Supporting Information

An ESEEM Analysis of Multi-Histidine Coordination in Model Complexes, Peptides and Amyloid-β

K. Ishara Silva, Brian C. Michael, Steven J. Geib, Sunil Saxena*

Department of Chemistry, University of Pittsburgh, PA 15260

sksaxena@pitt.edu

AUTHOR EMAIL ADDRESS sksaxena@pitt.edu

* To whom correspondence should be addressed. Phone: (412) 624-8680. Fax: (412) 624-8611

E-mail: sksaxena@pitt.edu

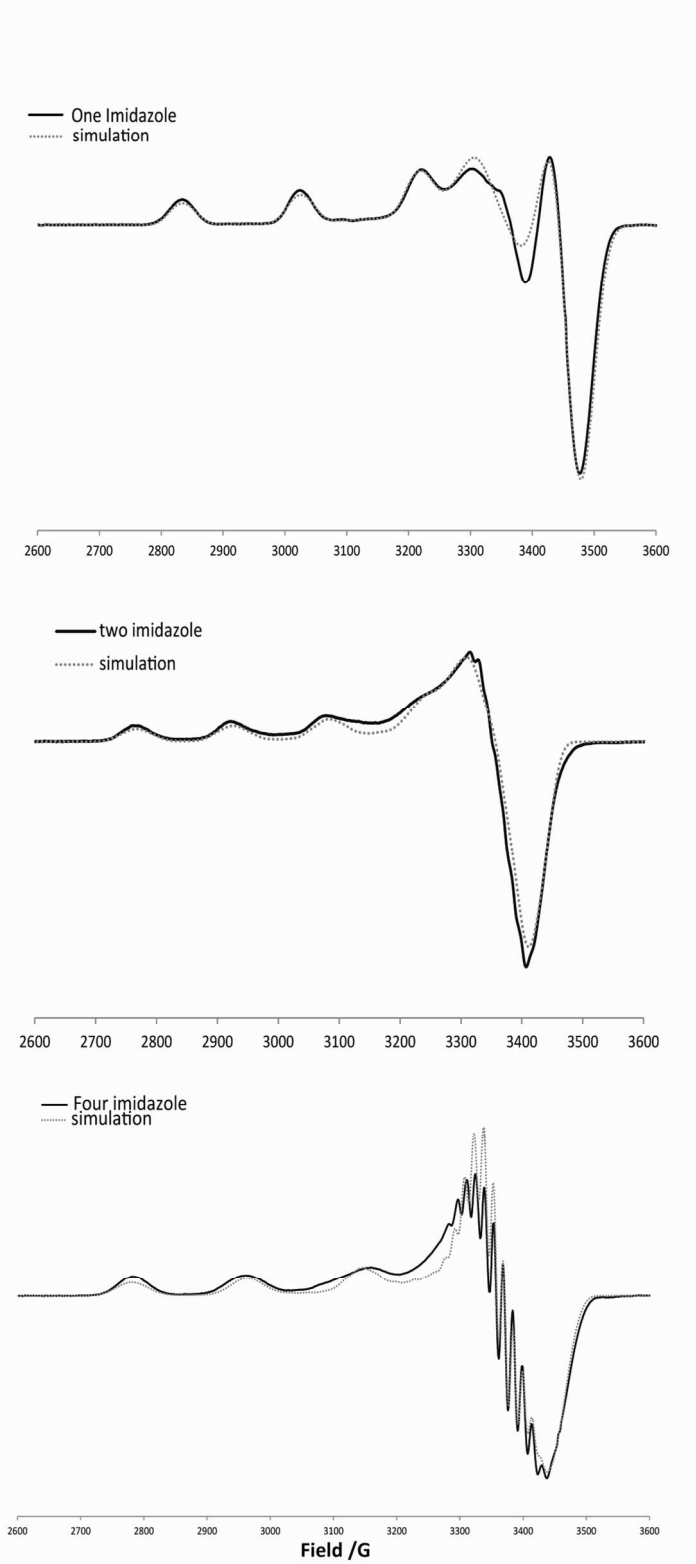

Figure S1. Simulated CW spectra for Cu(II) – imidazole complexes. Experimental spectra are shown in solid lines and simulated ones are shown in dashed.

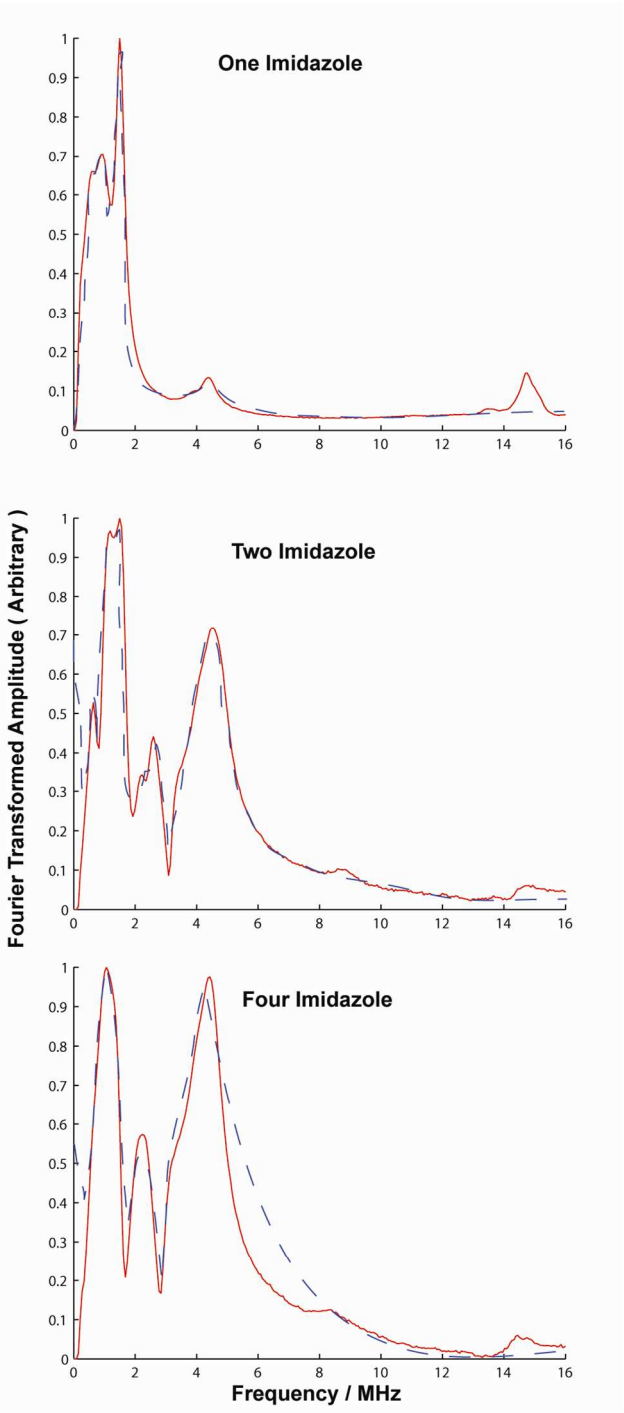

Figure S2. Experimentally obtained and simulated three-pulse ESEEM spectra of the model complexes

Table S1. Parameters used for ESEEM simulation in model complexes

Parameter	One Imidazole	Two Imidazole	Four Imidazole
η	0.67±0.02	0.72±0.02	0.67±0.02
A_{iso}	1.70±0.03	1.84±0.04	1.87±0.04
T_{dip}	0.14±0.01	0.14±0.01	0.12±0.01
α	75°±5	60°±5	45°±5
β	90°±5	30°±5	30°±5
κ	1.59	1.64	2.80

Error calculation in ESEEM

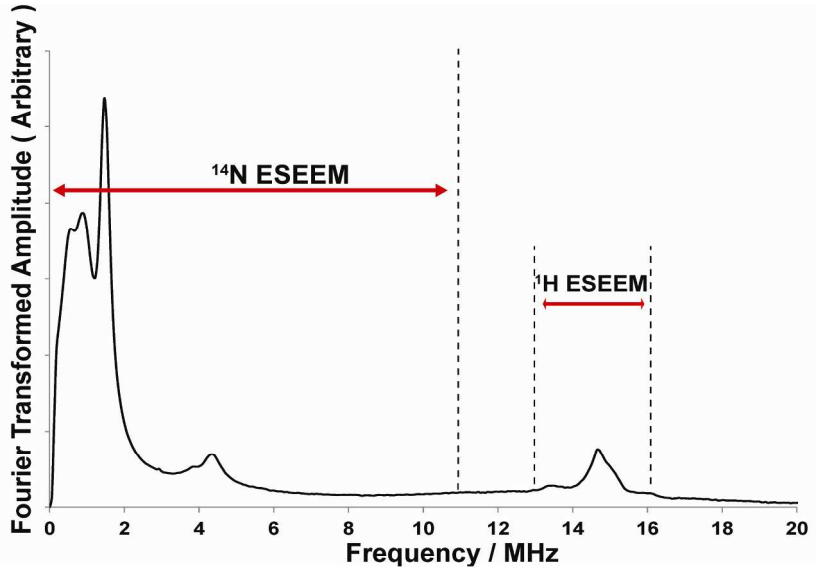

Figure S3. Regions used to calculate the integrated intensities of ^{14}N-ESEEM and ^{1}H-ESEEM

1. The frequency region from 20 MHz – 30 MHz was used to calculate the standard deviation of the baseline. (s)
2. Then, the sum of the region from 0 – 11 MHz was calculated for ^{14}N-ESEEM. The error was calculated using the standard deviation from the step 1. The number of points used for the integration is given as "n". (For 14N-ESEEM region n = 145)

$$Sum_{14N\ ESEEM} \pm \sqrt{n} \times s^2$$

3. Same procedure was used to calculate the integration intensity (for 13 – 16 MHz region) and the error for the ^{1}H-ESEEM regions. The number of points (n) used was 50.
4. The propagation of error was used to calculate the final error associated with the ^{14}N-ESEEM/^{1}H-ESEEM.

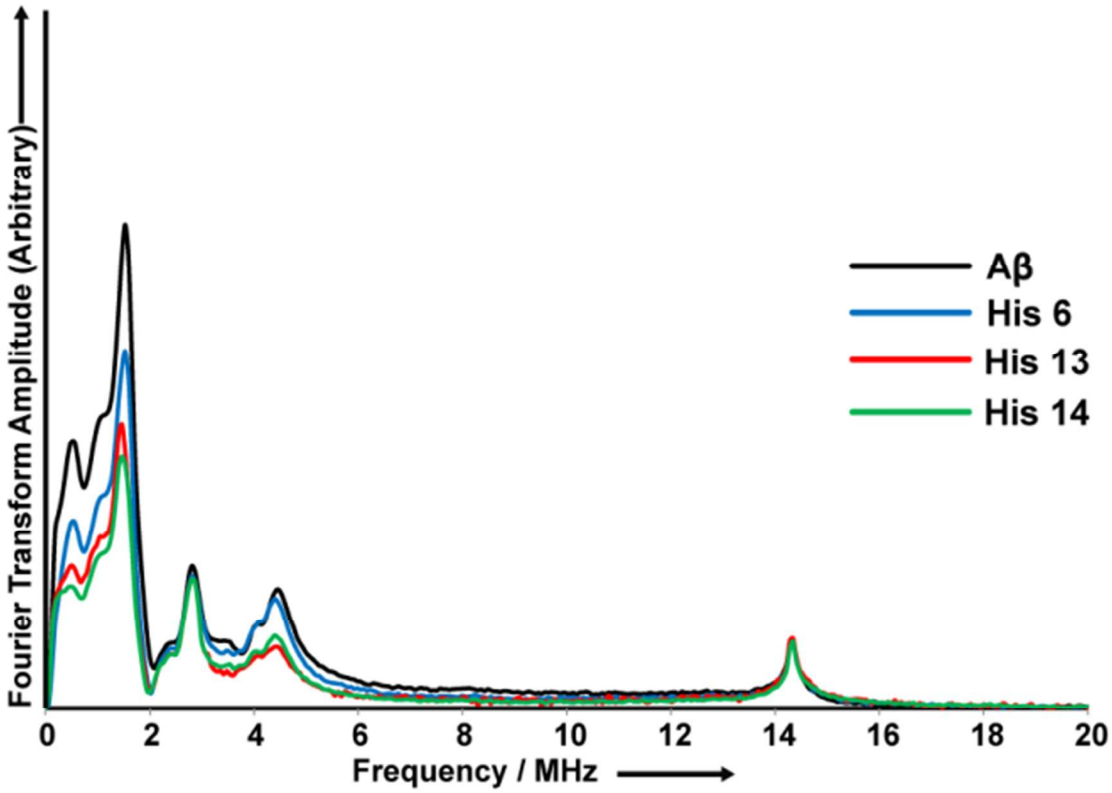

Figure S4. Three-pulse ESEEM spectra of the nonlabeled and single ^{15}N labeled Aβ(1 – 16) variants mixed with equimolar amounts of Cu(II) and Zn(II) at 3355 G at pH 8.7. The decrease in intensity below 8 MHz in ^{15}N labeled Aβ(1 – 16) variants gives the contribution of each histidine residue for component I in Aβ(1 – 16)-Cu(II).

Table S2 : Relative integrated intensities of ESEEM spectra of the nonlabeled and ^{15}N- double labeled Aβ(1 – 16) variants at pH 8.7 mixed with an equimolar amount of Cu(II) at the ^{14}N-ESEEM region (0 – 11 MHz) and ^{1}H-ESEEM region (13 – 16 MHz) and the relative contribution from each histidine residue

Sample	^{14}N-ESEEM	^{1}H-ESEEM	$^{14}N/^{1}H$	% involvement
Aβ	10767 ± 7	951 ± 5	11.32 ± 0.1	
His 6,13	4270 ± 7	950 ± 5	4.49 ± 0.1	39.6 ± 1
His 6,14	4313 ± 7	950 ± 5	4.54 ± 0.1	40.1 ± 1
His 13,14	2795 ± 7	950 ± 5	2.94 ± 0.1	25.9 ± 1

Table S3 : Relative integrated intensities of ESEEM spectra of the nonlabeled and ^{15}N- single labeled Aβ(1 – 16) variants at pH 8.7 mixed with an equimolar amount of Cu(II) at the ^{14}N-ESEEM region (0 – 11 MHz) and ^{1}H-ESEEM region (13 – 16 MHz) and the relative contribution from each histidine residue

Sample	^{14}N-ESEEM	^{1}H-ESEEM	$^{14}N/^{1}H$	% reduction
Aβ	5847 ± 4	515 ± 3	11.35 ± 0.07	
His 6	4673 ± 3	508 ± 2	9.20 ± 0.04	18.9 ± 0.7
His 13	3329 ± 5	500 ± 4	6.66 ± 0.05	41.3 ± 0.8
His 14	3431 ± 3	466 ± 2	7.36 ± 0.03	35.1 ± 0.7