Automated direct monitoring of H/D exchange reactions for mapping conformational landscapes of proteins

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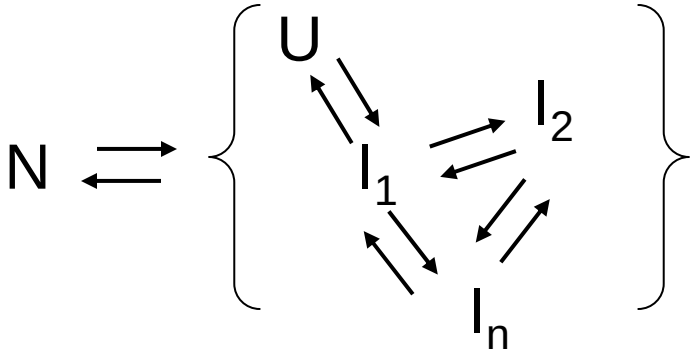
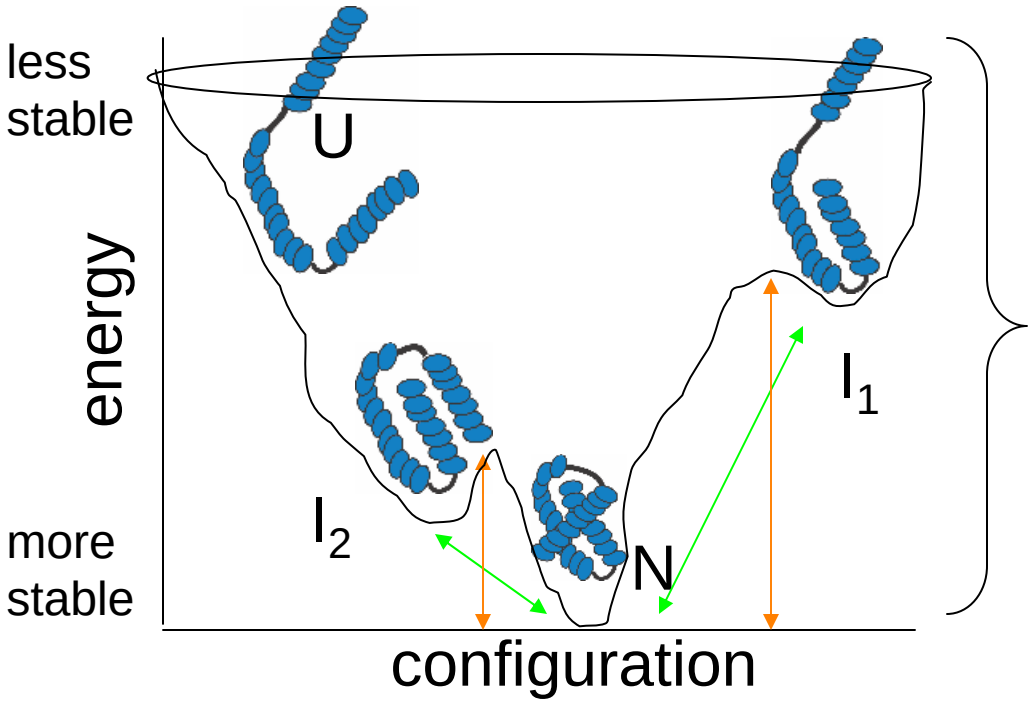
What are Conformational Landscapes and why map them?

"Native" protein (N) also populates an ensemble of non-N conformations

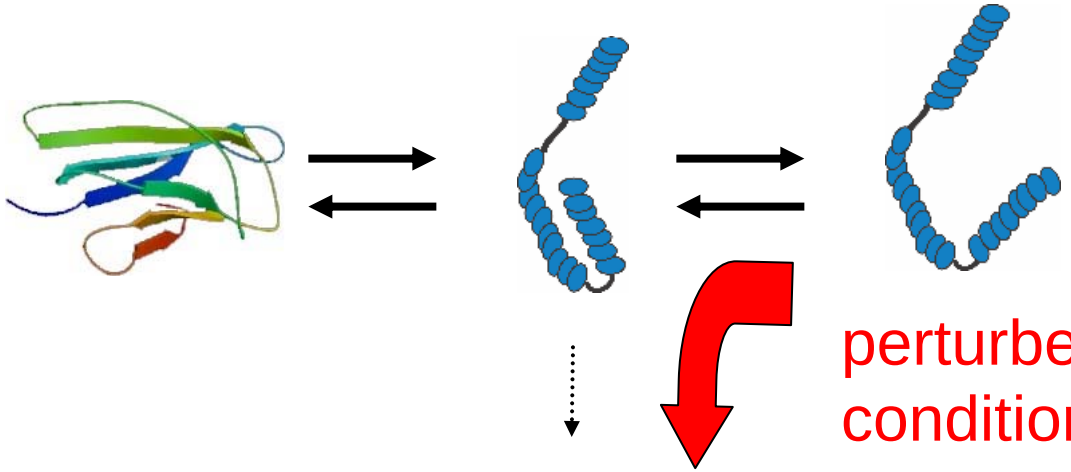


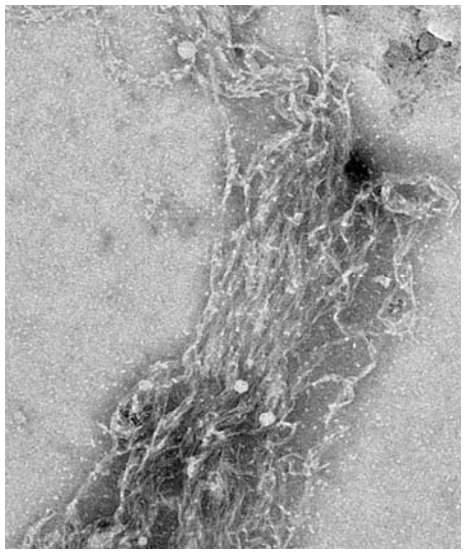


Funnel Landscape

Entire equilibrium determines function

Why do we want to detect intermediates?



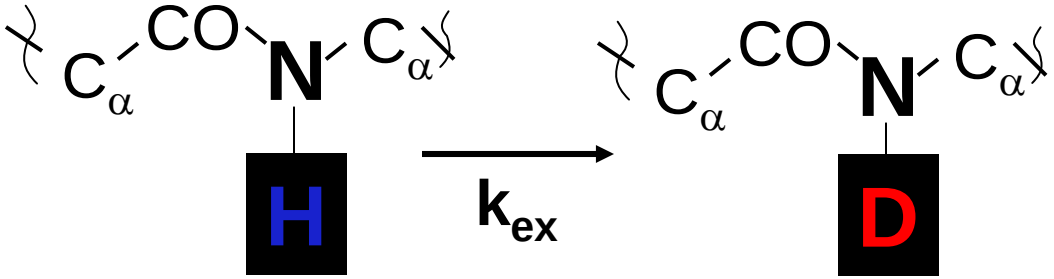


B2microglobulin fibers.
C. Eakin, Yale University

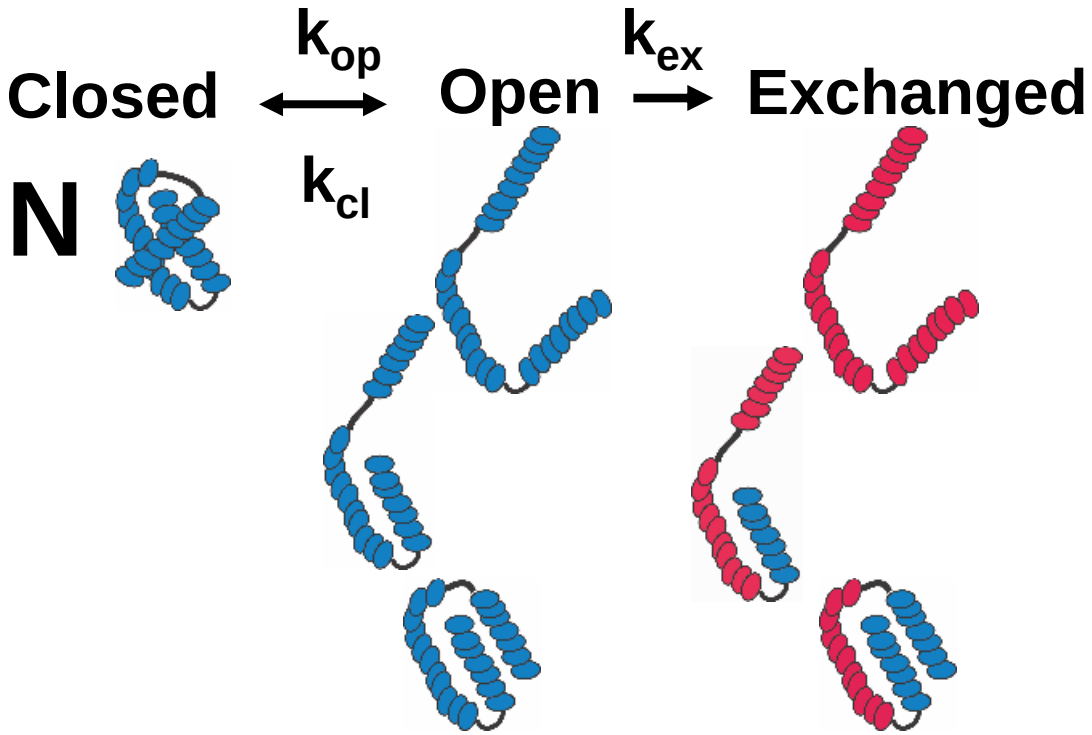
Amyloid fiber formation: aggregation-prone intermediate is stabilized

Native State Hydrogen Exchange to map conformational landscapes of proteins

Accessible amide protons exchange with (deuterated) solvent with known k_{ex}

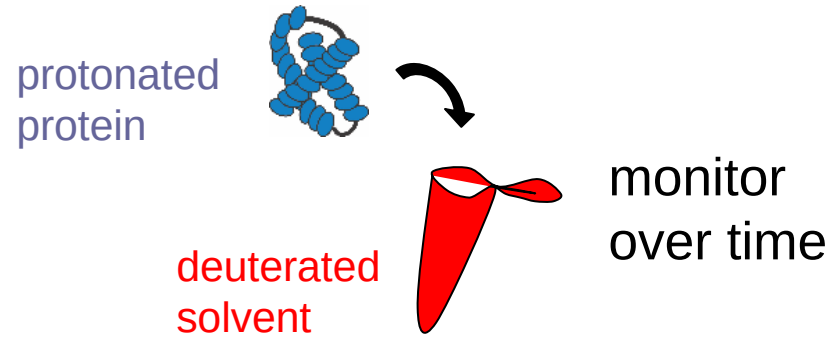


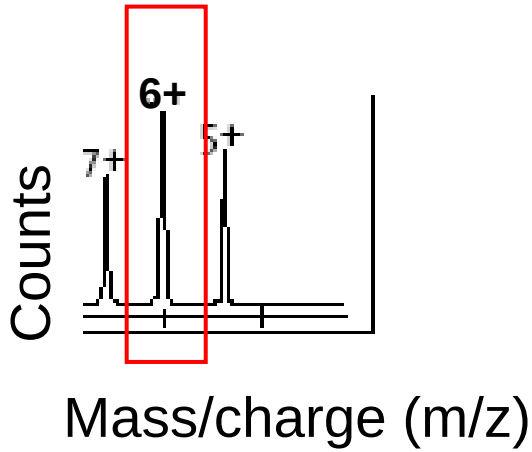
Protons buried in folded proteins exchange in unfolding events:

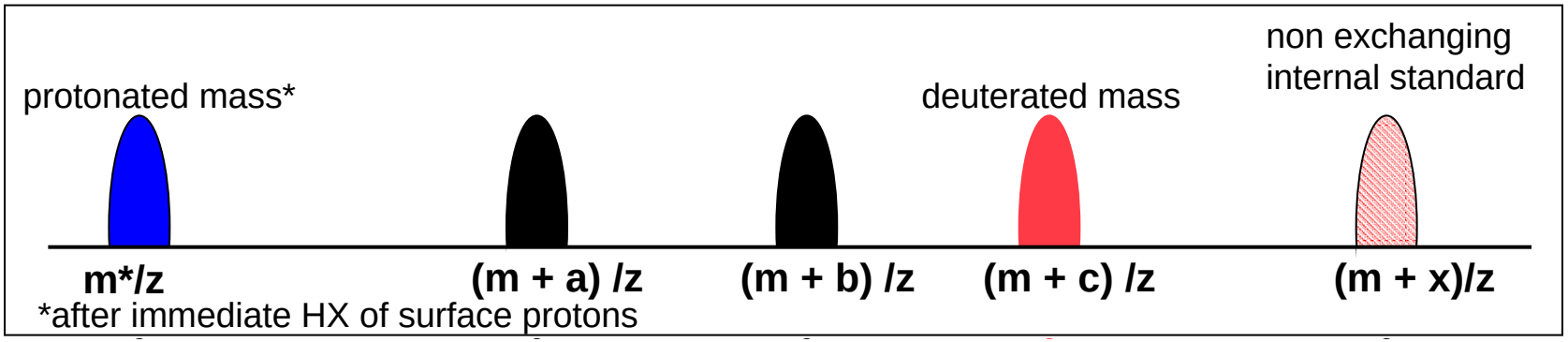


HX protection pattern identifies N vs. I_1 vs. I_2 vs. U

Electrospray Ionization MS to monitor H/D Exchange



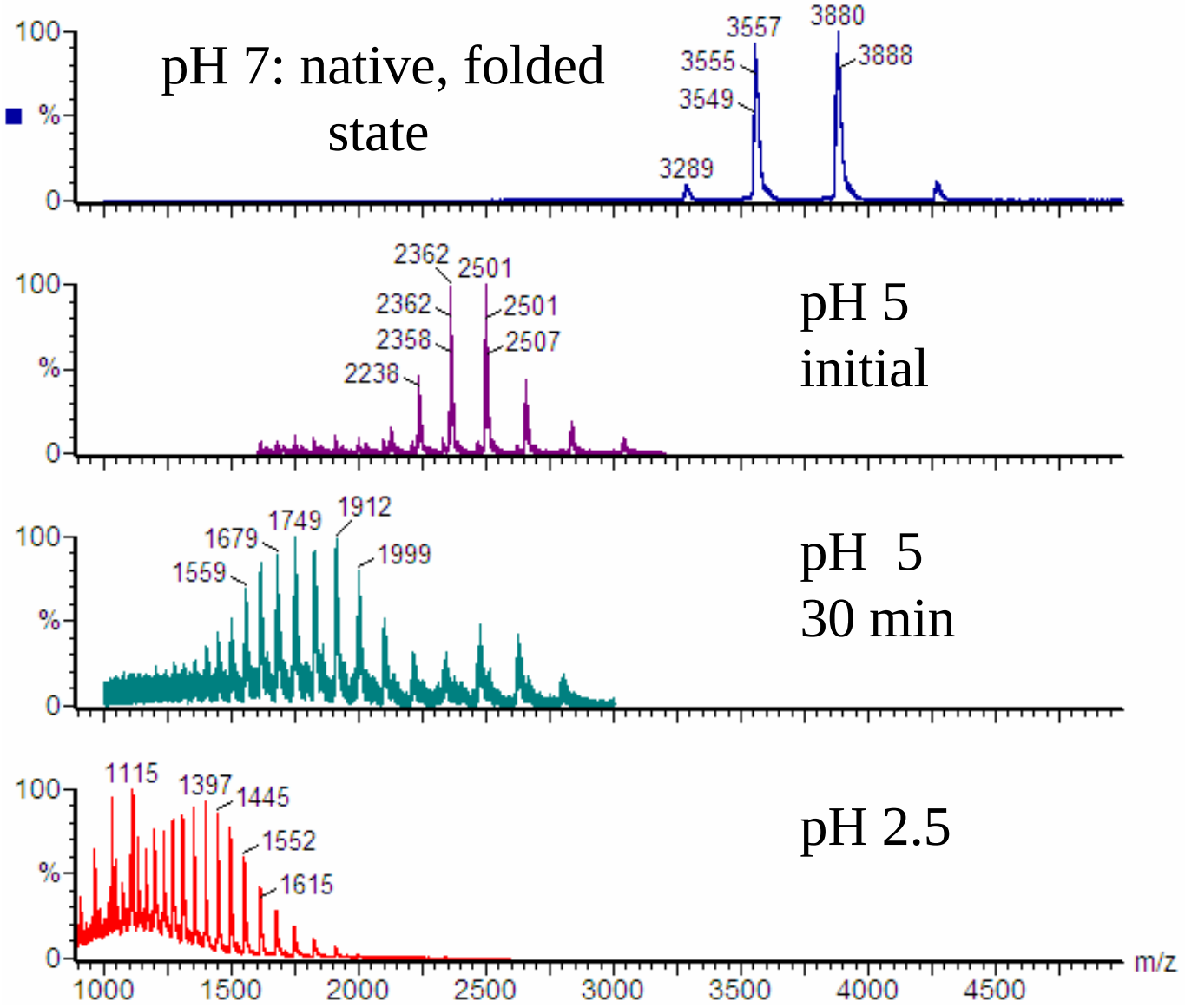




t=0 ⟶ t= end

what happens in between?

NanoMate sprays Actin in 5 mM ammonium acetate, pH 7



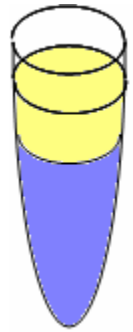
As the protein is denatured, it acquires more charges and shifts to lower m/z values.

“MRM”: 5 H/D exchange rxns, 36 timepoints, 48hr

timepts	AP	BP	CP	DP	EP	A	B	C	D	E
mix time						**A 10**	**B 40**	**C 72**	**D 109**	**E 135**
1	AP 0	BP 2	CP 4	DP 6	EP 8	A 11	B 41	C 73	D 110	E 136
2	AP 22	BP 57	CP 62	DP 106	EP 171	A 12	B 42	C 74	D 111	E 137
3	AP 179	BP 201	CP 203	DP 205	EP 207	A 14	B 44	C 76	D 113	E 139
4	AP 231	BP 233	CP 235	DP 237	EP 244	A 16	B 46	C 78	D 115	E 141
5	AP 261	BP 263	CP 265	DP 267	EP 269	A 18	B 48	C 80	D 117	E 143
6	AP 293	BP 295	CP 297	DP 299	EP 301	A 20	B 50	C 82	D 119	E 145
7	AP 497	BP 499	CP 501	DP 503	EP 505	A 25	B 55	C 87	D 124	E 150
8	AP 977	BP 979	CP 981	DP 983	EP 985	A 30	B 60	C 92	D 129	E 155
9	AP 1337	BP 1339	CP 1341	DP 1343	EP 1345	A 35	B 65	C 97	D 134	E 160
10	AP 3017	BP 2991	CP 2993	DP 2995	EP 2997	A 40	B 70	C 102	D 138	E 165
11						A 52	B 81	C 112	D 149	E 175
12						A 67	B 90	C 122	D 159	E 185
13						A 84	B 100	C 132	D 169	E 195
14						A 94	B 114	C 147	D 184	E 210
15						A 104	B 130	C 162	D 199	E 225
16						A 126	B 144	C 177	D 214	E 240
17						A 152	B 161	C 192	D 229	E 255
18						A 157	B 190	C 222	D 259	E 285
19						A 187	B 220	C 252	D 289	E 315
20						A 216	B 250	C 282	D 319	E 345
21						A 242	B 280	C 312	D 349	E 375
22						A 291	B 340	C 372	D 409	E 435
23						A 342	B 400	C 432	D 469	E 495
24						A 402	B 460	C 492	D 529	E 555
25						A 462	B 520	C 552	D 589	E 615
26						A 522	B 640	C 672	D 709	E 735
27						A 642	B 760	C 792	D 829	E 855
28						A 762	B 880	C 912	D 949	E 975
29						A 882	B 1000	C 1032	D 1069	E 1095
30						A 1002	B 1120	C 1152	D 1189	E 1215
31						A 1122	B 1240	C 1272	D 1309	E 1335
32						A 1242	B 1480	C 1512	D 1549	E 1575
33						A 1842	B 1840	C 1872	D 1909	E 1935
34						A 2202	B 2200	C 2232	D 2269	E 2295
35						A 2562	B 2560	C 2592	D 2629	E 2655
36						A 2922	B 2920	C 2952	D 2989	E 3015

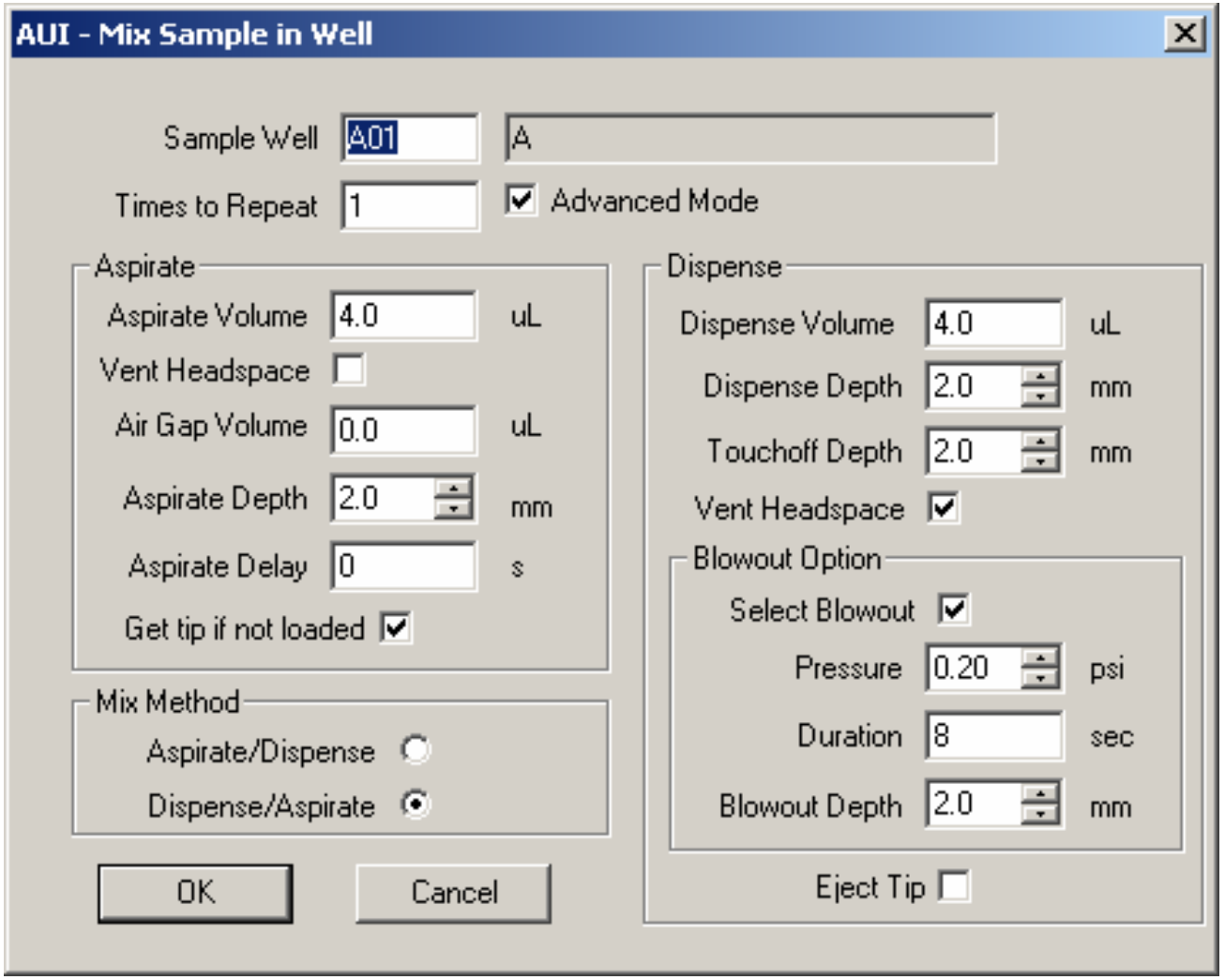
A,B,C,D,E: 0,2,4,6,8% ACN; P: corresponding protonated controls

Hydrocarbon layering as an evaporation barrier

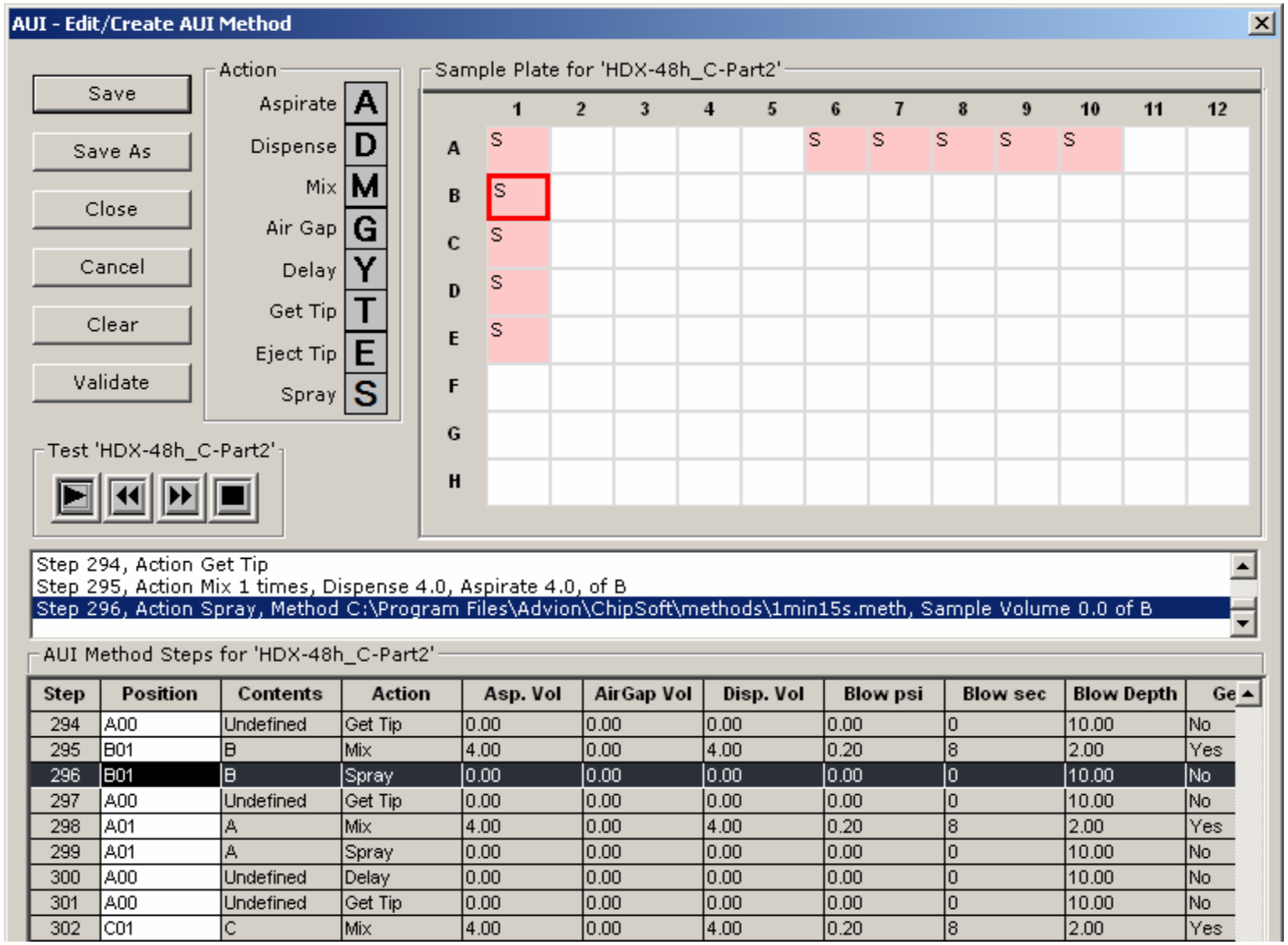


	Density (g/mL, 25°C)	**Bp (°C)**	**vapor pressure**
Octane (C_8)	0.70	125-127	11mm Hg (20°C)
Decane (C_{10})	0.73	174	1mm Hg (16.5°C)
Dodecane (C_{12})	0.75	215-217	1mm Hg (47.8°C)
Tetradecane (C_{14})	0.76	252-254	1mm Hg (76.4°C)
Hexadecane (C_{16})	0.77	287	1mm Hg (105.3°C)

Sample pickup with hydrocarbon layering: “dispense”, then aspirate



AUI Method: get tip, “mix”, spray, (delay)



Step	Position	Contents	Action	Asp. Vol	AirGap Vol	Disp. Vol	Blow psi	Blow sec	Blow Depth	Ge
294	A00	Undefined	Get Tip	0.00	0.00	0.00	0.00	0	10.00	No
295	B01	B	Mix	4.00	0.00	4.00	0.20	8	2.00	Yes
296	B01	B	Spray	0.00	0.00	0.00	0.00	0	10.00	No
297	A00	Undefined	Get Tip	0.00	0.00	0.00	0.00	0	10.00	No
298	A01	A	Mix	4.00	0.00	4.00	0.20	8	2.00	Yes
299	A01	A	Spray	0.00	0.00	0.00	0.00	0	10.00	No
300	A00	Undefined	Delay	0.00	0.00	0.00	0.00	0	10.00	No
301	A00	Undefined	Get Tip	0.00	0.00	0.00	0.00	0	10.00	No
302	C01	C	Mix	4.00	0.00	4.00	0.20	8	2.00	Yes

- **Spray parameters: 0.2 psi, 1.9 kV**

Sampling Sequence:
750 AUI steps,
230 tips/nozzles

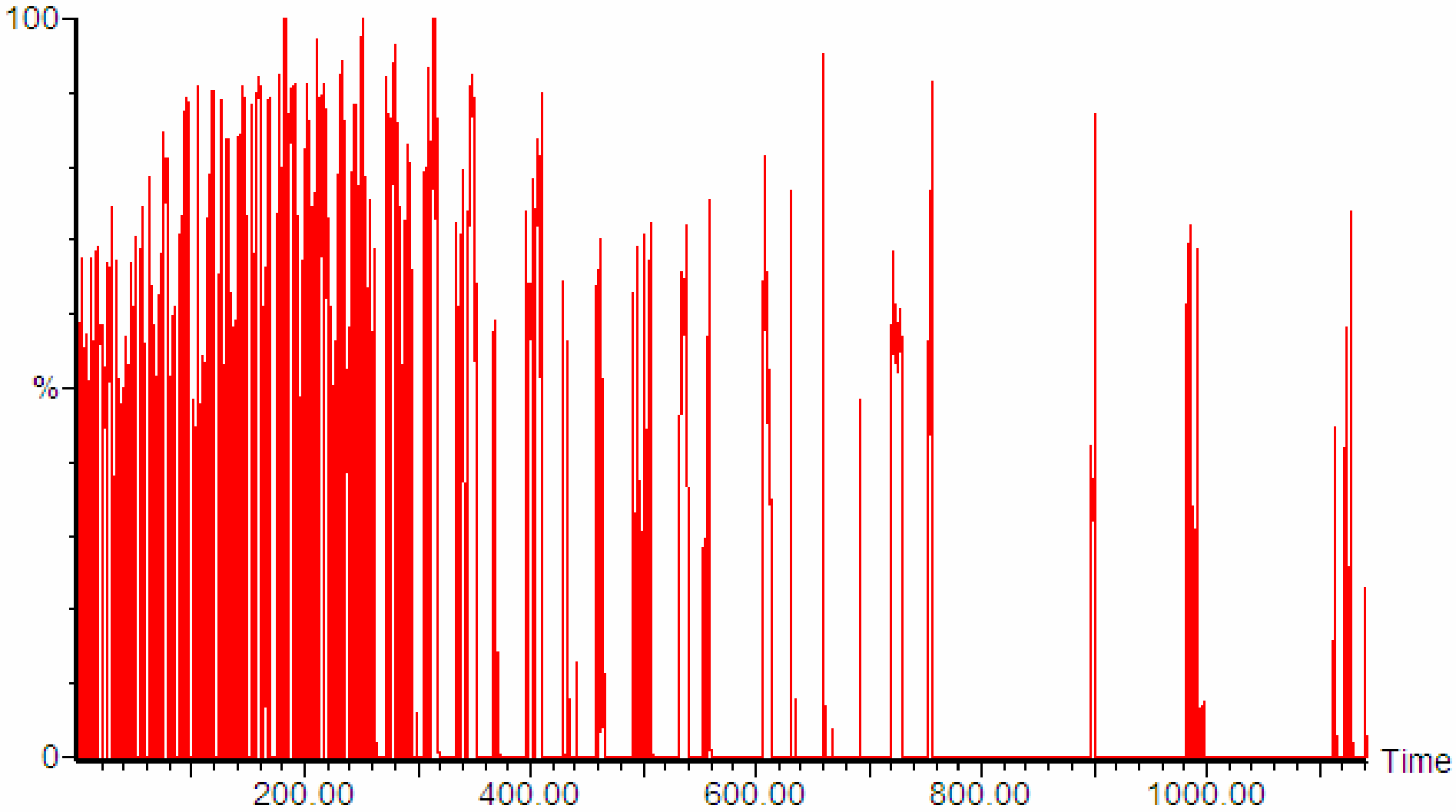
Change 96-tip trays at 3h, 16h,
Re-synchronize timing

tip#	AUI Step (spray)	Sample	Time (min)	Time btwn samples	Spray Time
1	3	AP	0	2	1:15
2	6	BP	2	2	1:15
3	9	CP	4	2	1:15
4	12	DP	6	2	1:15
5	15	EP	8	2	1:15
6	3	A	**10**	1	0:30
7	6	A	11	1	0:30
8	9	A	12	2	0:45
9	12	A	14	2	1:15

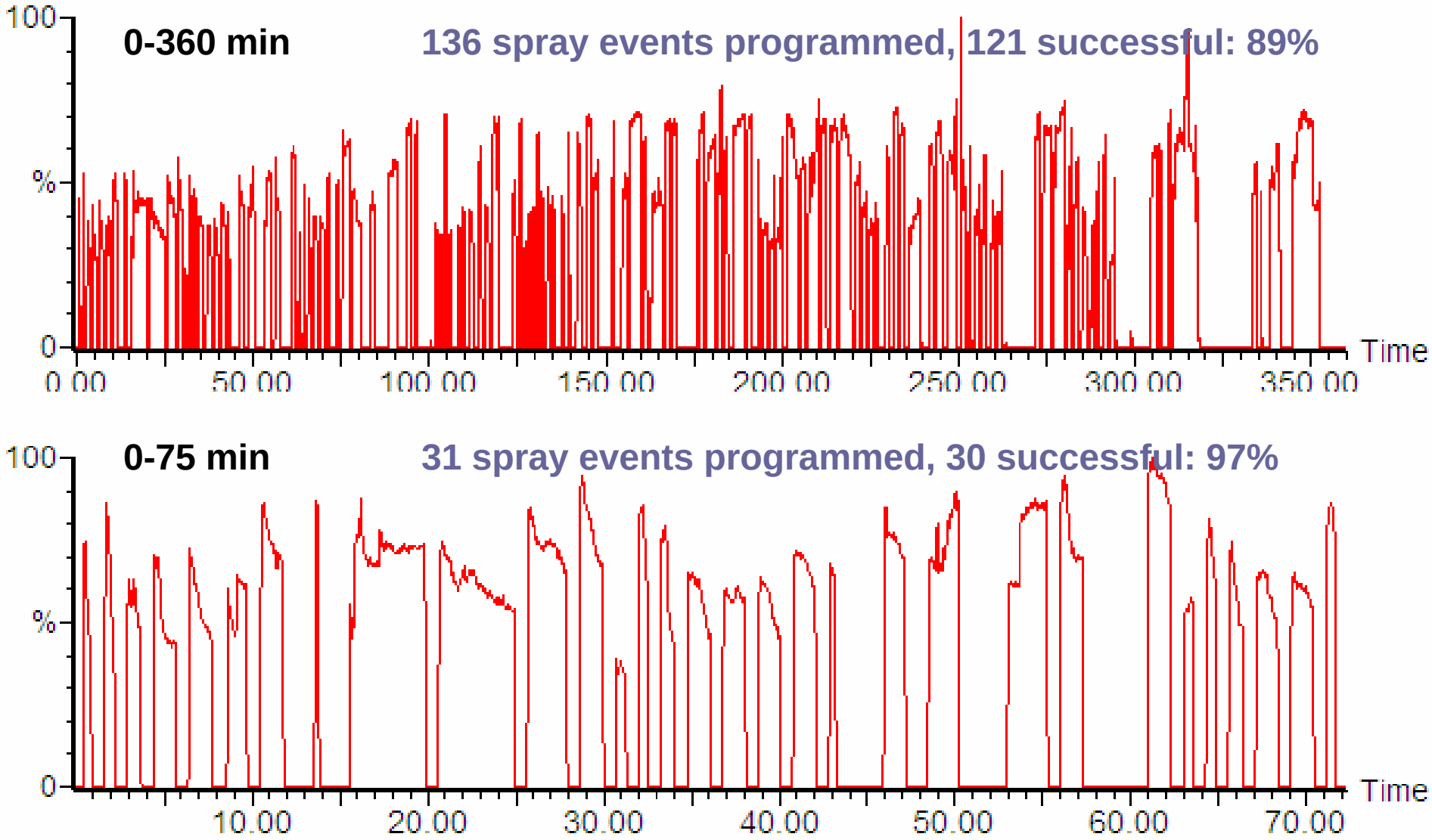
mix

mix

Total Ion Chromatogram of Time Course

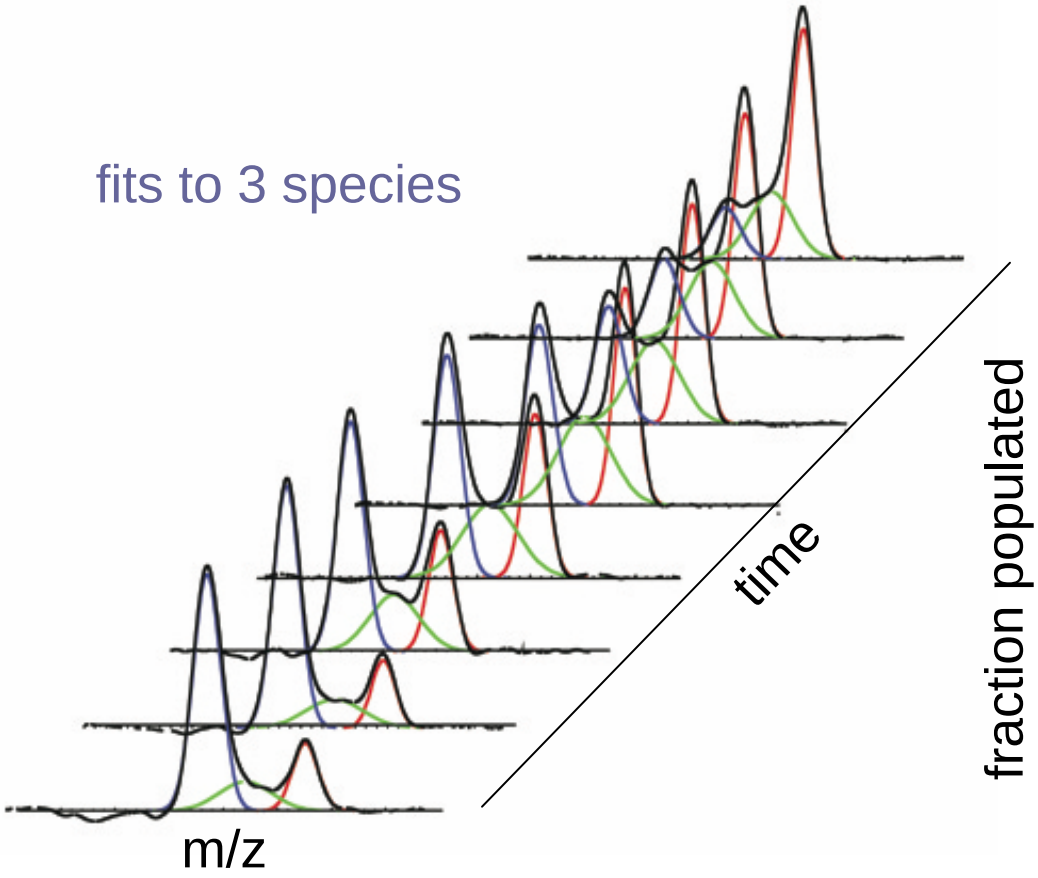


Total Ion Chromatogram of Time Course

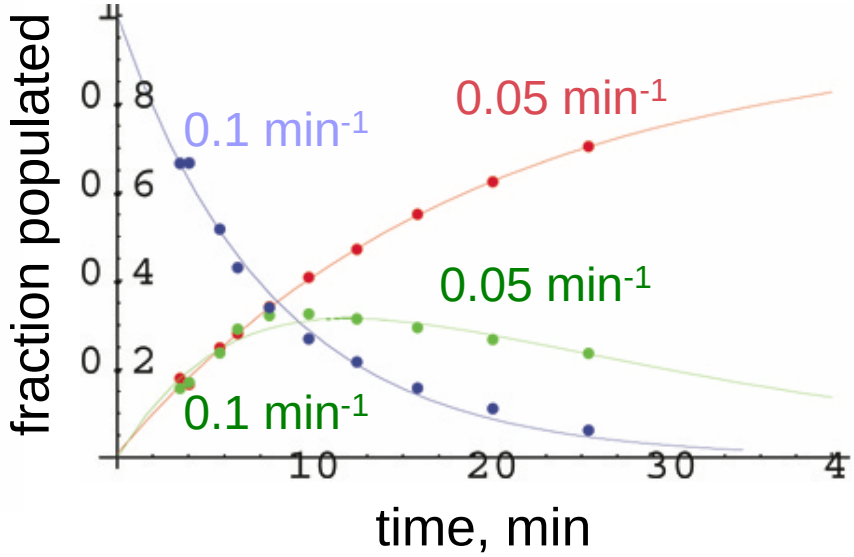


HXMS reveals an intermediate populated by amyloidogenic precursor protein

fits to 3 species



shows population shift



N ⇌ I ⇌ U

Conclusion

Automated monitoring of reactions enabled by NanoMate & hydrocarbon layering

- Future work
  - Refine “Multiple Reaction Monitoring” method
  - Automate data analysis
- Looking forward to
  - Stepwise “AUI-like” control in real time
  - 386-tip trays
- Thanks to
  - Beckman Foundation
  - Vincent & Stella Coates Foundation
  - Dennis Price, Gary Schultz, Dave Bajkowski, Colleen van Pelt, Mike Lees