LINX Membrane Protein Workshop

25th – 26th May, 2021

EUROPEAN SPALLATION SOURCE

Towards Neutron Crystallography: Insights into production of deuterium-labelled OmpF

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Proton

Electron

Neutron

LUND UNIVERSITY

UNIVERSITÉ Grenoble Alpes

MARIE CURIE ACTIONS

Importance of Neutron crystallography

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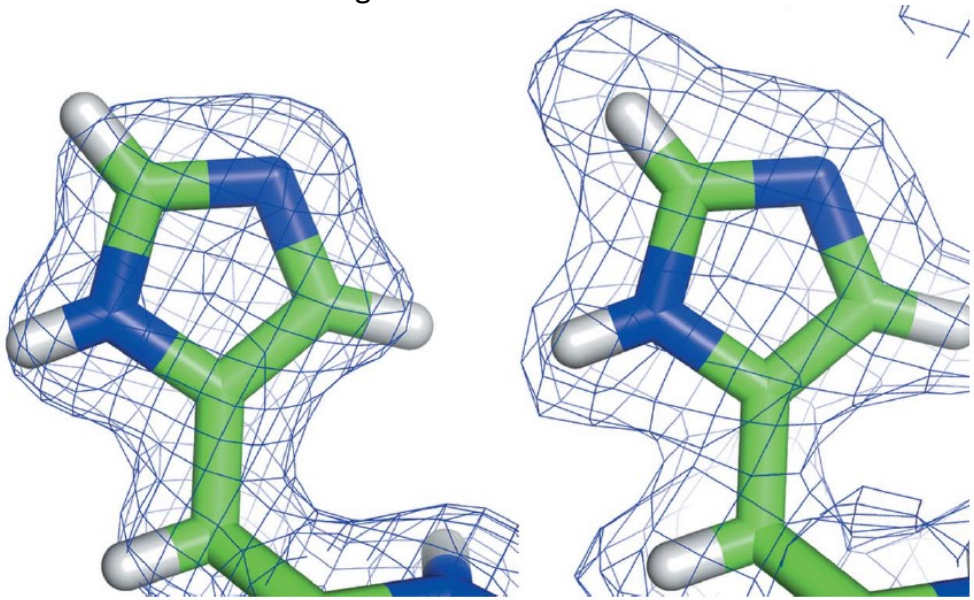
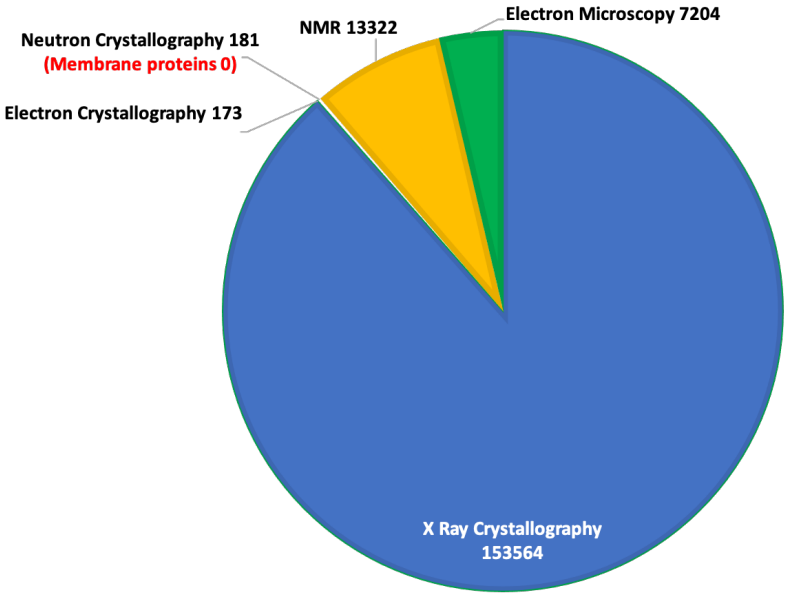
- Neutrons interact with nuclei and independent of atomic number
- Hydrogens maybe visible even when the crystals diffracting X-rays to (sub-)atomic resolution
- Understand the protonated state of chemical groups, orientation of methyl and hydroxyl groups
- Data collection can be performed at room temperature
- No radiation damage



X-ray (left) and neutron (right) 2mFo-DFc maps (contoured at = 1.0) of His187 in the TIM structure. Kelpsas et al, 2018



Reported protein structures in PDB obtained by using different experimental methods

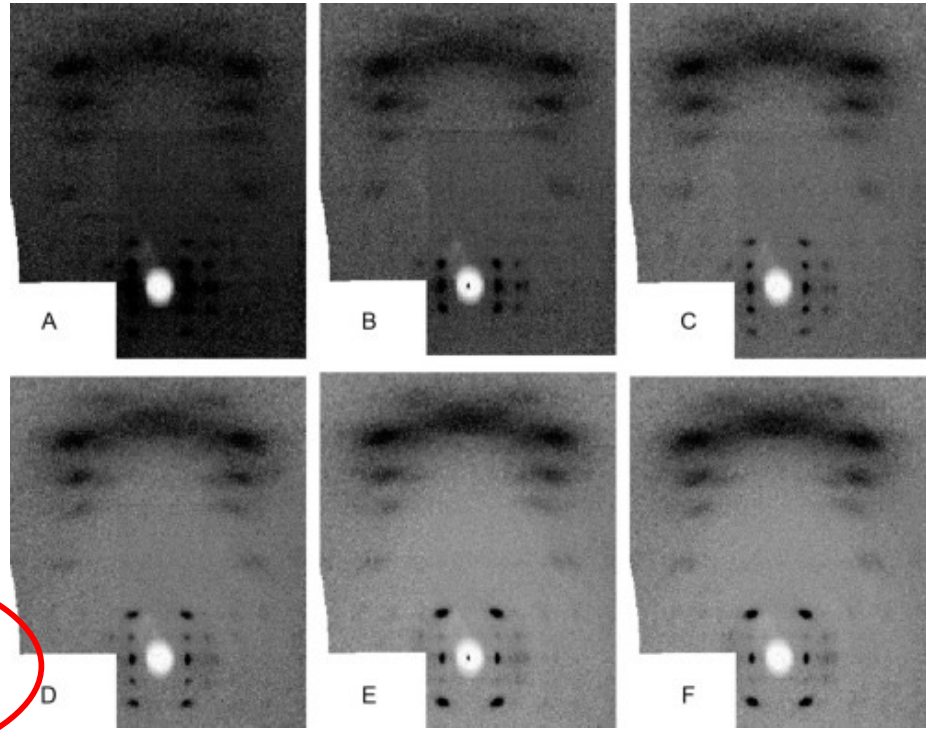
Bottlenecks:
Weak flux of neutron beam
Large crystals
Negative coherent scattering of Hydrogen

Purpose of D-labelling

- Incoherent scattering from hydrogen reduces the signal-to-noise ratio of the data
- Scattering length of deuterium is similar in sign and magnitude to those of C, N and O (Table 1).
- Deuterium should be as visible as carbon at 2.0 A ° resolution.
- Perdeuterated crystals allow suitable neutron diffraction data at lower crystal volume (<1 mm^3).

Atom	Atomic Number	Incoherent Cross-section(barn)	Neutron Scattering Length (fm)
Hydrogen	1	79.6	-3.74
Deuterium	1	2	6.67
Carbon	12	0	6.65
Nitrogen	14	0.3	9.3
Oxygen	16	0	5.8

Table 1. Neutron scattering length and cross sections of elements. (Adapted from Sears, 1992)



Improvement in the visibility of the coherent diffraction peaks of A-DNA as the hydration of the sample is changed from H_2O to D_2O (Forsyth et al, 2016).

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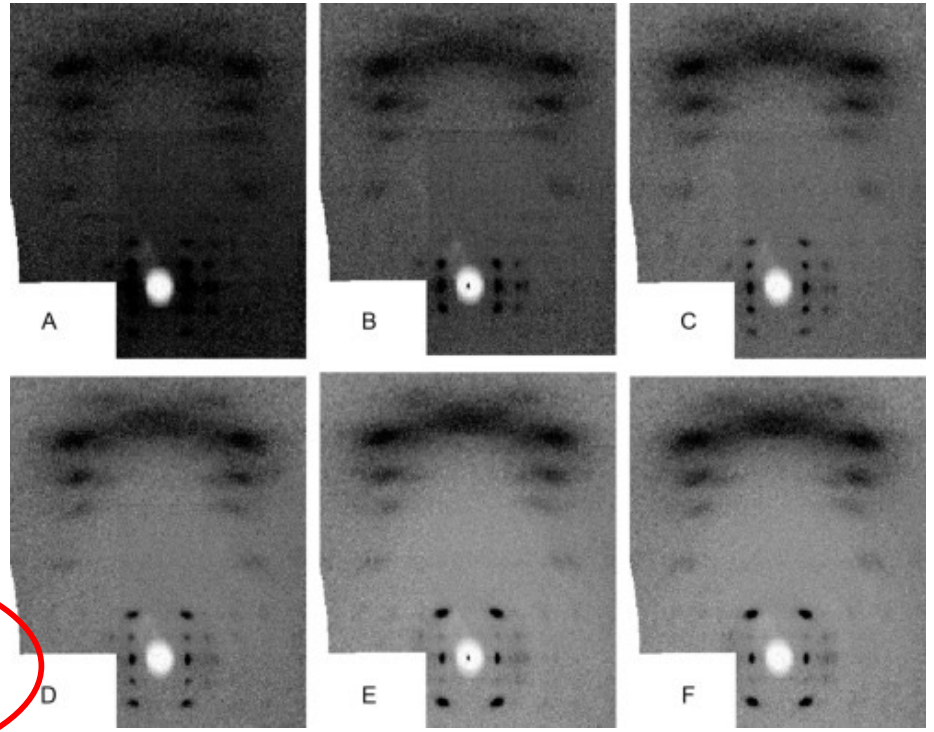

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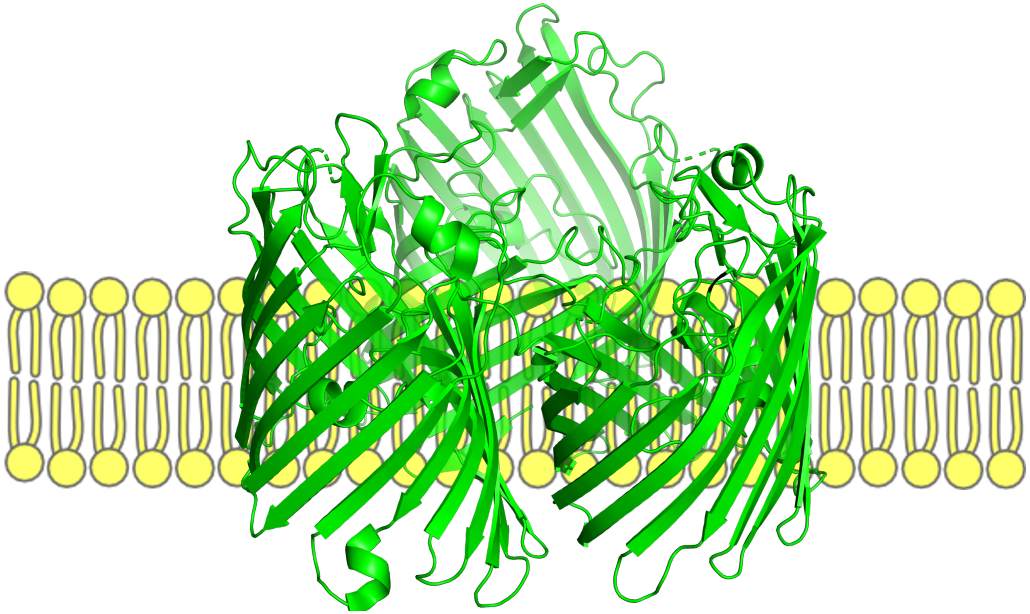
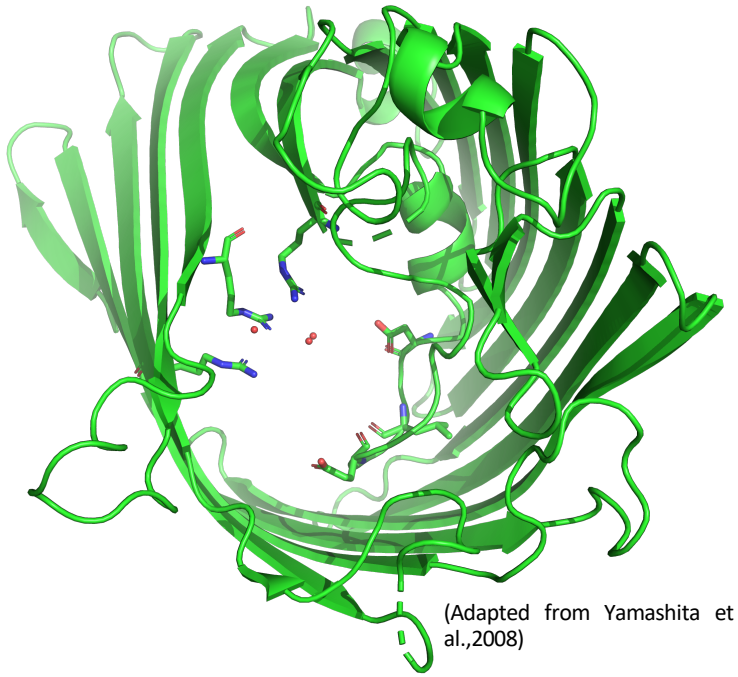
Labelling can be done partially by H/D exchange or fully by producing deuterated protein

OmpF: Model membrane protein system





(Adapted from Yamashita et al.,2008)

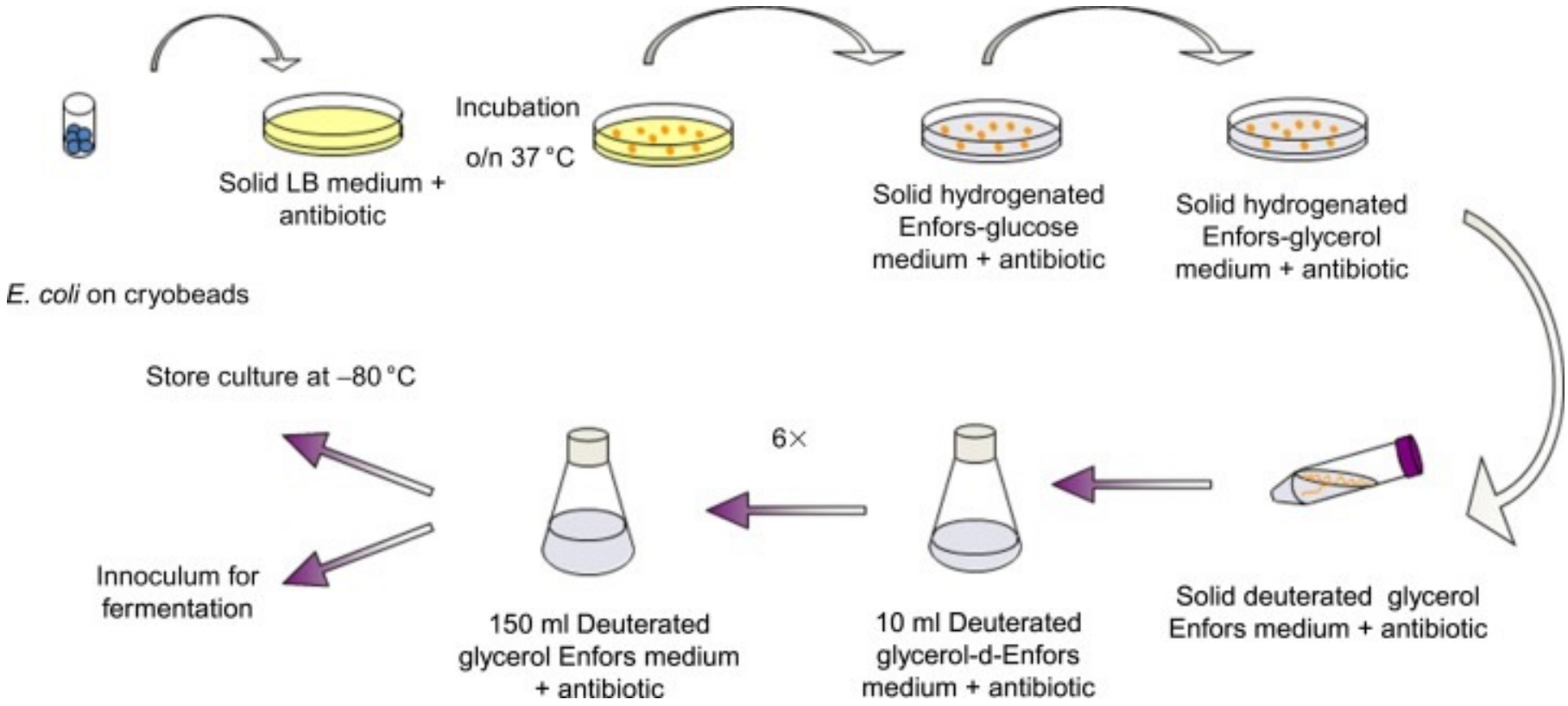
Characteristics

- It constitutes around 7% of the total protein mass of the bacteria
- X-Ray structure at 1.8 A° resolution
- Well studied and highly stable membrane protein

Research Interest

- Hydrogen bonding between each monomer in the trimeric structure
- Understand the flexibility of water channel that involves a network of acidic and basic residues
- Potential role in drug resistance of bacteria

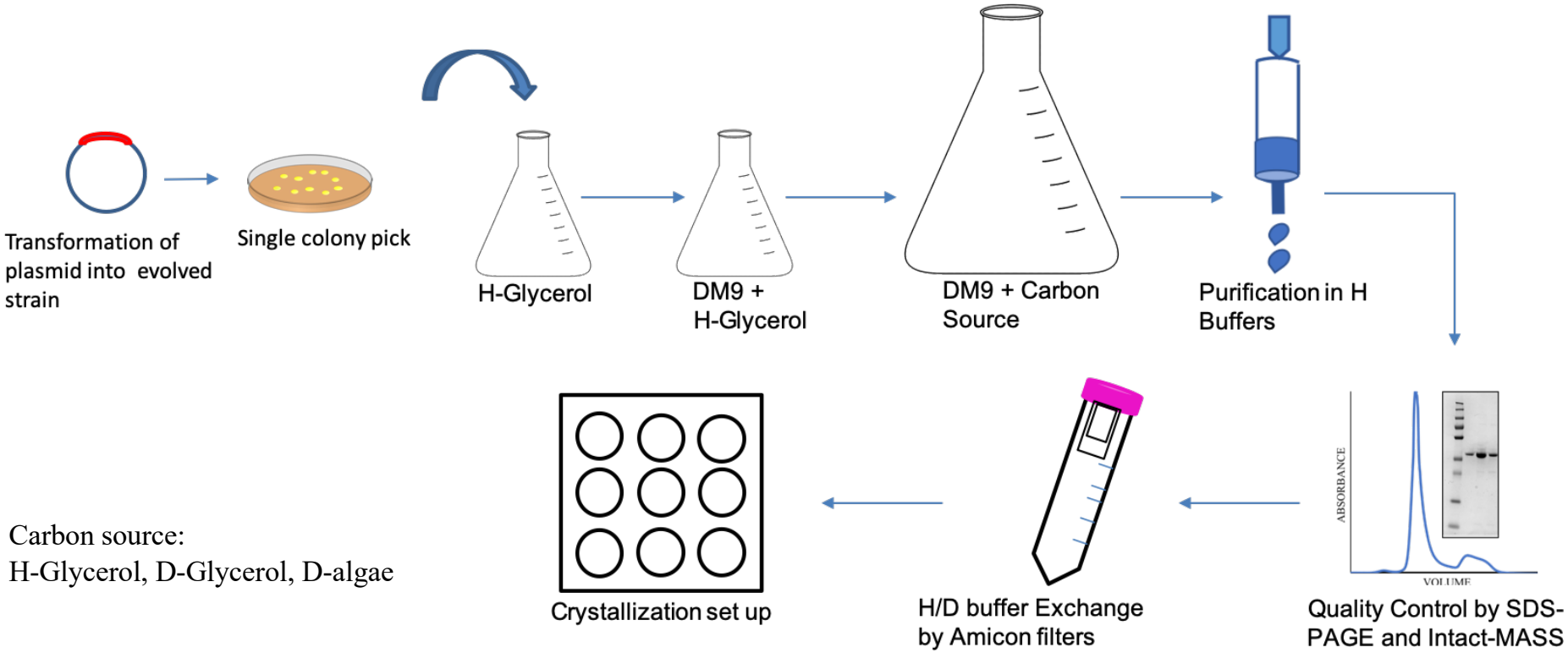
Current adaptation Strategy to produce deuterated proteins



Adapted from Haertlein et al, 2015

E.coli as the preferred host

Production of Deuterated proteins in *E. coli* using adapted strain



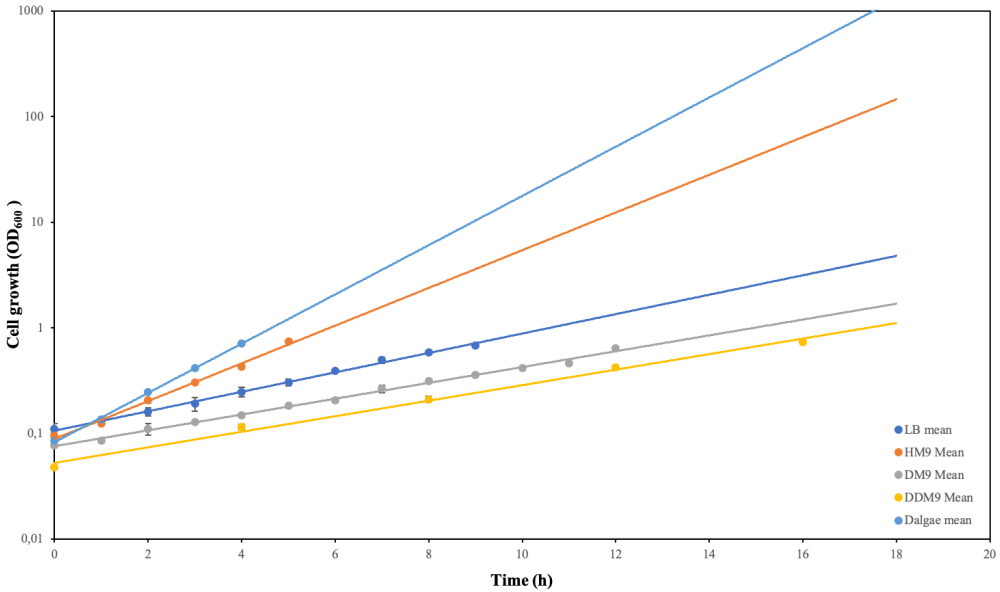
Carbon source:
H-Glycerol, D-Glycerol, D-algae

Major requirement

- Kanamycin resistance plasmid
- T7 RNA Polymerase

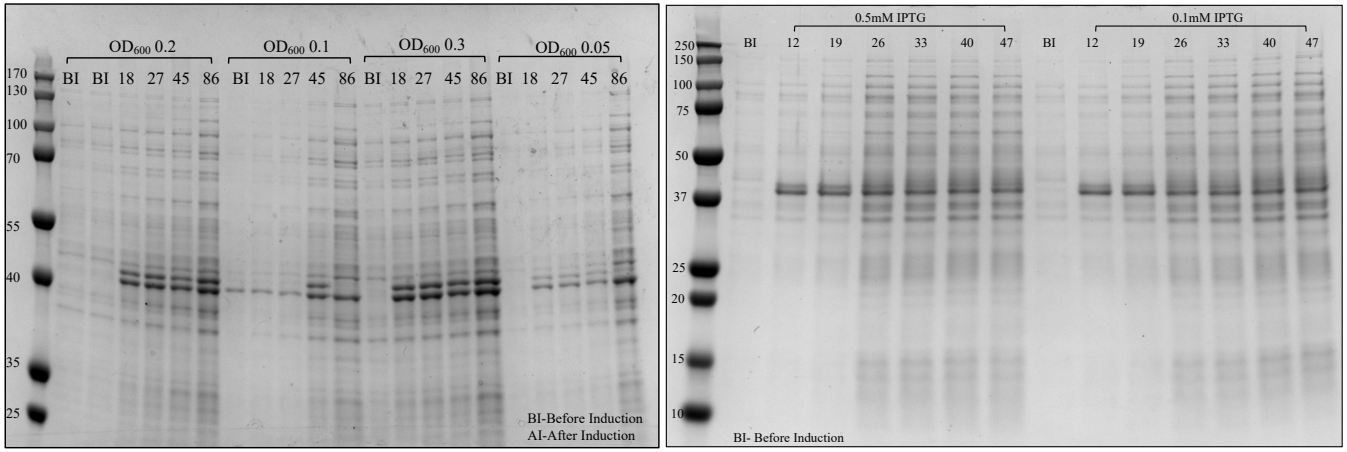
Kelpšas, et al, *Sci Rep* **9,** 17694 (2019)

Expression trials are necessary



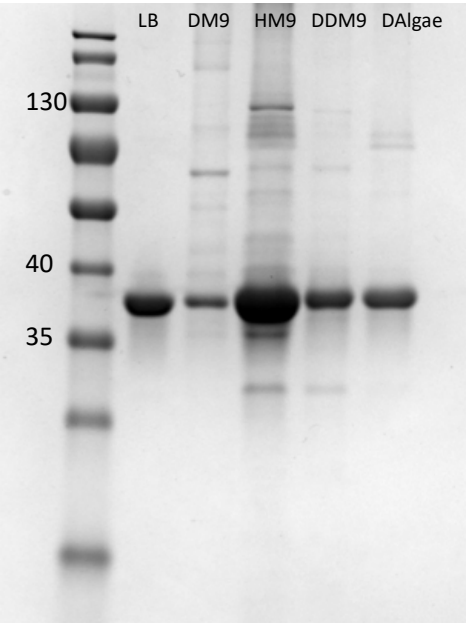
Growth Curve *E.coli* DA1(DE3) transformed with *ompF* in pET-24(+) grown in minimal media

- Bacterial growth in deuterated media is significantly reduced
- Optimal expression time is important
- Long incubation time had no effect on protein yield
- Starting culture optical density had a significant role in protein yield



ompF expression level test in deuterated minimal media

Does labelling has significant effect on membrane protein stability?



SDS-PAGE analysis of purified OmpF protein samples produced using different media components

OmpF yield comparison in different media

Growth media used	Protein yield (mg/l)
LB	1.5
M9	1
DM9	1
DDM9	1
DAlgae	1

Theoretically and experimentally calculated mass of hydrogenated and deuterated OmpF in different media components.

Deuterated Protein Sample	Calculated Molecular weight (Da)	Mass from MS (Da)	% Deuterated
DM9 (H-Glycerol)	39539.6	38759.7	90
DDM9 (D-Glycerol)	39539.6	39054.8	100
Dalgae	39539.6	38860.7	99

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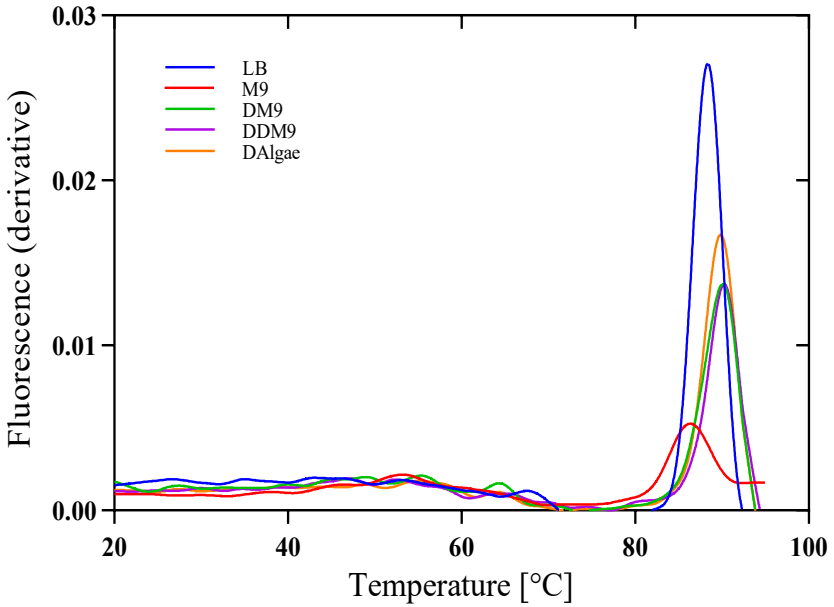
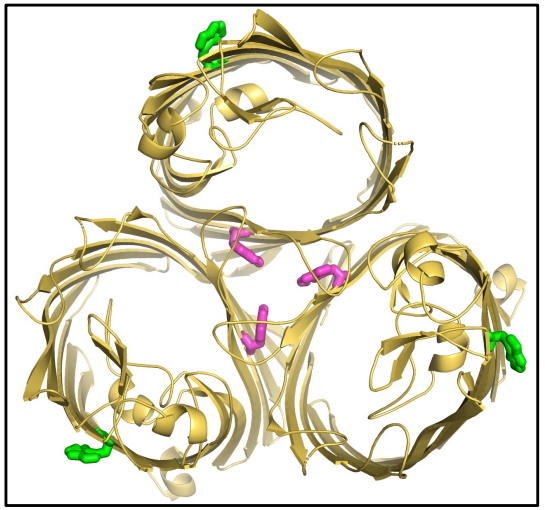


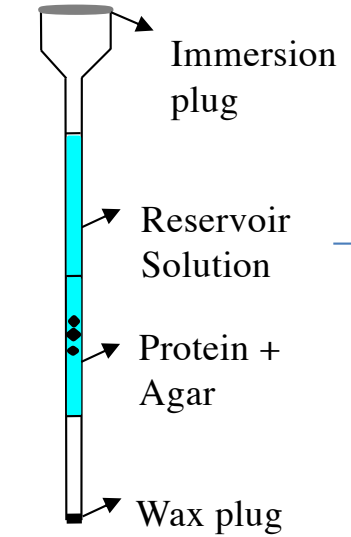


- Thermal unfolding temperature was higher for deuterated protein samples
- Temperature determines oligomeric structure of protein

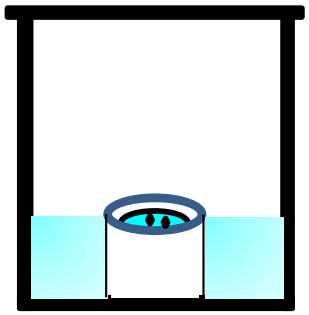
Protein Sample	Protein Unfolding Temperature (°C)
LB	88.3
M9	86.5
DM9	90.0
DDM9	90.2
DAlgae	89.9

Protein unfolding temperatures (Tm in ºC) as determined by Nano-DSF

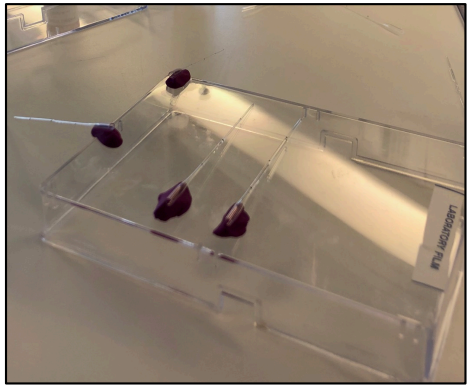
Techniques used for growing large crystals

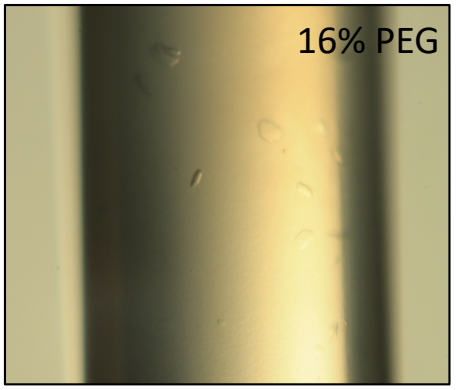


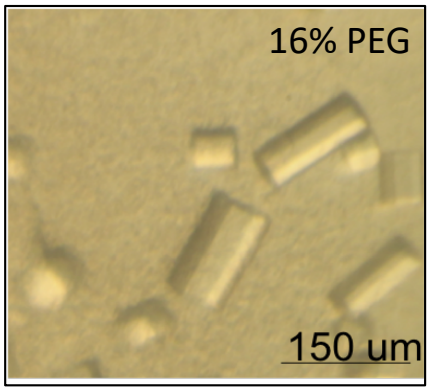
Capillary counter diffusion

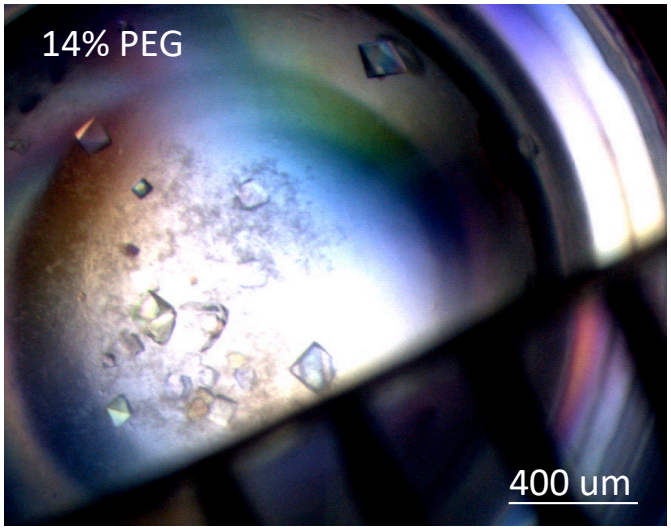


Vapour diffusion

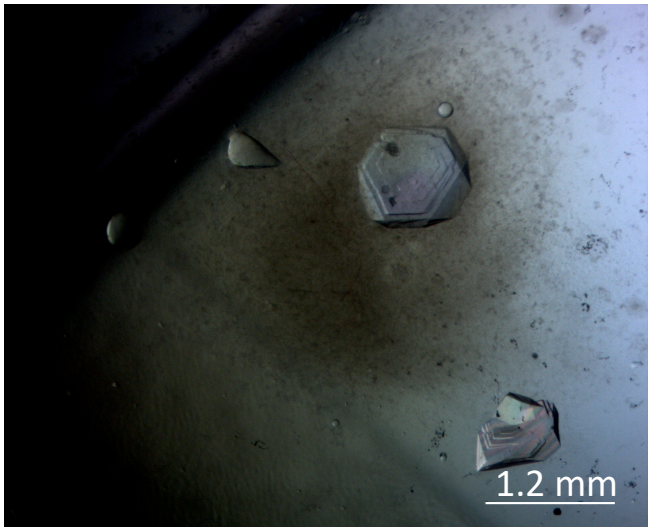








H/D Exchange



Hydrogenated

Crystallization trials of OmpF in heavy water using vapor diffusion

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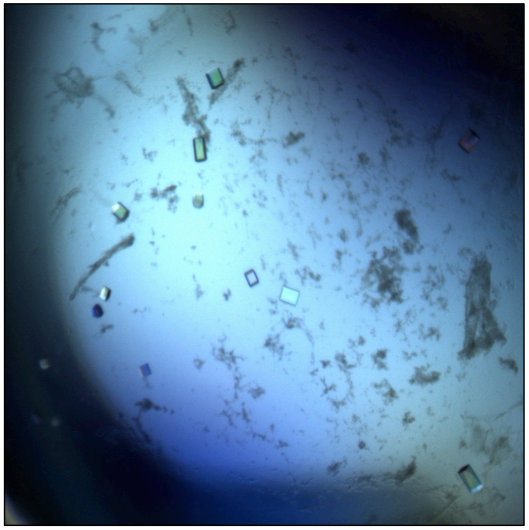
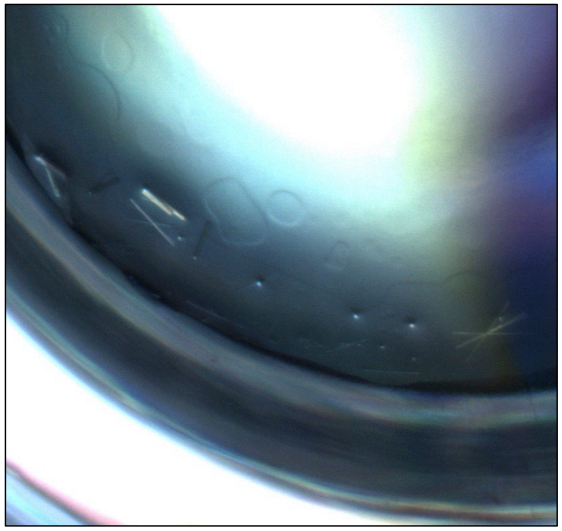
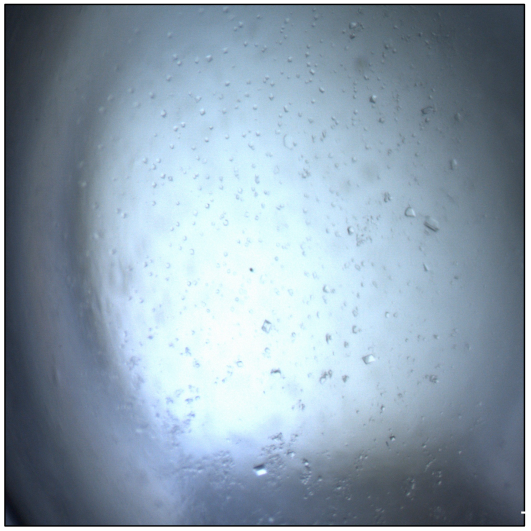


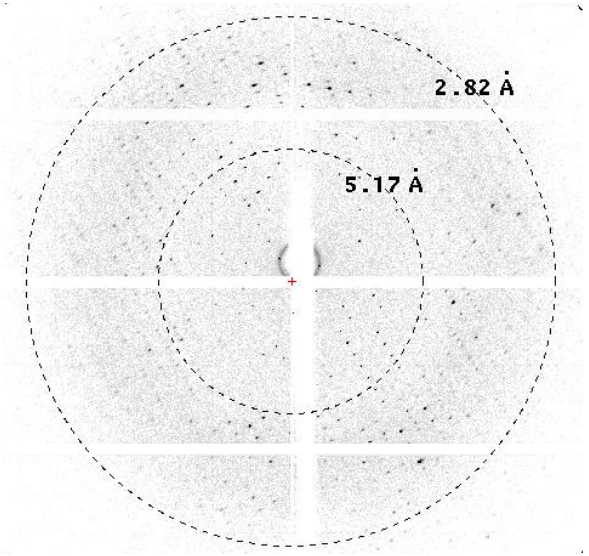
HM9



DM9



DDM9



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Conclusions

- The protocol developed in this study serves as a convenient way to produce and improve the yield of perdeuterated membrane protein without using large fermenters.
- Perdeuteration has subtle effects upon the physicochemical properties of proteins.
- Deuteration affects the secondary structure motifs and hydrophobic surface exposure during the unfolding events.
- Further challenges to reoptimise crystallization conditions in D_2O.
- The limitations of large crystal size and small unit cell volumes might not hold with the construction of ESS in Lund, Sweden.

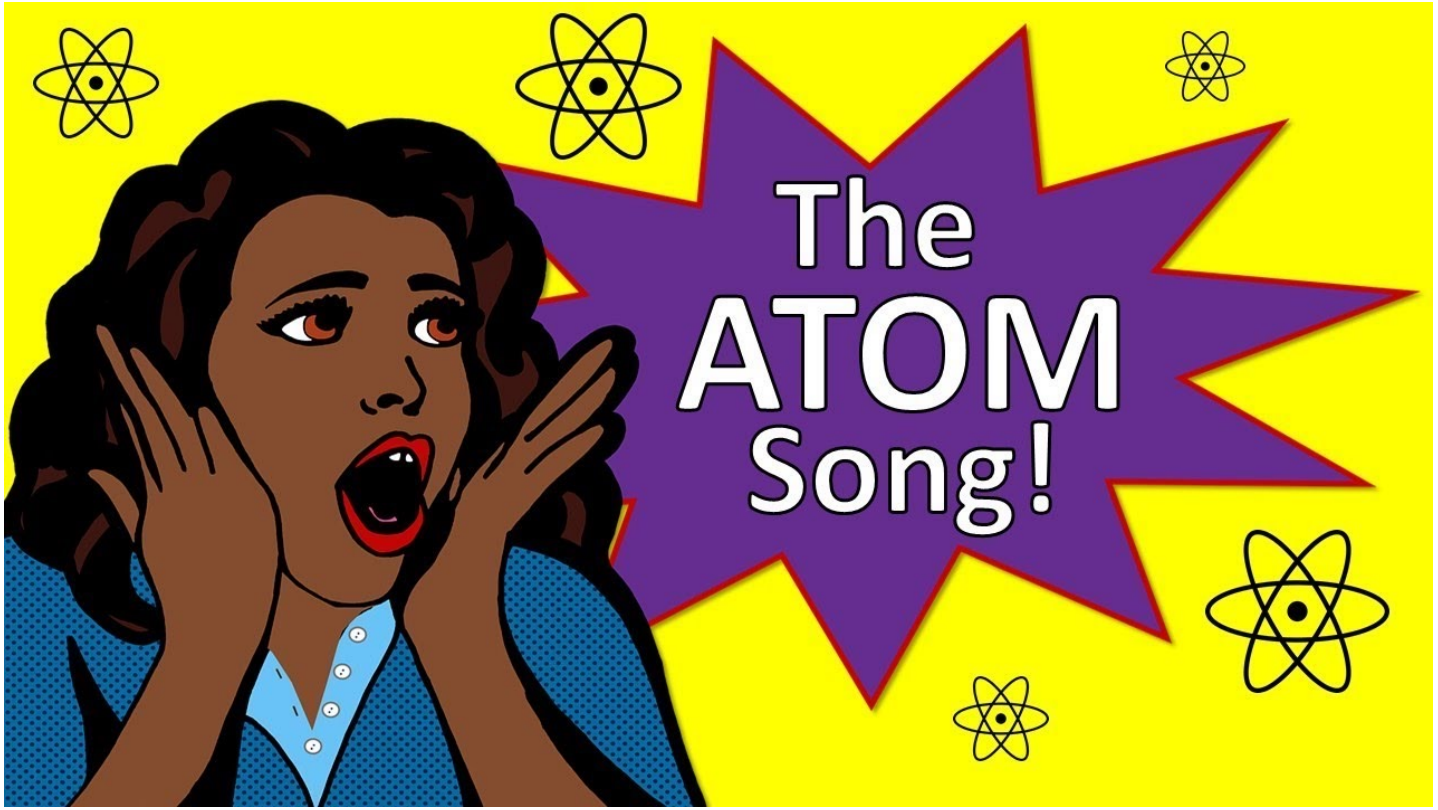
Acknowledgements

- Esko Oksanen, ESS, Sweden
- Claes Von Wachenfeldt, Lund University
- Zoë Fisher, ESS, Sweden
- Vinardas Kelpsas, Lund University
- Maria Gourdon and Celeste, LP3
- Large scale facilities- ILL and MAXIV- for beamtime and support during measurements
- Prof. Poul Nissen, Samuel Hjort Jensen, Sofia Trampari and Caroline Neumann during my secondment at Aarhus University
- Thesis Committee members: Monika Spano, Dr. Jean Luc Ferrer and Prof. Adrian Goldman

RAMP Members

This project has received funding from the European Union's Horizon 2020 research and Innovation programme under the Marie Skłodowska-Curie Grant agreement No 722687

Thank You for your attention!!! ☺



Questions and Suggestions??