Fabrication of polypyrrole nano-arrays in lysozyme single crystals†

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Supplementary Information

Experimental methods

Experimental

Water with a resistivity of 18.2 Ω cm^{-1} was used in all experiments, and all chemicals were purchased from Sigma-Aldrich. Hen egg white lysozyme 0.2 g (20 mg mL^{-1}) was added with rapid stirring to 10 mL of a crystallization solution of NaCl (60 mg mL^{-1}) and NaOAc buffer (8.2 mg mL^{-1}) maintained at pH 4 using 50% acetic acid. The crystallisation solution was then filtered using a 45 µm syringe filter into a glass vial containing 5-10 mL of dichloromethane (DCM), so that the solution was layered on top of the organic solvent. Crystals of lysozyme were produced by leaving the vial undisturbed for up to 48 hours at room temperature. The aqueous supernatant and DCM layer were then removed, and a 10 - 20 mL cross-linking solution (8.2 mg mL^{-1} NaOAc, 60mg mL^{-1} NaCl, pH 4) containing 1-5 % glutaraldehyde was added. The vial was left to stand for a 24-48 hours at room temperature, until the crystals had turned a pale yellow colour. The crystals were then removed and washed with water, and dried under vacuum for 3 hours.

Polypyrrole (Ppy) was synthesized within the solvent channels of the cross-linked crystals as follows. The crystals were initially soaked in aqueous 0.12 M ammonium persulfate (APS) for three days at room temperature until the colouring had changed from yellow to red. The oxidant-infiltrated crystals were then washed with distilled water, dried under vacuum for 3 hours, and subsequently placed in an unsealed glass petri-dish positioned inside a desiccator, which also contained an unsealed vial or glass petri-dish of pyrrole (approximately 10 mL.) The crystals were then exposed for 48 hours to the pyrrole vapour at room temperature. Alternatively, the crystals were placed in the dessicator, and a vacuum pump used to remove the initial build-up of pyrrole vapour; the pump was then switched off and the crystals left inside the dessicator with the pyrrole for 24 hours at room temperature. When the crystals had changed colour from red to black, they were removed from the desiccator, and washed in water to remove excess pyrrole. Surface material was removed from the polymer-infiltrated crystals by submerging them in 10-20 mL water placed inside sealed petri-dishes, and shaking at 300 rpm for up to 7 days at room temperature, with the water being changed as necessary.

For the synthesis of PPy films, a 0.12 M APS solution was prepared in a glass petri-dish, with a glass slide placed at the bottom. The petri-dish was then placed into a desiccator, which also

contained an unsealed vial or glass petri-dish of pyrrole (approximately 10 mL) The solution was left to stand in the desiccator at room temperature for up to two days and then removed. The glass slide was removed from the petri-dish, pulling off the PPy film with it. This was then washed with water, and dried under vacuum at room temperature for 1-3 hours.

Optical images were taken using a Cannon EOS 500D digital camera. Optical microscopy was carried out on a Carl Zeiss Stemi SV 11 optical microscope. TEM analysis was performed using a JEOL JEM 1200 EX microscope in bright field mode, and images were recorded using a Mega View II digital camera and Soft Imaging Systems GmbH analysis 3.0 analysis software. Samples for TEM were ground using a pestle and mortar, and a dilute suspension in water was pipetted onto carbon-coated copper grids, which were then dried over night. High resolution TEM analysis was undertaken on microtome sections of PPy-CLLCs cut from crystals embedded in an epoxy resin matrix, and then transferred onto carbon coated EM grids. SEM analysis was carried out using a JEOL 5600LV scanning electron microscope. Samples were fixed onto double-sided adhesive carbon discs on aluminium stubs, and coated with a 15 nm layer of gold using an Agar High Resolution Sputter Coater. UV-Vis spectra of conducting polymers were taken using a Perkin Elmer Lambda 25 UV/Vis spectrometer, and analysed using WinLab software. Samples for SEM were ground to a fine powder, and sonicated in water for 15 minutes to produce a suspension. UV-Vis spectra of crystal samples were taken using a Perkin Elmer Lambda 35 UV/vis spectrometer, and UV WinLab software was used for analysis. Fourier transform infrared spectroscopy was done using a Perkin Elmer Spectrum One. Samples were ground using a pestle and mortar with potassium bromide (KBr), and then pressed into discs, which were then used for analysis. For electrical conductivity studies at 295 K, the free-standing PPY films were processed into 4 x 4 mm^2 planar devices. The 4-terminal (Van der Pauw) measurements of the films were performed with Ag-contacts. Individual CLLC and PPy-CLLCs were measured in 2-terminal configuration with top and bottom Ag contacts. All graphs were drawn in Excel.

Force spectroscopy measurements were performed in contact mode using a Bruker Multimode atomic force microscope with a Veeco Multimode V controller fitted with a picoforce module and scanner running on Nanoscope version 7.2 software. Measurements were conducted in air or in water using a dedicated liquid cell. AlTap300 rectangular cantilevers with a 10 nm radius were used and tip force constants were determined using a thermal tune built into the controller. All force curves were acquired from inclined {101} surfaces under water, as the AFM tips were not able to deform the surfaces of dried samples. Analysis was performed using the Nanoscope software package. The numerical data were obtained by fitting the data from the cantilever approach to the Hertzian equation $f(x) = KR^{0.5}.x^{1.5}$, where $f(x)$ is the force (N) at a distance (x nm), R the diameter of the tip (nm) and K the force constant (N/nm^2). The latter is proportional to the increase in the gradient of the plots, and was used as a numerical value of the force required to deform the surface. The displacement distance at which the force reached zero was compared to the depth of the initial deformation, and used to calculate the depth of permanent deformation on the sample surfaces.

Supplementary Information

Figures

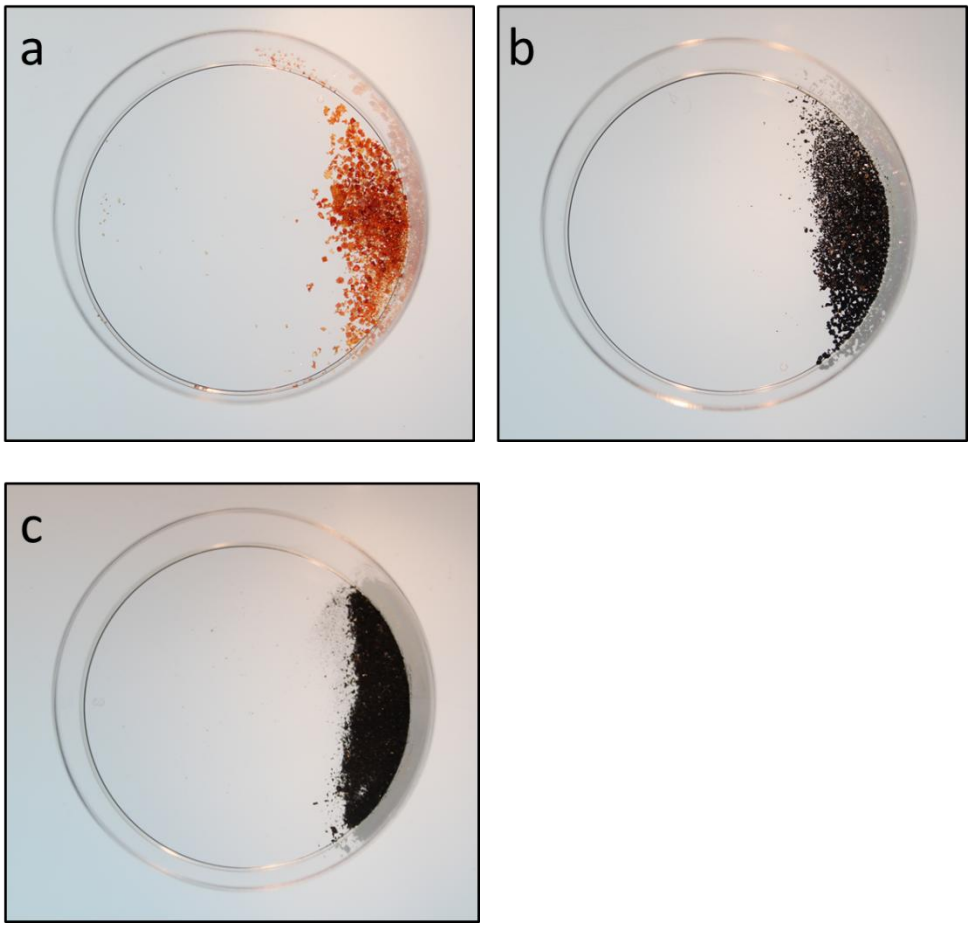

Figure S1 Optical photographs of bulk APS-CLLCs, (b) PPy-CLLC, and (c) control PPy powder prepared by exposure of APS solution to pyrrole vapour.

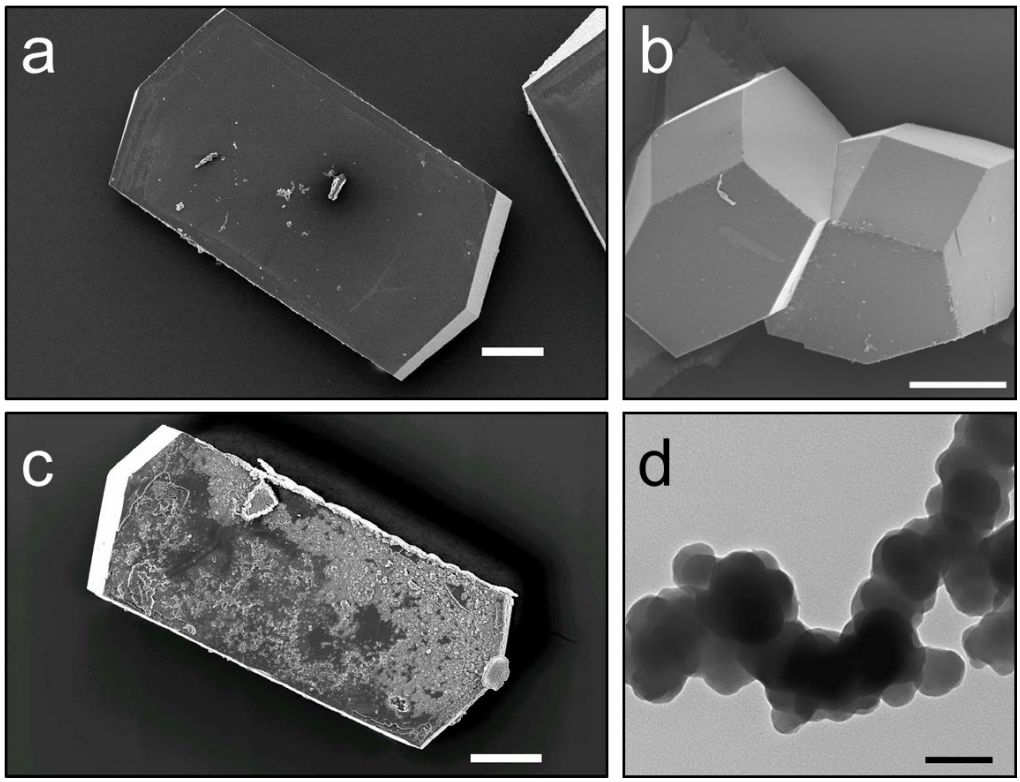

Figure S2 (a-c) SEM images of (a) single CLLC, (b) individual APS-loaded CLLCs, and (c) unwashed PPy-CLLC. Scale bars = 50 μm (d) TEM of washed particles of PPy prepared in the absence of CLLCs. Scale bar = 250 nm.

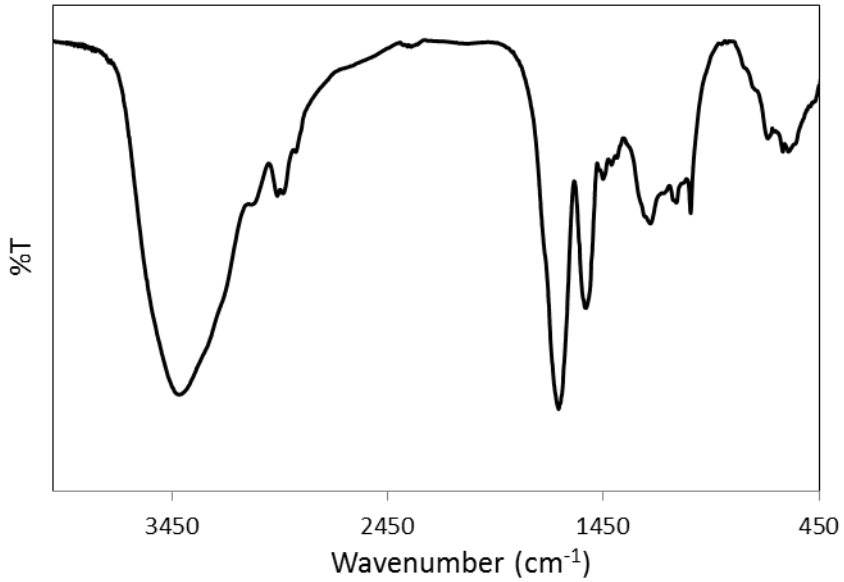

Figure S3 FT-IR spectrum of APS-CLLC. Strong absorbances at 1654 and 1535 cm^{-1} correspond to protein amide I and II bands. Peaks in the region 1350-450 cm^{-1} (mainly associated with intercalated APS) are assigned below.

Peak (cm^{-1})	**Origin**	**Assignment**
1220-1270	APS, Protein	SO_3, Amide III
1123	Protein	Side chains
1045	APS	S-O-O-S Shifted from 1055 cm^{-1}
754	Protein	Side chains Shifted from 745 cm^{-1}
688	APS	NH_2 wag
618	Protein	Side chains
593	APS	SO_3^- bend, N-H deformation
562	APS	S-O stretch

Note: the change in colour from yellow to red/orange crystals after infiltration with APS was indicative of an interaction between APS and side-chain amino acid residues without significant disruption of the 3-D protein structure or subsequent reaction with pyrrole.

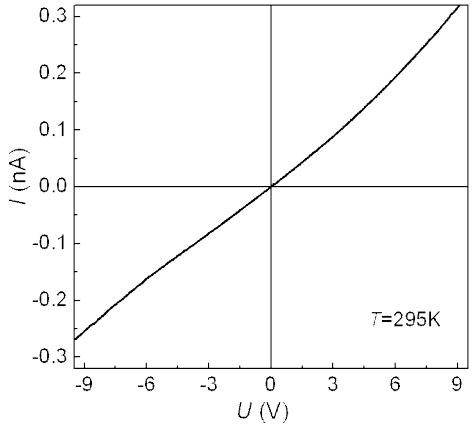

Figure S4 I/V curve for individual PPy-CLLCs at T = 295 K.

Table S1: FT-IR peak assignment for PPy-CLLLCs: protein bands

Peak (cm^{-1})	**Assignment**
3400	N-H/O-H stretch
2962	C-H stretch
2930	C-H stretch
2875	C-H stretch
1654	Protein Amide I
1534	Protein Amide II
1451	C-H scissor
1236	Protein Amide III
Broad, minimum at 610	Protein/polymer overlap

Table S2: FT-IR peak assignment for PPy-CLLLCs: PPy bands

Peak (cm^{-1})	**Assignment**
3400	N-H/O-H stretch
(i) 1260	C-H or C-N in-plane deformation modes (shifted from 1280-1300 cm^{-1} possibly due to confinement within channels)
(ii) 1096	In-plane deformation vibration of NH+ formed on PPy chains by protonation[33]
(iii) 1044	C-H and N-H in-plane deformation vibration
(iv) 968	C-C out of plane ring deformation
(v) 932	C-H out of plane ring deformation vibration (shifted from ca. 900 cm^{-1} due to overlap with protein bands)
(vi) 807	C-H out of plane ring deformation vibration of oxidized form of Ppy[33] (shifted from 780 cm^{-1} possibly due to confinement within channels)
(vii)Broad, minimum at 610	Protein/polymer overlap