Supplemental Data

Unraveling the interactions of the physiological reductant flavodoxin with the different conformations of the Fe protein in the nitrogenase cycle

Natasha Pence, Monika Tokmina-Lukaszewska, Zhi-Yong Yang, Rhesa N. Ledbetter, Lance C. Seefeldt, Brian Bothner, John W. Peters

Supplemental Data includes:

Table S1. Peptides observed to appear and disappear in time-resolved limited proteolysis experiments.
Table S2. Peptides observed to appear and disappear in chemical cross-linking experiments.
Figure S1. Representative MALDI-TOF mass spectra for digestion of the Fe protein.
Figure S2. Representative MALDI-TOF mass spectra for digestion of Fld.

Table S1. Peptides observed to appear and disappear in time-resolved limited proteolysis experiments. **Peptides** that appear in response to the different nucleotide conditions and/or complex formation are highlighted in bold. Residues highlighted in gray represent amino acids modifications: carbamidomethyl (Cys) and oxidation (Met), $^{\#}$ peptides identified by MALDI-TOF. The amino acid sequences for the Fe protein and flavodoxin are provided for reference.

Time-resolved limited proteolysis experiments: Nitrogenase Fe protein

Nitrogenase Fe protein amino acid sequence:
AMRQCAIYGKGGIGKSTTTQNLVAALAEMGKKVMIVGCDPKADSTRLILHSKAQNTIME
MAAEAGTVEDLELEDVLKAGYGGVKCVESGGPEPGVGCAGRGVITAINFLEEEGAYE
DDLDFVFYDVLGDVVCGGFAMPIRENKAQEIYIVCSGEMMAMYAANNISKGIVKYANS
GSVRLGGLICNSRNTDREDELIIALANKLGTQMIHFVPRDNVVQRAEIRRMTVIEYDPKA
KQADEYRALARKVVDNKLLVIPNPITMDELEELLMEFGIMEVEDESIVGKTAEEV

Peptide sequence	Residue number	Expected mass	m/z
AMRQCAIYGKGGIGK	**1-15**	1551.80	1551.58$^{\#}$
GGIGKSTTTQNLVAALAEMGK	11-31	2046.08	683.37
GGIGKSTTTQNLVAALAEMGKK	11-32	2174.17	726.06
GGIGKSTTTQNLVAALAEMGKK		2190.16	731.40
STTTQNLVAALAEMGK	16-31	1633.83	817.92
STTTQNLVAALAEMGK		1649.82	825.93
STTTQNLVAALAEMGKK	**16-32**	1761.93	1761.77$^{\#}$
			881.97
STTTQNLVAALAEMGKK		1777.92	889.98
VMIVGCDPKADSTR	**33-46**	1490.72	1490.50$^{\#}$
VMIVGCDPKADSTR		1547.74	773.87
VMIVGCDPKADSTR		1563.73	522.58
VMIVGCDPKADSTRLILHSK	33-52	2182.16	2182.85$^{\#}$
ADSTRLILHSK	42-52	1239.69	620.85
AQNTIMEMAAEAGTVEDLELEDVLK	**53-77**	2719.29	2719.29$^{\#}$
			1361.17
AQNTIMEMAAEAGTVEDLELEDVLK		2735.28	1369.17
AQNTIMEMAAEAGTVEDLELEDVLK		2751.27	1377.15
AQNTIMEMAAEAGTVEDLELEDVLKAGYGGVK	**53-84**	3351.62	3351.73$^{\#}$
AGYGGVKCVESGGPEPGVGCAGR	78-100	2105.96	2105.63$^{\#}$
CVESGGPEPGVGCAGR	85-100	1473.63	1473.34$^{\#}$
GIVKYANSGSVR	167-178	1249.68	1249.40$^{\#}$
			625.85
YANSGSVR	171-178	852.41	852.17$^{\#}$
			428.23
LGGLICNSR	179-187	931.49	931.24$^{\#}$
NTDREDELIIALANK	188-202	1713.89	1713.56$^{\#}$
			857.96
NTDREDELIIALANKLGTQMIHFVPR	188-213	2993.58	2993.53$^{\#}$
LGTQMIHFVPR	203-213	1297.70	1297.49$^{\#}$
			649.86
LGTQMIHFVPR		1313.69	657.85
LGTQMIHFVPRDNVVQR	203-219	2009.06	2009.72$^{\#}$

LGTQMIHFVPRDNVVQR		2025.05	1006.05 676.36
MIHFVPR	207-213	914.47	458.24
DNVVQRAEIR	214-223	1198.64	600.32
DNVVQRAEIRR	214-224	1354.74	1354.44# 452.58
RMTVIEYDPK RMTVIEYDPK	224-233	1250.63 1266.62	626.33 634.32
RMTVIEYDPKAK RMTVIEYDPKAK	224-235	1449.76 1465.75	1449.46# 725.89 489.59
RMTVIEYDPKAKQADEYR	224-241	2212.09	2212.81#
RMTVIEYDPKAKQADEYRALAR	224-245	2623.35	2623.10#
MTVIEYDPK MTVIEYDPK	225-233	1094.53 1110.52	548.27 556.27
MTVIEYDPKAK MTVIEYDPKAK	225-235	1293.66 1309.65	1293.37# 647.84 655.84
MTVIEYDPKAKQADEYR MTVIEYDPKAKQADEYR	225-241	2055.99 2071.98	2055.73# 1029.51 1037.50
MTVIEYDPKAKQADEYRALAR	225-245	2467.25	2467.97#
AKQADEYR	234-241	979.47	979.22#
AKQADEYRALAR	234-245	1390.73	696.37
QADEYRALAR	236-245	1191.60	1191.33# 596.80
QADEYRALARK	236-246	1319.69	1319.41# 440.90

Time-resolved limited proteolysis experiments: Flavodoxin

Flavodoxin amino acid sequence:
AKIGLFFGSNTGKTRKVAKSIKKRFDDETMSDALNVNRVSAEDFAQYQFLILGTPTLGE
GELPGLSSDCENESWEEFLPKIEGLDFSGKTVALFGLGDQVGYPENYLDALGELYSFF
KDRGAKIVGSWSTDGYEFESSEAVVDGKFVGLALDLDNQSGKTDERVAAWLAQIAPE
FGLSL

Peptide sequence	Residue number	Expected mass	m/z
AKIGLFFGSNTGK	1-13	1338.73	1338.44#
AKIGLFFGSNTGKTR	**1-15**	1595.88	1595.55#
AKIGLFFGSNTGKTRK	1-16	1723.97	1723.65#
AKIGLFFGSNTGKTRKVAK	1-19	2022.17	2022.66#
IGLFFGSNTGK	**3-13**	1139.60	570.80
KRFDDETMSDALNVNR	23-38	1925.89	642.97
RFDDETMSDALNVNR RFDDETMSDALNVNR	24-38	1781.80 1797.79	1781.53# 899.91
FDDETMSDALNVNR FDDETMSDALNVNR	25-38	1625.70 1641.69	1625.42# 813.86 821.86
IEGLDFSGK	81-89	964.49	964.27#

			483.25
TVALFGLGDQVGYPENYLDALGELYSFFKDR	**90-120**	3496.72	3496.67[#]
IVGSWSTDGYEFESSEAVVDGK	124-145	2361.06	1182.05
FVGLALDLDNQSGK	146-159	1475.76	738.89
FVGLALDLDNQSGKTDER	**146-163**	1976.98	1976.70[#] 660.33

Table S2. Peptides observed to appear and disappear in cross-linking experiments. **Peptides** that disappear due to cross-linking in response to the different nucleotide conditions and/or complex formation are highlighted in bold. Residues highlighted in gray represent amino acid modifications: carbamidomethyl (Cys) and oxidation (Met), *single oxidation of multiple methionine residues. The amino acid sequences for the Fe protein and flavodoxin are provided for reference.

Cross-linking experiments: Nitrogenase Fe protein

Nitrogenase Fe protein amino acid sequence:
AMRQCAIYGKGGIGKSTTTQNLVAALAEMGKKVMIVGCDPKADSTRLILHSKAQNTIMEMAA
EAGTVEDLELEDVLKAGYGGVKCVESGGPEPGVGCAGRGVITAINFLEEEGAYEDDLDFVFY
DVLGDVVCGGFAMPIRENKAQEIYIVCSGEMMAMYAANNISKGIVKYANSGSVRLGGLICNS
RNTDREDELIIALANKLGTQMIHFVPRDNVVQRAEIRRMTVIEYDPKAKQADEYRALARKVVD
NKLLVIPNPITMDELEELLMEFGIMEVEDESIVGKTAEEV

Peptide sequence	Residue number	Expected mass	m/z
QCAIYGK	**4-10**	838.40	420.21
GGIGKSTTTQNLVAALAEMGKK	**11-32**	2190.16	731.40
STTTQNLVAALAEMGKK	16-32	1761.93	882.48
STTTQNLVAALAEMGKK		1777.92	889.98
STTTQNLVAALAEMGK	16-31	1633.83	817.93
STTTQNLVAALAEMGK		1649.82	826.43
KVMIVGCDPKADSTR	**32-46**	1618.82	811.42
KVMIVGCDPK	32-41	1145.59	573.81
KVMIVGCDPK		1161.58	581.80
VMIVGCDPK	33-41	1017.50	509.76
VMIVGCDPK		1033.49	517.76
LILHSK	47-52	709.45	355.73
AQNTIMEMAAEAGTVEDLELEDVLK	**53-77**	2719.29	1361.17
AQNTIMEMAAEAGTVEDLELEDVLK		2735.28	1369.17
AQNTIMEMAAEAGTVEDLELEDVLK		2751.27	1377.17
AGYGGVKCVESGGPEPGVGCAGR	**78-100**	2220.00	741.35
CVESGGPEPGVGCAGR	**85-100**	1587.67	794.86
DVLGDVVCGGFAMPIR	**125-140**	1704.83	853.43
DVLGDVVCGGFAMPIR		1720.82	861.43
DVLGDVVCGGFAMPIRENK	125-143	2076.01	1039.51
DVLGDVVCGGFAMPIRENK		2092.00	1047.52
AQEIYIVCSGEMMAMYAANNISKGIVK	**144-170**	2990.44	998.50
AQEIYIVCSGEMMAMYAANNISK	144-166	2593.17	1298.10
AQEIYIVCSGEMMAMYAANNISK		2609.16	1306.11*
AQEIYIVCSGEMMAMYAANNISK		2641.14	1322.09
AQEIYIVCSGEMMAMY	144-159	1894.80	948.91
AQEIYIVCSGEMMAMY		1942.77	972.90
IVCSGEMMAMYAANNISK	149-166	1988.88	995.95
IVCSGEMMAMYAANNISK		2020.86	1011.95*
IVCSGEMMAMYAANNISK		2036.85	1019.94
GIVKYANSGSVR	**167-178**	1249.68	625.85
YANSGSVR	171-178	852.41	427.21
LGGLICNSR	179-187	988.51	495.27

NTDREDELIIALANK	188-202	1713.89	857.96
NTDREDELIIALANKLGTQMIHFVPR NTDREDELIIALANKLGTQMIHFVPR	188-213	2993.58 3009.57	749.66 753.91
EDELIIALANK	192-202	1227.67	614.85
LGTQMIHFVPR LGTQMIHFVPR	203-213	1297.70 1313.69	649.85 657.86
RMTVIEYDPK RMTVIEYDPK	224-233	1250.63 1266.62	626.33 634.33
MTVIEYDPK MTVIEYDPK	225-233	1094.53 1110.52	548.28 556.27
LLVIPNPITMDELEELLMEF LLVIPNPITMDELEELLMEF	252-271	2358.21 2390.19	1180.63 1196.61
GIMEVEDESIVGK GIMEVEDESIVGK	272-284	1404.68 1420.67	703.35 711.35

Cross-linking experiments: Flavodoxin

Flavodoxin amino acid sequence:
AKIGLFFGSNTGKTRKVAKSIKKRFDDETMSDALNVNRVSAEDFAQYQFLILGTPTLGEGELP
GLSSDCENESWEEFLPKIEGLDFSGKTVALFGLGDQVGYPENYLDALGELYSFFKDRGAKIV
GSWSTDGYEFESSEAVVDGKFVGLALDLDNQSGKTDERVAAWLAQIAPEFGLSL

Peptide sequence	Residue number	Expected mass	m/z
AKIGLFFGSNTGK	1-13	1338.73	670.38
IGLFFGSNTGK	3-13	1139.60	570.81
KRFDDETMSDALNVNR	23-38	1925.89	642.98
RFDDETMSDALNVNR RFDDETMSDALNVNR	24-38	1781.80 1797.79	891.91 900.41
FDDETMSDALNVNR FDDETMSDALNVNR	25-38	1625.70 1641.69	813.86 821.86
RFDDETMSDAL	24-34	1314.53	658.28
VSAEDFAQYQFL	39-50	1416.66	709.34
ILGTPTLGEGELPGLSSDCENESWEEFLPK	51-80	3303.55	1653.30
GTPTLGEGELPGLSSDCENESWEEFLPK	53-80	3077.38	1540.21
IEGLDFSGK	81-89	964.49	483.25
TVALFGLGDQVGYPENYLDALGELY	**90-114**	2716.33	1359.68
GDQVGYPENYLDALGELYSFFK	**97-118**	2524.18	1348.67
SFFKDR	**115-120**	798.40	400.21
IVGSWSTDGYEFESSEAVVDGK	124-145	2361.06	1182.05
STDGYEFESSEAVVDGK	129-145	1818.78	910.40
EFESSEAVVDGK	134-145	1295.59	648.81
FVGLALDLDNQSGKTDER	**146-163**	1976.98	990.00
FVGLALDLDNQSGK	**146-159**	1475.76	739.90
VGLALDLDNQSGK	**147-159**	1328.69	665.36
ALDLDNQSGK	**150-159**	1059.52	530.77
VAAWLAQIAPEFGLSL	164-179	1684.92	843.47

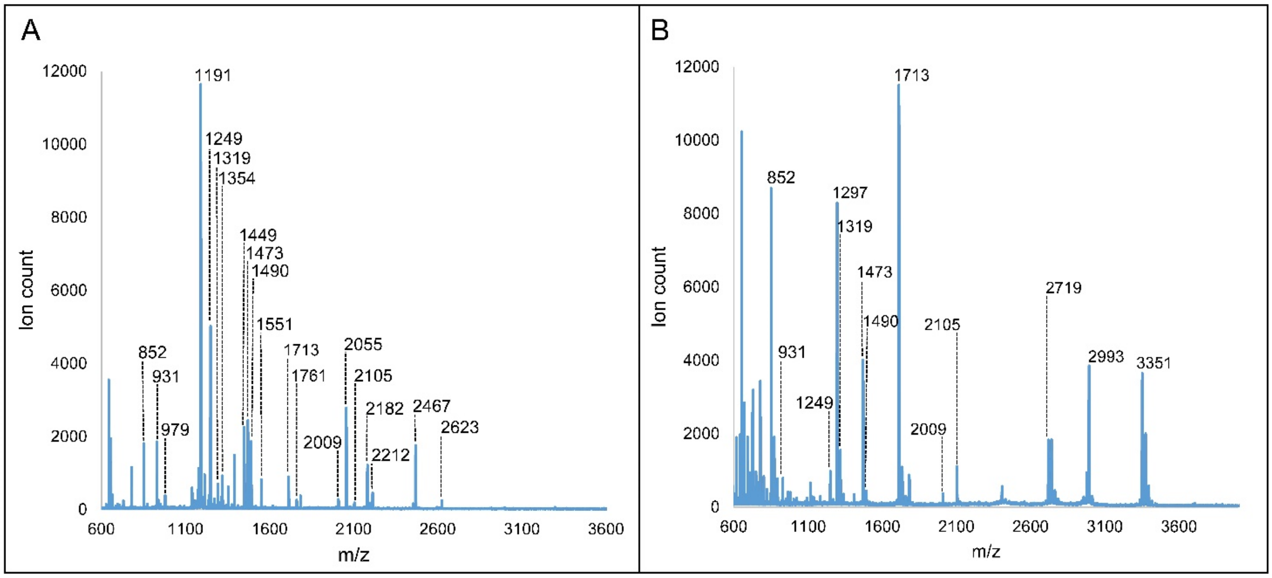

Figure S1. Representative MALDI-TOF mass spectra for digestion of the Fe protein. *A,* representative MALDI-TOF spectrum of Ion count vs m/z for the Fe protein after 10 minutes digestion with labelled ions: 852 (YANSGSVR), 931 (LGGLICNSR), 979 (AKQADEYR), 1191 (QADEYRALAR), 1249 (GIVKYANSGSVR), 1319 (QADEYRALARK), 1354 (DNVVQRAEIRR), 1449 (RMTVIEYDPKAK), 1473 (CVESGGPEPGVGCAGR), 1490 (VMIVGCDPKADSTR), 1551 (MRQCAIYGKGGIGK), 1713 (NTDREDELIIALANK), 1761 (STTTQNLVAALAEMGKK), 2009 (LGTQMIHFVPRDNVVQR), 2055 (MTVIEYDPKAKQADEYR), 2105 (AGYGGVKCVESGGPEPGVGCAGR), 2182 (VMIVGCDPKADSTRLILHSK), 2212 (RMTVIEYDPKAKQADEYR), 2467 (MTVIEYDPKAKQADEYRALAR), 2623 (RMTVIEYDPKAKQADEYRALAR). *B,* representative MALDI-TOF spectrum of Ion count vs m/z for the Fe protein after 240 minutes digestion with labelled ions: 852 (YANSGSVR), 931 (LGGLICNSR), 1249 (GIVKYANSGSVR), 1297 (IGTQMIHFVPR), 1319 (QADEYRALARK), 1473 (CVESGGPEPGVGCAGR), 1490 (VMIVGCDPKADSTR), 1713 (NTDREDELIIALANK), 2009 (LGTQMIHFVPRDNVVQR), 2105 (AGYGGVKCVESGGPEPGVGCAGR), 2719 (AQNTIMEMAAEAGTVEDLELEDVLK), 2993 (NTDREDELIIALANKLGTQMIHFVPR), 3351 (AQNTIMEMAAEAGTVEDLELEDVLKAGYGGVK).

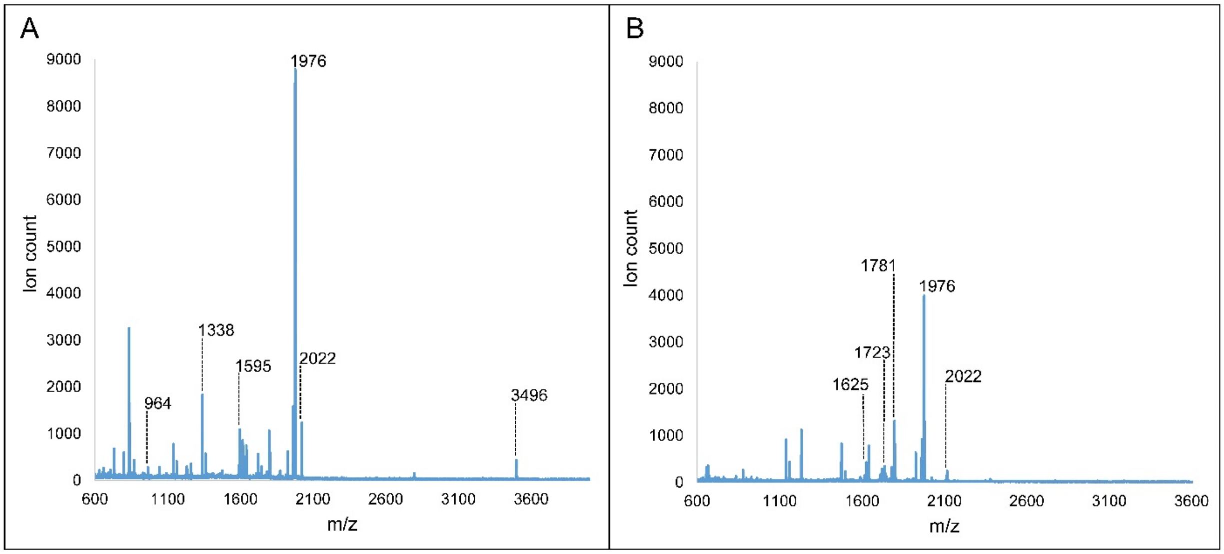

Figure S2. Representative MALDI-TOF mass spectra for digestion of Fld. *A*, representative MALDI-TOF spectrum of Ion count vs m/z for Fld after 20 minutes digestion with labelled ions: 964 (IEGLDFSGK), 1338 (AKIGLFFGSNTGK), 1595 (AKIGLFFGSNTGKTR), 1976 (FVGLALDLDNQSGKTDER), 2022 (AKIGLFFGSNTGKTRKVAK), 3496 (TVALFGLGDQVGYPENYLDALGELYSFFKDR). *B*, representative MALDI-TOF spectrum of Ion count vs m/z for Fld after 240 minutes digestion with labelled ions: 1625 (FDDETMSDALNVNR), 1723 (AKIGLFFGSNTGKTRK), 1781 (FDDETMSDALNVNR), 1976 (FVGLALDLDNQSGKTDER) and 2022 (AKIGLFFGSNTGKTRKVAK).