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| DEUNET Member and Advisory Panel Meeting |

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| Date | Location |
| March 5th 2018 | Medicon Village, Lund, Sweden (Host: ESS) |
| Attendees |  |
| See participants list  **Apologies**  Trevor Forsyth  Thomas Hellweg |  |

**Action List** (see minutes for more details)

1. Anna Leung/Hanna Wacklin-Knecht: Prepare a template/questions for the advisory panel to complete after the meeting for the report we have requested for Parma.

DUE Friday 23th March

1. ISIS: to complete the deliverable report (5.7) that is due ASAP and sent it to Miriam.

DUE Friday 16th March

1. Anna Leung/Hanna Wacklin-Knecht: To contact Peixun for an exhaustive list of the MAG, DAG and TAGs produced at ISIS and discuss which others would be most useful for users.

DUE Thursday 29th March – done

1. All members: Start updating P1 report from June 2017.

DUE at Parma meeting, Tuesday 5th June

1. All members: to contribute to dissemination materials.

e-learning material:

DUE 9 APRIL (list of potential contributions and responsibles to Hanna)

DUE 30 APRIL (first draft of contributions to Hanna)

DUE 20 MAY (draft material to upload to e-neutrons.org)

1. Giovanna Fragneto: to contact someone at ILL to discuss a regular DEUNET newsletter.

DUE Friday 16th March - done

1. ISIS: To assess their next proposal round for non-standard requests/requests they cannot (closes 15th April) they should forward them to the network.

DUE Friday 15th May (or when proposal assessment is completed)

1. FZJ: to contact other network members for any information/assistance they need for an application for potential funding.

DUE minimum 3 weeks before any application deadline

1. Hanna Wacklin-Knecht/Anna Leung: to draft a charter for the DEUNET and circulate to members for comment.

DUE Friday 158h May

1. Hanna Wacklin-Knecht: create discussion pages/mailing lists for amino acids and ionic liquids (lipids?) to put those labs working or wanting to work in this area in contact.

DUE Thursday 29th March

# Minutes

# Welcome and Introduction to the Advisory Panel (Anna Leung)

The DEUNET welcomed members and the advisory panel members and thanked them for their attendance and contributions.

The organization of the DEUNET was presented to inform the advisory panel members, including member labs participating in the SINE2020 grant WP5 (ISIS, ILL, FZJ, ESS and ANSTO as observer), and the aims of the network. The DEUNET has existed since 2016. J-PARC joined the network in 2017 as a non-grant funded member.

Some activities of the DEUNET since the last member meeting in Oxford 2017 were summarized and the charge to the advisory panel was presented. DEUNET is seeking advice from the panel member on the following questions:

* What would a sustainable DEUNET look like and what should it do?
* (How) Could DEUNET function as a user platform for providing coordinated access to deuteration?
* How could we involve the neutron facilities in supporting DEUNET?
* How can we involve the user community actively in DEUNET?
* What is the most significant impact DEUNET could make?

# ILL Update: Lipid extraction at the ILL – Progress report since Jan 2017 (Giovanna Fragneto)

Rachel Morrison: investigation different methods of cell pre-treatment (sonication, freeze-drying and grinding, glass bead homogenisation) and extraction (Blight Dyer, Folch, Soxtec extraction) for optimal extraction of lipid groups. Freeze-drying and grinding/sonication are equally good; glass beads not as good. Blight Dyer and Folch similarly effective so Blight Dyer used. Soxtec gave lots of lyso-phospholipids, probably because of the heat (60 C).

Focus changed from single molecular species separation to lipid class separation. Methods investigated for this: 1) solid phase extraction – could not replicate separation reported in the literature. Could be a problem of scale. Things co-eluted. 2) HPLC (ongoing) – silica column: partial separation with isocratic solvent system (hexane, isopropanol, phosphate, ethanol, acetic acid) and gradient flow rate. Now investigating acetonitrile, methanol, phosphoric acid. Diol column also evaluated: two different solvent systems; at 65 C; some separation but the results were not reproducible with higher quantities.

ILL is at present successful at producing deuterated cell paste from which model membranes can be reconstructed, these are more representative cell membrane mimics than single phospholipids but more complex to work with.

GC-MS (Shimadzu GC2010-Plus, same as at ESS in Lund) to be installed 7-8th March 2018.

Future work: fatty acid incorporation into phospholipids (yeast grown, supplemented with FA of interest to increase proportion of lipids of interest).

Rachel Morrison working until end of April 2018. New postdoc Krishna Batchu to begin 1st June 2018. Next deliverable 5.9 to be postponed from month 36 to month 42.

Questions: Has the yeast species been decided? Originally planned to use spent biomass from D-lab protein expressions, but now aimed at best species for lipid production. Nothing better than Pichia pastoris found yet.

The differences between the hydrogenous and deuterated lipid mixtures tend to be at the level of unsaturation (deuterated are less polyunsaturated). Factors are being investigated to try to mitigate this – solvent/temperature to affect different phase transitions.

# ESS Update (Anna Leung)

Update on ESS lab space, new equipment (analytical + preparative HPLC, rotating bed reactor, pH Stat titrator). DEUNET activities: presented DEUNET at J-PARC workshop October 2017, and JPARC joined the DEUNET as observer member; deuteration survey carried out and results analysed with help of Rachel at ILL. Deliverables update: 5.3 (deuterated enantiopure lactic acid) completed; work on polymerisation continues at FZJ, neutron beam time applied for by user. Work will continue using the same enzyme system but unnatural substrates – need to identify useful ones that can be easily deuterated/isolated.

Enzyme catalysis being extended from oxo-reductases to other enzymes – lipases and phospholipases first. Both commercially available immobilised and but we are also establishing methods to immobilise specific enzymes in-house. Application of these systems to glycerides, phospholipids will be explored during MSc thesis of Oliver Bogojevic (LU).

First DEMAX proposal round will be opened within the next 12 months. Preparation for this is beginning. For chemical deuteration: well-defined proposal round with molecules that can be requested on the basis of what methods have already been tested.

Questions/comments:

ZF: It is likely that we don’t see a kinetic effect in the lactate dehydrogenase case because we are well below optimal conditions i.e. well below the optimal Kcat. Should compare the rate we see with Kcat to determine whether this is the case.

PH: Should not be too prescriptive with molecules that can be requested for initial proposal rounds. Also allow expressions of interest to see what kinds of molecules are desired so that you don’t exclude things you haven’t thought of.

ISIS: they get requests for very expensive commercially available lipids and monomers because the STFC gives users a consumables allowance. They procure these instead of making them but they are not counted in some of their reporting as a result.

# STFC/ISIS Update (Kun Ma)

Making hydrogeneous lipoproteins with their peptide synthesiser. Often, having the acyl chains deuterated in e.g. aminoacid-based surfactants provides enough contrast so the amino acids do not always need to be deuterated (Jian Lu’s comment). ISIS are working on the synthesis of deuterated amino acids too, but the results are not very promising yet.

STFC has a H-cube (one month old) for flow-through hydrogenation. Commercially available catalyst columns (Pt/C, Pd/C, raney Ni) are used; can also pack their own columns but they haven’t. Functionalised crown ethers synthesized.

MicroWave synthesiser being used for:



180 ℃, D% 84% after 1 hour

200 ℃, D% ~ 40% after 6 hours

Completed 8 European access proposals.

Sulfonate surfactants synthesized.

# FZJ Update (Jürgen Allgaier)

Novel route to deuterated isoprene completed, after investigating several routes (D5.3, month 15).

Synthesis of deuterium-labelled polythiophene based block copolymers completed (D5.5, month 20) using GRIM polymerisation. Very high molecular weights and narrow distribution. Deuteration possibilities presented.

Poly(lactic acid): ongoing in collaboration with RWTH. Lactide formation completed at Jülich; using toluene and zeolite beta. Yields were low because of the presence of water in toluene so lots of unreacted lactic acid; Dean-Stark apparatus was modified to include a molecular sieve-containing tube between the condenser and reaction vessel. Re-crystallisation also needs to be done in dry toluene otherwise the lactide is hydrolysed to lactic acid.

Polymerisation results: mostly III>>>>>>IIS/SII/ISI. 96% conversion.

I = isotactic triad; S = syndiotactic triad.

Polymerisation of ethylene oxide (both h and d). End-group functionalisation to azide or acid from terminal alcohol; or to maleimide.

Karen Edler: interest in deep eutectic solvents with PEGs.

# ANSTO Update (Peter Holden)

New capabilities for chemical deuteration: 1) tail-deuterated cholesterol-d15 (used in combination with deuterated phospholipids to produce liposomes for drug delivery applications).

DDM (n-beta-D-dodecyl maltoside) protocol being extended – method modified to increase production scale. Published results with collaborators in 2018: Mitgaard et al the FEBS journal.

Phytantriol-d39 synthesized for liquid crystals, from phytanic acid in 6 steps.

Janus work with gold nanoparticles (Nature Communications) with Francesco Stellacci – using deuterated alkanethiols(?)

# Future Projects and Reporting – Discussion (Hanna Wacklin)

Advisory board requested a template/questions for in order to complete the report we have requested for before the SINE2020 Annual meeting in Parma (June 6-7). Hanna/Anna to send this in the next few weeks, for report completion by 21 May.

ISIS to complete the deliverable report (5.7) that is due ASAP and sent it to Miriam. Templates are available from ESS.

ILL will define D5.9 but also write to Miriam and request a delay from M36 to M42 in light of time lost with maternity leave/delayed starting date of new postdoc (1st June 2018). Will also consider D5.10 since beamtime will need to be applied for in September.

ESS deliverable 5.11 (with ISIS) needs to be defined: three proposals presented:

a) synthesis of modified triglycerides using enzymatic synthesis and perdeuterated fatty acids – ISIS currently produced tail-deuterated and perdeuterated mono-, di- and tri-acylglycerides but the di- and tri- always contain the SAME FA. So ESS should focus on mixed chains. Are there users at ISIS who would find some of these useful? Simon Titmuss, Adam Squires, Tommy Nylander potentially interested.

b) synthesis of lactic acid containing molecules such as alkyl lactylactates (used as food emulsifiers). Not established in the ESS lab so requires method development. Also have not identified users who would profit from this at the moment.

c) Synthesis of oleic acid at ESS using perdeuterated nonanoic acid and azelaic acid produced at ISIS would be feasible using existing methods and would allow work load and costs to be shared for producing this popular precursor to lipids. Also discussed: amino acids – summer student working on this at ISIS at the moment.

ISIS and ESS agreed upon a). Anna Leung to define project further, liaising with ISIS about suggestions.

FZJ: 5.8 poly(lactic acid) due M30 May 2018 – on track to succeed.

D5.12 (ESS) Final Report of INE2020 WP5 – will require significant input from all members. To be discussed in depth at Parma in June. In preparation, all members should start updating P1 report from June 2017.

Dissemination (with WP2) also remaining – contribution welcome from all members for both e-learning material about deuterium labelling and industry-specific outreach material. All members should contribute content.

Regular DEUNET newsletter raised. Could be comprised on the blog posts on the website. Giovanna to ask Alison Mather at ILL if she could help with this.

We are reminded that we need to publish in open access journals (at least self-archiving). Pay open access fees if necessary.

All members to list dissemination and outreach activities funded by and related to Deunet/SINE2020, including:

* Networking events, annual meetings
* Conferences, meetings and visits of members funded from grant
* Presentations (talks and posters)
* Publications (open access)
* DEUNET and SINE2020 webpage articles
* @Deunet twitter account
* neutronsources.org
* facility newsletters, annual reports, interviews.

John Webster: ISIS sometimes receive non-standard requests/requests they cannot fulfil. If this happens in their next proposal round (closes 15th April) they will forward them to the network.

Jülich management for Soft matter group has indicated interest to support DEUNET, which may help with further funding opportunities for deuteration 2020-2030.

Possibility of ISIS supplying deuterated compounds as an in kind contribution to ESS raised. We do not at present know about in kind contributions to ESS operations, but the issue has been discussed in other contexts as well.

Access to deuteration at ESS – should his be only for members countries? Member country subscription fee one possibility, or an instrument subscription fee? Would most likely need to include different options for members with different user communities – e.g. unlimited access fee for big user groups or one-off sample payment in other cases.

Comments

PH: Currently, our meetings focus on technical/capability development. Need to focus on value generation – what impact will the techniques developed have on the scientific output and societal impact of neutron facilities?

Other molecules of interest: Karen Edler - non-ethoxylated non-ionic surfactants, tweens (Bob Thomas has synthesised these but commercial tween always a mixture so difficult to replicate). DDM – ANSTO published synthesis in Feb 2018.

JA: PEG-based materials would be a good collaboration topic in the future, as they have many different uses in polymers, surface functionalisation, particle stabilisation etc.

GF: polyelectrolytes would be of interest as increasing no. or groups use them at ILL – what kind of PE? To be investigated further by GF.

HW: Lipid transformations and/or purification of Glyco or sphingolipids would be of interest to many users – collaboration with ILL?

Need to consider how it works in practise when a user wants something from two different labs – easiest way is to forward propsals.

HW: It is of no significance to users which lab provided the deuterated compounds and if it is the same as the facility where they performed the neutron experiment, in practise this is already so – but important to ensure that deuteration work is acknowledged in its own right by neutron facilities as it has at least an equivalent impact to one instrument.

PH: Consider how deuteration work is acknowledged (without neutrons) when different facilities make the molecule and perform the neutron experiment. Remember it is the outcome of the science that is valuable, not simply performing the neutron experiment. Impact should focus on what science we have enabled and not a list of molecules made or neutron experiments performed.

# SINE2020 General Assembly (Hanna Wacklin)

All members to send at least one representative to Parma, registration and accommodation possibilities available at <https://indico.frm2.tum.de/event/93/overview>.

Sessions to focus on the Advisory panel report, planning of remaining deliverables and M36 (October 2018) and final reports). Should also look for further funding opportunities to discuss here.

John Webster and Anna Leung to chair sessions.