Hybridization of single PNA-peptide conjugate



A 500 nM solution of the compound was used for hybridization on a microarray with 125 DNA oligos which are complementary to the 125 PNA oligos of the split-and-mix library (see general hybridization procedure above). The PNA hybridizes to the expected DNA oligomer (**ATGGATTGATGACTCTCT**) (1), while a weak signal is detected at a 10-mer complementary DNA oligo (GGGGATTGATGACTCTCT) (2). Fluorescently labelled DNA oligos were printed at various positions as controls (M). Each spot was printed in a 4 x 1 subarray format (Fig. 1). We expect that under competitive conditions (in the presence of the other library members), the selectivity should be enhanced.



Figure 1

Melting temperature PNA-DNA studies.

To a solution of the PNA-peptide library **2b** ($AA^4 =$ Phe and therfore125 different PNA oligos) in buffer (60mM NaCl, 6mM citric acid, 0.7% w/v *N*-lauroylsarcosine sodium salt, 0.1mM EGTA, pH 7.4) was added a mixture of their complementary 125 DNA oligos (see Library 4 DNA 384-well plate.xls file) in order to study PNA-DNA melting properties of this library. The study was carried out using a CARY 400 Scan UV Spectrophotometer, showing a sigmoidal shape curve (Figure 2a). Additional to this experiment the same mixture of DNA oligos was treated with PNA-peptide library **3** (unlabelled) which were non-complementary to these DNA sequences, no melting curve was observed (Figure 2b)





Figure 2. Melting curves of a) 125 complementary DNA-PNA sequences, b) 125 non-complementary DNA-PNA sequences





Scheme 1.Structure and Synthesis of the control peptide-PNA conjugate 2 and PNA encoded peptide libraries, protease library **1** and kinase library **2**. PNA code used for each library, * N-C sense (i) 1 Split (ii) NH2OH.HCl/imidazole, (iii) Dde-PNA(Mmt)-OH (5.5 eq.), PyBop (5 eq.), NEM (11 eq.) in DMF (0.1 M) 3h.; repeat (i) and (ii) two times (iv) 20% piperidine in DMF; (v) Fmoc-AA-OH (5.5 eq.), PyBop (5 eq.), DIPEA (16 eq.), HOBt (5.5eq) in DMF (0.08 M), 3h; (vi) mix and split; repeat (ii)-(vi); (vii) if necessary, labeling was carried out just before cleavage from the resin TFA/TIS/CH2Cl2 (90/5/5), 1 h.

DNA microarray production.

3'-amino modified DNA oligos were purchased from Bioneer (Korea). DNA printing was performed using a Robot Microarrayer (Genetix QMini, UK). 15 Solid pins were used in order to print 3'-amino modified DNA 18 mers on aldehyde-coated glass slides (Genetix) keeping humidity at 70%. Two different patterns were used generating microarrays with: a) 6x5 subarrays, where each DNA was printed five times or b) 6x4 subarray, in this case each DNA was printed four times. Then, the microarrays were kept overnight in a chamber containing 3M NaCl solution. Following imine reduction with NaBH₄, the chips were stored in dark before hybridization was carried out.

Hybridization onto DNA microarrays.

GenHYB buffer (Genetix) was demonstrated to give most selective hybridization. Before hybridization, solutions were heat denaturated at 90 °C for 5 min, then. 100μ L of the solution was poured on the DNA chip, which was covered with a glass cover-slide and kept in a hybridization chamber at 55 °C. Hybridization temperature was then slowly lowered to 20 °C during two hours, after which hybridization was carried out for further 90 min. Afterwards the chips were rinsed with saline buffer (30min), rinsed with dH₂O and spin-dried by centrifugation.

Scanning.

Microarrays were scanned with a CCD based fluorescence Scanner (Bioanalyser 4F, LaVision Biotech, Germany) using FITC filters. Analysis of the microarray images was made using FIPS software (LaVision BioTech).

Protease experiment (Pepsin).

A pool ($AA^4 = Leu$, therefore containing 125 members) of the PNA-peptide library **1** was diluted in a 0.2 M sodium citrate buffer (pH = 4.5) to give a final concentration of 500 nM. The solution was split in two equal aliquots adding pepsin solution (100nM final concentration) to one of them (solution B) while the other aliquot was kept as a control (solution A). Following two hours incubation at 37 °C both solutions were diluted with hybridization buffer (see above) to a final concentration of 50 nM followed by hybridization on DNA microarrays (Fig. 3).

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Figure 3 (a) Microarray (5x1 pattern) hybridized with library 1 ($AA^4 = Leu$); (b) Microarray hybridized with library 1 following pepsin incubation in solution; (c) Relative fluorescence increase of the 30 peptides most readily cleaved.

Interpretation of data. Median values from the five different features containing the same oligomer were calculated. In order to normalise the data, fluorescence intensities from DNA markers were compared (Intensity_{solution B}/Intensity_{solution A}), being 0.9 the factor obtained. Therefore, median values of the microarray hybridised with solution B were divided by 0.9. Relative fluorescence increases were calculated by dividing [Norm. Median Intensityⁿ_{solution B}/ Median Intensityⁿ_{solution A}]

Enzymatic abl assay. A pool (AA^4 = Phe, therefore containing 125 members) of the PNA-peptide library **2b** was incubated in the following buffer at a final concentration of 50 μ M : 120 U abl (New England Biolabs), 5 mM ATP, abl-buffer (10 x) diluted 1:10

(New England Biolabs), 0.1 % BSA (Sigma), at 30 °C for 24 h. Before hybridization, 1 volume of this buffer was diluted with 9 volumes of hybridization buffer (see above). After hybridisation, the slide was treated with FITC labeled anti-phosphotyrosine antibody (SIGMA) (diluted 1:10) in a saline phosphate buffer (pH 7.4) supplemented with 0.5 % tween and 1 % bovine serum albumine (SIGMA) for 1 h. The slide was washed 3 times with this buffer and 3 times with water, spin-dried by centrifugation before scanning.

Interpretation of data: only oligos having Median values above 10000 (background) for both **2a** and **2b** were considered. The ratio Median value (fluorescently labelled pool **2b** $(AA^4 = Phe))$ / Median value (unlabelled pool **2a** $(AA^4 = Phe)$) was used to calculate kinase activity.





Kinase library micro-array screening. Each spot was printed as 4 x 1 subarray, some fluorescently labeled DNA oligos were printed as controls (M) (a) Fluorescently labeled PNA-peptide control library; (b) PNA-peptide library after kinase treatment and detection with a fluorescent labeled anti-phosphotyrosine antibody;