Supercharged Separations on the Microscale – Ions to Bacteria

University of Texas
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Analytical Science

Separation (including Mass Spectrometry)

Spectroscopy

Electrochemistry
Bioparticles are incredibly diverse in size and characteristics

Bioparticles range in size from a few nm to over 100 µm

Human body consists of over 30 trillion cells

Human microbiome is diverse and abundant

Image credits: Rocky Mountain Laboratories, NIAID, http://commons.wikimedia.org
General Introduction to Separations and Our Work
1) Diffusion (and dispersion) is the enemy of separations

\[ D = \frac{\Delta G}{a} = 1.35 \text{ cal/mol} \]

Fat peaks are bad, even if you have the thermodynamics (and kinetics) right.

Diffusion is our mortal enemy & it can only be controlled, not eliminated.

Separatory Forces: push centroids apart

Dispersive Forces: relax concentration gradients

\[ \Delta(\Delta G) \]

\[ \alpha = 1.35 \text{ cal/mol} \]

Time or space
Applying separation science to bioparticles

1. Starting Sample (Ampholyte and Proteins)

2. Transient State

3. Steady State
Gradients are better

- Concentrate while separating
- Need opposing forces, at least one selective

Problem:
- to create narrow peaks, need steep gradient
- steep gradients tend to run out of ‘realistic’ values quickly
- dynamic range limited
Electrophoresis (EP) can be used to move charged particles

A uniform electric field produces a force that acts upon suspended particles with a net charge

\[ F = qE \]
Dielectrophoresis (DEP) can be used to move polarizable particles. A non-uniform electric field produces a force that moves a dipole either towards or away from higher field strength.

\[ F_{\text{DEP}} = 2\pi r^3 \varepsilon_m f_{CM} \nabla |E|^2 \]

A non-uniform electric field produces a force that moves a dipole either towards or away from higher field strength.
Insulator constrictions shape electric fields

Insulator geometries often used in microfluidic channels include:

A) Posts
B) Hurdles
C) Angular features
Our Device Design
Opposing forces create capture zones near a gate
Constructing a gradient insulator-dielectrophoresis (g-iDEP) microchannel
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Data from Our Approach
Blood is a clinically-interesting and complex sample matrix

- **Clinical interest**

- **Blood cell characteristics vary with disease state**

- **Exploring g-iDEP capability**

- **Cells are relatively large (6-8 µm diameter) and fragile**

- **Blood is a complex, heterogeneous mixture**

Cells labeled with fluorescent dye:

invitrogen Vybrant DiO
Capture of blood cells

- At large-pitch gates (A) no capture occurs
- Capture observed at 90 µm (B) and 27 µm (C) gates, 200 V and above
- This occurs in the presence of blood plasma, and in isotonic buffers

*Escherichia coli* bacteria serve as an interesting analytical target

- *E. coli* strains range from commensal to pathogenic

One serotype (O157:H7) is responsible for 70,000 illnesses, 2000 hospitalizations, and 60 deaths per year

Three serotypes were cultured and labeled with fluorescent dye (Invitrogen Vybrant DiO):
- O157:H7
- O55:H7
- O6:K1:H1

Source: Centers for Disease Control and Prevention 2011
Expected and observed capture of a single *E. coli* strain

- Illustration of expected forces that lead to capture at a gate

Negative DEP capture of O6:H1:K1 at 27 µm gates

\[ V_{\text{app}} = 700 \text{ V} \]

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- Illustration of expected forces that lead to capture at a gate

Negative DEP capture of O6:H1:K1 at 27 µm gates

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Fluorescence Intensity (FI) data for O6:K1:H1

- Capture only occurs above a threshold value of field properties
- Above that threshold, material accumulates continuously

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FI at a constant time point, but varied field values ($E_{\text{app}}$)

The field value where capture begins is strain-specific

Serotypes can be differentiated

- Field values required for capture differ for all three serotypes
- Capture occurs at three different gate pitches, differentiation at two
- This indicates that all three serotypes can be distinguished using g-iDEP

How Could This be Very Important (and What Else do We Have to do to Make it Happen)?
Possible Impact: Early Pathogen Identification

How do we correctly identify pathogens and their susceptibility early and accurately?

Why does it take so long now?

What is the best way to move this line from here...

...to here...

...and gain this ‘wellness’?
An Ideal Pathogen Identification Scheme

Pretty simple really:

✓ Fast: allow meaningful action
✓ Cheap: cost effective (<$1 per test)
✓ Available: widely distributed, low power use, simple operation and readout, etc.
✓ Accurate & Precise: sensitive and selective
Current Workflow of Pathogen Identification

Symptoms & Sample → Phenotype → DNA/RNA → Culture/Metabolic → Susceptibility test

Time

CORRECT Identification including susceptibility

Susceptibility test → Culture Negative

No actionable result

Vetting/Confirmatory
Current Workflow of Pathogen Identification

- Symptoms & Sample
  - Phenotype
  - DNA/RNA
  - Culture/Metabolic

- Time
  - CORRECT Identification including susceptibility
  - Susceptibility test
  - Lacking DNA/RNA sequence or Immuno Reagent for pathogen
  - Bias Loss
  - Failure to thrive in artificial environment
  - Culture Negative

- Vetting / Confirmatory

- No actionable result
Current Workflow of Pathogen Identification

- Symptoms & Sample
- Phenotype
- DNA/RNA
- Culture/Metabolic
- Hyperfine Isolation
- Susceptibility test
- Bias Loss
- Culture Negative
- No actionable result
Current Workflow of Pathogen Identification

Symptoms & Sample

Confirmatory / Vetting

Time

CORRECT Identification including susceptibility

Hyperfine Isolation

Susceptibility test

Bias Loss

Culture/Metabolic

DNA/RNA

Phenotype

Susceptibility test

Culture Negative

No actionable result
How do we Improve our System? How Good Can It be?
Greater resolution provides more information
Resolution in a g-iDEP microchannel

**Low resolution** – capture of a single analyte is “spread” across multiple gates

This leads to overlap between multiple analytes

**Improved resolution** – multiple analytes are captured in distinct zones
The fundamental approach to resolution

Zone width: how wide is the band?

Depends on competing forces: focusing and spreading

\[ \sigma^2 = \frac{D_T}{a} \]

Resolution: to what degree are analytes “separated?”

Depends on the degree of intrinsic difference between two analytes

\[ R = \frac{\Delta X}{4\sigma} \]

Source: Giddings, J.C., 1991
Building a theoretical understanding of g-iDEP resolution

To improve resolution:

Minimize $D_T$
Maximize local slope of $E$ and $\nabla |E|^2$

Source: Jones, P.V., et al., *Electrophoresis* 2015
Building a theoretical understanding of g-iDEP resolution

To improve resolution:

Minimize $D_T$
Maximize local slope of $E$ and $\nabla |E|^2$
Minimize gate-to-gate change in $E$ and $\nabla |E|^2$

Source: Jones, P.V., et al., Electrophoresis 2015
Initial (V1) design typically captured material at final gates

- **Red blood cells**
- **Amyloid fibrils***
- **Escherichia coli**

Proved suitable for both capture and differentiation of bioparticles

Not ideal for separation of bioparticles

*not shown here today, published works, Staton et al. 2011
Designing a higher-resolution g-iDEP microchannel

- V1 microchannel

- V2 microchannel
Antibiotic resistance (AR) is increasingly a problem

Humanity is engaged in an antibiotic arms race

AR epidemiology in the US: morbidity = 2,000,000/year; mortality = 23,000/year

Testing takes time: Common tests such as diffusion testing require multiple culturing steps (24 - 72 h each)

Source: Centers for Disease Control and Prevention 2013
Staphylococcus epidermidis: friend or foe?

*S. epidermidis* is a commensal participant in human skin microbiome.

*S. epidermidis* has increasingly emerged as a cause of multi-resistant nosocomial infections. It is now the leading cause of infections associated with medical prostheses and implants.

Gentamicin-resistant and gentamicin-susceptible *S. epidermidis* were cultured and labeled with fluorescent dye.
Individual strains were examined in V1 microchannel

Capture at 27-µm gate, $V_A = 1200$ V

Above threshold $V_A$, bacteria accumulate with time.

Fluorescence intensity (FI) was integrated across the capture zone (yellow box)
Individual strains were examined in V1 microchannel

Capture at 27-µm gate, $V_A = 1200$ V

Above threshold $V_A$, bacteria accumulate with time.

Fluorescence intensity (FI) was integrated across the capture zone (yellow box)

Integrated FI

Green line: $t_A = 0$ s
Yellow line: $t_A = 15$ s
Red line: $V_A = 0$ V
Amount of analyte captured depends upon $V_A$

Bacteria only accumulate at gate above threshold $V_A = c$

For $V_A > c$, increasing amounts are both delivered and captured
Amount of analyte captured depends upon $V_A$

Data described by piecewise function:

$$N(V_A) = \begin{cases} 
0 & \text{if } V_A < c \\
 m(V_A - c) & \text{if } V_A \geq c 
\end{cases}$$

Baseline calculated from first 4 points

Significant capture when $N > 2\sigma_{\text{baseline}}$

Points representing capture fitted with linear regression

Allows estimation of $c$, which can be related to intrinsic analyte properties

Bacteria only accumulate at gate above threshold $V_A = c$

For $V_A > c$, increasing amounts are both delivered and captured
Differentiation of resistant and susceptible *S. epidermidis* in V1 microchannel

Gentamicin-resistant
- $c = 443 \pm 59$ V
- $\mu_{EK}/\mu_{DEP} = (4.6 \pm 0.6) \times 10^9$ V/m$^2$

Gentamicin-susceptible
- $c = 881 \pm 38$ V
- $\mu_{EK}/\mu_{DEP} = (9.2 \pm 0.4) \times 10^9$ V/m$^2$
Simultaneous capture in V2 microchannel

V2 g-iDEP microchannel

Inlet

Outlet

Gentamicin-resistant

Gentamicin-susceptible
Remaining Challenges and Approaches.
How hard will it be & how good can we get?

Possible physical changes and their effect on electrokinetics

- **CAPSULE**
  - charges
  - sugars
  - thickness

- **PEPTIDOGLYCAN LAYER**
  - structural changes
  - rigidity
  - charge/charge distribution

- **CELL WALL**
  - membrane proteins/lipids
  - permeability
  - ion channels

- **CYTOPLASMA MEMBRANE**
  - fluidity/viscosity
  - proteins, structural elements

- **CYTOPLASM**
  - number or structure of inclusions, ribosomes, plasmids
  - overall viscosity or conductivity

Overall shape and size unlikely to significantly change

Primarily EP,
Some effects on DEP

Primarily DEP
How hard will it be & how good can we get?

<table>
<thead>
<tr>
<th>Cellular Structure</th>
<th>Alteration</th>
<th>Electrokinetic Effect</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Electrophoresis</td>
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<td></td>
<td></td>
<td>Dielectrophoresis</td>
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<td>Conductivity</td>
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<td></td>
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<td>Assymmetry</td>
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<td>permanent dipole (multipole)</td>
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<td></td>
<td>Charge</td>
<td>Surface Viscosity</td>
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<td></td>
<td>m&lt;sub&gt;EP&lt;/sub&gt;</td>
<td>b</td>
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<td>h&lt;sub&gt;surf&lt;/sub&gt;</td>
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<td></td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>CASULE</td>
<td>charge increase (decrease)</td>
<td>+ (-)</td>
</tr>
<tr>
<td></td>
<td>distribution</td>
<td></td>
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<tr>
<td></td>
<td>glycosylation increase (decrease)</td>
<td>+ (-)</td>
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<tr>
<td></td>
<td>thickness increase (decrease)</td>
<td>+ (-)</td>
</tr>
<tr>
<td>PEPTIDOGLYCAN LAYER</td>
<td>structural changes</td>
<td></td>
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<tr>
<td></td>
<td>rigidity : more (less)</td>
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<tr>
<td></td>
<td>thickness: increase (decrease)</td>
<td>+ (-)</td>
</tr>
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<td>CELL WALL</td>
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</tr>
<tr>
<td></td>
<td>distribution</td>
<td></td>
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<tr>
<td>CYTOPLASMA MEMBRANE</td>
<td>fluidity, increased viscosity increase (decrease)</td>
<td>- [+]</td>
</tr>
<tr>
<td></td>
<td>proteins, structural elements increase (decrease)</td>
<td>- [+]</td>
</tr>
<tr>
<td>CYTOPLASM</td>
<td>number or structure of inclusions, ribosomes, plasmids increase (decrease)</td>
<td>- [+]</td>
</tr>
<tr>
<td></td>
<td>overall viscosity or conductivity increase (decrease)</td>
<td>- [+]</td>
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</tbody>
</table>
Future Work

Differing length-scale structures ‘shim’ the particle trajectory and improve capture efficiency

no escape of any particles from these traps (new data)
Theoretical ‘best’

Minimum Resolvable Change in Radius at Maximum Field and Gradient

Smallest Resolvable Dielectrophoretic Mobility at Maximum Field and Gradient

In terms of radius, 50 pm for 1 micron nominal

In terms of electrical properties, 1:10^8 or 1:100,000,000
Boat in a Specific Slip Analogy

Each slip represents a unique strain and susceptibility.

There is a possibility of generating millions of biophysically unique ‘slips’.

Thanks to Dr. McLaren for analogy idea.
Stretching the Analogy

Thanks to Dr. Mclaren for analogy idea : to his credit, he did not stretch it this far!

Anything in the harbor : infection
Specific dock : species/strain
Specific slip: susceptibility

Traditional techniques usually go: harbor, dock, slip.

We can directly address the slip and then compare to known pathogens.
Summary

A new paradigm for separations: punctuated microgradients

Potentially enables identifying pathogens, including susceptibility

Based on biophysics and a break-through technology advance in bioparticle separations

Compares very favorably to current techniques: faster, cheaper, no bias, compatible with all existing strategies

Early in the development cycle—core capabilities theoretical described, seminal data obtained.
Acknowledgements

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“Pathogen Isolation and Concentration for Phenotypic Subtyping” NIH 2012-2014  
“Isolating Viral Particles from Whole Blood” NIH 2012-2014
End
## How do we compare? : Specifics

<table>
<thead>
<tr>
<th>Desirable Features</th>
<th>Aspects to be avoided or minimized</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Accurate and precise (sensitive &amp; selective)</td>
<td>• Cold Chain Supplies</td>
</tr>
<tr>
<td>• Multiple Targets Simultaneously</td>
<td>• Costly reagents</td>
</tr>
<tr>
<td>• Sense unknown/undocumented</td>
<td>• Long analysis times</td>
</tr>
<tr>
<td>• Simple operation</td>
<td>• Bias</td>
</tr>
<tr>
<td>• Fast</td>
<td>• Expertise required</td>
</tr>
<tr>
<td>• Inexpensive device and operation</td>
<td></td>
</tr>
<tr>
<td>• Proven technology/strategy</td>
<td></td>
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</tbody>
</table>
How do we compare?: Global

“Radar Plot”

Our Strategy:

Many categories numerically plotted in easy to see plot.

Many other categories could be imagined, these are reasonably representative.
How do we compare?: Global & current techniques

Our Strategy:

- Proven
- Multiple targets
- Cost
- Prior molecular knowledge
- Cold chain
- Culture negative
- Costly reagents
- Unknown target

Current Techniques:

- Proven
- Multiple targets
- Cost
- Prior molecular knowledge
- Cold chain
- Culture negative
- Costly reagents
- Unknown target

Legend:
- Culture / metabolic
- DNA/RNA
- Phenotype