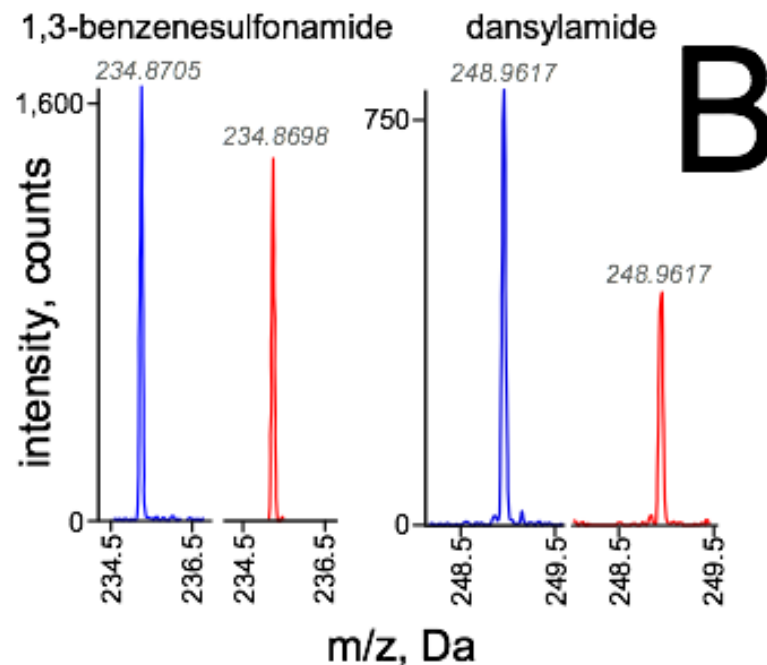
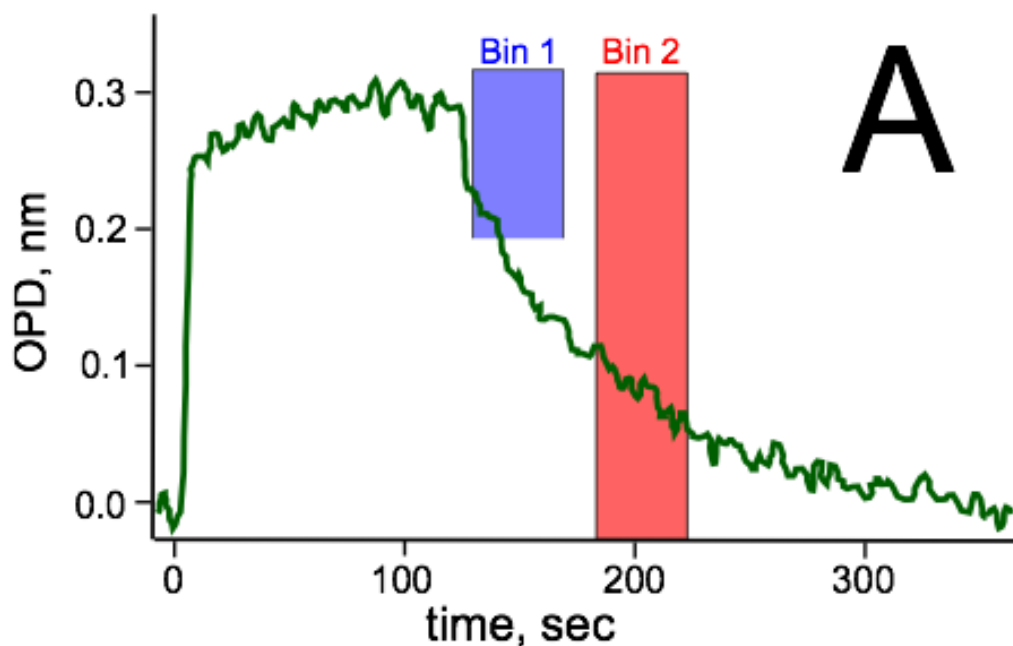


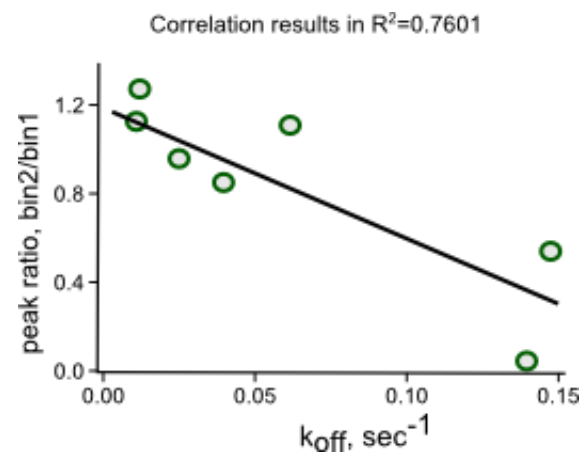
# AC-MS: Small Molecule Screening 2 of 3

- Prepare CAII surface using COOH chips
- Apply equimolar mixture of 8 sulfonamides at 33  $\mu\text{M}$
- Collect two MS-bins at different times after dissociation from CAII



# AC-MS: Small Molecule Screening 3 of 3

Analyze the MS signal ratio in the two bins, compare with  $k_{\text{off}}$ 's known from separate, single molecule at a time measurements on SKi Pro



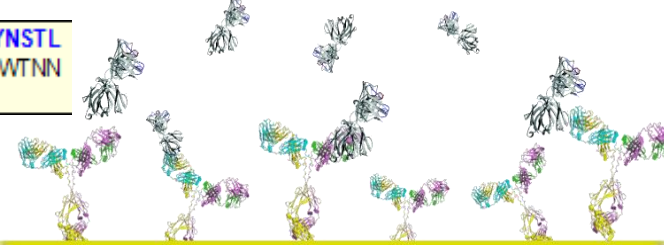
Sulfonamide	Peak (Bin 1)	Peak (Bin 2)	$k_{\text{on}}$	$k_{\text{off}}$	$K_D$ ( $\mu\text{M}$ )	bin2 - bin1	bin2 / bin1
Sulpiride	109	5.5	395	0.1	362	-104.1	0.05
Furosemide	142	181	12,400	0	0.98	39	1.27
Dansylamide	37	20.2	55,000	0.2	2.73	-16.74	0.55
1,3-benzene-disulfonamide	68	77	12,500	0	1.04	9	1.13
Acetazolamide	24	23.9	376,000	0	0.07	-0.7	0.97
Sulfanilamide	22	18.9	9,700	0	4.33	-3.1	0.86
Benzenesulfonamide	223	248	54,400	0.1	1.18	25	1.11

# AC-MS: Antigen Capture and Detection 1 of 3

1. Study the tryptic fragments of a known antigen (mouse IgG Fc Region).

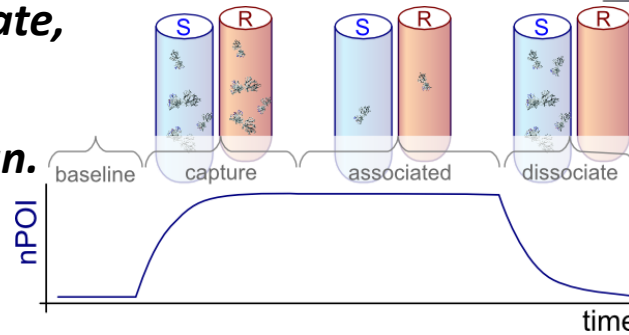
```
EPRGPTIKPCPPCKCAPNLLGGPSVFIFPPKIKDVLMSLSPVTCVWVDVSEDDPDVQISWVFVNNVEVHTAQTQTTHREDYNSTL  
RVVSAALPIQHQDWMMSGKEFKCKVNNKDLPAPIERTISKPKG SVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNN  
GKTELNYKNTEPVLDSGSGFMYSKLRVEKKNWVERNSSCSWHEGLHNHHTTKSFSRTPGK
```

2. Capture the known antigen with an IgG molecule.  
using polyclonal anti mouse IgG.



Ski Sensor npoSi Chip

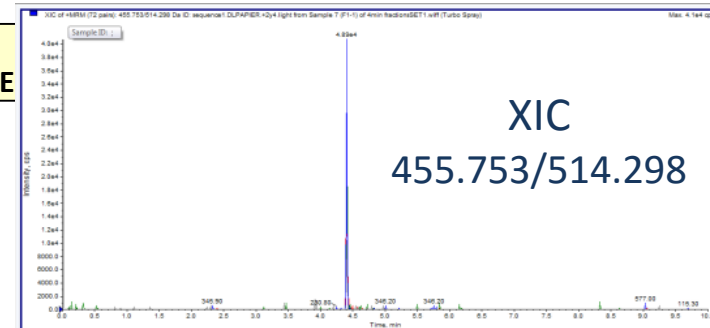
3. Capture, associate, dissociate,  
while collecting fractions  
from an affinity capture run.



4. Digest fractions, alkylate cysteines.

```
R.GPTIKPCPPCK.C [3,13], K.CPAPNLLGGPSVFIFPPK.I [14,31], R.EDYNSTLR.V [79,86]  
K.DLPAPIER.T [112,119], R.TISKPK.G [120,125], K.TELNYK.N [172, 177], R.NSSCSVVHE
```

5. Analyze by LC-MS using known MRM transitions.



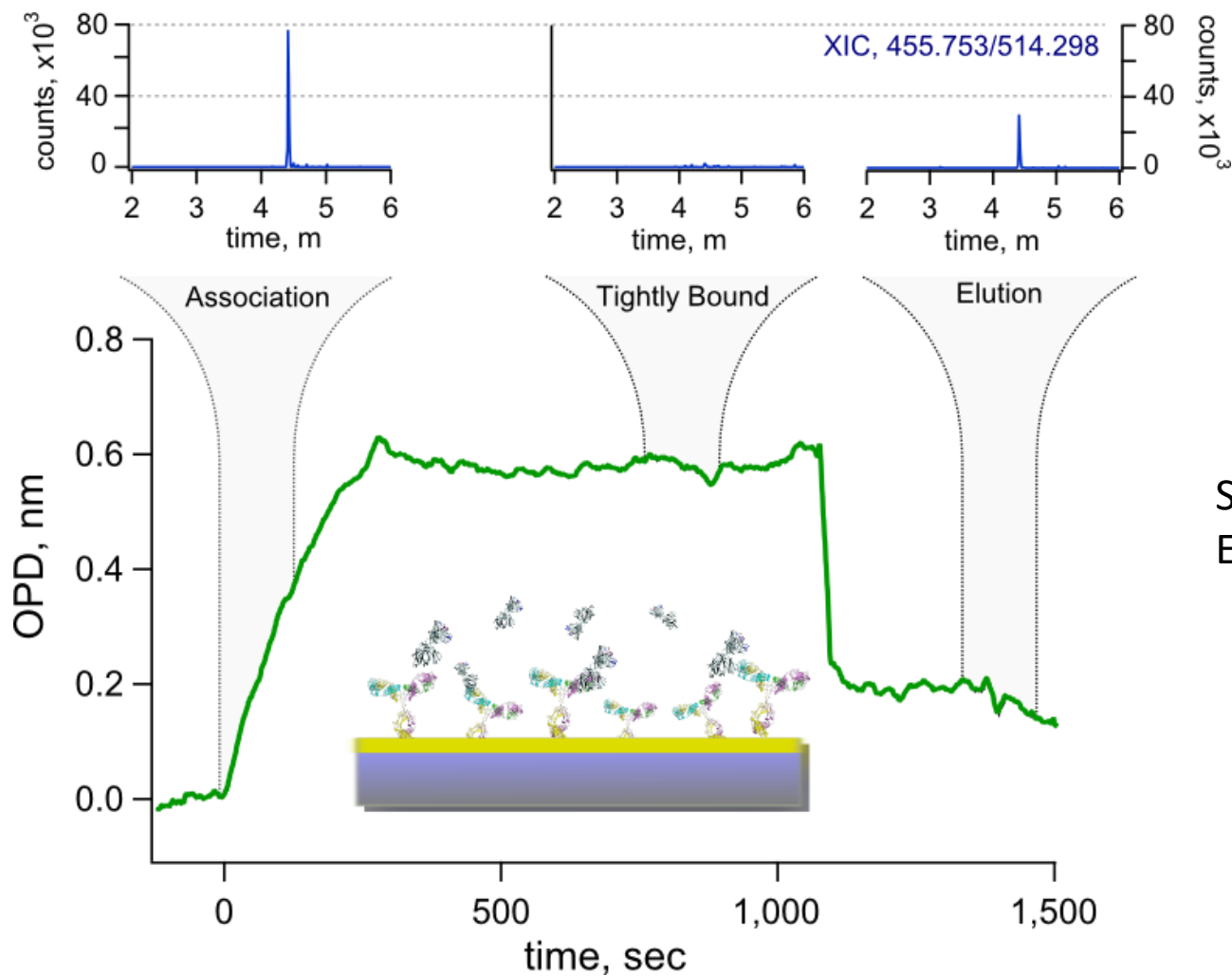
# AC-MS: Antigen Capture and Detection 2 of 3



Data taken at PharmaCadence  
Hatfield, PA, USA

- **SKi Pro X10 w/ SKi Bridge**
- **Eksigent Express HT**
- **AB Sciex Qtrap5500**

# AC-MS: Antigen Capture and Detection 3 of 3



Starting [mIgG] 10  $\mu\text{g}/\text{mL}$   
Elution pulls out  $\sim 4 \mu\text{g}/\text{mL}$

# Thank You



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