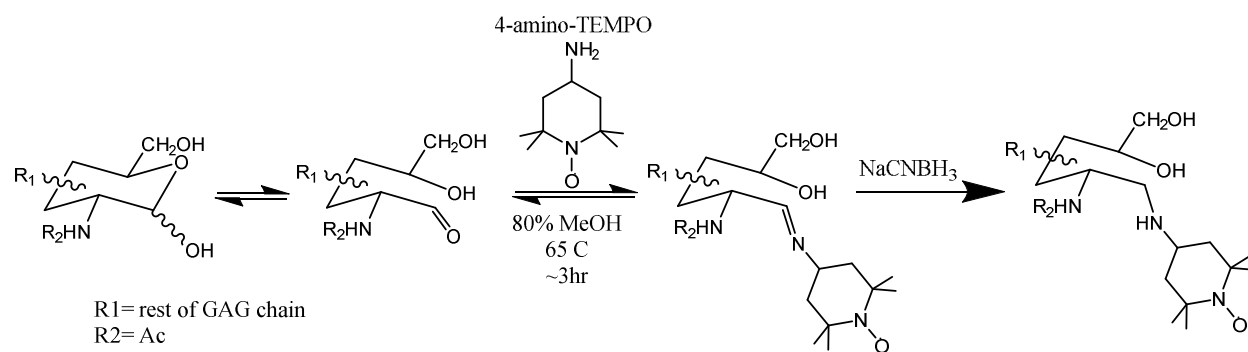
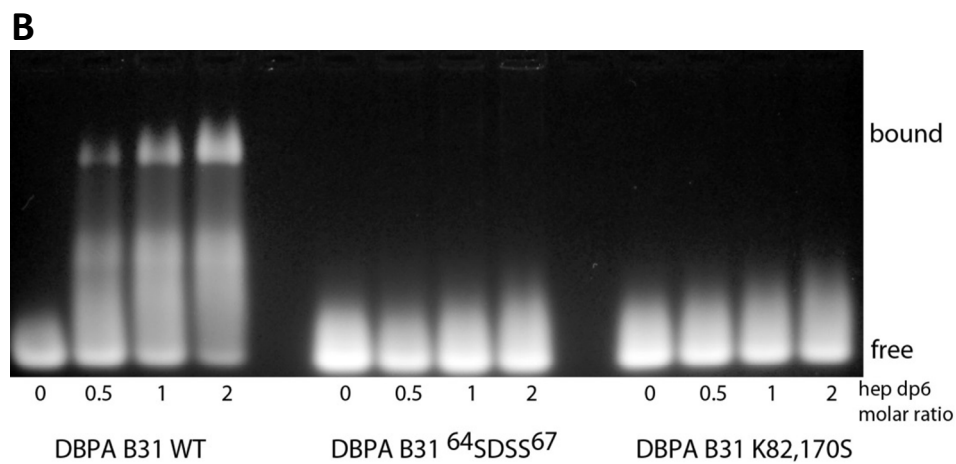
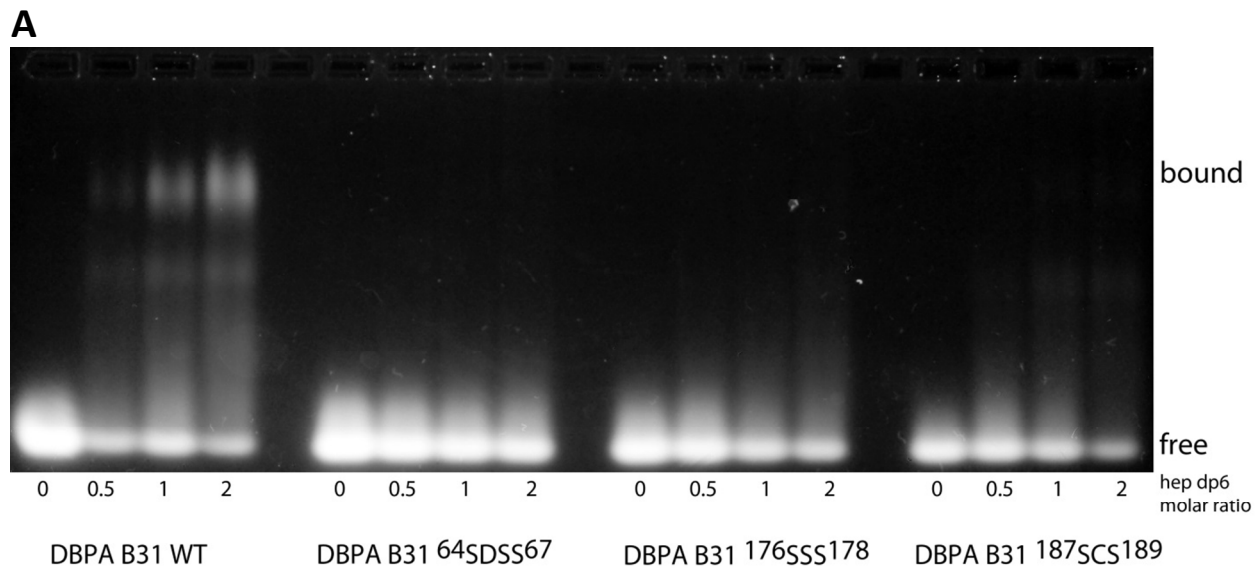


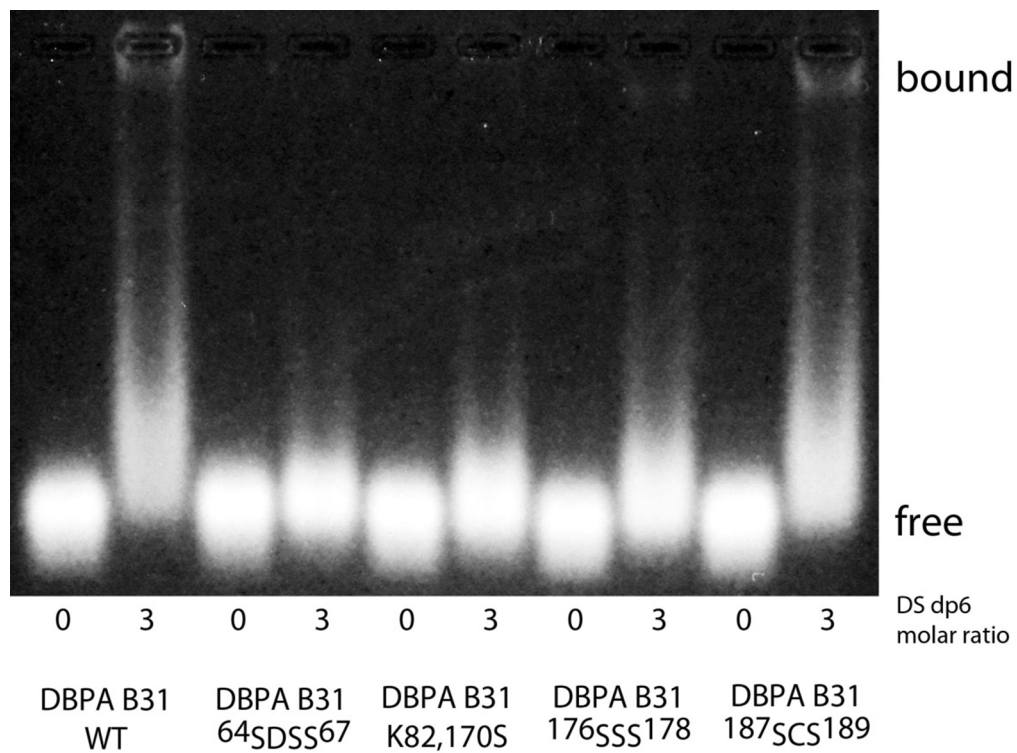
**Supplementary Figure 1.** SDS-PAGE gel showing the purification of B31 DBPA. Lanes 1 and 3 contain the before induction samples while lanes 2 and 4 show the production of Ubi-B31 after induction. Lane 5 shows the flow-through from the first  $\text{Ni}^{2+}$  column. Purification with a second  $\text{Ni}^{2+}$  column after digestion resulted in the purified protein (Lane 6) and elution fractions containing undigested B31 and ubiquitin (Lanes 7 and 8).



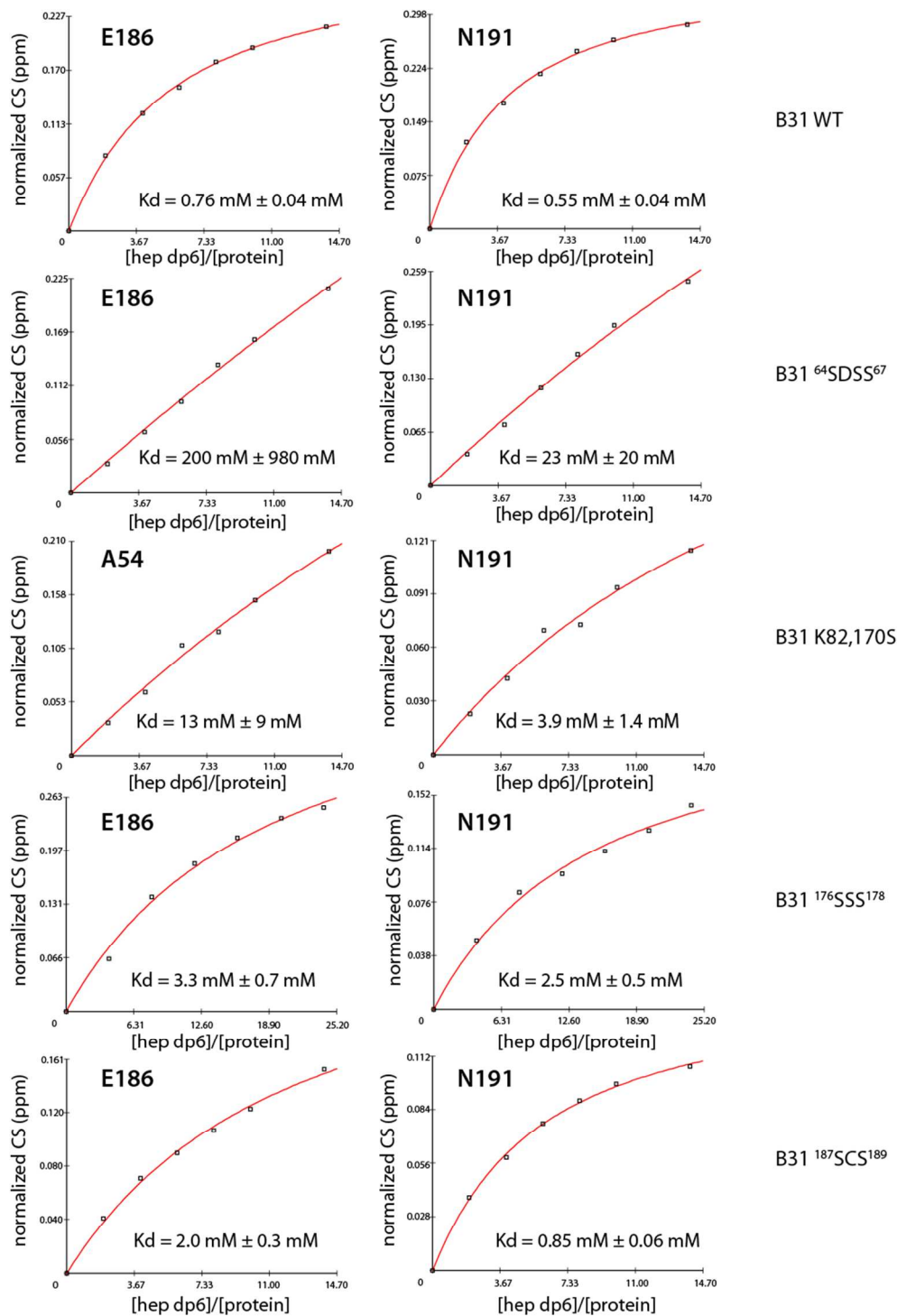
**Supplementary Figure 2.** Reducing end TEMPO labeling of heparin fragments through reductive amination.



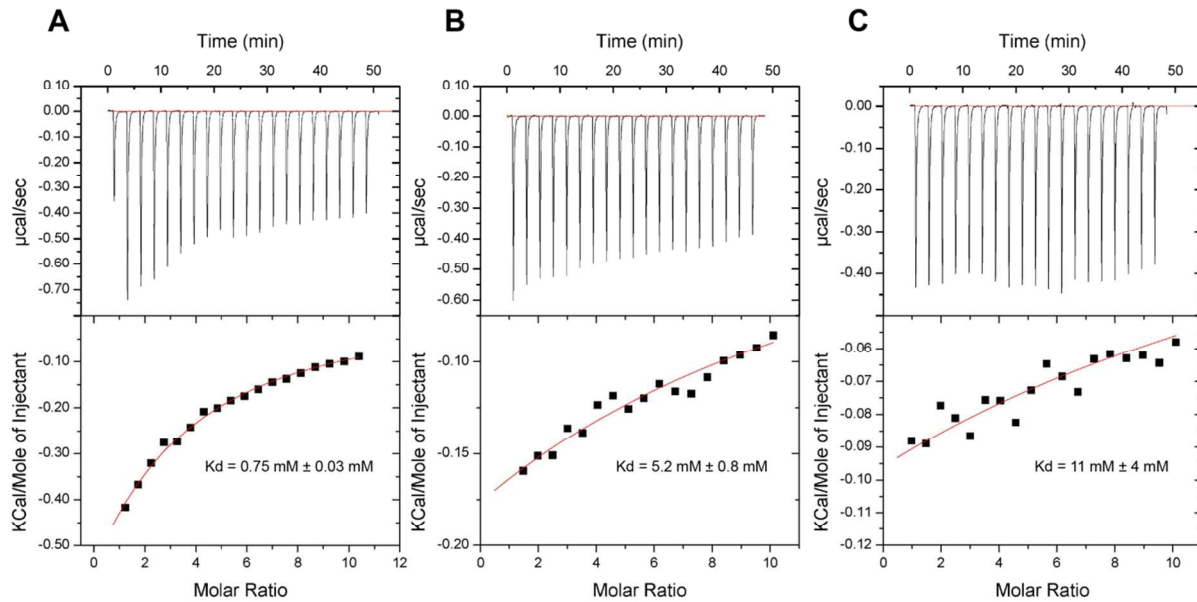
**Supplementary Figure 3.** Gel mobility shift assay of heparin dp6 in the presence of increasing concentrations of (A) B31 WT, <sup>64</sup>SDSS<sup>67</sup>, <sup>176</sup>SSS<sup>178</sup>, and <sup>187</sup>SCS<sup>189</sup> and of (B) B31 WT, <sup>64</sup>SDSS<sup>67</sup>, and K82,170S.



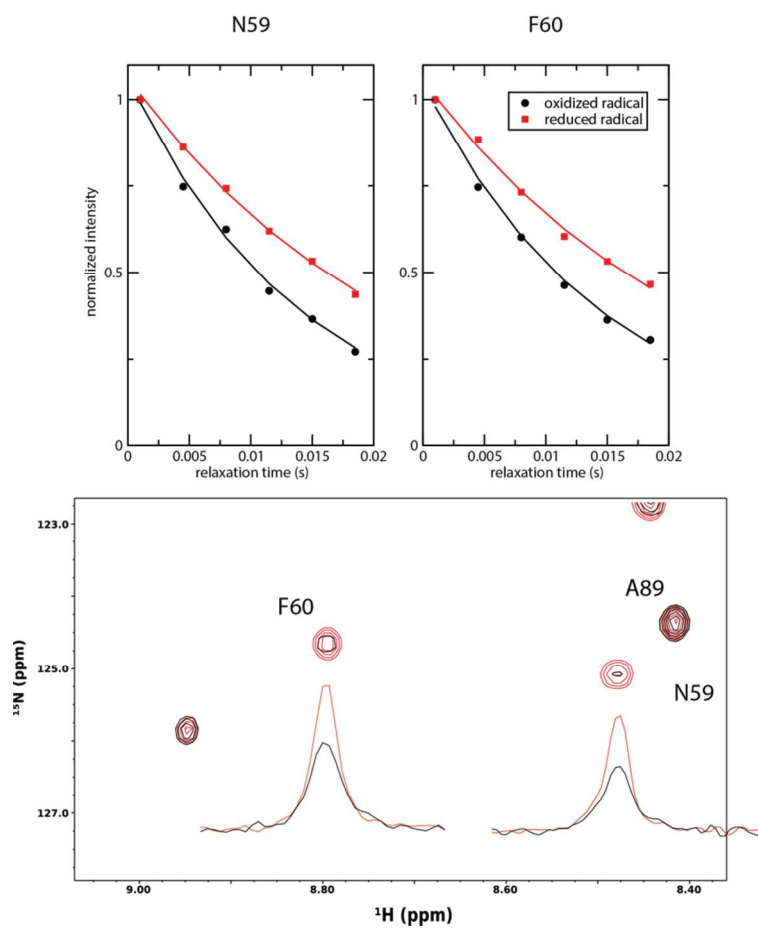
**Supplementary Figure 4.** Gel mobility shift assay of DS dp6 in the absence and in the presence of B31 WT, <sup>64</sup>SDSS<sup>67</sup>, K82,170S, <sup>176</sup>SSS<sup>178</sup>, and <sup>187</sup>SCS<sup>189</sup>.



**Supplementary Figure 5.**  $K_D$  curves of two residues for each B31 wildtype and mutants. These residues (A54, E186, and N191) experienced the greatest linear peak migration when titrated with heparin dp6, and these peaks were analyzed to give the  $K_D$  for each B31 variant. Residue A54 was used in place of E186 for the K82,170S mutant due to poor signal-to-noise from E186 of the mutant.



**Supplementary Figure 6.** ITC data for DBPA (A) B31 WT, (B)  $^{64}\text{SDSS}^{67}$ , and (C) K82,170S. The  $K_{D,S}$  from this data show the same trend quantified by NMR.

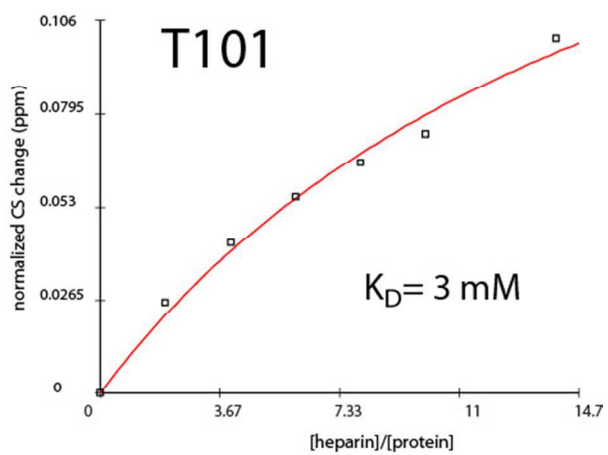
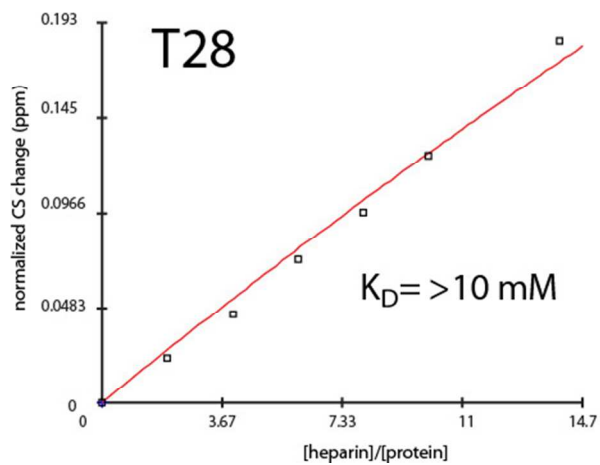


**Supplementary Figure 7.** Transverse  $^1\text{H}$  magnetization relaxation curves and HSQCs of residues N59 and F60 in the presence of oxidized (black) and reduced (red) radical. The maximum intensities have been normalized to 1.

B31		----	MIKCNNKTFNNLLKLTILVNLLISCGLTGATKIRLERSAKDITDEIDAIAIKKDAALK	56
297		----	MIKCNNKTFNNLLKLTILVNLLISCGLTGATKIKLESSAKAIVDEIDAIAIKKKAASM	56
N40			MNKYQKTFKIFNFKNLLKLSLLV-ALISCGLKGETKIILERSAKDITDEINKIKKDAADN	59
PBr		-----	MIKYNKILLKLSLIVSLLVACGLTGETKIRLESSAQEIKDEINKIKANAKKE	52
VS461		-----	MIKYNKIILTLTLLASLLAACSLTGK--ARLESSVKDITNEIDKAIKAAKDA	50
			: :*. * : : . * : * . * . * ** * . : * : * * :	*
B31	57		GVNFDAFKDKKKTGSGVSENP-FILEAKVRATTVAEKFVIAIEEEATKLKETGS-SGEFSA	114
297	57		GVNFDAFKDKKKTGSGVSENP-FILEAKVRATTVAEKFVIAIEEEATKLKETGS-SGEFSA	114
N40	60		NVNFAAFDSETGSKVSENS-FILEAKVRATTVAEKFVTAIEGEATKLKKTGS-SGEFSA	117
PBr	53		GVKFEAFNTQTGSKI SEKPEFILKAKIKAIQVAERFVKAIKEEAELKKSGS-SGAFSA	111
VS461	51		GVNTDAFTETQTGGKVAGSQ--IRDAKKLVADLTIEFLKATEEETITFKENGAGEDEFSG	108
			* : ** . : * * . : : . * . * * : : . * : * : * : . * * . * * .	
B31	115		MYDLMFEVSKPLQKLGIEQMTKTVSDAAEENPPTTAQGVLEIAKKMREKLQRVHTKNYCT	174
297	115		MYDLMFEVSKPLQELGIEQMTKTVSMAAEENPPTTAQGVLEIAKKMREKLQRVHKKNQDT	174
N40	118		MYNMMLEVSGPLEELGVL RMTKTVTDAAEQHPTTTAEGILEIAKIMKTKLQRVHTKNYCA	177
PBr	112		MYDLMIDVSKPLEEIGIQKMTGTVKEAAQKTPATTADGIIAIAQAMEDKLN NVNKKQHDA	171
VS461	109		IYDLIYRTAEAVEKIGMK-VKQAVEDTAKENPKTTANGIIAIVKVMKAKVENIKEKQTKN	167
			* : * : * : : . : : * : * : * : * : * : * : * : * : * : * : * :	
B31	175		LKKKENSTFTDEKCKNN	191
297	175		LKKKNTEDSTAKS----	187
N40	178		LEKKKNPNFTDEKCKNN	194
PBr	172		LKNLKEKAKTATTT---	185
VS461	168		QK-----	169
			*	

**Supplementary Figure 8.** Sequence alignment of DBPA variants found in B31, 297, N40, PBr and VS461 strains for *Borrelia*. Strain B356 is not shown because it has 99 % sequence identity with strain N40.





**Supplementary Figure 9.**  $K_D$  curves of N-terminal residues T28 and T101 of wildtype B31.