Supramolecular "Click Chemistry" for Targeting in the Body

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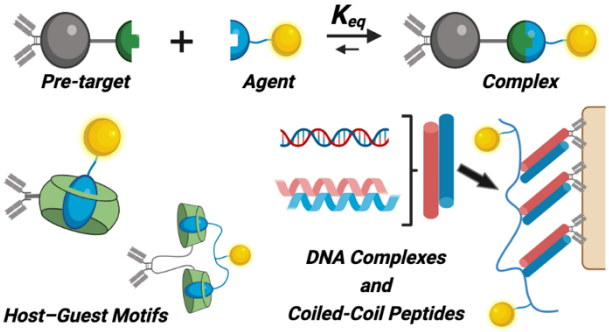
ABSTRACT: The fields of precision imaging and drug delivery have revealed a number of tools to improve target specificity and increase efficacy in diagnosing and treating disease. Biological molecules, such as antibodies, continue to be the primary means of assuring targeting of various payloads. However, molecular-scale recognition motifs have emerged in recent decades to achieve specificity through the design of interacting chemical motifs. In this regard, an assortment of bioorthogonal covalent conjugations offer one possibility for *in situ* complexation under physiological conditions. Herein, a related concept is discussed that leverages interactions from non-covalent or supramolecular motifs to facilitate *in situ* recognition and complex formation in the body. Classic supramolecular motifs based on host–guest complexation offer one such means of facilitating recognition. In addition, synthetic bioinspired motifs based on oligonucleotide hybridization and coiled-coil peptide bundles afford other routes to form complexes *in situ*. The architectures to include recognition of these various motifs for targeting enable both monovalent and multivalent presentation, seeking high affinity or engineered avidity to facilitate conjugation even under dilute conditions of the body. Accordingly, supramolecular "click chemistry" offers a complementary tool in the growing arsenal targeting improved healthcare efficacy.



KEYWORDS: Drug delivery; Diagnostics; Therapeutics; Supramolecular Chemistry; Biomaterials

1. INTRODUCTION

A primary goal in drug delivery is the pursuit of technologies to increase the fraction of drug delivered to a site of need.[1] One key characteristic of an active pharmaceutical agent is its therapeutic index, a ratio of its toxic dose (TD_{50}) to its effective dose (ED_{50}). Accordingly, drug delivery technologies seek to increase the therapeutic index through two parallel mechanisms: *i)* attenuating the systemic activity of a drug through encapsulation and/or prodrug methodologies to enable higher dosing without toxicity,[2,3] and *ii)* ensuring a larger fraction of the delivered agent reaches the physiological site of need to increase effectiveness of the therapeutic agent.[4] Drug delivery can be achieved through passive accumulation of drug carriers, sometimes taking advantage of leaky vasculature that is a pathophysiological hallmark of certain diseases.[5,6] The first FDA-approved nanoscale drug delivery technology, Doxil®, was a PEGylated liposomal formulation of doxorubicin that functioned through such a mechanism.[7] Accordingly, early efforts in the field of drug delivery often sought to increase the therapeutic index through a combination of sequestering toxic agents within nanoscale carriers and leveraging physiologic features of diseased tissue to promote preferential accumulation.

Another strategy broadly explored in the field of drug delivery to increase the therapeutic index is active targeting. These routes commonly leverage affinity from biological molecules such as antibodies to localize a therapeutic to a site of need, targeting on the basis of a disease-relevant biomarker.[8–10] Several antibody-drug conjugates have been recently FDA-approved,[11] consisting of a therapeutic agent attached *via* a labile linker to a monoclonal antibody with affinity for specific biomarkers.[12] Antibodies or aptamers can likewise be used for active targeting of nanoscale drug carriers.[13–15] These and other methods of active targeting are often limited by the availability of targeting antibodies specific

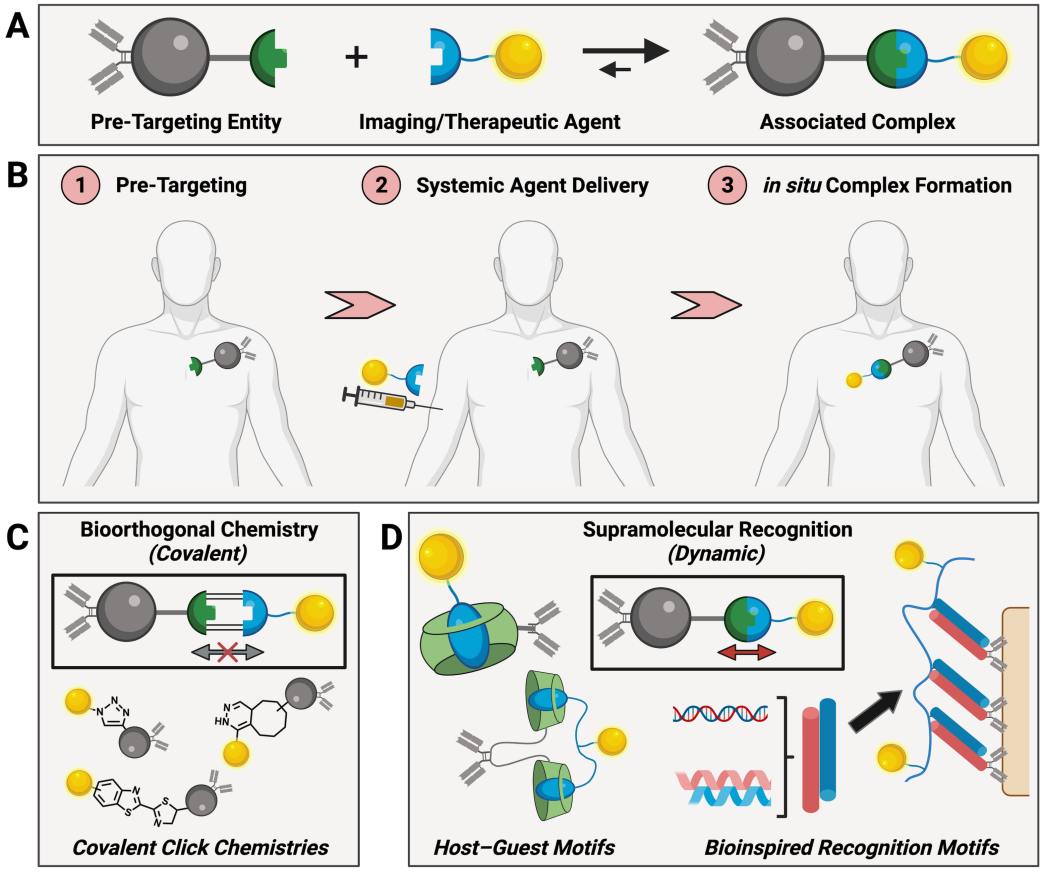


Figure 1: (A) Schematic of dynamic supramolecular interactions used for *in situ* association, leveraging complementary motifs with one attached to an entity for pre-targeting and another attached to the desired agent to be delivered. **(B)** Generalized overview of this two-component approach to targeting via *in situ* complex formation. **(C)** Examples of common covalent bioorthogonal reactions used in pre-targeting applications for *in situ* covalent bond formation. **(D)** Overview of the dynamic supramolecular motifs described herein, which form association complexes through non-covalent molecular recognition.

to the disease of interest; the use of larger constructs or drug carriers also limits tissue perfusion, carries risk of off-site accumulation, and may lead to prolonged circulation while shedding active drug systemically.[16,17] For example, only 0.001-0.01% of an injected monoclonal antibody, and by logical extension an antibody-drug conjugate, localizes to a tumor site in humans.[18,19] Meanwhile, nanoparticles targeted with a clinically validated antibody have demonstrated local accumulation of <1% *in vivo*.[20] As such, there remains a need to explore new technologies in order to more effectively deliver therapeutics to sites of need.

In the field of bioconjugate chemistry, a molecular scale pre-targeting approach has been demonstrated using different bioorthogonal ligations to capture circulating agents at specific sites in the body through spontaneous formation of covalent bonds.[21–24] Spatial localization can be achieved within the body by covalent bond formation *in situ* using two-step application of a pre-targeted entity bearing one component of a bioorthogonal motif followed by application of the second motif attached to a drug or imaging agent (***Fig 1B-C***).[25–29] The attachment of a drug to a motif for click chemistry offers certain prodrug benefits of attenuated systemic activity; such agents also incorporate labile linkages for subsequent release of the active therapeutic *via* linker hydrolysis following local accumulation. Others have demonstrated so-called "click-to-release" and "catch and release" chemistries wherein an active agent releases from its bioorthogonal motif-bearing prodrug precursor by spontaneous ring isomerization simultaneous to *in situ* formation of a covalent bond.[30,31]

The present review focuses on related molecular-scale approaches akin to bioorthogonal click chemistry, instead using non-covalent supramolecular interactions for *in situ* recognition in the body (***Fig 1A***). These synthetic motifs are attractive in the development of drug delivery platforms due to their scalable, tunable, and molecularly well-defined characteristics.[32–36] The various motifs used for non-covalent recognition in the body include host–guest macrocycle complexes, complementary oligonucleotide segments, and coiled-coil peptide

assemblies (***Fig 1D***). The mechanisms that underlie recognition incorporate pseudo-specificity through unnaturally high affinity and/or high effective affinity through engineering multivalent motifs to enable avidity. While certain of these motifs (*e.g.*, host–guest) are subject to competition from naturally occurring compounds and thus not fully bioorthogonal, outcomes resembling orthogonality can be realized through motif selection and design to tune affinity well in excess of naturally present competitors, or by engineering avidity to gain advantage.[35]

Molecular-scale approaches to drug targeting through both *in situ* covalent bioconjugation and non-covalent recognition offer certain distinctions relative to drug delivery methods using active biological targeting. In one manifestation of this two-step molecular-scale approach, pre-targeting a site of interest with an antibody or related biomolecule maintains the benefits of biological recognition of disease biomarkers. However, separating the drug from the antibody reduces the risk of undesired release during prolonged circulation. In other uses, pre-targeting and capture of a circulating therapeutic using a localized material suffers from a requirement for *a priori* knowledge in applying the pre-targeting material to guide subsequent administration of the agent, and as such may be more limited in its practical application. Small molecule prodrugs, prepared by modifying a therapeutic with a molecular-scale targeting motif, offer the benefits of attenuated activity in systemic circulation, more extensive tissue perfusion, and rapid clearance owing to small size relative to antibodies or even larger nanoscale carriers. As such, the general concept introduced here for non-covalent molecular recognition of synthetic motifs in the context of *in situ* targeting of therapeutics and imaging agents should be framed with these benefits and drawbacks in mind. With the aim of specifically focusing on uses of synthetic non-covalent molecular recognition motifs, this review will also (by necessity) not cover voluminous work in the areas of biomolecular-based recognition using antibodies, aptamers, peptides, or other common biomolecular affinity agents. Instances where such affinity agents are used in the context of pre-targeting a synthetic motif for subsequent non-covalent recognition-mediated targeting will be discussed.

2. THERMODYNAMICS OF RECOGNITION

The propensity for a non-covalent complex to form in the dilute environment of the body is governed by the thermodynamics of the particular interaction. In the simplest case of a monovalent interaction, the dynamic process of recognition proceeds as follows:

$$[A] + [B] \rightleftarrows [A{\bullet}B]$$

where $[A]$ and $[B]$ are the concentrations of the free binding pairs and $[A{\bullet}B]$ is the concentration of the formed complex. From the law of mass action, an equilibrium constant, K_{eq} (sometimes denoted K_A), can be derived as follows:

$$K_{eq} = \frac{[A \cdot B]}{[A][B]}$$

This quantity has standard units of [M^{-1}] for a 1:1 monovalent interaction. K_{eq} is commonly referred to as the affinity of an interaction. It is conventional in some systems to express the reciprocal of this value, $1/K_{eq}=K_D$, yielding units of [M] and serving to define the dilution concentration for spontaneous dissociation of a complex. A number of synthetic host molecules have been reported to bind with an array of complementary guest motifs, enabling K_{eq} to be tuned by molecular design or in response to a biologically relevant stimulus.[37] A higher value of K_{eq} thus signifies a more stable complex that exhibits preferential formation even under dilute conditions. The value of K_{eq} is also related to the rates of dynamic formation, k_{on}, and dissociation, k_{off}, of the complex, as follows:

$$K_{eq} \approx \frac{k_{on}}{k_{off}}$$

For a 1:1 monovalent interaction, k_{on} has units of [$M^{-1}s^{-1}$] and k_{off} has units of [s^{-1}]. The rate of complex formation for some supramolecular motifs has been found to occur near the diffusion limit (~10^8 $M^{-1}s^{-1}$);[38] this suggests a possible benefit of fast association for supramolecular motifs when compared to common covalent bioorthogonal conjugations such as azide–DBCO (2.3 $M^{-1}s^{-1}$; from [39]) and tetrazene–*trans*-cyclooctene (3100 $M^{-1}s^{-1}$; from [40]). Typically, higher affinity interactions will have concomitantly slower k_{off} and thus have a longer lifetime of complexation once formed.

The effective doses of different therapeutics vary, but an assumption for serum concentrations on the order of ~[nM] for most drugs defines (*roughly*) the target K_{eq} needed for complex formation when considering the use of a particular motif in targeting therapeutics; this implies K_{eq} may need to be greater than ~10^8 M^{-1} to drive complex formation *in vivo*. Given this extent of dilution expected for uses in the body, as well as a variety of possible competitors for certain classes of interactions, monovalent affinity may thus not be sufficient for some recognition motifs to facilitate efficient supramolecular complexation. Accordingly, other design approaches may couple multiple lower affinity interactions on a defined scaffold to achieve a higher effective affinity, a phenomenon referred to as avidity. The complexes formed between antibody and antigen, with multivalent

display of a specific binding epitope on the antibody, illustrates the use of avidity in nature.[41] Binding events in multivalent systems do not necessarily occur simultaneously, but they are likewise not completely independent. The physical tethering of multiple binding motifs creates an elevated local concentration through the close proximity of binding sites to drive complex formation.[42] In other instances, both motifs may be presented on multivalent scaffolds, leading to an overall reduction in the effective k_{off} given the asynchronous timescale of dynamic complex exchange for individual binding sites as multiple dynamic interactions drive greater complexation between the two scaffolds.[43] In this way, the use of multivalent systems may compensate for the low affinity of an individual motif to facilitate recognition even under the conditions of dilution expected for applications in the body.

3. HOST–GUEST RECOGNITION

Host–guest chemistry, characterized as the non-covalent association of a small molecule guest within the portal of a host macrocycle, is among the most recognizable of supramolecular motifs. The affinity of different interactions can vary substantially, though complexes have been demonstrated that form at high affinity (*e.g.*, K_{eq} >10^{10} M^{-1}) and are therefore resistant to dilution and native competition, in pursuit of various bioconjugation-based applications.[35,44,45] Many synthetic macrocyclic host molecules are known, including crown ethers, cryptands, cyclodextrins (CD), cucurbit[n]urils (CB[n]), calix[n]arenes, and pillar[n]arenes.[32] High-affinity designer molecules have also been revealed from host–guest complexes that form highly fluorescent and stable dyes.[46,47] Of the motifs used in the context of drug delivery, CD macrocycles prepared enzymatically from starch constitute the most broadly explored and readily available macrocycles.[48,49] CDs have rigid conical geometry and are comprised of different numbers of glucopyranoside subunits, to include α-CD (6), β-CD (7), and γ-CD (8), enabling size-mediated selectivity in their binding to different guests. Their hydrophobic interiors and hydrophilic exteriors allow guest encapsulation within the cavity, taking advantage of both hydrophobic and Van der Waals interactions.[50] Binding between CDs and their guests occur with K_{eq} values not typically exceeding ~10^5 M^{-1}, the order expected for binding between β-CD and an adamantane guest.[51,52] The CB[n] family of macrocycles, composed of [*n*] repeating glycoluril subunits, constitutes another useful macrocycle for guest binding in water.[53–56] Glycoluril subunits afford a symmetric macrocycle with a rigid hydrophobic cavity and two identical carbonyl-fringed portals. CB[7] macrocycles bind to adamantane-class guests with K_{eq} up to ~10^{17} M^{-1}, well in excess of what is achievable by other macrocycles or even natural motifs such as biotin-avidin.[57–59] High-affinity binding is possible through a combination of the hydrophobic association and volume-filling of the macrocycle cavity coupled along with electrostatic interactions between aliphatic-adjacent

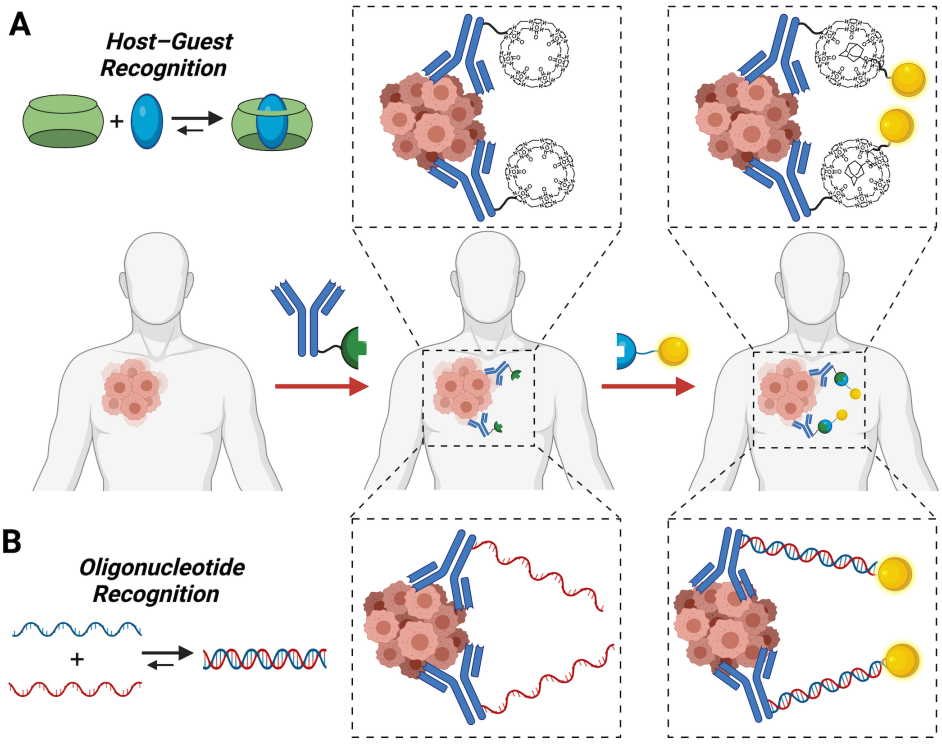


Figure 2: Antibody-based pre-targeting for delivery to sites of disease bearing a specific biomarker, coupled with a secondary delivery approach and *in situ* targeting driven by (**A**) monovalent host–guest motifs or (**B**) complementary oligonucleotide sequences designed for hybridization. (Panel A inspired by refs. 69 and 70; Panel B inspired by ref. 99)

protonating groups and the electronegative carbonyl-fringed portal.[60] Accordingly, the differing spectrum of affinity offered by CD and CB[n] macrocycles affords distinct opportunities for host–guest recognition and complex formation in the conditions of the body, as described herein.

3.1 Monovalent Host–Guest Recognition

In the context of *in situ* recognition, protein-based motifs have been extensively explored, yet can exhibit slow biodistribution and clearance.[61–63] Long circulation times to reach a target may limit their use to deliver short-lived isotopes for radioimaging and increase the possibility for enzymatic degradation in circulation.[64,65] Host–guest motifs, with small molecule guests on the order of ~200 g/mol and macrocycles on the order of 1200 g/mol, may thus offer a variety of possible benefits. CB[n] macrocycles, and in particular the water-soluble and high-affinity CB[7] variant, have been most explored in the context of monovalent host–guest recognition in the body. The types of guest molecules useful for this purpose are amine-containing ferrocene and adamantane derivatives, exhibiting K_{eq} values in the range of $10^{10\text{-}15}$ M^{-1} in binding CB[7].[66–68] Recognition using these motifs has thus been explored for a variety of imaging and therapeutic applications.

The use of supramolecular host–guest motifs for *in situ* targeting typically comprises a pre-targeting step followed by subsequent administration of an agent for imaging or therapy. In this context, an antibody may be used for the initial pre-targeting to deposit either a host or guest at the site of interest, followed by subsequent addition of the desired agent attached to the complementary binder (***Fig 2A***).[69] Using pre-targeting principles, *in situ* formation of host–guest complexes have been explored in live nematodes (*C. elegans*) and mice.[70] The studies in nematodes coupled complementary FRET pairs to CB[7] and guest, verifying sequential administration of the motif resulted in complex formation *in situ*. This system was then explored in mice for *in vivo* cancer imaging. CB[7] was covalently attached to cetuximab, an antibody recognizing epidermal growth factor receptor that is used clinically in treatment of colorectal, neck, and lung cancers. Following pre-targeting with the CB[7]-antibody conjugate, adamantane linked to a near-infrared cyanine dye was found to accumulate at the tumor site for selective tumor imaging.[70]

Pre-targeting has also been achieved by local injection of a polymer hydrogel presenting CB[7], with subsequent systemic administration of a guest-linked agent.[71] A series of guests ranging in K_{eq} from ~10^9 to 10^{12} M^{-1} fused to a

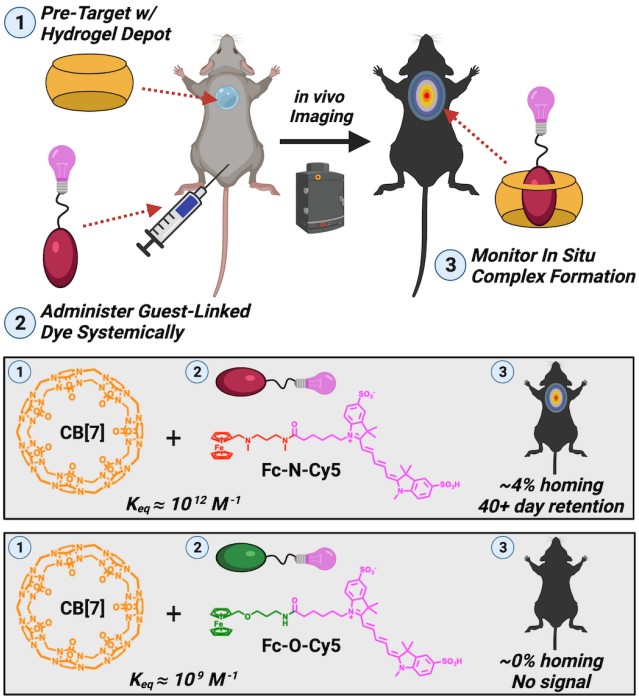


Figure 3: Methodology to assess the affinity needed for *in situ* recognition and targeting. A hydrogel presenting pendant CB[7] macrocycles was implanted locally at a site. Subsequently, a near-infrared fluorescent probe (Cy5) modified with two ferrocene guests having different affinities for CB[7] (Fc-N:10^{12} M^{-1} vs. Fc-O:10^9 M^{-1}) was administered systemically. Through *in vivo* imaging, the amount of dye localized and retained at the site presenting CB[7] was then quantified. Subtle differences in the guest structure, altering their resulting affinity for CB[7], led to dramatic changes in the effectiveness of *in situ* complexation. (Figure inspired by ref 71)

near-infrared fluorescent dye were explored to assess the role of affinity for *in situ* complex formation at the site of the CB[7]-rich depot (***Fig 3***). These studies identified complexes between CB[7] and an amino-ferrocene guest with K_{eq} of ~10^{12} M^{-1} that achieved substantial localization, whereas the dye bound to a different ferrocene guest with K_{eq} of ~10^9 M^{-1} showed no accumulation. For the high-affinity case, ~4% of the administered agent homed within a few hours; the remainder was rapidly cleared over this same time. This figure is impressive in context of the typical targeting efficiency achieved by antibodies, referenced previously here. The depot site could be serially reloaded, with site retention of the bound agent for multiple weeks following administration. This same high-affinity guest motif was then conjugated to the chemotherapeutic doxorubicin to create a prodrug for integration with supramolecular targeting. By injecting the CB[7]-rich hydrogel near a tumor, the therapeutic efficacy of supramolecular homing was evaluated in comparison to a prodrug variant with no affinity for CB[7]. In this case, the targeted prodrug demonstrated a

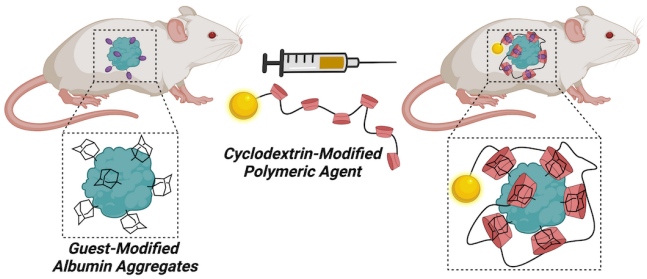


Figure 4: Schematic overview of the use of guest-modified serum albumin aggregates, which following administration preferentially accumulate at sites of disease. Subsequently, a cyclodextrin-modified polymer was administered to enable multivalent *in situ* complexation and agent delivery to the site bearing these guest-modified albumin aggregates. (Figure inspired by ref. 72)

significant reduction in tumor growth rate, with the effect extending for weeks following initial dosing.

3.2 Multivalent Host–Guest Recognition

The uses of CD for *in situ* complex formation have primarily leveraged multivalent constructs to introduce avidity, thereby compensating for the relatively low K_{eq} of a monovalent CD host–guest complex compared to those observed for CB[7]. In one such design, adamantane-functionalized albumin aggregates were used to pre-target sites for subsequent delivery of β-CD-modified polymers carrying agents for either fluorescence or SPECT imaging modalities (***Fig 4***).[72] Pre-targeting with the multivalent albumin aggregates followed by multivalent agent delivery offered a ~16-fold increase in the accumulation of the agent in the liver and 4.5-fold in the lungs when compared using SPECT imaging to pre-targeting with unmodified albumin aggregates. Further studies using albumin aggregates to pre-target a β-CD-modified polymer leveraged dual-isotope imaging (^{99m}Tc on the albumin particles and ^{111}In on the polymer) to validate co-localization of the two components *in vivo*.[73] As such, multivalent scaffolds presenting both host and guest enable the use of CD macrocycles in spite of its modest monovalent affinity.

4. OLIGONUCLEOTIDE HYBRIDIZATION

The association of complementary strands of DNA, forming its canonical double helix, is one of the most recognizable non-covalent motifs in the living world. Synthetic oligonucleotides thus offer a tunable and biologically relevant affinity motif for non-covalent complex formation, toward many therapeutic uses.[74–76] This is highlighted by the decades of work evaluating the therapeutic potential of small interfering RNAs (siRNA), where therapeutic function arises specifically from recognition and binding to target mRNA to transiently inhibit protein expression.[77,78] Oligonucleotide strand complexation, a process known as hybridization, is driven by Watson-Crick base pairing with lateral hydrogen bond formation between complementary bases offering an enthalpic driving force.[79–81] The vertical stacking of aromatic bases in the formed helical structure also contributes a favorable driving force for hybridization *via* hydrophobic and π-π interactions.[82] The number of base pairs, and by extension the number of hydrogen bonds and π-π interactions, dictates the binding affinity between oligonucleotide strands; this affinity is highly dependent on environmental conditions such as temperature, concentration, and osmolarity.[83,84] For example, the complexation of model 10-base strands in 3 mM buffer exhibits a K_{eq} of ~$5*10^7$ M^{-1} at 15°C, reducing to ~$3*10^5$ M^{-1} at 35°C as non-covalent interactions become less favorable.[84] Meanwhile, 20-base strands have K_{eq} values (~10^8 M^{-1}) that are much less temperature-dependent over the same range. For both lengths, affinity also increases by ~1-2 orders of magnitude for interactions in a buffer of higher salt (10 mM). Accordingly, the design of oligonucleotide sequences for recognition in the body must account for specific operating conditions to ensure reliable complex formation. As the focus here is on the use of synthetic non-covalent recognition motifs for targeting applications, the many important uses of aptamers for recognition of biomolecules falls outside the present scope of this review; the reader is encouraged to explore other relevant reviews on this topic.[85–87]

4.1 Monovalent Oligonucleotide Hybridization

One benefit of oligonucleotide-based recognition arises from its ease of synthetic modification with molecular cargo.[88–91] This design tool enables an array of therapeutics or imaging agents to be appended to oligonucleotide strands. One salient example of this approach is found in the field of molecular beacons, wherein binding to a target DNA or RNA strand triggers a hybridization-mediated unfolding of the beacon and typically an increase in fluorescence relative to a quenched state in the folded form.[92,93] Early work using this technology *in vivo* relied on aptamer-mediated recognition to facilitate beacon rearrangement for imaging.[94,95] Related aptamer-targeted technologies have also been used to deliver drugs bound *via* intercalation with double-stranded regions of the probe.[96] Other technologies evaluated *in vitro* suggest the possibility that aptamer-based constructs with a pendant oligonucleotide tail can be used for pre-targeting, with subsequent delivery of a probe coupled to the complementary oligonucleotide strand.[97] A similar pre-targeting approach was also demonstrated *in vitro* using copper-free click chemistry to modify the cell surface with oligonucleotides, subsequently delivering a

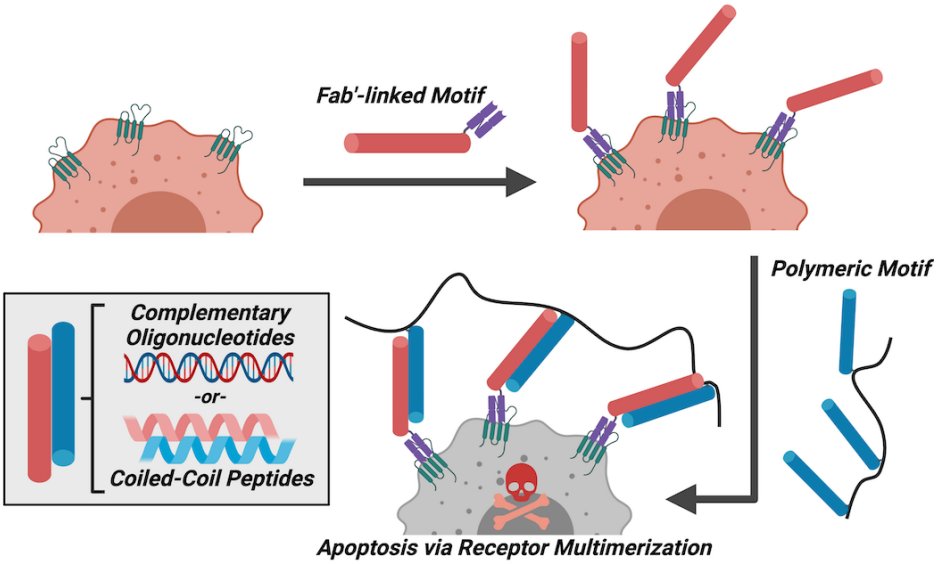


Figure 5: Schematic overview of the concept of drug-free macromolecular therapeutics. This concept has been demonstrated for motifs derived from both oligonucleotide hybridization and coiled-coil peptide association. A cancer cell of interest was first pre-targeted with a reagent for biological recognition, an antibody or antibody fragment, that was modified with one-half of the desired recognition motif. Subsequently, a multivalent polymer bearing the complementary recognition motif was administered. The polymer scaffold, by simultaneously binding multiple surface-presented motifs, serves to non-covalently link the receptors on the cell leading to triggered cell death. (Figure inspired by works from Kopecek and colleagues, such as refs. 106 and 130)

complementary strand linked to a probe for imaging.[98] However, the use of oligonucleotide hybridization specifically for targeting molecular beacons *in vivo* has been less commonly explored.

Targeting *via* monovalent oligonucleotide hybridization has been demonstrated in the context of antibody-mediated pre-targeting for PET-CT imaging (***Fig 2B***).[99] In this work, the cetuximab antibody was modified with a 17-mer L-DNA segment. Subsequently, a mirror-image 17-mer L-DNA segment connected to a ^{64}Cu radionuclide chelator was administered for localization by *in situ* hybridization. Biodistribution studies performed *in vivo* demonstrated significant tumor accumulation and contrast enhancement when using this pre-targeting approach for radionuclide delivery.

In a related context, synthetic oligonucleotide analogues known as peptide nucleic acids (PNAs) may also enable recognition in the context of targeting. The nucleobases of PNAs form stable duplexes with DNA or RNA segments, and may also be designed to recognize other PNAs.[100–103] Accordingly, PNA recognition has been used in the context of a two-step pre-targeting.[104] In this work, a PNA-modified protein was first administered for passive accumulation at sites of infection or tumors, and subsequently a PNA radiolabelled with ^{99m}Tc was administered for localization by *in situ* hybridization.

4.2 Multivalent Oligonucleotide Hybridization

Efforts to increase the effective binding affinity of complementary oligonucleotide strands have entailed developing multivalent scaffolds to introduce avidity into the process of targeting. In one example, recognition *via* oligonucleotide hybridization of complementary oligonucleotides has been demonstrated to refill a locally applied hydrogel depot.[105] In this design, an alginate hydrogel modified with oligodeoxynucleotide (ODN) strands was applied locally. Subsequent systemic application of alginate modified with the complementary ODN strands enabled local accumulation at the depot through strand recognition. A control of non-complementary ODN sequences exhibited no increased accumulation. The ODN-targeted platform was evaluated for functional use *in vivo* in the delivery of a chemotherapeutic, doxorubicin, to the site of a tumor. Mice treated weekly by systemic application of ODN-modified alginate strands conjugated to doxorubicin showed a significant reduction in tumor growth compared to controls, attributable to ODN hybridization localizing the drug-modified polymer to the site of the depot.

Certain therapeutic benefits arise when using multivalent scaffolds apart from increasing the effective K_{eq} of recognition. One such example is found in efforts to pre-target using oligonucleotide-modified antibodies followed by subsequent recognition on the cell surface of a multivalent oligonucleotide scaffold (***Fig 5***).[106,107] The therapeutic effect of this approach arises from induction

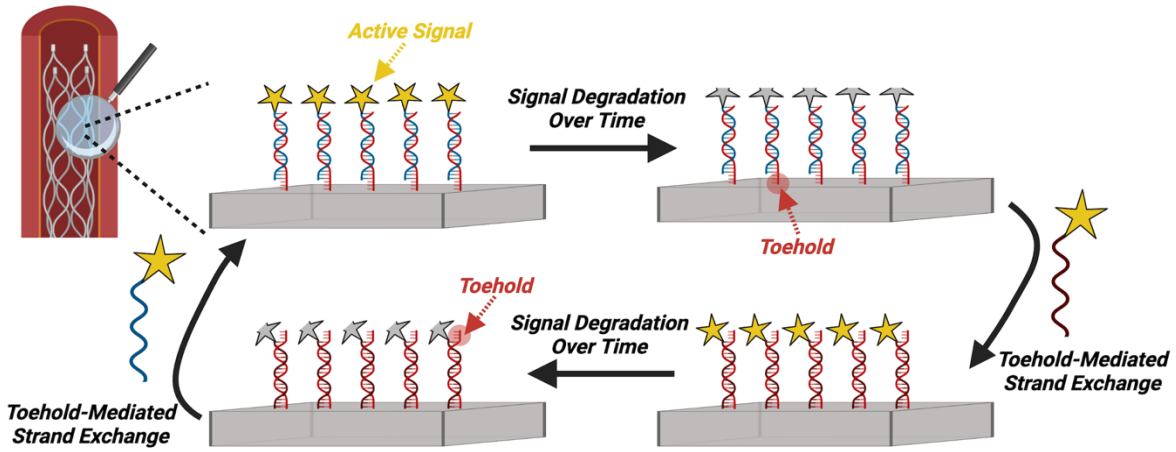


Figure 6: Schematic of *in situ* surface regeneration using a toehold-mediated strand exchange approach. This general strategy illustrates a route to use designed recognition motifs to regenerate the presentation of active sites on a device surface. (Figure inspired by ref. 112)

of apoptosis due to receptor multimerization on the cell surface, leading to demonstrations for a new class of drug-free macromolecular therapeutics.[108] Efforts to prepare these constructs with oligonucleotides have relied on morpholino oligomers, synthetic analogs of oligonucleotides consisting of DNA bases attached to a backbone of methylenemorpholine rings linked through phosphorodiamidate groups, intended to facilitate enhanced stability in serum.[109] The first design leveraged an antibody fragment (Fab') against a marker for B-cell lymphoma (CD20), fusing this to a morpholino strand for pre-targeting cancer cells. A polymer based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) with pendant complimentary morpholino strands was then administered to multimerize the CD20 cell surface receptors and induce apoptosis.[106] These constructs demonstrated therapeutic function in a disseminated B-cell lymphoma model in mice, demonstrating a key benefit of this approach against metastatic disease. Subsequent work on this concept utilized an intact anti-CD20 antibody (Obinutuzumab) for pre-targeting with morpholino strands and induced multimerization with morpholino-modified human serum albumin.[107] By combining the intact antibody with multivalent crosslinking, this approach enabled two synergistic modes to induce apoptosis.

4.3 In Situ Strand Displacement

Oligonucleotide complexes can be designed to engage in strand displacement through binding to unhybridized segments flanking a double-stranded segment, an approach used to facilitate polymer de-gelling, site-specific drug release, and surface regeneration.[110–112] This displacement is often initiated through toehold-mediated strand exchange, wherein a single-stranded oligonucleotide binds to an exposed portion of its complementary strand that is otherwise engaged in a double helix, triggering dissociation of the initial complex as the replacement strand hybridizes.[113] Recently, this mechanism was utilized to regenerate antithrombotic functionality of a surface (***Fig 6***).[112] To combat degradation of an antithrombotic agent presented on the device, strand displacement was designed to replace the degraded agent and restore antithrombotic functionality of the surface. This approach demonstrated a significant reduction in fibrin formation. Though not used *in vivo*, recognition-mediated strand displacement offers many possible opportunities to externally control the properties of biomedical device interfaces *in situ*.

5. PEPTIDE COILED-COIL FORMATION

Engineered coiled-coil peptides, characterized by the arrangement of alpha-helical peptides into a superhelix bundle, afford recognition properties with utility in the design of functional materials and systems.[114,115] Their biological relevance as a common structural motif found in nature have inspired significant study into both the mechanisms of formation and strategies for sequence manipulation to realize coiled-coils motifs comprised of a different number (*n=2-6*) of both homo- or hetero-[n]meric alpha-helical peptides.[116–119] Various naturally derived and *de novo* designs have thus been demonstrated for coiled-coil recognition, with some synthetic heterodimeric variants having K_{eq} values up to 10^{14} M^{-1}.[120–123] Such interactions are thus comparable to (*or higher than*) high-affinity host–guest or oligonucleotide motifs. The predictable nature of these associations has been used to recreate the complex higher-ordered structures of natural proteins with synthetic variants, for instance in the preparation of discrete cage-like assemblies.[124,125] In addition, coiled-coil motifs have been incorporated as a

modular associating unit in non-covalent preparation of modular drug carriers.[126–129] Accordingly, these interactions offer another class of synthetic non-covalent interactions with promise for *in situ* recognition in the body.

5.1 Multivalent Coiled-Coil Recognition

As with work in oligonucleotide systems, coiled-coil interactions have been explored in conjunction with routes for pre-targeting as well as scaffolds for multivalent presentation toward the concept of drug-free macromolecular therapeutics.[108] In one example, one component of a heterodimeric coiled-coil was attached multivalently to HPMA with the complementary alpha-helical segment attached to a Fab' with reactivity against CD20 (***Fig 5***).[130] This platform showed *in vivo* efficacy in a mouse model of B-cell lymphoma, functioning by crosslinking the surface-bound Fab' on cell surface receptors to induce apoptosis.[131] The immunogenicity of this platform was studied *in vitro* and *in vivo*, pointing to no specific immunogenicity for the coil-forming peptide motif; this study explored the same motif prepared from *D*-isomer peptides and found the enantiomeric peptide coiled-coils to behave similarly to the originally used *L*-amino acids.[132] Multi-fluorophore imaging of this system further verified *in situ* assembly of the two components on B-cell membranes when administered by this two-step pre-targeting approach, noting the importance of the delay time between administration of the first and second component to enable localization.[133] This system was also found to function when the multivalent HPMA component was replaced with human serum albumin modified with multiple copies of one of the coil-forming peptide segments.[134] Related work demonstrated the ability to target cancer cells presenting one-half of a coiled-coil motif with liposomes presenting the complementary peptide, demonstrating *in situ* homing in a zebrafish model.[135] Accordingly, systems based on pre-targeting and multivalent recognition may also use synthetic coiled-coil motifs to facilitate recognition in the body.

6. CONCLUSIONS

In the continued pursuit of new routes to enhance efficacy in diagnosing and treating disease, strategies for recognition on the molecular scale hold promise. In particular, the use of these synthetic motifs offers new routes to reliably and efficiently perform *in situ* conjugation under dilute conditions in the body, while in the presence of salts, proteins, lipids, and other "sticky" biological entities. The use of small molecules affords rapid and extensive tissue perfusion. To date, bioorthogonal covalent conjugations have offered one means of achieving this outcome of *in situ* recognition. Herein, a related concept leveraging the noncovalent association of synthetic supramolecular motifs is described. Through motif selection and design, high-affinity interactions can be realized to enable quasi-specificity and orthogonality in the body. Many of these motifs offer kinetic advantages over traditional bioorthogonal chemistries, such as the ability to associate with diffusion-governed interaction rates. Moreover, the synthetic origins of these motifs enable facile multivalent display on polymers, nanoparticles, or related scaffolds to engage avidity and further enhance recognition specificity. This approach has even revealed a new therapeutic class based on drug-free macromolecular architectures.

There remain challenges that must be navigated to more fully exploit the potential of these supramolecular tools for *in situ* targeting. The two-step targeting used in many systems, while advantageous in limiting off-site accumulation and systemic drug shedding of often toxic drugs, introduces complexities and variability with respect to the timing of administration of each component. The benefit of broader and biologically specific systemic surveillance when pre-targeting is done using antibodies is not captured in cases where a locally implanted material depot is used as the pre-targeting entity. This requirement for *a priori* knowledge of the desired site of action also limits uses for the latter case in disseminated diseases such as metastatic cancer, yet may remain relevant for applications in regenerating active signals on implanted biomedical devices. There are also remaining challenges to better integrate supramolecular targeting motifs with relevant methods in prodrug chemistry, such as incorporating analyte- or enzyme-sensitive linkers for site-specific drug activation following homing.

The emerging concept to use non-covalent association of supramolecular motifs offers inspiration to reimagine the diagnosis and treatment of disease. With nature as inspiration for specific non-covalent recognition in physiological conditions, recreating these concepts using synthetic tools is a path primed for many possible applications. Accordingly, the concept of supramolecular "click chemistry" for *in situ* targeting offers a promising direction ripe for further evaluation.

ASSOCIATED CONTENT

Supporting Information

There is no online supporting information for this work.

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Notes

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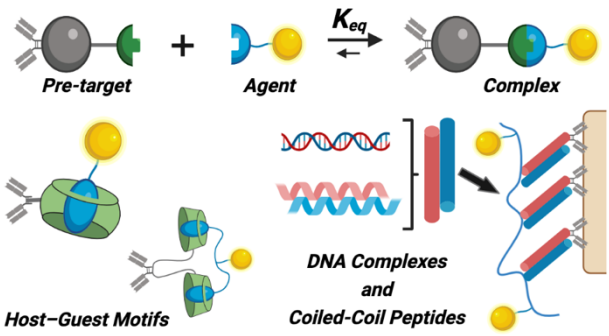
SYNOPSIS & TOC FIGURE



Synthetic supramolecular recognition motifs offer new routes for pre-targeting and *in situ* complexation, yielding a means for site-specific targeting of drugs or imaging agents.