Protein Residues that Control the Reaction Trajectory in *S*-Adenosylmethionine Radical Enzymes: Mutagenesis of Asparagine 153 and Aspartate 155 in *Escherichia coli* Biotin Synthase

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Supporting Information

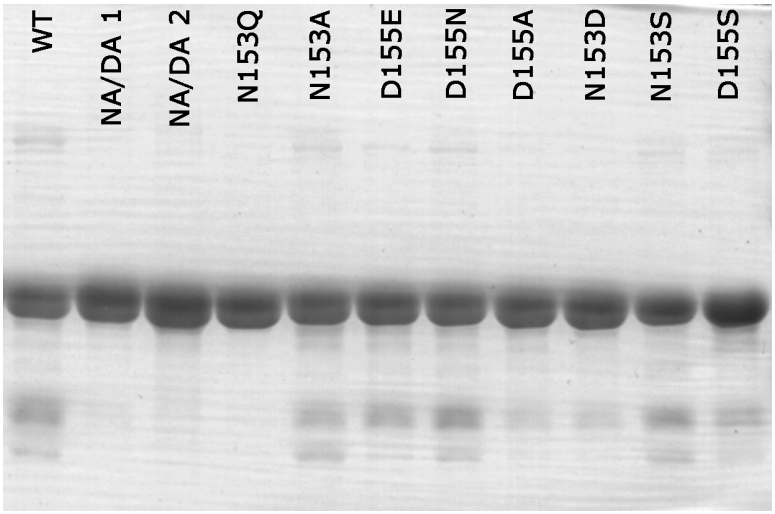

Figure S1. 10% SDS-PAGE of 10 μg of WT and nine mutant BioB proteins expressed in and purified from *E. coli*. The gel was stained with Coomassie and scanned on an Epson scanner. The main band for each sample ran at ~40 kDa. The gel was over loaded in order to emphasize any minor contaminating bands. In all samples, there are some low molecular weight bands (less than 20 kDa) and some high molecular weight bands (approximately 70-80 kDa). While almost all of the mutant proteins contain these bands, they are no more prevalent than what is also present in the WT BioB protein, indicating that the presence of the mutations does not greatly affect the stability of this protein.

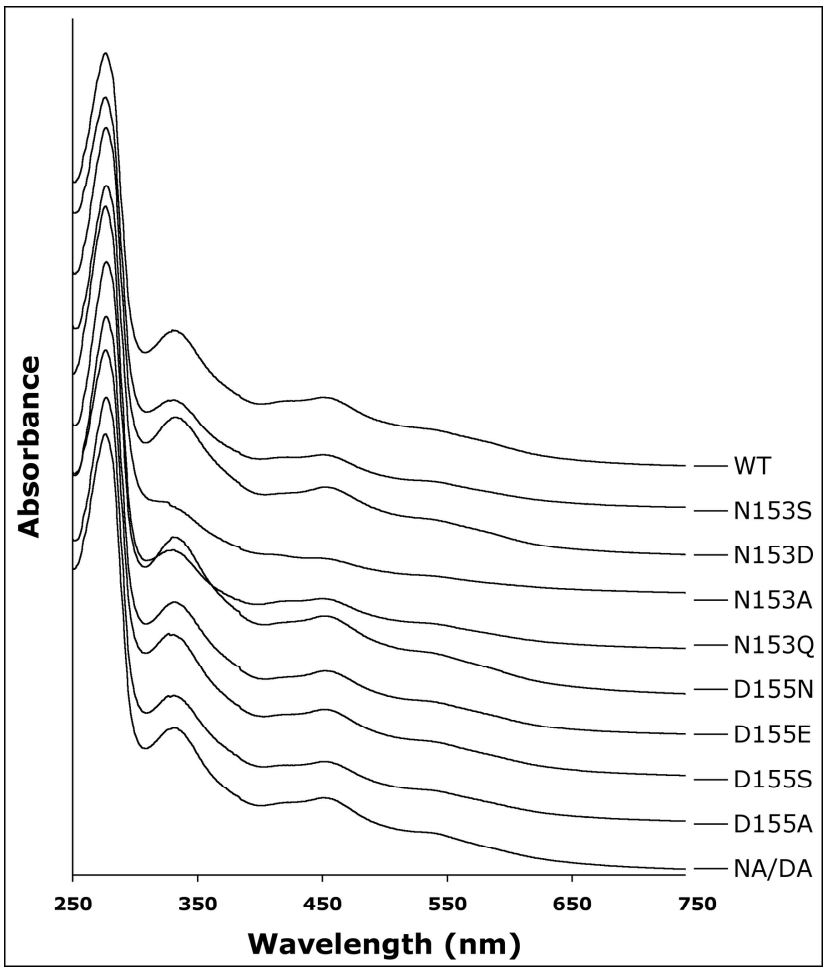

Figure S2. UV/Vis scans of WT and mutant BioB proteins (at approximately 1 mg/ml) expressed in and purified from *E. coli*. Aerobically purified proteins were diluted to ~10 μM and scanned from 250-750 nm using a Cary 50 UV-visible spectrophotometer. All of the proteins show the characteristic peaks at 332 and 452 nm that are normally associated with the $[2Fe\text{-}2S]^{+}$cluster. However, in some of the Asn153 mutants, such as the alanine, glutamine, and serine mutants, these peaks appear somewhat diminished. These same mutants also showed lower levels of iron in analytical iron analyses.

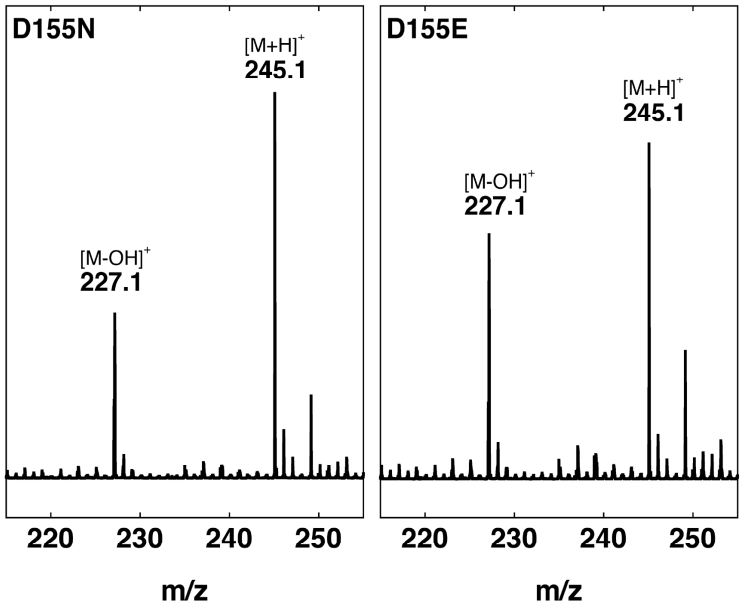

Figure S3. Mass spectrum of biotin observed in two BioB mutant proteins' final reaction mixtures. The catalytic activity of BioB proteins was measured under standard assay conditions. After acid precipitation of the proteins, the reactionary products present in the sample mixtures were detected using ESI-MS. Both the Asp155Asn and Asp155Glu mutants do not show a detectable level of biotin by HPLC, however, these samples do show trace amount of biotin by ESI-MS, as shown

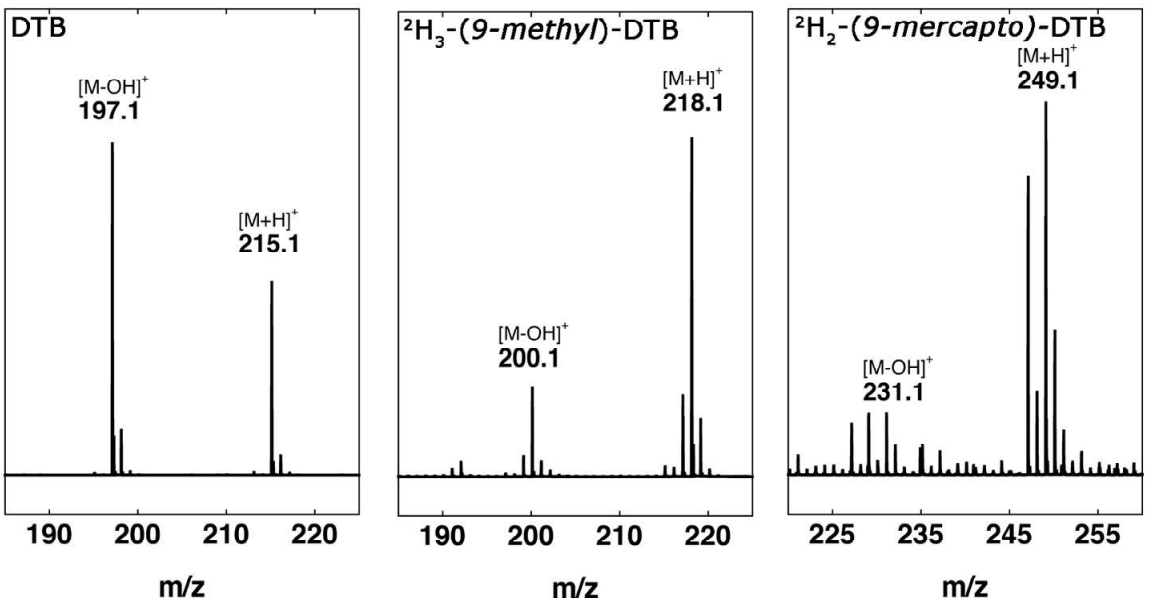

Figure S4. Mass spectra of DTB, $^{2}H_{3}$-(*9-methyl*)-DTB, and MDTB observed as a reactionary product of the Asn153Ser mutant using $^{2}H_{3}$-(*9-methyl*)-DTB as a substrate. BioB activity assay was performed using synthetic DTB that had been predominantly deuterated at the C9 position. The major peak observed in the mass spectrum had a $[M+H]^{+}$ of 218.1, which is shifted +3 mass units from unlabeled DTB, which had a $[M+H]^{+}$ of 215.1. If the first step in biotin formation involves abstraction of a hydrogen/deuterium atom from C9, then one deuterium should be transferred to 5´-deoxyadenosine and the intermediate formed from labeled DTB should retain only two deuterium atoms. The major peak in the mass spectrum of the reaction product formed had a $[M+H]^{+}$ of 249.1, which is shifted by only +2 mass units from the unlabeled sample with a $[M+H]^{+}$ of 247.1, indicating that the thiol has been incorporated at the C9 position.

Sample	Peak Area (μV.sec) Total	Filtrate	Conc. (μM) Total	Free	% Bound	Average % Bound
9-MDTB						
WT	116	54	13.9	6.5	53.4%	46.2%
	110	58	13.2	7.0	47.3%	
	131	80	15.7	9.6	38.9%	
Asn153Ser	393	371	47.2	44.5	5.9%	7.2%
	425	387	51.0	46.4	9.8%	
	382	362	45.8	43.4	5.5%	
					% retained	
DTB only	403	397	100.8	99.3	1.5%	
(no BioB)	401	400	100.3	100.0	0.3%	

Table S1. Binding of 9-MDTB produced by the Asn153Ser mutant and WT BioB. Following a 2 hr activity assay, the assay mixture was passed through a 10 KDa centrifugal concentrator under anaerobic conditions. The concentration of 9-MDTB in the eluted fraction was compared to the total concentration in the assay mixture by HPLC. The difference in concentration is presumed to be due to tight binding to the enzyme. Control samples of DTB with no protein demonstrated that there is negligible binding to the filter alone. WT enzyme produced ~0.1 equiv 9-MDTB and 46% is tightly bound to the protein, while the remainder is found in solution due to slow dissociation during the assay. In contrast, the Asn153Ser mutant enzyme produced ~0.5 equiv 9-MDTB and that only 7% is bound to the protein, while the remainder is found free in solution. We conclude that the Asn153Ser mutation increases the rate at which 9-MDTB dissociates from the protein, such that binding of the second equiv of AdoMet and formation of the C6-S bond cannot kinetically compete and do not occur.