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COMMUNICATION

Discovery of an entropically-driven small molecule streptavidin binder from nucleic acid-encoded libraries†

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Dehydrocholic acid was identified as a selective streptavidin binder from a PNA-tagged library. Isothermal calorimetry titration measurements showed this interaction to be entropically driven. Peptides tagged with dehydrocholic acid can be captured on a streptavidin resin and released under thermal conditions.

The interaction between biotin and streptavidin is amongst the strongest non-covalent interactions reported for a small molecule-protein complex.[1,2] This incredibly strong and selective host–guest interaction has been harnessed in countless applications and is of critical importance in many technologies.[3,4] The unique nature of this interaction coupled to the breadth of applications in which it has been implemented has inspired many research groups to find alternative streptavidin binders. To date, phage display and ribosomal display technologies have yielded low mM to nM binders with 3 to 18mer peptide[5–7] ligands (Fig. 1). SELEX technology has also been used to identify streptavidin binding aptamers from large libraries.[8,9] Several technologies to encode libraries of small molecules with nucleic acid tags have emerged over the past decade with the aim of facilitating and accelerating ligand discovery.[10–13] Screening of a DNA-encoded small molecule library led to the identification of a truncated version of biotin[14] and peptide-based small molecule with affinities of 1.3 nM and 0.35 μM respectively (Fig. 1).[15] We and others have opted for peptide nucleic acid (PNA)[16,17] to tag libraries based on its compatibility with classical solid phase split and mix combinatorial synthesis.[18–22] We have shown that the fittest ligands from such libraries could be selected against a

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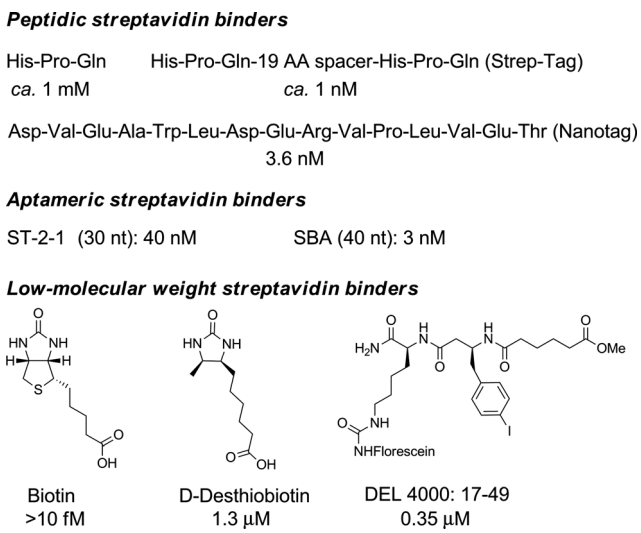

Fig. 1 Selected streptavidin binders. Peptidic binders: tripeptide,[5] streptag[7] and nanotag;[6] aptameric binders: ST-2-1,[8] SBA;[9] Small molecule binders: biotin, desthiobiotin,[14] DEL4000:17–49.[15]

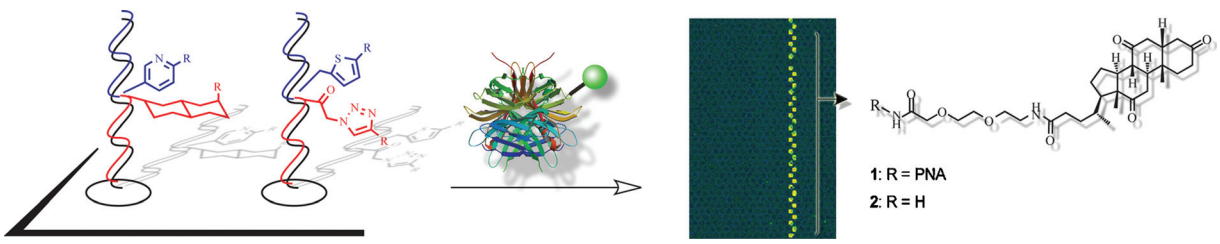

Fig. 2 Screening of self-assembled PNA-encoded library for streptavidin binder. The PNA-encoded library was hybridized onto a DNA microarray to produce 62 500 entities which were incubated with fluorescently labeled streptavidin. A fragment corresponding to dehydrocholic acid clearly emerges as the unique streptavidin binder within this library (bright green spots correspond to the microarray features containing the dehydrocholic acid fragment in conjunction with the different fragment pair).

targeted protein by different affinity purification methods.[23,24] It has also been shown that PNA encoded libraries can be selected for cell permeability and affinity to cell surface receptors.[25,26] More recently, we have shown that such PNA-encoded small molecule libraries could be combinatorially assembled onto a library of DNA templates[27] and displayed as adjacent pairs to select for drug fragments which bind cooperatively to a target.[28,29] Conversely, libraries can be hybridized directly onto a microarray containing the complementary DNA sequences and the microarray can be interrogated with a labelled protein for binders (Fig. 2). Screening for binders on a microarray does present differences compared to a selection of the fittest binders present as single entities in solution. In the microarray format, the potential ligands are present at a very high local concentration within a given spot rather than evenly distributed in solution. We had shown that this format was suitable to identify low affinity carbohydrate ligand pairs for lectins.[30] Microarray based screening of PNA encoded libraries has also been shown to be effective for whole cell assays.[31] Herein we applied this technology on a broader scale with a microarray containing 62 500 ligand pairs resulting in the identification of a novel streptavidin binding chemotype.

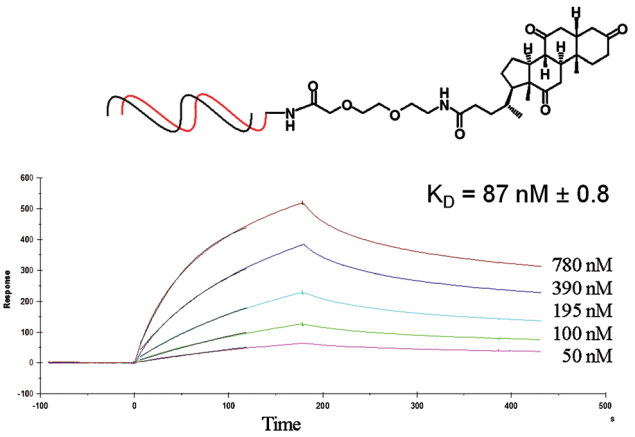

Fig. 3 SPR sensogram for DNA-PNA-dehydrocholic acid conjugate **1**.

The library of PNA-encoded small molecules containing various heterocycles and bioactive small molecule fragments[28] was hybridized to a custom array containing the complementary 62 500 sequences (Custom array containing up to 1 million unique oligonucelotides are now readily available through

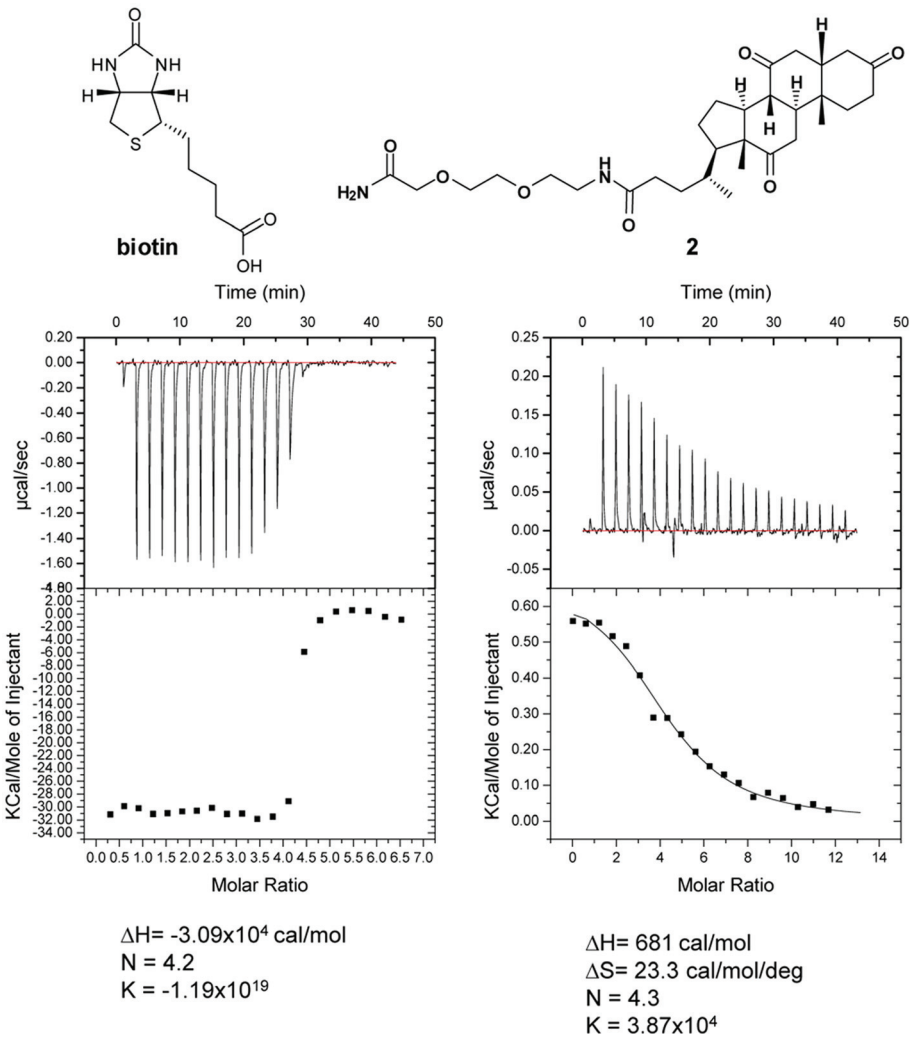

Fig. 4 Isothermal calorimetry (ITC) titration of streptavidin with biotin and PEG-dehydrocholic acid conjugate **2**.

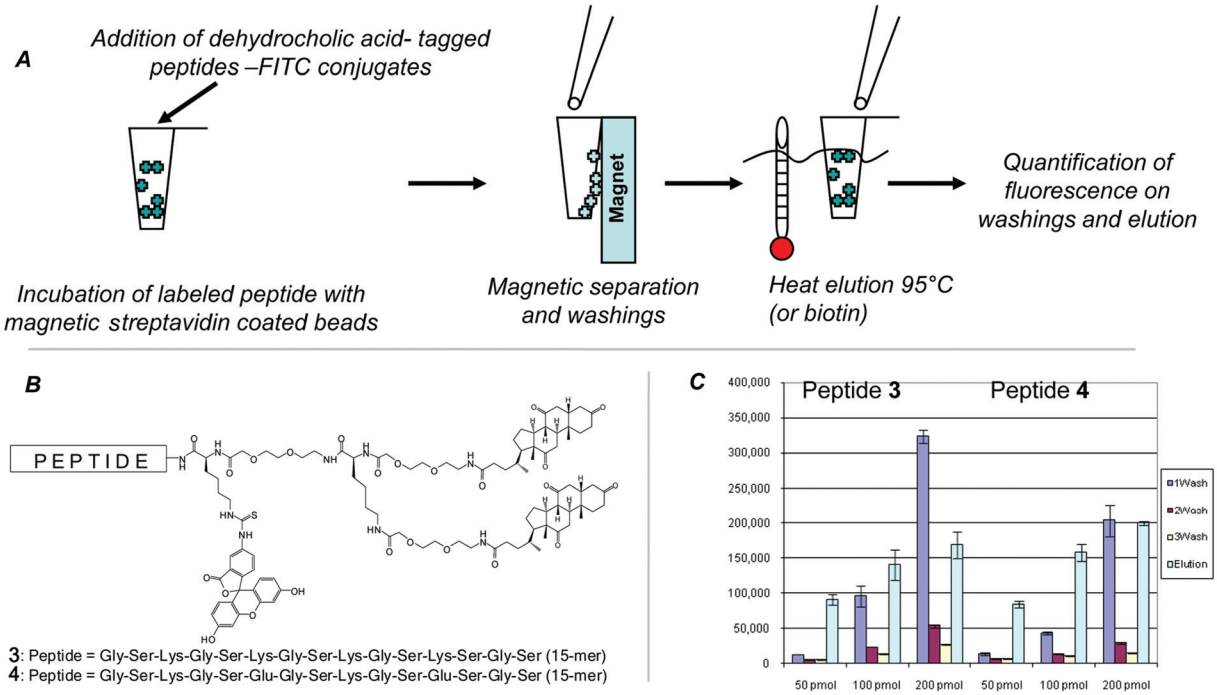

Fig. 5 Immobilization of dehydrocholic-tagged peptides on streptavidin coated magnetic beads. A. The peptides **3** and **4** are incubated with streptavidin coated magnetic beads, washed twice and heat denatured to recover the tagged peptide. Alternatively, the peptides could be eluted with biotin. B. Chemical structure of peptides **3** and **4**. C. Florescence measurements of washes and recovery for peptides **3** and **4** using 50 μL of streptavidin beads (theoretical loading of biotin is 35 pmol), error bars represent the standard deviation of three experiments.

commercial services: http://www.genomics.agilent.com/). The array was then interrogated for novel streptavidin binders using a 16 pM (1 ng mL^{-1}, 300 μL) solution of streptavidine-Dylight conjugate (Pierce) diluted in PBS buffer containing 0.05% Tween with 0.5% BSA as illustrated in Fig. 2. The fact that sub-nanogram quantities of protein are required for a screen attests to the miniaturization of this format. The microarray was exposed to this solution for 15 min with agitation at room temperature, the solution was removed and the array was washed three times with the same buffer (PBS-T) and one wash with mQ water. The microarray slide was dried by centrifugation and scanned (Dylight Ex. 635 nm; Em. 673 nm). Analysis of the microarray data showed that a single fragment was highly selected (see Fig. 2 for microarray picture and structure, see ESI for enlarged image†). Similar experiments carried out with other proteins (HSP90, HSP70, MEK ABL AXL kinases, carbonic anhydrase and bromodomains) did not show any binding for this fragment suggesting it was specifically targeting streptavidin. To our surprise, the selected fragment, dehydrocholic acid (a synthetic bile acid), has no structural or functional similarity to biotin. Two other steroid derivatives (prednisone and abietic acid) are present in the library but showed no detectable binding. Other lipophilic molecules known to bind promiscuously such as bexarotene neither showed detectable binding to streptavidin suggesting that the observed binding was not the product of unspecific interactions. To confirm the validity of this unexpected interaction, the PNA dehydrocholic adduct from the library was hybridized to a complementary DNA (14mer) and the assembly was used directly to measure kinetics of binding by surface plasmon resonance (SPR). As shown in Fig. 3, a K_d of 87 nM was measured for this interaction (a control with the PNA-DNA adduct devoid of dehydrocholic ligand showed no binding). Next, we compared the binding of the selected dehydrocholic PEG-amide derivative (**2**) with biotin by isothermal calorimetry (ITC). While the high concentration of ligand required for this experiment necessitated the use of 5% DMSO as a co-solvent, streptavidin was found to be functional under these conditions. As shown in Fig. 4, while the binding of biotin is highly exothermic ($\Delta H = -3.09 \times 10^4$ cal mol^{-1}),[32] compound **2** showed an endothermic binding ($\Delta H = 681$ cal mol^{-1}) with a favourable entropy $\Delta S = 23.3$ cal mol^{-1}/deg. As for biotin, a ligand stoichiometry of 4 was obtained for compound **2**, consistent with the tetrametric nature of streptavidin. The fact that this interaction results in a positive change in entropy suggests that it is the product of changes in solvation. While a dissociation constant of 26 μM was calculated under these conditions, which is nearly three orders of magnitude higher than measured for the PNA adduct by SPR, the discrepancy can be rationalized by the fact that the high level of DMSO used for the ITC effected the change in solvation and hence the changes in entropy upon binding.

We next asked whether dehydrocholic acid could be used as an affinity tag to isolate molecules of interest. To this end, two randomly selected peptides (15mer) containing a fluorescein (FITC) were synthesized by standard Fmoc-based chemistry, derivatized with a lysine bearing a short polyethylene spacer (PEG) and capped with two units of dehydrocholic acid using DIC/HOBt coupling. The peptides were cleaved from the resin and their capture on magnetic beads coated with streptavidin (Promega) was investigated. To this end, solutions containing 50 to 200 pmol of the peptides were incubated with 50 μL of streptavidin beads (theoretical loading of 35 pmol), washed with buffer and then heat eluted. As can be seen in Fig. 5, the peptide

is sequestered efficiently in both cases at the theoretical loading capacity of the resin. While addition of an excess of the peptide leaves fluorescence in the first and second washes, the third wash is essentially devoid of fluorescence which is recovered with high efficiency upon heat denaturation. Control peptides lacking the dehydrocholic tag were not retained on the streptavidin beads. Treatment of the streptavidin beads with 200 pmol of biotin led to the release of the peptide from the beads suggesting that biotin binds competitively with dehydrocholic acid derivative.

Conclusions

Dehydrocholic acid derivative **2** was identified as a new streptavidin binder from PNA-encoded libraries which were self-assembled onto a DNA microarray. The identified ligand does not bear structural similarities to biotin and in contrast to the latter, its binding is entropically driven and endothermic. The identified ligand was shown to be useful as an affinity purification tag with streptavidin resin.

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