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DNA display of PNA-tagged ligands

A versatile strategy to screen libraries and control geometry of multidentate ligands

Nicolas Winssinger

Institut de Science et Ingénierie Supramoléculaires; Université de Strasbourg; CNRS; Strasbourg, France

Over the past decade, several technologies have emerged to access nucleic acid-tagged libraries and select the fittest compound within such libraries. This perspective focuses on recent development with PNA-tagged small molecules displayed on DNA templates for screening purposes and to probe the optimal geometry in multivalent interactions.

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Correspondence to: Nicolas Winssinger;
Email: winssinger@unistra.fr

The discovery of small molecules that bind selectively to a target and perturb its function lies at the core of chemical biology and drug discovery. While high throughput screening technologies have proven effective, there is a clear need to further accelerate this discovery process while reducing its cost. The emergence of technologies based on nucleic acid encoding of small molecules presents a new paradigm for the discovery of small molecule binders.[1,2] The nucleic acid tags offer two opportunities, first and foremost, it provides a robust and extremely sensitive means of identifying selected molecules; second, it can be used to program the assembly of multiple ligands or fragments thereof that act cooperatively in their interactions with a target. The fact that the inter-ligand geometry can be fine-tuned by changing the template sequence (assembly instructions) makes this latter point particularly appealing. At the core of these nucleic acid encoding technologies is the ability to prepare diverse small molecules with their nucleic acid tags. To this end, peptide nucleic acids[3,4] (PNA) stand out for their synthetic compatibility.[5,6] Based on the fact that PNA oligomers are prepared using peptidic coupling and are compatible with standard peptide chemistry, it is the only nucleic acid tag which can be co-synthesized with small molecules by traditional solid phases synthesis. As illustrated in **Figure 1A**, this makes PNA-encoded synthesis (PES) compatible with the powerful technique of split and mix whereby a batch of resin is split into different pools at each step a synthon of the library is introduced. The resin is then mixed and the cycle reiterated thus ultimately providing a library with all possible permutations of products. In PES, each steps resulting in addition of diversity is accompanied by the addition of a PNA codon assigned to that fragment of diversity. PES has thus far been used to prepare libraries of protease substrates,[7,8] mechanism-based protease inhibitors,[9-11] peptides,[11-14] carbohydrates[15] and heterocycles (triazoles, pyrazoles and pyrimidines).[16] Early screening efforts were leveraged on a selection of the fittest PNA-encoded compounds within a library by affinity and de-coding of their identity by hybridization of the selected members onto a DNA microarray.[9,10] This strategy has been elegantly extended to other forms of selections such as the identification of cell-penetrating peptides.[14]

Over the past three years, several reports describing the use of DNA to display PNA-tagged molecules (**Fig. 1B**) that interact cooperatively with their target were published. In the first one, DNA display was used to emulate the complex carbohydrate epitope of HIV's glycoprotein (GP120).[17] This epitope emanates from multiple copies a high mannose undecasaccharide. Based on evidences that only the terminal mannose disaccharides of this complex glycan were involved

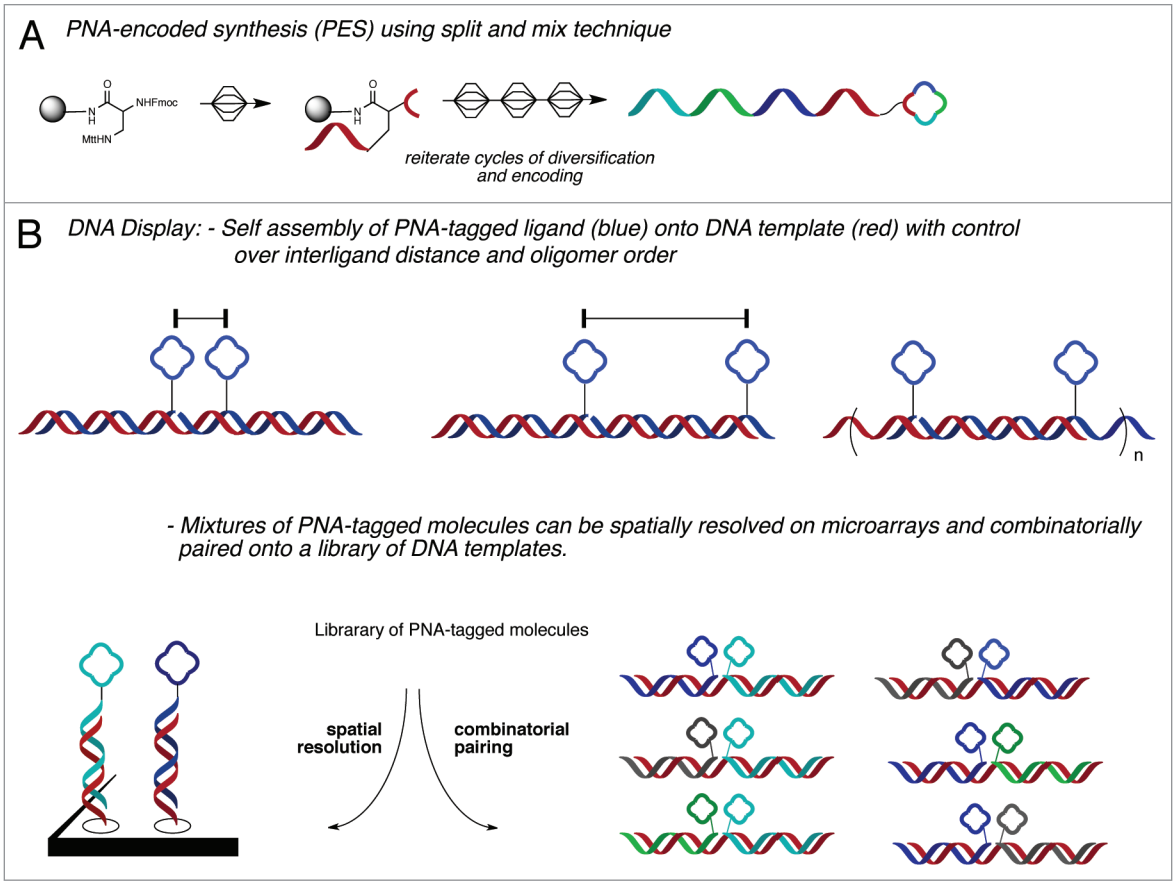

Figure 1. Synthesis and DNA display of PNA-tagged molecules. (**A**) Libraries of diverse PNA encoded molecules can be prepared by split and mix synthesis thus facilitating access to large libraries without recourse to complex infrastructure. (**B**) DNA display offers a simple means to build: (1) assemblies presenting multiple ligand with controlled density (inter-ligand distance) and oligomer order; (2) Organize the ligands on a surface or combinatorially pair ligand on a template. Ligands used in these assemblies can be derived from PNA-encoded synthesis as shown in **A** (a unique color is used for each ligand in **B** to simplify their representation). For the sake of clarity, only one example of each display is shown but other permutations are possible. For examples, it is possible to combinatorially pair ligands using a DNA microarray which includes different ligand pair and different inter-ligand distance or oligomer order.

in the preponderant interactions, it was hypothesized that this epitope could be mimicked using DNA to control the assembly of PNA tagged glycans containing strictly these terminal mannose fragments. As a proof of principle, 2G12, an antibody that broadly neutralizes HIV by tightly binding to the GP120 glycan epitope, was used for affinity measurements using surface plasmon resonance (SPR). A wide range of assemblies were evaluated from the combination of 14 different PNA-tagged glycans with different DNA templates thus providing diversity in the ligand pairs and inter-ligand distances. The optimal geometry afforded assemblies with a low micromolar affinity. It was clearly demonstrated that there was a relationship between the inter-ligand distance and the affinity for the target. The optimal inter-ligand distance could be controlled by changing the program of assembly with the DNA template. Subsequently, it was shown that DNA microarrays could be used to combinatorially assemble glycan fragments and that these fragments interacted cooperatively with lectins (concanavalin A and peanut lectin).[15] More recently, a similar strategy was used to evaluate the multivalent presentation of LacNAc (a disaccharide) and its impact on the affinity for the *Erythina cristagalli* lectin (ECL).[18] Impressively, the strategy was found productive to address binding sites on opposite faces of the protein with a 65 Å inter-site distance (requiring a linker of nearly 100 Å to accommodate the required bend around the protein). Interestingly, higher affinities were obtained by using a DNA template containing an unpaired region that provided increased flexibility.

Oligomerization of receptors induced or stabilized by polyvalent ligands is a fundamental principle in cellular recognition and signal transduction. Beyond glycans, nucleic-acid programmed self assembly was also used to investigate oligomers of a macrocylic peptides (16mer) which selectively bind to DR5, a member of the TRAIL receptor family.[19] It was demonstrated that the different oligomer architectures had significant impact on the kinetics of binding with the best assembly achieving dissociation half-life in excess of 30 min (10-fold enhancement). Most recently, this concept was used to target the $\alpha_v\beta_3$ integrin receptor with a cyclic RGD motif.[20] This receptor is involved in cellular adhesion and mobility and has been

implicated in metastasis of some cancer. The ability to rapidly produce systematically varied assemblies over a broad range of valancies and geometries allowed a fast optimization, which afforded assemblies with 100 fold enhancement in binding compared with the cyclic peptide alone. Most importantly, these assemblies were found to be efficacious in vivo, resulting in 50% reduction in tumor colonies following melanoma cells injection.

These examples illustrate the potential of cooperative interactions programmed through the instructions of DNA templates. Building on this concept and based on the fact that the DNA instructions of the assemblies can be readily amplified by PCR, this strategy has been harnessed for a more extensive combinatorial display of PNA-encoded fragments followed by a selection of the fittest assembly by affinity panning against an immobilized target. This idea was first reduced to practice with a screen against a representative target, carbonic anhydrase, by iterative cycles of affinity selection, amplification of DNA template and "translation" back into selected library members (**Fig. 2**).[16] In this example, the combinatorial output of the fragments produced a library of 62,500 combinations. Following the selection for the best binders, the DNA-based instructions were amplified by PCR using a biotinylated primer thus enabling the templating strand to be captured on a streptavidin resin. Exposure of this template to the library of PNA-encoded fragment led to the recapture of selected fragments, the remainder being removed with washing steps. Release from the streptavidin resin afforded the assemblies that were selected in the previous round. Thus the PCR-amplified templated could be converted back into the selected assemblies ("translation"). It was shown that reiteration of the cycle of selection/amplification provided a convergence toward a fragment set which, upon resynthesis as a covalent adduct had an affinity of 87 nM for carbonic anhydrase (neither fragment had an affinity below 1 μM). The same strategy was used to optimize binding to DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin), a tetrameric lectin implicated in interactions with a broad array of pathogens, using libraries of modified mannose disaccharides.[21] In this example, screening a library of over 37,000 members led to the identification of an assembly with 30-fold improved binding over the unmodified mannose assembly. A dendrimer derivatized with the identified ligand was able to outcompete the interaction of HIV's gp120 with dendritic cell at 10 μM. The ability to perform multiple rounds of selection/amplification has been the key to the remarkable success of biochemical selection systems such as phage display and SELEX, to identify binders from peptide and nucleic acid libraries. Taken together, these examples illustrate that DNA-display extends the scope of iterative selection/amplification technologies to glycans and small molecules broadly recognized as versatile pharmacophores. Most recently, a strategy to convert the PNA-tags of compounds selected in a screen into DNA for further amplification by PCR was reported. In this case, selected PNA were hybridized to a library of complementary ssDNA.[13] Unhybridized ssDNA was then degraded with a single-strand specific nuclease thus leaving only the DNA corresponding to the complementary PNA, which could be amplified. This strategy was used to discover new ligands for cell surface receptors using human cells overexpressing either integrins or the CCR6 receptor with a 10,000 compound library.

There are now a number of examples demonstrating that fittest molecules from a PNA-encoded libraries can be selected by different methods. Displaying the library onto DNA templates reconciles one of the foremost limitations of PNA, namely that it cannot be amplified by PCR. There are also several examples demonstrating that the optimal geometry in a multivalent interaction can be rapidly probed and optimized using a DNA template to display the PNA-tagged ligand. The recent demonstration that such constructs are functional in a whole organism represents an important step forward. We hope that these examples will stimulate further development in the area.

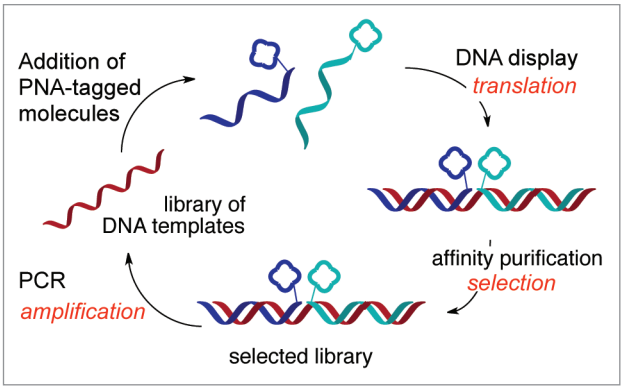

Figure 2. Selection and amplification of PNA-tagged molecules using DNA display.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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