Supporting Materials and Methods

Analysis of the TCR-MHCp biphasic association/dissociation time courses. To examine the causes for the observed biphasic pattern of MHCp–TCR association/dissociation time courses the following models were analyzed:

Two-Step (Induced-Fit) Model. First, we examined whether the observed time courses can be fitted by a two-step model:

$$\mathrm{MHCp + TCR} \underset{k_{21}}{\overset{\text{Step1}\ k_{12}}{\rightleftarrows}} \mathrm{MHCpTCR^{int}} \underset{k_{32}}{\overset{\text{Step2}\ k_{23}}{\rightleftarrows}} \mathrm{MHCpTCR^{st}} \qquad \textbf{[1]}$$

This reaction starts by formation of an intermediate (int) complex, which is stabilized upon conversion to the stable (st) conformation in the second reaction step. The following system of ordinary differential equations describes this mechanism:

$$\frac{d([MHCp])}{dt} = -k_{12}[MHCp][TCR] + k_{21}[MHCpTCR^{tr}] \qquad \textbf{[2]}$$

$$\frac{d([MHCpTCR])^{tr}}{dt} = k_{12}[MHCp][TCR] - (k_{21} + k_{23})[MHCpTCR^{tr}] + k_{32}[MHCpTCR^{st}] \qquad \textbf{[3]}$$

$$\frac{d([MHCpTCR])^{st}}{dt} = k_{23}[MHCpTCR^{tr}] - k_{32}[MHCpTCR^{st}] \qquad \textbf{[4]}$$

Because TCR concentration in the SPR experiments remains essentially constant, the above linear system allows a simple numerical solution. Rate constants providing the best fit were found by using the GLSA optimization program (Alango Ltd, Haifa, Israel). Although this model allows tolerable fit of the individual time-courses (Fig. 4 and Table 3) it cannot fit the whole set of the experimental data because the k_2 and k_{-2} values exhibited at 4°C a systematic decrease in the studied concentration ranges along with increasing the analyte concentration. This model also yields an abnormal temperature behavior of the k_2 value, which was found to be faster at 4°C than at 25°C. In addition, the conformational transition step, which is expected to stabilize a final complex, reduced 3 to 5-fold the overall affinity. Therefore, we conclude that the above model does not allow satisfactory explanation for the minor phase of the association and dissociation time-courses.

Two-Conformers Model. The second model we examined was a model involving a preequilibrium between two conformers of the unbound analyte; an active (TCR) and inactive (TCR^*):

$$TCR^{*} \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} TCR + MHCp \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} MHCpTCR \quad \textbf{[5]}$$

This model does not allow accounting for the slow dissociation component in the experimental data. In order to accomplish this task we have to introduce a third step accounting for additional stabilization of the MHCpTCR complex:

$$TCR^{*} \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} TCR + MHCp \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} (MHCpTCR)^{tr} \underset{k_{43}}{\overset{k_{34}}{\rightleftarrows}} (MHCpTCR)^{st} \quad \textbf{[6]}$$

This model, containing two additional rate constants in comparison with the induced-fit one (Eq. **1**), provides a slightly better fit to the data, but suffers from the same above-mentioned drawbacks. Therefore, we concluded that the observed biphasic character of the experimental time courses is neither due to the preequilibrium of two TCR conformations in the unbound state nor the induced-fit stabilization of the intermediate MHCpTCR complex.

Two-Species Reversible, Single Step Model. Then, we examined a two-species model accounting analyte heterogeneity (TCR_1 and TCR_2).

$$MHCp + TCR_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} MHCpTCR_1 \quad \textbf{[7]}$$

$$MHCp + TCR_2 \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} MHCpTCR_2 \quad \textbf{[8]}$$

This model is described by the following kinetic equations:

$$\frac{d([MHCp])}{dt} = -k_1[TCR_1][MHCp] + k_{-1}[MHCpTCR_1] - k_2[TCR_2][MHCp] + k_{-2}[MHCpTCR_2] \quad \textbf{[9]}$$

$$\frac{d([MHCpTCR_1])}{dt} = k_1[TCR_1][MHCp] - k_{-1}[MHCpTCR_1] \quad \textbf{[10]}$$

$$\frac{d([MHCpTCR_2])}{dt} = k_2[TCR_2][MHCp] - k_{-2}[MHCpTCR_2], \quad \textbf{[11]}$$

where $TCR_1 = fr \cdot TCR$ and $TCR_2 = (1\text{-}fr) \cdot TCR$ are concentrations of two TCR's fractions, and MHCp is the concentration of the immobilized MHCp ligand. This model predicts competition between TCR_1 and TCR_2 for binding to the limited number of MHCp-

binding sites upon increasing the total TCR concentrations. This model allowed a satisfactory fit for both sets of data monitored at 25 °C and 4°C (Fig. 5). The evaluated rate constants are listed in Table 4. It is noteworthy that both components' association rate constants, k_1 and k_2, were found to have similar values, whereas their dissociation rate constants differed by about an order of magnitude. The binding-rate constant of the major component exhibited a considerable temperature dependence decreasing by 39 fold from 7×10^3 to 2×10^2 $M^{-1}\cdot s^{-1}$ upon lowering temperature from 25 °C to 4°C, whereas the minor component's binding rate constant exhibited only a 9-fold decrease. The calculations also revealed that the slow dissociating fraction, comprising 2% at 25°C and 8 – 25% at 4°C, possesses 10 to 15-fold higher affinity than the major one.

Because only the two-species model provides a satisfactory interpretation of the experimental data, we suggested that the minor component of the biphasic binding time courses belongs to binding of a high affinity TCR fraction. This finding could be due to traces of TCR dimers present in the immonoaffinity-purified samples produced by leakage of the column. The dimers exhibited, as expected, a significantly higher affinity than the monomers, due to their bivalent binding to the immobilized MHCp ligand. The inhomogeneity of MHCp immobilization could be a result of the tetravalent nature of streptavidin molecules coating the chip surface. Alternatively, the ligand inhomogeneity could be due to binding two MHCp to two neighboring streptavidin molecules separated by a distance smaller than 12 – 15 nm so that mAb–TCR dimers could interact with them bivalently. The major component is attributed to the interaction of monomeric TCR with its ligand.