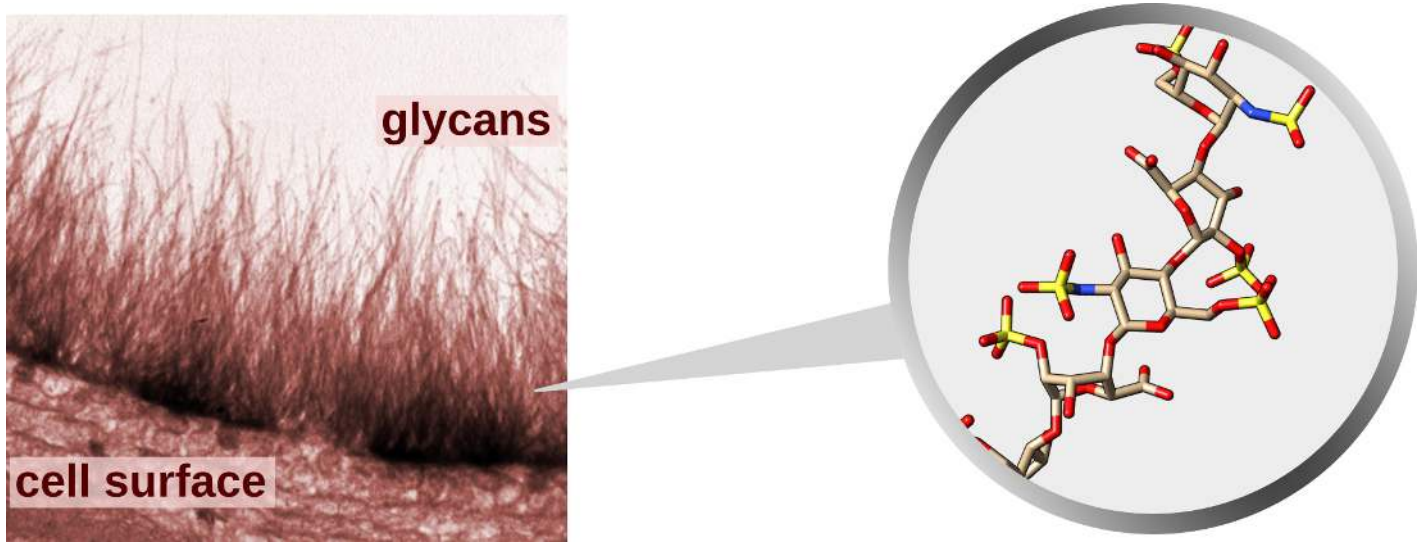
Introduction to single-particle cryo-EM and its use to study glycosyltransferase complexes

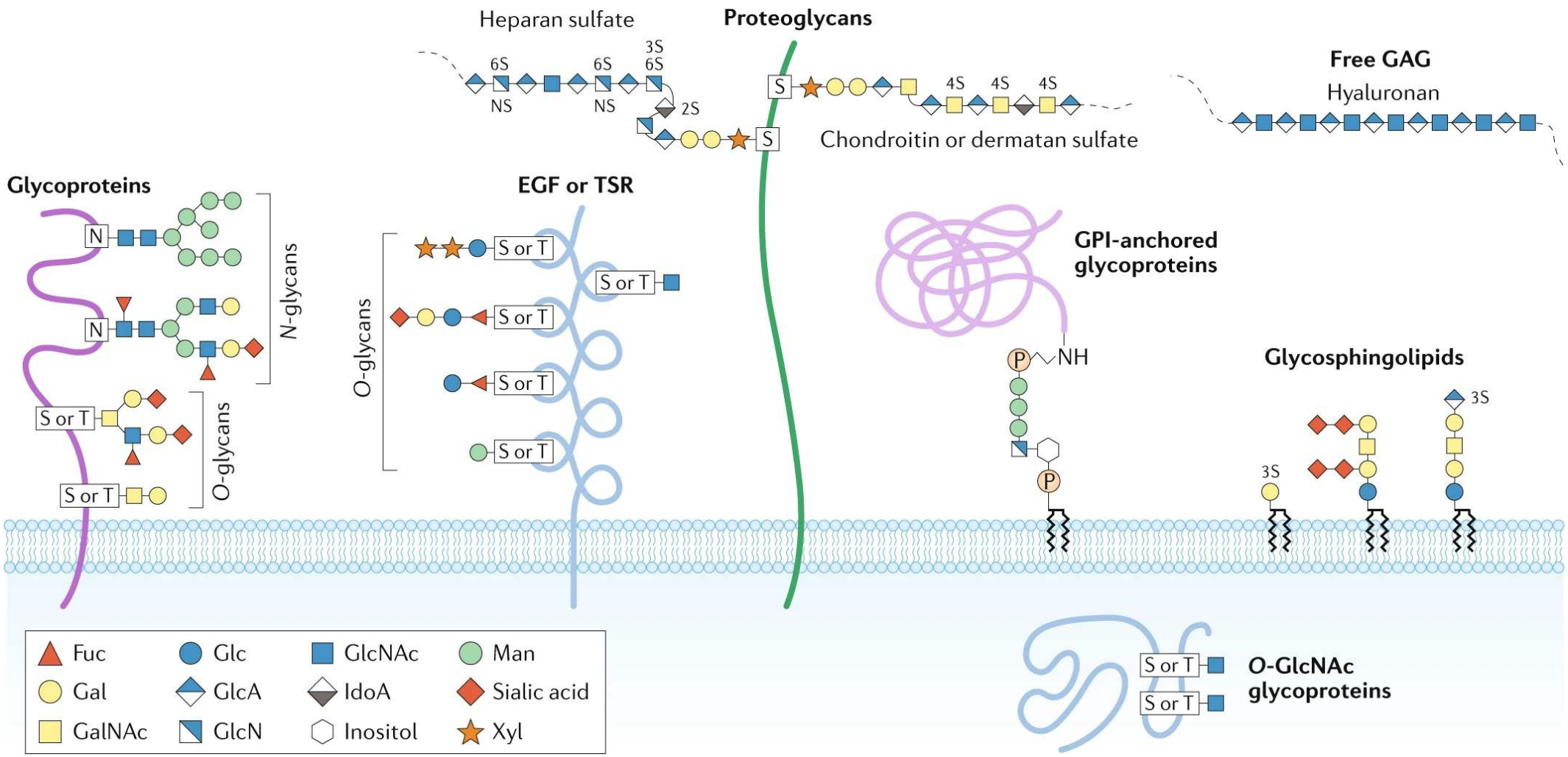
Structural Glycoscience Summer School

06.06.2023

Rebekka Wild

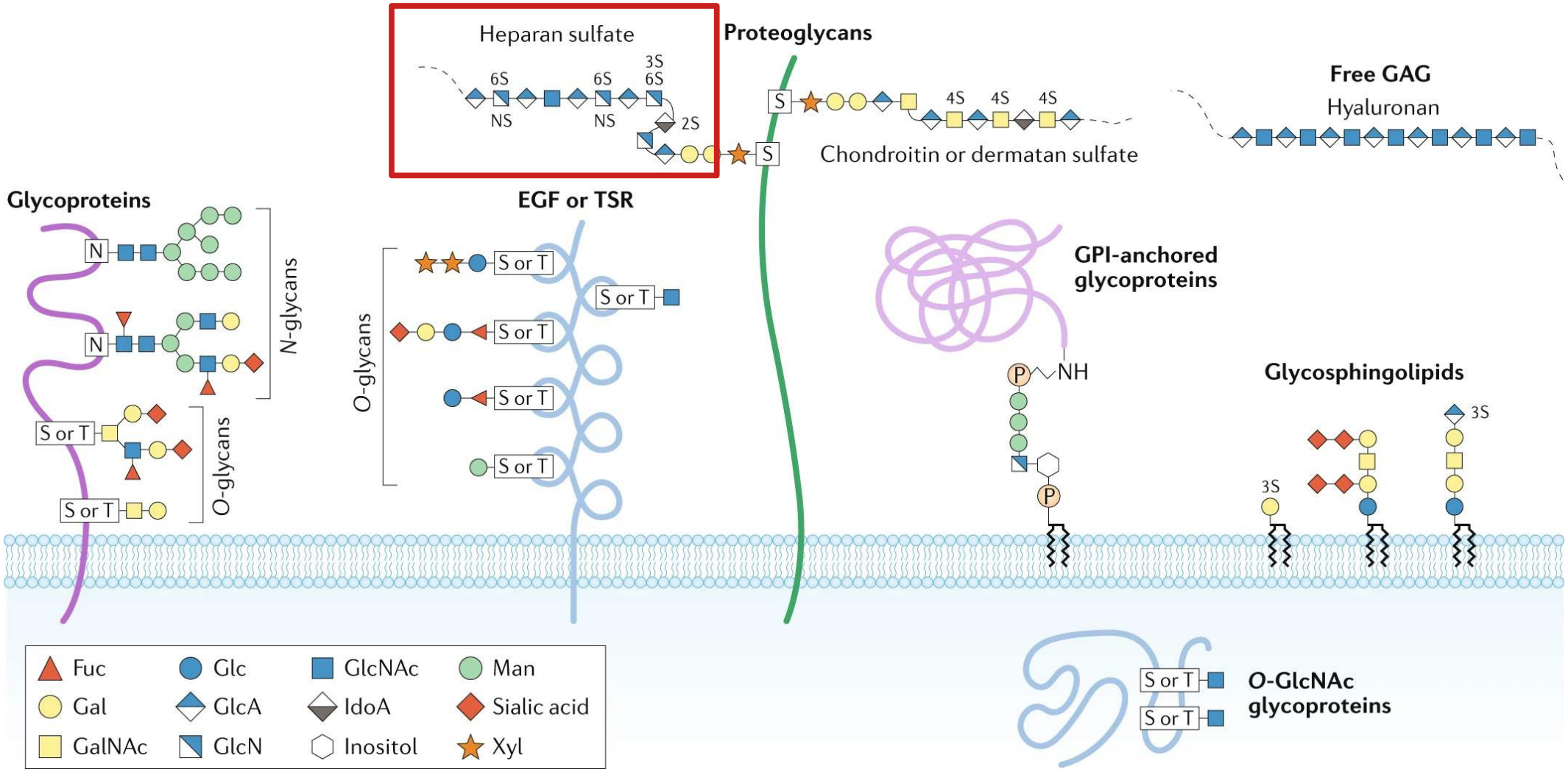


Overview on major types of glycosylation in humans



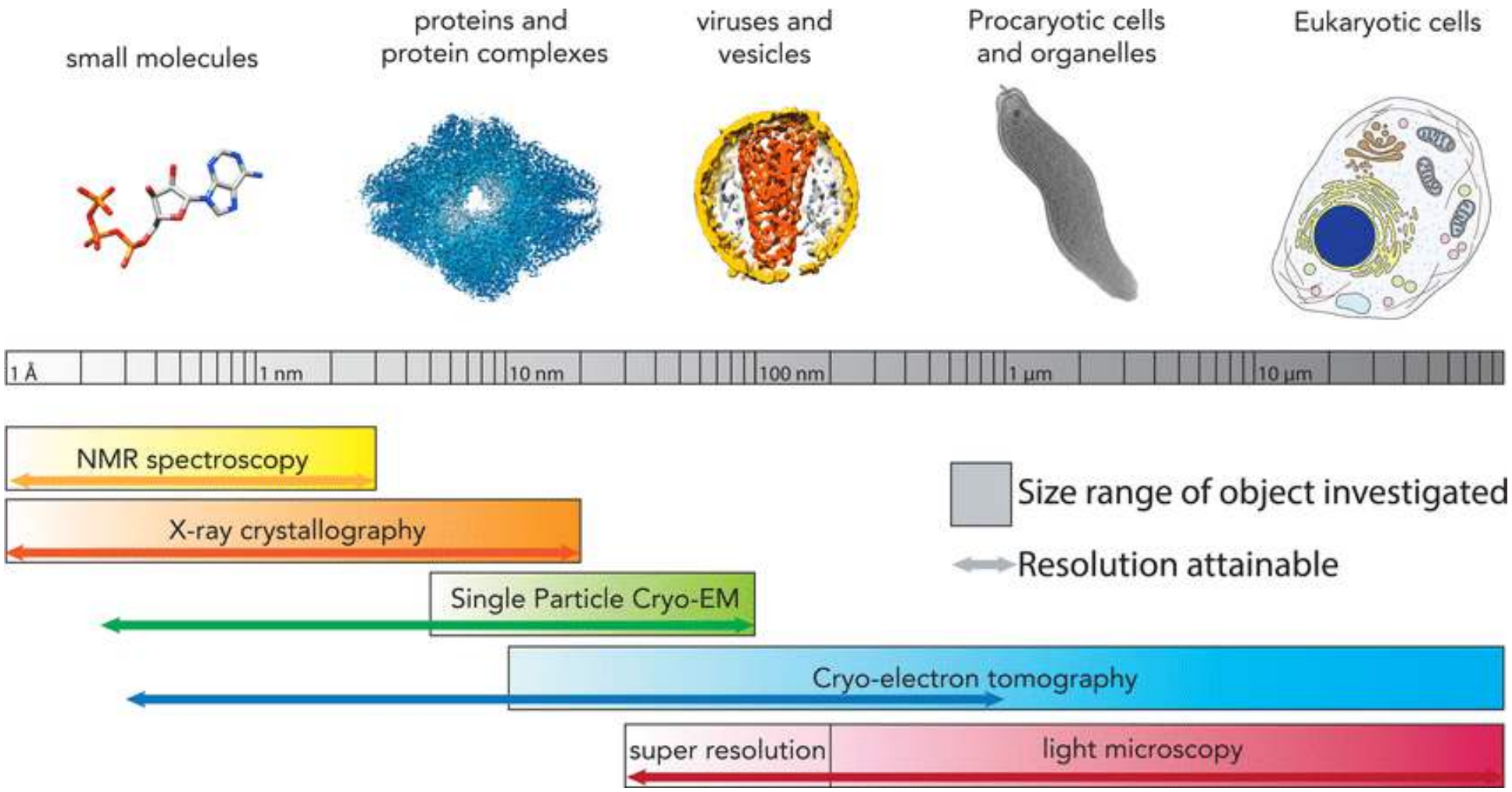
Reily et al., 2019

Overview on major types of glycosylation in humans



Reily et al., 2019

Overview on structural biology techniques



Hutchings & Zanetti, 2018

Why using cryo-EM to study glycosyltransferases?

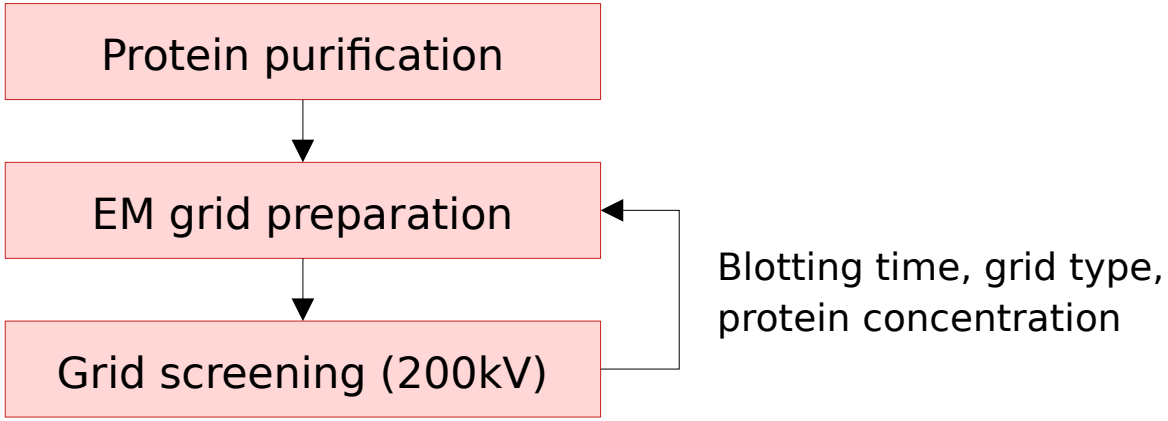
Advantages

- Requires low amounts of proteins
- Large complexes
- Less sensitive to sample heterogeneity
- Possible for flexible proteins (limits)
- Information on sample quality
- Intermediate resolution for most samples
- Data processing more and more user-friendly
- …

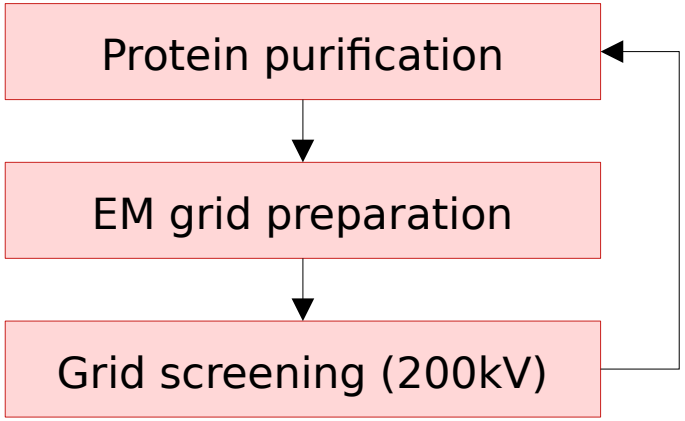
Disadvantages

- Difficult to reach very high resolution (1-2Å)
- Minimum size of protein (>60-100 kDa)
- Not possible for highly flexible proteins
- Large amounts of data to store/process
- Access to electron microscopes limited
- …

Workflow cryo-EM structure determination

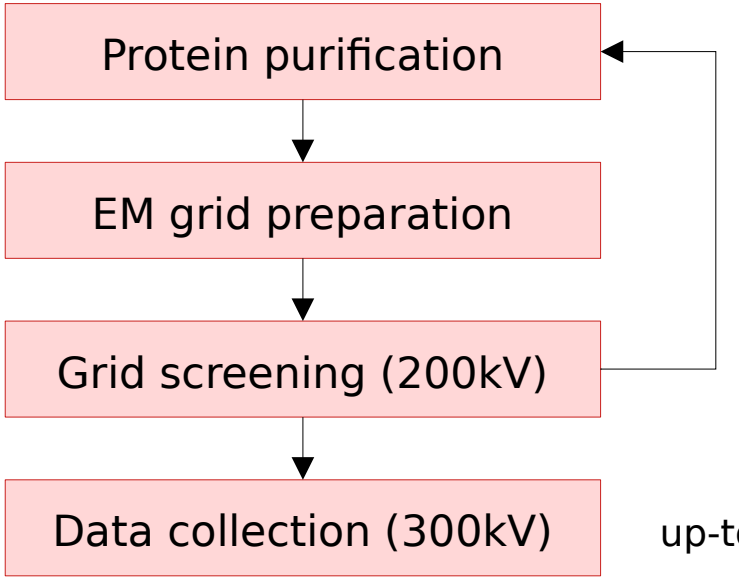


Workflow cryo-EM structure determination



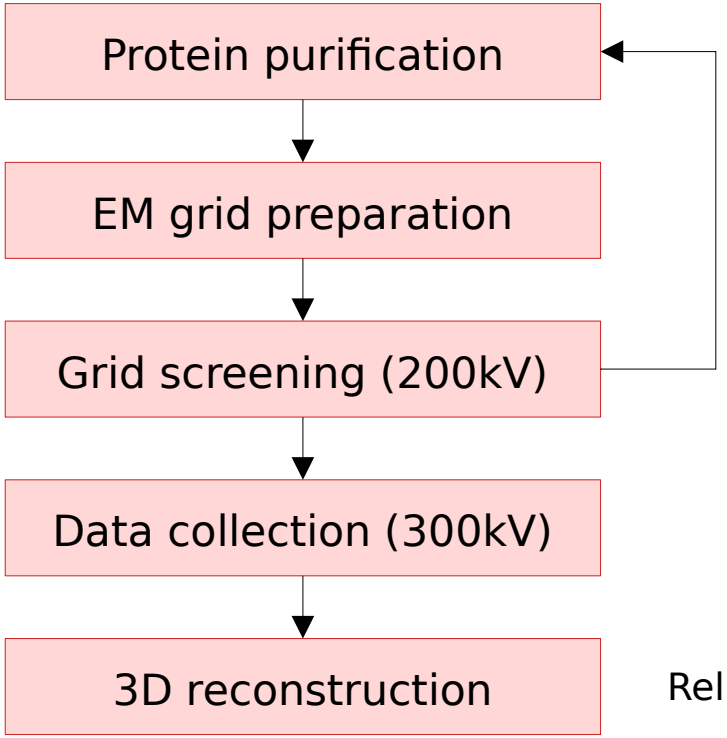
Protein aggregation, preferred orientation, protein concentration, sample heterogeneity

Workflow cryo-EM structure determination



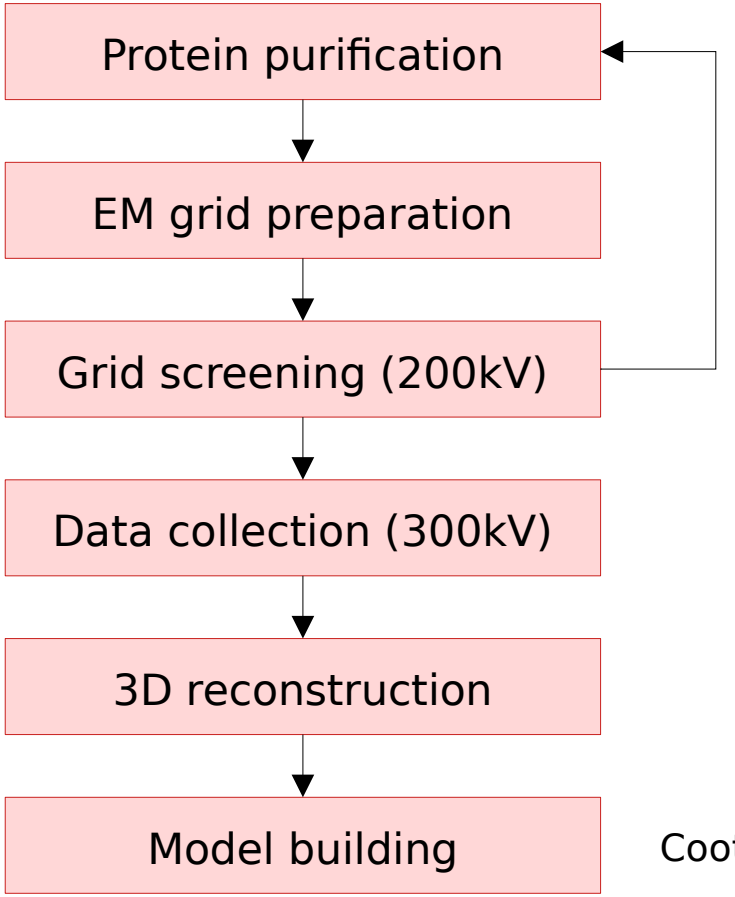
up-to 10.000 movies

Workflow cryo-EM structure determination



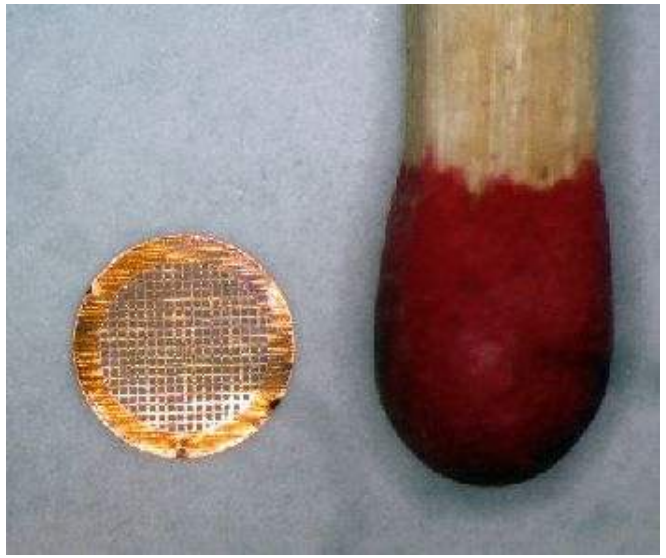
Relion, cryoSPARC, ...

Workflow cryo-EM structure determination



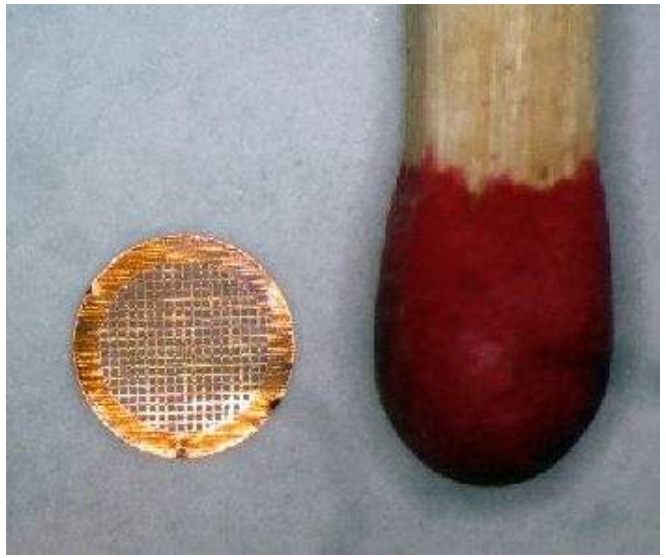
Coot, Phenix, ccpem, ..., AlphaFold!

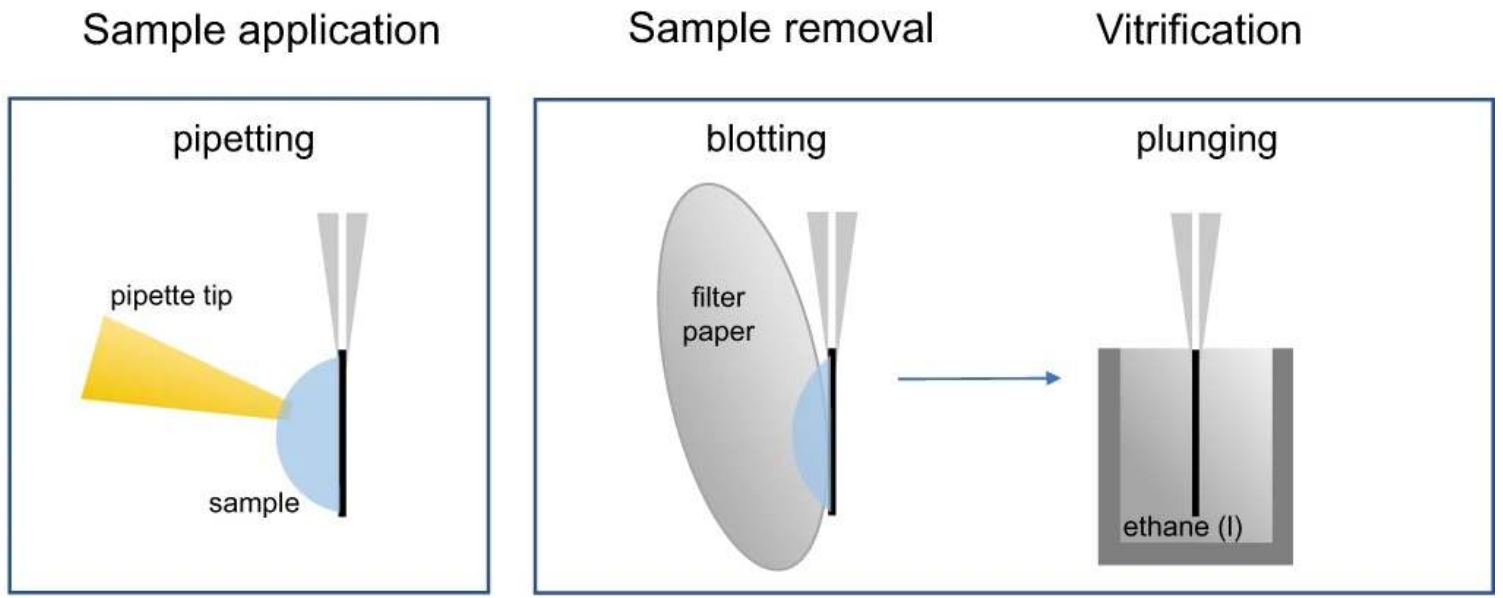
Cryo-EM grid preparation



Tiago et al., 2017
Koning et al., 2022

Cryo-EM grid preparation





Tiago et al., 2017
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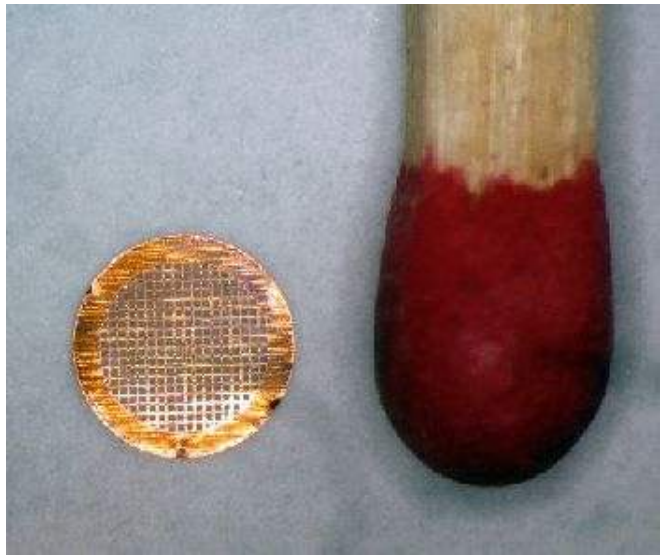
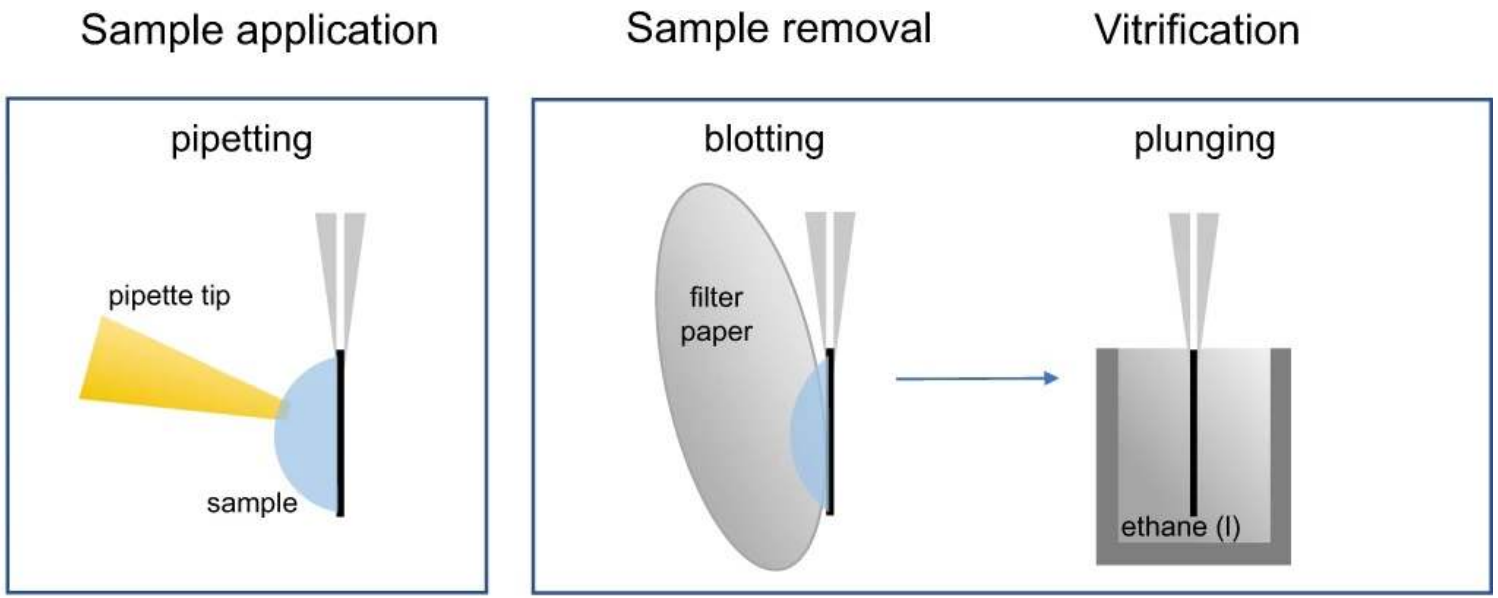
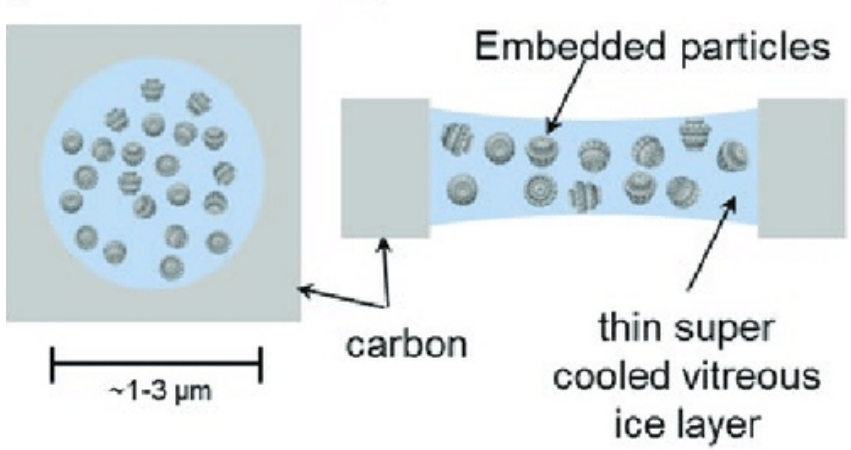


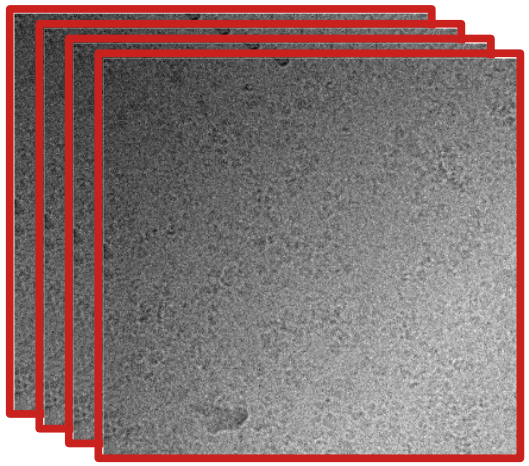




Tiago et al., 2017
Koning et al., 2022

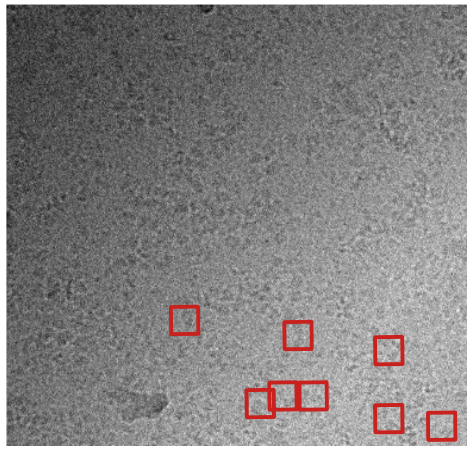
EM data processing

8656 movies



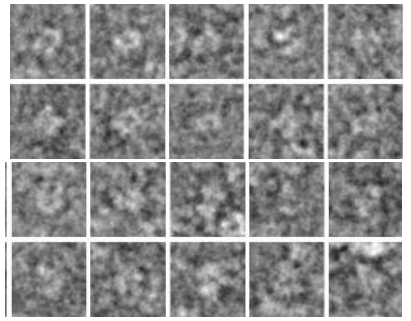
Motion correction

8656 micrographs



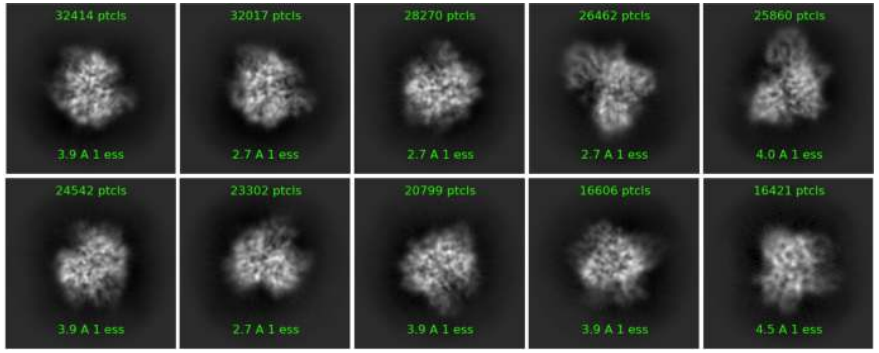
Particle picking

611911 particles



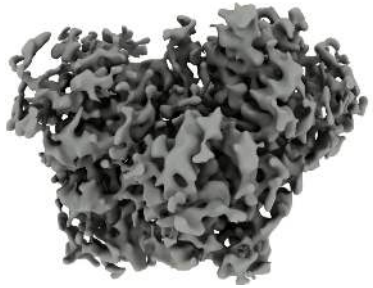
Several rounds of 2D classification

261348 particles

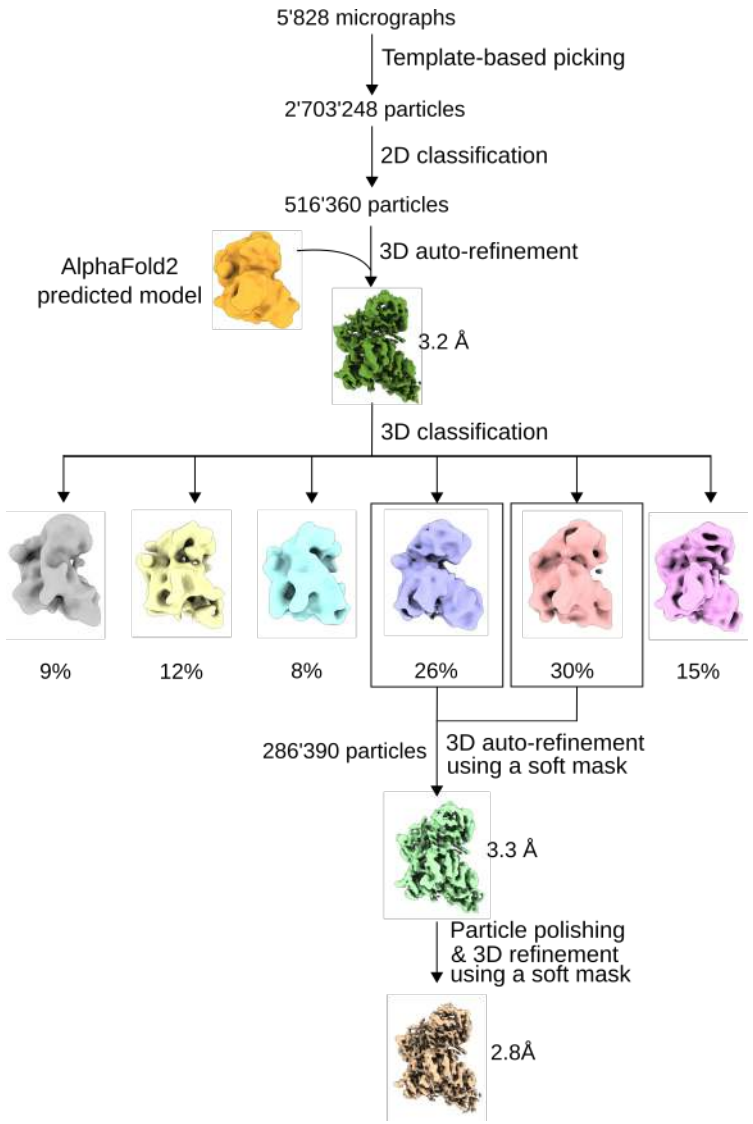


Ab initio reconstruction

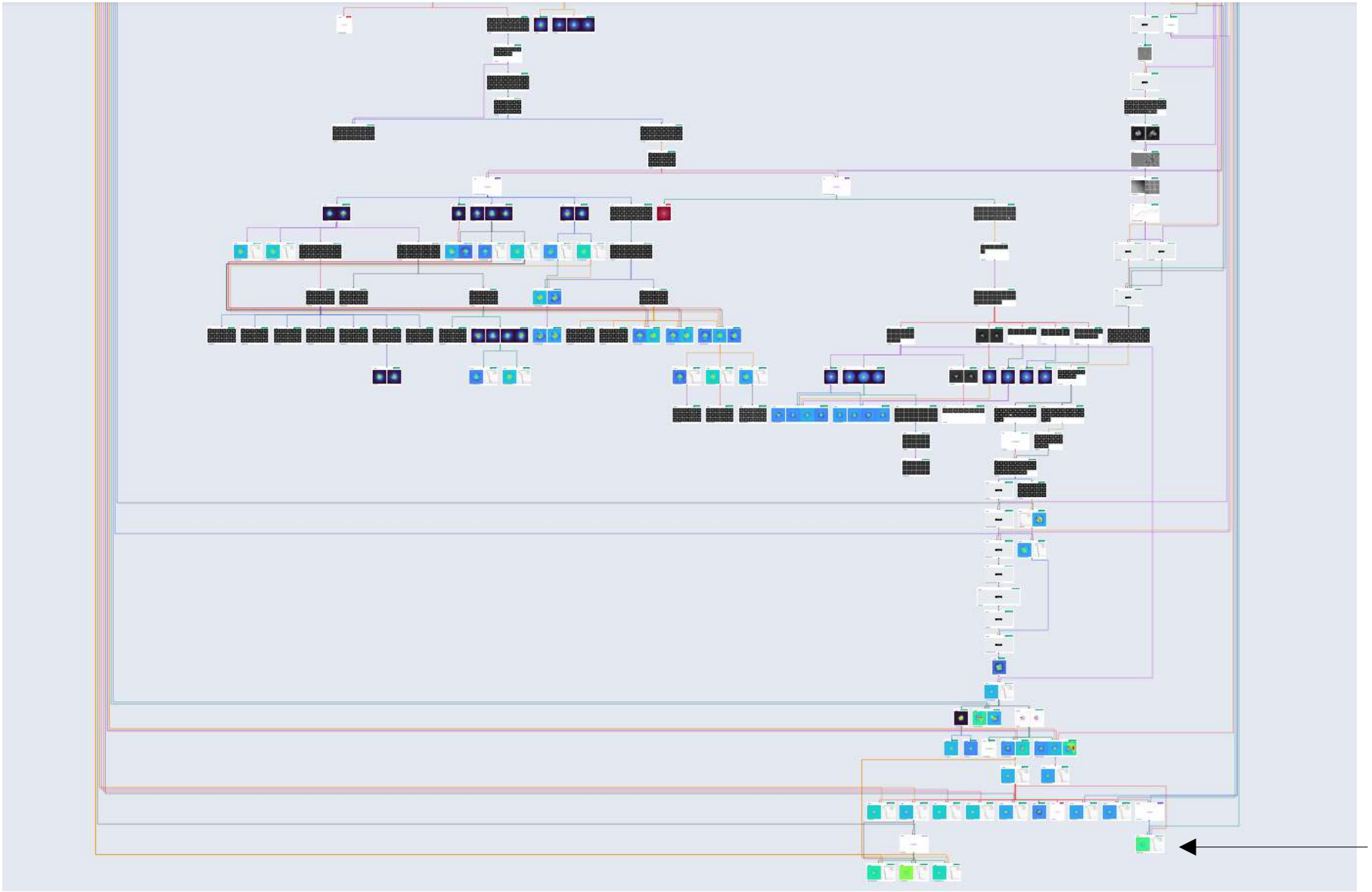
3D refinement



EM data processing - continued



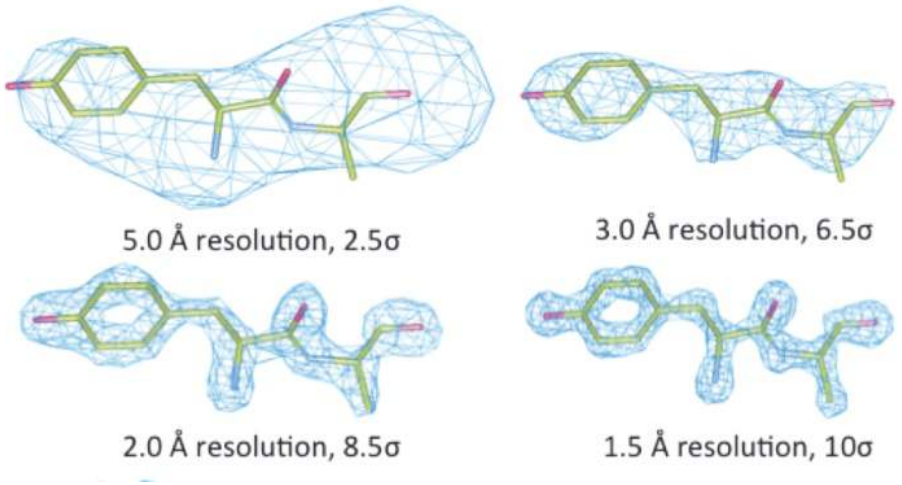
EM data processing – the reality...



Job327

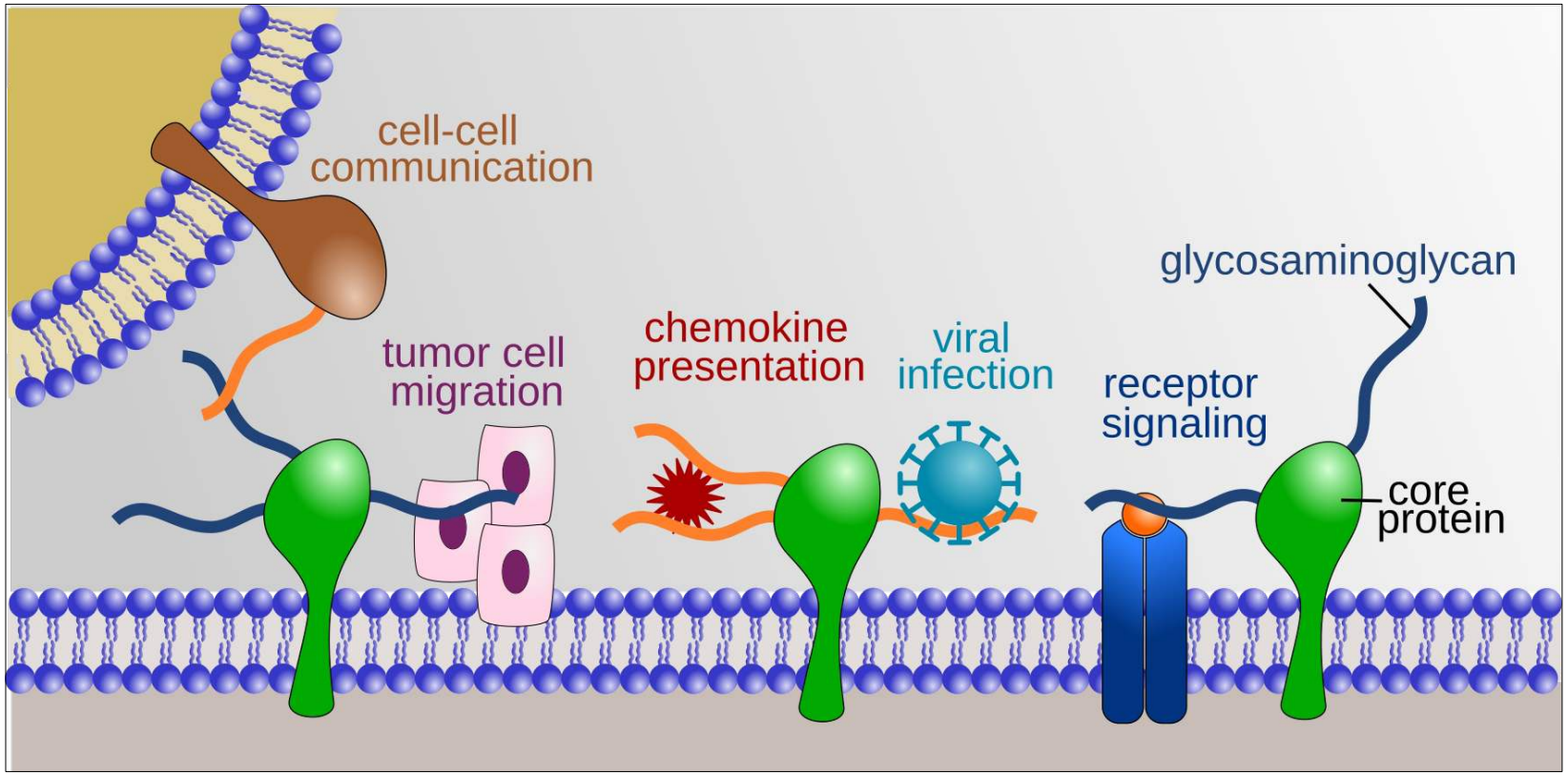
3D map interpretation & model building

- Assess the quality of your map
- *De novo* building in Coot (high resolution)
- Docking/fitting of predicted model (lower resolution)
- Phenix refinement, validation, deposition



Kuster et al., 2015

Cryo-EM study of the heparan sulfate polymerase complex

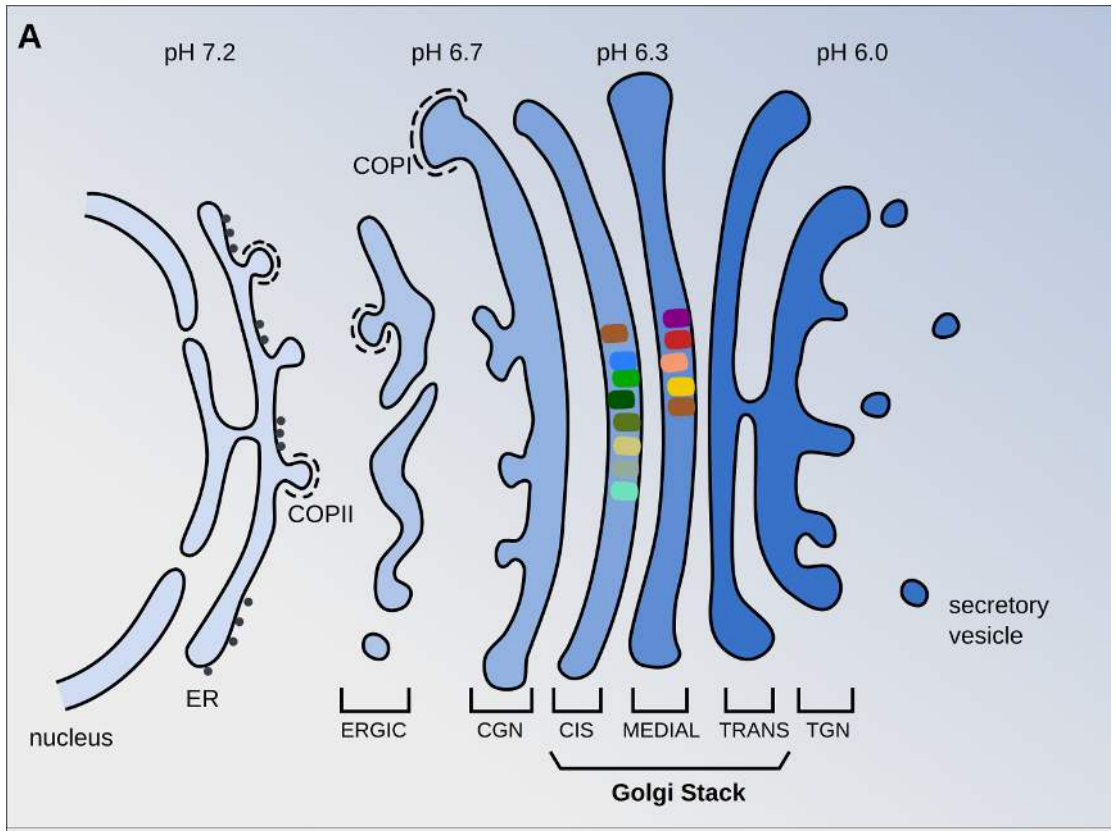


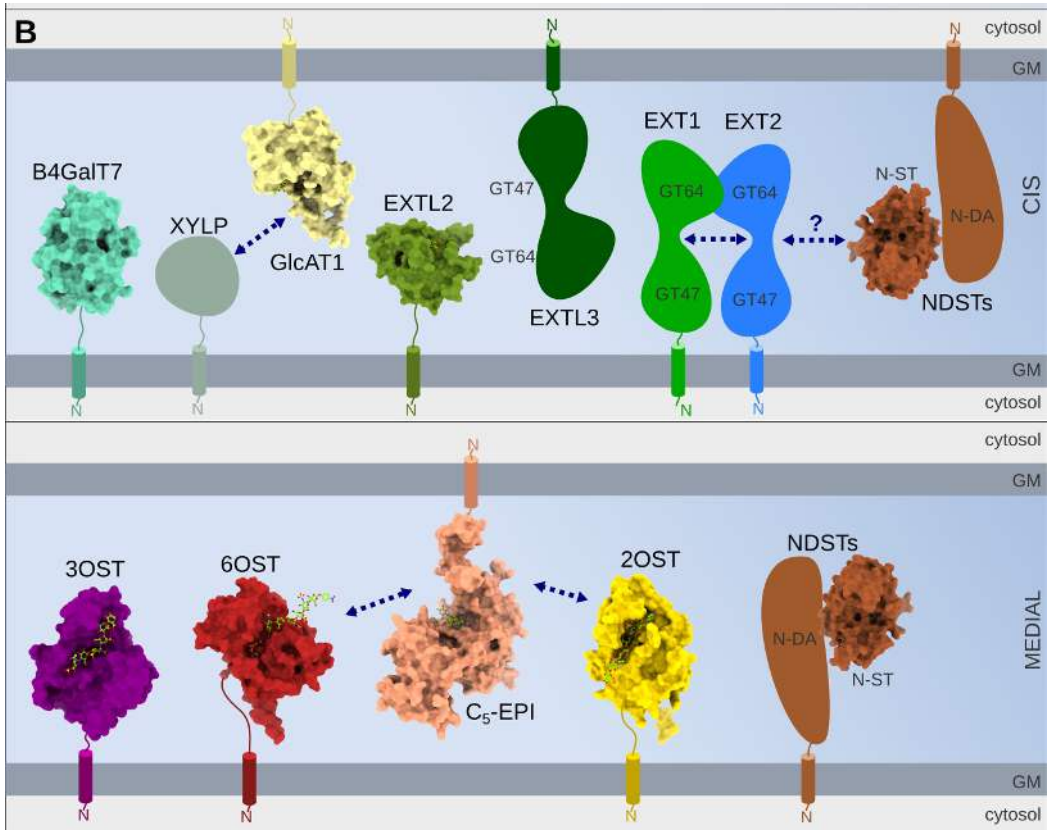
Annaval, Wild, et al., 2020

GAG classes:

- Heparan sulfate
- Heparin
- Chondroitin sulfate
- Dermatan sulfate
- Keratan sulfate
- Hyaluronic acid

Heparan sulfate biosynthesis takes place in the Golgi

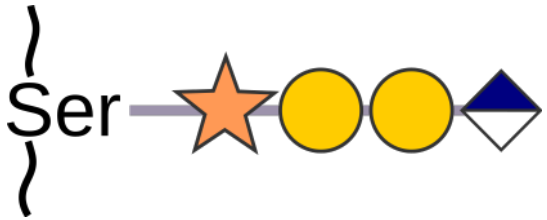




Annaval, Wild, et al., 2020

Heparan sulfate biosynthesis is a multi-step process

Initiation



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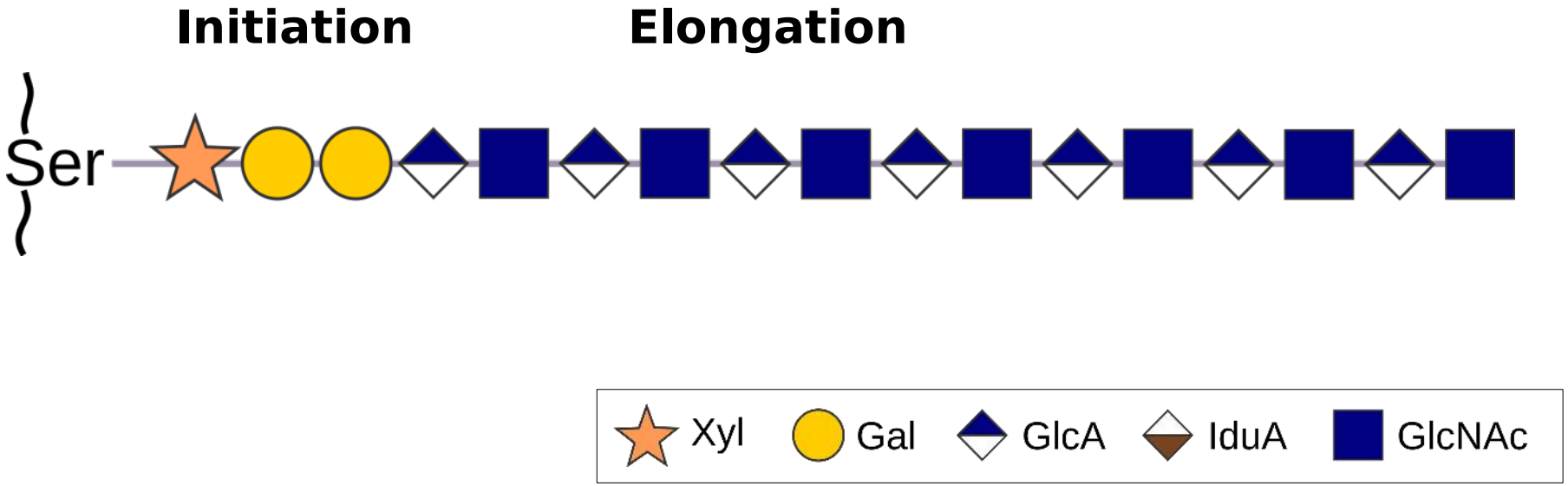

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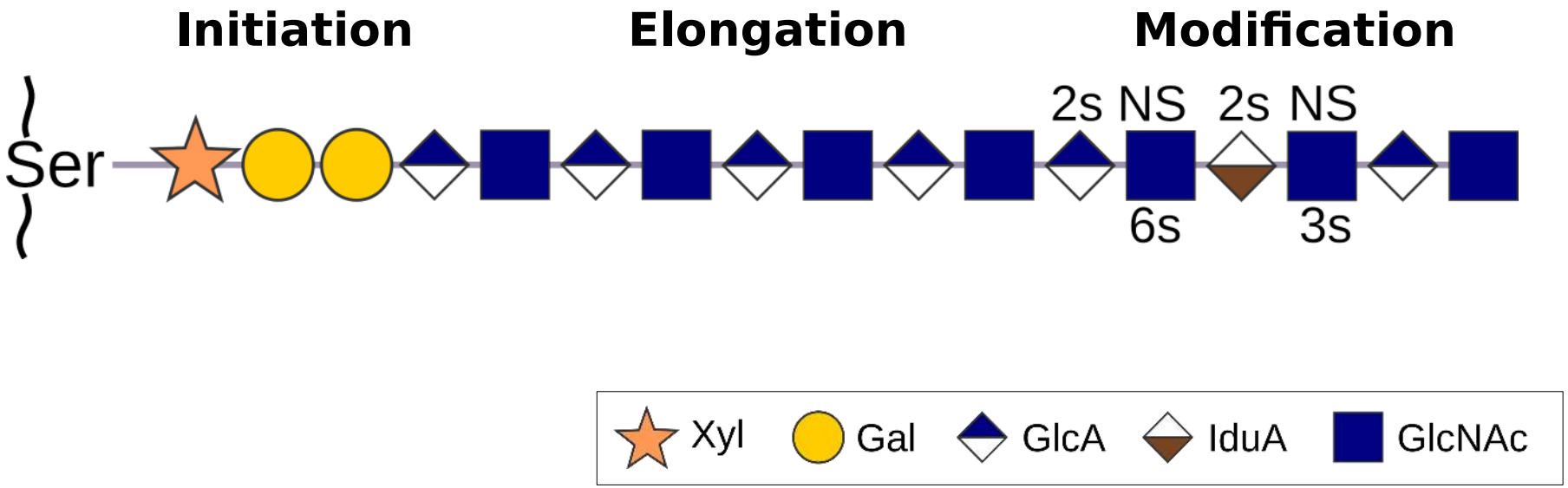

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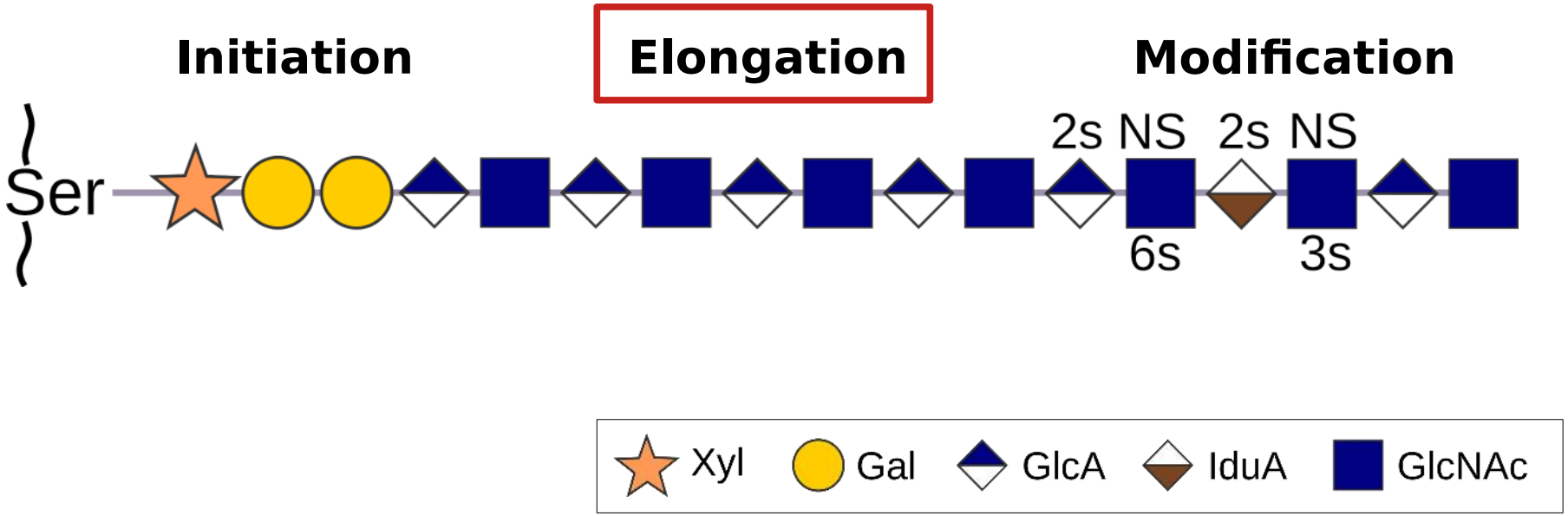
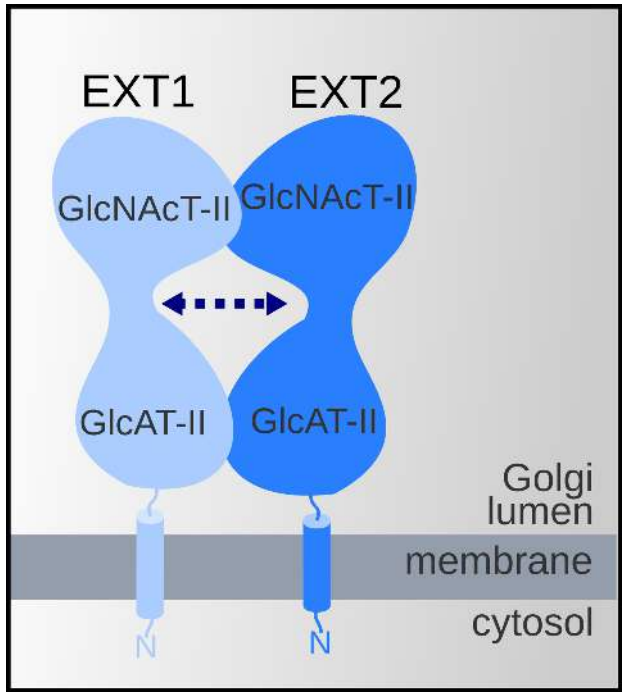
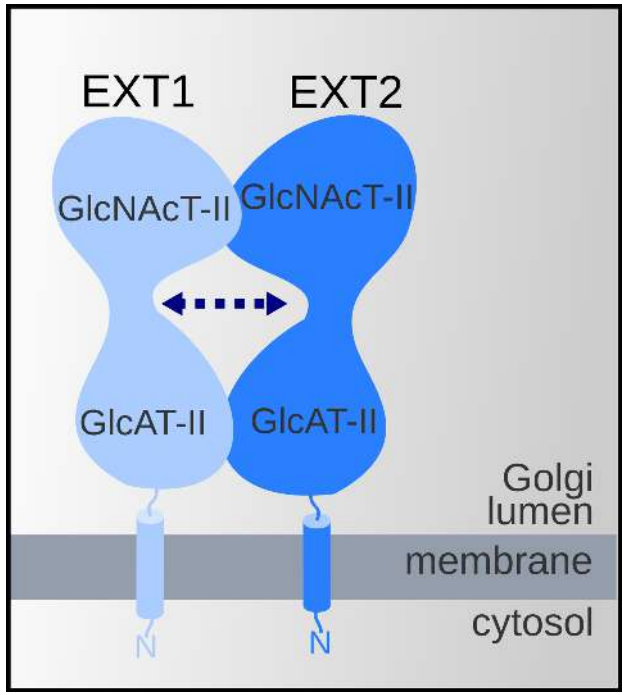


Heparan sulfate chain polymerization is carried out by EXT1 & EXT2



- Exostosin-1 and 2
- Mutations lead to HME
- Hetero-dimeric complex?
- Mechanism of chain polymerization?

Heparan sulfate chain polymerization is carried out by EXT1 & EXT2



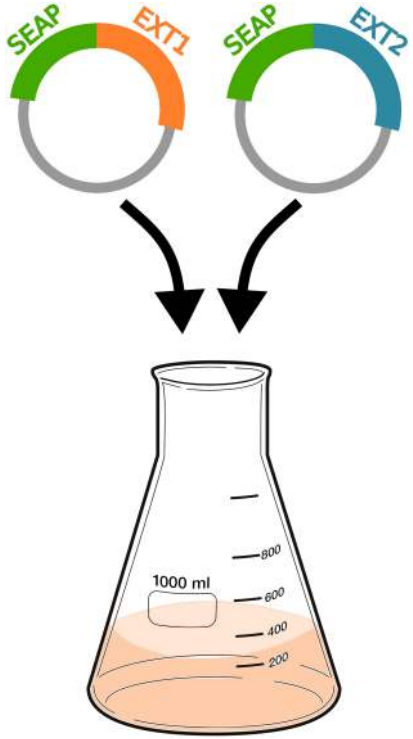
- Exostosin-1 and 2
- Mutations lead to HME
- Hetero-dimeric complex?
- Mechanism of chain polymerization?

Aim: Functional and structural characterization of the EXT1-EXT2 complex

Hetero-dimeric EXT1-EXT2 complex can be expressed and purified

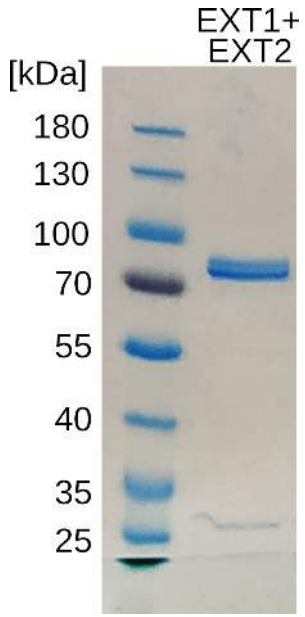
Construct design

Co-expression

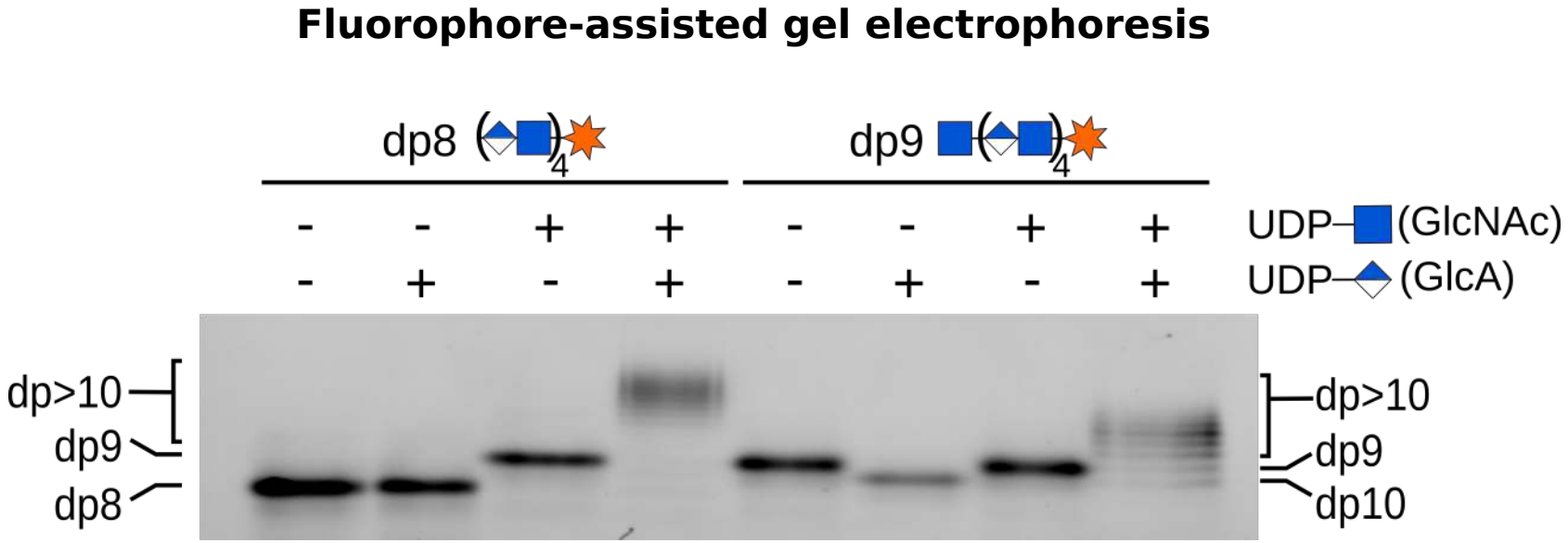


HEK 293F cells

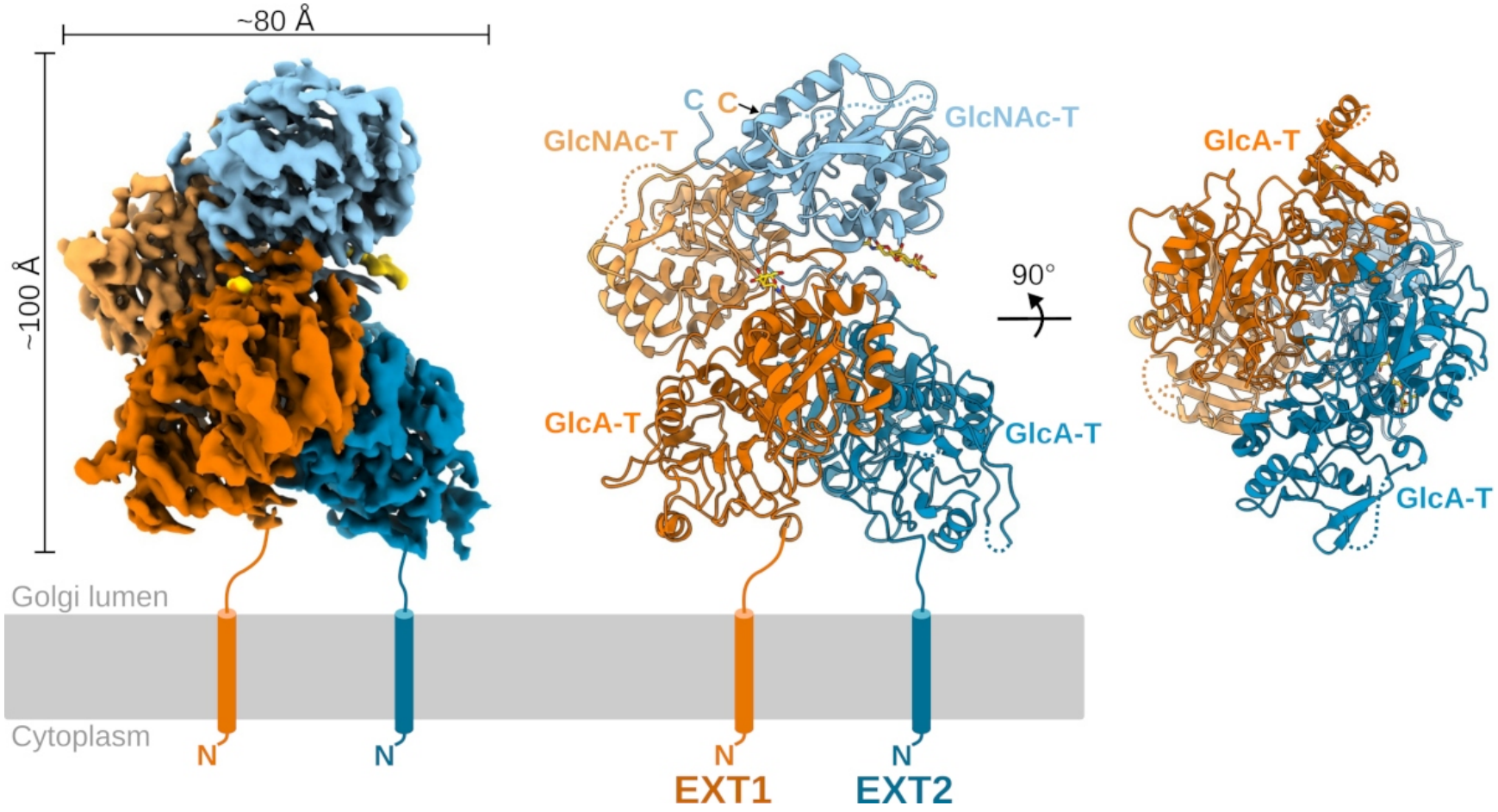
SDS-Page



EXT1-EXT2 complex activity is highly specific

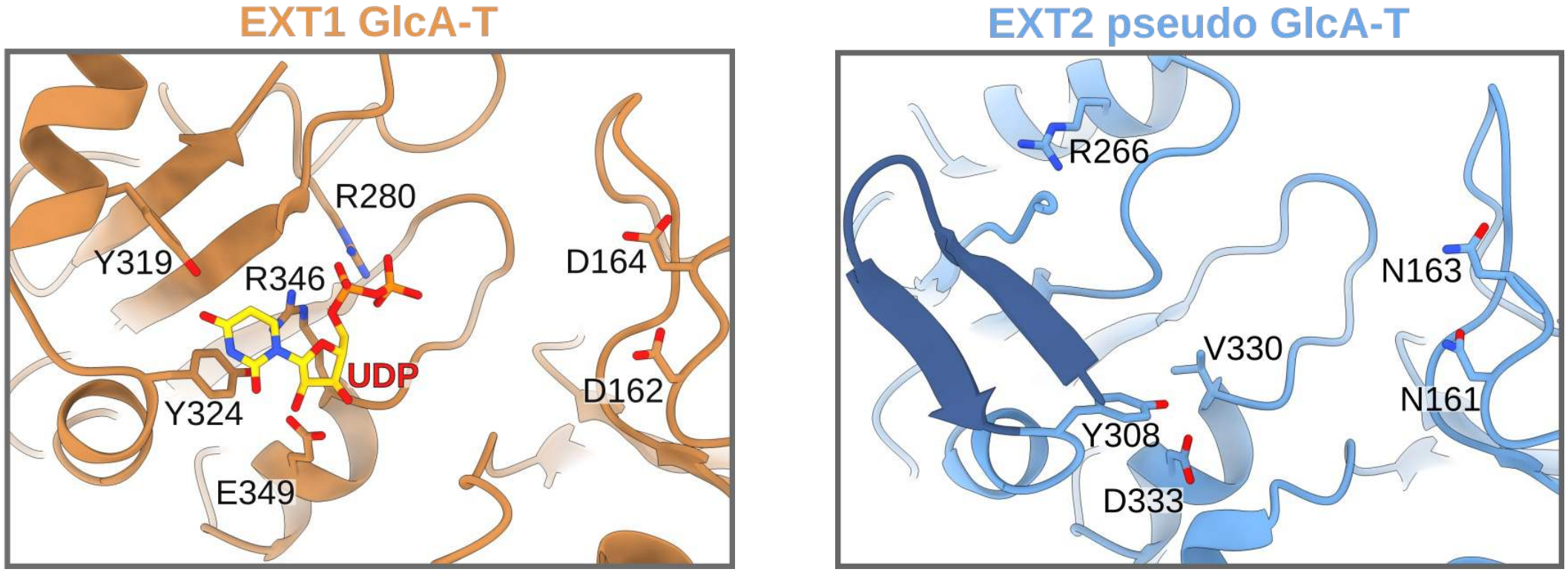


EXT1-EXT2 cryo-EM structure reveals a tightly packed complex



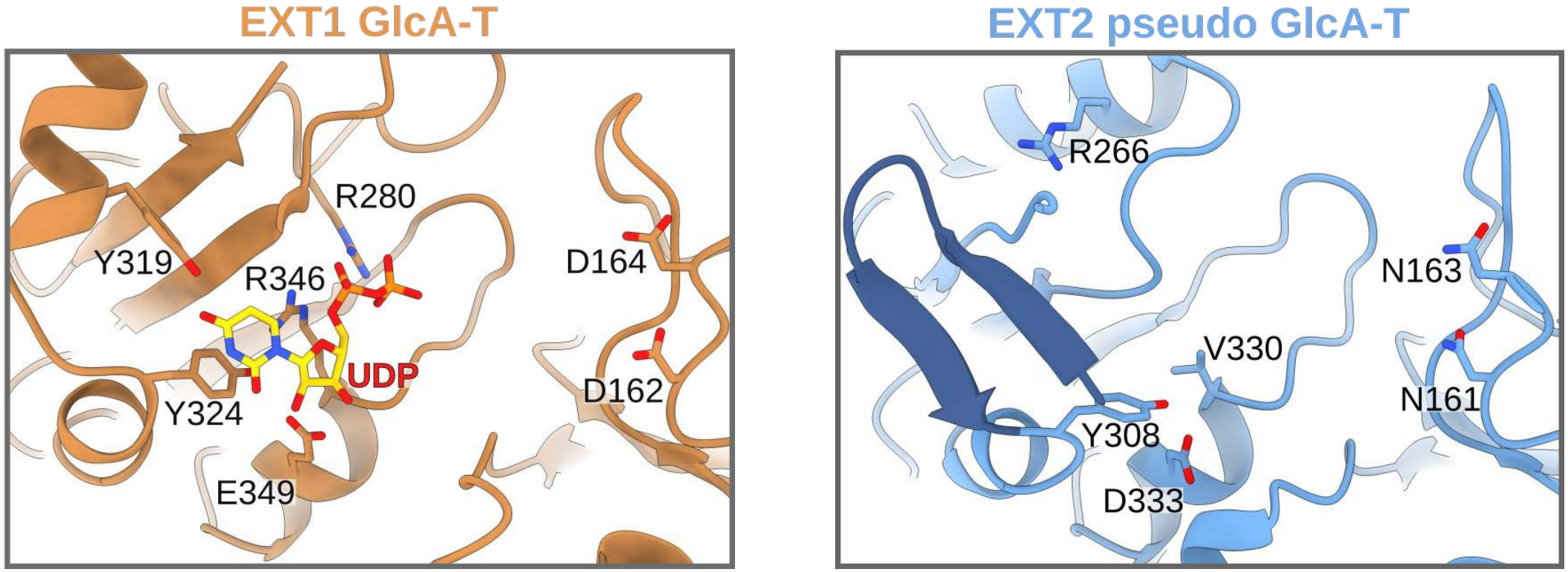
Leisico, Omeiri, …, Wild, Nature Communications (2022)

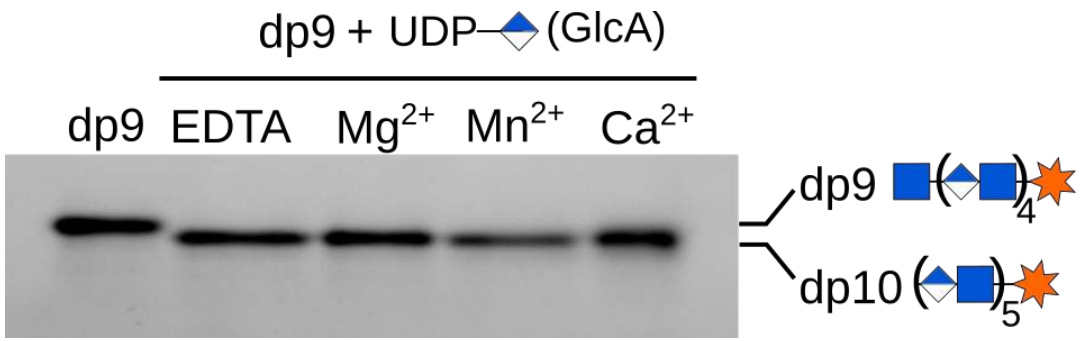
Characterization of the putative GlcA-transferase catalytic sites



- UDP bound in EXT1
- EXT2 catalytic site blocked by β-hairpin

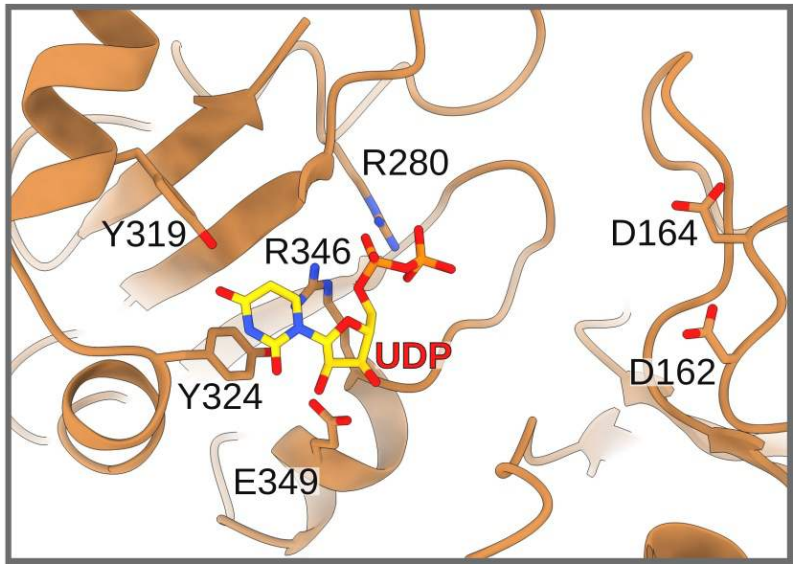
Characterization of the putative GlcA-transferase catalytic sites



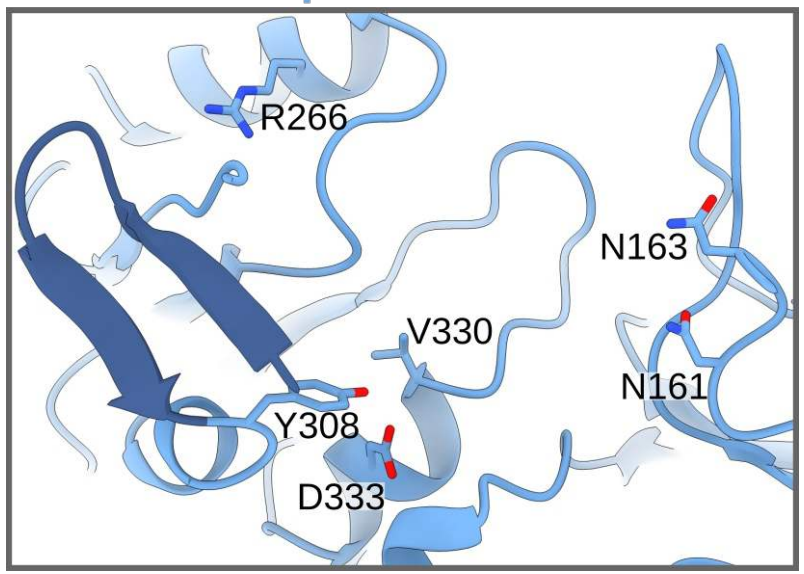


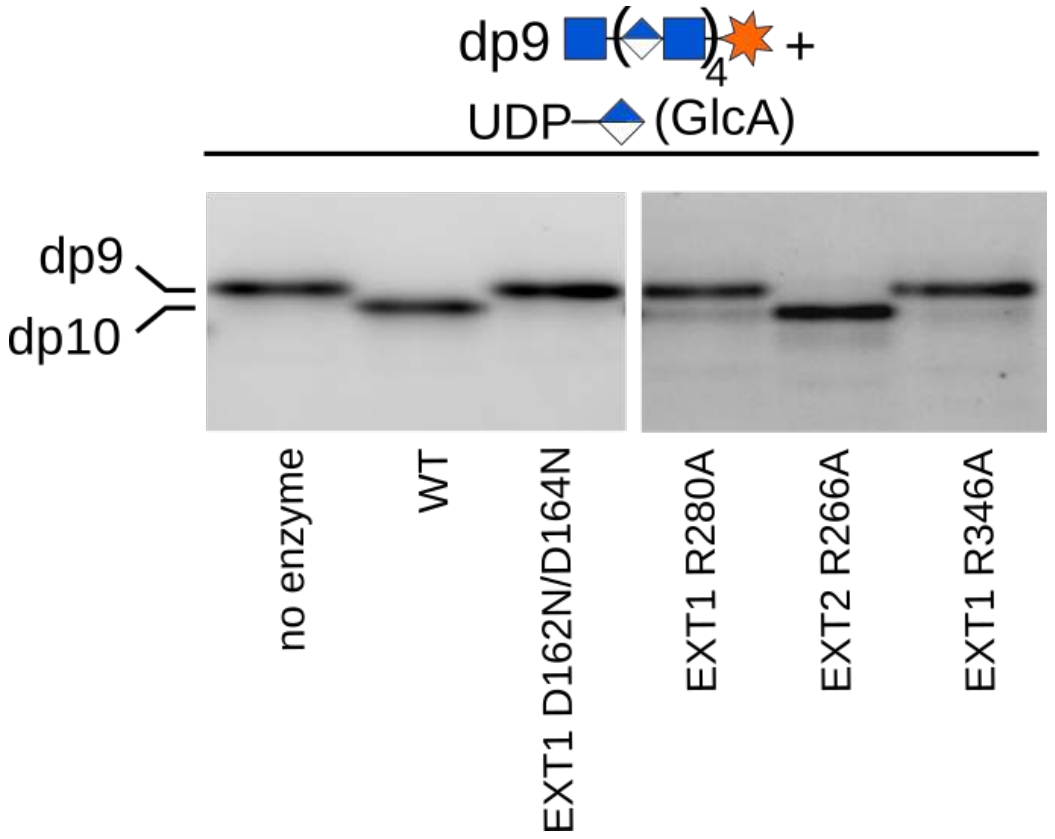
Only mutations in the EXT1 GlcA-T site affect activity *in vitro*

EXT1 GlcA-T

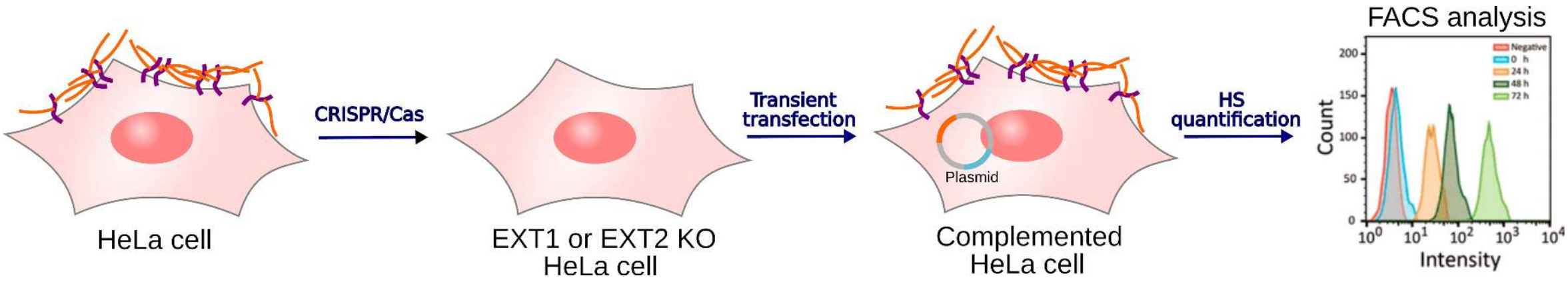


EXT2 pseudo GlcA-T



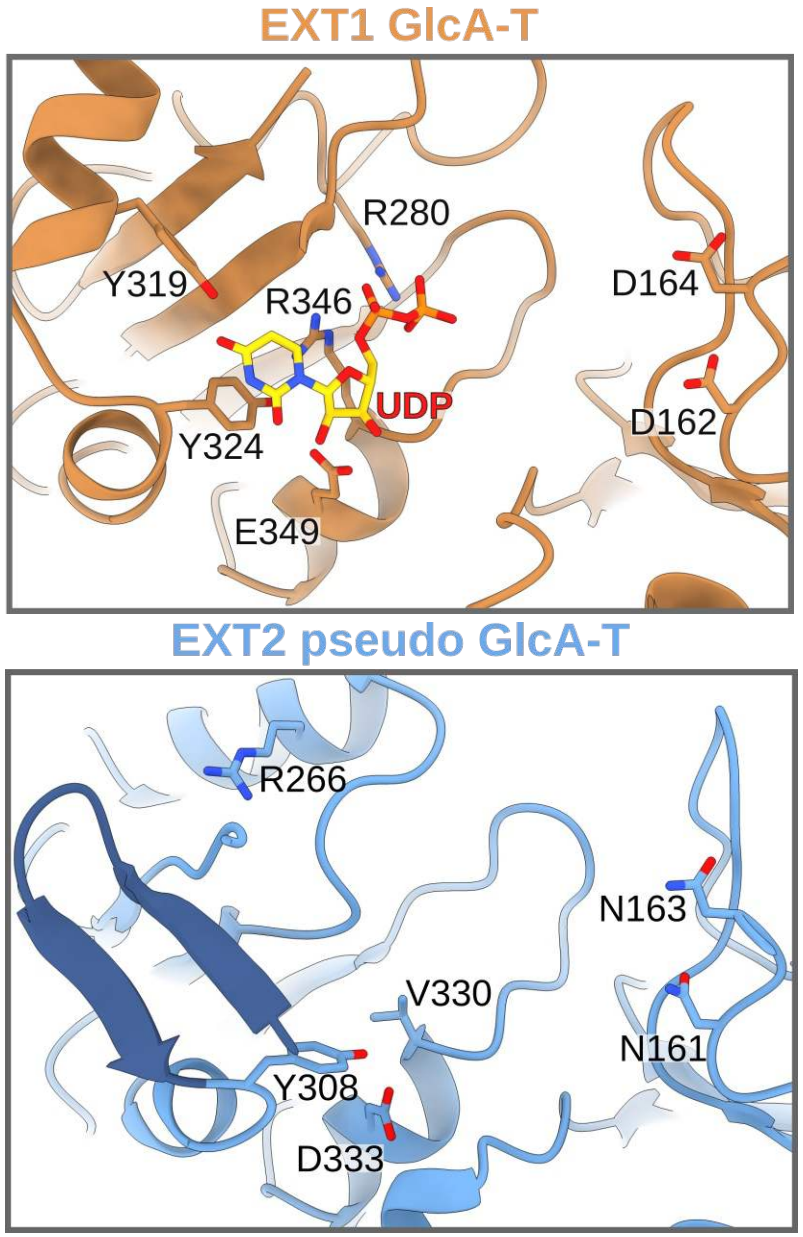


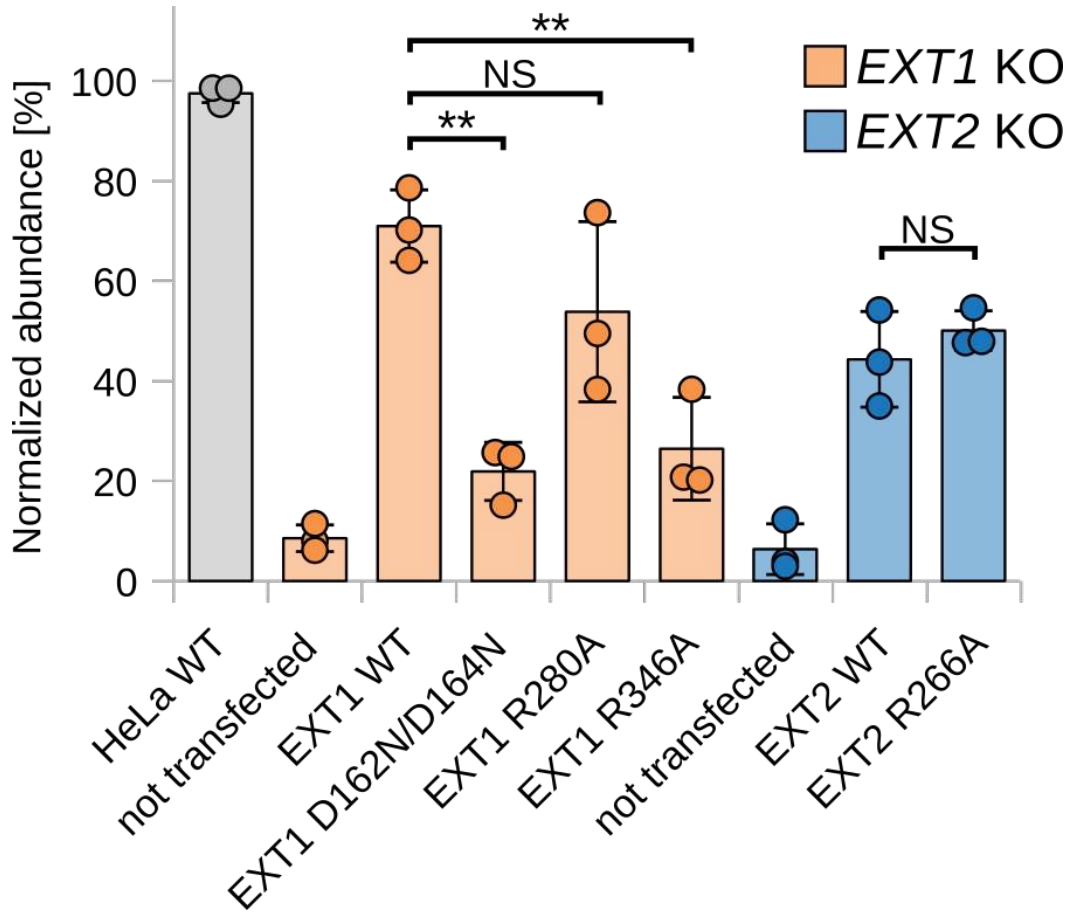
Studying importance of EXT1-EXT2 activity *in cellulo*



- EXT1 and EXT2 KO cells have no heparan sulfate
- Complementation using wt and mutant EXT1/EXT2 genes
- Cell surface HS detected using specific antibodies

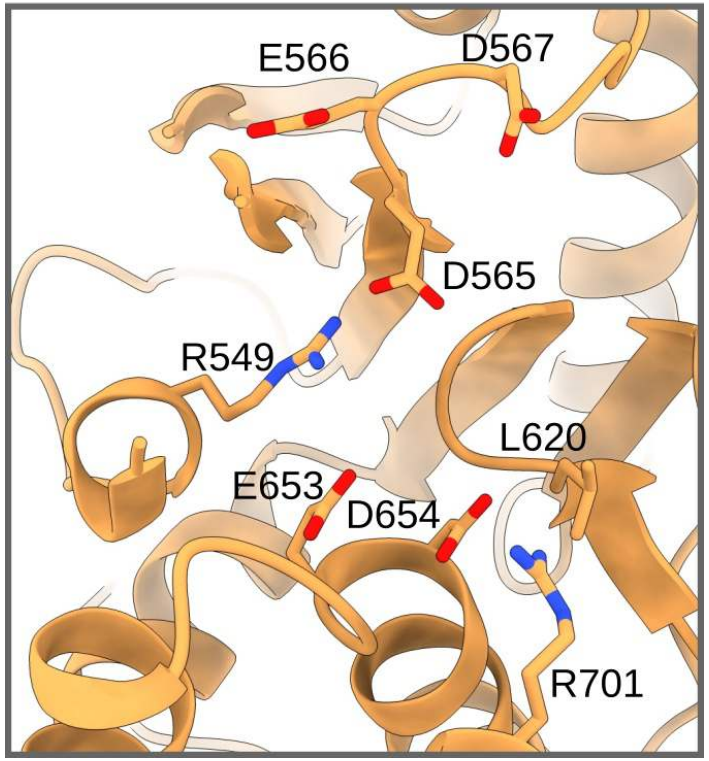
Only mutations in the EXT1 GlcA-T site affect activity *in cellulo*



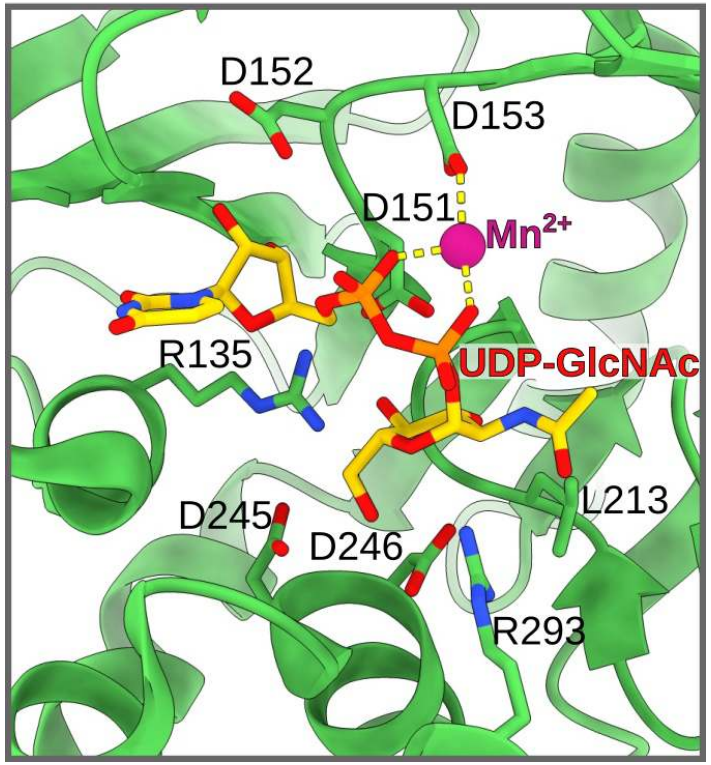


Characterization of the putative GlcNAc-T active sites

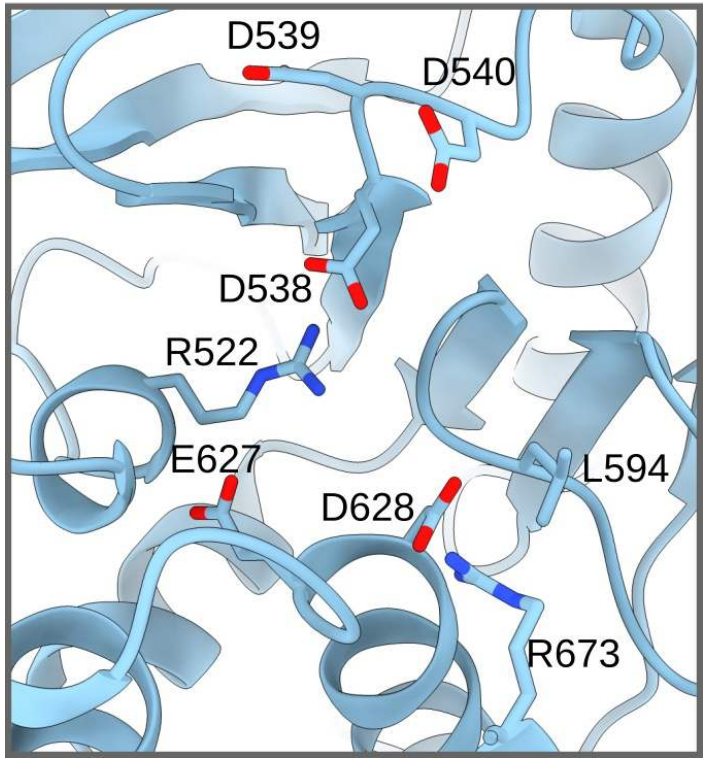
EXT1 GlcNAc-T

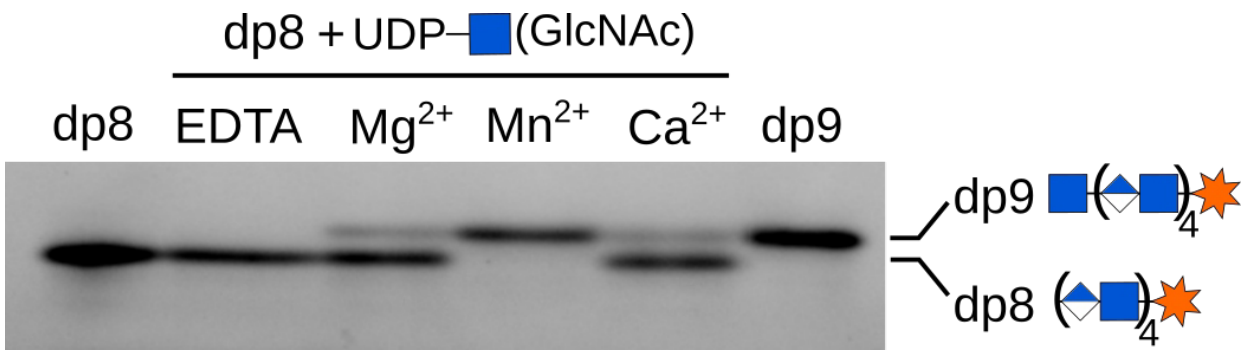


EXTL2

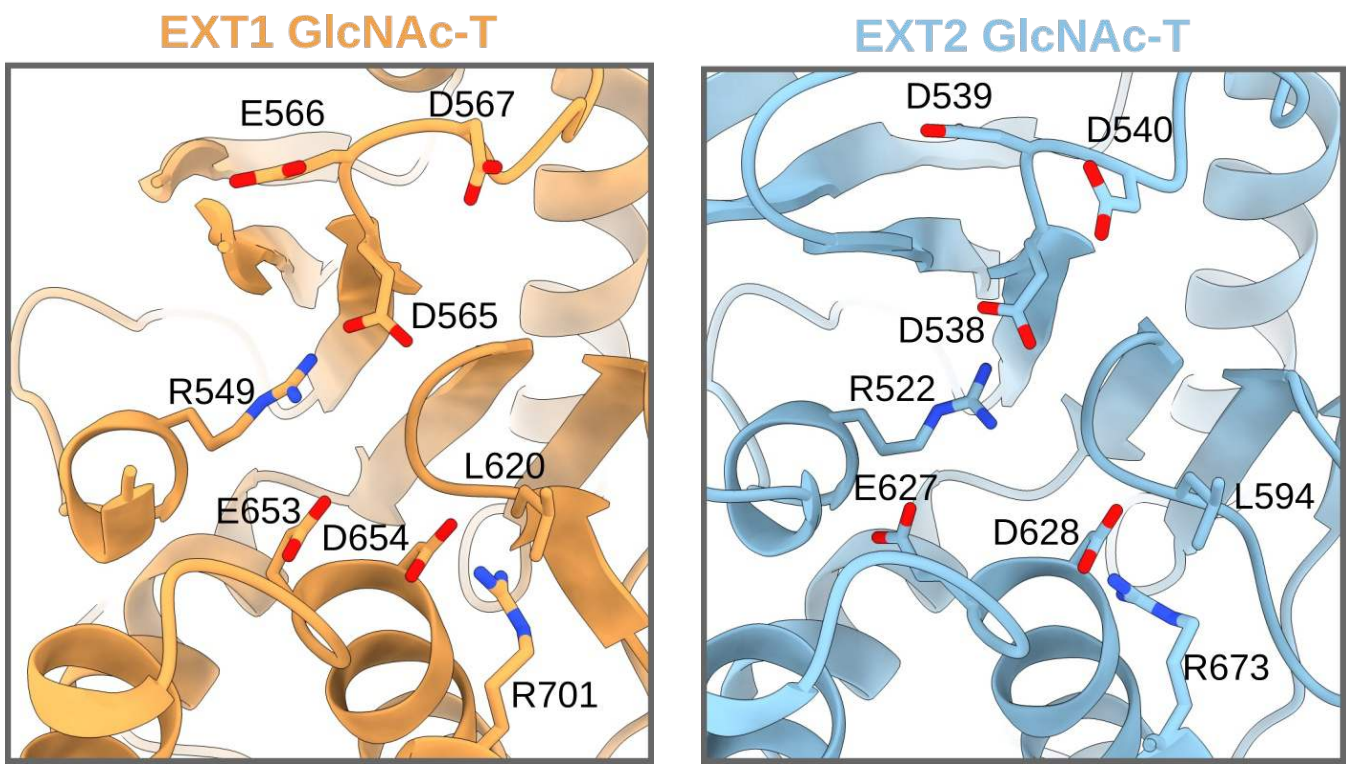


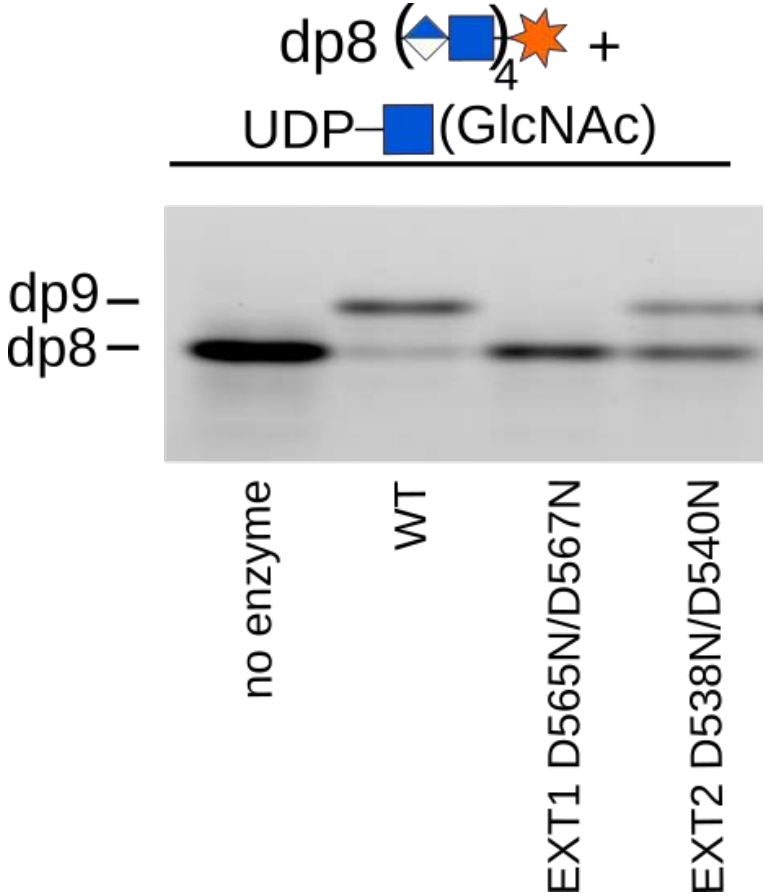
EXT2 GlcNAc-T



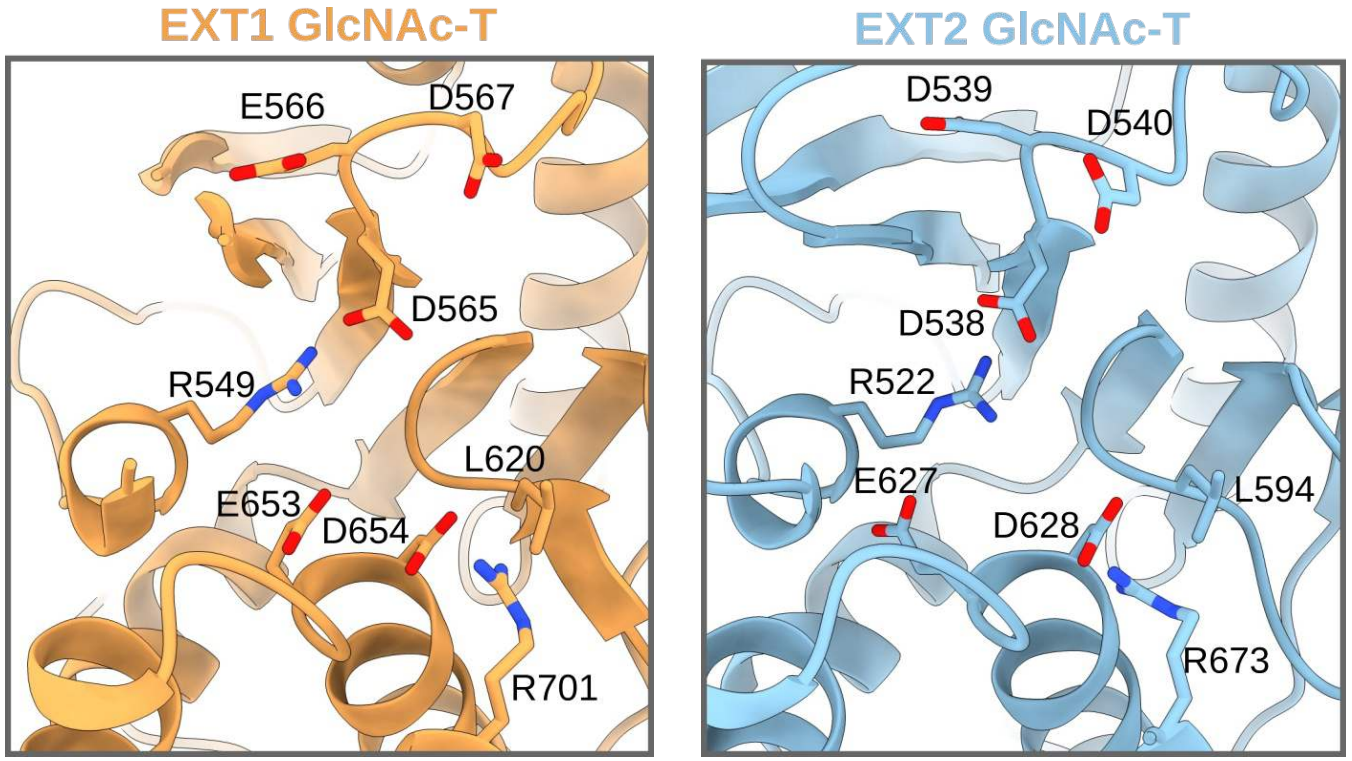


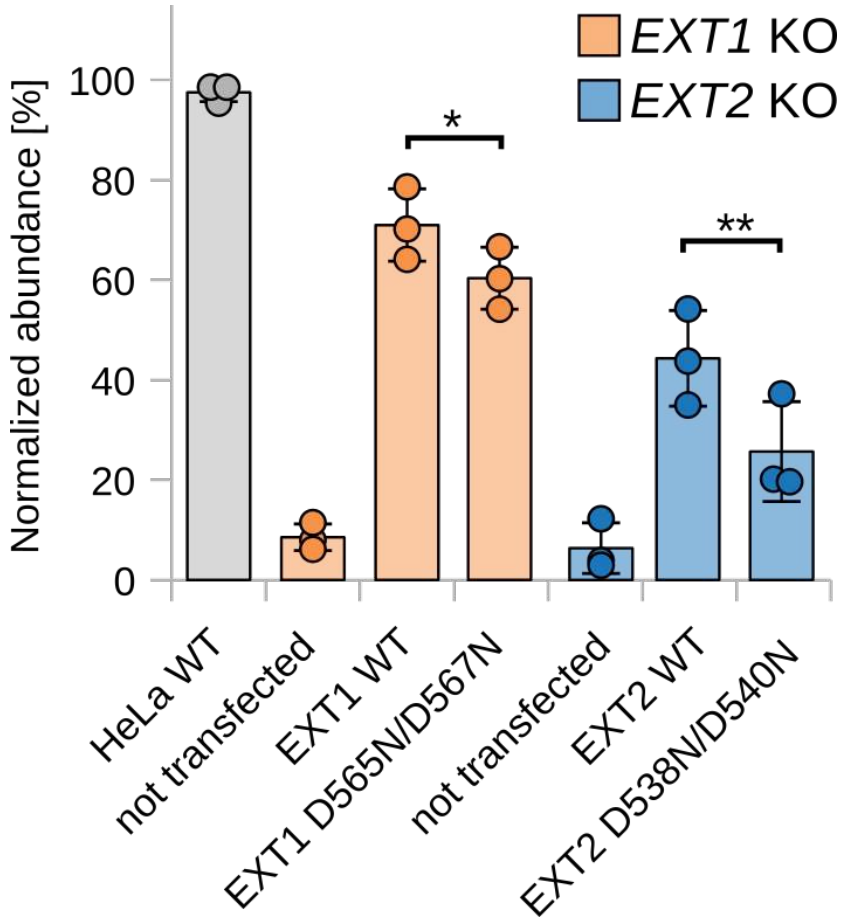
Mutations in the both GlcNAc-T sites affect activity *in vitro*



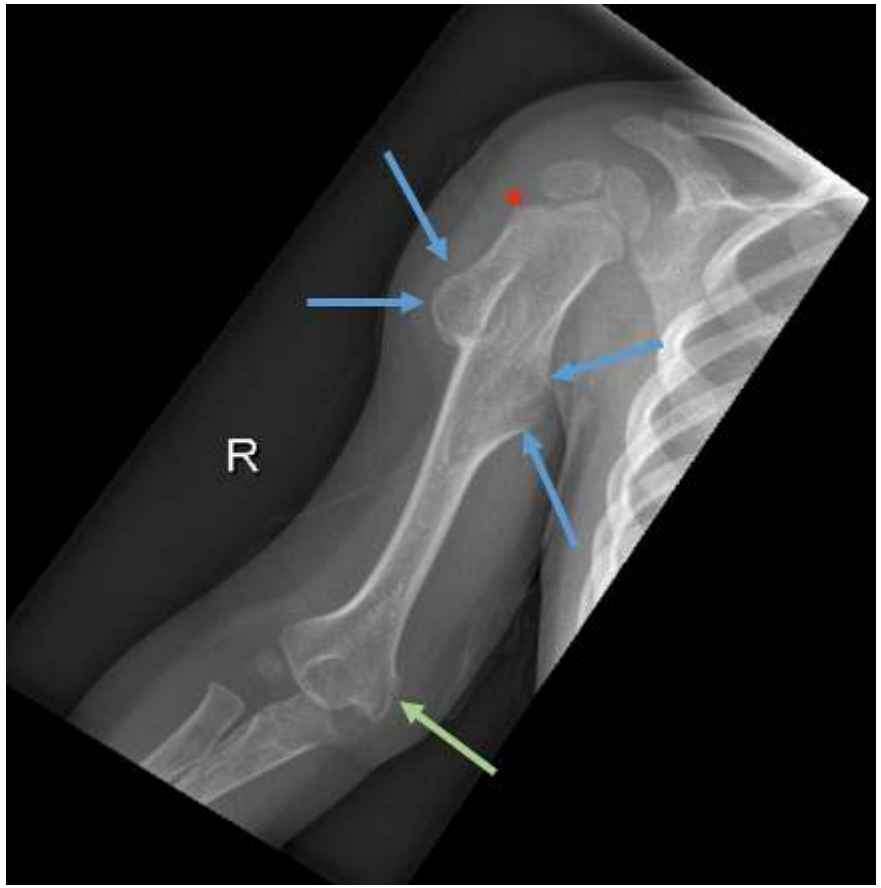


Mutations in the both GlcNAc-T sites affect activity *in cellulo*





Mutations in the ext1 or ext2 gene can lead to HME

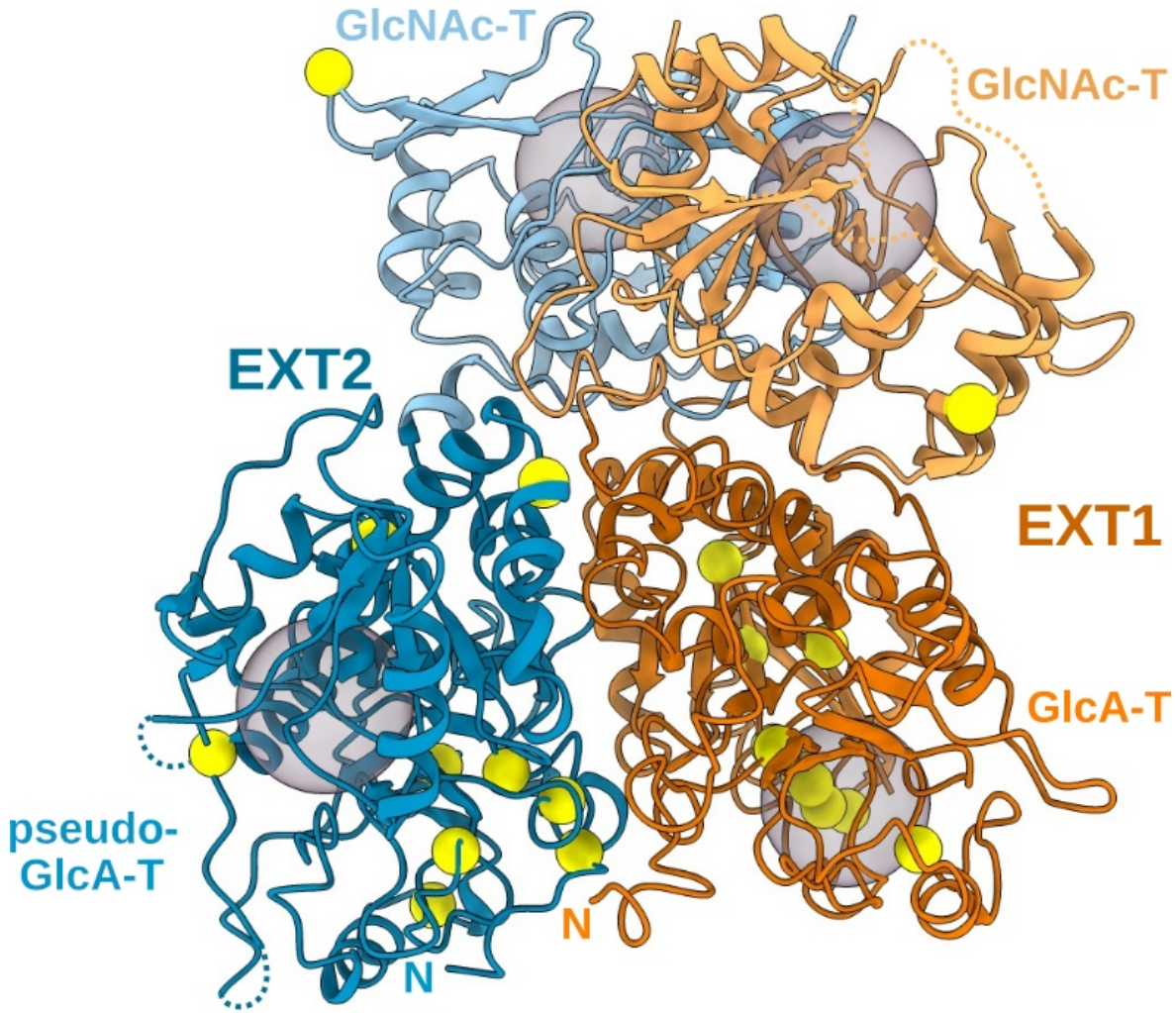


Funk et al., Consultant (2021)

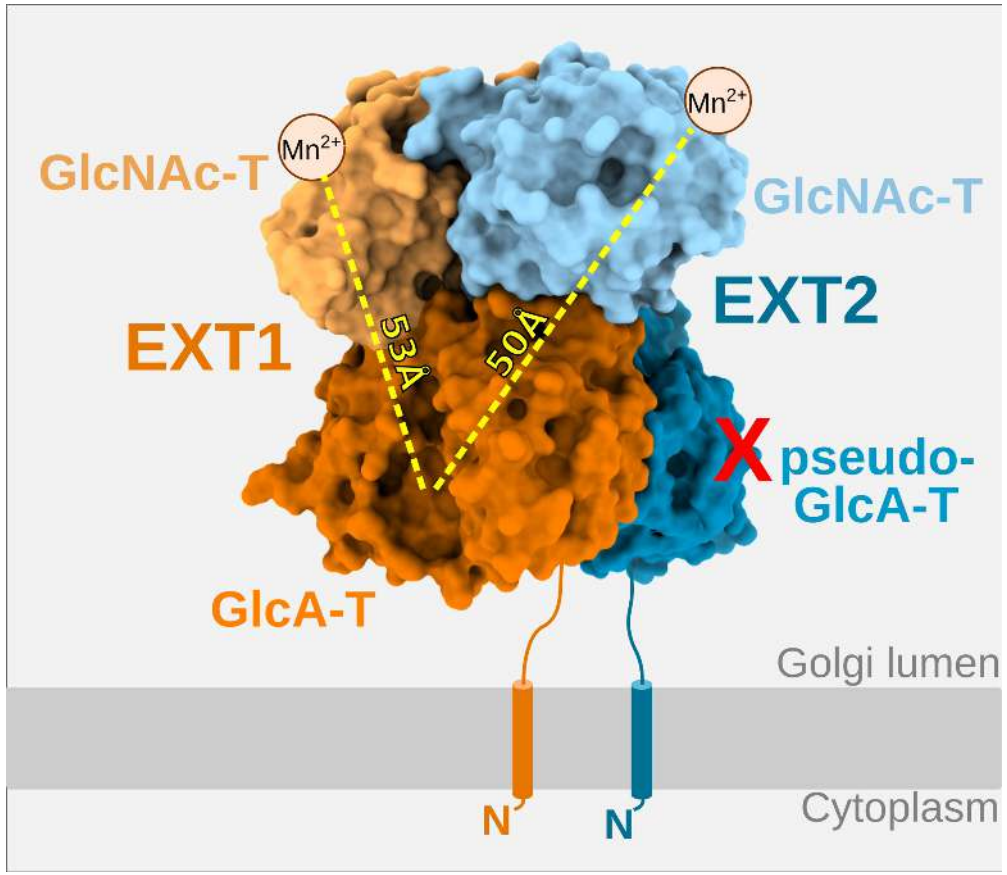
Hereditary multiple exostoses

- Benign bone tumors
- Incidence of 1 in 50,0000
- Autosomal dominant disorder

Mutations in HME patients locate to EXT1 GlcA-T active site

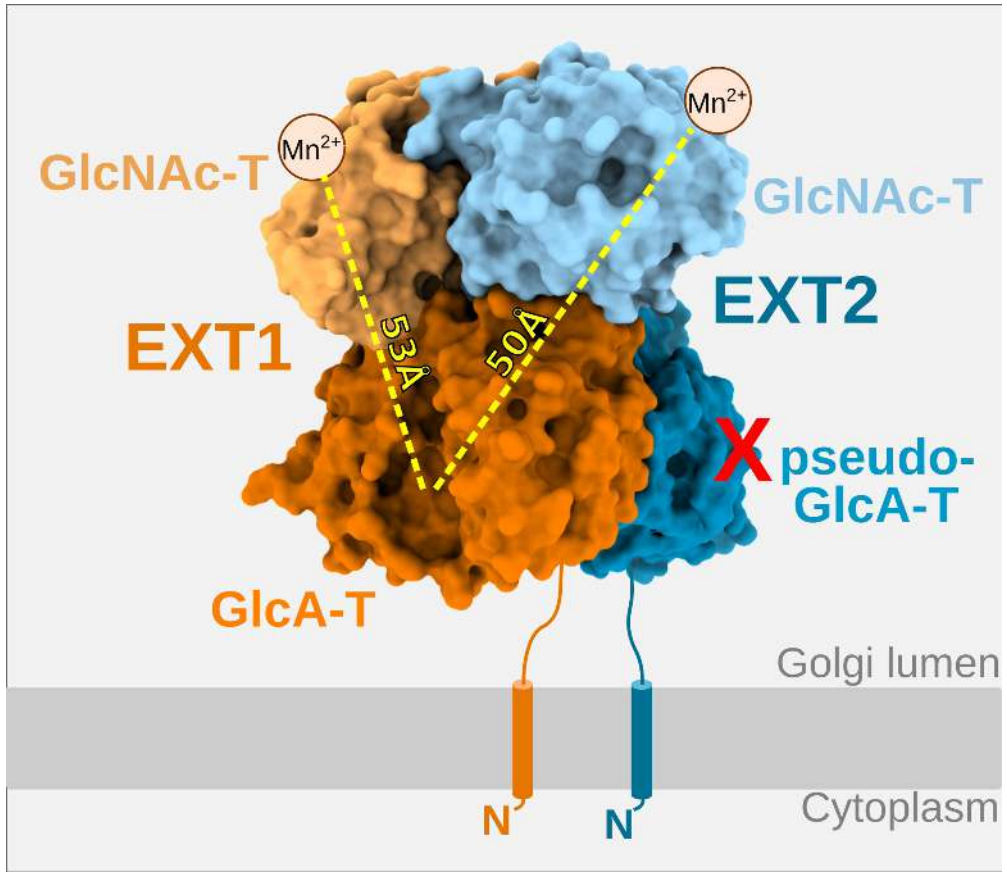


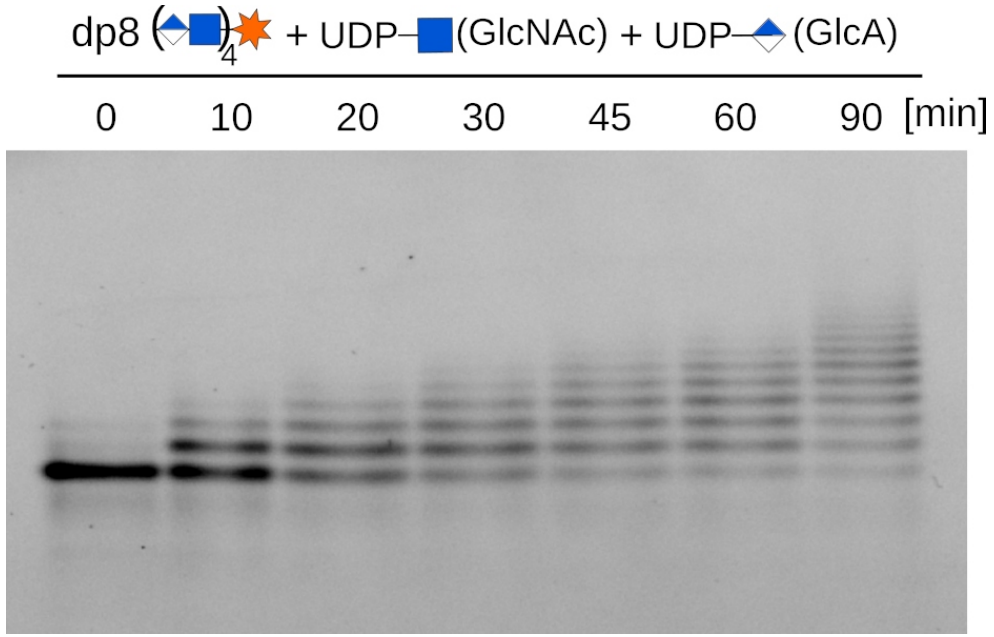
Proposed mechansim for heparan sulfate chain elongation



- GlcA transfer is catalyzed by EXT1
- GlcNAc transfer assured by both proteins
- Active sites are far from each other
- Reaction is disruptive rather than processive

Proposed mechansim for heparan sulfate chain elongation





Take home messages

- Cryo-EM is a powerful technique to study difficult protein targets
- However, better sample will result in a better reconstruction
- EXT1-EXT2 structure reveals mechanism for heparan sulfate chain elongation

Acknowledgment

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Rebekka Wild
Francisco Leisico
Poushalee Dutta
Marie Bourgeais
Margot Weber
Juneina Omeiri (Alumni)
Farah Fouladkar

SAGAG Group (IBS)
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MS&MT Group (ICMMO)
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