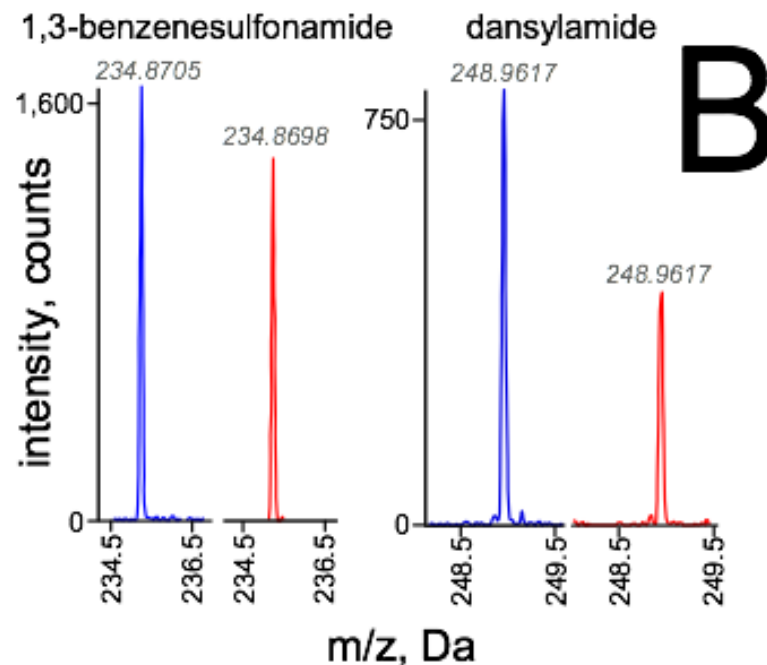
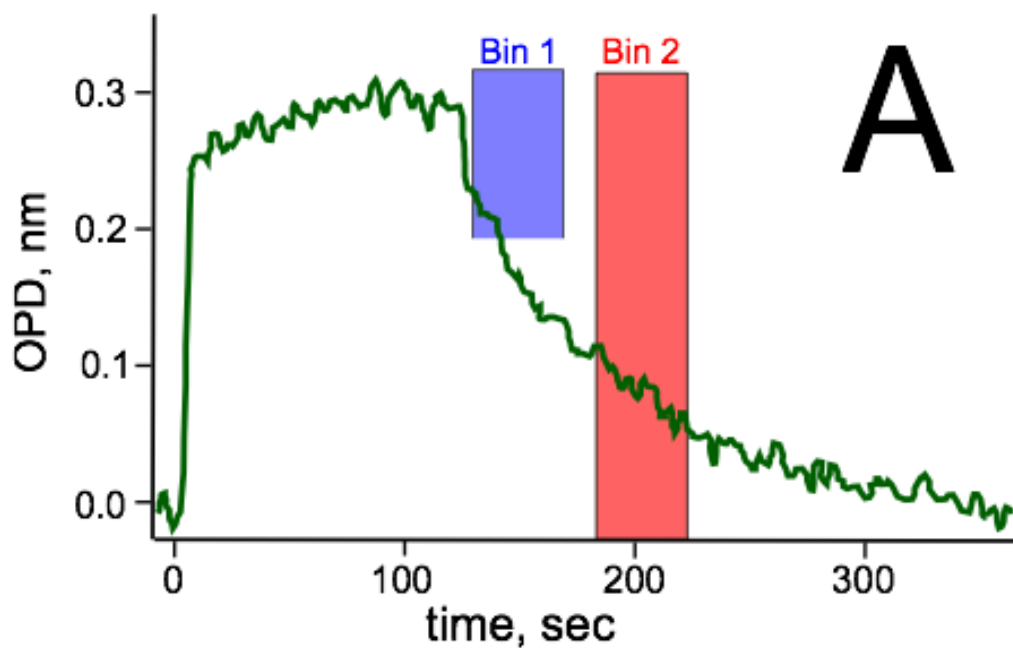


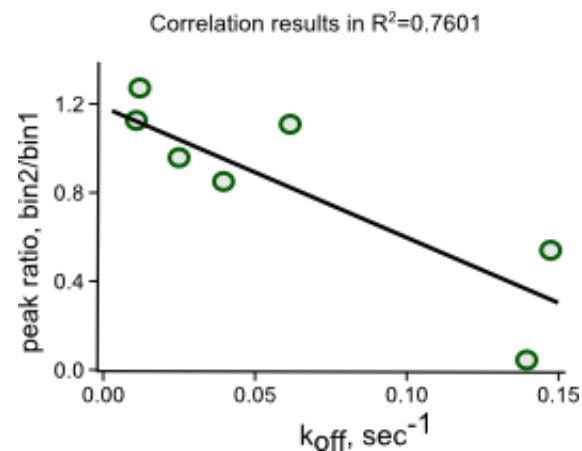
# AC-MS: Small Molecule Screening 2 of 3

- 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

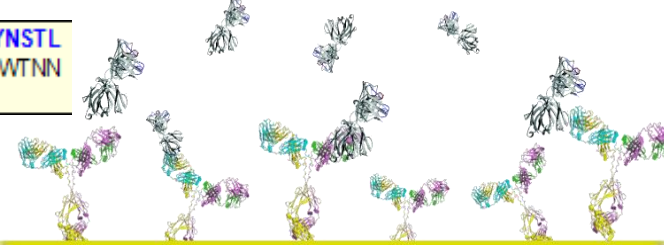


Sulfonamide	Peak (Bin 1)	Peak (Bin 2)	$k_{\text{on}}$	$k_{\text{off}}$	$K_D$ ( $\mu\text{M}$ )	bin2 - bin1	bin2 / bin1
Sulpiride	109	5.5	395	0.1	362	-104.1	0.05
Furosemide	142	181	12,400	0	0.98	39	1.27
Dansylamide	37	20.2	55,000	0.2	2.73	-16.74	0.55
1,3-benzene-disulfonamide	68	77	12,500	0	1.04	9	1.13
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).

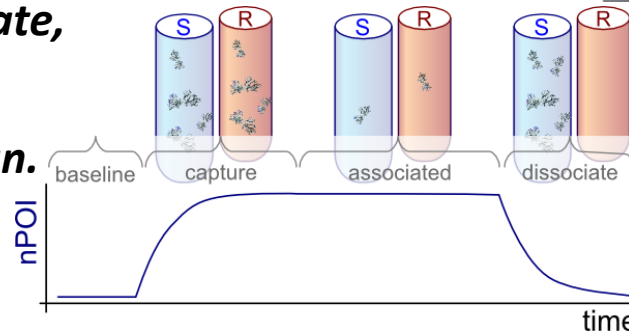
EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVWVDVSEDDPDVQISWVFVNNVEVHTAQTQTTHREDYNSTL  
RVVSAALPIQHQDWMMSGKEFKCKVNNKDLPAPIERTISKPKG SVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNN  
GKTELNYKNTEPVLDSGFSMYSKLRVEKKNWVERNSSCSWHEGLHNHHTTKSFSRTPGK



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

Ski Sensor npoSi Chip

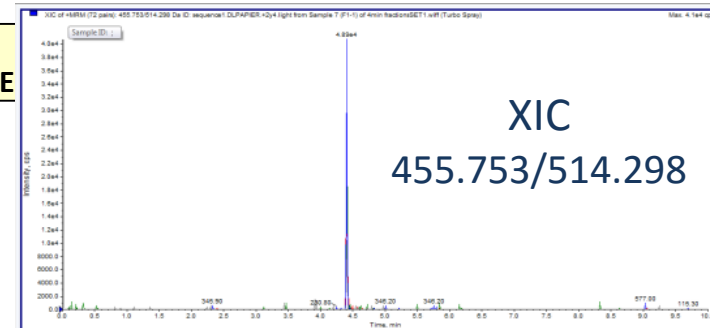
## 3. Capture, associate, dissociate, while collecting fractions from an affinity capture run.



## 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.



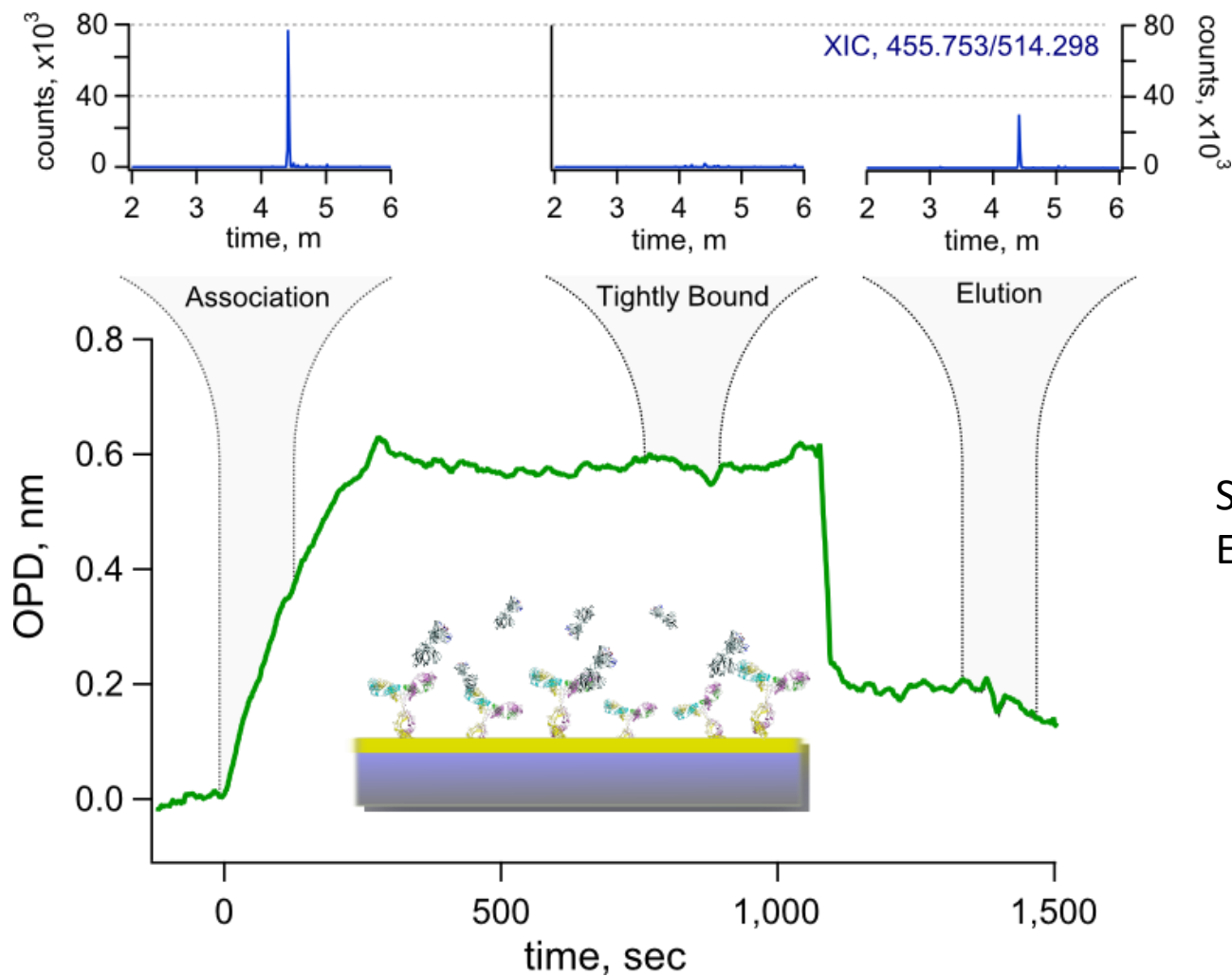
# AC-MS: Antigen Capture and Detection 2 of 3



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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