SUPPLEMENTARY MATERIAL

Elucidating the structural and conformational factors responsible for the activity and substrate specificity of alkanesulfonate monooxygenase

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Figure S1. Description of the surface of SsuD in terms of hydrophobic/hydrophilic areas. The GRID analysis of the enzyme surface was performed by using two different probes: WATER (dipolar interactions and hydrogen bond formation), DRY (hydrophobic interactions). The method describes and quantifies the different interactions as Molecular Interaction Fields (MIFs). MIFs are visualised as isopotential surfaces. Hydrophilic MIFs were calculated with the WATER probe (visualised at -2.5 kcal/mol) and are displayed in cyan. Hydrophobic MIFs were calculated with the DRY probe (visualised at -0.3 kcal/mol) and they are visualized in yellow. Active site is highlighted in the red circle.

Figure S2. Two projections of the secondary structure of SsuD. Subunit A is in cyan and subunit B in yellow. The picture on the left shows the opening of the active site of subunit A and the back side of subunit B. Projection on the right represents the opposite perspective.

Figure S3. Active sites of the two subunits of SsuD: the closed conformation for Subunit A is on the left whereas the open Subunit B is on the right. Arg297 interacts with Asp111 in the closed conformation of Subunit A and with Glu20 in the open conformation of Subunit B.

Figure S4. Superimposition of the highly conserved residues (in sticks mode) of the three enzymes (1m41, 1luc, and 3b9o). Red for SsuD, yellow for bacterial luciferase and cyan for LadA. The protein structure in the background is SsuD.

Figure S5. Upper figure: superimposition of SsuD, Mer, LadA and Luciferase enzymes to compare the relative position of active site and cofactor accommodation. Cofactor FMN (green, stick mode) is co-crystallized in the LadA structure. Cofactor F₄₂₀ (pink, stick mode) is co-crystallized in Mer structure. Lower figure: Mer crystal structure (in cyan) with F₄₂₀ cofactor (sticks mode). The picture highlights the different loop of SsuD (in raspberry) and its conserved Arg (sticks mode) after superimposition of the two structures,

Figure S6. GRID analysis of the active site of SsuD. MIFs were calculated with DRY probe (-2.5 kcal/mol) in yellow. The hydrophobic area (yellow) around Trp196 is clearly visible. The cyan area represents the MIFs calculated with the WATER probe.

Figure S7. The salt bridge between Arg297 and Asp111 in subunit A prevents the expulsion of the cofactor by moving a protein loop (from Thr108 to Glu114) and thus locking the cofactor inside the cavity

Figure S8. Active site of Subunit A with the docked cofactor FMNH₂ and substrate **1**. The conformation corresponds to that used for the construction of the PLS model. The cofactor is located in the correspondence of the hydrophobic area in proximity of Trp196. The sulfonic substrate interacts with His11.

TIP-TIP vectors

Figure S9. Example of how the GRIND TIP/TIP descriptors (first principal component) are calculated for substrate **1** (R=aliphatic long chain, high specificity) and substrate **8** (R=cyclic moiety, low specificity). Crosses represent the points of the MIFs (TIP probe) selected for the generation of the descriptors.

Table SI. Full detail of the evaluation of the distances between Arg297 - Asp111 (in grey) and Asp111 - Glu20 (in black) during ten 20 ns molecular dynamics simulations of Subunit A and Subunit B.