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Fortebio Octet® at Biogen Idec

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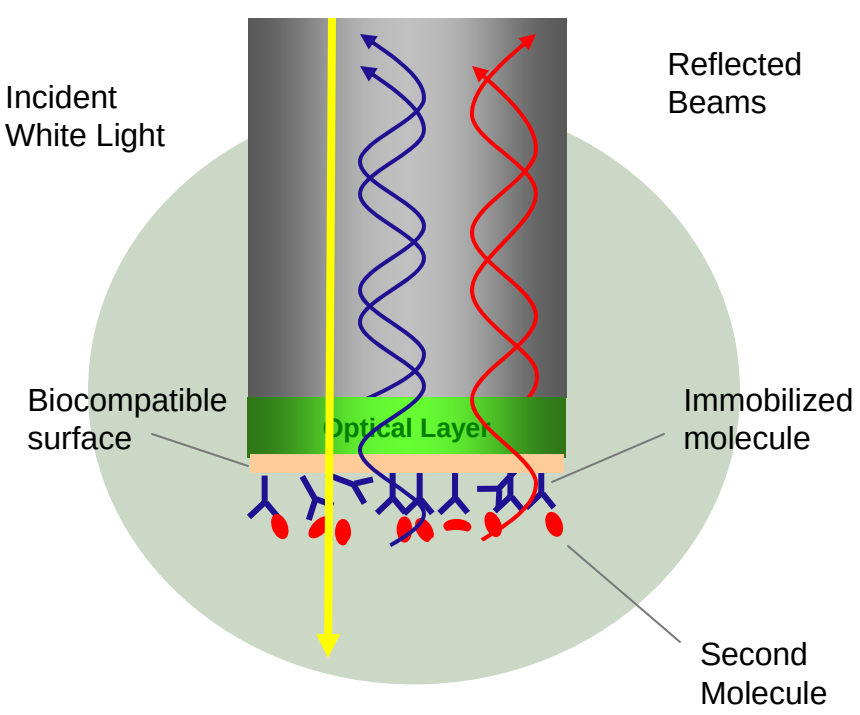
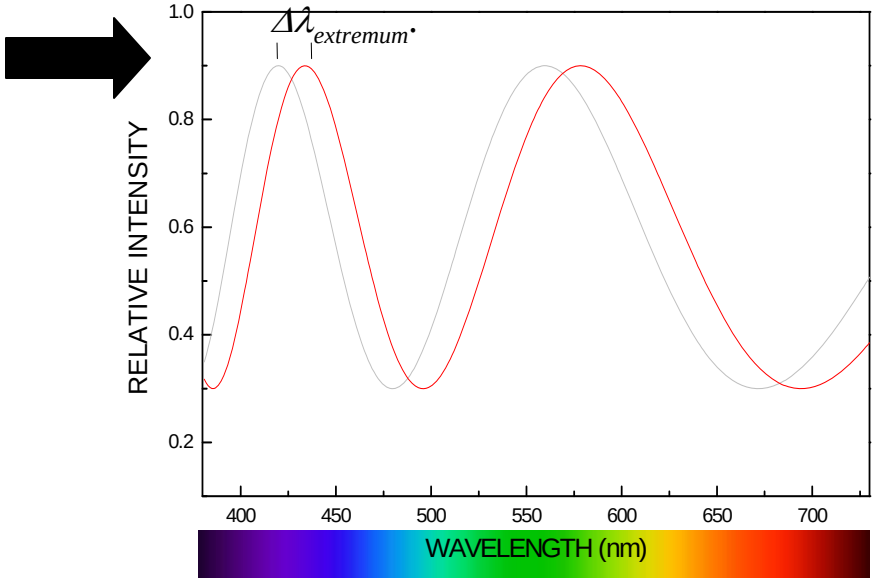
Bio-Layer Interferometry (BLI)

Proprietary new technology for label-free detection

- A layer of molecules attached to the tip of an optic fiber creates an interference pattern at the detector
- Any change in the number of molecules bound causes a measured shift in the pattern





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Octet QK vs Octet Red

- The Octet Red is roughly twice the cost of the Octet QK. However, the Red has much lower noise levels (100X) and eight-fold higher sampling rate.
- The instruments use different acquisition and analysis software. Methods, data file format and sensor tips (mostly) are the same between the two instruments. Data generated on one instrument can be analyzed by the other instruments software.

Fortebio Instruments at BIIB's San Diego Campus

- Octet QK
  - 18 months experience
  - Shared between Antibody Engineering and Research Cell Engineering
- Octet Red
  - 3 months experience
  - Protein Biochemistry

An additional Octet Red is on order for use in Cambridge

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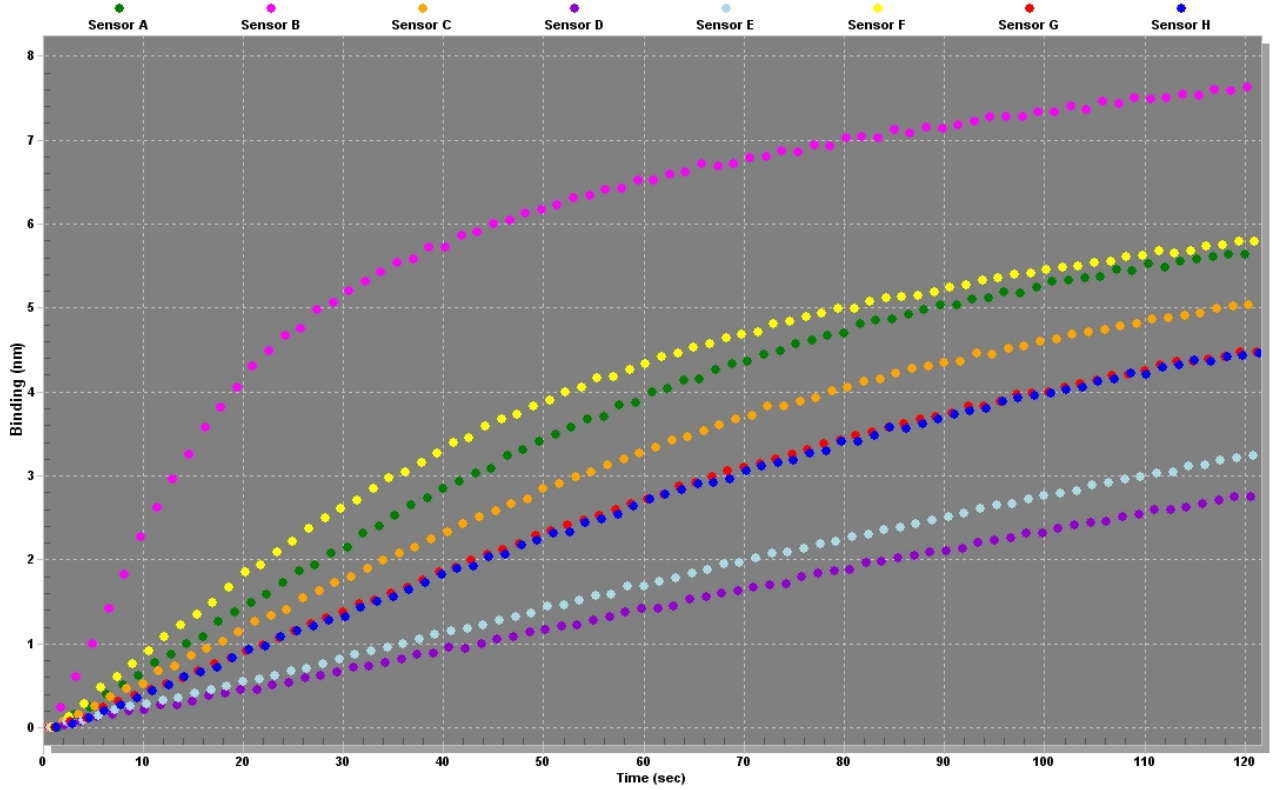
Main Octet Functions at BIIB

- Quantitation
- Off rate screening of crude samples
- MAb cross-blocking
- Epitope scanning
- Affinity ranking with purified material

Quantitation

- Easy instrument to train users on and also to operate
- Fast- ~3 minutes per 8 samples. For most samples, no preparation or dilution is needed
- Can easily make custom quantitation sensors with biotinlyated ligand and SA sensors



Increasing Protein Concentration

Protein A quantitation of a human IgG.

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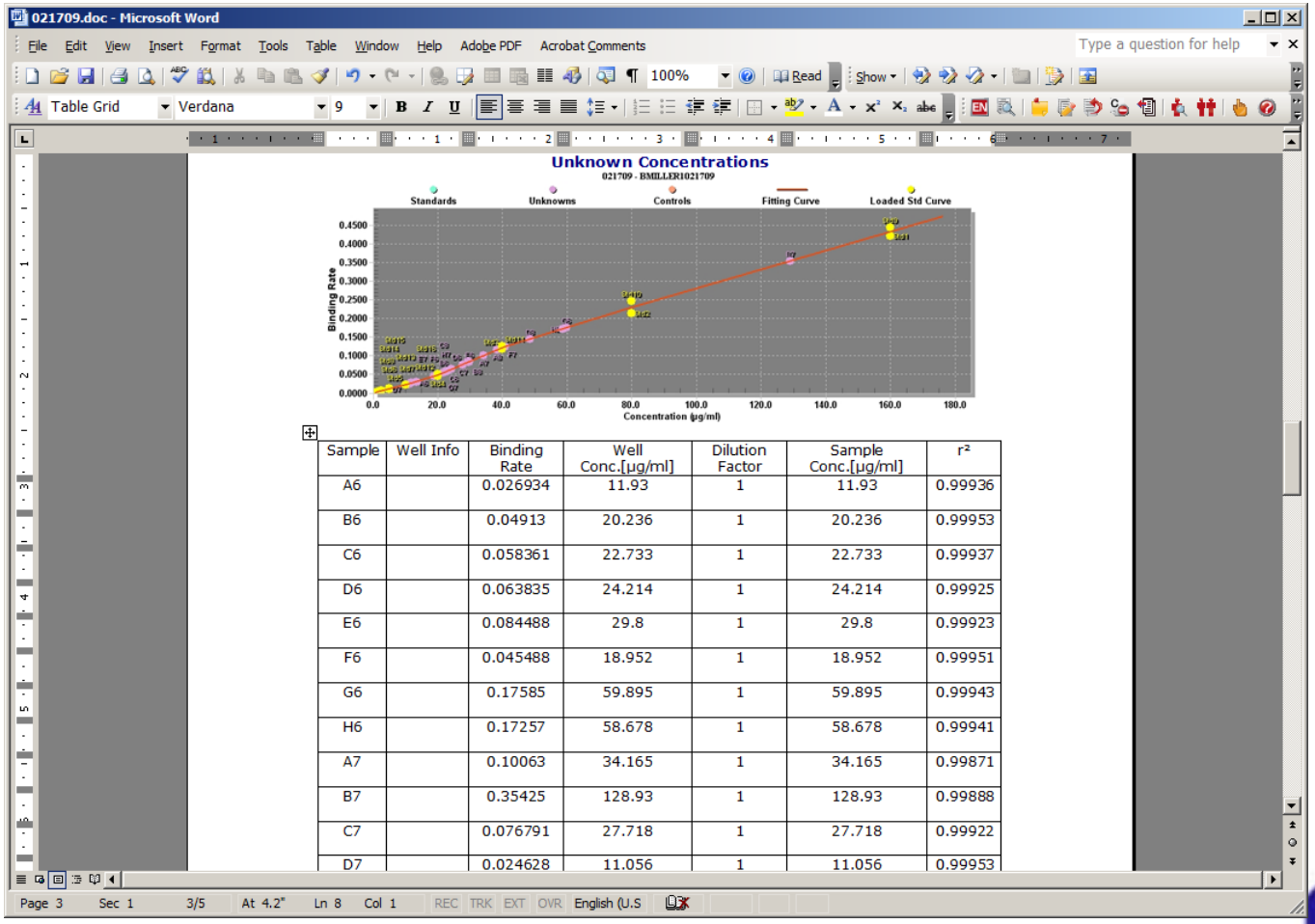
Quantitation Report

Single read per sample is accurate enough
Pre-generated standard curves used for the majority of applications



Sample	Well Info	Binding Rate	Well Conc.[µg/ml]	Dilution Factor	Sample Conc.[µg/ml]	r²
A6		0.026934	11.93	1	11.93	0.99936
B6		0.04913	20.236	1	20.236	0.99953
C6		0.058361	22.733	1	22.733	0.99937
D6		0.063835	24.214	1	24.214	0.99925
E6		0.084488	29.8	1	29.8	0.99923
F6		0.045488	18.952	1	18.952	0.99951
G6		0.17585	59.895	1	59.895	0.99943
H6		0.17257	58.678	1	58.678	0.99941
A7		0.10063	34.165	1	34.165	0.99871
B7		0.35425	128.93	1	128.93	0.99888
C7		0.076791	27.718	1	27.718	0.99922
D7		0.024628	11.056	1	11.056	0.99953

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Quantitation Applications

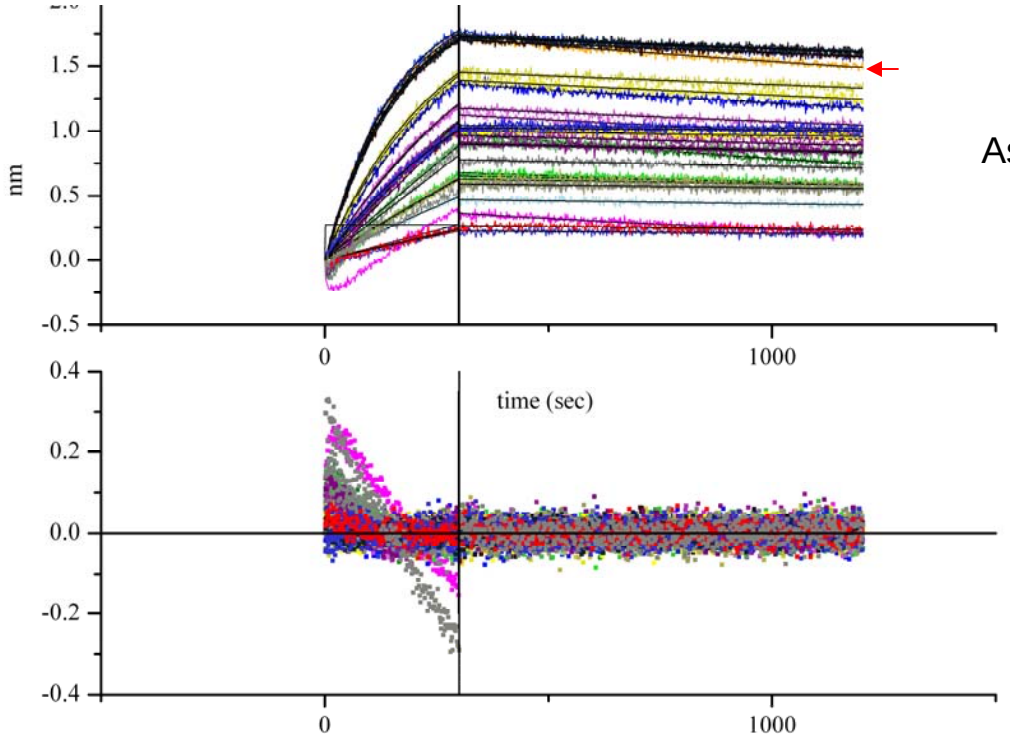
- Clone/Media/Host ranking. Also used for following cultures over time to determine quantity of protein
- Determining titer for downstream purification

We tend to use the Octet to determine relative titer, and are less often interested in highly accurate absolute titer. However, with the appropriate standards the Octet is at least as accurate as previous methods used in-house.

Off-rate Screening for Library Triage

16 mutant culture supernatents assayed in triplicate on Ag-Fc fusion protein bound to Prot. A sensors. Approximately 3 hour run time.

On-rate analyzed qualitatively, off-rates quantitatively.



Assoc./Dissoc.

Residuals from fit

These crude samples are unsuitable for Biacore analysis due to media composition and unfilterable cell debris.
Rank by off-rate as a first pass triage, eliminating 5 out of 16 mutants by this criterion.

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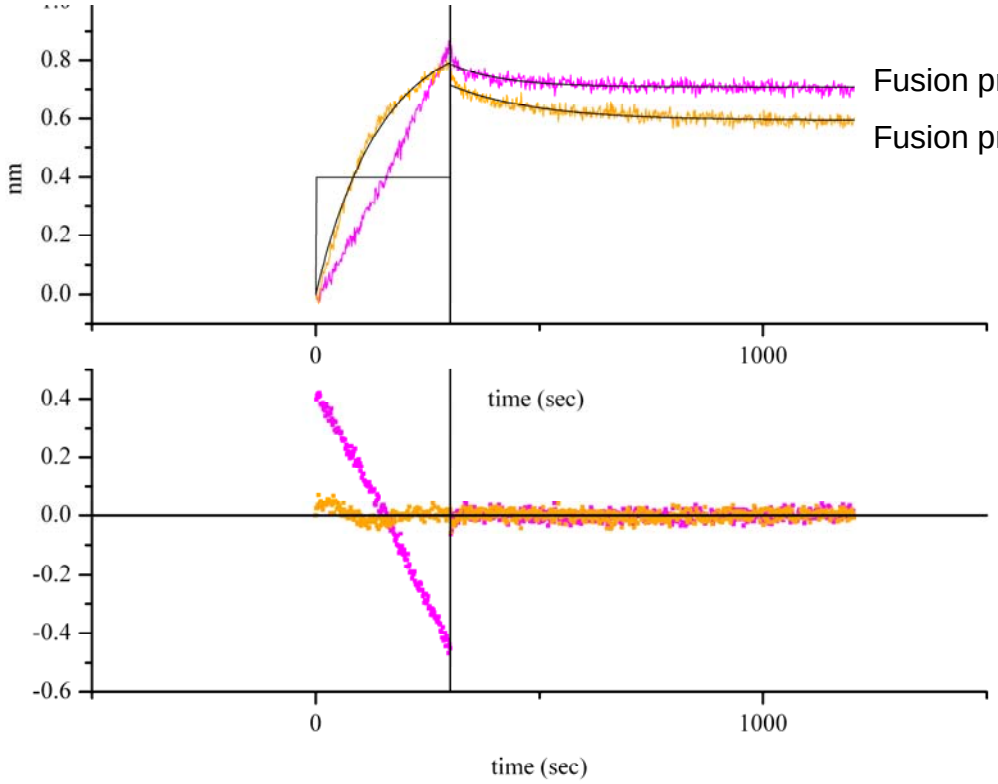
Caveat for Crude Samples

Protein aggregates can give odd looking binding curves.

Crude culture supernatents assayed on Ag-Fc fusion proteins bound to anti-human Fc sensors.



These two fusion proteins differ only in the linker sequences between the fused domains. Fusion 1 was found to contain a much higher level of soluble aggregates when compared to Fusion 2.

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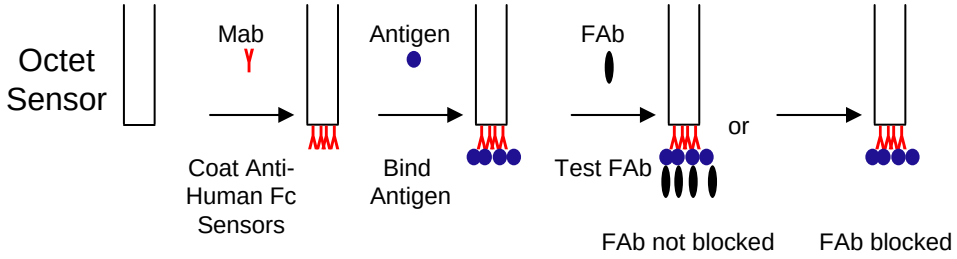
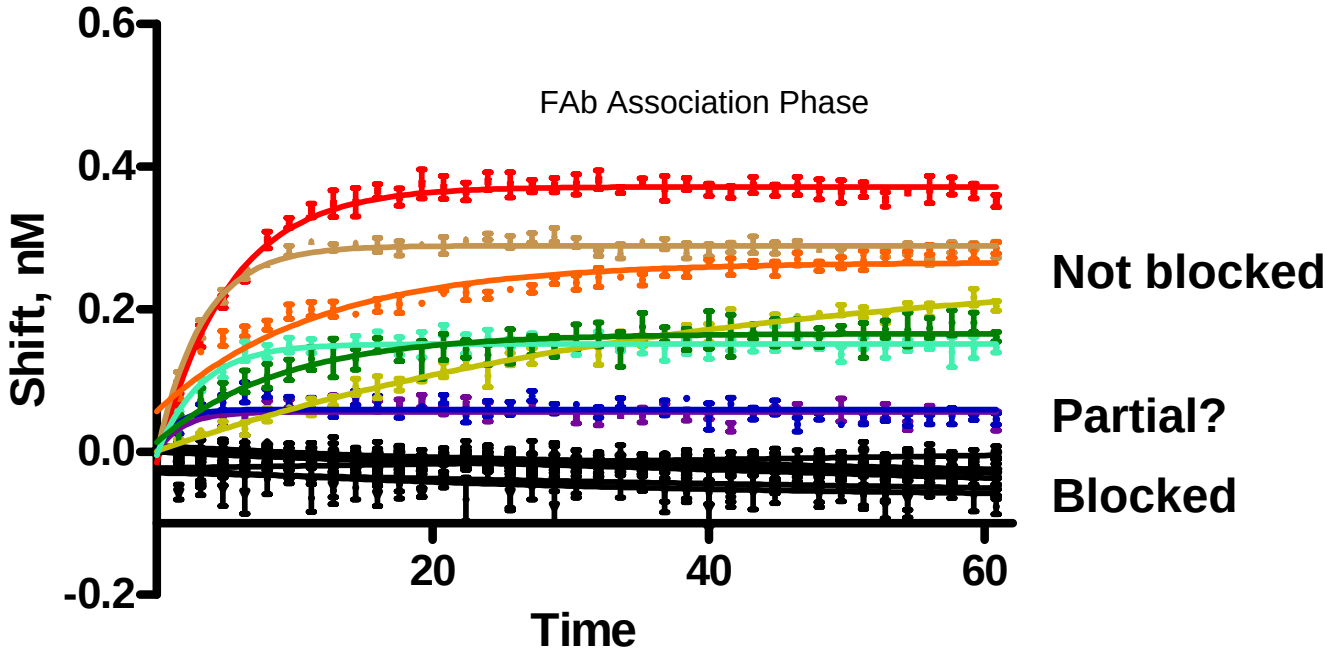
Off-rate Screening

- The throughput of the Octet allows for us to rapidly assess the off-rate of 50-100 samples per day as a rapid means of triaging mutant proteins
- The Octet is *relatively* insensitive to detergent, non-specific proteins, nucleic acids, etc. in crude samples. Changes in refractive index between sample and buffers has no effect

Cross-blocking: Sandwich Assay





Octet allows for rapid analysis of cross-blocking MAb's

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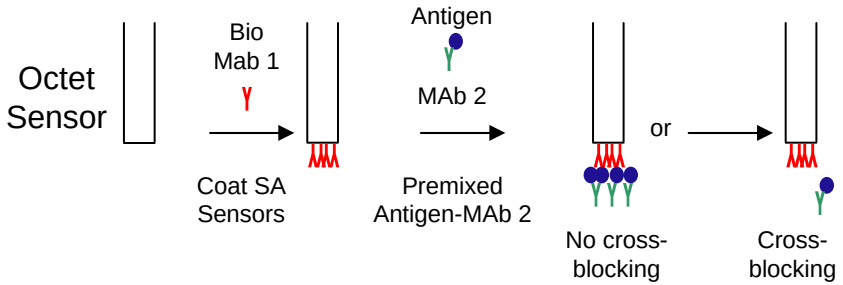
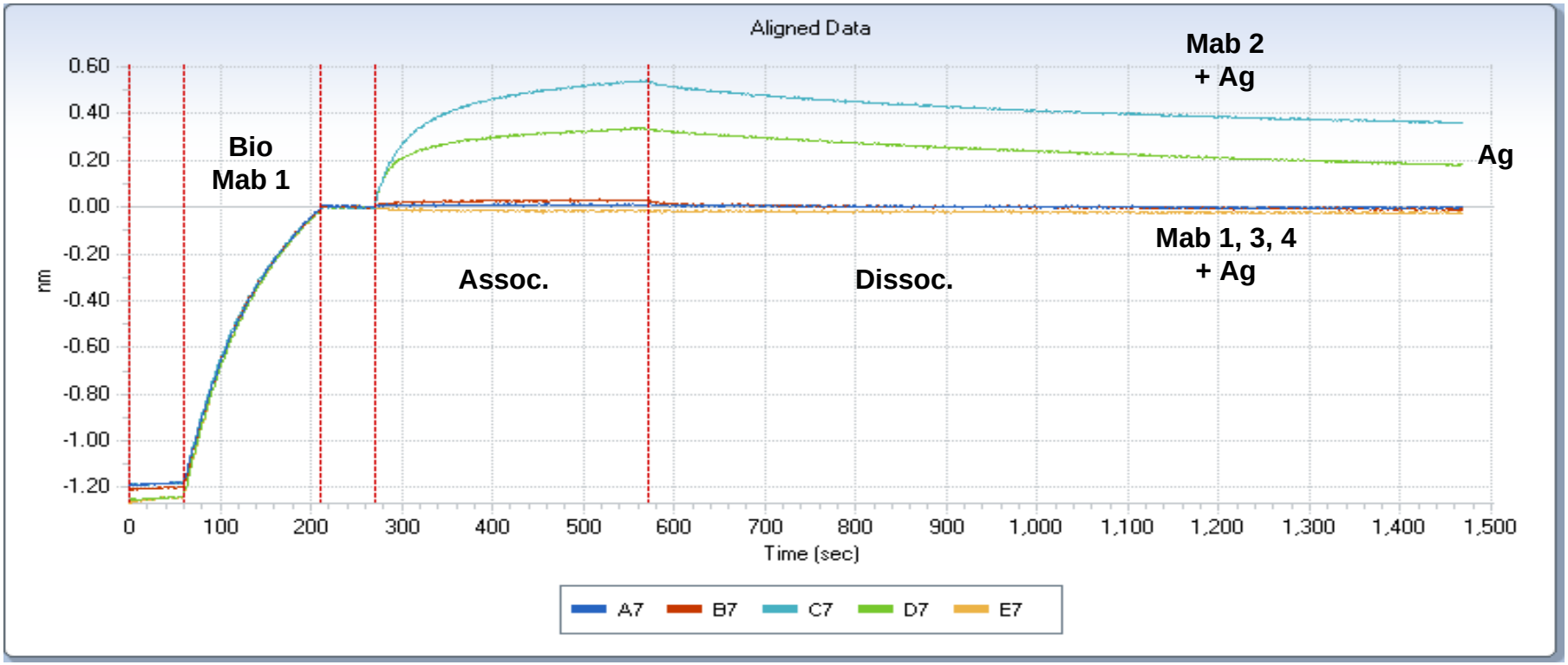
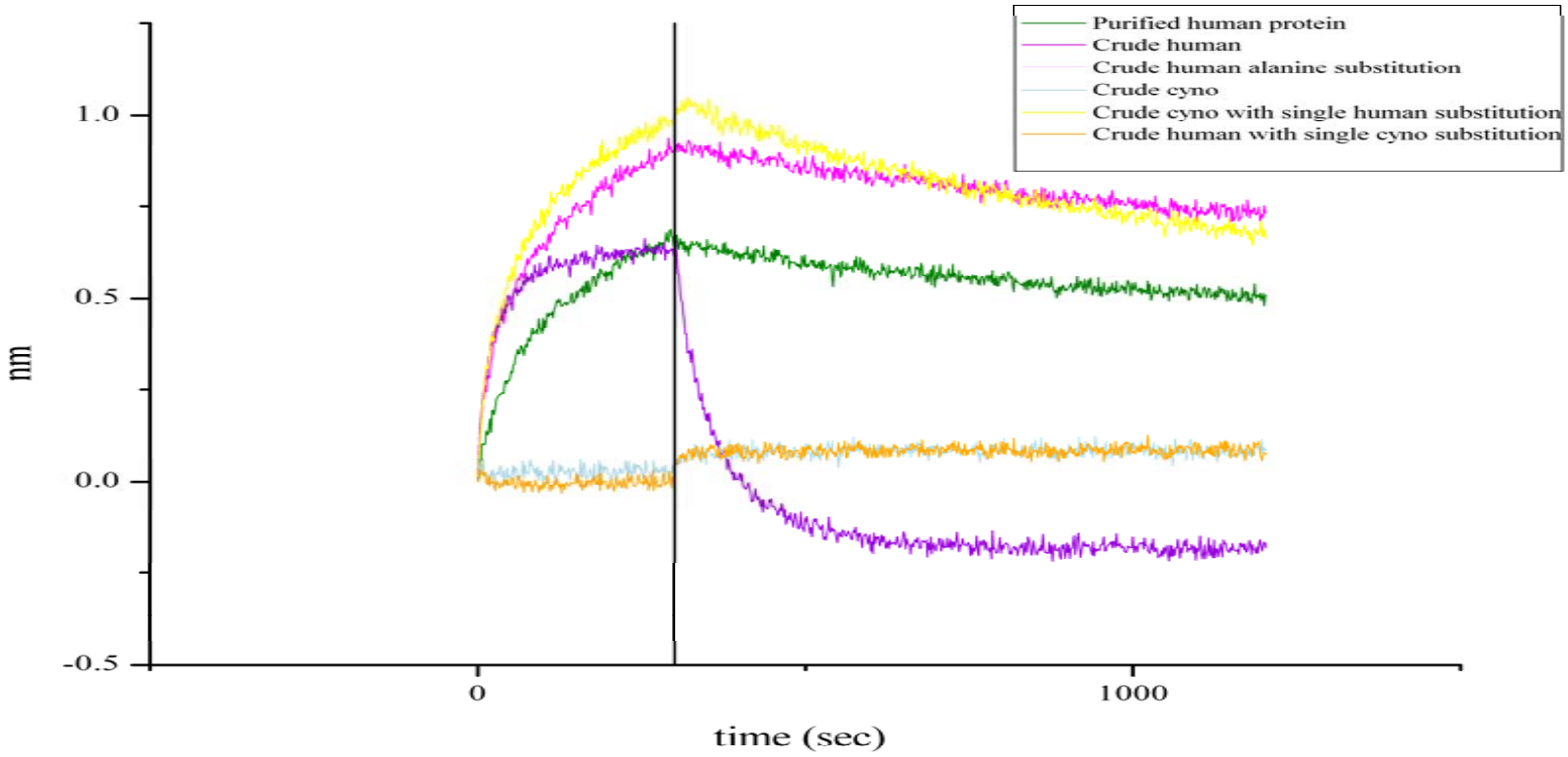
Cross-blocking: Solution Competition Assay





Epitope Scanning

Test case: MAb that binds human but not cyno antigen. Substitute single cyno residues into human sequence, express in Pichia. A single position was found that confers the majority of species specificity in this MAb.



Protein samples assayed using biotinylated Ag bound to SA sensors

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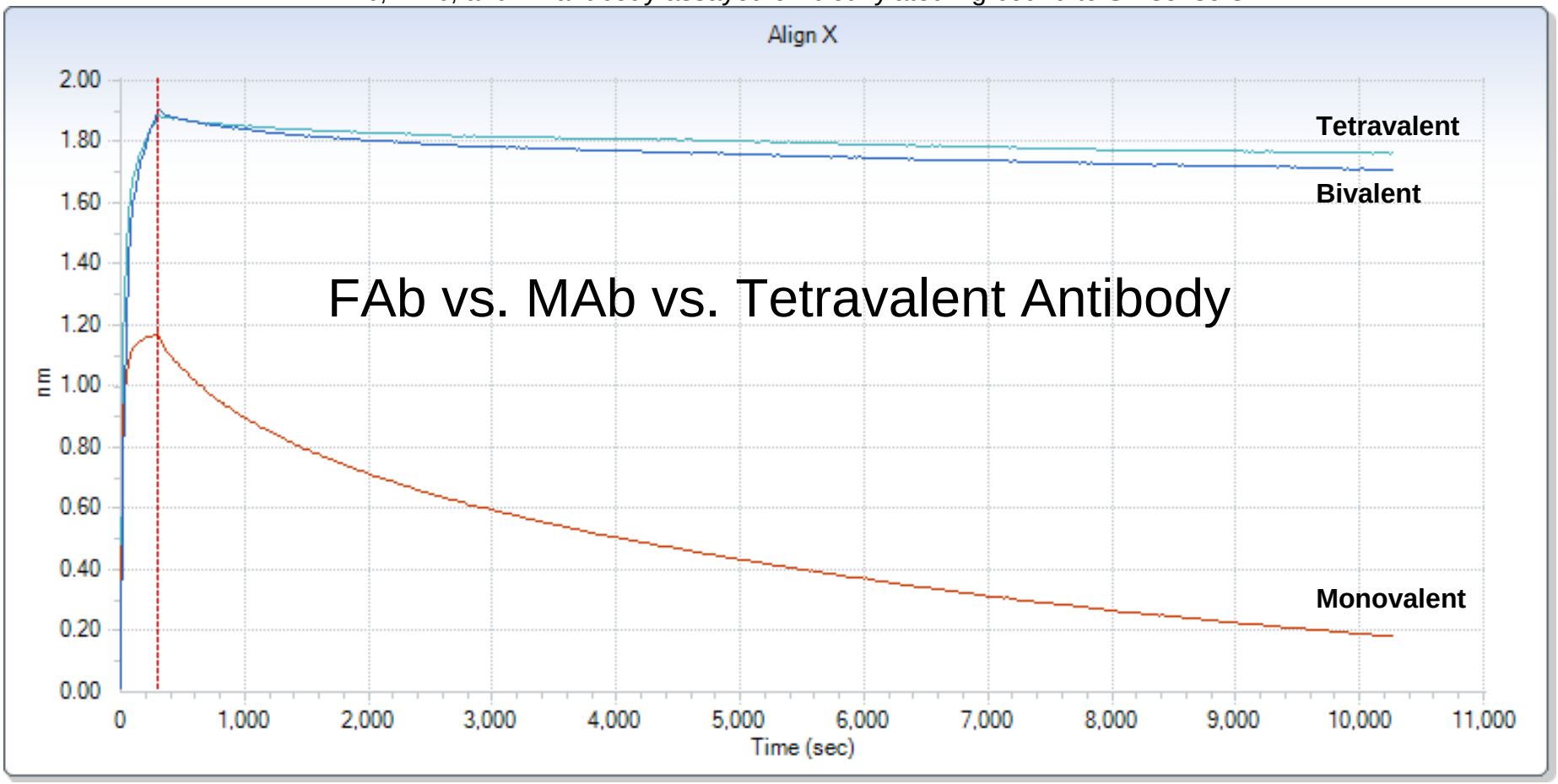
Epitope and Cross-blocking Summary

- The Octet allows for rapid determination of which MAb's cross-block each other
- Large numbers of epitope scanning mutations can be analyzed kinetically for their effects on MAb binding without the need for purification of each mutant protein

K_D from Kinetic Analysis: Pure protein

FAb, MAb, and Tv antibody assayed on biotinylated Ag bound to SA sensors



Apparent affinity increases with valency

1 nM → 50 pM → ~10 pM

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Conclusions

- The Octet has rapidly become an integral part of workflows in multiple groups with BIIB Research:
  - Research Cell Engineering: Quantitation
  - Antibody Engineering: Rapid library triage on crude supernatants, epitope mapping, cross-blocking
  - Protein Biochemistry: K_D determination with purified proteins, cross-blocking
- Assays are easily and rapidly developed. Regeneration methods developed for Biacore experiments are transferable to Octet experiments
- Sensor costs are minimal, especially in light of FTE cost savings

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