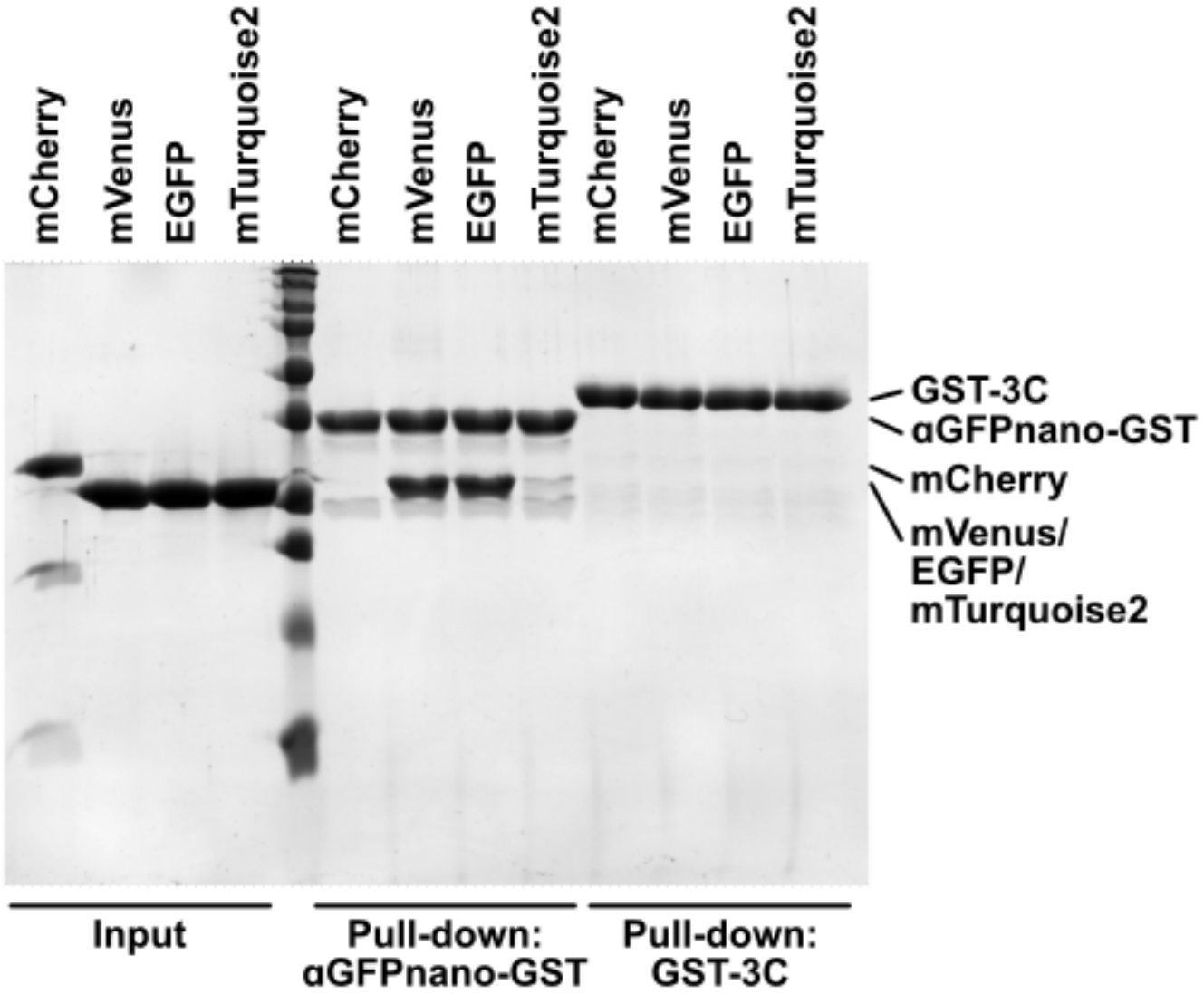
UNIVERSITY OF CAMBRIDGE

Biochemistry and SPR workshop

Wednesday 29th March

GST pull-down



SPR – CM5 chemistry

- Store at 4°C – allow to equilibrate at room temperature for ~30 min before opening to prevent condensation

Ligand capture

- Using a CM5 surface and EDC/NHS chemistry
- Between 10 to 50 µg/mL is a good starting [ligand]
- Did the following dilutions:

Protein	[Stock] mg/mL	Dilution
aGFPnano-GST	13.5	1 uL per mL for 13.5 ug/mL
GST	8.2	1.5 uL per mL for 12.3 ug/mL

- Diluted the proteins in 10 mM Sodium Acetate pH 5.0 for the ligand capture

Ligand pre-concentration

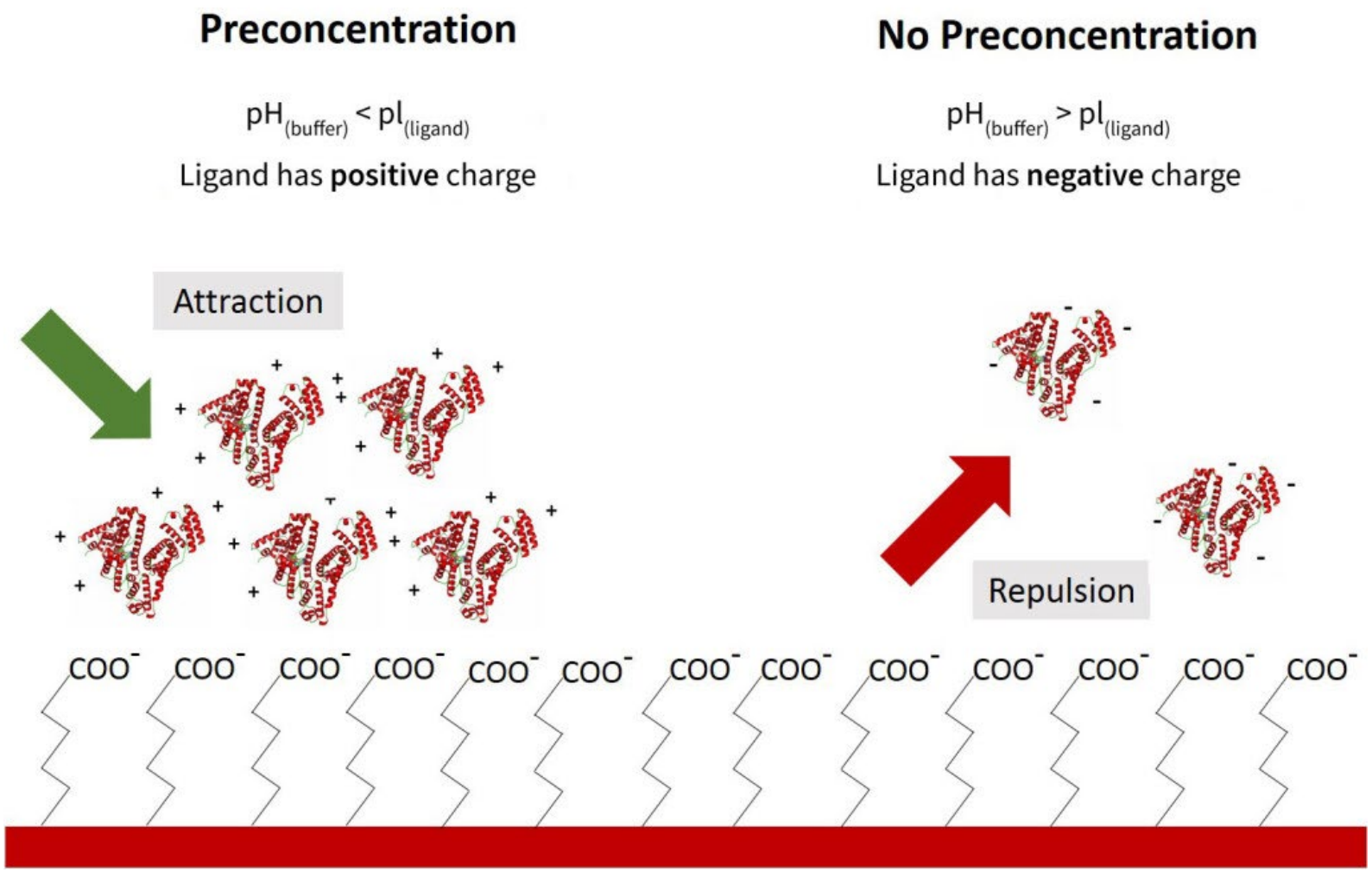


Image: https://nicoyalife.com/blog/spr-tips/save-protein-samples-preconcentration-surface-plasmon-resonance/

SPR: Flow cell set-up

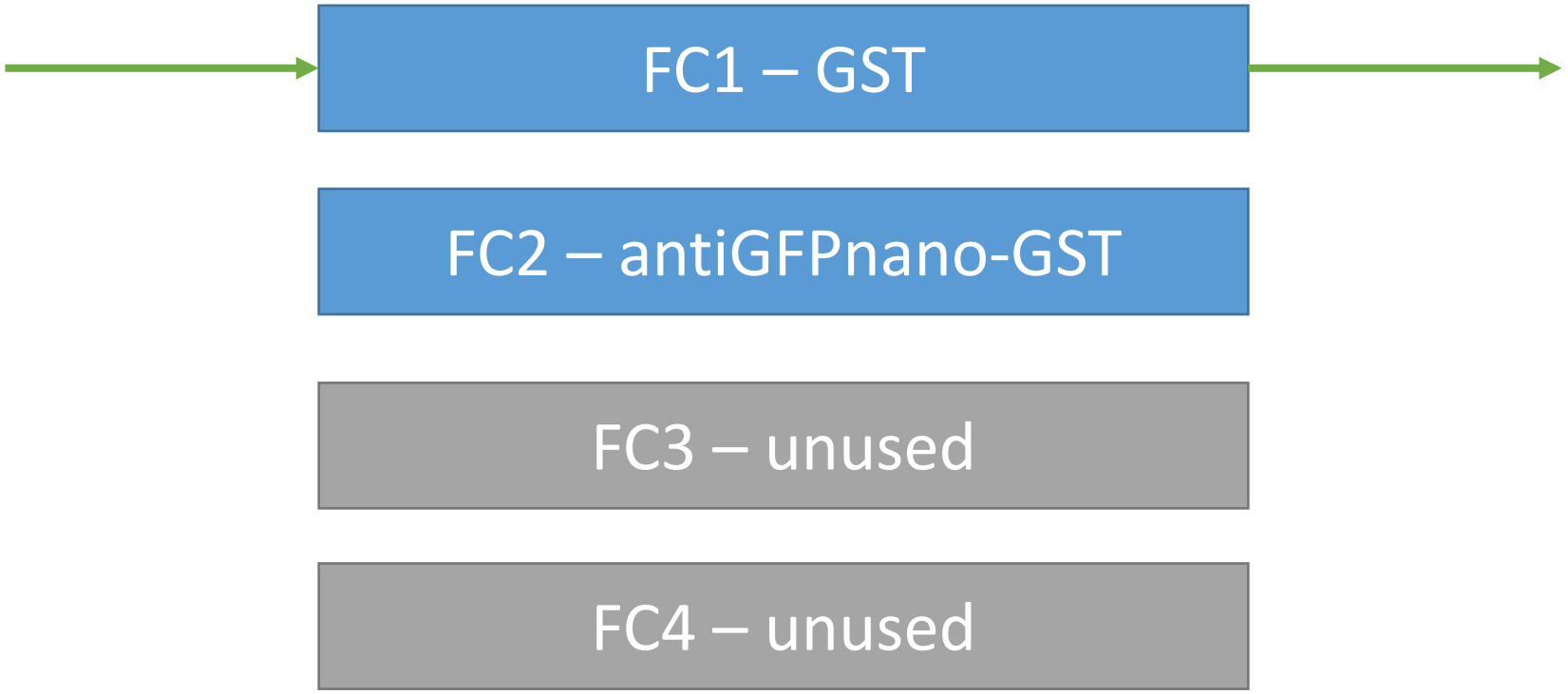
FC1 – GST

FC2 – antiGFPnano-GST

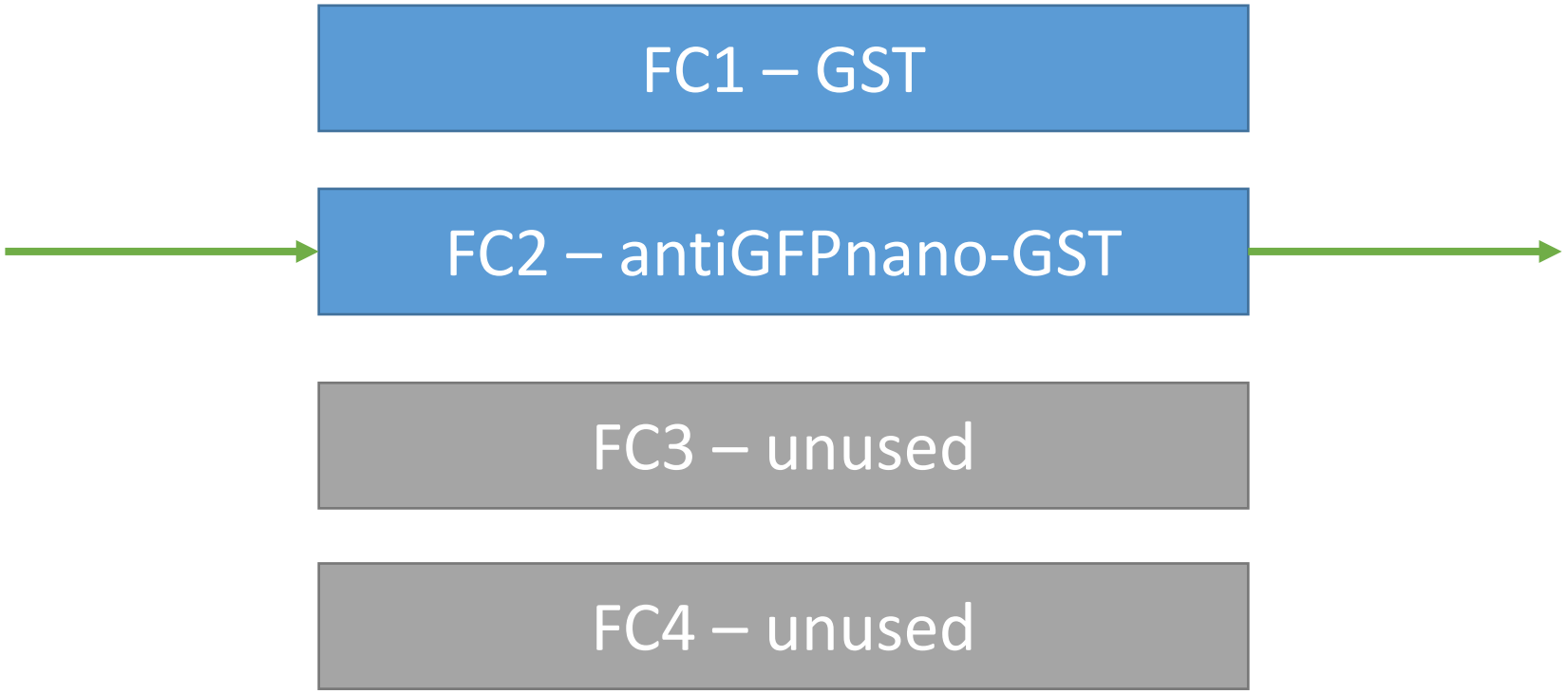
FC3 – unused

FC4 – unused

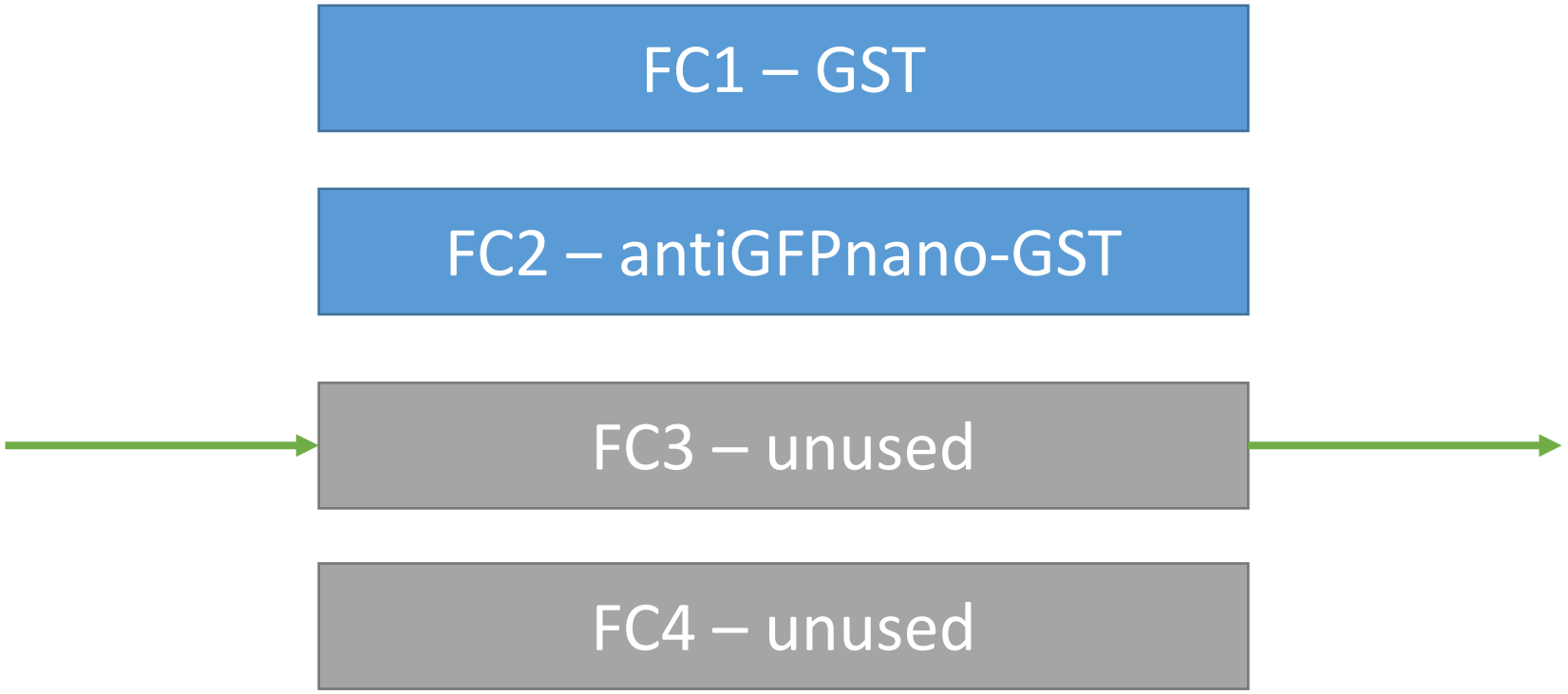
SPR: Flow paths



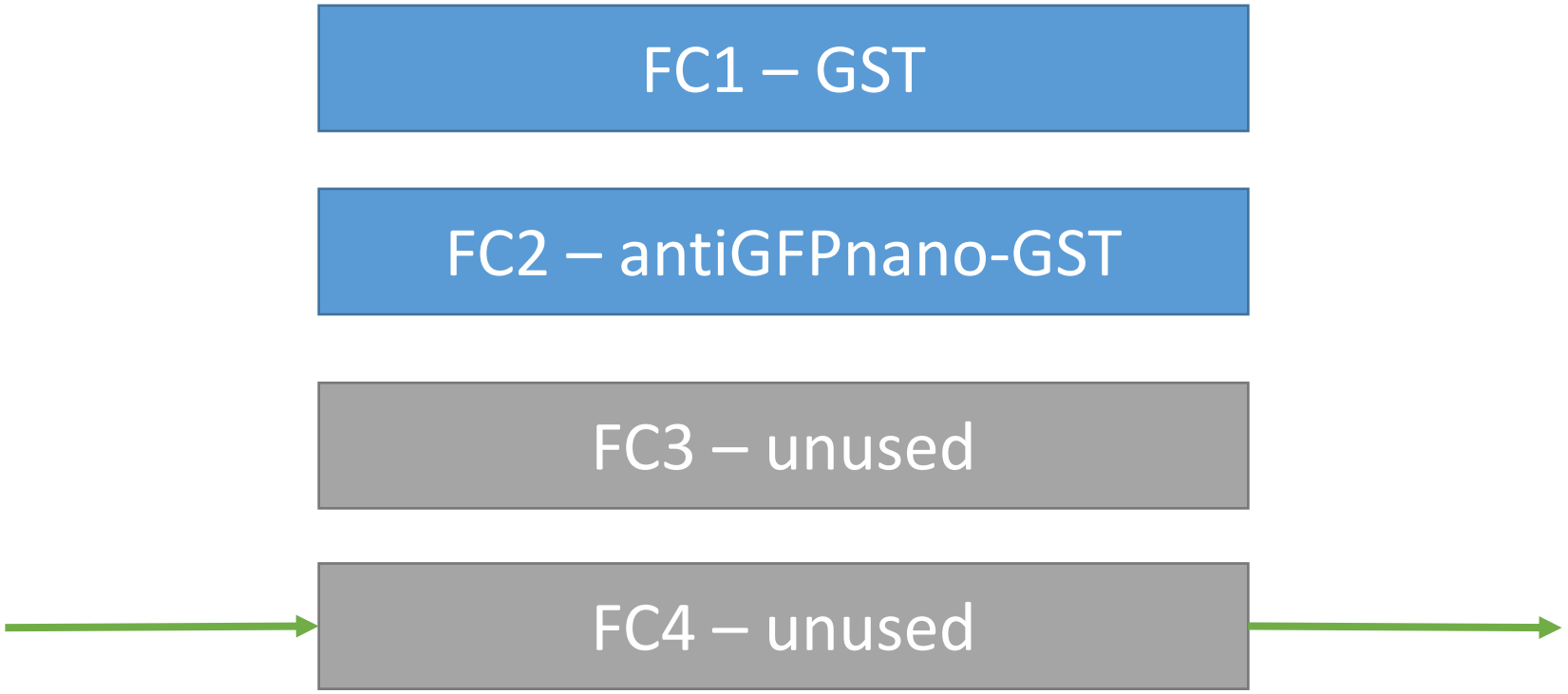
SPR: Flow paths



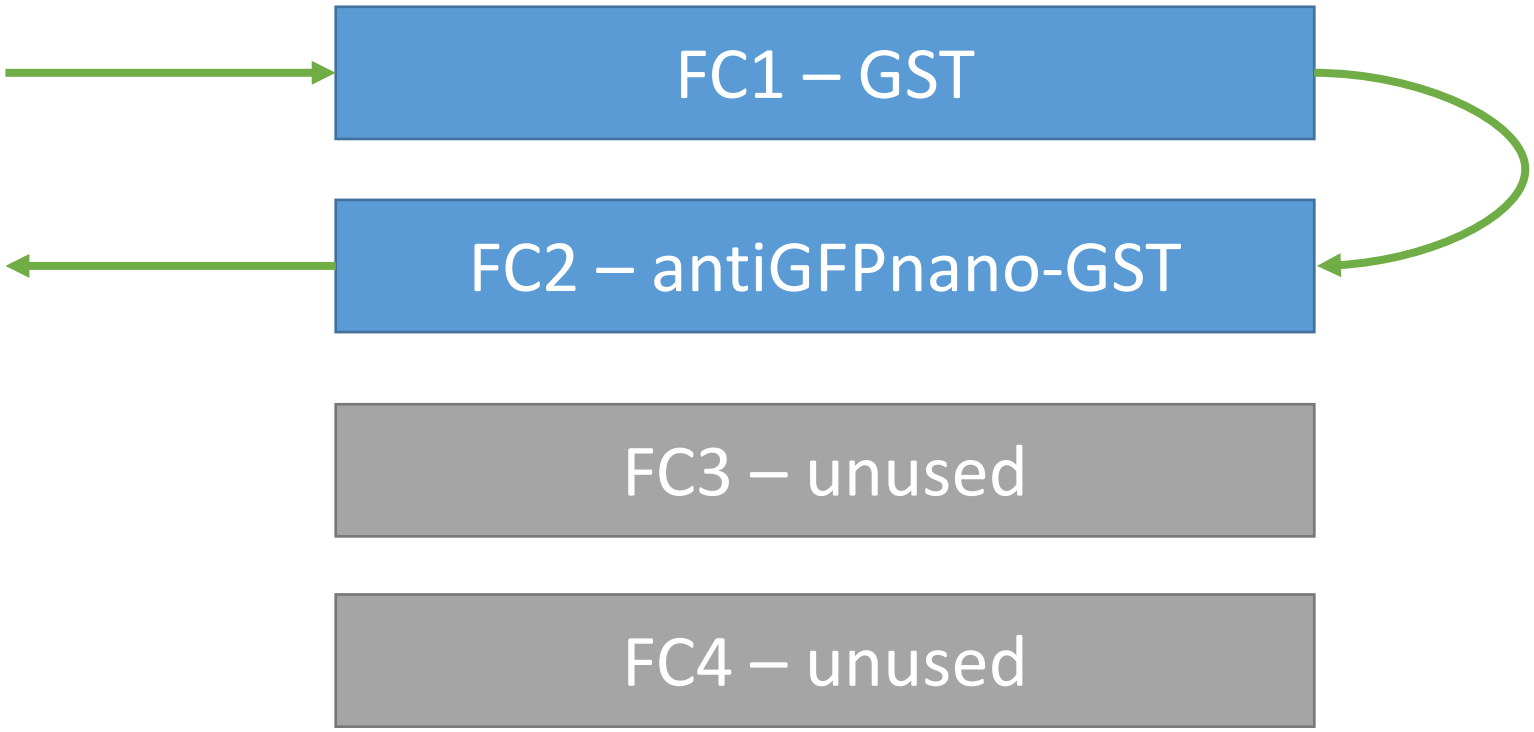
SPR: Flow paths



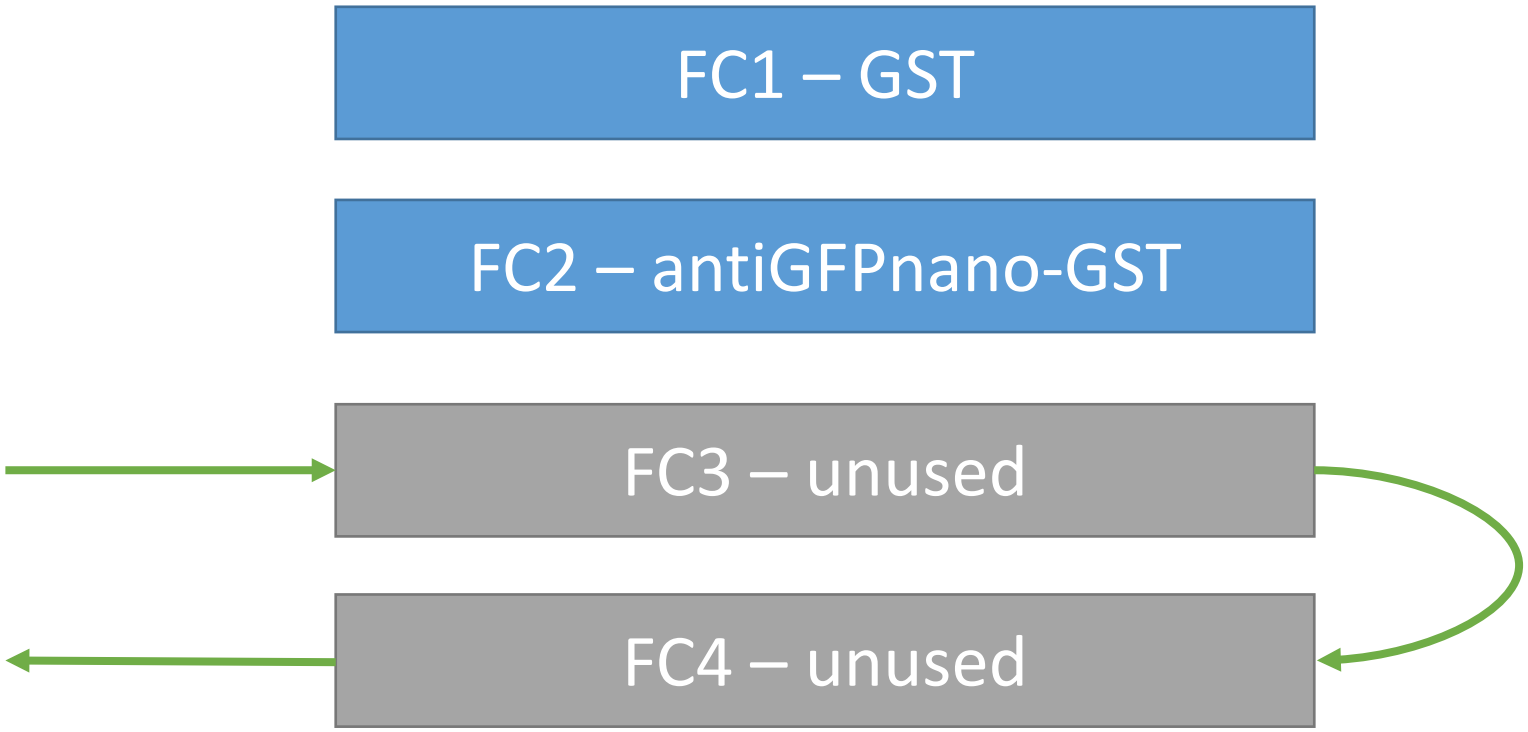
SPR: Flow paths



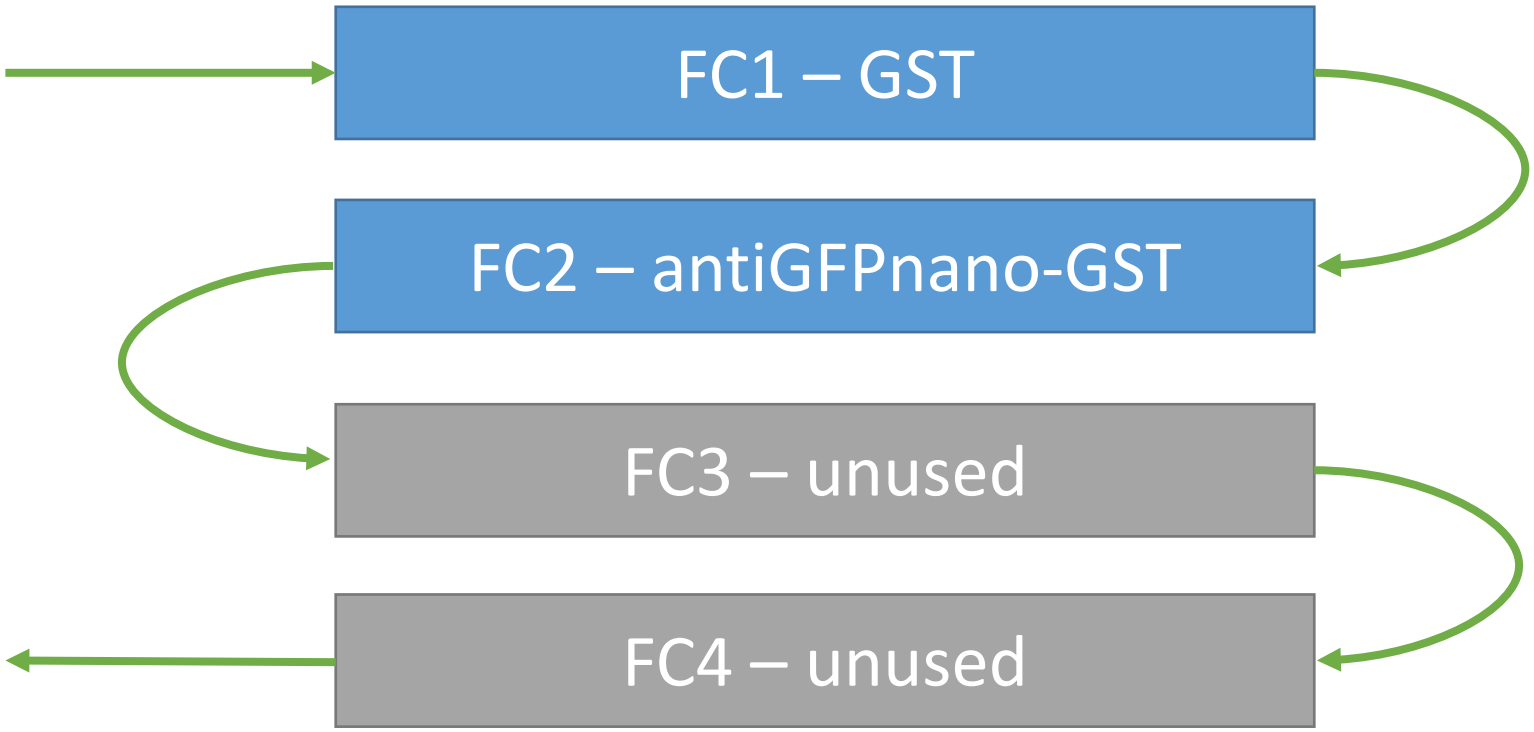
SPR: Flow paths



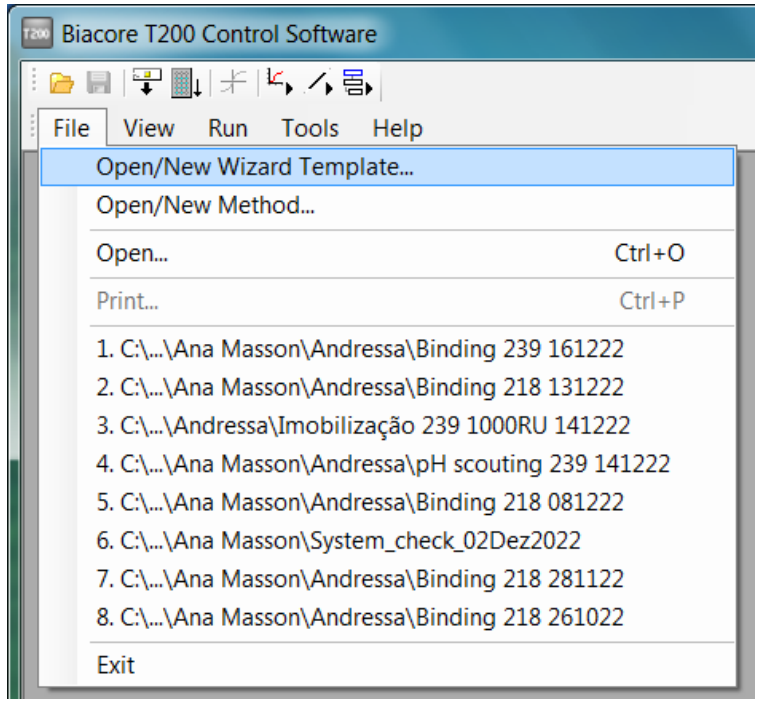
SPR: Flow paths

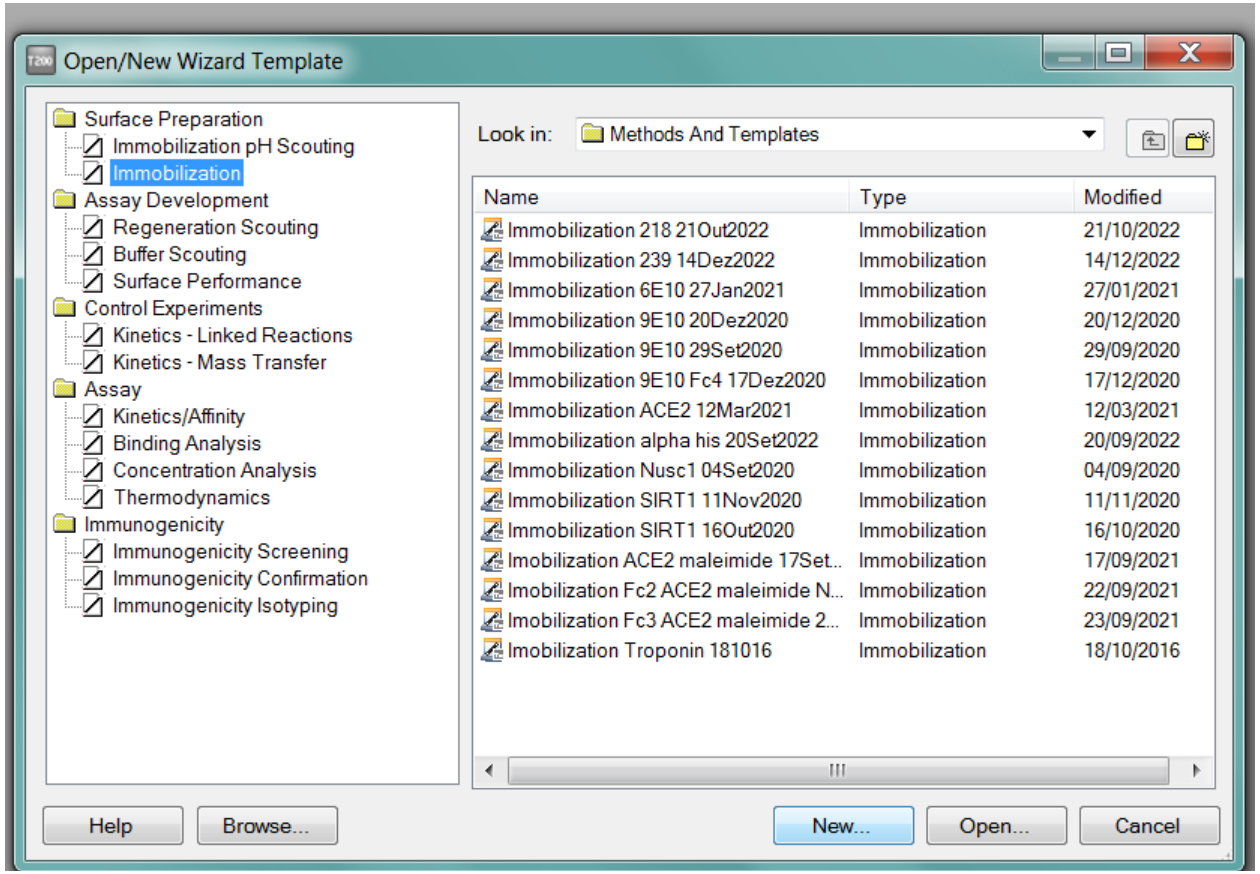


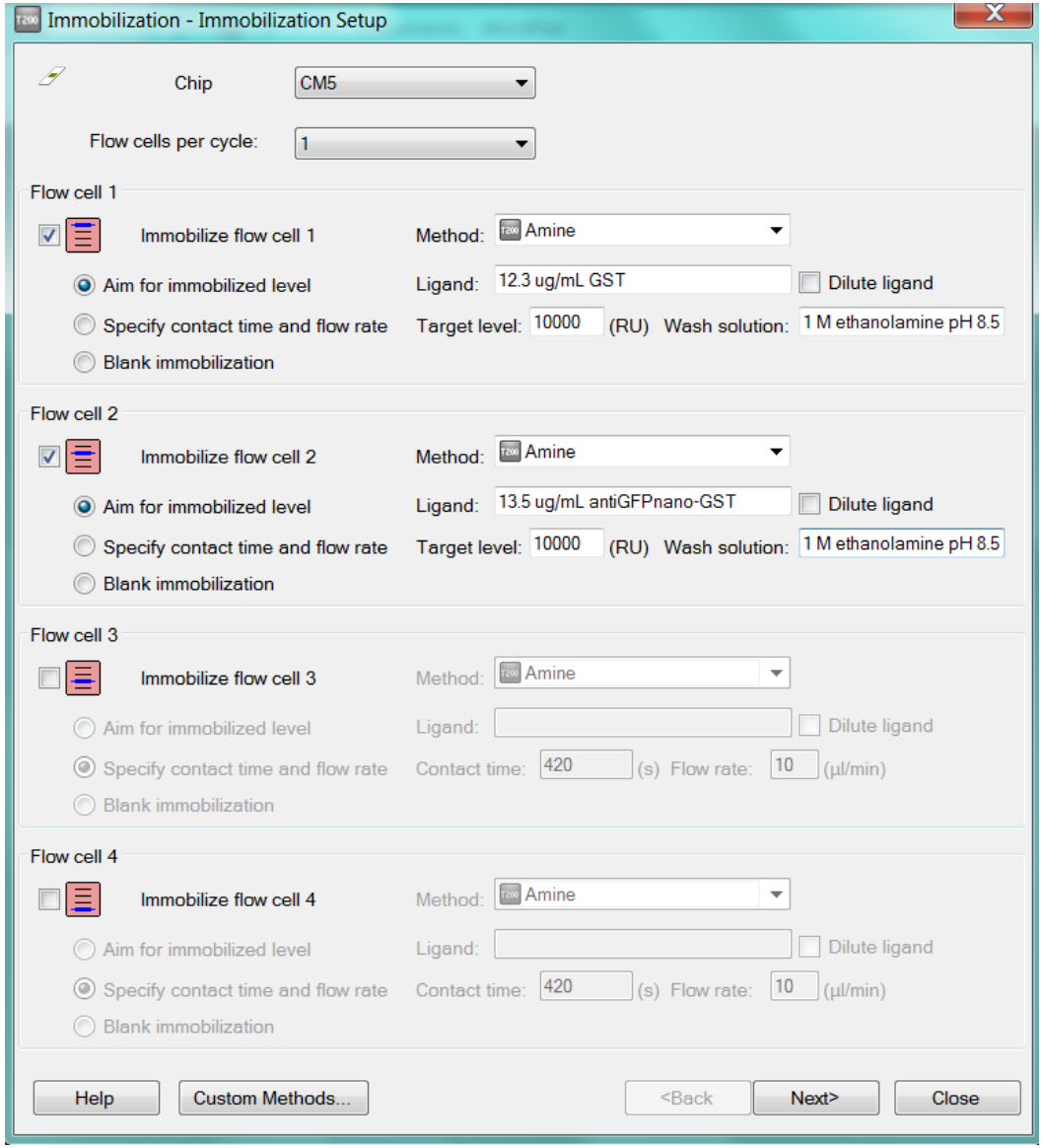
SPR: Flow paths

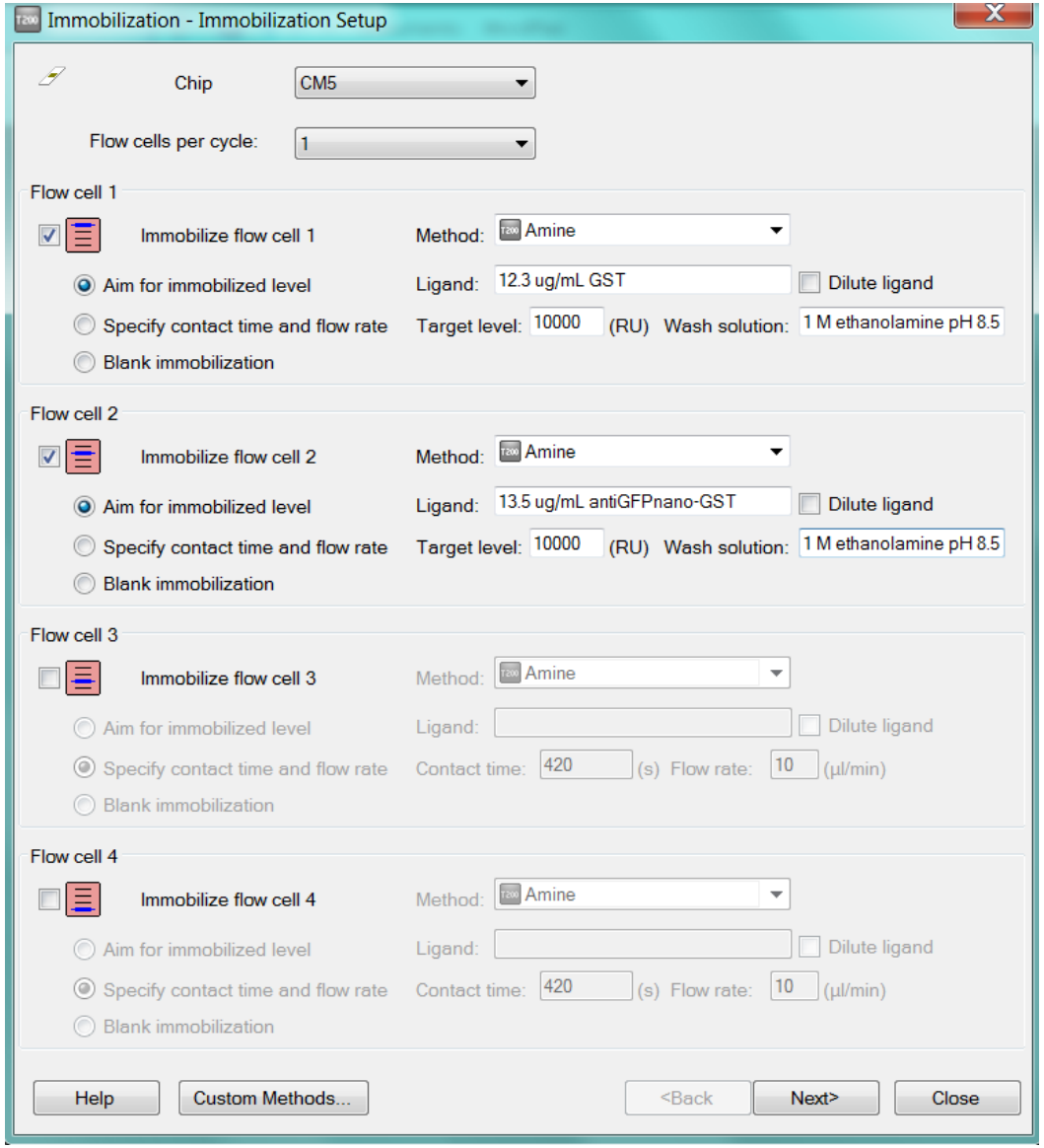


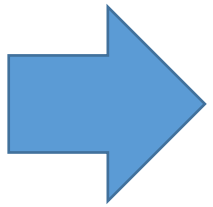
Ligand capture – setting up the experiment

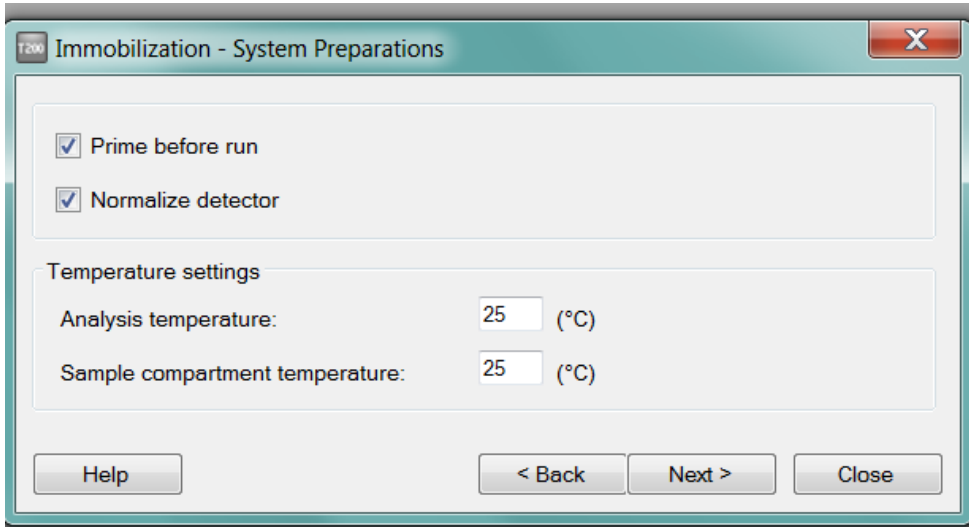




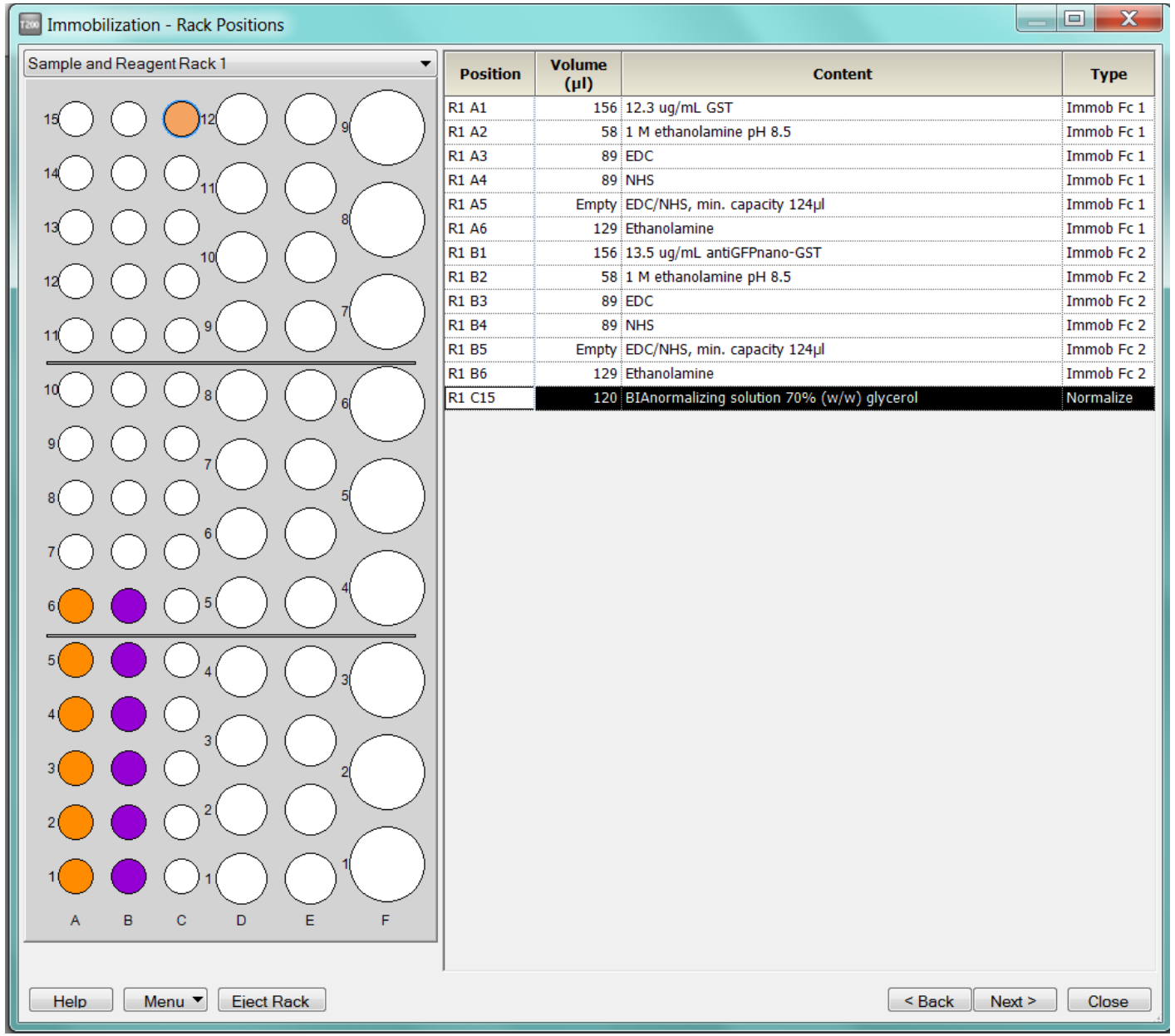

Immobilization - Immobilization Setup
Chip
CM5
Flow cells per cycle:
1
Flow cell 1
Immobilize flow cell 1
Method:
Amine
Aim for immobilized level
Ligand:
12.3 ug/mL GST
Dilute ligand
Specify contact time and flow rate
Target level:
10000
(RU)
Wash solution:
1 M ethanolamine pH 8.5
Blank immobilization
Flow cell 2
Immobilize flow cell 2
Method:
Amine
Aim for immobilized level
Ligand:
13.5 ug/mL antiGFPnano-GST
Dilute ligand
Specify contact time and flow rate
Target level:
10000
(RU)
Wash solution:
1 M ethanolamine pH 8.5
Blank immobilization
Flow cell 3
Immobilize flow cell 3
Method:
Amine
Aim for immobilized level
Ligand:
Dilute ligand
Specify contact time and flow rate
Contact time:
420
(s)
Flow rate:
10
(µl/min)
Blank immobilization
Flow cell 4
Immobilize flow cell 4
Method:
Amine
Aim for immobilized level
Ligand:
Dilute ligand
Specify contact time and flow rate
Contact time:
420
(s)
Flow rate:
10
(µl/min)
Blank immobilization
Help
Custom Methods...
<Back
Next>
Close



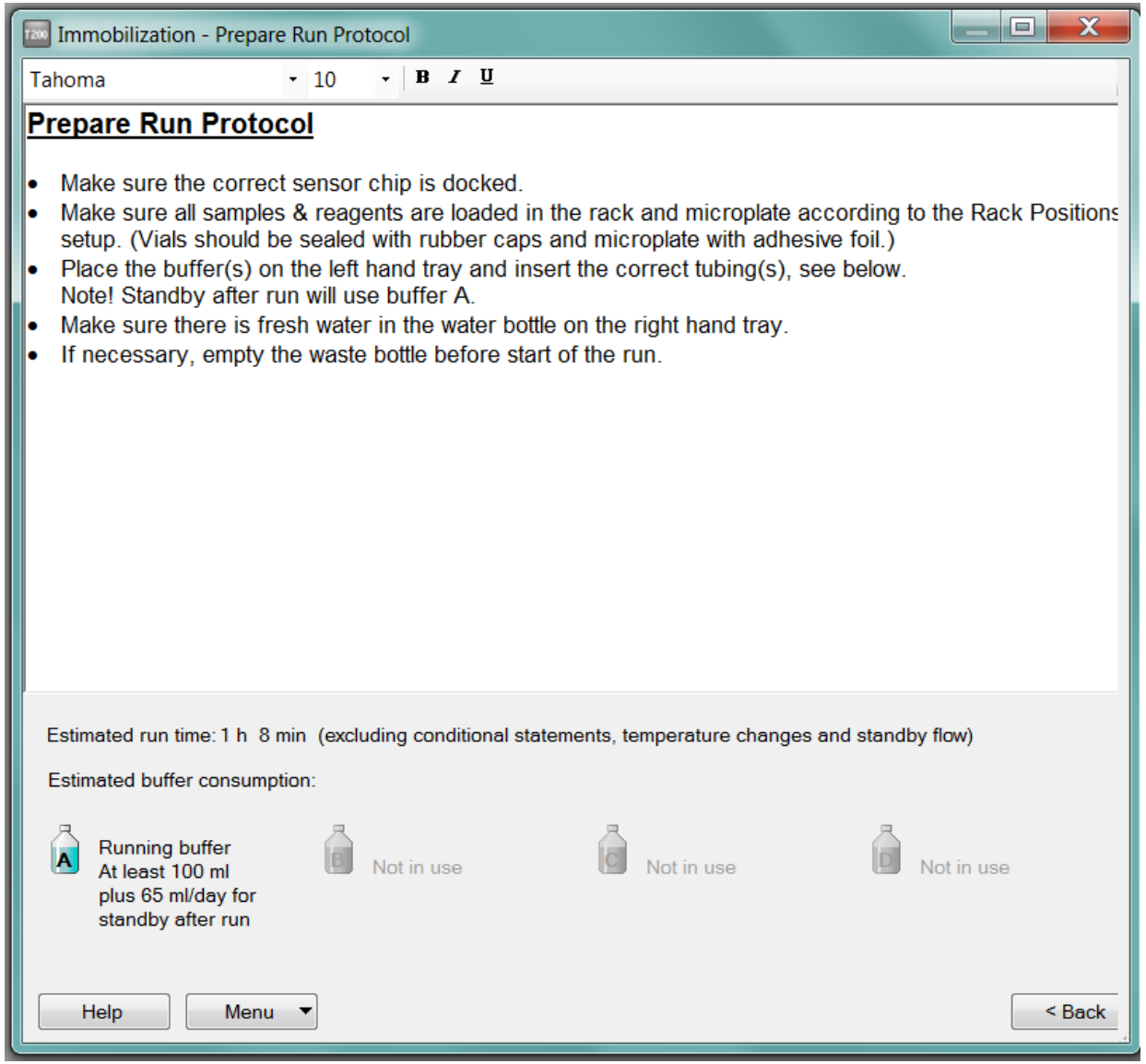




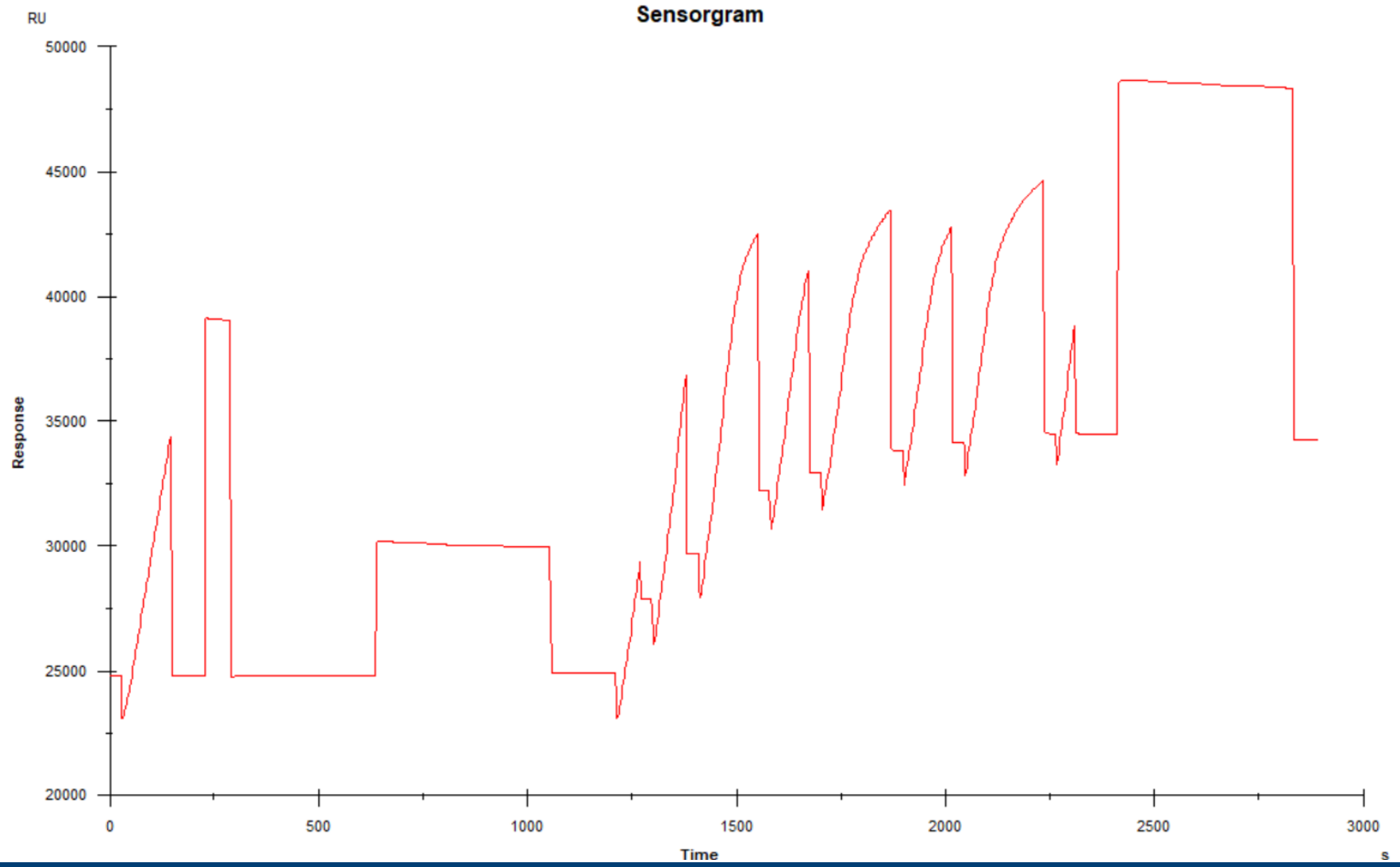
Important: Only need to normalise the detector for a new flow-cell once, don't re-normalise again after you have captured ligand.

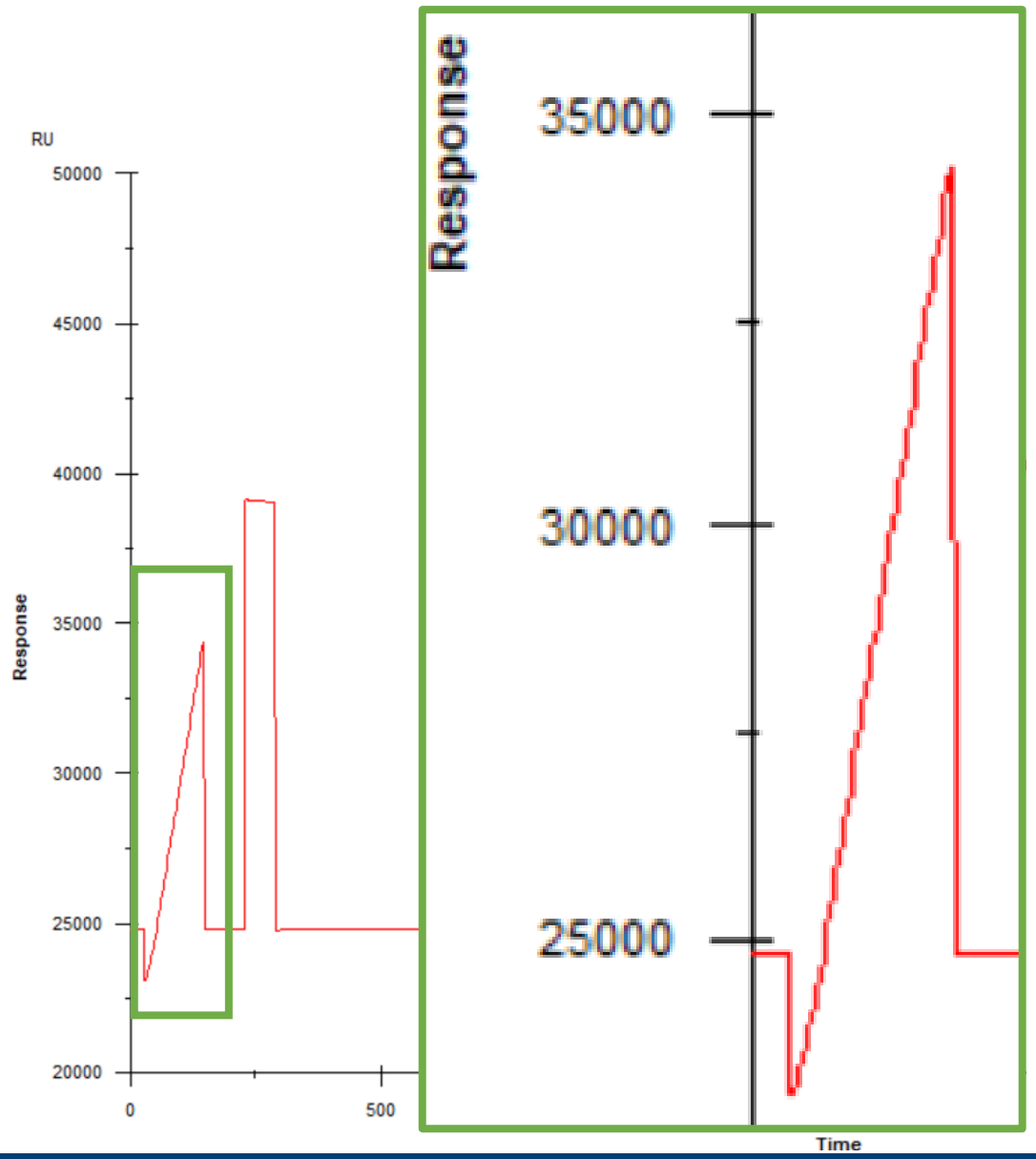


Position	Volume (µl)	Content	Type
R1 A1	156	12.3 ug/mL GST	Immob Fc 1
R1 A2	58	1 M ethanolamine pH 8.5	Immob Fc 1
R1 A3	89	EDC	Immob Fc 1
R1 A4	89	NHS	Immob Fc 1
R1 A5	Empty	EDC/NHS, min. capacity 124µl	Immob Fc 1
R1 A6	129	Ethanolamine	Immob Fc 1
R1 B1	156	13.5 ug/mL antiGFPnano-GST	Immob Fc 2
R1 B2	58	1 M ethanolamine pH 8.5	Immob Fc 2
R1 B3	89	EDC	Immob Fc 2
R1 B4	89	NHS	Immob Fc 2
R1 B5	Empty	EDC/NHS, min. capacity 124µl	Immob Fc 2
R1 B6	129	Ethanolamine	Immob Fc 2
R1 C15	120	BIAnormalizing solution 70% (w/w) glycerol	Normalize

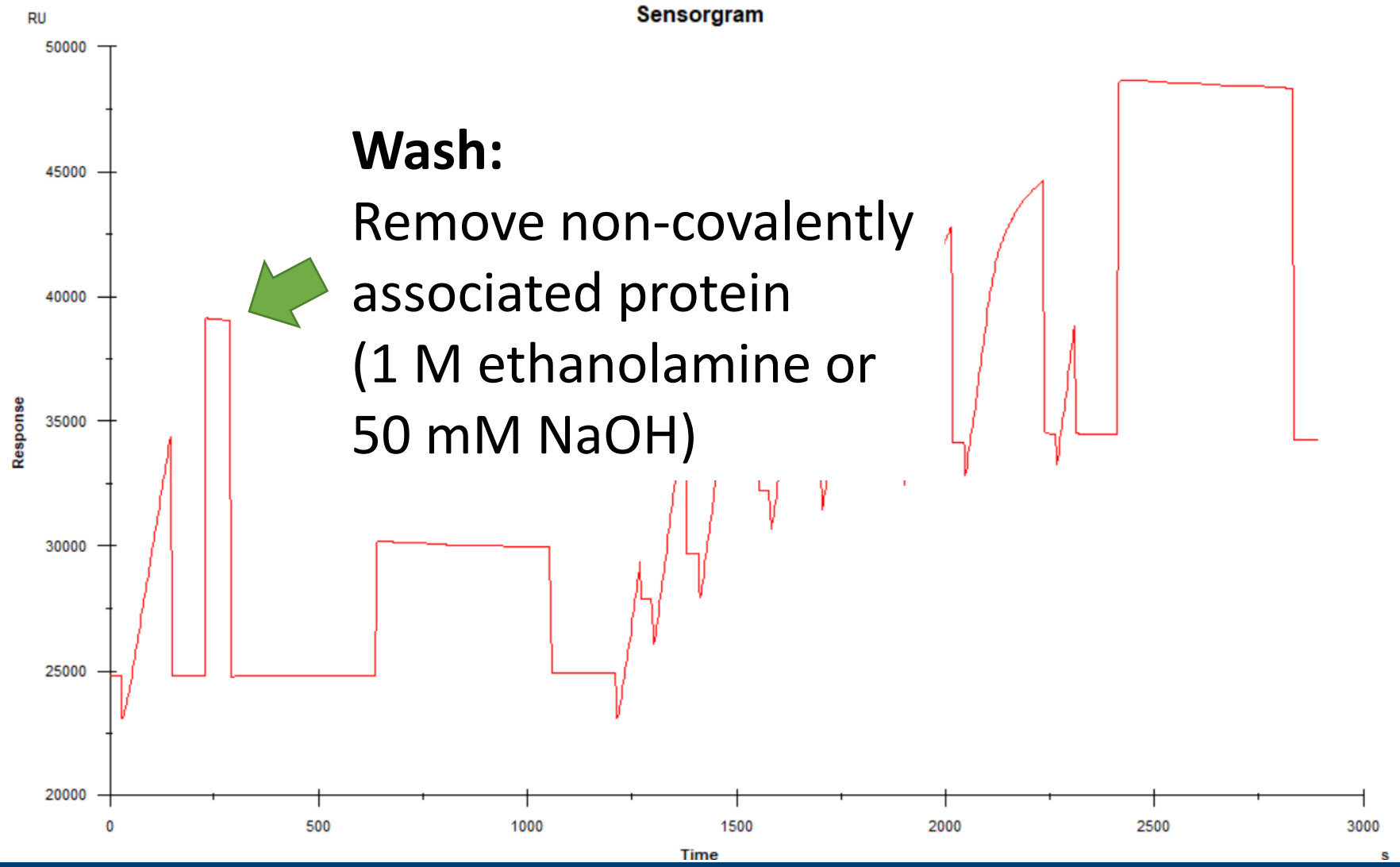

Immobilization - Prepare Run Protocol
Tahoma
10
B I U
Prepare Run Protocol
Make sure the correct sensor chip is docked.
Make sure all samples & reagents are loaded in the rack and microplate according to the Rack Positions setup. (Vials should be sealed with rubber caps and microplate with adhesive foil.)
Place the buffer(s) on the left hand tray and insert the correct tubing(s), see below.
Note! Standby after run will use buffer A.
Make sure there is fresh water in the water bottle on the right hand tray.
If necessary, empty the waste bottle before start of the run.
Estimated run time: 1 h 8 min (excluding conditional statements, temperature changes and standby flow)
Estimated buffer consumption:
A
Running buffer
At least 100 ml
plus 65 ml/day for
standby after run
B
Not in use
C
Not in use
D
Not in use
Help
Menu
< Back

Ligand capture – FC2 (antiGFPnano-GST)

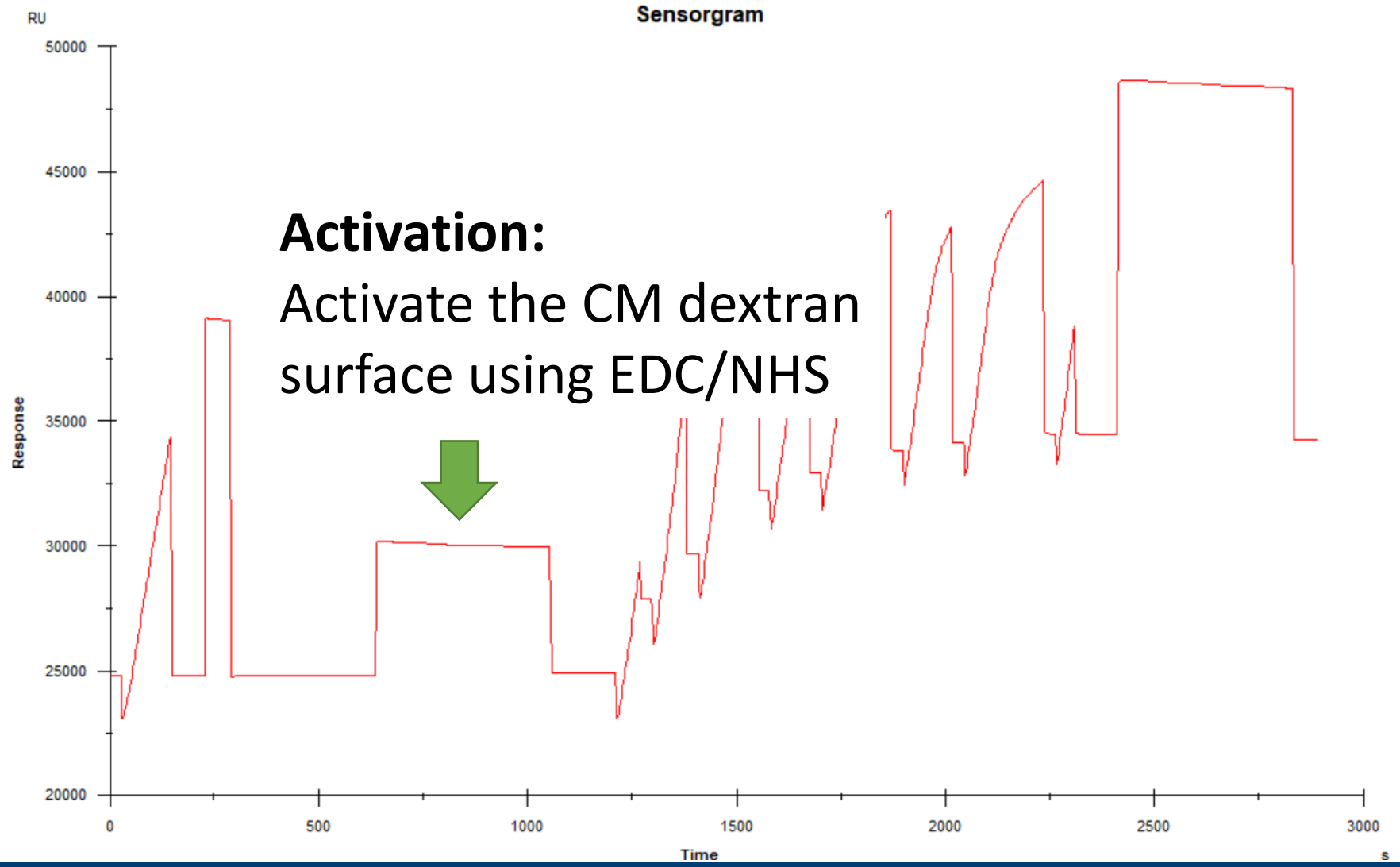




Pre-concentration:
Protein is non-covalently accumulating in the carbodymethyldextran surface (ionic interaction)



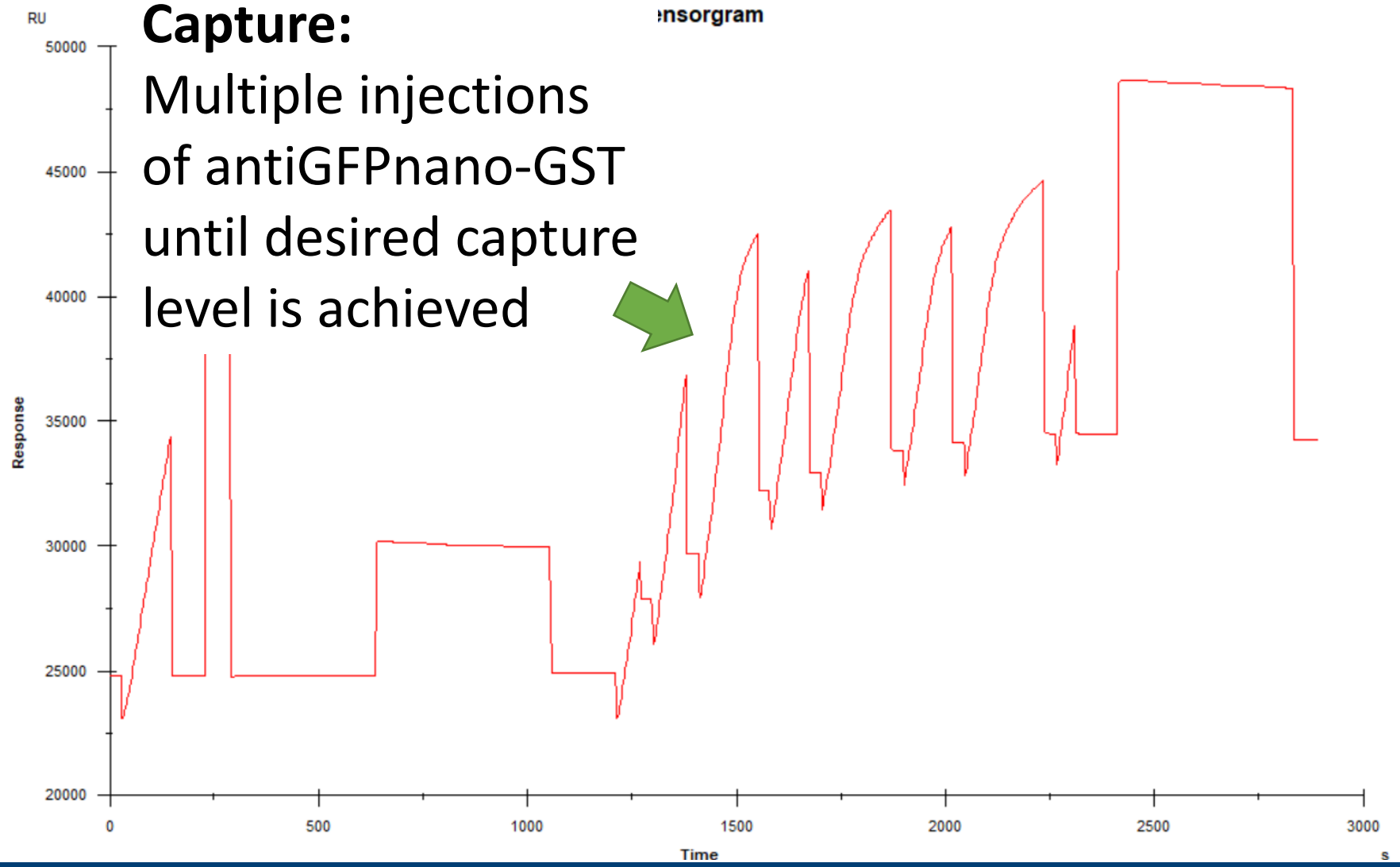
Wash:
Remove non-covalently associated protein
(1 M ethanolamine or 50 mM NaOH)

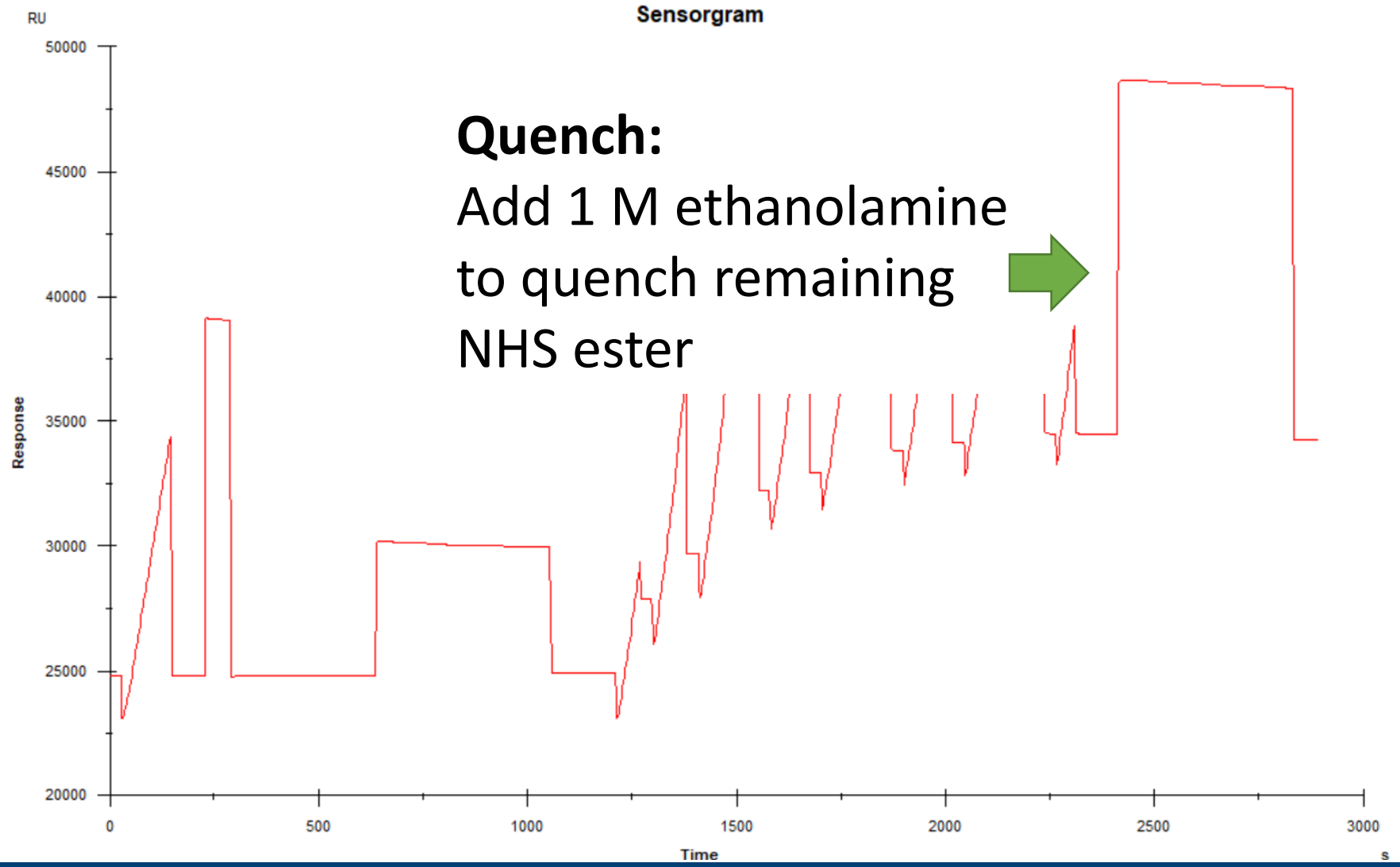


Activation:

Activate the CM dextran surface using EDC/NHS

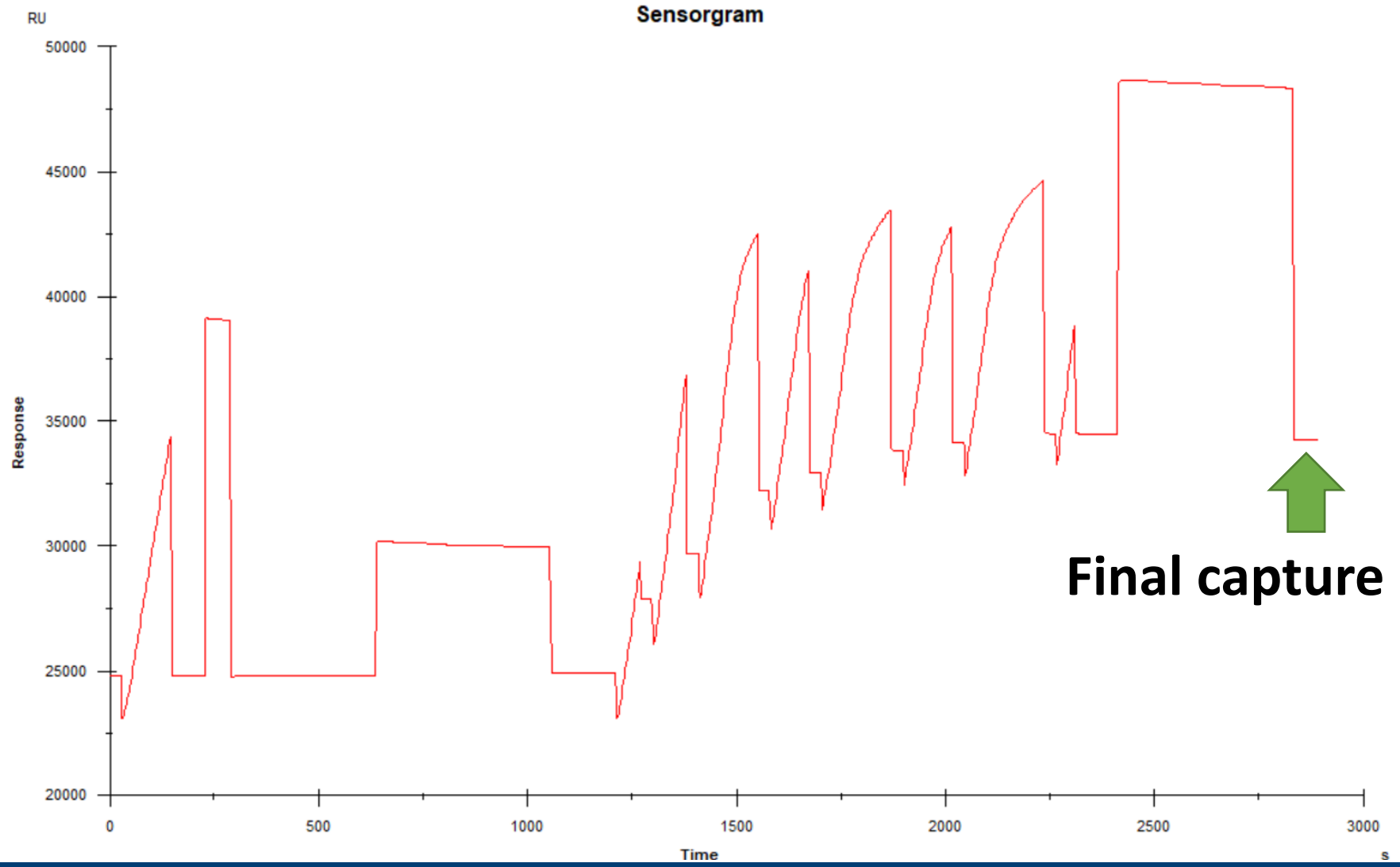
Capture:
Multiple injections of antiGFPnano-GST until desired capture level is achieved



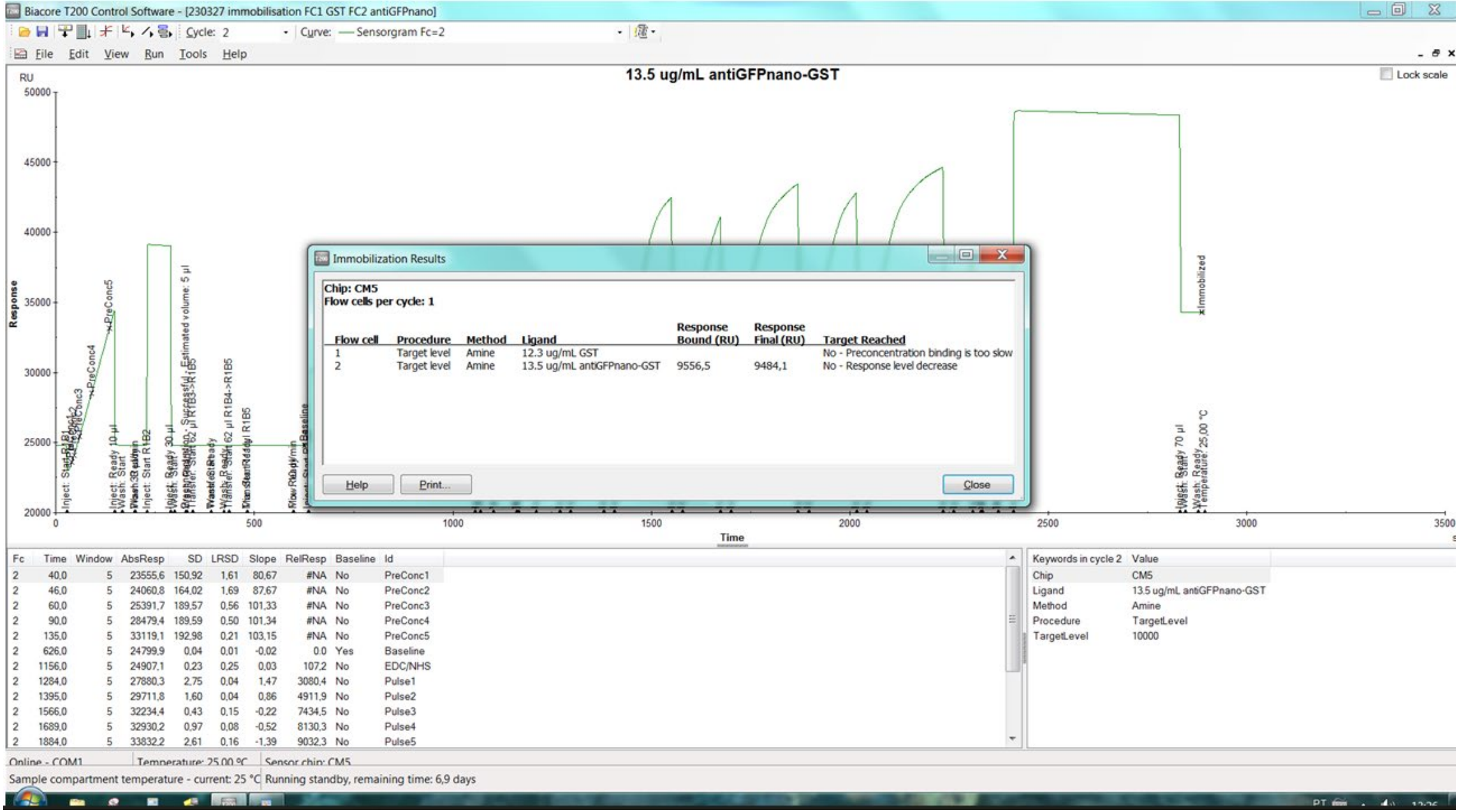


Quench:

Add 1 M ethanolamine to quench remaining NHS ester



Final capture level



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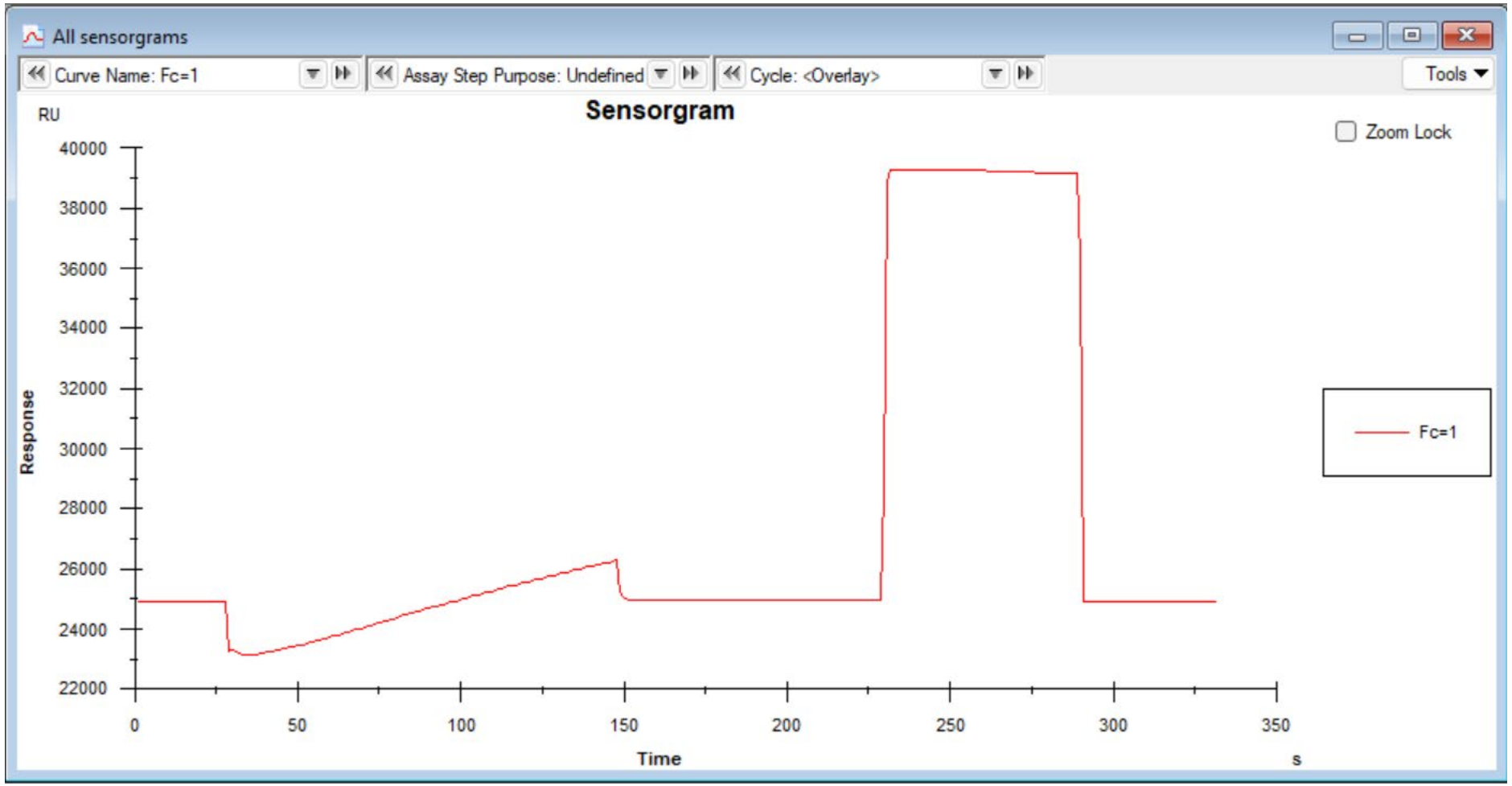
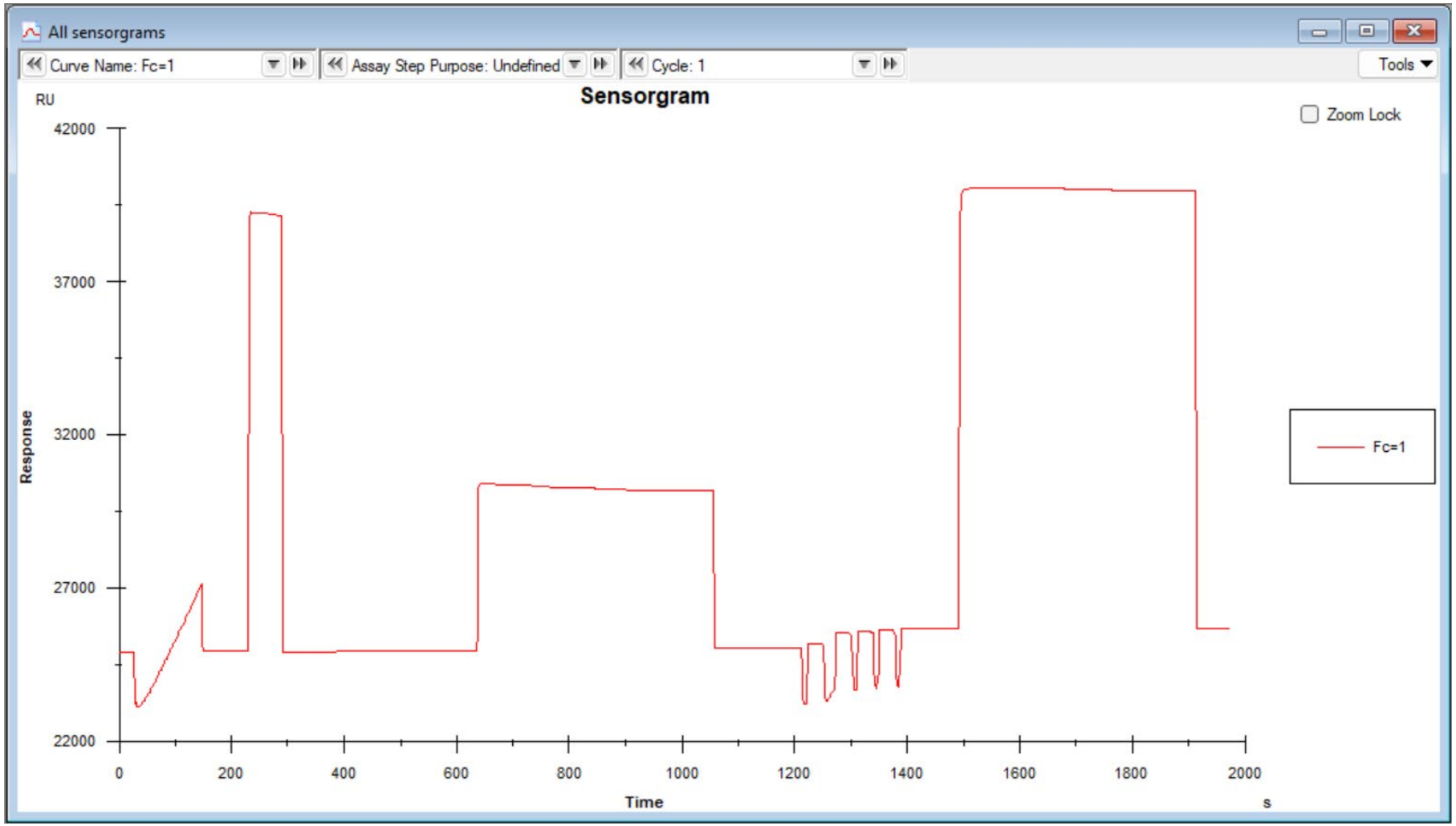
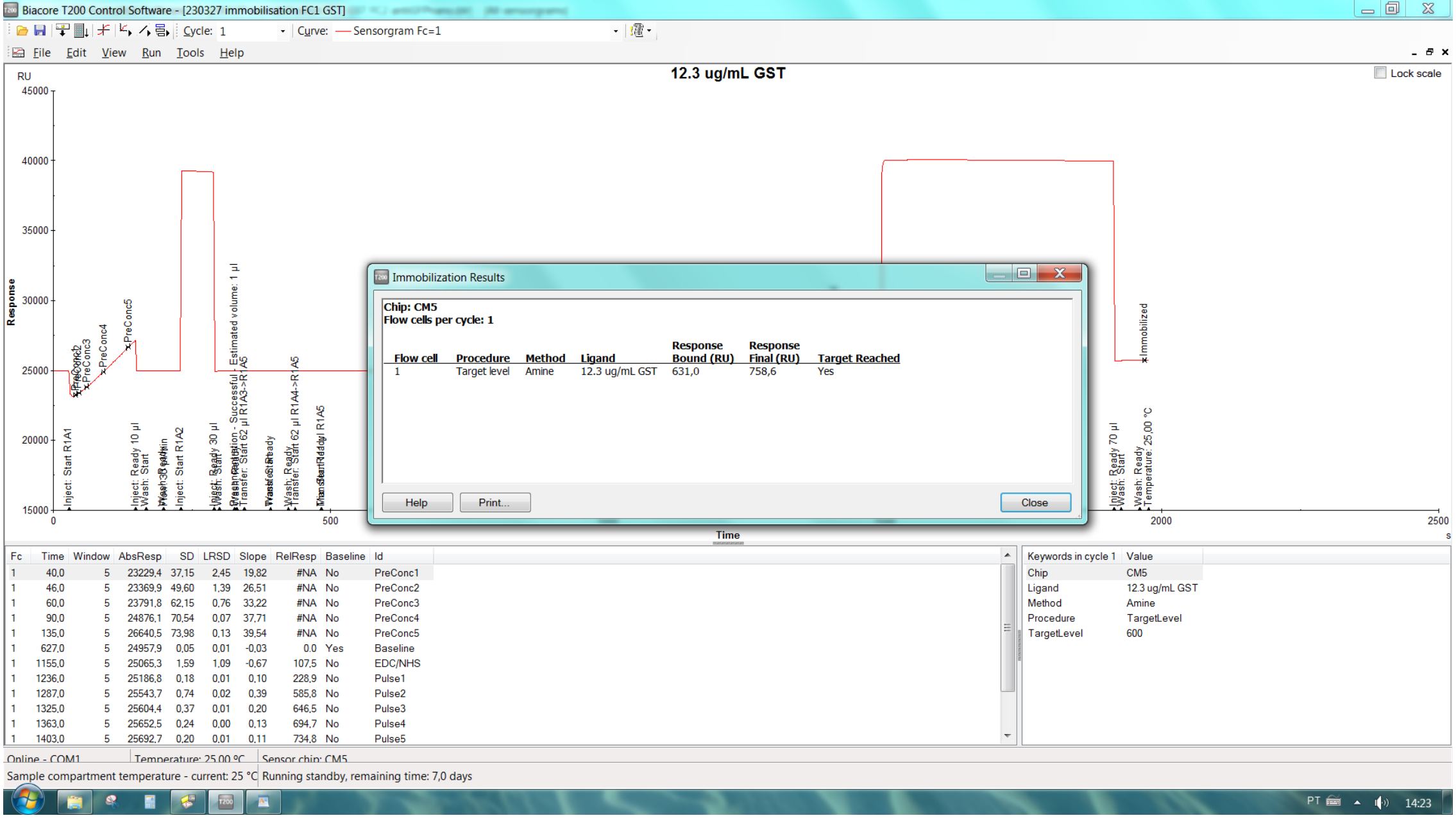
GST ligand capture



GST ligand capture - repeat





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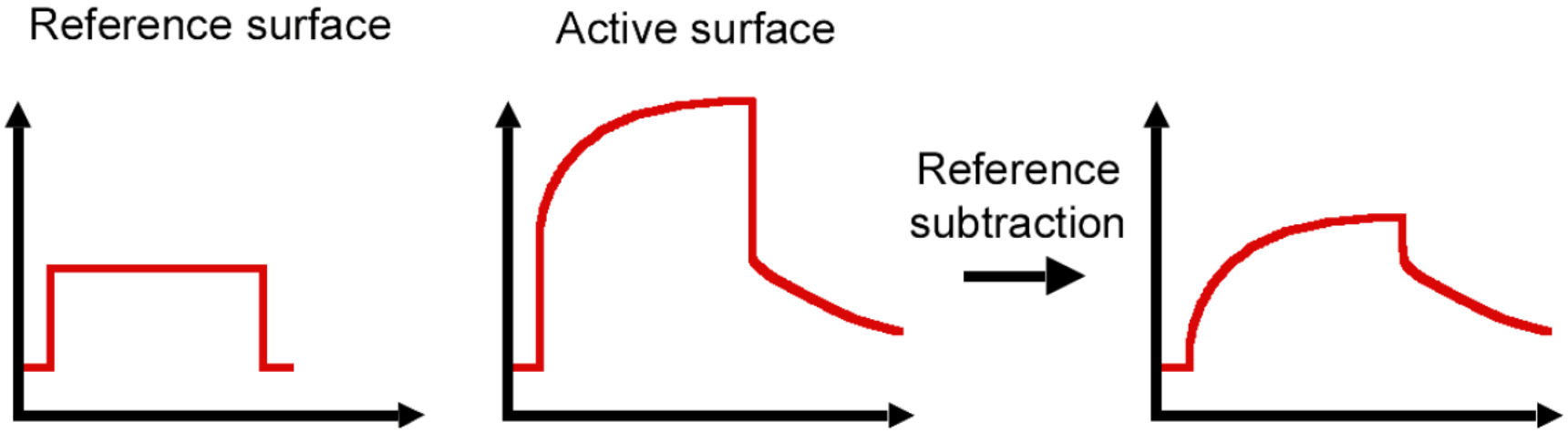
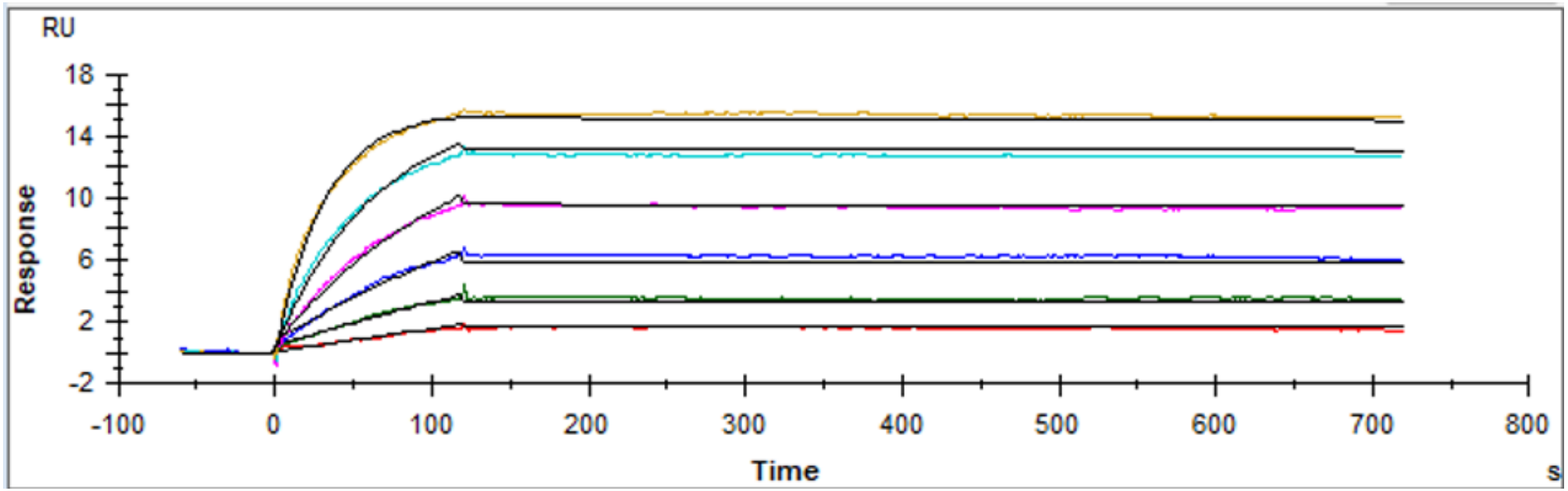
Why use a mock flow cell? Reference subtraction



- Changing the buffer in the flow cell changes the refractive index, which changes the SPR signal
- Subtract reference (mock) signal from experimental signal when injecting analytes

Image: Cytiva

How much ligand should I capture?



- R_{max} = amount if signal (RU) if all ligand sites are occupied by analyte
  - Relative signal, so baseline (0) is the surface before injection of analyte

How much ligand should I capture?

- For good kinetics experiments, you want R_{max} to be as low as possible to still get good signal
  - Minimise mass transport and rebinding
  - Minimise aggregation and steric hindrance

$$R_{max} = \frac{\text{analyte MW}}{\text{ligand MW}} \times \text{immobilized amount} \times \text{stoichiometric ratio}$$

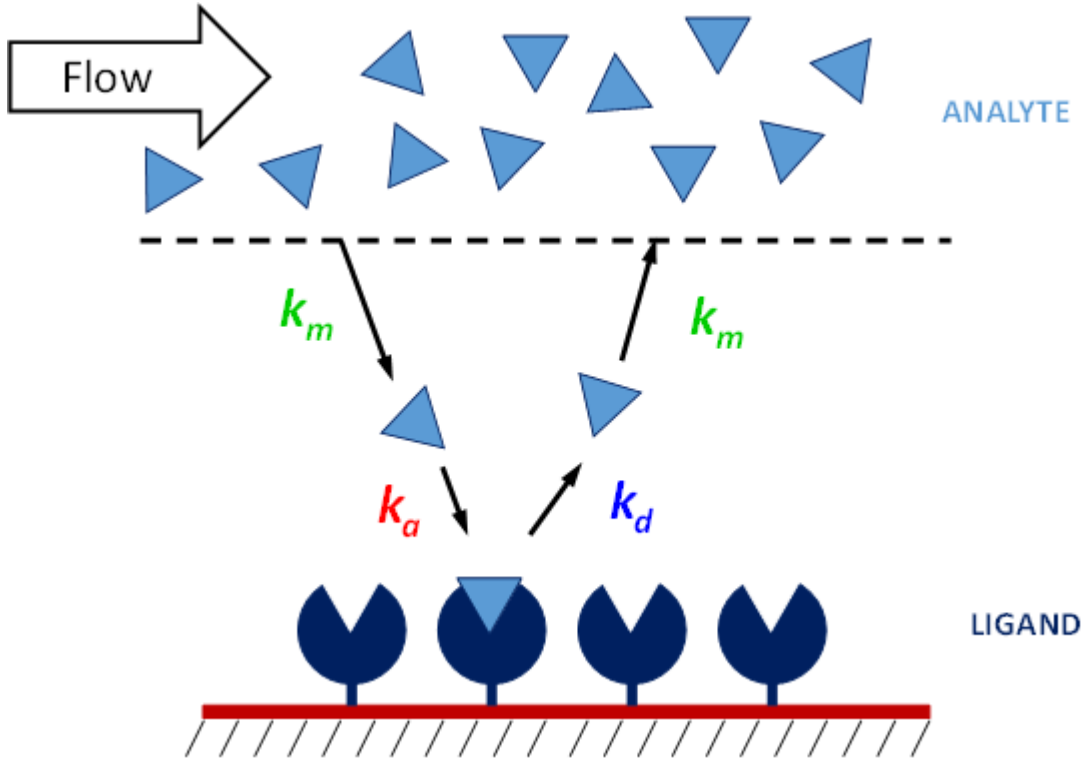
- For protein interactions, aim for ~50-150
  - For small molecule interactions, aim for as low at 10!

Mass transport

$$A_o \underset{k_m}{\overset{k_m}{\rightleftharpoons}} A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

mass transport coefficient

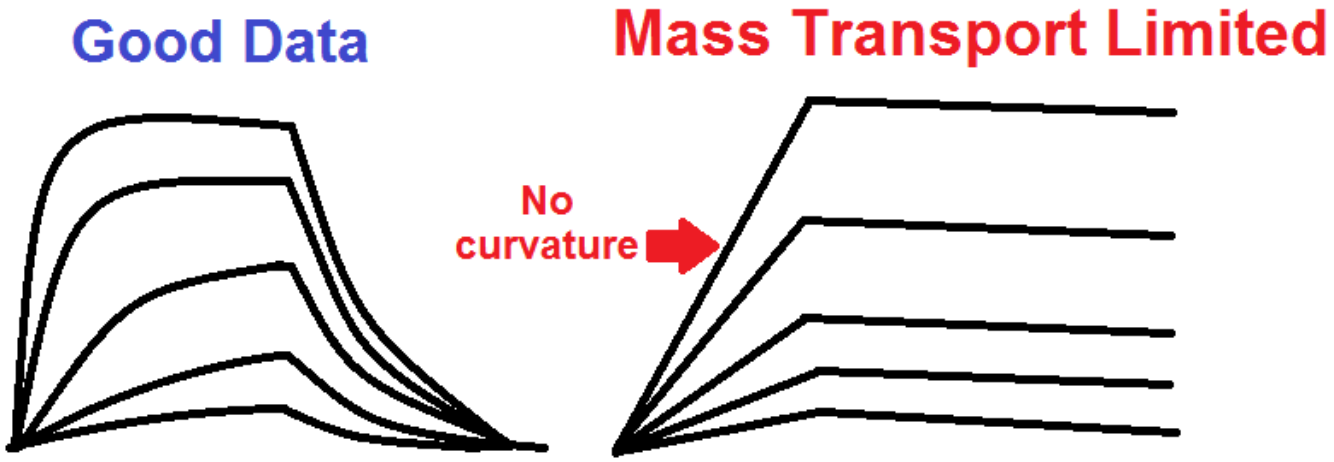
intrinsic kinetics



- If the diffusion of analyte into the CM matrix is slower than the association, you will get local depletion of the analyte
  - Mass transport limited association

Image: Nicoya

Dealing with mass transport

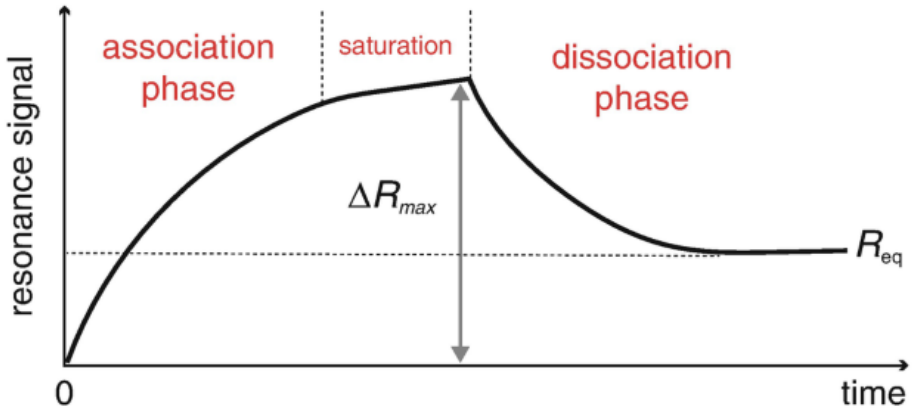


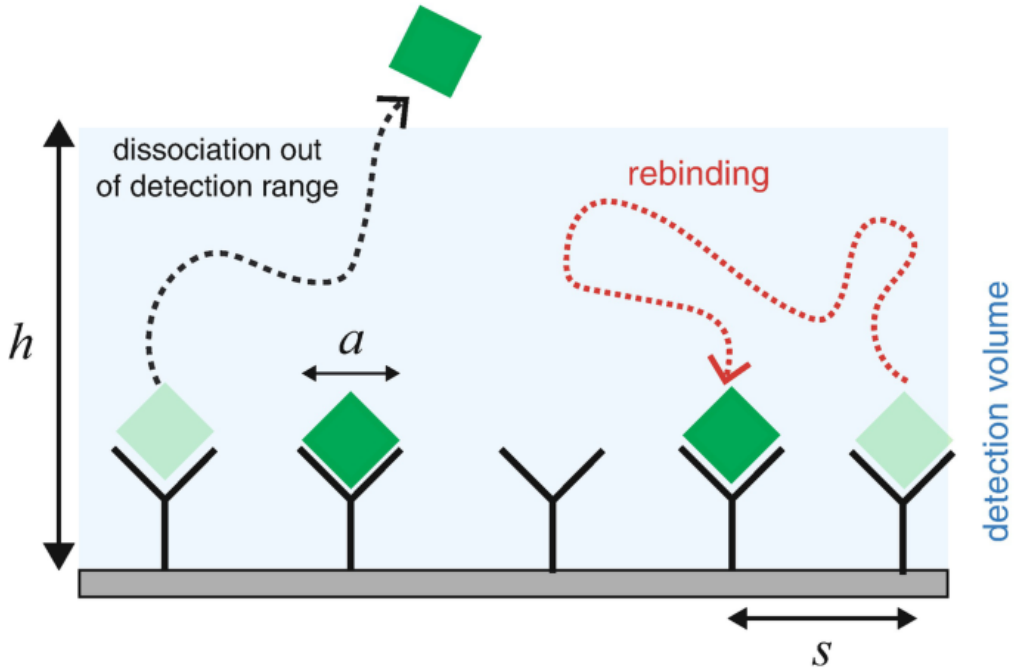
- Increase flow rate
  - Flow rate at edge of flow cell is lower than in centre
  - Higher flow rate consumes more analyte
- Decrease ligand capture
- Use mass transport corrected fitting model
  - Better to avoid the problem than correct the data, if possible!

Image: Nicoya

Rebinding

- During dissociation, analyte re-binds to ligands on the surface rather than flowing away
- No longer see exponential decay of analyte during dissociation phase
  - Incorrect calculation of the dissociation rate constant
- Avoid by using high flow rates and low [ligand]





Erbaş and Inci (2022) Methods in Molecular Biology, vol 2385. https://doi.org/10.1007/978-1-0716-1767-0_11

How much ligand did we capture?

$$R_{max} = \frac{\text{analyte MW}}{\text{ligand MW}} \times \text{immobilized amount} \times \text{stoichiometric ratio}$$

- For flow cell 2 (antiGFPnano-GST):
  - $MW_{analyte}$ = 26 kDa (approx. for EGFP)
  - MW_{ligand} = 39.2 kDa
  - Immobilised amount = 9500
  - Stoichiometric ratio = 1
  - **R_{max}** ≈ 6300 RU
- *You'll see later that our R_{max} is much lower…why??*

Regeneration

- Aim to remove all of the analyte from the surface, while not damaging any of the ligand

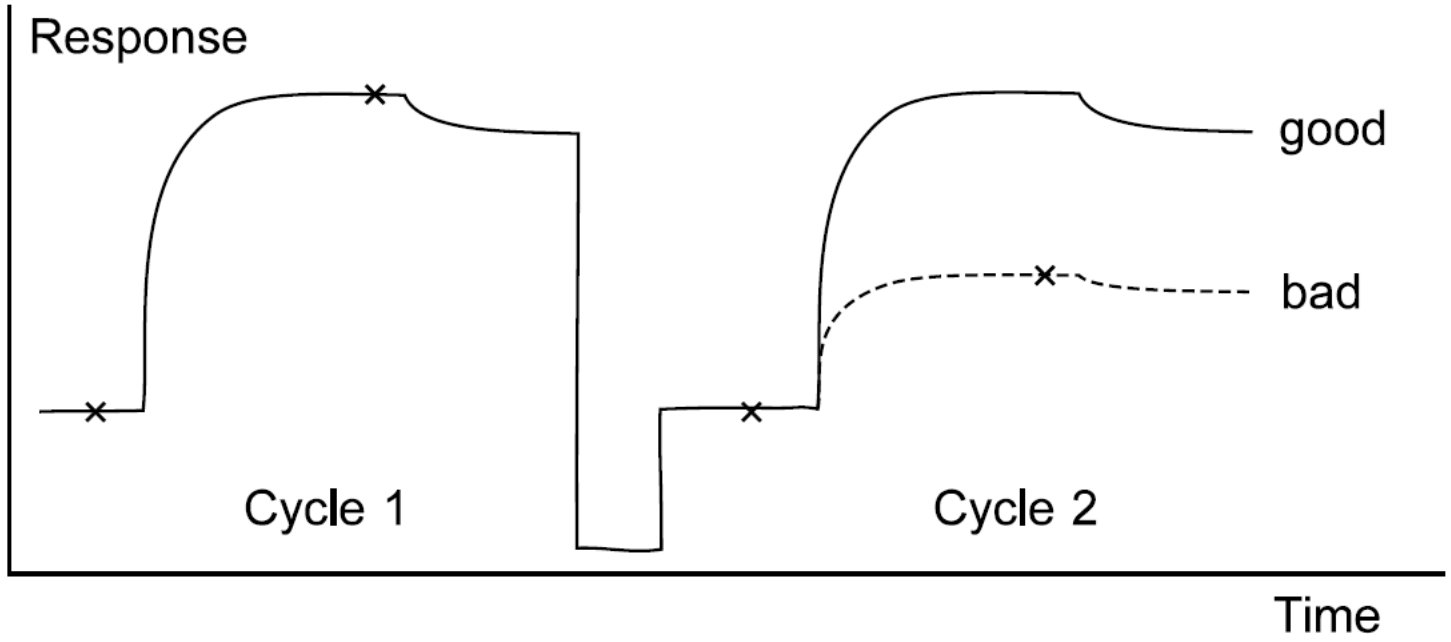


Image: Cytiva

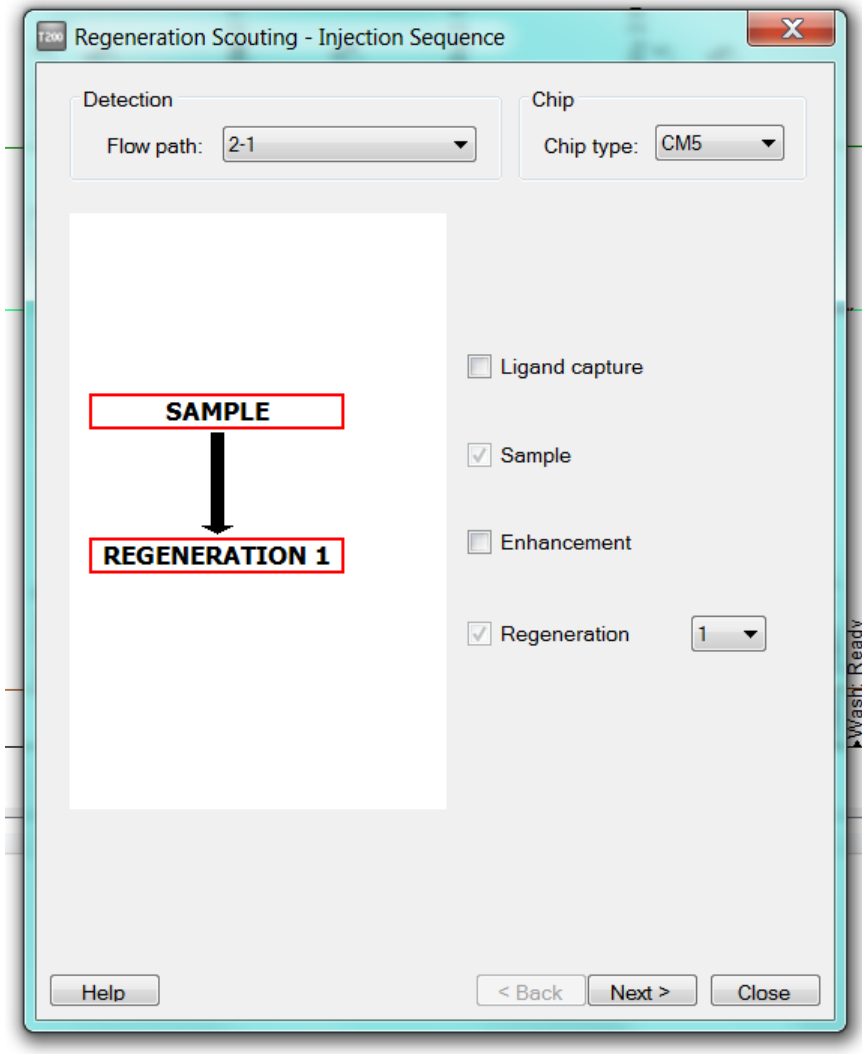
Regeneration

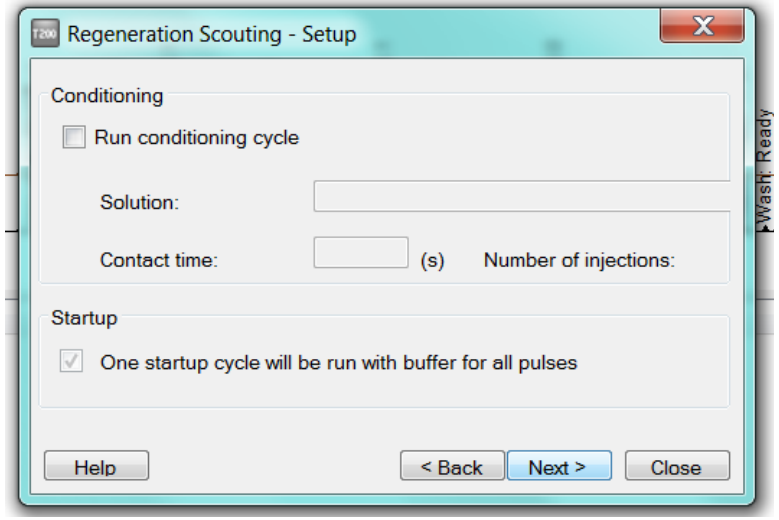
Type of bond	Acidic	Basic	Hydrophobic	Ionic
Strength				
Weak	**pH > 2.5**	**pH < 9**	**pH < 9**	1 M NaCl
	HCl	10 mM HEPES/NaOH	50 % ethylene glycol	
	10 mM Glycine/HCl			
Intermediate	**pH 2-2.5**	**pH 9-10**	**pH 9-10**	2 M $MgCl_2$
	formic acid	NaOH	50 % ethylene glycol	
	HCl	10 mM Glycine/NaOH		
	10 mM Glycine/HCl			
	H_3PO_4			
Strong	**pH < 2**	**pH > 10**	**pH > 10**	4 M $MgCl_2$
	formic acid	NaOH	25-50 % ethylene glycol	
	HCl	6 M guanidinechloride		
	10 mM Glycine/HCl			
	H_3PO_4			

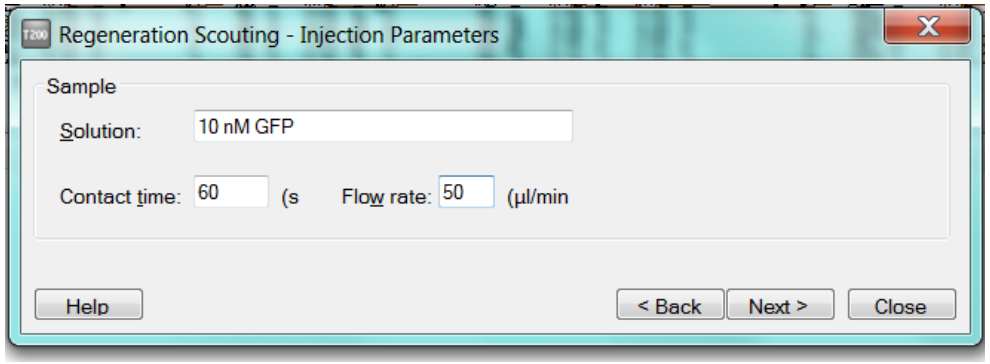
Table: Nicoya

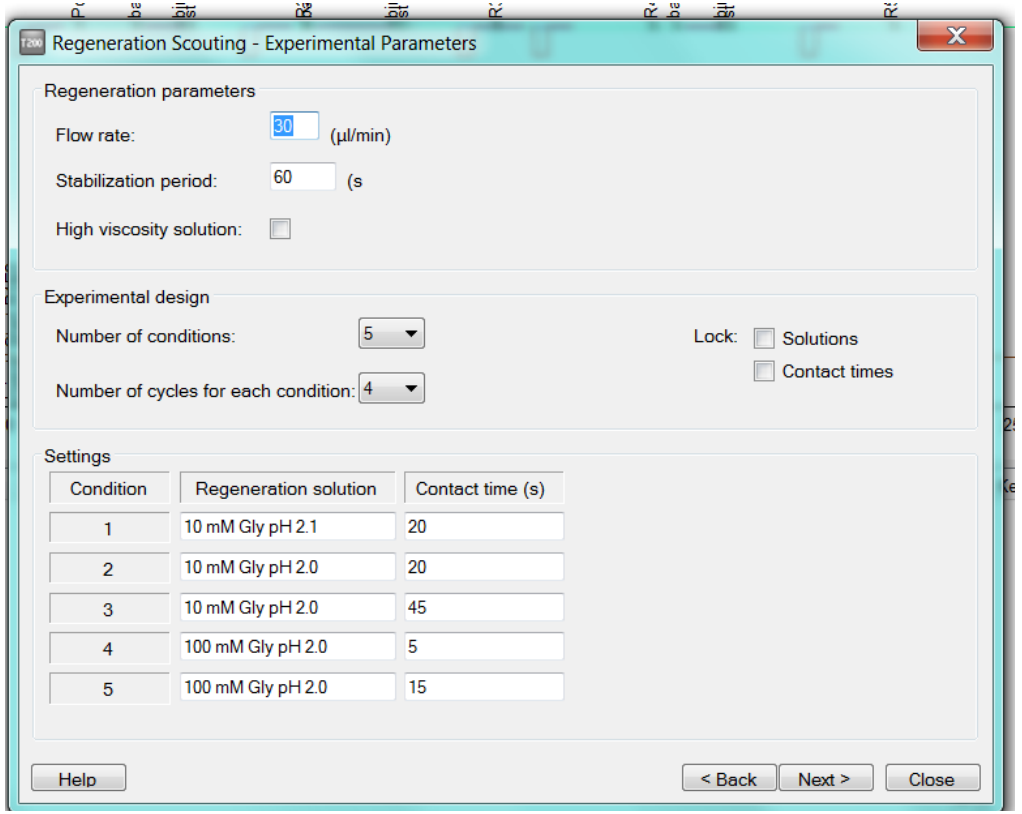
Regeneration scouting

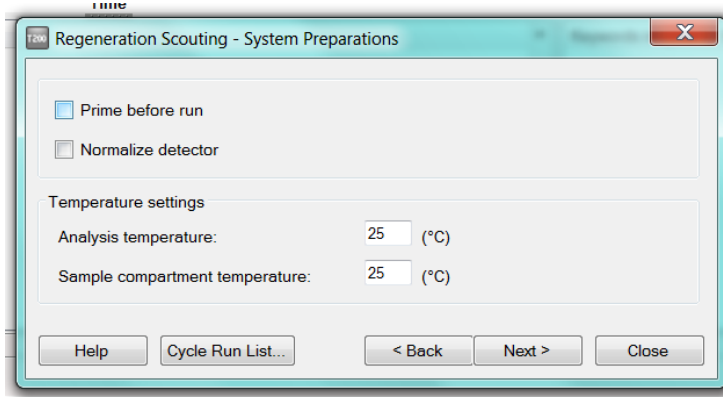
- Aim to discover optimal regeneration conditions
  - For antibody:antigen interactions low pH glycine is a good choice
- First attempt:
  - 10 mM Glycine pH 2.2 for 20 s
  - 10 mM Glycine pH 2.1 for 20 s
  - 10 mM Glycine pH 2.0 for 20 s
  - 100 mM Glycine pH 2.0 for 5 s
  - 100 mM Glycine pH 2.0 for 15 s
- Analyte was 10 nM EGFP
  - Use a relatively high concentration of analyte for good sensitivity


Regeneration Scouting - Injection Sequence
Detection
Flow path:
2-1
Chip
Chip type:
CM5
SAMPLE
REGENERATION 1
Ligand capture
Sample
Enhancement
Regeneration
1
Help
< Back
Next >
Close
Wash Ready


Regeneration Scouting - Setup
Conditioning
Run conditioning cycle
Solution:
Contact time:
(s)
Number of injections:
Startup
One startup cycle will be run with buffer for all pulses
Help
< Back
Next >
Close
Wash Ready


Regeneration Scouting - Injection Parameters
Sample
Solution:
10 nM GFP
Contact time:
60
(s
Flow rate:
50
(µl/min
Help
< Back
Next >
Close


Regeneration Scouting - Experimental Parameters
Regeneration parameters
Flow rate:
30
(µl/min)
Stabilization period:
60
(s
High viscosity solution:
Experimental design
Number of conditions:
5
Number of cycles for each condition:
4
Lock:
Solutions
Contact times
Settings
Condition
Regeneration solution
Contact time (s)
1
10 mM Gly pH 2.1
20
2
10 mM Gly pH 2.0
20
3
10 mM Gly pH 2.0
45
4
100 mM Gly pH 2.0
5
5
100 mM Gly pH 2.0
15
Help
< Back
Next >
Close


Regeneration Scouting - System Preparations
Prime before run
Normalize detector
Temperature settings
Analysis temperature:
25
(°C)
Sample compartment temperature:
25
(°C)
Help
Cycle Run List...
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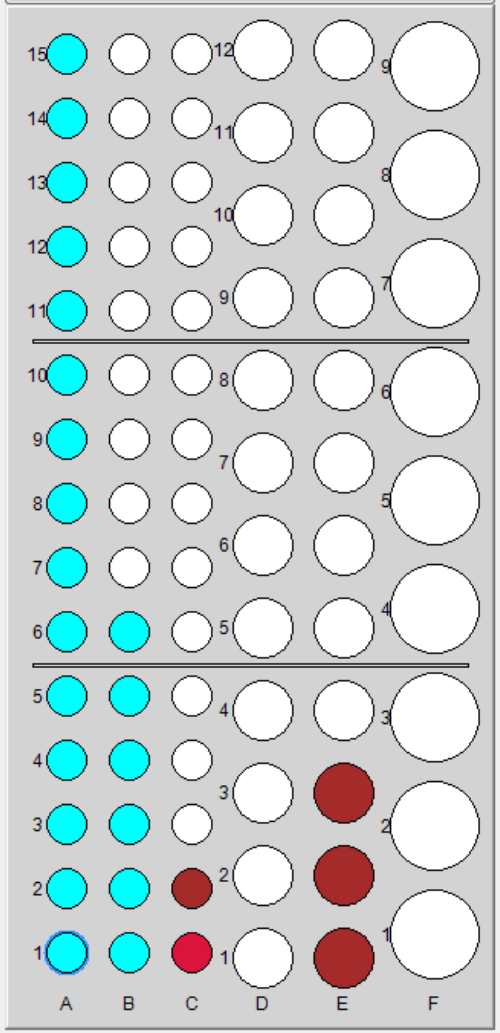
Close

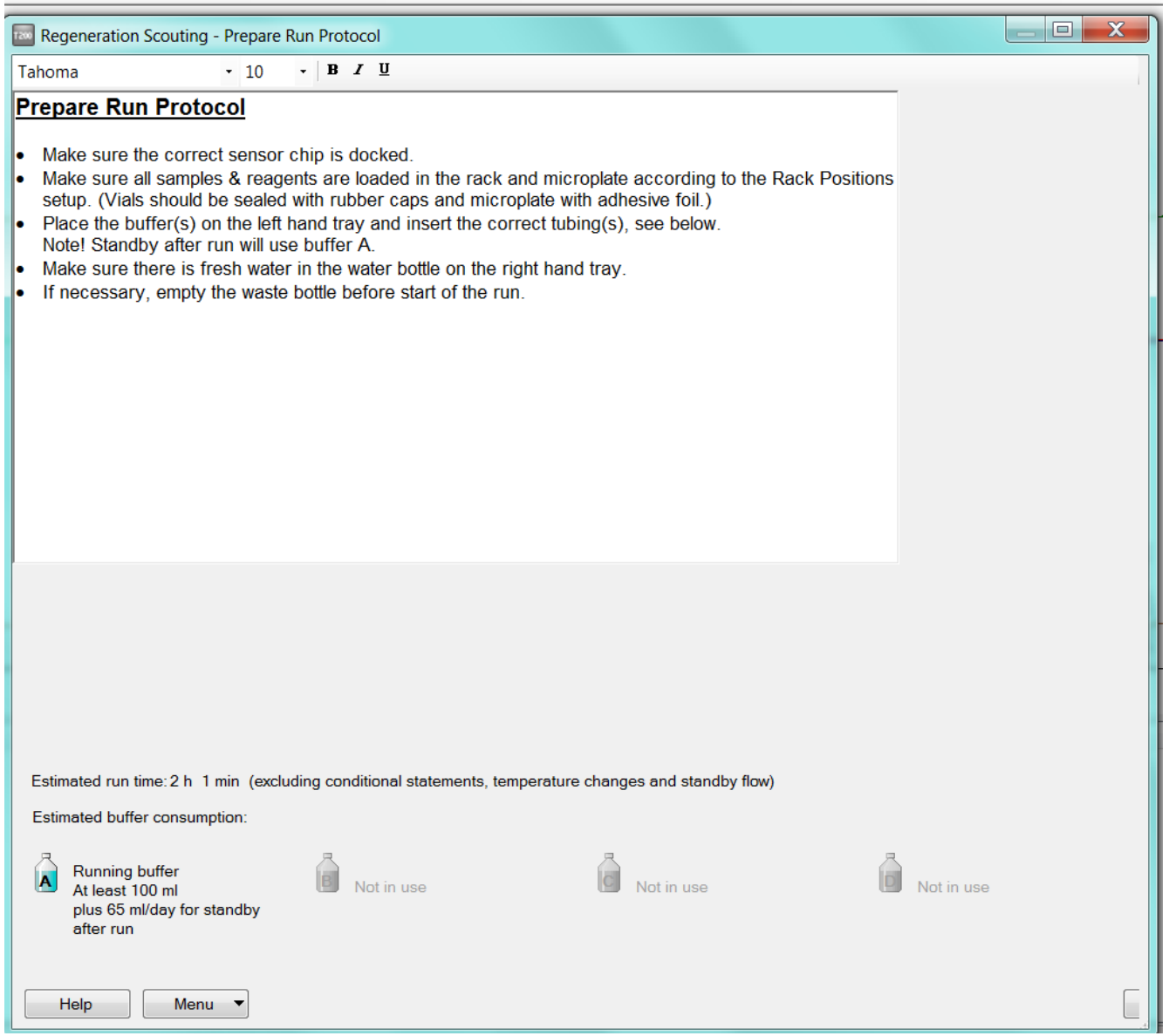
Regeneration Scouting - Rack Positions

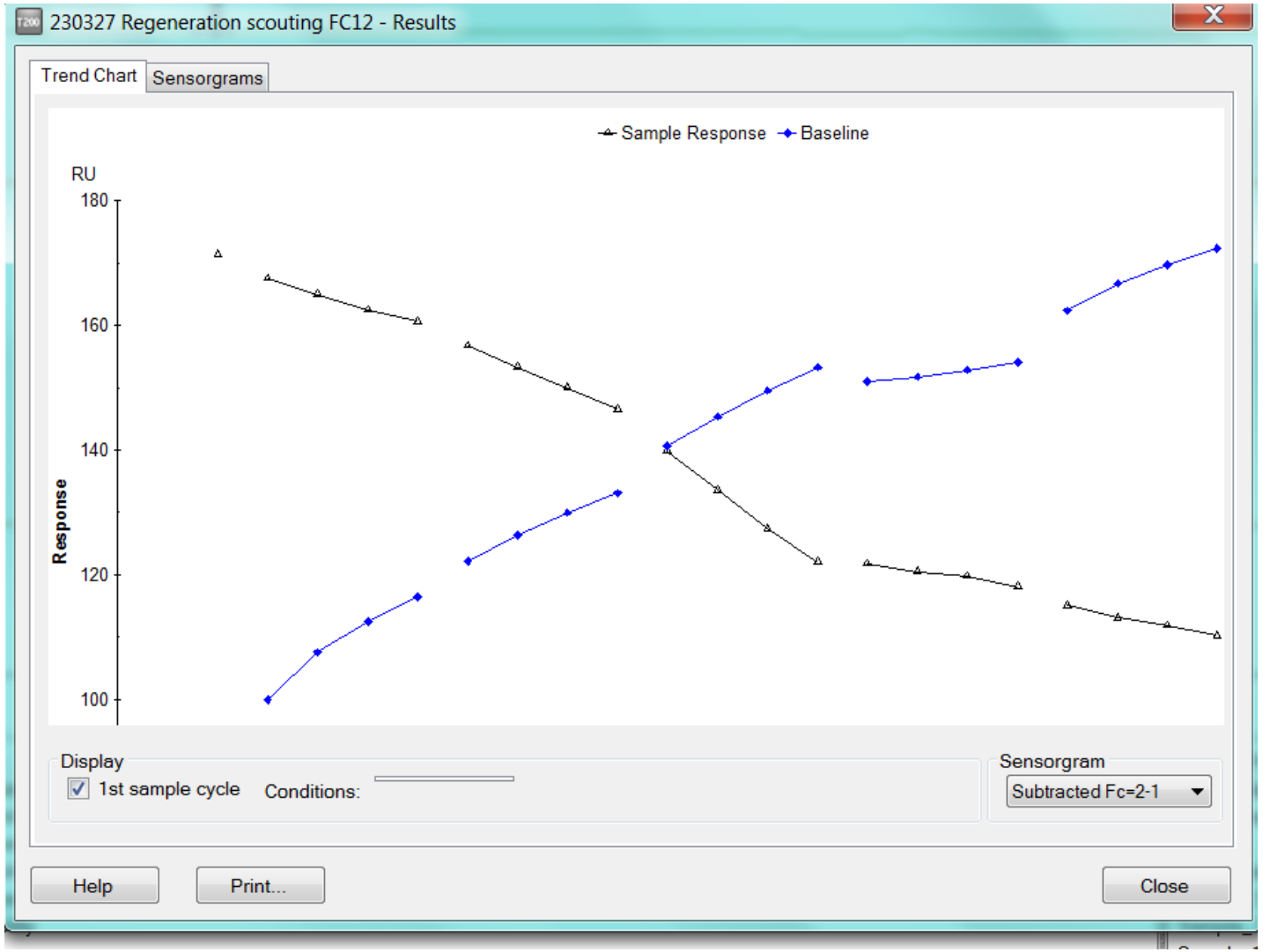
Sample and Reagent Rack 1



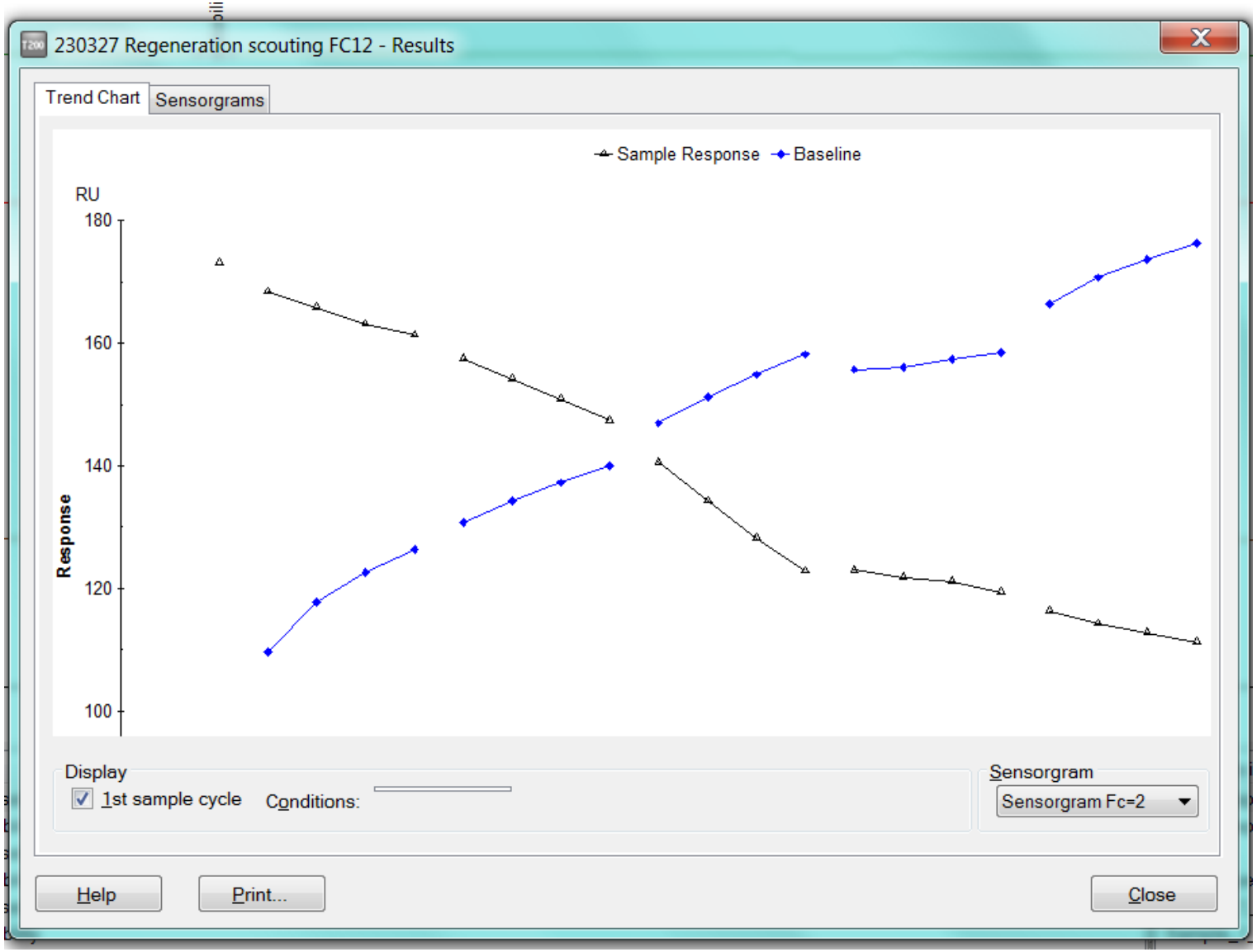
Position	Volume (µl)	Content	Type
R1 A1	78	10 nM GFP	Sample
R1 A2	78	10 nM GFP	Sample
R1 A3	78	10 nM GFP	Sample
R1 A4	78	10 nM GFP	Sample
R1 A5	78	10 nM GFP	Sample
R1 A6	78	10 nM GFP	Sample
R1 A7	78	10 nM GFP	Sample
R1 A8	78	10 nM GFP	Sample
R1 A9	78	10 nM GFP	Sample
R1 A10	78	10 nM GFP	Sample
R1 A11	78	10 nM GFP	Sample
R1 A12	78	10 nM GFP	Sample
R1 A13	78	10 nM GFP	Sample
R1 A14	78	10 nM GFP	Sample
R1 A15	78	10 nM GFP	Sample
R1 B1	78	10 nM GFP	Sample
R1 B2	78	10 nM GFP	Sample
R1 B3	78	10 nM GFP	Sample
R1 B4	78	10 nM GFP	Sample
R1 B5	78	10 nM GFP	Sample
R1 B6	78	10 nM GFP	Sample
R1 C1	78	Buffer	Startup
R1 C2	52	Buffer	Regeneration
R1 E1	404	10 mM Gly pH 2.0	Regeneration
R1 E2	188	10 mM Gly pH 2.1	Regeneration
R1 E3	356	100 mM Gly pH 2.0	Regeneration

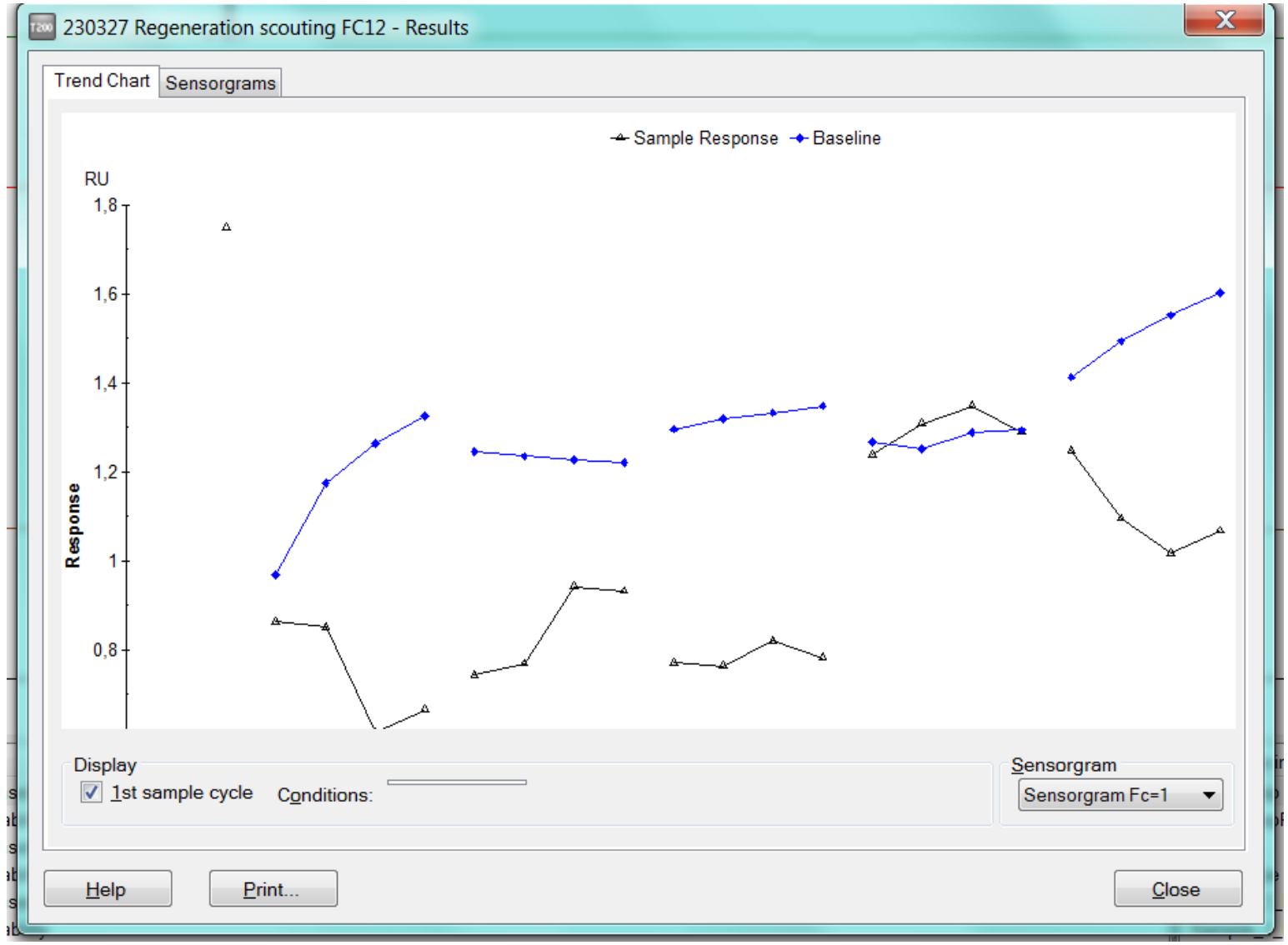
Help | Menu | Eject Rack | < Back | Next > | Close


Regeneration Scouting - Prepare Run Protocol
Tahoma
10
Prepare Run Protocol
Make sure the correct sensor chip is docked.
Make sure all samples & reagents are loaded in the rack and microplate according to the Rack Positions setup. (Vials should be sealed with rubber caps and microplate with adhesive foil.)
Place the buffer(s) on the left hand tray and insert the correct tubing(s), see below.
Note! Standby after run will use buffer A.
Make sure there is fresh water in the water bottle on the right hand tray.
If necessary, empty the waste bottle before start of the run.
Estimated run time: 2 h 1 min (excluding conditional statements, temperature changes and standby flow)
Estimated buffer consumption:
A
Running buffer
At least 100 ml
plus 65 ml/day for standby
after run
B
Not in use
C
Not in use
D
Not in use
Help
Menu

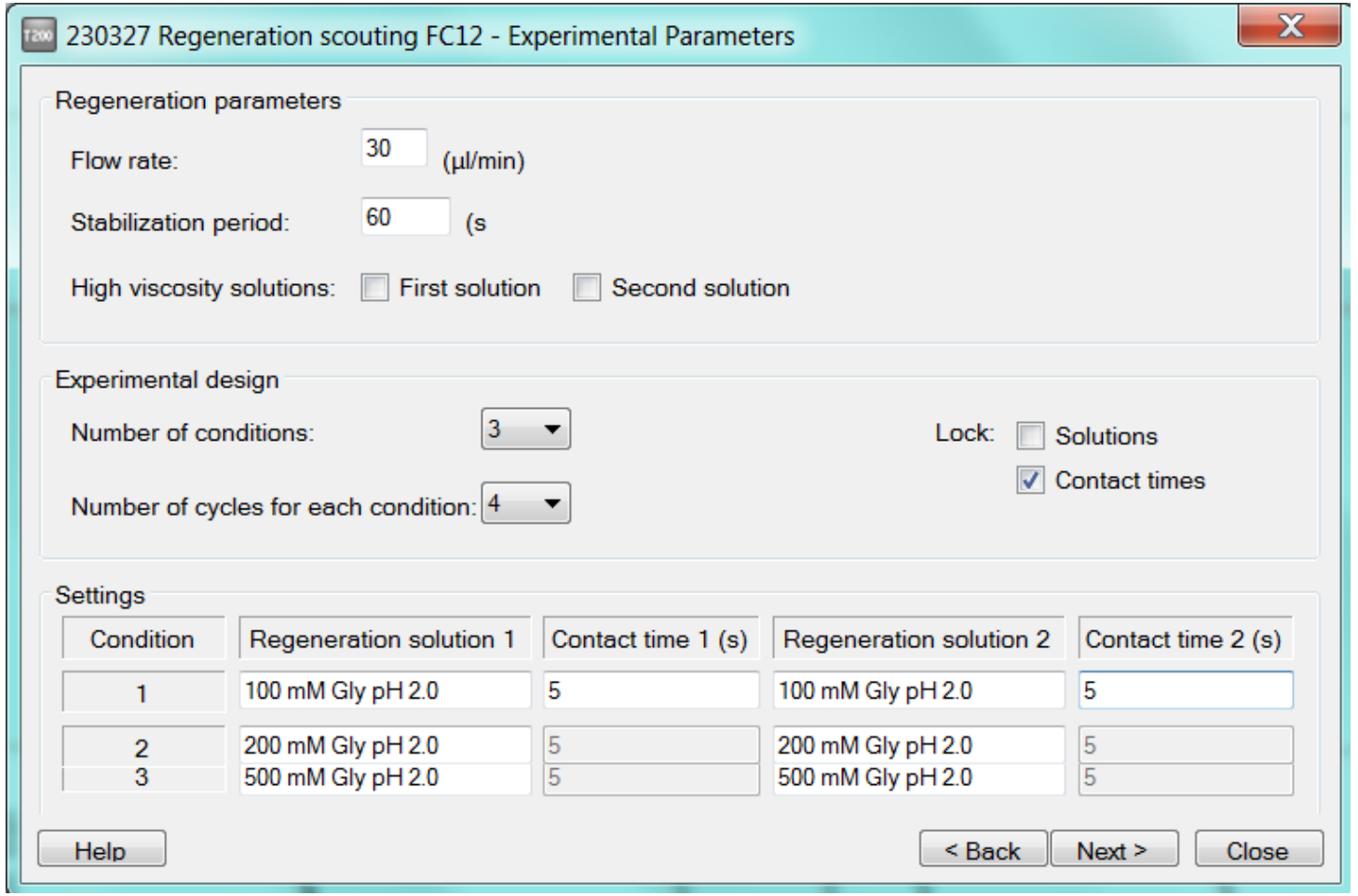


- Don’t close this window before inspecting…you can never get it back!

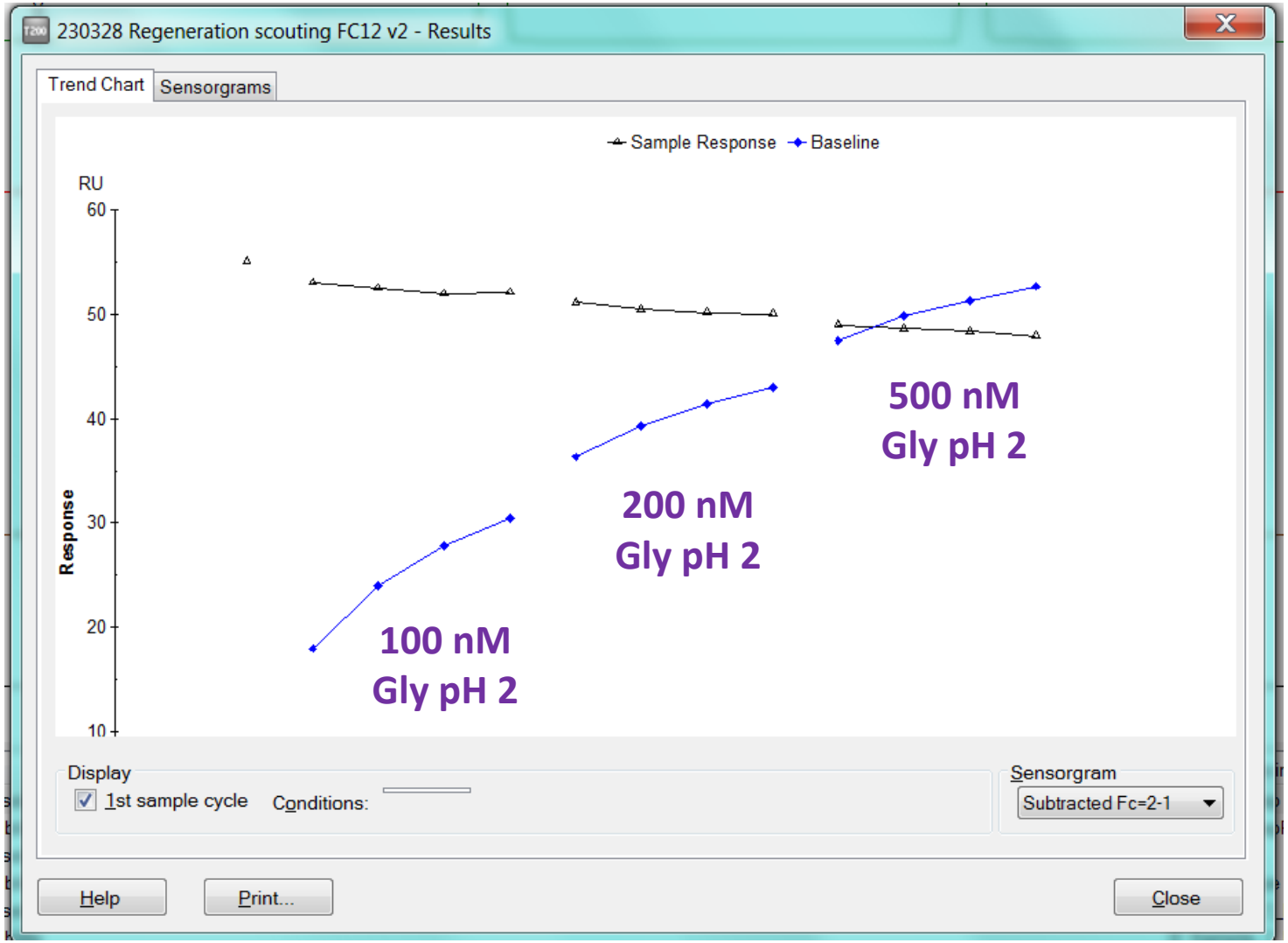

230327 Regeneration scouting FC12 - Results
Trend Chart
Sensorgrams
Sample Response
Baseline
RU
180
160
140
120
100
Response
Display
1st sample cycle
Conditions:
Sensorgram
Sensorgram Fc=2
Help
Print...
Close


230327 Regeneration scouting FC12 - Results
Trend Chart
Sensorgrams
Sample Response
Baseline
RU
1,8
1,6
1,4
1,2
1
0,8
Response
Display
1st sample cycle
Conditions:
Sensorgram
Sensorgram Fc=1
Help
Print...
Close

Regeneration scouting – second attempt

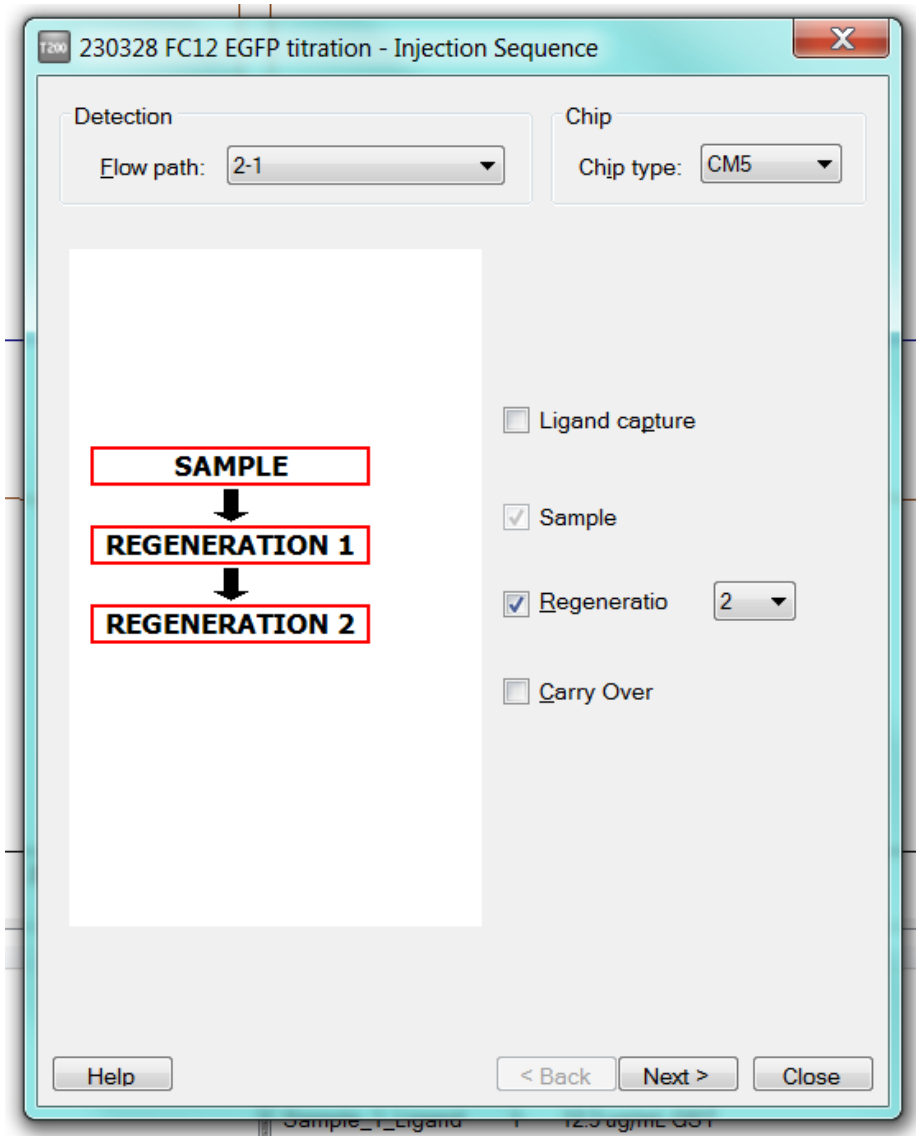


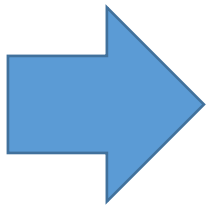
Regeneration scouting – second attempt

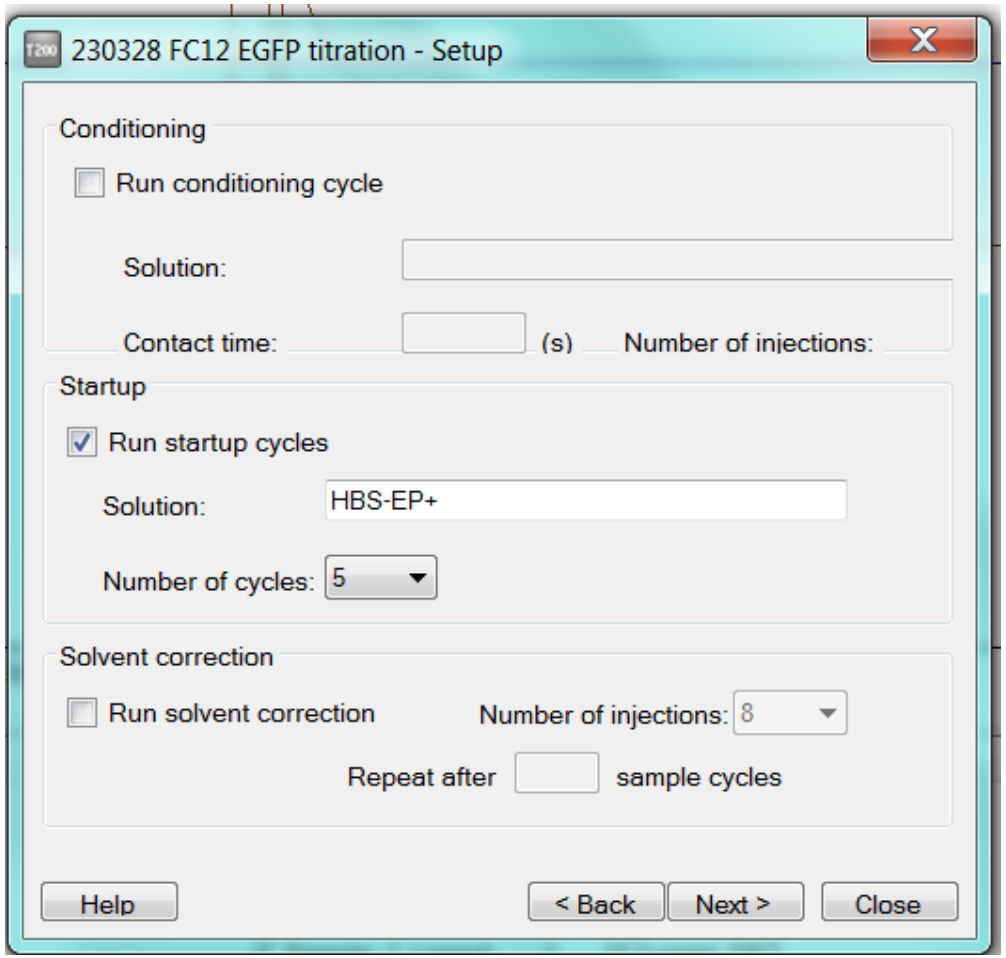


Kinetic titrations

- Aim to span a concentration range from $10 \times K_D$ to $0.1 \times K_D$
- Association phase must be long enough to get reasonable shape in association curve (2-5 min)
- Dissociation phase also long enough to get good shape in curve
  - For tight interactions (low off rate, k_d), leave as long as possible
  - Length of dissociation is determined by the machine hardware (10 min)
- Faster flow rates are better for kinetic analysis
  - But use more analyte
- Inject a ‘zero’ concentration too (buffer only)
- Inject samples from lowest to highest concentration


230328 FC12 EGFP titration - Injection Sequence
Detection
Flow path: 2-1
Chip
Chip type: CM5
SAMPLE
REGENERATION 1
REGENERATION 2
Ligand capture
Sample
Regeneratio 2
Carry Over
Help
< Back
Next >
Close




230328 FC12 EGFP titration - Setup
Conditioning
Run conditioning cycle
Solution:
Contact time: (s) Number of injections:
Startup
Run startup cycles
Solution: HBS-EP+
Number of cycles: 5
Solvent correction
Run solvent correction
Number of injections: 8
Repeat after sample cycles
Help
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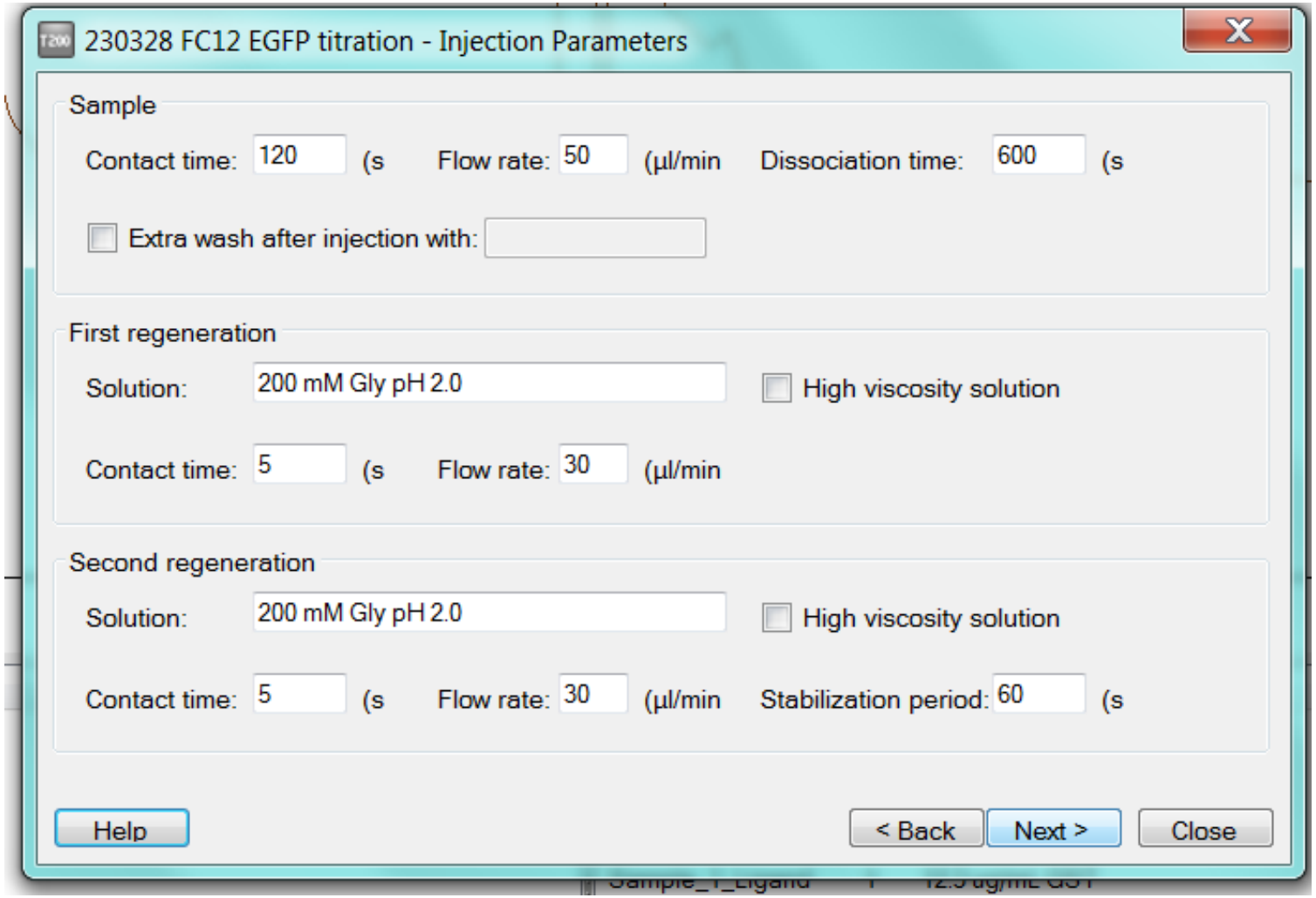
Next >
Close


230328 FC12 EGFP titration - Injection Parameters
Sample
Contact time: 120 (s
Flow rate: 50 (µl/min
Dissociation time: 600 (s
Extra wash after injection with:
First regeneration
Solution: 200 mM Gly pH 2.0
High viscosity solution
Contact time: 5 (s
Flow rate: 30 (µl/min
Second regeneration
Solution: 200 mM Gly pH 2.0
High viscosity solution
Contact time: 5 (s
Flow rate: 30 (µl/min
Stabilization period: 60 (s
Help
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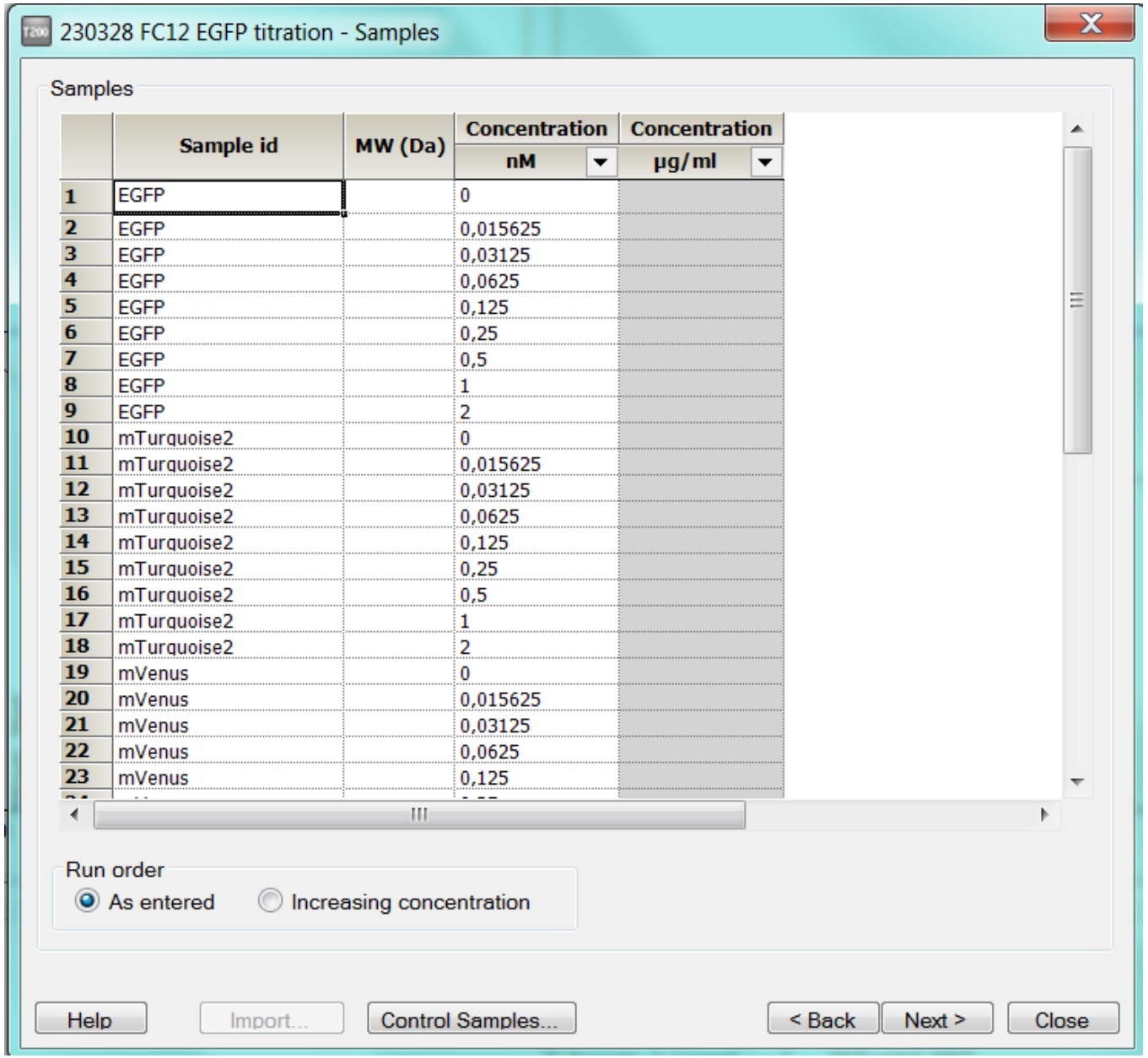
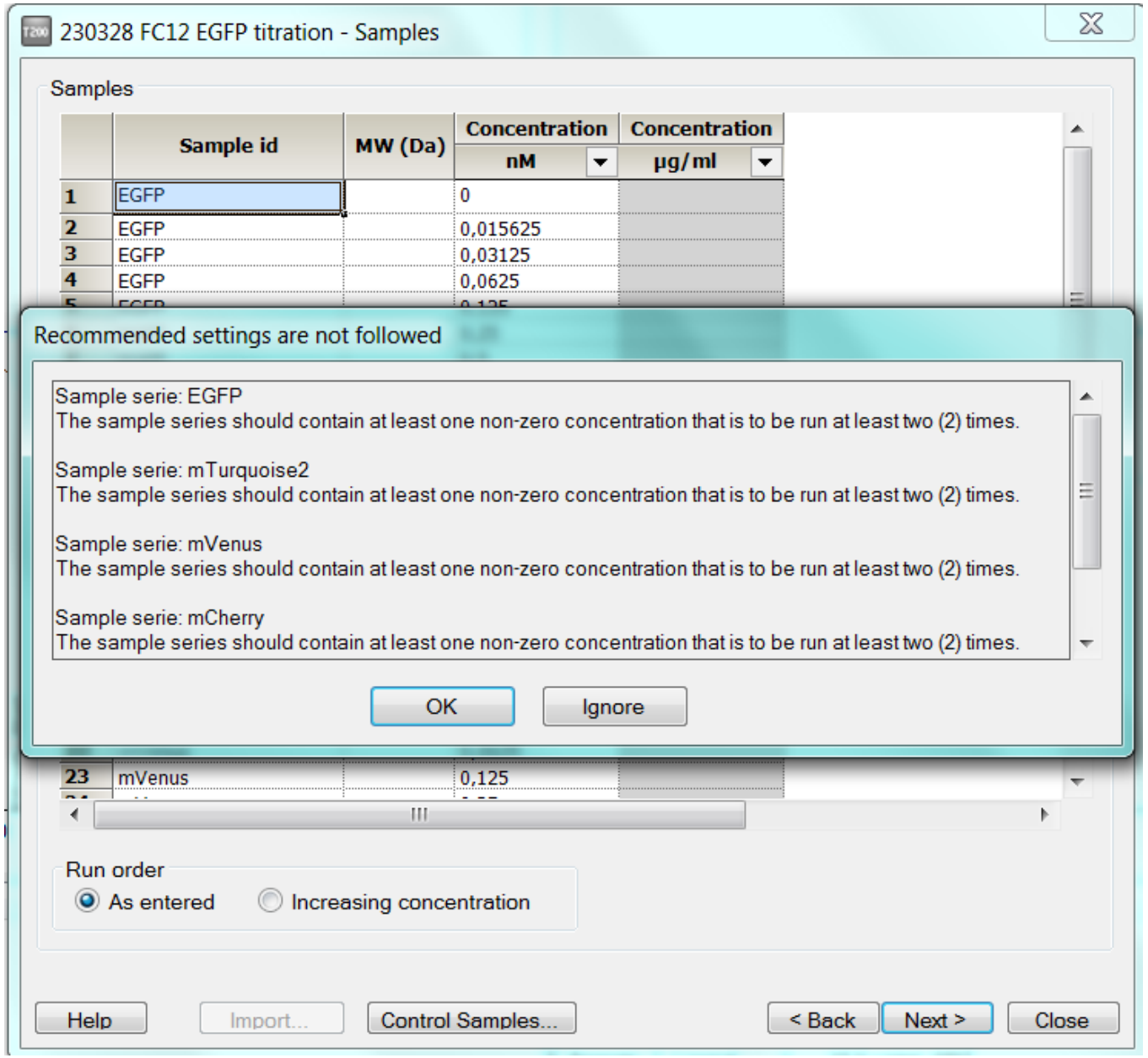
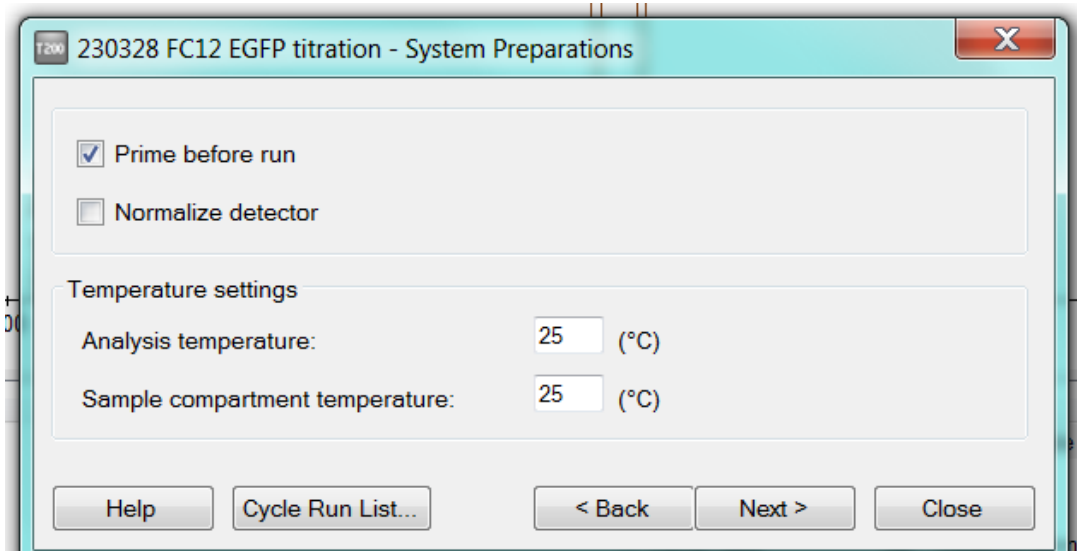
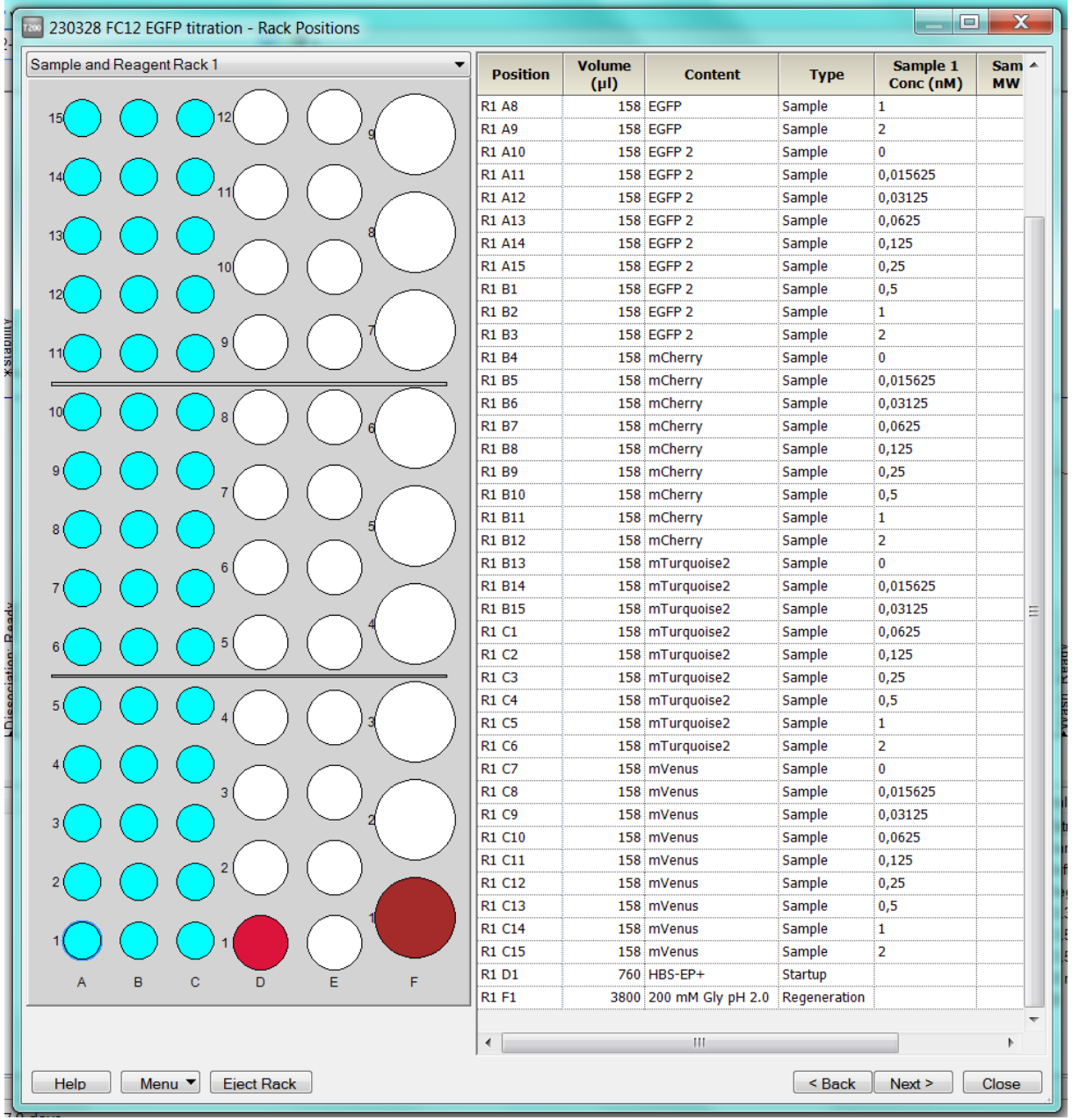
Close

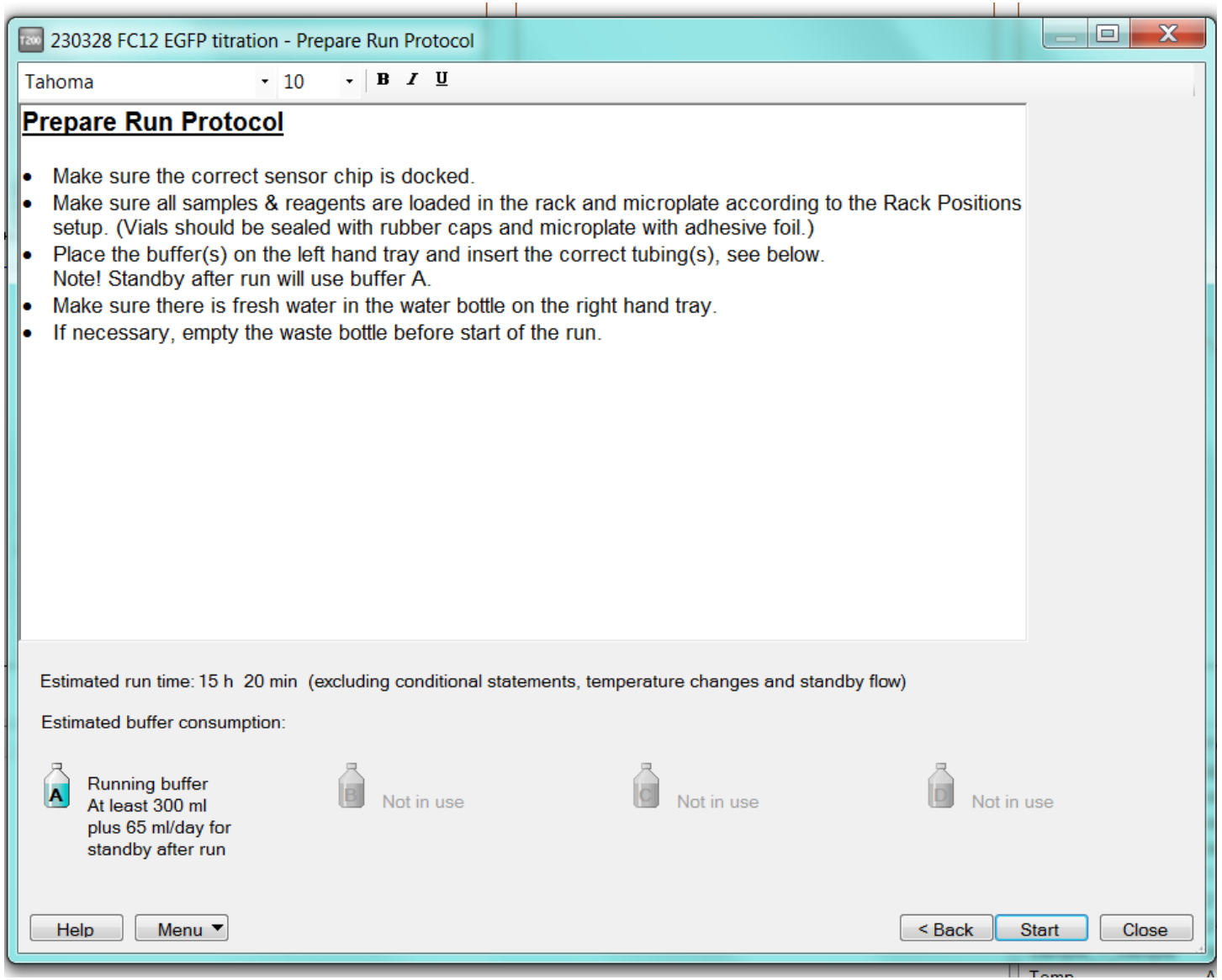

230328 FC12 EGFP titration - Samples
Samples
Sample id
MW (Da)
Concentration
nM
Concentration
µg/ml
1 EGFP 0
2 EGFP 0,015625
3 EGFP 0,03125
4 EGFP 0,0625
5 EGFP 0,125
6 EGFP 0,25
7 EGFP 0,5
8 EGFP 1
9 EGFP 2
10 mTurquoise2 0
11 mTurquoise2 0,015625
12 mTurquoise2 0,03125
13 mTurquoise2 0,0625
14 mTurquoise2 0,125
15 mTurquoise2 0,25
16 mTurquoise2 0,5
17 mTurquoise2 1
18 mTurquoise2 2
19 mVenus 0
20 mVenus 0,015625
21 mVenus 0,03125
22 mVenus 0,0625
23 mVenus 0,125
Run order
As entered
Increasing concentration
Help
Import...
Control Samples...
< Back
Next >
Close

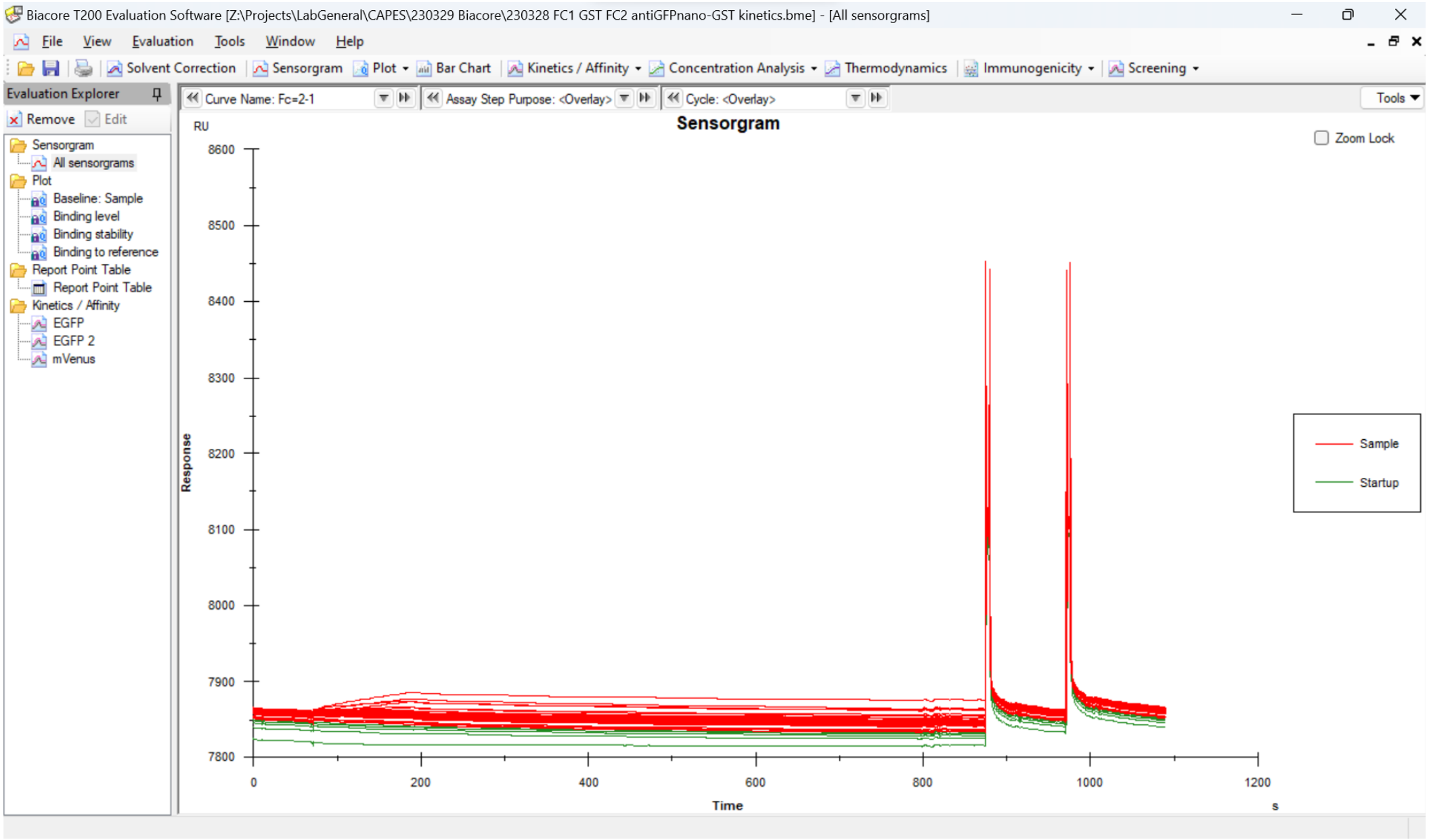

230328 FC12 EGFP titration - Samples
Samples
Sample id
MW (Da)
Concentration
nM
Concentration
µg/ml
1 EGFP 0
2 EGFP 0,015625
3 EGFP 0,03125
4 EGFP 0,0625
Recommended settings are not followed
Sample serie: EGFP
The sample series should contain at least one non-zero concentration that is to be run at least two (2) times.
Sample serie: mTurquoise2
The sample series should contain at least one non-zero concentration that is to be run at least two (2) times.
Sample serie: mVenus
The sample series should contain at least one non-zero concentration that is to be run at least two (2) times.
Sample serie: mCherry
The sample series should contain at least one non-zero concentration that is to be run at least two (2) times.
OK
Ignore
23 mVenus 0,125
Run order
As entered
Increasing concentration
Help
Import...
Control Samples...
< Back
Next >
Close


230328 FC12 EGFP titration - System Preparations
Prime before run
Normalize detector
Temperature settings
Analysis temperature:
25
(°C)
Sample compartment temperature:
25
(°C)
Help
Cycle Run List...
< Back
Next >
Close



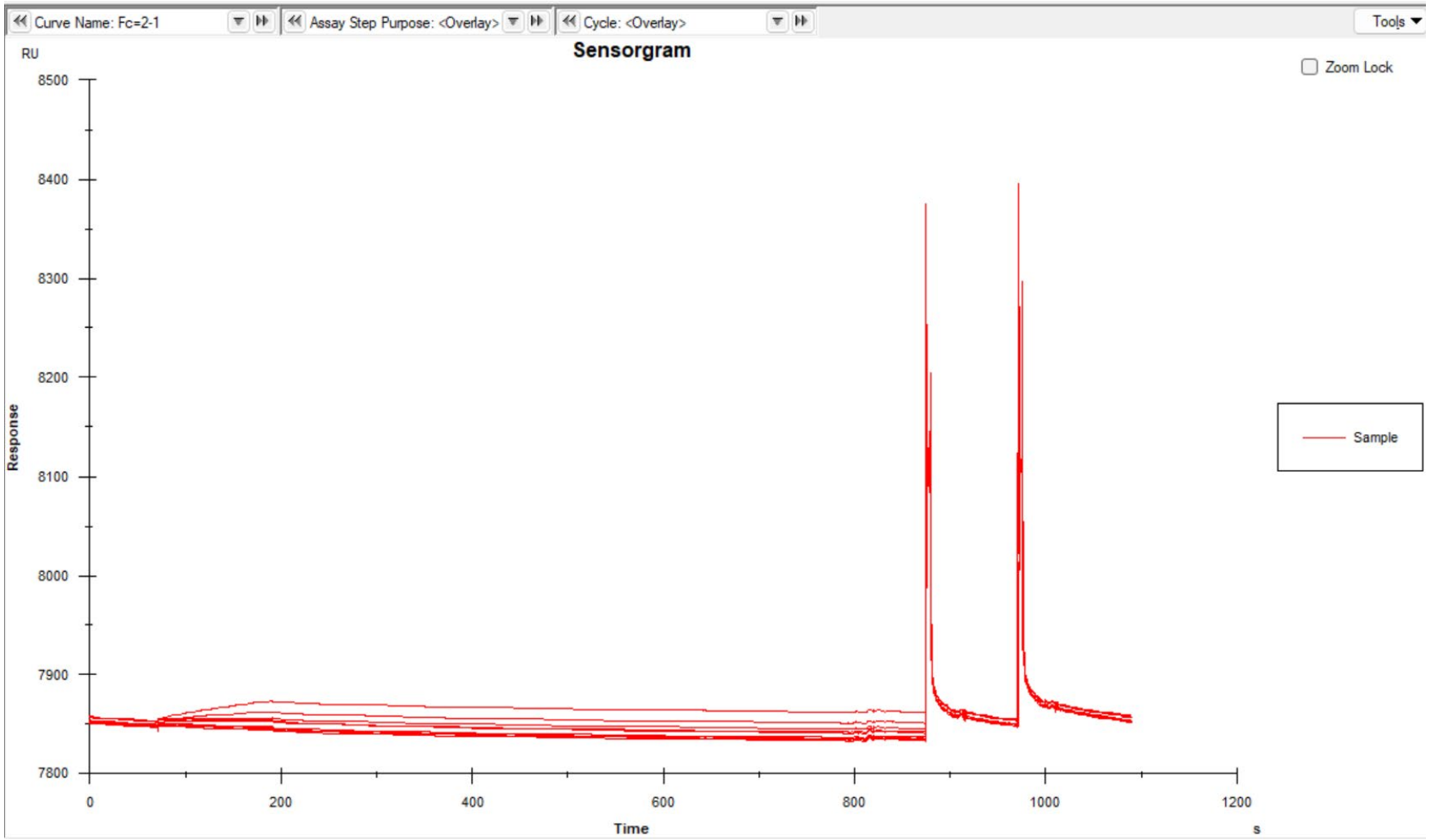
Position	Volume (µl)	Content	Type	Sample 1 Conc (nM)	Sam MW
R1 A8	158	EGFP	Sample	1	
R1 A9	158	EGFP	Sample	2	
R1 A10	158	EGFP 2	Sample	0	
R1 A11	158	EGFP 2	Sample	0,015625	
R1 A12	158	EGFP 2	Sample	0,03125	
R1 A13	158	EGFP 2	Sample	0,0625	
R1 A14	158	EGFP 2	Sample	0,125	
R1 A15	158	EGFP 2	Sample	0,25	
R1 B1	158	EGFP 2	Sample	0,5	
R1 B2	158	EGFP 2	Sample	1	
R1 B3	158	EGFP 2	Sample	2	
R1 B4	158	mCherry	Sample	0	
R1 B5	158	mCherry	Sample	0,015625	
R1 B6	158	mCherry	Sample	0,03125	
R1 B7	158	mCherry	Sample	0,0625	
R1 B8	158	mCherry	Sample	0,125	
R1 B9	158	mCherry	Sample	0,25	
R1 B10	158	mCherry	Sample	0,5	
R1 B11	158	mCherry	Sample	1	
R1 B12	158	mCherry	Sample	2	
R1 B13	158	mTurquoise2	Sample	0	
R1 B14	158	mTurquoise2	Sample	0,015625	
R1 B15	158	mTurquoise2	Sample	0,03125	
R1 C1	158	mTurquoise2	Sample	0,0625	
R1 C2	158	mTurquoise2	Sample	0,125	
R1 C3	158	mTurquoise2	Sample	0,25	
R1 C4	158	mTurquoise2	Sample	0,5	
R1 C5	158	mTurquoise2	Sample	1	
R1 C6	158	mTurquoise2	Sample	2	
R1 C7	158	mVenus	Sample	0	
R1 C8	158	mVenus	Sample	0,015625	
R1 C9	158	mVenus	Sample	0,03125	
R1 C10	158	mVenus	Sample	0,0625	
R1 C11	158	mVenus	Sample	0,125	
R1 C12	158	mVenus	Sample	0,25	
R1 C13	158	mVenus	Sample	0,5	
R1 C14	158	mVenus	Sample	1	
R1 C15	158	mVenus	Sample	2	
R1 D1	760	HBS-EP+	Startup		
R1 F1	3800	200 mM Gly pH 2.0	Regeneration		

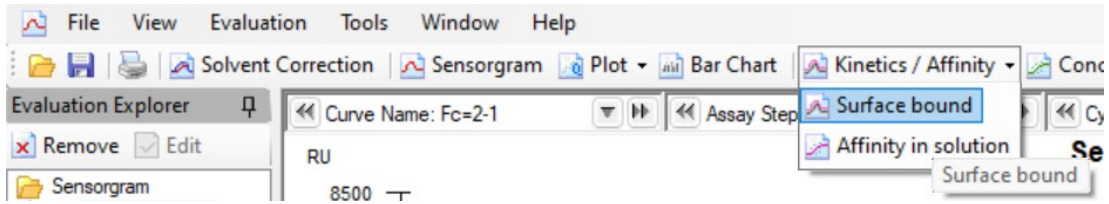

230328 FC12 EGFP titration - Prepare Run Protocol
Tahoma
10
Prepare Run Protocol
Make sure the correct sensor chip is docked.
Make sure all samples & reagents are loaded in the rack and microplate according to the Rack Positions setup. (Vials should be sealed with rubber caps and microplate with adhesive foil.)
Place the buffer(s) on the left hand tray and insert the correct tubing(s), see below.
Note! Standby after run will use buffer A.
Make sure there is fresh water in the water bottle on the right hand tray.
If necessary, empty the waste bottle before start of the run.
Estimated run time: 15 h 20 min (excluding conditional statements, temperature changes and standby flow)
Estimated buffer consumption:
A
Running buffer
At least 300 ml
plus 65 ml/day for
standby after run
B
Not in use
C
Not in use
D
Not in use
Help
Menu
< Back
Start
Close

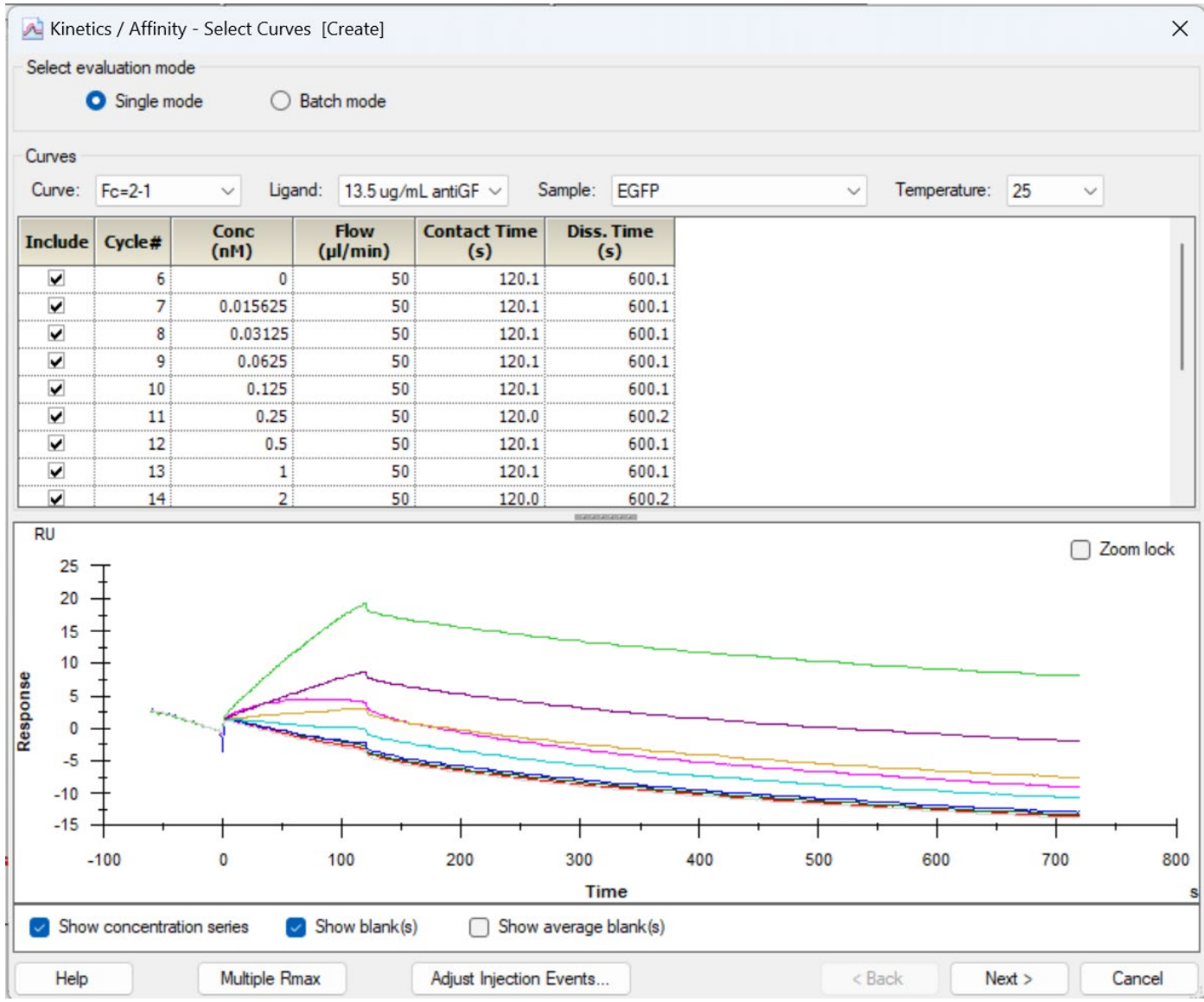

Biacore T200 Evaluation Software [Z:\Projects\LabGeneral\CAPES\230329 Biacore\230328 FC1 GST FC2 antiGFPnano-GST kinetics.bme] - [All sensorgrams]
File View Evaluation Tools Window Help
Solvent Correction Sensorgram Plot Bar Chart Kinetics / Affinity Concentration Analysis Thermodynamics Immunogenicity Screening
Evaluation Explorer
Remove Edit
Sensorgram
All sensorgrams
Plot
Baseline: Sample
Binding level
Binding stability
Binding to reference
Report Point Table
Report Point Table
Kinetics / Affinity
EGFP
EGFP 2
mVenus
Curve Name: Fc=2-1
Assay Step Purpose: <Overlay>
Cycle: <Overlay>
Tools
Sensorgram
RU
Zoom Lock
Response
8600
8500
8400
8300
8200
8100
8000
7900
7800
0
200
400
600
800
1000
1200
Time
s
Sample
Startup

Cycle: <Overlay>

Included	Cycle#	Assay Step Purpose	Sample Name	Conc.	MW
Yes	5	Startup	HBS-EP+		
Yes	6	Sample	EGFP	0	
Yes	7	Sample	EGFP	0.015625	
Yes	8	Sample	EGFP	0.03125	
Yes	9	Sample	EGFP	0.0625	
Yes	10	Sample	EGFP	0.125	
Yes	11	Sample	EGFP	0.25	
Yes	12	Sample	EGFP	0.5	
Yes	13	Sample	EGFP	1	
Yes	14	Sample	EGFP	2	


Curve Name: Fc=2-1
Assay Step Purpose: <Overlay>
Cycle: <Overlay>
Tools
Sensorgram
RU
Zoom Lock
8500
8400
8300
8200
8100
8000
7900
7800
Response
Sample
0
200
400
600
800
1000
1200
Time
s


File
View
Evaluation
Tools
Window
Help
Solvent Correction
Sensorgram
Plot
Bar Chart
Kinetics / Affinity
Surface bound
Affinity in solution
Surface bound
Evaluation Explorer
Remove
Edit
Sensorgram
Curve Name: Fc=2-1
Assay Step
RU
8500


Kinetics / Affinity - Select Curves [Create]
Select evaluation mode
Single mode
Batch mode
Curves
Curve: Fc=2-1
Ligand: 13.5 ug/mL antiGF
Sample: EGFP
Temperature: 25
Include | Cycle# | Conc (nM) | Flow (µl/min) | Contact Time (s) | Diss. Time (s)
6 | 0 | 50 | 120.1 | 600.1
7 | 0.015625 | 50 | 120.1 | 600.1
8 | 0.03125 | 50 | 120.1 | 600.1
9 | 0.0625 | 50 | 120.1 | 600.1
10 | 0.125 | 50 | 120.1 | 600.1
11 | 0.25 | 50 | 120.0 | 600.2
12 | 0.5 | 50 | 120.1 | 600.1
13 | 1 | 50 | 120.1 | 600.1
14 | 2 | 50 | 120.0 | 600.2
RU
Zoom lock
Response
Time
s
Show concentration series
Show blank(s)
Show average blank(s)
Help
Multiple Rmax
Adjust Injection Events...
< Back
Next >
Cancel

Kinetics / Affinity - Select Curves [Create]

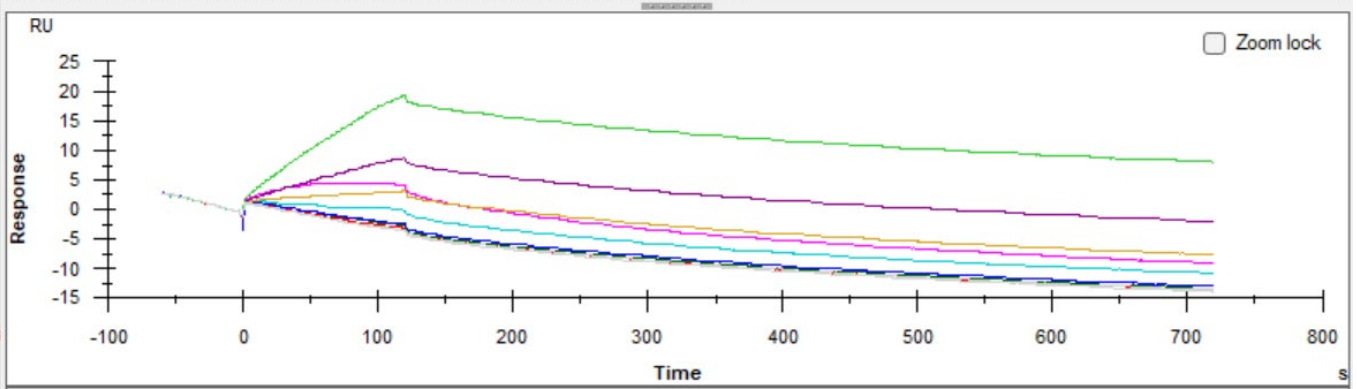
Select evaluation mode

(●) Single mode ( ) Batch mode

Curves

Curve: Fc=2-1 Ligand: 13.5 ug/mL antiGF Sample: EGFP Temperature: 25

Include	Cycle#	Conc (nM)	Flow (µl/min)	Contact Time (s)	Diss. Time (s)
☑	6	0	50	120.1	600.1
☑	7	0.015625	50	120.1	600.1
☑	8	0.03125	50	120.1	600.1
☑	9	0.0625	50	120.1	600.1
☑	10	0.125	50	120.1	600.1
☑	11	0.25	50	120.0	600.2
☑	12	0.5	50	120.1	600.1
☑	13	1	50	120.1	600.1
☑	14	2	50	120.0	600.2
	Blanks from other sample series				
☑	15	0	50	120.1	600.1
☐	24	0	50	120.1	600.0
☐	33	0	50	120.1	600.1
☐	42	0	50	120.1	600.1



☑ Show concentration series ☑ Show blank(s) ☐ Show average blank(s)

Help | Multiple Rmax | Adjust Injection Events... | < Back | Next > | Cancel

Kinetics / Affinity - Select Curves [Create]

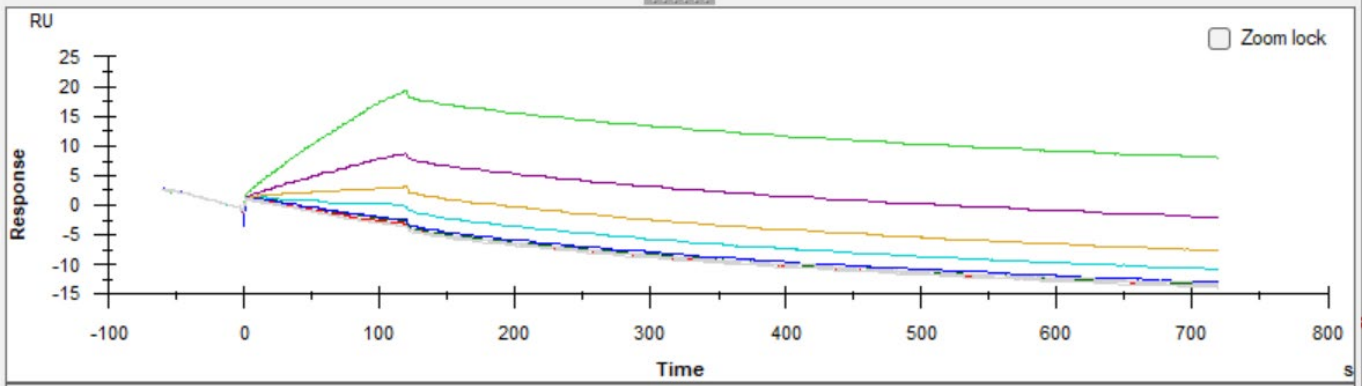
Select evaluation mode

(●) Single mode ( ) Batch mode

Curves

Curve: Fc=2-1 Ligand: 13.5 ug/mL antiGF Sample: EGFP Temperature: 25

Include	Cycle#	Conc (nM)	Flow (µl/min)	Contact Time (s)	Diss. Time (s)
☑	6	0	50	120.1	600.1
☑	7	0.015625	50	120.1	600.1
☑	8	0.03125	50	120.1	600.1
☑	9	0.0625	50	120.1	600.1
☐	10	0.125	50	120.1	600.1
☑	11	0.25	50	120.0	600.2
☑	12	0.5	50	120.1	600.1
☑	13	1	50	120.1	600.1
☑	14	2	50	120.0	600.2
	Blanks from other sample series				
☑	15	0	50	120.1	600.1
☑	24	0	50	120.1	600.0
☐	33	0	50	120.1	600.1
☐	42	0	50	120.1	600.1



☑ Show concentration series ☑ Show blank(s) ☐ Show average blank(s)

Help | Multiple Rmax | Adjust Injection Events... | < Back | Next > | Cancel

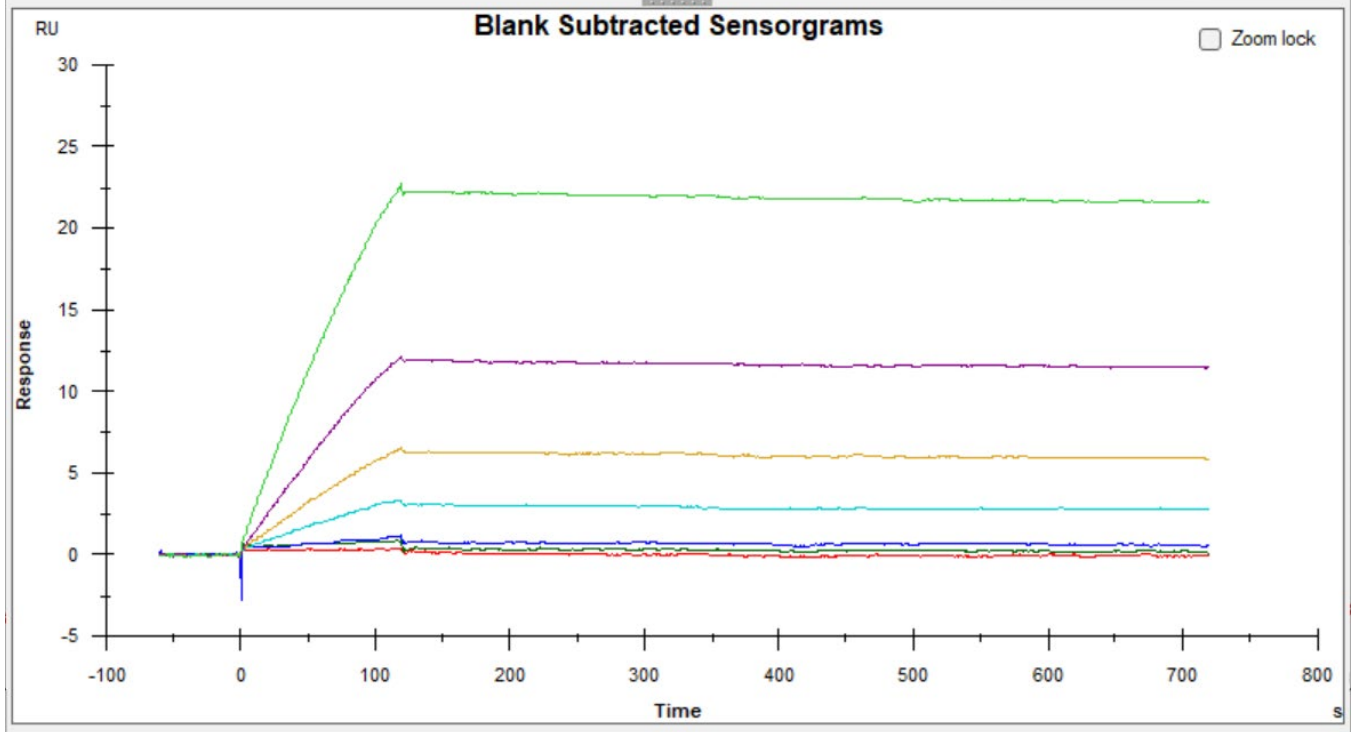
Kinetics / Affinity - Select Data [Create]

Curves

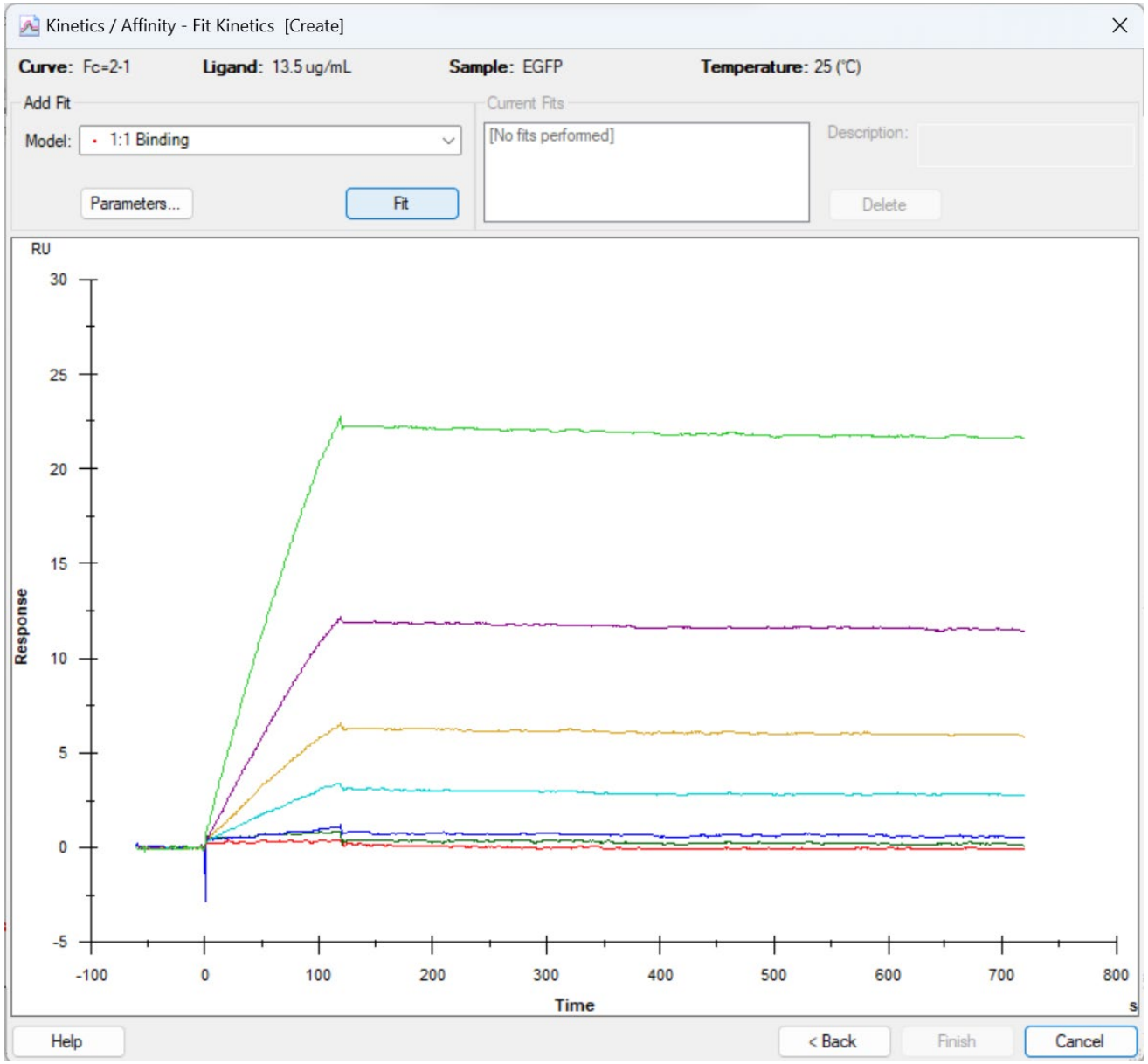
Curve: Fc=2-1 **Ligand:** 13.5 ug/mL **Sample:** EGFP **Temperature:** 25 (°C)

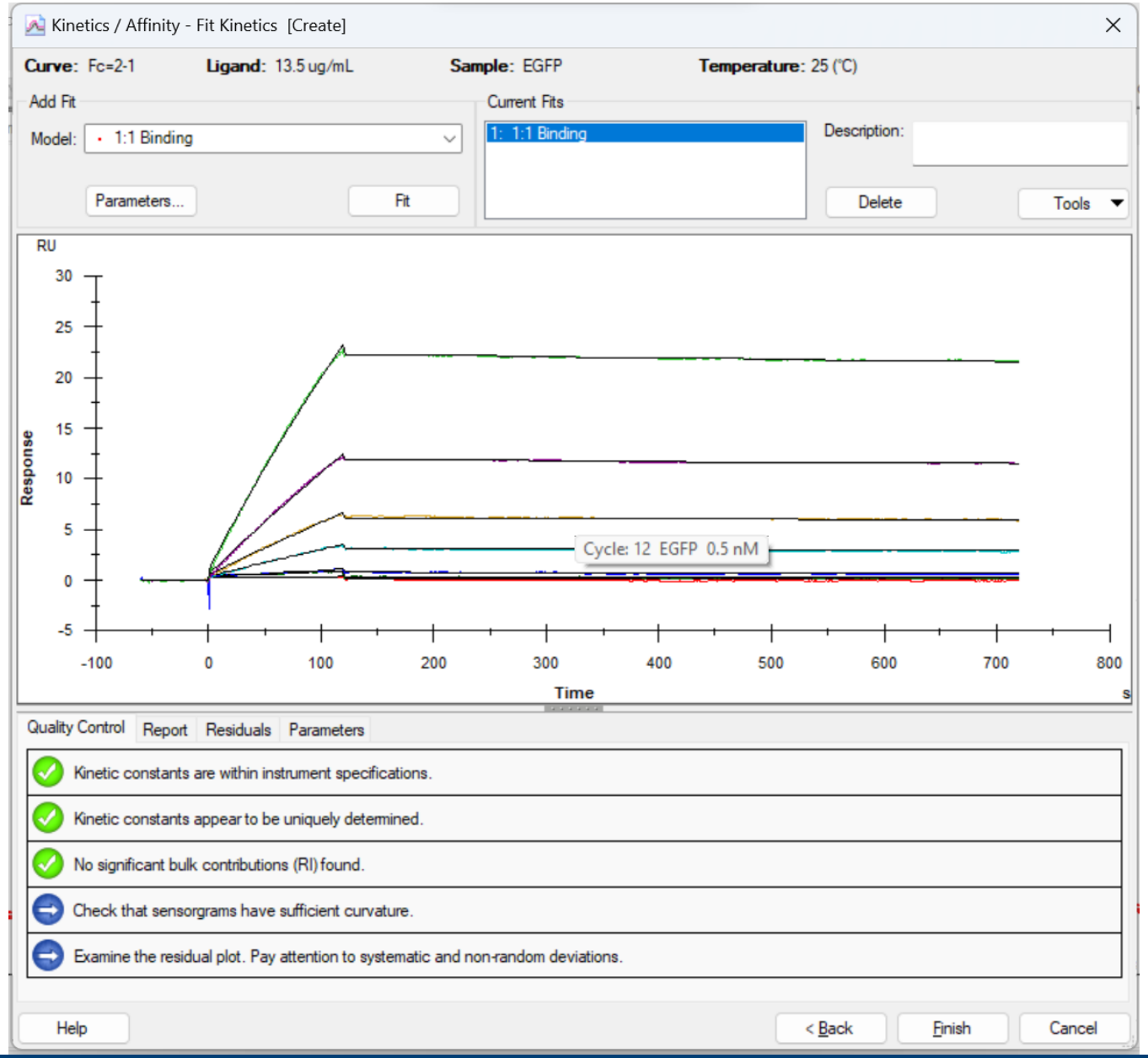
Edit	Cycle#	Curve	Conc (nM)	Flow (µl/min)	Contact Time (s)	Diss. Time (s)
☑	7	Fc=2-1	0.015625	50	120.1	600.1
☑	8	Fc=2-1	0.03125	50	120.1	600.1
☑	9	Fc=2-1	0.0625	50	120.1	600.1
☑	11	Fc=2-1	0.25	50	120.0	600.2
☑	12	Fc=2-1	0.5	50	120.1	600.1
☑	13	Fc=2-1	1	50	120.1	600.1
☑	14	Fc=2-1	2	50	120.0	600.2

Remove Selection Undo



Help < Back Affinity > Kinetics > Cancel


Kinetics / Affinity - Fit Kinetics [Create]
Curve: Fc=2-1
Ligand: 13.5 ug/mL
Sample: EGFP
Temperature: 25 (°C)
Add Fit
Model: 1:1 Binding
Parameters...
Fit
Current Fits
[No fits performed]
Description:
Delete
RU
30
25
20
15
10
5
0
-5
Response
-100
0
100
200
300
400
500
600
700
800
Time
s
Help
< Back
Finish
Cancel

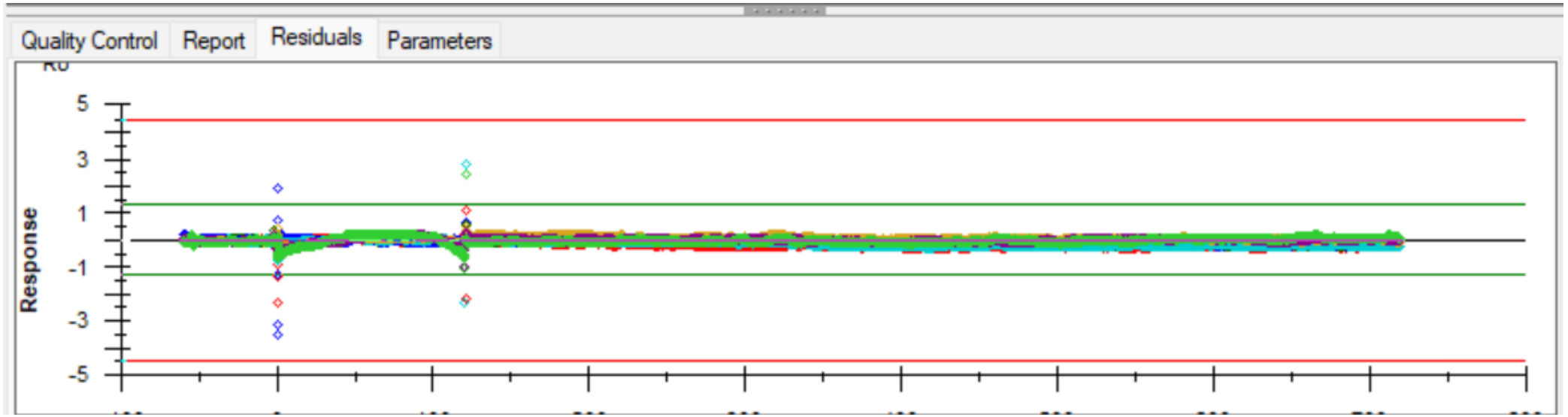

Kinetics / Affinity - Fit Kinetics [Create]
Curve: Fc=2-1
Ligand: 13.5 ug/mL
Sample: EGFP
Temperature: 25 (°C)
Add Fit
Model: 1:1 Binding
Parameters...
Fit
Current Fits
1: 1:1 Binding
Description:
Delete
Tools
RU
Response
Time
s
Cycle: 12 EGFP 0.5 nM
Quality Control
Report
Residuals
Parameters
Kinetic constants are within instrument specifications.
Kinetic constants appear to be uniquely determined.
No significant bulk contributions (RI) found.
Check that sensorgrams have sufficient curvature.
Examine the residual plot. Pay attention to systematic and non-random deviations.
Help
< Back
Finish
Cancel

Quality Control | Report | Residuals | Parameters

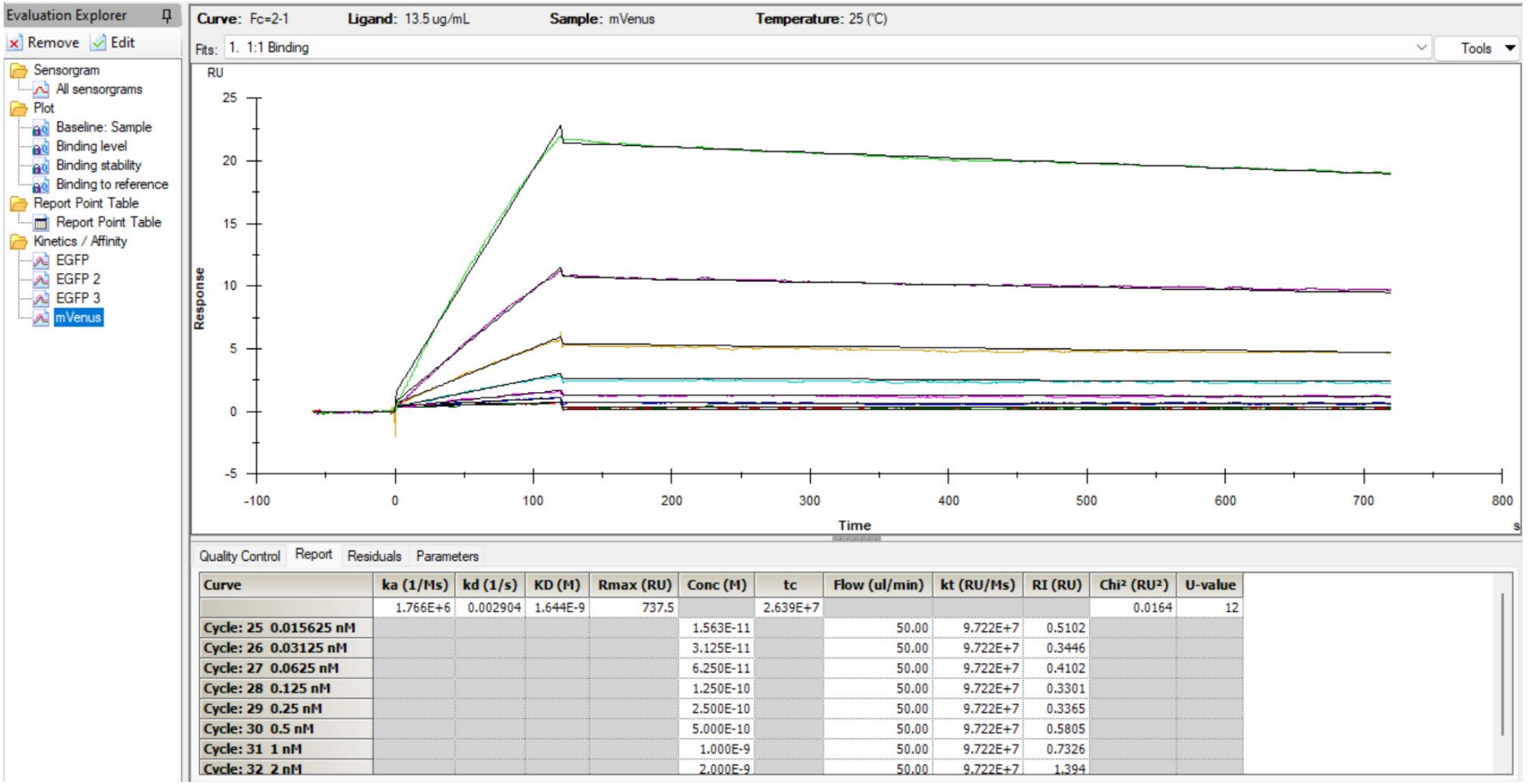
Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)
	1.797E+6	7.261E-5	4.040E-11	78.04		1.169E+8			
Cycle: 7 0.015625 nM					1.563E-11		50.00	4.307E+8	0.2150
Cycle: 8 0.03125 nM					3.125E-11		50.00	4.307E+8	0.4908
Cycle: 9 0.0625 nM					6.250E-11		50.00	4.307E+8	0.3390
Cycle: 11 0.25 nM					2.500E-10		50.00	4.307E+8	0.4108
Cycle: 12 0.5 nM					5.000E-10		50.00	4.307E+8	0.5581
Cycle: 13 1 nM					1.000E-9		50.00	4.307E+8	0.6197
Cycle: 14 2 nM					2.000E-9		50.00	4.307E+8	1.076

Quality Control | Report | Residuals | Parameters

Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)
	1.797E+6	7.261E-5	4.040E-11	78.04		1.169E+8			
Cycle: 7 0.015625 nM					1.563E-11		50.00	4.307E+8	0.2150
Cycle: 8 0.03125 nM					3.125E-11		50.00	4.307E+8	0.4908
Cycle: 9 0.0625 nM					6.250E-11		50.00	4.307E+8	0.3390
Cycle: 11 0.25 nM					2.500E-10		50.00	4.307E+8	0.4108
Cycle: 12 0.5 nM					5.000E-10		50.00	4.307E+8	0.5581
Cycle: 13 1 nM					1.000E-9		50.00	4.307E+8	0.6197
Cycle: 14 2 nM					2.000E-9		50.00	4.307E+8	1.076

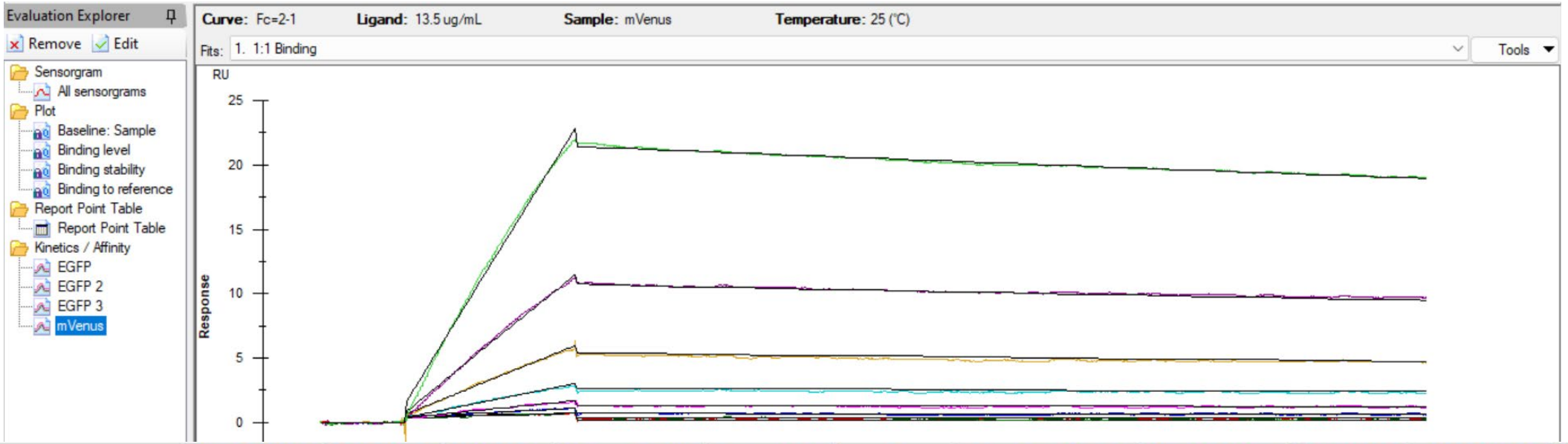


mVenus



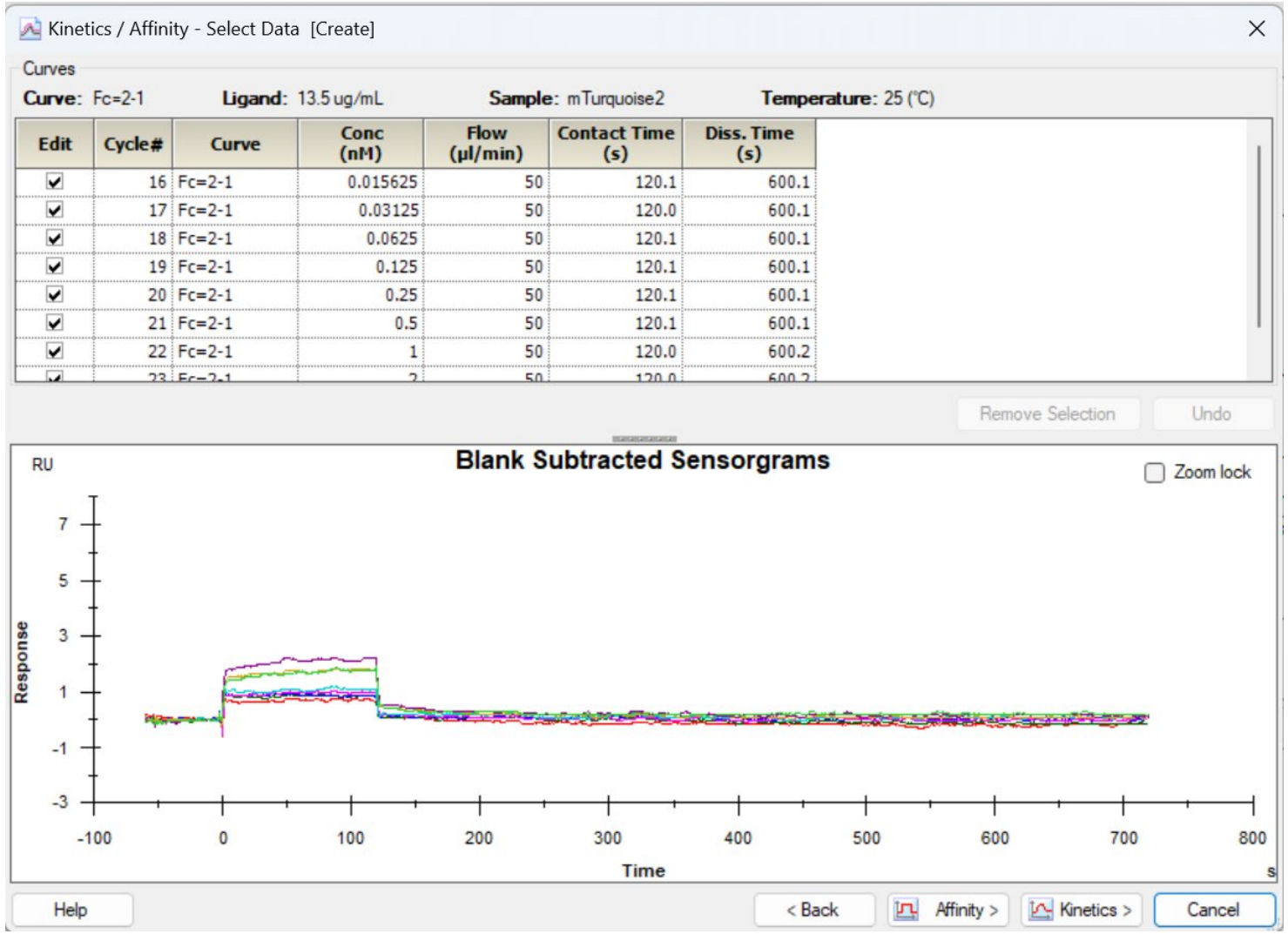
Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	1.766E+6	0.002904	1.644E-9	737.5		2.639E+7				0.0164	12
Cycle: 25 0.015625 nM					1.563E-11		50.00	9.722E+7	0.5102		
Cycle: 26 0.03125 nM					3.125E-11		50.00	9.722E+7	0.3446		
Cycle: 27 0.0625 nM					6.250E-11		50.00	9.722E+7	0.4102		
Cycle: 28 0.125 nM					1.250E-10		50.00	9.722E+7	0.3301		
Cycle: 29 0.25 nM					2.500E-10		50.00	9.722E+7	0.3365		
Cycle: 30 0.5 nM					5.000E-10		50.00	9.722E+7	0.5805		
Cycle: 31 1 nM					1.000E-9		50.00	9.722E+7	0.7326		
Cycle: 32 2 nM					2.000E-9		50.00	9.722E+7	1.394		

mVenus

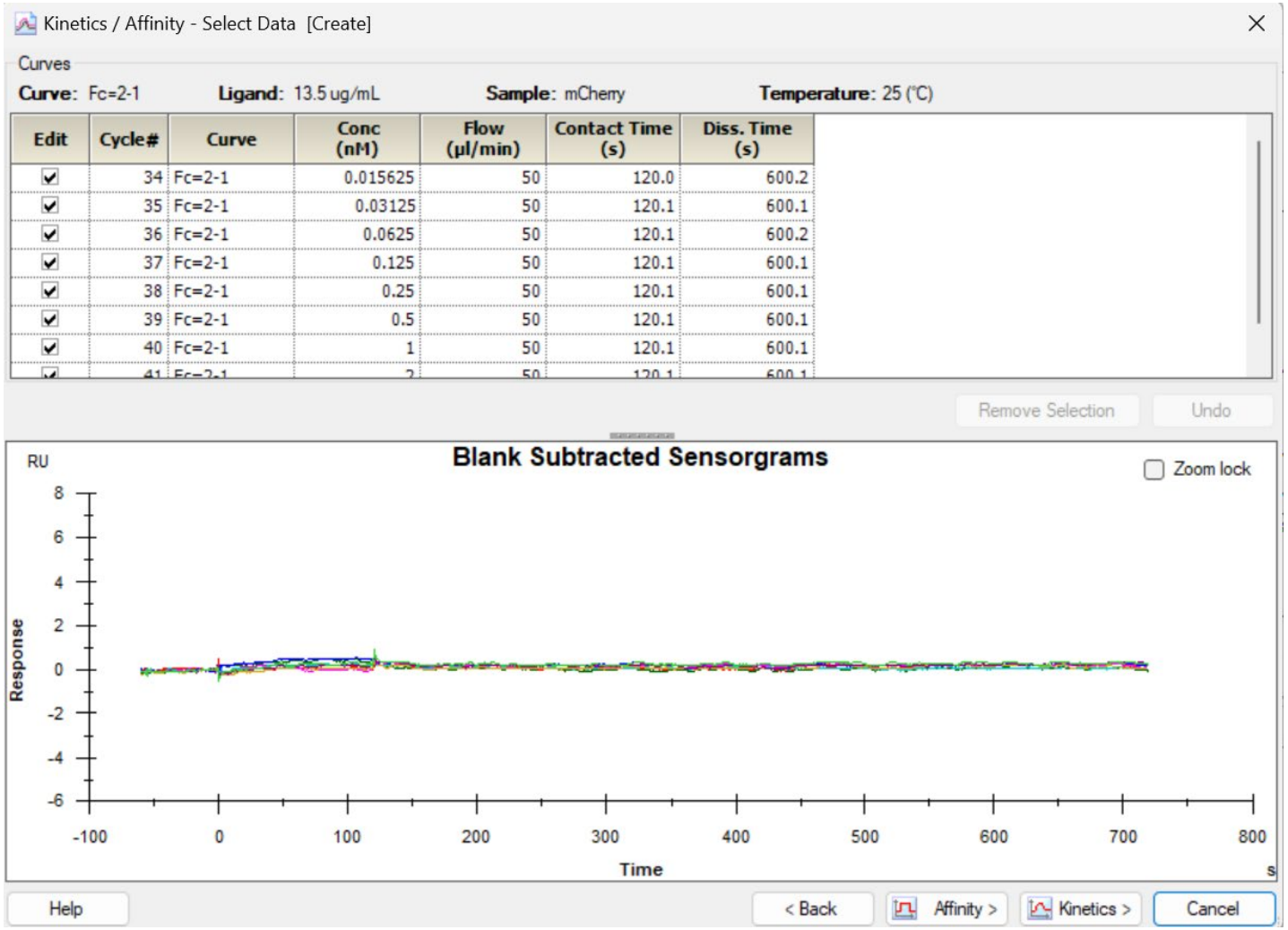


Curve	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Conc (M)	tc	Flow (ul/min)	kt (RU/Ms)	RI (RU)	Chi² (RU²)	U-value
	1.766E+6	0.002904	1.644E-9	737.5		2.639E+7				0.0164	12
Cycle: 25 0.015625 nM					1.563E-11		50.00	9.722E+7	0.5102		
Cycle: 26 0.03125 nM					3.125E-11		50.00	9.722E+7	0.3446		
Cycle: 27 0.0625 nM					6.250E-11		50.00	9.722E+7	0.4102		
Cycle: 28 0.125 nM					1.250E-10		50.00	9.722E+7	0.3301		
Cycle: 29 0.25 nM					2.500E-10		50.00	9.722E+7	0.3365		
Cycle: 30 0.5 nM					5.000E-10		50.00	9.722E+7	0.5805		
Cycle: 31 1 nM					1.000E-9		50.00	9.722E+7	0.7326		
Cycle: 32 2 nM					2.000E-9		50.00	9.722E+7	1.394		

mTurquoise2



mCherry


Kinetics / Affinity - Select Data [Create]
Curves
Curve: Fc=2-1
Ligand: 13.5 ug/mL
Sample: mCherry
Temperature: 25 (°C)
Edit | Cycle# | Curve | Conc (nM) | Flow (µl/min) | Contact Time (s) | Diss. Time (s)
34 | Fc=2-1 | 0.015625 | 50 | 120.0 | 600.2
35 | Fc=2-1 | 0.03125 | 50 | 120.1 | 600.1
36 | Fc=2-1 | 0.0625 | 50 | 120.1 | 600.2
37 | Fc=2-1 | 0.125 | 50 | 120.1 | 600.1
38 | Fc=2-1 | 0.25 | 50 | 120.1 | 600.1
39 | Fc=2-1 | 0.5 | 50 | 120.1 | 600.1
40 | Fc=2-1 | 1 | 50 | 120.1 | 600.1
Remove Selection
Undo
Blank Subtracted Sensorgrams
RU
Zoom lock
Response
Time
s
Help
< Back
Affinity >
Kinetics >
Cancel

How did we do?

	k_{on}	k_{off}	K_D
EGFP 1	1.82E+06	7.42E-05	4.09E-11
EGFP 2	1.80E+06	7.26E-05	4.04E-11
EGFP 3	1.28E+07	2.68E-05	2.10E-12

Interferometry using Octet Biosensor

k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_d (k_{off}/k_{on}) (nM)
8.84×10^4	1.24×10^{-4}	1.40

Surface plasmon resonance

k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_d (k_{off}/k_{on}) (nM)
No data	No data	0.32[b]
7.68×10^5	1.74×10^{-4}	0.23[c]

Quartz crystal microbalance[d]

k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_d (k_{off}/k_{on}) (nM)
2.45×10^5	1.45×10^{-4}	0.59 ± 0.11

Kubala et al (2010) Protein Sci 19:2389-401. doi: 10.1002/pro.519

What might we improve for next time?

- Ligand (antiGFPnano-GST)
  - Repeat with non-truncated/digested ligand
  - Capture at higher pH or further optimise regeneration to ensure we aren't damaging ligand (unclear why observed R_{max} was so much lower than theoretical)
- EGFP
  - Inject fixed concentration at different flow rates to test for mass transport
  - Use longer analyte injection so we see more curvature of traces (5 min)
- mVenus
  - Repeat with higher concentrations of mVenus (>20 nM)
- mTurqouise2
  - Repeat with much higher concentrations of mTurquoise2 (µM concentrations for an equilibrium analysis), perhaps with higher ligand capture
- mCherry
  - No binding!!

Further reading

- Cytiva manuals
  - Lots of very good reference material for the theory and practice of SPR
- SPR pages (https://sprpages.nl/)
  - Very extensive website with lots of useful tips and tricks
- Harvard Centre for Molecular Interactions (https://cmi.hms.harvard.edu/surface-plasmon-resonance)
  - Handy manuals that give you a good starting protocol for setting up your experiments
- Institut de Biologie Structurale handbook (https://www.isbg.fr/IMG/pdf/biacore_t200_getting_started_guide.pdf)