

Supramolecular Chemistry Targeting Proteins

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ABSTRACT: The specific recognition of protein surface elements is a fundamental challenge in the life sciences. New developments in this field will form the basis of advanced therapeutic approaches and lead to applications such as sensors, affinity tags, immobilization techniques, and protein-based materials. Synthetic supramolecular molecules and materials are creating new opportunities for protein recognition that are orthogonal to classical small molecule and protein-based approaches. As outlined here, their unique molecular features enable the recognition of amino acids, peptides, and even whole protein surfaces, which can be applied to the modulation and assembly of proteins. We believe that structural insights into these processes are of great value for the further development of this field and have therefore focused this Perspective on contributions that provide such structural data.

INTRODUCTION

Proteins are the key regulators in the cell, involved in cell signaling processes, catalysis, and communication.¹ Most proteins function as homo- or hetero-oligomeric assemblies.² Concomitantly, interest in protein–protein interactions (PPIs) has grown in the drug development and chemical biology fields³ because PPIs act orthogonally to conventional drug targets such as enzymes and transcription factors.⁴ In contrast to these conventional drug targets, the modulation of PPIs often requires the targeting of larger protein elements or multiple hotspots on the surface of the proteins,⁵ rendering them challenging targets for small molecules.⁶ Hydrophobic and electrostatic interactions play a significant role in PPIs, where shape complementarity and organization of chemical components implement selectivity.^{1,7,8}

Supramolecular host–guest chemistry in water greatly resembles the hotspot-driven interaction between proteins.^{4,9} Several synthetic host molecules have been reported in the literature that recognize either short peptides or molecular elements at protein interfaces through supramolecular host–guest interactions: crown ethers,¹⁰ pillararenes,¹¹ porphyrins,¹² cucurbiturils,^{13,14} cyclodextrins,¹⁵ calixarenes,¹⁶ and molecular tweezers.¹⁷ Similar to PPIs, the binding energetics of these supramolecular host–guest systems in water is largely driven by the hydrophobic effect, with other non-covalent interactions, electrostatics and hydrogen bonding, playing a secondary, modulatory role. These thermodynamic constraints therefore demand a different design strategy compared with the well-studied interactions of supramolecular systems in organic solvents. An optimal balance between hydrophilic and hydro-

phobic interactions needs to be reached¹⁸ in order to ensure sufficient solubility in water while repelling water from the protein recognition elements to enable additional secondary interactions such as electrostatics and hydrogen bonding.¹⁹ Such water-based systems add to the complexity, diversity, and robustness of supramolecular systems and create opportunities for their application to proteins.¹⁹ Over the past decade, structural data acquired from X-ray crystallography and NMR spectroscopy have dramatically increased our understanding of the complexation of synthetic supramolecular elements with amino acids, peptides, and, more recently, protein interfaces.¹³ Now we are at the stage where the structure-guided design of these synthetic hosts can not only enhance our knowledge of the supramolecular recognition of proteins but also greatly advance their applicability as PPI modulators.

This Perspective will focus on the use of synthetic supramolecular systems for the recognition of proteins and their subsequent effect on the modulation of PPIs and protein assembly (Figure 1). Small-molecule approaches for the inhibition^{20–22} or stabilization^{4,23,24} of proteins and their

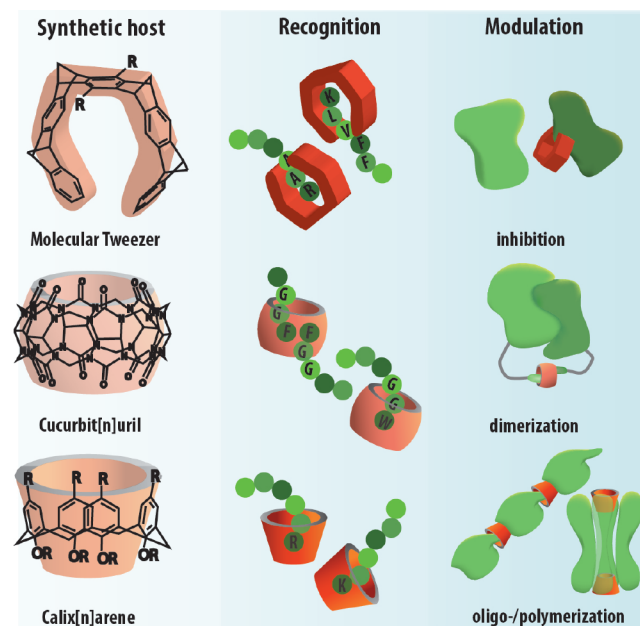


Figure 1. Synthetic host molecules can selectively complex with amino acids and peptides and modulate protein function. A selection of the most studied supramolecular hosts, representative peptide sequence recognition, and protein modulation mechanisms are schematically depicted.

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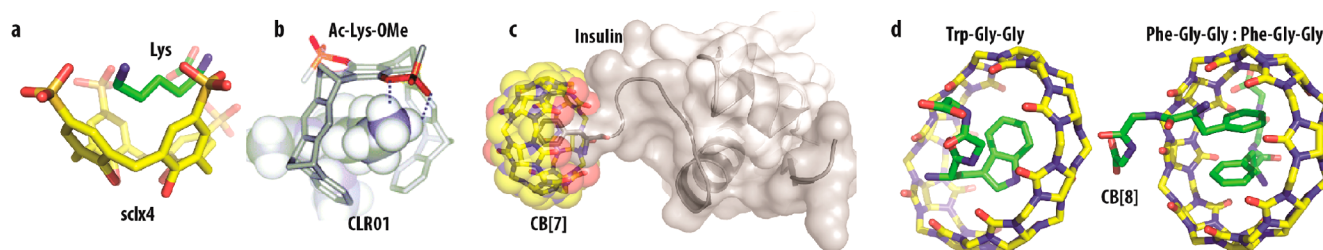


Figure 2. (a) Crystal structure of sclx4 complexed with Lys (CCDC no. 140210). (b) Monte Carlo simulation of CLR01 complexed with Ac-Lys-OMe. Adapted from ref 38. Copyright 2013 American Chemical Society. (c) Crystal structure of CB[7] complexed with the N-terminal Phe of insulin (PDB entry 3Q6E). (d) Crystal structure of CB[8] complexed with one WGG peptide (left) and two FGG peptides (right) (CCDC nos. 628234 and 628235).

assemblies will not be covered. Different host–guest interactions will be discussed on the levels of single amino acids and peptides but above all on their effect on whole proteins. Primarily because of their instructive quality, priority will be given to examples from the literature that provide structural information. For the assembly of proteins, several supramolecular approaches to induce protein dimerization, oligomerization, or polymerization will be put into perspective.

■ AMINO ACID AND PEPTIDE RECOGNITION

The recognition properties of synthetic host molecules are deployable for many applications, including catalysis,²⁵ drug delivery,²⁶ surface immobilization,^{27,28} and sensing.²⁹ Here we discuss a small selection of cavitands, namely, calixarenes, cucurbiturils, and molecular tweezers, that display specific, well-characterized recognition properties toward both amino acids and peptides. Despite their structural differences, the complexation of these cavitands with amino acids and peptides is based on a number of common features: (1) a highly preorganized structure, (2) a hydrophobic cavity, and (3) accessibility for chemical modifications. Structural data will be used to clarify the influence of factors that provide a differentiated recognition of amino acid elements, such as portal size, cavity volume, peripheral groups, and flexibility upon complexation. These binding preferences also translate at the protein level, which sets the stage for the future development of even more specific and high-affinity binding hosts.

Calixarenes are cyclic oligomeric host molecules formed through a condensation reaction between phenol and formaldehyde. They can exist in different ring sizes, each bearing a hydrophobic interior, though most studies have been performed on the four-monomer ring system calix[4]arene (Figure 1). The upper and lower rims of this cavitand can be modified with small functional groups, including sulfonate and guanidinium, with more complex peptides and nucleotides, and with bridging linkers to control the rigidity of the cavitand. This combination of modifications allows a wide range of biologically relevant targets to be addressed via often multivalent supramolecular interactions with the modified calixarene rims.³⁰ The modification of the rims with polar moieties allows for additional electrostatic interactions or hydrogen bonds that synergistically work with the hydrophobic effect via the aromatic core. Naphthylidene conjugates of calixarenes can recognize cysteine, histidine, aspartic acid, and glutamic acid dependent on an additionally bound metal ion.^{31,32} Sulfonate conjugates of calixarenes such as *p*-sulfonatocalix[4]arene (sclx4) (Figure 2a) bind aromatic and cationic amino acid residues,³³ resulting in a millimolar binding affinity toward methylated arginine and a micromolar affinity toward

methylated lysine present in certain short peptide sequences with neighboring polar side chains.^{9,34} A 70-fold selectivity for trimethyllysine (Lys_{Me₃}) over every other natural amino acid was reported to be mainly driven by a large favorable enthalpic component and a smaller entropic component, as determined by isothermal titration calorimetry (ITC). Via upfield chemical shift trends in *N*-methyl groups and molecular modeling, the side chains of Lys_{Me₃} were shown to bind deep within the hydrophobic cavity of sclx4.⁹ Methylated arginine (Arg) derivatives also showed a significant upfield shift for the *N*-methyl groups but did not bind as deep within the cavity according to energy-minimized structures. A recent crystal structure of sclx4 complexed with lysozyme-Kme₂ endorses the *in silico* and NMR data. In this example, the central positioning of the Lys116-Kme₂ residue inside sclx4 maximizes cation– π interactions.⁵ By contrast, a side-on binding mode to the same calixarene was reported for unmodified Lys³⁵ and Arg⁵ in solved X-ray cocrystal structures (Figure 2a). This mode of binding facilitates the formation of a salt bridge between the amino acids' positively charged side chains and the sulfonate substituents on sclx4 and displaces water from the hydrophobic calixarene core. Additionally, these calixarenes were found in a more pinched cone formation to increase van der Waals interactions between the carbons of the residue. The pivotal role of methylated cationic amino acid residues is not limited to calixarenes but is also observed for other cavitands.³⁶

Schrader and co-workers designed a molecular tweezer (MT), which consists of a hydrophobic cavity composed of alternating norbornadiene and benzene rings. To a central hydroquinone ring, different functionalities can be introduced (Figure 1). This MT can bind biologically relevant cationic peptides with binding affinities in the micromolar range.^{37–39} The cationic amino acid residues thread through the cavity of the MT, which stabilizes the host–guest complex via CH– π , van der Waals, electrostatic, and hydrogen-bonding interactions (Figure 2b).^{38,39} The effect of different functionalities has been extensively studied by NMR, ITC, fluorescence titration, crystallography, and molecular simulation studies. Long alkyl tethers were found to induce host–host self-inclusion, while linkers including esters or carboxylates outperformed ethers and alcohols by keeping the cavity open.³⁷ The strong contribution of hydrophobic interactions was demonstrated through the monitoring of shifts in characteristic ¹H NMR peaks in methanol. In this case, host–guest complexation was dominated by electrostatic interactions with anionic linkers rather than the hydrophobic effect, leading to peripheral complexation with the tweezer.³⁸ The phosphate-functionalized tweezer CLR01 in particular has been shown to bind cationic

amino acid residues with a slight (2-fold) preference for Lys residues over Arg residues due to the entropy-driven overcompensation of the rigid delocalized guanidinium ion.³⁸

Cucurbiturils (CBs) are relatively rigid macrocycles made from glycoluril monomers connected via methylene bridges (Figure 1). CBs with six to eight monomers have been mainly used as synthetic receptors for the recognition of protein elements because of the high shape complementarity between the amino acid residues and the cavities of these CBs.⁴⁰ The double-sided carbonyl rims, together with the hydrophobic interior, dictate the preference of CB to bind cationic and hydrophobic elements. CB[6], consisting of six glycoluril monomers, has a relatively small cavity (Vol = 164 Å³) that binds moderately strongly to Lys residues (10⁴ M⁻¹) and more weakly to other amino acid residues (10³ M⁻¹) in water as a result of the formation of inclusion complexes.^{13,41} The size of the CB[7] cavity (Vol = 279 Å³) is sufficient to accommodate larger single amino acid residues, which maximizes the energetic contribution from the release of high-energy water according to molecular dynamics (MD) simulations.⁴² Similar to sclx4, CB[7] can very selectively recognize methylated lysine and arginine. Interestingly, ion–dipole interactions and the hydrophobic effect are the main contributors instead of cation– π interactions, as shown by the decrease in association upon systematic replacement of the methyl groups with hydrogen atoms at the terminal ammonium group ($K_{\text{LysMe}_3} = 10^6$ M⁻¹, $K_{\text{LysMe}_2} = 10^4$ M⁻¹, $K_{\text{LysMe}} = 10^3$ M⁻¹, $K_{\text{Lys}} = 10^2$ M⁻¹).⁴³ Also, the aromatic amino acids (phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp)) form strong complexes with CB[7] with association constants in the 10⁵ M⁻¹ range.^{13,44} The free ammonium group of these single amino acids contributes to the high binding affinity and selectivity of CB[7] for aromatic dipeptides. This was shown by ITC experiments performed by Rekharsky et al.⁴¹ where a 2000–23000-fold increase in affinity was measured for N-terminal aromatic amino acids compared with their C-terminal counterparts. These observations are supported by work performed by Urbach and co-workers, who obtained structural information on the binding of CB[7] with the N-terminal Phe of insulin via X-ray cocrystallography (Figure 2c).⁴⁵ In the crystal structure, CB[7] binds this solvent-exposed N-terminal aromatic residue of insulin with a high selectivity over proteins that lack an N-terminal Phe. The phenyl ring and peptide backbone are clearly observed in the hydrophobic cavity, while the N-terminal ammonium group stabilizes the Phe–CB[7] complex by interacting with the carbonyl oxygens at the rim of CB[7]. CB[7] buries 200 Å² of the solvent-exposed surface of insulin, resulting in a substantial displacement of the N-terminal Phe from its native position. The high selectivity and structural information for this native protein sequence can facilitate the development of CB[7] as an interesting orthogonal protein tag⁴⁶ and the exploration for new chemical receptors as well as noncanonical amino acid guests with improved binding affinities.⁴⁷

CB[8] (Vol = 479 Å³) can host even larger hydrophobic residues or multiple amino acid side chains through the formation of homo- or heteroternary complexes.⁴⁰ CB[8] recognizes single amino acid residues through the binding of auxiliary guests like methyl viologen (MV) with Phe-, Tyr-, and Trp-containing peptides.⁴⁸ Alternatively, CB[8] can also form homoternary complexes directly with Trp and Phe in a noncooperative and cooperative manner, respectively ($K_{\text{ter}} =$

10⁹–10¹¹ M⁻²).⁴⁹ Complexation of the small WGG tripeptide with CB[8] (Figure 2d) is stabilized by multiple interactions with the carbonyl oxygens at the CB[8] rim. These interactions include (1) ion–dipole interactions and hydrogen bonds with the N-terminal ammonium cation, (2) bifurcated dipole–dipole interactions with the indolic NH of the tryptophan side chain, and (3) a hydrogen bond and bifurcated dipole–dipole interactions with the peptidic backbone. In this manner, six of the eight available carbonyl groups are occupied at one portal, thus explaining the increase in affinity of the tripeptide compared with monomeric Trp.⁴⁹ The crystal structure of CB[8] with FGG shows the homoternary complex with the two phenyl groups in a parallel orientation at the center of the CB[8] cavity, engaging in π – π stacking and extensive van der Waals contact (Figure 2d). Also, all of the ordered water molecules have been expelled, and similar to WGG, an ion–dipole interaction between the ammonium groups and carbonyl oxygens and additional polar interactions between the backbone and carbonyl oxygens are observed. Combined, these observations account for the positive cooperativity observed for this system and clarify the selectivity of CB[8] for binding to N-terminal hydrophobic residues.^{49,50} Recently, Urbach and colleagues convincingly showed the very strong binding of the Tyr-Leu-Ala tripeptide via CB[8]-induced folding and inclusion of neighboring side chains.⁵¹ This result provides an entry toward antibody-like specificity for supramolecular host recognition. The recognition of internal peptide motifs with a preformed MV–CB[8] complex using a phage-display-based approach⁵² led to the discovery of a three amino acid motif that bound with micromolar affinity to MV–CB[8]. Impressively, this host–guest binding persisted when incorporated into the immunoglobulin-like Tn3 protein, creating further opportunities for selective protein recognition against this target and others.

PROTEIN RECOGNITION

The fact that protein assembly is typically governed by hydrophobic and electrostatic interactions^{1,7,8} presents the opportunity to modulate these processes using synthetic supramolecular systems.⁵³ The design of supramolecular systems for protein surface recognition is, however, complicated by the need to account for the topology and chemistry of neighboring amino acids. Analysis of the mode of action of supramolecular protein recognition events is therefore of importance for subsequent attempts at the rational design of selective host ligands.

Hamilton and co-workers were the first to make use of calix[4]arenes to target proteins and functionally inhibit PPIs by covering a large protein surface (>600 Å²).⁵⁴ Inspired by the structure of antibodies, these authors linked several peptide loops to the upper rim of calix[4]arene. This generated scaffolds that presented multiple binding groups over a large surface to induce high-affinity binding via cooperative supramolecular interactions. The cone-shaped geometry and charge-complementary peptides were designed to cap the positively charged belt close to the heme edge of cytochrome *c* (cyt-*c*). As a result, the binding of cyt-*c* to its natural protein partner, cytochrome *c* peroxidase (CCP), was blocked.⁵⁵ By tuning of the peptide loops, several different proteins and their functionalities could be targeted by calixarenes. Prominent examples include the inhibition of the proteolytic activity of α -chymotrypsin⁵⁶ and binders to vascular endothelial growth

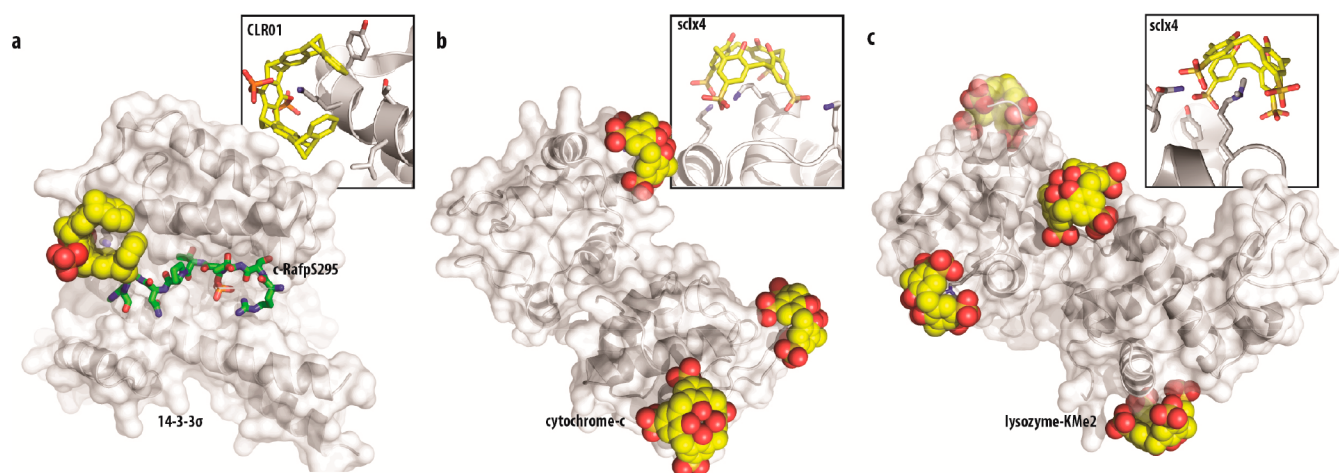


Figure 3. (a) Crystal structure of CLR01 (yellow) bound to 14-3-3 σ (gray) (PDB entry 4HQW) and superimposition with C-RafpS295 (green), revealing the steric clash with a zoom in on the CLR01 binding site with Lys214. (b) Crystal structure of sclx4 bound at three positions to an asymmetric cyt-*c* dimer with a zoom in on the calixarene binding site with Lys89 (PDB entry 3TYI). (c) Crystal structure of the asymmetric dimethyllysine lysozyme dimer bound to sclx4 with a zoom in on the calixarene binding site with Lys116-Me₂ (PDB 4N0J).

factor and platelet-derived growth factor that blocked the binding to their correspondent receptors.^{57–59}

Metal complexes are another current supramolecular host for the recognition of protein surface elements.⁶⁰ The recent work of Wilson and co-workers on ruthenium complexes is of particular interest because of the detailed thermodynamic and structural analysis of the mode of action for protein–surface recognition.⁶¹ Carboxylate-functionalized tris(bipyridine)-ruthenium(II) (Ru^{II}(bpy)₃) complexes were employed for the recognition of cyt-*c* and inhibition of the cyt-*c*–CCP interaction. Via a luminescence quenching assay and subsequently van't Hoff analyses, the *K_d* and thermodynamic parameters for complexation of cyt-*c* with different carboxylate-functionalized Ru^{II}(bpy)₃ complexes were determined. The binding event was shown to be driven by electrostatic interactions and entropically favorable. Increasing the number of carboxylate groups in Ru^{II}(bpy)₃ resulted in a higher affinity for cyt-*c* through an increased enthalpic contribution. Structural solution-based data were obtained via ¹H–¹⁵N HSQC NMR spectroscopy, revealing a similar binding site for cyt-*c* by the metal complexes as observed for CCP, indicating an orthosteric mode of inhibition.

The binding of CB to peptides or proteins can be used to modulate the activity of biomolecules. Data from tandem MS analysis suggest that the binding of CB[6] to several Lys residues on the surface of ubiquitin leads to changes in the conformation of this protein.⁶² CB[7]'s high binding affinity for aromatic residues formed the basis of the inhibition of aminopeptidase N,^{63,64} type-II endonuclease,⁶⁵ and amyloid fibrillation.⁶⁶ Host molecules are particularly well suited as inhibitors for amyloid fibrillation. Besides CB[7],⁶⁶ cyclodextrins,⁶⁷ calixarenes,⁶⁸ and especially molecular tweezers¹⁷ have been reported to inhibit amyloid fibrillation. The tweezer CLR01 was shown to efficiently inhibit the self-assembly and/or toxicity of multiple amyloids by disrupting electrostatic and hydrophobic interactions that are important contributors to the formation of amyloids.^{69–73} By targeting the whole amyloid formation process rather than a specific protein, CLR01 has potential therapeutic benefit for multiple amyloidosis-related diseases.⁷⁴ CLR01 was also effective in animal models of Alzheimer's⁷⁵ and Parkinson's⁷⁶ disease, making it a promising

lead for clinical trials.^{17,77} However, the exact mechanisms behind these inhibitory effects frequently remain unknown. More structural data will certainly help to bridge this knowledge gap and boost the applicability of host molecules for protein inhibition.

In 2013, the cocrystal structure of CLR01 with a 14-3-3 protein dimer was reported.⁷⁸ In accordance with MD simulations, the binding mechanism of CLR01 with 14-3-3 was resolved. Remarkably, only a single host–guest complex was formed at a single surface-exposed Lys214 residue (Figure 3a). In the crystal structure, the CLR01 host almost completely embraces this Lys214. One of the two phosphate groups of CLR01 interacts with the Lys residue, while the second phosphate moiety remains solvated. CLR01's preferential binding of Lys214 can be explained by the unique environment surrounding the amino acid residues. The protein shields CLR01 from bulk water via three neighboring apolar residues, and Lys214 is without neighboring Lys or Arg residues, thereby providing an optimal molecular fit. Lys214 is positioned close to the binding region of many 14-3-3 binding partners. The binding of CLR01 to Lys214 inhibits the subsequent binding of 14-3-3 with its interaction partners such as the proteins C-RafpS259 and ExoS. The interaction with CLR01 provides a steric blockade for the interaction partners, as revealed by superposition of 14-3-3 with C-RafpS259 (Figure 3a, green peptide). Information on the specific requirements for supramolecular complex formation with proteins, garnered from similar crystallographic studies, will aid in the design of stronger and more specific protein modulators, for example through redesign of the phosphate groups in the case of CLR01.

In 2012, the first crystal structure of a calixarene-bound protein, specifically sclx4 bound to cyt-*c*, was reported.⁷⁹ By the combination of crystallography data with binding data obtained in solution from NMR titration experiments, the dynamic peripheral superficial binding of sclx4 with cyt-*c* was explored. The crystal structure implicates the presence of three different binding sites, all involving a Lys side chain (Figure 3b). All three Lys side chains adopt a bent conformation, thereby enabling the formation of salt bridges with the sulfonate substituents and displacement of water from the hydrophobic

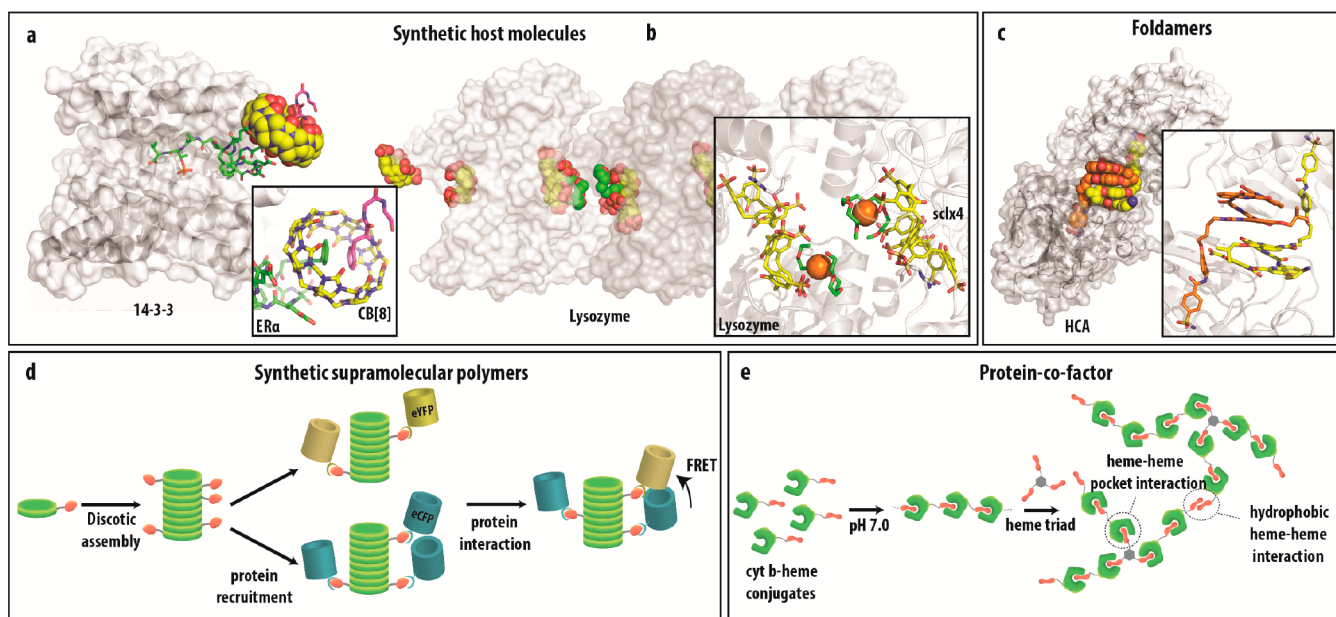


Figure 4. Supramolecular-mediated assembly of proteins. (a) Crystal structure of the 14-3-3/FGG-ER α /CB[8] complex (PDB entry 5N10). (b) Crystal structure of the linear assembly of lysozyme tetramers by sclx4 (PDB entry 4PRQ). (c) Crystal structure of foldamer-induced dimerization of HCA (PDB entry 4LP6). (d) Dynamic supramolecular platform for protein assembly and interactions. (e) Self-assembly of heme-functionalized hemoproteins into protein nanowires.

calixarene interior. Dependent upon the position of the Lys, additional polar contacts were made by sclx4 with neighboring polar residues or the backbone amides. For example, two of the sulfonates form a salt bridge with Lys89 while a third salt bridge interacts with the neighboring Lys5 residue (Figure 3b, zoom). The sulfonate-bearing rim also induces the formation of hydrogen bonds with Asn92 and the amide backbone of Lys89. Furthermore, cation- π interactions with the C^e of Lys89 are present. The multiple binding sites observed by X-ray crystallography, along with NMR mapping studies, inspired the authors to construct a model in which sclx4 explores the surface of cyt-c, thereby camouflaging the charge-rich, solvent-exposed surface of cyt-c. Recently, this transient alteration of the protein surface has been shown on human ubiquitin for supramolecular anions like sclx4 and CLR01.⁸⁰ The gained structural information casts light on the interactions that are important for the development of (small) supramolecular host molecules for protein-surface recognition. Unsymmetrical sclx4 calixarene analogues, larger cavities,⁸¹ or functionalized peripheries promise to increase the specificity of these host molecules.

Sclx4 binds selectively to methylated lysines over regular Lys, as was recently shown by Crowley and co-workers.⁸² A dimethyllysine lysozyme variant was studied for specific calixarene recognition using crystallography and NMR spectroscopy. Of the six available Lys_{Me2} residues present on the lysozyme surface, only Lys116_{Me2} was buried in the calixarene cavity as a result of its steric accessibility and an advantageous local charge environment. In contrast to Lys-sclx4 complexation, Lys_{Me2} binds with one of its methyl groups in the center of the calixarene to maximize cation- π bonds (Figure 3c). Besides the binding of Lys116_{Me2}, sclx4 was also found by X-ray crystallography to bind to Arg14 despite the 50-fold greater affinity for Lys_{Me2} amino acids in comparison with free Arg.⁹ This work perfectly highlights how both protein surface topology and the molecular structure of the host molecule

can influence the supramolecular protein binding. Structural data can therefore be useful to further elucidate the role of neighboring moieties on the recognition by supramolecular systems in order to assist in the design of supramolecular PPI modulators.

■ PROTEIN ASSEMBLY AND MODULATION

Many proteins form assemblies to execute various functions in the cell. Different supramolecular approaches to control assembly of proteins and modulate their correlated function are under development. In this context, structural insights acquired through the structural elucidation of interactions between cellular components will help with the design of more complex assembly systems.

Mendoza and co-workers were one of the first to use designed supramolecular elements to modulate protein assembly and elucidate the role of the supramolecular host molecules in the assembly process. Two calix[4]arenes, each with four cationic guanidiniumethyl groups on their upper rim and loops on their lower rim, rescued the tetramer formation of p53 from the tetramer-destabilizing R37H mutation.⁸³ The hydrophobic surface of the calix[4]arene fits cooperatively in the hydrophobic slit between two monomers while the guanidine moieties on the upper rim enhance the tetramerization via ion pairing and hydrogen bonding with glutamate residues on the surface of the protein. The tetramerization could be further improved by exchanging the loops on the lower rim for the more flexible propyl side chain, demonstrating the importance of side-chain flexibility for the proper molecular recognition of motifs.⁸⁴ Kamada et al. showed that a calix[6]arene modified with imidazoles on the upper rim could stabilize the tetramerization of the p53 mutant under physiological conditions, thereby enhancing the *in vivo* transcriptional activity of the protein.⁸⁵

The capacity of CB[8] to simultaneously bind two N-terminal Phe's laid the foundations for its use as a supra-

molecular inducer of protein dimerization. Introduction of genetically encoded N-terminal FGG motifs into fluorescent proteins enabled their reversible assembly into 1:2 ternary complexes with CB[8].⁸⁶ Subsequently, the addition of CB[8] resulted in energy transfer between two proteins, of either the homo-FRET or hetero-FRET type, in line with homo- or heterodimerization of the different fluorescent proteins. Via additional engineering of the protein surface, bivalent interplay between an intrinsic affinity for the protein to dimerize and supramolecular dimerization was possible, resulting in the formation of well-defined protein tetramers.⁸⁷ Eventually, the functionality of the CB[8]-induced protein dimerization could be brought into play to control the activity of enzymes. By equipping inactive monomeric caspase-9 with an N-terminal FGG motif, its assembly into an active dimer could be controlled by CB[8].⁸⁸ Addition of CB[8] to a solution of caspase-9 led to 50-fold enhanced enzymatic activity. Alternatively, CB[8] was also used as a supramolecular platform to induce cooperative heterodimerization of a split luciferase. The two inactive halves of the split luciferase were assembled into a functionally reinstated enzyme upon addition of CB[8] with the capability of iterative on and off switching as well.⁸⁹ Supramolecular platforms such as CB[8] thus show great potential for stabilization of PPIs.

De Vink et al. further explored CB[8]-mediated stabilization, this time targeting the dimeric 14-3-3 adapter protein.⁹⁰ By fusion of the FGG motif to 14-3-3 binding epitopes of estrogen receptor α (ER α), a binary and bivalent protein assembly platform was constructed. The two FGG-functionalized 14-3-3 binding epitopes were shown to govern the formation of this platform, which was synergistically directed by the CB[8] host and 14-3-3 protein dimer, as shown by fluorescence spectroscopy assays. Additionally, a cocrystal structure of the 14-3-3/FGG-ER α /CB[8] complex was solved, showing the binding of FGG-ER α to one 14-3-3 dimer and CB[8] via the FGG motif (Figure 4a). A second FGG motif, from a symmetry related 14-3-3 dimer, also bound to CB[8] in a similar mode. Interestingly, additional polar and hydrophobic contacts between CB[8] and FGG-ER α were observed, as well as a water network established by amino acid residues from the 14-3-3, which lock CB[8] into place in the crystal.

Foldamers are another interesting class of synthetic supramolecular systems, inspired by natural folding biomolecules.^{91,92} While they are heavily used to mimic folded peptides, their exploration for use as orthogonal supramolecular inducers of protein assembly has just been ignited. Huc and co-workers made use of an aromatic foldamer to induce the dimerization of human carbonic anhydrase II (HCA).⁹³ A nanomolar HCA ligand was tethered to the helical aromatic oligoamide foldamers to allow for the screening and visualization of foldamer–protein interactions and acquire key features for protein–foldamer recognition. After a small screening campaign, a crystal structure was solved showing a substantial number of interactions between the foldamers and proteins themselves as well as between the protein and foldamer (Figure 4c). The foldamers protrude from the HCA to establish interactions with the opposite HCA protein and induce a clipped conformation between the first quinoline units of the foldamers. Additionally, protein–protein interactions occur through the formation of hydrogen bonds, bridged by water molecules, and the coordination of Zn²⁺, which induce the formation of the quaternary complex.

Recently, structural solution studies using NMR spectroscopy revealed an interesting buffer-dependent formation of HCA–foldamer dimers.⁹⁴ Additionally, there is a strong foldamer sequence dependence of the assembly behavior.⁹⁵ Novel protein–foldamer stoichiometries were observed with, as an exceptional highlight, the structural elucidation of a protein–foldamer complex consisting of two proteins and three foldamers. Two of the foldamers are bound to the HCA via a ligand, and the third foldamer is sandwiched between these two foldamers as a kind of molecular glue.

Besides the formation of well-defined protein oligomers, many proteins assemble to form larger oligomers, for example by interacting with cofactors, substrates, inhibitors, or protein partners. As a result, interprotein interactions have been widely explored for the development of supramolecular protein polymers.^{94,96} Alternatively, as considered in this next section of the Perspective, the protein assembly process can be induced or steered by synthetic supramolecular systems, providing an orthogonal entry to and control over novel types of architectures. The sulfonated calix[4]arene *scx4* can induce the linear assembly of lysozyme tetramers under appropriate conditions, as clarified by a crystal structure (Figure 4b).⁹⁷ *Scx4* binds to the highly exposed C-terminal Arg128 in a side-on binding mode, as previously seen for Lys.³⁵ The side-on conformation allows for the formation of salt bridges between the solvent-exposed guanidinium group and two of the upper-rim sulfonate groups. The partially cationic C^δ sits in proximity to the phenyl rings of *scx4*, suggesting additional cation– π interactions. A second *scx4* forms a complex with a Mg²⁺ cation and a poly(ethylene glycol) fragment. Two *scx4*–Mg²⁺–PEG complexes from neighboring tetramers are within range for van der Waals contact, thereby possibly connecting two tetramers. Recent work by Crowley and co-workers⁸¹ also shows the ability of *p*-phosphonatocalix[6]arene (*pclx6*) to mediate protein dimerization as a result of the dimeric packing of two *pclx6*. The assemblies of *pclx6* with *cyt-c* were structurally (i.e. X-ray crystallography) as well as dynamically characterized and shown to bind more specifically than *scx4* by nestling at only a single site on the protein surface.

Liu et al. used the ternary complexation of FGG with CB[8] to construct supramolecular protein materials.⁹⁸ Nanowires were obtained by combining the CB[8]-based assembly with glutathione S-transferase (GST) recognition. A GST homodimer protein was provided with N-terminal FGG tags. Addition of CB[8] resulted in supramolecular polymerization into the nanowires, as was shown for example by atomic force microscopy (AFM). The GST scaffold could be functionalized with an additionally incorporated glutathione peroxidase mimic to generate nanowires with antioxidant properties. A similar design strategy was used to develop a self-assembling protein nanospring.⁹⁹ By generation of a fusion protein of GST with Ca²⁺-sensitive recoverin and the FGG tag, a fully reversible supramolecular protein nanospring was generated that extends or contracts depending on the binding or release of Ca²⁺. To circumvent the spatial limitation of the FGG tag on proteins, Liu and co-workers¹⁰⁰ designed a maleimide-functionalized FGG tag. This tag could site-selectively introduce FGG moieties over the protein surface, thereby expanding the number of possible configurations of host–guest-driven protein assemblies. Different morphologies ranging from nanorings to superwires could be obtained by controlling the concentration of FGG-modified GST and the stoichiometry between the protein and CB[8].

Synthetic supramolecular systems can also be covalently attached to proteins to steer interactions between the proteins by creating a dynamic assembly platform (Figure 4d). Flat disc-shaped molecules were conjugated to proteins, resulting in the formation of self-assembled columnar structures with proteins at their periphery. In this manner, different fluorescently labeled streptavidin constructs could be brought into proximity, as evidenced by efficient energy transfer.¹⁰¹ The reversible supramolecular platform allowed for reorganization of the assembled protein upon the addition of additional supramolecular components. Via this approach the distance between a cyan fluorescent protein and yellow fluorescent protein could be tuned with resulting changes in the energy transfer efficiency.¹⁰²

The natural supramolecular elements of proteins can also be manipulated to generate supramolecular protein materials. Hayashi and co-workers used the interactions between a heme protein and its heme cofactor to design different supramolecular polymeric protein networks (Figure 4e).¹⁰³ Fibrous, one-dimensional self-assembled protein polymers were generated by covalently attaching a heme to a heme protein, resulting in a head-to-tail self-assembly motif, as confirmed by AFM. The structure could be controlled thermodynamically or via the addition of native heme and could be regulated by varying the pH, thus rendering them promising stimuli-responsive functional nanobiomaterials.^{103,104} Intermixing of heme protein with a heme triad induced the formation of two-dimensional networks. The branching pattern of the network could be controlled by alternating the stoichiometry between the protein and heme triad building blocks.¹⁰⁵ Also, linear heterotropic protein assemblies of alternating dimeric myoglobin and tetrameric streptavidin were realized with a heme–biotin conjugate designed as the artificial cofactor.¹⁰⁶ These examples show that, especially at the interface of synthetic supramolecular systems and protein engineering, many opportunities exist to control protein assembly and make new protein materials by taking the best of both worlds.

CONCLUSIONS

Synthetic supramolecular host molecules have already shown applications in the life sciences, notably as drug delivery^{107–109} and scavenger reagents (e.g., sugammadex).¹¹⁰ As highlighted in this Perspective, supramolecular host molecules are now also becoming of interest as drug concepts themselves. Synthetic host molecules show particular promise as an approach to modulating PPIs. This is illustrated by encouraging results obtained using the CLR01 molecular tweezer in amyloid fibrillation studies, which are currently progressing toward clinical trials.^{75,76}

To develop this field and its application further, an exact understanding of the molecular interplay of supramolecular systems with proteins is required. Structural data obtained from techniques like NMR and crystallography have provided first insights into the supramolecular forces at play. Especially the crystal structures of host–protein complexes have been of great value for understanding the roles of topology and the chemistry of neighboring amino acids. This has opened the way for the development of supramolecular binders with high affinities and specificities. Tuning of the molecular structure of the host molecule or generation of multimeric hosts are open avenues for supramolecular chemists to follow in order to address the plethora of proteins and their PPIs. Alternatively, the combination of the fields of supramolecular chemistry and

protein engineering has resulted in novel modes of control over protein assembly and the formation of protein materials. These systems provide novel tools to study the effects of protein assembly in a controlled and reversible manner and to generate responsive protein materials for advanced applications in signal amplification and sensing. Supramolecular chemistry targeting proteins has a bright future ahead.

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Notes

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