Supplementary methods

Determine the dissociation constants through a competitive binding assay:

This method was first described by Gao et al (高振霆 et al, 中国科学 B 辑，化学，2004，34(6):441-448) for the measurement of the binding affinity of a non-fluorescent ligand (L) towards a receptor (R) through its competitive binding of the same binding site on the receptor with a fluorescent probe (P).

Two equilibriums are established in competitive binding.

$$R + P \xleftrightarrow{Kd} P\,R \qquad\qquad R + L \xleftrightarrow{Ki} L\,R$$

K_d: dissociation constant of R and P, Ki: dissociation constant of L and R, R: receptor, P: fluorescent probe, L: ligand, P R: complex of receptor and probe, L R: complex of receptor and ligand. In the following deduction, [R], [P] and [L] represent the concentration of the free receptor, free probe and free ligand, respectively. [P R] and [L R] represent the concentration of the free complex of receptor and probe and free complex of receptor and ligand, respectively. $[R]_t$, $[P]_t$ and $[L]_t$ represent the concentration of the total receptor, total probe and total ligand, respectively. If the receptor has only one binding site, five equations can been constructed according to above equilibrium.

$$[P]_t = [P] + [P\,R] \qquad (1)$$

$$[L]_t = [L] + [L\,R] \qquad (2)$$

$$[R]_t = [R] + [P\,R] + [L\,R] \qquad (3)$$

$$Kd = \frac{[P] * [R]}{[P \cdot R]} \qquad (4)$$

$$Ki = \frac{[L] * [R]}{[L \cdot R]} \qquad (5)$$

Before the competitive ligand is added into equilibrium solution, the $[L]_t = [L\,R] = 0$. Rearrange the (1) and (3),

$$[P] = [P]_t - [P\,R] \qquad (6)$$

$$[R] = [R]_t - [P\,R] \qquad (7)$$

(6) and (7) are brought in (4),

$$Kd = \frac{([P]_t - [P \cdot R]) * ([R]_t - [P \cdot R])}{[P \cdot R]} \qquad (8)$$

Rearrange (8),

$$[P \cdot R] = \frac{K_d + [\mathrm{P}]_t + [R]_t - \sqrt{(K_d + [\mathrm{P}]_t + [R]_t)^2 - 4 * [\mathrm{P}]_t * [R]_t}}{2} \quad (9)$$

In competitive binding, $[R]_t$, $[P]_t$ and Kd is constant. The initial complex [P R] can be figured out according to (9).

After the competitive ligand is added into equilibrium solution, rearrange (1) and (2),

$[P] = [P]_t - [P\ R]$ (6)

$[L] = [L]_t - [L\ R]$ (10)

(6) and (10) are brought in (4) and (5), respectively.

$$[R] = \frac{Kd * [P \cdot R]}{[P]_t - [P \cdot R]} \quad (11)$$

$$[R] = \frac{Ki * [L \cdot R]}{[L]_t - [L \cdot R]} \quad (12)$$

Rearrange (3),

$[L\ R] = [R]_t - [R] - [P\ R]$ (13)

(11) is brought in (13),

$$[L\ R] = [R]_t - [P\ R] - \frac{Kd * [P \cdot R]}{[P]_t - [P \cdot R]} \quad (14)$$

(11) is brought in (12),

$$\frac{Kd * [P \cdot R]}{[P]_t - [P \cdot R]} = \frac{Ki * [L \cdot R]}{[L]_t - [L \cdot R]} \quad (15)$$

Rearrange (15),

$$[L]_t = [L\ R] * \left(\frac{Ki * ([P]_t - [P \cdot R])}{Kd * [P \cdot R]} + 1\right) \quad (16)$$

(14) is brought in (16),

$$[L]_t = \left([R]_t - [P\ R] - \frac{Kd * [P \cdot R]}{[P]_t - [P \cdot R]}\right) * \left(\frac{Ki * ([P]_t - [P \cdot R])}{Kd * [P \cdot R]} + 1\right) \quad (17, \text{Equation II})$$

In this equation, except [P R], all parameters are constant. $[P\ R]_o$: initial concentration of the free complex of receptor and probe, F: fluorescence, F_o: initial fluorescence.

$$[P\ R] = \frac{F}{F_o}[P\ R]_o \qquad (18, \text{ Equation III})$$

By non-linear curve fit using (17), Ki can be figured out.