Human glucosylceramide synthase at work, as provided by "in silico" Molecular Docking, Molecular Dynamics and Metadynamics

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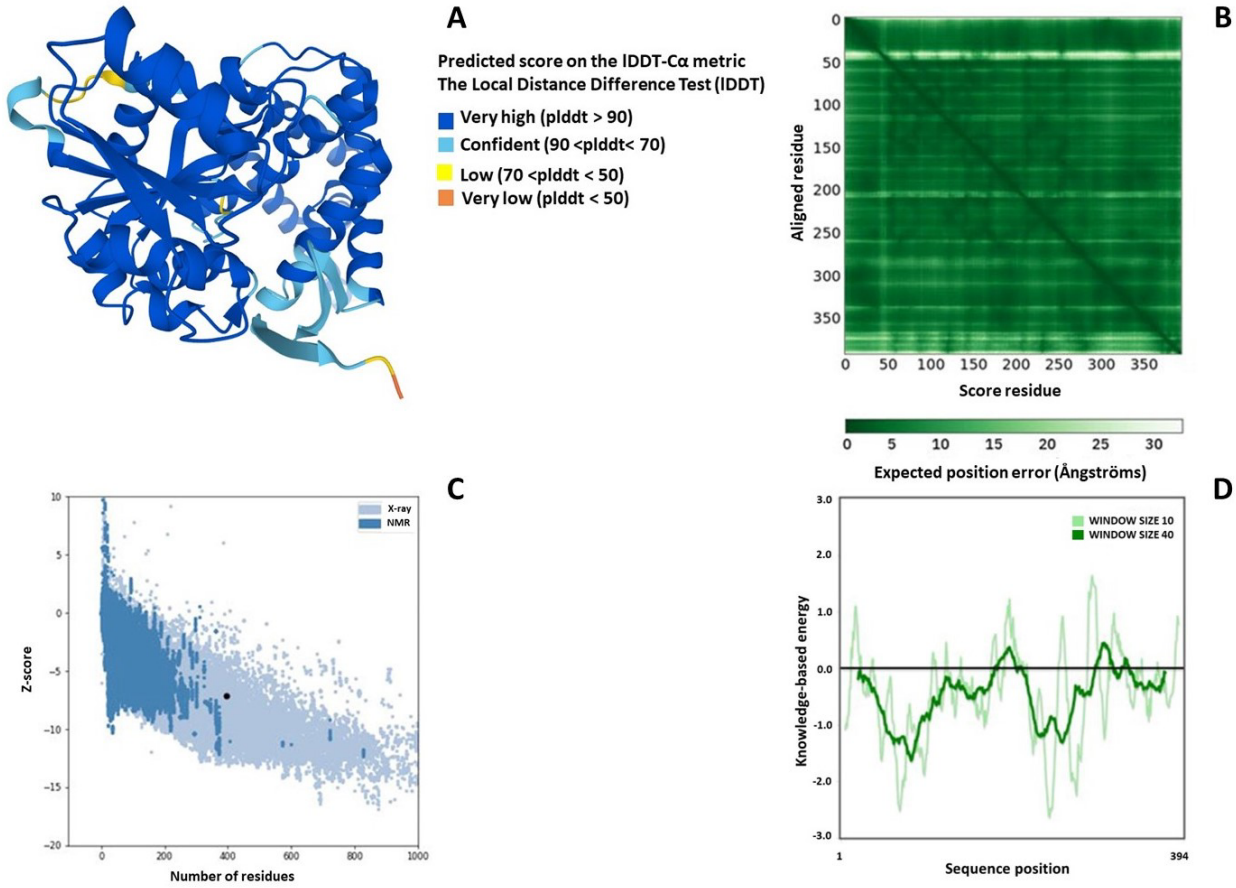
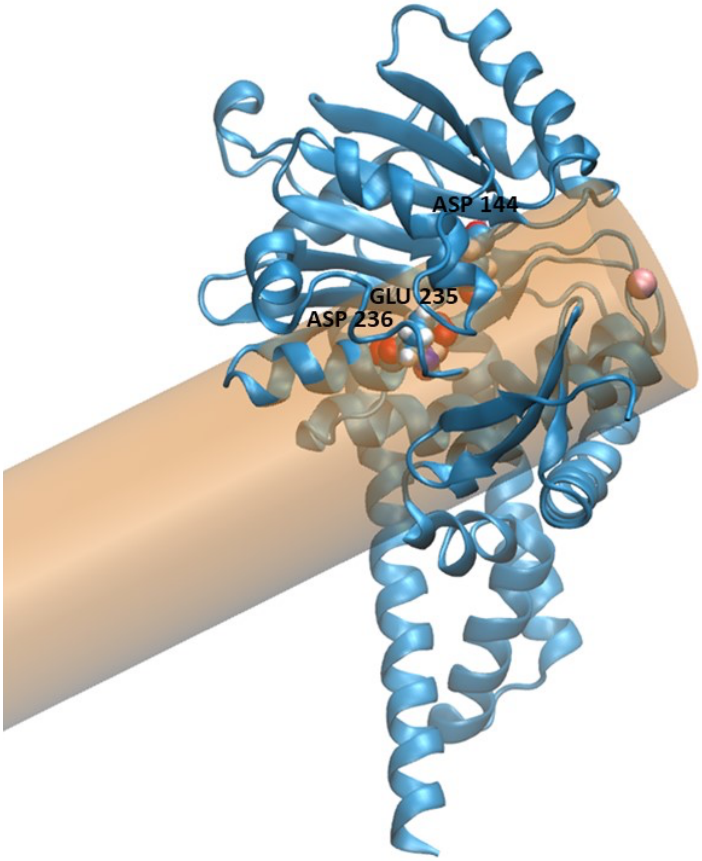
Figure S1



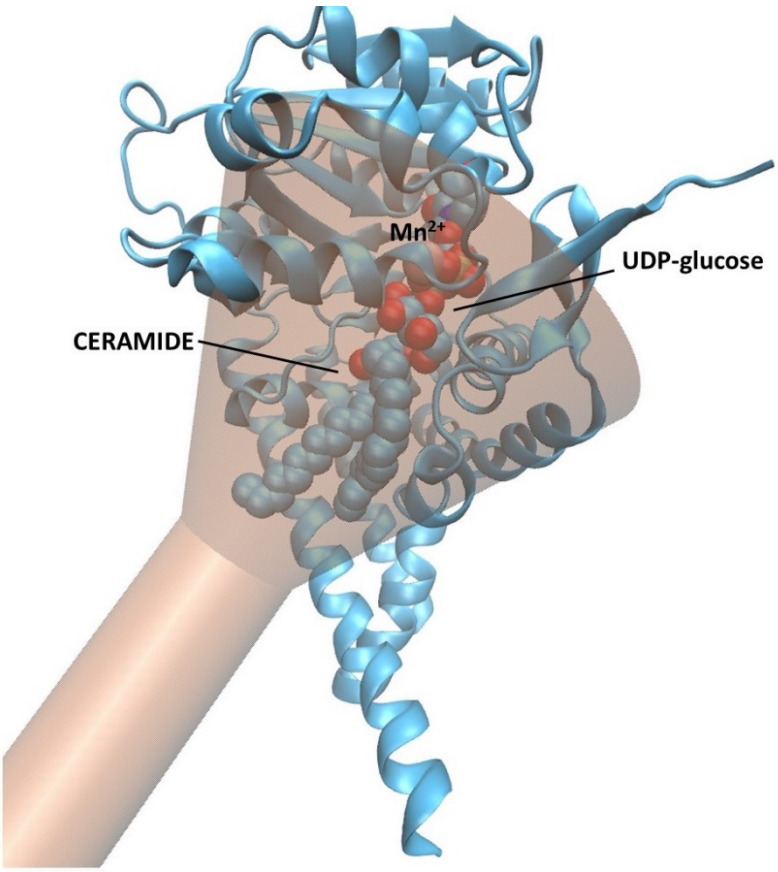
(A) AlphaFold [1] model confidence. (B) Predicted Aligned Error, low value indicates that AlphaFold is confident about the relative domain positions. (C) (z-score) Model quality according to ProSa-web [2], z-score = -7.17. (D) Local model quality according to ProSa-web. Energy values are plotted as a function of amino acid sequence. The plot is smoothed by calculating the average energy over each 40-residue fragment s (i,i + 39), which is then assigned to the 'central' residue of the fragment at position i + 19 (thick line). A second line with a smaller window size of 10 residues is shown in the background of the plot (thin line).

Figure S2



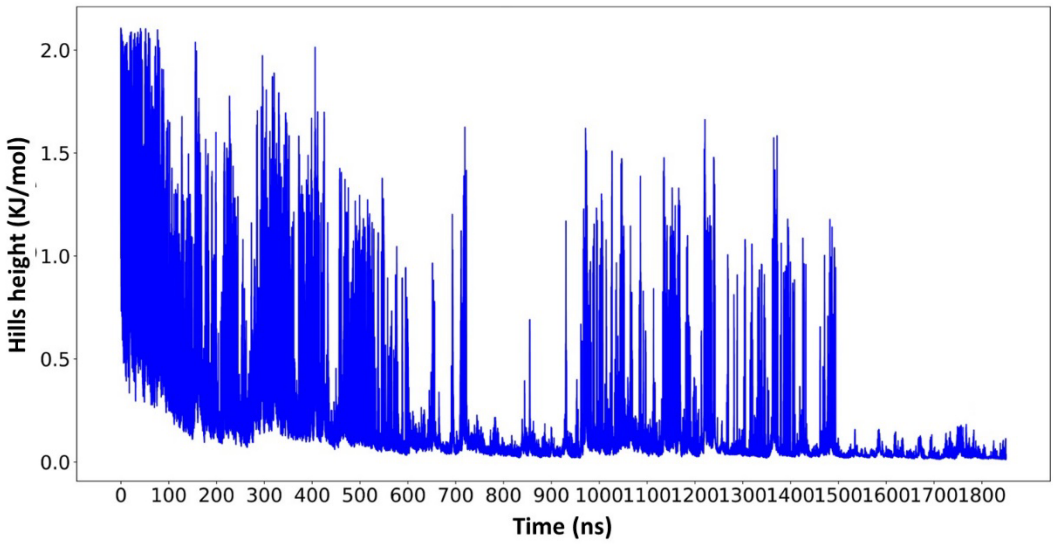
Representation of the cylinder used to sample the position of divalent ions in the active site with a classical metadynamics. The starting position is represented as a pink sphere at the edge of the cylinder.

Figure S3



Representation of the Funnel Metadynamics (FM) [3] system used to analyze the location and conformation of the GCS substrates. The Mn^{2+} is represented as magenta sphere, the UDP-glucose and ceramide as blue and red spheres.

Figure S4



Plot of evolution of hills heights as a function of time in FM [3] simulation with GCS substrates. The Gaussian heights tend to zero during the time course, indicating that the WTM has converged.

References

(1) Varadi, M et al. AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Research.***2022,** *50(D1),* D439-D444, DOI: 10.1093/nar/gkab1061.

(2) Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res*. **2007**;*35 (Issue suppl_2)*, W407–W410, DOI: https://doi.org/10.1093/nar/gkm290

(3) Raniolo S, Limongelli V. Ligand binding free-energy calculations with funnel metadynamics. *Nat Protoc*. **2020,***15(9)*,2837-2866, DOI: 10.1038/s41596-020-0342-4.