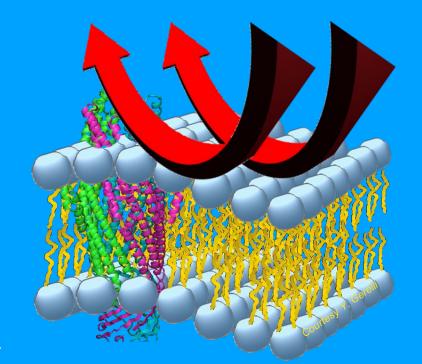
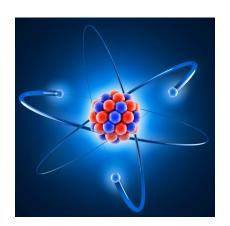


## Neutron Scattering – Exploring Cell Membrane Mechanisms

Giovanna Fragneto **European Spallation Source ERIC** 





### **Neutrons**

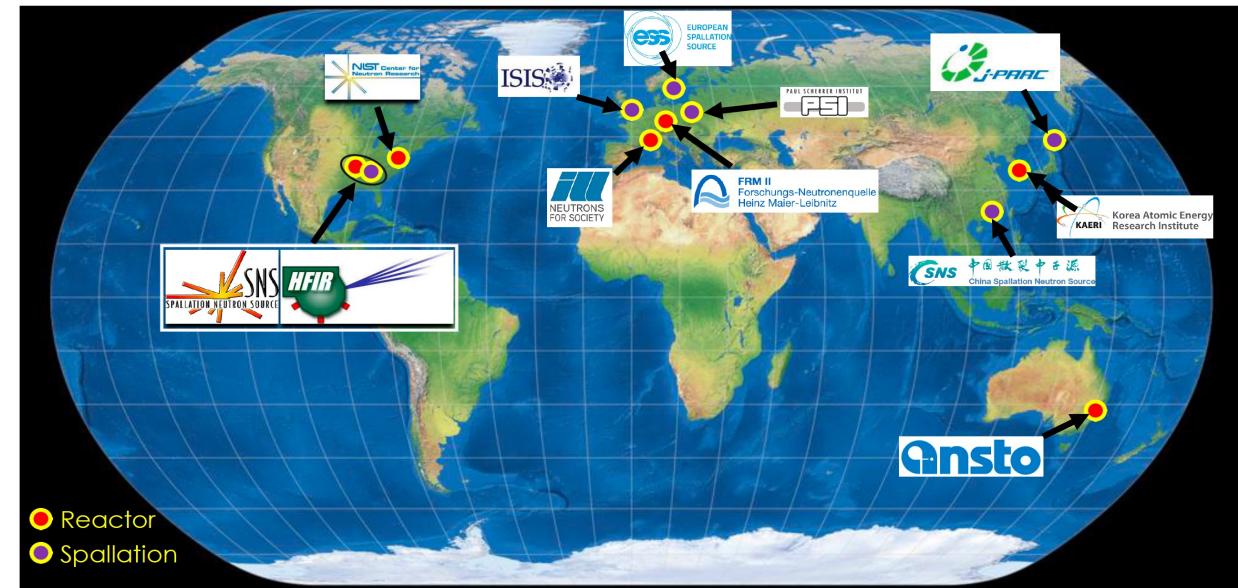


- Subatomic particles found in atomic nuclei
- Discovered in 1932 by Chadwick
- Mass slightly higher than that of protons ( $\sim 1.67 \times 10^{-27} \text{ kg}$ )
- Uncharged
- Lifetime ~15 mins
- Wave-particle duality (v = 2.2 km/s at RT)
- Wavelength similar to atomic distances ( $\lambda = 0.18$  nm at RT)
- Energies similar to motion of molecules (~ 0.025 eV)
- Possess a spin

## **Generating neutrons**

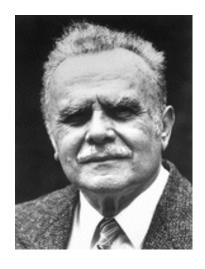


Neutron Sources around the world



### **Nobel Prize in Physics, 1994**

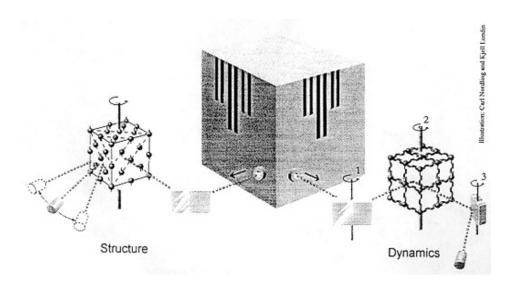
A unique probe of 'where atoms are and what atoms do' - to paraphrase the citation for the Nobel Prize in Physics awarded to Brockhouse and Shull in 1994



Bert Brockhouse Dynamics (Neutron Spectroscopy)

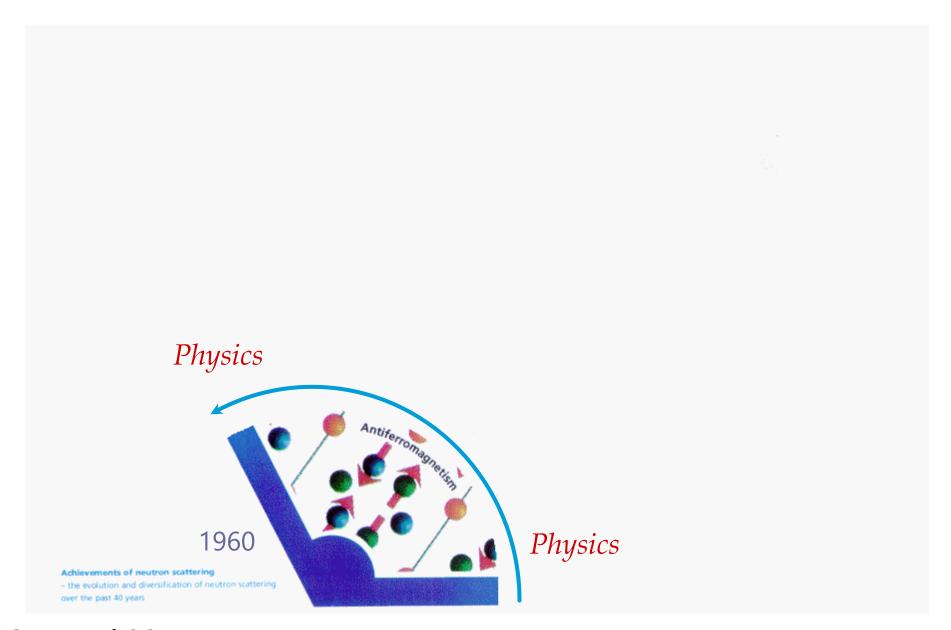


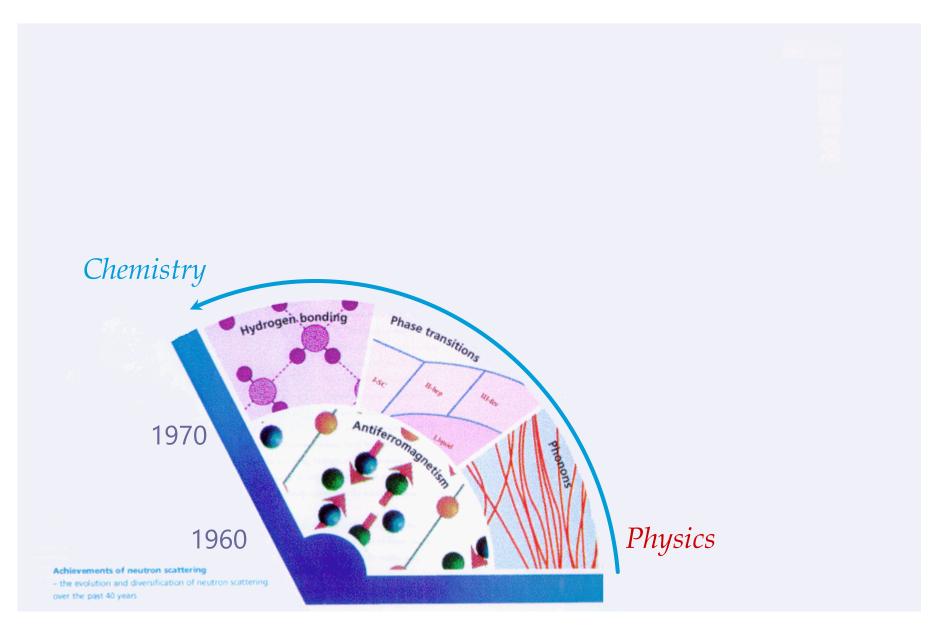
Cliff Shull Structure (Neutron Diffraction)

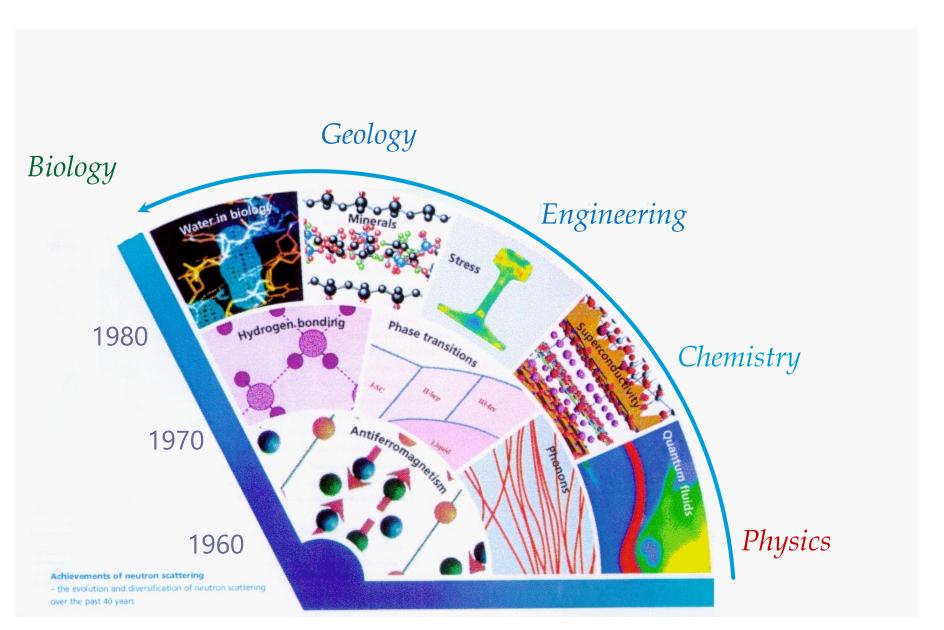


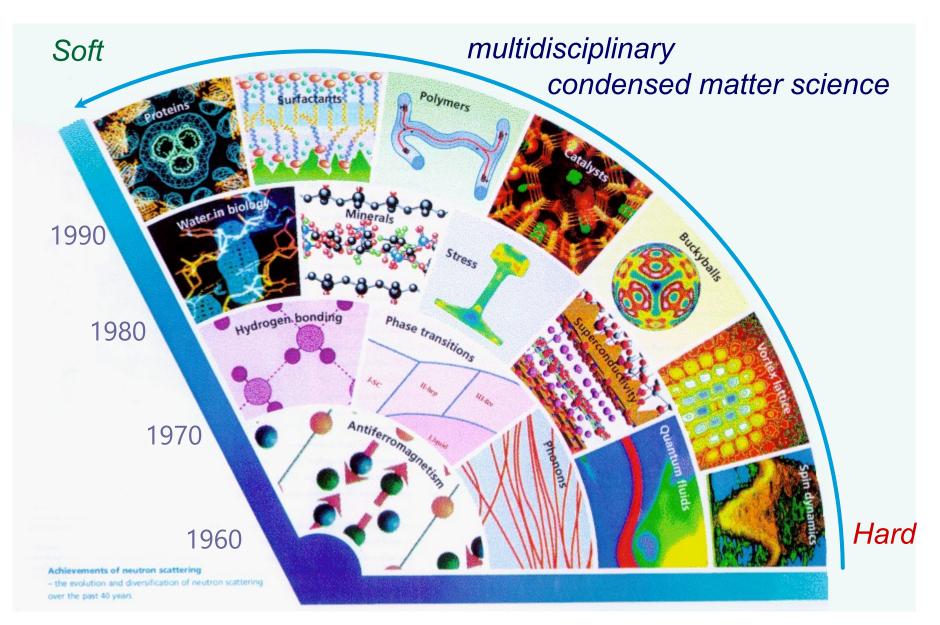
### 12 Oct 1994 Press Release

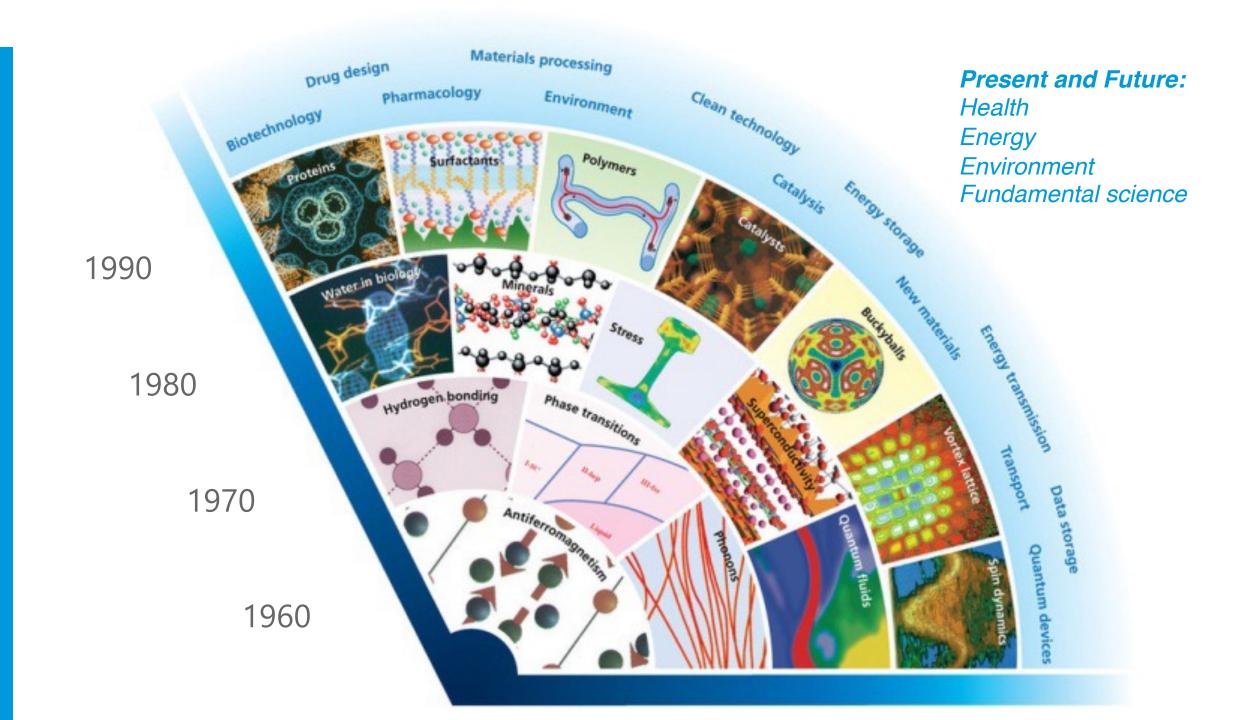
(...) Both methods are based on the use of neutrons flowing out from a nuclear reactor. When the neutrons bounce against (are scattered by) atoms in the sample being investigated, their *directions* change, depending on the atoms' relative positions. This shows how the atoms are arranged in relation to each other, that is, the structure of the sample. Changes in the neutrons' *velocity*, however, give information on the atoms' movements, e.g. their individual and collective oscillations, that is their dynamics.





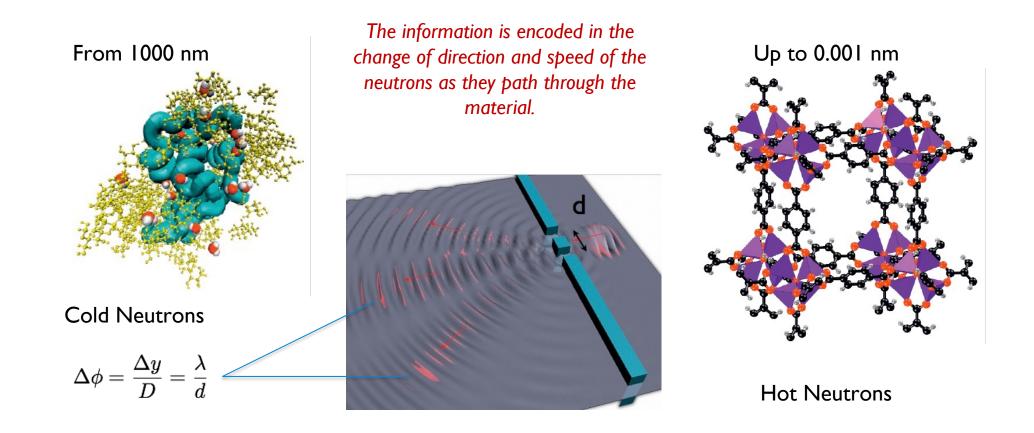






## Neutrons: An ideal probe at the atomic scale

- Like X-rays thermal neutrons possess the right wavelengths (structures  $\mathring{A}$  to  $\mu m$ ).
- They possess the ideal energies for spectroscopy of thermal fluctuations (dynamics ps to hr).

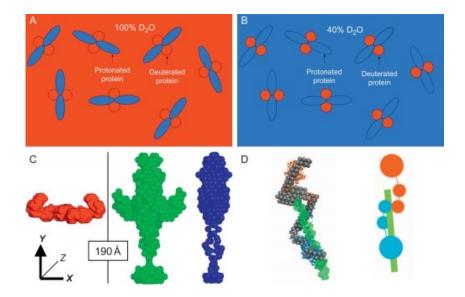


### Why use neutrons to study soft an biological material?

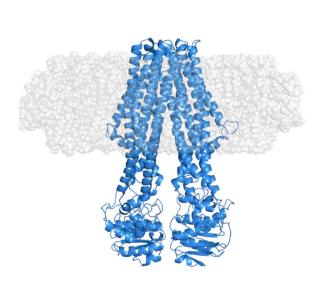


### Neutrons interact with nuclei

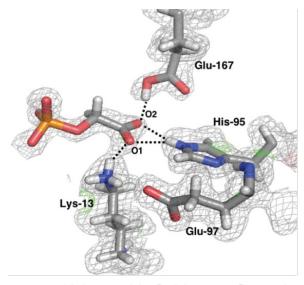
- are sensitive to light atoms, particularly hydrogen
- can exploit isotopic substitution, especially H/D
- 'see' materials differently to X-rays, complementary
- no markers



Haertlein et al. Methods in Enzym. 2016.



losts et al. Structure 2018



Xrays

neutrons

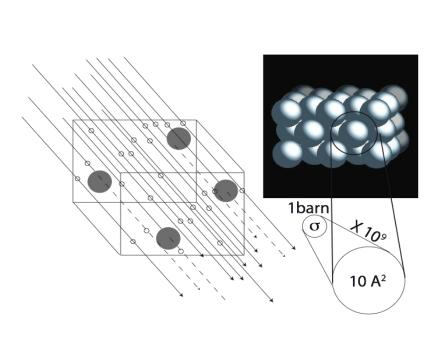
Kelpsas, V., Caldararu, O. et al. (2021) *IUCrJ* **8** 633-643

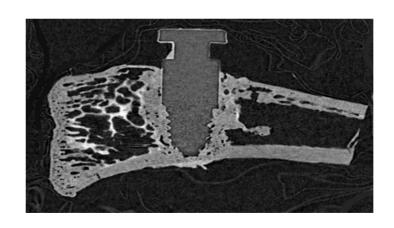
## Neutrons are a neutral particle

0 sec

• are highly penetrating - imaging of light elements deeply buried in materials

• can be used as non-destructive probes



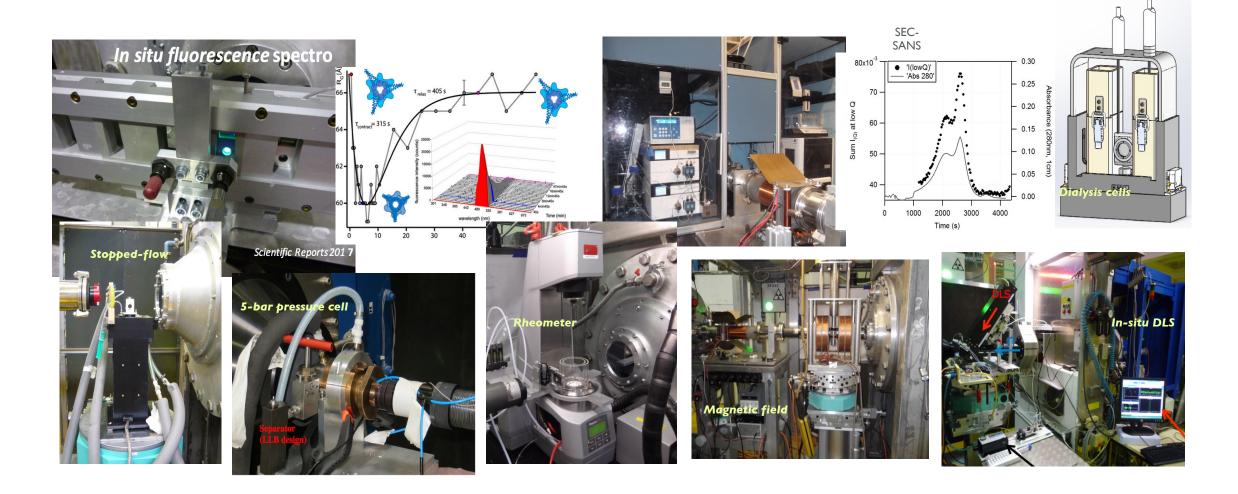


Bone Structures + implants



## Neutrons are a neutral particle

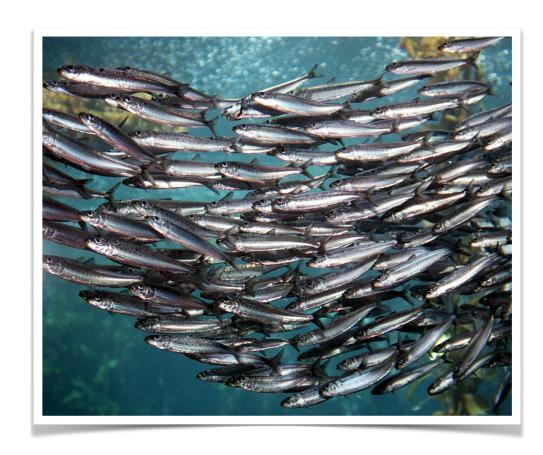
- are highly penetrating  $\rightarrow$  buried interfaces
- can be used to study samples in extreme environments and allow use of in-situ complementary techniques



### Why use neutrons to study soft and biological material?

### Non destructive

Possibility to work in physiological conditions





**Neutrons** 

VS.

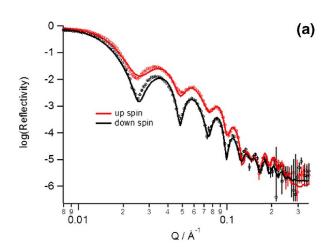
Some other methods

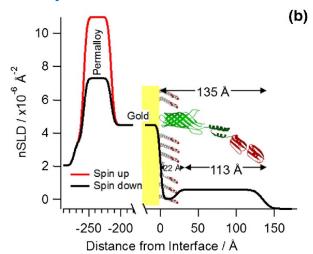
## Neutrons have a magnetic moment and spin

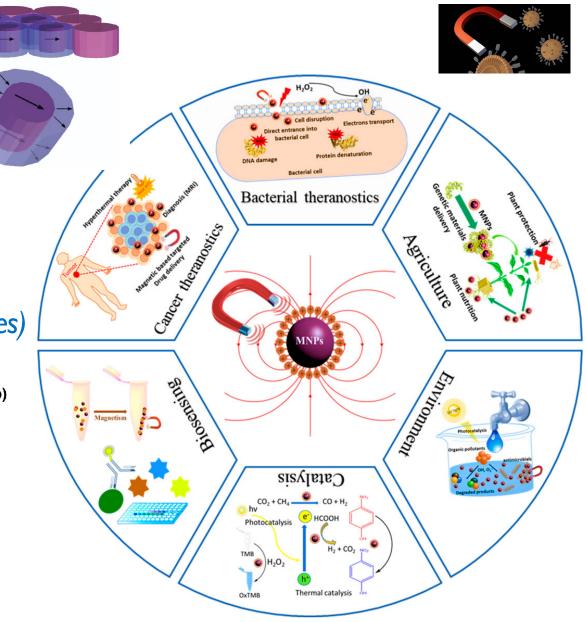
- study microscopic magnetic structures
- study magnetic fluctuations, and
- develop magnetic materials
- formed into polarised beams,
- study nuclear (atomic) orientation, and
- separate coherent from incoherent scattering

• magnetic systems in soft matter (magnetic nanoparticles)

• magnetic contrast in reflectometry

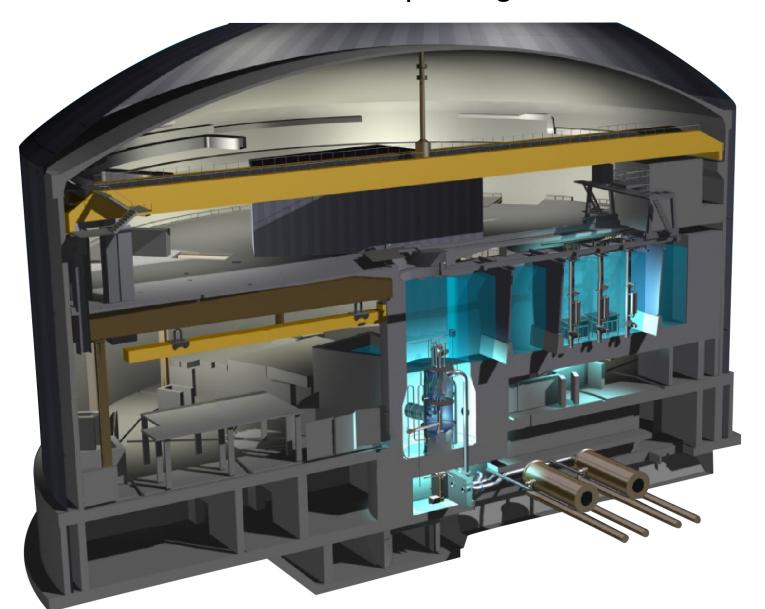


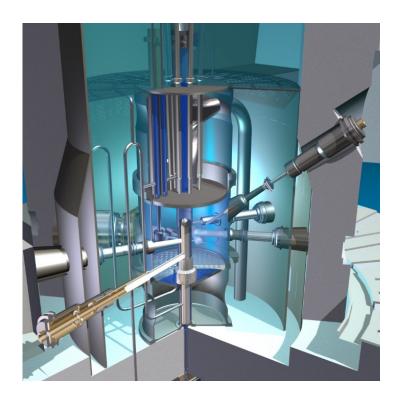




## The ILL benefits from the world's most intense continuous neutron source operating since 1971



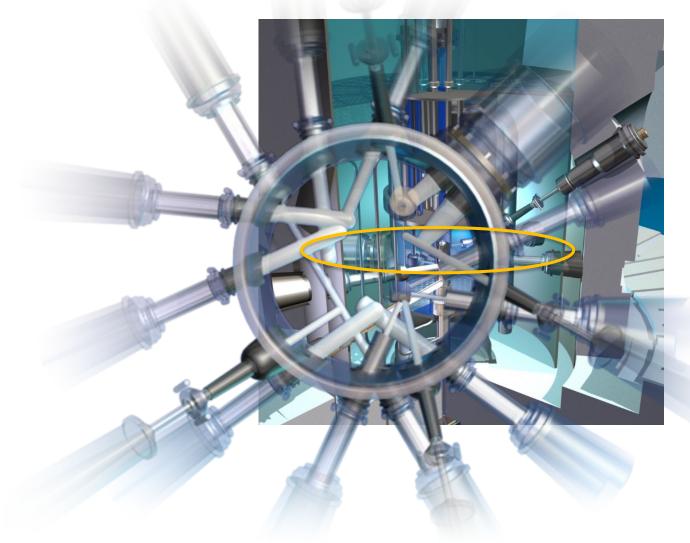




A neutron source generating  $5 \times 10^{18}$  fast neutrons/sec at a max power of 58 MW

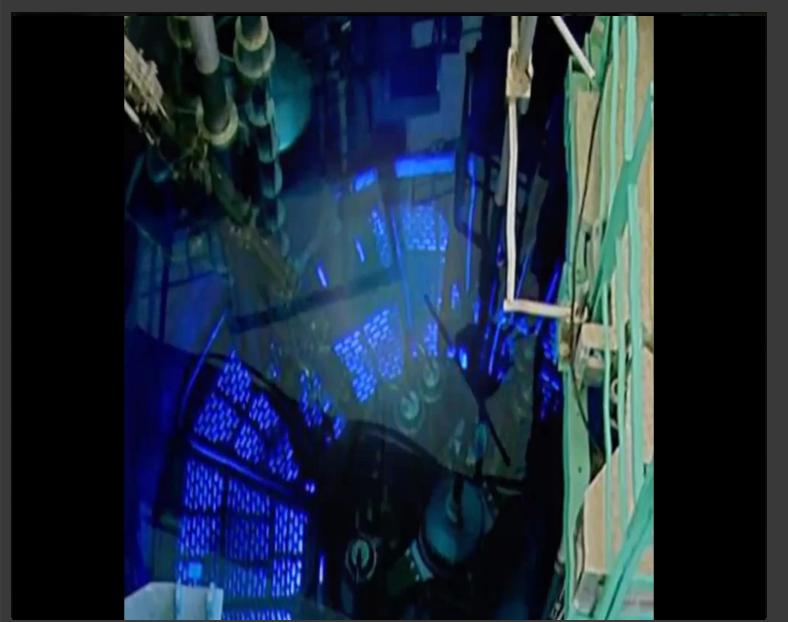
### HOW NEUTRONS ARE EXTRACTED AND GUIDED

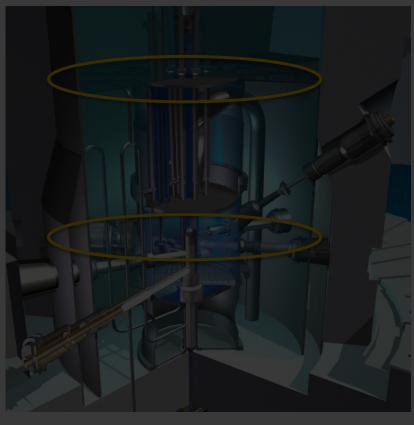




## HOW NEUTRONS FEED THE INSTRUMENT SUITE

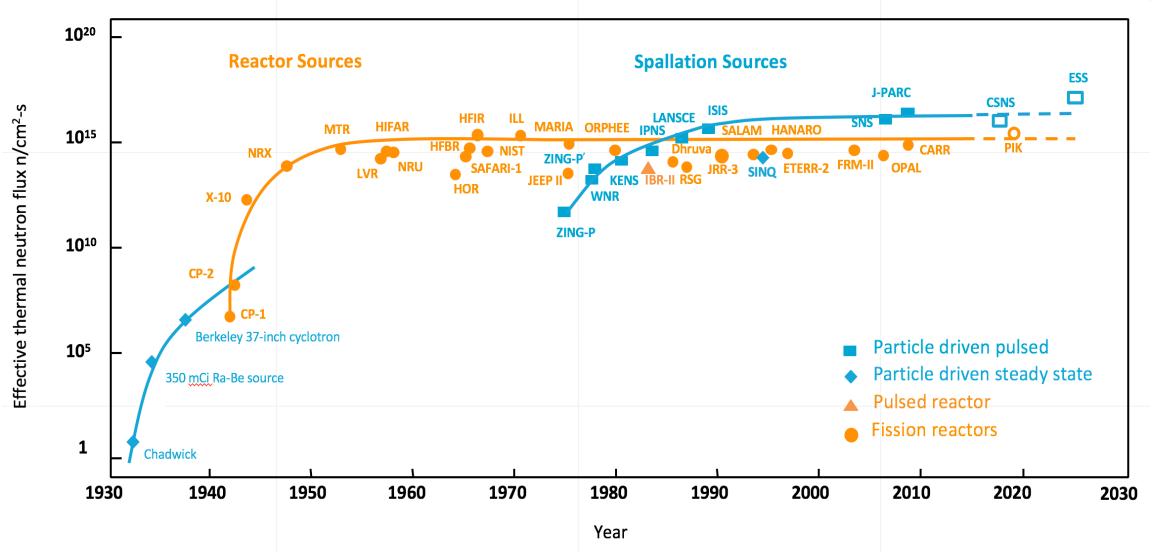






## Neutron Source Brightness





## The European Spallation Source Project



2027-2028 Where, when, why, how, .... User science program 2014 Construction starts on green 2025 2009 field site First neutrons Decision to site **ESS** in Lund 2019 Start of initial 2012 operations phase ESS design update phase complete

2003
European design
of ESS completed

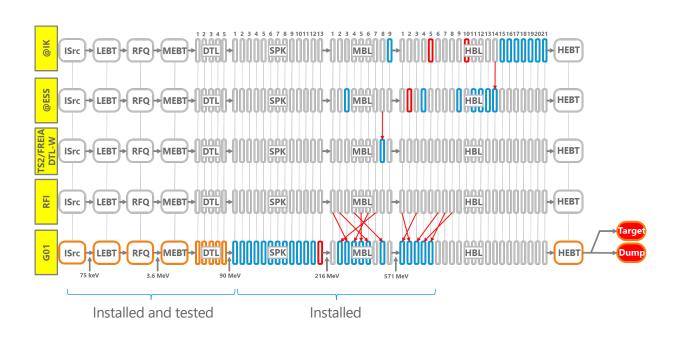
Long-pulse driven by linear accelerator pulse length 2.86 milliseconds

14 Hz, 0.8 – 2 GeV, 2-5 MW proton beam power TARGET MONOLITH 4.9 TONNES **TARGET WHEEL** High energy neutrons are The neutrons are slowed released from the target. down, focused and sent down the beamlines. EXPERIMENTAL SCIENTIFIC INSTRUMENT **ACCELERATOR** ...until they Particles are They are accelerated produced in to 96% of the speed hit the target. the ion source. of light in the 600 m long accelerator... LABORATORIES EXPERIMENTAL HALL 2 When the neutrons arrive at the EXPERIMENTAL SCIENTIFIC instruments, the researchers can use them to explore materials down to an atomic level.

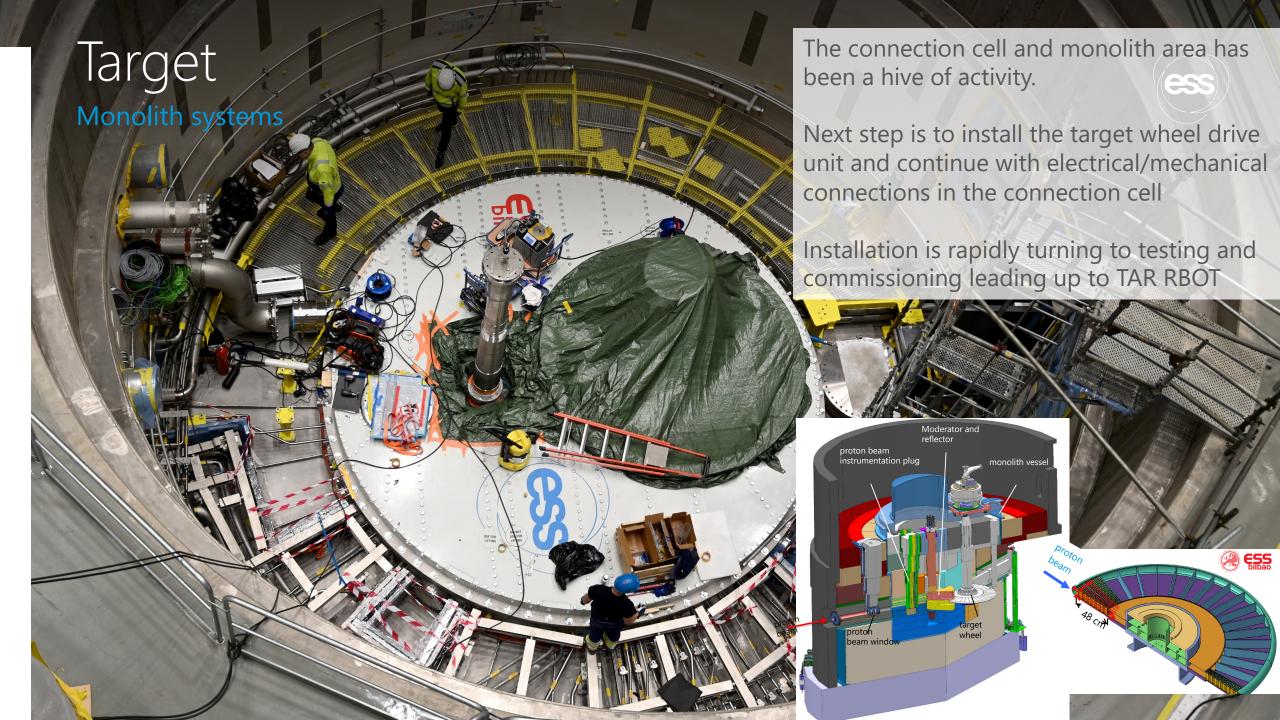
## Accelerator

### Installations progressing

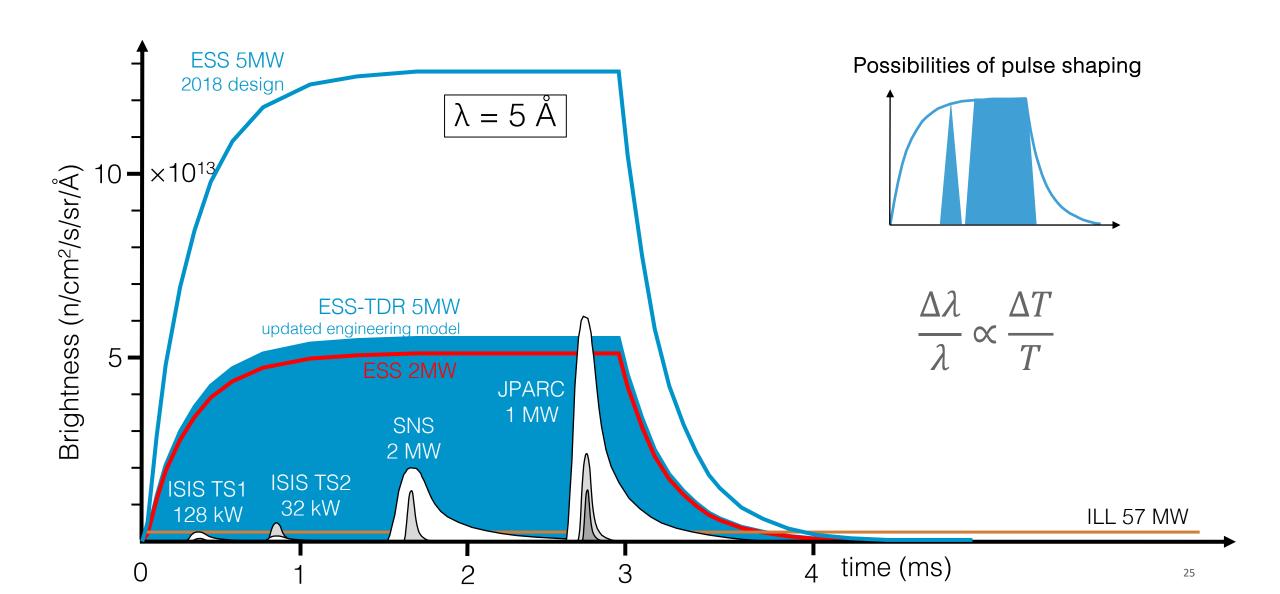
- Almost a full compliment of CMs ready for BOD/BOT
- Gallery support systems are in good shape. All RF racks needed for BoT have been energized, now being soak tested.
- A2T region nearing completion







## Long-pulse Performance and Flexibility



## Neutron Instruments

Andersen, K. H.; Argyriou, D. N.; Jackson, A. J. et al. The Instrument Suite of the European Spallation Source. Nuclear Instruments and Methods in Physics Research Section A: 2020, 957, 163402.

https://doi.org/10.1016/j.nima.2020.163402.

#### 15 instruments + Test Beamline

Diffractometers (DREAM, MAGIC, HEIMDAL) SANS (LoKI, SKADI)

Reflectometers (Estia, FREIA)

Imaging (ODIN)

Engineering Diffraction (BEER)

Macromolecular Crystallography (NMX)

Spectrometers (CSPEC, T-REX, BIFROST, MIRACLES, VESPA)

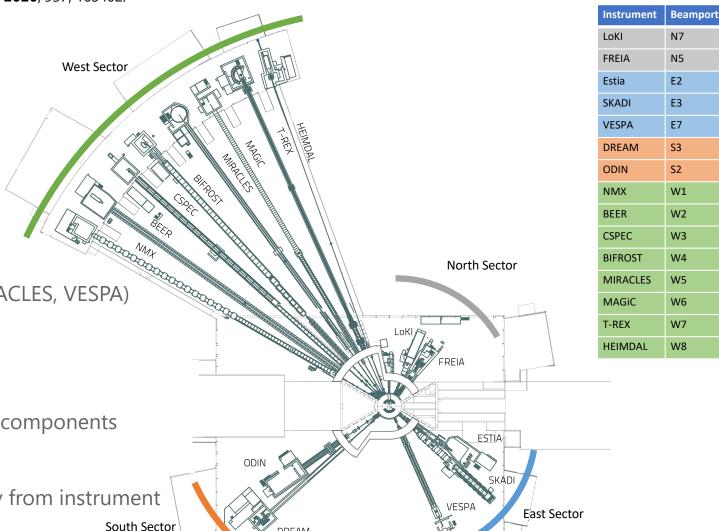
*Novel detector technologies and geometries* Complex pulse-shaping

Shared neutron bunker – common space for components

Common timing system for facility

Single controls infrastructure (EPICS)

Control and data recording running remotely from instrument



N7

N5

E2

E3

E7

S3

S2

W1

W2

W3

W4

W5

W6 W7

W8

26

## Neutron Instruments for soft/bio science

Andersen, K. H.; Argyriou, D. N.; Jackson, A. J. et al. The Instrument Suite of the European Spallation Source. Nuclear Instruments and Methods in Physics Research Section A: 2020, 957, 163402.

https://doi.org/10.1016/j.nima.2020.163402.

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*Novel detector technologies and geometries* 

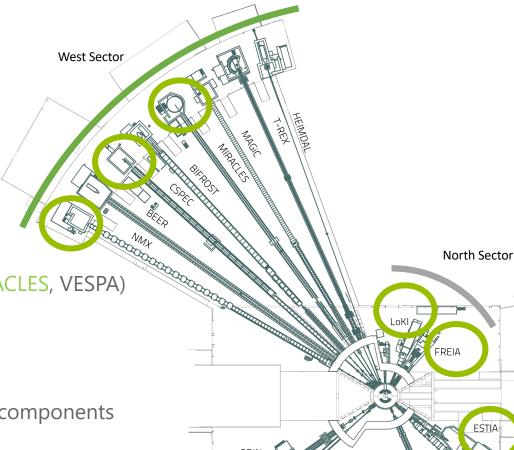
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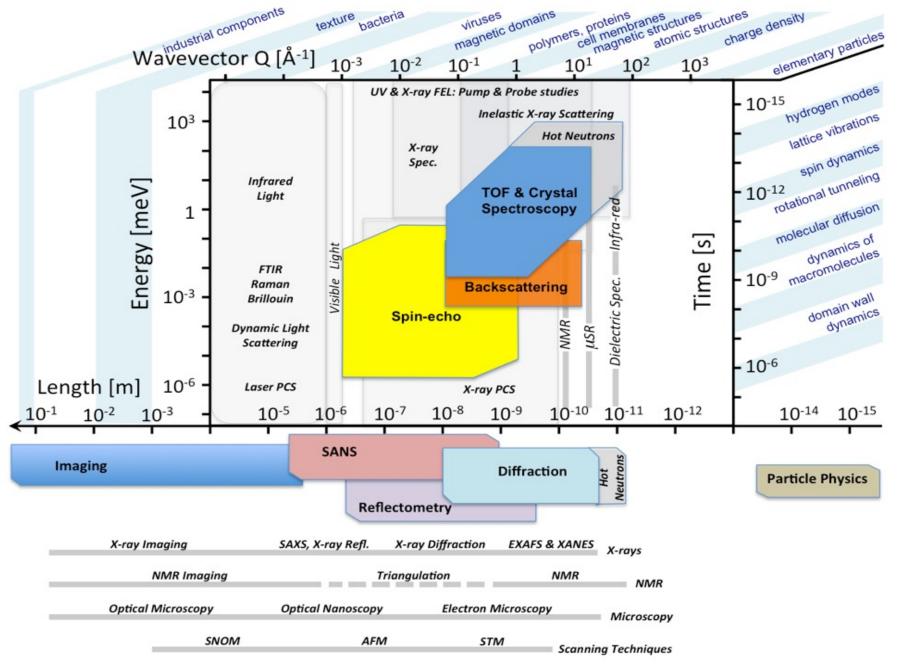


South Sector



Instrument	Beamport
LoKI	N7
FREIA	N5
Estia	E2
SKADI	E3
VESPA	E7
DREAM	S3
ODIN	S2
NMX	W1
BEER	W2
CSPEC	W3
BIFROST	W4
MIRACLES	W5
MAGIC	W6
T-REX	W7
HEIMDAL	W8

East Sector

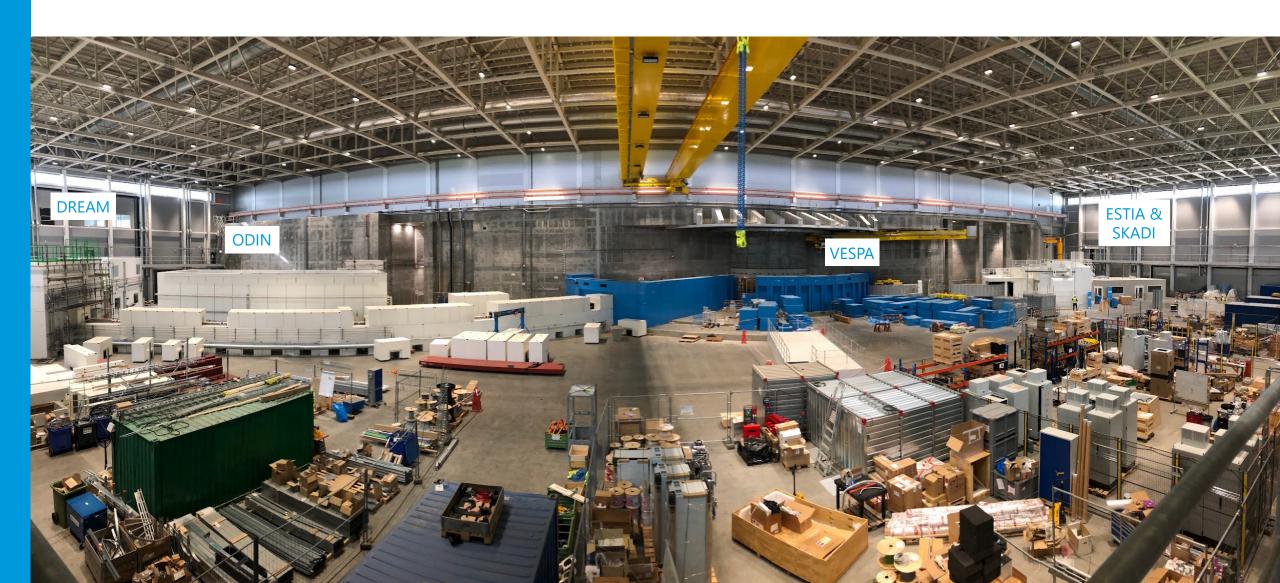


Instruments are optimised to access the desired length and time scales.

## Neutron Instruments at ESS

D01 side

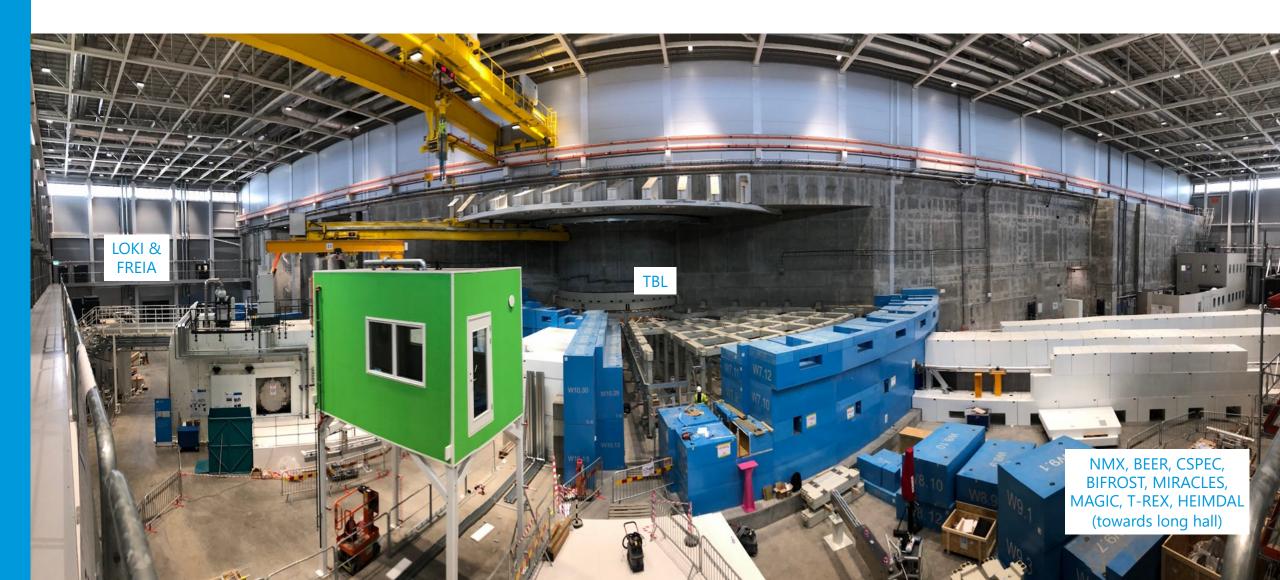




## Neutron Instruments at ESS

D03 side









The current focus is getting LOKI, BIFROST, ODIN, DREAM, NMX and TBL ready for BOT

In bunker components for later instruments are also being prioritized to limit future bunker work

Cave installations for ESTIA, SKADI and MAGIC ongoing





### materials and bio-science

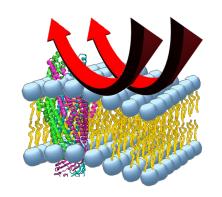


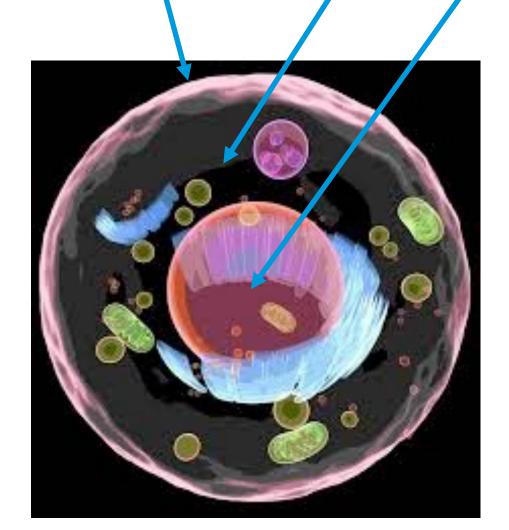
## Mechanisms at cell membranes

Cells are the smallest living units of an organism

All cells have three things in common, no matter what type of cell they are:

a cell membrane, cytoplasm and DNA





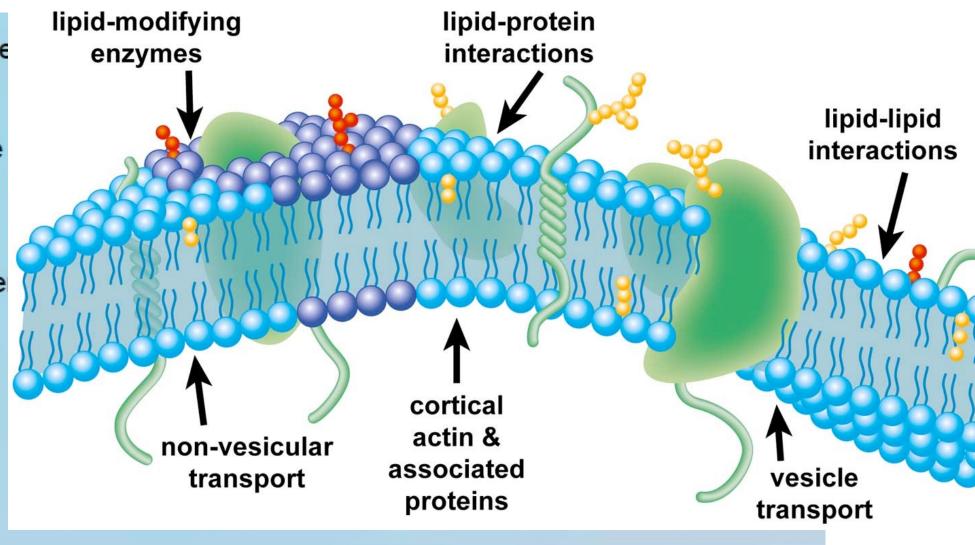
## Mechanisms at cell membranes

Complex assemblie of lipids & proteins

Exhibit short range but not long range order

High degree of late heterogeneity

Transversely asymmetric





Lipid scaffold composed by a large variety of lipid species and levels of chain unsaturation, often difficult to synthesise chemically. Because of the complexity model membrane systems are used for fundamental studies.

### Why so much interest for membranes and lipid bilayers

### Total surface of membranes covers an area of 30,000 m<sup>2</sup> in our body

Function of membrane proteins: dependent on membrane composition, lipid-protein interaction, lipid mediated protein-protein interaction

Pharmacological interest: Drug transport through membranes (dependent on physico-chemical membrane properties), anti-microbial peptides

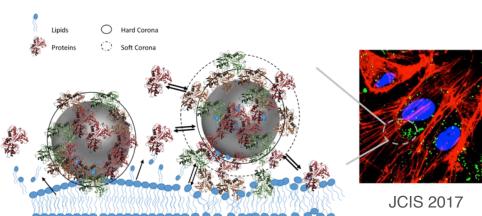
Membranes may play a direct role in signal transduction

Diseases associated with changes in lipid composition (diabetes, schizophrenia, Tay-Sachs syndrome, Alzheimer, Parkinson)

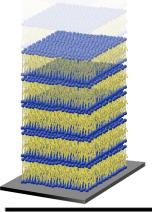
### Cell adhesion

Nano-biotechnology applications (biosensors, bio-coatings)

and .... fascinating chemistry and physics!

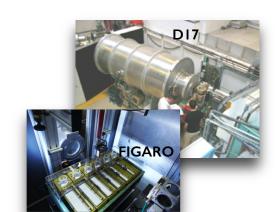


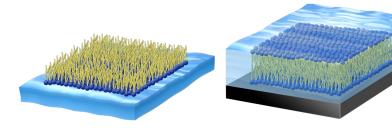


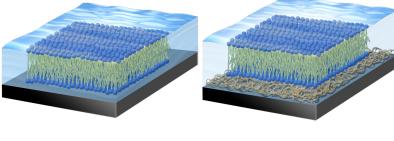


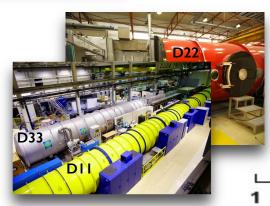
DIFFRACTION

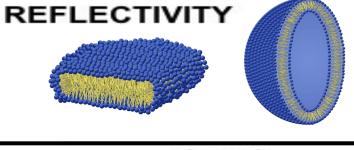
## Model membranes and scattering techniques







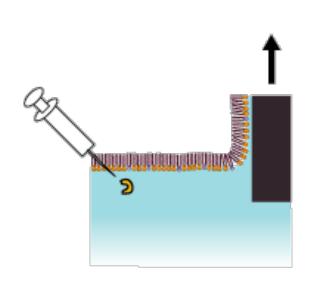


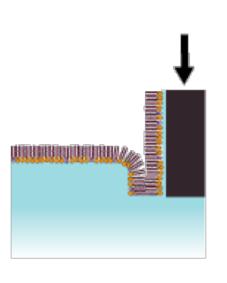


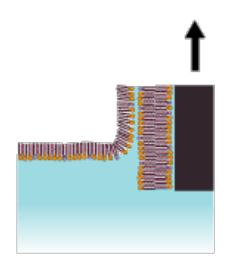
SANS

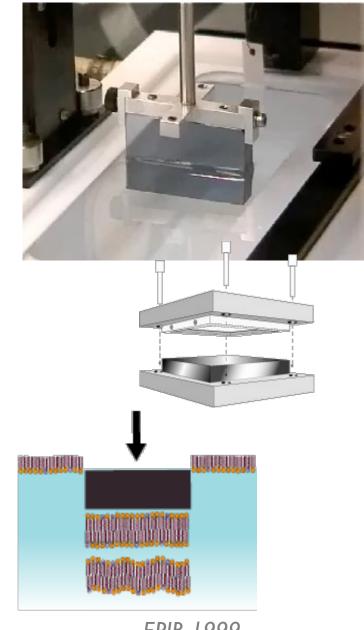
1 10 100 1000 Thickness

# Floating bilayers prepared by Langmuir-Blodgett Langmuir-Schaefer techniques







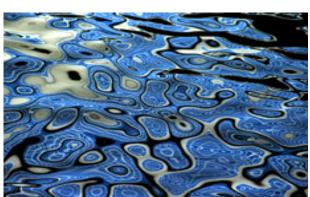


EPJB 1999

## Looking at Surfaces with Neutron Reflectometry = Skipping Stones

"Skipping stones is the act of throwing a (usually) flat stone into a body of water in a manner such that the stone ricochets off the top layer of water, appearing to "skip" along the water's surface."





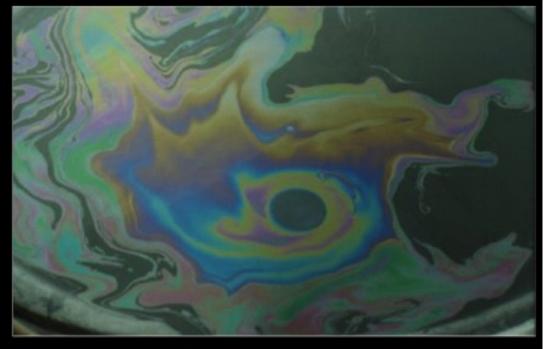


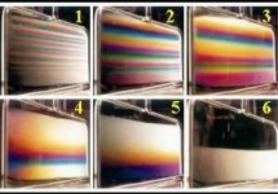




#### Two well known examples of light interference from thin films

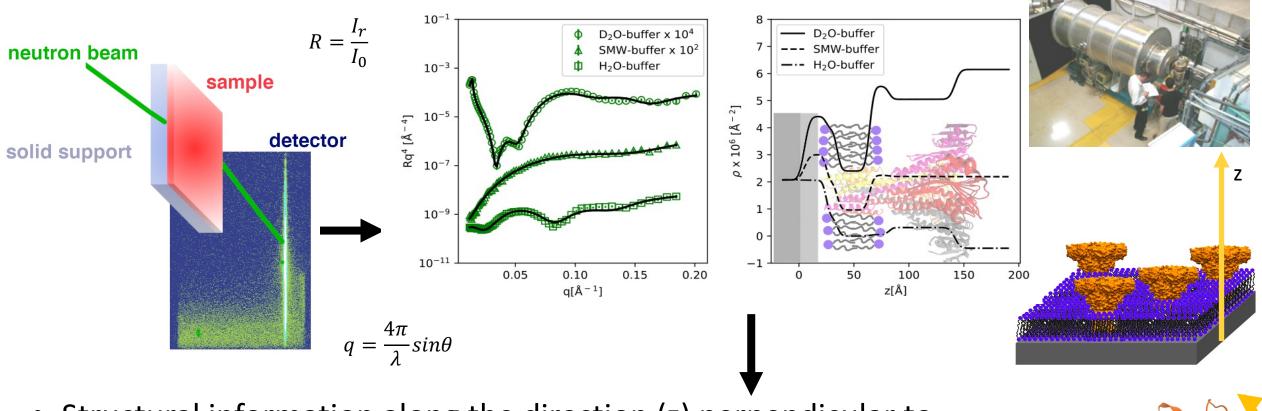
Oxidized oil film on water



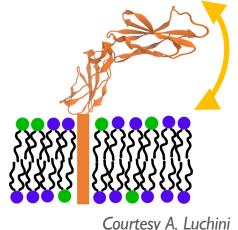


Drainage of soap film. Note the absence of reflection in the top half of the film in 6.

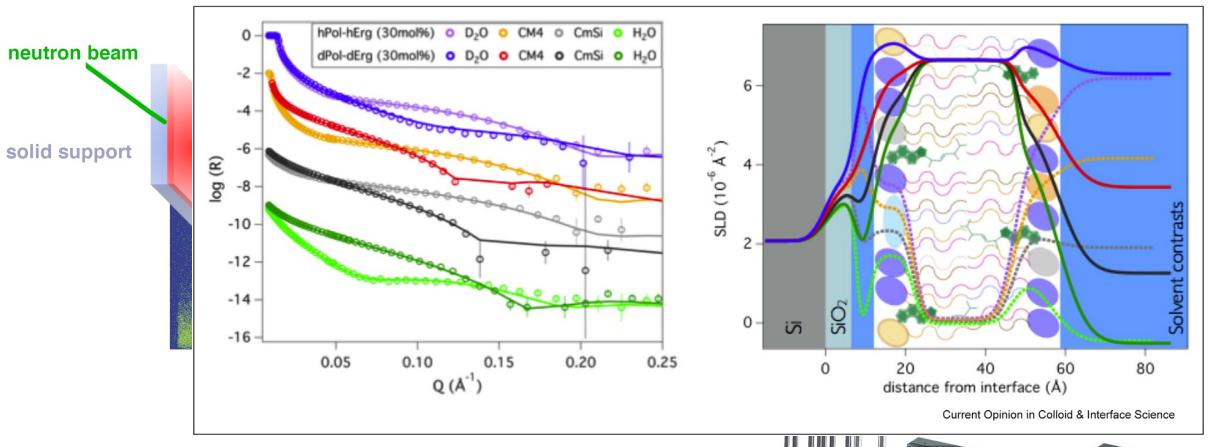
#### Neutron Reflectometry and biological membranes



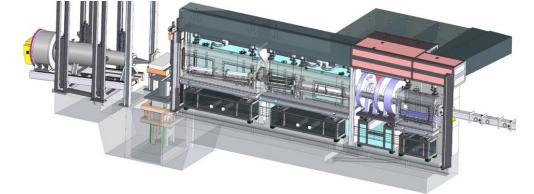
- Structural information along the direction (z) perpendicular to the membrane surface.
- relevant direction for investigating protein structure in a membrane and interactions at membrane surface



#### Specular reflectivity measurements

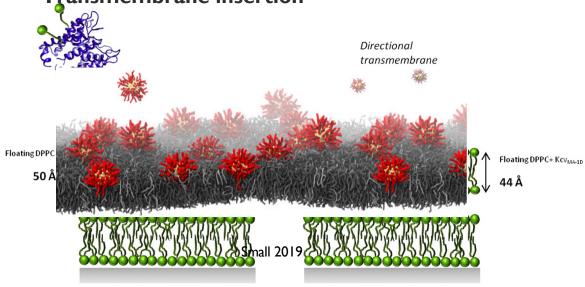


 Isotopic substitution and multiple contrast measurements used to improve resolution

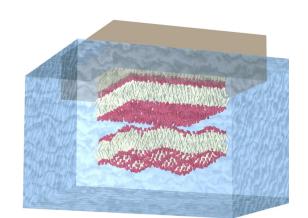


### Neutron and synchrotron radiation studies of structure and fluctuations of floating bilayers

- Effect of temperature (giant swelling)
- Effect of charges
- Effect of AC current
- Interaction with gene delivery complexes
- Effect of domain forming molecules/asymmetry
- Lipid flip-flop
- Interaction with nano particles
- Transmembrane insertion

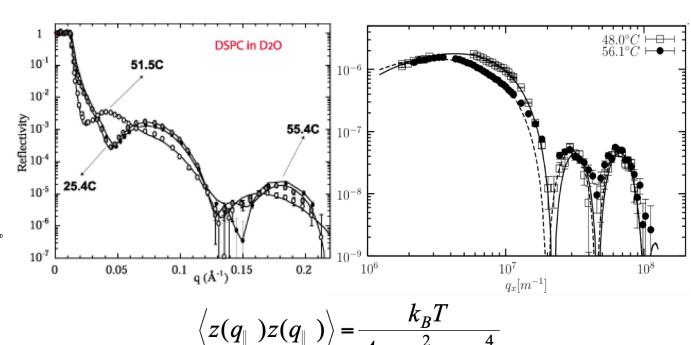


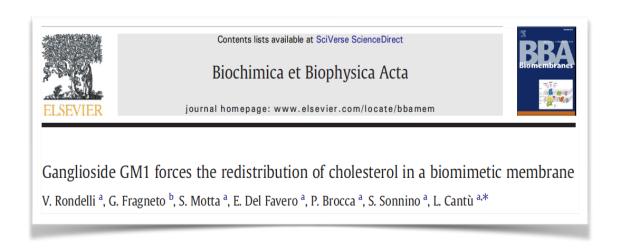
Mukhin Room chellije Chalen BBAh 20.18 ett. 2019



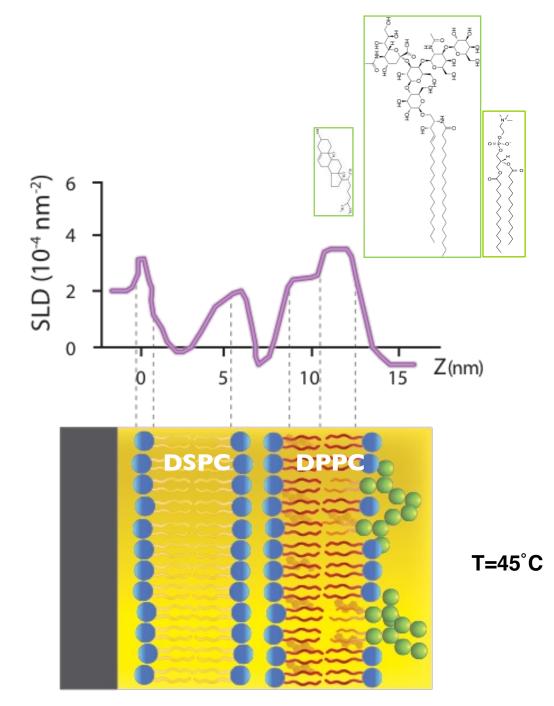
EPL 2001 Langmuir 2001 Langmuir 2003 Langmuir 2005 Langmuir 2005 PNAS 2005 EPJE 2006 Soft Matter 2007 Langmuir 2009 BBA 2012 PNAS 2012 Langmuir 2012 EPJE 2013 Soft Matter 2015 PRL 2016 BBA 2018 Small 2019 | Chem Phys Lett 2019 J. Coll. Int. Sci. 2021

**EPIB** 1999





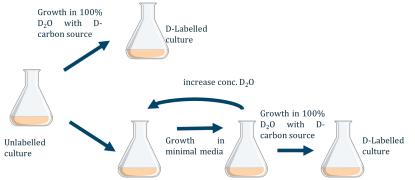
- The presence of ganglioside forces asymmetry in cholesterol distribution, opposite to what happens for a ganglioside-free membrane where a full symmetrisation of cholesterol distribution is observed.
- A preferential asymmetric distribution of ganglioside and cholesterol is attained revealing that a true coupling between the two molecules occurs.



Moving towards complexity:

deuterated (and non) lipid extraction and purification:

better mimic of real membranes, easier deuteration

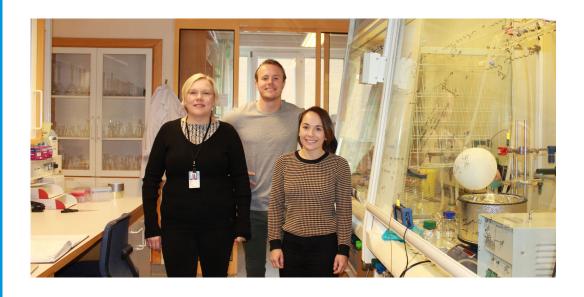






# Yeast lipid production at DEMAX

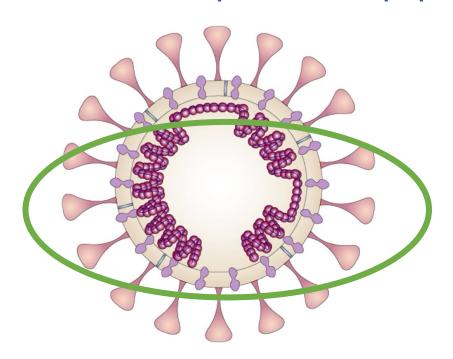
The Deuteration and Macromolecular Crystallisation (DEMAX) platform supports life science and soft matter research users of neutron instruments.



https://europeanspallationsource.se/science-support-systems/demax

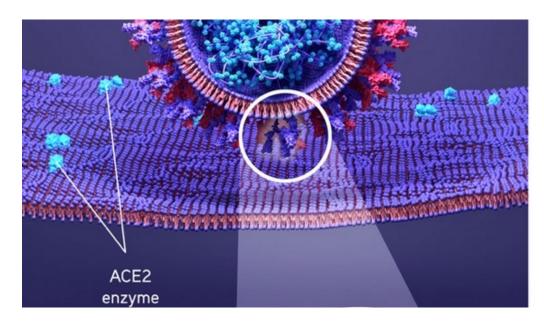
#### SARS-CoV-2 VIRAL FUSION MECHANISM

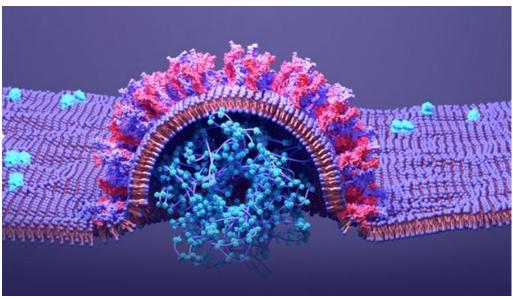
- Interaction of spike protein with ACE2 receptor containing membranes
- Interaction of spike fusion peptides with biological model membranes



Schematic of the membrane fusion process

#### Viral infection and spike proteins

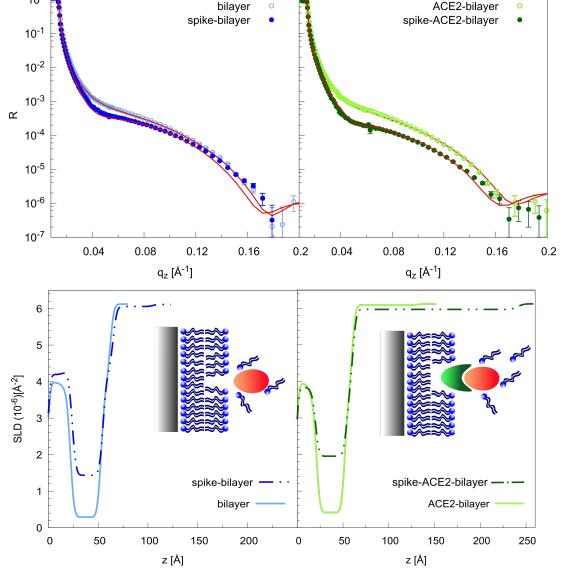




- SARS-CoV 2 is an eveloped virsus
- The viral envelope contains a large fraction of glycoproteins, also known as Spike proteins
- The Spike proteins play a fundamental role in favouring the fusion between in the virus evelope and the host cell
- Attachment of the virus to the host cell membrane can occur via binding of the Spike proteins to the ACE2 receptor

What is the interaction of the Spike proteins with the lipid membrane of the host cell?

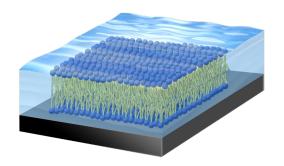
#### Interaction of spike protein with ACE2 receptor containing membranes





spike protein from IBS

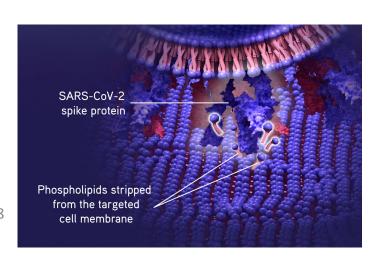
Bilayers formed from natural lipids in presence and absence of ACE-2



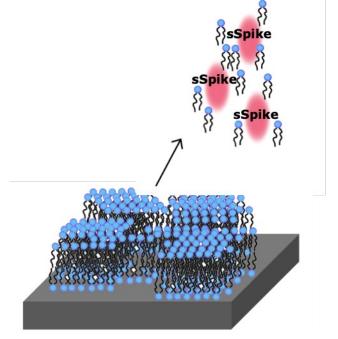
OPEN Lipid bilayer degradation induced by SARS-CoV-2 spike protein as revealed by neutron reflectometry

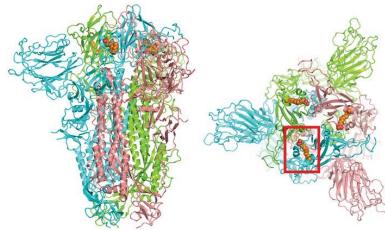
Alessandra Luchini<sup>1,5</sup>, Samantha Micciulla<sup>1,5</sup>, Giacomo Corucci<sup>2</sup>, Krishna Chaithanya Batchu<sup>3</sup>, Andreas Santamaria, Valerie Laux<sup>2</sup>, Tamim Darwish<sup>3</sup>, Robert A. Russell<sup>3</sup>, Michel Thepaut<sup>4</sup>, Isabelle Bally<sup>4</sup>. Franck Fieschi<sup>4</sup> & Giovanna Fraoneto<sup>263</sup>

www.nature.com/scientificreport



#### Interaction of spike protein with ACE2 receptor containing membranes





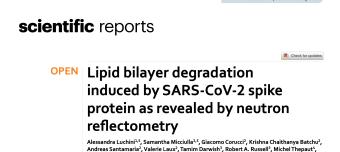
Lipid pockets in the extramembrane domain of sSpike



spike protein from IBS

The spike protein interacts with model membranes either in presence or absence of ACE-2 receptor in the membrane.

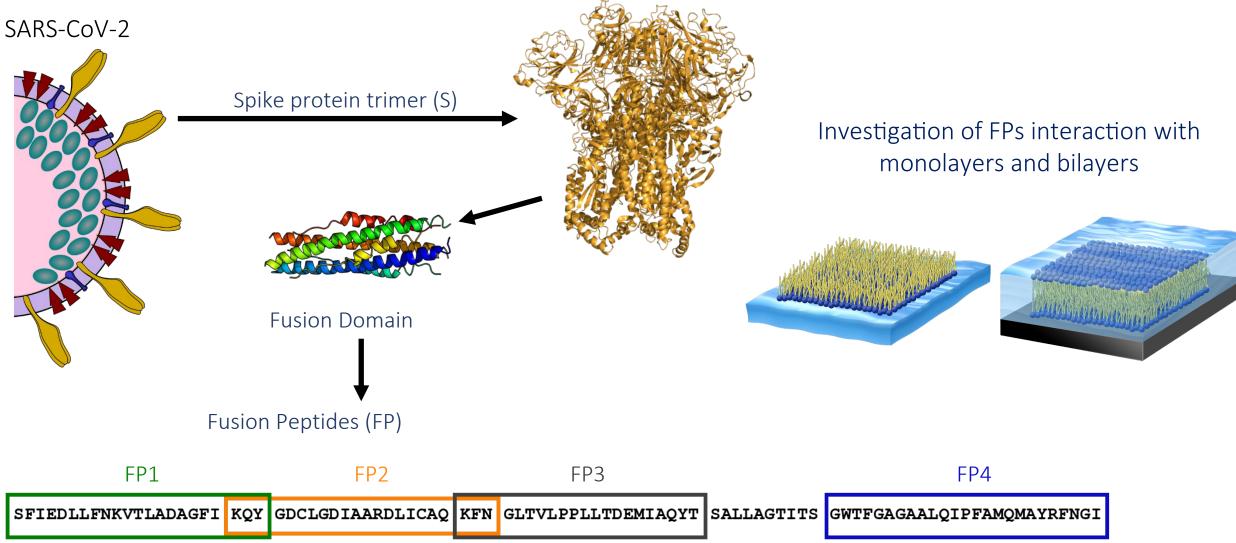
In both cases it promotes lipid removal although to a bigger extent in presence of receptor where a very diluted layer of spike on the surface of the membrane can be resolved at Å level



SARS-CoV-2
spike protein

Phospholipids stripped
from the targeted
cell membrane

#### Interaction of spike protein fusion peptides with model membranes

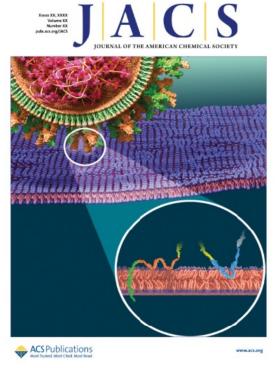


# 1. Viral membrane SARS-CoV-2 FP4 FP2 FP1 Plasma membrane Eukaryotic cell

#### Model for fusion mechanism

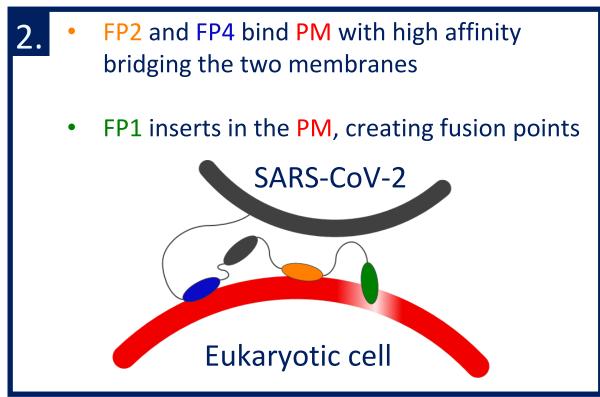
#### Strikingly Differed by Neutron Scat

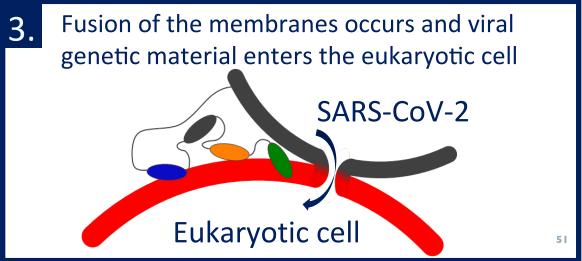
Andreas Santamaria, Kri Francesca Natali, Tilo S Robert A. Russell, Giaco

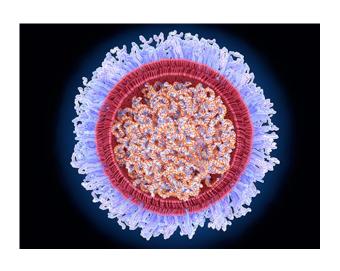


#### ptides Uncovered

st, Daniela Russo, ein, Tamim A. Darwish, ,,\* and Nathan R. Zaccai\*



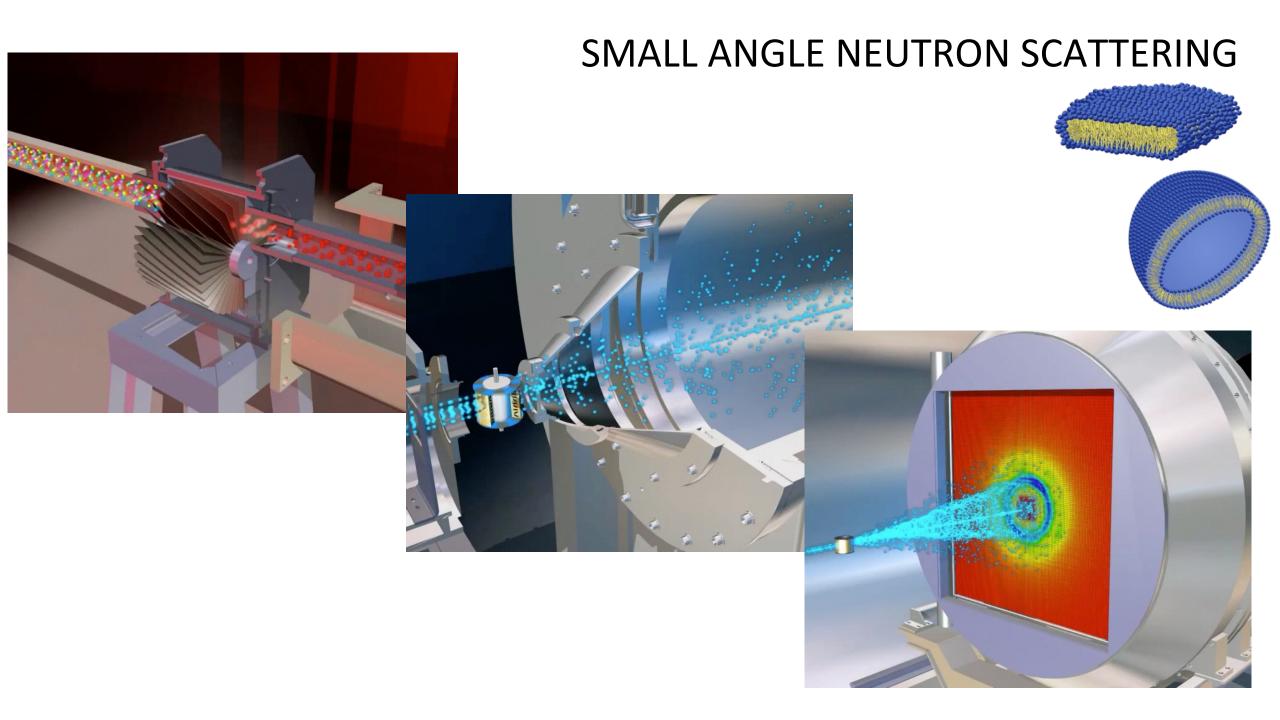




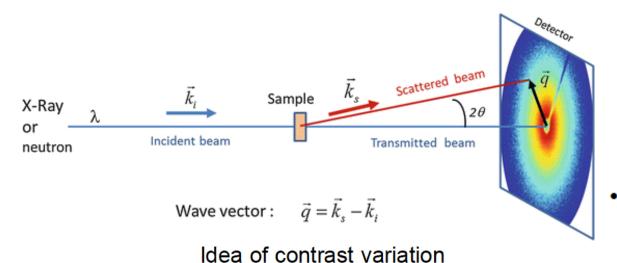
#### SANS for mRNA VACCINES







#### SANS & contrast variation

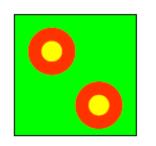




Vary scattering behaviour of (parts of) solutes with respect to solvent

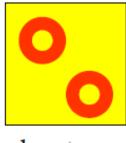


- Contrast Matching
  - reduce the number of phases 'visible'

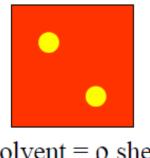


becomes...

or

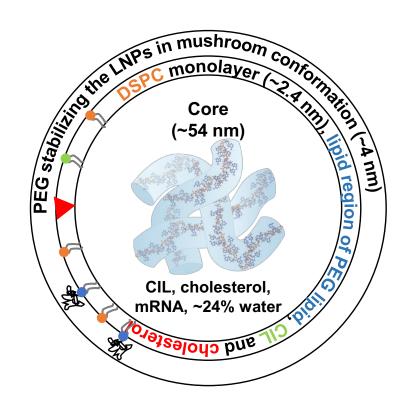


 $\rho$  solvent =  $\rho$  core (shell visible)



 $\rho$  solvent =  $\rho$  shell (core visible)

#### Samples



mRNA-LNP composition

CIL:DSPC:Chol:DMPE-PEG

50:10:38.5:1.5

CIL:nucleotide 3:1

LNP – lipid nanoparticle
CIL - cationic ionizable lipid: a
critical component that quickly
concentrates in the liver upon
intravenous LNP administration

MCH prepared with matched out-cholesterol

→ localise cholesterol

MCHPC with a combination of matched out-cholesterol and d-DSPC

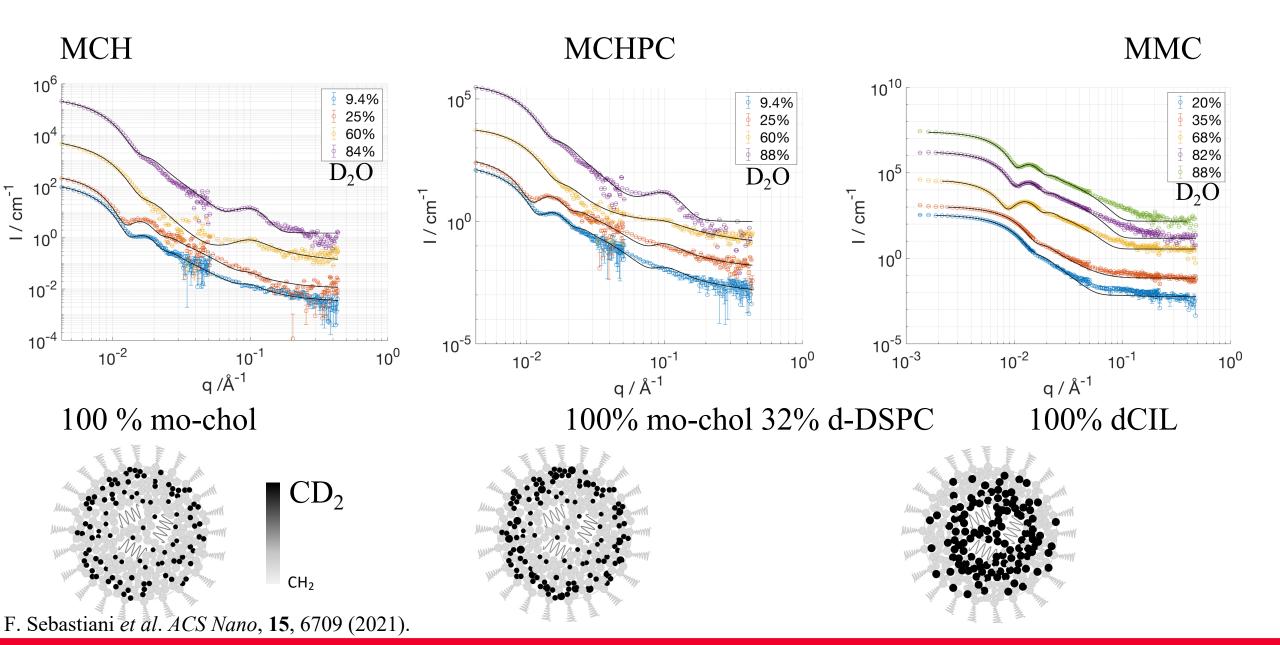
→ highlight the shell

MMC with deuterated CIL

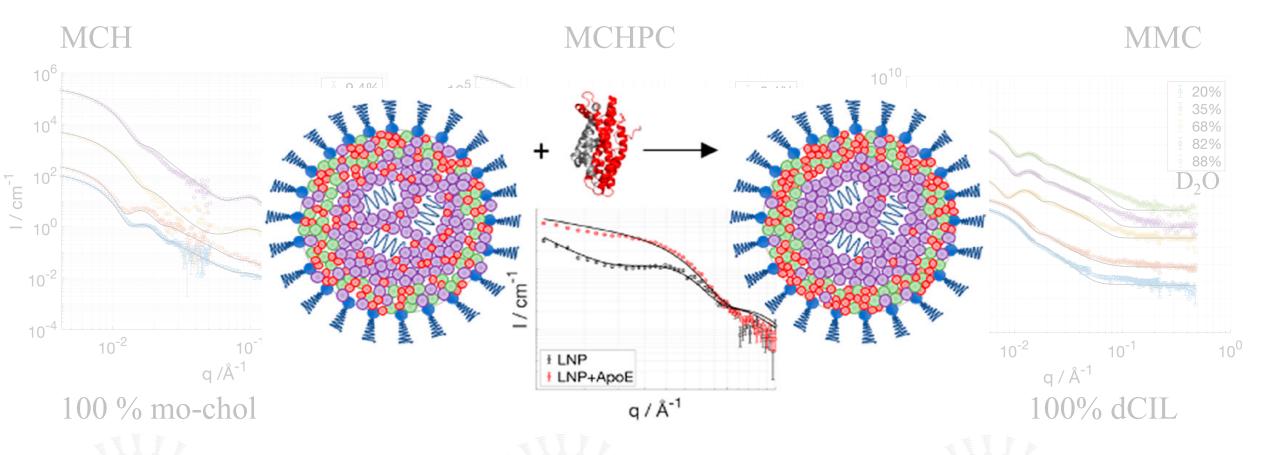
→ localise CIL

M. Yanez-Arteta *et al.* PNAS, **115**, E3351 (2018). F. Sebastiani *et al. ACS Nano*, **15**, 6709 (2021).

#### Results



#### Results



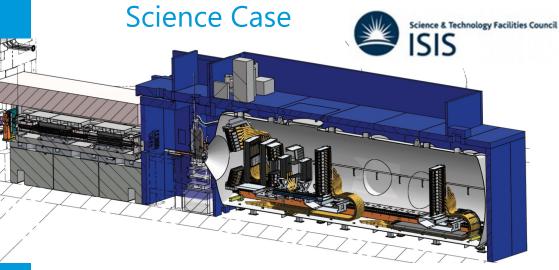
apolipoproteinE (ApoE), being responsible for fat transport in the body, plays a key role in the LNP's plasma circulation time.

CH<sub>2</sub>



#### Future opportunities at ESS

#### LoKI: Broad Band SANS



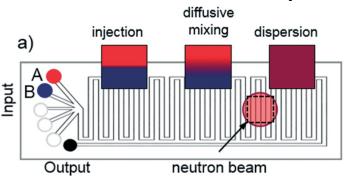
→ high flux, wide simultaneous size range, and a flexible sample area.

#### ABILITIES:

- Investigate multiple length scale systems (simultaneously 0.5-300 nm)
- Perform "single-shot" kinetic measurements on sub-second timescales.
- Perform experiments that use flow e.g. rheology
   & microfluidics with small beam sizes
- High throughput of regular SANS measurements

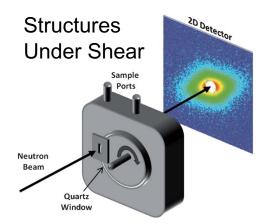
#### Microfluidic SANS

High Throughput Mixing & Tailored Flow Geometry



Lab Chip, 2017, 17, 1559

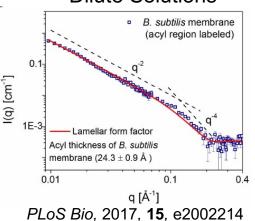
#### **Rheo-SANS:**



Soft Matter, 2011, 7, 9992

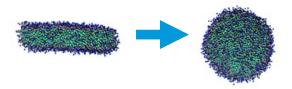
#### **Biological Samples:**

Weak Scatterers & Dilute Solutions



#### **Non-Equilibrium Studies:**

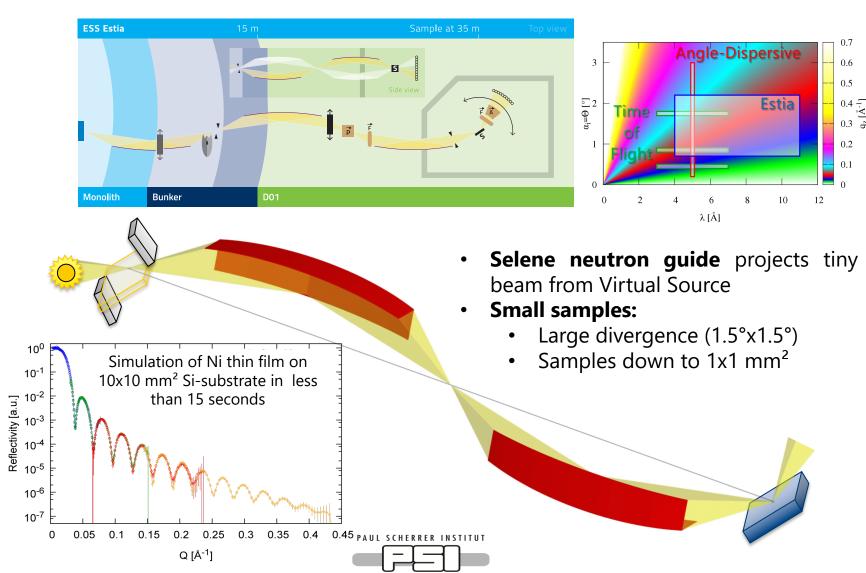
Self-Assembly & Kinetics



Colloid Polym Sci, 2010, 288, 827

#### Estia

#### Focussing Polarised Reflectometer for Tiny Samples





]	Estia Quick Facts.			
	Estia Quick Facts			
-	Instrument Class	Reflectometry		
Z [V] Z	Moderator	Cold		
	Primary Flightpath	35 m		
	Secondary Flightpath	4 m		
	Wavelength Range	3.75–28 Å		
	Polarised Incident Beam	Optional		
	Polarisation Analysis	Optional		
	Sample Orientation	Vertical		
	Total Q-Range	0.001 to 3.15 $\mbox{\normalfont\AA}^{-1}/-0.001$ to $-0.3~\mbox{\normalfont\AA}^{-1}$		
	Standard Mode (14 Hz)			
	Bandwidth	7 Å		
	Flux at Sample at 2 MW <sup>a</sup>	$6 \times 10^{8} \text{ n s}^{-1} \text{ cm}^{-2}$		
	Relative Q-Range	$Q_{\text{max}} = 2.85 \times Q_{\text{min}}$		
	Q-Resolution $\Delta Q/Q$	7.8%–3.0% over Q-range		
	2-Pulse Skipping Mode (4.7 Hz)			
	Bandwidth	21 Å		
	Flux at Sample at 2 MW <sup>a</sup>	$2 \times 10^{8} \text{ n s}^{-1} \text{ cm}^{-2}$		
	Relative Q-Range	$Q_{\text{max}} = 6.6 \times Q_{\text{min}}$		
	Q-Resolution ΔQ/Q	7.8%–1.3% over Q-range		

<sup>&</sup>lt;sup>a</sup>Full-divergence beam averaged over  $5(H) \times 10(V) \text{ mm}^2$ .

For the study of surfaces and interfaces including magnetic layers

#### FREIA Horizontal Reflectometer

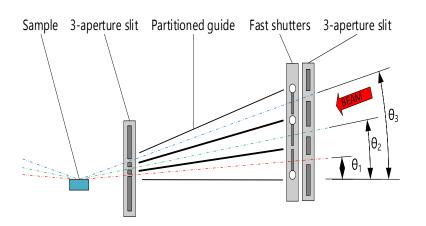


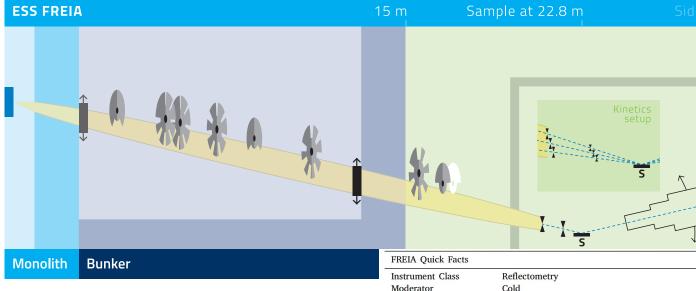


FREIA is a flexible instrument optimised for **time- resolved** and high throughput studies:

- Wide vertical divergence; extended simultaneous Q range & avoids slow sample movements
- Downward orientation for **liquid interfaces**
- **Flexible** Collimation options
- High flux ( $d\lambda/\lambda = 3-20$  %) or high resolution ( $d\lambda/\lambda < 3$ %) modes

Wide ranging science case in **soft-matter and biosciences** 





#### **Fast Shutter development**

Rapidly change angles without moving sample... allows full Q-range measurement with collimated beam with subsecond time resolution

Representative Incident Beam Angles	$0.45^{\circ},~0.9^{\circ},~3.4^{\circ}$ (full range $0.2^{\circ}3.7^{\circ}$ depending on angular resolution)
Standard Mode (14 Hz)	
Wavelength Range Flux at Sample at 2 MW	2-10 Å $1 \times 10^5$ , $5 \times 10^5$ , $7 \times 10^6$ n s <sup>-1</sup> cm <sup>-2</sup> [high res (WFM mode] $1 \times 10^6$ , $4 \times 10^6$ , $6 \times 10^7$ n s <sup>-1</sup> cm <sup>-2</sup> [high flux mode $1 \times 10^8$ n s <sup>-1</sup> cm <sup>-2</sup> [full divergence mode]
Q-Range	0–1 Å <sup>-1</sup> (solid samples)

Available as a foreseen upgrade

22.8 m

3.0 m

Horizontal

Primary Flightpath Secondary Flightpath

Sample Orientation

Q-resolution

Polarised Incident Beam

	Q-resolution	3%–3.5% [high res (WFM) mode] 5%–23% (across free-liquid Q-range) [high flux mode] 2%–6% [full divergence (WFM) mode]
Pulse Skipping Mode (7 Hz)		Hz)
		0

Table onlyping mode (* 112)				
Wavelength Range	2–18 Å			
Flux at Sample	$5 \times 10^5$ , $2 \times 10^6$ , $3 \times 10^7$ n s <sup>-1</sup> cm <sup>-2</sup> [high flux mode]			
Q-Range	0–1 Å <sup>-1</sup> (solid samples)			
	$0.002 \cdot 0.29 \cdot \text{Å} - 1 \text{ (free liquids)}$			

3%-23% (across free-liquid Q-range) [high flux mode]

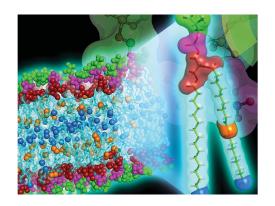
 $0.0045-0.38 \text{ Å}^{-1}$  (free liquids)

#### FREIA

#### Science Case



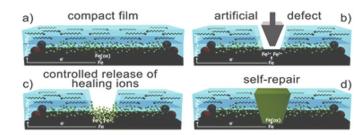
In-situ time-resolved reflectometry for soft condensed matter, life science and functional materials



Instrument characteristics to allow very fast measurements:

- Very high flux
- Horizontal sample geometry Broad
- Flexible collimation
- Variable resolution
- Broad simultaneous Q
- No sample movement

#### **Applications**

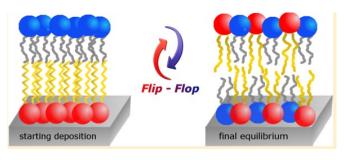


Design



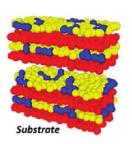
- response to external stimuli
- in situ and in operando
- complex sample environments

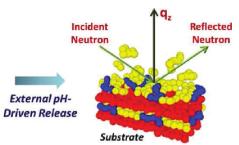
#### **Dynamics**



- deposition, structure and phase behavior
- adsorption, self-assembly and reactions
- gas/liquid/solid interfaces

#### **Function**





Consider using neutrons for your research!

Thank you!



