DEMAX Activities, collaborations, projects

Hanna Wacklin-Knecht, Zoe Fisher, Anna Leung, Oliver Bogojevic

DEMAX & the ESS instrument suite

Chemical Deuteration:

- Synthesis of surfactants and lipids
- H/D exchange, chemical synthesis, purification and analysis
- Enzyme immobilisation and enzymatic catalysis
- Lipid purification/analysis from biomass

Biological Deuteration:

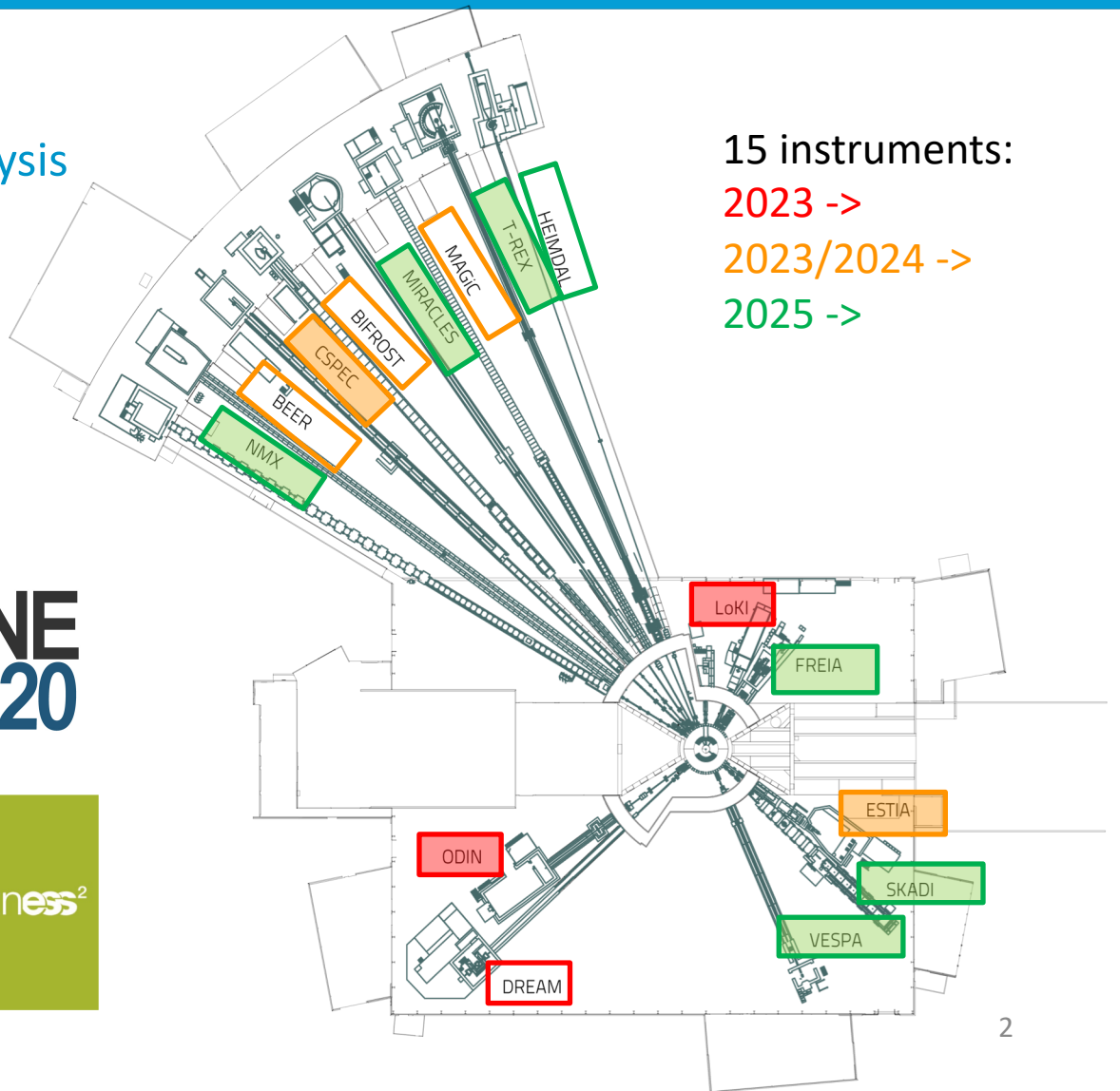
- Protein & lipid biodeuteration
- Cell culturing of bacteria, yeasts, algae
- Protein purification/characterisation
- Protein crystallisation

Collaborations

- SINE2020 & DEUNET
- Brightness^2
- LENS WG3

Grant Projects:

- LU VR grant
- LU PhD student projects

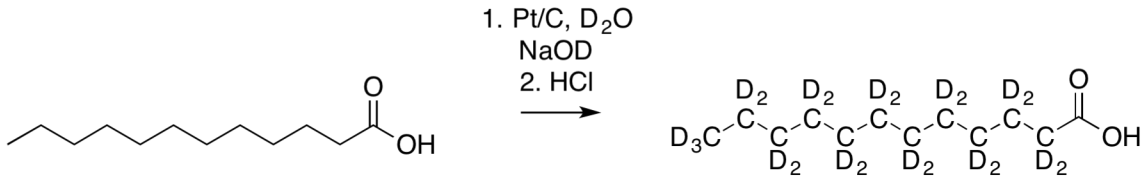


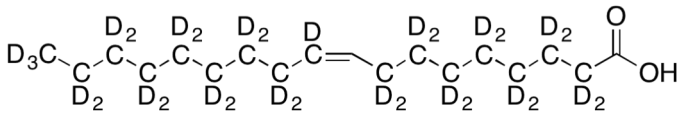
15 instruments:
2023 ->
2023/2024 ->
2025 ->

Chemical Deuteration

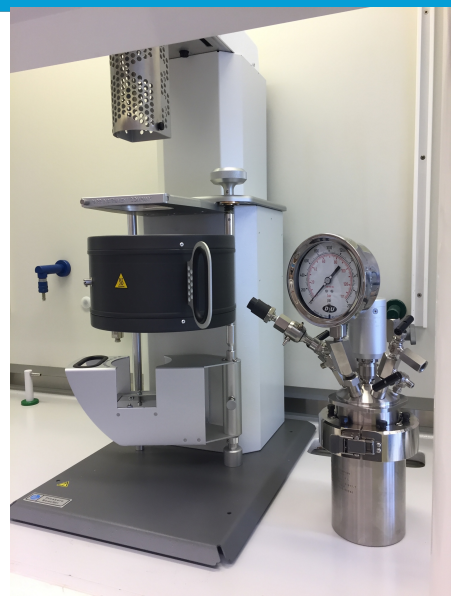
H/D exchange, chemical synthesis of fatty acids/surfactants

- Perdeuteration of saturated fatty acids (C9, C12, C16, C18:1):

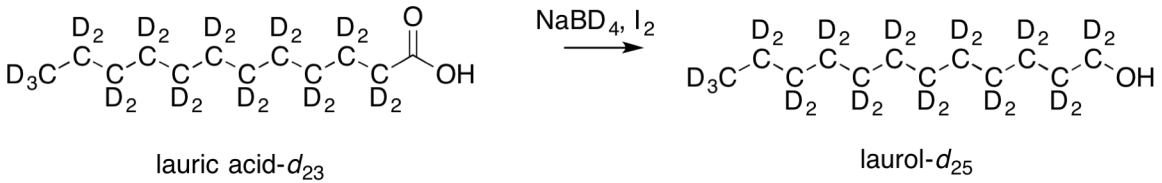




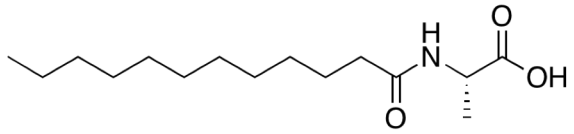
oleic acid-d_{32} (on-going)



- Method of reducing deuterated carboxylic acids to deuterated alcohols without $LiAlD_4$ (no longer commercially available!):



- Synthesis of chiral amino acid surfactants



N-lauroyl-alanine

Chemical Deuteration

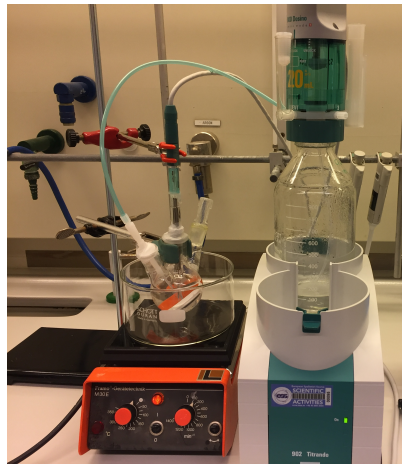
SINE2020 WP5: Immobilised enzyme catalysis for biopolymer synthesis

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654000

EUROPEAN SPALLATION SOURCE

SINE2020

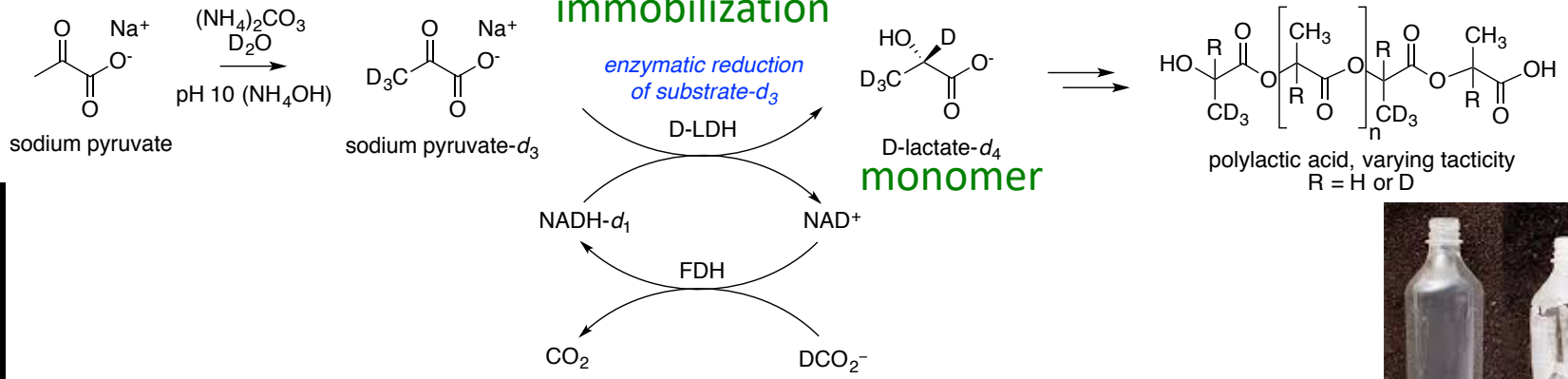
The enzymatic synthesis of perdeuterated D- and L-lactic acid-d4 and polymerisation of their lactides to polylactic acid.



Anna E. Leung[1], Andreas Raba[2#], Klaus Beckerle[3], Jürgen Allgaier[2*], Hanna P. Wacklin-Knecht[1,4*]

[1]European Spallation Source ERIC, PO Box 176, Lund, Sweden
[2]Jülich Research Centrer, Jülich, Germany
[3]Institute for Inorganic Chemistry, RWTH Aachen University, Germany
[4]Division of Physical Chemistry, Department of Chemistry, Lund University, Sweden

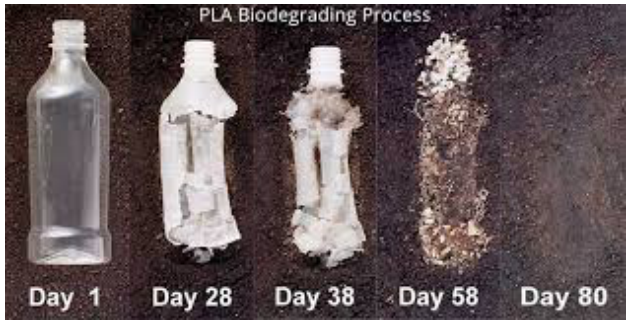
chemical deuteration of substrate



coupled enzymatic oxidation of NAD+ using deuterated cofactor

FZJ/RWTH: lactide & polymer

polylactic acid, varying tacticity
R = H or D



Chemical Deuteration

Immobilised enzymes for lipid synthesis – Oliver Bogojevic

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654000

EUROPEAN SPALLATION SOURCE

SINE2020

Enzymatic Synthesis

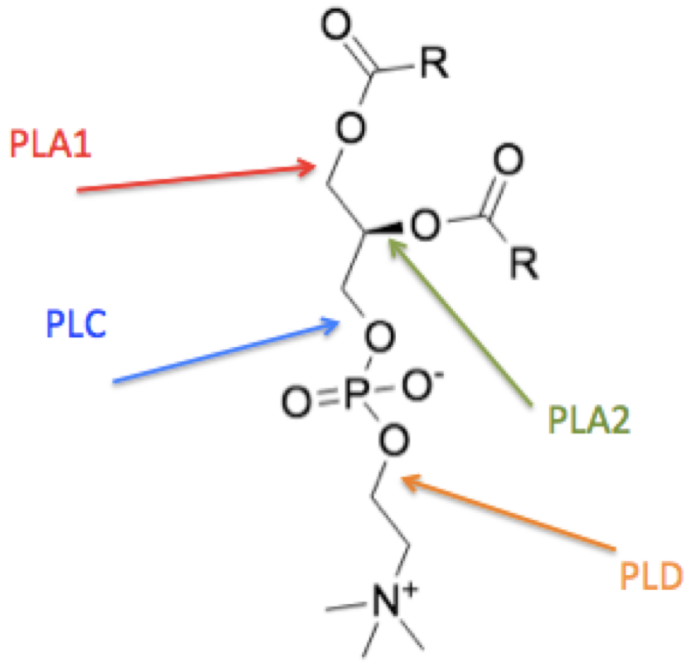
+ CLEAN and GREEN – few by products/no toxic chemicals
+ Highly specific – shortens reactions/purifications
+ Immobilised enzymes can be reused

Application to lipid deuteration:

- different enzymes attack selectively in different positions
- Can be used to swap d-fatty acids h-fatty acids

Commercial enzymes available:

- Lipases (1,3 specific), PLA_2, PLA_1



R = fatty acid chain

brightness²

WP2 A strategy to deliver neutrons for Europe and beyond

- Task 2.3B: Deuteration For Soft Matter and Life Sciences ESS-STFC

i) chemical and/or microbial production of perdeuterated fatty acids and lipids, followed by
ii) enzymatic synthesis of complex novel deuterated compounds.

BrightnESS² is funded by the European Framework Programme for Research and Innovation Horizon 2020, under grant agreement 823867

Chemical Deuteration

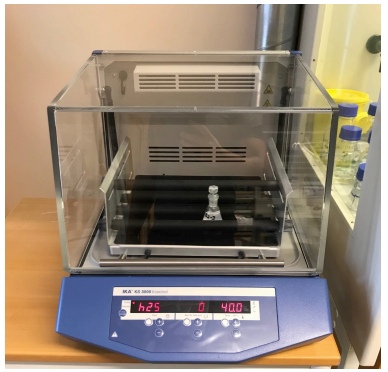
Immobilised enzymes for lipid synthesis – Oliver Bogojevic

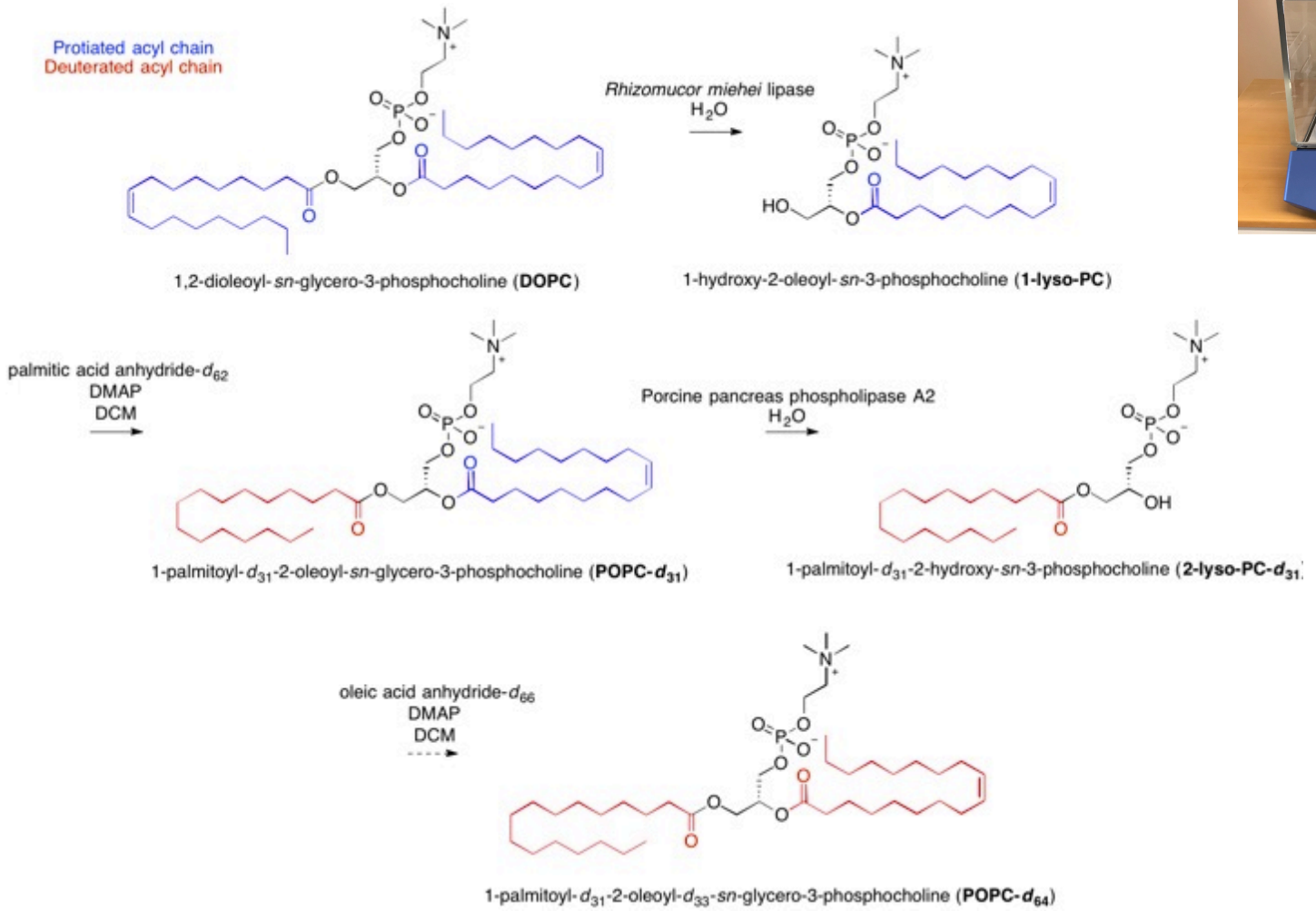
BrightnESS² is funded by the European Framework Programme for Research and Innovation Horizon 2020, under grant agreement 823867

EUROPEAN SPALLATION SOURCE

brightness²

Combined enzymatic/chemical approach for facile POPC synthesis (100mg):





Practical considerations:

- ✓ Acyl-migration
- ✓ Enzyme activity
- ✓ Solubility
- ✓ Mixing
- ✓ Reaction monitoring

Lipid Extraction, purification, analysis

Biodeuterated lipids from Pichia Pastoris yeast biomass

R.Delhom ESS/ILL PhD 2014-2017

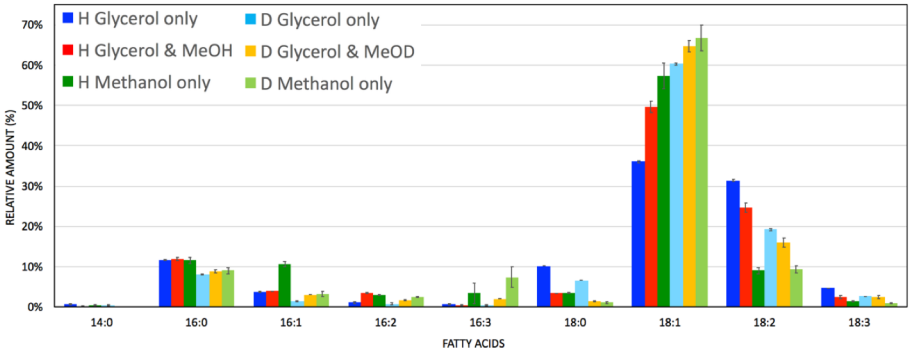
Current capabilities:

- ✓ Total lipid extracts
- ✓ Non-polar lipid separation
- ✓ Total phospholipid extracts
- ✓ Sterols
- ✓ Analysis, %d (TLC, GC, MS)

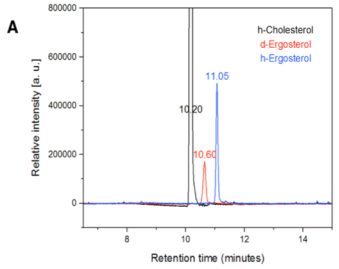
On-going:

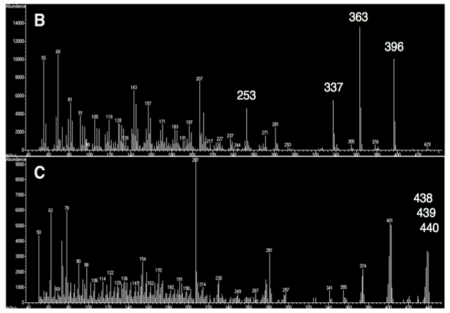
- Separation of phospholipid classes
- Reverse-phase HPLC

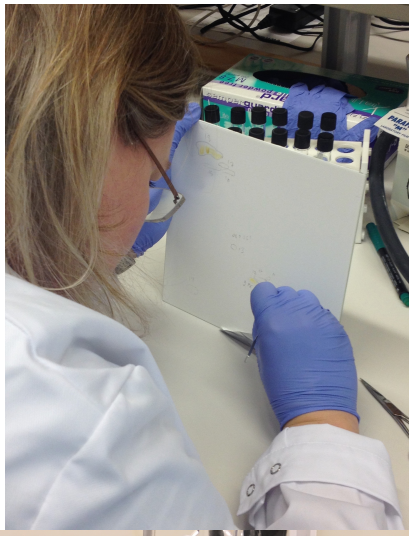
Effect of carbon source on lipid composition

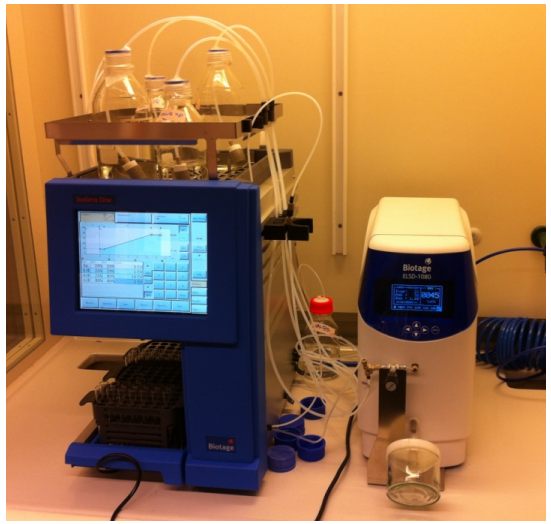


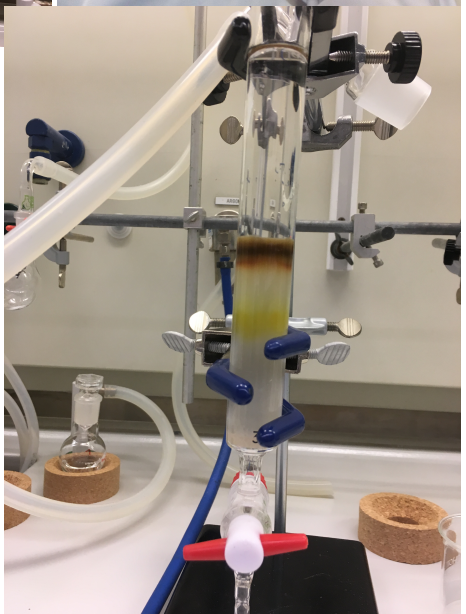
d-ergosterol











Biological deuteration

Crude Biomass – yeast, algae, bacteria

EUROPEAN SPALLATION SOURCE

Extracted products: recombinant proteins (*E. coli*), total lipid extract (*P. pastoris*)

Type	How?	Level of D incorporation	Application	$
H/D exchange	In vitro	25-30% labile H	Crystallography	$
Partial deuteration	In vivo	65-80 % (unlabeled C-source, recycled or fresh D_2O)	Matched-out product for SANS, NR, crystallography – spectroscopy? imaging?	$$
Perdeuteration	In vivo	Minimal media, D-carbon source, fresh D_2O	SANS, NR, crystallography, spectroscopy, QENS, NSE etc.	$$$
Perdeuteration	In vivo	Rich media, D-algal extract, fresh D_2O	SANS, NR, crystallography, spectroscopy, QENS, NSE etc.	$$$

Biological deuteration

– projects with LP3, Katarina Koruza, Manuel Orozco (LU), Akos Vegvari (KI); LANL (algae)

Developed cost-effective methods to maximize protein yield and D-incorporation in *E. coli*, including biophysical characterization of recombinant proteins

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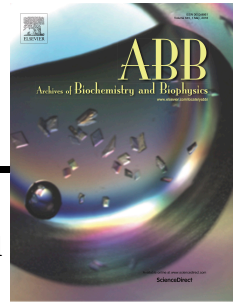
Archives of Biochemistry and Biophysics

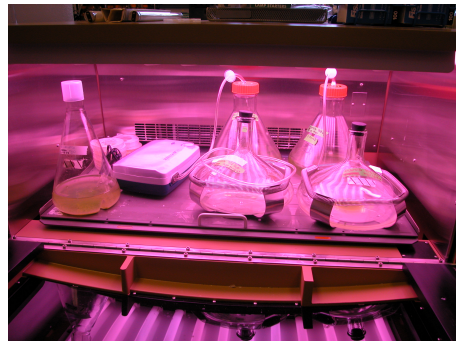
journal homepage: www.elsevier.com/locate/yabbi

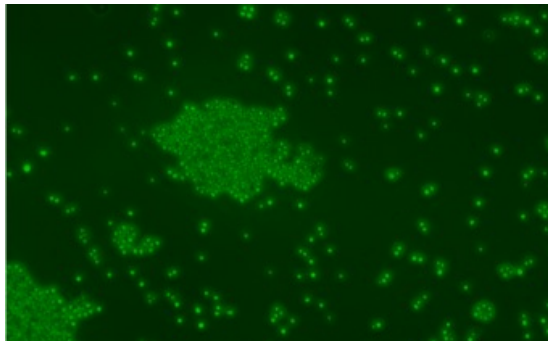


Deuteration of human carbonic anhydrase for neutron crystallography: Cell culture media, protein thermostability, and crystallization behavior

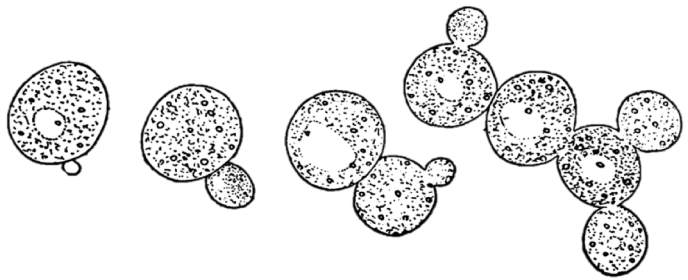
K. Koruza[a], B. Lafumat[a], Á. Végvári[b], W. Knecht[a], S.Z. Fisher[a,c,*]

[a] Department of Biology & Lund Protein Production Platform, Lund University, Sölvegatan 35, Lund 22362, Sweden
[b] Department of Medical Biochemistry & Biophysics, Karolinska Institute, Scheeles väg 2, Stockholm 17177, Sweden
[c] Scientific Activities Division, European Spallation Source ERIC, Tunavägen 24, Lund 22100, Sweden





Growing algae as source of D-nutrients for rich broth preparation.
Presently: *Botryococcus braunii*
Coming soon: *Scenedesmus obliquus*



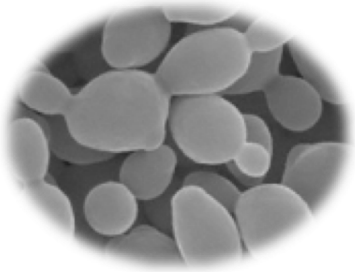
Yeast – growing *P. pastoris* under perdeuterated conditions for total lipid extract
Future: protein expression

Lipid BioDeuteration in yeast

Yeast cell cultures and biomass production at LP3

Yeasts contain all major lipid phospholipid classes (PC, PE, PI, PS, CL), sterols, glycerolipids, sphingolipids

Composition depends on
i) Species and strain
ii) Growth conditions
iii) GM

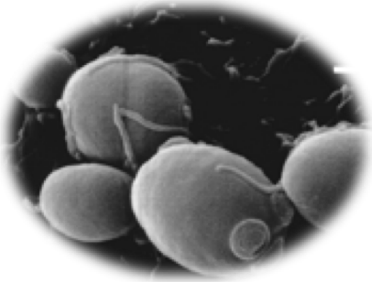


Pichia pastoris

Widely used for protein and lipid production

Rhodotorula glutinis

Oleaginous yeast, high producer of triglycerides



Candida glabrata

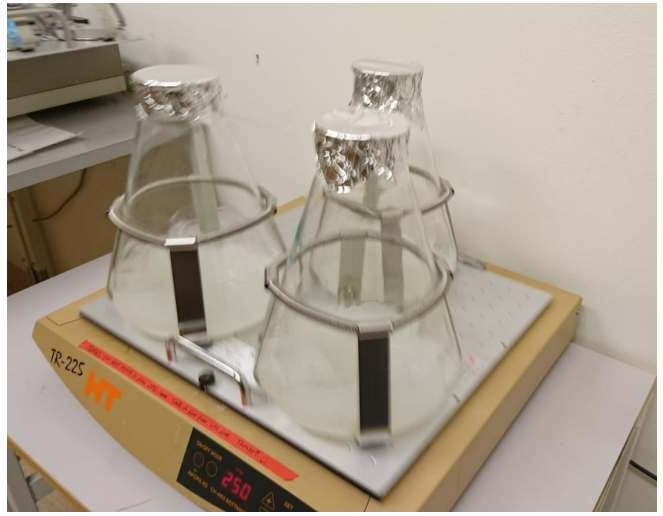
Human pathogen, drug resistance model

Current capabilities:

- ✓ Shaker flask cultures
- ✓ Up to 500mg per-deuterated total lipid extracts from P. Pastoris
- ✓ Up to 50mg perdeuterated lipid extracts from C. glabrata

On-going/next steps:

- Growth conditions for oleaginous yeasts (e.g. R. glutinis)
- Optimisation of lipid production in bioreactors (pH control)



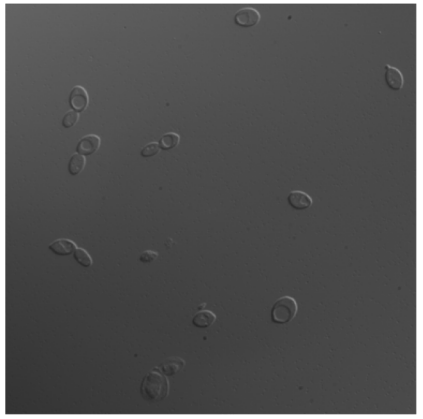
Lipid BioDeuteration in yeast

Oleaginous yeasts for glycerolipid production

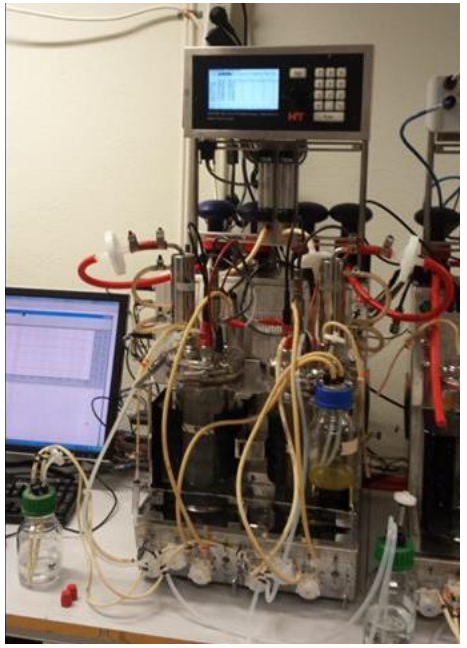
Oleaginous yeasts can produce up to 60wt% as storage fats – mainly triglycerides

FA composition depends on
i) Species and strain
ii) Growth conditions

Control of pH and nutrients important for high-fat content - fermentor cultures



Hydrogenated Rhodotorula glutinis



Rhodotorula glutinis

Oleaginous yeast,
high producer of linoleic acid

Current capabilities:

✓ Shaker flask cultures

✓ Fermentor cultures (^{1}H) of *R. glutinis and P. Pastoris*

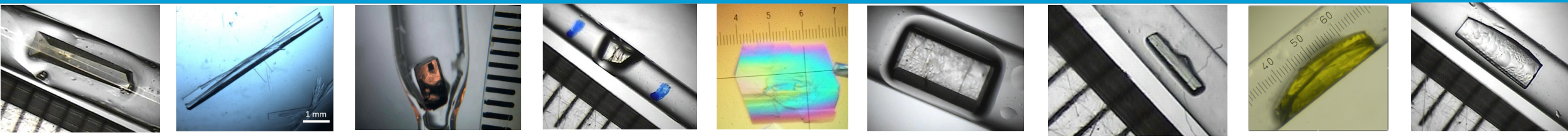
On-going/next steps:

- Growth conditions for further oleaginous yeasts (e.g. Yarrowina)
- Optimal carbon source €€€
- Selection of strains suitable for perdeutration

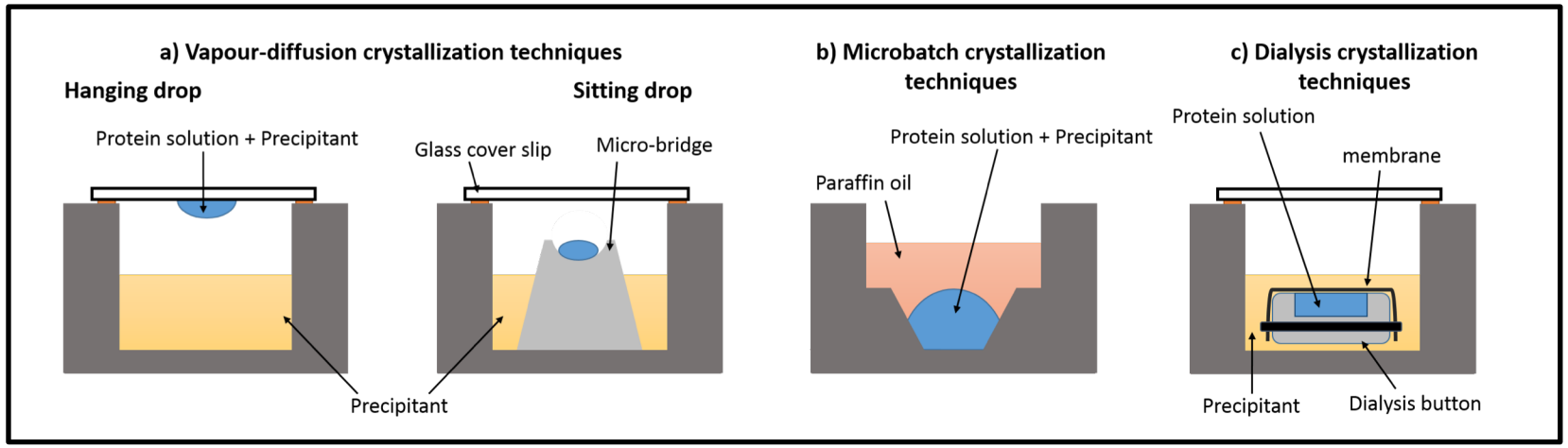
EU Internship chem lab assistant from Berlin LM School August 2019 for lipid analysis

Crystallization

– large single crystals (> 0.5 mm3 today)



- Xtallisation: micro & macroseeding, crystal feeding, dialysis, large volume sitting drop vapour diffusion, (macro)batch (with/out oil), temperature control/pH/precipitant



<u>Characterization of proteins</u>: ESI-MS & MALDI-TOF (D-incorporation, intact mass), DLS, Nanotemper Thermofluor (stability, aggregation), low and high-throughput screening (Oryx8, Mosquito, by hand), large crystal growth, X-ray testing/data collection at BioMAX.

Crystallization

– methods developed & services offered (in collaboration with LP3)

EUROPEAN SPALLATION SOURCE

Journal of Structural Biology 205 (2019) 147–154

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Using neutron crystallography to elucidate the basis of selective inhibition of carbonic anhydrase by saccharin and a derivative

Katarina Koruza[a], Brian P. Mahon[b,f], Matthew P. Blakeley[c], Andreas Ostermann[d], Tobias E. Schrader[e], Robert McKenna[f], Wolfgang Knecht[a], S. Zoë Fisher[a,g,*]

research papers

Acta Cryst. D STRUCTURAL BIOLOGY

ISSN 2059-7983

Perdeuteration, crystallization, data collection and comparison of five neutron diffraction data sets of complexes of human galectin-3C

Francesco Manzoni,[a,b] Kadhirvel Saraboji,[a]‡ Janina Sprenger,[a] Rohit Kumar,[a] Ann-Louise Noresson,[c] Ulf J. Nilsson,[c] Hakon Leffler,[d] Zoë Fisher,[e]§ Tobias Schrader,[f] Andreas Ostermann,[g] Leighton Coates,[h] Matthew P. Blakeley,[i] Esko Oksanen[a,b] and Derek T. Logan[a]*

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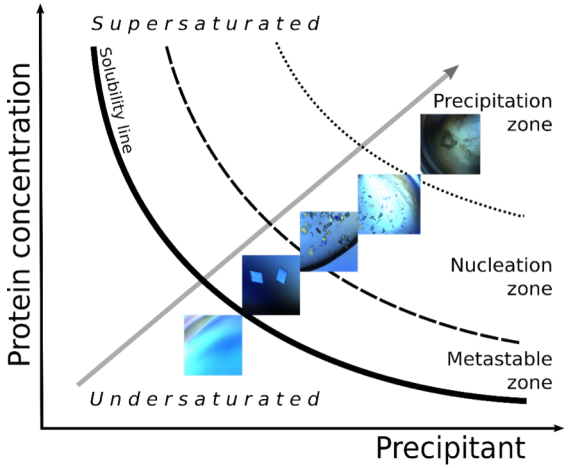

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Neutron Crystallographic Studies Reveal Hydrogen Bond and Water-Mediated Interactions between a Carbohydrate-Binding Module and Its Bound Carbohydrate Ligand

S. Zoë Fisher,[†] Laura von Schantz,[‡,⊥] Maria Håkansson,[§] Derek T. Logan,[§,∥] and Mats Ohlin*[,‡]

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[‡]Department of Immunotechnology, Lund University, Medicon Village, S-223 81 Lund, Sweden
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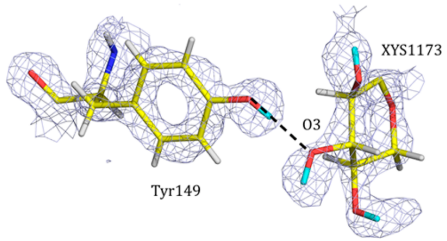


- Systematic optimization, phase diagram mapping, microseeding, batch methods.
- Making complexes with ligands: soaking vs. dry co-crystallization

crystals

MDPI

Article

From Initial Hit to Crystal Optimization with Microseeding of Human Carbonic Anhydrase IX—A Case Study for Neutron Protein Crystallography

Katarina Koruza [1,*], Bénédicte Lafumat [1], Maria Nyblom [1], Wolfgang Knecht [1] and Zoë Fisher [1,2,*]

Collaborations

WP5 Chemical Deuteration (ESS, ILL, STFC, FZJ)
- DEUNET

WP6 XTALGEN (ILL, ESS, FZJ)
- Phase diagram characterisation for proteins (ESS, FZJ)

2015-2019

WP2 A strategy to deliver neutrons for Europe and beyond
- Task 2.3B: Deuteration For Soft Matter and Life Sciences ESS-STFC

2019 - 2021

i) chemical and/or microbial production of perdeuterated fatty acids and lipids, followed by
ii) enzymatic synthesis of complex novel deuterated compounds.

WG3 Working Group 3: Synergies in technological development and operation
- Task 3.x Deuteration Technologies (Chem, Bio, Xtal) ESS, ILL, STFC, FZJ

DEUNET – SINE2020 Sustainability report

DEUNET achievements enabled by SINE2020:

1. Establishment of a new chemical deuteration laboratory at ESS
2. Access to STFC deuteration facility to European users
3. Development of methods for lipid deuteration, and separation from cell cultures at ILL
4. R&D in enzymatic and chemical synthesis of chiral biopolymers and lipids at FZJ and ESS.

Currently funded ESS	FTE	STFC	FTE	ILL	FTE	FZJ	FTE
2 scientists	2	4 scientists	4	1 technician	0.2	-	-
		1 technician	1				
		2 Post-docs	2				
		3 PhD students	2				

Conclusions and recommended actions:

1) Continued staffing resources for a sustainable DEUNET

2) Inclusion of biodeuteration/macromolecular crystallisation facilities in DEUNET

3) Continued R&D and international networking to facilitate innovation in neutron science

4) A cross-facility working group on inter-facility access to deuteration

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 654000

DEUNET – Deuteration Network

- next meeting 25-26 April Lund @ LINXS

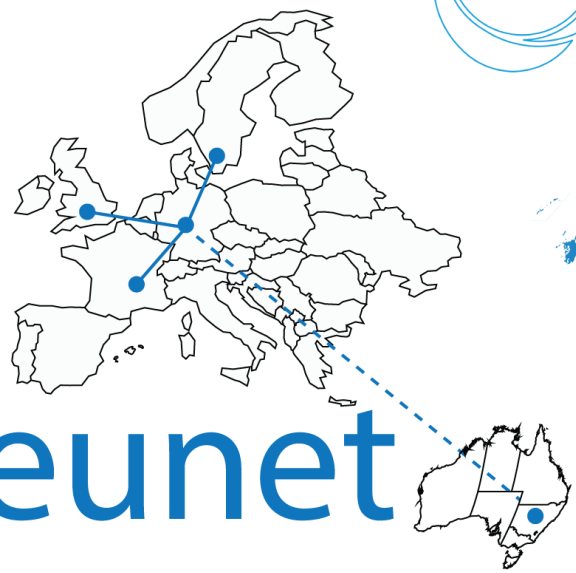
New members: ANSTO NFD, JPARC-MLZ, LP3

DBI Net possible new collaboration with ORNL/US deuteration

Larodan Lipids first industrial partner interested in distributing deuterated (and non-deuterated) products

Discussion on post-SINE2020 DEUNET and LENS

Continuation funding – seeking opportunities for new projects



A sustainable DEUNET

WG3 : Synergies in technological development and operation

- Task 3.x Deuteration Technologies (Chem, Bio, Xtal) ESS, ILL, STFC, FZJ

4 Pillars:

- chemical deuteration
- biological deuteration
- macromolecular crystallisation
- networking and synergies

Priorities aligned to outcomes of SINE2020 WP5 and WP6:

1. Identifying new R&D projects and collaborations aligned to future research themes and priorities in Europe
2. Networking with international deuteration facilities
3. Cross-facility working group on deuteration user access in Europe

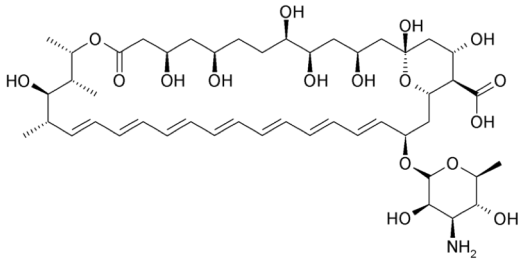
Lipid composition and antibiotic resistance in *C. glabrata* (0.3FTE HWK)

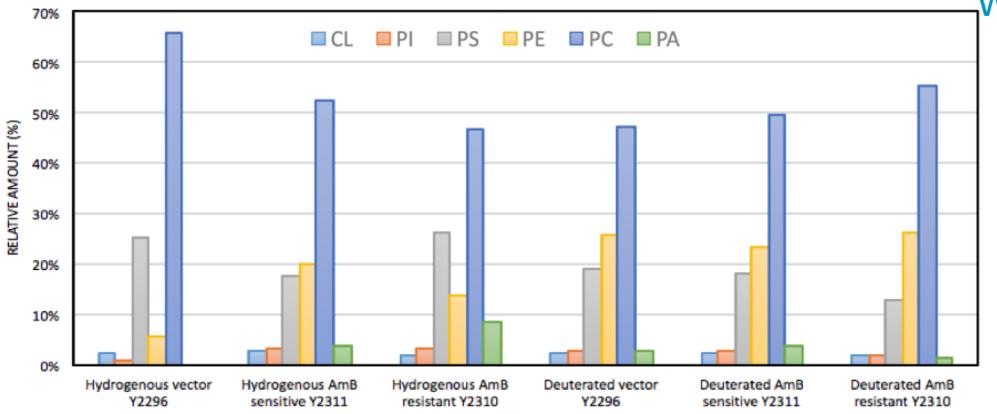
3-year project funded by Swedish Research Council VR grant nr. 2016-01164 (2017-2020)

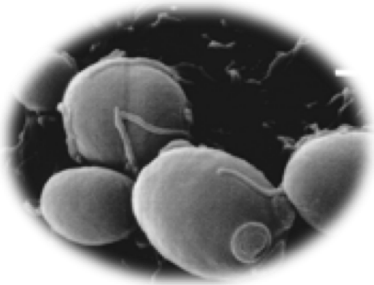
W. Knecht

(Olena Ishchuk)

iRNA used to up/downregulate genes – strains chosen for increased/decreased Amphotericin B resistance







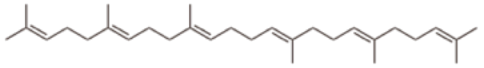
Candida glabrata

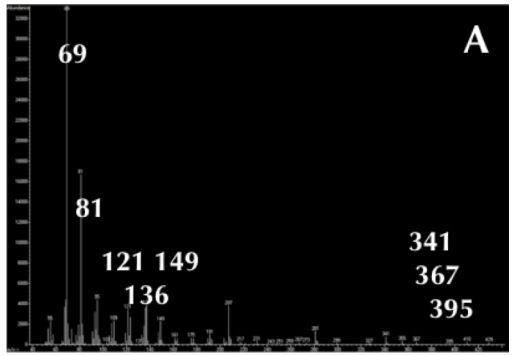
Human pathogen

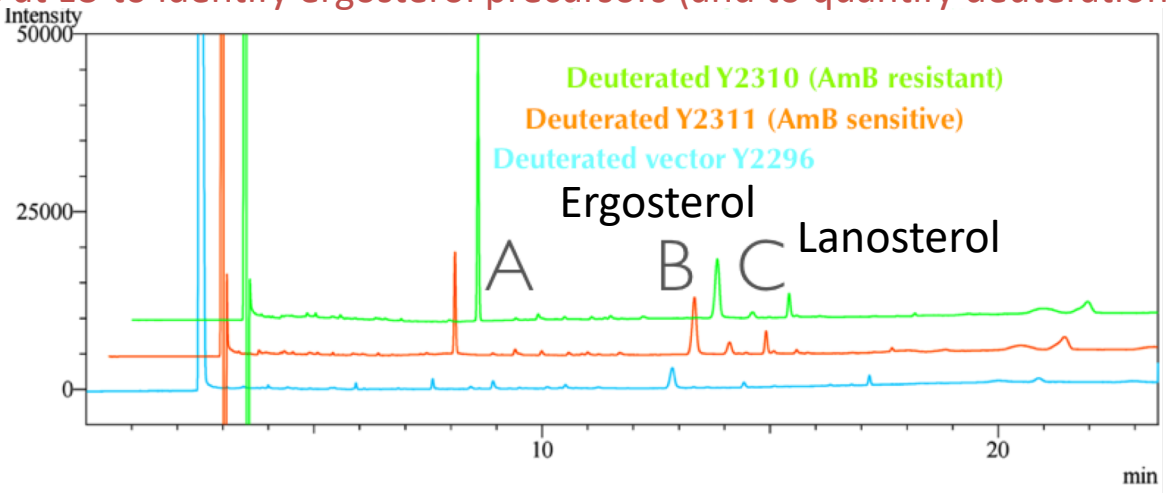
Squalene

Detailed GC-MS analysis at LU to identify ergosterol precursors (and to quantify deuteration)

Alterations in sterol biosynthesis lead to accumulation of squalene - NR show SQ to be located in the centre of membranes where it prevents AmB insertion.





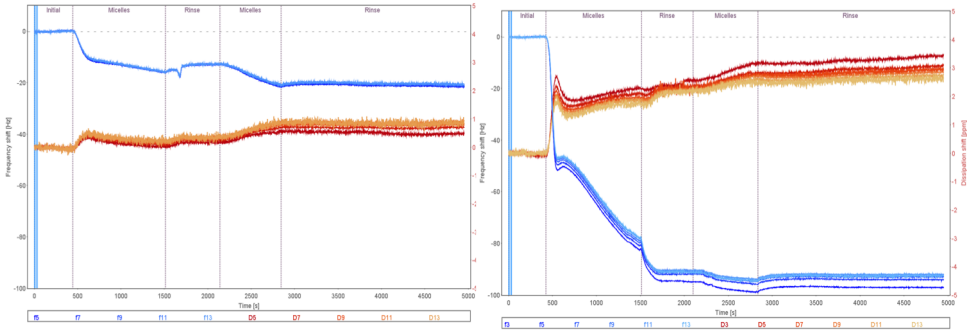


GC-MS difficult to access in LU teaching labs

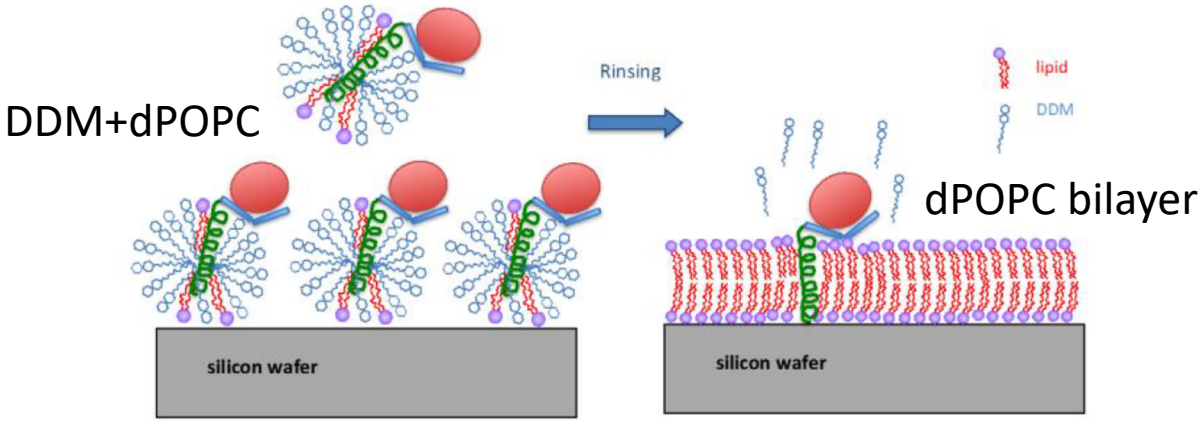
User-provided strains can be cultured, but further GM work would require a molecular biologist.

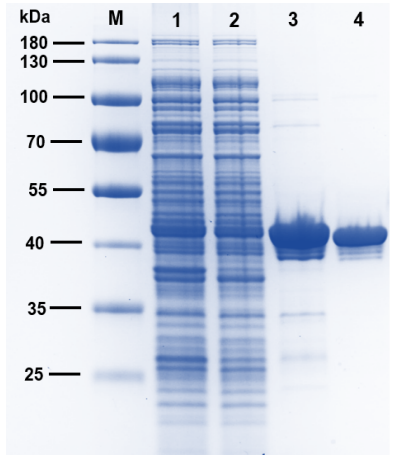
Reconstitution and function of human DHODH in membranes (Manuel Orozco PhD LU 2018-2022)

- Purification of full length DHODH and solubilisation with DDM for SANS and NR studies of lipid-DDM reconstitution process into supported lipid bilayers

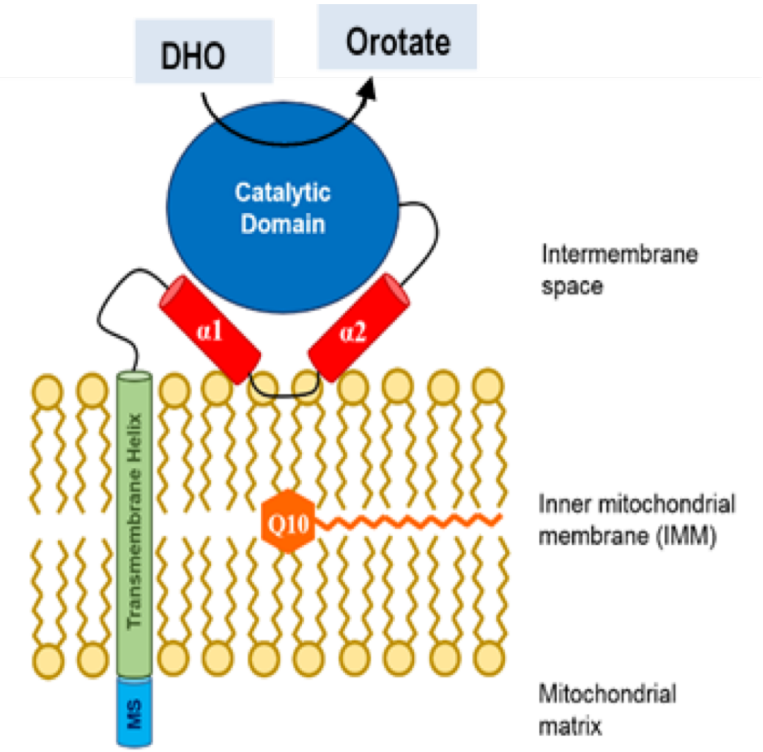


Reconstitution of full-length DHODH into supported lipid bilayers by detergent/lipid micelle adsorption monitored by QCM-D. *Left*: lipids only (80% POPC, 10% cardiolipin and 10% Q_{10}). *Right*: lipids and DHODH (10:1 mol/mol).





Purification of full-length hDHODH from *E. coli*.
1 crude cell lysate.
2 solubilized fraction
3 metal affinity column eluate.
4,purified DHODH in DDM.



Questions to STAP:

- Commercial access to deuterated products?

i) For industrial neutron users

ii) Sale of products to commercial vendors

E.g. Larodan Lipids (SE) is interested in purchasing or distributing excess deuterated (and non-deuterated) products from DEMAX.