Supporting Information

Enzyme Stabilization by Virus-Like Particles

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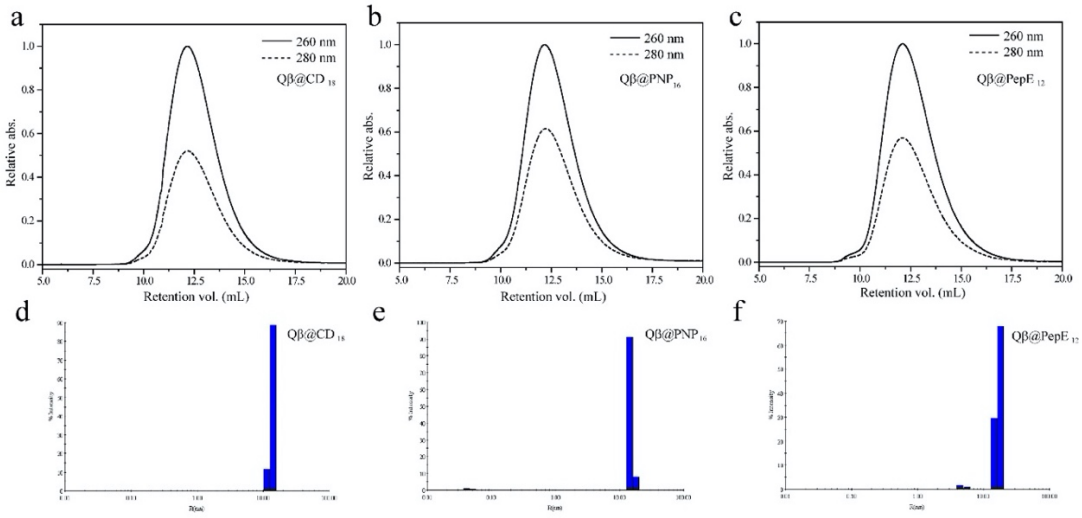

Figure S1. Characterization of enzyme-containing Qβ nanoparticles. (a-c) Size-exclusion chromatography of particles containing CD, PNP, and PepE, respectively. (d-f) Dynamic light scattering of particles containing CD, PNP, and PepE, respectively.

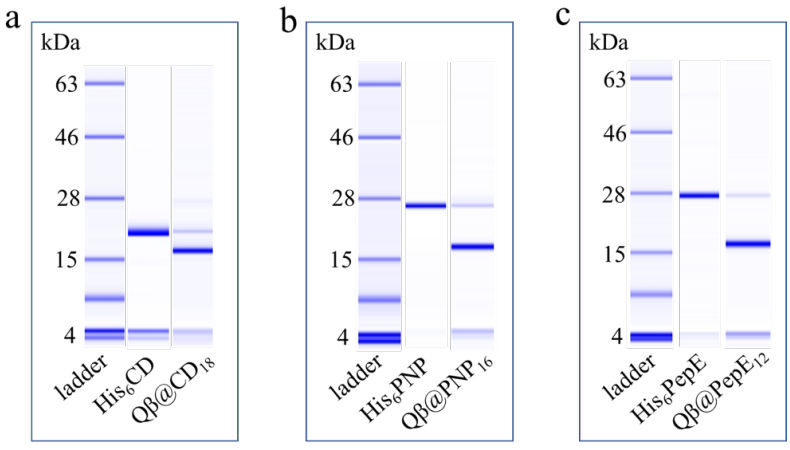

Figure S2. Denaturing microfluidic electrophoresis (Bioanalyzer) analysis of free and packaged (a) cytosine deaminase, (b) purine nucleoside phosphorylase, and (c) peptidase E.

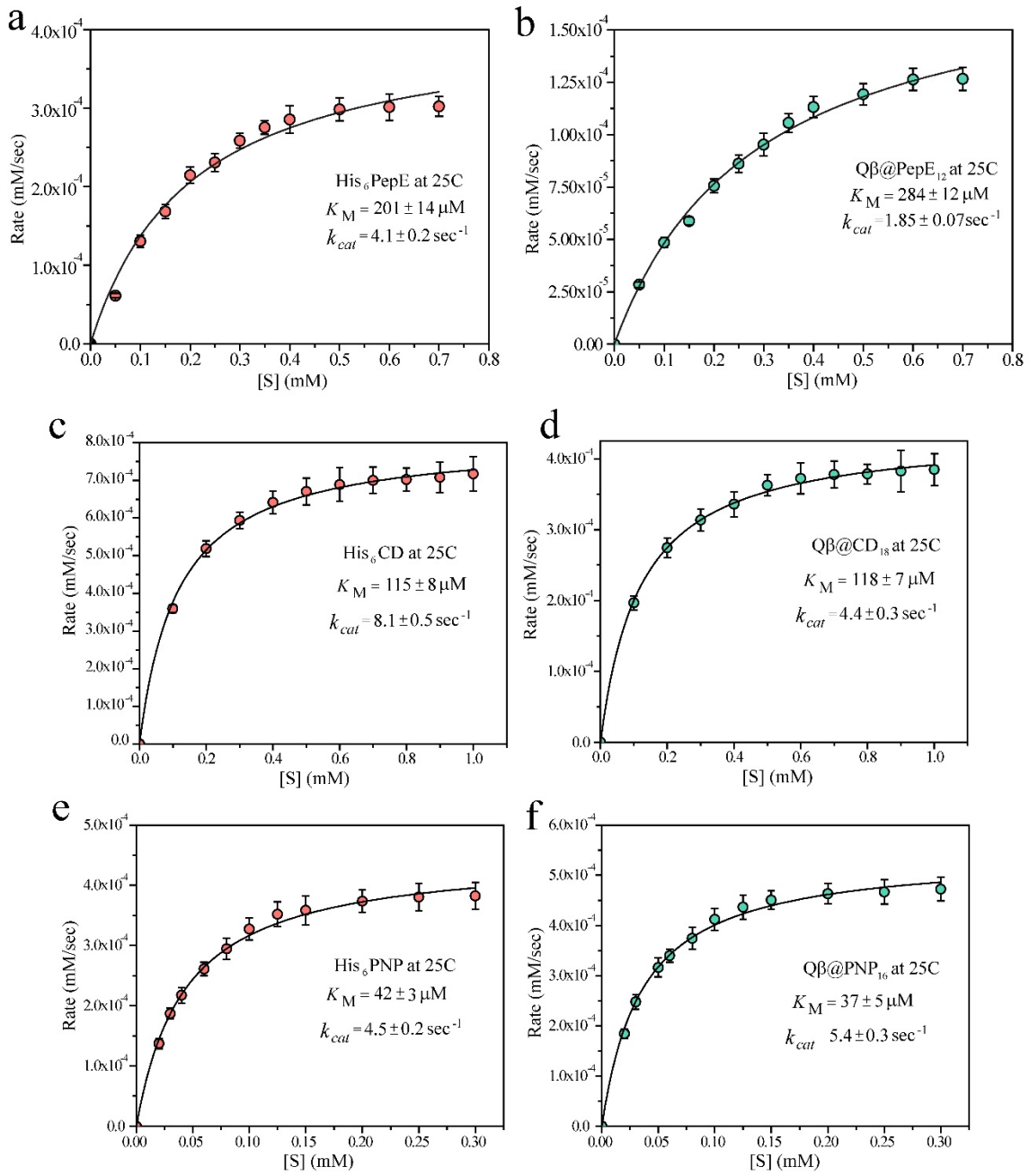

Figure S3. Reactivity of (a) free and (b) packaged PepE enzymes, measured against the substrate Asp-AMC at 25 ºC in 100 mM phosphate buffer (pH 7.4). Reactivity of (c) free and (d) packaged CD enzymes, measured against the deamination reaction of 5-FC to 5-FU at 25 °C in 100 mM phosphate buffer (pH 7.4). Reactivity of (e) free and (f) packaged PNP enzymes measured against the substrate MESG in presence of 1 mM potassium phosphate at 25 ºC in 50 mM Tris buffer (pH 7.4). Values are the mean of initial velocities ± standard deviation vs. substrate concentration [S]. Lines are the best fit to the Michaelis-Menten kinetics equation (R^2>0.99).

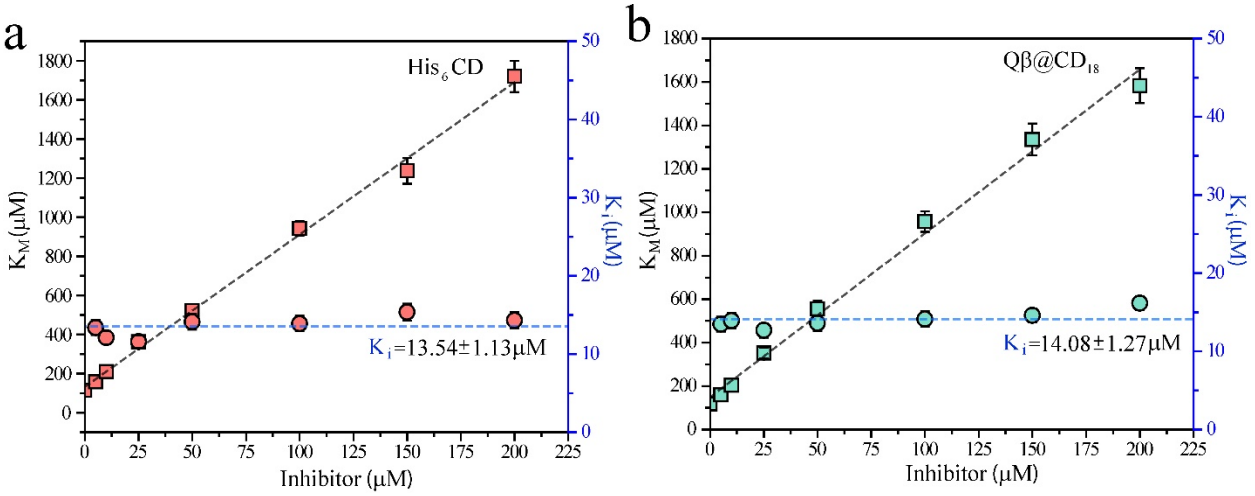

Figure S4. Plot of apparent K_M and inhibition constant K_i of CD enzymes against different inhibitor concentrations: (a) His_6CD and (b) $Q\beta@CD_{18}$. The CD enzyme inhibition assay was performed with 2-hydroxypyrimidine, a well-known competitive inhibitor of CD. The inhibition constant K_i was determined from the Lineweaver-Burk plot and compared with the free enzyme. The free and encapsulated CD exhibits K_i values without statistically significant difference. The 2-hydroxypyrimidine had a K_i of 13.54 ± 1.13 μM against free CD while the K_i was 14.08±1.27 μM against packaged CD.

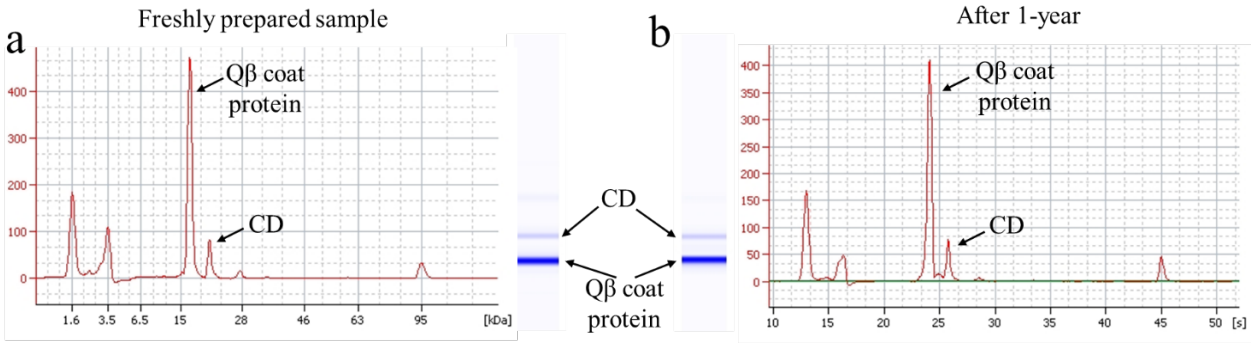

Figure S5. Denaturing electropherograms of (a) freshly prepared $Q\beta@CD_{18}$ and the same sample (b) after one year.

Table S1. Values of k_{cat} of free and Qβ packaged cytosine deaminase and peptidase E vs. temperature.

Temperature [°C]	k_{cat} [sec^{-1}]			
	His_6CD	Qβ@CD_{18}	His_6PepE	Qβ@$PepE_{12}$
20	6.1 ± 0.2	3.6 ± 0.2	-	-
25	8.3 ± 0.4	4.6 ± 0.3	4.06 ± 0.2	1.8 ± 0.1
30	12.0 ± 0.9	11.6 ± 0.5	4.8 ± 0.3	2.5 ± 0.6
35	17.8 ± 1.1	19.2 ± 1.6	7.7 ± 0.7	3.1 ± 0.8
40	25.9 ± 2.1	24.4 ± 1.8	10.0 ± 0.9	3.7 ± 1.2
45	18.3 ± 2.0	29.7 ± 2.5	-	5.8 ± 1.4
50	-	43.1 ± 2.9	-	8.5 ± 1.3
55	-	55.7 ± 3	-	-
60	-	47.1 ± 2.8	-	-

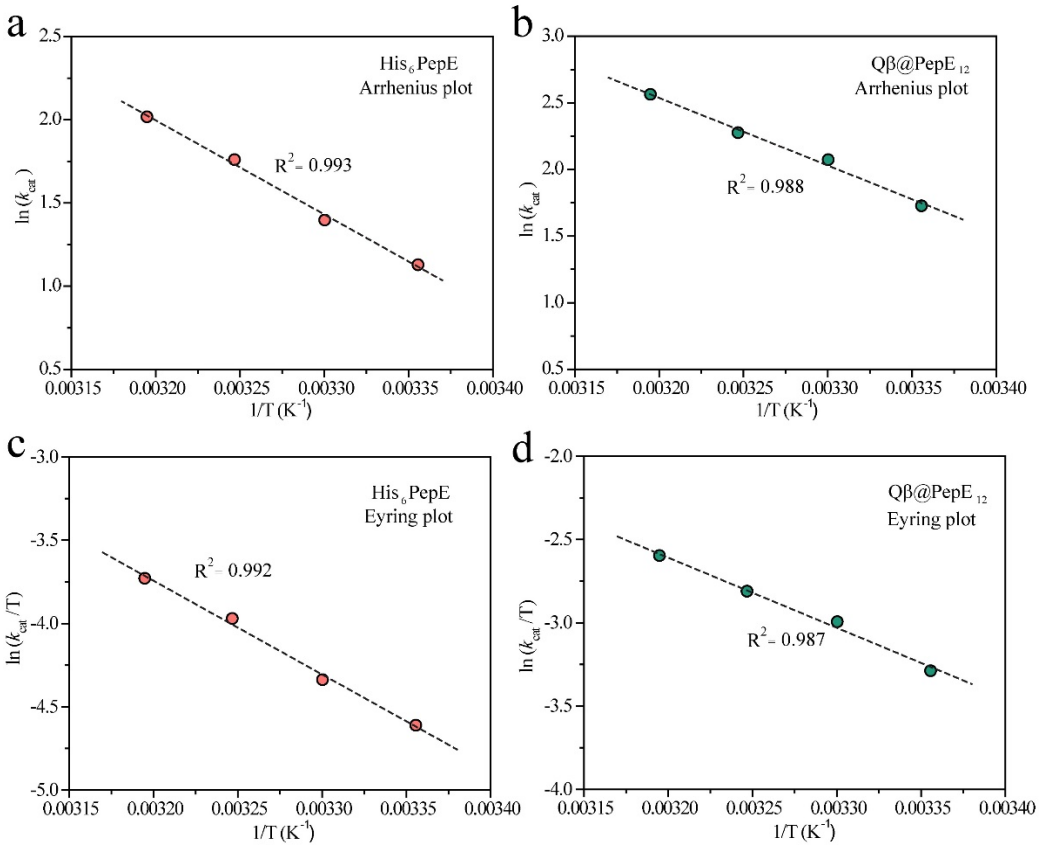

Figure S6. Kinetic analyses. Arrhenius plots: (a) free His_6PepE, (b) packaged PepE. Eyring plots: (c) free His_6PepE, (d) packaged PepE.

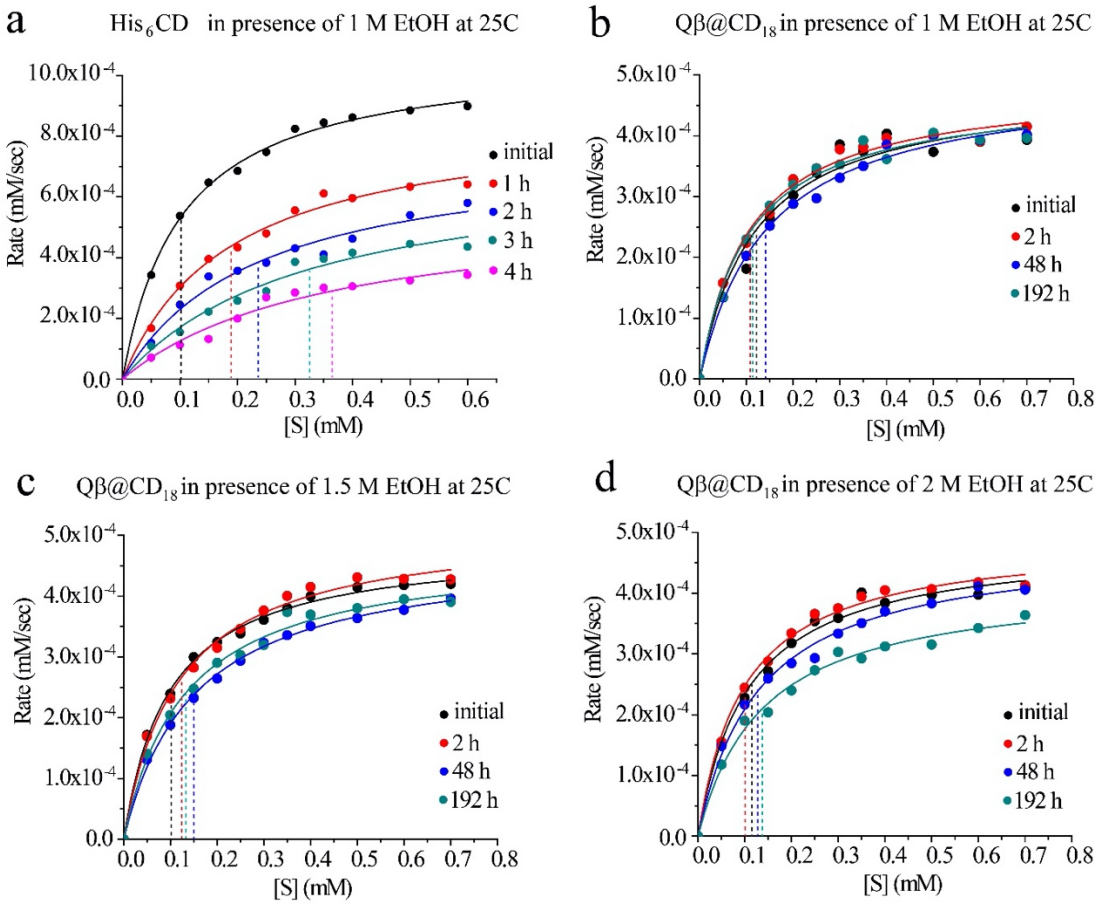

Figure S7. Michaelis–Menten analyses of cytosine deaminase at 25 °C. (a) free enzyme, (b-d) packaged enzyme in the presence of different concentrations of EtOH. Dotted lines indicate the corresponding K_M value.

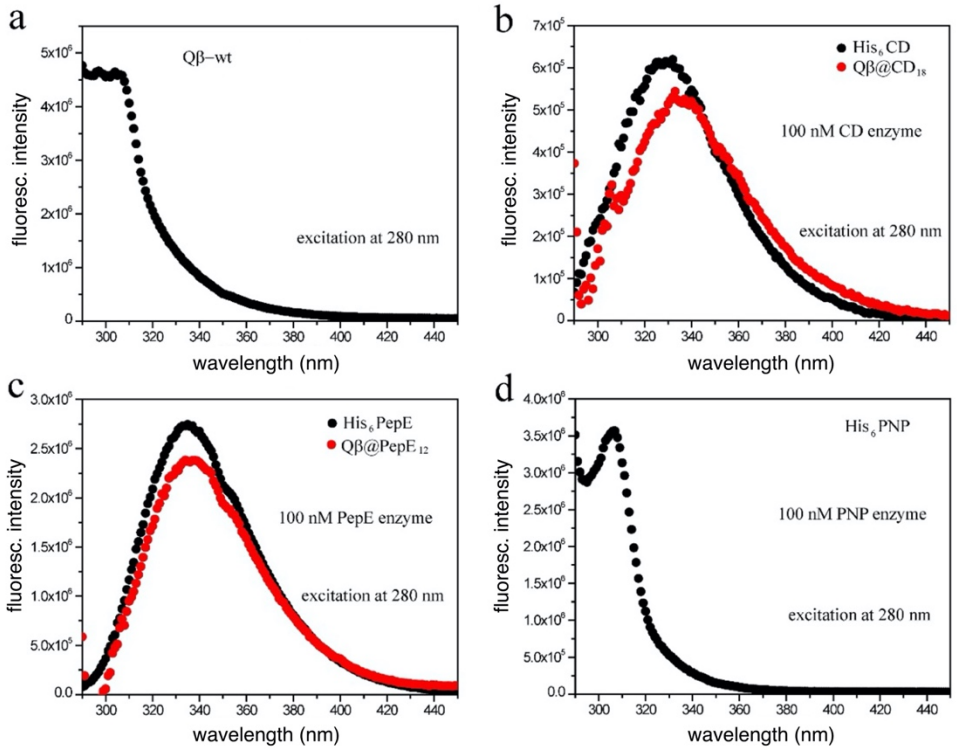

Figure S8. Fluorescence emission spectra of the proteins (excitation 280 nm). (a) Qβ-wt at the same particle concentration (0.014 mg/mL) as used for Qβ@enzyme samples to provide a 100 nM concentration of enzyme in 0.1 M phosphate buffer, pH 7.4. (b) His_6-tagged and Qβ-packaged CD enzyme after background subtraction. (c) 100 nM His_6-tagged and Qβ-packaged PepE enzyme after background subtraction. (d)100 nM His_6-tagged PNP enzyme after background subtraction.

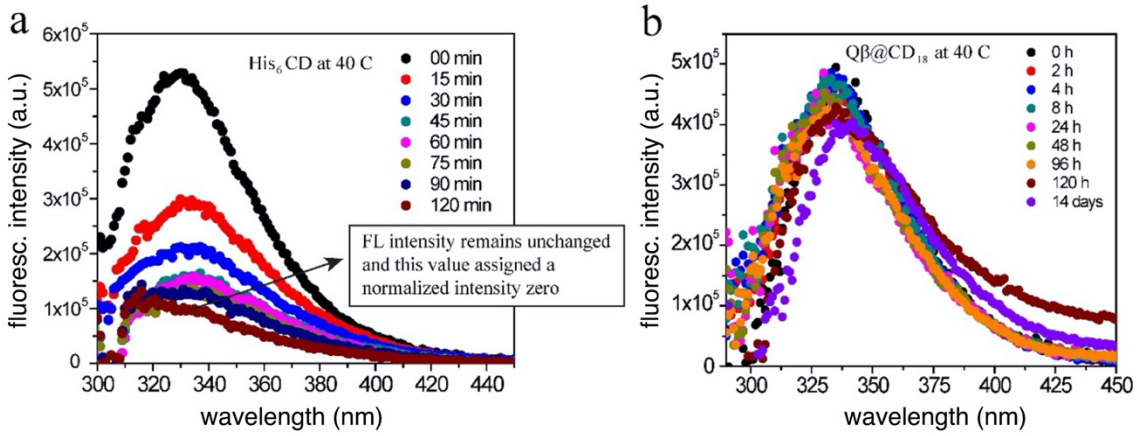

Figure S9. Monitoring CD at 40 °C by intrinsic fluorescence (λ_{ex} = 280 nm, λ_{em} = 335 nm). Background-corrected fluorescence emission spectra from (a) free enzyme and (b) packaged enzyme at different time points.

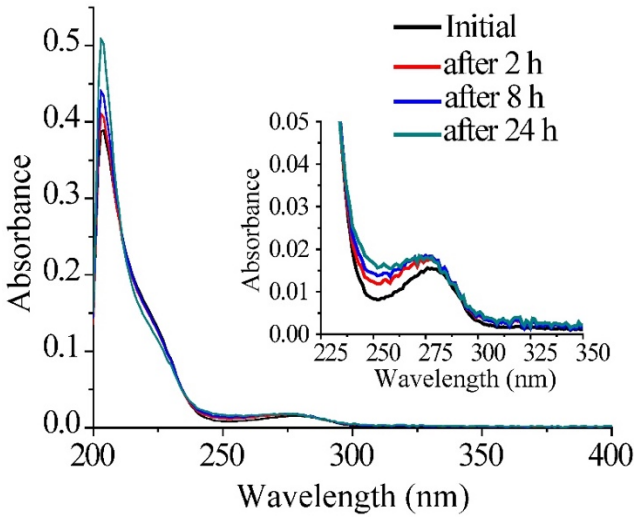

Figure S10. Absorption spectra of free His_6CD at 40 °C incubation. The nearly unchanged nature of absorption at 280 nm shows that no precipitation occurred during the experiment.

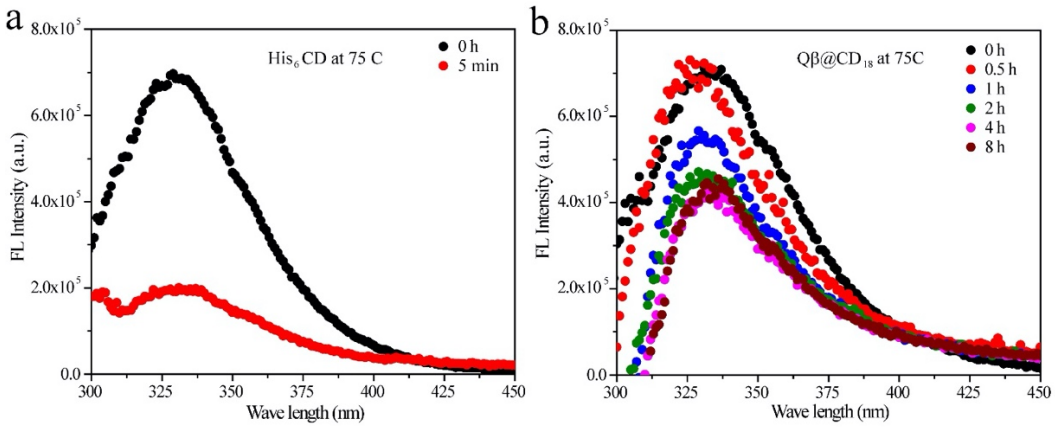

Figure S11. Background-corrected intrinsic fluorescence spectra (λ_{ex} = 280 nm) from (a) free CD enzyme and (b) packaged CD incubated at 75 °C for the indicated lengths of time. At this temperature, both forms of the enzyme were completely inactivated before the first spectrum was acquired.

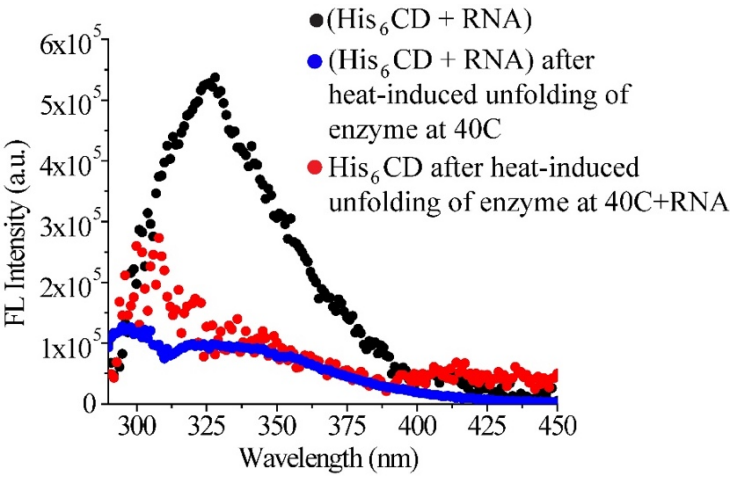

Figure S12. Intrinsic fluorescence (λ_{ex} = 280 nm, λ_{em} = 335 nm) of free His_6CD unfolding at 40 °C in presence of high concentration of RNA. enzyme and fluorescence spectra recorded (red).

To His_6CD in 100 mM phosphate buffer (pH 7.4) was added freshly extracted RNA from Qβ-wt to final concentrations of 100 nM enzyme and 1.4 mg/mL RNA. After acquiring the initial fluorescence spectrum (Figure S12, black), the sample was incubated at 40 ºC for 2 h, cooled to room temperature, and fluorescence spectra recorded again (blue). Separately, 200 nM fresh His_6CD without added RNA was unfolded by incubation at 40 ºC for 2 h and then mixed with freshly extracted RNA to final concentrations of 1.4 mg/mL RNA and 100 nM (Figure S12, red).

Note that, while this experiment employs a high RNA concentration (approximately 4 mM in nucleotide), it is impossible to replicate RNA concentrations inside standard Qβ VLPs if one defines the interior of the particle where both packaged enzyme and RNA are contained (approximately 0.7 M RNA nucleotide).

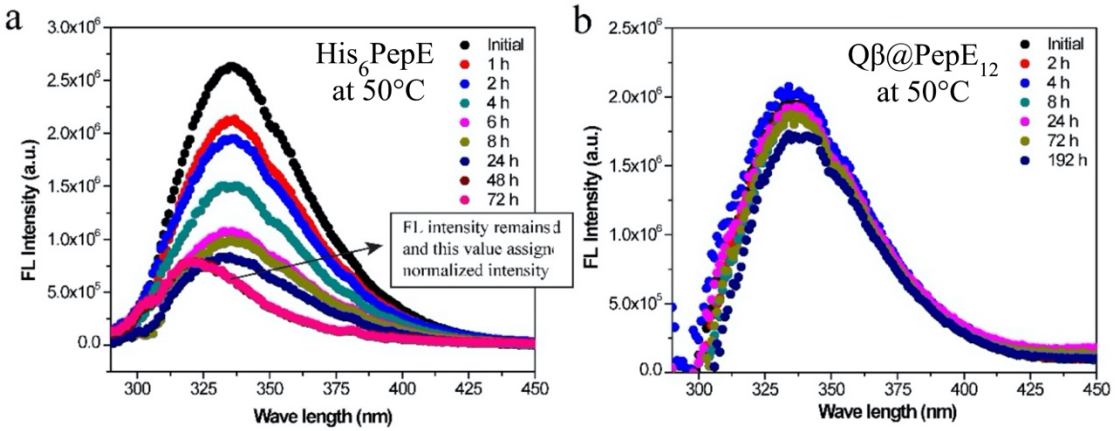

Figure S13. Stability of PepE at 50 °C. (a,b) Background-corrected intrinsic fluorescence emission (λ_{ex} = 280 nm) of (a) free and (b) packaged enzymes at different time points. The corresponding plots of relative activity and decay in fluorescence emission intensity are shown in Figure 7c,d.

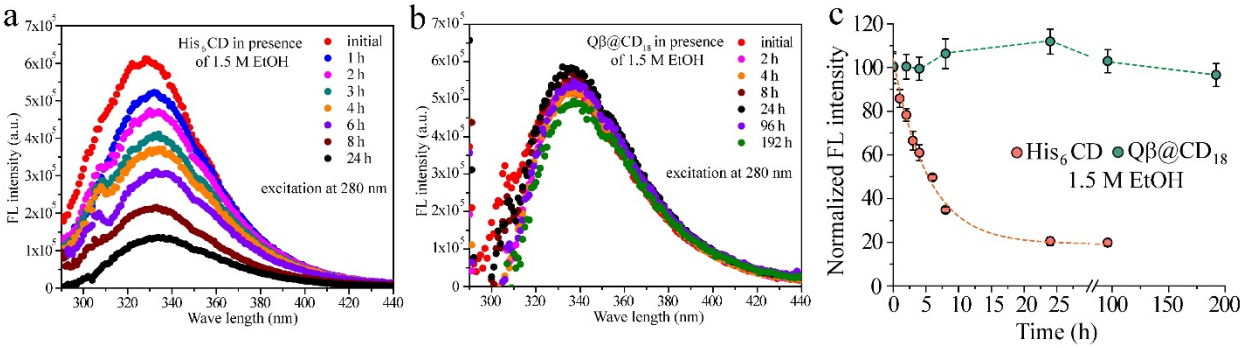

Figure S14. Monitoring enzyme unfolding in presence of 1.5 M ethanol by intrinsic fluorescence (λ_{ex} = 280 nm, λ_{em} = 335 nm). (a,b) Background corrected fluorescence emission spectra from free (panel a) and packaged (panel b) cytosine deaminase at different time points. (c) Decay in fluorescence intensity of free and packaged CD over time.

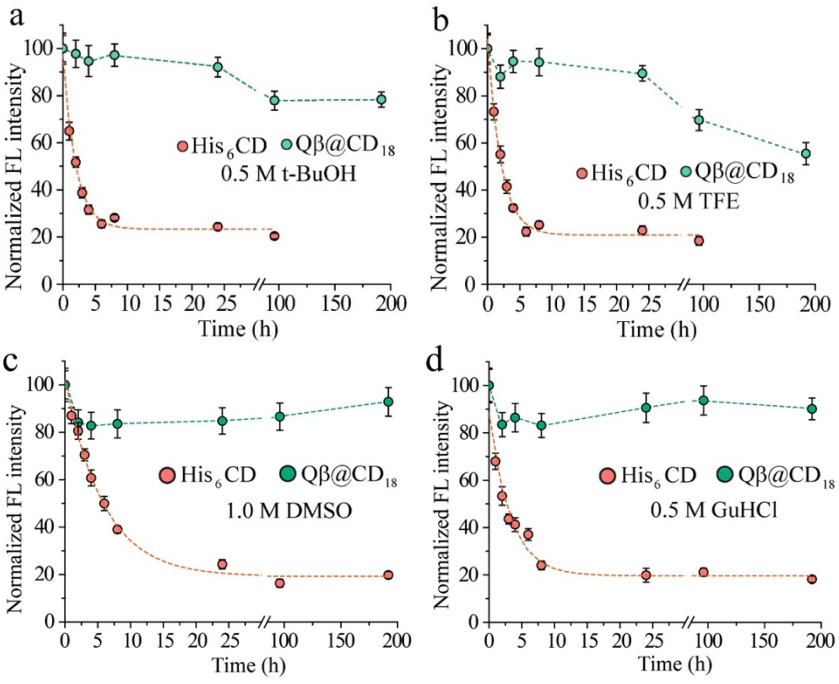

Figure S15. Monitoring enzyme unfolding by intrinsic fluorescence (l_{ex} = 280 nm, l_{em} = 335 nm). Decay in fluorescence emission intensity over time at 25°C of free and packaged CD in the presence of (a) 0.5 M *t*-BuOH, (b) 0.5 M 2,2,2-trifluoroethanol (TFE), (c) 1.0 M DMSO, (d) 0.5 M GuHCl.

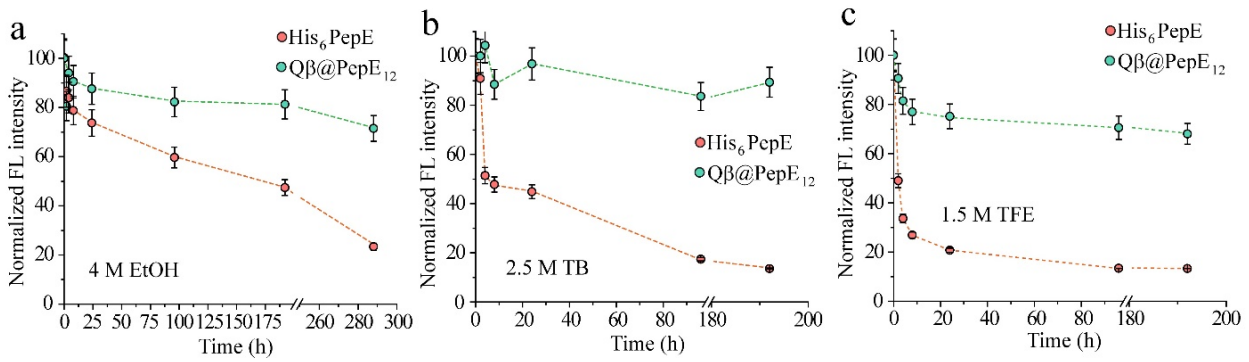

Figure S16. Monitoring enzyme unfolding by intrinsic fluorescence (λ_{ex} = 280 nm, λ_{em} = 335 nm). Decay in fluorescence emission intensity over time at 25°C of free and packaged PepE in the presence of (a) 4 M EtOH, (b) 2.5 M *t*-BuOH, and (c) 1.5 M TFE.

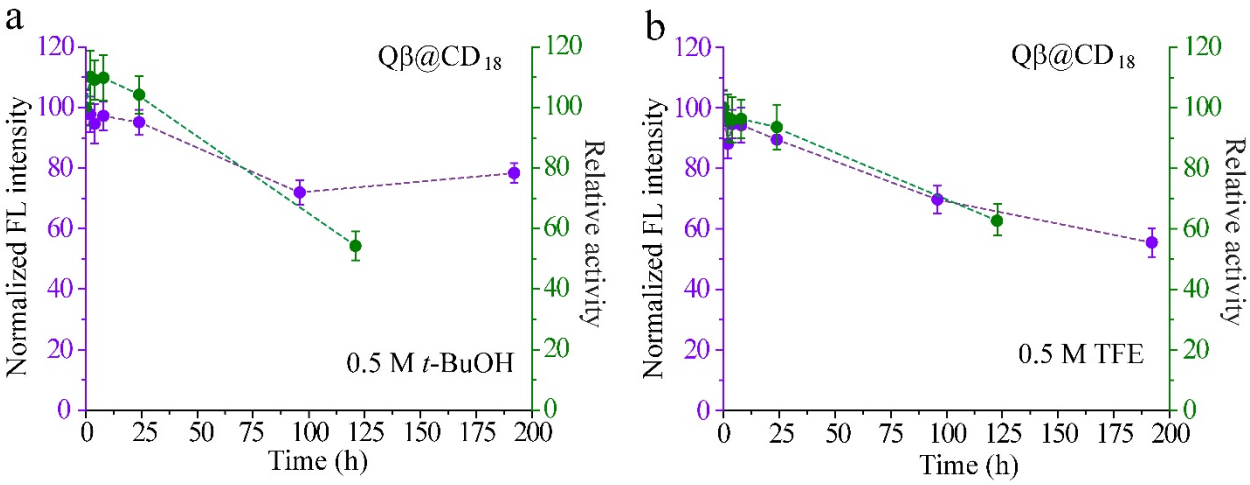

Figure S17. Monitoring enzyme unfolding by intrinsic fluorescence (ex. 280 nm, em. 335 nm) vs. relative enzymatic activity. (a) packaged CD in presence of 0.5 M *t*-BuOH, (b) packaged CD in presence of 0.5 M TFE. Free his-tagged CD loses enzyme activity within 30 minutes in 0.5M *t*-BuOH; its rapid fluorescence decay profile (within 5 hours) in that mixture is shown in Figure S15a.

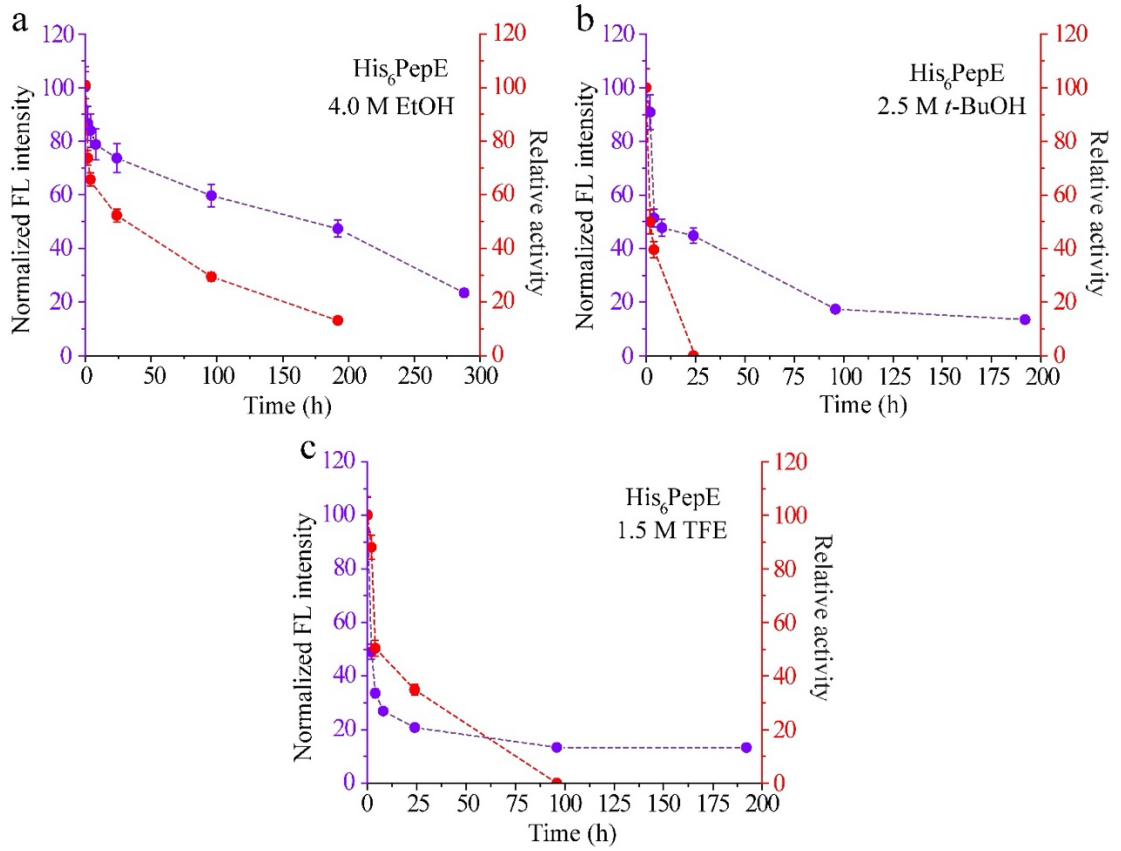

Figure S18. Extended monitoring of intrinsic fluorescence (ex. 280 nm, em. 335 nm) and relative enzymatic activity for free His_6-tagged peptidase E. (a) in the presence of 4 M EtOH, (b) in the presence of 2.5 M *t*-BuOH, (c) in the presence of 1.5 M TFE.

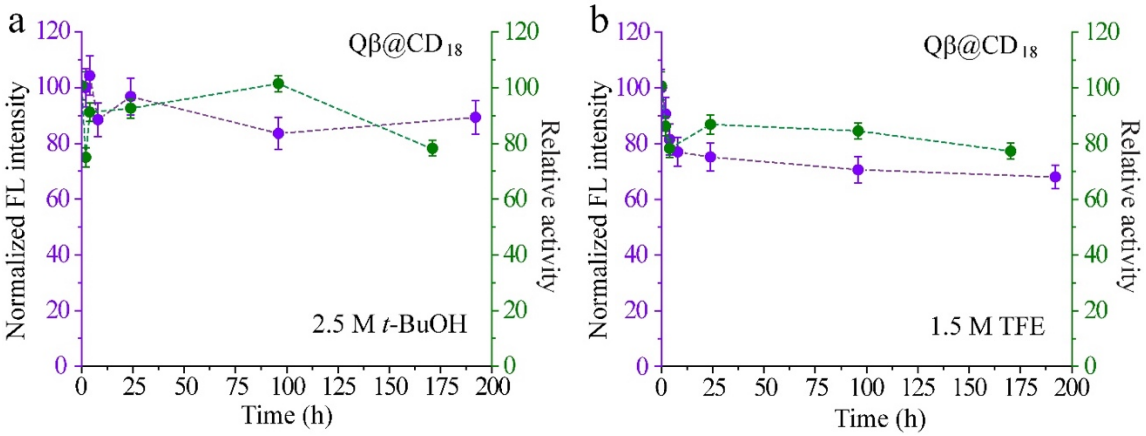

Figure S19. Monitoring enzyme unfolding by intrinsic fluorescence (ex. 280 nm, em. 335 nm). Relative activity and decrease in fluorescence intensity for (b) packaged PepE in presence of 2.5 M *t*-BuOH and (b) packaged PepE in presence of 1.5 M TFE.