Discovery of G-quadruplex stabilizing ligands through direct ELISA of a one-bead-one-compound library

James E. Redman, Sylvain Ladame, Anthony P. Reszka, Stephen Neidle, Shankar Balasubramanian

Supporting Information

Oligonucleotide sequences
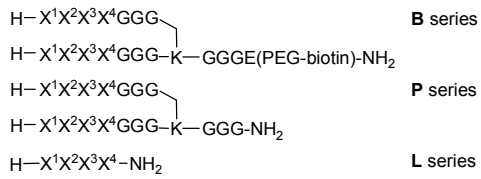
Htelo 5´ Biotin-GTT AGG GTT AGG GTT AGG GTT AGG GTT AGG 3´

Bck 5´ Biotin-AGT TAG AGT TAG AGT TAG AGT TAG AGT TAG 3´

Dup 5´ Biotin-GGC ATA GTG CGT GGG CGT TAG C 3´

5´ GCT AAC GCC CAC GCA CTA TGC C 3´

F21T 5´ FAM-GGG TTA GGG TTA GGG TTA GGG-TAMRA 3´

Polyamide structures and sequences, structures of amino acids

H—X¹X²X³X⁴GGG⌐   **B series**

H—X¹X²X³X⁴GGG⌐—K—GGGE(PEG-biotin)-NH₂

H—X¹X²X³X⁴GGG⌐   **P series**

H—X¹X²X³X⁴GGG⌐—K—GGG-NH₂

H—X¹X²X³X⁴—NH₂   **L series**

Number	Tetrapeptide sequence XXXX
1	YRHY
2	WR-Py-Y
3	FRKV
4	Y-Im-Py-R
5	Y-Im-KV
6	Y-Im-HR
7	WK-Py-Y
8	WKKR

E(PEG-biotin)

Im

Py

H—GGG⌐

H—GGG⌐—K—GGGE(PEG-biotin)-NH₂   **B0**

Acr
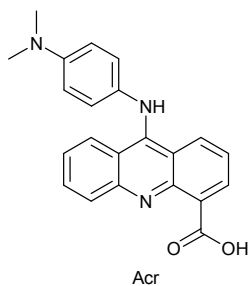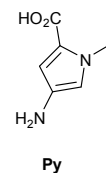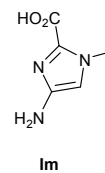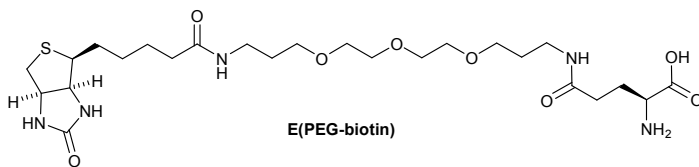
Amino acid analysis of peptides **B0** – **B8**, **P1** – **P8** and **L1** – **L8**.

Compound	Observed m/z	r.t. (min)^a	Amino acid analysis
B0	1217 [M+H]$^+$	12.8	E 1.03(0.03), G 8.97(0.02), K 1.00(0.01)
B1	2455 [M+H]$^+$	13.1	E 1.09(0.01), G 9.56(0.01), Y 3.65(0.01), H 1.88(0.01), K 1.08(0.01), R 1.73(0.01)
B2	2471 [M+H]$^+$	14.9	E 1.14(0.04), G 9.39(0.01), Y 1.86(0.01), K 1.04(0.00), R 1.56(0.02)
B3	1139.7 [M+2H]$^{2+}$	13.4	E 1.13(0.01), G 8.85(0.00), V 1.98, F 1.98(0.01), K 3.07(0.01), R 1.99 (0.00)
B4	1173.6 [M+2H]$^{2+}$	14.0	E 1.06(0.02), G 9.21(0.04), Y 1.76 (0.00), K 1.03(0.00), R 1.94(0.02)
B5	1122.6 [M+2H]$^{2+}$	13.9	E 1.09(0.00), G 9.09(0.00), V 1.97, Y 1.84(0.01), K 3.01(0.00)
B6	2375 [M+H]$^+$	13.2	E 1.17(0.01), G 9.57(0.07), Y 1.55(0.03), H 1.85(0.01), K 1.05(0.01), R 1.80(0.01)
B7	2415 [M+H]$^+$	14.7	E 1.08(0.01), G 9.34(0.01), Y 1.97(0.00), K 2.61 (0.00)
B8	2413 [M+H]$^+$	13.1	E 1.10(0.01), G 9.14(0.01), K 4.89(0.00), R 1.88(0.00)
P1	949.5 [M+2H]$^{2+}$	12.4	G 9.03(0.00), Y 4.07 (0.00), H 1.99(0.00), K 0.94(0.00), R 1.96(0.00)
P2	958.0 [M+2H]$^{2+}$	15.4	G 9.10(0.03), Y 1.98(0.02), K 0.95(0.00), R 1.96(0.01),

P3	860.5 $[M+2H]^{2+}$	12.7	G 8.81(0.01), V 2.02, F 2.01(0.00), K 3.10(0.01), R 2.05(0.00)
P4	894.4 $[M+2H]^{2+}$	14.1	G 9.39(0.01), Y 1.77(0.01), K 0.95(0.01), R 1.88(0.01)
P5	843.5 $[M+2H]^{2+}$	14.0	G 8.94(0.02), V 2.00, Y 1.97(0.00), K 3.08(0.00)
P6	909.4 $[M+2H]^{2+}$	12.3	G 9.26(0.03), Y 1.85(0.01), H 1.92(0.00), K 1.00(0.01), R 1.96(0.02)
P7	929.5 $[M+2H]^{2+}$	15.4	G 9.10(0.01), Y 1.93(0.01), K 2.97(0.00)
P8	929.0 $[M+2H]^{2+}$	12.5	G 8.98(0.00), K 5.05(0.00), R 1.97(0.00)
L1	637.3 $[M+H]^{+}$	12.6	Y 2.02(0.00), H 0.99(0.00), R 0.99(0.00)
L2	645.4 $[M+H]^{+}$	17.0	Y 0.99(0.01), R 1.01(0.01)
L3	548.4 $[M+H]^{+}$	12.8	V 1.00, K 1.00(0.00), R 1.01(0.00)
L4	582.3 $[M+H]^{+}$	15.5	Y 0.99(0.01), R 1.01(0.01)
L5	531.3 $[M+H]^{+}$	15.2	V 1.00(0.01), Y 0.99(0.00), K 1.01(0.00)
L6	597.3 $[M+H]^{+}$	12.3	Y 0.97(0.00), H 1.01(0.00), R 1.02(0.00)
L7	617.3 $[M+H]^{+}$	16.7	Y 0.98(0.00), K 1.02(0.00)
L8	616.4 $[M+H]^{+}$	11.9	K 2.04(0.00), R 0.96 (0.00)

[a]HPLC methods: **B** series, 0 – 2.5 min 5% B, 20.0 min 60% B; **P** series, 0 – 2.5 min 5% B, 20.0 min 50% B; **L** series, 0 – 2.5 min 5% B, 20 min 40% B. For all methods: Phenomenex C18 Luna 5 μM, 250 × 4.6 mm column, flow rate 1 cm^3 min^{-1}, detection by absorbance at 220 nm. Solvent A = H_2O, 0.1% TFA, solvent B = MeCN, 0.1% TFA.
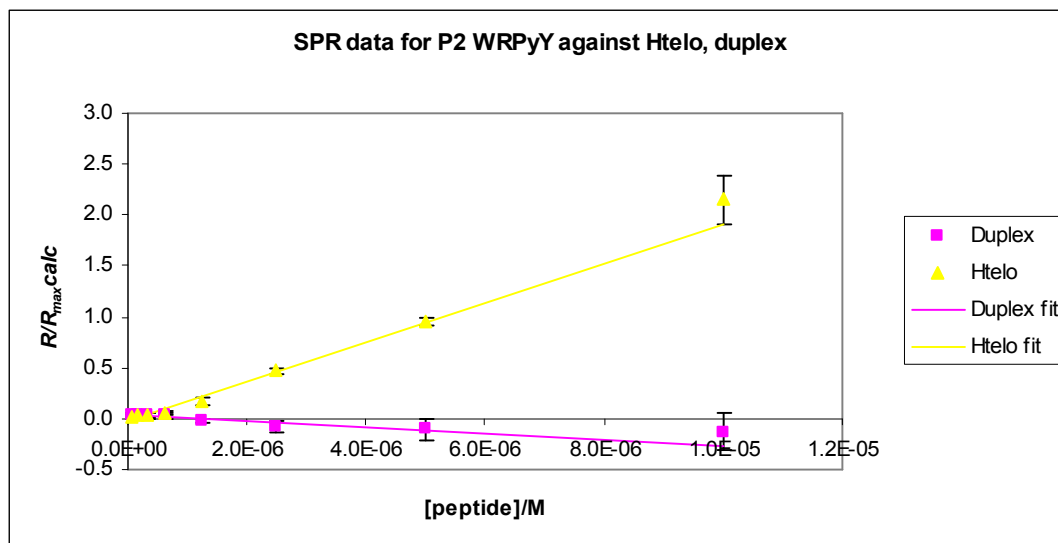
Surface plasmon resonance data and regression

SPR data for P1 YRHY against Htelo, duplex
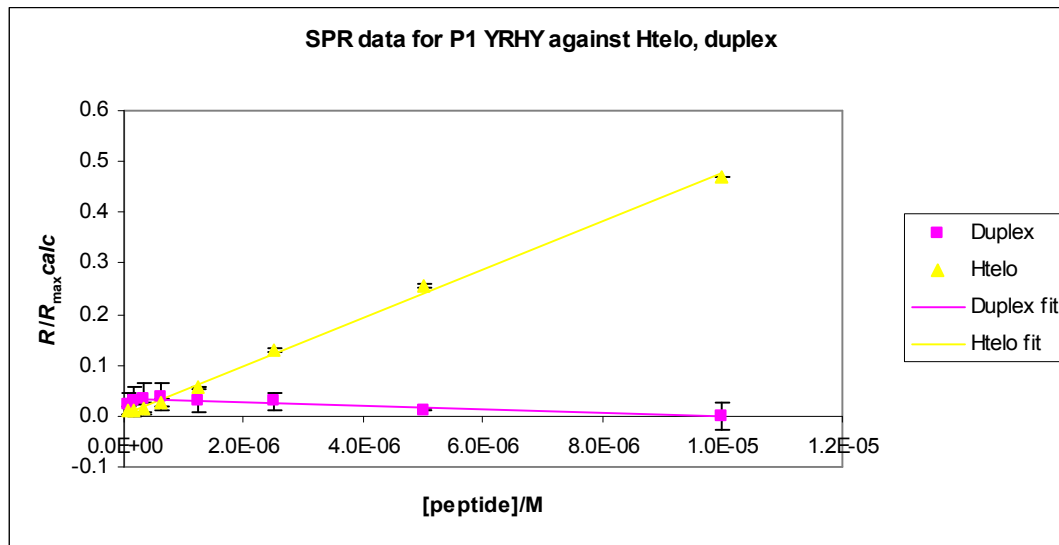
Duplex

Slope (K_a)	-3.3×10^3	Intercept	3.3×10^{-2}
Std error	2.3×10^3	Std error	9.4×10^{-3}
R^2	0.065	F statistic	2.1
DoF	30		

Htelo

Slope (Ka)	4.742×10^4	Intercept	3.9×10^{-3}
Std error	8.3×10^2	Std error	3.4×10^{-3}
R^2	0.99	F statistic	3.2×10^3
DoF	30		

SPR data for P2 WRPyY against Htelo, duplex

Duplex

Slope (Ka)	-3.0×10^4	Intercept	3.0×10^{-2}
Std error	1.0×10^4	Std error	2.3×10^{-2}
R^2	0.24	F statistic	8.4
DoF	26		

Htelo

Slope (Ka)	1.932×10^5	Intercept	-2.6×10^{-2}
Std error	5.8×10^3	Std error	1.3×10^{-2}
R^2	0.98	F statistic	1.1×10^3
DoF	26		

SPR data for P3 FRKV against Htelo, duplex
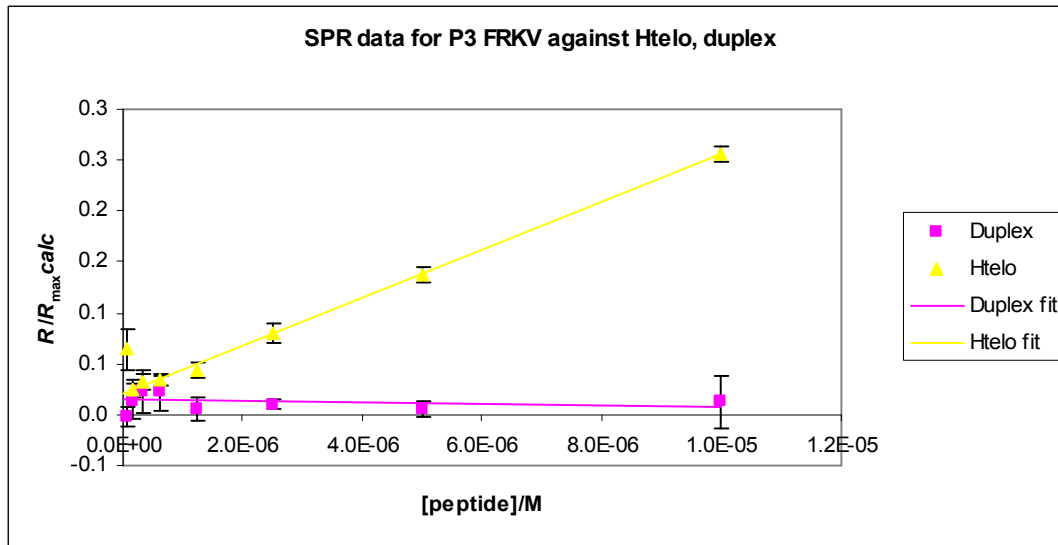
Duplex

Slope (Ka)	-7×10^{2}	Intercept	1.51×10^{-2}
Std error	1.7×10^{3}	Std error	7.6×10^{-3}
R^{2}	6.2×10^{-3}	F statistic	1.6×10^{-1}
DoF	26		

Htelo

Slope (Ka)	2.352×10^{4}	Intercept	2.01×10^{-2}
Std error	8.1×10^{2}	Std error	3.5×10^{-3}
R^{2}	0.97	F statistic	8.4×10^{2}
DoF	26		
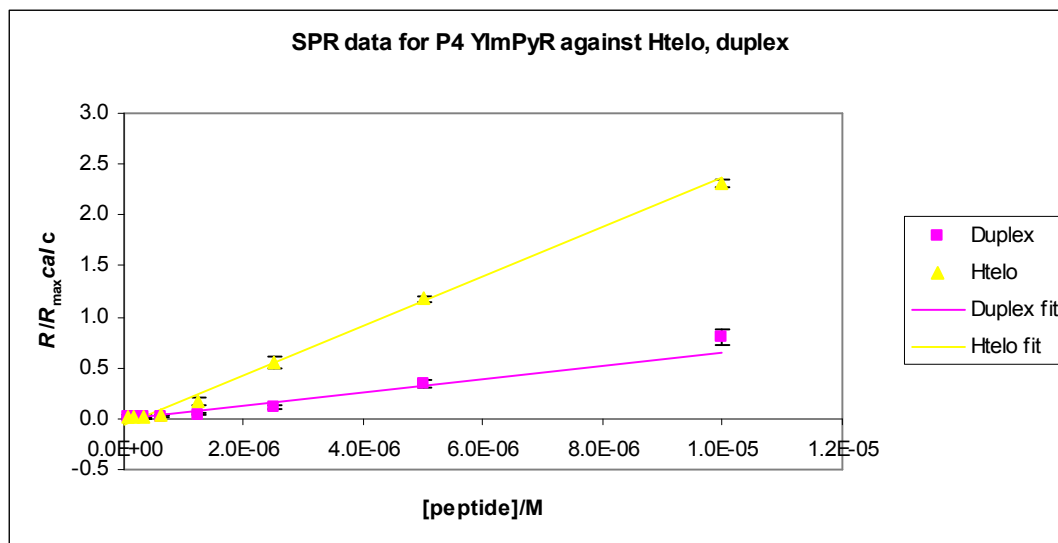
Duplex

Slope (Ka)	6.53×10^4	Intercept	-8×10^{-3}
Std error	5.5×10^3	Std error	1.2×10^{-2}
R^2	0.84	F statistic	1.4×10^2
DoF	26		

Htelo

Slope (Ka)	2.443×10^5	Intercept	-6.5×10^{-2}
Std error	7.6×10^3	Std error	1.7×10^{-2}
R^2	0.98	F statistic	1.0×10^3
DoF	26		
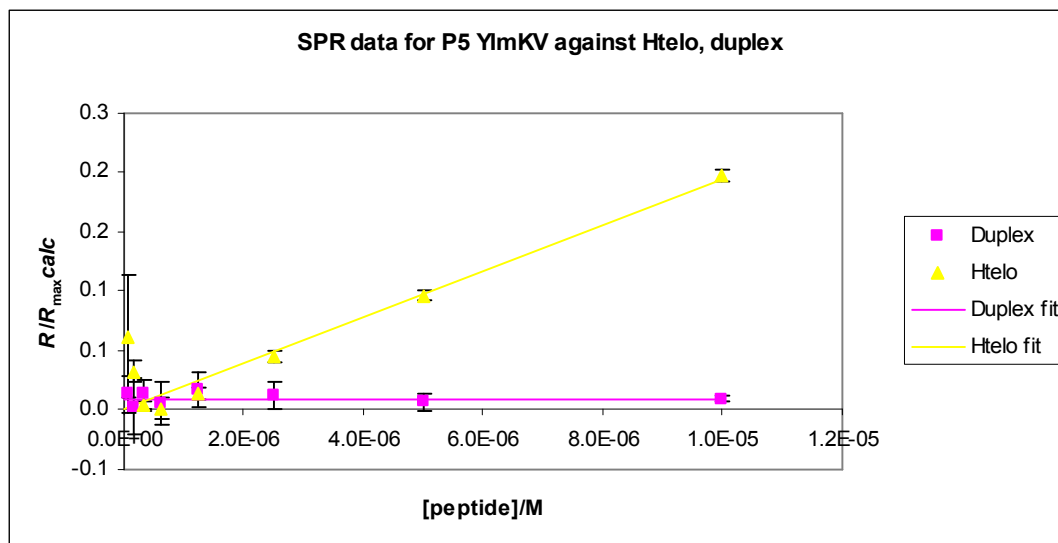
Duplex

Slope (Ka)	-3×10^1		Intercept	9.6×10^{-3}
Std error	1.5×10^3		Std error	6.6×10^{-3}
R^2	1.6×10^{-5}		F statistic	4.3×10^{-4}
DoF	26			

Htelo

Slope (Ka)	1.927×10^4		Intercept	1.1×10^{-3}
Std error	9.7×10^2		Std error	4.2×10^{-3}
R^2	0.94		F statistic	3.92×10^2
DoF	26			

SPR data for P6 YImHR against Htelo, duplex
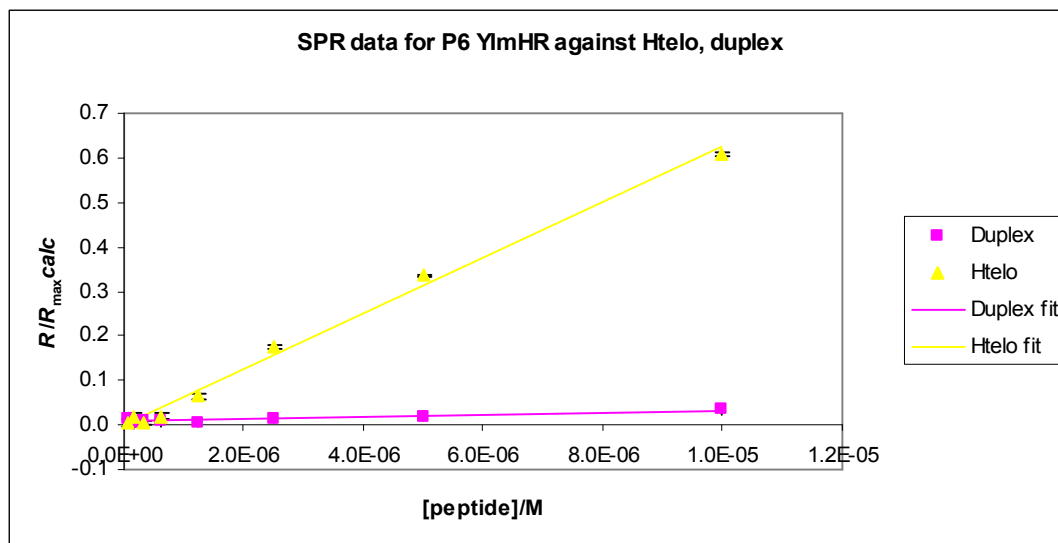
Duplex

Slope (Ka)	2.5×10^3		Intercept	7.3×10^{-3}
Std error	1.1×10^3		Std error	4.5×10^{-3}
R^2	0.15		F statistic	5.3
DoF	30			

Htelo

Slope (Ka)	6.25×10^4		Intercept	-1.3×10^{-3}
Std error	1.1×10^3		Std error	4.3×10^{-3}
R^2	0.99		F statistic	3.4×10^3
DoF	30			
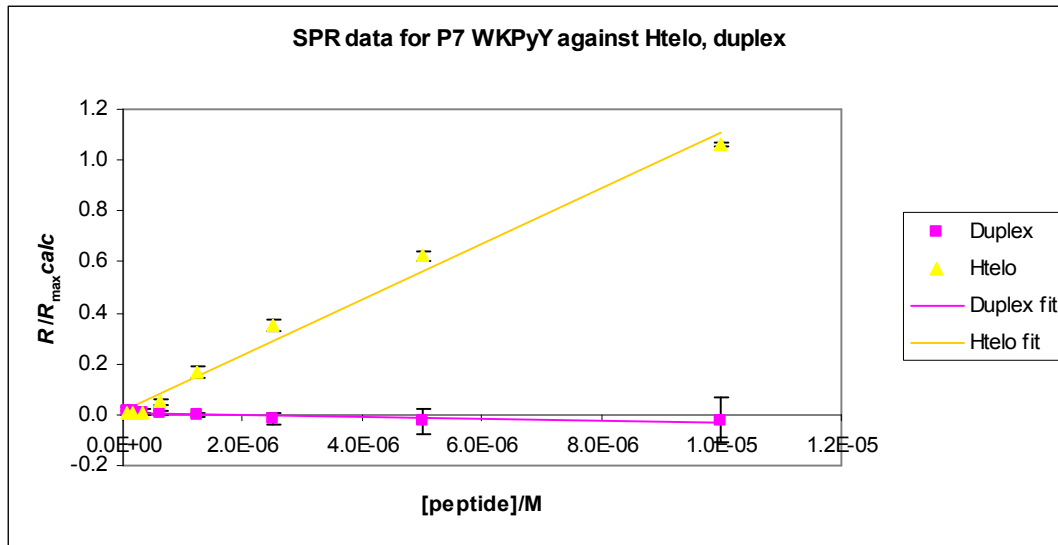
Duplex

Slope (Ka)	-3.7×10^3	Intercept	6×10^{-3}
Std error	3.7×10^3	Std error	1.5×10^{-2}
R^2	3.2×10^{-2}	F statistic	9.8×10^{-1}
DoF	30		

Htelo

Slope (Ka)	1.098×10^5	Intercept	1.1×10^{-2}
Std error	2.8×10^3	Std error	1.1×10^{-2}
R^2	0.98	F statistic	1.6×10^3
DoF	30		

SPR data for P8 WKKR against Htelo, duplex
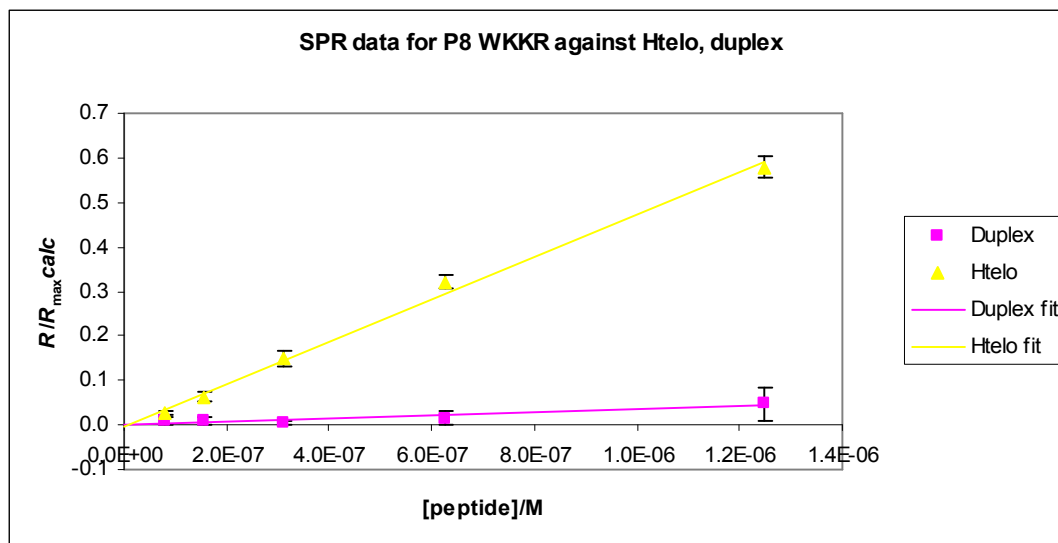
Duplex

Slope (Ka)	3.5×10^4	Intercept	-7×10^{-4}
Std error	1.8×10^4	Std error	1.2×10^{-2}
R^2	0.17	F statistic	3.7
DoF	18		

Htelo

Slope (Ka)	4.74×10^5	Intercept	2×10^{-3}
Std error	1.7×10^4	Std error	1.1×10^{-2}
R^2	0.98	F statistic	7.6×10^2
DoF	18		

SPR data for L1 YRHY against Htelo, duplex
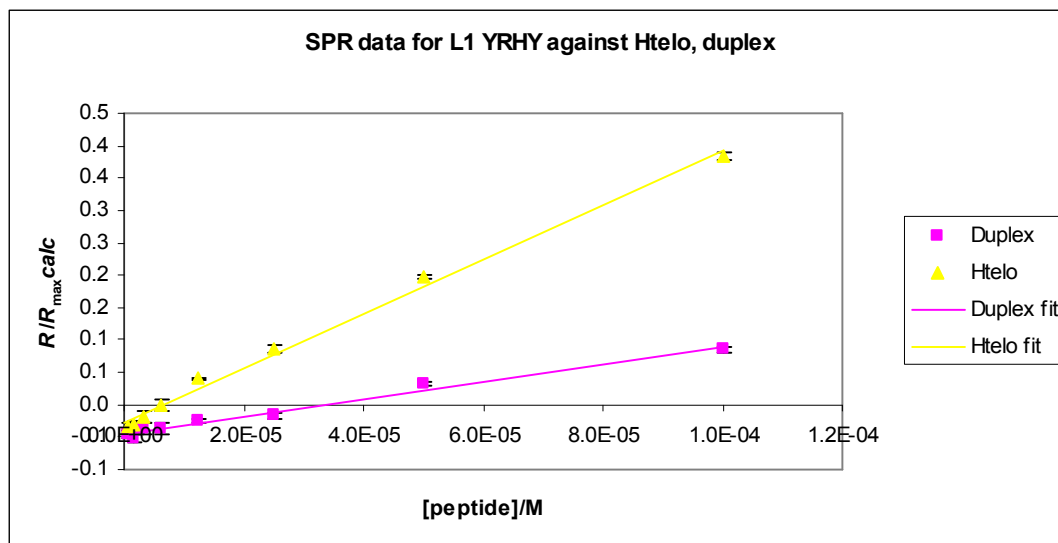
Duplex

Slope (Ka)	1.346×10^3	Intercept	-4.56×10^{-2}
Std error	6.6×10^1	Std error	2.7×10^{-3}
R^2	0.97	F statistic	4.2×10^2
DoF	14		

Htelo

Slope (Ka)	4.215×10^3	Intercept	-2.71×10^{-2}
Std error	8.7×10^1	Std error	3.6×10^{-3}
R^2	0.99	F statistic	2.3×10^3
DoF	14		
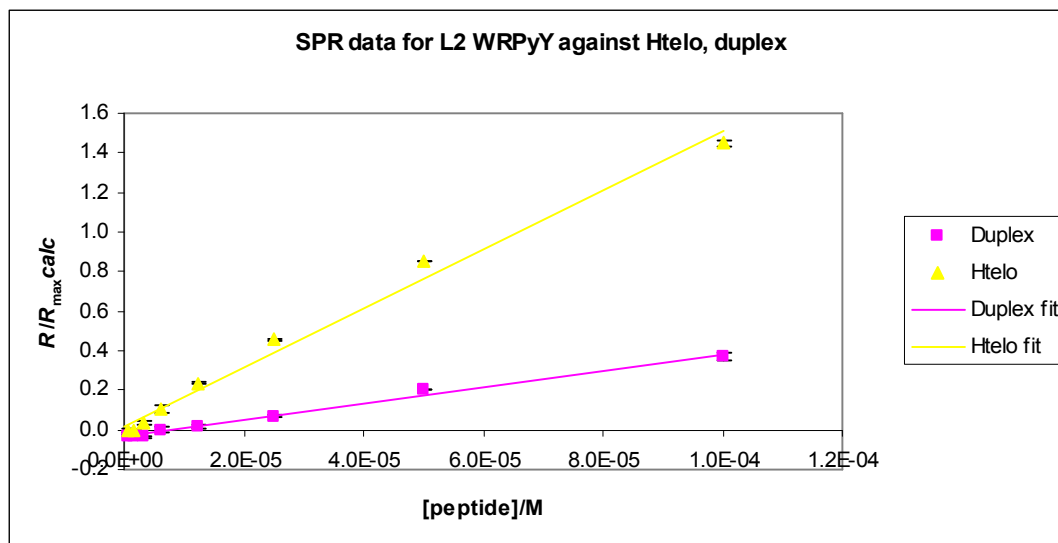
Duplex

Slope (Ka)	4.16×10^3		Intercept	-3.36×10^{-2}
Std error	1.3×10^2		Std error	5.3×10^{-3}
R^2	0.99		F statistic	1.0×10^3
DoF	14			

Htelo

Slope (Ka)	1.489×10^4		Intercept	2.0×10^{-2}
Std error	4.2×10^2		Std error	1.7×10^{-2}
R^2	0.99		F statistic	1.2×10^3
DoF	14			

SPR data for L3 FRKV against Htelo, duplex
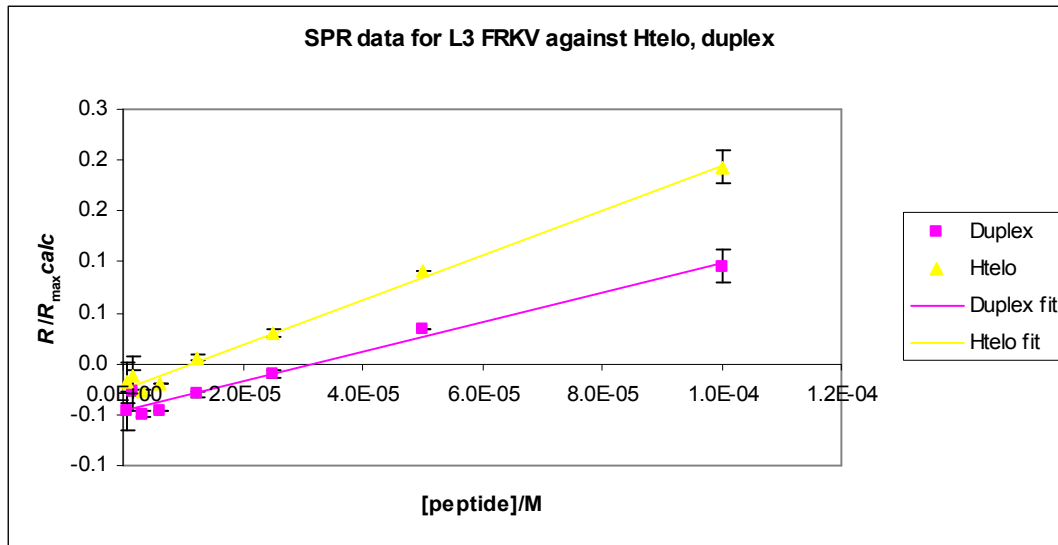
Duplex

Slope (Ka)	1.44×10^3	Intercept	-4.57×10^{-2}
Std error	1.2×10^2	Std error	4.8×10^{-3}
R^2	0.92	F statistic	1.5×10^2
DoF	14		

Htelo

Slope (Ka)	2.20×10^3	Intercept	-2.46×10^{-2}
Std error	7.0×10^1	Std error	2.8×10^{-3}
R^2	0.99	F statistic	1.0×10^3
DoF	14		

SPR data for L4 YImPyR against Htelo, duplex
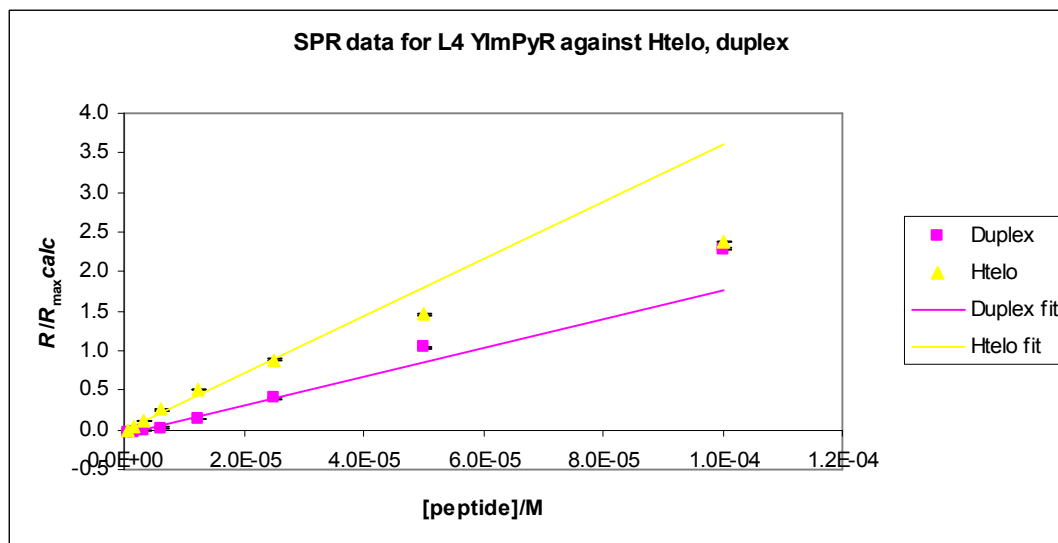
Duplex

Slope (Ka)	1.823×10^4	Intercept	-6.73×10^{-2}
Std error	6.6×10^2	Std error	7.8×10^{-3}
R^2	0.99	F statistic	7.7×10^2
DoF	10		

Htelo

Slope (Ka)	3.61×10^4	Intercept	4×10^{-3}
Std error	1.0×10^3	Std error	1.2×10^{-2}
R^2	0.99	F statistic	1.3×10^3
DoF	10		

SPR data for L5 YlmKV against Htelo, duplex
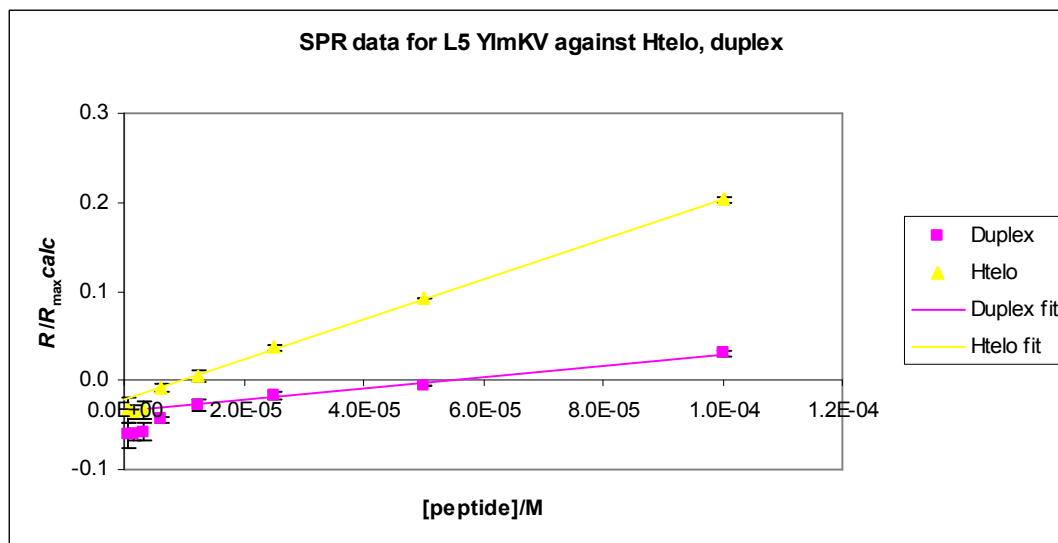
Duplex

Slope (Ka)	6.49×10^2	Intercept	-3.55×10^{-2}
Std error	5.8×10^1	Std error	3.3×10^{-3}
R^2	0.95	F statistic	1.3×10^2
DoF	6		

Htelo

Slope (Ka)	2.256×10^3	Intercept	-2.17×10^{-2}
Std error	4.9×10^1	Std error	2.8×10^{-3}
R^2	0.997	F statistic	2.1×10^3
DoF	6		

SPR data for L6 YImHR against Htelo, duplex
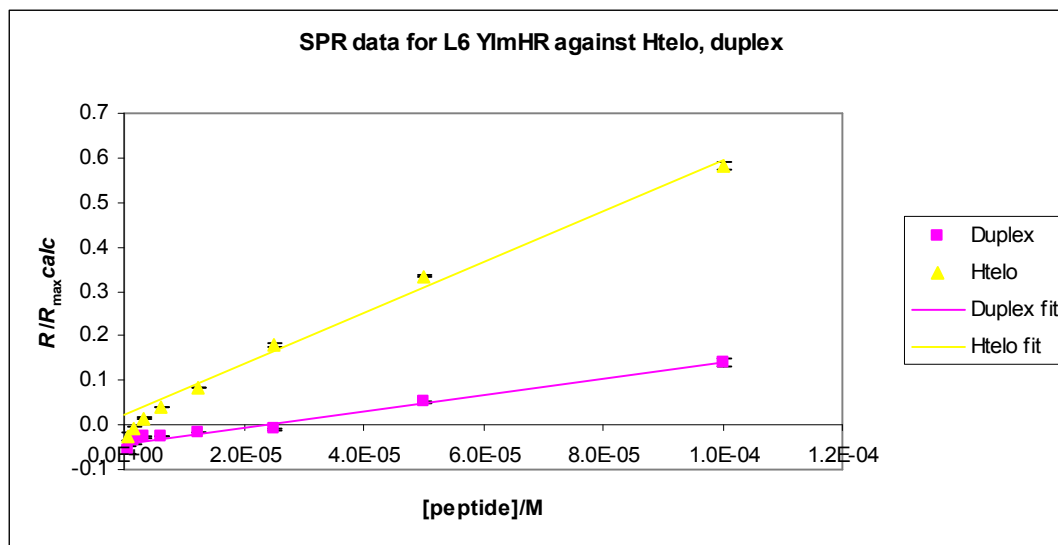
Duplex

Slope (Ka)	1.838×10^3	Intercept	-4.28×10^{-2}
Std error	8.2×10^1	Std error	4.2×10^{-3}
R2	0.98	F statistic	5.1×10^2
DoF	8		

Htelo

Slope (Ka)	5.74×10^3	Intercept	2.16×10^{-2}
Std error	1.8×10^2	Std error	9.2×10^{-3}
R2	0.99	F statistic	1.0×10^3
DoF	8		

SPR data for L7 WKPyY against Htelo, duplex
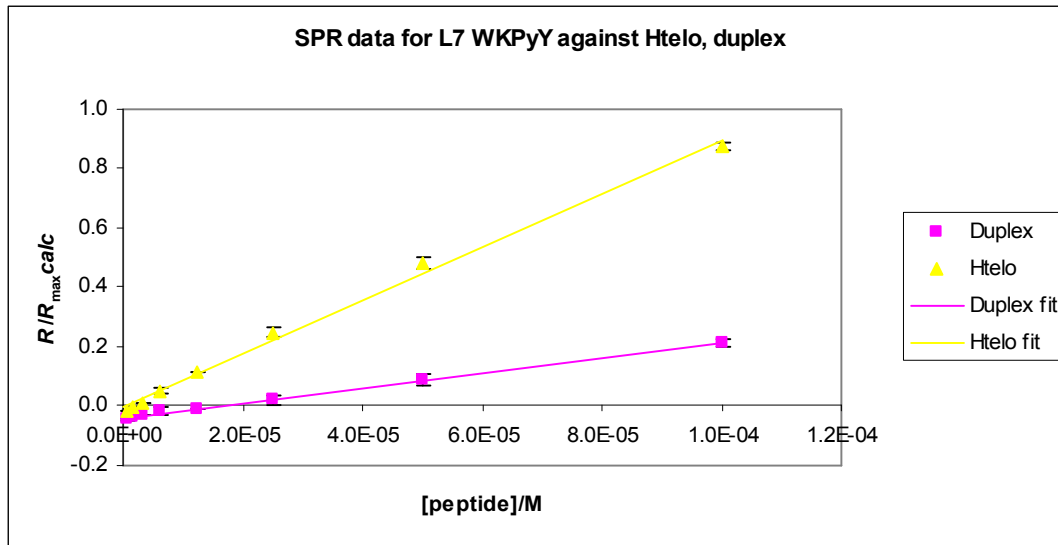
Duplex

Slope (Ka)	2.538×10^3	Intercept	-4.02×10^{-2}
Std error	9.2×10^1	Std error	3.8×10^{-3}
R^2	0.98	F statistic	7.6×10^2
DoF	14		

Htelo

Slope (Ka)	9.02×10^3	Intercept	-4.0×10^{-3}
Std error	1.7×10^2	Std error	7.0×10^{-3}
R^2	0.99	F statistic	2.7×10^3
DoF	14		

SPR data for L8 WKKR against Htelo, duplex
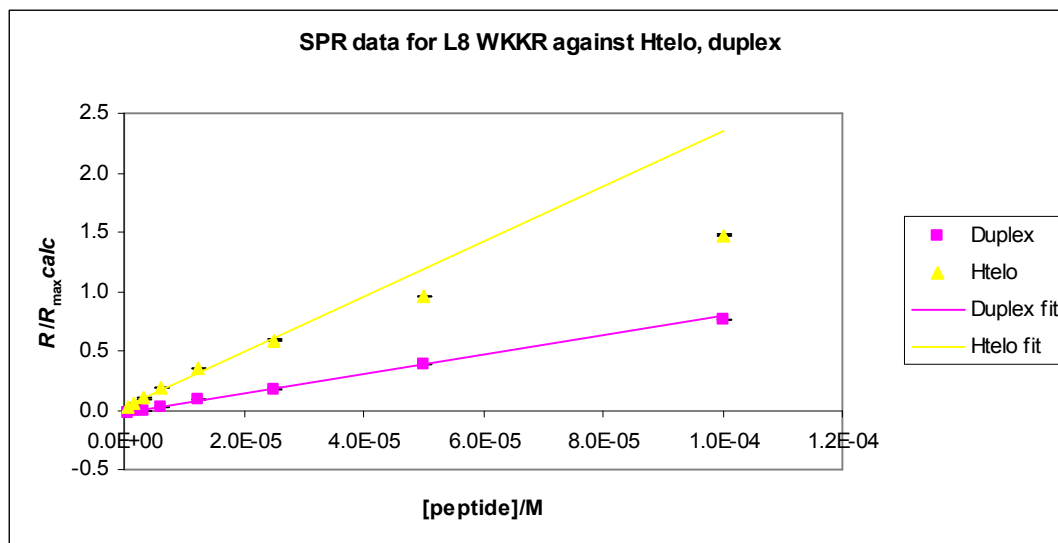
Duplex

Slope (Ka)	8.23×10^{3}	Intercept	-2.30×10^{-2}
Std error	2.37×10^{2}	Std error	2.8×10^{-3}
R^2	0.99	F statistic	1.2×10^{3}
DoF	10		

Htelo

Slope (Ka)	2.334×10^{4}	Intercept	2.60×10^{-2}
Std error	8.2×10^{2}	Std error	9.7×10^{-3}
R^2	0.99	F statistic	8.1×10^{2}
DoF	10		

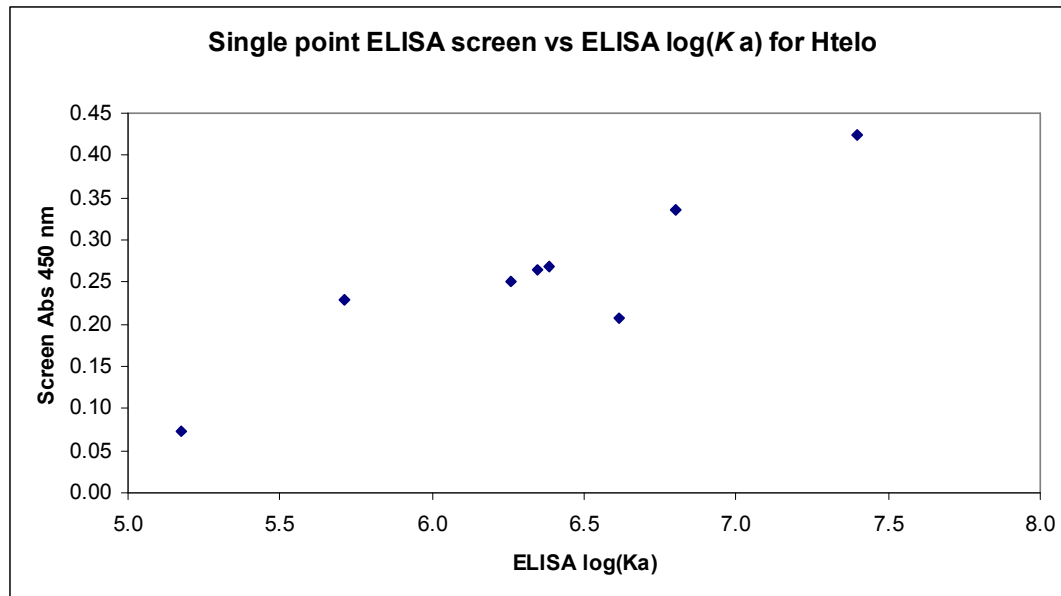**Summary of apparent association constants of linear peptides for quadruplex, and duplex DNA.**

Sequence		SPR $\log(K_a)$ **L** series	
		Htelo	Dup
L1	YRHY	3.6	3.1
L2	WR-Py-Y	4.2	3.6
L3	FRKV	3.3	3.2
L4	Y-Im-Py-R	4.6	4.3
L5	Y-Im-KV	3.4	2.8
L6	Y-Im-HR	3.8	3.3
L7	WK-Py-Y	4.0	3.4
L8	WKKR	4.4	3.9

Apparent association constants, with standard errors, for branched polyamides determined by ELISA.

	Sequence	ELISA **B** series log(K_a) ± std. error
B1	YRHY	5.7 ± 0.1
B2	WR-Py-Y	6.3 ± 0.1
B3	FRKV	6.8 ± 0.1
B4	Y-Im-Py-R	6.6 ± 0.1
B5	Y-Im-KV	5.2 ± 0.3
B6	Y-Im-HR	6.3 ± 0.1
B7	WK-Py-Y	6.4 ± 0.1
B8	WKKR	7.4 ± 0.3

Scatterplot of single point ELISA screen responses for crude peptides against Htelo versus log(K_a) determined by ELISA titration using purified compounds B1 - B8.

Kendall τ 0.71
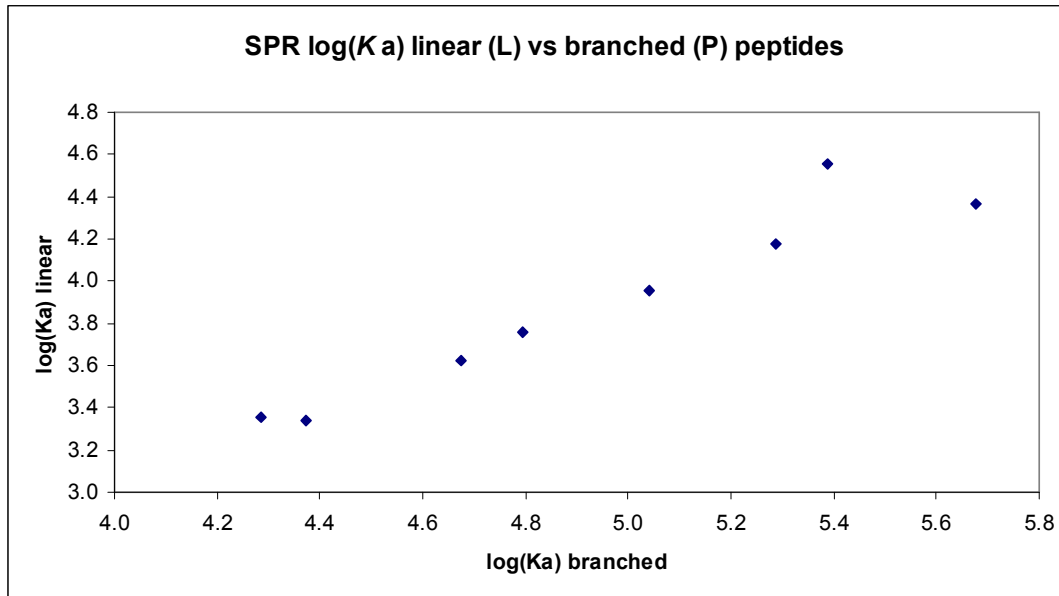
Two sided significance 0.013

Scatterplot of ELISA log(K_a) and SPR log(K_a) for ligand binding to Htelo quadruplex

Mean ELISA log(K_a) plotted vs SPR log(Ka)
Branched peptides vs Htelo quadruplex
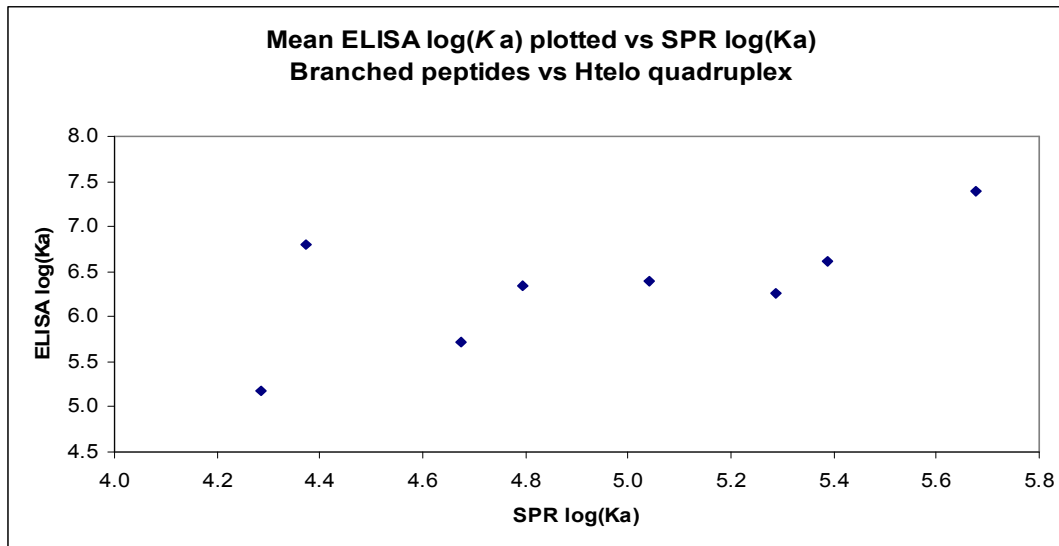
ELISA determinations are for biotinylated compounds (**B** series), whereas SPR relates to the corresponding unbiotinylated compound (**P** series). Regression statistics are given below.
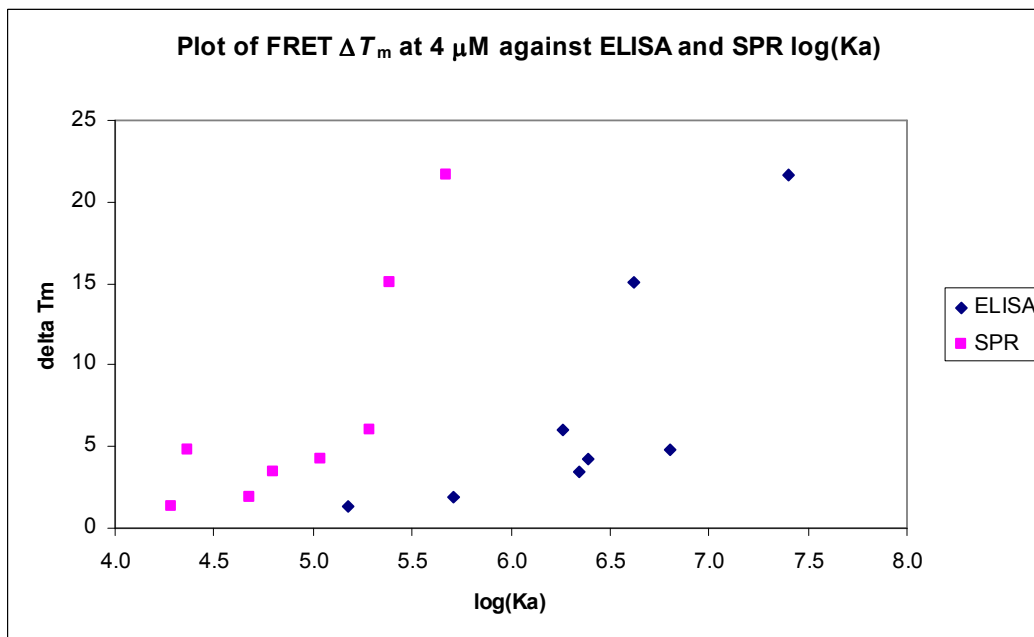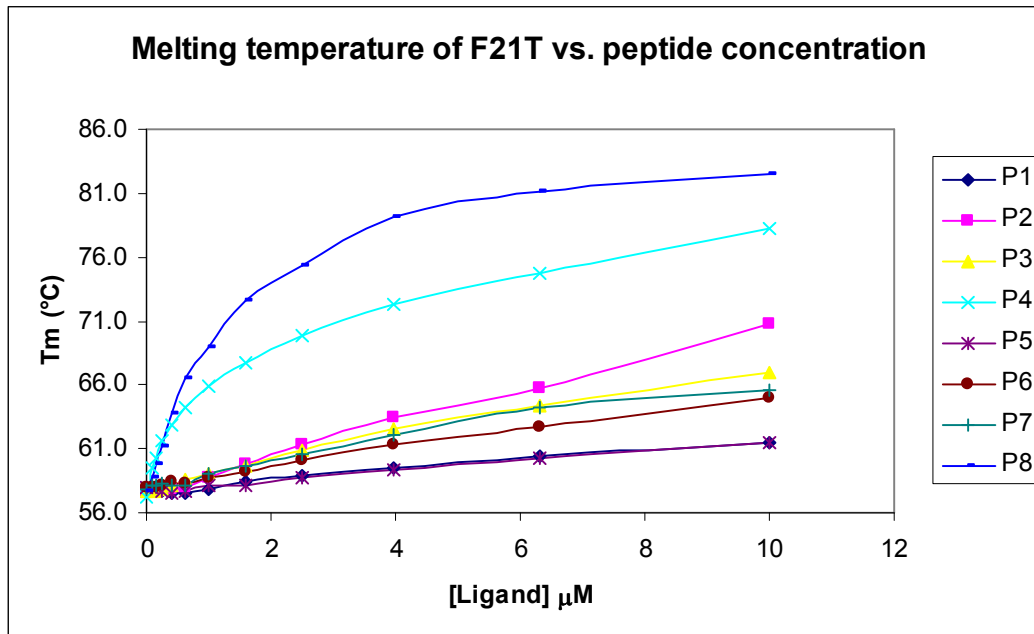
Slope	1.05	Standard Error	0.48
Intercept	1.1	Standard Error	2.4
R2	0.44	Pearson R	0.66
F	4.7	Degrees of Freedom	6
t	2.2	Significance (2 tailed)	0.07

Scatterplot of SPR Htelo log(K_a) for linear peptides (L1 – L8) against branched peptides (P1 – P8)

Slope	0.88	Standard Error	0.10
Intercept	−0.45	Standard Error	0.51
R2	0.92	Pearson R	0.96
F	73	Degrees of Freedom	6
t	8.5	Significance (2 tailed)	0.00014

Melting temperature of F21T quadruplex on ligand addition

Melting temperature of F21T vs. peptide concentration

Plot of FRET ΔT_{m} at 4 µM against ELISA and SPR log(Ka)

ΔT_{m} at 4 µM vs. ELISA log(K_{a})

Kendall τ 0.71   Two sided significance 0.013

ΔT_{m} at 4 µM vs. SPR log(K_{a})

Kendall τ 0.79   Two sided significance 0.0065