The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

A Case Study With Novozymes, Denmark

Philip Buckle
26th April 2013
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

We have seen many examples of how the Octet systems can measure concentrations directly from crude samples.

Quantitation of HIS-tagged 40 kDa protein in CHO cell spent media
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

We have also seen before how off-rate ranking can be performed on Octet systems using crude samples. But, for full KD determination in crude samples, the concentration must be known.

Since off-rate is independent of concentration,

But, for full KD determination in crude samples, the concentration must be known.

In this presentation, I will show how both concentration AND kinetics data can be obtained from the same crude protein samples.
In this presentation I will show how the Octet RED96 system has been used in both kinetics and quantitation assays using crude samples in fermentation media.

A set of protein variants were prepared on 2 separate occasions: 2012 and 2013.

Kinetics assays were performed on each set of variants, with (for the 2013 variants) concentration measurements directly from the crude samples in fermentation media being used in the subsequent KD determinations. For the 2012 variants, concentrations were determined by affinity chromatography.

All data presented here is courtesy of Novozymes, Denmark.
Both the 2012 and 2013 assays involved kinetics analysis of protein variants binding to a receptor immobilised on AR2G sensors, for which typical immobilisation profiles are shown below.

EDC/NHS Activation | Receptor, 10μg/ml at pH 5 | Ethanolamine Blocking
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Kinetics Analysis of samples performed in November 2012

- Dilution series of Reference protein, WT protein and a series of 7 variants were prepared
  - Stock samples were in crude fermentation medium
  - All dilutions were prepared in blank medium
  - Concentrations of each sample were obtained by affinity chromatograph
  - Pre-immobilised sensors were prewetted in blank fermentation medium for a minimum of 10 minutes
  - Association data was collected for 120s
  - Dissociation data was collected for 300s
  - Regeneration was performed for 20s

- The curves obtained were analysed using a 1:1 fit
Results obtained for 2012 Variants

Reference Protein

Variant 1

WT Protein

Variant 2
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Variant 7

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>KD (nM)</th>
<th>Ka (/Ms)</th>
<th>Kd (/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>169</td>
<td>2.53e4</td>
<td>4.28e-3</td>
</tr>
<tr>
<td>WT</td>
<td>82</td>
<td>5.09e4</td>
<td>4.18e-3</td>
</tr>
<tr>
<td>Variant 1</td>
<td>Weak Binder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variant 2</td>
<td>131</td>
<td>3.04e4</td>
<td>3.98e-3</td>
</tr>
<tr>
<td>Variant 3</td>
<td>15.6</td>
<td>3.46e4</td>
<td>5.4e-4</td>
</tr>
<tr>
<td>Variant 4</td>
<td>24.1</td>
<td>2.70e4</td>
<td>6.5e-4</td>
</tr>
<tr>
<td>Variant 5</td>
<td>Weak Binder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variant 6</td>
<td>266</td>
<td>2.58e4</td>
<td>6.86e-3</td>
</tr>
<tr>
<td>Variant 7</td>
<td>28.1</td>
<td>1.99e4</td>
<td>5.6e-4</td>
</tr>
</tbody>
</table>
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Kinetics Analysis of new sample batches performed in April 2013

- Here, concentration Measurements for each sample were performed using the Octet RED96 system rather than HPLC
- Concentration measurements were performed using an antibody, specific for an epitope unaffected by protein variations.
- The antibody was available with a biotin label, and was therefore loaded onto streptavidin sensors for the concentration assays
- Kinetics analysis was performed with protein variants binding to immobilised receptor
Measuring concentrations in fermentation medium

- SA sensors were preloaded with biotin labelled Protein-specific antibody at 10ug/ml
- Loaded sensors were prewetted in fermentation medium for at least 10 minutes
- Standards, in fermentation medium, were run in a dilution series, from 200ug/ml down in 2x dilutions
- Standard Quant assay (200rpm, 120 binding)
- An average from 3 dilutions (1/4,1/8 and 1/16 in fermentation medium) for each sample was used in the kinetics assays
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Typical immobilisation profile for SA coupling of biotinylated antibody (8 sensors in parallel) using Octet RED96
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Standards Binding in fermentation medium to Captured Antibody
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Standard Curve and Samples (5 parameter equation)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Reference</th>
<th>WT</th>
<th>Variant 1</th>
<th>Variant 2</th>
<th>Variant 3</th>
<th>Variant 4</th>
<th>Variant 5</th>
<th>Variant 6</th>
<th>Variant 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc</td>
<td>143 000</td>
<td>151</td>
<td>62.9</td>
<td>141</td>
<td>146</td>
<td>139</td>
<td>154</td>
<td>167</td>
<td>156</td>
</tr>
</tbody>
</table>
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Kinetics Analysis of new sample batches performed in April 2013

- Dilution series of Reference protein, WT protein and a series of 7 variants were prepared
- Stock samples were in crude fermentation medium
- Concentrations of each sample were obtained using Octet RED96
- All dilutions were prepared in blank medium
- Pre-immobilised sensors were prewetted in blank fermentation medium for a minimum of 10 minutes
- Association data was collected for 120s
- Dissociation data was collected for 300s
- Regeneration was performed for 20s

- The curves obtained were analysed using a 1:1 fit
Both the 2012 and 2013 assays involved kinetics analysis of protein variants binding to a receptor immobilised on AR2G sensors, for which typical immobilisation profiles are shown below.
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Results obtained for 2013 Variants

Reference Protein

WT Protein

Variant 1

Variant 2
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Variant 3

Variant 4

Variant 5

Variant 6
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

### Variant 7

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>KD (nM)</th>
<th>Ka (/Ms)</th>
<th>Kd (/s)</th>
<th>Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference 2012</td>
<td>169</td>
<td>2.53e4</td>
<td>4.28e-3</td>
<td>6</td>
</tr>
<tr>
<td>Reference 2013</td>
<td>213</td>
<td>3.27e4</td>
<td>6.95e-3</td>
<td>6</td>
</tr>
<tr>
<td>WT 2012</td>
<td>82</td>
<td>5.09e4</td>
<td>4.18e-3</td>
<td>4</td>
</tr>
<tr>
<td>WT 2013</td>
<td>59</td>
<td>2.39e4</td>
<td>1.40e-3</td>
<td>4</td>
</tr>
<tr>
<td>Variant 1 2012</td>
<td>Weak Binder</td>
<td></td>
<td></td>
<td>8=</td>
</tr>
<tr>
<td>Variant 1 2013</td>
<td>Weak Binder</td>
<td></td>
<td></td>
<td>7=</td>
</tr>
<tr>
<td>Variant 2 2012</td>
<td>131</td>
<td>3.04e4</td>
<td>3.98e-3</td>
<td>5</td>
</tr>
<tr>
<td>Variant 2 2013</td>
<td>77</td>
<td>1.65e4</td>
<td>1.27e3</td>
<td>5</td>
</tr>
<tr>
<td>Variant 3 2012</td>
<td>15.6</td>
<td>3.46e4</td>
<td>5.4e-4</td>
<td>1</td>
</tr>
<tr>
<td>Variant 3 2013</td>
<td>42</td>
<td>1.0e4</td>
<td>4.19e-4</td>
<td>2</td>
</tr>
<tr>
<td>Variant 4 2012</td>
<td>24.1</td>
<td>2.70e4</td>
<td>6.5e-4</td>
<td>2</td>
</tr>
<tr>
<td>Variant 4 2013</td>
<td>30</td>
<td>1.05e4</td>
<td>3.1e-4</td>
<td>1</td>
</tr>
<tr>
<td>Variant 5 2012</td>
<td>Weak Binder</td>
<td></td>
<td></td>
<td>8=</td>
</tr>
<tr>
<td>Variant 5 2013</td>
<td>Weak Binder</td>
<td></td>
<td></td>
<td>8=</td>
</tr>
<tr>
<td>Variant 6 2012</td>
<td>266</td>
<td>2.58e4</td>
<td>6.86e-3</td>
<td>7=</td>
</tr>
<tr>
<td>Variant 6 2013</td>
<td>Weak Binder</td>
<td></td>
<td></td>
<td>8=</td>
</tr>
<tr>
<td>Variant 7 2012</td>
<td>28.1</td>
<td>1.99e4</td>
<td>5.6e-4</td>
<td>3</td>
</tr>
<tr>
<td>Variant 7 2013</td>
<td>47</td>
<td>1.52e4</td>
<td>1.86e-5</td>
<td>3</td>
</tr>
</tbody>
</table>
The Octet and Blitz Systems: Obtaining Kinetics From Crude Media

Reference Protein 2012

KD = 169nM

Reference Protein 2013

KD = 213nM

WT Protein 2012

KD = 82nM

WT Protein 2013

KD = 59nM

Variant 2 2012

KD = 131nM

Variant 2 2013

KD = 77nM

Variant 3 2012

KD = 16nM

Variant 3 2013

KD = 42nM

Variant 4 2012

KD = 24nM

Variant 4 2013

KD = 30nM

Variant 7 2012

Variant 7 2012
The Octet RED96 system was able to provide valuable kinetics data for a series of protein variants in crude fermentation medium with no prior sample purification or filtration steps.

The kinetics values obtained showed good agreement between two separate production batches (2012 and 2013)

It was possible to obtain the concentrations of the crude samples using direct quantification assays using the Octet RED96 system, allowing a significant saving in time and resource over the chromatographic methods employed previously.
Thank You