Structural biology with neutrons at the European Spallation Source

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Macromolecular Crystallography

Outline

- **Neutrons in structural biology**
- Structural biology at ESS

Why neutrons for biological structures?

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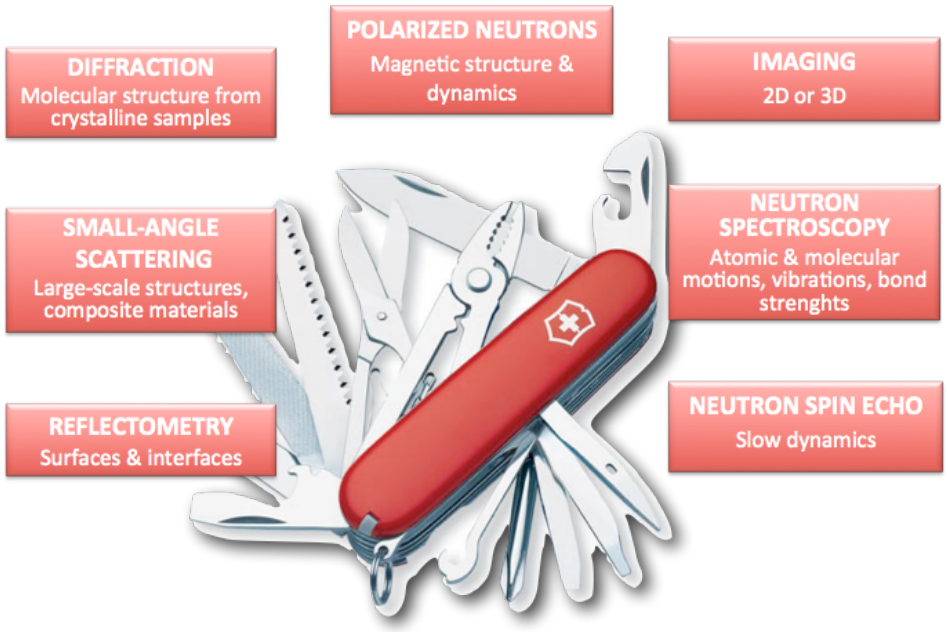
Neutron scattering is not a technique!



- We can see light atoms → hydrogen positions

  Crystallography

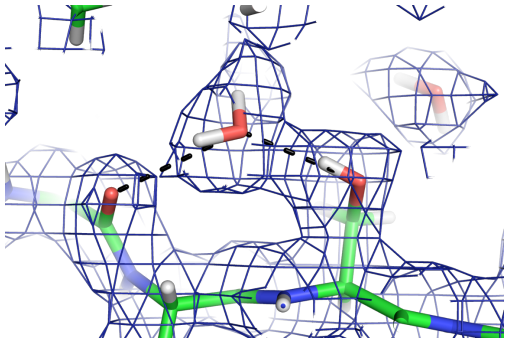
- We can use isotope labelling to create contrast → protein-protein complexes

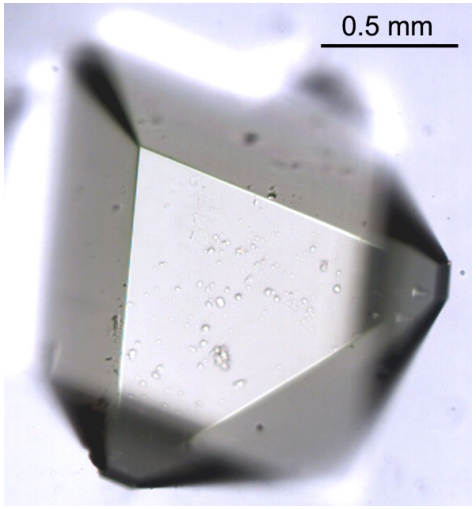
  Small angle scattering, reflectometry

- We can observe dynamics with inelastic scattering → relating dynamics to function

  Neutron spectroscopy

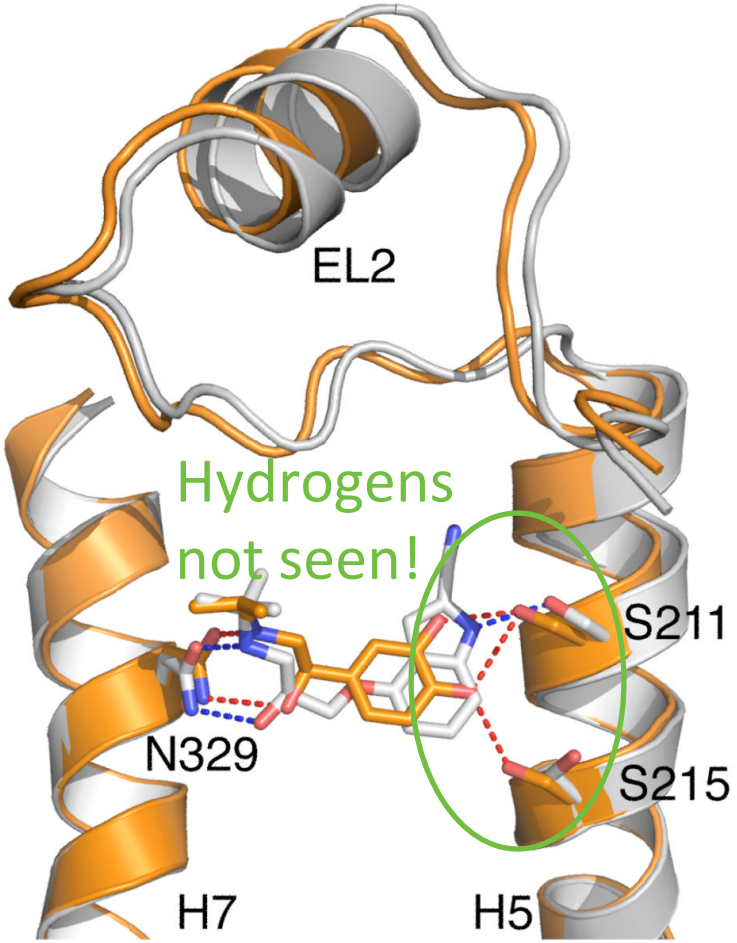
Neutron Macromolecular Crystallography





- ☺Hydrogens are visible
- ☺No radiation damage
- ☹Large crystals needed
- ☹Data collection takes weeks
- ☹Few instruments available

Where are hydrogens important?

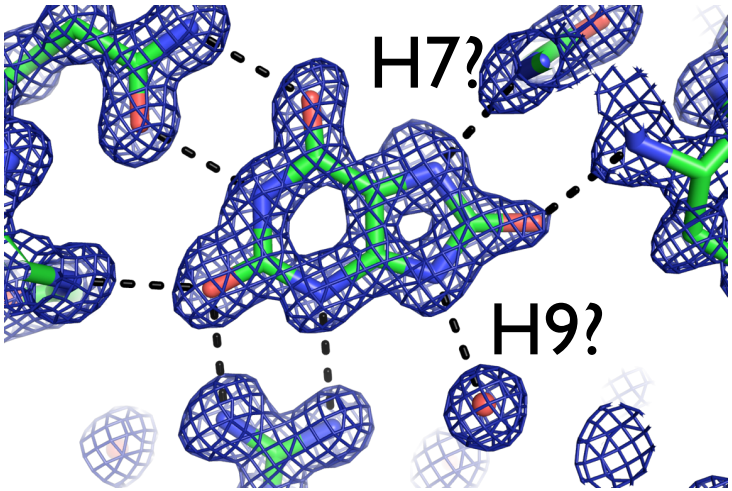


Oksanen, E *et al. J. R. Soc. Interface* **2009**, *6 Suppl 5*, S599-610.

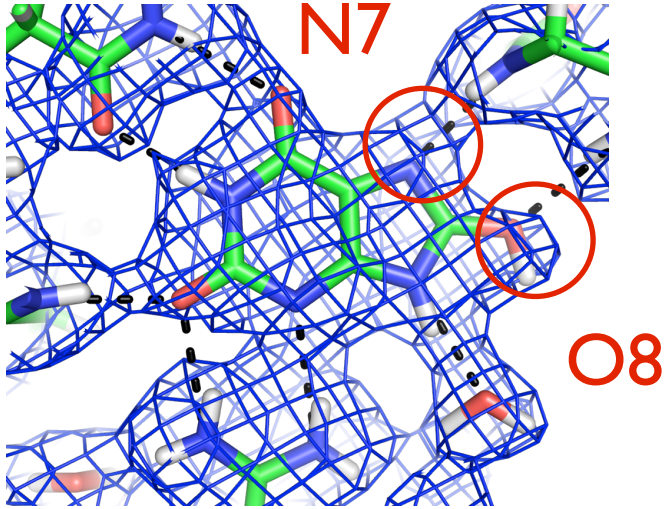
Why is hydrogen interesting?

1. Enzyme mechanism

Urate oxidase



Mono- or dianion?



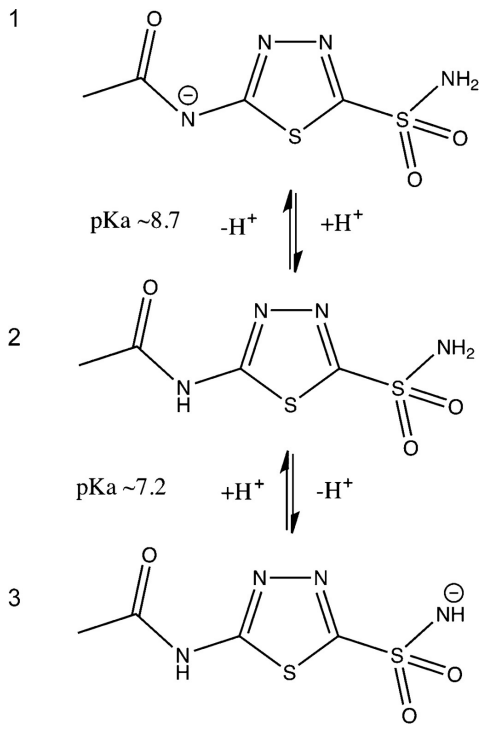
Unexpected enol form
(8-hydroxyxanthine)

Oksanen, E.; Blakeley, M. P.; El-Hajji, M.; Ryde, U.; Budayova-Spano, M. *PLoS ONE* (2014), ***9***, e86651

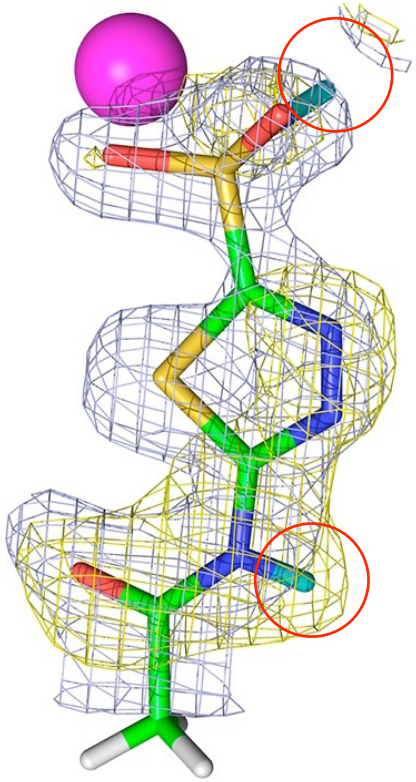
Why is hydrogen interesting?

2. Ligand binding and protonation states
Acetazolamide in Human Carbonic Anhydrase II

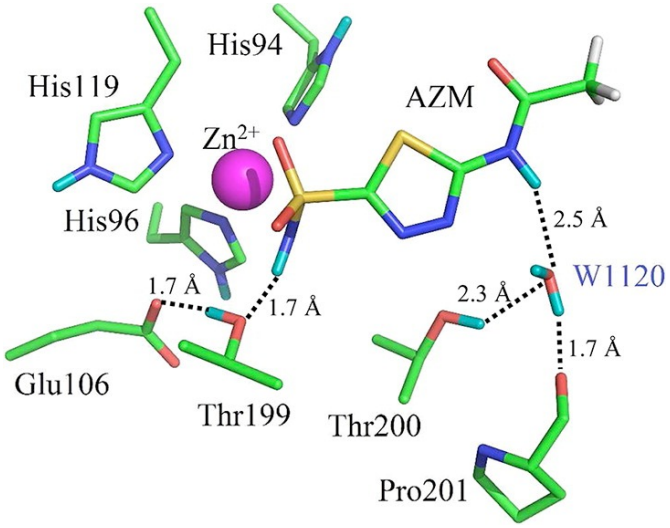
Three possible protonation states at physiological pH



Protonation state clearly determined by neutrons



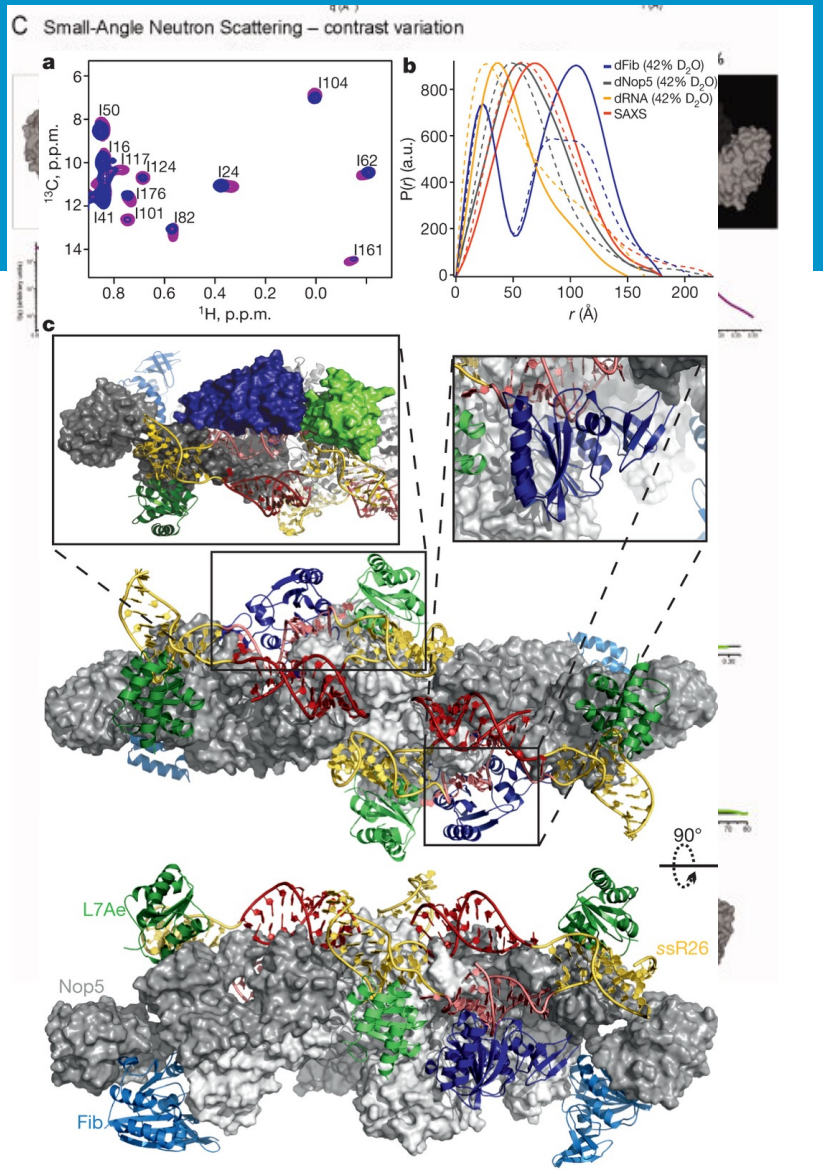
Provides full picture of ligand binding



Fisher SZ *et al.* JACS (2012);**134**:14726-14729

Small Angle Neutron Scattering

☺Solution structure
☺Complexes resolved by contrast variation
☹Requires D-labelling
☹Sample volumes larger than SAXS (~200 µl/measurement)



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

Neutron reflectometry

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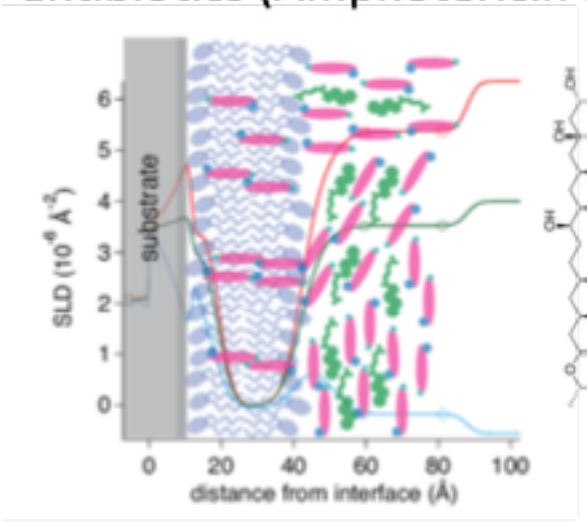
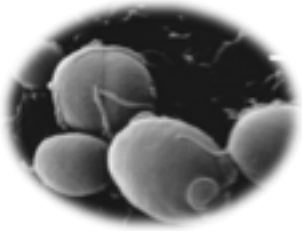
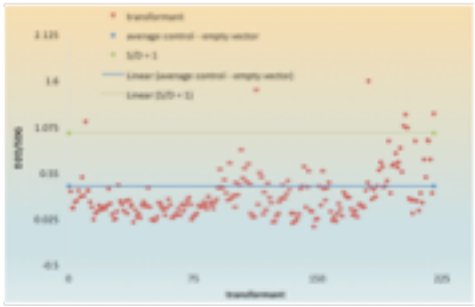
- ☺Can study surfaces in solution
- ☺Membrane composition with Å resolution
- ☹Information only along normal
- ☹Deuterated compounds essential

Mechanism of membrane-binding antibiotics (Amphotericin B):



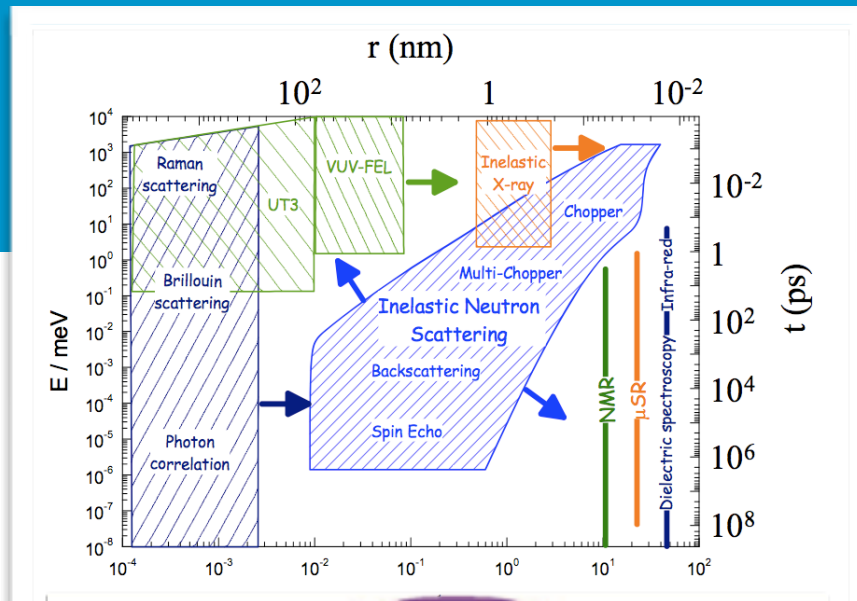


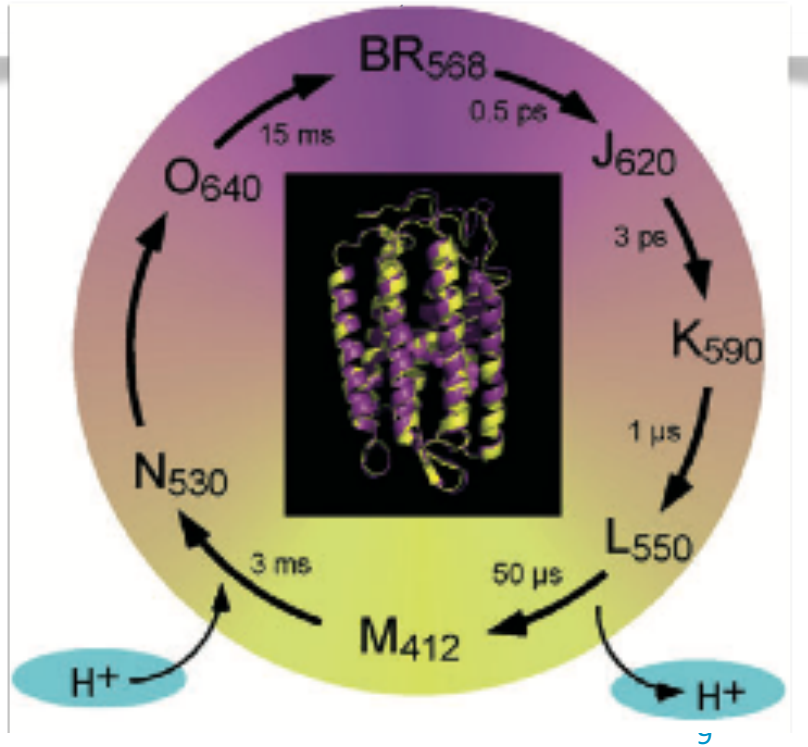


Human pathogen -> Membrane model for screening virulence genes/AmB resistance

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

Outline

- Neutrons in structural biology
- **Structural biology at ESS**

The world's brightest neutron source

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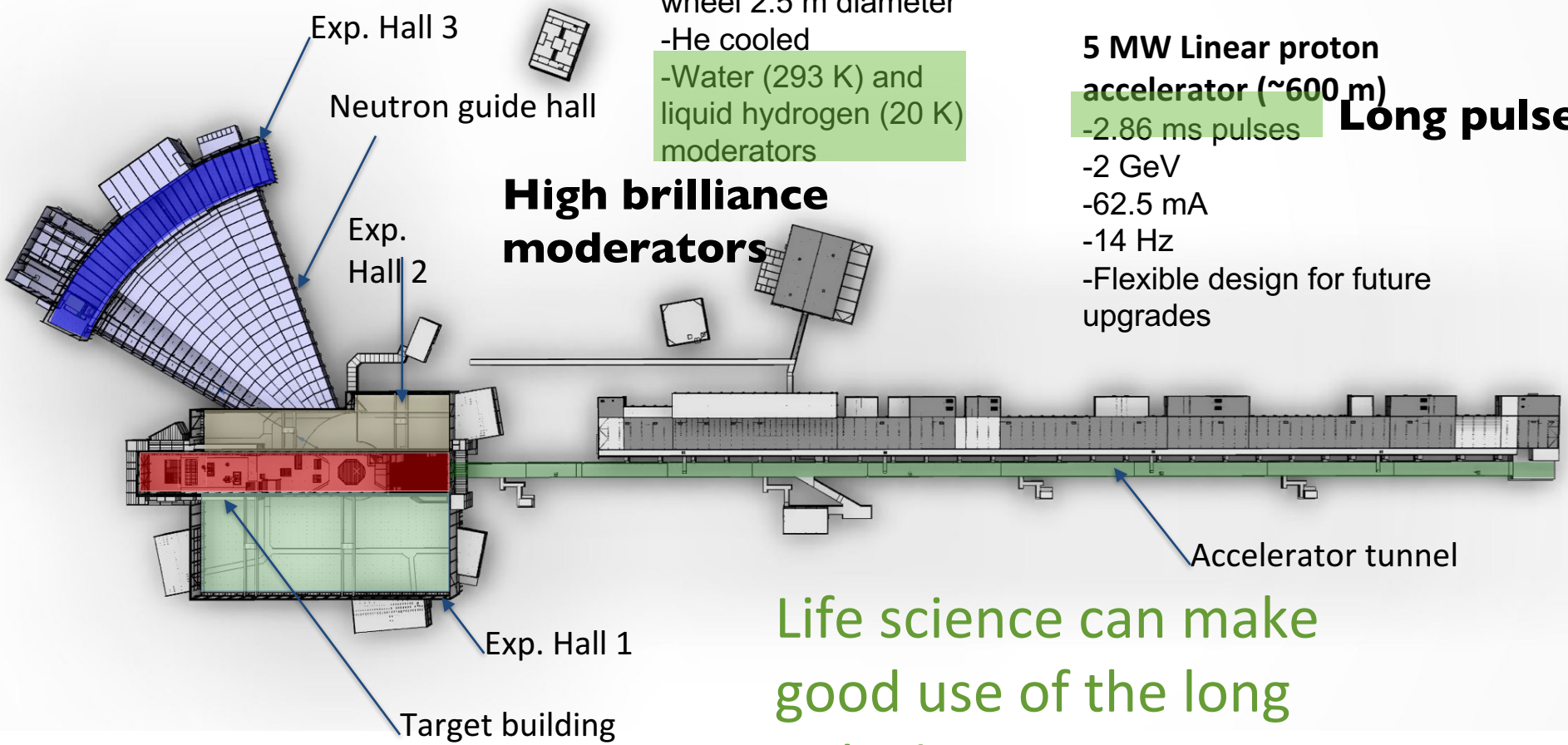
Target station
-Rotating tungsten wheel 2.5 m diameter
-He cooled
-Water (293 K) and liquid hydrogen (20 K) moderators

5 MW Linear proton accelerator (~600 m)
-2.86 ms pulses
-2 GeV
-62.5 mA
-14 Hz
-Flexible design for future upgrades

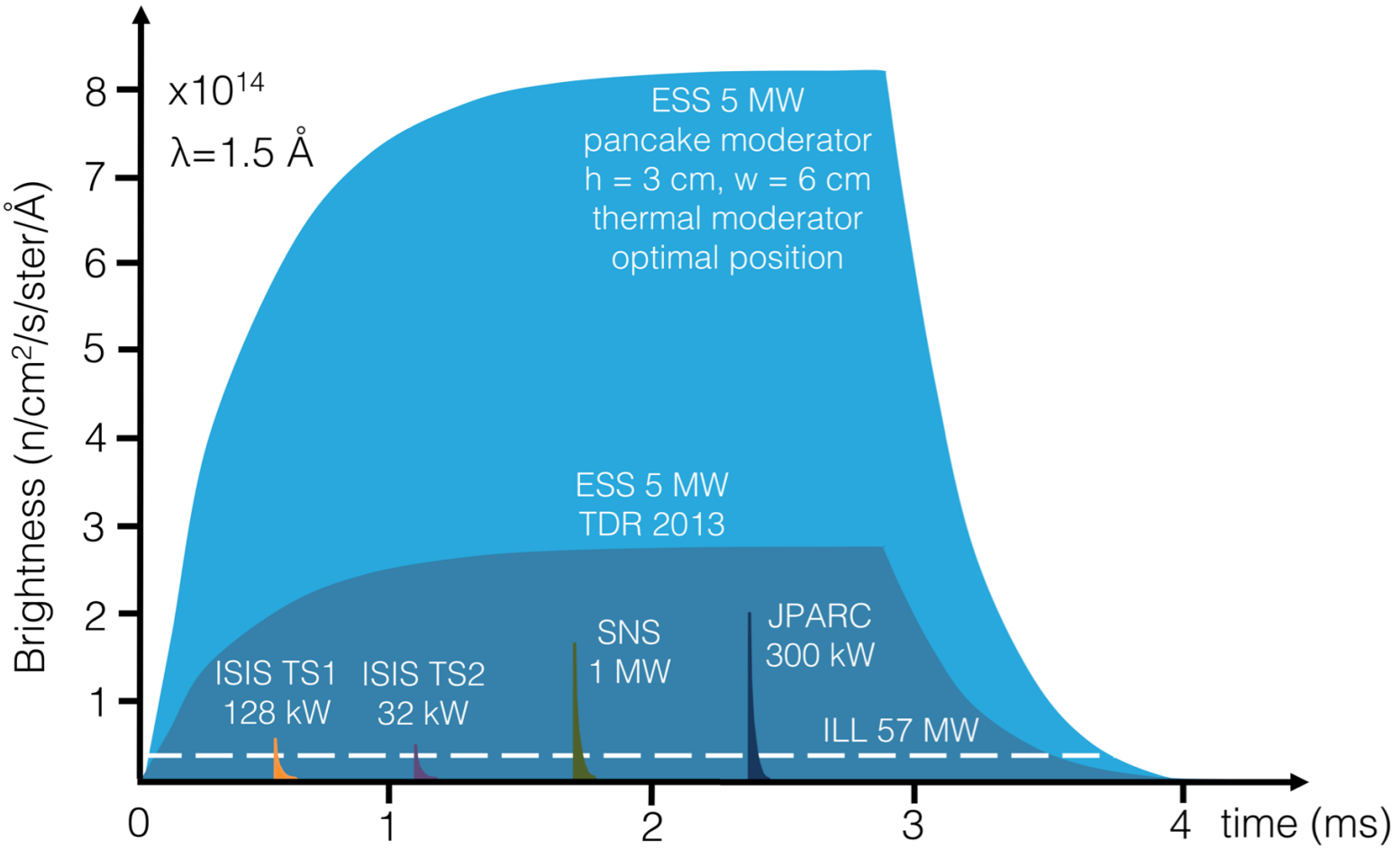
Long pulse

High brilliance moderators

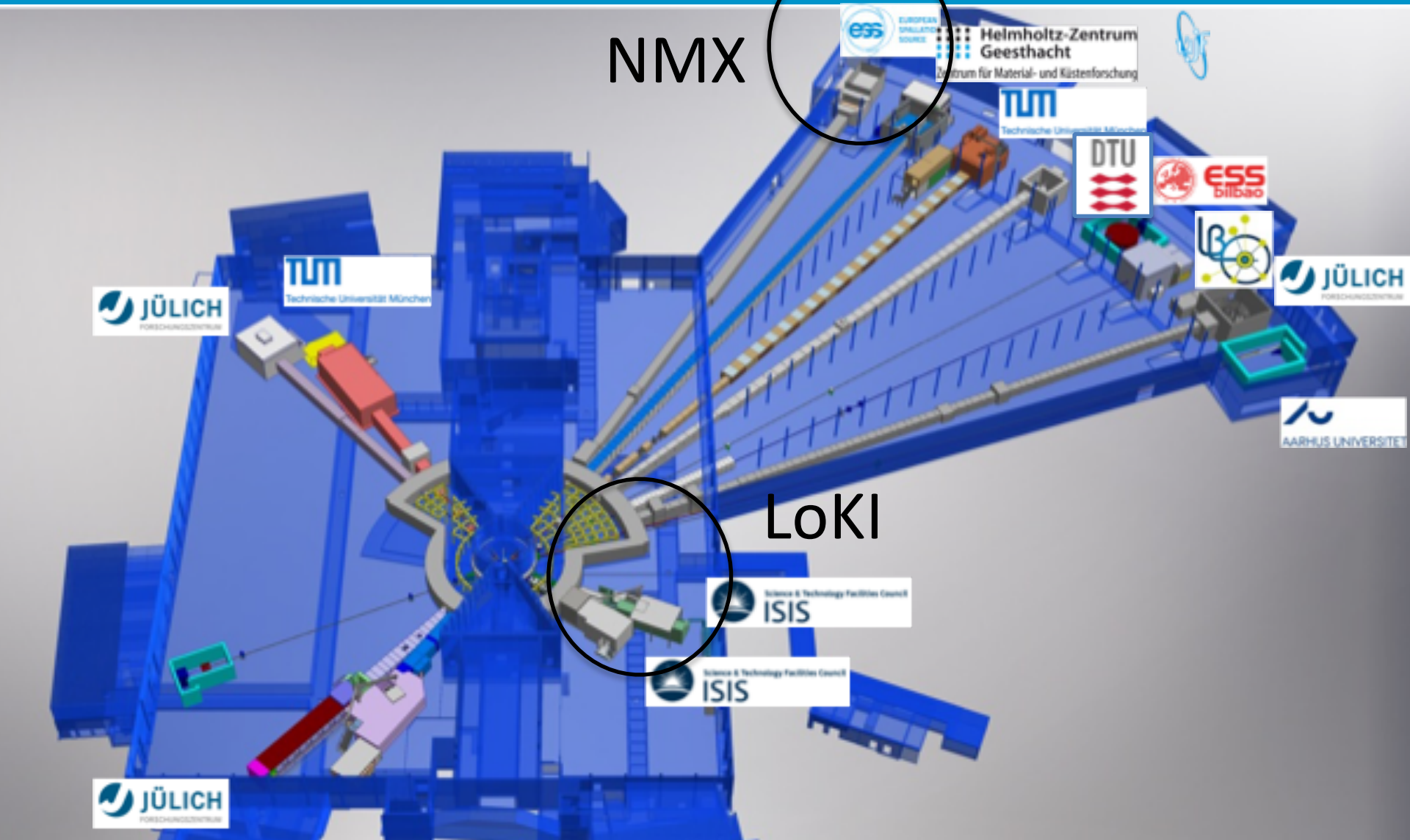


Life science can make good use of the long pulse!

ESS Long Pulse



Instrument suite is taking shape



NMX – Macromolecular crystallography

Partners

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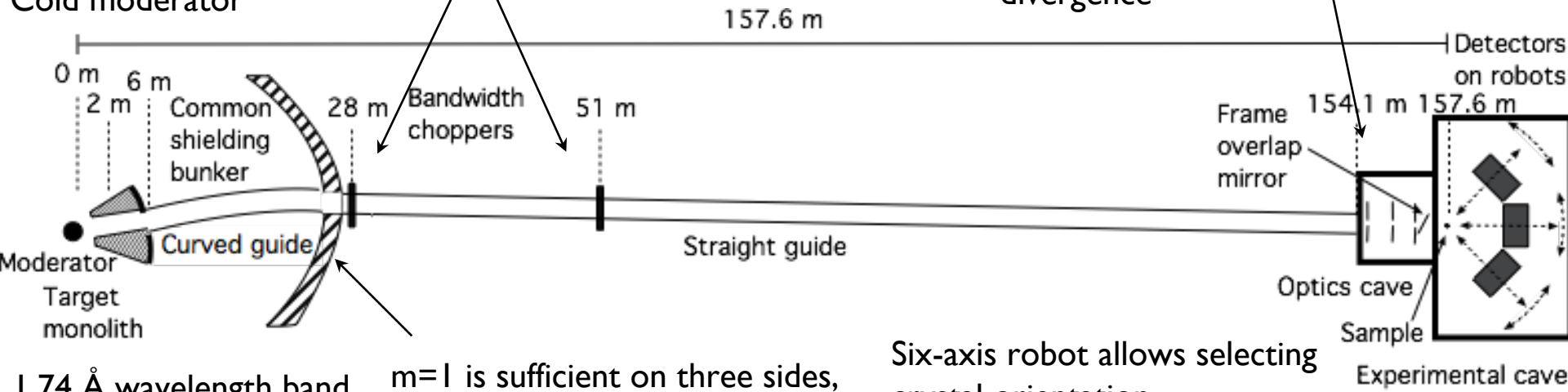
NMX – conceptual view

Adjustable opening chopper allows flexible bandwidth selection.

Collimation section giving flexibility in beam size and divergence

Cold moderator



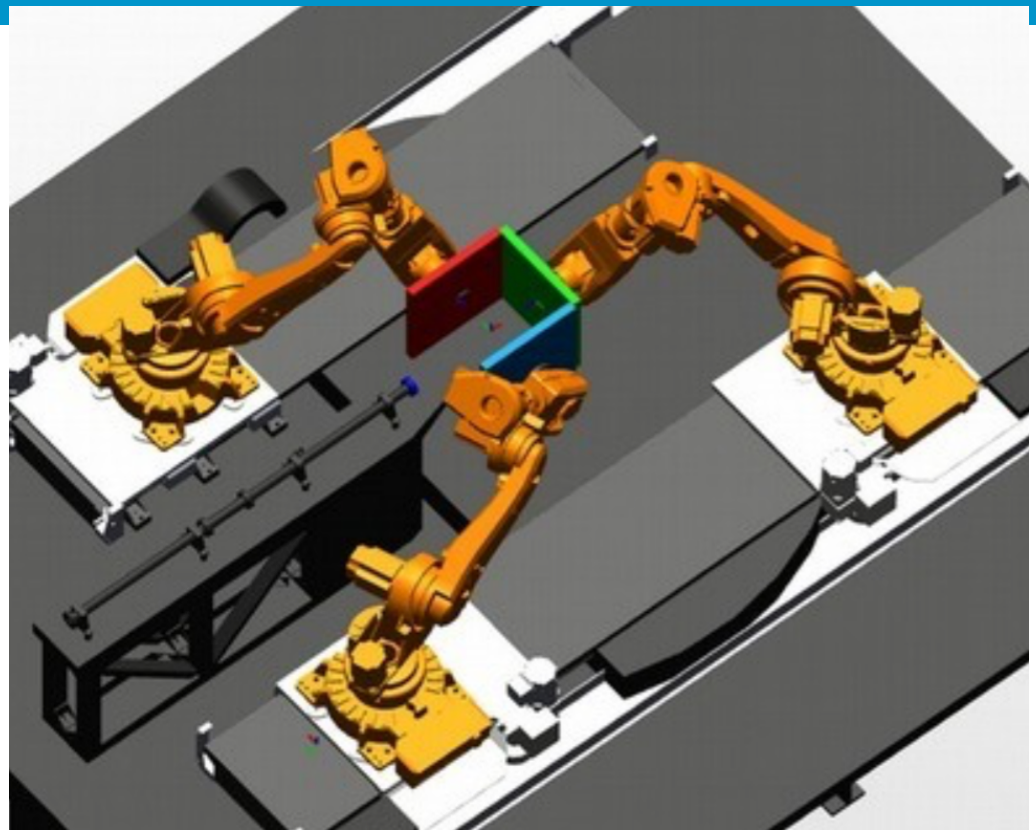
1.74 Å wavelength band (1.8-3.55 Å)

m=1 is sufficient on three sides, m=2.2 for curving

Six-axis robot allows selecting crystal orientation

Three 60 x 60 cm detectors with 0.2 mm spatial resolution
Variable sample-detector distance (0.2-1.0 m)
Variable 2θ angle (0-110°)

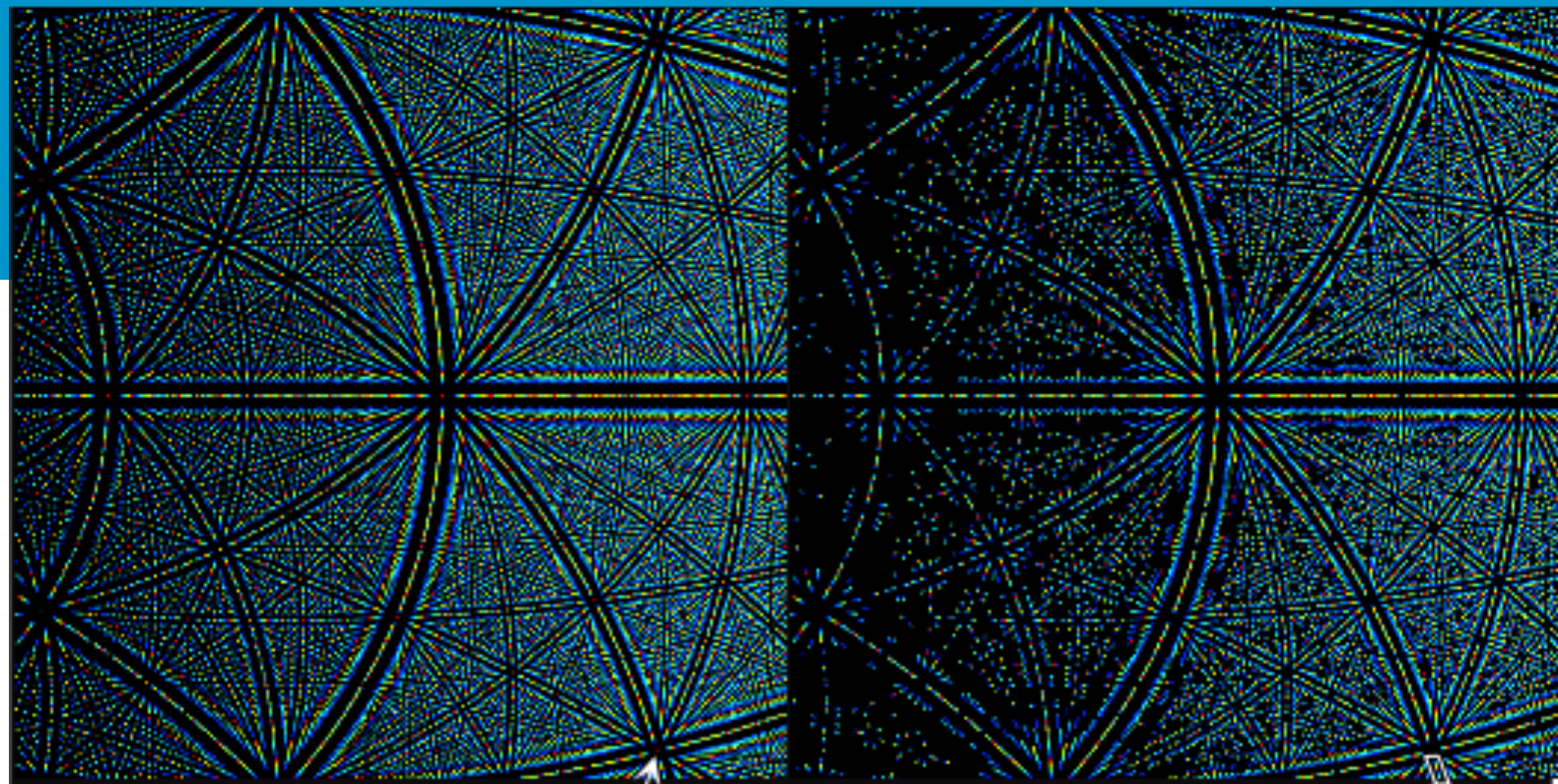
NMX Detector geometry



Three 60 x 60 cm detectors with 0.2 mm spatial resolution
Sample-detector distance (0.2-1.0 m) and 2θ angle (0-110°) variable by robotic positioning

- Solid angle coverage can be traded for unit cell size
- Large unit cells will take longer to collect

Bovine heart
cytochrome c oxidase
$P2_12_12_1$
a = 182.59 Å
b = 205.40 Å
c = 178.25 A
Detector distance 1 m



All reflections

14 28 42 (3.409 Å, 134.4 ms) 21 35 49 (2.809 Å, 110.8 ms)
15 29 43 (3.309 Å, 130.5 ms) 22 36 50 (2.739 Å, 108.0 ms)
16 30 44 (3.215 Å, 126.8 ms) 23 37 51 (2.672 Å, 105.4 ms)
17 31 45 (3.124 Å, 123.2 ms) 24 38 52 (2.608 Å, 102.9 ms)
18 32 46 (3.040 Å, 119.9 ms) 25 39 53 (2.548 Å, 100.5 ms)
19 33 47 (2.959 Å, 116.7 ms) 26 40 54 (2.489 Å, 98.2 ms)
20 34 48 (2.882 Å, 113.6 ms)

- 1.800 to 2.019 Angstroms
- 2.019 to 2.237 Angstroms
- 2.237 to 2.456 Angstroms
- 2.456 to 2.675 Angstroms
- 2.675 to 2.894 Angstroms
- 2.894 to 3.112 Angstroms
- 3.112 to 3.331 Angstroms
- 3.331 to 3.550 Angstroms

Spatial overlaps only

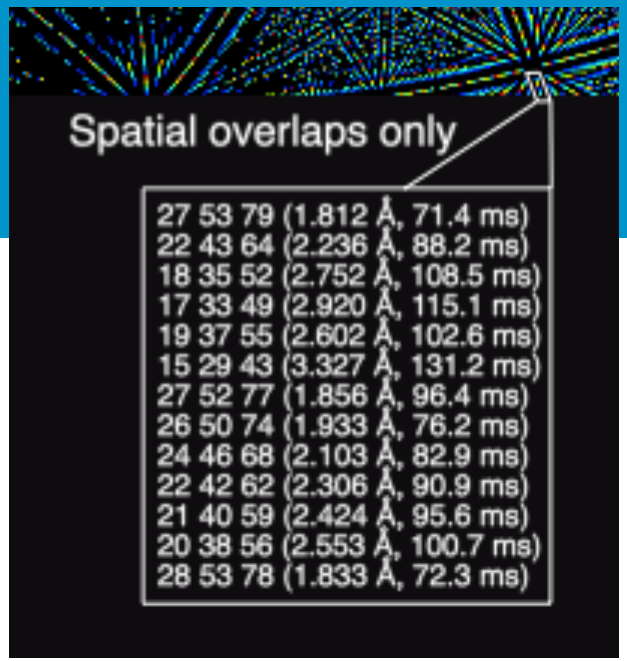
27 53 79 (1.812 Å, 71.4 ms)
22 43 64 (2.236 Å, 88.2 ms)
18 35 52 (2.752 Å, 108.5 ms)
17 33 49 (2.920 Å, 115.1 ms)
19 37 55 (2.602 Å, 102.6 ms)
15 29 43 (3.327 Å, 131.2 ms)
27 52 77 (1.856 Å, 96.4 ms)
26 50 74 (1.933 Å, 76.2 ms)
24 46 68 (2.103 Å, 82.9 ms)
22 42 62 (2.306 Å, 90.9 ms)
21 40 59 (2.424 Å, 95.6 ms)
20 38 56 (2.553 Å, 100.7 ms)
28 53 78 (1.833 Å, 72.3 ms)

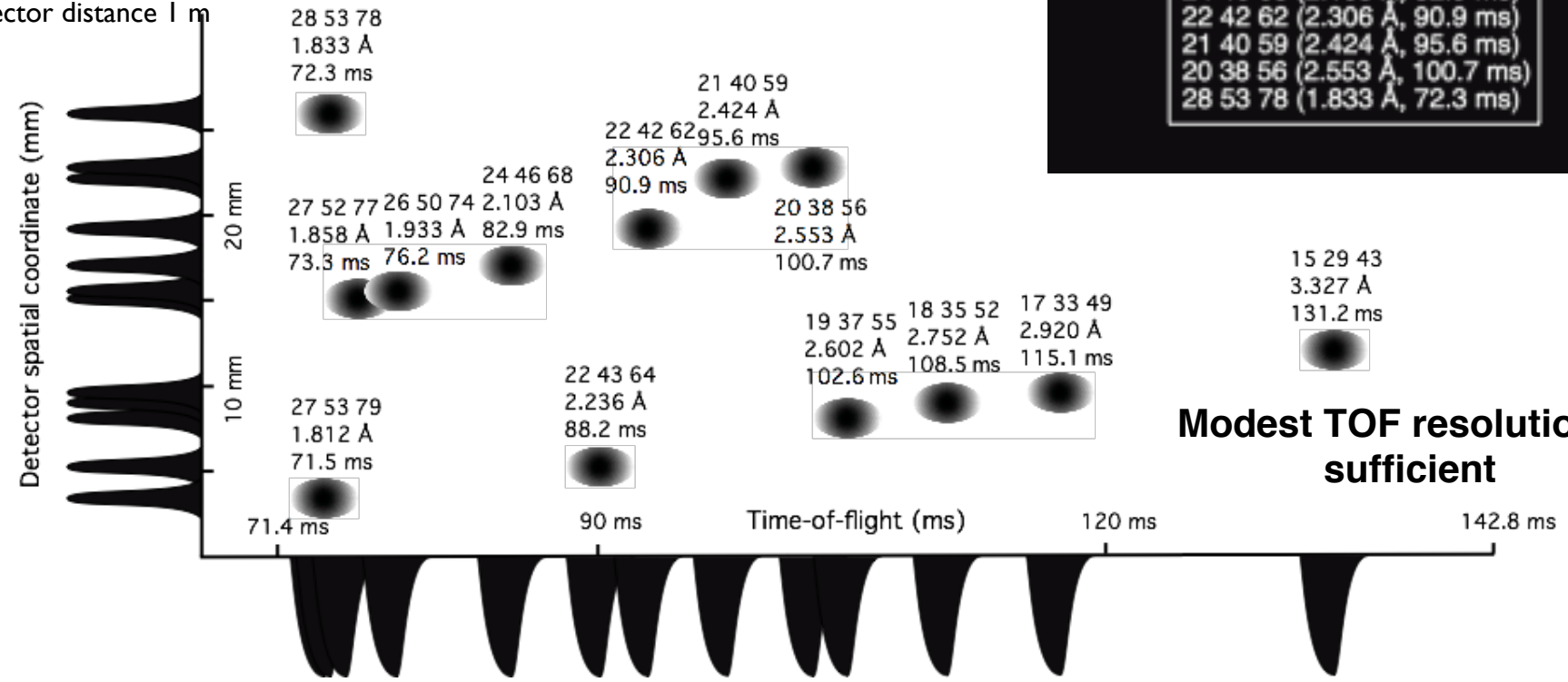
Generated using the Daresbury Laue Suite

Campbell et al. J. Appl. Cryst. (1998). 31, 496-502
Artz et al. J. Appl. Cryst. (1999). 32, 554-562
Helliwell, J.R. et al. J. Appl. Cryst. (1989) 22, 483–497

Overlap separation with TOF

Bovine heart
cytochrome c oxidase
$P2_12_12_1$
a = 182.59 Å
b = 205.40 Å
c = 178.25 A
Detector distance 1 m





Modest TOF resolution is sufficient

Sub-mm spatial resolution needed to integrate intensities

LoKI – Small angle scattering

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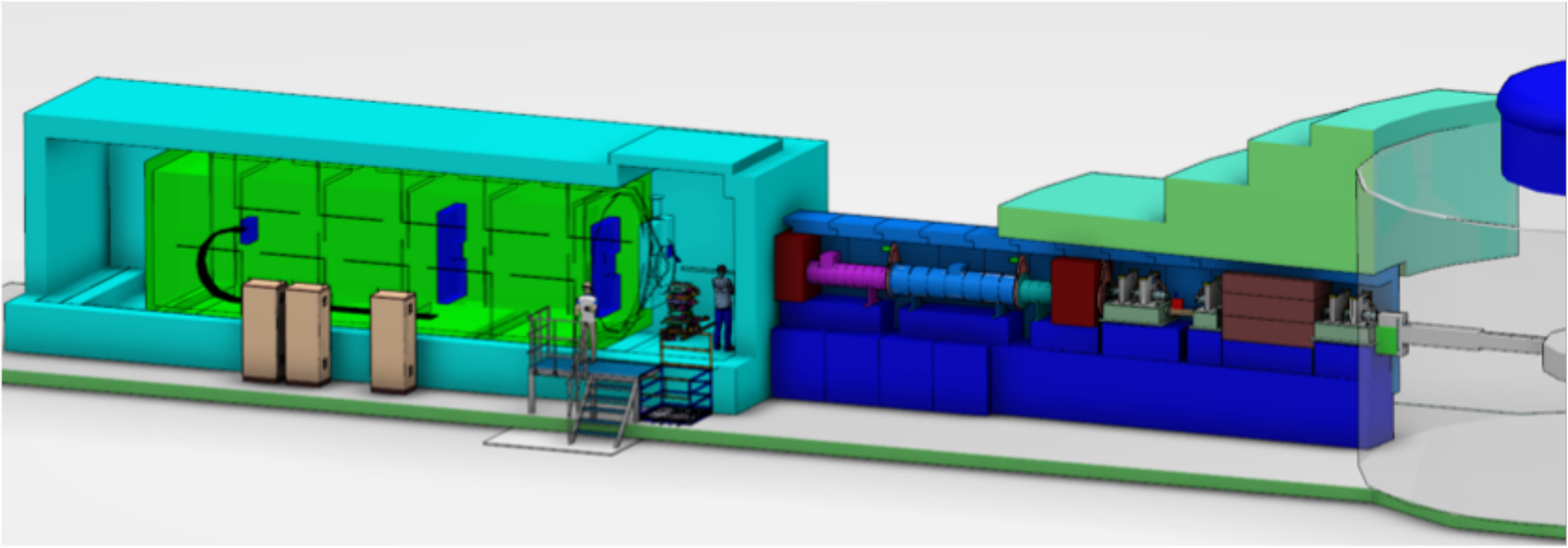
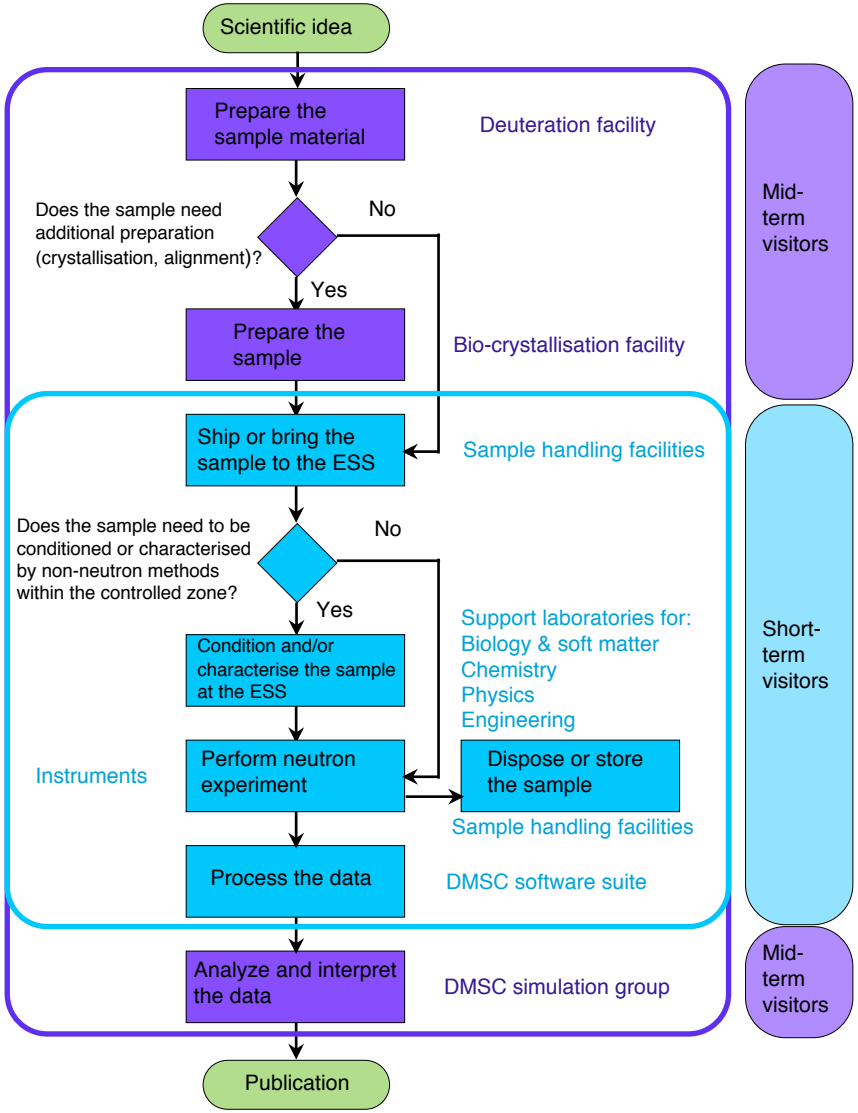
A broad Q range, high flux SANS instrument for soft matter, materials and bio-science

Science & Technology Facilities Council



Supporting facilities



DEMAX platform together with Lund University

LUND UNIVERSITY

LP3
Lund Protein-Production Platform

DEMAX platform

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LP3 Lund Protein-Production Platform

LUND UNIVERSITY

- Support for (bio, chem) deuteration and macromolecular crystallisation
- Core mission: to deliver user support labs, access, expertise for biological deuteration and protein crystallization
- These labs aim to support users in the fields of soft matter & life science research.
- Goal: be ready to prepare samples for hot commissioning and pre-operations activities. Also be ready to ensure early scientific success on first beamlines by supplying appropriate samples.

What will be different at the ESS

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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)

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Questions?