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Supporting Material

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Exploring the Conformational States and Rearrangements of *Yarrowia lipolytica* Lipase

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F. Bordes and S. Barbe have equally contributed to the work.

The particular structure of chain G in the lid region may be explained by structural constraints imposed by the crystal packing around this molecule. Although the N-terminal part of the lid (L91-T96) is maintained as an α-helix, its C-terminal end (region D97-105) is completely distorted and residues D97- I100 do not appear as an α-helix anymore (Fig. S2). As a result of this rearrangement, the lid is found in a more closed conformation than what is observed for the other chains of the asymmetric unit. For instance, d(I95-Cα/I286-Cα) is only 7.1 Å in the case of chain G instead of 8.5 Å for other molecules (Fig. S3 *a*).

Noticeable differences were also observed within the D61-D67 loop which is found in close contact to the lid region in all chains but G. To prevent clashes between the side chain of D97 and that of L64 in chain E, the lid region is rearranged and stabilized by electrostatic interactions involving side chains of residues D97 and R99 from chain G and D67 from chain E. The D61-D67 loop also rearranged to avoid steric conflicts between L64 and the side chain of D97 from chain E. In addition, the region 60-63 is stabilized by a network of hydrophobic interactions with the 63-65 region of molecule E and the regions 61-67 and 89-97, which correspond to the beginning of the lid, of chain B. Finally, due to the modification of the lid conformation, D61 is oriented in an opposite direction compared to other chains where it is stabilized by intramolecular interactions with atoms I65 N and T88 O, whereas in the other chains, atom T88 OG interacts with S68 OG and D61 O.

The predominant structural modifications described above induce in turn the reorganization of loops 32-37, 44-46, and 237-240 and of helix 284-290. When all these regions are excluded from the superimposition, the rmsd obtained when comparing chain G with other molecules decreased from 0.8 to 0.4 Å, what is very similar to the rmsd value obtained after superposing chains A to F.

Supporting figures

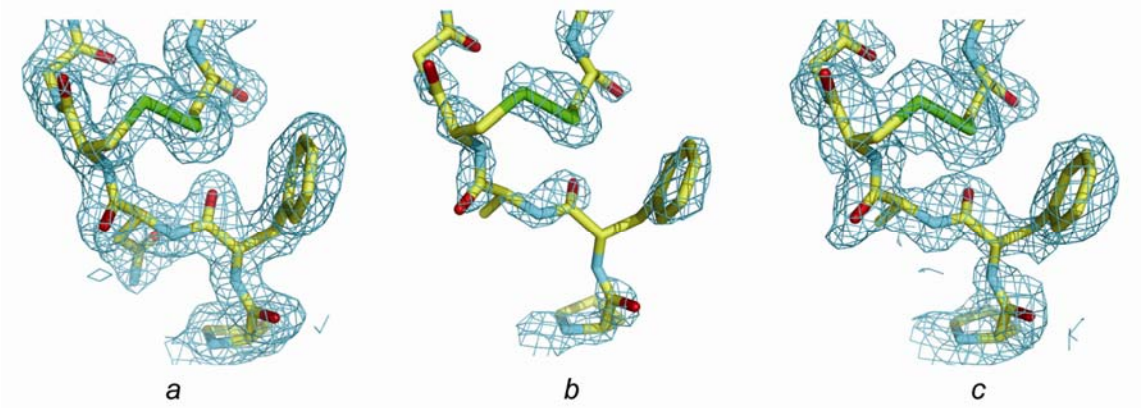

FIGURE S1 SigmaA weighted 2Fo-Fc electron density map around residues 40 to 47 of *(a)* chain A at a contouring level of 1 sigma, *(b)* chain G contoured at 1 sigma, *(c)* chain G contoured at 0.5 sigma.

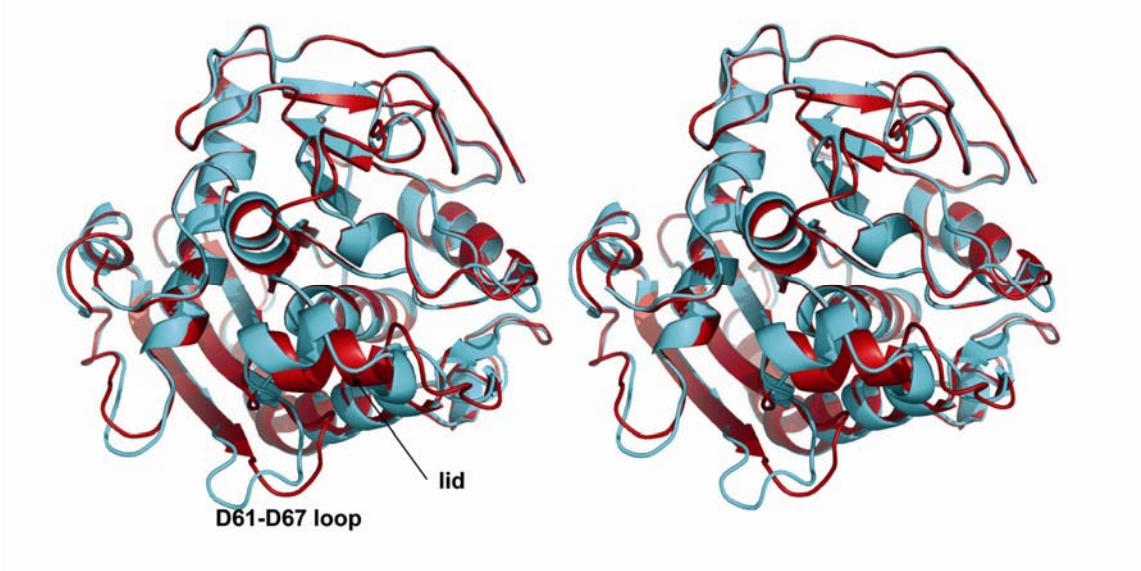

FIGURE S2 Stereoview of chain A (red) superposed on chain G (cyan). The main differences are observed around the lid region (L91-L105) and in the D61-D67 loop.

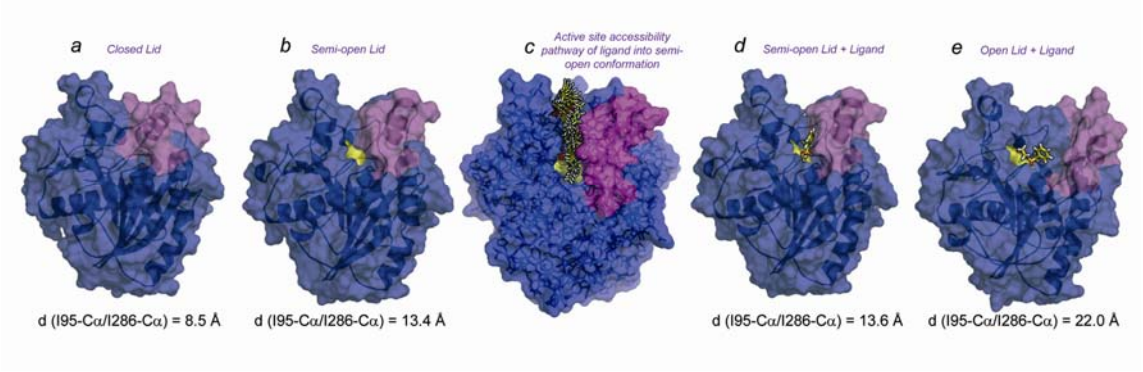

FIGURE S3 Representation of lid movements occurring in Lip2 during MD simulations carried out at an octane/water interface. *(a)* Closed x-ray structure of Lip2. *(b)* Semi-open conformation obtained after 8 ns of MD simulation started from the x-ray structure shown in *(a)*. *(c)* Representation of the exit trajectory of (*S)* ethyl 2-bromophenylacetate computed by BioMove3D from the catalytic site (yellow) to the surface of Lip2. The conformation of Lip2 used for calculation is that obtained after 8 ns of MD simulation in an octane/water interface. The lid region is colored in magenta. The different conformations adopted by the substrate along the exit pathway are shown in stick. *(d)* Covalent complex between Lip2 in the semi-open form and (*S*) ethyl 2-bromophenylacetate. *(e)* Fully open conformation obtained after 20 ns of MD simulation started from the complex shown in *(d)*. The lid region and the catalytic triad are colored in magenta and yellow, respectively. The substrate is shown in stick mode.