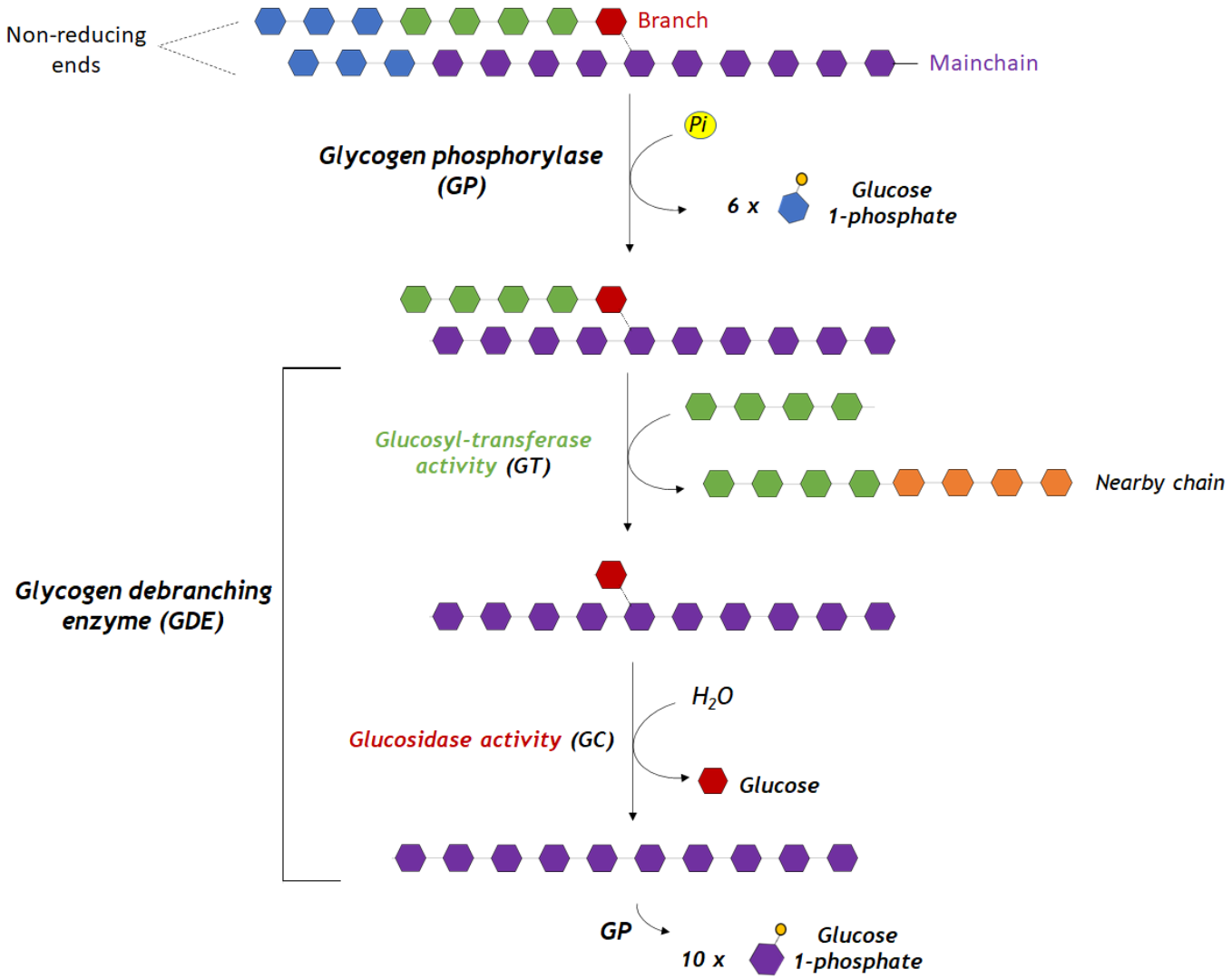
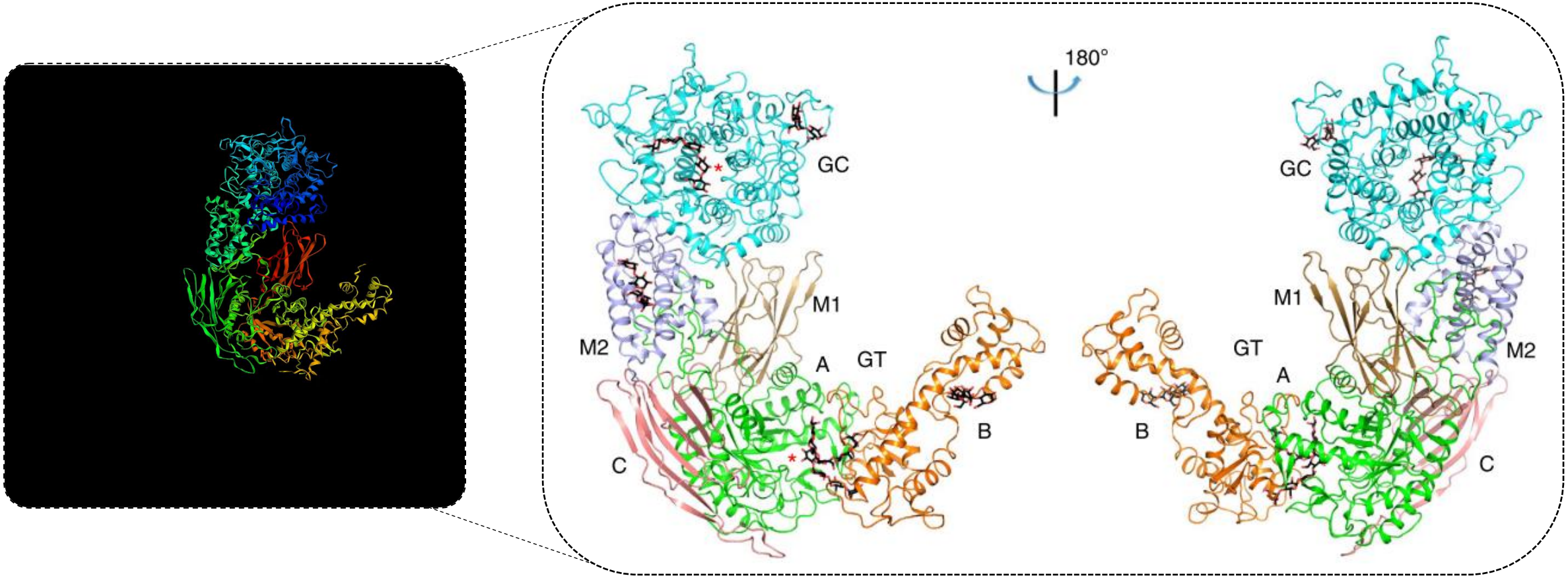
Glycogen degradation



Crystal structure of glycogen debranching enzyme (GDE)

Crystal structure from *Candida glabrata* GDE (CgGDE) expressed in *E. coli*, 38% sequence identity to human GDE



Oligo-1,4-glucantransferase (**GT**) and *amylo-1,6-glucosidase* (**GC**), both of which are responsible for removing glycogen branches during cytoplasmic glycogenolysis.

Reynolds CR, Islam SA, Sternberg MJE (2018). "EzMol: A web server wizard for the rapid visualisation and image production of protein and nucleic acid structures." *J Mol Biol*.

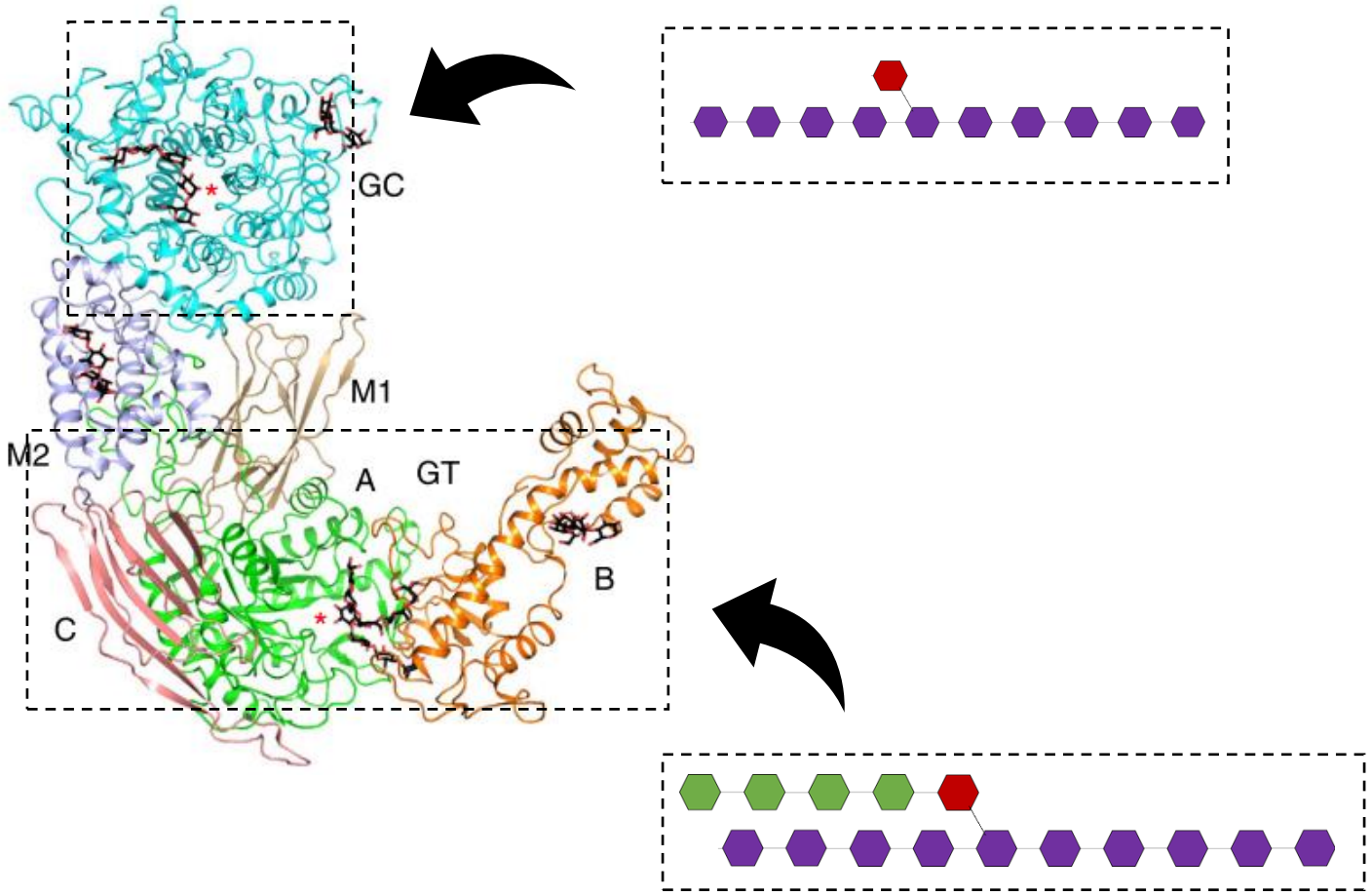
Crystal structure of glycogen debranching enzyme (GDE)

Crystal structure from *Candida glabrata* GDE (CgGDE), 38% sequence identity to human GDE

Substrate specificity

GT – accommodate branched structures, with branches shorter than **5 glucose residues** (6 glucose residues cause steric clashes with the protein)

GC – accommodate the mainchain and the branch with only **one glucose residue**; more than one glucose residues can create issues with protein stability.



Zhai, L., Feng, L., Xia, L., Yin, H. & Xiang, S. Crystal structure of glycogen debranching enzyme and insights into its catalysis and disease-causing mutations. *Nat. Commun.* **7**, 1–11 (2016).

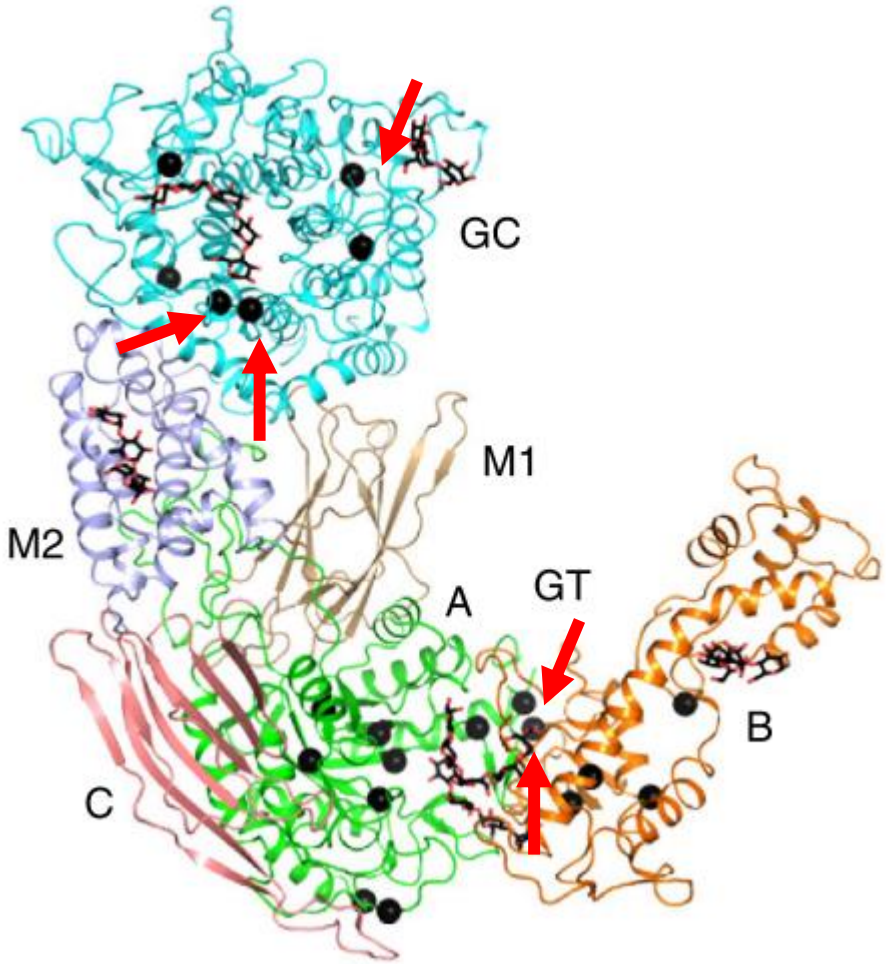
Genetic mutations in GDE and the effects on the amount of glucose released

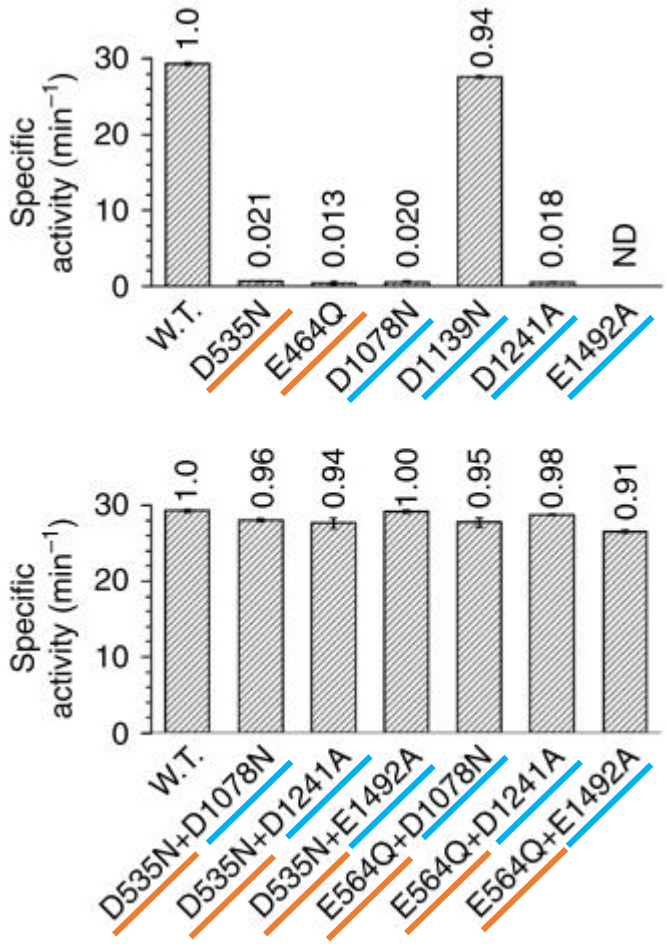
Effects of mutations at GT and GC sites

Mutations at the GT and GC catalytic site dramatic decreases the release of glucose

↓

The products contain branching linkages or they are too short to be substrates of the GP





Mutations at GC site

Mutations at GT site

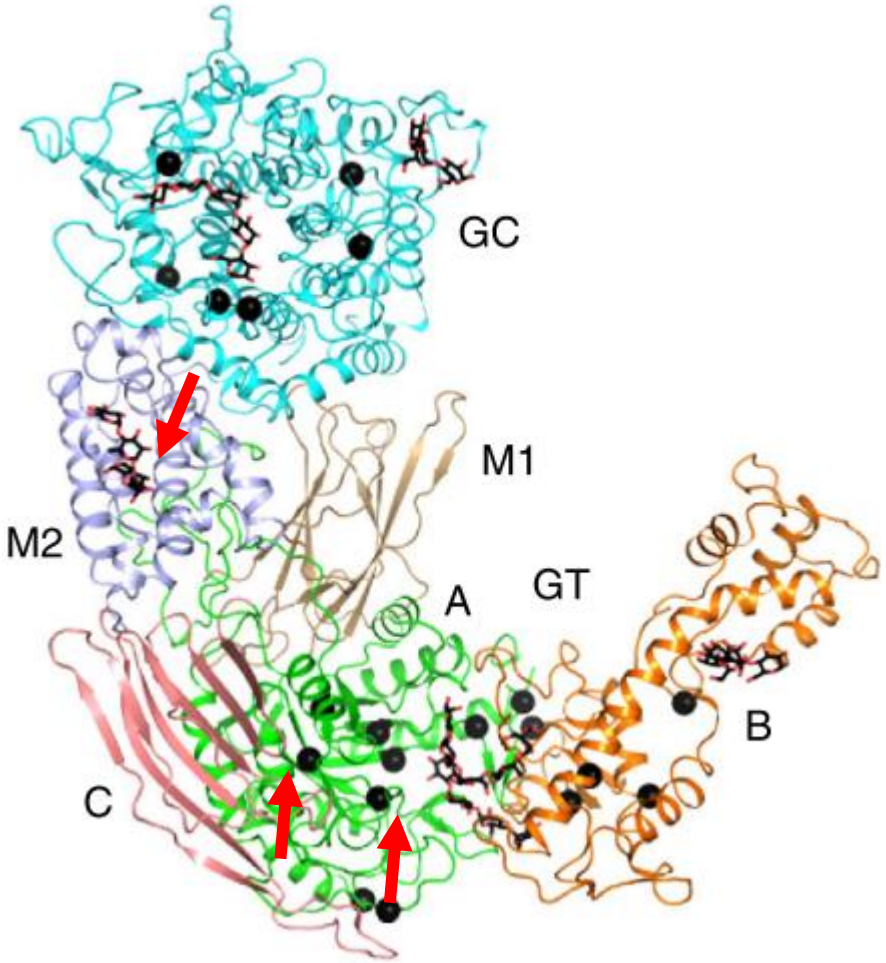
Zhai, L., Feng, L., Xia, L., Yin, H. & Xiang, S. Crystal structure of glycogen debranching enzyme and insights into its catalysis and disease-causing mutations. *Nat. Commun.* **7**, 1–11 (2016).

Genetic mutations in GDE and the effects on the amount of glucose released

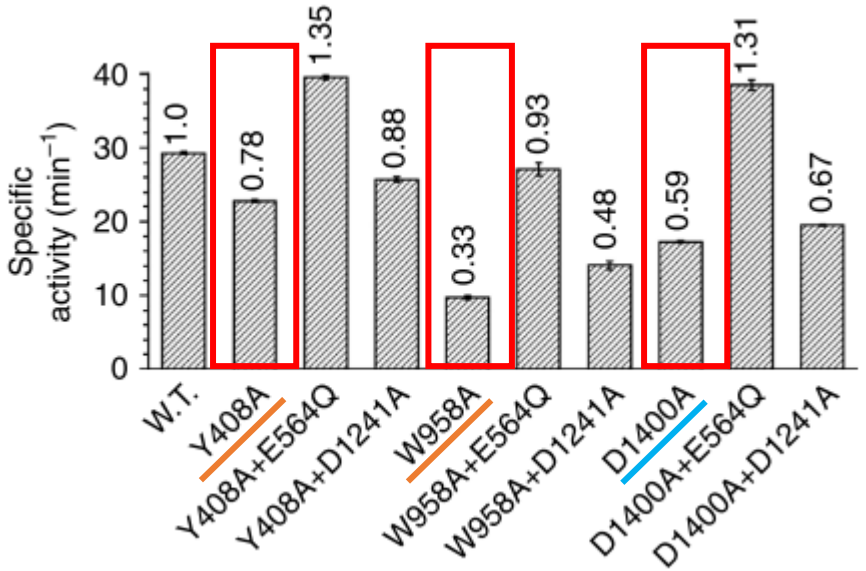
Role of glycogen

Mutations at specific amino acids involved in the association with glycogen cause significant decrease in the glucose availability.

GDE requires anchoring to glycogen for its activity

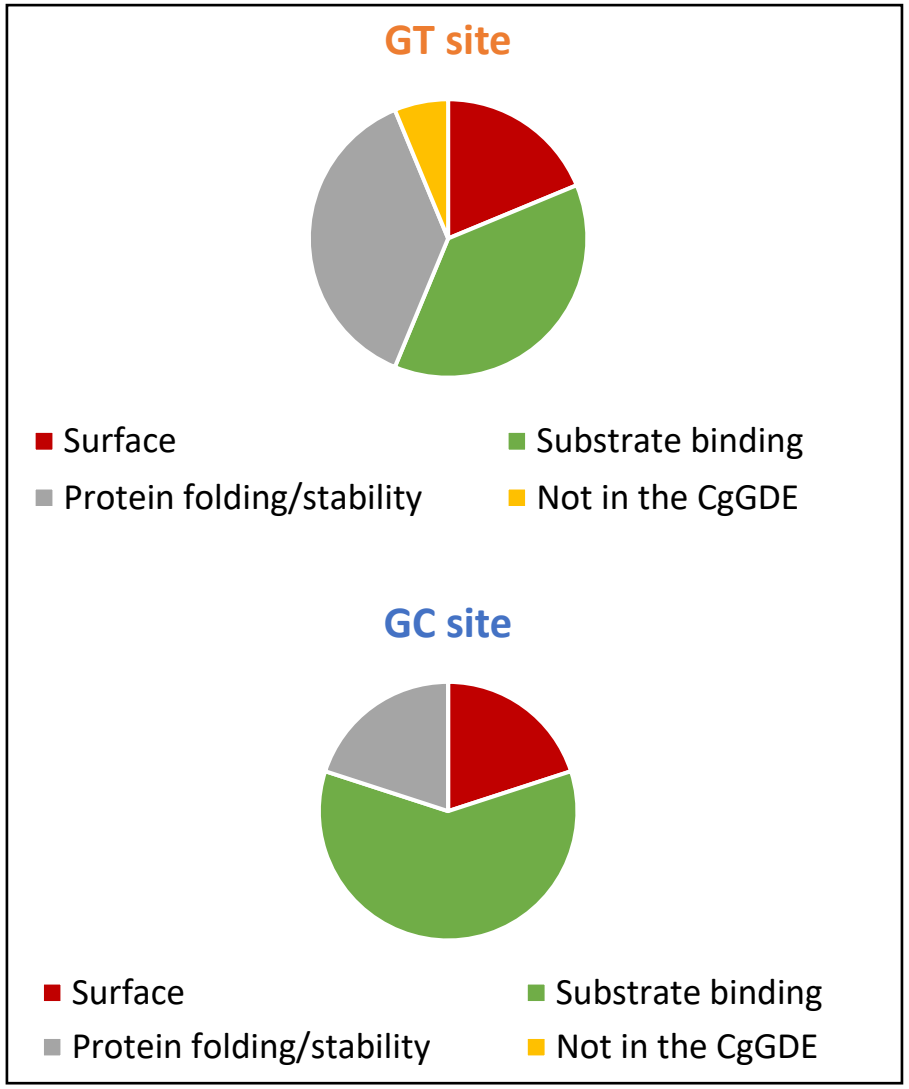


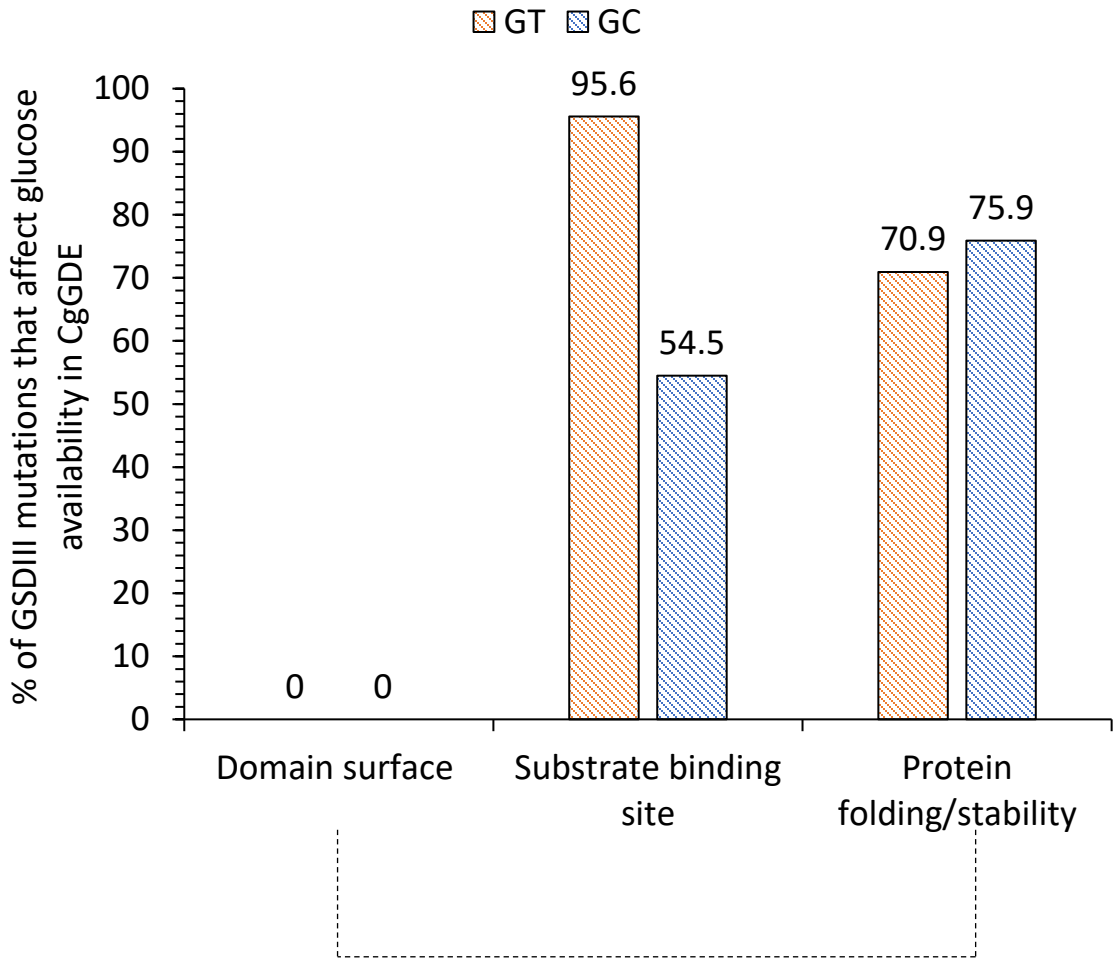
Reduced association with glycogen leads to a decrease in the GDE activity



Zhai, L., Feng, L., Xia, L., Yin, H. & Xiang, S. Crystal structure of glycogen debranching enzyme and insights into its catalysis and disease-causing mutations. *Nat. Commun.* **7**, 1–11 (2016).

Mutations associated with GSDIII expressed in *Cg*GDE*





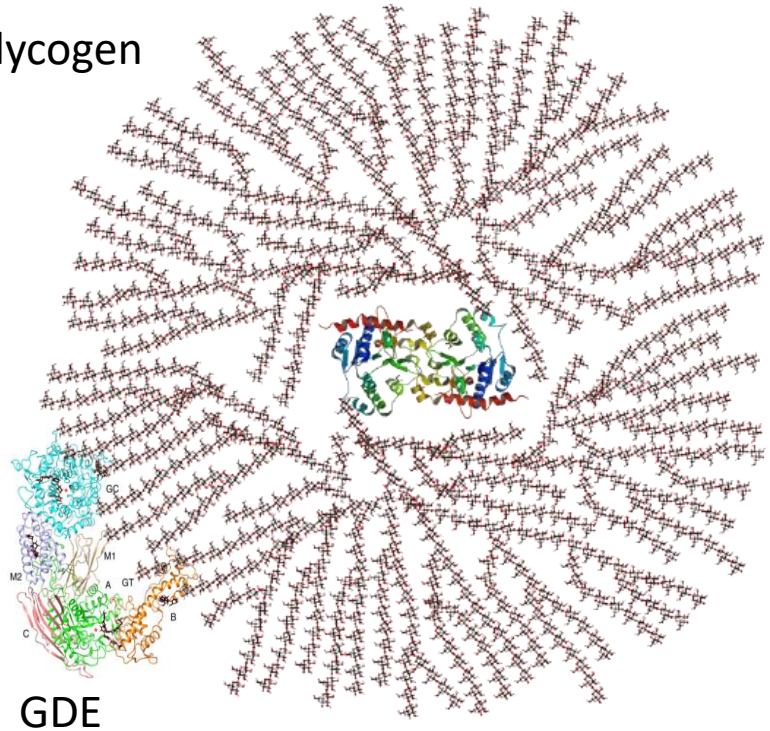
6 mutations associated to GSDIII patients did not affect the CgGDE activity

**on a total of 21 miss-sense mutations associated with GSDIII*

Zhai, L., Feng, L., Xia, L., Yin, H. & Xiang, S. Crystal structure of glycogen debranching enzyme and insights into its catalysis and disease-causing mutations. *Nat. Commun.* **7**, 1–11 (2016).

Sum up

Glycogen



GDE

Brief summary:

- ✓ GSDIII affects GDE activity
  - ✖ Glucose availability
  - ✖ Use of glycogen as source of energy
- ✓ Products from defected GDE are not substrates for the GP = amount of glucose available is reduced.

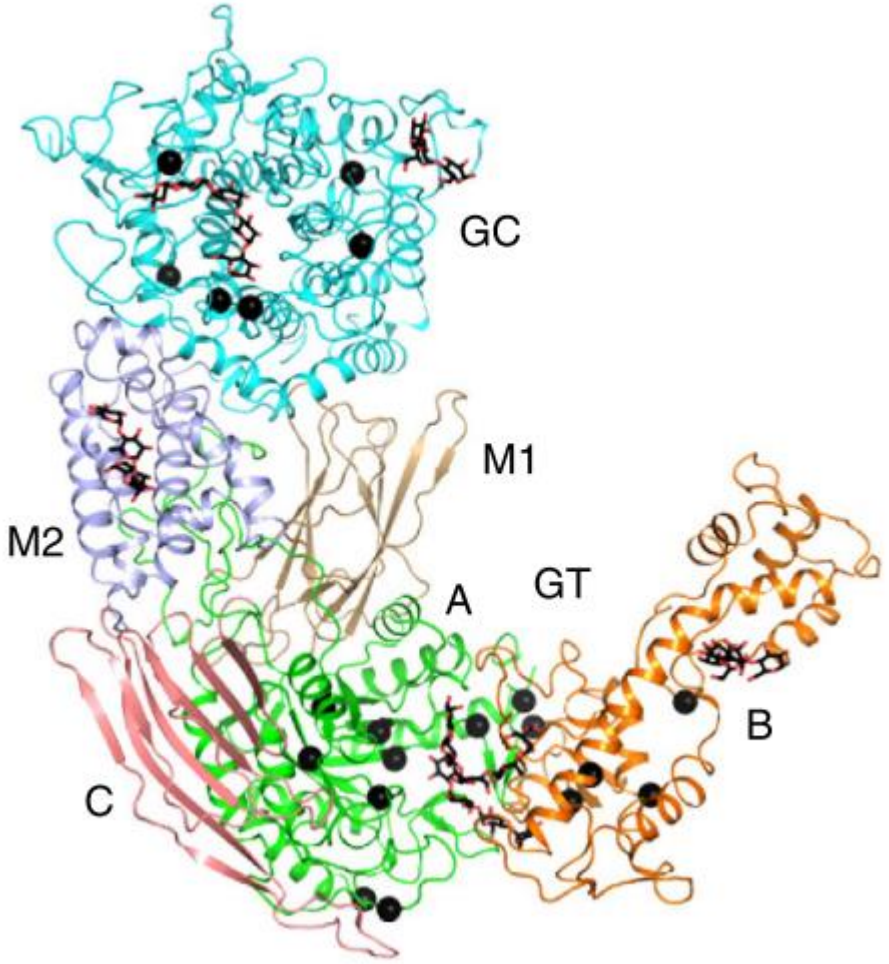
Question:

GDE → release one glucose molecule at a time, the percentage of branches is 6-10% on the overall glycogen molecule

GP → release an extensive amount of glucose compared to GDE

The amount of glucose available should not be a problem. As we know, it is a problem ☹

Sum up



Therefore:

? **In which way the GSDIII is affecting the GDE activity and the main substrate (glycogen)?**

? **How can the activity of GDE be investigated?**

? **How can we identify GSDs effects and discriminate what is "normal" and "abnormal" in glycogen structure?**

To have a complete understanding of GSDIII, we have to:

1. **Investigate glycogen structure**
2. **Analysis of GDE activity**

➢ <u>Determine the changes that occurs in glycogen structure as consequence of the GDE activity.</u>

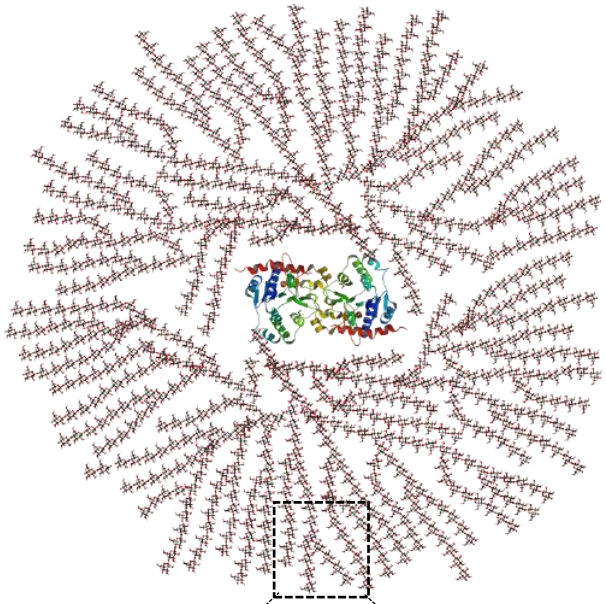
Investigate glycogen structure

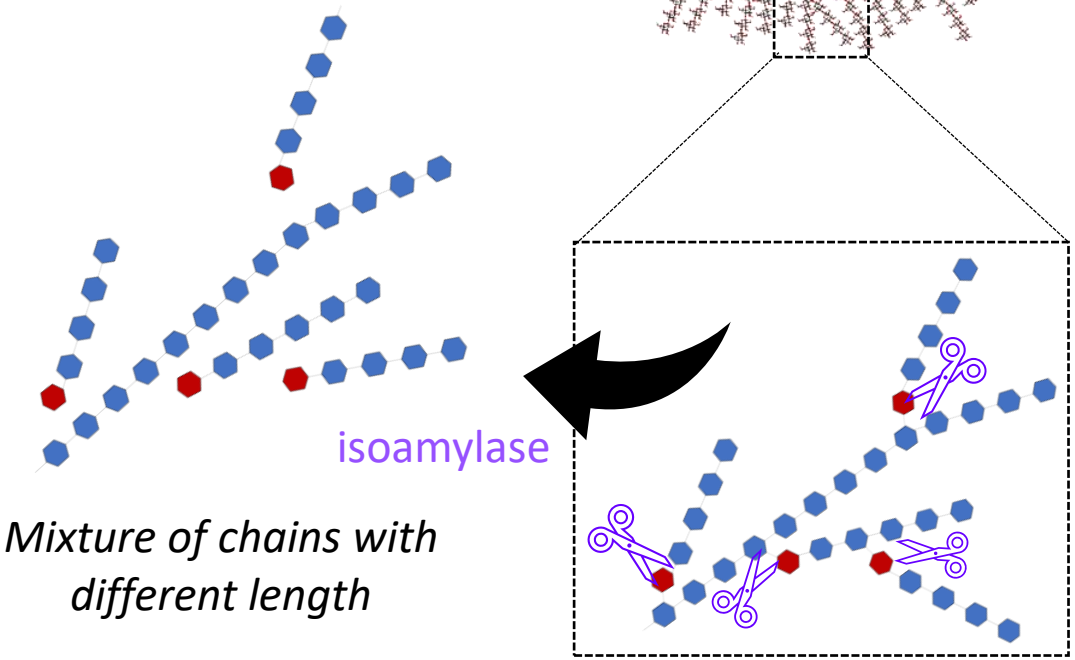
Activity of isoamylase

- ✓ Cleavage of branches
- ✓ Release of linear oligosaccharides

Analysis of the products

- ✓ Chain length that characterise glycogen structure (degree of polymerization)
- ✓ Amount of branches that characterise each glycogen species (degree of branching)
- ✓ Chains promptly available for glycogenolysis and release of glucose



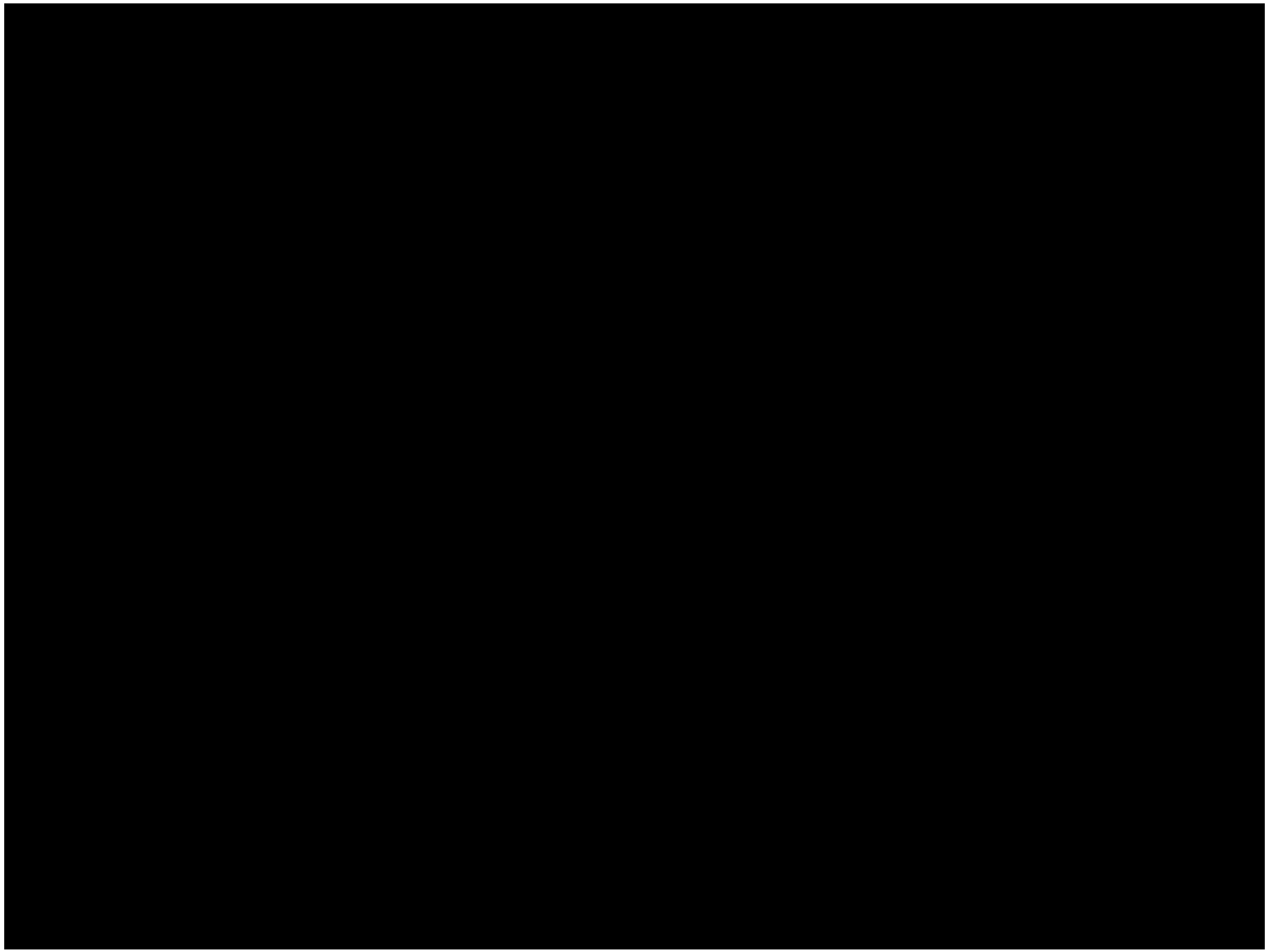


Mixture of chains with different length

Investigate glycogen structure

..Why isoamylase?

Think about the ultimate goal: **understand the effects of GSDIII on GDE activity**



…Thanks for your attention!