A newly crystallized structure of human formylglycine-generating enzyme

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Human formylglycine-generating enzyme (FGE) is key to posttranslational modification of the active site of all known human sulfatases by oxidizing a cystein to a formylglycine residue. Lack of sulfatase modification due to mutations in the gene encoding FGE lead to the rare disease multiple sulfatase deficiency.[1] Previous structures of human FGE miss a surface loop which was cleaved off by treatment with elastase prior to crystallization.[2,3] With regard to a future crystallographic fragment screening campaign, crystallization of human FGE was optimized and elastase treatment is no longer needed.

Expression & Purification[4]

antibiotic free Hi5 cell culture
His-trap
IEX

Crystallization

MRC 2-well 96 well sitting drop
12-14 mg/ml
0.1 M Tris pH 9.0
0.2 M $CaCl_2$
0-10 % DMSO
26-33 % PEG 4000

Cryoprotection

10 % glycerol

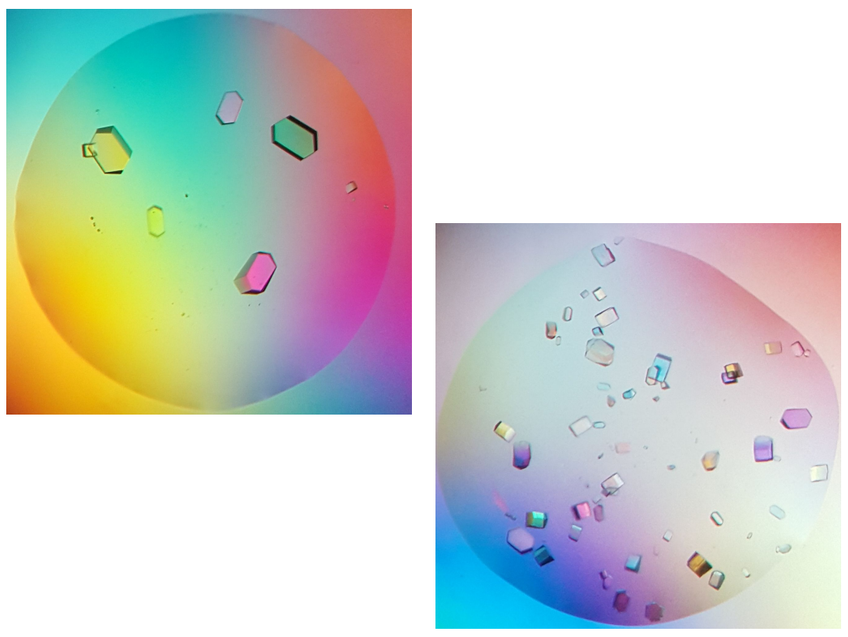

Fig. 1: Crystals of human FGE grown under optimized conditions in a 2:1 protein to reservoir solution ratio.

Tab. 1: Crystallographic data statistics for the solved FGE structure.

	Overall (outer)
Resolution / Å	53.47 – 1.50 (1.56 – 1.50)
Space group	P 2_1 2_1 2
Unit cell a, b, c / Å α, β, γ / °	61.39, 108.81, 43.43 90, 90, 90
Unique reflections	46369 (3763)
Completeness / %	96.41 (64.08)
<I/σ(I)>	19.34
R_{work} / %	16.54
R_{free} / %	20.53

Fig. 2: Final model of the human FGE structure with active site cysteines (yellow), structural calcium ions (pink) and the new surface loop (red).

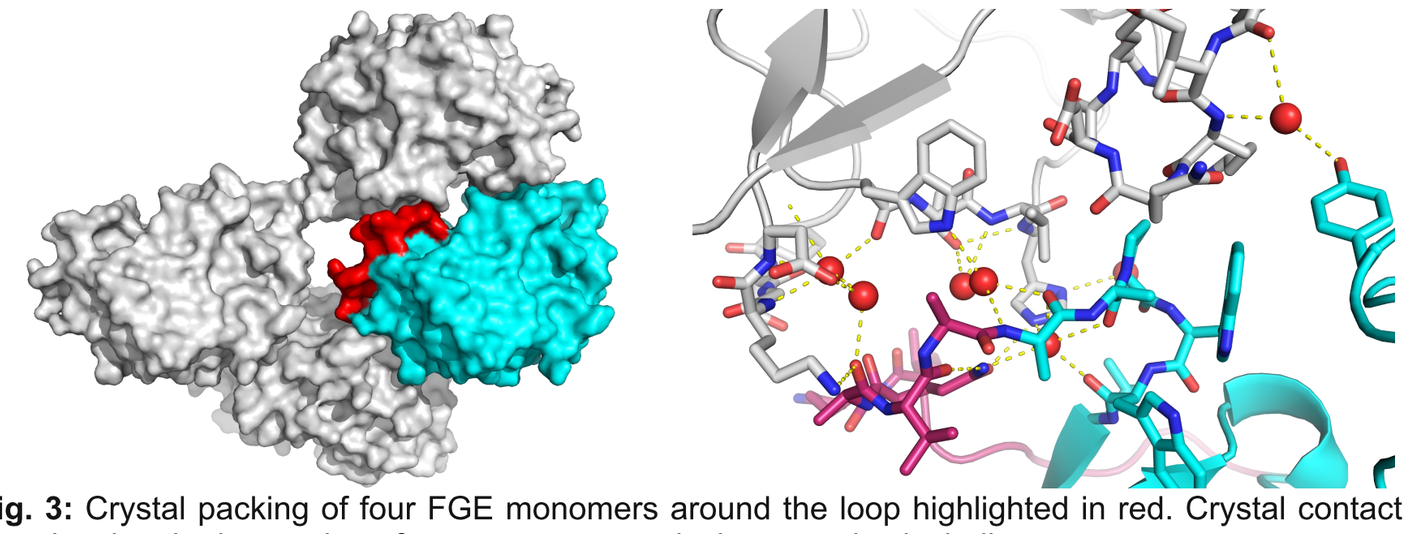

Fig. 3: Crystal packing of four FGE monomers around the loop highlighted in red. Crystal contact site showing the interaction of two symmetry equivalent proteins including waters.

Compared to previously crystallized human FGE crystals, packing and unit cell dimensions are isomorphous. The new surface loop takes part in the formation of a crystal contact with three amino acids.

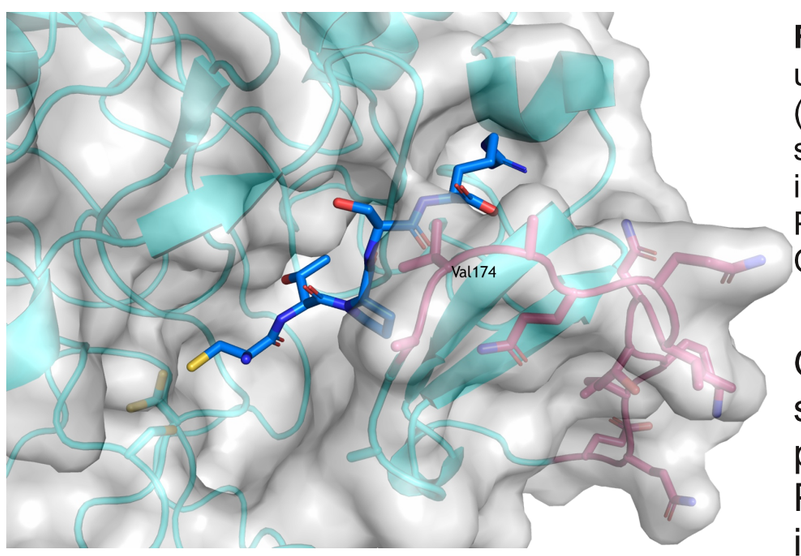

Fig. 4: Superposition of the untreated FGE with loop (cyan & red) and a CTPSR substrate peptide (blue) inside the active site groove. Peptide position from FGE-C336S[3], PDB: 2AIJ.

Overlaying the new structure with the peptide-bound structure PDB: 2AIJ, reveals an interference of Val174 with the substrate binding site. The loop seems to be flexible since it partially covers the peptide binding site when no substrate is found.

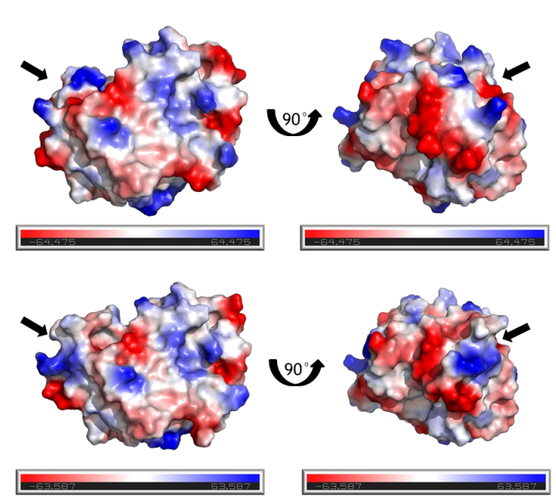

Fig. 5: Comparison of the vacuum electrostatic surface potential calculated with *Pymol*.
a) Elastase treated FGE[2], PDB: 1Z70.
b) Untreated FGE.

In the structure without the loop, a big part of positive potential is missing. In respect to fragment-based drug design, a missing loop creates an artificial surface at this part of the protein, which does not properly represent the protein surface in vivo.

Human FGE was crystallized without the need for elastase-treatment for the first time. The now present surface loop could be modelled into the electron density and additionally contributes to a crystal contact, which was also observed in previous structures. It appears to be flexible but does not hinder crystallization. For a crystallographic fragment screen with the aim to develop pharmacological chaperones to stabilize instable FGE variants causing MSD, the presented structure will serve as a principal model.

[1] Dierks T. et al., *Cell* (2005), 541-552.
[2] Roeser D. et al., *Acta Cryst D* (2005), 1057-1066.
[3] Roeser D. et al., *Proc Natl Acad Sci USA* (2006), 81-86.
[4] Peng J. et al., *FEBS J.* (2015), 3262-3274.

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