Loop size and dynamics modulate substrate specificity in chitin deacetylases

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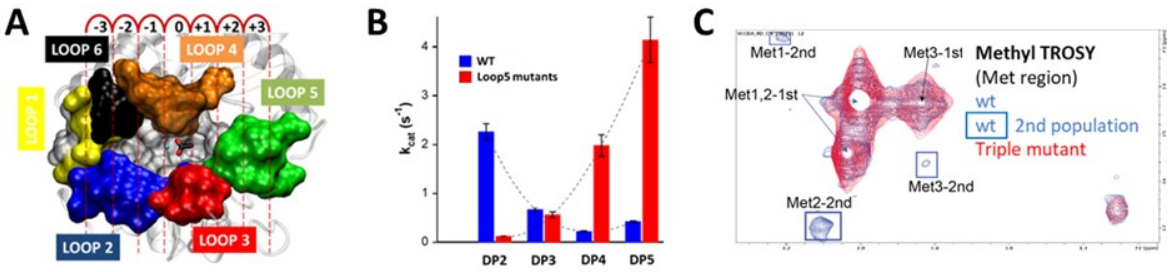
Chitin deacetylases (CDA) are members of family 4 carbohydrate esterases (CE4) which deacetylate chitooligosaccharides (COS) with different deacetylation patterns [1]. We are interested in understanding the structural bases of substrate specificity by CE4 enzymes and the use of engineered variants as biocatalysts [2]. Because of the influence of different acetylation patterns in signaling events (*i.e.* pathogenic fungi-host interactions), the availability of a panel of CDAs with defined specificities will provide sequence-defined partially deacetylated COS with applications in biomedicine and biotechnology.

Most CDAs follow a multiple attach mechanism but few are specific for deacetylating a single position of COS. The 3D structure of *Vibrio cholerae* CDA in complex with COS substrates showed six surface loops that shape the binding site cleft and guide deacetylation to a single position of short COS [3]. We proposed the "subsite capping model" to rationalize the differential accessibility of substrates to the binding cleft mediated by these non-conserved loops within CE4 enzymes [3,2]. After identifying Loop5 in *Vc*CDA as the structural element controlling specificity for short substrates [4], here we report on a rational design to modify specificity towards larger substrates. By biochemical, NMR side-chain (CH_3) relaxation and MD studies, we show that loops dynamics are coupled and their modulation strongly alters specificity. The mutations shift ligand binding from a conformational selection to an induce fit mechanism, with a large impact in specificity and activity.



A) Loops of VcCDA that shape the binding site. B) Specificity change from wt to a triple mutant at Loop5. C) NMR methyl TOSY of wt and triple mutant.

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