SUPPORTING INFORMATION

Dynamic Encapsulationand Activation of Carbonic Anhydrase inmultivalent Dynameric Host Matrixes

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1. General methods

Reagents were obtained from Manchester organics (trialdehyde**1**) and Sigma Aldrich (compounds **2-5**) and used as received. ^{1}H NMR spectra were recorded on an ARX 300 MHz Bruker. Chemical shifts are reported as δ values (ppm) with $CDCl_3$ (^{1}H NMR δ 7.26) as an internal standard. Fluorescence spectra were recorded in Perkin Elmer LS-55, using a quartz cuvette (2 ml), with excitation and emission slit width at 8. UV-vis spectra were obtained from Shimadzu UV-2401PC, using a quartz cuvette (1 ml).Circular dichroism (CD) spectra were acquired by BioLogic MOS 450 CD spectrometer (France). The scan speed was set at 30 nm·min^{-1}. The measurements of solution were taken using a 1.0 mm path length quartz cuvette.

2.Typical synthesis of the dynamic dynamers

Benzene-1,3,5-tricarbaldehyde (**1**, 0.1 mmol) or isopthalaldehyde (**5**, 0.1 mmol) was added into a flask with 0.1 mmol poly(ethylene glycol) bis(3-aminopropyl) terminated (**2**,Mn~1500) and 5 mL solvent MeOH, together with 0.1 mmol corresponding amide functionalized amine*N*-(2-aminoethyl)acetamide (**3**) or dendrimer PAMAM (**4**, ethylenediamine core, generation 1.0). The reaction mixture was stirred at 60°C for 3-4 days, and monitored by ^{1}H NMR until the equilibrium was reached. The solvent was thereafter removed and 10 mL mini-Q H_2O was subsequently added to prepare the stock solution for further analysis.

3. ^{1}H **NMR spectra of the dynamers 6-8:** ^{1}H NMR experiments were performed on an ARX 300 MHz Bruker spectrometer in $CDCl_3$ with the use of the residual solvent peak as reference.

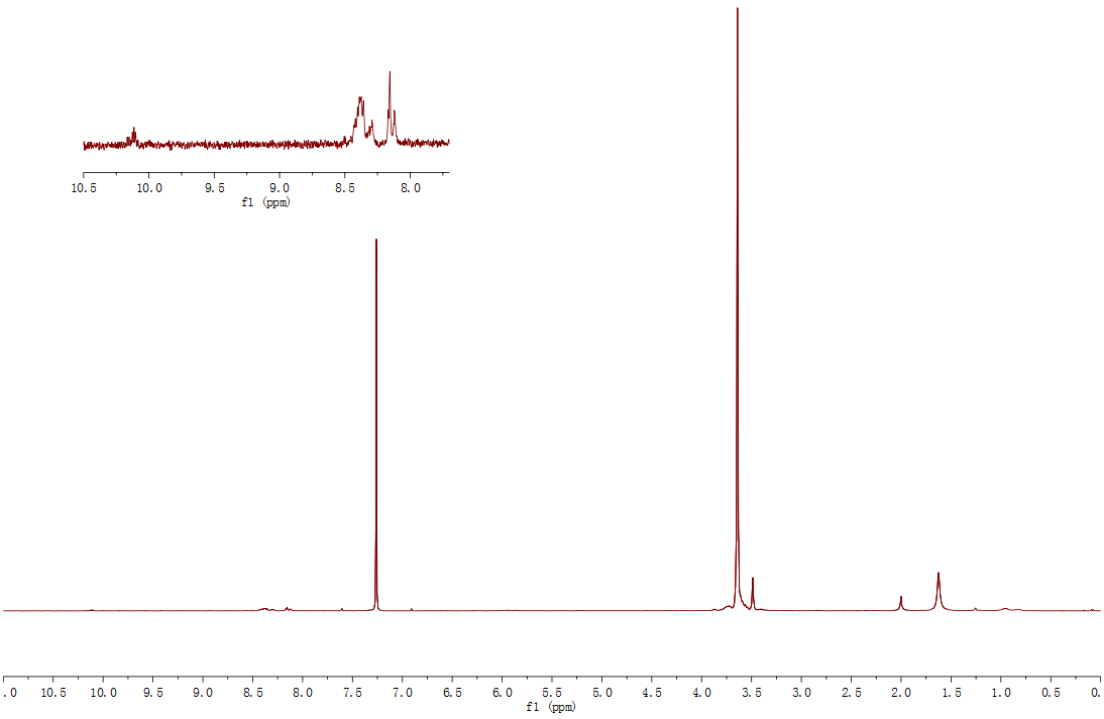


Figure S1. ^{1}H NMR spectrum of dynamer **6**

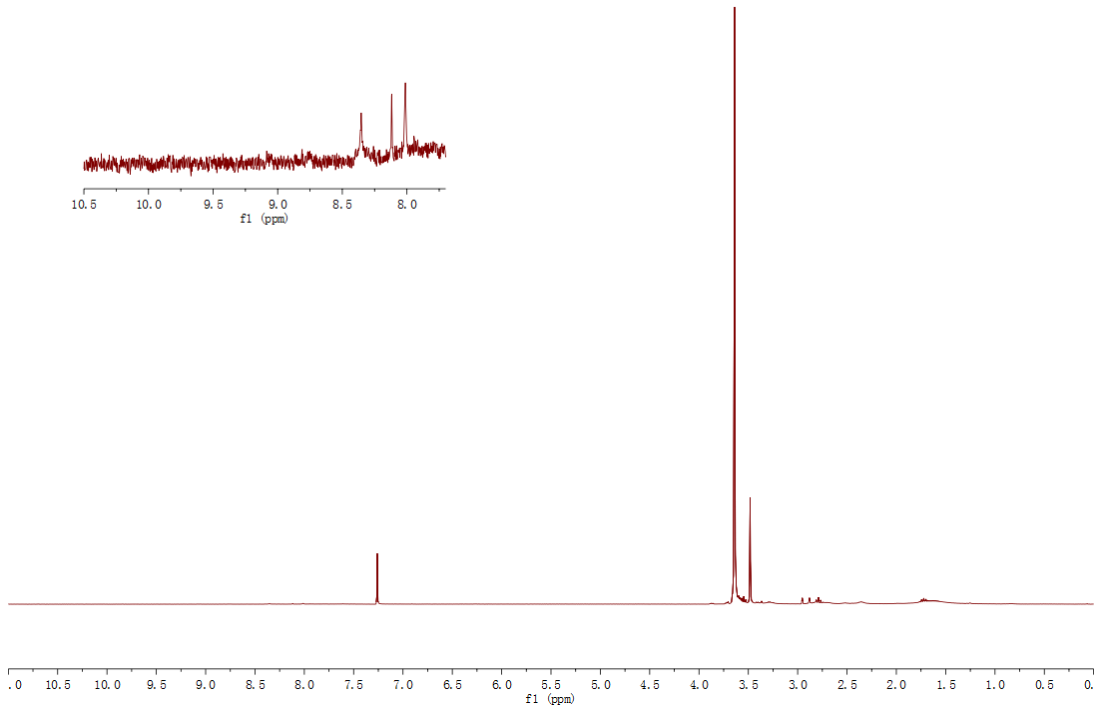


Figure S2. ^{1}H NMR spectrum of dynamer **7**.

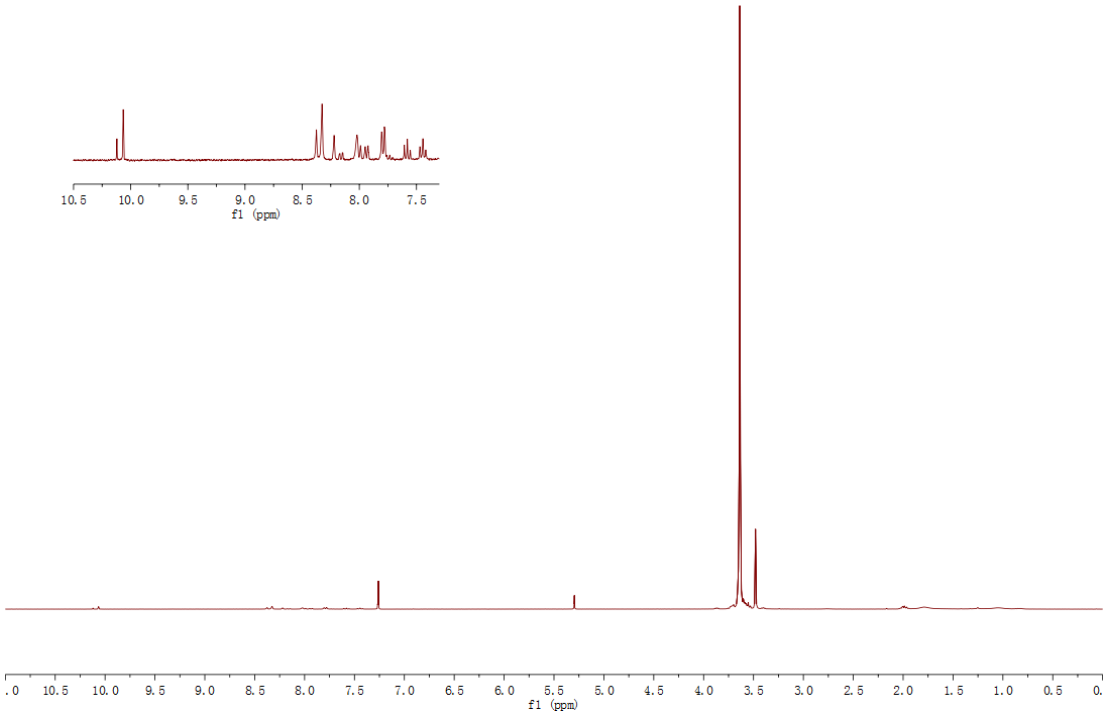


Figure S3. ^{1}H NMR spectrum of dynamer **8**.

2D-DOSY (Diffusion-Ordered SpectroscopY) experiments were performed on an ARX 300 MHz Bruker spectrometer in D_2O with the use of the residual solvent peak as reference.

DOSY NMR experiments were performed at 298 K with a Bruker Dual z-gradient probe head capable of producing gradients in the z direction with strength 55 G cm^{-1}.The NMR tube was not spun. The DOSY spectra were acquired with pulsed-gradient stimulated echo (LED-PFGSTE) sequence, using a bipolar gradient (ledbpgp2s pulse program: 2D sequence for diffusion measurement using echo and led with bipolar gradient pulse: D. Wu, A. Chen & C.S. Johnson Jr., J. Magn. Reson. A 115, 260-264 (1995).

All spectra were recorded with 8 Ko time domain data points in the F2 Frequency axis and 32 experiments (F1). The gradient strength was logarithmically incremented in 32 steps from 2% up to 95% of the maximum gradient strength. All measurements were performed with a diffusion delay (□) of 120 ms in order to keep the relaxation contribution to the signal attenuation constant for all samples. The gradient pulse length (δ) was 5 ms in order to ensure full signal attenuation. The diffusion dimension of the 2D DOSY spectra was processed by means of the Bruker Topspin software (version 2.1).

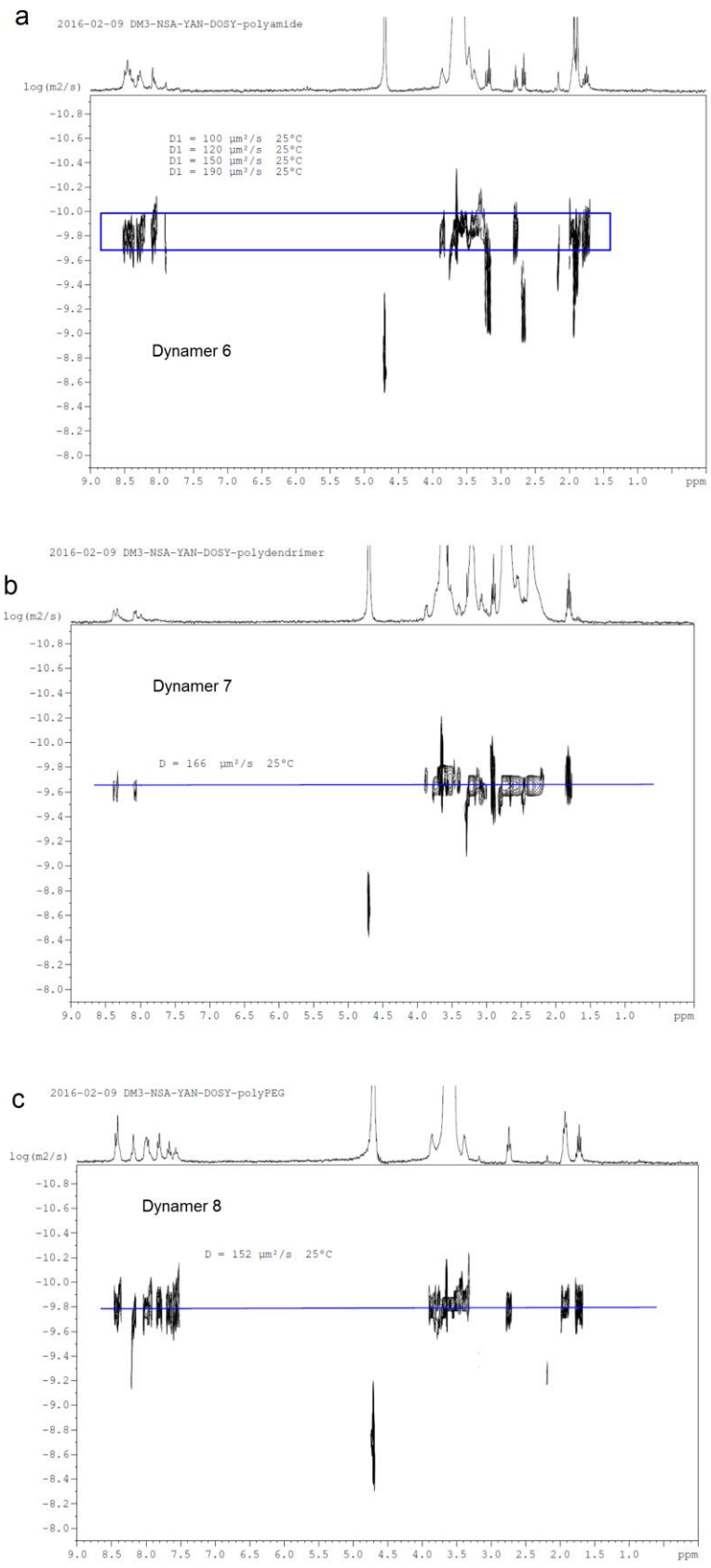


Figure S4. DOSY spectra of dynamers a) **6** b) **7** and c) **8**

Table S1. DOSY diffusion coefficients for dynamers **6-8**.

Dynamer	$D_{estimated}$* (NMR) ($\mu m^2.s-1$)	MM Calculated (g/mol)
6	100	69589
	120 (main component)	40706
	150	21117
	190	10536
7	165	15954
8	152	20310

*S. Floquet, S. Brun, J.-F. Lemonnier, M. Henry, M.-A. Delsuc, Y. Prigent, E Cadot, and F. Taulelle, J. Am. Chem Soc. **2009**, *131*, 17254–17259.

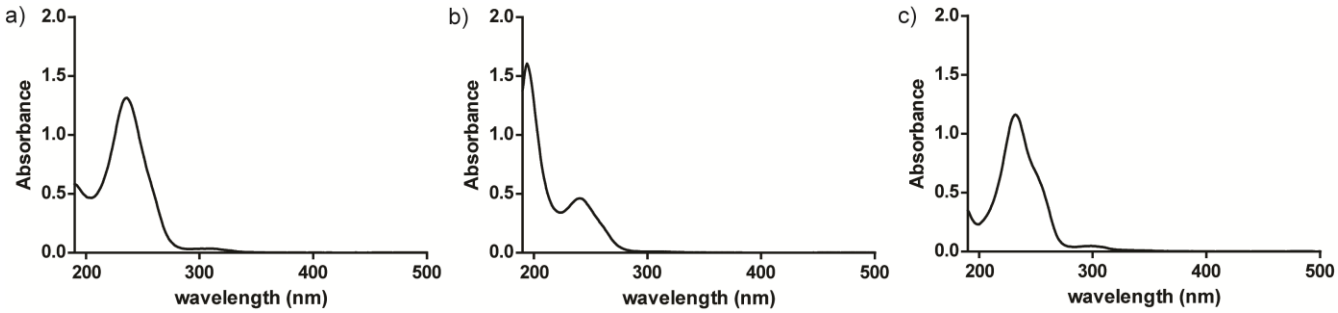


Figure S5. UV-vis full spectra of a) dynamer **6**; b) dynamer **7** and c) dynamer **8** as references for fluorescence quenching and UV-vis kinetic studies.

4. Fluorescence studies ofthe binding between dynamers 6-8 and bCA

Stock solution of bCA(0.1 mM) was prepared by adding 3 mg of bCA powder (molecular mass 29 kDa) into 1 mL of PBS buffer solution (100 mM, pH 7.0). Stock solution of dynamer/monomer (10 mM) was prepared by adding 0.01 mmol of chemicals into 1 mL mini-Q H_2O.

The procedure was started by adding 2 μL of bCA stock solution to 2 mL of PBS buffer (100 mM, pH 7.0) in a 2 mL quartz cuvette. The initial fluorescence of bCA alone was firstly recorded at λ_{exc} = 280 nm and λ_{em} from 280 to 500 nm.Then increasing amount of dynamer/monomer solution was added into the same cuvette to measure the intensity change.

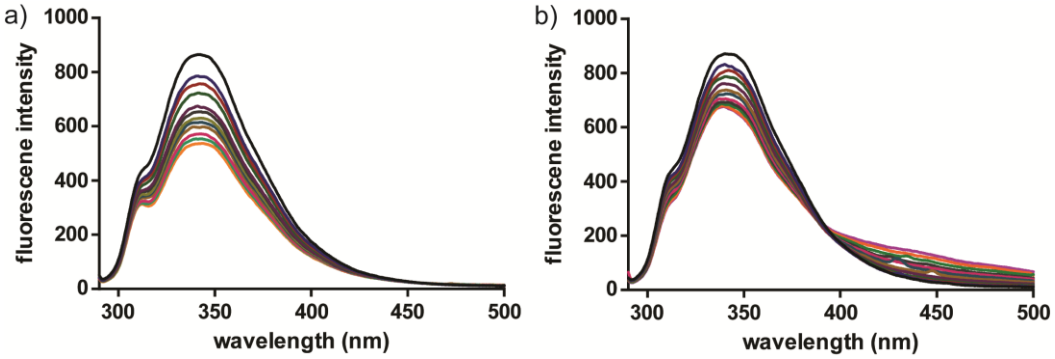


Figure S6. Fluorescence quenching of bCA upon addition of increasing amount of a) amide **3**; b) dendrimer **4**. The concentrations of dynamers are 0 mM, 0.01 mM, 0.025 mM, 0.05 mM, 0.10 mM, 0.15 mM, 0.20 mM, 0.25 mM, 0.30 mM, 0.40 mM, 0.50 mM and 0.60 mM respectively from top to bottom.

To calculate the association constant (K_a), the highest fluorescence intensityat 343 nmwas used and applied into the plot of Stern-Volmer relation ($I_0/I = 1+K_a$ [P]), where I_0 is the initial fluorescence intensity of bCA alone; I is the intensity of bCA with the presence of dynamers (**6**-**8**) or monomers (**3**-**4**); [P] is the concentration of added dynamers or monomers. For **3** and **4**, the Stern-Volmer relation does not fit well the experimental data.

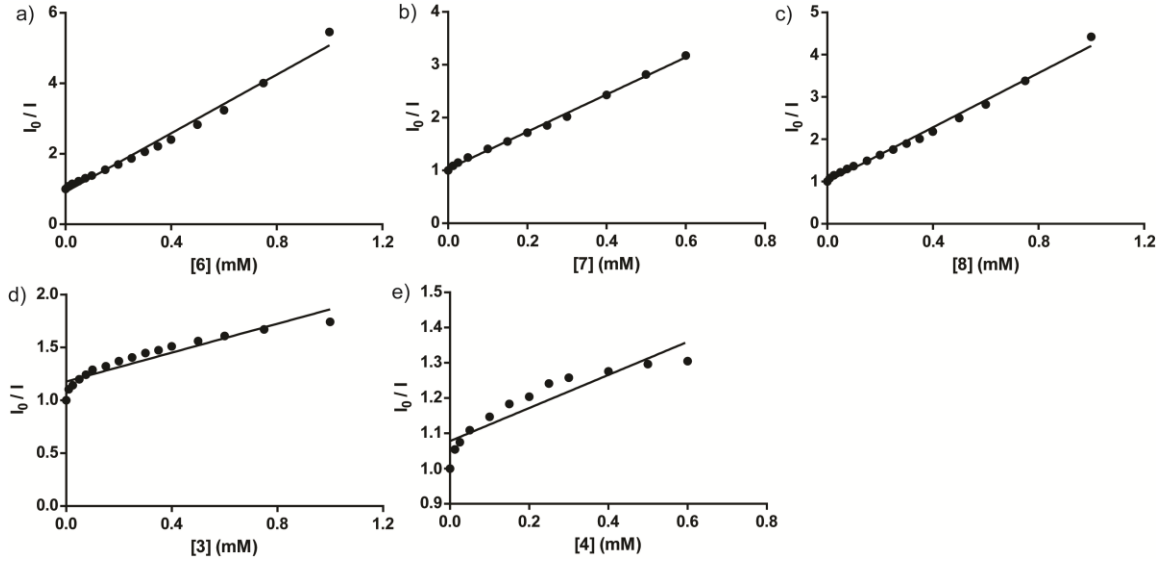
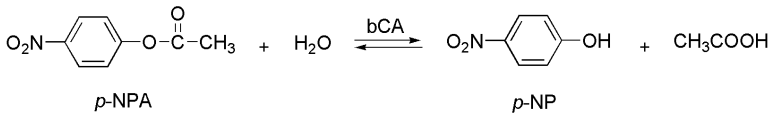


Figure S7. Plots of Stern-Volmer relation for the determination of association constant between bCA and a) dynamer**6**; b) dynamer**7**; c) dynamer **8**; d) monomer **3**; e) monodendrimer**4**.

5. UV-vis studies of the activation effects of the dynamers

The catalytic reactivity of bCA was studied using UV-vis spectroscopy, based on the hydrolysis reaction of *p*-nitrophenyl acetate (*p*-NPA, Scheme S1). The formation of product *p*-nitrophenol (*p*-NP) can be followed by UV absorbance at the wavelength of 400 nm.



Scheme S1. Hydrolysis reaction of *p*-NPA catalyzed by bCA.

The same stock solution of bCA (0.1 mM) in PBS (pH 7.0) prepared for fluorescence study was also used here. Substrate solution of *p*-NPA (3.4 mM) was prepared by adding 2.5 mg *p*-NPA in 4 mL CH_3CN. The enzymatic reaction was initiated by adding 100 µL *p*-NPA (3.4 mM) to a quartz cuvette containing a mixture of 20 µL bCA (0.1 mM) stock solution, PBS (pH 7.0) buffer and increasing amount of dynamers/monomers, with the total volume of 1.0 mL.Time-drive analysis method of UV-vis spectroscopy was employed to monitor the formation of *p*-NP over the first 8 min at the wavelength of 400 nm. Control experiments without the presence of bCA were also carried out to check the hydrolysis ability of the dynamers/monomers alone.

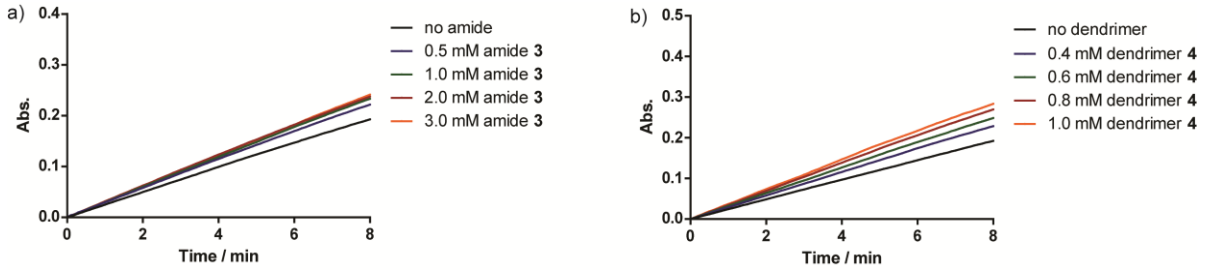


Figure S8. Activity change of bCA on hydrolysis reaction of *p*-nitrophenyl acetate, with the gradual addition of a) monoamide**3**; b) dendrimer **4**, followed UV-vis spectroscopy.

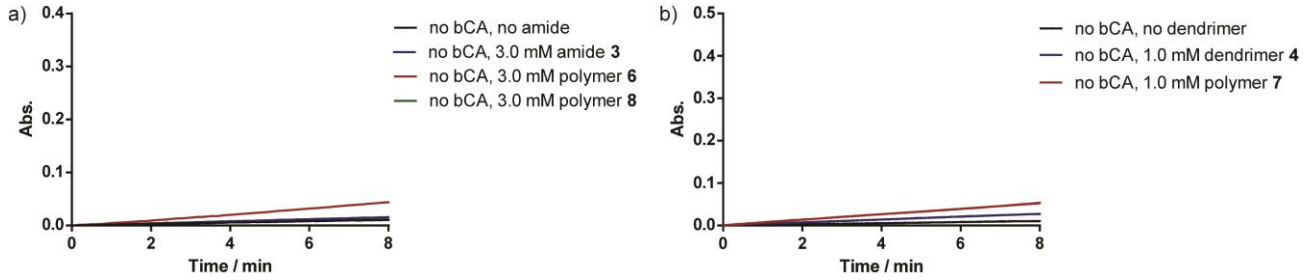


Figure S9. Control experiments of hydrolysis reaction of *p*-nitrophenyl acetate in the absence of bCA, with the addition of a) monoamide **3**, dynamer **6** and **8**; b) dendrimer **4** and dynamer **7**, followed UV-vis spectroscopy.

6. Determination of activation constants (K_A) of dynamers 6 and 7

The activation constant (K_A) was defined similarly with the inhibition constant (K_I), can be obtained based on Michaelis-Menten equation ($v = v_{max}/ \{1 + K_M/[S](1+[A]_f /K_A)\}$), using the fitting of non-linear least squares by PRISM 6 (GraphPad, San Diego, CA, USA).

To quantitatively measure the activation effect of the dynamer **6**, the same UV-vis kinetic method was applied by varying the concentration of *p*-PNA from 0.14 mM to 0.41 mM while the concentration of dynamers **6** was fixed to 1.0 mM, 2.0 mM and 3.0 mM respectively. The same measurements were used for dynamer **7** except the concentration of **7** was fixed to 0.26 mM, 0.52 mM and 0.78 mM respectively.

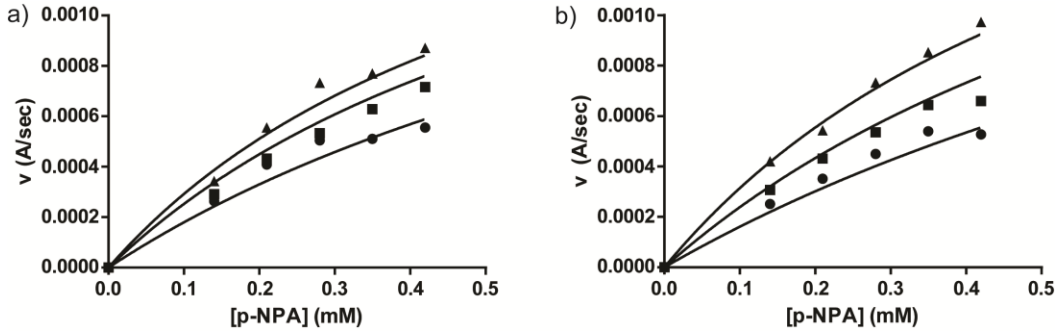


Figure S10. Estimation of activation constants of a) dynamer **6** and b) dynamer **7** using non-linear regression analysis (modified competitive inhibition equation by replacing [I]/K_I with K_A/[A]) in GraphPad.

7. Circular dichroism (CD) studies on protein secondary structure of bCA

The CD spectra were recorded at wavelengths between 180 and 250 nm, using Xe lamp (100 W), and 1.0 mm path length quartz cuvette, at a speed of 30 nm·min^{-1}. All observed CD spectra were baseline subtracted for pH 7.0 PBS buffer (100 mM).

The CD spectrum of bCA protein alone was recorded by transferring 0.5 mL of 3.4 μM bCA in PBS (pH 7.0) buffer solution into the cuvette. Then increasing amount of dynamers **6**-**8** (0.04 - 0.20 mM) were added into the solution respectively. The final data were averaged from three measurements and subsequently smoothed.

To further determine the bCA structure modifications and the related influence on the activity enhancement, circular dichroism (CD) spectroscopy in the far-UV region was applied to study the effect of dynamers on the protein secondary structure of bCA. As it can be observed in Fig. S11 with gradual addition of **6**-**8**, the characteristic negative peak at 215 nm which represents the β-sheet structure in bCA showed significantly decreasing ellipticity in CD spectra. The spectra of pure dynamers **6**-**8** interfere with the CD reading as well they present smaller positive peaks in this spectral region when compared with the enzyme CD response (Fig. S12). Probably we should rule out on minor non-specific assay interferences. Importantly, the shape of the specific peak of bCA gave comparatively preserved typical β-sheet curve even at higher concentrations of dynamers **6** and **7**. This is reminiscent with the preservation of the enzyme structure.

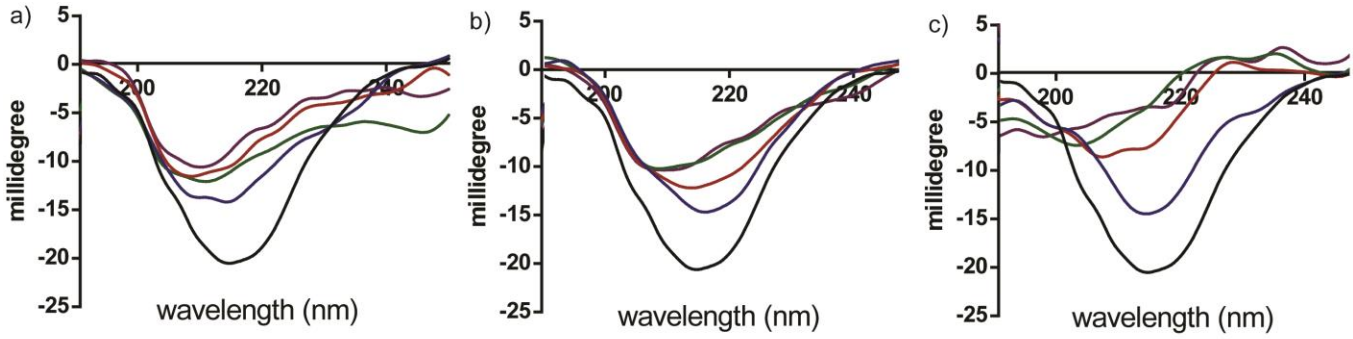


Figure S11. Circular dichroism spectroscopy of bCA upon addition of increasing amount of dynamers a) **6**; b) **7**; c) polymer **8**. The concentrations of polymers are 0, 0.04, 0.08, 0.12 and 0.20mM from bottom to top.

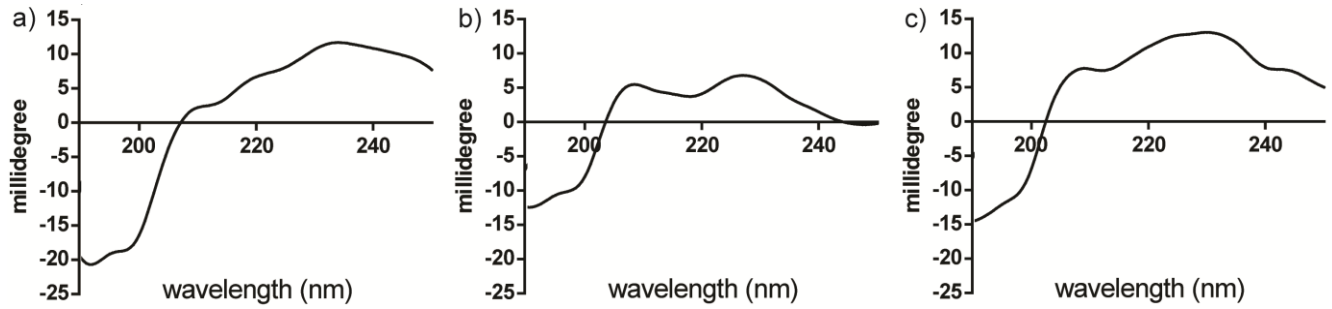


Figure S12. Circular dichroism spectra of solutions containing 0.20 mM of a) dynamer **6**; b) dynamer **7** and c) dynamer **8**.