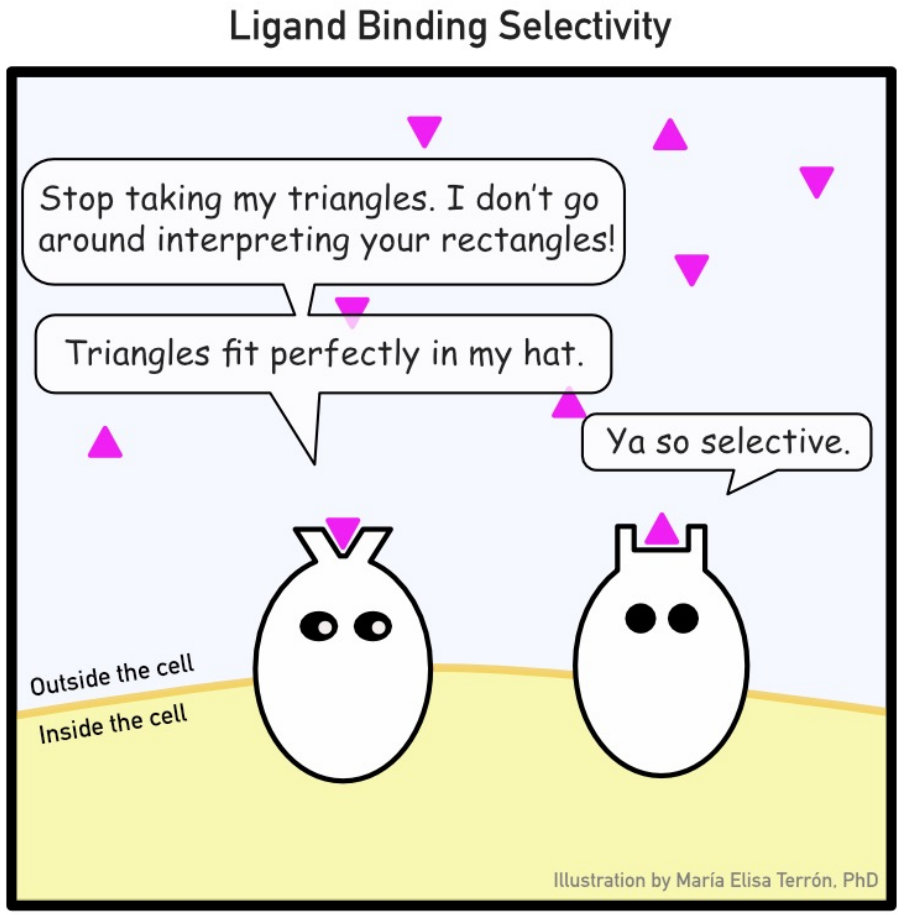
M2D5: Perform secondary assay to test putative small molecule binders

1. Prelab discussion
2. Set up plates
3. Read plates on Octect



Module 2 Roadmap

Determine putative PF3D7_20109-F21 binders via high throughput screening (SMM)

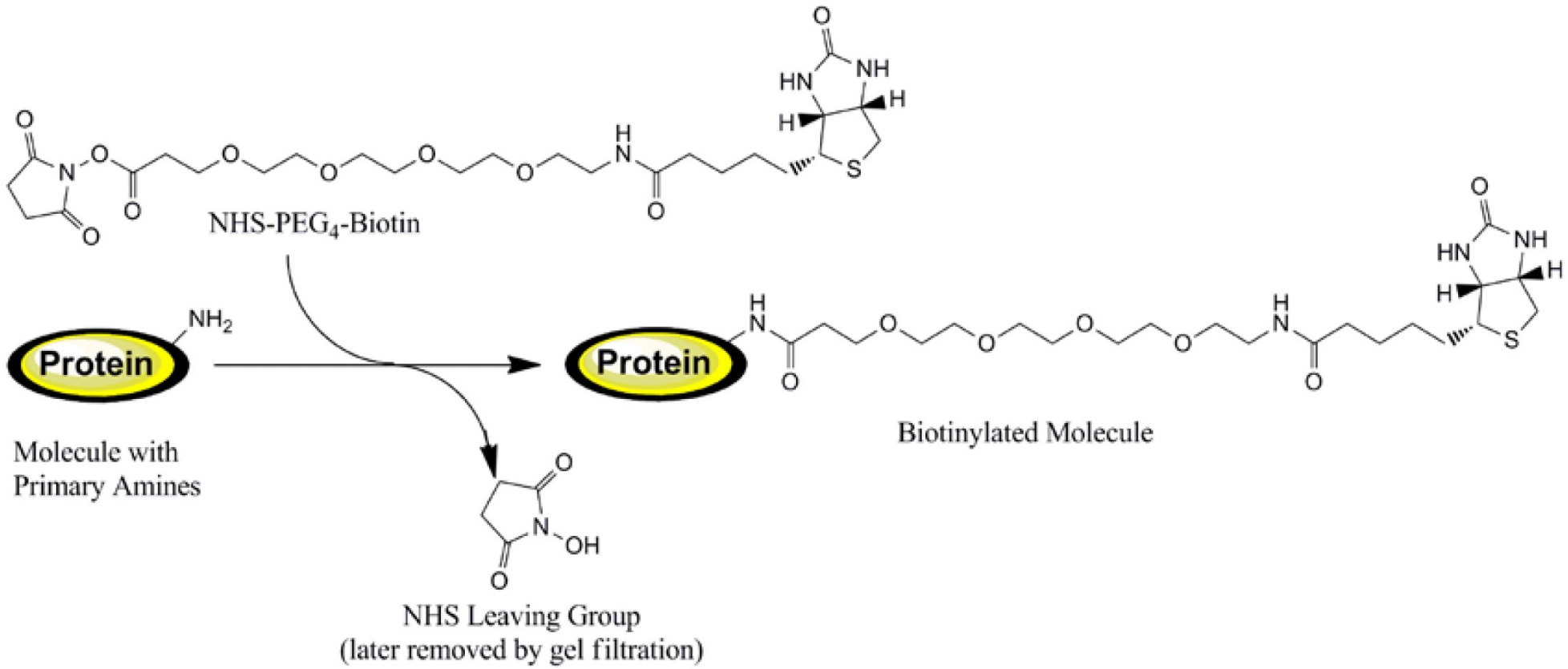
Create plasmid of PF3D7_20109-F21 to use in validation assays

Express PF3D7_20109-F21 (from plasmid) in bacteria and purify protein

Assess purity and concentration of purified protein

Use purified protein in BLI assay to validate binding of small molecules identified in SMM

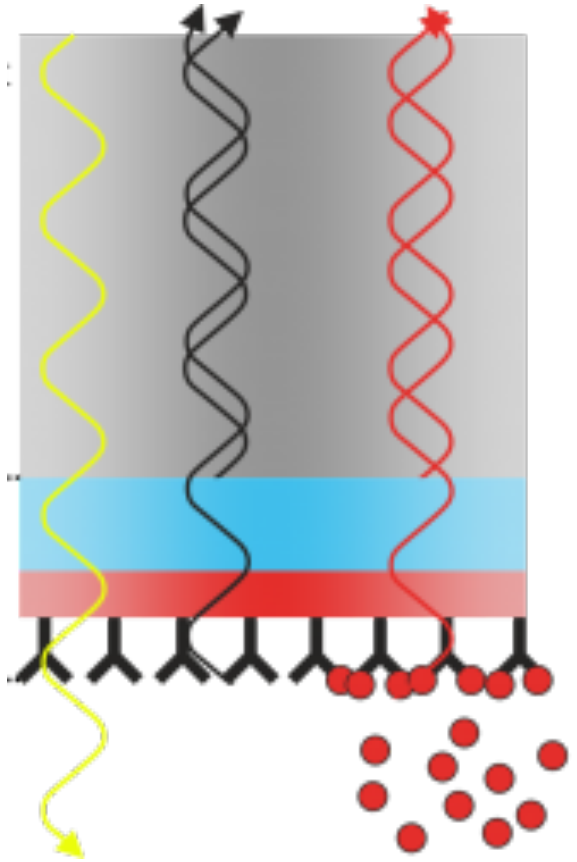
Step one of BLI experiment: biotinylate PF3D7_20109-F21



- Add biotin on PEG linker to primary amines of purified protein
- Allows us to immobilize our protein of interest to probe used in BLI experiment

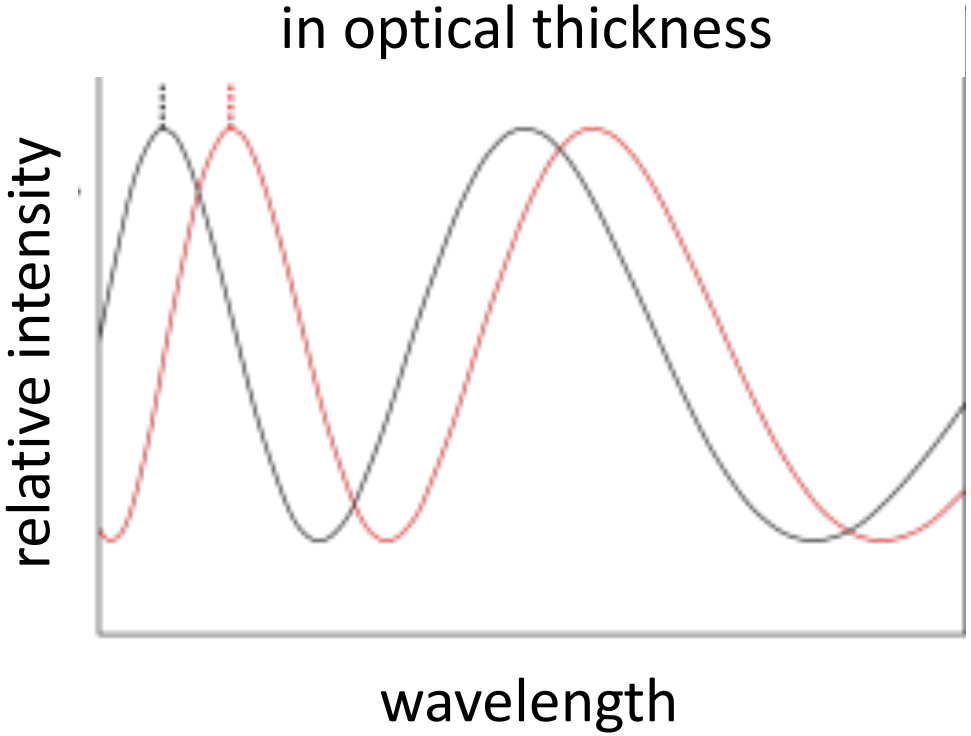
BioLayer Interferometry (BLI) by Octet

SSA (super streptavidin) Probe

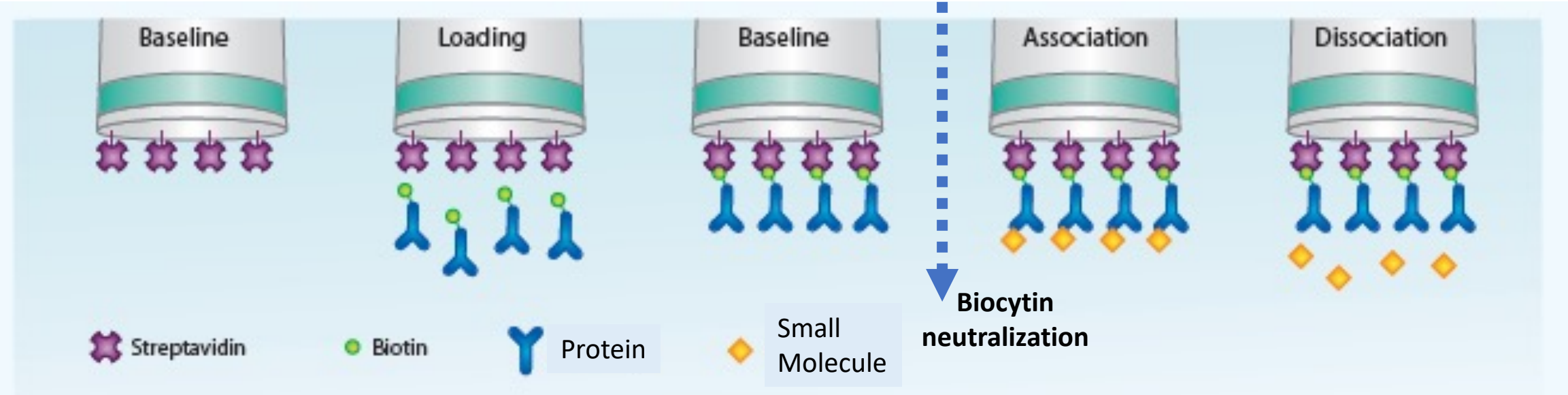


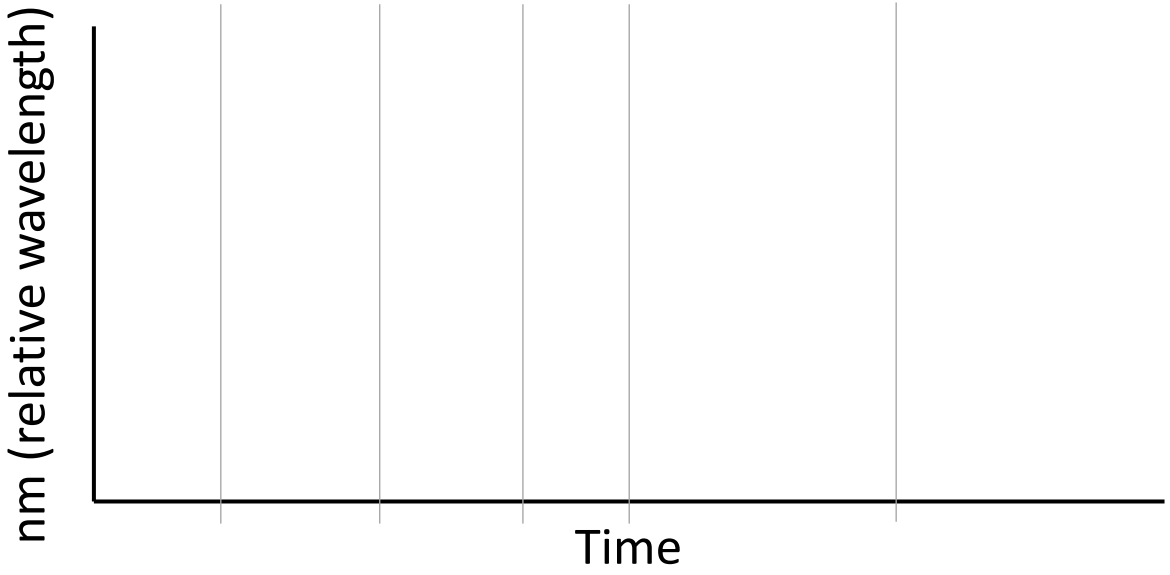
BLI
signal
processing

$\Delta\lambda$ Spectral shift due to change in optical thickness

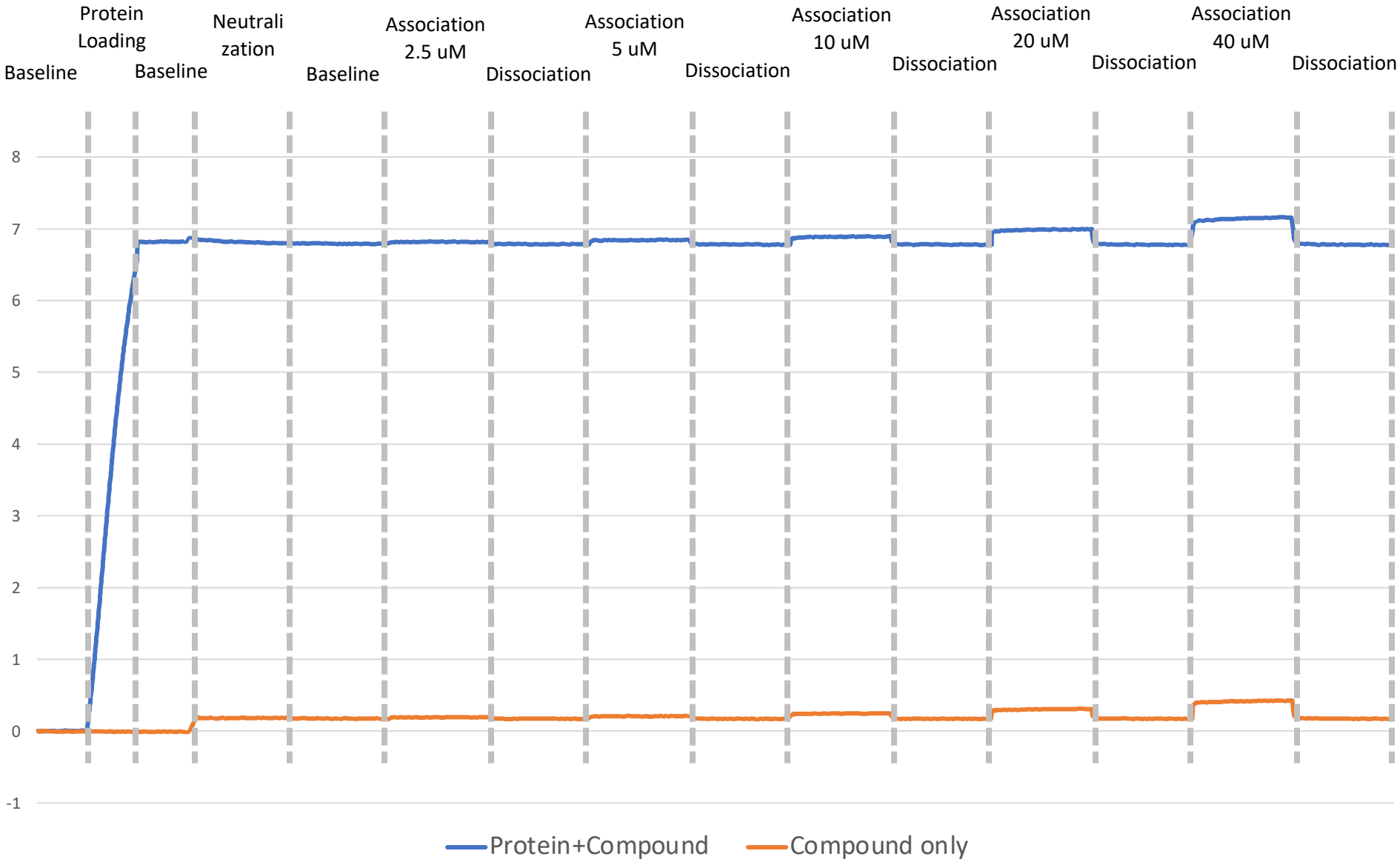


BLI run of protein-ligand binding assay





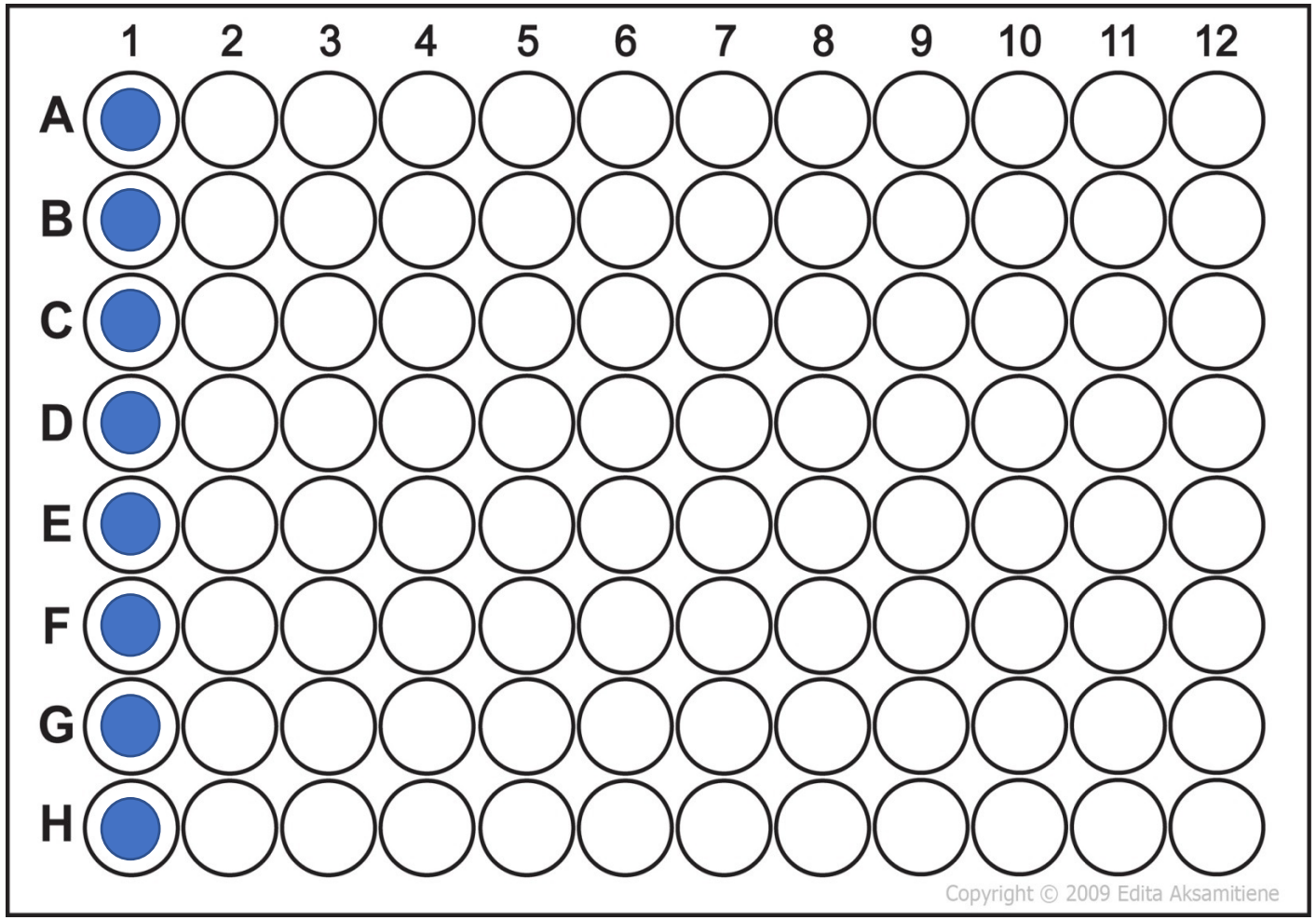
Raw data from experimental run



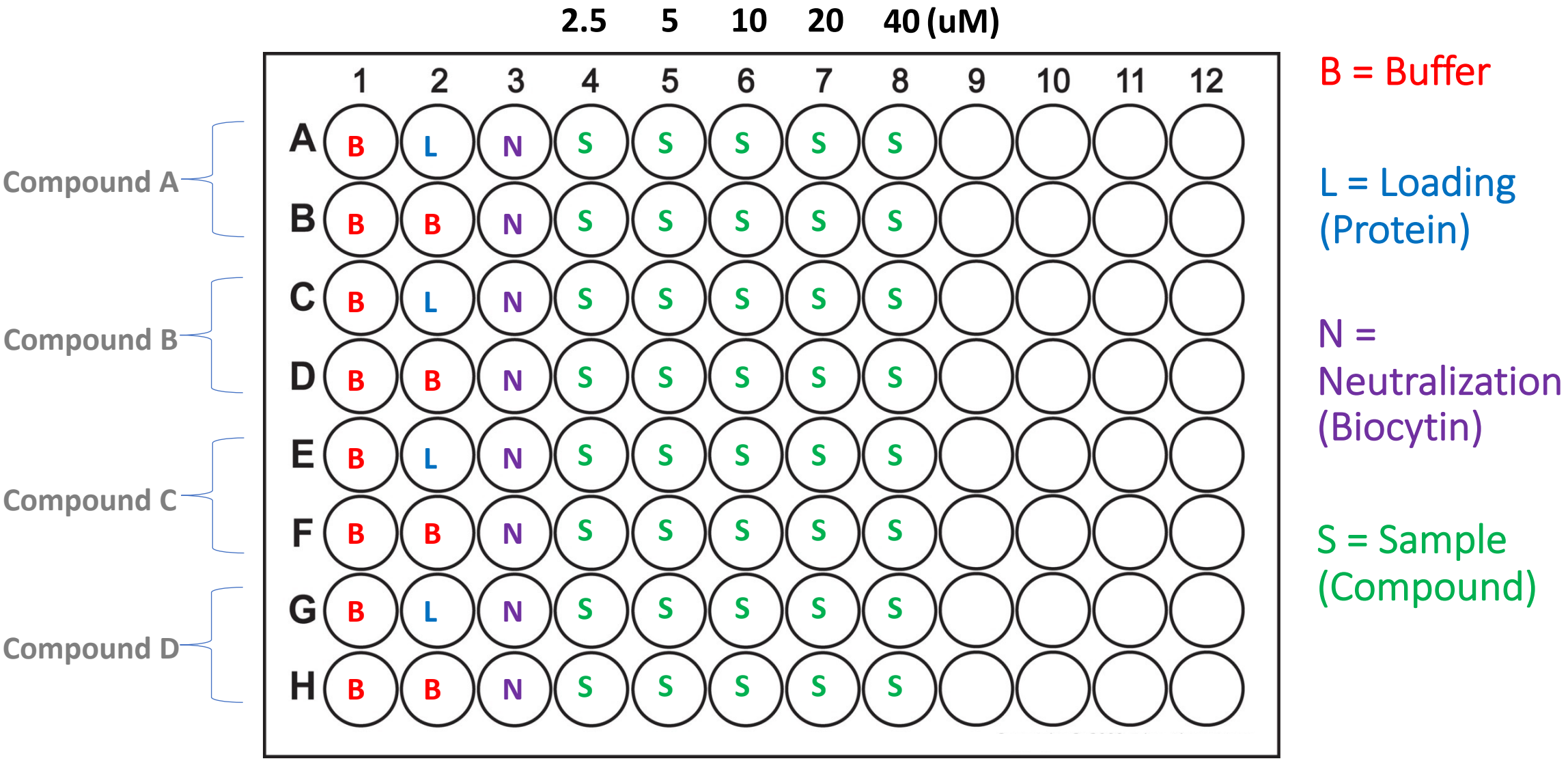
Octet Red can run 8 probes at one time

- The probe moves from column to column
- You dictate which column the probes will go on your plate.

8 pins at most for one run -



It is essential to follow the plate map



B = Buffer

L = Loading (Protein)

N = Neutralization (Biocytin)

S = Sample (Compound)

Solutions provided

1. Buffer (1 x PBS+1 mM TCEP)
2. Protein solution {(0.5 uM in buffer (1 x PBS+1 mM TCEP)}
3. Biocytin {1ug/mL in buffer (1 x PBS+1 mM TCEP)}
4. Compounds {40 uM in buffer (1 x PBS+1 mM TCEP)}

Protocol Overview

1. Make serial dilution of the compounds in the buffer (1 x PBS+1 mM TCEP) from 40 uM to 2.5 uM in Eppendorf tubes.
   - Keep these on ice until ready to use!

2. Dispense 200 uL of solutions in each well according to the plate map.
   - At front bench

3. Bring the plate to the Octet machine and start the experiment.

Groups will share plate runs

		Run Time
Plate 1:	**Positive control** **Negative control** **Red Team** (Compound 1) **Orange Team** (Compound 2)	2:15-2:45pm
Plate 2:	**Yellow Team** (Compound 3) **Gray Team** (Compound 4)	2:45-3:15pm
Plate 3:	**Green Team** (Compound 1) **Blue Team** (Compound 2) **Pink Team** (Compound 3) **Purple Team** (Compound 4)	3:30-4:00pm 4:00-4:30pm

For today…

1. Collect your reagents and set up Eppendorf tubes in accordance with group map
2. Set up plate for Octet at front bench
3. Go get you some data!

For M2D4…

- Write outline of your Introduction for the Research Article
  - Use same framework as you did for Background and Motivation from the Data Summary
  - See Wiki homework and assignment page for RA for more information