# PNA as a Biosupramolecular Tag for Programmable Assemblies and Reactions

Sofia Barluenga and Nicolas Winssinger*

Department of Organic Chemistry, NCCR Chemical Biology, University of Geneva, 30 quai Ernest Ansermet, Geneva, Switzerland

CONSPECTUS: The programmability of oligonucleotide hybridization offers an attractive platform for the design of assemblies with emergent properties or functions. Developments in DNA nanotechnologies have transformed our thinking about the applications of nucleic acids. Progress from designed assemblies to functional outputs will continue to benefit from functionalities added to the nucleic acids that can participate in reactions or interactions beyond hybridization. In that respect, peptide nucleic acids (PNAs) are interesting because they combine the hybridization properties of DNA with the modularity of peptides. In fact, PNAs form more stable duplexes with DNA or RNA than the corresponding natural homoduplexes. The high stability achieved with shorter oligomers (an 8-mer is sufficient for a stable duplex at room temperature) typically results in very high sequence fidelity in the hybridization with negligible impact of the ionic strength of the buffer due to the lack of electrostatic repulsion between the duplex strands. The simple peptidic backbone of PNA has been shown to be tolerant of modifications with substitutions that further enhance the duplex stability while providing opportunities for functionalization. Moreover, the metabolic stability of PNAs facilitates their integration into systems that interface with biology. Over the past decade, there has been a growing interest in using PNAs as biosupramolecular tags to program assemblies and reactions. A series of robust templated reactions have been developed with functionalized PNA. These reactions can be used to translate DNA templates into functional polymers of unprecedented complexity, fluorescent outputs, or bioactive small molecules. Furthermore, cellular nucleic acids (mRNA or miRNA) have been harnessed to promote assemblies and reactions in live cells. The tolerance of PNA synthesis also lends itself to the encoding of small molecules that can be further assembled on the basis of their nucleic acid sequences. It is now well-established that hybridization-based assemblies displaying two or more ligands can interact synergistically with a target biomolecule. These assemblies have now been shown to be functional in vivo. Similarly, PNA-tagged macromolecules have been used to prepare bioactive assemblies and three-dimensional nanostructures. Several technologies based on DNA-templated synthesis of sequence-defined polymers or DNA-templated display of ligands have been shown to be compatible with reiterative cycles of selection/amplification starting with large libraries of DNA templates, bringing the power of in vitro evolution to synthetic molecules and offering the possibility of exploring uncharted molecular diversity space with unprecedented scope and speed.
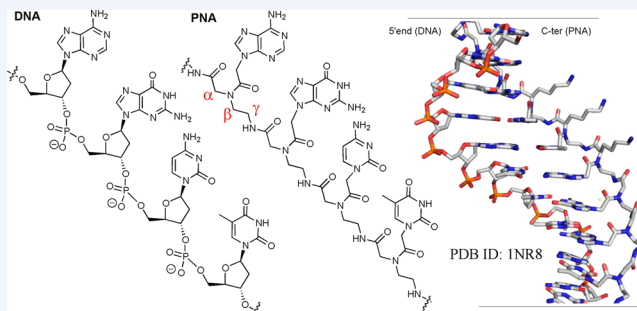
■ PNA SYNTHESIS, MODIFICATION, AND CONJUGATION

The success of peptide nucleic acid (PNA)[1,2] has stimulated many studies exploring the effect of modifications or substitutions on the original N-(2-aminoethyl)glycine (aeg) backbone. Several modifications are well-tolerated and even enhance duplex formation, providing further opportunities for modulation of PNA properties (e.g., duplex stability, electrostatic charge, cell permeability, and derivatization with functional molecules).[3] Substitution at the α position (replacement of glycine with other amino acids)[4] was shown to be tolerated, preferably with D-amino acids. Importantly, an arginine at this position (with a guanidinium group, giving guanidinium PNA (GPNA); Figure 1A) was shown to significantly improve the cellular permeability while slightly enhancing the duplex stability.[5] While achiral aeg-PNAs do not have well-defined conformational folds, an (S)-γ substituent (defined on the basis of a methyl substituent; Figure 1A) generates a steric bias that

favors right-handed helicity with base stacking, resulting in enhanced duplex stability with DNA.[6−8] The introduction of a short poly(ethylene glycol) (PEG) at this position (MPEG-PNA; Figure 1A) dramatically improves the solubility and reduces aggregation compared with unmodified PNA while maintaining the uncharged state of the oligomer.[9] A lysine[7,10,11] or cysteine[12] side chain at the γ position has also proven useful for the conjugation of small molecules (Figure 1A). The combination of chirality at the α and γ positions within a single PNA unit provides additive duplex stability with an overriding influence of the γ stereochemistry.[13] Alternate linkages between the PNA units have also been investigated (Figure 1B). Amine linkages resulting from reductive amination[14] and triazole linkages resulting from Cu-catalyzed azide−alkyne cycloaddition (CuAAC) have been reported to have minimal effects on the
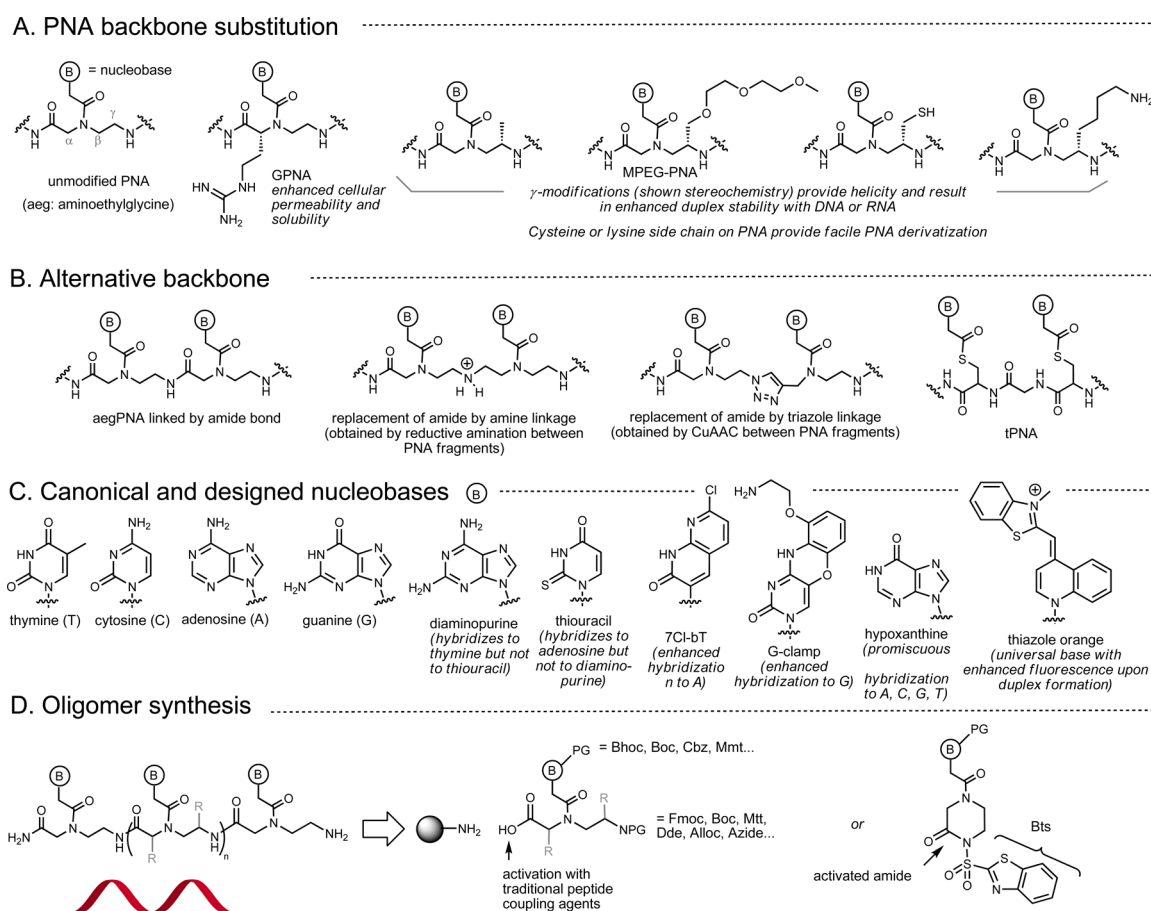
Figure 1. Modifications and synthesis of PNA.

hybridization of the oligomer.[15] A modified PNA backbone linking the nucleobases via thioesters was used to create dynamic PNA (tPNA; Figure 1B) that can exchange nucleobases via transthioesterification reactions.[16]

In addition to modifications to the PNA backbone, many alternative nucleobases beyond the canonical A, C, G, and T have been designed and can provide valuable benefits in biosupramolecular assemblies (Figure 1C). For example, replacement of adenine by 2,6-diaminopurine was shown to increase DNA binding and sequence discrimination.[17] Thiouracil hybridizes to the complementary A but not to 2,6-diaminopurine because of steric clash.[18] This latter point could also be important to bias a given secondary structure within PNA. Analogues of thymine (7-Cl-bT)[19] and cytosine (G-clamp)[20] have been shown to increase the duplex stability by increasing the base-pair stacking. Conversely, indiscriminate behavior can also be engineered with hypoxanthine, which can pair with any nucleobase.[21] A fluorescent base (thiazole orange) has also been reported as a universal base pair; this base exhibits enhanced fluorescence upon duplex formation.[22]

PNA is typically synthesized by iterative cycles of coupling/deprotection with monomers protected orthogonally on the nucleobases and N-terminus (Figure 1D). As an alternative synthetic strategy for oligomerization, a cyclic PNA monomer with a reactive amide (activated via the sulfonamide BTS; Figure 1D) has been shown to yield high-purity oligomers.[23] However, the synthetic hurdles to access modified PNA monomers have limited their implementation and, regrettably, curtailed their full

potential. Clearly, innovative synthetic technologies that facilitate access to substituted PNA would be empowering.

The compatibility of PNA synthesis with traditional solid-phase peptide synthesis and combinatorial chemistry makes it an appealing tag to encode small molecules (Figure 2). For peptide synthesis, this process can be performed via linear synthesis combining reiterative amino acid couplings with PNA couplings. Illustrating the efficiency of the chemistry, 16-mer macrocyclic peptides with 10-mer PNA tags were prepared by automated linear solid-phase synthesis.[24] For small-molecule conjugation, amines and acids can be coupled through standard amide formation. As a more general strategy, nitrophenyl carbamate has been used to couple hydroxyl- or amine-functionalized small molecules, including anilines and phenols (Figure 2A).[25] The use of an α or γ substitution within a PNA provides the opportunity for conjugation at different positions within the oligomer. An alternative to the use of nitrophenyl carbamate for the conjugation of amine-functionalized molecules has been reported with squaric esters (Figure 2A).[11,26] For glycan conjugation, nucleophilic substitution of a chloroacetamide with readily available glycosyl thiols provided high yields with diverse oligosaccharides.[27,28] Glycans have also been conjugated onto PNA–peptide adducts by CuAAC.[29,30] In addition, PNA can be designed to contain a single functionality addressable with chemoselective conjugation methods for derivatization in solution post cleavage, as is most often done with DNA; however, this process is generally more laborious.

Clearly, an advantage of PNA synthesis relative to DNA is the flexibility of the protecting group strategy and its compatibility
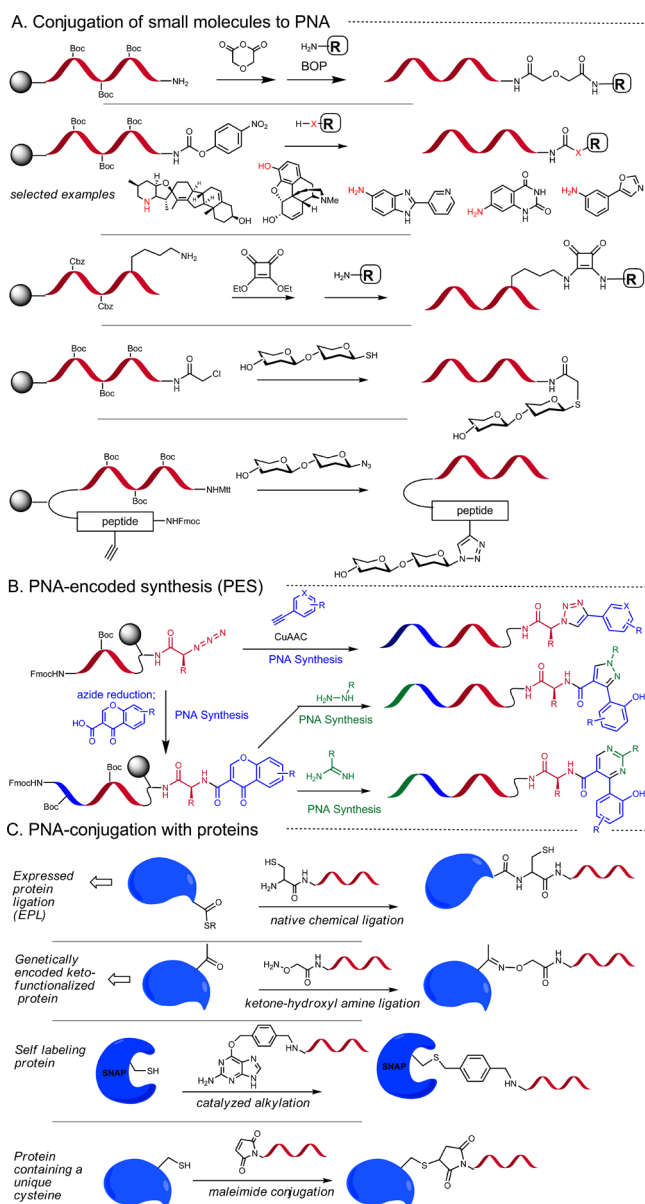
Figure 2. PNA conjugation and PNA-encoded synthesis (PES).

predefined position in the protein may be desirable. Site-specific conjugation of PNA has been accomplished via chemoselective oxime formation[50] using a protein containing a genetically encoded ketone-functionalized unnatural amino acid (*p*-acetylphenylalanine).[51] It should be noted that this conjugation proved to be significantly more efficient with GPNA than with unmodified PNA because of GPNA's higher solubility. Protein–PNA conjugates have also been prepared using the SNAP tag technology.[52] SNAP is a widely used self-labeling protein that operates under physiological conditions. Finally, proteins with a unique or engineered cysteine can be tagged with a PNA–maleimide adduct.[53]

■ PNA-BASED REACTIONS TEMPLATED BY HYBRIDIZATION

Nucleic acid-templated reactions are accelerated by the preorganization and high effective concentration of the reactive moieties achieved upon hybridization (Figure 3). Shortly after
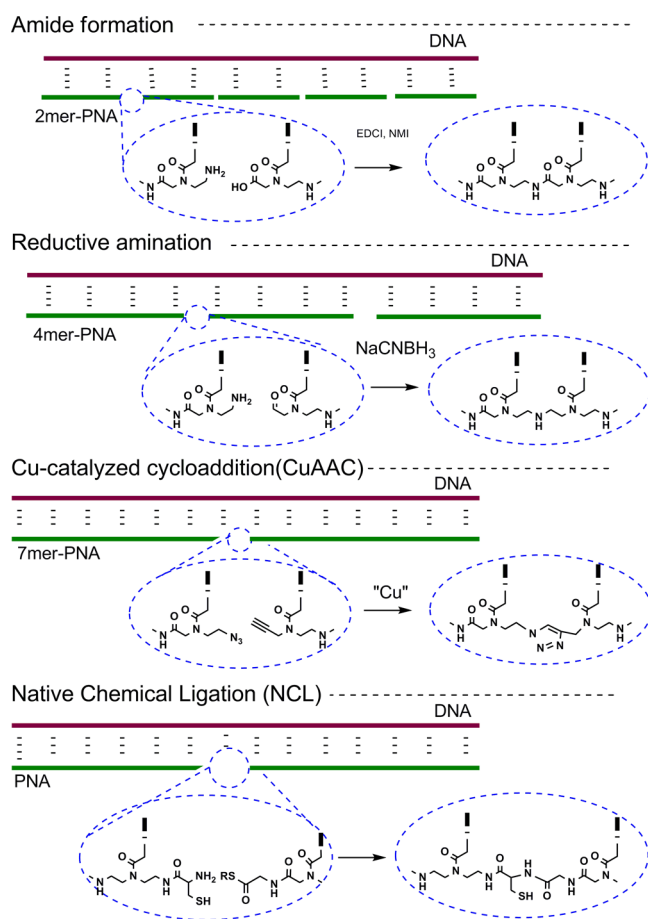

Figure 3. Templated ligations with PNA.

with the cosynthesis of biologically relevant small molecules.[31−35] This asset is best exemplified by the use of PNA in split and mix combinatorial synthesis, in which each diverse synthon used in the small-molecule synthesis is encoded by a PNA codon.[36−38] Reiteration of this process yields a library in which the synthetic history and hence the structure of each molecule are encoded in the PNA tag (see Figure 2B for a representative example of a divergent synthesis of heterocycles[25]). This PNA-encoded synthesis (PES) has been successfully used to prepare peptide-based libraries[35,39−45] and peptidoglycans[29,30] as well as heterocycles[25] or other druglike small molecules.[46,47]

In addition to small-molecule encoding, PNAs have also been used to tag proteins to direct assemblies of macromolecules, paralleling advances with DNA tagging (Figure 2C).[48] Seitz and co-workers used native chemical ligation (NCL) to achieve conjugation between an intein fusion protein and a cysteine-derivatized PNA, yielding a conjugate at the C-terminal position of the protein.[49] In the context of self-assemblies, a tag at a

the discovery of PNA, it was shown that DNA could template the condensation of PNA fragments (Figure 3).[54] Although this pilot experiment demonstrated information transfer from DNA to PNA, its applicability was not further studied. Other reactions that closely mimic the linkage of native PNA were developed, such as reductive amination,[14] CuAAC,[15] and NCL[55] (Figure 3). Liu and co-workers ingeniously harnessed a DNA-templated reductive amination to translate a DNA sequence into a polymer of modified PNA.[56,57] PNA aldehyde tetramer or pentamer units functionalized with one or multiple side chains at various
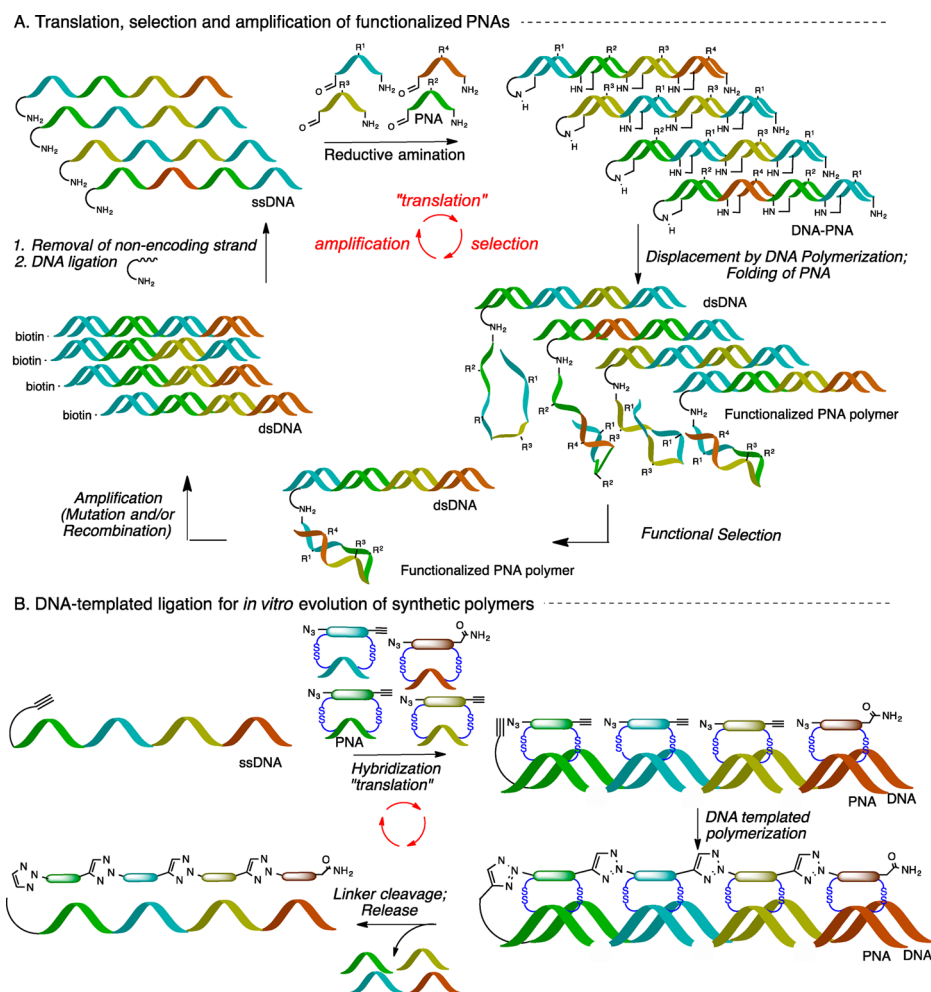
Figure 4. Translation of DNA into synthetic polymers.

positions within the PNA fragments were condensed onto DNA templates (Figure 3). Impressively, this reaction was used to ligate eight PNA fragments, affording 66−90% yields of 40-mer PNAs, a length that would be challenging to obtain via chemical synthesis.[56] It was further demonstrated that a DNA library could be translated into diverse PNA oligomers for iterative cycles of translation/selection (Figure 4A). When a hairpin architecture is used in the DNA template, the PNA oligomer remains covalently linked to its template. DNA polymerization can be used to displace the PNA oligomer, allowing it to adopt a folded structure. The ability to cycle through the translation of DNA into a PNA oligomer, selection, and amplification, was demonstrated using a PNA fragment derivatized with biotin, thus establishing a precedent for the evolution of synthetic polymers.[57] This general concept was further developed for an enzyme-free translation of DNA into sequence-defined synthetic polymers that are structurally unrelated to nucleic acids (Figure 4B).[58] For this purpose, sequence-defined polymer fragments were transiently tethered to each end of a PNA codon (5-mer) via disulfides and coupled by templated CuAAC, enabling ligation of up to 16 fragments with >60% conversion of the full-length product. Reduction of the disulfides yielded the DNA-encoded polymer. This methodology was used to prepare a 26 kDa oligomer composed of 90 β-amino acid residues, thus demonstrating the viability of the technology for the fabrication of synthetic polymer products with molecular weights comparable to those of biological origin. It was further shown

that following translation into a synthetic polymer, the DNA template could be amplified using polymerase chain reaction (PCR) to regenerate the synthetic polymer, again demonstrating that DNA-templated translation can support the laboratory evolution of a range of synthetic polymers and macromolecules beyond the reach of previous technologies.

DNA-templated NCL has also proven valuable (Figure 5). Seitz and co-workers used the high duplex stability of PNA–DNA to achieve a templated reaction starting with double-stranded DNA (dsDNA). Through the use of auxiliary PNA fragments flanking the sequence employed in the templated ligation, it was shown that the NCL reaction could be achieved following a thermal denaturation.[55] However, turnover in templated ligation is difficult because of product inhibition. An ingenious solution to this problem was to use an isocysteine in the NCL, which yields a destabilizing ligation product, thus reducing the product inhibition.[59] More recently, Roloff and Seitz[60] used a postligation reaction to cyclize the ligated product, thus preventing it from annealing to the template. It was shown that the ligation−cyclization sequence (NCL followed by iodoacetamide thiol reaction) on a substoichiometric template provided higher yields in shorter times than a ligation-only reaction.

Ladame and co-workers pioneered another interesting templated ligation that yields diverse fluorophores (Figure 6). The templated synthesis of a series of cyanine dyes through aldolization−elimination reactions affords fluorophores that
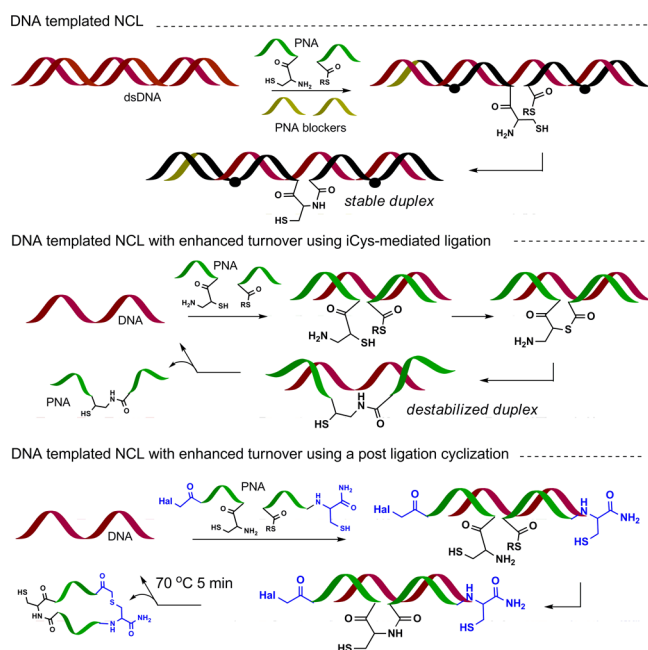
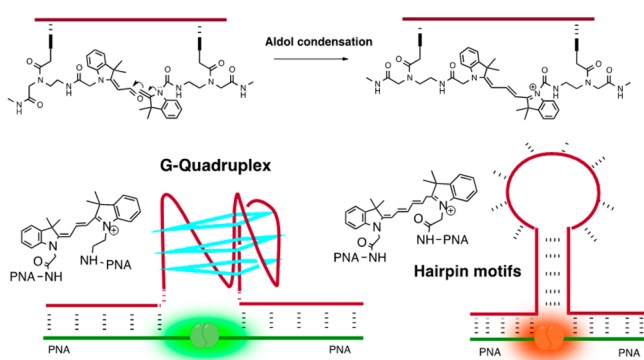**Figure 5.** Templated native chemical ligations (NCL).

Figure 6. Templated formation of cyanine dyes.

emit from 550 to 645 nm from nonfluorescent starting materials.[61−63] This reaction was useful for the detection of different DNA folds (G-quadruplex or hairpin) that affect the proximity of the PNA probes and hence the kinetics and yield of the fluorophore formation.

An alternative reaction design is to use templated transformations that do not lead to ligation, thus alleviating product inhibition. The inherent advantage of this strategy in terms of signal amplification through higher turnover renders it an appealing choice for nucleic acid sensing.[64,65] Through a simple modification of the reaction architecture, Seitz and co-workers used NCL to achieve a templated transfer rather than a ligation (Figure 7).[66,67] Attesting to the achievable signal amplification, a >400-fold turnover was calculated for a reaction performed at an extremely low template concentration (10 pM; 0.01% template loading) after 24 h. This reaction was also used for the templated transfer of a biotin group from one PNA strand to another strand bearing a His$_6$ tag, thus enabling the use of an enzyme-linked immunosorbent assay (ELISA) readout.[67] The double amplification (templated reaction/ELISA) enabled the detection of HIV-I RNA in amounts as low as 500 amol (1 amol = 10^{-18} mol). A second strategy for the consecutive amplification of the signal output from a templated reaction was also reported. It uses a

templated Wittig olefination to yield a product that participates in a fluorescence-enhancing host−guest interaction.[68] In addition to nucleic acid sensing, the templated NCL transfer reaction was used to synthesize a bioactive peptide in response to a template harboring an oncogenic mutation.[69] Tumor cells frequently overexpress a protein called X-linked inhibitor of apoptosis protein (XIAP) that confers resistance to apoptotic signals by inhibiting caspase-9. The templated reaction was designed to yield a tetrapeptide ligand that outcompetes this interaction and restores the apoptotic cascade in lysates.

The bioorthogonality of an azide reduction makes this transformation a compelling choice for templated reactions leveraged on cellular nucleic acids. Accordingly, coupling of an azide reduction to a fluorescent output has attracted significant attention. Although efforts using DNA and PNA probes have been pursued,[70−75] this discussion is restricted to recent reactions with PNA. We have pursued two strategies to translate nucleic acid-templated azide reduction into a functional output (fluorescence or bioactivity): the reduction of azide-based fluorogenic substrates and cleavage of an immolative linker (Figure 8). The biological stability of PNA coupled with the fact that modified PNAs (GPNAs) are cell-permeable lends itself to imaging of cellular nucleic acids in live cells. This process was first demonstrated using a templated Staudinger reduction of azidorhodamine in response to an mRNA coding for MGMT[76] or microRNAs (miRs).[77] The same reaction manifold was applied to the cleavage of an azide-based immolative linker used to mask a fluorophore (rhodamine) or a bioactive molecule (estradiol or doxorubicin).[78] However, a limitation of the Staudinger reduction is the propensity of fortuitous phosphine oxidation. Inspired by the discovery that Ru(bpy)$_3$Cl$_2$ can photocatalytically reduce azides,[79] this reaction was adapted for templated cleavage of the azide-based immolative linker or reduction of the azide-quenched profluorophore.[80] Compared with the Staudinger reaction, this ruthenium-catalyzed version benefits from the fact that only the azide component must be exchanged on the template to achieve reaction turnover, thus reducing competing hybridization with spent reagent. This reaction proceeded with high signal amplification (>4000 turnovers at 0.002% template loading; 5 pM).[81] Importantly, the reaction is compatible with live-cell imaging, suggesting that the redox cycle of the ruthenium catalyst is aligned with the cellular redox buffer. We have extended the scope of this templated reaction to detect protein dimerization or oligomerization using specific small-molecule ligands rather than nucleic acids as a means to increase the effective concentration of the reagents through biosupramolecular interactions.[82]

The combination of strand displacement and templated reactions offers unique opportunities to engineer nonlinear responses and logic gate operations. Using PNA derivatized with a photolabile group that does not cleave upon exposure to light with wavelengths above 350 nm and a complementary strand with a sensitizer that is excited at 405 nm, we demonstrated that the templated energy transfer could be used to cleave the photo labile group and unmask a fluorophore (rhodamine, λ_{ex} = 490 nm, λ_{em} = 530 nm). The templated sensitization of a nitrobenzyl photolabile group was used to investigate the behavior of a system with up to four components competing for mutual interactions (Figure 9). By adjusting the hierarchy of the interactions among the different duplexes through the number of base pairs, we obtained a system that responds positively and then negatively to increasing amounts of input. The unique
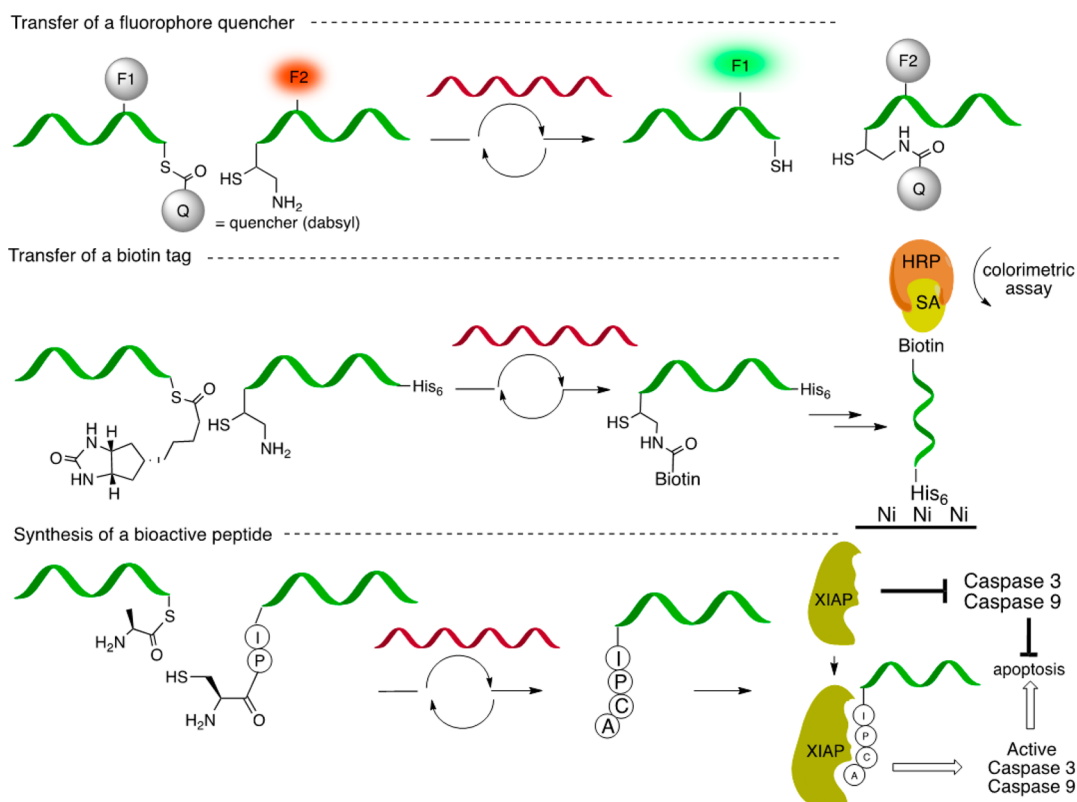
Figure 7. Applications of templated transfer reactions via native chemical ligation.

behavior of this system makes it amenable to diverse logic operations (AND, NOT, XNOR, and NOR).

■ PNA-BASED ASSEMBLIES WITH SMALL MOLECULES AND PROTEINS

The PNA tags provide a simple platform to "barcode" small molecules or macromolecules and program their assembly through self-complementarity or hybridization to a template. These assemblies offer the opportunity to capitalize on the synergy of the interactions between multiple ligands and a target (Figure 10). A compelling advantage of a hybridization-based dimerization or oligomerization relative to a covalent approach is that a double-stranded nucleic acid presents a relatively rigid scaffold with well-defined nucleobase spacing (3.4 Å). The interligand distance can be tailored through the positioning of multiple ligands along a single strand, which is rigidified upon duplex formation (the relative orientation of the ligands will depend on the position along the helix). Conversely, the interligand distance can be controlled by the pairing of disconnected sequences hybridizing at different positions of a template. In the latter case, a flexible region in the linker can be engineered using a single strand stretch in the template.

Pioneering work by Kobayashi and co-workers demonstrated that the oligomerization of half-slide complementary DNA fragments derivatized with galactose could be used to generate periodic glycoclusters.[83] Neri and co-workers used complementary strands to pair a library of small-molecule fragments.[84] Using a nucleic acid template to pair ligands rather than complementary sequences offers the advantage of modulating the interligand distance or ligand combination via template instructions. We first implemented this concept to recapitulate the epitope of HIV, which is composed of multiple copies of a high-mannose glycan on the surface of gp120. A pilot library of 14 PNA-tagged glycoconjugates that included mannose disaccharides joined by linkers with different lengths were paired through hybridization, varying the ligand combinations and interligand distances.[27] Measuring the affinity of 32 different assemblies against 2G12, an antibody directed at the glycan epitope, revealed a clear distance−affinity relationship that was consistent with the proposed binding of 2G12 with gp120 (Figure 11A). Notably, neither of the PNA-tagged fragments had measurable affinities for 2G12, thus demonstrating clear synergy of the templated fragments. The same strategy was used to gain insight into the optimal dimerization geometry for macrocyclic peptides targeting DR5, an oligomeric TRAIL receptor.[24] Concurrently, Chaput and co-workers reported a hybridization-based pairing of DNA-tagged peptide ligands (12-mers) to create high-affinity bivalent reagents[85] with a 1000-fold enhancement in affinity over the individual peptides. Seitz and co-workers further extended the scope of this approach by bridging binding sites on opposite faces of a lectin (65 Å apart) using a 100 Å DNA linker with an unpaired region to accommodate bending around the protein (Figure 11B).[12,86] Other examples include dimerization of ligands targeting the SH2 domain[87] and the estrogen receptor.[88] Although PNA and DNA have been used for hybridization-directed assemblies of small-molecule ligands, the high density of negative charges on DNA can affect the interaction of the assembly with the target. In two cases, a PNA-templated assembly had superior affinity compared with the corresponding DNA-templated assembly.[24,26] Appella and co-workers further demonstrated that hybridization-based assemblies targeting the $\alpha_v\beta_3$ integrin receptor with a PNA-tagged cyclic RGD motif are functional in vivo.[11] Optimal efficacy was obtained by screening a library of 52 different DNA-templated assemblies that systematically varied valences and geometries. When PNA−DNA duplexes derivatized with one to three ligands were compared, a
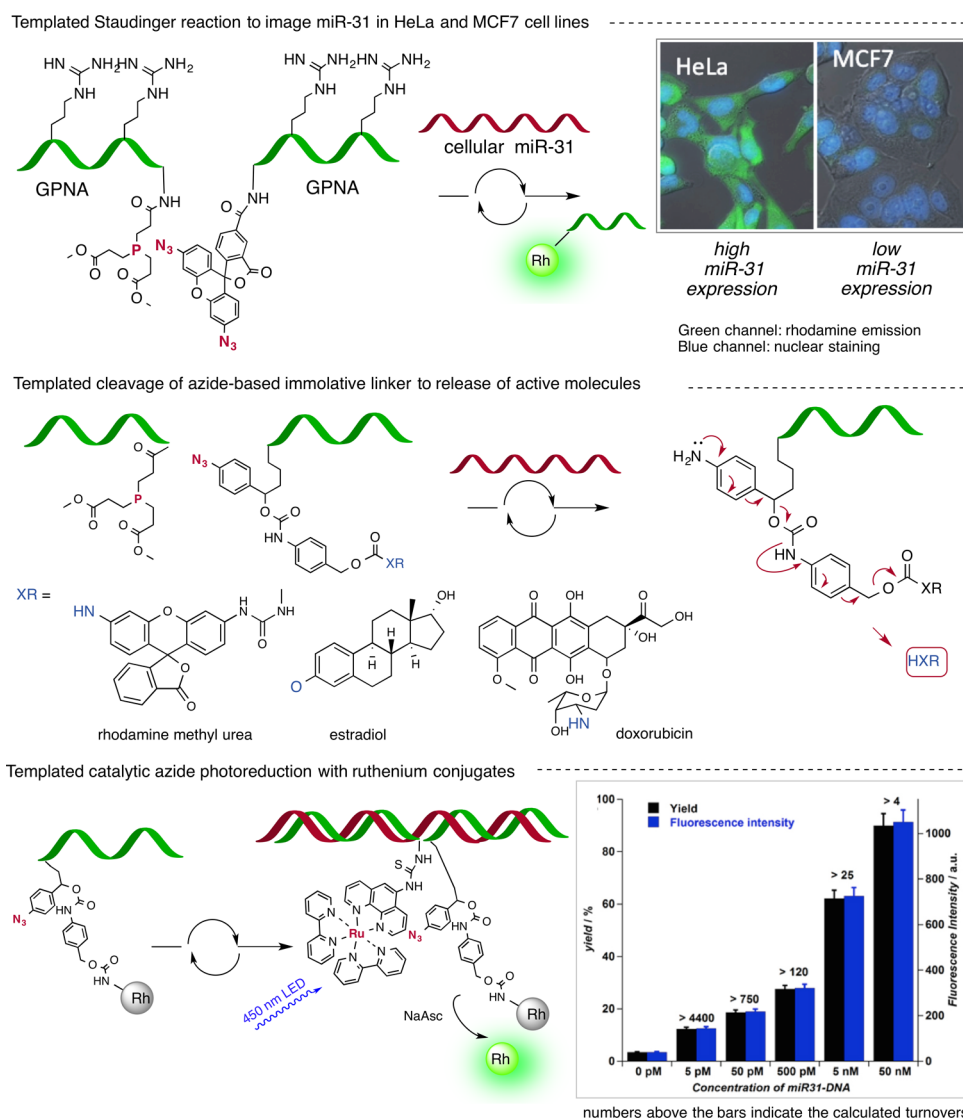
Figure 8. Templated azide reduction to unmask a fluorophore or bioactive molecule.
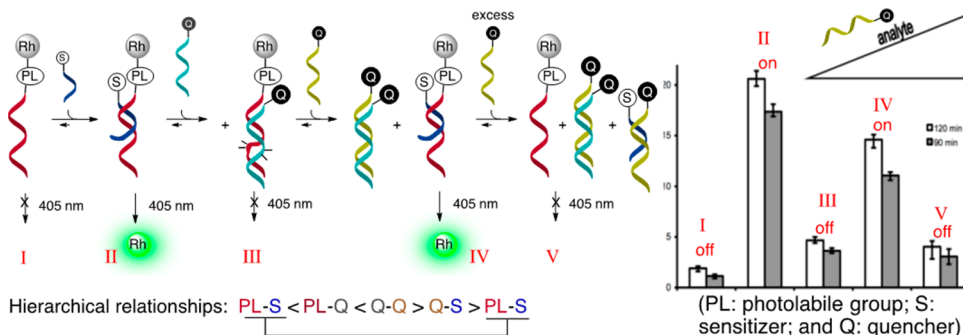

Figure 9. Four-component system based on template photo-deprotection.

progressive improvement in the IC$_{50}$ was observed, which was consistent with the model of three ligands simultaneously binding the trimeric integrin receptor (Figure 11C). The activity also increased with the number of consensus sequence repeats along longer DNA templates, suggesting that longer templates can span multiple receptor clusters. The assembly displaying five repeats of the trisubstituted RGD−PNA adduct was found to yield a 100-fold enhancement in binding compared with the

cyclic peptide alone and to afford a 50% reduction in tumor colonies in a mouse model. More recently, the same approach was used to dissect the multivalent effects of ligand binding to the A2A adenosine receptor (a drug target for neurodegenerative conditions belonging to the G-protein-coupled receptor family).[26]

An attractive opportunity presented by templated displays is the combinatorial pairing of ligands from PNA-encoded libraries
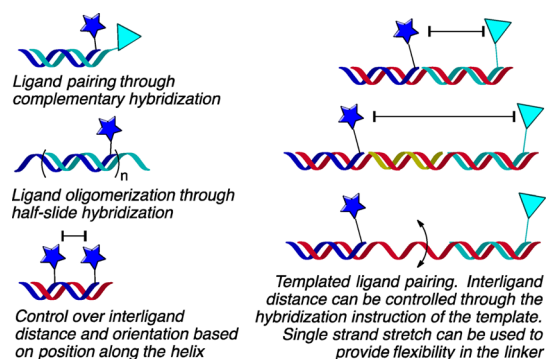
Figure 10. Hybridization-based assemblies to display small molecules.

using DNA templates in solution or in a microarray format (Figure 12). From a molecular diversity standpoint, the ability to combine different pharmacophore fragments affords a rapid entry into a large molecular diversity space. Since the templates corresponding to the fittest assemblies (fragment pairs) can be enriched following an affinity selection against a target of interest and amplified via PCR, the process can be used for reiterative cycles of selection/amplification (Figure 12A). We demonstrated this process with a screen against carbonic anhydrase.[25] It was shown that reiteration of the selection/amplification cycle starting with a library of 62500 assemblies provided convergence

toward a fragment set that upon resynthesis as a covalent adduct had an affinity of 87 nM for carbonic anhydrase (neither fragment had an affinity below the micromolar level). The same strategy was used to optimize binders to DC-SIGN using a glycan library.[89] DC-SIGN is a tetrameric lectin implicated in interactions with a broad array of pathogens, including HIV. In this example, screening a library of over 37000 members led to the identification of an assembly with 30-fold-improved binding over the unmodified mannose assembly (neither fragment had measurable affinity individually). Notably, a dendrimer derivatized with the identified ligand was shown to outcompete the interaction of HIV's gp120 with dendritic cells at 10 μM. These examples demonstrate that templated display extends the scope of reiterative selection/amplification technologies to glycans and small molecules broadly recognized as versatile pharmacophores.

Alternatively, the combinatorial pairing can be performed on a DNA microarray to afford spatially resolved assemblies (Figure 12B). The fittest ligand pair is then identified via incubation of the array with a tagged protein. We used this approach to assemble a diverse array of glycans from simpler libraries[28,30] or small-molecule fragments. The fittest fragment pairs can then be incorporated into a focused library, covalently pairing them through diverse likers. The focused library can be further enriched with closely related analogues to the selected fragments. This screening strategy led to the discovery of the lowest-affinity
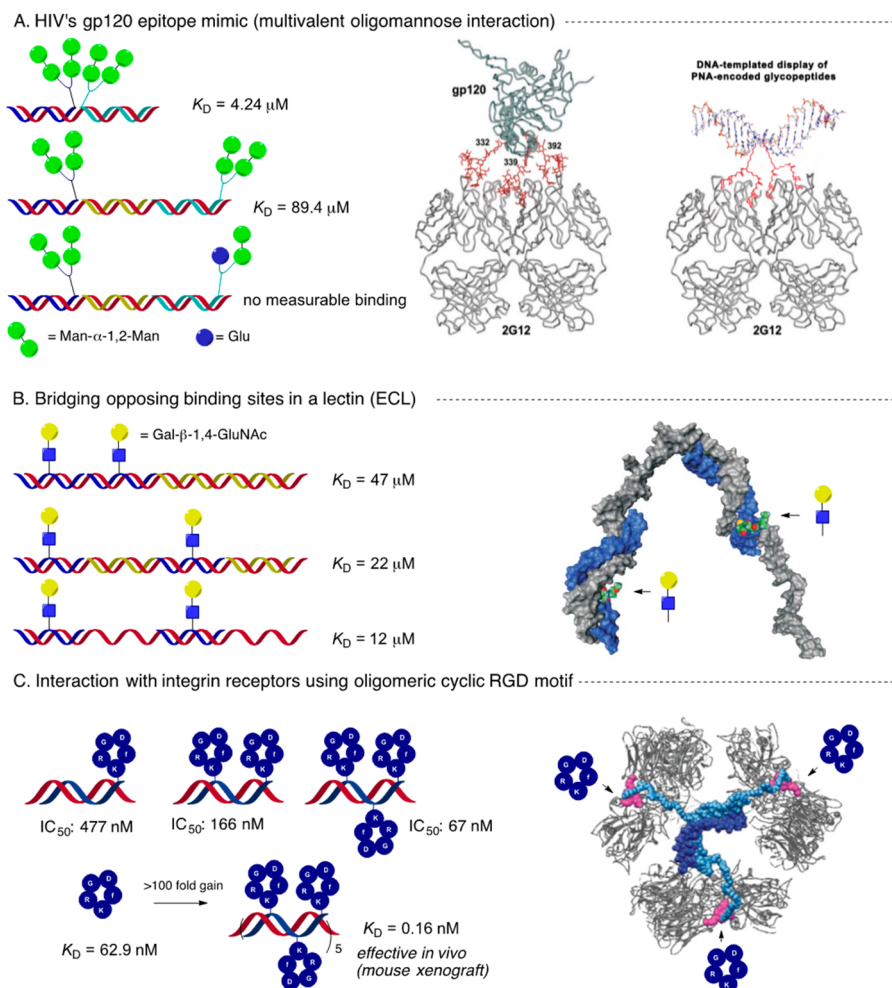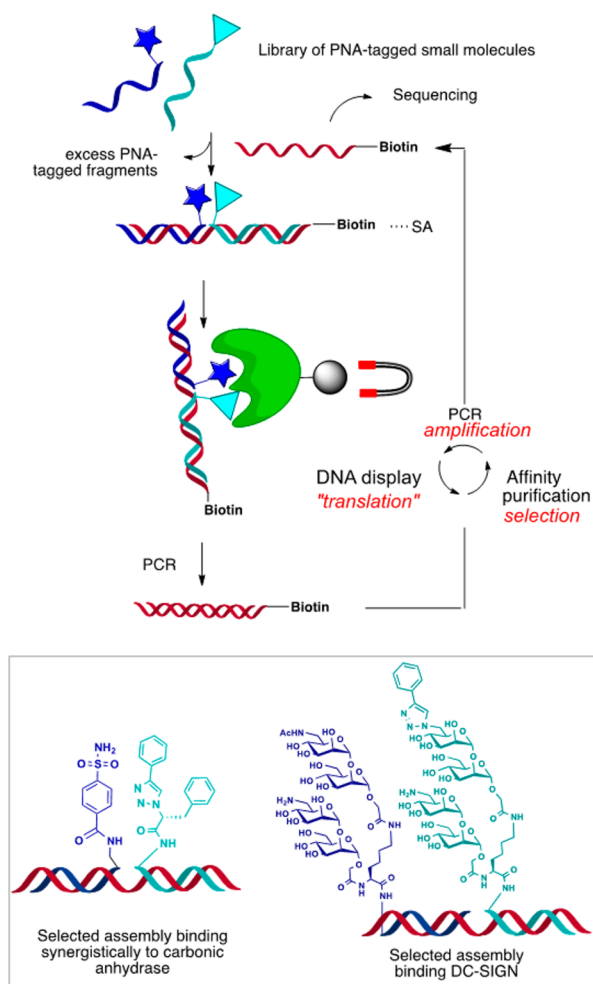

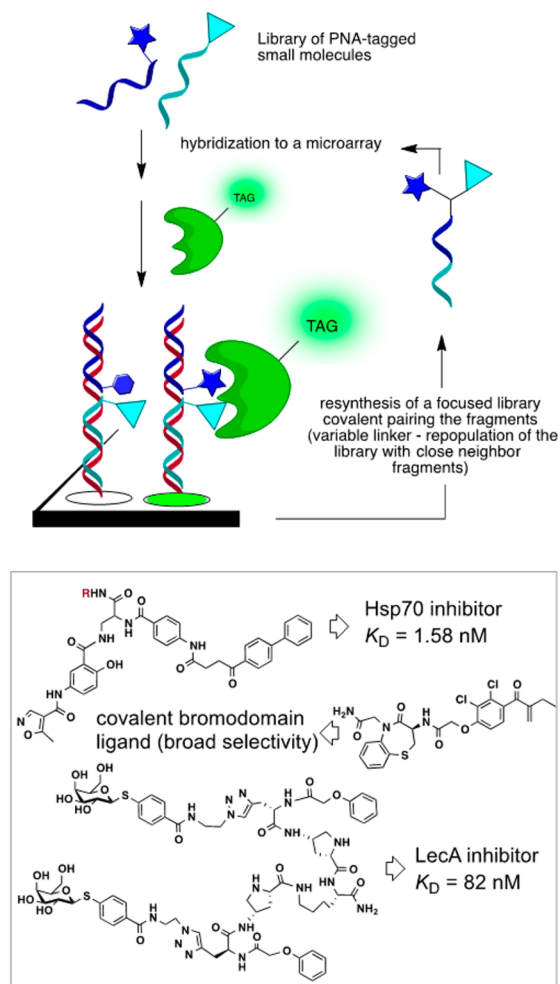**Figure 11.** Selected examples of hybridization-based small molecule displays.

Figure 12. Hybridization-based combinatorial pairing of ligands and selection.

ligands for Hsp70,[46] the first covalent inhibitor of bromodomains,[47] and a potent ligand of LecA.[29]

Tagging of macromolecules with DNA is well-established,[90] but the unique chemistry, duplex stability, and metabolic robustness of PNA can be advantageous. The compatibility of PNA with expressed protein ligation (EPL) tagging was exploited for DNA-directed immobilization of proteins in a microarray format (Figure 13A).[49] PNA tags have also been used to program the assembly of antibody fragments (Figure 13B).[50] On the basis of the interest in bispecific antibodies, a strategy to rapidly access and evaluate heterodimers (bispecific) or higher-order oligomers should facilitate the discovery of the optimal pairing, geometry, and valency. As a model system, site-selectively tagged Fab (oxime ligation) directed at Her2, a receptor overexpressed in several cancers, was self-assembled into well-defined multimeric complexes. A 12-mer PNA tag afforded sufficient duplex stability for the assembly to be analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and to withstand long incubation times in serum without degradation. The PNA-based αHer2 homodimer exhibited cellular activity comparable to that of trastuzumab (an FDA-approved antibody) and was more effective than the comparable DNA-based dimer. A higher-order oligomer (αHer2 tetramer) further enhanced the efficacy, achieving an EC_{50} of 4.6 nM in a cellular assay, which is 6 times more effective than

trastuzumab. The generality of this approach was applied to the generation of bifunctional heterodimers with αCD3 Fab (αHer2−αCD3 and αCD20−αCD3) that were shown to direct T-cell-mediated cytotoxicity to tumor cells in vitro (the unconjugated Fab fragment had marginal activity). These examples illustrate the fact the multimeric constructs can be rapidly generated by mixing the relevant Fab−PNA subunits combining "binders" and "effectors". Hybridization-based protein assembly was also used to direct the assembly of multiple PNA-tagged monomeric teal fluorescent protein (mTFP). Fluorescence resonance energy transfer (FRET) across the different fluorescent proteins was evaluated by anisotropy and showed a clear decrease from the monomeric unit to the four adjacent units (Figure 13C), which is consistent with homo-FRET.[91]

■ PNA IN DNA-BASED NANOSTRUCTURES

A straightforward strategy to direct molecules to discrete locations within a DNA-based assembly is to capitalize on hybridization.[92] To ensure sufficient thermal stability at room temperature, sequences of 15−20 nucleotides are typically used, which form 5−6.5 nm long dsDNA helices. However, this length can be prohibitive in smaller structures, such as tetrahedral DNA nanocages.[93] Fromme and co-workers demonstrated that such DNA nanocages can be assembled leaving a short unpaired
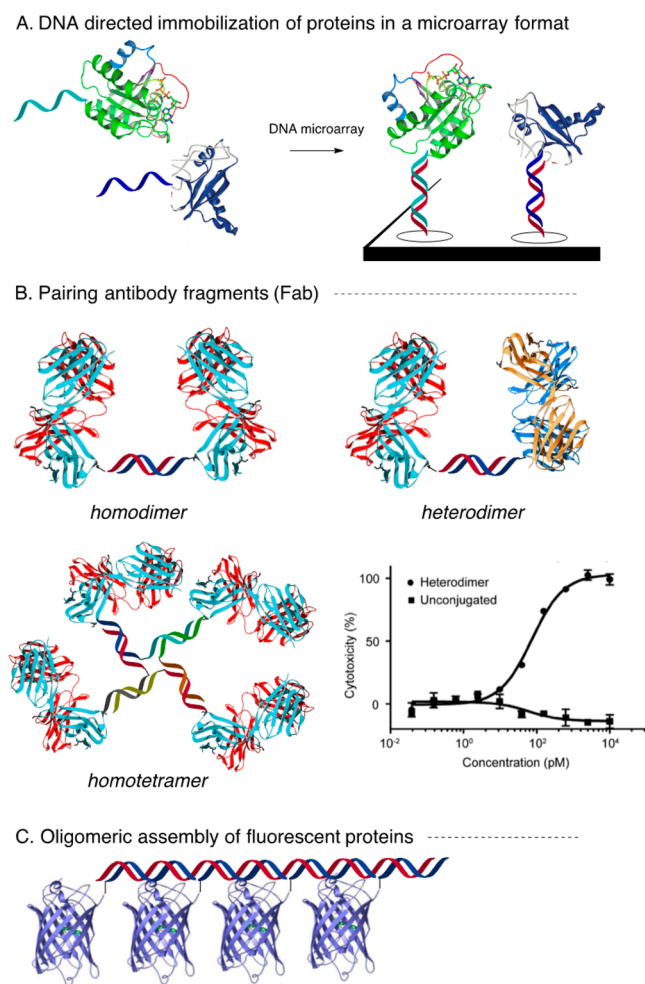
Figure 13. Hybridization-based assemblies of macromolecules.

region (eight nucleotides) that was sufficient to hybridize PNA derivatized with peptides, fluorophores, or proteins (Figure 14A), clearly benefiting from the high duplex stability of PNA.[53,94] The ability to introduce various proteins into a defined position within a 3D structure offers a robust approach for studying functional interactions of different proteins.

Komiyama and co-workers constructed DNA origami forceps that can be imaged using atomic force microscopy (AFM), enabling its use in the visualization of various binding events between biochemical targets (biotin–streptavidin, antigen–antibody, metal ions interacting with DNA) through the motion in the forceps (Figure 14B).[95] It was shown that actuation of the forceps could be achieved by outcompeting a DNA duplex interaction with the formation of a PNA–DNA triplex, triggering a drastic shape transition. AFM imaging and gel electrophoresis provided a clear observation of PNA invasion using nano-mechanical DNA origami devices.[96]

■ CONCLUSION

It is safe to predict that the use of nucleic acid-based supramolecular assemblies will continue to yield novel applications and advances. Although DNA has been at the center of most assemblies and enjoys a greater economy of scale than PNA, there are a growing number of examples in which the unique properties of PNA have been empowering. The fact that PNA-based templated processes can be interfaced with biology for in cellulo or in vivo applications paves the way to new developments in therapeutic and diagnostic applications. Several PNA-based technologies are compatible with reiterative cycles of selection/amplification, extending the scope of in vitro evolution to synthetic molecules.

We hope that the examples presented in this Account will encourage practitioners in supramolecular chemistry, systems chemistry, and DNA nanotechnologies to embrace and further develop the use of PNA as programmable supramolecular tags.
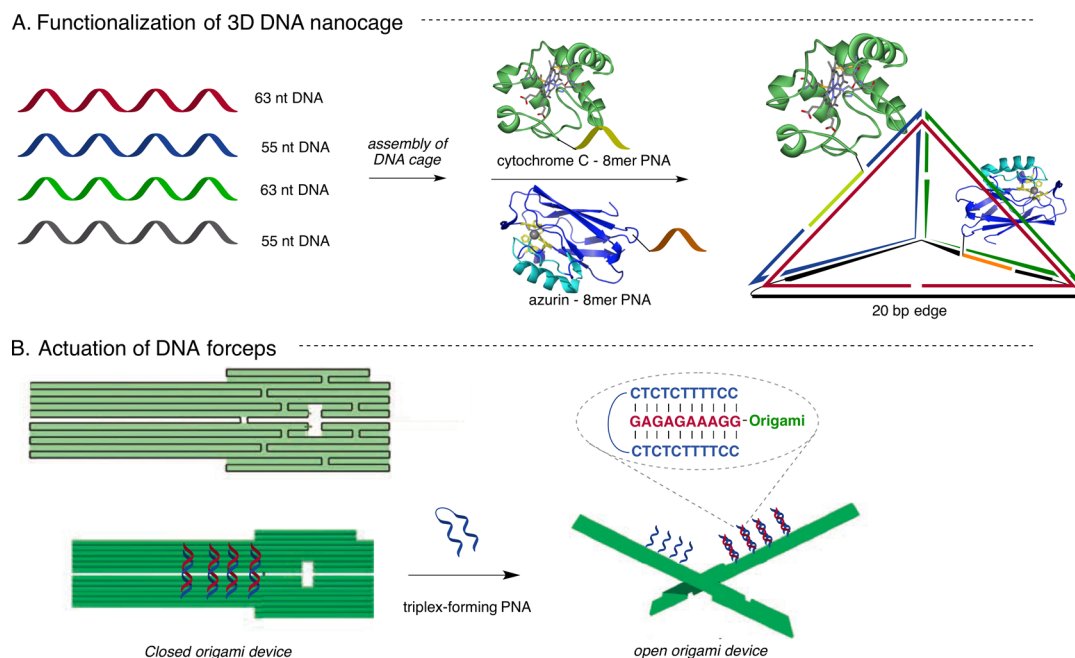

Figure 14. PNA in DNA-based nanostructures.

AUTHOR INFORMATION

Corresponding Author

*E-mail: nicolas.winssinger@unige.ch.

Notes

The authors declare no competing financial interest.

Biographies

Sofia Barluenga received her Ph.D. with F. Aznar and J. Barluenga before joining the group of K. C. Nicolaou for postdoctoral training. She is currently a senior researcher at the University of Geneva.

Nicolas Winssinger received his Ph.D. with K. C. Nicolaou and postdoctoral training with P. G. Schultz. He started his independent career at the University of Strasbourg (Institut de Science et Ingénierie Supramoléculaires) before moving to the University of Geneva.

ACKNOWLEDGMENTS

REFERENCES

(1) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. PNA hybridizes to complementary oligonucleotides obeying the Watson–Crick hydrogen-bonding rules. *Nature* **1993**, *365*, 566–568.

(2) Nielsen, P. E. Peptide nucleic acid. A molecule with two identities. *Acc. Chem. Res.* **1999**, *32*, 624–630.

(3) Sugiyama, T.; Kittaka, A. Chiral Peptide Nucleic Acids with a Substituent in the *N*-(2-Aminoethyl)glycine Backbone. *Molecules* **2013**, *18*, 287–310.

(4) Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. Peptide nucleic acids (PNAs) containing thymine monomers derived from chiral amino acids: Hybridization and solubility properties of D-lysine PNA. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1939–1942.

(5) Zhou, P.; Wang, M.; Du, L.; Fisher, G. W.; Waggoner, A.; Ly, D. H. Novel binding and efficient cellular uptake of guanidine-based peptide nucleic acids (GPNA). *J. Am. Chem. Soc.* **2003**, *125*, 6878–6879.

(6) Dragulescu-Andrasi, A.; Rapireddy, S.; Frezza, B. M.; Gayathri, C.; Gil, R. R.; Ly, D. H. A simple γ-backbone modification preorganizes peptide nucleic acid into a helical structure. *J. Am. Chem. Soc.* **2006**, *128*, 10258–10267.

(7) Englund, E. A.; Appella, D. H. Synthesis of γ-substituted peptide nucleic acids: A new place to attach fluorophores without affecting DNA binding. *Org. Lett.* **2005**, *7*, 3465–3467.

(8) Yeh, J. I.; Shivachev, B.; Rapireddy, S.; Crawford, M. J.; Gil, R. R.; Du, S. C.; Madrid, M.; Ly, D. H. Crystal Structure of Chiral γPNA with Complementary DNA Strand: Insights into the Stability and Specificity of Recognition and Conformational Preorganization. *J. Am. Chem. Soc.* **2010**, *132*, 10717–10727.

(9) Sahu, B.; Sacui, I.; Rapireddy, S.; Zanotti, K. J.; Bahal, R.; Armitage, B. A.; Ly, D. H. Synthesis and Characterization of Conformationally Preorganized, (*R*)-Diethylene Glycol-Containing γ-Peptide Nucleic Acids with Superior Hybridization Properties and Water Solubility. *J. Org. Chem.* **2011**, *76*, 5614–5627.

(10) Englund, E. A.; Appella, D. H. γ-Substituted peptide nucleic acids constructed from L-lysine are a versatile scaffold for multifunctional display. *Angew. Chem., Int. Ed.* **2007**, *46*, 1414–1418.

(11) Englund, E. A.; Wang, D. Y.; Fujigaki, H.; Sakai, H.; Micklitsch, C. M.; Ghirlando, R.; Martin-Manso, G.; Pendrak, M. L.; Roberts, D. D.; Durell, S. R.; Appella, D. H. Programmable multivalent display of receptor ligands using peptide nucleic acid nanoscaffolds. *Nat. Commun.* **2012**, *3*, No. 614.

(12) Scheibe, C.; Bujotzek, A.; Dernedde, J.; Weber, M.; Seitz, O. DNA-programmed spatial screening of carbohydrate–lectin interactions. *Chem. Sci.* **2011**, *2*, 770–775.

(13) Sforza, S.; Tedeschi, T.; Corradini, R.; Marchelli, R. Induction of helical handedness and DNA binding properties of peptide nucleic acids (PNAs) with two stereogenic centres. *Eur. J. Org. Chem.* **2007**, 5879–5885.

(14) Rosenbaum, D. M.; Liu, D. R. Efficient and sequence-specific DNA-templated polymerization of peptide nucleic acid aldehydes. *J. Am. Chem. Soc.* **2003**, *125*, 13924–13925.

(15) Chouikhi, D.; Barluenga, S.; Winssinger, N. Clickable peptide nucleic acids (cPNA) with tunable affinity. *Chem. Commun.* **2010**, *46*, 5476–5478.

(16) Ura, Y.; Beierle, J. M.; Leman, L. J.; Orgel, L. E.; Ghadiri, M. R. Self-assembling sequence-adaptive peptide nucleic acids. *Science* **2009**, *325*, 73–77.

(17) Haaima, G.; Hansen, H. F.; Christensen, L.; Dahl, O.; Nielsen, P. E. Increased DNA binding and sequence discrimination of PNA oligomers containing 2,6-diaminopurine. *Nucleic Acids Res.* **1997**, *25*, 4639–4643.

(18) Lohse, J.; Dahl, O.; Nielsen, P. E. Double duplex invasion by peptide nucleic acid: A general principle for sequence-specific targeting of double-stranded DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11804–11808.

(19) Eldrup, A. B.; Christensen, C.; Haaima, G.; Nielsen, P. E. Substituted 1,8-naphthyridin-2(1*H*)-ones are superior to thymine in the recognition of adenine in duplex as well as triplex structures. *J. Am. Chem. Soc.* **2002**, *124*, 3254–3262.

(20) Ausin, C.; Ortega, J. A.; Robles, J.; Grandas, A.; Pedroso, E. Synthesis of amino- and guanidino-G-clamp PNA monomers. *Org. Lett.* **2002**, *4*, 4073–4075.

(21) Sanders, J. M.; Wampole, M. E.; Chen, C. P.; Sethi, D.; Singh, A.; Dupradeau, F. Y.; Wang, F.; Gray, B. D.; Thakur, M. L.; Wickstrom, E. Effects of Hypoxanthine Substitution in Peptide Nucleic Acids Targeting KRAS2 Oncogenic mRNA Molecules: Theory and Experiment. *J. Phys. Chem. B* **2013**, *117*, 11584–11595.

(22) Kohler, O.; Seitz, O. Thiazole orange as fluorescent universal base in peptide nucleic acids. *Chem. Commun.* **2003**, 2938–2939.

(23) Lee, H. N.; Jeon, J. H.; Lim, J. C.; Choi, H.; Yoon, Y. H.; Kim, S. K. Peptide nucleic acid synthesis by novel amide formation. *Org. Lett.* **2007**, *9*, 3291–3293.

(24) Gorska, K.; Beyrath, J.; Fournel, S.; Guichard, G.; Winssinger, N. Ligand dimerization programmed by hybridization to study multimeric ligand–receptor interactions. *Chem. Commun.* **2010**, *46*, 7742–7744.

(25) Daguer, J. P.; Ciobanu, M.; Alvarez, S.; Barluenga, S.; Winssinger, N. DNA-templated combinatorial assembly of small molecule fragments amenable to selection/amplification cycles. *Chem. Sci.* **2011**, *2*, 625–632.

(26) Dix, A. V.; Moss, S. M.; Phan, K.; Hoppe, T.; Paoletta, S.; Kozma, E.; Gao, Z. G.; Durell, S. R.; Jacobson, K. A.; Appella, D. H. Programmable Nanoscaffolds That Control Ligand Display to a G-Protein-Coupled Receptor in Membranes To Allow Dissection of Multivalent Effects. *J. Am. Chem. Soc.* **2014**, *136*, 12296–12303.

(27) Gorska, K.; Huang, K.-T.; Chaloin, O.; Winssinger, N. DNA-Templated Homo- and Heterodimerization of Peptide Nucleic Acid Encoded Oligosaccharides That Mimick the Carbohydrate Epitope of HIV. *Angew. Chem., Int. Ed.* **2009**, *48*, 7695–7700.

(28) Huang, K. T.; Gorska, K.; Alvarez, S.; Barluenga, S.; Winssinger, N. Combinatorial self-assembly of glycan fragments into microarrays. *ChemBioChem* **2011**, *12*, 56–60.

(29) Novoa, A.; Eierhoff, T.; Topin, J.; Varrot, A.; Barluenga, S.; Imberty, A.; Roemer, W.; Winssinger, N. A LecA Ligand Identified from a Galactoside-Conjugate Array Inhibits Host Cell Invasion by *Pseudomonas aeruginosa*. *Angew. Chem., Int. Ed.* **2014**, *53*, 8885–8889.

(30) Novoa, A.; Machida, T.; Barluenga, S.; Imberty, A.; Winssinger, N. PNA-Encoded Synthesis (PES) of a 10000-Member Hetero-Glyco-conjugate Library and Microarray Analysis of Diverse Lectins. *ChemBioChem* **2014**, *15*, 2058–2065.

(31) Diaz-Mochón, J. J.; Bialy, L.; Bradley, M. Full orthogonality between Dde and Fmoc: The direct synthesis of PNA−peptide conjugates. *Org. Lett.* **2004**, *6*, 1127−1129.

(32) Winssinger, N.; Harris, J. L.; Backes, B. J.; Schultz, P. G. From split-pool libraries to spatially addressable microarrays and its application to functional proteomic profiling. *Angew. Chem., Int. Ed.* **2001**, *40*, 3152−3155.

(33) Debaene, F.; Winssinger, N. Azidopeptide nucleic acid. An alternative strategy for solid-phase peptide nucleic acid (PNA) synthesis. *Org. Lett.* **2003**, *5*, 4445−4447.

(34) Pothukanuri, S.; Pianowski, Z.; Winssinger, N. Expanding the scope and orthogonality of PNA synthesis. *Eur. J. Org. Chem.* **2008**, 3141−3148.

(35) Chouikhi, D.; Ciobanu, M.; Zambaldo, C.; Duplan, V.; Barluenga, S.; Winssinger, N. Expanding the Scope of PNA-Encoded Synthesis (PES): Mtt-Protected PNA Fully Orthogonal to Fmoc Chemistry and a Broad Array of Robust Diversity-Generating Reactions. *Chem.—Eur. J.* **2012**, *18*, 12698−12704.

(36) Zambaldo, C.; Barluenga, S.; Winssinger, N. PNA-encoded chemical libraries. *Curr. Opin. Chem. Biol.* **2015**, *26C*, 8−15.

(37) Harris, J. L.; Winssinger, N. PNA encoding: From solution-based libraries to organized microarrays. *Chem.—Eur. J.* **2005**, *11*, 6792−6801.

(38) For parallel work with DNA encoding, see: Franzini, R. M.; Neri, D.; Scheuermann, J. DNA-Encoded Chemical Libraries: Advancing beyond Conventional Small-Molecule Libraries. *Acc. Chem. Res.* **2014**, *47*, 1247−1255.

(39) Debaene, F.; Mejias, L.; Harris, J. L.; Winssinger, N. Synthesis of a PNA-encoded cysteine protease inhibitor library. *Tetrahedron* **2004**, *60*, 8677−8690.

(40) Harris, J.; Mason, D. E.; Li, J.; Burdick, K. W.; Backes, B. J.; Chen, T.; Shipway, A.; Van Heeke, G.; Gough, L.; Ghaemmaghami, A.; Shakib, F.; Debaene, F.; Winssinger, N. Activity profile of dust mite allergen extract using substrate libraries and functional proteomic microarrays. *Chem. Biol.* **2004**, *11*, 1361−1372.

(41) Debaene, F.; Da Silva, J. A.; Pianowski, Z.; Duran, F. J.; Winssinger, N. Expanding the scope of PNA-encoded libraries: Divergent synthesis of libraries targeting cysteine, serine and metalloproteases as well as tyrosine phosphatases. *Tetrahedron* **2007**, *63*, 6577−6586.

(42) Winssinger, N.; Damoiseaux, R.; Tully, D. C.; Geierstanger, B. H.; Burdick, K.; Harris, J. L. PNA-encoded protease substrate microarrays. *Chem. Biol.* **2004**, *11*, 1351−1360.

(43) Diaz-Mochón, J. J.; Bialy, L.; Bradley, M. Dual colour, microarray-based, analysis of 10 000 protease substrates. *Chem. Commun.* **2006**, 3984−3986.

(44) Urbina, H. D.; Debaene, F.; Jost, B.; Bole-Feysot, C.; Mason, D. E.; Kuzmic, P.; Harris, J. L.; Winssinger, N. Self-Assembled Small-Molecule Microarrays for Protease Screening and Profiling. *ChemBioChem* **2006**, *7*, 1790−1797.

(45) Pouchain, D.; Diaz-Mochón, J. J.; Bialy, L.; Bradley, M. A 10,000 member PNA-encoded peptide library for profiling tyrosine kinases. *ACS Chem. Biol.* **2007**, *2*, 810−818.

(46) Daguer, J. P.; Zambaldo, C.; Ciobanu, M.; Morieux, P.; Barluenga, S.; Winssinger, N. DNA display of fragment pairs as a tool for the discovery of novel biologically active small molecules. *Chem. Sci.* **2015**, *6*, 739−744.

(47) Daguer, J. P.; Zambaldo, C.; Abegg, D.; Barluenga, S.; Tallant, C.; Muller, S.; Adibekian, A.; Winssinger, N. Identification of Covalent Bromodomain Binders from DNA Display of Small Molecules. *Angew. Chem., Int. Ed.* **2015**, DOI: 10.1002/anie.201412276.

(48) Meyer, R.; Giselbrecht, S.; Rapp, B. E.; Hirtz, M.; Niemeyer, C. M. Advances in DNA-directed immobilization. *Curr. Opin. Chem. Biol.* **2014**, *18*, 8−15.

(49) Lovrinovic, M.; Seidel, R.; Wacker, R.; Schroeder, H.; Seitz, O.; Engelhard, M.; Goody, R. S.; Niemeyer, C. M. Synthesis of protein−nucleic acid conjugates by expressed protein ligation. *Chem. Commun.* **2003**, *39*, 822−823.

(50) Kazane, S. A.; Axup, J. Y.; Kim, C. H.; Ciobanu, M.; Wold, E. D.; Barluenga, S.; Hutchins, B. A.; Schultz, P. G.; Winssinger, N.; Smider, V.

(51) Wang, L.; Zhang, Z. W.; Brock, A.; Schultz, P. G. Addition of the keto functional group to the genetic code of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 56−61.

(52) Gholami, Z.; Hanley, Q. Controlled Assembly of SNAP−PNA−Fluorophore Systems on DNA Templates To Produce Fluorescence Resonance Energy Transfer. *Bioconjugate Chem.* **2014**, *25*, 1820−1828.

(53) Flory, J. D.; Simmons, C. R.; Lin, S.; Johnson, T.; Andreoni, A.; Zook, J.; Ghirlanda, G.; Liu, Y.; Yan, H.; Fromme, P. Low Temperature Assembly of Functional 3D DNA−PNA−Protein Complexes. *J. Am. Chem. Soc.* **2014**, *136*, 8283−8295.

(54) Bohler, C.; Nielsen, P. E.; Orgel, L. E. Template Switching between PNA and RNA Oligonucleotides. *Nature* **1995**, *376*, 578−581.

(55) Ficht, S.; Mattes, A.; Seitz, O. Single-nucleotide-specific PNA−peptide ligation on synthetic and PCR DNA templates. *J. Am. Chem. Soc.* **2004**, *126*, 9970−9981.

(56) Kleiner, R. E.; Brudno, Y.; Birnbaum, M. E.; Liu, D. R. DNA-templated polymerization of side-chain-functionalized peptide nucleic acid aldehydes. *J. Am. Chem. Soc.* **2008**, *130*, 4646−4659.

(57) Brudno, Y.; Birnbaum, M. E.; Kleiner, R. E.; Liu, D. R. An in vitro translation, selection and amplification system for peptide nucleic acids. *Nat. Chem. Biol.* **2010**, *6*, 148−155.

(58) Niu, J.; Hili, R.; Liu, D. R. Enzyme-free translation of DNA into sequence-defined synthetic polymers structurally unrelated to nucleic acids. *Nat. Chem.* **2013**, *5*, 282−292.

(59) Dose, C.; Ficht, S.; Seitz, O. Reducing product inhibition in DNA-template-controlled ligation reactions. *Angew. Chem., Int. Ed.* **2006**, *45*, 5369−5373.

(60) Roloff, A.; Seitz, O. Reducing Product Inhibition in Nucleic Acid-Templated Ligation Reactions: DNA-Templated Cyclization. *ChemBioChem* **2013**, *14*, 2322−2328.

(61) Koripelly, G.; Meguellati, K.; Ladame, S. Dual Sensing of Hairpin and Quadruplex DNA Structures Using Multicolored Peptide Nucleic Acid Fluorescent Probes. *Bioconjugate Chem.* **2010**, *21*, 2103−2109.

(62) Meguellati, K.; Koripelly, G.; Ladame, S. DNA-Templated Synthesis of Trimethine Cyanine Dyes: A Versatile Fluorogenic Reaction for Sensing G-Quadruplex Formation. *Angew. Chem., Int. Ed.* **2010**, *49*, 2738−2742.

(63) Sleiman, M. H.; Ladame, S. Synthesis of squaraine dyes under mild conditions: applications for labelling and sensing of biomolecules. *Chem. Commun.* **2014**, *50*, 5288−5290.

(64) Shibata, A.; Abe, H.; Ito, Y. Oligonucleotide-Templated Reactions for Sensing Nucleic Acids. *Molecules* **2012**, *17*, 2446−2463.

(65) Gorska, K.; Winssinger, N. Reactions Templated by Nucleic Acids: More Ways to Translate Oligonucleotide-Based Instructions into Emerging Function. *Angew. Chem., Int. Ed.* **2013**, *52*, 6820−6843.

(66) Grossmann, T. N.; Seitz, O. DNA-catalyzed transfer of a reporter group. *J. Am. Chem. Soc.* **2006**, *128*, 15596−15597.

(67) Grossmann, T. N.; Roglin, L.; Seitz, O. Target-catalyzed transfer reactions for the amplified detection of RNA. *Angew. Chem., Int. Ed.* **2008**, *47*, 7119−7122.

(68) Chen, X. H.; Roloff, A.; Seitz, O. Consecutive Signal Amplification for DNA Detection Based on de Novo Fluorophore Synthesis and Host−Guest Chemistry. *Angew. Chem., Int. Ed.* **2012**, *51*, 4479−4483.

(69) Erben, A.; Grossmann, T. N.; Seitz, O. DNA-triggered synthesis and bioactivity of proapoptotic peptides. *Angew. Chem., Int. Ed.* **2011**, *50*, 2828−2832.

(70) Cai, J. F.; Li, X. X.; Yue, X.; Taylor, J. S. Nucleic acid-triggered fluorescent probe activation by the Staudinger reaction. *J. Am. Chem. Soc.* **2004**, *126*, 16324−16325.

(71) Pianowski, Z. L.; Winssinger, N. Fluorescence-based detection of single nucleotide permutation in DNA via catalytically templated reaction. *Chem. Commun.* **2007**, 3820−3822.

(72) Franzini, R. M.; Kool, E. T. 7-Azidomethoxy-coumarins as profluorophores for templated nucleic acid detection. *ChemBioChem* **2008**, *9*, 2981−2988.

(73) Franzini, R. M.; Kool, E. T. Efficient nucleic acid detection by templated reductive quencher release. *J. Am. Chem. Soc.* **2009**, *131*, 16021−16023.

(74) Abe, H.; Wang, J.; Furukawa, K.; Oki, K.; Uda, M.; Tsuneda, S.; Ito, Y. A reduction-triggered fluorescence probe for sensing nucleic acids. *Bioconjugate Chem.* **2008**, *19*, 1219−1226.

(75) Shibata, A.; Ito, Y.; Abe, H. RNA-templated molecule release induced protein expression in bacterial cells. *Chem. Commun.* **2013**, *49*, 270−272.

(76) Pianowski, Z.; Gorska, K.; Oswald, L.; Merten, C. A.; Winssinger, N. Imaging of mRNA in live cells using nucleic acid-templated reduction of azidorhodamine probes. *J. Am. Chem. Soc.* **2009**, *131*, 6492−6497.

(77) Gorska, K.; Keklikoglou, I.; Tschulena, U.; Winssinger, N. Rapid Fluorescence Imaging of miRNAs in Human Cells Using Templated Staudinger Reaction. *Chem. Sci.* **2011**, *2*, 1969−1975.

(78) Gorska, K.; Manicardi, A.; Barluenga, S.; Winssinger, N. DNA-templated release of functional molecules with an azide-reduction-triggered immolative linker. *Chem. Commun.* **2011**, *47*, 4364−4366.

(79) Chen, Y.; Kamlet, A. S.; Steinman, J. B.; Liu, D. R. A biomolecule-compatible visible-light-induced azide reduction from a DNA-encoded reaction-discovery system. *Nat. Chem.* **2011**, *3*, 146−153.

(80) Rothlingshofer, M.; Gorska, K.; Winssinger, N. Nucleic acid templated uncaging of fluorophores using Ru-catalyzed photoreduction with visible light. *Org. Lett.* **2012**, *14*, 482−485.

(81) Sadhu, K. K.; Winssinger, N. Detection of miRNA in Live Cells by Using Templated Ru[II]-Catalyzed Unmasking of a Fluorophore. *Chem.—Eur. J.* **2013**, *19*, 8182−8189.

(82) Sadhu, K. K.; Eierhoff, T.; Romer, W.; Winssinger, N. Photoreductive Uncaging of Fluorophore in Response to Protein Oligomers by Templated Reaction in Vitro and in Cellulo. *J. Am. Chem. Soc.* **2012**, *134*, 20013−20016.

(83) Matsuura, K.; Hibino, M.; Yamada, Y.; Kobayashi, K. Construction of glyco-clusters by self-organization of site-specifically glycosylated oligonucleotides and their cooperative amplification of lectin-recognition. *J. Am. Chem. Soc.* **2001**, *123*, 357−358.

(84) Melkko, S.; Scheuermann, J.; Dumelin, C. E.; Neri, D. Encoded self-assembling chemical libraries. *Nat. Biotechnol.* **2004**, *22*, 568−574.

(85) Williams, B. A. R.; Diehnelt, C. W.; Belcher, P.; Greving, M.; Woodbury, N. W.; Johnston, S. A.; Chaput, J. C. Creating Protein Affinity Reagents by Combining Peptide Ligands on Synthetic DNA Scaffolds. *J. Am. Chem. Soc.* **2009**, *131*, 17233−17241.

(86) Scheibe, C.; Wedepohl, S.; Riese, S. B.; Dernedde, J.; Seitz, O. Carbohydrate−PNA and Aptamer−PNA Conjugates for the Spatial Screening of Lectins and Lectin Assemblies. *ChemBioChem* **2013**, *14*, 236−250.

(87) Eberhard, H.; Diezmann, F.; Seitz, O. DNA as a Molecular Ruler: Interrogation of a Tandem SH2 Domain with Self-Assembled, Bivalent DNA−Peptide Complexes. *Angew. Chem., Int. Ed.* **2011**, *50*, 4146−4150.

(88) Abendroth, F.; Bujotzek, A.; Shan, M.; Haag, R.; Weber, M.; Seitz, O. DNA-Controlled Bivalent Presentation of Ligands for the Estrogen Receptor. *Angew. Chem., Int. Ed.* **2011**, *50*, 8592−8596.

(89) Ciobanu, M.; Huang, K.-T.; Daguer, J.-P.; Barluenga, S.; Chaloin, O.; Schaeffer, E.; Mueller, C. G.; Mitchell, D. A.; Winssinger, N. Selection of a synthetic glycan oligomer from a library of DNA-templated fragments against DC-SIGN and inhibition of HIV gp120 binding to dendritic cells. *Chem. Commun.* **2011**, *47*, 9321−9323.

(90) Niemeyer, C. M. Semisynthetic DNA−Protein Conjugates for Biosensing and Nanofabrication. *Angew. Chem., Int. Ed.* **2010**, *49*, 1200−1216.

(91) Gholami, Z.; Brunsveld, L.; Hanley, Q. PNA-Induced Assembly of Fluorescent Proteins Using DNA as a Framework. *Bioconjugate Chem.* **2013**, *24*, 1378−1386.

(92) Pal, S.; Deng, Z. T.; Ding, B. Q.; Yan, H.; Liu, Y. DNA-Origami-Directed Self-Assembly of Discrete Silver-Nanoparticle Architectures. *Angew. Chem., Int. Ed.* **2010**, *49*, 2700−2704.

(93) Goodman, R. P.; Schaap, I. A. T.; Tardin, C. F.; Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. *Science* **2005**, *310*, 1661−1665.

(94) Flory, J. D.; Shinde, S.; Lin, S.; Liu, Y.; Yan, H.; Ghirlanda, G.; Fromme, P. PNA−Peptide Assembly in a 3D DNA Nanocage at Room Temperature. *J. Am. Chem. Soc.* **2013**, *135*, 6985−6993.

(95) Kuzuya, A.; Sakai, Y.; Yamazaki, T.; Xu, Y.; Komiyama, M. Nanomechanical DNA origami single-molecule beacons directly imaged by atomic force microscopy. *Nat. Commun.* **2011**, *2*, No. 449.

(96) Yamazaki, T.; Aiba, Y.; Yasuda, K.; Sakai, Y.; Yamanaka, Y.; Kuzuya, A.; Ohya, Y.; Komiyama, M. Clear-cut observation of PNA invasion using nanomechanical DNA origami devices. *Chem. Commun.* **2012**, *48*, 11361−11363.