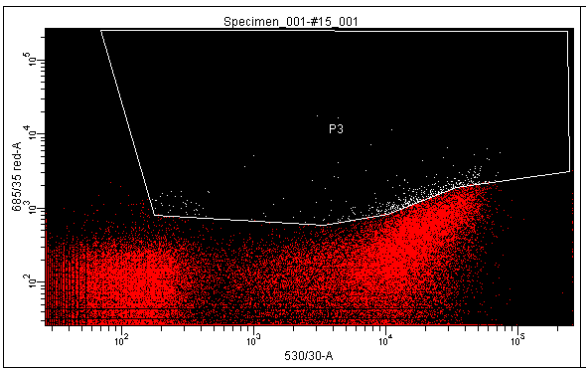
Supporting Information for "A Variable Light Domain Fluorogen Activating Protein Homodimerizes to Activate Dimethylindole Red"

Supporting Information Figure S1 Directed evolution FACS enrichment data of $M8V_L$ and $M8V_HV_L$.

Directed evolution FACS enrichments of $M8V_HV_L$ with DIR.(Panels A-C)

Panel A. First sort of a library of yeast cells expressing error prone PCR mutants of $M8V_HV_L$. The x-axis indicates the cell surface expression of the scFvs as determined by fluorescent c-myc epitope labeling with anti-cmyc antibody (Roche) and Alexa 488 (Invitrogen). The y-axis shows the increasing fluorescence signal of the yeast library interrogated with 0.5nM DIR. Gate P3 (white box and white cells) was drawn to isolate and expand 0.5% of the total library yeast library with the greatest DIR fluorescence.

A



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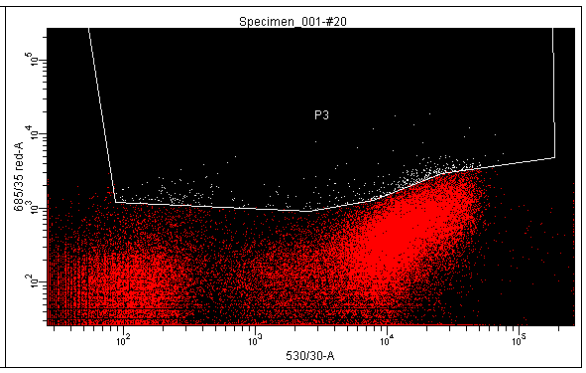

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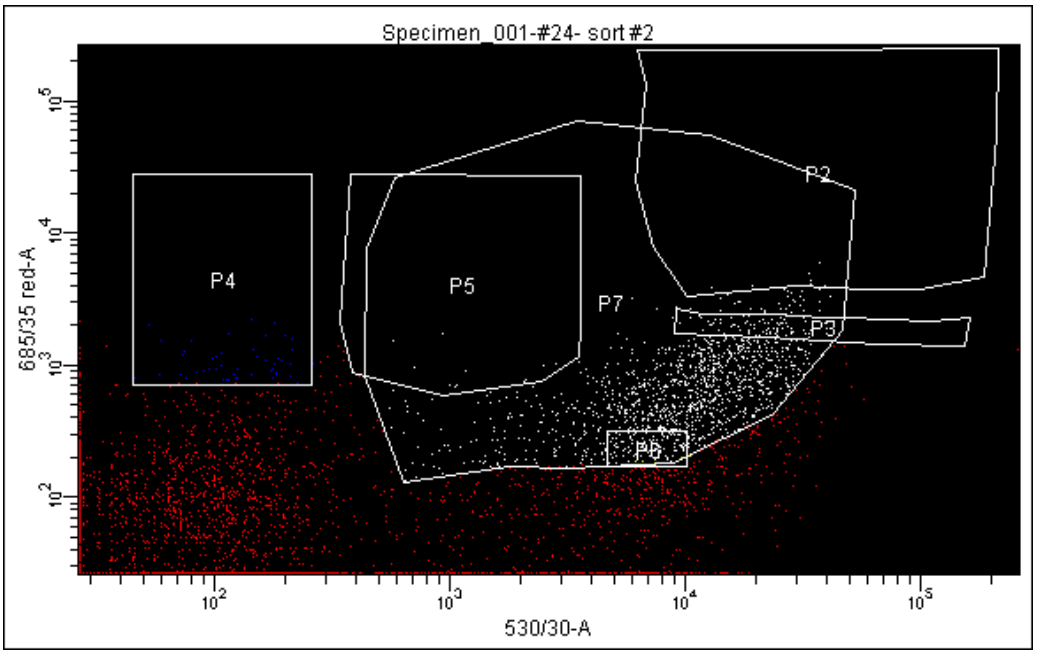


Panel B. Second sort of a library of yeast cells expressing error prone PCR mutants of $M8V_HV_L$. The x-axis indicates the cell surface expression of the scFvs as determined by fluorescent c-myc

epitope labeling with anti-cmyc antibody (Roche) and Alexa 488 (Invitrogen). The y-axis shows the increasing fluorescence signal of the yeast library interrogated with 0.25nM DIR. . Gate P3 (white) was drawn to isolate and expand 0.5% of the total library yeast library with the greatest DIR fluorescence.

Panel C. Third sort of a library of yeast cells expressing error prone PCR mutants of $M8V_HV_L$. The x-axis indicates the cell surface expression of the scFvs as determined by fluorescent c-myc epitope labeling with anti-cmyc antibody (Roche) and Alexa 488 (Invitrogen). The y-axis shows the increasing fluorescence signal of the yeast library interrogated with 0.25nM DIR. Different gates were drawn in order to isolate single yeast cell that represented distinct populations with different expression and fluorescence profiles. For example, gate P2 (top right hand corner) was drawn to isolate clones with the greatest DIR fluorescence and scFv expression. Another example is gate P7 that was drawn around clones with strong DIR fluorescence but less robust yeast surface expression as indicated by reduced fluorescence from the c-myc epitope labeling. Clones were isolated from each of the 7 gates and plated as single cells onto yeast induction media containing plates with agar and 10nM DIR for visual inspection.

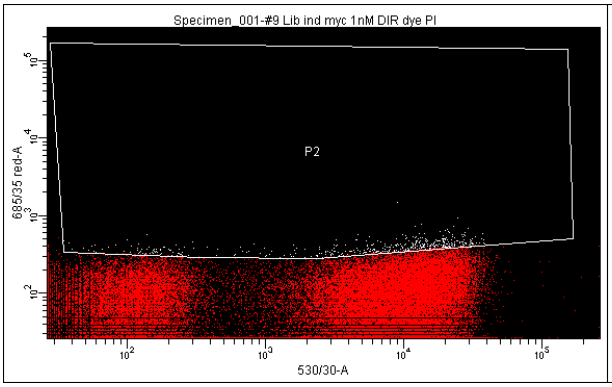
Directed Evolution FACS enrichments for $M8V_L$ (Panels D-F)

Panel D. First sort of a library of yeast cells expressing error prone PCR mutants of $M8V_L$. The x-axis indicates the cell surface expression of the scFvs as determined by fluorescent c-myc epitope labeling with anti-cmyc antibody (Roche) and Alexa 488 (Invitrogen). The y-axis shows the increasing fluorescence signal of the yeast library interrogated with 1.0nM DIR. Gate P2 (white) was drawn to isolate and expand 0.5% of the total library yeast library with the greatest DIR fluorescence.

Panel E. Second sort of a library of yeast cells expressing error prone PCR mutants of $M8V_L$. The x-axis indicates the cell surface expression of the scFvs as determined by fluorescent c-myc epitope labeling with anti-cmyc antibody (Roche) and Alexa 488 (Invitrogen). The y-axis shows the increasing fluorescence signal of the yeast library interrogated with 1.0nM DIR. Gate P2 (white) was drawn to isolate and expand 0.5% of the total library yeast library with the greatest DIR fluorescence.

Panel F. Third sort of a library of yeast cells expressing error prone PCR mutants of $M8V_L$. The x-axis indicates the cell surface expression of the scFvs as determined by fluorescent c-myc epitope labeling with anti-cmyc antibody (Roche) and Alexa 488 (Invitrogen). The y-axis shows the increasing fluorescence signal of the yeast library interrogated with 1.0 nM DIR. Different gates were drawn in order to isolate single yeast cell that represented distinct populations with different expression and fluorescence profiles. For example, gate P3 was drawn to isolate clones with the greatest DIR fluorescence and scFv expression. Cells from each gate were plated as colonies containing single cells onto yeast induction media containing plates with agar and 10nM DIR for visual inspection.

D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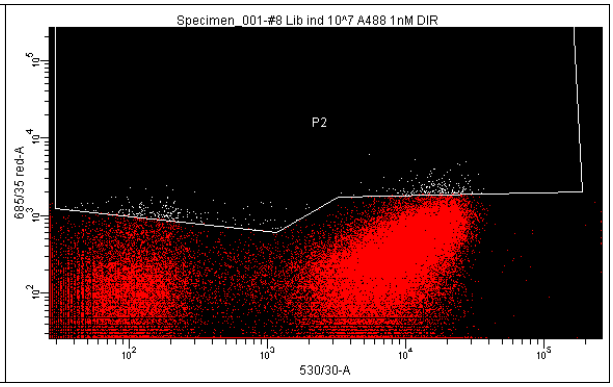
P2
685/35 red-A
530/30-A

E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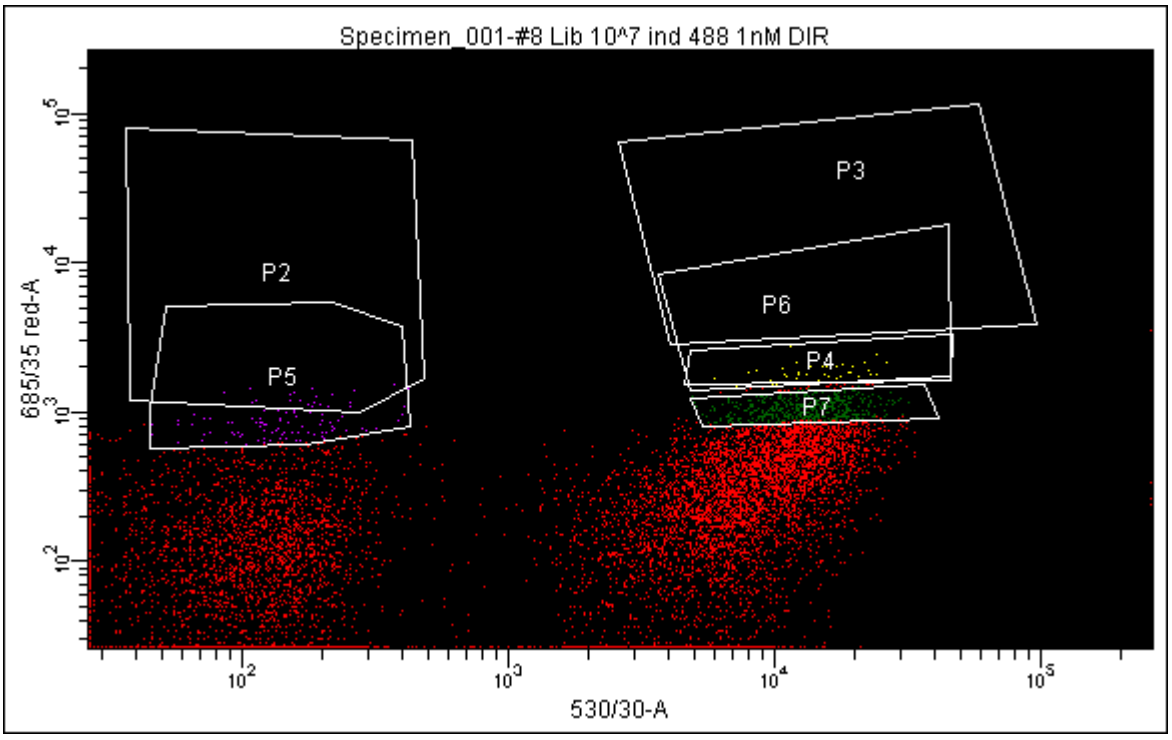
P2
685/35 red-A
530/30-A

F

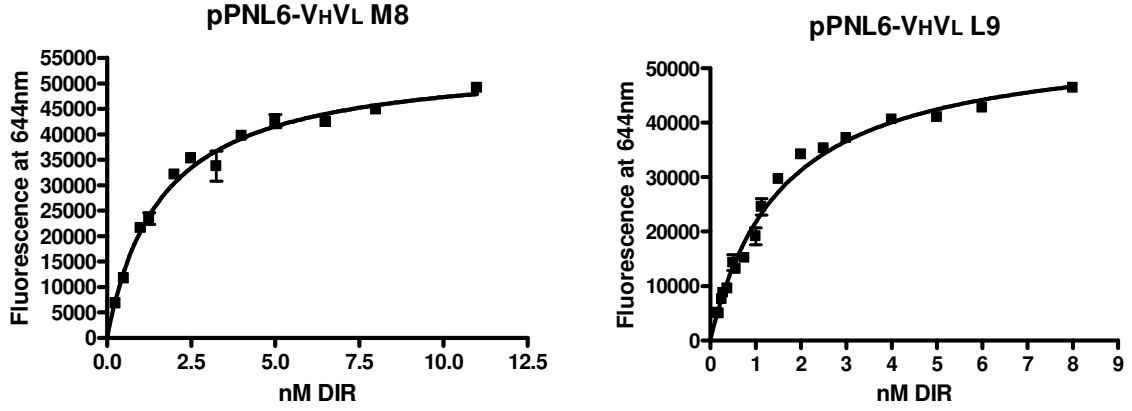

Specimen_001-#8 Lib 10^7 ind 488 1nM DIR
P2
P3
P4
P5
P6
P7
685/35 red-A
530/30-A

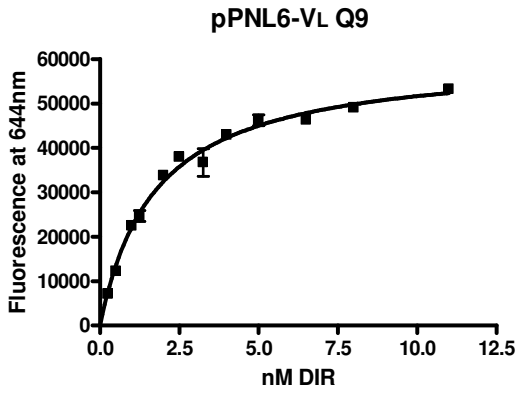
Supporting Information Figure S2. Characteristics of clones isolated from directed evolution of V_H-V_L M8 and M8V_L FAPs and fluorescence equilibrium affinity determinations on the yeast cell surface**.**

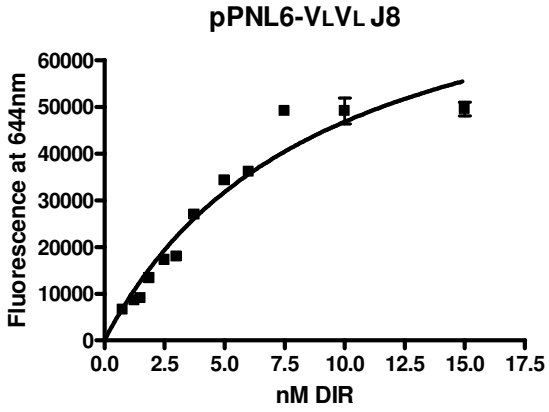
Table S2. Characteristics of FAPs before and after directed evolution.

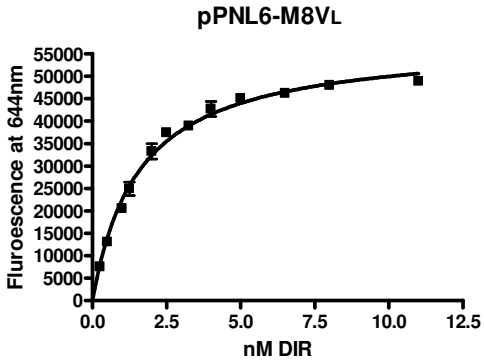
Clone name	Format	surface K_D(nM)	Mutations in sequence different from wild type (V_H-V_L M8 or M8V_L)
M8	V_H-V_L	1.2 ± 0.2	
L9	V_H-V_L	1.6 ± 0.1	$F^{H29}S$, $W^{H36}C$, $A^{H93}V$, $I^{H113i}T$
Q9	V_L	1.9 ± 0.1	$Q^{L1}R$, $T^{L14}I$,$D^{L30}G$,$Q^{L37}R$, $S^{L55}P$, $F^{L62}L$, $S^{L80}P$
J8	V_L-V_L	10.4 ± 1.4	VL#1: $D^{L85}N$, $K^{L103}T$, $L^{L107}S$, $I^{L108i}T$ VL#2: $S^{L9}P$, $S^{L32}P$
M8V_L	V_L	1.8 ± 0.1	
A4	V_L	1.0 ± 0.2	$S^{L55}P$

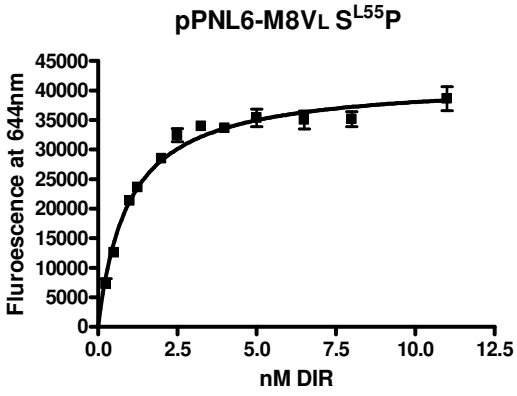
Affinity titrations represented in Table S2. Serial dilutions of DIR were added to 10^6 yeast cells induced to display the protein of interest in buffer (PBS pH 7.4 with 2mM EDTA, 0.1% w/v Pluronic F-127). All pPNL6 titles on figures indicate yeast surface display clones with the clone name after the pPNL6. The yeast surface proteins (black squares) are shown. All measurements were performed in duplicate in 96 well black-bottom plates (Whatman) in Tecan Safire^2 fluorimeter and excited at 602nm. The fluorescence measurements of DIR titrations into 10^6 uninduced yeast cells in buffer were subtracted from raw data prior to curve fitting. Fluorescence was recorded at 644nm and data analyzed using GraphPad fit to a one-site binding equation $y=F_{max}* [X]/(K_d+[X])$, where [X] is the DIR concentration.




pPNL6-VL Q9
Fluorescence at 644nm
60000
50000
40000
30000
20000
10000
0
0.0
2.5
5.0
7.5
10.0
12.5
nM DIR


pPNL6-VLVL J8
Fluorescence at 644nm
60000
50000
40000
30000
20000
10000
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
nM DIR


pPNL6-M8VL
Fluroescence at 644nm
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0.0
2.5
5.0
7.5
10.0
12.5
nM DIR


pPNL6-M8VL S L55 P
Fluroescence at 644nm
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0.0
2.5
5.0
7.5
10.0
12.5
nM DIR

Supporting Information Figure S3. Amino acid sequence alignment of V_H-V_L M8 wild type (top) compared to clones isolated after directed evolution that contain both V_H and V_L domains. Clone names are listed on the left hand side with conserved residues shown as stars, conserved mutations listed by ":", semi-conserved by ".", and a blank space indicates no conserved match (ClustalW).

```
VHVLM8          QVQLVESEGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEGVATIKQDGSEKYY 60
L13             QVQLVESEGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEGVATIKQDGSEKYY 60
L9              QVQLVESEGGLVQPGGSLRLSCAASGFTSSSYWMSCVRQAPGKGLEGVATIKQDGSEKYY 60
O3              QVQLVECEGGLVQPGGPLRLSCAASGFTFSSYWMSWVRQAPGKGLEGVATIKQDGSEKYY 60
P15             QVQLVESEGGLVRPGGSLRLSCAASGFTFSSCWMSWVRQAPGKGLEGVATIKQDGSEKYY 60
O5              QVQLVESEGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEGVATIKQDGSEKYY 60
K6              QVQLVESEGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEGVATIKQDGSEKYY 60
R2              QVQLVESEGGLVQPGGSLRLSCAASGFAFSSYWVSWVRQAPGKGLEGVATIKQDGSEKYY 60
                ******.*****:***.**********: ** *:* ************************

VHVLM8          VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRLVRETGGDYRGLDLWGQGTT 120
L13             VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRLVRETGGDYRGLDLRGQGTT 120
L9              VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCVRDRLVRETGGDYRGLDLWGQGTT 120
O3              VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRLVRETGGDYRGLDLWGQGTT 120
P15             VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRLVRETGGDYRGSDLWGQGTT 120
O5              VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRLVRETGGDYRGLDLWGQGTT 120
K6              VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRLVRETGGDYRGLDLWGQGTT 120
R2              VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRPVRETGGDYRGLDLWGQGTT 120
                ************************************.*** ********** ** *****

VHVLM8          VTVSSASTKGPSGILGSGGGGSGGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 180
L13             VTVSSASTKGPSGILGSGGGGSGGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 180
L9              VTVSSASTKGPSGTLGSGGGGSGGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 180
O3              VTVSSASTRGPSGILGSGGGGSGGGGSGGGGSQPALTQSPSVSGTPGQKVTIFCSGSSSN 180
P15             VTVSSASTRGPSGILGSGGGGSGGGGSGGGGSQPALTQSPSVSGTPGQKVTIFCSGGSSN 180
O5              VTVSSASTKGPSGILGSGGGGSGGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 180
K6              VTVSSASTKGPSGILGSGGGGSGGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 180
R2              VTVSSASTKGPSRILGSGGGGSGGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 180
                ********:***  ********************.**********************.***

VHVLM8          VEDNSVYWYQQFPGTTPKVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 240
L13             VEDNSVYWYQQFPGTTPKVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 240
L9              VEDNSVYWYQQFPGTTPKVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 240
O3              VEDNSVYWYQQFPGTTPKVLIYNDDRRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 240
P15             VEDNSVYWYQQFPGTTPKVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 240
O5              VGDNSVYWYQQFPGTTPKVLIYNDDRRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYC 240
K6              VEDNSVYWYQQFPGTTPKVLIYNDDRRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 240
R2              VEDNSVYWYQQFPGATPKVLIYNDDRRPSGVPDRFSGSKSGXSASLXISGLRSEDEADYY 240
                * ************:************.************* **** ************

VHVLM8          CLSWDDSLNGWVFGGGTKVTVL 262
L13             CLSWDDSLNGWVFGGGTKVTVL 262
L9              CLSWDDSLNGWVFGGGTKVTVL 262
O3              CLSWDDSLNGWVFGGGTKVTVL 262
P15             CLSWDDSLNGWVFGGGTKVTVL 262
O5              CLSWDDSLNGWVFGGGTKVTVL 262
K6              CLSWDDSLNGWVFGGGTKVTVL 262
R2              CLSWDDSLNGWVFGGGTKVTVL 262
                **********************
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Supporting Information Figure S4. Amino acid sequence alignment of V_H-V_L M8 wild type (top) to compared clones isolated from affinity maturation that contained a single V_L domain after directed evolution. Clone names are listed on the left hand side with conserved residues shown as stars, conserved mutations listed by ":", semi-conserved by ".", and a blank space indicates no conserved match (ClustalW).

```
VHVLM8          QVQLVESEGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEGVATIKQDGSEKYY 60
R5              ------------------------------------------------------------
T6              ------------------------------------------------------------
N8              ------------------------------------------------------------
Q9              ------------------------------------------------------------

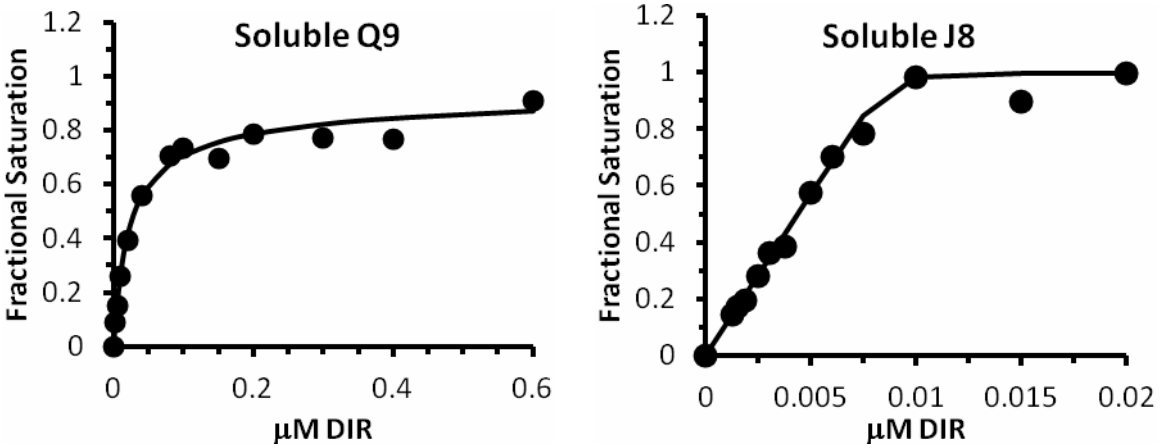
VHVLM8          VDSVKGRITISRDNAKNSLNRQINSLRAEDTAVYYCARDRLVRETGGDYRGLDLWGQGTT 120
R5              ------------------------------------------------------------
T6              ------------------------------------------------------------
N8              ------------------------------------------------------------
Q9              ------------------------------------------------------------

VHVLM8          VTVSSASTKGPSGILGSGGGGSGGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 180
R5              -----------------GGGGSGGCGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 43
T6              -----------------GGGGSCGGGSGGGGSQPVLTQSPSVSGTPGQKVTIFCSGSSSN 43
N8              -----------------GGGGSGGGGSGGGGSRPALTQSPSVSGTPGQKVTIFCSGSSSN 43
Q9              -----------------GGGGSGGGGSGGGGSRPVLTQSPSVSGIPGQKVTIFCSGSSSN 43
                                 ***** * *******:*.********* ***************

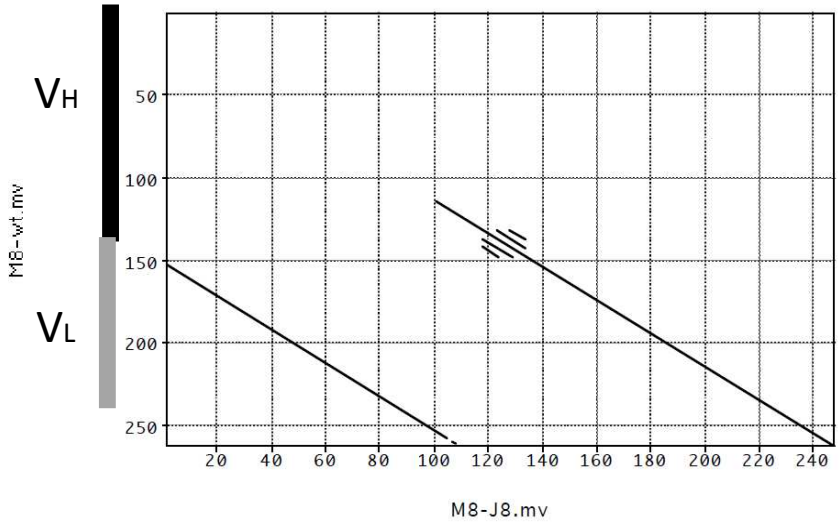
VHVLM8          VEDNSVYWYQQFPGTTPKVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 240
R5              VEDNSVYWYQQFPGTTPKVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 103
T6              VEDNSVYWYQQFPGTTPKVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRSEDEADYY 103
N8              VEDNSVYWYQQFPGTAPEVLIYNDDRRSSGVPDRFSGSKSGTSASLAISGLRPEDEADYY 103
Q9              VEGNSVYWYRQFPGTTPKVLIYNDDRRPSGVPDRLSGSKSGTSASLAISGLRPEDEADYY 103
                **.******:*****:*:*********.******:*****************.*******

VHVLM8          CLSWDDSLNGWVFGGGTKVTVL 262
R5              CLSWDDSLNGWVFGGGTKVTAL 125
T6              CLSWDDSLNGWVFGGGTKVTVL 125
N8              CLSWDDNLNGWVFGGGTKVTVL 125
Q9              CLSWDDSLNGWVFGGGTKVTVL 125
                ******.*************.*
```

Supporting Information Figure S5. Affinity titrations represented in Table 1. Serial dilutions of DIR were added to soluble protein in buffer for Q9 (33 nM) and J8 (9 nM) (PBS pH 7.4 with 2mM EDTA, 0.1% w/v Pluronic F-127). All measurements were performed in duplicate in 96 well black-bottom plates (Whatman) in Tecan Safire^2 fluorimeter and excited at 602nm. The fluorescence measurements of DIR in buffer were subtracted from the raw data prior to curve fitting. Fluorescence was recorded at 644nm and data analyzed using in-house software as described in methods section of the manuscript.



Supporting Information Figure S6. Protein scoring matrix (MacVector) of V_H-V_L M8 compared to V_L-V_L J8 pseudodimer clone from directed evolution. Scoring matrix shows identify between two light domains in V_L-V_L J8 and the V_H-V_L M8 light domain. Left-hand axis shows the relative position of the V_H or V_L domains relative to the J8 clone. Boxes along the left hand side of the diagram indicate (black) sequence homology to the M8 V_H or (grey) sequence homology corresponding to the M8V_L. Small lines represent the (G_4S) repeats in the linker region between domains.



Supporting Information Figure S7. Extension of Kabat nomenclature used for V_H-V_L M8 wild type parent clone. The residues listed below are cannot be assigned in the Kabat nomenclature. They are listed as residue L108 with a lower-case letter to indicate sequence order.

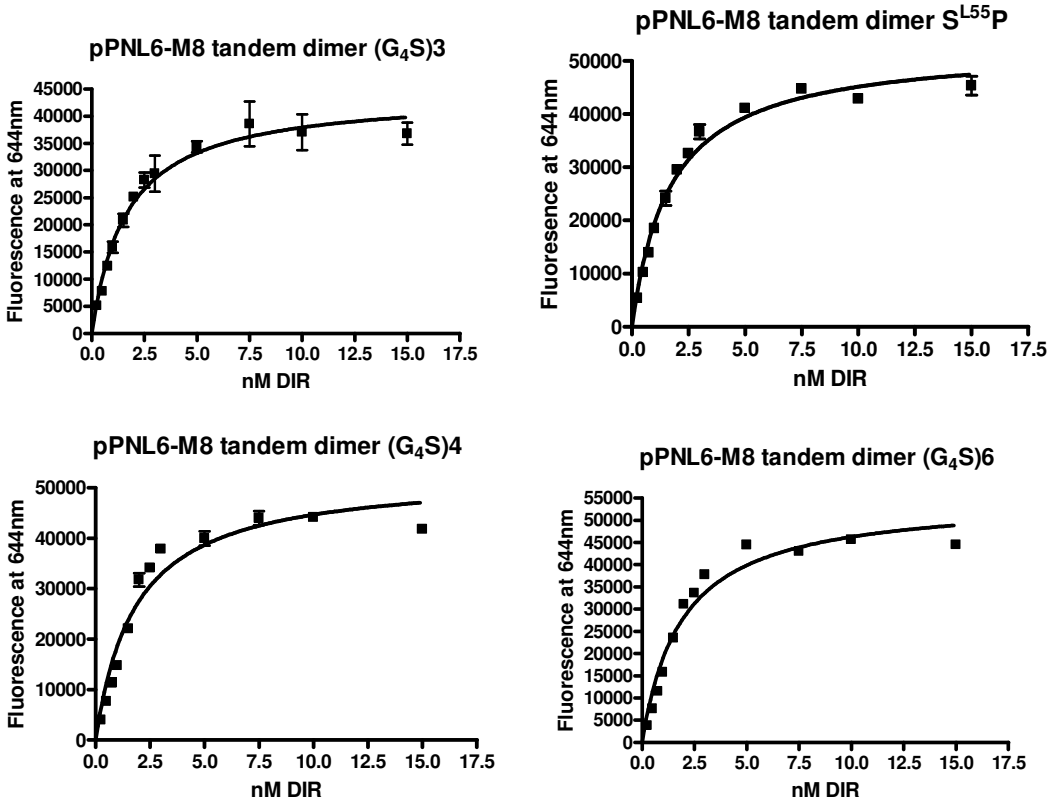
A^{L108a} ,S^{L108b} , T^{L108c} , K^{L108d}, G^{L108e}, P^{L108f}, S^{L108g}, G^{L108h}, T^{L108i}, L^{L108j}, G^{L108k}

Supporting Information Figure S8. Results of altering glycine-serine rich linker length in tandem homodimers created from the $M8V_L$ and fluorescence equilibrium affinity titrations.

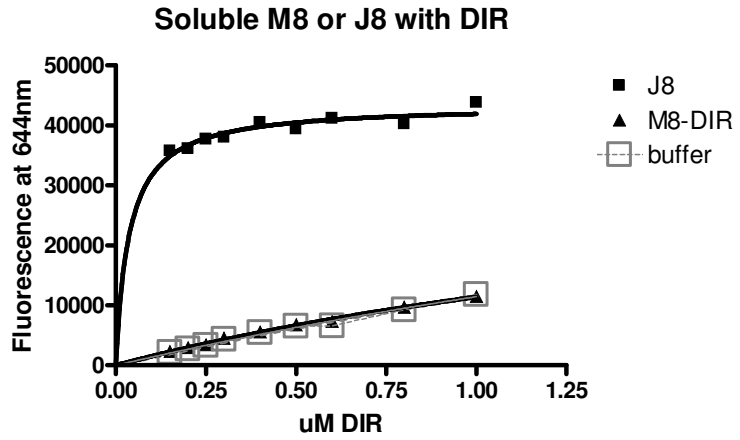
Table S8. Characteristics of covalent homodimers with different linker lengths (yeast surface).

Clone	(G_4S) repeats in linker	Surface K_D (nM)
$dM8V_L(G_4S)_3$	3	1.7 ± 0.2
$dM8V_L(G_4S)_4$	4	1.9 ± 0.3
$dM8V_L(G_4S)_6$	6	2.0 ± 0.2
$dM8V_LS^{L55}P(G_4S)_3$	3	1.8 ± 0.1

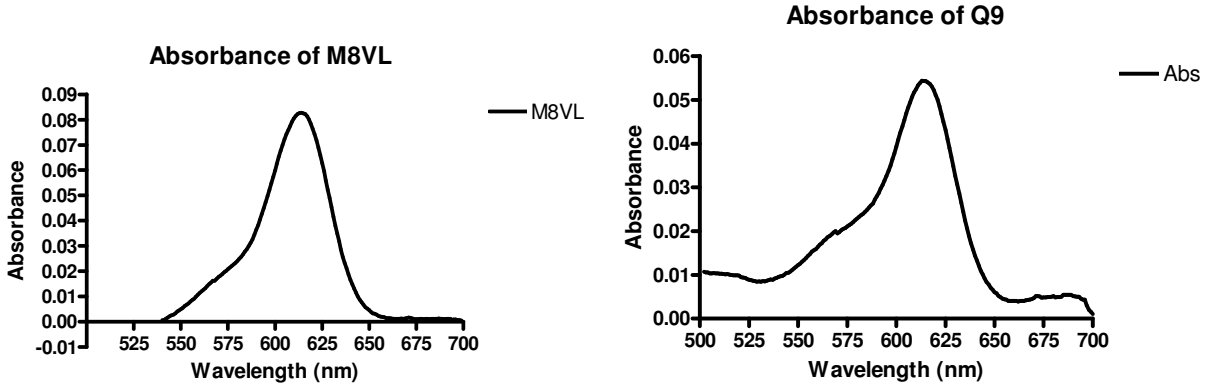
Fluorescence affinity titration represented in Table S8. Serial dilutions of DIR were added to 10^6 yeast cells induced to display the protein of interest in buffer (PBS pH 7.4 with 2mM EDTA, 0.1% w/v Pluronic F-127). pPNL6 titles on figures indicate yeast surface display clones with the clone name after the pPNL6. The fluorescence from yeast surface proteins (black squares) are shown. All measurements were performed in duplicate in 96 well black-bottom plates (Whatman) in Tecan Safire[2] fluorimeter and excited at 602nm. The fluorescence measurements of DIR titrations into 10^6 uninduced yeast cells in buffer were subtracted from the raw data prior to curve fitting. Fluorescence was recorded at 644nm and data analyzed using GraphPad fit to a one-site binding equation $y=F_{max}* [X]/(K_d+[X])$, where [X] is DIR concentration.



Supporting Information Figure S9. Fluorescence titration curve of DIR into soluble V_H-V_L M8 (black triangle), soluble pseudodimer V_L-V_L J8 (black square) or buffer (grey triangle). Serial dilutions of DIR were titrated into 10nM soluble protein, excited at 602nm and the fluorescence recorded at 644nm on TECAN Safire^2 plate reader. Curves are intended to guide the eye.



Supporting Information Figure S10. Absorbance spectra for two monomeric V_L fluoromodules; 5.0uM M8V_L with 0.5uM DIR that gives a high quantum yield (71%) and 0.5uM Q9 with 0.1uM DIR that has a low quantum yield (19%). Data obtained on a Cary 300 Bio UV-Visible spectrophotometer at 20°C.



Supporting Information S11

S11A: Effect of Protein Concentration on Data Fitting: Nine synthetic data sets were constructed using protein concentrations of 100 nM, 10 nM, and 1 nM and K_D values of 1 nM, 0.1 nM, and 0.01 nM. The error in the measured fluorescence in these data sets was 10%. Each data set consisted of 50 independent binding curves. The dependence of the χ^2 as K_D is varied provides information on the robustness of the data fitting (Figure S11A). These plots show that well defined minima in χ^2 can be obtained even if the protein concentration is considerably higher than K_D (panels A and B). In particular, protein concentrations as high as 100 nM can be used in titrations to measure K_D values as low as 1 nM. However, more robust fits are clearly obtained when the protein concentration is on the order of the K_D value (or lower). We found that the average K_D values obtained for fits which showed a well defined minimum in χ^2 for all 50 members of the data set were within a factor of 2 of the expected K_D value (see Table S11). Panel B & C (Figure S11A) indicate that at protein concentrations of ~1 nM (or higher), K_D values less than 0.1 nM cannot be reliably obtained by fitting.

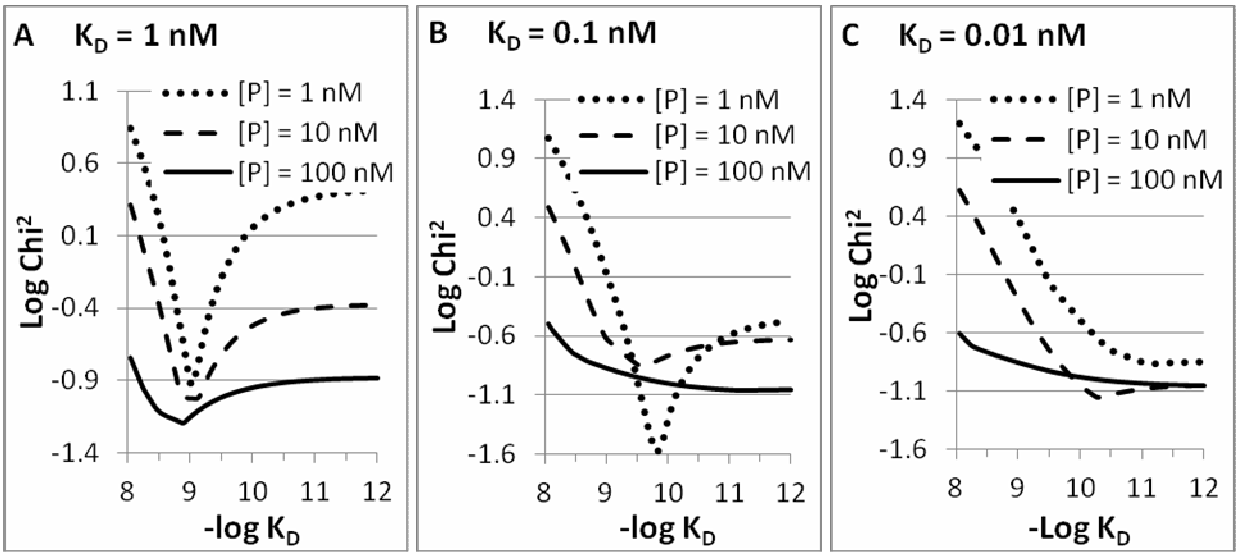
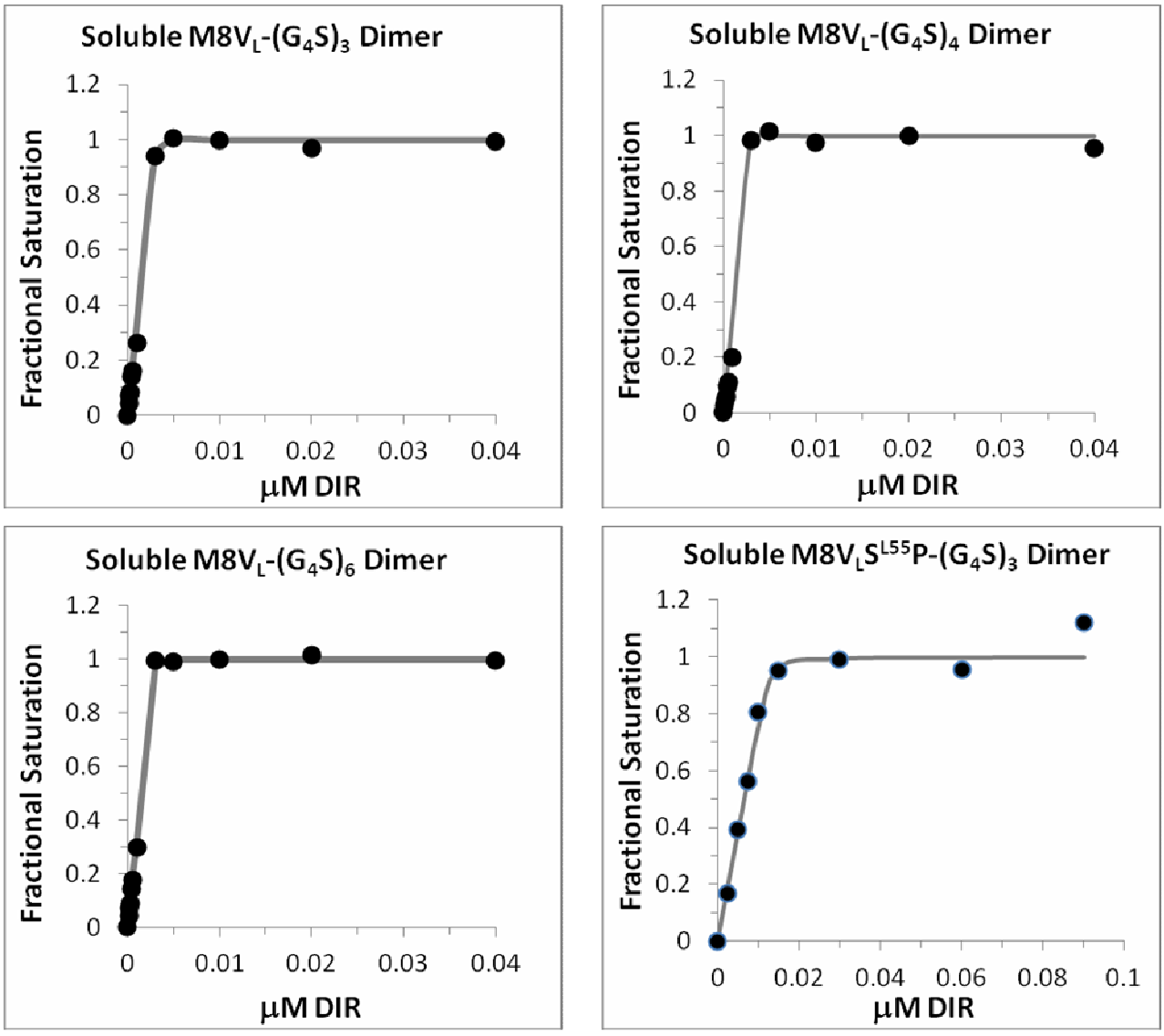


Figure S11A. The effect of varying protein concentration on the ability to obtain K_D values from data fitting. For each of the K_D values (A=1.0, B= 0.1, C= 0.01 nM) and protein concentrations (100, 10, 1 nM), a total of 50 synthetic data sets were constructed assuming an error of 10% in the measurement of the fluorescence signal. Plotted curves are a representative plot of the log of the square of the difference between the actual and predicted data (χ^2) for one of the 50 data sets associated with each condition. Panels A and B indicate that a clear minima in the χ^2 value can be observed for measurements where the protein concentration is 10 fold higher than the K_D. Panel C shows that reliable values for K_D values less than ~0.01 nM cannot be obtained if the protein concentration is 1 nM or higher.

Table S11 – Effect of Protein Concentration on Fitting Accuracy.

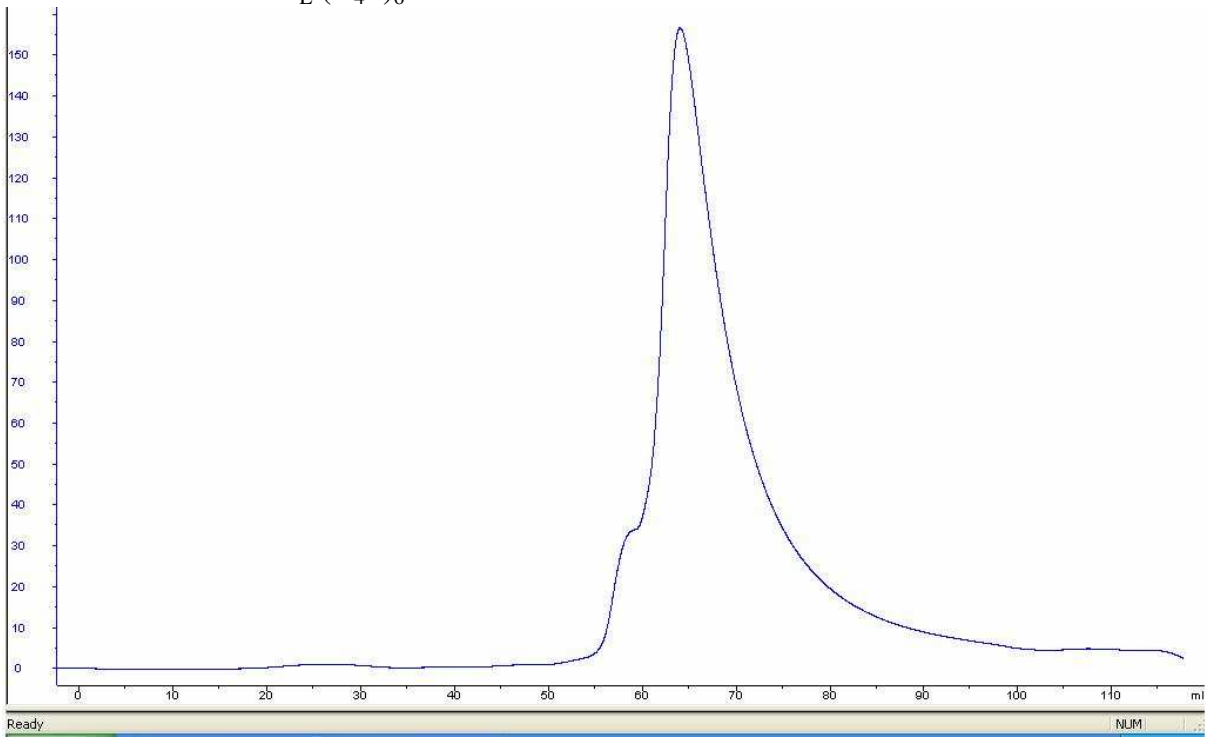
	Fitted K_D		
K_D (Expected)	**[P]=100 nM**	**[P]=10 nM**	**[P]=1 nM**
1.00 nM	1.42 ±1.00	0.94 ± 0.27	1.00±0.09
0.10 nM	No χ^2 minimum.	0.18±0.10	0.150±0.030

S11B: Fluorescence affinity titrations represented in Table 2 for synthetic dimers of $M8V_L$ and $M8V_LS^{L55}P$. These were performed in PBS, pH 7.4, with 2mM EDTA, 0.1% w/v Pluronic F-127 by titrating DIR into protein solutions. Concentrations of proteins were 3nM, 3nM, 3nM, and 12 nM for $M8V_L(G_4S)_3$, $M8V_L(G_4S)_4$, $M8V_L(G_4S)_6$, $M8V_LS^{L55}P$, respectively. All measurements were performed in triplicate in 96 well black-bottom plates (Whatman) in Tecan Safire^2 fluorimeter and excited at 602nm. Fluorescence was recorded at 644nm and data, corrected for background fluorescence in the absence of protein, and fit to a single binding site using the quadratic equation with in-house software. The solid grey line shows the best fit.

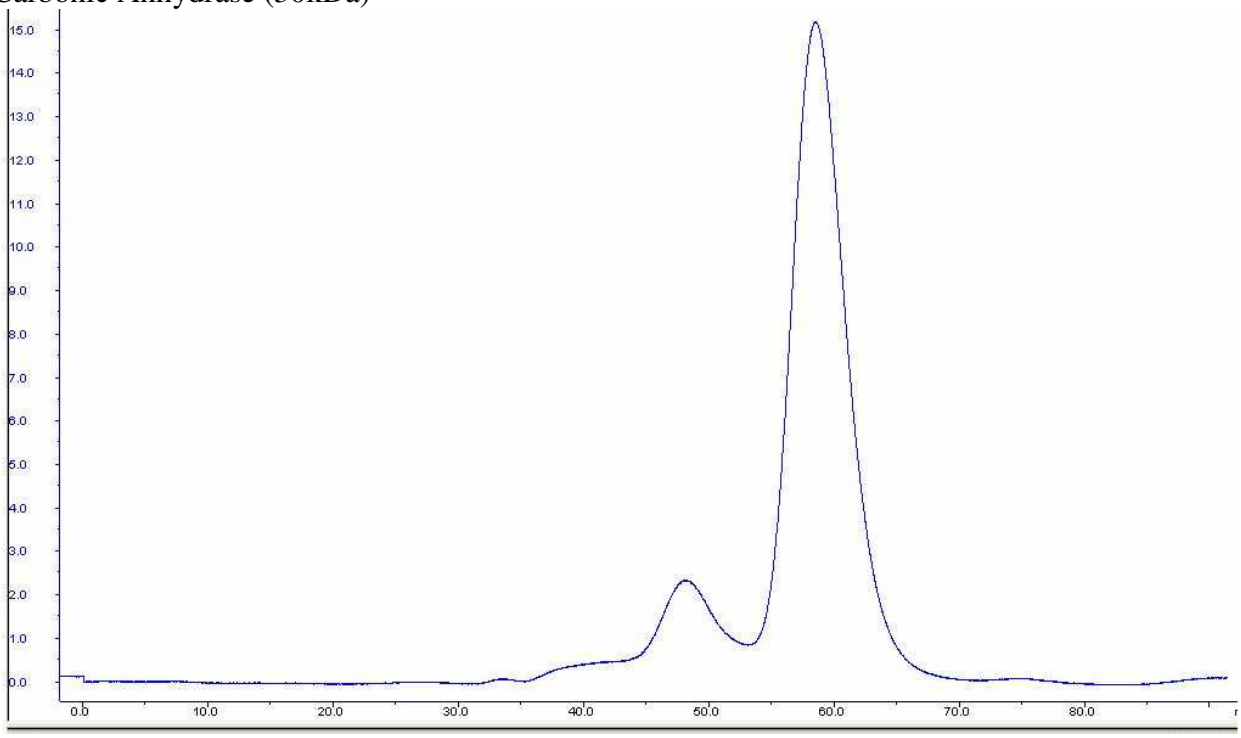


Supporting Information S12. Size exclusion analysis of the tandem homodimer $M8V_L$ with $(G_4S)_6$ linker repeats. Four milligrams of purified protein was injected into a HiPrep 16/60 Sephacryl S-100 column (GE Healthcare) in buffer (10mM Tris pH 8.0, 300mM NaCl) run at 1mL/min.. The single peak of tandem dimer $M8V_L$ $(G_4S)_6$ correspondes to a molecular weight of 26kDa (elution of 65mLs). For comparision two molecular weigth standards are shown, conalbumin 76kDa (elution 45mLs) and carbonic anhydrase B 30kDa (elution 60mLs).

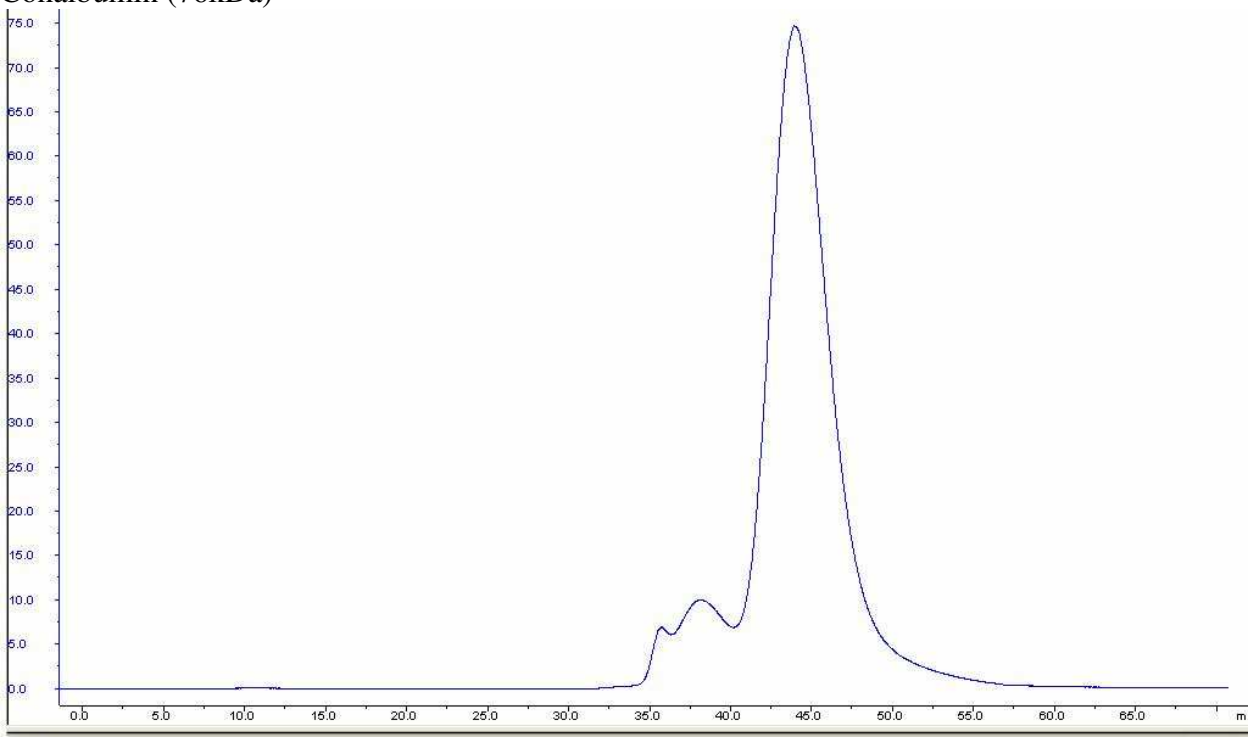
Tandem dimer of $M8V_L$ $(G_4S)_6$



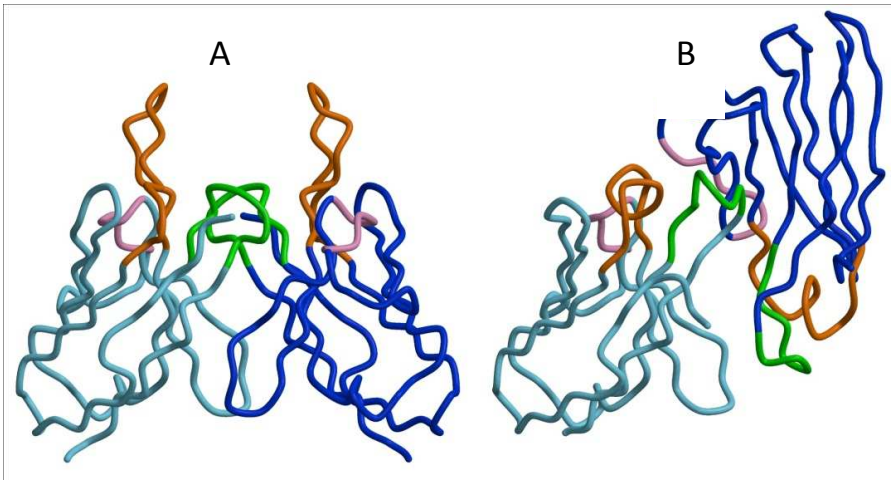
Carbonic Anhydrase (30kDa)



Conalbumin (76kDa)



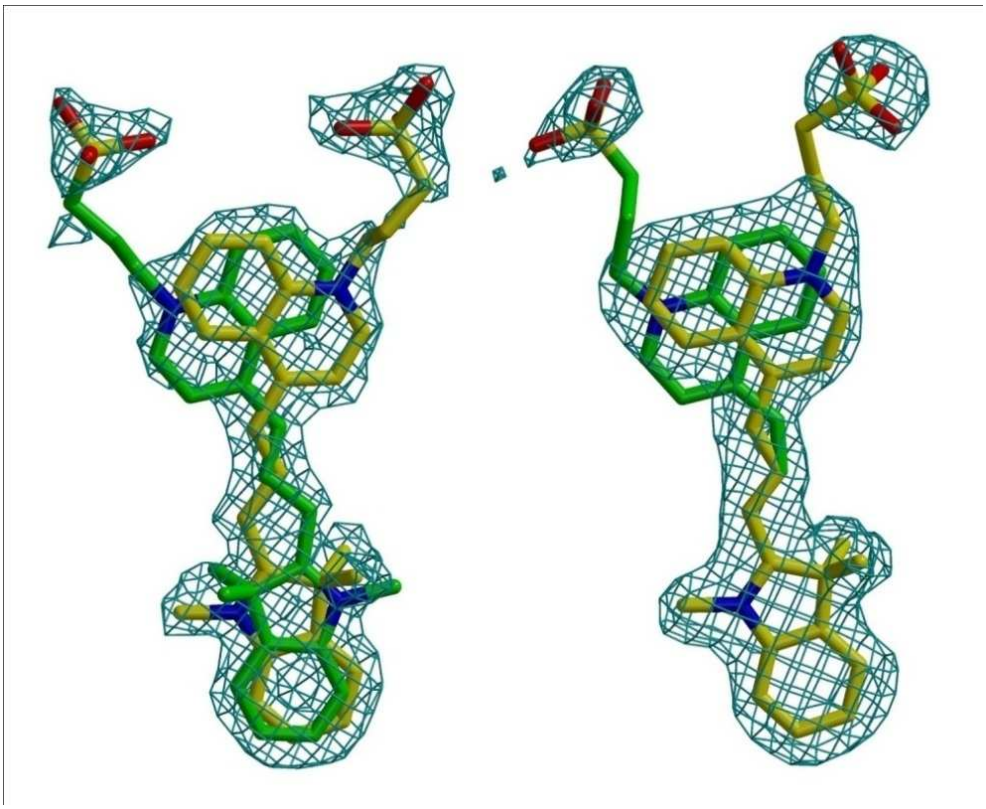
Supporting Information Figure S13. Comparison of V_L dimeric arrangement. (A) The 'typical' light chain dimer LEN has a V_L-V_L arrangement very similar to that seen in a Fab V_L-V_H variable domain. (B) The M8V_LS^{L55}P dimer is shown, oriented with the A chain (light blue) superimposed on the A chain of LEN. The B chain (dark blue) is rotated by 133° from the position of the B chain in LEN. The B chain of the M8V_L structure (not shown) is rotated 131° from the position in LEN.



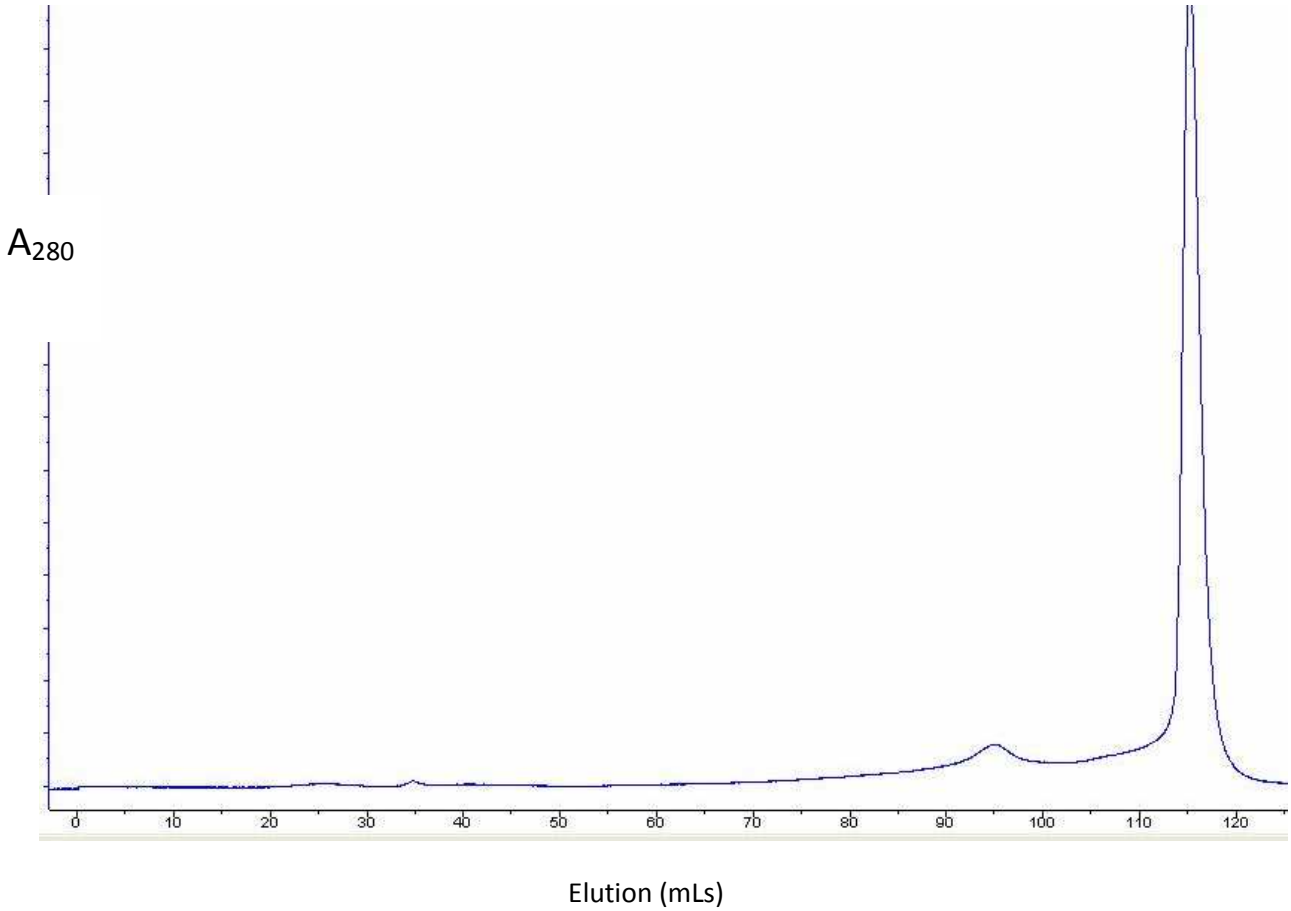
Supporting Information Table S14. Table of angles calculated between key contact residues and different functional groups of DIR in two conformations. Also shown are distances between key contact residues and the different functional groups of DIR. The DIR atoms used to estimate the plane and center of gravity of the quinoline ring were C14, C15, C19, C20, C21, C22. This is the 6-atom ring closest to the Tyr^{L49} ring. DIR atoms used for the indole ring plane and center of gravity were N7, C, C2, C3, C8, C9, C30, C31, C32. The Tyr and Phe ring planes and centers of gravity used the 6 atoms of the main ring, and the Trp ring plane and center of gravity was calculated using all indole atoms. Distances are between the centers of gravity of the ring systems.

Table S14. Angles and distances between aromatic residues and DIR									
		M8V_L				M8V_LS^{L55}P			
		Angle (°)		Distance (Å)		Angle (°)		Distance (Å)	
DIR atoms	FAP atoms	DIR conf. 1	DIR conf. 2	DIR conf. 1	DIR conf. 2	DIR conf. 1	DIR conf. 2	DIR conf. 1	DIR conf. 2
quinoline	Tyr49A	5.0	7.6	4.0	3.6	7.1	10.7	3.9	3.7
quinoline	Tyr49B	10.4	6.5	3.7	4.1	11.3	7.6	4.3	4.2
indole	Tyr34A	36.4	36.7	5.8	5.9	31.7	31.7	5.6	5.6
indole	Tyr34B	34.1	32.9	5.9	5.8	36.3	36.3	6.1	6.1
indole	Trp96A	75.4	74.0	5.6	5.5	78.6	78.6	5.5	5.5
indole	Trp96B	71.1	70.3	5.7	5.7	72.2	72.2	5.6	5.6
indole	Phe98A	67.3	66.8	6.7	6.8	67.6	67.6	6.9	6.9
indole	Phe98B	64.1	62.7	7.0	6.9	65.7	65.7	6.8	6.8

Supplemental Information S15. Electron density for DIR ligands. (a) Electron density (1σ contour) in the $M8V_L$ complex shows two alternate conformations for DIR that are related by the approximate non-crystallographic 2-fold symmetry. (b) Electron density (1σ contour) for DIR in the $M8V_LS^{L55}P$ complex shows one conformation for the indole ring, and two conformations for the quinoline ring that are related by the approximate non-crystallographic 2-fold axis.



Supporting Information Figure S16. Gel filtration chromatography of purified $M8V_L$ monomer protein on Sephacryl S-100 column (GE Healthcare) with a 120mL column volume. The $M8V_L$ elutes in a large peak at approxiamtely 116mLs, an elution volume much reduced when compared to low molecular wieght kit of protein gel filtration standards (GE Healthcare). This unusual elution pattern was not altered by several buffer conditions tested.



Supporting Information Figure S17. NMR data of mean T_2 relaxation times, in addition to the theoretical and experimentally derived global correlation times (τ_m).

	$M8V_L$	**$M8V_L$ in the presence of DIR**
Mean T_2 (ms)	95.80	57.17
Theoretical τ_m (ns)	9.36	16.38
Experimental τ_m (ns)	9.90	15.87