Substrate Pathways in the Nitrogenase MoFe Protein by Experimental Identification of Small Molecule Binding Sites

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Movie

In this movie, the MoFe protein is rotated to show the location of all pathways discussed in the manuscript, including newly identified and previously published pathways (blue: AI/IS pathway; magenta: AII pathway; forest green: BI pathway; green: CI pathway; orange: DI pathway/interstitial water channel; brown: center water channel; yellow: ammonia egress pathway; red: molecular dynamics pathway). The α subunits of the protein are shown as green and magenta ribbon, and the β subunits are shown in cyan and yellow ribbon. One FeMo-cofactor and P-cluster are shown as ball and sticks colored by element (yellow: sulfur; orange: iron; cyan: molybdenum; red: oxygen; grey: carbon). The two black Fe atoms in the FeMo-cofactor are Fe2 and Fe6, which bind the reversible inhibitor, carbon monoxide, in a bridging fashion. The three large black spheres are Xe binding sites in Av1 and Cp1. For clarity, the pathways, cofactors, and Xe binding sites are only displayed in one αβ.

Table S1. RMSD of MoFe Protein Xe Binding Pockets Compared to Native Structures

	Av1-Xe structure compared to Av1 (1.0 Å, 3U7Q)	Cp1-Xe structure compared to Cp1 (1.08 Å, 4WES)
RMSD of whole protein (Å)	0.192	0.215
RMSD of Xe1 binding site (Å)*	0.153	0.210
RMSD of Xe2 binding site (Å)*	0.151	0.232
RMSD of Xe3 binding site (Å)*	0.119	0.211

* Residues within 6 Å of the Xe site were used in the RMSD calculations.

Table S2. Small Molecules and Ions in Published MoFe Protein Structures

Protein (resolution)	PDB ID	Small molecule (total number observed)	Binding location
Av1 (1.0 Å)	3U7Q[1]	IMD (4)	Surface
		Mg^{2+} (2)	Surface
		IMD	Interior
Av1 (1.5 Å)	4TKV[2]	CO (3)	Surface
			Interior (2)
		S	Interior
Cp1 (1.08 Å)	4WES[3]	MPD	Surface
Kp1 (1.60 Å)	1QGU, 1QH8[4]	Mg^{2+} (5)	Surface
		EDO (14)	Surface
		EDO	Surface
		EDO	Interior

Table S3. Residues Involved in Proposed Pathways from Previous and Current Studies

Pathway target	Pathway	Residues lining pathway (Av1 numbering)	
FeMo-cofactor	AI	α	Val70, Val71, Trp72, Ile75, His195, His196, Asn199, Asp200, Val202, Arg203, Tyr229, Trp253, Ser254, Cys275, Ser278, Met279, Tyr281, Ile282
	AII	α	Ile59, Tyr354, Ile355, Glu380, Gly422, Ser423, Gly424, Lys426, Glu427, Arg439, Glu440, Met441, His442, Asp445, Ser447, Tyr450, Phe459, Asp462, Met463
		β'	Thr360
	BI	α	Ala65, Gly66, Gly69, Val70, Gln90, Tyr91, Ser92, Arg93, Ala94, Gly95, Arg96, Gln191, His195
		β	Ala67, Lys68, Ala69, Leu67, Tyr102, Arg105, His106, Phe107, His193, Phe230, Thr232, Gly368, Asp369, Phe372, Asn445, Tyr447, Gly470, Phe471, Pro472, Phe474, Thr484
P-cluster	CI	α	Gly61, Cys62, Tyr64, Ala65, Gln90, Tyr91, Ser92
		β	Ala67, Lys68, Ala69, Leu77, Gly94, Ala97, Tyr98, Ser100, Tyr102, Arg105, His106, Phe107, His193, Phe230, Glu231, Gly368, Asp369, Phe372, Asn445, Tyr447, Gly470, Phe471, Pro472, Phe474, Thr484
FeMo-cofactor	Interstitial water channel[5]/DI	α	Lys68, Gly69, Gly73, Ser92, Arg93, Ala94, Gly95, Arg96, Arg97, Asn98, Tyr99, Tyr100, Ile101, Thr104, Val110, Thr111, Ile231, His442, Ser443, Tyr446
		β	Leu16, Lys21, Leu24, Arg28, Tyr98, Arg105, Phe450, Arg453
		β'	Gln513, Ala514, Asp516, Tyr517, Asn518, His519, Asp520, Leu521, Val522
	IS	α	Val70, Val71, Trp72, Ile75, Met78, Arg96, Val179, His195, Val202, Trp205, Lys209, Tyr229, Trp253, Ile262, Glu263, Met279
	NH_3 egress[6]	α	Gln53, Met57, Thr58, Ile59, Arg60, Ala65, Gln191, Glu380, Asp403, Lys426
		β	Gln93, Gly94, Ser115, Ser117
	MD simulation[7]	α	Asn49, Gly66, Val70, Ser190, Gln191, Ser192, Leu193, His195, His196, Asn199, Arg277, Ser278, Met279, Asn280, Tyr281, Gly357, Phe381, Ala382, His383

Table S4. IMD, CO, S, and EDO Binding Sites in Av1 and Kp1

Crystal (PDB ID)	Site	Displaced species in native protein	Occupancy (%)	Distance* to (Å)	
				FeMo-cofactor	surface
Av1 (3U7Q)	IMD	HOH	100	11.5	11.6
Av1 (4TKV)	CO	HOH	60%	17.0	8.7
Av1 (4TKV)	S	Empty pocket	100%	19.0	10.9
Kp1 (1QGU)	EDO	HOH	100	11.6	10.1

* Distances were measured from the closest substrate atom to the closest metal in the FeMo-cofactor or to the closest non-solvent atom on the protein surface.

Table S5. Conservation of Specific Residues and Residue Type in Av1 and Cp1

Residue conservation	Native protein			Xe/PRL binding pockets and AI, AII, BI, CI pathways
	All residues	Non-surface residues	Surface residues	
Conservation of specific residue	38.0%	38.4%	24.8%	62.5%
Conservation of residue type (hydrophobic or hydrophilic)	73.7%	63.4%	53.2%	85.6%

Table S6. Close Contacts and Residue Conservation for Av1-Xe1 (Protein Interior)

	Residue	**Atom**	**Distance from Xe (Å)**	**Corresponding residue in Cp1**
1	α-Val71	CG1	3.88	α-Val60
2	α-Trp72	CE3	4.48	α-Met61
3	α-Ile75	CD1	3.29	α-Ile64
4	α-Ala198	O	5.95	α-Ala187
5	α-Val202	CG2	3.59	α-Val191
6	α-Trp253	CE3	4.34	α-Leu238
7	α-Ser254	CB	4.31	α-Thr239
8	α-Ile262	CD1	4.59	α-Val247
9	α-Met279	CE	4.49	α-Ile264
Hydrophobic residues: 89% Conservation of specific residue: 44% Conservation of residue type*: 100%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S7. Close Contacts and Residue Conservation for Av1-Xe2 (Protein Surface)

	Residue	**Atom**	**Distance from Xe (Å)**	**Corresponding residue in Cp1**
1	α-His196	CD2	5.20	α-His185
2	α-Asn199	O	3.98	α-Asn188
3	α-Asp200	OD1	3.08	α-Asn189
4	α-Arg203	NE	4.17	α-Met192
5	α-Tyr281	O	3.67	α-Tyr266
6	α-Ile282	CD1	3.87	α-Ile267
7	α-His285	CB	4.18	α-Met270
Hydrophobic residues: 29% Conservation of specific residue: 57% Conservation of residue type*: 71%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S8. Close Contacts and Residue Conservation for Av1-Xe3 (Protein Surface)

	Residue	**Atom**	**Distance from Xe (Å)**	**Corresponding residue in Cp1**
1	β-Tyr233	OH	5.24	β-Gly180
2	β-Arg468	O	5.61	β-Arg408
3	β-Ile469	CD1	4.36	β-Phe409
4	β-Ser482	CB	4.50	β-Asn422
5	β-Thr483	C	4.81	β-Pro423
6	β-Thr484	O	3.92	β-Lys424
7	β-Gly489	O	5.10	β-Gly429
8	β-Gln492	CB	4.19	β-Arg432
9	β-Ile493	CG1	3.83	β-Leu433
10	β-Thr496	OG1	4.44	β-Glu436
Hydrophobic residues: 40% Conservation of specific residue: 20% Conservation of residue type*: 90%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S9. Close Contacts and Residue Conservation for Cp1-Xe1 (Protein Surface)

	Residue	**Atom**	**Distance from Xe (Å)**	**Corresponding residue in Av1**
1	α-Asp107	OD2	5.84	α-Asp117
2	α-Lys120	CE	5.99	α-Lys130
3	α-Glu127	OE1	5.40	α-Glu137
4	β-Arg12	O	3.81	β-Arg59
5	β-Lys13	C	4.11	β-Glu60
6	β-Ala14	N	3.93	β-Ala61
7	β-Leu15	O	3.51	β-Leu62
8	β-Arg16	NH1	3.73	β-Thr63
9	β-Ile17	N	5.81	β-Val64
10	β-Glu371	O	5.84	β-Gly424
11	β-Asp373	OD2	5.09	β-Asp426
Hydrophobic residues: 27% Conservation of specific residue: 64% Conservation of residue type*: 91%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S10. Close Contacts and Residue Conservation for Cp1-Xe2 (Protein Surface)

	Residue	**Atom**	**Distance from Xe (Å)**	**Corresponding residue in Av1**
1	α-Asp254	O	3.57	α-Lys269
2	α-Leu255	CB	4.30	α-Leu270
3	α-Pro278	CG	5.41	α-Pro293
4	α-Cys300	O	3.38	α-Lys315
5	α-Phe301	CZ	3.84	α-Phe316
6	α-Val406	CG1	3.90	n/a
7	α-Ile407	CD1	4.18	n/a
Hydrophobic residues: 71% Conservation of specific residue: 43% Conservation of residue type*: 80%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S11. Close Contacts and Residue Conservation for Cp1-Xe3 (Protein Interior)

	Residue	Atom	Distance from Xe (Å)	Corresponding residue in Av1
1	α-Phe460	CE1	4.63	α-Gly422
2	α-Lys477	CG	3.95	α-Arg439
3	α-Leu479	CD2	5.55	α-Met441
4	α-Asp483	OD2	5.94	α-Asp445
5	α-Tyr488	OH	3.61	α-Tyr450
6	α-Asn496	O	5.39	α-Ile458
7	α-Phe497	CD1	3.29	α-Phe459
8	α-Gly498	N	5.37	α-Ala460
9	α-Glu500	CB	3.83	α-Asp462
10	α-Leu501	N	3.71	α-Met463
Hydrophobic residues: 60% Conservation of specific residue: 30% Conservation of residue type*: 90%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S12. Close Contacts for Cp1-PRL

	Residue	Atom	Distance from ligand (Å)	Corresponding residue in Av1
1	β-Tyr30	CZ	3.7	β-Leu77
2	β-His59	NE2	3.7	β-His106
3	β-Phe60	CZ	3.8	β-Phe107
4	β-Phe178	CE2	3.4	β-Phe230
5	β-Val179	CA	3.5	β-Glu231
6	β-Gly180	C3	3.4	β-Thr232
7	β-Asp183	OD2	4.1	β-Asn236
8	β-Glu323	OE2	3.0	β-Phe375
9	β-Gly410	O	3.8	β-Gly470
10	β-Phe411	C1	3.8	β-Phe471
11	β-Lys424	NZ	2.7	β-Thr424
Conservation of specific residue: 46% Conservation of residue type*: 73%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S13. Close Contacts for Av1-IMD

	Residue	Atom	Distance from ligand (Å)	Corresponding residue in Cp1
1	α-Ala94	O	4.1	α-Gly85
2	α-Gly95	N	5.2	α-Gly86
3	α-Arg96	O	3.1	α-Arg87
4	α-Arg97	CA	3.9	α-Arg88
5	α-Asn98	ND2	3.9	α-Phe89
6	α-Tyr99	CE1	3.3	α-Lys90
7	α-Tyr100	CE2	4.2	α-Pro91
8	α-Val110	CG1	4.2	α-Asn102
9	α-Thr111	CG2	3.9	α-Glu103
10	β'-Asp516	O	5.7	β'-Glu451
11	β'-Tyr517	C5	4.1	β'-Glu452
12	β'-Asn518	N	6.0	β'-Asp453
13	β'-His519	C	5.4	β'-Phe454
14	β'-Asp520	OD1	3.7	β'-Glu455
15	β'-Leu521	N	4.9	β'-Val456
Conservation of specific residue: 20% Conservation of residue type*: 60%				

***** Residues are categorized as hydrophilic or hydrophobic

Table S14. Residues Lining the AI Pathway in Av1 and Cp1

AI pathway in Av1	**AI pathway in Cp1**
α-Val70	α-Val59
α-Val71	α-Val60
α-Trp72	α-Met61
α-Ile75	α-Ile64
α-His195	α-His184
α-His196	α-His185
α-Asn199	α-Asn188
α-Asp200	α-Asn189
α-Val202	α-Val191
α-Arg203	α-Met192
α-Tyr229	α-Tyr214
α-Trp253	α-Leu238
α-Ser254	α-Thr239
α-Cys275	α-Cys260
α-Ser278	α-Ser263
α-Met279	α-Ile264
α-Tyr281	α-Tyr266
α-Ile282	α-Ile267
Conservation of specific residue: 67% Conservation of residue type*: 94%	

***** Residues are categorized as hydrophilic or hydrophobic

Table S15. Residues Lining the AII Pathway in Cp1 and Av1

AII pathway in Av1	**AII pathway in Cp1**
α-Ile59	α-Ala48
α-Tyr354	α-Tyr340
α-Ile355	α-Val341
α-Glu380	α-Glu366
α-Gly422	α-Phe460
α-Ser423	α-Ala461
α-Gly424	α-Gly462
α-Lys426	α-Lys464
α-Glu427	α-Glu465
α-Arg439	α-Lys477
α-Glu440	α-Gln478
α-Met441	α-Leu479
α-His442	α-His480
α-Asp445	α-Asp483
α-Ser447	α-Asn485
α-Tyr450	α-Tyr488
α-Phe459	α-Phe497
α-Asp462	α-Glu500
α-Met463	α-Leu501
β'-Thr360	β'-Gln308
Conservation of specific residue: 45% Conservation of residue type*: 90%	

***** Residues are categorized as hydrophilic or hydrophobic

Table S16. Residues Lining the BI Pathway in Cp1 and Av1

BI pathway in Av1	BI pathway in Cp1
α-Ala65	α-Ala54
α-Gly66	α-Gly55
α-Gly69	α-Gly58
α-Val70	α-Val59
α-Gln90	α-Phe79
α-Tyr91	α-Tyr80
α-Ser92	α-Thr81
α-Arg93	α-Trp82
α-Ala94	α-Gly83
α-Gly95	α-Gly84
α-Arg96	α-Arg85
α-Gln191	α-Gln180
α-His195	α-His184
β-Ala67	β-Ala20
β-Lys68	β-Lys21
β-Ala69	β-Thr22
β-Leu67	β-Tyr30
β-Tyr102	β-Val55
β-Arg105	β-Arg58
β-His106	β-His59
β-Phe107	β-Phe60
β-His193	β-His146
β-Phe230	β-Phe178
β-Thr232	β-Val179
β-Gly368	β-Gly319
β-Asp369	β-Asp320
β-Phe372	β-Glu323
β-Asn445	β-Asn392
β-Tyr447	β-Tyr394
β-Gly470	β-Gly410
β-Phe471	β-Phe411
β-Pro472	β-Pro412
β-Phe474	β-Met414
β-Thr484	β-Lys424
Conservation of specific residue: 68% Conservation of residue type*: 82%	

***** Residues are categorized as hydrophilic or hydrophobic

Table S17. Residues Lining the CI Pathway in Cp1 and Av1

CI pathway in Av1	CI pathway in Cp1
α-Gly61	α-Gly50
α-Cys62	α-Cys51
α-Tyr64	α-Tyr53
α-Ala65	α-Ala54
α-Gln90	α-Phe79
α-Tyr91	α-Tyr80
α-Ser92	α-Thr82
β-Ala67	β-Ala20
β-Lys68	β-Lys21
β-Ala69	β-Thr22
β-Leu77	β-Tyr30
β-Gly94	β-Gly47
β-Ala97	β-Ser50
β-Tyr98	β-Tyr51
β-Ser100	β-Thr54
β-Tyr102	β-Val55
β-Arg105	β-Arg58
β-His106	β-His59
β-Phe107	β-Phe60
β-His193	β-His146
β-Phe230	β-Phe178
β-Glu231	β-Val179
β-Gly368	β-Gly319
β-Asp369	β-Asp320
β-Phe372	β-Glu323
β-Asn445	β-Asn392
β-Tyr447	β-Tyr394
β-Gly470	β-Gly410
β-Phe471	β-Phe411
β-Pro472	β-Pro412
β-Phe474	β-Met414
β-Thr484	β-Lys424
Conservation of specific residue: 66% Conservation of residue type*: 81%	

***** Residues are categorized as hydrophilic or hydrophobic

Table S18. Residues Lining the DI Pathway/Interstitial Water Channel in Cp1 and Av1

DI pathway in Av1	DI pathway in Cp1
α-Lys68	α-Lys57
α-Gly69	α-Gly58
α-Gly73	α-Gly62
α-Ser92	α-Thr81
α-Arg93	α-Trp82
α-Arg96	α-Arg85
α-Arg97	α-Arg86
α-Asn98	α-Ser89
α-Tyr99	α-Lys90
α-Tyr100	α-Pro91
α-Ile101	α-Glu92
α-Thr104	α-Thr95
α-Val110	α-Asn100
α-Thr111	α-Glu101
α-Ile231	α-Ile216
α-His442	α-His480
α-Ser443	α-Ser481
α-Tyr446	α-Tyr482
β-Leu16	n/a
β-Lys21	n/a
β-Leu24	n/a
β-Arg28	n/a
β-Tyr98	β-Tyr50
β-Arg105	β-Arg57
β-Phe450	β-Phe397
β-Arg453	β-Arg400
β'-Gln513	n/a
β'-Ala514	β'-Cys449
β'-Asp516	β'-Glu451
β'-Tyr517	β'-Glu452
β'-Asn518	β'-Asp453
β'-His519	β'-Phe454
β'-Asp520	β'-Glu455
β'-Leu521	β'-Val456
β'-Val522	β'-Val457
Conservation of specific residue: 50% Conservation of residue type*: 87%	

***** Residues are categorized as hydrophilic or hydrophobic

Table S19. Residues Lining the IS Pathway in Av1 and Cp1

IS pathway in Av1	IS pathway in Cp1
α-Val70	α-Val59
α-Val71	α-Val60
α-Trp72	α-Met61
α-Ile75	α-Ile64
α-Met78	α-Met67
α-Arg96	α-Arg85
α-Val179	α-His168
α-His195	α-His184
α-Val202	α-Val191
α-Trp205	α-Glu194
α-Lys209	α-Lys198
α-Tyr229	α-Tyr214
α-Trp253	α-Leu238
α-Ile262	α-Val247
α-Glu263	α-Gln248
α-Met279	α-Ile264
Conservation of specific residue: 56% Conservation of residue type*: 94%	

***** Residues are categorized as hydrophilic or hydrophobic

Table S20. Residues Lining Dance's NH_3 Egress Pathway in Av1 and Cp1[6]

NH_3 egress pathway in Av1	**NH_3 egress pathway in Cp1**
α-Gln53	α-Val42
α-Met57	α-Ile46
α-Thr58	α-Thr47
α-Ile59	α-Ala48
α-Arg60	α-Arg49
α-Ala65	α-Ala54
α-Gln191	α-Gln180
α-Glu380	α-Glu366
α-Asp403	α-Asp441
α-Lys426	α-Lys464
β-Gln93	β-Gln46
β-Gly94	β-Gly47
β-Ser115	β-Thr68
β-Ser117	β-Ser70
Conservation of specific residue: 71% Conservation of residue type*: 93%	

***** Residues are categorized as hydrophilic or hydrophobic

Table S21. Residues Lining Smith's Molecular Dynamics Pathway in Cp1 and Av1[7]

MD pathway in Av1	MD pathway in Cp1
α-Asn49	α-Asn38
α-Gly66	α-Gly55
α-Val70	α-Val59
α-Ser190	α-Ser179
α-Gln191	α-Gln180
α-Ser192	α-Ser181
α-Leu193	α-Ala182
α-His195	α-His184
α-His196	α-His185
α-Asn199	α-Asn188
α-Arg277	α-Arg262
α-Ser278	α-Ser263
α-Met279	α-Ile264
α-Asn280	α-Asn265
α-Tyr281	α-Tyr268
α-Gly357	α-Gly343
α-Phe381	α-Phe367
α-Ala382	α-Ala368
α-His383	α-His369
Conservation of specific residue: 90% Conservation of residue type*: 100%	

***** Residues are categorized as hydrophilic or hydrophobic

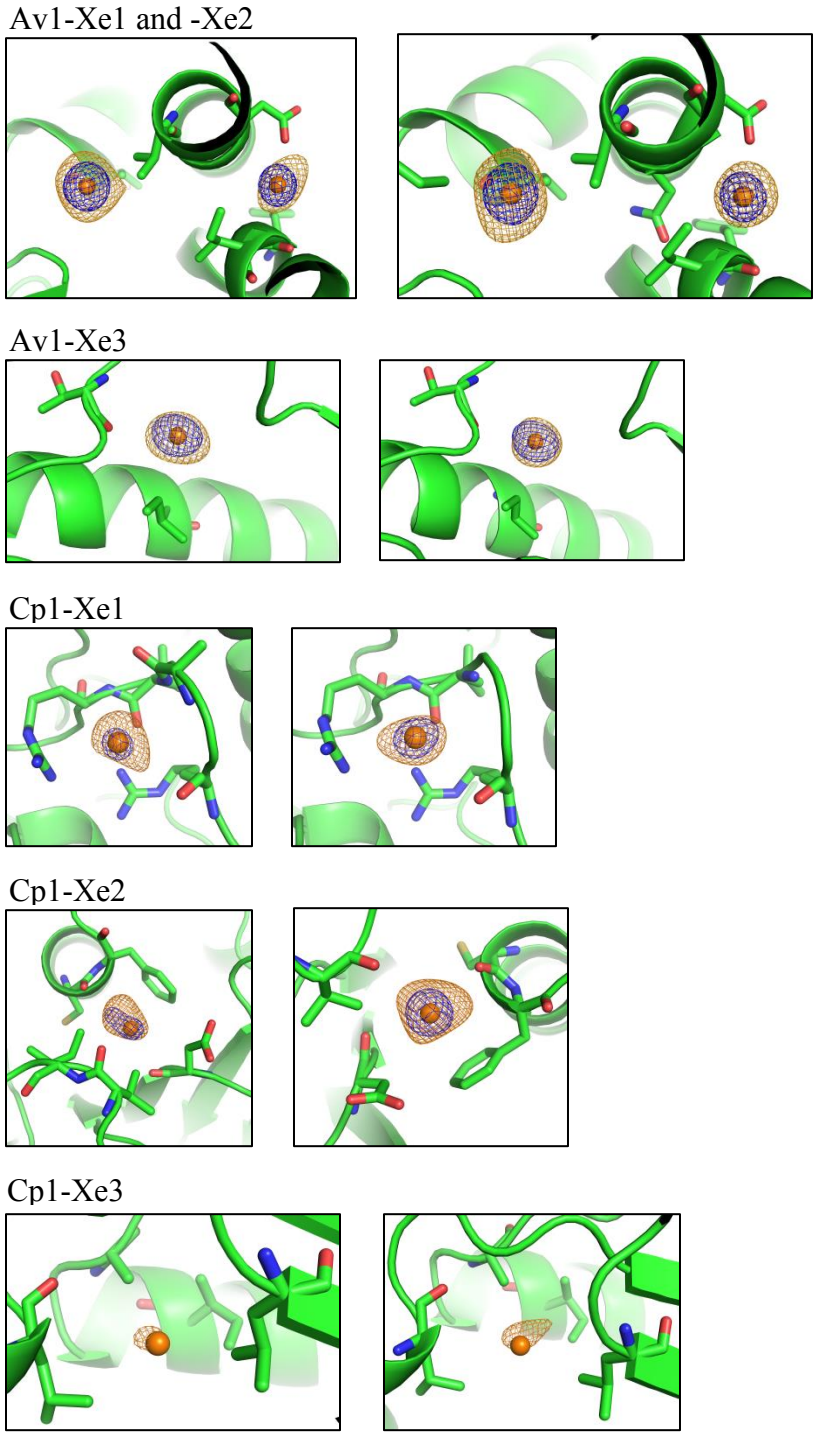

Figure S1. Electron density maps (blue mesh) and anomalous difference maps (orange mesh) for each Xe binding site, including noncrystallographically-related pairs, and their surrounding environment. Both maps are contoured to 3 σ. Residues with at least one atom that is 4 Å or closer to the Xe atom are shown in sticks. Note that the electron density for Cp1-Xe3 is not visible above the orange Xe atom at 3 σ due to low site occupancy.

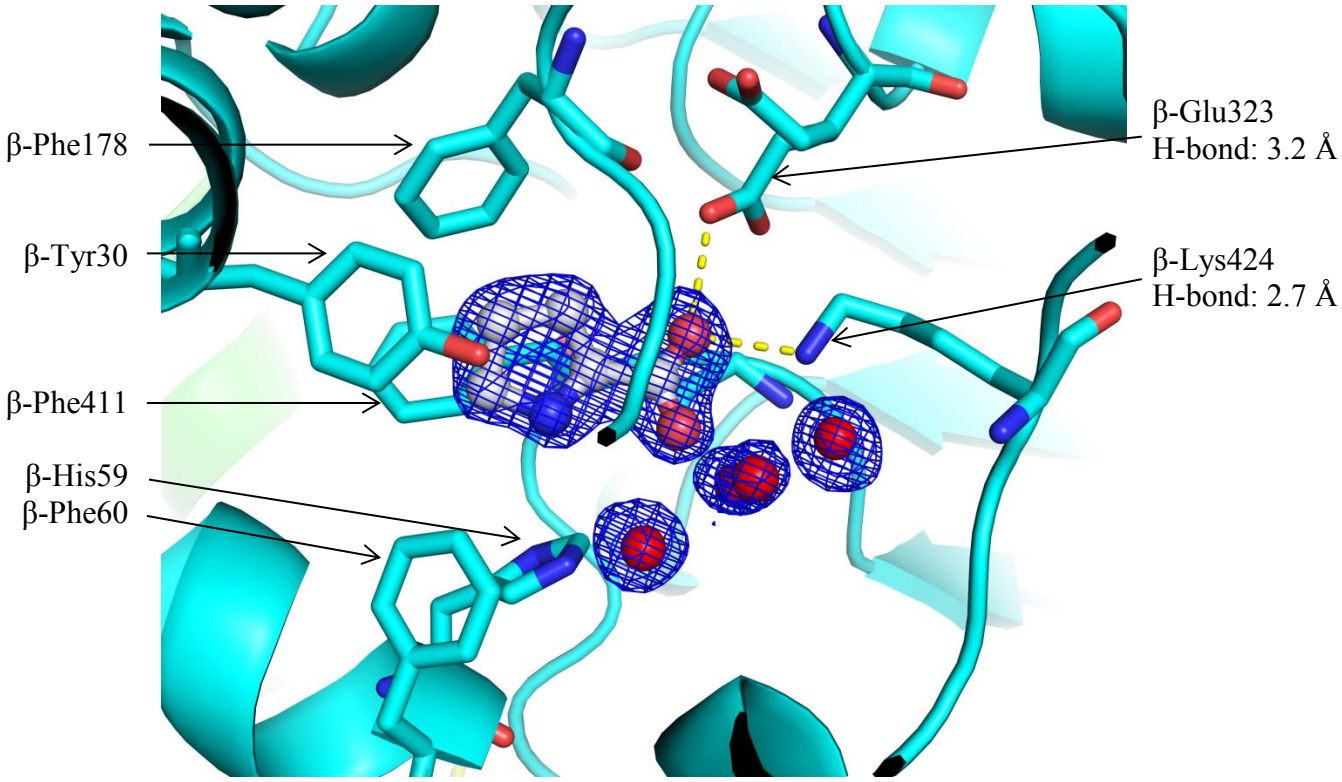

Figure S2. PRL binding site with the neighboring residues in sticks. The PRL molecule is shown with spheres and sticks and is colored by element. The electron density of the PRL and nearby water molecules are shown in blue mesh contoured to 2.0 σ. Distances between the closest N atom of PRL and the N of β-Lys424 and OE2 of β-Glu323 are given. Note the well-defined electron density of the water molecules. See Experimental section of the main text for further discussion.

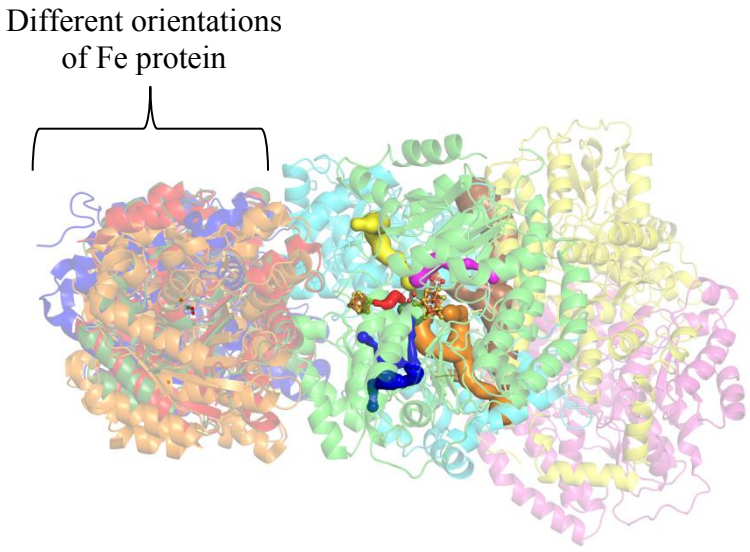

Figure S3. Overlays of four different Av1-Av2 complexes: ADP·AlF_4^- stabilized Av1-Av2 (PDB ID 1M34, red cartoon), nucleotide-free Av1-Av2 (PDB ID 2AFH, blue cartoon), MgAMPPCP-bound Av1-Av2 (PDB ID 2AFK, forest green cartoon), and MgADP-bound Av1-Av2 (PDB ID 2AFI, orange cartoon). The docking of the Fe protein does not block any of the proposed substrate pathways[8, 9].

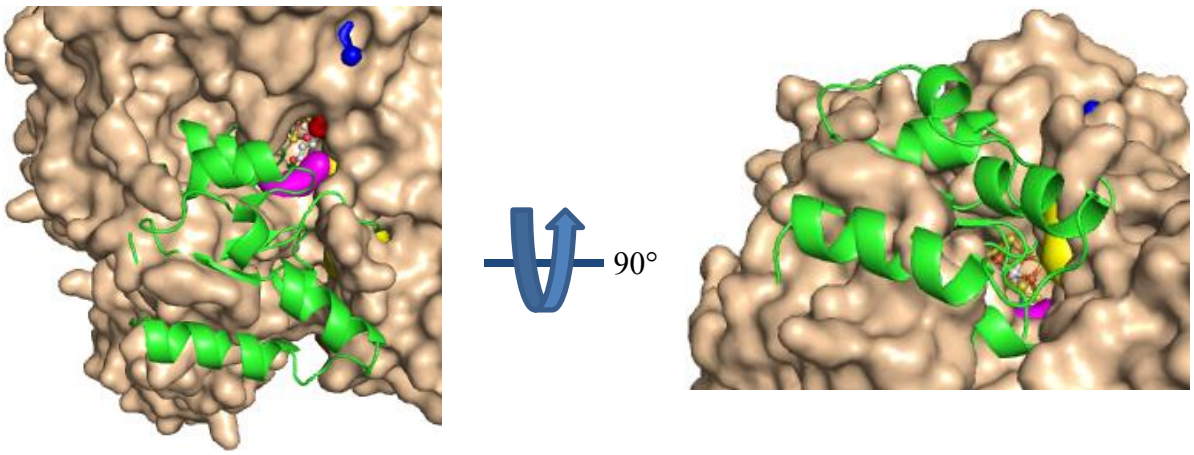

Figure S4. Overlay of the molecular surface of the FeMo-cofactor-deficient Av1 protein (wheat surface, PDB ID 1L5H)[10]. The channel used for transportation of the FeMo-cofactor is visible as the hole in the wheat surface that connects the protein surface to the location of the FeMo-cofactor in the mature protein. The green protein backbone is the α-subunit of the Av1-Xe structure, and the other colored surfaces belong to the substrate access pathways. Three are visible: the AI (blue surface), AII (magenta surface), and NH_3 egress (yellow surface) pathways. Part of the AII pathway overlaps with the FeMo-cofactor insertion pathway.

References

1. Spatzal, T., Aksoyoglu, M., Zhang, L., Andrade, S. L., Schleicher, E., Weber, S., Rees, D. C., and Einsle, O. (2011) Evidence for interstitial carbon in nitrogenase FeMo-cofactor, *Science 334*, 940.
2. Spatzal, T., Perez, K. A., Einsle, O., Howard, J. B., and Rees, D. C. (2014) Ligand binding to the FeMo-cofactor: Structures of CO-bound and reactivated nitrogenase, *Science 345*, 1620-1623.
3. Zhang, L.-M., Morrison, C. N., Kaiser, J. T., and Rees, D. C. Nitrogenase MoFe-protein from *Clostridium pasteurianum* at 1.08 Å resolution: Comparison to the *Azotobacter vinelandii* MoFe-protein., *Acta Crystallogr., Sect. D: Biol. Crystallogr. In press*, doi: 10.1107/S1399004714025243.
4. Mayer, S. M., Lawson, D. M., Gormal, C. A., Roe, S. M., and Smith, B. E. (1999) New insights into structure-function relationships in nitrogenase: A 1.6 Å resolution X-ray crystallographic study of *Klebsiella pneumoniae* MoFe-protein, *J. Mol. Biol. 292*, 871-891.
5. Barney, B. M., Yurth, M. G., Dos Santos, P. C., Dean, D. R., and Seefeldt, L. C. (2009) A substrate channel in the nitrogenase MoFe protein, *J. Biol. Inorg. Chem. 14*, 1015-1022.
6. Dance, I. (2013) A molecular pathway for the egress of ammonia produced by nitrogenase, *Sci. Rep. 3*, 3237.
7. Smith, D. M. A., Danyal, K., Raugei, S., and Seefeldt, L. C. (2014) A substrate channel in nitrogenase revealed by a molecular dynamics approach, *Biochemistry 53*, 2278-2285.
8. Schmid, B., Einsle, O., Chiu, H.-J., Willing, A., Yoshida, M., Howard, J. B., and Rees, D. C. (2002) Biochemical and structural characterization of the cross-linked complex of nitrogenase: Comparison to the ADP-AlF_4^--stabilized structure, *Biochemistry 41*, 15557-15565.
9. Tezcan, F. A., Kaiser, J. T., Mustafi, D., Walton, M. Y., Howard, J. B., and Rees, D. C. (2005) Nitrogenase complexes: multiple docking sites for a nucleotide switch protein, *Science 309*, 1377-1380.
10. Schmid, B., Ribbe, M. W., Einsle, O., Yoshida, M., Thomas, L. M., Dean, D. R., Rees, D. C., and Burgess, B. K. (2002) Structure of a cofactor-deficient nitrogenase MoFe protein, *Science 296*, 352-356.