Numerical Solving of the Induced-Fit Model's Kinetic Equations

Alternatively, the rate constant of this two-step mechanism can be calculated by fitting the SF time-courses using the following system of kinetic equations:

$$\dot{y}_1 = -k_{12}[TCR] + k_{21}y_2 \tag{1}$$

$$\dot{y}_2 = k_{12}[TCR] - (k_{21}+k_{23})y_2 + k_{32}y_3 \tag{2}$$

$$\dot{y}_3 = k_{23}y_2 - k_{32}y_3 \tag{3}$$

where y_1 is a concentration of free pMHC molecules and y_2 and y_3 are concentrations of the transient $(TCR\cdots pMHC)^{tr}$ and the stabilized $(TCR\cdot pMHC)^{st}$ complexes, respectively.

The SF experimental data represent FRET quenching of fluorescein-labeled pMHC by TMR-labelled TCR. The quenching amplitude is proportional to the sum of concentrations of $(TCR\cdots pMHC)^{tr}$ and $(TCR\cdot pMHC)^{st}$ complexes:

$$y_1(t) = y_1(0) - [y_2(t) + \alpha\, y_3(t)] \tag{4}$$

where α accounts for the difference in FRET efficiency in the transient and stabilized complexes. Although the crystal structure of this TCR·pMHC complex has not yet been determined, and therefore the difference in the distances between the donor and acceptor molecules in the transient and stabilized complexes are not yet known, we assume that similarly to other known structures (1,2) α is to be 1.2 -1.4. In the following calculations, $\alpha = 1.3$ was used.

In order to reduce the number of variables and thereby to simplify fitting of the data, the value of k_{32} was taken the same as the dissociation constant found in the SPR experiment $(0.44s^{-1})$. The three remaining rate constants k_{12}, k_{21} and k_{23} can be evaluated by fitting the experimental time-course (Supporting Fig. 1). The fitting yields $k_{12} \approx 3\cdot10^5$ $M^{-1}s^{-1}$; $k_{21} \approx 30\ s^{-1}$ and $k_{23} \approx 4\ s^{-1}$. These calculations also show that this time-course is determined predominantly by the second step forward rate constant k_{21}.

Using the rate constants determined here, one can calculate the overall equilibrium dissociation constant: $K_d = \dfrac{k_{21}\, k_{32}}{k_{12}\, k_{23}} \approx 7.6 \mathrm{M}^{-1}$, which is close to the equilibrium dissociation constant calculated from the SPR experiment ($5.3\ \mathrm{M}^{-1}$). The second step stabilization factor is $\dfrac{k_{32}}{k_{23}} \approx 9$. Thus, the first and second evaluation methods yield similar values for the induced-fit rate: $k_{23} = 2 - 4\ \mathrm{s}^{-1}$ and the complex stabilization factor 6 – 9-fold.

1. Reiser JB, Darnault C, Grégoire C, Mosser T, Mazza G, Kearney A, van der Merwe PA, Fontecilla-Camps JC, Housset D, Malissen B (2003) *Nat Immunol* 4:241-247.

2. Reiser JB, Darnault C, Guimezanes A, Grégoire C, Mosser T, Schmitt-Verhulst AM, Fontecilla-Camps JC, Malissen B, Housset D, Mazza G (2000) *Nat Immunol* 1:291-297.