Enzymatic deglycation of proteins: Computational characterization and *in silico* engineering of Amadoriase I

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Rationale

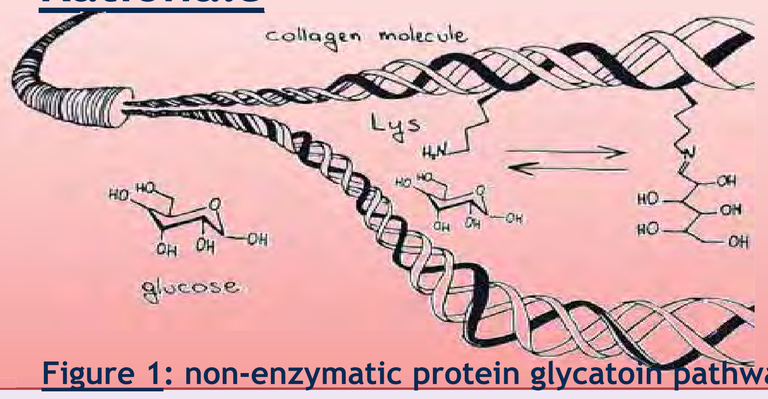

Figure 1: non-enzymatic protein glycatoin pathway

- In protein pathological glycation (AGEs and diabetes) amino-acids side chains are frequent glycation sites
- Amadoriases are enzymes that catalyze de-glycosylation of single fructosyl amino acids (FAOX enzyme)
- Amadoriase I is specifically active on ε-fructosamine (as AA side-chains)
- Crystal structures of apo and bound forms obtained by our group [pdb code 4WCT, 4XWZ]

In silico wild type characterization of Amadoriase I

✓ **What is the tunnel geometry to reach catalytic site?**

Method: Random Accellerated Molecular Dynamics and CAVER software
Results:
- Single possible tunnel connecting bury active pocket and bulk solvent:
  bottleneck ≈2.8 Å- depth ≈18 Å
- Exit defined by two loops and two α-helices (in red)
- internal available volume rather large, with L-shape

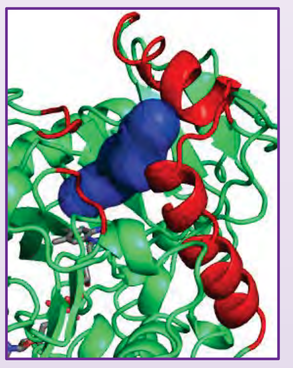

Figure 4: single ligand entry tunnel

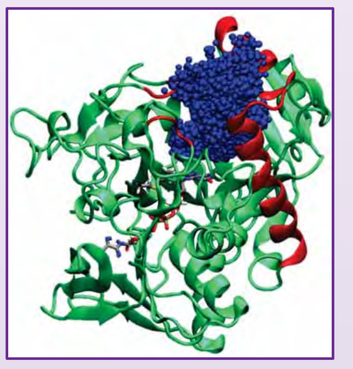

Figure 5: internal available volume

✓ **What is ligand conformation inside the active pocket?**

Method: Classic MD simulations: ACEMD software, AMBER- GAFF forcefield, 1μs
Results:
- Cluster of FLY conformations in the active pocket shows different conformations of lysine-tail inside the active site
- Main preserved interactions between ligand and residues Glu285, Arg418, Gly371, Glu59, Asn371
- strong interactions of sugar ring with enzyme and high flexibility of the FLY tail

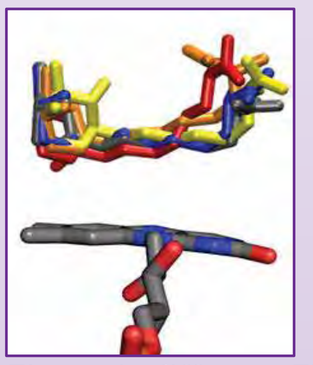

Figure 6: different FLY conformation (top) on FAD rings plane (bottom) in binding pocket

✓ **What is fly entry strategy and binding energy?**

Method: METADYNAMICS (free energy profile) and alchemical free binding energy (FEP) dynamics using "double decoupling" method
Results:
- Two FLY entry strategies:

I. head-sugar ring first: angle of 110° with respect to FAD
no rearrangement needed, favoured pathway

II. tail-lysine first: angle of 50° with respect to FAD need reorientation within the larger inner volume before reaching active pocket

- Absolute binding free energy = -8.4 ± 2.0 kcal/mol

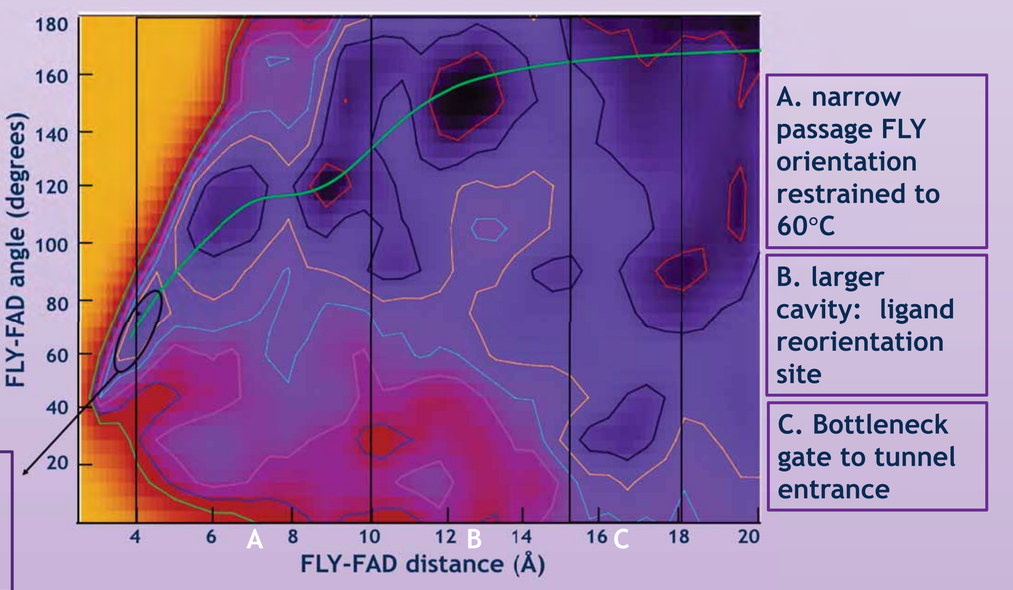

Figure 7: Free energy surface as a function of 2 collective variables: FLY-FAD distance (x-axis) and angle between NεFLY, FAD sugar rings, N-FAD (y-axis)

In silico engineering of Amadoriase I

✓ **Change bottleneck structures to enlarge access tunnel**

Method: TCL script
Results:
- Comparing wild structure with pdb database to find similar folding structure with more open gate structures
- Using resulting structures as template to build new mutants

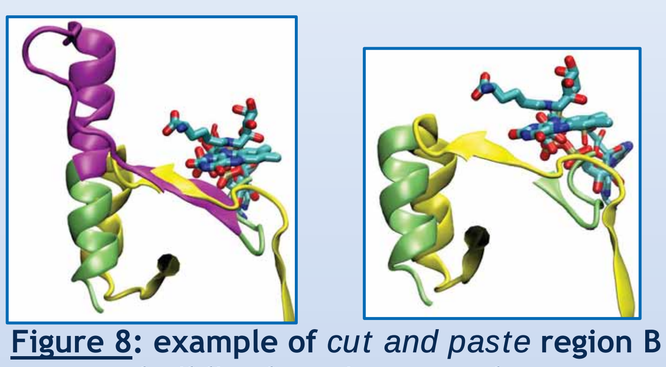

Figure 8: example of *cut and paste* region B mutants building by using natural enzyme template

✓ **Design of new mutants**

Method: XML script and Rosetta design
Results:
- Optimize sequence of mutants in order to enhace solubility and folding
- Define design regions (new loop regions, 6.0 A shell around loop in wild conformation, residues now exposed to solvent) and frozen regions (catalytic and Flavin binding residues highly conserved residues)
- Using a combinations of Position-Specific Scoring matrix and Rosetta design methods

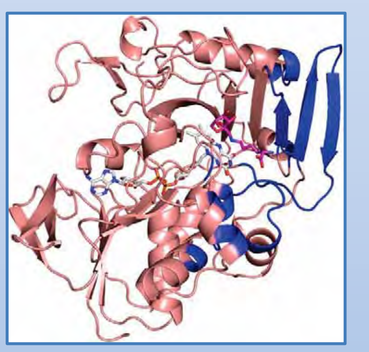

Figure 9: example of new design mutant with a wider open pocket (designable residues in blue)

✓ **Computational screening and experimental test of best mutants**

Method: energy and docking protocols, stopped flow technique for enzymatic activity assays
- select the best mutants through an in silico screening and test them experimentally (on-going activity)