Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Label-free detection of Biotoxins via Photo-Induced Force Spectrum at Single-molecular level

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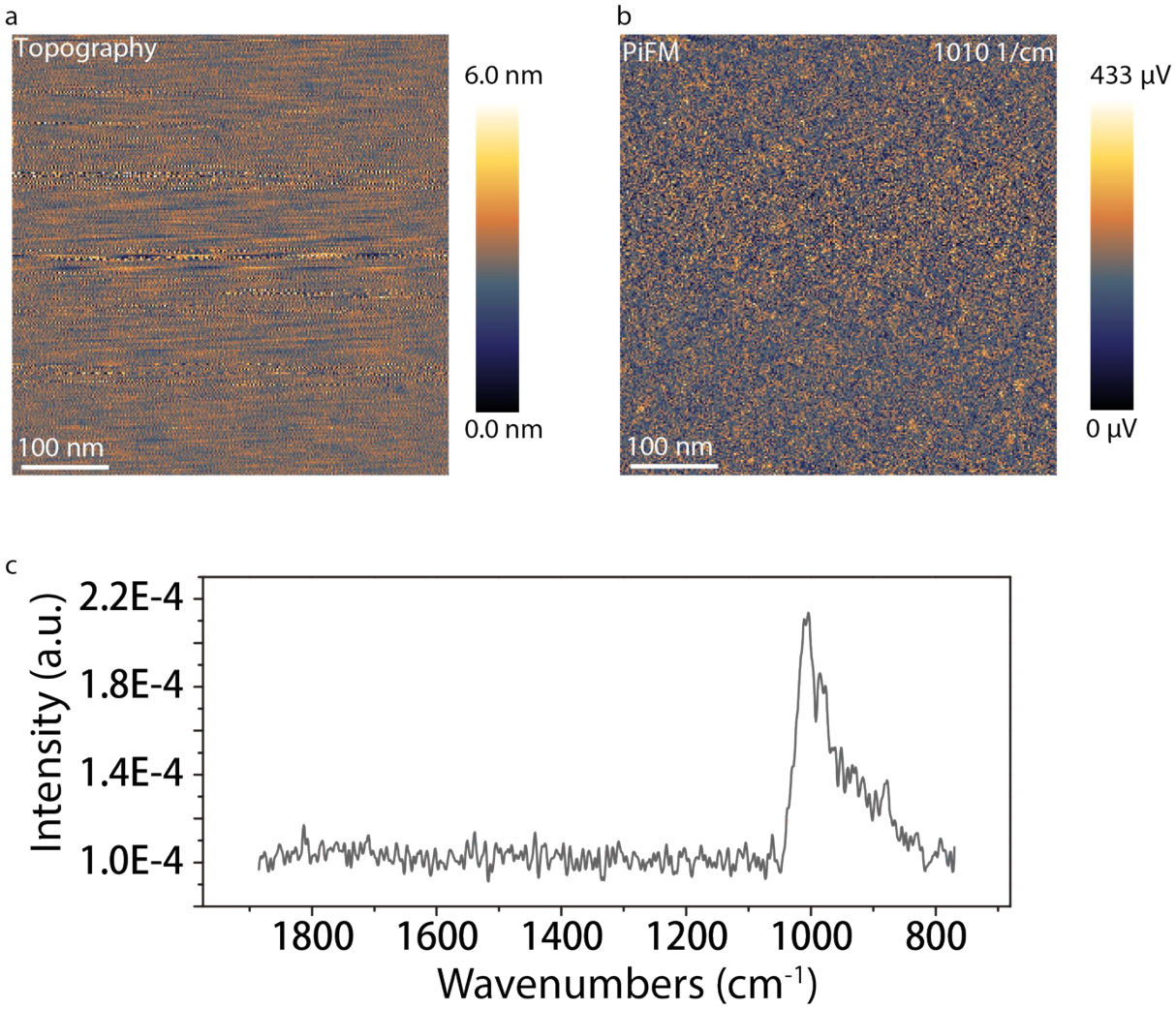

Figure S1. The (a) Topography, (b) PiFM image and (c) Photo-induced force spectra of APTES-mica. No obvious particles are shown in the topography. PiFM signal in 1010 cm^{-1} was collected in situ with topography. And an average signature photo-induced force spectra show that no obvious bands in amide areas or other protein structure signature areas except the range near 1000 cm^{-1}.

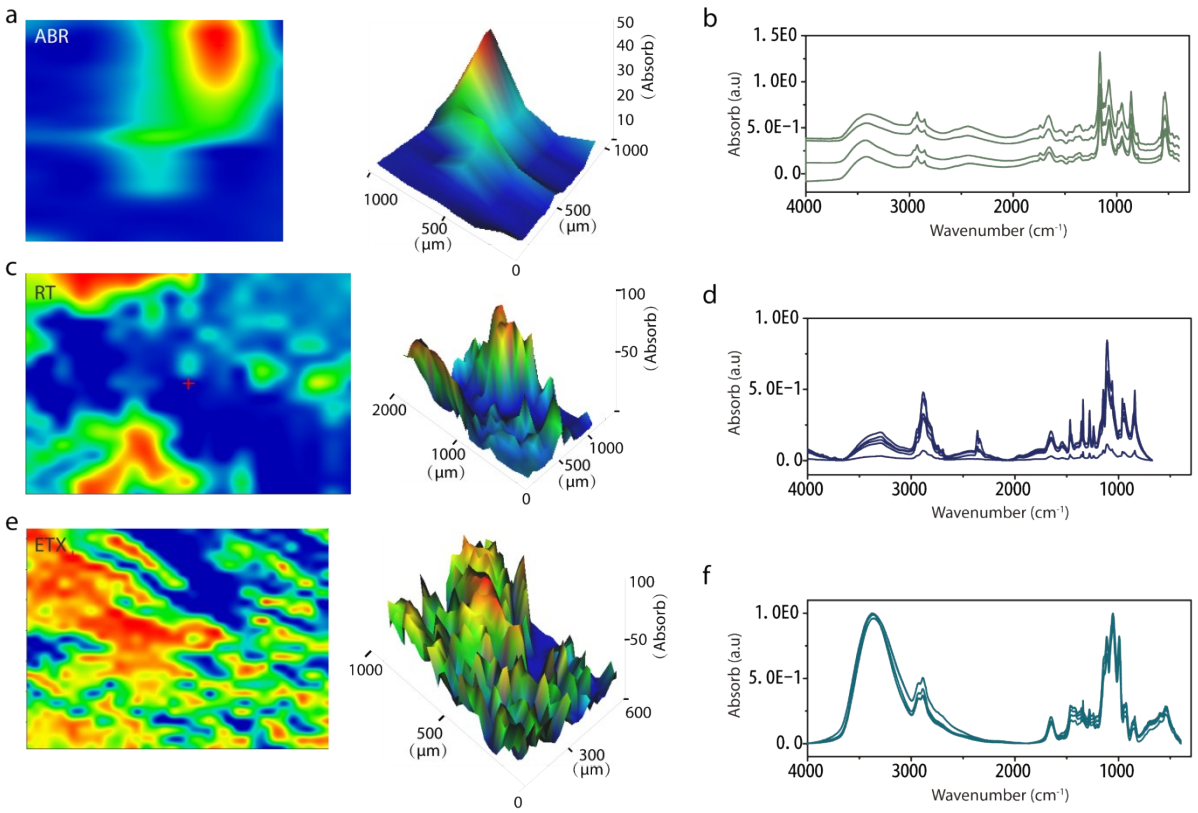

Figure S2. FTIR imaging with high concentration of three proteins. a,b)ABR fourier Infrared Imaging and Infrared absorption Spectrum; c, d) RT fourier Infrared Imaging and Infrared absorption Spectrum; e, f) ETX fourier Infrared Imaging and Infrared absorption Spectrum. The red region represents the strong infrared absorption and the blue region represents the KBr substrate. Data acquisitions of the right spectral profile are from the acquisition point in the infrared absorption region.

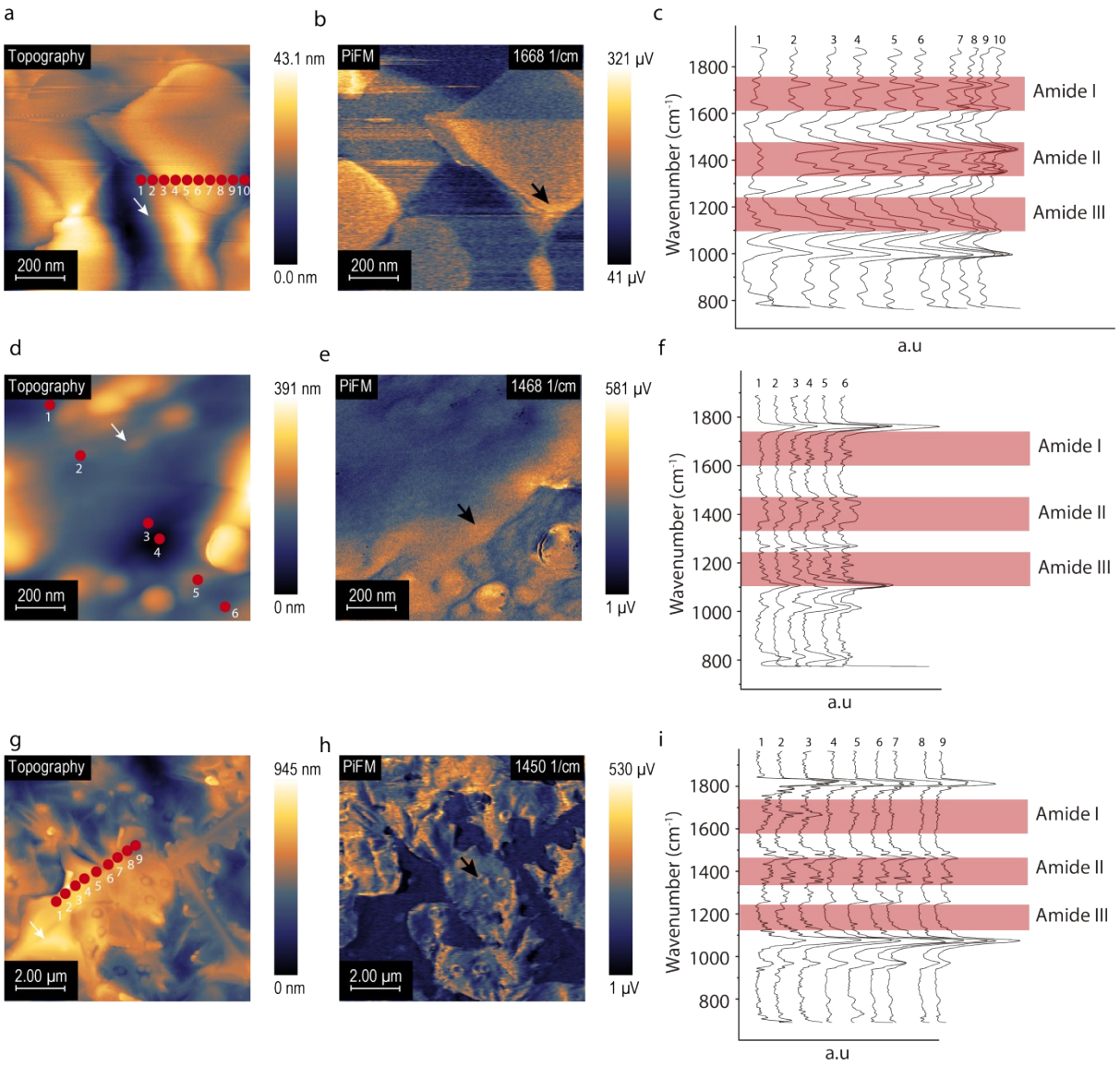

Figure S3. PiFM imaging with high concentration of three proteins. a) The AFM image of 100μg/mL ETX protein solution drips on mica surface after drying; b) The PiFM image of ETX in absorb 1668cm-1 (amide I); c) The photo-induced infrared spectrum from 10 points in topography respectively; d) The AFM image of 100μg/mL ABR protein solution drips on mica surface after drying; e) The PiFM image of ABR in absorb $1468^{cm\text{-}1}$ (amide II); f) The photo-induced infrared spectrum from 10 points in topography respectively; g) The AFM image of 100μg/mL RT protein solution drips on mica surface after drying; h) The PiFM image of RT in absorb $1450^{cm\text{-}1}$ (amide II); i) The photo-induced infrared spectrum from 10 points in topography respectively . The white arrow indicates the salt composition in those samples. The red arrows indicate protein components in those samples.

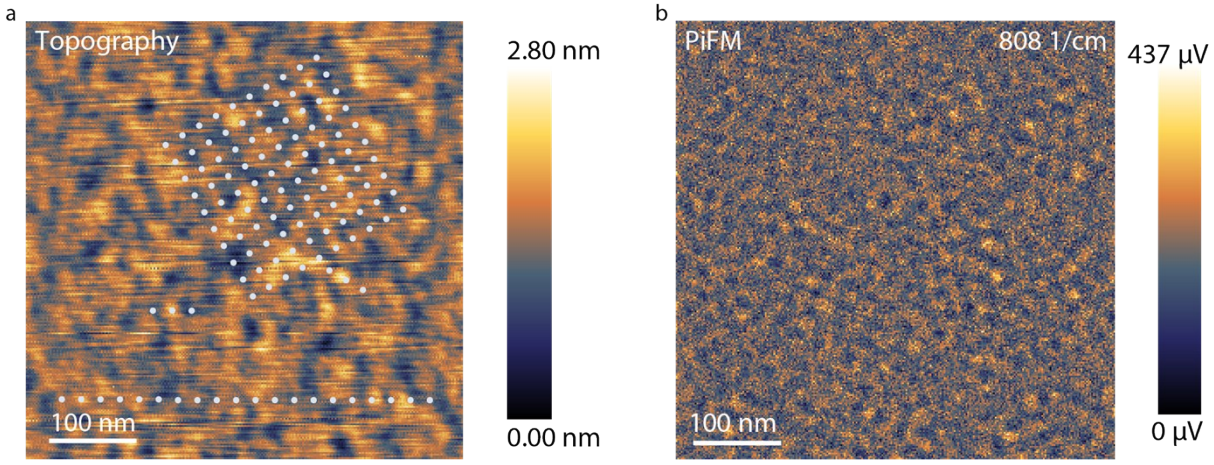

Figure S4. The (a) Topography and (b) PiFM image of mixed sample (molar ratio of ABR: RT: ETX is 2:3:5). PiFM signal in 1660 cm^{-1} was collected. Total 140 points in the coverage area was collected in each of 15nm spacing (the array in Figure S2a).

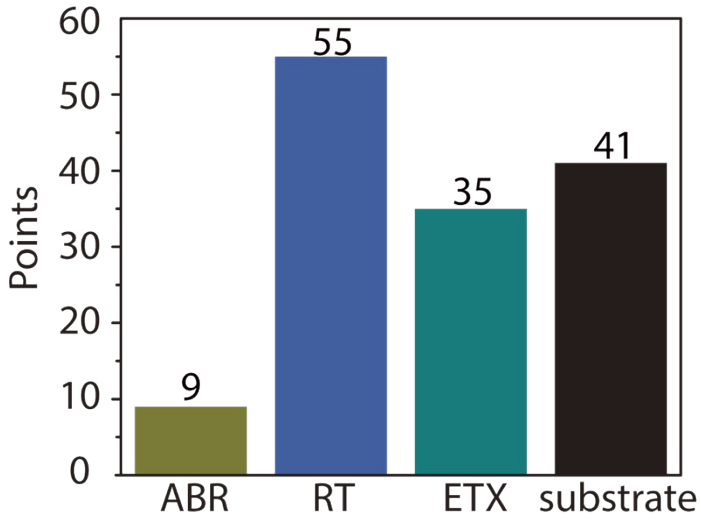

Figure S5. The PCA scores distribution statistics of the 140 data set in mixed samples. As shown in figure, 9 points contain signals with AT component, 55 points contain signals with RT components, 35 points contain signal with ETX components and the remaining 41 points does not contain any protein signal.

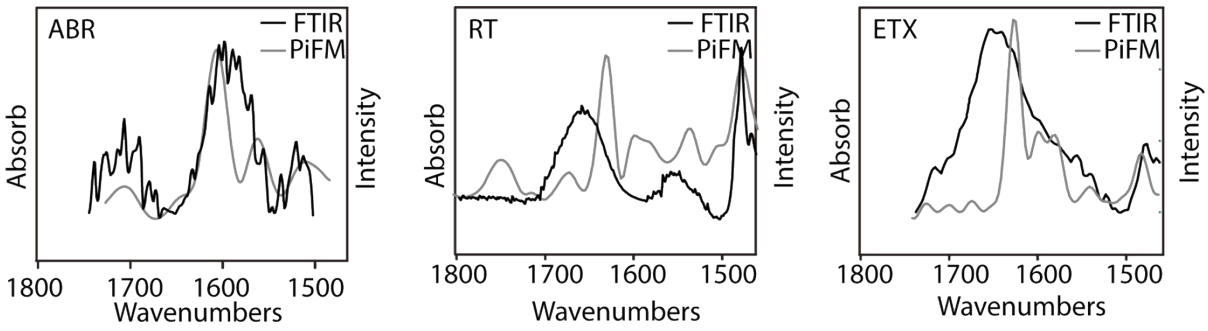

Figure S6. Comparison of toxins in range of amides between FTIR and PiFM. The vertical of FTIR and PiFM represent absorbance and intensity, respectively. Occasionally, shifts in peak wavenumber and amplitude are observed in PiFM spectra, arising from the extreme sensitivity of PiFM to localized populations of molecules.

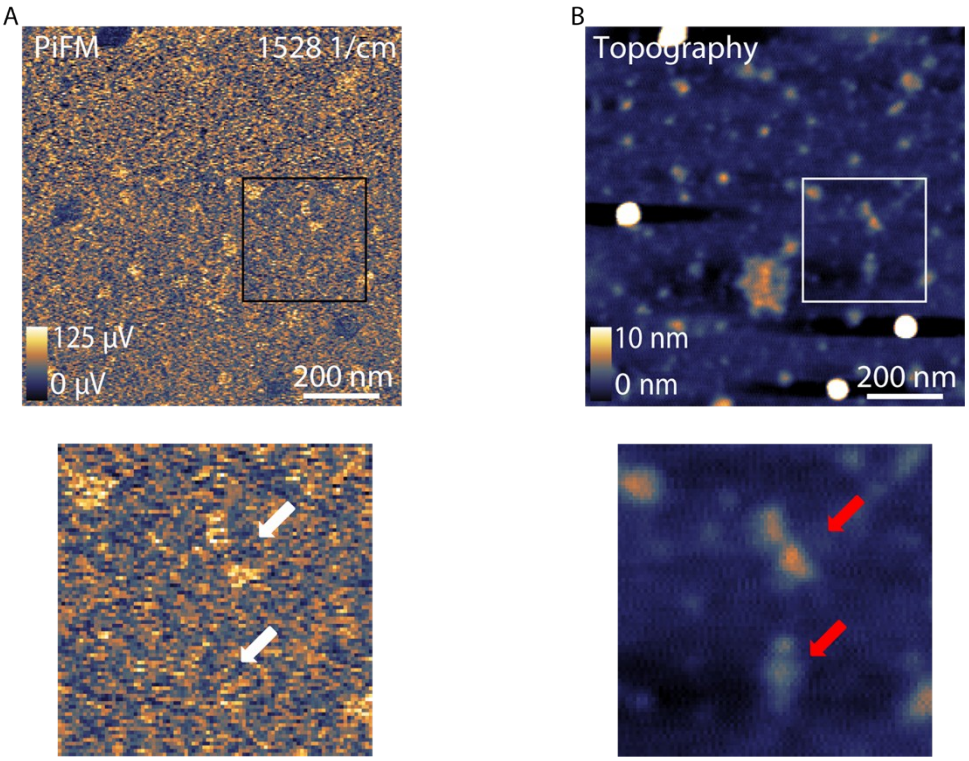

Figure S7. Spatial distributions of (A) PIFM and (B) topography spectral information of AT molecules measured simultaneously on resonance at 1528 cm^{-1} wavenumber vibrational modes. Arrows indicate the difference of AT molecules at the same location under the point spectrum. The red arrows indicate that the AT molecules have similar height and size, and the white arrows indicate that they have different PiFM feedback.

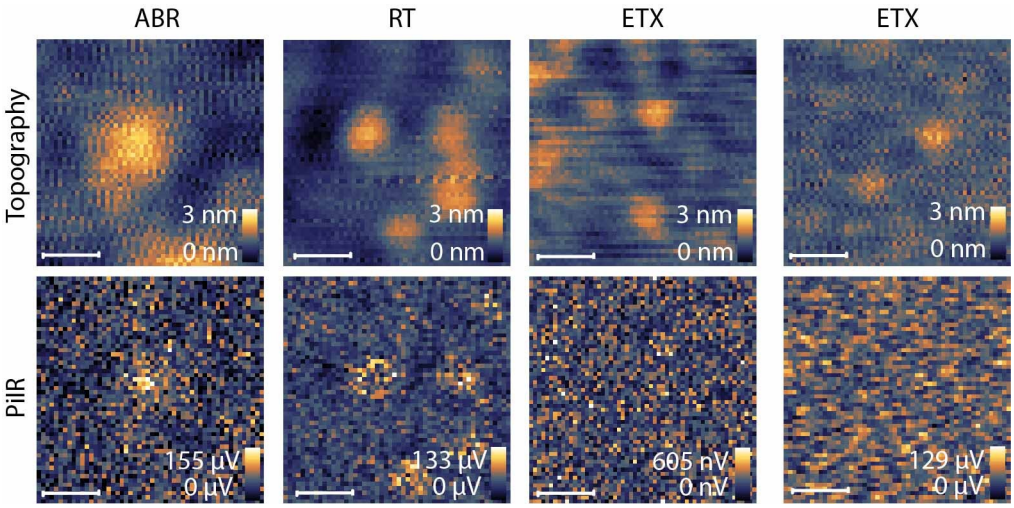

Figure S8. Topography and PIFM image of three toxin proteins. Both AT and RT have good PiFM feedback under similar laser power. However, ETX does not perform PiFM imaging very well. The two ETX image represent 50% (lift) and 100% (right) QCL power. (Scale bar: 25 nm).

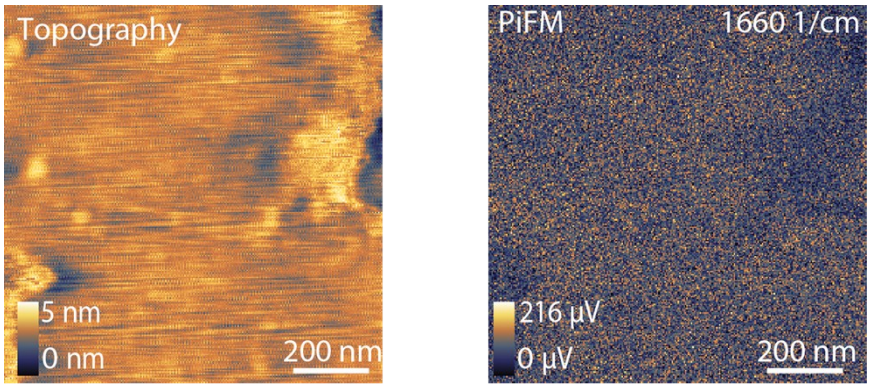

Figure S9 the topography and PiFM of high concentration (100nM) ETX. The diluted protein solution was incubated on the mica surface for 30 min and then washed three times with ultrapure water. 100% QCL power were adopted here to excite the PiFM signal.

Table S1. 10 intensity bands and protein secondary structure components

Wavenumber (cm^{-1})	808	1328	1386	1413	1467	1529	1610	1669	1731	1797
Related protein secondary structure or amino acid assignment	—	Amide III	—	Glutamine	—	Amide II	β-sheet	Amide I	Side chains	—

Table S2. Scores plots of three proteins in different principal components

ABR	PC1-PC2 Scores Plot	0.00015<ABR<0.0006
	PC1-PC3 Scores Plot	-0.00002<ABR<0.00003
	PC2-PC3 Scores Plot	-0.00002<ABR<0.000005
RT	PC1-PC2 Scores Plot	0.0006<RT<0.0016
	PC1-PC3 Scores Plot	-0.00001<RT<0.00004
	PC2-PC3 Scores Plot	-0.000025<RT<-0.000005
ETX	PC1-PC2 Scores Plot	0.0007<ETX<0.001
	PC1-PC3 Scores Plot	-0.00001<ETX<0.00009
	PC2-PC3 Scores Plot	-0.000005<ETX<0.000022