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Section D

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Supporting information for article:

Visualization of a substrate-induced productive conformation of catalytic triad of the *Neisseria meningitidis* peptidoglycan *O*-acetylesterase reveals mechanistic conservation in the SGNH esterase family member

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S1. Biophysical characterization of Ape1

There appears to be a weak interaction between the N-terminal domains of molecule A and B, which most likely is an artifact of crystal packing (Fig. S1). Gel filtration chromatography experiments indicated that Ape1 is most likely a monomer. We confirmed this finding experimentally using sedimentation velocity using three different concentrations of Ape1 (1, 8 and 27 μM). At these concentrations only one major species could be detected, and the sedimentation coefficient ($S_{20,w}$) extrapolated at null concentration gave a value of 3.3 ± 0.1 S with a frictional ratio (f/f_0) of 1.3, suggesting a slightly elongated shape. A molecular mass for Ape1 of 38.4 ± 1.5 kDa was calculated from these experimental values showing that Ape1 was monomeric in these conditions.

The hydrodynamic characteristics of the Ape1 were calculated from the crystal structure, giving a sedimentation coefficient of 3.36 S, which is in excellent agreement with the analytical ultracentrifugation experimental value (3.3 ± 0.1 S). The dimensions and shape of the complex are therefore consistent with that of the complex in solution.

Most of Ape1 residues were successfully modeled into the structure with the exception of the first 25 residues which appear to be unstructured or flexible at the N-terminus (Fig. S3). We confirmed by Edman degradation and electrospray mass spectrometry that our protein for both the native and selenomethionine samples were intact. Ape1 is localized to the inner-leaflet of the outer-membrane and in some bacteria appear to have a distinct lipoprotein signal. It is possible these residues are involved in tethering Ape1 to the inner-leaflet of the outer-membrane.

S2. The electrostatic surface of Native Ape1

The surface of Ape1 near the active site cleft reveals other major grooves that may be involved in PGN substrate interactions. Notably, the surface contour of the catalytic cleft appears consistent with the anticipated binding mode of the sugar backbone of polymeric PGN (Fig. S6). The electrostatic surface, calculated using the Adaptive Poisson-Boltzman Software program,

pinpoints several basic patches in proximity of the catalytic triad and may be ideally positioned to provide charge complementarity to the peptide cross-links, which consists of an acidic glutamate moiety (Fig. S6).

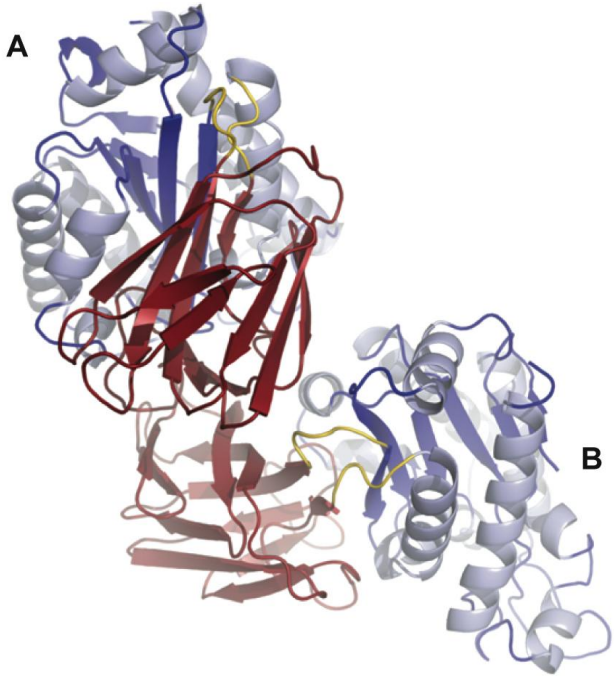

Figure S1 Ape1 forms an artificial dimer (A, B) in the crystalline form.

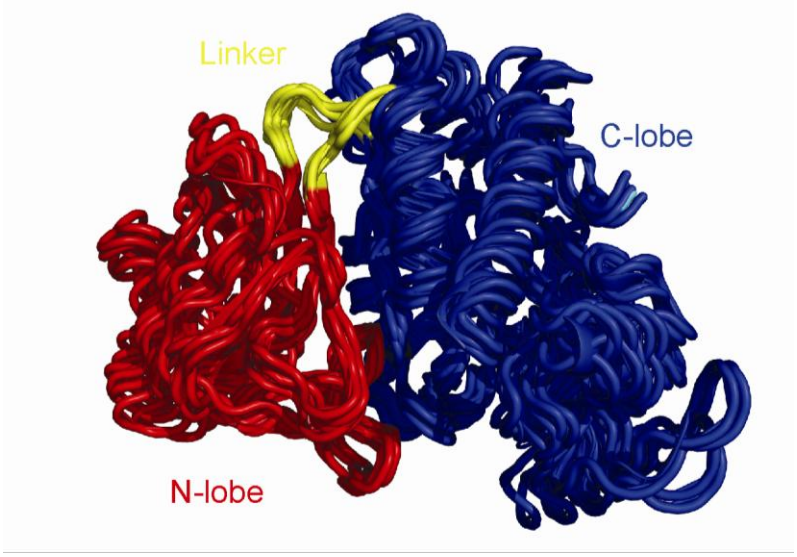

Figure S2 Normal mode analysis of Ape1 native structure by elNemo output ensembles reveal no interdomain mobility between the N and C Lobe.

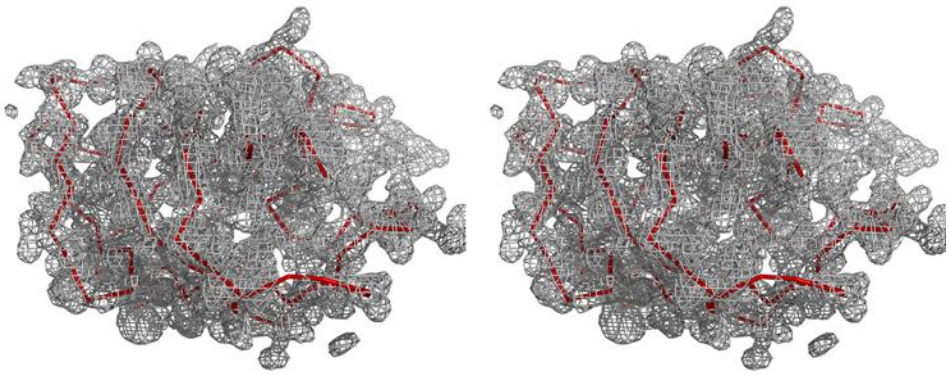

Figure S3 Stereo representation of the 2fofc map corresponding to the N-lobe of Ape1 contoured at 1.4 σ.

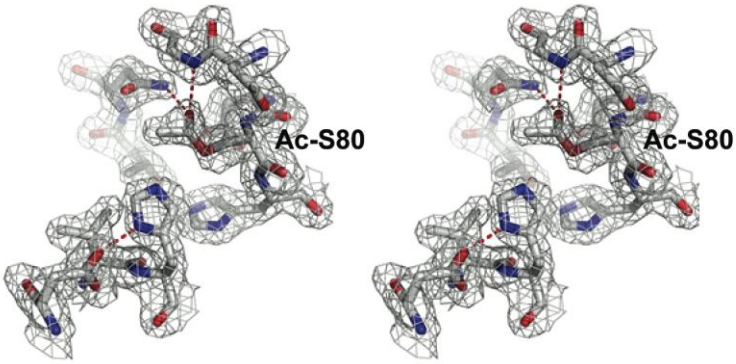

Figure S4 Stereo view of the 5-min trapped acyl enzyme intermediate.

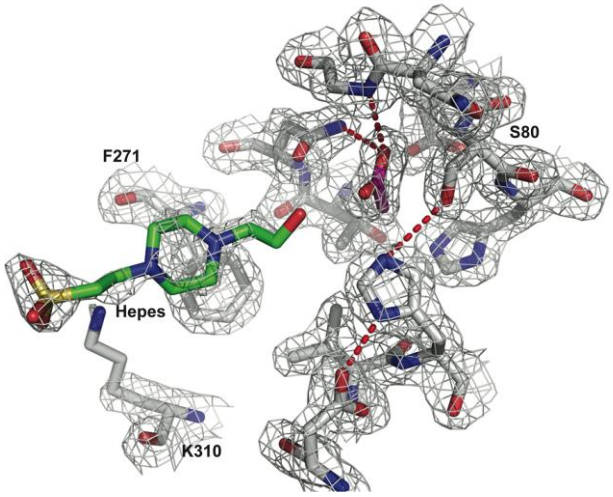

Figure S5 HEPES buffer molecule bound in the active site region of Ape1. Additional residues (F271 and K310) potentially involved in substrate binding are highlighted.

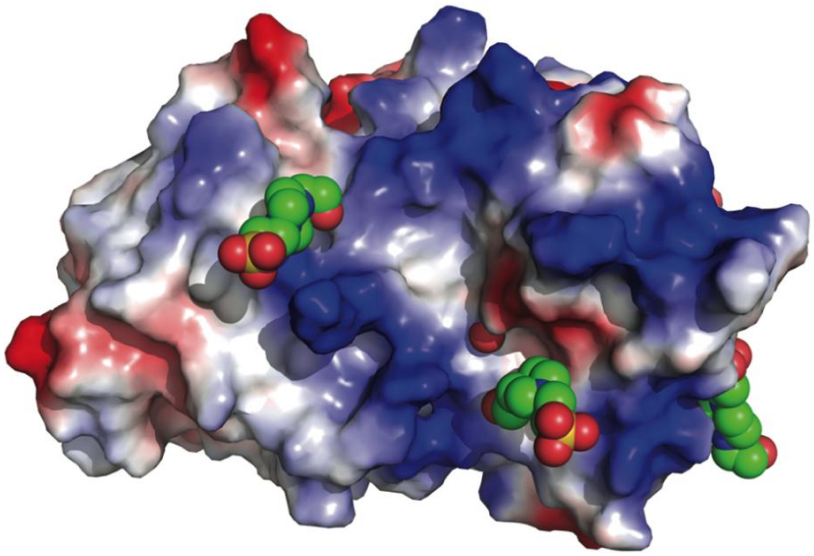

Figure S6 The electrostatic surface of Ape1. Bound HEPES molecules are colored in green.