

Supporting Information for Özhalici-Ünal, et al.

“A Rainbow of Fluoromodules: A Promiscuous scFv Protein Binds to and Activates a Diverse Set of Fluorogenic Cyanine Dyes”

Selection of Yeast Surface Display Clones that Bind and Activate DIR

A yeast cell surface display library, composed of ca. 8×10^8 recombinant human scFv's derived from cDNA representing a naïve germline repertoire, was obtained from Dane Wittrup, MIT. This library is currently available from Pacific Northwest National Laboratory (PNNL, <http://www.sysbio.org/dataresources/singlechain.stm>). EBY100 was the host to the yeast display library. For studies of individual FAP genes, pPNL6 plasmids were isolated from yeast using Zymoprep I kit (Zymoresearch) and transferred to Mach1™-T1^R or TOP10 *E. coli* (Invitrogen).

Enrichment and cloning of DIR-activating scFvs from the surface display library was essentially as described previously.^{1,2} Briefly 10^{10} induced yeast cells from the surface display library (ca. 10X over-sampling) were incubated with 1 μ M biotinylated DIR³ with gentle mixing for 60 minutes at room temperature, followed by a 10 minute incubation on ice. The cells were pelleted and washed once in modified PBS buffer (PBS pH 7.4, 2 mM EDTA, 0.1% w/v Pluronic F-127 (Molecular Probes, Invitrogen). The cells were pelleted and resuspended in 5 ml modified PBS to which was added 200 μ l streptavidin MACS microbeads (Miltenyi Biotec).⁴ The cells were held on ice for 30 minutes with gentle inversion every 2 minutes. The cells were pelleted and resuspended in 50 ml modified PBS for collection on a MidiMACS column as described by Chao.⁵ The collected cells (ca. 3.5×10^6) were dispersed into low pH growth media and grown to saturation. The MACS isolation was repeated on these cells after induction except that anti-biotin MACS microbeads were used in the second isolation.⁴ 3.7×10^7 cells were isolated in the second selection and grown to saturation in low pH growth media. From this population, yeast cells that directly activate fluorescence from DIR after induction were isolated by two successive cycles of 2-color FACS enrichment based on enhanced fluorescence of the fluorogen and high surface expression of scFvs.² FACS enrichment was carried out on a Becton Dickinson FACSVantage SE with FACSDiva option. In a final third FACS sort, cells were autocloned onto agar plates comprised of solidified induction media containing 10 μ M DIR (Y. Creeger, manuscript in preparation). After 5 days of growth at 20°C, this plate was imaged under 635x20 nm band pass illumination from red LEDs, using an Andor DU434-BR-DD CCD camera fitted with a 680x25 nm band pass filter. Colonies showing brightest DIR fluorescence signal were picked for further analysis.

Determination of fluorogen binding affinity to yeast surface displayed FAPs.

10^7 induced yeast cells in 150 μ L modified PBS (1X PBS with 2mM EDTA, 0.1 % pF127, pH 7.4) containing fluorogen over a concentration range were assayed in triplicate for fluorescence in 96 well microplates on a Tecan Safire² reader. Fluorescence was corrected by subtracting the fluorescence of a dye only sample. As controls, uninduced EBY100 cells which do not express the scFvs were assayed with the same conditions. The following one site saturation binding algorithm equation was used to fit the resulting saturation binding curves and to calculate the binding affinities using Origin 6.1:

$$y = B_{\max} * x / (K_d + x)$$

where x is the concentration of the fluorogen.

Expression and purification of soluble K7

The scFv (K7) genes were subcloned into the periplasmic expression vector pAK400 introducing a His6-tag in *E. coli* cells. IPTG induced cells were harvested for osmotic shock and extensively dialyzed. Expressed protein was purified by nickel-nitrilotriacetic acid chromatography (Ni-NTA, Qiagen) according to manufacturer's instructions. Eluted fractions were assayed for protein content using a NanoOrange protein quantitation kit (Invitrogen), and analyzed by SDS gel electrophoresis.

Determination of fluorogen binding affinity to soluble K7

The DIR binding affinity to soluble FAP was determined using a Photon Technologies International fluorimeter. A saturation binding curve was generated by titrating 2 nM aliquots of a DIR solution into a 10 nM K7 solution in 1 mL total volume of modified PBS. Fluorescence was corrected by subtracting the fluorescence obtained by titration of DIR into buffer. Titrations were performed in triplicate. Spectra were obtained by exciting the samples at 580 nm and measuring the fluorescence intensity from 600 to 750 nm. Fluorescence at 636 nm was monitored for the generation of the binding curve. The bandpass for both excitation and emission monochromators were 5nm. Resulting binding curves were fitted with the one site binding algorithm as described above. Analogous experiments were performed with other dyes shown in Figure 3 (dyes obtained from Invitrogen).

Microscopy

The image in Figure 2 was acquired on an Olympus Fluoview 1000 scanning laser confocal microscope using a 100x oil immersion objective. Yeast were incubated with 100 nM DIR for 30 min on ice. Sample was excited with a 633 nm HeNe laser and emission was monitored in the Cy5 window.

Sequences of DIR scFvs

K7

QVQLVQSGAEVKKPGASVKVSCKVSGYTLSESPMHWVRQAPGKGLEWMGHFDPEDGEKIYAAQ
KFQGRVTLTDDTSTDTVYLELSSLTSEDVAVYYCATETGWGPGTLVTVSSGILGSGGGGSGGGG
SGGGGSNFMLTQPRSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTIIYEDHQRPSPV
DRFSGSIDSSNSASLTIAGLRTEDEADYFCQSYDANDVVFVGGGTKLTVL

A8

QVQLVESGGRLVKPGGSLRLSCEASGFTFSDYYMGWIRQAPGKGLEWVSYINGDSSYTNYTDSV
KGRFSISRDNKNSLYLQLSRLRAEDTAVYYCVTSRGSSDSWYRVHWGQGTTLVTVSSGILGSGG
GGSGGGGSGGGGSSYELTQSPSASGTPGQRVTISCSGSSSNIGSQYVYWYQQLPGTAPKLLIYKN
NQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGPVFVGGGTQLTVL

K10

QVQLQQSGPGLAKPSQTLSTCAISGDSVSSNTATWNWIRQSPSSGLEWLGRITYYRSKWHNDYE
VSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARSGYFQEYLQHWGQGTTLVTVSSGILGSGG
GGSGGGGSGGGGSGPVLTVQSPSVSGTPGQKVTIFCSGSSSNVEDNSVYWYQQFPGTTPKVLIYND
DRRSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCLSWDDSLNGWVFGGGTKVTVL

J6

QVQLVQSGAEVKKPGASVKVSCRVSRYRLSDLSIHWVRQAPGKGLDWVGSFDPEAGETIYDQK
FQGRVTLTDDTSTDTAYMELRRLTSDDTAIYYCATDQAAA WGQGTTLVTVSSGILGSGGGGSGG
GGSGGGGSNFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRP
GVPDRFSGSIDSSNSASLTVSGLKTEDEADYYCQSYDSSNHA VFGGGTKLTVL

M8

QVQLVESEGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEGVATIKQDGSEKYYVDS
VKGRITISRDNKNSLRQINSLRAEDTAVYYCARDRLVRETGGDYRGLDLWGQGTTLTVSSAS
TKGPSGILGSGGGGSGGGGSGGGGSGPVLTVQSPSVSGTPGQKVTIFCSGSSSNVEDNSVYWYQQF
PGTTPKVLIYND RRRSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCLSWDDSLNGWVFGGGT
KTVL

Supplementary Figures

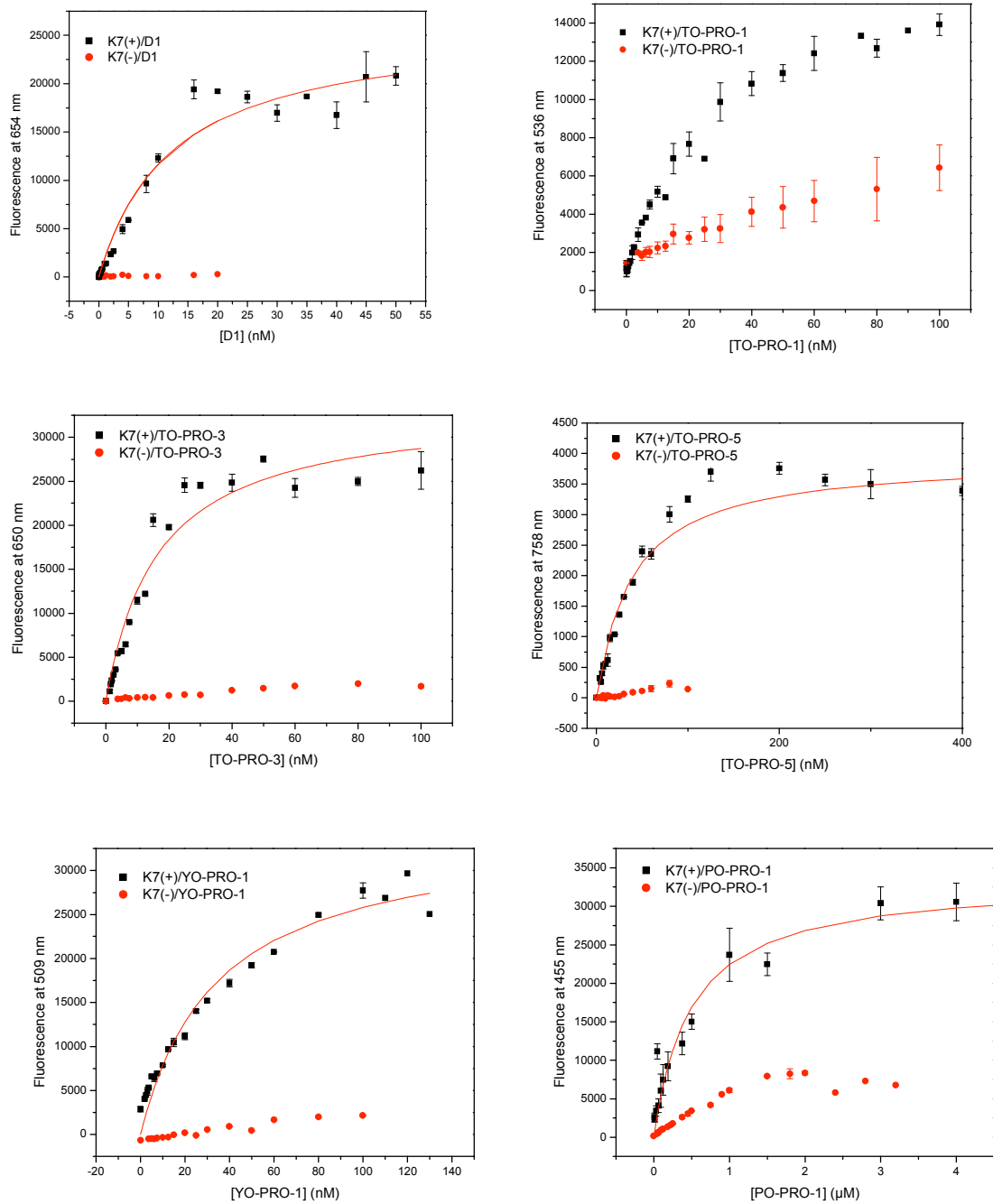


Figure S1. Saturation binding curves generated by titrating fluorogens into cells expressing K7 (black data points), and into cells which do not express K7 (red data points). Both binding curves were corrected for fluorescence of fluorogens in buffer. Data points for induced cells were fitted with one site binding algorithm (red line). Shown are binding curves for D1, TO-PRO-1, TO-PRO-3, TO-PRO-5, YO-PRO-1, and PO-PRO-1. Note that TO-PRO-1 and PO-PRO-1 do show non-negligible fluorescence with uninduced cells.

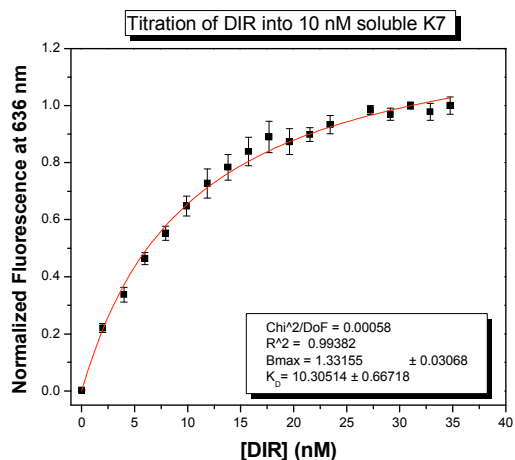


Figure S2. Fluorescence saturation binding curve generated by titrating aliquots of DIR into a 10 nM solution of K7.

References

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