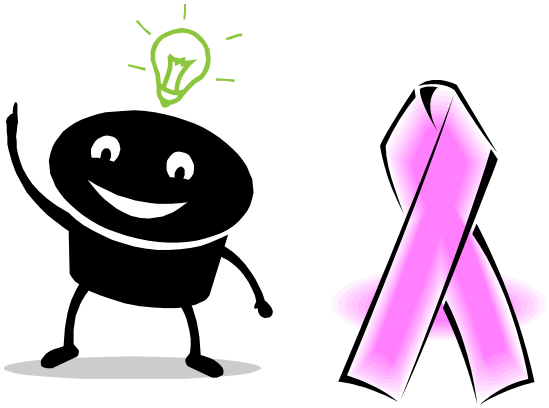
# How to Write a Successful Neutron Proposal

*Victoria Garcia Sakai*

*JSTS*

sample

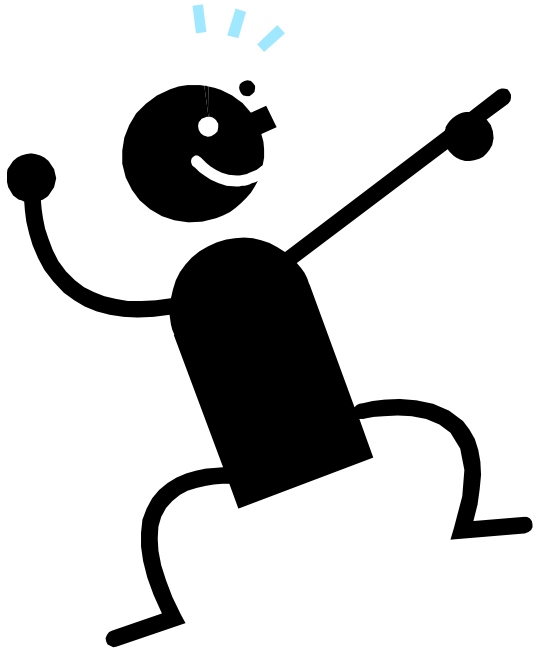


Idea & Research problem

Pre- characterisation

the unique information obtained from neutron experiments

Can ~~neutrons~~ help me?

Are you sure?

Can you obtain the information with a different technique?

Are you completely sure?

# Before writing a proposal

- Literature review on similar experiments

# Before writing a proposal

- Literature review on similar experiments
- Talk to colleagues

# Before writing a proposal

- Literature review on similar experiments
- Talk to colleagues
- Research available instruments worldwide

# Where should I go to get my neutrons?

Sources http://neutronsources.org/


# Where should I go to get my neutrons?

- Where can I do the best science?
  - Instrument specs
  - Flux
  - Sample environment
  - Technical/user support
  - Laboratory space/facilities
  - PhD programmes
  - Software
- Proximity/ease of access
- Funding
- Personal connections/collaborations
- Food/Scenery

# Before writing a proposal

- Literature review on similar experiments
- Talk to colleagues
- Research available instruments worldwide
- Contact instrument scientist and ask questions!
    - *instrument configuration*
    - *sample environment*
    - *time required*
    - *…*

# Before writing a proposal

- Literature review on similar experiments
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    - *instrument configuration*
    - *sample environment*
    - *time required*
    - *…*
- Decide on proposal type

# Access Types

- **Normal proposal rounds** – twice per year
- **Rapid access** – for urgent studies or ‘hot topics’, submit at any time
- **Xpress access**, including postal service
- **Industrial access** (collaborative or for cash)
- **Back door** – collaboration/tests with institute scientists
- **Programme access** – long time proposals

# Before writing a proposal

- Literature review on similar experiments
- Talk to colleagues
- Research available instruments worldwide
- Contact instrument scientist and ask questions!
    - *instrument configuration*
    - *sample environment*
    - *time required*
    - *…*
- Decide on proposal type
- Preliminary sample characterisation?

# Before writing a proposal

- Literature review on similar experiments
- Research available instruments worldwide
- Talk to colleagues
- Contact instrument scientist and ask questions!
    - *instrument configuration*
    - *sample environment*
    - *time required*
    - *…*
- Decide on proposal type
- Preliminary sample characterisation?
- How will neutrons answer my questions?

# Before writing a proposal

- Contact instrument scientist and ask questions!
- Have I done preliminary characterisation?
- Will neutrons answer my questions?

# the Proposal Process (in general)

- Two proposal calls per year
- Deadline is real!
- Technical reviews (by facility scientists) – feasibility, safety…
- Scientific Review
  - Classification is done by subject or technique
  - At least 2 reviewers per proposal by external experts
  - Panel meetings at facilities
  - Time recommended
- Final balance (eg. national funding)
- Letters sent out to PI's

# Things to keep in mind…

Scientific reviewers are not always experts in your specialty since science at the facilities is so diverse. So, don't assume they know everything.

Most reviewers spend 5-8 minutes per proposal! Many will not have time to read through the references!

So, you must get all relevant information in the proposal.

Make your point, clearly and succinctly.

# Proposal Ingredients (Part I)

- User/participant information
- Title and abstract
- Sample description
- Sample environment requirements
- Instrument specs requested and time
- Publications, student thesis, scientific area, grants, submission status, safety…

# JCNS, Munich

**Proposal No. : 8744**
**Proposer :**
**Affiliation :**
**Short Name :**

**Subject**

| | |
|---|---|
| Title | |
| Scientifique area | Soft Condensed Matter |
| Grand Challenges | Soft Matter, Macromolecules, Complex fluids |
| Instrument | KWS-1 |
| Continuation of experiment No. | 7211 |
| Resubmission of proposal nr. | |

Rapid Access only available for instruments KWS-2, PGAA and SPODI.
Each accepted Rapid Access proposal will receive up to a maximum of 12 hours of beamtime.

| | |
|---|---|
| Rapid Access Proposal? | No |
| Internal beam time | No |
| Did you submit this proposal also to another facility? | No |
| Measuring time [days] | 1 |
| Abstract (max 200 words) | |

Title

Instrument

Time

Abstract

**Experimental team**

| | |
|---|---|
| Co-authors name, affiliation | |
| Local contact | |

User info

**Sample**

| | |
|---|---|
| Substance | deuterium oxide |
| Elemental formula | D2O |
| Sample type | liquid |
| sample size [mm] weight [mg] | 1 (mm) thickness, 20 (g) |
| Number of samples | 2 |
| Availability of samples | 2013-07-19 |
| Space group | |
| unit cell parameters | |

Sample info

**Sample environment**

| | |
|---|---|
| No sample environment needed | Yes |
| Cryostat | |
| High temperature furnace | |
| Pressure cell | |
| Magnetic field | |
| other sample environment | shear cell (Anton Paar) |
| Temperature range | |
| Temperature stability | |
| Pressure range | |
| Magnetic field | |

Sample environment info

**Security aspects**

| | |
|---|---|
| Toxic | No |
| explosive | No |
| radioactive | No |
| Sample gets activated | No |
| activity after experiment [Bq / isotope] | |
| Other risks | |

**Miscellaneous**

| | |
|---|---|
| Sample preparation laboratory (neutron guide hall) | No |
| Typ of work, materials, equipment in use | |
| Special technical support | No |
| Details(e.g. own equipment, special configurations, mechanics, control, software) | |

# ISIS, UK

Science & Technology Facilities Council

ISIS

## Experiment Proposal

Experiment Number: 920168

| | | |
|---|---|---|
| Principal investigator(*) | Dr V Garcia Sakai, STFC, United Kingdom | |
| Co-investigator | | |
| Co-investigator | | |
| Co-investigator | | |
| Co-investigator | | |
| Co-investigator | | |
| Co-investigator | | |
| Co-investigator | | |
| Co-investigator | | |
| Experiment Title | | |
| Instrument | IRIS/ OSIRIS | Days Requested: 7 |
| Access Route | Direct Access - Resubmission | Previous RB Number: - |
| Science Areas | Biology and Bio-materials | |
| Sponsored Grant | No | Sponsor: - |
| Grant Title | - | |
| Grant Number | - | |
| | Start Date: - | Finish Date: - |
| EU Access? | | |
| Similar Submission? | No | |
| Abstract | | |
| Publications | | |

User info

Title

Time & Instrument

Abstract

Instrument: IRIS Days Requested: 7 Experiment Number: 920168 Page: 1

## ISIS Sample record sheet

| | |
|---|---|
| Principal contact | Dr V Garcia Sakai, Victoria.garcia-sakai@stfc.ac.uk, Tel: 00-44-1235-446703 |
| Instrument | IRIS/ OSIRIS, 7 days, preferred contact is Garcia Sakai, V (Victoria.garcia-sakai@stfc.ac.uk) |
| Special requirements | - |

**SAMPLES**

| | |
|---|---|
| Material | protein |
| Formula | - |
| Forms | Solid |
| Volume | 1 cc |
| Weight | - |
| Container / substrate | - |
| Storage requirements | - |
| Xtal details | |

Sample info

**SAMPLE ENVIRONMENT**

| | |
|---|---|
| Equipment | CCR |
| Temperature range | 10-330 K |
| Pressure range | - |
| Magnetic field range | - |
| Special equipment | - |

Sample environment info

**SAFETY**

| | |
|---|---|
| Hazards | - |
| Hazard details | - |
| Sample sensitivity | - |
| Experimental hazards | - |
| Sample prep hazards | - |
| Equipment hazards | - |
| Prep lab needed | Yes |
| Special equip reqs | - |
| Sample will be | Removed By User |

# NCNR, USA

**NIST Center for Neutron Research**

**Proposal for Neutron Beam Experiment**

Submission ID:13104 Proposal Number: E23-19

**Experiment Title**

**Title:** Dynamics of phospholipid vesicles in the presence of bioprotectants

Title

**Proposal Type:** New Proposal

**Time Received:** 21-MAR-08 17:52

**Scheduling**

**Desired Dates:** 07-01-2008 to 12-31-2008

**Impossible Dates:**

**Estimated Duration:** 6 days

Time

**Participants**

User info

| | Name | Address | Country | Telephone/e-mail |
|---|---|---|---|---|
| Principal Investigator | Garcia-Sakai, Victoria | Rutherford Appleton Laboratory<br>ISIS Facility<br>Chilton, Didcot<br>Oxon, OX11 0QX | United Kingdom | 000-000-0000<br>victoria.garcia-sakai@stfc.ac.uk |
| User 2 | Nanda, Hirsh | National Institute of Standards and Technology<br>NIST Center for Neutron Research<br>100 Bureau Drive, MS6102<br>Gaithersburg, MD<br>20899-6102 | United States | hirsh.nanda@nist.gov |

**Instrument**

| | |
|---|---|
| Instrument Requested: | NG-5 -- NSE, Neutron spin echo spectrometer (CHRNS) |
| Suggested Local Contact: | Antonio Faraone |
| Instrument Resolution: | |
| Instrument Configuration: | Default instrument configuration |

Instrument

**Sample Description**

| | Sample 1 |
|---|---|
| Name | DPPC/D2O/maltose |
| Chemical Formula | |
| Mass (grams) | |
| Form | Liquid |



Sample info

| | |
|---|---|
| Temperature Measurement Range (K) | 300-330 |
| Number of Runs | |
| Total Collection Time (hrs) | |
| Sample Availability | 2008-03-01 00:00:00.0 |

| | Sample 2 |
|---|---|
| Name | DPPC/D2O/sucrose |
| Chemical Formula | |
| Mass (grams) | |
| Form | Liquid |
| Temperature Measurement Range (K) | 300-330 |
| Number of Runs | |
| Total Collection Time (hrs) | |
| Sample Availability | 2008-03-01 00:00:00.0 |

**Sample Environment**

**Sample Environment Equipment:**

Sample environment info

**Special Requirements**

Please describe any non-routine needs for sample temperature, magnetic field, etc., or other ancillary equipment. Specify any equipment needed at NIST for sample loading, treatment, storage, etc. (inert atmosphere, refrigeration, dry box, etc.). Also describe any equipment you plan to bring to NIST.

**Safety**

Check *at least* one box that describes your sample

[X] No Hazards

[] Toxic [] Corrosive [] Radioactive [] Explosive [] Flammable

If there are any hazards associated with your proposed experiment, please indicate how any risks are to be handled.

**Categorization**

For reporting purposes, please categorize your proposal:

| | |
|---|---|
| Research Area: | Biomolecular Science |
| Funding Agency: | NRC and STFC UK |

**Publications**

# Proposal Ingredients (Part II)

Two-page description of proposed research (incl. references)

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- Brief background, state the problem clearly and why the experiment is important, why it will make a difference – **Why should one care?**

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- Clear justification of need for neutrons and particular instrument- **why do you need beamtime on X?**

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- Evidence team's productivity and experience – **Will they publish in a timely manner?**

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- Description of data analysis/modelling – **What will you do with the data?**
- Evidence team's productivity and experience – **Will they publish in a timely manner?**
- **Be clear and specific – not vague and general!**
- **Think of yourself as a reviewer! What would annoy you?**

# 2-page case including references and figures/tables

FRM II
Forschungs-Neutronenquelle
Heinz Maier-Leibnitz

User Office
Lichtenbergstr. 1, 85748 Garching/ Germany
Tel.: +49.(0)89.10703/ 10794
Fax: +49.(0)89.10799
Email: userinfo@frm2.tum.de Web: user.frm2.tum.de

## SUBMISSION OF A PROPOSAL

**Experiment Title**

**Proposer**

Name
Email
Affiliation
Co-Proposers

**Scientific background and detailed description of the proposed experiment**

**Abstract (~100 words)**

**Introduction**

**Reference**

[1] K. Sadakane, A. Onuki, K. Nishida, S. Koizumi, and H. Seto, *Phys. Rev. Lett.*, **103**, 167803 (2009). [2] A. Onuki, *J. Chem. Phys.*, **128**, 224704 (2008).

**Previous results**



FRM II
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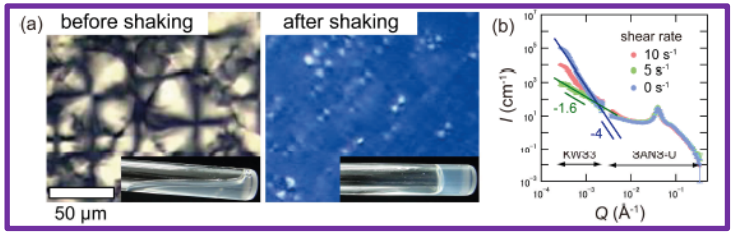
Email: userinfo@frm2.tum.de Web: user.frm2.tum.de




**Aim of proposed work**

**Proposed experiments**

Here is our experimental plan:

1) Instrument: KWS1 with rheo-meter (Anton Paar)
2) Shear-rate: 0 $s^{-1}$, 0.1 $s^{-1}$, 1 $s^{-1}$, 3 $s^{-1}$, 5 $s^{-1}$, 7 $s^{-1}$, 10 $s^{-1}$, 50 $s^{-1}$, 100 $s^{-1}$, 1000 $s^{-1}$
3) The measured spatial domain: $Q$ = 0.003 $Å^{-1}$ to 0.3 $Å^{-1}$
4) Sample: (i) $D_2O$ / 3-methylpydine / $NaBPh_4$
   (ii) $D_2O$ / $C_{14}E_5$
5) Temperature: 298 K

We assume that one measurement takes 45 minutes (15 minutes at high-$Q$ and 45 minutes at low-$Q$ region).Then, the total measurement time is estimated as
0.75 (hours) × 10 (shear rate) × 2 (samples) × 1 (temperature) = 15 (hours).
Additionally, we need 8 hours for setting rheo-meter and changing the detector length.
Threfore, we request 1 days beam-time.

**Your publication record (give references to papers published in the last two years arising from experiments at FRM II instruments)**

There is no paper arising from experiments at FRM II instruments.

# 2-page case including references and figures/tables

SANS Study (

Proposers:

**Introduction**

**Experiment**



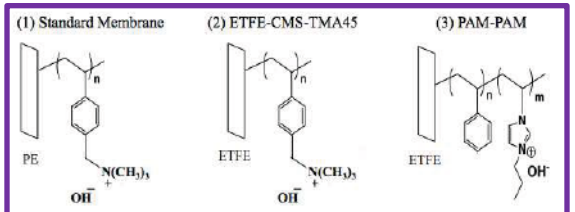



Figure 1. Molecular structure of the membranes used in this study.

Table 1. Characterization of the samples.

| Sample | Grafting degree (%) | IEC (mmol/g) | σ (mS/cm²) | Density (Dry) (g/cm³) |
|---|---|---|---|---|
| (1) | 103 | 1.8 | 15 | 1.02 |
| (2) | 45 | 1.2 | 30 | 1.56 |
| (3) | 80 | 2.0 | 10 | 1.47 |

# 2-page case including references and figures/tables

**Changes in lipid dynamics induced by melittin absorption on membrane surfaces**

The rise of infectious bacterial strains resistant to current antibiotic treatments is a growing concern universally. This has spurred an intensified interest both in the discovery and understanding of naturally occurring anti-microbial agents and the molecular mechanism by which they function. Most anti-microbial compounds associate with the cellular membrane and disrupt the delicate electro-chemical balance required for bacterial cellular life. One such naturally occurring molecule is melittin (MLT), found in the venom of honeybees. MLT posses many characteristics shared among known anti-microbial peptides. It is a single domain a-helix with a strong amphipathic quality (Fig. 1a). Structural studies from X-ray diffraction experiments [1] show partitioning into the lipid membrane of cells - intercalating with the headgroup region (Fig. 1b). Significant perturbations to the lipid chains are also observed: a thinning of the hydrocarbon region as well as a broadening of the terminal methyl distribution suggest an increase in chain disorder due to MLTs presence. At higher concentrations, MLT fully penetrates the membrane as self-assembled helical bundles that form large pores in the membrane, leading to cell death.

Detailed structural data from diffraction experiments has helped elucidate the function of MLT. However the mechanism for biological activity stems from the dynamics. We propose to use quasielastic neutron scattering (QENS) to characterize the changes in mobility of a model dioleoylphosphatidylcholine (DOPC) phospholipid membrane, in the presence of MLT. The protein:lipid system will be divided into three major components, the phospholipid headgroups, the lipid hydrocarbon tails and the MLT itself. Selective deuteration will allow us to follow the mobility of each of the three components separately. Regions of lipid that interact with MLT the most will be identified by comparison of dynamical changes with the pure DOPC bilayer measurements. Furthermore a study combining molecular dynamic (MD) simulations with neutron results on a similar system [2] suggests that regulating the mobility of phospholipid headgroups controls melting transitions. Measuring the effect on the membrane Tm provides another method for probing the balance between headgroup and chain interactions with MLT.

Previous QENS measurements of ordered lipid systems have used a combination of several dynamic models to describe motions in the ps to ns range of accessible time scales [1,3-4]. Given the sub ns dynamic range of the IRIS backscattering instrument our experiments will primarily be sensitive to methyl rotations, dihedral isomerization and localized diffusion (Fig. 2a). Despite the use of selective deuteration, the dynamical processes are still complex and may prove difficult to dissect into their individual contributions. Therefore, we will use an experimentally validated MD simulation [5] to provide a powerful method for aiding in the interpretation of QENS data, since there is total overlap in time and length scales accessed by both methods. Preliminary analysis of a DOPC/MLT simulation already provides some insights into potential perturbation in lipid dynamics caused by the peptide. Fig. 2b shows a snapshot of the simulation in which lipids within the vicinity of the protein are either highly kinked or extended. Furthermore the less mobile headgroups adjust their packing behavior around MLT. The results already suggest a possible framework for interpreting QENS data for this system.

We propose to perform experiments on the following samples:

(1) Fully hydrogenated DOPC [hh-DOPC]
(2) Fully hydrogenated DOPC with melittin [hh-DOPC+h-melittin]
(3) Hydrogenated head-group DOPC [hd-DOPC]
(4) Hydrogenated head-group DOPC with melittin [hd-DOPC+h-melittin]

The experiments proposed are presented in turn below:

(a) Elastic window scans (10-350K): elastic scans will give us a number of preliminary results. A comparison of the scans of the non-labeled lipid with and without MLT (samples 1,2), will show changes in Tm and in the dynamic regimes within the timescale of the IRIS spectrometer. Comparing head labeled with fully hydrogenated (signal dominated by tail protons) DOPC will indicate if the gel-to-fluid transition is characteristic to a specific part of the lipid (samples 1,3). Addition of the MLT to the labeled DOPC will show any differences in mobility in the presence of MLT that are specific to the individual components of the lipid (samples 2,4). Finally, mean-square displacement data for all samples will reveal changes in the mobility of all three components in the system (all samples). Elastic scans will require 3 days.

(b) Dynamic runs: we propose to measure the dynamics of each of samples 3-6 at two temperatures, below and above Tm. The measurements will allow analysis of the mobility of the DOPC head and tail groups quantitatively (samples 1,3), allowing for precise assessment of their response to the addition of MLT (samples 2,4). These experiments require 4 days (assuming 12hr per temperature run based on sample quantities).

The samples will consist of multilayers of DOPC and DOPC/MLT mixtures containing 1.5 mol % MLT per mol DOPC, plated onto a series of silicon wafers. Around 15 wafers are stacked in an aluminium slab-shaped cell with the face area of the same dimensions as the neutron beam. Such a cell has already been used for experiments on the backscattering spectrometer at the NIST Center for Neutron Research. The cell is contained in a humidity chamber and the samples are kept at 66 % r.h. with a NaNO2 solution in D2O. Use of D2O allows minimization of incoherent scattering from the buffer and also from the exchangeable protons in the MLT. The concentration of MLT and the humidity is chosen to match the MD simulations and diffraction experiments.

We propose to use the IRIS spectrometer with the PG002 configuration at a resolution of 8.8 ueV (HWHM) and an energy range of 1.0 meV, giving us access to timescales between ca. 0.5-100 ps. The Q-range accessible is 0.3- 1.8 inv. Ang. These distances and times are directly comparable to the MD simulations. For the completion of the proposed work we are requesting a total of 7 days.

We note that this is a resubmission of RB 0720585 which was awarded 7days. Since then we have been trying to synthesize MLT and encountered some difficulties, thus we have not used our beamtime and we thought it would be better to resubmit. We now have a successful route for expressing MLT and will be ready to perform the experiment.

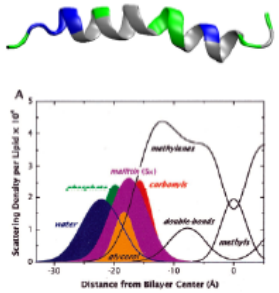



**Figure 1:** (a) The amphipathic MLT monomer is shown with polar residues in green, basic residues in blue and non-polar residues in grey. b) X-ray scattering length density profiles show MLT partitioning into the lipid headgroup region of a dioleoylphosphatidylcholine (DOPC) bilayer [1].

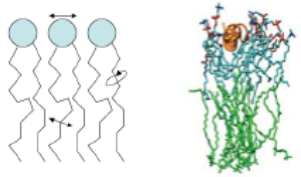


**Figure 2:** (a) A schematic of accessible motions on the sub ns time scale. Straight arrows represent localized mobility and circular arrows represent dihedral isomerization or terminal methyl rotation. (b) Snapshot of a DOPC/MLT MD simulation. Perturbation to lipid tail conformation and packing defects in lipid headgroups are evident.


References:

[1] K. Hristova et al, *Biophysical J.* **80** 801 (2001).
[2] M. Doxastakis et al, *Biophysical J.* **92** 147 (2007).
[3] S. König et al, *J. Phys II France* **2** 1589 (1992).
[4] S. König et al, *Biophysical J.* **68** 1871 (1995).
[5] R. W. Benz et al, *Biophysical J.* **91** 3617 (2006).

# 2-page case including references and figures/tables

*(If you have been allocated beam time through proposals to the NCNR during the past three years, please list the instrument and resulting publications)*


Local Polymer Dynamics in Polymer-C60 Mixtures J. M. Kropka, V. Garcia Sakai, and P. F. Green, Nanoletters (in press). Role of Hydration Water in Dynamics of Biological Macromolecules, A. P. Sokolov, J. H. Roh, V. García Sakai, and E. Mamontov, Chem. Phys. (in press). Dynamics of PEO in blends with PMMA: study of the effects of blend composition via quasi-elastic neutron scattering, V. García Sakai, J.K. Maranas, I. Peral, and J.R.D. Copley, Macromolecules (accepted). Direct Analysis of the Ion-Hopping Process Associated with the alpha-relaxation in Perfluorosulfonate Ionomers using Quasielastic Scattering, K. A. Page, J. K. Park, R. B. Moore, and V. Garcia Sakai, Macromolecules (submitted) Confinement Induces Both Higher Free Volume and Lower Molecular Mobility in Hydrogen Bonded Glass-Former, D. Kilburn, P. E. Sokol, V. García Sakai, and M. A. Alam, Applied Physics Letters (submitted) Role of Hydration Water in Dynamics of Biological Macromolecules, A. P. Sokolov, J. H. Roh, V. García Sakai, and E. Mamontov, Chem. Phys. (in press) Observation of a Dynamic Crossover in Water Confined in Double-Wall Carbon nanotubes, X.-Q. Chu, A. I. Kolesnikov, A. P. Moravsky, V. García Sakai, and S.-H. Chen, Phys. Rev. E (2008). Dynamics and Structure of Hydration Water on Rutile and Cassiterite Nano-powders Studied by Quasielastic Neutron Scattering and Molecular Dynamics Simulations, E. Mamontov, L. Vleck, D. J. Wesolowski, P. T. Cummings, W. Wang, L. M. Anovitz, J. Rosenqvist, C. M. Brown, and V. García Sakai, J. Phys. Chem. C, 111, 4328-4341 (2007). Microscopic Protein Diffusion at High Concentrations, S. Busch, W. Doster, S. Longeville, V. García Sakai, and T. Unruh, in Quasi-Elastic Neutron Scattering Conference 2006 (QENS 2006), edited by Paul E. Sokol, H. Kaiser, D. Baxter, R. Pynn, D. Bossev, and M. Leuschner (Mater. Res. Soc., Warrendale, PA, 2007), 107-114. Diffusion of Water in the Na0.3CoO2.1.4H2O Superconductor, V. García Sakai, E. Mamontov, J. W. Lynn, L. Viciu and R. J. Cava, Physical Review B, 75, 014505 (2007). A Molecular View of Melting in Anhydrous Phospholipidic Membranes M. Doxastakis, V. García Sakai, S. Ohtake, J. K. Maranas and J. J. de Pablo, Biophysical Journal, 92, 147-161 (2007). Local Dynamics of Syndiotactic Poly(methyl methacrylate) Using Molecular Dynamics Simulation, C. Chen, J. K. Maranas, and V. García Sakai, Macromolecules, 39, 9630-9640 (2006). The composition dependence of the segmental dynamics of poly(methyl methacrylate) in miscible blends with poly(ethylene oxide), J. Liu, V. García Sakai, J. K. Maranas and Z. Chowdhuri, Macromolecules, 39, 2866-2874 (2006). Local dynamics of syndiotactic poly(methyl methacrylate) using molecular dynamics simulation, C. Chen, J.K. Maranas, and V. Garcia Sakai, Macromolecules 39, 9630 (2006). Dynamic transition in tRNA is solvent induced, G. Caliskan, R. M. Briber, D. Thirumalai, V. García Sakai, S. A. Woodson, and A. P. Sokolov, Journal of American Chemical Society, 128, 32-33 (2006). Miscible Blend Dynamics and the Length Scale of Local Compositions, V. García Sakai, J. K. Maranas, Z. Chowdhuri, I. Peral, and J. R. D. Copley, J. Polym. Sci. Part B: Polym. Phys., 43, 2914 (2005).


## Description of Proposed Research

*(Please include scientific context; relevance of proposed experiment; preliminary work performed using neutron scattering and other techniques; details of proposed experimental approach; appropriate references.)*

Background: The interactions of sugars with biological molecules have been investigated thoroughly in an effort to understand the preservation and stability of biomolecules. Biological systems such as proteins, vaccines or cells must often be stored for extended periods of time and this is done by lyophilisation in solutions of lyoprotectants, which results in products that are stable under ambient conditions.[1-3] Phospholipids are one of the major structural components of cell membranes and have been studied extensively, primarily as phospholipidic liposomes, which serve as a simple membrane model. The stabilization of these liposomes by lyophilisation has been studied to gain insight into their behaviour at low hydration [2,4-9] It is suggested that the stabilization by sugars is achieved by their ability to prevent the increase in the gel-to-fluid transition temperature Tm, associated with dehydration [5]. Sugars prevent the occurrence of a phase transition during hydration and dehydration [10] which is crucial, since this leads to leakage across the bilayer membrane [11].

Despite the many experimental and numerical studies on sugar bioprotection and stabilisation, the molecular mechanisms remain unclear. There are three proposed mechanisms: (a) formation of a glass that maintains the conformation of the membrane and prevents dehydration-induced stresses and fusion (vitrification) [1, 6-7], (b) preferential exclusion of the sugars cause an increase in the surface tension of water, allowing the free water to hydrate the biomolecule [12] and water replacement hypothesis [13] where the sugars directly hydrogen bond with the polar head groups of the lipids and compensate with the loss of hydrogen bonds upon dehydration.

In the recent years, computational [14-16] and experimental studies [17-19] have provided new insights into lipid-sugar interactions. Simulations suggest that the interactions occur along the surface of the membrane and that the sugar preferentially partitions to the headgroup region, where it increases the density. The area per headgroup is only slightly altered. In contrast, sugars induce significant changes to the dynamics. Lipid mobility is reduced considerably as a result of the sugar molecules binding to the lipids. In particular, rotational and translational modes of the membrane are slowed down which suggests that the sugar inhibits changes to the lateral organisation of the bilayer component. This is vital in preventing leakage. Such studies were performed at low hydration.

Amongst the common sugars, trehalose has superior preservative effects [2] and accumulates to high concentrations in many anhydrobiotic organisms. Other dissacharides, such as sucrose, which is found in high concentrations in plants and maltose, also reduce the Tm but are not as effective as biopreservatives [13,22]. Although the three sugars have the same chemical formula, they have different structures, which could account for their differing preservation effectiveness and could provide insight to lipid-sugar interactions.

We have recently carried out experiments using the neutron spin-echo spectrometer on fully hydrated unilamellar vesicles of 1,2-dipalmitoylphosphatidylcholine (DPPC) in D2O with and without trehalose at temperatures below and above the melting transition (Tm = 42C) of DPPC. The data was analyzed using the Zilman-Granek theory to obtain values of the bending modulus and look for changes in the bending elasticity of the vesicles. The results are shown in figure 1. At all temperatures measured, trehalose stiffens the bilayer suggesting strong interactions between trehalose and the lipid bilayer. Trehalose appears to broaden the melting transition although it does not change the Tm. This agrees with observations using differential scanning calorimetry [18].

Objective:
With the proposed experiment we aim to characterize the changes induced by sucrose and maltose on the bending elasticity of hydrated DPPC vesicles and compare the results with those for trehalose. NSE will give us bending modulus as a function of sugar. We aim to confirm a possible relationship between preservation effectiveness and lipid-sugar interactions in the bilayer. We will conduct measurements at temperatures below and above the Tm of DPPC, so as to characterize the changes in each of the lipid phases. In this experiment we will focus on hydrated bilayers; lower hydrations will be measured at a later stage.

Experimental details:
We plan to use 100nm diameter uninamellar vesicles prepared by the extrusion method which gives a narrow size distribution of vesicles and almost entirely unilamellar vesicles. We will measure DPPC/D2O/sugar solutions of a lipid concentration of 1% by weight, and lipid to sugar molar ratio of 1:5. We plan to measure a total of six temperatures per sample, each temperature

taking approximately 9hrs. Including time to measure the resolution, the solvent and four temperatures for the pure lipid solution, we estimate we will need 7 days of beamtime for the completion of the experiment.

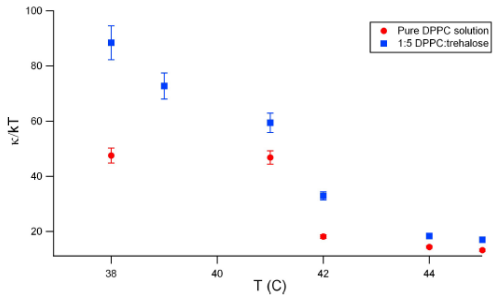



FIGURE 1. The effect of trehalose on the bending elasticity of DPPC unilamellar vesicles. The plot shows the temperature dependence of the bending modulus.

# Do's and Don't's'

- ✓ Use all space allocated
- ✓ Add readable figures/graphs
- ✓ Justify need for neutrons
- ✓ Add references
- ✓ Check before submission

- ✗ Use miniture font
- ✗ Include if they do not add to proposal
- ✗ Use generic arguments
- ✗ Expect reviewer to read
- ✗ Make silly mistakes

# Proposal Submission

- Online
- Read guidelines for given facility system
- Follow instructions carefully
- Meet the deadline (don’t play tricks!)


INSTITUT MAX VON LAUE - PAUL LANGEVIN (ILL)

**Guidelines for the scientific background and detailed description of the proposed experiment**

**(For electronic proposal submission only)**

**Please remove this first page before creating your post-script file**

The two pages of this form are to be filled in by all users or groups of users who apply for beamtime for experiments at the ILL via the Internet. Please print pages two and three of this document into a postscript file and attach it to your proposal on the **E**lectronic **P**roposal **S**ystem. This two-page description will be reduced by the system to a one-page, A4 format in black & white, and will be attached to your web proposal.

**When preparing your description, please follow the instructions below:**

- *Give a brief statement of the **background** and the general importance of the research.*
- *Give a clear account of the **aims of the proposed experiment** and a detailed description of the experiment; keep in mind that not all of the subcommittee members are experts in the field.*

# Success …

… depends on many factors:

- Quality of proposal
- Days available
- Oversubscription
- Committee's feeling about high risk-high reward proposal versus unexciting but definite publication
- Mood, tiredness…
- Country balances
- …