Antibody Fold and Protein Binding
Domains of IgG
IgG contains 2 $F_{\text{AB}}$ and $F_{\text{C}}$
Neuraminidase With Fab
Neuraminidase With Fab

Sidechains to neuraminidase
And between Fab variable

Homodimer, mostly hydrophobic
Side chains
Meuraminidase is a target of drugs
Blocking Neuramindase Shortens Flu

Reduction in influenza type A virus titer values after administration of TAMIFLU (n=56) and placebo (n=13)\textsuperscript{9,10}

![Graph showing reduction in influenza virus titer over time with placebo and TAMIFLU treatments. The graph indicates a significant reduction in virus titer with TAMIFLU compared to placebo.](image-url)
How an antibody fold is designed to bind antigens
Topology of IgG Variable Domain

Names of beta strands and topological positions of CDR's in V domains.
How many ways can beta strands connect to fold into a domain?

<table>
<thead>
<tr>
<th>Strands</th>
<th>Number of Motifs</th>
<th>Strands</th>
<th>Number of Motifs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>5</td>
<td>960</td>
</tr>
<tr>
<td>6</td>
<td>11520</td>
<td>7</td>
<td>161280</td>
</tr>
<tr>
<td>8</td>
<td>2580480</td>
<td>9</td>
<td>46448640</td>
</tr>
<tr>
<td>10</td>
<td>928972800</td>
<td>11</td>
<td>2.0437 \times 10^{10}</td>
</tr>
</tbody>
</table>

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0032461
Relations of CDR and domains
Constant Domains Interface

Flat rigid & very hydrophobic
Pairing Two IgG Fab’s- Nest of CDR Loops
Variable Domains Interface
Do CDR loops have wildly varying 3D structures?

• Amino acid sequences hyper variable
• hyper variable loops fall into few classes
• They have similar structures
• They have concerved anchor residues from which the loops project
Features of CDR’s

<table>
<thead>
<tr>
<th>Loop</th>
<th>SEQUENCE</th>
<th>Key Framework</th>
<th>Insertions-</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1- 4 types</td>
<td>26-32</td>
<td>2,25,33,71</td>
<td>insertions at 31</td>
</tr>
<tr>
<td>L2- 1type</td>
<td>50-62</td>
<td>48, 64</td>
<td>none</td>
</tr>
<tr>
<td>L3 – 3 types</td>
<td>91-96</td>
<td>95, 90</td>
<td>variable at 96</td>
</tr>
<tr>
<td>H1 - 1 type</td>
<td>26-32</td>
<td>34,94</td>
<td>None</td>
</tr>
<tr>
<td>H2- 4 types</td>
<td>52-55</td>
<td>52,54, 55,71</td>
<td>insertions at 52</td>
</tr>
</tbody>
</table>
## Comparing CDR contacts to one antigen

<table>
<thead>
<tr>
<th>Fab</th>
<th>L 30</th>
<th>L 50</th>
<th>L 90</th>
<th>H 30</th>
<th>H 50</th>
<th>H 90</th>
<th>No. of lysozyme or peptide side chains in contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>HyHel-10</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>D-1.3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>HyHEL-5</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>gp120</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>V3-14A A peptide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Other binding proteins have IgG fold
CD4 domain has Structure of Fab Variable Domain
Associations between two immune cells
Peptide in HLA presented to TCR
complex of CD8 (red and blue) HLA, peptide, microglobulin, yellow
HLA-A W68 A245 V, binds CD8 poorly. V 245 does not interact with CD8 but twists the 223-229 loop.
HLA and CD8

CDR loops and associations of the CD8’s like two V domains of Fab

From GEORGE F. GAO Nature 387, 630 - 634 (1997) Crystal structure of the complex between human CD8 and HLA-A2
Part of Growth Hormone Receptor
Variant of the IgG constant domain topology
Nearly symmetric dimer of GHR
Compare IL4 IL4R and GH GHR
Crystal structure shows changes in SAS

Hormone

Receptor

asymmetric

symmetric
Figure 1. Reaction (A) and kinetics (B) for binding of hGH or (G120R)hGH to the (S237C)hGHbp coupled to the BIAcore™ biosensor. The (S237C)hGHbp was immobilized on the thiol-dextran matrix (A) at a level of 1220 RUs, which corresponds to 1.2 ng/mm². In the example, binding profile (B) hGH (open symbols) or (G120R)hGH (filled symbols) was injected at saturating concentrations (5 μM) to follow association and establish the limiting amount of bound hormone from which the stoichiometry was calculated. After saturation, the injector loop was switched to buffer to follow dissociation (indicated by the arrow). See text and Materials and Methods for further details.
Figure 2. Reaction (A) and kinetics (B) for binding of hGH (open symbols) or the (G120R)hGH (filled symbols) to the (S201C)hGHbp coupled to the BIAcore™ biosensor. The (S201C)hGHbp was immobilized at a level of 1480 RUs (1.48 ng/mm²) on the biosensor as described in Materials and Methods. Binding conditions and profiles are analogous to those in Fig. 1.
## Table 1

Kinetic constants for binding of wild-type (WT) or (G120R)hGH to (S237C)hGHbp or (S201C)hGHbp immobilized on the thiol-matrix of the BIAcore™ biosensor

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Matrix</th>
<th>Stoichiometry (hormone : hGHbp)</th>
<th>On-rate (s⁻¹ M⁻¹)</th>
<th>Off-rate (s⁻¹)</th>
<th>Kₐ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>(S237C)hGHbp</td>
<td>0.40</td>
<td>4.0 × 10³</td>
<td>&lt;1.0 × 10⁻³</td>
<td>ND</td>
</tr>
<tr>
<td>G120R</td>
<td>(S237C)hGHbp</td>
<td>0.70</td>
<td>2.6 × 10³</td>
<td>4.3 × 10⁻⁴</td>
<td>1.6</td>
</tr>
<tr>
<td>WT</td>
<td>(S201C)hGHbp</td>
<td>0.84</td>
<td>3.0 × 10³</td>
<td>2.7 × 10⁻⁴</td>
<td>0.9</td>
</tr>
<tr>
<td>G120R</td>
<td>(S201C)hGHbp</td>
<td>0.82</td>
<td>1.4 × 10³</td>
<td>3.7 × 10⁻⁴</td>
<td>2.7</td>
</tr>
</tbody>
</table>

On-rate and off-rate profiles were measured at 25°C and analyzed for hGH and (G120R)hGH. Average standard errors for on-rate, off-rate and affinities on the same biosensor chip are 12%, 11% and 16% of the value reported, as described in Materials and Methods. Stoichiometries of binding were calculated from data in Figs 1B and 2B according to the following formula:

\[
\left( \frac{RU_{\text{max}} \text{ hormone}}{RU_{\text{max}} \text{ hGHbp}} \right) \times \left( \frac{M_{\text{hGHbp}}}{M_{\text{hormone}}} \right)
\]
Ala mutants- Changes in Off Rates, not On Rates

**Figure 4.** Relative change in off-rate (A) or on-rate (B) for alanine mutants at contact residues. Data taken from Table 2.
Free Energy change for mutants

Figure 7. Relationship between the change in binding affinity upon alanine substitution and the change in buried surface area ($\AA^2$, A) or number of van der Waals' (VDW) contacts (B) for atoms in contact side-chains beyond the $\beta$-carbon. Filled circles show residues buried at the interface that make hydrogen bonds or salt bridges with the receptor at site 1, and open circles show residues that do not. Data are plotted from Table 2.
Transcription Factors

• Common features weak binding
• Helix formation and stabilization
VP16 constructs and Assays

A

<table>
<thead>
<tr>
<th>VP16 C</th>
<th>452</th>
<th>490</th>
<th>TAF binding</th>
<th>Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP16 452-474</td>
<td>452</td>
<td>474</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>VP16 469-490</td>
<td>469</td>
<td>490</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>VP16 469-485</td>
<td>469</td>
<td>485</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>VP16 474-485</td>
<td>474</td>
<td>485</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VP16 469-480</td>
<td>469</td>
<td>480</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

B

![Image of gel electrophoresis](image)

C

![Image of gel electrophoresis](image)
VP16C has no secondary structure.

HSQC of VP16C & 1HN-1HC coupling constants
NOESY Spectra and Summary

A

B

C
Sequences VP16C, p53, NF-κB p65- aligned by FXXΦΦ
MDM2 and P53
Details of Helix Interactions
Small MDM2 inhibitors Nutlins

L T Vassilev et al. Science 2004;303:844-848
Phosphorylation & Assembly
ARA70 & SRC2-3 Reveal Dimorphic AF2

Is SARMS function mediated by dimorphic coactivator site?
Helix 3, 4, 5 and 12 of AR

Changes To The Coregulator Binding Site Of AR On Binding The FXXFL Biomotif

Eugene Hur