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Supporting Information

Fluorescent Probes to Characterise FK506-Binding Proteins

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Appendix 1:

The equation:

$$[EC_{50}] = \tfrac{1}{2}[L]_t + K_d \qquad (1.)$$

Equation *(1.)* can be deduced as follows: At equilibrium, $K_d = [L] \times [R] / [LR]$ *(2.)*, $[L]_t = [L] + [LR]$ *(3.)*, and $[R]_t = [R] + [LR]$ *(4.)*, with *[L]*, *[R]*, and *[LR]* being the concentrations of free ligand, free receptor and the ligand-receptor complex and $[L]_t$ and $[R]_t$ being the total concentrations of ligand and receptor, respectively.

In FP binding experiments, the EC_{50} is defined as the total concentration $[R]_t^{50}$ where equal amounts of free ligand and bound ligand are present, assuming identical intrinsic fluorescence intensities for free and bound ligands. Therefore, at the EC_{50}, equation *(3.)* simplifies to $[L] = [LR] = \tfrac{1}{2}[L]_t$ *(5.)* and equation *(2.) to* $K_d = [R]$ *(6.)*. At the EC_{50}, equations *(4.)*, *(5.)* and *(6.)* therefore yield $[R]_t^{50} = K_d + \tfrac{1}{2}[L]_t$ *(1.)*.

Appendix 2:

A mathematical term describing the ligand-receptor complex (LR) in binding assays was described by Wang et. al. 1992 [45].

$$[LR] = [([R]_t + [L]_t + K_d)/2] - \{[(R]_t + [L]_t + K_d)/2]^2 - [L]_t[R]_t\}^{0.5}\} \qquad (7.)$$

The fluorescence anisotropy (A) is direct proportional to the increase of [LR] [46]. This relation is representated by a linear function, raising from the minimal measured anisotropy (A_{min}) to the maximal measured anisotropy (A_{max}) with a slope of $\Delta A/[L]_t$

$$A = (\Delta A / [L]_t)\,[LR] + A_{min} \qquad (8.)$$

Eq.(9.) follows from inserting (7.) into (8.) and was used to fit K_d values from binding curves.

$$A = [(A_{max} - A_{min}) / [L]_t] \times \{[([R]_t + [L]_t + K_d)/2] - \{[(R]_t + [L]_t + K_d)/2]^2 - [L]_t[R]_t\}^{0.5}\} + A_{min} \qquad (9.)$$

Appendix 3:

A mathematical expression describing competitive binding of two different ligands to a protein molecule was described by Wang et. al. 1995 [30].

The mathematical term for the competition of a titrated compound with a tracer for the binding to a protein was used to describe competition curves. The conversion into anisotropy values was done by insertion into (8.), the resulting formula was used to fit K_i values.

$$A = (A_{max} - A_{min}) / [L]_t \mathrm{~x~} (([L]_t \mathrm{~x~} ((2\mathrm{x}((K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)\text{\textasciicircum}2 - 3\mathrm{x}(K_{comp} \mathrm{~x~} ([L]_t - [R]_t) + K_{lig} \mathrm{~x~} ([I]_t - [R]_t) + K_{lig} \mathrm{~x~} K_{comp}))\text{\textasciicircum}0.5\mathrm{x}\ \mathrm{COS}(\mathrm{ARCCOS}((-2\mathrm{x}(K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)\text{\textasciicircum}3 + 9\mathrm{x}(K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t) \mathrm{~x~} (K_{comp} \mathrm{~x~} ([L]_t - [R]_t)+ K_{lig} \mathrm{~x~} ([I]_t - [R]_t) + K_{lig} \mathrm{~x~} K_{comp}) - 27\mathrm{x}(-1\mathrm{x}\ K_{lig} \mathrm{~x~} K_{comp} \mathrm{~x~} [R]_t)) / (2\mathrm{x}\ ((((K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)\text{\textasciicircum}2 - 3\mathrm{x}\ (K_{comp} \mathrm{~x~} ([L]_t - [R]_t) + K_{lig} \mathrm{~x~} ([I]_t - [R]_t) + K_{lig} \mathrm{~x~} K_{comp}))\text{\textasciicircum}3)\text{\textasciicircum}0.5))) / 3)) - (K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t))) / ((3\mathrm{x}\ K_{lig}) + ((2\mathrm{x}((K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)\text{\textasciicircum}2 - 3\mathrm{x}(K_{comp} \mathrm{~x~} ([L]_t - [R]_t) + K_{lig} \mathrm{~x~} ([I]_t - [R]_t) + K_{lig} \mathrm{~x~} K_{comp}))\text{\textasciicircum}0.5\mathrm{x}\mathrm{COS}(\mathrm{ARCCOS}((-2\mathrm{x}(K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)\text{\textasciicircum}3 + 9\mathrm{x}(K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t) \mathrm{~x~} (K_{comp} \mathrm{~x~} ([L]_t - [R]_t) + K_{lig} \mathrm{~x~} ([I]_t - [R]_t) + K_{lig} \mathrm{~x~} K_{comp}) - 27\mathrm{x}(-1\mathrm{x}\ K_{lig} \mathrm{~x~} K_{comp} \mathrm{~x~} [R]_t)) / (2\mathrm{x}((((K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)\text{\textasciicircum}2 - 3\mathrm{x}(K_{comp} \mathrm{~x~} ([L]_t - [R]_t) + K_{lig} \mathrm{~x~} ([I]_t - [R]_t) + K_{lig} \mathrm{~x~} K_{comp}))\text{\textasciicircum}3)\text{\textasciicircum}0.5)))/3)) - (K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)))) + A_{min} \tag{10.}$$

In this equation K_{lig} and K_{comp} stand for the K_d values of the used tracer or competing inhibitor, $[I]_t$ is referring to the total concentration of the titrated inhibitor.