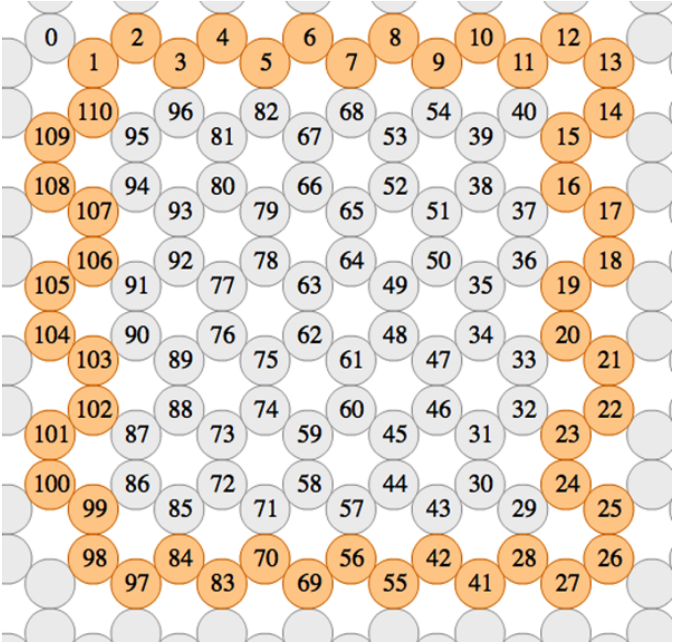
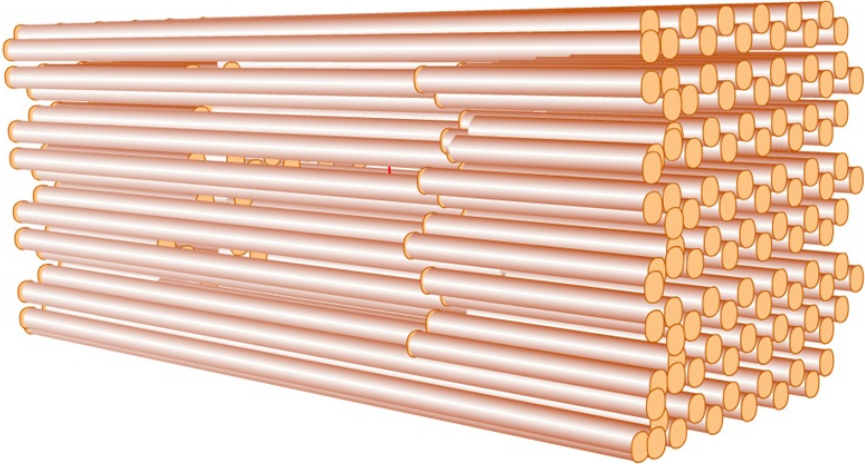


Supplementary Figures

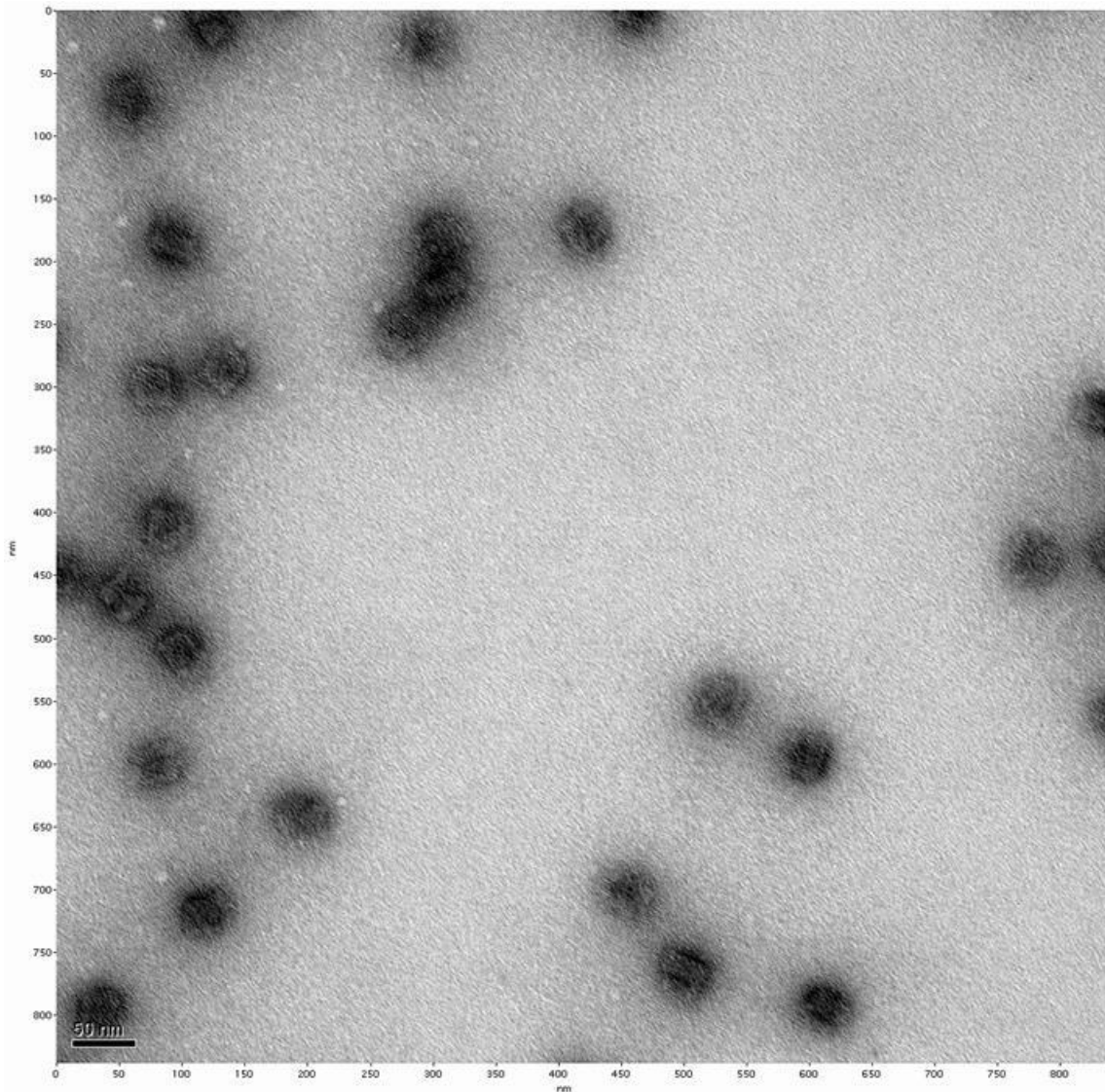
Cross-sectional view



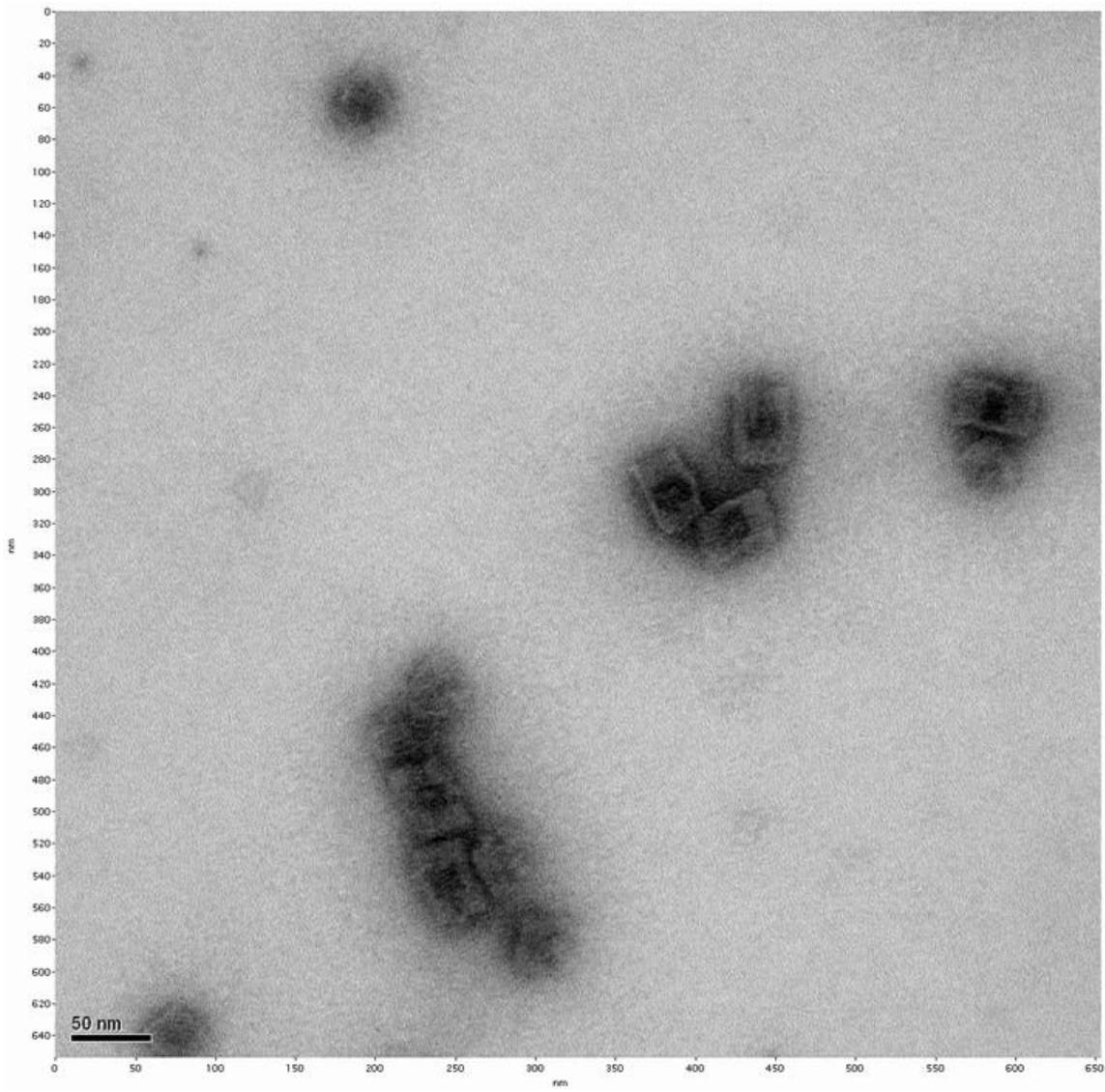
3D View



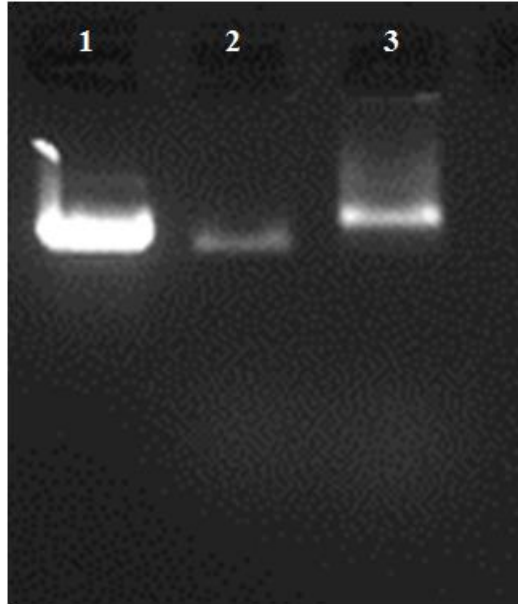
Supplementary Figure 1: Design of SH full-cage (honeycomb lattice), including cross-sectional view and 3D view.



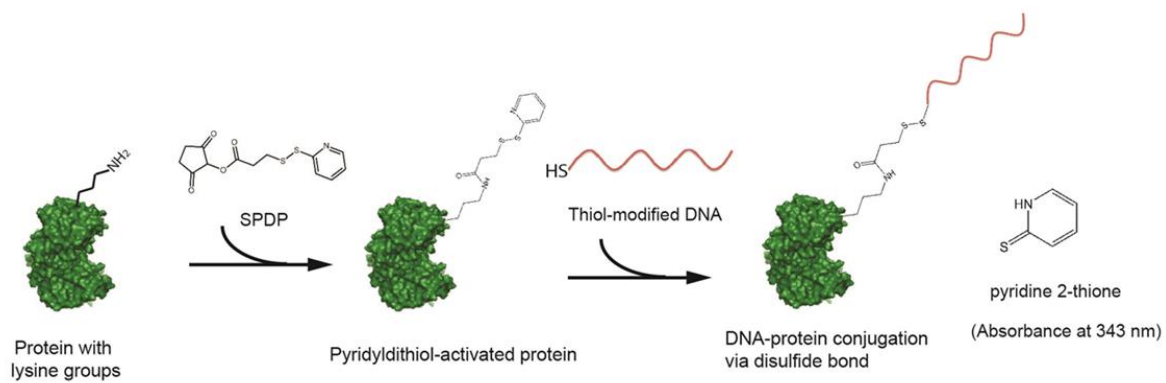
**Supplementary Figure 2:** A representative TEM image of the half-cage structure (scale bar: 50 nm).



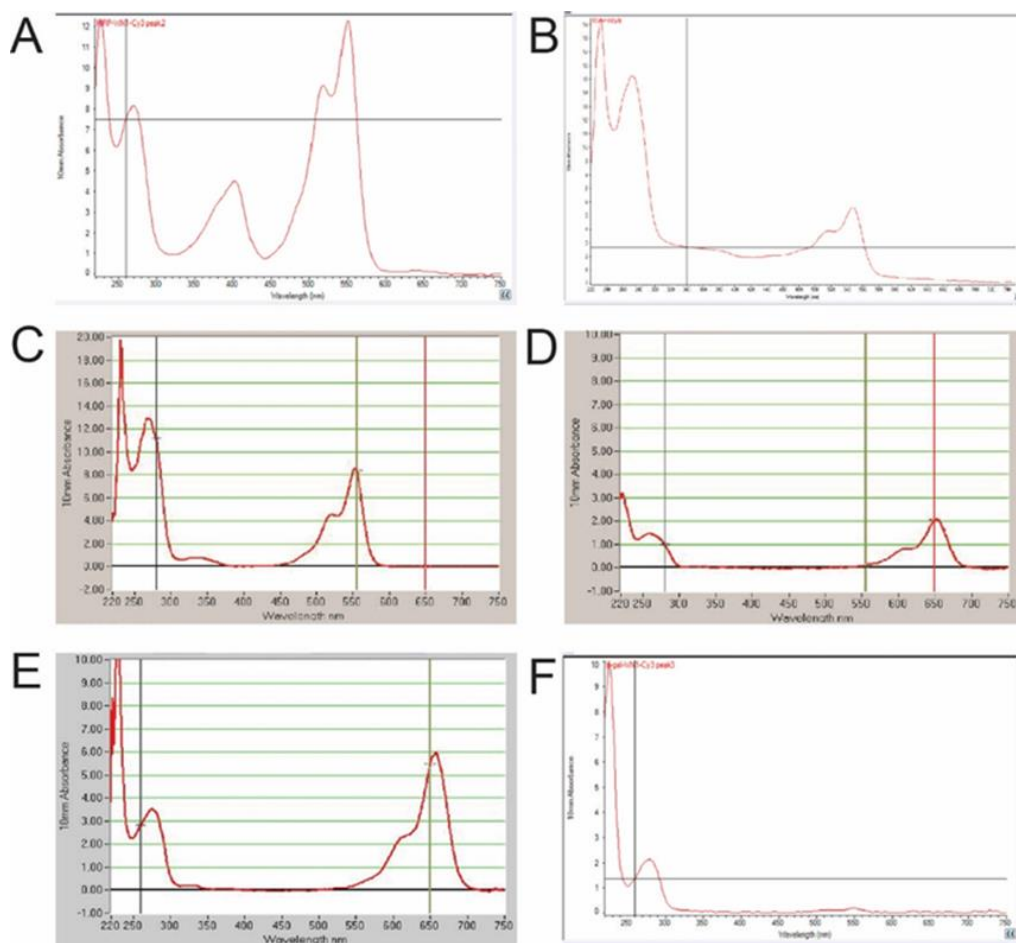
**Supplementary Figure 3:** A representative TEM image of the full-cage structure (scale bar: 50 nm).



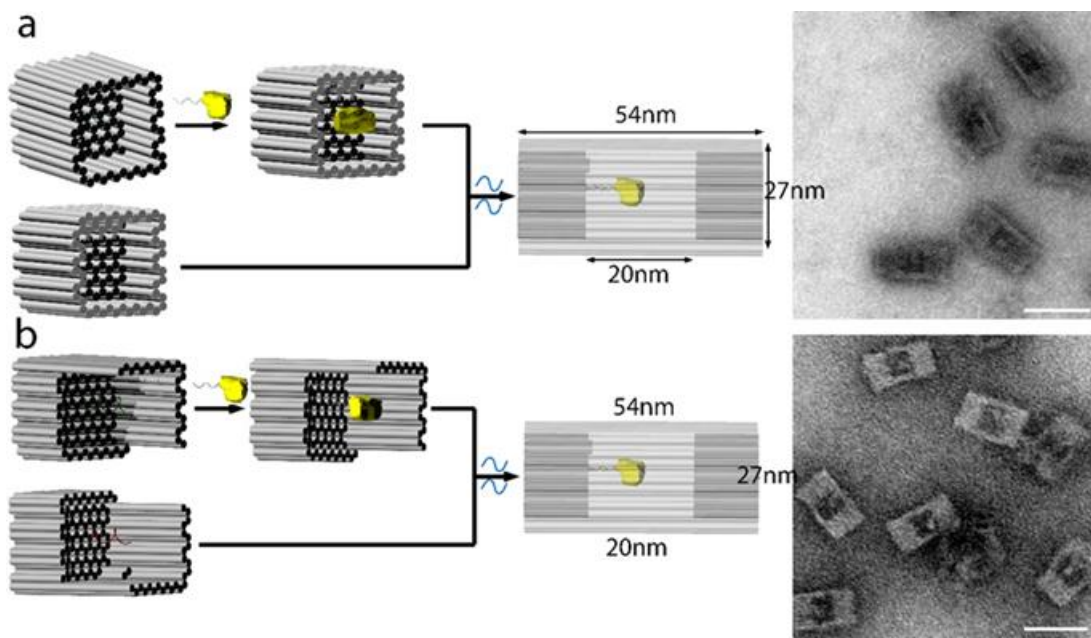
**Supplementary Figure 4:** Agarose gel electrophoresis (AGE) to characterize the full-cage structure (lane 1: M13 DNA, lane 2: half-cage; lane 3: full-cage). According to the gel band intensity, the assembly yield of the full-cage was higher than 90%.



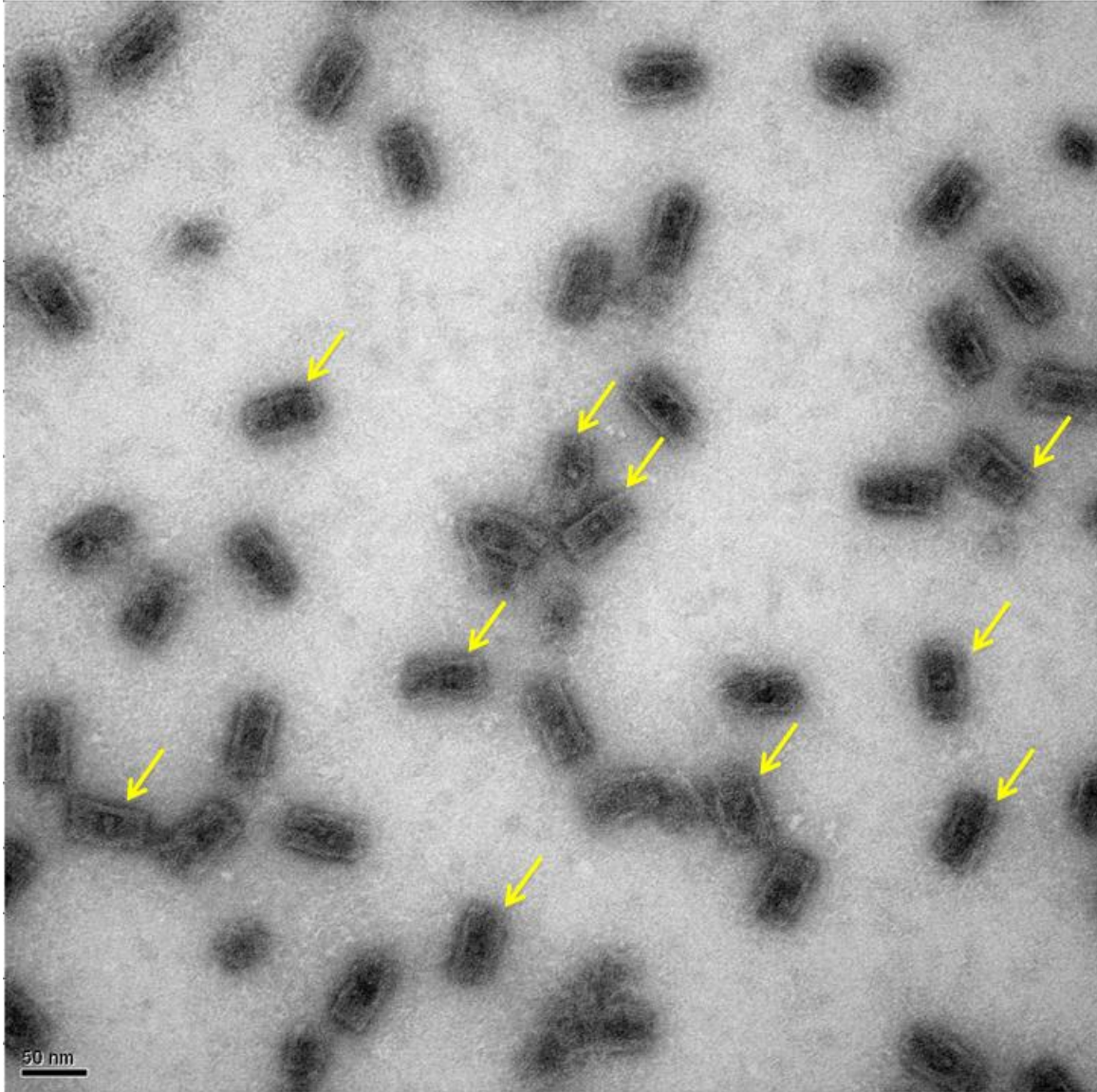
**Supplementary Figure 5:** Schematic illustration of the SPDP conjugation chemistry used for protein—DNA conjugation.



**Supplementary Figure 6: Quantification of fluorescent dye-labeled enzyme-DNA conjugates using UV-Vis absorbance spectroscopy.** (A) Cy3-labeled HRP-TTTTTCCCTCCCTCC with an average dye-to-protein ratio of  $\sim 1.8$ ; (B) Cy3-labeled GOx-TTTTTCCCTCCCTCC with an average dye-to-protein ratio of  $\sim 1.5$ ; (C) Cy3-labeled G6pDH-TTTTTCCCTCCCTCC with an average dye-to-protein ratio of  $\sim 1.6$ ; (D) Alexa Fluor 647-labeled MDH-TTTTTGGCTGGCTGG with an average dye-to-protein ratio of  $\sim 1.2$ ; (E) Alexa Fluor 647-labeled LDH-TTTTTGGCTGGCTGG with an average dye-to-protein ratio of  $\sim 1.7$ ; (F) Cy3-labeled ( $\beta$ -Gal)-TTTTTCCCTCCCTCC with an average dye-to-protein ratio of  $\sim 0.6$ .

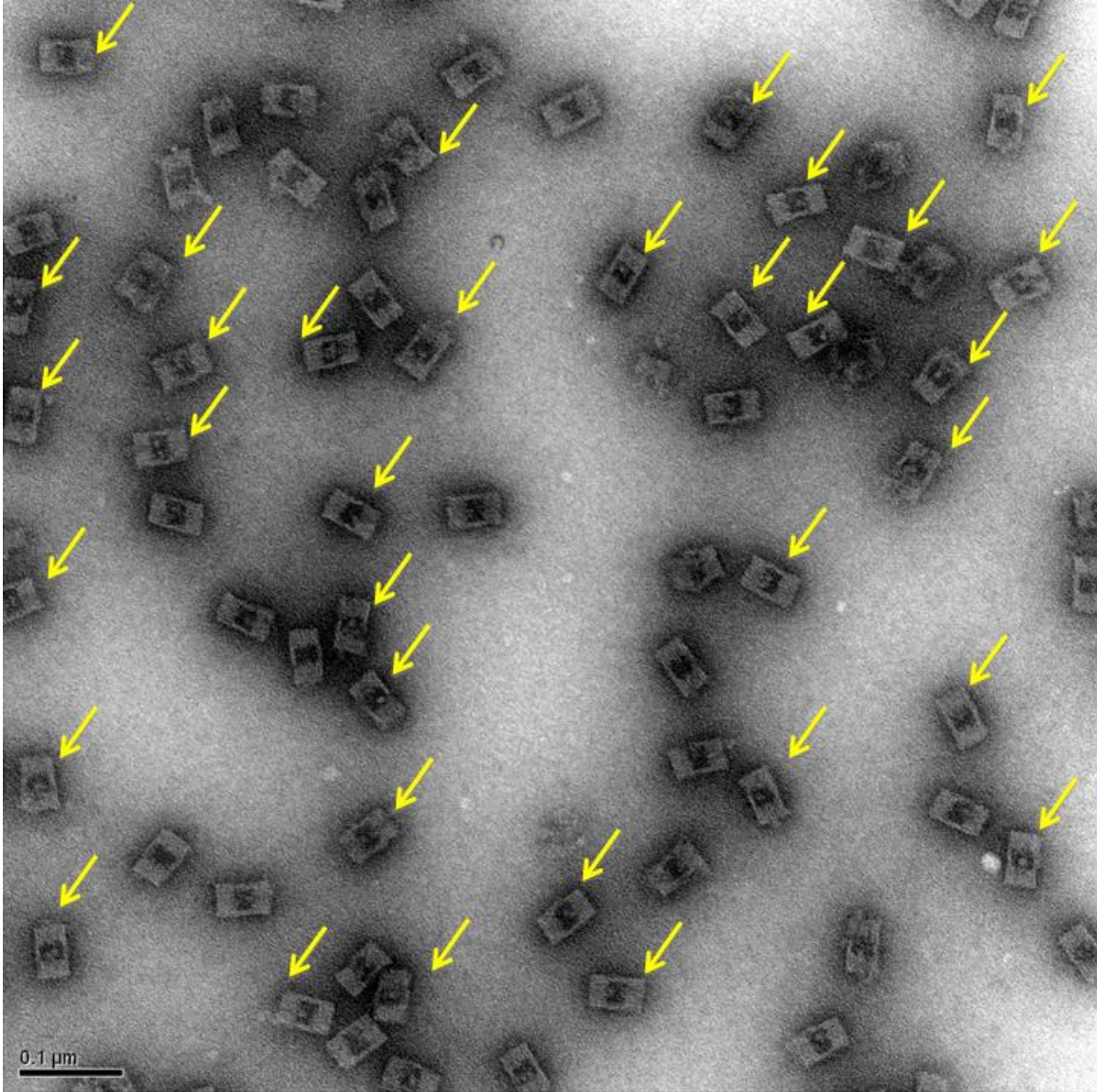


**Supplementary Figure 7:** Two different designs for the cage structure with different encapsulation yields (see Supplementary Figures 8 and 9), assembled with GOx. (a) Cage with closed-wall design. (b) Cage with open-wall design.

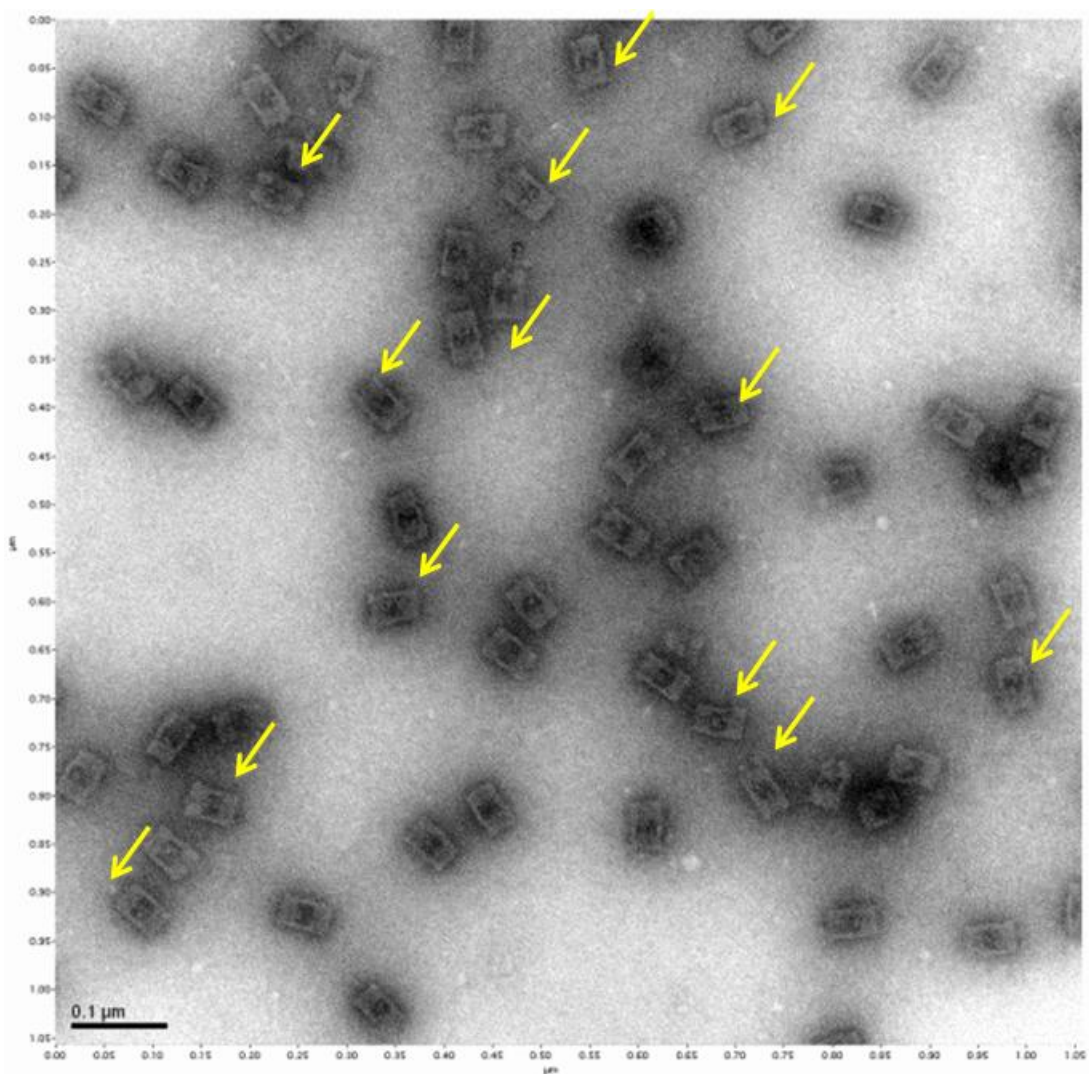


**Supplementary Figure 8:** TEM image of full-cages with closed-wall design (Supplementary Figure 7a) encapsulating GOx. An encapsulation yield of 38% was estimated from similar images containing ~230 DNA cages by dividing the number of cages with a discernible protein inside by the total number of the cages counted (yellow arrow indicates DNA cage with enzyme inside).

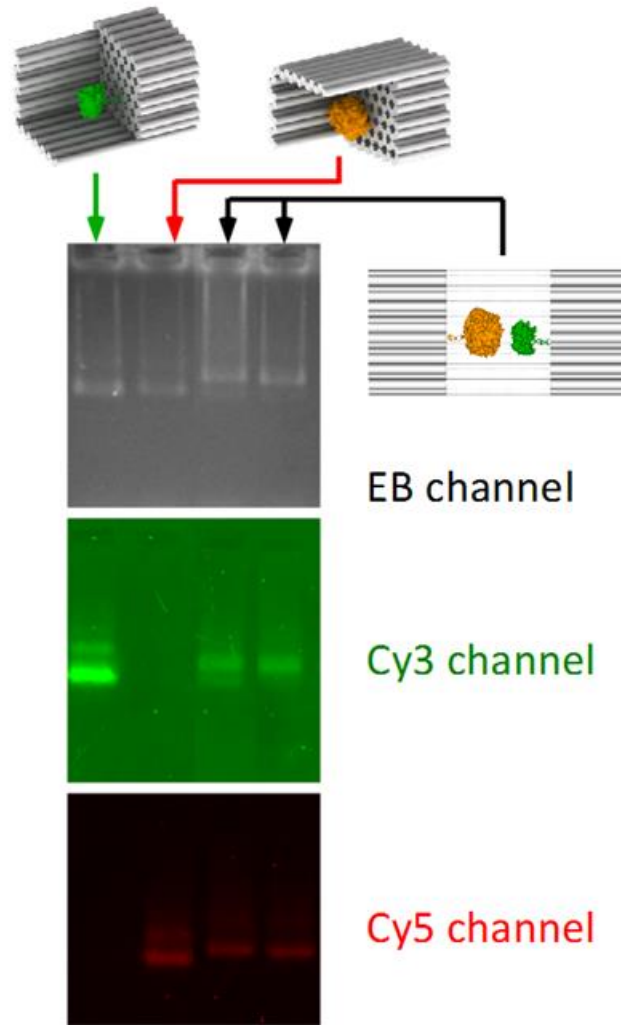




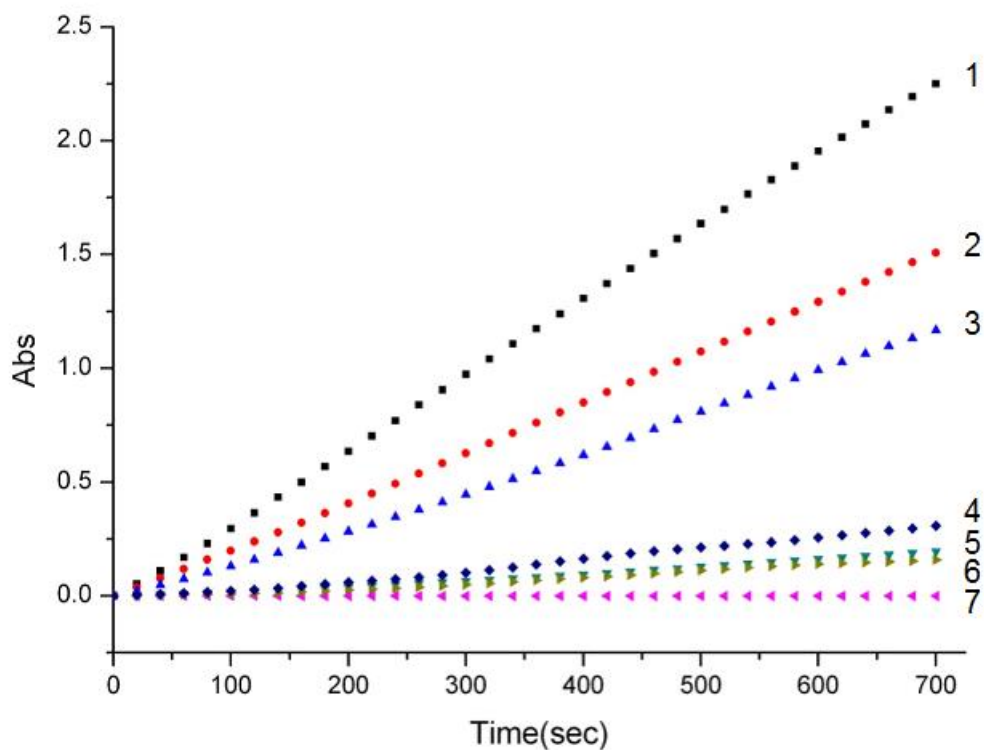
**Supplementary Figure 9:** TEM image of full-cage with open-wall design (Supplementary Figure. 7b) encapsulating GOx. An encapsulation yield of 77% was estimated from similar images containing ~300 DNA cages by dividing the number of cages with a discernable protein inside by the total number of cages counted (yellow arrow indicates DNA cage with enzyme inside).



**Supplementary Figure 10:** TEM image for HRP-GOx enzyme pairs encapsulated in DNA full-cage. Despite variable quality of staining across the field of view, the inner cavity of many nanocages appeared to contain two bright spots, which we interpreted as intact HRP-GOx enzyme pairs (yellow arrow indicates DNA cage with enzyme pair inside).

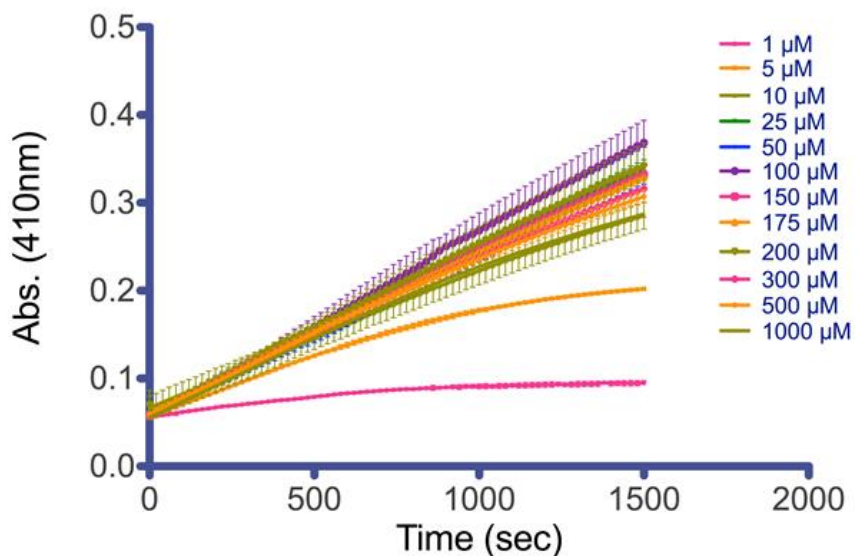


**Supplementary Figure 11:** Native AGE characterization of a DNA nanocage encapsulating a GOx/HRP pair. GOx and HRP were conjugated with Cy3 and Cy5, respectively. Lane 1 (from left): half-cage assembled with GOx-Cy3, lane 2: half-cage assembled with HRP-Cy5, lanes 3 and 4: full-cage with GOx/HRP. “EB” indicates ethidium bromide staining of the gel to visualize all DNA bands.

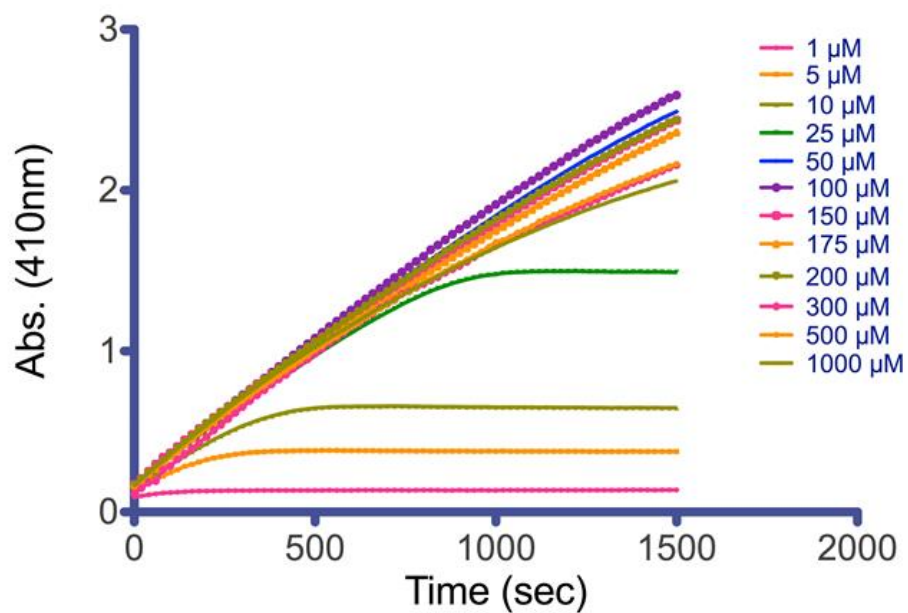


**Supplementary Figure 12:** Raw activity for a set of DNA cage-encapsulated enzymes. 1: Full[H+G], a full cage-encapsulated GOx and HRP; 2: Full[H] + Full[G], a full cage-encapsulated HRP and a full cage-encapsulated GOx; 3: half[H] + half[G], a half cage-encapsulated HRP and a half-cage encapsulated GOx; 4: Full + H + G, a full cage incubated with a pair of free HRP and GOx; 5: H + G fresh control, a fresh solution of free HRP and GOx; 6: H + G annealing control, a solution of free HRP and GOx that is incubated using the same thermal program as the DNA cage-encapsulated enzymes; 7: substrate background control. Assay conditions: 1 nM enzyme or enzyme-encapsulating DNA cage, with 1 mM Glucose, 2 mM ABTS in pH 7.5, 1×TBS buffer. Absorbance is monitored at 410 nm.

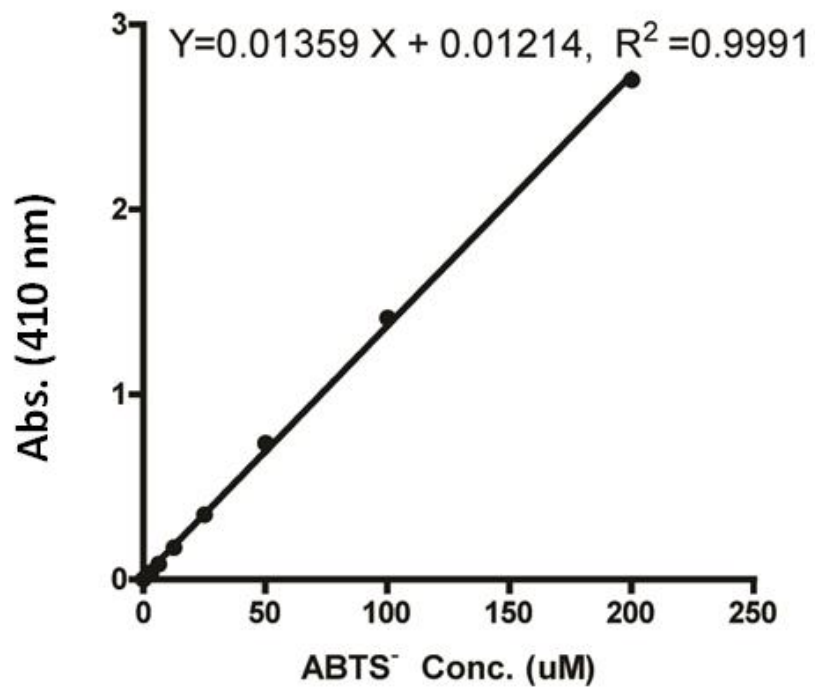
### Determination of the Michaelis-Menten constants for enzymes-HRP



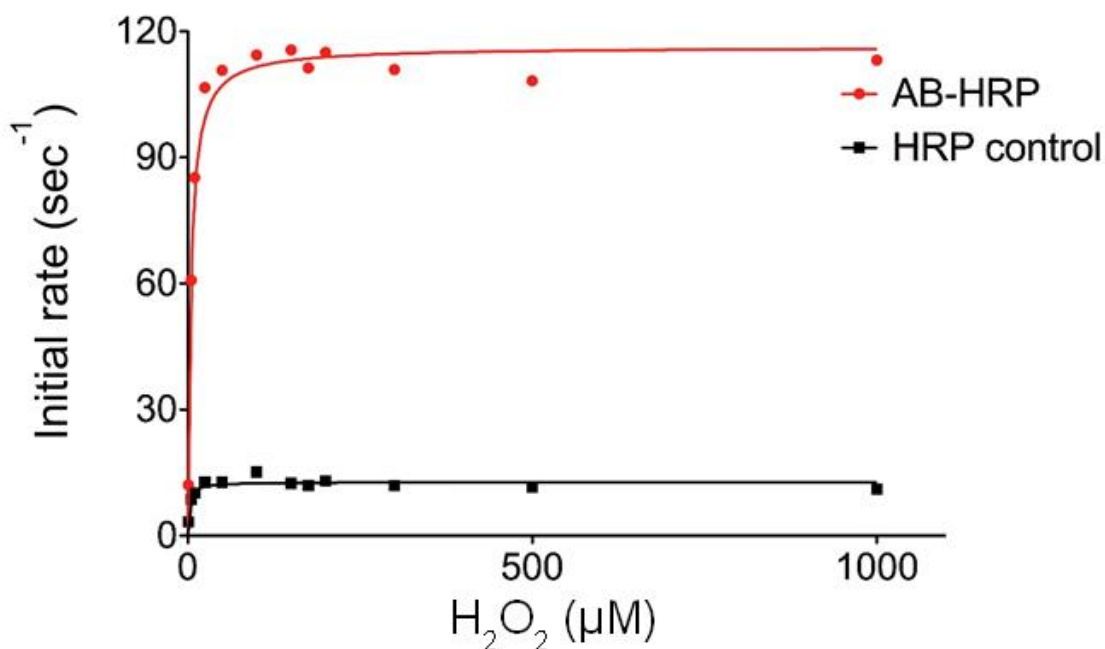
**Supplementary Figure 13:** Raw activity for free enzyme solution of DNA-conjugated HRP (0.5 nM) with H<sub>2</sub>O<sub>2</sub> concentration varied from 1 μM to 1000 μM, and 2 mM ABTS, monitoring absorbance at 410 nm. Error bars were calculated from the standard deviation of at least three replicates.



**Supplementary Figure 14:** Raw activity for DNA cage-encapsulating HRP (0.5 nM) with H<sub>2</sub>O<sub>2</sub> varied from 1 μM to 1000 μM and 2 mM ABTS, monitoring absorbance at 410 nm. Error bars were calculated from the standard deviation of at least three replicates.



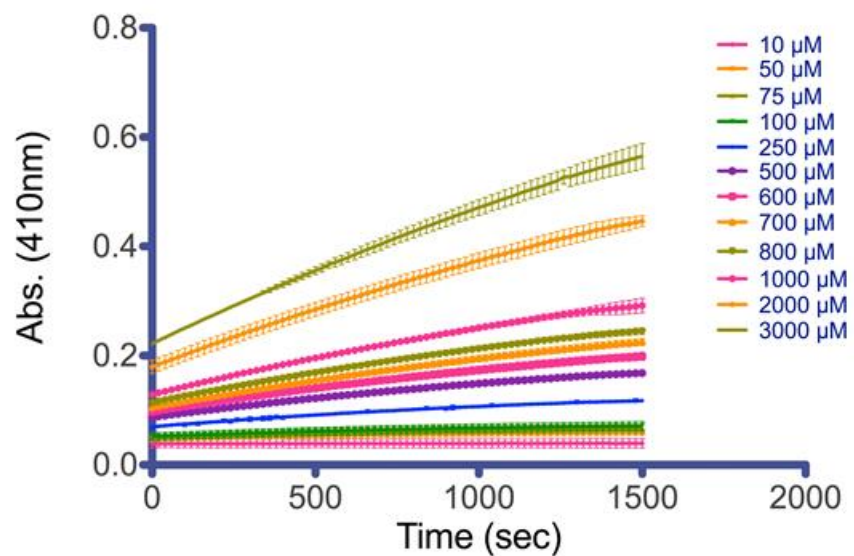
**Supplementary Figure 15:** ABTS standard curve to calculate  $k_{cat}$  value ( $Y=0.01359X+0.01214$ ).



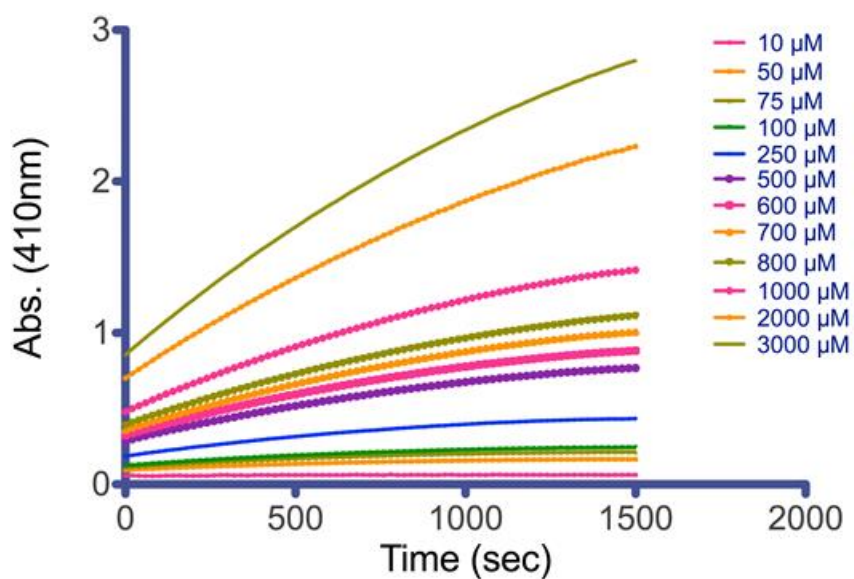
	$K_M$ (μM)	$k_{cat}$ (s <sup>-1</sup> )
<b>Full Cage[HRP]</b>	4.3±0.6	290±5
<b>Free HRP</b>	2.3±0.5	32±1

**Supplementary Figure 16:** Michaelis-Menten plot of HRP encapsulated within a full-cage (Full-Cage[HRP], red circles), compared with that of free HRP (HRP control, black squares) using H<sub>2</sub>O<sub>2</sub> as the substrate. The solid lines represent fits of the Michaelis-Menten model to the data. Enzyme assay conditions: 0.5 nM enzyme or DNA-cage-encapsulated enzyme, 2 mM ABTS with different concentrations of H<sub>2</sub>O<sub>2</sub> ranging from 1 μM to 1000 μM, in 1×TBS buffer (pH 7.5, 1 mM MgCl<sub>2</sub>), absorbance monitored at 410 nm. The table lists the fit parameters. Full-cage encapsulation of the enzyme caused a ~2-fold increase in  $K_M$  and a ~9-fold increase in  $k_{cat}$ .

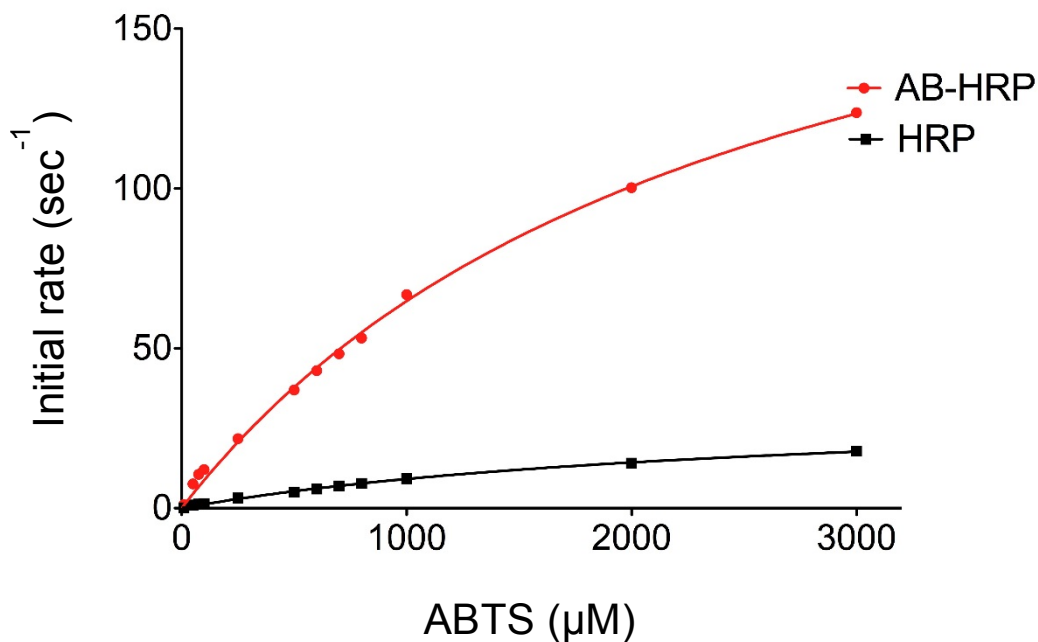




**Supplementary Figure 17:** Raw activity measurement of Full-Cage [HRP] (0.5 nM) with ABTS concentration varied from 10  $\mu\text{M}$  to 3000  $\mu\text{M}$  and 2000  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , monitoring absorbance at 410 nm. Error bars were calculated from the standard deviation of at least three replicates.

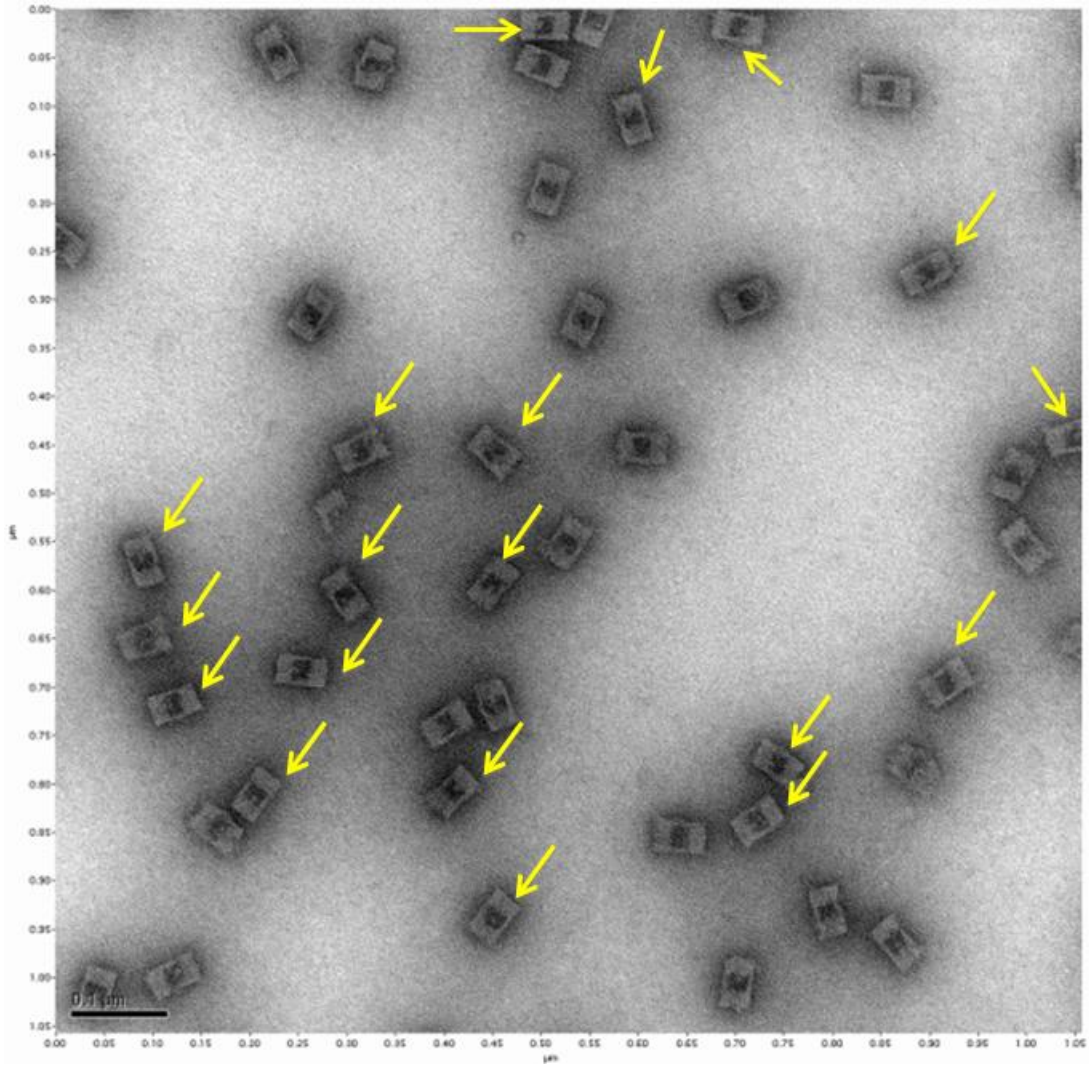


**Supplementary Figure 18:** Raw activity measurement of free DNA-conjugated HRP (0.5 nM) with ABTS concentration varied from 10  $\mu\text{M}$  to 3000  $\mu\text{M}$ , and 2000  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub>, monitoring absorbance at 410 nm. Error bars were calculated from the standard deviation of at least three replicates.

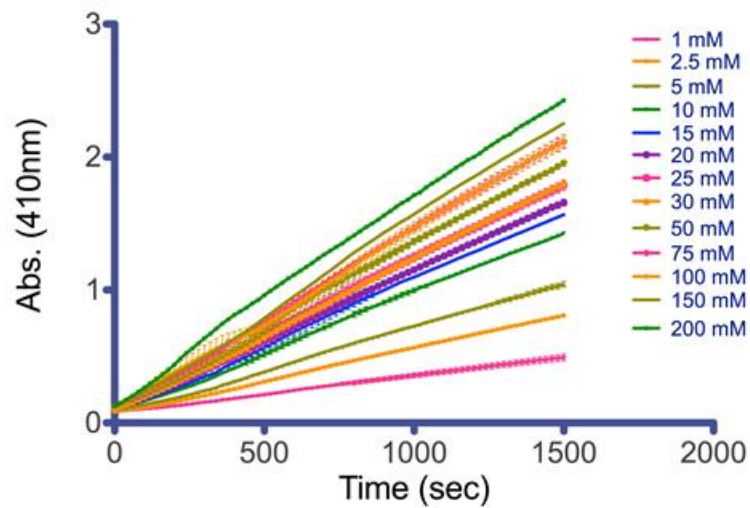


	$K_M$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
<b>AB-HRP</b>	$2500 \pm 200$	$560 \pm 20$
<b>HRP control</b>	$2600 \pm 400$	$59 \pm 5$

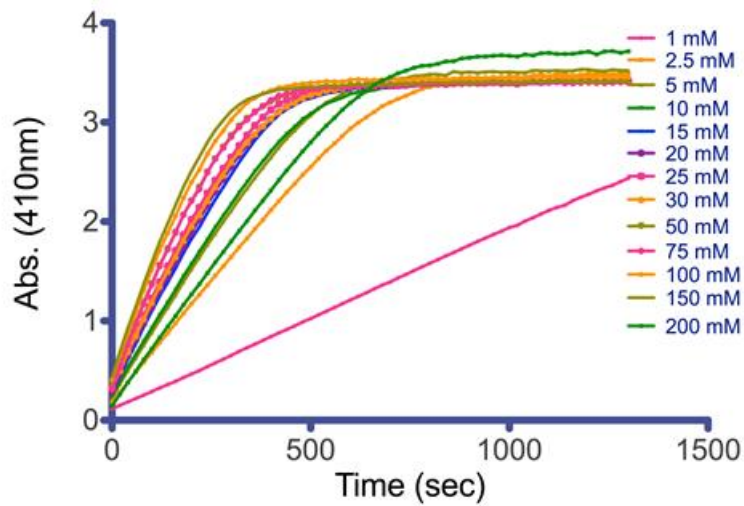
**Supplementary Figure 19:** Michaelis-Menten plot for HRP encapsulated within a full-cage (AB-HRP, red circles), compared with that of free HRP enzyme (HRP control, black squares) using ABTS as the substrate. The solid lines represent fits of the Michaelis-Menten model to the data. Enzyme assay conditions: 0.5 nM enzyme or full-cage-encapsulated enzyme, 2000  $\mu\text{M}$   $\text{H}_2\text{O}_2$  with different concentrations of ABTS, ranging from 10  $\mu\text{M}$  to 3000  $\mu\text{M}$ , in 1 $\times$ TBS buffer (pH 7.5, 1 mM  $\text{MgCl}_2$ ), monitoring absorbance at 410 nm. The table lists the fit parameters. DNA encapsulation of the enzyme caused no change in  $K_M$  and a  $\sim 9$ -fold increase in  $k_{cat}$ .



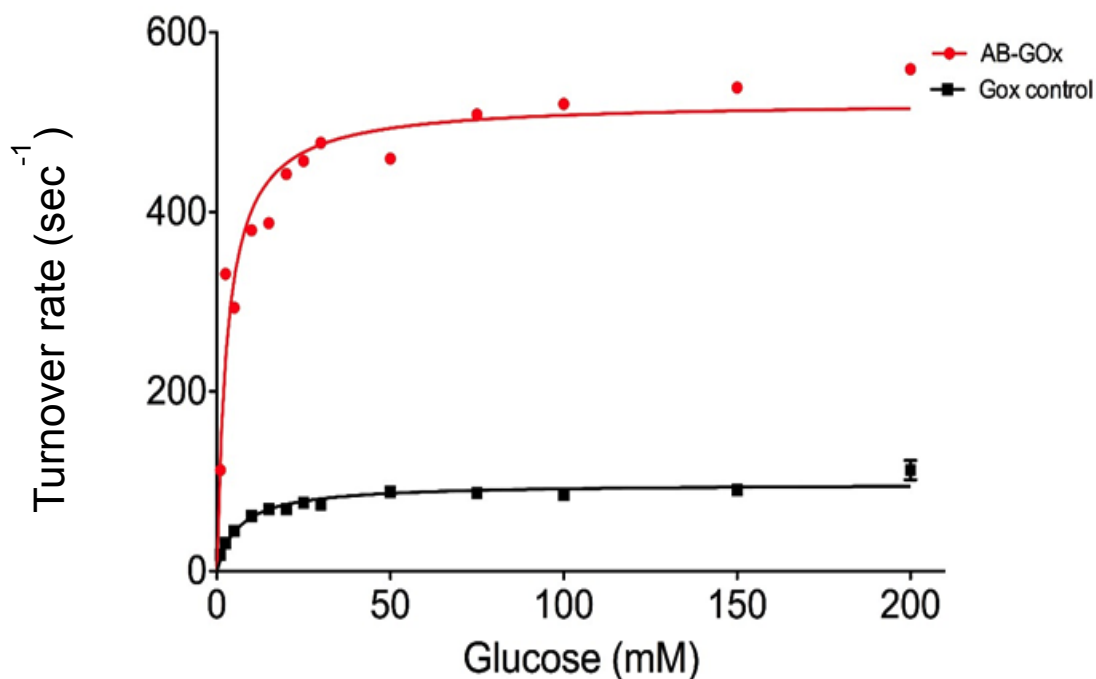
**Supplementary Figure 20:** TEM image for the purified DNA full-cage with only HRP enzyme inside. Scale bar: 100 nm. The majority of cages showed one lighter spot inside the cavity, representing the enzyme. Despite variable quality of staining across the field of view, the inner cavity of many nanocages appeared to contain one bright spot, which we interpreted as intact one HRP enzyme (yellow arrow indicates DNA cage with enzyme inside).



**Supplementary Figure 21:** Raw activity for free DNA-conjugated GOx (0.5 nM) with different concentrations of glucose ranging from 1 mM to 200 mM. 2 mM ABTS and 100 nM HRP were used to quickly convert H<sub>2</sub>O<sub>2</sub> to detectable signal that was monitored at 410 nm. Error bars were calculated from the standard deviation of at least three replicates.

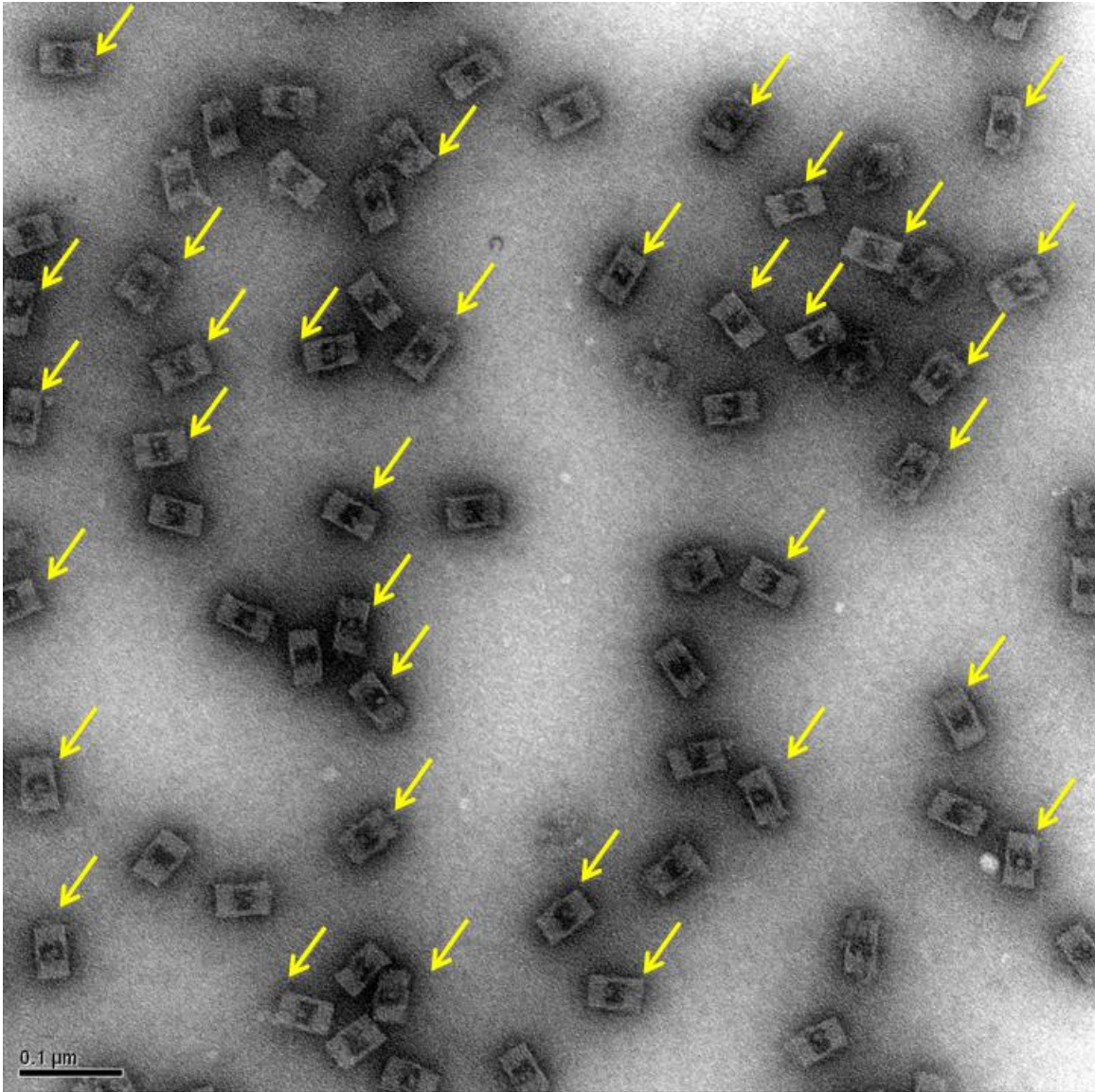


**Supplementary Figure 22:** Raw activity for DNA cage-encapsulating GOx (0.5 nM) with different concentrations of glucose ranging from 1 mM to 200 mM. 2 mM ABTS and 100 nM HRP were used to quickly convert H<sub>2</sub>O<sub>2</sub> to detectable signal that was monitored at 410 nm. Error bars were calculated from the standard deviation of at least three replicates.



	$K_M$ ( $\mu\text{M}$ )	$K_{cat}$ ( $\text{s}^{-1}$ )
<b>AB-GOx</b>	3000±600	1300±50
<b>GOx control</b>	6200±900	240±10

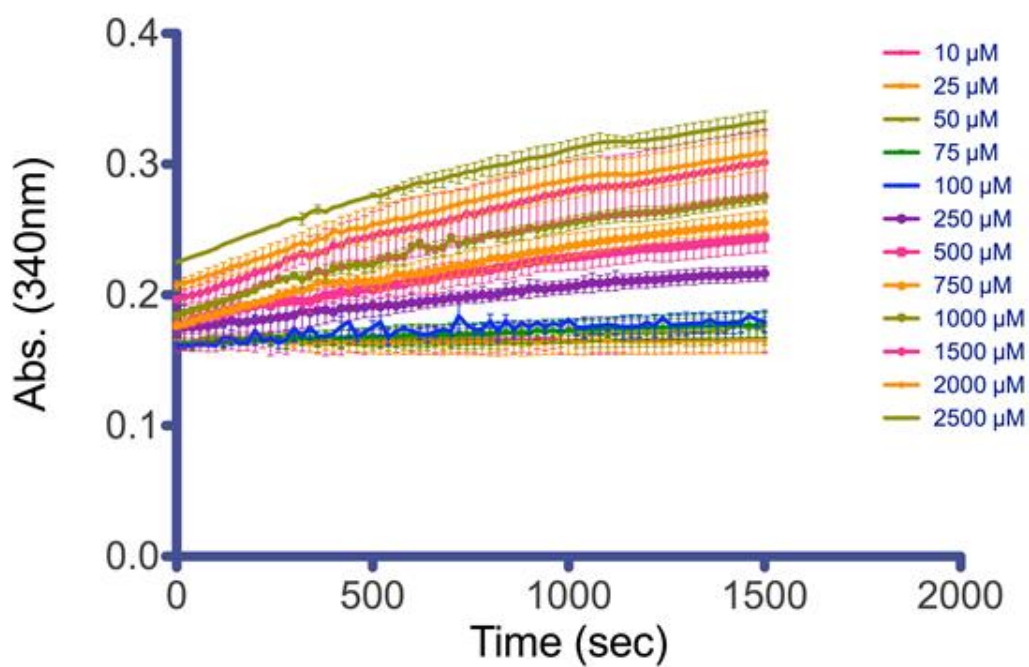
**Supplementary Figure 23:** Michaelis-Menten plot of GOx inside the full-cage (AB-GOx, red circles), compared with that of free GOx enzyme (GOx control, black squares) using glucose as the substrate. The solid lines represent fits of the Michaelis-Menten model to the data. Enzyme assay conditions: 0.5 nM enzyme or DNA cage encapsulated enzyme, 2 mM ABTS, 100 nM HRP with different concentrations of glucose ranging from 1 mM to 200 mM, in 1×TBS buffer (pH 7.5, 1 mM MgCl<sub>2</sub>) monitoring absorbance at 410 nm. The table lists the fit parameters. DNA encapsulation of the enzyme caused a ~2-fold decrease in  $K_M$  and a ~5-fold increase in  $k_{cat}$ .



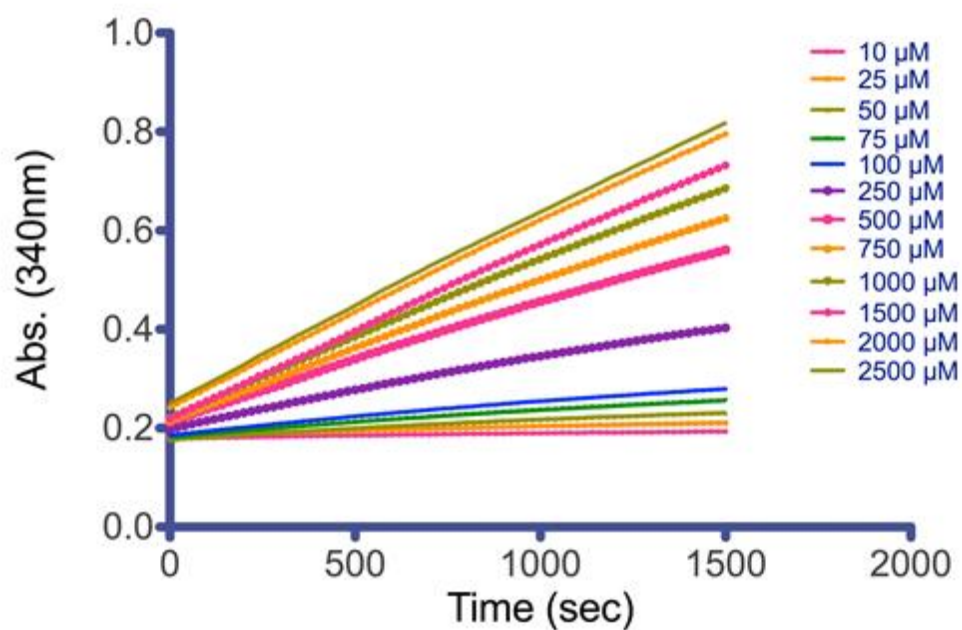
**Supplementary Figure 24:** TEM image of the purified DNA full-cage with only GOx inside (yellow arrow indicates DNA cage with enzyme inside).



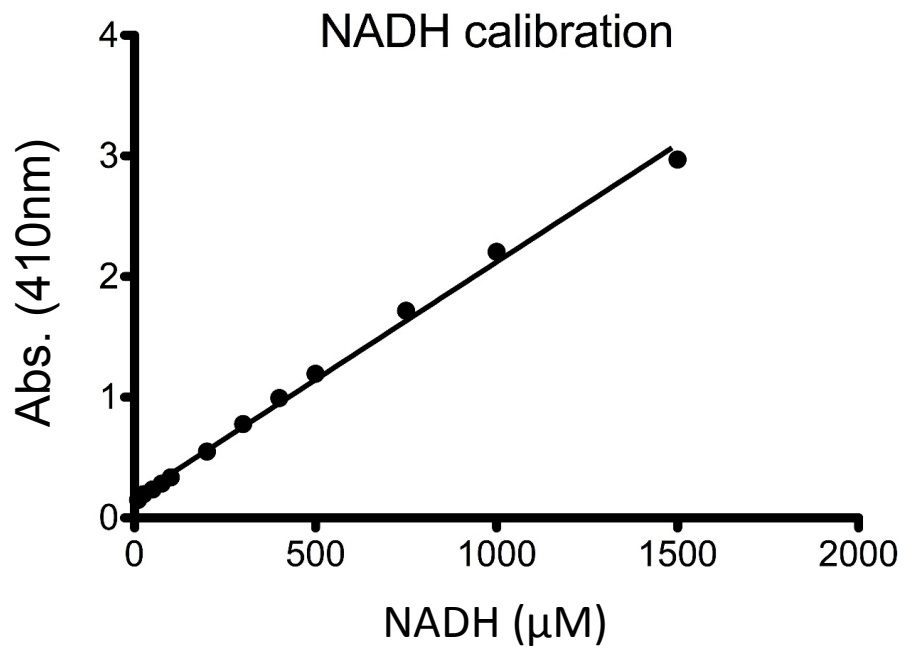
## Determination of the Michaelis-Menten constants for enzymes-G6PDH



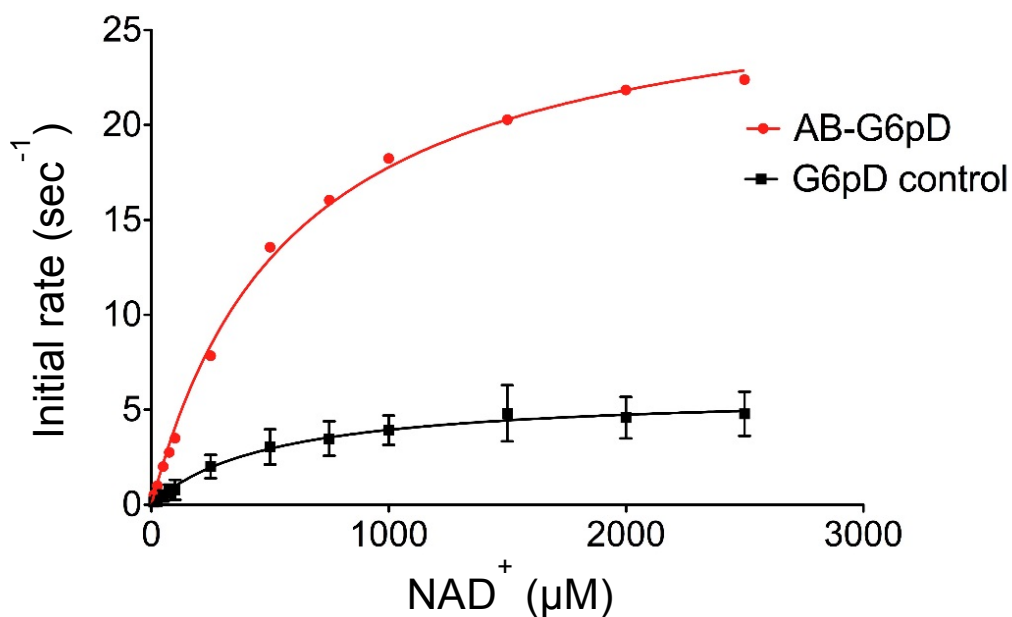
**Supplementary Figure 25:** Raw activity for free DNA-modified G6pDH (0.5 nM) with 10-2500 μM NAD<sup>+</sup> and 1 mM glucose 6-phosphate, monitoring absorbance at 340 nm. Error bars were calculated from the standard deviation of at least three replicates.



**Supplementary Figure 26:** Raw activity for Full-Cage [G6pDH] (0.5 nM) with 10-2500 μM NAD<sup>+</sup> and 1 mM glucose 6-phosphate, monitoring absorbance at 340 nm. Error bars were calculated from the standard deviation of at least three replicates.

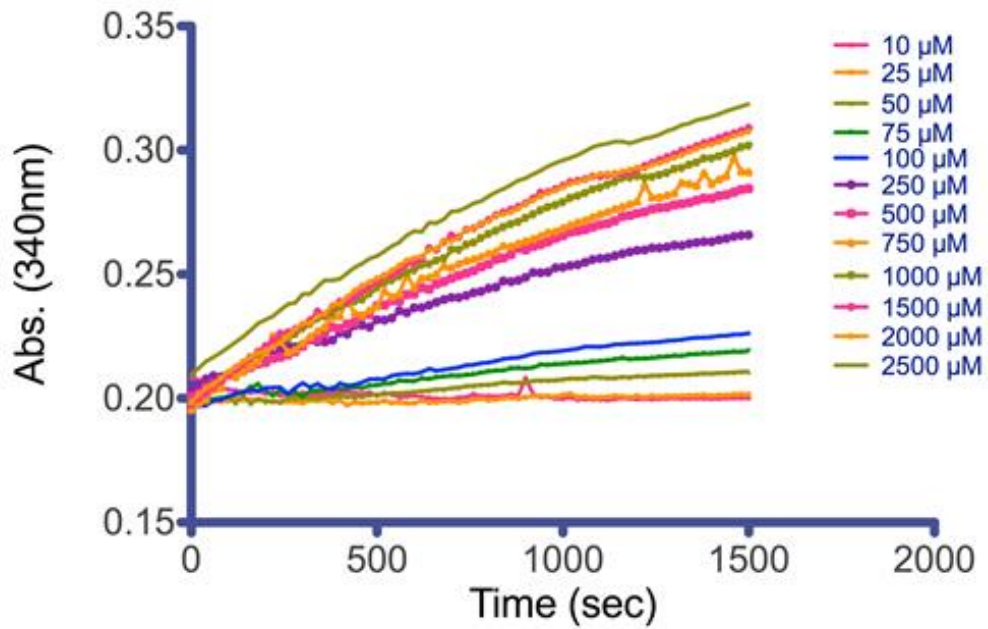


**Supplementary Figure 27:** NADH absorbance standard curve to calculate  $k_{cat}$  ( $Y=0.001951X+0.1694$ ).

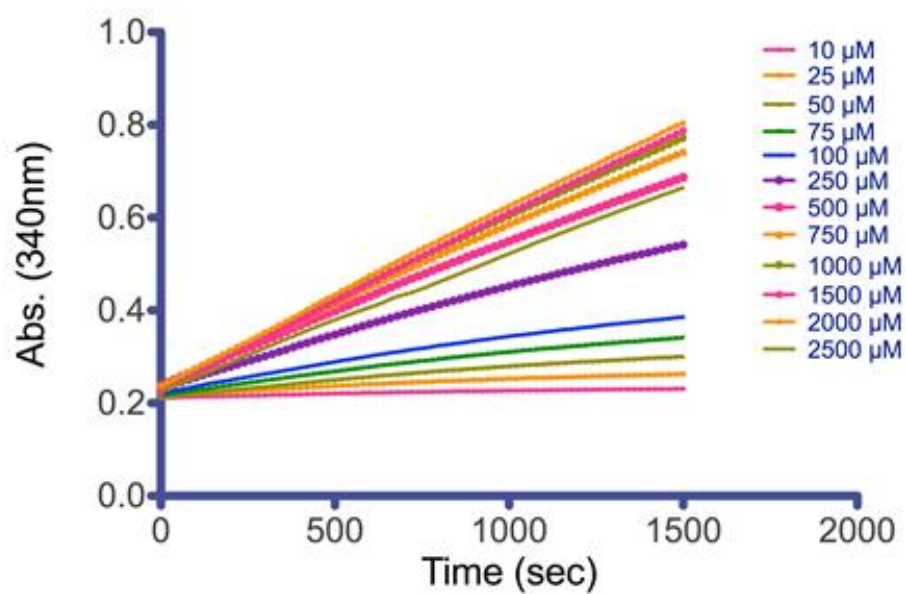


	$K_M$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
Full[G6pDH]	$590 \pm 40$	$480 \pm 10$
G6pDH control	$510 \pm 50$	$100 \pm 3$

**Supplementary Figure 28:** Michaelis-Menten plot of Full-Cage[G6PDH] (red circles) compared with that of free G6pDH (black square), using  $\text{NAD}^+$  as the varying substrate. The solid lines represent fits of the Michaelis-Menten model to the data. Enzyme assay conditions: 0.5 nM enzyme or DNA cage-encapsulated enzyme, 1 mM glucose 6-phosphate, with different concentrations of  $\text{NAD}^+$  ranging from 10  $\mu\text{M}$  to 2500  $\mu\text{M}$ , in 1×TBS buffer (pH 7.5, 1 mM  $\text{MgCl}_2$ ), monitoring absorbance at 340 nm. The table lists the fit parameters. Full-cage encapsulation of the enzyme caused little change in  $K_M$  and a ~5-fold increase in  $k_{cat}$ . Error bars were calculated from the standard deviation of at least three replicates.

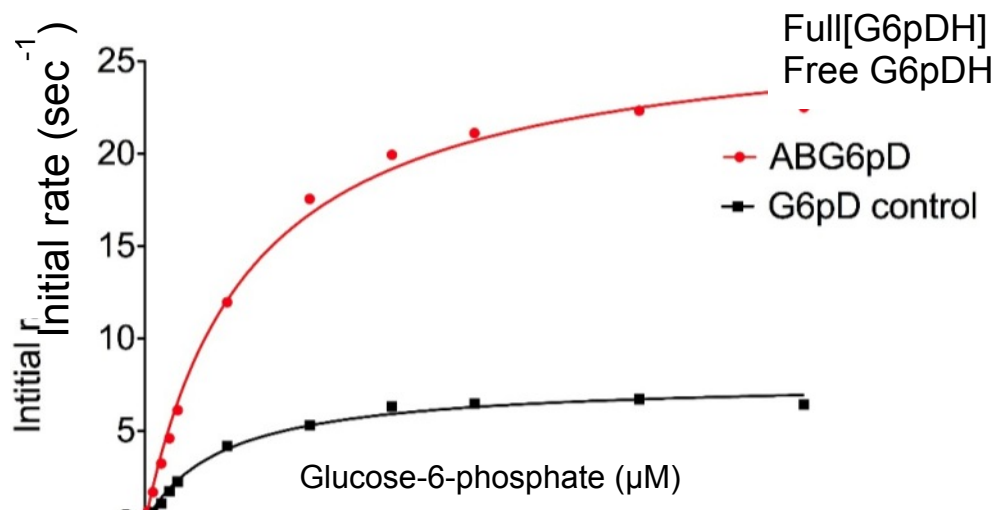


**Supplementary Figure 29:** Raw activity for free DNA-modified G6pDH (0.5 nM) with glucose 6-phosphate varied from 10  $\mu\text{M}$  to 2500  $\mu\text{M}$ , and 1 mM  $\text{NAD}^+$ , monitoring at 340 nm. Error bars were calculated from the standard deviation of at least three replicates.



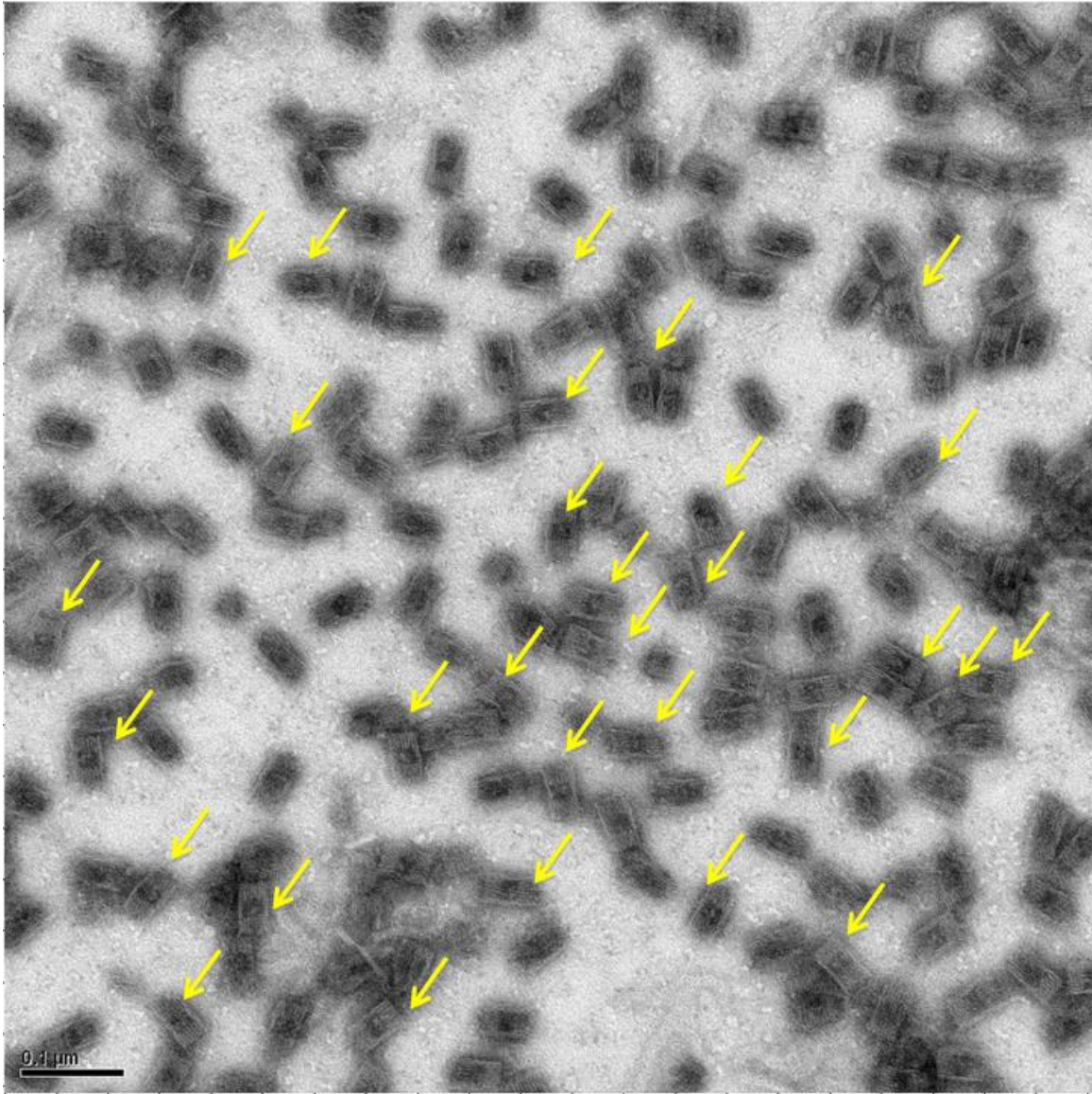
**Supplementary Figure 30:** Raw activity for Full-Cage [G6pDH] (0.5 nM) with glucose 6-phosphate varied from 10 μM to 2500 μM, and 1 mM NAD<sup>+</sup>, monitoring absorbance at 340 nm. Error bars were calculated from the standard deviation of at least three replicates.

## G6pD enzyme kinetics-G6p



	$K_M$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
<b>Full[G6pDH]</b>	$310 \pm 30$	$460 \pm 10$
<b>G6pDH control</b>	$220 \pm 20$	$130 \pm 3$

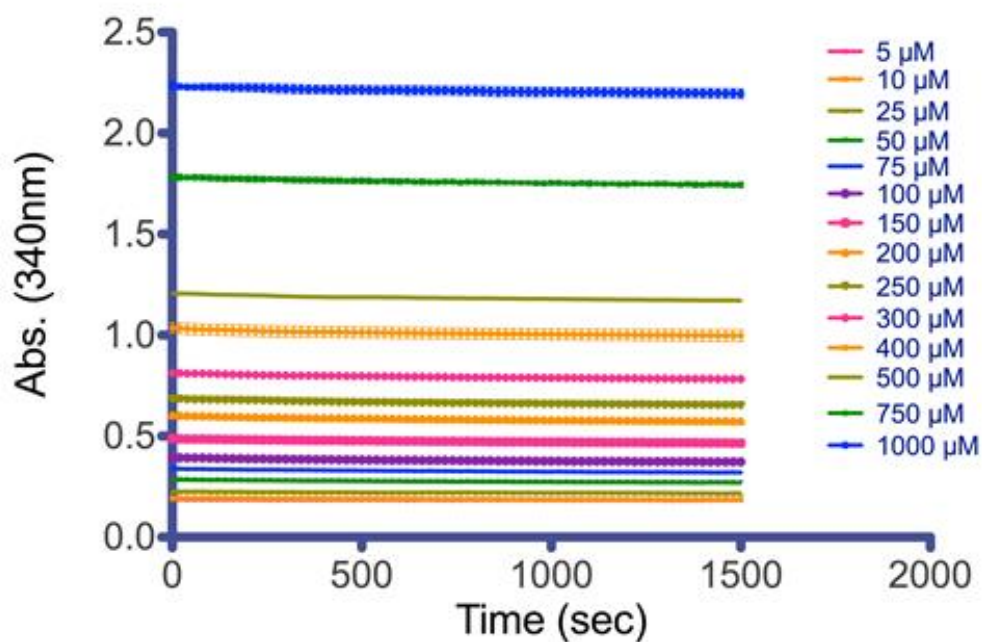
**Supplementary Figure 31:** Michaelis-Menten plot of Full-Cage [G6pDH] (red circles), compared with that of the free G6pDH enzyme (black squares), using glucose 6-phosphate as the substrate. The solid lines represent fits of the Michaelis-Menten model to the data. Enzyme assay conditions: 0.5 nM enzyme or DNA cage-encapsulated enzyme, 1mM  $\text{NAD}^+$ , with different concentration of glucose-6-phosphate ranging from 10  $\mu\text{M}$  to 2000  $\mu\text{M}$ , in 1 $\times$ TBS buffer (pH 7.5, 1 mM  $\text{MgCl}_2$ ) monitoring absorbance at 340 nm. The table lists the fitting parameters. DNA encapsulation of the enzyme caused a  $\sim 1.4$ -fold increase in  $K_M$  and a  $\sim 4$ -fold increase in  $k_{cat}$ .



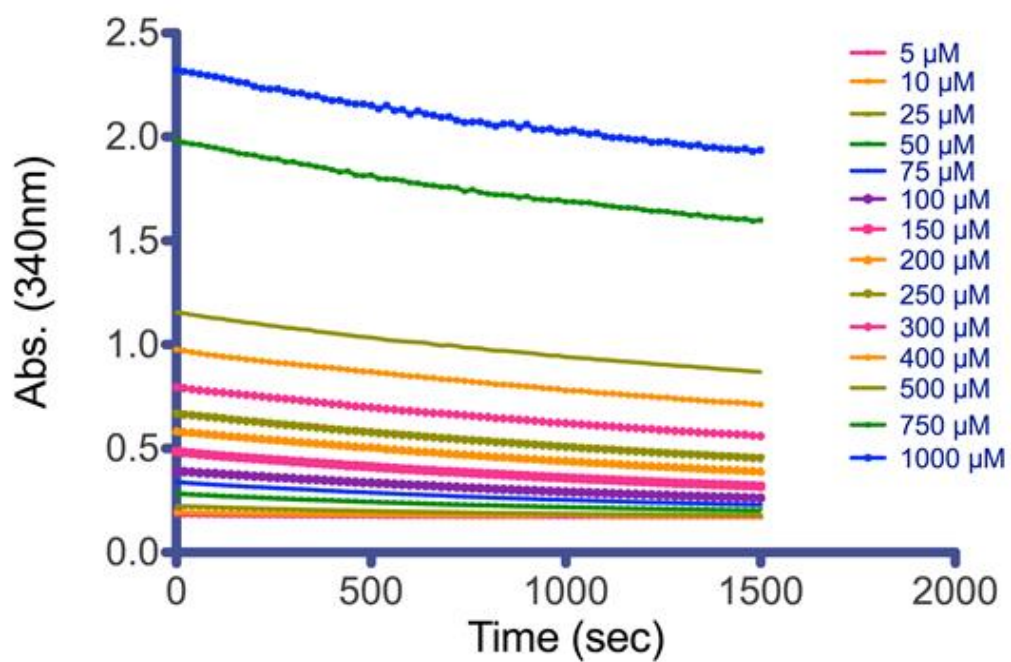
**Supplementary Figure 32:** TEM image of AGE-purified DNA full-cages with G6pDH inside (yellow arrow indicates DNA cage with enzyme inside).



### Determination of the Michaelis-Menten constants for enzymes-MDH



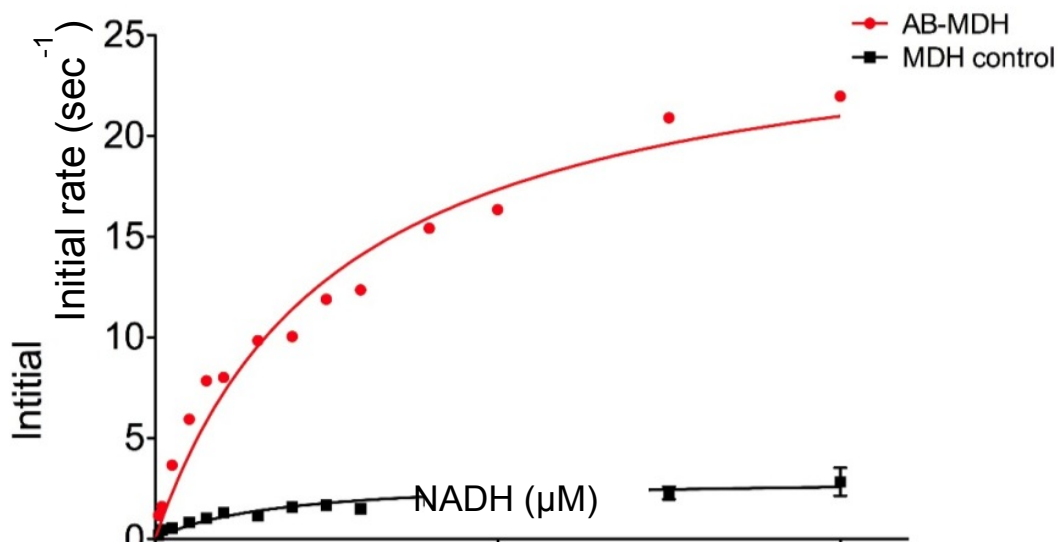
**Supplementary Figure 33:** Raw activity for free DNA-modified MDH (0.5 nM) with 5-1000 μM NADH and 2 mM OAA, monitoring absorbance at 340 nm. Error bars were calculated from the standard deviation of at least three replicates.



**Supplementary Figure 34:** Raw activity for Full-Cage [MDH] (0.5 nM) with 5-1000  $\mu\text{M}$  NADH and 2 mM OAA, monitoring absorbance at 340 nm. Error bars were calculated from the standard deviation of at least three replicates.

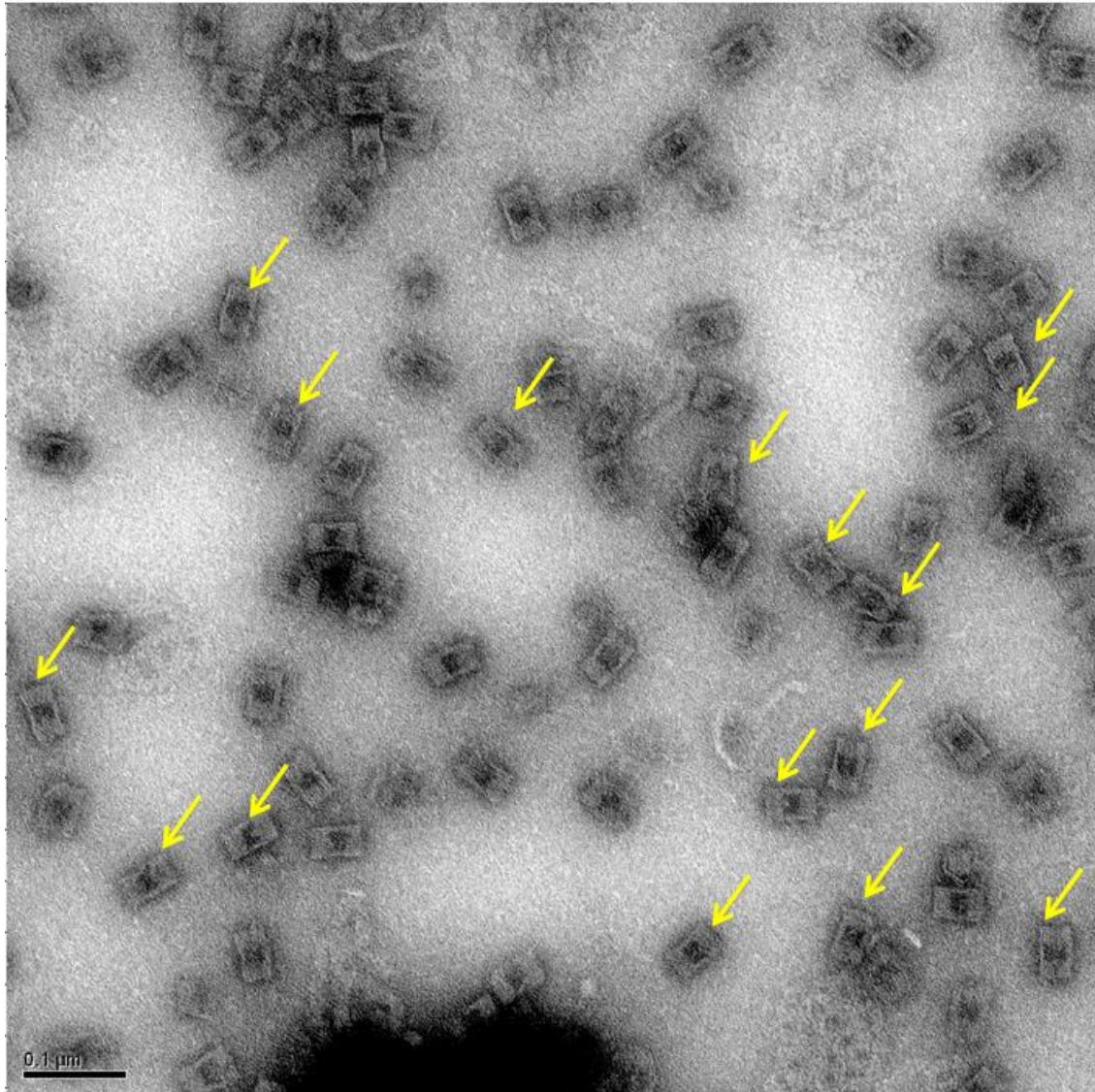
## MDH enzyme kinetics-NADH

Full[MDH]  
Free MDH



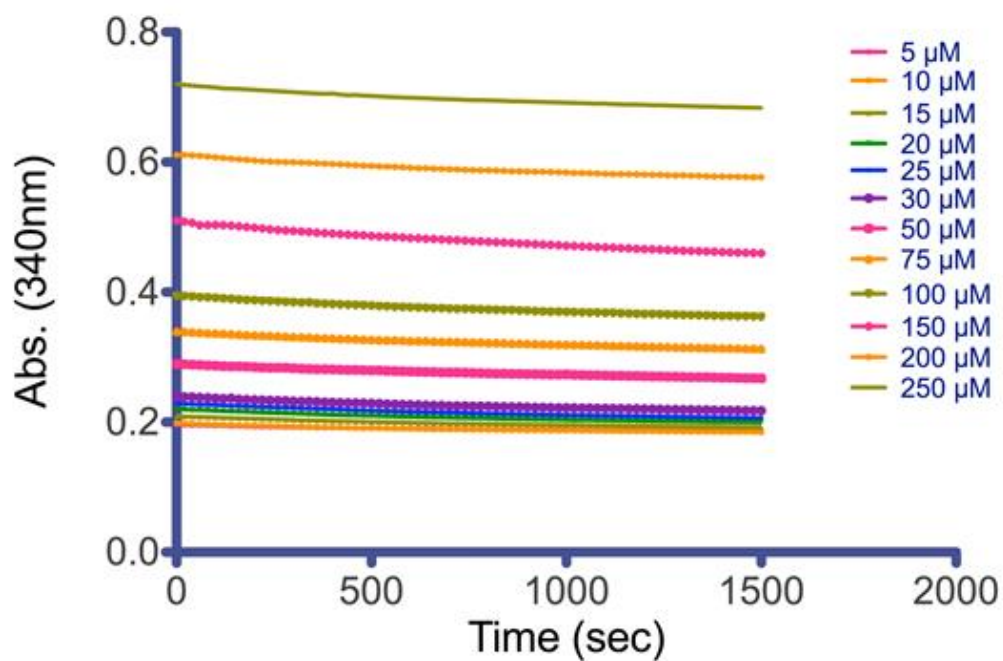
	$K_M$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
<b>Full[MDH]</b>	$270 \pm 50$	$460 \pm 30$
<b>MDH control</b>	$180 \pm 50$	$51 \pm 5$

**Supplementary Figure 35:** Michaelis-Menten plot of Full-Cage [MDH] (red circles), compared with that of free MDH (black squares) using NADH as the varying substrate. The solid lines represent fits of the Michaelis-Menten model to the data. Enzyme assay conditions: 0.5 nM enzyme or DNA cage-encapsulated enzyme, 2 mM OAA, with different concentration of NADH ranging from 5  $\mu\text{M}$  to 1000  $\mu\text{M}$ , in HEPES buffer (pH 7.5, 1 mM  $\text{MgCl}_2$ ) monitoring absorbance at 340 nm. The table lists the fit parameters. DNA encapsulation of the enzyme caused a  $\sim 1.5$ -fold increase in  $K_M$  and a  $\sim 9$ -fold increase in  $k_{cat}$ .

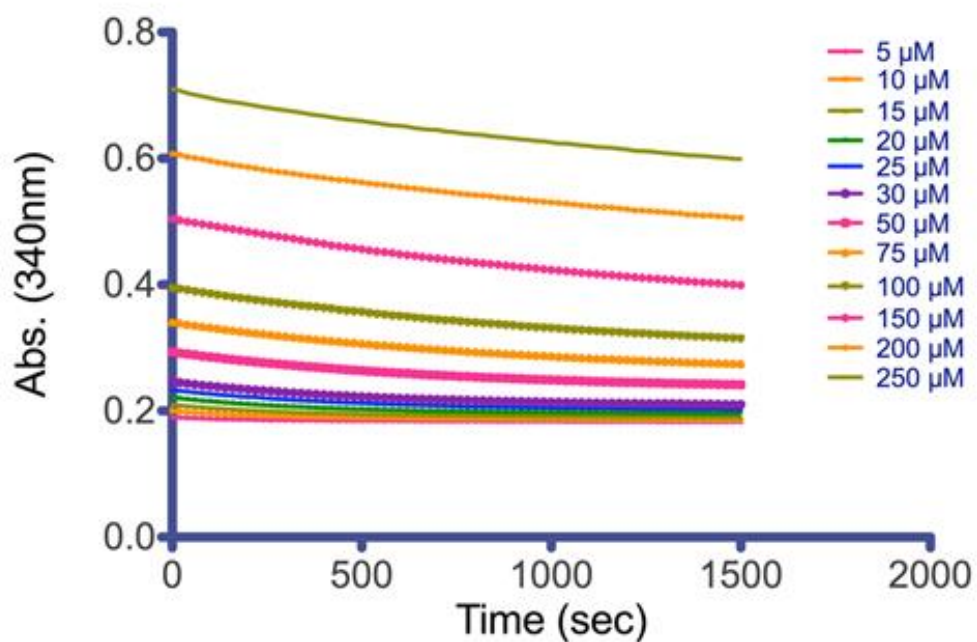


**Supplementary Figure 36:** TEM image for DNA full-cages with MDH inside (yellow arrow indicates DNA cage with enzyme inside).

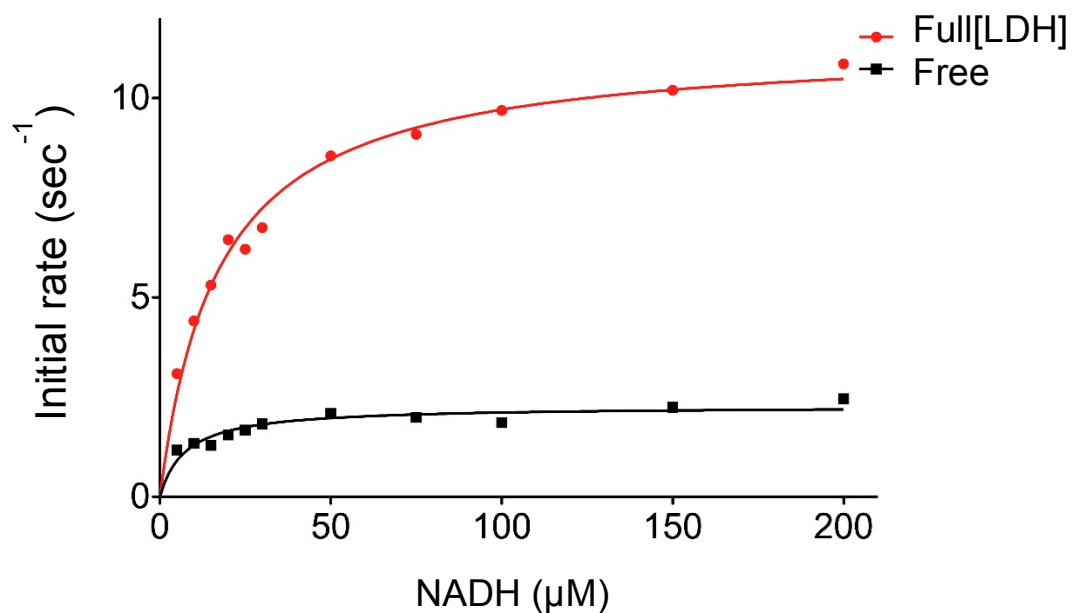
### Determination of the Michaelis-Menten constants for enzymes-LDH



**Supplementary Figure 37:** Raw activity for free DNA-modified LDH (0.5 nM) with 5-250 μM NADH and 2 mM pyruvate, monitoring absorbance at 340 nm. (Error bars were calculated from the standard deviation of at least three replicates)



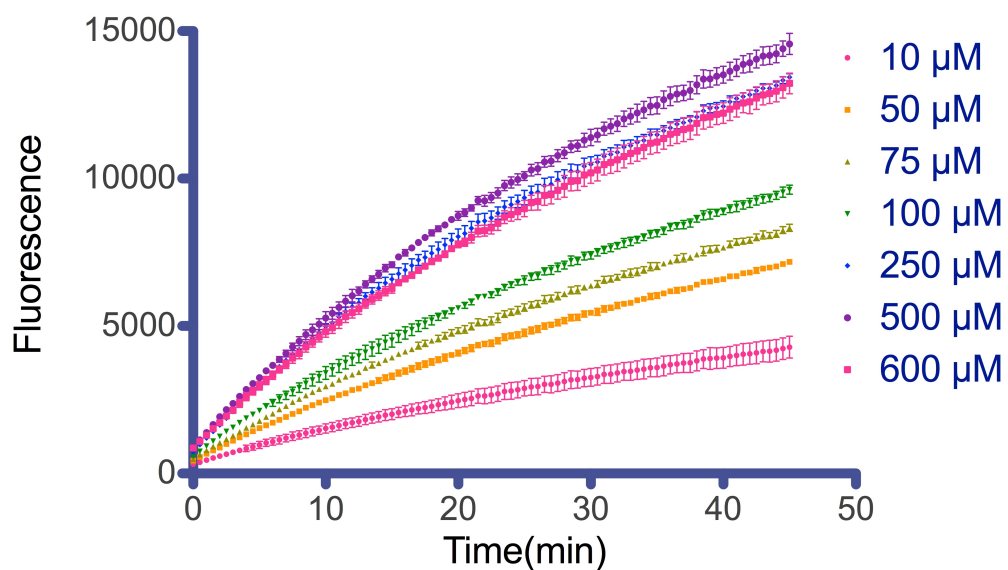
**Supplementary Figure 38:** Raw activity for full-cage [LDH] (0.5 nM) with 5-250 μM NADH and 2 mM pyruvate, monitoring absorbance at 340 nm. (Error bars were calculated from the standard deviation of at least three replicates)



	$K_M$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
<b>Full[LDH]</b>	$17.0 \pm 1.5$	$190 \pm 5$
<b>LDH control</b>	$7.2 \pm 1.3$	$46 \pm 2$

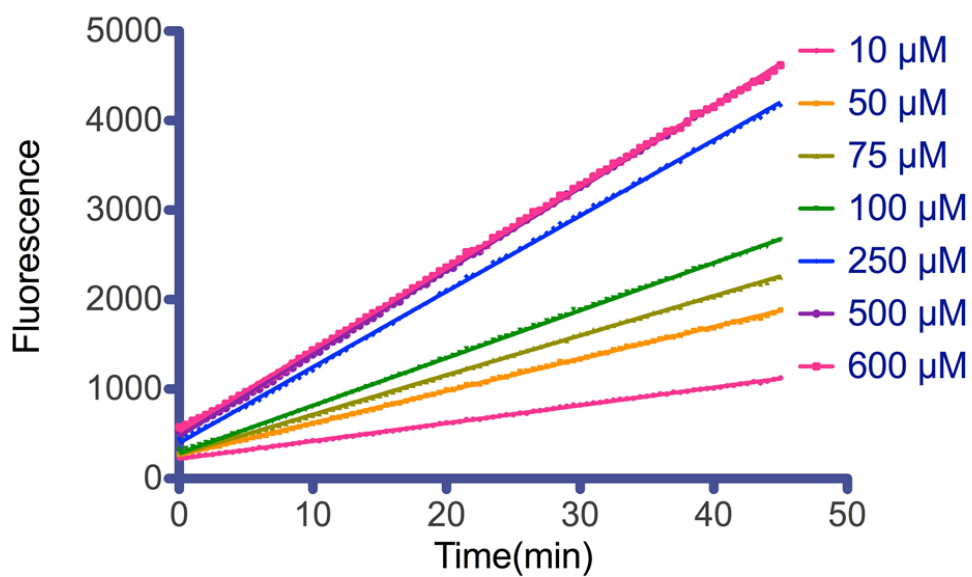
**Supplementary Figure 39:** Michaelis-Menten plot of Full-Cage [LDH] (red circles), compared with that of free LDH (black squares), using NADH as the varying substrate. The solid lines represent fits of the Michaelis-Menten model to the data. Enzyme assay condition: 0.5 nM enzyme or DNA cage encapsulated enzyme, 2 mM pyruvate, with different concentration of NADH ranging from 5  $\mu\text{M}$  to 200  $\mu\text{M}$ , in HEPES buffer (pH 7.5, 1 mM  $\text{MgCl}_2$ ) monitoring absorbance at 340 nm. The table lists the fit parameters. DNA encapsulation of the enzyme caused a  $\sim 2.4$ -fold increase in  $K_M$  and a  $\sim 4$ -fold increase in  $k_{cat}$ .

## Determination of the Michaelis-Menten constants for enzymes - $\beta$ -Gal

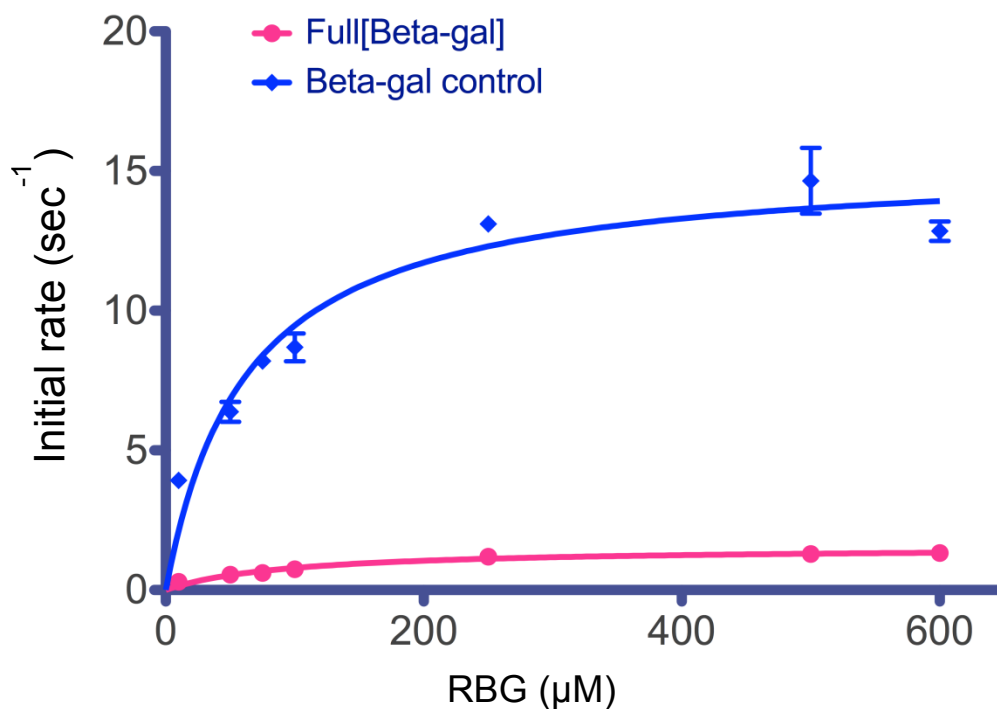


**Supplementary Figure 40:** Raw activity for free DNA-modified  $\beta$ -Gal (0.5 nM) with different concentration of, ranging from 10  $\mu$ M to 600  $\mu$ M RBG, monitoring fluorescence at 590 nm (excitation 532 nm). Error bars were calculated from the standard deviation of at least three replicates.



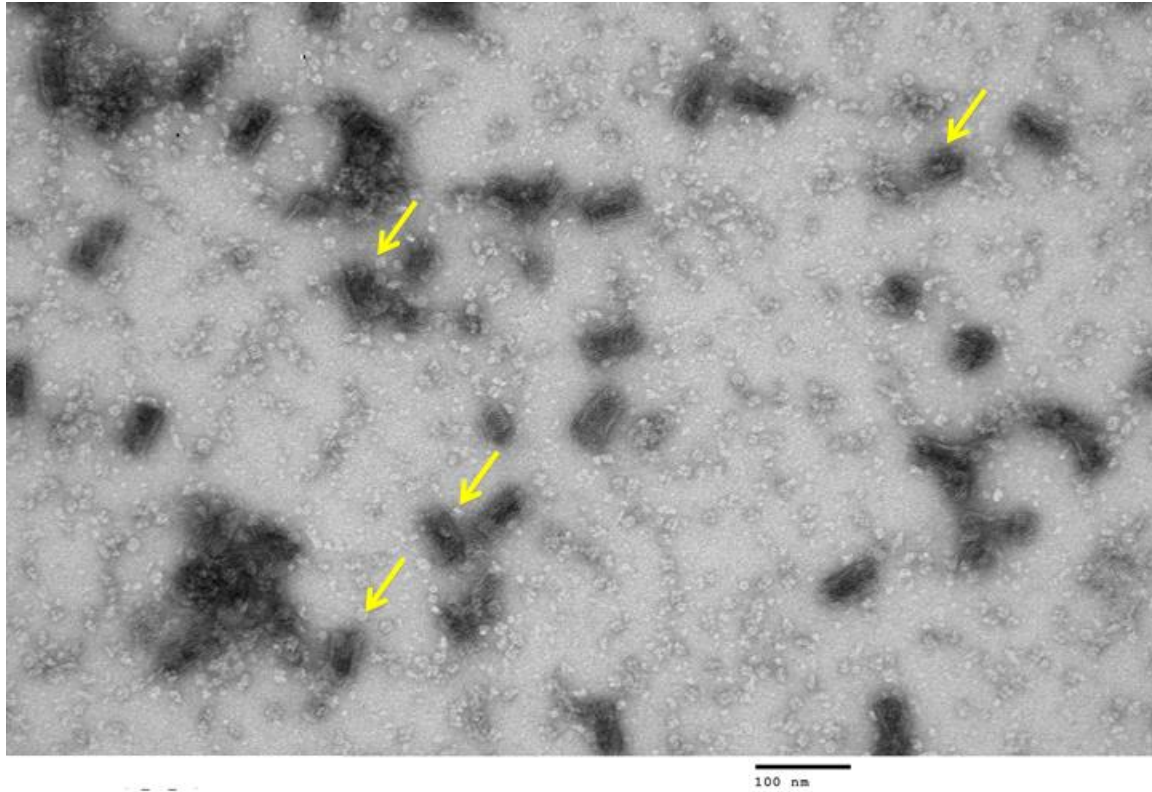


**Supplementary Figure 41:** Raw activity for full-cage [β-Gal] (0.5 nM) with different concentration of, ranging from 10 μM to 600 μM RBG, monitoring fluorescence at 590 nm (excitation 532 nm). Error bars were calculated from the standard deviation of at least three replicates.

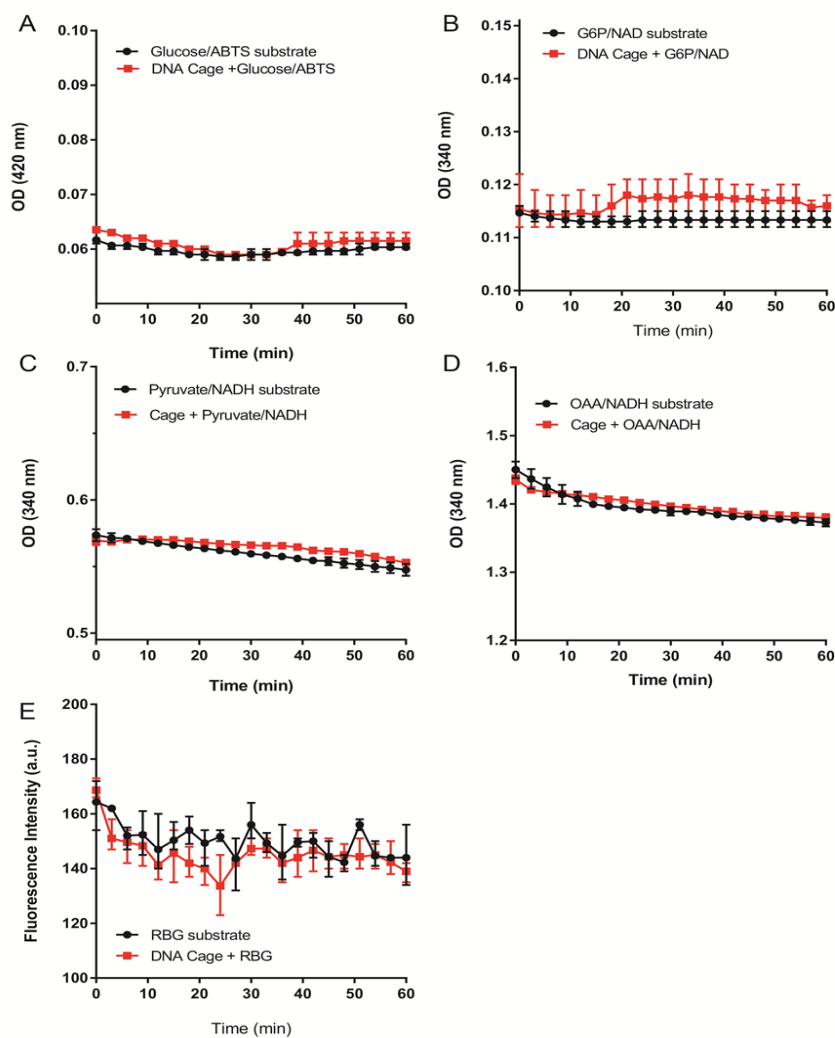


	$K_M$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
<b>Full[<math>\beta</math>-Gal]</b>	$95.5 \pm 18.9$	$1.6 \pm 0.1$
<b><math>\beta</math>-Gal control</b>	$58.7 \pm 16.0$	$8.5 \pm 0.6$

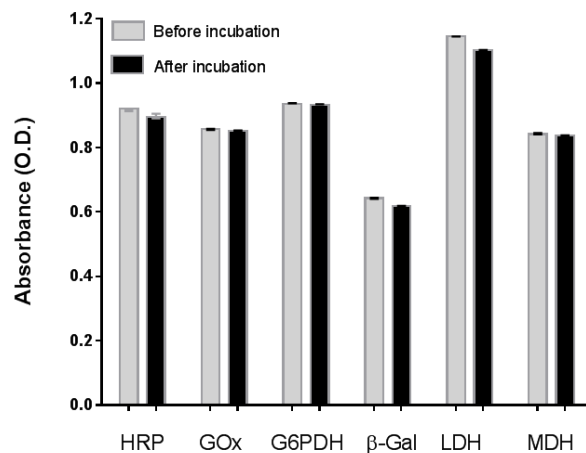
**Supplementary Figure 42:** Michaelis-Menten plot of full-cage [ $\beta$ -Gal] (red circle), compared with that of the fresh free MDH enzyme (blue square) using RBG as the substrate. The solid line is the fitting curve using the Michaelis-Menten model. Enzyme assay condition: 0.5 nM enzyme or DNA cage encapsulated enzyme, with different concentration of RBG, ranging from 10  $\mu\text{M}$  to 600  $\mu\text{M}$ , in TBS buffer (pH 7.5, 1 mM  $\text{MgCl}_2$ ) monitoring fluorescence at 532/590 nm. The table lists the fitting parameters. DNA encapsulation of the enzyme caused a  $\sim 1.6$ -fold increase in  $K_M$  and a  $\sim 81\%$  decrease in  $k_{cat}$ . Error bars were calculated from the standard deviation of at least three replicates.



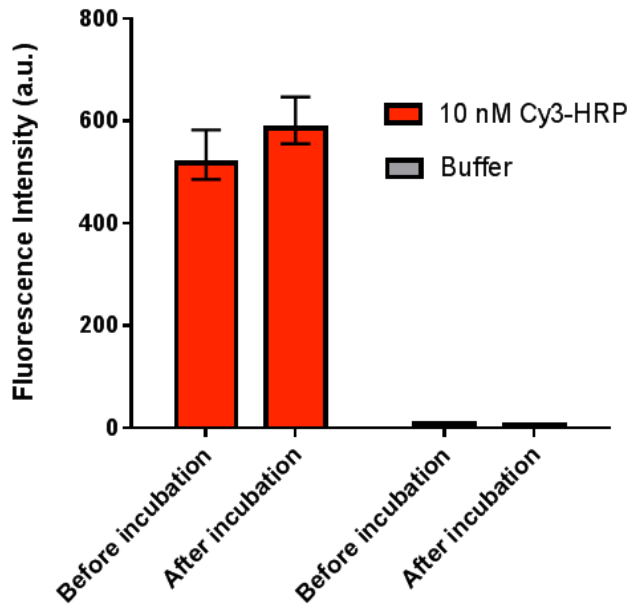
**Supplementary Figure 43:** TEM image for the DNA full-cages with  $\beta$ -Gal inside (yellow arrow indicates DNA cage with enzyme inside).



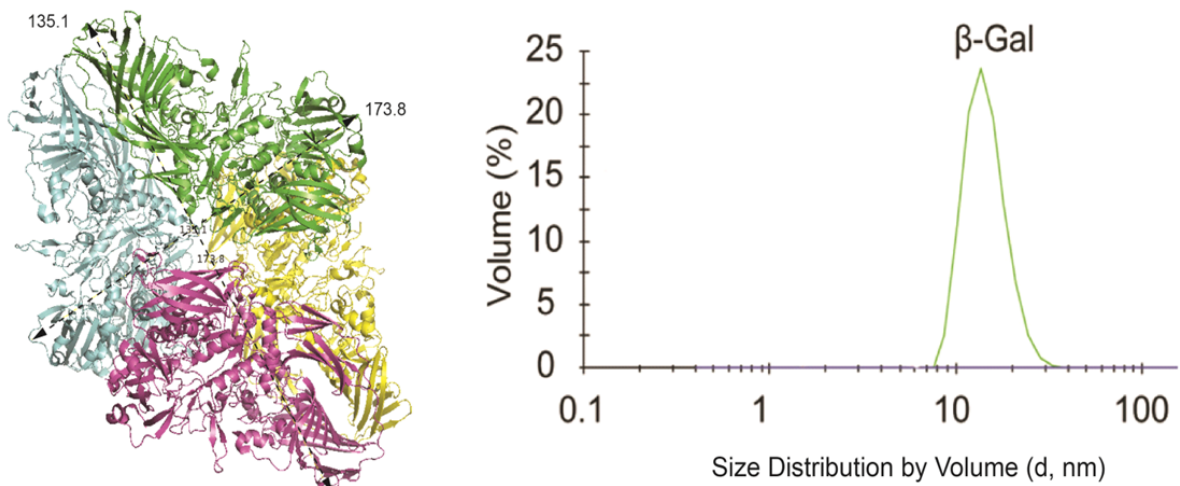
**Supplementary Figure 44:** Control experiments in which DNA cages were incubated with enzyme substrates. (A) Red curve: 1 nM Cage was incubated with 1 mM glucose and 2 mM ABTS (GOx/HRP substrates) in 1×TBS, pH 7.5; Black: Autocatalysis of 1 mM glucose and 2 mM ABTS (GOx/HRP substrates) in 1×TBS, pH 7.5. (B) Red: 0.5 nM Cage was incubated with 1 mM glucose-6-phosphate and 1 mM NAD<sup>+</sup> (G6PDH substrates) in 1×TBS, pH 7.5; Black: Autocatalysis of 1 mM glucose-6-phosphate and 1 mM NAD<sup>+</sup> in 1×TBS, pH 7.5. (C) Red: 0.5 nM Cage was incubated with 2 mM pyruvate and 0.25 mM NADH (LDH substrates) in 1×TBS, pH 7.5; Black: Autocatalysis of 2 mM pyruvate and 0.25 mM NADH in 1×TBS, pH 7.5. (D) Red: 0.5 nM Cage was incubated with 2 mM oxaloacetate (OAA) and 1 mM NADH (MDH substrates) in 1×TBS, pH 7.5; Black: Autocatalysis of 2 mM OAA and 1 mM NADH in 1×TBS, pH 7.5. (E) Red: 0.5 nM Cage was incubated with 100 μM resorufin beta-D-galactopyranoside (RBG, β-Gal substrate) in 1×TBS, pH 7.5; Black: Autocatalysis of 100 μM RBG in 1×TBS, pH 7.5, 532 nm (excitation)/590 nm (emission). Error bars were calculated from the standard deviation of at least three replicates. All above results indicate that DNA cages at our experimental concentrations do not significantly catalyze substrate conversion.



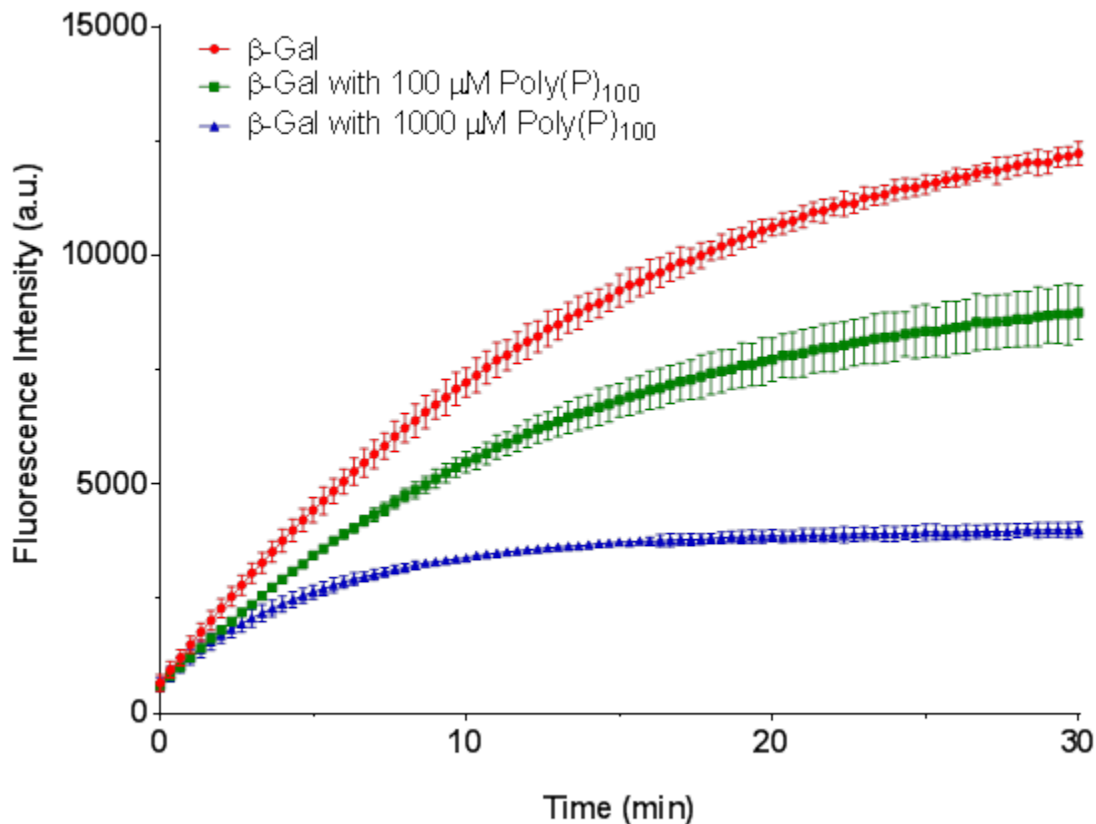
**Supplementary Figure 45:** Test of the nonspecific adsorption of enzymes onto a plastic 96-well plate. Enzyme concentrations are quantified by UV-VIS spectrometer using the following extinction coefficients: HRP ( $E_{405\text{ nm}} \sim 100,000\text{ M}^{-1}\text{ cm}^{-1}$ ), GOx ( $E_{280\text{ nm}} \sim 267,200\text{ M}^{-1}\text{ cm}^{-1}$ ), G6pDH ( $E_{280\text{ nm}} \sim 118,450\text{ M}^{-1}\text{ cm}^{-1}$ ),  $\beta$ -Gal ( $E_{280\text{ nm}} \sim 972,093\text{ M}^{-1}\text{ cm}^{-1}$ ), LDH ( $E_{280\text{ nm}} \sim 202,640\text{ M}^{-1}\text{ cm}^{-1}$ ), MDH ( $E_{280\text{ nm}} \sim 19,600\text{ M}^{-1}\text{ cm}^{-1}$ ). The UV-Vis absorbance of 100  $\mu\text{L}$  of each enzyme solution was measured before adding to the plates, as well as after one hour incubation within the plates in the dark. These conditions are the same as those of the enzyme activity assay. As shown in the Figure, all enzyme solutions showed only a very slight decrease in absorbance after incubation in the plates, suggesting very weak nonspecific adsorption of enzymes onto the plastic plates. Error bars were calculated from the standard deviation of at least three replicates.



**Supplementary Figure 46:** Testing for nonspecific adsorption of low nanomolar concentrations of enzymes onto plastic 96-well plates was tested using Cy3-labeled HRP. 100  $\mu$ L of 10 nM Cy3-labeled HRP was assayed for fluorescence intensity, and then the plate was incubated inside a plate reader for one hour. The remaining fluorescence intensity was tested again. A slight increase of fluorescence intensity was observed, possibly due to the buffer evaporation during the incubation. This result suggests that there is very little nonspecific adsorption of Cy3-HRP onto the 96-well plate. Error bars were calculated from the standard deviation of at least three replicates.

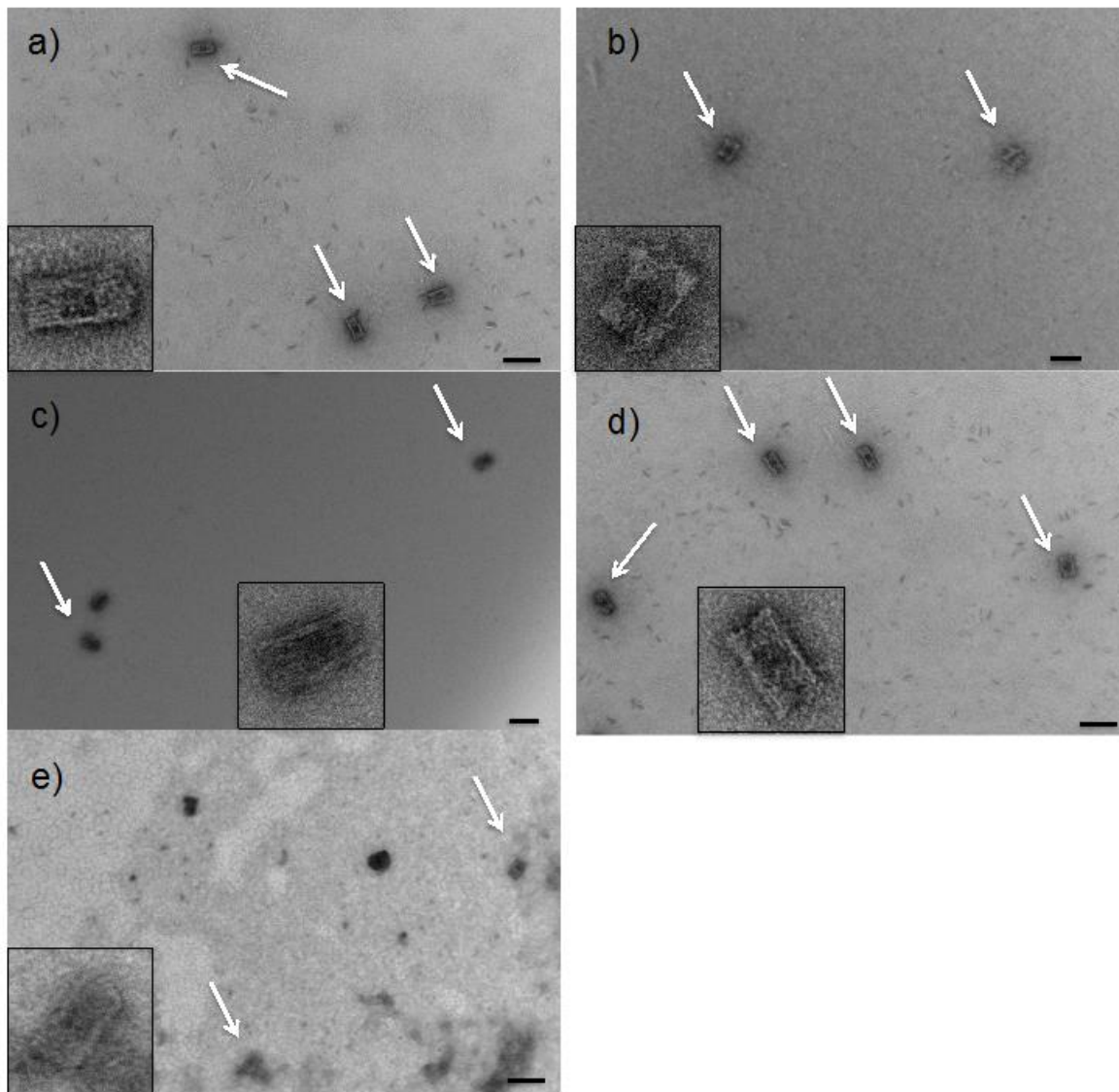


**Supplementary Figure 47:** The crystal structure of  $\beta$ -Gal shows its dimensions to be  $\sim 17 \text{ nm} \times 14 \text{ nm}$  (left) (Jacobson, R. H. et al. *Nature* **369**, 761-766 (1994)). Dynamic Light Scattering measures a hydrodynamic diameter of  $\sim 14 - 18 \text{ nm}$ .

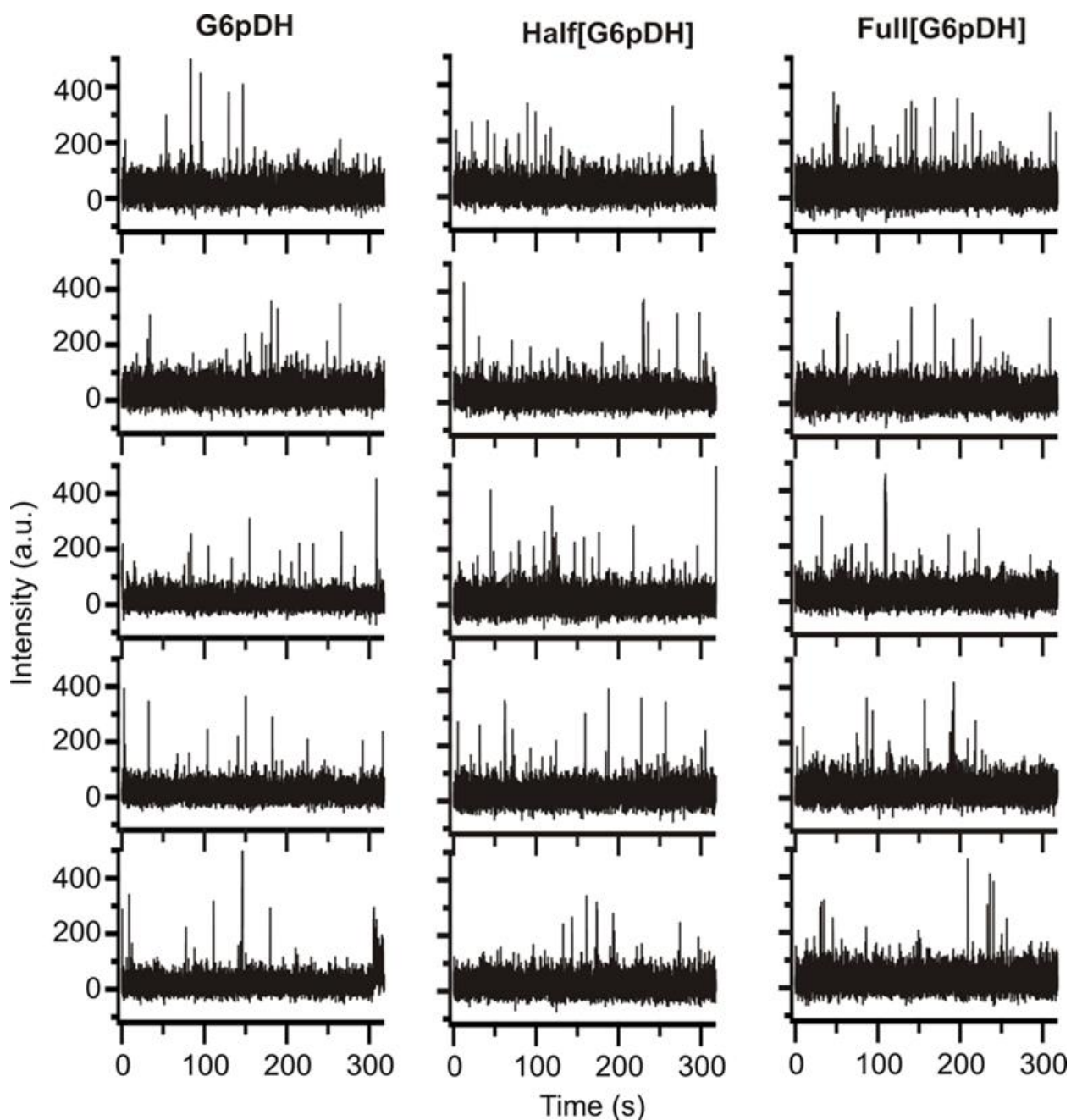


**Supplementary Figure 48:** Inhibition of  $\beta$ -Gal activity by 100-mer polyphosphate (Poly(P)<sub>100</sub>) in solution. Assay condition: 0.25 nM  $\beta$ -Gal and 100  $\mu$ M RBG in pH 7.4, 50 mM HEPES buffer. For inhibition assay,  $\beta$ -Gal was first incubated with Poly(P)<sub>100</sub> for half an hour, then RBG substrate was added before measuring the activity. The control  $\beta$ -Gal was run at the same condition except for the incubation with buffer for half an hour. The activity of  $\beta$ -Gal was significantly inhibited by 1000  $\mu$ M Poly(P)<sub>100</sub>. Error bars were calculated from the standard deviation of at least three replicates.

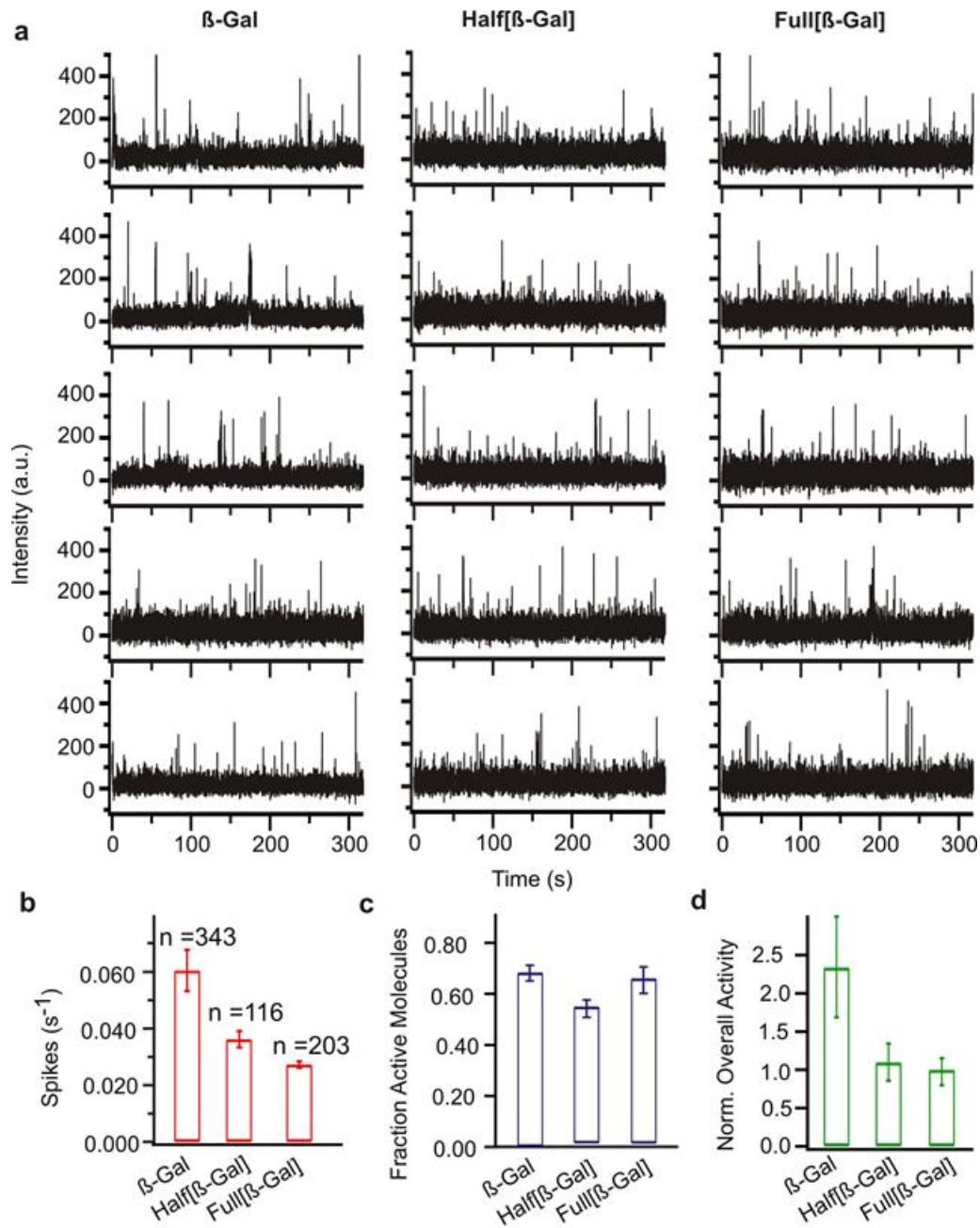




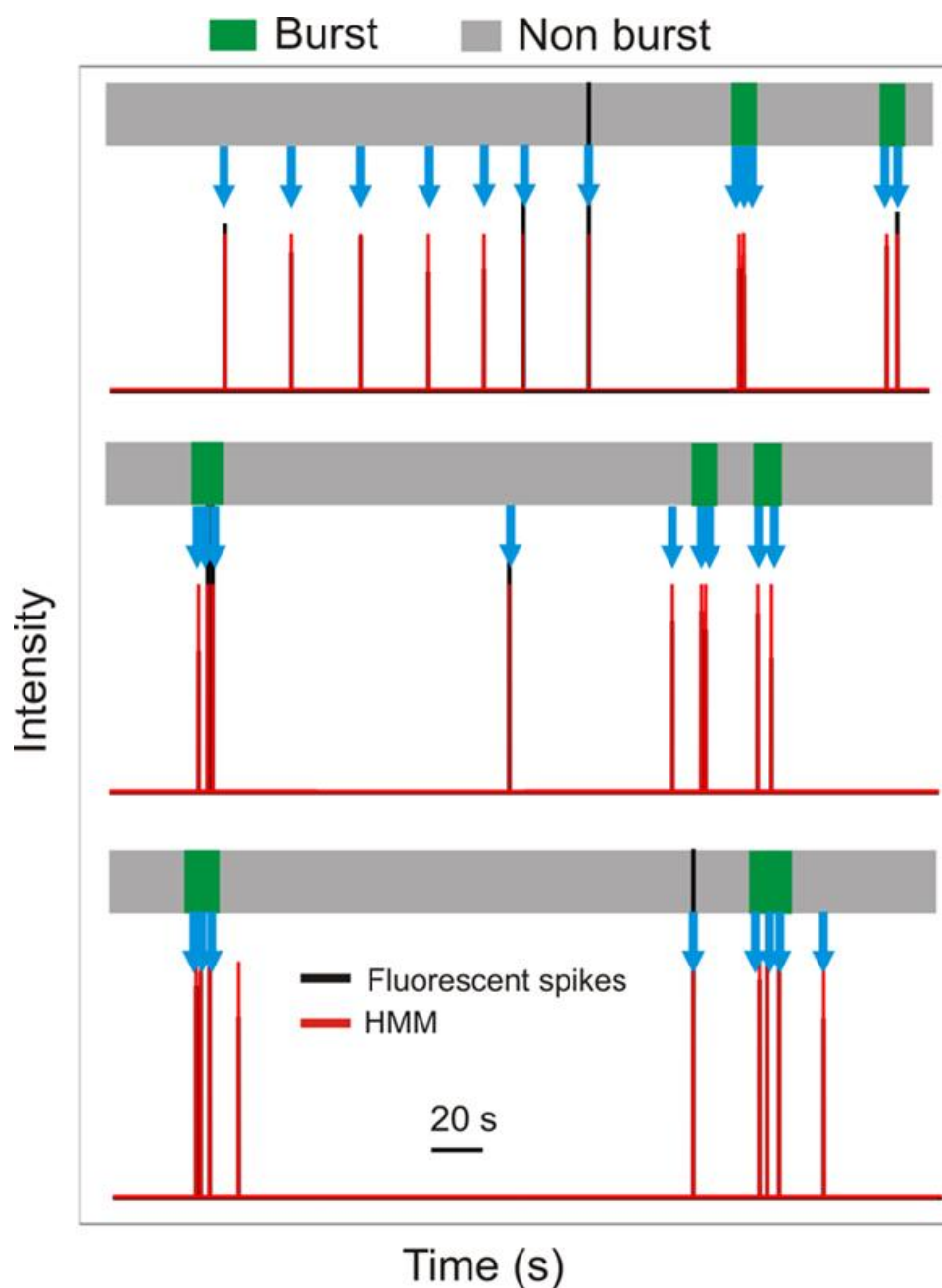
**Supplementary Figure 49:** TEM image of DNA cages after 1h incubation with a) GOx-HRP enzymatic reaction (conditions: 50 mM HEPES, pH 7.5, 1mM MgCl<sub>2</sub>, 1mM glucose, 2mM ABTS, 1nM GOx-HRP, 0.5nM DNA cage), b) G6pDH enzyme reaction (conditions: 50 mM HEPES, pH 7.5, 1mM MgCl<sub>2</sub>, 1mM glucose-6-phosphate, 1mM NAD<sup>+</sup>, 1nM G6pDH, 0.5nM DNA cage), c) MDH enzyme reaction (conditions: 50 mM HEPES, pH 7.5, 1mM MgCl<sub>2</sub>, 2mM OAA, 1mM NADH, 1nM MDH, 0.5nM DNA cage), d) LDH enzyme reaction (conditions: 50 mM HEPES, pH 7.5, 1mM MgCl<sub>2</sub>, 2mM pyruvate, 1mM NADH, 1nM LDH, 0.5nM DNA cage), e) β-gal enzyme reaction (conditions: 50 mM HEPES, pH 7.5, 1mM MgCl<sub>2</sub>, 1mM RBG 1nM Beta-gal, 0.5nM DNA cage). (Scale bars: 50 nm)



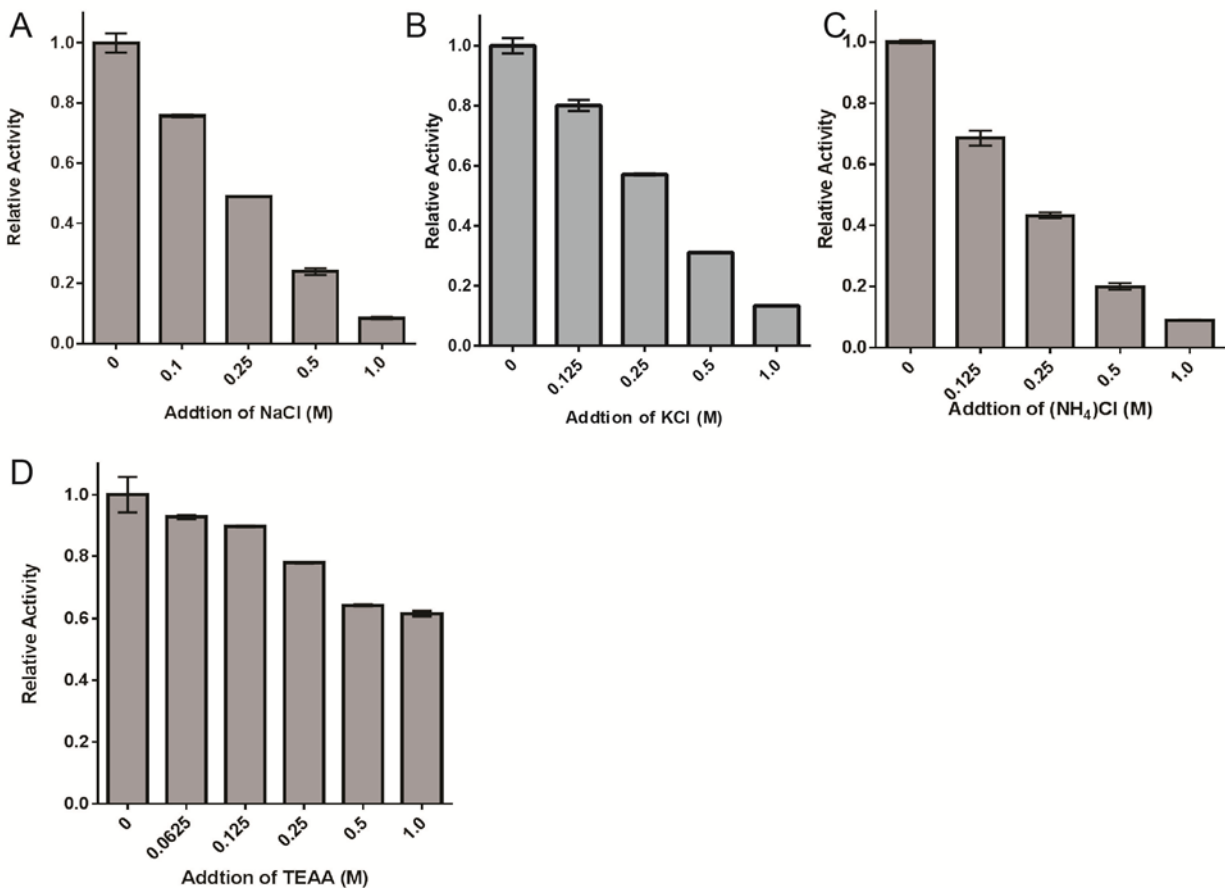
**Supplementary Figure 50: Raw enzyme activity data of single G6pDH molecules.** Representative fluorescence-time traces of free-, half-cage and full-cage G6pDH. Five representative molecules are shown for each sample. The fluorescence intensity of enzyme reaction on the microscope slide was recorded for ~5 min at 35 ms time resolution. The average spikes per molecule for different samples are compared in Fig. 5. All experiments were carried out at room temperature in  $1\times$  TBS buffer in presence of  $1\text{ mM Mg}^{2+}$ , pH 7.5 (Supplementary Table S4).



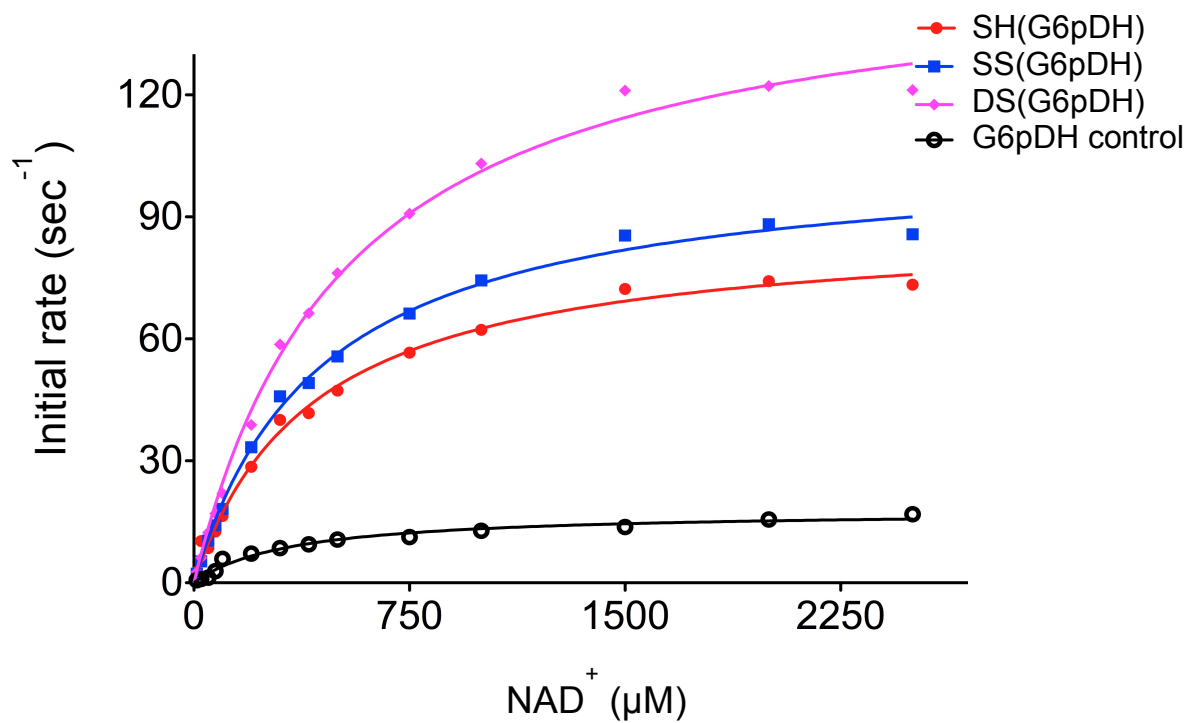
**Supplementary Figure 51: Enzyme activity data of single  $\beta$ -Gal molecules.** (a) Representative raw fluorescence-time traces of free-, half-cage and full-cage  $\beta$ -Gal. Five representative molecules are shown for each sample. The fluorescence intensity of enzyme reaction on the microscope slide was recorded for  $\sim 5$  min at 35 ms time resolution. (b,c,d) Statistics of spike frequency, fraction of active molecules, and overall observed enzyme activity. The number of active molecules analyzed is denoted by ‘n’ in b. The standard deviations for spike frequency and fraction of active molecules were calculated after randomly assigning the active molecules into three groups. The standard deviation for the normalized overall activity was estimated from the propagation of errors. All experiments were carried out at room temperature in  $1\times$  TBS buffer, pH 7.5 in presence of 1 mM  $Mg^{2+}$  and 10% (w/v) PEG 8000.



**Supplementary Figure 52:** Representative intensity-time traces (black) of full-cage enzyme after background correction and Hidden Markov Model (HMM) idealization to a two-state model (red). The fluorescence-time traces of the enzyme reaction on the microscope slide were recorded at 35 ms time resolution over ~5 min.

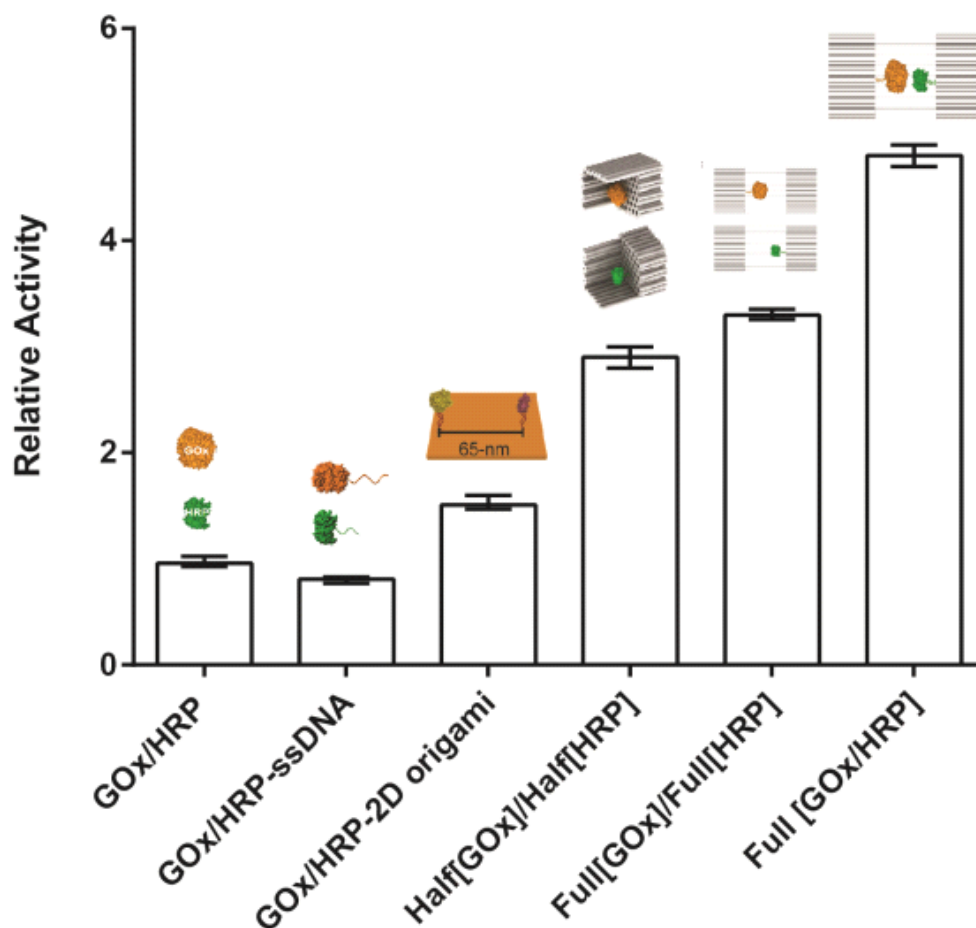


**Supplementary Figure 53:** Titrations showing the effects of (A) NaCl, (B) KCl, (C) NH<sub>4</sub>Cl and (D) Triethylammonium acetate (TEAA) on the activity of free G6pDH. Assay conditions: 0.5 nM enzyme was incubated with a series of ion concentrations from low to high. Enzyme activity was monitored by absorbance at 340nm with the addition of 1 mM Glucose-6-phosphate and 1 mM NAD<sup>+</sup> in 1×TBS buffer (pH 7.5). The results show that high concentration of salts containing small cations such as Na<sup>+</sup>, K<sup>+</sup> and NH<sub>4</sub><sup>+</sup> significantly reduce the activity of G6pDH, possibly due to the chaotropic ion effect that disrupts hydrogen-bonded water structures as reported in the previous studies (Zhao, H. *Journal of Molecular Catalysis B: Enzymatic* 2005, 37, 16; Leberman, R. and Soper, A. K. *Nature* 1995, 378, 364.). Conversely, the salt containing a bulky organic cation (kosmotropic), triethylammonium, does not strongly inhibit enzyme activity, even at high concentrations. Error bars were calculated from the standard deviation of at least three replicates.



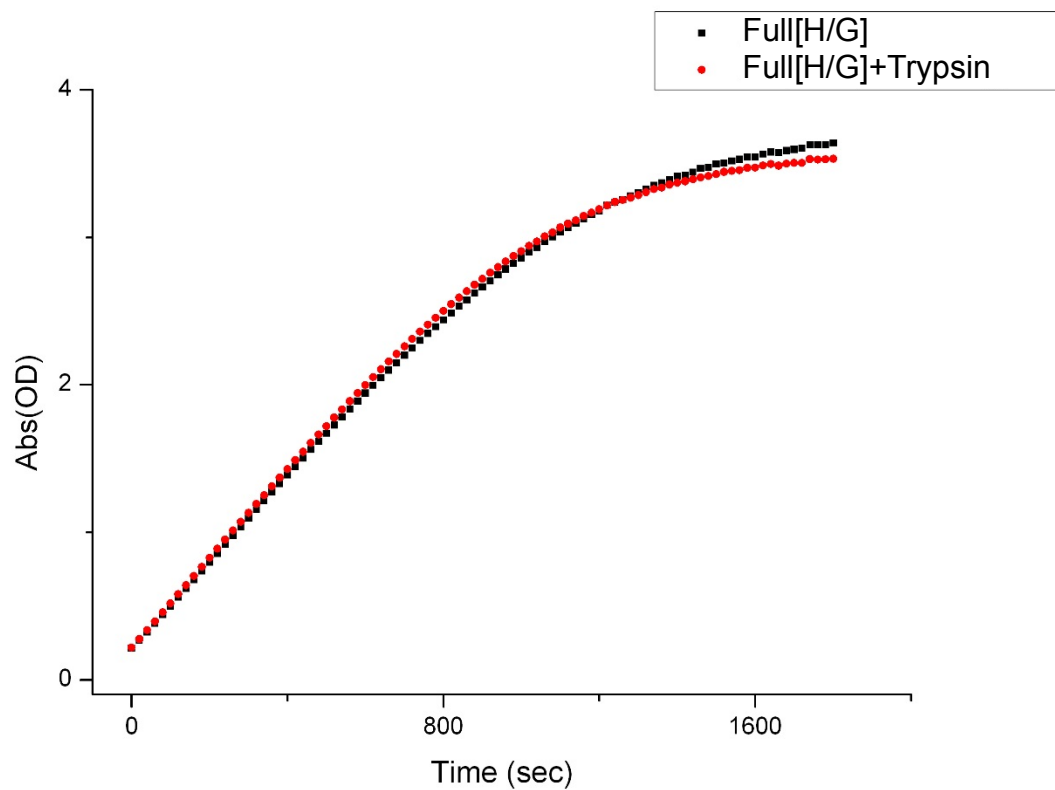
	$K_M$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )
SH-G6pDH	$411 \pm 32$	$520 \pm 10$
SS-G6pDH	$436 \pm 26$	$620 \pm 10$
DS-G6pDH	$527 \pm 37$	$900 \pm 20$
G6pDH control	$340 \pm 47$	$100 \pm 10$

**Supplementary Figure 54:** Comparison of G6pDH activity inside three different DNA full-cages, compared with that of free G6pDH, using  $\text{NAD}^+$  as the varying substrate. The SH, SS and DS cages are described in the main text. Enzyme assay conditions: 0.5 nM enzyme or DNA-cage-encapsulated enzyme, 1 mM glucose 6-phosphate, with different concentration of  $\text{NAD}^+$  ranging from 10  $\mu\text{M}$  to 2500  $\mu\text{M}$ , in  $1 \times \text{TBS}$  buffer (pH 7.5, 1 mM  $\text{MgCl}_2$ ) monitoring absorbance at 340 nm. The table lists the fit parameters. Encapsulation of the enzyme in different DNA full-cages caused a  $\sim 1.2$ - to  $1.5$ -fold increase in  $K_M$  and a  $\sim 5$ - to  $9$ -fold increase in  $k_{cat}$ .



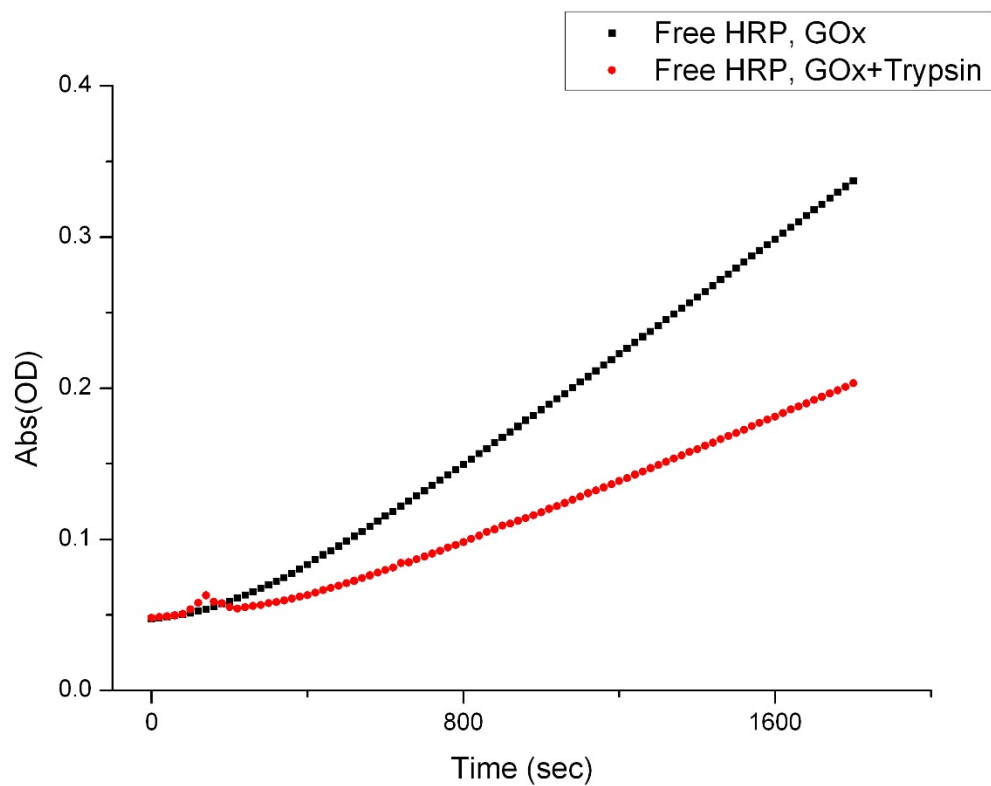
**Supplementary Figure 55:** The relative activity of a GOx/HRP pair when attached to a variety of DNA scaffolds: enzyme wildtypes (GOx/HRP), ssDNA (GOx/HRP-ssDNA), 2D rectangular DNA origami (GOx/HRP-2D origami), separate 3D half cages (Half[GOx]/Half[HRP]), separate full cages (Full[GOx]/Full[HRP]) and the same full cage (Full [GOx/HRP]). Enzyme activity is positively correlated to the density of DNA helices within the scaffolds, and partially or fully caged enzymes exhibit activity several-fold higher than that of free and unconjugated enzymes. Error bars were calculated from the standard deviation of at least three replicates. The value for GOx/HRP-2D origami is extracted from our previously published article (Fu, J. et al. *JACS* **2012**, *134*, 5516–5519).

We concluded that the boosted activities of Full[GOx/HRP] cannot be simply attributed to a single factor of DNA density or close proximity, but may be induced by both of the high DNA density and close proximity within a DNA cage.

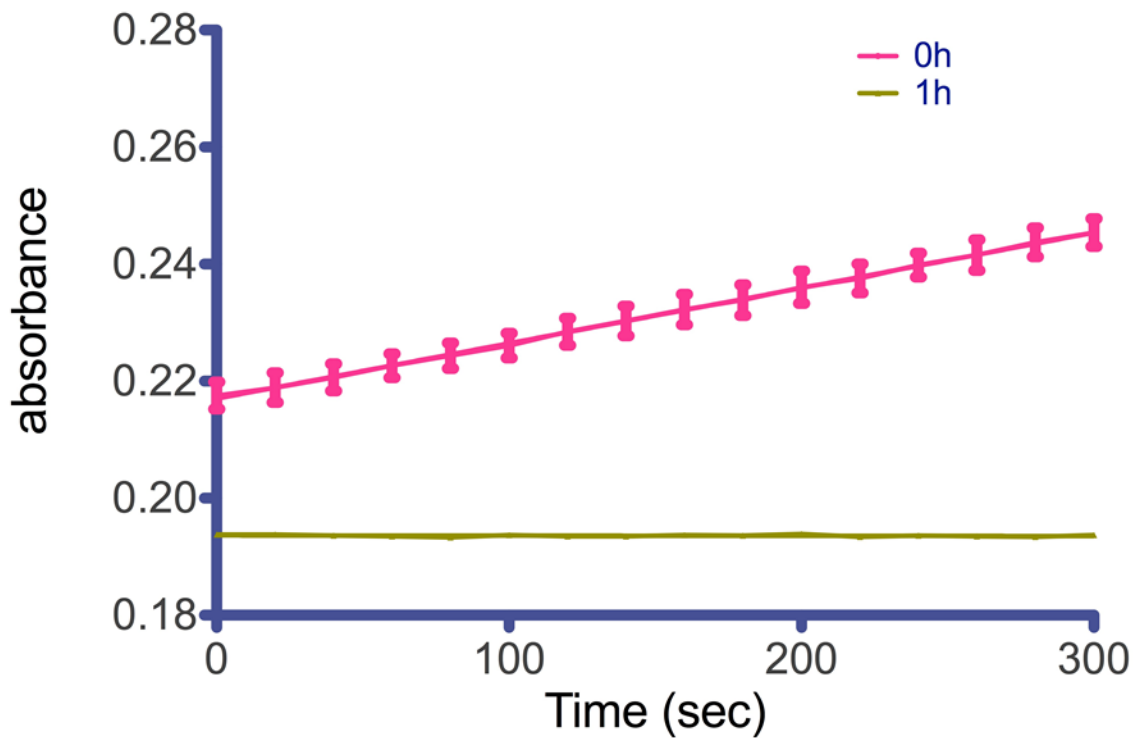


**Supplementary Figure 56:** Raw activity for full-cage [HRP/GOx] (0.5 nM) before and after trypsin digestion for 24 hours at 37 °C in 1 × TBS buffer (pH 7.5).

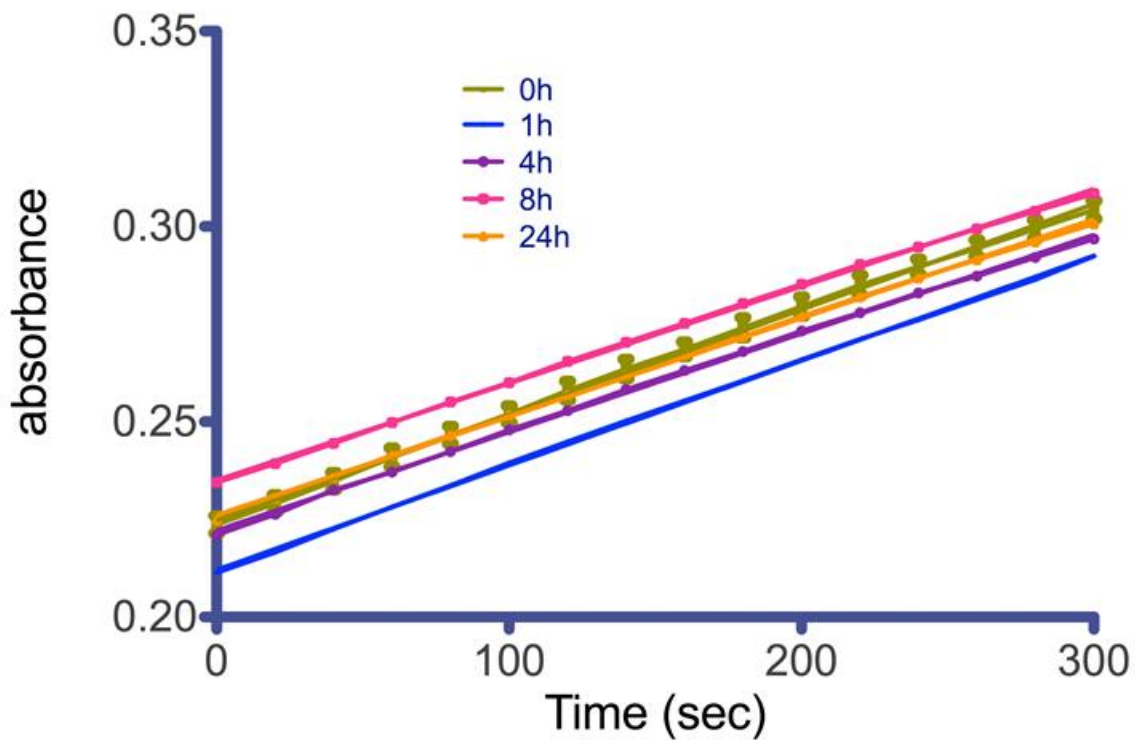




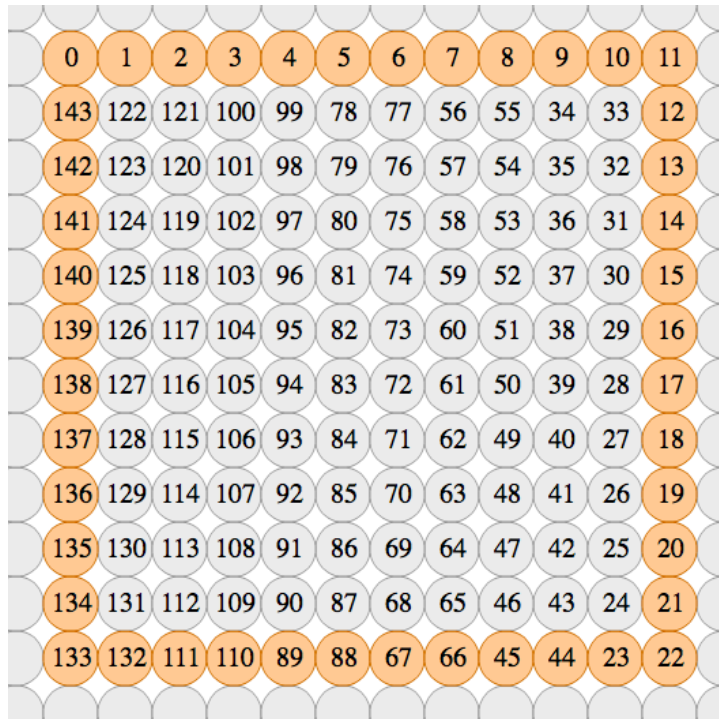
**Supplementary Figure 57:** Raw activity of a free pair of HRP and GOx (0.5 nM) before and after trypsin digestion for 24 hours at 37 °C in 1 × TBS buffer (pH 7.5).



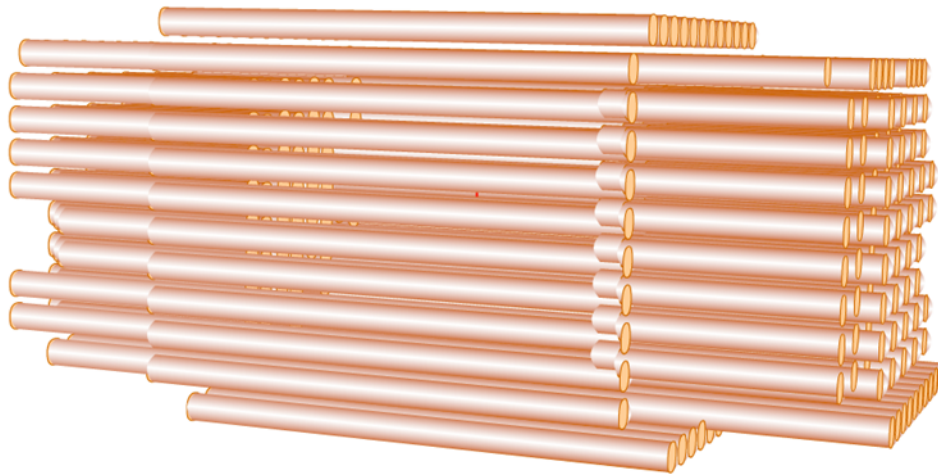
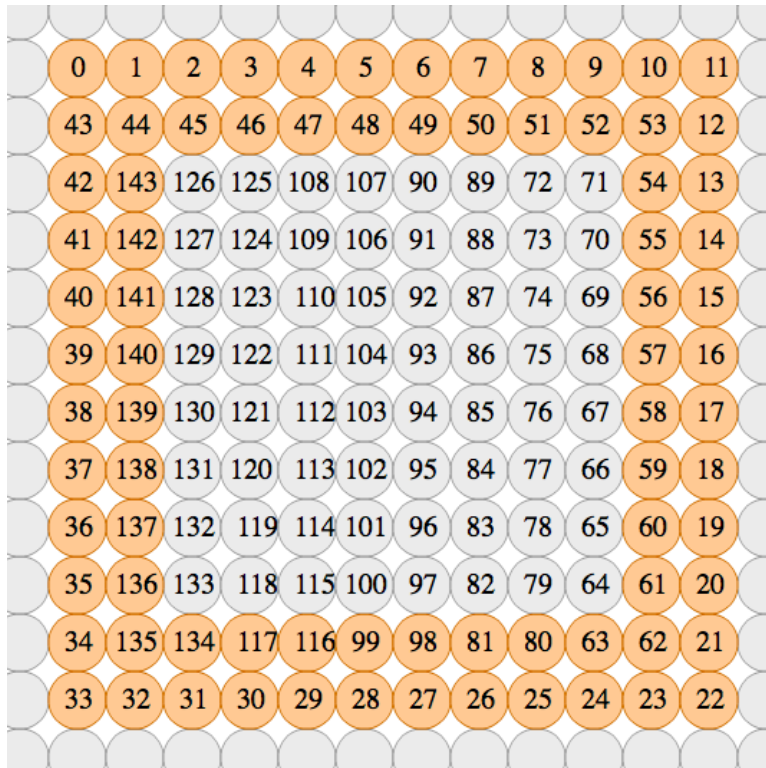
**Supplementary Figure 58:** Raw activity data for free G6pDH (0.5nM) before and after trypsin digestion for 1h at 37 °C in 1 × TBS buffer (pH 7.5). Error bars were calculated from the standard deviation of at least three replicates.



**Supplementary Figure 59:** Raw activity data for Full[G6pDH] (0.5nM) timecourse trypsin digestion from 0 h to 24 h at 37 °C. Error bars were calculated from the standard deviation of at least three replicates.



**Supplementary Figure 60:** Design of SS cage (square lattice arrangement), including cross-sectional view and 3D view.



**Supplementary Figure 61:** Design of DS cage (square lattice arrangement), including cross-sectional view and 3D view.

## Supplementary Tables

**Supplementary Table 1.** Estimation of the concentration and DNA labeling ratio of the purified DNA-conjugated enzymes by measuring the absorbance at 260 and 280 nm

DNA	A260/A280	$\epsilon_{260}$ ( $M^{-1} cm^{-1}$ )	$\epsilon_{280}$ ( $M^{-1} cm^{-1}$ )	Protein	A260/A280	$\epsilon_{260}$ ( $M^{-1} cm^{-1}$ )	$\epsilon_{280}$ ( $M^{-1} cm^{-1}$ )	Sample	A260/A280	A260	A280	DNA - to- Protein Ratio	Protein Conc. ( $\mu M$ )	Dye ( $\mu M$ )
P1-Cy3	1.27	115200	90709	Gox	0.63	168336	267200	Gox-P1-Cy3	1.18	13.50	14.10	3.09	25.77	37.00
P1-Cy3	1.27	115200	90709	$\beta$ -Gal	0.59	573534.9	972093	$\beta$ -Gal-P1-Cy3	0.63	1.34	2.11	0.74	2.03	1.10
P1-Cy3	1.27	115200	90709	G6pDH	0.52	61594	118450	G6pDH-P1-Cy3	1.00	11.15	11.17	2.30	34.17	53
P2-AF647	1.60	130100	81313	MDH	0.72	14112	19600	MDH-P2-AF647	1.49	1.47	0.99	1.63	6.49	8
P2-AF647	1.60	130100	81313	LDH	0.57	115504.8	202640	LDH-P2-AF647	0.83	2.83	3.41	0.84	12.59	22

DNA	A260/A280	$\epsilon_{260}$ ( $M^{-1} cm^{-1}$ )	$\epsilon_{280}$ ( $M^{-1} cm^{-1}$ )	Protein	A260/A405	$\epsilon_{260}$ ( $M^{-1} cm^{-1}$ )	$\epsilon_{405}$ ( $M^{-1} cm^{-1}$ )	Conjugates	A260/A280	A260	A280	A405	DNA conc. ( $\mu M$ )	DNA - to- Protein Ratio	Protein Conc. ( $\mu M$ )	Dye
P1-Cy3	1.27	115200	90709	HRP	0.38	38000	100000	HRP-P1-Cy3	1.24	7.32	5.89	4.53	45.50	1.01	45.26	81 $\mu M$

$$A_{260}(DNA - protein) = \epsilon_{260}(protein) * Conc.(protein) + \epsilon_{260}(DNA) * Conc.(DNA)$$

$$A_{280}(DNA - protein) = \epsilon_{280}(protein) * Conc.(protein) + \epsilon_{280}(DNA) * Conc.(DNA)$$

$$Ratio \left( \frac{DNA}{protein} \right) = \frac{Conc.(DNA)}{Conc.(protein)}$$

Concentration of HRP-P1-Cy3 was estimated by the unique absorbance at 405 nm.

**Supplementary Table 2.** Enzyme encapsulation efficiency calculation

	$N$	$N_{coloc}$	$N_{right}$	$N_{coloc}/N_{right}$
<b>HRP</b>	176	156	165	0.94
<b>GOx</b>	205	197	201	0.98
<b>G6pDH</b>	218	209	214	0.98
<b>LDH</b>	1229	826	1008	0.82
<b>MDH</b>	363	335	348	0.96
<b><math>\beta</math>-Gal</b>	284	115	179	0.64

Enzyme encapsulation was calculated by taking the ratio of the number of colocalized molecules (i.e., both enzyme and **right** half-cage) to the total number of molecules containing the **right** half-cage.  $N$  is the number of particles analyzed,  $N_{coloc}$  is the number of particles containing both fluorophores, and  $N_{right}$  is the number of particles showing evidence of the **right** half-cage.

**Supplementary Table 3.** Calculation of enzyme copies per DNA nanocage

	$N$	<u>Cy3 Steps (% molecules)</u>			$\mu_{\text{Cy3\_Enca}}$ $p$	<u>Cy3 Steps (% molecules)</u>			$\mu_{\text{Cy3\_Unenc}}$ $ap$	$N_{\text{enz}}$
		<i>One</i>	<i>Two</i>	<i>Three</i>		<i>One</i>	<i>Two</i>	<i>Three</i>		
HRP	176	86	13	1	1.15	92	8	0	1.08	1.0
G6pD H	218	87	10	3	1.16	93	7	0	1.07	1.1
$\beta$ -Gal	284	93	6	1	1.08	88	9	3	1.15	0.9

The percentage of molecules exhibiting a given number of Cy3 photobleaching steps “Cy3 Steps” for both the encapsulated and unencapsulated enzymes are provided. The mean number of enzymes per cage ( $N_{\text{enz}}$ ) was calculated by taking a ratio of  $\mu_{\text{Cy3\_Encap}}$  to  $\mu_{\text{Cy3\_Unencap}}$ .  $N$  is the total number of particles analyzed.



**Supplementary Table 4.** Conditions for the single-molecule enzyme activity assay

<b>Solution</b>	<b>Concentration</b>
10× TBS, pH 7.5	1×
Resazurin	50 nM
Glucose-6-phosphate (G6p)	1 mM
Phenazine Methosulfate (PMS)	12.5 μM
Mg <sup>2+</sup> (MgCl <sub>2</sub> )	1 mM
NAD <sup>+</sup>	1 mM
PEG 8000	10% (w/v)

## Supplementary Notes

### Supplementary Note 1: Preparation, purification, and characterization of protein-DNA conjugates

**Protein-DNA conjugation:** As shown in Supplementary Figure 5, SPDP conjugation chemistry was used to couple enzymes to oligonucleotides as reported previously<sup>1,2</sup>:

- a) Enzymes (GOx, HRP, G6pDH, LDH, MDH and  $\beta$ -Gal) were first conjugated with SPDP at enzyme-to-SPDP ratios of 1:5, 1:20, 1:3, 1:5, 1:5, and 1:5, respectively, in HEPES buffer (50 mM HEPES, pH 8.5) for 1 h at room temperature. Different values of SPDP-to-Protein ratio were used due to the varied number of accessible surface lysine residues for each protein. Excess SPDP was removed by washing with 50 mM HEPES buffer using Amicon centrifugal filters (30 kD cutoff). The SPDP coupling efficiency was evaluated by monitoring the increase in absorbance at 343 nm due to the release of pyridine-2-thione (extinction coefficient:  $8080 \text{ M}^{-1} \text{ cm}^{-1}$ ).
- b) TCEP-treated thiolated DNA (/5ThioC6-/TTTTTCCCTCCCTCC (P1), or /5ThioC6-D-/TTTTTGGCTGGCTGG (P2)) was incubated with the SPDP-modified enzymes at an enzyme-to-DNA ratio of 1:10 in 50 mM HEPES buffer (pH 7.4) for 1 h in the dark. Excess unreacted oligonucleotide was removed by ultrafiltration using Amicon 30 kD cutoff filters: washing one time with 50 mM HEPES (pH 7.4) containing 1 M NaCl and three times with 50 mM HEPES (pH 7.4). The high salt concentration in the first washing buffer helps remove DNA nonspecifically bound to the surface of the protein due to electrostatic interactions.
- c) The absorbance values at 260 nm and 280 nm ( $A_{260}$  and  $A_{280}$ ) were recorded to quantify the enzyme-DNA complex concentrations and the labeling ratios using a Nanodrop spectrophotometer (Thermo Scientific) (Supplementary Figure 6 and Supplementary Table 1). Extinction coefficients of DNA oligonucleotides were received from IDT-DNA, and extinction coefficients of enzymes were obtained from published data.
- d) Dye labeling of DNA-conjugated proteins: The DNA-conjugated proteins were further labeled with spectrally distinct fluorescent dyes, which allow us to use native gel electrophoresis and single-molecule fluorescence to confirm the encapsulation of proteins within DNA nanocages. NHS-ester-modified dyes were reacted with the purified DNA-conjugated proteins from the above steps at a 20:1 ratio in 50 mM HEPES buffer, pH 8.5. Cy3 was directly labeled to the lysine residues on the protein surface. Excess dyes were then removed using 3-kD cutoff Amicon filters. The UV-Vis absorbance spectra of the purified dye-labeled proteins are shown in Supplementary Figure 6 and were used together with the extinction coefficients of the dye ( $150,000 \text{ M}^{-1} \text{ cm}^{-1}$  for Cy3 at 546 nm;  $250,000 \text{ M}^{-1} \text{ cm}^{-1}$  for Alexa647 at 647 nm ) and of the protein-DNA conjugates to quantify the concentration and labeling ratio of the dye-labeled proteins.
- e) Conjugate proteins to Cy3-labeled DNA: In order to perform the single-molecule enzyme activity assay, selected enzymes (G6PDH and  $\beta$ -Gal) were conjugated to a Cy3-labeled DNA. First, NHS-ester-modified dyes were reacted with the 3'-amine of oligonucleotides at a 20:1 ratio in 50 mM HEPES buffer, pH 8.5. Excess dyes were then removed using 3-kD cutoff Amicon filters. Dye-modified oligonucleotides were then conjugated to proteins *via* the 5'-thiol using the SPDP chemistry described above. Fast Protein Liquid Chromatography (FPLC) was used to purify the protein-DNA-Cy3 conjugates for removing excess DNA-Cy3, and characterized with the UV-Vis absorbance spectra.

### **Enzyme-DNA cage assembly, purification and characterization:**

- a) The purified DNA half-cage containing capture strands was mixed with one of several enzyme-DNA conjugates at a 1:15 cage:enzyme ratio and annealed from 37°C to 4°C over 2 h in 1×TAE-Mg<sup>2+</sup> buffer (containing 12.5 mM Mg(OAc)<sub>2</sub>).
- b) Twenty-four single-stranded DNA linkers were mixed with the two purified half-cages at a 5:1 linker:cage ratio to connect the two half-cages together by incubating at room temperature for 3 h.
- c) Agarose gel electrophoresis (2%, 1×TAE-Mg<sup>2+</sup>) was employed to remove excess free enzymes (70V, 2h). The band of the DNA cage containing the enzyme was cut from the gel and extracted using a Freeze 'N Squeeze column (Bio-Rad). The DNA origami concentration was quantified by measuring the absorbance at 260 nm ( $A_{260}$ ) using an extinction coefficient of 0.109 nM<sup>-1</sup>cm<sup>-1</sup>.

### **Supplementary Note 2: Single-molecule fluorescence microscopy for characterizing DNA cage-encapsulating enzymes.**

**Yield estimation by TIRF colocalization:** All single-molecule measurements were performed at room temperature using a total internal reflection fluorescence (TIRF) microscope on PEGylated fused silica microscope slides. To passivate the microscope slides and functionalize the surface with biotin for selective immobilization of nanocages, a biotin- and PEG-coated surface was prepared by silylation with APTES, followed by incubation with a 1:10 mixture of biotin-PEG-SVA 5k:mPEG-SVA 5k as described previously.<sup>3</sup> A flow channel was constructed as described elsewhere.<sup>3</sup> To prepare the surface for enzyme or nanocage binding, a solution of 0.2 mg/mL streptavidin in T50 buffer (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA) was injected in to the flow channel, incubated for 10 min, and the excess streptavidin was flushed out thoroughly first with T50, then with 1× TAE-Mg.

The right half of the DNA origami cage was labeled with Cy5 dye inside the cavity, *via* hybridization of Cy5-labeled DNA to complementary handles incorporated into the structure. Each of the ssDNA conjugated enzymes (HRP, GOx, G6pD, LDH, MDH and β-Gal) was covalently labeled with Cy3 as described in section 3 (Cy3-Enzyme-5'-TTTTCCCTCCCTCC), and then linked to the left half of the DNA origami cage via hybridization with complementary handles. Because Cy3 was directly labeled onto the enzyme surface, any observed Cy3 signal of the immobilized DNA nanocages came from the encapsulated enzymes. Linker strands were added to a 1:1 mixture of the two half-cages to encapsulate the enzymes in a full-cage. To capture DNA-modified enzymes in the absence of nanocage (as control) the microscope slide was incubated with 10-20 nM biotin-modified complementary DNA oligonucleotide (5'-biotin-TTTTTGGAGGGAGGG) for 3 min, followed by 10 min incubation with 20-50 pM enzyme sample in 1×TAE-Mg buffer. Excess enzyme was flushed out with ~400 μL buffer (channel volume ≈ 30 μL). For the nanocage experiments, the samples were diluted to 20-50 pM in 1× TAE-Mg and immobilized on the streptavidin-coated PEG surface for 1 min, and the excess sample was flushed out with ~400 μL of 1× TAE-Mg. The DNA-modified enzymes were imaged with illumination at 532 nm (~15 W/cm<sup>2</sup>), and the nanocage-encapsulated enzymes were imaged

with simultaneous illumination at both 532 nm ( $\sim 15$  W/cm<sup>2</sup>) and 640 nm ( $\sim 40$  W/cm<sup>2</sup>) as described.<sup>4</sup> Particle-finding and colocalization analysis were performed using custom-written scripts in IDL and MATLAB, using a threshold of 150 counts per frame for particle identification (typical particles showed 500-1,000 counts per frame in each detection channel). The enzyme encapsulation yield, defined as the fraction of assembled nanocages containing enzyme(s), was estimated by dividing  $N_{coloc}$  by the total number of particles containing a right half-cage,  $N_{right}$  (Supplementary Table 2).

**Estimation of enzyme copy number per nanocage:** The number of enzyme copies per nanocage ( $N_{enz}$ ) was determined by single-molecule photobleaching (SMPB). First, the number of Cy3 photobleaching steps was determined separately for unencapsulated as well as half-cage- and full-cage-encapsulated enzymes. For this, the donor channel data of all single molecules were idealized in QuB (<http://www.qub.buffalo.edu>) using a six-state model<sup>5</sup>. The histogram of the photobleaching steps was then acquired using a custom-written MATLAB script. Representative intensity traces exhibiting one, two, and three photobleaching steps are shown in Supplementary Figure 2c (more than three photobleaching steps were rarely seen). Finally, the number of enzyme molecules per cage was estimated by dividing the mean number of Cy3 photobleaching steps of the full-cage ( $\mu_{Cy3\_Encap}$ ) by the mean number of Cy3 photobleaching steps for the unencapsulated enzyme ( $\mu_{Cy3\_Unencap}$ ). Results are summarized in Supplementary Table 3.

### Supplementary Note 3: Single-molecule enzymology

**Single-molecule enzyme activity assay:** Prior to single-molecule activity measurement, streptavidin-modified slides were incubated for  $\sim 2$  min with neutravidin-coated fluorescent beads (Invitrogen, 0.04  $\mu$ m diameter, excitation/emission; 550/605 nm) at  $10^6$ -fold dilution and the excess flushed out with  $1\times$ T50 buffer. These beads ( $\sim 5$ -8 per field of view) were used as fiducial markers to correct for drift of the microscope stage and/or slide (Fig. 5a,c). Following complete photobleaching of Cy3 in a field of view, the activity of single unencapsulated or nanocage-encapsulated enzyme molecules was imaged on the same field of view. During analysis of the movies, the coordinates of the initial photobleaching movie were registered with those of subsequent movies using the fiducial markers (visible throughout all sequential movies) in a custom-written MATLAB script. This approach allowed us to infer the locations (x- and y-coordinates) of all individual enzymes/nanocages in the field of view even after bleaching Cy3, and to monitor enzyme turnovers ( $\approx$  resorufin formation) at these specific coordinates.

To image enzyme activity, 300  $\mu$ L of substrate solution in  $1\times$ TBS buffer (pH 7.5, 1 mM Mg<sup>2+</sup>, and 10% (w/v) PEG8000) (Supplementary Table 4) was injected into the flow channel. Movies were recorded for  $\sim 5$  min (9,091 frames) at 35 ms frame exposure time immediately after injecting the substrate solution. In case of G6pDH, the activity was measured in the same field of view under identical laser illumination and microscope settings, with or without glucose-6-phosphate (G6p) (Fig. 5c). Enzyme activity for  $\beta$ -Gal was measured similarly using a 500 nM solution of resorufin  $\beta$ -D-galactopyranoside (RBG) as substrate, which is hydrolyzed by  $\beta$ -Gal into fluorescent resorufin. Fluorescence fluctuations over time were measured for unencapsulated enzyme as well as half- and full-cage-encapsulated enzyme (Supplementary

Figures 57 and 58), and the fluorescence time traces were analyzed for intensity spikes using custom-written MATLAB script. The script allowed us to measure the background intensity of single-molecule traces and set a threshold (mean + 8 standard deviations) to subtract from the raw intensity. Since we often observed one or two spikes above this intensity threshold in the control experiments, only those molecules with  $\geq 4$  spikes were counted as active molecules (Supplementary Figure 59) and considered for burst analysis. Due to the low concentration of resazurin (Supplementary Table 4), the criteria we used to determine the fraction of active molecules might have excluded some molecules that are not highly active.

**Burst analysis:** Burst analysis was carried out using a modified Rank Surprise (RS) method<sup>6</sup> recently utilized to analyze the binding of fluorescent DNA probes to a riboswitch<sup>7</sup>. Briefly, Interspike Intervals (ISIs) were determined by calculating the time in between individual fluorescent spikes for each molecule (Supplementary Figure 59). The RS method was used to demarcate the start and end points of bursts after collecting ISIs for all molecules. Only intensity spikes characterized by an ISI of  $\leq 5$  seconds were considered part of a burst; any other intensity spikes are counted as non-bursts.

**Comparing bulk and single-molecule enzyme activity:** Unlike our single-molecule assay, the bulk measurement of enzyme activity cannot explicitly determine the fraction of active enzyme molecules present in the solution (it is well known that a fraction of enzyme molecules loses their activity during oligonucleotide conjugation, buffer exchange and the purification process). However, the observed bulk activity is contributed not only by enzyme turnover rate but also by the fraction of enzyme molecules that are still active. Both of these contributing factors need to be accounted for to directly compare the single-molecule enzyme activity with the bulk measurements. Therefore, in the single-molecule experiment, the overall activity of free, half-cage and full-cage enzymes were calculated by multiplying the turnover rate with the fraction of active molecules for the given sample.

#### Supplementary Note 4: DNA sequences of the designed nanocages.

##### Sequences of staple strands in the SH Full-Cage-Left cage

5[18] GGTGGAGAGGCGGTTTGCCTTTT  
 11[18] CGAGTTGGGTAACGCCAGGTTTT  
 13[9] TTTTTCGCCATTCAGG  
 17[9] TTTTGCCAGCTTTCATCAACATTCGT  
 21[9] TTTTGGAGCAAACAAGAGAATCGGAAGATTAGC  
 25[9] TTTTGGGAGAAGCCTTTATTTCAAAAAGGGACAG  
 31[5] GGTGGCATCAATTCATGGGCGCGACCTGTTTGTATAAGCAAATTTT  
 36[16] ATATAAAGTAGTAGATGGGCGCTTTT  
 43[18] AATCATACTAATAGTAGTAGCATTTT  
 54[17] GCTGTCATAGCACCGAGCTCGAATTCGTTTT  
 55[2] TTTTGGAGGACTAAAGACTTCAACACTAAGG  
 67[18] CGGTTTTGCTTTGCGCTAGTGAGCTAACTCACATTTT  
 69[2] TTTTGAAGGATTAGGATTAGCGGTAGCAACGCGA  
 83[2] TTTTAAAAGGGCGACATTCAACCAGGC

95[18] T G A C T A A T A T G T T T G A T G T T T G C C C C A G C A G G C T T T T  
 97[2] T T T T A G G C T T A T C C G G T A T T C T A G T T T  
 108[20] C T C A A C A A G T A T C A C A T A A T T T A T T A A A G T T C C A G T T T G G A A C A T T T T  
 1[5] T T T T A G A G T C C A C A C T A G A A A A T T  
 3[5] T T T T G A A A A T C C T C A G A G A G A T T T T  
 5[5] T T T T A T T G G G C G G A G C C A C C A T T T T  
 7[5] T T T T A A T T G C G A A A C A A C T T T T  
 9[5] T T T T A A T C A T G G C T C A T T C A G T T T T  
 11[5] T T T T G T T T T C C C A G T C A T T T T T  
 15[9] T T T T A T C G T A A C C G T G G C A A A G C G C C A T T T T  
 19[9] T T T T A T T T A A A T T G T G G C C T T C C T G T A T T T T  
 23[9] T T T T G A G A C A G T C A A A T G C C T G A G A G T C T T T T  
 27[2] T T T T A G C C T C A G A G C A T A A A G C T T A A T A C T T T T G C T T T T  
 29[2] T T T T A A C A T C C A A T A T T A A G C A A T A A T T T T  
 33[2] T T T T A A A T G G T C A A T A A G C T G A A A A T T T T  
 35[5] T T T T A T T C C C A A T G A T A C A T T T C G C T T T T  
 37[2] T T T T A A A T A T G C A A C T A A C A G T T G T T T T  
 39[5] T T T T G C G G A T G G C C T C A A C A T G T T T T  
 41[2] T T T T G T T T A C C A G A C G A C G A T A A T A G C A A A A A A T C A T T G A G A A A G G C C G T T T T  
 43[2] T T T T A C A T A A C G C C A A A T C A T A A C C C T C T T T T  
 45[5] T T T T A G A A A G A T A C T A A T G C A G A T T T T  
 47[2] T T T T G G A A G A A A A A T C T A T T A C A G G T T T T  
 49[5] T T T T G A A T T A C C T G T C A G G A C G T T G T T T T  
 53[5] T T T T G A A T A A G G T A A A T T G G G C T T T T  
 57[2] T T T T C A C C C T C A G C A G G C T A C A G A G G C T T T T  
 59[5] T T T T A T A T T C G G T T T G C G G G A T C G T T T T  
 61[2] T T T T G A T A C C G A T A G T C A T A A C C G A T T T T  
 63[5] T T T T A A T T G T A C T T A A A C A G C T T T T  
 65[2] T T T T A A T A A T T T T T T A A G G A G C C T T T T  
 67[5] T T T T C A A C A G T T A G G A A T T G C G A A T T  
 71[2] T T T T C G G A A C C T A T G A C T C C T C A A G A T T T T  
 73[5] T T T T G T C A G T G C C C C C C T G C C T A T T T T  
 75[2] T T T T C A T A C A T G G C T T T T A A C G G G T T T T  
 77[5] T T T T C A T T A A A G C T T C C A G T A A G C G T T T T  
 79[2] T T T T A G G T T G A G G C A G A T A A A T C C T T T T  
 81[5] T T T T C C C T C A G A G A G C A T T G A C A G G T T T T  
 85[2] T T A A G T T T A T T T T G A G C G C C A A A G A C T T T T  
 87[5] T T T T A T A C A T A C A A C A C C A C G G A A T T T T  
 89[2] T T T T G A A C T G G C A T G A A C G T A G A A A T T T  
 91[5] T T T T C A A A G T T A C G A A T A C C C A A A A T T T T  
 93[2] T T T T G C A A T A G C T A T C A T A G C C G A A T T  
 95[5] T T T T A A C C C A C A A A A C A A T G A A A T A T T T T  
 99[2] T T T T C G A G A A C A A G C A A T C A G A T A T A G A T T T T

101[5] TTTTAGAAACCAATACCGCACTCATTTTT  
103[2] TTTTACGCGCCTGTTTCGAGCATGTTTT  
105[5] TTTTATAAAGTACAGCTAATGCAGATTTT  
107[2] TTTTTGAGAATCGCCATAAGAGAATTTTT  
109[5] TTTTAGCCTGTTTGTAGGGCTTAATTTTT  
1[28] GACTCCAATAAACACCAGGGAAGCGCATAAGTCAGCGGCAAATGCAGCA  
1[77] GAACCATCGTAAAGCACTAAACTTGACG  
2[34] TAGGGTCCGAAATAGGGTAAACAAATC  
2[55] GGTCAAAGAATAGAGGGCGAAAAACCGTAAATAAGAGAATTAA  
3[49] ATAAAAGGAACACCACCACCGG  
3[77] GGGAAAGGGGCGCTGGCAAGTCGCTGCGCGTAACCTTGACGA  
4[34] AGCGGTCCTTTTCACCCTCAGATTTAGC  
4[69] GTAGCTCTTTAGAGTCGGAACATGGCCCACTACGT  
6[34] CGGCCAACGCTTTCTTTTCTGAATGGCT  
6[48] CTGCATTGACGGGCAGAGAGTATCCCTT  
6[76] GCACGTAAGCTAAACAGGAGGTTTATAATCAGTGGTAAAAG  
7[25] GCCCGCGGGGTTTTTCACGCTGGGTGGTTGAGTGTGAACGTG  
7[49] GTCGTTTTTCCAGAGTAATCTTG  
8[34] TGGGGTGTGTGACAAATCACTCGAAC  
8[69] AGGCCATGAGCGGGTAACGTGCTGGTCA  
9[77] AGTCTGTCACTTGCCTGAGTAATCCAGAACAATATACGCTCA  
9[88] CACGAAGTGCCGATTAGGTTGCTACCACACGGCG  
10[34] GAGGATCAAACGACAATTGCTCAGTTTGTAGGTCAAATGTGAATAATT  
10[48] GCAGGTCATCCGCTTAAAGTGGAAACCT  
11[49] TTGCACGTCAGGATGTATCGGGGCGGATCGTCGGAACCAATA  
12[34] TGCAAGGACTGTTGGTGCCGAAACCAGCATCTGCCCTTTTGTAA  
12[69] GAAATATTGGTAATGAAGAACACACCGA  
12[76] ATCGTCTATTTACACAGAGATAGCGCAC  
13[21] CTGCGCACGATTAACGTTGTACCCGGGTTGTTTCCCCTAATGCACT  
13[42] CGATCGGAAAGGGGGCCAAGC  
13[84] ATTCACCCATTTTGTACCGCCATAACATCCAT  
14[48] CGGCACCGACGACATAGA  
15[63] AAGATAACCCTTCTAGCCCTAATTA AAAACGCTGAGAGCTCA  
15[91] AGAATACGTCTTTAACCAGCAAACACCGCCTGCAAAAATCTA  
16[24] TGGTCGGTGTATATAATAGGACAGACCAGAAAAATCTAAAG  
16[69] AAACAGGAACAAACCCTCAGGTCCAGCCCTCTTCGCTGGATT  
17[42] AACAACTGACCGTGACTTCAAATAT  
17[84] ACGAACCATGCGCGAACTGATGACCTGATGGCCAATTGGCAG  
18[27] CGCGTCTAAACGTTAGTTTCAACGAGTAACTTTGTTTT  
19[63] AATCAGCCAGCAGCAATCAACAATTGAGGATTTAGAAGGAGA  
19[91] AAGCATCTCAATATATATCTTTCAATAGATAATACACAATTC  
20[69] AGTTGTGTACCCCGTTTGTATTTTTTATTCTCCGTGTCGCC  
21[42] GTAAAACATCAGAACAGAAAACGAGA

21[84] ATCTAAACTGGTCAGTTGGCAAAATGAACAGTGCCATACCGA  
22[27] AGGTCATTACCATTCAATTTGACTGCGGTAACGGATTGA  
22[48] GAGAGATACCGTTCTAGCTGATGCCTGAGAACCCTTGGGAAGG  
23[63] ATGCCGTATTAGACATCATT  
23[91] GACAACTTTTAAAAAATTATCCATCAATATAATCCTGATTGTACCAGAA  
24[69] TGCGGTAAATGCAATAAATTAGGGTAGCTCAATCATAAAAGG  
25[84] GGAGCGGGTTTGAGTAACATTTTTACAAATTTGAGGAAGGTT  
26[27] GACCCTGAAATCGGAAAGAATAAACCAAGTAAGAGTTCA  
26[41] CCTACAAGATAAAAATTTTTAGTAATGTTTTGCCAGAGGG  
26[48] GTTAGAACAAAATTGTAGATTTTCAGAGGCTTTGCTTGTACCAACATAT  
26[62] TGAATAACATATATTTAACAAAGAAACCTTGGATTATACTTC  
29[25] GCATAGCGTCTACGAGGGAATACCACAGCATAGTTAAAAAAC  
29[35] AAGAAGTGTAGGTAATATTCACTACAAAGGTAATC  
30[53] GGTAATAGTAAACCAACCTAAAAAC  
32[46] TGAATCCCCCTCAAATGTTCAATATGAAGATTCACGCAAGACATTAT  
33[25] TATTAGTTTGATAAAAACGGCTCATACAATGATTTCGAGGATAC  
34[34] CAGGTTTATCTTTAAACAGTTAAGCCCCTGTTAAACATCAAAGCGAGT  
35[35] CCCTGACAAAAGATTAAGAGAAATATTTAAAAACAGATGAACGGCTATC  
36[57] AAAGCGGATTTAAATTGTTGATATAGCATGTATTTTT  
37[35] CCCGAAAAATGGGAAGGGGACGCTTCTGGGAAGGG  
38[53] CGCGTTTTAATTGA  
39[25] TTGACGTAAGTACGAGTATGGGAAGTGAGAAACCGCCCAGA  
39[35] CAGACCGTACCTTTGGCCAGTGATGTGC  
40[44] GAGGAAGCAAGGATATTATCAAGACGTTAGTTCTAAAGCCTC  
40[57] GGGCCTTGCTACGCCAGCTGGCGTGCGGGCAGCTTTC  
43[35] GAAGGCAATGTTTAATAAATATTCAT  
44[53] GAAAGAGGCAAAAGAGGGTTGATA  
45[25] GATAACCATCGGCTTGCTACTGGTACAGTGCCAGTATGGGCA  
45[35] TCATCTTAAGTACAACGGAACAATCGTCGACTGGAAGTGCAA  
46[57] TACCAAGCGCGAAACATGACCCCCAGCGATTA  
48[16] CCATATGCGAAAACTTAATCATTGT  
49[25] AAGAAAAAAGATCAGCTATATTCAGAAAGCGAGAAAAGAAAC  
49[35] TACTTAGGAACCGAGTGTACACGAGCTTCAAAGGATGGGAAG  
50[57] ACGGTCAATGTCAGAAGC  
51[2] GAGATGGTTAATTTCAAGGCTGTAGTTAGAGCATAAGAGGTCA  
52[34] TAGGCACATGAACGACTGACCGACTTTA  
52[43] CCACAACGCCTGTA  
53[35] GACCTTCCATTACCAATTGTTGACTCTA  
54[57] ACCCACACATCAAATATTGCCT  
55[25] TGAACGAGGGGGTTTTGTATTAAGGATTGAGTCATATGAGAACGCCCAA  
55[35] TCCATTAATACGTAATGCCTAAATAGCGAGGTTAACGTCAGGGGTAAA  
55[42] AACGGAGTACCAAGTTACAAGGCGGAGAGGAAGTT  
57[18] AAGACATTCATCATCAGACAACATTACGTTAACCATTATCTGCGATTCC



57[35] GCCGTCGAGAATACACTAAAGCAACTAC  
58[53] TAAGTATAGCCCCACCGTCACCGA  
60[46] TTTAGTACCGCCACCCTACTTAACAC  
61[35] CCGCCACCGCGACCTGCTCCTGAGATTTGTATCATCAAAAAT  
62[16] TTTTCGGTTTGTCTCCAACACGTTGCGAGTAGCTTGCCCAA  
63[35] GGGATAGTGAGTTTCGTCAAAAACATGT  
66[23] GAAGATTGGCCAGAGCAGCCCTTAATAAGCAACGCCGCCAACG  
66[53] GCATTCCACAGACA  
67[35] GTAACGAAAATGAACAGTCGGTAAAGCC  
68[57] CTAGAATCAACGAGCCGGAAGCACACAATTAAGAACCCTCCAACA  
70[16] TGATATTCTGGGCCGCTTCGCTGAGCCCACGTGCGCCGTATA  
70[34] CATTAAAGCTCAGTACCAAATCGCGCAGAAGACGGA  
71[25] ATGATAAACACAATAGAAAAGAAATTTATTTGGTATTA  
72[34] AGAGCGTAAAAGGTGAATTATGGAATAGGTGTAGGCGTAAAGT  
72[53] CTTGAGCCATTTTCGGGAGGTTTTG  
74[34] CAATGTGAGTCACCGTACTCAGGAGG  
74[57] ATTAGCAAGGCCGGAACAGTAGCACCATTACC  
75[25] AGGCTCTGAATCCTTATACGCAATATAGATATAAACAA  
76[34] GTTTGGTAGAAACCATCGATACACCACCCTCATCTCACAGAA  
76[53] TCAGTAGCGACAACGAGCGTCTTT  
79[35] CGTTTGCCAGCCCTCATAGAGCCCCAGTACAAACTAGGCGCA  
80[43] TCAAATAGCAGCCT  
81[25] ACCTTGAGCGGTTAAGCCCGGAATTATGCGTTATACAA  
81[35] ACCGGAAGCCGCCACCAGTGAATGAAT  
82[44] AGACCAGAGCCTGAACATAGACGGGGCGTTATGACCTA  
82[57] AAGATTGCCCTTTCCTCGGCCAG  
83[42] TATTGAAAATTACATTTAATAGCGAAATGGAGGGAAGGTAAAAATTATT  
84[16] ACCTCACAAATGTTAATGTTGAGTAAATAAGTTTTGATGTGAA  
86[53] AAGCCTTAAATCGAGTGAATAATTTTCCATTCC  
87[25] ACACCCATCCTCGGCTGTCTTTCCTTATCCTAAGAAAA  
88[46] CAATTTTATCCTGAATCCGCCAGCAAAAATCACACGTAC  
89[18] GACTTTACCGCAGAATGCAAACAAGTCAGACCAACTAATCAG  
90[53] CCAGAGCCTAATGTGAATTTTAACTCCAGACGACGACAAAAGTCCTG  
91[18] AGGTAAGCAGTTACCGACGCCGCCCGCCACACCCTCACCAG  
91[25] CGATTTTCGAGAGGTAAAGTAATTCTGTCCGGAGAGGCA  
92[34] TTTAATACACCTTTAGCGTCACATAGCCCCCTTTGTGTTTCA  
92[57] TCCAATAAGAAACGAATATTATTTATCCCAA  
94[34] AAAACAATTCGTCAAAAATGATTTTCATAATCACACTATTAG  
94[53] TTACAGAGAGAAAAAGAACATTTTCAT  
96[57] CTCCCCGAACCGCCTGGCCCTGAACAGCTCCGCCTCTTTTGTCTG  
97[42] TCATTTGTCAATATATTCATT  
98[23] TAGCAAGCAAAGCCGTTTCGCAAAGTAAAGGTTTAGCAATTAA  
99[35] AGGAAGTAAGATTAGTTGCTAAACCTCCCGACTTGGGGAATT

100[20] AACCAAGTCAATAATAATTTAATCAACAAATAACGCAGA  
 100[34] AAGAACGTCATCGTACCGCGCGAGGCGTTTCAATT  
 102[34] ATAATATTATATTTTGCACCCAGCTA  
 103[35] AACAAATTGCCAGTTACAAATATTACCAACGCTAGAATCAA  
 104[20] CATGTTCCGACAAACCAGTAATATTTAAAGCAAGAGAAT  
 106[34] GAGGCATGGAAATAAACAGCCTTTTTTG  
 108[41] CTTACCAGTATAAAAACATGTAATTTACTAACATA  
 109[35] ATTCTTCAATAAGAACGTCAACCCGAGA  
  
 4[86] CTACCGGCGAGAGGTGCCACCCAAATCAAGTTTT  
 12[107] TTTTGCTCATGGAAATACCTAAGTCACATAAAAGGGACATTCAAGCGTA  
 91[46] TATAATCGCACTTAGGTTGGGTATACCTTTTATCAAATCATAGTTTT  
 101[49] CTTGAAATATTAATTAACCTTGCTTCTGTTTT  
 102[65] TTTTATAGATTAAGACGCTGAGAAGAGTCTAGAATC  
 106[65] TTTTAATGCTGATGCAAATCCTTATCCCAA  
 109[49] AATTTAATTAGTTAGCGAGAAAACCTTT  
 110[67] TTTTCCGACCGTGTGATCTATCACCTAAAG  
 2[96] TTTTGGGGTACGTGGCGAGAAAGGAATTTT  
 4[107] TTTTAGAAAGCGAAAGGAGCGCCGCGCTTAATGCGTTTT  
 6[107] TTTTACAGGGCGCGTACTATAAGGGATTTTAGACAGGTTTT  
 8[107] TTTTGTACGCCAGAATCCTGAGCAAATTAACCGTTGTATTTT  
 10[107] TTTTACTTCTTTGATTAGTAAGCCATTGCAACAGGAAATTTT  
 42[62] TTTTACCTTTTACATCGATGAATATACAGTATTTT  
 70[62] TTTTATTACCTGAGCAAAGGCGAATTATTCATTTTT  
 98[62] TTTTAAACAGTACATAAAAATTACCTTTTTTAATTTTT

**Sequences of staple strands in the SH-right cage**

14[176] TTTTATAACATCACAATATTACTTTT  
 16[176] TTTTCGCCAGCCATTGCAACTCCAGAACTTGCCTACTTCTTTGATTAGTATTTT  
 18[176] TTTTATCGTCTGAGGACATTCTTTTT  
 20[176] TTTTGGCCAACAGAGATAGAATAAAAGAATGGATTACATTTTGACGCTCATTTT  
 22[176] TTTTAATATTTTTTAAAAATACTTTT  
 24[176] TTTTCGAACGAACCACCAGCTCGCCATGAATGGCAATACGTGGCACAGACTTTT  
 26[176] TTTTCCTGCAACAGTGCCACGTCAGTATTAACACCGTTTT  
 28[169] TTTTACCTCAAATCAAATCAACTTTT  
 30[169] TTTTAGTTGAAAGGAGCACTAACAATTTT  
 32[172] TTTTCTAATAGATTAGGAAGTATTATTTT  
 34[169] TTTTGACTTTACACCGAACGTTATTTT  
 36[172] TTTTAATTTTTAAAAGTAACCACCAGTTTT  
 38[169] TTTTAAGGAGCGGGGCAATTCATCATTTT  
 40[172] TTTTATATAATCCTGATGTTTTTATAATTTT  
 42[169] TTTTAAACATAGCATAGTGAATTTT  
 44[169] TTTTATCAAATCATATTAGAGTCAGATAGCTCCCTTAGAATCCTTGATTTT

46[172] TTTTAACCTCCGGCTGATGCATTTT  
48[169] TTTTAATCCAATCATATATTTTAGTTTT  
50[172] TTTTAAATTCATCTTCGTGTGATATTTT  
52[169] TTTTAATAAGGCGCTAGAAAAAGCCTTTT  
54[172] TTTTGTTTAGTATCAGAGCGGGAGCTATTTT  
56[169] TTTTGGAATCATTAAAGGCTTATTTT  
58[169] TTTTCCGGTATTCACCTTGCGGGAGGTTTT  
60[172] TTTTGAAGCCTTTACAATTTT  
62[169] TTTTATCCTGAATCTAATTTGCCAGTTTT  
64[172] TTTTACAAAATAAAAACGATTTT  
66[169] TTTTGTTTAAACGAATAACATAAAATTTT  
68[172] TTTTAACAGGGAAGCGGGCGCCGCTACAGTTTT  
70[169] TTTTGACATTCAAAAATTATTCTTTT  
72[169] TTTTATTAAAGGTGGAATTAGAGCCTTTT  
74[172] TTTTAGCAAATCACCCGTCACCAATTTT  
76[169] TTTTGAAACCATTCAAGTTTGCCTTTT  
78[172] TTTTAGCGTCAGACAGCCCCCTTTT  
80[169] TTTTATTAGCGTTCAGAGCCACCCTTTT  
82[172] TTTTCGGAACCGCCTCAGCGGGCGCTAGTTTT  
84[169] TTTTAAGAGAAGGGCGGATAAGTTTT  
86[169] TTTTGCCGTCGAGTATCACCGTACTTTT  
88[172] TTTTCAGGAGGTTTAAACCGCCACCTTTT  
90[169] TTTTCTCAGAGCCTAGGAACCCATGTTTT  
92[172] TTTTACCGTAACTGTAGCATTCTTTT  
94[169] TTTTCACAGACAGGTCGTCTTTCCATTTT  
96[172] TTTTGACGTTAGTAAAGCCCCGATTTATTTT  
98[169] TTTTAAAATACGCAGCGATTATTTT  
100[169] TTTTACCAAGCGGACGGTCAATCATTTT  
102[172] TTTTAAGGGAACCGAGTAATCTTGTTTT  
104[169] TTTTACAAGAACCCTTGAGATGGTTTT  
106[172] TTTTAATTTCAACTTCTACGTTAATTTT  
108[169] TTTTAAAACGAAGATACATAACGCTTTT  
110[172] TTTTCAAAGGAATTAGAACCATCACCTTTT  
1[154] ACTACGTCGAGGCAAAGTTTTCCCTCATAACGCCTGAGTTTCGACA  
2[132] AGTGTTGAGGGCGAAAAACCGCTATCATTGAGAAT  
3[133] GATAGACTGCTAAAGCCGCCACCAGATCCCCTCAGGGAAGGGTGCGCGT  
5[158] AGGCCTCAGAACAGAGAGTCAAAAAATAAGACAGCCATTTTT  
6[139] AAGAGTTGCAGCAAATCCTGTTTGAAAAACCGCCAGCGCTA  
7[133] TGAGACCGAACACCTTAATTGAGAATACATTCTTAGTGCTTTAGACAGG  
7[151] CGCGTCACGCAAGAAAGGGCGAACGAACCCTCGAGGTGATGGCCC  
7[158] AATCATTAGAATAATTATTAATATAACCGACCTGA  
10[139] AAAATCGGCCAACGAGGGTGTTTTTACCCAGTATAATTATT  
10[160] ATTAAGTGAGAAGTTGTTTGGGTAATAAGGAAAAAATACCTATTTAC

11[133] TAATGCGATAATGGCAATTCCAATCATGCCCCGGGCGGCCAG  
13[147] ACCGTTGAAGAGTCAGAATCCGGATTTTCCTCGTTTTGACGACCGC  
14[132] GAGCCGGCCGCTCAAAGGGTTAGAAC  
14[153] AGAACTCAAACCTACCAAATTA  
15[147] CCTTGCTGATTATAGATTATCTATAACAACGGA  
16[153] ACGCTCGTTGCGGAATCA  
18[132] TGCCAAGCACGACGAGATGAATATAC  
18[153] ATTGGCATCACACGACATTATATTAATAAATTTAGAAAACCTATTA  
20[153] TGACCATCATTTGACGACAACAATAAAAAGAACATTTTGCACGC  
21[158] AAGTATTAGTCTTTAATATAGCCCAATAGATTAAA  
22[132] GGAAGGGCGCCATTTCAATTTCAATTA  
22[146] GCGCGCAGAAAGGGGGTGA  
24[128] GTGTTTCATTAACAATAATTTCCAACAATAATCATAATAGTATGTAGTT  
24[153] AAAACGCATCTGGTGGAAAGGTGCTGAGATACGAGCCAAATCAGCGA  
25[158] GCGGCTGAGAGCCAGCAAATCTAACCTC  
26[132] CCAGTTTTGGGCGCAGTACATCTGTAAACAATTG  
26[146] GCAAAGTCTCCAGCCAAGAGG  
27[67] ATTCTCCGTGGGAACAA  
27[84] ACGGCGGTAAATGTAAATAATTTTTGTTAATCAGAGGTA  
27[105] GATAGGTGAAGCCAGCTTTCATCAACATATTGACCGTAATGG  
28[149] AATTAATTTTTAGATTAAAGCCGTCCAA  
30[139] ATCAAGATGAATTACCTTTATTTTCCGGCGAACTG  
31[121] CCTGAGCAAAAAGAA  
31[140] TGAAATCAAGAGGCGAATTATCAGGCTGCACCGCTGATCGCAGCATCTG  
31[151] ATATTGGGTTCCATCCTGATTAGTTAGC  
33[140] GCGCATAGTGCTGCACACCAGGATTCGATACCGAGCTCATGG  
35[121] AGTAACAGTACCAAAGTACCGACA  
35[140] TCGGGGTCAAGGTTTAAACGTCTTGTA AAAAGGCGAAGCTGGCACTGTTG  
37[128] AAATTGCGTAGATTTTCTTAATTCGTACATCGG  
38[149] TATTTTGAAAAGAATAACAATCCAATGAAAAGCAT  
39[121] CTACCATATCAAAAAGCCAACGCT  
39[140] TGCACCTCTTCTGAGTACGCCTGTCCATCATTGCGCTCACTGGCTGCAT  
41[99] GGCCTTCCTGTGTTTGTAAAGGAAGAGTAACAAGAGCATT  
42[125] TAAACGTCCTTATCATTAAATTAC  
42[139] AGTACCGTCGTCGCTATTAATCATTAAATGGAAACATCGTAACCTGAAA  
45[131] CATAACGGAATACC  
47[117] ATGCAGAACCCTGATTGC  
47[140] ACAATATTCAGAAACAATAACCAAAAATC  
49[158] CAAGCAAGACTGTAAATGCTTAGGTCTTTAGGAATTGACAGTTGGATCA  
50[156] CCTCCTTTGCAACAATTGGATTTAAGCCGTCTAAAACAAGAAGATTGAG  
52[139] TATTTAATTCGAGCCAGTAGGTCGTAAAACAGAAATAAAG  
53[121] CAACAGTAGGGCCTGAACAAAGTC  
53[151] ATCCGGGAGAAGCCTTTGCCGCCAAAAATCATCTG

54[156] CGTAGATGATAATTATCACAAAGATTGAGTAACCAGTAACCCTTCGCGT  
55[126] ATTTACTCGCAAAGAATAGAATTACCAT  
56[86] ATCAGGTCATATGCCGGGTAGGTATTTTTAGAATACTTGAGCATA  
56[97] GATGAACAAAGCCCCAAAAACAATTTCGCATTAAATTCGCGTCT  
56[149] TAGACCAGCGAGGGAGGGTATTAATTAGCGGTGAGGAAACTACGAA  
57[117] TAGGAATAATTGTATAAGCAAAT  
57[140] GCAACAGATGTAGATAATATCATAGATAAGTCCTGAAGATGA  
58[149] GCGTAAATACACCGTCTTGCTCAGATATAATCATCTTAAGTACA  
59[121] CAAAAGAACTGGTG  
59[151] GAACCATTACACTTGAGCACCCCTCAGCCCGGAACTTTGCGGAACGA  
60[139] GCAATAAAGAAAAAACAATGAACGGGTATTA ACTACAAAC  
62[139] TTTAAGAAAGGAAACCGAGCTGCCGACGACAATAATTTATCA  
62[149] TAAAGCAAGGAGCACCGCCCACTCATTTTGACCTTCCATTACC  
63[121] ATAGCAATAGCTCACAAACAATA  
64[139] AAGAGCAATTCTGTCCAGAGAATATAAGAGAATATTTTTACA  
64[149] TTTCGTCTTTCGTTTTCCAGTAGCGTCACCAGATA  
65[128] GAATTGAGTTAAGCCCACCACCGCCA  
67[121] AGAGGGTAATTGACCCTCAGAGCC  
67[140] ATATCGCATTAACTACCACACGCACGTATACTTTTCACCAGTCTGGCCC  
69[140] ATGGTTTCAATATAAAAAGAAACATCGAGAACAAGCAGAACCA  
70[111] ACCGTTCTGAGAAACATTCAACGCAAGGATAAAAAAAGATTCA  
70[125] CAATATGTATAAACTAGCAAACG  
71[117] CGATATTCAATTTTGTACAATCACACCACGAAAATACGGCTGTCT  
71[130] AATCTTGAGTTTTGCGGGGCTTGCCAAAAGACATCGCC  
72[139] TCAGTGCGCCCCCTGCCTAAGGTTCTTATTACGCAAAGGTG  
73[131] GTAATGACAACAAC  
73[140] TTTAACGCAACAGGAGTGTACCATGATTAAGACTTTGGAAC  
77[121] AATCCTCATTAATCTCCAAAAAAA  
77[140] ATGGAATATGGCCTTGATATTATCTTACCGAAGAGATATAAT  
77[158] GAACGATAGCCCGGAAAAGTAGCACCTCCCGTAAGAACGATATAGACCG  
79[140] CGATTCGTAAGAGAGATAACCCACAA  
79[151] TTTTTTCATAAACTACAGTTAGCTTGGGAAAACAACA  
81[121] ACCACCCTCAGACAACCTTCAACA  
81[158] AACTGCCATCCGGTCATTGTAGCGCCAGAGCCTTACCAACCCAGCAAAT  
83[99] AAAGGGTAGCTGATAAATTATGCCTGAGAGTCTGGAGAATC  
83[126] AAATCATAACAGCATTGAGGACAACGAAA  
84[139] GCGAAAGTCTGAAACATGAAAGATTCGGAACCTAAATTCAT  
85[117] AAAGGGTAGGGCCGGAGACAGTC  
87[121] CATCGCCACGCAACGGTGACCTGCT  
87[140] ATATAATAGAGTTGCGCCGACAATAAGT  
89[117] TGAATTTCTTAAACAGCAGCTTGGACCAGGAAAGCTG  
89[140] CCGATAATAAAGCGCAGTCTCGCTTTTGATGATTTCCGCCCTT  
90[156] CCCCCTCAGAGTACCGCCATTTGGAATTATTTGACGGCCGA

91[121] AGGCTCCAAAAGTTAAGAACTGACGA  
91[140] TAATTGGGTTACGTTGAAAAAGCCAGA  
91[151] GCATGTGAATAGTAGTAA  
93[128] TGCGAATAATAATTTTACAAAACCACCAGAGGTCAGA  
95[121] GTTTCAGCGGAGAACCCTCGTTGAGA  
95[140] AGAAAAAGGGATTTTAAATCGGTGGCGAGATGGTGGTTCCGAAGCCCCGA  
96[149] TATCGATCTATAGTAAGATTCAAC  
97[98] AGAAGCCTTTATACAAATTAAGCAATAACATCCAATAAATCAAATAACC  
97[119] GAGGCTTCGGAACGGGCCGCTAACAGTGCC  
98[125] GAAAAGAATTAGCAGGCTACA  
98[145] GGCACCAAACACGTTTCGGTCGCTGAGATCGTCACCCTTTACCGGGG  
100[104] TGTTTAGTACATTTAAGTTTCGTAGCTCAACATGTAGAGAGT  
100[118] TTGTGTCTACAGGCAAGGCGGAGGGAGTTA  
100[125] TGATAAAATCCGCGTAACTAAAGTACGGTGTCTGGCGCAAATGGTCGAA  
100[146] ACGGAGAACTTAGCAAAGAGGGCTGGCTCAGTATCGGTTTATCTTGATA  
101[133] CCATGTTTTTGTATATACACTAACCTAATAAAGACTTTTTTCAGGCAGCA  
102[90] ATAACAGGTTTGACCATTAGACTATATTGCATTAAGCCTCATTGCGGG  
104[104] ACCTTTACTCCAACGAAGCCCTATTATAGTCAGAACATTGAA  
104[125] CTCATTCAGTGATTTTTAAATATGCACACTTTTCGAGG  
104[146] CAAATCACAGAACGTACCTTACAGGACGGTGGAACAACCTAAAGGAAT  
105[133] GAAACACACGTAACCGCATAGACAGATGATAACCG  
105[155] ATTGGGGGATATTATCAAGAACTGACCAATAGGTGAGGGTTGTAC  
106[90] TTCAAATAGACCGGAAGCAAAATTGCTCTAATGCTATTCCAT  
106[111] TTAAGAGAGGTCAGGAATAAGGCTTGCCCTGCATC  
107[119] GGATTGGCTCATTAAAGATTCATGTCATAAATATTGCAAAGCAAAAAGA  
108[104] TCCCCCTTGGATAGACCAAAAATAGCGAG  
108[146] TTATTACATACCACAGCAACATCTATCACCGTAAAGCGGTTG  
109[133] TTTAGGAAGGTAGATACCAGTTGCGATTGAGCCTT  
109[154] TAATGCACTAACGGGAAAAATTAATCATAGCCCAAACCA  
110[90] AGGCTTTTAAATGTTTAGACCAAATGCCCTGACGAAAGAC  
110[118] AGACGACGATAAAACGTCCAATACTGCGGAATCCAGTTTACC

1[105] TTTTGAACGTGGACTCCAACGTCAATTCCAGTTTGGAAACAAGAGTCTTTT  
15[116] TTTTAAATTGTTATAAAGCATAAAGTGTAAGCCTTTT  
17[116] TTTTGGTCGACTCTAGAGGATGTCATAGCTGTTTCCTGTTTT  
19[116] TTTTCCCAGTCTTGCATGCCTTTT  
23[116] TTTTAAGCGCCATTCGATCGGTGCTTTT  
41[80] TTTTCAAGAGCGAGTAACAACCCGTTTT  
55[67] TTTTATGTACCCCGTTGATAAATTTT  
69[81] TTTTATCGTAAAACTAGCATGTCATTTT  
83[67] TTTTCAATGCCTGAGTAATGTAGATTTT  
97[65] TTTTAAAAACATTATGACCCTGTAACCCTCATATATTTTATTTT  
109[70] TTTTAGAGGGGGTAATAGTGCAAAGAAGTTTTGTTTT

13[106] TTTTCCTAATGAGTGAGCTAACTCACATTAATTGCGCGAACATAC  
 21[116] TTTTCTCTTCGCTATTACGCCTTAAGTTCAA  
 25[116] TTTTAGTATCGGCCTCAGGAATCTGTTTT  
 29[117] ATTATATGTCACGTTGGTGTAGAGAGGGGACGATTTT  
 33[117] TTTGAATACGGGTAACGCCTTTT  
 43[117] TTTAATATTGAATAACCTTGCTTAAATCAATTAACAACCGGAAACCA  
 98[83] AAGCTAATACTAATAGTAGTATTCATTTGGGGCGCGAGCTGAAAATTTT  
 101[70] TTTAACGAGTAGATTTATTGATTCTTAATTG  
 103[84] CTGAATACTTTTGATAAGAGGTCATTTTTGCTTTT  
 105[70] TTTTCTTCAAAGCGAACCATCGCGTAAATCAG  
 107[84] GTCTTTATTTAAACAGTTCAGAAAACGAGAATTTT  
  
 2[172] TTTTAAATCAAGTTTTTTGGGGTAAAGGGATGAATTTCCGG  
 4[172] TTTTGAGCTTGACGGGGAAAGCCCGAA  
 6[172] TTTTGGCGCTGGCAAGGTAGCGGCTT  
 8[172] TTTTGGCGCGTACTATGGTTGCTAGAATCATATG  
 10[172] TTTAACAGGAGGCCG  
 12[172] TTTTCAGTGAGGCCACCGAGTAATAGCAATGAGTAGA  
 28[156] AACAGCATCACCTTGCTGATTTT  
 56[156] CGCTTTTATTTTCATCGTATTTT  
 60[156] CAAAAGGTCTGAGAGACTACCTTTT  
 70[156] TTGCCAAAGACAAAAGGGCTTTT  
 85[158] CAGATTAGGAGAGGCTGAGACTCCTCTTTT  
 101[155] GGCGCACGAAACATGACCCCTAATGCCGTTTCCATTAACGGGTTTT

**AB-Linker strands**

1[97] TTTTTAAACACTATTT  
 3[108] GGTATAAATCAAAAAGAATAATCGGCAAAATCCCTGA  
 5[108] CCTGCCCCAGCAGGCGAAGCGGTCCACGCTGGTTGC  
 7[108] AAGATTGCCCTTCACCGCGAGACGGGCAACAGCTCG  
 9[108] GCTTGCGTATTGGGCGCCCCGCGGGGAGAGGCGGTAA  
 11[109] AGAAACCTGTCGTGCCACCCGCTTTCCAGTCGGAC  
 14[105] GGGAGTAACGACCGTG  
 16[115] TGTTTTGAATGGCTATTAGTGGCACAGACAATATTG  
 18[115] TGTGAGGCGGTCAGTATTGAAGATAAAACAGAGGCA  
 20[115] AGAATATCAAACCCTCAAACCTTGCTGAACCTCAGG  
 22[115] GGTAATAGATTAGAGCCGTAGGAGCACTAACAACGC  
 24[115] GGCCCGAACGTTATTAATCGTATTAATCCTTTGCA  
 26[115] CGTCAGATGATGGCAATTATCATATTCTGATTAAC  
 28[66] TCGAAATAAAGAAATTGCATTTGCACGTA AACAGG

41[63] ACCCAATAGGAACGCCACAGCTCATTTTTTAAAG  
 56[66] ATGCCTGATTGCTTTGAAAAACAATAACGGATTCCA  
 69[63] TTGAGATCTACAAAGGCTGGGTAGCTATTTTTGACA  
 84[66] AATCAAGAAAACAAAATTGATGATGAAACAAACATG  
 99[63] TATGGCATCAATTCATCGGTTGTACCGG  
 100[66] GGAATCGTCGCACATAGCGATAGCG  
 103[63] GTATGGCTTAGAGCCCAATTCTGCT  
 104[66] GGCTGAGAGACTATAACTATATGAG  
 107[63] TCACCATAAATCAATTTAATTCGTA  
 108[66] TGAAATATATTTGGTTTGAAATACC

**SH-probe strands.** The red- and green-colored portions of the sequences are complementary to the ssDNA conjugated to the enzymes, and are located in the Left and Right half-cages, respectively.

34[53] ATGACCATAAATCGCCTGATAAAT **GGAGGGAGGG**  
 48[53] TGTGTCGAAATCCCTCAGAACCGC **GGAGGGAGGG**  
 62[53] CACCCTCAGAGCGCAGCACCGTAA **GGAGGGAGGG**

51[117] TTTAGGCAGAGGCATTCAACGCCAACATGTAA **CCAGCCAGCC**  
 61[117] CGAACAAAGTTACCAGAAAGTAAGCAGATAGC **CCAGCCAGCC**  
 75[117] GTAAGCGTCATACATGTGAATTTACCGTTCCA **CCAGCCAGCC**



### Sequences of staple strands in the SS-left half-cage

0[55] TTGCTTTGACGAGCACGTA  
0[79] GCCGCTACAGGGCGCGTGGTCAAT  
1[37] TAACGTGCTTTCAATTCTACCACCGAGTAAAAGTT  
1[72] AACCTGTTTAGCTAGCTTAGTTGACCATTAG  
1[104] AGGGCGCTGAACGTGGCGAGAAAGGGGAGCCCCGATTTAGGTCGAGG  
2[55] GGTGGCATCCTCGTTAGAATCAAATACTATGG  
2[87] GAAATATTTTCATTTGAGTACGGTGCTGAATA  
3[37] ATAGTAGTAGCCTAAATCGAAACTATC  
3[72] GCAAGGCAAAGAAGGAGCTTAATTGTCTGGAA  
4[55] GCATAAAGATTAACATCATGAGTCTGTCCATCAGCAAAATCAC  
4[87] ATCTTAGCAAAATTAACAGGATTAATTCGAGC  
4[103] TGCCGTAAGTCTATCAGTGAACCATTGGAACAAGAGTCCAAAAGAATA  
5[37] GTACCAAAAAACAAAATTTTAATACCTA  
5[72] GGGAACGTCAAAGGGCGCGTTTTAGAGAGTAC  
6[55] CAAGGATAATTATGACCCGTGCTGGTAATATCGCGCAGTCTCT  
6[87] GAACGTGGACTCCAGATAGTCAGACGAGAATG  
7[37] AACCTCATATAGGCCGAGAGGGGGTGCTTTTGCTATTCGGTTATT  
7[72] TGTGGCAAAATCCCTTTCAGAAAAAGCAAAGC  
7[104] GCCCGAGAGCAGGCGAAAATCCTGAGAGAGTTGCAGCAAGTTTTTCTT  
8[55] GGTGAGAAATTTTAAACAGTGACGCTCAATCGGGGATAGCAAG  
8[79] AAATCGTAAGCGTCCACCAGACGA  
9[37] AGTCAAATCACCTATTTTTTATTTTTGATGTCAATCATAT  
9[72] TAGGCCCTTACCAGCCCTCGTTTAATACTGCG  
10[55] GAGGGTAGCATCAATAAGCGAGAGAATAGTAA  
10[79] TGATTCTGCTAATGCAGAGAATCGGAAGATTGTATTAACACATTAA  
10[103] TTCACCAGGCGGGGAGAGGCGGTTTTCGCTATTTCCAGTCG  
11[37] AGATCTACAAAGGCTATCAAACCTAGCAATATTTA  
11[72] TCTGGAGCAAAAATCGGCCAACGCTGAGACGGGCAACAGC  
12[63] TAATCGTAGGTCATTGATGCCGGA  
13[35] GTACCCCGTTGATAATCAGAATATTTTGAGATGCGA  
13[96] TTGCGTTGGTTCGTGCCAGCTGCATTAATGCAAGATACATAACAACATT  
14[63] AAACGTTAAAAGCCCCTTCATCAGTTGAGGGCCGC  
14[95] CTAATGAGTGAGCAAGAGTCAGGAGGTTTAAT  
15[35] TAAATTTTTGTAAATCAGCTTAATTCGCTTGGTAAC  
15[80] GTTATCCGCATAGCTGGCTTGCCCTCTTGACA  
16[63] ATCAAAAACATTTTTTGTGAATTACCTTAAGAAGC  
17[35] GTAGCCAGCTTTCATCAACATTCGTTGGGCCGAACCTGCGCAGACGACG  
17[80] TGCCAAGCACGACGTTAACGGTGTGACCTGCT  
17[96] CTGCAGGTAATTCGTAATCATGGTCTCACAATTCCACACATGGGGTGC  
18[63] TCGGATTCTAAATGTGTACCCAAATCAACCTGCGG  
19[35] TTGACCGTAATGGGATAGGTCCATCTGCCGACCCCA  
20[63] TAACCGTGACGTTGGTGAACGAGGACCAACTT

20[95] TGGGAAGGAGCTGGCGAAAGGGGGCAGGGTTTTCCAGTCTTGCATGC  
 21[35] GACGACAGTATCGGCCCTCAGGAAGATCGCACTCCAGCGCGCATCG  
 21[88] CTTCTGGTGCCGAAAGCAACTGT  
 44[71] ATACATTTGCAACTAAGGGCGCGATCATAACAG  
 45[88] GTTTCATTGAGTAGATGAAAGGAGCCGCCGCGCTTAATGC  
 46[71] TAATGCTGCAACAGGTGCAATAAAACTTTTTGC  
 47[48] GGCCTGAAGCAAACCTCTAGCTCAACCAATAAAGCTGAAAA  
 47[88] CTTTAATTGGCTTAGAACCTAAAGAAGGGAA  
 48[23] CCAGCCATTCACTTGCCCATATTTAAGGCTTACAATAGCACGAATTCA  
 48[71] TTCAAAGCGACTATTAAGCCTTTACTGAGTAA  
 49[48] CATTTGTCTTTACCCTGAACCAGACCTGTAATGCCTCAGA  
 49[88] GGATTGCACAAATATCGAAAAACCAGCACTAA  
 50[23] TACATTGGTGCAACAGTAATTTTCTTAATTGAAAAGCCAAGAGGACGA  
 50[71] ACCATAAAGACTGGATGGTAAAGAAACCGTTC  
 51[56] AATGTTTATCAAAAATTGCAATGCTTTCAACG  
 51[88] GAATCGTCTAACAGTATAAATCACTATTAAA  
 52[23] AACAGAGAAGTAATAAGGATTATATCGTCGCTAGTGAATATAGCCCTC  
 52[71] CGATAAAAACATTCAAATAAATTACCTGAGAG  
 53[56] GGAATACCACCAAATGATATTCTTCAAAG  
 53[92] CAAATCATAACCTGGCCCTGTTTGATGGTGGTTCCG  
 54[19] CCCTAATCCTGACAGATGATCTATTGAT  
 54[31] GCGAACTGTACGTGGCTTCTGGCC  
 54[43] AGTCCCACCAGCTTAAAATTCGCAT  
 54[79] ATTACAGGATTATACCCAAATATTGTGAAATT  
 55[56] TTTTAAGAAGTGGCTCTAGAAAGAAAAACAGATGAACGG  
 56[31] CGGTCAGTAAAAATACAAGGCCGCTTGCGCAT  
 56[79] TTCAACTTTGAATAAGTTTCCTGTACGGCCAG  
 57[56] AAAGCTGCTCATTAGTAATCATTAACCAATATAAATTGT  
 58[19] ATCTATCATTTTAATTTTAATAAAAAATC  
 58[31] CCCTCAATAAATGAAACCACCAGATTTTGCGGTTTCTTAA  
 58[79] AGAACCGGGACAGATGGTAAAACGTCTTCGCT  
 59[64] TGAAAGAGATATTCATAGCGAGTAGGAACGCC  
 60[31] AGGTTATCTCAACAGTTAAAGACTGCGGAACAGTATGCGT  
 60[79] CCATGTTACGAAACAATGCGGGCCCAGCTTTCCGGCACCG  
 61[24] GTCAACACCTACGAAGTTTTCATGTTTTTCAC  
 61[56] GCGATTATACCAAGCGCTTAGCCGGTAGATGGACAACCCG  
 62[23] AATACATTATTCGACAGCACCAACAAGATTGCTTTGAATATCATTCA  
 62[40] GGCAAAAAGAAATATAGAT  
 64[50] TTGGTAAAATACGTT  
 66[50] CTACAGAGGCTTCCATTAAGTCAATCATCATCTTTAGTTTGAGGGGAC  
 67[8] TTGTAACATTGGTTT  
 67[40] GGTAGCAAAACCTCAATAAGGGAAAACAAACGGCGGA  
 68[50] GAAAGACAGCATCGGAAAAAATCTAAAAGGTGAGG

70[50] TGAGGCTTGCACCCTCAGCTAAAACAGGCATCACCGTCTGGCCTTCCT  
71[32] AACCGATAAAAAGAAGACAGACAAGAG  
72[44] GACAACAACCATCGCCATTTAAGGGACAGGATTATT  
73[24] ACCGATAGCCGTAACAATTACCCT  
74[23] ACAGCCCATATATGTGTAATGGAAAGTGAATT  
74[44] CTTTCGAGGTGAAGATCGTCAGGGAGTTACGAACGAATTTAATGC  
75[24] GAAATTCGACCTTTTTCTGAGTTTTTTAGTAC  
76[23] AGATTTTCATTTAACACATCAAGATTAGGCGG  
76[44] GGAGCCTTTAATTAAGACGAG  
77[24] TCCAAAAAAGTTTTGTATTTTCATTCCCAAATC  
78[23] GTTGACAGGAAACAAAATTACCTGTGATGCAA  
78[44] TGCGAATAATAATAGGAAGTTTTGAGGACTGAAAGGAATATCAAA  
79[24] GCCTGGAATAAACAACCGTCTTTCTTGCTCAG  
81[24] TTTCAACATCCATCGCAAGACAAAGTTAATTTTGAAACATCCAAGTCC  
82[50] TTTCTGTATGGTTTT  
84[50] AGTTAGCGTAAAGTAAATGAAT  
86[50] AACGCCTGTAGCATTCAATTGTTTATCAGCTTG  
89[32] CATTTTCATCTGAAATTTTTGCCAGAC  
90[44] GCCACCCTCAGAGCCACCAATGAAAAACGTATTACCG  
91[24] AGAACCGCACCAGTATGAAGCCAG  
92[23] CGCCACTTCATATGCGTACTAGAAAAAGTACC  
92[44] CGTACTCAGGAGGCGTCACCACCCATGTATTGCGCCGACAAT  
93[24] TATCAGGAGTACTGGTTTATAACAATTGAGGCA  
94[23] ATAGGTCTATCATAATCGTTAAATCATCCCTC  
95[24] ATAAGTGCAAATAAGGAATAAGTTCCAAAGGT  
96[23] TACCAGGTTTTGAAATCATCTTCTTCAACAAT  
96[44] GATTAGCGGGGTTTCAGACGTTTCGATCTAAAAAGGCTCCAAAA  
97[24] ATCCATCATATTATTCACCGACCGCTCAGAAC  
100[50] TTTCCCTGCCTATTT  
102[50] GAGTAACAGTGCCCGTGATCGTCGAGAGGGTT  
106[50] GAATTTACCGTTCCAGCTTCACCCTCAGAACC  
107[32] AATGGAAACAGAACAACACTCATGGATAG  
108[44] AAATAAATCCTCATTAAATCGCTGAGTAGTAACCGTT  
109[24] TTGGCCTTAGCAAGGCTCCGGGAA  
110[23] GGTCAGCAACCGCGCCTTTATTTTAGATTAGT  
111[24] GACAACCAAAGCCGTTTCGGAAACGATTGACGG  
112[23] AAAGTAATACCGCACTCCAAGAACCGCAAATT  
113[24] AGAGCCCACTGTAGCCATCGAGAACATTTTG  
114[23] CGCCAGTTTTATCATTCAATCAATCCAGTTAC  
114[44] CAGAGCCGCCACCTGTATAAACAGTTAATGCC  
115[24] AGATACCGCCATCTTTGCGTTTTCCACAATCA  
116[23] TGAACAAGGTAGAAACTCATAATCCGCAAACA  
118[50] TTTGCCCCCTTATTT

120[50] AGTTTGCCTTTTTTCGGTCATA  
122[50] TAGCAGCACCGTAATCAGCGAGCCGCCGCCAG  
124[50] CAGTAGCACCAGAAACCATCGA  
125[32] TTAGAGCCACGCAAATAAGAACTCGTT  
126[44] CACCGACTTGAGCCATTTGGTTTTATAATAAGGGATT  
127[24] TTAAAGGTATAATAAGTACCGAAG  
128[44] AGGGAAGGTAAATTCACCAATTTACCATTGATATTCACAAAC  
130[44] AGACAAAAGGGCGACAAGTAGCGACAGAATCA  
132[23] ATAGATAATACAGAGAGTCAAAAAT  
132[44] AAGTTATTTTTGTATCGGCATAGCGTCAGCACCCCTCAGAGCC  
134[23] GCCATATTTGTTTAACATACATAA  
135[32] AGGTGGCACAAACGTAGACACCACGGAAT  
  
45[5] TTTTCAGAATCCTGAACTTCTTTAGATATAGAACAACGCCAACATTTT  
65[8] TTTTTTGCCCGACTTTAGGAGCACTTTTT  
69[8] TTTTTCATATCCGCCTGCAACAGTTTTT  
73[3] TTTTGAAGGGTTAGAACGGCAATTCTTTT  
75[3] TTTTGCACGTAAAACAAATTATCATTTT  
77[3] TTTTGATGAATATACAGAAGTTTGATTTT  
79[3] TTTTGGGAGAAACAATATAAATCCTTACAAACATGAGGATTTAGAAGTATTTT  
83[8] TTTTAAGATGATTACCTTTTACATCTTTT  
85[8] TTAATTACAGGTTTAACGTCATTTT  
87[8] TTTTTAAATCAATATCAAAATTATT  
91[3] TTTTGAAAACATAGCGAACCTTGCTTTTT  
93[3] TTTTGAGAAGAGTCAATACAGTACA  
95[3] TTTAACCTCCGGCAAACAAAATTTT  
97[3] TTTTATATGTAAATGCAGCAAAAGCGAATTATCCAAGTTACAAAATCGTTTT  
101[8] TTTTAATGGTGGGTTATATAACTTTT  
103[8] TTTTACACCGGAGAGAGACTACCTTTTTT  
105[8] TTTTTTTAGTATAGATTAAGACGCTTTT  
107[8] TTTTAGTAGGGCCCTTAGAATCCTTTTTT  
109[3] TTTTTGTAATTTAGGCACGCTCAACTTTT  
111[3] TTTTAATAAGAGAATATAAAGCCTGTTTT  
113[3] TTTTCGACAATAAACAAAAGAATAATTTT  
115[3] TTTTACGCGCCTGTTTAGACCTAAAATATTTTAGAACGCGAGAAAACCTTTTTT  
119[8] TTTTGTCTTTCCAGCTAATGCAGATTTT  
121[8] TTTTAACCAAGTTCTGTCCAGACGATTTT  
123[8] TTTTGAATCATTTTTTTCGAGCCAGTTTTT  
127[3] TTTTTTAGCGAACCTCCAGCAAATCTTTT  
129[3] TTTTAAGCCTTAAATCACATCGTAGTTTT  
131[3] TTTTTGAATCTTACCAAGGGTATTATTTT  
133[3] TTTTAGAGCCTAATTTGAATCGGCTACGAGCATAAAAATAATATCCCATTTTTT  
137[8] TTTTGCAGCCTTCGAGCGTCTTTCCTTTT

139[8] TTTTATTAAGTGTACAATTTTATCCTTTT  
141[8] TTTTAGATAACCGCGGGAGTTTTGTTTT  
143[32] TTTTCCCTTTTTGCCGATTACAGTGAGGCTATTTT  
  
44[23] TTAGACAGGAACGGTAATAGCAATAACGCGAGGCGTTTTTT  
46[23] GTAGCAATGAAGTGTTATTCTAAGAGCTATCTAGCAAGAAACAATGAATTTT  
47[5] TTTTAGTAATAACA  
50[15] CAGATTCATCTGTAAACTTCTGAATAATGTTTT  
51[5] TTTTGTACACGACCTAGAACCATCAATATAAA  
53[5] TTTTGGACCTGAAAGCGTAAGAAATAG  
55[13] TTTTACATCGCCATTATTAACACCTGATTATAGGAGCGGGAAATAAA  
57[3] TTTTGGCACGCTGAGAGCCAGCAGCCAAT  
59[13] TTTTCAGTTGGCAAATAAAATATACGTTATTACTCGTATACGGATTC  
61[3] TTTTAAACAATAATAGATTAGAGCC  
63[0] TTTTTTAGACTT  
81[0] TTTTCGCAGAGG  
99[0] TTTTTTTCAAAT  
117[0] TTTTCCTAATTT  
128[23] AAATCCTCACAAGAAAGCGCTAATATCAGAGTTTT  
130[23] CACCAGCAACACCCTCGCATTAGACGGGAGATTTT  
135[0] TTTTATAAGAAA  
136[14] GAAAATACGATTTTTATTTATCCCAATCCAATTTT  
  
2[122] TTTTGGAAAGCCGGCGGCAAGTGTAGTTTT  
4[122] TTTTAAGTTTTTTGGGAGCTTGACGGTTTT  
6[122] TTTTGTGTTCAGTTCACCCAAATCTTTT  
8[122] TTTTGGTTTGCCCATAGGGTTGAGTTTTT  
10[122] TTTTCGCCAGGGTGGCGGTCCACGCTTTTT  
14[119] TTTTCATAAAGTGTAAGCCACATACGAGCCGGAAGTTTT  
16[119] TTTTCCGGGTACCGAGCTCGCGACTCTAGAGGATCCTTTT  
18[119] TTTTTTAAGTTGGGTAACGCATGTGCTGCAAGGCGATTTT  
20[119] TTTTTCGCCATTACAGGCTGCCAGGCAAAGCGCCATTTT  
56[92] TTTTGAGATCGTTGGTTTT  
58[93] TTTTGAGTAATGACGTTTT  
60[92] TTTTTCCGCACAGACTTTT  
0[122] TTTTCGGTCACGCTGCGCGTAACCACCACACCGGGCGCT  
12[111] GGAAACCTCGCTCACTGCCCGCTTTTTT  
19[80] ATTACGCCGCGATCGGAGTACAACGGAGATTTT  
45[56] TTAAATATCGCAGAGCGGGAGCTAAACAGGAGAAGAAAAGTTTT  
54[93] TTTTAAACGGAACGC  
80[40] TTTTGAGAATAGACTAAAACGTAATGCCATAAAACACATTGAGGA  
98[40] TTTTGGCTGAGACGTTTCAGCGATTTTGGCAACTAAAGGAAT  
116[40] TTTTCCAGAGCCAGAAAGTATTCGGAACCAGAGAAGGATTAG

129[24] TGCTATTCGGTAATTGTTGAGTTAAGCAGTTACCAGAAGGTTTT  
 131[24] TTTTCATATGGTCAGGGAAGGAACAAAGTCAATAACGGAATACC  
 133[24] AAAATAAAGAAAATACGAATAACATAAAAGACTCCTTATTTTTT  
 134[40] TTTTTAAAAGAAAAAATCACAGCGTTTGGAACCGCCTCCCT  
 136[48] TTTTGTATGTTAG  
 138[48] TTTTGAACTGGCATGATTAAATTACCAGCGCCAA  
 140[48] TTTTGAGGAAACGCAATAGAGAACCGATTGAGGG  
 142[48] TTTTAGATAGCCGAACAACCAGAATTATCACCGT

**Sequences of staple strands in the SS-right half-cage**

22[116] GCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCACCAGCTTGCTTC  
 23[88] ATCGGCCAGGAAACAGAATTTATCCAGACGAC  
 24[71] TGTCGAAAATCCTGTTTGATGGTCAAAGAATA  
 24[116] ATTTGAATTACCTTTTTTAATACGCGCGCCAGCTGC  
 25[56] GCCCGAGAAGTCCACTATTAAAGAGTCTATCAGAACCATCGTAAAGCA  
 25[88] AAACAAAAAGATGATATTTACGATGAAAATA  
 26[71] GGAACAAGTAGGGTTGTTTCAGCTAAGACGCTG  
 26[116] TTTCAATTACCTGAGCAAAAGTTAATTACATTCTGTCAAAAATCAT  
 27[88] TACAAAATGCCTGATTTGAGCGCTTCACCGAC  
 28[55] CTAATCGGACGGGAAAGCCGGCAAGGAGCGGGCGCTAGTAACCACC  
 28[71] GAGGTGCCACCCAAATGAATAACACAAGAAAA  
 28[116] GGGAGAAACAATAACGATTCCGCGCAGAGTCAAAAAGCATGTAG  
 29[88] GAATATACAGATTTTCAGCAGCACTAAGTTTT  
 30[71] GAAAGCGAGAACGTGGTTAGAGCCCCCTGAAC  
 30[116] ACAGAAATAAAGAAATTGCGTAGTAACAGTATCACCGAATATCAG  
 31[56] ACACCCGCATGGTTGCTTTGACGAGAGCGGGAGCTAAACA  
 31[88] CTACCATATCTGAATAATTAAGAGAGGAGCGGCCGAACGT  
 31[104] TATTTGCACGTAAAT  
 32[71] CGCGTACTCGCGCTTATGAGTAACAACGTCAC  
 32[116] CCTGATTGTTGGATTATACTTCAAATTACTGGTAACGTAATCA  
 33[64] GGAGGCCGATTAATATCTACAGGG  
 33[96] ATGGCAATCCACCAGAGCTTATAC  
 34[79] TCAAGGGATTTTAGACCCTATTATTAGCG  
 34[124] TCATTTTGCGGAACAAAGAAATCATCAATATAAT  
 35[64] CCACCGAGTTGTAGCAATACTTCTAAGAACTCAAATATCCGCCAGCC  
 35[96] TATTAATTGTATTAAGAATCATGAGGAAGTTCAG  
 36[79] ATTAACCGTAAAAGAGATTAGGATTCTGAAACCAGT  
 36[124] TTACAAACAATTCGACAACCTTAAAAGTGACCCCA  
 37[96] TACATTTGTAGATTAGCAGAGGCCGCTTTTGCAAT  
 38[63] ATTGCAACGACGCTCAATCGTCTGTACACGACCAGTAATCCTTCTGA  
 38[79] AATATTACGGCCTTGCAGGAGGTTGAGGGTTG  
 38[124] TTTAGGAGCACTAACAATAAAGGATTTAACTAAAGA  
 39[96] AGGAATTGTCAGTTGGCATTGCTT

40[79] TTCACCAGAAATGGATAACCCATGCCTCAGAG  
40[124] AAACCCTCAATCAATATCTGGAGGAAGGTTGCAGGGA  
41[64] CCTGAAAGAATGGCTATTAGTCTTCATTAATAAATACCGAACGAACCAC  
41[96] AAGCATCAAGCCAGCACAGCGGAG  
42[124] CTGCAACAGTGCCACGCTGAGCCTTGCTGCGGTTTAT  
43[80] CAGCAGAAACAGACAATATTTTTGCGTAAGAAAGTTTTGTCTGTAGCA  
43[96] AGAGGTGAGGCGGTCAGTATTAACACCGC  
44[143] CCATTCGCGAATAATAAAAGCTGCATTCATTAACCCACC  
45[136] CGCTTCTGGCACTCCAAGTGAATAGCCAGAGGAGAGGCTTTGCGAATA  
48[143] GGGACGCATAGTAAAACGGTGTCTTGTTTTAAGAAATCCG  
49[136] GCATCGTATAGGTCATTCATTCCGGTAAAGAAATGCAATTCAGTTG  
56[127] GAAATTGTCATGGTCAACCGTGTGATAAA  
60[127] CTCACATTTGGGGTGCAAGACAAAGAACG  
62[95] ATTAATGATGTAATCCAATAGTGTACATAAACATCAAGA  
62[119] CGGGAAACGAGACTACCTTTTTAATTAGTACC  
63[72] AGAAGAGTGTGCTATTGAATAACTGAGACGGGCAACAGCTGATTGCC  
63[104] AGGTCTGACTGTCGTGGGAGAGGCGGTTT  
64[135] ACGGTCCGCAGAAAAGTGAGCTAA  
65[115] TATATAACTATATGAGGCATTCAACGCCAAAGCCGTTTTTAT  
67[115] CGAGAAAACCTTTTTATGGCTTAATTGAGAATC  
68[135] CGGAGAATTTGTAAATCCTGTGT  
69[115] TTTTCATCTTCTGAATTCTTACTTTAGTATAGAACGCGAGGCG  
73[109] GAAAAAGCCTGCAGTATAAAGC  
75[109] CAACGCTCAACAGTAGTTCACCGCGCCCAATA  
77[109] GCCATATTTAATTTCGAGCCAGT  
79[109] AATAAGAGAATATAAAAGCATCATTCCAAGAA  
80[87] GACAATAACCATCCTAGAAACAAATACCAAGT  
80[119] GACAAAAGCAATAATCGGCTGTCTTCGAGAAACGATTTTTCCCACAAG  
81[72] ATAATATCACAACATGAGTGTGTTCAATATA  
81[104] AAACCAATGTAAAGTAATTTAACAATTC  
82[135] TAACACTTAATAAAGCTTTAGGCAGTAAATGC  
83[115] CGGGTATTAAACCGTCAAACAGCCATATTATT  
83[136] CACTCGAATGTACCAACTCAGAGCATCGATGA  
84[135] GAACAAGCACATGTAAGAAGCCTTTCAAGGGT  
85[136] AACCTGTTTTGCGGGAAAACATTATCACAAAT  
86[135] AATGGATTAACGCAAGTTATACAACCTAAATT  
87[136] GGCTTCGAATATTTAGATAAAAAATTAATGC  
88[135] GTATTCTACATATGCGTTCTAAAC  
89[115] TTTTAGCGAACCTCCCGAAGTGTGGTGTCTCCGTG  
91[109] AATCAAGATTAGTTGCGTAAACTGGCATGATT  
95[109] TTGCCAGTTACAAAATAGGCTTTTTAAGAAAA  
97[109] TATCCCAATCCAAATACGTCAATAATAAGAGC  
98[87] GCAGCCTTAGGGTAATGCTTTGAACGTCAGAT

99[72] AAAGTCAGTACAGAGACAAGTTTTTCCAGTTT  
 99[104] AGAGATAATGTTTAACGGCGAATTATTCA  
 99[120] AATTGAGTCATAATTATTCATTAAGAATCAA  
 100[135] ATTGCGCCTTTAATTCTCCAACAGAAGTACCG  
 101[115] AAGAAACAATGAAAACCGATTGCCAAAGACGTTTGCCATCTT  
 101[136] AGCTAGTCAAGCAAACGAGCTTCAAGTAGCAT  
 102[135] CCGAAGCCATTAGAGACTAACGAGATCTCAAT  
 103[136] GTTCAGAAAAGAGGTCGTACCTTTGCTATCGA  
 104[135] GCTTTACATACCAACGAATATAATATATAGAA  
 105[115] GAAACCGAGGAAAAAGACACCGTGGCAACCGCCACCCTCAGA  
 105[136] TAACGGGAAATTGCTGATTTTTGCATTTTCGCA  
 106[135] CCCAAAAGGCTCAACAGGACTTGC  
 107[115] AAGACTCCTTATTACGCATAAAGGCGATTAGATGGGC  
 111[109] TTATTTTGTCAACAATCCATGAACCAGAGCCAC  
 113[109] GGTTTACCAGCGAGGGAGGGAA  
 115[109] GGTAATATTGACGGACAGTCAGACTGTAGCG  
 116[87] TTGAGCCACCATCGATAGGTTAAGTTAGAAC  
 117[72] CAATGAAATTTGGGAACGAGAAAGTTGGGGTC  
 117[104] GTAGCGACAGGTGAATTACCTTTTACATC  
 117[120] GTTTGCCCTCCTTTTTGATGATACAAACAAAG  
 118[135] ATAAAGCGTTGAGATTTCGACATTCATAGCAAT  
 119[115] CGTTTTTCATCGGCCCGGAATT  
 119[136] TCATAATACAGATACATAGGAATACAAAGCGG  
 120[135] CTTATTAGCAAAGGGAGCAACACCGAAAACA  
 121[115] TTCATAATCAAATCCTCATTCTTGATATTCGGTCGAAACAGCT  
 121[136] GTGAATTAATAGTAAGTAACGCCACAGTCTTA  
 122[135] TTTAACCGAACCTCGGAAACGCACGCAATAA  
 123[115] CACCGGAACCGCCGACGGAGG  
 123[136] AGCCGGAGAAATAGCGTTTACCAGCCTCAAAT  
 124[135] CTCAGAACATATAAAAGGGGTATG  
 125[115] GCCACCACCTCAGAGCCTTCGCCAGCTTGGGGATGT  
 127[109] GCCGCCAGCATTGACAAAGGAGCCTTCAACTAAA  
 128[119] TTGAGGCAAATTTCTTCTGAGGCTTATCTAAAATATC  
 129[109] CAGACGATTGGAAAGCCAGGGGATCGTCTTTGAGGGAAGTATTAGACT  
 131[109] GGAAAGCGCAGTCTCTAACTACAGAGGCACCCTCA  
 132[119] TACCGTTCTCCATTAATCATCTTTTTGAGTAACATTA  
 133[109] TAAGCGTCATACATGGGCTTAAAACACACGGGTAA  
 134[75] GCCTATGCGCCGGAAGGGAA  
 134[87] AACGGGGTATGAAAGTATGGAAGGTCCTGATTATCAGATG  
 135[104] CAAGCGCGAGGAGTGT  
 135[120] TACAACGGAGATTTGTGAATACACCCATGTTATAAGGGAAATTTTCGG  
 136[103] GCGATGAGACTCCTCAATAGCCCGTCTTTGCATAGATAA  
 137[80] ATATAAGTAGAGAAGGTCTGTCCAAATTATCA



137[128] AATACGTAGCAACGGCTGACCAACCAAATAAATCATCATT  
138[103] CTTTTTAGGTGTACTCATTTTAGCCGTCACAGTTGAA  
139[80] CCACCACCCCGTACTCTGGTAATATCACGCAA  
139[128] GCAGCGAACCGATATATTCACAAAGTAATCTTTCCCTCAG  
140[103] GTTAAGGATAGCAAGCACAGCCCTCAAATCAAAAAATCTA  
140[143] ACGCATAAAGACAGCAGTACAGACTTTGAAAGATTGCCCC  
141[80] TTCCACAGCCAATAGGTATTTACATCCAGAAC  
141[104] TTTCGAGGTGGGTTT  
141[128] TGATACCGTCCAAAAGAACCGGATTCAGCCAC  
142[103] CAGCTAGTTAGCGTAAAACAGTTTGCAAATGAGATAAAAC  
142[143] AAAAAGGCATAGTTGCCATCAAGACAGGCGCAATTTCAAC  
143[66] GGGATTTTGCTAAACAACCTTCCGATCTAATACGTGGCTTGGCAGA  
143[104] TTTGAGAATAGCCTT  
143[128] GGAATTGCCATTCAGGTCCGGCAC  
46[154] TTTTTCAGGAAGATCGTGCCGAAACGTAACATTTTTTCACGTTTTTT  
50[154] TTTTCCGTAATGGGAACCGTGCCTAAAGTATGTTTAGACTGGATTTT  
54[151] TTTTCTGGCCTTAAAGGCCGAGACTTTT  
56[151] TTTTCCAATAGGTGATATTCAACCGTTTT  
58[151] TTTTTAATATTTTTGAGAGATCTACTTTT  
60[151] TTTTATTGTATACCTGAGAGTCTGGTTTT  
64[156] TTTTAGCAAACAAGAGAATAAAGCTTTTT  
66[156] TTTTAAAGGCTATCAGGTGACCCTGTTTT  
68[156] TTTTTTCTAGCTGATAATTTTTAGATTTT  
70[156] TTTTAGTCAAATCACCAGCCTGAGTTTTT  
74[151] TTTTACCCTCATGTAGATTTAGTTTTTTTT  
76[151] TTTTTAATACTTTAGCTATATTTTCTTTT  
78[151] TTTTAAATCGGTAAGGTGGCATCAATTTT  
80[148] TTTTCAAGGCAAAGAATTAGCAAACCTAATCGTAAAACCTAGCATGTTTT  
82[156] TTTTTTCTACTAATAGTAAGCGAACTTTT  
84[156] TTTTATTTGGGGCGCGAAATTGCTCTTTT  
86[156] TTTTGACCATTAGATACGGATGGCTTTTT  
88[156] TTTTTTCCCAATTCTGCATATGCAATTTT  
92[151] TTTTLAGAGCTTATCGTCATAAATATTTT  
94[151] TTTTCTTTTGATAACGAGAATGACCTTTT  
96[151] TTTTCAGACCGGTTTACCCTGACTATTTT  
98[148] TTTTAAAGCCGAAAGACTTCAAATATTCTCCAATAAATCATACAGGTTTT  
100[156] TTTTTTATAGTCAGAAGCCACATTTCTTTT  
102[156] TTTTATAAATCAAAAATAAAGGAATTTTTT  
104[156] TTTTTTCATTGAATCCCACGACGATTTTT  
106[156] TTTTLAGCGTCCAATACTTGCAAAATTTT  
110[151] TTTTAAAAACCATAGTAAATTGGGCTTTT  
112[151] TTTTACGAGGCCCTTATGCGATTTTTTTT  
114[151] TTTTAACTAATGCCAGTCAGGACGTTTTT

116[148] TTTTATTATTACAGGTAGAAAAGATTTAAATCAAAAAGATTAAGAGGTTTT  
118[156] TTTTTGGGAAGAAAAATGAACGAGGTTTT  
120[156] TTTTAAAGAACTGGCTCAGGACAGATTTT  
122[156] TTTTTTGAGATGGTTTATAGGCTGGTTTT  
124[156] TTTTCGAGAAACACCAGCCAAATCTTTT  
128[151] TTTTCTGACCTTGCCGACAATGACATTTT  
130[151] TTTTTGAACGGTTCGTTTT  
132[151] TTTTCGCAGACGACGAAGGCACCAATTTT  
134[148] TTTTAAATTGTGTGCAAATCCGCGATTAACGAACTAACGGAACAACCTTTT  
136[156] TTTTCCTAAAACGAAAGAGGCAAAAATCATCGCCTGATTTTT  
138[146] TTTTGAACGAGGGTAATGCCACTGTCAATCACTTAGCCGCTACGTTATTTT  
44[154] TTTTCAGGCAAAGCG  
47[136] GCTGACGACAGTATCGGAAGTTTTAGGCTTGCCCTGATTTT  
48[154] TTTTGCCAGTTTGAG  
51[136] GGAACAAATAACAACCAGGGTGAGCCTGTAGCCAAAAATAATTCGCGTTTTT  
51[144] CGGCGGATAATGTGTAATATAACAGTTGATTTT  
62[148] TTTTCAATCATATGTACCCCGTTGATCCAGT  
65[136] TGATGTTGAGCAAATACCCCAAAAACAGGAAGTTTT  
67[136] AGCTATTTTGTAAAATTTAAATTGTAAACGTTTTT  
69[136] TAATGATAAACGCCATTCAGCTCATTTTTTAATTTT  
71[115] TAAGGCGTTAAATAAGAAAAACGTCGGATATTAATGTGAGCGAGTTTT  
140[156] TTTTACAACCATCGCCC  
142[156] TTTTGAAAATCTCCAAA  
23[32] TTTTTTGCAAGCGGTCCCCTGGCCCTGAGAGAGTTTT  
25[32] TTTTAATCCCTTATAAATCAGTTCGAAATCGGCAATTTT  
27[32] TTTTCAAAGGGCGAAAAACCACGTGGACTCCAACGTTTTT  
29[32] TTTTCCCGATTTAGAGCTTGAACCCTAAAGGGAGCTTTT  
31[32] TTTTGCGGTCACGCTGCGCGGGCGCTGGCAAGTGTATTTT  
37[40] TTTTCACTTGCCTGAGTAGTTGATTAGTAATAACATTTT  
39[40] TTTTGAAATACCTACATTTTAGGAAAAACGCTCATGTTTT  
41[40] TTTTCCAACAGAGATAGAACAAAAGGGACATTCTGGTTTT  
43[40] TTTTAGCCCTAAAACATCGCTAATGCGCGAACTGATTTTT  
53[114] TTTTCTAGAGGATCAACGCATGCCTGCAGGTTTT  
55[111] TTTTATTCGTAATTATCCGCTTTTT  
59[111] TTTTGTAAAGCCAATTGCGTTTTT  
63[58] TTTTAGATTAATGCATTTT  
81[59] TTTTCTGAATAAAAATTTT  
99[58] TTTTGAACAAGCAATTTT  
117[59] TTTTCCGGAAGTGCCTTTT  
135[58] TTTTTCGGAAAGGAACGGCAGTGAGGTTTT  
137[67] TTTTGTGATAGTACTTTT  
139[66] TTTTCGCCACTACCGTTTT  
141[67] TTTTAACGCCGTCTTTTTT

22[55] C TTCACCGACGCTGGTTTGCCCCAGCAGGGAGTAATTAATTTTCCCTTTT  
 33[32] T TTTTTTCCTCGT  
 34[47] A ATCCTGATAGAATCAGCACGTATAACGTGCTTTTT  
 35[40] T TTTGAAGTGTTTTTATAATTACGCCAG  
 45[114] G GGGCGACCACCAGAGAAAGGAAAATTGTATAACCTCAAATATCTTTT  
 47[114] C CCAGCTCGTAGAAAATATTTTT  
 49[114] G GTTTTCTTGAAGCCTTATTTTT  
 51[114] C CCAGTGCATAAATACTATTTTT  
 57[111] T TTTTTCCACACAACATATTTATATTTTAGTTAA  
 61[111] T TTTTCACTGCCCGCTTTAATGCTTAGGTTGGGT  
 66[135] C CCAATCGCCTAATGAGTTCGCATTAACGAGCCGGAAGCATTTT  
 70[135] A AAATACCGTAGCTGTTTCAGCTTTCATCCCCGGGTACCGAGTTTT  
 72[127] A ACCGGAATCCAAGCTTGACGTTGTAAAACTTTT  
 90[127] G GGGAGGTTCCAGTCACAAGTTGGGTAACGTTTT  
 108[127] T TAGCAAAGGCGAAAGGGCCTCTTCGCTATTTTT  
 126[127] C CAGAACCATCGGTGCGCTGCGCAACTGTTTTTT

### SS-linker strands

44[114] G GGTCCCAATTCTGCGAACCCATATAACAGTTGATAA  
 46[114] T TAGGTCATTTTTGCGGATGCTCCTTTTGATAAGACG  
 48[114] C CCAGAAGCCCGAAAGACTTTCAAAAAGATTAAGAGGG  
 50[114] G GACTCCCCCTCAAATGCTTATAAATATTCATTGAAGG  
 52[114] T TCGAGTAAGAGCAACACTAAGGAATTACGAGGCATAC  
 54[111] C CTCTTAATAAAAACGAACTGAAGAAAAATCTACGGA  
 56[111] C CACGTAGTAAATTGGGCTTAGAAACACCAGAACGAAA  
 58[111] T TAAGCTGACCTTCATCAACAGGCGCATAGGCTGAG  
 60[111] T TGCATAAATTGTGTCGAAATTTGTATCATCGCCTGGC  
 63[40] A AAAACATAGCGATAGCTTTTAGAATCCTTGAAAGA  
 81[40] G GGACAACAATAGATAAGTTCGAACGCGCCTGTTTATGT  
 99[40] T TAAGACGGGAGAATTAACCAGGGAAGCGCATTAGA  
 117[40] C CGGATTACCATTAGCAAGGAATCACCAGTAGCACCAA  
 135[40] A ACATGCCCCCTGCCTATTCGTATAAACAGTTAATA  
 137[48] A ACGCAGGCGGATAAGTGCCGGTTTTGCTCAGTACCA  
 139[48] C CAACGCCACCCTCAGAACCGCCACCCTCAGAACAA  
 141[48] A AAACACCAGTACAACTACTAACACTGAGTTTCGTCC  
 143[48] T TAAATGAATTTTCTGTATTCCAGACGTTAGTAAGC

**SS-probes.** The red- and green-colored portions of the sequences are complementary to the ssDNA conjugated to the enzymes, and are located in the Left and Right half-cages, respectively.

94[44] G ATATAAGTATAGTGACACAGACAGCCCTCATGGAGGGAGGG  
 104[50] C TTTTGATGATGTCAGTGCCTTGGAGGGAGGG  
 110[44] C ATTGACAGGAGGATTTAAGCGTCATACATGGGGAGGGAGGG

87[115] GCAAGCAAATCAGGCTTATTTTGCACCCAGCTCCAGCCAGCC  
 93[109] ACAATTTTATCCAGAGCCTAATCCAGCCAGCC  
 103[115] GTAAGCAGATAGCTATAATAGAAAATTCATATCCAGCCAGCC

## DS Full-Cage design

### Cross-sectional view

#### Sequences of staple strands in the DS-left half-cage

1[16] TTTTCAGTACAAACTACAACCACTGAGTTTCGTCACTTTT  
 3[16] TTTTAATTTTCTCAGCTTCCGGCATT  
 5[16] TTTTTCACGTTGGAGATCTTTTT  
 7[16] TTTTATACCGATAGTTGCGCTTCTTAAACAGCTTGTTTT  
 11[13] TTTTCCATTAAACGGCAAGCGCGAAATTTT  
 12[31] GATTATACGTAAAATATGTTTAGAGTCACCCTGTAAAGGCCGCTTTTTTTTT  
 13[13] TTTTCAAAGTACAACAACCGAACTGATTTT  
 14[31] CATAAGGGGGAGATTTAAGAAGTTTTGCCTTTT  
 15[13] TTTTCCAACCTTTGAAAACGTAACAAATTTT  
 16[31] CCCAAATCAGAGGACACCCTCGTTTACCATTTT  
 17[13] TTTTGCTGCTCATTTCATGCGATTTTATTTT  
 18[31] ATTACCTTAGTGAATATACGAGGCATAGTTTTT  
 19[13] TTTTAGAACTGGCTCCGGTTTTT  
 20[39] TAATAAAACGAACTAAATTATACCGATTTAGGAATACTTTT  
 21[21] TTTTAACAACATTATGCTTCAAATTCAAAATAGAGAGTACCTTTATTTT  
 23[19] TTTTACATTCAACTAATGAAAAAGATTAAGAGGAATTTT  
 25[19] TTTTAAGAGCAACACTAGACTATTAATCAAATCAACATGTTTTATTTT  
 27[19] TTTTGACGACGATAAAAACGACAGTTCAGAAAACGATTTT  
 29[19] TTTTAGAGGGGGTAATAATAAATATAGCGTCCAGTAGATTTAGTTTTT  
 31[21] TTTTGATTCATTGAATCCTTTT  
 33[13] TTTTCCCTCAAATGCTTTAAGGTGTGTCTGGAAGTTTTTTT  
 35[13] TTTTGAATGACCATATAGTCAGAAGCTTTT  
 37[13] TTTTAAAGCGGATTGCATCAACAGGTCATTTTTGCGTTTTT  
 39[13] TTTTGCCCGAAAGACGCGTTTTT  
 41[21] TTTTAACCAGACCGGACATTATGAAAGCTAATCAACGCAAGGATTTTT  
 43[19] TTTTATTGCTCCTTTTGCATAAATTAAGCAATAAAGTTTTT  
 45[19] TTTTGATGGCTTAGAGCCCAATAAATACTAATATGAGAAAAGGCCGGTTTTT  
 47[19] TTTTAATATGCAACTAAAAACGCGAGCTGAAAAGGTTTTT  
 49[19] TTTTTCATTCCATATAAGTCAATAAACCATTAGATTT  
 51[21] TTTTTTGCCTGTTTAGCTTTTTT  
 53[13] TTTTATATTTTCATTTGGGGTCCAATATGATATTCATTTT

55[13] TTTTGGCATCAATTCTCATAACAGGCATTTT  
57[13] TTTTAGGCAAAGAATTAGCAAGCATATATTTTAAATTTTT  
59[13] TTTTCCTCAGAGCAT  
61[25] TTTTCCCTGTACATTTTTCATTAAATCTGGCCTTCCTGTTTTT  
63[19] TTTTAAAAATTTTAGATCCTAAACGTTAATATTTTTTTTT  
65[19] TTTTGCAATGCCTGAGTAAACAGGAGTTGATAATTGACCGTAATGTTTT  
67[19] TTTTAGACAGTCAAATCTGTACCCCTTTT  
69[19] TTTTACCGTTCTAGCTGGAGCAAACATCAGGTCACTC  
70[27] TTGAAAAATCTCGCGAATAATAATTTTTTTTTT  
71[17] TTTTAAAGGCTAAGAGAATCGATTTT  
73[13] TTTTGAACGGTAATCGTAAAACTGCATCTGCCAGTTTTT  
76[23] AGATTGTATAATTTT  
77[13] TTTTGCAAATATTTAAATTGTTCCCGTCGGATTCTTTTT  
79[13] TTTTGTTAAAATTCG  
81[25] TTTTACCAATAGTCGACTCAGTGCCAAGAAATTGTTATCCTTTT  
83[19] TTTTAGCCAGCTTTCATATACAGTCACGACGTTGTATTTT  
85[19] TTTTCCGTGGGAACAAACAAGGCGAAGCTGGCGAACTCACATTAATTTTT  
87[19] TTTTGGATAGGTCACGTGCTCGGTGCGGGCCTCTTCTTTT  
89[19] TTTTTTGAGGGGACGACGCCATTACGGAAACCCGTATTGGGCGTTTT  
90[27] CAGCGTATGGGACAGACGTTAGTAAATGTTTT  
91[11] TTTTCCGCTTCTGGTGCGGCTGCGCAACTTTT  
93[13] TTTTTGTTGGGAAGGGCGATATTGTCGTGCCAGCTGTTTT  
95[13] TTTTGCTATTACGCCTTAAGTTGGGTTTTT  
97[13] TTTTAAACGCCAGGGTTTTCCAAAGTGTAAGCCTGGTTTT  
99[13] TTTTAAACGACGGCCTAGAGGATCCCCGTTTT  
101[11] TTTTGGTACCGAGCTCGAATTCGTACAAAGGGCATTAAAGA  
103[19] TTTTGCTCACAATCCATGTTGTTTCAGAATAGC  
105[19] TTTTGGTGCCTAATGAGCGAAATCGGAAAATCC  
107[19] TTTTTCGTTGCGCTCAAGCGGTCCCCTGGCCC  
109[19] TTTTCATTAATGAATCGAGACGGCAACAGCTGATTTTTT  
111[21] TTTTCCAGGGTGGTTTTTCTTTTTACCGTAAGCCTGTAG  
113[13] TTTTGCCCTTACCGACGCTGGTTTGTTTT  
115[13] TTTTCCCAGCAGGCGCAAATCCCTTTTT  
117[13] TTTTTATAAATCAAACAGTTTGGAACTTTT  
119[13] TTTTAAAGAGTCCACT  
121[24] TTTTAAAAACCGTCTATCATCCAACGTATCATGG  
0[55] CCAATAGGAACCCATGATAACGTGTTAGAGAGG  
1[40] CATTCCACAGTTTGTTTAAAAATCCATCAGGA  
1[72] TCGAGAGGTCAGTACCAGGCGGATTAACAGTG  
1[88] AAGTATAGACCCTCAGAGCCACCACCCTCATTTTCAGGGAAAGTGCCG  
2[55] CGATCTAAAGACAGCCAAGGGATTCTTTCCTCGTTTTGAC  
3[40] AACAACTTAACAACCTAGAACCTACTAAGGAGAG  
3[72] CCCGTATAGGGTCAGTGCCTTGAGCACAAACAAATAAATCGATTGGCC

4[55] TAGAAAGGTCAACAGTTTCAGCGGTAGCGTAA  
4[87] TTTTAACGAACAGTTAATGCCCCATTAGCGGGGTTTTGCGTTGATAT  
5[40] AGGCTCCATTGCTTTCATTTTAGTTGAATTCTGC  
6[55] TTATCAGCAAAGGAGCAACAGAAACATA  
6[71] TTGATATTCGCCTCCCTCAGAGCCGAGCCACCACCGGAACCAGTAGCG  
7[40] ACAACAACCTGAGGCTCATTACCGCTTATCC  
7[88] CAGAACCGTTGAGGCAGGTCAGACCTCATTAAGCCAGAAGTAATAAG  
8[55] TTCGGTCGCATCGCCCTAATGGTTTAAT  
9[40] AAAGACAGCTTTGAGGCACTACGA  
9[72] ACAGAATCATAGCAGCGTGAATTATCACCGTCAAATTATT  
10[31] CTTTTTCATGAGGAAGGCGGGATC  
10[55] TACAGAGGCATCGGAAATAGAAGGCGCCCAATTTTT  
10[87] AACCATCGAAGTTTGCCTTTAGCGAAAATCACCGGAACCAGCCACCCT  
11[48] AGGCACCAAACACTCGCGTTTTAGCGAA  
11[64] CGAAAGAGACCGTAATGCAACGGC  
12[63] ATACACTAACCTAAAAAATCAGATCGAGGGTACCGATATA  
12[79] CATTAAAGTTTATTTTGTACAATGACACCACGGAATAAGTACCCAAA  
12[95] ATTGACGGACCGACTTGAGCCATTGAAACGTCACCAATGA  
13[48] AATTGTGTCCGGAACGATTTTGAAGCCTTA  
15[48] ACCAGGCGTTGACAAGTATCCTGAATCTT  
15[64] GGCTGACCCTCCATGTTACTTAGCCGAAATCCGCGACCTGGCAAAGA  
15[80] AGAACTGGCGCAATAATAACGGAAAGAGCAAGAAACAATGGTTAAGCC  
15[96] AGACTCCTATAAAAGAAACGCAAACAATAGAAAATTCATAAGGTAAAT  
17[48] GAAACACCTAATTTCAATTCAGATATTATTTAACG  
18[63] AGATGGTTAGAACGAGTAGTAAATTTTCATCAAGAGTAATCCATAGGCT  
18[79] CAATAATAAAAACAGGGAAGCGCATTAGACGGGAGAATTAACCCACA  
18[95] AGAATTGAAAATAGCAATAGCTATAAGGAAACCGAGGAAACATGATTA  
19[58] GAGAGAAACAGCCAGCCTAATTTGCCAGTT  
22[39] TCAGTTGAAGTCAGGACATTGTGA  
22[71] ACAAATAATAACATATGGGCTTG  
23[40] CATAAGTCACTTTAATCGTTGGGAAGACTTTACA  
24[38] AAGGAATAGGCTTGCATTCATTA  
24[60] ACCAACGCTAACGATCCTAAT  
25[41] ACAATTTAACCGGATCCTGACGA  
26[41] TTTGCACTAAGATGAACGCGGTCAAT  
26[60] AATCAAGATTAGTATAATCGG  
27[39] TAGCGAAGGGGCGCAGAGTGTACAG  
28[37] TTGCAAGTATCATCCCCCAGC  
28[60] CCTCCGACTTGCCACTCATCTGTCTTTGTATCATATGCGT  
29[42] CGCGAGATCTTTGAGCCTGATA  
30[40] GGTATTAACGTAATGCACTAAAGA  
32[51] CATCACCGACCGACCGGAATACGCGAGAATAACTATTTTT  
32[66] AGCCGTTTTTAAGCAAGCA

33[56] GAGAACAAGAATAAACTGTGATAAATAAGGCG  
36[55] TTACGAGCATAAAGCCAACGC  
38[55] TGTTTATCACGCCAACTAATAAGAATTAATTAACCTTGCTCTTTTTTA  
39[52] CGCCAAACAACAAAAGTACCGACAAAAGAGTGAATA  
40[39] TAATTCGATACAGGTAGAAAAGCCAATCTACGT  
42[38] AGGATTATCGCGTTTATAAGTCCTGCAGATA  
42[71] GGCATTTTCGAGCCAGATGTAATTTAGGCAGA  
43[41] TTTAACAAACAATAGTAATGCAGATCATTCA  
44[40] AGAATCAGAAGAAAAATTTACCCTTCACCAGCT  
44[60] TCAACAGTAGGGCACGCTGAGATTTTCCCAAAC  
45[39] CTGAATAGTATGTAGAAAATATCCCAGCCGCCAA  
46[37] TGTAGCATCAGGTCTTCCAAGAACCAAAA  
46[60] TATACAAATCTTCTTTTTAAAAAATCATTACAAAATTGAG  
47[41] CTGTTTACCTTATCAACCAATCATGCTAT  
48[40] ACTAGAACGATTAACCGAATCGTCGTAAGAA  
48[71] TTTTTTAAATAAGCA  
49[39] TTCCCAAATGTAGGAATAAGTACCGGGGGAGGCTT  
50[37] GAACGAATACTGCGTGCAGGGACAGCAGCG  
50[63] CCTAAATTACGCATAAGTATCGGT  
51[52] TTCAAAACCTTTAATTGCGTAGATT  
51[56] TTTTTTTCTTCTGA  
54[66] GGTTGGGTTATTTTT  
57[56] TTTTAAGAGTCAATA  
61[39] TGCGGGTACTCTGTAAATACCAAAAAAGCAAACCTCCAATATTGTTTCAGC  
61[56] ATGGAAACCTAATAGATTTAGAAGAATCAACACAATCAATATCTGGTC  
62[38] CTTTATTATCGGTTGCTTGAAAAATAGCCATA  
63[41] ATCAAGATTAGAATCTCGTCGCTGAACAGGTC  
63[52] AAAATTCATTTGTTTGAGGATTAGAGCCAGGAAGGT  
64[40] GATGAACCTCGATAGCTTATTAACATTTAATTG  
64[51] CAAATTTTAAAAACAATTCCAAACCCTGTTG  
65[39] AGGTAAAGTAGGTCTGAAGATTAAGTTAATTG  
66[38] AAAAGGGGTAGTAGCGCTGATGCAGTAAAAGC  
66[71] GGATTCGCCTGATTGCCGGGAGAAATTCATCA  
67[42] AGTACCATGTAAATGAGACTACACCATAATGC  
67[52] ACATTTTGAATAGAGCGGAAGCGGAACATCTAAAGCATCAC  
68[39] ATATAATCAATCGCAACGCAAATGCAGTTGA  
68[60] TTCAGGTTTAACTACTTCTGATATAATCATTAAACACCGCCT  
69[39] AATGCCAGATCAAATATGACAAAGACATAATT  
70[38] GGTAGCTATACATTTGAGGTGAACGACAATG  
70[62] CACGTAACCTTAATTAGTGAGAA  
71[52] TCAAATAATGGGCAGAAGATAAAA  
71[56] TTTTTTTAATTATTT  
81[39] CATCAATTGGTCAATAGAATCAGCTATACTTT

81[56] TATCTAAATTGACGCT  
82[38] TTCGCGTTTTTGTAGTATTAACCACAAAC  
82[71] AGTTGGCATATTTT  
83[40] AATGTTATGACAACCTCATAATACAAATAGAAGC  
84[38] GTAACAAGCCCGAACAGCCCCAAAATGTGT  
84[60] CTTGCTGAACCTCCAGAGATAGATTCACCTGGTAATATCCAG  
85[42] GAAAAAAGAAACCGTTATTAAGAAGAT  
86[39] CCAGCCGGATCAGAAACAATCATAACCCAGTAAC  
86[60] GCAACAGTGCCACAGAATACGGAAC  
87[39] GATGGGAGTCTGATTGTACCAGAAGCCAAGATTC  
88[37] TAACCGTAGCATGTAGAGTCTGATAAATT  
88[60] CAGAGGTGAGGCGACTGATAGTGGCACAGAGTAAAAGAGTCT  
89[39] TCGGCCCAAAGGGTTATTGGATTATCAGATGA  
90[38] AGATCGCATTGCCTGAAGGAATTCAAAAAA  
90[52] TTTACGAAACCGATTT  
92[55] CCCTAAAAAGGAACGGCAGTGAGGATGCGCCGTAACCACC  
96[51] CCTTGATTAGTAACTATCGGCGGCGAACGATTTAGA  
101[39] TCATAGGCCTGAAATGGGCCTGCAGGGAACGC  
102[37] CTGTGTGCTTGCATGACCAGTACAACATTA  
102[60] AACAATATTACCGCACTAAATTTTTGGGG  
103[39] TACGAGTGCAGTCACACATTATTTAGAAAAATAA  
104[38] GCATAAAGGGACATTATGTGCTGCGGAGCAAAT  
105[42] TTCTTTCTGACCTGCTGGCCAAAAGAGCGA  
105[64] TTTTTTACTTGCCT  
106[39] TTGTAGCTAAAGGGGGTTTGAATGTGGTGTA  
106[60] GTCCATCACGCAACGCTGGCAAAGCGAAA  
107[39] CTTTCCCCGACAATATTAAGCGTAGCTGAGAG  
108[38] GAAACCTAGTCTTTACGCCATTTCGACAGTA  
109[39] CGCGGGGACCATCGCCAATGCGCGAGTCCGCATCG  
109[64] TTTTTTAAATCCTGA  
110[38] CGGTTTGAGGCAAAGCGTCTTTCTTTTGCTA  
112[47] GAGCACGTCGCGCTTACCAAGTCGG  
113[32] TGAGAGAGCACCAGTGGCCAACG  
114[47] ACACCCGCCGCTAGGGATTAACCG  
115[32] TGTTTGATTTGCAGCACTGCCCCG  
116[47] GGAGCGGGGGGAAAGCCCTCCGGAA  
117[32] CCGAGATAGGTGGTTCTGAGCAATAC  
118[47] GCTTGACGCCGTAAAGCCACTGTTTC  
119[32] ACGTGGACGGGTTGAGCACAACA  
0[111] TTTTCACCCTCAGAACCGCCCCGGAATAGGTGTATTTTT  
2[111] TTTTCAAGAGAAGGATTAGGTGCCTATTTTCGGAACCTTTT  
4[111] TTTTATACAGGAGTGTACTGTGGAAAGCGCAGTCTCTTTT  
6[111] TTTTCAGCATTGACAGGAGGCCACCCTCAGAGCCACTTTT



8[111] TTTTCCATCTTTTCATAATCTCAGACTGTAGCGCGTTTTT  
 10[111] TTTTCAAGGCCGTGGGAATTAGAGCCAGTTTT  
 12[119] TTTTCCGATTGAGGGAGGGATGGTTTACCAGCGCCATTTT  
 14[119] TTTTATAAAGGTGGCAACATTATTACGCAGTATGTTTTTT  
 16[119] TTTTCGAACAAAGTTACCAGCTTACCGAAGCCCTTTTTTT  
 18[119] TTTTCTAATATCAGAGAGATACTGAACACCCTGAACTTTT  
 20[62] TTTTGAAAATAGCAGCAAAATCCAAATAAGAAACGACGACAATTTTT  
 40[71] TTTTTCCAGACGATTTTTTGT  
 80[71] TTTTACTAACAAAGTACATATTTT  
 82[51] TTTTAAAGCATTGGCACAATCGTCATTGCAACAGGAAAAATTTT  
 104[71] TTTTGAGTAGAAGAACTCAAATAACATCAGGGAAGAAGTGTAGCTTTT  
 108[71] GAAGTGTTTTTATAATTACGCCAGCTATGGTTGTTAGAATCAGAGCGGTTTT  
 110[71] TTTTAAACAGGAGGCCGATTACTCATAGTTAGCAAGCTTTT  
 114[63] TTTTGCTGCGCGCTACAGGGTTTT  
 118[63] TTTTGAGCCCCCGTGGCGAGTTTT  
 120[47] TCGAGGTGCGATGGCCACTACGTTTTT  
 120[63] TTTTATCAAGTTCGGAACCCTTTT

**Sequences of staple strands in the DS-right half-cage**

1[136] TTTTATTGACGGACCGACTTTTTT  
 3[136] TTTTTTTGGGAAACCATTAGTTTT  
 5[136] TTTTCGGAAACGATCAGTAGTTTT  
 7[136] TTTTAATCAAGTATCGGCATTTTT  
 9[136] TTTTTCATAGCCAAAATCACCTTTT  
 11[134] TTTTGAGCCACCACCGGAACCGAGCCGCCACCGTAACAGCAAGCCCCAGACGT  
 13[134] TTTTCCTCAGAGCCACCACCCTACCAGAACCACCACCAGTTTT  
 15[134] TTTTGCCAGCATTGACAGGAGGTTGAGAGATCAGAACCGCCAC  
 17[134] TTTTCAAACAAATAAATCCTCAAATGGAAAGCGCAGTCTCTTTT  
 19[134] TTTTTACCGTTCCAGTAAAGCGTCATACAGCGGGGTTTTGCTCA  
 20[119] TTTTTTTTAAACGAAACATGAAAGTATTATTTGAGG  
 21[96] TTTTGGAACCTATTATTCTGGGGTTCAGT  
 25[136] CCGTACTCTTGGCCTTGATTTT  
 29[136] CCCATGTACCCTCAGAACTTTT  
 40[135] CAGCTTGCAGAGGCTGAGACTCCTATACAGGAGTTTTT  
 41[79] TTTTAAACCATCGCCACGCATTTTTTAAGAAGTGGCTCATTTT  
 41[104] TATTCGGTTTTAAACAGCTTGATACTTTT  
 61[96] TTTTAAAAATCTACGTTAATGAATTACCTTATGCGAAACCGATA  
 81[78] TTTTGAACGAGTAGATTTAGTTTTGTAAACGTTAATATTTTTTT  
 81[104] AGATACATGGAAGTTTCATTCCATTTTTT  
 101[80] TTTTTTCGCATTAAATTTTTCTATTAAATTTT  
 110[155] GCAACATTAAAGATTCAACCGATTGAGGGAGGGAAGTTTT  
 111[88] TTTTGTGCTGCAAGGCGATTAAGTTGGGGCGATCGGTGCGGGCCTCTTCGCTTTTT  
 113[88] TTTTTGGTCATAGCTGTTTCGCATGCCTGCAGGTCGTTTT

115[88] TTTTGCTTTCCAGTCGGGAAAGCCTGGGGTGCCTAATTTT  
117[88] TTTTCGCCTGGCCCTGAGAGGCCAGGGTGGTTTTTTTT  
119[88] TTTTGTTCAGTTTGGAACACGAAATCGGCAAAATCTTTT  
121[70] TTTTGCGAAAAACCGTCTATCAATGGCCACTACGTGAAGAGTCCAGTTAAATC  
20[103] GCCTTGAGTAACAGTGCCCGTATAAATTTT  
1[168] ATAGAAAAAATAAGTTCTGGTCAGAGGTTAT  
2[151] TCACCGTCAAATTATTAGCGCCATAAGAACTCTAATAACA  
3[168] ACATATAAGAAAATACTTGCTTTGTTAATCCCCC  
4[151] GCACCATTTTAGAGCCGCC  
5[168] TCCTTATTCAAAGAAAAATATATATGGTTT  
6[151] GCACCGTATCACCAATCAGTTCAGAAAAC  
7[168] ACCGAGGAGCCGAACACCAAGAACAAGCA  
8[151] GCGTTTTCTTGCTTTCATCGCCTGATAA  
10[151] TCATAATCCCCTTATTACT  
12[160] AAGACTCAGCCTCCATTCAGTACAAAGCGTTTACTGTAGC  
14[162] ACACCCCCGCCAGAGTGACAGGGATACTGAGTTTCCCTCATAACGC  
16[161] CAGAGGCAGGTCAGACGAAGGAGGTTTCGGAATAGATTTTTT  
18[161] CCAAAGCCAGTTAAATAAGTATAGCCTAGTACCGAGTGAGAAAACA  
20[151] TTTTGATGCAAGAGAAGGATTAGGATACCTTTAA  
22[168] CTACAAAGCCTAATTTGCCCAAT  
23[147] GTACCAGGCGGATAACGAAAATC  
24[165] ATATAAGAAACGATCCTTTA  
26[167] AACGCCCCATAACATAACTGA  
27[147] CCTCAGAACCGCCGAGATGAATT  
28[165] CATTTTACAAAGTCAACCCAC  
30[168] AGCCGTCACGAGTTAAGCAATAGCTCCATCTTT  
31[149] ACAGTTAGCGTAACGATCTAAAGT  
31[156] CTGTATTTGTATAGCGTCAGCGATAGCA  
33[131] TTTGTCGTCTTTCAATAGGAA  
33[151] TAGTAACATTTATACCAAGCGC  
35[141] GGATTTTGCTATAGAAAGGAACAACTAAAGGA  
35[156] ACTTACTACGAATACACTAAAAGAGGAAGGGAACCAGCGTCCAATACT  
37[131] ATTGCGAATAATAGTGTATCA  
37[151] CACGTTATGAGTTTCCATTA  
38[149] TCCAAACGGCTACAACAGCATCCACCAGA  
39[141] AAGGCTCCAAAAGGAGTAAAGCG  
40[119] TGAATTTCCGCTGAGGCTTGCAGGCAACTTTA  
40[149] TTGTATTTGCGGGATCGTCACCGATAGTAAATTGGGCTTAGAAAAGA  
42[155] AAAGGAGGCTTTTAAGGCTTTAACAAAGTATCATAACCCTC  
42[168] AACATGAGCAGTACCGACAATAAACAAGTGCC  
43[136] TTTTGGTAGCAAAAA  
43[167] AAAGACAGGGACGACGACAAAAGGTCACCCAG  
44[165] TGAGGATTCAGCTATTCAGCGCCAGAGGCGT

45[147] CGGGTAAAATACGTTACAAGATTCATGGTAAACCAAACAGAGGGGTAAGAAAGA  
 GCCCCAGGAAG  
 45[170] AAATTCCAATAGATATGCAGAAGAAAGGGTTG  
 46[167] TATGCTGCTCAACAGTTAATTTACACCCTCA  
 47[136] TTTTCGAAAGAGATG  
 47[167] CATCTTGGATCCCATCCAAGTCCTGATTCTAAG  
 48[165] CAGCGAGTAGAAACACAGACAGCGTTTTTATT  
 49[147] GAAACAAAGTACAGCCGGAACCCGCGACCGCTT  
 49[170] TGTGATTTATCATTCAATCAATCAACCACCCT  
 50[168] GAAATAGAGAGCATTCCAAGTTACCATCTTACC  
 51[139] ATTGTGTCGAAATGAGGCGCAGAC  
 53[141] GGTC AATCATA CAGATGAAAGTTTTGCATAGCGAGGCGAACC  
 55[131] AGGCGCATAGGCTACCTAAAA  
 57[131] TATTCATTACCCAAATCAACGGCCCTGACCATA  
 59[141] AAAGGAACGAGGGCCGCTTCGGTTTAT  
 59[151] ACGAGTAGCTTTAGGAACAAA  
 60[119] ATCATTGTA AACGAACTAACGGACTAAAGTACGGTGTCTTTCGCAA  
 60[135] TTTAATTTGAGTTAAA  
 61[152] TTCATCAGGATCTGTATAATGTATAAAAAGGTGGCATC  
 62[168] CGTCGCAGATTAGATTATCAGTGAAGAGGACT  
 63[136] TTTTTGCAGATAGAG  
 63[148] ACGTACCACATGCTGAATAGCTCAACATTTTCATT  
 63[156] AGGATCTGATAACTTTTGAAATACAGGCGCCT  
 63[167] GGCATACAAAATTTATCAGACGCTGCAACGCC  
 64[165] CAACACCTGCTCATCCTCCGGCTAAGTTATAC  
 65[147] GTTACCAGACGACAGGAAGCAA  
 65[170] TGAATCTTTTTTAAAAAATCATAGTTTTTTCA  
 66[168] TAACAATAATGGGTTATCAACTTTGAAACACT  
 67[167] TAAAATTGAAAATCCAAATAACTATTAGTATCA  
 68[165] CTGGATGAACTGACGTTACTTAACGCCGACCG  
 69[147] GCGGAATCGTCATGACTATTAATCAAAAAATG  
 69[167] CATTGATTA CTTTTTCTCGCAAGAACCTGACCCC  
 70[155] TAAAGAAACCATCACCAGTA  
 70[165] TCAAATTGCTCCATCTGGCATGAGAAGGAA  
 71[139] GAGAATGACCATATAGTCAGATTTAGAACTATTTCAAATATTCA  
 75[151] AGACCGAAAAGCTAAATCGGTT  
 76[149] ACTCCAGCAATAAAAAGGCAAAGAATCGA  
 77[131] AGAGAGTACCTTTAATGCTCGAGGTCATTTTTGCGGATGGC  
 79[131] TTAGAGCTTAATTTCAACTAAATTACAGGTGAGATGG  
 81[120] TGGTCAATAAACAGGAAGATTGTATTTTAACCAATAGGAA  
 81[136] TAGCTATATGTTTTAAATATGCAAACAACATT  
 81[152] TGGGGCGCGCGACCCCGG  
 82[168] GAACAAC TGCAGATGATATTATACTATTACGA

83[147] AATTCTACTAATAAGAGTAATCGTAAAACCTAG  
83[167] CATTAATAATTGTTTGGGGCAATTCTGTAAAT  
84[165] TAAATCTAATGGAAACGTAAAACGATTCATT  
85[156] CAGAAAGGCTATGTAGCTATGCGCATCGTAACC  
85[170] GACAACCTTATTTGCGGGTTAGAAATGTAAGAG  
86[168] TTACAATAATAAAGAAAATATACAGGTAATAG  
87[147] GTACCAAAAACATAAGCTAGCTGATAAATTA  
87[167] CTGTAATAGTCAGATGATTGCGTAGTTACATT  
88[155] CCTTCCTCATATAGGGTGAGGTAATGTGCCAG  
88[165] GCGGGATACCTTTTTTTACCCTAAATATT  
89[136] TAAAAATTAGCAAAGCGGATT  
89[170] AGGAGCTCGCCTGAACATCGGGTGAGTTTAGA  
90[155] CAATAGCAAAATGTGAATTA  
90[168] CTAAAATTAATCAGGTCATACATAAATTAAGAC  
91[149] TGAAAAGGCCGGCACCCTGATCGCACCAGTGAGGAATCCTGA  
93[131] CACCATCAATATGCGCAAGGA  
93[151] ACCGTTTCATCTCAGGAATCTGGTGCTTGATTAGAACTATC  
95[141] TGCCGGAGAGGCAGGTCATTAGG  
97[131] GGAGCAAACAAGAGAATTAGC  
97[151] TGAACGAGATGGGAACAGTTGGTGTGGTTGCTTGAATCAGA  
101[104] AGCTCATTTAAGCAAATATTTAAATGACCATT  
101[128] CGCCATCAAAAATAATATCAGAAAAGCCCCAAAACCTGTT  
102[168] ACGTGGAGCTGATAGCCCCACCAGCGTAGTAG  
103[147] CATTAAATGTGAGGGAGCGGGTGCGCGTA  
103[167] CAACCCAGACGAACGAACATAAATTTGCG  
104[165] TCTCCGTAAAACAGGATCTACAGCAACAATTC  
105[136] TAATGGGATGCCTGAGAGTCT  
105[148] TCACAACGGCGGGGTCACGCCGCTAGGG  
105[170] ACACGACGCCTGCAAGGTGAGGTATCATCAA  
106[168] AGATTCTGGTTTTGAGAAAAATCTATATGACC  
107[147] GTGCATCTGCCAGTACGCCAGCCACCGAG  
107[167] GGACGAACGGCAAATGAACAGTGCCTTAGACT  
108[165] ATCGGCCACCTTGCGATTCAAATTTATCTTT  
109[170] ACAGGAATCAATATTGAACCTCCAATACTTTT  
110[119] TGGGAAGGTAACGCCAGGCCAGTGCCAAGCTTCTGTGTGA  
110[135] CAGGCTGCGCAACTGT  
111[136] CTGAGTAGTCGCCATTGCTTTCCGGAGACAGTCAAAT  
111[160] GGCCTTGACGGGCGACCACAATCA  
112[143] TCACTTGCTTTATAATTCCAGCCA  
113[112] AATTGTTAGAAGCATAAAGTGTAACCTGTCGTGCCAGCTGCGGTTTG  
113[120] TCCGCTCACAATTCCAGACGTTGTAACGACGGGTTTTCCAGTCAC  
113[160] TAAAAGAGATACTTCTCGGCATTGCA  
114[143] GAAGTGTTTCGTGCTTTCTCGTTATGACGAGC

115[160] GCGGGAGCAGGAACGGTTTGAGG  
116[111] CGTATTGGAGTTGCAGCAAGCGTTGTTTGATGGTGGTTCGGTGCCGT  
116[135] CAACGCGCGGGGAGAGGCATTAATGAATCGGCCACAACATACGAGCCG  
116[143] ACGTATAAAGTGTAGCATTGACCG  
117[160] ACCACCACCGTACTATAGAACCAGTC  
118[143] CGCTGGCAGGGAGCCCCGATTTA  
119[112] AAAGCACTAGTTTTTTGGGGTCGAACCATCACCCAAATCA  
119[120] AAATCGGAACCCTAAACAGCAGGCGAAAATCCCCACGCTGGTTTGCCC  
119[152] GAGCTTGACGGGGAAAAAGCGAAACGAGTAA  
99[141] CATGTCAATCATATGTAACCAGCTTTCATCAA  
  
0[186] TTTTTACCAGCGCCAAAGACAAATGGTAATATCCAGTTTT  
2[186] TTTTAGACACCACGGTTCATATGGTTTTTTT  
4[186] TTTTAGCAAACGTAAAGAAACGCAATTTT  
6[186] TTTAACGGAATACCACGCAGTATGTTTTT  
8[186] TTTTGTAAGCAGATAAACGCAATAATTTTT  
9[168] GAAGCCCTTGAAATAGCCCAATAATAAGAGCATTTT  
10[183] TTTTAGAAACAATTTTAAGAAAATTTT  
12[183] TTTTCTAATATCGTAGGAATCATTATTTT  
14[183] TTTTTTAGACCGAATCAGATATAGATTTT  
16[183] TTTTAAAATGAAGAACCTCCCAGCTTTTT  
18[183] TTTTCAGCCATATTAATCAAGATTTTTT  
20[180] TTTTCAACGCTAACGAGCGTCTTTCAGATGGC  
22[188] TTTTAGTTGCTATTTTGAAAGTAATTTTT  
23[167] GTCGAGGCCCTATTTATCCAGTTACAAAATAAATTTT  
24[188] TTTTTCGCGGAGGTTTTTCGCGCCTGTTTT  
25[170] TTTAGCAATAGCAGTTTTTTGTTTAAACGTCATTTT  
26[188] TTTTAGGCTTATCCGGTAACAAGAATTTT  
27[167] GAGCCAGCAGAGAATTAACAGGGAAGCGCATTTT  
28[188] TTTTCCGCGCCCAATAGAATCGGCTTTTT  
29[170] TTCATCAGAGAGATAGAGGGTAATTGAGCGTTTT  
30[191] TTTTACCGCACTCATCGAGAGGGTATTAGTCTTTCCAAATAAGGCGTTATTTT  
31[184] AACCAAGTTTTTTTTT  
34[183] TTTTAAATAATAATCATAACTACTATTTT  
36[183] TTTTTTTATCAATTACCAGTATAAATTTT  
38[183] TTTTCTGTCCAGCTTAATTGAGAATTTT  
40[180] TTTTTTTCGAGCCAGTAATAAGAGAATTTTTTATCCTGAATCTTACTTTT  
42[188] TTTTTCGCCATATTTAAAGAAGAGTTTTT  
44[188] TTTTGCCAACGCTCAACAGGTCTGATTTT  
46[188] TTTTGAAAAAGCCTGTTATGTAAATTTTT  
48[188] TTTTAAATAAGAATAAACCAAAGAACTTTT  
50[191] TTTTTTCTGACCTAAATTTATTTAGTTAGCGAGAAAATTTCAATTACCTTTTT  
51[184] ATTCATCTTTTTTTTT

54[183] TTTTGCTGATGCAACAAACATCAAGTTTT  
56[183] TTTTGAGACTACACCTTTTTTAATGTTTT  
58[183] TTTTCAATAGTGTATATGTGAGTGATTTT  
60[180] TTTTTAGAATCCTTGAAAACATAGCCTCTAATTTAGGCAGAGGCATTTTT  
62[188] TTTTATAACCTTGCTTCATCAATATTTTT  
64[188] TTTTGAAACAGTACATAACCTACCATTTT  
66[188] TTTTAAAACAAAATTAATTTTCAGTTTT  
68[188] TTTTGAGCAAAAGAAGAAGAAACAATTTT  
70[191] TTTTATCGCGCAGAGGCGAAAATACCAATAACGGATACTAACAATAATTTTT  
71[184] GTTACAAATTTTTTTTT  
74[183] TTTTGTTAACGATAATACATTTGATTTT  
76[183] TTTTTATCAAAATCGTATTAAATCCTTTT  
78[183] TTTTAATCCTGATTTTAAAAGTTTGT  
80[180] TTTTCGGAATTATCATCATATTCCTTTGTATTAATTAATTTTCCCTTTTT  
82[188] TTTTAGTAACATTATCATCGCCATTTTTT  
84[188] TTTTTTTGCCCCAACGTCGGTCAGTTTTT  
86[188] TTTTGGATTTAGAAGTAACGCTGAGTTTT  
88[188] TTTTAGATTAGAGCCGTAATATCATTTT  
90[191] TTTTTGAAAGGAATTGAGGATTGGCAAAAACCCTCAAAAACGCTCATGGTTTT  
91[184] TCAACAGTTTTTTTTT  
94[183] TTTTAGCCAGCACTCAATCGTCTGATTTT  
96[183] TTTTATTAACACCAGTAATAAAAGTTTT  
98[183] TTTTAAAATACGATAGAACCCTTCTTTT  
100[149] TTGATATCGGCTGGCCTTCCTGTACAGACAATATTTTTGATTTT  
100[180] TTTTATGGCTATTAGTCTTTAATGCGAGAGAAACCACCAGAAGGAGTTTT  
102[188] TTTTTGACCTGAAAGCGACGTGGCGTTTT  
104[188] TTTTGGACATTCTGGCCCGCTTAATTTTT  
106[188] TTTTAATGGATTATTTAAGGCCGATTTTT  
108[188] TTTTAAATACCTACATTTACGCAATTTT  
110[180] TTTTAAACAATATTACCGCCAGCAATAGGTAAATATTTTTGTAGGTGGCA  
112[183] TTTTATTAACCGTTGTAGCATCTGTCCATTGCGACAGT  
114[183] TTTTTAAAGGGATTTTAGACTAAACAGGCATTGGC  
116[183] TTTTGCGCCGCTACAGGGCGACCCGCCGAACGTCGGAT  
118[183] TTTTAGAAAGGAAGGGAAGAGCCGCGATAAGAAT

**DS-linker strands**

0[135] GTATACCGCCACCCTCAGAACCGC  
2[135] GAGTATTAAGAGGCTGAGACTCCTCACCGTACTCAGGAGGTTTAGAAT  
4[135] CAACGTCATACATGGCTTTTGATGTATTATTCTGAAACATGAAAGCCA  
6[135] CGAAACCACCACCAGAGCCGCCGCTGAATTTACCGTTCCAGTAAGGGC  
8[135] TTTTAGCCCCCTTATTAGCGTTTGCACCCTCAGAGCCGCCACCAGCAG  
10[134] GGAAGTAGCACCATTACCATTAGTTTCATCGGCATTTTCGGTCACGG  
12[133] CGCGCGACATTCAACAAAATCACCACCA

14[133] GCCAAAATACATACAAGACAAAAGGCAC  
 16[133] TATAAGCAGATAGCAGCAAACGTAGGCC  
 18[133] TGAGTAATTGAGCGTTAAGAAAAGTTCA  
 20[77] CAGAACGTCAAAAAT  
 20[133] GTACTGGTAATAAGAAAGTCAGAGGATT  
 21[72] TTTTAAATGCCCCCTGCCTATTTCCGATAGTTGCGCCGACAATGACTG  
 60[78] TTATCAATATATGTGGTAAAGTAATTCAAC  
 61[72] AATACCAGTCAGGACGTTGGGAAGATAACAGTTGATTCCCAATTCAGC  
 100[77] TGTATACCTACATTATATCTTTAGGTGC  
 101[64] CGCTCATGGAATAAAA  
 110[87] ATTACGCCAGCTGCTA  
 111[72] GAGGCGAAAGGGGGATACTCTAGAGGATCCCCGGGTAGTA  
 113[64] CGCCCGAGCTCGAATTCGTAATCATGAGTGAGCTAACTCACATTACAC  
 115[64] GGTATTGCGTTGCGCTCACTGCCCTCTTTTCACCAGTGAGACGGGGGA  
 117[64] AAACAACAGCTGATTGCCCTCACCTTATAAATCAAAGAATAGAGG  
 119[64] TAACCCGAGATAGGGTTGAGTGTTGAACGTGGACTCCAACGTCAACAA  
 121[56] TGAACCATCACCAGG

**DS-probes.** The red- and green-colored portions of the sequences are complementary to the ssDNA conjugated to the enzymes, and are located in the Left and Right half-cages, respectively.

64[71] ATTCATTTCAATTACCCGCGCAGAGGCCGAATTTTTGGAGGGAGGG  
 74[76] TCAGATGATGGCAACAATAACTTTGGAGGGAGGG  
 76[66] ATTATCATTTTTATCATCATATTCCTGATTATTTGGAGGGAGGG  
  
 34[149] TTCTGTGCAAAAAGAAGGCACCAGGCTGACCGTAATCTTGACAAGAACCGGATTTTC  
 CAGCCAGCC  
 67[136] GCAAAAGACGGTGTACAGACCTTTCCAGCCAGCC  
 73[131] GCATCAAAAAGATTAAGAGGAACCTCAAATATCGCGTTTTAATTTCCAGCCAGCC

## Supplementary Methods.

**Enzymes and substrates:** Glucose-6-phosphate dehydrogenase (G6PDH, *Leuconostoc mesenteroides*), malic dehydrogenase (MDH, *porcine heart*), lactate dehydrogenase (LDH, *rabbit muscle*), glucose oxidase (GOx, *Aspergillus niger*), horseradish peroxidase (HRP) and  $\beta$ -galactosidase ( $\beta$ -Gal, *E. coli*) were purchased from Sigma (St. Louis, MO). Pyruvate, oxaloacetate (OAA), glucose 6-phosphate (G6P), glucose, resorufin  $\beta$ -D-glucopyranoside (RBG),  $\beta$ -nicotinamide adenine dinucleotide (NAD), resazurin (RESA) and phenazine methosulfate (PMS) were obtained from Sigma-Aldrich. ABTS (2,2'-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) was purchased from Pierce (Rockford, IL), polyphosphate (100) is ordered from Kerafast.

**DNA strands:** Single-stranded M13mp18 DNA was purchased from New England Biolabs. Staple strand oligonucleotides were obtained from Integrated DNA Technologies (IDT) on 96-well plates and used without further purification. Thiol-modified DNA oligonucleotides were also purchased from IDT, and were purified by denaturing PAGE before use.

**Crosslinking reagents:** *N*-Succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and tris(2-carboxyethyl)phosphine (TCEP) were obtained from Pierce. Dimethyl sulfoxide (DMSO) was purchased from Sigma.

**Buffers:** Phosphate buffered saline (PBS), HEPES sodium salt, Tris buffered saline (TBS), Tris base, acetic acid, EDTA, and magnesium acetate were purchased from Sigma.  $1\times$ TAE/Mg<sup>2+</sup> buffer (pH 8.0) is prepared by 40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate.

**Dye-labeling reagents:** NHS-Cy3, Cy5 amine reactive dyes were purchased from GE Healthcare Life Sciences. NHS-AlexaFluor®555 and AlexaFluor®647 amine reactive dyes were obtained from Life Technologies.

**Amicon centrifugal filters** were purchased from Millipore.

**PEG 8000** was purchased from Promega.

**Surface PEGylating reagents:** APTES (3-Aminopropyl)triethoxysilane was purchased from Sigma-Aldrich. mPEG-SVA 5k and biotin-PEG-SVA 5k were obtained from Laysan Bio, Inc.

**TEM imaging:** TEM grids (400 mesh, copper grid coated with ultrathin carbon, Ted Pella) were glow discharged (Emitech K100X). 2  $\mu$ l concentrated samples were deposited onto the grids for 1 min, washed with 10  $\mu$ l DI water for 5 sec, stained with 10  $\mu$ l 1% uranyl formate twice (2 sec for the first time and 15 sec for the second time), and imaged using Philips CM12 transmission electron microscope.

**Enzyme activity assay:** A 96-well-plate reader was used to monitor enzyme activity through absorbance changes of the samples. The enzyme samples and substrates were loaded in the wells of the 96-well plate with a final concentration of caged enzymes  $\sim$  0.5 nM in  $1\times$  TBS (Tris buffered saline with 1 mM MgCl<sub>2</sub>, pH 7.5) for most assays. The DNA cage concentration was determined by the A<sub>260</sub> value as described above. For a typical GOx and HRP assay, 1 mM Glucose and 2 mM ABTS was used as substrate and enzyme activity was measured by monitoring the increase in absorbance at 410 nm (ABTS<sup>-1</sup>). For a typical G6pDH assay, 1 mM G6P and 1 mM NAD<sup>+</sup> were used as substrates, and enzyme activity was measured by monitoring the increased absorbance at 340 nm due to the reduction of NAD<sup>+</sup> to NADH. For a typical LDH



assay, 2 mM pyruvate and 1 mM NADH were used as substrates, and enzyme activity was measured by monitoring the decreased absorbance at 340 nm due to the oxidation of NADH to NAD<sup>+</sup>. For a typical MDH assay, 2 mM OAA and 1 mM NADH were used as substrates, and enzyme activity was measured by monitoring the decrease in absorbance at 340 nm. For a typical  $\beta$ -Gal assay, 100  $\mu$ M RBG was used as substrate and enzyme activity was measured by monitoring fluorescence intensity, with excitation at 532 nm and emission at 590 nm.

**Trypsin assay:** Enzyme activity was measured after incubation with or without trypsin (1  $\mu$ M) at 37 °C for 24 h in 1 $\times$ TAE-10mM Mg buffer (pH 8.0). Activity assay conditions: 1 mM Glucose, 1 mM ABTS, 1 nM of free GOx and HRP in pH 7.5, 1 $\times$ TBS buffer containing 1 mM MgCl<sub>2</sub>, and monitoring absorbance at 410 nm. In the DNA cage experiment, all conditions were the same except for incubating 1 nM DNA cage-encapsulated GOx and HRP with trypsin.

## Supplementary References

1. Fu, J., Liu, M., Liu, Y., Woodbury, N. W. & Yan, H. Interenzyme Substrate Diffusion for an Enzyme Cascade Organized on Spatially Addressable DNA Nanostructures. *J. Am. Chem. Soc.* **134**, 5516–5519 (2012).
2. Liu, M., Fu, J., Hejesen, C., Yang, Y., Woodbury, N. W., Gothelf, K., Liu, Y. & Yan, H. A DNA Tweezer-Actuated Enzyme Nanoreactor. *Nature Commun.* **4**, 1-5 (2013).
3. Abelson, J. *et al.* Conformational dynamics of single pre-mRNA molecules during in vitro splicing *Nat. Struct. Mol. Biol.* **17**, 504-512 (2010).
4. Michelotti, N. *et al.* A bird's eye view tracking slow nanometer-scale movements of single molecular nano-assemblies. *Methods Enzymol.* **475**, 121-148 (2010).
5. Blanco, M. & Walter, N. G. Analysis of Complex Single-Molecule FRET Time Trajectories. *Method. Enzymol.* **472**, 153-178 (2010).
6. Gourévitch, B. & Eggermont, J. J. A nonparametric approach for detection of bursts in spike trains. *Journal of Neuroscience Methods* **160**, 349-358 (2007).
7. Rinaldi, A. J., Lund, P. E., Blanco, M. R. & Walter, N. G. The Shine-Dalgarno sequence of riboswitch-regulated single mRNAs shows ligand-dependent accessibility bursts. *Nat. Commun.*, 8976 (2015).