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

Jean-Pierre Daguer,[a] Mihai Ciobanu,[a] Sofia Barluenga,[a] Nicolas Winssinger*[a]

Supporting Information

The microarray was custom designed and purchased from Agilent Technologies. A short spacer of 5 nucleotides (CTCGT) was included at the 3' end of the sequence (oligonucleotides are immobilized through the 3') before the 28 mers (2 x 14mers complementary to our previously designed PNA codon system) [1,2] and followed by a common 5mer (GTCGT).

The PNA encoded libraries were obtained as previously described [1] and hybridized on the microarray slide at 5 nM (concentration of individual probes) for 12 hours at 50 °C in SSC buffer (A 20X stock solution consists of 3 M sodium chloride and 300 mM trisodium citrate, adjusted to pH 7.0 with HCl) with 40 % formamide.[2] Upon cooling, the solution was removed and the array was washed by immersion in SSC buffer (30 sec) followed by a wash with mQ water.

Microarray Screen

Streptavidin, DyLight 650 Conjugated (Pierce) (SA-Dylight) diluted 1 ng/ mL on PBS-T (50 mM Na phosphate, 150 mM NaCl, 3mM KCl, 0.05% Tween 20) with 0.5%BSA, was incubated on the microarray for 15 min with agitation at room temperature. The excess SA-Dylight was washed by immersion in three baths with PBS-T (10 sec), one wash with mQ water and the microarray slide was dried by centrifugation (4000 g). The array was scanned at 635 nm wavelength (red laser excitation, filter at 670DF40) on a GenePix 3100 Personal Scanner.

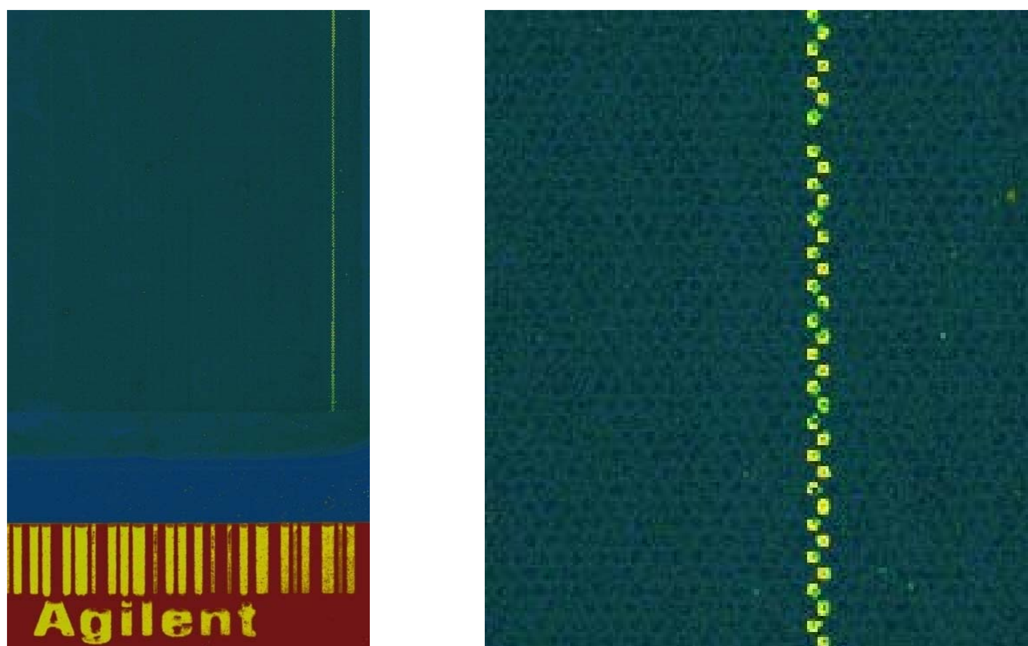


Fig S1 Fluorescence image of full array (left), an a blow up of a portion of the array (right).

Surface Plasmon Resonance

PNA conjugate 1/DNA hybrids were prepared by mixing equimolar amounts of both molecules to a final concentration of 5 µM in HBS buffer (10 mM Hepes, pH 7.2, 150 mM NaCl, 3 mM EDTA, 0.005% Tween-20). Streptavidin was immobilized on a CM5 Biacore TM chip (EDC-NHS activated) according to the procedure recommended by the manufacturer using a 200 µg/ml streptavidin solution in a sodium acetate buffer, pH, 4.5, approximately 7000 RU of protein were immobilized.

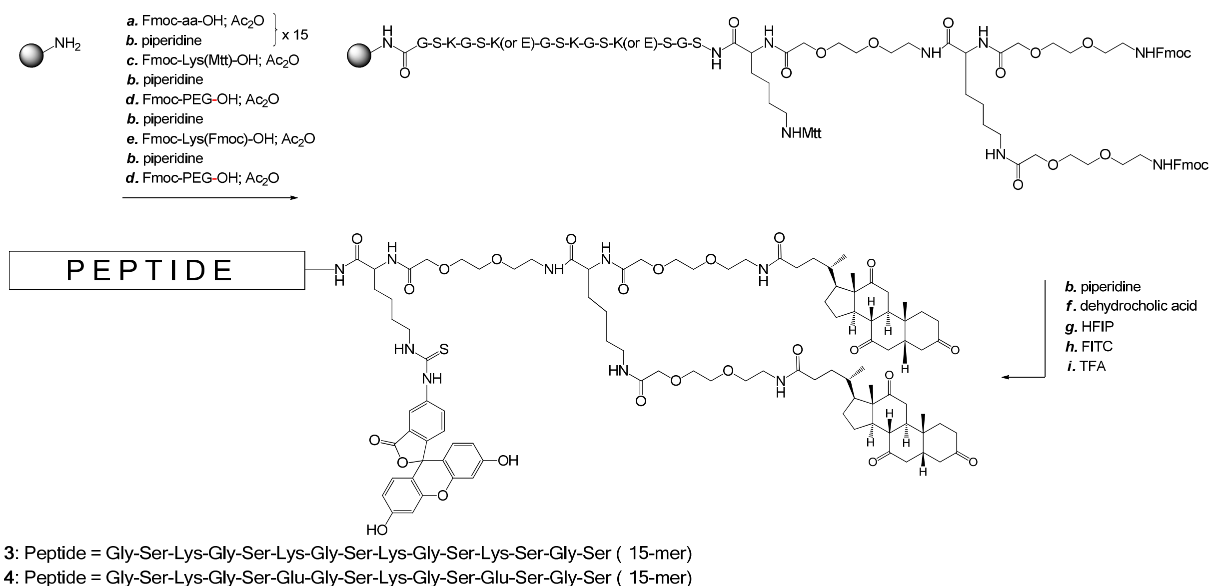
All experiments were performed on a Biacore 3000 at 25°C with a flow rate of 20uL/min. The PNA/ DNA hybrids were injected at increasing concentrations in order to determine the kinetic constants of the interaction.

ITC

The affinities were measured using a microcalorimetric ITC 200 (Microcal). Streptavidin was dissolved at 30 µM in 0.1M Tris pH 8.8 (Trizma base adjusted with HCl), 0.3M NaCl buffer, 5% DMSO. Biotin was prepared at 300 µM in the same buffer and DHCA and DHCA-PEG were prepared at 2 mM. The ITC curves were obtained by treatment of the data with Origin 7 software (OriginLab).

DHCA labeled peptide affinity purification

Two different peptides were synthesized by standard Fmoc-based chemistry as shown in scheme S1.



Scheme S1. Synthesis of peptides **2** and **3**.

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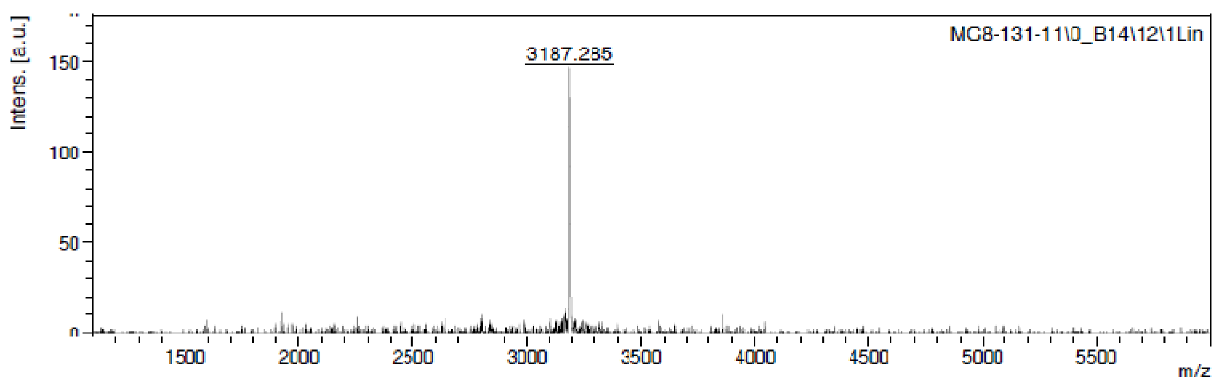

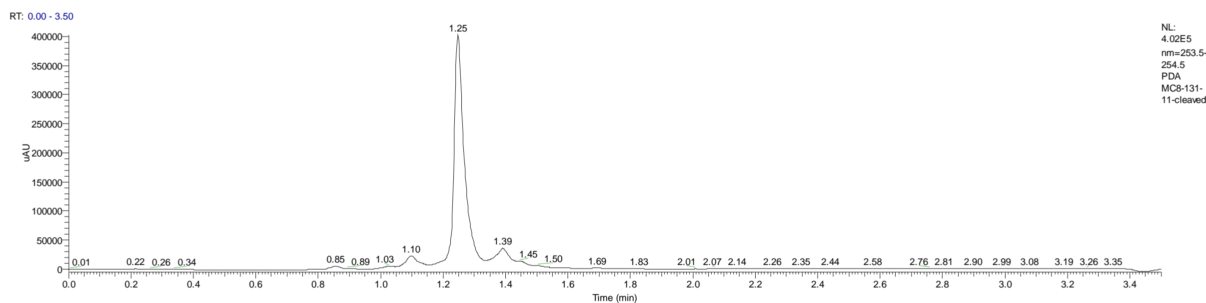

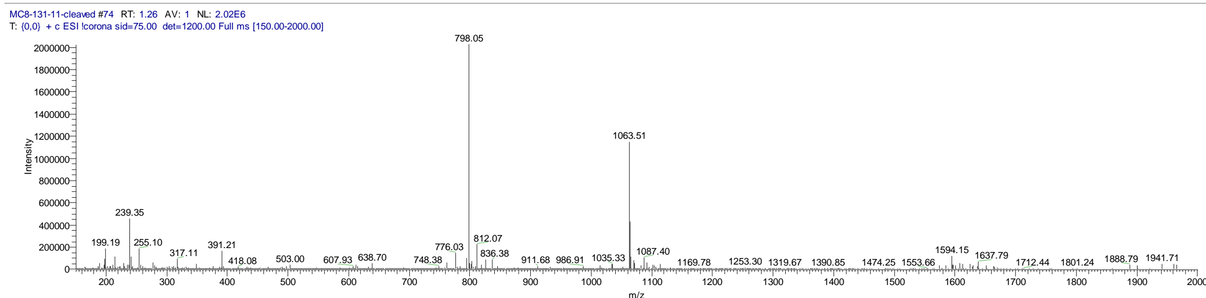

3: Gly-Ser-Lys-Gly-Ser-Lys-Gly-Ser-Lys-Gly-Ser-Lys-Ser-Gly-Ser (MW = 3186.37): MALDI analysis (top), HPLC trace (middle; Thermo C18 (5 cm x 2.1 mm, 1.9 µm particles) Hypersil gold column, linear elution gradient for 95% H_2O 0.01% TFA to 90% MeCN 0.01% TFA in 3.6 minutes at a flow rate of 1.0 $mLmin^{-1}$), ESI analysis of major peak (bottom, Surveyor MSQ Plus spectrometer)

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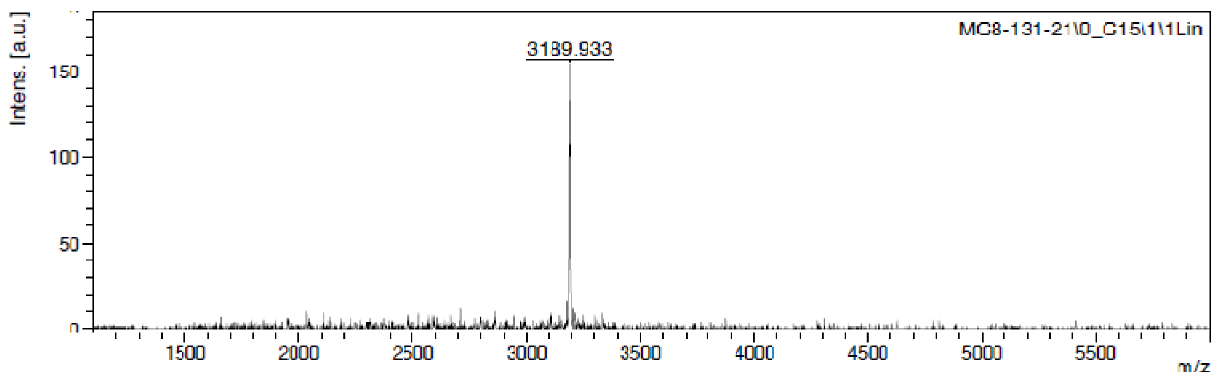

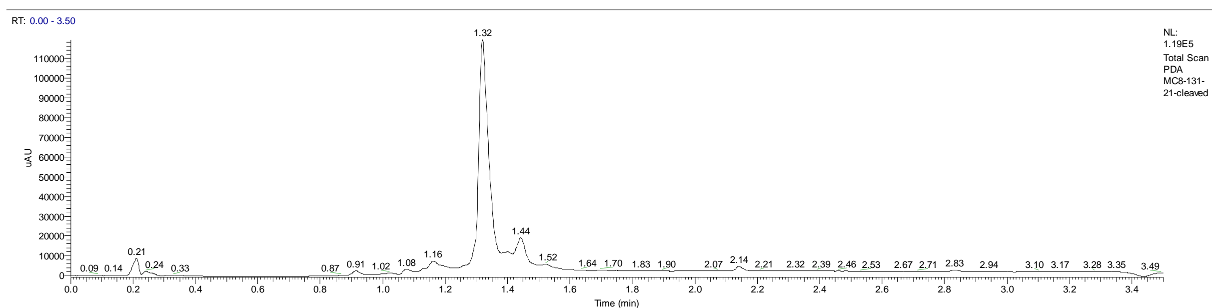

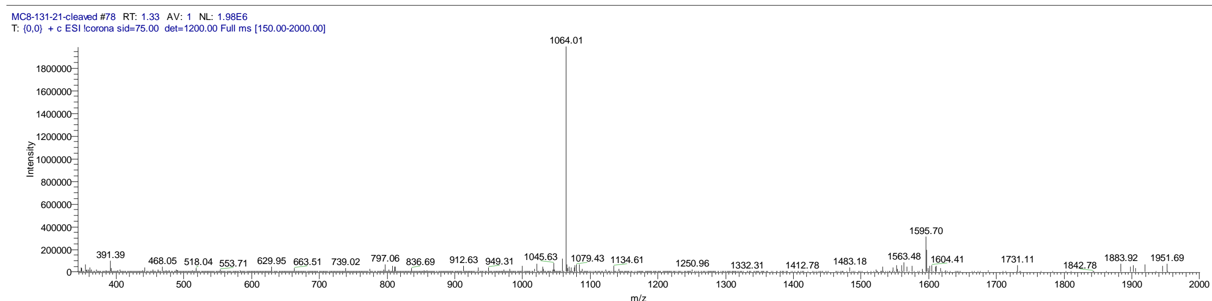

4: Gly-Ser-Lys-Gly-Ser-Glu-Gly-Ser-Lys-Gly-Ser-Glu-Ser-Gly-Ser (MW = 3188.27). MALDI analysis (top), HPLC trace (middle; Thermo C18 (5 cm x 2.1 mm, 1.9 µm particles) Hypersil gold column, linear elution gradient for 95% H_2O 0.01% TFA to 90% MeCN 0.01% TFA in 3.6 minutes at a flow rate of 1.0 $mLmin^{-1}$), ESI analysis of major peak (bottom, Surveyor MSQ Plus spectrometer)

Streptavidin affinity purification on DHCA-peptides

50 µl of Promega Paramagnetic SA beads were rinsed three times with PBS-T buffer to replace the storage buffer and then 50, 100 or 200 pmol of peptide **2** and **3** diluted in 100 µL of PBS-T (0.5, 1, 2 µM) were incubated with agitation at room temperature with the Streptavidin coated beads for 1 hour. Then the magnetic coated beads were washed three times with PBS-T and finally the ligands were eluted at 95°C for three minutes. The fluorescence intensity of the different fractions was measured a Spectramax fluotrometer (Molecular Devices).

1. J. P. Daguer, M. Ciobanu, S. Alvarez, S. Barluenga and N. Winssinger, *Chemical Science*, 2011, **2**, 625-632.

2. H. D. Urbina, F. Debaene, B. Jost, C. Bole-Feysot, D. E. Mason, P. Kuzmic, J. L. Harris and N. Winssinger, *ChemBioChem*, 2006, **7**, 1790-1797.
3. S. Melkko, C. E. Dumelin, J. Scheuermann and D. Neri, *Chem Biol*, 2006, **13**, 225-231.