



Substrate and mechanistic investigation of the tannases *CbTan1* and 2 – serine hydrolases active on gallotannins

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Background

Tannases (tannin acyl hydrolases, EC 3.1.1.20) are serine α/β -hydrolases which act on gallotannin ester bonds [1]. **Tannins** are complex secondary metabolites that inhibit the growth of microbes and are enriched in bark and wood. Organisms that express tannases can survive tannin-rich microenvironments.

We recently expressed and characterized the biochemical activity of three tannases from the soil/gut bacterium *Clostridium butyricum* (*CbTan1*–3) [2], which was the first example of 3 tannases characterized from the same organism. Each enzyme displayed different substrate preferences, which suggested they have differing biological functions.

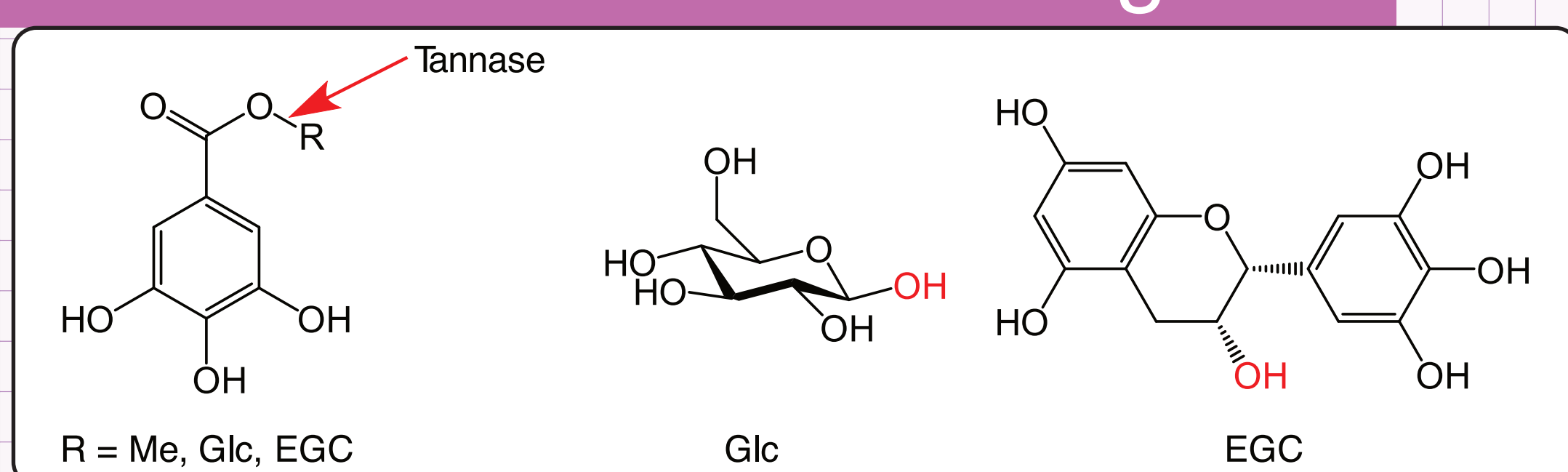
Tannases are phylogenetically categorized into subtypes A and B based on the absence or presence (respectively) of an acidic residue in the canonical Ser-His-Asp catalytic triad. *CbTan1* contains an amidic residue (Gln) while *CbTan2* and 3 contain a catalytic Asp, making these useful enzymes for investigating the mechanistic differences between subtypes of tannases.

CbTan1 contains a long, possibly uncoiled domain which may be a cap domain. *CbTan2* contains a hairpin-style β -sheet cap which is probably involved in substrate binding. The cap of each enzyme is inserted into a different part of the α/β -hydrolase fold. It is currently unclear how the cap of each tannase impacts its preferred substrates.

Research questions

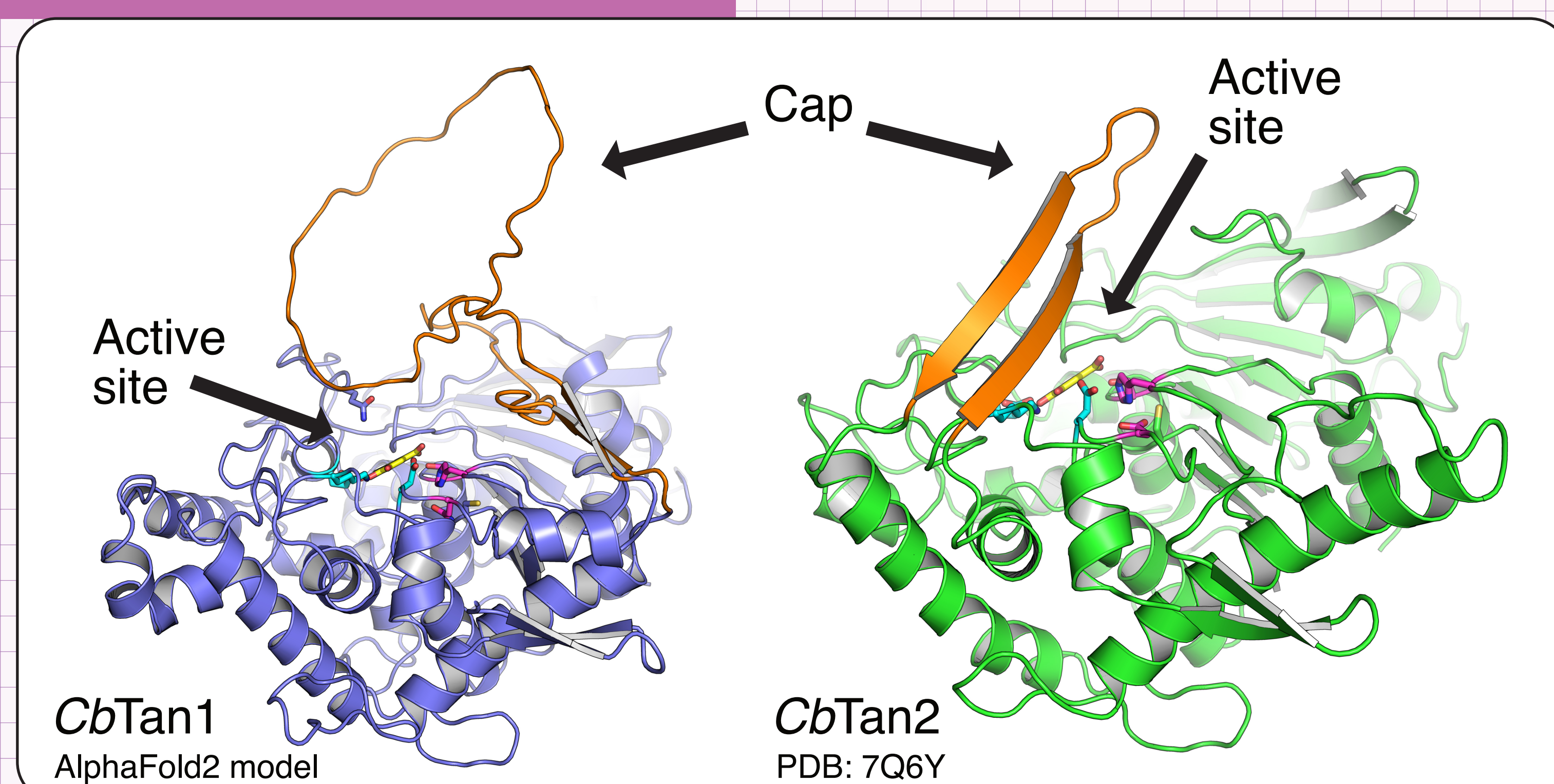
- What role does the cap domain play in tannase **substrate binding**?
- Can the enzyme activity of subtype A tannases (eg, *CbTan1*) be improved by “restoring” the **catalytic acidic residue**?

Tannase substrates investigated

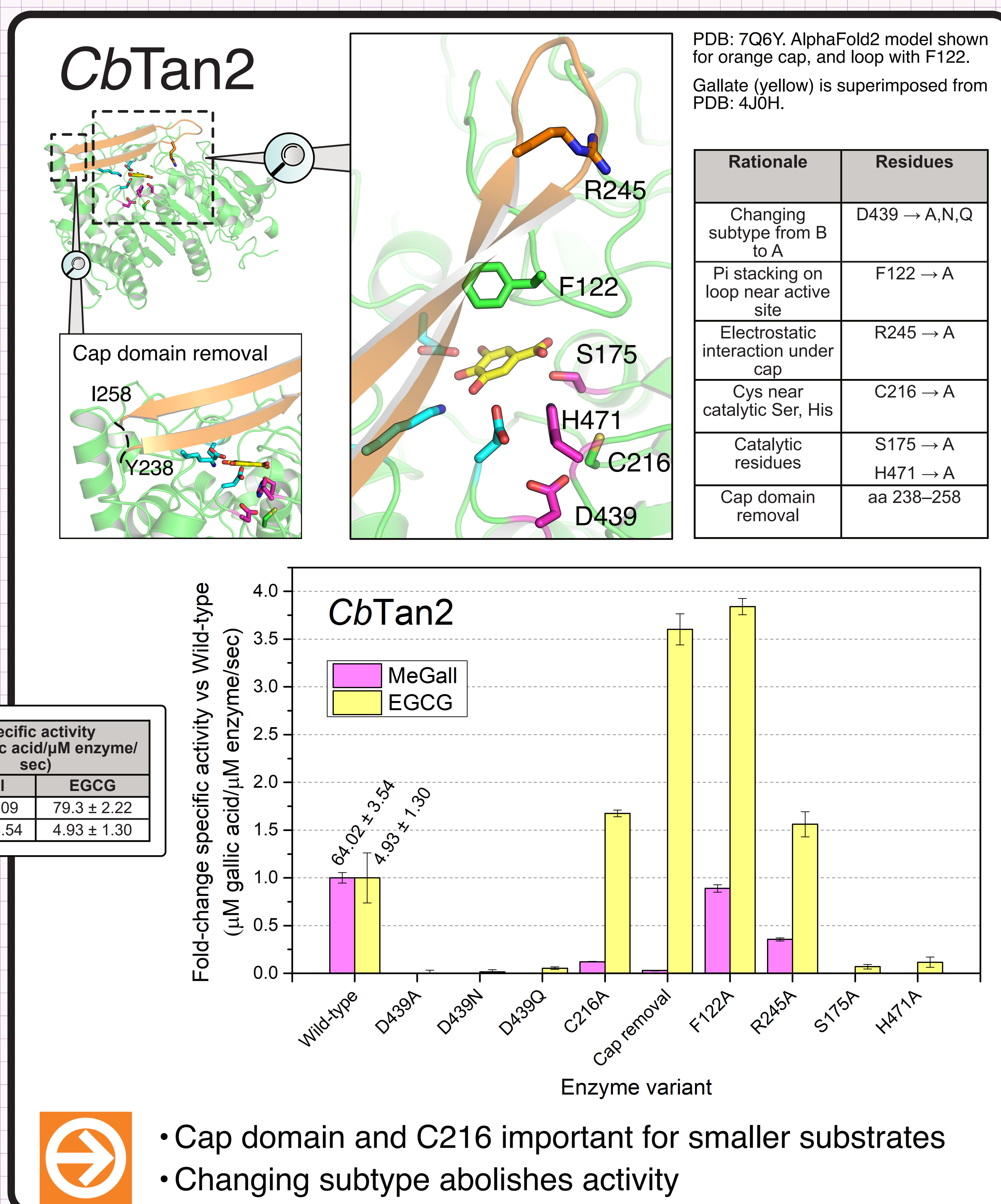
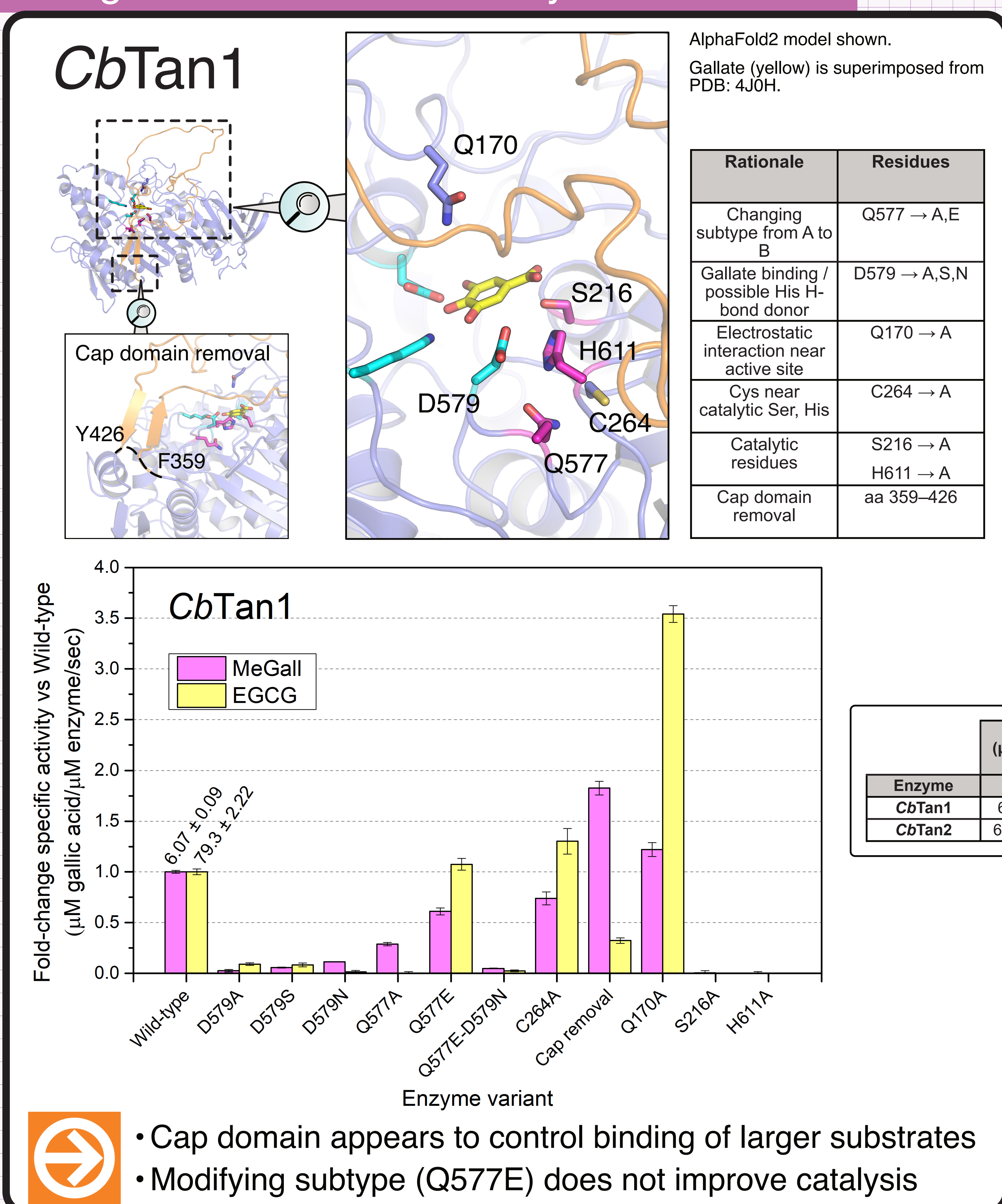


Left: General structure of gallotannins. The red arrow indicates the ester bond broken by tannase enzymes. **Right:** Structure of gluco (Glc) and epigallocatechin (EGC) moieties. The hydroxyl groups shown in red are linked to the gallate moiety.

Tannase overall structure

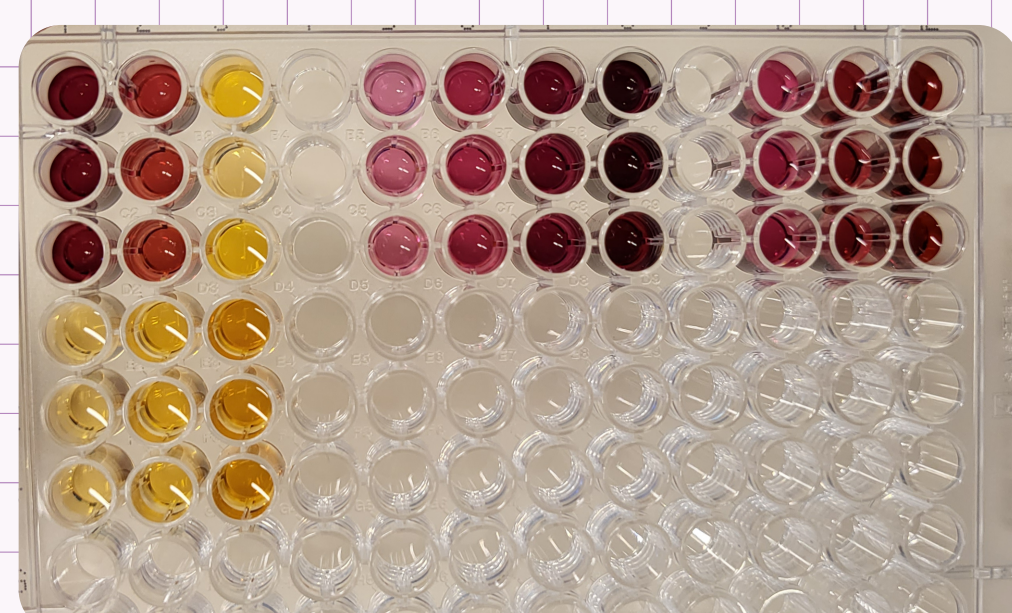


Mutagenesis sites and activity determination



Activity measurement

- Activity determined using 1 mM substrate using the rhodanine assay [3], in triplicates with 5 min reaction time
- Absorbance at 520 nm measured



Key findings

- The role of the catalytic acidic/amide residue is nontrivial in tannases – subtypes require further mechanistic investigation
- *CbTan1* well adapted for EGCG; can be engineered to improve activity
- *CbTan2* well adapted for MeGall; EGCG activity can be improved

Future directions

- Characterization with β -glucogallin substrate
- Crystal structures of substrate-bound tannases

References

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