doi:10.2533/chimia.2025.128 Chimia 79 (2025) 128–136

Control of Therapeutic Activity through Programmed Assembly

Millicent Dockerill* and Nicolas Winssinger*

Abstract: This review explores the control of therapeutic activity through programmed assembly of supramolecular systems. We examine the use of nucleic acids as scaffolds to create tailored assemblies of ligands, including glycan and peptide-based systems, drug-like small molecules or reagents for proximity-induced reactions. We discuss the principles of cooperativity in multivalent interactions, emphasizing their potential to enhance binding affinity and therapeutic efficacy and the opportunity to control their activity through strand displacement. We highlight seminal studies and illustrative case examples and address the challenges faced in translating these designs into clinical applications. Furthermore, we explore recent advancements that demonstrate successful *in vivo* applications, particularly in the context of anticoagulation therapies. This review aims to provide insights into the future of responsive therapeutic systems that leverage the programmability of supramolecular assemblies to develop potent and adaptable therapeutics.

Keywords: Cooperativity · Programmed assembly · Reversal · Supramolecular therapeutics

Millicent Dockerill received her master's degree in chemistry from the University of Edinburgh (UK). She spent her final year research project in the medicinal chemistry department at AstraZeneca in Cambridge. In 2020, she joined the group of Professor Winssinger at the University of Geneva (Switzerland) to pursue her PhD. Her interests lie in using supramolecular assemblies to develop potent reversible therapeutics.

Nicolas Winssinger is a Professor of Chemistry at the University of Geneva. His research interests lie at the intersection of molecular sciences and life sciences. His lab leverages nucleic acids as supramolecular tags to encode molecules, program the spatial organization of ligands, and drive reactions through proximity within assemblies. A long-term objective of his research is to extend these principles toward the development of responsive systems that harness the programmability of logic-gated assemblies and reactions.

1. Introduction

It is now well established that the binding affinity to a target of interest can be substantially enhanced through the synergistic interactions of ligands conjugated to oligonucleotides and paired *via* hybridization (Fig. 1.A). The inherent programmability of hybridization offers precise control over key parameters, including inter-ligand distance, the number and composition of ligands, the thermodynamic stability of the assembly, and, importantly, dynamic regulation through strand displacement.[1–3] Developments in DNA-encoded libraries facilitates the discovery of ligands that interact synergistically with a target,[4–9] while advancements in hybridization-based circuits have provided robust strategies for controlling assembly processes *via* strand displacement. Together, these technologies present a promising framework for creating highly adaptable and programmable molecular interfaces. Are we there yet? Although numerous elegant designs have been described in the literature, their practical implementation faces significant challenges and few have been implemented in animal studies, let alone clinical development. A major obstacle is the inherent metabolic lability of DNA and its potential to induce immune activation through (TLR) interaction.[10] Although chemical modifications to DNA or the use of analogues such as locked nucleic acids (LNAs) and peptide nucleic acids (PNAs) can mitigate these challenges, their adoption is often hindered by the need for specialized expertise and resources. In contrast, commercial DNA enjoys widespread accessibility due to its unmatched economy of scale and straightforward out-of-the-box conjugation chemistry. In this review, we examine fundamental principles, highlight seminal studies in the field, present illustrative case studies, and explore potential future advancements.

2. Gain of Function Through Multimerization

Numerous biological processes are regulated by cooperative multivalent interactions, where individually low-affinity binding events confer reversibility and provide an inherent error-correcting mechanism. Drawing inspiration from these natural systems, researchers have developed synthetic platforms that leverage multivalent interactions for a range of applications, including protein inhibition and the prevention of viral adhesion. The benefits from the chelate effect of multivalent interactions has been extensively studied.[11] A prominent example of a designed trivalent ligand-receptor interaction yielded one of the most stable organic receptor-ligand pairs involving small molecules that is known.[12] Whitesides *et al*. reported the binding of a trivalent analogue of vancomycin to a trivalent D-Ala-D-Ala construct which displayed a K_D of 4 x 10^{-17} M (Fig. 2.A). This example shows near perfect cooperativity as the binding of the individual fragments display a K_D of 10^{-6} M giving the trivalent construct a theoretical K_D of

*Correspondence: M. Dockerill, E-mail: Millicent.Dockerill@unige.ch; Prof. N. Winssinger, E-mail: nicolas.winssinger@unige.ch
Department of Organic Chemistry, University of Geneva, CH-1211 Geneva.

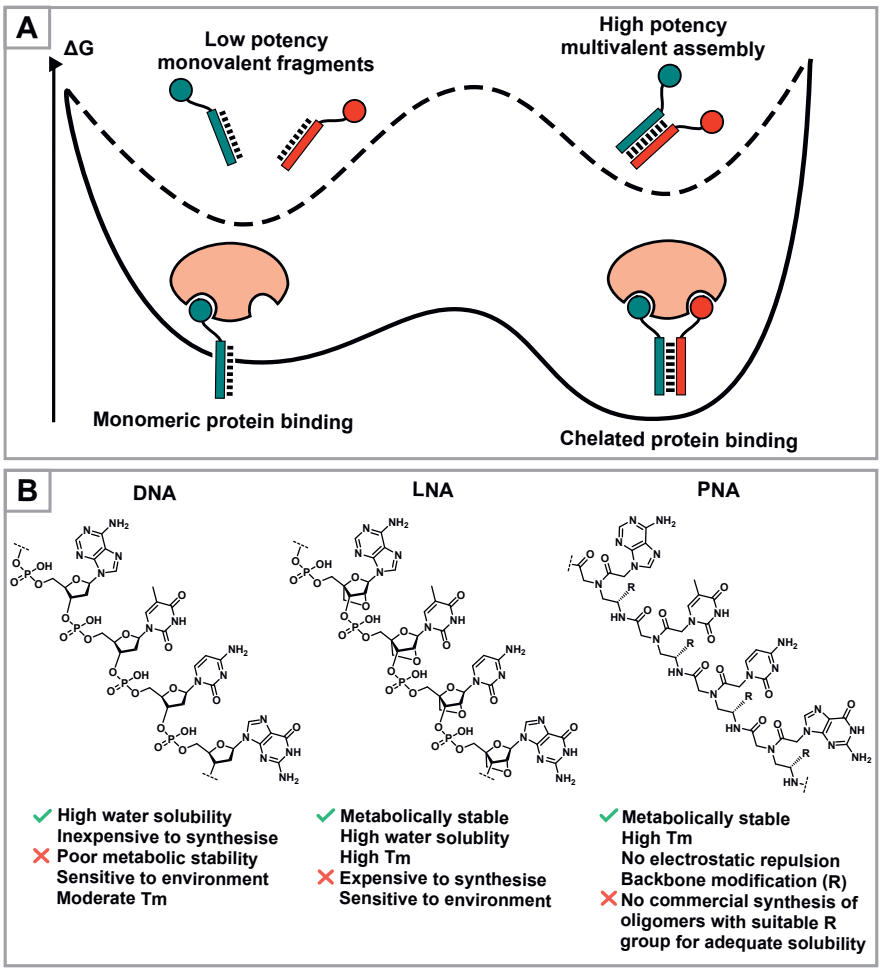

Fig. 1. A) View of a thermodynamic profile of the chelate effect on self-assembled fragment pairing using short oligonucleotides that do not form a stable duplex. B) Chemical structure of different nucleic acids: DNA (left), LNA (centre), PNA (right) with their pros and cons.

10^{-18} M. While this work is an example of rigorous engineering, the design of individual covalent systems requires laborious synthesis and design. Supramolecular assemblies based on nucleic acids provides a general platform where the individual ligands can be easily varied, and the inter-fragment distances are programmed by the nucleic acid sequences.[13–15] These assemblies can also differ in the nucleic acids that are used (*e.g.* DNA, LNA,[16] PNA[17–19]) providing an array of desirable properties (Fig. 1.B).

2.1 *Glycan-Based Assemblies*

One of the first examples of the use of nucleic acids to prepare assemblies of biologically relevant ligands was published by Kobayashi and co-workers in 2001.[20] They developed a DNA-based 'Glyo-cluster' that displays galactose ligands at specific distances programmed by a complementary DNA strand (Fig. 2.B1). The self-assembly showed cooperative binding to RCA_{120} lectin. The multivalent display of glycans has since seen a range of applications as protein-carbohydrate interactions, which are often multivalent, are of high therapeutic significance. Our lab previously prepared a pilot library of oligosaccharide-tagged PNA which can assemble a instructed by a DNA strand (Fig. 2.B2).[21] The library was designed to mimic the carbohydrates present on the glycoprotein 120 of HIV (gp120). A range of oligosaccharide compositions and distances were easily prepared using a combinatorial approach and tested on SPR against 2G12, an antibody that interacts with gp120. One architecture displayed a K_D of 4.2 μM whilst the monomer showed no detectable binding. This technology was further developed by preparing a DNA templated library through self-assembly; 105 glycan-PNAs, prepared by split-and-mix were paired with 357 other glycan-PNAs, resulting in 37,485 possible combinations, encoded by the DNA template.[22] This larger library was screened against the DC-SIGN, a lectin present on dendritic cells and macrophage. This tetrameric lectin also binds to gp120 present on HIV and facilitates viral entry. The library yielded a 127 nM hit which inhibited binding of gp120 to dendritic cells when incorporated into a dendrimer. Similarly, Seitz *et al.* used a DNA-PNA-glycan assembly technology to probe the spatial organisation of lectin binding sites.[23] They showed by experiments and modelling that the rigidity of the nucleic acid complexes is necessary to increase the effective molarity of the ligands, which was not observed with a flexible polyethylene glycol (PEG) linker. The Seitz lab then extended their system to target adjacent HA trimers on the viral surface. They achieved this by rolling-circle amplification (RCA) of a template DNA strand. This technique enables the preparation of long DNA strands (~750–1,825 nucleotides) which display repeats of the same sequence (15–46 repeats).[24] PNA- glycan strands could therefore be displayed along the oligo, either linearly or branched *via* an adaptor strand (Fig. 2.B3). These assemblies showed 107-fold enhancement over the monovalent sugar as well as selectivity between viral strains. The previously discussed examples cover linear or branched assemblies, but other architectures have been reported. Ebara and Matsui have developed carbohydrate-modified DNA three-way junctions which bind to lectins concanavalin A and RCA_{120},[25] followed by a second generation which was designed to interact with influenza virus HA.[26] Our lab also developed a PNA architecture which encircles *Ralstonia solanacearum* lectin (RSL) by binding to all six binding sites (Fig. 2.B4).[27] SPR measurements showed a >1,000 fold gain in binding for the hexameric assembly versus the monomer. The PNA was then shortened from 8 to 4-mer to increase the dynamicity of the system. The K_D of the fucose monomer to RSL, as well as the K_D of the 4-mer PNA interaction are in the μM range, however the assembly displayed 11 nM affinity for RSL. The dynamic assembly also showed inhibition of *Burkholderia ambifaria*

lectin (BambL) to epithelial lung cells, with a 723-fold enhancement compared to the fucose monomer. Considering the ability of dynamic assemblies to potently inhibit lectin binding, we proceeded to make a PNA-based dynamic combinatorial library (PDCL) to screen against lectins.[28] The library was designed with a 4-mer PNA which could display glycan based ligands on the same or opposite ends. The library was selected against *Aspergillus fumigatus* lectin (AFL) and RSL with mass readout using Matrix-assisted laser desorption ionization (MALDI) (Fig. 2.B5). A 95 nM AFL binding assembly was identified where the glycan alone displayed a K_D of 41 μM.

2.2 *Peptide-Based Assemblies*

Whilst glycan assemblies may seem the most obvious to mimic the multivalent properties found in biology, other molecule classes have since been exploited for programmed assembly. Peptides,

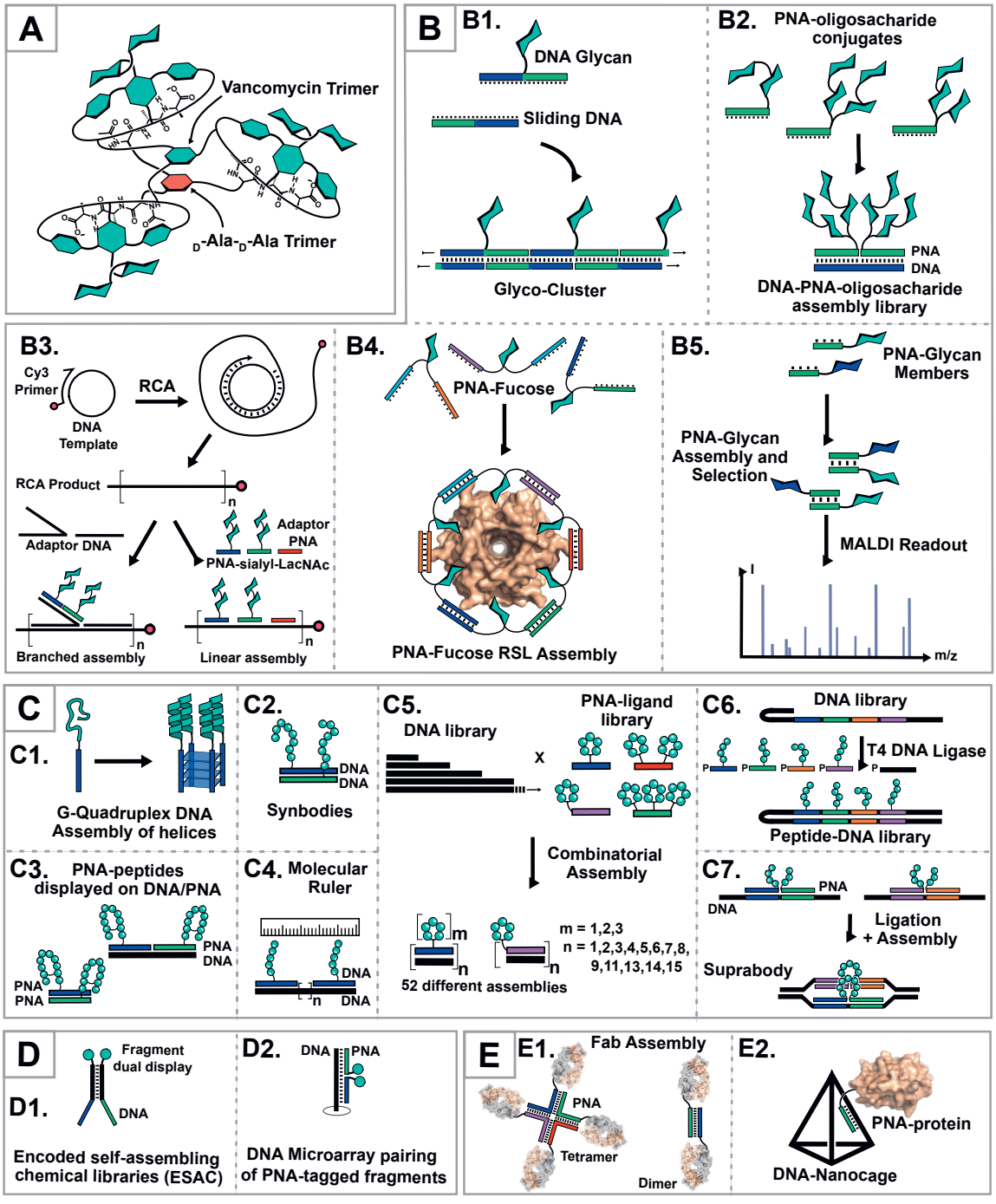

Fig. 2. **Gain of function through multimerization**. A) Cartoon representation of vancomycin trimer binding to D-Ala-D-Ala trimer. B) **Glycan display** (1) Assembly of DNA-Glycans on sliding DNA to form a 'Glyco-cluster'. (2) PNA-oligosaccharide library assembled on a DNA library. (3) RCA as a method to prepare long DNA strands for PNA-sialyl-LacNAc assembly. (4) Assembly of Fucose-PNA conjugates around RSL. (5) Dynamic combinatorial library of PNA-Glycans. C) **Peptide display** (1) Assembly of a four helix bundle on a G-Quadruplex scaffold. (2) Peptide-DNA conjugates assembled into 'synbodies'. (3) Display of PNA-Peptides on DNA or self-assembly of PNA-peptides. (4) DNA-Peptides displayed on a DNA template to evaluate the distance between two binding sites (molecular ruler). (5) Combinatorial library of PNA-*c*(RGDfK) on DNA templates. (6) DNA-templated polymerisation of pentanucleotide-peptide conjugates to form a large library amenable to multiple rounds of selection. (7) Suprabody library of DNA displaying PNA-Peptide loops formed by templated ligation. D) **Small molecule supramolecular display** (1) Encoded self-assembling chemical libraries for dual fragment display. (2) Dual Fragment display of PNA-fragment libraries on a DNA microarray. E) **Protein assembly** (1) Fab fragments assembled into dimers or tetramers *via* PNA assembly. (2) PNA-protein assembled onto a DNA nanocage (Cytochrome c).

which are seeing a renewed interest in recent years, have been used in multiple supramolecular scaffolds.[29] Hamilton and coworkers, who previously reported a DNA G-quadruplex scaffold displaying anionic hydrophobic fragments for cytochrome *c* binding,[30] used a similar scaffold to mimic a four helix bundle, but did not show any biological activity (Fig. 2.C1).[31] Other examples of alpha helix assemblies have been reported including one by Wengel, Jensen and coworkers which displayed a short coiled coil peptide on an oligonucleotide triplex.[32] More recently, we simplified a three-helix mini protein by PNA-helix supramolecular hybrids that showed picomolar binding to the receptor binding domain of the COVID-19 Spike protein.[33] Further work by Sleiman and coworkers also demonstrated the assembly of three peptides into a coiled-coil motif using branched DNA trimers.[34]

Whilst nucleic acid supramolecular assemblies of alpha helices have the added supramolecular templating of the individual coils, other peptide scaffolds have also been studied. The Chaput lab developed *synbodies*, DNA-tagged short peptides which can bivalently assemble to mimic antibodies (Fig. 2.C2).[35] Individual peptides were screened against the yeast regulatory protein Gal180 *via* a peptide microarray and the hits were combined at different distances along a DNA scaffold. Individual DNA-peptides displayed K_Ds in the μM range, while the best *synbody* had a K_D of 4.2 μM.

Similarly, our lab developed bivalent peptide assemblies but rather than using DNA to tag the peptides, we used PNA which could easily be synthesised alongside the peptide *via* solid phase peptide synthesis (SPPS).[36] The PNA-peptides were then hybridised on a DNA template or to each other *via* PNA-PNA hybridisation (Fig. 2.C3). The dual PNA display of cyclic peptides that bind and activate the TRAIL death receptor (DR5), showed improved binding (23.8 nM) compared to its DNA display counterpart (103.49 nM) and the monomeric ligand (129 nM). It was theorised that the neutral backbone of PNA was favoured, whilst DNA suffered from electrostatic repulsion. Subsequently, the Seitz lab developed a bivalent DNA-peptide assembly as a molecular ruler to study the distance and flexibility of two binding sites of the SH2 domains of the Syk kinase (Fig. 2.C4).[37,38] They showed that the binding of the double stranded DNA-peptide display and nicked double stranded DNA-peptide were highly dependent on the number of nucleotides whereas the more flexible single stranded DNA showed strong binding at most lengths. The optimal length for all constructs was found to be 11 nucleotides (37 Å) which corroborates the distance observed in the crystal structure.

Appella *et al.* used a PNA-DNA nanoscaffold to arrange cyclic RGDfK ligands, antagonists of $\alpha_v\beta_3$ integrins (overexpression linked to metathesis). The ligand was attached to the PNA backbone or *N*-terminus with one, two or three ligands per strand (12-mer).[39] These conjugates were assembled on a library of 13 DNA templates of different lengths. They were designed to hybridise with 1 to 15 PNA strands (each containing 1 to 4 ligands) resulting in 52 different assemblies displaying 1 to 45 ligands per DNA template (Fig. 2.C5). The IC_{50} values improved as the number of ligands increased: 477, 169 and 67 nM for one, two and three ligands per PNA strand (1 per DNA), respectively. The activity also increased as the number of PNA strands per DNA increased, but plateaued at 5 PNA per DNA for the trivalent PNA (15 ligands), whilst it plateaued at 11 PNA per DNA for monovalent PNA (11 ligands). DNA:PNA-D_5, where 5 trivalent PNA strands are assembled, was tested in a cellular competition assay and displayed a K_D of 0.16 nM versus 62.9 nM for the cyclic peptide alone (393-fold enhancement). This nanoscaffold was also validated *in vivo* using a mouse melanoma cell line which typically metastasize and form lung tumours. The assembly showed 50% decrease in tumour colonies at 0.026 μmol of cyclic peptide loading whilst the cyclic peptide alone only showed 30% decrease at 92 times higher dose (2.4 μmol). DNA-peptide conjugates have also been used to craft a precisely controlled extracellular matrix for cell adhesion and dynamic control over multiple signals.[40]

Whilst DNA and/or PNA assemblies seem to be the most exploited, LNA has also been used. Wengel and coworkers studied LNA-peptide conjugates which hybridise with DNA or RNA templates. Interestingly, they observed that the peptide shields the oligonucleotides from degradation providing increased serum stability.[41]

Whilst the previously mentioned work usually starts with known binders which are improved by multimerization, the combinatorial output of this technology lends itself to fragment pairing and preparation of large libraries. One example was published by Hili *et al.* where they developed a DNA-templated polymerisation of pentanucleotide-peptide conjugates in a sequence dependent manner (Fig. 2.C6).[42] The DNA template was translated into peptide-DNA polymers and selected for a biologically relevant target (His-tag peptide and cobalt beads were used as a model system). The selected members were then PCR amplified and regenerated to perform multiple cycles of selection akin to Darwinian evolution.[8] Our lab has also previously developed a DNA encoded library scaffold that can undergo multiple selection cycles.[43] Peptide loops prepared by PNA:DNA templated ligation can be displayed on a DNA template and assembled to display two loops in a multivalent fashion (Fig. 2.C7). The large library was selected against Programmed Death Ligand 1 (PD-L1), a relevant oncology target, and a bivalent binder with a K_D of 70 nM was discovered (versus 904 nM and 2,057 nM for the individual loops).

2.3 *Small Molecule-Based Assemblies*

DNA Encoded Libraries (DEL) that allow the preparation of large combinatorial libraries which can benefit from cooperative binding are not restricted to peptides and were initially exploited for the discovery of small molecule therapeutics. In 2004, Neri *et al.* developed Encoded Self-Assembling Chemical (ESAC) libraries,[44,45] based on a fragment pairing approach (Fig. 2.D1).[4] Initially, they performed affinity maturation on a known binder for both human serum albumin (HSA) and bovine carbonic anhydrase. Neri, Scheuermann, Zhang and Li have further developed the technology to prepare large combinatorial libraries of fragments displayed in multiple geometries.[46–48] Our lab has also developed multiple PNA-based DEL technologies for the pairing of small molecule fragments.[6] One such method is the pairing of PNA-fragment libraries on a DNA template which can be amplified post-selection for sequencing or performing multiple rounds of selection.[49] A 625,000-member library was screened against carbonic anhydrase and the best hit displayed 87 nM affinity versus 2.2 μM for the individual sulfonamide fragment. This technology has also been applied to a microarray workflow where the DNA template is present on a microarray and the protein selection is done on the array rather than by affinity pull down (Fig. 2.D2). We have applied this technique to a range of targets including HSP70 (chaperone protein),[50] PTP1B (phosphatase),[51] and mSin1 (pH domain).[52] Whilst very prominent in the DEL field, the multivalent display of small molecules is not limited to encoded libraries. Similarly to their multivalent display technology for integrin peptides (Fig. 2.C5), Appella and coworkers published DNA:PNA assemblies with small molecule ligands. They programmed assemblies of xanthine amine congeners (XAC) for antagonism of the GPCR A_{2A} adenosine receptor (A_{2A}AR),[53] followed by (±)-2-(*N*-phenethyl-*N*-propyl)amino-5-hydroxytetralin ((±)-PPHT) for agonism of the dopamine D_2 receptors (D_2R).[54] In both cases, activity improved with increase in valency, and theoretical models were developed, confirming the importance of binding receptor dimers of A_{2A}AR.

2.4 *Assemblies of Other Modalities*

Most of the nucleic acid based supramolecular assemblies have been developed to display glycan, peptides and small molecules. Nonetheless, a number of studies displaying other modalities also exist. The Schultz, Smider labs and ourselves, disclosed self-assembled antibody multimers (Fig. 2.E1).[55] Antibody Fab fragments were coupled to oligonucleotides *via* incorporation of *p*-acetylphenylalanine which can be reacted with aminooxy groups to form stable oxime adducts. Initially, the assemblies were trialled as dimers with DNA but showed poorer activity than the monomeric Fabs in a Her2 phosphorylation assay. By replacing the DNA (30-mer) with PNA (12-mer), which has a neutral backbone and higher Tm at shorter lengths, an increased activity upon dimerization was observed. A tetramer was also developed which displayed better activity than the full IgG antibody (trastuzumab). Heterodimers that target multiple proteins were also prepared (Her2-CD3 and CD20-CD3), these showed impressive cytotoxicity compared to the monomer alone.

Another example was reported by Fromme and coworkers who used PNA-tagged proteins for self-assembly on DNA nanocages.[56] They conjugated PNA (8-mer) to a protein *via* NHS and cysteine chemistry (using a SMCC) linker). They showed that the charge on the proteins tested (azurin (–) *vs* cytochrome *c* (+)) affects the repulsion with the DNA nanostructure. Assembling cytochrome *c* on the cage did not affect its catalytic activity or secondary structure and a mild decrease of redox potential was observed.

Another modality class that has been investigated for therapeutic supramolecular assembly is aptamers. Multiple examples of bivalent aptamers have been developed for thrombin,[57,58] a key protease in the blood coagulation pathway. These examples use covalent linkage between aptamers that bind to different binding sites on thrombin.

2.5 *In Vivo Efficacy of Multimeric Assemblies and Outlook*

The previous examples detail the large diversity of ligands that can be supramolecularly displayed on different scaffolds. However, with the exception of the c(RGDfK)-PNA-DNA scaffold developed by Appella *et al.* which was tested against an *in vivo* metathesis model, these supramolecular assemblies were not progressed to *in vivo* studies, let alone to clinical development. We will discuss two case studies which have broken this trend, interestingly, both are in the field of anticoagulation.

Recently, RNA-origami has been developed for the display of thrombin binding aptamers. The exosite I and exosite II binding RNA aptamers ($RNA_{R9D\text{-}14T}$ and Toggle-25t, respectively) were appended to the origami with two copies of each aptamer (Fig. 3.A). To increase the stability of the nanostructure, 2′-fluoromodified RNA was used resulting in storage stability of >3 months and stability in human plasma for >24 hours.[59] The authors also state that this modification decreases the immunological response.[60,61] The construct was tested in a range of *in vitro* and *in vivo* assays and showed efficient prolongation of clotting time in activated partial thromboplastin time (aPTT) assays (in human, porcine and murine plasma) and in a murine liver laceration model (1 mg/kg).[61]

Another example of a supramolecular assembly that functions *in vivo* is a bivalent PNA-peptide thrombin inhibitor.[62] Inspired by small flexible proteins from blood sucking insects that bridge multiple binding sites on thrombin, we developed a bivalent construct that binds cooperatively to the active site and exosite II (Fig. 3.B). The peptides were synthesised linked to PNA strands (8-mer) *via* SPPS. Individually, the exosite II peptide-PNA showed no inhibition (at 1 µM) and the active site peptide-PNA displayed a moderate K_i of 58.7 nM. When simply mixed in the same well, the supramolecular assembly inhibited thrombin with a K_i of 74 pM, an 800-fold improvement over the individual fragments. The PNA length could be decreased to 6- and 4-mer, but cooperativity was still observed at concentrations below the K_D of hybridisation. The best inhibitor was tested in an *in vivo* needle injury model and showed a similar decrease in fibrin intensity and thrombus size to Argatroban (approved drug) at 22 times lower dose.

2.6 *Drug Inactivation by Strand Displacement and Supramolecular Blocking*

As seen from the range of examples covered in this review, supramolecular assemblies tend to exhibit strong binding, whilst the individual components do not. Breaking the link between the ligands *via* strand displacement would result in a return to the monovalent system and therefore rapid reversal of activity. Whilst strand displacement is commonly used in nucleic acid systems, it has only been applied *in vivo* as an off-switch in the two anticoagulant systems mentioned before.

In the case of the RNA aptamer, the authors used a classic technique in aptamer chemistry where the complementary strand is used to block the aptamer binding interface and therefore restores thrombin activity (Fig. 3.A). PNA antidotes showed in-

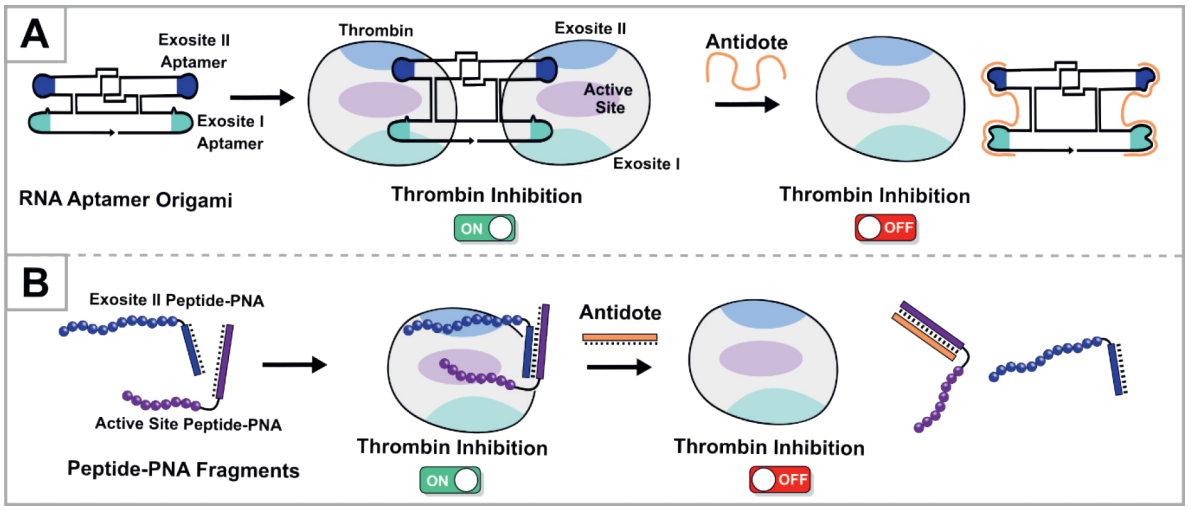

Fig. 3. **Supramolecular anticoagulants**. A) RNA Aptamers of exosite I and exosite II on thrombin, displayed on an RNA origami. The inhibition can be reversed by adding the aptamer complementary strand. B) Bivalent thrombin inhibitor composed of an active-site peptide-PNA and an exosite II peptide-PNA which cooperatively inhibit blood clotting. The inhibition can be reversed by the simple addition of a PNA antidote which breaks the PNA interaction of the drug *via* toe-hold strand displacement.

creased reversal, but the authors claim that DNA antidotes are preferred for clinical development as the synthesis of long DNA chains remains more accessible.[60,61] The DNA antidote showed rapid reversal in aPTT assays, as well as a murine liver laceration model (10 equivalents of antidote).[62]

In the case of our bivalent thrombin inhibitor, the activity is reliant on the hybridisation of the two fragments. A 4-mer toehold is included in the PNA of the active-site fragment which allows for strand displacement using a simple 12-mer PNA antidote (Fig. 3.B). The antidote reversal was also tested in the needle injury model and showed similar thrombus size and fibrin intensity to the untreated control (at 5 equivalents of antidote). There is a clear case for rapid reversal of action for anticoagulants, but it is not the only indication that would require such a property. Patients under immuno-suppression (*e.g.* cancer, autoimmune disorders, organ transplant) are at risk of infection or CAR-T therapy patients, at risk of a cytokine storm, would require rapid reversal, but no antidotes exist. Multimerization of ligands and their reversal is a simple strategy with a wealth of scaffolds to inspire the next generation drugs with on-demand activity.

3. Alternatives to Dimerization for Gain of Function

3.1 *Activation Through Hybridisation-Induced Folding*

Drug multimerization is not the only method to turn on a therapeutic effect. Our lab has previously disclosed a PNA-peptide system where the hybridisation of the PNA folds the peptide into the active form (Fig. 4.A).[63] Inspired by phosphatase enzymes, a library of 1,000 PNA-peptides were prepared on beads and incubated with a phosphate-caged-fluorophore (quinazolinone precipitating dye (QPD) – $OPO3^{2-}$). The fluorescent beads, which successfully uncaged QPD-$OPO3^{2-}$ to QPD-OH, were picked individually and the peptide was deconvoluted by mass (MALDI). A selected peptide had 25-fold increased phosphate hydrolysis activity when folded *vs* non folded. Since the 'on' is dependent on nucleic acid hybridisation, the activity can easily be switched on and off using strand displacements.

3.2 *Drug Uncaging by Templated Reaction*

DNA circuitry has been used in previous examples to display ligands and reverse activity. It has also been developed for systems responding to specific cues.[3]

Our lab previously developed a DNA-templated release of functional molecules in response to a specific oligonucleotide.[64] Two PNA strands, complementary to a template DNA, were tagged with a phosphine group (TCEP) and a *p*-azidobenzyl linker attached to a functional molecule. Upon PNA dimerization on the DNA template (microRNA21 (miR21) sequence), a templated Staudinger reaction reduced the azide, cleaving the linker to release a functional molecule (Fig. 4.B1). The system was tested with a fluorophore (rhodamine methyl urea) to monitor release, and two therapeutically relevant molecules (estradiol, a transcription factor activator, and the cytotoxic molecule doxorubicin). All three molecules showed fast kinetics of release upon azide reduction (<21 minutes) and the release was conditionoal to the correct DNA sequence (compared to mismatched, random and no DNA). The templated Staudinger reaction has more recently been applied to a hairpin-chain reaction system.[65] Similar to the previous example, a *p*-azidobenzyl linker attached to a functional molecule was incorporated into a hybridization chain reaction (HCR) hairpin, whilst a phosphine was incorporated on the other hairpin. Upon miR21 detection, an HCR circuit was initiated which in turn induced molecule release. Disulfide exchanges ensuring an HCR was also investigated to uncage bioactive molecules.[66]

Other templated chemistries have been developed, including ruthenium-mediated photocatalytic reduction of an immolative pyridinium linker.[67] This system has been incorporated into a bioluminescence resonance energy transfer (BRET) sensor due to the spectral overlap of emission of nanoluciferase (NLuc) and the excitation spectrum of the ruthenium complex. The full sensor, termed Luciferase-based Photocatalysis Induced *via* Nucleic acid template (LUPIN), is 'on' in the absence of free methotrexate but 'off' in the presence of methotrexate.[68] In the 'on' state, NLuc and the ruthenium are in close proximity, resulting in BRET followed by cleavage of a PNA-pyridinium-prodrug to release the active molecule (Fig. 4.B2). The short PNA strand incorporated into the sensor and into the effector molecule allows for multiple turnover cycles. Our lab has used this templated reaction in multiple other settings, including a DNA-templated quadratic amplification system, with two hairpin-ruthenium strands that open upon DNA template binding with a toe-hold that binds to PNA-pyridinium-prodrug releasing the drug (anticancer drug 5-fluorouracil (5-FU)) (Fig. 4.B3).[69] Another example of this chemistry is the development of a molecular network that releases the effector molecule upon receptor dimerization (Fig. 4.B4).[70]

Whilst our lab has mainly focused on the release of known molecules, the Seitz lab 'uncages' their molecules by ligating two fragments to reconstitute the active compound or by reducing a functional group to recover activity. The first technique includes an RNA templated native chemical ligation (NCL) reaction with DNA/PNA-tagged drug fragments to reconstitute benzanilide containing drugs (*e.g.* ponatinib and GZD824, which are both kinase inhibitors).[71] However, the employed chemistry is not traceless, leaving behind a thiol which is not present in the commercial drugs (Fig. 4.B5). This modification did not impact Ponatinib or GZD824 but increased the K_D of nilotinib for Abl1 kinase by 225-fold. A second technique used by Seitz and coworkers is the caging of a Smac (second mitochondria-derived activator of caspases) peptidomimetic compound (SMC) by swapping the N-terminus amine for an N-terminus azide.[72] The compound was tagged with a PNA and a second PNA was prepared containing a reducing agent (either phosphine for Staudinger reaction, or a ruthenium complex for photoreduction). Upon RNA sensing, the two PNA strands bring the reactive groups into close proximity to reduce the azide, resulting in the active drug. Whilst the system was effective *in vitro*, its implementation in a cell-based assay is limited by the poor cellular permeability of the constructs. More recently, the Inverse Electron Demand Diels Alder Reaction (IEDDA) has been exploited by Deiters and coworkers for a range of DNA logic gates.[73] They developed OR, AND, and an OR-AND circuit which uncages a fluorophore as an output adding to prior work levering energy transfer between a sensitiser and photolabile group.[74]

3.3 *Drug Release Through Strand Displacement*

Whilst DNA circuits typically rely on toehold displacements or templated reactions to generate a functional output, the lab of Jayawickramarajah have developed a Host-Guest driven strand displacement. Firstly, they used a split ATP binding aptamer where one strand is linked to cucurbituril(7) (CB7), a host moiety which binds an adamantane-drug and shields it from protein binding.[75] The other strand contains an adamantane headgroup. Upon ATP binding the aptamer is formed, bringing the DNA-cucurbituril and DNA-adamantane into close proximity. This interaction increases the effective molarity of the adamantane which therefore releases the adamantane drug (a carbonic anhydrase II inhibitor in this case) (Fig. 4.C1). This technique was further developed to respond to different cues, rather than only ATP.[76] Inspired by base pair toehold-mediated strand displacement (BP-TMSD), they replaced the DNA toehold with a host-guest driven toehold-mediated strand displacement (HG-TMSD) system (Fig. 4.C2).[76] They applied the technology to a more complex system for the detection of microRNA. Host–guest complexes, particu-

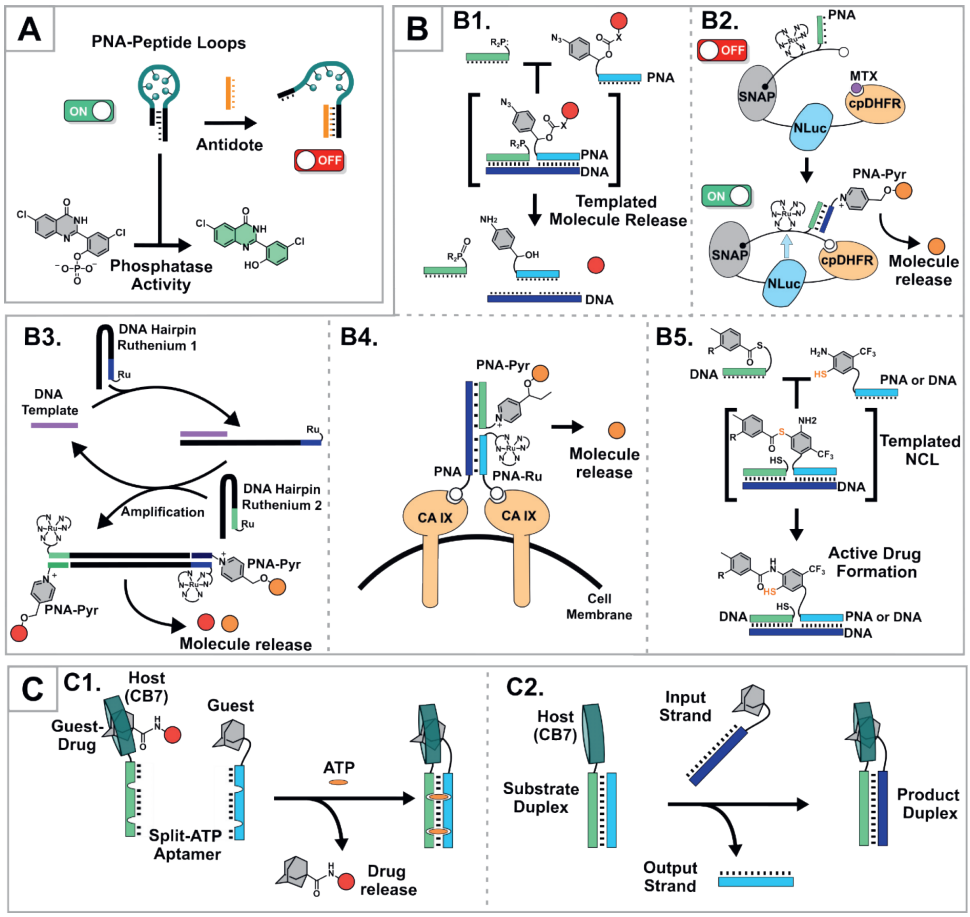

Fig. 4. **Alternatives to multimerization for gain of function**. A) **Hybridisation induced activation**. PNA peptide loops that display phosphatase activity upon folding. B) **Drug uncaging through templated reaction**. (1) DNA:PNA templated Staudinger reaction for functional molecule release. (2) LUPIN sensor for release of functional molecules. (3) A quadratic amplification system using HCR-Ruthenium strands for catalytic cleavage of functional molecules. (4) Dimerization of membrane proteins leading to functional molecule release. (5) PNA:DNA templated NCL for the formation of functional molecules. C) **Host-guest strand displacement**. (1) ATP sensor resulting in release of a functional molecule. (2) Host-guest toehold mediated strand displacement.

larly those involving CB7, are of significant interest due to their high affinity for guest molecules in aqueous environments, making them promising candidates for drug delivery and biomedicine applications. However, challenges remain in achieving guest release using biological triggers.

4. Limitation of DNA Assemblies *In Vivo*

This review has covered a wealth of supramolecular assemblies that dimerise, fold, and uncage ligands for a functional output. However, their application *in vivo* remains limited to a handful of examples. Many of the examples mentioned rely on DNA as a template which limits their applicability in complex systems. Nonetheless, the field of DNA nanotechnology has provided successful *in vivo* examples but also important lessons to be learnt. Whilst DNA origamis are sensitive to the environment (ionic strength) and nucleases,[77] modifications on the backbone,[59] encapsulation,[78] or special coatings can overcome these limitations.[79] Their limitations and applications *in vivo* have been extensively reviewed.[80,81]

Ryu, Shih and coworkers used DNA origami to display CpG oligonucleotides in a multitude of organisms to probe the best display as a vaccine adjuvant.[82] Other success stories of DNA nanotechnologies *in vivo* include the use of DNA nanorobots to deliver payloads. Church and co-workers loaded antibody fragments into their nanorobot which released the cargo upon 'key' antigen binding (based on aptamer 'locks').[83] Another example *in vivo* is the release of thrombin from the interior of a tubular nanorobot upon nucleolin sensing, resulting in inhibition of tumour growth in mice.[84]

Advances have been made in the world of DNA to improve the function of DNA or RNA-based assemblies in biological systems, as mentioned previously. An alternative to the modifications mentioned above is exchanging DNA or RNA for non-natural oligonucleotides. PNA, as seen from the wealth of examples presented in this review, is a favoured alternative. Following the discovery of PNA, their metabolism and *in vivo* distribution were evaluated in mice using PNA radio nucleic labelling,[85] showing rapid clearance, (50% eliminated in about 2 hours) with more than 99% of the PNA cleared after 24 hours; however, the PNA were retained on beads with a complementary sequence implanted subcutaneously, demonstrating the PNAs remained functional. More recent studies with fluorescent probes concur these findings.[86] While much effort has been invested in translational application of PNA's antisense activity, their inability to cross cellular membranes precludes meaningful activity as illustrated in a recent study aiming at splice correction,[87] unless specifically modified and formulated.[88] This has made them an attractive choice for radio nuclei delivery with pre-targeting of affinity proteins,[89–91] and *in vivo* diagnostic analysis of circulating DNA,[86] where the rapid clearance of unhybridized PNA is an asset rather than a liability.

5. Conclusions and Outlook

Supramolecular assemblies represent a promising platform for the development of intelligent therapeutics that exploit the principles

of cooperativity and proximity-induced reactions. These assemblies, enabled by the programmability of hybridization, can be dynamically formed, and deactivated on-demand through strand displacement, offering precise control over reaction kinetics and equilibria. Notably, strand displacement reactions exhibit rate constants as high as $10^6\ M^{-1}s^{-1}$, surpassing the kinetics of current bioorthogonal chemistries and rivalling with the rates of antibody-ligand interactions. The incorporation of multivalency through programmed assembly provides a straightforward approach to modulate therapeutic activity. Numerous examples highlighted in this review demonstrate significant progress in enhancing binding affinities and creating responsive systems across various oligonucleotide platforms. Despite these advancements, translating sophisticated laboratory designs into practical *in vivo* applications has been achieved in only a few examples. A first-in-class therapeutic with on-demand control of activity would establish a critical milestone in the field. The development of antibody-drug conjugates, antisense oligonucleotides, and synthetic peptides exceeding 30 residues underscores that the synthetic complexity is not an insurmountable barrier. The clinical success of these modalities has inspired a wave of novel candidates. The initial applications of self-assembled, responsive drugs are likely to target extracellular pathways, circumventing the challenges associated with intracellular delivery of oligonucleotide conjugates. While this strategy narrows the repertoire of accessible targets, it simultaneously reduces complications related to liver or cellular metabolism. Additionally, a smaller pool of accessible targets minimizes the risk of off-target effects, enhancing therapeutic safety. High-affinity and efficacious bidentate assemblies allow for the use of low drug concentrations, further mitigating the likelihood of off-target toxicity. However, the development of multi-component therapeutics necessitates individual evaluation of each component, potentially increasing development costs. Nevertheless, this challenge is comparable to the evaluation of metabolites in small-molecule drug development and should not be considered a prohibitive barrier. While there is no absorption, distribution, metabolism and excretion (ADME) data on such supramolecular assemblies, unmodified oligonucleotides typically have short circulation times and are renally cleared. Including a moiety for albumin binding should enable long circulation times for injectable therapeutics.

While the field of supramolecular therapeutics remains in its early stages, ongoing research into programmed assembly strategies holds immense potential to establish a novel therapeutic modality. We hope that the precedents and concepts presented in this review will inspire further advancements and innovation in this promising area of drug development. As illustrated with anticoagulants, an appealing application of the technology is for targets where adverse effects greatly benefit from an antidote.

Acknowledgements

We thank our collaborators who have contributed to our work in the area of responsive self-assemblies as well as the funding agencies (Swiss National Science Foundation, NCCR Chemical Biology, ERC, and HFSP).

Received: January 7, 2025

[1] P. Yin, H. M. T. Choi, C. R. Calvert, N. A. Pierce, *Nature* **2008**, *451*, 318, https://doi.org/10.1038/nature06451.
[2] D. Y. Zhang, G. Seelig, *Nat. Chem.* **2011**, *3*, 103, https://doi.org/10.1038/Nchem.957.
[3] E. E. Watson, S. Angerani, P. M. Sabale, N. Winssinger, *J. Am. Chem. Soc.* **2021**, *143*, 4467, https://doi.org/10.1021/jacs.0c12970.
[4] J. Scheuermann, D. Neri, *Curr. Opin. Chem. Biol.* **2015**, *26*, 99, https://doi.org/10.1016/j.cbpa.2015.02.021.
[5] M. Wichert, N. Krall, W. Decurtins, R. M. Franzini, F. Pretto, P. Schneider, D. Neri, J. Scheuermann, *Nat. Chem.* **2015**, *7*, 241, https://doi.org/10.1038/Nchem.2158.
[6] C. Zambaldo, S. Barluenga, N. Winssinger, *Curr. Opin. Chem. Biol.* **2015**, *26*, 8, https://doi.org/10.1016/j.cbpa.2015.01.005.
[7] F. V. Reddavide, M. Cui, W. Lin, N. Fu, S. Heiden, H. Andrade, M. Thompson, Y. Zhang, *Chem. Commun.* **2019**, *55*, 3753, https://doi.org/10.1039/C9CC01429B.
[8] M. Dockerill, N. Winssinger, *Angew. Chem. Int. Ed.* **2023**, *62*, e202215542, https://doi.org/10.1002/anie.202215542.
[9] Y. Zhou, W. Y. Shen, Y. Gao, J. Z. Peng, Q. R. Li, X. Y. Wei, S. H. Liu, F. S. Lam, J. Mayol-Llinàs, G. X. Zhao, G. Li, Y. Z. Li, H. Z. Sun, Y. Cao, X. Y. Li, *Nat. Chem.* **2024**, *16*, 543, https://doi.org/10.1038/s41557-024-01442-y.
[10] N. A. Lind, V. E. Rael, K. Pestal, B. Liu, G. M. Barton, *Nat. Rev. Immunol.* **2022**, *22*, 224, https://doi.org/10.1038/s41577-021-00577-0.
[11] C. Fasting, C. A. Schalley, M. Weber, O. Seitz, S. Hecht, B. Koksch, J. Dernedde, C. Graf, E. W. Knapp, R. Haag, *Angew. Chem. Int. Ed.* **2012**, *51*, 10472, https://doi.org/10.1002/anie.201201114.
[12] J. H. Rao, J. Lahiri, L. Isaacs, R. M. Weis, G. M. Whitesides, *Science* **1998**, *280*, 708, https://doi.org/10.1126/science.280.5364.708.
[13] T. MacCulloch, A. Buchberger, N. Stephanopoulos, *Org. Biomol. Chem.* **2019**, *17*, 1668, https://doi.org/10.1039/c8ob02436g.
[14] S. B. Yeldell, O. Seitz, *Chem. Soc. Rev.* **2020**, *49*, 6848, https://doi.org/10.1039/d0cs00518e.
[15] A. Novacek, B. Ugaz, N. Stephanopoulos, *Biomacromolecules* **2024**, *25*, 3865, https://doi.org/10.1021/acs.biomac.4c00372.
[16] P. H. Hagedorn, R. Persson, E. D. Funder, N. Albæk, S. L. Diemer, D. J. Hansen, M. R. Møller, N. Papargyri, H. Christiansen, B. R. Hansen, H. F. Hansen, M. A. Jensen, T. Koch, *Drug Discovery Today* **2018**, *23*, 101, https://doi.org/10.1016/j.drudis.2017.09.018.
[17] S. Barluenga, N. Winssinger, *Acc. Chem. Res.* **2015**, *48*, 1319, https://doi.org/10.1021/acs.accounts.5b00109.
[18] J. Saarbach, P. M. Sabale, N. Winssinger, *Curr. Opin. Chem. Biol.* **2019**, *52*, 112, https://doi.org/10.1016/j.cbpa.2019.06.006.
[19] L. M. Carson, E. E. Watson, *ChemPlusChem* **2024**, *89*, e202400305, https://doi.org/10.1002/cplu.202400305.
[20] K. Matsuura, M. Hibino, Y. Yamada, K. Kobayashi, J. *Am. Chem. Soc.* **2001**, *123*, 357, https://doi.org/10.1021/ja001945j.
[21] K. Gorska, K. T. Huang, O. Chaloin, N. Winssinger, *Angew. Chem. Int. Ed.* **2009**, *48*, 7695, https://doi.org/10.1002/anie.200903328.
[22] M. Ciobanu, K. T. Huang, J. P. Daguer, S. Barluenga, O. Chaloin, E. Schaeffer, C. G. Mueller, D. A. Mitchell, N. Winssinger, *Chem. Commun.* **2011**, *47*, 9321, https://doi.org/10.1039/c1cc13213j.
[23] C. Scheibe, A. Bujotzek, J. Dernedde, M. Weber, O. Seitz, *Chem. Sci.* **2011**, *2*, 770, https://doi.org/10.1039/C0SC00565G.
[24] V. Bandlow, S. Liese, D. Lauster, K. Ludwig, R. R. Netz, A. Herrmann, O. Seitz, *J. Am. Chem. Soc.* **2017**, *139*, 16389, https://doi.org/10.1021/jacs.7b09967.
[25] M. Matsui, Y. Ebara, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6139, https://doi.org/10.1016/j.bmcl.2012.08.028.
[26] M. Yamabe, K. Kaihatsu, Y. Ebara, *Bioconjugate Chem.* **2018**, *29*, 1490, https://doi.org/10.1021/acs.bioconjchem.8b00045.
[27] T. Machida, A. Novoa, E. Gillon, S. S. Zheng, J. Claudinon, T. Eierhoff, A. Imberty, W. Römer, N. Winssinger, *Angew. Chem. Int. Ed.* **2017**, *56*, 6762, https://doi.org/10.1002/anie.201700813.
[28] L. Farrera-Soler, J. P. Daguer, P. Raunft, S. Barluenga, A. Imberty, N. Winssinger, *Bioorg. Med. Chem.* **2020**, *28,* 115458, https://doi.org/10.1016/j.bmc.2020.115458.
[29] N. Stephanopoulos, *Bioconjugate Chem.* **2019**, *30*, 1915, https://doi.org/10.1021/acs.bioconjchem.9b00259.
[30] D. M. Tagore, K. I. Sprinz, S. Fletcher, J. Jayawickramarajah, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2007**, *46*, 223, https://doi.org/10.1002/anie.200603479.
[31] B. A. Rosenzweig, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2009**, *48*, 2749, https://doi.org/10.1002/anie.200804849.
[32] C. G. Lou, N. J. Christensen, M. C. Martos-Maldonado, S. R. Midtgaard, M. Ejlersen, P. W. Thulstrup, K. K. Sorensen, K. J. Jensen, J. Wengel, *Chem. Eur. J.* **2017**, *23*, 9297, https://doi.org/10.1002/chem.201700971.
[33] a) M. Imiolek, N. Winssinger, *ChemBioChem* **2023**, *24*, https://doi.org/10.1002/cbic.202200561; b) B. Brennecke, B. Civili, P. M. Sabale, S. Barluenga, B. Meyer, N. Winssinger, *Proc. Natl. Acad. Sci. U. S. A.* **2025**, *122*, e2412850122, https://doi.org/10.1073/pnas.2412850122.
[34] F. Z. Zhao, M. Frandsen, S. Capodaglio, H. F. Sleiman, *J. Am. Chem. Soc.* **2024**, *146*, 1946, https://doi.org/10.1021/jacs.3c08984.
[35] B. A. R. Williams, C. W. Diehnelt, P. Belcher, M. Greving, N. W. Woodbury, S. A. Johnston, J. C. Chaput, J. *Am. Chem. Soc.* **2009**, *131*, 17233, https://doi.org/10.1021/ja9051735.
[36] K. Gorska, J. Beyrath, S. Fournel, G. Guichard, N. Winssinger, *Chem. Commun.* **2010**, *46*, 7742, https://doi.org/10.1039/c0cc02852e.
[37] H. Eberhard, F. Diezmann, O. Seitz, *Angew. Chem. Int. Ed.* **2011**, *50*, 4146, https://doi.org/10.1002/anie.201007593.
[38] V. Bandlow, D. Lauster, K. Ludwig, M. Hilsch, V. Reiter-Scherer, J. P. Rabe, C. Böttcher, A. Herrmann, O. Seitz, *ChemBioChem* **2019**, *20*, 159, https://doi.org/10.1002/cbic.201800643.

[39] E. A. Englund, D. Y. Wang, H. Fujigaki, H. Sakai, C. M. Micklitsch, R. Ghirlando, G. Martin-Manso, M. L. Pendrak, D. D. Roberts, S. R. Durell, D. H. Appella, *Nat. Commun*. **2012**, *3*, 614, https://doi.org/10.1038/ncomms1629.
[40] R. Freeman, N. Stephanopoulos, Z. Alvarez, J. A. Lewis, S. Sur, C. M. Serrano, J. Boekhoven, S. S. Lee, S. I. Stupp, *Nat. Commun.* **2017**, *8*, 15982, https://doi.org/10.1038/ncomms15982.
[41] I. K. Astakhova, L. H. Hansen, B. Vester, J. Wengel, *Org. Biomol. Chem*. **2013**, *11*, 4240, https://doi.org/10.1039/c3ob40786a.
[42] C. Guo, C. P. Watkins, R. Hili, *J. Am. Chem. Soc*. **2015**, *137*, 11191, https://doi.org/10.1021/jacs.5b07675.
[43] B. R. Vummidi, L. Farrera-Soler, J. P. Daguer, M. Dockerill, S. Barluenga, N. Winssinger, *Nat. Chem*. **2022**, *14*, 141, https://doi.org/10.1038/s41557-021-00829-5.
[44] S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, *Nat. Biotechnol*. **2004**, *22*, 568, https://doi.org/10.1038/nbt961.
[45] S. Melkko, C. E. Dumelin, J. Scheuermann, D. Neri, *Chem. Biol*. **2006**, *13*, 225, https://doi.org/10.1016/j.chembiol.2005.12.006.
[46] Y. Zhou, C. Li, J. Peng, L. Xie, L. Meng, Q. Li, J. Zhang, X. D. Li, X. Li, X. Huang, X. Li, J. *Am. Chem. Soc*. **2018**, *140*, 15859, https://doi.org/10.1021/jacs.8b09277.
[47] a) S. Oehler, L. Plais, G. Bassi, D. Neri, J. Scheuermann, *Chem. Commun*. **2021**, *57*, 12289, https://doi.org/10.1039/D1CC04306D; b) L. Plais, A. Lessing, M. Keller, A. Martinelli, S. Oehler, G. Bassi, D. Neri, J. Scheuermann, *Chem. Sci.* **2022**, *13*, 967, https://doi.org/10.1039/DISC05721A.
[48] M. Y. Cui, D. Nguyen, M. P. Gailez, S. Heiden, W. L. Lin, M. Thompson, F. V. Reddavide, Q. C. Chen, Y. X. Zhang, *Nat. Commun.* **2023**, *14*, 1481, https://doi.org/10.1038/s41476-023-37071-1.
[49] J. P. Daguer, M. Ciobanu, S. Alvarez, S. Barluenga, N. Winssinger, *Chem. Sci*. **2011**, *2*, 625, https://doi.org/10.1039/c0sc00574f.
[50] J. P. Daguer, C. Zambaldo, M. Ciobanu, P. Morieux, S. Barluenga, N. Winssinger, *Chem. Sci*. **2015**, *6*, 739, https://doi.org/10.1039/c4sc01654h.
[51] S. Barluenga, C. Zambaldo, H. A. Ioannidou, M. Ciobanu, P. Morieux, J.-P. Daguer, N. Winssinger, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1080, https://doi.org/10.1016/j.bmcl.2015.11.102.
[52] A. Gonse, J. Gajić, J.-P. Daguer, S. Barluenga, R. Loewith, N. Winssinger, *ACS Chem. Biol*. **2024**, *19*, 2502, https://doi.org/10.1021/acschembio.4c00597.
[53] A. V. Dix, S. M. Moss, K. Phan, T. Hoppe, S. Paoletta, E. Kozma, Z. G. Gao, S. R. Durell, K. A. Jacobson, D. H. Appella, *J. Am. Chem. Soc.* **2014**, *136*, 12296, https://doi.org/10.1021/ja504288s.
[54] A. V. Dix, J. L. Conroy, K. M. G. Rosenker, D. R. Sibley, D. H. Appella, *ACS Med. Chem. Lett*. **2015**, *6*, 425, https://doi.org/10.1021/ml500478m.
[55] S. A. Kazane, J. Y. Axup, C. H. Kim, M. Ciobanu, E. D. Wold, S. Barluenga, B. A. Hutchins, P. G. Schultz, N. Winssinger, V. V. Smider, *J. Am. Chem. Soc*. **2013**, *135*, 340, https://doi.org/10.1021/ja309505c.
[56] J. D. Flory, C. R. Simmons, S. Lin, T. Johnson, A. Andreoni, J. Zook, G. Ghirlanda, Y. Liu, H. Yan, P. Fromme, *J. Am. Chem. Soc.* **2014**, *136*, 8283, https://doi.org/10.1021/ja501228c.
[57] Y. Kim, Z. Cao, W. Tan, *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 5664, https://doi.org/10.1073/pnas.0711803105.
[58] H. Hasegawa, K.-i. Taira, K. Sode, K. Ikebukuro, *Sensors* **2008**, *8*, 1090, https://doi.org/10.3390/s8021090.
[59] A. Krissanaprasit, C. Key, M. Fergione, K. Froehlich, S. Pontula, M. Hart, P. Carriel, J. Kjems, E. S. Andersen, T. H. LaBean, *Adv. Mater*. **2019**, *31*, 1808262, https://doi.org/10.1002/adma.201808262.
[60] A. Krissanaprasit, C. M. Key, K. Froehlich, S. Pontula, E. Mihalko, D. M. Dupont, E. S. Andersen, J. Kjems, A. C. Brown, T. H. LaBean, *Adv. Healthcare Mater*. **2021**, *10*, 2001826, https://doi.org/10.1002/adhm.202001826.
[61] A. Krissanaprasit, E. Mihalko, K. Meinhold, A. Simpson, J. Sollinger, S. Pandit, D. M. Dupont, J. Kjems, A. C. Brown, T. H. LaBean, *Mol. Ther*. **2024**, *32*, 2286, https://doi.org/10.1016/j.ymthe.2024.05.002.
[62] M. Dockerill, D. J. Ford, S. Angerani, I. Alwis, L. J. Dowman, J. Ripoll-Rozada, R. E. Smythe, J. S. T. Liu, P. J. B. Pereira, S. P. Jackson, R. J. Payne, N. Winssinger, *Nat. Biotechnol*. **2024**, https://doi.org/10.1038/s41587-024-02209-z.
[63] T. Machida, S. Dutt, N. Winssinger, *Angew. Chem. Int. Ed*. **2016**, *55*, 8595, https://doi.org/10.1002/anie.201602751.
[64] K. Gorska, A. Manicardi, S. Barluenga, N. Winssinger, *Chem. Commun*. **2011**, *47*, 4364, https://doi.org/10.1039/c1cc10222b.
[65] K. Morihiro, Y. Tomida, D. Fukui, M. Hasegawa, A. Okamoto, *Angew. Chem. Int. Ed.* **2023**, *62*, https://doi.org/10.1002/anie.202306587.
[66] A. L. Prinzen, D. Saliba, C. Hennecker, T. Trinh, A. Mittermaier, H. F. Sleiman, *Angew. Chem. Int. Ed*. **2020**, *59*, 12900, https://doi.org/10.1002/anie.202001123.
[67] a) D. Chang, E. Lindberg, N. Winssinger, *J. Am. Chem. Soc*. **2017**, *139*, 1444, https://doi.org/10.1021/jacs.6b12764; b) L. Holtzer, I. Oleinich, M. Anzola, E. Lindberg, K. K. Sadhu, M. Gonzalez-Gaitan, N. Winssinger, *ACS Cent. Sci,* **2016**, *2*, 394, https://doi.org/10.1021/acscentsci.6b00054.
[68] E. Lindberg, S. Angerani, M. Anzola, N. Winssinger, *Nat. Commun*. **2018**, *9*, 3539, https://doi.org/10.1038/s41467-018-05916-9.
[69] K. T. Kim, S. Angerani, D. L. Chang, N. Winssinger, *J. Am. Chem. Soc*. **2019**, *141*, 16288, https://doi.org/10.1021/jacs.9b05688.
[70] S. Angerani, N. Winssinger, *J. Am. Chem. Soc*. **2020**, *142*, 12333, https://doi.org/10.1021/jacs.0c04469.
[71] R. Houska, M. B. Stutz, O. Seitz, *Chem. Sci*. **2021**, *12*, 13450, https://doi.org/10.1039/d1sc00513h.
[72] Y. Altrichter, P. Bou-Dib, C. Kuznia, O. Seitz, *J. Pept. Sci*. **2023**, *29,* e3477, https://doi.org/10.1002/psc.3477.
[73] C. Emanuelson, A. Bardhan, A. Deiters, *ACS Synth. Biol*. **2024**, *13*, 538, https://doi.org/10.1021/acssynbio.3c00474.
[74] M. Röthlingshöfer, K. Gorska, N. Winssinger, *J. Am. Chem. Soc*. **2011**, *133*, 18110, https://doi.org/10.1021/ja2086504.
[75] X. Zhou, X. Y. Su, P. Pathak, R. Vik, B. Vinciguerra, L. Isaacs, J. Jayawickramarajah, *J. Am. Chem. Soc*. **2017**, *139*, 13916, https://doi.org/10.1021/jacs.7b07977.
[76] D. V. D. W. Kankanamalage, J. H. T. Tran, N. Beltrami, K. Meng, X. Zhou, P. Pathak, L. Isaacs, A. L. Burin, M. F. Ali, J. Jayawickramarajah, *J. Am. Chem. Soc*. **2022**, *144*, 16502, https://doi.org/10.1021/jacs.2c05726.
[77] J. Hahn, S. F. J. Wickham, W. M. Shih, S. D. Perrault, *ACS Nano* **2014**, *8*, 8765, https://doi.org/10.1021/nn503513p.
[78] S. D. Perrault, W. M. Shih, *ACS Nano* **2014**, *8*, 5132, https://doi.org/10.1021/nn5011914.
[79] N. Ponnuswamy, M. M. C. Bastings, B. Nathwani, J. H. Ryu, L. Y. T. Chou, M. Vinther, W. A. Li, F. M. Anastassacos, D. J. Mooney, W. M. Shih, *Nat. Commun*. **2017**, *8*, 15654, https://doi.org/10.1038/ncomms15654.
[80] S. Alexander, M. G. Moghadam, M. Rothenbroker, L. Y.T. Chou, *Adv. Drug Delivery Rev*. **2023**, *199*, 114898, https://doi.org/10.1016/j.addr.2023.114898.
[81] Y. Xiao, Z. Liang, M. Shyngys, A. Baekova, S. Cheung, M. B. Muljadi, Q. Bai, L. Zeng, C. H. J. Choi, *Adv. Mat*er. **2025**, *37*, 2314232, https://doi.org/10.1002/adma.202314232.
[82] Y. C. Zeng, O. J. Young, C. M. Wintersinger, F. M. Anastassacos, J. I. MacDonald, G. Isinelli, M. O. Dellacherie, M. Sobral, H. Q. Bai, A. R. Graveline, A. Vernet, M. Sanchez, K. Mulligan, Y. Choi, T. C. Ferrante, D. B. Keskin, G. G. Fell, D. Neuberg, C. J. Wu, D. J. Mooney, I. C. Kwon, J. H. Ryu, W. M. Shih, *Nat. Nanotechnol*. **2024**, *19*, https://doi.org/10.1038/s41565-024-01615-3.
[83] S. M. Douglas, I. Bachelet, G. M. Church, *Science* **2012**, *335*, 831, https://doi.org/10.1126/science.1214081.
[84] S. P. Li, Q. Jiang, S. L. Liu, Y. L. Zhang, Y. H. Tian, C. Song, J. Wang, Y. G. Zou, G. J. Anderson, J. Y. Han, Y. Chang, Y. Liu, C. Zhang, L. Chen, G. B. Zhou, G. J. Nie, H. Yan, B. Q. Ding, Y. L. Zhao, *Nat. Biotechnol*. **2018**, *36*, 258, https://doi.org/10.1038/nbt.4071.
[85] G. Mardirossian, K. Lei, M. Rusckowski, F. Chang, T. Qu, M. Egholm, D. J. Hnatowich, *J. Nucl. Med*. **1997**, *38*, 907.
[86] Z. C. Xiang, J. H. Lu, Y. Ming, W. S. Guo, X. Y. Chen, W. J. Sun, *Adv. Sci.* **2024**, *11*, 2310225, https://doi.org/10.1002/advs.202310225.
[87] C. Brolin, E. W. K. Lim, S. Grizot, C. H. Olsen, N. Yavari, T. O. Krag, P. E. Nielsen, *Nucleic Acid Ther*. **2021**, *31*, 208, https://doi.org/10.1089/nat.2020.0856.
[88] E. Quijano, R. Bahal, A. Ricciardi, W. M. Saltzman, P. M. Glazer, *Yale J. Biol. Med*. **2017**, *90*, 583.
[89] H. Honarvar, K. Westerlund, M. Altai, M. Sandström, A. Orlova, V. Tolmachev, A. E. Karlström, *Theranostics* **2016**, *6*, 93, https://doi.org/ 10.7150/thno.12766.
[90] A. Leonidova, C. Foerster, K. Zarschler, M. Schubert, H. J. Pietzsch, J. Steinbach, R. Bergmann, N. Metzler-Nolte, H. Stephan, G. Gasser, *Chem. Sci.* **2015**, *6*, 5601, https://doi.org/10.1039/c5sc00951k.
[91] J. X. Yan, P. Zhao, Y. Y. Li, J. Wang, X. Yang, H. B. Li, L. A. Zhuo, W. Liao, W. Q. Fan, Y. D. Jia, H. Y. Wei, Y. Chen, *Nucl. Med.: Commun*. **2024**, *45*, 901, https://doi.org/10.1097/Mnm.0000000000001877.

License and Terms

The definitive version of this article is the electronic one that can be found at https://doi.org/10.2533/chimia.2025.128