Electronic Supplementary Material (ESI) for Chemical Science.

An N···H···N low-barrier hydrogen bond preorganizes the catalytic site of aspartate aminotransferase to facilitate the second half-reaction

Victoria N. Drago,[1] Steven Dajnowicz,[1,2] Jerry M. Parks,[3] Matthew P. Blakeley,[4] David A. Keen,[5] Nicolas Coquelle,[4] Kevin L. Weiss,[2] Oksana Gerlits,[6] Andrey Kovalevsky[2]* and Timothy C. Mueser[1]*

[1]*Department of Chemistry and Biochemistry, University of Toledo, Toledo, OH 43606, U.S.A.*
[2]*Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, U.S.A.*
[3]*Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, U.S.A.*
[4]*Large Scale Structures Group, Institut Laue–Langevin, 71 Avenue des Martyrs, 38000 Grenoble, France*
[5]*ISIS Facility, Rutherford Appleton Laboratory, Harwell Campus, Didcot, OX11 0QX, UK*
[6]*Department of Natural Sciences, Tennessee Wesleyan University, Athens, TN 37303, U.S.A.*

Table S1. Neutron and X-ray data collection and refinement Statistics

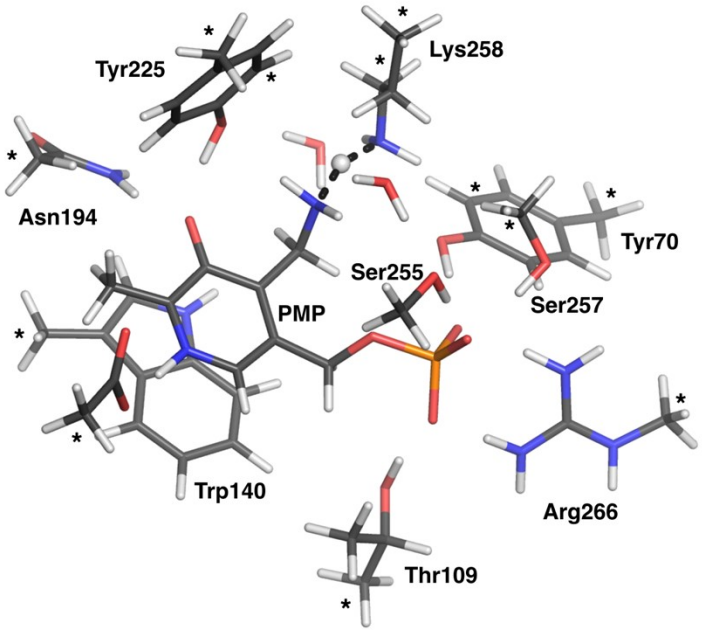
	AAT-PMP PDB ID 7TUR	
Data Collection	**Neutron**	**X-ray**
Beam line	LADI-III, ILL	FIP-BM30A, ESRF
Wavelength(s), Å	3.10 – 4.15	0.97
Temperature (K)	293	293
Resolution (Å)	55.21-2.20 (2.32-2.20)	47.30-1.70 (1.76-1.70)
Space group	P 21 21 21	
Unit cell *a,b,c* (Å)	55.71, 125.03, 130.90	
Unit cell α,β,γ (°)	90.0, 90.0, 90.0	
No. of reflections (total)	182856	292311
No. unique reflections	33902 (4048)	96556 (9507)
Multiplicity	5.4 (4.3)	3.0 (2.2)
Completeness (%)	75.0 (61.6)	95.3 (96.5)
R_{merge}	0.161 (0.308)	0.039 (0.37)
I/σ(I)	7.5 (4.4)	18.3 (3.3)
$CC_{1/2}$	0.988 (0.901)	0.999 (0.685)

Refinement	**Joint X-ray/Neutron**
Resolution (neutron, Å)	40.0 – 2.20
Resolution (X-ray, Å)	40.0 – 1.70
Data rejection criteria	No observation and $\lvert F \rvert = 0$
Sigma cutoff	2.5
No. reflections (neutron)	31861
No. reflections (X-ray)	87497
R_{work} / R_{free} (neutron)	0.237 / 0.265
R_{work} / R_{free} (X-ray)	0.203 / 0.233
R_{work}/R_{free} (joint XN)	0.217 / 0.244
No. of atoms	
Protein, including H/D	13030
Cofactors	67
Water	1128 (*i.e.*, 376 D_2O molecules)
B-factors	
Protein	28.30
Cofactors	24.32
Water	41.96
R.m.s. deviations	
Bond length (Å)	0.008
Bond angles (°)	1.017

Statistics for the highest-resolution shell are shown in parentheses.

Table S2. Geometric parameters of H bonds made by the PMP with the active site residues of AAT. A and B refer to the heavy atoms and D is deuterium.

Joint X-ray/Neutron Structure				
H bond bond / Type	A…D (Å)	D…B (Å)	A…B (Å)	∠ A,D,B (°)
N_{SB}···D···Nζ(Lys258) / cationic	1.4	1.4	2.8	155
N1(PMP)-D···Asp222 / zwitterionic	1.0	1.8	2.7	171
O3′···D-Tyr225 / anionic	1.7	1.0	2.6	172
O3′···D-Asn194 / anionic	1.8	1.0	2.8	169
DFT Calculations (Reactant Structure)				
Bond	A…H (Å)	H…B (Å)	A…B (Å)	∠ A,D,B (°)
N_{SB}-H···Nζ(Lys258) / cationic	1.11	1.56	2.67	177.9
N1(PMP)-H···Asp222 / zwitterionic	1.10	1.49	2.60	175.8
O3′···H-Tyr225 / anionic	1.59	0.99	2.56	164.9
O3′···H-Asn194 / anionic	1.85	1.02	2.86	165.6
DFT Calculations (Transition State Structure)				
Bond	A…H (Å)	H…B (Å)	A…B (Å)	∠ A,D,B (°)
N_{SB}···H···Nζ Lys258 / cationic	1.25	1.32	2.57	179.3
N1(PMP)-H···Asp222 / zwitterionic	1.10	1.51	2.61	176.0
O3′···H-Tyr225 / anionic	1.59	0.99	2.57	166.5
O3′···H-Asn194 / anionic	1.83	1.02	2.83	165.7
DFT Calculations (Product Structure)				
Bond	A…H (Å)	H…B (Å)	A…B (Å)	∠ A,D,B (°)
N_{SB}···H-Nζ Lys258 / cationic	1.58	1.10	2.68	178.3
N1(PMP)-H···Asp222 / zwitterionic	1.09	1.52	2.61	176.1
O3′···H-Tyr225 / anionic	1.60	0.99	2.59	169.2
O3′···H-Asn194 / anionic	1.80	1.03	2.81	166.5



Supplemental Figure 1. Quantum mechanical cluster built from AAT-PMP neutron structure. The 151-atom minimal cluster is composed of PMP, ten active site residues, and two crystallographic waters. The residues were truncated to remove the backbone and methyl capped. From this model, the Lys258-protonated (reactant) and PMP-protonated (product) cluster models were built. Asterisks indicate positions that were constrained in the geometry optimization. The oxygen positions in the crystallographic waters were also frozen.