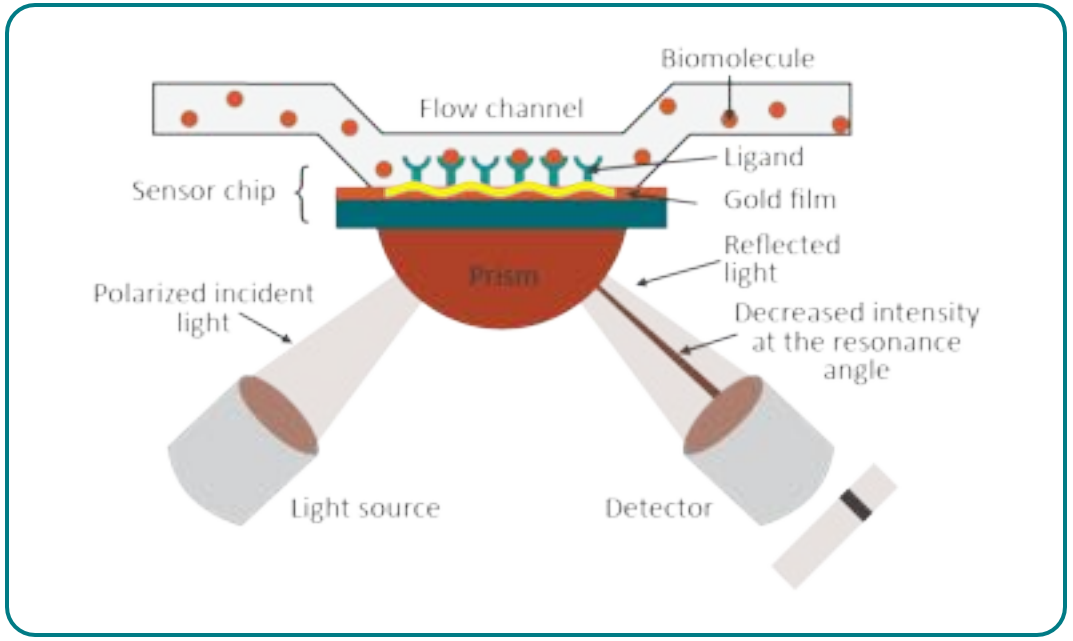
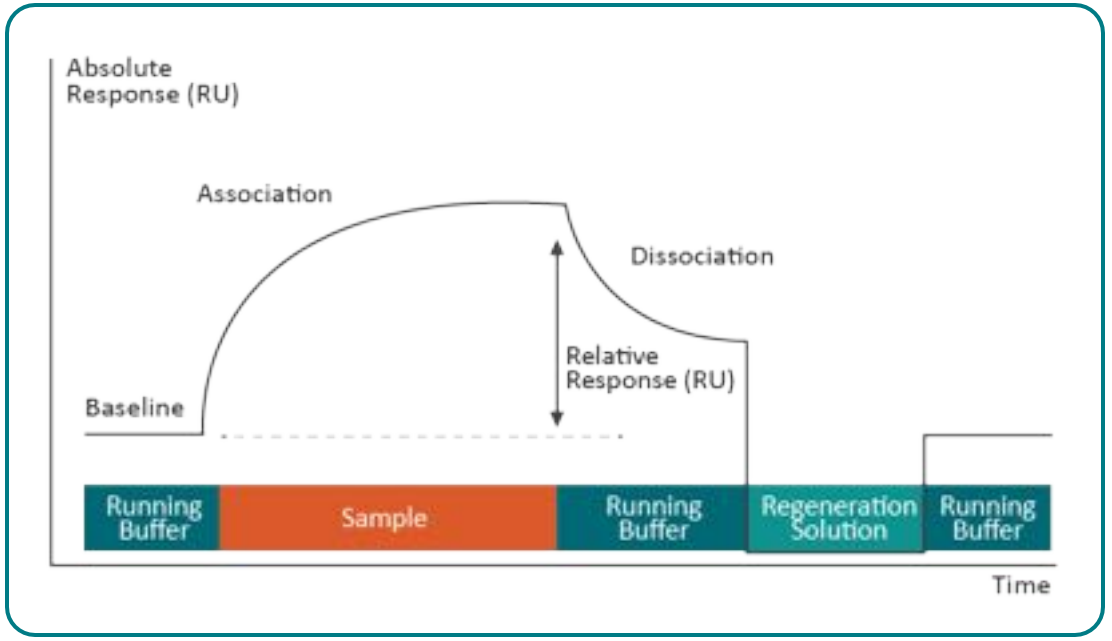
Application of SPR technology for assessment of immunogenicity of a dual-peptide cancer vaccine

Daniel Worms | 26th February 2025

BioAgilytix

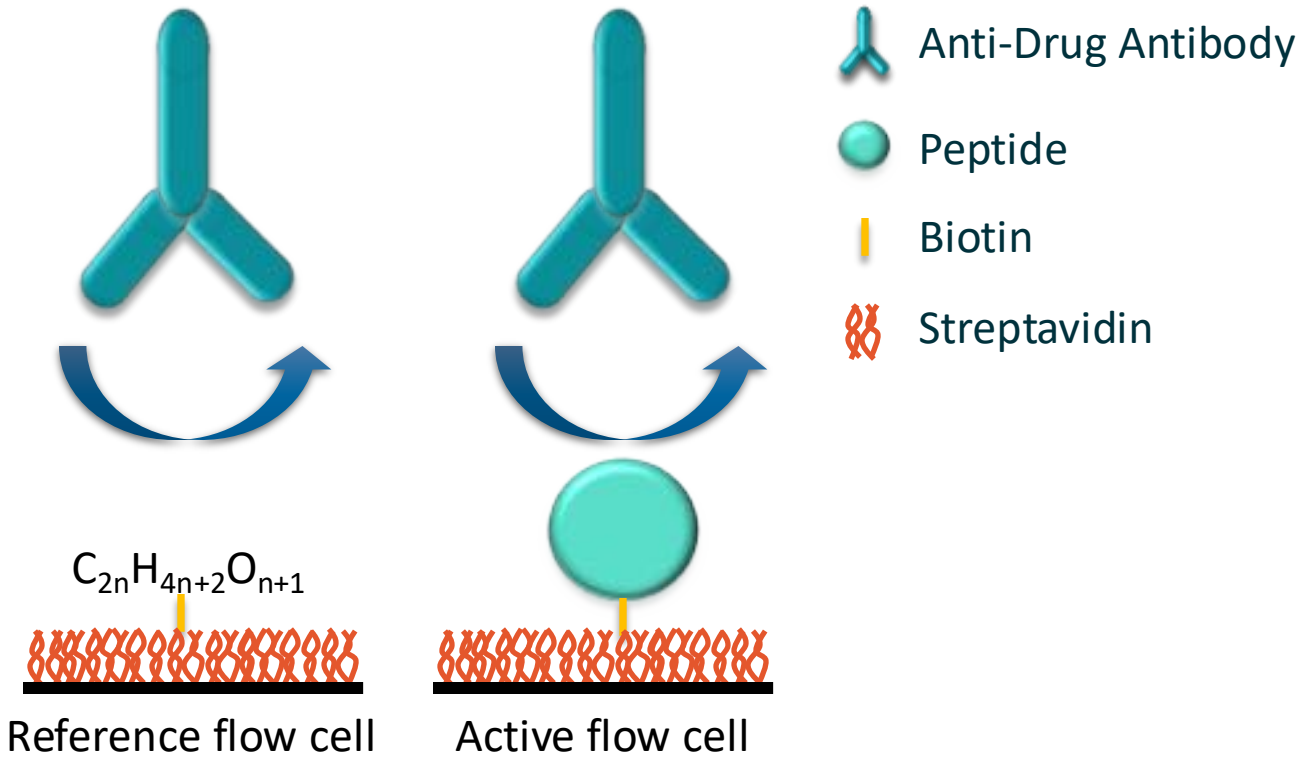
Surface Plasmon Resonance





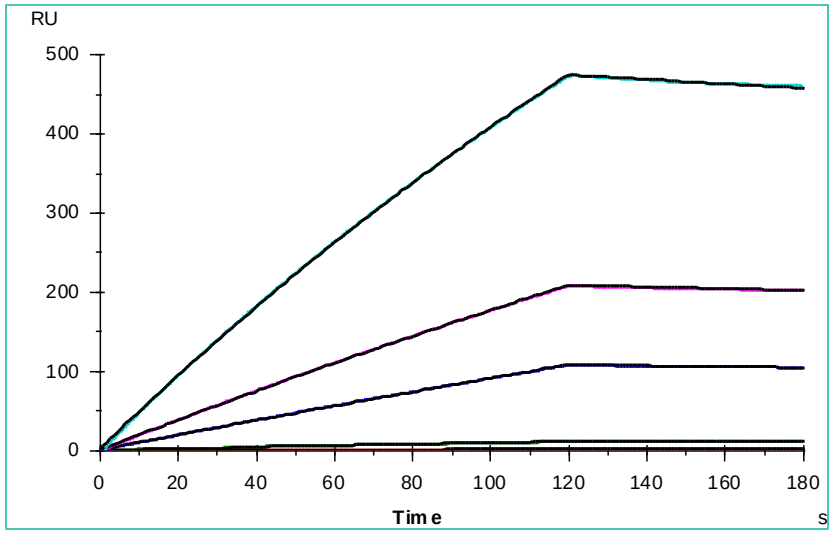
Label-free detection of molecular interactions in real-time

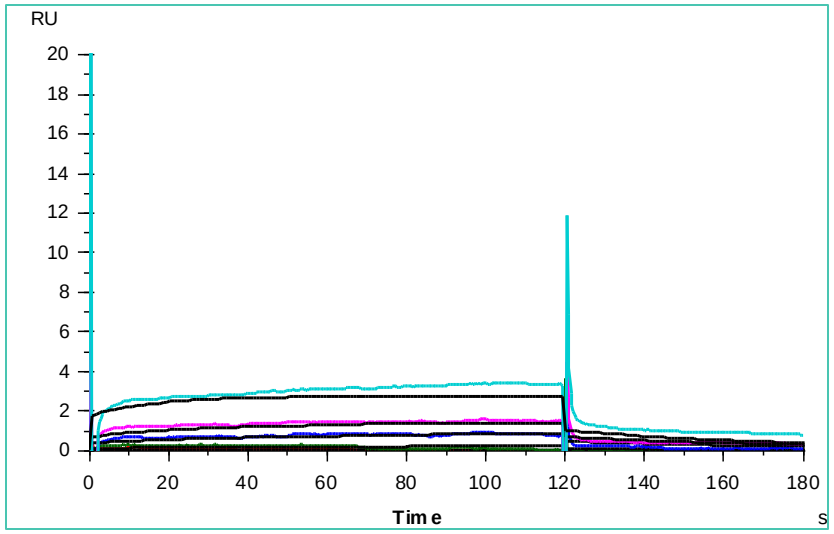
Assay Setup



- Drug: two peptides with different properties
- Four flow cells, ADAs against each of the peptides can be detected in parallel with one sample (multiplex)
- Duplicate sample injections for replicate measurements
- Same setup for ADA assay and kinetic characterization of SPCs
- Orthogonal assay setup avoids avidity effects of the antibody and was used for verification of kinetic results

Kinetic Analysis





- Almost all SPCs showed medium to high affinity (K_D < 50 nM)
- Antibodies produced in rabbit have generally higher affinity than antibodies from rat
- Highly specific, no cross-reactivity observed
- Affinity can be used as a starting point for SPC selection, but does not precisely predict assay performance

Development on MSD platform

- Sulfo-tag conjugated peptides generated at external vendor
- Site-specific biotin label incorporated via *de novo* peptide synthesis
- Parameters tested:
  - Various concentrations, ratios and isomers of labeled peptides for capture and detection (‚Checkerboard‘)
  - Various MRDs (1-fold to 20-fold)
  - Standard, High Bind, Streptavidin plates
  - All available SPCs
  - Acid dissociation for reducing matrix interference

MSD – Checkerboard

S/N at 100 ng/ml SPC

Bio-Peptide A	10.0 µg/ml	5.0 µg/ml	2.5 µg/ml	1.0 µg/ml	0.5 µg/ml	0.25 µg/ml	Sulfo-Peptide A
MSD SA	1	2	3	4	5	6	
A	1.5	2.1	1.2	1.0	1.0	1.1	2.0 µg/ml
B	1.4	1.2	1.1	0.9	0.9	1.2	1.0 µg/ml
C	1.1	1.2	1.1	1.0	1.2	0.9	0.5 µg/ml
D	1.0	1.0	1.1	1.2	1.1	1.0	0.25 µg/ml
E	0.9	1.1	0.9	0.9	1.1	1.1	0.125 µg/ml
F	1.0	1.0	1.2	1.2	0.7	0.9	0.0625 µg/ml
G	1.0	1.0	0.8	1.1	1.0	0.8	0.03125 µg/ml
H	0.8	1.0	1.0	1.0	0.9	0.9	0.0 µg/ml

+ Acid Dissociaton

Bio-Peptide A	5.0 µg/ml	2.5 µg/ml	1.0 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 µg/ml	Sulfo-Peptide A
MSD Standard	1	2	3	4	5	6	
A	2.4	1.2	1.6	2.5	1.6	1.7	2.0 µg/ml
B	2.5	2.5	2.2	1.3	0.7	2.7	1.0 µg/ml
C	2.4	1.8	1.2	1.2	1.7	1.4	0.5 µg/ml
D	1.3	1.5	2.3	1.7	2.5	1.6	0.25 µg/ml
E	1.8	1.4	1.5	1.3	1.3	3.0	0.125 µg/ml
F	1.3	1.0	2.0	2.1	0.6	1.4	0.0625 µg/ml
G	1.5	1.4	2.0	1.4	1.7	1.9	0.03125 µg/ml
H	0.8	0.9	0.9	0.8	0.9	1.0	0.0 µg/ml

Bio-Peptide B	10.0 µg/ml	5.0 µg/ml	2.5 µg/ml	1.0 µg/ml	0.5 µg/ml	0.25 µg/ml	Sulfo-Peptide B
MSD SA	1	2	3	4	5	6	
A	0.9	1.3	1.0	1.0	1.1	1.2	2.0 µg/ml
B	0.9	0.9	0.8	1.0	1.0	1.1	1.0 µg/ml
C	0.8	1.0	0.9	1.1	0.9	1.0	0.5 µg/ml
D	0.8	0.8	0.9	0.9	1.1	1.1	0.25 µg/ml
E	0.9	1.0	0.8	1.0	1.0	1.1	0.125 µg/ml
F	0.9	1.0	0.9	0.9	0.9	1.2	0.0625 µg/ml
G	0.9	0.9	0.9	0.9	0.9	1.0	0.03125 µg/ml
H	0.9	1.2	1.1	0.8	0.9	1.0	0.0 µg/ml

Bio-Peptide B	5.0 µg/ml	2.5 µg/ml	1.0 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 µg/ml	Sulfo-Peptide B
MSD Standard	1	2	3	4	5	6	
A	1.0	1.4	1.2	0.9	0.9	0.9	2.0 µg/ml
B	1.4	1.3	1.2	1.0	1.1	1.1	1.0 µg/ml
C	1.6	1.4	1.2	1.1	1.0	1.0	0.5 µg/ml
D	1.4	1.5	1.6	1.3	1.4	1.1	0.25 µg/ml
E	1.1	1.6	1.5	1.4	1.1	0.9	0.125 µg/ml
F	1.3	1.1	1.4	1.4	1.0	1.1	0.0625 µg/ml
G	1.0	1.1	1.2	1.2	1.1	1.2	0.03125 µg/ml
H	0.9	1.0	1.0	1.0	0.9	1.1	0.0 µg/ml

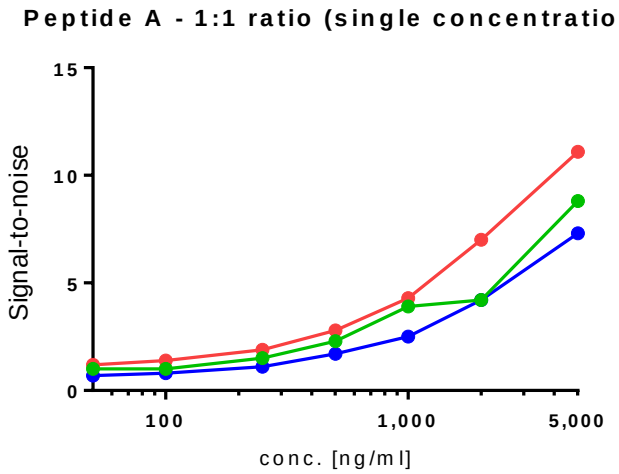
→High background signal

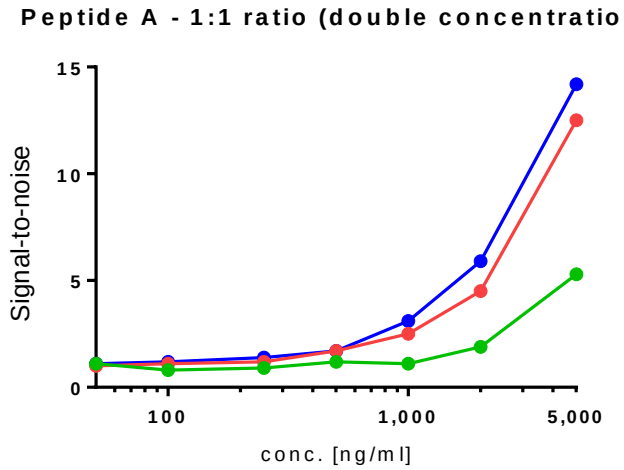
→Poor signal-to-noise ratio

→Low sensitivity

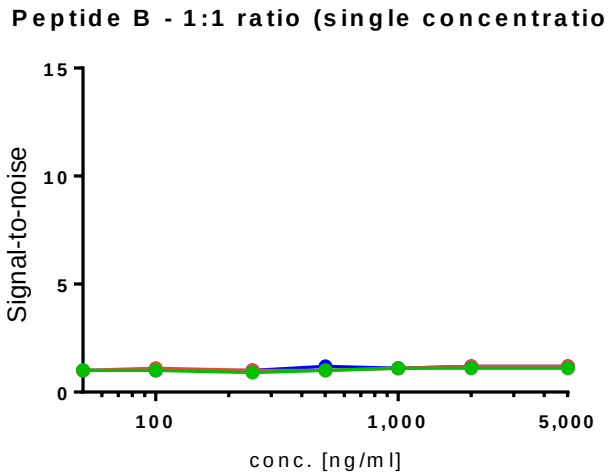
→Very little improvement through acid dissociation

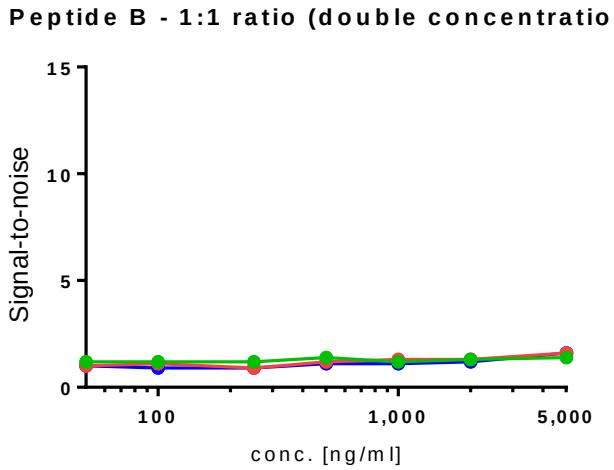
MSD – Checkerboard + Sensitivity Curve





MRD 5
MRD 2
MRD 1





→High background signal
→Poor signal-to-noise ratio
→Low sensitivity

Development on Biacore platform

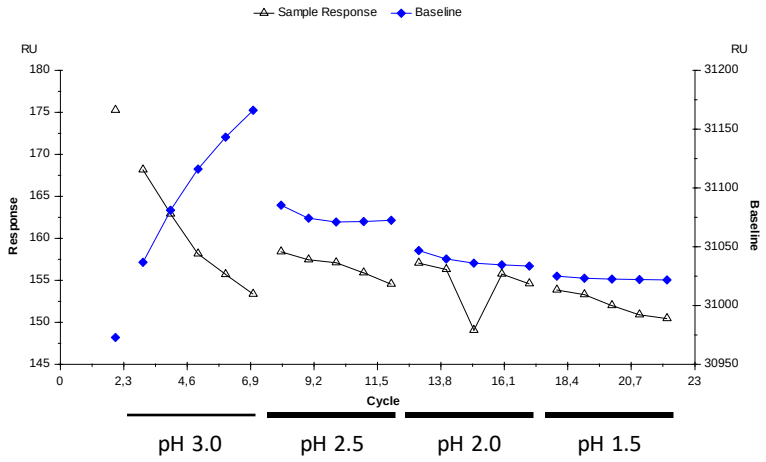
- ~~Sulfo-tag conjugated peptides generated at external vendor~~
- Site-specific biotin label incorporated via *de novo* peptide synthesis
- Parameters tested:
  - Peptide immobilization level
  - Various MRDs (2-fold to 20-fold)
  - Extensive regeneration scouting & surface stability testing
  - SPEAD pretreatment (reducing matrix interference)
  - Heat inactivation (reducing matrix interference)
- Strategy for cut point assessment

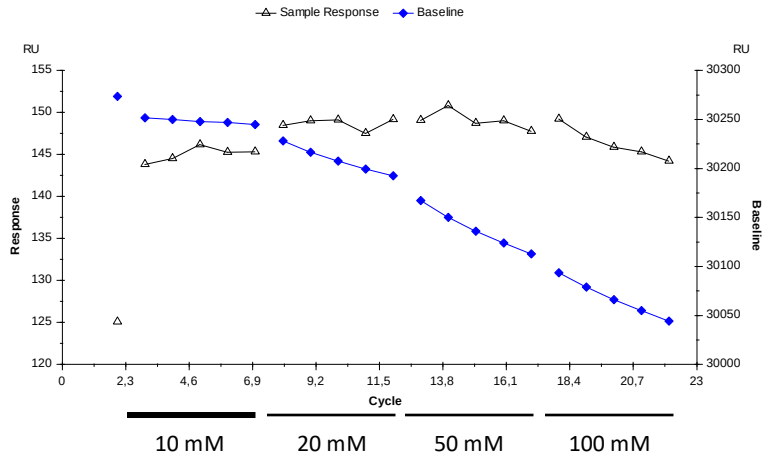
Biacore – Regeneration Scouting

Glycine-HCl

NaOH

A





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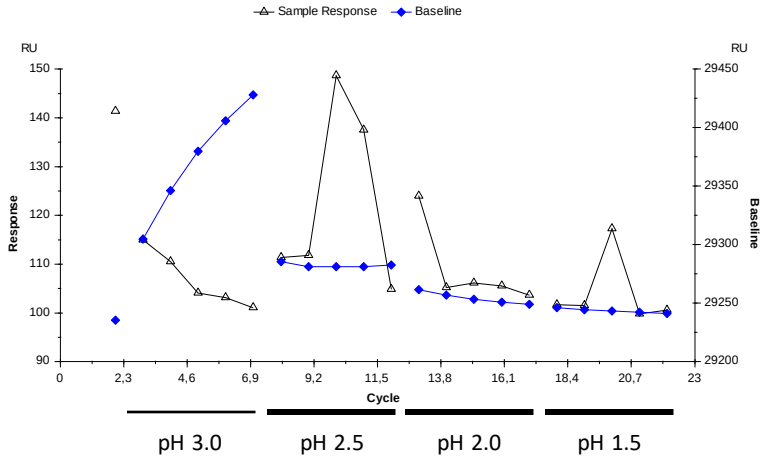
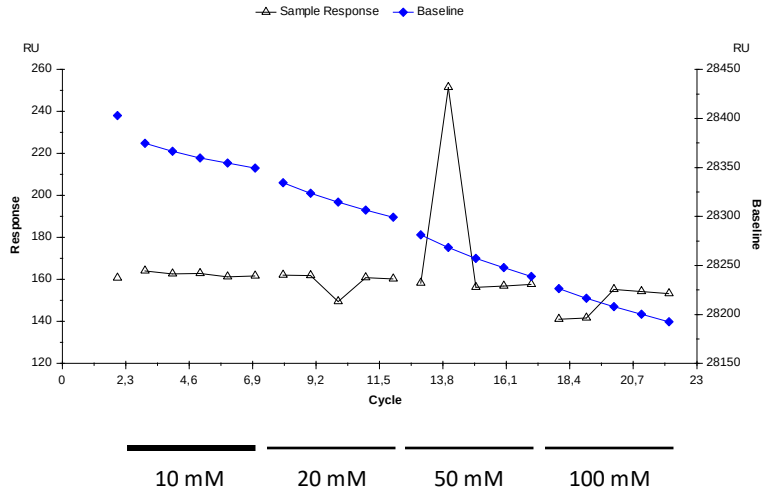
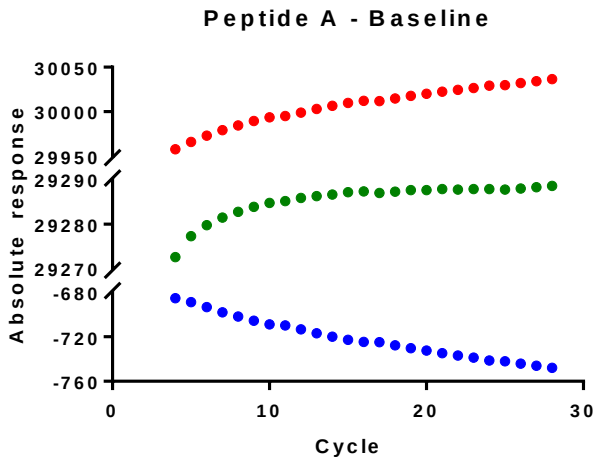


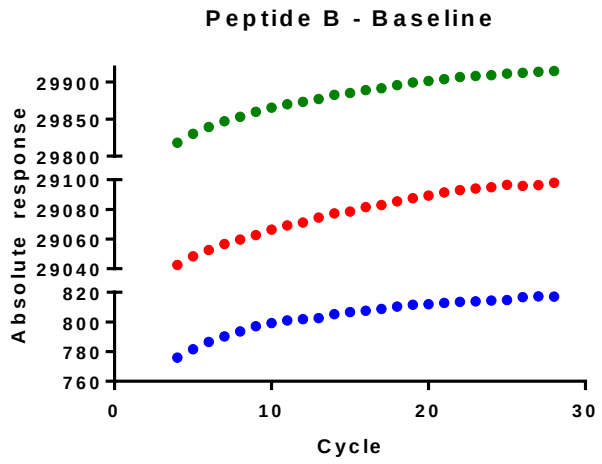


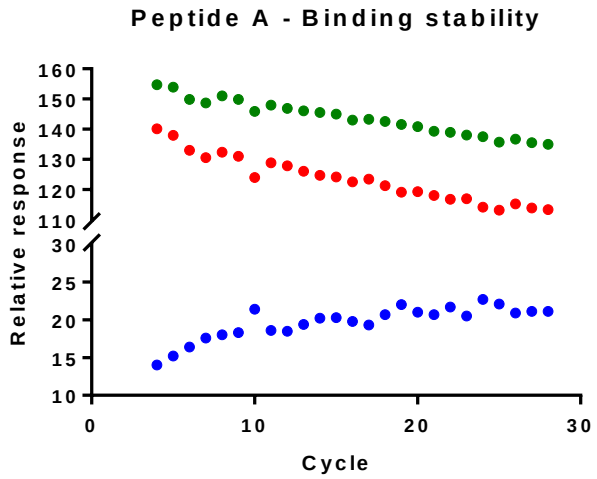
Parameters tested

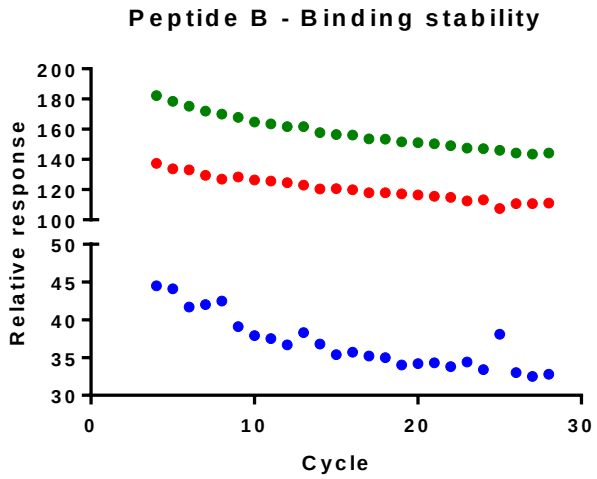
- NaCl, SDS, $MgCl_2$, DMSO
- Injection duration (10-60 sec)
- Multiple injections
- Various combinations

Biacore – Surface Stability









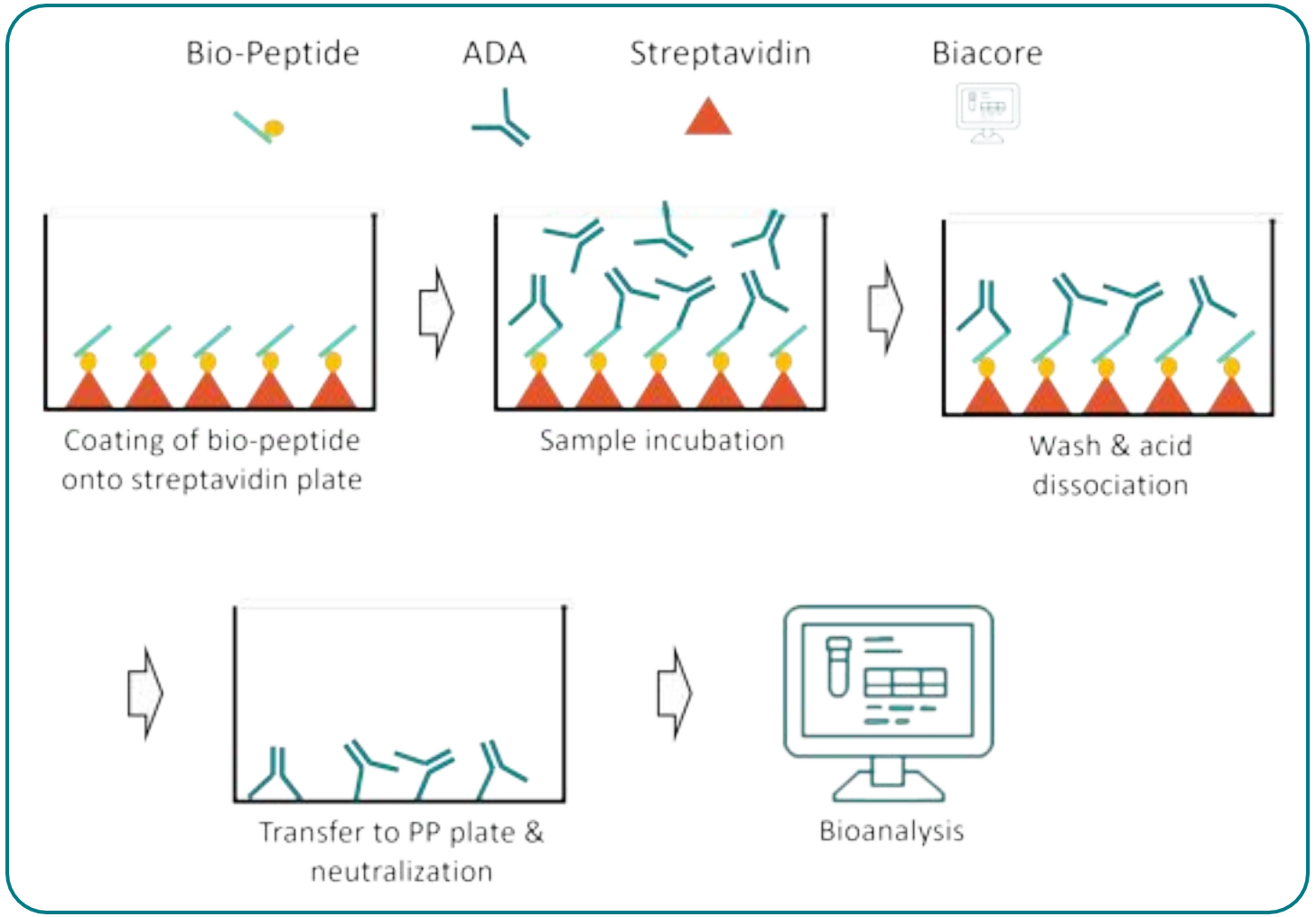
- Fc1 or Fc3 (reference)
- Fc2 or Fc4 (active)
- Fc2-1 or Fc4-3 (ref. subtracted)

Peptide A – Baseline
Ø increase per cycle: 3.1 (Fc1) vs. 0.6 (Fc2)

Peptide B – Baseline
Ø increase per cycle: 2.2 (Fc3) vs. 3.9 (Fc4)

→ Incomplete surface regeneration

Biacore – Solid Phase Extraction and Acid Dissociation

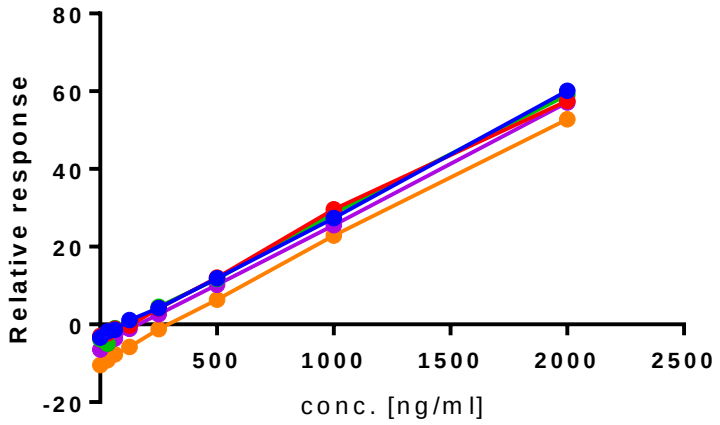


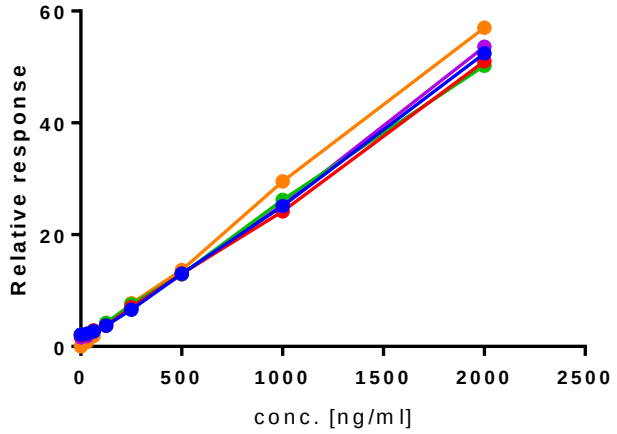
- Extract ADAs from matrix to remove potentially interfering components
- Use of established regeneration protocol from Kinetics/Affinity
- Single extraction: both peptides coated onto one plate

Biacore – Heat Inactivation

– SPEAD **+ SPEAD**

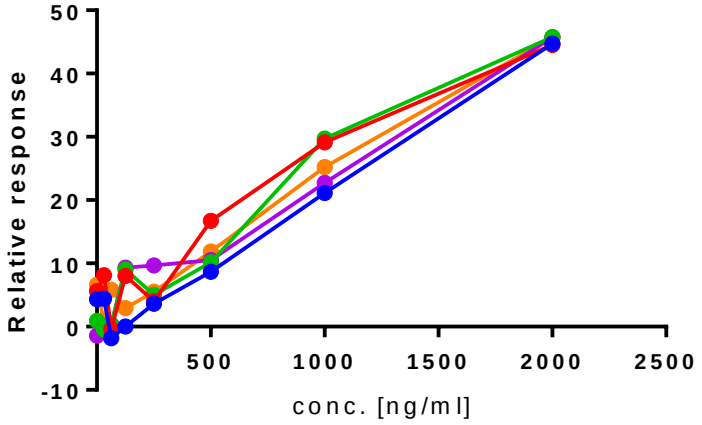
A

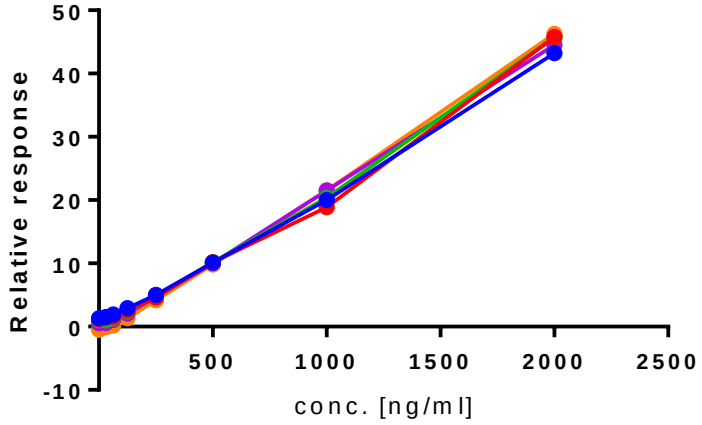




- 90 min
- 75 min
- 60 min
- 45 min
- 30 min

B





- Heat inactivation improved surface regeneration (optimum at 60 °C)
- No difference between 30 and 90 min
- Subsequent SPEAD reduces variability

Biacore – Heat Inactivation & SPEAD

		Reference Fc	Active Fc
Peptide A	Standard	3.3 RU/cycle	0.6 RU/cycle
	+ SPEAD	3.3 RU/cycle	1.3 RU/cycle
	+ Heat & SPEAD	**0.4 RU/cycle**	**1.0 RU/cycle**
Peptide B	Standard	2.2 RU/cycle	3.9 RU/cycle
	+ SPEAD	1.1 RU/cycle	1.1 RU/cycle
	+ Heat & SPEAD	**0.5 RU/cycle**	**0.7 RU/cycle**

Combination of heat inactivation and SPEAD pretreatment reduced variability and improved surface regeneration performance.

Biacore - Selectivity

Sample ID		Conc. SPC [ng/ml]	Mean RelResp	SD	%CV	delta RU
Peptide A	1	0.0	**-58.8**	0.5	-0.8	0.0
		500.0	-31.7	0.1	-0.4	**27.1**
	2	0.0	**-31.9**	0.1	-0.2	0.0
		500.0	-2.5	0.2	-8.7	**29.4**
	3	0.0	**-6.4**	0.1	-1.1	0.0
		500.0	18.2	0.2	1.2	**24.5**
Peptide B	1	0.0	**-46.4**	0.3	-0.6	0.0
		500.0	-29.7	0.1	-0.2	**16.8**
	2	0.0	**-13.8**	0.0	0.0	0.0
		500.0	1.5	0.1	4.9	**15.3**
	3	0.0	**11.7**	0.1	0.6	0.0
		500.0	24.9	0.1	0.6	**13.3**

- Variable baseline signal between individuals
- Negative response values cannot be log-transformed for cut point analysis
- Relative distance of SPC-spiked and unspiked signal $(delta\ RU)$ is less variable between individuals
- This data can be used to set a cut point at a specific SPC concentration

Biacore – Cut Point Assessment

Cut point assessment from unspiked and SPC-spiked samples using $delta\ RU$

- Screening & Titration Cut Point: $\Delta\ RU = mean\ RelResp_{SPC-spiked} - mean\ RelResp_{SPC-unspiked}$
- Confirmatory Cut Point: $Inhibition\ Ratio = \frac{\Delta\ RU_{drug-unspiked}}{\Delta\ RU_{drug-spiked}}$
- Lower quantile of the spiked positive population for setting fixed cut points
- Preliminary sensitivity: 250 ng/ml (Peptide A) and 500 ng/ml (Peptide B)
- Preliminary cut point assessment confirmed appropriateness
- Sample analysis: pre-dose and all post-dose samples of a patient in one run to calculate $delta\ RU$

Conclusions

- We developed a multiplex assay for the detection of ADAs against each of the peptides with one sample injection.
- A combination of heat inactivation and SPEAD reduced variability and improved surface performance.
- The workflow included a simplified single SPEAD extraction step with both peptides reducing handling time and errors.
- The signal difference between SPC-spiked (post-dose) and unspiked (pre-dose) sample can be employed for cut point setting.