Binding Properties and Stoichiometry of the T Cell Receptor

Jennifer Stone
Summer School on Theoretical and Experimental Immunology
September 2010
Outline

• T Cell Receptor Complex
  – Components, Assembly, and Organization

• Ligand binding measurements
  – Surface Plasmon Resonance
  – Peptide-MHC Multimers
  – In Situ 2D Binding Measurements
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Components of the TCR Complex

T Cell Receptor complex

CD4 or CD8

binding site

variable constant

membrane

ITAMs

cytoplasm

LCK

LCK
T cell recognition of peptide-MHC ligands

- APC
  - Class II MHC
  - Peptide
  - CD4

- APC
  - Class I MHC
  - Peptide
  - CD8
Structures of MHC, TCR, and co-receptors

2C/H-2K\textsuperscript{b}  H-2K\textsuperscript{b}/CD8\textalpha\textalpha  I-A\textsuperscript{k}/CD4  HA1.7/HLA-DR4

Rudolph et. al. *Annu Rev Biophys Biomol Struct* 2002
The T Cell Receptor Complex

No complete structure of the TCR complex has been solved

Stoichiometry and surface expression/distribution of subunits still a subject of study

Assembly of the TCR-CD3 complex.
Characterization of TCR-CD3 complex from T cells

Alarcon et. al. EMBO Rep 2006
Early evidence for a Bivalent TCR

Fernandez-Miguel et. al.
PNAS 1999

Double TCR-Tg mice

Blotted with: \(\text{anti-}V_\beta\,8\)  
\begin{tabular}{cc}
Spleen & Thymus \\
8 & 2 \end{tabular}

\begin{tabular}{cc}
Spleen & Thymus \\
8 & 2 \end{tabular}

Immunoprecipitate: C 8 2 C 8 2

D

145
83
60
50
35 kDa
Early evidence for a Bivalent TCR

Double TCR-Tg mice
Donor quencing of FITC detection

Fernandez-Miguel et. al. PNAS 1999
Model of Bivalent TCR

Fernandez-Miguel et. al. PNAS 1999
Cholesterol-dependent TCR multimers

BN-PAGE: proteins labeled with Coomassie Blue G-250

Doesn’t disrupt membrane protein interactions like SDS

TCR microclusters are present before activation
TCR complex dimerization

Intracellular Erythropoietin Receptor Signaling Domain Dimerization Assay

Kuhns et. al. PNAS 2010
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Surface Plasmon Resonance

\[ A + B \rightleftharpoons AB \]

\[ \frac{d[AB]}{dt} = k_{on}[A][AB_{\text{max}} - [AB]] - k_{off}[AB] \]

Issues to be concerned about:
- Temperature
- Mass Transport artifacts
- Surface heterogeneity
- Decay of immobilized analyte
- Multivalency or aggregation of solution-phase ligand

Affinity ranges:
- very high (pM) affinities can be difficult to measure
- estimate only above \( \sim 50 \, \mu\text{M} \)
- \( t_{1/2} \) ranges
  - qualitative only under 5-10 seconds
  - extremely long dissociation can be problematic

de Mol and Fischer, & Schuck and Zhao, *Methods Mol Biol*, 2010
Correlation of binding parameters with stimulatory capacity of interaction

Relatively few peptide-MHC/TCR interactions measured by SPR.
Furthermore, most SPR measurements are taken at 25°C, while T cell activation occurs at 37°C.

Stone et. al. *Immunology* 2009
T cell activation correlates with $K_D$ and $t_{1/2}$
Correlation of $K_D$ with T cell stimulation

Comparison of wild-type and high-affinity TCR

Stone et. al. *Immunology* 2009
Kinetic Proofreading model

Short $t_{1/2}$ would not be predicted to allow full zeta phosphorylation

Longer $t_{1/2}$ would be predicted to be fully phosphorylated and cause T cell activation

Jones et. al., *J Immunol*, 2008

Kersh et. al., *Science*, 1998
Longer $t_{1/2}$ does not always result in better stimulation

Extended $t_{1/2}$ results in less potent stimulation in the case of the OT-1 TCR binding to OVA(G4)/K$^b$

Stone et. al. *Immunology* 2009
Optimal Dwell-time model

Peptide-MHC/TCR interactions with intermediate $t_{1/2}$ are the most potent.

Based on the hypothesis of serial triggering

Valitutti et. al., *Nature*, 1995

*Cells with engineered TCRs with $t_{1/2}$ 100- to 1000-fold longer than wild-type are efficiently and sensitively triggered.*

Kalergis et. al., *Nat Immunol*, 2001
Molecular Flexibility ($\Delta C_p$)

Rigid body adjustments

Alterations to reach transition state

Alterations to attain fully bound complex

Qi et al., PNAS, 2006
Molecular Flexibility ($\Delta C_p$)

Measure $\Delta C_p$ by SPR or Isothermal Titration Calorimetry

$$\Delta G^o = \Delta H^o - T\Delta S^o = -RT \ln K$$

$$\Delta G^o_{T} = \Delta H^o_{T_0} + \Delta C_p^o (T - T_0) - T\Delta S^o_{T_0} - T\Delta C_p^o \ln \left( \frac{T}{T_0} \right)$$

$$E_{a_{assoc}} = -RT \ln k_a$$

$$E_{a_{diss}} = -RT \ln k_d$$

Boniface et. al., *PNAS*, 1999

Krogsgaard et. al., *Mol Cell*, 2003
Molecular Flexibility ($\Delta C_p$)

$$t^{2D}_{1/2} = t^{3D}_{1/2} A \exp[-B\Delta C_p]$$

Qi et al., *PNAS*, 2006
Peptide-MHC Confinement Time

Aleksic, Dushek, et. al. *Immunity* 2010
Peptide-MHC Confinement Time

\[ k_{\text{off}}^* = \frac{1}{T_c} = \frac{k_- k_{\text{off}}}{(k_{\text{on}}^* + k_-)} \]

Aleksic, Dushek, et. al. *Immunity* 2010
Peptide-MHC Confinement Time

Aleksic, Dushek, et. al. *Immunity* 2010
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Peptide-MHC Multimers

IgG or Fc fusion (dimer)

Streptavidin-linked Tetramer

Ultimer (hexamer)

X-Link (dimer-octamer)

Pentamer

Binding of peptide-MHC multimers to T cells

Complications:

Relatively high threshold of sensitivity (up to 10% receptors bound)

“Steady state” measurements do not represent a true equilibrium

Binding affected by multiple factors, including co-receptor

*this may be an advantage

Stone et. al. *Immunology* 2009
TCR panel with various binding parameters

<table>
<thead>
<tr>
<th>TCR</th>
<th>$t_{1/2}$ (s)</th>
<th>$K_D$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S51αA</td>
<td>86</td>
<td>15</td>
</tr>
<tr>
<td>m33</td>
<td>46</td>
<td>16</td>
</tr>
<tr>
<td>Y26αA</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>N27βA</td>
<td>33</td>
<td>40</td>
</tr>
<tr>
<td>Y49αA</td>
<td>58</td>
<td>47</td>
</tr>
<tr>
<td>S51αA/Y48βA</td>
<td>11</td>
<td>540</td>
</tr>
<tr>
<td>Y48βA</td>
<td>2</td>
<td>2900</td>
</tr>
<tr>
<td>N30βA</td>
<td>3</td>
<td>8200</td>
</tr>
<tr>
<td>Y50αA</td>
<td>0.5</td>
<td>7000</td>
</tr>
<tr>
<td>2C</td>
<td>0.9</td>
<td>36,000</td>
</tr>
</tbody>
</table>

View toward TCR from perspective of MHC

Chervin, Stone et. al. *J Immunol* 2009
Peptide-MHC tetramer $t_{\frac{1}{2}, \text{tet}}$ values

Chervin, Stone et. al. *J Immunol* 2009
Are $K_{D,tet}$ values valid?
Are $K_{D,\text{tet}}$ values valid?

<table>
<thead>
<tr>
<th>TCR</th>
<th>$K_{D,\text{SPR}}$ (nM)</th>
<th>$K_{D,\text{tet}}$ (nM)</th>
<th>Enhancement Factor ($K_{D,\text{SPR}}/K_{D,\text{tet}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S51_{\alpha}A$</td>
<td>15 ± 2</td>
<td>0.96</td>
<td>15.6</td>
</tr>
<tr>
<td>$Y26_{\alpha}A$</td>
<td>17 ± 8</td>
<td>1.4</td>
<td>12.1</td>
</tr>
<tr>
<td>m33</td>
<td>16 ± 12</td>
<td>1.56 ± 0.43</td>
<td>10.2</td>
</tr>
<tr>
<td>N27pA</td>
<td>40 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y49_{\alpha}A</td>
<td>47 ± 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S51_{\alpha}A/Y48_{\beta}A$</td>
<td>540 ± 56</td>
<td>14</td>
<td>38.6</td>
</tr>
<tr>
<td>Y48pA</td>
<td>2,900 ± 2,100</td>
<td>26</td>
<td>111</td>
</tr>
<tr>
<td>2C WT</td>
<td>22,800 ± 6,000</td>
<td>11 ± 1</td>
<td>2,160</td>
</tr>
</tbody>
</table>

Chervin, Stone et. al. *J Immunol* 2009
Co-receptor and peptide-MHC tetramers

TCR $K_D$: 30, 80, >250$\mu$M

Wooldridge et. al. *Immunology* 2009
The effect of CD8 co-receptor binding on MHC tetramer dissociation

Wooldridge et al. J Biol Chem 2005
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2D Rates of binding and diffusion

**FRAP:** Fluorescence Recovery After Photobleaching
2D Rates of binding and diffusion

Wu et. al. Biophys J 2008
2D Rates of binding and diffusion

Rapid initial phase of recovery due to diffusion of unbound molecules

Slower second phase of recovery due to unbinding, diffusion, and re-binding for reaction-limited kinetics
2D Rates of binding and diffusion

In Situ FRET: Fluorescence Resonance Energy Transfer

<table>
<thead>
<tr>
<th></th>
<th>$K_D$ (µM)</th>
<th>$t_{1/2}$ (s$^{-1}$)</th>
<th>$k_{on}$ (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In situ</td>
<td>5</td>
<td>0.1</td>
<td>$1.4 \times 10^6$</td>
</tr>
<tr>
<td>In vitro</td>
<td>40</td>
<td>1.2</td>
<td>$1.4 \times 10^4$</td>
</tr>
</tbody>
</table>

Huppa et. al. *Nature* 2010
2D Rates of binding and diffusion

In Situ Cell Adhesion Assay – controlled contact time and area

$m_r m_l A_c K_a$ and $k_{off}$

$A_c k_{on} = A_c K_a \times k_{off}$

Huang et. al. *Nature* 2010
Correlation of 2D Binding Parameters with T Cell Recognition

2D off-rates up to 8300-fold faster than 3D off-rates

Broader dynamic range of values seen

Huang et al. *Nature* 2010
Reference List


- Schuck P, Zhao H. **The role of mass transport limitation and surface heterogeneity in the biophysical characterization of macromolecular binding processes by SPR biosensing.** Methods Mol Biol. 2010;627:15-54.

- Stone JD, Chervin AS, Kranz DM. **T-cell receptor binding affinities and kinetics: impact on T-cell activity and specificity.** *Immunology.* 2009 Feb;126(2):165-76.

- Kersh EN, Shaw AS, Allen PM. **Fidelity of T cell activation through multistep T cell receptor zeta phosphorylation.** *Science.* 1998 Jul 24;281(5376):572-5.

- Jones LL, Colf LA, Stone JD, García KC, Kranz DM. **Distinct CDR3 conformations in TCRs determine the level of cross-reactivity for diverse antigens, but not the docking orientation.** *J Immunol.* 2008 Nov 1;181(9):6255-64.


Reference List


