The Electron Bifurcating FixABCX Protein Complex from *Azotobacter vinelandii*: Generation of Low-Potential Reducing Equivalents for Nitrogenase Catalysis

Rhesa N. Ledbetter[1], Amaya M. Garcia Costas[2], Carolyn E. Lubner[3], David W. Mulder[3], Monika Tokmina-Lukaszewska[2], Jacob H. Artz[4], Angela Patterson[2], Timothy S. Magnuson[5], Zackary J. Jay[6], H. Diessel Duan[7], Jacquelyn Miller[2], Mary H. Plunkett[8], John P. Hoben[7], Brett M. Barney[8], Ross P. Carlson[6], Anne-Frances Miller[7], Brian Bothner[2], Paul W. King[3], John W. Peters[2,4], and Lance C. Seefeldt[1]*

[1]Department of Chemistry and Biochemistry, Logan, UT 84322
[2]Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717
[3]National Renewable Energy Laboratory, Golden, CO 80401
[4]Institute of Biological Chemistry, Washington State University, Pullman, WA 99163
[5]Department of Biological Sciences, Idaho State University, Pocatello, ID 83201
[6]Department of Chemical and Biological Engineering, Center for Biofilm Engineering and Thermal Biology Institute, Montana State University, Bozeman, MT 59717
[7]Department of Chemistry, University of Kentucky, Lexington, KY 40506
[8]Department of Bioproducts and Biosystems Engineering and Biotechnology Institute, University of Minnesota, St. Paul, MN 55108

*Corresponding author: Lance C. Seefeldt, 0300 Old Main Hill, Logan, Utah, 84322
Phone: +1.435.797.3964, Email: lance.seefeldt@usu.edu

Table S1. Primers for Δ*fix* mutant generation.

Primer name	Sequence	Purpose
fix101	5'-GGTGGGAATTCCGATCTGCATGGCGCCCG-3'	Construct
fix102	5'-CGGCGGATCCGGCATAGGAAGTGGCCAGGGT-3'	Construct
fix103	5'-TCAAGGATCCCTTCATCGCCTCACGTTCG-3'	Construct
fix104	5'-CGTGAAGCTTCTGCGGGCGCTGTACTTC-3'	Construct
fix107	5'-ACATTCGGTGCAATGGTCGG-3'	Verification
fix108	5'-CCATCATGCGCCAGGGTG-3'	Verification
fix105	5'-GTCACGTCTGGGTCTGCAT -3'	Verification
fix106	5'-CGGTCAGTGCCGGCAGG-3'	Verification
fix109	5'-GCCACCGACAGCGCCAG-3'	Verification

Table S2. Primers for overexpression of FixABCX.

Primer name	Sequence	Purpose
BB2290	5’- NNNTCTAGACATATGCCCTACAAGATCAACGGTTCC-3’	Construct
BB2291	5’-NNNGGATCCTCAGTTCTTGGCCGAGCGG-3’	Construct
BBP2286	5’-CTGGAAGGCACCAATGAAATGCG-3’	Verification
BBP2287	5’-GCTGTGCACCATCCTCACCCAG-3’	Verification
BBP2288	5’-CGCCATCGAGAAGATCATTCCCGAC-3’	Verification
BBP2289	5’-GTCAACGGCATCCATCGCGAAG-3’	Verification
BB2283	5’-TCAGAGGGCTTGGTAGCGCGGTAC-3’	Verification

Table S3 (inserted at the end of this document due to size). Protein-protein interactions captured, (A) the inter-cross-links and (B) intra-cross-links, within FixABCX complex during cross-linking reaction with BS3. Mapping of intra-subunit cross-links onto the homology models was consistent with the expected spanning distance of 20-25 Å between C-alpha chains. It should be noted that a subset of cross-links mapped to regions spanning 26-30 Å (for Lys-Lys) and higher. This is to be expected and has been explained by protein flexibility using known systems.[1]

Table S4. Overview of the EPR signals observed for FixABCX from *A. vinelandii*.

Signal Name	*g*-values	T_{opt} (K)	Samples present	Percent contribution	Possible source
Fast relaxing, broad	*	5 K	As-purified NADH	* *	Unknown
Rhombic 2.07	2.072, 1.940, 1.895	15 K	NADH Na-dithionite	86 87	$[4Fe\text{-}4S]^{1+}$
Axial 2.04	2.041, 1.944, 1.944	5 – 10 K	NADH Na-dithionite	9 12	$[4Fe\text{-}4S]^{1+}$
Isotropic 2.005	2.005	> 50 K	As-purified NADH Na-dithionite	* 1 1	Flavin radical
Axial 2.03	2.030, 2.00, 2.00	10 K	NADH	4	Interacting flavin radical and $[4Fe\text{-}4S]^{1+}$

*Not determined

Table S5. Calculated Gibbs free energies of three possible reactions catalyzed by the Fix complex and three possible non-enzymatic reactions.

Reaction	$\Delta \mathbf{G}^{\circ}_{\mathbf{rxn}}$ (kJ/mol)
$2\ NADH + 2\ Fld^{Sq} + CoQ = 2\ Fld^{Hq} + CoQH_2 + 2\ NAD^+$	-36.7
$NADH + CoQH_2 + 4\ Fld^{Sq} = 4\ Fld^{Hq} + CoQ + NAD^+ + 3\ H^+$	118
$NADH + 2\ Fld^{Hq} + 2\ CoQ + 3\ H^+ = 2\ CoQH_2 + 2\ Fld^{Sq} + NAD^+$	-154
$2\ Fld^{Sq} + CoQ + 2\ H^+ = 2\ Fld^{Ox} + CoQH_2$	-36.7
$NADH + CoQ + H^+ = CoQH_2 + NAD^+$	-63.7
$NADH + 2\ Fld^{Ox} = 2\ Fld^{Sq} + NAD^+ + H^+$	-27.0

$Fld^{Sq} + e^- = Fld^{Hq}$, E°= -460 mV; $Fld^{Ox} + e^- = Fld^{Sq}$, E°= -180 mV; $CoQ + 2\ e^- = CoQH_2$, E°= + 10 mV; $NAD^+ + 2\ e^- = NADH$, E°= -320 mV

Table S6. Fitting of the ASQ kinetic curves for *R. rubrum* FixAB and *A. vinelandii* FixABCX reveal a biphasic decay, with one component having a lifetime of tens of picoseconds and a slightly longer lifetime of one thousand picoseconds. Fitting was performed in Igor Pro using a double exponential fit function.

Sample	**ASQ τ_1**	**ASQ τ_2**
R. rubrum FixAB	14 ps	970 ps
A. vinelandii FixABCX	14 ps	1140 ps

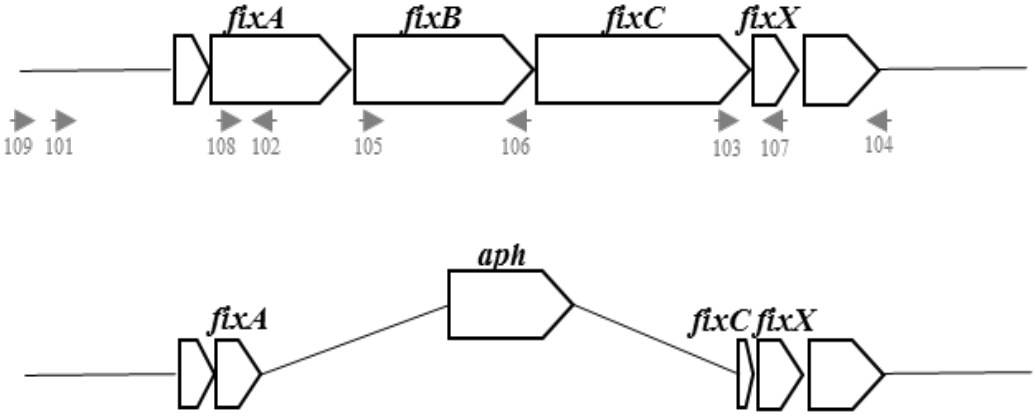

Figure S1. Scheme for *Δfix* mutant generation.

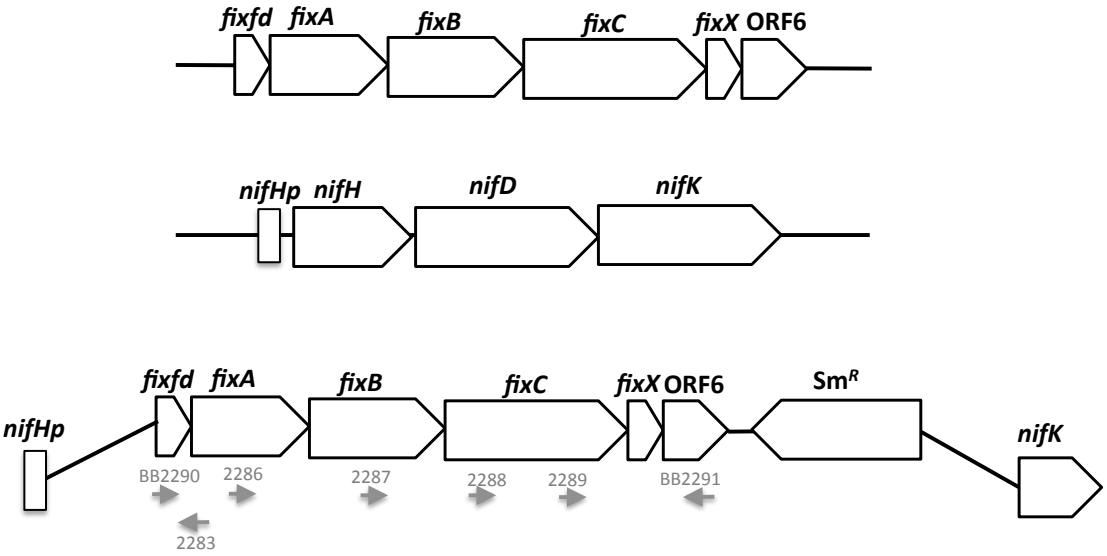

Figure S2. Scheme for overexpression of *fix* genes under the *nifH* promoter in *A. vinelandii*.

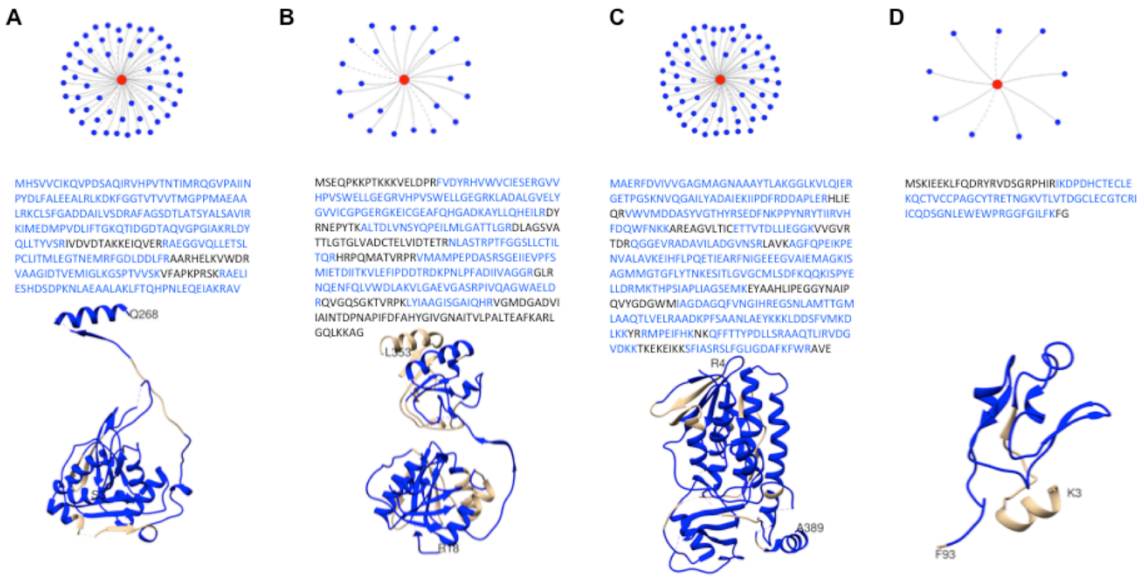

Figure S3. Protein identification within FixABCX complex purified from *A. vinelandii*. The identity of FixA (panel A), FixB (panel B), FixC (panel C), and FixX (panel D) subunits was confirmed based on 54, 22, 59, and 9 unique tryptic peptides that correspond to 79%, 63%, 85%, and 73% sequence coverage (top row). Peptides and proteins are represented by blue and red spheres, respectively. Identified peptides were rendered in blue on primary sequence (middle row) and homology models of corresponding subunits (bottom row). Homology models were generated based on following protein templates: FixA, 4L2I; FixB, 3CLR; FixC, 4K2X; FixX, 2GMH.

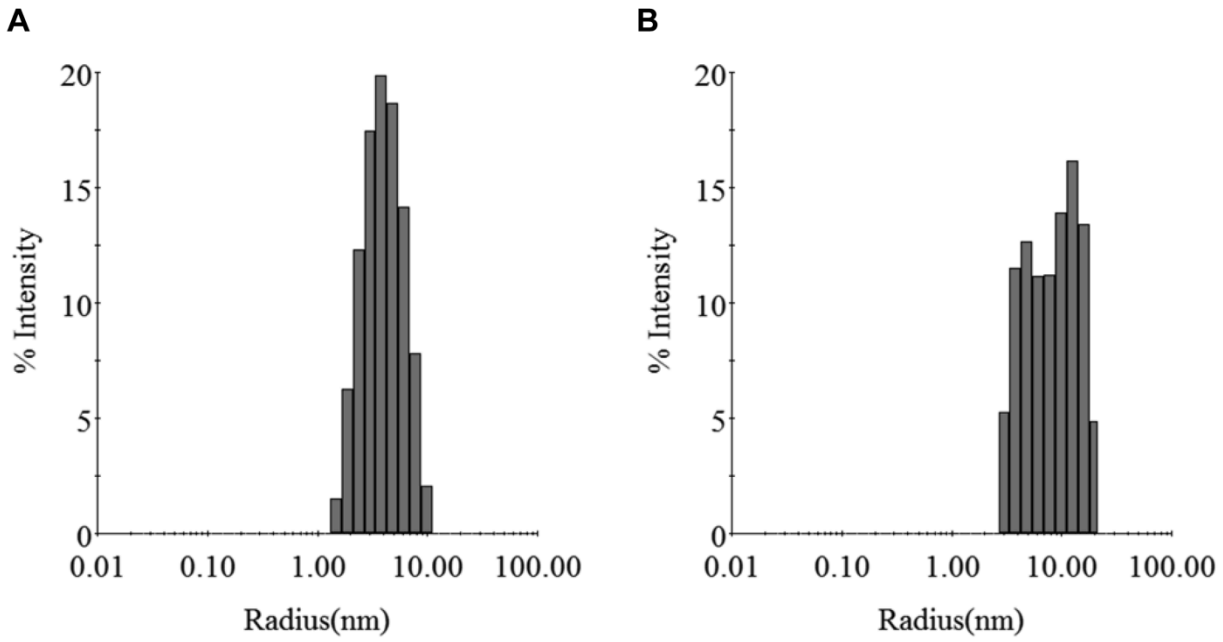

Figure S4. Size distribution of protein complexes as revealed by dynamic light scattering, comparing buffer containing DDM (negative control) and the purified FixABCX complex. (A) Buffer containing 0.02% DDM (w/v) with a determined hydrodynamic radius of 4.32 nm. (B) 1.0 mg/mL of FixABCX complex with a determined hydrodynamic radius of 4.69 nm accounted for 99.4% of the total population. Triplicate measurements yielded an average hydrodynamic radius of 5.1 ± 0.3 nm.

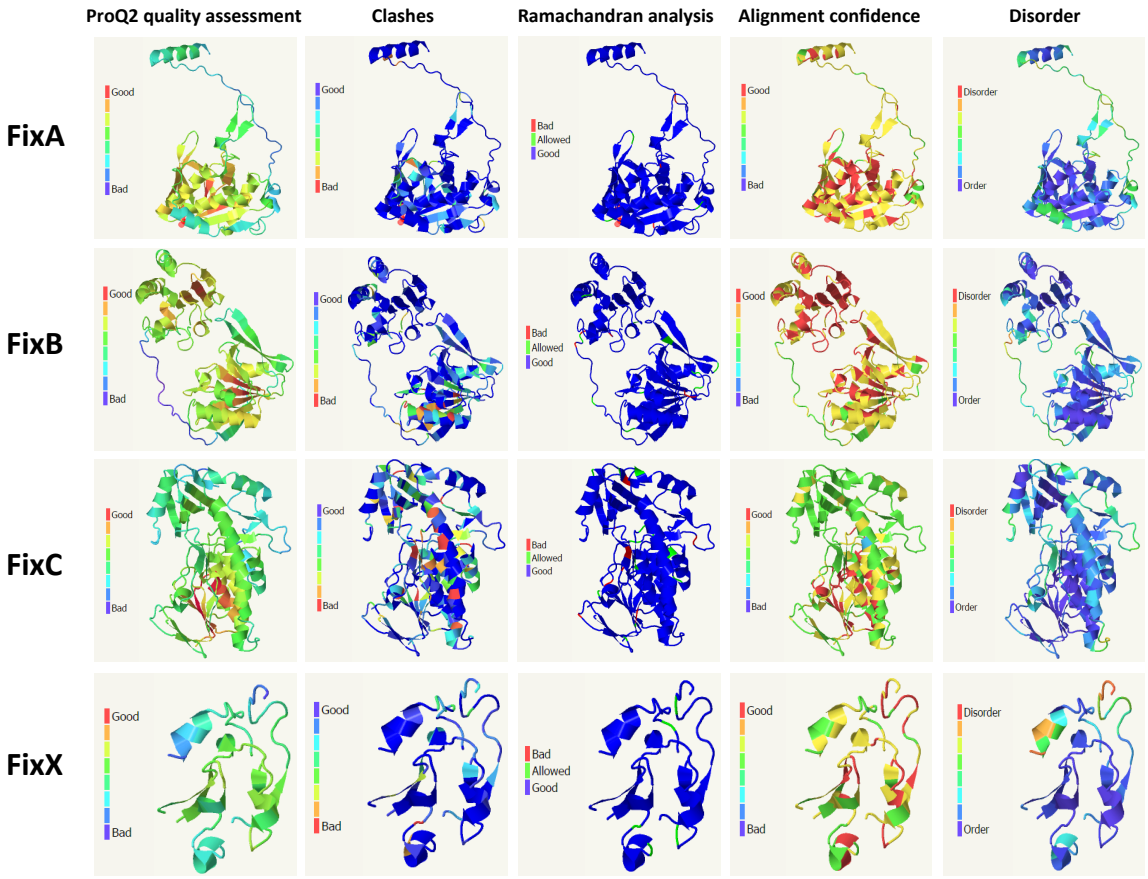

Figure S5. Evaluation of subunit homology models. *ProQ2 quality assessment:* a support vector machine is used to predict local and global quality of the model;[2] *Clashes:* indicates which residues lie too close to one another due to incorrect side chain and/or backbone placement; *Ramachandran analysis*: provides information on residues position (favorable/allowed/disallowed) in the model and potential problems with the backbone phi/psi angles; *Alignment confidence:* reliability of the pair-wise query-template alignment as reported by HHsearch. The confidence values are obtained from the posterior probabilities calculated in the forward-backward algorithm; *Disorder:* identifies dynamically flexible regions. This prediction has been made by the knowledge-based Disopred method (PSIPRED server).[3] Overall, models for each subunit were obtained with the highest template confidence of 100% (with the exception

of 99.8% for FixX). However, it was impossible to attain complete sequence coverage, primarily due to insufficient structural information in the database. For example, sequence coverage for FixA, FixB, FixC, and FixX were 92%, 88%, 82%, and 96%, respectively.

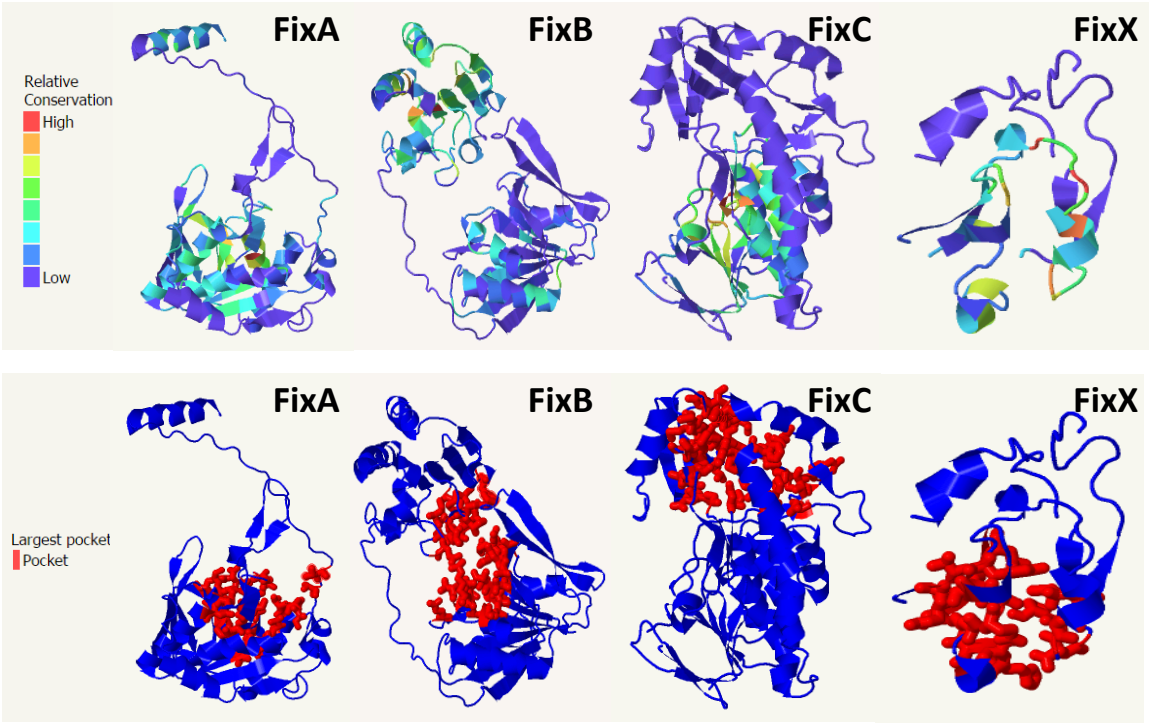

Figure S6. Predicted structural features in Fix subunits. *Conservation:* sequence analysis based on Jensen-Shannon divergence;[4] *Largest pocket:* the pockets are detected by the fpocket2 program and highlighted in red.[5] This provides a tool for tracking small molecule binding sites, pockets for molecular docking or detection of subpockets of conformational ensembles. The highest degree of conservation (from green to red color) is associated with cofactor binding sites. A helix (Ala338-Leu353) on the C-terminal domain of FixB appears to be critical for A and B subunit interactions. Further, large pockets are detected around FixA, FixB and FixX cofactor binding sites. In addition to flavin binding sites, two large cavities are present between the N-terminal and C-terminal domain of FixB (important for interactions with FixA) and near the predicted transmembrane region (Met299-Leu325) on FixC.

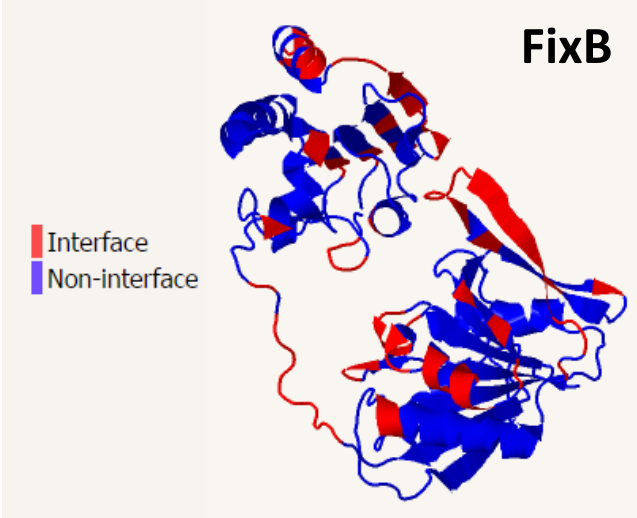

Figure S7. Protein interface. PI-site interface residues[6] and ProtinDB interface residues provide information on prediction of protein-protein interaction sites based on information extracted from protein complexes stored in the Protein Data Bank. Interface residues are colored in red.

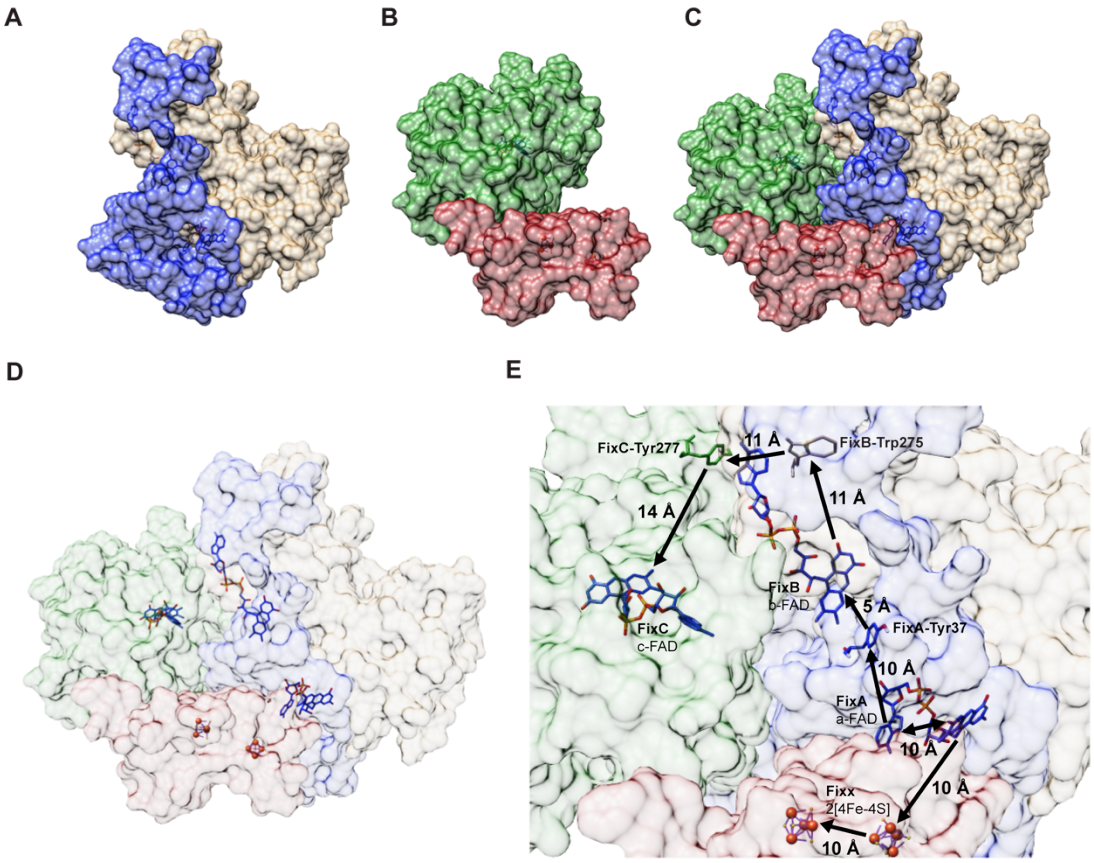

Figure S8. Structural model of FixABCX from *A. vinelandii*. Fix subunit homology models were built using Phyre2 and had the closest match to following templates from the Protein Data Bank (PDB): FixA, 4L2I; FixB, 3CLR; FixC, 4K2X; FixX, 2GMH. Chemical cross-linking data was used to produce distance constraints for generating structural models. (A) ClusPro2 docking model of FixAB subunits (visualized in Chimera). FixA in blue, FixB in tan. (B) ClusPro2 docking model of FixCX subunits (visualized in Chimera). FixC in green, FixX in red. (C) FixABCX complex model generated by docking (ClusPro2) four homology models (Phyre2). Restraints derived from the chemical cross-linking experiment. The final model was evaluated and satisfied two requirements: 1) Most of the cross-links could be explained by the model and 2) cofactor distances of <14 Å that enable electron transfer. (D) Cofactors and protein

residues that could function in electron transfer. (E) Distances between cofactors taking into account the presence of conserved Trp and Tyr side chains that may convey electrons between cofactors. FixA-Tyr37 is conserved as Tyr in 44 of the 53 FixAs of which we are aware, FixB-Trp275 is conserved as Trp in 53 out of 53 FixBs of which we are aware, and FixC-Tyr277 is Tyr in 8/8 sequences examined.

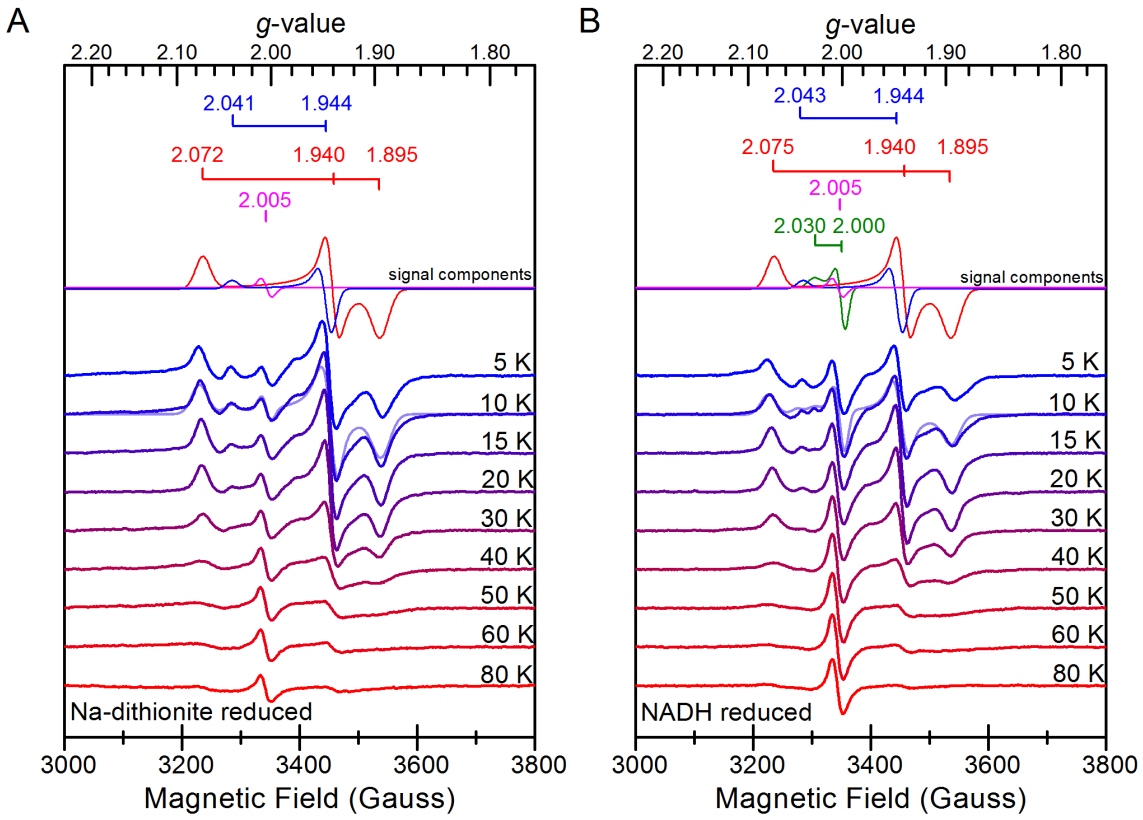

Figure S9. EPR temperature profiles of FixABCX from *A. vinelandii* reduced with either Na-dithionite or NADH. (A) FixABCX (100 μM) reduced with Na-dithionite (10 mM). (B) FixABCX (100 μM) reduced with NADH (1 mM). The spectra were recorded at 5, 10, 15, 20, 30, 40, 50, 60 and 80 K. Simulations of the overall spectra at 10 K are shown in the lighter shade of blue. The individual signal components and *g*-values comprising the overall simulated spectra at 10 K are shown at the top (blue, axial 2.04; red, rhombic 2.07 magenta, isotropic 2.0; green, axial 2.03) and varied slightly for each treatment (Table S4). The overall signal intensities for NADH (0.40 spins/mol protein) and Na-dithionite treatments (0.45 spins/mol protein) were similar at 10 K, indicating partial reduction of the protein complex. Microwave frequency, 9.38 GHz; microwave power, 1 mW; modulation frequency, 100 kHz; modulation amplitude, 10.0 G.

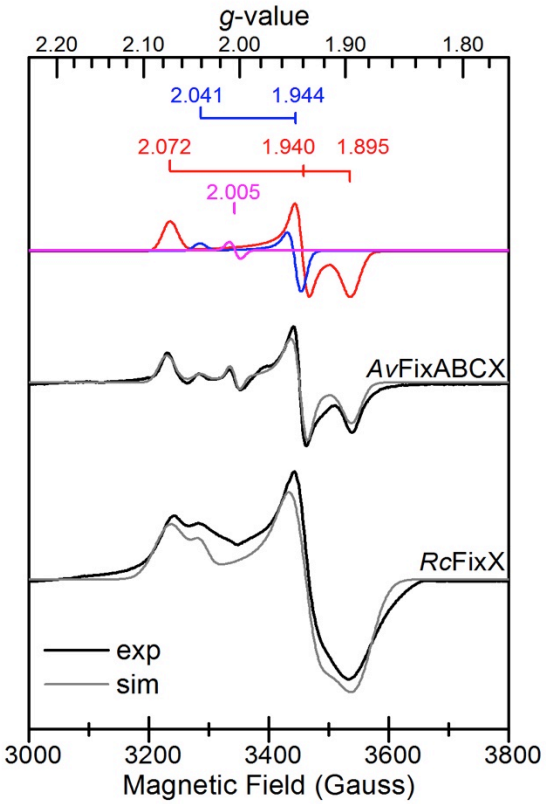

Figure S10. Comparison of EPR spectra of reduced FixABCX from *A. vinelandii (Av)* and the reduced, individual subunit FixX from *Ro. castenholzii (Rc)*. FixABCX from *A. vinelandii* (100 μM) was reduced 10 mM Na-dithionite. FixX from *Ro. castenholzii* (200 μM) was reduced with 5 mM Na-dithionite. Signal intensities were normalized for sample concentration. Experimental (exp) spectra are colored black and simulated (sim) spectra are colored gray. The individual signal components and *g*-values comprising the overall simulated spectra are shown at the top (blue, axial 2.04; red, rhombic 2.07 magenta, isotropic 2.0; green, axial 2.03). Microwave frequency, 9.38 GHz; microwave power, 1 mW; modulation frequency, 100 kHz; modulation amplitude, 10.0 G; sample temperature, 10 K.

Supplemental information references

(1) Merkley, E. D., Rysavy, S., Kahraman, A., Hafen, R. P., Daggett, V., and Adkins, J. N. (2014) Distance restraints from crosslinking mass spectrometry: mining a molecular dynamics simulation database to evaluate lysine-lysine distances. *Protein Sci. 23*, 747–759.

(2) Ray, A., Lindahl, E., and Wallner, B. (2012) Improved model quality assessment using ProQ2. *BMC Bioinformatics 13*, 224.

(3) Buchan, D. W. A., Minneci, F., Nugent, T. C. O., Bryson, K., and Jones, D. T. (2013) Scalable web services for the PSIPRED Protein Analysis Workbench. *Nucl. Acids Res. 41*, W349–W357.

(4) Capra, J. A., and Singh, M. (2007) Predicting functionally important residues from sequence conservation. *Bioinformatics 23*, 1875–1882.

(5) Schmidtke, P., Bidon-Chanal, A., Luque, F. J., and Barril, X. (2011) MDpocket: open-source cavity detection and characterization on molecular dynamics trajectories. *Bioinformatics 27*, 3276–3285.

(6) Higurashi, M., Ishida, T., and Kinoshita, K. (2009) PiSite: a database of protein interaction sites using multiple binding states in the PDB. *Nucl. Acids Res. 37*, D360–D364.

A

	Experimental M+H	Primary Score	ppm	Peaks Matched	Peaks Matched	Peptide Sequence (additional mass modifications indicated)	Protein 1	Protein 2	Cross-linked residue	Cross-linked residue	Cα to Cα Distance
A with B				Protein 1	Protein 2				Protein 1	Protein 2	
	4839.566	10.77	8.1	86	20	DYRNEPYTKALTDLVNSYQPEILMLGATTLGR - KEIQVERR	FIXB	FIXA	103	158	25.4
	5727.943	7.62	4.4	44	31	GKEICGEAFQHGADKAYLLQHEILRDYR - VAAGIDTVEMIGLKGSPTVVS(156.0786)K	FIXB	FIXA	84	227	30.8
	4185.177	7.82	5.5	62	15	HVWVC(57.02146)IESERGVVHPVSWELLGEGRK - IVDVDTAKK	FIXB	FIXA	31	157	33.9
	5727.943	7.62	4.4	44	31	GKEICGEAFQHGADKAYLLQHEILRDYR - VAAGIDTVEMIGLKGSPTVVS(156.0786)K	FIXB	FIXA	84	225	34.8
	6080.245	7.73	11.1	35	41	HVWVC(57.02146)IESERGVVHPVSWELLGEGRK - VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K	FIXB	FIXA	31	225	35.8
	5727.939	12.75	5.1	61	65	GKEICGEAFQHGADKAYLLQHEILRDYR - VAAGIDTVEMIGLKGSPTVVS(156.0786)K	FIXB	FIXA	71	227	37.2
	5969.066	12.13	10.5	62	58	HVWVC(57.02146)IESERGVVHPVSWELLGEGRK - LK(156.0786)DKFGGTVTVVTMGPPMAEAALRK	FIXB	FIXA	31	51	37.4
	5969.066	12.83	10.5	62	65	HVWVC(57.02146)IESERGVVHPVSWELLGEGRK - LKDK(156.0786)FGGTVTVVTMGPPMAEAALRK	FIXB	FIXA	31	49	39.1
	4186.197	8.51	13.7	60	24	NLASTRPTFGGSLLC(57.02146)TILTQR - IVDVDTAKKEIQVER	FIXB	FIXA	166	157	42.1
	5727.939	12.65	5.1	61	64	GKEICGEAFQHGADKAYLLQHEILRDYR - VAAGIDTVEMIGLKGSPTVVS(156.0786)K	FIXB	FIXA	71	225	42.2
	6949.570	14.36	8.2	79	63	FGGTVTVVTMGPPMAEAALRKC(57.02146)LSFGADDAILVSDR - HVWVC(57.02146)IESERGVVHPVSWELLGEGRK	FIXA	FIXB	72	31	42.9
	4034.089	9.55	6.2	58	36	KLADALGVELYGVVICGPGER - KIMEDMPVDLIFTGK	FIXB	FIXA	49	107	46.5
A with C											
	6080.255	9.52	8.9	52	42	ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR - QGVPAIINPYDLFALEEALRLKDK	FIXC	FIXA	246	49	13.5
	4112.079	7.03	14.9	58	11	ESITLGVGCMLSDFKQQKISPYELLDR - KEIQVER	FIXC	FIXA	243	158	20.9
	7025.418	15.05	19.0	74	75	KC(57.02146)LSFGADDAILVSDRAFAGSDTLATSYALSAVIR - ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR	FIXA	FIXC	72	246	21.5
	4094.064	7.72	11.1	47	29	DKFGGTVTVVTMGPPMAEAALR - THPS(156.0786)IAPLIAGSEMK	FIXA	FIXC	51	272	24.4
	4034.089	9.05	9.6	43	46	MHSVVCIKQVPDSAQIR - ESITLGVGCMLSDFKQQK	FIXA	FIXC	1	246	25.9
	4112.079	7.64	14.9	64	11	ESITLGVGCMLSDFKQQKISPYELLDR - KEIQVER	FIXC	FIXA	251	158	26.1
	5727.934	10.04	5.3	74	25	DKFGGTVTVVTMGPPMAEAALRKC(57.02146)LSFGADDAILVS(156.0786)DR - KLDDSFVMKDLK	FIXA	FIXC	75	351	26.1
	6080.245	8.11	7.3	49	31	ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR - QGVPAIINPYDLFALEEALRLKDK	FIXC	FIXA	249	49	26.3
	5727.934	10.66	5.3	80	25	DK(156.0786)FGGTVTVVTMGPPMAEAALRKC(57.02146)LSFGADDAILVSDR - KLDDSFVMKDLK	FIXA	FIXC	72	351	27.1
	4112.079	7.13	14.9	59	11	ESITLGVGCMLSDFKQQKISPYELLDR - KEIQVER	FIXC	FIXA	246	158	27.4
	4029.078	7.34	11.9	41	31	AADK(156.0786)PFSAANLAEYKK(156.0786)K - QTIDGDTAQVGPGIAKR	FIXC	FIXA	349	137	27.8
	4839.566	11.05	0.1	67	42	LKDK(156.0786)FGGTVTVVTMGPPMAEAALRK - QGGEVRADAVILADGVNSR	FIXA	FIXC	49	165	28.0
	7025.418	14.85	19.0	66	81	KC(57.02146)LSFGADDAILVSDRAFAGSDTLATSYALSAVIR - ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR	FIXA	FIXC	75	249	30.0
	5727.934	10.05	8.9	68	31	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - ES(156.0786)ITLGVGC(57.02146)MLSDFKQQK	FIXA	FIXC	207	243	30.2
	7025.418	15.66	19.0	74	81	KC(57.02146)LSFGADDAILVSDRAFAGSDTLATSYALSAVIR - ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR	FIXA	FIXC	72	249	30.9
	4094.064	7.21	2.4	35	36	AFAGSDTLATSYALSAVIR - ESITLGVGC(57.02146)MLSDFKQQK	FIXA	FIXC	98	246	31.0
	5471.799	7.31	0.8	53	19	QQKISPYELLDRMKTHPS(156.0786)IAPLIAGS(156.0786)EMK - CLSFGADDAILVS(156.0786)DR	FIXC	FIXA	260	75	31.2
	4839.566	12.08	13.6	101	18	EAGVLTICETTVTDLLIEGGKVVGVRTDRQGGEVR - VFAPKPRSK	FIXC	FIXA	139	241	31.2
	6949.570	14.65	2.2	89	56	FGGTVTVVTMGPPMAEAALRKC(57.02146)LSFGADDAILVS(156.0786)DR - GETPGSKNVQGAILYADAIEKIIPDFR	FIXA	FIXC	72	41	31.4
	4190.183	8.41	2.6	58	25	SKRAELIESHDS(156.0786)DPKNLAEAALAK - ADAVILADGVNSR	FIXA	FIXC	242	165	31.8
	4112.079	7.74	14.9	65	11	ESITLGVGCMLSDFKQQKISPYELLDR - KEIQVER	FIXC	FIXA	249	158	31.9
	4094.064	7.21	2.4	35	36	AFAGSDTLATSYALSAVIR - ESITLGVGC(57.02146)MLSDFKQQK	FIXA	FIXC	102	246	32.3
	6949.570	14.35	2.2	87	55	FGGTVTVVTMGPPMAEAALRKC(57.02146)LSFGADDAILVS(156.0786)DR - GETPGSKNVQGAILYADAIEKIIPDFR	FIXA	FIXC	75	42	33.1
	4094.064	7.13	12.7	58	12	AADKPFSAANLAEYKK(156.0786)KLDDSFVMK - QVPDSAQIR	FIXC	FIXA	349	13	33.6
	5727.934	9.85	8.9	68	29	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - ES(156.0786)ITLGVGC(57.02146)MLSDFKQQK	FIXA	FIXC	207	246	33.6
	6949.570	14.55	2.2	89	55	FGGTVTVVTMGPPMAEAALRKC(57.02146)LSFGADDAILVS(156.0786)DR - GETPGSKNVQGAILYADAIEKIIPDFR	FIXA	FIXC	72	42	33.7
	5969.066	12.13	17.4	55	65	GETPGSK(156.0786)NVQGAILYADAIEKIIPDFR - LKDK(156.0786)FGGTVTVVTMGPPMAEAALRK	FIXC	FIXA	41	49	34.0
	4839.566	11.38	13.6	101	11	EAGVLTICETTVTDLLIEGGKVVGVRTDRQGGEVR - VFAPKPRSK	FIXC	FIXA	139	238	34.5
	5969.066	11.93	17.4	53	65	GETPGSKNVQGAILYADAIEK(156.0786)IIPDFR - LKDK(156.0786)FGGTVTVVTMGPPMAEAALRK	FIXC	FIXA	42	49	34.8
	4094.064	7.10	2.4	34	36	AFAGSDTLATSYALSAVIR - ESITLGVGC(57.02146)MLSDFKQQK	FIXA	FIXC	92	246	35.4
	4094.064	7.14	12.7	58	12	AADKPFSAANLAEYKKK(156.0786)LDDSFVMK - QVPDSAQIR	FIXC	FIXA	350	13	37.0
	4185.174	7.15	2.7	48	22	FDVIVVGAGMAGNAAAYTLAKGGLK - RAELIESHDS(156.0786)DPK	FIXC	FIXA	25	249	38.2
	4094.064	7.25	12.7	59	12	AADKPFSAANLAEYKKKLDDS(156.0786)FVMK - QVPDSAQIR	FIXC	FIXA	351	13	38.5
	5727.949	7.11	6.3	48	22	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - ES(156.0786)ITLGVGC(57.02146)MLSDFKQQK	FIXA	FIXC	227	246	38.6
	4112.079	8.04	9.8	49	30	DKFGGTVTVVTMGPPMAEAALR - AADKPFSAANLAEYKK	FIXA	FIXC	51	349	39.5
	3340.753	7.03	1.1	57	12	TDRQGGEVRADAVILADGVNSR - KEIQVER	FIXC	FIXA	165	158	40.1
	5967.053	7.11	4.3	57	13	DKFGGTVTVVTMGPPMAEAALRKCLS(156.0786)FGADDAILVSDR - VHFDQWFNKK(156.0786)AR	FIXA	FIXC	72	115	40.4
	5727.934	9.94	5.3	74	24	DKFGGTVTVVTMGPPMAEAALRKC(57.02146)LSFGADDAILVSDR - KLDDS(156.0786)FVMKDLK	FIXA	FIXC	51	351	40.7
	5471.799	7.31	7.5	62	10	DKFGGTVTVVTMGPPMAEAALRKCLS(156.0786)FGADDAILVSDR - KLDDS(156.0786)FVMK	FIXA	FIXC	85	351	40.9
	4185.174	7.11	16.2	54	16	GETPGS(156.0786)KNVQGAILYADAIEKIIPDFR - IVDVDTAKK	FIXC	FIXA	42	157	42.4
	4185.174	7.22	16.2	55	16	GETPGSK(156.0786)NVQGAILYADAIEKIIPDFR - IVDVDTAKK	FIXC	FIXA	41	157	42.6
	4094.064	7.45	11.1	41	32	AFAGSDTLATSYALS(156.0786)AVIR - NKQFFTTYPDLLS(156.0786)R	FIXA	FIXC	98	375	42.6
	6080.245	7.91	17.0	51	27	VHFDQWFNKK(156.0786)AREAGVLTICETTVTDLLIEGGK - MHSVVCIKQVPDS(156.0786)AQIR	FIXC	FIXA	115	8	46.2
	4094.064	7.13	11.1	36	34	AFAGSDTLATS(156.0786)YALS(156.0786)AVIR - NKQFFTTYPDLLSR	FIXA	FIXC	92	375	46.3
	5471.799	7.31	0.8	53	19	QQKISPYELLDRMKTHPS(156.0786)IAPLIAGS(156.0786)EMK - CLS(156.0786)FGADDAILVSDR	FIXC	FIXA	260	85	47.8
	4190.194	9.74	10.9	53	43	VAAGIDTVEMIGLKGSPTVVSK - AADK(156.0786)PFSAANLAEYKK	FIXA	FIXC	232	349	50.1
	4839.566	11.27	1.9	87	24	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - LDDSFVMKDLK	FIXA	FIXC	227	359	49.7
	4190.194	9.96	10.9	57	41	VAAGIDTVEMIGLKGSPTVVS(156.0786)K - AADKPFSAANLAEYKK	FIXA	FIXC	227	349	50.5
	4839.566	10.86	10.2	77	30	KIMEDMPVDLIFTGKQTIDGDTAQVGPGIAK - VLQIERGETPGSK	FIXA	FIXC	107	41	51.8
	6080.245	7.91	17.0	51	27	VHFDQWFNKK(156.0786)AREAGVLTICETTVTDLLIEGGK - MHSVVCIKQVPDS(156.0786)AQIR	FIXC	FIXA	115	3	51.9
	4190.194	10.06	10.9	58	41	VAAGIDTVEMIGLKGS(156.0786)PTVVSK - AADKPFSAANLAEYKK	FIXA	FIXC	225	349	52.0
	4112.079	7.53	9.8	49	25	DKFGGTVTVVTMGPPMAEAALR - AADKPFSAANLAEYKK	FIXA	FIXC	51	338	52.9
	4112.079	7.34	9.8	49	23	DKFGGTVTVVTMGPPMAEAALR - AADKPFSAANLAEYKK	FIXA	FIXC	51	341	53.2
	6080.245	8.12	17.0	51	29	VHFDQWFNKK(156.0786)AREAGVLTICETTVTDLLIEGGK - MHSVVCIKQVPDS(156.0786)AQIR	FIXC	FIXA	115	1	53.7
	4029.076	7.03	12.5	33	36	AADK(156.0786)PFSAANLAEYK(156.0786)KK - QTIDGDTAQVGPGIAKR	FIXC	FIXA	350	137	53.8
	6080.252	7.29	5.4	34	38	GETPGSKNVQGAILYADAIEK(156.0786)IIPDFR - VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K	FIXC	FIXA	42	227	54.3
	5412.792	8.42	18.5	67	16	VWDRVAAGIDTVEMIGLKGSPTVVSKVFAPK(156.0786)PR - AADKPFSAANLAEYK	FIXA	FIXC	233	341	55.7

	5412.792	8.83	18.5	67	20	VWDRVAAGIDTVEMIGLKGSPTVVSKVFAPK(156.0786)PR - AADKPFSAANLAEYK	FIXA	FIXC	233	338	56.8
	6080.252	7.19	5.4	36	35	GETPGSKNVQGAILYADAIEK(156.0786)IIPDFR - VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K	FIXC	FIXA	41	225	57.0
	4034.089	9.55	17.8	57	37	VAAGIDTVEMIGLKGSPTVVSK - AADKPFSAANLAEYKK	FIXA	FIXC	232	338	57.2
	6949.570	14.24	13.2	127	14	MKTHPSIAPLIAGSEMKEYAAHLIPEGGYNAIPQVYGDGWMIAGDAGQFVNGIHR - GS(156.0786)PTVVSK	FIXC	FIXA	260	232	57.2
	4094.064	7.24	16.3	55	16	MAERFDVIVVGAGMAGNAAAYTLAKGGLK - MHS(156.0786)VVCIK	FIXC	FIXA	25	1	58.0
	4700.564	7.94	6.4	71	7	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIERGETPGSK - GSPTVVSK	FIXC	FIXA	29	227	59.4
	4839.612	14.20	17.4	103	37	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIER - GSPTVVSKVFAPKPR	FIXC	FIXA	29	227	59.4
	4190.183	8.35	13.4	53	29	VAAGIDTVEMIGLKGSPTVVS(156.0786)K - AADKPFSAANLAEYKK	FIXA	FIXC	225	341	60.0
	4190.183	8.96	13.4	55	33	VAAGIDTVEMIGLKGSPTVVS(156.0786)K - AADKPFSAANLAEYKK	FIXA	FIXC	227	338	60.4
	4700.564	7.83	6.4	71	6	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIERGETPGSK - GSPTVVSK	FIXC	FIXA	25	232	60.9
	4190.183	8.75	13.4	53	33	VAAGIDTVEMIGLKGSPTVVS(156.0786)K - AADKPFSAANLAEYKK	FIXA	FIXC	225	338	61.7
	4029.075	7.01	12.8	35	34	AADK(156.0786)PFSAANLAEYKK(156.0786)K - QTIDGDTAQVGPGIAKR	FIXC	FIXA	341	137	62.8
	4029.076	7.02	12.5	33	36	AADKPFSAANLAEYK(156.0786)K(156.0786)K - QTIDGDTAQVGPGIAKR	FIXC	FIXA	338	137	63.6
	6080.255	9.23	0.4	55	36	HELKVWDRVAAGIDTVEMIGLK(156.0786)GSPTVVS(156.0786)K - FDVIVVGAGMAGNAAAYTLAKGGLK	FIXA	FIXC	227	25	64.9
	5727.939	12.96	8.2	84	44	HELK(156.0786)VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - ESITLGVGC(57.02146)MLSDFKQQK	FIXA	FIXC	225	233	65.3
	6080.252	7.19	0.9	42	29	HELK(156.0786)VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - FDVIVVGAGMAGNAAAYTLAKGGLK	FIXA	FIXC	225	25	67.5
B with C												
	5471.799	7.72	3.5	45	31	VLGAEVGASRPIVQAGWAELDRQVGQS(156.0786)GK - ES(156.0786)ITLGVGC(57.02146)MLSDFKQQK	FIXB	FIXC	267	246	13.7
	5727.944	8.53	17.9	55	29	ES(156.0786)ITLGVGCMLSDFKQQKIS(156.0786)PYELLDR - TVRPKLYIAAGIS(156.0786)GAIQHR	FIXC	FIXB	246	292	30.2
	5727.944	8.93	17.9	59	29	ESITLGVGCMLSDFK(156.0786)QQKIS(156.0786)PYELLDR - TVRPKLYIAAGIS(156.0786)GAIQHR	FIXC	FIXB	249	292	31.2
	5727.943	8.23	16.7	44	37	QQKISPYELLDRMKTHPSIAPLIAGSEMK - NLASTRPTFGGSLLC(57.02146)TILTQR	FIXC	FIXB	249	166	34.1
	5500.014	14.17	16.3	78	62	VLQIERGETPGSKNVQGAILYADAIEK - NLAS(156.0786)TRPTFGGSLLC(57.02146)TILTQR	FIXC	FIXB	42	166	37.4
	6949.570	14.97	2.8	121	27	DLAGSVATTLGTGLVADC(57.02146)TELVIDTETRNLASTRPTFGGSLLC(57.02146)TILTQR - QQKIS(156.0786)PYELLDR	FIXB	FIXC	131	249	37.7
	4185.177	7.33	8.9	50	22	VLGAEVGASRPIVQAGWAELDR - LDDS(156.0786)FVMKDLK(156.0786)K	FIXB	FIXC	267	359	40.1
	5727.949	7.03	5.0	56	13	K(156.0786)LADALGVELYGVVICGPGERGKEIC(57.02146)GEAFQHGADK - QQKIS(156.0786)PYELLDR	FIXB	FIXC	71	249	45.1
	7024.433	7.81	10.2	40	37	LADALGVELYGVVICGPGERGKEICGEAFQHGADK - ESITLGVGC(57.02146)MLSDFKQQKIS(156.0786)PYELLDR	FIXB	FIXC	71	246	43.5
	4034.089	8.95	16.4	58	30	NLASTRPTFGGSLLCTILTQR - QQKIS(156.0786)PYELLDR	FIXB	FIXC	158	249	44.0
	4185.177	7.02	8.9	50	19	VLGAEVGASRPIVQAGWAELDR - KLDDS(156.0786)FVMK(156.0786)DLK	FIXB	FIXC	267	351	44.8
	4839.566	13.12	3.3	57	72	FDVIVVGAGMAGNAAAYTLAKGGLK - NLASTRPTFGGSLLC(57.02146)TILTQR	FIXC	FIXB	25	166	47.3
	4185.177	8.35	0.3	52	30	VLGAEVGASRPIVQAGWAELDR - AADKPFS(156.0786)AANLAEYK	FIXB	FIXC	267	338	49.3
	4839.566	11.48	4.6	82	31	VLGAEVGASRPIVQAGWAELDRQVGQSGK - AADKPFSAANLAEYKK	FIXB	FIXC	285	338	50.8
	4190.194	10.65	9.3	66	39	KLADALGVELYGVVICGPGERGK - THPS(156.0786)IAPLIAGSEMK	FIXB	FIXC	49	272	60.9
	5471.799	7.43	10.0	49	24	IS(156.0786)PYELLDRMKTHPSIAPLIAGSEMK - SGEIIEVPFS(156.0786)MIETDIITK	FIXC	FIXB	260	198	61.8
	4839.566	12.30	3.3	57	64	FDVIVVGAGMAGNAAAYTLAKGGLK - NLASTRPTFGGSLLC(57.02146)TILTQR	FIXC	FIXB	25	158	63.3
	4112.079	7.05	11.3	49	20	MPEIFHKNKQFFTTYPDLLS(156.0786)R - VMAMPEPDASR	FIXC	FIXB	375	196	72.2
	5412.792	8.12	14.8	64	16	KLADALGVELYGVVICGPGERGKEIC(57.02146)GEAFQHGADK - QFFTTYPDLLSR	FIXB	FIXC	71	386	72.3
	4185.186	7.32	11.5	37	35	SGEIIEVPFSMIETDIITK - GGLKVLQIERGETPGS(156.0786)K	FIXB	FIXC	198	29	79.5
	4112.079	7.24	19.7	36	35	SGEIIEVPFSMIETDIITK - AADKPFSAANLAEYKKK	FIXB	FIXC	198	350	82.1
A with X												
	7078.324	10.42	1.7	98	5	QCTVCCPAGCYTRETNGKVTLVTDGCLECGTC(57.02146)RIICQDSGNLEWEWPR - GSPTVVSKVFAPKPR	FIXX	FIXA	54	232	18.7
	7078.324	10.32	1.7	98	4	QCTVCCPAGCYTRETNGKVTLVTDGCLECGTC(57.02146)RIICQDSGNLEWEWPR - GSPTVVSKVFAPKPR	FIXX	FIXA	54	233	19.5
	5727.934	9.94	0.2	58	40	VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - VDS(156.0786)GRPHIRIKDPDHCTECLEK	FIXA	FIXX	225	25	20.9
	5727.934	10.34	0.2	62	40	VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - VDS(156.0786)GRPHIRIKDPDHCTECLEK	FIXA	FIXX	227	25	21.9
	7063.375	7.41	11.7	42	31	ETNGKVTLVTDGC(57.02146)LECGTCRIIC(57.02146)QDSGNLEWEWPR - VWDRVAAGIDTVEMIGLKGS(156.0786)PTVVSK	FIXX	FIXA	54	225	22.6
	7078.324	10.72	1.7	98	8	QCTVCCPAGCYTRETNGKVTLVTDGCLECGTC(57.02146)RIICQDSGNLEWEWPR - GSPTVVSKVFAPKPR	FIXX	FIXA	54	227	24.7
	7429.417	18.85	12.3	100	87	KCLSFGADDAILVSDRAFAGSDTLATSYALSAVIRK - IKDPDHCTECLEKQCTVC(57.02146)CPAGC(57.02146)YTRETNGK	FIXA	FIXX	72	36	28.4
	7078.324	10.72	1.7	98	8	QCTVCCPAGCYTRETNGKVTLVTDGCLECGTC(57.02146)RIICQDSGNLEWEWPR - GSPTVVSKVFAPKPR	FIXX	FIXA	54	238	29.2
	7024.429	11.92	13.2	58	60	K(156.0786)IMEDMPVDLIFTGK(156.0786)QTIDGDTAQVGPGIAKR - IK(156.0786)DPDHCTECLEKQCTVCCPAGC(57.02146)YTR	FIXA	FIXX	137	36	33.4
	4700.564	8.13	3.9	63	17	VWDRVAAGIDTVEMIGLKGSPTVVSKVFAPKPR - VDSGRPHIR	FIXA	FIXX	233	17	34.3
	7429.417	18.75	6.1	142	44	QCTVCCPAGCYTRETNGKVTLVTDGC(57.02146)LECGTCRIIC(57.02146)QDSGNLEWEWPR - KCLSFGADDAILVS(156.0786)DR	FIXX	FIXA	54	72	34.7
	4700.564	8.43	3.9	66	17	VWDRVAAGIDTVEMIGLKGSPTVVSKVFAPKPR - VDSGRPHIR	FIXA	FIXX	238	17	37.8
	5727.934	9.94	0.2	58	40	VWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - VDSGRPHIRIK(156.0786)DPDHCTECLEK	FIXA	FIXX	225	17	38.3
	5727.944	8.54	8.6	63	21	FGGTVTVVTMGPPMAEAALRKC(57.02146)LS(156.0786)FGADDAILVSDR - MSKIEEK(156.0786)LFQDR	FIXA	FIXX	72	3	41.5
	5727.943	7.41	5.4	39	34	YRVDSGRPHIRIKDPDHC(57.02146)TECLEK - VFAPKPRSKRAELIESHDS(156.0786)DPK	FIXX	FIXA	25	249	53.9
B with X												
	7429.417	19.05	6.4	100	89	KLADALGVELYGVVICGPGERGKEICGEAFQHGADK - IKDPDHCTECLEKQC(57.02146)TVCCPAGC(57.02146)YTRETNGK	FIXB	FIXX	49	36	34.8
	5471.799	7.31	1.4	35	37	IICQDSGNLEWEWPRGGFGILFKFG - NLAS(156.0786)TRPTFGGSLLC(57.02146)TILTQR	FIXX	FIXB	92	166	36.8
	7429.417	18.94	6.4	100	88	KLADALGVELYGVVICGPGERGKEICGEAFQHGADK - IKDPDHC(57.02146)TECLEKQCTVCCPAGC(57.02146)YTRETNGK	FIXB	FIXX	49	25	44.8
	7429.417	18.96	14.0	106	82	ETNGKVTLVTDGCLECGTCRIIC(57.02146)QDSGNLEWEWPR - VMAMPEPDASRSGEIIEVPFSMIETDIITK	FIXX	FIXB	54	207	53.5
	7429.417	19.35	6.4	105	87	KLADALGVELYGVVICGPGERGKEICGEAFQHGADK - IKDPDHCTECLEKQC(57.02146)TVCCPAGC(57.02146)YTRETNGK	FIXB	FIXX	71	25	56.0
	4112.079	7.11	13.5	55	15	NQENFQLVWDLAKVLGAEVGAS(156.0786)RP - IEEKLFQDR	FIXB	FIXX	258	7	56.9
	7024.433	7.71	9.6	42	34	VDS(156.0786)GRPHIRIKDPDHCTECLEKQCTVCCPAGCYTR - NQENFQLVWDLAKVLGAEVGAS(156.0786)RP	FIXX	FIXB	25	258	58.0
C with X												
	7024.433	7.69	15.5	42	34	EGSNLAMTTGMLAAQTLVELRAADKPFSAANLAEYKK - IKDPDHCTECLEKQC(57.02146)TVCCPAGCYTR	FIXC	FIXX	349	25	34.0
	5727.944	8.53	2.0	69	15	EGSNLAMTTGMLAAQTLVELRAADKPFS(156.0786)AANLAEYKK - MSKIEEKLFQDR	FIXC	FIXX	338	7	35.2
	7025.418	14.86	18.8	70	77	IKDPDHCTECLEK(156.0786)QCTVCCPAGC(57.02146)YTRETNGK - ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR	FIXX	FIXC	25	246	38.3
	7024.433	7.90	15.5	44	34	EGSNLAMTTGMLAAQTLVELRAADKPFSAANLAEYKK - IKDPDHCTECLEKQC(57.02146)TVCCPAGCYTR	FIXC	FIXX	341	25	40.1
	6949.570	14.16	1.5	93	47	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIERGETPGS(156.0786)K - YRVDSGRPHIRIKDPDHCTECLEK	FIXC	FIXX	29	17	40.2
	5727.944	8.64	2.0	69	16	EGSNLAMTTGMLAAQTLVELRAADKPFS(156.0786)AANLAEYKK - MSKIEEKLFQDR	FIXC	FIXX	338	3	41.1
	7024.433	8.10	15.5	46	34	EGSNLAMTTGMLAAQTLVELRAADKPFSAANLAEYKK - IKDPDHCTECLEKQC(57.02146)TVCCPAGCYTR	FIXC	FIXX	338	25	42.2
	7063.375	7.41	8.9	39	34	VLQIERGETPGSKNVQGAILYADAIEK(156.0786)IIPDFR - IK(156.0786)DPDHCTECLEKQCTVCC(57.02146)PAGCYTR	FIXC	FIXX	41	36	44.8
	7024.433	7.80	15.5	46	31	EGSNLAMTTGMLAAQTLVELRAADKPFSAANLAEYKK - IKDPDHCTECLEKQC(57.02146)TVCCPAGCYTR	FIXC	FIXX	338	36	45.2

	7024.405	9.94	17.4	43	55	IKDPDHCTECLEKQCTVC(57.02146)CPAGCYTRETNGK - ESITLGVGCMLSDFK(156.0786)QQKIS(156.0786)PYELLDR	FIXX	FIXC	36	249	45.8
	7063.375	7.51	8.9	40	34	VLQIERGETPGSKNVQGAILYADAIEK(156.0786)IIPDFR - IK(156.0786)DPDHCTECLEKQCTVCC(57.02146)PAGCYTR	FIXC	FIXX	42	36	47.3
	7024.405	9.94	17.4	44	54	IKDPDHCTECLEK(156.0786)QCTVC(57.02146)CPAGCYTRETNGK - ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR	FIXX	FIXC	25	249	47.8
	7063.375	7.22	3.2	47	24	ETNGKVTLVTDGCLECGTCRIIC(57.02146)QDSGNLEWEWPR - AADKPFSAANLAEYKKKLDDS(156.0786)FVMK	FIXX	FIXC	54	338	49.1
	4185.174	7.20	14.5	52	19	KAREAGVLTICETTVTDLLIEGGK - IKDPDHCTECLEK	FIXC	FIXX	116	25	52.4
	7024.405	9.94	1.9	59	39	ETNGKVTLVTDGC(57.02146)LECGTCRIICQDSGNLEWEWPR - GETPGSKNVQGAILYADAIEKIIPDFR	FIXX	FIXC	54	41	54.8
	7024.405	10.04	1.9	59	40	ETNGKVTLVTDGC(57.02146)LECGTCRIICQDSGNLEWEWPR - GETPGSKNVQGAILYADAIEKIIPDFR	FIXX	FIXC	54	42	57.5
	7024.405	9.63	1.9	59	36	ETNGKVTLVTDGC(57.02146)LECGTCRIICQDSGNLEWEWPR - GETPGSKNVQGAILYADAIEKIIPDFR	FIXX	FIXC	54	56	61.4
	6949.570	14.44	15.6	127	16	THPS(156.0786)IAPLIAGSEMKEYAAHLIPEGGYNAIPQVYGDGWMIAGDAGQFVNGIHR - VDSGRPHIR	FIXC	FIXX	275	17	66.4

B

	Experimental M+H	Primary Score	ppm	Peaks Matched	Peaks Matched	Peptide Sequence (additional mass modifications indicated)	Cross-linked residue	Cross-linked residue	Cα to Cα Distance
FixC				Peptide 1	Peptide 2		Peptide 1	Peptide 2	
	7078.324	9.81	14.0	47	50	ISAGMMGTGFLYTNKESITLGVGCMLSDFK(156.0786)QQK - ISAGMMGTGFLYTNKESITLGVGC(57.02146)MLSDFK	231	233	5.4
	5471.803	7.03	11.1	49	20	ISPYELLDRMKTHPS(156.0786)IAPLIAGS(156.0786)EMK - MK(156.0786)THPS(156.0786)IAPLIAGSEMK	260	272	9.4
	4186.197	8.42	16.7	51	32	THPSIAPLIAGSEMKEYAAHLIP - ESITLGVGCMLSDFK	275	233	10.5
	6080.252	7.29	0.9	57	15	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIERGETPGS(156.0786)K - YTIIRVHFDQWFNKK	25	115	13.6
	6080.255	9.32	1.4	64	28	FDVIVVGAGMAGNAAAYTLAKGGLK(156.0786)VLQIERGETPGSK - YTIIRVHFDQWFNKK	41	115	14.1
	6080.252	7.29	0.9	57	15	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIERGETPGS(156.0786)K - YTIIRVHFDQWFNKK	29	115	17.1
	4034.089	9.14	0.6	59	31	TDRQGGEVRADAVILADGVNSR - K(156.0786)LDDSFVMKDLK	165	359	18.5
	4029.075	7.12	11.0	60	10	VVGVRTDRQGGEVRADAVILADGVNSR - KLDDSFVMK	165	351	21.1
	4185.176	7.63	5.1	58	17	VVGVRTDRQGGEVRADAVILADGVNSR - KLDDS(156.0786)FVMK	165	351	21.1
	4112.079	7.32	6.1	40	32	GETPGSKNVQGAILYADAIEK - ES(156.0786)ITLGVGC(57.02146)MLSDFK	42	243	21.4
	6080.255	9.70	5.9	58	38	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIER - VVGVRTDRQGGEVRADAVILADGVNSR	29	165	23.2
	4112.079	7.13	14.9	38	32	GETPGSKNVQGAILYADAIEK - MKTHPSIAPLIAGSEMK	42	272	23.9
	4839.612	14.39	4.4	105	37	FDVIVVGAGMAGNAAAYTLAKGGLK(156.0786)VLQIER - VLQIERGETPGSK	25	41	24.7
	4839.566	12.08	14.0	85	34	FDVIVVGAGMAGNAAAYTLAK(156.0786)GGLKVLQIER - VLQIERGETPGSK	29	41	25.4
	3165.743	2.60	3.9	25		KAREAGVLTICETTVTDLLIEGGKVVGVR	116	139	26.9
	6080.255	9.31	11.2	40	52	MAERFDVIVVGAGMAGNAAAYTLAKGGLK - GETPGSKNVQGAILYADAIEK(156.0786)IIPDFR	25	42	27.6
	4112.079	7.52	14.9	38	36	GETPGSKNVQGAILYADAIEK - MKTHPSIAPLIAGSEMK	42	260	29.0
	4094.064	7.14	4.2	37	33	ESITLGVGC(57.02146)MLSDFKQQK - LDDSFVMKDLK(156.0786)KYR	246	359	29.0
	4186.197	8.43	0.8	67	16	GETPGSKNVQGAILYADAIEKIIPDFR - MPEIFHKNK	41	373	29.8
	4112.079	7.42	14.9	37	36	GETPGSKNVQGAILYADAIEK - MKTHPSIAPLIAGSEMK	41	260	30.5
	4186.197	8.33	0.8	66	16	GETPGSKNVQGAILYADAIEKIIPDFR - MPEIFHKNK	42	373	30.5
	6949.570	14.16	4.6	69	71	AADKPFSAANLAEYK(156.0786)KKLDDSFVMKDLKK - ESITLGVGCMLSDFK(156.0786)QQKIS(156.0786)PYELLDR	362	249	30.8
	4094.064	7.12	4.2	36	34	ESITLGVGC(57.02146)MLSDFKQQK - LDDSFVMKDLKK(156.0786)YR	233	362	31.1
	4186.197	8.44	3.6	76	7	ESITLGVGCMLSDFKQQKIS(156.0786)PYELLDR - DLKKYR	249	362	33.4
	4186.197	8.53	9.2	57	27	VHFDQWFNKKAREAGVLTIC(57.02146)ETTVTDLLIEGGK - ES(156.0786)ITLGVGC(57.02146)MLSDFK	115	243	33.7
	4034.089	9.86	4.0	50	47	QGGEVRADAVILADGVNSR - MKTHPS(156.0786)IAPLIAGSEMK	165	260	36.2
	5727.943	7.61	9.5	56	19	ISAGMMGTGFLYTNKESITLGVGCMLS(156.0786)DFK(156.0786)QQK - KLDDSFVMKDLK(156.0786)K	231	359	37.1
	4190.194	10.06	10.4	68	31	FDVIVVGAGMAGNAAAYTLAKGGLK - ESITLGVGC(57.02146)MLSDFK	25	233	38.0
	4186.197	9.03	12.3	44	45	ESITLGVGC(57.02146)MLSDFKQQK - AADKPFSAANLAEYKK(156.0786)K	233	349	40.0
	5727.943	7.61	2.3	58	17	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIERGETPGSK - VWVMDDASYVGTHYR	25	83	40.0
	6949.570	14.16	13.8	73	67	ISAGMMGTGFLYTNKES(156.0786)ITLGVGCMLSDFKQQK - AADKPFSAANLAEYKK(156.0786)KLDDS(156.0786)FVMK	218	351	40.1
	4190.194	10.37	10.4	68	34	FDVIVVGAGMAGNAAAYTLAKGGLK - ESITLGVGC(57.02146)MLSDFK	25	243	40.5
	5727.943	7.51	9.5	56	18	ISAGMMGTGFLYTNKESITLGVGCMLS(156.0786)DFK(156.0786)QQK - KKLDDSFVMK(156.0786)DLK	231	351	40.8
	4186.197	8.32	12.3	46	36	ESITLGVGC(57.02146)MLSDFK(156.0786)QQK - AADKPFSAANLAEYKKK	233	350	41.7
	5727.943	7.52	15.5	43	31	IS(156.0786)PYELLDRMKTHPSIAPLIAGS(156.0786)EMK - YTIIRVHFDQWFNKK(156.0786)AR	260	115	43.1
	5727.943	7.41	9.5	56	17	ISAGMMGTGFLYTNKESITLGVGCMLS(156.0786)DFK(156.0786)QQK - KKLDDSFVMK(156.0786)DLK	231	350	43.6
	5727.939	13.36	13.3	110	22	FDVIVVGAGMAGNAAAYTLAKGGLK(156.0786)VLQIERGETPGS(156.0786)K - QFFTTYPDLLSR	25	386	44.5
	5727.943	7.40	2.3	56	17	FDVIVVGAGMAGNAAAYTLAKGGLKVLQIERGETPGSK - VWVMDDASYVGTHYR	29	83	45.6
	4839.566	11.18	15.4	74	36	EAGVLTICETTVTDLLIEGGKVVGVRTDR - ESITLGVGC(57.02146)MLSDFK	139	233	47.5
	5727.939	12.64	3.2	104	21	EGSNLAMTTGMLAAQTLVELRAADKPFS(156.0786)AANLAEYK(156.0786)K - ISPYELLDRMK	338	251	48.5
	5727.939	13.44	13.3	111	22	FDVIVVGAGMAGNAAAYTLAK(156.0786)GGLKVLQIERGETPGS(156.0786)K - QFFTTYPDLLSR	29	386	49.7
	5969.066	12.36	16.4	71	51	QQK(156.0786)ISPYELLDRMKTHPSIAPLIAGSEMK - FDVIVVGAGMAGNAAAYTLAKGGLK	260	25	52.4
	6950.565	8.31	2.4	49	33	ISAGMMGTGFLYTNKESITLGVGCMLSDFKQQK - GGLKVLQIERGETPGSKNVQGAILYADAIEK	218	29	54.3
FixA									
	5727.934	10.14	16.6	77	23	FGGTVTVVTMGPPMAEAALRKCLSFGADDAILVS(156.0786)DR - CLSFGADDAILVS(156.0786)DR	72	75	5.1
	4185.176	7.35	15.6	33	39	MHSVVC(57.02146)IKQVPDS(156.0786)AQIR - KC(57.02146)LSFGADDAILVS(156.0786)DR	8	72	11.9
	4185.176	7.04	15.6	33	36	MHSVVC(57.02146)IKQVPDS(156.0786)AQIR - KC(57.02146)LS(156.0786)FGADDAILVSDR	8	85	13.0
	4185.174	7.15	5.3	47	23	NLAEAALAKLFTQHPNLEQEIAK - AELIESHDS(156.0786)DPK	264	249	14.3
	5471.799	7.51	4.0	65	9	DK(156.0786)FGGTVTVVTMGPPMAEAALRKCLS(156.0786)FGADDAILVSDR - HELKVWDR	72	207	14.8
	5969.066	12.64	8.1	95	30	DKFGGTVTVVTMGPPMAEAALRKCLS(156.0786)FGADDAILVSDR - KIMEDMPVDLIFTGK	85	107	14.9
	2866.561	3.21	7.7	31		VWDRVAAGIDTVEMIGLKGSPTVVSK	225	232	15.2
	5500.014	14.58	17.7	94	50	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - MHSVVC(57.02146)IKQVPDSAQIR	207	3	15.7
	4185.177	7.76	15.7	39	37	MHSVVC(57.02146)IKQVPDS(156.0786)AQIR - KC(57.02146)LSFGADDAILVS(156.0786)DR	8	75	16.0
	5471.799	7.31	4.0	63	9	DKFGGTVTVVTMGPPMAEAALRK(156.0786)CLSFGADDAILVS(156.0786)DR - HELKVWDR	75	207	16.1
	4185.176	7.45	15.6	34	39	MHSVVC(57.02146)IKQVPDS(156.0786)AQIR - KC(57.02146)LSFGADDAILVS(156.0786)DR	3	72	16.8
	4185.174	7.03	7.9	57	12	MHSVVC(57.02146)IKQVPDSAQIRVHPVTNTIMR - GSPTVVS(156.0786)K	8	227	19.0
	5500.014	14.39	17.7	104	38	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - MHSVVC(57.02146)IKQVPDSAQIR	227	8	19.0
	5500.014	14.78	17.7	94	52	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - MHSVVC(57.02146)IKQVPDSAQIR	207	1	19.5
	4185.176	7.65	15.6	36	39	MHSVVC(57.02146)IKQVPDS(156.0786)AQIR - KC(57.02146)LSFGADDAILVS(156.0786)DR	1	72	20.7
	5727.934	10.16	5.6	74	26	FGGTVTVVTMGPPMAEAALRKCLS(156.0786)FGADDAILVSDR - KIMEDMPVDLIFTGK	72	107	23.1
	5727.934	9.95	5.6	72	26	FGGTVTVVTMGPPMAEAALRKCLSFGADDAILVS(156.0786)DR - KIMEDMPVDLIFTGK	75	107	24.1
	6080.245	8.03	9.6	49	30	VAAGIDTVEMIGLKGSPTVVSKVFAPK(156.0786)PR - VFAPK(156.0786)PRSKRAELIESHDS(156.0786)DPK	233	242	25.8
	5471.799	7.62	4.0	66	9	DKFGGTVTVVTMGPPMAEAALRK(156.0786)CLS(156.0786)FGADDAILVSDR - HELKVWDR	51	207	26.3
	4112.079	7.03	9.0	38	31	DKFGGTVTVVTMGPPMAEAALR - QTIDGDTAQVGPGIAKR	51	137	26.6
	5969.066	12.05	8.1	89	30	DKFGGTVTVVTMGPPMAEAALRK(156.0786)CLSFGADDAILVSDR - KIMEDMPVDLIFTGK	51	107	26.6
	5727.939	12.64	1.6	84	41	C(57.02146)LSFGADDAILVSDRAFAGSDTLATSYALS(156.0786)AVIR - IVDVDTAKK(156.0786)EIQVER	75	157	27.1

	6080.245	7.93	9.6	48	30	VAAGIDTVEMIGLKGSPTVVSKVFAPK(156.0786)PR - VFAPK(156.0786)PRSKRAELIESHDS(156.0786)DPK	232	242	28.7
	5500.014	14.69	17.7	95	50	HELKVWDRVAAGIDTVEMIGLKGSPTVVS(156.0786)K - MHSVVC(57.02146)IKQVPDSAQIR	225	3	30.9
	6080.255	9.21	12.0	48	43	HELKVWDRVAAGIDTVEMIGLKGSPTVVSK - PMAEAALRKCLS(156.0786)FGADDAILVSDR	232	72	34.1
	6949.570	14.33	17.6	76	66	VWDRVAAGIDTVEMIGLKGSPTVVSKVFAPKPR - KIMEDMPVDLIFTGKQTIDGDTAQVGPGIAK	233	107	36.4
	5500.014	14.07	10.9	92	47	VAAGIDTVEMIGLKGSPTVVSKVFAPK(156.0786)PRSK - K(156.0786)CLSFGADDAILVS(156.0786)DR	225	75	36.5
	6080.255	9.14	10.0	59	31	C(57.02146)LSFGADDAILVSDRAFAGS(156.0786)DTLATSYALSAVIR - GSPTVVSK(156.0786)VFAPKPRSK(156.0786)R	102	238	37.3
	6080.245	7.82	9.6	46	31	VAAGIDTVEMIGLKGSPTVVSKVFAPK(156.0786)PR - VFAPKPRSKRAELIES(156.0786)HDS(156.0786)DPK	227	242	39.0
	6080.245	8.03	9.6	49	30	VAAGIDTVEMIGLKGSPTVVSKVFAPK(156.0786)PR - VFAPKPRS(156.0786)KRAELIESHDS(156.0786)DPK	233	249	46.0
	4190.183	8.44	5.6	49	34	LDYQLLTYVSRIVDVDTAKK - SKRAELIESHDSDPK	157	241	52.0
	5500.014	14.88	2.1	73	74	NLAEAALAKLFTQHPNLEQEIAK(156.0786)RAV - VAAGIDTVEMIGLKGSPTVVS(156.0786)K	264	227	53.4
	5500.014	14.88	2.1	73	74	NLAEAALAKLFTQHPNLEQEIAK(156.0786)RAV - VAAGIDTVEMIGLKGSPTVVS(156.0786)K	264	225	58.2
	6080.245	7.72	9.6	43	33	VAAGIDTVEMIGLKGSPTVVSK(156.0786)VFAPK(156.0786)PR - VFAPKPRSKRAELIESHDS(156.0786)DPK	225	249	60.1
FixB									
	6080.255	9.13	13.3	58	32	NLAS(156.0786)TRPTFGGSLLCTILTQRHRPQMATVRPR - TVRPKLYIAAGIS(156.0786)GAIQHR	166	292	21.6
	4034.089	9.04	14.3	62	27	NLASTRPTFGGSLLC(57.02146)TILTQR - GKEICGEAFQHGADK	166	71	38.8