Application of the Octet Red96 system to characterize protein interactions

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Protein interaction analysis

Proteins are fundamental molecules in biology

Interactions are essential for protein functions

Protein-protein
(Ab-Ag), (ligand-receptor)

Protein-carbohydrate
(lectin-sialic acid)

Protein-lipid
(protein-phospholipid)

Early stage characterization	**Later stage characterization**
Rapid, easy to use	Rapid, easy to use
Relatively crude preparation	Purified samples
Qualitative	Quantitative
Rough ranking	Publishable results

Octet Red96 biolayer interferometry

Rapid qualitative antigen-antibody interaction characterization

1. Hybridoma produced IgG

2. Stably expressed IgG of phage display Ab

IgGs are secreted in tissue culture media

For early stages:
Use crude tissue culture media containing IgG

Quantify IgG production?

Does the IgG bind the antigen?

Rapid qualitative antigen-antibody interaction characterization

IgG quantification (reproducible test)

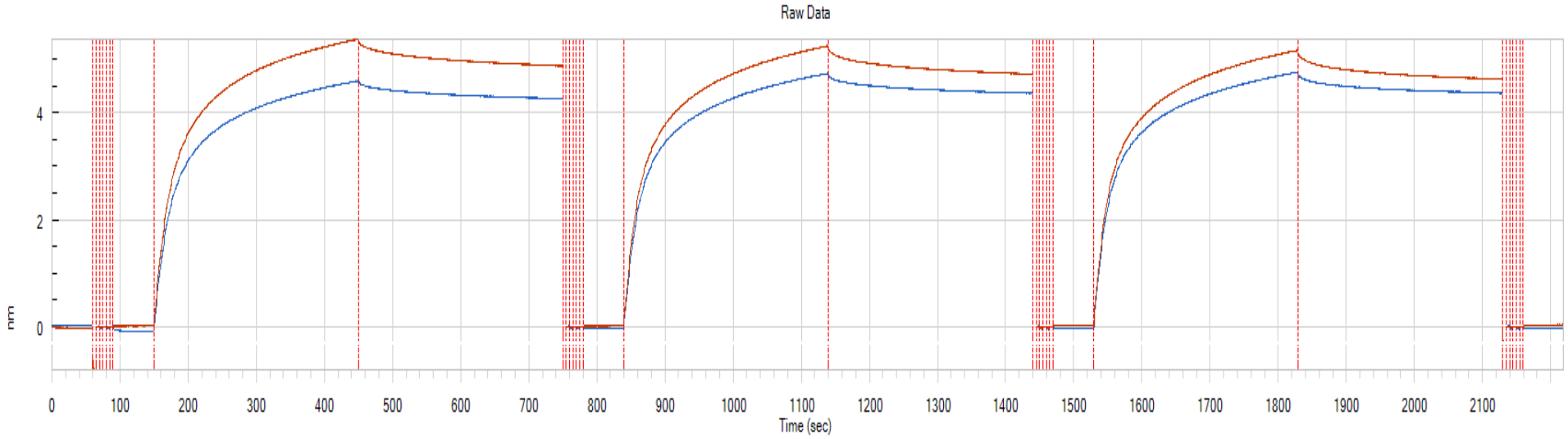
Ab: Human phage display IgG1
IgG produced from serum free media (no purification)

Probe: protein A (ProA) biosensor

Well: IgG containing serum free media

Repeated measurement of IgG using proA probe after subsequent acid washes (100mM citric acid pH3)

	1	2	3	4	Probe
A	Hyb Med	IgGZ	100mM citric acid pH3	100mM HEPES pH7.5	proA
B	..	..	..	..	..
	Baseline		Regenerate	Neutralization	



Both lanes generated curves of similar level

Proceed with further characterization

Rapid qualitative antigen-antibody interaction characterization

Hybridoma produced IgG-Ag interaction

Ab: mouse IgG1, mouse IgG2
IgG produced from serum free hybridoma media (no purification)

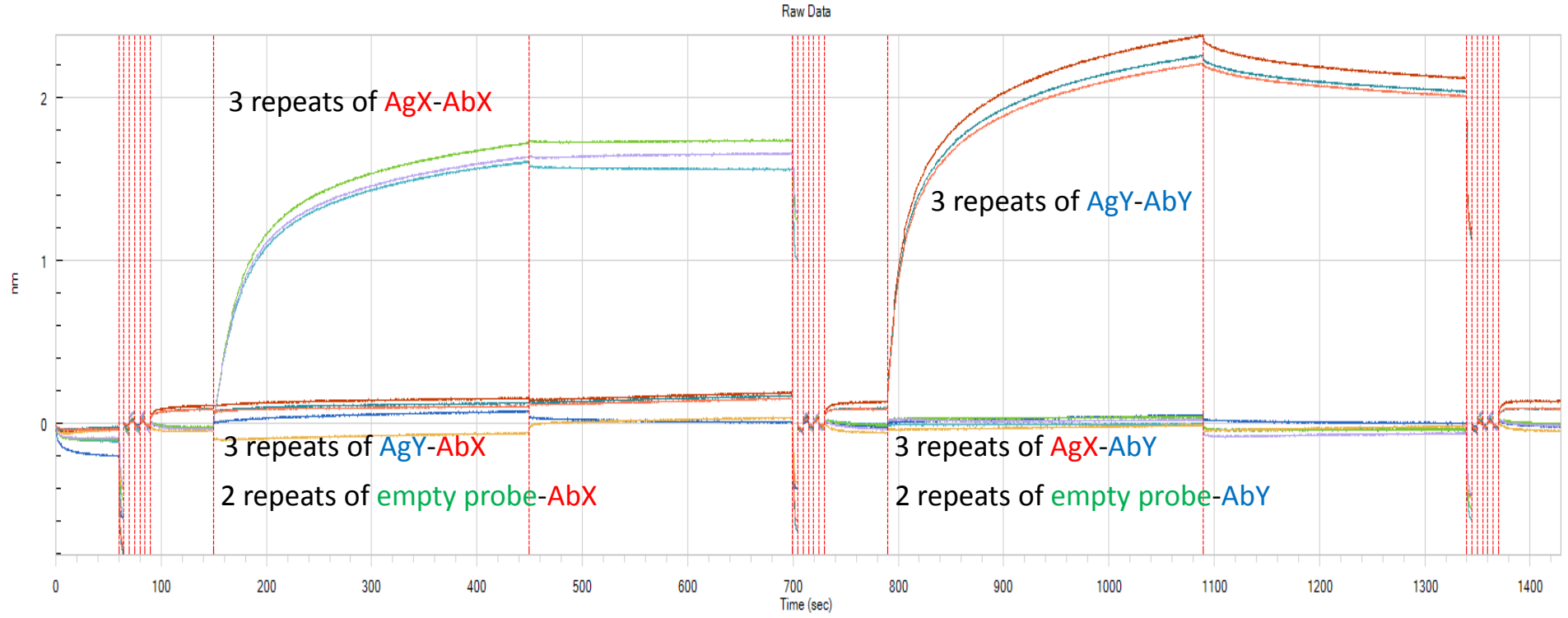
Ag: untagged purified soluble glycoproteins
gel filtration purified (>95%) antigens expressed in HEK293 cells
biotin label Ag on primary amines

Probe: streptavidin (SA) biosensor (externally loaded biotin-Ag)

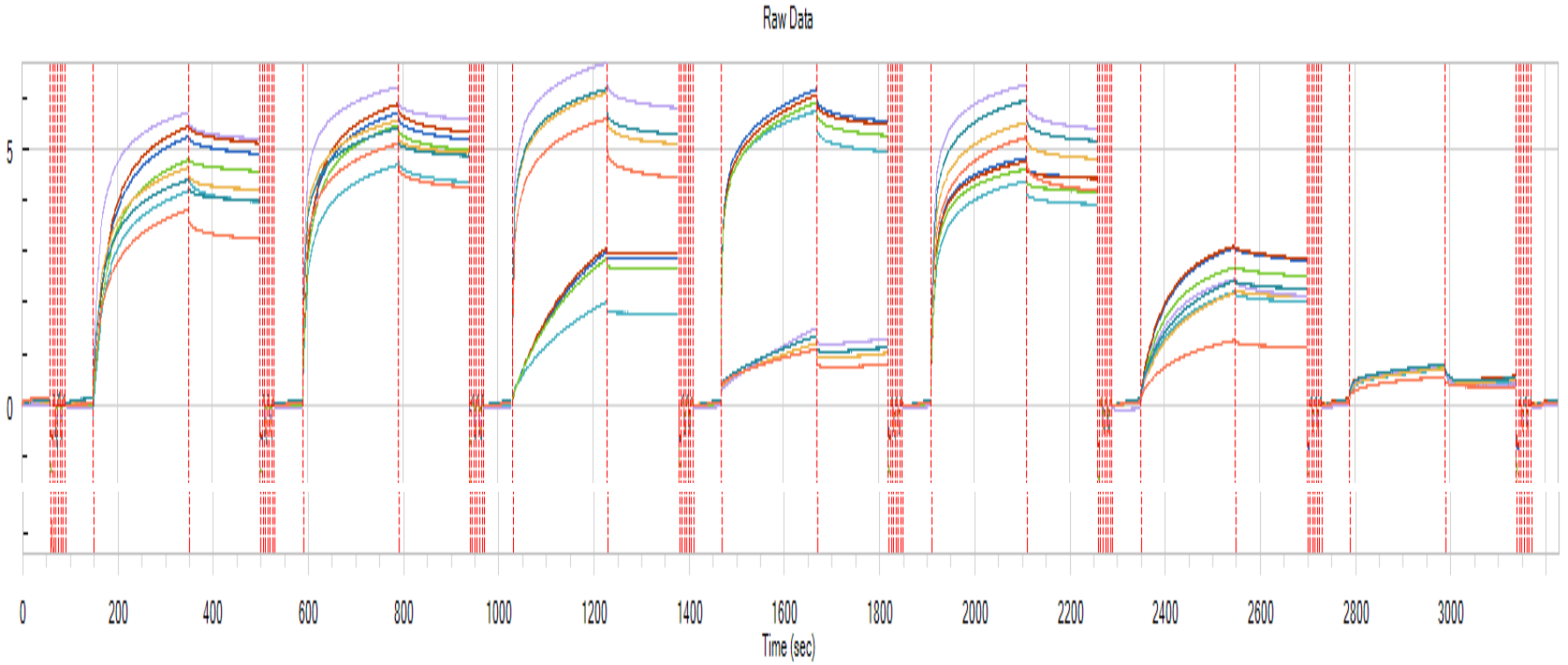
Well: crude hybridoma media

	1	2	3	4	5	Probe
A	Hyb Med	IgGX	IgGY	50mM Acetic acid pH 4.5	100mM HEPES pH7.5	SA: Empty
B	..	..	..	..	..	SA: AgX
C	..	..	..	..	..	SA: AgY
D	..	..	..	..	..	SA: AgY
E	..	..	..	..	..	SA: Empty
F	..	..	..	..	..	SA: AgY
G	..	..	..	..	..	SA: AgX
H	..	..	..	..	..	SA: AgX

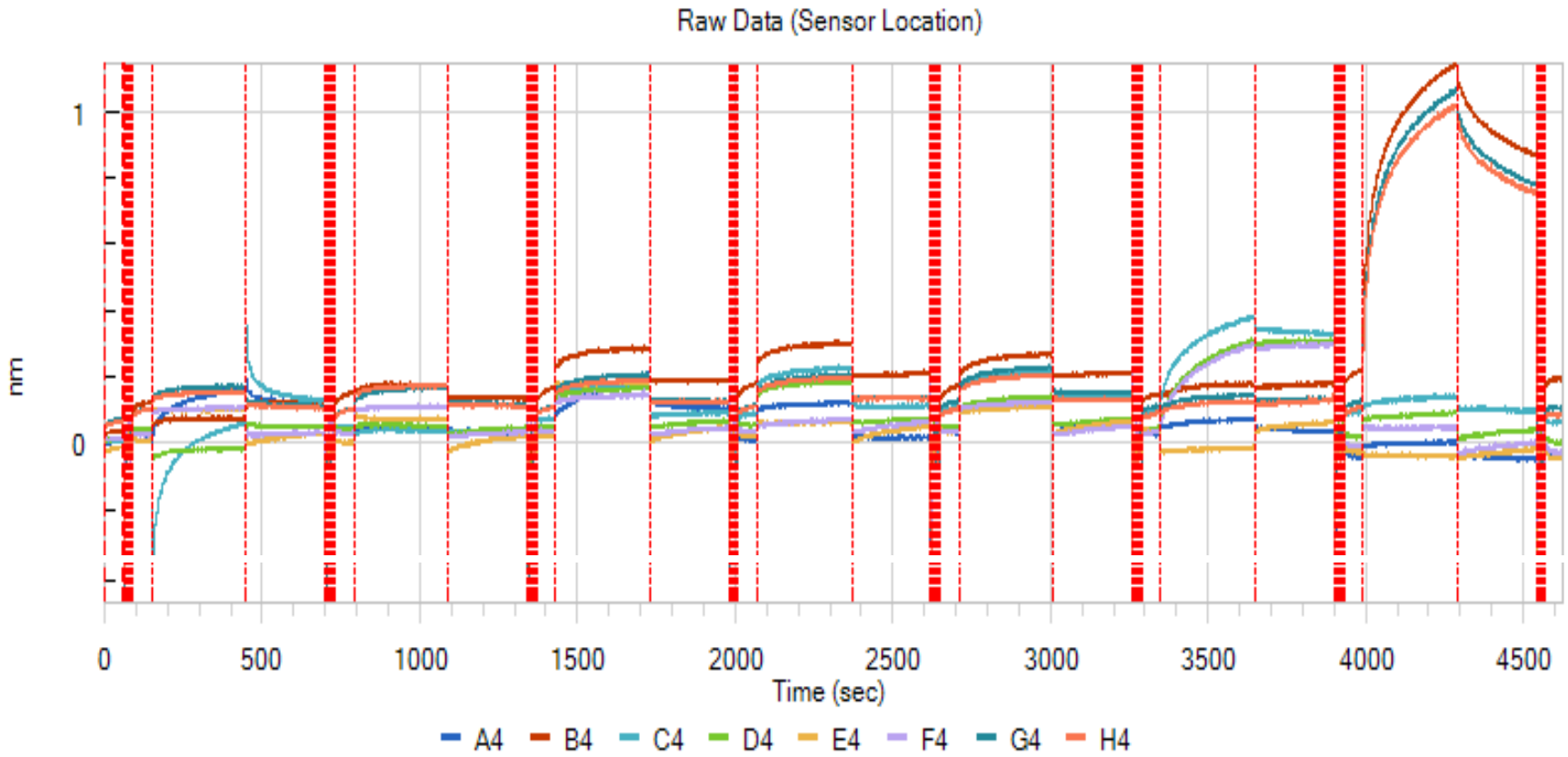
Baseline Regeneration Neutralization



	1	2	3	4	5	6	7	8	9	10	Probe
A	100mM Citric Acid pH3	100mM HEPES pH7.5	SFM	IgGA	IgGD	IgGF	IgGH	IgGJ	IgGL	IgGM	proA
B	..	..	..	..	..	..	..	..	..	..	..
C	..	..	..	..	..	..	..	..	..	..	..
D	..	..	..	..	..	..	..	..	..	..	..
E	..	..	..	IgGB	IgGE	IgGG	IgGI	IgGK	..	..	..
F	..	..	..	..	..	..	..	..	..	..	..
G	..	..	..	..	..	..	..	..	..	..	..
H	..	..	..	..	..	..	..	..	..	..	..



	1	2	3	4	5	6	7	8	9	10	Probe
A	100mM Acetic Acid pH4.5	100mM HEPES pH7.5	SFM	IgGA	IgGD	IgGF	IgGH	IgGJ	IgGL	IgGM	SA: Empty
B	..	..	..	..	..	..	..	..	..	..	SA: AgX
C	..	..	..	..	..	..	..	..	..	..	SA: AgY
D	..	..	..	..	..	..	..	..	..	..	SA: AgY
E	..	..	..	IgGB	IgGE	IgGG	IgGI	IgGK	..	..	SA: Empty
F	..	..	..	..	..	..	..	..	..	..	SA: AgY
G	..	..	..	..	..	..	..	..	..	..	SA: AgX
H	..	..	..	..	..	..	..	..	..	..	SA: AgX



Quantitative protein-protein interaction characterization

Protein-protein interaction

Can we obtain binding constants of protein-protein interaction using purified proteins?

Quantitative protein-protein interaction characterization

Mammalian cell line produced protein-protein interaction

Protein 1: untagged purified soluble glycoprotein
gel filtration purified (>95%) proteins expressed in HEK293 cells

Protein 2: untagged purified soluble glycoproteins
gel filtration purified (>95%) proteins expressed in HEK293 cells
biotin label Ag on primary amines

Probe: streptavidin (SA) biosensor (externally loaded biotin-protein 2)

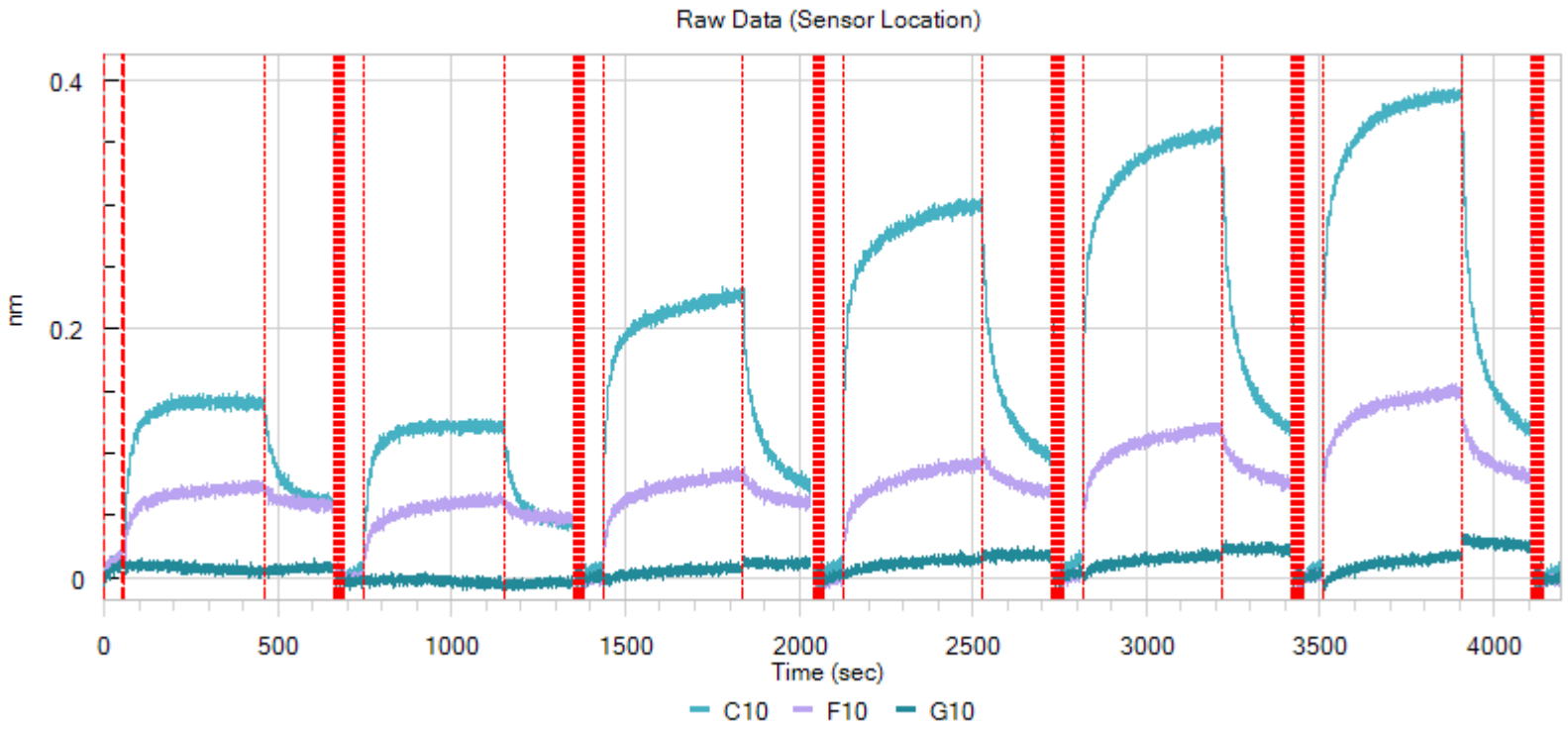
Negative control: Biotin-non-interacting protein 3

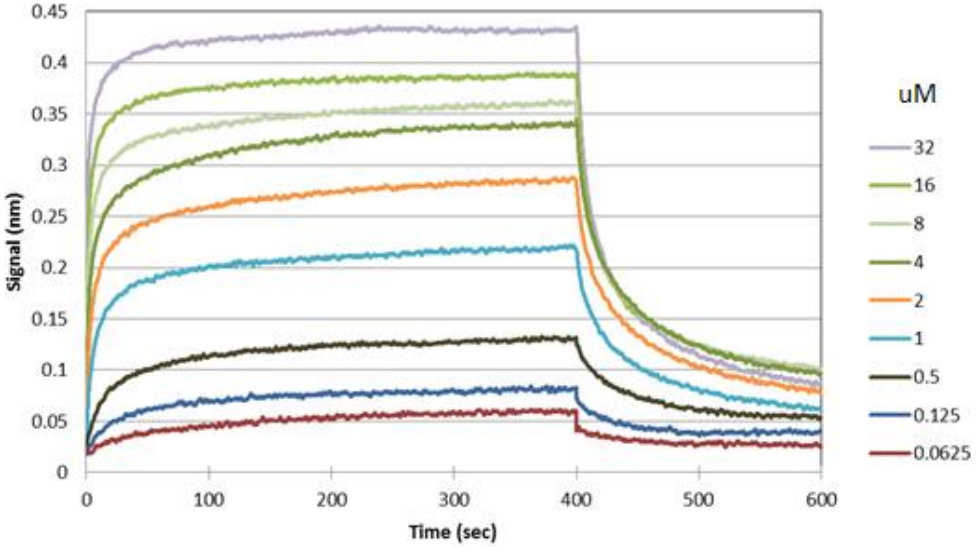
Well: titration of purified protein 1

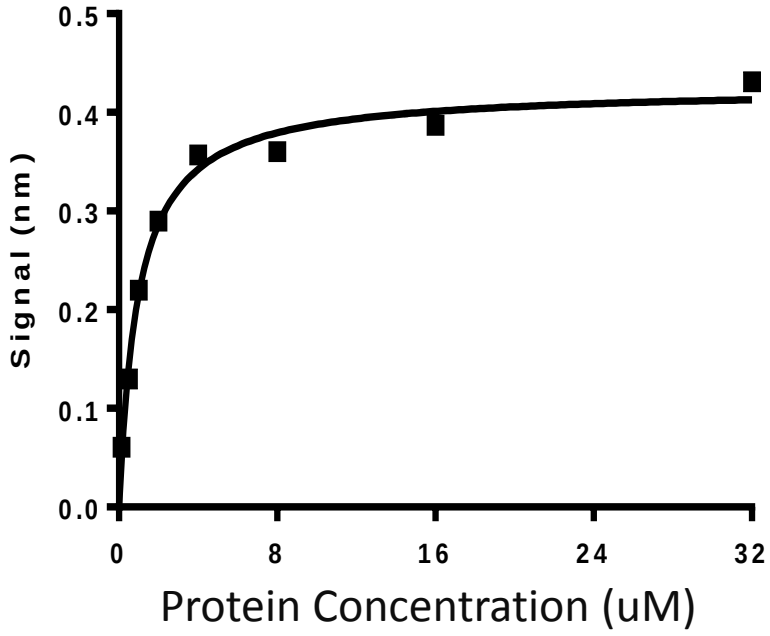
	1	2	3	4	5	6	7	8	9	10	11	12	Probe
C	50mM Citric acid pH 4	100mM HEPES pH7.5	100mM HEPES 100mM NaCl pH7.5	0.5uM protein 1	100mM HEPES 100mM NaCl pH7.5	1uM protein 1	100mM HEPES 100mM NaCl pH7.5	2uM protein 1	100mM HEPES 100mM NaCl pH7.5	4uM protein 1	100mM HEPES 100mM NaCl pH7.5	8uM protein 1	SA: Protein 2
F	..	..	..	..	..	..	..	..	..	..	..	..	SA: Non-interacting protein 3
G	..	..	..	..	..	..	..	..	..	..	..	..	SA: Empty

Regeneration

Neutralization




0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Signal (nm)
0
100
200
300
400
500
600
Time (sec)
uM
32
16
8
4
2
1
0.5
0.125
0.0625


0.5
0.4
0.3
0.2
0.1
0.0
Signal (nm)
0
8
16
24
32
Protein Concentration (uM)

Kd = 0.98 uM

Quantitative lectin-carbohydrate interaction characterization

Lectin-carbohydrate interaction

Can we obtain binding constants of describing protein-carbohydrate interaction using purified lectins?

Quantitative lectin-carbohydrate interaction characterization

Lectin-sialic acid interaction

Lectin: sialic acid binding soluble glycoprotein
Gel filtration purified (>95%) lectin expressed in HEK293 GnT1^{-} cells (no sialic acid on the lectin)

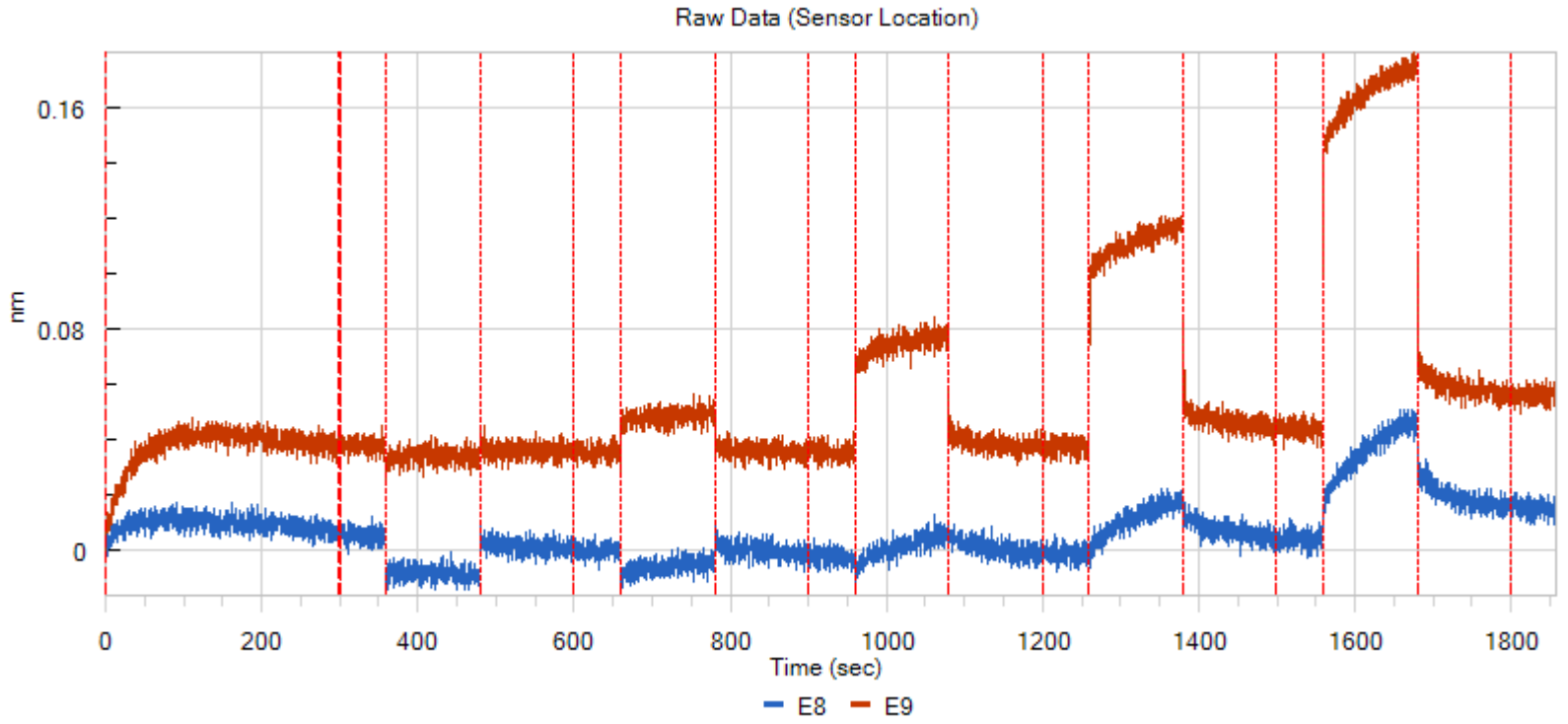
Carbohydrate: soluble silaic acid containing glycoproteins
Biotin label silaic acid containing glycoproteins on primary amines

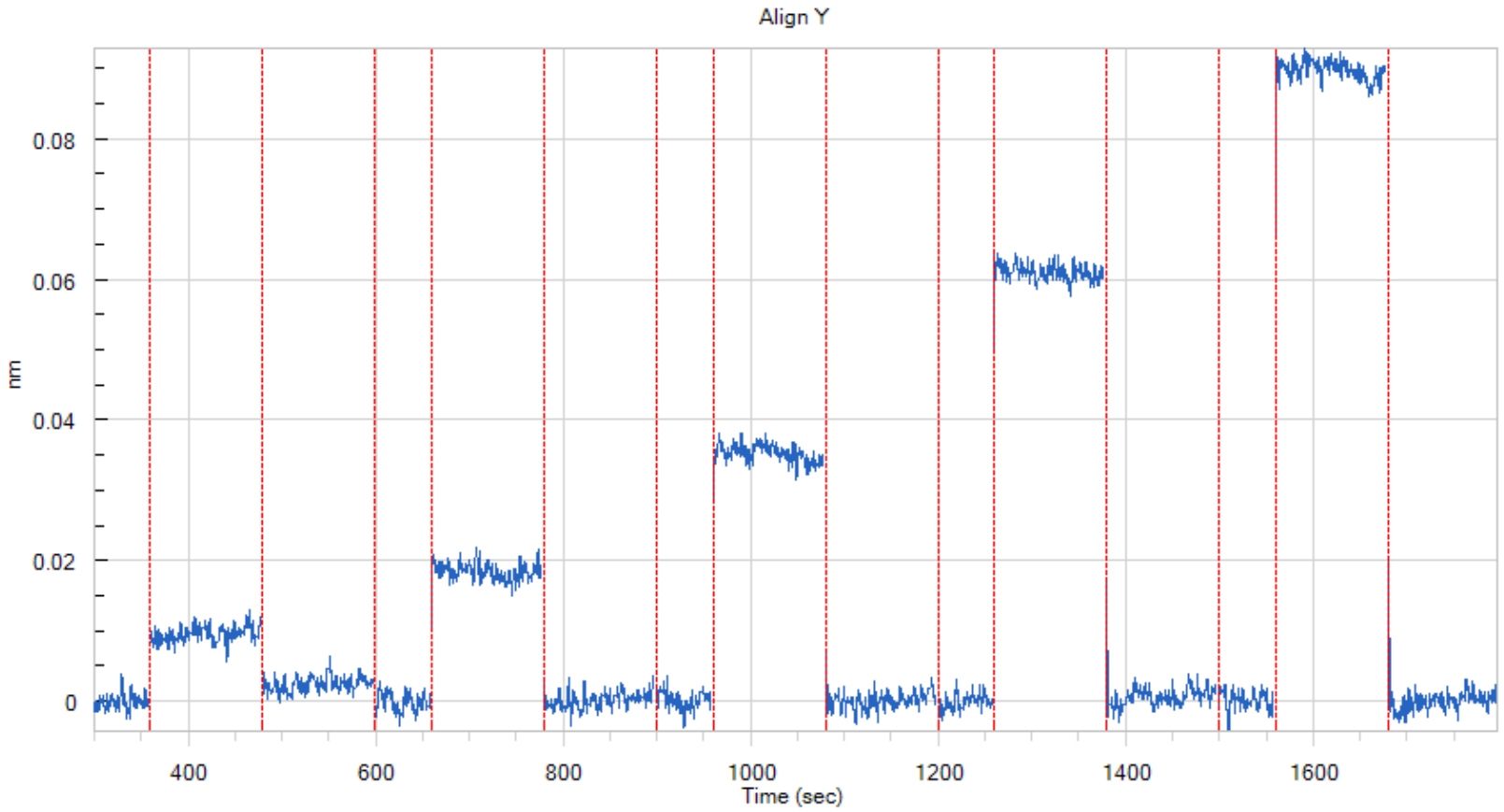
Probe: streptavidin (SA) biosensor (externally loaded biotin- sialic acid containing glycoprotein)

Negative control: probe incubated with neuraminidase (external)

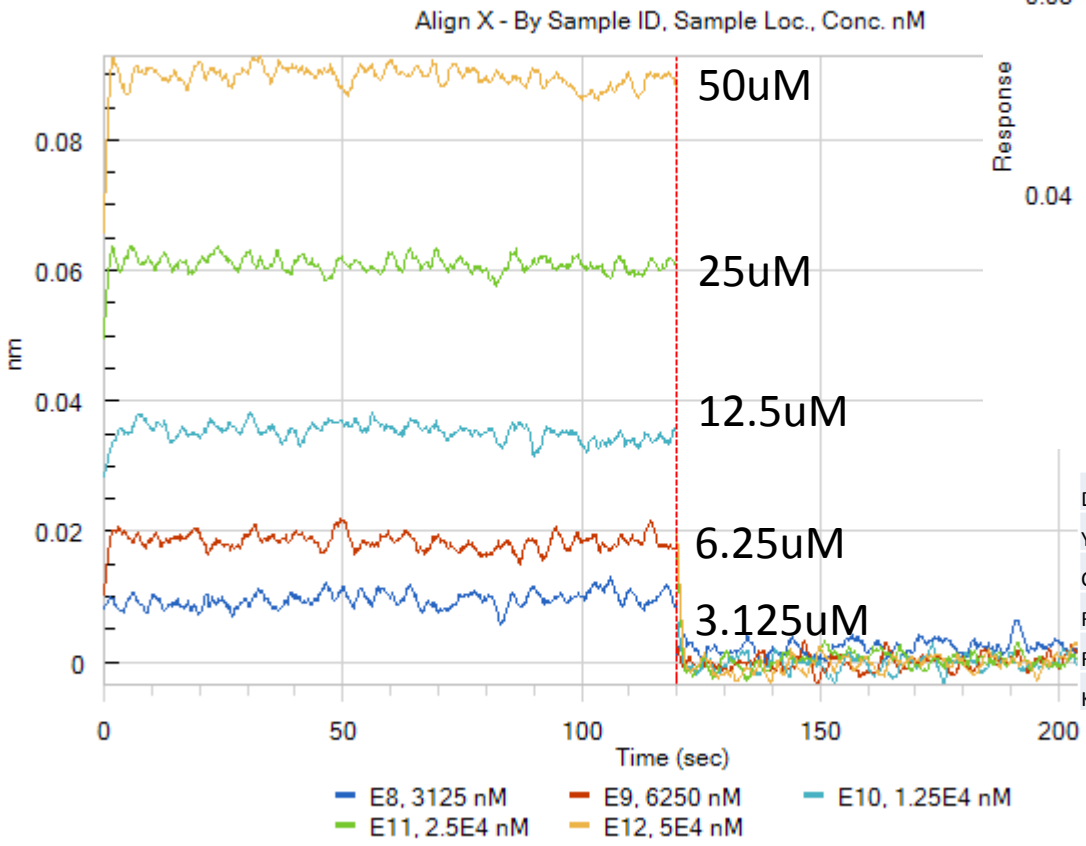
Well: titration of lectin

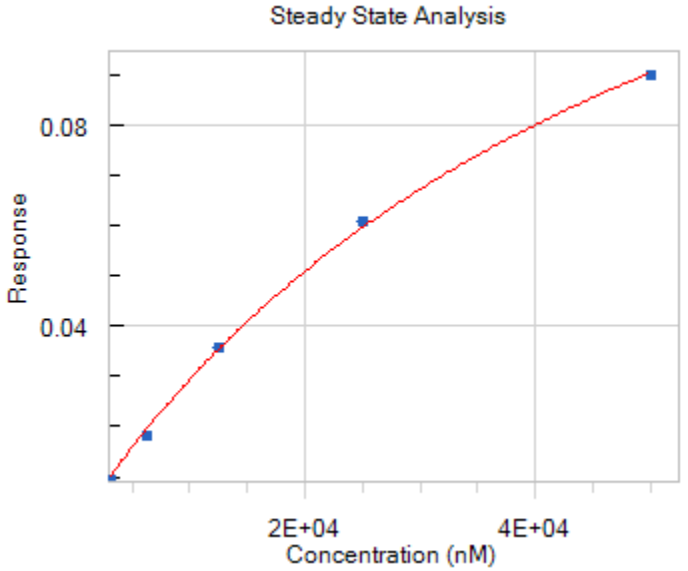
	1	2	3	4	5	6	7	8	9	10	11	12	Probe
E9	100mM HEPES 100mM NaCl pH7.5	3.125uM Lectin	100mM HEPES 100mM NaCl pH7.5	6.25uM Lectin	100mM HEPES 100mM NaCl pH7.5	3.125uM Lectin	100mM HEPES 100mM NaCl pH7.5	12.5uM Lectin	100mM HEPES 100mM NaCl pH7.5	25uM Lectin	100mM HEPES 100mM NaCl pH7.5	50uM Lectin	SA: Glycoprotein
E8	..	..	..	..	..	..	..	..	..	..	..	..	SA: Neuraminidase treated glycoprotein




Align Y
0.08
0.06
0.04
0.02
0
nm
400
600
800
1000
1200
1400
1600
Time (sec)

Take response value at 20 to 100 sec





Data Used	Response	
Y Weighting		
Chi^2/DoF	1.47857E-06	
R^2	0.998973223	
RMax	0.187001208	0.007974
KD	5.30E-05	±3.7E-06M

KD from BLI = 53uM

KD from SPR = 45 uM

Qualitative protein-lipid interaction characterization

Protein-lipid interaction

Can we obtain detect binding of protein with phospholipid in a liposome?

Qualitative protein-lipid interaction characterization

Protein-liposome interaction

Protein: Phospholipid binding protein, multiple lipid binding sites
Gel filtration purified (>95%) protein expressed in E.coli

Liposome: biotin labeled liposome

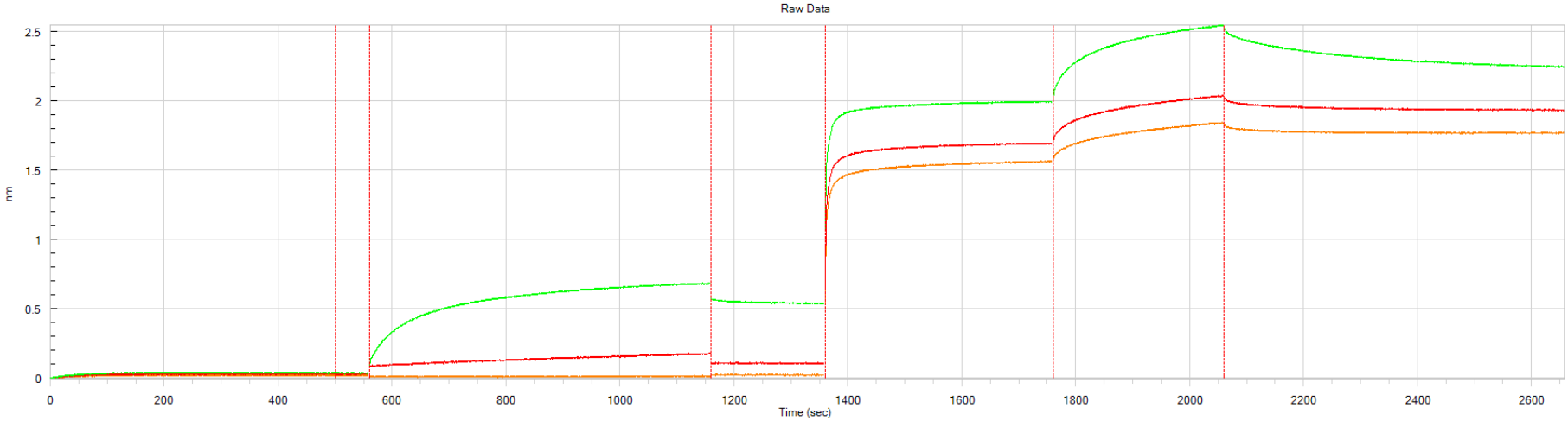
Probe: streptavidin (SA) biosensor

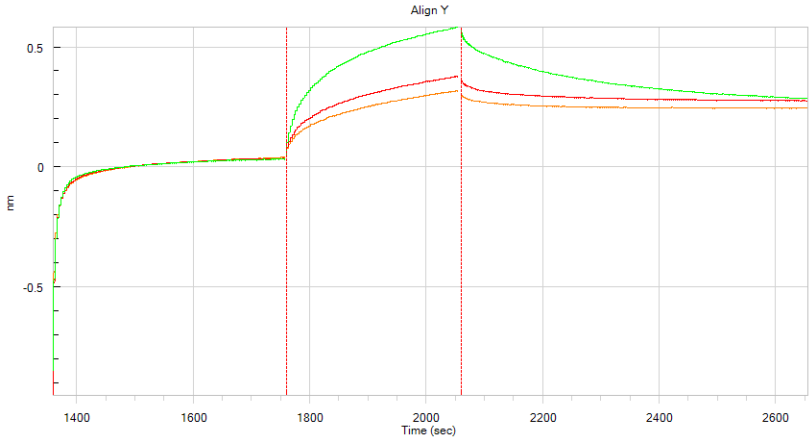
Load: liposome with biotin labeled PE

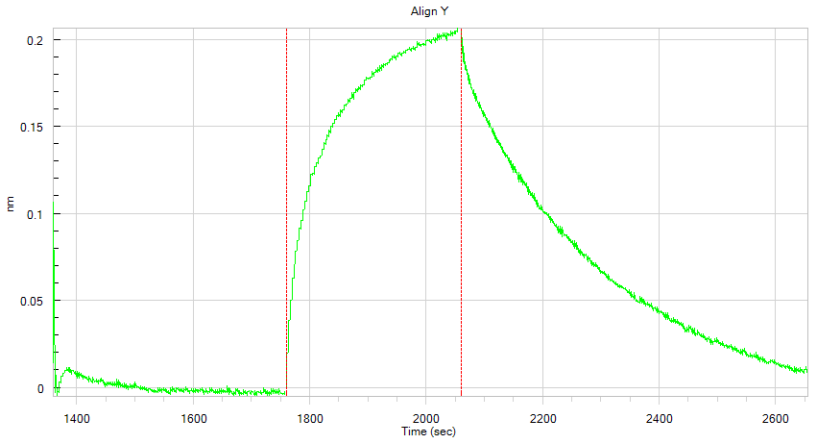
Negative control: liposome with normal PE

Well: titration of phospholipid binding protein

	1	2	3	4	Probe
A	100mM HEPES 100mM NaCl pH7.5	67%PC / 30%PS / 3% Biotin-PE	Skim Milk	2uM Protein in skim milk	SA
B	..	67%PC / 30%PS / 3%PE	..	..	..
C	..	100mM HEPES 100mM NaCl pH7.5	..	..	..







Conclusion

Octet RED 96 system can be used for:

Detection of Ab production and rapid identification of Ag binding at early stage
Alternatives: Anti-Mouse IgG quantification and anti-mouse Fc capture probes

Determine protein-protein and protein-carbohydrate binding properties

Possible to characterize protein-lipid interaction
Alternatives: Aminopropylsilane probes

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