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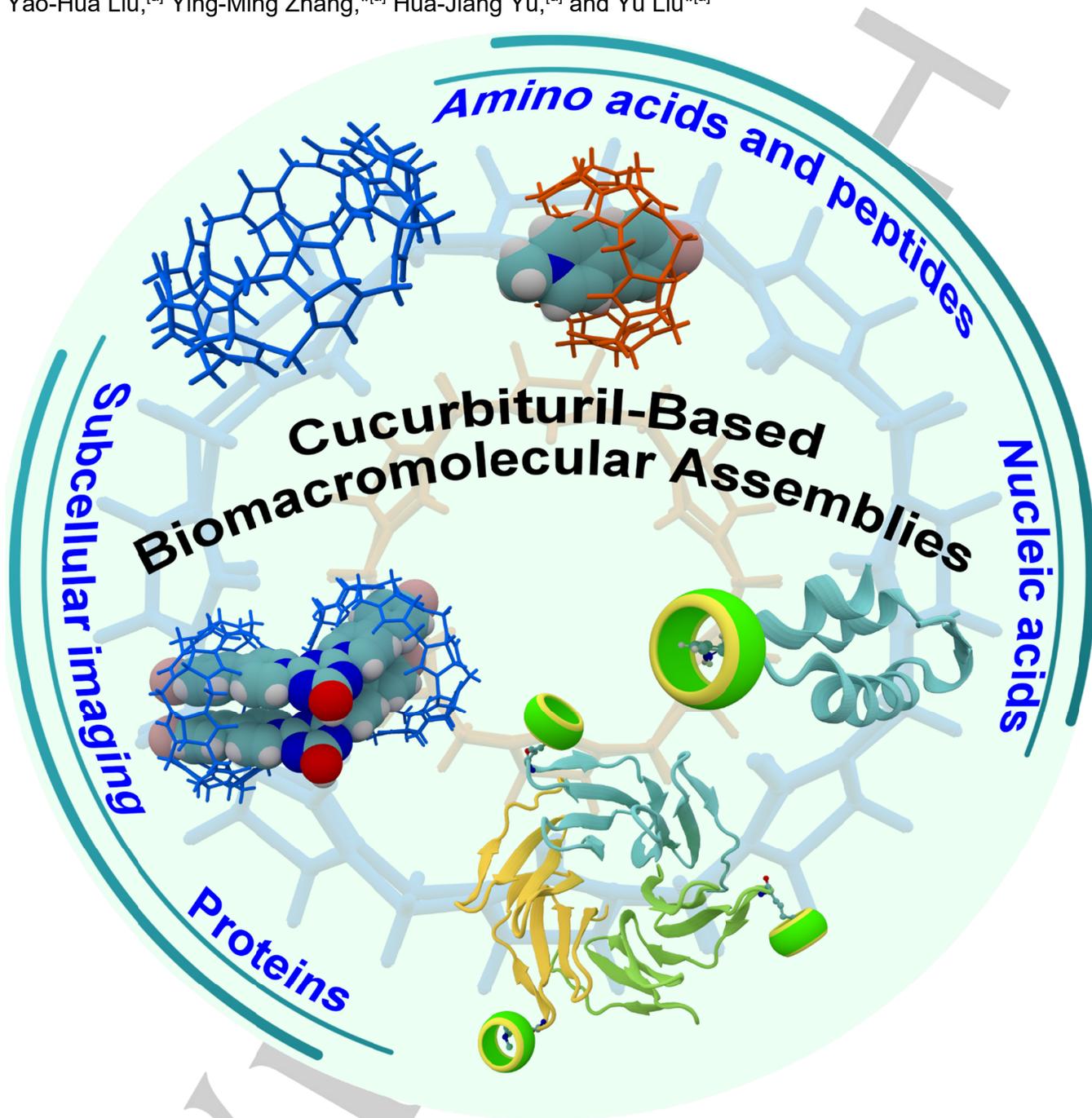
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Cucurbituril-Based Biomacromolecular Assemblies

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Abstract: The construction of controlled biomacromolecular assemblies has become a thriving area of supramolecular chemistry. In this respect, cucurbiturils (CBs), a class of macrocyclic receptors having robust skeletons, hydrophobic cavities, and carbonyl-laced portals, have been drawn into the limelight because of their advantageous molecular recognition characteristics with a variety of biomacromolecules, including peptides, nucleic acids, and proteins. In this minireview, we focus on the impressive advances in CB-based biomacromolecular assemblies, such as biosensors and assays, the regulation of biochemical reactions, and the treatment of serious diseases. CB-promoted subcellular bioimaging has also been demonstrated in different organelles. The case studies presented herein demonstrate the numerous applications, from fundamental research to translational applications, of diverse CB-based supra/biomacromolecular architectures.

1. Introduction

Cucurbit[*n*]urils (CBs, *n* = 5–8, 10, and 14), which are formed by the acid-catalyzed condensation between glycoluril and formaldehyde, are a family of macrocyclic receptors in host–guest chemistry (Figure 1a).^[1] Among various synthetic macrocyclic hosts, CBs stand out because of their great versatility in molecular recognition and self-assembly.^[2] Because of the highly rigid molecular skeleton, CBs can encapsulate a vast number of inorganic and organic guest molecules with highly tunable binding affinities.^[3] Moreover, ultrastable homo- and heteroternary inclusion complexes can be readily obtained by using large CBs, which offers a convenient route to prepare multidimensional nanoarchitectures.^[4]

In the realm of CB science, many pioneering works have promoted the practical application of CBs in chemistry, biology, medicine, and materials science.^[5] Meanwhile, a large number of new supramolecular receptors, such as acyclic CBs, inverted CBs, hemiCBs, bambusurils, and taurauril, have been created by the synthetic transformation of CBs and show improved molecular recognition properties in water. Meanwhile, diverse topologically intriguing nanostructures, including oligomeric and coordination complexes, (pseudo)rotaxanes, amphiphiles, cross-linked networks, as well as supramolecular polymers and organic frameworks, can be derived from the intermolecular self-assembly of CBs, which has greatly broadened the research scope for supramolecular chemistry (Figures 1b–1f).^[6] More importantly, CB-based analytical methods and biosensing devices have been successively established for the qualitative and quantitative analyses of many bioactive molecules at different scales, ranging from small-molecule detection, controllable protein modification and enrichment to advanced NMR spectroscopy techniques.^[7]

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Ying-Ming Zhang received his Ph.D. degree in organic chemistry from Nankai University under the guidance of Professor Yu Liu in 2010. Now he is an associate professor at the College of Chemistry of Nankai University. His research interests focus on the bioactive nanoassemblies based on cyclodextrins and cucurbiturils.



Yu Liu graduated from the University of Science and Technology of China in 1977 and received his Ph.D. degree from Himeji Institute of Technology, Japan, in 1991. In 1993, he moved to Nankai University as a full professor. His research focuses on molecular recognition and assembly of macrocyclic receptors.



In recent years, with the rapid development of bioactive nanoassemblies, the modulation of the topological structures and mimicking of the biological functions of naturally occurring biomacromolecules have become focal points of supramolecular chemistry. These biomacromolecules, including polypeptides, nucleic acids, proteins, and polysaccharides, are fundamental structural units in cells and play critical roles in maintaining physiological functions in living systems.^[8] By leveraging controllable host–guest interactions, the co-assembly of biomacromolecules with cavity-bearing synthetic macrocycles, especially CBs, has proven to be an effective and powerful strategy to prepare simplified but realistic biological systems, decipher molecular binding modes, construct innovative biomaterials, and develop new disease treatments.^[9]

In this minireview, inspired by our ongoing interest in bioactive supramolecular nanostructures, we highlight the recent achievements of CB-based biomacromolecular assemblies, with an emphasis on the molecular design, assembly principles, and their concomitant potential in biosensing and detection, control over biochemical and enzymatic reactions as well as supramolecular therapies for the treatment of diabetes, cancer, and neurodegenerative diseases. We also present some

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intriguing multicomponent assemblies that can serve as targeted bioimaging agents at the subcellular level. Finally, the challenges and perspectives for the creation of functional CB–biomacromolecule assemblies are discussed with the ultimate goal of promoting their translational application in advanced biomedical fields.

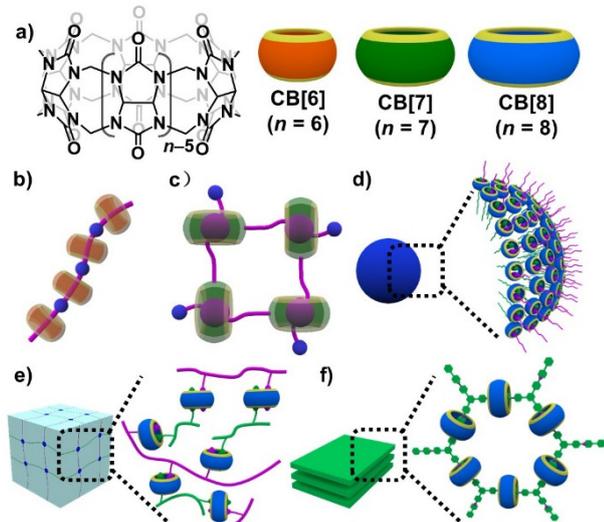


Figure 1. Typical CB-based nanoarchitectures: a) molecular structures of CB[*n*]s (*n* = 6, 7, and 8), b) (pseudo)rotaxanes, c) oligomeric complexes, d) amphiphiles, e) cross-linked network, and f) supramolecular organic frameworks.

2. Amino Acids and Peptides

The wide variety of sequences and spatial distributions of native amino acids results in a vast number of polypeptides and proteins in living organisms. In CB-based host–guest chemistry, a plethora of studies have aimed to identify and develop reliable supramolecular systems that can exclusively recognize amino acid residues in peptides and proteins for wider biological applications.

2.1. Selective Molecular Recognition

Generally, in the solid state, the crystal structures of inclusion and exclusion complexes of CBs with amino acids and peptides are stabilized by multipoint complementary interactions.^[10] In aqueous buffer solutions, binding mechanism studies have revealed that the expulsion of high-energy water, guest desolvation, and entropic penalties arising from the loss of conformational freedom upon complexation are the crucial factors determining the binding strengths of CBs and amino acids.^[11] In the gas phase, mass spectrometric investigation has demonstrated that ion–dipole interactions are the dominant driving forces under water-free conditions, which is strikingly distinct from the hydrophobic effect in the solvated state.^[12] Early examples of the molecular recognition of CBs for amino acids and oligopeptides came from Buschmann's work, in which the intramolecular association process was governed in a thermodynamically favorable way with moderate binding strengths.^[13] Meanwhile, Inoue and Kim also reported that CB[7], an achiral host, exhibits high diastereoselectivities toward Phe-containing dipeptides.^[14] Thereafter, the guest scope of CB-mediated peptide recognition has been extended from aromatic amino acids to their corresponding N-terminal peptides.

Urbach's group has made pioneering contributions in the CB-based sequence-specific molecular recognition of natural amino acids and peptides.^[15] Having validated the charge-mediated recognition of N-terminal tryptophan using methyl viologen (MV²⁺) as an auxiliary guest, they observed the CB-promoted dimerization of N-terminal aromatic tripeptides, confirming that CB[8] could selectively and tightly bind to Trp–Gly–Gly (WGG) and Phe–Gly–Gly (FGG) peptides in aqueous solution (Figures 2a and 2b).^[16] The N-terminal FGG tripeptide motif, which combines the structural merits of the terminal ammonium site, hydrophobic aromatic terminal group, and appropriate molecular size, is regarded as a reliable candidate for the formation of a stable 2:1 homoternary complex with CB[8]. The association constant (K_a) for the FGG–CB[8] complex can reach up to $1.5 \times 10^{11} \text{ M}^{-2}$ via ion–dipole and π -stacking interactions. The latest study revealed that two staggered FGG molecules could be encapsulated in the CB[8] cavity with a noncooperative binding scenario.^[17]

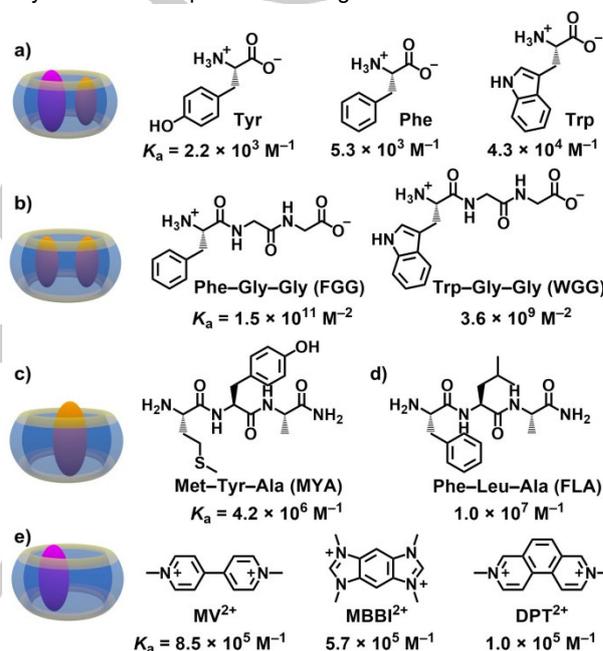


Figure 2. CB[8]-based molecular recognition and corresponding K_a values: a) aromatic amino acids with the CB[8]–MV²⁺ complex, b) 2:1 ternary complexation with FGG and WGG, c) 1:1 binary complexation with MYA and FLA, and d) 1:1 complexation with representative auxiliary guests.

Notably, both structural and environmental issues should be considered in the pursuit of high affinity CB–peptide complexes. As observed for the Tyr–Leu–Ala tripeptide, rather than forming homo- or heteroternary complexes, both the N-terminal tyramine residue and neighboring side chain are encapsulated in the CB[8] cavity to form a clear 1:1 binary complex.^[18] Augmenting the existing molecular recognition of aromatic residues, submicromolar affinity has been achieved for CB[8] by the subtle design of nonaromatic methionine-terminated peptides (Figure 2c).^[19] The key structural features of these methionine-bearing peptides is the N-terminal charge, nonbranched bulky side chain, and a tail with low steric bulk. These works demonstrate that CB[8] has great potential in targeting methionine-terminated proteins and protecting them from aminopeptidase attack. In a local three-dimensional structure, Scherman and Herrmann dissected the molecular recognition of cyclic peptide sequences expressed within a protein domain, pointing out that the steric

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constraints in the peptide skeleton and the whole protein conformation are responsible for the formation of a stable heteroternary complex with CB[8] and MV^{2+} .^[20] In a recent report, using a Phe–Leu dipeptide and relevant polypeptides as examples, Scherman et al. revisited the sequence-specific molecular recognition of oligopeptides with CB[8] and verified a switchable binding process from a 1:1 stoichiometric complex to a 2:1 complex, depending on the host–guest molar ratios (Figure 2d).^[21] The diverse CB–peptide complexation is the molecular basis for the construction of CB–protein nanosystems, as presented in Sections 4.1 and 4.2.

2.2. Bioactive CB–Peptide Systems

The discoveries of sequence-selective CB–peptide binding pairs has provided researchers with opportunities for the fabrication of functional nanomaterials, establishment of analytical methods, and modulation of biochemical reaction processes. Many innovative applications stem from the specific 1:1 binding of amino acid residues with CB[6] and CB[7], such as the isolation and identification of peptides, inhibition of enzymatic digestion, protein recognition in the serum, and protein analysis by mass spectrometry.^[22] Moreover, morphological interconversion can be realized by inclusion complexation between CB[7] and the N-terminal amino acid residue. Specifically, a tubular supramolecular polymer was disassembled when CB[7] was attached to the polyethylene glycol (PEG)-modified cyclic peptide containing two Phe units, and this structure could be reversibly assembled upon addition of adamantanamine (Figure 3a).^[23]

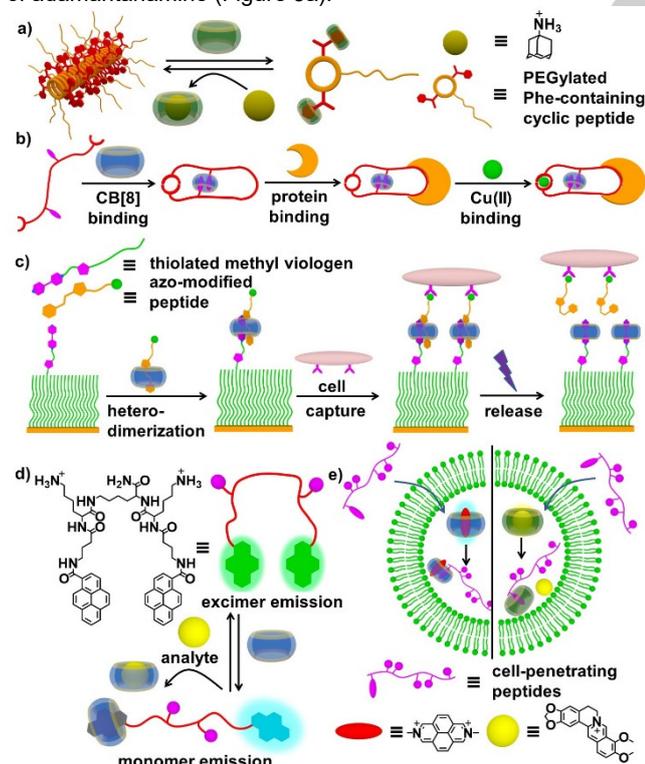


Figure 3. a) CB[8]-induced morphological regulation of cyclic peptide assembly.^[23] b) Biomarker detection by CB[8]-involved multivalent peptide assembly.^[24] c) Photocontrolled cell capture and release by a CB[8]-based ternary inclusion complex.^[25] d) Fluorescence detection of biological substrates by CB[8] and amphiphilic peptides.^[26] e) Monitoring of peptide transport pathways by CB–dye reporters.^[28]

By using ternary inclusion complexation comprising CB[8] and amino acid residues, multivalent ensembles can be endowed with unique physicochemical properties and wide adaptability for target substrates. On the one hand, for 2:1 homoternary inclusion complexation, cross-linked supramolecular systems with good rheological performance and biocompatibility have been constructed. For instance, the catalytic ability of a peptide–Cu(II) complex can be activated by the synergistic ligand binding of CB[8] and integrin. The conformational rearrangement from linear to loop structures in these designed peptides also facilitates the signal conversion and amplification in a bioassay of overexpressed integrin as a tumor invasive biomarker (Figure 3b).^[24] On the other hand, for 1:1:1 heteroternary inclusion complexation, CB[8] and MV^{2+} are frequently employed for peptide separation, cytotoxicity modulation, and protein detection. Moreover, light-triggered cell adhesion onto and release from the bioactive surface can be reversibly manipulated by the co-encapsulation of CB[8] with MV^{2+} and arylazopyrazole-modified peptide (Figure 3c).^[25] Meanwhile, in addition to MV^{2+} , various π -conjugated dicationic components have been introduced as auxiliary chromophores to achieve the optical chemosensing of amino acids, peptides, and proteins (Figure 2e). In an alternative study, a pyrene-functionalized peptide was synthesized for the ratiometric fluorescence monitoring of tryptophan derivatives, FGG, and insulin (Figure 3d).^[26] These analytes could initially form heteroternary complex with pyrene inside the CB[8] cavity and finally occupy the whole CB[8] cavity to form a homoternary complex, thus resulting in spectral changes from the pyrene monomer to excimer emission.

Remarkably, by making full use of the large disparities in binding affinities between the substrates and products, Nau and coworkers developed a supramolecular tandem assay by competitive displacement of a fluorescent dye as an indicator.^[27] This operating principle has been widely applied for the determination of the enantiomeric excesses of amino acids and in monitoring the enzymatic activities of protease and amino acid decarboxylases. In a recent study, different CB–dye reporter pairs have been selected to conduct supramolecular membrane transport assays. The membrane transport of N-terminal aromatic peptides into phospholipid vesicles could be monitored in real time by either dye deportation from the CB[7] cavity or dye co-encapsulation inside the CB[8] cavity (Figure 3e).^[28] Given the advantages of label-free and user-friendly fluorescence-based assays, it is conceivable that this work can be developed as a promising analytical method to detect the uptake of bioorganic analytes by living systems.

3. Nucleic Acids

The classic mode of interactions between CBs and nucleic acids largely arises from the condensation and delivery of DNA and RNA by positively charged guests. Early studies revealed that the efficiency of DNA condensation by a two-dimensional pseudopolyrotaxane containing CB[6] and β -cyclodextrin is dependent on the amount of CB[6].^[29] The opposing effects of CB-induced charge shielding and structural rigidity are believed to be the decisive factors in CB-induced DNA condensation. The theoretical basis can be explicitly interpreted from the viewpoint of complexation-assisted pK_a shift. For example, the complexation of butanediamine with CB[6] results in a pronounced positive pK_a shift of up to 1 unit.^[30] The content of

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doubly protonated species and the density of positive charges notably increased, which facilitates RNA binding and gene transfection in malignant cells.

Furthermore, CB–nucleic acid nanoconjugates have provided valuable theoretical models and analytical methods for the simulation and quantification of many fundamental biological events.^[31] For instance, Seitz et al. recently conducted a systematic study using spatial screening for DNA-programmable adamantane–CB[7] complexation, revealing that the limits of bivalent interactions largely relied on the contact distance, scaffold flexibility, and monovalent binding strength.^[32] These findings established standards for the design of bivalent probes and inhibitors where unintended multimolecular cross-linking should be avoided. Meanwhile, the combined utilization of complementary nucleotide sequences and dynamic inclusion complexation gives rise to aptamer-based supramolecular sensing platforms that can specifically recognize and precisely detect target biomolecules with high affinity and good sensitivity. In a recent study, CB[7] and adamantyl groups were covalently tethered onto a single strand of DNA aptamer, respectively, and then the included small molecular protein inhibitor (adamantyl benzenesulfonamide) was released once the duplex architecture had formed using adenosine triphosphate (ATP) as the input signal (Figure 4a).^[33] This CB–DNA hybrid nanoconjugate with ATP-triggered self-assembly behavior is amenable to use with other biomarkers by functional displacement of the aptamer domain.

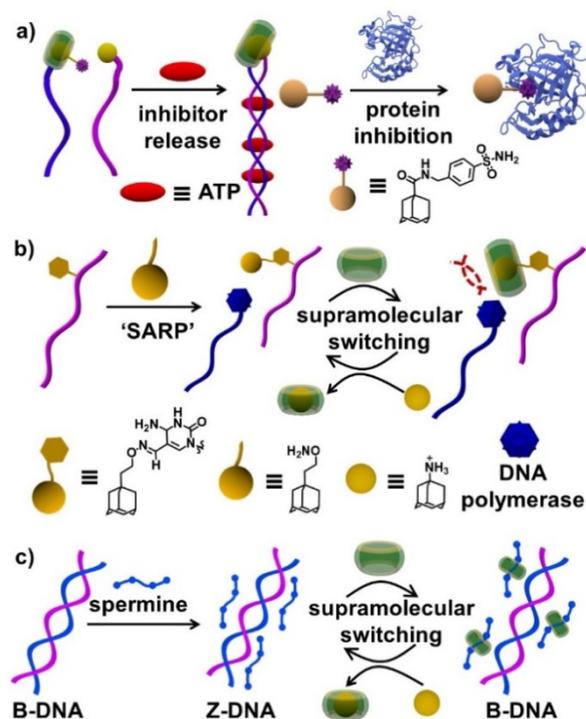


Figure 4. Schematic illustration of a) ATP-triggered release of protein inhibitor from CB[7]-based DNA aptamer.^[33] b) Modulation of 5-formylcytosine-targeted biochemical reactions by CB[7]-adamantane complexation.^[35] c) DNA chiral transition by the competitive binding with CB[7] and spermine.^[36]

More excitingly, CB-mediated host–guest complexation can also be used as a sturdy tool to regulate ligand–DNA interactions and control the molecular conformation and biochemical activities of nucleic acids.^[34] In this respect, Zhou and colleagues proposed the concept of a supramolecular

aldehyde reactive probe (SARP) to introduce an adamantyl moiety site-selectively into 5-formylcytosine through aldoxime ligation (Figure 4b).^[35] Consequently, three types of 5-formylcytosine-involved biochemical reactions, including DNA polymerase elongation, restriction endonuclease digestion, and polymerase chain reaction, were terminated by the host–guest interaction of the bulky CB[7] with the exposed adamantyl site and then restored by competitive complexation with adamantylamine. Subsequently, the spermine–CB[7] binding pair was employed to reversibly control the DNA chiral transition by the same team (Figure 4c).^[36] The spermine-induced switchable transition between the right- and left-handed DNA helices could be efficiently controlled by the alternating addition of CB[7] and adamantylamine. These studies demonstrate that both the structures and bioactivities of DNA can be adjusted by strong and dynamic CB–guest interactions, which is beneficial for the construction of advanced genetic materials with biorecognition and environmentally responsive capabilities.

4. Proteins

With the increased knowledge about the molecular binding behavior of peptides and nucleic acids, it has been possible to modulate the biological functions of proteins using CB-based supramolecular strategies.^[37] There are two general construction strategies: CB–protein hybrid systems, which rely on the CB-involved complexation/interaction at the specific sites of proteins in a straightforward fashion, and CB-mediated supramolecular protein systems, which can be indirectly achieved via chemically synthetic molecules/groups as intermediary guests.

4.1. CB–Protein Biohybrids

To attain well-defined CB–protein biohybrids, the recognition motifs on natural protein skeletons for potential CB binding must be screened out, either by inclusion complexation with the interior cavity or exclusion interaction with the exterior surface. For example, the association and dissociation of supra-biomolecular nanoassemblies of CBs with bovine hemoglobin and serum albumin can be reversibly controlled, as confirmed by competitive binding with MV^{2+} and adamantylamine, respectively.^[38] Nevertheless, direct communication between CBs and proteins has been sporadically reported by crystallographic analysis at the molecular scale. The scarcity of such reports is due in part to the intrinsically dynamic structures of biomacromolecules and the lack of appropriate groups that can be selectively associated with the CB cavity.

For the preparation of crystallographic CB–protein biohybrids, CB[7] is frequently employed because of its high water solubility, peculiar symmetry, and clear 1:1 binding stoichiometry toward aromatic amino acids. In one case, by combining the clustering effect of gregarious CB[7] and host–guest interaction with dimethyllysine, Crowley et al. constructed a multicomponent biohybrid using a dimethylated β -propeller protein (Figure 5a).^[39] Owing to the side chain accessibility, CB[7] selectively bound to the most exposed dimethyllysine site with extensive hydrogen-bonding interactions and simultaneously formed trimeric and tetrameric clusters in two different space groups. In their subsequent work, this group continued to study a CB[7]-directed crystalline biohybrid by introducing a mutant β -propeller protein rich in dimethyllysine groups as extra binding sites, thus leading to a dramatic

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increase in the CB content and the formation of high-molecular-weight aggregates in solution (Figure 5b).^[40] In contrast to the established inclusion complexation with aromatic amino acid residues and on account of the biological significance of protein methylation, dimethyllysine and trimethyllysine can be exploited as valuable host-guest binding counterparts in the modulation of topological structures and biological functions of proteins.

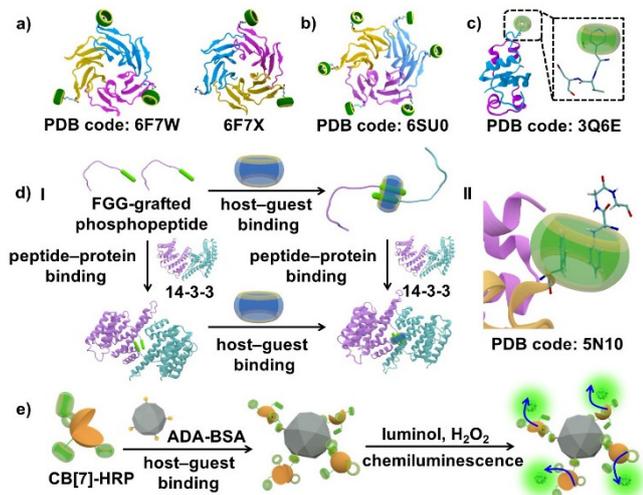


Figure 5. Crystalline biohybrids of a) CB[7] and dimethylated β -propeller protein,^[39] b) CB[7] and mutant β -propeller protein,^[40] and c) CB[7] and human insulin.^[41] d) CB-mediated protein assemblies of (I) CB[8], phosphopeptide, and protein 14-3-3 and (II) its crystal structure.^[45] e) Detection of ADA-BSA using CB[7]-HRP-catalyzed oxidation of luminol by H_2O_2 .^[48] The crystal data in (a–d) are deposited in Protein Data Bank (PDB).

Achieving one of the most important accomplishments in CB-protein biohybrids, Urbach et al. adopted the N-terminal binding methodology to associate CB[7] with the Phe site of human insulin strongly, and the single-site binding constant with CB[7] was as high as $1.5 \times 10^6 \text{ M}^{-1}$.^[41] The crystallographic superstructure of the insulin complex revealed that the first few residues are in the unfolded state, thus making sufficient space to accommodate CB[7] (Figure 5c). This unique conformational change is the key structural factor preventing insulins from forming inactive amyloid aggregates and has been successfully applied in the supramolecular PEGylation of insulin for diabetes treatments, as described in Section 4.3.

4.2. CB-Mediated Protein Structure

The incorporation of exogenous substituent groups into protein skeletons is a broadly applicable method for tuning the assembly modes and functions of proteins. It can provide anchoring points for CB encapsulation while maintaining the intact structure of pristine proteins. Previously, research on CB-protein assemblies primarily focused on the fabrication of multifunctional and stimuli-responsive biointerfaces for protein separation, immobilization, fluorescent patterning, and sensing assays.^[42] In later studies, some scientists diverted their attention to the modulation of protein-protein interactions using CB-based supramolecular approaches.^[43]

Among the pioneering studies, Brunsveld and colleagues fabricated a series of protein assemblies based on CB[8]-induced homo- and heterodimerization. As early as 2010, an N-terminal FGG tripeptide motif was genetically engineered onto a monomeric fluorescence protein, thus realizing controlled energy

transfer behavior in the presence of CB[8].^[44] More excitingly, a binary bivalent assembly was constructed by the synergistic fusion of 2:1 host-guest complexation and 2:1 peptide-protein recognition (Figure 5d).^[45] Specifically, the dimerization of 14-3-3 adapter proteins was significantly enhanced by CB[8] after being equipped with FGG motifs onto the N-terminus of the estrogen receptor α -phosphopeptide. The cocrystal structure revealed that the obtained nanoarchitecture was favorably stabilized by multiple intermolecular connections. By rational replacement with many other biomacromolecules and functional pendant groups, a similar design principle involving FGG-CB[8] homoternary complexation was successfully utilized for the visualization of protein aggregation and immobilization, construction of highly ordered superstructures, and control over enzymatic activities, which offers a supramolecular perspective for the modulation of protein-protein interactions and paves a new route to the fabrication of innovative biomaterials.^[46]

In addition to the frequently encountered N-terminal FGG moiety, some organic cations, including linear polyamines and fluorescent photosensitizers, have also been employed with CBs to improve the aggregation behavior and medicinal activities of target proteins.^[47] In turn, the photophysical performance of the included chromophoric guest molecules could be considerably enhanced by the mutual cooperation between CBs and proteins. Meanwhile, the emergence of chemically modified CBs has opened up great opportunities to widen the biological applications of multicomponent CB-protein assemblies. For example, the chemical modification of hydroxylated CBs has been readily realized through nucleophilic substitution under acidic conditions (Figure 5e).^[48] To explore the practicability of protein labeling and detection, one of the transformation products, monocarboxylated CB[7], was conjugated with horseradish peroxidase (HRP) via amide condensation. Consequently, the adamantane-grafted bovine serum albumin (ADA-BSA) was visually detected with assistance of CB[7]-grafted HRP (CB[7]-HRP), which was jointly attributed to the tight host-guest association and the amplified chemiluminescent signal output triggered by H_2O_2 . This practical synthetic methodology can remove the roadblocks in the site-selective functionalization of the CB skeleton, which may allow the creation of a library of biosensing platforms based on the chemically active CB derivatives.

4.3. CB-Protein Assemblies as Therapeutic Agents

Along with the unprecedented progress in supramolecular theranostics, CB-protein nanoassemblies have emerged as powerful candidates in the battle against many acute and chronic diseases, such as diabetes, cancers, and neurodegenerative diseases. Three functional biomacromolecules, insulin, microtubules (MT), and amyloid- β ($A\beta$) protein, have recently stimulated an upsurge in research interest because of their intimate involvement in many living and pathological processes.

In the wake of the exciting discovery of Phe-CB[7] complexation with human insulin, considerable efforts have been devoted to exploring drug nanoformulations using CB[7] as a pharmaceutical excipient.^[49] In 2016, a platform approach was established to realize the supramolecular PEGylation of therapeutic proteins.^[50] The structural stability of protein drugs dramatically increased with the assistance of PEG-CB[7] conjugates, and their pharmacological properties could be altered in a controlled fashion depending on the molecular

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weight of appended PEG chains. Thereafter, having a clear understanding of the impact of PEGylation on biological therapies, Appel et al. optimized the formulation for insulin analogs by mixing with PEGylated CB[7] and other inactive ingredients, thus enabling the enrichment of stable monomeric species with shorter duration of action compared to the commercially available formulations.^[51] Very recently, complexation-stabilized diabetes drugs have been developed by the co-administration of insulin and pramlintide with PEGylated CB[7] in diabetic rats and pigs (Figure 6a).^[52] The therapeutic windows of insulin and pramlintide overlap sufficiently with each other upon the complexation of PEGylated CB[7] with the aromatic amino-acid residues of insulin and pramlintide at their protonated N- and amidated C-termini, respectively. This supramolecular dual-hormone therapy improves the pharmacokinetic biocompatibility, suppresses postprandial glucagon secretion, and might eventually relieve the burden of multiple daily doses reliant on medication. This inventive work has shown great advances in preclinical trials for supramolecular nanomedicines in the treatment of patients with diabetes.

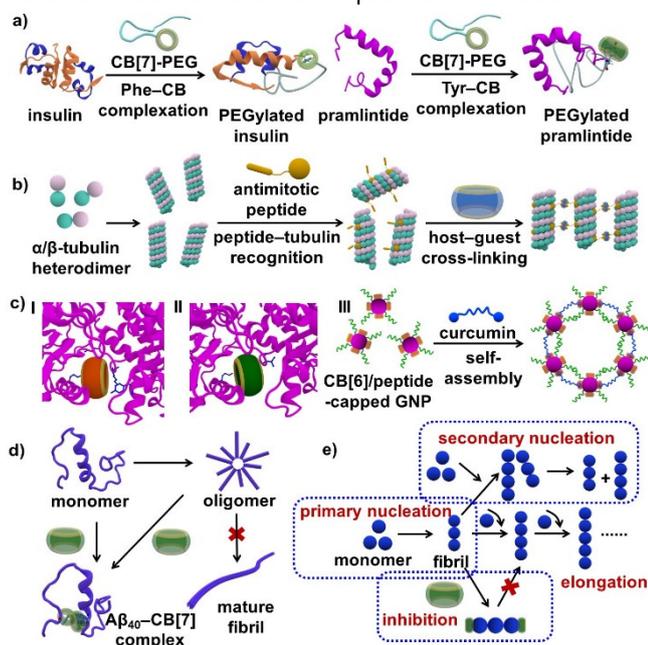


Figure 6. a) Co-formulation of CB[7]-PEGylated insulin and pramlintide to improve the pharmacokinetic profiles and therapeutic effect in diabetes management.^[52] b) Crosslinked MT aggregation by CB[8] and antimimetic peptide to enhance cell apoptosis.^[53] c) Molecular docking of MT with (I) CB[6] and (II) CB[7], and (III) CB[6]-based nanocapsules for MT disruption.^[54] d) (I) CB[7]-assisted inhibition of $A\beta_{40}$ fibrillation,^[55] and (II) the proposed fibril-end binding mechanism.^[58]

Filamentous MTs are dynamically assembled by the alternating arrangement of globular α/β tubulin heterodimers and are regarded as promising molecular targets in cancer drug discovery. After validating the cyclodextrin-mediated photocontrollable MT aggregation and disaggregation behavior, Liu and coworkers fabricated a ternary CB-tubulin supramolecular assembly, which was achieved by the synergistic interactions involving tubulin-tubulin heterodimerization, peptide-tubulin targeting recognition, and benzimidazolium-CB[8] inclusion complexation (Figure 6b).^[53] Benefiting from the CB[8]-induced intertubular aggregation, significant cell apoptosis and tumor ablation were observed both

at the cellular level and in the mouse body. In comparison, no curative effect was found in the monovalent binding with CB[7], indicating that the CB[8]-involved multivalent cross-linkage can be developed in modulating protein-protein interactions and interfering with cellular fate. Moreover, CB[6] and CB[7] also exhibited biological activity against tubulin polymerization through strong interactions with tubulins close to the vinblastine-binding pocket (Figure 6c, I and II).^[54] Therefore, a bottom-up approach was implemented to fabricate CB[6]-capped gold nanoparticles (GNPs) equipped with curcumin and a peptide targeted toward the neuropilin-1 receptor of cancer cells (Figure 6c, III). Consequently, apoptosis and tumor-growth suppression have been achieved by the perturbation of tubulin polymerization and glutathione-triggered drug release. These results clearly demonstrate that different CB homologs have tremendous potential to modulate tubulin polymerization and MT aggregation by means of either inclusion complexation within the CB cavity or exclusion interaction with the CB skeleton, which may hold great promise in the diagnosis and treatment of MT-related diseases.

The extracellular deposition of $A\beta$ fibrils in the brain is a pathological hallmark of neurodegenerative diseases. It is imperative to prevent the amyloid fibrillation process and dissolve the existing $A\beta$ fibrils in a noninvasive manner. Interestingly, a supramolecular inhibition strategy was presented for the amyloid fibrillation of $A\beta_{40}$ and $A\beta_{42}$; that is, the hydrophilic-hydrophobic balance in the fibrillation process is disrupted by the host-guest complexation of CB[7] with Phe residues, which resulted in the inhibition of amyloid fibrillation and dissolution of the previously formed fibrils (Figure 6d).^[55] On the basis of this work, a CB[6]-induced amyloid self-assembly was reported by the same group, in which various amyloidogenic proteins underwent a phase transition from soluble to insoluble states upon complexation with a lysine residue, accompanied by a dramatic morphological changes with controlled length and low homogeneity.^[56] It was also found that CB[7] could bind to the $A\beta_{25-35}$ fragments, leading to the modulation of oligomer structures and suppression of higher-order oligomer aggregation through encapsulation with lysine residues and the N-termini of the $A\beta$ monomer.^[57] This inhibitory effect of CB[7] on fibrillar formation was preserved even in the presence of polyphenol as a stimulatory ligand. More remarkably, with an exposed Phe termini, $A\beta_{4-16}$ could form extremely strong inclusion complexes with CB[7] and CB[8], whereas no apparent binding strength was observed in the case of $A\beta_{1-16}$ (Figure 6e).^[58] Compared to $A\beta_{1-40}$ with weaker binding affinity, the cytotoxicity of $A\beta_{4-40}$ fibrils was largely reduced upon incubation with CB[7] and CB[8], and the cell viability was recovered in a dose-dependent manner. By virtue of the intermolecular communication with amino-acid residues, the commonly used CB[n]s ($n = 6-8$) all exert good inhibitory effects on different $A\beta$ species, which improves our molecular-level understanding of the self-assembly behavior of amyloid proteins and expedites the development of supramolecularly therapeutic methods for amyloidosis.

5. CB-Assisted Subcellular Imaging

Considering the biosafety of the parent CBs and their binding preference for π -conjugated cationic guests, CB-guest complexes can be readily internalized in the cellular environment, and the resultant nanoconstructs have been exploited for

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targeted cell imaging. Cell-permeable CB-based nanostructures can be conveniently endowed with luminescent properties by the attachment of functional chromophores.^[59] For instance, Park and Kim reported a bio-orthogonal supramolecular latching strategy based on the extremely strong binding affinities of CB[7] with adamantane and ferrocene (Figure 7a), by which the examined proteins could selectively and reversibly label cell surfaces, the intracellular compartments, and animal bodies.^[60]

Meanwhile, CBs can maximize the spectroscopic potentials of chromophoric guests via host-guest complexation. In this respect, near-infrared (NIR) fluorescence with strong emission beyond 650 nm shows enormous advantages in live-cell imaging because it causes minimal phototoxicity and avoids undesirable interference from auto-fluorescence in the medium. One intriguing example is a cube-like nanoassembly by CB[8] and a carbazole-conjugated pyridinium salt, resulting in enhanced production of reactive oxygen species and lysosome-targetable NIR fluorescence emission at 662 nm (Figure 7b).^[61] In addition, Liu et al. recently developed a highly emissive system by the sequential fusion of two types of macrocycles (Figure 7c).^[62a] The NIR fluorescence emission of an anthracyl pyridinium guest at 655 nm showed a sudden leap by co-assembly with CB[8] and dodecyl-modified sulfonatocalix[4]arene (SC4AD), accompanied by the morphological conversion from nanorods to nanoparticles. The obtained assembly exhibited selective subcellular distribution and lysosome imaging with negligible cytotoxicity. This unusual cell imaging performance was attributed to the combination of the conformational restriction by CB[8] and hydrophobic environment for the fluorophores produced by the amphiphilic calixarene. In subsequent work, the same calixarene was employed to fabricate a light-harvesting platform, which exhibited a high antenna effect and targeted imaging in the Golgi apparatus.^[62b] By integrating different kinds of macrocycles into a single self-assembled entity, these studies provide a facile modular approach for the creation of ultrasensitive bioagents with precise subcellular localization.

Because many π -conjugated organic cations are capable of stacking with nucleobases and intercalating into the grooves of DNA, CB-pyridinium assemblies are prone to selective accumulation in the cell nucleus. The direct complexation between CBs and known nucleus-staining dyes has been proven as a feasible means to improve the organelle-targeting visualization process.^[63] In addition, owing to aggregation-induced emission and strong binding affinity with DNA, a framework-like ternary assembly constructed by CB[8] and π -extended pyridinylmethylene has been utilized as both a nucleus tracker and cell visualizer.^[64] Meanwhile, the photosensitization of cationic porphyrins has been improved and efficient anticancer activity is achieved in the nucleus by the CB[7]-anchored polymer vesicles.^[65] Collectively, possessing appropriate size and surface charges, the CB-based cell nucleus-specific nanocomposites hold promise for enhancing the existing efficacy of targeted phototherapeutics.

The room-temperature phosphorescence characteristics including long emission lifetime and large Stokes shift represent some of the most appealing luminescence properties, exceeding those of conventional fluorescence emitters for bioimaging.^[66] Recently, benefitting from the promotion of intersystem crossing and suppression of nonradiative decay, CB-based phosphorescent enhancement was realized by the strict host-guest complexation with phenylmethylpyridinium salts (Figure 7d).^[67] Subsequently, motivated by the distinct photophysical properties of discrete oligomeric CB complexes,

Ma et al. designed a triazine-bridged pyridinium guest, which could form a 2:2 charge-transfer inclusion complex with CB[8] (Figure 7e).^[68] Satisfactorily, the obtained quaternary assembly showed preferential aggregation in the endosome. However, because of the dissolved oxygen, which acts a quencher, and free molecular motion in water, it is still challenging to fabricate such purely organic phosphorescent soft materials with high photoluminescence efficiency in aqueous solution.

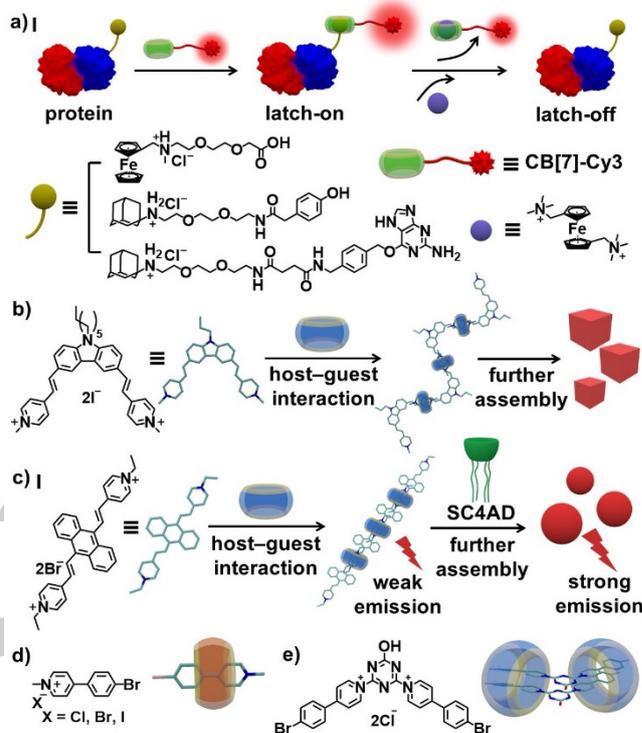


Figure 7. a) Supramolecular latching system for visualization of intracellular proteins and subcellular organelles.^[60] b) Lysosome-targeted CB-carbazole assembly.^[61] c) Two-step assembly with CB[8] and calixarene for lysosome cell imaging.^[62a] d) CB[6]-enhanced phosphorescence emission in the solid state^[67]; e) CB[8]-mediated 2:2 inclusion complex for cell imaging in endosome.^[68]

6. Summary and Outlook

In this minireview, we have summarized the advances made in the construction and functionalization of CB-based supramolecular assemblies with different biomacromolecules, including polypeptides, nucleic acids, and proteins. Their cell-imaging behavior in particular organelles are also introduced to stimulate further studies on the visualization and quantification of key events with biomacromolecules. The steady development of CB-mediated nanosystems largely relies on the exploration of pairwise host-guest complexes and the understanding of dynamic life processes at the molecular level.

Thus, the following challenges should be addressed: (a) concerns about cytotoxicity and nonspecific clearance in vivo can be partially eliminated by camouflage protection via supramolecular complexation; however, the biosafety of commonly used guest molecules, such as ferrocene and bipyridinium salts, remains problematic. Therefore, in addition to the known peptide sequences, more biocompatible guests, such as noncanonical amino acids and new proteins, should be explored to enrich the store of substrates in molecular recognition processes. In addition, when these components are

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used in drug delivery systems, the interference substances in cell culture media and unstable linker groups may reduce the binding strength and delivery potency.^[69] (b) The biological outputs of CB-peptide nanoassemblies are mostly restricted to the biosensing and detection in the laboratory, and, thus, miniaturized devices and practical applications are urgently required. (c) Despite the distinct advantages of RNA in gene coding and enzymatic catalysis, there are tremendous challenges facing the development of functional CB-RNA nanoconjugates compared to the hybrid CB-DNA systems. In addition, at present, the performance-directed construction of CB-nucleic acid nanoassemblies is still in its infancy and there is plenty of room for tuning the bioactivity of nucleic acids using CB-guest conjugates. (d) For better CB-protein association, more bioorthogonal chemical reactions that can site-selectively incorporate functional groups into target sites in proteins should be exploited. Moreover, the molecular binding characteristics in supramolecular assemblies, such as self-inclusion, self-exclusion, amphiphilicity, and multivalency, should be fully considered when analyzing the precise binding mode between CBs and proteins. In addition, it is sensible to take both sides of macrocycle-induced aggregation and disaggregation abilities in the construction of macrocycle-protein biohybrids.^[70] (e) At the subcellular level, the bioimaging mechanism of CB-based emissive nanostructures is not very clear, and it is, thus, necessary to clarify their metabolic pathway and action of modes with biomolecules, which may provide guidance for establishing new photoactivated therapeutic methods.

In short, over the past decades, we have witnessed a profound evolution in CB science from single inclusion complexes to multicomponent biomacromolecular assemblies at different scales and dimensions, which can not only generate a multitude of topologically fascinating structures but also bring about many promising theranostics against life-threatening diseases. Benefitting from the substantial development of synthetic biology and interdisciplinary research, CB-based supra-biomacromolecular assemblies have the potential to accelerate the creation of more advanced bioinspired nanomaterials and eventually promote their translational application in human health.

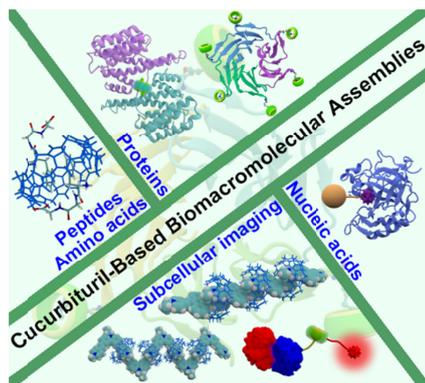
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This minireview highlights the significant progress and emerging applications in cucurbituril-based biomacromolecular assemblies with peptides, nucleic acids, and proteins. This is a challenging and rapidly developing area that involves precise control over intermolecular communication at multidimensional levels and holds great promise for the creation of innovative biomaterials and therapeutic methods.