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## Structural and functional characterization of tautomerase and aspartase/fumarase superfamily enzymes

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# Stellingen

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## STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF TAUTOMERASE AND ASPARTASE/FUMARASE SUPERFAMILY ENZYMES

van

Harshwardhan Poddar

1. X-ray crystallography still remains the preferred technique to study enzyme mechanisms at the molecular level. (This thesis)
2. It is very gratifying when the proposed mechanism of an enzymatic reaction is validated with high-resolution crystal structures. (Chapters 2 and 5)
3. For small ligands such as acetaldehyde it is imperative to obtain ultra high-resolution enzyme structures, to be certain of their presence in the active site. (Chapter 2)
4. The rearrangement of the active site of 4-OT, caused by the M45Y/F50A mutations was difficult to predict. The crystal structure of the double mutant highlights the importance of structural information to properly assess the impact of mutations. (Chapter 3)
5. Although unconventional, the mutability landscape method is a powerful way to carry out engineering of an enzyme and presents an effective alternative to the more commonly used structure guided approach. (Chapter 3)
6. The presence of a bound metal in an enzyme does not always mean that it is needed for the enzyme's structural integrity or activity. (Chapter 4)
7. You know you have a winner when you can express, purify, crystallize and solve the structure of an enzyme, all in the same week. (EDDS lyase, Chapter 5)
8. Sometimes in protein crystallography you have to just follow your gut feeling and go ahead with an experiment, no matter how ridiculous it sounds. This helped me to get different ligand-bound structures of EDDS lyase. (Chapter 5)
9. Challenging and difficult projects are the ones that teach you the most in your research career. The same is true for life. Difficult times improve you as a person and although you may still end up making mistakes, it is ultimately how you rise to the challenge that matters.