Ligand dimerization programmed by hybridization to study multimeric ligand – receptor interactions

Katarzyna Gorska, [†a] Julien Beyrath, [†b] Sylvie Fournel,[b] Gilles Guichard[b,c] and Nicolas Winssinger*[a]

Supplementary Information

S1. Preparation of PNA-peptide conjugates

Loading of the first amino acid residue with reduction of resin loading to 0.2 mmol/g.

NovaPEG Rink amide resin (0.44 mmol/g, NovaBiochem) was swollen in CH_2Cl_2 for 20 min. The first Fmoc-protected amino acid residue (0.45 equiv., reduction of the resin loading to 0.2 mmol/g) was dissolved in anhydrous NMP and HOBt (2.5 equiv) followed by diisopropylcarbodiimide (DIC, 7.5 equiv) were added. The mixture was stirred for 15 min prior the addition to the resin and then shaken for 3 hours with the resin. The unreacted amino groups were capped (20 min) with a solution of acetic anhydride (5.3 equiv) and 2,6-lutidine (6.4 equiv) in DMF. Then, the resin was washed extensively with DMF and CH_2Cl_2 and dried.

Automated PNA synthesis.

PNAs were synthesized on an Intavis MultiPep instrument in a fully automated fashion in 500 μL fritted tubes. The resin (10 mg, 2 μmol, 1.0 equiv per column) was swollen in CH_2Cl_2 (300 μL) for 20 min and deprotected with 20% piperidine in DMF (2x5 min). The resin was then washed with DMF and CH_2Cl_2 and treated with a preactivated (5 min) solution of the corresponding PNA monomer or amino acid or PEG spacer (5.0 equiv), HATU (4.4 equiv), DIPEA (5.0 equiv) and 2,6-lutidine (7.5 equiv) during 20 min in NMP. This process was repeated once (double couplings). Each coupling was followed by a capping step with Ac_2O (5.3 equiv), 2,6-lutidine (6.4 equiv) in NMP (100 μL).

Mtt deprotecion.

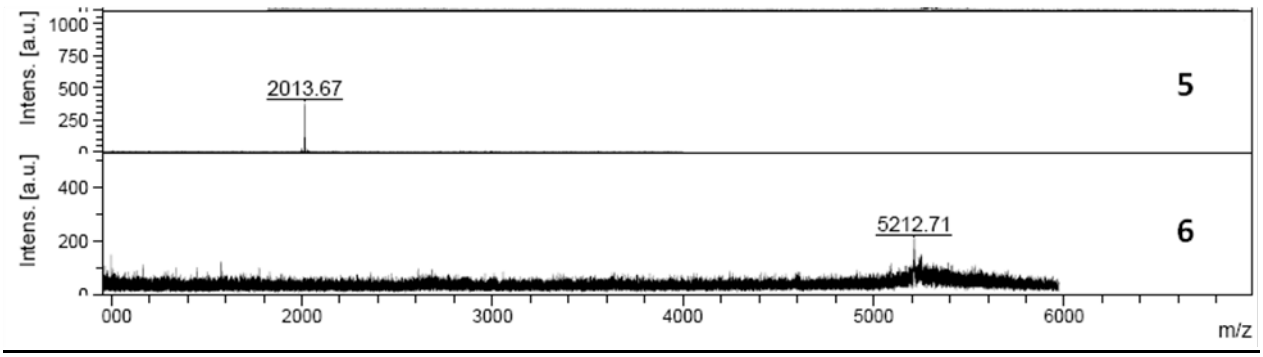
The resin (10 mg, 2 μmol, 1.0 equiv), was swollen in CH_2Cl_2 for 20 min and washed with a solution 1:1 of hexafluoroisopropanol (HF*i*P) in dichloroethane (100 μL) 15 cycles of 30 sec washes followed by a last incubation of 5 min. The resin was then washed with DMF and CH_2Cl_2.

Procedure 4. Cleavage from the resin.

The resin was treated with TFA (95% in H_2O, 500 μL for 2 μmol of resin) for 4 hours. The TFA solution was precipitated in Et_2O (10 times TFA volume) and centrifuged to recover the product as a pellet.

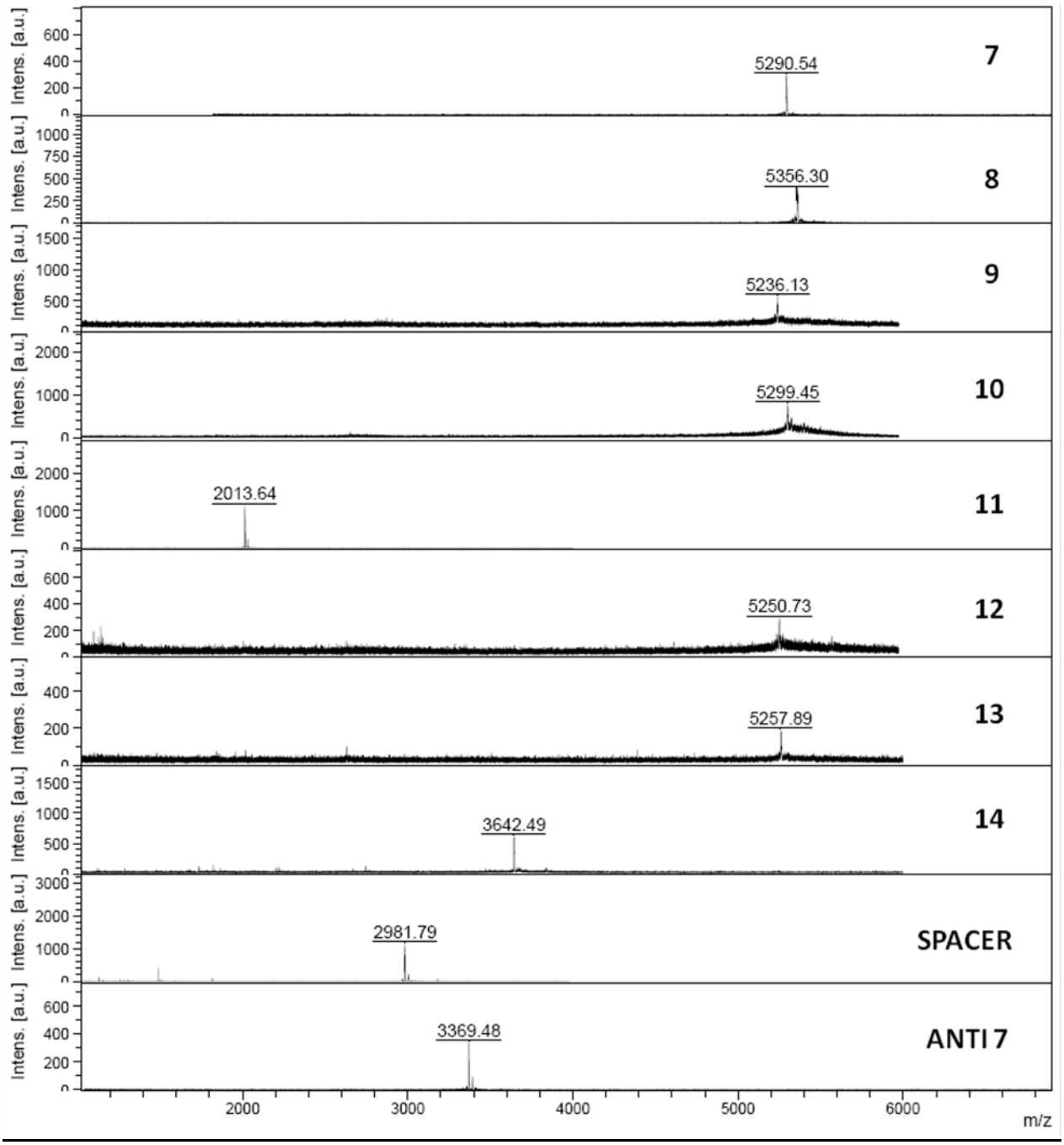
S2. Characterisation of PNA-peptide hybrids and auxiliary PNA:

Name	Peptide precursor[1]	PNA tag [2]	MW calculated	MW found MALDI TOF
5	NH2CO-LKVCQRRGIRNDLCDWAc	-	$[M+H]^+$= 2013.99	$[M+H]^+$= 2013.67
6	NH2CO-LKVCQRRGIRNDLCDWAc	GA*ACG*GCA*GC -$ArgNH_2$	$[M+H]^+$= 5212.42	$[M+H]^+$= 5212.71
7	NH2CO-LKVCQRRGIRNDLCDWAc	AEEA- GC*TGC*CGT*TC-$ArgNH_2$	$[M+H]^+$= 5289.45	$[M+H]^+$= 5290.54
8	NH2CO-LKVCQRRGIRNDLCDWAc	AEEA-GA*ACG*GCA*GC -$ArgNH_2$	$[M+H]^+$= 5356.49	$[M+H]^+$= 5356.30
9	NH2CO-LRVCQRKGIRNDLCDWAc	AA*GGC*GAC*GA-$ArgNH_2$	$[M+H]^+$= 5236.43	$[M+H]^+$= 5236.13
10	NH2CO-LRVCQRKGIRNDLCDWAc	AEEA- CG*TCT*GGC*AC-$ArgNH_2$	$[M+H]^+$= 5299.47	$[M+H]^+$= 5299.45
11	NH2CO-LRVCQRRGIKNDLCDWAc	-	$[M+H]^+$= 2013.99	$[M+H]^+$= 2013.64
12	NH2CO-LRVCQRRGIKNDLCDWAc	GC*CGT*GGG*TG-$ArgNH_2$	$[M+H]^+$= 5250.39	$[M+H]^+$= 5250.73
13	NH2CO-LRVCQRRGIKNDLCDWAc	AEEA-TC*CCA*GGC*TCA-$ArgNH_2$	$[M+H]^+$= 5257.44	$[M+H]^+$= 5257.89
14	NH2CO-LKAc	AEEA-GA*ACG*GCA*GC -$ArgNH_2$	$[M+H]^+$= 3642.68	$[M+H]^+$= 3642.49
SPACER	-	H2NCO-GTC*GTGG*CGA-NH_2	$[M+H]^+$= 2981.23	$[M+H]^+$= 2981.79
ANTI-7	-	NH2CO-ArgGA*ACG*GCA*GC-$ArgNH_2$	$[M+H]^+$= 3369.51	$[M+H]^+$= 3369.48



[1] Sequence written C to N, underlined residue to which PNA tag was attached

[2] Sequence written C to N, * denotes residues where GPNA was used (for GPNA see: (a) P. Zhou, M. Wang, L. Du, G. W. Fisher, A. Waggoner and Danith H. Ly, *J. Am. Chem. Soc.*, 2003, **125**;(b) Z.Pianowski, K. Gorska, L. Oswald, C. A. Merten and N.Winssinger, *J. Am. Chem. Soc.*, 2009, **131**, 6492)



S3. Assembly of supramolecular complexes.

PNA-peptide hybrids were dissolved in deionized water to obtain 1mM stock solutions. To break potential pre-existing secondary structures stock solutions where first kept at 45°C for 1h and then heated to 60°C. DNA templates were dissolved in deionized water to obtain 1mM stock solutions. To assemble supramolecular complexes appropriate volume of PNA stock solutions were added to sterile 1x PBS previously heated to 60°C, followed by (if applicable)

DNA. Resulting solution was kept at 60^{o}C for 5minutes and then slowly cooled to room temperature. Final complex concentration – 100 μM.

S4. Nucleic acid sequences for supramolecular complexes:

Entry	PNA tagged segment 1	PNA tagged segment 2	DNA sequence
C1	**12**	**12**	TCGGCACCCACCGGCACCCACT
C2	**12**	**9**	TCGGCACCCACTTCCGCTGCTT
C3	**12**	**6**	TCGGCACCCACCTTGCCGTCGT
C4	**12**	**7**	TCGGCACCCACATCACGCGCCT
C5	**12**	**10**	TCGGCACCCACGCAGACCGTGT
C6	**12**	**13**	TCGGCACCCACAGGGTCCGAGT
C7	**9**	**9**	TTTCCGCTGCTTTCCGCTGCTT
C8	**9**	**6**	TTTCCGCTGCTCTTGCCGTCGT
C9	**9**	**7**	TTTCCGCTGCTATCACGCGCCT
C10	**9**	**10**	TTTCCGCTGCTGCAGACCGTGT
C11	**9**	**13**	TTTCCGCTGCTAGGGTCCGAGT
C12	**6**	**6**	TCTTGCCGTCGCTTGCCGTCGT
C13	**6**	**7**	TCTTGCCGTCGATCACGCGCCT
C14	**6**	**10**	TCTTGCCGTCGGCAGACCGTGT
C15	**6**	**13**	TCTTGCCGTCGAGGGTCCGAGT
C16	**7**	**7**	TATCACGCGCCATCACGCGCCT
C17	**7**	**10**	TATCACGCGCCGCAGACCGTGT
C18	**7**	**13**	TATCACGCGCCAGGGTCCGAGT
C19	**10**	**10**	TGCAGACCGTGGCAGACCGTGT
C20	**10**	**13**	TGCAGACCGTGAGGGTCCGAGT
C21	**13**	**13**	TAGGGTCCGAGAGGGTCCGAGT

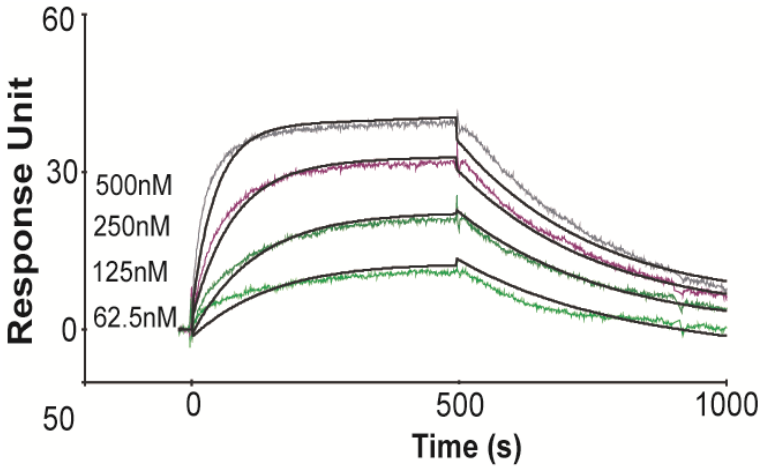
Nucleic acid sequences for supramolecular complexes prepared for detailed kinetic screen:

Entry	PNA tagged segment 1	PNA tagged segment 2	Auxiliary PNA	DNA sequence
3	14	14	-	TCTTGCCGTCGCTTGCCGTCGT
4	14	13	-	TCTTGCCGTCGAGGGTCCGAGT
5	13	13	-	TAGGGTCCGAGAGGGTCCGAGT
6	13	13	SPACER	TCTTGCCGTCGCAGCACCGCTCTTGCCGTCGT
7	7	7	-	TATCACGCGCCATCACGCGCCT
8	7	13	-	TATCACGCGCCAGGGTCCGAGT
9	7	8	-	-
10	7		Complementary to 7 (ANTI-7)	-

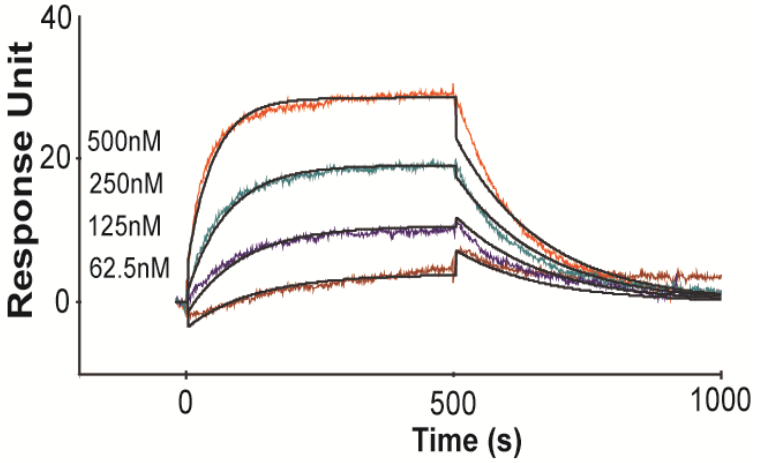
S5. Kinetic parameters of binding of the various compounds on DR5:

DR5 was immobilized on a CM5 sensor chip (Research Grade , Biacore AB) using amine coupling at 5µg/mL in 10mM acetate buffer (pH 5.0) according to the manufacturer's instructions. RANK, another receptor of the TNF superfamily was immobilized in the control channel according to the same procedure. The chip was then flushed with 1M ethanolamine hydrochloride (pH 8.5) (Biacore AB) and 25mM HCl to eliminate unbound protein. Biosensor assay were performed at 25°C in HBS-EP buffer [10mM HEPES (pH 7.4) containing 0.15M NaCl, 3.4mM EDTA, and 0.005% (v/v) surfactant P20] as running buffer. The compounds were injected sequentially in both channels (kinetic mode) at a flow rate of 30µL/min for 8 min and allowed to dissociate for an additional 10 min. The channels were then regenerated for 45 s with 25mM HCl. The RANK protein was considered as negative control, thus the control sensograms of RANK channel were subtracted from the DR5 one and analyzed with BIAevaluation version 4.1 using the simple 1:1 Langmuir binding model.

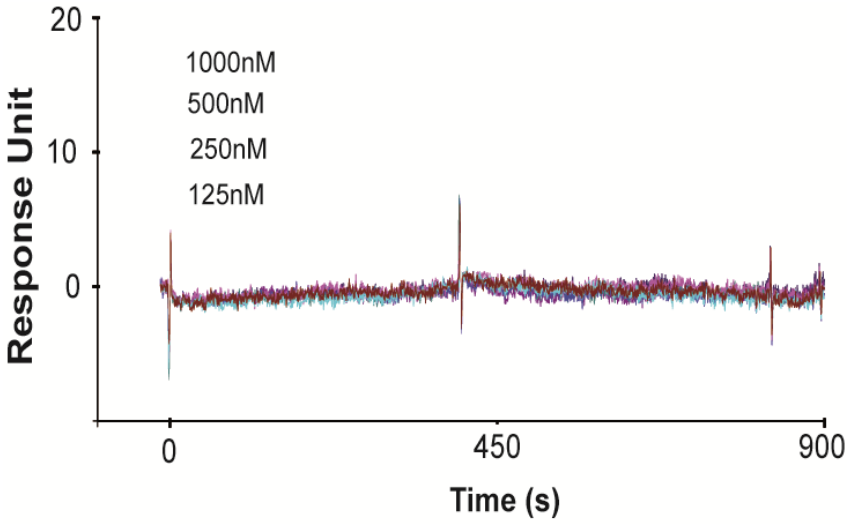
S6. Sensograms from detailed kinetic screen:



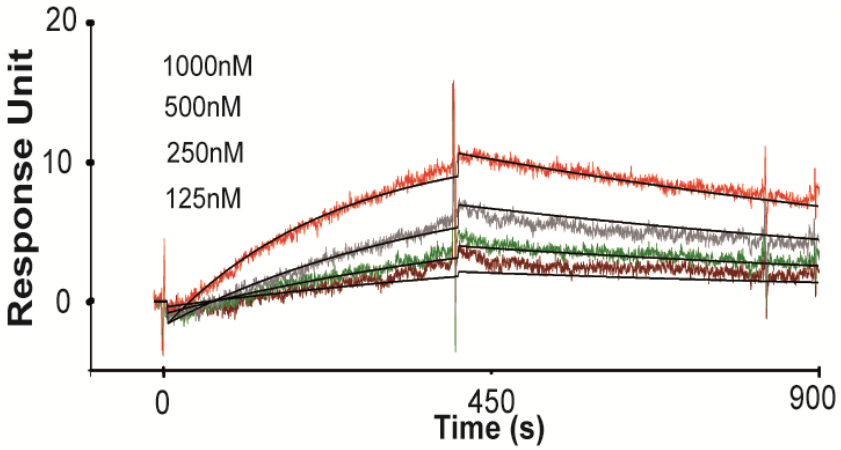
Entry 1: Compound 5 (chi^2=4.51).



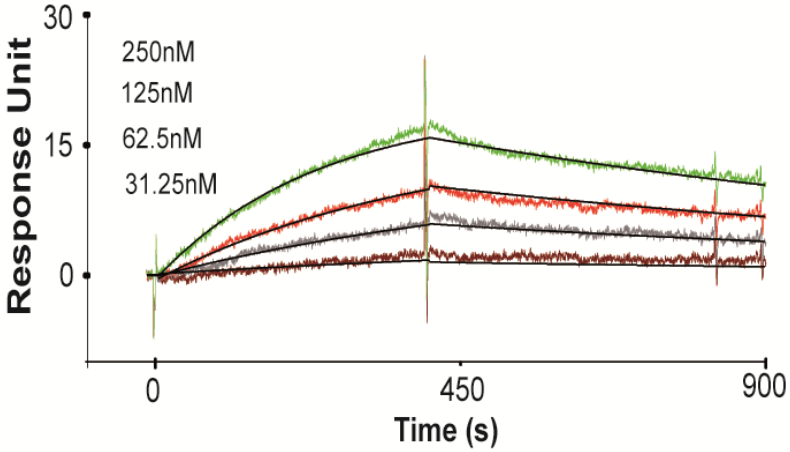
Entry 2: Compound 11 (chi^2=0.91).



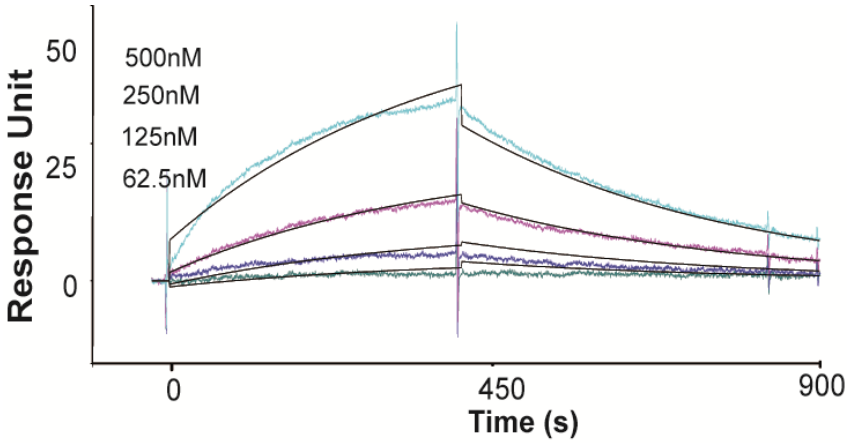
Entry 3: Compounds 14 + 14+ DNA template.



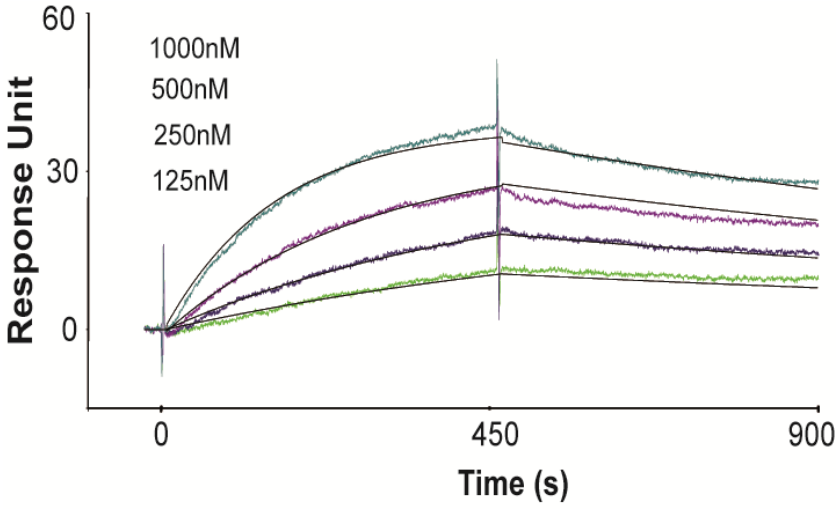
Entry 4: Compounds 13 + 14+ DNA template (chi^2=0.25).



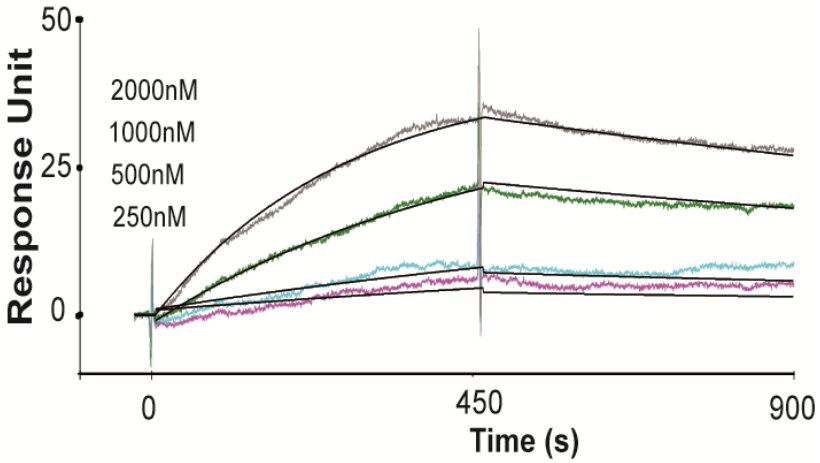
Entry 5: Compounds 13 + 13+ DNA template (chi^2=0.26).



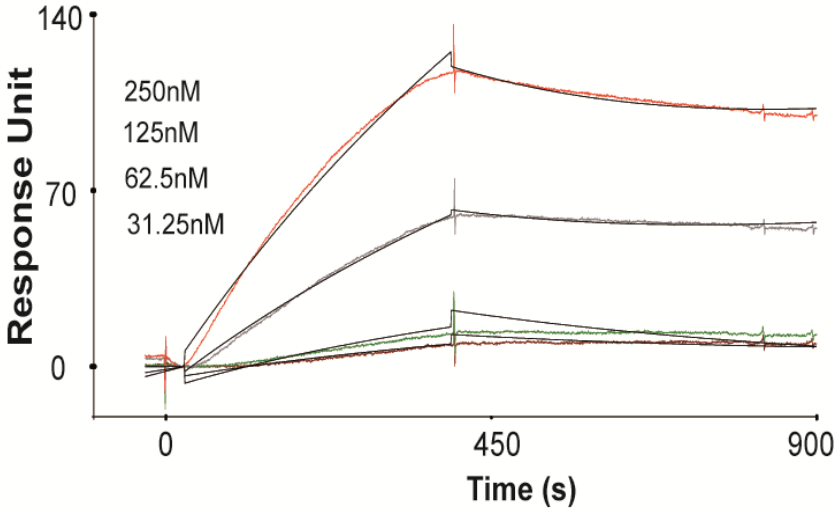
Entry 6: Compounds 13 + SPACER+13+ DNA template (chi^2=1.11).



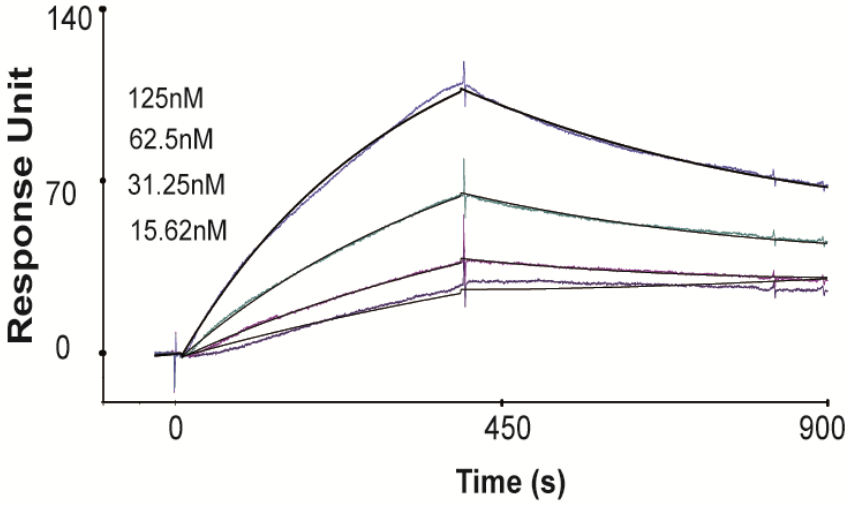
Entry 7: Compounds 7 + 7+ DNA template (chi^2=1.11).



Entry 8: Compounds 7 + 13 + DNA template (chi^2=1.21).



Entry 9: Compounds 7 + 8 (chi^2=4.50).



Entry 10: Compound 7 + complementary PNA – ANTI-7 (chi^2=1.74).