SUPPORTING INFORMATION FOR

"Kinetic Discrimination of DNA Quadruplex Targets by Guanine-Rich PNA Probes"

by Roy, S. et al

Experimental

Materials. DNA oligonucleotides were purchased from Integrated DNA Technologies (www.idtdna.com) and used without further purification. t-Boc protected peptide nucleic acid monomers were purchased from Applied Biosystems and used for standard solid phase synthesis of the PNA oligomers.[1, 2] (PNA monomers are no longer sold by this company. Presently they can be purchased from ASM Research Chemicals; Hannover, Germany). The PNA oligomers were purified by reverse phase HPLC and verified by MALDI-TOF mass spectrometry (Applied Biosystems, Voyager DE sSTR) using sinapinic acid as the matrix $\mathbf{P_{myc}}$: expected m/z for $[M+H]^+$, 2460.2; found, 2462.52. $\mathbf{P_{telo}}$: expected 2701.47; found, 2703.28; $\mathbf{P_{eg2}}$**:** expected 2185.2; found, 2188.72)

All DNA and PNA concentrations were determined by measuring the absorbance at 260nm at 85°C on a Cary 3 Bio spectrophotometer. At high temperatures the bases are assumed to be unstacked and the extinction coefficient of the oligomer is estimated as the sum of the individual bases. For the DNA oligomers the extinction coefficients were used as reported in literature.[3] The PNA extinction coefficients at 260nm were obtained from Applied Biosystems. (A: 13700 $M^{-1}cm^{-1}$; C: 6600 $M^{-1}cm^{-1}$; G: 11700 $M^{-1}cm^{-1}$ and T: 8600 $M^{-1}cm^{-1}$).

Circular Dichroism Spectropolarimetry. CD measurements were performed on a Jasco J-715 CD spectropolarimeter equipped with water circulating temperature controller. Samples were prepared by mixing all components together in 10mM Tris-HCl (pH 7), 0.1mM Na_2EDTA and 150 mM KCl. Samples were annealed by heating to 95°C for five minutes and then slowly cooling to room temperature. All spectra were collected at 37°C by equilibrating the solutions at

this temperature for 10min prior to recording. Each spectrum represents an average of 10 scans, collected at a rate of 100nm min^{-1}. The spectra were baseline corrected.

Surface Plasmon Resonance Experiments. Surface plasmon resonance (SPR) measurements were performed by using a Biacore 2000 system with four-channel, streptavidin-coated sensor chips (SA) for all experiments. This chip consists of a gold surface and streptavidin covalently immobilized on a carboxymethylated dextran layer at the surface. 5'-Biotinylated DNA was immobilized on the surface by noncovalent capture to streptavidin. To prepare sensor chips for use, they were conditioned with three consecutive 1 min injections of 1 M NaCl in 50 mM NaOH followed by extensive washing with HEPES buffer, pH 7.4 (0.01 M Hepes, 0.15 M LiCl, 3 mM EDTA, and 50 µl/liter Surfactant P20). Biotinylated oligonucleotide (25nM) in coupling buffer (10 mM HEPES, pH 7.4, 3 mM EDTA, and 150 mM LiCl) was heated at 95 °C for 5 min and cooled slowly to room temperature, and then injected at a flow rate of 2 µL/min to achieve long contact times with the surface and to control the amount of the DNA that was immobilized. For direct SPR measurements (Figure 1 and 4), 100 response units (RU) of biotinylated DNA was immobilized. For competition SPR, a much higher level of Myc19 DNA, >1000 RU, was used to maximize mass transfer. PNA samples were dissolved in water at approximately 1 mM and then diluted to a working stock solution of 10 µM in the Tris buffer: 10 mM Tris (pH 7.4), 1 mM EDTA, and 100 mM KCl, which was used in all binding determinations. Samples of varying PNA concentration were then prepared in filtered and degassed Tris buffer by serial dilutions from the stock solution.

Initial rates of PNA hybridization to low density chips were determined based on the amount of time required to reach 3.0 response units of bound PNA. This value for the slowest target (**hTelo22**) was used to normalize the values for the other targets, giving the relative initial rates of hybridization indicated in Figure 1.

Solution Competition Analysis.[4] *(A) Construction of a Standard Curve.* All competition BIAcore studies were carried out at 25 °C using the $\mathbf{P}_{\mathbf{myc}}$ PNA sequence. Serial dilutions of this PNA into Tris buffer gave concentrations of 0-30nM. Each sample was injected over the Myc19 DNA biosensor surface at flow rates of 10-30 µL/min in different experiments (see Figure S4). The biosensor surface was regenerated after each PNA sample with a 50 mM NaOH wash for 0.5 min to dissociate the PNA-DNA complex. The slope for each $\mathbf{P}_{\mathbf{myc}}$ concentration in the linear association region was obtained by averaging the results over a 20 s window beginning 50 s post injection. Data from the first 50 s of each sensorgram were omitted due to effects at the start of injections, such as sample dispersion and depletion. A plot of the average slopes versus concentration of $\mathbf{P}_{\mathbf{myc}}$ yields a standard curve which can be used to determine the concentration of free $\mathbf{P}_{\mathbf{myc}}$ PNA in equilibrium with nonbiotinylated Myc19, hTelo22 or rHT3 DNA.[4] A calibration function was obtained by a linear least-squares fit of the standard curve data. The results are linear over the desired concentration range of 0-30 nM and the linear function can be used to determine free PNA concentration in the competition experiments described below.

(B) Solution Competition for Determination of Binding Constants. A range of concentrations of nonbiotinylated DNA, determined from preliminary studies to define the competition curve, were serially diluted into Tris buffer and mixed with a constant total concentration of 15 nM PNA. Samples were allowed to equilibrate for approximately 2 h at 25°C and then injected over the calibrated Myc19-biosensor surface with flow rates of 30 µL/min. (Figure S4 shows results for competition with soluble hTelo22.) Control experiments demonstrated that no significant binding of DNA to the surface is observed in the experimental time range and the experiment thus provides a measure of the free concentration of $\mathbf{P}_{\mathbf{myc}}$. The biosensor surface was regenerated after each sample using NaOH. Sensorgrams for each PNA concentration were obtained, and the slopes of free $\mathbf{P}_{\mathbf{myc}}$ binding to the biosensor surface were determined as described above. Free $\mathbf{P}_{\mathbf{myc}}$ concentrations, determined from the calibration curve, were then subtracted from the total PNA concentration in solution to determine the bound $\mathbf{P}_{\mathbf{myc}}$

concentration and r, moles of $\mathbf{P_{myc}}$ bound/ total moles of DNA in solution. Equilibrium dissociation constants ($K_d = 1/K_A$) were obtained from the nonlinear least squares best fit of r versus free compound concentration (C_{free}):

$$r = n * (K_A * C_{free}) / (1 + K_A * C_{free}) \quad (1)$$

where $n = 2$ for binding to two equivalent sites on the quadruplex DNA target. (The data were fit poorly with a single site model corresponding to 1:1 binding, Figure S6.)

Table S1. Sequences of DNA quadruplex targets and PNA probes.

DNA	Sequence
Myc19	5'-AGG GTG GGG AGG GTG GGG A-3'
VEGF	5'-GGG GCG GGC CGG GGG CGG GG-3'
cKit22	5'-AGG GAG GGC GCT GGG AGG AGG G-3'
Bcl2	5'-GGG CGC GGG AGG AAT TGG GCG GG-3'
hTelo22	5'-AGG GTT AGG GTT AGG GTT AGG G-3'
rHT3	5'-AGG GTT AGG GTT AGG GTT AGG G-3' (ribo-G residues are underlined)
PNA	
$\mathbf{P_{myc}}$	H-GGG AG GGG-Lys-NH_2
$\mathbf{P_{telo}}$	H-GGG TTA GGG-Lys-NH_2
$\mathbf{P_{eg2}}$	H-GGG $(eg)_2$ GGG-Lys-NH_2

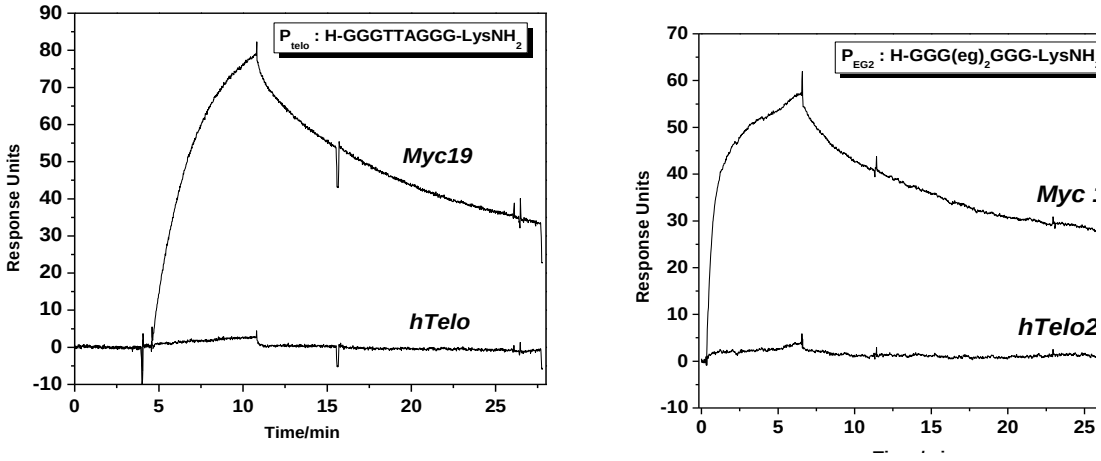

Figure S1. SPR binding curves for binding of 40 nM $\mathbf{P_{telo}}$ and $\mathbf{P_{eg2}}$ to immobilized Myc19 and hTelo22. Equal amounts of the two DNA targets were immobilized, allowing direct comparison of the amount of PNA bound in each experiment.

We also performed SPR analysis of binding of Ptelo to hTelo22 at higher PNA concentrations. As shown in the figure below, the PNA exhibited similarly slow dissociation from this target as from Myc19 (Figure S1), consistent with a kinetic discrimination during the association phase.

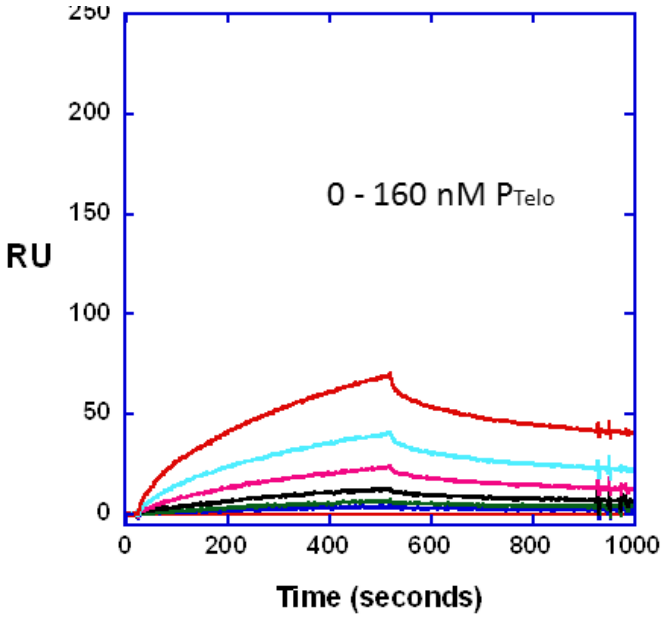

Figure S2. SPR sensorgrams for binding of $\mathbf{P_{telo}}$ (0-160 nM) to **hTelo22**.

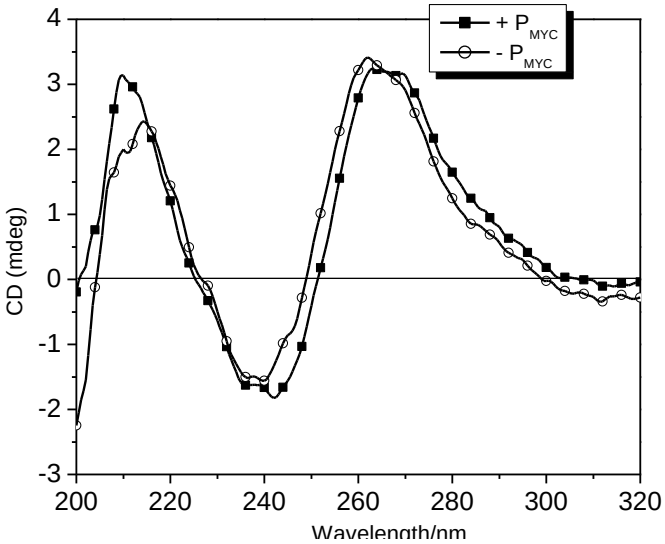

Figure S3. CD spectra recorded for rHT3 quadruplex in absence (open circles) and presence (filled circles) of $\mathbf{P_{myc}}$. [rHT3] = 1.0 μM, [$\mathbf{P_{myc}}$] = 2.0 μM. Buffer contained 10 mM Tris.HCl and 0.1 mM Na_2EDTA.

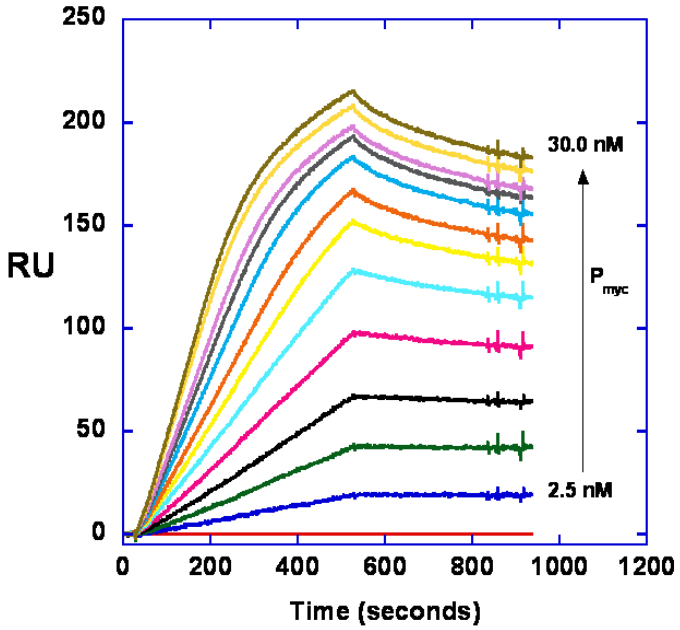

Figure S4. Concentration dependence of $\mathbf{P_{myc}}$ binding to immobilized **Myc19**. PNA concentration ranged from 2.5-30.0 nM.

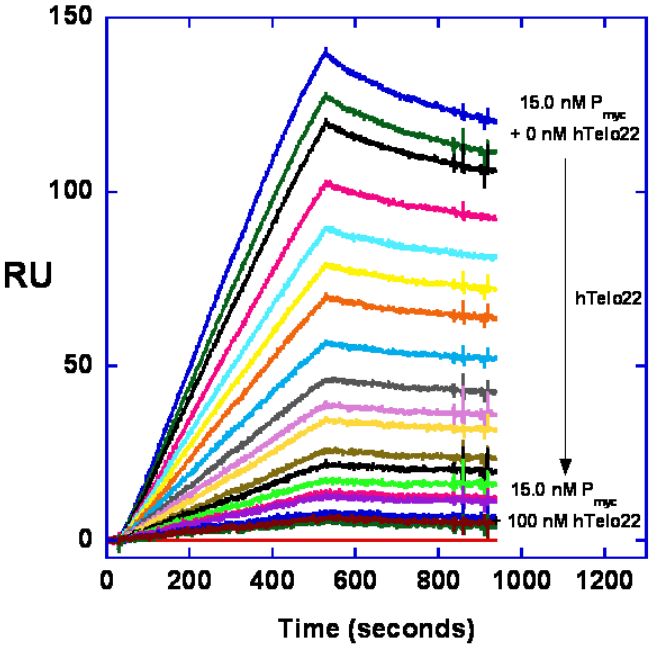

Figure S5. Competition experiment showing that soluble **hTelo22** inhibits binding of $\mathbf{P_{myc}}$ to immobilized **Myc19**. PNA concentration was held constant at 15.0 nM, while **hTelo22** concentration ranged from 0-100 nM.

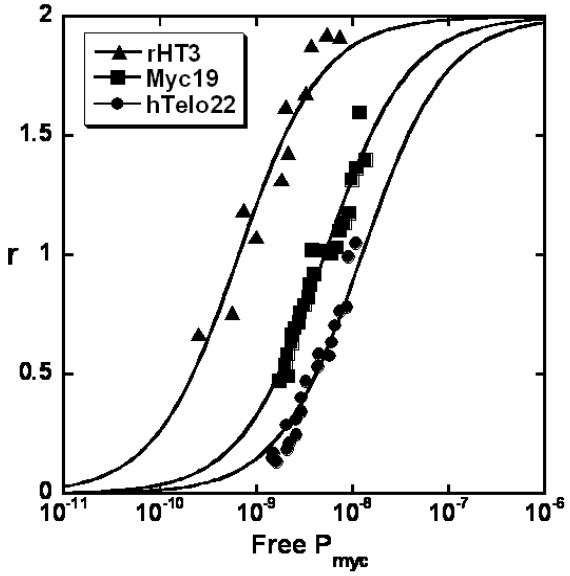

Figure S6. Binding isotherms for hybridization of $\mathbf{P_{myc}}$ to **Myc19**, **rHT3** and **hTelo22** quadruplexes in solution, as determined by competition SPR. Lines correspond to fits to 2:1 equivalent site binding model.

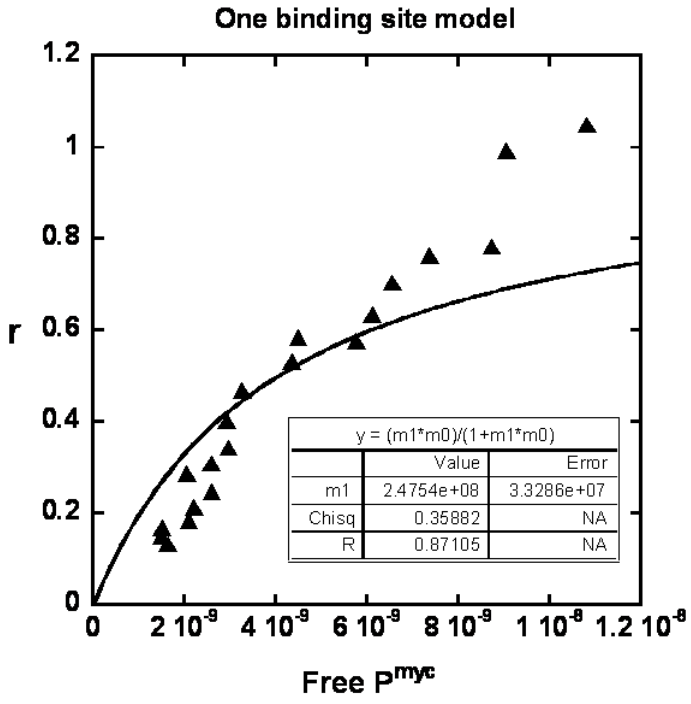

Figure S7. Binding isotherm for **hTelo22**-$\mathbf{P_{myc}}$ interaction as determined by competition SPR. Fitting to a one-site model yields a poor fit compared to the two-site model shown in Figure S5.

References

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