Supporting Information for

Mapping Protein-Protein Interactions by Localized Oxidation: Consequences of the Reach of Hydroxyl Radical[†]

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Example MS/MS of O-ECAT peptides:

Both side chain carbonyl tagging (Figure s1) and N-terminal carbonyl tagging (Figure s2) were observed.

(R)I**R**QEAK

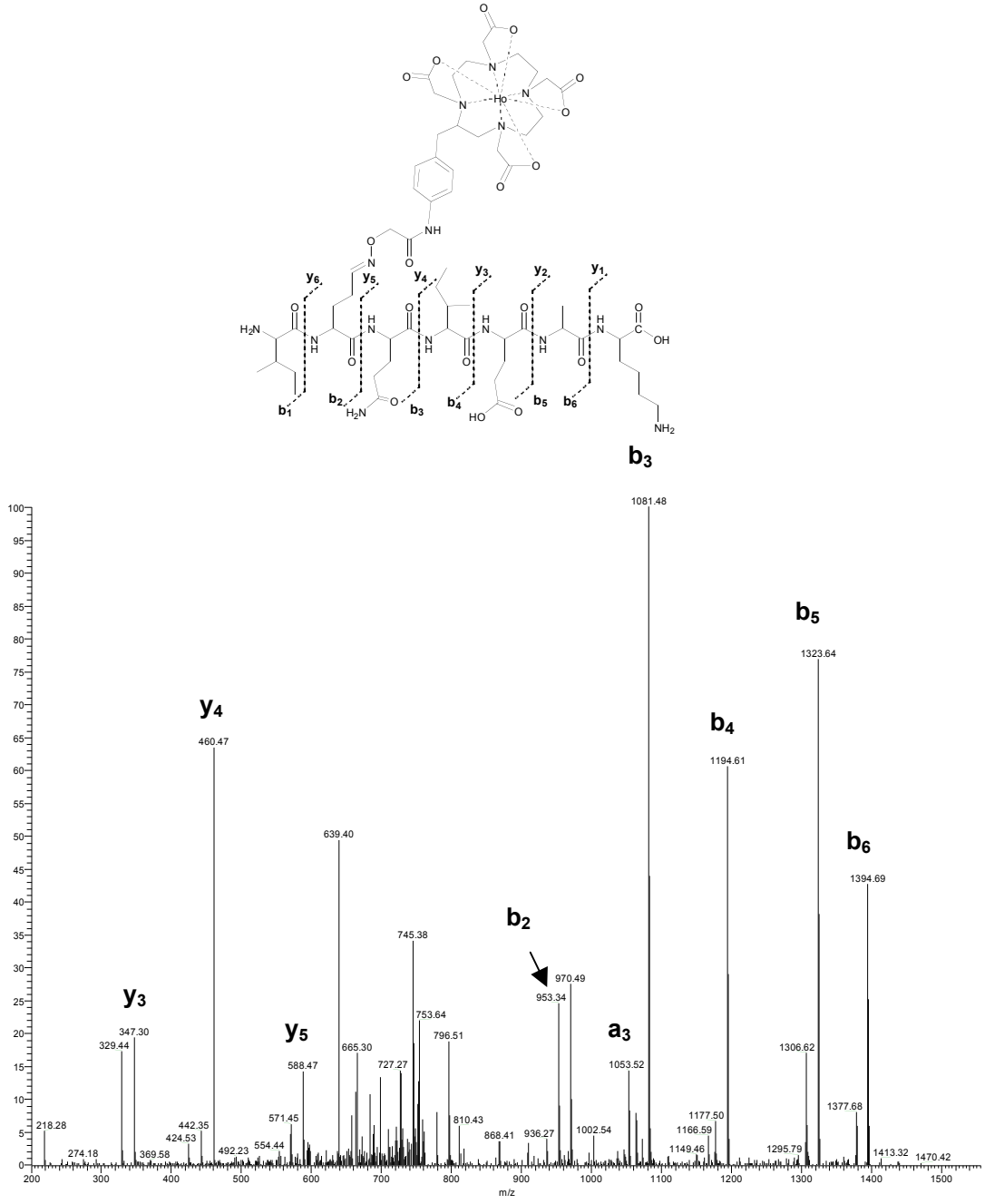

Figure s1. MS/MS spectrum of a side chain O-ECAT tagged peptide. The side chain of arginine is oxidized to a carbonyl and subsequently tagged with O-ECAT.

RS*HATAQEEILK

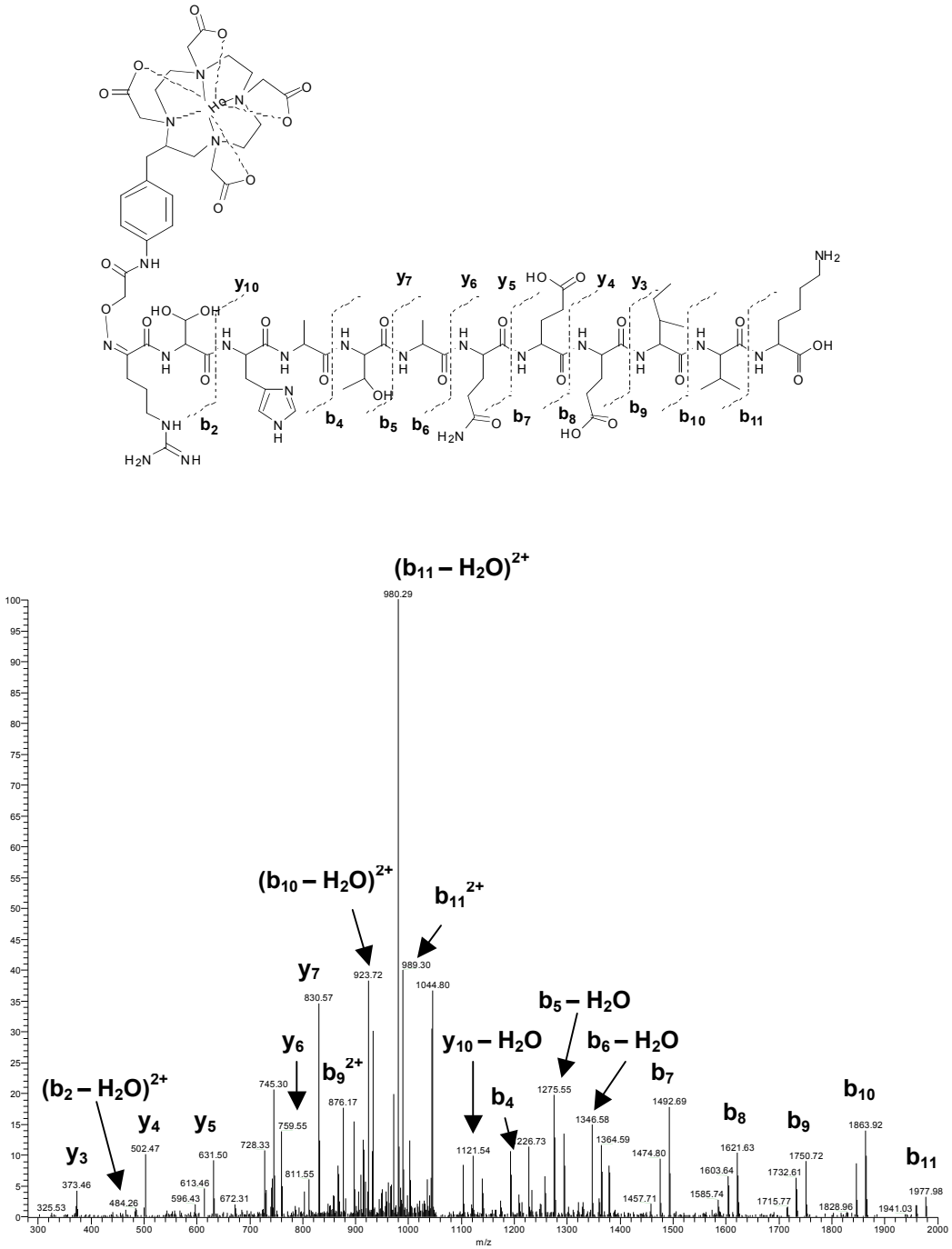

Figure s2. MS/MS spectrum of a N-terminal O-ECAT tagged peptide. The peptide backbone was cleaved by a hydroxyl radical, resulting in a N-terminal α-ketoacyl derivative. This carbonyl was tagged by O-ECAT.

Determination of ratios of (M1)AOD and (M2)AOD tagged peptides using mass spectrometry:

A FeBABE/ascorbate (final 1 mM)/hydrogen peroxide (final 10 mM) oxidized 376C sample was tagged with equimolar PrAOD and HoAOD and the identified oxidized and tagged peptides were quantitated as a control.

Table s1. Oxidized peptides labeled with MAOD identified on FeBABE σ^{70} 376 C following treatment with ascorbate and hydrogen peroxide. The sequence of the identified peptide, along with the preceding amino acid residue, is listed with the oxidized, tagged residue indicated in bold. Any other oxidative modifications (e.g. +16amu, +32amu) are indicated with an asterisk. During this experiment the oxidized protein was tagged with equimolar HoAOD and PrAOD so the theoretical HoAOD/PrAOD ratio was 1.

Sequence	Modified AA	HoAOD intensity	PrAOD intensity	HoAOD/PrAOD
(K)LLVT**R**GK	R15	2.67E3	2.07E3	1.3
(K)FAEL**R**AQ	R226	1.18E3	1.34E3	0.9
(R)V**R**TQER	R281	1.43E4	1.54E4	0.9
(R)SIADQA**R**TIR	R448	1.23E3	1.30E3	1.0
(R)EPT**P**EELAER	P480	1.67E3	1.61E3	1.0
(K)QFDVT**R**ER	R584	4.88E2	4.60E2	1.1
(R)I**R**QEAK	R588	5.98E3	5.61E3	1.1

Sigma self-oxidation data:

Table s2. Summary of all σ^{70} self-oxidation data. The sequence of the identified peptide, along with the preceding amino acid residue, is listed with the oxidized, tagged residue indicated in bold. Any other oxidative modifications (e.g. +16amu, +32amu) are indicated with an asterisk. A comparison of intensities of PrAOD tagged peptides from FeEDTA and HoAOD tagged peptides from FeBABE conjugates is included. If the oxidized residue was identified using a 2IT FeBABE Sigma library a "Y" is indicated.

Cys mutant	Sequence	Modified AA	Distance from Cys (Å)*	Intensity ratio FeBABE/FeEDTA	2IT FeBABE Sigma**
132C	-.M*EQNPQSQLK	P5		8.1	Y
	-.MEQNPQSQLK	P5			Y
	(R)EGEIDIAKR	K121	16.4		
	(Y)LLEQYDRVEAEEAR	R150	24.5		Y
	(R)EKFAELR	E219	23.1	7.1	Y
	(R)AQYVVTRDTIK	R232	19.9		
	(K)LHDVSEEVHRALQK	R339	32.7		Y
	(R)MS*IGEAKAR	K371	14.1		Y
	(K)AVDKFEYR	A415	23.9	2.5	Y
	(K)AVDKFEYR	K418	11.1		
	(K)AVDKFEYRR	R422	20.5		
	(R)QAITRSIADQAR	R441	36.8		Y
	(R)EPTPEELAER	P478		13.2	Y
	(R)M*LMPEDKIR	K493			Y
	(R)AATHDVLAGLTAREAK	R554			Y
	(K)QFDVTRER	R584			
	(R)IRQIEAK	R588		29.3	Y
	(R)QIEAKALR	K593			
376C	-.M*EQNPQSQLK	P5		1.4	Y
	-.MEQNPQSQLK	P5		2.1	Y
	-.ME*QNPQSQLKL	P5			Y
	(V)LSSVESEIGRT	R93			
	(R)EGEIDIAKR	K121	10.7		
	(R)EGEIDIAKR*	K121	10.7	2.0	
	(R)EKFAELR	E219	35.9		Y
	(R)EKFAELR	K220	32.6		Y
	(R)AQYVVTRDTIK	R232	38.4		

Table s2 is continued on next page.

Table s2, continued.

Cys mutant	Sequence	Modified AA	Distance from Cys (Å)*	Intensity ratio FeBABE/FeEDTA	2IT FeBABE Sigma**
376C	(G)RS*HATAQEEILK	R240	39.2(H242)		
	(A)TAQEEILK	E247	34.5	0.23	
	(K)QFRLVPK	R260	21.1	2.0	
	(K)LSVEQSKM*PK	K296	45.1		Y
	(M)NKPWSEK	N323	54.2	0.74	Y
	(M)NKPWSEK	K324	52.8		
	(K)LHDVSEEVHRALQK	R339	42.1		
	(R)MS*IGEAKAR	K371	8.61		Y
	(K)AVDKFEYR	A415	10.0		Y
	(K)AVDKFEYRR	R422	16.8		
	(R)QAITRSIADQAR	R441	23.6		
	(G)REPTPEELAER	R476			
	(R)EPTPEELAER	E477			Y
	(R)EPTPEELAER	P478		12.1(+3), 12.6 (+2)	Y
	(R)EPTPEELAER	T479			
	(R)EPTPEELAER	P480		12.6	
	(R)KVLKI*AK	K496			Y
	(R)AATHDVLAGLTAREAK	R554			Y
	(R)IRQIEAK	R588		2.4	Y
	(R)QIEAKALR	K593			
422C	-.M*EQNPQSQLK	P5		9.8(+3), 9.3(+2)	Y
	-.MEQNPQSQLK	P5		0.64(+3), 0.75(+3)	Y
	(R)EGEIDIAKR	E114	13.7	0.4	
	(R)EGEIDIAKR	K121	9.5	2.6	
	(R)EKFAELR	E219	28.8		Y
	(R)EKFAELR	K220	25.7	2.6	Y
	(R)AQYVVTRDTIK	R232	37.8		
	(G)RS*HATAQEEILK	R240	39.2(H242)	3.5	
	(K)LSVEQSKM*PK	K296	58.0		Y
	(R)MSI*GEAKAR	K371	19.4		Y
	(R)MS*IGEAKAR	K371	19.4		Y
	(R)QAITRSIADQAR	R441	28.6		Y
	(R)SIADQARTIR	R448			
	(R)EPTPEELAER	E477			Y
	(R)EPTPEELAER	P478		5.4	Y
	(R)EPTPEELAER	P480		5.4	
	(R)EPTPEELAER	E482		2.8	
	(K)QFDVTRER	R584		7.0	
	(R)IRQIEAK	R588			Y

* Distance from the α-carbon of the Cys residue to the α-carbon of the oxidized and tagged amino acid residue.
**The oxidized residues found using the 2IT FeBABE library not found during studies with single cys Sigma mutants: R15, A248, R274, R279.

Sigma-RNAP oxidation data:

Table s3. Inferred distances from the α carbon of the σ70 cysteine to which FeBABE was attached, to the α carbon of each oxidized and tagged amino-acid residue on the E. coli RNAP core enzyme. These data were determined using the crystal structure of Taq RNAP holoenzyme (pdb file 1L9U) and sequence homology.

Single-Cys σ^{70}	RNAP subunit	Identified E.coli AA	T.aquaticus Sequence #	Inferred Distance to Single Cys (Å)
376C	β	1163T	986V	110.5
396C	β′	41P	29P	13.9
517C	β′	2K	2K	23.2
		27P	14P	33.1
		41P	29P	25.9
		47R	35R	25.5
		339R	628R	23.2
		392R	671K	35.4
		1188E	1 aa gap; (1305K)	57.8
517C	β	191K	179S	50.6
		233R	>10 aa gap; (207L)	N/A
		470R	350R	32.2
		530R	420R	22.4
		1044P	817P	25.3
		1065K	837K	32.9
		1323F	1085F	16.9
581C	β′	27P	14P	63.8
		41P	29P	58.4
		47R	35R	56.2
		270R	545R	63.2
		282L	557L	72.0
581C	β	1241D	1003D	66.8