Supporting Information for

NMR Localization of Protons in Critical Enzyme Hydrogen Bonds

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Materials and Methods

The procedures for preparing the ^{15}N-labeled PLP and the model aldimines are described in Ref. 6. The protected aspartic acid (Boc-Asp-OtBu) was purchased from Bachem GmbH.

The ^{15}N NMR broadband ^{1}H-decoupled spectra of ^{15}N-PLP embedded in *E. coli* AspAT in 10% D_2O was collected using a Bruker Avance 600 MHz (14 Tesla) liquid state spectrometer (60.8 MHz for ^{15}N) at 282 K (9 °C). The 90° pulse for nitrogen was 25 μs by using a recycle time of 3 s and more than 40000 scans were recorded. In order to reference the ^{15}N chemical shifts, we recorded under the same ^{2}H field locking conditions ^{15}N spectra of neat nitromethane containing a capillary with D_2O; the nitromethane scale was converted into the solid external $^{15}NH_4Cl$ scale. Solid state ^{15}N spectra of ^{15}N-PLP in *e. coli* AspAT as lyophilized apoenzyme (to verify the ^{15}N chemical shift of the backbone signal at natural abundance) and microcrystalline holoenzyme un- and liganded with maleate (inhibitor) with ^{15}N-PLP, were performed on a Varian Infinity Plus 600 MHz (14 Tesla) solid state NMR spectrometer (60.8 MHz for ^{15}N) at 225 K (-50 °C). Standard cross polarization $^{15}N\{^{1}H\}$ CP RAMP MAS NMR experiments were performed under magic angle spinning (MAS) conditions. In the latter case, the spinning rate was 7 kHz. The 90° pulse for protons was 4 μs, the cross polarization contact time 1 ms, by using a recycle time of 3 s. For each spectrum more than 50000 scans were recorded. An echo sequence was employed to minimize artifacts from long radiofrequency pulses. The 180° pulse for nitrogen was 19 μs by an echo delay of one rotor period. The spectral resolution increases significantly when microcrystalline samples are used. The external standard was glycine (95%, ^{15}N-enriched) which was converted into the external solid $^{15}NH_4Cl$ scale. The liquid state ^{15}N NMR spectra of the model complexes in the polar liquid were measured using a Bruker AMX 500 spectrometer (500.13 MHz for ^{1}H, 50.68 MHz for ^{15}N) equipped for low-temperature NMR down to 100 K.

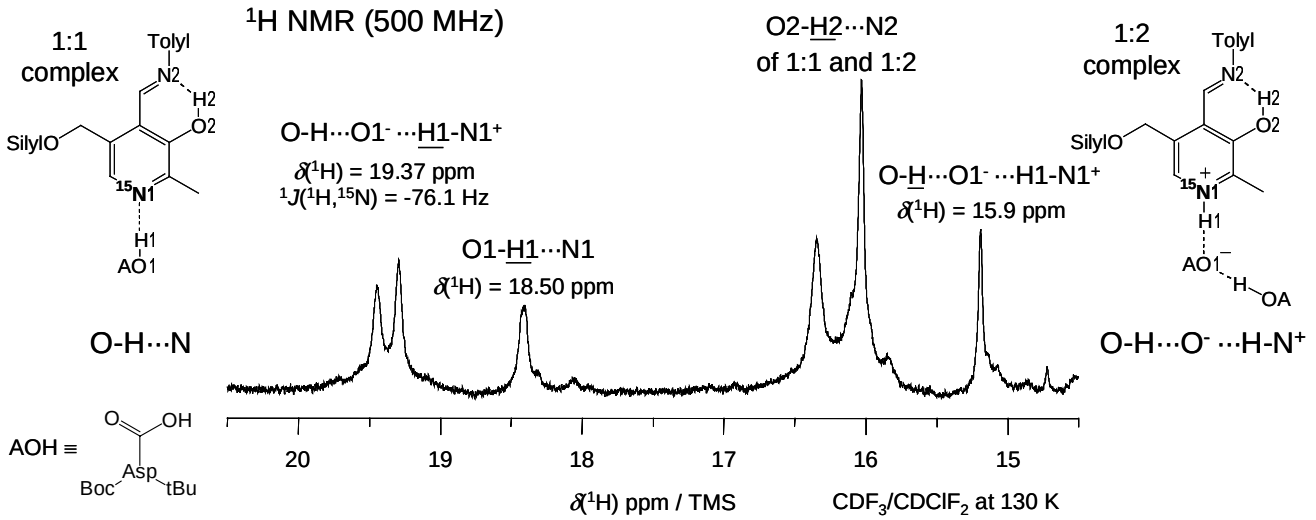

Figure S1. ^{1}H NMR spectrum of the ^{15}N ring labeled aldenamine model with protected aspartic acid (Boc-Asp-OtBu), which mimic the Asp222 side-chain in AspAT, in the freon mixture at 130 K. Shown are the 1:1 and 1:2 complexes.