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Supporting Information

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Supporting Information

“DNA-controlled reversible switching of peptide conformation and bioactivity”

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General procedures and materials

Automated solid phase synthesis was carried out by using a microscale ResPep-Synthesizer (Intavis AG) in microscale columns for PNA synthesis (Intavis AG). DMF, NMP, Piperidine and TFA were purchased in peptide synthesis grade and used without further purification. Commerical reagents were used without further purifications. PNA-monomers were obtained from Perseptive Biosystems, standard Fmoc/tBu protected amino acids were purchased from Senn chemicals. HCTU was purchased from Iris Biotech and the Rink-amide resin as well as the phosphotyrosine building block Fmoc-Tyr($PO(NMe_2)_2$-OH were obtained from Novabiochem.

Yields of the PNA-peptide-PNA syntheses have been calculated using the extinction coefficients given below. Extinction coefficients of the PNA-peptide-PNA chimeras have been calculated using Proligos "Oligos Parameter Calculation"- tool (http://proligo2.proligo.com/Calculation/calculation.html) entering only the Nucleobases neglecting the small phosphotyrosine extinction coefficient at 260 nm. The extinction coefficients of FAM and phosphotyrosine are values taken from the literature (FAM: Invitrogen: Web edition of "The Handbook – A Guide to Fluorescent Probes and Labeling Technologies, Tenth edition", Data Table – 1.5 Fluorescein, Oregon Green and Rhodamine Green Dyes; pTyr: R. C. Cousins-Wasti, R. H. Ingraham, M. M. Morelock, C. A. Grygon, *Biochemistry* **1996**, *35*, 16746-16752)

Analytical HPLC-MS was performed on an Agilent 1100 HPLC-MS system equipped with a UV/Vis-detector and a VL-quadrupole mass spectrometer using a thermostated (55 °C) analytical CC 125/4 Nucleodur-C18 gravity, 3µ column (Macherey-Nagel) and a detection wavelength λ=260 nm. Eluents A (H_2O:MeCN:HCOOH = 98.9:1:0.1 (v:v:v)) and B (MeCN:H_2O:HCOOH = 98.9:1:0.1 (v:v:v)) were used in a linear gradient at a flow rate of 1 mL/min.

Gradient A: 3-30 % B in 15 min.

Gradient B: 3-60 % B in 15 min.

Preparative HPLC was performed on an Agilent 1100 HPLC system equipped with a multi wavelength detector using a semipreparative 250/10 C18-Polaris, 5µ column (Varian) and a detection wavelength λ=260 nm. Eluents A (H_2O:MeCN:TFA = 98.9:1:0.1 (v:v:v)) and B (MeCN:H_2O:TFA = 98.9:1:0.1 (v:v:v)) were used in a linear gradient from 3 % B to 80 % B in 30 minutes at a flow rate of 6 mL/min.

Automated solid-phase synthesis of PNA-phosphopeptide-PNA-chimera

Automated solid phase synthesis of PNA-phosphopeptide-PNA-chimera was performed in a 2 µMol scale using a Fmoc-Glycine preloaded tentagel Rink resin (NovaSyn TGR, Novabiochem) with a loading of 0.15 mmol/g, Fmoc/Bhoc-protected PNA-monomers and Fmoc-Ile-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH and Fmoc-Lys(Boc)-OH (Senn Chemicals). Phosphotyrosine was coupled as Fmoc-Tyr($PO(NMe_2)_2$)-OH, which was obtained from Novabiochem. For coupling of PNA-monomers the desired monomer was preactivated for 1 minute using 4 eq. of monomer, 3.6 eq. HCTU and 8 eq. *N*-methylmorpholine. Amino acids were preactivated for 30 seconds using 6 eq. amino acid, 5.4 eq. HCTU and 12 eq. *N*-methylmorpholine. Double couplings were used after assembly of the *C*-terminal PNA-segment. After completion of the linear assembly the *N*-Fmoc-protected chimera was cleaved of the resin by treatment with 1 mL TFA/H_2O/TIS/*m*-cresole (85:5:5:5/v:v:v:v). The resin was washed twice with 200 µL TFA. For the removal of the phosphotyrosine protecting group 10-Vol % H_2O was added to the solution. After shaking for 12-16 h the solution was concentrated under reduced pressure and the crude product precipitated with diethyl ether. After purification by preparative HPLC the *N*-terminal Fmoc-protecting group was removed by treatment with 200 µL piperidine/H_2O (1:1/v:v) for 3 minutes followed by acidification with 200 µL TFA and subsequent precipitation with diethyl ether. After purification by preparative HPLC the product was characterized by analytical HPLC-MS.

H-Gly-Lys-gcgtata-Gln-pTyr-Glu-Glu-Ile-ccaatac-Lys-Gly-NH_2 (1)

Yield: 34.0 OD_{260} (236.1 nMol. 11.7 %); ε_{260}= 144,000 $M^{-1}\cdot cm^{-1}$

HPLC/ESI-MS:	calculated:	4932.76
	found:	4932.29
	t_R: 3.39 min (Gradient A)	

$C_{197}H_{261}N_{96}O_{58}P$ (4932.76)

H-Gly-Lys-cgcgtata-Gln-pTyr-Glu-Glu-Ile-ccaatacg-Lys-Gly-NH_2 (2)

Yield 26.0 OD_{260} (166.4 nMol. 8.3 %); ε_{260}= 156,600 $M^{-1}\cdot cm^{-1}$

HPLC/ESI-MS:	calculated:	5435.25
	found:	5434.60
	t_R: 3.34 min (Gradient A)	

$C_{217}H_{287}N_{106}O_{64}P$ (5435.25)

H-Gly-Lys-tcgcgtata-Gln-pTyr-Glu-Glu-Ile-ccaatagca-Lys-Gly-NH_2 (3)

Yield 25.4 OD_{260} (141.7 nMol. 6.3 %); ε_{260}= 179,200 $M^{-1}\cdot cm^{-1}$

HPLC/ESI-MS: calculated: 5976.77

found: 5976.22

t_R: 3.45 min (Gradient A)

$C_{239}H_{314}N_{117}O_{70}P$ (5976.77)

Manual peptide synthesis

For the manual synthesis of the reference peptides standard Fmoc-based synthesis procedures were applied. The peptides were synthesized on a tentagel Rink resin (Tentagel S RAM, Iris Biotech) with a loading of 0.25 mmol/g. After each step the resin was washed with 3xDMF, 3xCH_2Cl_2, 3xDMF. Deprotection was achieved by treating the resin with 20 % piperidine in DMF for 2 minutes and subsequent washing with the same solution twice. Couplings were performed by shaking the resin for 30 minutes in a solution of 4 eq. of monomer, 3.6 eq. HCTU and 8 eq. *N*-methylmorpholine (2 minutes preactivation). The phosphotyrosine building block was Fmoc-Tyr(PO(NMe_2)$_2$-OH (Novabiochem), the other amino acids were incorporated using Fmoc/*t*Bu-protected building blocks. For capping the resin was treated with 40 % Ac_2O in pyridine. The FAM fluorophore (when needed) was incorporated as 5(6)-carboxyfluoresceine by double coupling of 20 eq. FAM, using 20 eq. PyBOP, 40 eq. of *N*-methylmorpholine and in situ activation. After completion of the linear synthesis the peptide was released by treatment of the resin with 1 mL TFA/H_2O/TIS/*m*-cresole (85:5:5:5/v:v:v:v). The resin was washed twice with 200 µL TFA. For the removal of the phosphotyrosine protecting group 10-Vol % H_2O was added to the solution. After shaking for 12-16 h the solution was concentrated under reduced pressure and the crude product precipitated with diethyl ether. Purification was performed by preparative HPLC.

FAM-Gly-pTyr-Glu-Glu-Ile-Ala-NH_2 (6) (5 µmol scale)

Yield 57.2 OD_{490} (733.3 nMol, 14.6 %); ε_{492}= 78,000 $M^{-1}\cdot cm^{-1}$

HPLC/ESI-MS: calculated: 1117.99

found: 1117.40

t_R: 10.00 min (Gradient B)

$C_{51}H_{56}N_7O_{20}P_1$ (1117.99)

Ac-Gln-pTyr-Glu-Glu-Ile-NH_2 (7) (20 µmol scale)

Yield 0.785 OD_{267} (1.20 µMol, 6.0 %); ε_{267}= 652 $M^{-1}\cdot cm^{-1}$

HPLC/ESI-MS:	calculated:	801.73
	found:	801.30
	t_R: 8.12 min (Gradient A)	

$C_{32}H_{48}N_7O_{15}P_1$ (801.73)

Overexpression and Purification of GST-Src-SH2

The Src-SH2 domain (ggSrc PubMed: aaa70194, 142-246, N243T corresponding to human Src 145-249, A168P, N224S, T246N) was cloned into the expression vector pGEX-4T1 to produce it as glutathione-S-transferase (GST) fusion protein in *Escherichia coli*. We purified GST-Src-SH2 by glutathione sepharose affinity chromatography, concentration with centrifugal concentrators (Vivaspin 10 kDa MWCO, Viva Science) and size exclusion chromatography (Superdex 75, Pharmacia, Uppsala, Sweden) to obtain a final purity of at least 98 %. The purified protein solution was aliquoted and stored at -80 °C after freezing in liquid nitrogen. Thawed aliquots were stored at 0 °C and used for a maximum of three days.

Thermal denaturation experiments

Denaturation experiments were performed on a Cary 100 Bio UV/Vis spectrophotometer (Varian) equipped with a peltier thermostated cell changer. We used a probe concentration of 1 µM in a freshly degassed 20 mM NaH_2PO_4, 100 mM NaCl buffer at pH 7.4 and a heating rate of 0.5 °C/min. The given T_M-value is the mean of the maximum of the first derivative of at least three denaturation curves. As an example the thermal denaturation curves and derivatives of the thermal denaturation of the DNA complexes of chimera **1** are shown in Figure S1.

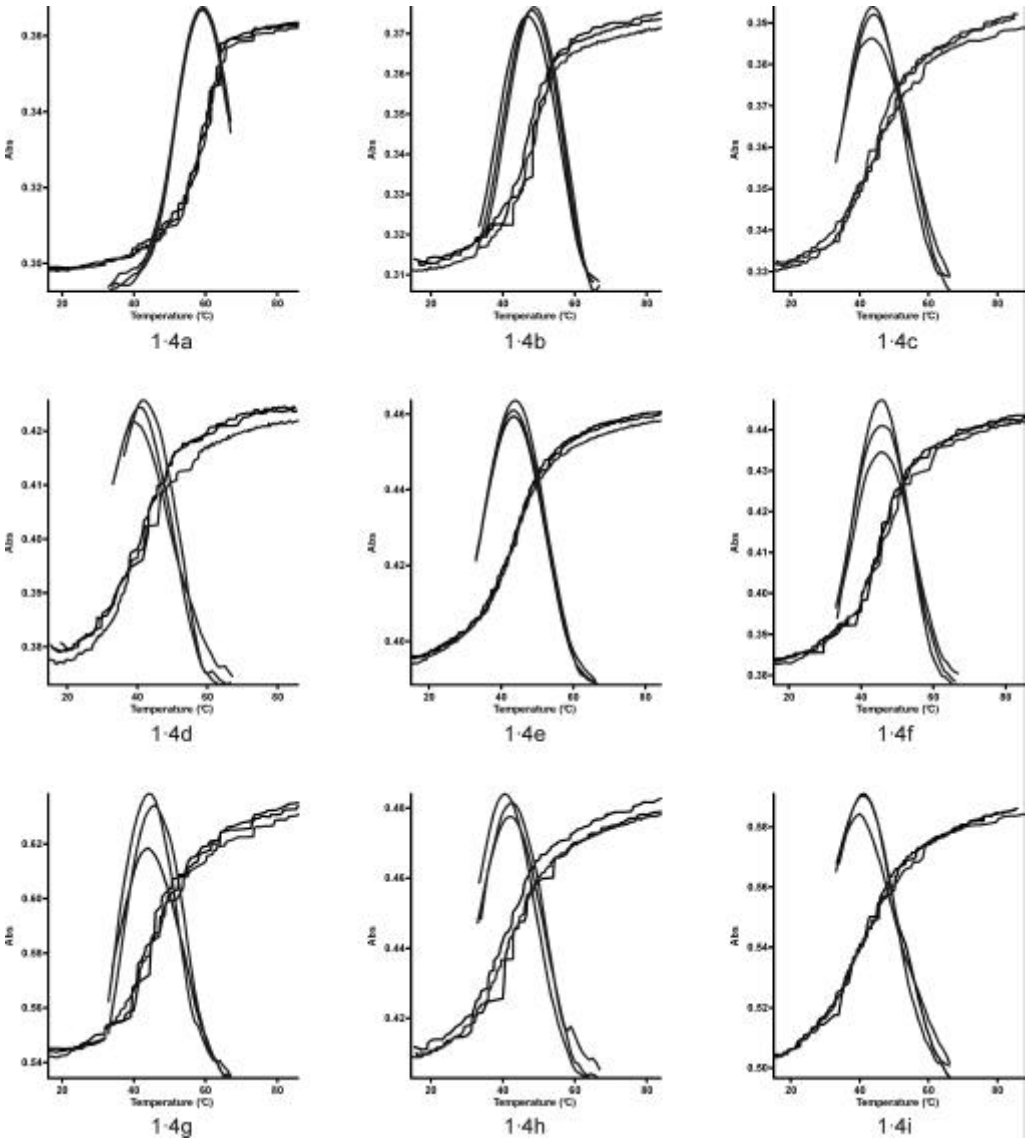

Figure S1: Thermal denaturation curves and their derivatives of the DNA complexes of chimera **1**.

Fluorescence polarization (anisotropy) experiments

Fluorescence anisotropy experiments were performed on a SPEX Fluoromax 3 fluorescence spectrometer (HORIBA Jobin Yvon) equipped with a peltier thermostated single cell holder (set to 25 °C) and automated polarizers. The buffer system was derived from that described by Lynch et al. (*Anal. Biochem.* **1997**, *247*, 77-82) but contained BSA instead of the more expensive BGG and was comprised of 20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT at pH 7.4, 0,1 % BSA. Slits were set to yield an intensity of approximately $1.0 \cdot 10^6$ counts with both polarizers set to vertical orientation.

Determination of IC_{50}-values

To 3 mL buffer (20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4, 0.1 % BSA) was added FAM-labeled reference peptide **6** from a 10 µM stock solution in 10 % Na_2CO_3 to a final concentration of 20 nM. The fluorescence polarization was measured. This value corresponded to fractional inhibition fi = 1, which is obtained if reference peptide **6** is free in solution. To the solution was added GST-Src-SH2 (from a 1.7 mM stock solution in 20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4) to a final concentration of 700 nM. The measured fluorescence polarization of the quantitatively formed peptide-SH2 complex corresponds to fractional inhibition fi=0. The PNA-phosphopeptide chimera **2** was added (from a stock solution in 10 % Na_2CO_3) to a final concentration of 250 nM. The measurement of the fluorescence polarization allowed the determination of the fractional inhibition value of the free chimera. In the subsequent experiments, 3 eq. of each DNA (**4a-i**) were added (from stock-solutions (175 µM – 300 µM) in 20 mM NaH_2PO_4, 100 mM NaCl, pH 7.4) to 200 µL aliquots of this solution. After equilibration the fluorescence polarization was recorded and the fi-value of the chimera•DNA duplexes was calculated. This procedure was repeated with 11-14 different concentrations of chimera **2** in a range of 250-4000 nM. Each datapoint is independent from the other datapoints. The calculated inhibition values were used to plot the fraction of bound reference peptide against the logarithmic concentration of the chimera or chimera-DNA duplex. Data analysis was performed using a sigmoidal dose response model with variable slope in GraphPad version 4.03. For the analysis the “top” parameter has been set as global (i.e. shared between all datasets) and “bottom” parameter has been fixed to 0.

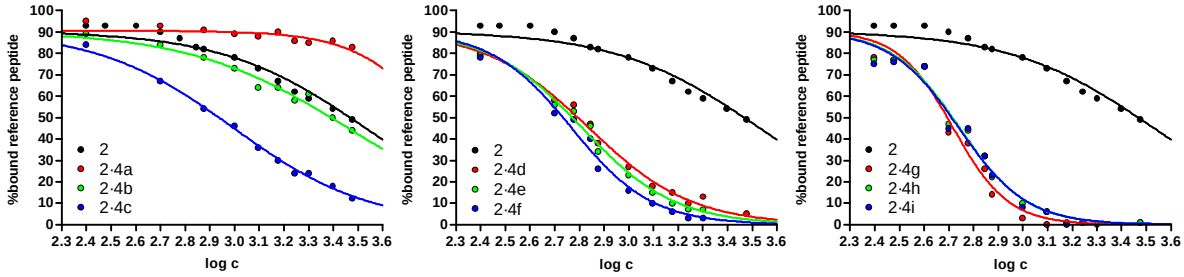

Figure S2: Inhibition curves of chimera **2** and the corresponding DNA-duplexes.

Figure S2 shows the inhibition curves of chimera **2** and its DNA duplexes. For clarity the inhibition curve of chimera **2** is shown in each graph together with the inhibition curves of three DNA complexes. The IC_{50}-values and other results of the fit are shown in table S1.

Table S1. IC_{50}-values and other parameters resulting from the fit of the inhibition values

	2	2·4a	2·4b	2·4c	2·4d	2·4e	2·4f	2·4g	2·4h	2·4i
IC50 / nM	3374	6920	2866	986,7	688	646,6	585,6	521,4	554,3	549,6
log IC50	3,528	3,84	3,457	2,994	2,838	2,811	2,768	2,717	2,744	2,74
Hill slope	-1,499	-2,584	-1,354	-1,582	-2,083	-2,322	-2,668	-3,88	-3,201	-3,147
top	90,55	90,55	90,55	90,55	90,55	90,55	90,55	90,55	90,55	90,55
					Std. Errors					
log IC50	0,02682	0,1108	0,026	0,02086	0,01596	0,01509	0,01452	0,01042	0,01148	0,01162
Hill slope	0,1568	0,9684	0,138	0,119	0,1595	0,1903	0,2384	0,3201	0,2481	0,2423
top	1,261	1,261	1,261	1,261	1,261	1,261	1,261	1,261	1,261	1,261

We wish to note that in case of chimera•DNA duplexes this procedure affords estimates of IC_{50}-values. Exact measurements are complicated due to the concentration dependence of the stability of chimera•DNA duplexes (which will have an effect at low concentrations). It is also difficult to account for the effect of the SH2 protein on the DNA affinity of the peptide chimera. The Hill slopes of up to -3.9 for the complex with DNA **4g** reflect these problems. Nevertheless the IC_{50}-values are of sufficient accuracy to select DNAs for the central switching experiments and at the concentration used for the strand exchange and reversible switching experiments (1.25 µM chimera) any DNA duplex should be formed quantitatively.

For the analysis of the binding properties of the unlabeled reference peptide Ac-Gln-pTyr-Glu-Glu-Ile-NH_2 (**7**) a solution conatining 20 nM reference peptide **6** and 700 nM GST-Src-SH2 was titrated twice with a 100 µM solution of **7**. The mean values of both titrations were fitted as described above yielding an IC_{50}-value of 5.51 µM and a Hill-slope of -1.0. The corresponding graph is shown in figure S3.

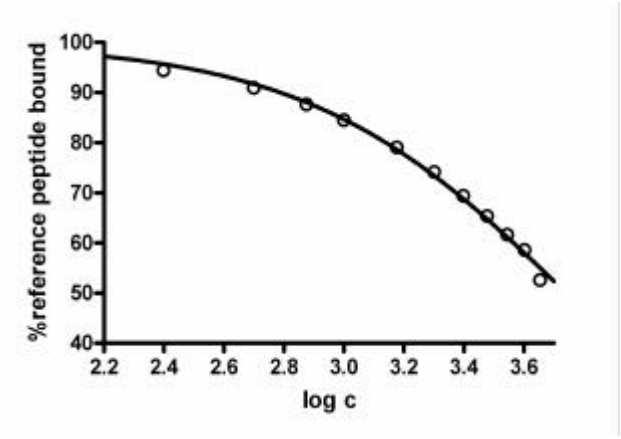

Figure S3. Inhibition curve of the unlabeled reference peptide **7**.

To explore the importance of the single stranded DNA parts opposite to the peptide part we also determined the IC_{50}-value of chimeras in which each of the left and right PNA arms was annealed with seperated DNA strands. We chose chimera **3** which has 9mer arms to provide for sufficient stability of

PNA-DNA hybrids. In the experiment PNA-phosphopeptide chimera 3 was allowed to hybridize with the DNA oligomers 5´-TGCTATTGG-3´ and 5´-TATACGCGA-3´ under the same conditions given for the reference peptide 7. The resulting IC_{50}-value is 0.98 µM with a hill slope of -1.8. The corresponding graph is shown in Figure S4.

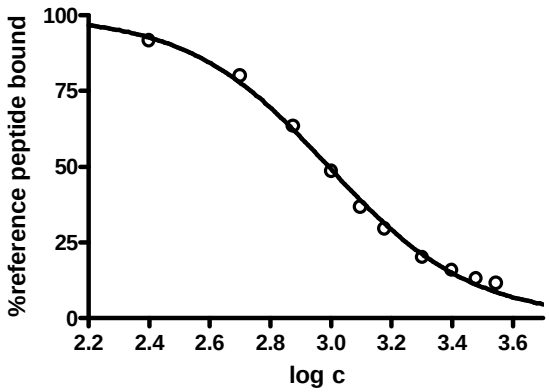

Figure S4. Inhibition curve of chimera **3** with PNA arm segments complexed to DNAs 5´-TGCTATTGG-3´ and 5´-TATACGCGA-3´.

These results indicate that there is also an activating effect of shorter DNA oligomers that hybridize to the PNA arm segments. Two explanations are possible for this fact. First, it might be possible that charge repulsion between the negatively charged duplex arms increase the population of molecules that adopt an extended conformation. Second, it is feasible that the DNA phosphoribose backbone slightly interacts with positively charged patches of the Src-SH2-domain.

Switching experiments

To 1 mL buffer (20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4, 0.1 % BSA) was added FAM-labeled reference peptide **6** from a 10 µM stock solution in 10 % Na_2CO_3 to a final concentration of 20 nM. The fluorescence polarization was measured. This value corresponded to fractional inhibition fi = 1, which is obtained if reference peptide **6** is free in solution. To the solution was added GST-Src-SH2 (from a 1.7 mM stock solution in 20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4) to a final concentration of 700 nM. The measured fluorescence polarization of the quantitatively formed peptide-SH2 complex corresponds to fractional inhibition fi = 0. The PNA-phosphopeptide chimera **1** was added (from a stock solution in 10 % Na_2CO_3) to a final concentration of 1.25 µM. The anisotropy was measured for 2.25 minutes (Figure 3). Data acquisition was paused for 30 s. In this period 1 equivalent of DNA **4a** or **4g** was added and the solution was mixed by pipetting up and down several times before

continuing data acquisition. To probe allosteric regulation 500 nM unlabeled pentapeptide Ac-Gln-pTyr-Glu-Glu-Ile-NH_2 **7** instead of chimera **1** was added to 20 nM FAM-labeled reference peptide **6**. The measurement of the fluorescence polarization revealed that neither DNA **4a** nor DNA **4g** has an effect on the resulting fractional inhibition value (Figure S5).

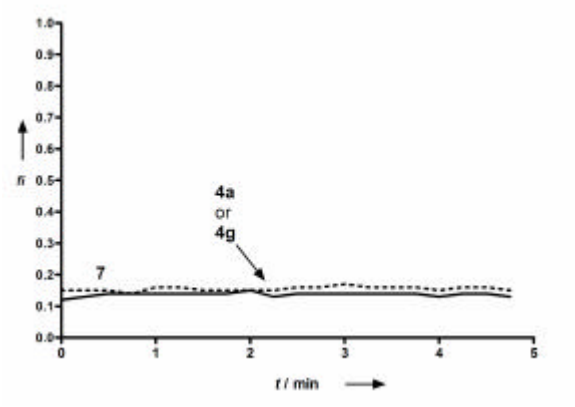

Figure S5. Control to rule out allosteric effects of DNA on the Src-SH2 protein

Strand exchange experiment (Figure 4)

To 0.8 mL buffer (20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4, 0.1 % BSA) was added FAM-labeled reference peptide **6** from a 10 µM stock solution in 10 % Na_2CO_3 to a final concentration of 20 nM. The fluorescence polarization was measured. This value corresponded to fractional inhibition fi=1, which is obtained if reference peptide **6** is free in solution. To the solution was added GST-Src-SH2 (from a 1.7 mM stock solution in 20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4) to a final concentration of 700 nM. The measured fluorescence polarization of the quantitatively formed peptide-SH2 complex corresponds to fractional inhibition fi = 0. A preformed complex of chimera **1** and DNA **5a** was added (from a stock solution of 100 µM **1** and 160 µM **5a** in mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4, 0.1 % BSA) to a final concentration of 1.25 µM **1•5a**. After 2.25 min (10 measurement cycles) data acquisition was paused for 30 s, in this period 10 µM of DNA **4g** were added and the data acquisition was continued after mixing the solution by pipetting up and down several times.

Reversible switching experiments (Figure 5)

To the solution (0.8 mL) of 20 nM FAM-labeled reference peptide **6** and 700 nM GST-Src-SH2 in buffer (20 mM NaH_2PO_4, 100 mM NaCl, 2 mM DTT, pH 7.4, 0.1 % BSA) was added chimera **1** to 1.25 µM concentration. One equivalent of DNA **4g** was added. The fractional inhibition value was determined as described before. Alternating addition of DNAs **4g** and **4g´** (from stock solutions of 224.7 µM (4g) and 202.5 µM (**4g´**) in 20 mM NaH_2PO_4, 100 mM NaCl, pH 7.4) in increasing concentrations (see Table S2)

each leading to an excess over the previous added DNA allowed four complete switching cycles without dramatic loss of dynamic range (Figure 5). Further DNA addition was avoided to minimize dilution effects.

Table S2. Reversible switching with increasing amounts of added DNA

	n added in nmol	Vol. added in µL	Concentration in µM	Total vol. added in µL	Dilution	Time of addition in min
DNA 4g	1,00	4,45	1,25	4,45	0,6%	2,25
DNA 4g´	2,00	9,88	2,50	14,33	1,8%	7,25
DNA 4g	2,00	8,90	3,75	23,23	2,9%	33,50
DNA 4g´	2,80	13,83	6,00	37,05	4,6%	38,50
DNA 4g	3,40	15,13	8,00	52,19	6,5%	64,75
DNA 4g´	4,80	23,70	12,00	75,89	9,5%	69,75
DNA 4g	5,60	24,92	15,00	100,81	12,6%	96,00
DNA 4g´	6,40	28,48	20,00	129,29	16,2%	102,25
DNA 4g´	4,00	17,80	25,00	147,10	18,4%	124,75

Further increases of the number of switching cycles were achieved by using only a constant excess of the switching oligonucleotide. Thus, after adding 1.25 µM DNA **4g** to the 1.25 µM solution of chimera **1** to achieve the first activation in the subsequent additions 3 µM of DNAs **4g´** and **4g** (from the same stock solutions as given above) were added (for details see Table S3). This allowed 10 complete switching cycles before reaching the self imposed volume alteration limit (Figure S6).

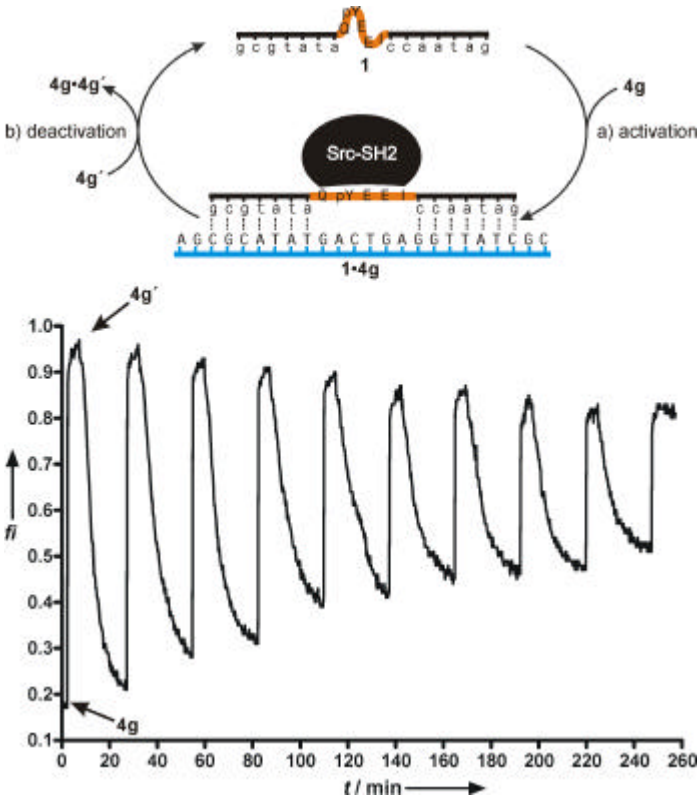

Figure S6. Reversible switching of peptide affinity by alternate addition of DNAs **4g** and **4g´** keeping the excess constant to achieve more switching cycles.

Table S3. Reversible switching by adding DNA in constant amounts.

	n added in nmol	Vol. added in µL	Concentration in µM	Total vol. added in µL	Dilution	Time of addition in min
DNA 4g	1,20	5,34	1,50	5,34	0,7%	2,25
DNA 4g´	2,40	11,85	3,00	17,19	2,1%	7,25
DNA 4g	2,40	10,68	4,50	27,87	3,5%	27,25
DNA 4g´	2,40	11,85	6,00	39,73	5,0%	32,25
DNA 4g	2,40	10,68	7,50	50,41	6,3%	54,75
DNA 4g´	2,40	11,85	9,00	62,26	7,8%	59,75
DNA 4g	2,40	10,68	10,50	72,94	9,1%	82,25
DNA 4g´	2,40	11,85	12,00	84,79	10,6%	87,25
DNA 4g	2,40	10,68	13,50	95,47	11,9%	109,75
DNA 4g´	2,40	11,85	15,00	107,32	13,4%	114,75
DNA 4g	2,40	10,68	16,50	118,00	14,8%	137,25
DNA 4g´	2,40	11,85	18,00	129,86	16,2%	142,25
DNA 4g	2,40	10,68	19,50	140,54	17,6%	164,75
DNA 4g´	2,40	11,85	21,00	152,39	19,0%	169,75
DNA 4g	2,40	10,68	22,50	163,07	20,4%	192,25
DNA 4g´	2,40	11,85	24,00	174,92	21,9%	197,25
DNA 4g	2,40	10,68	25,50	185,60	23,2%	219,75
DNA 4g´	2,40	11,85	27,00	197,45	24,7%	224,75
DNA 4g	2,40	10,68	28,50	208,14	26,0%	247,25

In both cases there is no technical reason why further switching cycles should not be possible but in this measurement setup a large volume increase should be avoided since the concentration of GST-Src-SH2 has a large influence on the anisotropy value. It should be noted, that the decrease in the dynamic range that can bee seen in Figure S6 originates from the intrinsic error of the DNA stock concentration determination. An estimated error of around 10 % accumulates fast in this setup which is based on adding exact equal amounts of switching DNA oligonucleotides. The dilution has no direct impact on the fluorescence anisotropy itself since it is a ratio quantity with no nominal dependence on dye concentration. (Invitrogen: Web edition of “The Handbook – A Guide to Fluorescent Probes and Labeling Technologies, Tenth edition”, Note – 1.5 Technical Focus: Fluorescence Polarization (FP)).