Supporting information

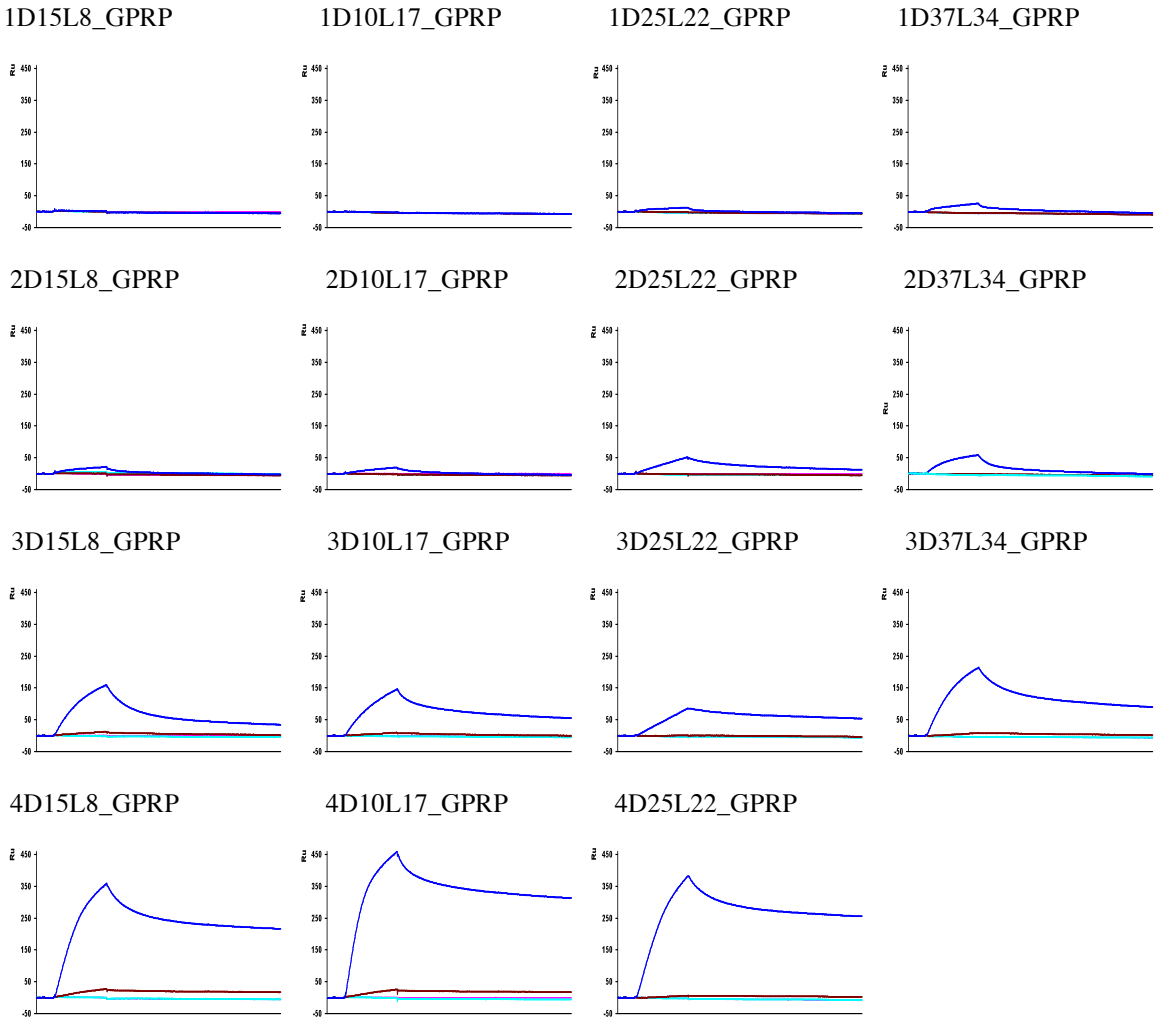

Figure S1 Panel of sensorgrams obtained from screening of binders for the D-dimer using 1 nM, 10 nM and 100 nM concentrations of binders. The injection time was 3 minutes and the clearance time was 10 minutes.

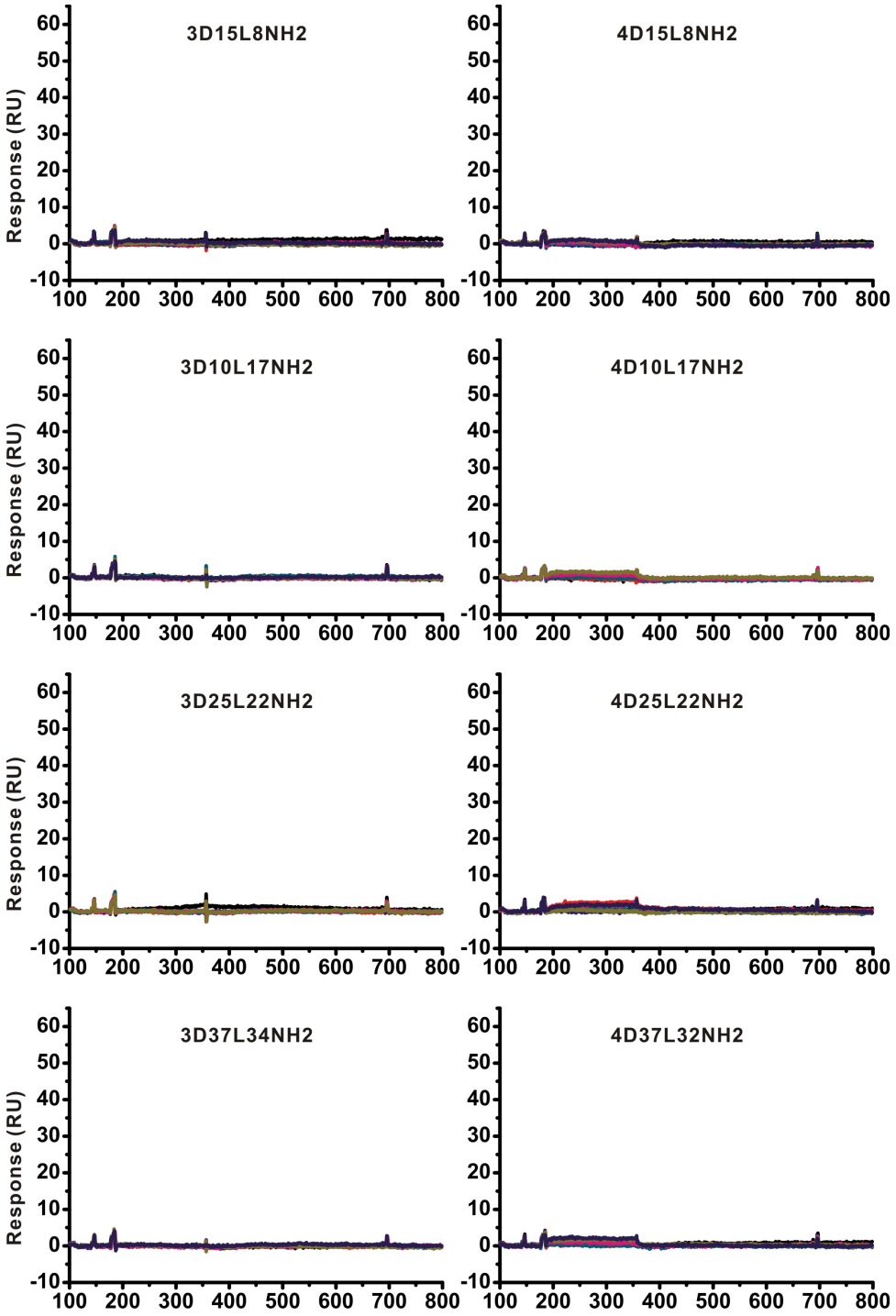

Figure S2. Panel of sensorgrams showing negligible binding of D-dimer by polypeptides corresponding to high affinity polypeptide conjugates but with no GPRP attached, in running buffer at pH 7.4 and 0nM, 5nM, 10 nM, 20 nM, 40 nM , 80 nM and 160 nM of polypeptide. The injection time was 3 minutes and the clearance time was 10 minutes.

Table S1 Dissociation constants, K_D, for the interaction between binder molecules and the D-dimer protein measured by SPR interaction analysis.

Binder	K_D (uM)	Binder	K_D (uM)
1D15L8_GPRP	NA	1D25L22_GPRP	NA
2D15L8_GPRP	10	2D25L22_GPRP	16
3D15L8_GPRP	0.16	3D25L22_GPRP	1.9
4D15L8_GPRP	0.48	4D25L22_GPRP	0.78
1D10L17_GPRP	NA	1D37L34_GPRP	5.0
2D10L17_GPRP	10	2D37L34_GPRP	14
3D10L17_GPRP	1.9	3D37L34_GPRP	1.2
4D10L17_GPRP	0.60	4D37L34_GPRP	ND

Binders are monomeric polypeptide conjugates and affinities were estimated using only three concentrations of binder molecule. Dissociation constants, K_D, were obtained by using the Biaevaluation program version 3.2 (Biacore) assuming a simple 1:1 Langmuir binding mechanism.

Table S2 Dissociation constants, K_D, for the interaction between selected high affinity binder molecules and the D-dimer protein measured by SPR interaction analysis.

PEG linked Binder Dimer	K_D (nM)	Chi 2	Binder	K_D (nM)	Chi 2
3D15L8_GPRP-PEG	11	3.9	3D15L8-GPRP	42	3.1
3D10L17_GPRP-PEG	24	2.5	3D10L17-GPRP	95	3.5
3D25L22_GPRP-PEG	4300	5.5	3D25L22- GPRP	36	5.1
3D37L34_GPRP-PEG	32	5.0	3D37L34- GPRP	15	11
4D15L8_GPRP-PEG	12	53	4D15L8- GPRP	5.8	46
4D10L17_GPRP-PEG	2.2	140	4D10L17- GPRP	6.6	252
4D25L22_GPRP-PEG	3.4	109	4D25L22- GPRP [2]	9.5	371

Binders are homodimers formed by conjugation with PEG bifunctional linker (3D15L8_GPRP-PEG, etc) or selected "best" monomeric binders (3D15L8-GPRP, etc). Dissociation constants, K_D, were obtained by using the Biaevaluation program version 3.2 (Biacore) and assuming a simple 1:1 Langmuir binding mechanism.

Confirmation of peptides and peptide conjugates. All peptides and conjugates were estimated to be >95% pure by analytical HPLC. Mass analysis was performed by MALDI-TOF mass spectrometry (Voyager-DE Pro, Applied Biosystem, USA) in the linear positive ion mode using α-cyano-4-hydroxycinnamic acid (CHCA) or 2-(4'-Hydroxybenzeneazo) benzoic acid (HABA) as matrix. Instrument calibration was done at regular intervals, however, factors such as the plate position of the sample slightly affected the obtained m/z values. This and the limited resolution of the linear mode gave an estimated ± 0.1 % maximum error. The calculated monoisotopic mass (M+H) and found m/z values are listed in Table S1.

Table S3: m/z values of the peptides and their respective conjugates.

Conjugate Name	Calc. M+H (peptide) *	m/z (peptide)*	Calc. M+H (conjugate)	m/z (conjugate)
1D15L8-GPRP	5165	5167	5673	5674
2D15L8-GPRP	5019	5021	5527	5527
3D15L8-GPRP	5046	5047	5553	5556
4D15L8-GPRP	5273	5275	5780	5777
1D10L17-GPRP	5022	5024	5533	5533
2D10L17-GPRP	4876	4878	5384	5382
3D10L17-GPRP	5046	5047	5554	5550
4D10L17-GPRP	5273	5275	5780	5781
1D25L22-GPRP	5107	5109	5615	5613
2D25L22-GPRP	4991	4993	5499	5503
3D25L22-GPRP	5018	5019	5525	5523
4D25L22-GPRP	5273	5275	5780	5779
1D37L34-GPRP	5049	5051	5557	5555
2D37L34-GPRP	4961	4963	5469	5467
3D37L34-GPRP	5047	5047	5554	5553
3D15L8-GPRP -PEG	-	-	11413	11412
3D10L17-GPRP-PEG	-	-	11413	11410
3D25L22-GPRP-PEG	-	-	11357	11353
3D37L34-GPRP-PEG	-	-	11414	11411
4D15L8-GPRP-PEG	-	-	11867	11863
4D10L17GPRP-PEG	-	-	11867	11858
4D25L22-GPRP-PEG	-	-	11867	11863
4D15L8-GPRP-Fluorescein	-	-	6207	6213

All N-terminals were acetylated, and the C-terminals were carboxylates in the 1- and 2-charge type series and amides in the 3- and 4- charge type series. An asterisk (*) indicates that cysteine was protected by acetylaminomethyl group.