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Aromatic Interactions by Molecular Tweezers and Clips in Chemical and Biological Systems

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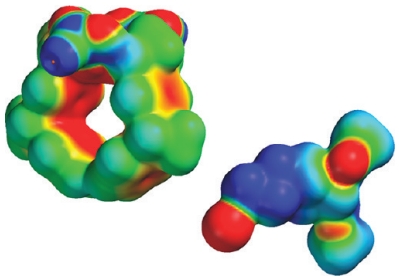

Noncovalent interactions involving aromatic rings, such as π-stacking and CH$-\pi$, occur throughout a range of fundamental processes including self-assembly and (bio)catalysis. Molecular clips and tweezers possess a central parallel or torus-shaped cavity with a surrounding belt of convergent aromatic rings; hence these structures exploit multiple aromatic interactions in a positively cooperative manner. Both clips and tweezers demonstrate selective binding of cationic or neutral guests that bear acceptor groups. The electrostatic surface potentials (ESP) explain this unexpected behavior: calculated ESPs were highly negative inside the tweezer or clip cavity, providing complementary profiles to the positive ESP plots of their preferred guest molecules. This Account presents more complex systems that use aromatic clips and tweezers to alter the reactivities of included guest species, to distinguish between guest enantiomers, and to interfere with biological processes such as enzymatic activity and protein aggregation.

Napthalene tweezers show potential applications in organocatalysis. When pyridinium moieties are bound within the spacious cavity of naphthyl-spaced tweezers, the resulting complex significantly influences the first step of single-electron reductions of (bi)pyridinium salts. In addition, the environment within the tweezer cavity strongly accelerates the Menshutkin reaction (the alkylation of pyridine derivatives).

Introduction of phosphonate, phosphate, or sulfate anions into the central aromatic bridge renders clips and tweezers water-soluble. Larger systems form extremely tight intertwined dimers that rely on the nonclassical hydrophobic effect for their stability. Smaller clips and tweezers with a simple benzene bridge remain monomeric in buffered aqueous solution and display a complementary binding profile. While the clips with parallel sidewalls prefer flat aromatic cations such as pyridinium salts, the torus-shaped tweezers bind to basic amino acids lysine and arginine via a threading process. These mutually exclusive binding modes make water-soluble clips and tweezers valuable tools for probing critical biological interactions with positively charged amino acid side chains and cofactors.

Molecular clips and tweezers can be employed for the complete inhibition of dehydrogenases. The clip extracts NAD^+ from its Rossman fold, while the tweezer complexes access strategic lysine residues around the active site. Our new enzyme inhibitors recognize the protein surface and thus offer additional targets for medicinal chemistry. Finally, the ability of molecular tweezers to cap critical lysine residues can be used to interfere with the pathology of protein misfolding diseases such as Alzheimer's disease, because many of them involve noncovalent interactions with these critical residues during their early stages. When the key protein produces a β-sheet-rich nucleus, this structure undergoes spontaneous polymerization into highly toxic oligomers, ultimately leading to mature fibrils. The benzene-spaced phosphate tweezer forms a specific complex with lysine residues 16 and 28 in Aβ42 and thus prevents the formation of misfolded oligomers rich in β-sheets. This entirely new process-specific mechanism that prevents pathologic protein aggregation also operates in many other related amyloidogenic proteins.

Introduction

Noncovalent intra- and intermolecular interactions involving aromatic rings are existent in many molecular and supramolecular chemical as well as biological systems. They play an important role in processes such as molecular recognition, self-assembly, chemical transport, and catalysis.[1] Three geometries of the benzene dimer (Figure 1), which are important for the understanding of aromatic interactions, were calculated at high level of theory to be attractive in nature favoring the parallel displaced and T-shaped edge-to-face

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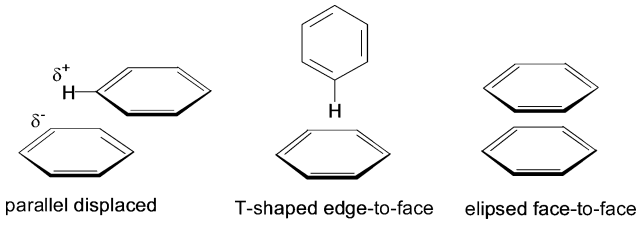


FIGURE 1. Noncovalent interaction geometries of two benzene molecules.

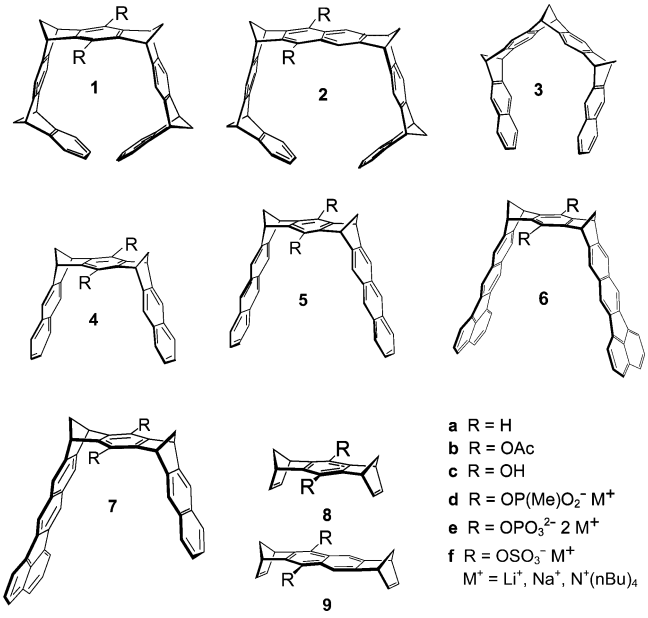


FIGURE 2. Structures of the molecular tweezers **1** and **2,** molecular clips **3**, **4**, **5**, **6**, and **7** and bridges **8** and **9**.

geometry.[2] The eclipsed face-to-face orientation (the so-called π stacking) is preferred if at least one benzene ring is substituted by electron-donating or -withdrawing groups.[3] Beside the electrostatic forces, dispersive interactions stabilize benzene dimers. Recently, Wheeler and Houk concluded from the calculation of electrostatic surface potential (ESP) maps that through-space effects (and not π resonance effects) of the substituents dominate the binding interactions between aromatic rings. This conclusion also rationalizes the excellent performance of molecular mechanics (MM) force fields for the description of $\pi-\pi$ interactions in host–guest complexes.[4]

More than 15 years ago, we started our own studies in this field with the simple question whether multiple arene–arene interactions lead to synergetic effects as it has been observed for multiple hydrogens bonds.[5] To answer this question, the parent and the substituted tetramethylene-bridged molecular tweezers **1a,b,c** and **2a,b**, the trimethylene-bridged molecular clip **3**, and the dimethylene-bridged clips **4a,b,c** and **5b,c** (Figure 2) were synthesized.[6] The synthesis and some fundamental supramolecular properties of these compounds (which function as host molecules in host–guest complexes) were already reviewed in *Accounts of Chemical Research* in 2003.[7] The dynamics of the host–guest complexes (the mutual complex dissociation/association and the guest rotation inside the host cavity) were reported in detail more recently.[8] These molecules are well preorganized because of their belt-type structures. Indeed, they all form host–guest complexes, for example, in chloroform solution, by multiple arene–arene and alkane–arene interactions. Generally, the complexes of the tweezers **1** and **2** with five aromatic binding sites are more stable than those of the molecular clips **3**, **4**, and **5** having only four or three aromatic moieties.[9] The benzene-spaced tweezers of type **1** prefer to bind alkane chains of aliphatic guest molecules inside their cavity via CH–π interactions, whereas the tweezers of type **2** bind aromatic guest molecules preferentially via CH–π and $\pi-\pi$ interactions, with the guest benzene ring oriented parallel to the central naphthalene spacer unit. The molecular clips of type **3**, **4**, and **5** bind aromatic guests by positioning the aromatic guest rings inside the host cavities parallel to the naphthalene or anthracene sidewalls.[10] The clips of type **5** and **6** (having larger anthracene or benzo[*k*]fluoranthene sidewalls) even bind polyaromatic guest molecules that are not complexed by clips of type **4** having only naphthalene sidewalls.[11]

All these host molecules only bind cationic or neutral guest molecules bearing acceptor groups. No complex formation could be detected with anionic or neutral guest molecules substituted with donor groups. This finding has been explained with electrostatic surface potential (ESP) contour plots, which were calculated by quantum chemical methods to be highly negative inside the tweezer or clip cavity and hence complementary to the positive ESP maps of the guest molecules that are bound by these hosts.[7]

This Account reports on more complex advanced systems, which pave the way toward numerous potential applications of clips and tweezers in materials science and biomedicine. We specifically discuss the host–guest complex formation of the molecular tweezers of type **2** with 4,4-bipyridinium salts bearing dendritic substituents and their influence on the reactivity of these salts in solution and gas phase The main focus of this report is, however, on the properties of water-soluble tweezers and clips and their remarkable effect on enzymatic reactions and protein aggregation. Therefore, we omit the discussion of molecular clips of type **4** carrying either one or two substituents at the tips of their naphthalene sidewalls. These compounds are

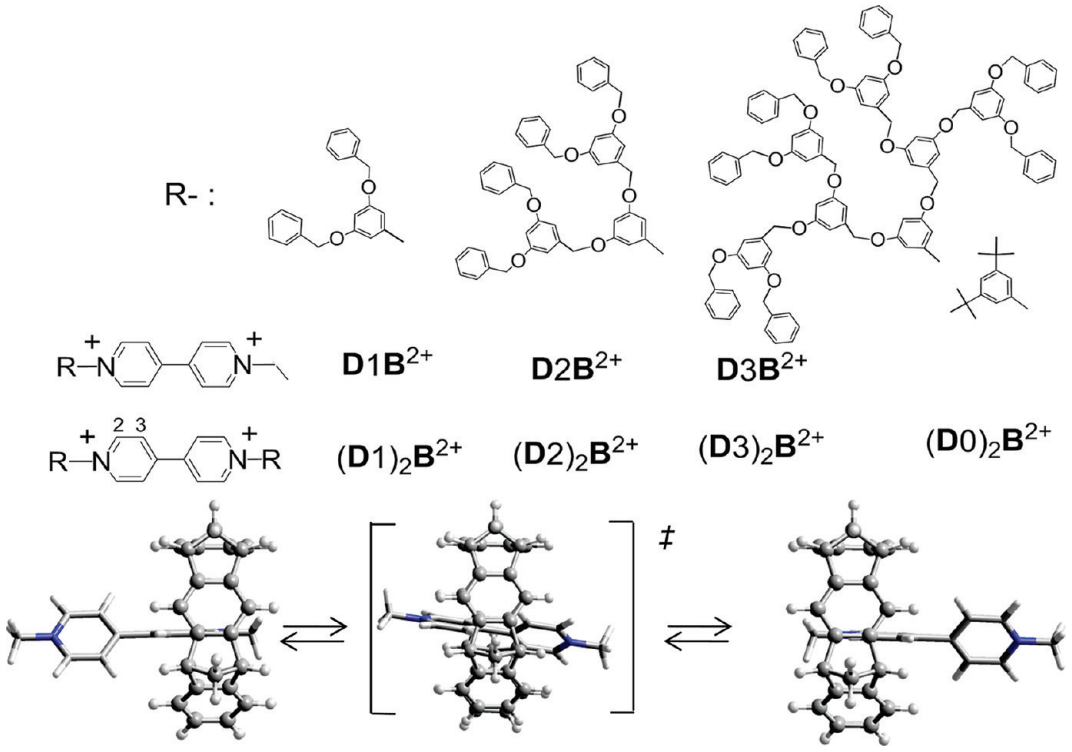


FIGURE 3. (top) Structures of dendrimers, which form stable host–guest complexes with naphthalene tweezer **2b**, and (bottom) the shuttling process of tweezer **2a** (R = H) along the axis of the *N*,*N*-dimethyl-bipyridinium dication calculated by force-field MMFF 94.

inherently chiral and can be used for the selective molecular recognition of enantiomers.[12]

Host–Guest Complexes of Molecular Tweezers with 4,4-Bipyridinium Salts

Because of their large structures and numerous functionalities dendrimers have been extensively used as hosts for a variety of guest molecules and metal ions. On the other hand, they can also be involved as guest molecules in molecular recognition phenomena. In such cases, the host species does not interact with the whole dendritic structure, but only with specific parts.[13] If the core of a symmetric dendrimer serves as the potential guest unit, host–guest complex formation with a ring-shaped host cannot take place, because it requires a threading process. Tweezers, however, are able to clip the dendritic core through the pincers' tips and, hence, to influence the reactivity of the dendrimer. In solution, tweezer **2b** (R = OAc) forms stable host–guest complexes with the bipyridinium core of the dendrimers shown in Figure 3 (top). The binding constants (determined by spectrofluorimetric titrations) are large in dichloromethane solution ($K_a > 15\,000\ M^{-1}$) but decrease with increasing size of the dendritic groups. They are solvent-dependent; in a more polar solvent mixture (CH_2Cl_2/CH_3CN, 1:3) they are smaller by almost 1 order of magnitude. Large ^{1}H NMR upfield shifts of the guest protons attached to pyridinium rings (up to 3 ppm) and temperature-dependent ^{1}H NMR experiments provide evidence for the fact that in the host–guest complexes the bipyridinium core is positioned inside the tweezer's cavity and the tweezer shuttles from one to the other pyridinium ring along the axis of the bipyridinium core as shown in Figure 3 (bottom).[14]

The bipyridinium core of the dendrimers is electroactive and undergoes two successive, reversible one-electron reductions. The half-wave potential of the first one-electron reduction moves to a more negative value upon addition of tweezer **2b** (e.g., for the reduction of $D1B^{2+}$ and $D1B^{2+}\cdot$**2b**, $B^{2+} \rightarrow B^+$, −0.29 or −0.36 V), whereas the peaks corresponding to the second reduction process remain practically unaffected ($B^+ \rightarrow B$, −0.77 or −0.77 V) by the tweezer. This finding indicates that the bipyridinium core is stabilized by complexation and that the complex of the radical cation (resulting from the first reduction) dissociates before the radical cation is further reduced. A similar strong effect of the tweezer **2b** was observed for the Menshutkin reaction of 4-cyanopyridine with methyl iodide leading to 1-methyl-4-cyanopyridinium iodide, which is accelerated in the presence of **2b** at 25 °C by a factor of 3000.[15] Obviously, the polarized transition state of this reaction is stabilized inside the electron-rich reaction chamber.

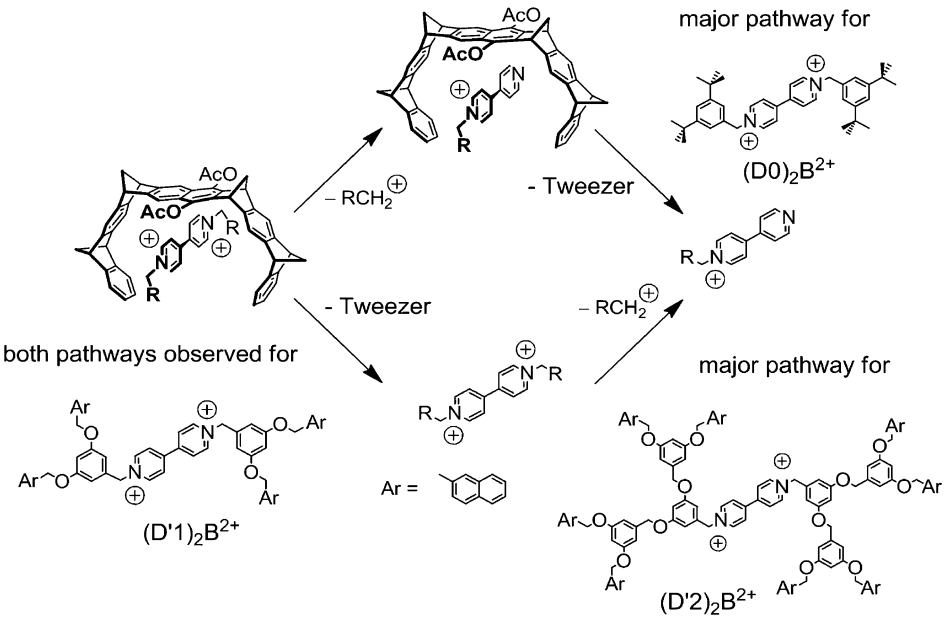


FIGURE 4. Pattern of fragmentation determined for tweezer–bipyridinium complexes by the use of electrospray ionization Fourier-transform ion-cyclotron-resonance (ESI-FT-ICR) mass spectroscopy in the gas phase.

The behavior of the tweezer–bipyridinium complexes could also be determined in the gas phase by using mass spectrometry (Figure 4).[16] The monoisotopic complex ions were isolated in an FT-ICR cell and subjected to collision with argon. In the tweezer complex of $(D0)_2B^{2+}\cdot$**2b**, the benzylic C–N bond is exclusively cleaved under these conditions leading to the tweezer–monocation complex, which subsequently dissociates the tweezer molecule, whereas the complex of the tweezer with the dendrimer of second generation (G2), $(D'2)_2B^{2+}\cdot$**2b**, loses the tweezer in the first step and then in a second step the benzylic C–N bond in the bipyridinium core is cleaved. In the complex with dendrimer of first generation (G1), $(D'1)_2B^{2+}\cdot$**2b**, a competition between these two reactions is observed in the first step. Evidently, dendritic substituents of increasing generation at the bipyridinium core provide increasing stability of the naked dication in the gas phase. This finding can be explained by force-field calculations (Figure 5). The G2 dendron is calculated to completely embrace the bipyridinium dication by back folding of the dendritic "arms" leading to an "intramolecular solvation" and hence to a stabilization of the bipyridinium core. The bipyridinium core is, however, calculated to be less efficiently embraced by folding of the G1 dendron and not embraced at all by the (D0) aryl groups leading more and more to the naked dication, which is calculated by quantum chemical methods to be not stable in the gas phase. This backfolding of the dendritic groups also occurs in CH_2Cl_2 solution and may well explain why tweezer **2b** affinities toward bipyridinium ions drop with increasing dendrimer generation (Figure 5).

The altered reactivity of included guest molecules inside the tweezer or clip cavities discussed in this section suggest their development into new photo- and redox-active reagents and organocatalysts with largely unexplored potential.

Supramolecular Properties of Water-Soluble Tweezers and Clips

Until 2002, all investigations of the above-mentioned clips and tweezers were conducted in organic solvents. A whole new door was opened when we decided to attach phosphonate, phosphate, or sulfate substituents onto the central benzene or naphthalene bridges, because these molecular tweezers of type **1** and **2** and clips of type **4**, **5**, and **7** are water-soluble. The general scheme of the synthesis of the benzene-spaced tweezers **1d,e,f** or clips **4d,e,f**, **5,d,f**, or **7d** starting from the corresponding hydroquinone **1c**, **4c**, **5c**, or **7c** is shown in Figure 6.[17–21,23] The naphthalene-spaced tweezer **2d** was not accessible by this route because of the instability of the naphthohydroquinone tweezer **2c**. The successful synthesis of **2d** started, therefore, from the naphthalene bridge of type **9** as precursor already substituted with phosphonate groups (Figure 2, R = OPO(Me)(OMe)).[22]

It was soon recognized that complexes of the anionic clips and tweezers with guest molecules in protic solvents are generally very stable; especially in water, hydrophobic

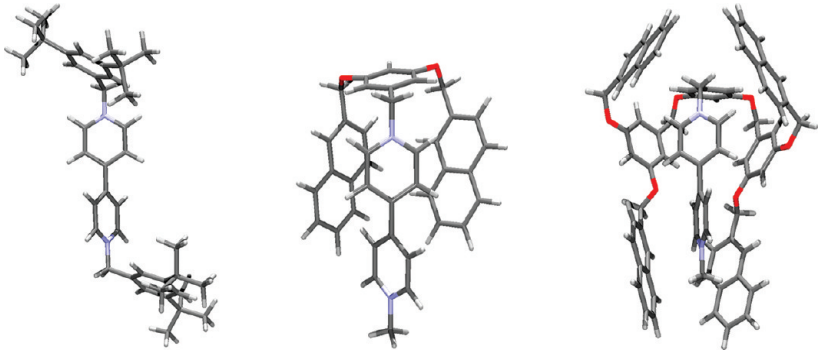


FIGURE 5. Monte Carlo simulation of bipyridinium structures (MMFF94): (left) $(D0)_2B^{2+}$; (middle) $(D'1)(Me)B^{2+}$; (right) $(D'2)(Me)B^{2+}$.

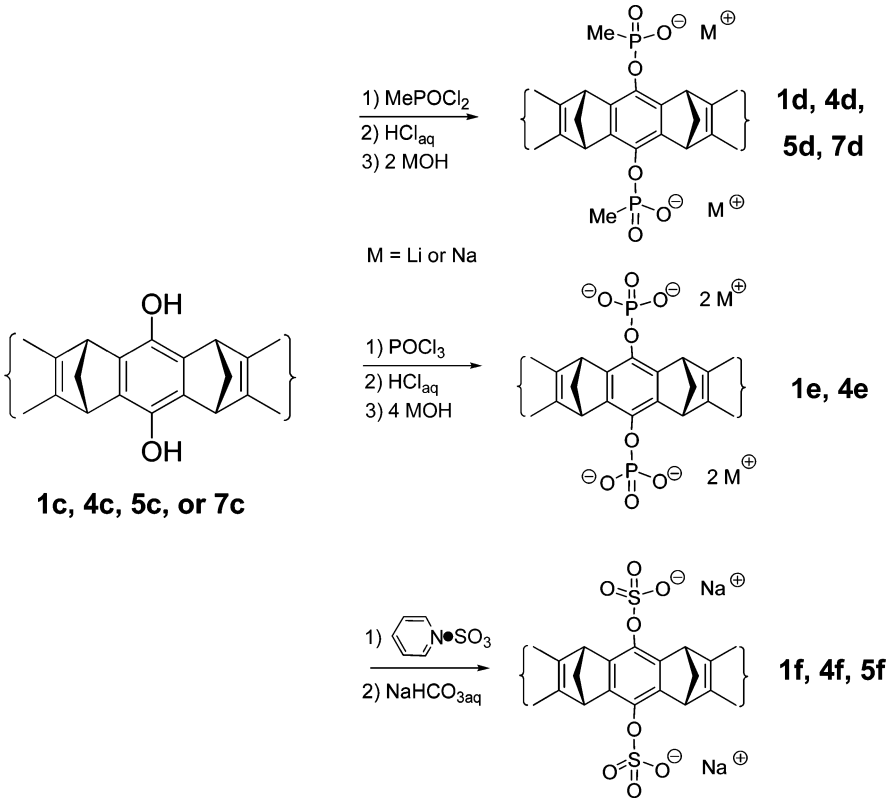


FIGURE 6. General synthesis scheme of water-soluble tweezers and clips.

forces complement $\pi-\pi$ interactions, and cationic guests are also attracted by the host anion. In water, the competition between self-association and guest inclusion strongly influences the host properties, and the size and shape of the water-soluble host determine its binding profile.

The naphthalene-bridged tweezer **2d**[22] and the clips **5d, f**[20,22] and **7d**[23] (having expanded anthracene or benzo[*k*]fluoranthene sidewalls) form highly stable self-assembled dimers in water, whereas dimers of the benzene-bridged tweezers **1d,e,f** and clips **4d,e,f** (having naphthalene sidewalls only) are weak. The structures of the dimers $(\mathbf{2d})_2$ and $(\mathbf{5d})_2$ (Figure 7) were assigned by comparison of their experimental chemical ^{1}H NMR shifts with those calculated by quantum chemical ab initio methods.[22] The ^{1}H NMR shift data of dimers $(\mathbf{5f})_2$ and $(\mathbf{7d})_2$ also suggest intertwined structures comparable to that of $(\mathbf{5d})_2$. All these systems exist as monomers in pure methanol. The negative values of the enthalpy and entropy of dimerization (Table 1) indicate that nonclassical hydrophobic interactions are responsible for the observed dimer formation in water.

Addition of acetylcholine to the aqueous solution of the dimeric clip $(\mathbf{5f})_2$ leads to the disruption of the dimer.[20] In the (1:1) adduct between monomeric **5f** and the dansyl dendrimer (Figure 8), formed in acidic methanol solution, a very

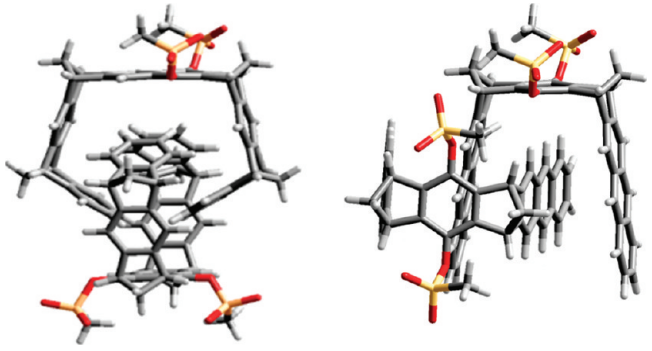


FIGURE 7. Structures of the dimers of phosphonate-substituted naphthalene tweezer (**2d**)$_2$ and anthracene clip (**5d**)$_2$ calculated by force field (MacroModel 6.5, Amber*/H_2O, Monte Carlo conformer search, 5000 structures).

TABLE 1. The Thermodynamic Parameters, log K_{Dim} [M^{-1}], ΔG [kcal mol^{-1}], ΔH [kcal mol^{-1}], and $T\Delta S$ [kcal mol^{-1}] determined for the Self-Assembled Dimerization of the Molecular Tweezers and Clips at 298 K in Aqueous Solution by ^{1}H NMR Spectroscopy at Variable Temperatures[a]

reaction	log K_{Dim}	ΔG	ΔH	$T\Delta S$
2 **1e** ⇆ (**1e**)$_2$	1.7			
2 **2d** ⇆ (**2d**)$_2$	**6.4**	**−8.7**	**−20.9**	**−12.2**
2 **4e** ⇆ (**4e**)$_2$	2.1			
2 **4f** ⇆ (**4f**)$_2$	2.5			
2 **5d** ⇆ (**5d**)$_2$	**5.2**	**−7.1**	**−13.8**	**−6.7**
2 **5f** ⇆ (**5f**)$_2$	**5.3**	**−7.2**	**−12.4**	**−5.2**
2 **7d** ⇆ (**7d**)$_2$	**6.0**	**−8.1**	**−15.2**	**−7.1**

[a]The thermodynamic data of tweezers and clips forming highly stable dimers are printed in bold type.

efficient energy transfer takes place from the excited clip anthracene units to the dansyl chromophores of the dendrimer. In dendrimers having light-harvesting antenna, an energy transfer from the excited dendrimer antenna to clip **5f** was observed driven by the addition of Zn^{2+} ions[24] or lanthanide ions.[25]

The nicotinamide adenine dinucleotides NAD^+ and $NADP^+$ are important cofactors for many enzymatic redox processes. In buffered aqueous solution at neutral pH, NAD^+ and $NADP^+$ (Figure 8) form stable host−guest complexes with the phosphonate-, phosphate-, and sulfate-substituted clips **4d,e,f** (NAD^+ $K_a = 4900$, 7100, or 1200 M^{-1}).[20,26] The structures of the host−guest complexes between clips **4d** or **4e** and NAD^+ were elucidated by quantum chemical ^{1}H NMR shift calculations.[26] For example, the complexation-induced ^{1}H NMR shifts, $\Delta\delta_{max}$, calculated for the structure shown in Figure 9 (left) agree well with the experimental data obtained for complex $NAD^+ \cdot$ **4d** indicating that the active site of NAD^+, the nicotinamide ring, is preferentially bound inside the clip cavity. Similar large $\Delta\delta_{max}$ values of the guest protons were found for the related complexes of NAD^+ with **4f** and $NADP^+$ with clips **4d,e**. The mononucleotides NMN and AMP, fragments of NAD^+, form complexes with the clips **4d** and **4e** but of lower stability. The AMP complex also displays large $\Delta\delta_{max}$ values of the guest protons indicating that the adenosine unit can also be incorporated inside the clip cavity, presumably in the $NAD(P)^+$ complexes to a minor extent. A systematic study of nucleosides, nucleotides, and derivatives (such as caffeine) reveals that in buffered aqueous solution, the phosphate-substituted clip **4e** binds, for example, caffeine more strongly than pyrimidine- or purine-containing nucleosides and nucleotides.[27] The phosphonate-substituted clip **4d** is less selective. The clips **4d,e** also bind other enzyme cofactors such as *S*-adenosylmethionine (SAM) and thiamine diphosphate (TPP).[18] To the best of our knowledge, our anionic clips are the only artificial host compounds which embrace these three biological cofactors at their chemically active functional groups. This renders them promising candidates for a potential external interference with cofactor-assisted biological processes.

The benzene tweezers **1d,e** do not bind NAD^+ within the limits of NMR detection. They bind, however, basic amino acids such as lysine and arginine. The complexes of the phosphate-substituted tweezer **1e** are substantially more stable than those of the phosphonate tweezer **1d**.[19,21] The large $\Delta\delta_{max}$ value observed for methylene protons attached to the ammonium function of the AcLysOMe provides experimental evidence for the inclusion of the aliphatic lysine side chain inside the tweezer cavity as indicated by the complex structure calculated by a Monte Carlo simulation (Figure 9, right). No other synthetic receptor molecule forms such a tight and well-defined complex with lysine; when peptides were titrated and also efficiently decorated with a tweezer on each lysine, it was only a question of time until the tweezers' profound influence on protein activities and protein−protein interactions were discovered. Today the great potential of molecular tweezers as diagnostic tools and therapeutic agents is explored in several national and international collaborations with protein chemists.

The Effect of Water-Soluble Tweezers and Clips on Enzymatic Reactions

Two case studies will be presented to explain how the water-soluble clips and tweezers introduced new mechanisms of enzyme inhibition: alcohol dehydrogenase (ADH) and glucose-6-phosphate-dehydrogenase (G6PD). Both enzymes use $NAD(P)^+$ as a cofactor and carry multiple lysines on their surfaces.

The phosphate clip **4e** inhibits the enzymatic oxidation of ethanol with NAD^+ catalyzed by alcohol dehydrogenase (ADH) with an IC_{50} value of 1500 μM and an almost

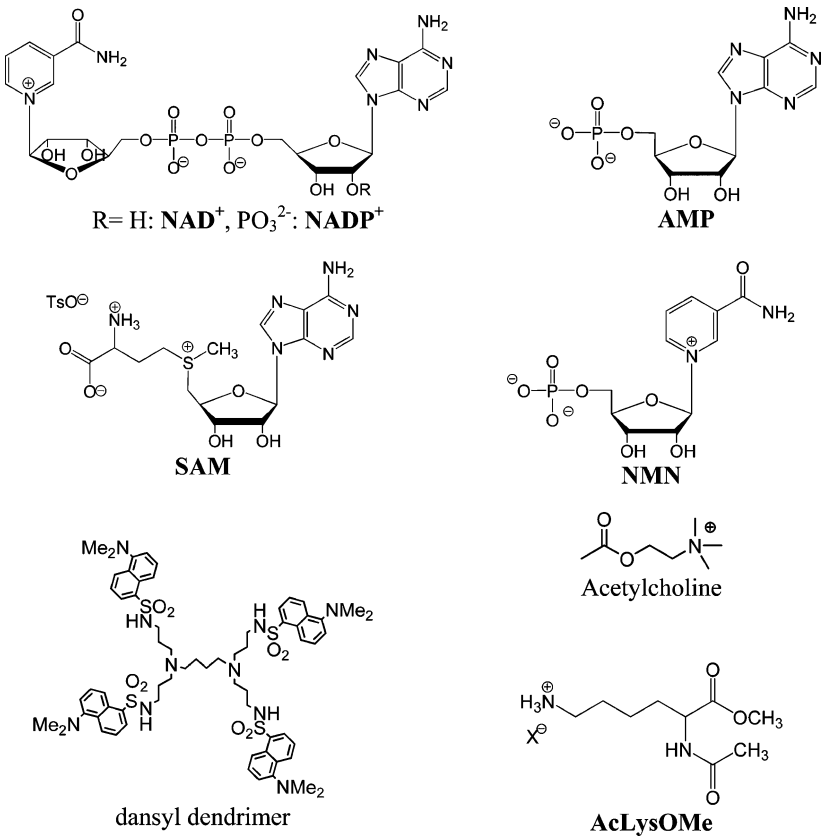


FIGURE 8. Structures of guest molecules, which interact with the water-soluble tweezers and clips.

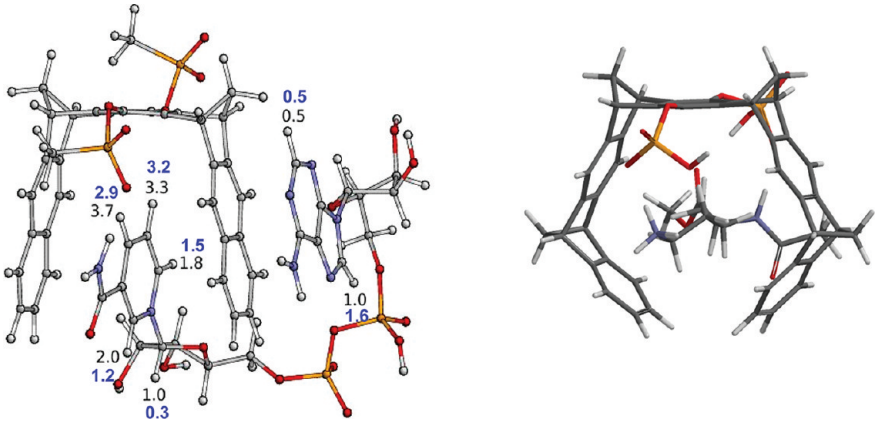


FIGURE 9. Structures of host–guest complexes calculated by force field (MacroModel 8.1, Monte Carlo conformer search, 5000 structures, AMBER*/H_2O): (left) $NAD^+ \cdot$ **4** (R = $OP(Me)O_2^-$), including comparison of the experimental complexation-induced ^1H NMR shifts, $\Delta\delta_{max}$, of the NAD^+ guest protons (in blue, obtained by NMR titration; in black, computed by quantum chemical methods at the GIAO-HF/SVP level); (right) AcLysOMe·**1** (R = $OP(OH)O_2^-$).

equimolar inhibitor/cofactor ratio ($IC_{50}/[NAD^+] = 0.8$; IC_{50} denotes the inhibitor concentration required to block 50% of the enzyme reaction). Kinetic analysis suggests a competitive behavior of clip **4e**, which depletes the cofactor level. In sharp contrast, phosphate tweezer **1e** readily inhibits this reaction with small substoichiometric amounts ($IC_{50} = 180\ \mu M$; $IC_{50}/[NAD^+] = 0.09$). Since the tweezer does not recognize NAD^+, we assume that it complexes lysine moieties of the enzyme close to the active site and most likely blocks the substrate entrance (noncompetitive with respect to the cofactor). Strong support comes from the discovery that the inhibited reaction can be switched on by addition of an

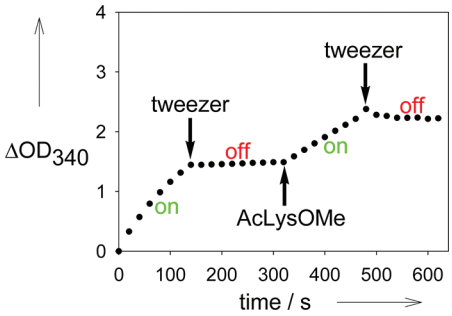
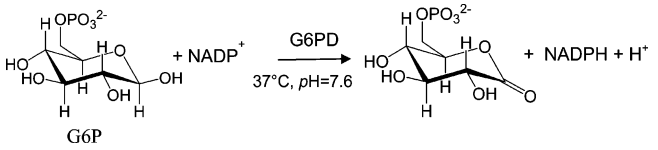


FIGURE 10. Alternating addition of tweezer **1e** and AcLysOMe turns ADH enzyme activity off and on (as characterized by the production of NADH measured by the change of the optical density at 340 nm, ΔOD_{340}, in the UV/vis spectrum of the reaction mixture).

external lysine derivative and again switched off by further addition of the tweezer (Figure 10).[21]

$$CH_3—CH_2—OH + NAD^+ \xrightarrow[37°C,\ pH=9.0]{ADH} CH_3—CH{=}O + NADH + H^+$$

Another example for supramolecular enzyme inhibition is the effect of the clips **4d,e** on the enzymatic oxidation of glucose-6-phosphate (G6P) with $NADP^+$ catalyzed by glucose-6-phosphate dehydrogenase (G6PD).[28] This reaction is the first step in the pentose-phosphate pathway and represents an essential source of reduction equivalents for the organism.



The phosphonate-substituted clip **4d** (required again in stoichiometric amounts) inhibits the enzymatic G6P oxidation, apparently by cofactor inclusion. Formal methyl−oxygen exchange in **4d** furnishes the closely related phosphate clip **4e**, which by virtue of its phosphate moieties is drastically more efficient (Table 2), even though both clips bind the cofactor $NADP^+$ with comparable binding constants (K_a = 5100 and 4500 M^{-1} for **4d** and **4e**, respectively).[28] This is also demonstrated by the study of the bridges **8d** and **8e** (Figure 2). Only the phosphate-substituted bridge **8e** shows a significant inhibition, which is, however, smaller than that of clip **4e**. This finding indicates that the clip sidewalls are also important for the inhibitor potency of these systems.

Clip **4e** produces a very rare Lineweaver−Burk plot in which the native and inhibition curves intersect in the positive *x,y* quadrant, in sharp contrast to clip **4d**, which shows the classical intersection of these two curves on the *y* axis indicative of competitive inhibition (Figure 11, top, left).

TABLE 2. Inhibition of the Enzymatic Oxidation of Glucose-6-phosphate (G6P, 2 mM) with $NADP^+$ (200 μM) Catalyzed by G6PD with the Phosphonate- and Phosphate-Substituted Molecular Clips **4d,e** and Bridges **8d,e** at 37°C in Aqueous Buffered Solution at pH = 7.6

inhibitor	IC_{50} [μM]	IC_{50}/[$NADP^+$]
clip **4d** (R = $OP(Me)O_2^-$ Li^+)	350	1.8
bridge **8d** (R = $OP(Me)O_2^-$ Li^+)	>500	>2.5
clip **4e** (R = OPO_3^{2-} 2 Li^+)	**7**	**0.03**
bridge **8e** (R = OPO_3^{2-} 2 Li^+)	53	0.27

According to theoretical studies by Dixon, Webb, and Whiteley,[29] the unusual slope found for clip **4e** is characteristic of "partial uncompetitive inhibition", characterized by formation of a ternary complex between enzyme, inhibitor, and cofactor. From detailed kinetic and thermodynamic studies, the following mechanism was proposed for the inhibition of the enzymatic G6P oxidation by the phosphate clip **4e**: According to the crystal structure, the enzyme offers two binding sites for clip docking, the so-called Rossman fold (cofactor binding cleft) and the substrate site. Gibbs enthalpies were determined for the first step when cofactor $NADP^+$ or clip **4e** binds to G6PD as well as for the subsequent complexation of clip or cofactor to the corresponding binary complexes. To this end, isothermal titration calorimetry (ITC) was combined with fluorometric titration experiments (Figure 11, top, right). Both pathways lead to a stable ternary complex consisting of the enzyme, one clip molecule, and one molecule of $NADP^+$.

On a second inhibition path, the substrate binding site is also (competitively) blocked by a clip molecule, adding to the partial uncompetitive first inhibition route. This dual mechanism makes the clip so efficient; it is supported by molecular dynamics calculations. The enzyme was calculated to form a stable ternary complex, in which the adenine unit of $NADP^+$ is bound inside the clip cavity (Figure 11, bottom, left) and the chemically active nicotinamide can hardly reach the substrate. A second very stable binary enzyme complex was calculated for clip **4e** occupying the substrate binding site (Figure 11, bottom, right). These findings provide evidence that the phosphate groups in clip **4e** are important for the binding of **4e** to the enzyme and hence for the efficiency of **4e** as inhibitor compared with the methanephosphonate groups in clip **4d**, which is a moderate inhibitor only binding to the cofactor NAD^+ but not to the enzyme. We assume that on one hand the methyl groups in **4d** block the binding of the clip to the enzyme sterically and that on the other hand in **4e** the higher negative charge of the phophate groups (compared with

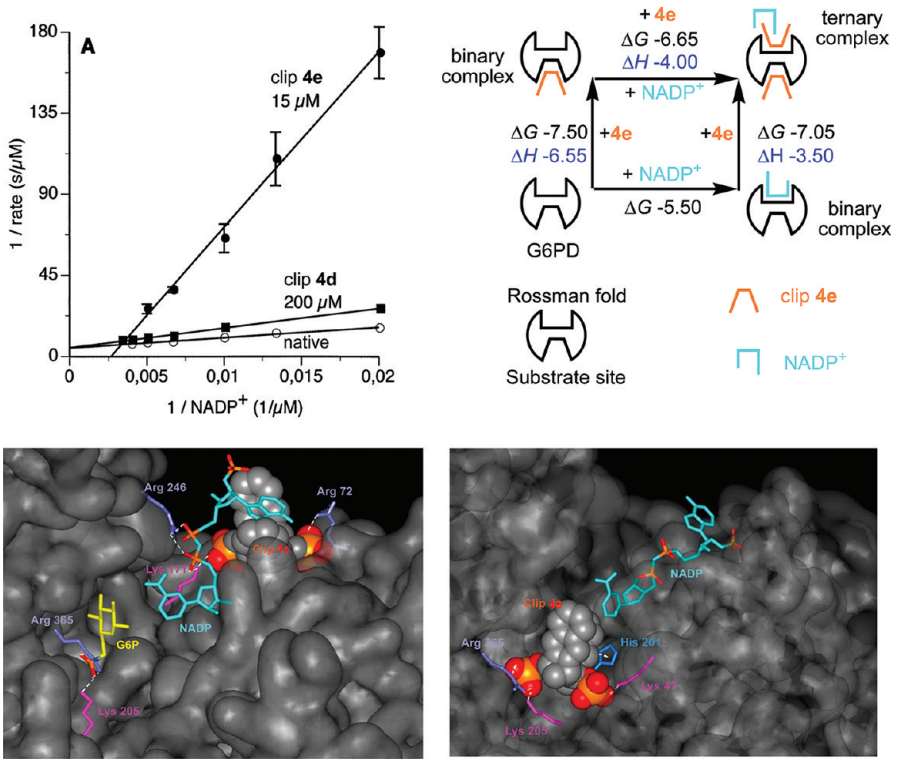


FIGURE 11. (top, left) Lineweaver–Burk plots for the reaction of G6P with $NADP^+$ catalyzed by G6PD, with phosphonate clip **4d** and phosphate clip **4e**. (top, right) Thermodynamic figure for successive complex formation between G6PD, clip **4e**, and $NADP^+$ (both in excess); ΔH, ΔG in kcal/mol. (bottom) MD simulations of ternary and binary complex illustrating the two inhibition modes of G6PD performed by clip **4e**.

the phosphonate groups in **4d**) favors the binding of **4e** to the enzyme.

The Effect of Water-Soluble Tweezers on Protein Aggregation

Lysine residues are also involved in the very early steps of aberrant protein misfolding and assembly, ultimately leading to severe neurological disorders. It is generally accepted that diseases such as Alzheimer's, Parkinson's, and prion diseases and type-2 diabetes are all started by nucleation-dependent polymerization of a critical protein, which produces very toxic oligomeric species as well as fibrils with a high β-sheet content.[30] After our original publication of the phosphonate tweezer's ability to complex lysine,[19] we were addressed by neurologists who suggested to try these molecules on amyloidogenic proteins, with the aim of preventing their early misfolding and assembly into toxic oligomers and fibrils.

Thus, we joined forces with the group of Gal Bitan at UCLA and studied the molecular interaction of our most potent lysine host (the phosphate-substituted tweezer **1e**) with Aβ, which carries two lysine residues (Figure 12).[31] Two-dimensional NMR experiments on $^{15}N/^{13}C$-labeled full-length Aβ revealed preferential complexation of Lys-16 and Lys-28, which was independently confirmed by drastic upfield-shifts of both lysine side chain signals in the ^1H NMR spectrum of an Aβ15–29 fragment. The NMR results are supported by mass spectrometric investigation (using ESI-FT-ICR). In CD experiments, β-sheet formation of monomeric Aβ was effectively suppressed by equimolar amounts of **1e** for several days. Subsequent dot–blot experiments with the oligomer-specific antibody A11 showed no immunoreactivity from the beginning and demonstrated that **1e** acts fast and precludes formation of toxic oligomers. Oligomer sizes were monitored by dynamic light scattering (DLS); while hydrodynamic radii of initially formed species were similar to those of Aβ alone, no particle growth was observed for over a month, implying that the phosphate tweezer stabilized nontoxic oligomeric Aβ populations but prevented their aggregation. In this and all other assays, the bridge **8e**, which lacks the sidewalls necessary for lysine inclusion, was always coexamined as a reference and furnished negative results as opposed to **1e**.

It was later shown that our molecular tweezer even dissolves preformed Aβ fibrils: in thioflavine T (ThT) experiments, the added dye fluoresces only in the presence of misfolded proteins and indicates the amount of β-sheets. If **1e** was added during fibril maturation, it dissolved

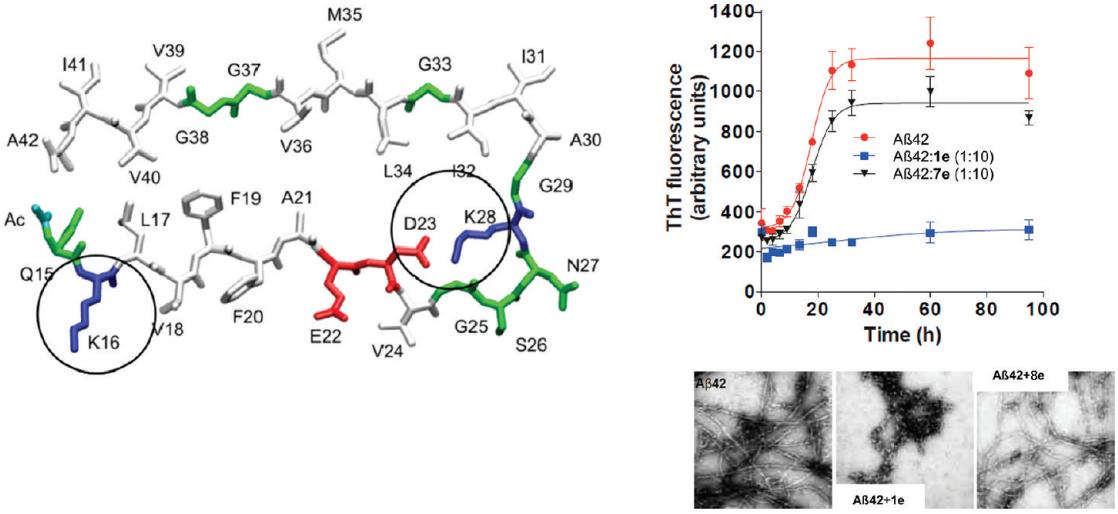


FIGURE 12. (left) Misfolded part of Aβ42 in fibrils with critical lysine residues K16 and K28, which are selectively complexed by phosphate tweezer **1e**.[32] (right) ThT experiments monitoring Aβ42 aggregation and inhibition by **1e**, but not by **8e**. (bottom) Corresponding TEM pictures demonstrating exclusive suppression of fibril formation by **1e**.

preformed aggregates, both in the elongation as well as in the lateral association phase.

After these very promising results with Aβ, the range of proteins was extended to nine other amyloidogenic representatives involved in various human diseases. Intriguingly, for all those that carry critical lysine residues fibrillogenesis was effectively inhibited in the presence of equimolar amounts of the phosphate tweezer. ThT fluorescence was complemented by turbidity measurements and accompanied by morphological examination of the aggregates by transmission electron miscroscopy (TEM). *In toto*, **1e** affected seven proteins and inhibited both their nucleation and fibril elongation in a dose-dependent manner. Finally, protection of cultured cells was investigated for the above-mentioned series of amyloidogenic proteins. MTT assays measure cell viability and were performed on differentiated PC-12 (Aβ, CT, β_2m, TTR, PrP), as well as RIN5fm cell lines (IAPP, insulin). In all cases, cells were rescued by **1e** with IC_{50} (half-maximal inhibition) values in the low micromolar range, reflecting the same order of magnitude as the protein concentration. In conclusion, the phosphate tweezer **1e** modulates the structure of early Aβ assemblies in a subtle way that renders them nontoxic and prevents further aggregation. Unlike almost all other small molecule inhibitors, however, its molecular interaction with Aβ was predicted by its high specificity for lysine side chains and could be unambiguously verified by spectroscopic methods. This process-specific mechanism interferes with aggregation and induced cell toxicity of a wide range of amyloidogenic proteins at a very early stage and seems to be a general phenomenon.

It might be argued, that many other essential proteins carry solvent-exposed lysine residues that might be complexed by the tweezer and thus lead to severe side effects. However, in biological terms **1e**'s affinity toward lysine is only moderate ($K_d = 20\ \mu$M) and is characterized by a high on−off rate. Thus, the tweezer seems not to interfere with normal cellular function until concentrations (micromolar) substantially higher than those needed for inhibition of protein aggregation (nanomolar). Toward both cell lines in this study, **1e** proved to be nontoxic up to concentrations of 0.4 mM.

Recent investigations demonstrated that the phosphate tweezer is indeed able to rescue Aβ-induced decrease of dendritic branching, as well as miniature excitatory postsynaptic currents. Finally, treatment with **1e** drastically reduced plaque loads in the mouse brain and improved spatial memory in AD transgenic mice. Similar highly promising results are being accumulated with α-synuclein[33] and IAPP, suggesting that the new mechanism of action also operates *in vivo*.

Conclusions

Molecular clips and tweezers with a convergent arrangement of aromatic rings are now well understood host molecules which include their guests by π stacking and CH−π interactions. Introduction of phosphonate, phosphate, or sulfate anions into their central aromatic unit renders them water-soluble and offers additional effects for guest attraction, namely hydrophobic interactions and Coulomb interactions. Their binding profile largely depends

on the shape of their cavity and can be fine-tuned by introduction of additional functional groups, for example, into the external aromatic ring (chiral hosts).

The advanced systems described in this Account point to two major areas of future applications: organocatalysis and biomedicine. Especially the larger tweezer skeletons offer enough space for reactive guest molecules and may be used for the selective stabilization of cationic transition states. This offers the advantage of a fast turnover rate, since the uncharged product molecule readily leaves the reaction chamber and simultaneously avoids product inhibition.

In light of the complementary binding profile of water-soluble benzene-spaced clips and tweezers both classes may be developed into artificial hosts for cofactors (clips) or for basic amino acids on proteins (tweezers). Specificity and affinity for a given target should be greatly strengthened by replacement of one phosphate or sulfate by a second recognition site, rendering the new unsymmetrical hosts selective for a given cofactor or protein. We would like to emphasize that the molecular tweezers are not designed for the active site but for sterically accessible lysine and arginine residues on the protein surface; thus they constitute an alternative approach to classical medicinal chemistry and, for example, may also be used to interfere with protein–protein interactions. This is especially true for the complexation of key players in protein misfolding diseases; our initial screening was performed against some aggregating proteins that are responsible for diseases that haunt modern society. A new therapeutic approach should therefore introduce a new mechanism of action and interfere at the earliest possible stage of the disease. The process-specific complexation of critical lysine residues may represent such a new strategy and redirect the aggregation of misfolded proteins into nontoxic species, which can be easily degraded by proteases. Research along these lines is underway in our laboratories and those of our collaboration partners.

We thank the colleagues, Ph.D. students, and postdoctoral research fellows cited in the references for the great collaboration.

BIOGRAPHICAL INFORMATION

Frank-Gerrit Klärner (b. 1941) studied chemistry at the University of Köln (Cologne, Germany) and received his Ph.D. there for work in the laboratory of Professor Emanuel Vogel in 1968. In 1974, he finished his "Habilitation" at the Ruhr-University of Bochum, was associate professor in Bochum from 1980 to 1992, and was visiting professor at University of Wisconsin, Madison, USA, in 1983. Since 1992, he has been full professor at the University of Duisburg-Essen, chaired the DFG center of supramolecular research at the Universities in Essen and Bochum ("Sonderforschungsbereich, SFB 452") from 1998 to 2005 and retired in 2006. His research interests are in the field of supramolecular chemistry (molecular tweezers and clips, molecular recognition, development of synthetic receptors for bioactive chemical compounds) and high-pressure chemistry (up to 14 kbar).

Thomas Schrader (b. 1958) studied chemistry at Bonn University (Germany) and received his Ph.D. degree for synthetic work with Prof. Wolfgang Steglich. After a postdoctoral stay at Princeton University in the group of Prof. Edward C. Taylor (1989–1990, total synthesis of antitumor drugs), he returned to Germany to work on his habilitation at Düsseldorf, associated with the group of Günter Wulff (1998, asymmetric umpolung and synthetic receptors with phosphates). In 2000, he was appointed associate professor at Marburg University, and moved to the University of Duisburg-Essen in 2006, where he now holds a chair of organic chemistry. His research focusses on the development of artificial receptor molecules which interfere with biological processes.

FOOTNOTES

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The authors declare no competing financial interest.

REFERENCES

1 Salonen, L. M.; Ellermann, M.; Diederich, F. Aromatic Rings in Chemical and Biological Recognition: Energetics and Structures. *Angew. Chem., Int. Ed.* **2011**, *50*, 4808–4842.

2 Sinnokrot, M. O.; Sherrill, C. D. High-Accuracy Quantum Mechanical Studies of $\pi-\pi$ Interactions in Benzene Dimers. *J. Phys. Chem. A* **2006**, *110*, 10656–10668. Review: Grimme, S. Do Special Noncovalent $\pi-\pi$ Stacking Interactions Really Exist? *Angew. Chem., Int. Ed.* **2008**, *47*, 3430–3434.

3 Wheeler, S. E.; Houk, K. N. Substituent Effects in the Benzene Dimer are Due to Direct Interactions of the Substituents with the Unsubstituted Benzene. *J. Am. Chem. Soc.* **2008**, *130*, 10854–10855.

4 Wheeler, S. E.; Houk, K. N. Through-Space Effects of Substituents Dominate Molecular Electrostatic Potentials of Substituted Arenes. *J. Chem. Theory Comput.* **2009**, *5*, 2301–2312.

5 Jorgensen, W. L.; Pranata, J. Importance of Secondary Interactions in Triply Hydrogen Bonded Complexes: Guanine-Cytosine vs Uracil-2,6-Diaminopyridine. *J. Am. Chem. Soc.* **1990**, *112*, 2008–2010. Murray, T. J.; Zimmerman, S. C. New Triply Hydrogen Bonded Complexes with Highly Variable Stabilities. *J. Am. Chem. Soc.* **1992**, *114*, 4010–4011.

6 The term "molecular tweezers" was used for noncyclic host molecules first by: Chen, C.-W.; Whitlock, H. W. Molecular Tweezers - A Simple-Model of Bifunctional Intercalation. *J. Am. Chem. Soc.* **1978**, *100*, 4921. Later by: Zimmerman, S. C. Rigid Molecular Tweezers As Hosts for the Complexation of Neutral Guests. *Top. Curr. Chem.* **1993**, *165*, 71. Harmata, M. Chiral Molecular Tweezers. *Acc. Chem. Res.* **2004**, *37*, 862–873. The term "molecular clips" by: Rowan, A. E.; Elemans, J. A. A. W.; Nolte, R. J. M. Molecular and Supramolecular Objects from Glycoluril. *Acc. Chem. Res.* **1999**, *32*, 995–1006. The distinction between molecular tweezers and clips in this work comes from their different guest binding mode.[8] The tweezers' "arms" are expanded and compressed during the guest complexation in a way comparable to the working principle of mechanical tweezers, whereas molecular clips form host–guest complexes by clipping flat guest molecules between their aromatic sidewalls.

7 Klärner, F.-G.; Kahlert, B. Molecular Tweezers and Clips as Synthetic Receptors. Molecular Recognition and Dynamics in Receptor–Substrate Complexes. *Acc. Chem. Res.* **2003**, *36*, 919–932.

8 Lobert, M.; Bandmann, H.; Burkert, U.; Büchele, U. P.; Podsadlowski, V.; Klärner, F. G. Dynamics in Host-Guest Complexes of Molecular Tweezers and Clips. *Chem.—Eur. J.* **2006**, *12*, 1629–1641.

9 The structures and binding energies of the host–guest complexes of tweezers and clips as host molecules calculated with DFT-D-BLYP agree well with those derived from NMR and X-rax analyses. Parac, M.; Etinski, M.; Peric, M.; Grimme, S. A Theoretical Investigation of the Geometries and Binding Energies of Molecular Tweezer and Clip Host-Guest Systems. *J. Chem. Theory Comput.* **2005**, *1*, 1110–1118.

10 Klärner, F.-G.; Kahlert, B.; Boese, R.; Blaeser, D.; Juris, A.; Marchioni, F. Synthesis and Supramolecular Properties of Molecular Clips with Anthracene Sidewalls. *Chem.—Eur. J.* **2005**, *11*, 3363–3374.

11 Branchi, B.; Balzani, V.; Ceroni, P.; Campañá Kuchenbrandt, M.; Klärner, F.-G.; Bläser, D.; Boese, R. Molecular Clips with Extended Aromatic Sidewalls as Receptors for Electron-Acceptor Molecules. Synthesis and NMR, Photophysical, and Electrochemical Properties. *J. Org. Chem.* **2008**, *73*, 5839–5851.

12 Fukuhara, G.; Madenci, S.; Polkowska, J.; Bastkowski, F.; Klärner, F.-G.; Origane, Y.; Kaneda, M.; Mori, T.; Wada, T.; Inoue, Y. Inherently Chiral Molecular Clips: Synthesis, Chiroptical Properties, and Application to Chiral Discrimination. *Chem.—Eur. J.* **2007**, *13*, 2473–2479. Klärner, F.-G.; Madenci, S.; Campañá Kuchenbrandt, M.; Bläser, D.; Boese, R.; Fukuhara, G.; Inoue, Y. Donor/Acceptor-Substituted Chiral Molecular Clips − Synthesis and Host−Guest Complex Formation. *Eur. J. Org. Chem.* **2012**, DOI: 10.1002/ejoc.201200112.

13 Review: Vögtle, F.; Richardt, G.; Werner, N. *Dendrimer Chemistry, Concepts, Syntheses, Properties, Applications*; Wiley-VCH: Weinheim, Germany, 2009.

14 Balzani, V.; Bandmann, H.; Ceroni, P.; Giansante, C.; Hahn, U.; Klärner, F.-G.; Müller, U.; Muller, W. M; Verhaelen, C.; Vicinelli, V.; Vögtle, F. Host−Guest Complexes between an Aromatic Molecular Tweezer and Symmetric and Unsymmetric Dendrimers with a 4,4′-Bipyridinium Core. *J. Am. Chem. Soc.* **2006**, *128*, 637–648. Giansante, C.; Mazzanti, A.; Baroncini, M.; Ceroni, P.; Venturi, M.; Klärner, F.-G.; Vögtle, F. Tweezering the Core of Dendrimers: Medium Effect on the Kinetic and Thermodynamic Properties. *J. Org. Chem.* **2009**, *74*, 7335–7343.

15 Verhaelen C. Ph.D. Dissertation, University of Duisburg-Essen, 2007.

16 Schalley, C. A; Verhaelen, C.; Klärner, F.-G.; Hahn, U.; Vögtle, F. Gas-Phase Host-Guest Chemistry of Dendritic Viologens and Molecular Tweezers: a Remarkably Strong Effect on Dication Stability. *Angew. Chem., Int. Ed* **2005**, *44*, 477–480.

17 Jasper, C.; Schrader, T.; Panitzky, J.; Klärner, F.-G. Selective Complexation of N-Alkylpyridinium Salts - Binding of NAD^+ in Water. *Angew. Chem., Int. Ed.* **2002**, *41*, 1355–1358. Fokkens, M.; Jasper, C.; Schrader, T.; Koziol, F.; Ochsenfeld, C.; Polkowska, J.; Lobert, M.; Kahlert, B.; Klärner, F.-G. Selective Complexation of N-Alkylpyridinium Salts: Binding of NAD+ in Water. *Chem.—Eur. J.* **2005**, *11*, 477–494.

18 Schrader, T.; Fokkens, M.; Klärner, F.-G.; Polkowska, J.; Bastkowski, F. Inclusion of Thiamine Diphosphate and S-Adenosylmethionine at Their Chemically Active Sites. *J. Org. Chem.* **2005**, *70*, 10227–10237.

19 Fokkens, M.; Schrader, T.; Klärner, F.-G. A Molecular Tweezer for Lysine and Arginine. *J. Am. Chem. Soc.* **2005**, *127*, 14415–14421.

20 Branchi, B.; Ceroni, P.; Balzani, V.; Cartagena, M. C.; Klärner, F.-G.; Schrader, T.; Vögtle, F. Fluorescent Water-Soluble Molecular Clips. Self-Association and Formation of Adducts in Aqueous and Methanol Solutions. *New J. Chem.* **2009**, *33*, 397–407.

21 Talbiersky, P.; Bastkowski, F.; Klärner, F.-G.; Schrader, T. Molecular Clip and Tweezer Introduce New Mechanisms of Enzyme Inhibition. *J. Am. Chem. Soc.* **2008**, *30*, 9824–9828.

22 Klärner, F.-G.; Kahlert, B.; Nellesen, A.; Zienau, J.; Ochsenfeld, C.; Schrader, T. Molecular Tweezer and Clip in Aqueous Solution: Unexpected Self-Assembly, Powerful Host-Guest Complex Formation, Quantum Chemical ^{1}H NMR Shift Calculation. *J. Am. Chem. Soc.* **2006**, *128*, 4831–4841; *J. Am. Chem. Soc.* **2010**, *132*, 4029.

23 Klärner, F.-G.; Schrader, T.; Polkowska, J.; Bastkowski, F.; Talbiersky, P.; Campañá Kuchenbrandt, M; Schaller, T.; de Groot, H.; Kirsch, M. Effect of Molecular Clips and Tweezers on Enzymatic Reactions by Binding Coenzymes and Basic Amino Acids. *Pure Appl. Chem.* **2010**, *82*, 991–999.

24 Branchi, B.; Ceroni, P.; Balzani, V.; Bergamini, G.; Klärner, F.-G.; Vögtle, F. Adducts between Dansylated Poly(propylene amine) Dendrimers and Anthracene Clips Mediated by Zn^{II} Ions: Highly Efficient Photoinduced Energy Transfer. *Chem.—Eur. J.* **2009**, *15*, 7876–7882.

25 Branchi, B.; Ceroni, P.; Balzani, V.; Klärner, F.-G.; Vögtle, F. A Light-Harvesting Antenna Resulting from the Self-Assembly of Five Components: A Dendrimer, Two Clips, and Two Metal Ions. *Chem.—Eur. J.* **2010**, *16*, 6048–6055.

26 Polkowska, J.; Bastkowski, F.; Schrader, T.; Klärner, F.-G.; Zienau, J.; Koziol, F.; Ochsenfeld, C. A Combined Experimental and Theoretical Study of the pH-Dependent Binding Mode of NAD^+ by Water-Soluble Molecular Clips. *J. Phys. Org. Chem.* **2009**, *22*, 779–790.

27 Bastkowski, F.; Polkowska, J.; Schrader, T.; Klärner, F.-G. Non Convalent Inclusion of Nucleosides and Nucleotides in Water-Soluble Molecular Clips. *Heterocycles* **2011**, 1549–1566.

28 Kirsch, M.; Talbiersky, P.; Polkowska, J.; Bastkowski, F.; Schaller, T.; de Groot, H.; Klärner, F.-G.; Schrader, T. A Mechanism of Efficient G6PD Inhibition by a Molecular Clip. *Angew. Chem., Int. Ed.* **2009**, *48*, 2886–2890.

29 Dixon, M.; Webb, E. C. *Enzymes*; Longman: London, 1979; pp 343−344. Whiteley, C. G. Enzyme Kinetics: Partial and Complete Uncompetitive Inhibition. *Biochem. Educ.* **2000**, *28*, 144–147.

30 Chiti, F.; Dobson, C. M. Protein Misfolding, Functional Amyloid and Human Disease. *Annu. Rev. Biochem.* **2006**, *75*, 333–366.

31 Sinha, S.; Lopes, D. H. J.; Du, Z.; Pang, E. S.; Shanmugam, A.; Lomakin, A.; Talbiersky, P.; Tennstaedt, A.; McDaniel, K.; Bakshi, R.; Kuo, P.-Y.; Ehrmann, M.; Benedek, G.-B.; Loo, J. A.; Klärner, F.-G.; Schrader, T.; Wang, C.; Bitan, G. Lysine-Specific Molecular Tweezers Are Broad-Spectrum Inhibitors of Assembly and Toxicity of Amyloid Proteins. *J. Am. Chem. Soc.* **2011**, *133*, 16958–16969.

32 Lührs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Dobeli, H; Schubert, D.; Riek, R. 3 D Structure of Alzheimer's Amyloid-beta (1−42) Fibrils. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17342–17347.

33 Prabhudesai, S.; Sinha, S.; Attar, A.; Kotagiri, A.; Fitzmaurice, A. G.; Lakshmanan, R.; Ivanova, M. I.; Loo, J. A.; Klärner, F.-G.; Schrader, T.; Stahl, M.; Bitan, G.; Bronstein, J. M. A Novel "Molecular Tweezer" Inhibitor of α-Synuclein Neurotoxicity in Vitro and in Vivo. *Neurotherapeutics* **2012**, DOI: 10.1007/s13311-012-0105-1.