Supporting Information

Catalytic Reaction Mechanism in Native and Mutant COMT from the Adaptive String Method and Mean Reaction Force Analysis

David Adrian Saez[†], Kirill Zinovjev[‡], Iñaki Tuñón[‡] and Esteban Vöhringer-Martinez[*,†].

[†]Departamento de Físico-química, Facultad de Ciencias Químicas, Universidad de Concepción, Chile.

[‡]Departament de Química Física, Universitat de València, 46100 Burjassot, Spain.

E-mail: evohringer@udec.cl

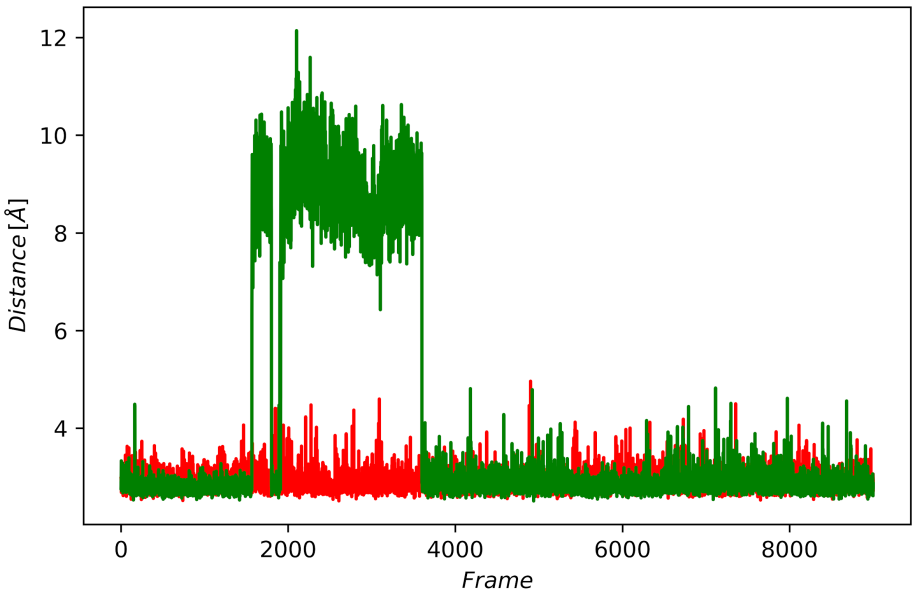


Figure S1. Distance between the side-chain nitrogen atom of Lys144 and the nucleophilic oxygen of DOP for the WT(red) and Y68A(green) enzymes obtained from five independent simulations of 450 ns. The short distance and most abundant conformation corresponds to the salt-bridge interaction between both atoms.

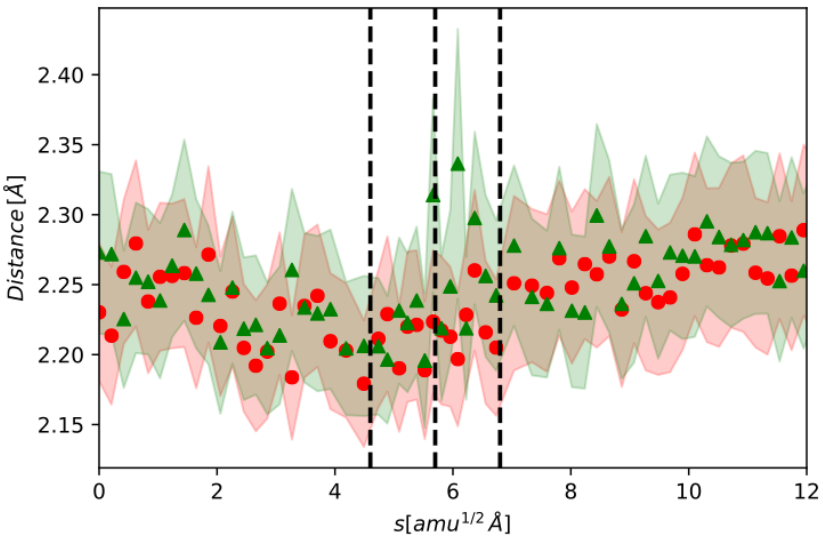


Figure S2. Distance between the Mg^{2+} ion and the hydroxyl oxygen atom of dopamine along the MFEP of the adaptive string method for the WT (red) and Y68A (green) systems. The shaded area shows the standard deviation.

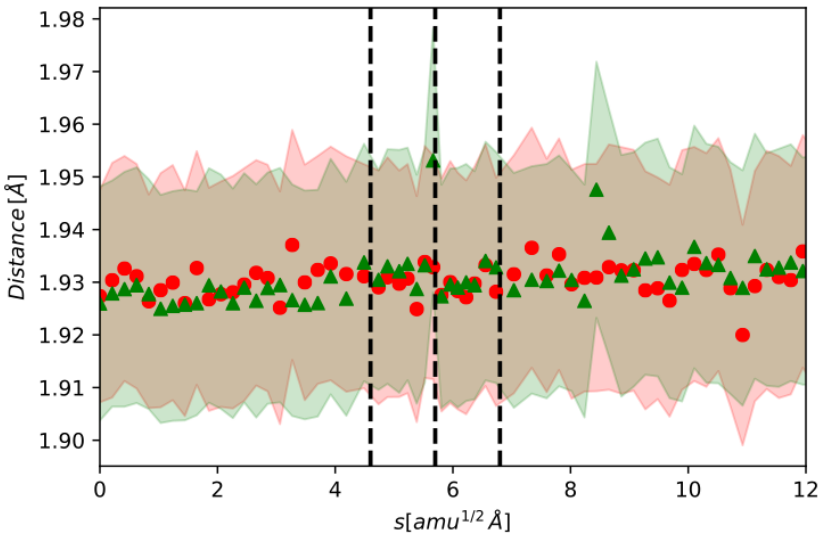


Figure S3. Distance between the Mg^{2+} ion and the coordinated oxygen atom of Glu199 along the MFEP of the adaptive string method for the WT (red) and Y68A (green) enzyme. The shaded area shows the standard deviation.

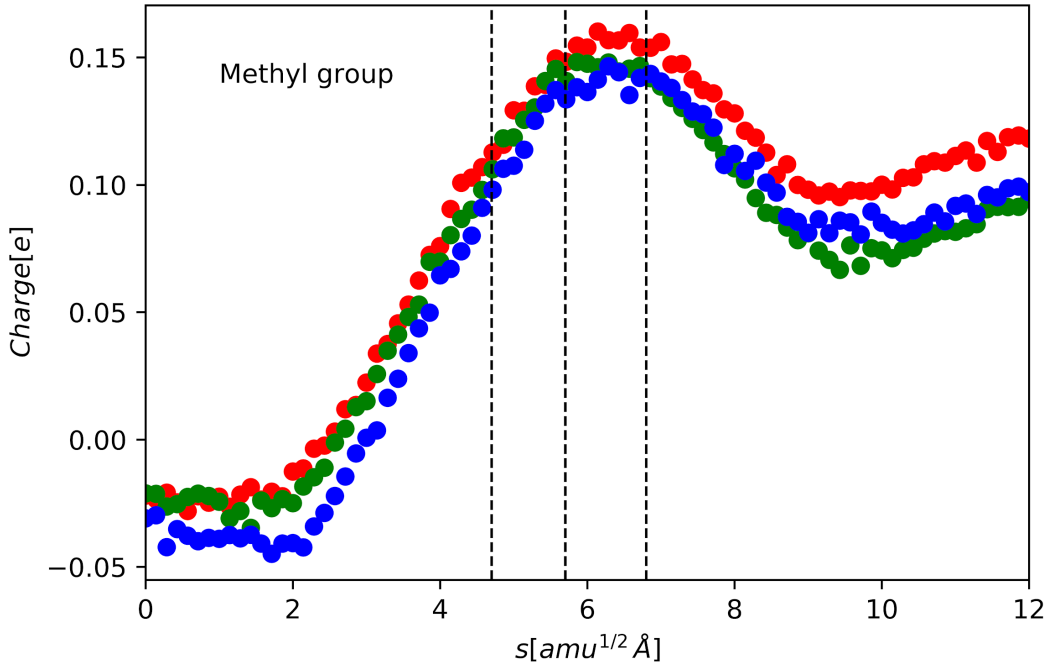
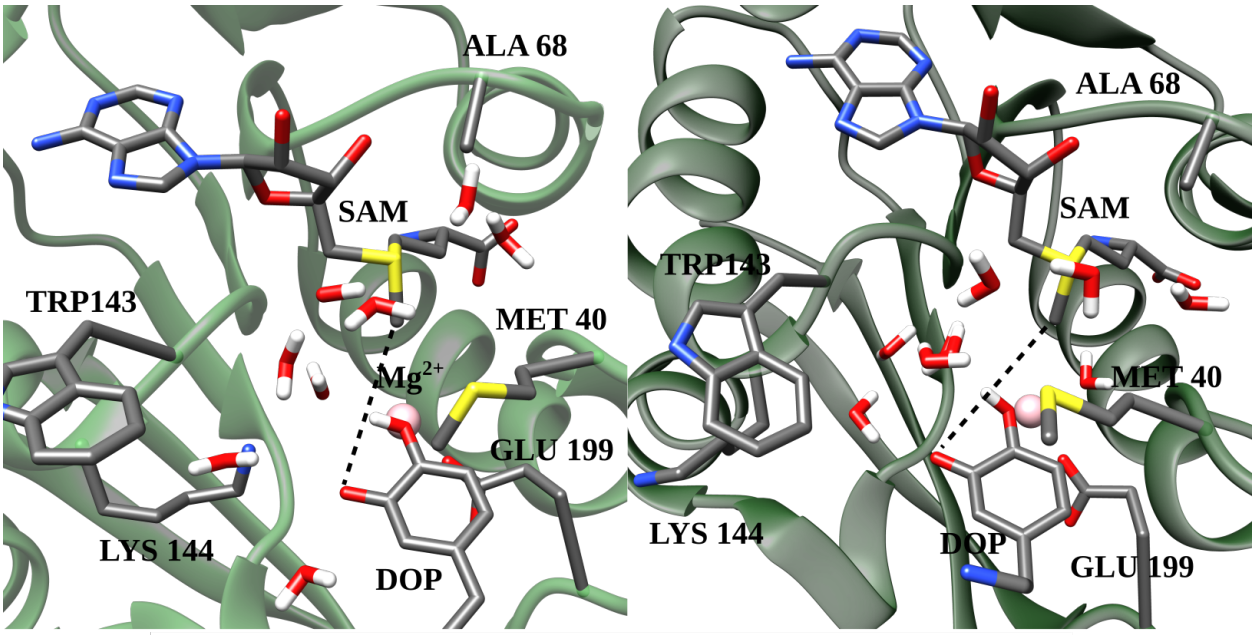


Figure S4. Atomic charge for atoms in the methyl group along the reaction path. WT (red), Y68A (green) and AQ (blue).



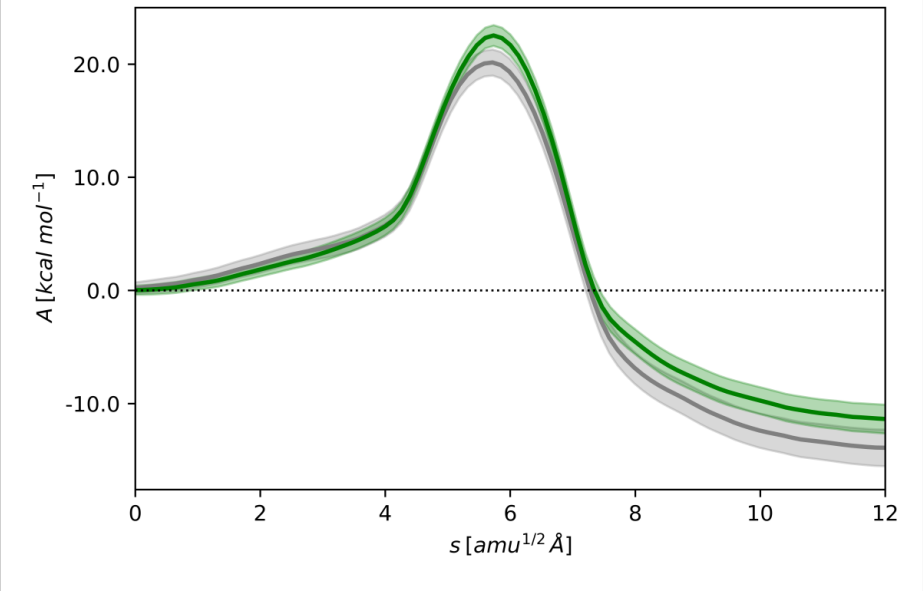


Figure S5. Up: active site configurations observed during the MM simulations of the mutant Y68A depicting the Lys 144 inside (left, most abundant conformation) and outside (right, alternative less abundant conformation) the active site.
Bottom: Free energy profile obtained for the different conformations of Lys 144 observed in the molecular dynamics of the mutant Y68A. The green line represents the most abundant conformation, grey line the alternative conformation. It can be seen that the free energy curve obtained in the unrestrained QM/MM stage is not greatly affected by the presence or absence of the Lys residue.

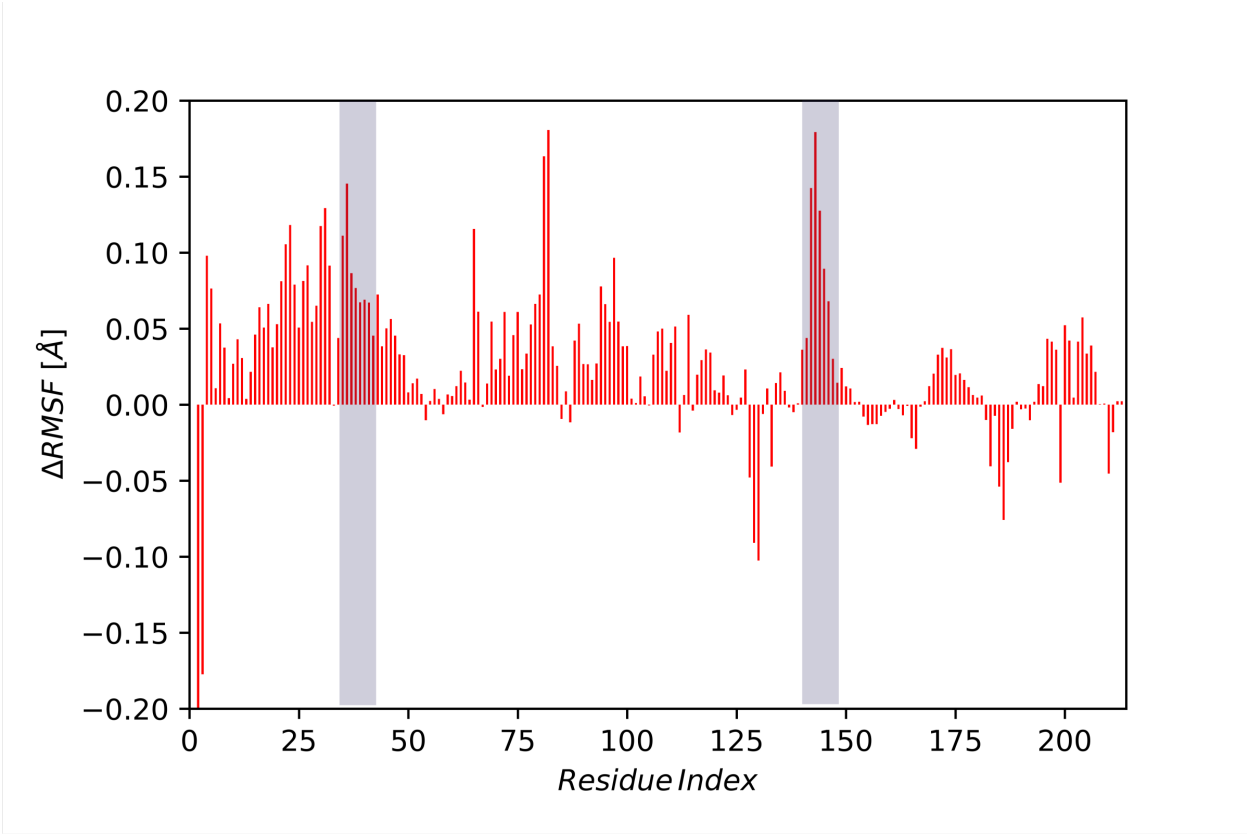


Figure S6. Difference in root-mean-square fluctuations (RMSF) of the backbone α-carbon atoms at the reactant state, calculated over 450ns of molecular dynamics simulations. The increased flexibility of residues around Trp143 and Met39 (shaded area) show increased flexibility of residues directly linked to the active site structure for the mutant than the wild-type enzyme.

System	CV1	CV2	CV3
WT	2.0	8.6	0.2
Y68A	2.3	10.0	0.2
AQ	2.9	10.7	0.3

Table S1. Free energy associated to W_1 contribution of each collective coordinate. Units are kcal mol^{-1}.

System	CV1	CV2	CV3
WT	6.0	4.0	0.3
Y68A	6.3	4.5	0.4
AQ	7.8	5.4	0.3

Table S2. Free energy associated to W_2 contribution of each collective coordinate. Units are kcal mol^{-1}.