Concentration-Enhanced Molecular Binding and Activity Assays using Microfluidic Biomolecule Concentrator

Jongyoon “Jay” Han

Department of Electrical Engineering and Computer Science
Department of Biological Engineering

Massachusetts Institute of Technology
Ion Concentration Polarization

Ubiquitous in many electrochemical devices

Caused by carrier mismatch at the interface  
(comparable to p-n junction in semiconductor)

Complicated, multiphysics / multiscale phenomenon  
(drift, diffusion, convection)
What is Ion Concentration Polarization?

Ion Depletion Zone
Ion Enrichment Zone

Video. Ion depletion/enrichment behavior
Electrokinetic Trapping of Molecules

\[ E(x) \]

\[ F_E = F_{\text{flow}} \]

\[ F_E < F_{\text{flow}} \]

\[ J = \sigma E \]

\[ \sigma(x) \sim C(x) \]

Electric Field Gradient

Ion Concentration Gradient
Nafion Surface Micropatterning for PDMS concentration device

Lee et al. Lab Chip 2008, 8, 596
Kim and Han Anal. Chem. 2008, 80, 3507
Ko et al. Lab Chip 2011, 11, 1351

Easy, ‘fab-less’ fabrication
Integration into cell microfluidics

Surface patterning of Nafion film
(150~200nm)

Cross section images

Simultaneous operation of 128 concentrators
Pre-binding Immunoassay Sensitivity / Kinetics Enhancement

- Sensitivity enhancement up to ~500 fold achieved
- Measurement was done with background molecules (1000X higher concentration)

**Lab on a Chip**

**Graph**

- Blue line: 30 min incubation, no preconcentration
- Green line: 60 min incubation, no preconcentration
- Yellow line: after 15 min preconcentration / binding
- Red line: after 30 min preconcentration / binding
- Black line: after 30 min preconcentration / binding followed by washing

**Reference**

Wang and Han, Lab Chip 2008
Enhancement of Enzyme Reaction for Low-Abundance Enzyme Activity Assay

\[
\frac{d[P]}{dt} \text{ (rate of reaction)} = \frac{k_2 [E_0][S]}{K_m + [S]}
\]

Increasing enzyme turnover rate by concentrating both substrates and (low level) enzyme and substrate

A generic method to boost biochemical reaction speed

Lee et al. Anal. Chem. 2008, 80, 3198

Enzyme: Trypsin (1 ng/mL)
Substrate: BODIPY FL casein (50 μg/mL)
Enhanced Enzyme Activity Assay (trypsin)

Significantly faster reaction kinetics!

- ~1000-fold enhancement of trypsin activity
- powerful tool for improving enzyme activity

Modeling Enzymatic Reactions in Concentration/Reaction Plug

Assumptions:
• Constant accumulation rate
• Reaction is plug is much faster than in reservoir supplying reactants
• Mass-transport limitations neglected

\[ E + S \xrightleftharpoons[k_2/k_2]{k_2} ES \xrightarrow{k_3} P + E \]

\[
\frac{d[E]}{dt} = -k_1[E][S] + (k_2 + k_3)[ES] + \alpha[E_0]
\]

\[
\frac{d[S]}{dt} = -k_1[E][S] + k_2[ES] + \alpha[S_0]
\]

\[
\frac{d[ES]}{dt} = k_1[E][S] + (k_2 + k_3)[ES]
\]

\[
\frac{d[P]}{dt} = k_3[ES]
\]
Modeling Enzymatic Reactions in Concentration/Reaction Plug

Aniruddh Sarkar (MIT)

- Non-linear in substrate concentration: Lower ‘effective’ $K_M$
- Linear in enzyme concentration: Can bias and tune amplification factor by collection/reaction time
Experimental data reproduces model prediction

Enzyme: β-Galactosidase
Substrate: FDG

Aniruddh Sarkar (MIT)
Concentration Enhanced ELISA (CELISA): Method A

Lih Feng Cheow (EECS, MIT) et al., Anal. Chem. 2010

(a) Concentration Enhanced ELISA

(b) FITC tracer

(c) 1000 pg/mL

1000 pg/mL

200 pg/mL

40 pg/mL

8 pg/mL

1.6 pg/mL

PSA detection from donkey serum
Aptamer-based Immunoassays(1)

Aptamers are ideal capture agents

high $K_D$ values hingers surface-bound immunoassay (inhomogeneous assays)

Homogeneous assays are preferred

Aptamer binding to target cause big shift in electrophoretic mobility, causing separate focusing in the concentration zone

Aptamer-based Immunoassays(2)

Target: Human IgE
Buffer: 10mM Tris-HCl, 1mM MgCl₂, 0.2mM BSA, pH=7.4

Limit of Detection (LOD) significantly lower than the $K_d$

Good separation of bound vs. unbound fractions leads to better sensitivity

IgE detection with 1% donkey serum background

Middle peak (caused by non-specific aptamer binding) appears

IgE detection with 10% donkey serum background, with 10μM non-specific oligonucleotides

Additional oligonucleotides eliminates non-specific binding peak

LOD: 48pM

Concentration-enhanced kinase activity assay

Kinase catalyze substrate phosphorylation

- Directly measure biological activity of active kinase in sample
- A large database (>8000) of kinase recognition sequences is available
- Simultaneous concentration and separation improves sensitivity

Lih Feng Cheow, 2012
Kinase activity signal from cell lysate

AKT activity in insulin stimulated (IS) and serum starved (SS) HepG2 cell lysate

Insulin stimulation upregulates AKT activity

Forskolin stimulation upregulates PKA activity

Detection approaching single cell sensitivity

Lih Feng Cheow, 2012
Single cell Akt activity assay

Lih Feng Cheow, 2012
- Correlation between total kinase activity and cell number
  (difference is statistically significant by Student’s t-test)
Spacer molecules for multi-kinase profiling

- Multiplexed kinase measurement can reveal the functional relationship between different kinases in the network

- Ampholyte: complex mixture of species with different pI values for isoelectric focusing.
  Different pI $\rightarrow$ Different $\mu$

Lih Feng Cheow, 2012
Synthetic peptides as spacer molecules

- Ampholyte cannot resolve all the bands.
- We can design peptide spacers with desired mobilities (different amino acid sequences)
- Peptide mobility can be approximated with the Offord Model $\mu = Q / M^{2/3}$

Fluorescent peptide substrates

- Add glycine (G) for mass
- Add glutamic acid (E) for charge

Lih Feng Cheow, 2012
6 bands / 3 kinases (AKT, MK2, PKA)
Single cell kinase activity measurement

Microfluidic pipette interface for single cell lysis

Confined reaction chamber for substrate turnover (single cell level sensitivity)

Integrated concentration device will add additional sensitivity

*Sarkar and coworkers, MicroTAS 2011 / 2012*

- Measured activities of three kinases from single cells

Controlled, selective cell lysis and capture

Single cell ~ 1ng of protein
Our Vision: Single cell signaling studies

Heterogeneous cell response to drug is well-known problem in cancer therapy

Minority cell populations could have distinct, crucial functionality

Study ‘cellular heterogeneity’

1. Application of drugs / chemical signals

2. Identification of diverse / unique (migrational) phenotypes in a given culture / tissue

3. Selective, localized single cell lysis and collection of cytoplasmic kinase content within a microfluidic device

4. Loaded single cell lysate will be reacted with specific biosensors (and inhibitors) for multiplexed (up to 5 targets) kinase activity assay, repeated for many individual cells
Continuous-flow biomolecule / bioparticle concentration

Nafion (cation selective membrane) was patterned with an angle (30°) to the main channel.

Application of electric field generated the ICP zone near the Nafion, which generated depletion force to charged molecules.

All charged molecules and ions were guided into concentrated channel by depletion force and hydraulic pressure.

Cell concentration (RBC)

Red Blood Cells (RBCs) and Escherichia coli (E. coli) concentration
✓ Fouling, clogging free ✓ Independent from the properties of sample (size, surface charge, flexibility, etc.)

Cell motion

Blocked and collected on Nafion → Climb Nafion to bifurcated point → Flow through concentrated channel

Q = 1 µm/min V = 90V

Sample flow

Buffer flow

Concentrated flow

Nafion

Filtered flow

RBCs

20-fold!

Concentrated outlet

Filtered outlet

Concentrator-droplet reactor interface

High sensitivity MMP (Matrix Metallo-Proteinase) activity measurement from cell culture supernatant

Chen et al. JACS 133, 10368 (2011)
Acknowledgement

• Team members
  Ragheb Khaja
  Lih Feng Cheow
  Aniruddh Sarkar
  Sha Hwang
  Rhokyun Kwak
  Lidan Wu
  Hyung Wan Do
  Bumjoo Kim (Postech)
  Sung Hee Ko (Postech)
  Yong-Ak Song, Ph.D.
  Sung Jae Kim, Ph.D. (SNU, Korea)
  Chia-Hung Chen, Ph.D. (NUS, Singapore)

• Research Support
  – NSF CBET- 0854026
  – NIH CDP (Cell Decision Processes) Center (GM68762)
  – NIH NIBIB (R01-EB005743), NCI (CA119402)
  – Harvard Catalyst grant
  – Singapore-MIT Advanced Research and Technology (SMART) Center, BioSyM IRG
  – SMART Innovation Center
  – DARPA DLT program / DARPA Cipher program

Prof. Doug Lauffenburger (BE, MIT)
Samuel Lin (Beth Israel Deaconess Medical Center)
Boris Zaltzman (Ben Gurion University, Israel)