

Applications of Synthetic Receptors in Bioanalysis and Drug Transport

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ABSTRACT: Synthetic receptors are powerful tools for molecular recognition. They can bind to guests with high selectivity and affinity, and their structures are tunable and diversified. These features, plus the relatively low cost and high easiness in synthesis and manipulation, support the feasibility of array-based molecular analysis with synthetic receptors for improved selectivity in the recognition of wide range of targets. More attractively, host:guest interaction is reversible and guest displacement allows biocompatible and gentle release of the host-bound molecules, simplifying the stimulation designs needed to control analyte sensing, enrichment and transportation. Here, we highlight a few recent advancements in using synthetic receptors for molecular analysis and stimulation, with the focus on macrocyclic receptors, and their applications in displacement sensing, separation, imaging, and drug transport.

INTRODUCTION

Small molecule synthetic receptors are a powerful alternative to large biomolecules for target recognition in bioanalysis, or as targeting agents and drugs in medical applications. Whereas large biomolecules such as antibodies are capable of very strong and highly selective molecular recognition of biologically important targets, their discovery and production are often lengthy and labor-intensive, and as a result, they are expensive and suffer from challenges in product characterization and quality control.^{1,2} In addition, antibodies are specific, so one antibody only targets a single analyte, with cross-reactivity often observed.³ Manipulations like immobilization or modification of these large biomolecules may also reduce target affinity and specificity.⁴ In contrast, small molecule receptors can be chemically synthesized at relatively low cost and with high scalability, can be easily derivatized and tuned to recognize a diverse series of targets.⁵ They do, however, show weaker affinity and selectivity than biomolecules, so represent a complementary, yet powerful alternative to antibodies, protein receptors, and aptamers in molecular recognition.

Synthetic receptors have been extensively employed in molecular detection by coupling the non-covalent host:guest interaction with a signal (often optical) output.^{6,7} To enhance target selectivity and differentiation, multiple receptors can form the so-called “chemical nose/tongue” sensor platform for differential sensing,⁸⁻¹⁰ in which binding between multiple individual receptors and each analyte results in a distinct response profile. Subjecting the combined response profiles to multivariate statistical tools such as principal component analysis (PCA) allows selective discrimination of different analytes.¹¹ Many elegant arrays have been reported for selective recognition of analytes with high structural similarity, such as neurotransmitters,¹² cannabinoids,¹³ and insect pheromones,¹⁴ and have been applied for diagnostics and drug discovery.¹⁵

Synthetic receptors have also been used to assist molecular separations, an approach they are ideally suited to, due to their small size, simplicity in surface immobilization, and reversible target binding.¹⁶ In addition, selective and controlled guest binding and release profiles can be used in applications such as drug delivery and analyte enrichment.¹⁷⁻¹⁹

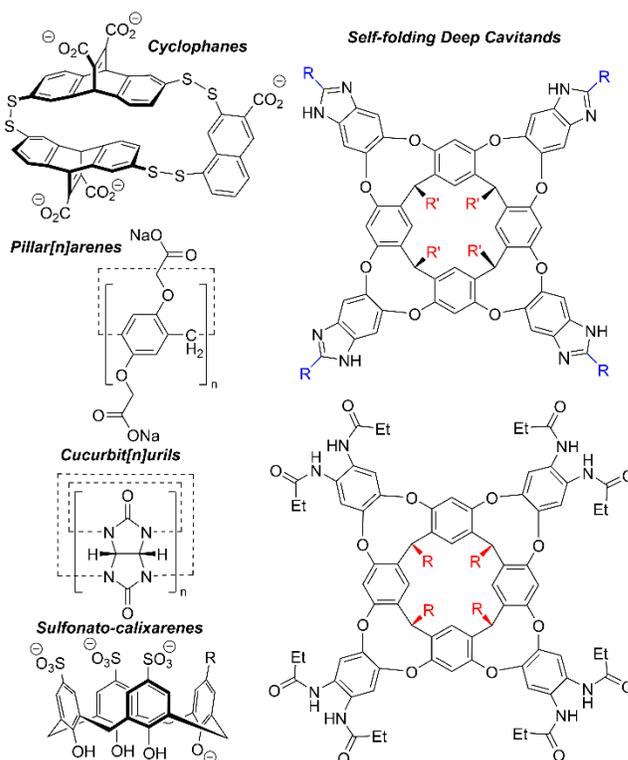


Figure 1. Common synthetic receptors used in bioanalysis and drug transport.

This topical review highlights recent advancements in employing macrocyclic receptors for the analysis and manipulation of biomolecules. As this is a concise review, the discussion must be limited, and so we have focused on molecular sensing via displacement assays, and some new developments in exploiting host:guest interactions for separation, intracellular imaging and drug transport, which are the emerging topics in the field. Notably, we have chosen to avoid applications involving cyclodextrins as receptors, as these are myriad and have been covered extensively in recent reviews.²⁰⁻²²

SYNTHETIC RECEPTORS FOR SPECTROSCOPIC SENSING OF BIOMOLECULES

The most common strategy employed when using synthetic receptors in molecular sensing is the indicator (or fluorescence) displacement assay (IDA/FDA).²³⁻²⁵ In these assays, the analyte of interest displaces the guest from the host, inducing an optical change. Many macrocyclic receptors have been employed in FDA or IDA, including cucurbit[n]urils (CB[n]), calix[n]arenes (CX[n]), pillar[n]arenes, cyclophanes and deep, water-soluble cavitands, etc.⁵ (Figure 1) These receptors offer a hydrophobic pocket for recognition of suitably sized targets, and the addition of charged or polar groups on the macrocycle can confer water-solubility to facilitate applications in aqueous environments. The binding properties of these hosts are often driven by the hydrophobic effect.²⁶ Alternative mechanisms, such as CH- π , cation- π and electrostatic interaction, can also be exploited, allowing detection of analytes that do not possess large hydrophobic surfaces for binding.^{27,28}

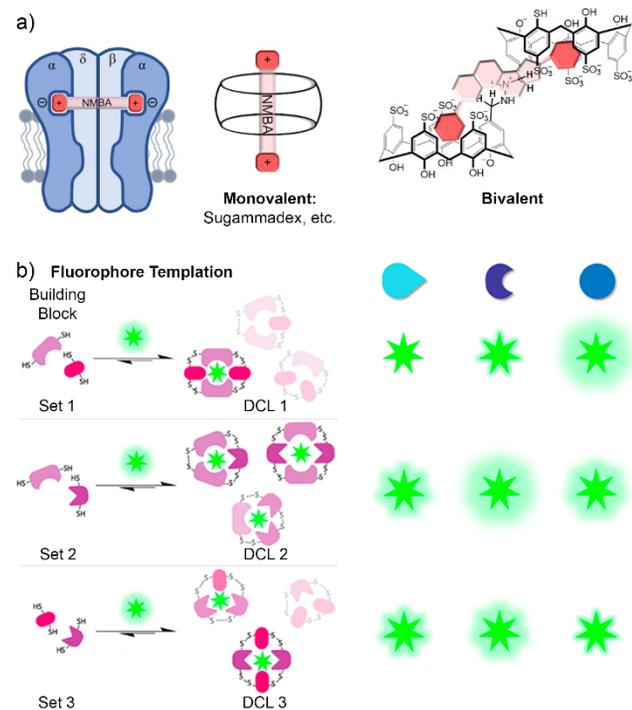


Figure 2. Novel design of supramolecular receptors. a) Left: Cross section of nicotinic acetylcholine receptors inhibited by an NMBA binding both acetylcholine sites; Center: Supramolecular reversal agents reported to date contain a single binding site; Right: NMBA-templated bivalent calixarene hosts with a flexible hydrazine linker. Reprinted with permission from ref 31, copyright 2021, Wiley. b) Formation of “imprint-and-report” DCLs followed by *in situ* fluorescent indicator displacement

analysis of targets, with the receptors colored in magenta, dyes in green, and analytes in cyan. Reprinted with permission from ref 36, copyright 2021, American Chemical Society.

Selective recognition of structurally similar targets can be challenging for simple receptors, but one of the most efficient methods of maximizing selectivity is templated synthesis.^{29,30} This concept was recently employed by the Hof group to create bivalent receptors for the recognition of neuromuscular blocking agents (NMBAs). NMBAs have diverse biscationic structures, but a common geometrically defined pharmacophore consisting of two alkyl ammonium ions separated by ~ 14 Å. Decamethonium, the paradigmatic NMBA, can bind to two calixarene units, one at each NMe_3^+ terminus. It was therefore employed as the template to link two highly anionic p-sulfonatocalixarene building blocks, which otherwise are strongly repulsed from each other (Figure 2a).³¹ After extraction of the decamethonium, the resultant bivalent calixarene receptors form 1 : 1 complexes with various NMBAs with micromolar dissociation constants (K_d), exhibit excellent selectivity over acetylcholine and hydrophobic monovalent ammonium ions, and provide a novel alternative to Sugammadex as NMBA reversal agents.

It can also be challenging to identify the correct receptor and dye pairs for differential sensing, because each pair should yield a detectable change in emission upon binding, and multiple such pairs are necessary to form the sensing array. Dynamic combinatorial chemistry (DCC) allows rapid multicomponent receptor synthesis and is a powerful strategy for quickly determining the “right” receptors for a specific target. Different building blocks can be combined within the same guest-templated synthesis reaction to form optimized cyclophane receptors in the presence of a guest.^{32,33} The receptors that show good affinity and/or are suitable for IDA can be revealed by dye displacement, HPLC, ESI-MS or NMR.^{34,35} To further enhance the discovery throughput, and speed up the selection of the proper receptor and dye combinations, the Waters group developed the “imprint-and-report” approach (Figure 2b).³⁶ This employs either the analyte or the fluorophore as the template for creation of dynamic combinatorial libraries (DCLs), which generated a large dataset comprised of distinct fluorescence signals. Subjecting this dataset to PCA revealed new analyte-specific receptors or receptor-dye pairs useful for IDA sensing, depending on whether the analyte or the dye was the template. Fluorophore templation was much faster and consumed less material, so more suitable for sensor array development. Since receptor purification is no longer needed in this approach, synthesis optimization is straightforward.³⁶ An array of receptor-dye pairs was successfully identified for the unprecedented, complete differentiation of all Arg and Lys methylation states in peptides.

These two studies focused on sensing biologically important targets with similar structural motifs: soft cations. The majority of analytes targeted in differential sensing with macrocyclic receptors contain “soft nitrogen-containing cations”, simply because they are the best substrates for aromatic receptors such as CB[n]s, calixarenes, and cyclophanes. Negatively charged analytes are far more challenging to sense, especially in aqueous solution. Some success has been achieved for analysis of small anions,³⁷ lipids,³⁸ sugars,³⁹ phosphates⁴⁰ and nucleotides,⁴¹ mainly relying on electrostatic interactions between the hosts and analytes. Still, it is challenging for synthetic receptors to provide strong and selective binding to macromolecules, as antibodies do, because their small, inflexible cavities limit the

size, shape and charge of groups that can be recognized. Recently, a group of more flexible, complex synthetic receptors, i.e. self-folding deep cavitands, were applied to recognize more difficult targets such as nucleic acid folded structures. These assays were built upon an indirect sensing mechanism: the hosts were employed as fluorescence modulators rather than the recognition element, using the *dyes* as the primary recognition motif, and adding the *hosts* as competitive sensing elements.

The deep cavitand array was applied to discriminate G-quadruplex (G4) topologies (Figure 3a).⁴² Although the secondary structures of nucleic acids have an important influence on their cellular functions, it is not a simple task to rapidly identify and classify different folded structures. The sensor array was formed using 5 deep cavitands and 2 cationic DNA-interacting dyes (which are good guests for the cavitands). Since the dyes can interact with both the cavitands and the DNA targets, several binding equilibria could occur simultaneously, including competitive binding of the dye between cavitand and DNA, and formation of heteroternary complexes among the dye, G4, and cavitand. These differential interactions result in distinct fluorescence patterns generated from G4 structures of identical

length but varying topological types. Different G4s that display the same folding topology can also be easily differentiated by the number of G-quartets and sequence differences. Treating the fluorescence patterns with Canonical Discriminant Analysis (CDA) can successfully classify the G4s by their topology, providing a simple method that is complementary to common techniques like Circular Dichroism (CD) in revealing the folding topology of any G4 sequences.

After addition of new dyes and new hosts, the array was challenged to detect more diverse DNA folding motifs like hairpins, i-motifs, triplexes, and other G4s, which included structures with the same broad folding category but carrying subtle differences, such as G4 with vacancies or bulges, and either parallel or antiparallel triplexes. By applying machine learning algorithms to the sensing data, we successfully predicted the folding state of unknown DNA strands (Figure 3b).⁴³ This design does not require the discovery and synthesis of specific binding ligands to the folded structures, but rather employs a simple multicomponent approach and selective molecular recognition to ensure both high selectivity and wide applicability.

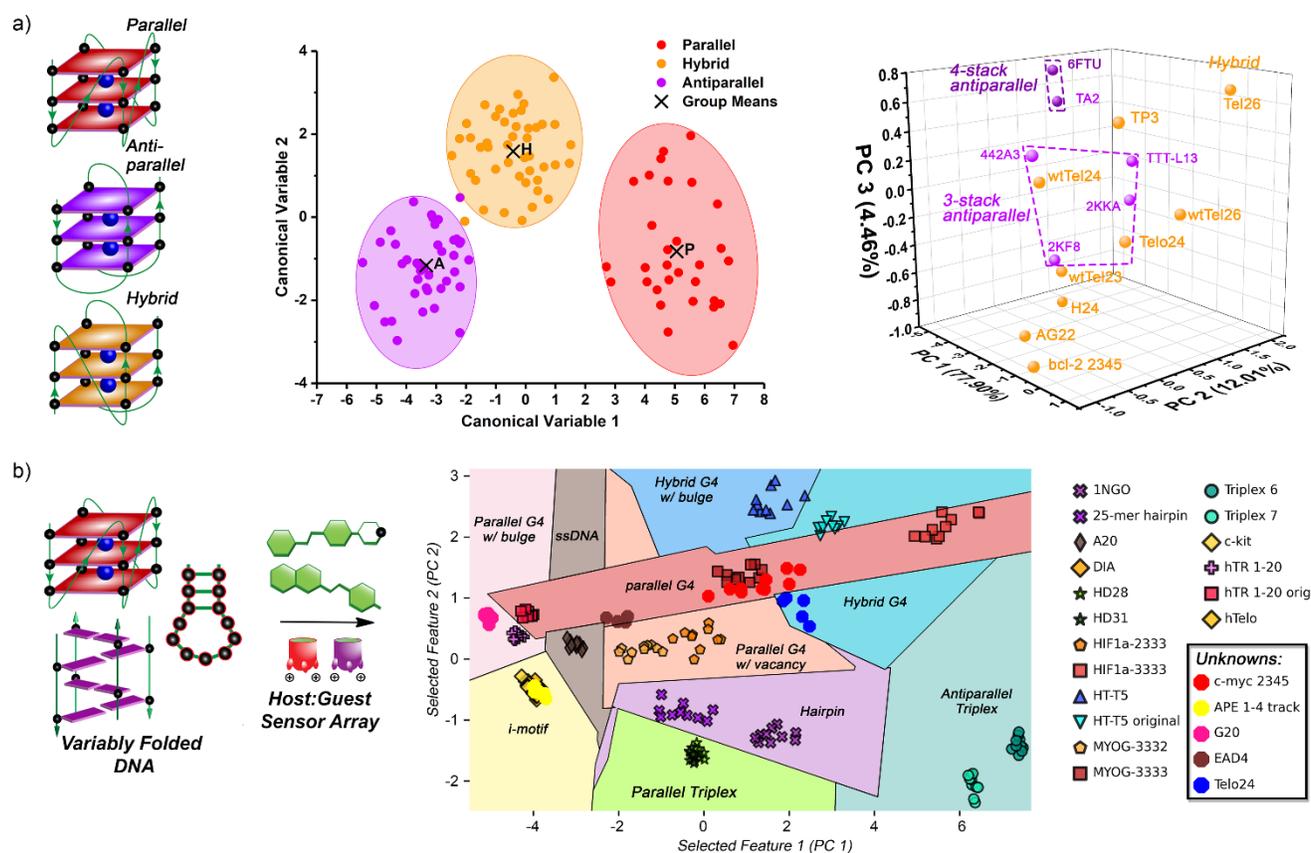


Figure 3. Host:guest sensor arrays for DNA structural differentiation. a) Left: Different G4 topologies. Center: CDA scores plot of 115 G4 samples, grouped by topology. Right: 3D PCA scores plot of 15 antiparallel and hybrid G4 strands with 10-component cavitand:dye sensing array. b) The arrayed host:guest fluorescence sensor system built with deep cavitands and DNA-binding dyes can discriminate among and classify multiple different noncanonical DNA structures. Decision region boundary plot using PC 1 and PC 2 obtained from subjecting the 16-element array data acquired from the 18-DNA pool by PCA-SVM-RFE. Five unknowns were projected to the regions representing the predicted folding structures. Reprinted with permission from ref 43, copyright 2021, American Chemical Society.

While IDA requires an indicator to convert the host:guest binding to a readable signal, a simpler approach is to build the fluorophore into the receptor, and modulate the fluorescence directly by host:guest interactions, as has been shown by the

Huang group.⁴⁴ They found that exposing the solid, non-fluorescent pillar[4]arene[1]quinone to the vapor of ethylenediamine can turn on the fluorescence in the solid state. This phenomenon was attributed to the restriction of intermolecular

π - π interactions among the hosts upon binding to ethylenediamine (EDA) that prevent aggregation-induced quenching. This method not only can help selective sensing of ethylenediamine in the presence of other aliphatic amines in gas phase, but also has important applications in the preparation of organic solid-state fluorescent materials through guest vapor adsorption (Figure 4a).

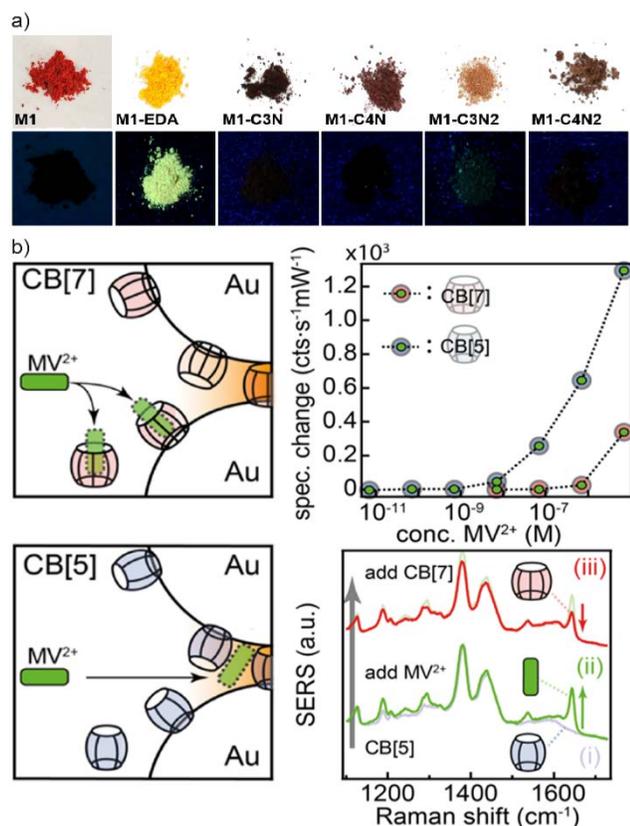


Figure 4. Receptors for gas phase detection and highly sensitive SERS detection. a) Photographs showing color changes under visible light and 365 nm UV irradiation when nonporous adaptive crystals were exposed to different aliphatic amine vapors. Reprinted with permission from ref 44, copyright 2020, American Chemical Society. b) Left: Analyte (methyl viologen (MV²⁺)) incorporation in plasmonic hotspots on AuNP conjugated with CB[7] and CB[5]. Right: Top – Integrated SERS spectral changes vs. MV²⁺ concentration. Bottom – SERS spectra showing the effect of adding (i) CB[5], then (ii) MV²⁺ resulting in a clear new peak at 1650 cm⁻¹, and subsequently (iii) CB[7], lowering the intensity of the peak at 1650 cm⁻¹ as CB[7] scavenges analytes away from the hotspot. Reprinted with permission from ref 45, copyright 2019, American Chemical Society.

Besides fluorescence, other optical signaling mechanisms have been used in synthetic receptor-based molecular sensing, most of them with the hosts immobilized on solid supports. A recent work showed that conjugating macrocyclic CB[n]s (n = 5–8) on Au nanoparticles (AuNP) produced high-quality substrates for surface-enhanced Raman spectroscopy (SERS) (Figure 4b).⁴⁵ SERS can produce fine spectroscopic fingerprints for specific analyte identification, but signal enhancement is strongly influenced by the location of substrate molecules relative to the plasmonic hotspots, which can be assisted by host:guest interactions, as recently demonstrated in single-molecule SERS.⁴⁶ Thus, this work employed CB[n] as the rigid spacer to precisely

control the interparticle spacing of AuNP aggregates, generating uniform plasmonic hotspots. Interestingly, CB[7], which has a proper cavity size to fit the analyte, rendered 10× lower SERS signals than CB[5], the cavity of which is too small for analyte inclusion. It was speculated that the analyte was interstitially incorporated into the space between the particles, i.e., the plasmonic hotspots, enhancing the SERS signals, as opposed to inside the CB cavity, which was too small for analyte binding. This design was applied to quantitatively detect tetrahydrocannabinol and its derivatives with high sensitivity.

SYNTHETIC RECEPTORS FOR MOLECULAR SEPARATION

Selective, non-covalent interactions between synthetic hosts and guests can help improve molecular separation. The receptors are much smaller and cheaper than antibodies, thus more suitable for use as additives in a separation matrix or column decorators to interact with analytes and manipulate retention. Host:guest interaction with fast on-and-off binding rates can help to provide high column efficiency, whereas strong and stable binding can be exploited in affinity separation.

Highly water-soluble synthetic hosts such as CB[n]s and calixarenes can be directly added to the aqueous running buffers to assist separation by capillary electrophoresis (CE). Recently, tetrasulfonatocalix[4]arene (CX4), hexasulfonato-calix[6]arene (CX6) and cucurbit[7]uril (CB[7]), were employed to help separate methylated peptides by CE.⁴⁷ Since they bind differentially to methylated lysines with different methylation states or at various sites on the peptide, they can resolve histone peptides carrying different lysine methylations effectively. The method was also exploited to analyze the activities of the methylation enzymes.⁴⁸

A remarkably stable host:guest pair is formed between cucurbit[7]uril (CB[7]) and adamantylammonium (AdA), with a $K_a > 10^{12} \text{ M}^{-1}$,^{49–51} comparable to that of the streptavidin-biotin or antibody-antigen complexes. CB[7] was recently employed to purify protein therapeutics (e.g. monoclonal antibody or cytokine proteins) site-specifically modified by AdA.⁵² Agarose beads conjugated with CB[7] captured the AdA labeled proteins, which can be easily recovered by addition of a stronger guest, N-(1-adamantyl) ethylenediammonium (Ad-EDA).

The Wang group also used CB[7] to mediate site-specific protein immobilization and biocompatible release of the captured proteins (Figure 5a).⁵³ CB[7] can bind to the N-terminal phenylalanine residues on proteins with micromolar K_a . An oligopeptide with a terminal Phe was then designed and covalently linked with a bait protein that can specifically recognize methyllysine on proteins. The peptide-bait conjugate was immobilized on the CB[7]-functionalized resin for enrichment of methyllysine proteins in cell lysates. Elution of the methylated proteins can be stimulated by AdA. These mild, non-denaturing conditions are much more desirable than the denaturing conditions such as acidic pH, chaotropic reagents and high temperatures usually needed to elute proteins from antibodies, protein receptors, or aptamers. This method was found to be highly compatible with mass spectrometry, and pro-

duced low background and high signal/noise ratios, outperforming the commonly used antibody enrichment or immobilized metal affinity (i.e. Ni(II)- nitrilotriacetic acid (NTA)) methods (Figure 5b).

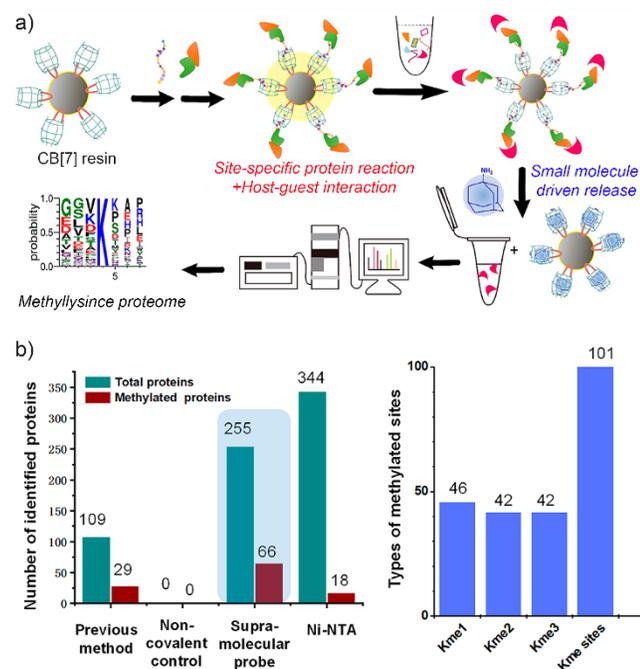


Figure 5. Host:guest interactions for affinity purification and selective enrichment of proteins. a) Overall flowchart - construction of supramolecular protein probes, selective enrichment of the methyllysine proteome, release by AdA displacement, and MS Analysis. Comparison of the HP1 β interactomes purified. b) Left: total numbers of proteins identified by different methods; Right: statistics of methyllysine sites identified by the present method. Reprinted with permission from ref 53, copyright 2020, American Chemical Society.

SYNTHETIC RECEPTORS FOR INTRACELLULAR APPLICATIONS

Synthetic receptors can be exploited for *in vitro* and *in vivo* imaging, with fluorescence still being the dominant signaling method and often employing IDA for signal production upon analyte recognition.⁵⁴ However, applying IDA for bioimaging is challenging, and a number of roadblocks still need to be overcome. These include high background due to insufficient indicator quenching, non-specific signals produced by interfering molecules present in biological environments, and low spatial resolution caused by the diffusion of the released indicators. Xiao and Guo developed a Förster resonance energy transfer (FRET)-assisted IDA to overcome these challenges, and the system allowed successful ATP imaging in live cells (Figure 6a).⁵⁵ In this platform, the amphiphilic supramolecular receptor, low-rim-dodecyl-modified guanidine-calix[5]arene (GC5A-12C), was assembled with amphiphilic poly(ethylene glycol) (PEG-12C) to form micellar nanoparticles. FRET donors

(perylene and 9,10-bis(2-phenylethynyl)anthracene) were trapped in the hydrophobic inner space of the nanoparticles, which greatly enhanced their fluorescence. In addition, FRET acceptors like fluorescein, orange G, and eosin Y, were also entrapped, quenching the fluorescence of the FRET donors. The donor/GC5A-12C/acceptor assembly can enter the cells efficiently via caveolae-mediated pathways and ATP-dependent micropinocytosis. Once in the cells, the intracellular ATP displaced the FRET acceptors and recovered the fluorescence of the donors. This work demonstrates that FRET can help enhance the applicability of IDA in bioimaging by minimizing the background noise, and donor encapsulation in micellar aggregates is an effective way to diminish signal diffusion, enhancing spatial resolution in imaging. More importantly, it illustrates the power of synthetic receptors in mediating signal stimulation through reversible host:guest interactions.

Synthetic hosts can aid drug delivery by stimulating rapid and on-demand control of the location and function of therapeutic compounds.⁵⁶ Guo and Chen proposed a strategy of biomarker displacement activation (BDA) and employed it to help deliver therapeutic peptides into cells. In this design, the micellar nanoparticles co-assembled by GC5A-12C and PEG-12C and used in the aforementioned FRET-assisted IDA was employed as drug carrier.⁵⁷ The receptor GC5A-12C can strongly bind to therapeutic phosphopeptides (K_a values $\sim 10^6$ – 10^8 M⁻¹) but not the unphosphorylated ones, and the binding can be reversed under high ATP concentrations. The phosphopeptides can disrupt abnormal protein-protein interactions and regulate cellular processes that could be applied in disease cures. However, they cannot enter cells easily due to the phosphoryl di-anionic charges. Since the ATP concentration in the extracellular environment is low (< 5 μ M), the pegylated hosts can help deliver the phosphopeptides across the cell membrane. Once inside the cells, the higher concentration of intracellular ATP (1~ 10 mM) then triggers the release of phosphopeptides (Figure 6b). This type of reversible host:guest interaction has also been exploited by the Zhang group⁵⁸⁻⁵⁹ and the Wang group⁶⁰ to trigger drug release by intracellular guests or environmental factors.

Macrocyclic receptors can also be used in combination with nanoparticles to enhance drug delivery.⁶¹⁻⁶³ One interesting approach that utilizes synthetic receptors to promote cell entry of NPs was recently reported.⁶⁴ In this work, AuNPs (diameter ~ 15 nm) coated with pyranine, a negatively charged ligand, induced strong protein adsorption in cell culture media. However, protein corona formation can be prevented by the addition of the oligocationic covalent cage A (Figure 6c), which can form a zwitterionic host:guest complex on the particle surface. Reduced protein adsorption effectively enhanced cellular uptake of the AuNPs. This strategy was also applied to control corona formation on Au nanorods (AuNRs, length \times width 29 \times 9 nm), a potential reagent for photothermal therapy. Once in the cells, these AuNRs induced local heating under irradiation of near-infrared light, and caused cytotoxicity.

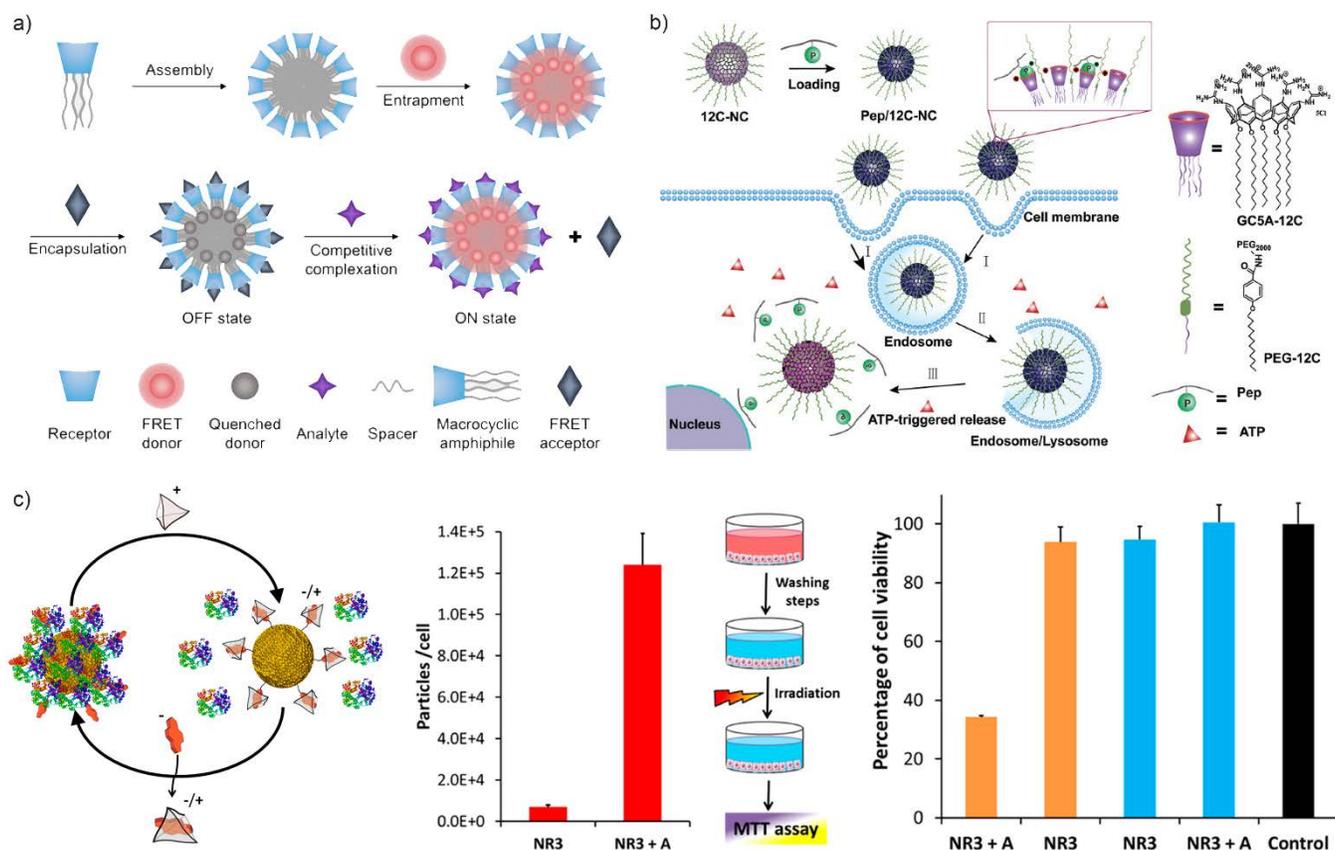


Figure 6. Host-guest system for intracellular applications. a) FRET-assisted IDA strategies for the development of molecular sensing systems. Reprinted with permission from ref 55, copyright 2021, Wiley. b) Intracellular transport of phosphopeptides via host-guest complexation, internalization of the peptide/12C-NC complex in endosomes, and the ATP-triggered release of phosphopeptides from the 12C-NC. Reprinted with permission from ref 57, copyright 2020, Royal Society of Chemistry. c) Cell uptake of gold nanorods NR3 by HeLa cells with or without cage A, NIR-laser hyperthermia experiments and cell viability results calculated using the MTT assay. Reprinted with permission from ref 64, copyright 2020, American Chemical Society.

CONCLUSIONS

As has been shown by the recent developments summarized above, supramolecular receptors have important roles in bioanalysis and drug transport. They provide selective binding to guests, as do macrobiomolecules (i.e. antibodies, protein receptors, aptamers, etc.), but they have other advantages such as smaller size, simpler design and production, and easier modification. They can either form a stand-alone sensor for rapid and sensitive target detection, or be combined to establish an array to differentiate a large group of analytes with high structural similarity. They can also be coupled to supporting materials without impeding their abilities in selective target recognition to facilitate spectroscopy measurement, imaging, separation, or drug transport. Their reversible binding with guests can be exploited to trigger the release of the bound analytes, under much milder conditions than those needed to overcome antibody-antigen, protein receptor-ligand, or aptamer-target interactions. Employing synthetic receptors in drug development can help alleviate obstacles in stability, biodistribution and delivery that hamper the clinical applications of traditional theranostic platforms.

Still, several challenges remain. These include applications in highly complex matrices for measurement of real-world mixtures, workload reduction during synthesis of new receptors and indicators, simplification in data collection and interpretation, and improvement in stability and reproducibility of sensing signals. Different sensing mechanisms than IDA should be proposed and tested to meet the demands in rapid, reliable, sensitive, specific, and/or long-term monitoring of diverse analytes of interest.

One final point is that the field is dominated by a series of privileged macrocyclic structures. Cucurbiturils, cyclodextrins, calixarenes and pillararenes have many benefits, and have provided a wealth of research targets, but their favored target motifs are quite similar. As the applications of receptors in complex biological environments become more popular, new types of host structure must be synthesized. The ideal targets would hopefully exploit orthogonal recognition mechanisms from those shown by electron rich toroidal (and shallow bowl-like) macrocycles: this would expand target scope exponentially, and allow new methods of minimizing interference from species present in cellular

matrices. This could enhance the utility of synthetic receptors in affinity purification and in intracellular applications like imaging and drug transport. Besides, the loading capacity and types of guest molecules, as well as the triggered release mechanisms should be improved to facilitate more diverse biomedical applications of synthetic receptors.

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Wenwan Zhong is a Professor of Chemistry at University of California – Riverside (UCR), specializing in separation and sensing. She received her B.S. degree in Applied Chemistry from University of Science and Technology of China, and her Ph.D. in Analytical Chemistry at Iowa State University under the direction of Prof. Edward S. Yeung. After finishing her postdoctoral training at the Los Alamos National Laboratory, she established her independent research group at UCR in 2006. The Zhong group focuses on development of analytical methods to study biomarkers and understand their functions, utilizing molecular tools like functional nucleic acids, synthetic or protein receptors, and nanomaterials.

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REFERENCES

- (1) Beck, A.; Wurch, T.; Bailly, C.; Corvaia, N. Strategies and challenges for the next generation of therapeutic antibodies. *Nat. Rev. Immunol.* **2010**, *10*, 345 – 352.
- (2) Nelson, A. L.; Dhimolea, E.; Reichert, J. M. Development trends for human monoclonal antibody therapeutics. *Nat. Rev. Drug Discov.* **2010**, *9*, 767 – 774.
- (3) Bradbury, A.; Plückthun, A. Reproducibility: Standardize antibodies used in research. *Nature* **2015**, *518*, 27 – 29.
- (4) Yu, Q.; Wang, Q.; Li, B.; Lin, Q.; Duan, Y. Technological development of antibody immobilization for optical immunoassays: progress and prospects. *Crit. Rev. Anal. Chem.* **2015**, *45*, 62 – 75.
- (5) Kolesnichenko, I. V.; Anslyn, E. V. Practical applications of supramolecular chemistry. *Chem. Soc. Rev.* **2017**, *46*, 2385 – 2390.
- (6) Xu, W.; Cheng, M.; Zhang, S.; Wu, Q.; Liu, Z.; Dhinakaran, M. K.; Liang, F.; Kovaleva, E. G.; Li, H. Recent advances in chiral discrimination on host–guest functionalized interfaces. *Chem. Commun.* **2021**, *57*, 7480 – 7492.
- (7) Kim, H. J.; Lee, M. H.; Mutihac, L.; Vicens, J.; Kim, J. S. Host–guest sensing by calixarenes on the surfaces. *Chem. Soc. Rev.* **2012**, *41*, 1173 – 1190.
- (8) You, L.; Zha, D.; Anslyn, E. V. Recent Advances in supramolecular analytical chemistry using optical sensing. *Chem. Rev.* **2015**, *115*, 7840 – 7892.
- (9) Sasaki, Y.; Kubota, R.; Minami, T. Molecular self-assembled chemosensors and their arrays. *Coord. Chem. Rev.* **2021**, *429*, 213607.
- (10) Anzenbacher, J. P.; Lubal, P.; Buček, P.; Palacios, M. A.; Kozelkova, M. E. A practical approach to optical cross-reactive sensor arrays. *Chem. Soc. Rev.* **2010**, *39*, 3954 – 3979.
- (11) Chen, Z.-H.; Fan, Q.-X.; Han, X.-Y.; Shi, G.; Zhang, M. Design of smart chemical ‘tongue’ sensor arrays for pattern-recognition-based biochemical sensing applications. *Trends Anal. Chem.* **2020**, *124*, 115794.
- (12) Mei, Y.; Zhang, Q.-W.; Gu, Q.; Liu, Z.; He, X.; Tian, Y. Pillar[5]arene-based fluorescent sensor array for biosensing of intracellular multi-neurotransmitters through host–guest recognitions. *J. Am. Chem. Soc.* **2022**, *144*, 2351 – 2359.
- (13) Gill, A. D.; Hickey, B. L.; Zhong, W.; Hooley, R. J. Selective sensing of THC and related metabolites in biofluids by host:guest arrays. *Chem. Commun.* **2020**, *56*, 4352 – 4355.
- (14) Hickey, B. L.; Chen, J.; Zou, Y.; Gill, A. D.; Zhong, W.; Millar, J. G.; Hooley, R. J. Enantioselective sensing of insect pheromones in water. *Chem. Commun.* **2021**, *57*, 13341 – 13344.
- (15) Geng, Y.; Peveler, W. J.; Rotello, V. M. Array-based “chemical nose” sensing in diagnostics and drug discovery. *Angew. Chem. Int. Ed.* **2019**, *58*, 5190 – 5200.
- (16) Albelda, M. T.; Frías, J. C.; García-España, E.; Schneider, H.-J. Supramolecular complexation for environmental control. *Chem. Soc. Rev.* **2012**, *41*, 3859 – 3877.
- (17) Webber, M. J.; Langer, R. Drug delivery by supramolecular design. *Chem. Soc. Rev.* **2017**, *46*, 6600 – 6620.
- (18) Ma, X.; Zhao, Y. Biomedical Applications of supramolecular systems based on host–guest interactions. *Chem. Rev.* **2015**, *115*, 7794 – 7839.
- (19) Dergham, M.; Lin, S.; Geng, J. Supramolecular self-assembly in living cells. *Angew. Chem. Int. Ed.* **2022**, e202114267.
- (20) Xu, W.; Li, X.; Wang, L.; Li, S.; Chu, S.; Wang, J.; Li, Y.; Hou, J.; Luo, Q.; Liu, J. Design of cyclodextrin-based functional systems for biomedical applications. *Front. Chem.* **2021**, *9*, 635507.
- (21) Gandhi, S.; Shende, P. Cyclodextrins-modified metallic nanoparticles for effective cancer therapy. *J. Control. Release*, **2021**, *339*, 41 – 50.
- (22) Fang, G.; Yang, X.; Chen, S.; Wang, Q.; Zhang, A.; Tang, B. Cyclodextrin-based host-guest supramolecular hydrogels for local drug delivery. *Coord. Chem. Rev.* **2022**, *454*, 214352.

- (23) Sedgwick, A. C.; Brewster, J. T.; Wu, T.; Feng, X.; Bull, S. D.; Qian, X.; Sessler, J. L.; James, T. D.; Anslyn, E. V.; Sun, X. Indicator displacement assays (IDAs): the past, present and future. *Chem. Soc. Rev.* **2021**, *50*, 9 – 38.
- (24) Guo, C.; Sedgwick, A. C.; Hiraio, T.; Sessler, J. L. Supramolecular fluorescent sensors: An historical overview and update. *Coord. Chem. Rev.* **2021**, *427*, 213560.
- (25) Norouzy, A.; Azizi, Z.; Nau, W. M. Indicator displacement assays inside live cells. *Angew. Chem. Int. Ed.* **2015**, *54*, 792 – 795.
- (26) Jordan, J. H.; Gibb, B. C. Molecular containers assembled through the hydrophobic effect. *Chem. Soc. Rev.* **2015**, *44*, 547 – 585.
- (27) Beatty, M. A.; Hof, F. Host–guest binding in water, salty water, and biofluids: general lessons for synthetic, bio-targeted molecular recognition. *Chem. Soc. Rev.* **2021**, *50*, 4812 – 4832.
- (28) Schneider, H.-J.; Yatsimirsky, A. K. Selectivity in supramolecular host–guest complexes. *Chem. Soc. Rev.* **2008**, *37*, 263–277.
- (29) Vickers, M. S.; Beer, P. D. Anion templated assembly of mechanically interlocked structures. *Chem. Soc. Rev.* **2007**, *36*, 211 – 225.
- (30) Anderson, S.; Anderson, H. L.; Sanders, J. K. M. Expanding roles for templates in synthesis. *Acc. Chem. Res.* **1993**, *26*, 469 – 475.
- (31) Selinger, A. J.; Cavallin, N. A.; Yanai, A.; Birol, I.; Hof, F. Template-directed synthesis of bivalent, broad-spectrum hosts for neuromuscular blocking agents. *Angew. Chem. Int. Ed.* **2022**, e202113235
- (32) Li, J.; Nowak, P.; Otto, S. Dynamic combinatorial libraries: From exploring molecular recognition to systems chemistry. *J. Am. Chem. Soc.* **2013**, *135*, 9222 – 9239.
- (33) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. Dynamic combinatorial chemistry. *Chem. Rev.* **2006**, *106*, 3652 – 3711.
- (34) Bravin, C.; Guidetti, A.; Licini, G.; Zonta, C. Supramolecular cages as differential sensors for dicarboxylate anions: guest length sensing using principal component analysis of ESI-MS and ¹H-NMR raw data. *Chem. Sci.* **2019**, *10*, 3523 – 3528.
- (35) Mullins, A. G.; St. Louis, L. E.; Waters, M. L. Using changes in speciation in a dynamic combinatorial library as a fingerprint to differentiate the methylation states of arginine. *Chem. Commun.* **2020**, *56*, 3947 – 3950.
- (36) Harrison, E. E.; Carpenter, B. A.; St. Louis, L. E.; Mullins, A. G.; Waters, M. L. Development of “imprint-and-report” dynamic combinatorial libraries for differential sensing applications. *J. Am. Chem. Soc.* **2021**, *143*, 14845 – 14854.
- (37) Gale, Philip A.; Howe, Ethan N. W.; Wu, X. Anion receptor chemistry. *Chem* **2016**, *1*, 351 – 422.
- (38) Herschede, S. R.; Gneid, H.; Dent, T.; Jaeger, E. B.; Lawson, L. B.; Busschaert, N. Bactericidal urea crown ethers target phosphatidylethanolamine membrane lipids. *Org. Biomol. Chem.* **2021**, *19*, 3838 – 3843.
- (39) Edwards, N. Y.; Sager, T. W.; McDevitt, J. T.; Anslyn, E. V. Boronic acid based peptidic receptors for pattern-based saccharide sensing in neutral aqueous media, an application in real-life samples. *J. Am. Chem. Soc.* **2007**, *129*, 13575 – 13583.
- (40) Hargrove, A. E.; Nieto, S.; Zhang, T.; Sessler, J. L.; Anslyn, E. V. Artificial receptors for the recognition of phosphorylated molecules. *Chem. Rev.* **2011**, *111*, 6603 – 6782.
- (41) Florea, M.; Nau, W. M., Implementation of anion-receptor macrocycles in supramolecular tandem assays for enzymes involving nucleotides as substrates, products, and cofactors. *Org. Biomol. Chem.* **2010**, *8*, 1033 – 1039.
- (42) Chen, J.; Hickey, B. L.; Wang, L.; Lee, J.; Gill, A. D.; Favero, A.; Pinalli, R.; Dalcanale, E.; Hooley, R. J.; Zhong, W. Selective discrimination and classification of G-quadruplex structures with a host–guest sensing array. *Nat. Chem.* **2021**, *13*, 488 – 495.
- (43) Chen, J.; Gill, A. D.; Hickey, B. L.; Gao, Z.; Cui, X.; Hooley, R. J.; Zhong, W. Machine learning aids classification and discrimination of noncanonical DNA folding motifs by an arrayed host:guest sensing system. *J. Am. Chem. Soc.* **2021**, *143*, 12791 – 12799.
- (44) Li, E.; Jie, K.; Fang, Y.; Cai, P.; Huang, F. Transformation of non-porous adaptive pillar[4]arene[1]quinone crystals into fluorescent crystals via multi-step solid–vapor postsynthetic modification for fluorescence turn-on sensing of ethylenediamine. *J. Am. Chem. Soc.* **2020**, *142*, 15560 – 15568.
- (45) de Nijs, B.; Carnegie, C.; Szabó, L.; Gryś, D.-B.; Chikkaraddy, R.; Kamp, M.; Barrow, S. J.; Readman, C. A.; Kleemann, M.-E.; Scherman, O. A.; Rosta, E.; Baumberg, J. J. Inhibiting analyte theft in surface-enhanced raman spectroscopy substrates: subnanomolar quantitative drug detection. *ACS Sens.* **2019**, *4*, 2988 – 2996.
- (46) Kim, N. H.; Hwang, W.; Baek, K.; Rohman, Md. R.; Kim, J.; Kim, H. W.; Mun, J.; Lee, S. Y.; Yun, G.; Murray, J.; Ha, J. W.; Rho, J.; Moskovits, M.; Kim, K. Smart SERS hot spots: Single molecules can be positioned in a plasmonic nanojunction using host–guest chemistry. *J. Am. Chem. Soc.* **2018**, *140*, 4705 – 4711.
- (47) Lee, J.; Perez, L.; Liu, Y.; Wang, H.; Hooley, R. J.; Zhong, W. Separation of methylated histone peptides via host-assisted capillary electrophoresis. *Anal. Chem.* **2018**, *90*, 1881 – 1888.
- (48) Lee, J.; Chen, J.; Sarkar, P.; Xue, M.; Hooley, R. J.; Zhong, W. Monitoring the crosstalk between methylation and phosphorylation on histone peptides with host-assisted capillary electrophoresis. *Anal. Bioanal. Chem.* **2020**, *412*, 6189 – 6198.
- (49) Barrow, S. J.; Kasera, S.; Rowland, M. J.; del Barrio, J.; Scherman, O. A. Cucurbituril-based molecular recognition. *Chem. Rev.* **2015**, *115*, 12320 – 12406.
- (50) Shetty, D.; Khedkar, J. K.; Park, K. M.; Kim, K. Can we beat the biotin–avidin pair?: cucurbit[7]uril-based ultrahigh affinity host–guest complexes and their applications. *Chem. Soc. Rev.* **2015**, *44*, 8747 – 8761.
- (51) Kim, K. L.; Sung, G.; Sim, J.; Murray, J.; Li, M.; Lee, A.; Shrinidhi, A.; Park, K. M.; Kim, K. Supramolecular latching system based on ultrastable synthetic binding pairs as versatile tools for protein imaging. *Nat. Commun.* **2018**, *9*, 1712.
- (52) An, J.; Kim, S.; Shrinidhi, A.; Kim, J.; Banna, H.; Sung, G.; Park, K. M.; Kim, K. Purification of protein therapeutics via high-affinity supramolecular host–guest interactions. *Nat. Biomed. Eng.* **2020**, *4*, 1044 – 1052.
- (53) Li, L.; Liu, M.; Yue, L.; Wang, R.; Zhang, N.; Liang, Y.; Zhang, L.; Cheng, L.; Xia, J.; Wang, R. Host–guest protein assembly for affinity purification of methyllysine proteomes. *Anal. Chem.* **2020**, *92*, 9322 – 9329.
- (54) Teunissen, A. J. P.; Pérez-Medina, C.; Meijerink, A.; Mulder, W. J. M. Investigating supramolecular systems using Förster resonance energy transfer. *Chem. Soc. Rev.* **2018**, *47*, 7027 – 7044.
- (55) Geng, W.-C.; Ye, Z.; Zheng, Z.; Gao, J.; Li, J.-J.; Shah, M. R.; Xiao, L.; Guo, D.-S. Supramolecular bioimaging through signal amplification by combining indicator displacement assay with Förster resonance energy transfer. *Angew. Chem. Int. Ed.* **2021**, *60*, 19614 – 19619.
- (56) Zhou, J.; Rao, L.; Yu, G.; Cook, T. R.; Chen, X.; Huang, F. Supramolecular cancer nanotheranostics. *Chem. Soc. Rev.* **2021**, *50*, 2839 – 2891.
- (57) Han, B.-B.; Pan, Y.-C.; Li, Y.-M.; Guo, D.-S.; Chen, Y.-X. A host–guest ATP responsive strategy for intracellular delivery of phosphopeptides. *Chem. Commun.* **2020**, *56*, 5512 – 5515.
- (58) Wang, H.; Xue, K.-F.; Yang, Y.; Hu, H.; Xu, J.-F.; Zhang, X. In situ hypoxia-induced supramolecular perylene diimide radical anions in tumors for photothermal therapy with improved specificity. *J. Am. Chem. Soc.* **2022**, *144*, 2360–2367.

- (59) Wu, H.; Wang, H.; Qi, F.; Xia, T.; Xia, Y.; Xu, J.-F.; Zhang, X. An activatable host-guest conjugate as a nanocarrier for effective drug release through self-inclusion. *ACS Appl. Mater. Interfaces* **2021**, *13*, 33962 – 33968.
- (60) Sun, C.; Wang, Z. Y.; Yang, K.; Yue, L.; Cheng, Q.; Ma, Y.-L.; Lu, S.; Chen, G.; Wang, R. Polyamine-responsive morphological transformation of a supramolecular peptide for specific drug accumulation and retention in cancer cells. *Small* **2021**, *17*, 2101139.
- (61) Tudisco, C.; Cambria, M. T.; Sinatra, F.; Bertani, F.; Alba, A.; Giuffrida, A. E.; Saccone, S.; Fantechi, E.; Innocenti, C.; Sangregorio, C.; Dalcanale, E.; Condorelli, G. G. Multifunctional magnetic nanoparticles for enhanced intracellular drug transport. *J. Mater. Chem. B* **2015**, *3*, 4134 – 4145
- (62) Sun, C.; Wang, Z.; Yue, L.; Huang, Q.; Cheng, Q.; Wang, R. Supramolecular induction of mitochondrial aggregation and fusion. *J. Am. Chem. Soc.* **2020**, *142*, 16523 – 16527.
- (63) Wang H.; Wu H.; Yi Y.; Xue K. F.; Xu J. F.; Wang H.; Zhao Y. L.; Zhang X. Self-Motivated Supramolecular Combination Chemotherapy for Overcoming Drug Resistance Based on Acid-Activated Competition of Host-Guest Interactions. *CCS Chem.* **2021**, *3*, 1413 – 1425.
- (64) Mosquera, J.; García, I.; Henriksen-Lacey, M.; Martínez-Calvo, M.; Dhanjani, M.; Mascareñas, J. L.; Liz-Marzán, L. M. Reversible control of protein corona formation on gold nanoparticles using host-guest interactions. *ACS Nano* **2020**, *14*, 5382 – 5391.

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