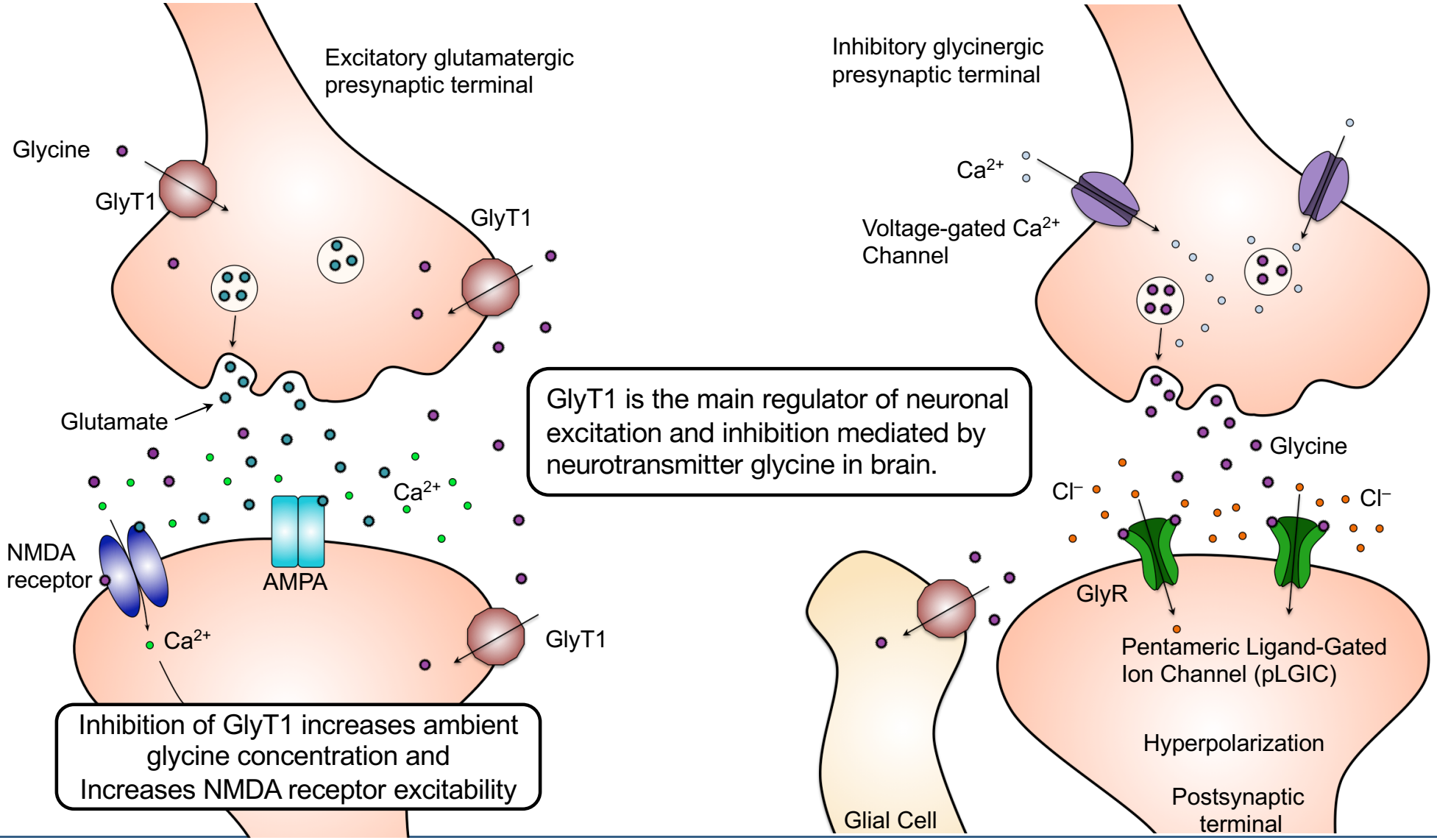
DANDRITE – Danish Research Institute of Translational Neuroscience

HALOS symposium
January 31st 2022

Unlocking the molecular mechanism of glycine reuptake inhibition

Azadeh Shahsavar


Excitatory glutamatergic presynaptic terminal
Glycine
GlyT1
GlyT1
Glutamate
Ca2+
NMDA receptor
AMPA
Ca2+
GlyT1
Inhibition of GlyT1 increases ambient glycine concentration and Increases NMDA receptor excitability
GlyT1 is the main regulator of neuronal excitation and inhibition mediated by neurotransmitter glycine in brain.
Inhibitory glycinergic presynaptic terminal
Ca2+
Voltage-gated Ca2+ Channel
Glycine
Cl−
Cl−
GlyR
Pentameric Ligand-Gated Ion Channel (pLGIC)
Hyperpolarization
Postsynaptic terminal
Glial Cell

- GlyT1 inhibitors achieve antipsychotic and pro-cognitive effects against schizophrenia

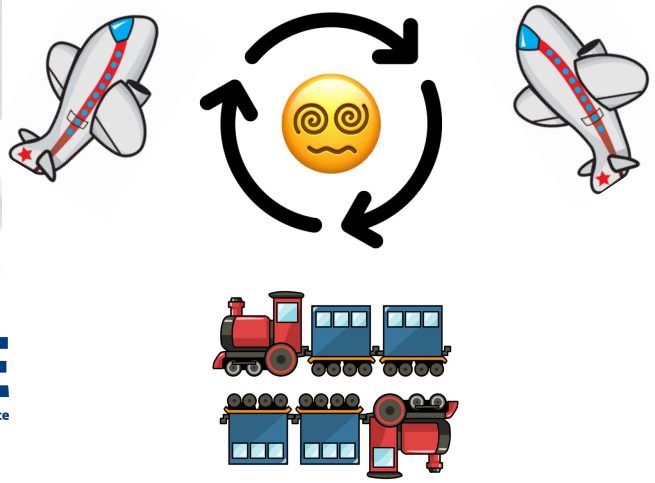
- Bitopertin, one of the most advanced GlyT1 inhibitors failed in a phase III clinical trial study

Roche

Roger Dawson

Poul Nissen

Thomas Schneider



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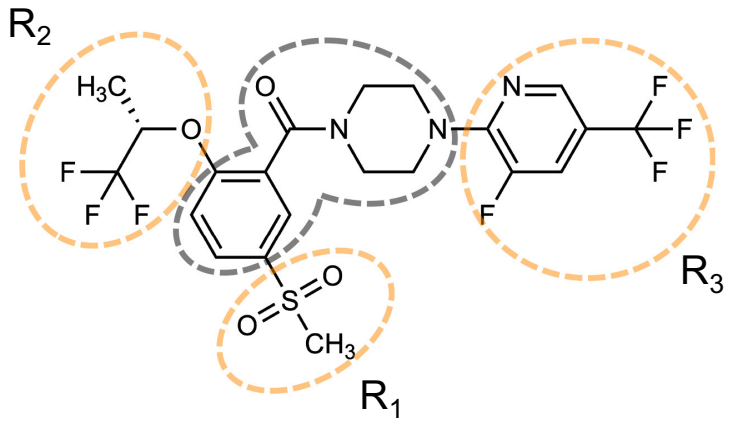
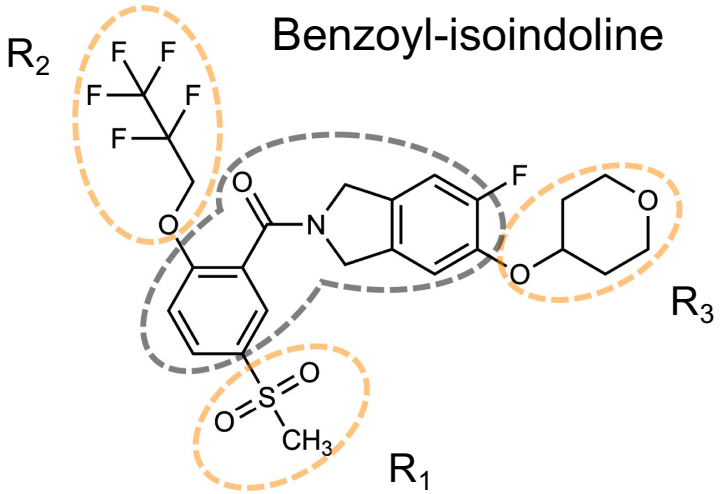
- GlyT1 inhibitors achieve antipsychotic and pro-cognitive effects against schizophrenia

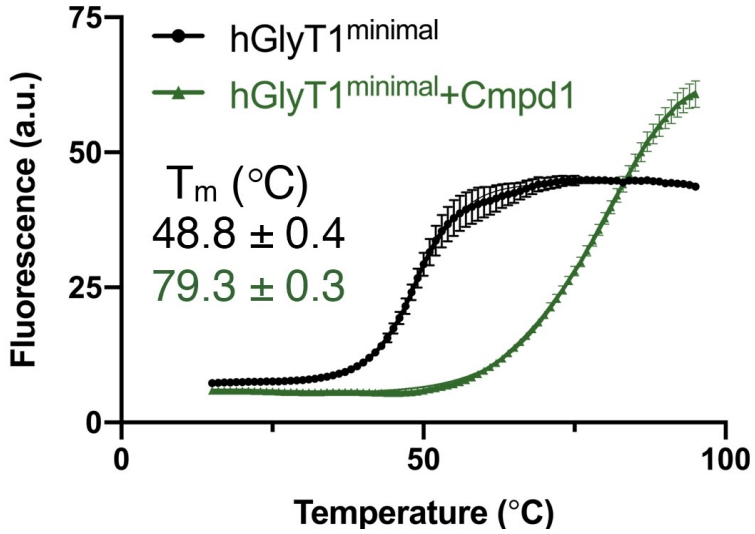
- Bitopertin, one of the most advanced GlyT1 inhibitors failed in a phase III clinical trial study

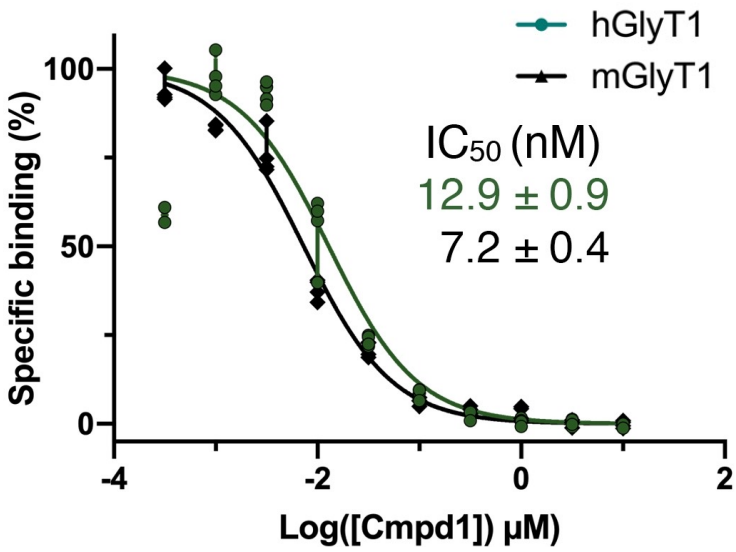
Bitopertin
Benzoyl-piperazine



Benzoyl-isoindoline







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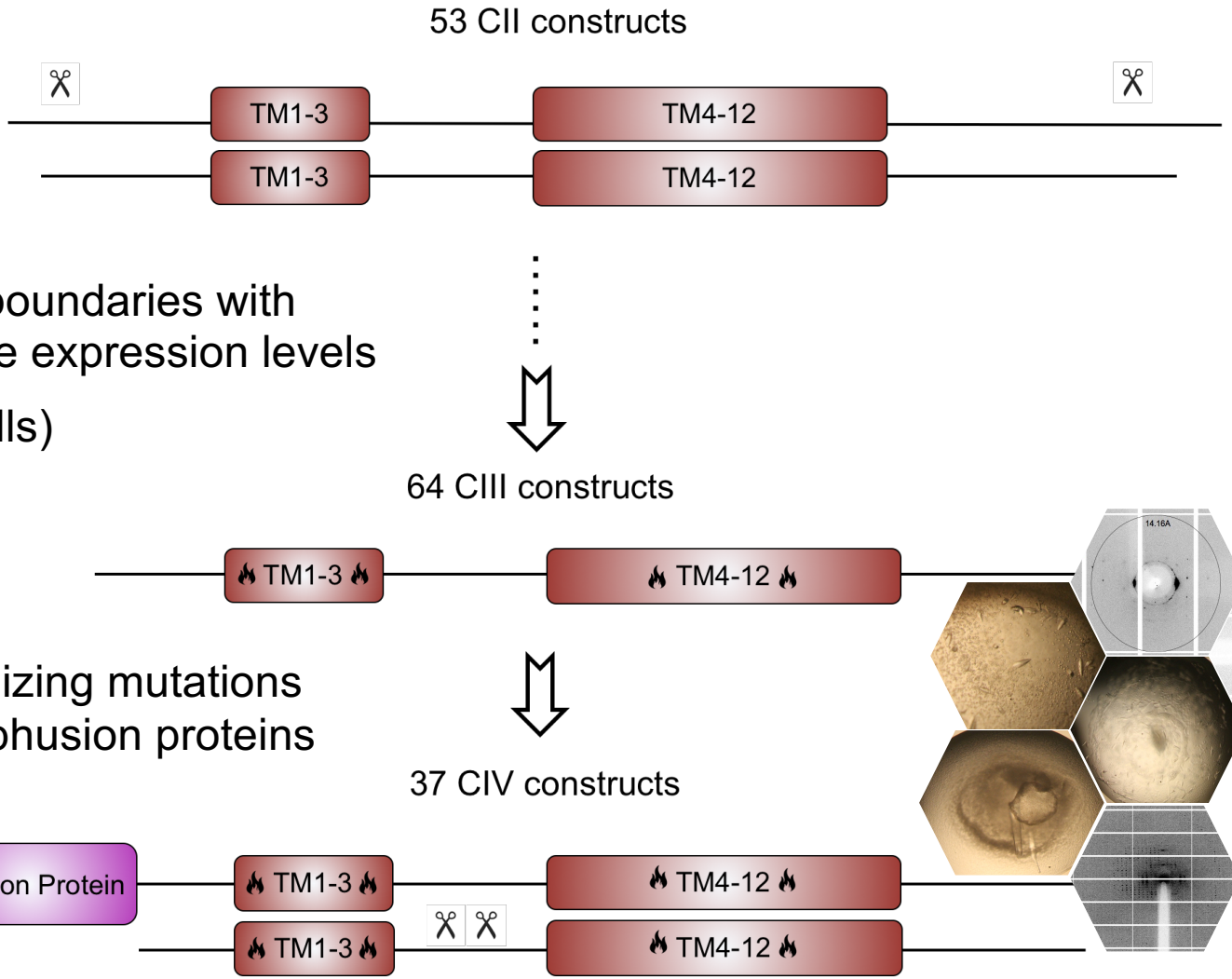
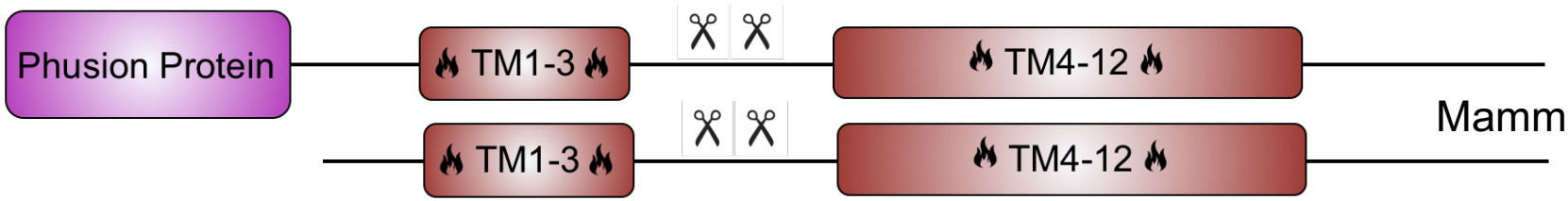
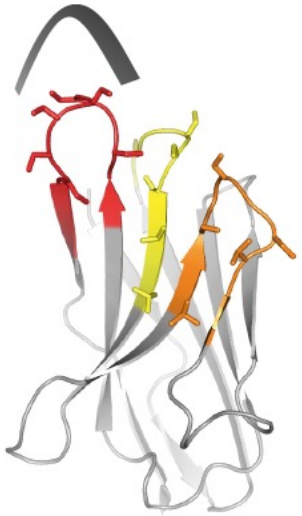
Construct Design of GlyT1



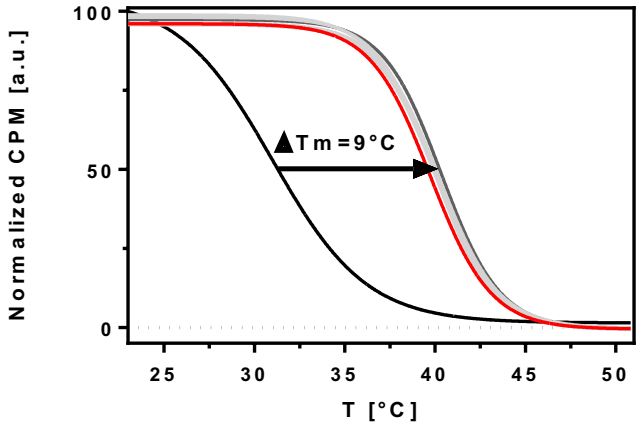
CV and CVI constructs of GlyT1



Synthetic single-domain antibodies (sybodies) for inhibitor-bound conformation of GlyT1



Inhibition state-specific Sybody



SEEGER LAB

Markus Seeger Iwan Zimmermann

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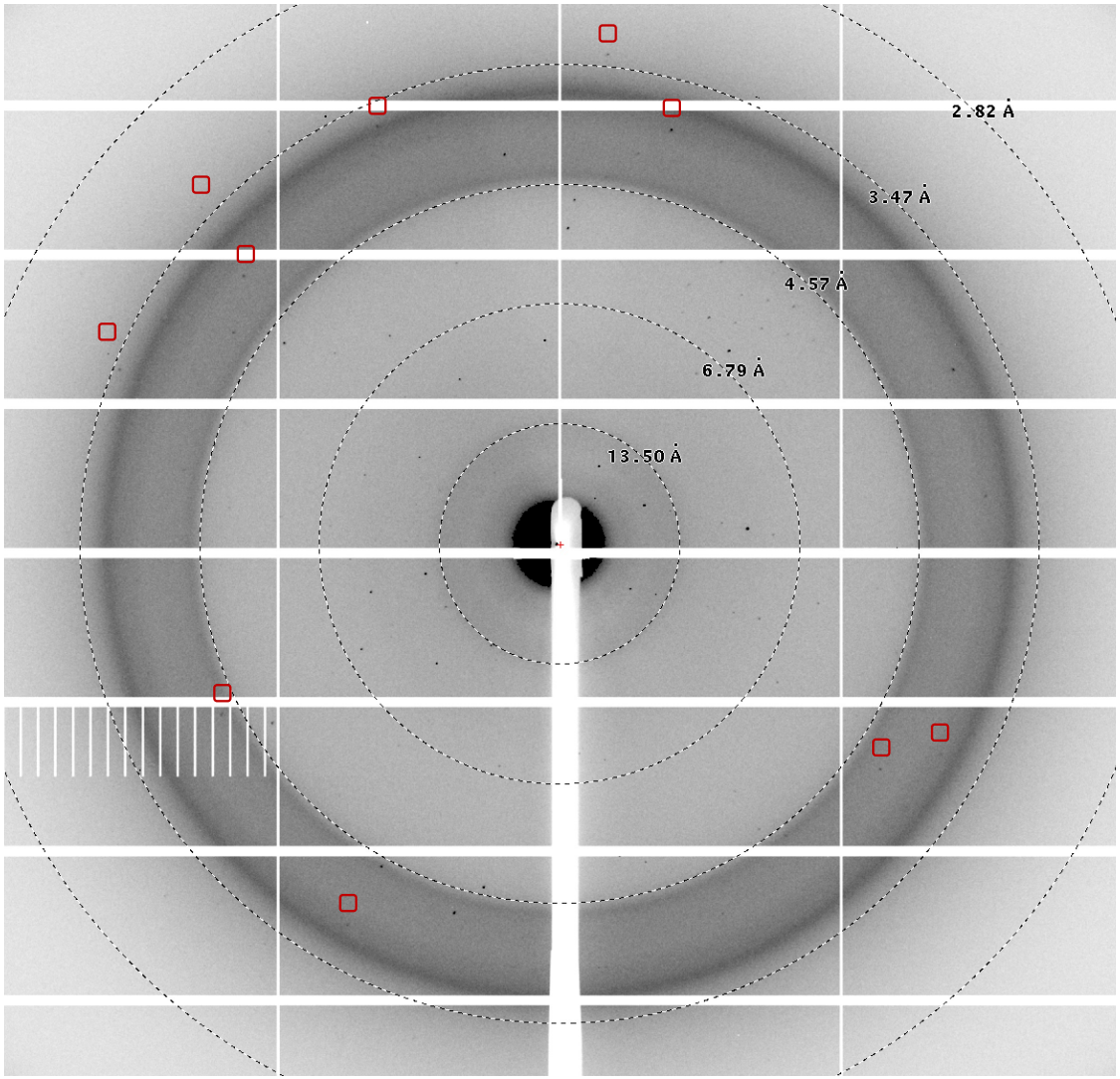
“960” crystallization conditions

⇩

“1” crystallization condition

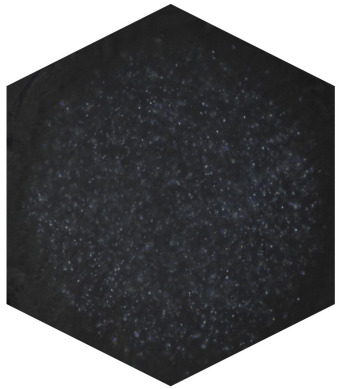


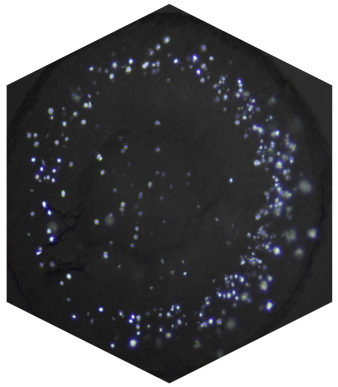
Micro crystals of GlyT1
in Meso phase

“960” crystallization conditions

⇩

“1” crystallization condition



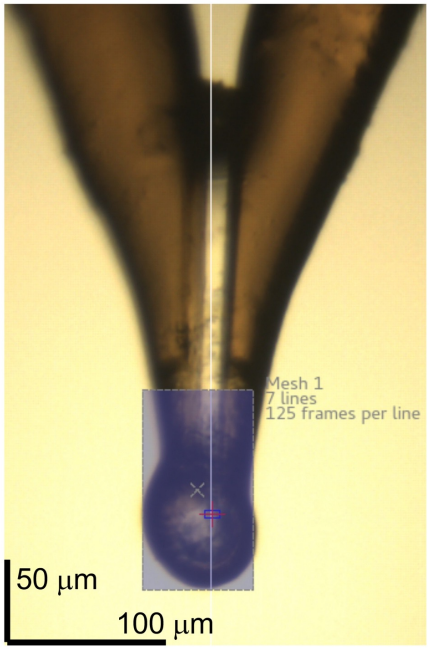


Micro crystals of GlyT1
in Meso phase

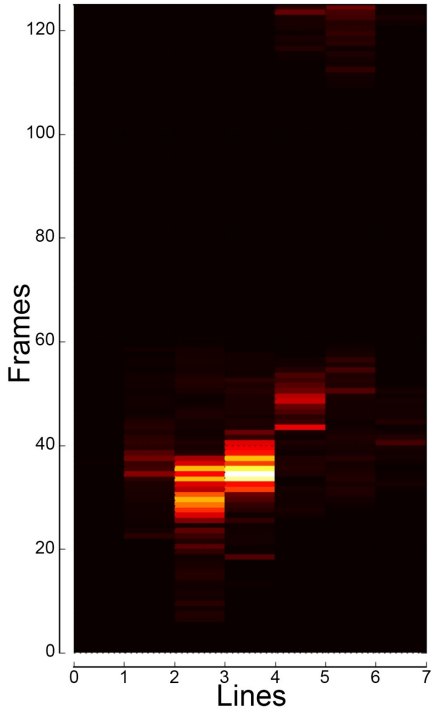
Serial helical line scan data collection @ EMBL P14 beamline

- 5 × 10 μm^2 beam size
- Flux: 1.3 × 10^{13} ph/sec
- 12.7 KeV

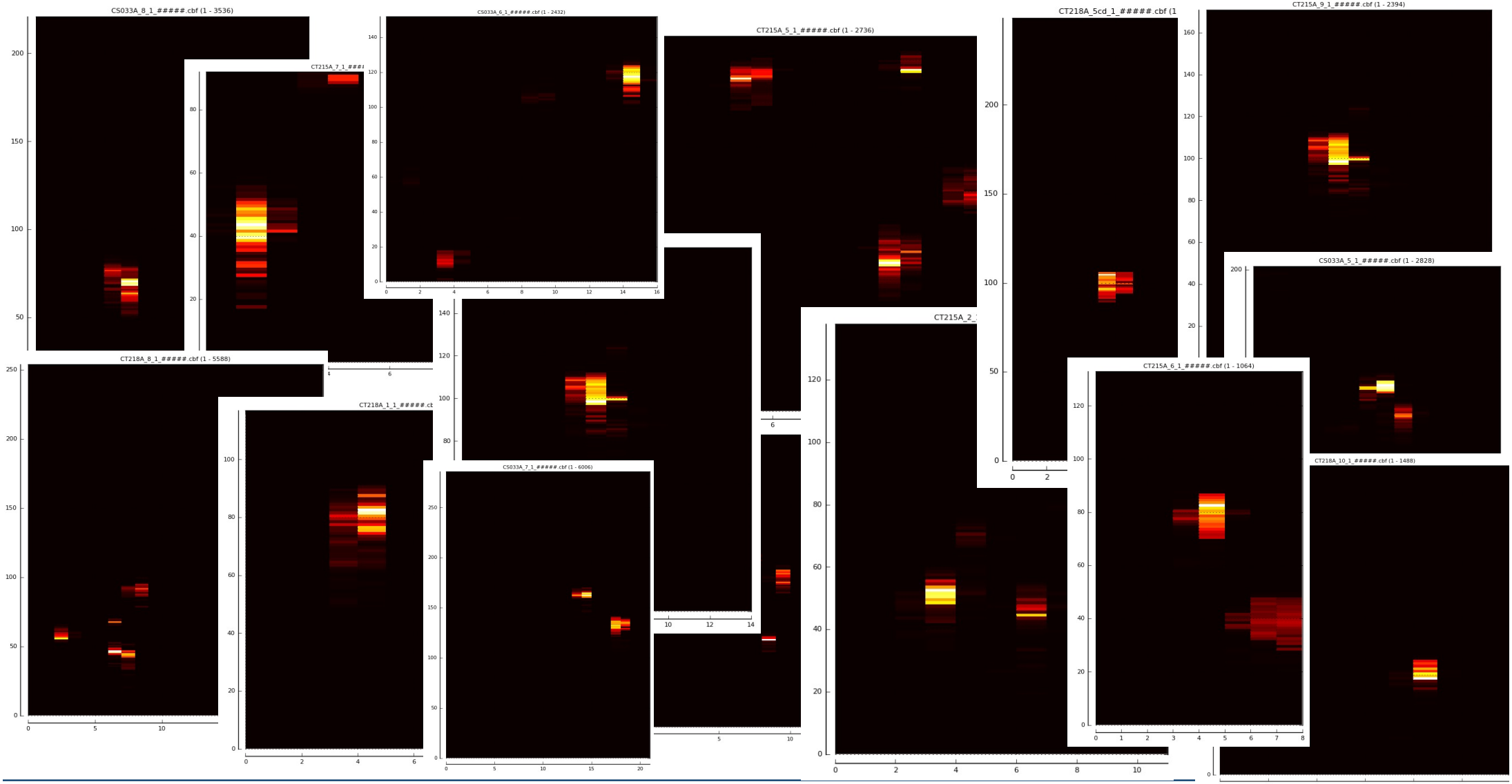
Region of interest:
60 × 14 – 290 × 340 μm^2
100 – 10,000 frames



Translation
& Rotation



The heat map indicating the location of crystals



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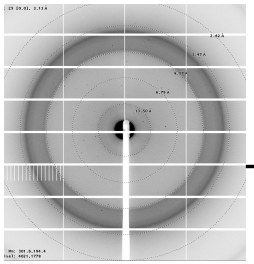
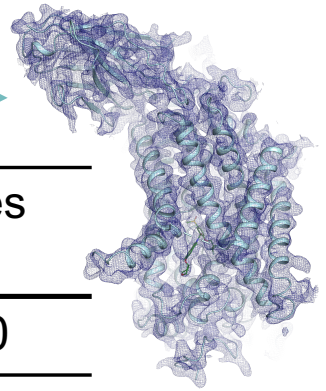


July 2019 → October 2019



Loops	ROIs	Diffraction patterns	Frames with >15 spots	Total mini data sets	data sets used	Frames used
409	514	1,365,232	30,837	229	207	3,400

Resolution Limit (Å)	Number of Reflections Observed	Unique	Possible	Completeness	R-Factor Observed	I/Sigma	R-meas	CC(1/2) (%)
25.0	183	30	31	96.8%	10.0%	15.99	10.9%	99.3
15.0	1445	221	222	99.5%	11.8%	17.59	12.8%	99.1
10.0	4595	670	675	99.3%	12.7%	16.15	13.8%	98.9
5.0	20247	3177	3211	98.9%	44.6%	5.19	48.6%	89.3
4.0	46129	7219	7283	99.1%	61.0%	4.41	66.3%	84.5
3.8	8249	1297	1311	98.9%	127.8%	2.16	139.1%	51.2
3.6	9726	1582	1606	98.5%	153.2%	1.50	167.3%	46.7
3.4	7500	1217	1227	99.2%	299.2%	1.00	326.4%	30.1
Total	153451	24071	24304	**99.0%**	41.5%	**4.38**	45.1%	**97.5**

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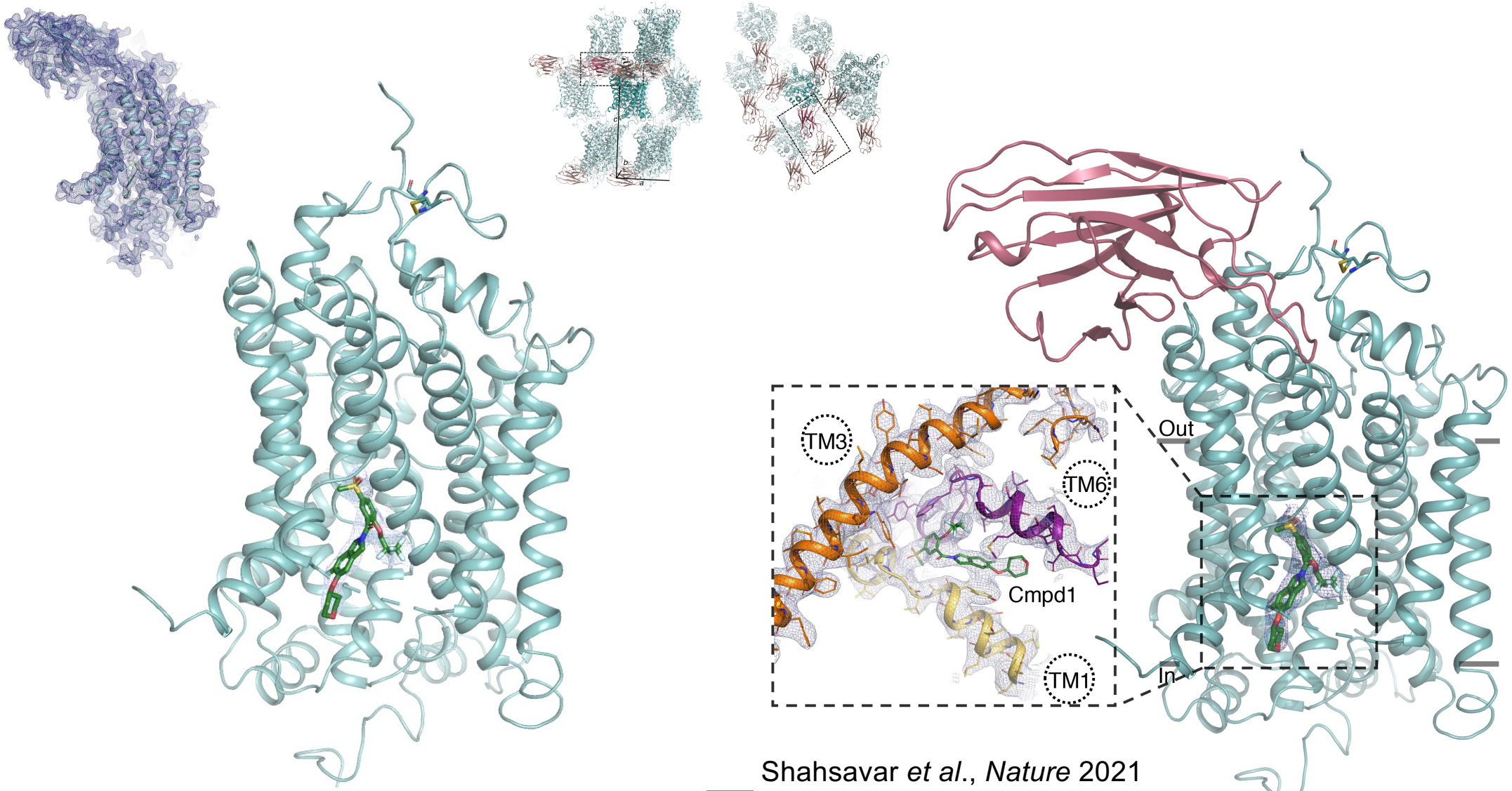

Shahsavar *et al.*, *Nature* 2021

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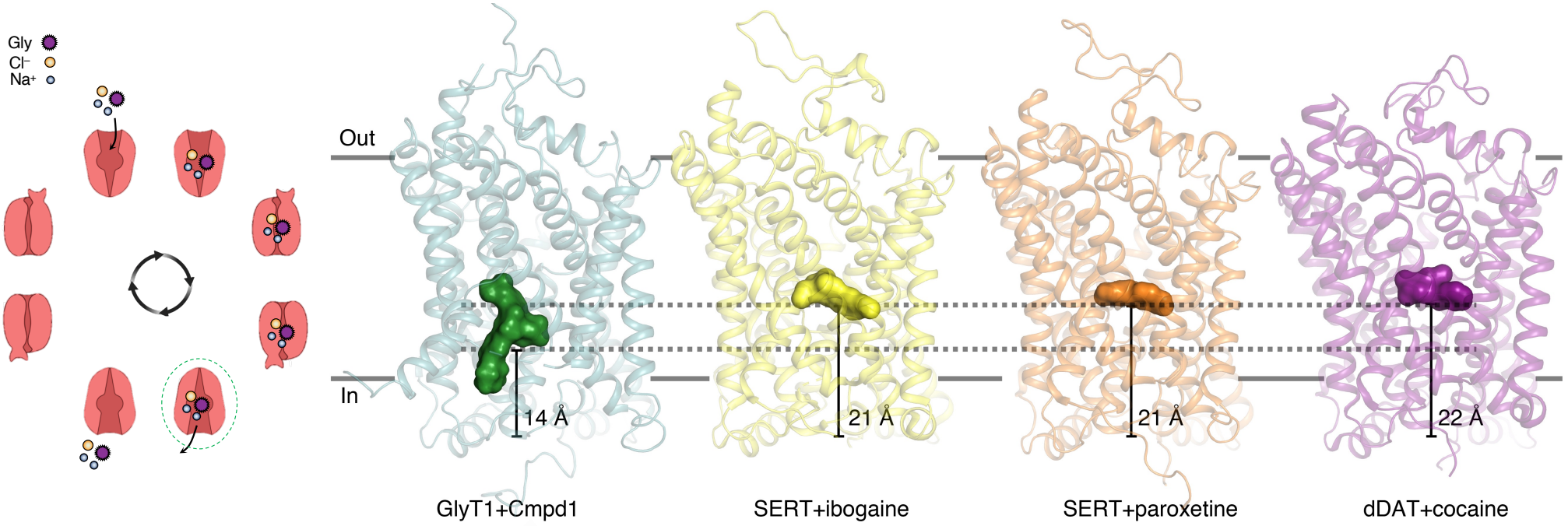
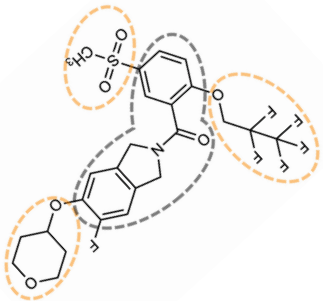
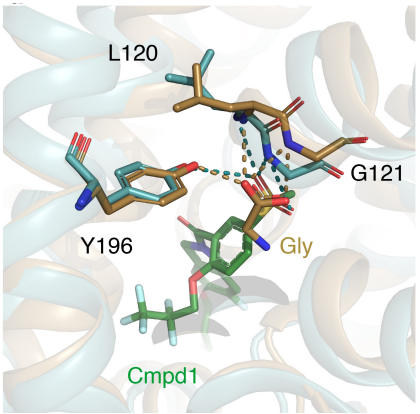


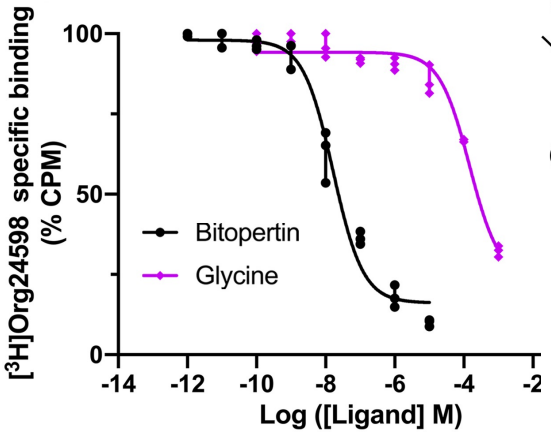


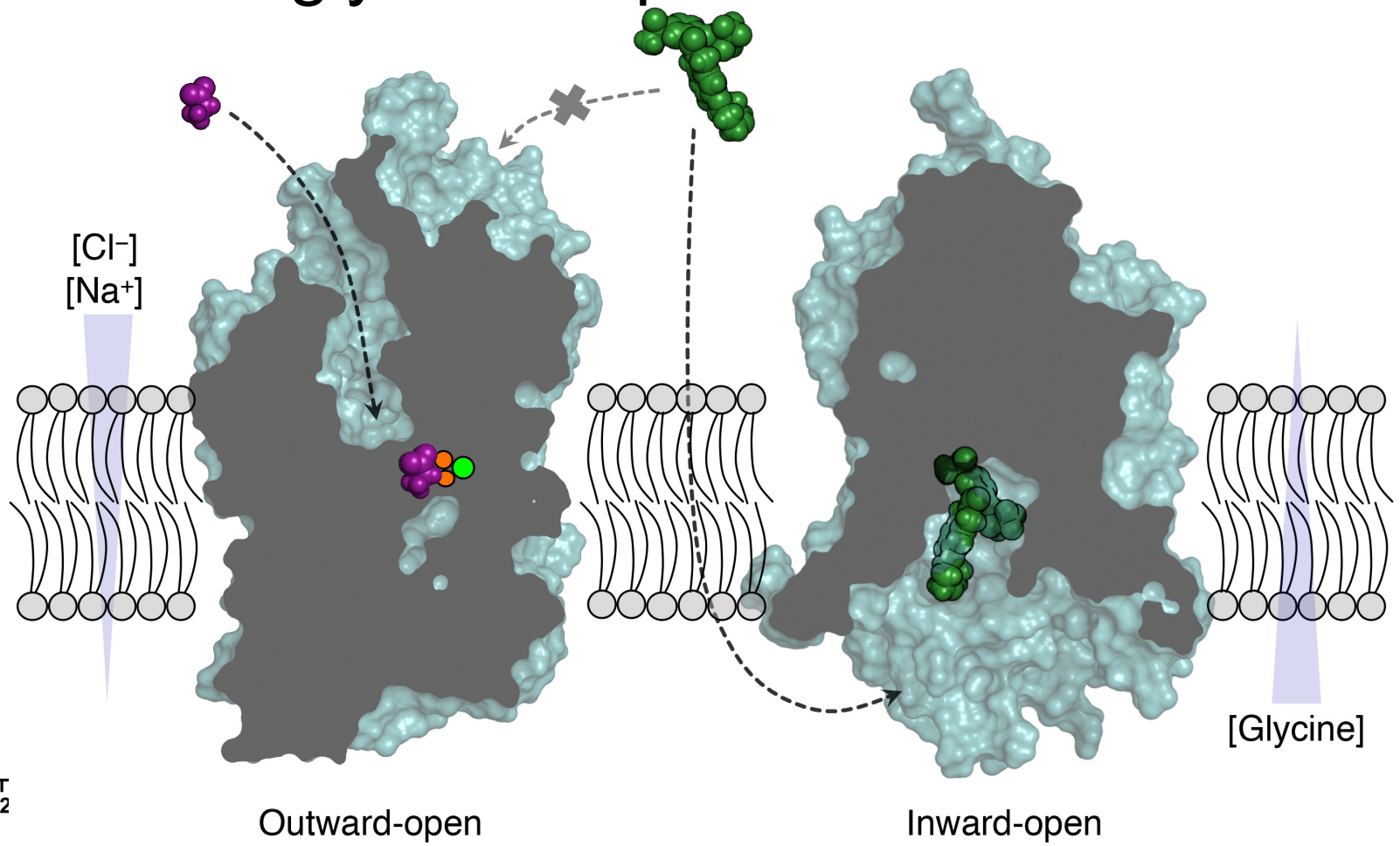


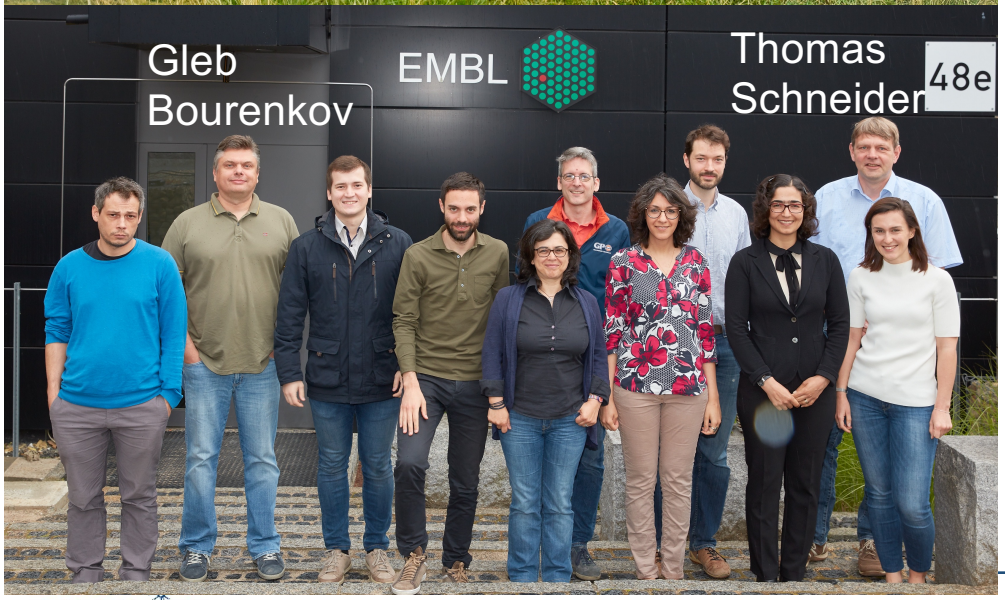
Bacterial homologue (LeuT) bound to Glycine

Mechanism of glycine reuptake inhibition

- Non-competitive inhibitor of functional transport
- Competitive binding to the substrate binding site of glycine







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Peter Stohler

Markus Seeger
Iwan Zimmermann

P14 beamline

Cryo-EM Aarhus:
Thomas Boesen
Andreas Bøgglid

SPC:
Maria Garcia Alai
Christian Guenther
Ioana-Maria Nemtanu

Funding: EI3POD programme fellowship under Marie Sklodowska Curie COFUND

Why GlyT1 inhibitors have not made it as a drug, yet?

- High membrane permeability
- GlyT1 in peripheral tissues (erythrocytes)

- Optimal GlyT1 occupancy?

- Higher placebo response?