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Supporting information for article:

Structural mechanism of *Escherichia coli* cyanase

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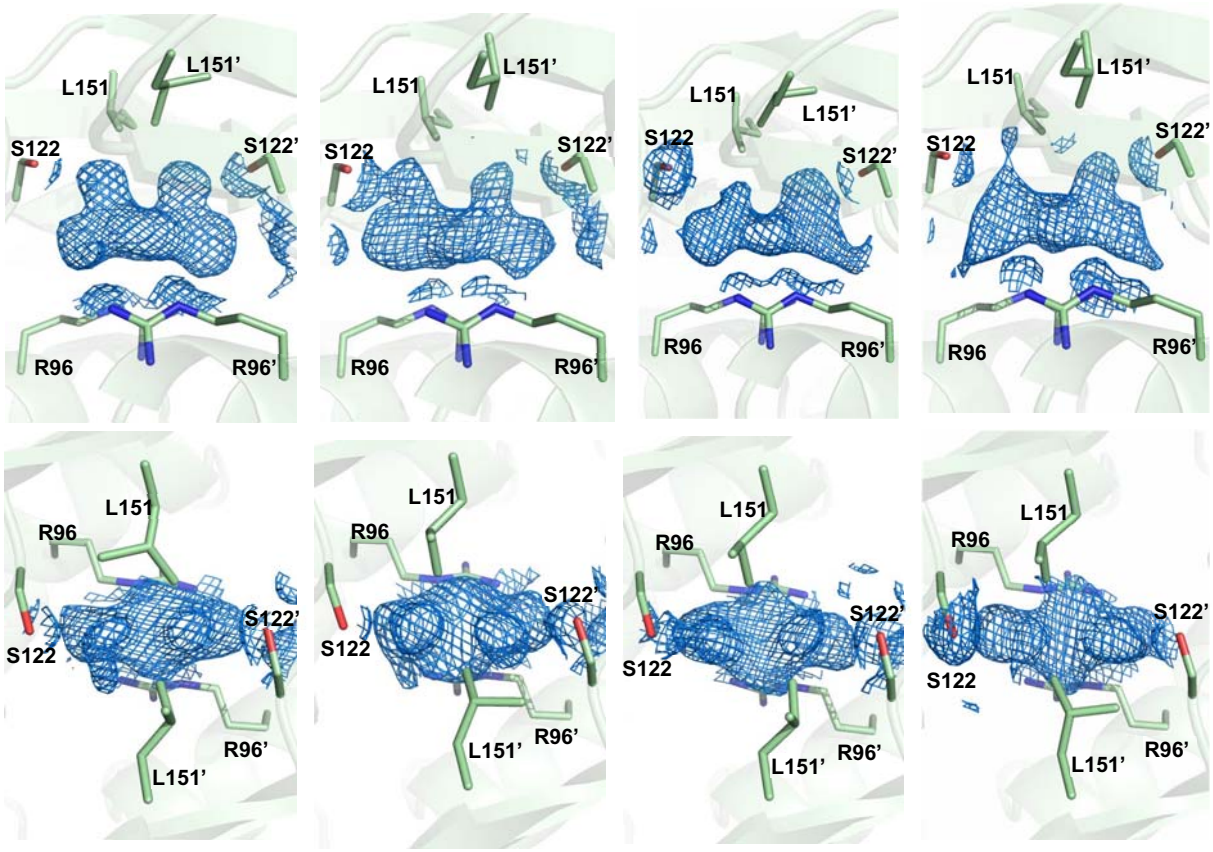

Figure S1 Simulated annealing omit map (blue mesh, 1σ) of the other four active site of native EcCYN (pale green) in two different views.

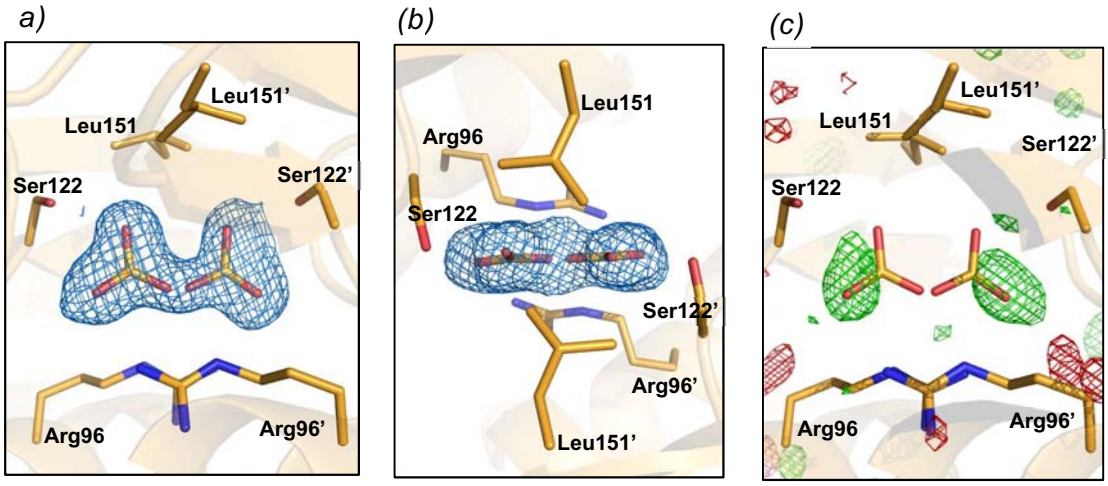

Figure S2 Bicarbonate binding state of EcCYN. (a) Front view of two bicarbonates binding to the active site (b) and top view. The 2Fo-Fc map (blue mesh, 1σ) for the symmetric bicarbonate model is depicted. Each bicarbonate has half occupancy to avoid clashes during refinement. Water molecules have been deleted for clarity. (c) The Fo-Fc map (contoured at ± 3σ and colored in green for positive values and red for negative values) for the half occupancy bicarbonate refined without water.

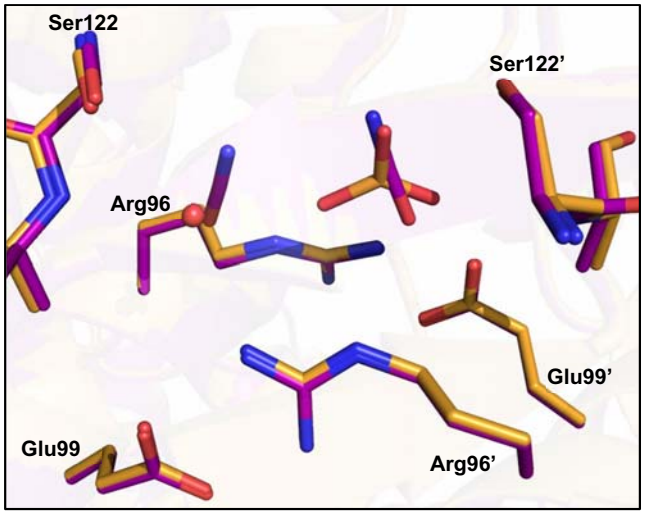

Figure S3 Comparison of cyanate and bicarbonate binding states of EcCYN. Superimposed active sites of EcCYN-cyanate (magenta) and EcCYN-bicarbonate (orange). Only one of the two bicarbonate models is shown for clarity. The Cα atoms of the structures are superimposed.

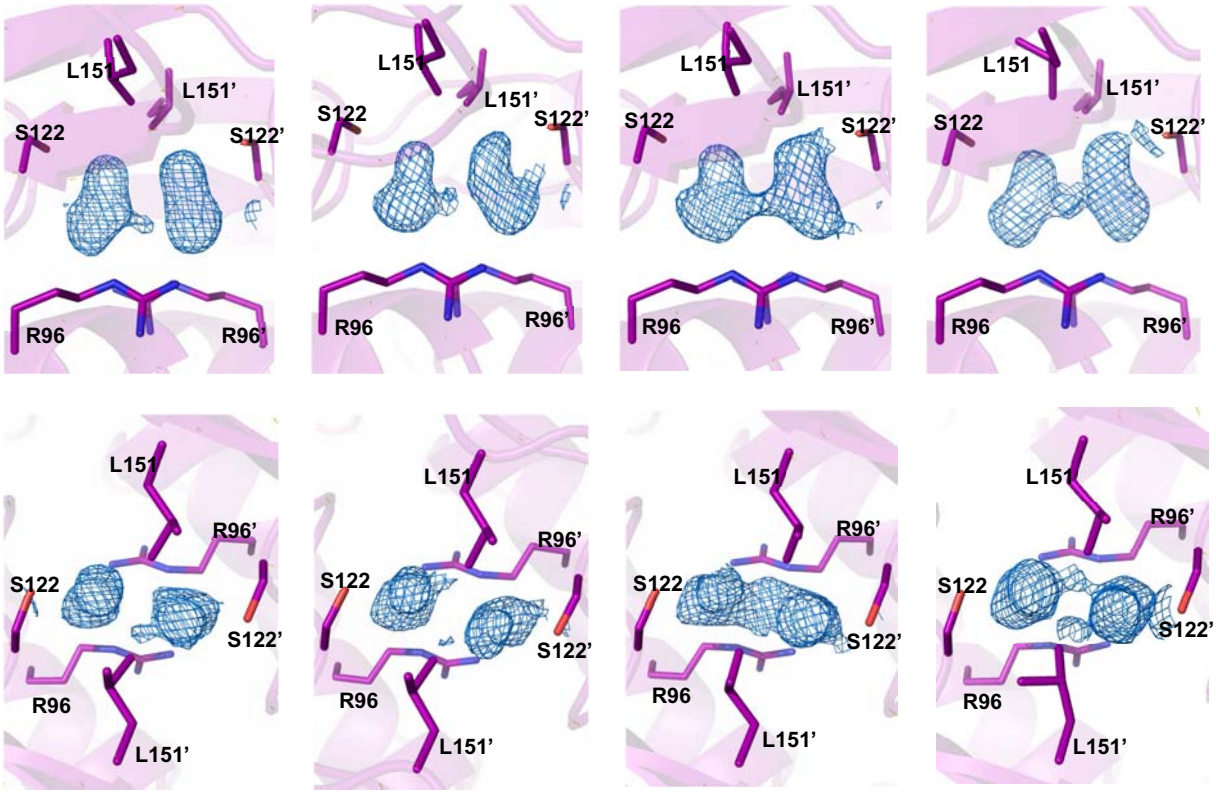

Figure S4 Simulated annealing omit map (blue mesh, 1σ) of the other four active site of EcCYN-cyanate (purple) in two different views**.**

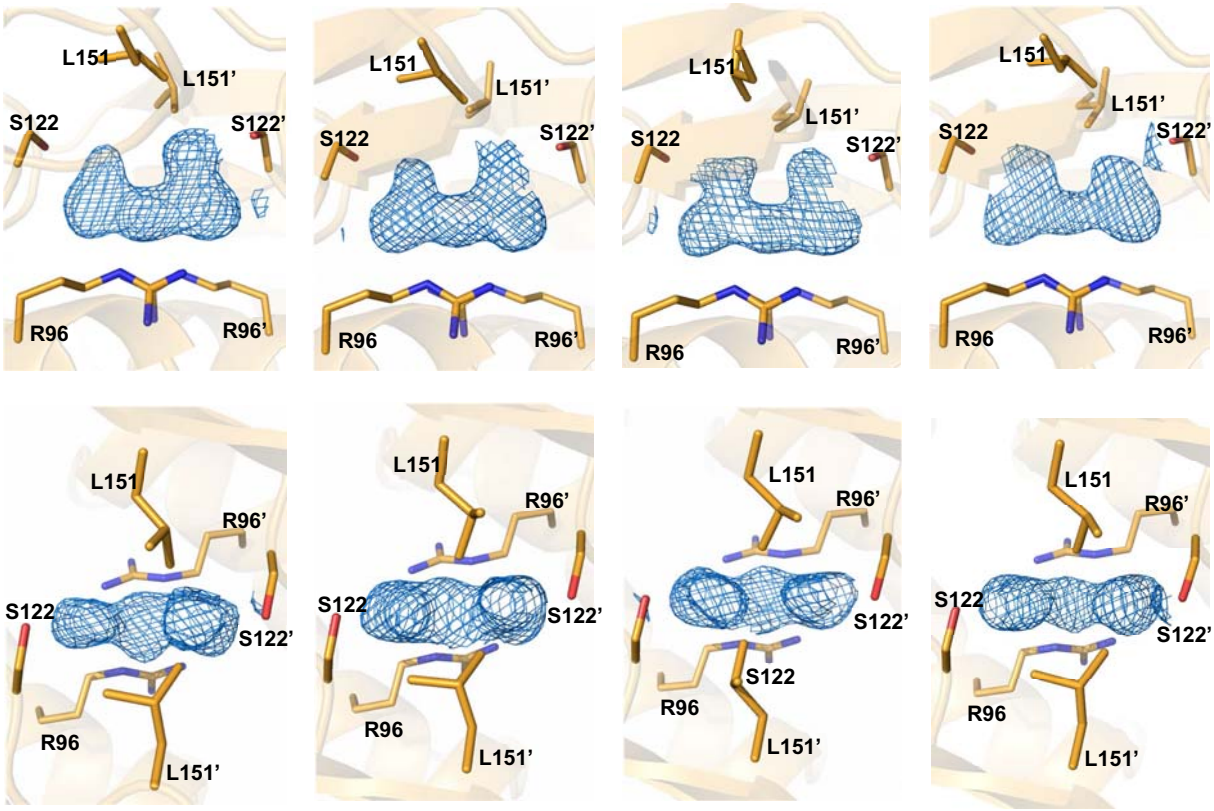

Figure S5 Simulated annealing omit map (blue mesh, 1σ) of the other four active site of EcCYN-bicarbonate (orange) in two different views**.**

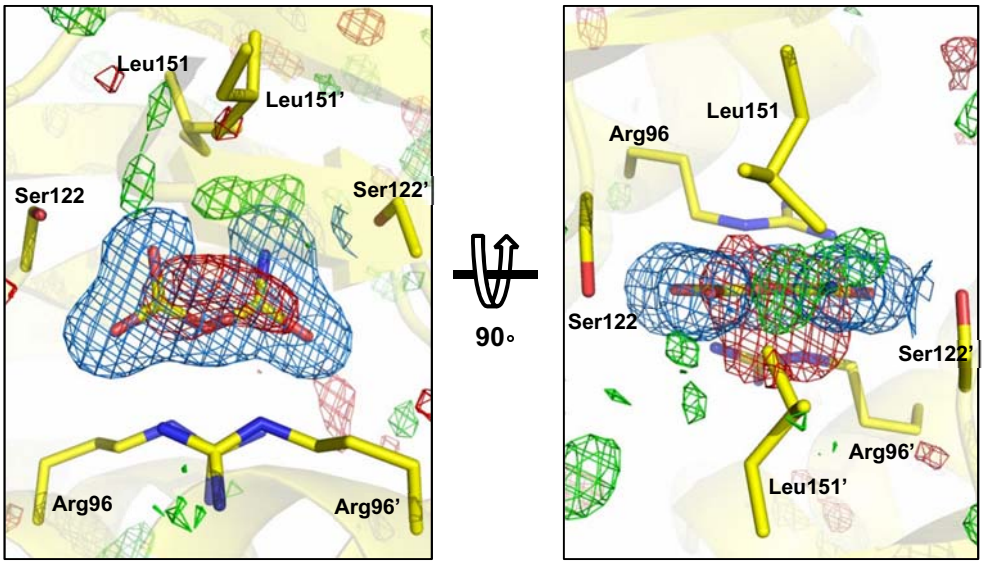

Figure S6 Electron density map of reaction state of EcCYN by SFX at 293K. The $2F_o$-Fc map (blue mesh, 1σ) and F_o-Fc map (green and red mesh for 3σ and -3σ, respectively) of the active site pocket in the EcCYN-reaction-SFX are shown.

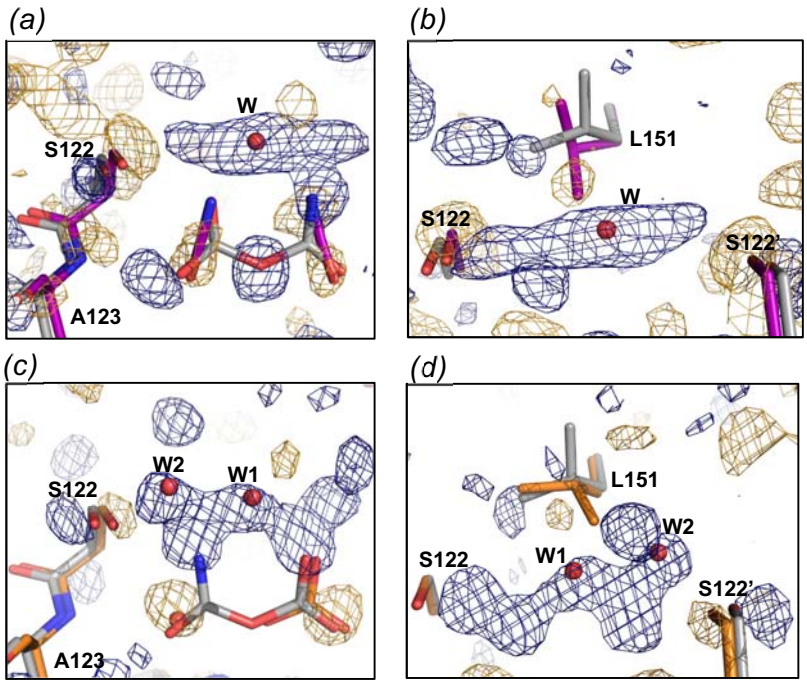

Figure S7

Figure S7 Difference Fourier maps of the EcCYN reaction. (A) ($F_{o(reaction)}$-$F_{o(cyanate)}$ map and superimposed structure of the EcCYN-reaction (gray) with EcCYN cyanate (purple). (B) ($F_{o\ (reaction)}$-$F_{o\ (bicarbonate)}$ map and superimposed structure of EcCYN-reaction (gray) with EcCYN bicarbonate (purple). (C) The $F_{o(reaction)}$-$F_{o(cyanate)}$ map showing the dynamics of Leu151. (D) The $F_{o(reaction)}$-$F_{o(bicarbonate)}$) map showing the dynamics of Leu151. Positive and negative difference Fourier maps are shown in blue (3.0 σ) and yellow (-3.0 σ), respectively. Water molecules are shown as red spheres.

Table S1 The active site residue distances between Ser122, Ala123 and Leu151.

The distances between Leu151 calculated based on the γ-carbon

The distances between Ser122 pair (Å)						
	Active site1	Active site2	Active site3	Active site4	Active site5	Average
EcCYN-native	7.96	8.08	8	7.97	8.01	8.004
EcCYN-cyanate	7.86	7.85	7.92	7.89	7.76	7.856
EcCYN-bicarbonate	8	8.18	8.08	8.05	8.13	8.088
EcCYN-reaction	8.48	8.74	8.49	8.54	8.7	8.59
SFX-EcCYN-reaction	8.46	8.62	8.51	8.84	8.38	8.562
The distances between amide of Ala123 pair (Å)						
	Active site1	Active site2	Active site3	Active site4	Active site5	Average
EcCYN-native	10.79	10.77	10.76	10.71	10.77	10.76
EcCYN-cyanate	10.41	10.32	10.41	10.39	10.32	10.37
EcCYN-bicarbonate	10.84	10.87	10.95	10.8	10.87	10.866
EcCYN-reaction	10.93	10.89	10.92	10.95	10.94	10.926
SFX-EcCYN-reaction	11.13	11.09	11.18	11.26	11.02	11.136

The distances between γ-carbon of Leu151 pair (Å)						
	Active site1	Active site2	Active site3	Active site4	Active site5	Average
EcCYN-native	6.1	5.51	5.28	6.07	5.68	5.728
EcCYN-cyanate	6.22	6.02	6.15	6.04	6.04	6.094
EcCYN-bicarbonate	5.25	5.41	5.12	5.34	5.27	5.278
EcCYN-reaction	5.36	5.35	5.86	5.46	5.35	5.476
SFX-EcCYN-reaction	5.18	4.85	5.05	5.42	5.1	5.12

The distances between Arg96 pair (Å)						
	Active site1	Active site2	Active site3	Active site4	Active site5	Average
EcCYN-native	3.62	3.65	3.65	3.66	3.63	3.642
EcCYN-cyanate	3.46	3.45	3.47	3.49	3.45	3.464
EcCYN-bicarbonate	3.4	3.46	3.48	3.35	3.36	3.41
EcCYN-reaction	3.3	3.36	3.34	3.32	3.47	3.358
SFX-EcCYN-reaction	3.25	3.26	3.23	3.32	3.18	3.248