Structural Biology with neutrons at the European Spallation Source

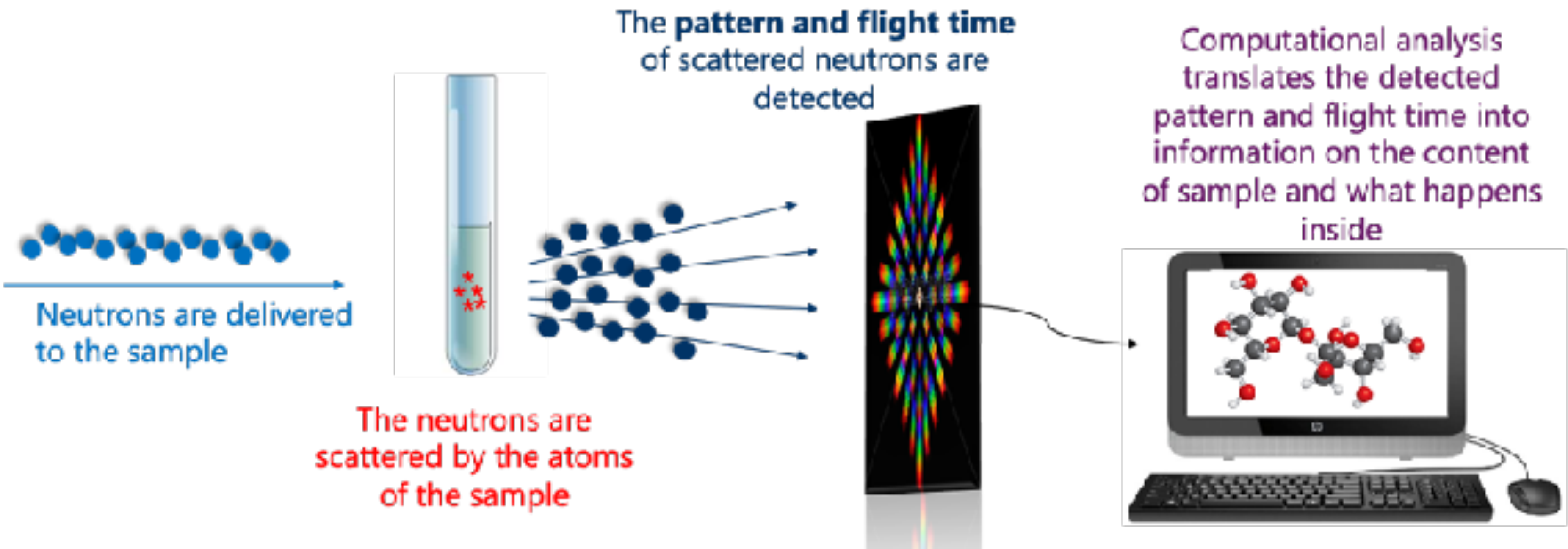
Infection Biology Across Scales

2025-02-03

Esko Oksanen Instrument Scientist,

Macromolecular Crystallography

Neutron scattering simplified



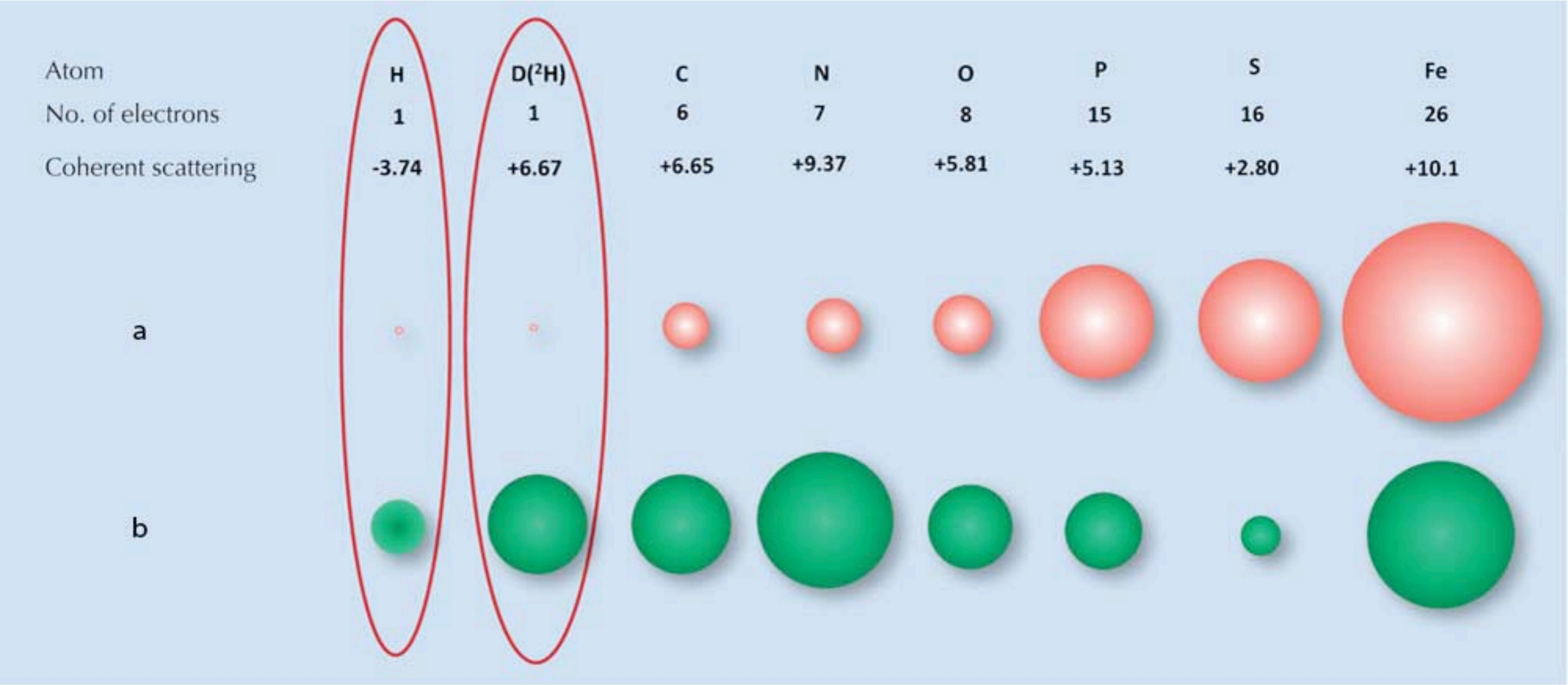
Sample environment to vary temperature, apply magnetic fields, pressure, humidity, in-operando processes like operating battery, welding, forging, etc

Neutrons tell us:
Where the atoms are (pattern)
What they are doing (flight time)

ESS will provide support in the form of data analysis packages, user support, data storage, etc.

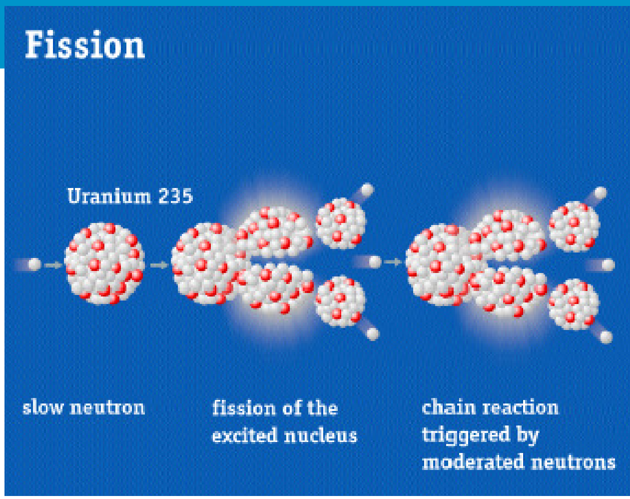
Neutrons as a probe for condensed matter

- They have wavelengths similar to atomic distances
- They have a magnetic moment
- They are non-destructive
- They see a completely different contrast to x-rays → isotope contrast & labelling



How to produce neutron beams

ILL - France



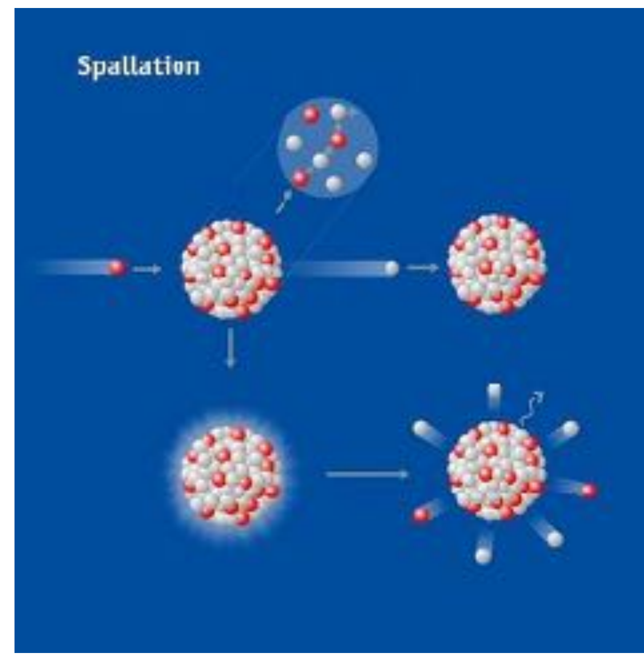
Typically continuous

Fission: One neutron in,
three neutrons out;
Use a nuclear reactor

Spallation: Up to 30 neutrons per proton;
Accelerator to propel proton onto target

ESS-Sweden

Typically pulsed



Why neutrons for biological structures?

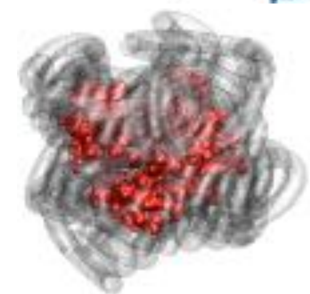
- We can see light atoms → hydrogen positions
- We can use isotope labelling to create contrast → protein-protein complexes, membranes
- We can observe dynamics with neutron energy changes → relating dynamics to function
- We can see through large objects → water transport

Crystallography

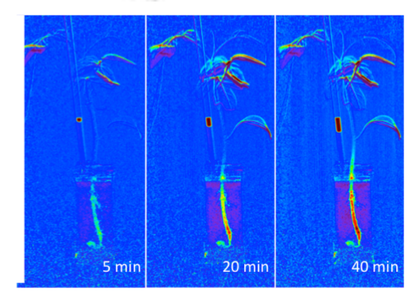
Small angle scattering

Reflectometry

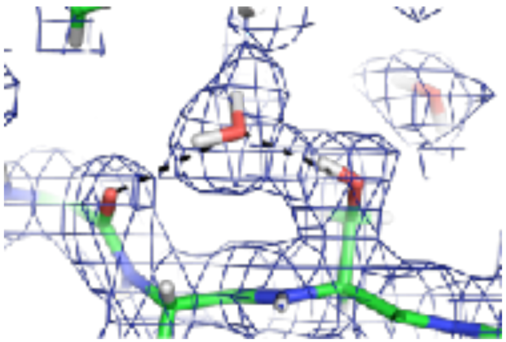
Inelastic scattering

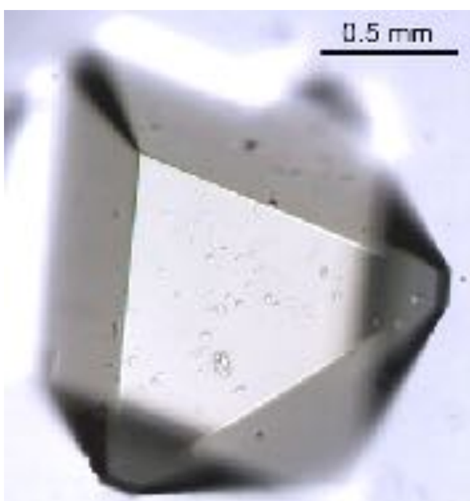


Imaging



Neutron Macromolecular Crystallography





Oksanen, E *et al. J. R. Soc. Interface* 2009, ***6*** *Suppl 5*, S599-610.

☺Hydrogens are visible

☺No radiation damage

☹Large crystals needed

☹Data collection takes weeks

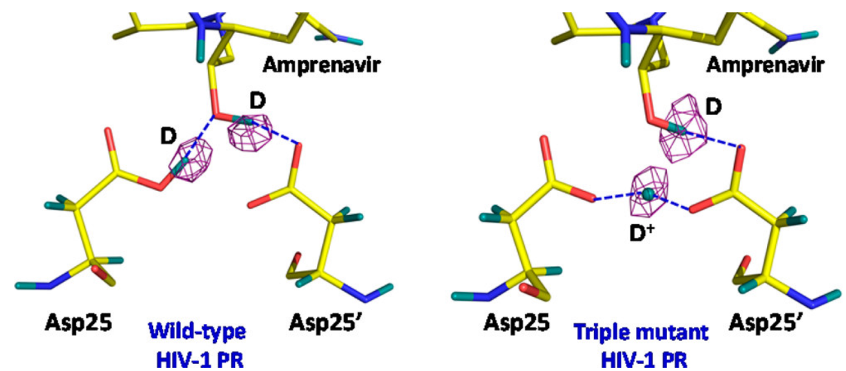
☹Few instruments available

Where are hydrogens important?

Enzyme mechanisms

Protein-ligand interactions

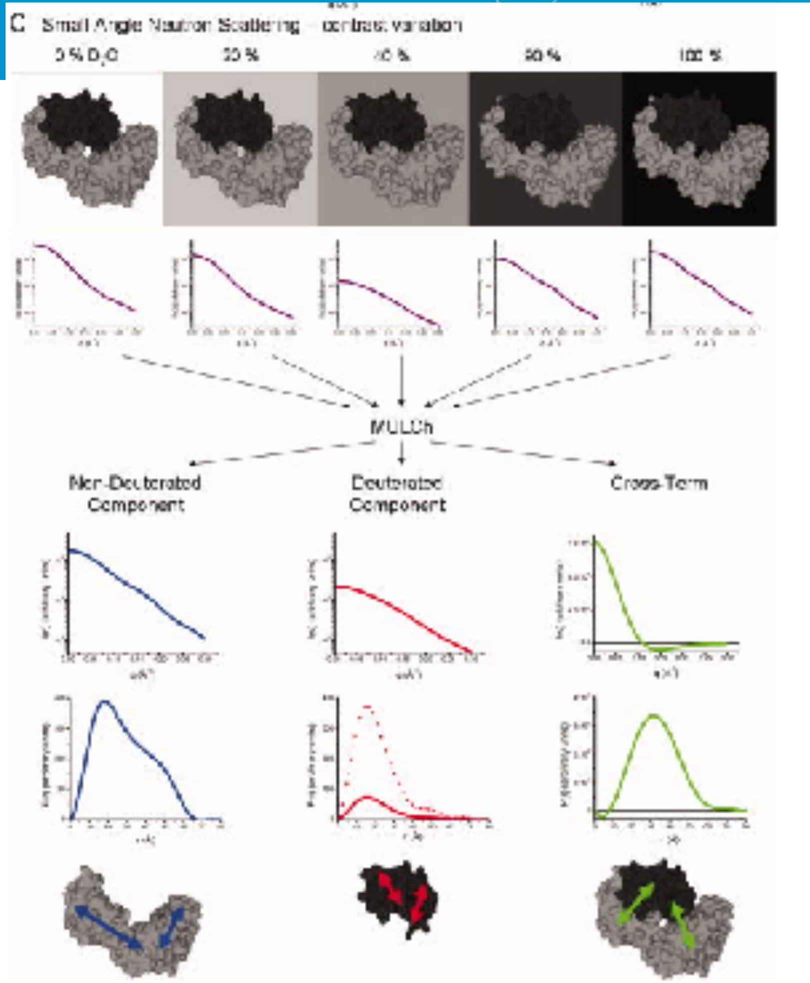
Proton transport across membranes



Gerlits et al., (2017) J. Med. Chem. **60**, p.2018

Small Angle Neutron Scattering

☺Solution structure
☺Complexes resolved by contrast variation
☺Membrane proteins can be studied with "invisible" micelles
☹Requires D-labelling



Jacques, D.A., Trewhella, J. (2010) *Protein Science* **19** 642-657

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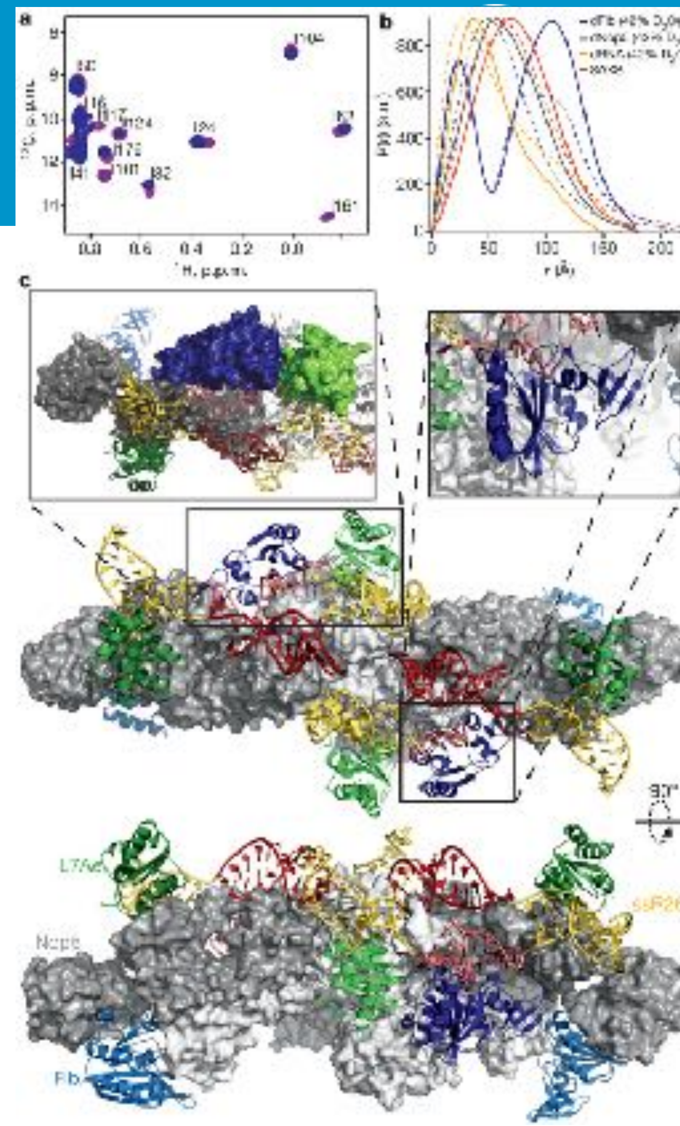

Lapinaite *et al.* (2013) *Nature*, **502** 519-523

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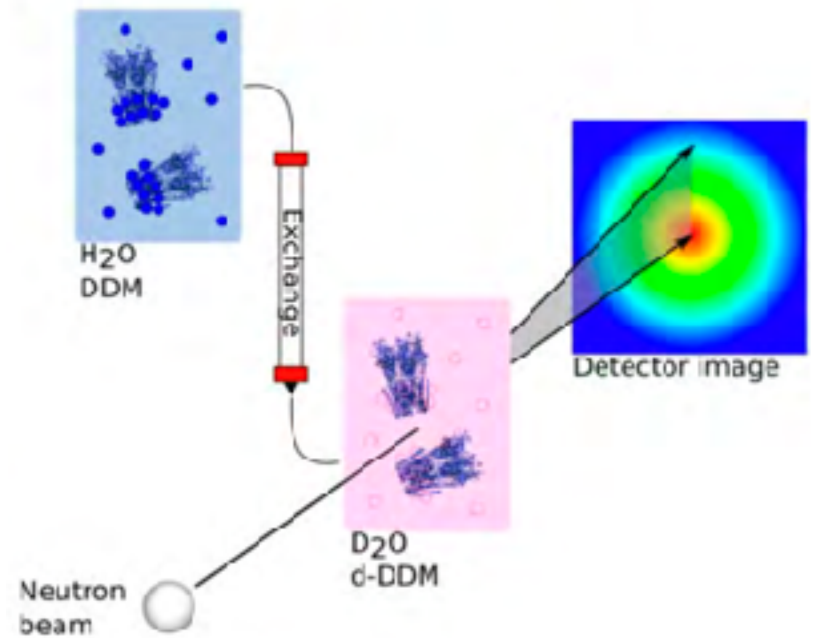
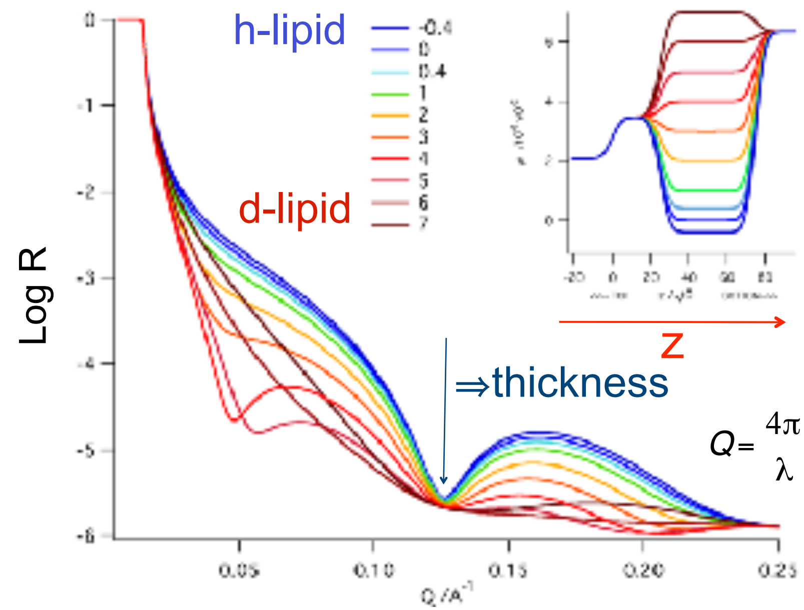


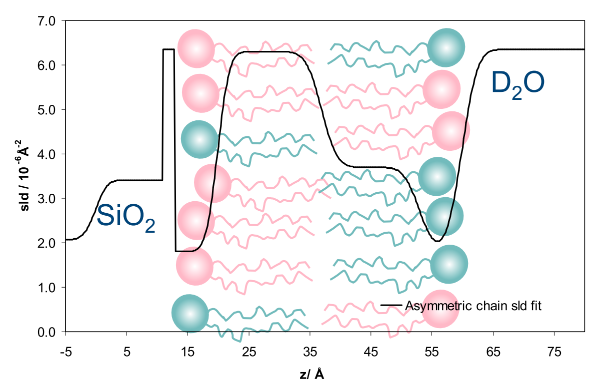
Lycksell *et al.* (2021) *PNAS*, **118** e2108006118

Neutron Reflectometry

EUROPEAN SPALLATION SOURCE

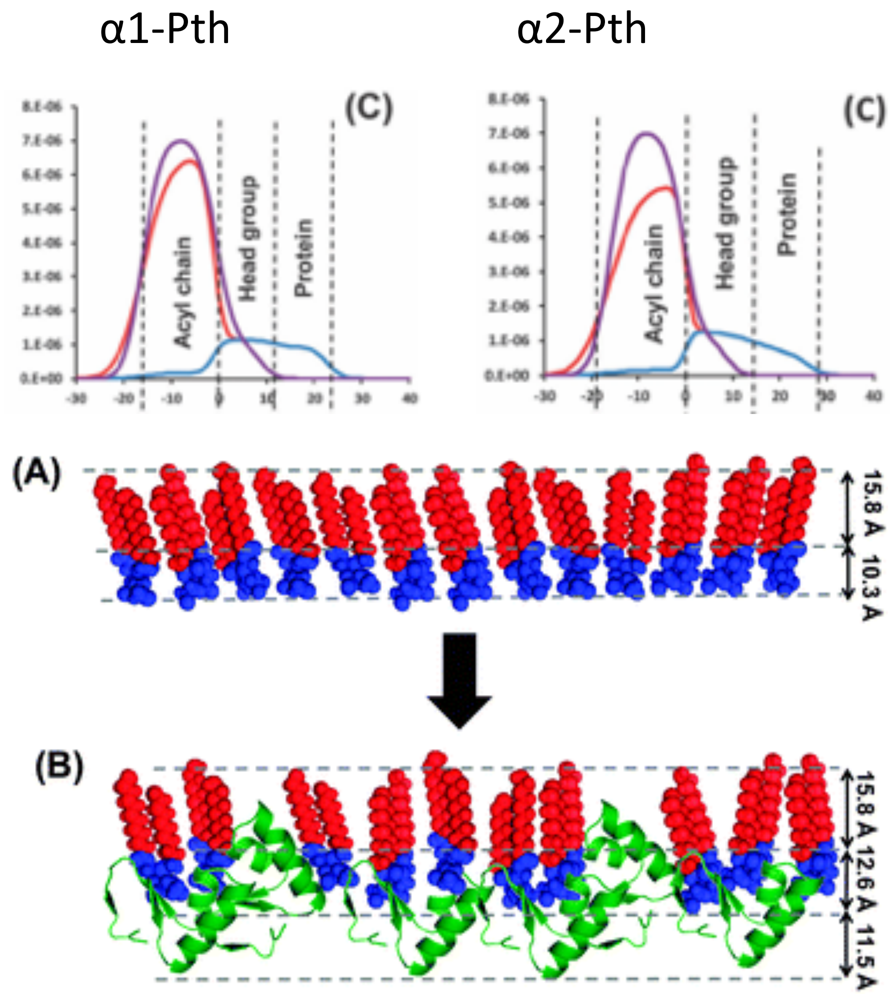
☺Membrane composition at atomic resolution
☺Can study surfaces in solution
☹Deuterated compounds essential
☹Information only along membrane normal





Neutrons as a tool to ”see” details of AMP penetration

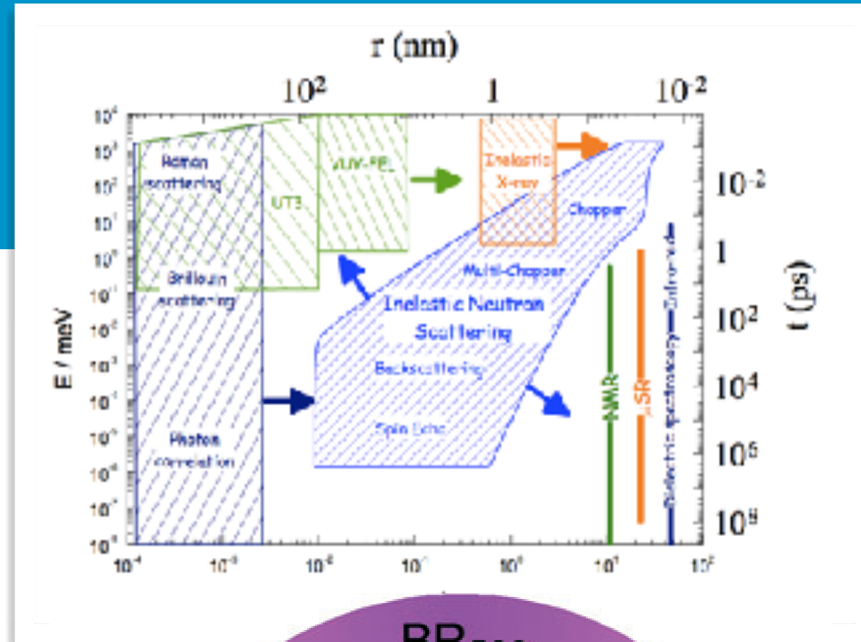
- •α1 and α2-purothionins (Pth) are produced by wheat in response to bacterial and fungal infections
- •Both disrupted in-plane structure of phospholipid monolayers: by adsorbing as a single protein layer, penetrating the lipid, and actually removing lipid.
- •α2 showed faster disruption and removed ~12% of the lipids – the hydrophobicity of the peptide made a huge difference in efficacy.

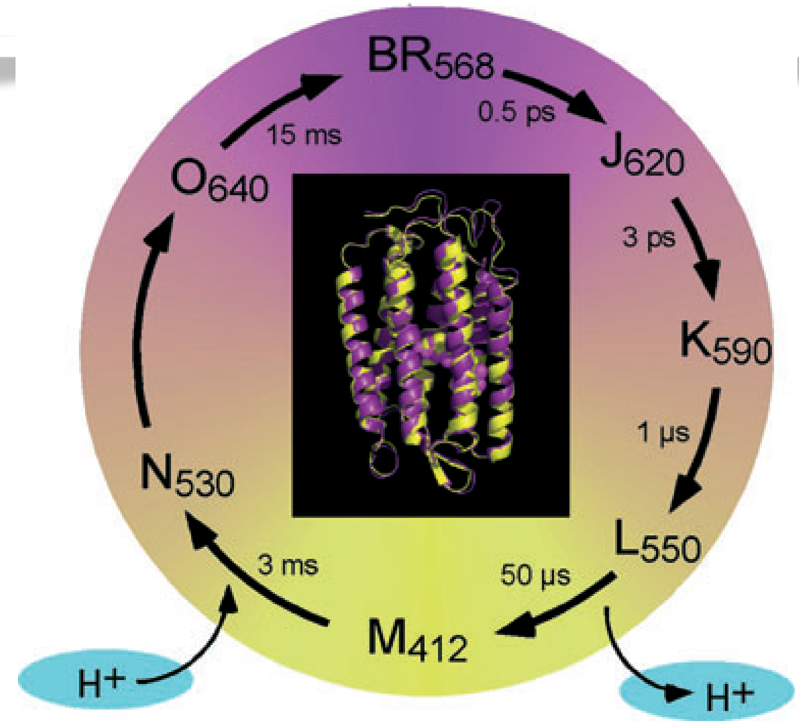


Clifton et al. *Phys. Chem. Chem. Phys.*, 2012, **14**, 13569-13579

Inelastic neutron scattering

- Dynamics information in time and length scales unaccessible by other techniques
- Directly comparable with MD simulations





Pieper et al. Photochem. Photobiol. 2009;85:590-597

A European Project

EUROPEAN SPALLATION SOURCE

Host countries

Sweden, Denmark

Construction
47.5%

Operations
15%

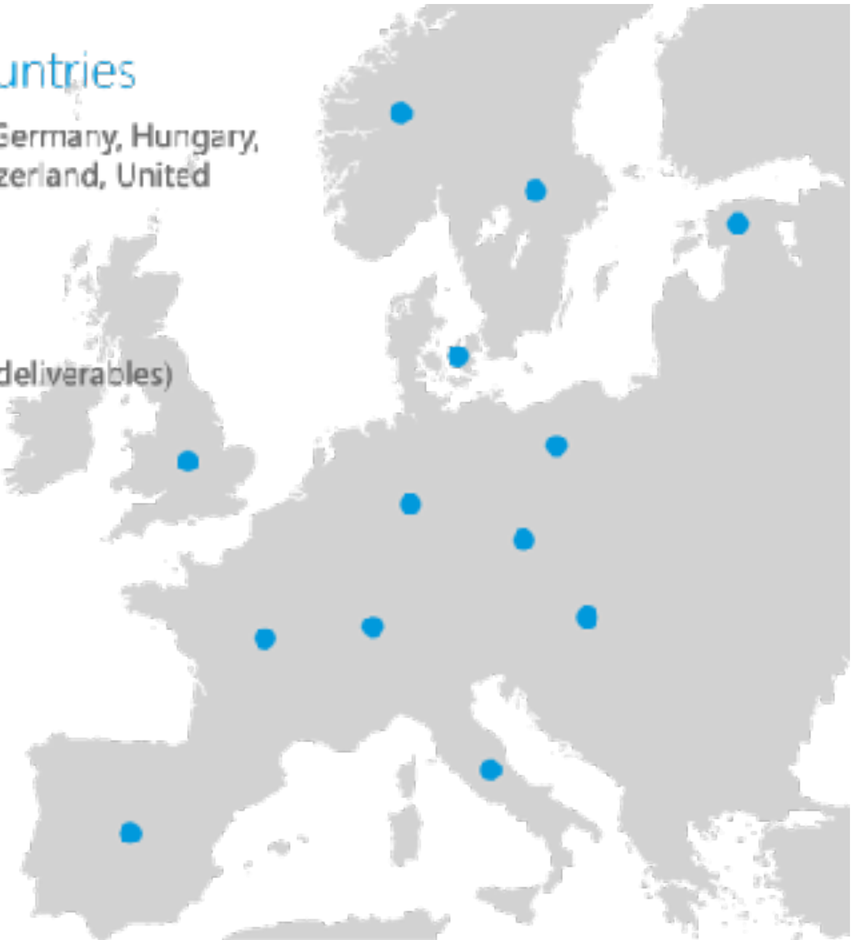
Budget for construction €1.84 billion
Estimated annual budget €140 million

Non host member countries

Czech Republic, Estonia, France, Germany, Hungary, Italy, Norway, Poland, Spain, Switzerland, United Kingdom.

Construction
52.5%
(of which 70% is in-kind deliverables)

Operations
85%



A European Project

EUROPEAN SPALLATION SOURCE

How will it be built?

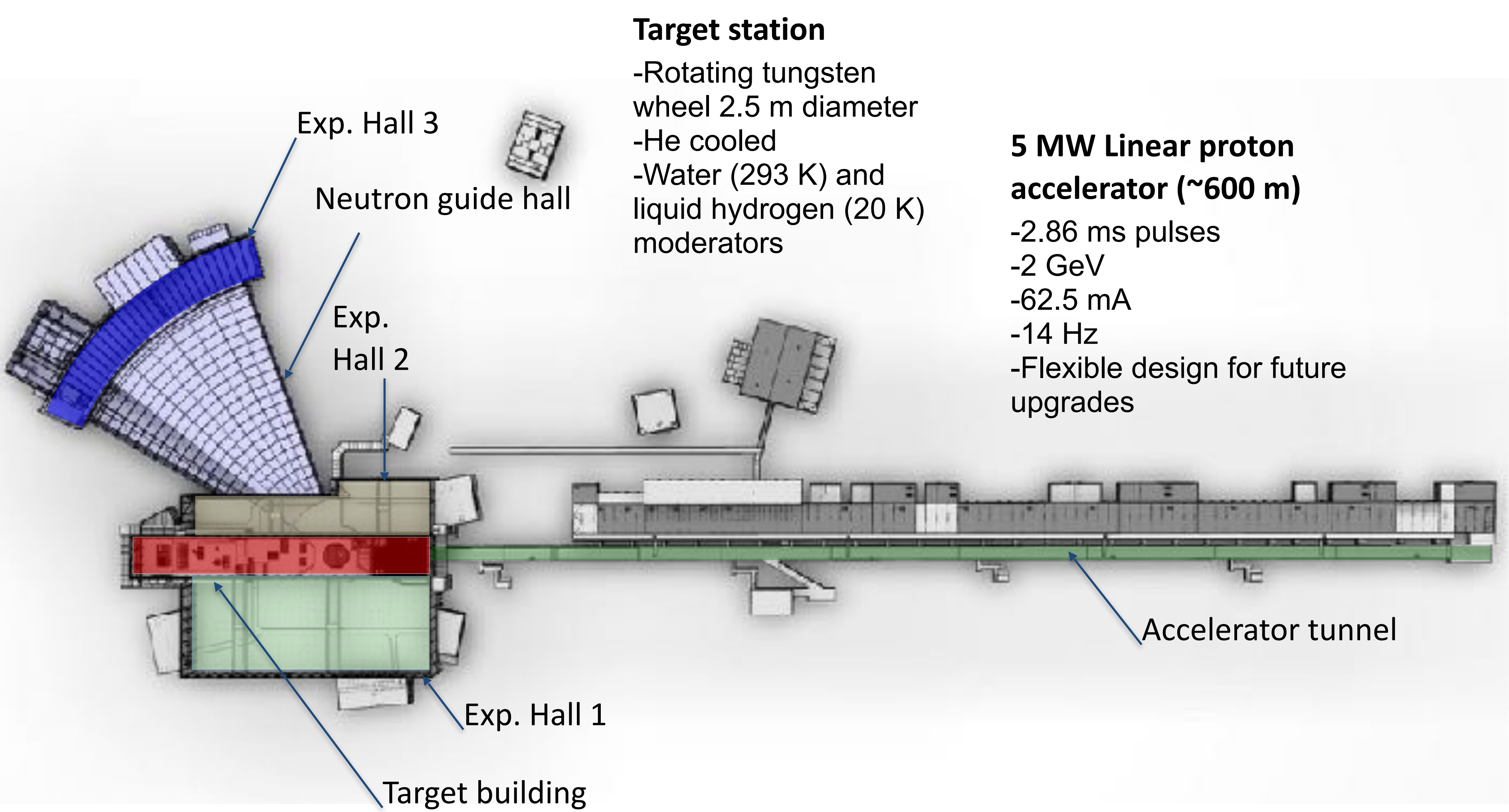
Aarhus University
Atomki - Institute for Nuclear Research
Bergen University
CEA Saclay, Paris
Centre for Energy Research, Budapest
Centre for Nuclear Research, Poland, (NCBJ)
CNR, Rome
CNRS Orsay, Paris
Cockcroft Institute, Daresbury
Elettra – Sincrotrone Trieste
ESS Bilbao
Forschungszentrum Jülich
Helmholtz-Zentrum Geesthacht
Huddersfield University
IFJ PAN, Krakow
INFN, Catania
INFN, Legnaro
INFN, Milan
Institute for Energy Research (IFE)
Rutherford-Appleton

Laboratory, Oxford (ISIS)
Copenhagen University
Laboratoire Léon Brillouin (CEA/CNRS/LLB)
Lund University
Nuclear Physics Institute of the ASCR
Oslo University
Paul Scherrer Institute (PSI)
Polish Electronic Group (PEG)
Roskilde University
Tallinn Technical University
Technical University of Denmark
Technical University Munich
Science and Technology Facilities Council
UKAEA Culham
University of Tartu
Uppsala University
WIGNER Research Centre for Physics
Wroclaw University of Technology
Warsaw University of Technology
Zurich University of Applied Sciences (ZHAW

ESS Timeline

- 2003: Concept design of ESS presented
- 2009: Decision to site ESS in Lund
- 2012: ESS design update phase complete
- 2014: Start of construction
- 2019: Start of initial operations phase
- 2025: Today
- 2025: First neutrons
- 2026: First science
- 2027: Construction phase completed

The world's brightest neutron source



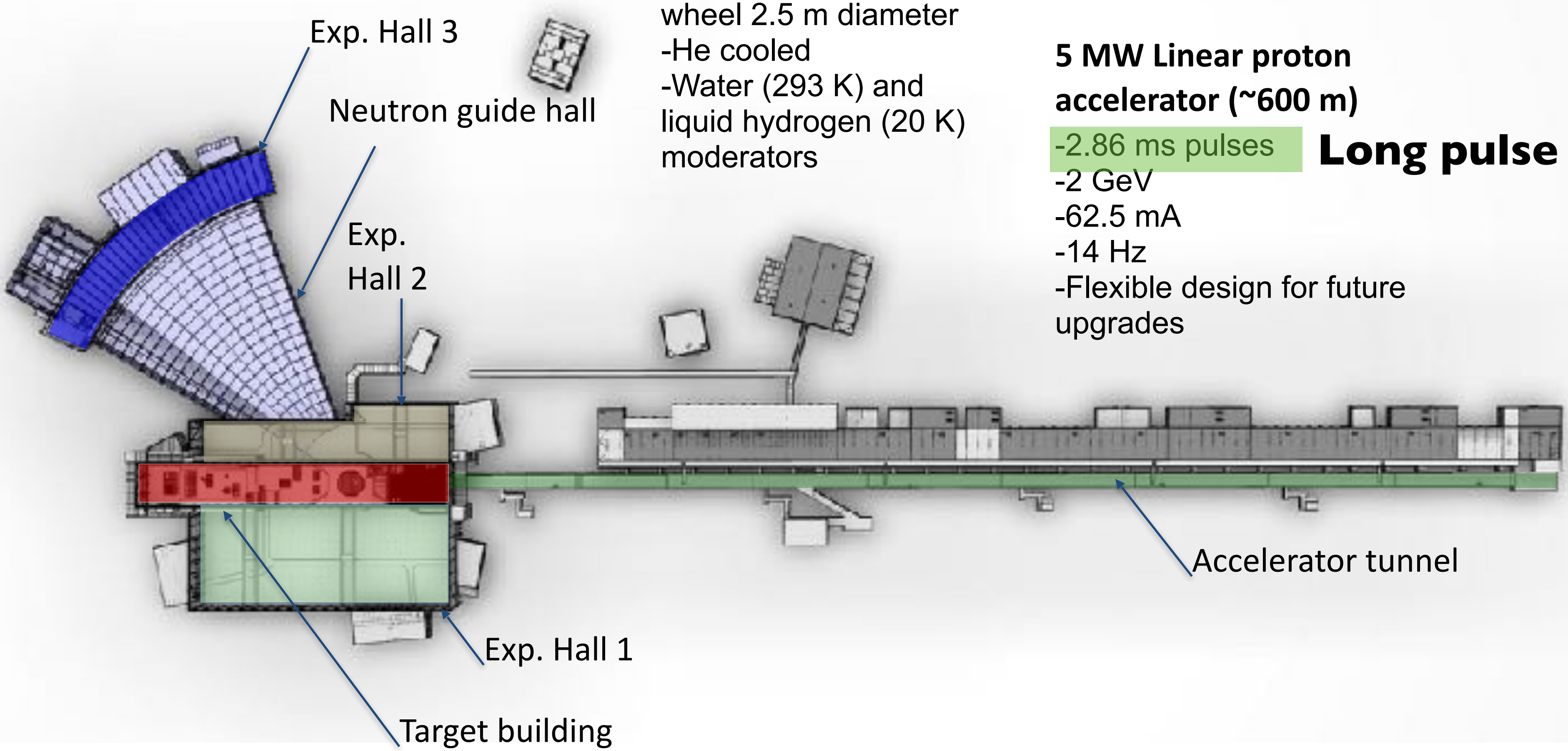
The world's brightest neutron source

Target station

-Rotating tungsten wheel 2.5 m diameter
-He cooled
-Water (293 K) and liquid hydrogen (20 K) moderators

5 MW Linear proton accelerator (~600 m)

-2.86 ms pulses **Long pulse**
-2 GeV
-62.5 mA
-14 Hz
-Flexible design for future upgrades



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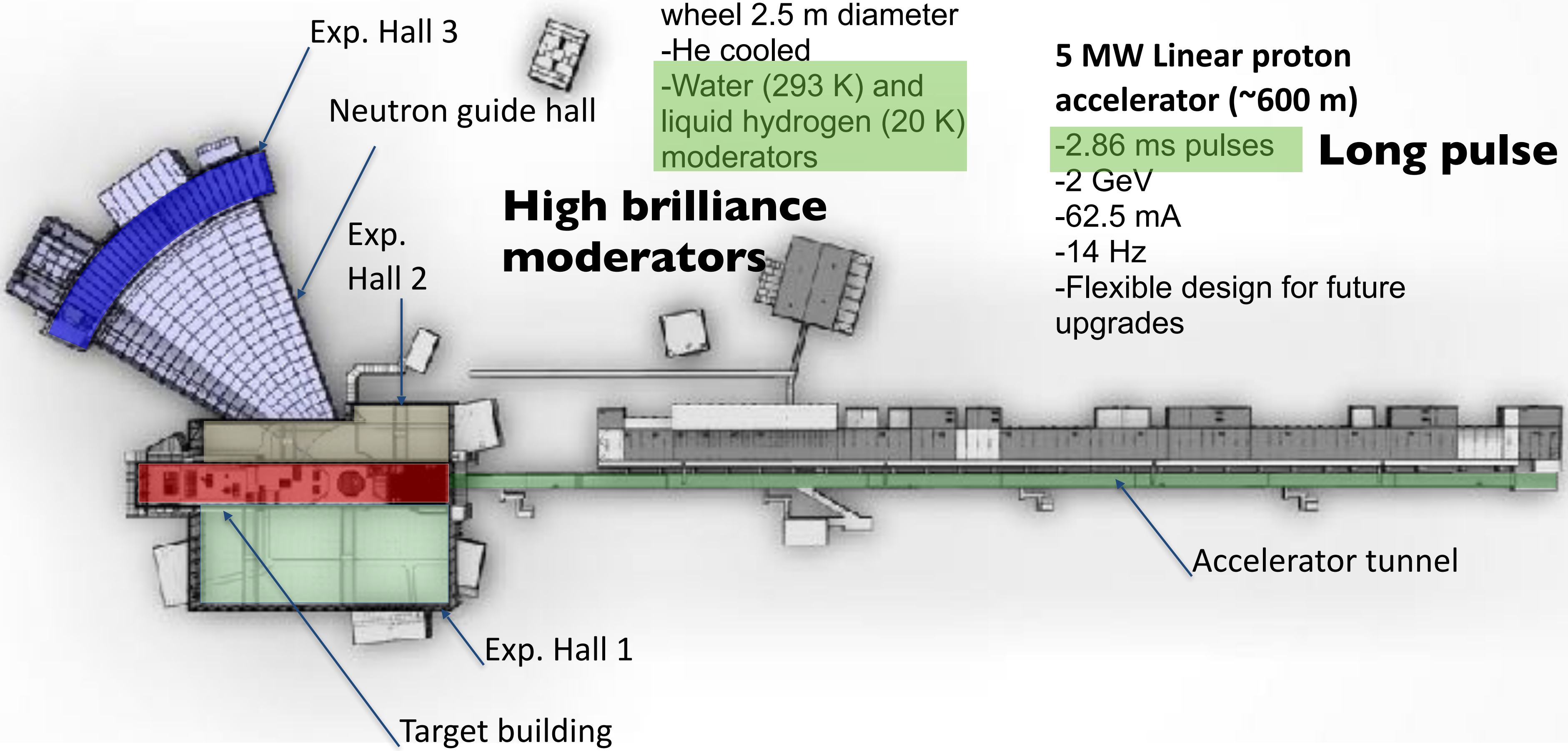

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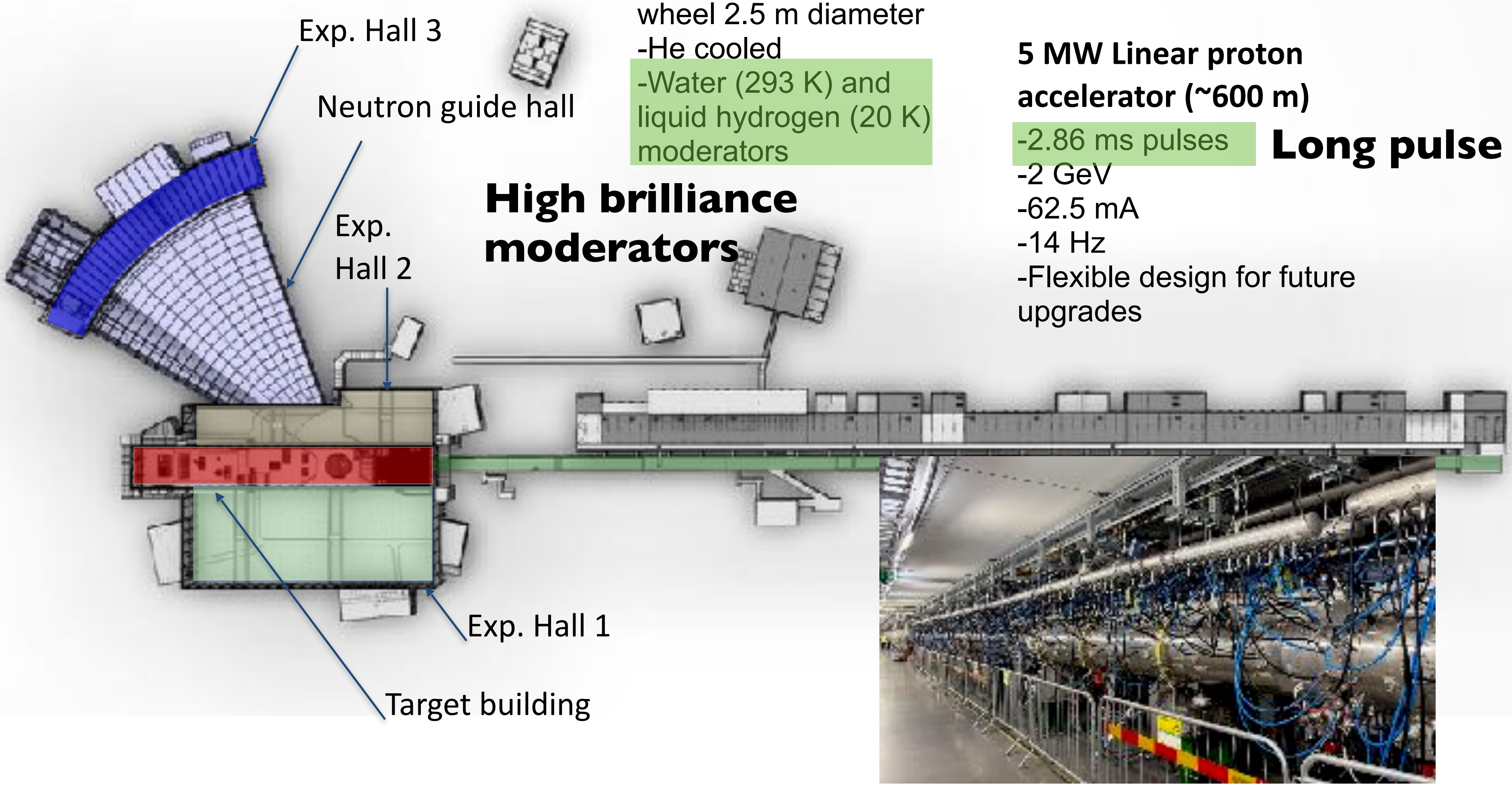

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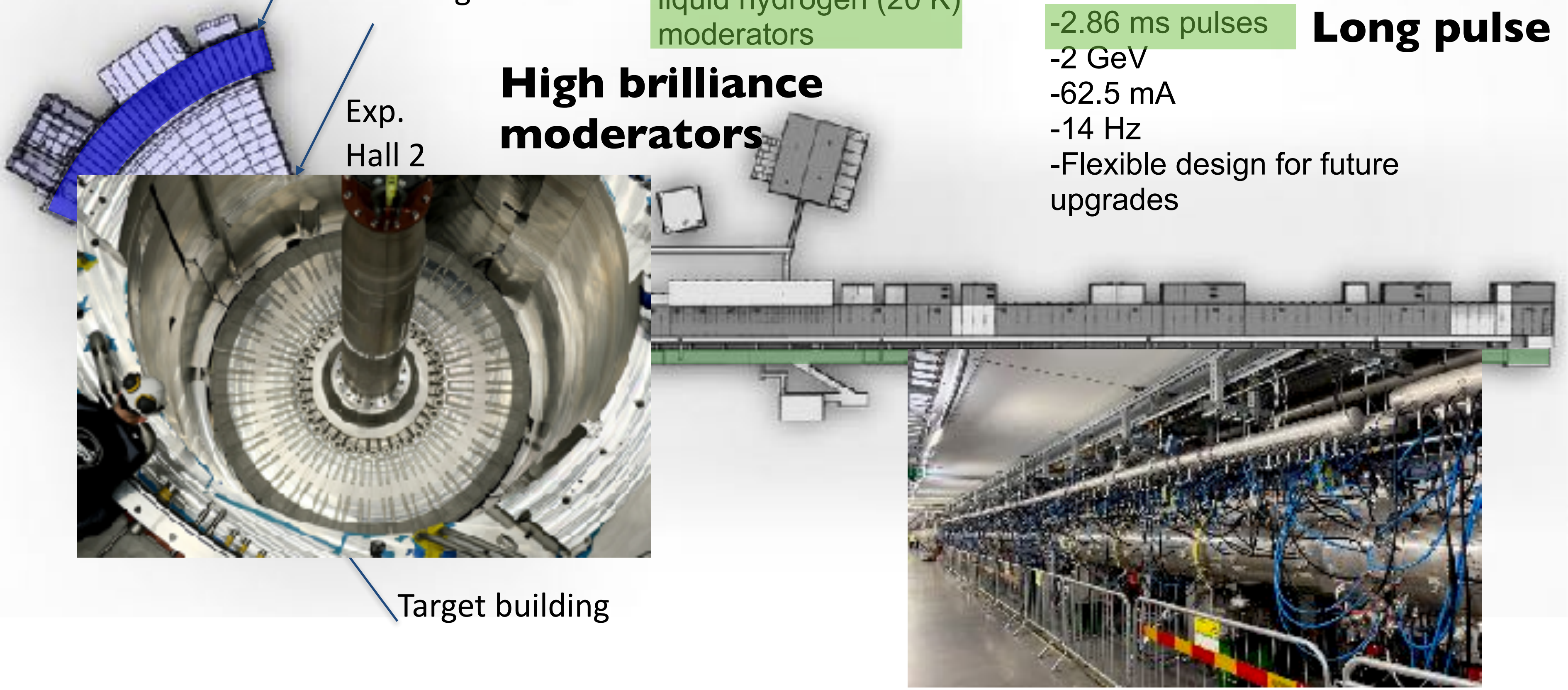
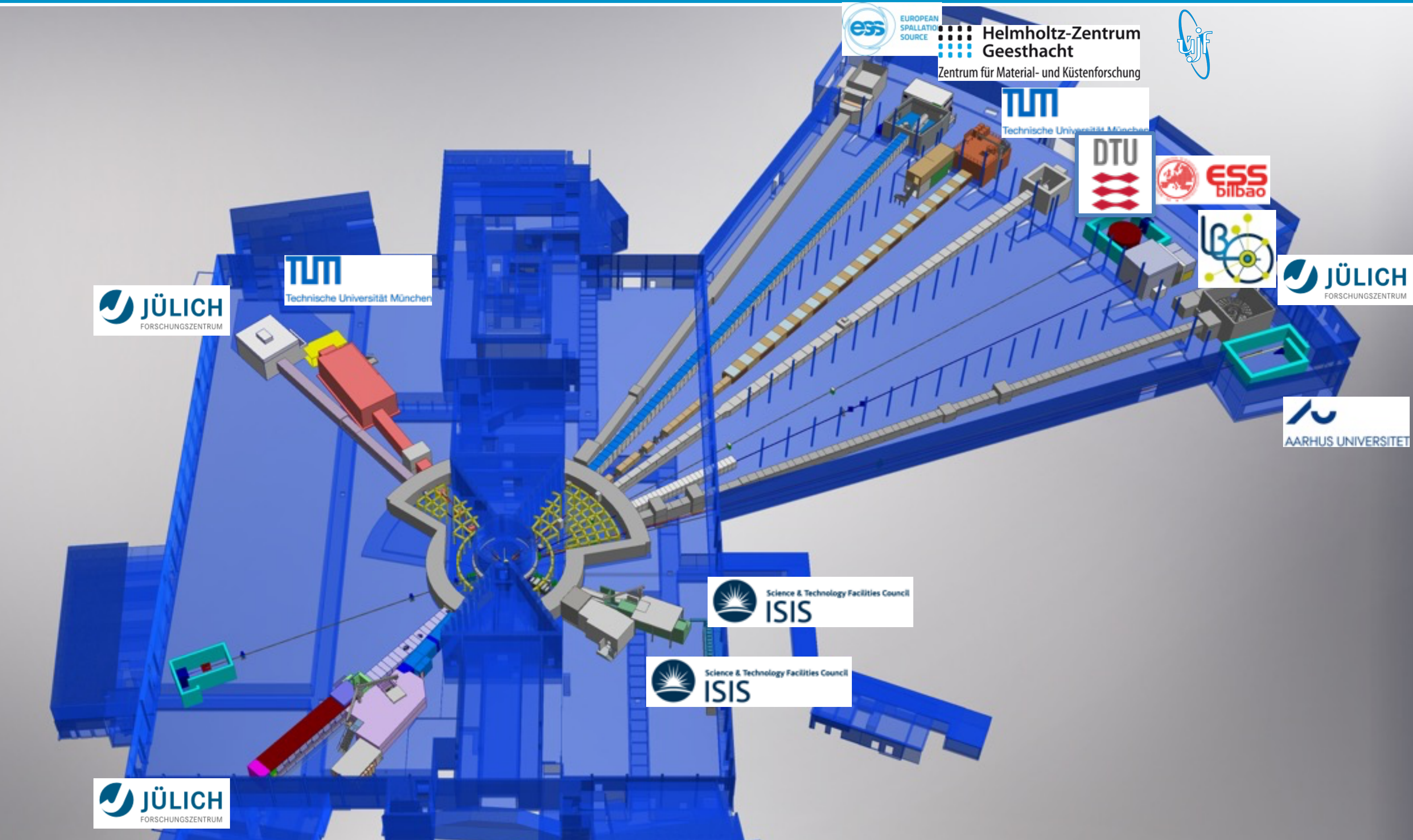
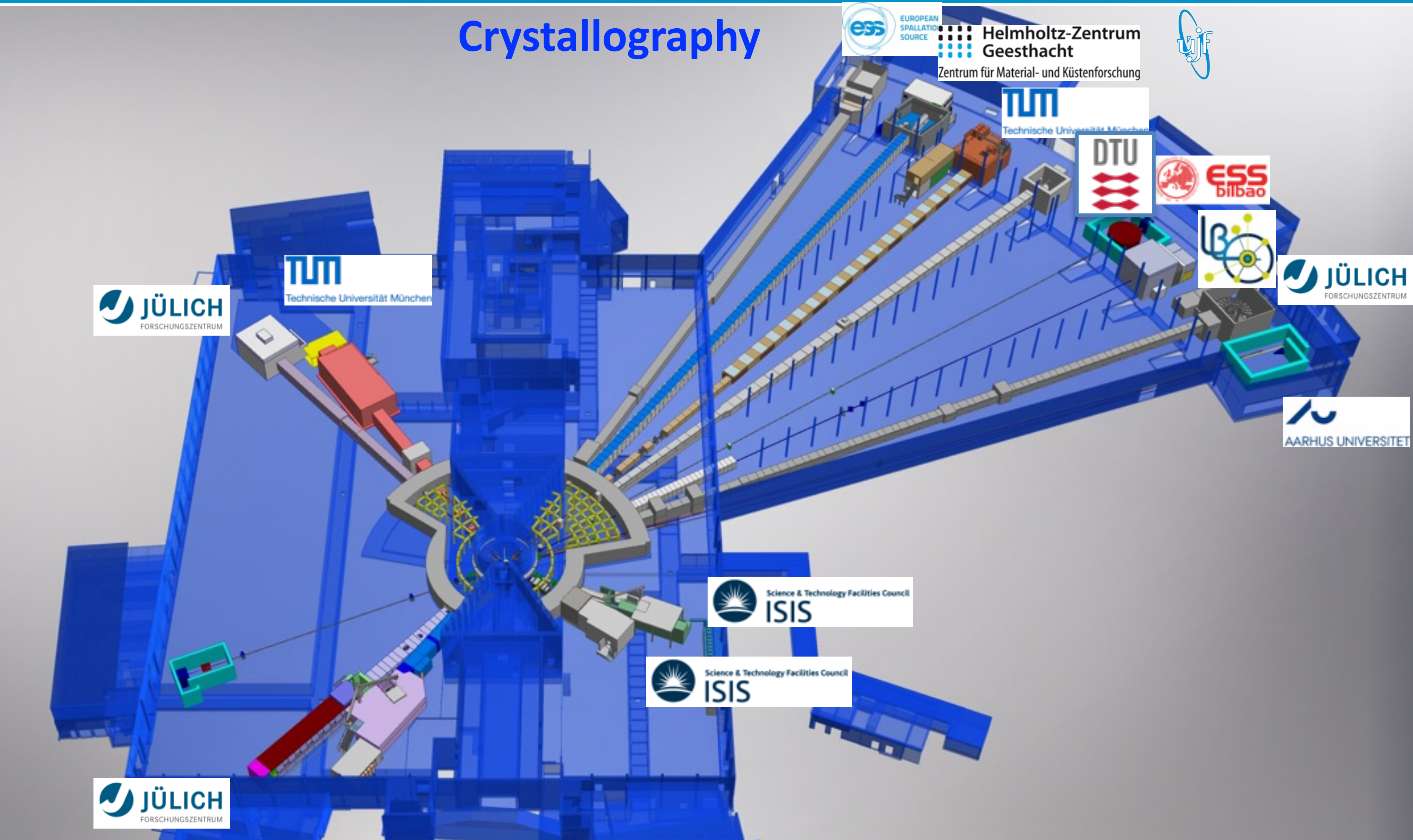


ESS Instrument suite



ESS Instrument suite

Crystallography



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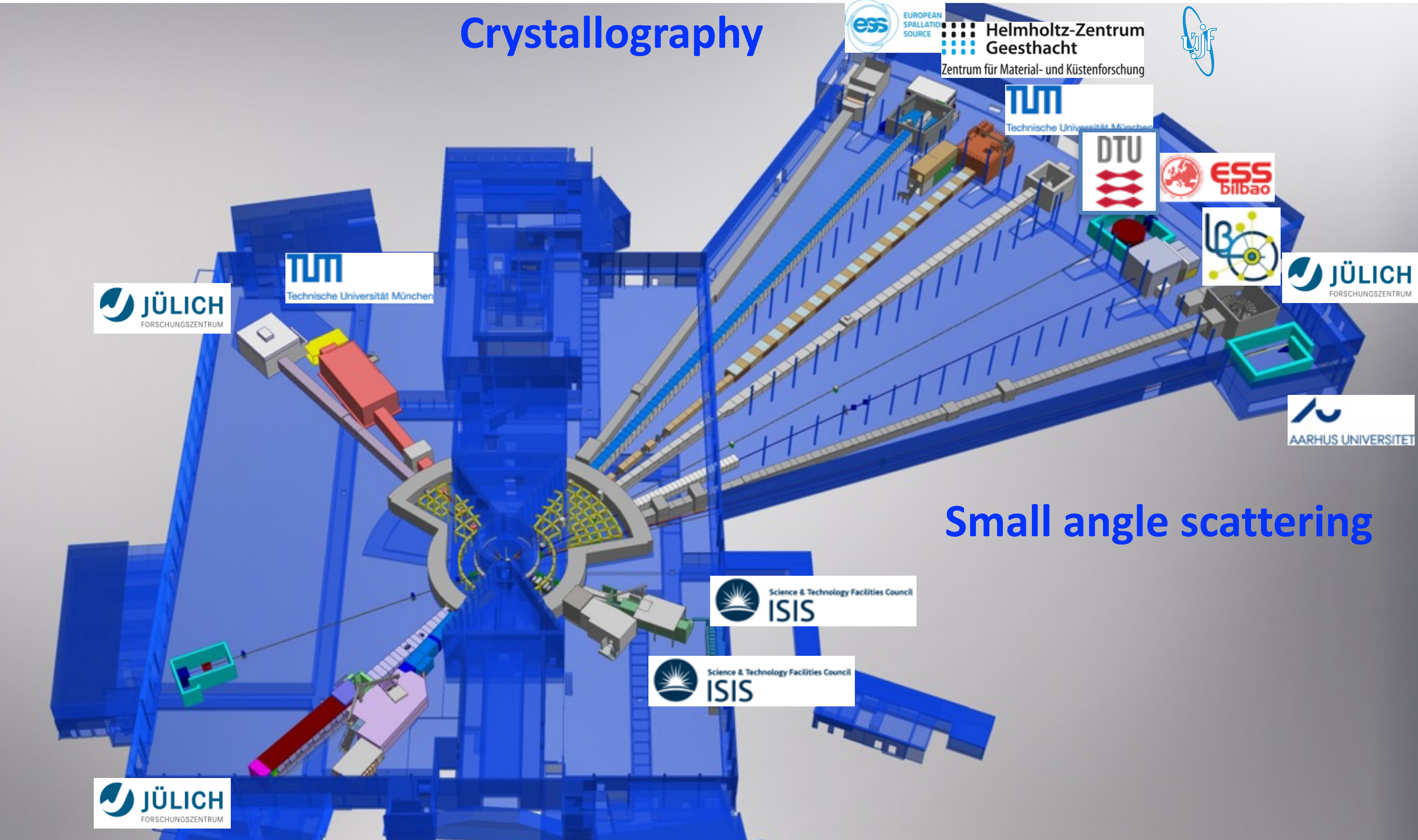

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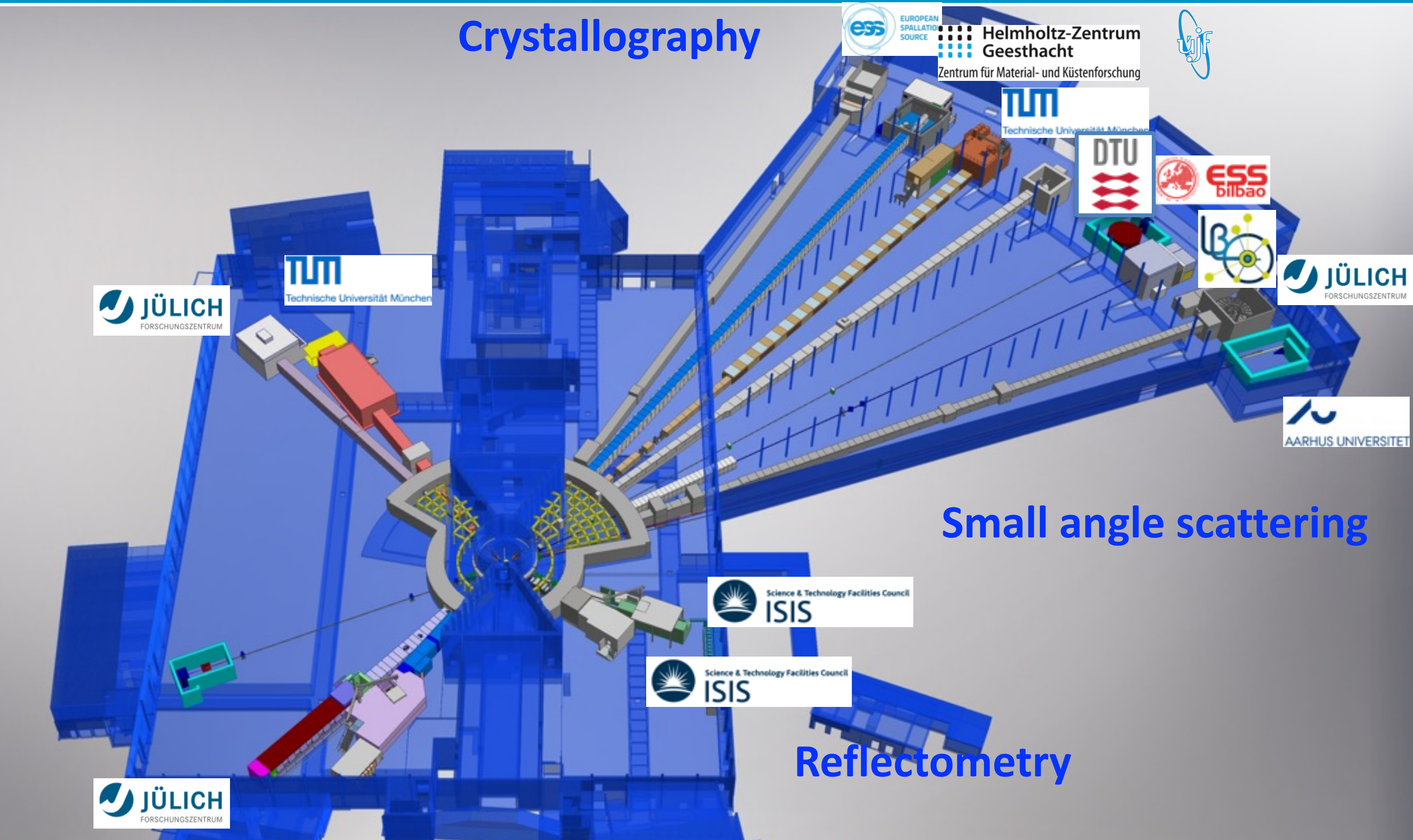

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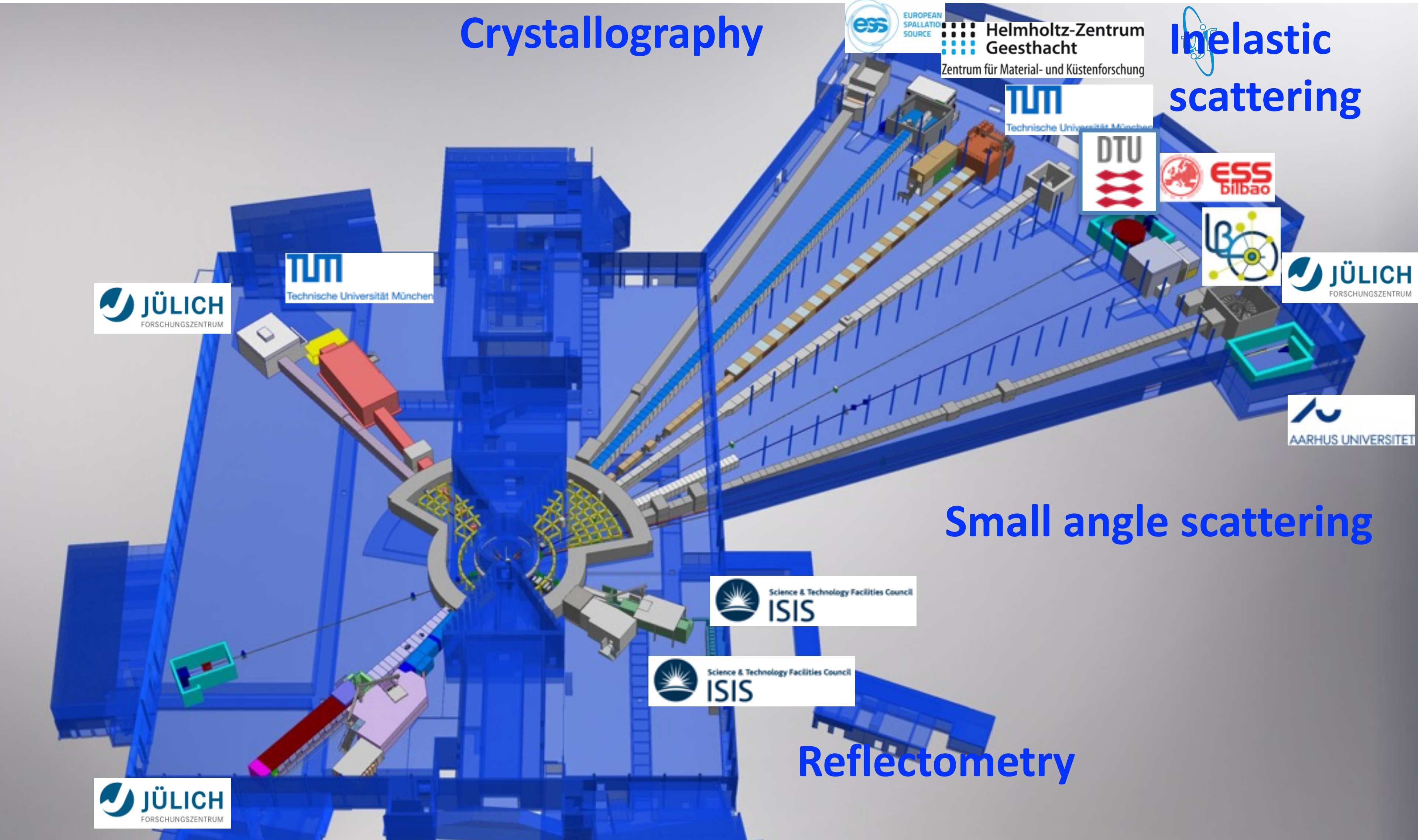

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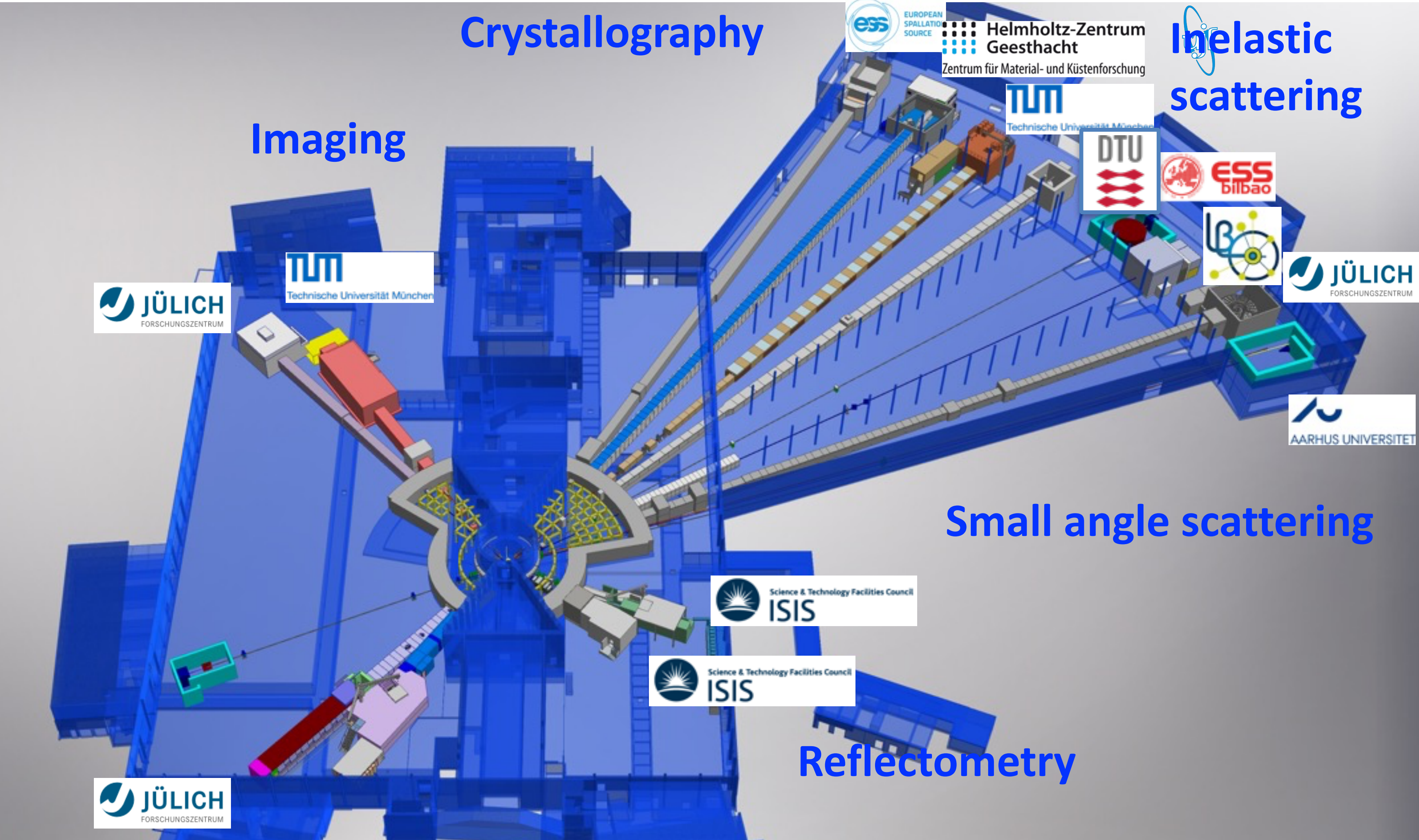


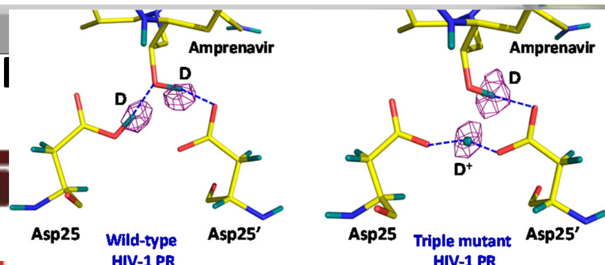
NMX – Macromolecular diffractometer at ESS

Where are hydrogens important?

Enzyme mechanisms

Protein-ligand interactions

Proton transport across membranes

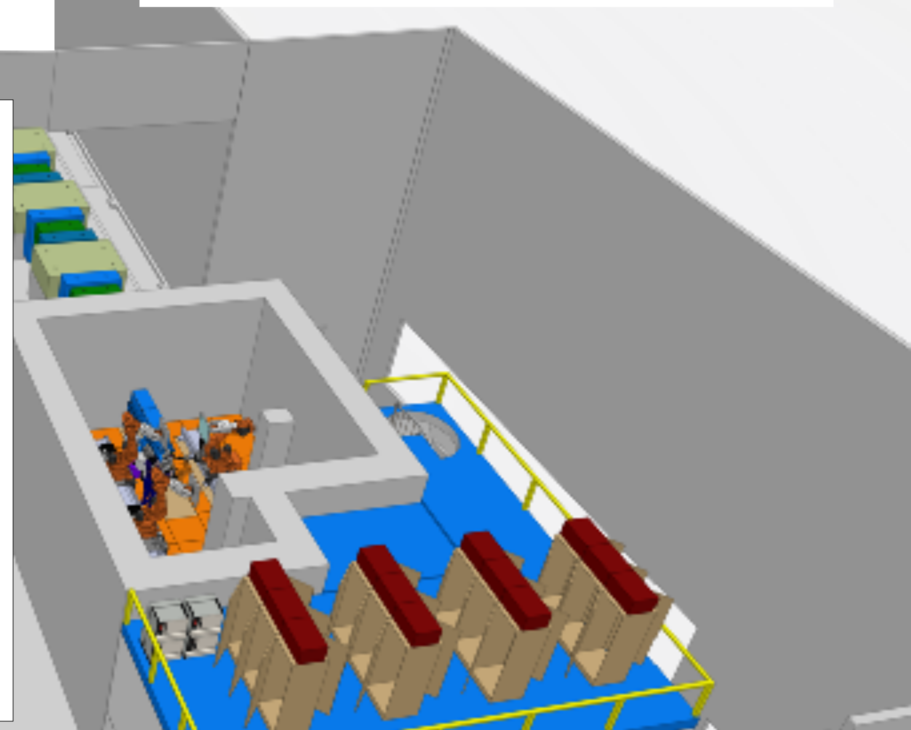


Key advantages of ESS Macromolecular Diffractometer

Smaller crystals needed (200 µm vs. 1 mm)

Data collection faster (days vs. weeks)

Larger unit cells possible (300 Å vs. 150 Å)



WIG

ibs Institut de Biologie Structurale

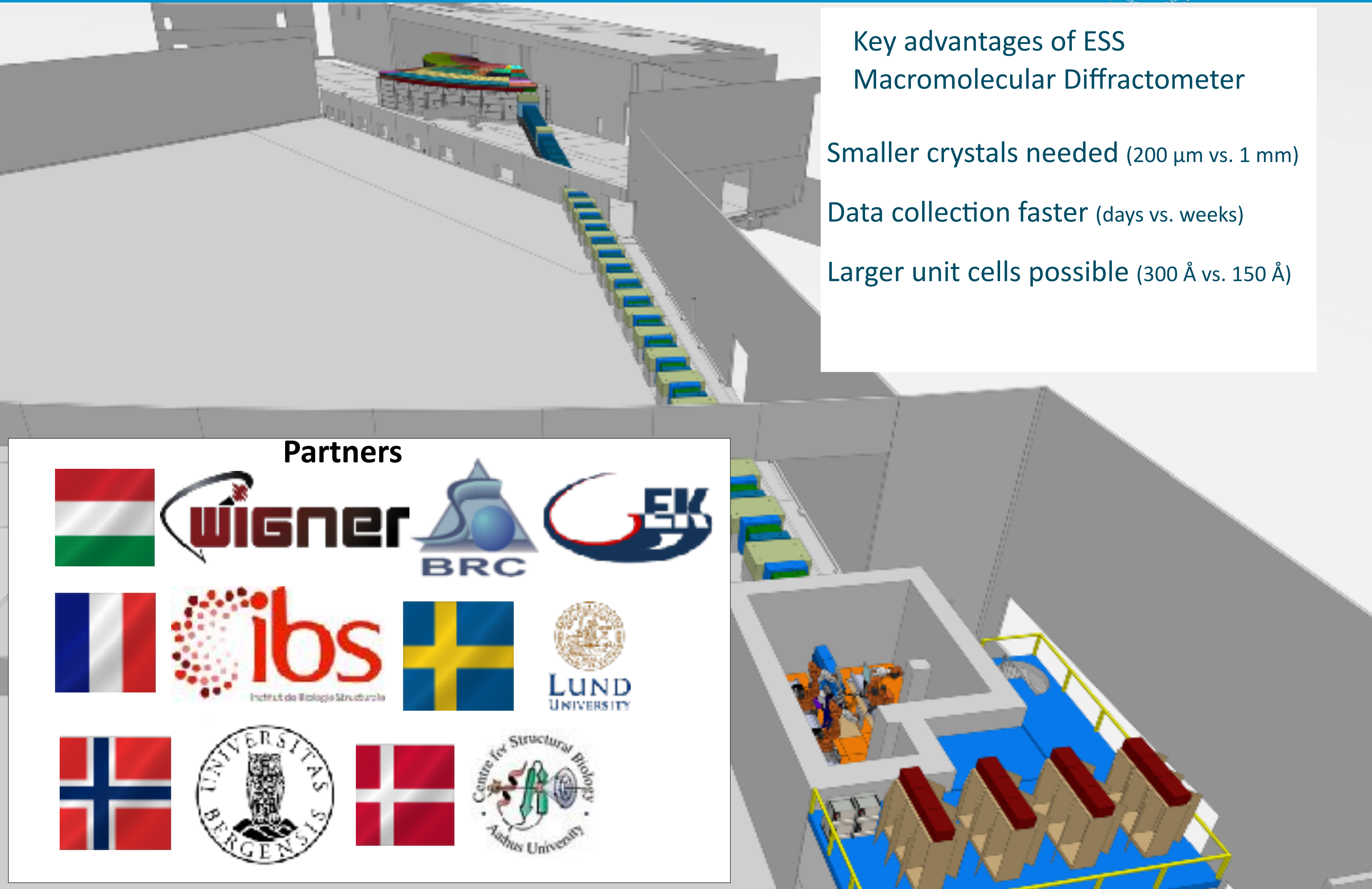
Lund University

Universitas Bergensis

Centre for Structural Biology Aarhus University

NMX – Macromolecular diffractometer at ESS

EUROPEAN SPALLATION SOURCE



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Partners

Wigner

BRC

EK

ibs Institut de Biologie Structurale

Lund University

Universitas Bergensis

Centre for Structural Biology, Aarhus University

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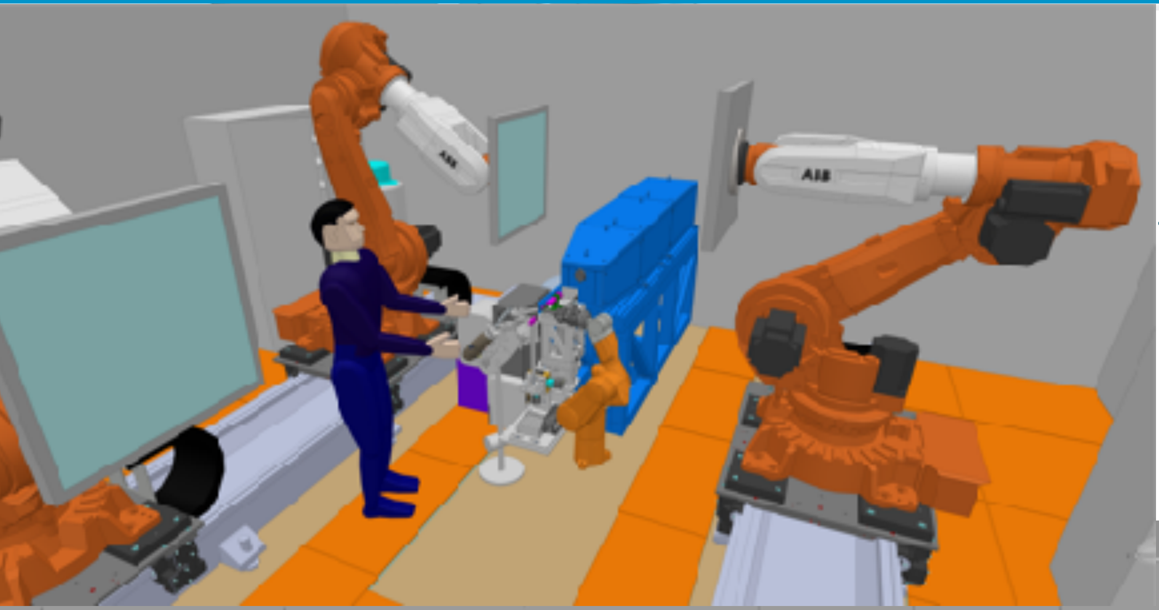

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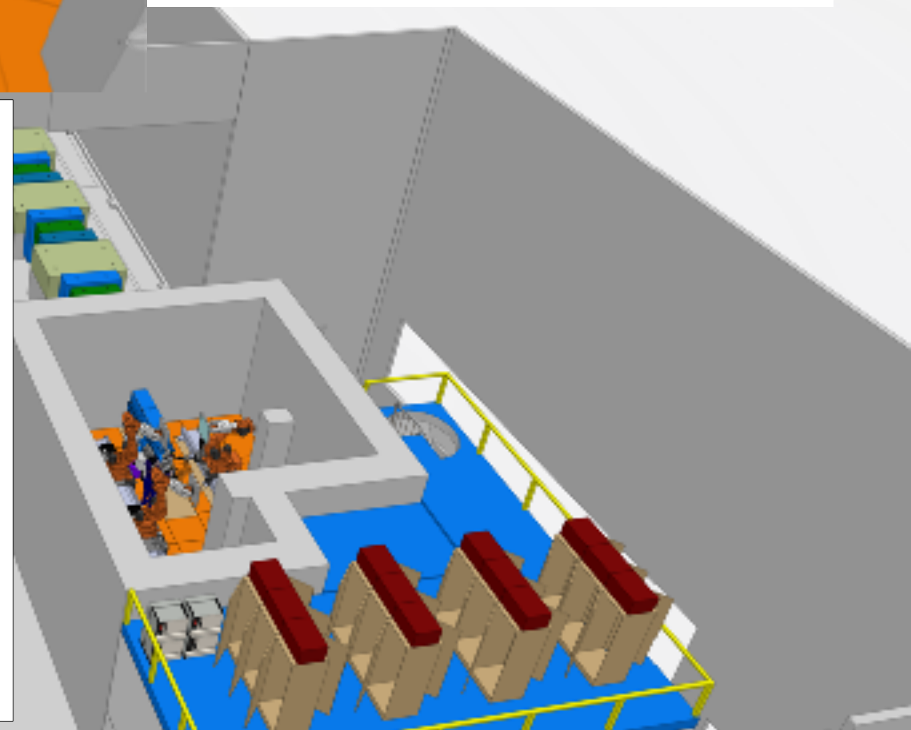

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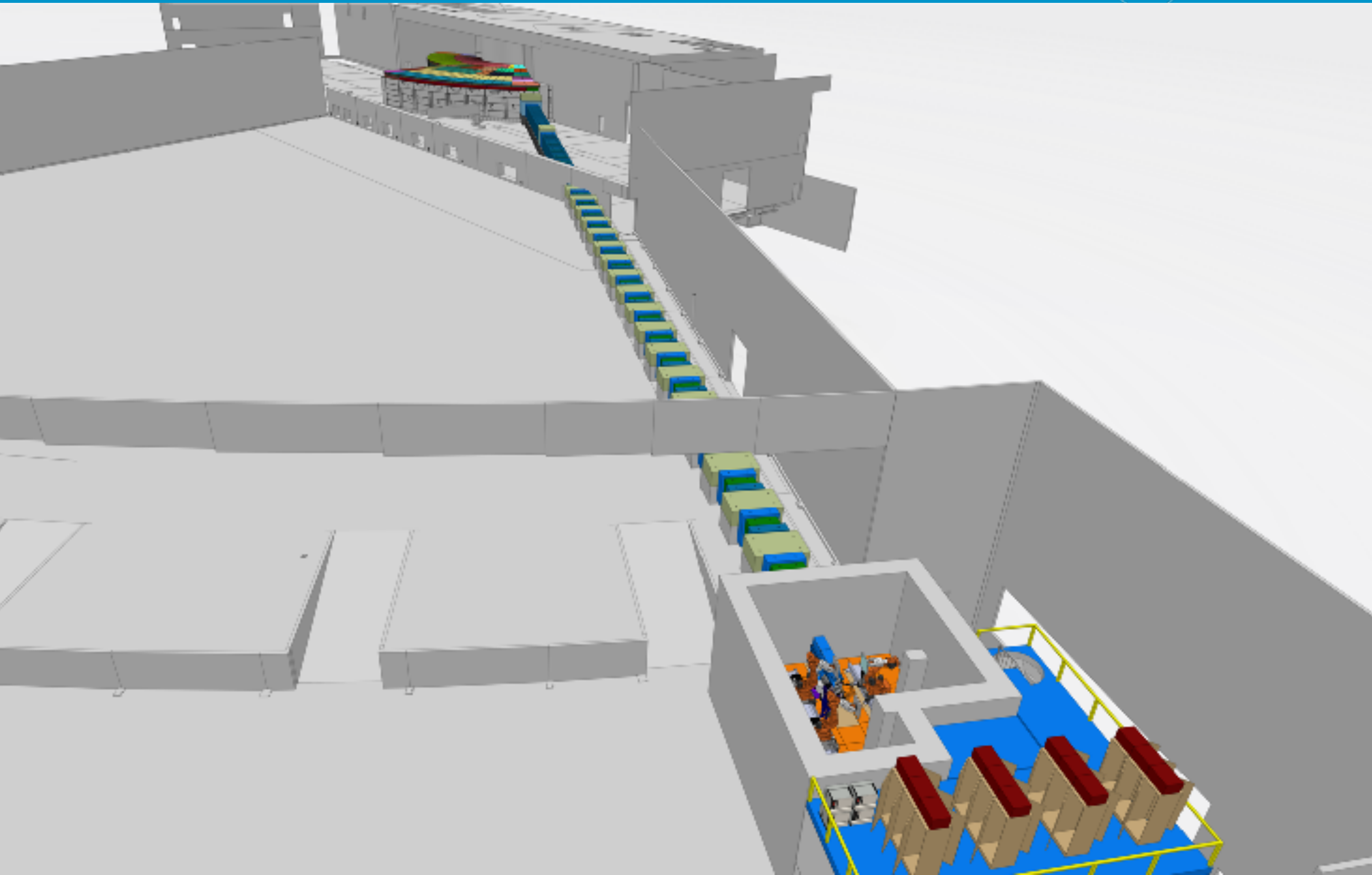

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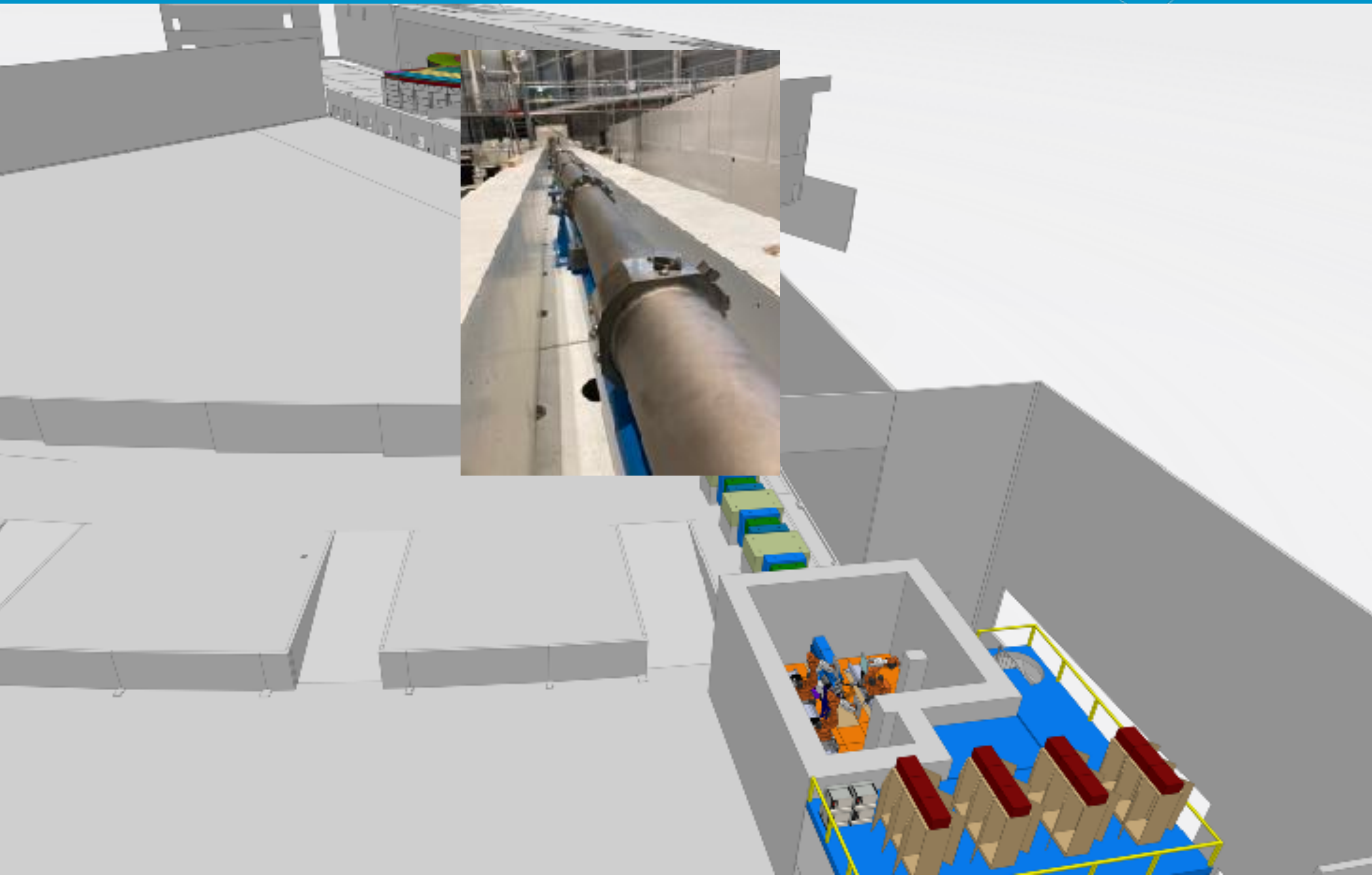
EUROPEAN SPALLATION SOURCE



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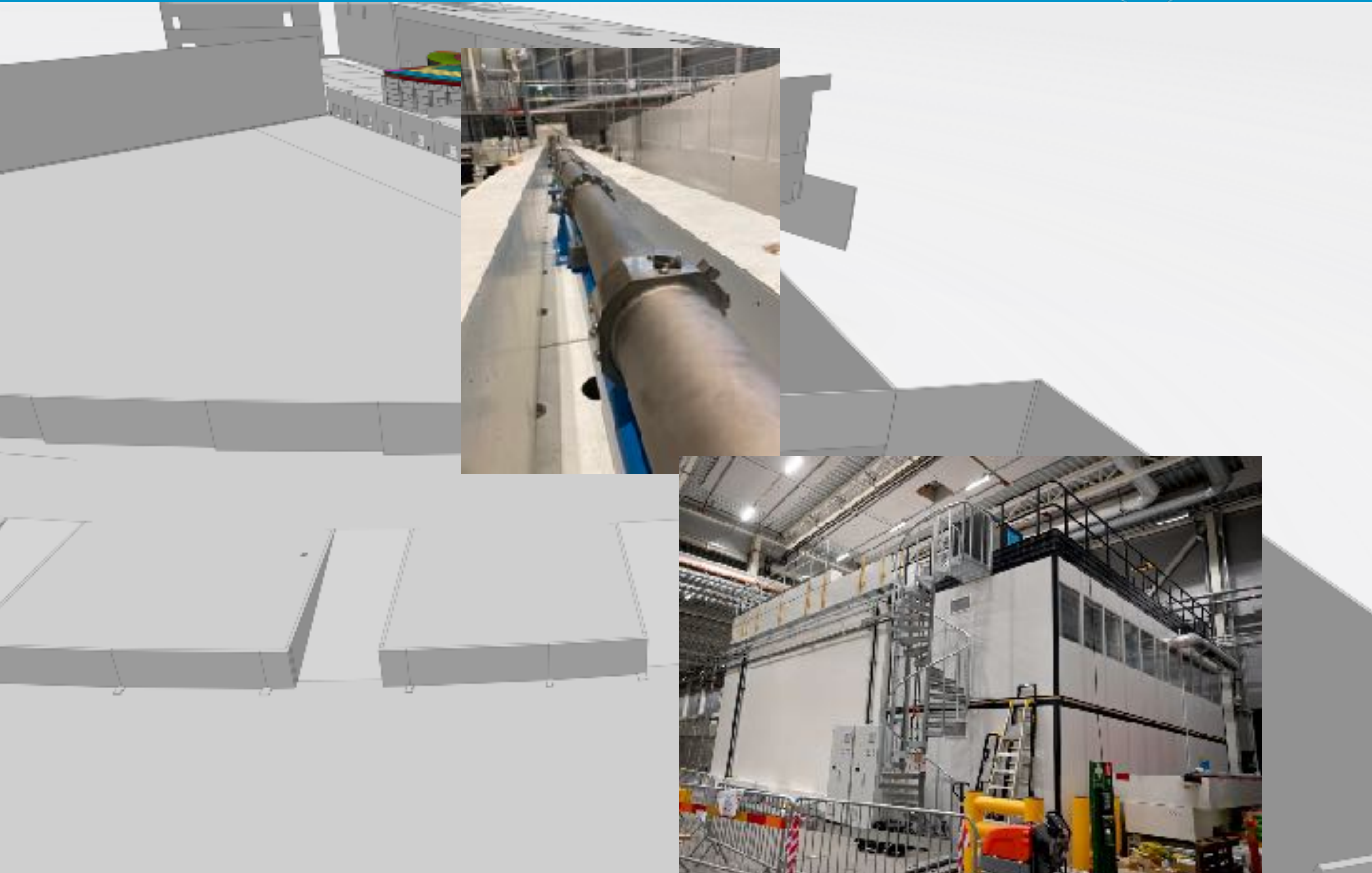

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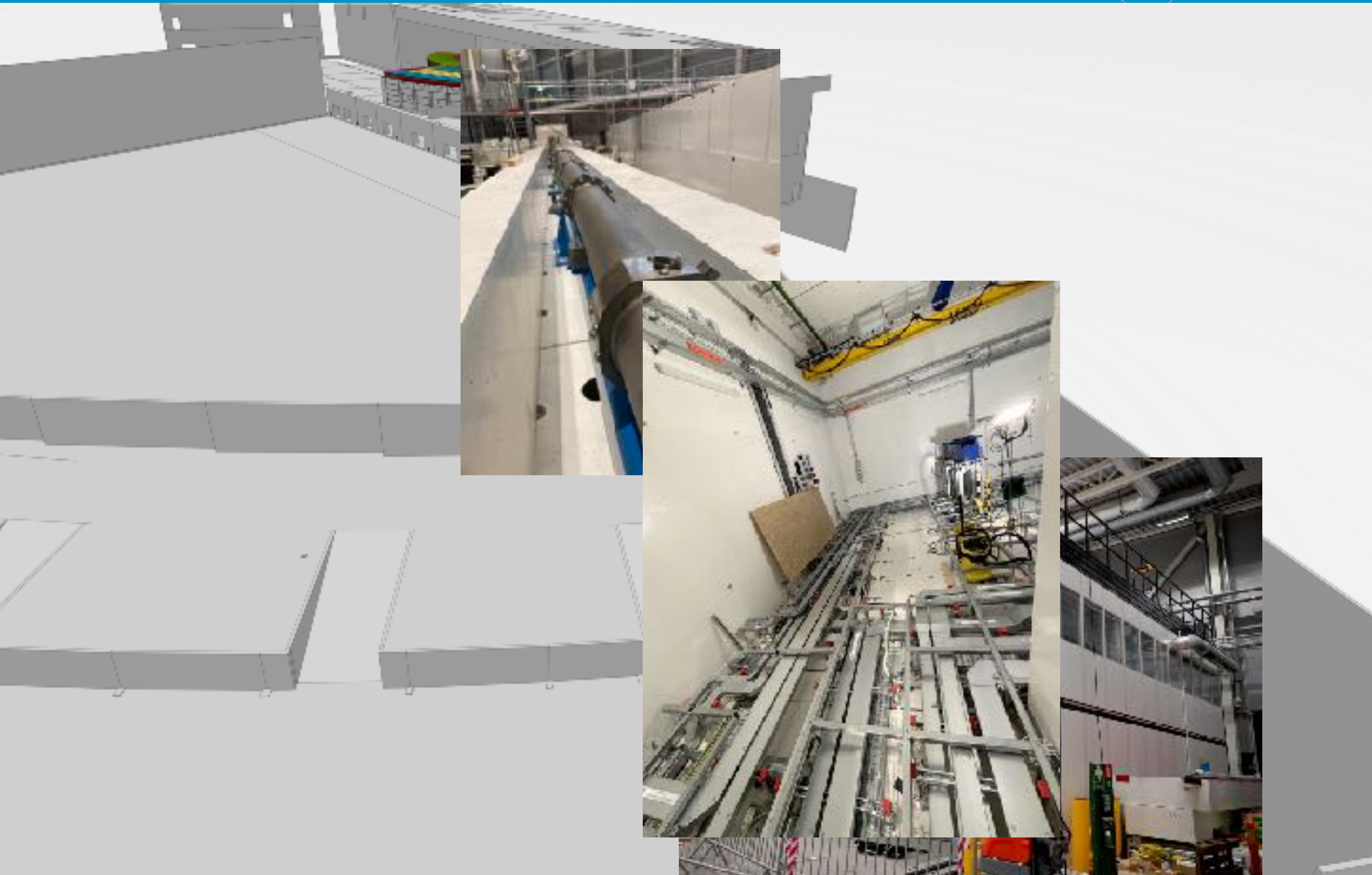

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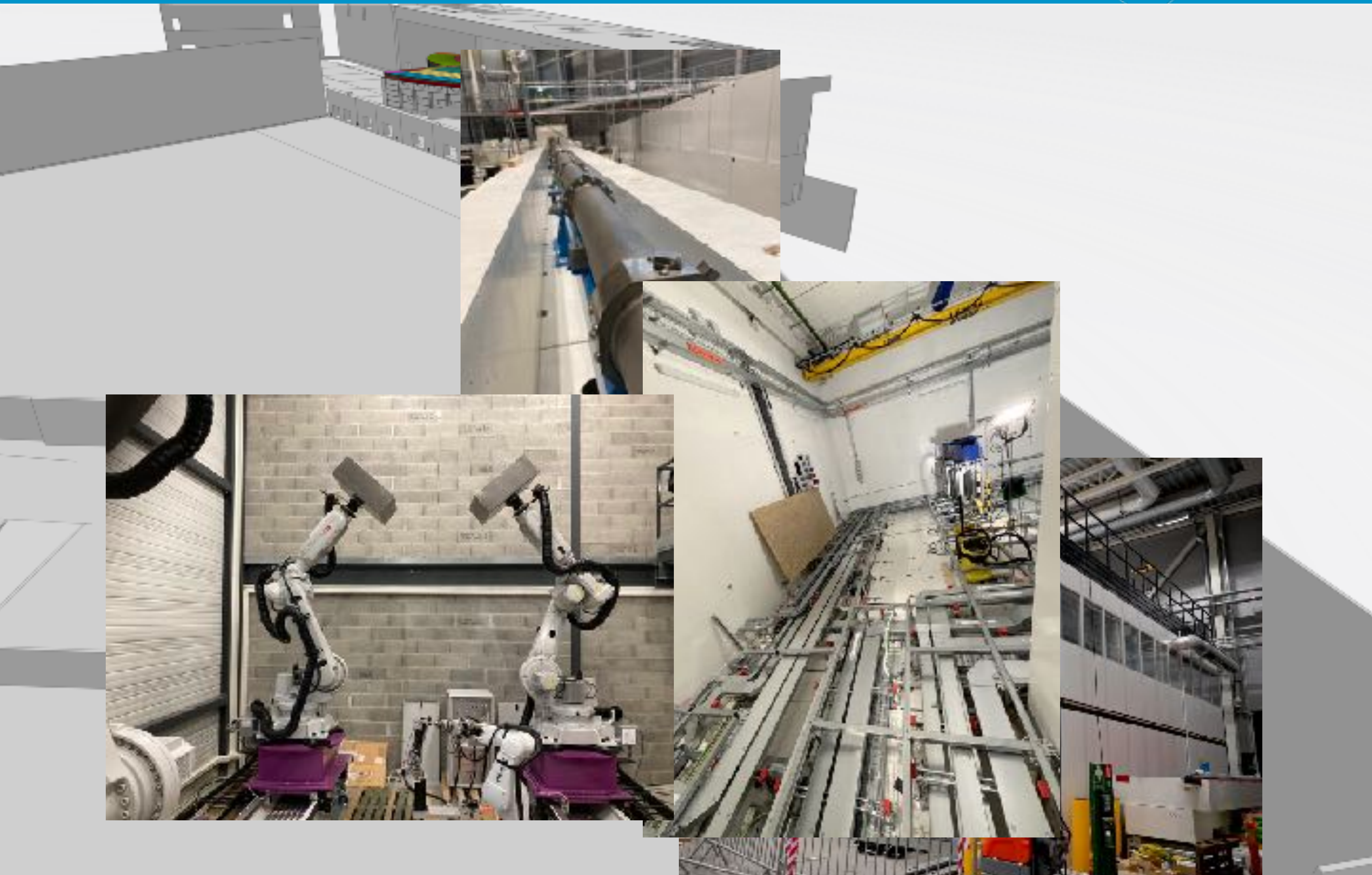
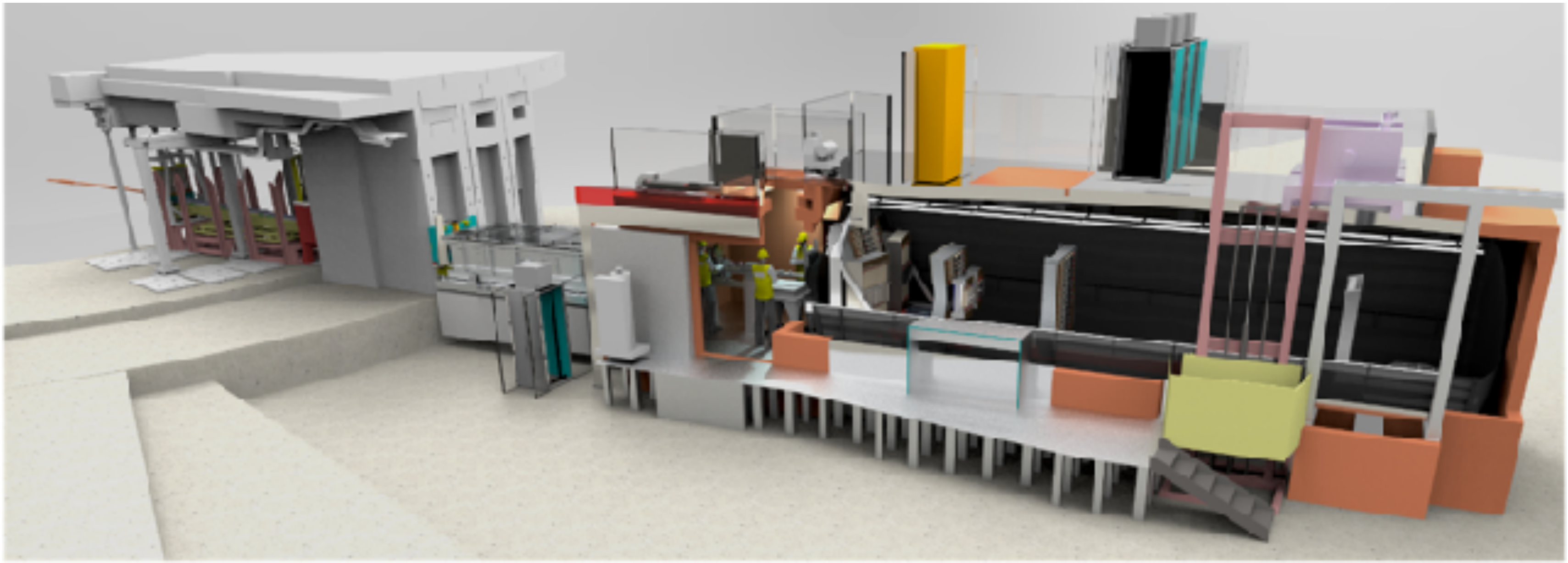


LoKI Broad Band SANS

- LoKI will have high flux, wide simultaneous size range, and a large sample area.
- LoKI will enable the use of small beams, making scanning experiments & microfluidics routine.
- LoKI aims to provide the ability to perform "single-shot" kinetic measurements on sub-LoKI will have high flux, wide simultaneous size range, and a large sample area.



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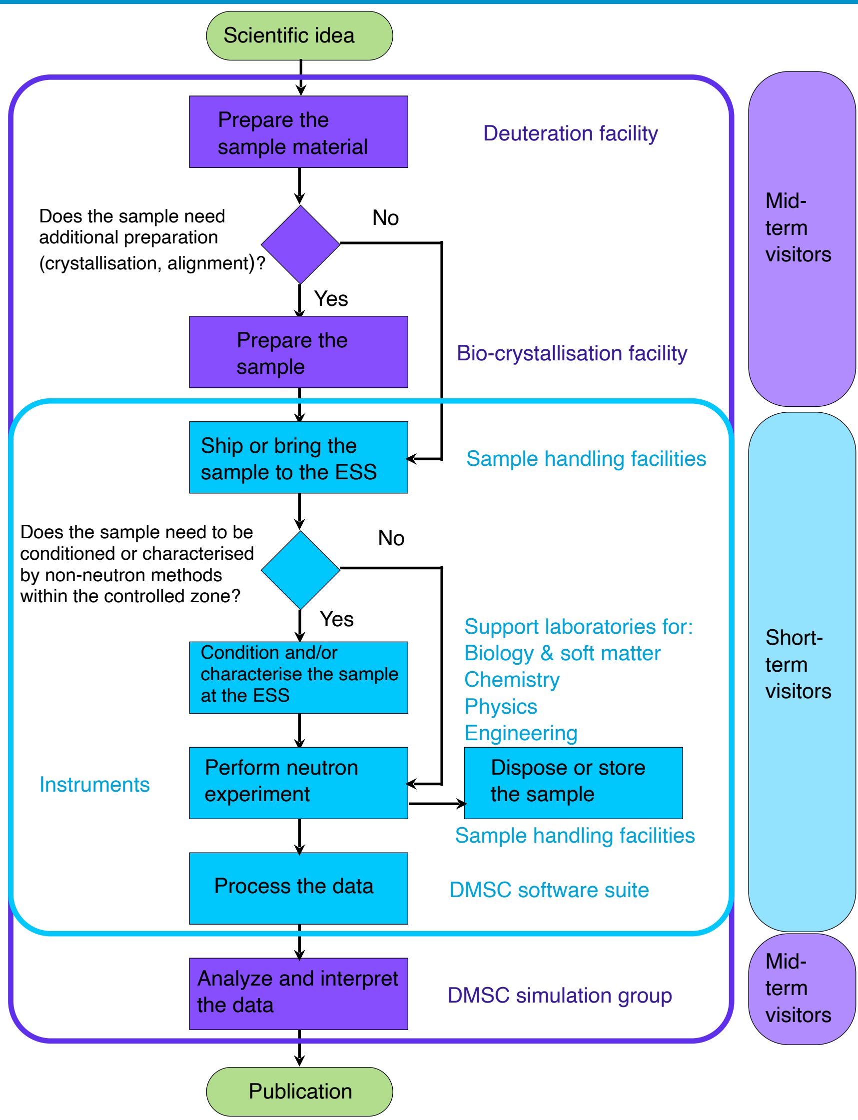
Supporting facilities



DEMAX platform together with Lund University

LUND UNIVERSITY

LP3 Lund Protein-Production Platform

What will be different at the ESS

EUROPEAN SPALLATION SOURCE

Macromolecular crystallography

- Smaller crystals (~200 µm)
- Larger unit cells (< 300 Å)
- Data collection in days, not weeks

Small-angle neutron scattering

- Smaller sample volumes (~10 µl)
- Higher throughput of samples
- Faster time resolution

Reflectometry

- Smaller samples (~1 cm^2, 10-100 µg)
- Kinetic studies faster (x10)

Inelastic neutron scattering

- Smaller samples (<5 mg)
- Longer length scales
- Broader dynamic range

Supporting facilities

- Sample preparation & characterisation laboratories
- Deuteration (biological & chemical)
- Crystal growth
- Computational support (DMSC Copenhagen)

EUROPEAN
SPALLATION
SOURCE

Questions?

esko.oksanen@ess.eu