Supplementary Information for

A prototype protein nanocage minimized from carboxysomes with gated oxygen permeability

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Supplementary Text

O2 sensing with an Ir probe sandwiched between HCcmL pentamers and QDs

A small-molecule O2 probe, a phosphorescent iridium (III) complex (Ir probe, SI Appendix, Figs. S20 and S21), was utilized to examine whether O2 can diffuse in the interfacial space between HCcmL pentamers and the QD core in QD@HCcmL cages. The phosphorescence of the Ir probe can be quenched by O2 by energy transfer to the lower excited states (1Δ and 1Σ) of O2 (1). The phosphorescence emission intensity of the Ir probe in an aqueous solution gradually decreased with increasing O2 exposure (SI Appendix, Fig. S22A). The decay curve revealed a linear relationship between the phosphorescence intensity of the Ir probe and the O2 level (SI Appendix, Fig. S22B).

To link the Ir probe site-specifically to the interior surface of the HCcmL pentamer, a mutant of HCcmL with D44C and C7S mutations was generated. The C44 residue served as the site of Ir probe cross-linking through maleimide chemistry (SI Appendix, Fig. S23). The Ir-labeled HCcmL mutant retained the ability of the parent species to coassemble with QDs and formed cage structures termed QD@Ir-HCcmL (SI Appendix, Fig. S24) indistinguishable from QD@HCcmL in Fig. 1I. It is worth mentioning that O2-insensitive ZnCdS/ZnS core/shell QDs were used here as the template for these experiments to eliminate interference from QD fluorescence changes (SI Appendix, Fig. S25).

Next, O2 sensing in QD@Ir-HCcmL cages was carried out under the conditions with and without Ni-NTA patches to the type 2 pores. When the cages treated with Ni-NTA (QD@Ir-HCcmL (patched); SI Appendix, Fig. S26) were challenged with 50% O2 for different lengths of time, their phosphorescence remained nearly unchanged within the tested 30 min, while that of the free Ir probe at an equivalent concentration (SI Appendix, Fig. S27) decreased to 53.8% of its initial value (SI Appendix, Fig. S28A, C, and D). In the case of nonpatched QD@Ir-HCcmL cages, the phosphorescence was quenched by the same O2 challenge by only 4% (SI Appendix, Fig. S28B and D), which was negligible. To rule out the possibility that cross-linking with HCcmL makes the Ir probe insensitive to O2, we examined the phosphorescence of Ir-HCcmL pentamers upon treatment with 50% O2 and confirmed that the Ir probe on the HCcmL pentamers was still O2 responsive (SI Appendix, Fig. S29). Taken together, the nearly complete protection of the Ir probe from O2 challenge in the nonpatched cages suggests that O2 cannot diffuse within the cage to reach the probe, indicating close interaction between HCcmL pentamers and the QD surface. The Ir-based O2 sensing data strengthen the explanation for the partial protection of the QD probe by the nonpatched cages.

Correlation between the quenching rate and the available surface area of QDs

To examine the correlation between the quenching rate and the available surface area of QDs, we performed O2 challenge experiments with free QDs and QD@HCcmL cages in a longer time range until the quenching reached equilibrium. Nearly complete quenching was observed for free QDs from 60 min on (SI Appendix, Fig. S30A), while the quenching of QD@HCcmL cages reached equilibrium around 60 min with ca. 65% fluorescence left (SI Appendix, Fig. S30B and C). On the basis of the sizes of QDs (ca. 5 nm in diameter) and type 2 pores (ca. 1.4 nm in diameter), the available surface area of a QD exposed by type 2 pores was estimated to be 39% of the total surface area (AQD) of the QD. The percentage was calculated as APore2/AQD × 100%, where APore2 is the area of the QD surface exposed by type 2 pores. Based on these data, there appears to be a good correlation between the O2-accessible surface of QDs (39%) and fluorescence quenching rate.
Protection of QD fluorescence from O2 quenching with other strongly adsorbed proteins

To investigate whether protection of QD fluorescence from O2 quenching by strongly adsorbed proteins is a real effect, we have also performed experiments with other proteins that can be strongly adsorbed on QDs. One is the major capsid protein of simian virus 40 (SV40), VP1. SV40 VP1 was fused with a polyhistidine tag (Histag) at its N-terminus, so it is termed HisVP1. HisVP1 pentamers can bind to QDs and assemble into a capsid-like structure surrounding a QD core (2). The other is a Cas9 protein with an Histag fused at the C-terminus, which is termed His-Cas9. Histag can mediate the binding of His-Cas9 onto QDs (3). When HisVP1-QD and His-Cas9-QD complexes were subjected to O2 sensitivity experiments, both proteins showed protection effects on QD fluorescence, however, to different extents (SI Appendix, Fig. S31A and B). In particular, after 30 min of O2 treatment, HisVP1-QD and His-Cas9-QD complexes remained ca. 60% and 47% (versus ca. 30% for free QDs) of the initial fluorescence intensity, respectively (SI Appendix, Fig. S31C). These results support that strongly adsorbed proteins on QDs can protect QD fluorescence from O2 quenching, which helps to explain the partial protection in the non-patched QD@HCcmL cages shown in Fig. 5E in the main text.

SI Methods

Plasmid construction. The coding sequence of CcmL was amplified from the genomic DNA of *Thermosynechococcus elongatus* BP-1 by using the primers 5’-GGAAATTTCCATGAAAATCGCGGAGTGTTG (P1) and 5’-CCGCTCGAGCTAATACTGGGTACGTTTGCT (P2) and was inserted into the pET32a(+) vector (Novagen) between the NdeI and XhoI restriction sites to generate pET32a-CcmL. The pET32a-HCcmL plasmid was constructed by site-directed mutagenesis with pET32a-CcmL serving as the template by using the primers 5’-GGGTGAGGACCATCACCATACCATGCGAATTTTTTACCGACTAC (P3) and 5’-AAAATTCGCCATGGATGGATGGTTGCTCTACACCCAAATATTGCAA (P4), resulting in the insertion of a pentahistidin tag. The mutant of HCcmL with D44C and C7S mutations was subcloned by following a method similar to that used for HCcmL. The His-Cas9 expression vector was constructed using a ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China), where the His-Cas9 coding sequence was amplified with primers Cas9-F (5’-CCCAAGAAGAAGAGGAGGTGATGATGGATAAGAAATACTCAATAGGCTTAG-3’) and Cas9-R (5’-GTCACCTCCTAGCTGAATACCTAATGAC-3’) and the pET28a(+)(5’-GTCACCTCCTAGCTGAATTACCTAATGAC-3’) and the pET28a(+) plasmid as the template. All constructs were verified by sequencing (Wuhan TSINGKE Biological Technology Co., Ltd.).

Expression and purification of CcmL and HCcmL. The pET32a-CcmL plasmid was transformed into *E. coli* BL21 (DE3) competent cells (Novagen). The strains were cultured at 37 °C to an OD600 of 0.6, and then expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 25 °C for 10 h. The cells were collected, washed with buffer A (20 mM Tris-HCl, pH 8.0), repelleted and resuspended in buffer A. The cells were lysed by sonication (JY92-IIN, Scientz) at a power of (100%-65%≈35%).
273 W with duty cycles of 10 s (on for 3 s and off for 7 s) for 240 cycles in a beaker placed in an ice water bath and then centrifuged at 12,000 × g for 30 min at 4 °C to remove the debris, and the resulting supernatant was subjected to ammonium sulfate precipitation. The CcmL protein was precipitated by 50% ammonium sulfate saturation at 4 °C, resuspended in buffer A and further purified by anion-exchange chromatography (HiTrap QHP column, GE Healthcare) using a salt gradient (0-1.0 M NaCl in 20 mM Tris-HCl, pH 8.0). The expression methods for HCcmL were similar to those for CcmL. HCcmL was purified on a nickel nitritotriacetic acid (Ni-NTA) Sepharose column (GE Healthcare) according to a previously established method (4). The protein concentration was determined using densitometry with a GS-900™ Calibrated Densitometer (Bio-Rad) after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie blue staining.

Preparation of quantum dots (QDs). CdSe/ZnS core/shell QDs coated with 3-mercaptopropionic acid (MPA) and MPA-coated ZnCdS/ZnS core/shell QDs were purchased from Suzhou NIR-Optics Technologies Co., Ltd., and Xingzi New Material Technology Development Co., Ltd., respectively. For O2 sensing inside the HCcmL cage, we encapsulated O2-sensitive Cds/ZnS core/shell QDs (Ocean NanoTech, Springdale, AR, USA) to achieve different cores with the same ZnS shell; water-soluble MPA-coated QDs were prepared as previously reported (4). All the QDs had the same ZnS shell.

Assembly and purification of CcmL-QD, QD@HCcmL, and QD@Ir-HCcmL. CcmL, HCcmL and Ir-HCcmL pentamers were incubated with QDs at a molar ratio of 10:1 at 4 °C overnight to allow adequate interactions. The coassembly products were then loaded onto a 10-50% sucrose density gradient and centrifuged at 38,000 rpm for 4 h at 4 °C in an SW41 Ti rotor (Beckman Coulter). The target products were collected and dialyzed using a regenerated cellulose (RC) dialysis membrane (molecular weight cutoff (MWCO) of 8-14 kDa) to remove sucrose, followed by ultrafiltration and concentration with an Amicon Ultra-15 Centrifugal Filter Unit (50 kDa MWCO, Millipore).

Transmission electron microscopy (TEM). For TEM characterization, a sample (20 μL) was transferred onto a carbon-coated copper grid (Beijing XXBR Technology Co., Ltd., Beijing, China), removed after 4 min with filter paper, and then negatively stained with 2% phosphotungstic acid (PTA) for 3 min. After blotting off the excess PTA with filter paper, the copper grid was naturally dehydrated overnight at RT. The samples were imaged using a Hitachi H7000 TEM (operated at 100 kV) equipped with an Olympus MegaView G2 camera. TEM image processing and data analysis were performed using ImageJ (NIH).

Optical property measurements. UV-Vis absorption spectra were acquired on a microplate reader (Synergy HT) for samples (100 μL) added to a well (volume: 205 μL) of a Corning® Half Area 96-well Clear Flat Bottom UV-Transparent Microplate with an optical pathlength of 5.7 mm. Fluorescence/phosphorescence spectra were measured on an LS 55 luminescence spectrometer (PerkinElmer) by adding a sample (2.0 mL) to a 3.5 mL quartz cuvette (optical pathlength, 10 mm). The QDs and the Ir probe were excited at 317 nm and 390 nm, respectively.

Affinity measurements. The binding kinetics of the CcmL or HCcmL pentamer with the QDs were analyzed using ForteBio® BioLayer Interferometry via an Octet® RED96 system (Pall Corporation, Port Washington, NY, USA). First, CcmL or HCcmL pentamers were biotinylated in PBS using the
reagent EZ-Link™ NHS-LC-LC-Biotin (Thermo Fisher) according to the manufacturer's instructions. Then, the mixture was dialyzed using an RC dialysis membrane (3.5 kDa MWCO) against 50 mM borate buffer (pH 8.4) to remove excess biotin. The biotinylated CcmL or HCcmL pentamer (62.5 μg/mL) was loaded onto streptavidin biosensors (Pall ForteBio) until reaching saturation. Next, the biosensors were washed and transferred into a series of QD solutions of different concentrations. All measurements were carried out in 50 mM borate buffer (pH 8.4) at 30 °C. The kinetic parameters (k_on and k_off) and affinities (K_D) were calculated from a nonlinear global fit of the data using Octet software (version 6.4.0.20) with a mass transport model.

**Dynamic light scattering (DLS).** DLS measurements of QD@HCcmL and QD@Ir-HCcmL in PBS were performed on a Zetasizer Nano ZS (Malvern Instruments) using a ZEN0040 cuvette, and the parameters were automatically optimized. Before measurement, the samples were filtered through a 0.1 μm syringe filter and then subjected to centrifugation at 10,000 rpm for 5 min at 25 °C.

**Preparation of isotope-labeled protein samples.** The method for preparing U-13C,15N- or 2-13C-glycerol-labeled HCcmL pentamers was performed as previously reported (5). Overexpression, purification, and quantification of the protein were performed by following a procedure similar to that used for HCcmL.

**ssNMR spectroscopy and data processing.** All ssNMR spectra of QD@HCcmL were acquired using a 3.2 mm E-free HCN MAS probe on a standard-bore Bruker Advance 800 MHz spectrometer with an MAS rate of 10.5 kHz and a temperature of 271 K. Experiments to determine the chemical shift assignments of HCcmL were performed using a 4-mm triple-resonance T3-HXY MAS probe on a wide-bore Varian VNMRS 600 MHz NMR spectrometer with an MAS rate of 8 kHz and a temperature of 271 K. Typical 90° pulse lengths of 3.3 μs (1H), 4.3 μs (13C) and 6.5 μs (15N) were used in the ssNMR experiments. During the 1H/15N CP period, the 15N rf field was kept at 45 kHz, and the 1H rf field was ramped linearly around the n = 1 Hartmann-Hahn condition (6) with a contact time of ~1 ms. During 15N/13Ca and 15N/13CO band-selective CP (7), the 15N rf field was ramped linearly around 2.5 ωr (spinning rate), and the 13Ca and 13CO rf fields were adjusted to ~1.5 ωr and 2.5 ωr, respectively, with a contact time of ~4 ms. Then, 90 kHz CW decoupling was used during the 15N/13Ca and 15N/13CO band-selective CP, and ~65 kHz 1H SPINAL64 decoupling was applied during the acquisition and indirect evolution periods in the 2D/3D experiments. The NMR data were processed with NMRpipe (8) and analyzed with Sparky (9). The carbon chemical shifts were referenced to adamantane by adjusting the position of the 13C adamantane downfield peak to 40.48 ppm (10).

**POMONA/CS-Rosetta CM protocol.** Calculations of the monomeric HCcmL structure were performed using the standard POMONA/CS-Rosetta CM protocol of the CS-Rosetta (11) package. As the starting point, only backbone 15N, 13Ca, 13C', and 13Cβ chemical shifts were used for the fragment and template selection procedures. For the subsequent structure generation procedure, only fragments and templates from PDB proteins with <30% sequence identity to the CcmL protein were used, and experimental restraints were also included to generate 10000 full-atom models, from which the 8 lowest-energy models were selected as the calculated structures (SI Appendix, Fig. S7).

**Dissociation of the QD@HCcmL assembly and HCcmL recovery.** First, purified QD@HCcmL assemblies were dialyzed using an RC dialysis membrane (8-14 kDa MWCO) against the dissociation buffer (20 mM Tris-HCl, pH 10.2, 200 mM NaCl, 16 mM EDTA). Then, the sample
was subjected to sucrose density gradient centrifugation (SDGC) for HCcmL separation. According to the analysis results of the ten fractions obtained from SDGC by SDS-PAGE, F1–F3 were combined and dialyzed using an RC dialysis membrane (3.5 kDa MWCO) against PBS and finally subjected to size-exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL Prepacked Tricorn™ column (GE Healthcare) for further analysis to confirm the oligomeric state of HCcmL.

De novo structure calculation of the HCcmL pentamer in QD@HCcmL with Xplor-NIH. We used all dihedral angle constraints and distance constraints obtained from the ssNMR spectra to perform iterative structure calculations using Xplor-NIH (12). Two different simulated annealing protocols were employed, one for refinement of the HCcmL monomer structure and the other for docking protein-protein complexes to form an HCcmL pentamer (13, 14). The details are described below:

During each round of structure refinement for the HCcmL monomer, we used only intramonomer restraints, and the distance restraints were disambiguated based on the previous structure model. Additional hydrogen bonding restraints were inferred using hydrogen bond calculations (ver. 1.1) (15). Cross-peaks from the ssNMR spectra provided only upper distance bounds. In this study, we set the upper distance bounds to 9 Å (16) during each round of monomer structure refinement, and we used a hard error-tolerant restraint potential. The protocol for the simulated annealing of the HCcmL monomer structure refinement is outlined below:

- Step 1: High-temperature dynamics in torsion angle space (3000 K, 16 ps or 8000 steps, whichever comes first).
- Step 2: Simulated annealing in torsion angle space (3000 to 25 K in 12.5 K increments, with 1.0 ps or 500 steps, whichever comes first, at every temperature).
- Step 3: Gradient minimization in torsion angle space.
- Step 4: Final gradient minimization in Cartesian space.

The potential terms used in the monomer structure refinement protocol are outlined below:

| Potential terms          | Force constant at different stages |
|--------------------------|-----------------------------------|
|                          | High temperature | Simulated annealing | Final minimization |
| Bonds (kcal.mol⁻¹.Å⁻²)   | 1                   | 1                   | 1                  |
| Angles (kcal.mol⁻¹.rad⁻²)| 0.4                 | 0.4–1               | 1                  |
| Improper (kcal.mol⁻¹.rad⁻²) | 0.1               | 0.1–1               | 1                  |
| NOE (kcal.mol⁻¹.Å⁻²)     | 1                   | 1–20                | 20                 |
| Torsion angles (kcal.mol⁻¹.rad⁻²) | 10               | 200                 | 200                |
| Repulsive vdw (kcal.mol⁻¹.Å⁻⁴) | 0.004             | 0.004–4             | 4                  |
| Torsion angle database potential | 0.002            | 0.002–2             | 2                  |

The docking protocol was designed for monomer-monomer docking using the lowest-energy monomer structure that we calculated previously. A symmetry potential (C5 symmetry) was used to enforce similarity between all monomers during the calculations, as required by our ssNMR data, which showed only one set of peaks for each residue. In each round of calculations, we fixed the structure of the monomer and used intermonomer distance restraints. We set the upper distance bounds to 10 Å and used a hard error-tolerant restraint potential. The protocol for the simulated annealing of the HCcmL pentamer structure refinement is outlined below:

- Step 1: Randomization of the positions and orientations of the proteins relative to one another.
- Step 2: Initial rigid body gradient minimization with experimental restraints.
- Step 3: Initial rigid body gradient minimization with experimental restraints and a van der Waals repulsion term.
- Step 4: Conjoined rigid body/torsion angle dynamics simulated annealing (from 3000 to 50 K in 60 K increments with 0.2 ps or 100 steps, whichever comes first, at every temperature).
- Step 5: Final conjoined rigid body/torsion angle minimization.

The potential terms used in the simulated annealing docking protocol are outlined below:

| Potential terms                  | Force constant at different stages |
|----------------------------------|-----------------------------------|
|                                  | Initial minimization  | Simulated annealing | Final minimization |
| NOE (kcal.mol\(^{-1}\).Å\(^{-2}\)) | 0.2                      | 0.2--20              | 20                 |
| Torsion angles (kcal.mol\(^{-1}\).rad\(^{-2}\)) | 10                       | 200                  | 200                |
| Hydrophobic contact potential    | 0                        | 1--50                | 50                 |
| Repulsive vdw (kcal.mol\(^{-1}\).Å\(^{-4}\)) | 0.01                     | 0.004--4             | 4                  |
| Torsion angle database potential | 0.002                    | 0.002--1             | 1                  |
| Rgyr potential (kcal.mol\(^{-1}\).Å\(^{-2}\)) | 0                        | 0.05--10             | 10                 |
| C5 symmetry (kcal.mol\(^{-1}\).Å\(^{-2}\)) | 5                        | 5                    | 5                  |
| “NCS” term                      | 100                      | 100                  | 100                |

**Cryo-electron microscopy data acquisition.** A QD@HCcmL solution was diluted to a final concentration of ~0.2 mg/mL. Three microliters of this solution were transferred to glow-discharged 200-mesh R2/1 Quantifoil grids. The grids were blotted for 3 s and rapidly cooled in liquid ethane using a Vitrobot Mark IV (Thermo Fisher) at 22 ℃ and 100% humidity. The samples were screened and imaged using a Talos Arctica cryo-electron microscope (Thermo Fisher) operated at 200 kV with a GIF energy filter (Gatan) at a magnification of 130,000× (corresponding to a calibrated sampling of 1.07 Å per pixel). The micrographs were recorded with a Gatan K2 Summit direct electron detector, and each image was composed of 30 individual frames, with an exposure time of 6 s and a specimen dose rate of 9 electrons per second per Å\(^2\). A total of 640 movie stacks were collected with a defocus range of -1 to -3 μm.

**Single-particle image processing and 3D reconstruction.** All micrographs were motion-corrected using MotionCor2 (17), and the contrast transfer function (CTF) was determined using CTFFIND4 (18). All particles were autopicked using the NeuralNet option in EMAN2 (19) and further checked manually, yielding 101,197 particles from 640 micrographs. The particle coordinates were then imported to RELION (20) for 2D classification. Since the particles were not quite homogeneous due to the QD binding, we selected the representative classes including 55,578 particles that displayed nearly circular outer cage for further 3D analysis. The 3D homogeneous refinement was then performed using the initial map generated in cryoSPARC (21) as the starting model, resulting in a map with a resolution of ~20 Å but poor outside cage density. The signal of the CdSe/ZnS QD density was then subtracted from the particle set using the “particle subtraction” option in cryoSPARC. Then, the subtracted particles were subjected to 2D classification and used for another round of initial model building, followed by heterogeneous refinement to remove the classes with distorted symmetric feature. The final 3D refinements were performed with or without the application of icosahedral symmetry, and corresponding 9.2-Å and 12.9-Å maps were achieved.
respectively. To be noted, the initial models for each round of homogeneous and heterogeneous refinement were low-pass filtered to 30-Å resolution. The resolution was estimated based on the Fourier shell correlation (FSC) of two independent particle sets at a threshold of 0.143.

**Molecular Dynamics Flexible Fitting (MDFF).** Based on the cryo-EM map, the HCcmL pentamer subunits were fitted to the cryo-EM density using the Situs package (22). As a result, a rigid docking structural model containing 12 pentamers was obtained. Then, the MDFF (23) method was performed using NAMD to fit the rigid docking model into a map. During the simulations, the particle mesh Ewald (PME) method was used to treat the long-range electrostatic interactions with a nonbonded cutoff of 10 Å, and the dielectric constant was set as 80. The CHARMM36 force field was employed with bonded interactions and nonbonded interactions of 1 fs and 2 fs, respectively (23). A Langevin thermostat couple was introduced for the whole system to maintain the temperature at 300 K and all heavy atoms at a damping constant of 5 ps. In addition, secondary structure restraints were applied with a force constant of 1 kcal/Å to avoid overfitting.

**Analysis of the HCcmL pentamer interfaces in QD@HCcmL cages.** To calculate the probability of the presence of a certain residue at the interpentamer interface, we performed the following steps: i) count the number of interpentamer interfaces of QD@HCcmL (30 interfaces in total); ii) count the number (N) of residues present at all interpentamer interfaces (30 interfaces in total); and iii) calculate the probability (N/30) of the selected residue appearing at each interface. Because we found that hydrogen bonds were the dominant type of interactions at the interfaces, we statistically analyzed the frequency of hydrogen bond formation by these interfacial residues. Hydrogen bonding was predicted by PyMOL software on the basis of the QD@HCcmL structure by considering the distance between two polar groups at the interpentamer interface. If the distance between H (O/N) and N/O was within 2.0 Å, we concluded that a hydrogen bond can form.

**Synthesis of the iridium(III) complex (Ir probe).** Before synthesizing the Ir probe, we first obtained the precursor [(DPPy)2Ir(N-N)] PF6+ according to a literature procedure (24, 25). To introduce maleimide groups onto this precursor, [(DPPy)2Ir(N-N)] PF6+ (3036 mg, 3.0 mmol), HBTU (1.14 g, 3.0 mmol) and DIPEA (1.04 mL, 6.0 mmol) were dissolved in 10.0 mL of anhydrous THF. 1-(2-Aminoethyl)-1H-pyrrole-2,5-dione (504 mg, 3.6 mmol) was then added in an ice bath. The reaction mixture was stirred at 0 °C for 10 min and then at room temperature for another 3 h. After removal of the solvent, the residue was purified by flash chromatography on silica gel using CH2Cl2/CH3OH (10:1) as the eluent to afford the Ir probe (683 mg, yield 65.8%). The Ir probe was characterized by 1H NMR spectroscopy and mass spectroscopy (Supplementary Figures).

**Patching and patch removal of HCcmL cages.** For patching, HCcmL cages were incubated with 50 mM Ni-NTA for 12 h at 4 °C. Then the mixture was dialyzed using an RC dialysis membrane (8-14 kDa MWCO) against PBS at 4 °C to remove excess Ni-NTA. To remove the Ni-NTA patches from the HCcmL cages, a sample was incubated with 500 mM imidazole for 12 h at 4 °C. Then the mixture was dialyzed using an RC dialysis membrane (8-14 kDa MWCO) against PBS at 4 °C to remove excess imidazole.

**O2 sensing with QDs and Ir complex.** Different O2 concentrations in the sample solutions were achieved by treating the solutions with mixtures of O2 and N2 containing different concentrations of O2 (v/v), namely, 0%, 20%, 40%, 50%, 60%, 80%, and 100%. To determine the O2 concentration in solutions after the treatment, a PBS (100 mL) solution sealed in a bottle, as a test, was treated with
different concentrations of O₂ for 20 min, and then the O₂ concentration was detected with a silver-zinc O₂ electrode connected to a JPSJ-605F O₂ meter (INESA Scientific Instrument Co., Ltd.). During the detection step, gas was added to the bottle above the liquid level to avoid disturbance of the solution as well as to maintain the O₂ concentration. In the following analysis with O₂ probes, the samples were treated in a similar manner. Free O₂ probes or HCCmL cages containing O₂ probes were dispersed in PBS, with the exception that the free Ir probe was dispersed in PBS supplemented with 20% DMSO. A flow chart of the permeability test with the O₂ probes is shown below. First, 1.98 mL of PBS sealed in a 4-mL cuvette was treated with N₂ (or 20%, 40%, 50%, 60%, 80%, or 100% O₂) for 20 min. Then, the probe or the HCCmL cage solution (20 μL) was injected via a microsyringe into the sealed cuvette through the plug and mixed well. Next, the cuvette was kept in the dark, and N₂ (or 20%, 40%, 50%, 60%, 80%, or 100% O₂) was continuously injected above the liquid level to maintain the O₂ concentration in the solution. Finally, the fluorescence or phosphorescence spectra were recorded every 5 min over a period of 30 min.

Examination of protection effects of strongly adsorbed proteins on QD fluorescence against O₂ challenge. Preparation of HisVP1 and its co-assembly with QDs were carried out as previously described (2). His-Cas9 was expressed in E. coli BL21 (DE3) carrying a pET28a(+) vector (Novagen) harboring the coding sequence of Cas9 with a Histag fused at the C-terminus. His-Cas9 protein was purified on a Ni-NTA Sepharose column (GE Healthcare). The complexes of His-Cas9 and QDs were prepared by mixing them in PBS at an His-Cas9/QD molar ratio of 50:1. The mixture was incubated for 12 h at 4 °C and then subject to O₂ challenge experiments. Fluorescence measurements under different conditions of O₂ gassing were performed as described for the QD@HCCmL cages.

Statistical analysis. All data are presented as the mean ± s.d., and statistical analysis and graph preparation were performed using OriginPro 8.0. For statistical analyses, the data were analyzed by one-way ANOVA followed by Fisher’s LSD test, as described in the figure legends. Statistical differences were defined as ***P< 0.001.
Supplementary Figures and Tables

**Fig. S1.** Image of an SDGC tube after the separation of free CdSe/ZnS QDs.

**Fig. S2.** SDS-PAGE analysis of HCcmL (11.5 kDa).

**Fig. S3.** DLS measurement of the hydrodynamic diameter of QD@HCcmL. QD@HCcmL showed a narrow hydrodynamic diameter distribution, with a peak at 14.9 nm. Due to the influence of counterion clouds on particle mobility (26) in DLS measurements, the value determined by DLS is usually slightly larger than the TEM data (14.9 nm versus 12.5 nm).
Fig. S4. Representative backbone walks for residues A54 to Q56 of QD@HCcmL using 3D NCAX, NCOCX, and CONCA spectra. These three experiments can be used to construct an extended spin system of Ni-CAi-COi-Ni+1-CAi+1. A $^{13}$C-$^{13}$C DARR mixing time of 100 ms was used for the NCACX and NCO CX spectra.

Fig. S5. 2D $^{13}$C-$^{13}$C correlation spectra of 2-$^{13}$C-glycerol-labeled QD@HCcmL. A DARR mixing time of 100 ms and PDSD mixing times of 300 ms and 500 ms were used.
**Fig. S6.** Analysis of the oligomeric state of HCcmL in QD@HCcmL. The QD@HCcmL sample purified from F6 (Fig. 1G) was treated with the dissociation buffer and then subjected to SDGC. (A) Image of the SDGC tube of QD@HCcmL after dissociation treatment. The image was taken under UV excitation (360 nm). (B) SDS-PAGE analysis of QD@HCcmL after dissociation treatment and SDGC separation. (C) SEC profile of the HCcmL sample from F1~3 in (B) compared with those of the pentamers of CcmL and HCcmL directly purified from *E. coli* and HCcmL treated with the dissociation buffer. SEC was performed on a Superdex™ 200 Increase 10/300 GL Prepacked Tricorn™ column (GE Healthcare) equilibrated with PBS.

**Fig. S7.** The eight CS-Rosetta structural models of the HCcmL monomer in QD@HCcmL with the lowest energies. Secondary structures are marked in different colors. Green: beta-sheet; red: alpha-helix; orange: loop.
**Fig. S8.** Overlay of 2D $^{13}$C-$^{13}$C correlation spectra of 2-$^{13}$C-glycerol-labeled HCCmL (red) and QD@HCCmL (green). The PDSD mixing time was 500 ms.

**Fig. S9.** The superposition of the ten lowest-energy HCCmL monomers and the superposition of the ten lowest-energy HCCmL pentamers in QD@HCCmL (512 structures calculated). Green: the segments giving rise to CP signals; red: the segments not giving rise to CP signals. Structural precision is shown in SI Appendix, Table S3.
Fig. S10. Workflow of the single-particle cryo-EM analysis.

Fig. S11. Superposition of the two 3D reconstructions with (cyan) and without (gray) icosahedral symmetry in two different views. The cross-correlation coefficient is 0.82.

Fig. S12. 3D reconstruction without symmetry in four different views.
Fig. S13. Analysis of the hydrogen bonding interactions at the interpentamer interfaces. (A) Some of the hydrogen bonds at the interpentamer interfaces. (B) Statistics of the frequency of occurrence for interfacial residue pairs forming hydrogen bonds (see details in SI Appendix, Table S4).

Fig. S14. The size distribution of pores in the QD@HCcmL cage. Each pore measurement reflects a specific single pore in the final model of the QD@HCcmL structure. (A) Radius distribution of central pores in the HCcmL pentamer (type 1 pores, Fig. 4E). (B) Radius distribution of pores surrounded by three HCcmL pentamers (type 2 pores, Fig. 4E). The pore size was measured using Hole software (27).
**Fig. S15.** The electrostatic potential distribution of HCcmL in QD@HCcmL. Positive potentials are shown in blue, and negative potentials are shown in red. Left: top view; right: bottom view. The electrostatic potential map was generated with the APBS Plug-in (28) for PyMOL software. During the calculation of the potential, we considered the pH value (7.4) of the environment in which the protein was located.

**Fig. S16.** Non-normalized emission spectra of QDs (A), QD@HCcmL (B), QD@HCcmL (patched) (C) and QD@HCcmL (patch removed) (D), corresponding to panels A, B, C, and D in Fig 5 in the main text, respectively.
Fig. S17. Responses of CdS/ZnS QDs to 30-min O₂ treatment. The fluorescence spectra of QDs in PBS were measured at increasing O₂ levels (0, 20, 40, 60, 80 and 100%) with excitation at 317 nm.

Fig. S18. Coassembly of CdS/ZnS QDs with HCcmL. (A) Image of an SDGC tube of HCcmL-QD coassembly products upon excitation at 360 nm. (B) TEM image of QD@HCcmL present in F6 in the SDGC tube. The inset shows the diameter distribution based on the TEM data (~240 particles). (C) DLS measurement of the hydrodynamic diameter of QD@HCcmL present in F6 (peaking at 14.6 nm). (D) Affinity measurement curves for interactions between the HCcmL pentamers and QDs by BLI.
Fig. S19. Absorption and fluorescence spectra of the QDs, QD@Hcml, and QD@Hcml (patched) at the same QD concentration. Solid and dashed lines of the same color represent the absorbance (left) and fluorescence (right) spectra, respectively, of the same sample. The absorption spectra of QDs, QD@Hcml, and QD@Hcml (patched) were normalized to the absorbance value of QD@Hcml at 402 nm; the fluorescence spectra of QDs, QD@Hcml, and QD@Hcml (patched) were normalized to the fluorescence value of QD@Hcml at 426 nm.

Fig. S20. The $^1$H NMR spectrum of the Ir probe in a CDCl$_3$ solution. The inset in the upper left shows the ChemDraw structure of the Ir probe. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 9.26 (s, 1), 9.01 (s, 2), 8.59 (s, 1), 8.49 (m, 1), 8.09 (m, 12), 7.56 (m, 8), 7.08 (m, 5), 6.73 (s, 2), 6.66 (m, 1), 6.37 (m, 1), 3.88 (m, 4). The bold numbers under the spectrum are the integrals of the corresponding peaks.
Fig. S21. The MALDI-TOF spectrum of the Ir probe. Mass (m/z): calcd for C\textsubscript{53}H\textsubscript{38}IrN\textsubscript{8}O\textsubscript{3}, exact mass: 1027.269; found: m/z 1026.730.

Fig. S22. Responses of the Ir probe to 30-min O\textsubscript{2} treatment. (A) Phosphorescence spectra of the Ir probe at increasing O\textsubscript{2} levels (0, 20, 40, 60, 80 and 100\%) with excitation at 390 nm. The probe was dissolved in PBS containing 20\% DMSO. (B) The decay curve of the peak phosphorescence intensity of the Ir probe based on the data in (A).
**Fig. S23.** Illustration of site-specific modification of the Ir probe on the interior surface of the HCcmL pentamers. (A) Structural model of the HCcmL pentamer with the Ir probe modification sites (D44C) highlighted. (B) Sketch map of the hybrid structure coassembled from Ir-HCcmL pentamers and QDs, illustrating that Ir probes are sandwiched between HCcmL pentamers and the QD core. This sketch map was prepared according to the structure of QD@HCcmL cages solved in this work.

**Fig. S24.** Coassembly of ZnCdS/ZnS QDs with Ir probe-labeled HCcmL. (A) Image of the SDGC tube of the coassembly products. The image was taken under UV excitation at 360 nm. (B) TEM image of QD@Ir-HCcmL present in F6 in the SDGC tube. The inset shows the diameter distribution based on TEM data (=250 particles). (C) DLS measurement of the hydrodynamic diameter of QD@Ir-HCcmL (with a peak at 14.6 nm).
**Fig. S25.** Optical properties of ZnCdS/ZnS QDs. Fluorescence spectra of ZnCdS/ZnS QDs were measured at increasing O$_2$ levels (0, 20, 40, 60, 80 and 100%) with excitation at 360 nm. The duration of O$_2$ exposure of QDs was 30 min.

**Fig. S26.** TEM image of QD@Ir-HCcmL (patched) purified via SDGC. The inset shows the diameter distribution based on the TEM data (~240 particles).

**Fig. S27.** Absorption and phosphorescence spectra of the Ir probe, QD@Ir-HCcmL, and QD@Ir-HCcmL (patched) at equivalent Ir probe concentrations. Solid and dashed lines of the same color represent the absorbance (left) and phosphorescence (right) spectra, respectively, of the same sample. The absorption spectra of the Ir probe, QD@Ir-HCcmL, and QD@Ir-HCcmL (patched) were normalized to the absorbance value of QD@Ir-HCcmL at 350 nm; the phosphorescence spectra of the Ir probe, QD@Ir-HCcmL, and QD@Ir-HCcmL (patched) were normalized to the phosphorescence value of QD@Ir-HCcmL at 607 nm.
**Fig. S28.** Responses of the Ir probe, QD@Ir-HCcmL and QD@Ir-HCcmL (patched) to O₂ treatment. (A), (B) and (C) Phosphorescence spectra of the Ir probe (A), QD@Ir-HCcmL (B) and QD@Ir-HCcmL (patched) (C) upon treatment with 50% O₂ for 30 min with excitation at 390 nm. (D) Histogram of the phosphorescence intensities of the Ir probe, QD@Ir-HCcmL and QD@Ir-HCcmL (patched) after 15 or 30 min of treatment with 50% O₂. Data were normalized to the phosphorescence values of the corresponding samples at 0 min and represent means ± SDs from three independent replicates. ****P < 0.0001. Statistical significance was assessed by one-way ANOVA followed by Fisher’s LSD test.

**Fig. S29.** Phosphorescence spectra of Ir-HCcmL. The phosphorescence was measured at 50% O₂ every 5 min for 30 min, with excitation at 390 nm.
**Fig. S30.** Responses of free QDs and QD@HCcmL to O2 treatment in a period of 80 min. (A) and (B) Fluorescence (FL) spectra of free QDs (A) and QD@HCcmL (B) upon treatment by 50% O2. Fluorescence quenching reached equilibrium around 60 min. (C) Histogram of the fluorescence intensities of QDs and QD@HCcmL after 70 min of treatment with 50% O2. Data were normalized to the fluorescence values of the corresponding samples at 0 min and represent means ± SDs from three independent replicates. ****P < 0.0001. Statistical significance was assessed by one-way ANOVA followed by Fisher’s LSD test.

**Fig. S31.** Responses of the HisVP1-QD and His-Cas9-QD complexes to O2 treatment. (A) and (B) Fluorescence (FL) spectra of HisVP1-QD (A) and His-Cas9-QD (B) complexes upon treatment with 50% O2 for 30 min. (C) Histogram of the fluorescence intensities of QDs, HisVP1-QD and His-Cas9-QD complexes after 30 min of treatment with 50% O2. Data were normalized to the fluorescence values of the corresponding samples at 0 min and represent means ± SDs from three independent replicates. ****P < 0.0001. Statistical significance was assessed by one-way ANOVA followed by Fisher’s LSD test.

**Fig. S32.** TEM image of QD@HCcmL (patched) purified via SDGC. The inset shows the diameter distribution based on the TEM data (=240 particles).
Fig. S33. ssNMR analysis of QD@HCcmL cages treated with Ni-NTA in comparison with the non-treated. (A) 2D $^{13}$C-$^{13}$C correlation spectrum with a DARR mixing time of 100 ms. Red: 2-$^{13}$C-glycerol-labeled QD@HCcmL; green: 2-$^{13}$C-glycerol-labeled QD@HCcmL treated by Ni-NTA. (B) Interpentamer interfaces in the QD@HCcmL cage. Blue: artificially introduced histidines at the interpentamer interfaces; red: disappeared residues after the introduction of Ni-NTA.

Fig. S34. TEM image of QD@HCcmL (patch removed) purified via SDGC. The inset shows the diameter distribution based on the TEM data (≈240 particles).
|      | CA  | CB  | CD  | CD1 | CD2 | CE  | CG  | CG1 | CG2 | CO  | N   |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A4   | 52.9| 22.0|     |     |     |     |     |     |     |     |     |
| R5   | 53.7| 28.1| 45.7|     |     |     | 27.0|     |     |     |     |
| V6   | 64.5| 31.8|     |     |     |     |     | 23.4| 22.5| 177.4| 127.4|
| C7   | 56.9| 31.8|     |     |     |     |     |     |     |     |     |
| G8   | 47.1|     |     |     |     |     |     |     |     |     |     |
| T9   | 61.3| 73.7|     |     |     |     |     |     |     | 22.6| 173.5| 113.4|
| V10  | 61.0| 36.0|     |     |     |     |     |     | 23.9| 23.1| 175.7| 121.7|
| T11  | 60.3| 72.0|     |     |     |     |     |     |     | 21.9| 175.1| 115.9|
| S12  | 58.9| 65.3|     |     |     |     |     |     |     |     |     |     |
| T13  | 63.4| 70.2|     |     |     |     |     |     |     |     |     |     |
| Q14  | 55.9| 30.2| 175.1|     |     |     | 34.3|     |     |     |     |     |
| K15  | 55.3| 35.5| 30.6|     |     | 43.3| 22.5|     |     |     |     |     |
| E16  | 58.0| 31.7| 181.6|     |     |     | 38.9|     |     |     |     |     |
| D17  | 58.2| 41.7|     |     |     |     | 179.9|     |     |     |     |     |
| T18  | 63.2| 70.1|     |     |     |     |     |     |     | 23.5| 176.5| 108.8|
| L19  | 54.2| 41.2|     |     | 23.9| 23.9| 26.7|     |     |     |     |     |
| T20  | 65.8| 69.4|     |     |     |     |     |     |     | 22.0| 175.9| 119.2|
| G21  | 46.2|     |     |     |     |     |     |     |     |     |     |     |
| V22  | 62.7| 32.4|     |     |     |     |     | 23.0| 22.2| 174.6| 121.1|
| K23  | 55.8| 33.9| 29.7|     |     | 42.9| 25.6|     |     |     |     |     |
| F24  | 55.7| 40.7|     |     |     |     |     |     |     |     |     |     |
| L25  | 53.4| 45.8|     |     | 23.3| 23.3| 27.5|     |     |     |     |     |
| V26  | 63.0| 32.3|     |     |     |     |     | 23.0| 22.2| 174.6| 121.3|
| L27  | 52.6| 44.7|     |     |     |     |     |     |     | 175.6| 127.0|     |
| H34–3B’| 50.8| 25.9|     |     |     |     |     |     |     |     |     |     |
| V47  | 61.4| 32.1|     |     |     |     |     | 22.4| 22.4| 175.2| 124.9|     |
| A48  | 50.2| 25.1|     |     |     |     |     |     |     | 176.2| 130.9|     |
| A49  | 52.2| 21.3|     |     |     |     |     |     |     | 176.6| 123.1|     |
| D50  | 55.0| 45.1|     |     |     |     | 179.2|     |     |     |     |     |
| T51  | 63.9| 70.1|     |     |     |     |     |     | 23.5| 176.6| 109.7|     |
| V52  | 59.1| 35.8|     |     | 23.5| 20.6| 176.8| 122.9|     |     |     |     |
| G53  | 46.6|     |     |     |     |     |     |     |     |     |     |     |
| A54  | 52.5| 21.5|     |     |     |     |     |     |     | 177.2| 128.0|     |
| G55  | 43.7|     |     |     |     |     |     |     |     |     |     |     |
| Q56  | 58.0| 28.7| 179.7|     |     |     | 32.9|     |     |     |     |     |
| D57  | 58.1| 40.2|     |     |     |     | 181.8|     |     |     |     |     |
| E58  | 59.2| 29.9|     |     |     |     | 39.5|     |     |     |     |     |
| W59  | 56.5| 30.4|     |     |     |     |     |     |     |     |     |     |
| V60  | 58.5| 36.7|     |     |     |     |     | 23.2| 19.0| 175.2| 114.5|     |
His tag H34–38 gave rise to five individual peaks in the NCA spectrum of QD@HCcmL but generated only one broad peak in the spectrum of HCcmL.

|    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|
| L61 | 53.2 | 45.2 | 25.1 | 25.1 | 26.7 | 176.2 | 116.2 |
| V62 | 59.5 | 35.9 |    |    | 23.8 | 20.7 | 175.8 | 123.2 |
| S63 | 57.4 | 66.4 |    |    | 174.0 | 124.5 |
| R64 | 55.8 | 34.1 | 43.7 |    | 28.4 | 177.8 | 123.9 |
| G65 | 45.6 |    |    |    |    | 175.8 | 110.0 |
| S66 | 61.2 | 63.0 |    |    |    | 178.1 | 118.5 |
| A67 | 56.4 | 21.3 |    |    |    | 179.7 | 125.6 |
| A68 | 54.8 | 19.4 |    |    |    | 176.7 | 115.3 |
| R69 | 56.9 | 30.2 |    | 29.5 |    | 176.6 | 109.3 |
| H70 | 57.2 | 28.2 |    |    |    | 176.5 | 114.8 |
| I71 | 62.6 | 38.7 | 14.0 |    | 23.2 | 18.7 | 176.8 | 113.4 |
| I72 | 63.2 | 38.0 | 14.0 |    | 28.9 | 17.4 | 176.5 | 127.1 |
| N73 | 55.1 | 38.3 |    |    | 178.8 |    | 177.5 | 119.5 |
| G74 | 46.9 |    |    |    |    | 175.5 | 106.7 |
| T75 | 66.0 | 70.2 |    |    |    | 22.4 | 175.5 | 110.5 |
| P78 | 62.8 | 51.8 | 28.0 |    |    | 175.8 | 133.3 |
| I79 | 60.1 | 42.4 | 14.4 |    | 25.2 | 19.3 | 174.0 | 116.4 |
| D80 | 52.8 | 43.7 |    | 179.7 |    | 177.3 | 121.3 |
| A81 | 52.9 | 21.3 |    |    |    | 176.3 | 118.8 |
| A82 | 51.7 | 23.9 |    |    |    | 176.1 | 122.9 |
| V83 | 63.9 | 33.2 |    | 23.8 | 21.9 | 176.6 | 123.2 |
| V84 | 60.9 | 33.6 |    | 21.9 | 17.7 | 175.1 | 115.1 |
| A85 | 52.9 | 22.0 |    |    |    | 176.0 | 119.0 |
| I86 | 61.8 | 39.1 | 14.3 |    | 28.9 | 18.3 | 178.0 | 122.2 |
| I87 | 64.7 | 39.5 | 16.0 |    | 29.8 | 19.2 | 174.8 | 134.1 |
| D88 | 56.4 | 42.3 |    |    |    | 176.0 | 126.1 |
| T89 | 60.3 | 74.3 |    |    |    | 22.5 | 173.9 | 107.1 |
| V90 | 62.8 | 36.1 |    | 23.2 | 21.6 | 173.8 | 118.3 |
| S91 | 56.3 | 65.0 |    |    |    | 173.8 | 120.0 |
| R92 | 57.9 | 36.0 |    | 34.3 |    | 173.6 | 127.8 |

**QD@HCcmL**

| CA | CB | CD | CD1 | CD2 | CE | CG | CG1 | CG2 | CO | N |
|----|----|----|-----|-----|----|----|-----|-----|----|----|
| A4 | 52.9 | 22.1 |    |    |    |    |    |    | 175.9 | 118.9 |
| R5 | 53.9 | 28.0 | 45.9 | 27.2 |    |    |    |    | 177.4 | 121.9 |
| V6 | 64.6 | 31.8 |    | 23.5 | 22.6 | 177.4 | 127.5 |
| C7 | 57.0 | 31.7 |    |    |    | 174.3 | 123.4 |
| G8 | 47.0 |    |    |    |    | 174.2 | 111.4 |
| T9 | 61.3 | 73.6 |    |    |    | 173.8 | 113.3 |
| V10 | 61.0 | 36.0 |    | 24.0 | 23.3 | 175.9 | 121.7 |
| T11 | 60.2 | 71.9 |    | 22.0 |    | 175.2 | 115.8 |
| Residue | ΔCA | ΔCB | ΔCO | ΔN | ΔCA | ΔCB | ΔCO | ΔN |
|---------|-----|-----|-----|----|-----|-----|-----|----|
| R69     | 56.8| 30.3| 44.7| 29.8| 176.9| 109.2|     |    |
| H70     | 56.7| 27.5|     |     | 176.0| 114.4|     |    |
| I71     | 62.6| 38.7| 14.2| 23.5| 18.6| 176.9| 113.3|    |
| I72     | 63.1| 38.1| 14.1| 29.0| 17.5| 176.7| 126.8|    |
| N73     | 55.3| 38.3|     | 179.0| 177.8| 119.7|     |    |
| G74     | 47.1|     |     |     |     | 175.9| 106.8|    |
| T75     | 66.7| 70.0|     |     | 175.5| 110.6|     |    |
| P78     | 62.8| 51.7| 28.2|     | 175.9| 133.6|     |    |
| I79     | 60.2| 42.4| 14.6| 25.3| 19.2| 174.2| 116.3|    |
| D80     | 52.8| 43.8|     | 179.8| 177.6| 121.3|     |    |
| A81     | 52.9| 21.4|     |     | 176.5| 118.6|     |    |
| A82     | 51.7| 24.0|     |     | 176.3| 122.8|     |    |
| V83     | 63.8| 33.2| 23.4| 176.9| 123.2|     |     |    |
| V84     | 61.0| 33.7| 21.9| 175.2| 115.3|     |     |    |
| A85     | 52.9| 22.0|     |     | 176.2| 119.1|     |    |
| B86     | 61.8| 39.2| 14.4| 29.0| 18.3| 178.2| 122.2|    |
| B87     | 64.7| 39.6| 16.2| 29.9| 19.2| 175.1| 134.2|    |
| D88     | 56.4| 42.4| 180.0|     | 176.3| 125.9|     |    |
| T89     | 60.3| 74.3| 22.6| 174.1| 107.0|     |     |    |
| V90     | 62.7| 36.1| 23.3| 174.0| 118.3|     |     |    |
| S91     | 56.2| 64.9|     |     | 173.9| 120.2|     |    |

**Table S2.** The statistics of the differences in chemical shifts between HCcmL and QD@HCcmL. The residues with significantly different chemical shifts in the two samples are labeled in red. R92 gave a signal in the NCA spectrum of HCcmL but no signal in the spectrum of QD@HCcmL.

### CS (QD@HCcmL) - CS (HCcmL)

| Residue | ΔCA  | ΔCB  | ΔCO  | ΔN  | ΔCA  | ΔCB  | ΔCO  | ΔN  |
|---------|------|------|------|-----|------|------|------|-----|
| A4      | 0    | 0.1  | 0    | 0   | Q56  | 0    | 0.2  | 0   |
| R5      | 0.2  | -0.1 | -0.1 | 0.1 | D57  | 0    | 0    | 0   |
| V6      | 0.1  | 0    | 0    | 0.1 | E58  | 0.1  | 0.3  | 0   |
| C7      | 0.1  | -0.1 | 0.1  | -0.1| W59  | 0    | 0    | 0   |
| G8      | -0.1 | 0    | -0.1 | 0.2 | V60  | 0    | -0.1 | 0.2 |
| T9      | 0    | -0.1 | 0.3  | -0.1| L61  | 0    | -0.1 | 0.6 |
| V10     | 0    | 0    | 0.2  | 0   | V62  | 0    | 0    | -0.2|
| T11     | -0.1 | 0    | 0.1  | -0.1| S63  | 0    | 0    | 0.1 |
| S12     | 0    | -0.1 | 0.1  | 0.1 | R64  | 0    | 0.2  | 0.3 |
| T13     | -0.1 | 0    | -0.1 | -0.3| G65  | 0    | 0    | 0.2 |
| Q14     | -0.1 | 0    | 0.2  | -0.1| S66  | -0.1 | 0    | -0.1|
| K15     | 0    | 0.1  | 0.2  | 0   | A67  | 0.1  | 0.2  | 0.2 |
| E16     | 0    | 0.1  | 0.2  | 0   | A68  | -0.1 | 0.1  | 0   |
| D17     | 0.1  | 0.1  | 0.2  | -0.2| R69  | -0.1 | 0.1  | 0.3 |
Table S3. Restraints and structure statistics of the HCcmL pentamers in QD@HCcmL.

| Number of restraints (per monomer) |       |
|-----------------------------------|-------|
| Intramonomer                      | 317   |
| Intraresidual (i - j) = 0         | 143   |
| Sequential (i - j) = 1            | 49    |
| Medium-range (2 ≤ |i − j| ≤ 5) | 26    |
| Long-range (|i - j| > 5)         | 98    |
| Intermonomer                      | 19    |
| Dihedral angle restraints (φ/ψ)   | 106 (53/53) |
| Hydrogen bonds restraints (intra) | 100   |

Restraints statistics

| Number of distance violations > 0.5 Å | 2 |
| Number of angle violations > 10°    | 0 |

Structural precision (residues 4-27, 47-75, 78-92)

| Backbone atoms (monomer) | 0.9 Å |
| Heavy atoms (monomer)    | 1.5 Å |
| Backbone atoms (pentamer) | 0.5 Å |
| Heavy atoms (pentamer)   | 1.4 Å |
Table S4. The statistics of residues involved in forming hydrogen bonds at the interpentamer interfaces in the QD@HccmL cage. There are thirty interpentamer interfaces (No. 1~30) and twelve pentamers in the cage. The twelve pentamers are represented as A, B… K, and L. The five chains in each HccmL pentamer are denoted 1, 2, 3, 4, and 5. The two interacting pentamers are labeled PART1 and PART2.

| No. | PART1          | PART2          | Distance (Å) |
|-----|----------------|----------------|--------------|
|     | Chain          | Residue        | Chain        | Residue       |             |
| 1   | A4 ARG92@NH2-HH22 | B2 ASP88@OD2   | 2            |
|     | A4 ARG92@OT-H01  | B2 ASP33@OD2   | 2.9          |
|     | A4 ARG92@OT-H01  | B2 THR89@OG1   | 2.9          |
|     | A4 ARG92@N-HN   | B2 THR89@OG1   | 2            |
|     | A4 HIS34@ND1-HD1| B2 HIS34@O     | 2.3          |
| 2   | A3 ARG92@NH2-HH22| G1 GLY8@O     | 2.4          |
|     | A3 ARG92@OT-H01  | G1 THR9@OG1    | 2            |
|     | A3 HIS37@ND1-HD1| G4 ASP33@OD1   | 2.6          |
|     | A3 HIS36@ND1-HD1| G4 HIS34@ND1-HD1| 2.5        |
|     | A3 SER91@O      | G4 HIS35@NE2-H01| 2.4       |
|     | A3 SER91@OG-HG1 | G4 THR89@OG1   | 2.3          |
|     | A3 HIS34@O      | G4 ARG92@N-HN  | 2.4          |
|     | A3 HIS34@ND1-HD1| G4 ARG92@OXT   | 2.2          |
|     | A4 THR9@OG1     | G4 ARG32@NE-HE | 2.5          |
|     | A4 THR9@OG1     | G4 ARG32@NH1-HH12| 2.4       |
| 3   | A5 ARG92@NE-HE | F3 PHE43@O     | 2.1          |
|     | A5 ARG92@ND1-HH12| F3 LEU44@O     | 2.5          |
|     | A5 VAL90@O     | F2 HIS35@NE2-H | 2.4          |
|     | A2 GLY39@O     | F2 ARG92@NH1-HH11| 2.4     |
| 4   | A2 ARG92@NH2-HH22| I4 VAL10@O    | 2.5          |
|     | A2 ARG92@NH1-HH11| I4 THR9@OG1    | 2.6          |
|     | A2 ARG92@NH1-HH11| I4 THR9@OG1    | 2.1          |
|     | A2 ARG92@O      | I3 HIS35@ND1-HD1| 2.8        |
| 5   | A1 ARG92@NH1-HH11| D2 THR9@OG1    | 2.5          |
|     | A1 ARG92@NH2-HH11| D2 THR9@OG1    | 2.6          |
|     | A1 ARG92@OT     | D5 HIS34@ND1-HD1| 2.6        |
|     | A1 HIS36@ND1-HD1| D5 HIS34@O     | 2.6          |
|   | A1  | HIS34@O | D5   | ARG92@O-H | 2.4 |
|   | A1  | HIS35@NE2-H01 | D5   | ARG92@O | 2.3 |
|   | A1  | THR89@O | D5   | SER91@OG-HG | 2.4 |
|   | A1  | THR89@OG-HG | D5   | VAL90@O | 2.3 |
|   | A5  | PHE41@O | D5   | ARG92@O-H | 2.8 |
| 6 | K5  | ARG92@N-HN | E4   | THR89@O | 2.4 |
|   | K5  | THR89@O | E4   | VAL90@N-HN | 2.6 |
|   | K5  | SER90@N-HN | E4   | SER91@OG | 2.2 |
| 7 | K1  | ARG92@NH1-HH11 | J3   | THR9@OG | 2.8 |
|   | K1  | ARG92@NH1-HH11 | J2   | GLU32@OE2 | 2.1 |
|   | K1  | ARG92@NH2-HH21 | J3   | THR9@OG1 | 2.6 |
|   | K1  | ARG92@OT-H01 | J3   | VAL10@O | 2.8 |
|   | K1  | SER91@O | J2   | TRP59NE1-HE1 | 2.1 |
|   | K5  | THR11@OG1 | J2   | ARG92@NH1-HH11 | 2.6 |
|   | K5  | THR11@OG1 | J2   | ARG92@NH2-HH21 | 2.1 |
|   | K5  | VAL10@O | J2   | ARG92@NH2-HH21 | 2.6 |
|   | K5  | THR9@OG1 | J2   | ARG92@NH2-HH22 | 2.4 |
| 8 | K4  | ARG92@NH1-HH12 | H2   | THR9@OG1 | 2.7 |
|   | K4  | ARG92@NH2-HH21 | H2   | PHE41@O | 2.3 |
|   | K4  | ARG92@NH1-HH11 | H2   | PHE41@O | 1.9 |
|   | K4  | ARG92@O | H5   | HIS34@N-HN | 2.1 |
|   | K4  | HIS35@ND1-HD1 | H5   | HIS34@O | 2 |
|   | K4  | THR89@O | H5   | SER91@OG-HG | 1.7 |
| 9 | K3  | ARG92@NH2-HH22 | C1   | GLY8@O | 2.1 |
|   | K3  | ARG92@O | C1   | THR9@OG-HG | 2.5 |
|   | K3  | SER91@O | C4   | VAL90@N-HN | 2.5 |
|   | K3  | HIS35@NE2-H01 | C4   | SER91@OG | 2.2 |
|   | K4  | GLU40@OE1 | C4   | HIS35@ND1-HD1 | 2.2 |
| 10 | K2  | HIS36@NE2-H01 | L5   | GLU40@OE2 | 2.9 |
|   | K2  | ARG36@NH2-HH21 | L5   | THR9@OG1 | 2.6 |
|   | K2  | ARG92@NH2-HH21 | L5   | VAL10@O | 2.4 |
|   | K2  | ARG92@OXT | L1   | TRP59@NE1-HE1 | 2.3 |
|   | K2  | ARG92@NH1-HH12 | L1   | THR89@OG | 1.8 |
|   |       |       |       |       |
|---|--------|-------|--------|-------|
| K2 | THR89@O | L1    | HIS35@ND1-HD1 | 2.6   |
| K3 | GLU40@OE1 | L1    | HIS36@NE2-H01 | 2.4   |
| K3 | PHE41@O | L1    | ARG92@NH1-HH12 | 2.5   |
| K3 | PHE41@O | L1    | ARG92@NE-HE | 2.7   |
| J5 | SER91@OG | E1    | HIS35@ND1-HD1 | 2.5   |
| J5 | HIS35@ND1-HD1 | E1 | HIS34@O | 2.3   |
| J5 | THR89@OG | E1    | ARG92@N-HN | 2.4   |
| J5 | HIS35@NE2-H01 | E1 | SER91@O | 2.5   |
| J5 | HIS35@NE2-H01 | E1 | ARG92@OXT | 2.5   |
| J2 | GLU40@OE2 | E1    | HIS36@NE2-H01 | 2.7   |
| J2 | PHE41@O | E1    | ARG92@NH1-HH12 | 2.6   |
| J1 | ARG92@NH2-HH21 | F5 | GLY8@O | 2.9   |
| J1 | HIS35@ND1-HD1 | F1 | SER91@OG | 2.4   |
| J5 | PHE41@O | F1    | ARG92@NH1-HH12 | 2     |
| J4 | ARG92@NE-HE | I5 | PHE41@O | 2.6   |
| J4 | ARG92@NH1-HH12 | I5 | PHE41@O | 2.3   |
| J4 | HIS36@O | I1    | HIS35@ND1-HD1 | 2.5   |
| J4 | ARG92@OT | I1 | HIS35@NE2-H01 | 2.2   |
| J4 | SER91@OG | I1    | THR89@OG-HG1 | 2.7   |
| J4 | HIS35@NE2-H01 | I1 | ARG92@OT | 2.4   |
| J1 | THR9@OG | I1    | ARG92@NH2-HH22 | 2     |
| J1 | VAL10@O | I1    | ARG92@NH2-HH21 | 2.3   |
| J3 | ARG92@NH1-HH11 | H5 | THR9@OG | 2     |
| J3 | ARG92@NH2-HH21 | H1 | GLU32@OE2 | 2.8   |
| J3 | ARG92@OT | H1    | HIS34@N-HN | 2.7   |
| J3 | ARG92@OT | H1    | HIS35@ND1-HD1 | 2.8   |
| J3 | ARG92@N-HN | H1 | GLU32@OE1 | 2.8   |
| J3 | ARG92@NE-HE | H5 | GLU40@OE2 | 2.1   |
| J3 | VAL90@O | H1    | THR89@OG-HG | 2.3   |
| J3 | SER91@OG-HG | H1 | THR89@O | 2.5   |
| J3 | HIS35@ND1-HD1 | H1 | SER91@OG | 2.5   |
| J3 | HIS35@N-HN | H1    | SER91@OG | 2.2   |
| J4 | PHE41@O | H1    | ARG92@NH1-HH11 | 2.7   |
|   | Residue 1   | Residue 2   | Residue 3   | Distance |
|---|-------------|-------------|-------------|----------|
| J4 | VAL10@OG    | H1          | ARG92@NH1-HH11 | 2.1      |
| G3 | ARG92@NH2-HH21 | B4         | VAL10@O    | 2.2      |
| G3 | ARG92@NE-HE | B4          | PHE41@O    | 2.3      |
| G3 | ARG92@NH1-HH12 | B4       | PHE41@O    | 2.1      |
| G3 | ARG92@OXT  | B3          | HIS35@NE2-H01 | 2.8      |
| G3 | HIS35@O    | B3          | HIS34@ND1-HD1 | 2.8      |
| G3 | HIS35@NE2-H01 | B3       | VAL90@O    | 2.6      |
| G3 | ASP33@O    | B3          | HIS36@NE2-H01 | 2.6      |
| G4 | THR9@OG   | B3          | ARG92@NE-HE | 2.7      |
| G2 | ARG92@NH2-HH21 | C3         | THR9@OG    | 2.8      |
| G2 | ARG92@NE-HE | C3          | VAL10@O    | 2.5      |
| G2 | ARG92@OT   | C2          | HIS34@N-HN  | 2.9      |
| G2 | HIS35@O    | C2          | HIS34@ND1-HD1 | 2.1      |
| G2 | HIS34@O    | C2          | HIS35@ND1-HD1 | 2.9      |
| G2 | HIS35@ND1-HD1 | C2     | SER91@OG   | 2.5      |
| G2 | THR89@OG  | C2          | HIS35@NE2-H01 | 2.6      |
| G3 | LEU42@O   | C2          | ARG92@NH1-HH12 | 2.6      |
| G3 | PHE41@N-HN | C2          | ARG92@OXT  | 2.1      |
| G3 | VAL10@O   | C2          | ARG92@N-HN  | 2.2      |
| G5 | ARG92@NH2-HH21 | H2         | GLU40@OE2  | 2.3      |
| G5 | HIS36@O   | H3          | HIS34@NE2-H04 | 2.6      |
| G5 | HIS35@ND1-HD1 | H3       | HIS35@O    | 2.7      |
| G5 | HIS34@ND1-HD1 | H3       | SER91@O    | 2.6      |
| G2 | PHE41@O   | H3          | ARG92@OT-H02 | 2.3      |
| G2 | PHE41@N-HN | H3          | ARG92@OXT  | 2.7      |
| G2 | THR9@OG  | H3          | ARG92@NH1-HH12 | 2.5      |
| G1 | ARG92@NH1-HH12 | I3         | THR9@OG    | 2.4      |
| G1 | ARG92@NE-HE | I3         | THR9@OG    | 2.3      |
| G1 | HIS36@NE2-H01 | I2         | HIS34@O    | 2.3      |
| G1 | VAL90@O  | I2          | HIS35@35@ND1-HD1 | 2.9      |
| G1 | THR89@OG-HG | I2         | SER91@OG   | 2.5      |
| G1 | GLU32@O  | I2          | SER91@OG-HG1 | 2.6      |
| G1 | HIS34@ND1-HD1 | I2       | SER91@O    | 2.3      |
| 19 | G4  | VAL10@O | I2  | ARG92@NH2-HH22 | 2.8 |
|----|-----|---------|-----|----------------|-----|
|    | H2  | ARG92@NH1-HH12 | C4  | THR9@OG       | 2.7 |
|    | H2  | ARG92@NE-HE   | C4  | THR9@OG       | 2.4 |
|    | H2  | ARG92@OXT     | C4  | PHE41@N-HN    | 2.5 |
|    | H2  | ARG92@OT-H03  | C3  | GLU32@OE1     | 2.8 |
|    | H2  | HIS35@NE2-H01 | C3  | VAL90@O       | 2.7 |
|    | H2  | HIS34@ND1-HD1 | C3  | ARG92@OXT     | 2.6 |
|    | H2  | HIS34@N-HN    | C3  | ARG92@OXT     | 2.6 |

| 20 | H4  | ARG92@OXT     | I5  | TRP59@NE1-HE1 | 2.2 |
|    | H4  | HIS34@ND1-HD1 | I5  | ASP33@O       | 2.6 |
|    | H4  | SER91@OG      | I5  | SER91@N-HN    | 2.7 |
|    | H4  | SER91@OG-HG   | I5  | SER91@OG      | 2.8 |
|    | H4  | SER91@N-HN    | I5  | SER91@OG      | 2.8 |
|    | H4  | HIS35@NE2-H01 | I5  | SER91@OG      | 2.6 |
|    | H4  | GLU32@OE2     | I5  | HIS35@ND1-HD1 | 2   |
|    | H1  | PHE41@O       | I5  | HIS35@NE2-H01 | 2.7 |
|    | H1  | PHE41@O       | I5  | ARG92@NH1-HH12| 2   |
|    | H1  | VAL10@O       | I5  | ARG92@NH2-HH22| 2.2 |

| 21 | I4  | ARG92@NH1-HH12 | F2  | PHE41@O       | 2.6 |
|    | I4  | ARG92@NE-HE   | F2  | PHE41@O       | 2.4 |
|    | I4  | HIS36@NE2-H01 | F2  | GLU40@OE1     | 2.7 |
|    | I4  | HIS34@NE2-H04 | F5  | ASP33@O       | 2.5 |
|    | I4  | HIS35@ND1-HD1 | F5  | HIS35@O       | 2.4 |
|    | I4  | HIS35@ND1-HD1 | F5  | HIS34@O       | 2 |
|    | I4  | GLU32@OE2     | F5  | SER91@OG-HG   | 2.5 |
|    | I4  | THR89@OG-HG   | F5  | VAL90@O       | 2.7 |
|    | I4  | HIS35@NE2-H01 | F5  | ARG92@OT      | 2.2 |
|    | I1  | THR9@OG       | F5  | ARG92@NH2-HH22| 2.4 |
|    | I1  | VAL10@O       | F5  | ARG92@NH2-HH22| 2.9 |

| 22 | D1  | ARG92@NH2-HH21 | F4  | VAL10@O       | 2.5 |
|    | D1  | ARG92@NH2-HH22 | F4  | THR9@OG       | 2.4 |
|    | D1  | ARG92@NH1-HH12 | F3  | ASP33@OD2     | 2.5 |
|    | D1  | ARG92@NE-HE   | F3  | ASP33@OD2     | 2.1 |
|   |   |   |   |
|---|---|---|---|
| D1 | ARG92@OXT | F3 | HIS34@N-HN | 2.1 |
| D1 | HIS35@ND1-HD1 | F3 | HIS34@O | 2.2 |
| D1 | HIS34@O | F3 | HIS36@ND1-HD1 | 2.3 |
| D1 | GLU32@OE1 | F3 | ARG92@OT-H02 | 2.3 |
| 23 | D4 | ARG92@OT | E2 | HIS35@NE2-H01 | 2.2 |
| 23 | D4 | SER91@OG-HG | E2 | SER91@OG | 2.9 |
| 24 | D3 | ARG92@NH1-HH12 | L3 | GLU40@OE2 | 2.5 |
| 24 | D3 | SER91@OG-HG | L2 | THR89@O | 2.7 |
| 24 | D3 | HIS35@NE2-H01 | L2 | ASP88@O | 2.6 |
| 24 | D3 | HIS35@NE2-H01 | L2 | SER91@O | 2.3 |
| 24 | D3 | HIS35@NE2-H01 | L2 | ARG92@OXT | 2.4 |
| 24 | D3 | HIS34@O | L2 | HIS34@N-HN | 2.3 |
| 24 | D4 | VAL10@O | L2 | ARG92@NH2-HH21 | 2.6 |
| 24 | D4 | THR9@OG | L2 | ARG92@NH1-HH11 | 2.7 |
| 25 | D2 | ARG92@OT-H03 | B2 | GLU40@OE2 | 2.6 |
| 25 | D2 | HIS35@NE2-H01 | B5 | ARG92@OXT | 2.3 |
| 25 | D3 | PHE41@O | B5 | ARG92@NH1-HH11 | 2.8 |
| 26 | L5 | SER91@OG | E3 | TRP59@NE1-HE1 | 2.3 |
| 26 | L5 | SER91@OG-HG | E3 | THR89@O | 2.4 |
| 26 | L5 | HIS34@O | E3 | HIS34@ND1-HD1 | 2.3 |
| 26 | L2 | VAL10@N-HN | E3 | ARG92@OXT | 2.3 |
| 26 | L2 | LEU42@O | E3 | ARG92@NH2-HH22 | 2 |
| 27 | L4 | ARG92@NH1-HH11 | C1 | ASP33@OD2 | 2.6 |
| 27 | L4 | ARG92@NH1-HH12 | C5 | PHE41@O | 2.8 |
| 27 | L4 | SER91@OG | C1 | THR89@OG-HG | 2.4 |
| 27 | L4 | GLU32@OE1 | C1 | HIS35@ND1-HD1 | 2.7 |
| 27 | L4 | HIS35@NE2-H01 | C1 | SER91@O | 2.3 |
| 27 | L4 | HIS35@NE2-H01 | C1 | ARG92 | 2.1 |
| 27 | L1 | PHE41@O | C1 | ARG92@OT-H03 | 2.5 |
| 27 | L1 | GLU40@OE2 | C1 | ARG92@NH1-HH11 | 2.1 |
| 27 | L1 | GLU40@OE2 | C1 | ARG92@NH2-HH21 | 2.4 |
| 28 | L3 | ARG92@NH1-HH11 | B5 | THR9@OG | 2.4 |
| 28 | L3 | ARG92@NH2-HH21 | B5 | THR9@OG | 2.5 |
|   | L3 | ARG92@NE-HE | B1 | THR89@OG | 2.5 |
|---|---|-------------|----|---------|----|
| L3 | ARG92@OT | B1 | HIS34@ND1-HD1 | 2.7 |
| L3 | ARG92@N-HN | B1 | GLU32@OE2 | 2.1 |
| L3 | ARG92@N-HN | B1 | THR89@O | 2.2 |
| L3 | SER91@OG | B1 | SER91@N-HN | 2.4 |
| L3 | HIS36@O | B1 | HIS35@N-HN | 2.0 |
| L3 | HIS35@NE2-H01 | B1 | HIS35@NE2-H01 | 2.4 |
| L3 | HIS35@NE2-H01 | B1 | ARG92@OXT | 2.1 |
| C5 | ARG92@OT-H03 | B1 | PHE41@O | 2.4 |
| C5 | SER91@OG | B4 | HIS34@N-HN | 2.5 |
| C5 | SER91@OG | B4 | TRP59@NE1-HE1 | 2.3 |
| C5 | SER91@OG | B4 | HIS35@NE2-H01 | 2.2 |
| C5 | SER91@OG-HG | B4 | THR89@O | 2.5 |
| C5 | ASP33@OD1 | B4 | HIS34@NE2-H01 | 2.9 |
| C5 | HIS34@O | B4 | HIS34@ND1-HD1 | 2.1 |
| C5 | HIS34@ND1-HD1 | B4 | ARG92@OXT | 2.3 |
| C2 | PHE41@O | B4 | ARG92@NH1-HH11 | 2.5 |
| C2 | THR9@OG | B4 | ARG92@NH2-HH22 | 2.3 |

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|   | E5 | HIS34@NE2-H04 | F1 | GLU40@OE2 | 2.3 |
|---|---|-------------|----|---------|----|
| E5 | HIS34@ND1-HD1 | F4 | ASP33@OD1 | 2.5 |
| E2 | PHE41@O | F4 | ARG92@NH1-HH12 | 2.2 |

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