Enhancement of Thermal Stability of Proteinase K by Biocompatible Cholinium-based Ionic Liquids

Rui Li,‡[a,b] Zhuo Liu,‡[b,c] Fan Jiang,[a,b] Yang Zhao,[d] Guangyu Yang[e*] and Liang Hong[a,b,c*]

[a]*School of Physics and Astronomy, Shanghai Jiao Tong University, Shanghai 200240, China*

[b]*Institute of Natural Sciences, Shanghai Jiao Tong University, Shanghai 200240, China*

[c]*Shanghai National Center for Applied Mathematics (SJTU center), MOE-LSC, shanghai Jiao Tong University, Shanghai 200240, China*

[d]*Institute of Biothermal Science and Technology, University of Shanghai for Science and Technology, Shanghai 200093, People's Republic of China*

[e]*State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Rd., Shanghai 200240, People's Republic of China*

Corresponding author and email address:

Liang Hong: hongl3liang@sjtu.edu.cn

Guangyu Yang: yanggy@sjtu.edu.cn

‡ These authors contributed equally to this work.

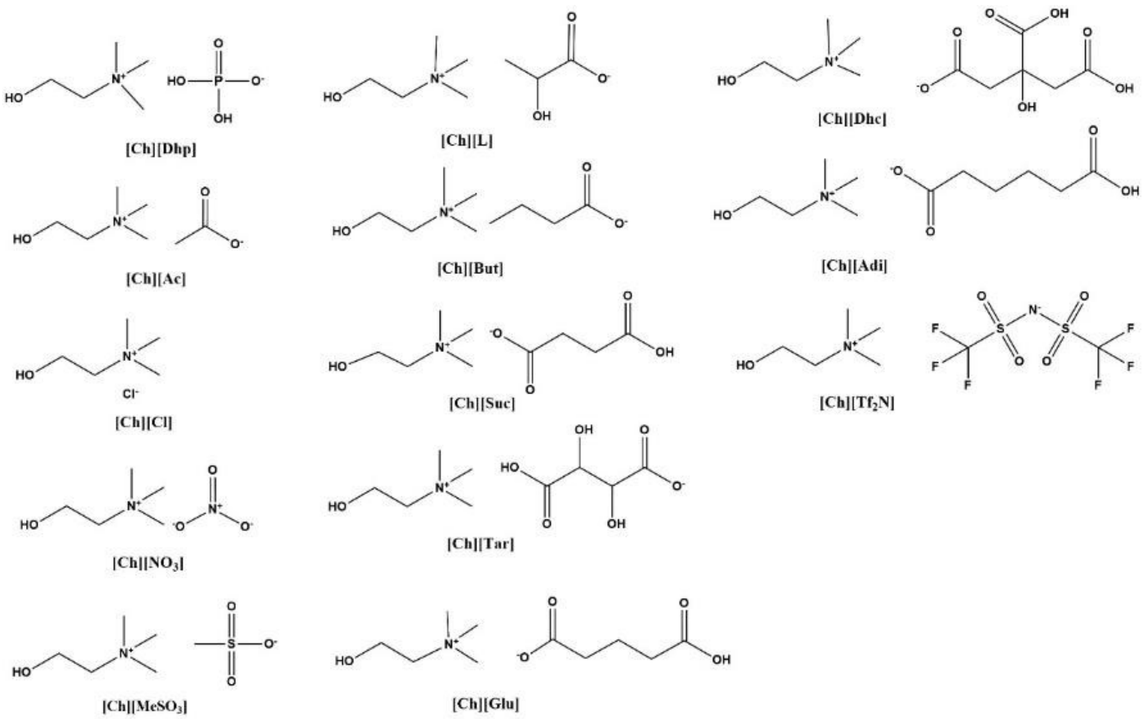


Fig. S1. Chemical structures of cholinium-based ILs.

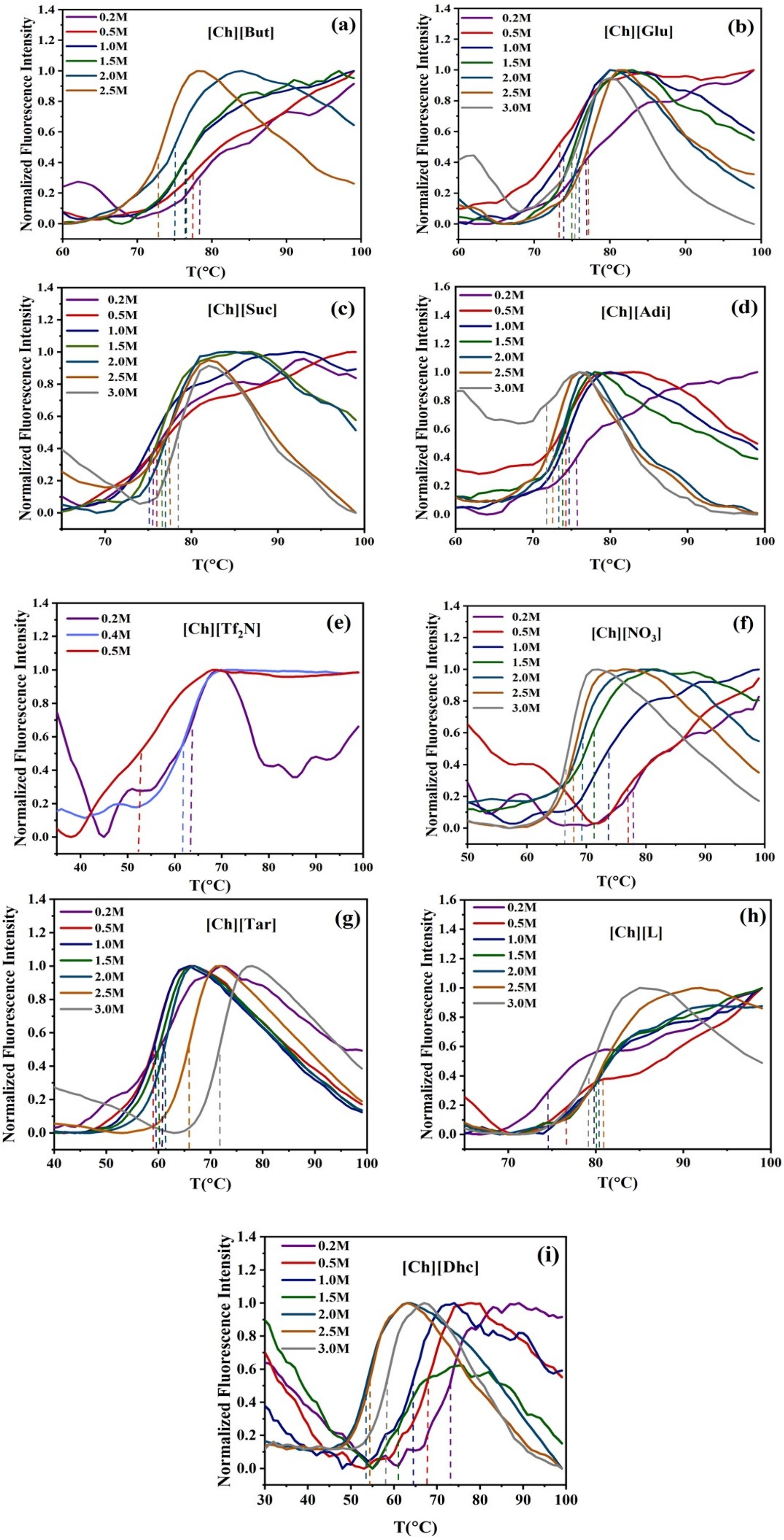

[Ch][But]
(a)
[Ch][Glu]
(b)
[Ch][Suc]
(c)
[Ch][Adi]
(d)
[Ch][Tf2N]
(e)
[Ch][NO3]
(f)
[Ch][Tar]
(g)
[Ch][L]
(h)
[Ch][Dhc]
(i)
0.2M
0.4M
0.5M
1.0M
1.5M
2.0M
2.5M
3.0M
Normalized Fluorescence Intensity
T(°C)

Fig. S2 Normalized fluorescence intensity obtained from qPCR in cholinium-based ILs at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M). (a) [Ch][But], (b) [Ch][Glu], (c) [Ch][Suc], (d) [Ch][Adi], (e) [Ch][Tf_2N], (f) [Ch][NO_3], (g) [Ch][Tar], (h) [Ch][L], and (i) [Ch][Dhc], respectively.

Table S1. The purity of materials in experiments.

Materials	Purity
Proteinase K	>95%
[Ch][DHP]	>97%
[Ch][Dhc]	>97%
[Ch][But]	>97%
[Ch][Glu]	>97%
[Ch][Adi]	>97%
[Ch][Suc]	>97%
[Ch][Tar]	>97%
[Ch][Ac]	>97%
[Ch][$MeSO_3$]	>98%
[Ch][NO_3]	>98%
[Ch][Cl]	>99%
[Ch][L]	>97%
[Ch][Tf_2N]	>99%

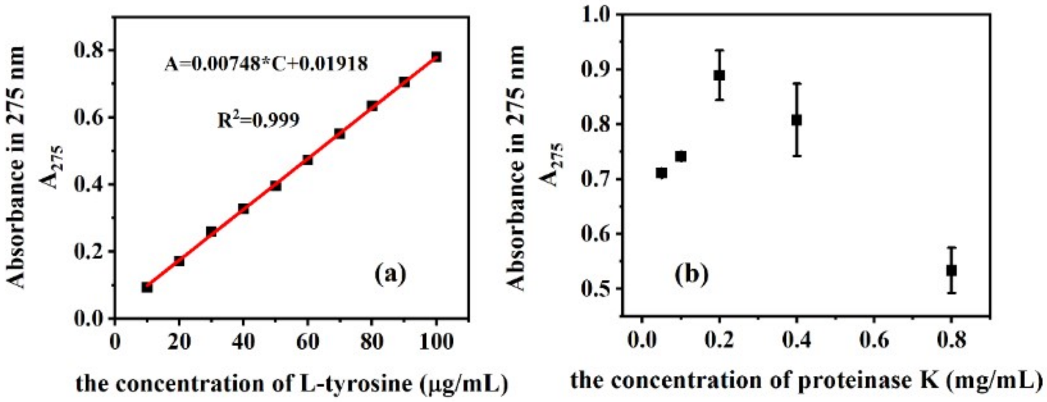


Fig. S3. (a) The tyrosine standard curve. From the curve, K=131.13 $\mu g/\ mL$, it is the concentration of L-tyrosine at which A_{275}=1. (b) The relationship between absorbance in 275 nm and the concentration of PK. We determined the concentration of PK in activity measurements as 0.1 mg/ mL.

Table S2. The Jones-Dole viscosity B coefficients of the anions obtained from ref. [1]

anions	$[Dhp]^-$	$[Ac]^-$	$[MeSO_3]^-$	$[Cl]^-$	$[NO_3]^-$	$[Na]^+$	$[Ca]^{2+}$
B coefficient	+0.34	+0.246	+0.127	-0.005	-0.043	+0.085	+0.284

The measurement of T_m of PK in buffer

During the experiment, we cannot obtain the *Tm* of PK in buffer by using qPCR because of its autolysis[2,3,4] making the fluorescence intensity disorderly and unsystematic, which make it impossible to fit the florescence intensity using Boltzmann equation to obtain the T_m. In order to inhibit the autolysis of PK, phenyl methane-sulfonyl fluoride (PMSF) was added into the sample mixture. We changed the concentration of PMSF to obtain the relationship between the T_m values and the concentration of PMSF. Finally, T_m in the absence of PMSF can be computed using extrapolation method. In addition, DSC was also carried out with a Nano-DSC (TA Instruments) at a heating rate 2.0 ℃/min from 25 ℃ to 90 ℃. The measurement was held at a constant temperature for 2 min between each scan. Data evaluation used the software provided by the manufacturer. Buffer–buffer baselines were subtracted from sample data.

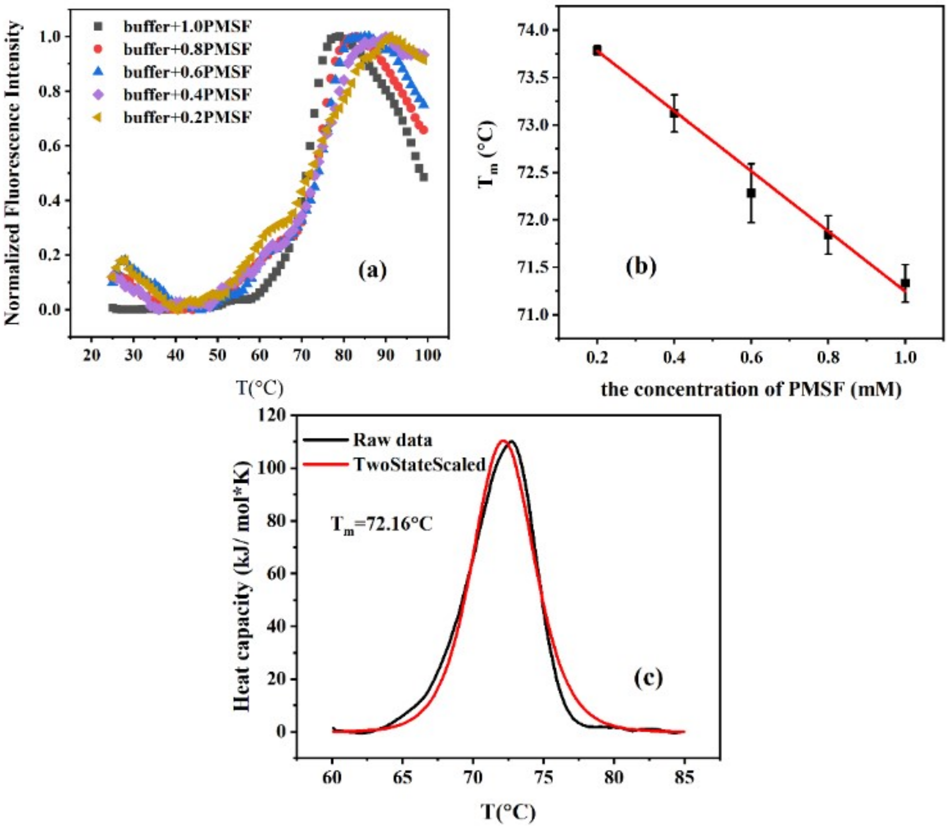


Fig. S4. (a) The normalized fluorescence intensities were obtained by qPCR at different concentration PMSF. (b) The relationship between T_m of PK in the presence of PMSF and the concentration of PMSF. T_m was obtained by fitting fluorescence intensities using Boltzmann equation. T_m of PK in the absence of PMSF can be calculated by extrapolation method. The value of T_m is 74.41℃ in the absence of PMSF. (c) The capacity of PK measured by DSC. The black line and red line are raw data and TwoStateScaled fitting data, respectively. The T_m is 72.16℃ obtained by DSC.

CD spectral analysis for PK in cholinium-based ILs

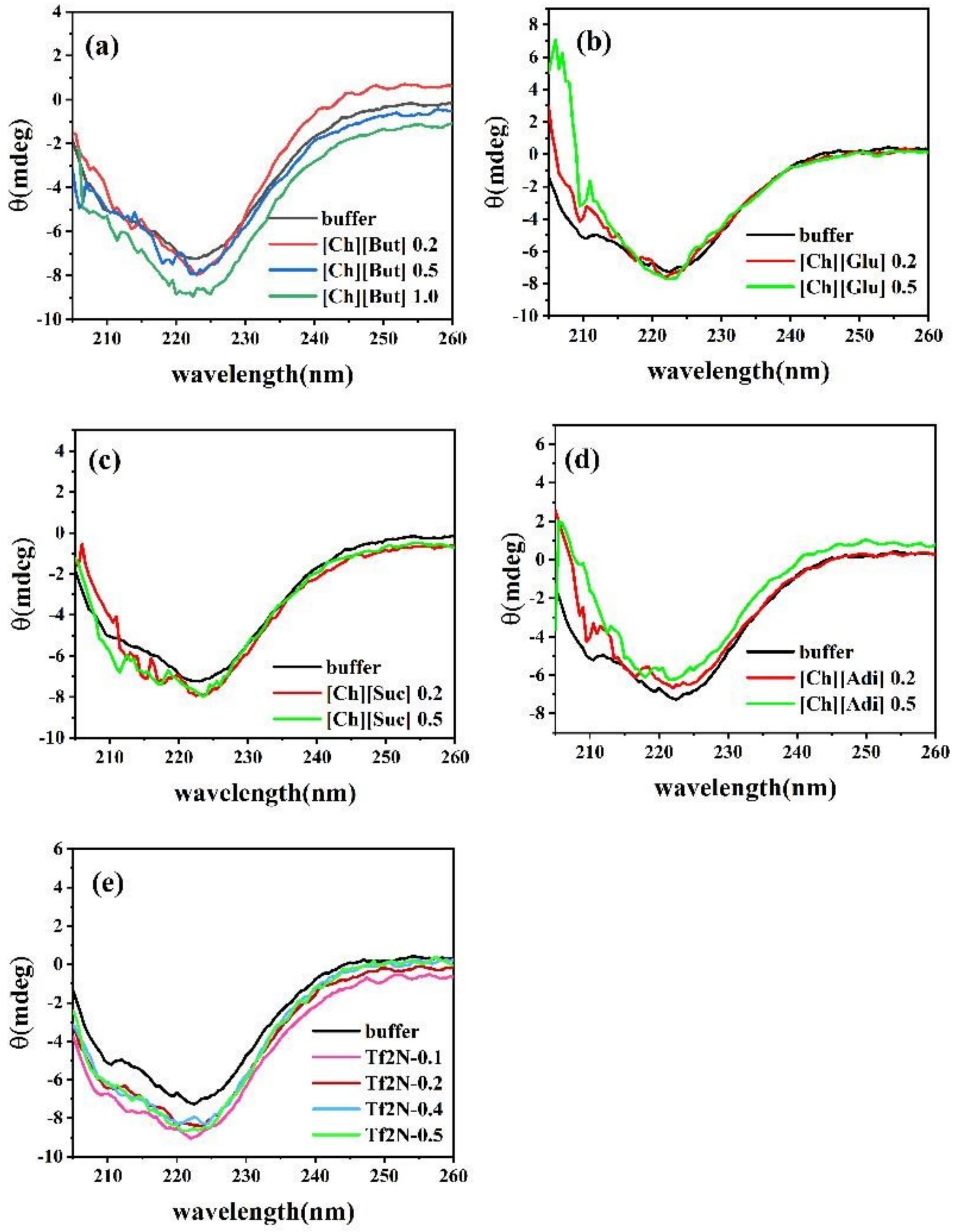


Fig. S5. CD spectra of PK in cholinium-based ILs (a) [Ch][But], (b) [Ch][Glu], (c) [Ch][Suc], (d) [Ch][Adi], and (e) [Ch][Tf_2N] at different concentrations.

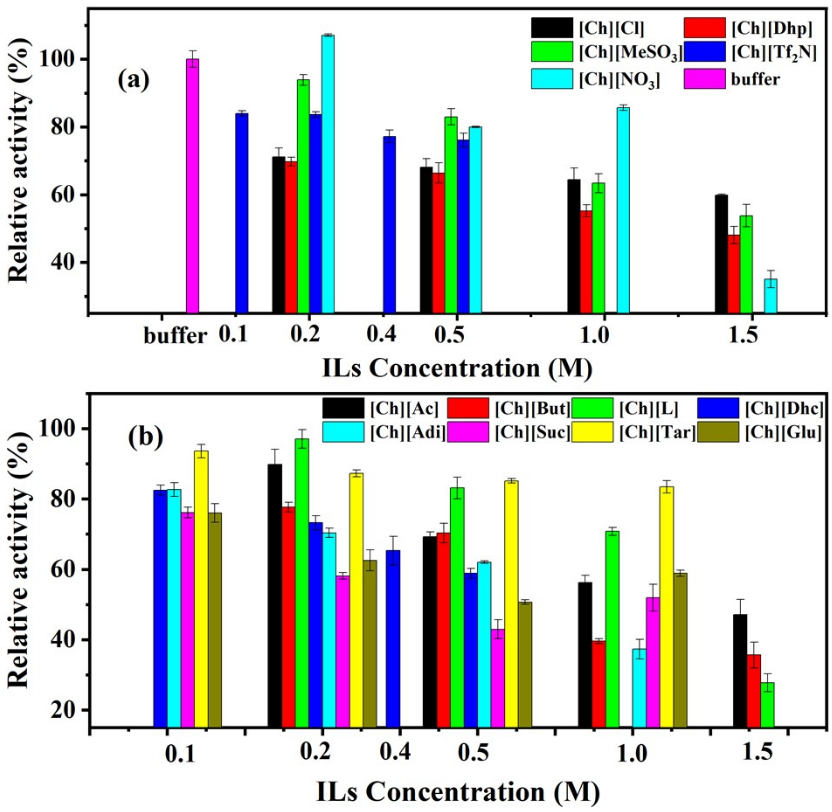
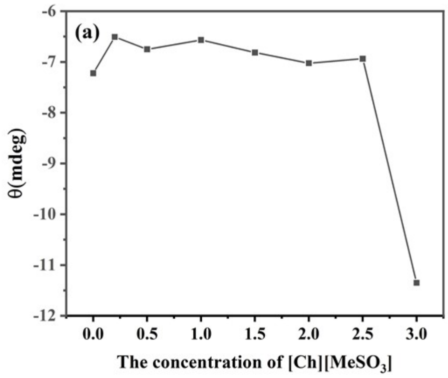


Fig. S6. The relative activity of PK in the absence and presence of ILs at various concentrations considering 100% of enzymatic activity in the buffer of PBS at pH 8.0. (a) The relative of activity of PK in general cholinium-based ILs at different concentrations. (b) The relative activity of PK in ILs whose anions are derived from carboxylic acids and dicarboxylic acids at different concentrations.



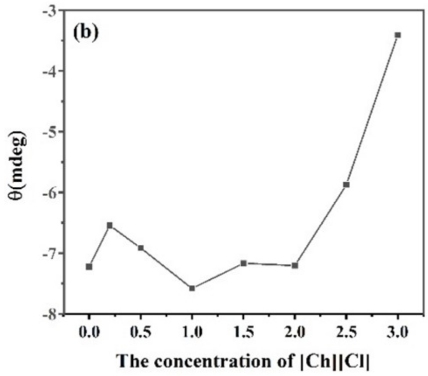


Fig. S7. The CD values at 222nm in (a) [Ch][$MeSO_3$] and (b) [Ch][Cl] at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M).

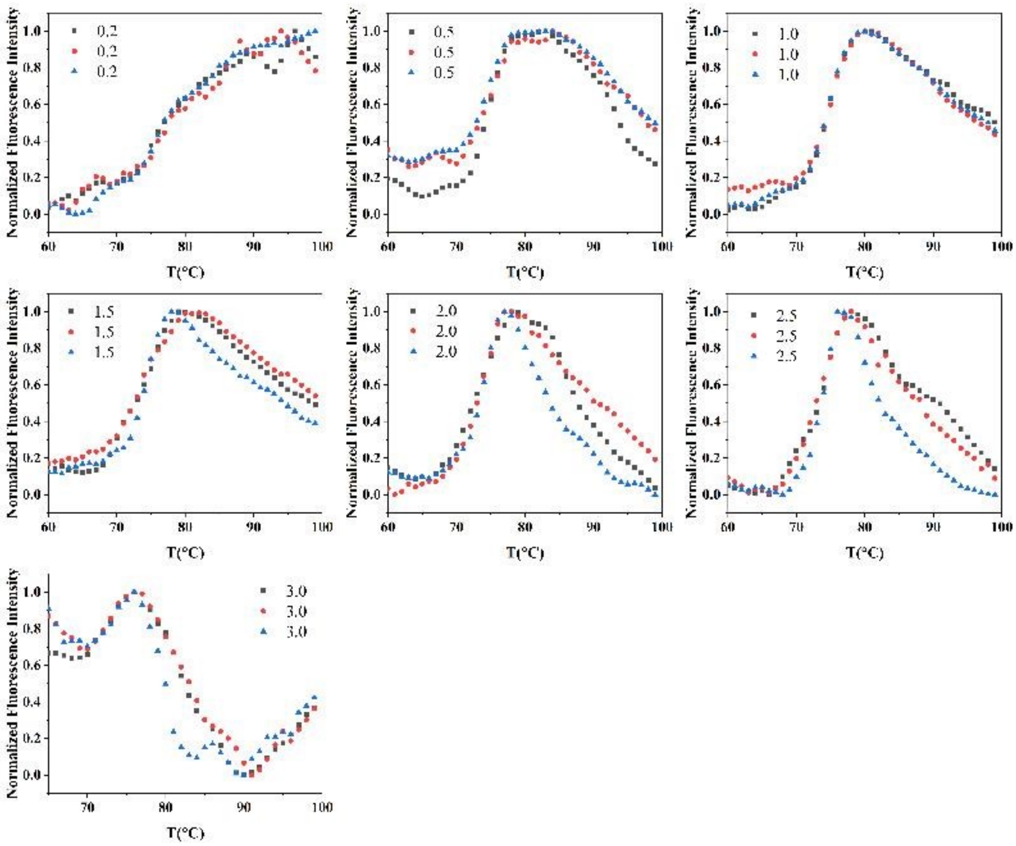


Fig. S8. Normalized fluorescence intensity of three repeated experiments obtained from qPCR in [Ch][Adi] at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M).

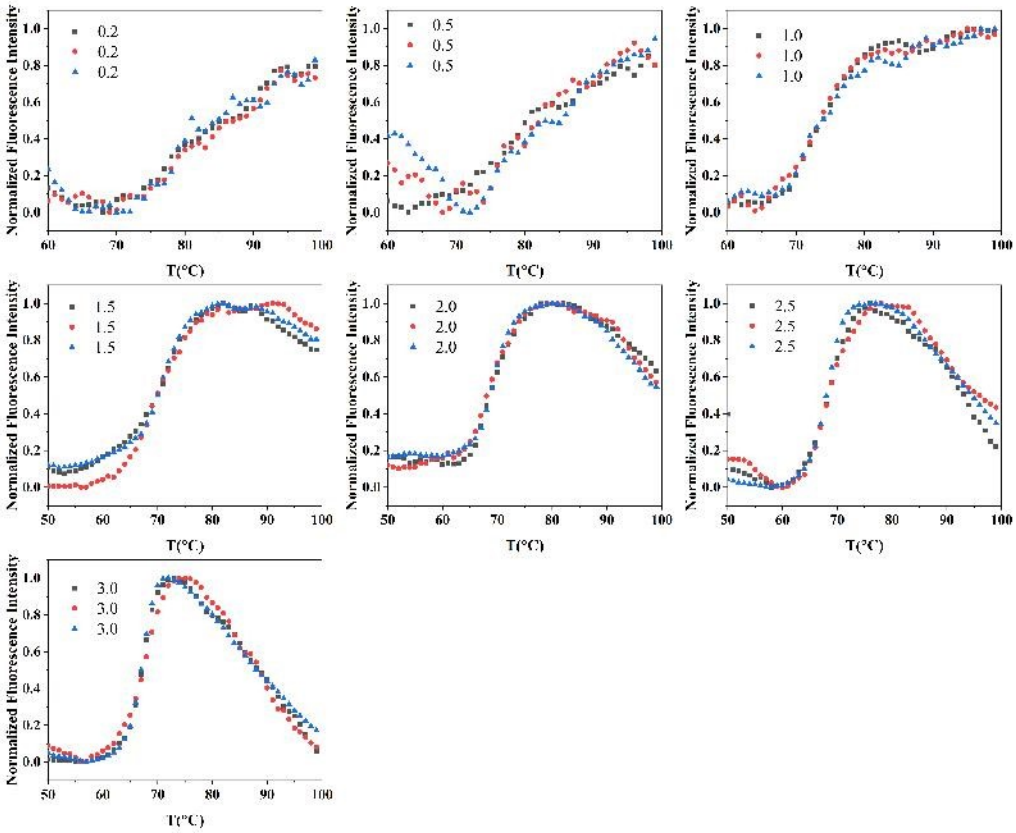


Fig. S9. Normalized fluorescence intensity of three repeated experiments obtained from qPCR in [Ch][NO_3] at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M).

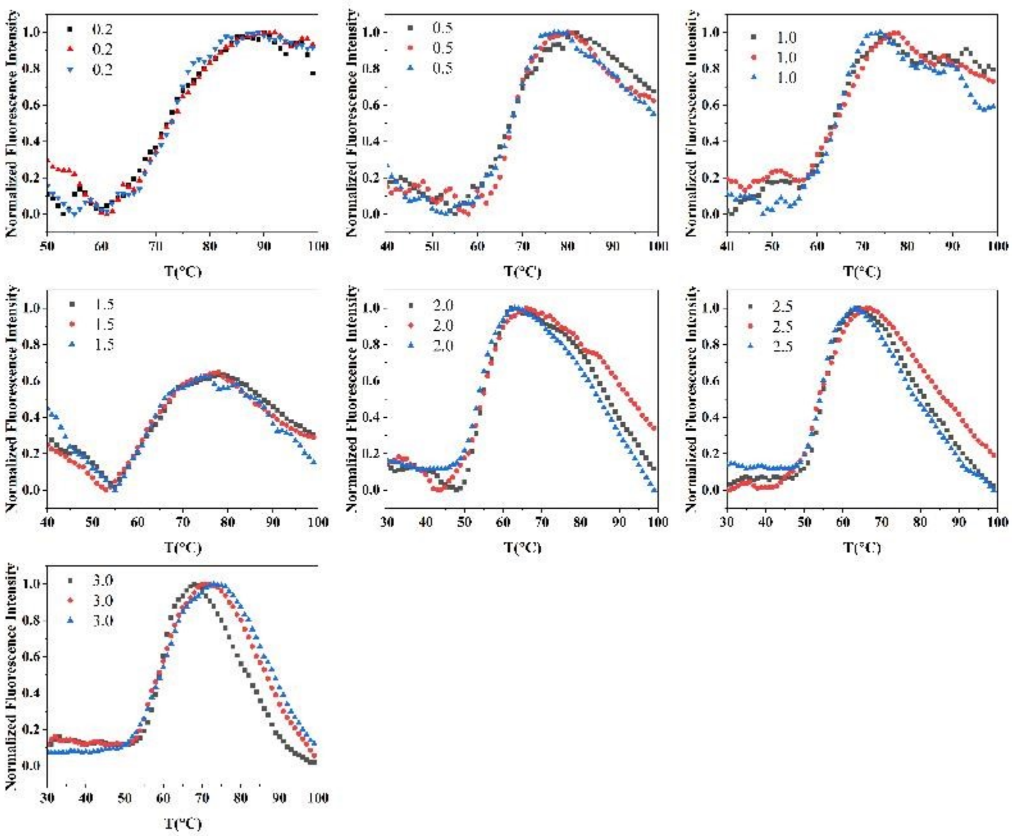


Fig. S10. Normalized fluorescence intensity of three repeated experiments obtained from qPCR in [Ch][Dhc] at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M).

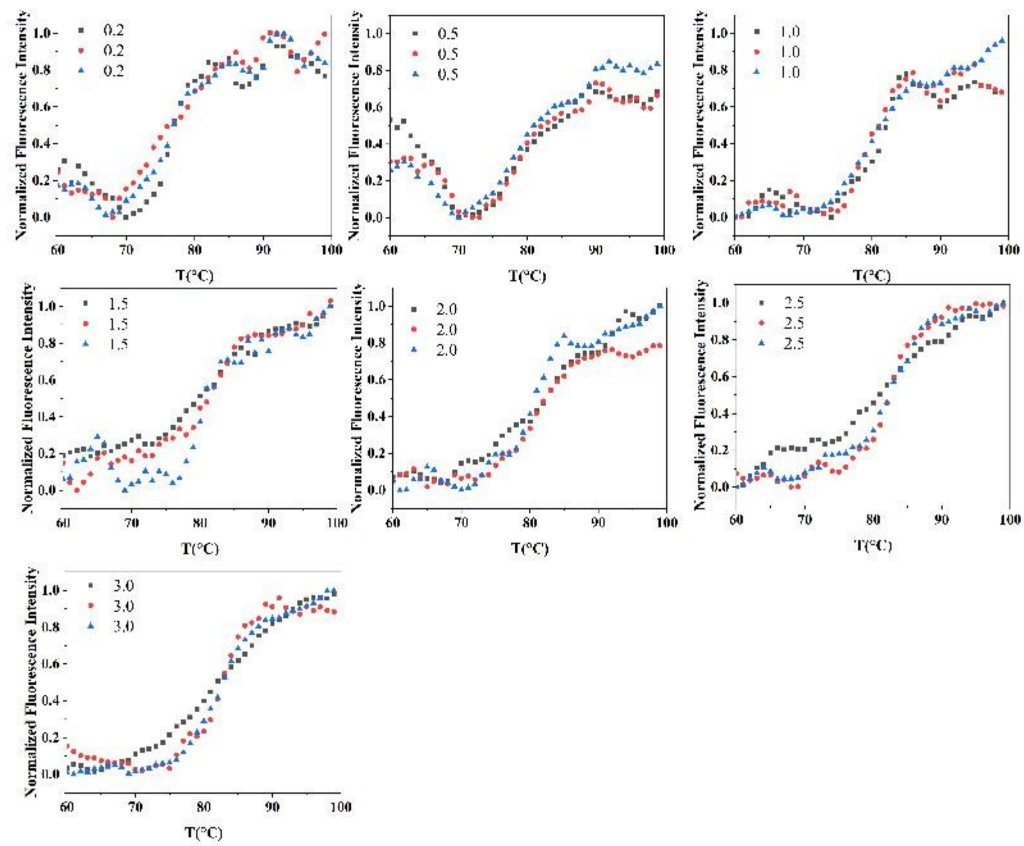


Fig. S11. Normalized fluorescence intensity of three repeated experiments obtained from qPCR in [Ch][$MeSO_3$] at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M).

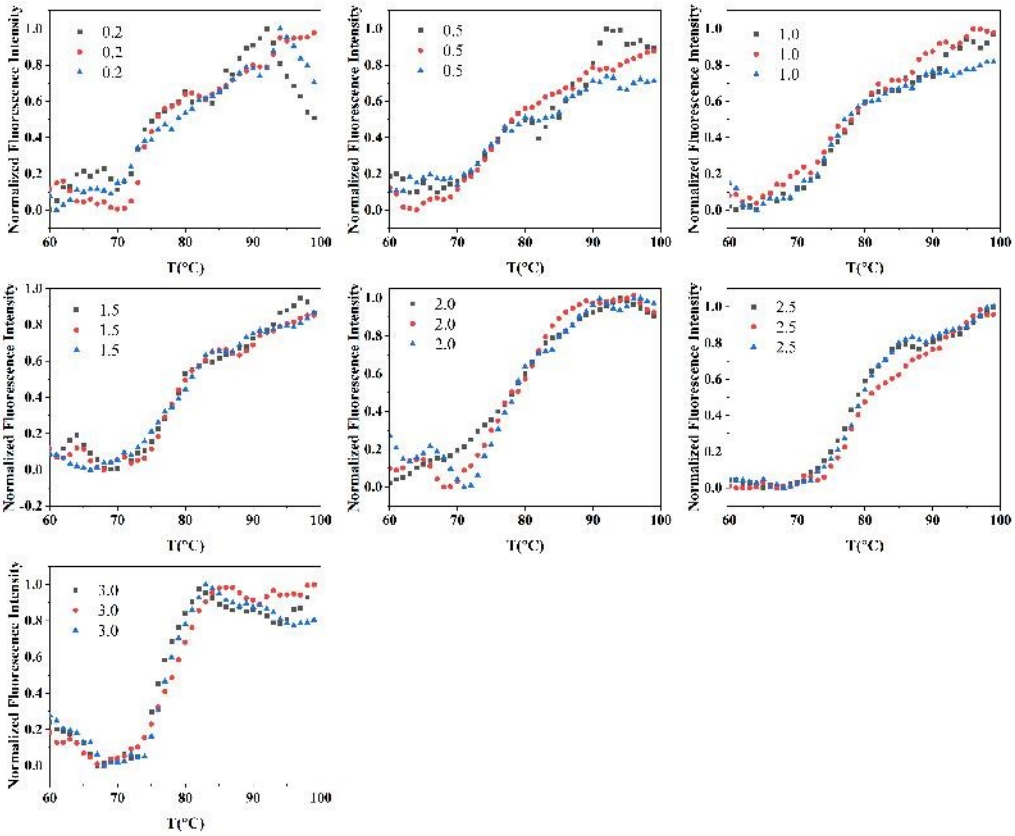


Fig. S12. Normalized fluorescence intensity of three repeated experiments obtained from qPCR in [Ch][Cl] at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 M).

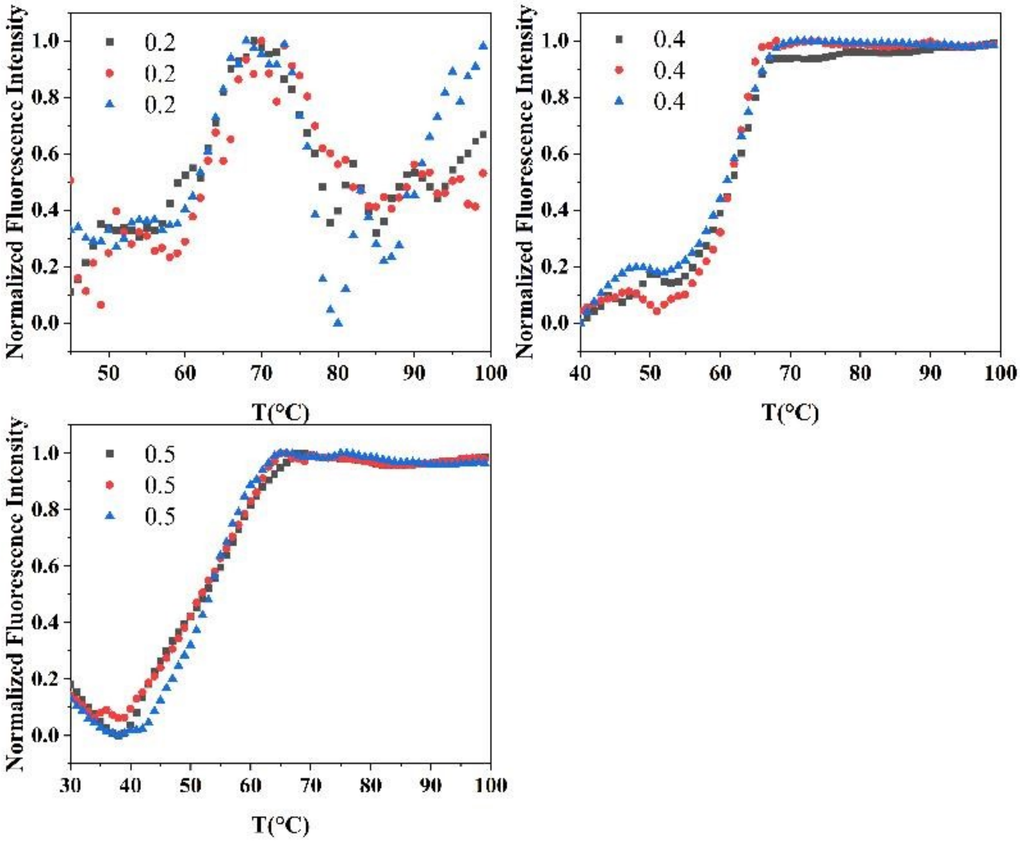


Fig. S13. Normalized fluorescence intensity of three repeated experiments obtained from qPCR in $[Ch][Tf_2N]$ at various concentrations (0.2, 0.4, and 0.5 M).

Table S3. The T_m values obtained by fitting three repeated experiments as mentioned above using Boltzmann equation.

	0.2 M (°C)	0.5 M (°C)	1.0 M (°C)	1.5 M (°C)	2.0 M (°C)	2.5 M (°C)	3.0 M (°C)
[Ch][Adi]	76.92±0.27	74.63±0.08	74.61±0.08	73.49±0.19	73.36±0.15	73.55±0.30	73.25±0.15
	76.66±0.32	74.84±0.20	74.90±0.07	73.69±0.12	73.65±0.20	73.40±0.15	73.40±0.14
	76.47±0.33	74.50±0.05	74.59±0.04	73.98±0.11	73.73±0.18	73.70±0.18	73.44±0.28
[Ch][NO_3]	78.49±0.32	76.43±0.30	73.44±0.13	70.89±0.16	69.41±0.13	68.29±0.09	67.21±0.07
	78.89±0.36	76.76±0.38	73.47±0.07	70.90±0.12	69.05±0.10	68.49±0.10	67.56±0.13
	78.79±0.36	76.78±0.30	73.27±0.24	70.86±0.08	69.41±0.04	68.02±0.04	67.05±0.06
[Ch][Dhc]	72.36±0.30	67.55±0.19	64.83±0.30	61.66±0.32	54.47±0.20	54.89±0.08	59.51±0.05
	72..78±0.37	67.75±0.16	64.37±0.20	61.23±0.31	54.42±0.12	54.56±0.13	59.61±0.17
	72.66±0.24	67.84±0.18	64.58±0.20	61.44±0.25	54.14±0.05	54.36±0.10	59.79±0.08
[Ch][$MeSO_3$]	76.74±0.18	78.20±0.15	80.22±0.34	81.48±0.31	81.02±0.36	83.04±0.27	82.74±0.24
	76.29±0.34	78.54±0.22	79.86±0.27	81.96±0.20	80.95±0.14	83.19±0.15	82.53±0.18
	76.06±0.22	78.14±0.11	80.05±0.20	81.59±0.27	81.03±0.18	83.43±0.16	82.21±0.20
[Ch][Cl]	73.78±0.27	74.68±0.27	75.86±0.23	76.73±0.17	77.76±0.25	77.70±0.09	76.44±0.16
	73.88±0.12	74.89±0.17	75.73±0.37	76.81±0.17	77.85±0.20	77.91±0.16	76.88±0.20
	73.55±0.29	74.70±0.26	75.37±0.37	76.98±0.23	77.47±0.30	77.81±0.10	76.93±0.17
	0.2 M (°C)	0.4 M (°C)	0.5 M (°C)				
[Ch][Tf_2N]	64.35±0.32	62.21±0.16	52.54±0.32				
	64.57±0.42	62.25±0.10	52.35±0.31				
	64.24±0.16	62.13±0.08	52.75±0.14				

Reference

1 H. Donald and B. Jenkins, "Viscosity S-Coefficients of Ions in Solution.pdf," Chem. Rev. 95, 2695-2724 (1995).

2 Jürgen Bajorath, Wolfram Saenger, and Gour Pada Pal, "Autolysis and inhibition of proteinase K, a subtilisin-related serine proteinase isolated from the fungus Tritirachium album Limber," Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology **954**, 176-182 (1988).

3 P. J. Sweeney and J. M. Walker, "Proteinase K (EC 3.4.21.14)," Methods Mol Biol **16**, 305-311 (1993).

4 W. Saenger, "Chapter 714 - Proteinase K", in *Handbook of Proteolytic Enzymes (Third Edition)*, edited by Neil D. Rawlings and Guy Salvesen (Academic Press, 2013), pp. 3240-3242.