National Institute of General Medical Sciences

Dorothy Beckett
Christina Liu
Alvin Yeh

Division of Biophysics, Biomedical Technology, and Computational Biosciences (BBCB), NIGMS

Miljan Simonovic

Division of Pharmacology, Physiology, and Biological Chemistry (PPBC), NIGMS

NIGMS Biomedical Technology Optimization & Dissemination (BTOD) Centers Program

Contact: NIGMS_BTODMailbox@nigms.nih.gov

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- **Today's Webinar:**

  - **What are the goals of BTOD Center Program?**
  - **What is the structural framework for achieving these goals?**
  - **How are the goals and structure implemented in TWO active BTOD Centers?**

  - **Send questions about application preparation to:**

NIGMS_BTODMailbox@nigms.nih.gov

OUTLINE

- BTOD Program Overview
- Descriptions of two active BTOD Centers by the Principal Investigators
- Q&A with PIs and NIGMS Staff
- Please type any questions related to the webinar in the Chatbox

NIGMS Biomedical Technology Development Pipeline

Untested Concept to Broad Utility

Proof of Concept	Prototype	Dissemination
Exploratory Research for Technology Development (R21)	Focused Technology Research and Development (R01)	Biomedical Technology Optimization and Dissemination Centers (RM1)

BTOD Program Goals

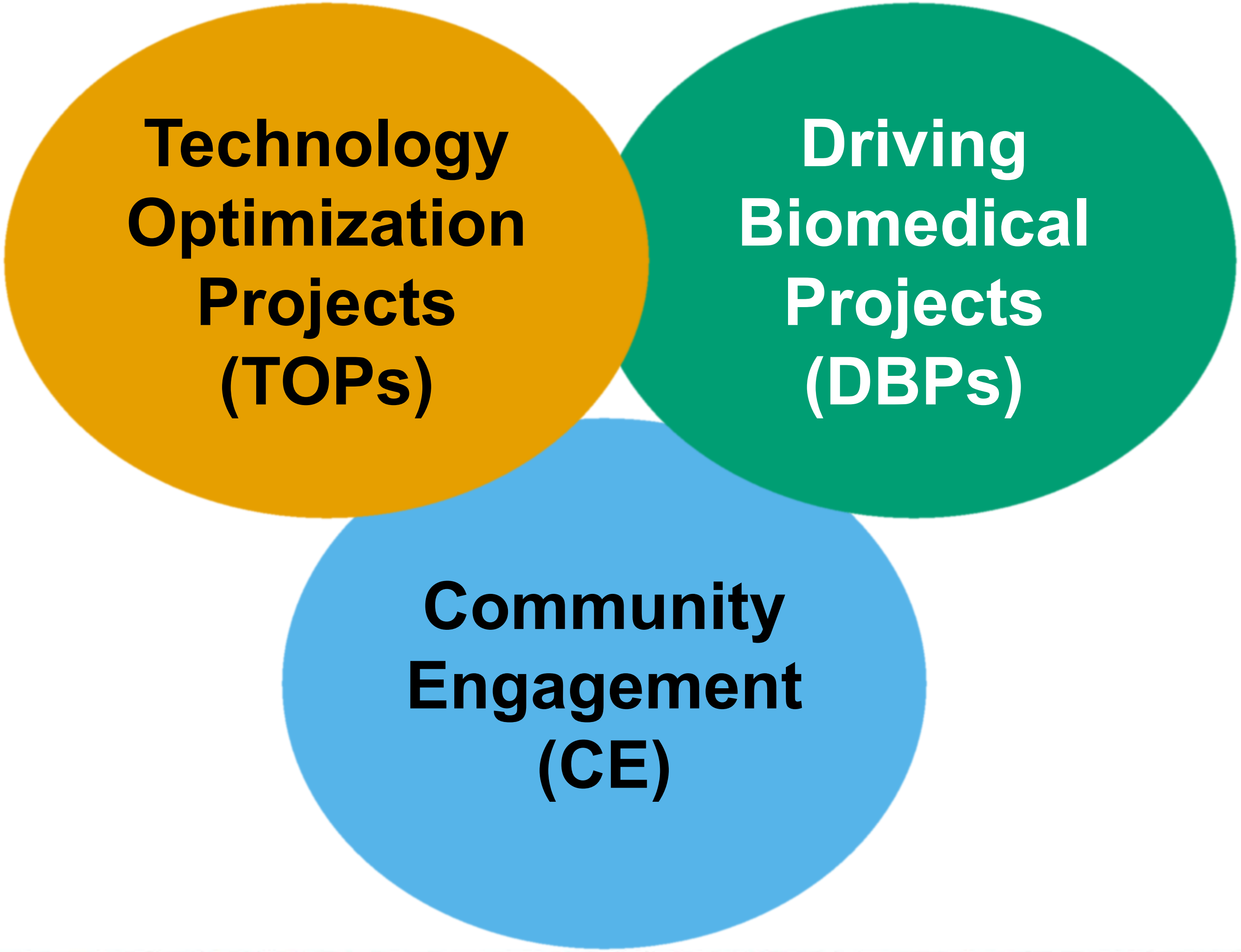
Optimize

state-of-the-art, late-stage technologies to enable broad use

Disseminate

the technologies for use by expert and non-expert biomedical researchers

Three-component Framework for Achieving BTOD Center Program Goals



Technology Optimization Projects (TOPs)

- Leading-edge, late-stage technologies that are in NIGMS mission

- Variations of a single technology or multiple technologies that support a single goal

- Potential for broad adoption by expert and non-expert biomedical researchers

DBPs

Driving Biomedical Projects (DBPs)

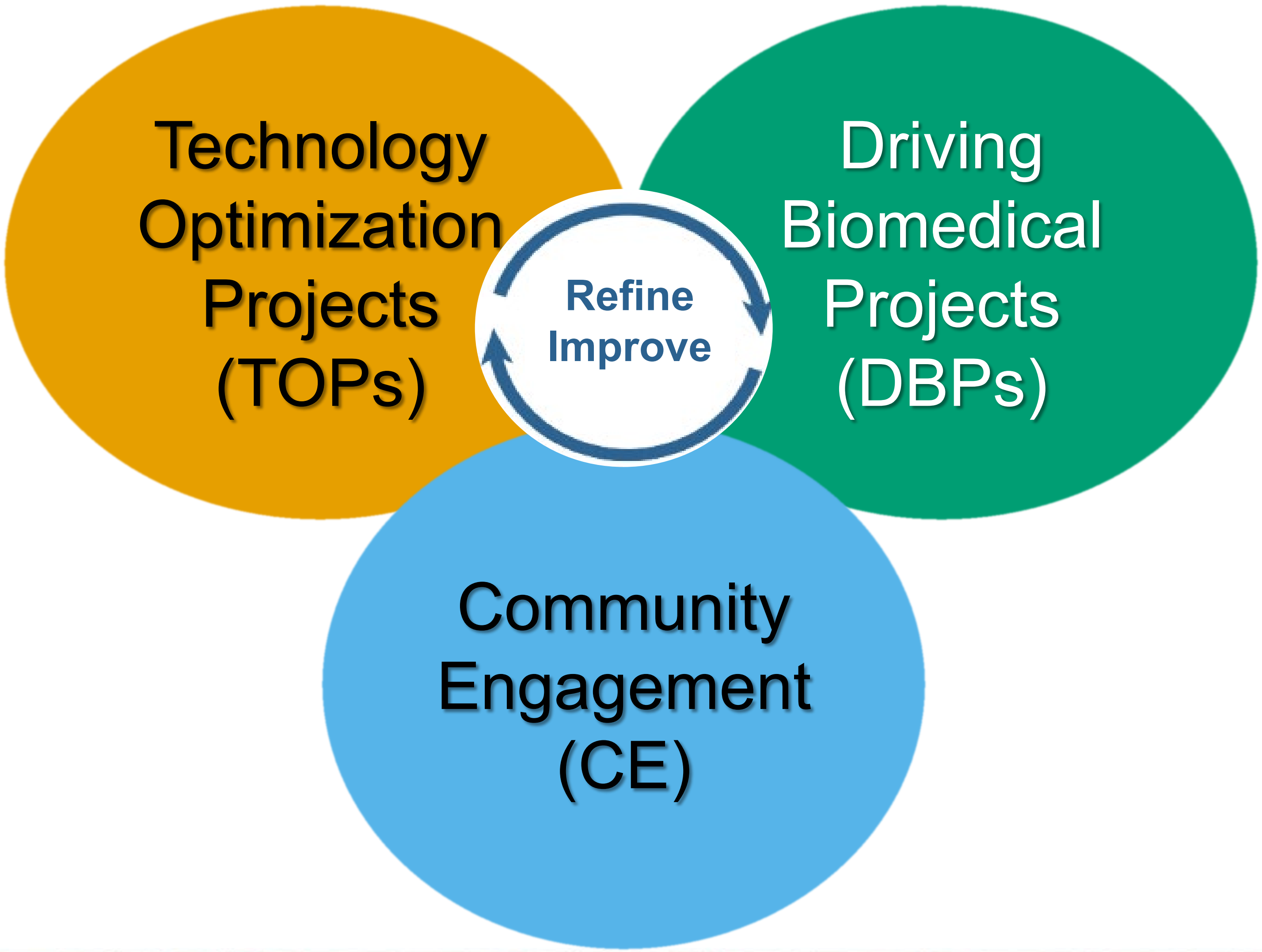
TOPs

- Independently funded research projects
- Challenge the Center technology to drive optimization
- Provide a range of scientific test-beds for the Center technology
- Drawn from diverse, nationally distributed research labs

Community Engagement (CE)

- Develop self-sustaining activities to disseminate technologies
- Host a user-friendly website
- Provide training for diverse research communities
- Engage in technology commercialization

BTOD Centers Integrate the Three Components



Features of BTOD Center Technologies

- Current state-of-the-art or unique technologies with demonstrated utility
- Technologies require optimization to make them broadly useful to the biomedical research community
- Potential utility for a variety of biomedical research problems
- In NIGMS mission

NIGMS Mission

The National Institute of General Medical Sciences (NIGMS) supports basic research that increases **understanding of fundamental biological processes** and lays the **foundation** for future advances in disease diagnosis, treatment, and prevention.

NIGMS also supports research in specific clinical areas that affect multiple organ systems, e.g., sepsis and anesthesiology

- NIGMS-funded scientists investigate how living systems work at a range of levels, from molecules and cells to tissues and organs, in research organisms, humans, and populations.

- NIGMS supports **research and technology development** aimed at understanding general principles, mechanisms, and processes.

Potential Technology Areas for BTOD Centers

- Bioanalytical Chemistry & Tools
- Chemical and Synthetic Biology
- Computational Tools for Biostatistics and Bioinformatics
- High-throughput Biochemistry
- Imaging Tools & Methods
- Microfluidics-based Tools for Biotechnology
- Single Cell Technologies
- Technologies for Structural Biology
- Multi-scale Computational Modeling of Biological Systems
- Spectroscopy and Spectrometry Tools for Interrogating Biological Systems

Current BTOD Centers

- **Center on Macromolecular Dynamics by NMR Spectroscopy (COMD/NMR)**
  New York Structural Biology Center
- **Center on Probes for Molecular Mechanotechnology (CPMM)**
  Emory University
- **National Resource for Advanced NMR Technology**
  Florida State University and the University of Florida
- **Native Mass Spectrometry Guided Structural Biology Center (nMS→ SB)**
  Ohio State University
- **The GCE4All Center: Unleashing the Potential of Genetic Code Expansion for Biomedical Research**
  Oregon State University
- **UTSW-UNC Center for Cell Signaling Analysis**
  UT Southwestern Medical Center and the University of North Carolina Chapel Hill

Two BTOD Centers presented by the Principal Investigators

- **The GCE4All Center: Unleashing the Potential of Genetic Code Expansion for Biomedical Research**
    - Oregon State University, Dr. Ryan Mehl, Principal Investigator

- **Native Mass Spectrometry Guided Structural Biology Center (nMS→ SB)**
    - Ohio State University, Dr. Vickie Wysocki, Principal Investigator

Ryan Mehl
GCE4All Research Center
Dept. of Biochemistry & Biophysics
Oregon State University

GCE4All

NIH
National Institute of General Medical Sciences
(RM1-GM144227)

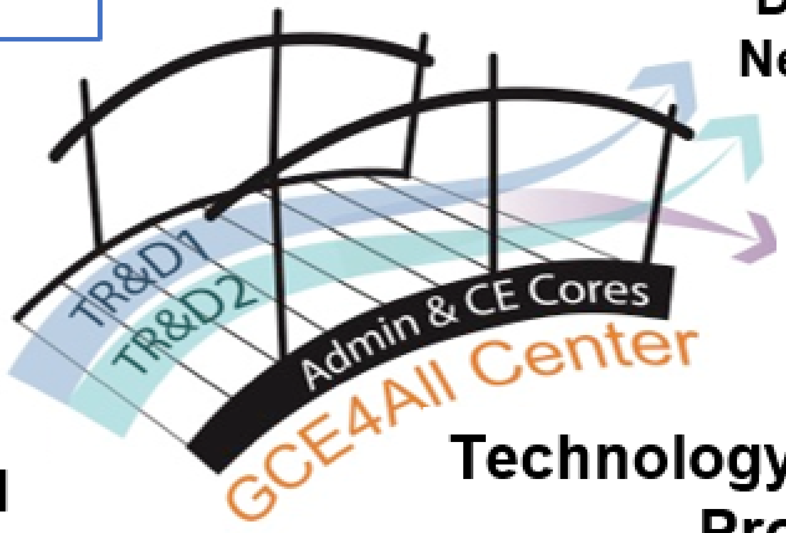
Oregon State University

GCE4All Center Structure

We aim to optimize and disseminate GCE technology such that it becomes a basic tool for protein research that is accessible **by all**

DBP Breakthroughs & New GCE Technologies

Community Engagement leading to widespread GCE awareness and use

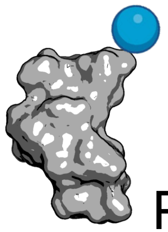


Driving Biomedical Projects (DBPs)

Technology Optimization Projects

What is Genetic Code Expansion Technology?

The ability to site-specifically encode new amino acid chemistry on a protein.

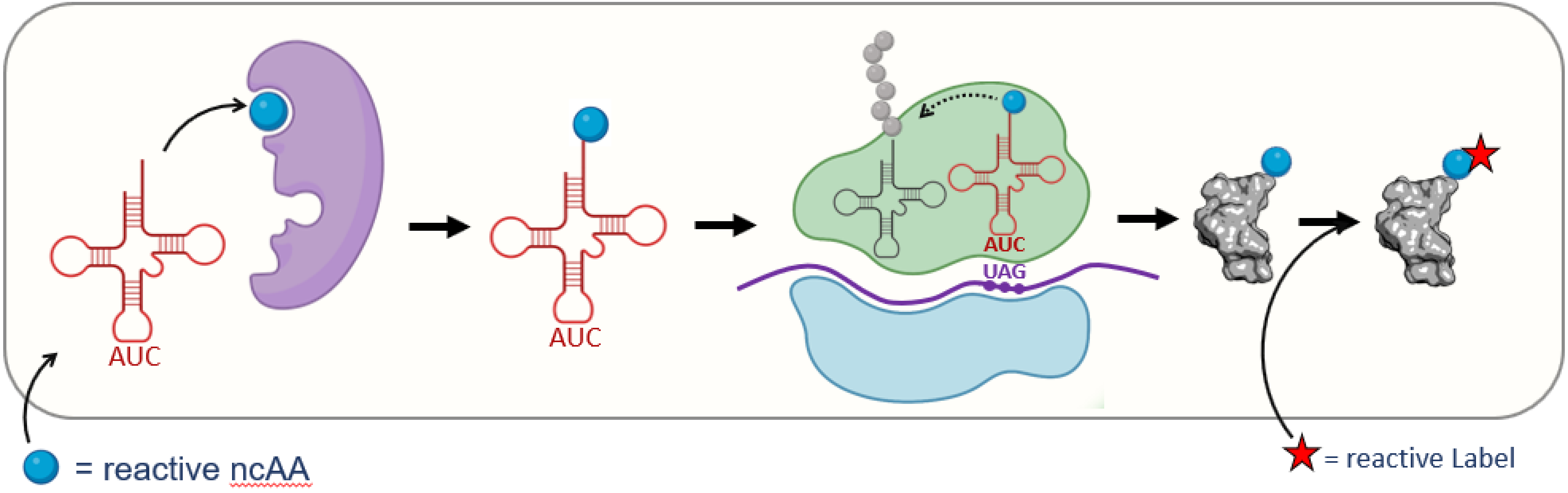


First in cell demonstration 2021.
More than 300 amino acids have been encoded.
Most model organisms have been expanded.

<u>No major equipment or software</u>
Chemical synthesis of amino acids
Directed evolution of translation
Optimization of evolved translation for needed organism
Optimization of protein scale
Optimization of amino acid functionality for intended use

Dissemination of optimized methods and increase access to required optimized reagents

GCE technology

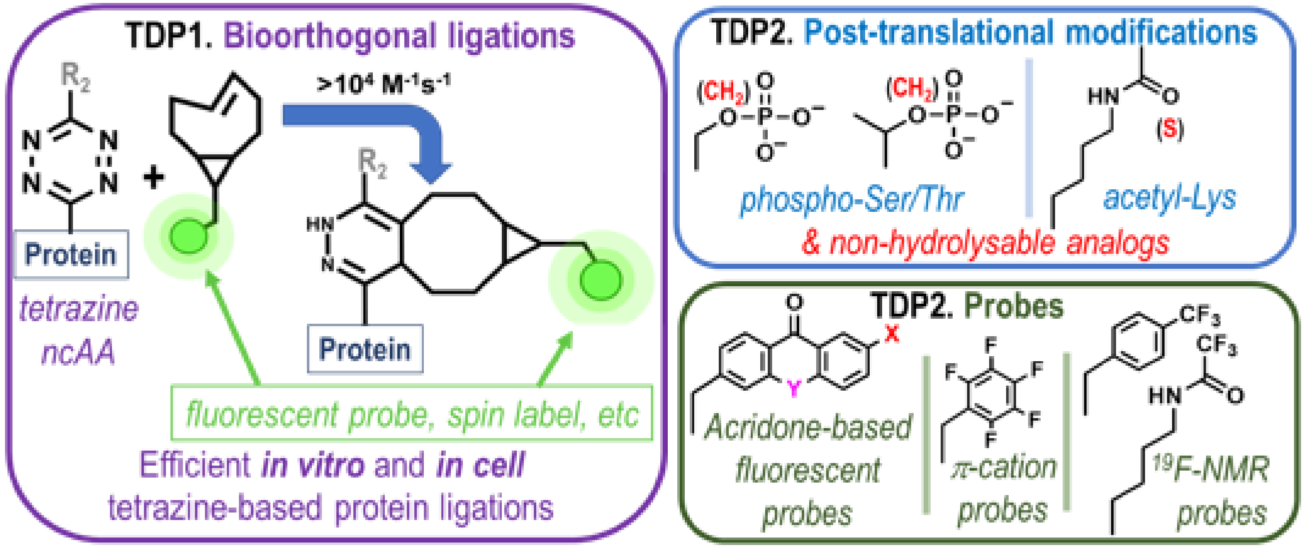


This is chemistry that must be optimized in live cells

What GCE to optimize?

What are NIH funded researchers needing?

TOP Cores



We selected amino acids that would have the greatest immediate impact for currently funded NIH researchers

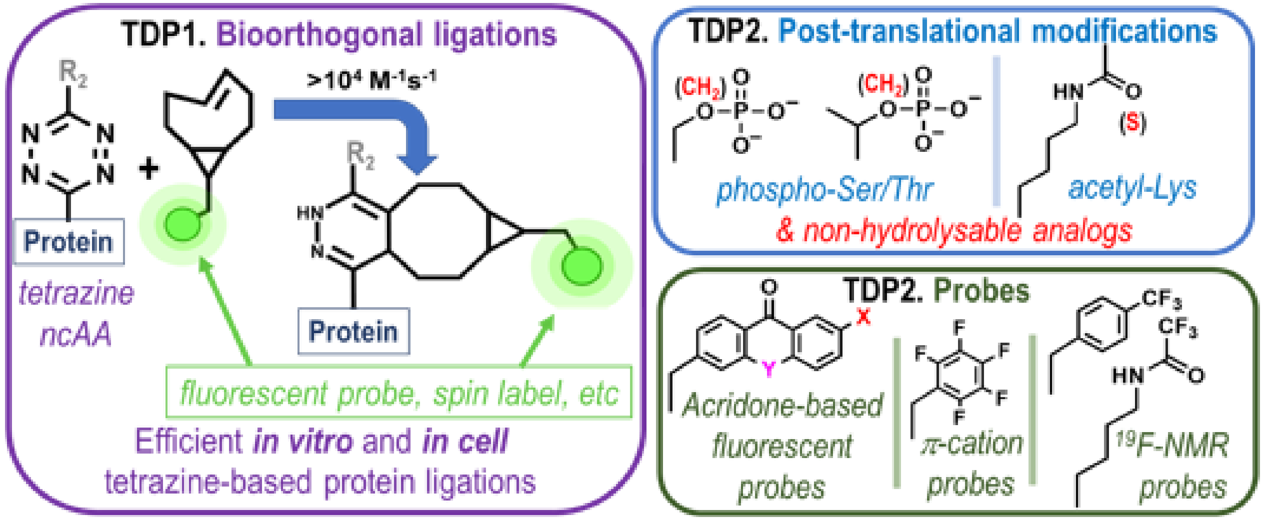
9 Driving Biomedical Projects (DBPs)

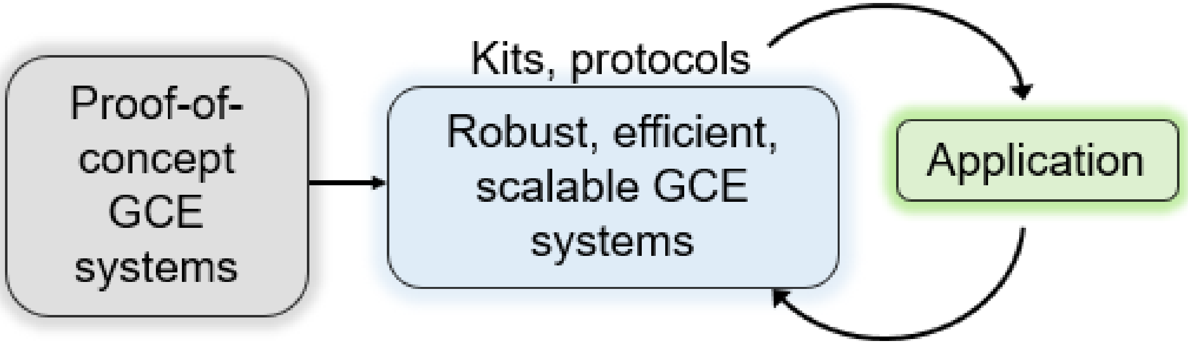
Investigator	Project
E. Gouaux (OHSU)	Direct On-grid Capture of Protein Complexes for CryoEM
C. Ahern (U of Iowa)	Functional Consequences of Disease Mutation in Membrane Proteins
S. Gordon & B. Zagotta (U of Wash)	Conformational Dynamics & Regulation of Ion Channels
M. Waters (UNC)	Engineering of Novel Histone eWriter Proteins & Studying Pi-Pi Interactions
Edwin Antony (SLU)	Deciphering the Assembly of Multi-Protein Complexes in DNA Metabolism
E.J. Petersson (UPenn)	Probing Protein Misfolding in Neurodegeneration
A. Gronenborn (U of Pitt)	Development of Efficient Eukaryotic In Cell ^{19}F NMR
J. Prescher (UC Irvine)	Genetically encodable cyclopropenones for bioorthogonal crosslinking
H. Arthanari (Harvard)	The use of GCE technologies to study the interaction of disordered section of the kinase Wee-1 with its structured domain

What GCE to optimize? (2)

What are NIH funded researchers needing?

TOP Cores





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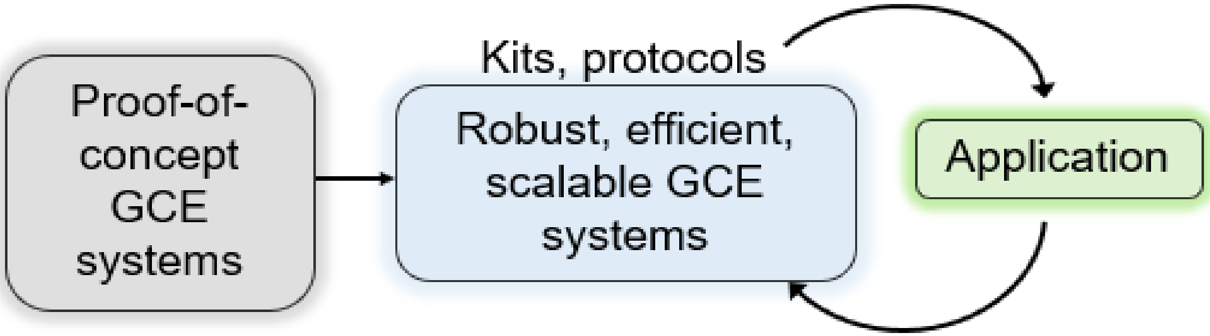
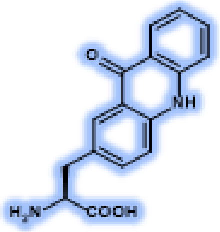
What GCE to optimize? (3)

What are NIH funded researchers needing?

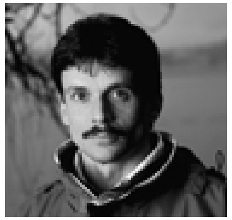




ACD

J. Petersson
UPenn

Dual in E. coli encoding of ACD with a good bioorthogonal ligation

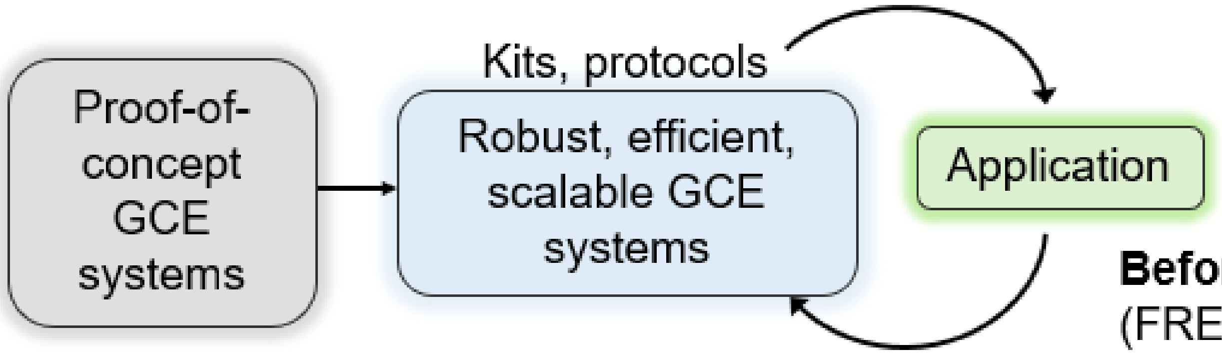


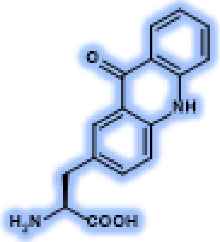
B. Zagotta S. Gordon
Univ. of Washington

ACD encoding in mammalian cells to resolve conformational distributions

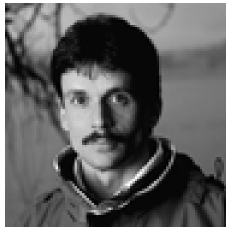
What GCE to optimize? (4)

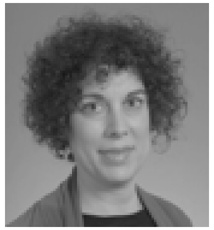
What are NIH funded researchers needing?





ACD





B. Zagotta S. Gordon
Univ. of Washington

ACD encoding in mammalian cells to resolve conformational distributions

Before GCE4All: fluorescence resonance energy transfer (FRET) approaches using a fluorescent noncanonical amino acid donor (Anap).

Acd's high quantum yield (2.5x) and photostability (10x). First eukaryotic FLIM studies with GCE because of its 15–16 ns fluoresce lifetime in water (2-3x that of fluorophores in use).

Jones et al. Chem. Sci., 2021, 12, 11955

We demonstrated that the long, single-exponential fluorescence lifetime of Acd is suitable for time-resolved measurements of the tmFRET efficiency allowing us to resolve conformational distributions of proteins in cells.

Zagotta et al. eLife 2021;10:e70236

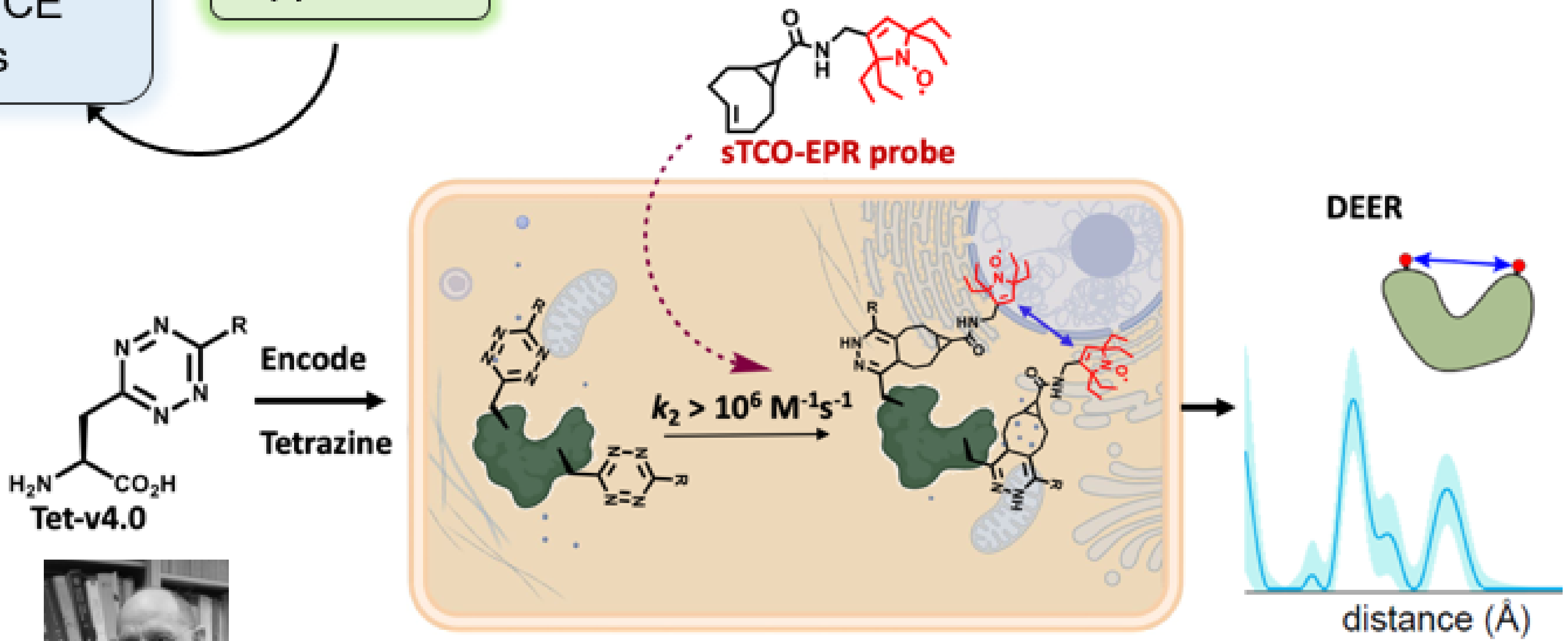
What GCE to optimize? (5)

What are NIH funded researchers needing?

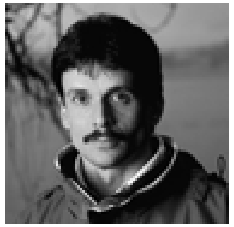
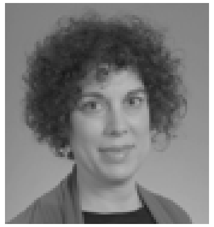
Proof-of-concept GCE systems → Robust, efficient, scalable GCE systems → Application

Kits, protocols

Measuring conformational changes by DEER spectroscopy in live mammalian cells is limited because EPR probes are reduced in cells.



Rapid Labeling In Live Eukaryotic cells

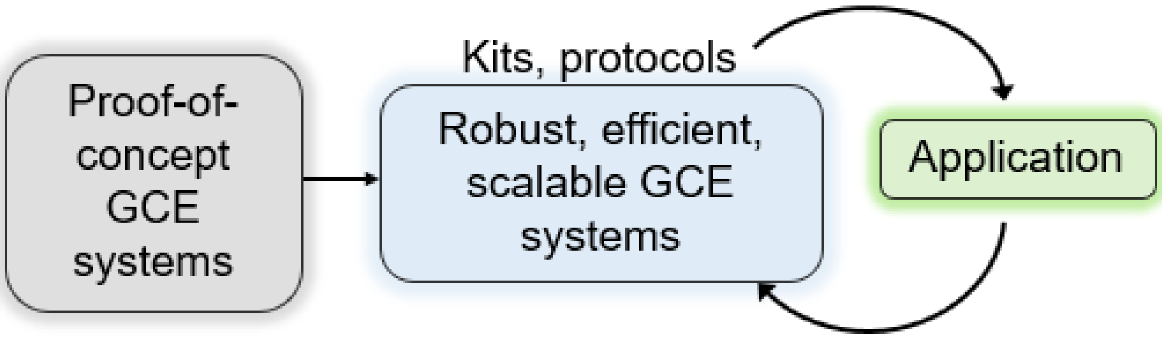




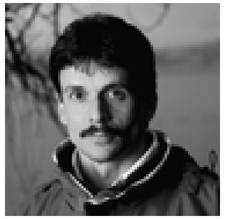

S. Stoll B. Zagotta S. Gordon A. Rajca

Univ. of Washington Univ. of Nebraska

What GCE to optimize? (6)

What are NIH funded researchers needing?



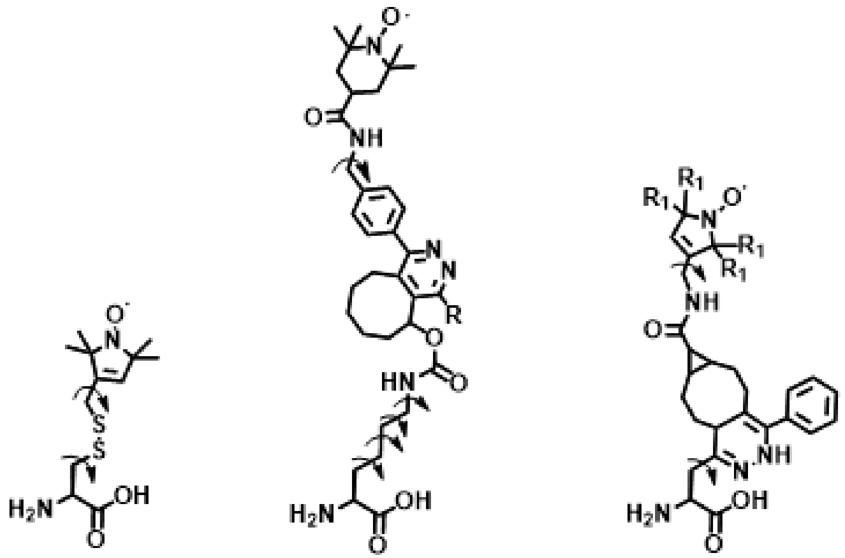

S. Stoll

B. Zagotta

S. Gordon

Univ. of Washington


A. Rajca

Univ. of Nebraska

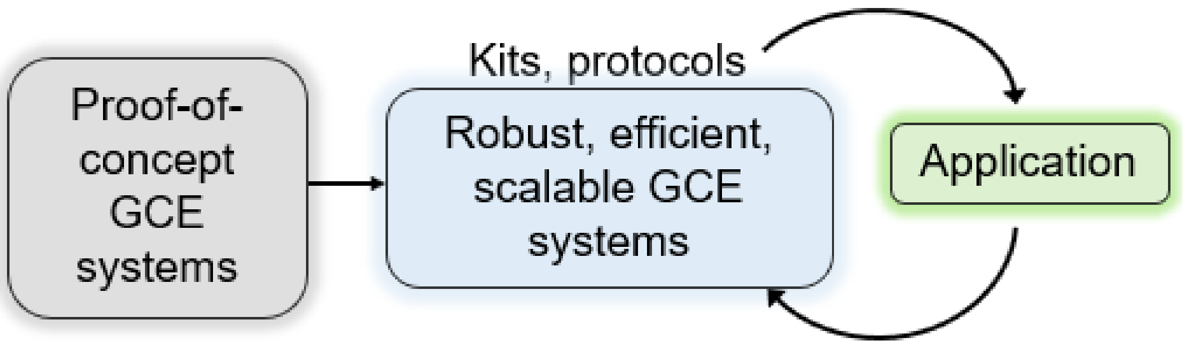


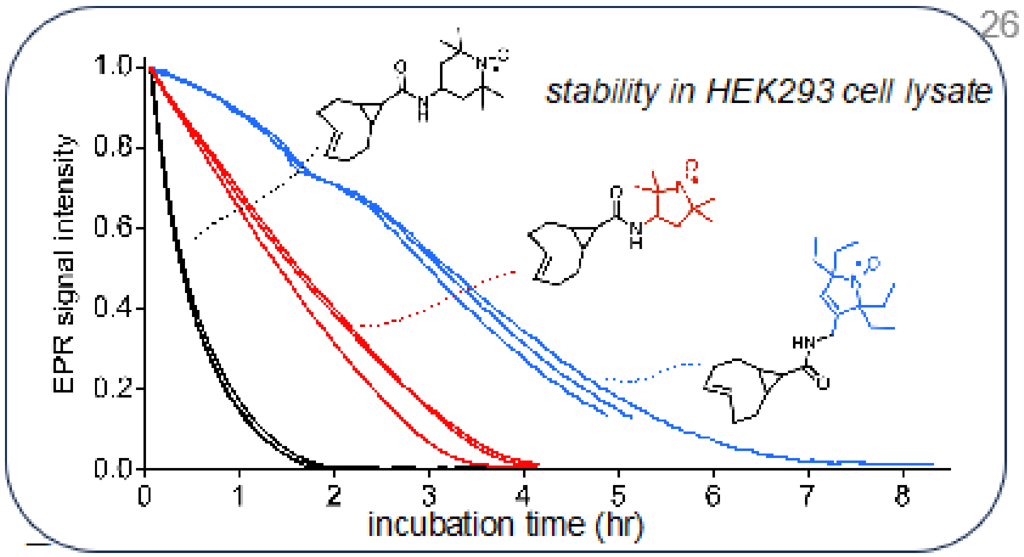
	Cysteine Gold standard	SCO-Lysine Previous work	Tet-v4.0Ph Present work
	In vitro	*In vitro*	In-cell
Site-specific	No	Yes	Yes
Labeling rate k_2 ($M^{-1}s^{-1}$)	10^5 (fast)	400 ± 200 (slow)	$4.0 \times 10^5 \pm 10^4$ (fast)
	Short	Long and more flexible	Short and less flexible

Improve label rate 1000x

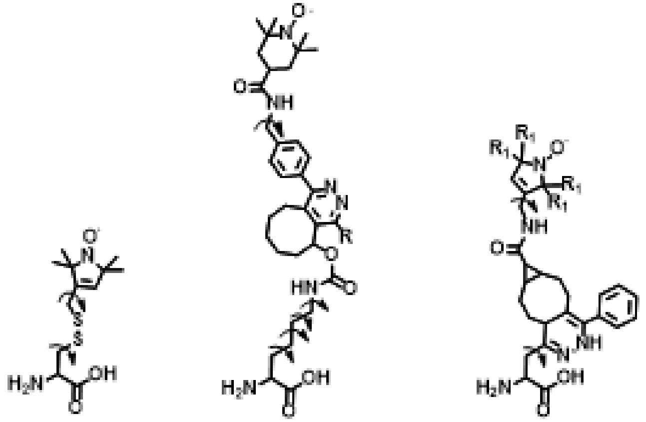
What GCE to optimize? (7)

What are NIH funded researchers needing?





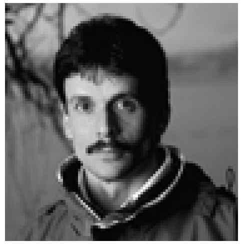
Improve label lifetime 15x

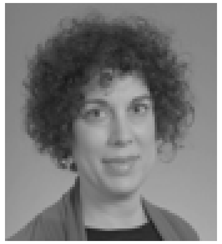


	Cysteine Gold standard *In vitro*	SCO-Lysine Previous work *In vitro*	Tet-v4.0Ph Present work In-cell
Site-specific	No	Yes	Yes
Labeling rate k_2 ($M^{-1}s^{-1}$)	10^5 (fast)	400 ± 200 (slow)	$4.0 \times 10^5 \pm 10^4$ (fast)
	Short	Long and more flexible	Short and less flexible

Improve label rate 1000x


S. Stoll


B. Zagotta


S. Gordon

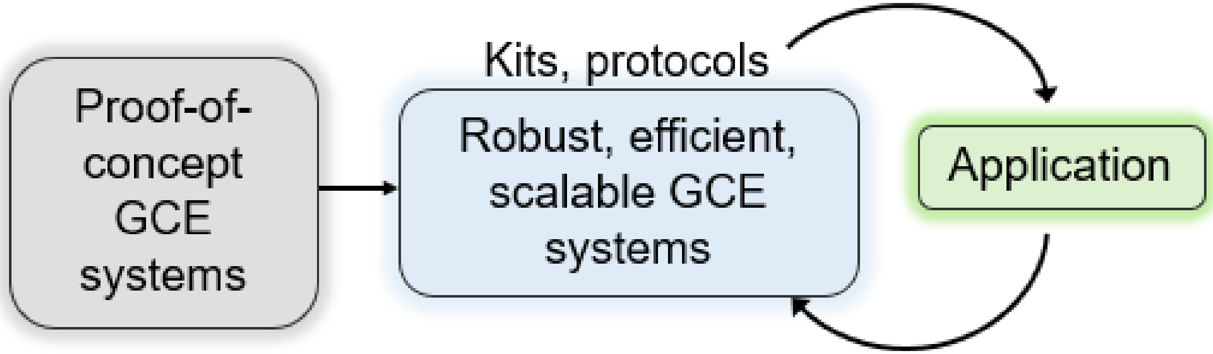

A. Rajca

Univ. of Washington

Univ. of Nebraska

What GCE to optimize? (8)

What are NIH funded researchers needing?



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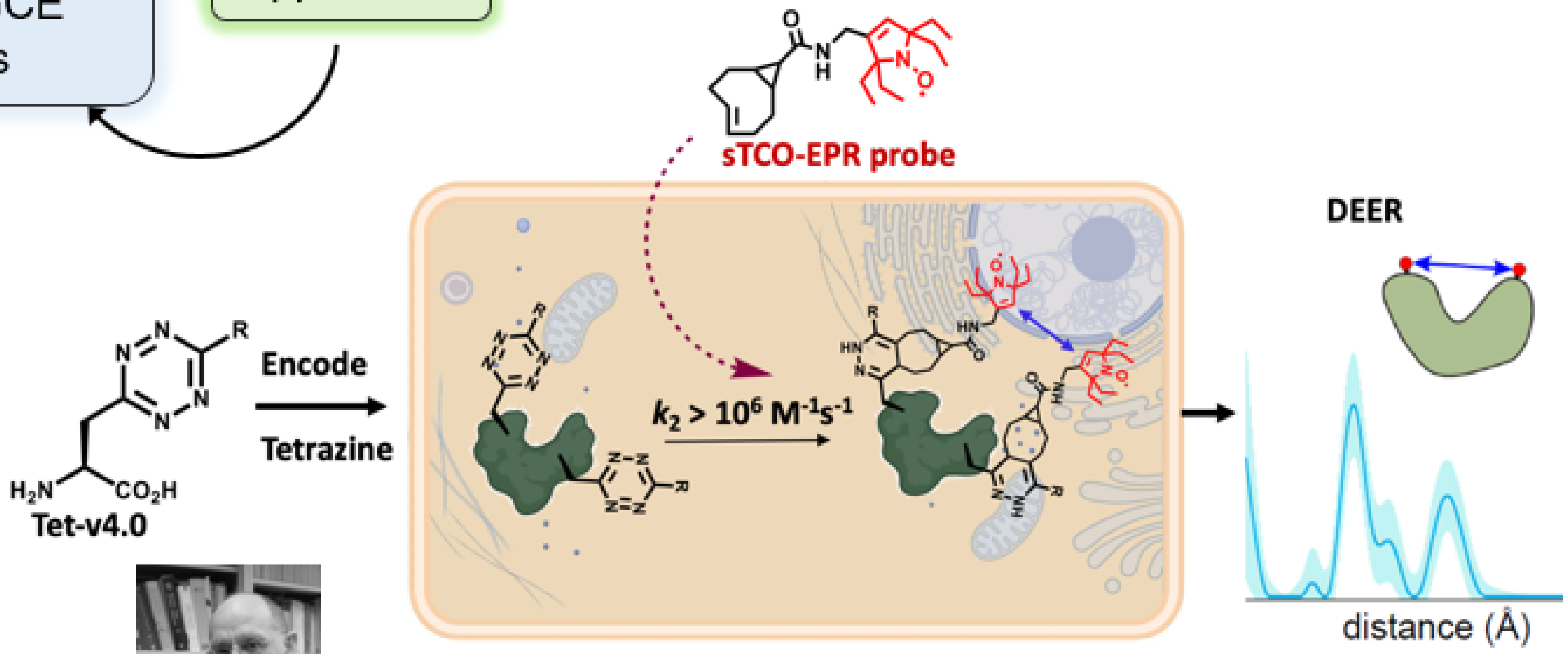
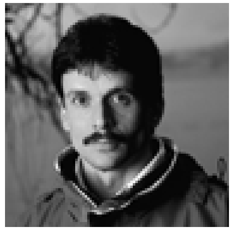
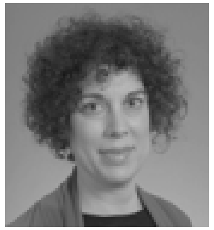


Rapid Labeling In Live Eukaryotic cells

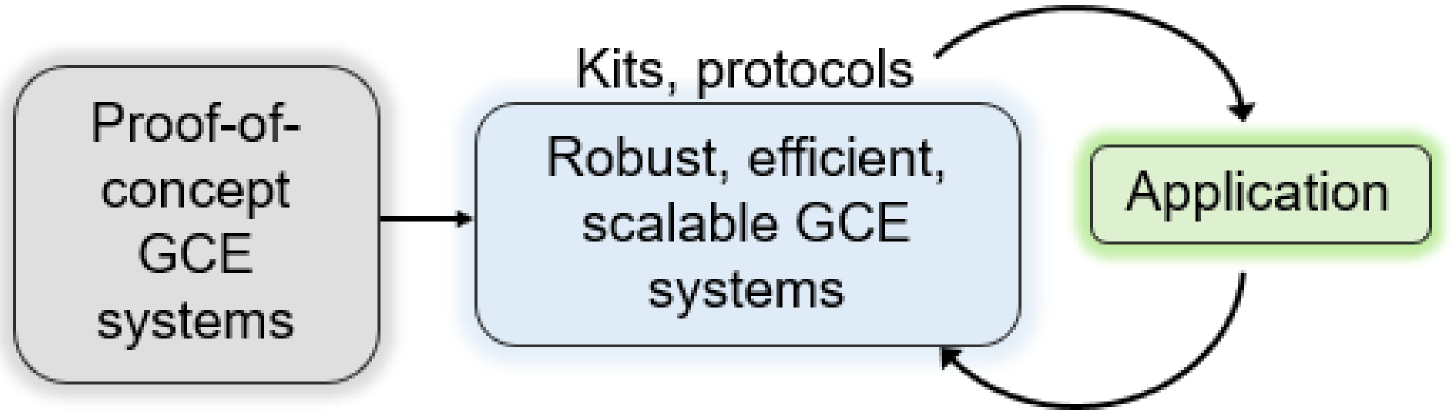
***Jana et al. J. Am. Chem. Soc.* 2023, 145, 27, 14608–14620**


S. Stoll B. Zagotta S. Gordon
University of Washington

A. Rajca
University of Nebraska

What GCE to optimize? (9)



What are NIH funded researchers needing (now)?

How to have the maximum impact with the fewest research areas?

Who drives the publication to completion?

How to cycle DBPs out and new DBPs in?

When is the optimized technology ready for broad distribution?

Community Engagement - (CE)

Website

One-stop GCE resource that supports Center administrative activities, provides up-to-date information about Center activities, and provides links to instructional/dissemination materials

gce4all.oregonstate.edu

GCE knowledgebase

One-stop resource for all things GCE planned for 2023

Training & Support

GCEbb listserve

A community forum for help trouble-shooting experiments and other networking

International GCE webinar

3rd Thursdays Oct-Jun

Workshops

2-4 each year hands-on support for applications using specific "certified" GCE tools

Online training modules

Available 24/7 worldwide

Dissemination

"Certified" Protocols & Materials

addgene

Publications & Presentations

GCE Conferences

August 8-11th, 2024 Oregon State University

Chairs: Kathrin Lang & Abhishek Chatterjee

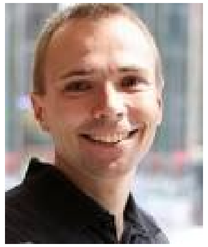
GCE4All

Oregon State University

NIH Biomedical Technology Optimization and Dissemination Center

Native Mass Spectrometry Guided Structural Biology

nativems.osu.edu



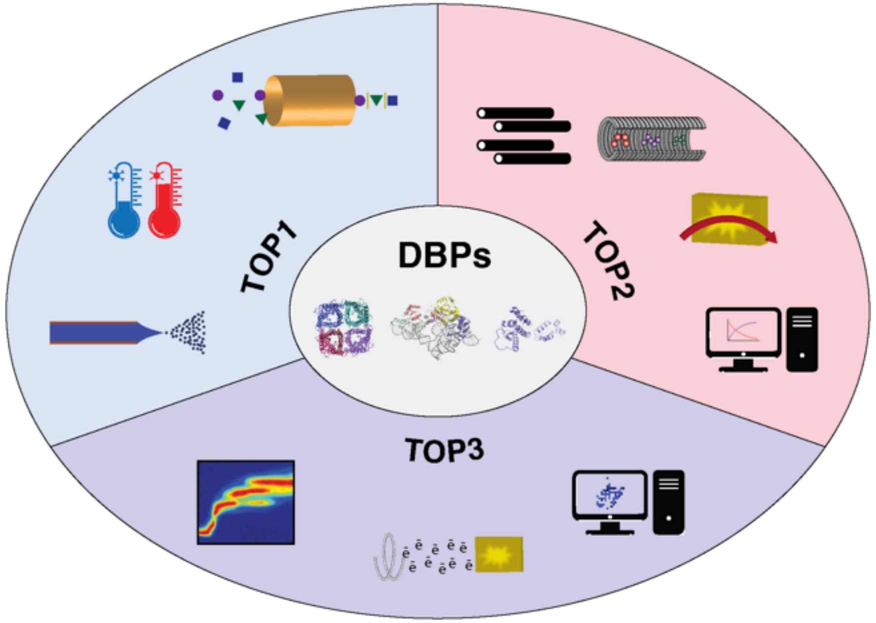
nMS→SB

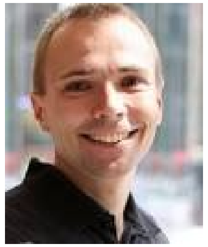
NATIVE MS GUIDED STRUCTURAL BIOLOGY

NIH Biomedical Technology Optimization and Dissemination Center

Native Mass Spectrometry Guided Structural Biology

nativems.osu.edu

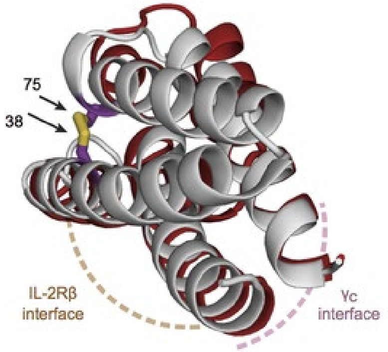


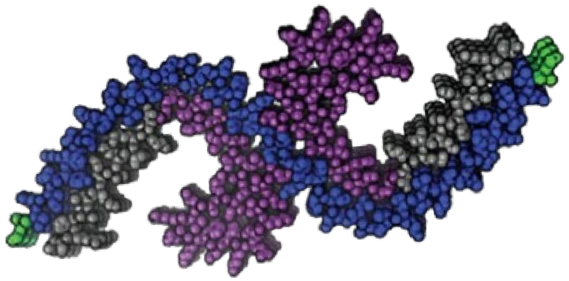


nMS→SB
NATIVE MS GUIDED STRUCTURAL BIOLOGY

DBPs for RM1: geographical and structural diversity

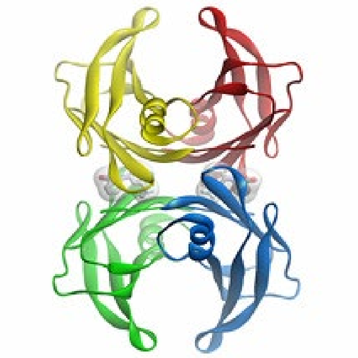
Baker (WA)
De Novo interleukin mimics for immunotherapy

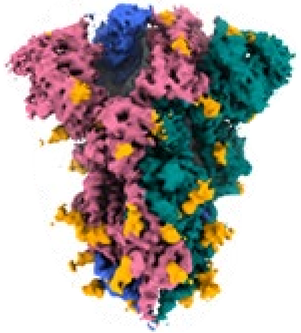




Sayed (U24)
Tau aggregates from Alzheimer's
(TX, OH, WA, CA)

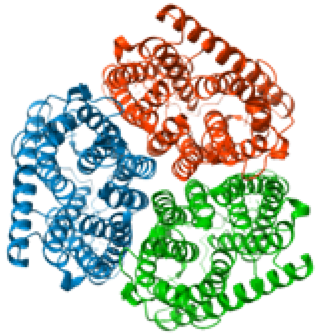
Kelly (CA)
Amyloid disease



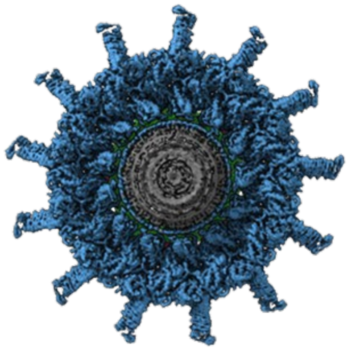


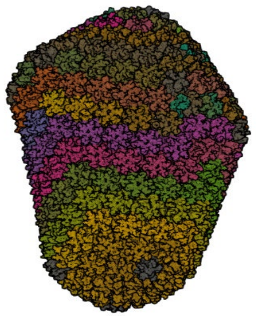
Ollman Saphire (CA)
Glycoproteins

Laganowsky (TX, NC, AZ)
Membrane protein lipid interactions



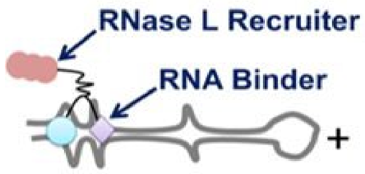
Cover and Ohi (MI, TN)
Type IV Protein Secretion

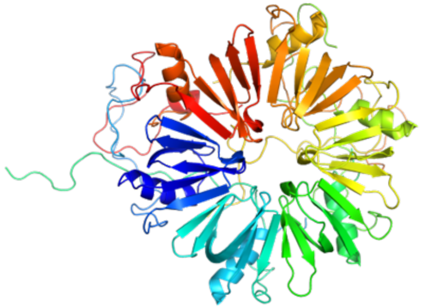




Torbett, Sarafianos (U54) Dynamics of HIV core interactions
(NC, WA, etc)

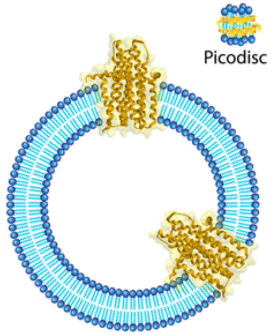
Disney (FL)
RIBOTACs





Woodson (MD)
RNA-protein complexes for genetic control

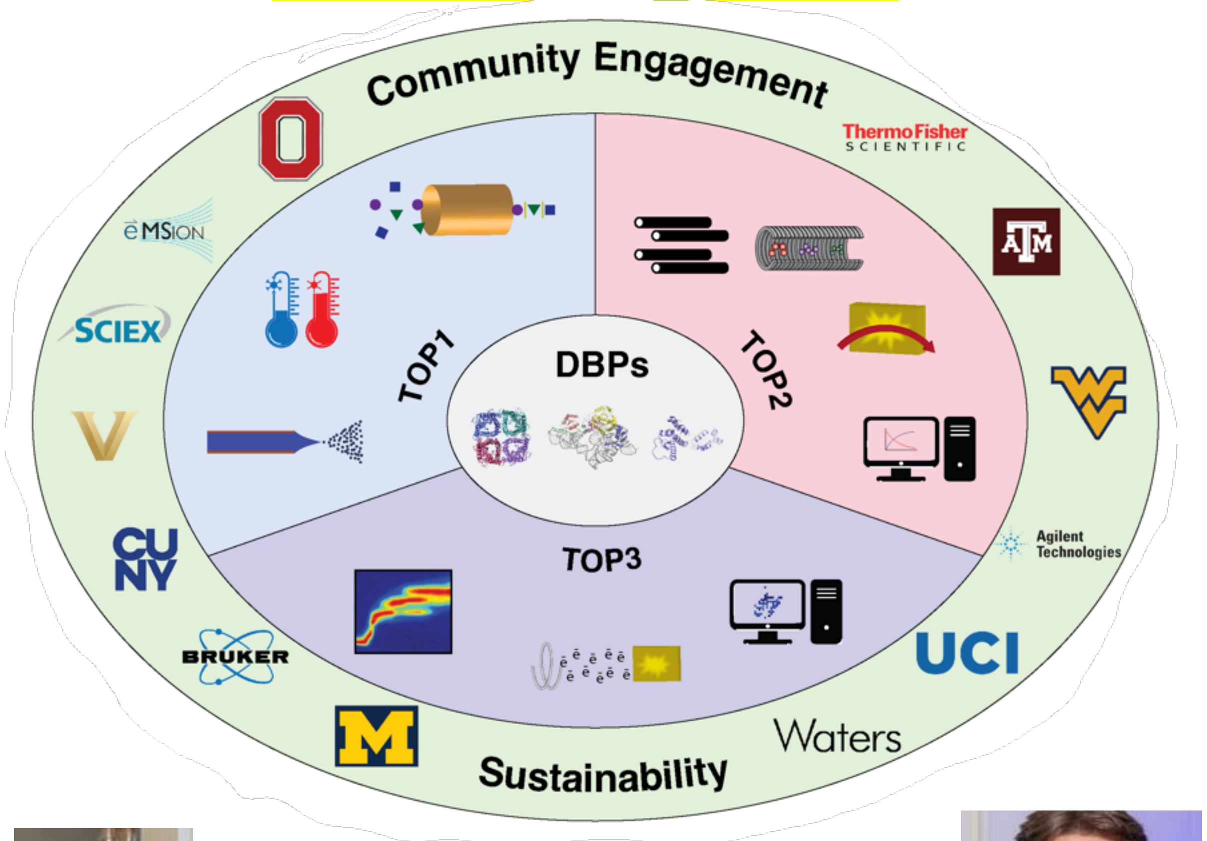
Gupta (CT)
Membrane proteins in lipid vesicles

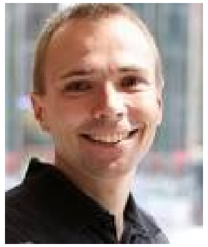


NIH Biomedical Technology Optimization and Dissemination Center

Native Mass Spectrometry Guided Structural Biology

nativems.osu.edu




nMS→SB
NATIVE MS GUIDED STRUCTURAL BIOLOGY



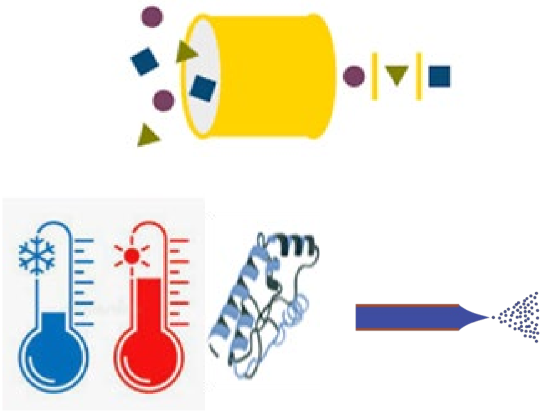
Native MS Guided Structural Biology Center

Goal: optimize user-compatible native MS technology to characterize the assembly and disassembly of macromolecular protein complexes

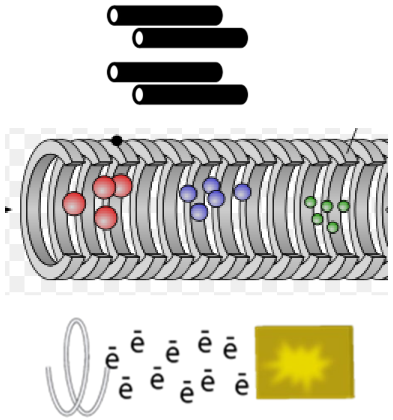
Native MS Guided Structural Biology Center

<u>Goal:</u> optimize user-compatible native MS technology to characterize the assembly and disassembly of macromolecular protein complexes

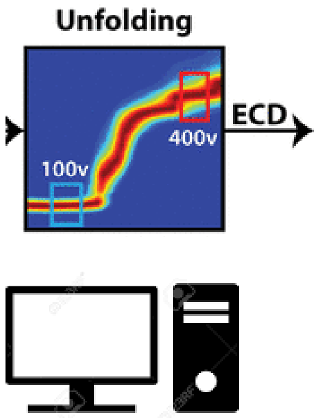
TOP1 Front End/ Solution Phase



TOP2 Ion Selection/ Separation/Manipulation

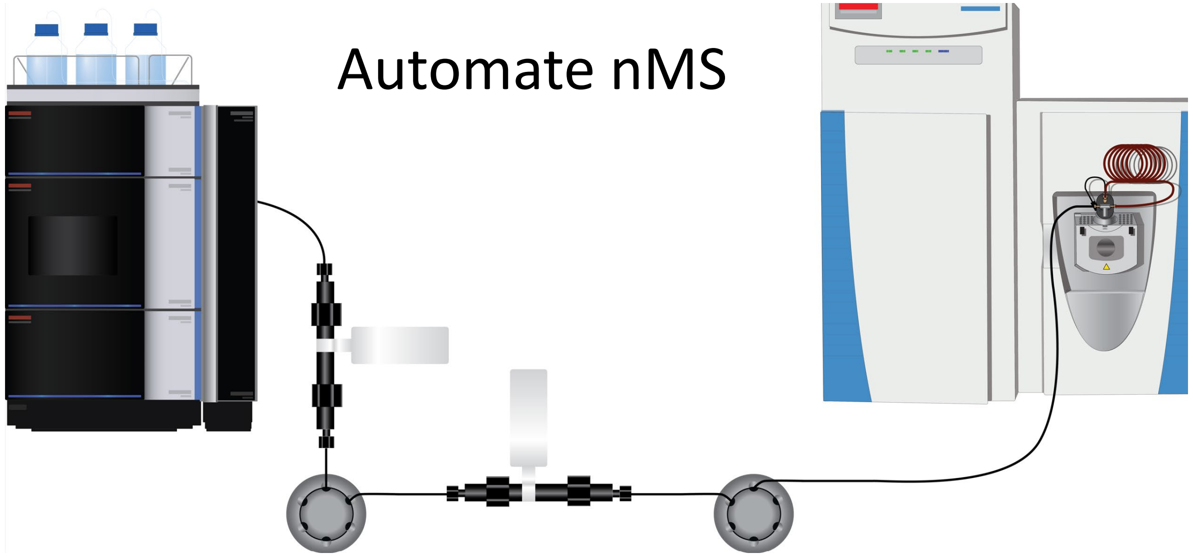


TOP3 Ion Stability/ Computations



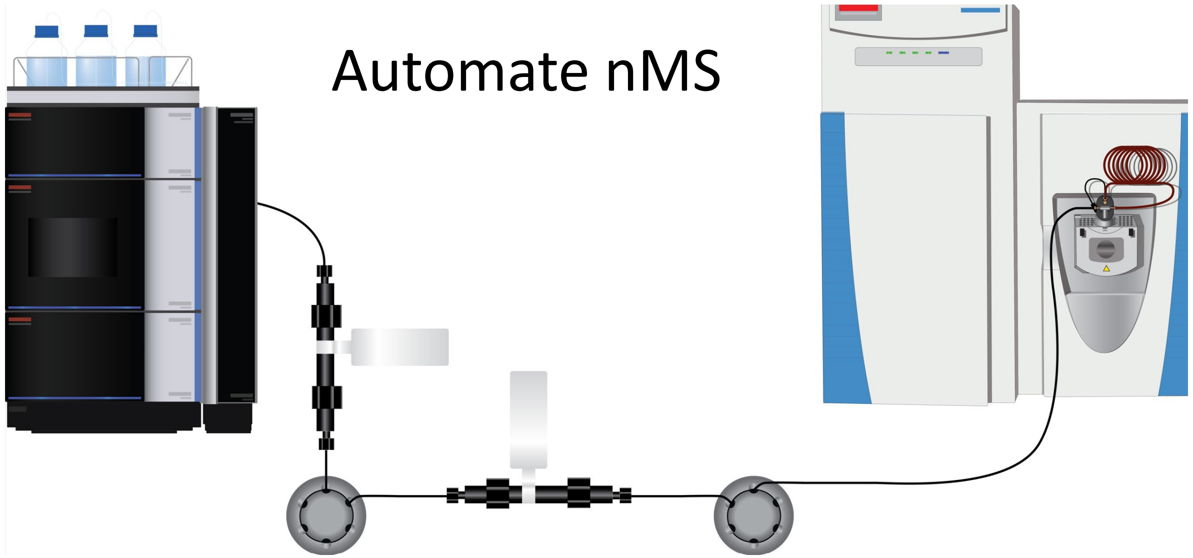
TOP1, Front End: 1D and 2D Online buffer exchange

Automate nMS



VanAernum, Busch, Jones, Jia, Chen, Boyken, Sahasrabuddhe, Baker, Wysocki. *Nature Protocols.* **2020**, 15, 1132-1157.

TOP1, Front End: 1D and 2D Online buffer exchange



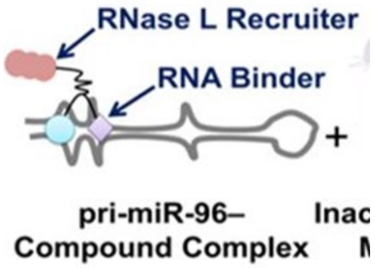
Automate nMS

Driving Biomedical Projects

Baker

INSTITUTE FOR Protein Design

Disney, Scripps



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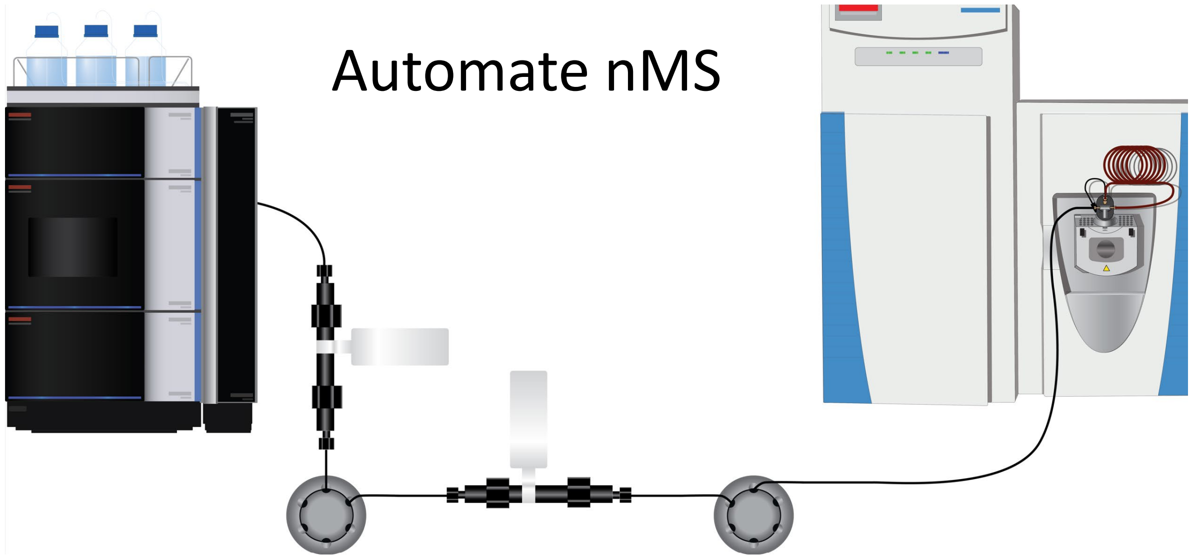

Automate nMS

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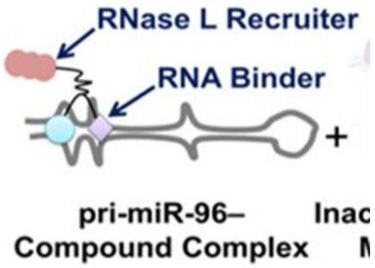
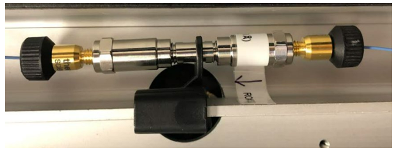


Why late stage? first column now commercial
adoption occurring in multiple labs

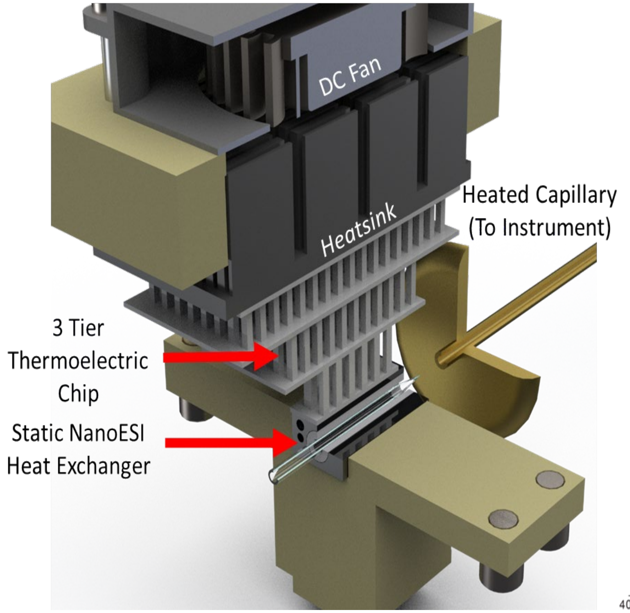
Thermo Scientific™ NativePac OBE-1™

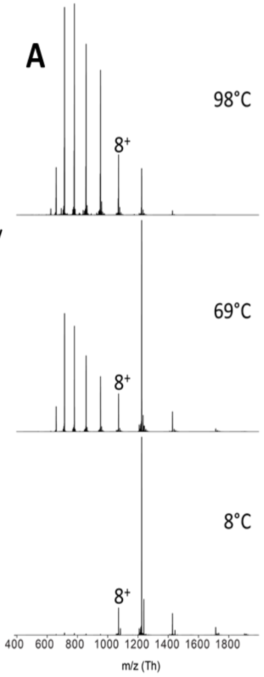


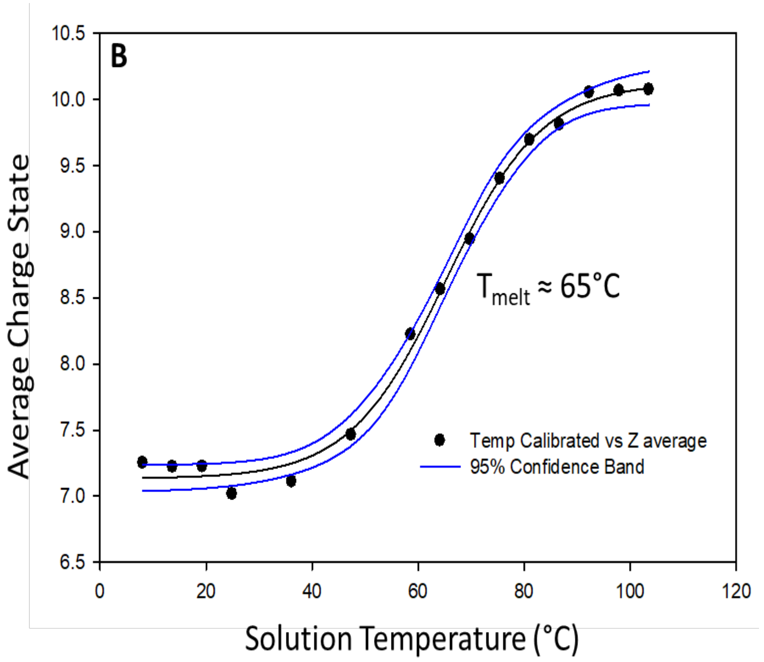
https://connect.acspubs.org/CENWebinar_Thermo_3_23_22

VanAernum, Busch, Jones, Jia, Chen, Boyken, Sahasrabuddhe, Baker, Wysocki. *Nature Protocols.* **2020**, 15, 1132-1157.

TOP1, Front End: Variable temperature ESI (vT-ESI)



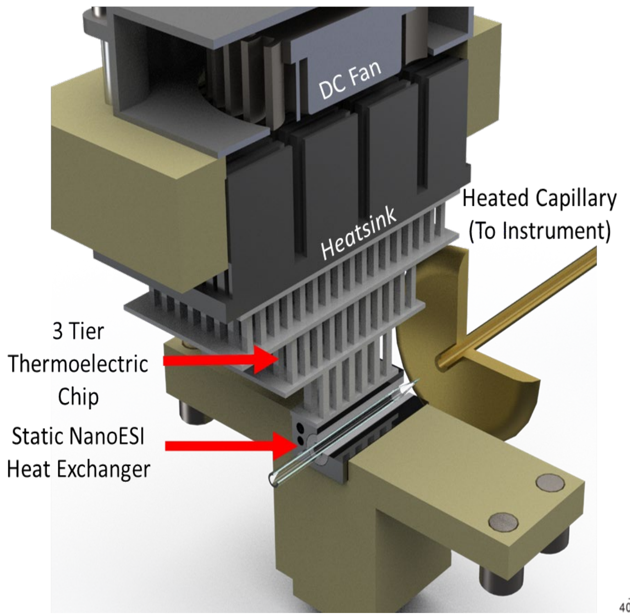


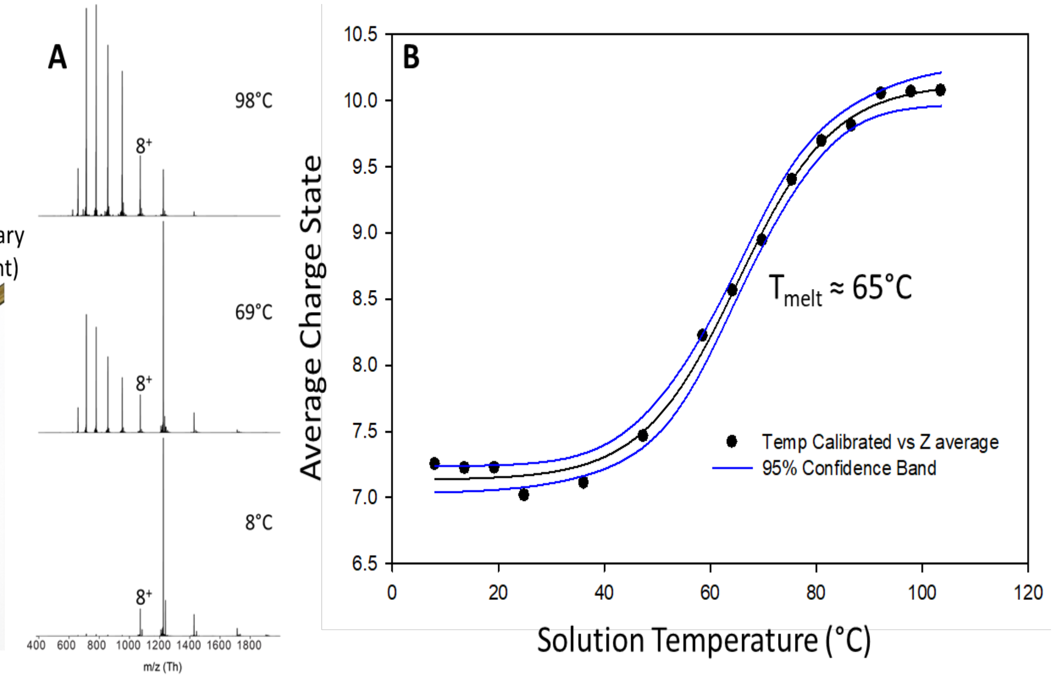


Dissecting the Thermodynamics of ATP Binding to GroEL One Nucleotide at a Time

T. E. Walker et al. ACS Cent Sci 2023, 9, 466-475.

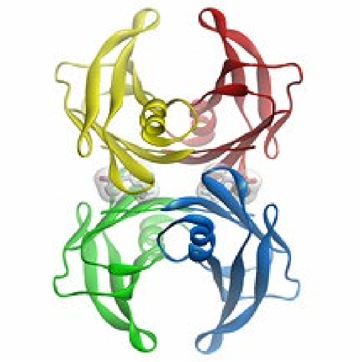
TOP1, Front End: Variable temperature ESI (vT-ESI)



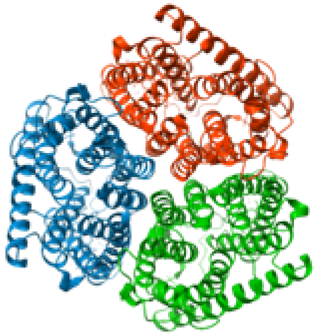


Driving Biomedical Projects

Kelly (CA)
Amyloid disease



Laganowsky (TX, NC, AZ)
Membrane protein lipid interactions



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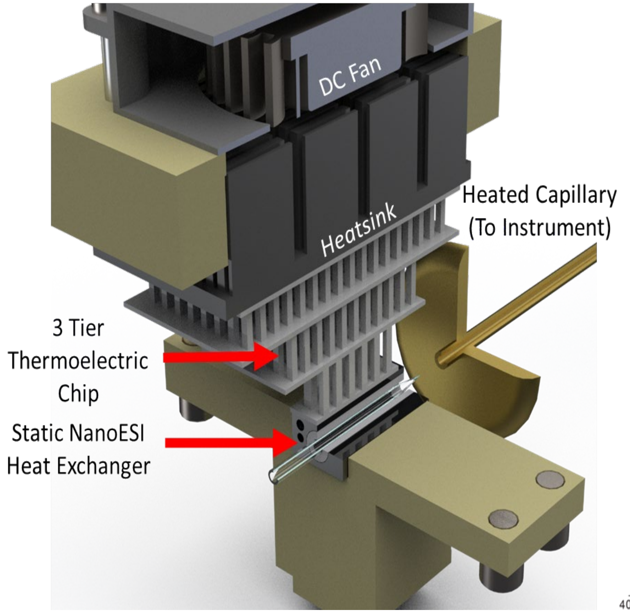
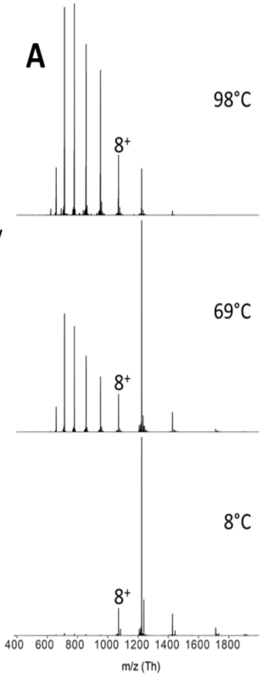
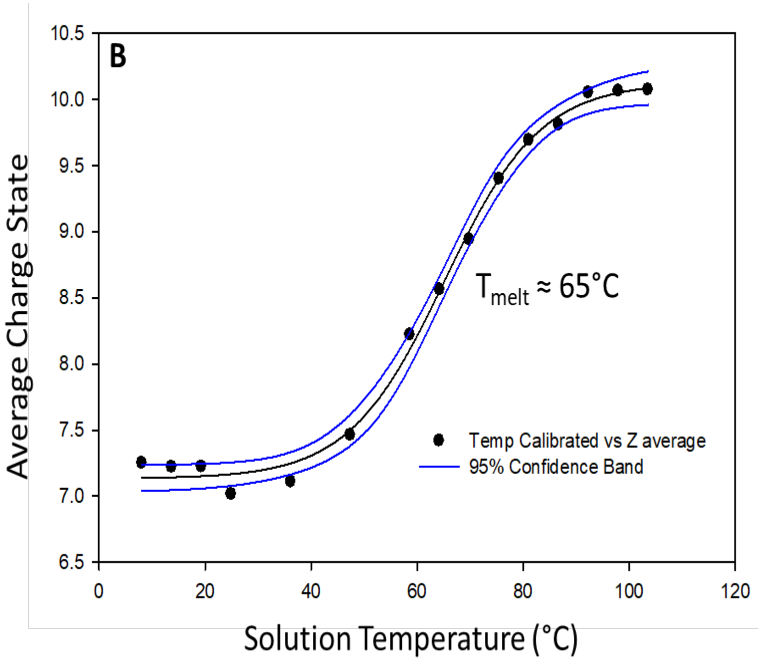
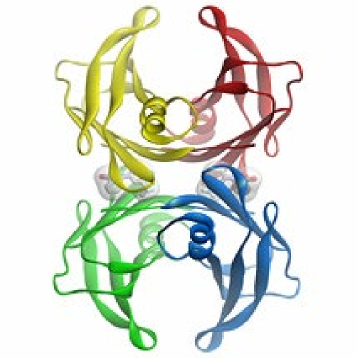




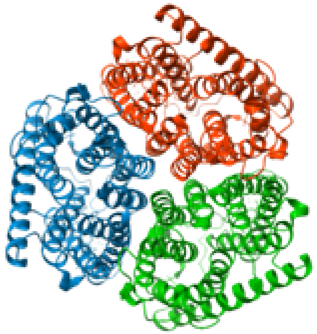


Driving Biomedical Projects

Kelly **(CA)**
Amyloid disease



Laganowsky **(TX, NC, AZ)**
Membrane protein lipid interactions

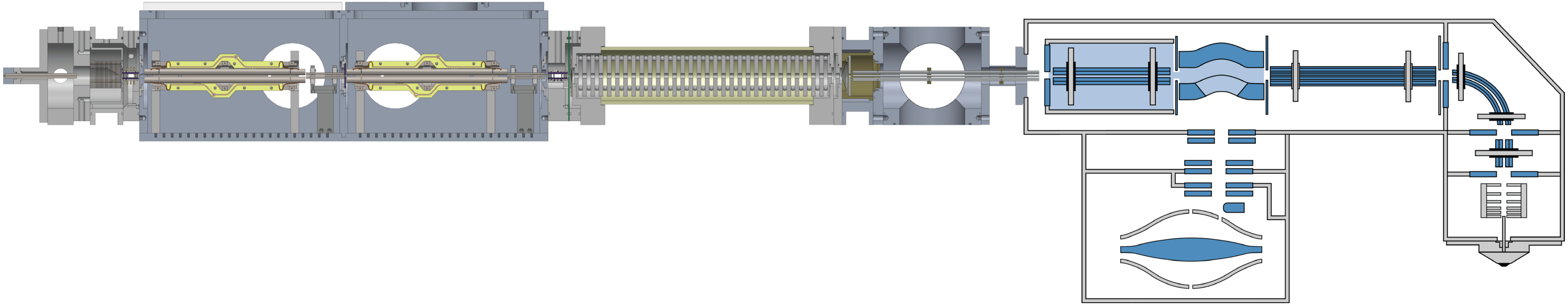


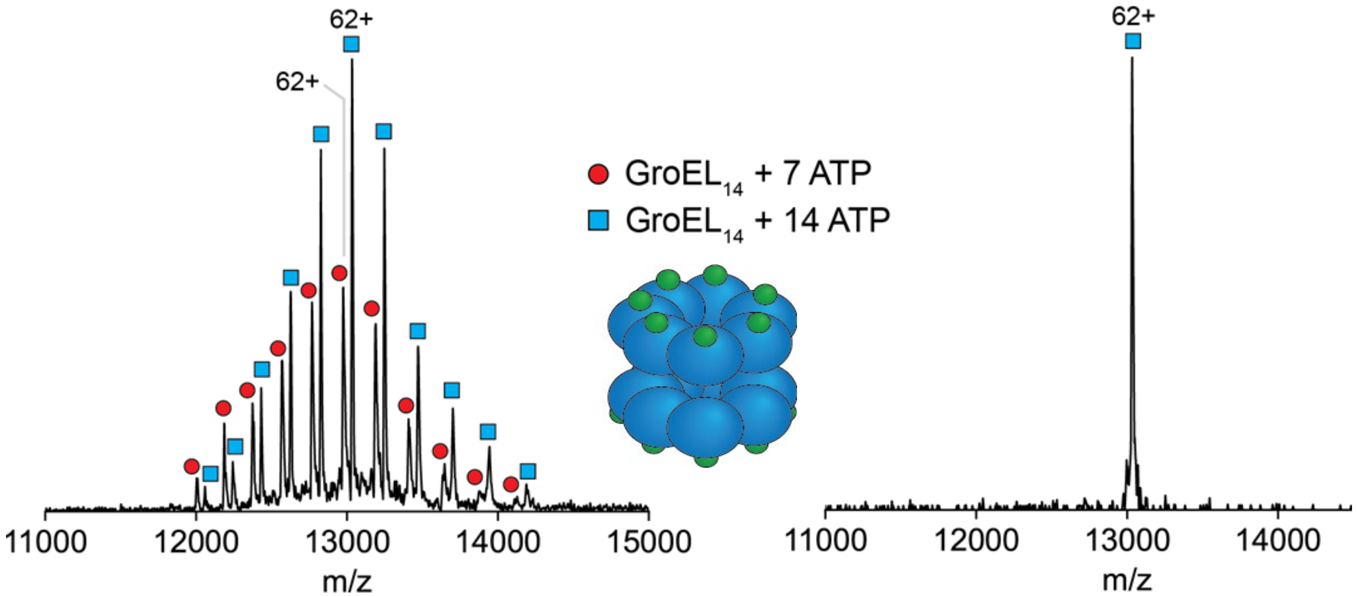
Why late stage? adoption in multiple labs
pharma collaborations
multi-vendor adoption possible

Dissecting the Thermodynamics of ATP Binding to GroEL One Nucleotide at a Time
T. E. Walker et al. ACS Cent Sci 2023, 9, 466-475.

TOP2, Ion Selection: Digital Quadrupole

Orbitrap Exactive Plus EMR





Optimization of a Digital Mass Filter for the Isolation of Intact Proteins in Stability Zone 1,1
R. L. Schrader et al. *Anal. Chem.* 2023, 95, 3062.

TOP2, Ion Selection: Digital Quadrupole

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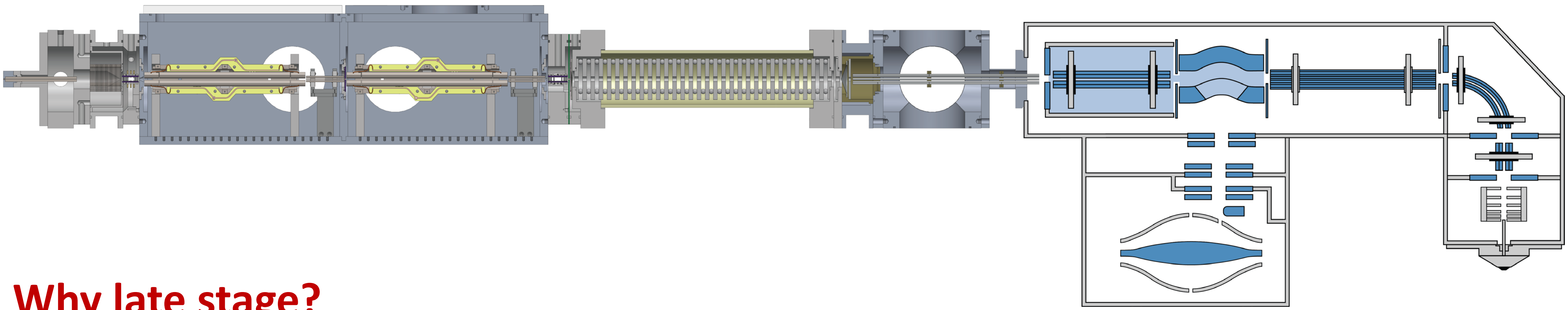
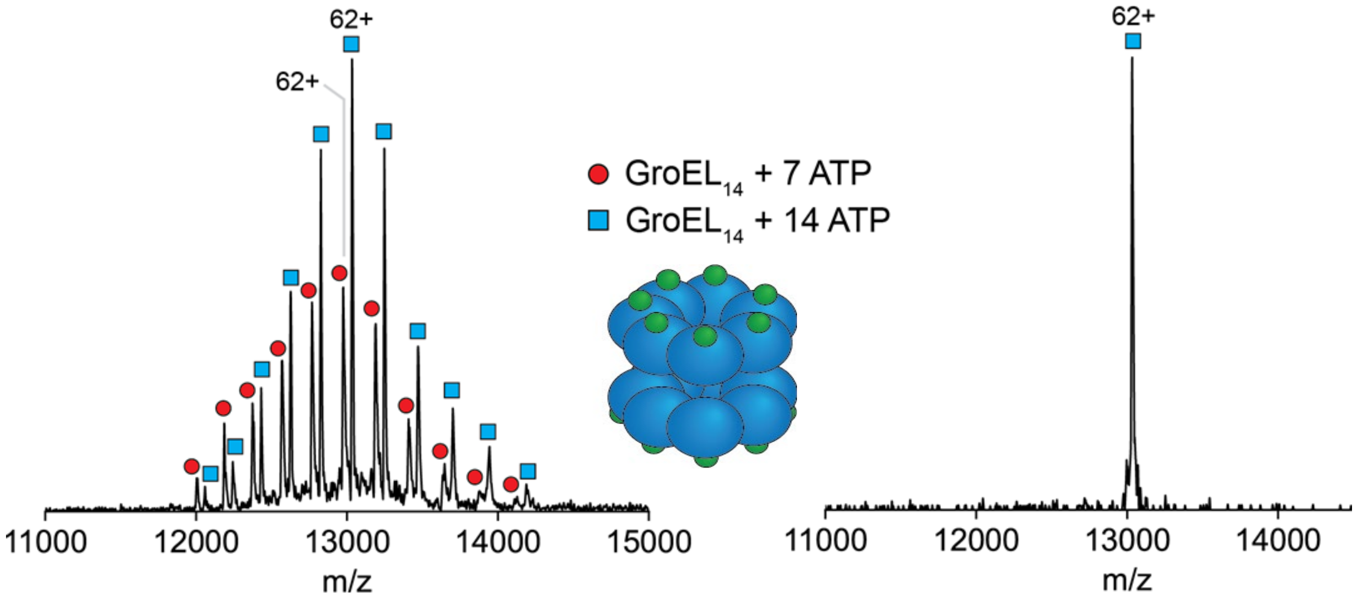


Why late stage?

Illustrated at TAMU & OSU

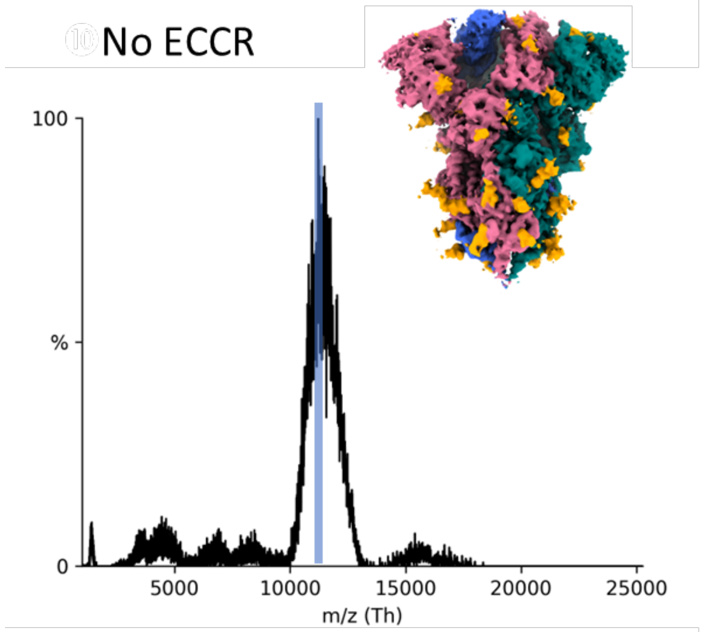
Illustrated in multiple vendors’ MS

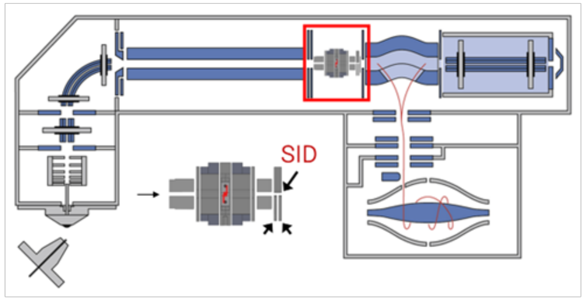


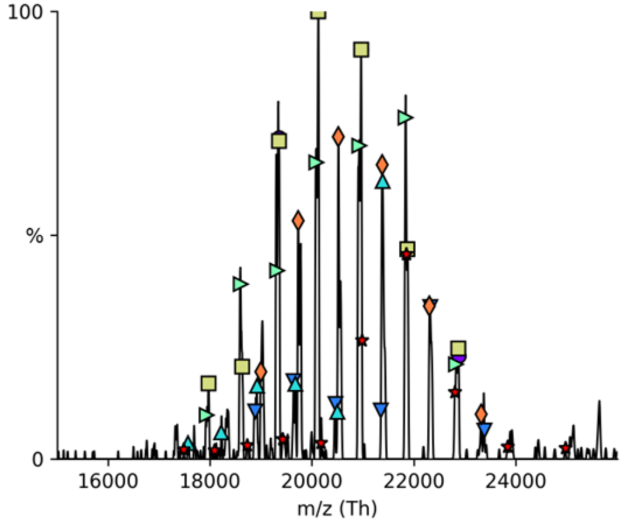
Optimization of a Digital Mass Filter for the Isolation of Intact Proteins in Stability Zone 1,1

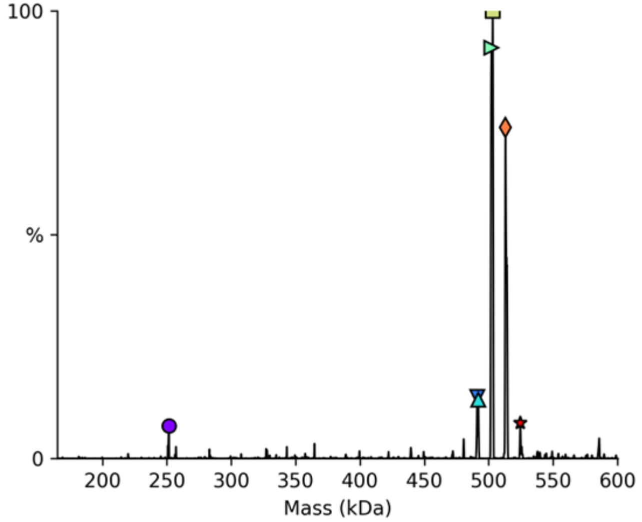
R. L. Schrader et al. *Anal. Chem.* 2023, 95, 3062.

TOP2, Electron Capture Charge Reduction-Surface Induced Dissociation









Driving Biomedical Projects

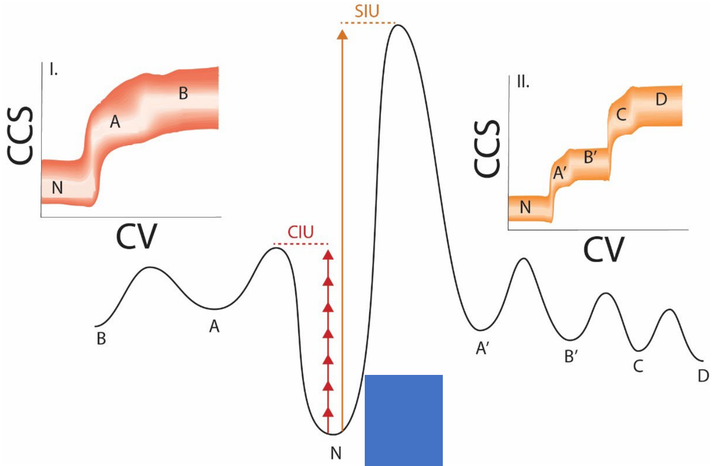
La Jolla Institute FOR IMMUNOLOGY

Ollman Saphire (CA)

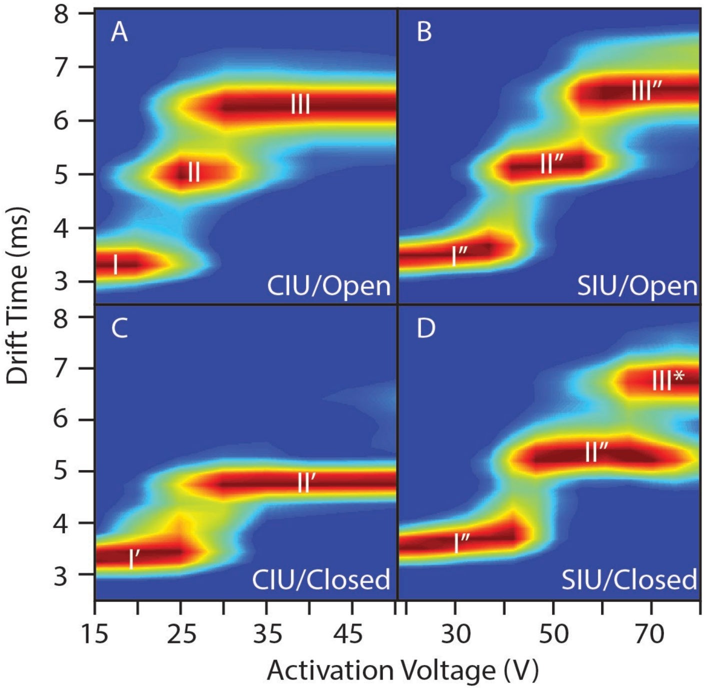
Glycoproteins

Unpublished

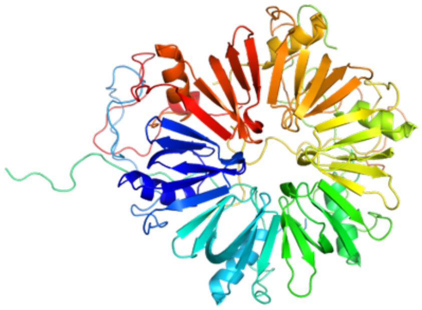
TOP3, Protein Stability: Collision induced unfolding



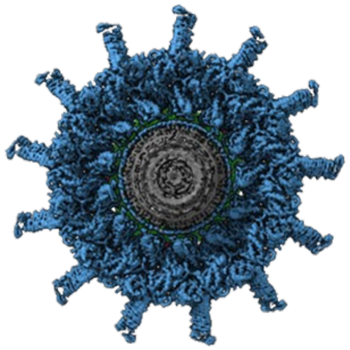
CIU SIU



Driving Biomedical Projects



Woodson (MD)
RNA-protein complexes for genetic control

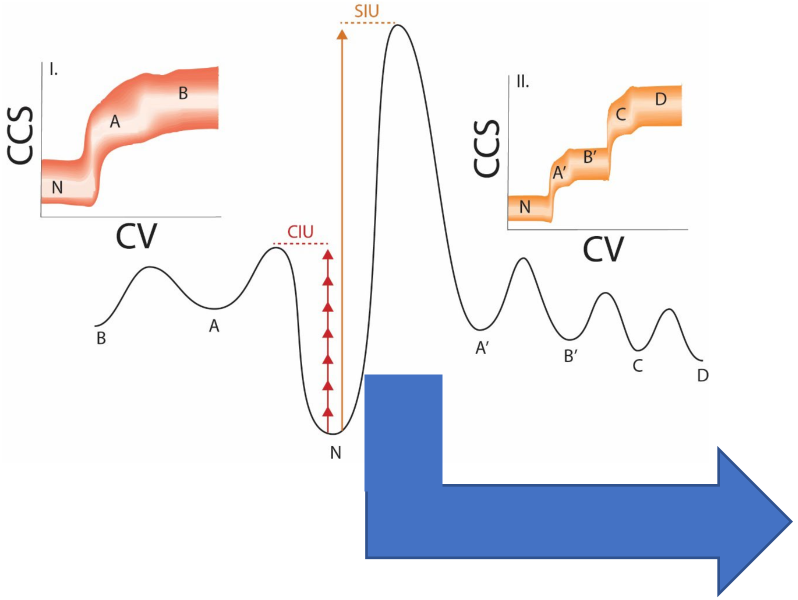


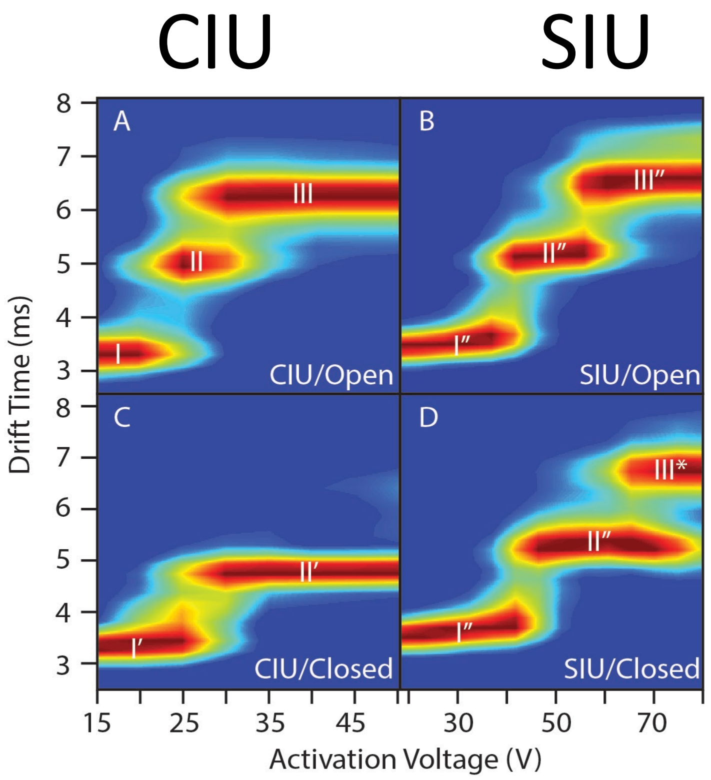
Cover and Ohi (MI, TN)
Type IV Protein Secretion

Dixit, Polasky, Ruotolo *Curr. Opin. Chem. Biol.* **2018**, 42, 93-100

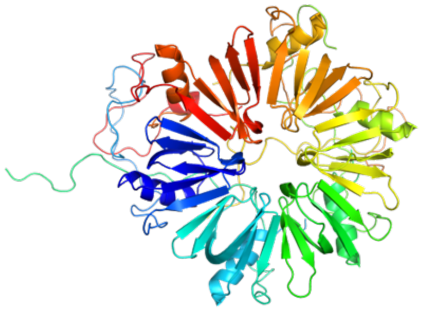
TOP3, Protein Stability: Collision induced unfolding

nMS→SB
NATIVE MS GUIDED STRUCTURAL BIOLOGY





Driving Biomedical Projects



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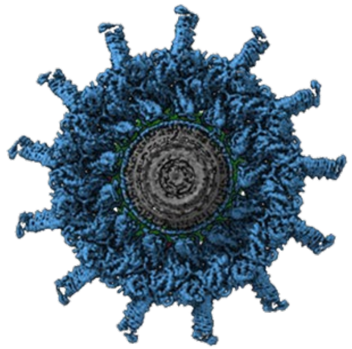
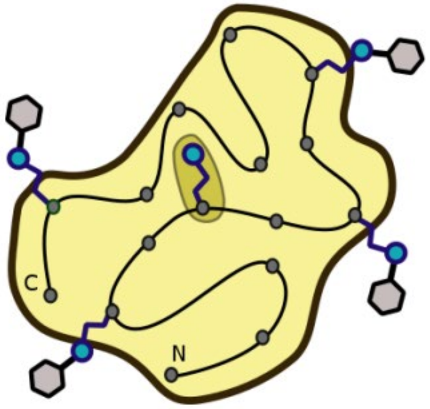


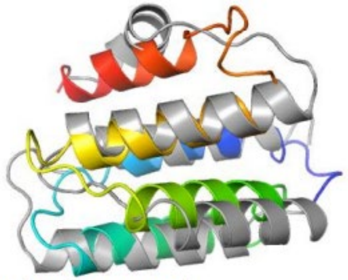
Cover and Ohi **(MI, TN)**
Type IV Protein Secretion

Why late stage? CIU option across dozens of labs worldwide
many pharma collaborations
multi-vendor adoption in progress

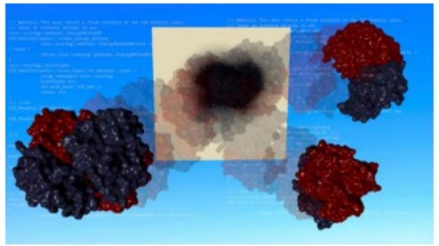
Dixit, Polasky, Ruotolo *Curr. Opin. Chem. Biol.* **2018**, 42, 93-100

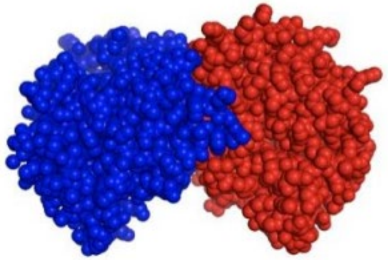
TOP2&3, Computations: Integrative Modeling for Quaternary Structure



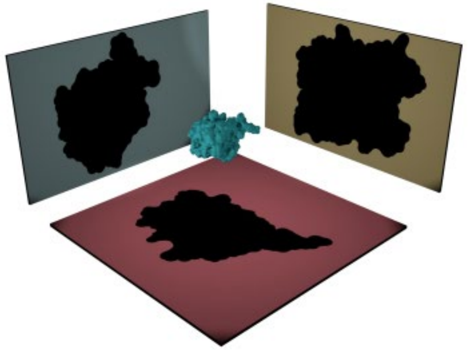


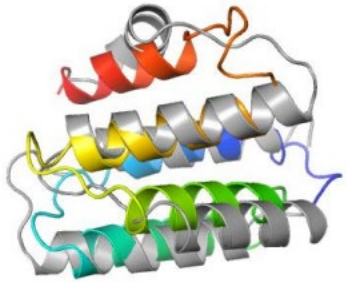
protein tertiary and
quaternary structure prediction





protein quaternary structure prediction



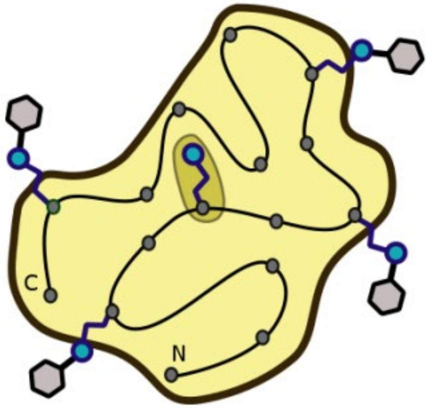


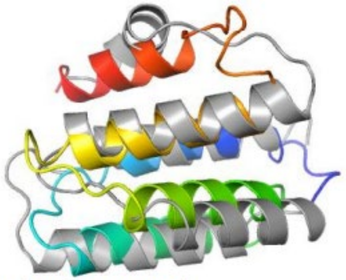
protein tertiary structure prediction

Combine MS and/or ion mobility data with complementary low resolution structural data for integrative modeling

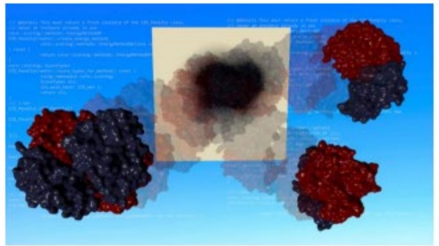
ACS Cent Sci. 2019, 5, 1330. Anal. Chem. 2021, 93, 7596. Nature Communications 2022, 13, 4377.

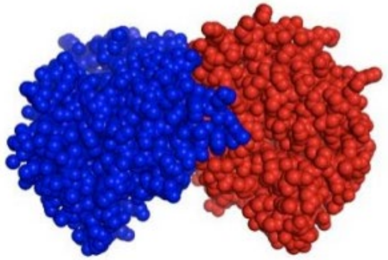
TOP2&3, Computations: Integrative Modeling for Quaternary Structure



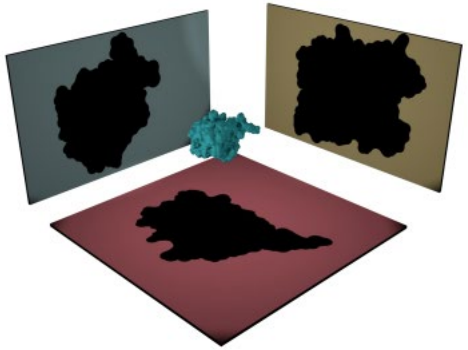


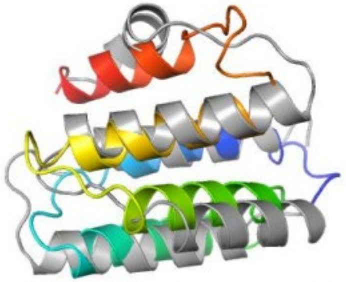
protein tertiary and
quaternary structure prediction





protein quaternary structure prediction





protein tertiary structure prediction

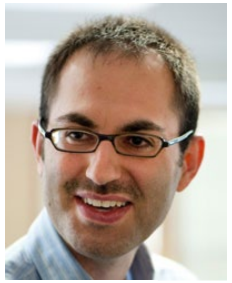
Combine MS and/or ion mobility data with complementary low resolution structural data for integrative modeling

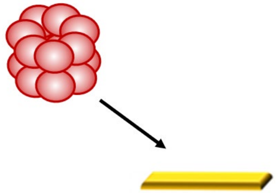
Why late stage?

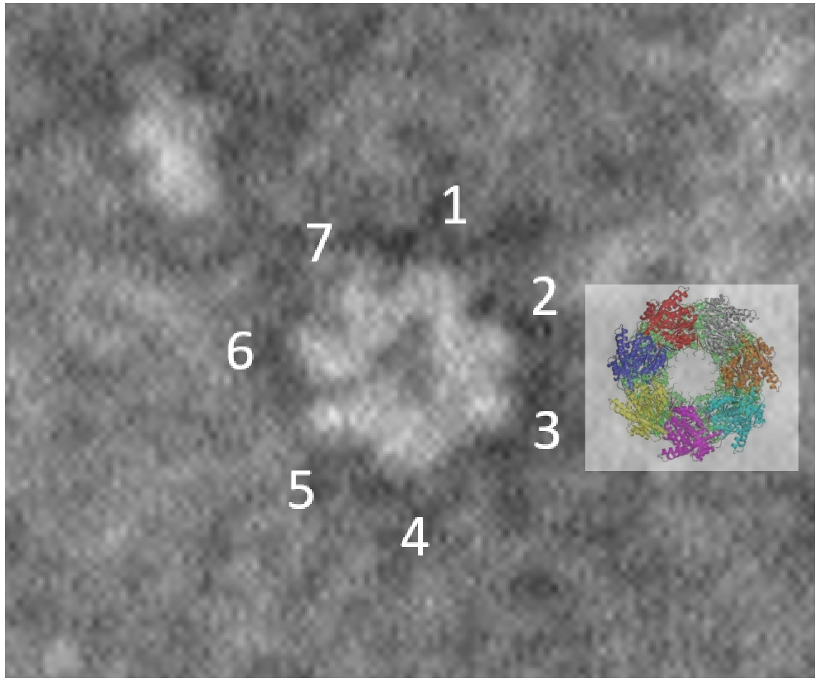
tools are beta tested and provided as developed, extended, and improved (Rosetta or OpenFold)

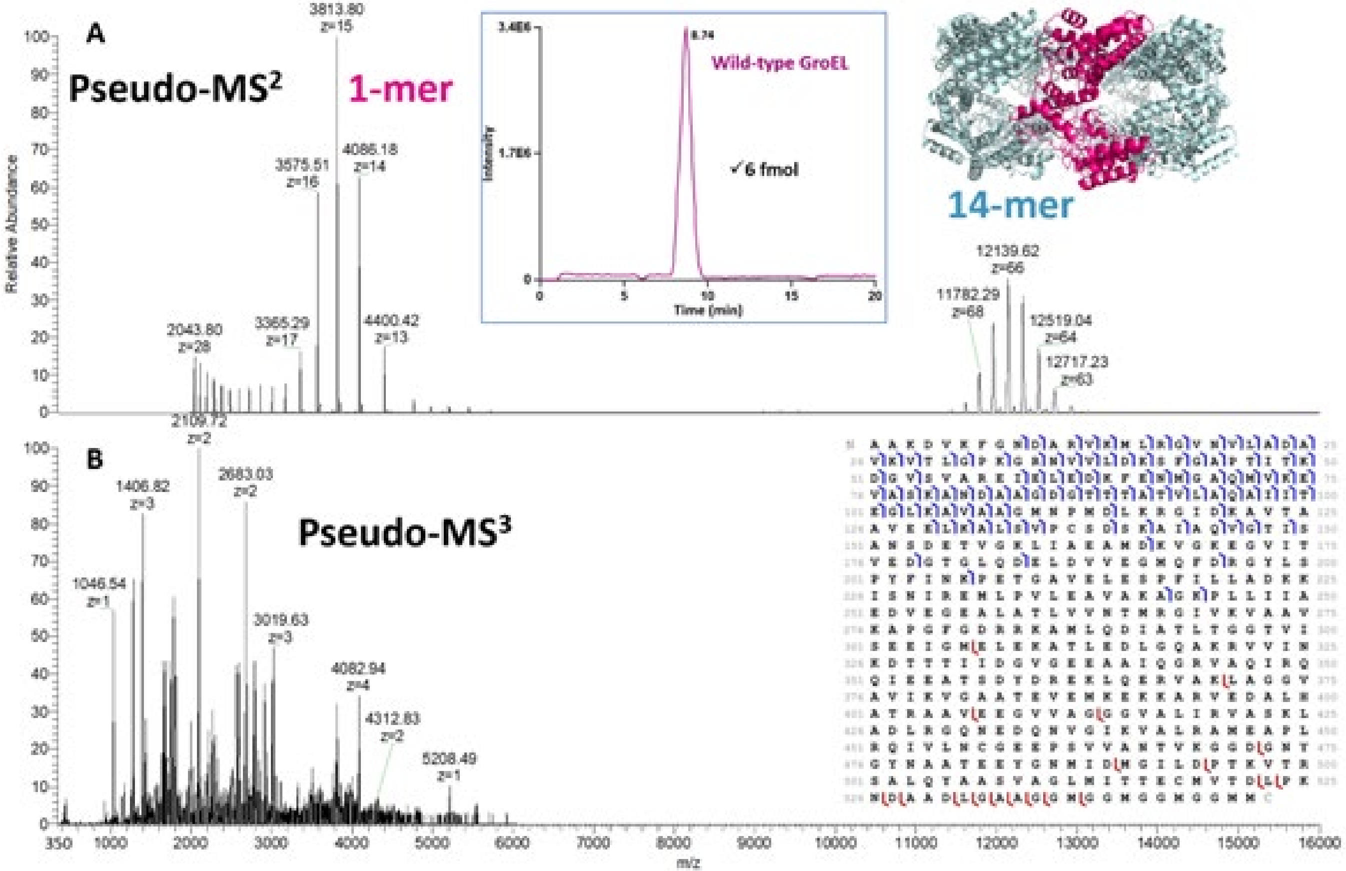
ACS Cent Sci. 2019, 5, 1330. Anal. Chem. 2021, 93, 7596. Nature Communications 2022, 13, 4377.

Technology Partnership Projects









Community Engagement (CE)

nMS→SB
NATIVE MS GUIDED STRUCTURAL BIOLOGY

Website

nativems.osu.edu

Overview
Details & News
Protocols
Contact link

Training & Support

Short Courses

Workshops

Webinars

Onsite and partner site technique training

Collaborator training: technique choice and data interpretation

Dissemination

Vendor Products

Prototypes to labs

Software

Publications & Presentations

Graduate Placements
Industrial Advisory Board

Move technology into core facilities

Question & Answer Session with PIs and NIGMS Staff

Please type your questions in the Chatbox.

Questions after the webinar? Send to

NIGMS_BTODMailbox@nigms.nih.gov

Application Submission

- PAR-23-110: Biomedical Technology Optimization and Dissemination Center (BTOD) (RM1-Clinical Trial Not Allowed)
- May/January receipt dates with next receipt date of January 26, 2024
- Send questions about application preparation to:
  NIGMS_BTODMailbox@nigms.nih.gov

For more information: NIGMS BTOD Website

- General descriptions of the BTOD Program and funded Centers
- FAQs

https://www.nigms.nih.gov/about/overview/BBCB/biomedicaltechnology/Pages/btdd.aspx