Supporting Information

Sweet Binders: Carbonic Anhydrase IX in Complex with Sucralose.

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Experimental Methods

CA IX-mimic was prepared as previously described by mutating seven active site residues of CA II in order to mimic the active site of CA IX.[1,2] The CA IX-mimic is more easily expressed and readily crystallizable than wild type CA IX and has been shown to maintain properties and interactions observed in wild type CA IX.[3] CA IX-mimic was expressed in BL21(DE3) competent cells and purified as previously described utilizing affinity chromatography with a *p*-(aminomethyl)benzenesulfonamide column.[2] Protein purity was verified via SDS-PAGE and the protein diluted to a concentration of 10 mg/mL. Crystals were then grown via the hanging drop vaporization method with a precipitant solution of 1.6 M Na-Citrate, 50 mM Tris, pH 7.8. Crystal growth was observed after 5 days. CA IX-mimic crystals were soaked in ~10 mM of sucralose 24 hrs prior to freezing. Crystals were transferred into a cryoprotectant solution of 20% glycerol and frozen in liquid nitrogen for shipment.

X-ray diffraction data was collected at the Cornell High Energy Synchrotron Source (CHESS) F1 beamline with a wavelength of 0.977 Å. Data sets were collected using a Pilatus 6M detector with a crystal-to-detector distance of 270 mm, 1° oscillation angle, and exposure time of 4 sec per image. A total of 180 images were collected. The data were indexed, integrated, and scaled using *HKL2000.* Data was scaled to the monoclinic $P2_1$ space group (crystallography statistics in Supplementary Table 1). Phases were determined via molecular replacement using PDB: 3KS3 as a search model. Refinements and ligand restraint files were generated in the program *Phenix*[4]. Modification to Models and ligand PDB files were performed in *Coot*[5], along with the measurement of bond lengths for observable interactions. All figures were generated using *PyMol*[6].

Supplementary Table 1. X-ray crystallography statistics

Sample	CA IX-mimic_Sucralose
PDB Accession Code	6CJV
Space Group **Cell Dimensions (Å;°)**	$P2_1$ a = 41.83, b = 41.46, c = 71.73; β = 103.9
Resolution (Å)	31.90 – 1.53 (1.56 – 1.53)
Total Reflections	48136
I/Iσ	23.7 (2.1)
Redundancy	3.1 (2.9)
Completeness (%)	99.8 (99.7)
Rcryst (%)	16.4 (22.9)
Rfree (%)	18.5 (24.3)
Rpim (%)	2.8 (37.1)
# of Protein Atoms	2077
# of Water Molecules	187
# of Ligand Atoms	23
Ramachandran Stats (%): Favored, Allowed, Generously Allowed	96.5, 3.5, 0.0
Avg. B factors (Å^2): Main-Chain, Side-Chain, Solvent, Ligand	16.6, 21.5, 27.6, 44.5
rmsd for Bond Lengths, Angles (Å,°)	0.008, 1.593

Data in parentheses correspond to the highest resolution shell

$R_{cryst} = (\sum|F_o - F_c|/\sum|F_o|) \times 100$

R_{free} is calculated similarly to R_{cryst} using data omitted from refinement (5%)

$R_{pim} = \sum_{hkl}\{1/[N(hkl) - 1]\}^{1/2} \times \sum_i|I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$

References

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