SUPPORTING INFORMATION

Reliable microspotting methodology for high hybridization efficiency peptide-nucleic acid

layers on gold SPR imaging chips

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Scheme S1. The chemical structure of the thiol modified C-terminal end of the peptide nucleic acid strands used in the study. The thiol group is linked to the PNA strand by the manufacturer through a $(CH_2)_6$ spacer and an ethyleneglycol linker (AEEA- 2-(2-Aminoethoxy)ethoxy]acetic acid).



Figure S1. Typical SPR image (A) and differential SPR image of PNA spots immobilized in different experimental conditions

Table S1. Typical 8x8 layout of the SPR chips used in this study. With the latex particles used as position markers for easier assignment of the measured spots.

	А	В	С	D	Е	F	G	Н
1		LAT	ГЕХ		5 μM phPNA 18mer in SSC			
2	1.25	µM phPNA	A 18mer in	SSC	5 μM phPNA 12mer in SSC			
3	5	uM ssPNA	18mer in SS	SC	5 μM phPNA 18mer in PBS			
4	5	uM ssPNA	12mer in SS	SC	5 μM phPNA 12mer in PBS			
5	5	uM ssPNA	18mer in Pl	BS	5 μM phPNA 18mer in BBS			

6	5 μM ssPNA 12mer in PBS	5 μM phPNA 12mer in BBS
7	5 μM ssPNA 18mer in BBS	5 μM nonspecific phPNA 18mer in PBS
8	5 μM ssPNA 12mer in BBS	LATEX



Figure S2. Typical pre-screening results obtained by SPR imaging from microspotted PNA chips. The change of surface coverage upon injection of 1 μ M miRNA208a is shown for PNA spots microspotted with solid pins from various concentration and composition PNA solutions as shown in the figure. The difference in the worst and best conditions is ca. 100 fold.



Figure S3. Unprocessed SPR signals upon injection of 140 fmol miRNA 208a on the 18-mer phPNA $(5\mu M, PBS)$ spot and the relevant mercaptohexanol blocked background