Complex Simplicity: The "Easy Button" Approach to Measurement Science

Tom Linz

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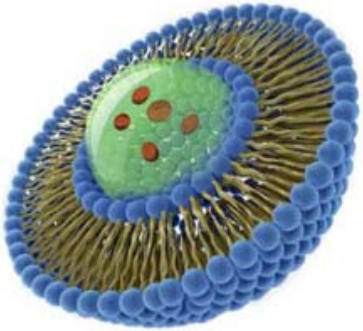
Wayne State University

Chemistry

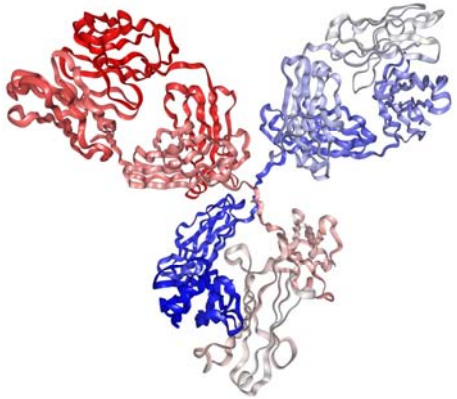
Biomedical Opportunities

Biopharmaceuticals

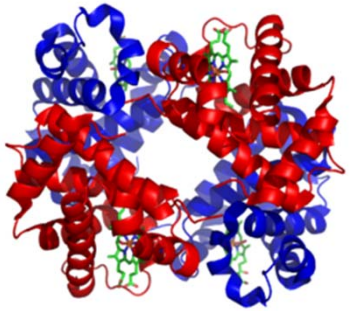
Clinical Diagnostics



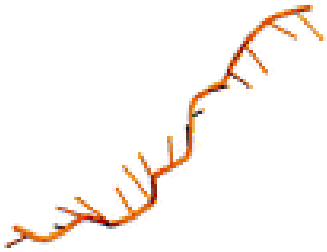
Liposomal Nucleic Acid Drugs



Protein Drugs



Protein Biomarkers



miRNA Biomarkers

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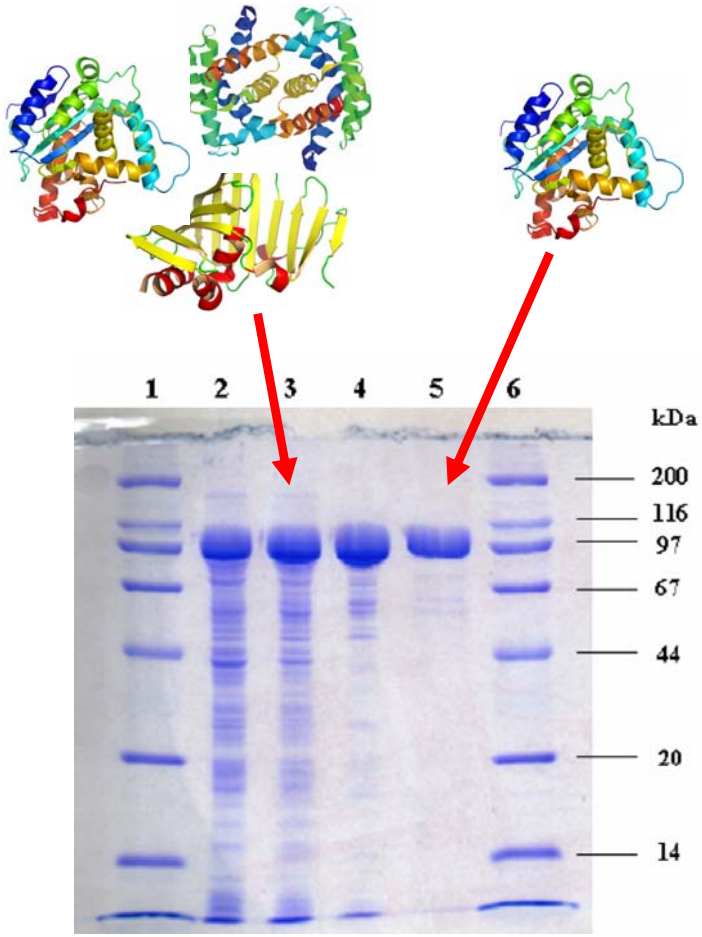
Analysis Performance Criteria

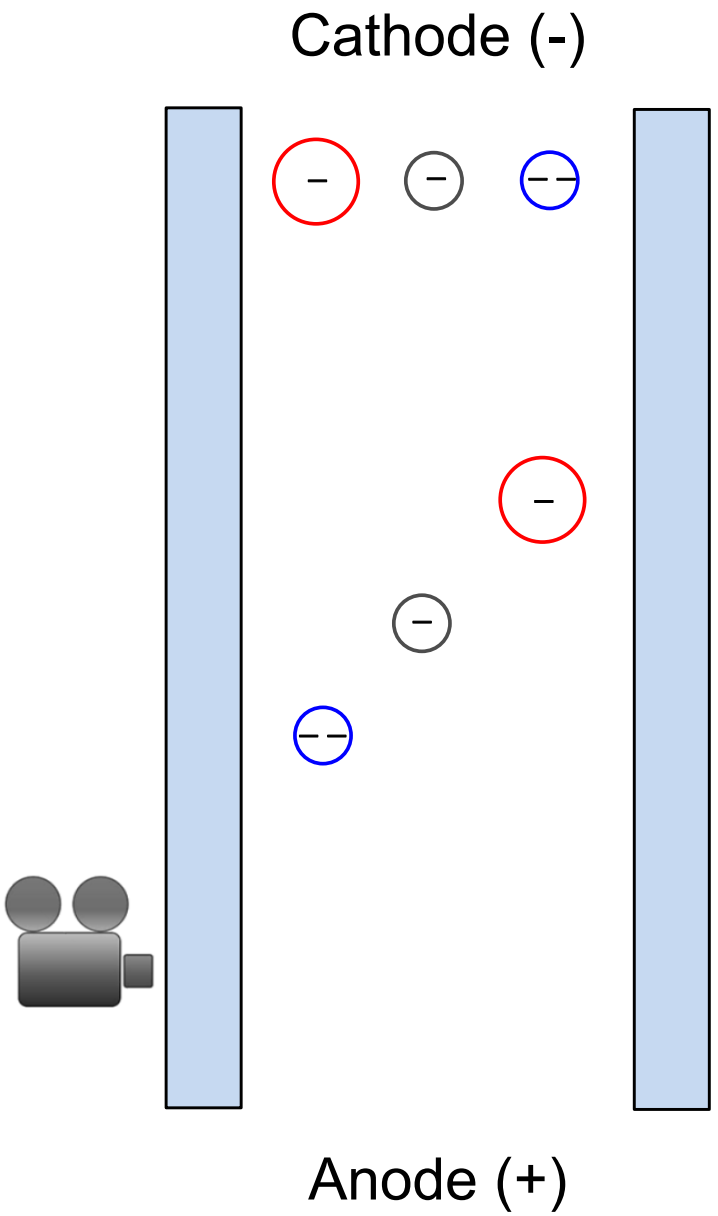
- Broadly applicable
- Inexpensive
- Rapid
- User-friendly
- High sensitivity
- High selectivity
- Low sample volume

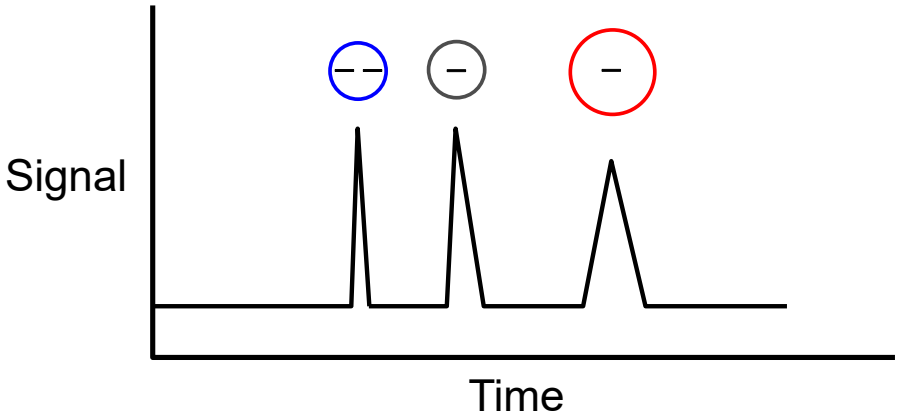
Gel Electrophoresis

- Determines the chemical purity of biological samples
  - Identify which analytes are present
- Slab gels have limited analytical performance
  - LOD and resolution are insufficient



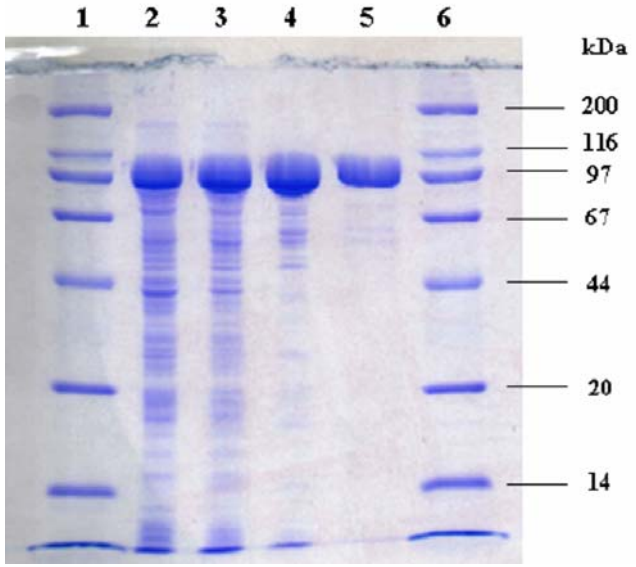
Electrophoresis Principles

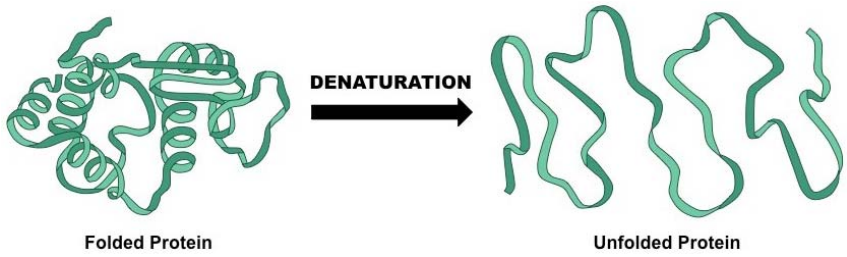


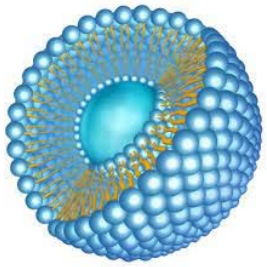


Gel Electrophoresis

- Determines the chemical purity of biological samples
  - Identify which analytes are present
- Slab gels have limited analytical performance
  - LOD and resolution are insufficient
- Slab gel analysis sacrifices biological information
  - SDS denatures proteins prior to mass measurement
  - Cannot analyze particles

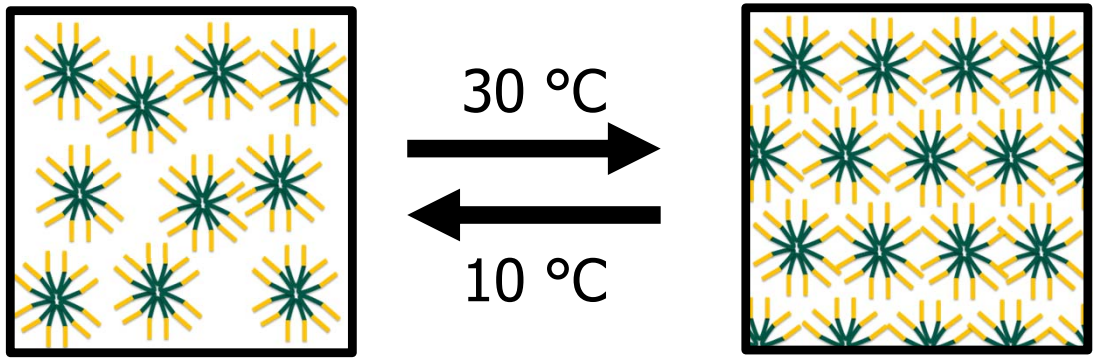






Thermal Gels

- Thermally responsive Pluronic polymers
  - Polymer micelles pack more densely at warm temperatures
    - Undergo a phase change
  - Viscosity is tunable with temperature



Adapted from *J. Pharm. Pharm. Sci.* 2006, *9*, 339-358

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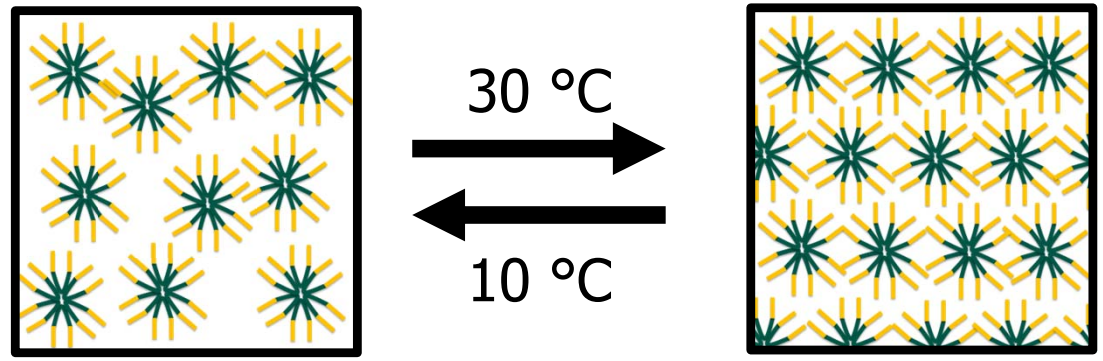
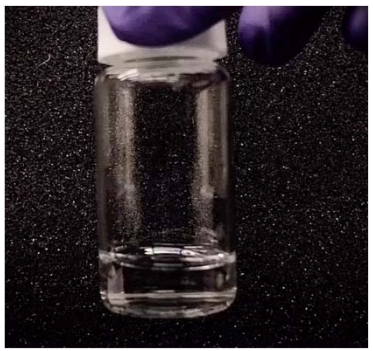
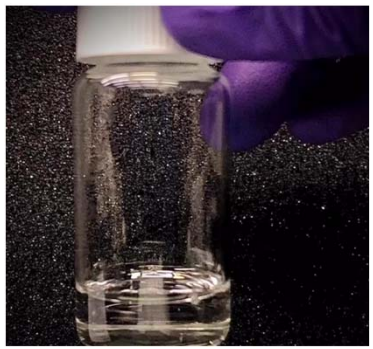


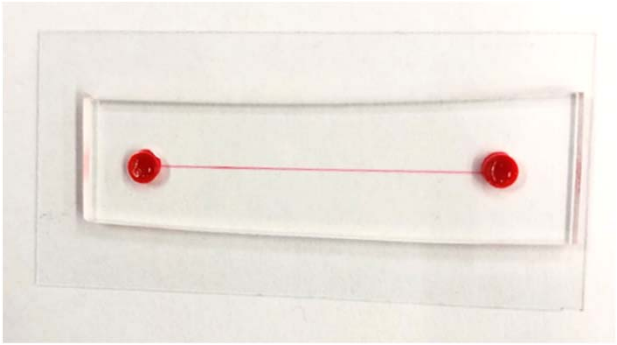




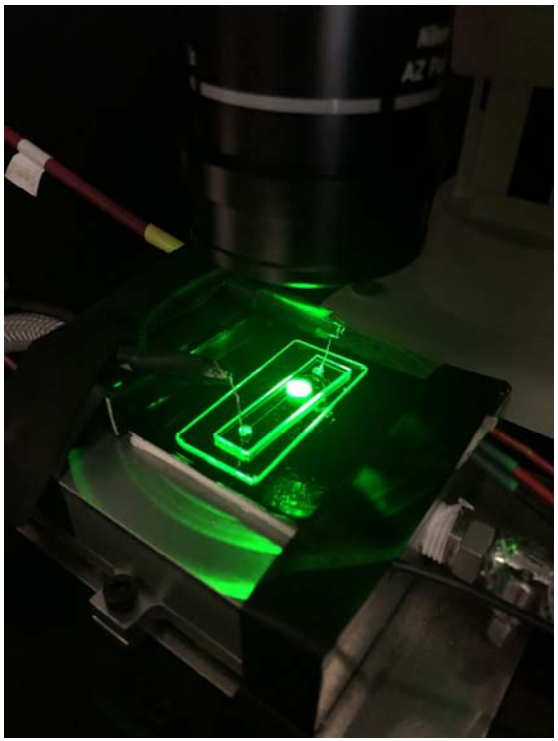
Adapted from *J. Pharm. Pharm. Sci.* 2006, *9*, 339-358

Microfluidic Device Operation

- Soft lithography used to fabricate PDMS microfluidic devices
  - 100 x 20 μm channels

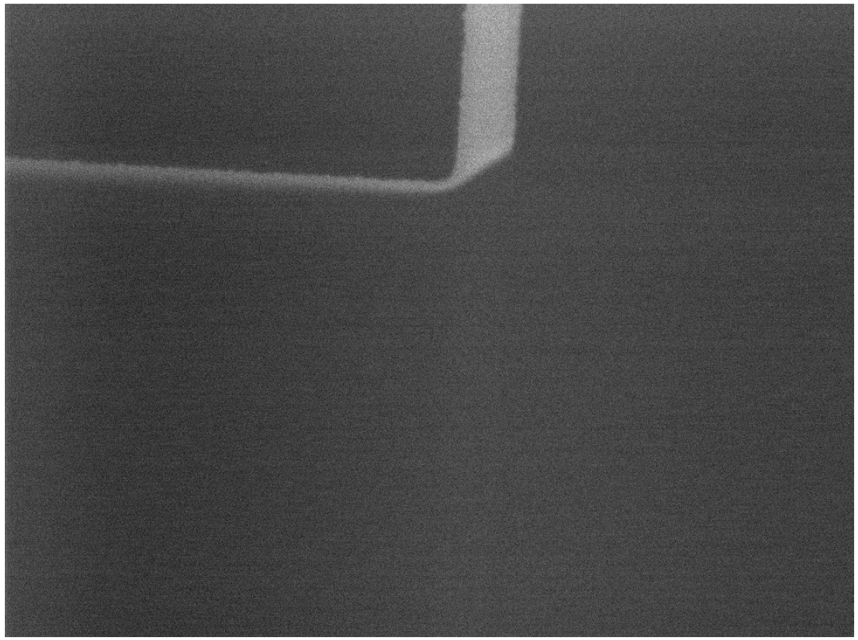


- Sample is directly cast into thermal gel
- Images acquired using an epifluorescence microscope
- Temperature controller integrated into microscope stage

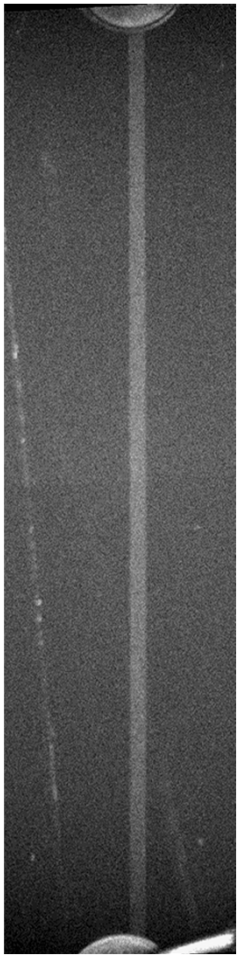


Microfluidic Separations

Gated Electrophoresis



Isotachophoresis

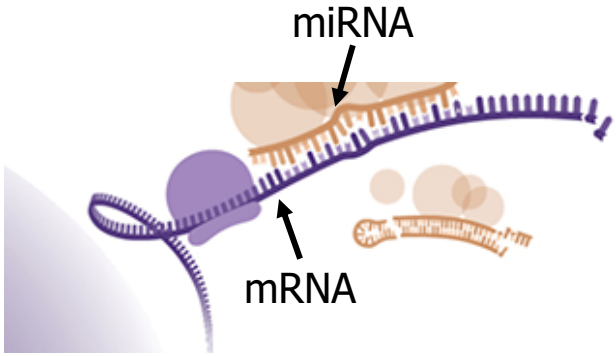


Thermal Gel Electrophoresis (TGE)

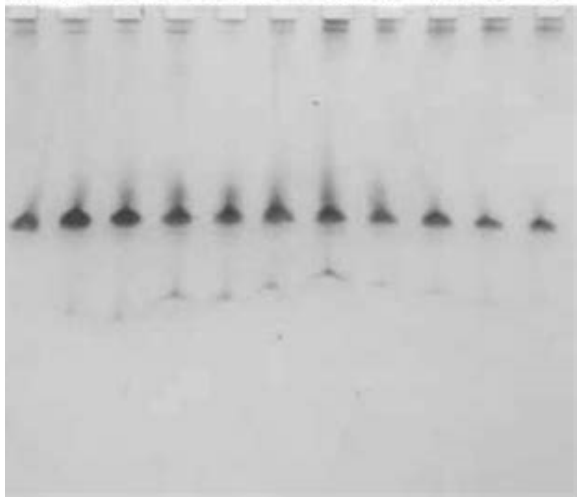
- Use simple single-channel microfluidic devices
  - Cast sample in thermal gel
  - Load thermal gel throughout channel
- Inline preconcentration and separation
  - No need to direct multiple fluid flows
  - No sample injection
  - No precise voltage switching
  - No user intervention

miRNA Analysis

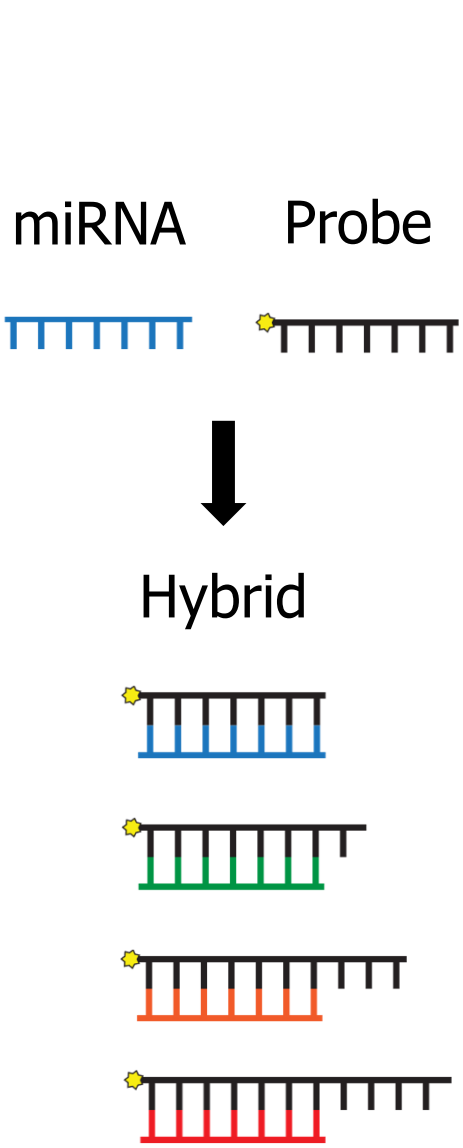
- MicroRNAs silence genes
  - Prevent protein translation from mRNA
  - Misregulation causes pathogenesis
    - Biomarkers for diseases
  - Being developed into therapeutics

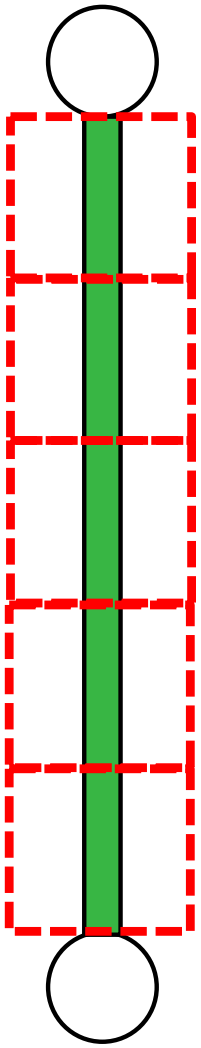


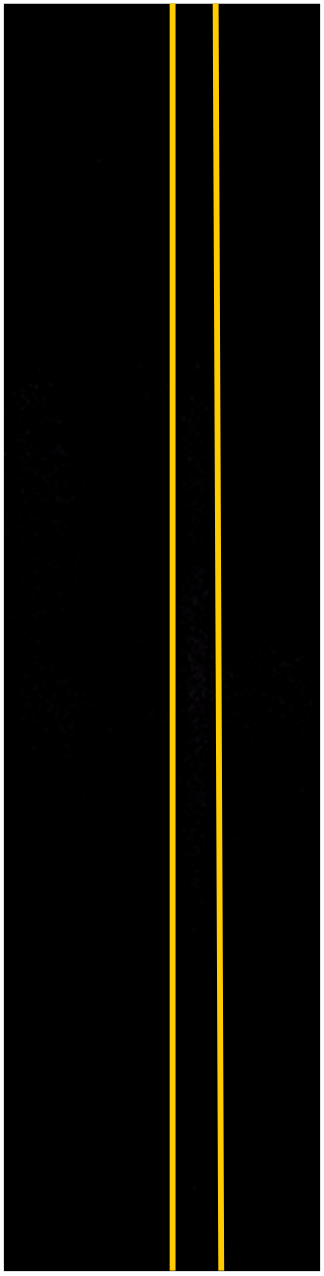
- miRNAs composed of ~22 nucleotides
  - Same-length sequences do not separate in gel electrophoresis
  - Cannot distinguish different species



Multiplexed miRNA TGE







M.A. Cornejo, T.H. Linz, *Anal. Chem.* 2022, 94, 14, 5674–5681

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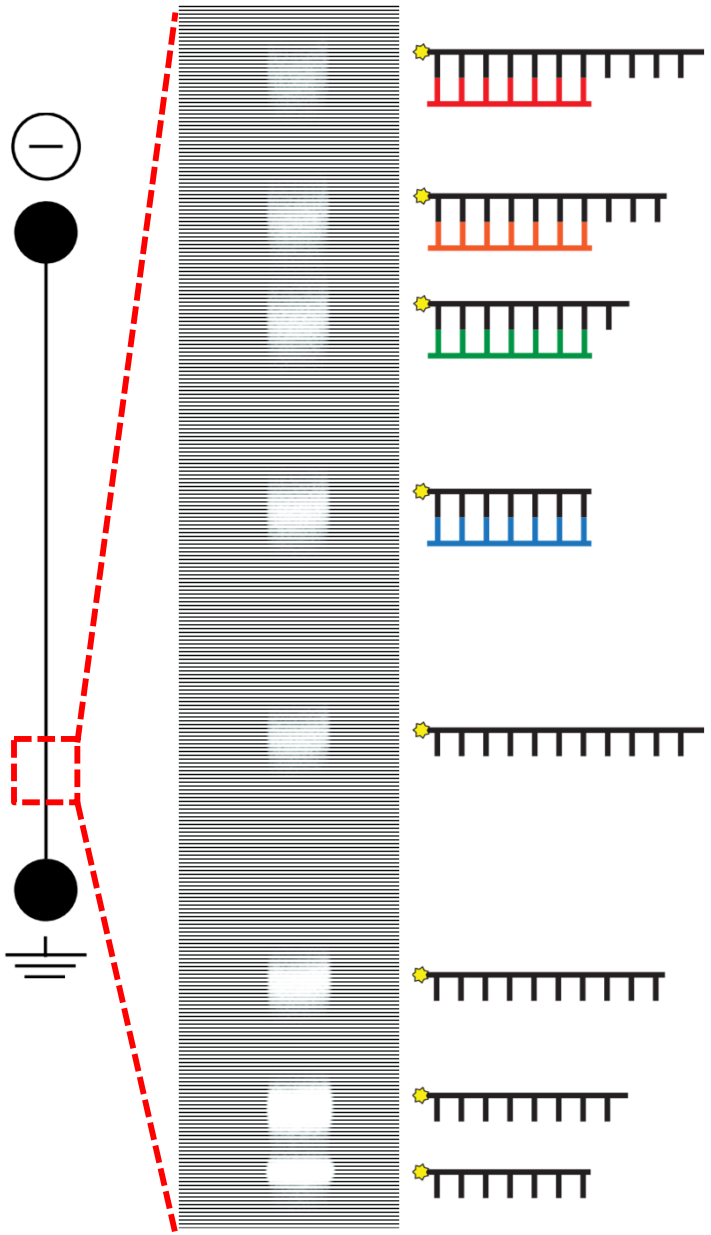
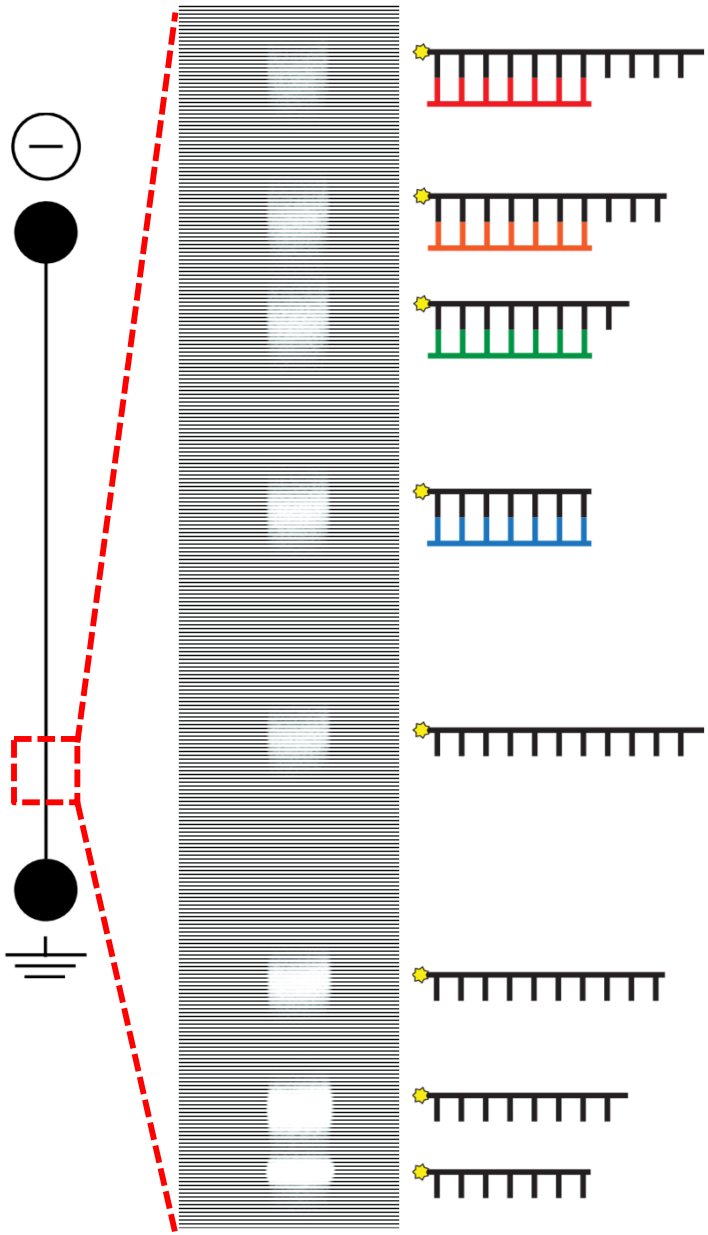
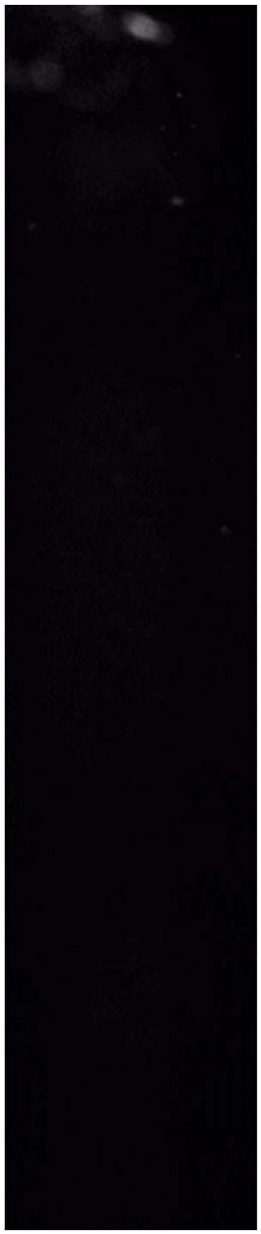
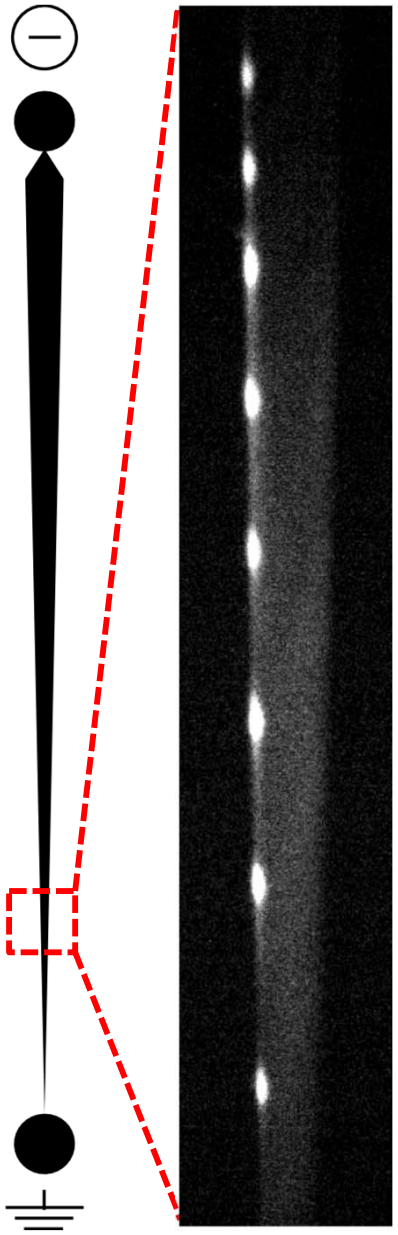


LOD ≈ 100 pM

Second Generation TGE Devices





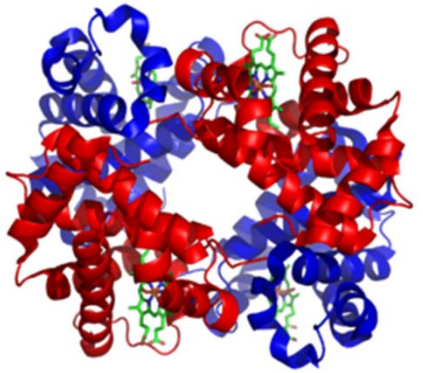


LOD ≈ 100 pM

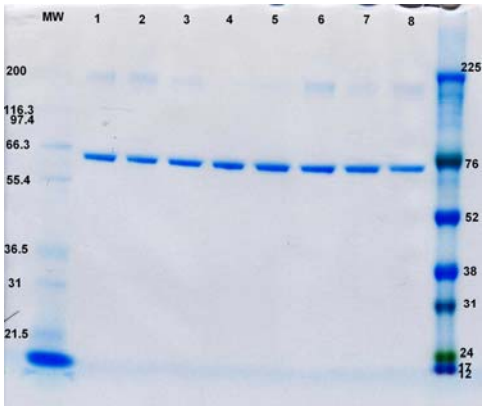
LOD ≈ 10 pM

Native Protein Analysis

- Proteins must remain correctly folded to exhibit proper bioactivity
  - Either inactive or harmful if misfolded or aggregated

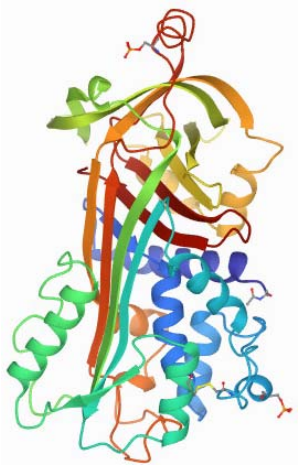


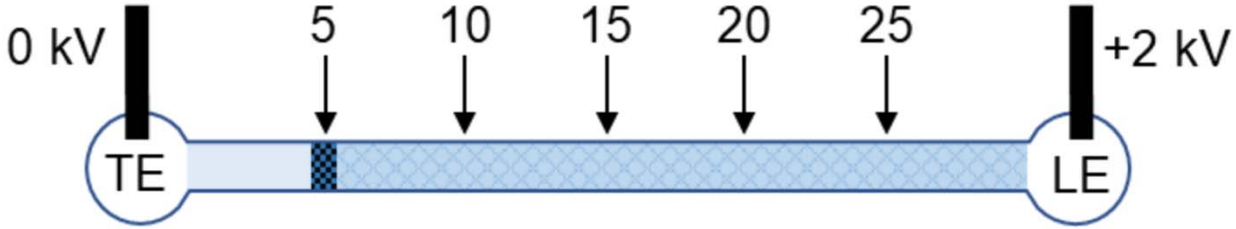
- Proteins must have proper multimeric units or complexes associated
  - Post-translation modifications must be present

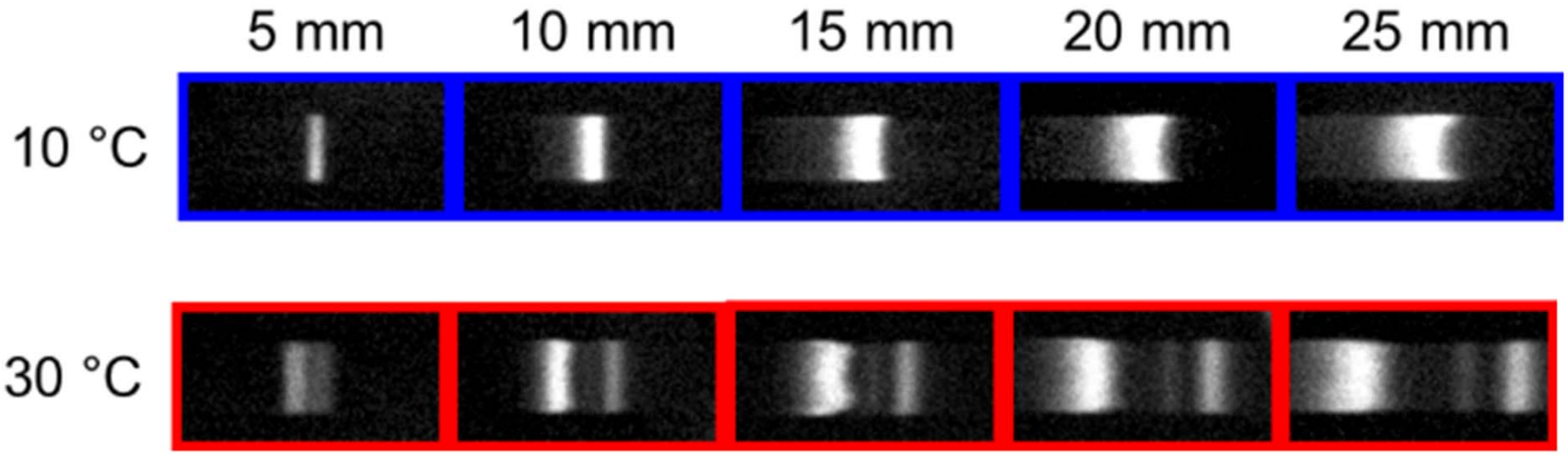


Native Protein TGE

- Fluorescent ovalbumin used as a model protein
  - Multiple folding and phosphorylation variants







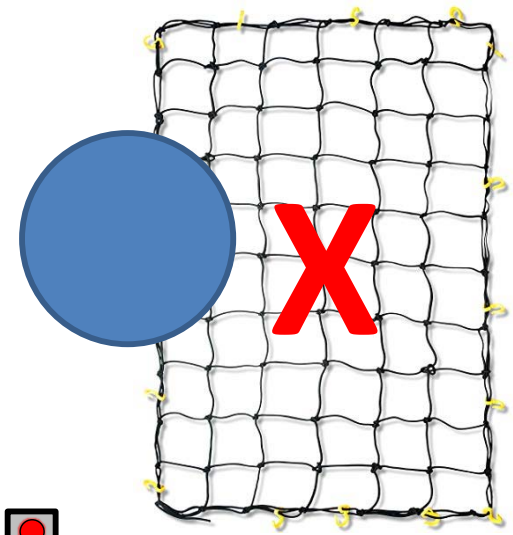
S.H. Peli Thanthri, C.L. Ward, M.A. Cornejo, T.H. Linz, *Anal. Chem.* 2020, 92, 6741-6747

Analysis Performance Criteria

- Broadly applicable ✓
- Inexpensive ✓
- Rapid ✓
- User-friendly ✓
- High sensitivity ✓
- High selectivity ✓
- Low sample volume ✓

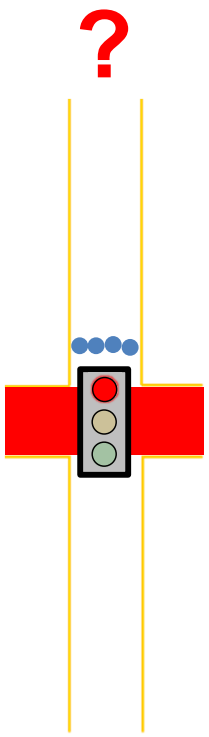
Vesicle Analysis

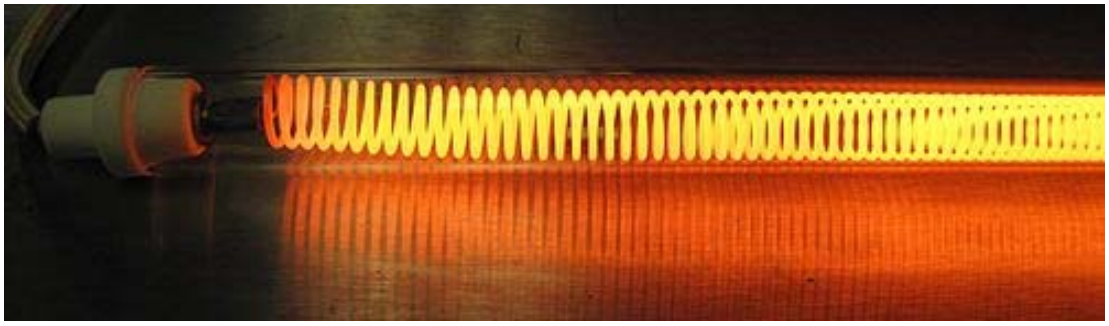
- Vesicles are lipid nanoparticles that contain biomolecules
- Liposomes are drug delivery vehicles that protect internal cargo
- Extracellular vesicles are secreted by cells for communication
- Need to interrogate intravesicular biomolecules



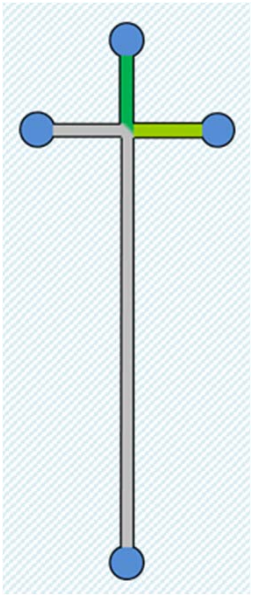
10 °C

Joule Heating Barriers





Cell Enrichment



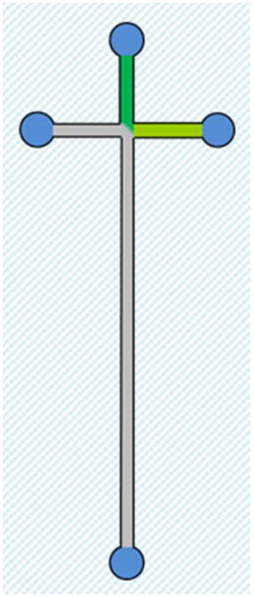
HEK 293 cells

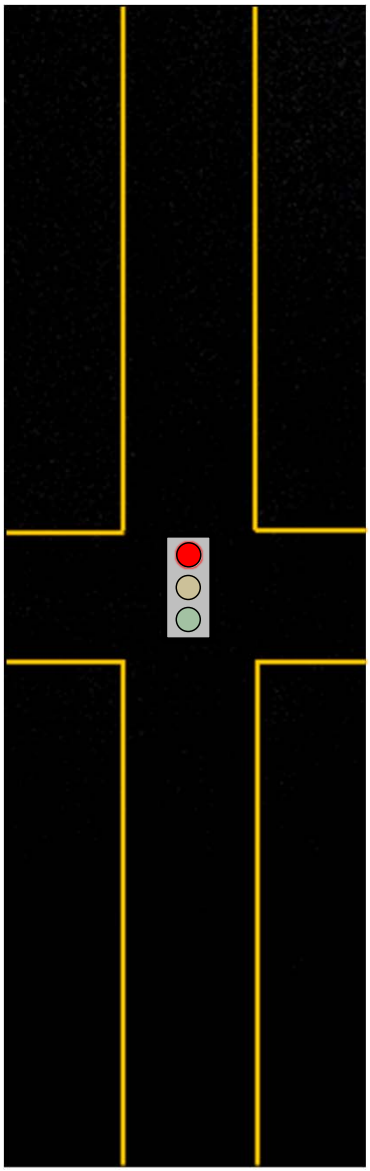
Stage = 10 °C

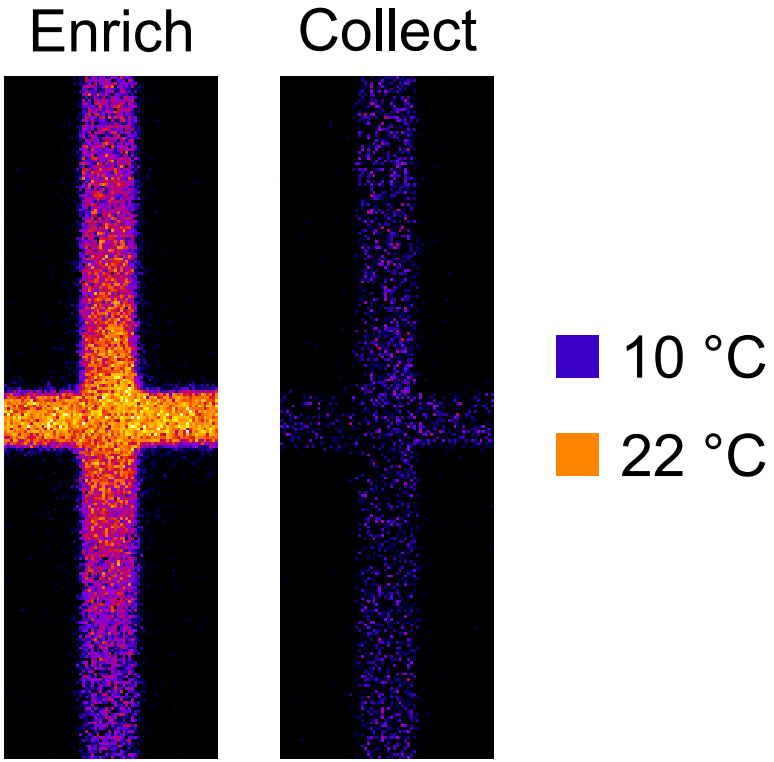
10x speed

M.A. Cornejo, T.H. Linz, *Electrophoresis*, 2021, 42, 1238-1246

Cell Enrichment







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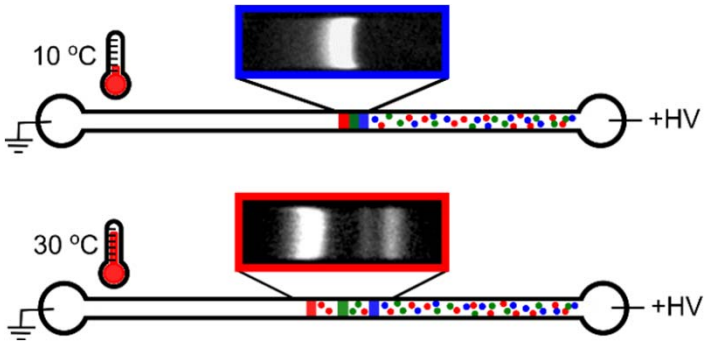
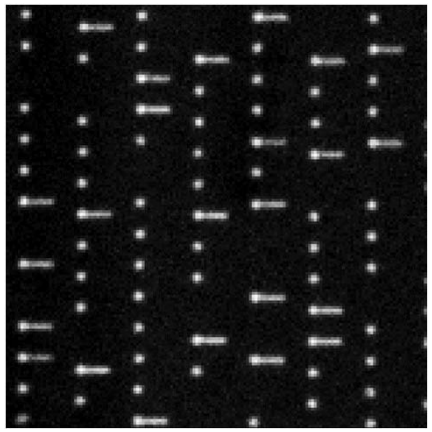
M.A. Cornejo, T.H. Linz, *Electrophoresis*, 2021, 42, 1238-1246

Conclusions

- Thermal gel electrophoresis provides inline enrichment and separation
  - Analyze proteins, nucleic acids, and vesicles in a user-friendly analysis



- Digital quantitative PCR provides single-molecule sensitivity and a large dynamic range
  - Quantify vesicles AND intravesicular biomolecules in a single analysis
  - Measure extracellular biomolecules with good LODs



Acknowledgements

- Linz Lab members
  - Bailey McCarthy Riley
  - Shakila Peli Thanthri
  - Mario Cornejo
  - Hao Mai
  - Courtney Cunningham
  - Brice Vanness

- Funding
  - NIH R21GM137278
  - NSF 2046487
  - SACP Starter Grant
  - Wayne State University

NIH National Institute of General Medical Sciences

NSF

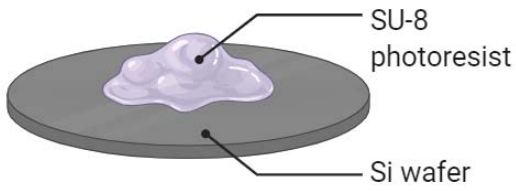
SACP Society for Analytical Chemists of Pittsburgh

Wayne State

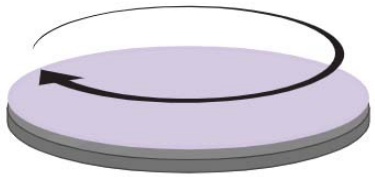
Microfabrication

1. Mold Fabrication

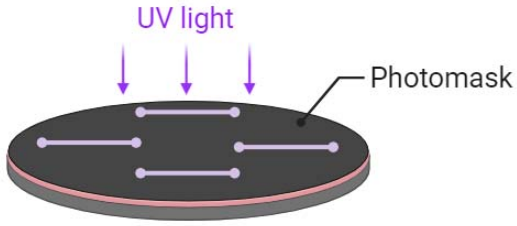
1. SU-8 photoresist application to Si wafer



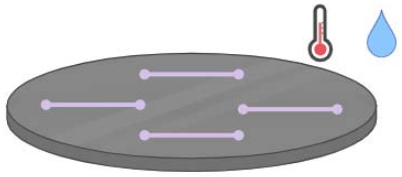
2. Spin-coating and soft baking



3. Photomask alignment and UV exposure

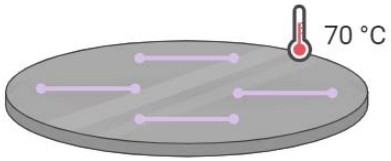


4. SU-8 development, baking, and rinsing

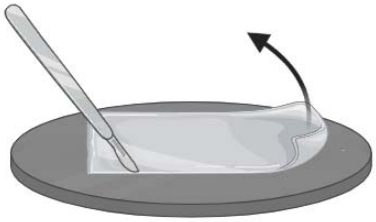


2. PDMS Device Fabrication

5. PDMS casting and thermal annealing



6. PDMS chip peeling-off



7. Creating ports

8. Lay glass slide onto PDMS chip