Appendix 1. What is the $K_{apparent}$ of the lifetime change of a fluorescent sensor, given its response properties? This simple treatment considers a two-state sensor, with an empty and full state of the binding site, and an intensity ratio **R** for the total fluorescence of the full state divided by the empty state. Provision is also made for cooperativity, in the form of a Hill coefficient. Terms are:

$N_0 =$ intensity of empty state

$N_1 =$ intensity of full state

$\boldsymbol{R} = N_1/N_0$

$\tau_0 =$ lifetime of empty state

$\tau_1 =$ lifetime of full state

$h =$ Hill coefficient

$\varphi = \frac{1}{1+\left(\frac{K}{c}\right)^h}$ is the fractional occupancy of the full state at [analyte] = *c*, where *K* is the $K_{apparent}$

The intensity change is then midway at [analyte] = *K*, because $\varphi = 0.5$ and thus

$$N = N_0(1-\varphi) + N_1\varphi = (N_1 + N_0)/2$$

At what [analyte] is the lifetime change midway?

We must solve for the *c* that gives $\tau(c) = (\tau_1 + \tau_0)/2$.

The mean lifetime is the photon-count-weighted average of the two states' lifetimes:

$$\tau(c) = \frac{\tau_0 N_0(1-\varphi) + \tau_1 N_1 \varphi}{N_0(1-\varphi) + N_1\varphi}$$

Solving for the midpoint:

$$\frac{(\tau_1 + \tau_0)}{2} = \frac{\tau_0 N_0(1-\varphi) + \tau_1 N_1 \varphi}{N_0(1-\varphi) + N_1\varphi}$$

gives:

$$(1-\varphi)/\varphi = N_1/\,N_0 = \boldsymbol{R}$$

And substituting the formula for φ gives:

$$\left(\frac{K}{c}\right)^h = \boldsymbol{R}$$

The *c* that satisfies this is equal to $K_{apparent} = K \cdot \boldsymbol{R}^{-\frac{1}{h}}$. So the shift in $K_{apparent}$ is a factor = $\boldsymbol{R}^{-\frac{1}{h}}$

For some sensors, the relative photon yield of the two states may be dependent on the excitation wavelengths. This would provide a way of shifting the midpoint of the lifetime dose-response, by using different excitation wavelengths.