



# Calculation of On- and Off-rates from Association and Dissociation Curves

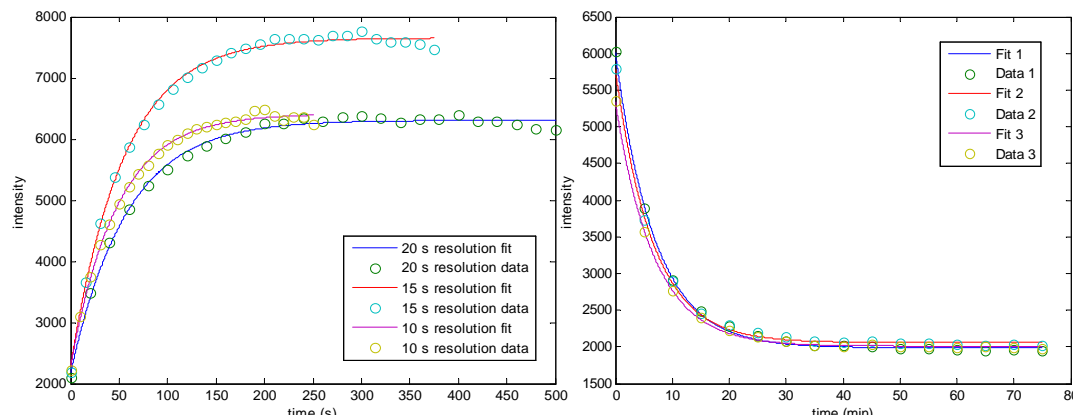
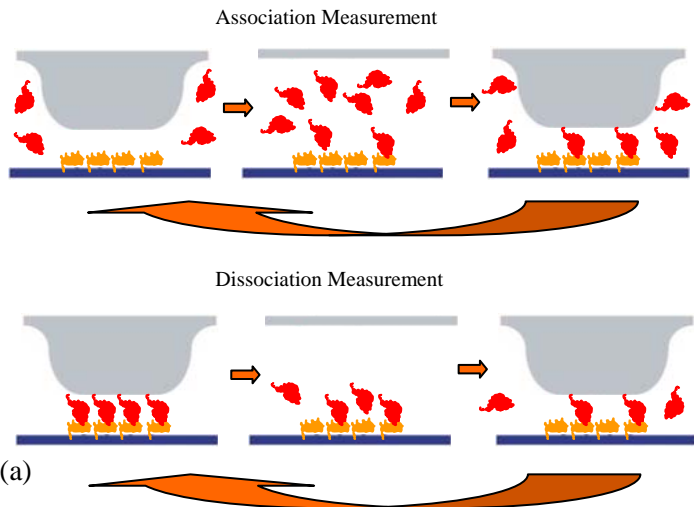
Stephen Quake, Stanford



A microfluidic “button” consisting of a valve actuated by pressure in the control layer of a PDMS device can be used to trap interactions [1]. Using the system for an out-of-equilibrium measurement establishes association and dissociation curves: With the first interacting partner (bait) immobilized on glass substrate using an epitope tag antibody, the second interaction partner (prey) is flowed in. Then the system is subjected to repeated rounds of lifting the button for a set time followed by flowing more prey to maintain a constant, known concentration (Fig. 1a, top panel), and the amount of prey bound is measured fluorescently. Starting with the fundamental kinetic equation (B=bait, P=prey, BP=prey bound to bait):

$$\frac{d[BP]}{dt} = k_{on}[B][P] - k_{off}[BP]$$

we solve to find an exponential decay up to a maximum, which we fit to the data to recover the association decay constant  $k_{on}[P]+k_{off}$  (Fig. 7.8b, left panel). The dissociation measurement reverses the process, with dissociated prey being washed out after each cycle of button opening, to maintain  $[P]=0$  (Fig. 7.8a, bottom panel). Under this condition the differential equation solves to simple exponential decay, and we can recover the dissociation decay constant  $k_{off}$ , which combined with the association decay constant yields  $K_d=k_{off}/k_{on}$ .



$$F = A + C(1 - \exp(-(k_{on}[P] + k_{off})t))$$

$$F = A + C \exp(-k_{off}ft)$$

**Figure 7.8:** (a) Schematic of process for using microfluidic membrane to establish time points for association and dissociation between bait bound to surface (orange) and fluorescently-labeled prey in solution (red). (b) Example of time points from association and dissociation experiments for T7-epitope/antibody interaction, and the fit to exponential decay curves.

[1] S.J. Maerkl and S.R. Quake, “A Systems Approach to Measuring the Binding Energy Landscapes of Transcription Factors” Science 315 233-237 (2007).