Droplet Microfluidics for High-Throughput Experimentation

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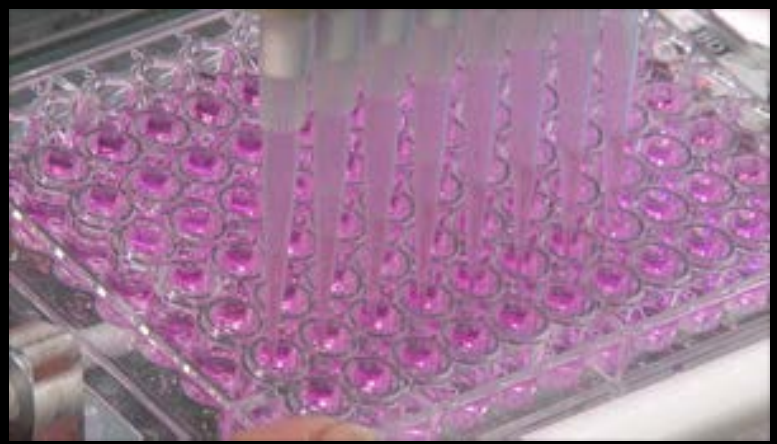
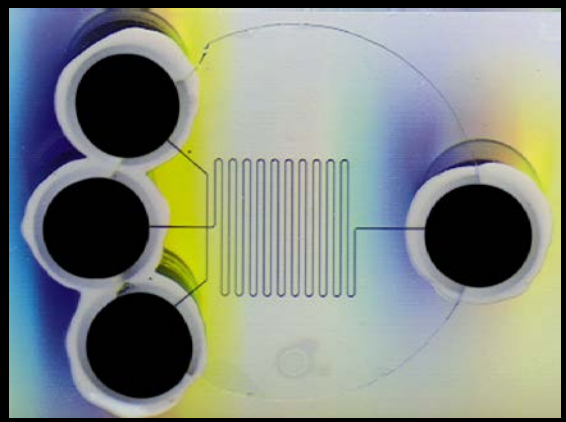
Routes to Automation and High Throughput

Wet Chemical Lab Procedures

Segmented flow analyzer (e.g. Technicon)



Multi-Well Plate and Robots



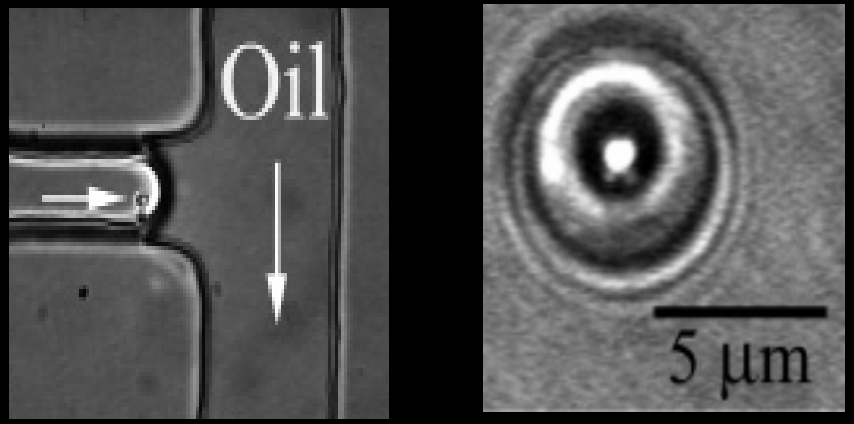
µchip - ORNL

Droplets in Microfluidic Systems

"micro" reactors or "test tubes"

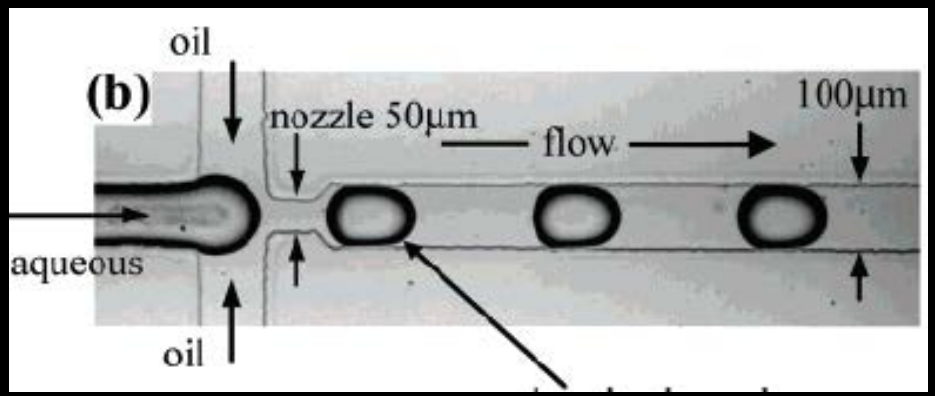
(Miniaturized "Segmented Flow Analysis")

Droplets



He, M.; et al. Anal. Chem. 2005, 77, 1539

Plugs (segmented flow)



Shim, et al. JACS 2007, 129, 8825

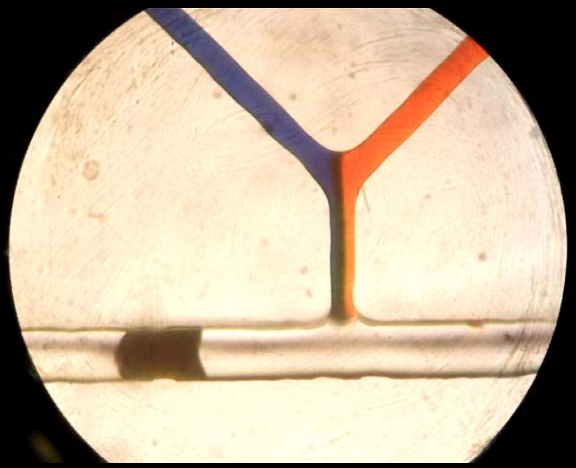
Processing Nanoliter Samples Using Droplet Microfluidics

Sample

Immiscible Fluid (fluorinated oil/ gas)

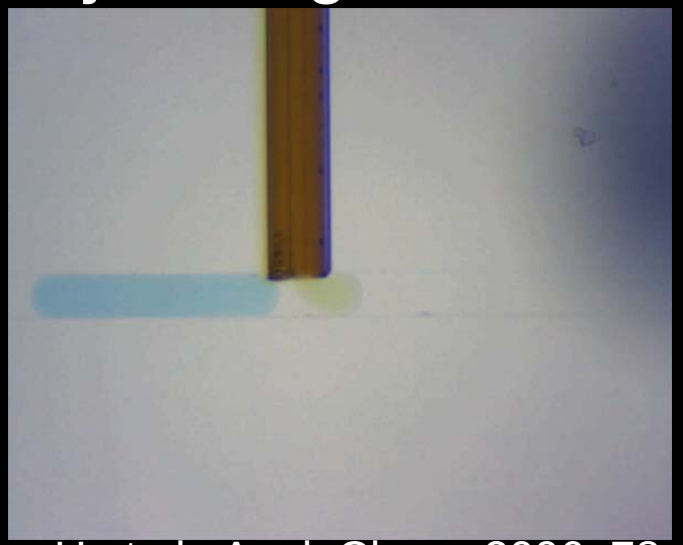
Garstecki, P. Lab on a Chip 2006, 6, 437

Mixing / Composition



Li, L et al., Proc. Natl. Acad. Sci. 2006, 103, 10342

Inject reagent / Dilute

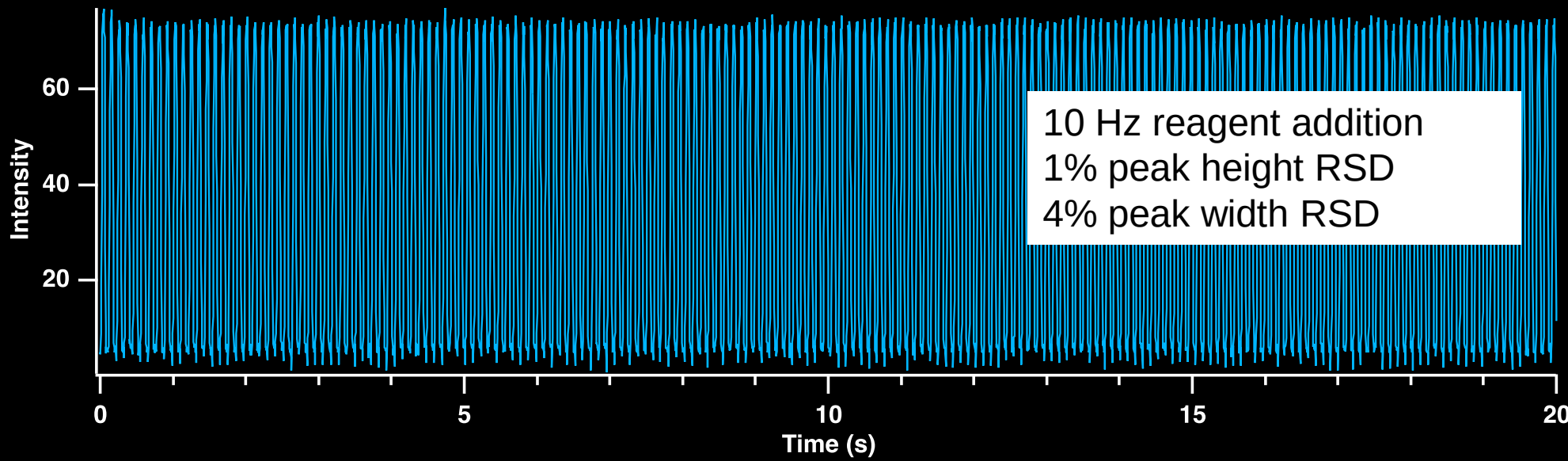


Song, H et al. Anal. Chem. 2006, 78, 4839

High Throughput Reagent Addition in Droplet Format

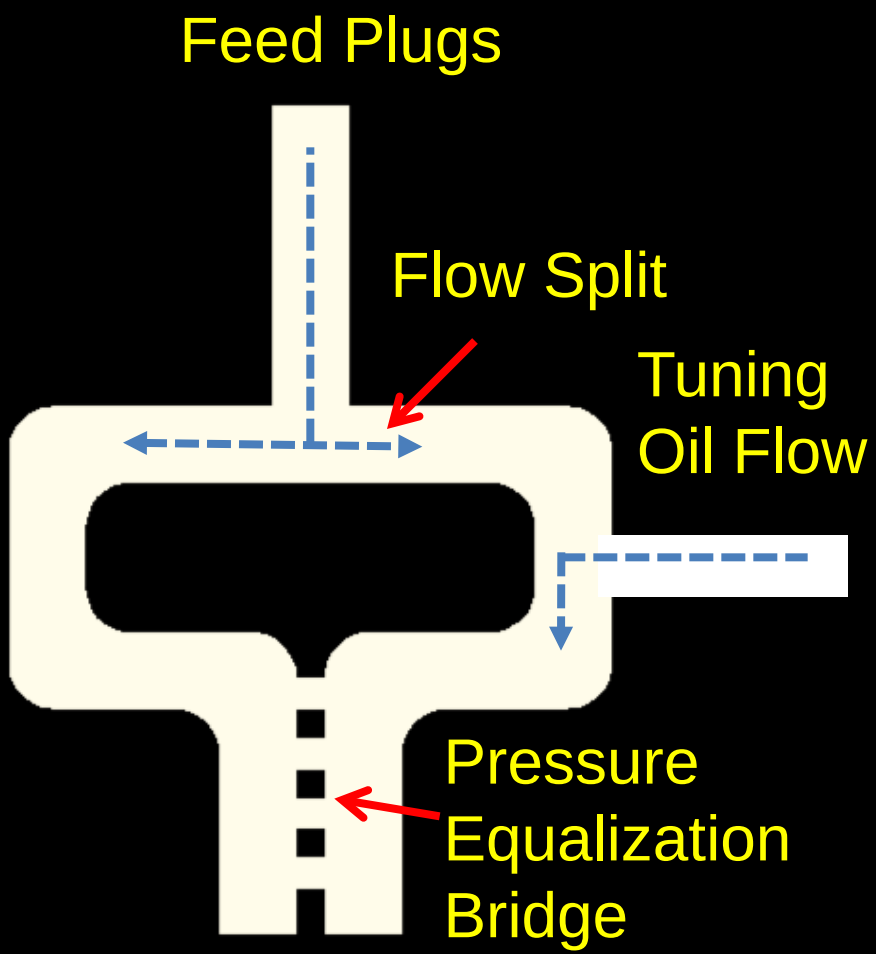
Real time

0.25x time

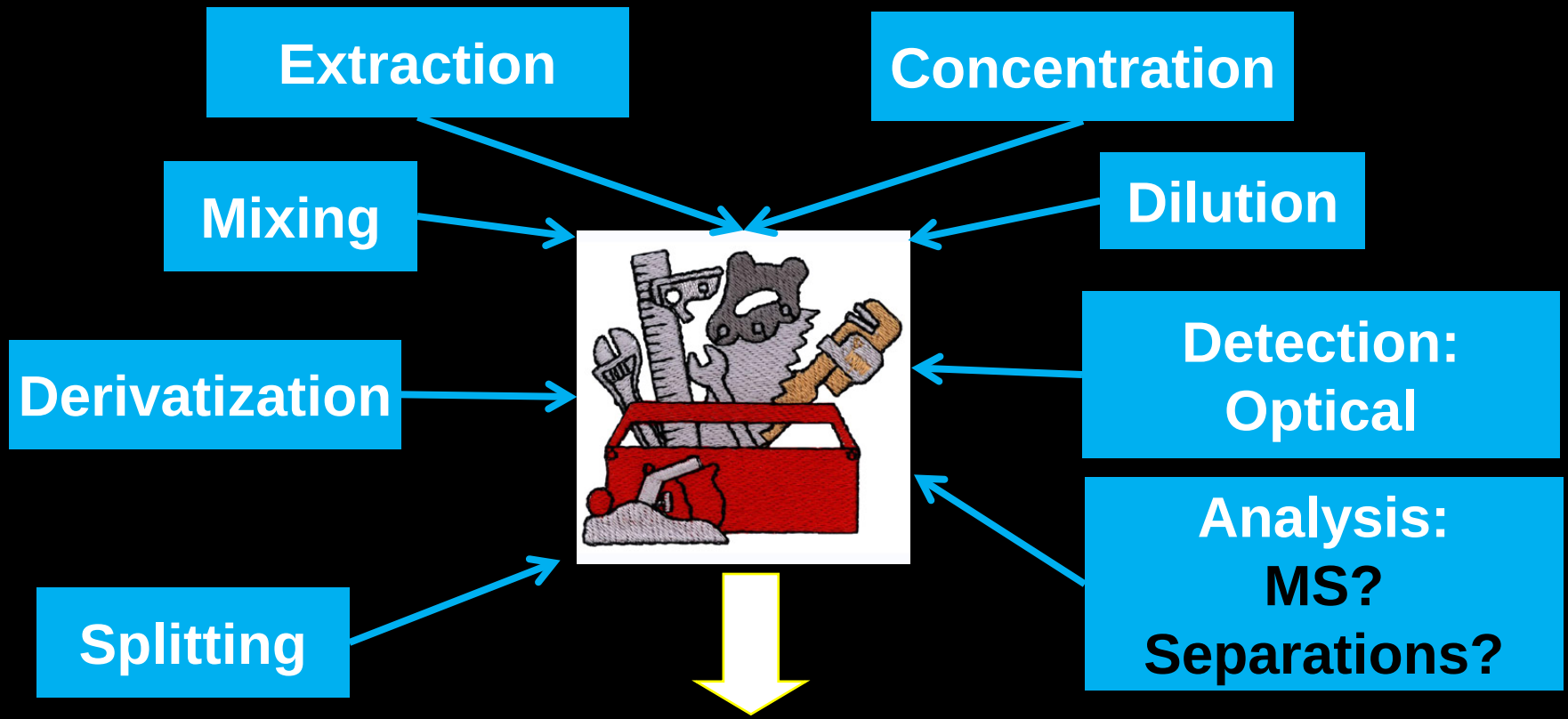


Song, H et al. Anal. Chem. 2006, 78, 4839

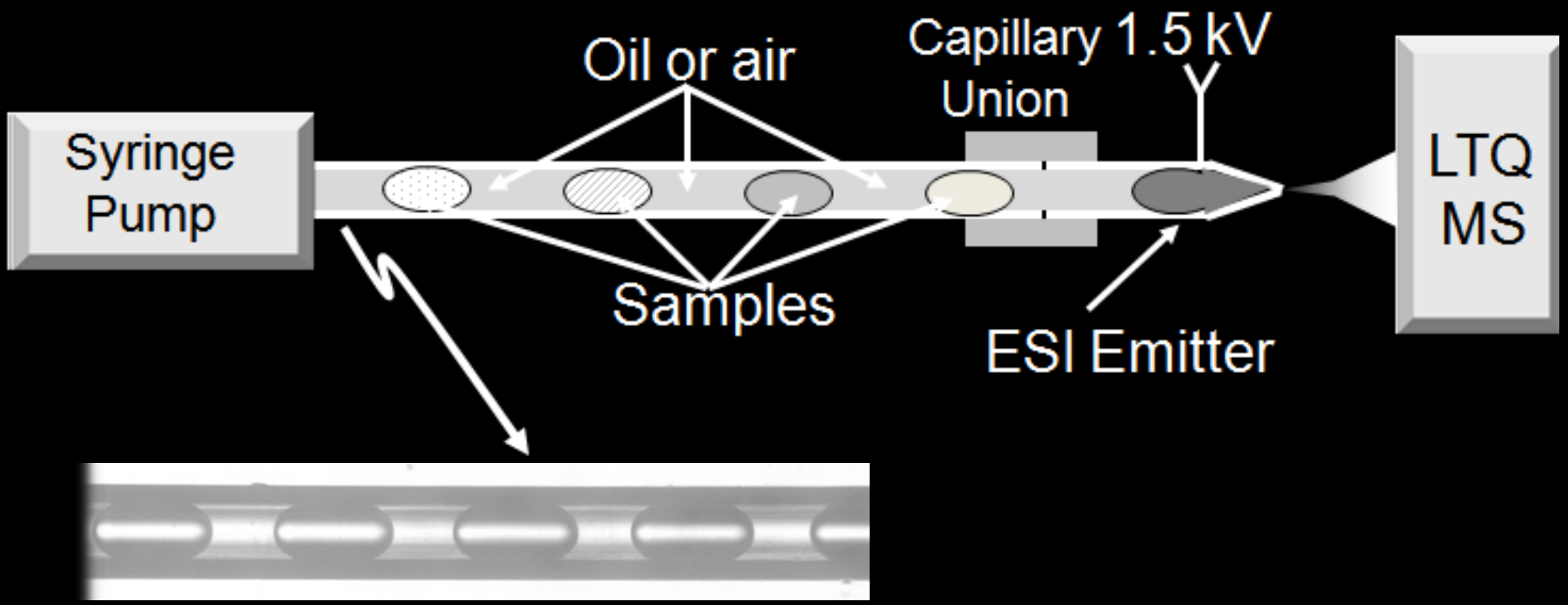
A Variable Nanoliter Pipetter



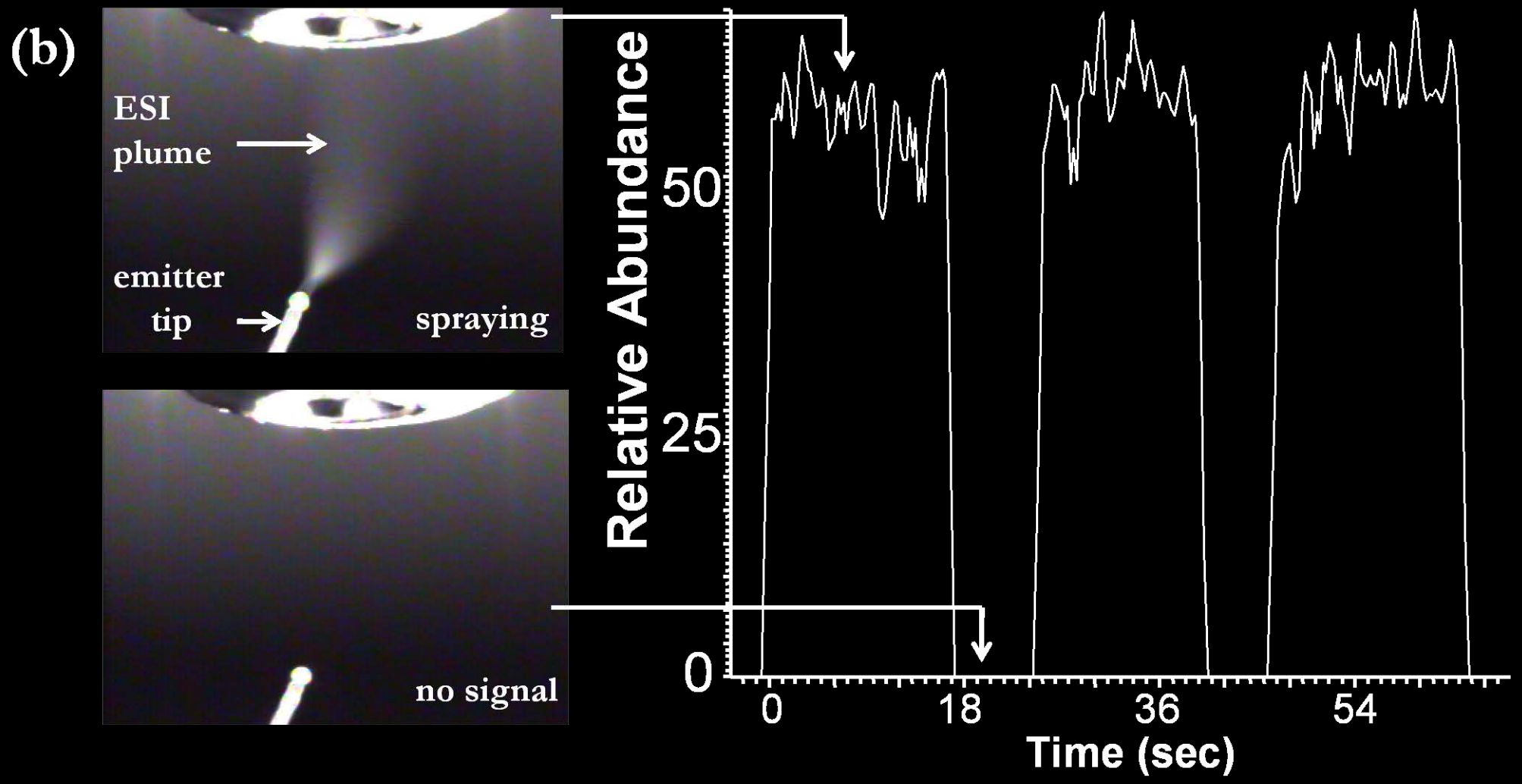
Droplet Microfluidic Toolbox



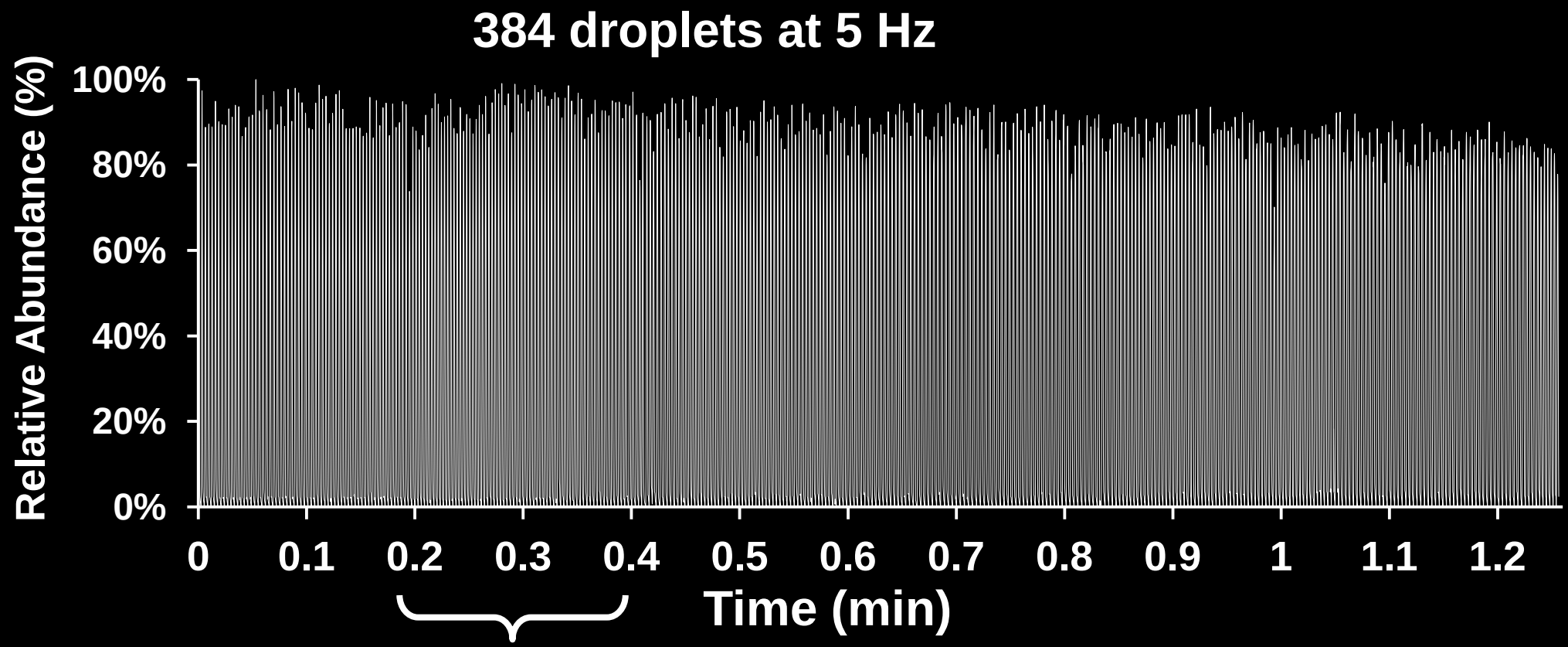
Analysis of Plug Contents: Direct ESI-MS

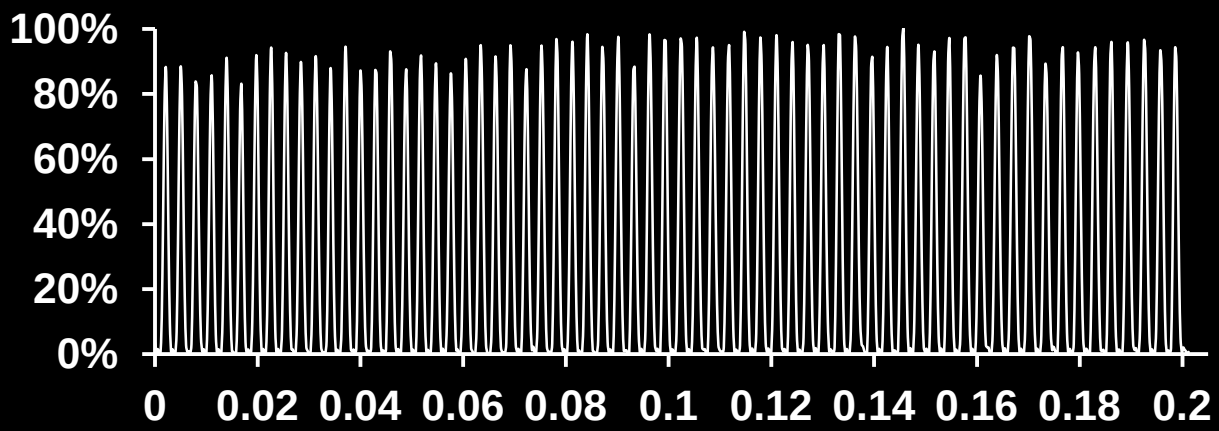


Direct ESI-MS of Segmented Flow



High Throughput MS Analysis of Plugs





- 30 μL/min
- 50 nL/droplet
- 15 μM Adenosine

Low Flow Rates Beneficial for Complex Samples: GABA in 150 mM Ionic Strength Saline (aCSF)

800 nL/min
30 µm spray tip
10 nL droplets

EIC/10^3

Time (min)

Stable Long Term Analysis by nESI-MS

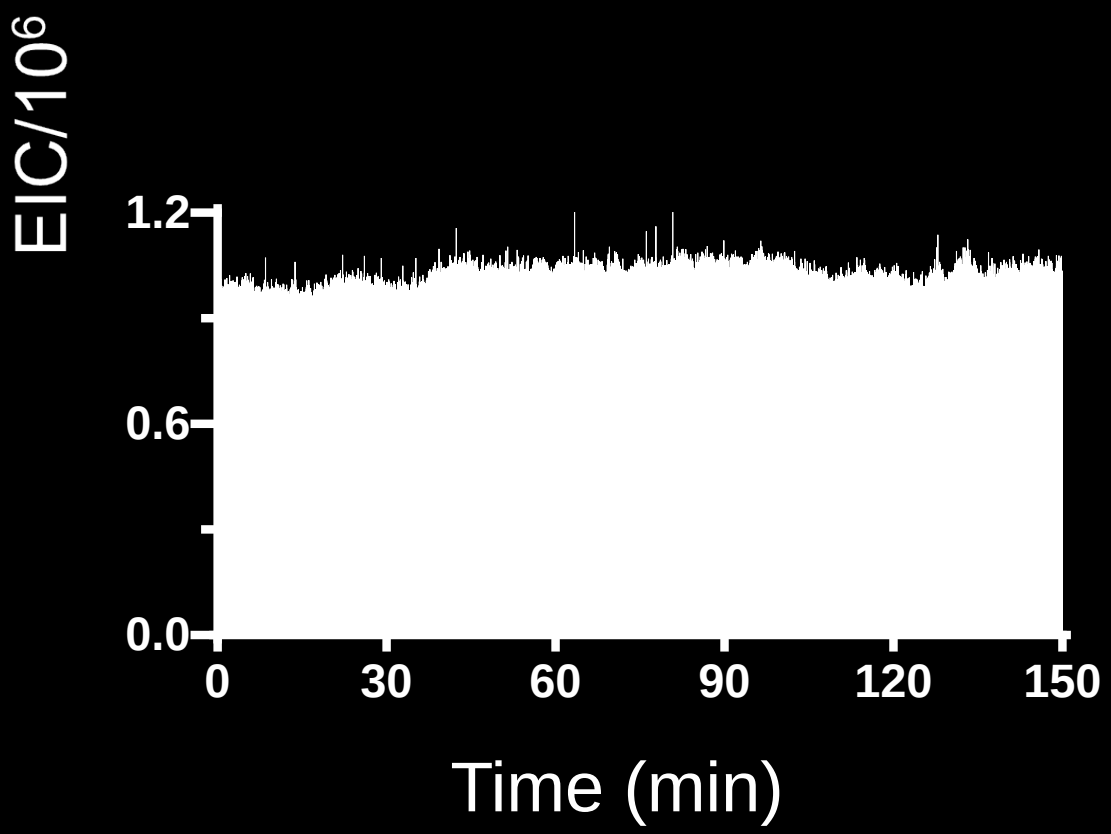
1.2 nL droplets

2.1 Hz throughput

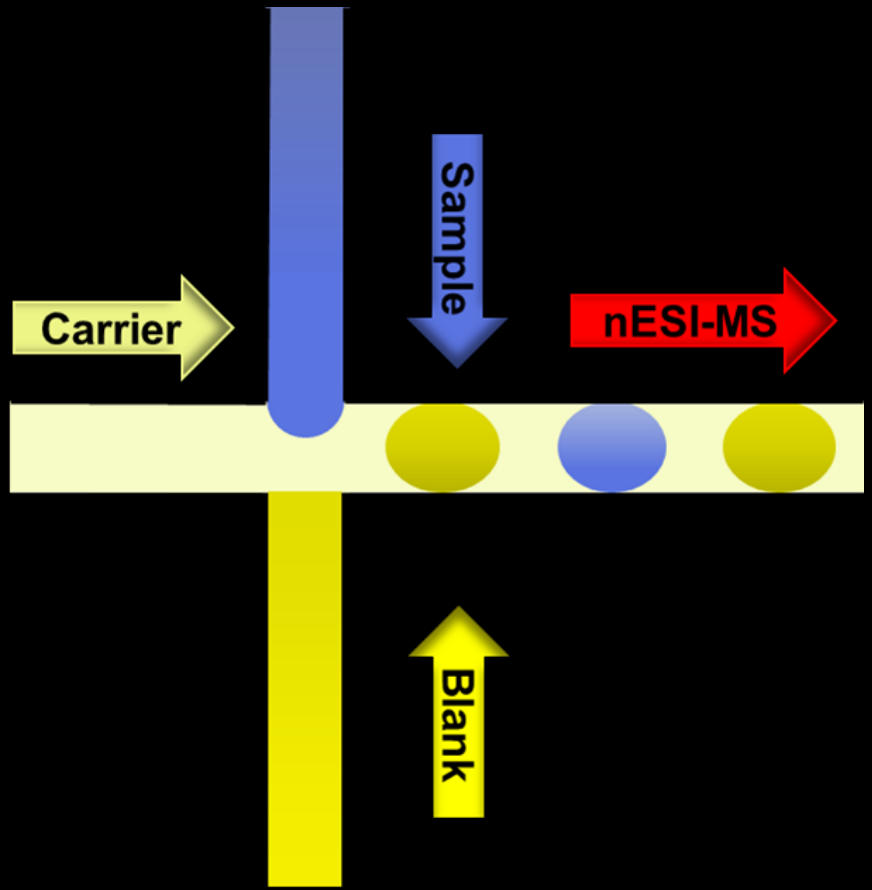
2.5 hours of continuous analysis

- >20,000 droplets

3.7% RSD in peak height



Low Droplet Carry-Over During nESI-MS



EIC/10^6

20x

Time (s)

High-Throughput at Low Volumes

300 pL – 6.2 Hz

EIC/10^6

Time (s)

300 pL – 9.5 Hz

EIC/10^6

Time (s)

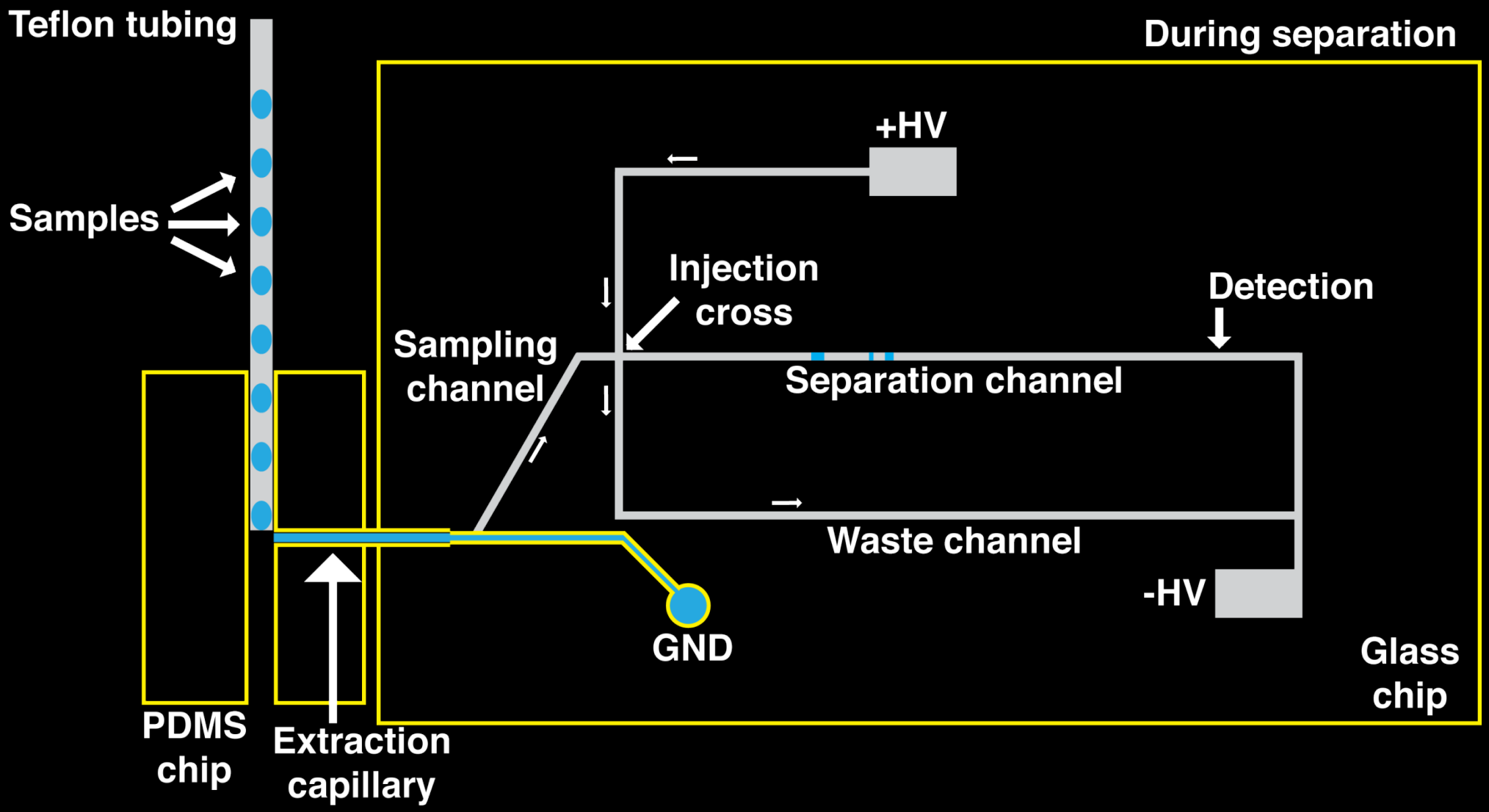
65 pL – 3.3 Hz

EIC/10^6

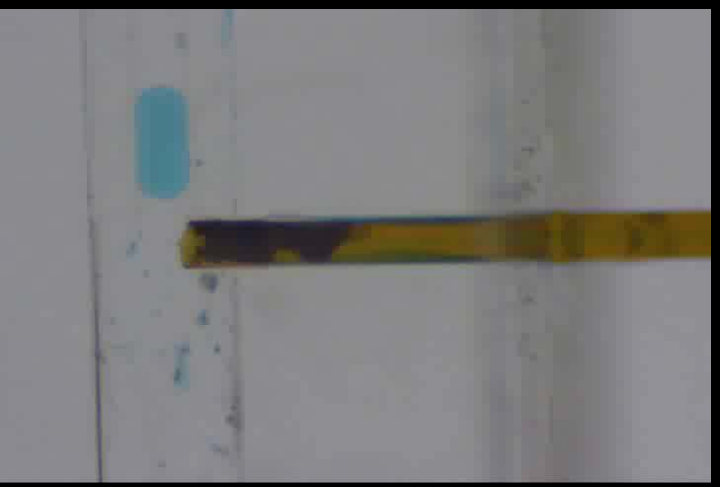
Time (s)

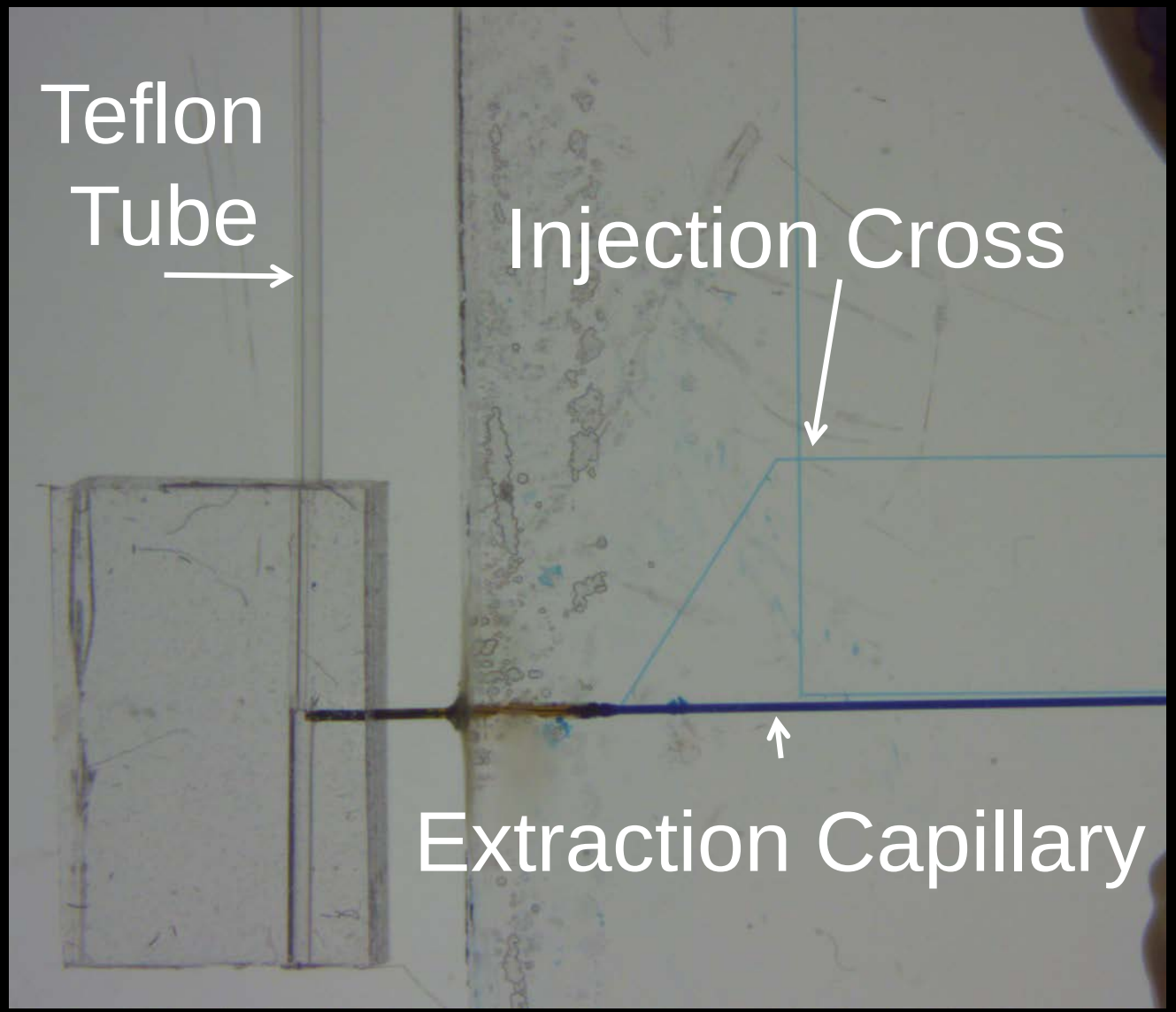
Droplet Volume	Flow Rate	Throughput	Peak Height RSD
65 pL	38 nL/min	3.3 Hz	4.7%
300 pL	225 nL/min	6.2 Hz	4.0%
300 pL	350 nL/min	9.5 Hz	7.1%
1.2 nL	500 nL/min	2.1 Hz	3.7%

Electrophoretic Analysis of Droplets

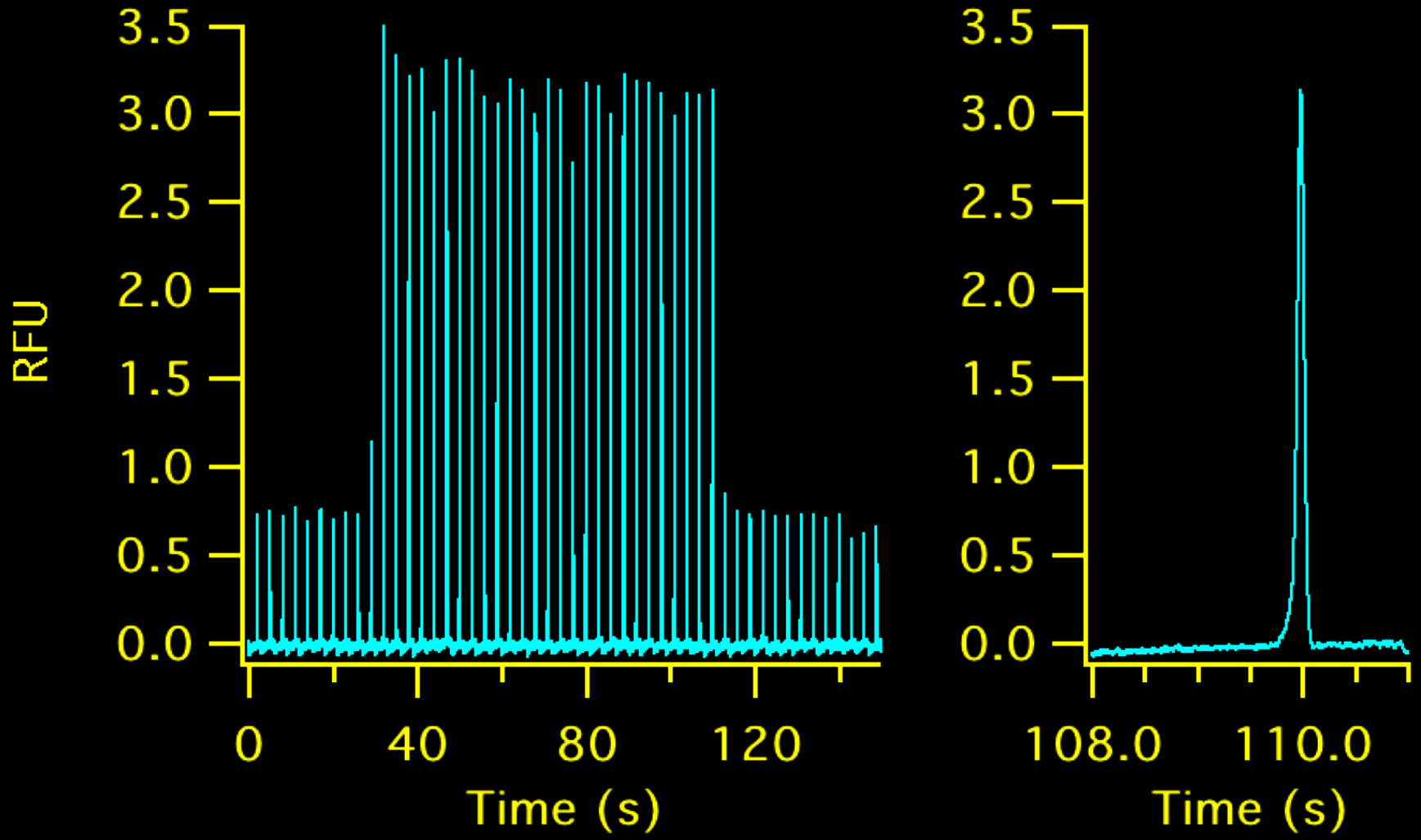


Dual chip droplet extraction scheme





Electrophoresis from Droplets



- alternating sets of 50 droplets containing either 20 nM or 100 nM fluorescein
- Detection at end of 2.5 cm separation channel
- 4 second separation with injection of every other droplet

Droplet Microfluidics in Chemical Analysis

- High Throughput Screening:
  CE, ESI-MS
- Sampling to Analysis (“Sensing”):
  In Vivo
  PAT
- Separation to Fractions (Capillary LC):
  Off-line MS, NMR
  Post-column reactions

"Sensing" in the Living Brain:

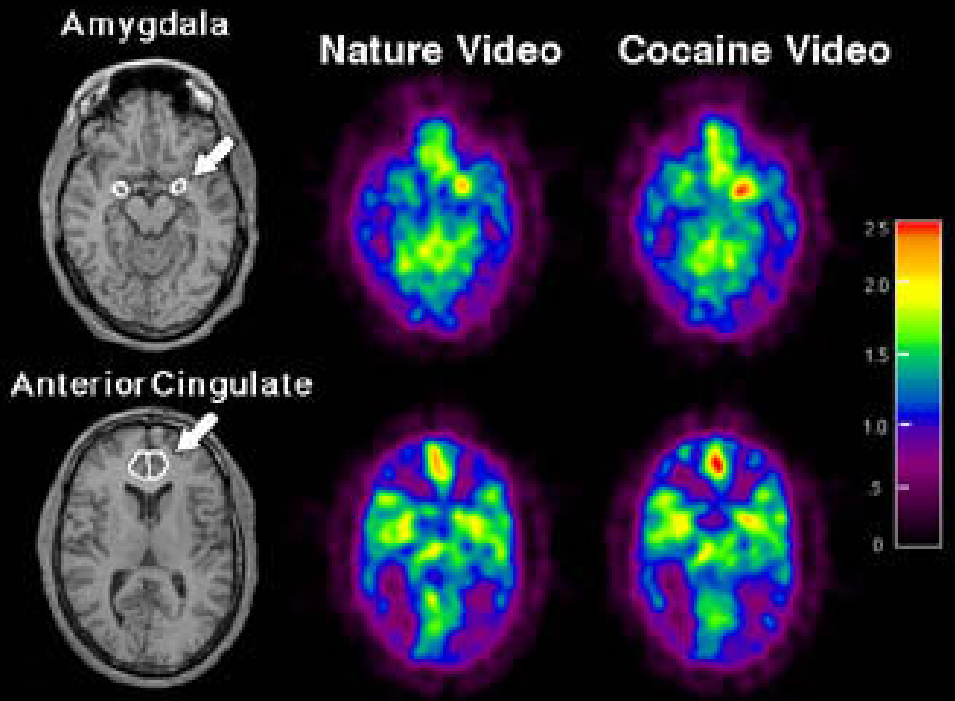
Identify chemical signals in behavior, learning, pharmacology, pathophysiology

Challenges:

- Rapid chemical changes
- Spatially heterogeneous
- Delicate tissue
- >100 neurotransmitters + metabolites
- Freely moving animals

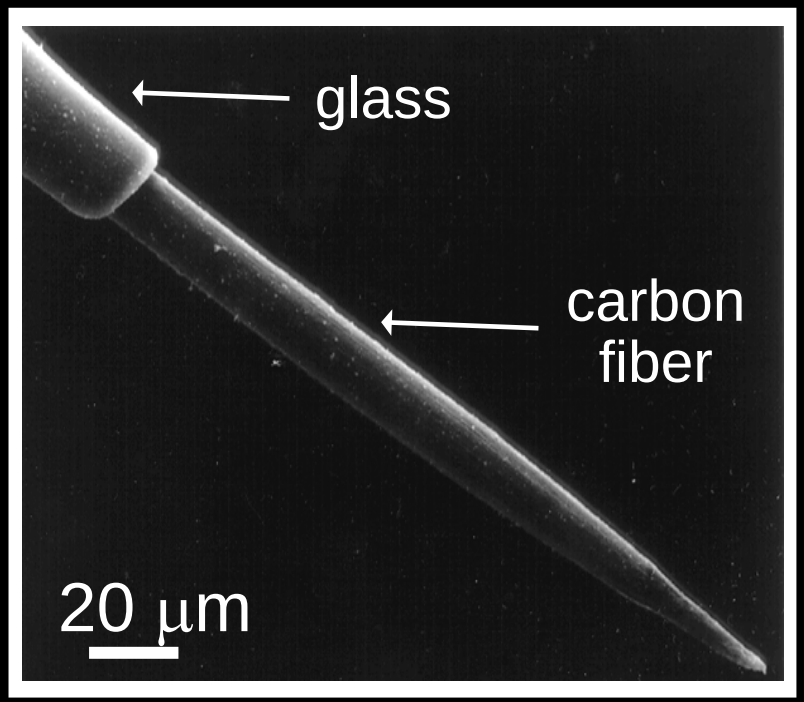
In Vivo Measurements of Neurotransmitters

PET



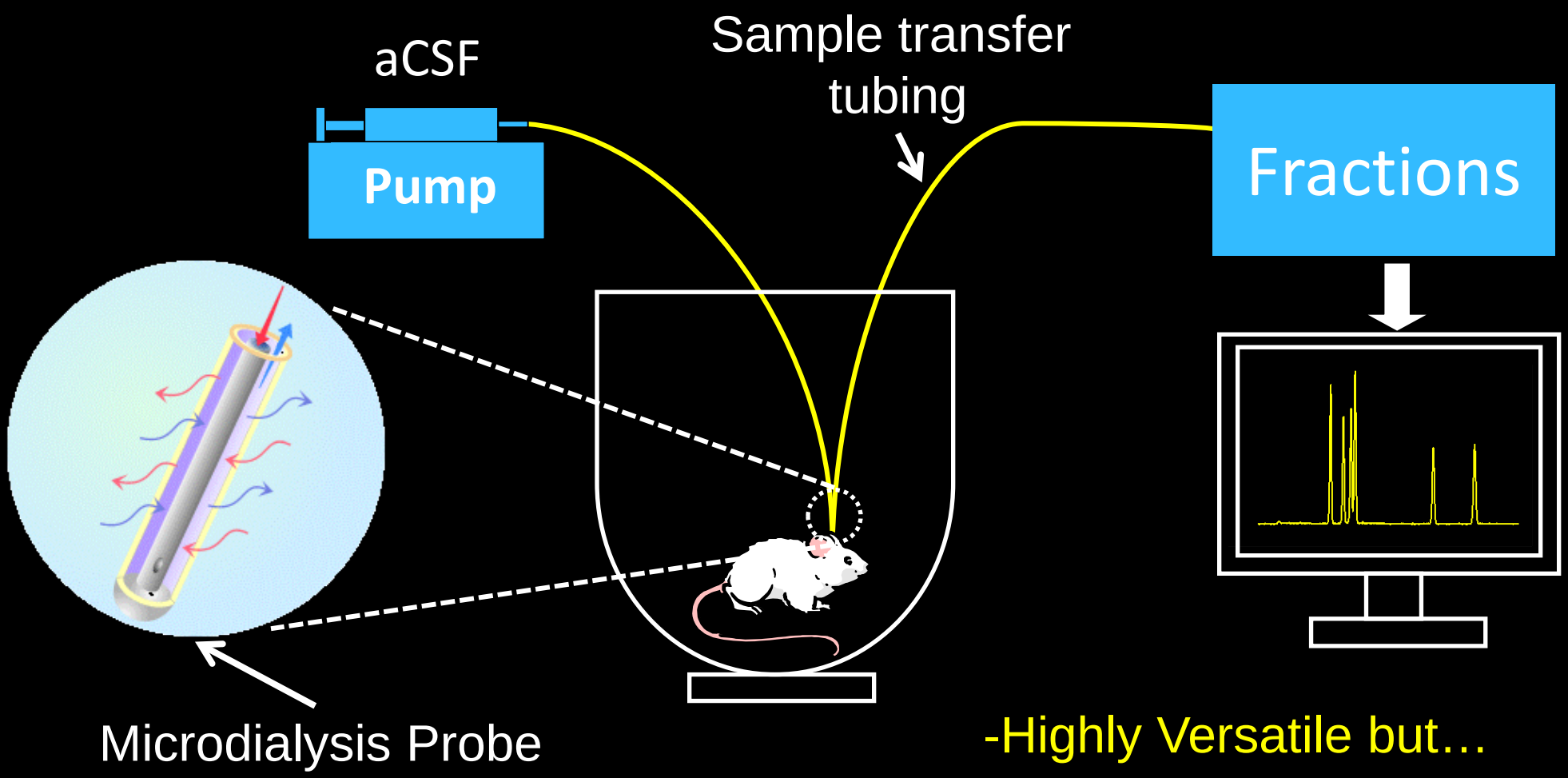
Some limits: Humans, single analyte, temporal and spatial resolution , interpretation

Implantable Sensors



Some limits: few analytes, single analyte, basal concentration

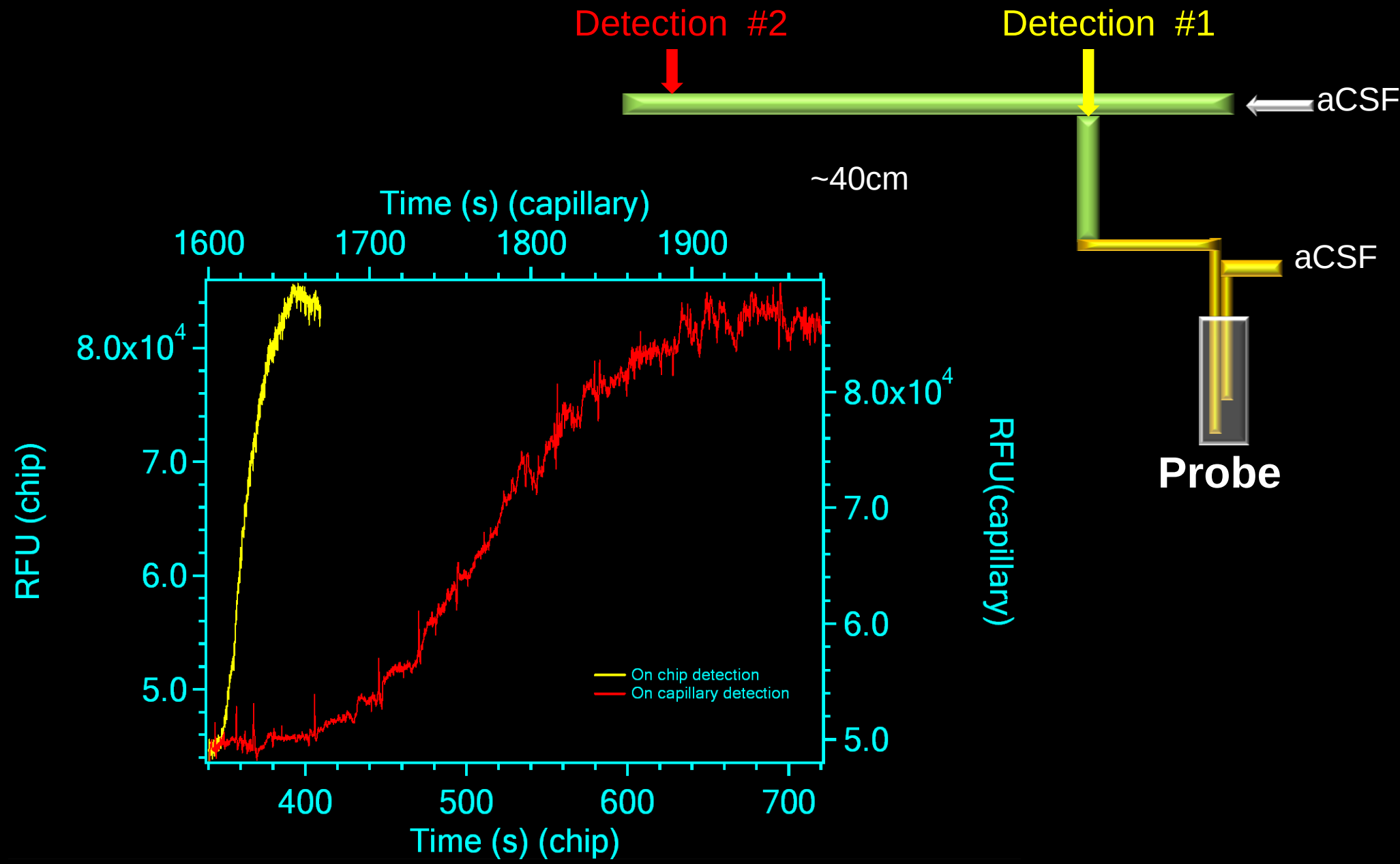
Microdialysis Sampling for In Vivo Monitoring



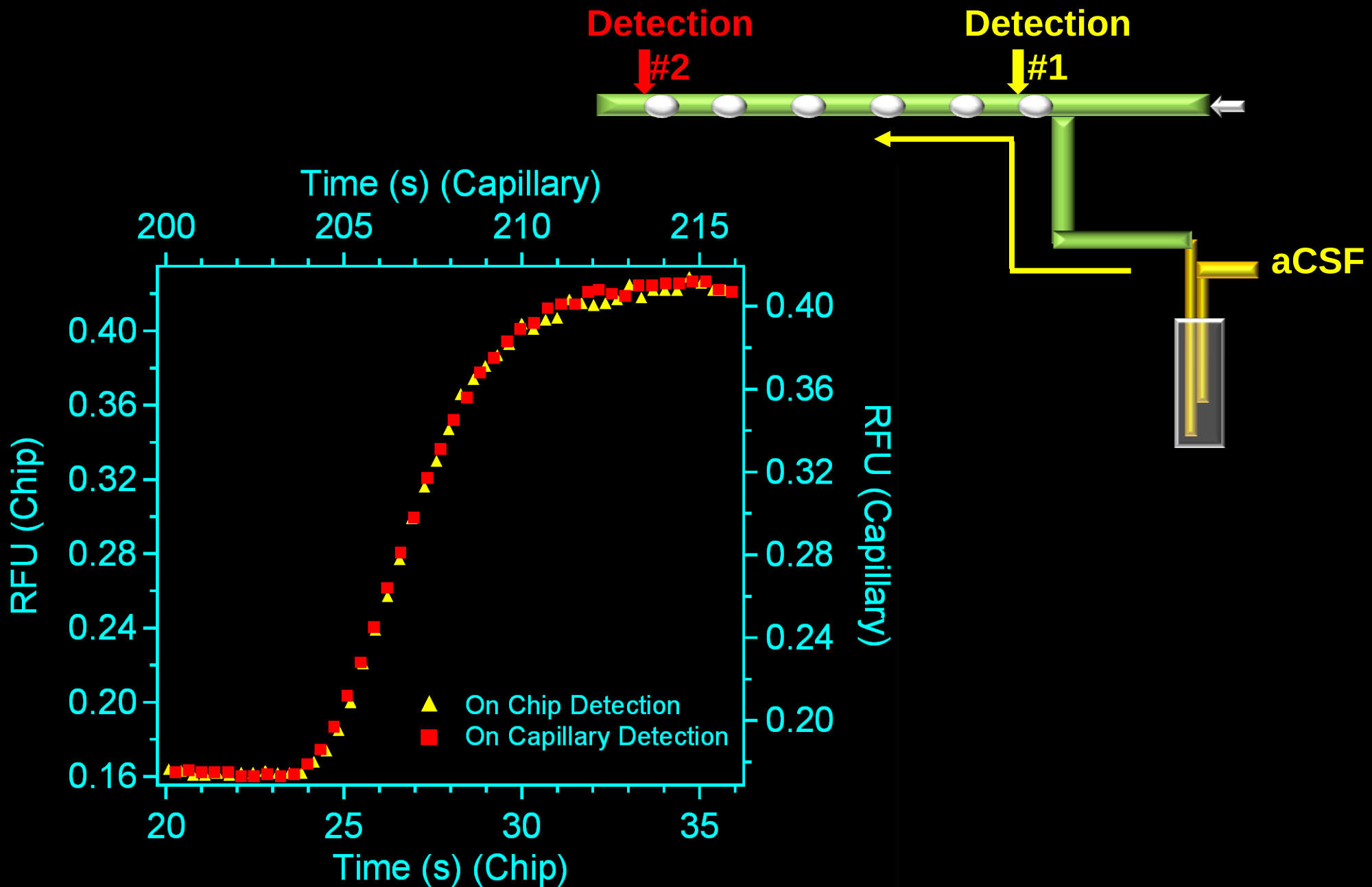
-Highly Versatile but…

-Low Temporal Resolution when coupled to HPLC because of large sample requirements (10 min)

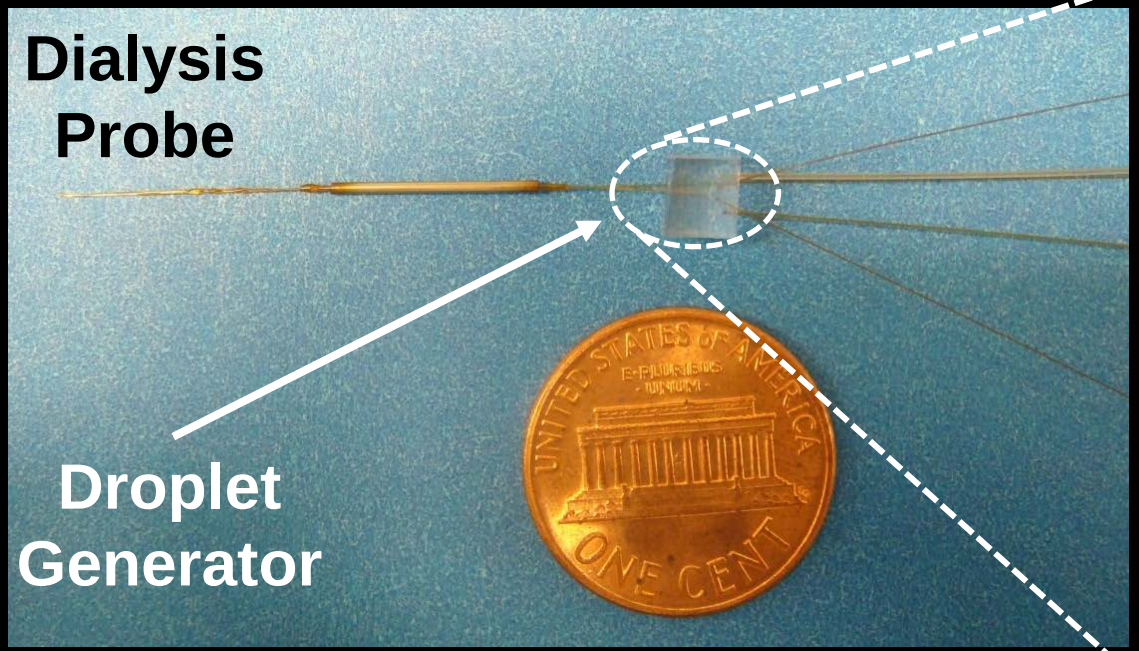
Temporal Resolution in Microdialysis Sampling: Impact of Transfer Tubing

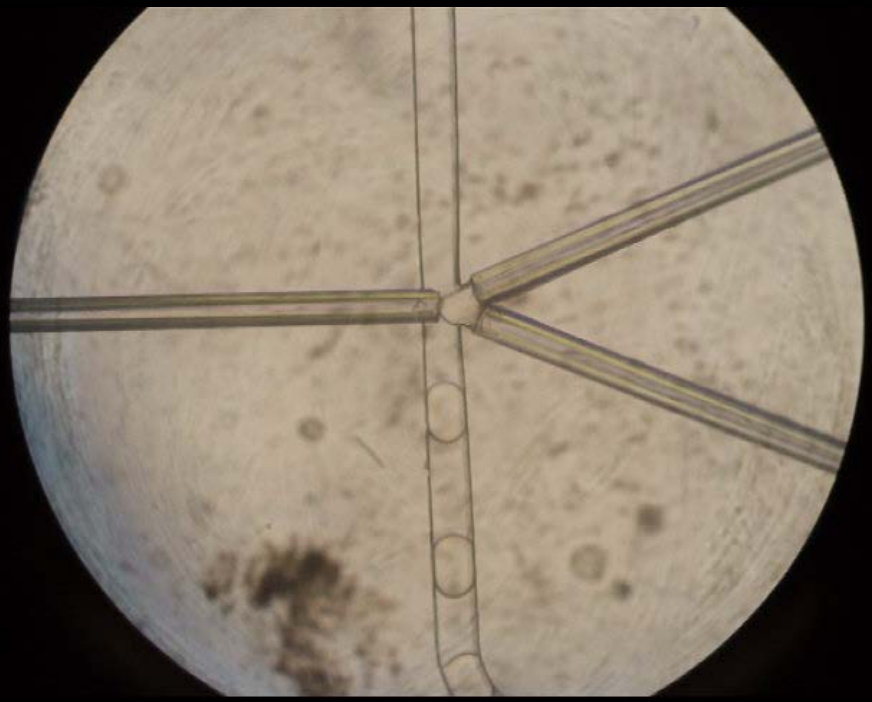


No Temporal Distortion with Segmented Flows



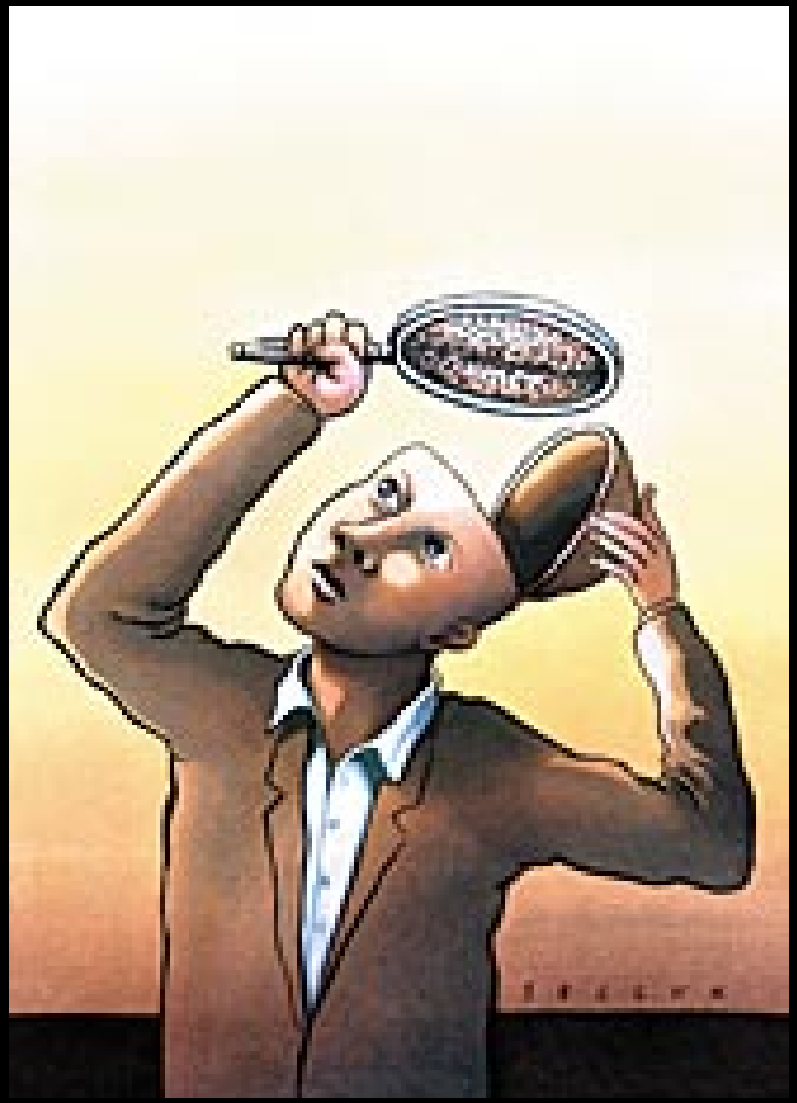
On-Board Droplet Generator for Awake Animal Experiments with Reagent Addition





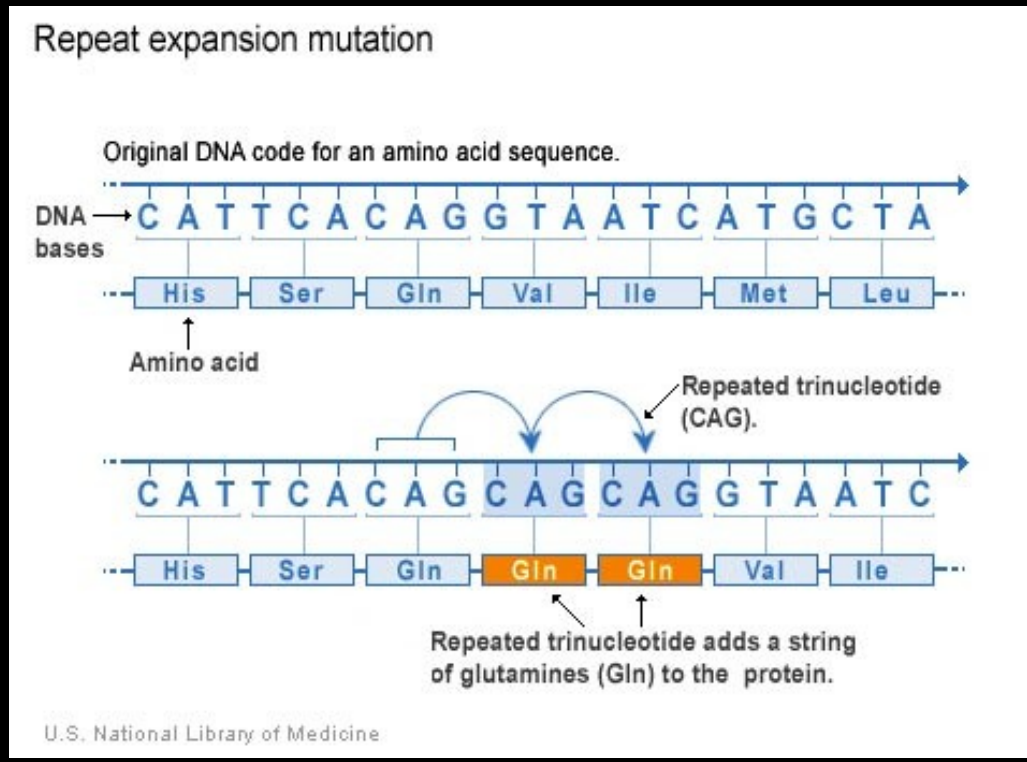
Applications

- Effect of drugs on the brain
- Changes in transmitters with diseases
- Changes in transmitters with behavior
- Changes in transmitters with learning and memory

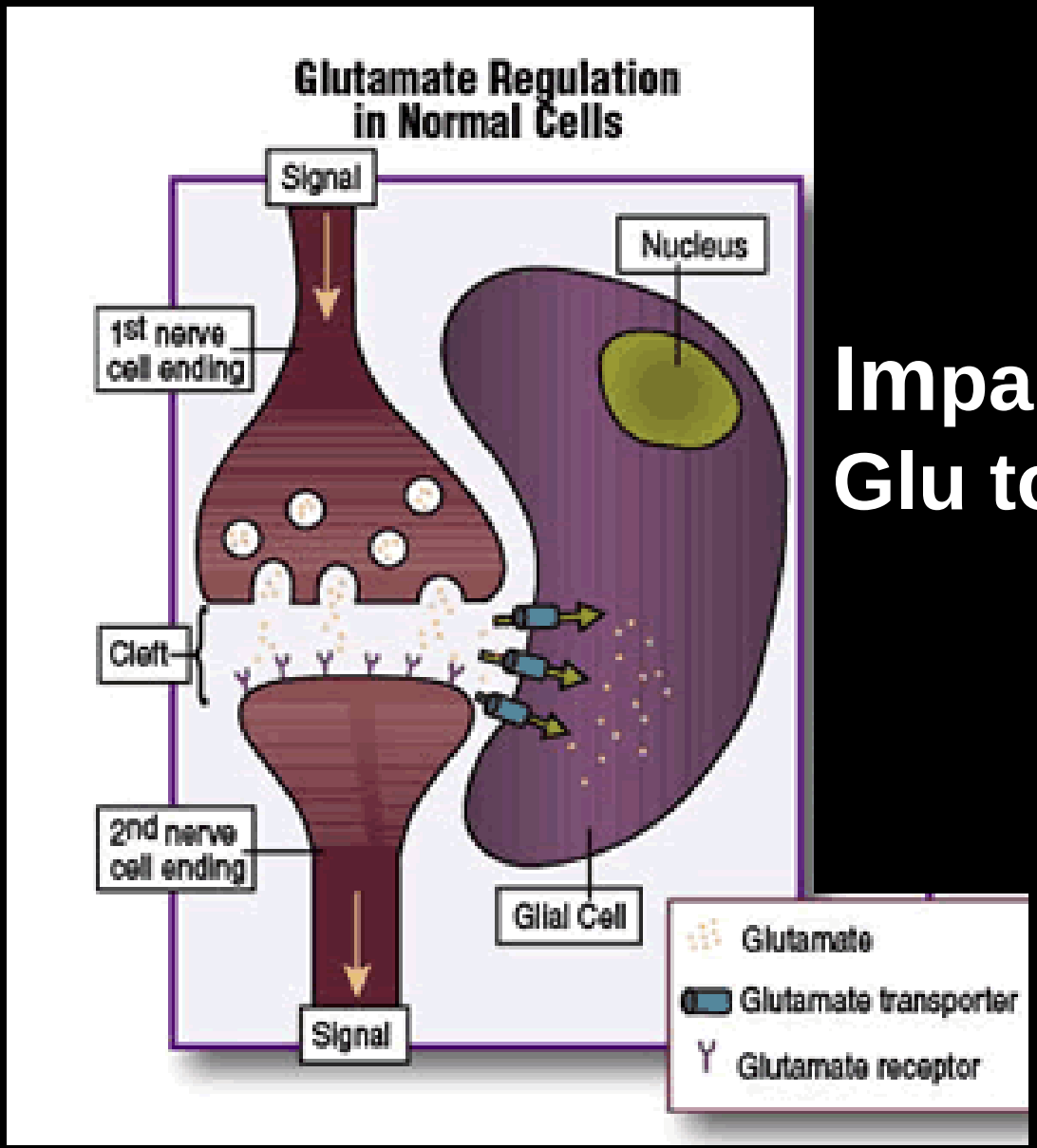


Huntington's Disease

- "CAG Repeat" disease
- Neurodegenerative
- Cognitive and motor impairment
- Death within 15 years
- ~30k cases in USA
- Little treatment available

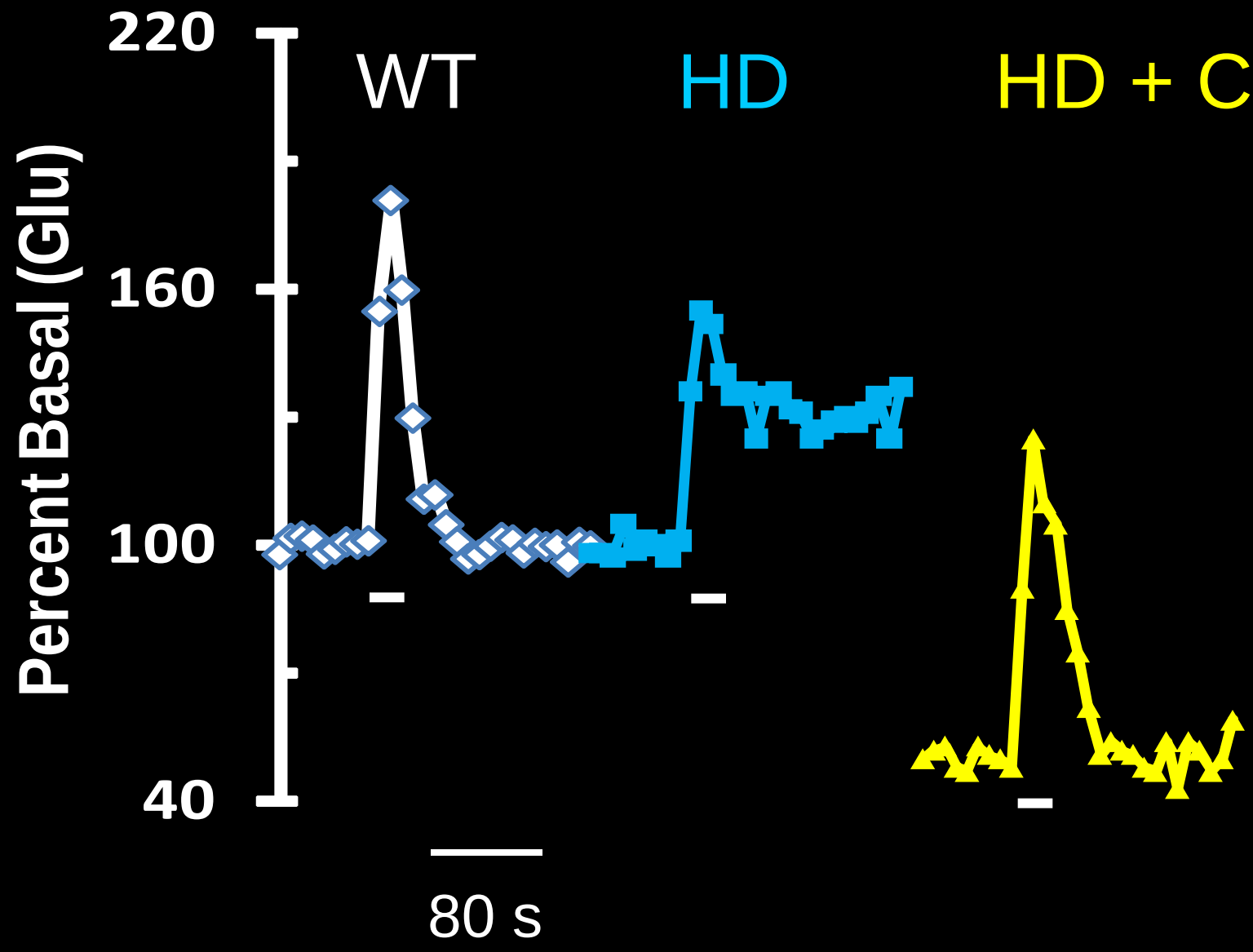


Glutamate Neurotransmission

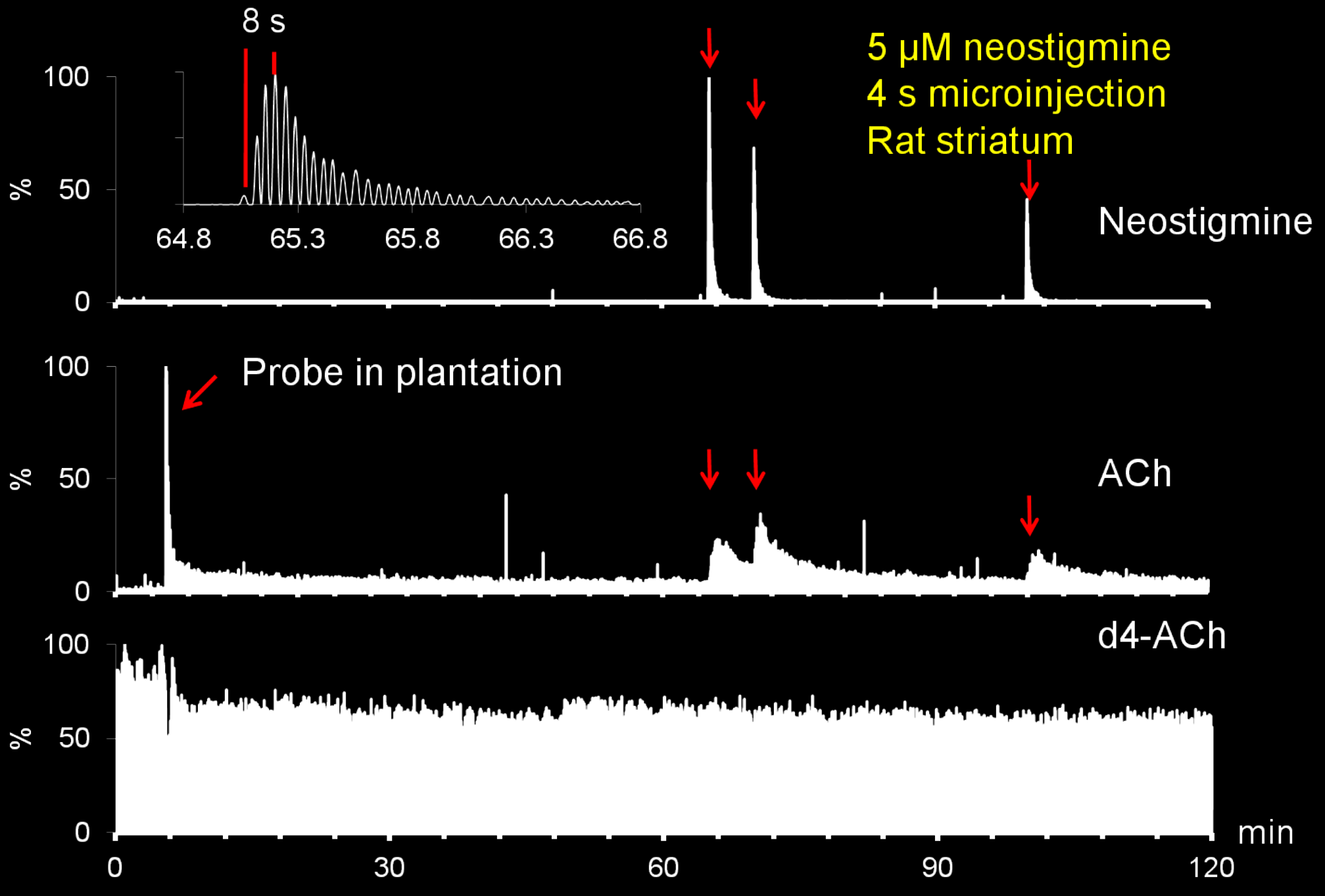


Impaired glutamate uptake
Glu toxicity?

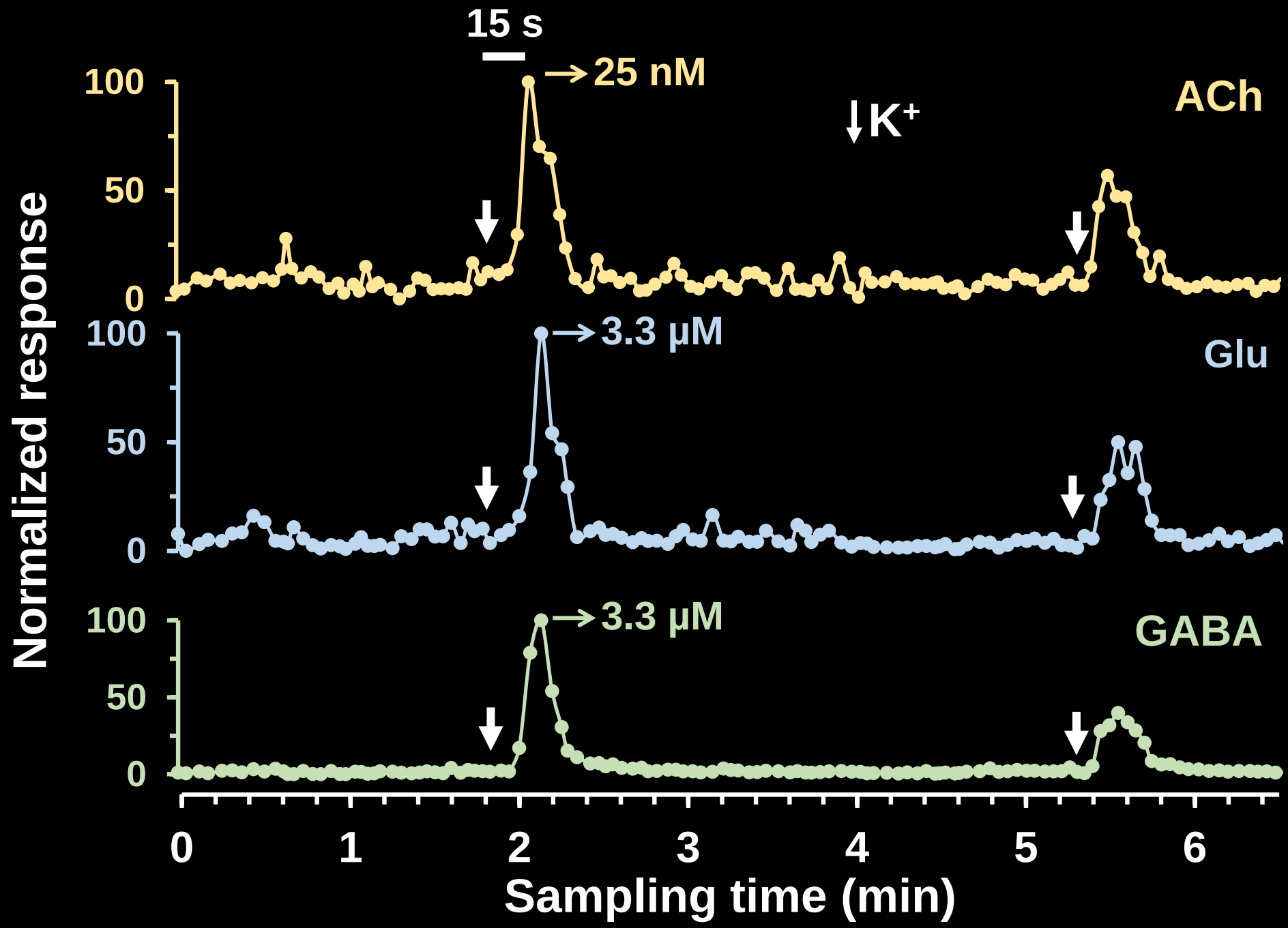
Recovery of Normal Glu Uptake in HD Mice with Cef



In Vivo Test – with MS Analysis



In Vivo Neurochemical Dynamics by Droplet MS



Droplet Microfluidics in Chemical Analysis

- High Throughput Screening:

  Drug Screening, Enzyme Evolution
- Sampling to Analysis (“Sensor”):
  In Vivo
  PAT
- Separation to Fractions (Capillary LC):

  Off-line MS, NMR

  Post-column reactions

High Throughput Screening (HTS) for Drug Discovery

- 100,000 to 2,000,000 candidates for one target
- High density well plates / Robots / Optical Readout

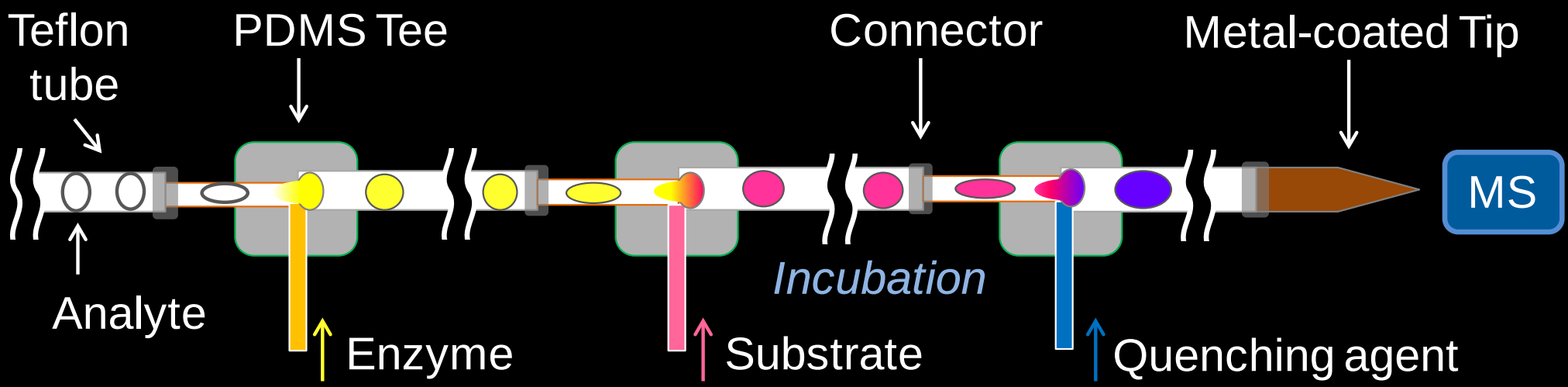
Sloan-Kettering Institute, www.mskcc.org

Problems:
-Assay development
-False Signals
-Reagent cost: $50k/screen (50 μL→7.5 L for 150,000 samples)

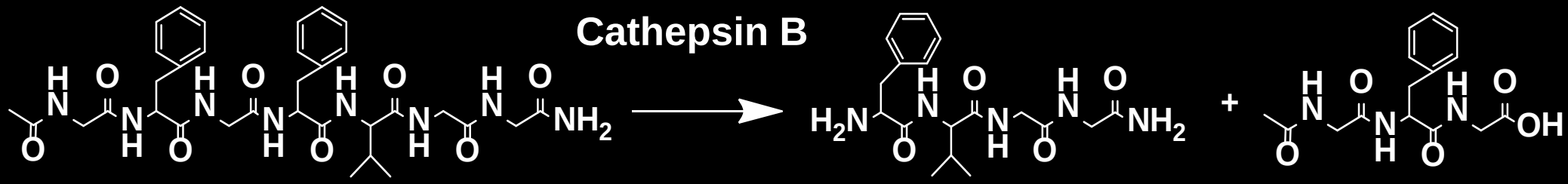
Droplet Mass Spectrometry for HTS

- Label free
- High Resolution: Complex assays (multi-product) or Multiplexed
- Enzyme and Binding reaction
- Potential for 1000-fold reduction in sample volume compared to multi-well plates

In-Droplet MS Assay (miniaturization)

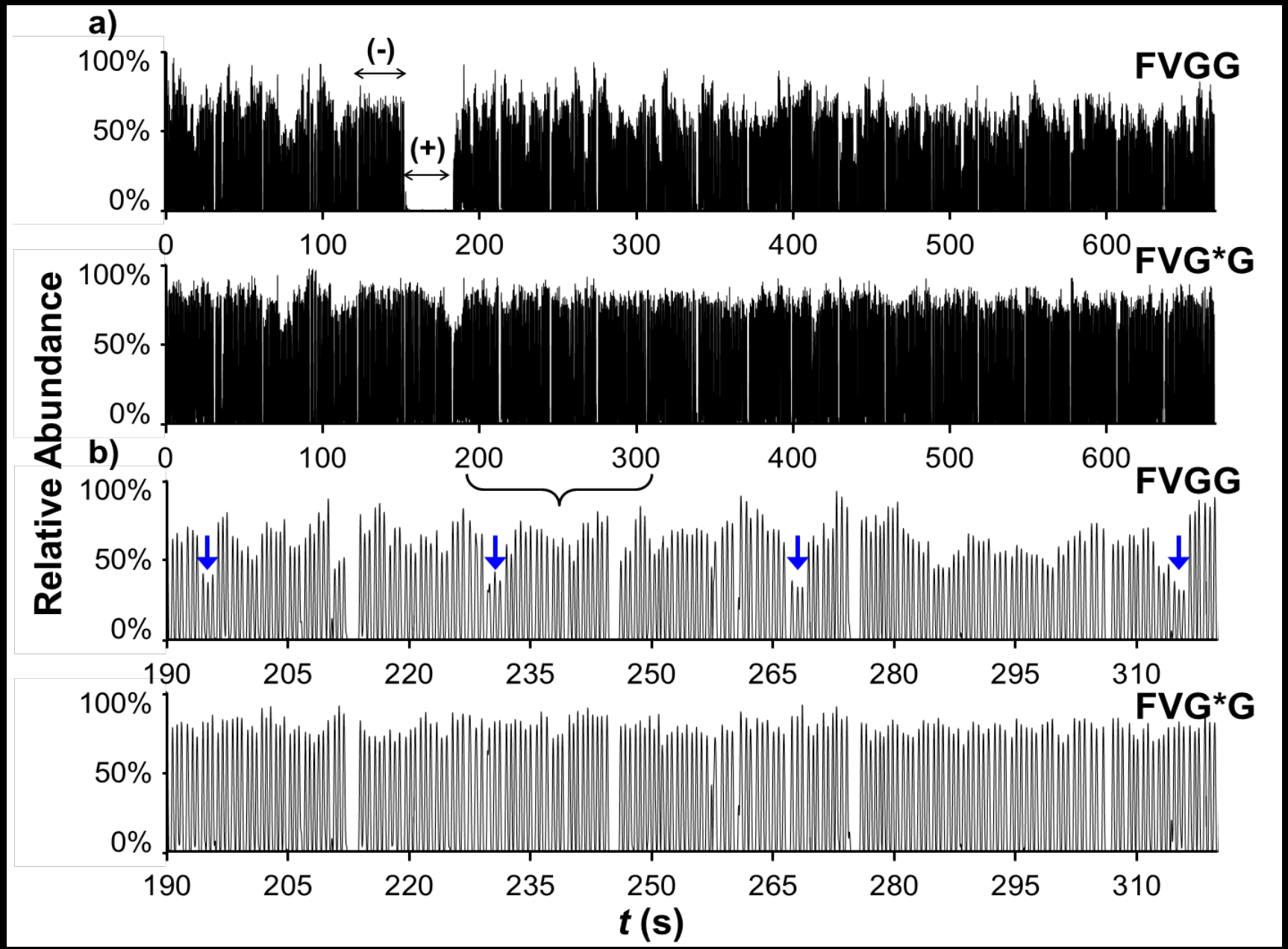


Can Droplet ESI-MS be Robust Enough to Screen Many Samples?



- Lysosomal cysteine protease
- Important in cartilage destruction
- Inhibitors of interest for some forms of arthritis, cancer, and infections

Raw Data from Prestwick Library Screen (1280 Compounds, 4,430 Assays)



Electrophoresis for High Throughput Screening? (Caliper)

✓ Fast and Low Sample Consumption

✓Do not have to engineer fluorescent change

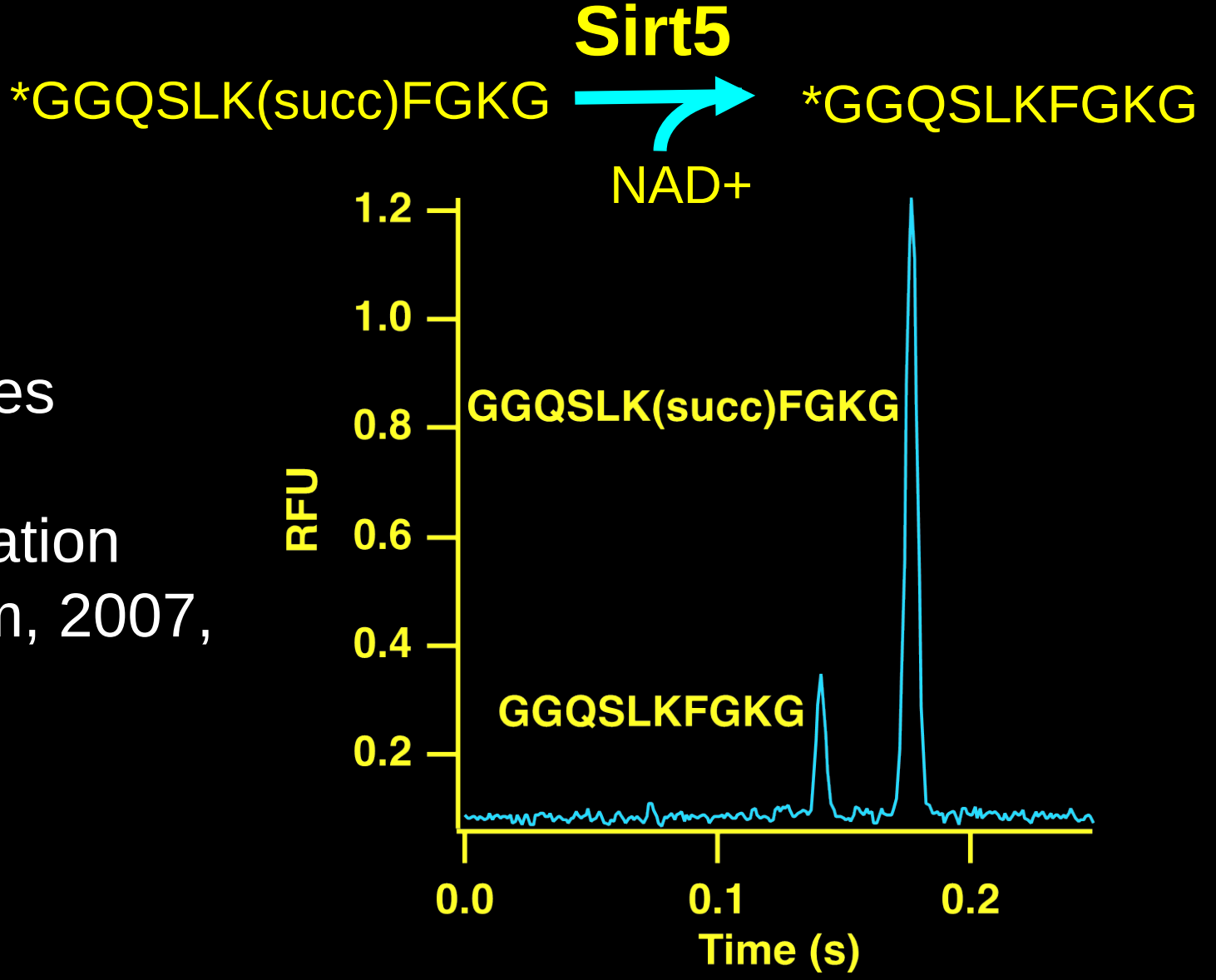
✓Versatile: Enzymes & Non-Covalent Complexes (protein-protein interaction)

✓ Multiplex

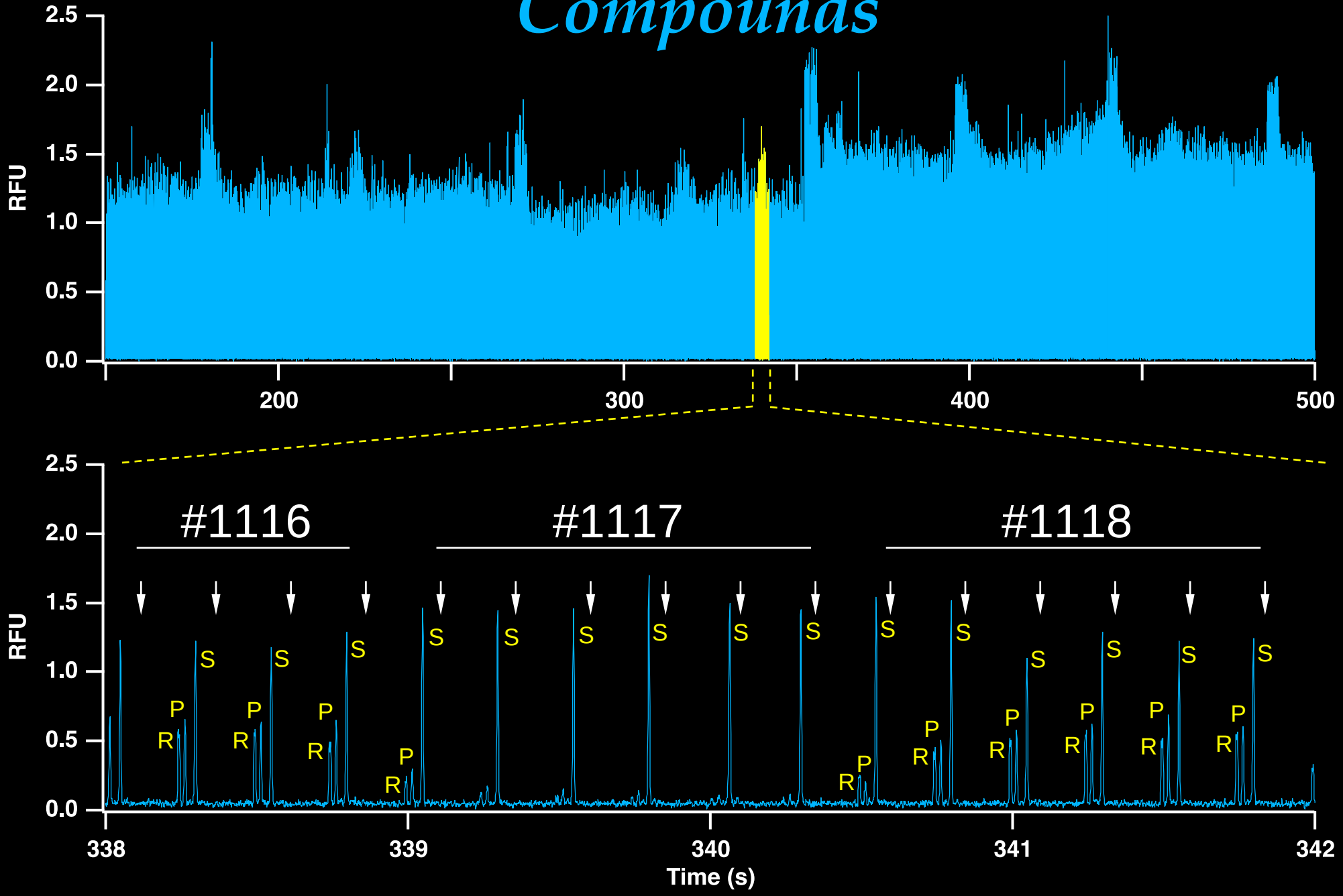
- Fast CE, but must get new samples onto chips

Electrophoresis for Screening: Fast Enzyme Assays

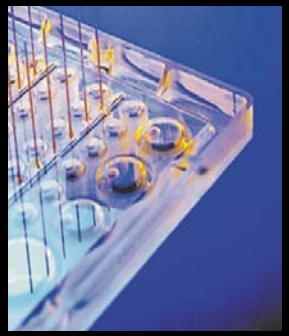
- Sirtuins
- Kinases
- Phosphatases
- Peptidases
- GPCR activation
  (Anal Chem, 2007, 79:1158)

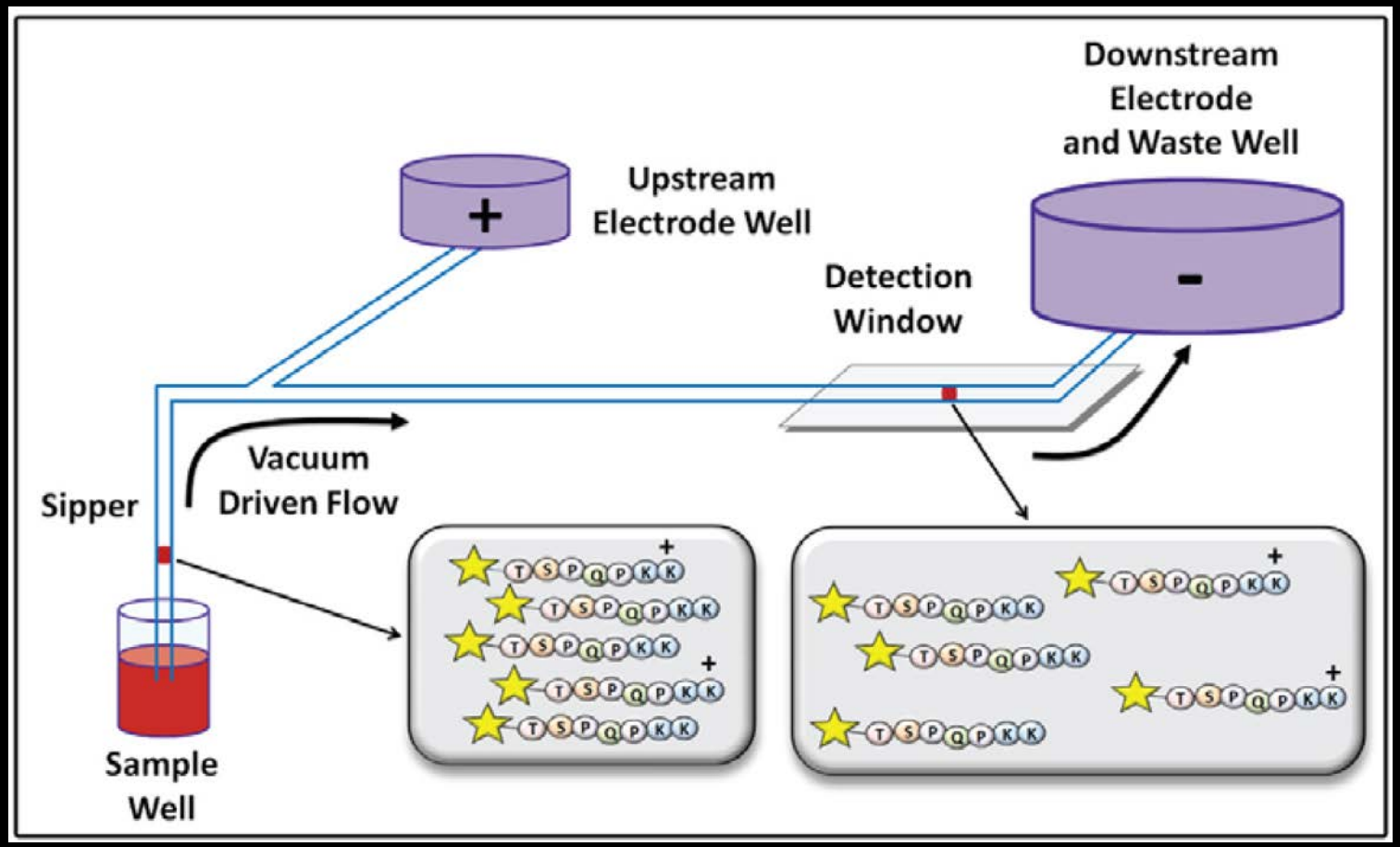


Large scale SIRT5 screening – 1280 Compounds



Fast Sample Introduction: Caliper High Throughput "Sipper Chip"

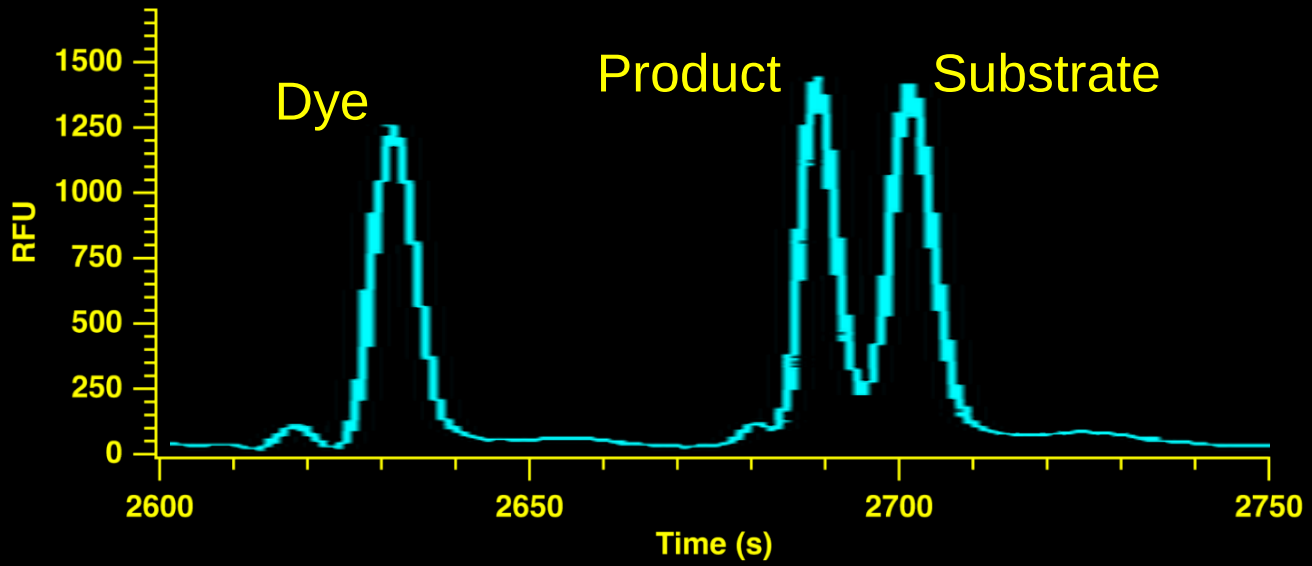




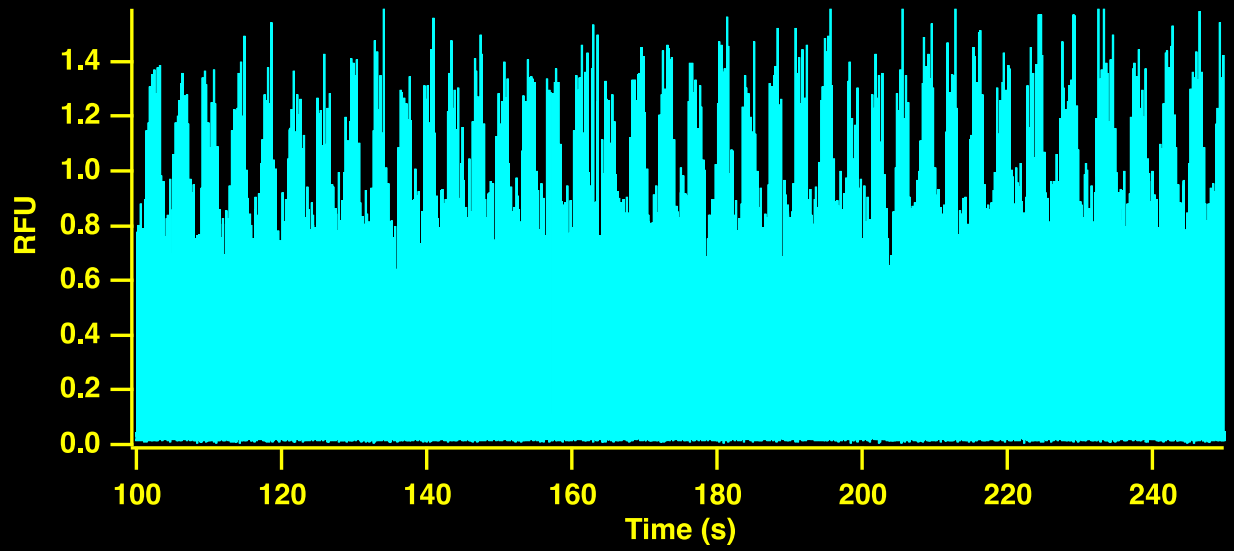
- Reads 384 Well Plate in 80 minutes
- Vacuum flow through channel is compromise

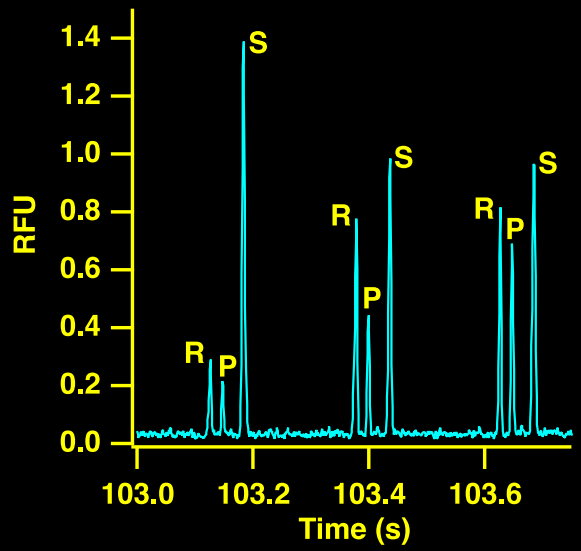
Comparison to LabChip System

Caliper LabChip (1 sample x 1 injection)



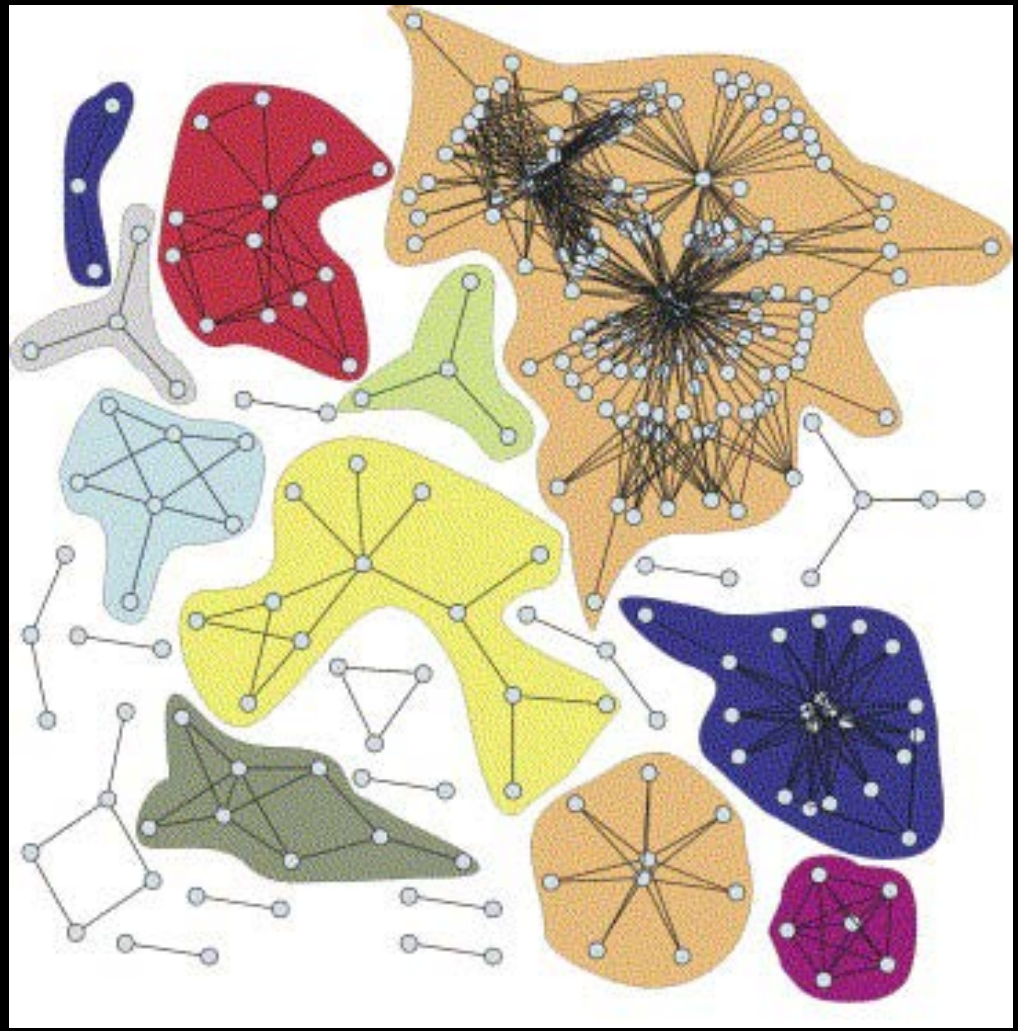
Droplet-CE (75 samples x 8 replicates = 600 injections)





Protein Protein Interaction as Drug Targets

- Long believed to be “undruggable”
- Large number of emerging targets
- Small molecule modulators identified recently



Part of human interactome

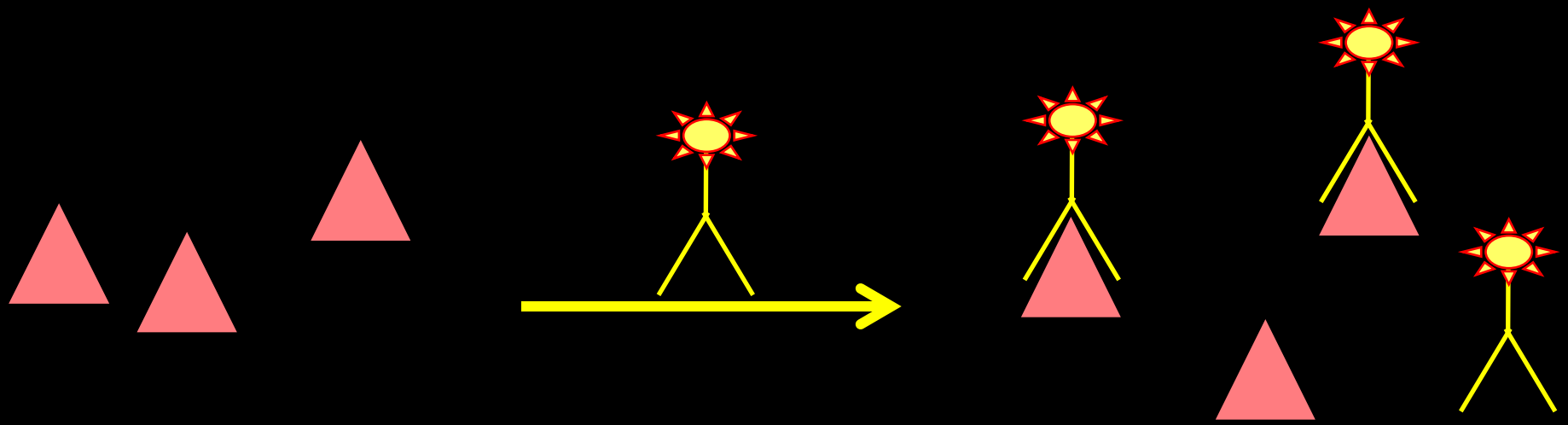
- Protein–Protein Interactions as New Drug Targets, Enno Klussmann, John Scott, Springer-Verlag Berlin Heidelberg, 2008.
- Aloy P, Russell RB, FEBS Lett. 2005 Mar 21;579(8):1854-8.

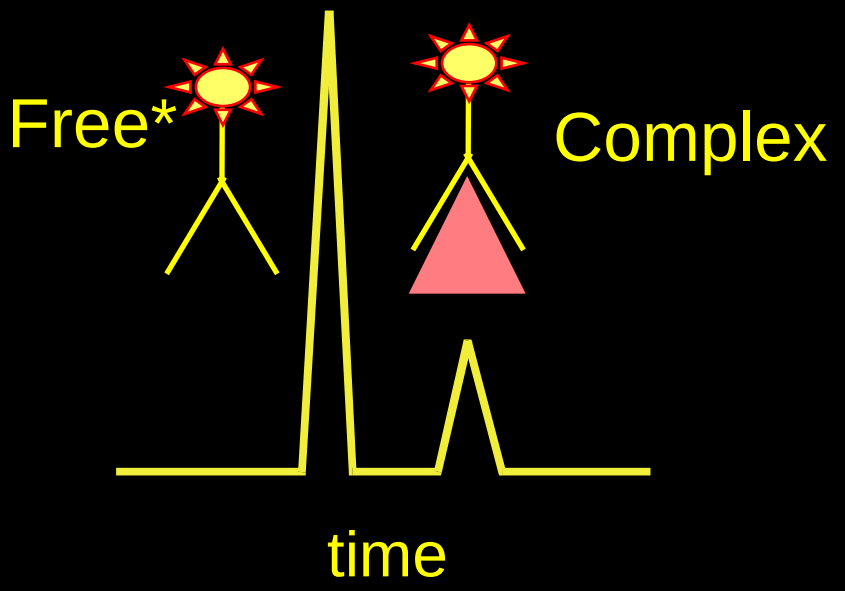
Protein-Protein Interaction Assays and Screens

Ideal: Fast, low sample consumption, label free, easy to adapt to new proteins, multi-protein complexes

- Surface Plasmon Resonance
- FRET
- Fluorescence Polarization
- Isothermal Calorimetry
- Bead & surface binding assays

Affinity Probe CE (Noncompetitive Affinity Assay)

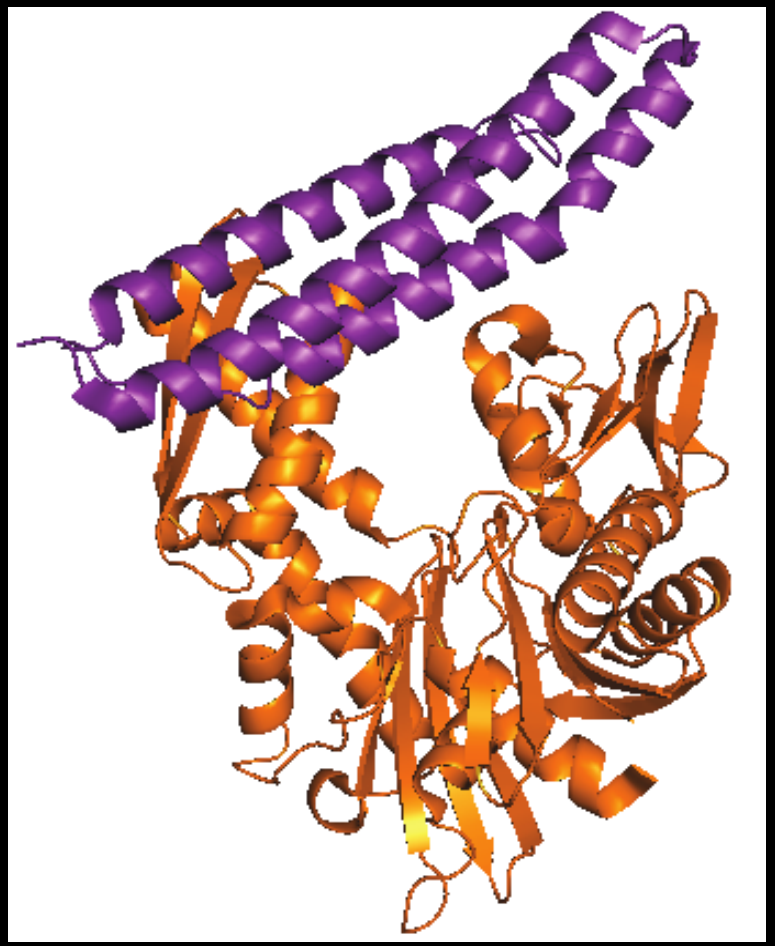




Antibody-Antigen
Aptamer-target
Protein-peptide
Ligand-receptor
DNA-protein
Drug-apoenzyme

Hsp70 and Bag3 as Cancer Target

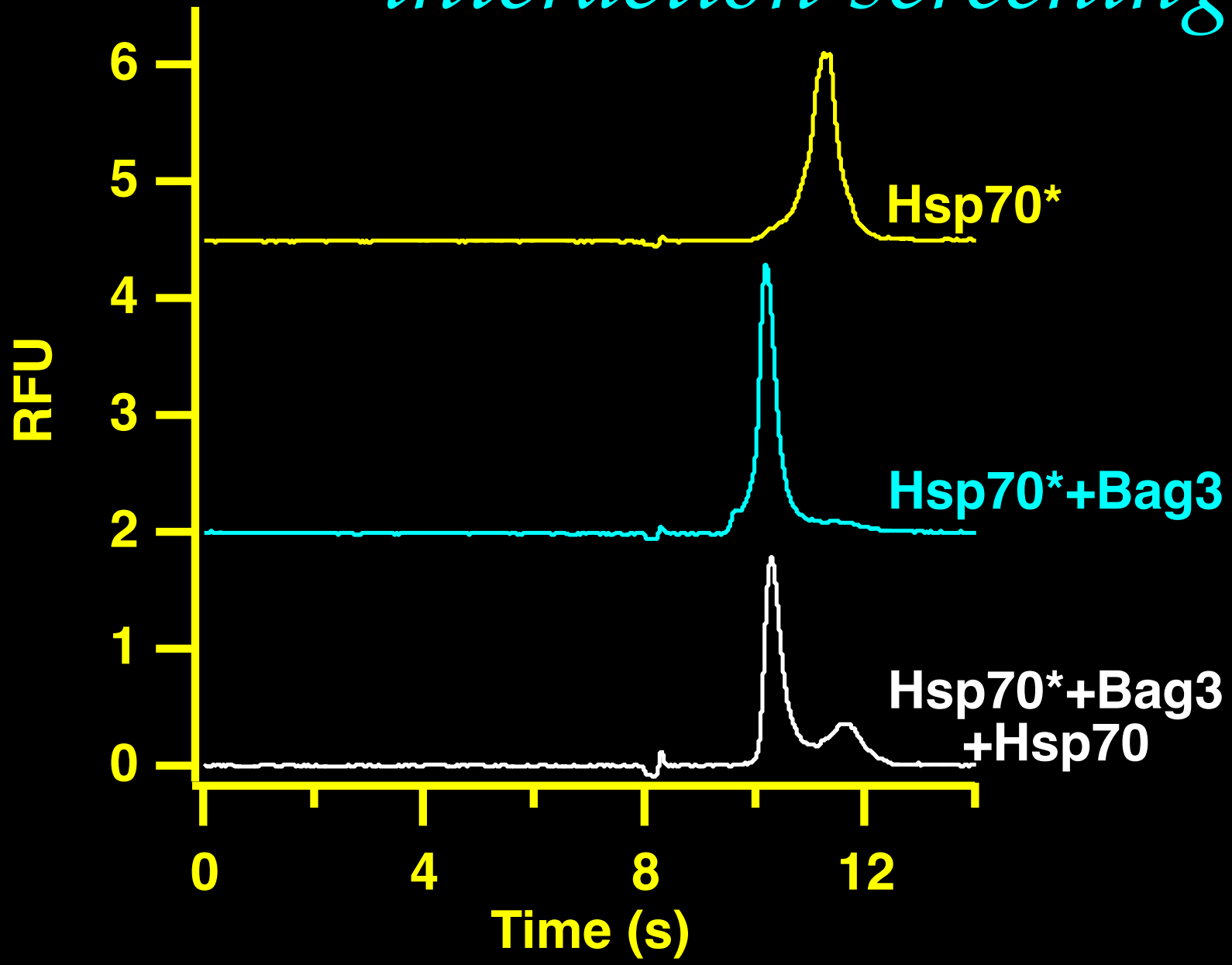
- **Hsp70s are chaperone proteins**
- **Bag3 binds Hsp70 with high affinity**
- **Bag3 has anti-apoptosis property**
- **Mechanism is Hsp70 dependent**
- **Interesting cancer target**



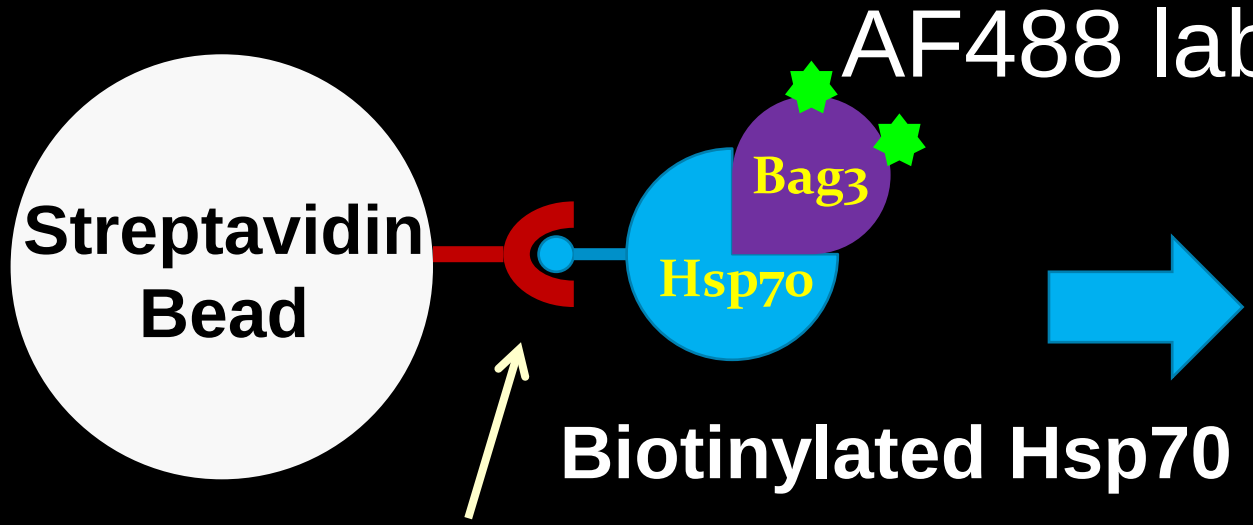
Crystal structure of Bag domain binding to Hsp70

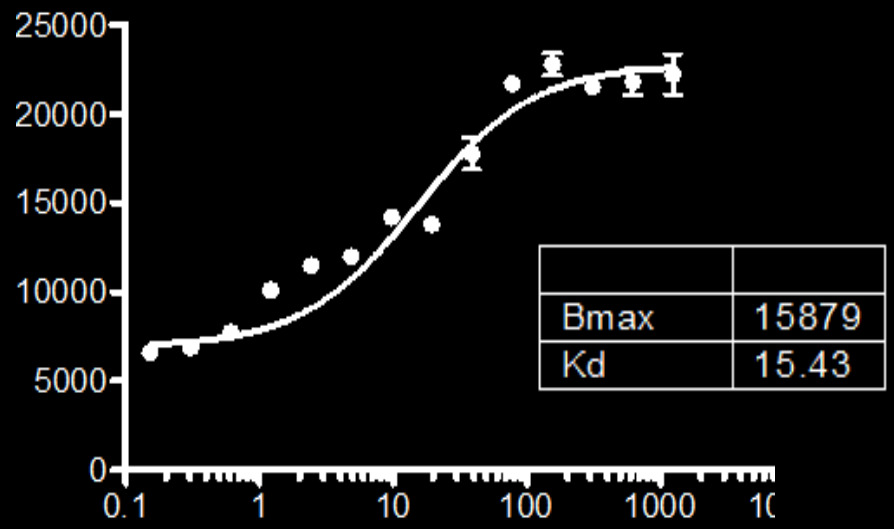
M. P. Mayer and B. Bukau, CMLS, Cell. Mol. Life Sci. Vol. 62, 2005

Rapid Separation for Protein-protein interaction screening



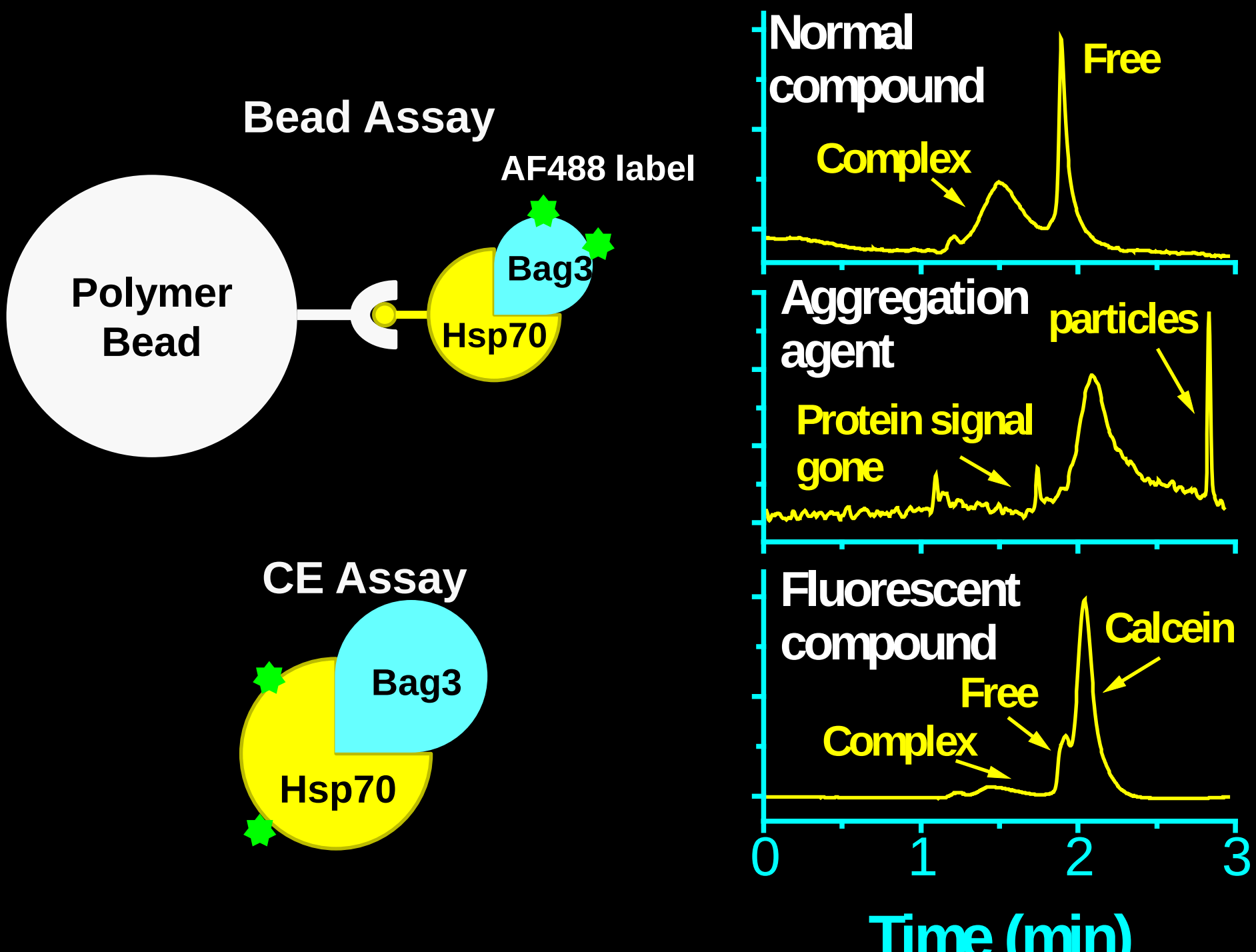
Current Standard Screen: Flow Cytometry Protein Interaction Assay





David L. Roman, et al, *Mol Pharmacol 71:169–175, 2007*

Why is CE More Selective?



Conclusion

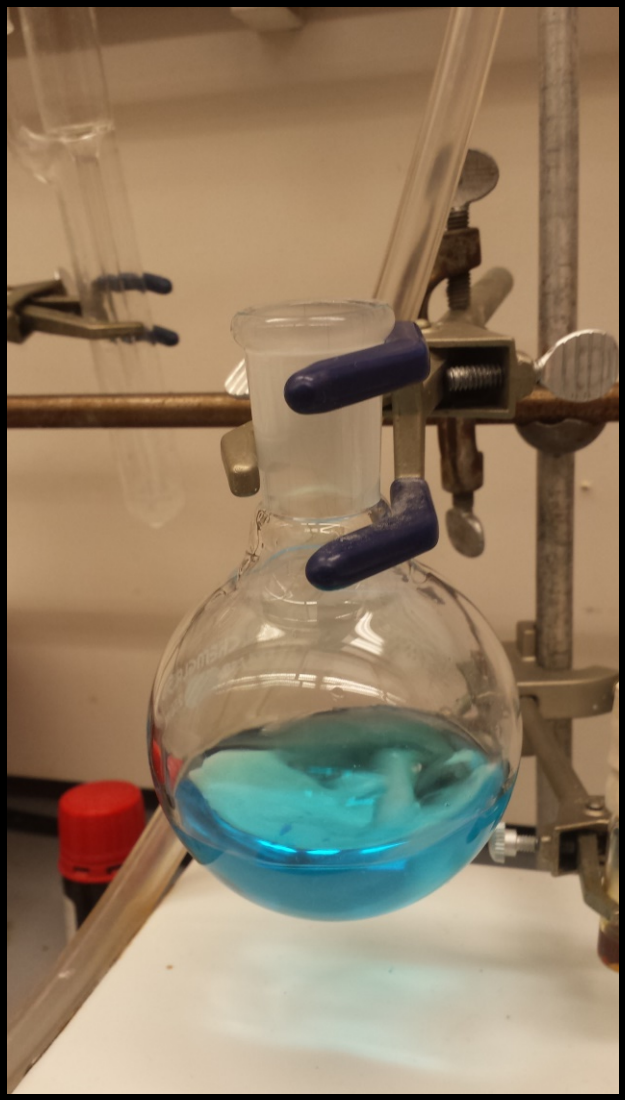
- CE and MS provide high quality hits
- Greatly reduced sample usage
- Enzyme and PPI possible

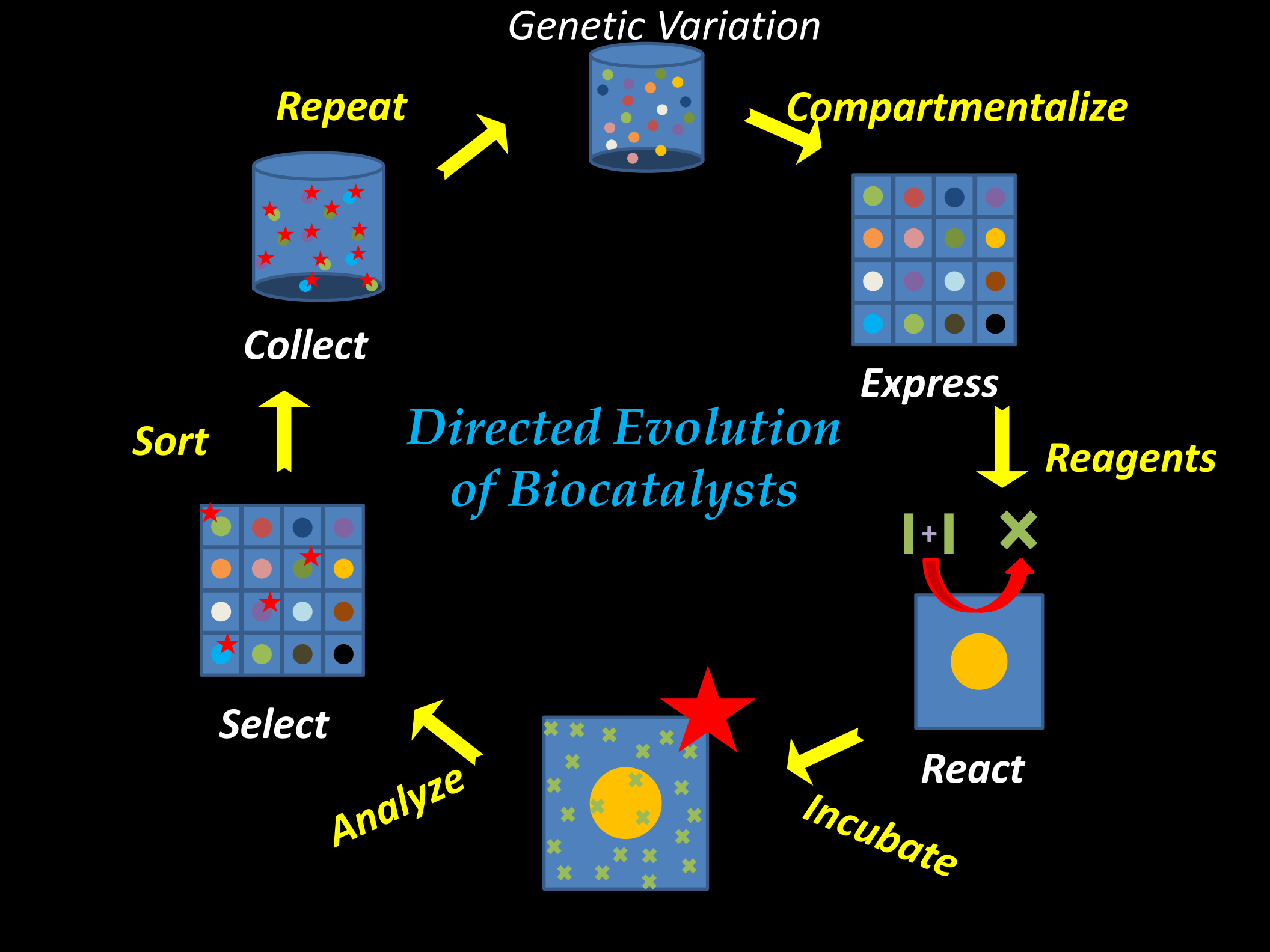
Enzyme Engineering For Catalysis

- Synthetic Catalysts
  - Labor intensive
  - Expensive
  - Toxic reagents
  - Variable stereoselectivity

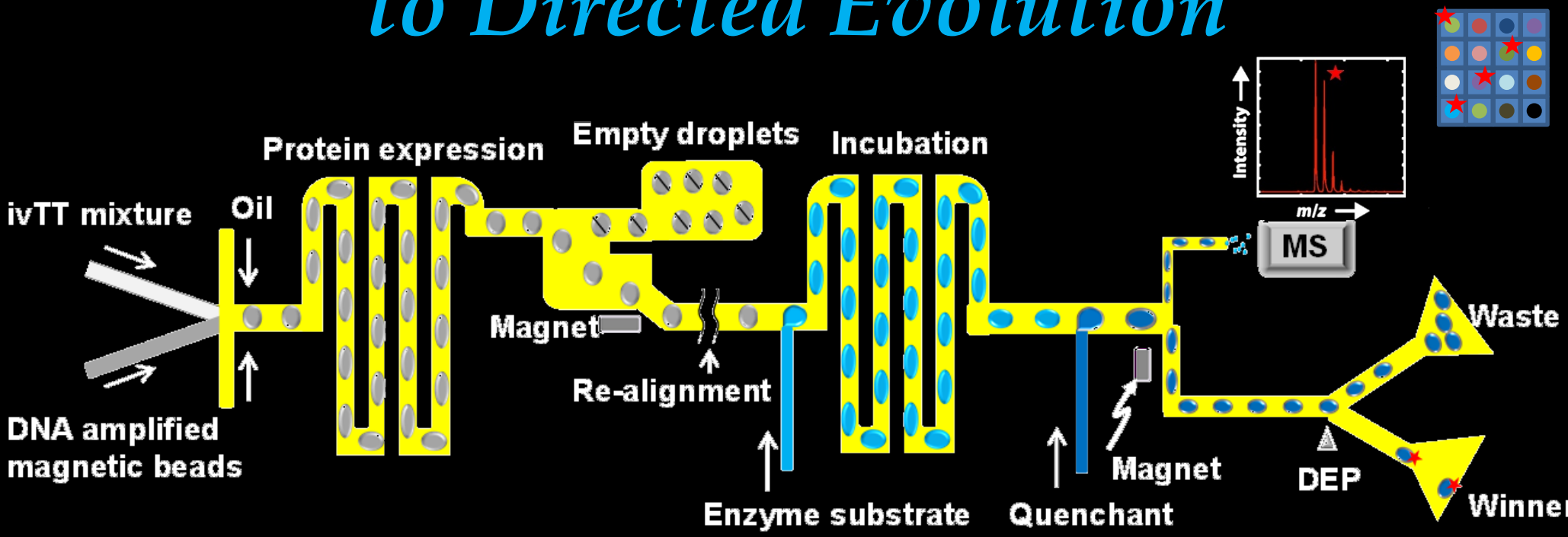
- Natural enzyme catalysts
  - Stereoselective
  - “Green”
  - Rapid, Efficient
  - Substrate specific

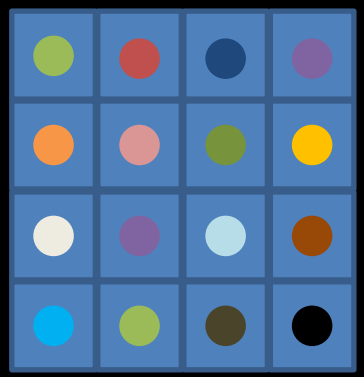
- Engineered Enzymes
  - Enzyme properties, for substrates of interest

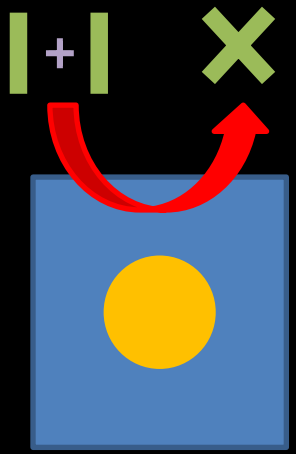


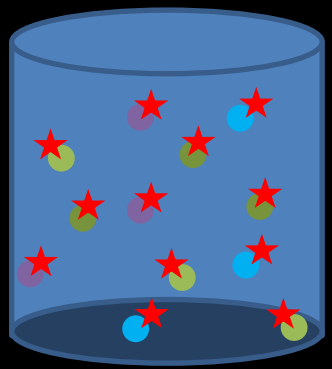

Genetic Variation
Compartmentalize
Express
Reagents
React
Incubate
Analyze
Select
Sort
Collect
Repeat
Directed Evolution of Biocatalysts

Adapting Droplet Microfluidics to Directed Evolution









Summary

- Segmented flow for high-throughput sample manipulation at low volume
- ESI-MS and CE interface
- Nanoliter lab
- HTS, in Vivo Sensing, Fraction collection

Acknowledgements

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NEW OBJECTIVE