# Structure and mechanism of action of two bacterial enzymes

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# Stellingen

behorende bij het proefschrift

**Structure and mechanism of action of two bacterial enzymes:**

**MltE from *Escherichia coli* and AspB from *Bacillus* sp YM55-1**

door

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1. The determination of binding affinity and stoichiometry can give a good estimate of the concentration and molar ratio to be used in protein-ligand co-crystallization experiments, but in the end, one must accept the verdict of the God of crystallization.

2. Crystallization conditions explained in research articles mostly mention only protein concentration, crystallization solution composition, temperature and the technique used for crystallization, but omit the X-factor, which may range from geographical location, presence of contaminants, to simply luck.

3. The presence of a 10x-His-tag in MltE allows an efficient single-step purification to give highly pure protein, and yet is still suitable for protein crystallization experiments. (Chapter 2)

4. *E. coli* has six exo-lytic transglycosylases to cleave the peptidoglycan chains starting from the reducing end, but only one endo-acting lytic transglycosylase, MltE. Nevertheless, MltE is not able to cleave glycan chains with peptide stems. (Chapter 2)

5. Although the sequence homology among the members of the aspartase-fumarase superfamily can be as low as 15%, they all are present as tetramers with a similar substrate-binding pocket and use a common catalytic mechanism. (Chapter 3)

6. *In-situ* proteolysis is a very powerful approach to improve the crystallization behavior of proteins with flexible domains (Wernimont and Edward (2009); Chapter 3).
   * Wernimont, A., and Edwards A. 2009. *In situ* proteolysis to generate crystals for structure determination: An update. PLoSONE 4: e5094.

7. *In-situ* proteolysis is not only a powerful approach to improve the crystallization behavior of a multi-domain protein, but it can also give valuable and unexpected functional insights on a proteolytically removed flexible domain. (Chapter 3)

8. Thin crystals, low temperature and fast soaking times are the key for obtaining native enzyme – substrate complex structures.