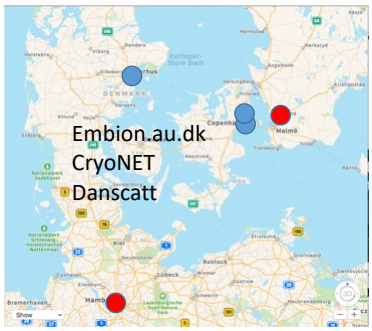
Great perspectives with microcrystals

Max IV workshop
– structural biology

Oct. 21st, 2021



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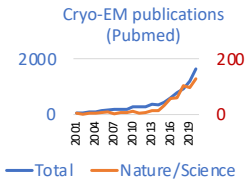
MicroMAX, BioMAX and other biosample beamlines

Unique resources:
MAX IV and ESS serve a general structural biology community using also e.g. cryo-EM
possible interactions also with e.g. EMBL-Hamburg/DESY and European XFEL

Structural biology 2021:
New, difficult structures will be based mostly on single-particle cryo-EM analysis
Small globular structures are highly guided by AlphaFold – but ligand binding?
- Time-resolved cryo-EM is still in its infancy and based on cryo-conditions
- In situ cryo-electron tomography is limited to sub-micrometer vitrified samples/sections



Large, important field for X-ray science:
Molecular and cellular structure/dynamics in biological context (time, temperature, 3D – living?)
Structure determination and time-resolved studies using serial crystallography/SAXS – *maintain crystallography/SAXS*
X-ray imaging of cells, organoids, native tissues – *support molecular cell biology, integrate with e.g. EMBL program*
Solution X-ray scattering studies is also an important complement to single-particle cryoEM
Integration of data and models – *support computing infrastructure for data analysis and modeling of complex dynamics*

Highly structured molecules/assemblies ***Dynamic structures*** ***Fully disordered molecules/assemblies***

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Advantages of serial crystallography/microcrystals

- Less sensitive to mechanical stress and strain
- Fast diffusion rates in soaking
- Uniform activation of photocaged compounds
- Possibility for both room temperature and cryo-crystallography
- Manageable in small-scale format

Potential applications

Time-resolved studies of e.g. photoactivated or diffusion-based reactions
Time-resolved studies of ligand binding reactions guiding drug design

(With microfocus: also X-ray absorption/spectroscopy/emission with subcellular resolution in imaging modalities)

Challenges:
More diversity of photochaged compounds - chemistry
Integral technologies at beamlines for pump-probe/stopped-flow, laser activation, sample delivery

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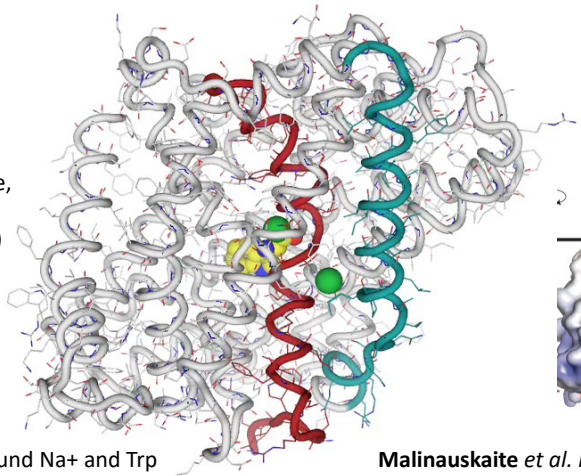
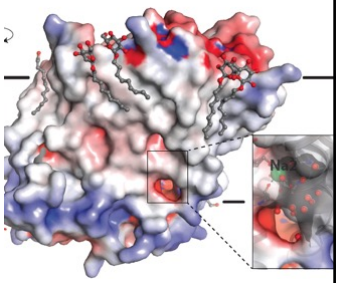
Structural biology – connecting structure and dynamics to function

SLC6 transporters
Neurotransmitter, monoamine and amino acid transporters
E.g. serotonin, dopamine, glycine, GABA transporters, B^0AT1
Bacterial orthologs (amino acids)
e.g. LeuT and MhsT





Amino acid transporter MhsT
(*B. halodurans*)
SLC6 family orthologue

- Occluded, inward-facing with bound Na+ and Trp
- N-term bound or unbound/TM5 unwound or continuous
- Solvation and dynamics of Na+ release coupled to TM5 and N-terminal dynamics

Malinauskaite *et al. Nature Struct Mol Biol.* 2014
Stolzenberg *et al. JBC* 2017
Gotfryd *et al. Nature Comm*. 2020
Focht, Neumann, Lyons *et al. EMBO J* 2021

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Article

Structural insights into the inhibition of glycine reuptake

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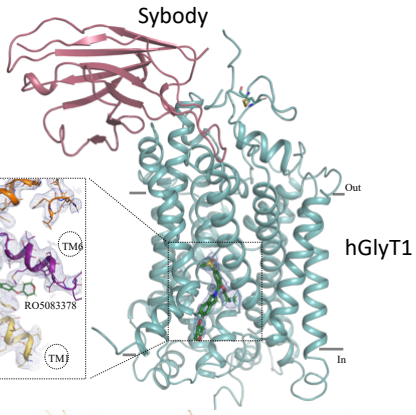
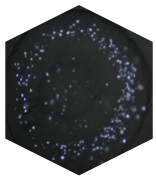
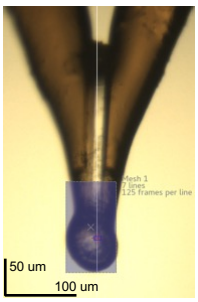
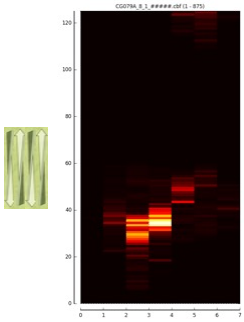
Azadeh Shahsavar[1,7], Peter Stohler[3], Gleb Bourenkov[2], Iwan Zimmermann[4,6], Martin Siegrist[3], Wolfgang Guba[3], Emmanuel Pinard[3], Steffen Sinning[6], Markus A. Seeger[4], Thomas R. Schneider[2✉], Roger J. P. Dawson[3,5✉] & Poul Nissen[1✉]



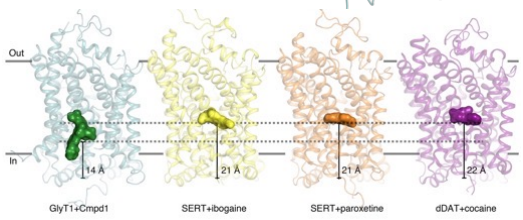


Microcrystals
in meso phase





Heatmap
in MxCuBE



Shahsavar *et al. Nature* 2021

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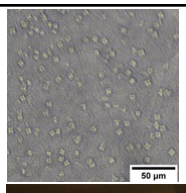
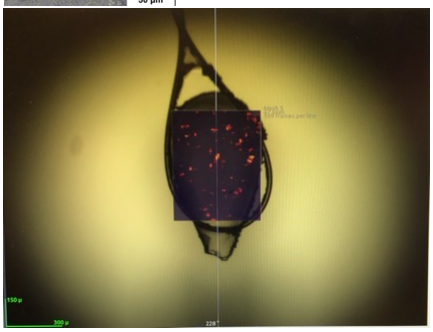


Serial crystallography – synchrotron sources and XFEL

Trampari *et al.*
in preparation

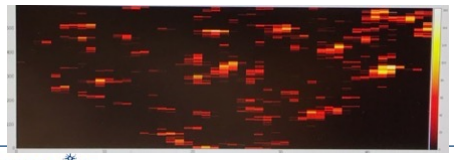
B. halodurans amino acid transporter MhsT-Met
Inhibited state (non-substrate amino acid)
Microcrystals, HiLiDe method



Serial Synchrotron X-ray crystallography (SSX):

- P14 beamline PETRA III
- 5 x 10 µm^2 microfocus beam

- 1 µm sample displacement
- 0.2° oscillation
- 0.025 sec exposure time
- 100% transmission



Data collected from 92 loops

	Ellipsoidal
Data collection	P14
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	44.27, 215.32, 51.02
α, β, γ (°)	90, 90.111, 90
Resolution range (Å)	28.42 - 2.7/3.1/3.0 (2.838 - 2.74)
Total reflections	703132 (66641)
Unique reflections	24985 (245)
R-meas (%)	74.33 (953.8)
R-pim (%)	13.7 (184.1)
R-merge (%)	73.0 (935.4)
CC (1/2)	0.993 (0.354)
Mean I/σI	5.50 (1.5)
Completeness (%)	81.2 (9.68)
Multiplicity	28.1 (26.3)
Wilson B	57.31

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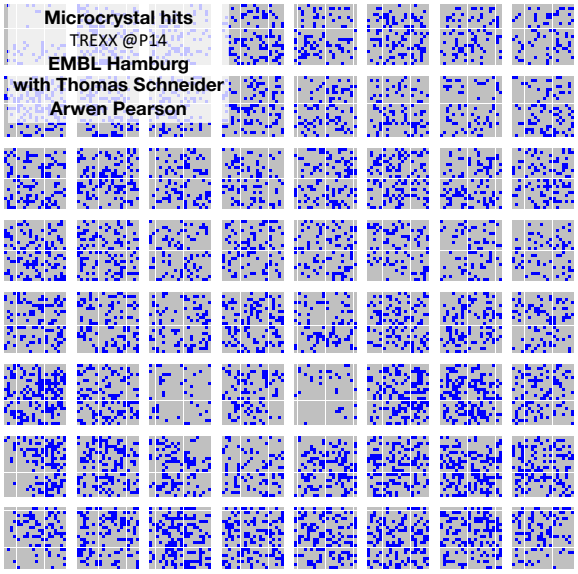
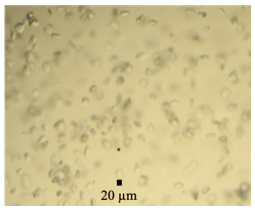
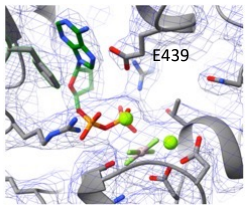
Warming up for time-resolved crystallography

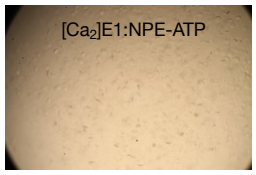


Samuel Hjort-Jensen
(EU-ITN RAMP)

SERCA phosphorylation @RT
$[Ca_2]E1.ADP\text{-}AlF_4^-.2\ Mg^{2+}$







Investing time in well-diffracting microcrystals

- Time-resolved binding/catalysis
- Drug discovery (intermediates)
- Need not be high resolution to restrain MD importantly

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