Electronic Supplementary Information

Correlating Bromelain's Activity with its Structure, Active-site Dynamics and Media's Physical Properties in a Hydrated Deep Eutectic Solvent

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***Content*:**

Table S1. Secondary structural parameters of untagged, CPM tagged and TMR tagged bromelain.

	α-helicity	β-sheet	β-turn	Random Coil
Untagged Bromelain	20.1	22.4	18.0	39.5
CPM-Bromelain	19.6	22.4	17.5	40.5
TMR-Bromelain	20.0	22.3	18.0	39.7

Table S2. Secondary structural parameters of native bromelain and back extracted bromelain from various DES composition.

DES Content % (v/v)	α-helicity	β-sheet	β-turn	Random Coil
0	20.5	23.4	17.6	38.5
10	20.4	23.5	17.6	38.5
20	20.4	23.4	17.8	38.4
30	20.2	23.8	17.8	38.2
40	20.0	24.0	17.7	38.3
50	19.7	24.1	17.7	38.5
60	19.1	24.3	17.8	38.8
70	18.3	24.8	17.8	39.1
80	16.1	26.4	17.8	39.7

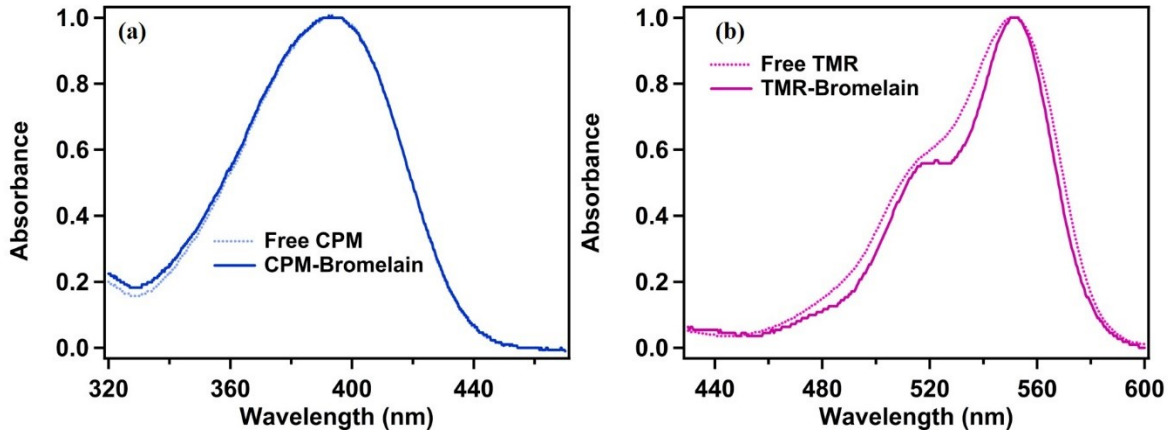

Figure S1. Absorption spectra of (a) free CPM and CPM tagged to bromelain; and (b) free TMR and TMR tagged to bromelain

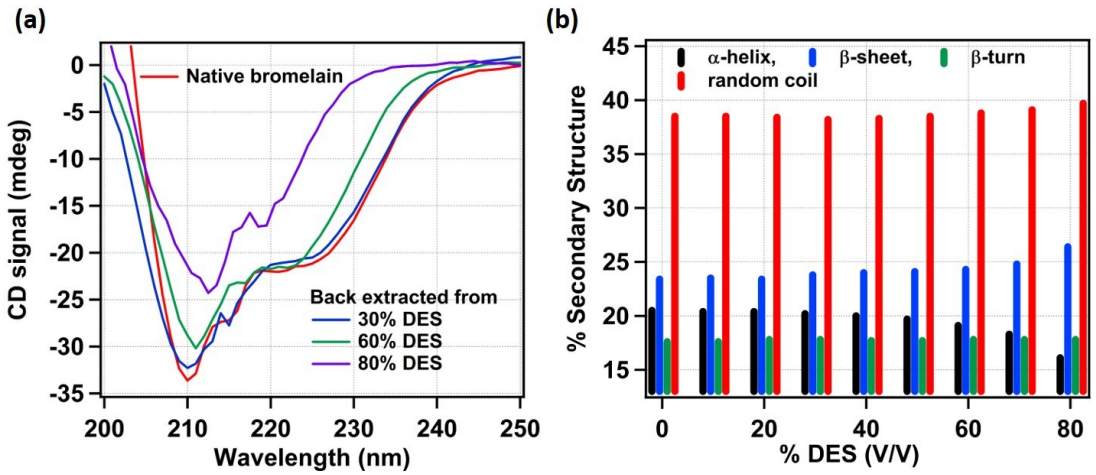

Figure S2. (a) CD spectra and (b) various secondary structural parameters of native bromelain in phosphate buffer (pH = 7.4) and back-extracted bromelain from various DES compositions. Bromelain concentration is 10 μM and path length is 2 mm.

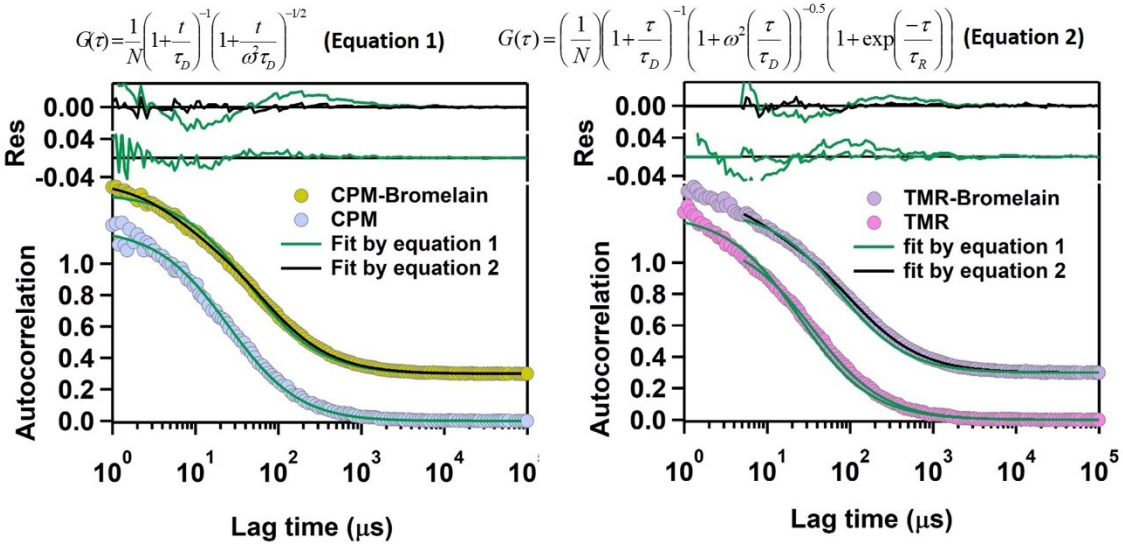

Figure S3. Fitting comparison of fluorescence autocorrelation traces of (a) CPM and CPM tagged to bromelain and (b) TMR and TMR tagged to bromelain. Equation 1 can fit the autocorrelation trace of CPM satisfactorily; but extra relaxation term is required to fit the autocorrelation trace of CPM-bromelain. Equation can fit the autocorrelation trace of TMR only when fitted from 5 μs. However, to fit autocorrelation trace of TMR-bromelain from 5 μs, equation 2 is required.

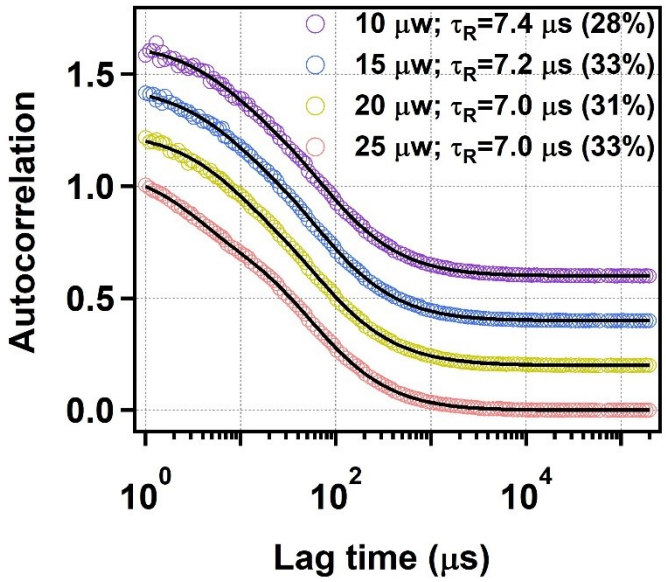

Figure S4. Autocorrelations traces of CPM tagged to bromelain at different laser power. The value of conformational fluctuation time and its contribution remains similar with the variation of power.

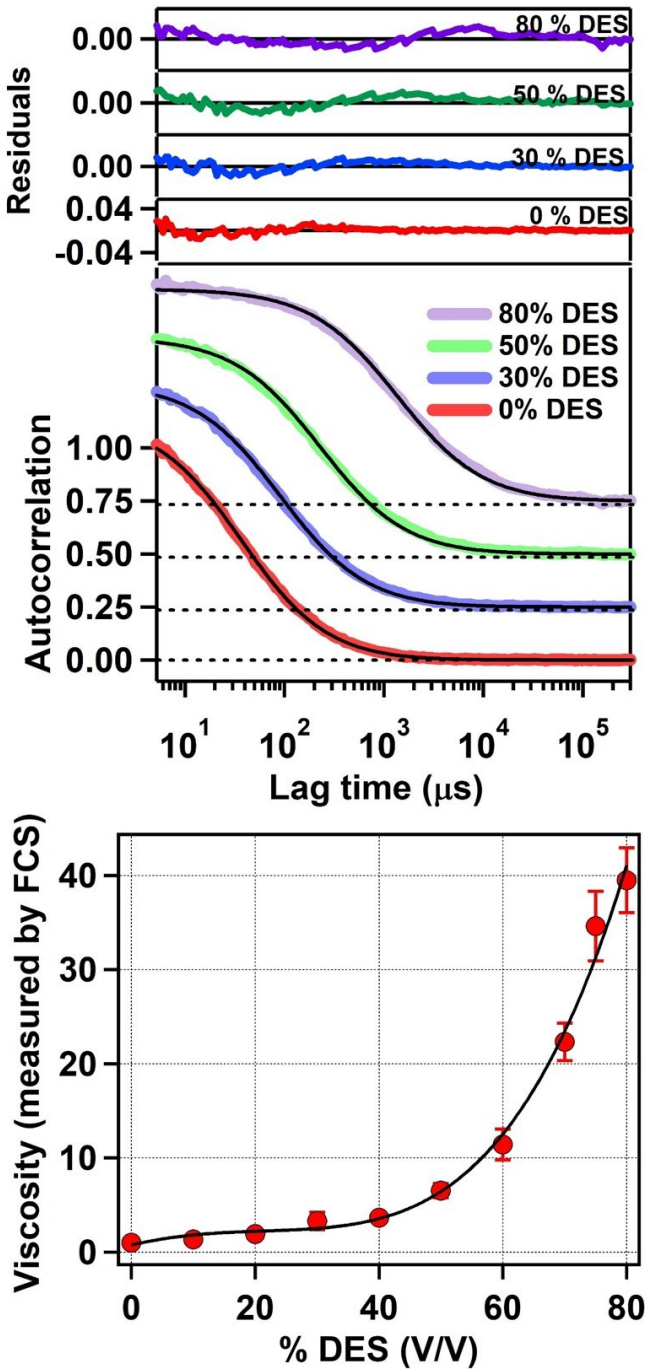

Figure S5. (a) Normalized fluorescence intensity autocorrelation curve for rhodamine-6G at some representative DES concentration. Fitting lines using equation 1 are shown by solid black lines. The residuals of fitting are also shown. (b) Variation of viscosity with increasing DES concentration