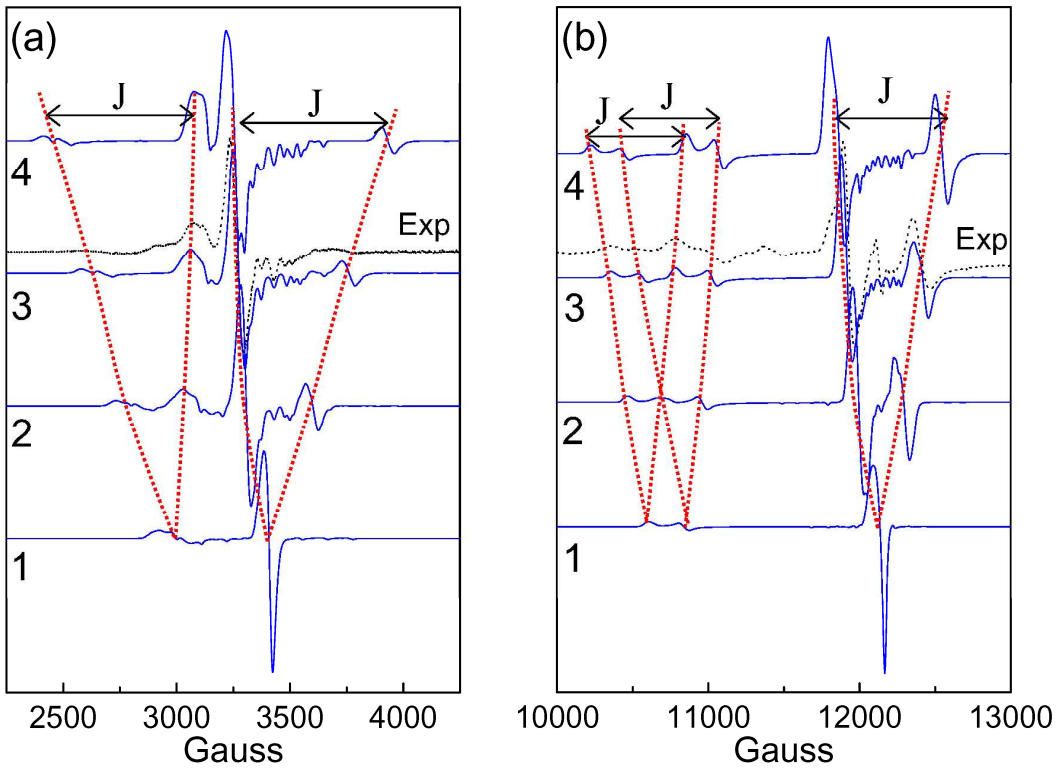

Figure SI: Effects of exchange coupling constant (J) on the EPR spectra of the bi-radical system in the reaction of 5,6-LAM with 4-thia-L-lysine. (a) X-band and (b) Q-band EPR spectra of experimental data (black dotted lines) and simulation data (blue lines). The g and A parameters used in the simulation for the substrate-PLP based radical and Co^{2+} are the same as those given in the legend of Figure 1.

Spectra **1** are simulation of the non-interacting (J = D = 0) substrate-PLP radical and Co^{2+} in cobalamin. The main features are the g_x and g_y components of Co^{2+} and the substrate-PLP radical.

Spectra **2** show upon introduction of exchange coupling (J = 300 G) and dipole-dipole interaction (D = 22 G), each transition in spectra **1** splits into two transitions (as guided by the red-dotted lines) and the separation between them is approximately equal to J. Since the g_x and g_y components are not well resolved in X-band, spectrum **2** of (a) exhibits four transitions, which is known as a four-line pattern for a weakly coupled bi-radical system.

Spectra **3** and **4** further show that upon increasing exchange coupling strength (J = 500 G for **3** and J = 700 G for **4**), the two outer transitions move apart and lose intensities while the inner transitions move together.

These data also demonstrate that while the X-band spectra accentuate the combined effects of ^{59}Co(I=7/2) hyperfine interaction and additional J+2D splitting in the g_z transitions of Co^{2+}, the Q-band spectra exhibit further splittings due to the rhombicity of the Co^{2+} g tensor (more sensitive to g-values) and provide more precise limits on the measurement of J.