dynamic BIOSENSORS

Theory Note

Association rate of two equimolar reactants in solution

Keywords: Association kinetics I solution phase I second order reaction

Association of two reactants in solution (no dissociation)	$A + B \rightarrow AB$	(1)
Both reactants are present at equal concentrations at t=0	$[A]_0 = [B]_0 = c_0$	(2)
Second order reaction rate law	$\frac{dc}{dt} = -k_{on}c^2$	(3)
Integrated second order rate law	$\frac{1}{c_t} = \frac{1}{c_0} + k_{on}t$	(4a)
c_t=c(t)= concentration of unbound reactant	$c_t = \frac{c_0}{1 + c_0 k_{on} t}$	(4b)
Units of rate constant k_{on}	$M^{-1}s^{-1}$	

For the measurement of the association reaction in the solution phase (e.g. stopped flow in a cuvette) it is assumed that a signal S (e.g. fluorescence, absorption) is monitored over time. At time t=0, the reactants are mixed (but could not react yet) and the signal is S0. At time t=∞, an equilibrium has established (all reactants are bound) and the signal is S∞. The change in signal S over time (St) is described by:

Signal at time t	$S_t = \underbrace{S_0}_{\substack{start \\ value}} + \underbrace{(S_\infty - S_0)}_{\substack{amplitude\ of \\ signal\ change \\ upon\ binding}} \cdot \underbrace{\left(\frac{c_0 - c_t}{c_0}\right)}_{\substack{fraction \\ bound}}$	(5)
	$S_t = S_0 + (S_\infty - S_0) \cdot \left(1 - \frac{c_t}{c_0}\right)$	
(4b) in (5b)	$S_t = S_0 + (S_\infty - S_0) \cdot \left(1 - \frac{1}{1 + c_0 k_{on} t}\right)$	(6a)
Signal as a function of time	$S_t = S_\infty - \frac{S_\infty - S_0}{1 + c_0 k_{on} t}$	(6b)

alternative representation of (6b)

$$S_t = S_0 + \frac{(S_\infty - S_0)c_0 k_{on} t}{1 + c_0 k_{on} t} \quad (6c)$$

Linear plot to determine k_{on}

$$\underbrace{\frac{1}{S_\infty - S_t}}_{y} = \underbrace{\frac{1}{S_\infty - S_0}}_{A} + \underbrace{\frac{c_0 k_{on}}{S_\infty - S_0}}_{B} \cdot \underbrace{t}_{x} \quad (7)$$

Example

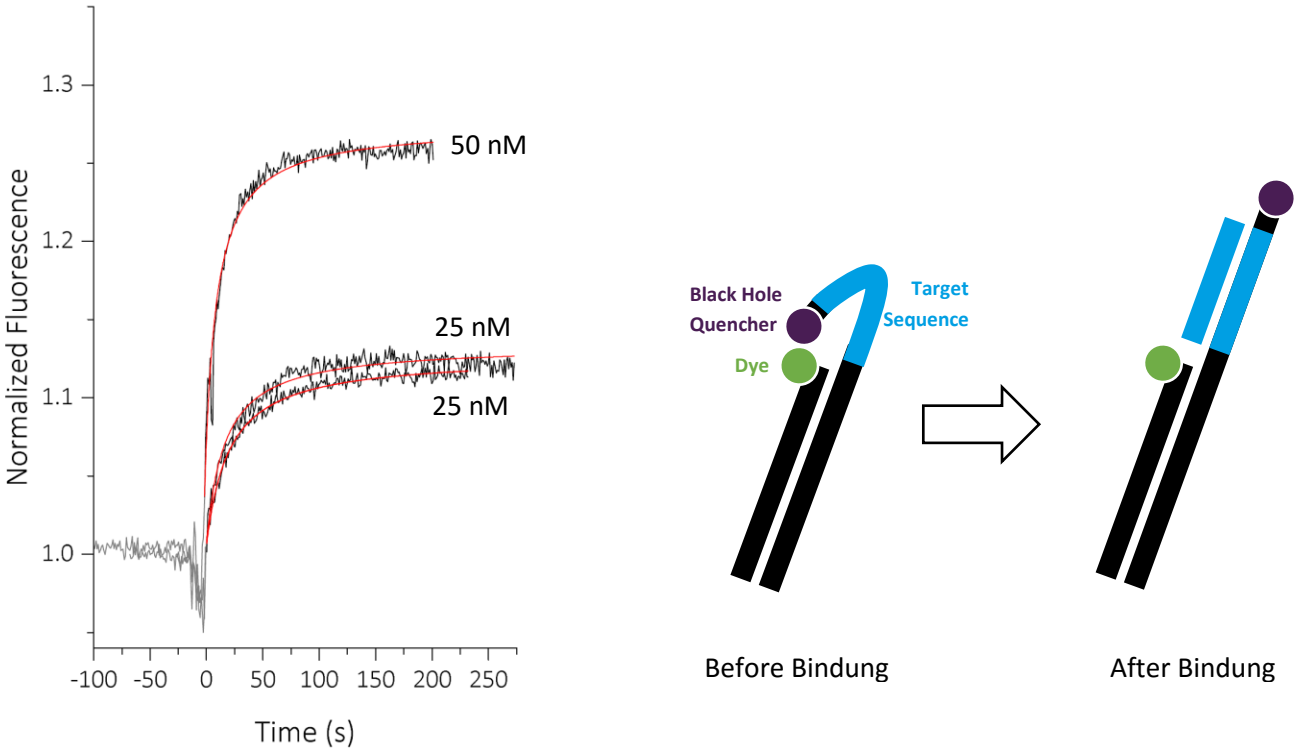

Figure 1 | Formation of a 10 bp DNA duplex in the solution phase.

Reactant 1: 17 base single stranded DNA, labelled with a green fluorophore at one end and a black hole quencher at the opposing end. Reactant 2: 10 base single strand, complementary to the overhang of reactant 1 (unlabeled). At t=0, reactant 2 was injected into a cuvette containing reactant 1 under intense stirring; final concentrations of both reactants were 25 nM (equimolar). The fluorescence increase (measured with a Shimadzu RF5301 spectrofluorophotometer) indicates the formation of the 10 bp duplex in real-time, because the quenching (FRET) efficiency decreases upon formation of duplex DNA, because it constitutes an effective rod-like spacer between the fluorophore and the quencher.

The solid red line is a non-linear least-squares fit with equation 6, yielding the association rate constant $k_{on} = 2.0\ E6\ M^{-1}s^{-1}$..

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