Supporting Information to

Evidence for Dramatic Acceleration of an C-H Bond Ionization Rate in Thiamin Diphosphate Enzymes by the Protein Environment.

By Sheng Zhang, Leon Zhou, Natalia Nemeria, Yan Yan, Zhen Zhang, Yu Zou and Frank Jordan

Table S1.

Ratio of ion intensities for a pair of compounds bearing $-CH_3$ or $-CD_3$ varies linearly with the concentration ratio.

Concentration Ratio $-CH_3/-CD_3$	Ion Intensity Ratio $-CH_3/-CD_3$
0.001957	0.002183
0.004891	0.004648
0.009783	0.008271
0.019566	0.017552
0.048914	0.046957
0.097828	0.090059
0.195657	0.18400

Figure legends.

Figure S1. Ionization response versus concentration ratio with a pair of model compounds with a CH_3 or CD_3 substituent.

Figure S2. MALDI-TOF mass spectrum of the lipoyl domain after 40 min incubation with PDHc-E1 (0.10 μM) and HEThDP (0.20 mM). The spectrum shows the molecular ions for acetylated (mass = 9022.73 Da) and unacetylated (mass= 8979.22 Da) lipoyl domain.

Inset shows time dependence of reductive acetylation of the lipoyl domain by HEThDP and PDHc-E1: curve (1) depletion of unacetylated lipoyl domain; curve (2) formation of reductively acetylated lipoyl domain.

Figure S3. Time-course of HEThDP-C2α-d_1 formation from HEThDP (2.5 mM) after incubation with PDHc-E1. In the inset (δ 1.62-1.66 ppm region of the ^{1}H NMR spectrum), the peaks of the doublet on the left (peaks A_l and A_r) show the spectrum prior to H/D exchange for the resonances corresponding to the C2βHs, with the integral of the left peak (A_l) equal to that of right peak (A_r). Replacement of C2αH by C2αD converts the C2βH_3 doublet to a singlet, and shifts the C2βH_3 resonance to higher field by about 3 Hz. The peaks on the right (after 15 hours) represent a mixture of HEThDP and HEThDP-d_1, the singlet peak of the C2β protons derived from HEThDP-d_1 overlaps with the right-hand peak of the doublet of the C2β protons derived from HEThDP. Therefore, the peak B_l corresponds to one half of the HEThDP concentration, and the peak B_r corresponds to one half of the HEThDP concentration plus the HEThDP-d_1 concentration. For quantification, the integral ($2 \times B_l$) represents the HEThDP, while the difference (B_r- B_l) the HEThDP-d_1 concentration.

Figure S1. S. Zhang et al.

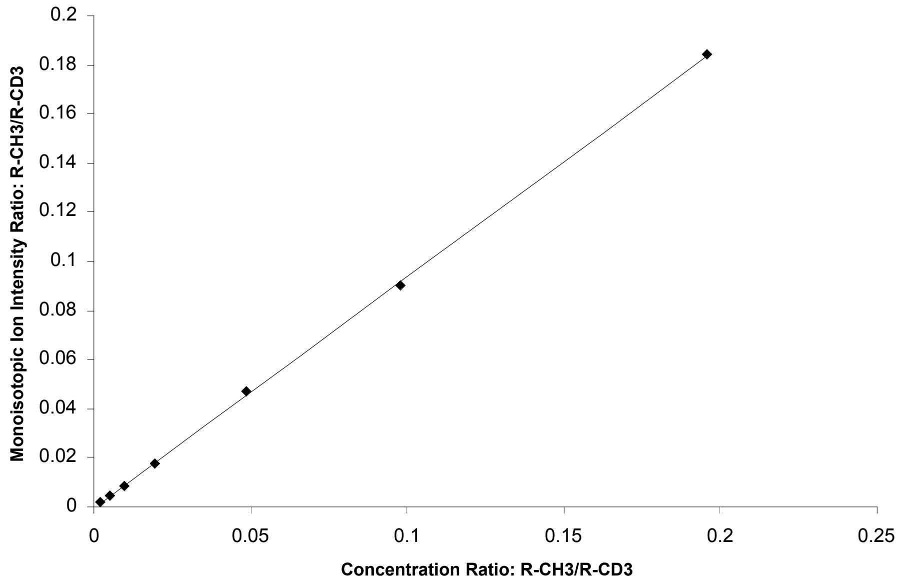

Figure S2. S. Zhang et al.

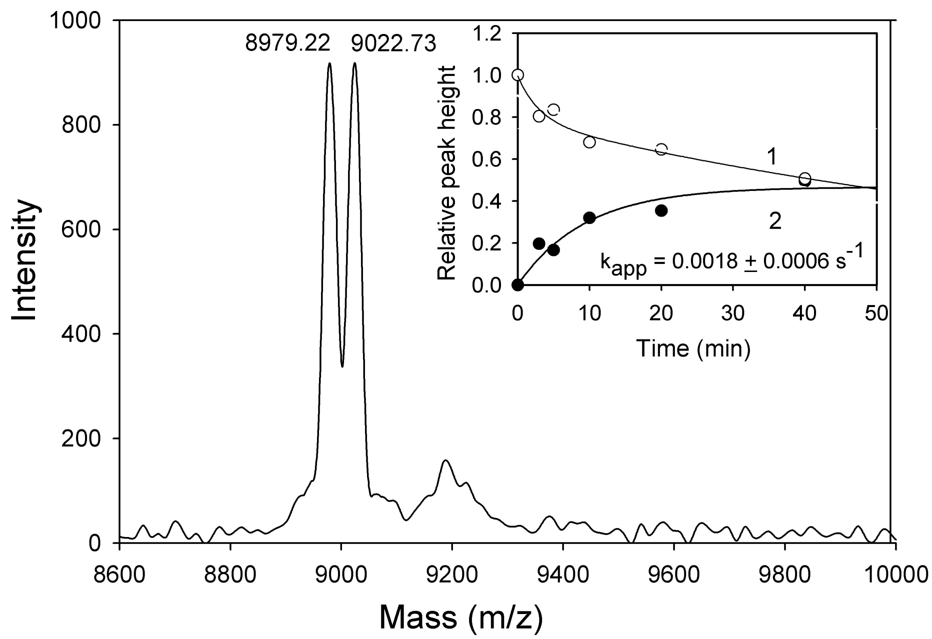

Figure S3. S. Zhang et al.