

Ice-mediated interfacial membrane synthesis of cargo-independent microcapsules

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Supporting Information

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Experimental Procedures

1. Materials and Methods

1.1 Materials

p-Phenylenediamine (PPD), trimesoyl chloride (TMC), catalase (CAT), horseradish peroxidase (HRP), glucose oxidase (GOx), amplex red, proteinase K, β -Nicotinamide adenine dinucleotide disodium salt hydrate (NAD^+), β -Nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH), ADH, FDH and FaldDH were purchased from Sigma-Aldrich. Rhodamine probes and rhodamine-dextran probes were purchased from Beijing Solarbio Science & Technology Co., Ltd. 2,2'-Bipyridine, dimer of pentamethylcyclopentadienylrhodium (III) chloride ($(\text{Cp}^*\text{RhCl}_2)_2$), triethanolamine (TEOA), Eosin Y, acetaldehyde and phosphate-buffered saline were purchased from Zancheng Tianjin Technology Co., Ltd. 3-(trimethylsilyl)-1-propane sulfonic acid and sodium salt (DSS) were purchased from Aladdin Reagent Co., Ltd. Cadmium sulfide (CdS) was purchased from Beijing Zhongke Leiming Technology Co., Ltd. Fluorescent silica and polystyrene microspheres were obtained from Jiangsu Zhichuan Technology Co., Ltd. Dimethyl sulfoxide, acetonitrile, tetrahydrofuran and ethyl acetate were purchased from Aladdin Reagent Co., Ltd. The mineral oil was purchased from Wuhan Jianmi Intelligent Control Technology Co., Ltd. p-Phenylenediamine, trimesoyl chloride and organic solvents were handled in a chemical fume hood with appropriate personal protective equipment.

1.2 Preparation of PA Microcapsules

Polyamide (PA) microcapsules were prepared through an ice-mediated interfacial reaction. To investigate the effects of preparation conditions on membrane formation, the PPD concentration was varied from 20 to 250 mmol L^{-1} , while the TMC concentration was maintained at 200 mmol L^{-1} (531.0 mg, 2.00 mmol dissolved in 10 mL of ethyl acetate). Reaction-time-dependent samples were prepared under the standard precursor concentrations while varying only the reaction time. Specifically, aqueous PPD precursor solutions containing 20, 50, 100, 150, 200 or 250 mmol L^{-1} were prepared by dissolving 4.3, 10.8, 21.6, 32.4, 43.3 or 54.1 mg of PPD, respectively, in 2.0 mL of deionized water. Unless otherwise stated, all other preparation parameters, including the microfluidic chip geometry, flow rates, reaction temperature and washing procedures, were kept identical.

The diameter of the PA microcapsules was tuned by varying the nozzle-orifice size and the flow rates of the continuous and dispersed phases. Using the same 80 μm nozzle orifice, reducing the aqueous-phase flow rate from 500 to 100 $\mu\text{L h}^{-1}$ while maintaining the mineral-oil flow rate at 1,000 $\mu\text{L h}^{-1}$ produced PA microcapsules with average

diameters of approximately 30 μm . Under the standard conditions (80 μm nozzle orifice; mineral-oil and aqueous-phase flow rates of 1,000 and 500 $\mu\text{L h}^{-1}$, respectively), PA microcapsules with average diameters of approximately 80 μm were obtained. PA microcapsules with average diameters of approximately 100 μm were prepared using a 120 μm nozzle orifice with the mineral-oil and aqueous-phase flow rates maintained at 1,000 and 500 $\mu\text{L h}^{-1}$, respectively. Reducing the mineral-oil and aqueous-phase flow rates to 200 and 100 $\mu\text{L h}^{-1}$, respectively, while using the same 120 μm nozzle orifice yielded PA microcapsules with average diameters of approximately 150 μm . Increasing the nozzle-orifice size to 150 μm while maintaining the same flow rates (200 and 100 $\mu\text{L h}^{-1}$ for the mineral-oil and aqueous phases, respectively) produced PA microcapsules with average diameters of approximately 200 μm . For larger capsules, a 300 μm nozzle orifice was used together with mineral-oil and aqueous-phase flow rates of 200 and 100 $\mu\text{L h}^{-1}$, respectively, yielding PA microcapsules with average diameters of approximately 500 μm .

After complete evaporation of the liquid nitrogen, the pre-cooled TMC solution in ethyl acetate was gently added to the container containing the frozen ice spheres. The reaction vessel was then maintained at $-5\text{ }^{\circ}\text{C}$ for 12 h without agitation to allow interfacial polyamidation at the ice–organic interface. During this process, PPD molecules released from the quasi-liquid layer at the ice surface reacted with TMC in the surrounding organic phase, resulting in the formation of a conformal PA membrane on the surface of each ice sphere.

After completion of the reaction, the organic phase was removed, and the samples were washed sequentially with pre-cooled ethyl acetate and pre-cooled ethanol at $-5\text{ }^{\circ}\text{C}$, followed by repeated washing with deionized water at room temperature to remove residual monomers, mineral oil and organic solvent. The resulting PA microcapsules were finally dispersed in deionized water or the corresponding reaction buffer for subsequent characterization and catalytic experiments.

For cargo-loaded PA microcapsules, enzymes, fluorescent molecules, quantum dots, nanoclusters, nanoparticles or microparticles were dissolved or uniformly dispersed in the aqueous PPD precursor solution before droplet generation. During freezing, these cargoes became confined within the ice templates and remained encapsulated after PA membrane formation and melting of the ice core.

1.3 Synthesis of $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$

Pentamethylcyclopentadienylrhodium(III) chloride dimer, $(\text{Cp}^*\text{RhCl}_2)_2$ (18.54 mg, 0.03 mmol), was dissolved in methanol (5.0 mL). Subsequently, 2,2'-bipyridine (9.37 mg, 0.06 mmol) was added. The initially turbid suspension gradually became clear,

yielding a yellow solution. Diethyl ether was then slowly added to the solution at 2 °C until precipitation was complete. The resulting yellow solid, $[\text{Cp}^*\text{Rh}(\text{bpy})\text{Cl}]\text{Cl}$ was collected and dried in an oven at 35 °C for 24 h. Upon dissolution in water, $[\text{Cp}^*\text{Rh}(\text{bpy})\text{Cl}]\text{Cl}$ underwent aquation through replacement of the coordinated chloride ligand by water, affording $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$. The resulting aqueous rhodium complex was used as the electron mediator for photocatalytic NADH regeneration and is hereafter denoted as [M].

2. Characterization

2.1 Fluorescence Analysis of PA Microcapsules

The fluorescence properties of the PA microcapsules were examined using a Zeiss LSM confocal microscope, where different excitation and emission wavelengths were used to analyze the fluorescence properties, particularly for enzyme labeling. The PA microcapsules themselves exhibited intrinsic green fluorescence, excited at 488 nm emitting in the range of 500-550 nm. The enzyme catalase (CAT) and horseradish peroxidase (HRP) were labeled with Alexa Fluor 568, excited at 560 nm and emitting at 570-620 nm. Additionally, glucose oxidase (GOx) was labeled with Alexa Fluor 405, excited at 405 nm and emitting at 450-490 nm. For the additional microspheres, silica (SiO_2) microspheres emitting blue fluorescence were excited at 405 nm and emitted at 450-490 nm. Polystyrene (PS) microspheres emitting yellow fluorescence were excited at 488 nm and emitted at 550-600 nm. This experimental setup allowed for the visualization of the spatial distribution and encapsulation efficiency of the fluorescently labeled enzymes inside the PA microcapsules.

2.2 SEM imaging and EDS analysis of PA microcapsules.

The morphology of PA microcapsules was characterized by scanning electron microscopy (SEM). Microcapsule suspensions were collected and washed with deionized water, then deposited onto a conductive SEM stub with carbon tape and dried under ambient conditions. To minimize charging, the samples were sputter-coated with a thin conductive layer of Au. SEM imaging was performed on a field-emission SEM (FE-SEM, Thermo Scientific Apreo 2) at an accelerating voltage of 5 kV.

Elemental analysis was carried out using an energy-dispersive X-ray spectroscopy (EDS) detector integrated into the SEM to verify the encapsulation of Pt-based catalysts within PA microcapsules. EDS spectra and elemental maps were acquired from selected regions of interest, and the presence of Pt was confirmed by the characteristic Pt signals in the EDS spectrum and the corresponding spatial distribution in the elemental maps.

2.3 High-resolution Scanning Transmission Electron Microscopy (HR-STEM) test

HR-STEM observation of specimens was carried out using an aberration-corrected

HR-STEM microscope (Spectra-300, Thermo Fisher, USA; equipped with a field emission gun) with a 300 kV electron beam accelerating voltage. The probe convergence angle is 24.5 mrad, the HAADF detector's angular range is from 57 to 200 mrad.

2.4 Scanning Tunneling Microscopy (STM) test

PA membranes were transferred onto an atomically flat Au substrate (Au-coated Si) and dried thoroughly prior to STM measurements. STM imaging was performed in an ultrahigh-vacuum STM system (Unisoku USM-1300) operated in constant-current mode. Electrochemically etched W tips were used and routinely conditioned on a clean Au surface before data acquisition. Topographic images were acquired at representative tunneling setpoints (sample bias in the sub-volt to volt range and tunneling current in the tens to hundreds of pA range), and multiple non-overlapping areas were collected for each sample to ensure statistical representativeness. Apparent opening-like lateral features were extracted from STM height maps by line-profile analysis across individual opening-like features using a consistent width criterion, and cross-validated by 2D image segmentation of pore-like depressions to obtain apparent opening-size distributions. Lateral calibration was verified on the Au surface, and potential tip-convolution effects were considered when reporting apparent opening dimensions.

2.5 Optical Microscopy for Capsule Size Measurement

The size of PA microcapsules was measured by optical microscopy using an upright microscope (Nikon Eclipse Ni-U). A drop of the microcapsule suspension was placed on a clean glass slide and covered with a coverslip to minimize evaporation and sample drift. Images were acquired under bright-field mode with appropriate objectives. Capsule diameters were determined from the acquired micrographs using image analysis software. For each size range, the diameters of at least 100 individual microcapsules were measured to generate size distributions and assess uniformity across the sample.

2.6 Nanoindentation Measurement of PA Microcapsules

The dried microcapsules were dispersed onto a clean silicon wafer and immobilized by gentle drying before measurement. Individual microcapsules with intact spherical morphology were selected under an optical microscope and compressed using a nanoindenter equipped with a spherical or flat-ended probe. Force–displacement curves were recorded under displacement-controlled loading, and the maximum indentation depth was kept small relative to the capsule diameter to avoid complete collapse of the shell. The apparent shell stiffness was obtained from the initial linear region of the loading curve. The apparent shell modulus was calculated using the Reissner thin-shell model, in which the PA microcapsule was treated as a hollow elastic spherical shell. The force–displacement relation is given by

$$F = \frac{4Eh^2}{R\sqrt{3(1-\nu^2)}}d$$

where F is the applied force, d is obtained by converting the engineering strain using the initial capsule diameter. E is the apparent shell modulus, h is the shell thickness, R is the capsule radius and ν is the Poisson's ratio. The slope of the initial linear region of the force–displacement curve was defined as the indentation stiffness $k=F/d$. Thus, the apparent shell modulus was calculated as:

$$E = \frac{kR\sqrt{3(1-\nu^2)}}{4h^2}$$

The shell thickness was about 200 nm, and capsule radius used was about 40 μm . A Poisson's ratio of 0.35 was assumed for the PA shell.

2.7 Low-Temperature Raman Spectroscopy

Raman spectra were recorded using a Horiba LabRAM HR Evolution Raman microscope with a 532 nm laser. Sample temperature was controlled using a customized Linkam THMS600 cold stage with a heating and cooling rate of 10 $^{\circ}\text{C min}^{-1}$. Briefly, 10 μL of aqueous p-phenylenediamine (PPD) solution was placed on the cryostage, frozen, and equilibrated at the target subzero temperature before spectral acquisition. Spectra were collected over the range of 400–4000 cm^{-1} , and time-dependent spectra were acquired during annealing at the indicated subzero temperatures.

2.8 Cryo-confocal in situ Observation of Ice Recrystallization

The ice recrystallization experiment was performed via the splat cooling method. The experiment apparatus was composed of a Zeiss LSM confocal microscope and a cryo-stage (Instec). The experimental procedure is described as follows: a droplet (approximately 10 μL) of the test solution containing eosin Y (at a concentration of about 10 $\mu\text{g/mL}$) was dropped onto a cover glass pre-cooled to -60°C from a height of 1.5 m to form a thin solid ice film. The temperature of the ice film was then raised to -5°C at a rate of 5 $^{\circ}\text{C min}^{-1}$, and the samples were annealed at -5°C for 40 minutes. Five experimental runs were conducted for each sample. Subsequently, the sizes of the 30 largest grains were measured. Among these 150 data, 100 corresponding to the largest grains were chosen to calculate the mean size.

2.9 Cryo-confocal in situ Observation of Eosin Y Release into the QLL During Ice Recrystallization.

To visualize the recrystallization-driven release of eosin Y into the quasi-liquid layer (QLL), experiments were conducted on the same Zeiss LSM confocal microscope equipped with an Instec cryo-stage, using the identical temperature program described in Section 2.8. Briefly, a 10 μL droplet of the test solution containing eosin Y ($\approx 10 \mu\text{g}$

mL⁻¹) was splat-cooled onto a cover glass pre-cooled to -60 °C to form a thin ice film. Immediately after ice formation, a 10 µL droplet of ethyl acetate was gently placed on top of the ice film to serve as an external liquid phase for collecting dye released from the ice-QLL interface. The temperature was then increased to -5 °C at 5 °C min⁻¹ and annealed at -5 °C for 40 min. Confocal imaging was used to monitor the emergence and accumulation of eosin Y fluorescence in the ethyl acetate droplet adjacent to the ice surface over time, which indicates the release of eosin Y from ice channels to the QLL and into the contacting ethyl acetate phase. Five independent runs were performed for each sample under identical conditions.

2.10 UV-vis Quantification of PPD Released into the QLL During Ice Recrystallization.

To quantify PPD released from ice spheres into the QLL during ice recrystallization, ice spheres were prepared from an aqueous PPD solution (100 mM) and transferred into pre-cooled ethyl acetate maintained at -5 °C, which served as an external collecting phase. During recrystallization/annealing, PPD transported through intercrystalline pathways to the QLL interface and subsequently partitioned into the surrounding ethyl acetate. At designated time points, the ethyl acetate phase was collected and analyzed by UV-vis spectroscopy using a UV-vis spectrophotometer (Shimadzu UV-2600) with 1 cm quartz cuvettes. Absorbance spectra were recorded over an appropriate wavelength range, and PPD was quantified using its characteristic absorption band at ~320 nm. A calibration curve was constructed from PPD standards prepared in ethyl acetate at known concentrations and measured under identical settings. The PPD concentration in the collected ethyl acetate samples was determined from the calibration curve with dilution correction when applicable. Each condition was measured in three independent replicates.

2.11 Thickness Measurement of PA Membranes.

The thickness of the PA membrane was measured by atomic force microscopy (AFM). PA microcapsules were mechanically disrupted at high speed to generate membrane fragments. The fragments were then deposited onto a clean silicon substrate and dried prior to measurement. AFM imaging was carried out on a Bruker Dimension Icon system in tapping mode using standard silicon probes. Membrane thickness was determined from height profiles across the edge of isolated fragments by measuring the step height between the Si surface and the membrane fragment. Multiple fragments from each sample were analyzed to obtain representative thickness values.

2.12 Thermogravimetric Analysis (TGA) of Enzyme-loaded PA Microcapsules.

Thermogravimetric analysis (TGA) was performed using a Mettler Toledo thermogravimetric analyzer to independently evaluate the enzyme content in PA microcapsules prepared from precursor solutions containing different enzyme concentrations. Dried capsule samples were heated from 25 to 1000 °C at a rate of 10 °C

min⁻¹ under a nitrogen atmosphere. Blank PA microcapsules prepared without enzyme were measured under identical conditions as the baseline control. Enzyme content was estimated from the additional mass-loss contribution of enzyme-loaded PA microcapsules relative to blank PA microcapsules over the corresponding temperature window. Each condition was measured using independently prepared samples.

2.13 Molecular-probe Permeability Assay.

To determine the diffusion kinetics of molecular probes across the PA membrane, preformed PA microcapsules were thoroughly washed and dispersed in PBS buffer (100 mM, pH 8.0) containing either rhodamine B or rhodamine-labelled dextran probes with defined molecular weights of 4, 10, 40 and 100 kDa. Probe transport into the aqueous core of the microcapsules was recorded over time by confocal laser scanning microscopy (CLSM) under identical imaging settings. For each time point, the fluorescence intensity inside the capsule lumen was quantified from regions of interest drawn within individual microcapsules after background subtraction. The internal fluorescence intensity was normalized to the equilibrium fluorescence intensity obtained after diffusion saturation. The half-diffusion time, $t_{1/2}$, was defined as the time required for the normalized internal fluorescence intensity to reach 0.5. Multiple microcapsules from at least three independently prepared batches were analyzed for each condition.

2.14 Diffraction Characterization

X-ray diffraction (XRD) patterns were collected on a Rigaku Ultima IV diffractometer using Cu K α radiation ($\lambda = 1.54059 \text{ \AA}$) at 40 kV and 15 mA. Data were recorded over $2\theta = 10\text{-}60^\circ$ with a scan rate of 5° min^{-1} .

2.15 Photobiocatalytic Conversion of Acetaldehyde into Ethanol

The photobiocatalytic conversion of acetaldehyde was performed in a glass reactor at 25 °C under irradiation with a $400 \pm 5 \text{ nm}$ LED light source, with light delivered into the reaction solution through a quartz light guide. In this section, ADH@PA refers to ADH-loaded PA microcapsules in which CdS quantum dots were co-encapsulated within the same aqueous compartment during microcapsule preparation, unless otherwise specified. The reaction mixture (3 mL total volume) contained 100 mM acetaldehyde, 1 mM NAD⁺, 0.5 mM [M], 400 mM TEOA, 10 mg mL⁻¹ ADH@PA microcapsules, and PBS buffer (100 mM, pH 8.0). At 0, 6, 15, 25, 60, 90, 120, 180, 240, 360, and 480 min, 100 μL aliquots were withdrawn and mixed with 400 μL deuterium oxide for NMR analysis. Ethanol was quantified by ¹H NMR (Bruker ARX) by referencing the ethanol signal to DSS at a known concentration.

Ethanol quantification by ¹H NMR. Ethanol was quantified using DSS as an internal standard. For each measurement, 100 μL of reaction aliquot was mixed with 400 μL of DSS-containing D₂O, giving a final DSS concentration of 2.5 mM in the NMR sample.

The methyl proton signal of ethanol at approximately 1.17 ppm was integrated against the trimethylsilyl proton signal of DSS at 0 ppm. The DSS signal corresponds to 9 protons, whereas the ethanol methyl signal corresponds to 3 protons. Therefore, the ethanol concentration in the NMR sample was calculated according to:

$$C_{\text{EtOH}} = \frac{I_{\text{EtOH}}}{I_{\text{DSS}}} \times \frac{9}{3} \times C_{\text{DSS}} \times \frac{V_{\text{total}}}{V_{\text{aliquot}}}$$

where I_{EtOH} and I_{DSS} are the integrated peak areas of ethanol and DSS, respectively, C_{DSS} is the final DSS concentration in the NMR sample, V_{total} is the total NMR sample volume and V_{aliquot} is the volume of the reaction aliquot. In this assay, $V_{\text{total}}/V_{\text{aliquot}} = 500/100 = 5$.

2.16 Photobiocatalytic Conversion of CO₂ into Methanol

The photobiocatalytic conversion of CO₂ into methanol was performed in a glass reactor at 25 °C under irradiation with a 400 ± 5 nm LED light source, with light delivered into the reaction solution through a quartz light guide. In this section, (FDH/FalddH/ADH)@PA refers to PA microcapsules in which CdS quantum dots were co-encapsulated with FDH, FalddH and ADH within the same aqueous compartment during microcapsule preparation, unless otherwise specified. The reaction mixture contained 1 mM NAD⁺, 0.5 mM rhodium electron mediator [M], 400 mM TEOA, 10 mg mL⁻¹ (FDH/FalddH/ADH)@PA microcapsules, and PBS buffer (100 mM, pH 8.0), with a total volume of 3 mL. Prior to illumination, CO₂ was bubbled through the reaction solution for 1 h to reach CO₂ saturation. At reaction times of 0, 1, 4, 8, and 24 h, 100 µL aliquots were withdrawn and immediately diluted with deionized water to a final volume of 800 µL in headspace vials for gas chromatography analysis.

Methanol production was quantified by headspace gas chromatography using a Trace 1300 GC system (Thermo Scientific) equipped with a flame ionization detector. Separation was performed on a TR-WAX capillary column with dimensions of 30 m × 0.32 mm × 0.50 µm. Before injection, samples were equilibrated under headspace conditions at 80 °C for 30 min. The injector temperature was set to 230 °C, and the detector temperature was set to 250 °C. The oven program was as follows: 50 °C for 1 min, followed by heating to 100 °C at 10 °C min⁻¹ and then to 200 °C at 40 °C min⁻¹. The flame ionization detector gas flows were set to 350 mL min⁻¹ for air and 35 mL min⁻¹ for hydrogen. Methanol concentrations were determined using an external calibration curve prepared from methanol standards and analyzed under the same headspace gas chromatography conditions.

2.17 Protein quantification and enzyme loading determination by Bradford assay

The enzyme loading of the microcapsules was quantified using a Bradford assay. A calibration curve was first established by measuring the absorbance at 590 nm for a series of enzyme standards with known concentrations after reaction with Bradford reagent. For capsule samples, 10 mg of enzyme-loaded microcapsules were collected and mechanically disrupted to release the encapsulated protein. The resulting

suspension was clarified, and an aliquot of the supernatant was mixed with Bradford reagent. The absorbance at 590 nm was recorded using a UV–vis spectrophotometer, and the enzyme concentration was determined from the standard curve. The enzyme loading was then calculated as the mass of enzyme quantified per mass of microcapsules.

2.18 Specific activity calculation for encapsulated ADH

The specific activity of encapsulated ADH was quantified from the initial kinetics of ethanol formation in the light-driven acetaldehyde reduction assay. The ethanol production rate (r_{EtOH}) was extracted from the linear regime of the concentration–time profile. The total mass of ADH in each reaction (m_{ADH}) was calculated from the measured enzyme loading of the capsules together with the capsule dose used in the assay.

Specific activity was reported as:

$$\text{Specific activity (U mg}^{-1}\text{)} = \frac{r_{\text{EtOH}}}{m_{\text{ADH}}}$$

where r_{EtOH} is expressed in $\mu\text{mol min}^{-1}$ (1 U = 1 $\mu\text{mol min}^{-1}$), and m_{ADH} is the total mass of ADH (mg) present in the reaction mixture.

2.19 Durability tests of ADH@PA microcapsules.

To evaluate the robustness of ADH@PA microcapsules, catalytic performance was assessed under graded thermal, pH, proteolytic, and organic-solvent stresses, using ethanol production as the indicator of residual ADH activity. For thermal stability, microcapsules were incubated at 40, 50, 60, 70, or 80 °C for 10 min and then cooled to room temperature. For pH stability, microcapsules were exposed to buffer solutions adjusted to pH 3, 4, 6, 9, or 11 for 30 min. For protease stability, microcapsules were incubated with proteinase K (5 mg mL⁻¹) for 60 min. For solvent stability, microcapsules were incubated for 30 min in 50% (v/v) solutions of acetonitrile, tetrahydrofuran, N,N-dimethylformamide, or dimethyl sulfoxide (each prepared by mixing the organic solvent with aqueous buffer). After each treatment, microcapsules were collected by gentle centrifugation, washed with PBS to remove residual acid, protease, or solvent, and then re-dispersed in PBS (100 mM, pH 8.0). Residual ADH activity was quantified by measuring ethanol concentration produced under standardized reaction conditions, and normalized to the untreated control to obtain the relative activity.

2.20 Catalytic recycling test of ADH@PA microcapsules.

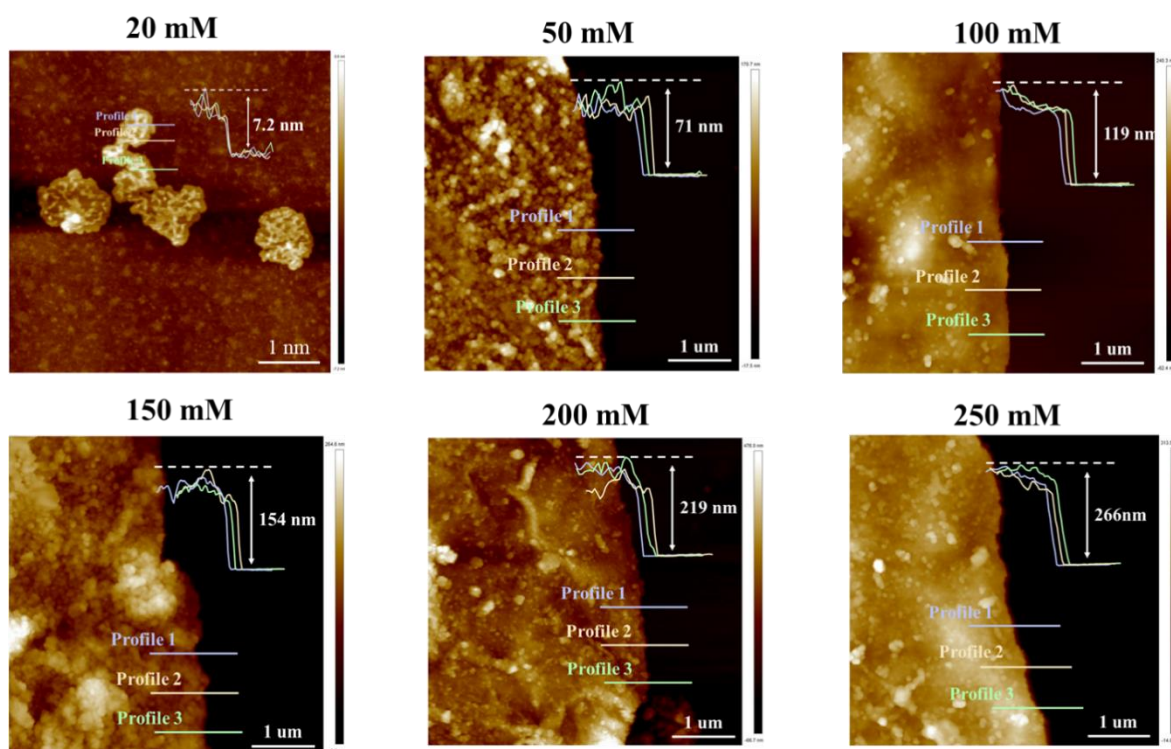
After each catalytic cycle, the reaction mixture was returned to room temperature, and the microcapsules were collected by centrifugation at $1,130 \times g$ for 5 min. After centrifugation, the supernatant was carefully removed along the wall of the centrifuge tube using a pipette, while a thin layer of residual liquid was left above the settled

microcapsules to avoid disturbing the pellet. PBS buffer (0.1 M, pH 8.0) was then added, and the microcapsules were gently redispersed by inverting the centrifuge tube, while vortexing was avoided. After the microcapsules settled, the upper liquid was carefully removed. This washing procedure was repeated five times to remove residual substrates and products. Finally, the washed microcapsules were redispersed in fresh PBS buffer (0.1 M, pH 8.0) for the next catalytic cycle.

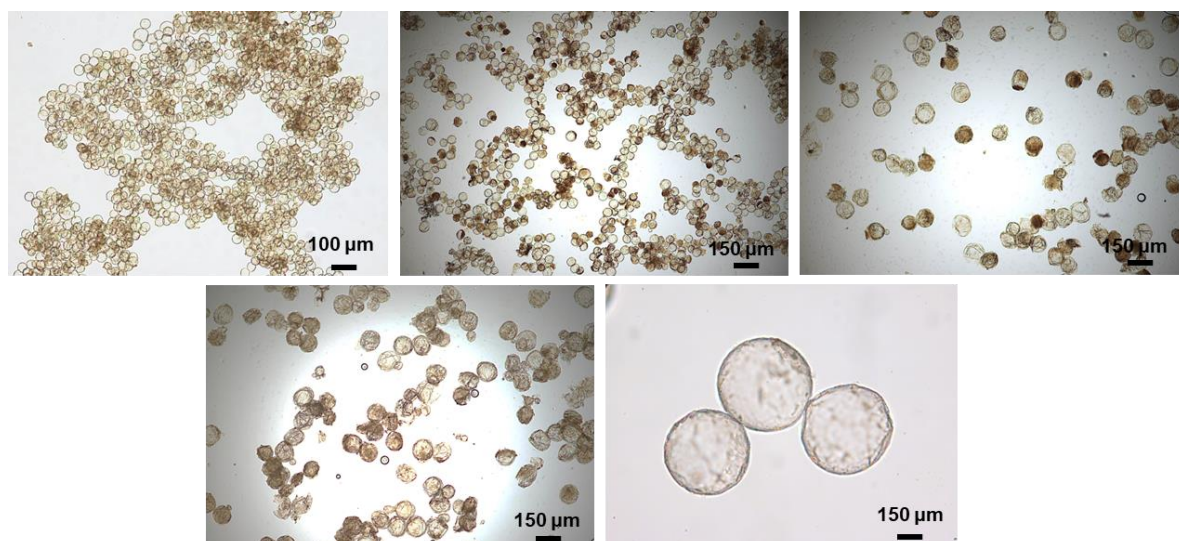
2.21 Molecular Dynamics Simulations

Molecular dynamics (MD) simulations were carried out with the OPLS-AA force field[1] using the GROMACS software package.[2] All molecular structures were first optimized using the ORCA program.[3] The 1.14*CM1A-LBCC atomic charges were generated with LiParGen.[4] During the simulations, the system temperature was maintained using the velocity-rescale thermostat,[5] while the pressure was controlled with the Parrinello-Rahman barostat.[6,7] A cutoff radius of 8.5 Å was applied for both van der Waals and Coulombic nonbonded interactions, and long-range electrostatics were handled using the particle-mesh Ewald (PME) method.[8] All hydrogen bonds were constrained with the LINCS algorithm.[9] The integration time step was 2 fs, and the neighbor list was updated every ten steps. Periodic boundary conditions were applied in all three directions. Initially, a block of ice Ih ($3.9 \times 3.7 \times 1.8 \text{ nm}^3$) was generated using the Genice program.[10] The ice block was equilibrated under the NPT ensemble at 268 K and 1 atm for 5 ns. The TIP4P/Ice force field was employed to describe water molecules.[11] In subsequent simulations, all oxygen atoms in the ice block were restrained to their equilibrium positions using soft harmonic springs. The systems contained 10, 20 and 30 TMC (or PPD) molecules and 300 ethyl acetate molecules, which were added into the simulation box of $3.9 \times 3.7 \times 12 \text{ nm}^3$. Systems containing 10 (0.3 mmol/mL), 20 (0.6 mmol/mL), or 30 (0.9 mmol/mL) PPD molecules were prepared in the simulation box. For systems containing 10, 20, or 30 PPD molecules, a 5 ns NVT simulation at 268 K was first performed, followed by a 500 ns NPT production run at 268 K and 1 atm. We performed simulations at five temperatures: 245 K, 250 K, 260 K, 268 K, and 270 K. The first 50 ns of each production trajectory was discarded as equilibration, and the remaining 50–500 ns interval was used for mass-density calculations. All visualization and image processing were carried out using VMD software.[12]

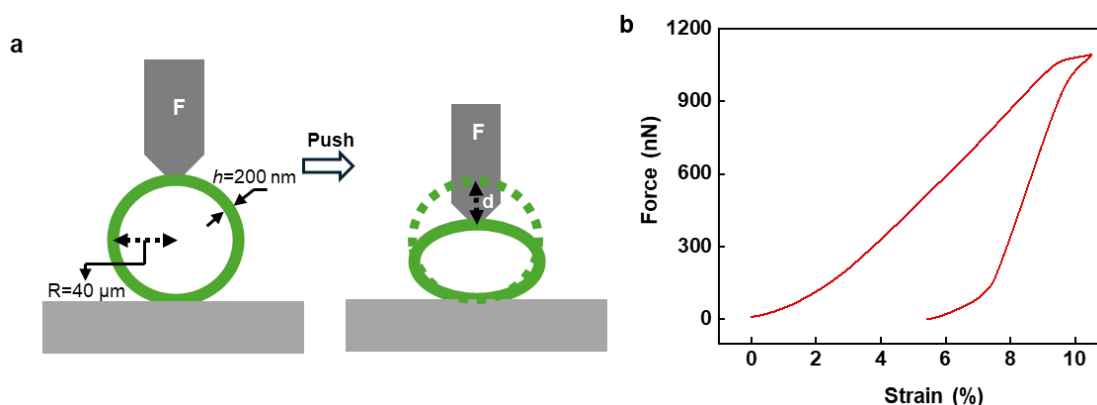
3. Supplementary Figures



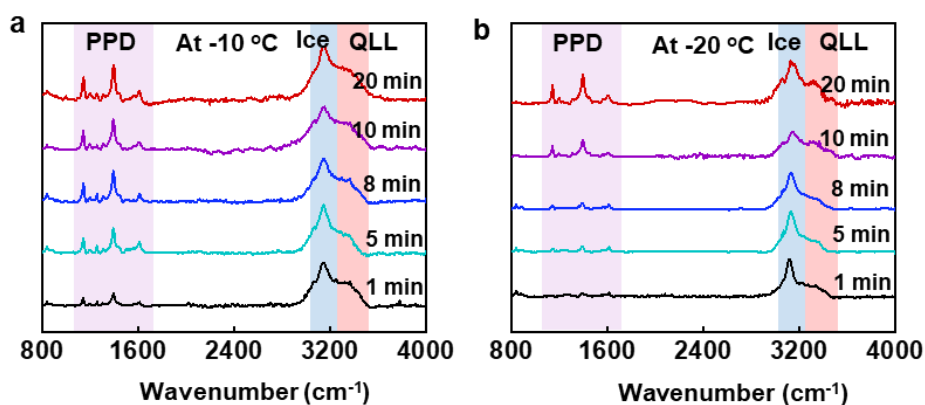
Supplementary Fig. 1 | AFM height image for thickness measurement of PA capsule membranes.



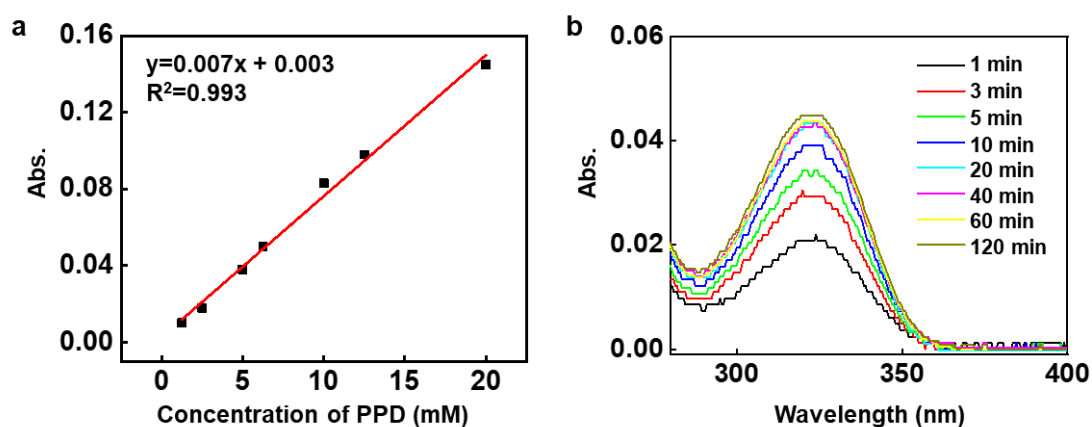
Supplementary Fig. 2 | Optical microscopy images of PA microcapsules with different sizes.



Supplementary Fig. 3 | (a) Schematic illustration of the nanoindentation test used to compress an individual dried PA microcapsule. (b) Representative force–displacement curve obtained during microcapsule compression.



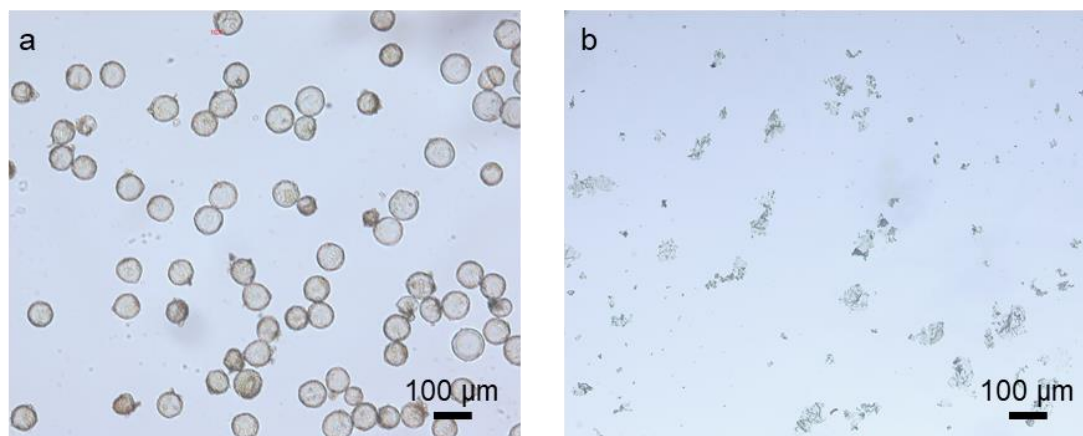
Supplementary Fig. 4 | Time-dependent Raman spectra showing PPD enrichment in the QLL during recrystallization at (a) -10°C and (b) -20°C .



Supplementary Fig. 5 | UV–vis calibration and time-resolved spectra for PPD release during ice recrystallization at -5°C . (a) Standard curve of PPD in ethyl acetate obtained

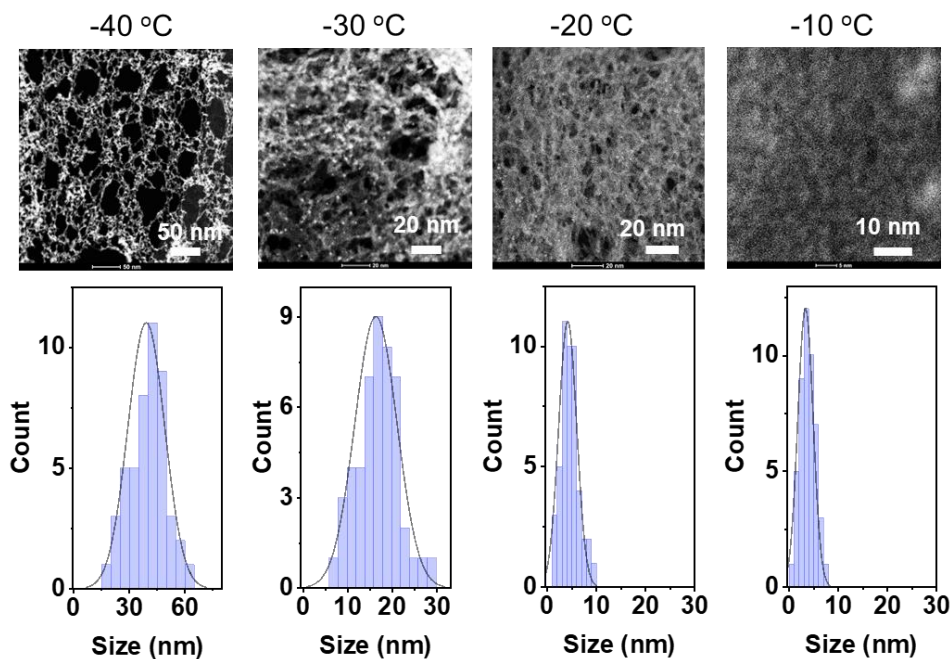
from UV–vis absorbance (linear fit: $y = 0.007x + 0.003$, $R^2 = 0.993$), where x is the PPD concentration (mM) and y is the absorbance. (b) Time-dependent UV–vis spectra of the ethyl acetate phase collected during recrystallization at $-5\text{ }^{\circ}\text{C}$.

To distinguish sustained internal replenishment from residual surface-accessible PPD, we performed two control experiments. PPD-loaded ice spheres still formed intact PA microcapsules after washing with pre-cooled ethyl acetate to remove surface-accessible PPD (Fig. S6a). In contrast, blank ice spheres briefly exposed to PPD-containing ethyl acetate produced only fragmented PA deposits after transferring into TMC-containing ethyl acetate and did not form closed microcapsules (Fig. S6b). These controls show that transient surface-accessible PPD is insufficient to sustain conformal shell growth. Therefore, closed microcapsule formation requires a continuously replenished internal PPD reservoir delivered during ice recrystallization.



Supplementary Fig. 6 | Surface-control experiments for PPD supply. (a) PA microcapsules formed after surface depletion of PPD-loaded ice spheres. (b) Fragmented PA deposits formed from blank ice spheres after brief exposure to external PPD.

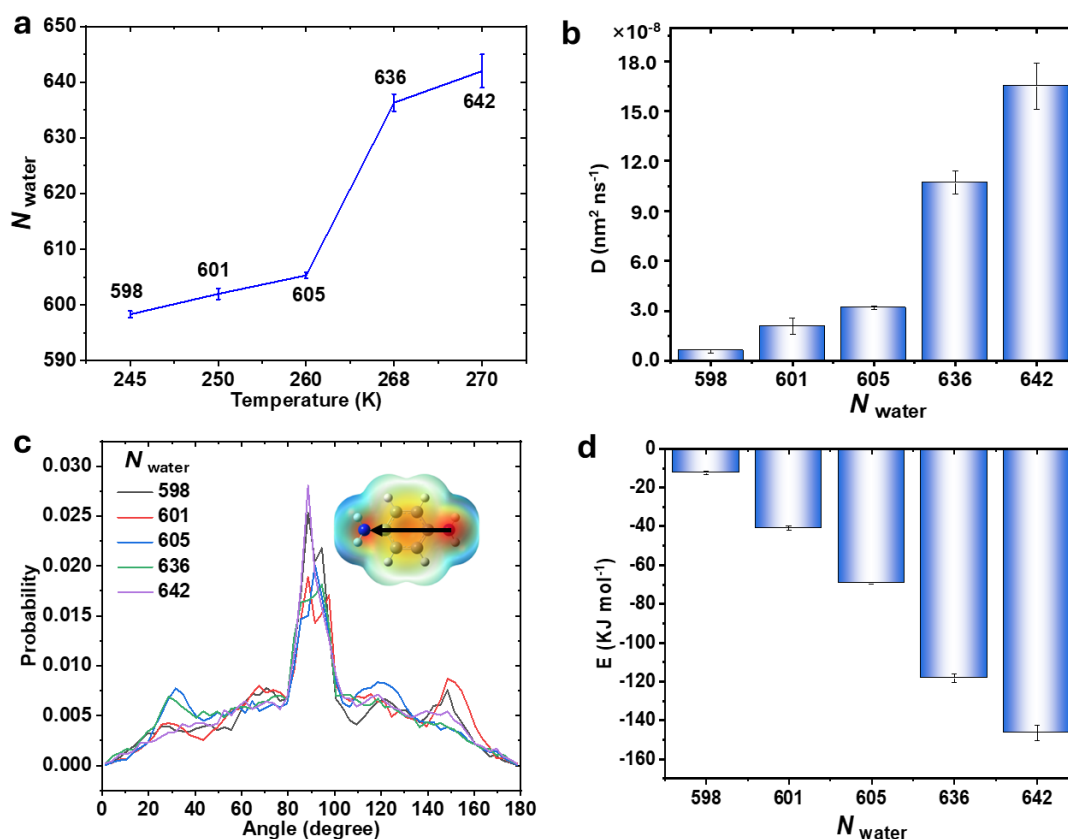
To examine how the subzero supply mode affects nanoscale PA network formation, polyamidation within a nanometric interfacial water layer can support continuous shell growth, whereas limited local monomer availability is expected to reduce network densification. Consistent with this interpretation, HR-STEM showed larger apparent nanoscale opening-like features under conditions with suppressed interfacial transport and slower PPD delivery (Fig. S7).



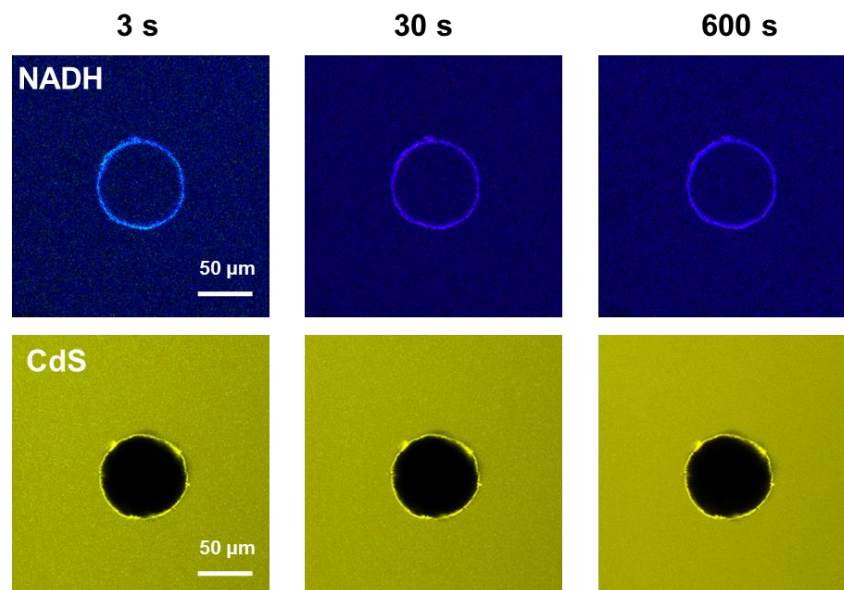
Supplementary Fig. 7 | HR-STEM images and apparent opening-size statistics of PA membranes prepared at different subzero temperatures.

As shown in Fig. S8a, increasing the number of QLL-associated water molecules expands the interfacial water environment between the ice and EA phases. These results are consistent with the previously reported trends in the variation of water molecules within the QLL.[13-15] This expanded interfacial region provides a more accessible pathway for PPD redistribution from the ice-side region toward the QLL–EA boundary. This result supports the view that the QLL-associated interfacial region acts as a transport-regulating zone that positions PPD at the reaction boundary. Consistently, the PPD diffusion coefficient also increases with the number of QLL-associated water molecules (Fig. S8b). We further analyzed PPD interfacial orientation and PPD–TMC interaction energy. The orientation analysis shows that PPD preferentially adopts an interfacial arrangement with its molecular long axis mainly parallel to the interface (Fig. S8c), supporting an organized monomer arrangement at the QLL–EA boundary. The PPD–TMC interaction energy becomes more favorable as the number of QLL-associated water molecules increases, indicating enhanced monomer contact near the reaction interface (Fig. S8d). Together, these analyses connect interfacial positioning with productive PPD-TMC contact before covalent polyamidation. Therefore, the increased PPD transfer in Fig. 3f can be assigned to a QLL-mediated interfacial transport process involving three coupled molecular events: enhanced PPD mobility, increased PPD accumulation in the EA-side interfacial region and strengthened PPD-TMC contact near the QLL-EA boundary. This configuration supports efficient delivery

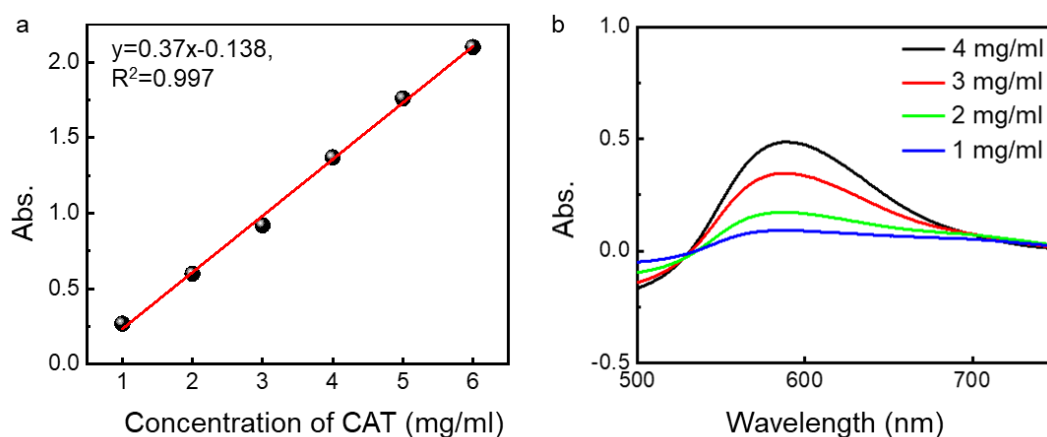
of PPD to the reaction boundary before interfacial polyamidation.



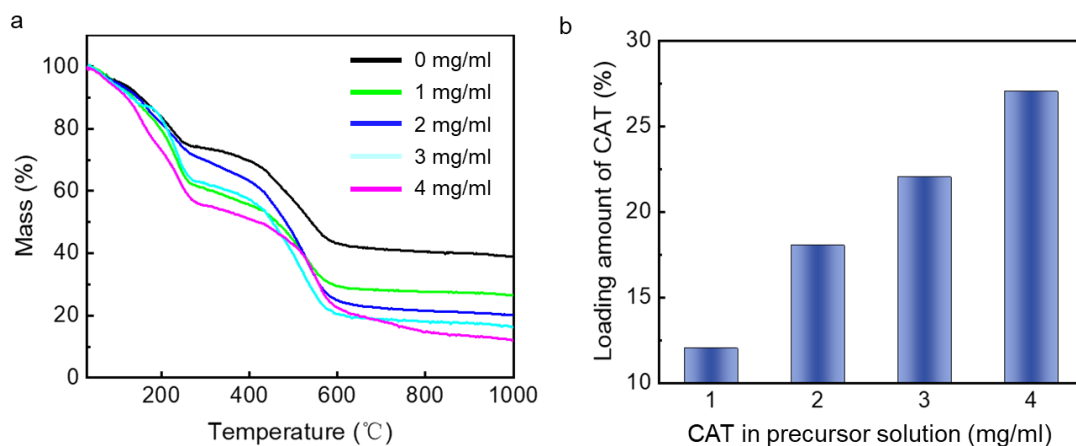
Supplementary Fig. 8 | Molecular dynamics analyses of the QLL-associated interfacial environment, PPD mobility, PPD orientation and PPD-TMC contact. (a) Variation in the number of QLL-associated water molecules across the simulated interfacial states. (b) Variation in the PPD diffusion coefficient (D) with the number of QLL-associated water molecules. (c) Orientation distribution of PPD molecules under different numbers of QLL-associated water molecules, defined by the angle between the molecular long axis of PPD and the z-axis. (d) PPD-TMC interaction energy as a function of the number of QLL-associated water molecules. The interaction energy includes van der Waals and electrostatic contributions.



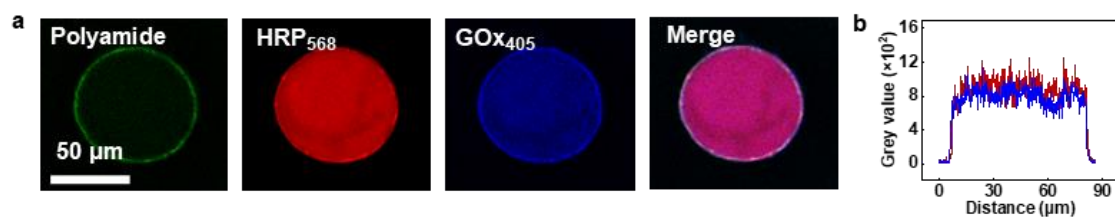
Supplementary Fig. 9 | Transport and retention assays show that the PA membrane allows exchange of NADH-related small molecules while retaining nanoscale photoredox components such as CdS quantum dots.



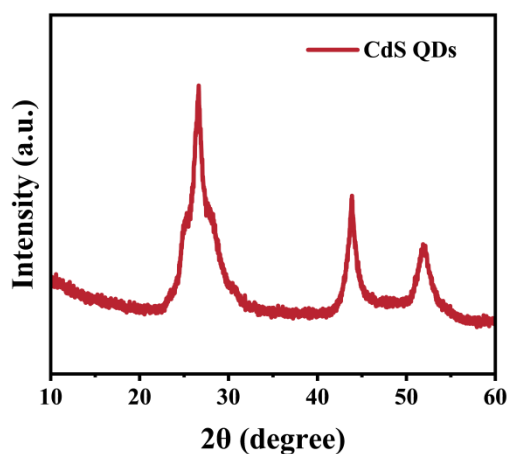
Supplementary Fig. 10 | UV-vis quantification of encapsulated enzyme using the Bradford assay. (a) Bradford calibration curve established from the absorbance of enzyme standards and fitted with a linear regression for concentration determination. (b) Representative UV-vis spectra of Bradford-developed samples obtained from PA microcapsules prepared with different enzyme loadings in the precursor solution.



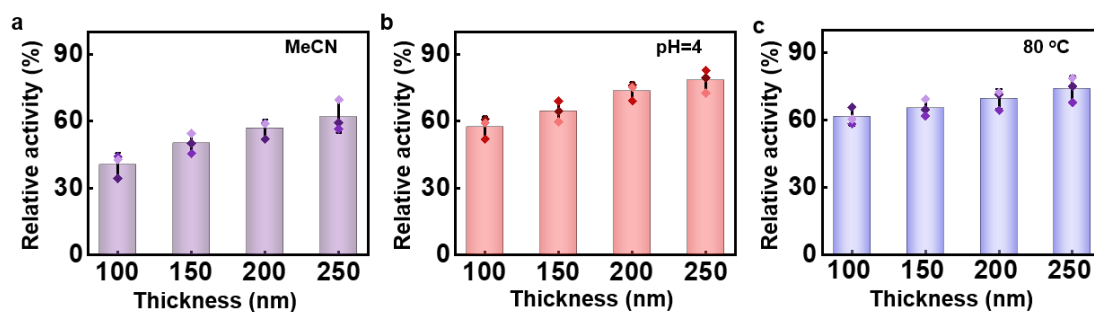
Supplementary Fig. 11 | (a) Thermogravimetric analysis (TGA) curves of PA microcapsules fabricated from precursor solutions containing different enzyme concentrations. (b) Enzyme content in the resulting microcapsules quantified from the corresponding TGA data.



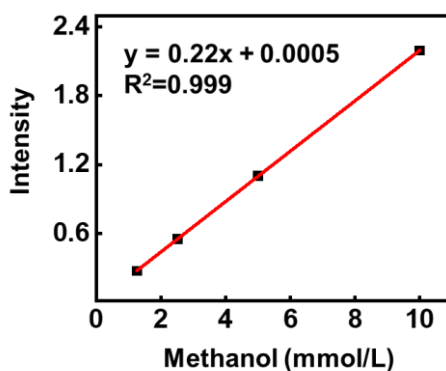
Supplementary Fig. 12 | (a) CLSM image of PA microcapsules co-encapsulating HRP₅₆₈ and GOx₄₀₅ at an HRP₅₆₈:GOx₄₀₅ ratio of 1:1 and (b) corresponding fluorescence intensity analysis.



Supplementary Fig. 13 | XRD patterns of CdS QDs.



Supplementary Fig. 14 | Membrane-thickness-dependent enzyme protection under (a) acetonitrile exposure, (b) acidic treatment and (c) thermal treatment. In panels a–c, data are presented as mean $\pm$ s.d. from $n = 3$ independent biological replicates. Each biological replicate corresponds to an independently prepared batch of PA microcapsules.



Supplementary Fig. 15 | Calibration curve for methanol quantification in the CO₂-to-methanol cascade reaction.

Tables S1–S15 list representative ^1H NMR integration datasets. The relative activity was calculated by normalizing the ethanol concentration after each treatment to that of the untreated ADH@PA control measured under identical catalytic conditions. The values plotted in Fig. 5b-k are mean values obtained from independent measurements.

Supplementary Table. 1 | ^1H NMR quantification of ethanol production from 100 μm , 250 nm-thick ADH@PA microcapsules using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	8.02	1.00	2.50	4.68
0.25	5.23	1.00	2.50	7.17
0.4	4.41	1.00	2.50	8.50
1	2.88	1.00	2.50	13.02
1.5	2.23	1.00	2.50	16.82
2	1.69	1.00	2.50	22.19
3	1.51	1.00	2.50	24.83
4	1.43	1.00	2.50	26.22
6	1.37	1.00	2.50	27.37
8	1.31	1.00	2.50	28.63

Supplementary Table. 2 | ^1H NMR quantification of ethanol production from 100 μm , 200 nm-thick ADH@PA microcapsules using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	10.70	1.00	2.50	3.50
0.25	4.77	1.00	2.50	7.86
0.4	4.35	1.00	2.50	8.62
1	3.13	1.00	2.50	11.98
1.5	2.92	1.00	2.50	12.84
2	2.53	1.00	2.50	14.82
3	2.51	1.00	2.50	14.94
4	2.47	1.00	2.50	15.18
6	2.13	1.00	2.50	17.61
8	2.08	1.00	2.50	18.03

Supplementary Table. 3 | ^1H NMR quantification of ethanol production from 100 μm , 150 nm-thick ADH@PA microcapsules using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	7.12	1.00	2.50	5.27
0.25	5.84	1.00	2.50	6.42

0.4	4.82	1.00	2.50	7.78
1	4.63	1.00	2.50	8.10
1.5	4.51	1.00	2.50	8.31
2	4.16	1.00	2.50	9.01
3	3.68	1.00	2.50	10.19
4	3.15	1.00	2.50	11.90
6	2.69	1.00	2.50	13.94
8	2.38	1.00	2.50	15.76

Supplementary Table. 4 | ^1H NMR quantification of ethanol production from 100 μm , 100 nm-thick ADH@PA microcapsules using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	23.57	1.00	2.50	1.59
0.25	15.43	1.00	2.50	2.43
0.4	9.72	1.00	2.50	3.86
1	8.88	1.00	2.50	4.22
1.5	7.15	1.00	2.50	5.24
2	5.70	1.00	2.50	6.58
3	4.90	1.00	2.50	7.65
4	4.25	1.00	2.50	8.82
6	4.08	1.00	2.50	9.19
8	3.89	1.00	2.50	9.64

Supplementary Table. 5 | ^1H NMR quantification of ethanol production from non-confined CdS/ADH system using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	32.43	1.00	2.50	1.16
0.25	21.30	1.00	2.50	1.76
0.4	15.57	1.00	2.50	2.41
1	11.24	1.00	2.50	3.34
1.5	10.11	1.00	2.50	3.71
2	9.52	1.00	2.50	3.94
3	9.19	1.00	2.50	4.08
4	8.74	1.00	2.50	4.29
6	8.34	1.00	2.50	4.50
8	8.25	1.00	2.50	4.55

Supplementary Table. 6 | ^1H NMR quantification of ethanol production from 30 μm , 250 nm-thick ADH@PA microcapsules using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	5.70	1.00	2.50	6.58
0.25	3.81	1.00	2.50	9.84
0.4	2.98	1.00	2.50	12.58
1	1.78	1.00	2.50	21.07
1.5	1.20	1.00	2.50	31.25
2	0.95	1.00	2.50	39.47
3	0.76	1.00	2.50	49.34
4	0.61	1.00	2.50	61.48
6	0.56	1.00	2.50	66.96
8	0.54	1.00	2.50	69.44

Supplementary Table. 7 | ¹H NMR quantification of ethanol production from 200 μm, 250 nm-thick ADH@PA microcapsules using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	17.99	1.00	2.50	2.08
0.25	6.12	1.00	2.50	6.13
0.4	4.95	1.00	2.50	7.58
1	3.39	1.00	2.50	11.06
1.5	3.29	1.00	2.50	11.40
2	2.92	1.00	2.50	12.84
3	2.53	1.00	2.50	14.82
4	2.36	1.00	2.50	15.89
6	2.13	1.00	2.50	17.61
8	2.00	1.00	2.50	18.75

Supplementary Table. 8 | ¹H NMR quantification of ethanol production from 500 μm, 250 nm-thick ADH@PA microcapsules using DSS as an internal standard.

Time (h)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
0	1.00	0.00	2.50	0.00
0.1	22.08	1.00	2.50	1.70
0.25	13.07	1.00	2.50	2.87
0.4	10.86	1.00	2.50	3.45
1	7.88	1.00	2.50	4.76
1.5	7.01	1.00	2.50	5.35
2	6.58	1.00	2.50	5.70
3	5.53	1.00	2.50	6.78
4	4.92	1.00	2.50	7.62
6	4.55	1.00	2.50	8.24
8	4.17	1.00	2.50	8.99

Supplementary Table. 9 | Residual catalytic activity of ADH@PA microcapsules after treatment in buffer solutions with different pH values.

pH	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
3	1.08	1.00	2.50	34.72
4	0.68	1.00	2.50	55.15
6	0.60	1.00	2.50	62.50
9	0.66	1.00	2.50	56.82
11	0.71	1.00	2.50	52.82

Supplementary Table. 10 | Residual catalytic activity of non-confined CdS/ADH system after treatment in buffer solutions with different pH values.

pH	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
3	2.56	1.00	0.16	0.96
4	1.61	1.00	0.16	1.53
6	0.86	1.00	0.16	2.86
9	1.05	1.00	0.16	2.34
11	1.51	1.00	0.16	1.63

Supplementary Table. 11 | Residual catalytic activity of ADH@PA microcapsules after treatment at different temperatures.

Temperature (°C)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
40	0.60	1.00	2.50	62.50
50	0.61	1.00	2.50	61.48
60	0.66	1.00	2.50	56.82
70	0.69	1.00	2.50	54.35
80	0.72	1.00	2.50	52.08

Supplementary Table. 12 | Residual catalytic activity of non-confined CdS/ADH system after treatment at different temperatures.

Temperature (°C)	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
40	0.68	1.00	0.16	3.61
50	0.92	1.00	0.16	2.67
60	1.15	1.00	0.16	2.14
70	1.65	1.00	0.16	1.49
80	1.82	1.00	0.16	1.35

Supplementary Table. 13 | Residual catalytic activity of ADH@PA microcapsules after treatment with different organic solvents and proteinase K.

Reagent	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
MeCN	0.91	1.00	2.50	41.21
THF	0.72	1.00	2.50	52.08
DMF	0.66	1.00	2.50	56.82

DMSO	0.63	1.00	2.50	59.52
PK	0.57	1.00	2.50	65.79

Supplementary Table. 14 | Residual catalytic activity of ADH after treatment with different organic solvents and proteinase K.

Reagent	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
MeCN	5.13	1.00	0.16	0.48
THF	2.48	1.00	0.16	0.99
DMF	1.25	1.00	0.16	1.97
DMSO	0.71	1.00	0.16	3.46
PK	9.79	1.00	0.16	0.25

Supplementary Table. 15 | Recycling performance of ADH@PA microcapsules over repeated catalytic cycles.

Cycle	DSS (Integration)	Ethanol (Integration)	DSS (mM)	Ethanol (mM)
1	0.56	1.00	2.50	66.96
2	0.57	1.00	2.50	65.79
3	0.59	1.00	2.50	63.56
4	0.60	1.00	2.50	62.50
5	0.61	1.00	2.50	61.48
6	0.62	1.00	2.50	60.48
7	0.63	1.00	2.50	59.52
8	0.63	1.00	2.50	59.52
9	0.63	1.00	2.50	59.52
10	0.64	1.00	2.50	58.59

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