Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary Information

for

Dynamic Ruffling Distortion of the Heme Substrate in Non-Canonical Heme Oxygenase Enzymes

Amanda B. Graves, Erik H. Horak, and Matthew D. Liptak

Contents

CD Spectroscopy.

Experimental

To determine the effect of the F23X mutations on the fold of MhuD, UV CD spectra were collected on WT MhuD–heme–CN, F23A MhuD–heme–CN, and F23W MhuD–heme–CN between 240 and 190 nm in quartz cuvettes. The cyanide-inhibited, heme bound species were prepared as described in the main text. The species were exchanged into 50 mM Kpi, pH 7.4 with no NaCl using a PD-10 column (GE Healthcare). The samples were then further diluted with Milli-Q water to contain 10 mM KPi, pH 7.4. The measurements were performed on a Jasco J-815 spectropolarimeter averaging 5 scans with a scanning speed of 20 nm/min, a bandwidth of 1 nm, a digital integration time of 8 s, and a data pitch of 0.5 nm.

Results

UV CD was utilized to monitor if the substitutions to Phe23 affected the secondary structure of the polypeptide. UV CD can be analyzed to assess the percentage of α-helices and β-sheets comprising the protein secondary (Figure S5).[1] The cyanide-inhibited versions of the species were studied to ensure the enzyme was inactive for the experiment. The UV CD spectra for WT, F23A, and F23W MhuD–heme–CN display a peak maximum of 191 nm. WT and F23W cross through the zero line at 198 nm and F23A is shifted to 197 nm. A negative band at 209 nm is observed for WT and F23A MhuD but is found at 208 nm for F23W MhuD. Because of the differences observed between WT, F23A, and F23W MhuD, the data was analyzed with SELCON3 using IBasis=4 in the CDPro software package,[2-6] and the newly developed BestSel program for deconvolution of the curves to percentage α helices and β-sheets (Table S_).[7] The WT SELCON3 data was previously compared to the X-ray crystal structure of WT MhuD–heme–CN (PDB 4NL5) and found to have no significant changes to the structure.[8] The SELCON3 analysis reveals a constant percentage of β-sheets between the species and a slight increase in α-helices from WT to F23A to F23W MhuD. The BestSel analysis provides UV CD WT fold values closer to the X-ray crystallography attained

values. The F23A and F23W MhuD variants change only by 2% at most compared to WT MhuD when analyzed by the BestSel program. As the mutations do not significantly affect the protein secondary structure the changes to the electronic structure of the heme are due to changes to the second coordination sphere and not due to the protein unfolding.

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3 Sreerama, N.; Woody, R. W. *Biochemistry* 1994, **33**, 10022-10025.
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6 Johnson, W. C. *Proteins* 1999, **35**, 307-312.
7 Micsonai, A.; Wien, F.; Kernya, L.; Lee, Y. H.; Goto, Y.; Refregiers, M.; Kardos, *P. Natl. Acad. Sci. USA.* 2015, **112**, 3095-3103.
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Table S1. F23A MhuD mutagenic primer

Primer	**Sequence**
Forward	5'-GCTGGAGAAGCGGGCCGCTCACCGCGCG -3'
Reverse	5'-CGCGCGGTGAGCGGCCCGCTTCTCCAGC -3'

Table S2. F23W MhuD mutagenic primer

Primer	**Sequence**
Forward	5'-GCTGGAGAAGCGGTGGGCTCACCGCGCG -3'
Reverse	5'-CGCGCGGTGAGCCCACCGCTTCTCCAGC-3'

Table S3. F23A MhuD gene sequence

M1	P2	V3	V4	K5	I6	N7	A8	I9
ATG	CCA	GTG	GTG	AAG	ATC	AAC	GCA	ATC
E10	V11	P12	A13	G14	A15	G16	P17	E18
GAG	GTG	CCC	GCC	GGC	GCT	GGC	CCC	GAG
L19	E20	K21	R22	A23	A24	H25	R26	A27
CTG	GAG	AAG	CGG	GCC	GCT	CAC	CGC	GCG
H28	A29	V30	E31	N32	S33	P34	G35	F36
CAC	GCG	GTC	GAG	AAC	TCC	CCG	GGT	TTC
L37	G38	F39	Q40	L41	L42	R43	P44	V45
CTC	GGC	TTT	CAG	CTG	TTA	CGT	CCG	GTC
K46	G47	E48	E49	R50	Y51	F52	V53	V54
AAG	GGT	GAA	GAA	CGC	TAC	TTC	GTG	GTG
T55	H56	W57	E58	S59	D60	E61	A62	F63
ACA	CAC	TGG	GAG	TCC	GAT	GAA	GCA	TTC
Q64	A65	W66	A67	N68	G69	P70	A71	I72
CAG	GCG	TGG	GCA	AAC	GGG	CCC	GCC	ATC
A73	A74	H75	A76	G77	H78	R79	A80	N81
GCA	GCC	CAT	GCC	GGA	CAC	CGG	GCC	AAC
P82	V83	A84	T85	G86	A87	S88	L89	L90
CCC	GTG	GCG	ACC	GGT	GCT	TCG	CTG	CTG
E91	F92	E93	V94	V95	L96	D97	V98	G99
GAA	TTC	GAG	GTC	GTG	CTT	GAC	GTC	GGT
G100	T101	G102	K103	T104	A105	G106	G107	V108
GGG	ACC	GGC	AAG	ACT	GCA	GGA	GGT	GTA
P109	R110	G111	K112	L113	A114	A115	A116	L117
CCA	CGA	GGT	AAG	CTT	GCG	GCC	GCA	CTC
E118	H119	H120	H121	H122	H123	H124		
GAG	CAC	CAC	CAC	CAC	CAC	CAC		

Table S4. F23W MhuD gene sequence

M1	P2	V3	V4	K5	I6	N7	A8	I9
ATG	CCA	GTG	GTG	AAG	ATC	AAC	GCA	ATC
E10	V11	P12	A13	G14	A15	G16	P17	E18
GAG	GTG	CCC	GCC	GGC	GCT	GGC	CCC	GAG
L19	E20	K21	R22	W23	A24	H25	R26	A27
CTG	GAG	AAG	CGG	TGG	GCT	CAC	CGC	GCG
H28	A29	V30	E31	N32	S33	P34	G35	F36
CAC	GCG	GTC	GAG	AAC	TCC	CCG	GGT	TTC
L37	G38	F39	Q40	L41	L42	R43	P44	V45
CTC	GGC	TTT	CAG	CTG	TTA	CGT	CCG	GTC
K46	G47	E48	E49	R50	Y51	F52	V53	V54
AAG	GGT	GAA	GAA	CGC	TAC	TTC	GTG	GTG
T55	H56	W57	E58	S59	D60	E61	A62	F63
ACA	CAC	TGG	GAG	TCC	GAT	GAA	GCA	TTC
Q64	A65	W66	A67	N68	G69	P70	A71	I72
CAG	GCG	TGG	GCA	AAC	GGG	CCC	GCC	ATC
A73	A74	H75	A76	G77	H78	R79	A80	N81
GCA	GCC	CAT	GCC	GGA	CAC	CGG	GCC	AAC
P82	V83	A84	T85	G86	A87	S88	L89	L90
CCC	GTG	GCG	ACC	GGT	GCT	TCG	CTG	CTG
E91	F92	E93	V94	V95	L96	D97	V98	G99
GAA	TTC	GAG	GTC	GTG	CTT	GAC	GTC	GGT
G100	T101	G102	K103	T104	A105	G106	G107	V108
GGG	ACC	GGC	AAG	ACT	GCA	GGA	GGT	GTA
P109	R110	G111	K112	L113	A114	A115	A116	L117
CCA	CGA	GGT	AAG	CTT	GCG	GCC	GCA	CTC
E118	H119	H120	H121	H122	H123	H124		
GAG	CAC	CAC	CAC	CAC	CAC	CAC		

Table S5. WT IsdI gene sequence.

M1	F2	M3	A4	E5	N6	R7	L8	Q9
ATG	TTT	ATG	GCA	GAA	AAT	AGA	TTA	CAA
L10	Q11	K12	G13	S14	A15	E16	E17	T18
TTA	CAA	AAA	GGC	AGT	GCG	GAA	GAA	ACG
I19	E20	R21	F22	Y23	N24	R25	Q26	G27
ATT	GAA	CGT	TTT	TAC	AAT	AGA	CAA	GGT
I28	E29	T30	I31	E32	G33	F34	Q35	Q36
ATT	GAA	ACT	ATT	GAA	GGC	TTC	CAA	CAA
M37	F38	V39	T40	K41	T42	L43	N44	T45
ATG	TTT	GTC	ACT	AAA	ACA	TTA	AAT	ACC
E46	D47	T48	D49	E50	V51	K52	I53	L54
GAG	GAT	ACA	GAC	GAA	GTT	AAA	ATC	TTA
T55	I56	W57	E58	S59	E60	D61	S62	F63
ACT	ATT	TGG	GAA	TCT	GAA	GAT	AGC	TTT
N64	N65	W66	L67	N68	S69	D70	V71	F72
AAT	AAT	TGG	TTG	AAT	TCC	GAT	GTA	TTT
K73	E74	A75	H76	K77	N78	V79	R80	L81
AAA	GAA	GCT	CAT	AAA	AAT	GTA	CGT	TTA
K82	S83	D84	D85	D86	G87	Q88	Q89	S90
AAA	AGT	GAT	GAC	GAT	GGA	CAG	CAA	AGT
P91	I92	L93	S94	N95	K96	V97	F98	K99
CCA	ATA	TTA	TCA	AAT	AAA	GTA	TTC	AAA
Y100	D101	I102	G103	Y104	H105	Y106	Q107	K108
TAT	GAT	ATT	GGC	TAC	CAC	TAT	CAA	AAA

Table S6. Secondary structure analysis by UV CD for MhuD–heme–CN variants

Species	Percent α helical	Percent β sheet
WT[a]	14.0	21.0
F23A[a]	23.0	22.0
F23W[a]	27.0	21.0
WT[b]	21.0	31.0
F23A[b]	22.0	29.5
F23W[b]	20.9	33.4
WT[c]	19.0	31.0

[a] Analyzed with Selcon3[2-6] [b] Analyzed with BestSel[7] [c] From X-ray crystal structure (PDB 4NL5)[8]

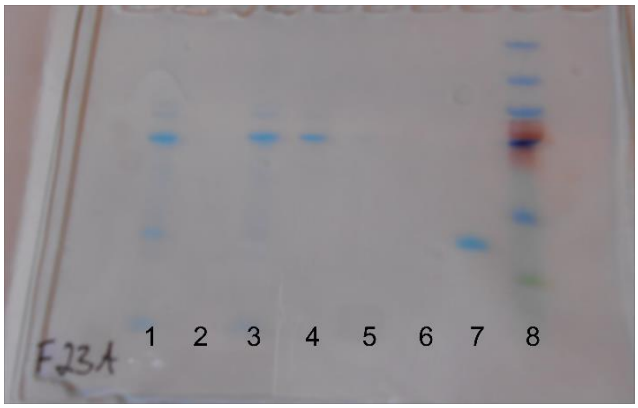

Figure S1. SDS-PAGE gel of F23A MhuD. 50 mM Tris, pH 7.4, 50 mM NaCl, 25 mM imidazole wash (1), 50 mM Tris, pH 7.4, 50 mM NaCl, 50 mM imidazole wash (2), 50 mM Tris, pH 7.4, 50 mM NaCl, 75 mM imidazole wash (3), 50 mM Tris, pH 7.4, 50 mM NaCl, 100 mM imidazole wash (4), 1/100 dilution of F23A MhuD (5), 1/10 dilution of F23A MhuD (6), Pure F23A MhuD (7), and molecular weight ladder (8).

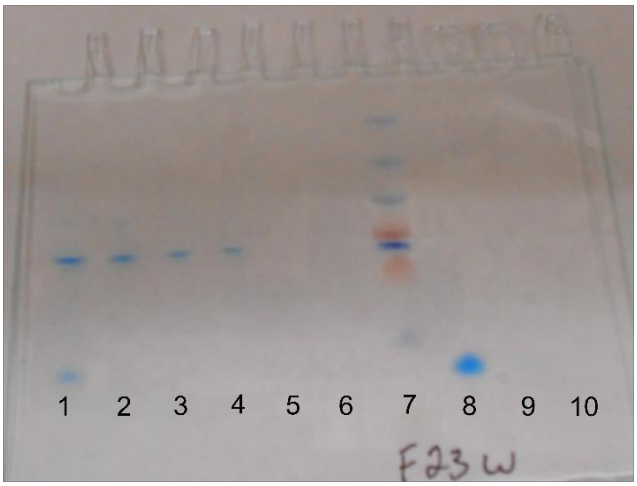

Figure S2. SDS-PAGE gel of F23W MhuD. Lysate (1), column flow through (2), 50 mM Tris, pH 7.4, 50 mM NaCl, 25 mM imidazole wash (3), 50 mM Tris, pH 7.4, 50 mM NaCl, 50 mM imidazole wash (4), 50 mM Tris, pH 7.4, 50 mM NaCl, 75 mM imidazole wash (5), 50 mM Tris, pH 7.4, 50 mM NaCl, 100 mM imidazole wash (6), and molecular weight ladder (7), pure F23W MhuD (8), 1/10 dilution of F23W MhuD (9), 1/100 dilution of F23W MhuD (10).

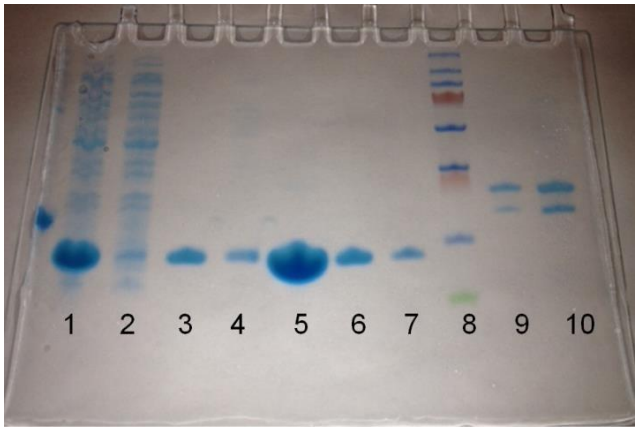

Figure S3. SDS-PAGE gel of Uncleaved WT IsdI. Lysate (1), column flow through (2), 50 mM Tris, pH 7.4, 10 mM imidazole wash (3), 50 mM Tris, pH 7.4, 150 mM NaCl, 50 mM imidazole wash (4), pure His-tagged WT IsdI (5), 1/20 dilution of pure His-tagged WT IsdI (6), 1/100 dilution of pure His-tagged WT IsdI (7), molecular weight ladder (8), TEV post-dialysis (9), TEV pre-dialysis (10).

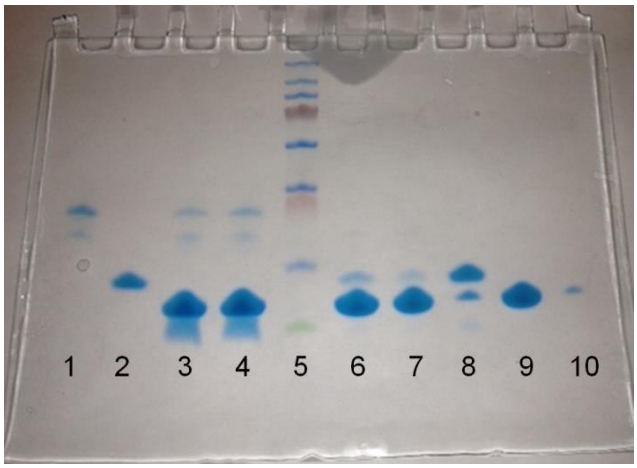

Figure S4. SDS-PAGE gel of WT IsdI cleavage with TEV. TEV post-dialysis (1), 1/20 dilution of pure His-tagged WT IsdI (2), IsdI/TEV reaction pre-dialysis (3), IsdI/TEV post-dialysis (4), molecular weight ladder (5), eluted WT IsdI in 50 mM Tris, pH 7.4 (6), 1st reloaded eluted WT IsdI (7), 50 mM Tris, pH 7.4, 10 mM imidazole wash (8), pure untagged WT IsdI (9), 1/20 dilution of pure untagged WT IsdI (10).

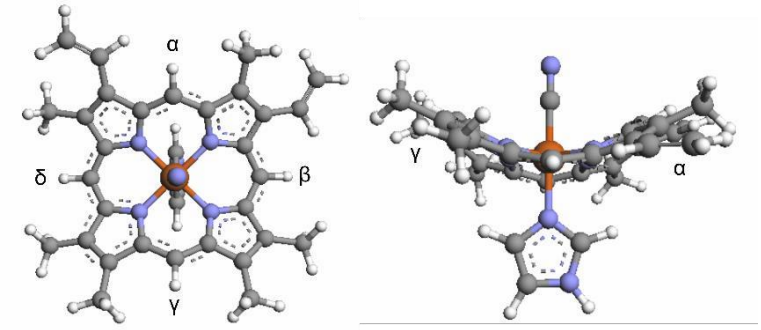

Figure S5. Computational model used for all calculations, shown with the heme ruffled to 2.2 Å. The α-meso–Fe–γ-meso angle was constrained to vary the degree of ruffling.

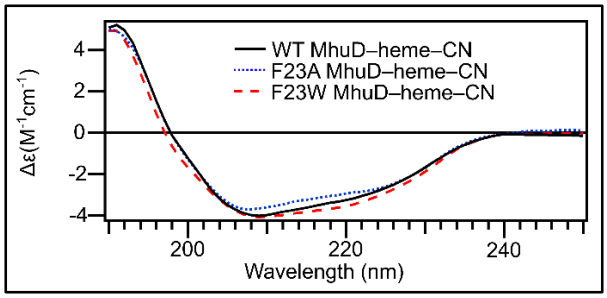

Figure S6. UV CD of WT (black, solid line), F23A (blue, dotted line), and F23W MhuD–heme–CN (red, dashed line) for secondary structural analysis. All collected in 10 mM KPi, pH 7.4