ForteBio Octet Kinetic Analysis as an Integral Step in the Therapeutic Antibody Screening and Selection Process.

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Director, Biochemistry Research
Integration of ForteBio technology into the KaloBios Humaneering™ Process

• Affinity considerations in therapeutic antibody development
• ForteBio Octet
• KaloBios and the *Humaneering™* process
• ForteBio Octet integration into the KaloBios screening cascade
• Examples of ForteBio data and uses in the antibody screening process
• Advantages of the ForteBio system
Affinity considerations in therapeutic antibody development

**In vivo Target**
- Receptor/ligand
- *In vivo* antigen concentration
- Therapeutic Function
  - Blocking/ADCC/Conjugate

**Lead identification**
- Rapid identification of high affinity Abs
- Detailed kinetics
  - High Ka / Low Kd

**Development**
- Efficacy
- Antibody structure:
  - IgG vs Fragment
- Pharmacokinetics
  - Pegylation/ Optimized Fc
- Dose concentration
- Dose regime
- Manufacture cost
  - Treatment cost
Measuring Protein: Protein Interactions with Biosensors

- Bio-Layer Interferometry (BLI) is capable of providing **real time**, **label free** monitoring for protein: protein interactions

- 8 interaction events can be measured simultaneously.

- Any change in the number of molecules bound to the biosensor tip changes the optical path, causing a shift in the interference pattern that can be measured in real time.

- Set-up for IgG quantification and **Kinetic analysis** of protein interactions.

- For more information see www.fortebio.com
KaloBios Pharmaceuticals

- **Novel antibodies to treat autoimmunity, infectious diseases and cancer**
  - Multiple antibody therapeutics in development
  - Clinical trials initiated 2006

- **Proprietary antibody platform technology**
  - Fuels KaloBios pipeline
  - High affinity, close to human germ-line sequences
  - Minimizes royalty stacking
  - Nine Antibodies *Humaneered™* to-date
Antibody Humaneering™
Libraries containing paired specificity determinants

Human Germline

VH

VL

V-segment generation and identification

Humaneered antibody library

Donor antibody

Minimum Specificity Determinant

Small epitope-focused libraries
Colony Lift Binding Assay (CLBA)

Antigen-coated membrane

Lift-membrane

Secreted Antibody Fragments

no expression

expression

Colonies

Binders

Antibody fragment detection

Fluor

HRP
Humaneering™: Screening cascade

- **ForteBio Octet of Reference Antibody**
  - Full Kinetic Analysis

- **Epitope focused Lib. Colony Lift Binding Assay**
  - (Clone no. 50K → 96)

- **Affinity Maturation (if required)**

- **Chase ELISA / Forte**
  - (Clone no. 96 → ~5)
  - Expression Media Kd Analysis

- **ForteBio Octet**
  - (Clone no. ~5)
  - Full Kinetic Analysis

- **Biacore**
  - (Clone no. ~5)
# Humaneering™: Typical Antibody Affinities

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$K_a$(M$^{-1}$s$^{-1}$)</th>
<th>$K_d$ (s$^{-1}$)</th>
<th>$K_D$</th>
<th>% Human*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Bacterial Murine</td>
<td>5.9E+5</td>
<td>6.2E-4</td>
<td>1.05nM</td>
<td>67</td>
</tr>
<tr>
<td>Humaneered</td>
<td>2.6E+5</td>
<td>1.7E-4</td>
<td>0.67nM</td>
<td>91</td>
</tr>
<tr>
<td>Anti-Cytokine Murine</td>
<td>2.64E+6</td>
<td>2.78E-5</td>
<td>10.5pM</td>
<td>65</td>
</tr>
<tr>
<td>Humaneered</td>
<td>1.75E+6</td>
<td>3.12E-5</td>
<td>17.8pM</td>
<td>93</td>
</tr>
<tr>
<td>Anti-cytokine Murine</td>
<td>4.52E5</td>
<td>2.96E-3</td>
<td>6.55nM</td>
<td>78</td>
</tr>
<tr>
<td>Humaneered</td>
<td>5.14E5</td>
<td>2.33E-3</td>
<td>4.53nM</td>
<td>96</td>
</tr>
</tbody>
</table>

*Calculated as amino acid sequence identity to closest human germline excluding CDR3.
Examples of KaloBios Octet Sensor Set-up

Streptavidin Sensors

- Biotinylated Protein
- Biotinylated Peptide
- Biotinylated IgG

Protein A

Amine Coupling

Purified Fab'

Expression Media

Kinetic measurements have been made using biotinylated intact protein antigens and peptide antigen binding to purified Fab' in solution and Fab' in protein mixtures such as bacterial expression media. IgG affinity measurements have been made with antigen in solution to compensate for avidity effects. Protein A and amine coupling sensors are also available.
**Purified Fab’ Kinetics: Humaneering™**

ForteBio Octet allows full kinetic analysis of 8 purified samples simultaneously.
ForteBio Octet software produces both visual and quantitative measures of binding kinetics and automatically exports results to MS Word and as Jpeg files.
Expression Media Fab’ Kd: Humaneering™

The Forte Octet has been used to rank-order Kd of Fab’s in bacterial expression media prior to purification.

<table>
<thead>
<tr>
<th>SampleID</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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</thead>
<tbody>
<tr>
<td>MolarConc[M]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>kobserved [1/s]</td>
<td>2.13E-3</td>
<td>2.96E-3</td>
<td>2.99E-3</td>
<td>6.13E-3</td>
<td>3.16E-3</td>
<td>7.67E-4</td>
<td>5.85E-4</td>
<td>1.58E-3</td>
</tr>
<tr>
<td>kobsErr</td>
<td>1.79E-5</td>
<td>1.51E-5</td>
<td>1.80E-5</td>
<td>5.57E-5</td>
<td>1.71E-5</td>
<td>2.77E-5</td>
<td>2.44E-5</td>
<td>1.45E-5</td>
</tr>
<tr>
<td>kdis [1/s]</td>
<td>1.03E-3</td>
<td>4.67E-4</td>
<td>9.28E-4</td>
<td>7.32E-4</td>
<td>5.85E-4</td>
<td>5.22E-4</td>
<td>9.24E-4</td>
<td>5.49E-4</td>
</tr>
<tr>
<td>kdisErr</td>
<td>2.26E-5</td>
<td>1.90E-5</td>
<td>1.74E-5</td>
<td>2.23E-5</td>
<td>2.26E-5</td>
<td>4.29E-5</td>
<td>1.42E-4</td>
<td>4.17E-5</td>
</tr>
<tr>
<td>kassoc [1/Ms]</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>K_D [M]</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td>--</td>
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</tr>
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</table>
Comparison of Dissociation Rates Generated from Purified Fab’ or Bacterial Expression Media

The values obtained from Fab’ dissociation-rates in a rank-ordering bacterial media screen compare favorably with subsequent measurements of pure Fab’ protein. This is even the case when Fab’ concentrations in the media vary widely between samples.

Over the course of many projects this type of analysis has allowed an off-rate (Kd) evaluation early in the selection process without the need for Fab’ purification.
### Forte Octet: Reproducibility

Forte Octet Runs of two Fab’ either 4 or 3 times on different days with independent set-up dilution.

<table>
<thead>
<tr>
<th></th>
<th>Ka (M⁻¹s⁻¹)</th>
<th>Kd (s⁻¹)</th>
<th>KD (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine Pure</td>
<td>1.76(+/-0.2)E5</td>
<td>5.84(+/-0.57)E-4</td>
<td>3.34(+/-0.35)E-9</td>
</tr>
<tr>
<td>N=4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HumaneerePd ure</td>
<td>2.81(+/-0.1)E5</td>
<td>4.98(+/-0.21)E-4</td>
<td>1.78(+/-0.11)E-9</td>
</tr>
<tr>
<td>N=3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- The data generated is extremely consistent with ~10% spread run to run
# Forte Octet vs Biacore: Kinetics Comparison

> Note: Biacore samples at 37°C and Forte samples 33°C
> *Biacore measurements carried out by D. Myszka, Biosensor tools.*

<table>
<thead>
<tr>
<th></th>
<th>Ka (m⁻¹s⁻¹)</th>
<th>Kd (s⁻¹)</th>
<th>KD (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Murine Pure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Forte</td>
<td>1.76E5</td>
<td>5.84E-4</td>
<td>3.34E-9</td>
</tr>
<tr>
<td>Mean Biacore</td>
<td>4.30E5</td>
<td>4.85E-4</td>
<td>1.05E-9</td>
</tr>
<tr>
<td><strong>Hu 1 Pure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Forte</td>
<td>2.00E5</td>
<td>3.29E-4</td>
<td>1.67E-9</td>
</tr>
<tr>
<td>Mean Biacore</td>
<td>3.00E5</td>
<td>2.00E-4</td>
<td>6.70E-10</td>
</tr>
<tr>
<td><strong>Hu 2 Pure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Forte</td>
<td>2.74E5</td>
<td>5.66E-4</td>
<td>2.08E-9</td>
</tr>
<tr>
<td>Mean Biacore</td>
<td>1.28E6</td>
<td>5.7(2)e-4</td>
<td>3.60E-10</td>
</tr>
<tr>
<td><strong>Hu 3 Pure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Forte</td>
<td>2.81E5</td>
<td>4.98E-4</td>
<td>1.78E-9</td>
</tr>
<tr>
<td>Mean Biacore</td>
<td>1.11E6</td>
<td>5.9E-4</td>
<td>3.01E-10</td>
</tr>
</tbody>
</table>

- Forte results are mean of 2-5 runs, Biacore are mean of 6 runs (3 high and 3 low antigen density on CM5)

- Overall, Ka is slower on the Forte Octet but kd is very consistent

- KD differs up 5 fold but overall rank-order the same
Advantages of the ForteBio Octet

• High throughput kinetic analysis of unpurified samples shortens the screening process

• The ForteBio Octet has been integrated into the screening process and allows for greater throughput

• The ForteBio low up-front and running costs make it immediately accessible

• The instrument and software enable detailed kinetic analysis with a minimum of training or expertise in protein kinetics
www.fortebio.com

A biopharmaceutical company applying its antibody engineering technology to the development of novel therapeutics.

www.KaloBios.com