min, 2.5 ECTS)
02/2018 – 05/2018	Enzyme and Binding Assays (13 x 75 min, 2.5 ECTS Points)
02/2018 – 03/2018	Supramolecular Chemistry Lab (6 x 255 min, 2.5 ECTS Points)

Fall 2017

09/2017 – 12/2017	Supramolecular Chemistry (28 x 75 min, 5.0 ECTS)
09/2017 – 10/2017	Scientific and Experimental Skills (18 x 75 min, 2.5 ECTS)

Fall 2016

10/2016 – 11/2016	Supramolecular Chemistry Lab (6 x 255 min, 2.5 ECTS)
09/2016 – 12/2016	Supramolecular Chemistry (28 x 75 min, 5.0 ECTS)
09/2016 – 10/2016	Scientific and Experimental Skills (18 x 75 min, 2.5 ECTS)

Spring 2016

02/2016 – 05/2016	Supramolecular Chemistry (28 x 75 min, 5.0 ECTS)
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Fall 2015

09/2015 – 10/2015	Scientific and Experimental Skills (18 x 75 min, 2.5 ECTS)
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Spring 2015

02/2015 – 05/2015	Supramolecular Chemistry (27 x 75 min, 5.0 ECTS Points)
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Fall 2006

09/2006 – 12/2006	Structure Elucidations by Spectroscopic Methods (Tutorial, 16 x 75 min)
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Supervised Students

02/2017 – 05/2017	Internship: MSc Maria LoPresti (from Valencia, Spain)
08/2016 – 04/2017	Scientific Visitor: Dr. Mostafa Ahmed (from Assiut University, Egypt)

07/2016 – 09/2016 Internship: MSc Aleksandra Engler (from Gdansk, Poland)
 since 06/2016 PhD thesis: MSc Yan-Cen Liu
 04/2016 – 08/2016 Internship: MSc Phoulinh Chanthavong (from L'Université Paris Descartes, France)
 01/2016 – 02/2016 Internship: BSc Sunhye Shin (from UNIST, South Korea)
 since 11/2015 PhD thesis: Mohammad Al-Najjar
 since 09/2015 PhD thesis: Shuai Zhang
 11/2015 – 10/2016 Internship: MSc Shu Peng (from Nankai University, Tianjin, China)
 09/2015 – 12/2015 Internship: BSc Yuchen Pan (from Nankai University, Tianjin, China)
 08/2015 – 10/2015 Internship: MSc Adam Lalewicz (with Prof. Nikolai Kuhnert, Jacobs University Bremen)
 06/2015 – 08/2015 Internship: MSc Can B. Uzundal (from Bilkent University, Ankara, Turkey)
 02/2015 – 07/2015 Internship: MSc Solène Collin (from Ecole Normale Supérieure de Lyon, France)
 10/2014 – 08/2015 MSc thesis: BSc Mohammed Nilam
 10/2014 – 05/2015 BSc thesis: Robert Hein
 03/2012 – 06/2015 Internship: Dipl.-Phys. Matthias Schnurr (part of PhD thesis with Dr. L. Schröder, FMP Berlin)
 06/2011 – 12/2015 PhD thesis: Dipl.-Chem. Jagoda Sloniec
 07/2012 Research project: BSc Marcel Steffin
 10/2010 – 04/2011 Diploma thesis: Jagoda Sloniec
 04/2006 – 10/2006 Research project: Garima Ghale
 09/2005 – 07/2007 BSc and MSc thesis: Mara Florea
 10/2004 Research project: Elvira Beitler
 03/2004 Research project: BSc Ahson J. Shaikh

Teaching Assistant for Lab Courses

2007 – 2009 Travaux Pratiques Chimie Organique (Master Level)
 2004 – 2006 Organic Chemistry (Sophomore Level)
 2003 Praktikum – Anorganische Chemie für Biologen (Freshman Level)
 2000 – 2003 Grundpraktikum – Organische Chemie (Sophomore Level)

Community Service

2018 – present Guest editor for special issue in “Molecules”:
 “Characterization Techniques in Supramolecular Chemistry”
 2016 – present Member of RSC Advances Reviewer Panel
 2011 – present Reviewer (Angew. Chem. Int. Ed., J. Am. Chem. Soc., Chem. Commun., Chem. Eur. J., ACS
 Appl. Mater. Interfaces, Bioconjugate Chem., RSC Advances, Org. Biomol. Chem., Bioorg. Med.
 Chem., Spectrochimica Acta, Molecules, Journal of Molecular Structure, Applied Surface
 Science, Int. J. Nanomedicine, and others)
 2008 – present Member of “Schweizerische Chemische Gesellschaft”
 2004 – present Member of “Gesellschaft Deutscher Chemiker e.V.”
 Member of the divisions “Photochemie” (since 2004) and “Analytische Chemie” (since 2010)

Language Skills

German (native)
 English (fluent, CEFR C1)
 French (basic knowledge, CEFR A1)

Invited Lectures

- 8 Aix-Marseille Université, 12.04.2019, Marseille (France), "Dynamic Host-Guest Chemistry in Complex Environments: A Tale From Supramolecular Sensors to Transporters and Materials"
- 7 BAM Federal Institute for Materials Research and Testing, 21.11.2016, Berlin (Germany), "The Supramolecular Displacement Principle in Analytical Chemistry".
- 6 X. International Workshop on Sensors and Molecular Recognition, 07.07.2016, Valencia (Spain), "The Supramolecular Approach to Enzyme and Membrane Transport Assays" (Plenary Lecture).
- 5 Czech Academy of Sciences, 01.02.2016, Prague (CZ), "Supramolecular Chemistry in Membrane Transport".
- 4 Kafrelsheikh University, 14.01.2016, Kafr El Sheikh (Egypt), "Supramolecular Chemistry for Bioanalytical Applications".
- 3 College of Chemistry, Nankai University, 13.10.2015, Taijin (China), "Biochemical Process Monitoring by Supramolecular Systems".
- 2 FMP Leibniz Institut für molekulare Pharmakologie, 15.02.2011, Berlin (D), "Fluorescent Derivatives of Cryptophan-A: Biomembrane Interactions of Potential Multimodal Contrast Agents".
- 1 4. DFG Exzellenzakademie Medizintechnik über Molekulare Bildgebung, 25.04.-01.05.2010, Aachen (D), "Eine neue Generation intelligenter optischer Fluoreszenzsonden".

Publications (>1900 citations, >39 Ø citations per article, h-index 21)

- 60 A. Hennig, W. M. Nau, Cucurbituril-Based Sensors and Assays, in: *Cucurbiturils and Related Macrocycles; Monographs in Supramolecular Chemistry Series*, K. Kim, Ed., RSC Publishing Cambridge, **2019**, in press.
- 59 A. Farcas, Y.-C. Liu, M. Nilam, M. Balan-Porcarasu, E.-L. Ursu, W. M. Nau, A. Hennig, Synthesis and Photophysical Properties of Inclusion Complexes Between Conjugated Polyazomethines with γ -Cyclodextrin and its Tris-O Methylated Derivative, *Eur. Polym. J.* **2019**, *113*, 236-243.
- 58 S. Zhang, K. I. Assaf, C. Huang, A. Hennig, W. M. Nau, Ratiometric DNA Sensing with a Host-Guest FRET Pair, *Chem. Commun.* **2019**, *55*, 651-674.
- 57 M. Nilam, M. Ahmed, M. A. Alnajjar, A. Hennig, Characterization of Mixed-Ligand Shells on Gold Nanoparticles by Transition Metal and Supramolecular Surface Probes, *Analyst* **2019**, *144*, 579-586.
- 56 S. Zhang, Z. Dominguez, K. I. Assaf, M. Nilam, T. Thiele, U. Pischel, U. Schedler, W. M. Nau, A. Hennig, Precise Supramolecular Control of Surface Coverage Densities on Polymer Micro- and Nanoparticles, *Chem. Sci.* **2018**, *9*, 8575-8581.
- 55 M. A. Alnajjar, J. Bartelmeß, R. Hein, P. Ashokkumar, M. Nilam, W. M. Nau, K. Rurack, A. Hennig, Rational Design of Boron-dipyrromethene (BODIPY) Reporter Dyes for Cucurbit[7]uril, *Beilstein J. Org. Chem.* **2018**, *14*, 1961-1971.
- 54 J. Döpfert, M. Schnurr, M. Kunth, H. M. Rose, A. Hennig, L. Schröder, Time-resolved Monitoring of Enzyme Activity with Ultrafast Hyper-CEST Spectroscopy, *Magn. Reson. Chem.* **2018**, *56*, 679-688.
- 53 S. Peng, A. Barba-Bon, Y.-C. Pan, W. M. Nau, D.-S. Guo, A. Hennig, Phosphorylation-Responsive Membrane Transport of Peptides, *Angew. Chem. Int. Ed.* **2017**, *56*, 15742-15745; Phosphorylierungs-regulierter Membrantransport von Peptiden, *Angew. Chem.* **2017**, *129*, 15948-15951.
- 52 A. Stahl, A. I. Lazar, V. N. Muchemu, W. M. Nau, M. S. Ullrich, A. Hennig, A Fluorescent Supramolecular Chemosensor to Follow Steroid Depletion in Bacterial Cultures, *Anal. Bioanal. Chem.* **2017**, *409*, 6485-6494.
- 51 M. Nilam, A. Hennig, W. M. Nau, K. I. Assaf, Gold Nanoparticle Aggregation Enables Colorimetric Sensing Assays for Enzymatic Decarboxylation, *Anal. Methods* **2017**, *9*, 2784-2787.
- 50 K. I. Assaf, A. Hennig, S. Peng, D.-S. Guo, D. Gabel, W. M. Nau, Hierarchical Host-Guest Assemblies Formed on Dodecaborate-Coated Gold Nanoparticles, *Chem. Commun.* **2017**, *53*, 4616-4619.
- 49 M. Nilam, P. Gribbon, J. Reinshagen, K. Cordts, E. Schwedhelm, W. M. Nau, A. Hennig, A Label-Free Continuous Fluorescence-based Assay for Monitoring Ornithine Decarboxylase Activity with a Synthetic Putrescine Receptor, *SLAS Discovery* **2017**, *22*, 906-914.
- 48 A. I. Lazar, F. Biedermann, K. R. Mustafina, K. I. Assaf, A. Hennig, W. M. Nau, Nanomolar Binding of Steroids to Cucurbit[n]urils: Selectivity and Applications, *J. Am. Chem. Soc.* **2016**, *138*, 13022-13029.

- 47 J. Sloniec-Myszk, U. Resch-Genger, A. Hennig, Chiral, J-Aggregate-Forming Dyes for Alternative Signal Modulation Mechanisms in Self-Immolative Enzyme-Activatable Optical Probes, *J. Phys. Chem. B* **2016**, *120*, 877-885.
- 46 R. Hein, C. B. Uzundal, A. Hennig, Simple and Rapid Quantification of Phospholipids for Supramolecular Membrane Transport Assays, *Org. Biomol. Chem.* **2016**, *14*, 2182-2185.
- 45 S. T. J. Ryan, R. M. Young, J. Henkelis, N. Hafezi, N. A. Vermeulen, A. Hennig, M. D. Krzyaniak, A. Karas, W. M. Nau, M. R. Wasielewski, J. F. Stoddart, O. A. Scherman, Ultrafast Energy Transfer in Water within a Series of Hexacationic Perylene Diimide/Cyclophane Catenanes, *J. Am. Chem. Soc.* **2015**, *137*, 15299-15307.
- 44 M. Schnurr, J. Sloniec-Myszk, J. Döpfert, L. Schröder, A. Hennig, Supramolecular Assays for Mapping Enzyme Activity by Displacement-Triggered Change in Hyperpolarized ^{129}Xe Magnetization Transfer NMR, *Angew. Chem. Int. Ed.* **2015**, *54*, 13444-13447; Supramolekulare Assays zur Lokalisation von Enzymaktivität durch Verdrängungs-induzierte Änderungen in Magnetisierungstransfer-NMR mit hyperpolarisiertem ^{129}Xe , *Angew. Chem.* **2015**, *127*, 13645-13648.
- 43 M. Kunth, C. Witte, A. Hennig, L. Schröder, Identification, Classification and Signal Amplification Capabilities of High-Turnover Gas Binding Hosts in Ultra-Sensitive NMR, *Chem. Sci.* **2015**, *6*, 6069-6075.
- 42 A. Hennig, P. M. Dietrich, F. Hemmann, T. Thiele, H. Borchering, A. Hoffmann, U. Schedler, C. Jäger, U. Resch-Genger, W. E. S. Unger, En Route to Traceable Reference Standards for Surface Group Quantifications by XPS, NMR and Fluorescence Spectroscopy, *Analyst* **2015**, *140*, 1804-1808.
- 41 P. Dietrich, A. Hennig, M. Holzweber, T. Thiele, H. Borchering, A. Lippitz, U. Schedler, U. Resch-Genger, W. Unger, Surface Analytical Study of Poly(acrylic acid)-Grafted Microparticles (Beads): Characterization, Chemical Derivatization and Quantification of Surface Carboxyl Group, *J. Phys. Chem. C* **2014**, *118*, 20393-20404.
- 40 A. Vargas Jentzsch, A. Hennig, J. Mareda, S. Matile, Synthetic Transporters that Work with Anion- π Interactions, Halogen Bonds and Anion-Macrodipole Interactions, *Acc. Chem. Res.* **2013**, *46*, 2791-2800.
- 39 J. Sloniec, U. Resch-Genger, A. Hennig, Photophysics and Release Kinetics of Enzyme-Activatable Optical Probes Based on H-Dimerized Fluorophores on Self-Immolative Linkers, *J. Phys. Chem. B* **2013**, *117*, 14336-14344.
- 38 A. Hennig, Supramolecular Enzyme Assays, in: *Supramolecular Systems in Biomedical Fields*, H.-J. Schneider, Ed., RSC Publishing Cambridge, **2013**, 355-396.
- 37 A. Hennig, S. Hatami, M. Spieles, U. Resch-Genger, Excitation Energy Migration and Trapping on the Surface of Fluorescent Poly(acrylic acid)-Grafted Polymer Particles, *Photochem. Photobiol. Sci.* **2013**, *12*, 729-737.
- 36 J. Sloniec, M. Schnurr, C. Witte, U. Resch-Genger, L. Schröder, A. Hennig, Biomembrane Interactions of Functionalized Cryptophane-A: Combined Fluorescence and ^{129}Xe NMR Studies of a Bimodal Contrast Agent, *Chem. Eur. J.* **2013**, *19*, 3110-3118.
- 35 A. Hennig, H. Borchering, C. Jaeger, S. Hatami, C. Würth, A. Hoffmann, K. Hoffmann, T. Thiele, U. Schedler, U. Resch-Genger, Scope and Limitations of Surface Functional Group Quantification Methods: An Exploratory Study with Poly(acrylic acid)-Grafted Micro- and Nanoparticles, *J. Am. Chem. Soc.* **2012**, *134*, 8268-8276.
- 34 B. Mizaikoff, M. Lämmerhofer, J. A. C. Broekaert, J. Popp, B. Lendl, K. Leopold, F. W. Scheller, A. Yarman, S. Schumacher, N. Plumeré, A. Hennig, W. M. Nau, C. Kranz, J. W. Einax, Trendbericht Analytische Chemie 2010/2011, *Nachr. Chem.* **2012**, *60*, 406-420.
- 33 R. N. Dsouza, A. Hennig, W. M. Nau, Supramolecular Tandem Enzyme Assays, *Chem. Eur. J.* **2012**, *18*, 3444-3459.
- 32 A. Hennig, A. Hoffmann, H. Borchering, T. Thiele, U. Schedler, U. Resch-Genger, Quantification of Surface Functional Groups on Polymer Microspheres by Supramolecular Host-Guest Interactions, *Chem. Commun.* **2011**, *47*, 7842-7844.
- 31 A. Hennig, A. Hoffmann, H. Borchering, T. Thiele, U. Schedler, U. Resch-Genger, Simple Colorimetric Method for Quantification of Surface Carboxy Groups on Polymer Particles, *Anal. Chem.* **2011**, *83*, 4970-4974.
- 30 U. Resch-Genger, T. Behnke, R. Brehm, M. Grabolle, L. Linck, A. Hennig, A. Hoffmann, K. Hoffmann, C. Lochmann, J. Pauli, M. Spieles, C. Würth, Funktionelle Chromophor-Systeme, innovative Validierungskonzepte und rückführbare Standards für die fluoreszenzbasierte multiparametrische Bioanalytik, in: *Multiparameteranalytik in Forschung und Praxis*, K. Conrad, D. Roggenbuck, W. Lehmann, U. Schedler, G. Peine, Eds., Pabst Science Publishers, Lengerich, **2011**, 102-124.
- 29 T. Takeuchi, J. Montenegro, A. Hennig, S. Matile, Pattern Generation with Counterion-Polyion Transporters for Fragrance Sensing in Fluorogenic Vesicles, *Chem. Sci.* **2011**, *2*, 303-307.

- 28 S. Matile, N. Sakai, A. Hennig, Transport Experiments in Membranes in *Supramolecular Chemistry: From Molecules to Nanomaterials*, J. W. Steed, P. A. Gale, E. V. Anslyn, B. D. Smith, Eds., Vol. 1: Concepts and Techniques, Wiley, Chichester, **2011**.
- 27 R. D. Dawson, A. Hennig, D. P. Weimann, D. Emery, V. Ravikumar, J. Montenegro, T. Takeuchi, S. Gabutti, M. Mayor, J. Mareda, C. A. Schalley, S. Matile, Functional Relevance of Anion- π Interactions: Evidence from Tandem Mass Spectrometry, Computational Studies, and Transmembrane Transport, *Nat. Chem.* **2010**, *2*, 533-538.
- 26 J. Montenegro-Garcia, A. Hennig, P. Geotti-Bianchini, G. A. Eggimann, D. Jeannerat, S. Matile, J. Misek, T. Schuster, N. A. Uhlich, A. Vargas, Functional Biosupramolecular Systems, *Chimia* **2009**, *63*, 881-884.
- 25 A. Hennig, L. Fischer, G. Guichard, S. Matile, Anion-Macrodipole Interactions: Self-Assembling Macrocyclic Anion-Selective Membrane Transporters that Respond to Membrane Polarization, *J. Am. Chem. Soc.* **2009**, *131*, 16889-16895.
- 24 W. M. Nau, G. Ghale, A. Hennig, H. Bakirci, D. Bailey, Substrate-Selective Supramolecular Tandem Assays: Monitoring Enzyme Inhibition of Arginase and Diamine Oxidase by Fluorescent Dye Displacement from Calixarene and Cucurbituril Macrocycles, *J. Am. Chem. Soc.* **2009**, *131*, 11558-11570.
- 23 A. Hennig, S. Hagihara, S. Matile, Hydrazinoanthrylboronic Acids as Supramolecular Exciton-Coupled Circular Dichroism (ECCD) Probes for Multivalent Catechols, Particularly Epigallocatechin Gallate, *Chirality* **2009**, *21*, 826-835.
- 22 S. M. Butterfield, A. Hennig, S. Matile, Colorful Methods to Detect Ion Channels and Pores: Intravesicular Chromogenic Probes that Respond to pH, pM and Covalent Capture, *Org. Biomol. Chem.* **2009**, *7*, 1784-1792.
- 21 A. Hennig, Development of Two Novel Fluorescent Enzyme Assays: Nano-TRF and Supramolecular Tandem Assays, *EPA Newsletters* **2008**, 122-124.
- 20 A. Hennig, S. Matile, Chirality Sensing with Pores: Reactive Amplifiers for Otherwise Inaccessible Small Molecules, *Chirality* **2008**, *21*, 145-151.
- 19 N. Banerji, R. Bhosale, G. Bollot, S. M. Butterfield, A. Fürstenberg, V. Gorteau, S. Hagihara, A. Hennig, S. Maity, J. Mareda, S. Matile, F. Mora, A. Perez-Velasco, V. Ravikumar, K. Ravuri, N. Sakai, D.-H. Tran, E. Vauthey, Artificial Tongues and Leaves, *Pure Appl. Chem.* **2008**, *80*, 1873-1882.
- 18 A. Hennig, S. Bhosale, N. Sakai, S. Matile, CD Methods Development at the Bio-Nano Interface, *Chimia* **2008**, *62*, 493-496.
- 17 A. Hennig, G. J. Gabriel, G. N. Tew, S. Matile, Stimuli-Responsive Polyguanidino-Oxanorbornene Transporters as Multicomponent Sensors in Complex Matrices, *J. Am. Chem. Soc.* **2008**, *130*, 10338-10344.
- 16 A. Hennig, S. Matile, Detection of the Activity of Ion Channels and Pores with Circular Dichroism Spectroscopy: G-Quartets as Functional CD Probes within Chirogenic Vesicles, *Chirality* **2008**, *20*, 932-937.
- 15 D. Bailey, A. Hennig, W. M. Nau, Supramolecular Tandem Enzyme Assays for Multiparameter Sensor Arrays and Enantiomeric Excess Determination of Amino Acids, *Chem. Eur. J.* **2008**, *14*, 6069-6077.
- 14 W. M. Nau, M. Florea, H. Sahoo, H. Bakirci, A. Hennig, D. Bailey, Nano-TRF und Tandem Assays für HTS und Multiparameteranalytik, in: *Multiparameteranalytik – Methoden, Applikationen, Perspektiven*, K. Conrad, W. Lehmann, U. Sack, U. Schedler, Eds., Pabst Verlag, Lengerich, **2008**, 147-162.
- 13 W. M. Nau, A. Hennig, A. L. Koner, Squeezing Fluorescent Dyes into Nanoscale Containers - The Supramolecular Approach to Radiative Decay Engineering, *Springer Series On Fluorescence*, Vol. 4, **2008**, 185-211.
- 12 A. Hennig, Development of Two Novel Fluorescent Enzyme Assays: Nano-TRF and Supramolecular Tandem Assays, *PhD Thesis* **2007**, Jacobs University Bremen, Germany.
- 11 H. Sahoo, A. Hennig, M. Florea, D. Roth, T. Enderle, W. M. Nau, Single-Label Kinase and Phosphatase Assays for Tyrosine Phosphorylation Using Nanosecond Time-Resolved Fluorescence Detection, *J. Am. Chem. Soc.* **2007**, *129*, 15927-15934.
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- 9 A. Hennig, H. Bakirci, W. M. Nau, Lysine Decarboxylase Assay with Cucurbituril, *Protocol Exchange* **2007**, doi:10.1038/nprot.2007.281.
- 8 A. Hennig, H. Bakirci, W. M. Nau, Label-free Continuous Enzyme Assays with Macrocyclic-Fluorescent Dye Complexes, *Nat. Methods* **2007**, *4*, 629-632.
- 7 A. Hennig, T. Schwarzlose, W. M. Nau, Bridgehead Carboxy-Substituted 2,3-Diazabicyclo[2.2.2]oct-2-enes: Synthesis, Fluorescent Properties and Host-Guest Complexation Behavior, *Arkivoc* **2007**, *8*, 341-357.

- 6 A. Hennig, G. Ghale, W. M. Nau, Effects of Cucurbit[7]uril on Enzyme Activity, *Chem. Commun.* **2007**, 1614-1616.
- 5 A. Hennig, M. Florea, D. Roth, T. Enderle, W. M. Nau, Design of Peptide Substrates for Nanosecond Time-Resolved Fluorescence Assays of Proteases: 2,3-Diazabicyclo[2.2.2]oct-2-ene as a Noninvasive Fluorophore, *Anal. Biochem.* **2007**, *360*, 255-265.
- 4 H. Sahoo, A. Hennig, W. M. Nau, Temperature-Dependent Loop Formation Kinetics in Flexible Peptides Studied by Time-Resolved Fluorescence Spectroscopy, *Int. J. Photoenergy* **2006**, Article ID 89638, 9 pages.
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- 1 A. Hennig, Analyse und Synthese von quorum-sensing aktiven N-Acylhomoserinlactonen, *Diplomarbeit* **2004**, TU Carolo Wilhelmina zu Braunschweig, Deutschland.

Patents

- 1 W. M. Nau, A. Hennig, H. Bakirci, Bestimmung von Konzentrationsänderungen mit Hilfe von supra-biomolekularen Tandem-Assays, 2007, PCT Int. Appl., WO 2007131696 A1 20071122.

Conference, Workshops, Presentations

P = Poster, V = Oral Presentation, W = Workshop (no contribution)

- 1W NRP Meeting: „Intellectual Property and Technology Transfer from the Viewpoint of Science and Research“, 12.06.2002, Bern (CH).
- 2P 37. Diskussionstagung der DGMS (Deutsche Gesellschaft für Massenspektrometrie), 07.03.-10.03.2004, Leipzig (D), „Massenspektrometrische Untersuchung derivatisierter N-Acyl-homoserinlactone“.
- 3W 2nd European Short Course on Principles & Applications of Time-Resolved Fluorescence Spectroscopy, 01.11.-05.11.2004, Berlin (D).
- 4P Fachgruppentagung „Photochemie“ der GDCh, 29.03.-31.05.2005, Jena (D), „Fast Time-Resolved Fluorescent (Fast TRF) Protease Assays“.
- 5P NRP Spring School: Supramolecular Functional Materials, 11.04.-15.04.2005, Murten (CH), „Supramolecular Radiative Decay Engineering (SRDE) with Cucurbituril“.
- 6V NRP Final Symposium: Supramolecular Functional Materials, 16.06.-18.06.2005, Murten (CH), „Enhancement on time-resolved energy transfer assays by supramolecular complex formation“.
- 8P Central European Conference on Photochemistry (CECP), 05.03.-09.03.2006, Bad Hofgastein (A), „Fluorescence Lifetime-Based Sensor Applications: Nano-TRF Assays & Lifetime Enhancers“, and „Nanosecond Time-Resolved Fluorescence (Nano-TRF) Assays – Application to Proteases“.
- 9P Joint International Symposium on Macrocyclic & Supramolecular Chemistry, 25.06.-30.06.2006, Victoria, BC (CDN), „Interactions of Cucurbituril with Peptide Substrates in Enzyme Assays: Potential Applications“.
- 10P ChemieContact – Innovation sucht Partner des VCI (Verband der Chemischen Industrie e.V.), 10.10.2006, Hamburg (D), „Enzym- und Katalysatorscreening mit Hilfe von supra-biomolekularen Tandem-Assays“.
- 11P Bremen Molecular and Marine Biology (BMMB) meeting, 26.01.-27.01.2007, Etelsen (D), „Enzyme and Catalyst Screening by Supra-Biomolecular Tandem Assays“.
- 12P II. International Symposium on Macrocyclic & Supramolecular Chemistry, 24.06.-28.06.2007, Salice Terme (IT), „Supramolecular Tandem Assays – Application Examples“.
- 13W CUSO's Summer School: Bottom-Up Approach to Nanotechnology, 26-30.08.2007, Villars (CH).
- 14P 20th International Symposium on Chirality (ISCD), 06.07.-09.07.2008, Geneva (CH), „CD Methods in the Nano-Biosciences – Beyond Structure Determination“.
- 15P SCS Fall Meeting, 11.09.2008, Zürich (CH), „Applications of CD Spectroscopy in the Nano-Biosciences – A Look Beyond Structural Studies“.

- 16P Frontiers in Chemical Biology, 25.01.-27.01.2009, Luzern (CH), "Methods Development at the Chemistry-Biology Interface".
- 17P CUSO's Summer School: Functional Biosupramolecular Systems, 23-27.08.2009, Villars (CH), "Anion- π Slides: Anion- π Interactions at Work"
- 18W 18th Swiss NMR Symposium, 09.09.2009, Geneva (CH).
- 19W 2. KISSWIN-Tagung: Lust auf wissenschaftliche Karriere in Deutschland! Wege, Förderungen und Netzwerke im Überblick, 25.02.2010, Berlin (D).
- 20V 4. DFG Exzellenz Akademie Medizintechnik über Molekulare Bildgebung, 25.04.-01.05.2010, Aachen (D), "Eine neue Generation intelligenter optischer Fluoreszenzsonden".
- 21V FMP Leibniz Institut für molekulare Pharmakologie, 15.02.2011, Berlin (D), "Fluorescent Derivatives of Cryptophan-A: Biomembrane Interactions of Potential Multimodal Contrast Agents".
- 22W Kolloidchemie: Grundlagen und moderne Entwicklungen, 14.03.-16.03.2011, Aachen (D).
- 23P International Bunsen Discussion meeting on Förster Resonance Energy Transfer in the Life Sciences, 27.03.-30.03.2011, Göttingen (D), "A FRET-Based Method to probe biomembrane interactions of multimodal contrast agents".
- 24V 2nd International Conference on Cucurbiturils (ICCB), 29.06.-02.07.2011, Cambridge (UK), "Quantification of surface functional groups by supramolecular host-guest interactions".
- 25W BAM-Fortbildung (hD): "Kommunikation und Zusammenarbeit", 23.07.-26.07.2012, Berlin (D).
- 26V 4th EuCheMS 2012, 26.08.-30.08.2012, Prague (CZ), "Scope and Limitations of Surface Group Quantification Methods".
- 27V Fachgruppentagung "Photochemie" der GDCh, 08.10.-12.10.2012, Potsdam (D), "Quantification of Surface Functional Groups by Spectroscopic Methods".
- 28W Fortbildung der Deutsche Akkreditierungsstelle GmbH (DAkkS), 23.06.-25.06.2014, Berlin (D), „Akkreditierungs- und Begutachtungstechnik für Inspektionsstellen“.
- 29W Fortbildung der Deutsche Akkreditierungsstelle GmbH (DAkkS), 06.11.-07.11.2014, Berlin (D), „Akkreditierungs- und Begutachtungstechnik für Prüflaboratorien“.
- 30W SupraChem 2015, 23.02.-24.02.2015, Berlin (D).
- 31V 4th International Conference on Cucurbiturils (ICCB), 08.10.-10.10.2015, Tianjin (China), "Molecular Imaging with ^{129}Xe NMR and Cucurbiturils – Highly Sensitive Contrast Agents for Detection of Enzyme Activity".
- 32V Kafrelsheikh University, 14.01.2016, Kafr El Sheikh (Egypt), "Supramolecular Chemistry for Bioanalytical Applications".
- 33V Czech Academy of Sciences, 01.02.2016, Prague (CZ), "Supramolecular Chemistry in Membrane Transport".
- 34V X. International Workshop on Sensors and Molecular Recognition, 07.-08.07.2016, Valencia (Spain), "The Supramolecular Approach to Enzyme and Membrane Transport Assays" (Plenary Lecture).
- 35V Annual Meeting of the German Biophysical Society (DGfB) and Satellite Meeting Dynamic Interactions at Biomembranes, 25.-29.09.2016, Erlangen (Germany), "Supramolecular Membrane Tandem Assays – A Facile Way to Monitor Membrane Transport".
- 36V BAM Federal Institute for Materials Research and Testing, 21.11.2016, Berlin (Germany), "The Supramolecular Displacement Principle in Analytical Chemistry".
- 37V SupraChem 2017, 12.02.-14.02.2017, Aachen (D), "Phosphorylation-Sensitive Peptide Recognition by Calixarenes Affords Selective Activation of Membrane Transport and Label-Free, Fluorescent Kinase Assays".
- 38W Workshop: „How to Write a Competitive Proposal for Horizon 2020“, 11.05.2017, Bremen (D).
- 39V 5th International Conference on Cucurbiturils (ICCB), 27.06.-30.06.2017, Brno (CZ), "Cucurbituril-Functionalized Polymer Microspheres for Traceable Bead-Based Assays".
- 40V 14th International Conference on Calixarenes, 20.8.-24.08.2017, Tianjin (China), "Phosphorylation-Responsive Membrane Transport with Amphiphilic Calixarenes".
- 41V Chemiedozententagung 2018, 05.03.-07.03.2018, Jena (Germany), "Precise Control of Surface Coverage Densities on Micro- and Nanoparticles by Supramolecular Host-Guest Chemistry".
- 42V SupraChem 2019, 24.02.-26.02.2019, Würzburg (D), "Efficient Cellular Delivery of Peptides by Amphiphilic Calixarenes".

Bremen, July 16th, 2019