Molecular Dynamics Simulations for the Michaelis Complex of Ectoine Synthase (EctC)

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Supplementary Materials

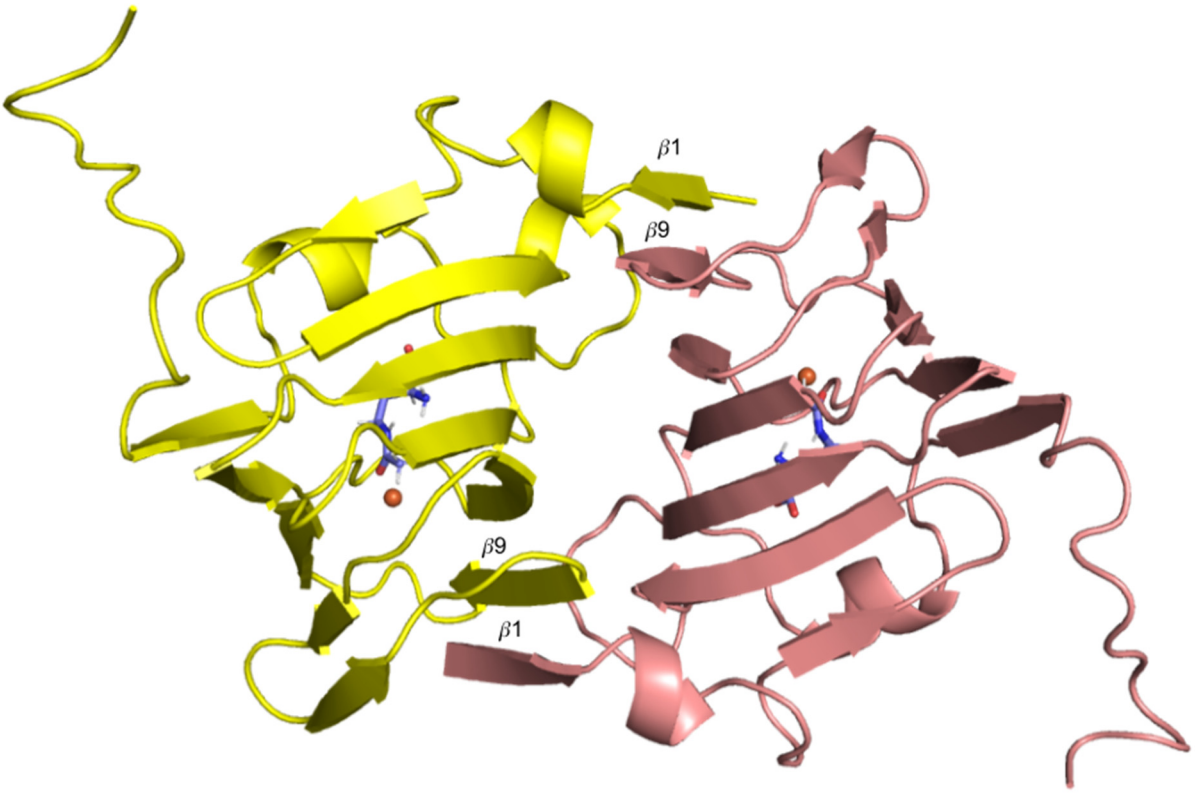


Figure S1. Ectoine synthase dimer from *P. Lautus* (PDB: 5ONN) with both monomers occupied by the ligand molecules. Interface of dimerization marked with labels of interacting β-strands.

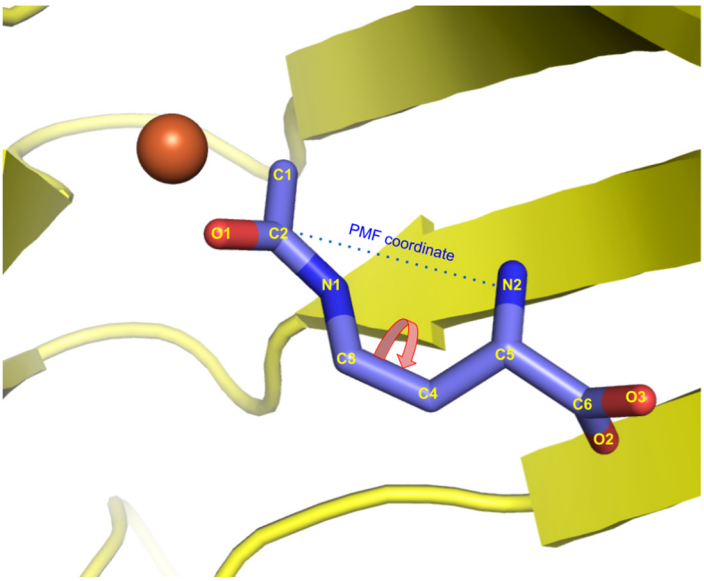


Figure S2. The substrate geometry from the 5ONN crystal structure. Atoms labelled and PMF reaction coordinate (C2-N2 distance) indicated with blue dotted line. N1-C3-C4-C5 dihedral marked with a red bent arrow.

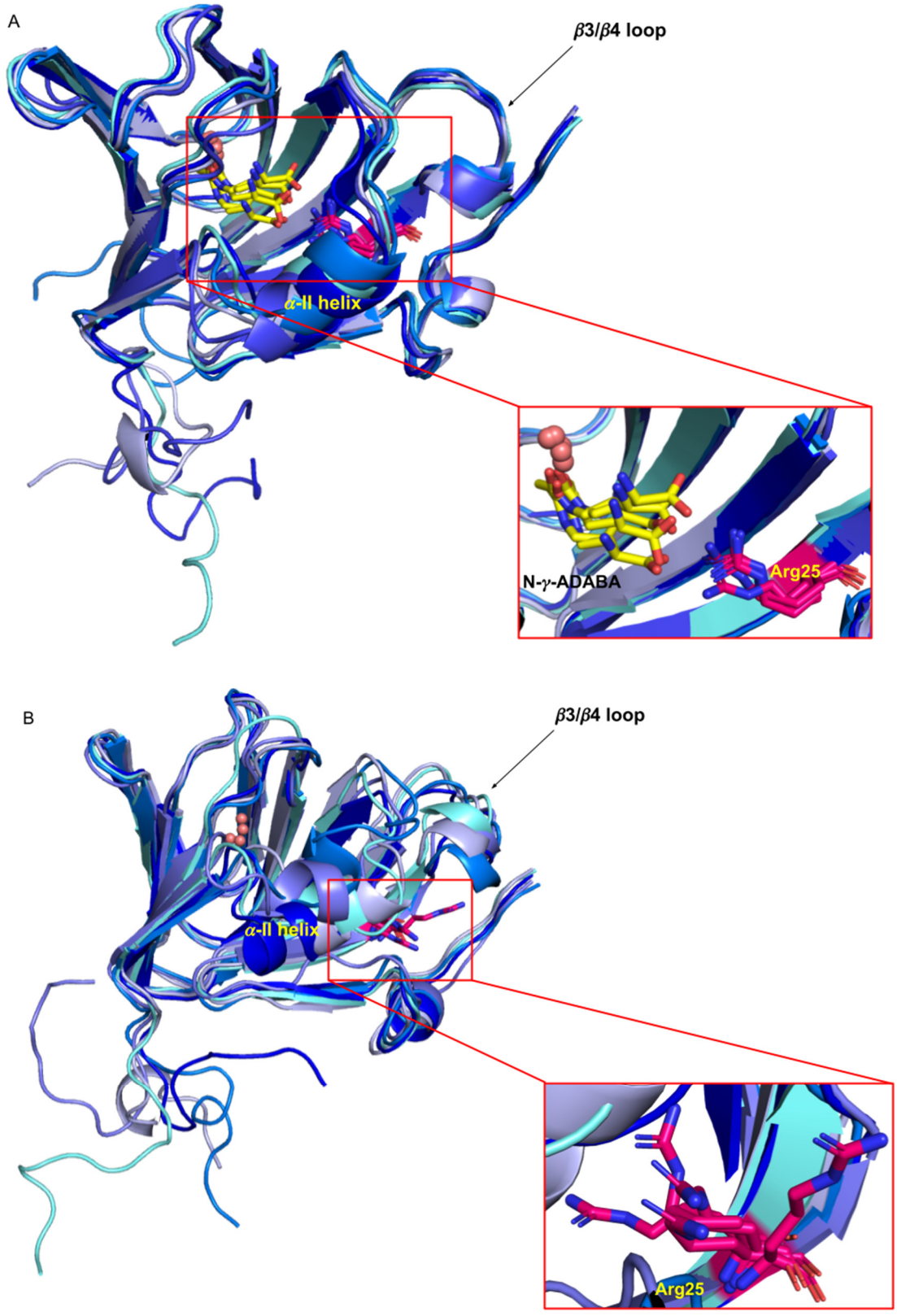


Figure S3. Structural alignment of five centroid geometries for the occupied (**A**) and empty (**B**) EctC monomers. **A**: Zoom into the Arg25-N-γ-ADABA mutual orientation. **B**: zoom into the multiple orientations of Arg25.

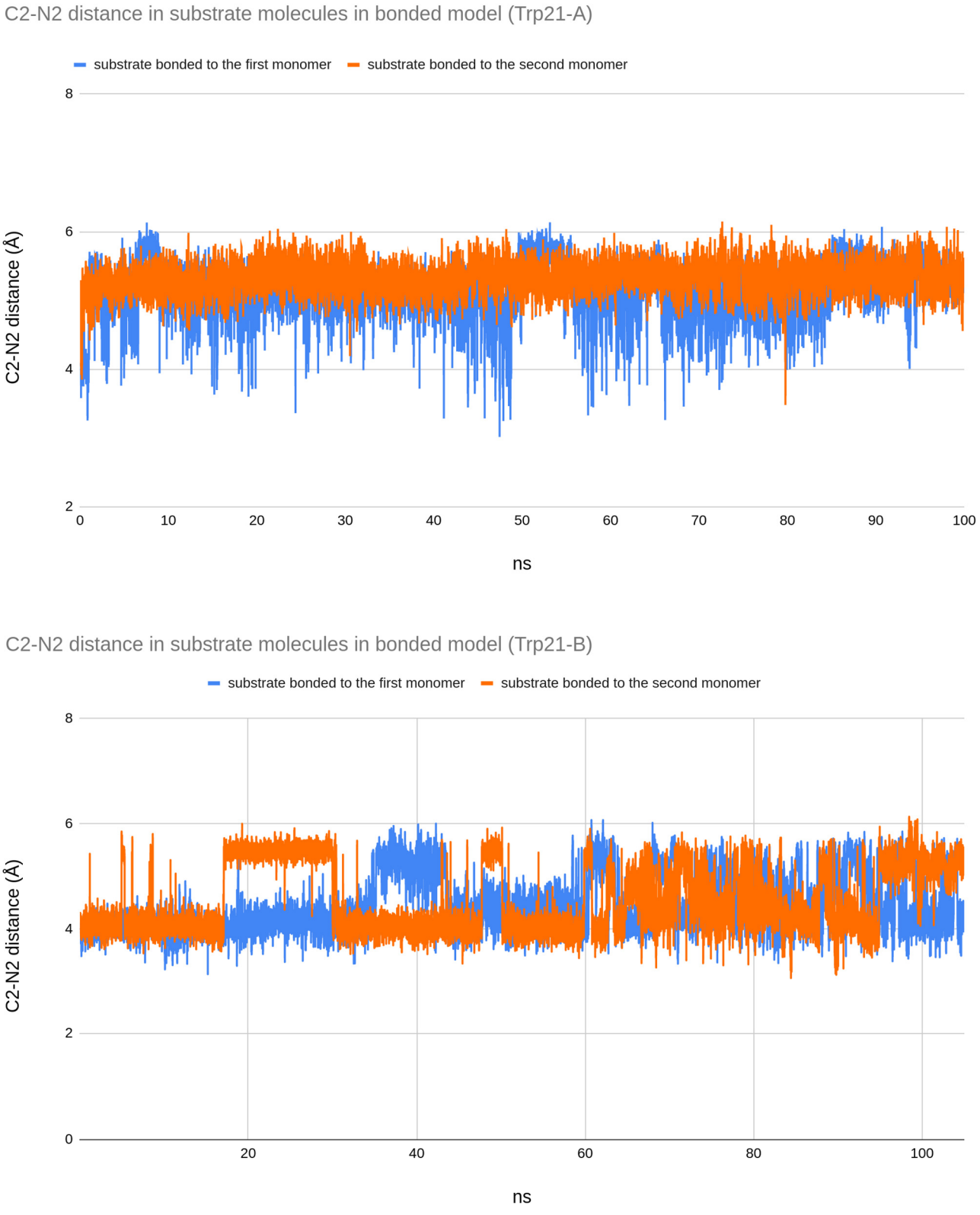


Figure S4. Graph of the C2-N2 distance in N-γ-ADABA molecules bonded to both monomers in the EctC dimer for the model γ with the Trp21-A or Trp21-B conformation. **Top**: Graph of the C2-N2 distance in N-γ-ADABA molecules bonded to both γ monomers in the EctC dimer for the model with the **Trp21-A** conformation. **Bottom**: Graph of the C2-N2 distance in N-γ-ADABA molecules bonded to both monomers in the EctC dimer for the model with the **Trp21-B** conformation. The measurements were extracted from the 100 ns long MD simulations for the bonded model with tetrahedral geometry around Fe^{2+}.

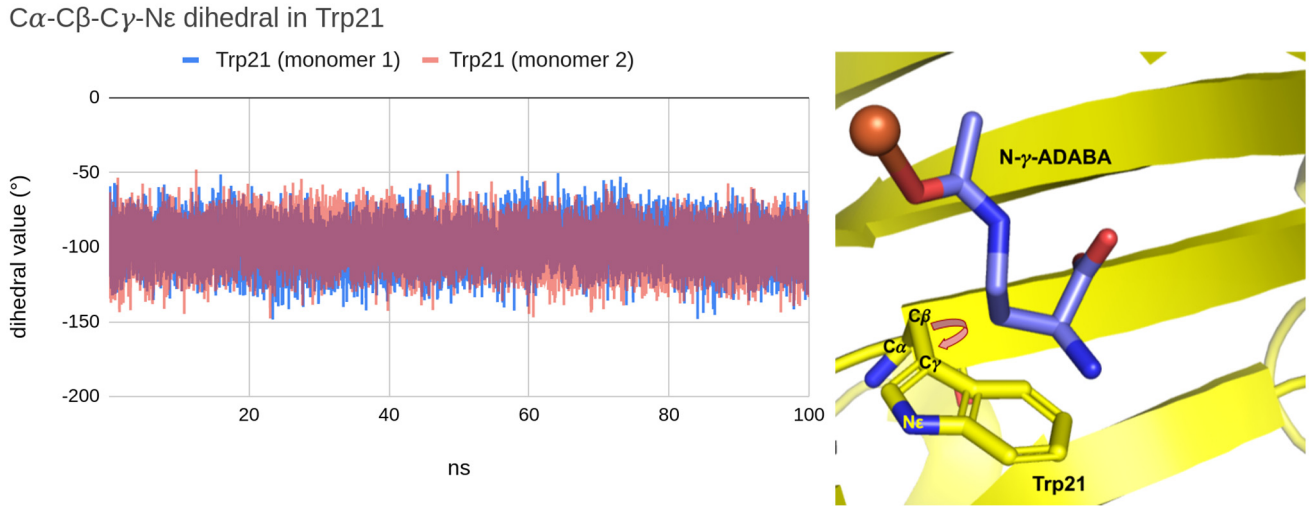


Figure S5. Conformation of Trp21 in MD for 4-coordinate bonded model. **Left**: the Cα-Cβ-Cγ-Nε dihedral values calculated for Trp21 in both monomers in MD for 4-coordinate bonded model. **Right**: Trp21 in the Trp21-A conformation with the Cα-Cβ-Cγ-Nε dihedral marked with red bent arrow.

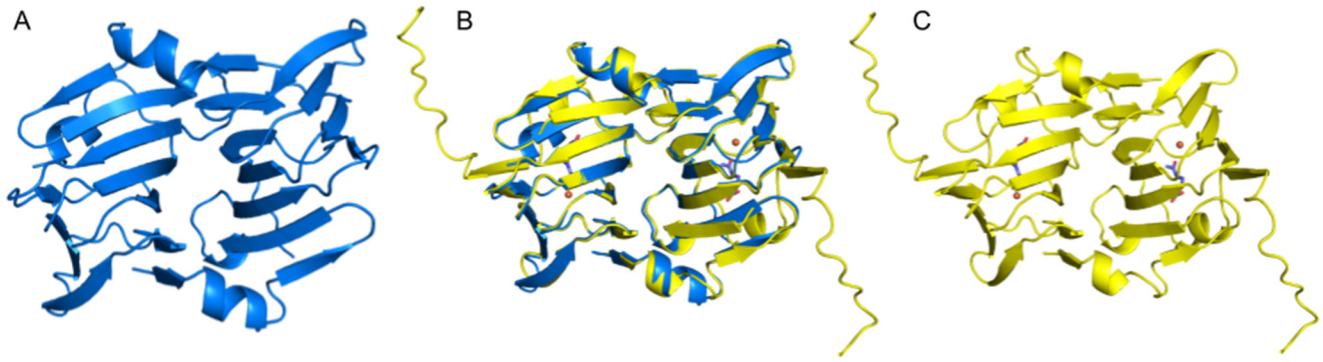


Figure S6. The homology modelling of *P*/EctC dimer. (**A**) The homology modelling template, EctC from S. alaskensis. PDB:5BXX [2](DOI:10.2210/pdb5BXX/pdb). (**B**) Structural alignment of the template (blue) and new model (yellow). (**C**) Two 5ONN monomers sequentially aligned with both monomers from the 5BXX dimer resulting in a new 5ONN dimer.

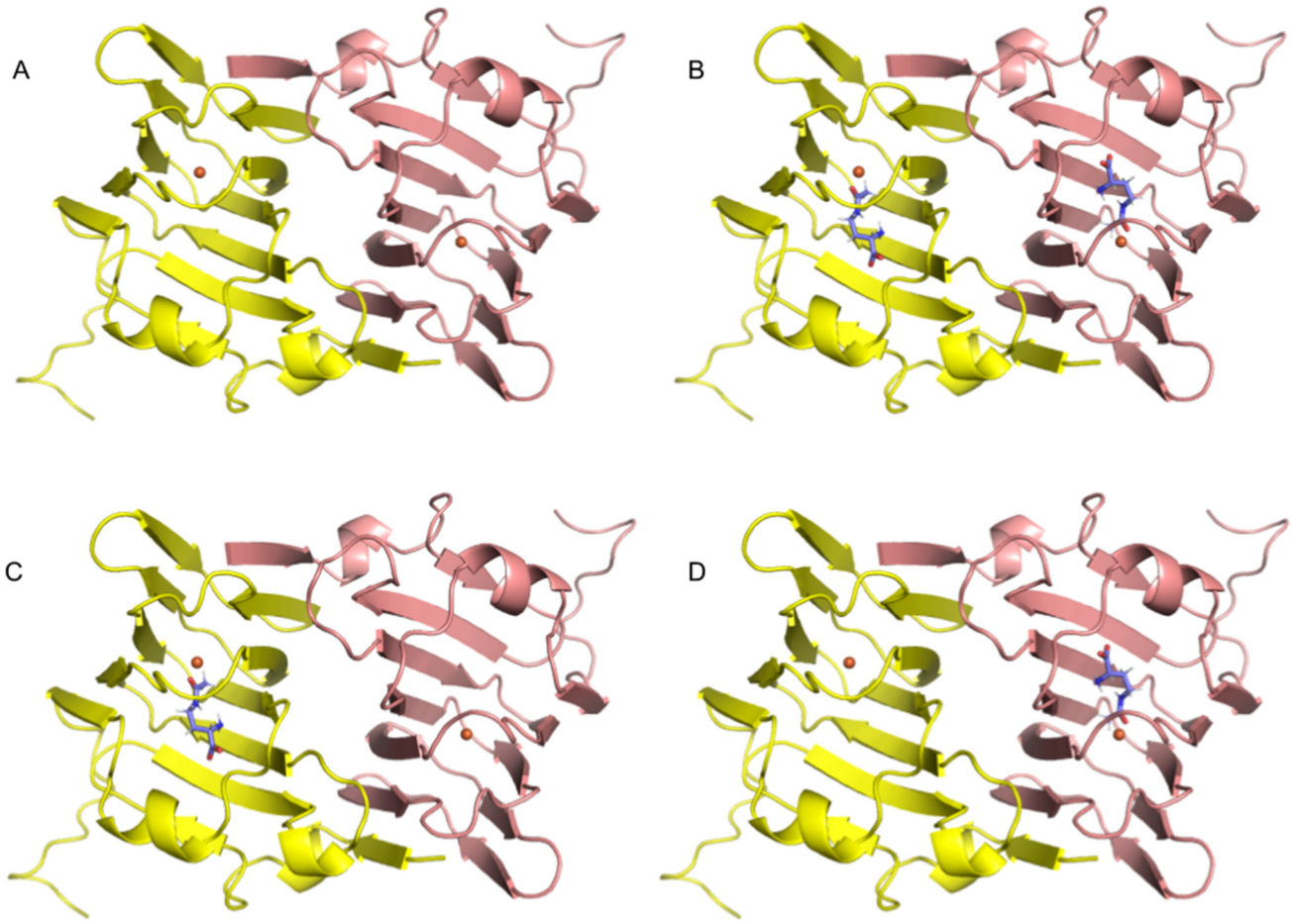


Figure S7. 6-coordinate Fe^{2+} models of EctC dimer with different monomer occupations: (**A**) both monomers empty, (**B**) both monomers occupied, (**C**) monomer A occupied; monomer B empty, (**D**) monomer A empty; monomer B occupied.

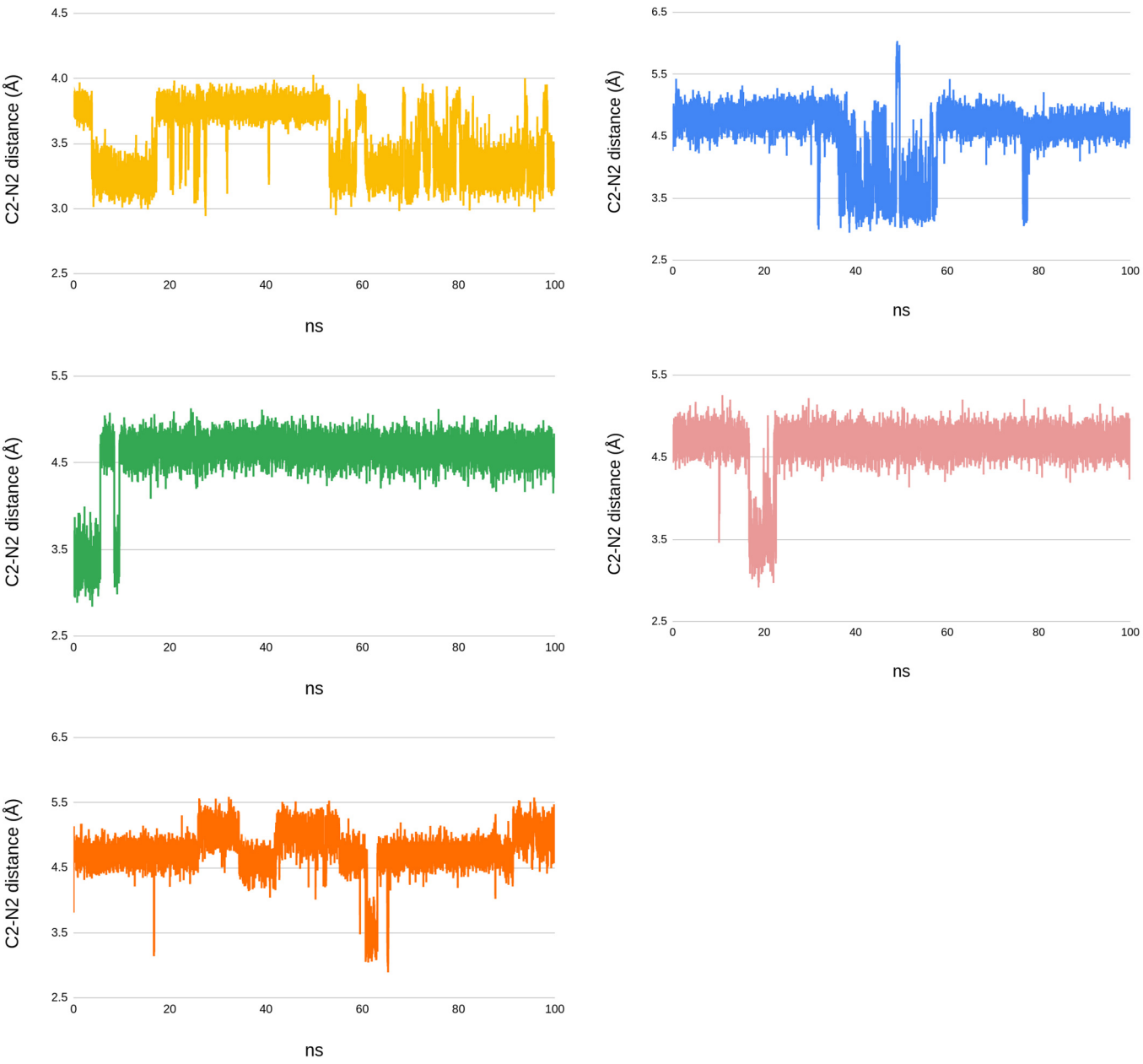
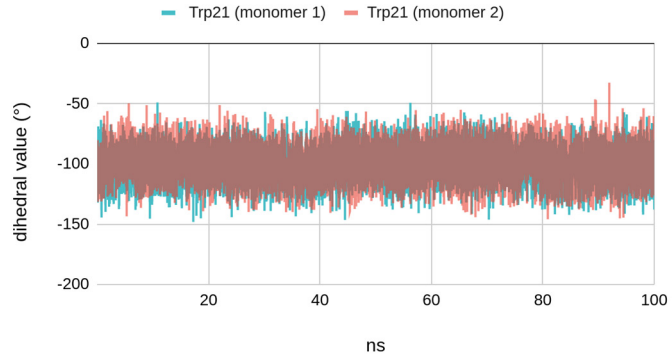
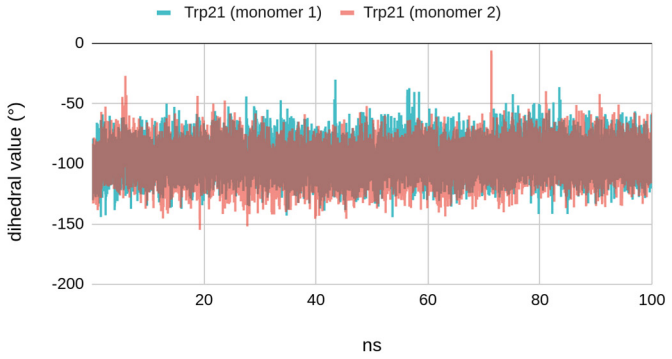


Figure S8. Graphs of the C2-N2 distances for MD trajectories for the dimers with at least one monomer in a form of the Michaelis complex. Some NAC states (C2-N2 oscillates around 3.2 Å) persisted for long periods during the simulation (yellow, blue) and some lasted shorter (green, pink, orange).

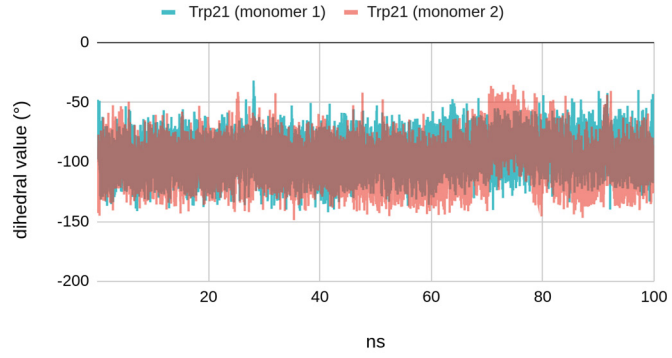
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers in holo form (1)



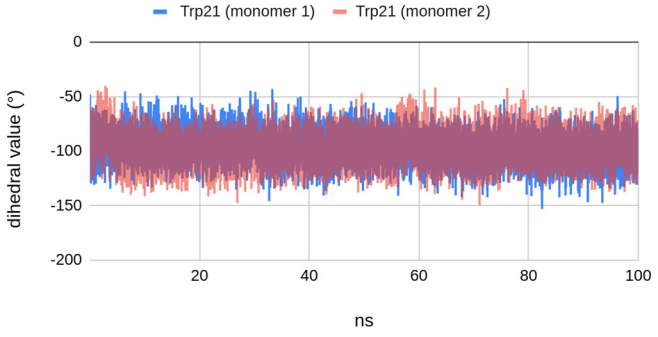
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers in holo form (2)



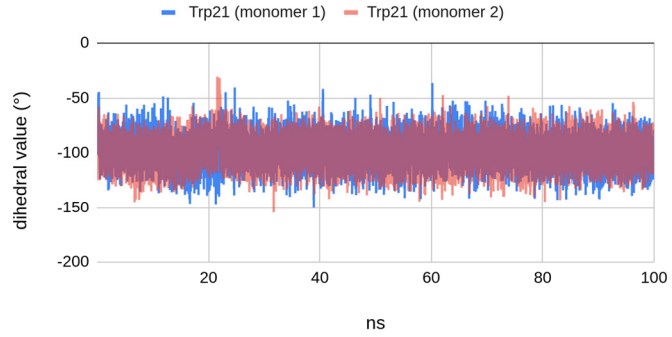
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers in holo form (3)



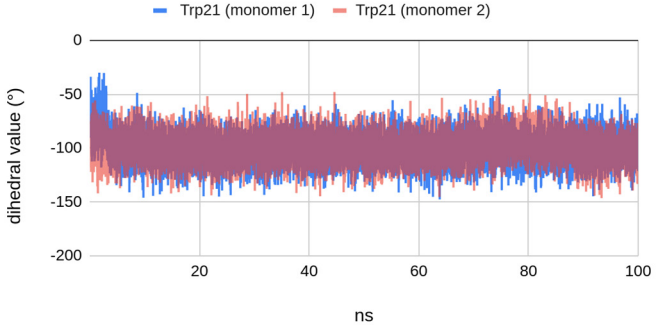
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers with N-γ-ADABA bound (1)



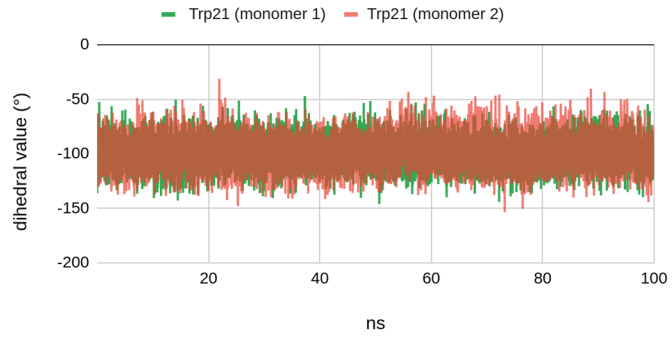
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers with N-γ-ADABA bound (2)



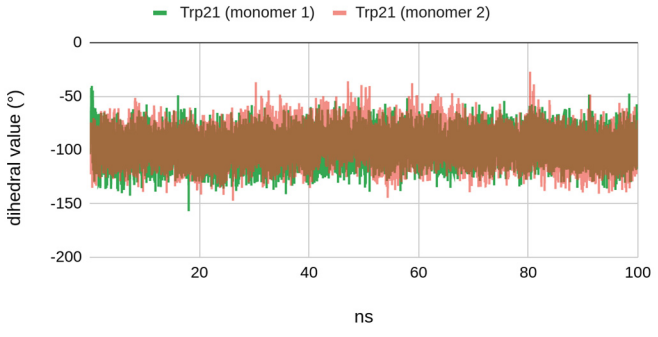
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers with N-γ-ADABA bound (3)



Cα-Cβ-Cγ-Nε dihedral in Trp21 - monomer 1 with N-γ-ADABA bound. monomer 2 in holo form (1)



Cα-Cβ-Cγ-Nε dihedral in Trp21 - monomer 1 with N-γ-ADABA bound. monomer 2 in holo form (2)



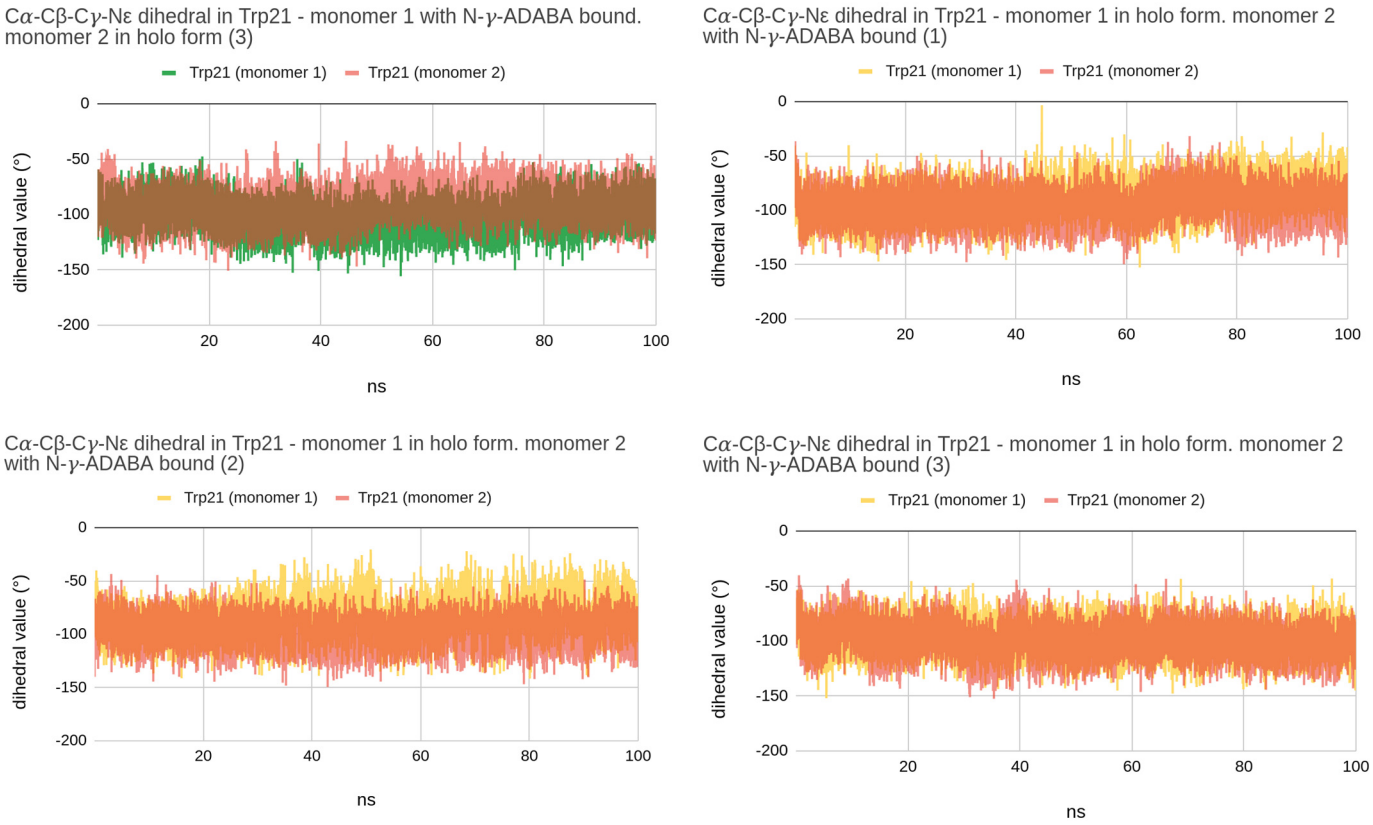


Figure S9. The Cα-Cβ-Cγ-Nε dihedral values for Trp21 in MD simulations for the 6-coordinate Fe^{2+} models of EctC dimer. The Cα-Cβ-Cγ-Nε dihedral values calculated for Trp21-A in both monomers in all twelve MD simulations for the 6-coordinate Fe^{2+} models of EctC dimer: first 3 MD for the model with both monomers empty, then 3 MD for the model with both monomers occupied , then 3 MD for the model with the first monomer occupied/second empty and last 3 MD for the model with the first monomer empty/second occupied. Trp-A conformation has values around −100 deg; Trp-B around +90 deg.

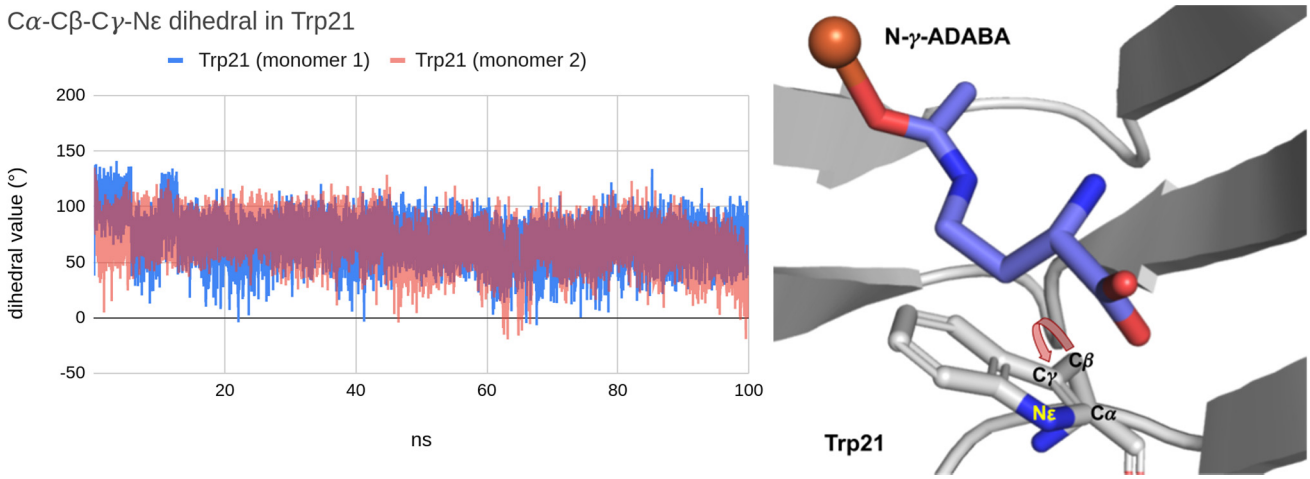
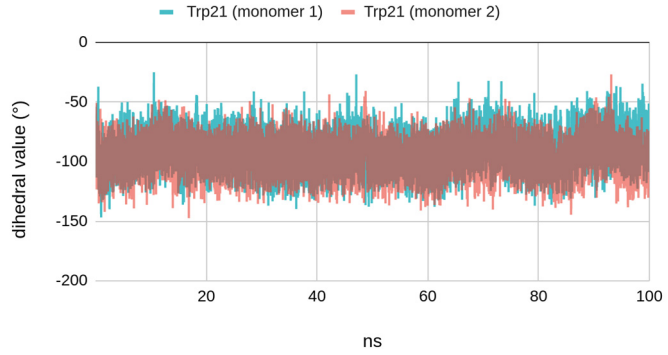
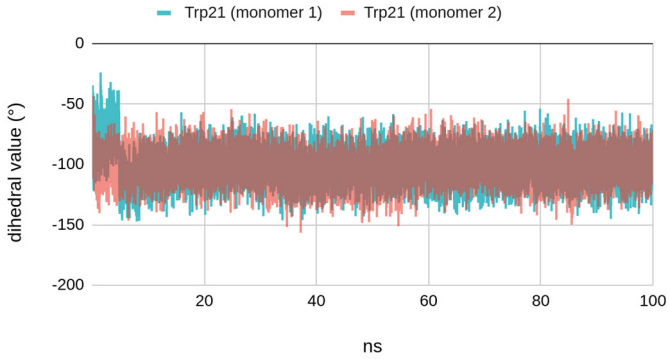


Figure S10. Conformation of Trp21(B) in MD for 4-coordinate bonded model. **Left**: Cα-Cβ-Cγ-Nε dihedral values for Trp21-B (both EctC monomers) in MD trajectories for 4-coordinate Fe^{2+} model. **Right**: Trp21 in the Trp21-B conformation with Cα-Cβ-Cγ-Nε dihedral marked with the red bent arrow.

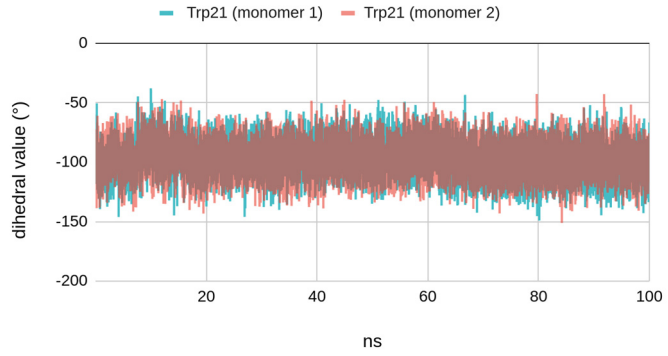
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers in holo form (1)



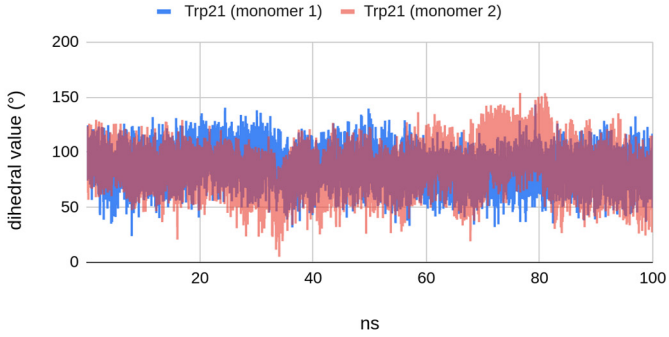
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers in holo form (2)



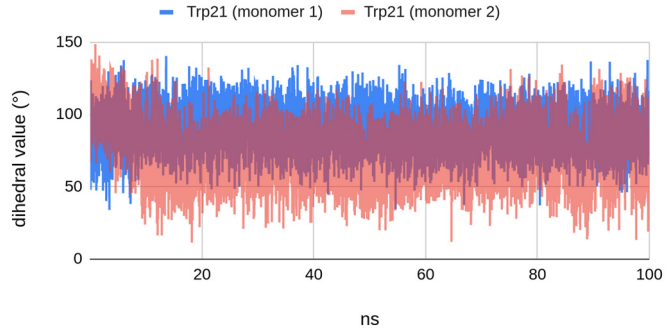
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers in holo form (3)



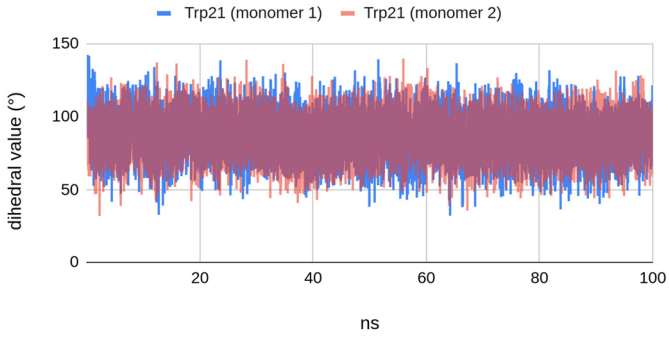
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers with N-γ-ADABA bound (1)



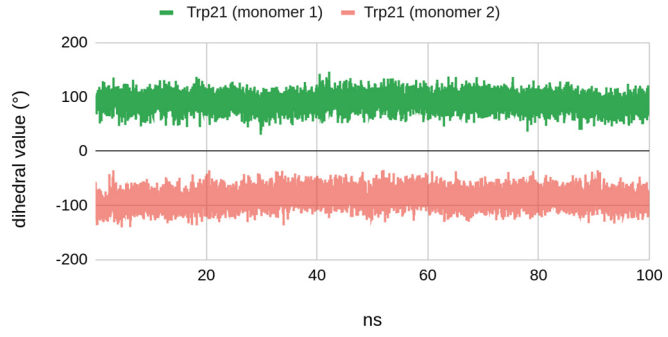
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers with N-γ-ADABA bound (2)



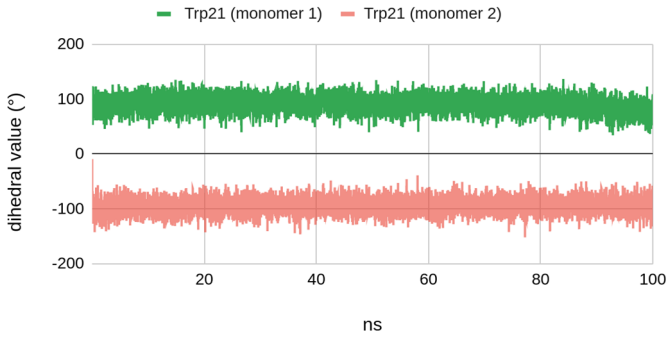
Cα-Cβ-Cγ-Nε dihedral in Trp21 - both monomers with N-γ-ADABA bound (3)



Cα-Cβ-Cγ-Nε dihedral in Trp21 - monomer 1 with N-γ-ADABA bound. monomer 2 in holo form (1)



Cα-Cβ-Cγ-Nε dihedral in Trp21 - monomer 1 with N-γ-ADABA bound. monomer 2 in holo form (2)



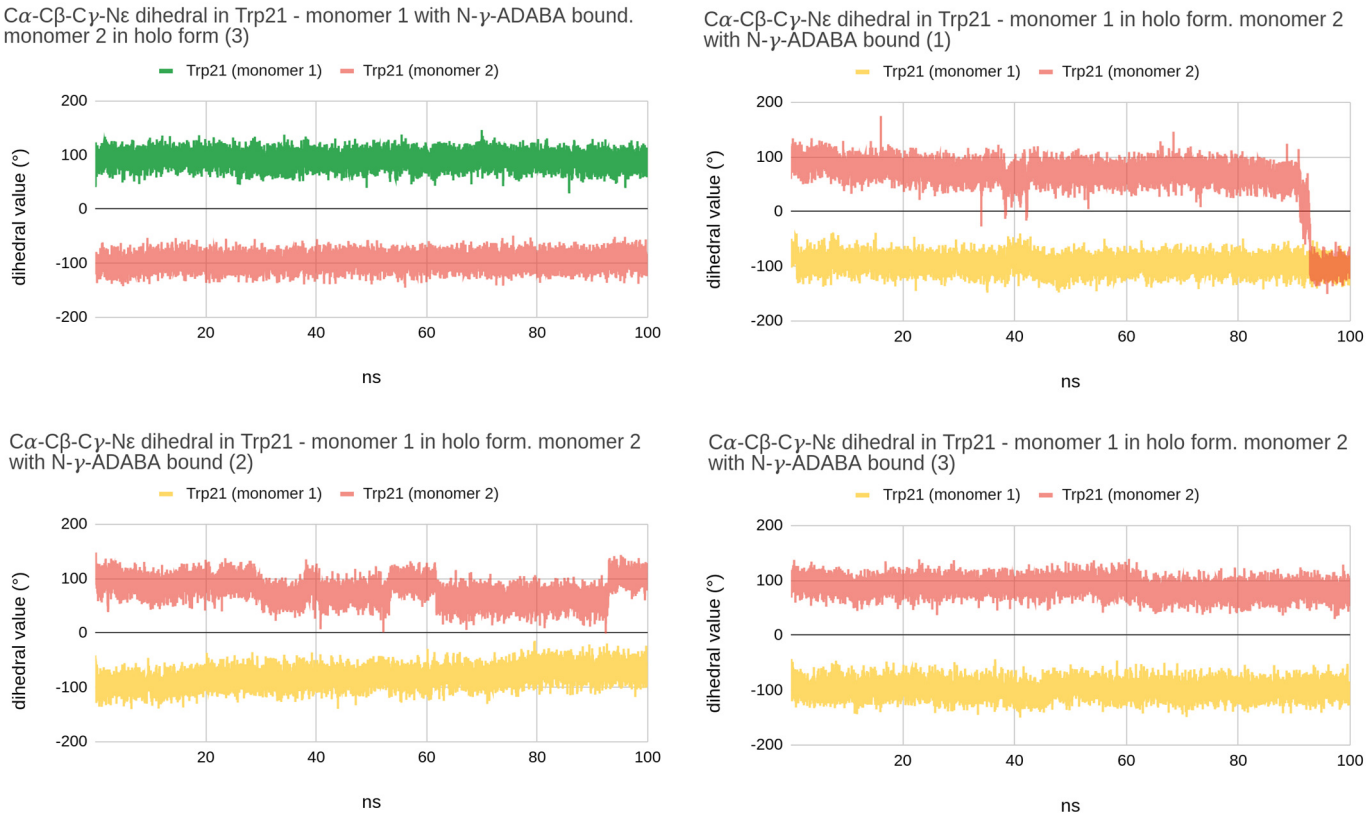


Figure S11. The Cα-Cβ-Cγ-Nε dihedral values for Trp21(B) in MD simulations for the 6-coordinate Fe^{2+} models of EctC dimer. The Cα-Cβ-Cγ-Nε dihedral values calculated for Trp21(B) in both monomers in all twelve MDs for the 6-coordinate Fe $^{2+}$ models of EctC dimer: first 3 MD for the model with both monomers empty, then 3 MD for the model with both monomers occupied, then 3 MD for the model with the first monomer in the complex occupied/second empty and last 3 MD for the model with the first monomer empty/second occupied. Trp-A conformation has values around −100 deg; Trp-B around +90 deg.

Table S1. Force field (bonded model) parameters describing the geometry around Fe^{2+} ion with tetrahedral geometry. Reference (equilibrium) values and force constants of bonds between Fe^{2+} and its ligands are listed in the "Bond" section: O2 stands for the carboxyl oxygen of Glu57, OH for the tyrosyl oxygen of Tyr84, O for the substrate carbonyl oxygen and NB for the -nitrogen atom of His92. Analogously, the "Angle'' section refers to the ε angles involving Fe^{2+}.

BOND	**r_eq [Å]**	**K [kcal/mol Å²]**
O2-FE	2.34	24.00
OH-FE	2.04	71.34
NB-FE	2.16	75.68
O-FE	2.41	24.00
ANGLE	**Θ_eq [deg]**	**K [kcal/mol rad²]**
O2-FE-OH	139.57	13.10
O2-FE-NB	123.16	9.26
O2-FE-O	123.72	12.00
CO-O2-FE	136.58	18.13
OH-FE-NB	107.16	21.00
OH-FE-O	120.72	20.08
C-OH-FE	145.37	9.00
CV-NB-FE	132.59	3.38
NB-FE-O	166.47	3.00
CR-NB-FE	126.40	3.45
FE-O-C	138.13	14.73