Probing the interaction of Aspergillomarasmine A (AMA) with metallo-β-lactamases NDM-1, VIM-2, and IMP-7

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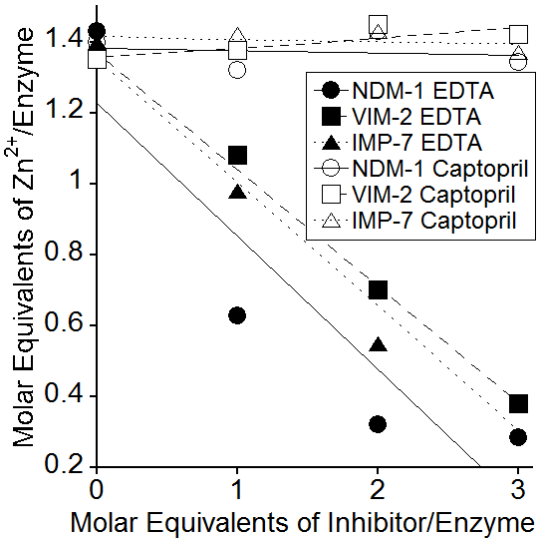

Figure S1. Effects of L-captopril and EDTA on Zn(II) content of NDM-1, VIM-2, and IMP-7. Each enzyme (100 µM) was incubated with 0, 100, 200, and 300 µM L-captopril or EDTA, dialyzed, then diluted (2 µM) in Nanopure water. ICP-AES was used to measure metal content of the resulting samples. EDTA titration results in the reduction of Zn(II) concentration with NDM-1, VIM-2, and IMP-7, whereas L-captopril produces insignificant variation in metal content.

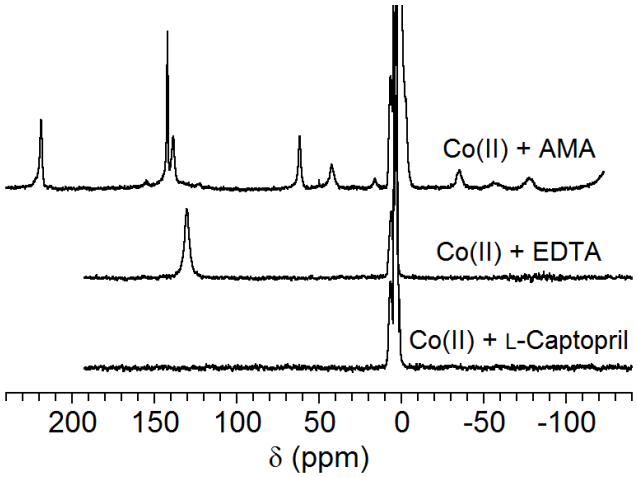

Figure S2. 200 MHz ^{1}H NMR control spectra of Co(II) and inhibitor in the absence of diCo(II)-substituted enzyme. Spectrum of 2 mM $CoCl_2$ and 5 mM L-captopril (bottom). Spectrum of 2 mM $CoCl_2$ and 5 mM EDTA (middle). Spectrum of 2 mM $CoCl_2$ and 4 mM AMA (top).

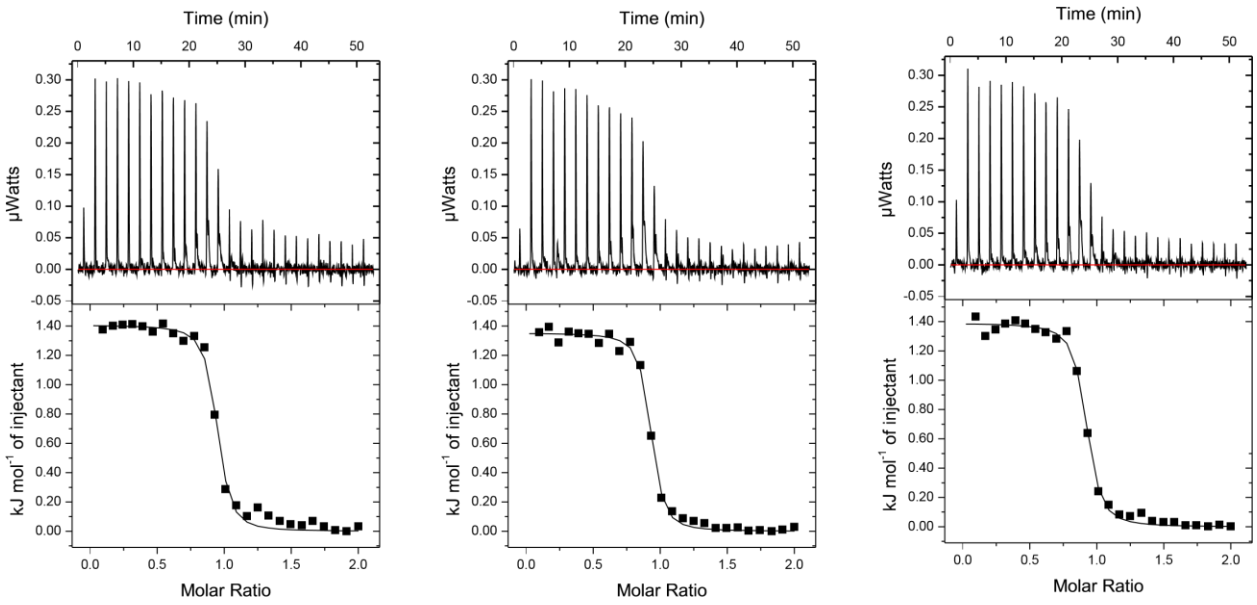

Figure S3. ITC thermograms of $CoCl_2$ titrated into AMA run in triplicate

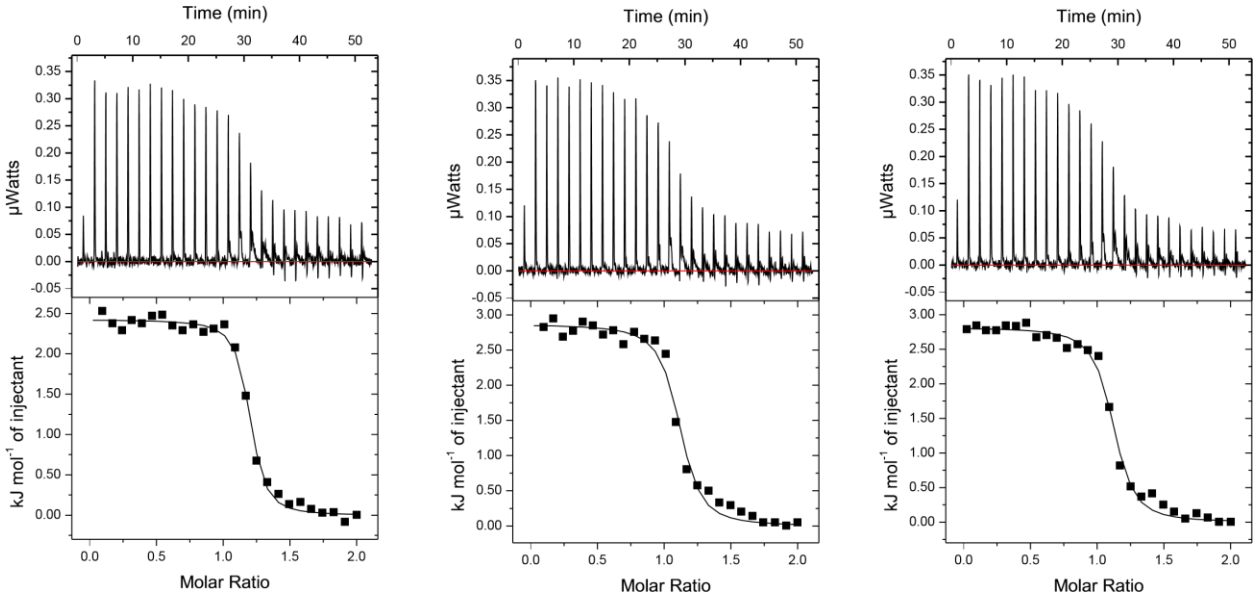

Figure S4. ITC thermograms of $ZnCl_2$ titrated into AMA run in triplicate

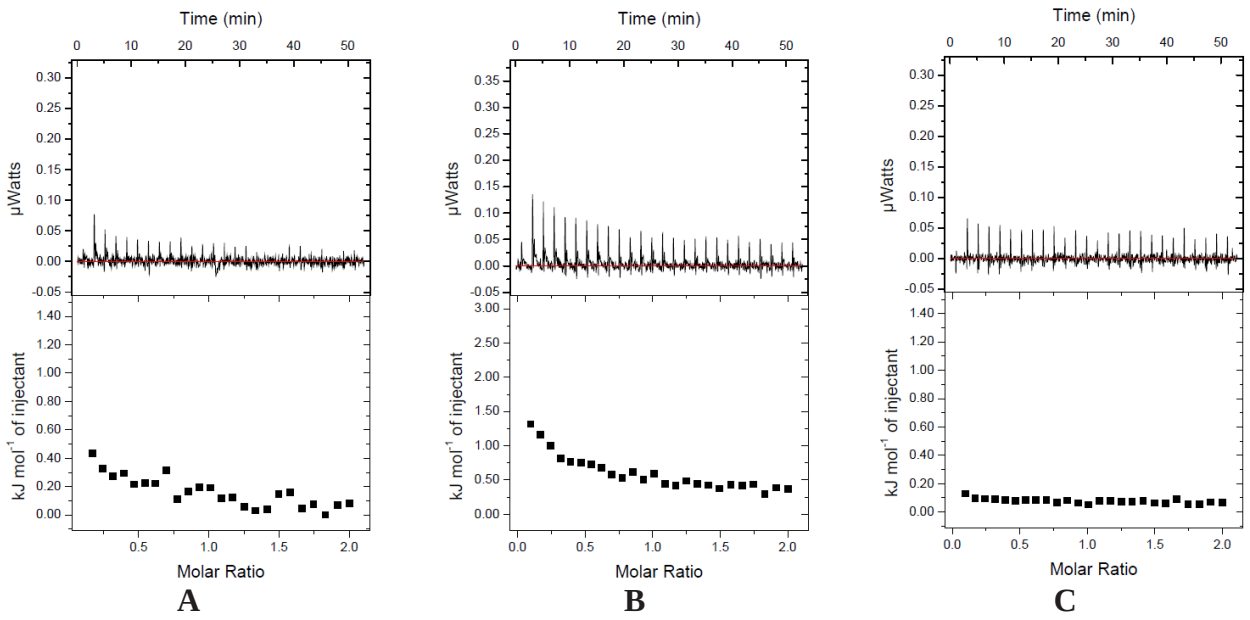

Figure S5. ITC thermograms of **A.** $CoCl_2$ titrated into HEPES buffer, **B.** $ZnCl_2$ titrated into HEPES buffer, and **C.** HEPES buffer titrated into AMA

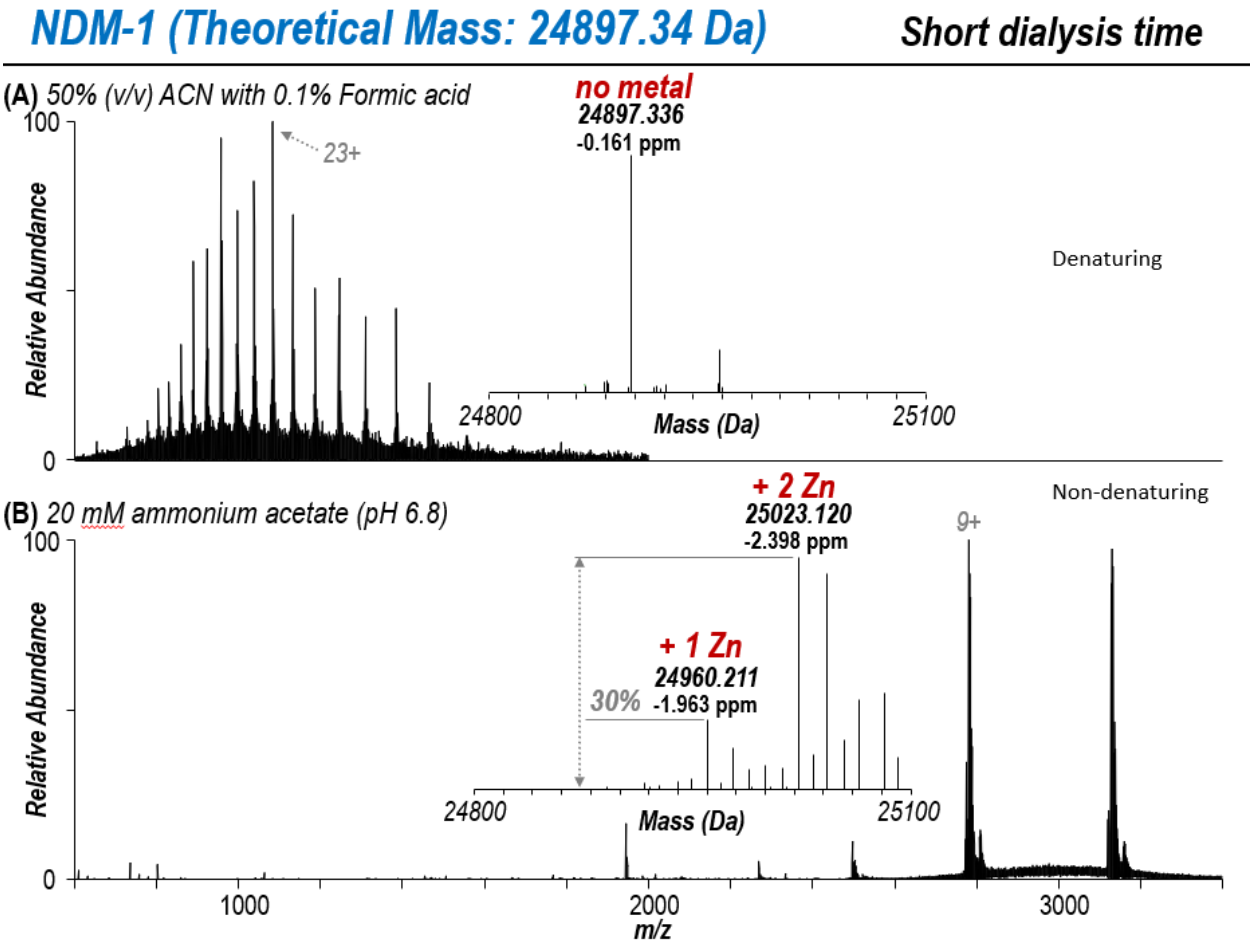

Figure S6. ESI-MS of NDM-1 after a short dialysis period obtained using (A) denaturing and (B) non-denaturing conditions. Insets give expanded views of the deconvoluted spectra (mass range 24800 Da – 25100 Da) labeled with the monoisotopic masses and mass accuracies relative to the theoretical mass of the protein bound to one or two zinc atoms. The singly bound species was 30% as abundant as the doubly bound species.

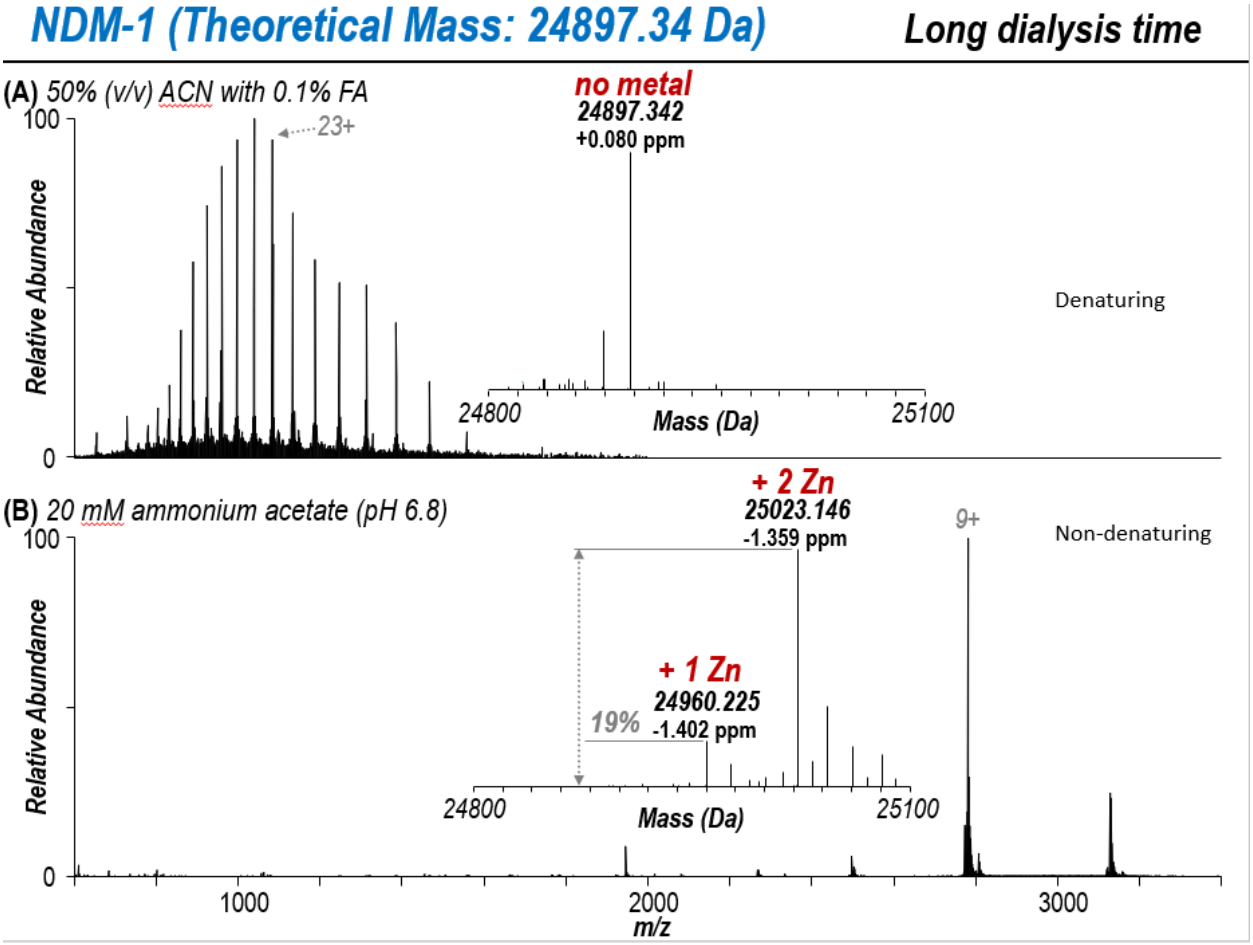

Figure S7. ESI-MS of NDM-1 after a long dialysis period obtained using (A) denaturing and (B) non-denaturing conditions. Insets give expanded views of the deconvoluted spectra (mass range 24800 Da – 25100 Da) labeled with the monoisotopic masses and mass accuracies relative to the theoretical mass of the protein bound to one or two zinc atoms. The singly bound species was 19% as abundant as the doubly bound species.

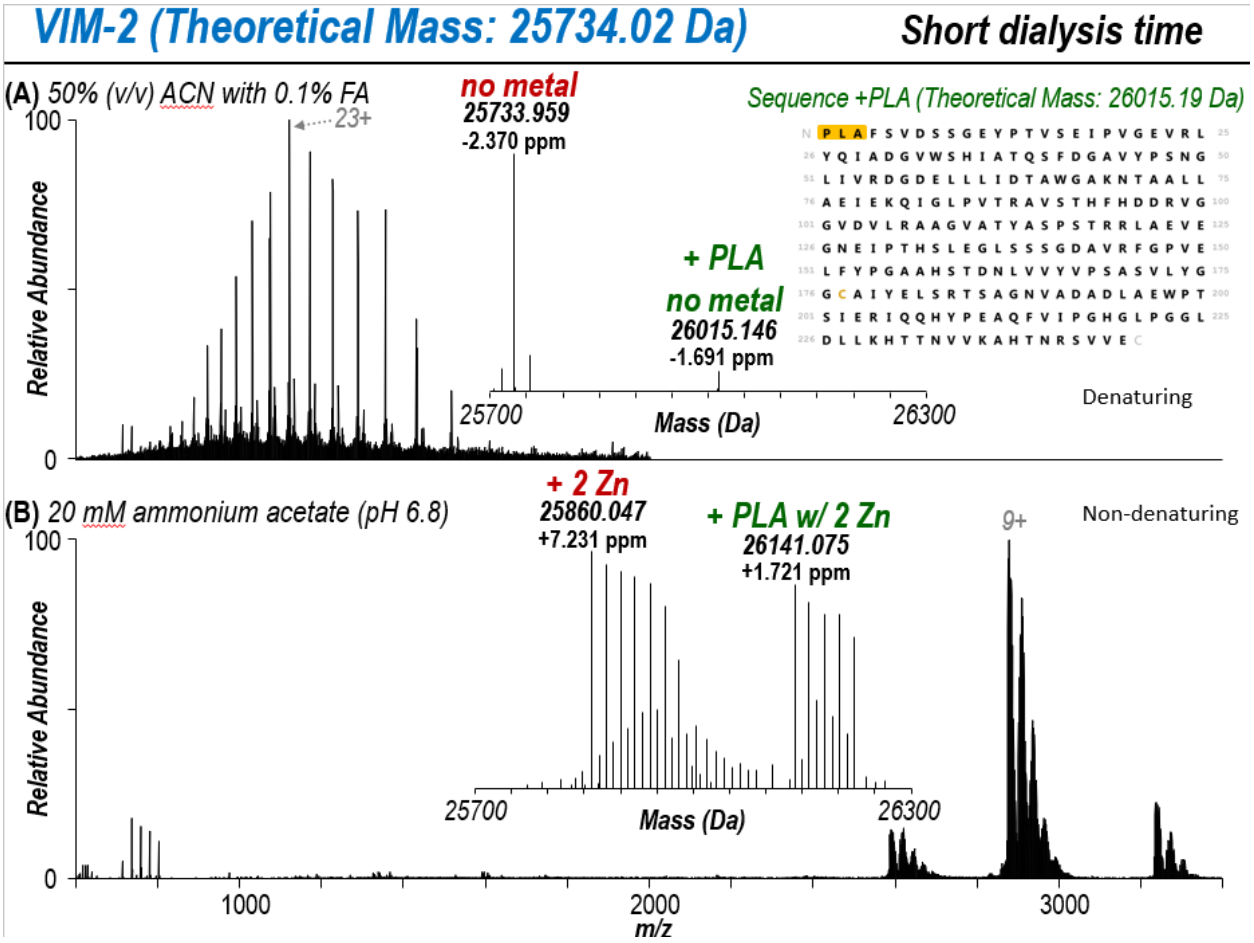

Figure S8. ESI-MS of VIM-2 after a short dialysis period obtained using (A) denaturing and (B) non-denaturing conditions. Insets give expanded views of the deconvoluted spectra (mass range 25700 Da – 26300 Da) labeled with the monoisotopic masses and mass accuracies relative to the theoretical mass of the protein bound to two zinc atoms. Additionally, a species with three additional amino acids (+ PLA) at the beginning of the sequence was observed.

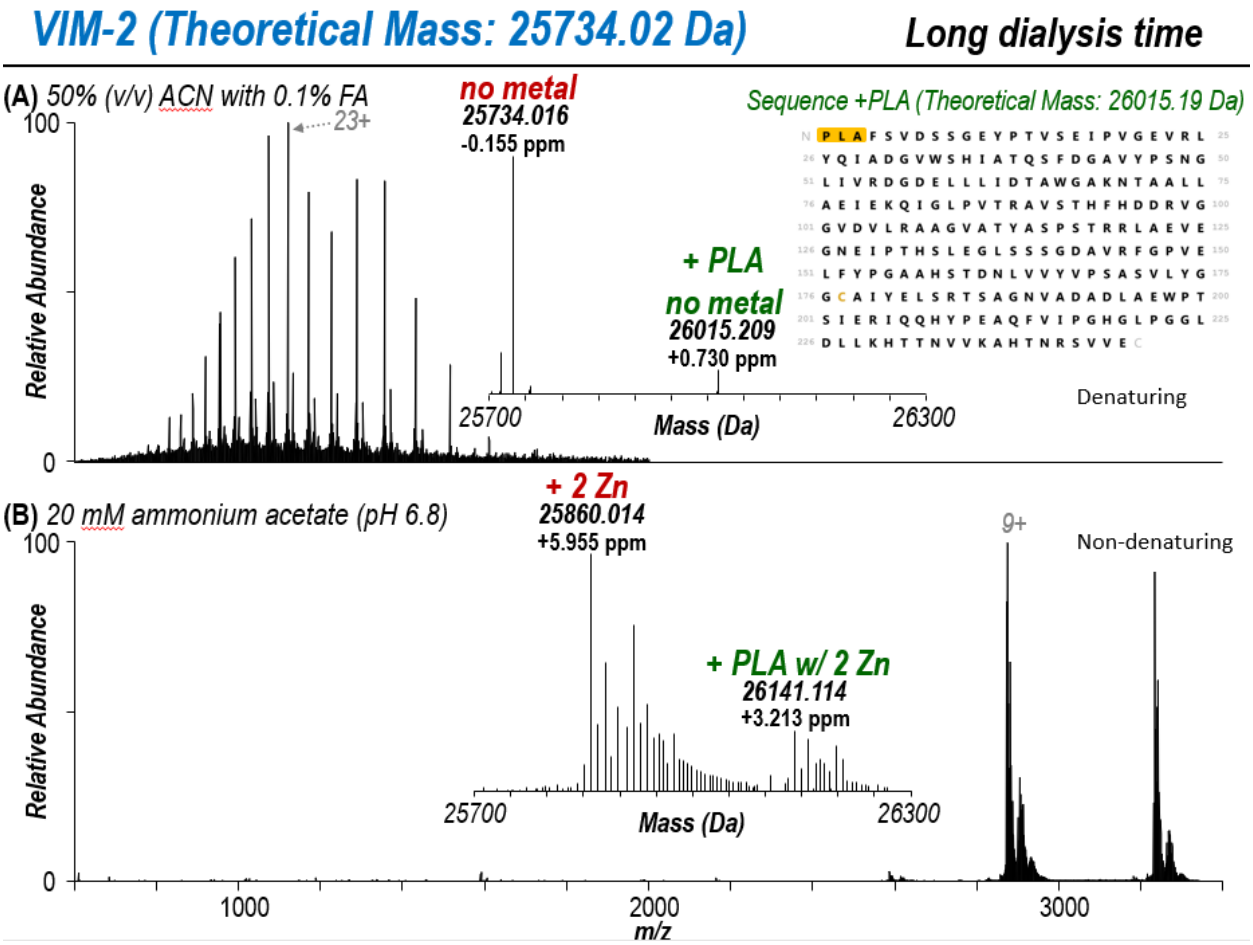

Figure S9. ESI-MS of VIM-2 after a long dialysis period obtained using (A) denaturing and (B) non-denaturing conditions. Insets give expanded views of the deconvoluted spectra (mass range 25700 Da – 26300 Da) labeled with the monoisotopic masses and mass accuracies relative to the theoretical mass of the protein bound to two zinc atoms. Additionally, a species with three additional amino acids (+ PLA) at the beginning of the sequence was observed.

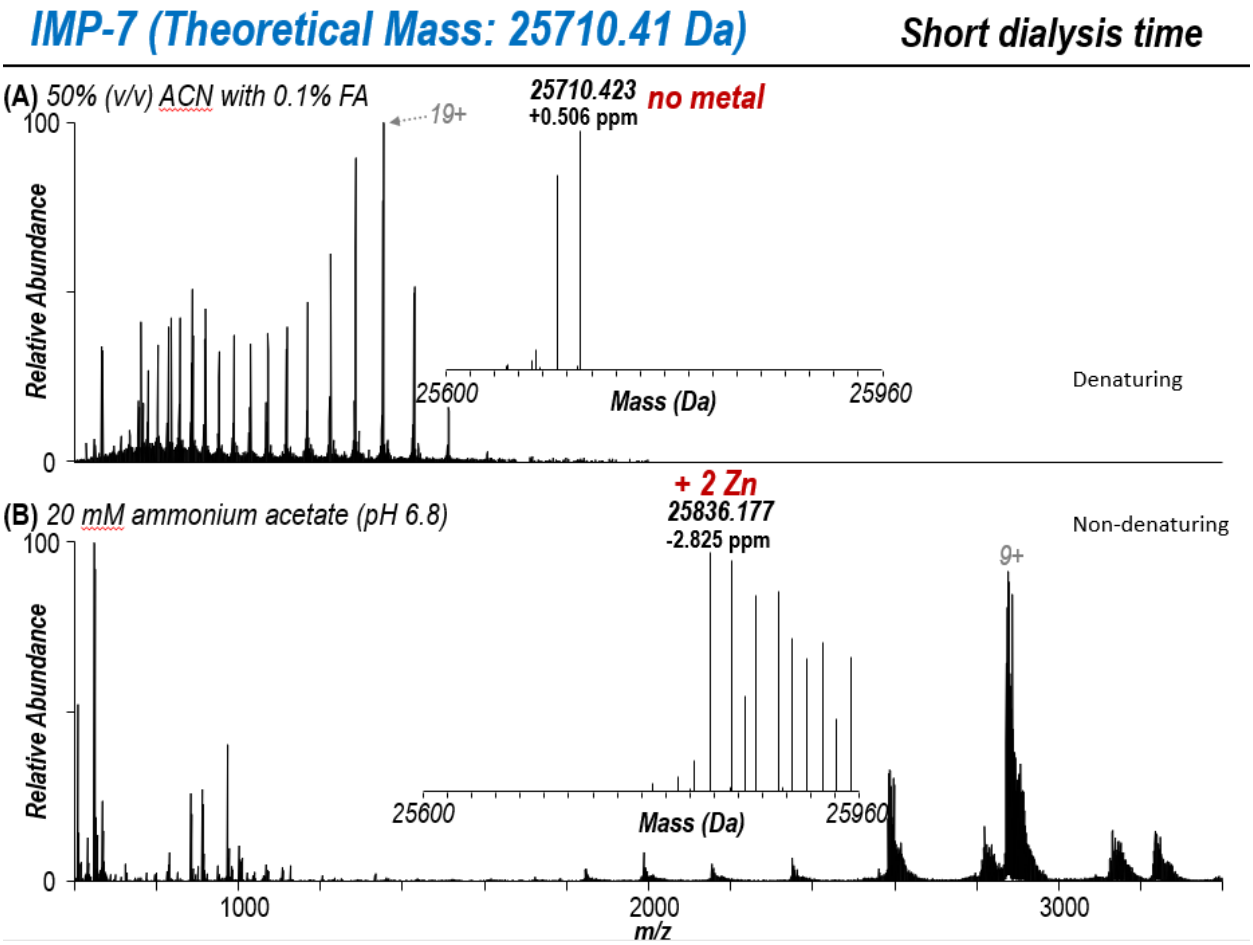

Figure S10. ESI-MS of IMP-7 after a short dialysis period obtained using (A) denaturing and (B) non-denaturing conditions. Insets give expanded views of the deconvoluted spectra (mass range 25600 Da – 25960 Da) labeled with the monoisotopic masses and mass accuracies relative to the theoretical mass of the protein bound to two zinc atoms.

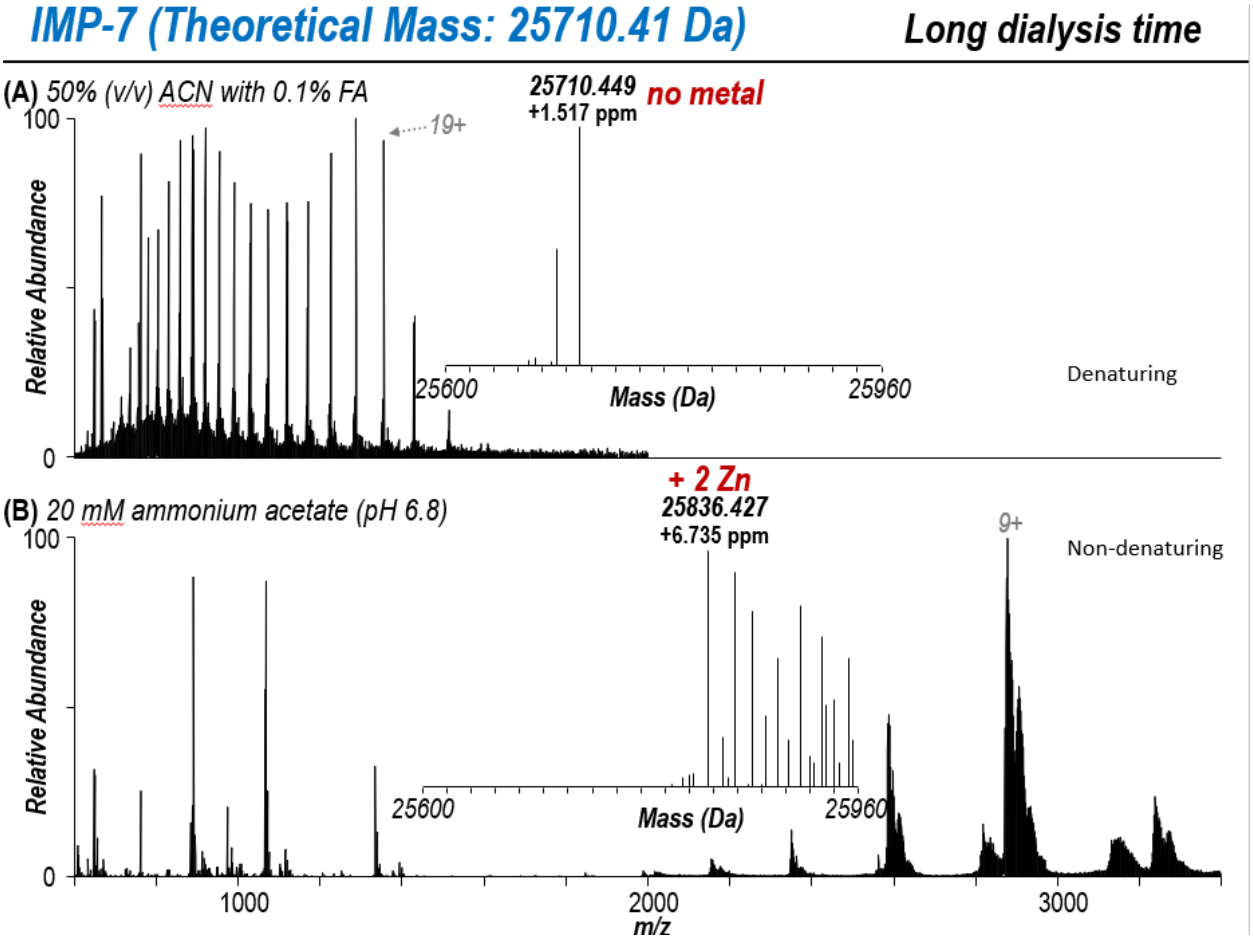

Figure S11. ESI-MS of IMP-7 after a long dialysis period obtained using (A) denaturing and (B) non-denaturing conditions. Insets give expanded views of the deconvoluted spectra (mass range 25600 Da – 25960 Da) labeled with the monoisotopic masses and mass accuracies relative to the theoretical mass of the protein bound to two zinc atoms.