OAK RIDGE National Laboratory

Bio-Deuteration

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Large Scale Structures Section
Neutron Scattering Division

ORNL is managed by UT-Battelle LLC for the US Department of Energy

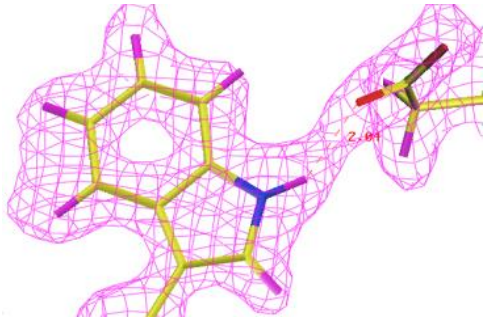
U.S. DEPARTMENT OF ENERGY

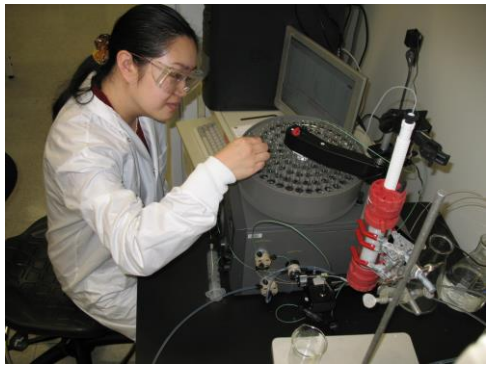
BER Structural Biology Resources
U.S. Department of Energy

The Bio-Deuteration Laboratory

Central facility and user program for H/D-labeling of macromolecules

- Dedicated to H/D-labeling of biomolecules in support of neutron structural biology.
- Develop efficient methods to produce deuterium labeled biological macromolecules
- Train research students, users, and staff in the application of these powerful techniques
- Assist in improving downstream efforts to exploit these reagents





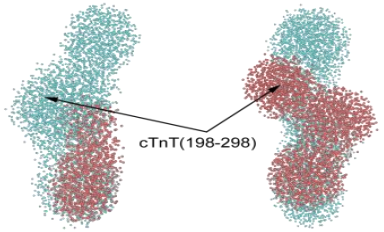
Deuterium in Structural Biology

Neutrons are excellent non-destructive structural probes that can discriminate between hydrogen and deuterium.

Characterizing Higher-order Macromolecular Complexes

Partial Deuteration

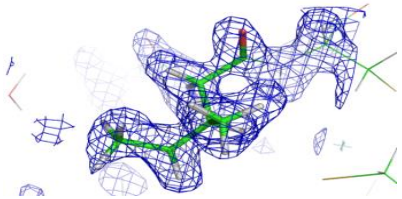
in situ structural information regarding individual components



Pinpointing Positions of Individual Hydrogen Atoms

Perdeuteration (Full Deuteration)

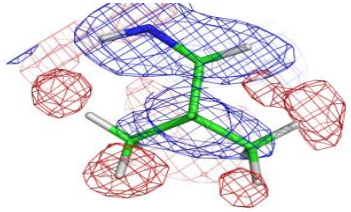
↑coherent scattering signal
↓incoherent background



Probing the Structure and Dynamics of Biological Macromolecules

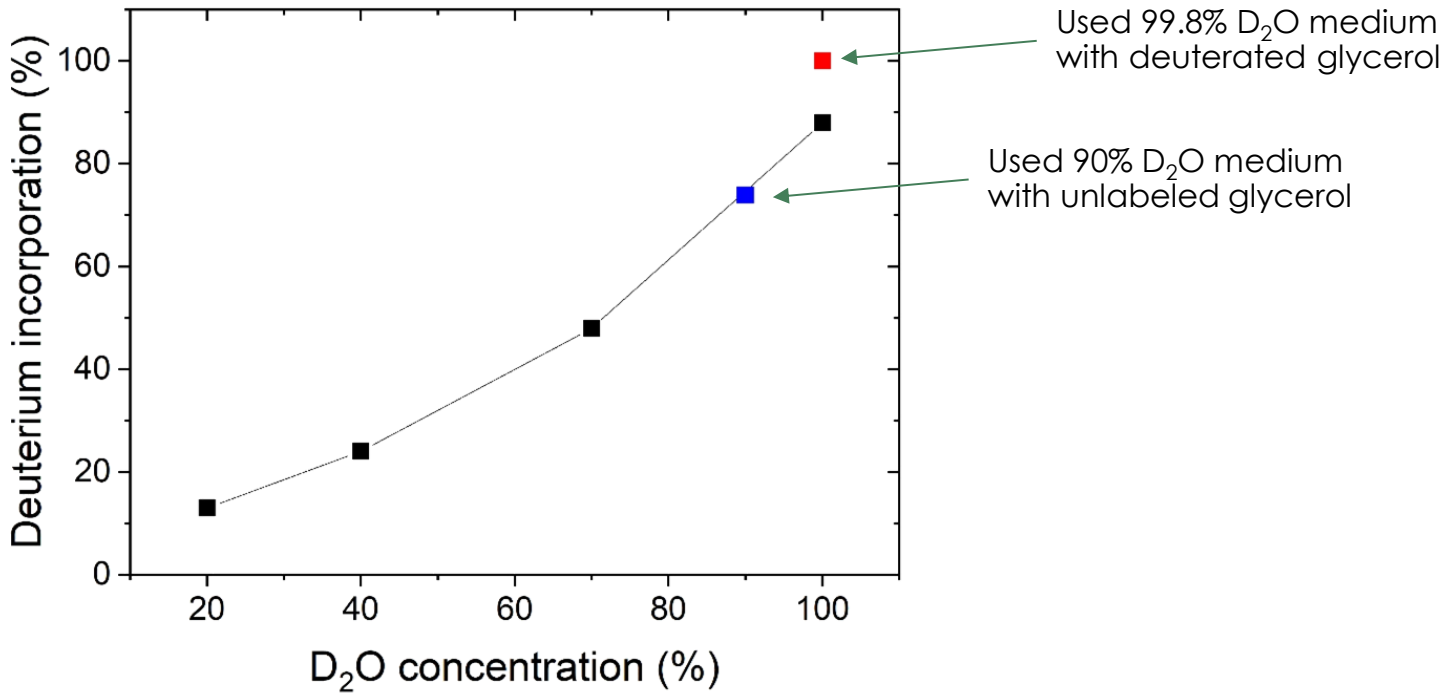
Residue-selective and Segmental Labeling

Allows the structure and dynamics of residues or domains to be highlighted against a perdeuterated background.



Bio-Deuteration

- Production of biomolecules in D_2O media
- Sometimes with a deuterated carbon source
- Deuteration of non-exchangeable positions



Weiss, K. L.; Fan, Y.; Abraham, P.; Odom, M.; Pant, S.; Zhang, Q.; O'Neill, H. Fed-Batch Production of Deuterated Protein in Escherichia Coli for Neutron Scattering Experimentation. In *Methods in Enzymology*; Elsevier, 2021; Vol. 659, pp 219–240. https://doi.org/10.1016/bs.mie.2021.08.020

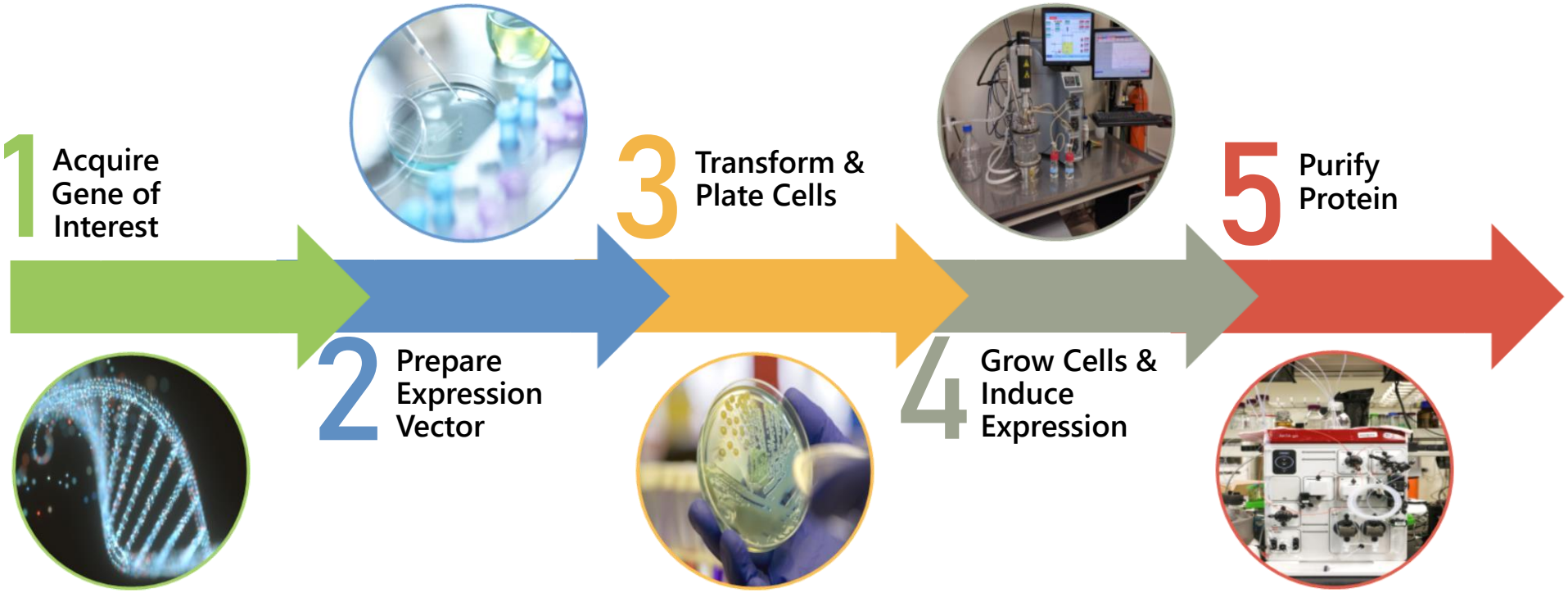
1960s: Early Bio-Deuteration Experiments

Katz & Crespi @ Argonne National Lab

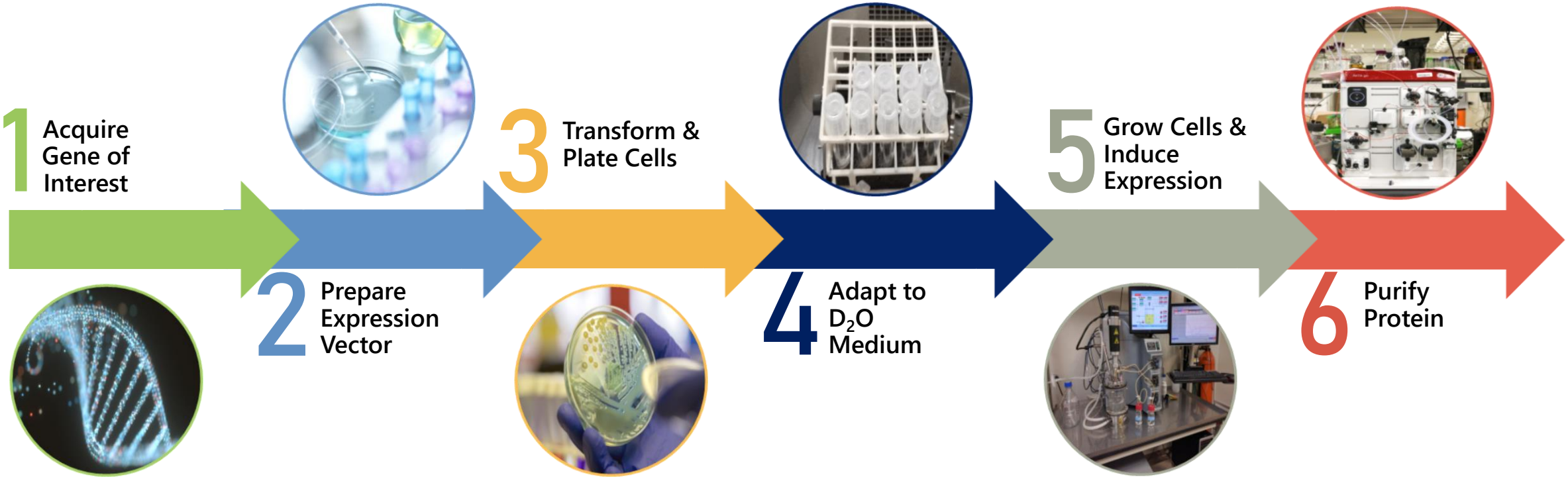
Green algae and cyanobacteria

- CO_2 = sole carbon source
- D_2O effects on the organisms
- Valuable source of fully deuterated materials
- Extracts useful for culturing other cell types in high percentages of D_2O

1970s: Recombinant DNA Technology



Deuterated Protein Expression and Purification



Where to start?

Optimize Unlabeled Production!

• Construct/Vector	Multiple options (pET)
• Choice of Strain	pLysS / BL21-AI / Rosetta
• Concentration of Inducer	0.2 mM → 1 mM IPTG
• Temperature	37˚C→30˚C→25˚C→15˚C
• Extraction and Purification	Methods / reagents
• Flask or Bioreactor	Aeration / pH
• Work with Minimal Media	Various recipes

Minimal Media

No extracts, amino acids, or precursors

M9 minimal medium (1L)

12.8 g $Na_2HPO_4 \cdot 7H_2O$
3 g KH_2PO_4
0.5 g NaCl
1 g NH_4Cl
2 mM $MgSO_4$
0.1 mM $CaCl_2$
4 g carbon source (e.g., glycerol, glucose, etc.)

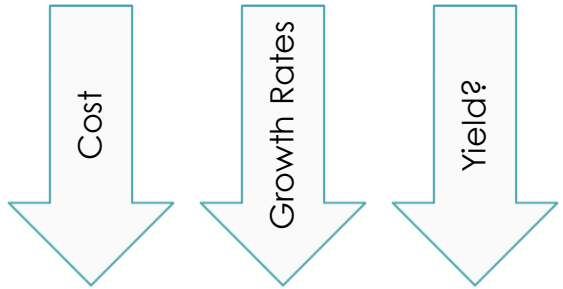
Perdeuterated M9 Medium (1L)

No H-containing salts, amino acids, or precursors

12.8 g $Na_2DPO_4 \cdot 7D_2O$
3 g KD_2PO_4
0.5 g NaCl
1 g ND_4Cl
2 mM $MgSO_4$
0.1 mM $CaCl_2$
4 g D8-Glycerol

per-
denoting the maximum proportion of one element in a compound

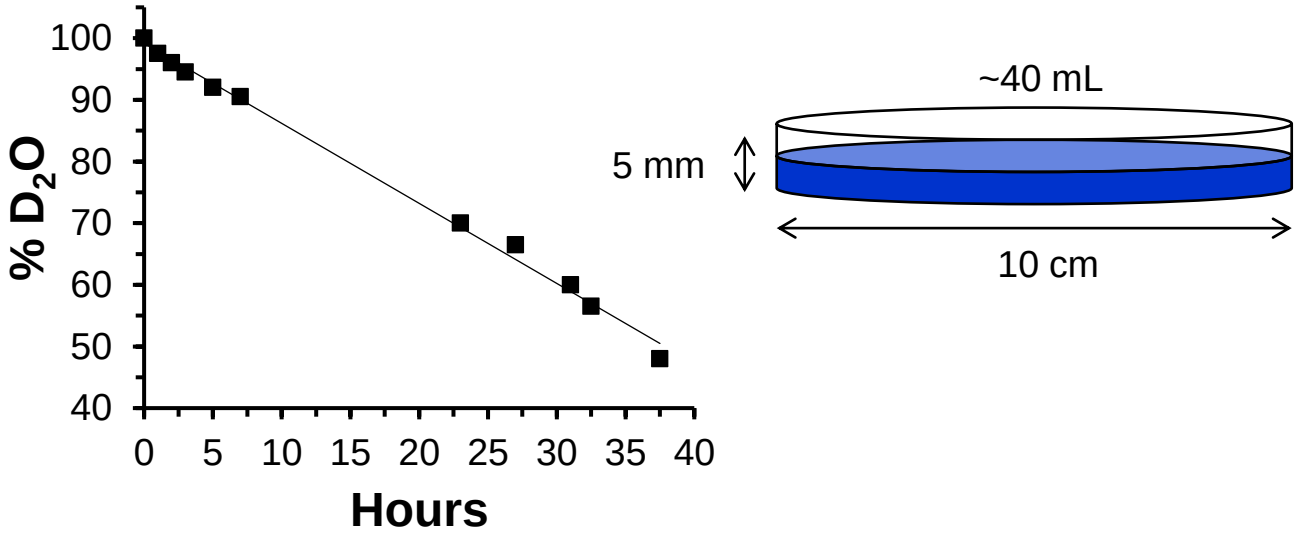
- D_7-Glucose
- D_8-Glycerol
- D_6-Succinic acid
- D_3-Sodium acetate



D-labeled amino acids (individual/mixtures) are also available.

Working with D_2O & D-Media

- Dry and sterile glassware
- Sterilize media components by filtration!
- Minimize prolonged exposure to air
- Dry the air used for fermentation

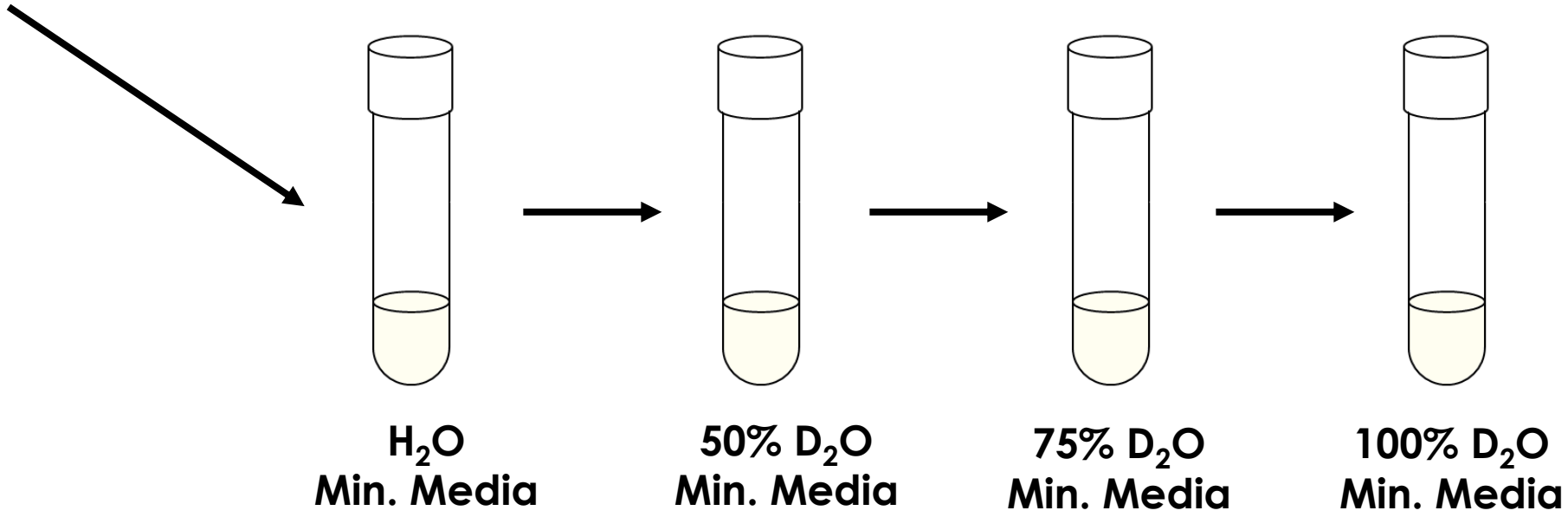


Deeney, F. A.; O'Leary, J. P. An Experimental Investigation of the Process of Isotope Exchange That Takes Place When Heavy Water Is Exposed to the Atmosphere. *European Journal of Physics* **2009**, *30* (4), 871–876. https://doi.org/10.1088/0143-0807/30/4/019.

Methods of D_2O Adaptation

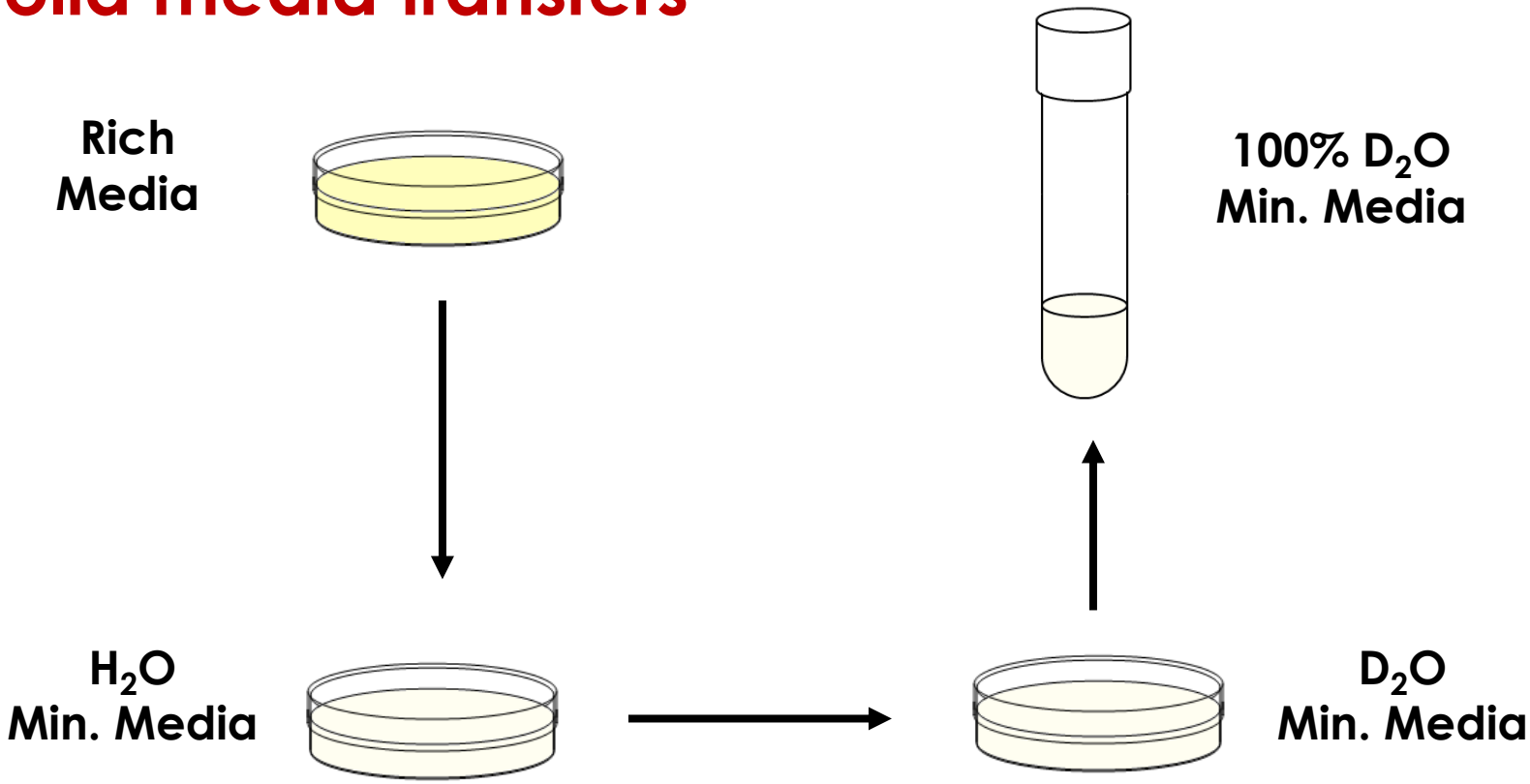
Rich Media

Liquid culture transfers



Methods of D_2O Adaptation

Solid media transfers



Artero, J.-B.; Härtlein, M.; McSweeney, S.; Timmins, P. A Comparison of Refined X-Ray Structures of Hydrogenated and Perdeuterated Rat γE-Crystallin in H_2O and D_2O. *Acta Crystallogr.* **2005**, *D61* (11), 1541–1549. https://doi.org/10.1107/S0907444905028532.

Deuterated Protein Expression and Purification

1 Acquire Gene of Interest

2 Prepare Expression Vector

3 Transform & Plate Cells

Bypass Adaptation

4 Adapt to D_2O Medium

5 Grow Cells & Induce Expression

6 Purify Protein

Bypassing Adaptation: "Rich" Deuterated Media

Solutions

- 10X BioExpress (algal)
- 1X Celtone-D complete (algal)
- Silantes OD2 (bacterial)

Powders

- Celtone-D base powder (algal)
- ISOGRO-D (algal)

Also used as minimal media additives

Questions?

Practical Tips/Suggestions

- Use fresh transformants
- Avoid using ampicillin resistance
- Centrifuge/resuspend cells before inoculation
- Use more inoculum (initial OD_{600} ~0.1-0.2)
- Adaptation: D_2O vs. D-carbon source
- Small scale testing in H- and then D- minimal media

Challenges of Deuteration

- Scale-up and Cost
- ↓ Growth Rates / Biomass
  Up to 50%
- Isotope Effects
- Media Limitations
- Host Limitations
  E. Coli, Pichia pastoris, & K. lactis

Examples

^{2}H-labeled Toho-1 β-lactamase

Expression Host

- *E.coli* [BL21(DE3)]
- Codon-optimized Gene
- Kanamycin Resistance

Adaptation

- D_2O agar to 100% D_2O

Fermentation Conditions

- 35°C in D_2O minimal media
- D8-glycerol: 5 g/L batch & 10g for 10% feed
- Induction coordinated with initiation of feed
- Harvested after 15 hr induction w/ 1 mM IPTG

Tomanicek, S. J.; Wang, K. K.; Weiss, K. L.; Blakeley, M. P.; Cooper, J.; Chen, Y.; Coates, L. The Active Site Protonation States of Perdeuterated Toho-1 β-Lactamase Determined by Neutron Diffraction Support a Role for Glu166 as the General Base in Acylation. *FEBS Lett* **2011**, *585* (2), 364–368. https://doi.org/10.1016/j.febslet.2010.12.017

Bioreactors / Control Software

- Higher Cell Densities
- Control of Variables / Data Logging
  - *pH*
  - *dissolved oxygen levels*
  - *temperature*
  - *mixing*
  - *off gas*
  - OD_{600}
  - *Other*
- Feeding Programs

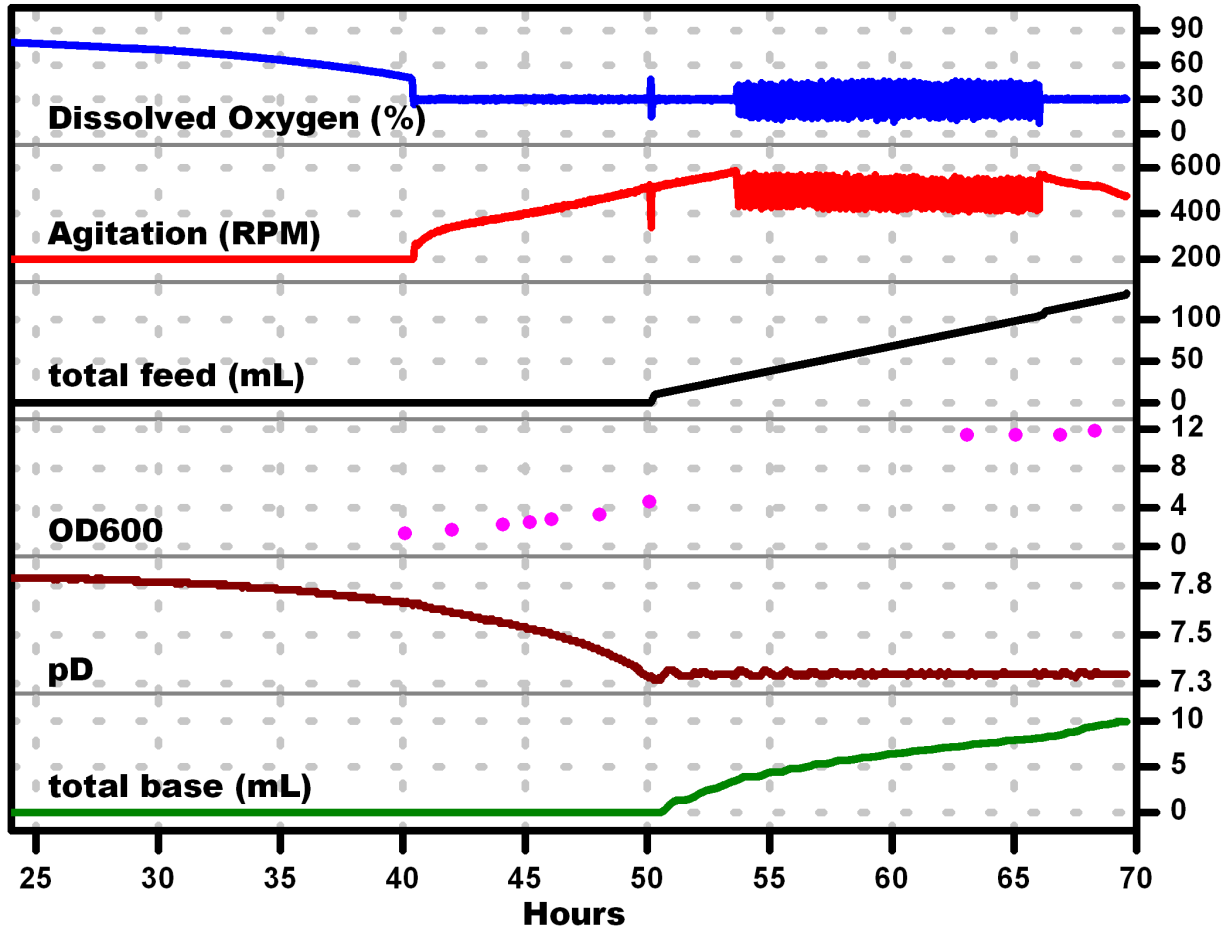
Minimal Medium for Fermentation

Salts	1,000 mL	Final Conc.
$(NH_4)_2SO_4$	7.00 g	53.0 mM
Na_2HPO_4	5.25 g	37.0 mM
KH_2PO_4	1.60 g	11.8 mM
$(NH_4)_2$-H-Citrate	0.50 g	2.2 mM
Glycerol	5.00 g	54.3 mM
20% $MgSO_4 \cdot 7H_2O$	1 mL	0.02%
1000X Trace Metals	1 mL	1X

Törnkvist, M.; Larsson, G.; Enfors, S.-O. Protein Release and Foaming in Escherichia Coli Cultures Grown in Minimal Medium. *Bioprocess Engineering* **1996**, *15* (5), 231–237. https://doi.org/10.1007/BF02391583


eppendorf
BIOFLO & CELLIGEN

Fed-Batch Fermentation



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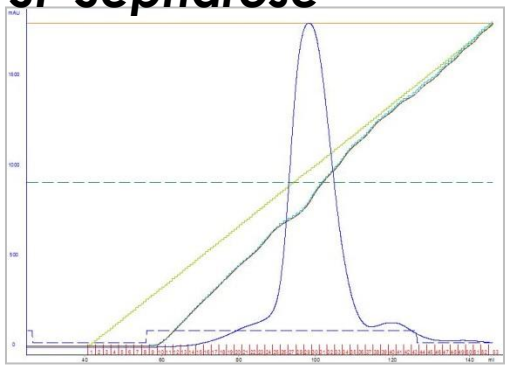
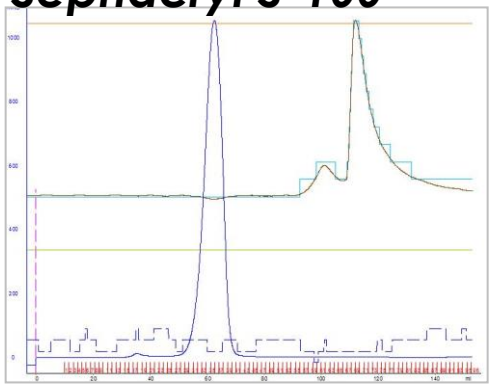
Protein Purification (This is done in H_2O!)

- Lysis
- Centrifugation
- Cation Exchange
- Gel Filtration

SP Sepharose



Sephacryl S-100



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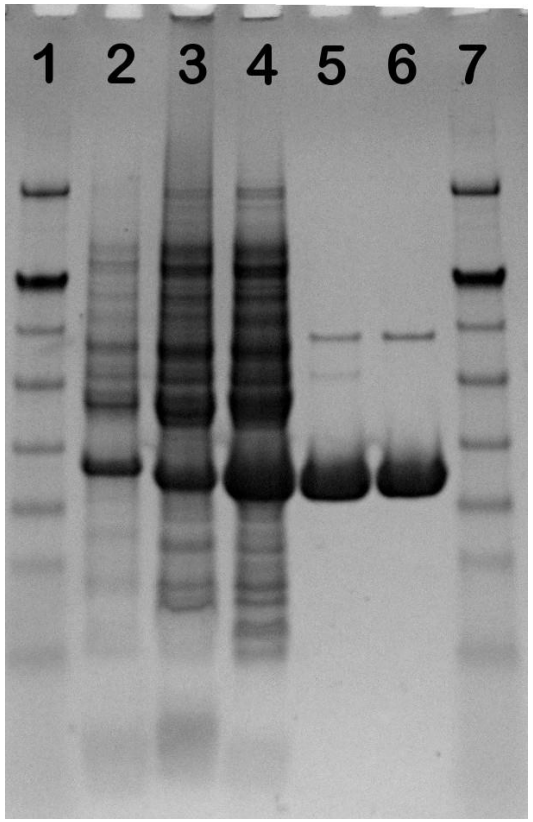
Results



1. MW marker
2. Induced 15 h with 1 mM IPTG
3. Lysis pellet
4. Lysate
5. SP Sepharose pooled fractions
6. S-100 pooled fractions
7. MW marker

Estimated yield

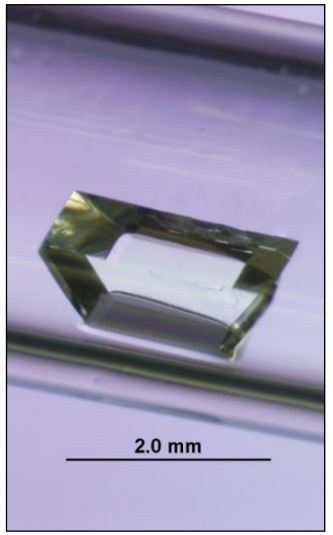
~16 g wet cell paste

~600 mg of purified protein/L

Tomanicek, S. J.; Wang, K. K.; Weiss, K. L.; Blakeley, M. P.; Cooper, J.; Chen, Y.; Coates, L. The Active Site Protonation States of Perdeuterated Toho-1 β-Lactamase Determined by Neutron Diffraction Support a Role for Glu166 as the General Base in Acylation. *FEBS Lett* **2011**, *585* (2), 364–368. https://doi.org/10.1016/j.febslet.2010.12.017

Sample Preparation

- Concentrate purified fractions
- Exchange the protein in D_2O buffer
- Crystallization (using D_2O)



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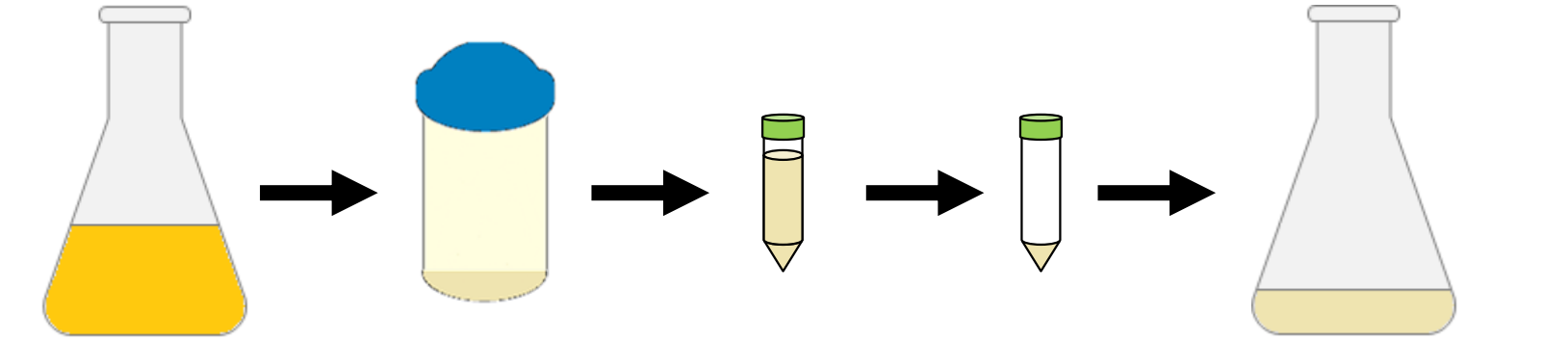
An Alternative Route to Deuterated Protein



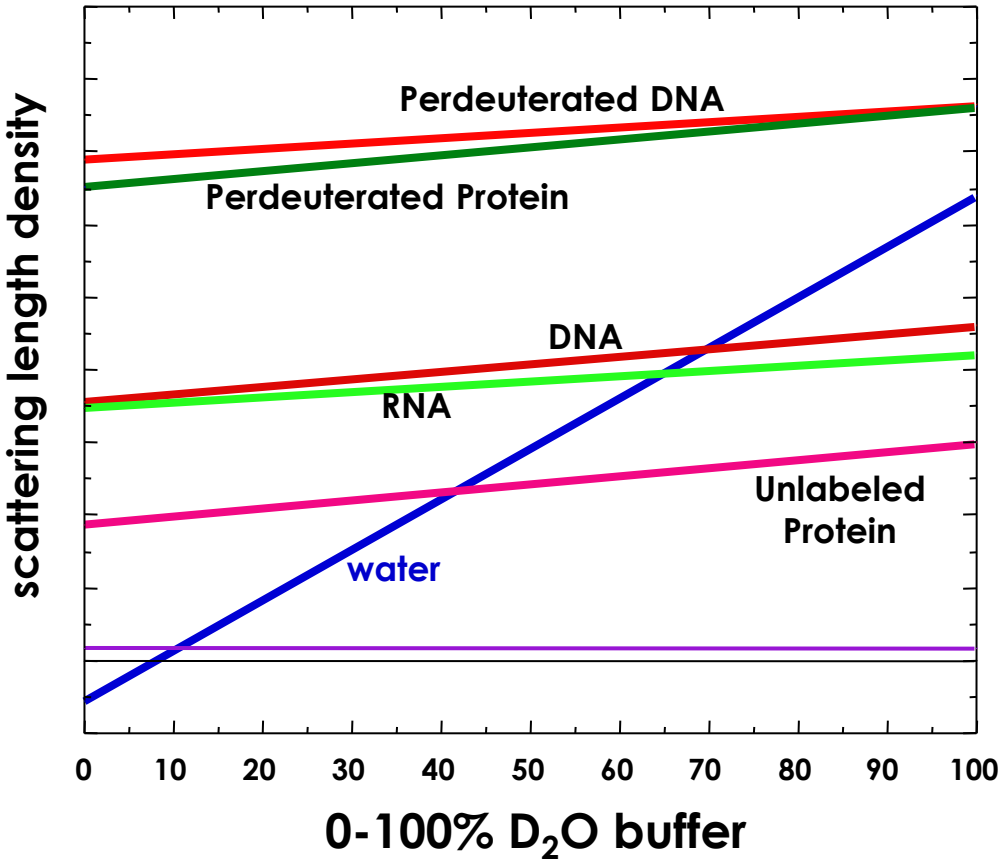
- No D_2O Adaptation
- 4X Induction Volume (conserves D_2O)
- Has been utilized in 75, 80, and 85% D_2O medium
- 85% D_2O medium => protein match point of ~100% D_2O

Marley, J.; Lu, M.; Bracken, C. A Method for Efficient Isotopic Labeling of Recombinant Proteins. *Journal of Biomolecular NMR* **2001**, *20* (1), 71–75. https://doi.org/10.1023/A:1011254402785

Bio-Deuteration & SANS

Contrast Variation

Used to separate the scattering contribution from individual components of a complex



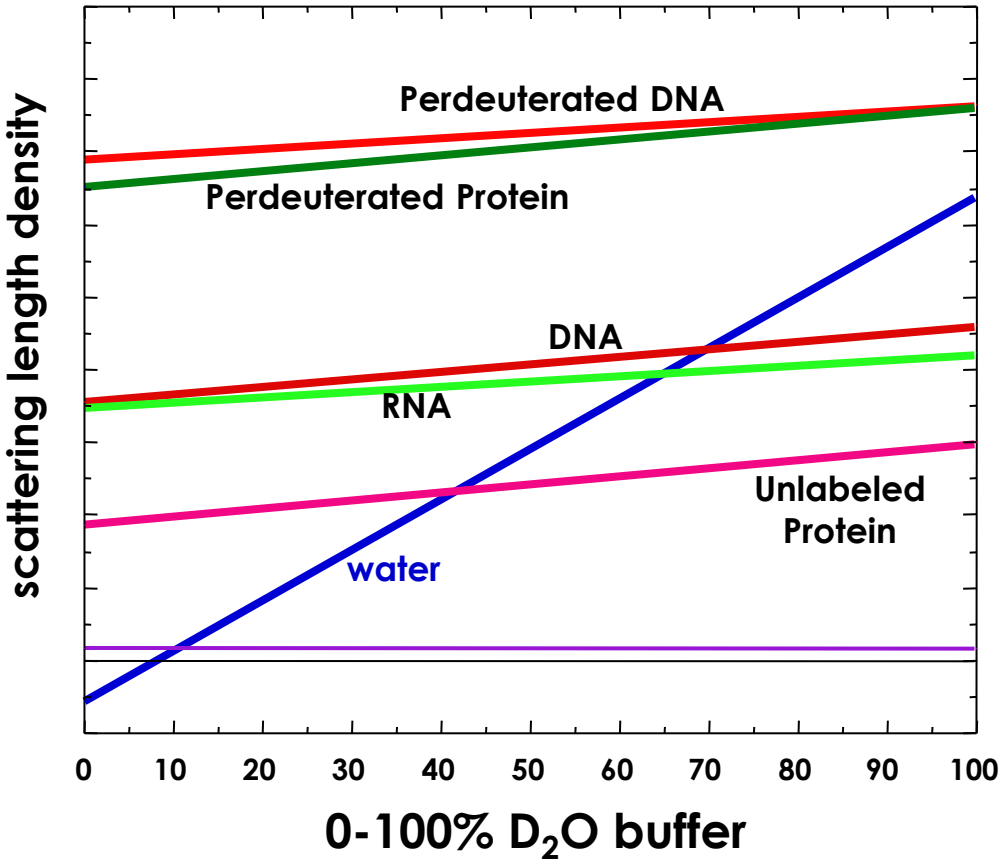
Examples

- Protein-nucleic acid
- Protein-lipid
- Protein-protein

%D_2O of buffer is varied to obtain information about individual components

Contrast Variation

Used to separate the scattering contribution from individual components of a complex



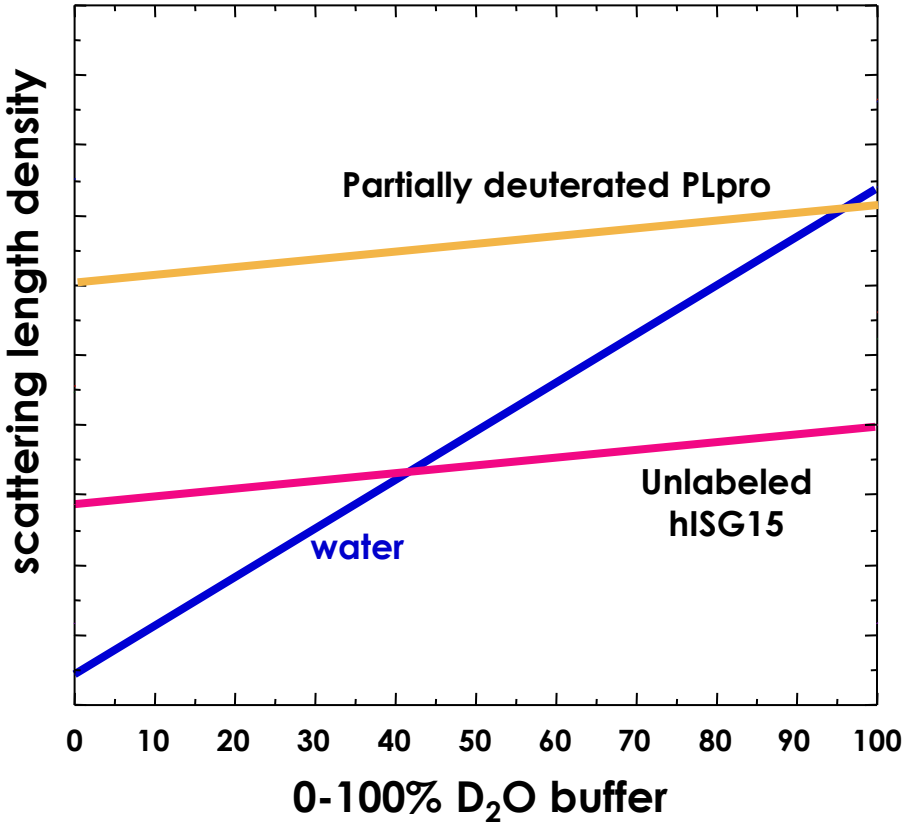
Examples

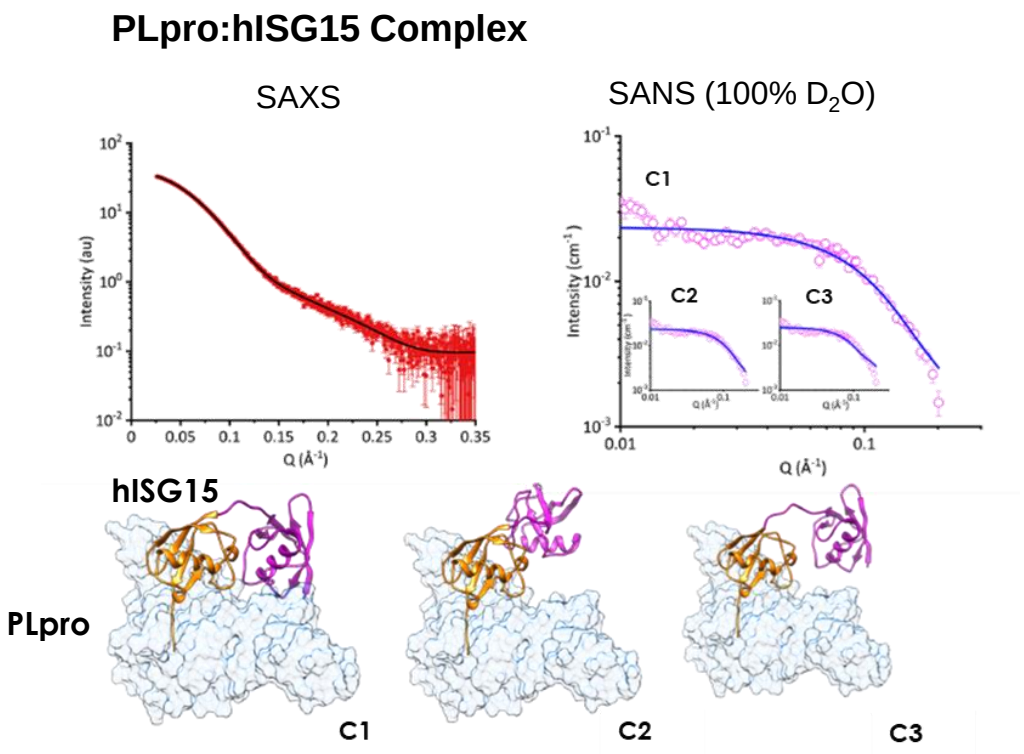
- Protein-nucleic acid
- Protein-lipid
- Protein-protein

Selective deuteration of individual components provides contrast within a class of biomolecules.

ISG15 & Papain-like Protease

Deuteration of SARS-CoV-2 papain-like protease enabled an investigation of the ISG15 conformational landscape

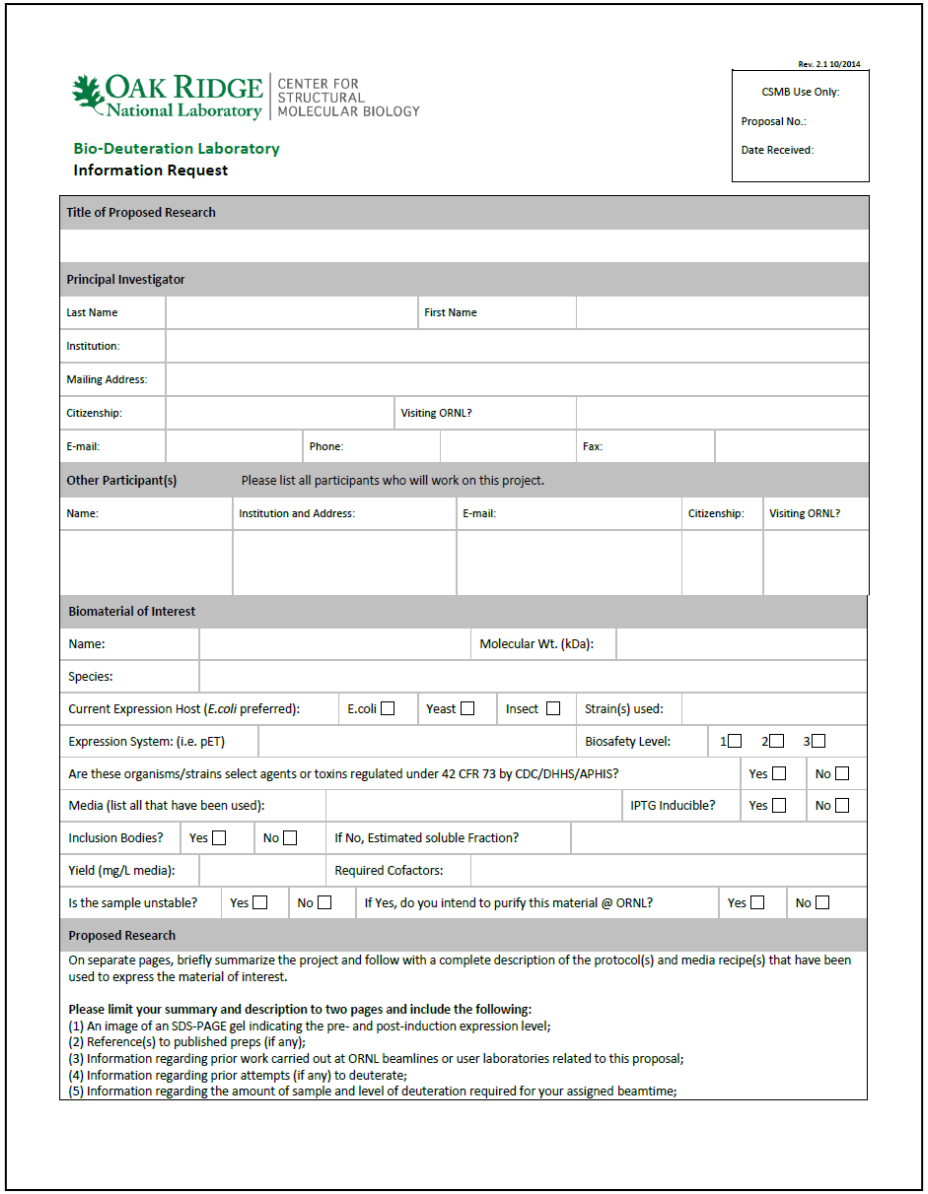




Leite, W. C.; Weiss, K. L.; Phillips, G.; Zhang, Q.; Qian, S.; Tsutakawa, S. E.; Coates, L.; O'Neill, H. Conformational Dynamics in the Interaction of SARS-CoV-2 Papain-like Protease with Human Interferon-Stimulated Gene 15 Protein. *J. Phys. Chem. Lett.* **2021**, *12* (23), 5608–5615. https://doi.org/10.1021/acs.jpclett.1c00831

Accessing the BDL

Access Model



OAK RIDGE National Laboratory | CENTER FOR STRUCTURAL MOLECULAR BIOLOGY

Rev. 2.1 10/2014

CSMB Use Only:
Proposal No.:
Date Received:

Bio-Deuteration Laboratory
Information Request

Title of Proposed Research					
Principal Investigator					
Last Name		First Name			
Institution:					
Mailing Address:					
Citizenship:		Visiting ORNL?			
E-mail:		Phone:		Fax:	
Other Participant(s)	Please list all participants who will work on this project.				
Name:	Institution and Address:	E-mail:	Citizenship:	Visiting ORNL?	
Biomaterial of Interest					
Name:		Molecular Wt. (kDa):			
Species:					
Current Expression Host (*E.coli* preferred):	E.coli ☐	Yeast ☐	Insect ☐	Strain(s) used:	
Expression System: (i.e. pET)		Biosafety Level:	1☐ 2☐ 3☐		
Are these organisms/strains select agents or toxins regulated under 42 CFR 73 by CDC/DHHS/APHIS?			Yes ☐	No ☐	
Media (list all that have been used):		IPTG Inducible?	Yes ☐	No ☐	
Inclusion Bodies?	Yes ☐	No ☐	If No, Estimated soluble Fraction?		
Yield (mg/L media):		Required Cofactors:			
Is the sample unstable?	Yes ☐	No ☐	If Yes, do you intend to purify this material @ ORNL?	Yes ☐	No ☐

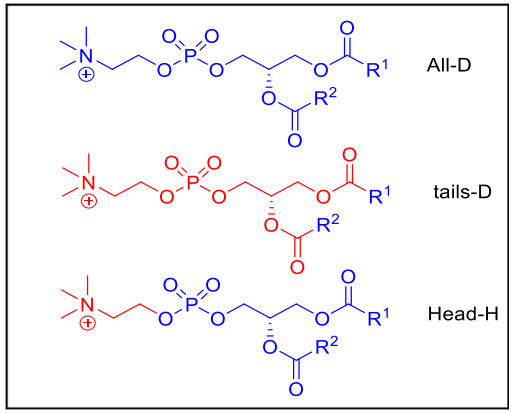
Proposed Research

On separate pages, briefly summarize the project and follow with a complete description of the protocol(s) and media recipe(s) that have been used to express the material of interest.

Please limit your summary and description to two pages and include the following:
(1) An image of an SDS-PAGE gel indicating the pre- and post-induction expression level;
(2) Reference(s) to published preps (if any);
(3) Information regarding prior work carried out at ORNL beamlines or user laboratories related to this proposal;
(4) Information regarding prior attempts (if any) to deuterate;
(5) Information regarding the amount of sample and level of deuteration required for your assigned beamtime;

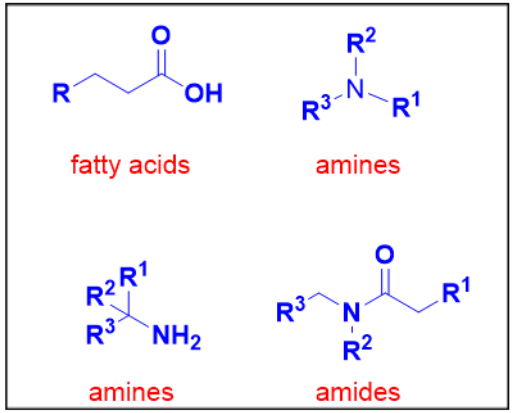
Chemical Deuteration

We also offer a variety of chemical deuteration capabilities to support the neutron scattering user program



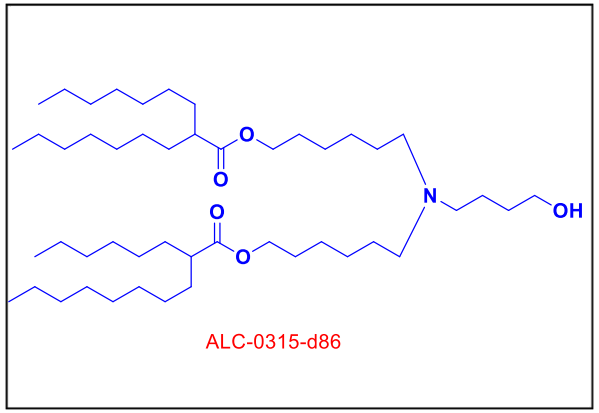
H/D-labeled PC

Selective labeling
Perdeuterated choline
Tunable contrast match point



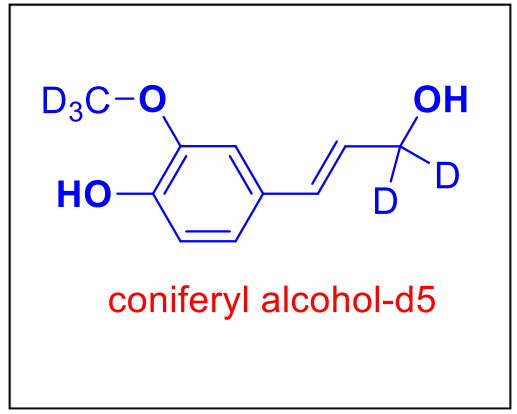
Building blocks

Deuterated fatty acids
Deuterated alkyl amines
Deuterated amides



Ionizable lipids

Controlled deuteration levels
Unique source, not commercially available



Monolignols

Selective deuteration
Lignin monomers

References

Leiting, B.; Marsilio, F.; O'Connell, J. F. Predictable Deuteration of Recombinant Proteins Expressed in Escherichia Coli. *Anal. Biochem.* **1998**, *265* (2), 351–355. https://doi.org/10.1006/abio.1998.2904.

Meilleur, F., Weiss, K. L. & Myles, D. A. A. Deuterium labeling for neutron structure-function-dynamics analysis, in *Methods in Molecular Biology* (eds. Lee, J. W. & Foote, R. S.) **544,** 281–292 (2009). https://www.ncbi.nlm.nih.gov/pubmed/19488706

Isotope Labeling of Biomolecules - Labeling Methods, in *Methods in Enzymology* (ed. Kelman, Z.) **565,** 2–640 (2015). https://www.ncbi.nlm.nih.gov/pubmed/26577745

Oliver, R. C.; Naing, S.-H.; Weiss, K. L.; Pingali, S. V.; Lieberman, R. L.; Urban, V. S. Contrast-Matching Detergent in Small-Angle Neutron Scattering Experiments for Membrane Protein Structural Analysis and Ab Initio Modeling. *Journal of Visualized Experiments* **2018**, No. 140. https://doi.org/10.3791/57901.

Weiss, K. L.; Fan, Y.; Abraham, P.; Odom, M.; Pant, S.; Zhang, Q.; O'Neill, H. Fed-Batch Production of Deuterated Protein in Escherichia Coli for Neutron Scattering Experimentation. In *Methods in Enzymology*; Elsevier, 2021; Vol. 659, pp 219–240. https://doi.org/10.1016/bs.mie.2021.08.020.

JoVE (Video Only)
https://www.ornl.gov/facility/csmb/tutorials/overview

Facility Acknowledgment Statement

- A portion of neutron scattering research presented as examples in this introduction used resources at the High Flux Isotope Reactor or Spallation Neutron Source, DOE Office of Science User Facilities, operated by the Oak Ridge National Laboratory.
- The Bio-SANS of the Center for Structural Molecular Biology at the High Flux Isotope Reactor is supported by the Office of Biological and Environmental Research of the U.S. DOE.