Supporting Information

Crystal Structures of Quinolinate Synthase in Complex with a Substrate Analogue, the Condensation Intermediate and Substrate-derived Product.

Anne Volbeda, Claudine Darnault, Oriane Renoux, Debora Reichmann, Patricia Amara, Sandrine Ollagnier de Choudens, Juan C. Fontecilla-Camps*

Table S1. Specific quinolinate synthase activity of NadA proteins from *T. maritima* and *E. coli.* Neither *Tm*NadA* Y107F nor *Tm*NadA*-PGH formed QA. The activities of *Tm*NadA and *Ec*NadA were assayed using oxaloacetate and ammonium sulfate as the iminoaspartate (IA) source. Activity values are reported for the same [4Fe-4S] cluster concentration. n.d = not detectable.

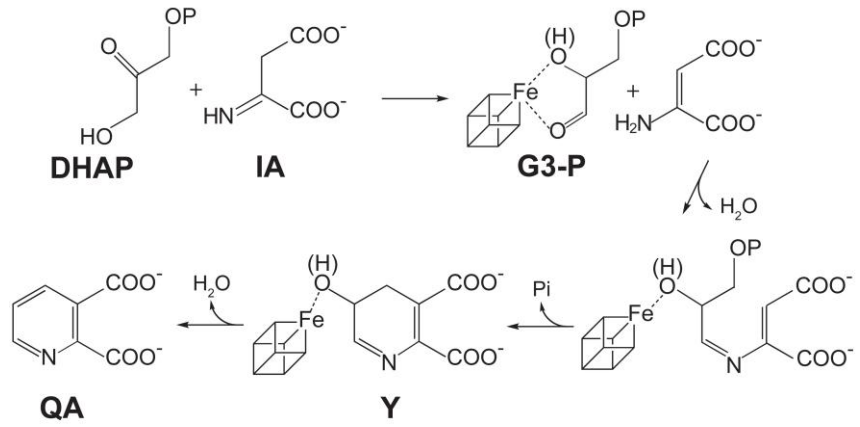
	Specific activity (nmoles/min/mg)
*Tm*NadA*	16.4
*Tm*NadA* Y21F	0.8
*Tm*NadA* Y107F	n.d
PGH-bound *Tm*NadA	n.d
*Ec*NadA	62.4
*Ec*NadA Y139F[1]	n.d
PGH-bound *Ec*NadA	6.1

[1]no activity was detected when using NadB and L-aspartate to produce IA

Table S2. X-ray data and refinement statistics.

Crystal (*Tm*NadA*)	**Y107F-W**	**Y21F-QA**	**Y107F-PGH**	**Y21F-citrate**	**Citrate**
PDB code	5F3D	5LQS	5F33	5LQM	5F35
Data collection:					
ESRF beamline	ID30A3	BM30A	ID30B	ID30B	ID23eh2
Wavelength (Å)	0.96770	0.979736	0.96862	0.96862	0.87260
Cell: a, b, c (Å)	53.9, 49.0, 60.5	52.1, 48.7, 60.3	52.1, 49.2, 60.2	52.8 48.9 60.4	49.0, 49.3, 62.7
Cell: β (°)	105.4	103.7	104.1	104.6	104.3
Resolution (Å)	45.2-1.90	43.7-1.90	37.6-1.45	37.5-1.62	49.3-1.57
(high resolution shell)	(1.96-1.90)	(1.97-1.90)	(1.50-1.45)	(1.68-1.62)	(1.62-1.57)
Measured reflections	98781 (9058)	83943 (7948)	143402 (14225)	87798 (5465)	218575 (18713)
Unique reflections	23542 (2157)	23026 (2228)	51724 (5059)	34681 (2297)	40043 (3641)
Redundancy	4.2 (4.2)	3.6 (3.6)	2.8 (2.8)	2.5 (2.4)	5.5 (5.1)
Completeness (%)	97.0 (89.1)	98.6 (97.8)	98.5 (98.7)	91.0 (62.0)	99.1 (99.1)
R_{merge} (%)	7.0 (89.3)	9.4 (58.8)	4.0 (55.5)	4.4 (45.4)	10.0 (84.1)
$CC_{1/2}$	0.997 (0.573)	0.989 (0.551)	0.998 (0.556)	0.998 (0.490)	0.997 (0.376)
$<I/\sigma_I>$	9.5 (1.4)	15.2 (2.9)	12.0 (1.7)	12.0 (1.8)	9.5 (1.5)
Refinement:					
Resolution (Å)	45.0-1.90	40.0-1.90	30.0-1.45	37.5-1.62	27.8-1.60
Used reflections	22317	21850	49128	32948	36433
R_{work} (%)	17.0	17.6	14.1	14.9	12.8
Reflections for R_{free}	1182	1175	2574	1733	1896
R_{free} (%)	21.1	21.4	18.6	20.1	17.7
Number of atoms	2779	2824	2826	2778	2872
Average B-factor (Å^2)	36.9	21.1	22.3	23.0	19.9
R.m.s. deviations					
Bond lengths (Å)	0.012	0.012	0.011	0.012	0.012
Bond angles (°)	1.5	1.6	1.5	1.5	1.6
Ligand occupancy*	1.00	0.65	0.75	1.00	2 x 0.50
$< B_{ligand} >$ (Å^2)*	45.2	17.5	21.1	21.6	15.4

*lower than 1.00 occupancies and/or higher than average <B>-factors for the ligands (**W**, QA, PGH and citrate) reflect the presence of some structural disorder/heterogeneity.



Scheme S1. Alternative mechanism (to the one presented in **Scheme 1**) for QA synthesis proposed by Begley et al.[1] In this mechanism dephosphorylation takes place after condensation, which is ruled out by our results.

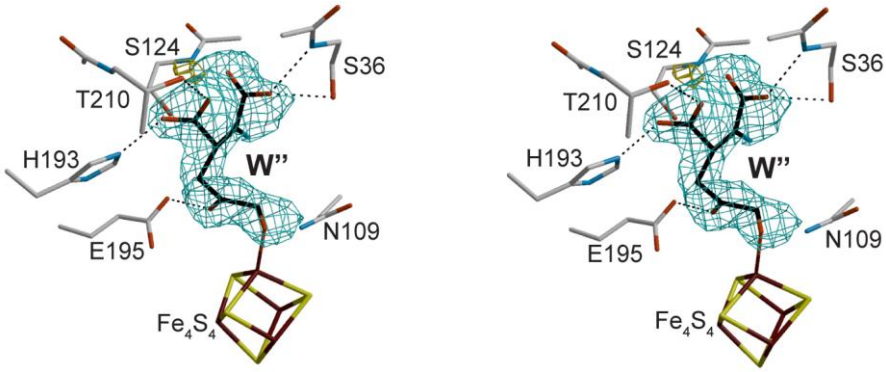
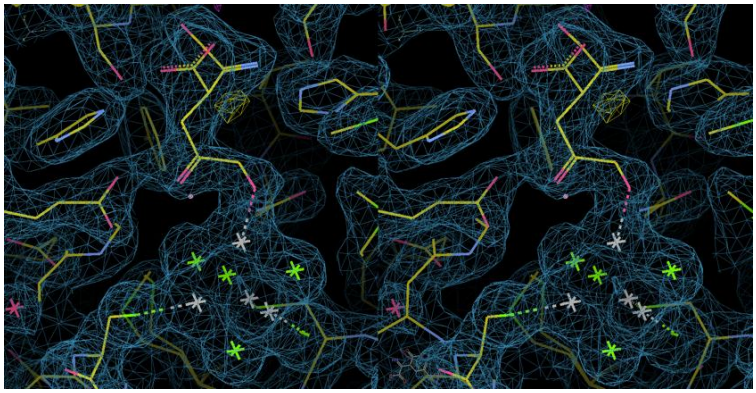
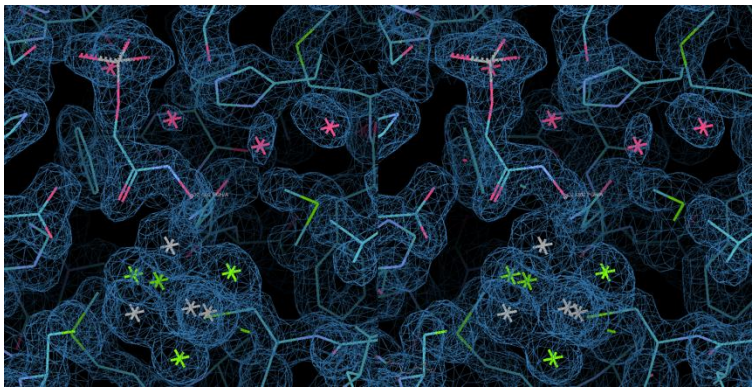


Figure S1. Stereo view of the model and electron density 'omit' map (in blue mesh) of a *Tm*NadA complex with **W"**: this species has been modeled as an alternative conformation of **W** with its N atom positioned in the residual electron density peak that was obtained with **W'** (**Figure 1** and main text). The small brown peak (contoured at 4 σ) represents residual electron density when **W"** is included in the calculated structure factors and phases. It is not possible to determine from the electron density whether the C2-bound group in this species is =NH or $-NH_2$ (or a mixture of the two species).

A



B



C

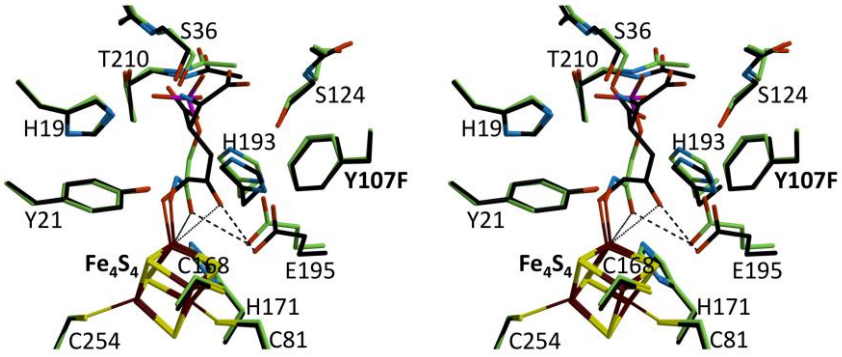


Figure S2. (A) *Tm*NadA* Y107-**W'** complex and matching (2Fo-Fc) electron density map (contoured at 1.0σ). The residual peak (yellow, contoured at 4.0σ) indicates the alternate position for the 2-imino/amino group closer to C6 (see main text). (B) *Tm*NadA* Y107-**PGH** complex and matching (2Fo-Fc) electron density map. (C) Superposition of **W'** (black carbons) and **PGH** (green carbons) crystal structures. In both cases, the –O(H) group, which is similarly oriented, interacts more tightly with the unique iron ion than the >C=O group. The phosphate moiety of **PGH** occupies the position corresponding to mostly one of the carboxylate groups of **W'**.

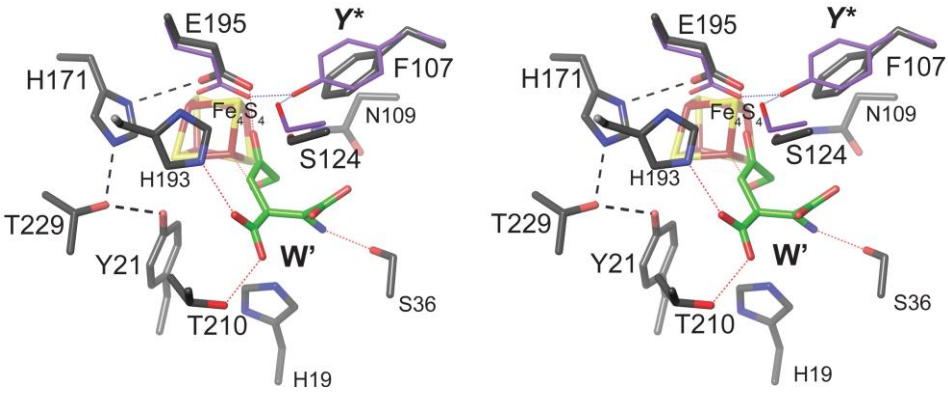


Figure S3. Stereo view of **Figure 8** of main text.

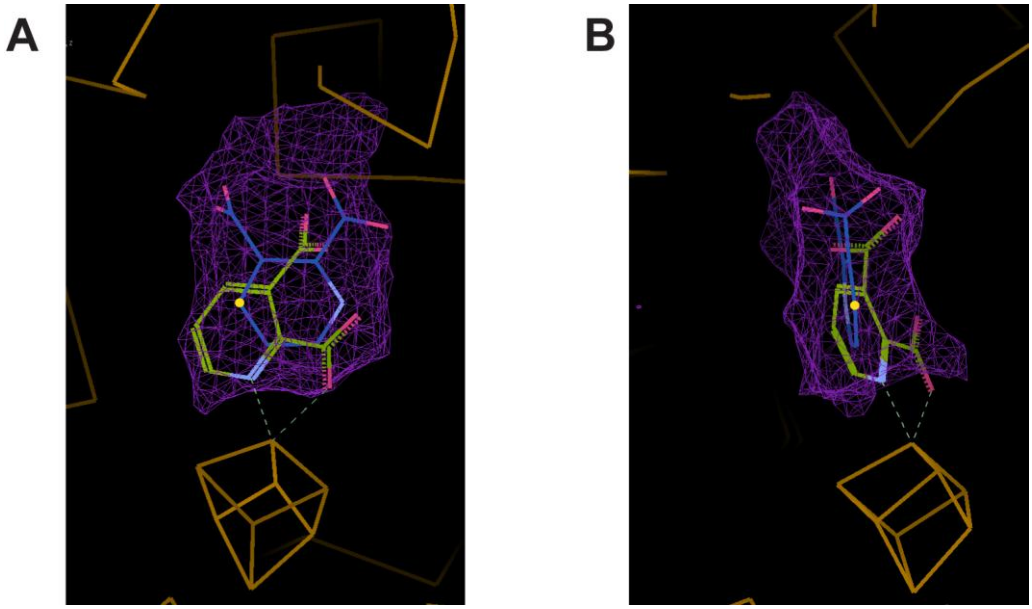


Figure S4. The active site of the *Tm*NadA* Y10F-**W** structure represented by a cavity map calculated with the CAVENV program developed by A. Volbeda and included in the CCP4 package[2] (purple mesh; probe radius = 1.0 Å), with solvent and **W** removed. (A) Superposition of QA bound as in the *Tm*NadA Y21F variant crystal (green carbon atoms, see also **Figure 4**) and its expected position immediately after the second dehydration (**Y** → QA in **Scheme 1**; shown here with blue carbon atoms). (B) The same superposition rotated by 90° about a vertical axis. Two simple rotations of QA, resulting from the observed **W** orientation (see main text and **Figure 1**), bring it to its observed binding mode in the crystal structure (**Figure 4**). An alternative **W** position, rotated about 180° from the one observed in our structure, would locate the QA N1 (**Scheme 1**) at the site corresponding to its C4 (indicated by a yellow dot on the modeled QA structure with dark blue carbon atoms). Starting from that alternative position, a 180° QA rotation about a vertical axis, in addition to the two simple rotations described above, would be required to reproduce its observed binding mode. The first (180°) rotation would cause severe clashes with the protein, as shown by the flattened shape of the active site cavity.

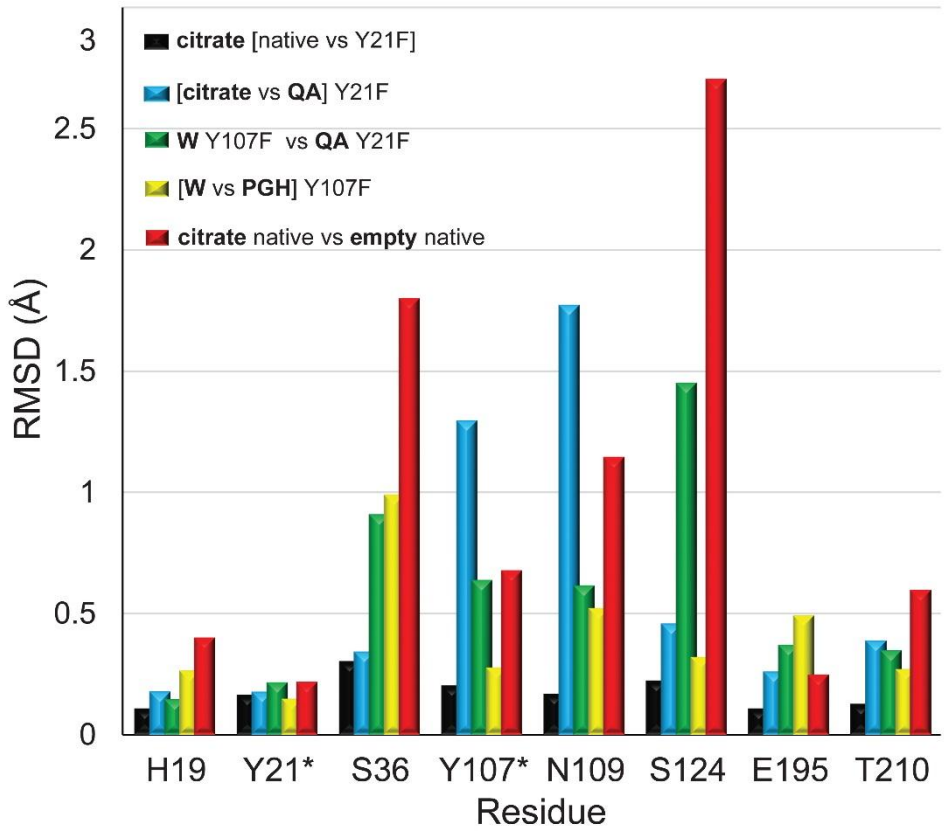


Figure S5. Root-mean square deviation (RMSD) calculated for selected residues at the active site between two forms differing by the nature of the ligand bound and/or the mutation at the active site. Y21* and Y107* are in some models (see inset) mutated into phenylalanine. Structural superpositions and calculations were carried out with the Schrödinger suite.[3] All X-ray models are from this study except for the "empty" K219R form that is the original *Tm*NadA* un-complexed open structure.[4]

References

(1) Begley, T. P., Kinsland, C.; Mehl, R. A.; Osterman, A.; Dorrestein, P. *Vitam. Horm.* **2001**, *61*, 103.
(2) Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G.; McCoy, A.; McNicholas S. J.; Murshudov G. N.; Pannu N. S.; Potterton E. A.; Powell H. R.; Read R. J.; Vagin A.; Wilson K. S. *Acta Crystallogr. D -Biol. Crystallogr.* **2011**, *67*, 235.
(3) Schrödinger, LLC, New York, NY, 2016.
(4) Cherrier, M. V.; Chan, A.; Darnault, C.; Reichmann, D.; Amara, P.; Ollagnier de Choudens, S.; Fontecilla-Camps, J.C. *J. Am. Chem. Soc.* **2014**, *136*, 5253.