Electronic Supplementary Material (ESI) for Nanoscale.

Enzyme-assisted mineralization of calcium phosphate: Exploring confinement for the design of highly crystalline nano-objects

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Electronic Supplementary Material

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1. Enzymatic activity in solution

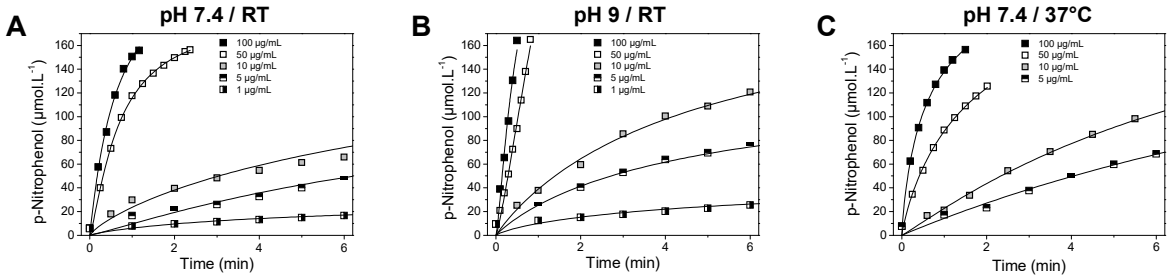

Fig. S1. Amount of p-Nitrophenol produced by ALP at different concentrations of ALP in water at (A) pH 7.4 (RT), (B) pH 9 (RT) and (C) pH 7.4 (37 °C) as a function of time**.** The measurements were performed at 410 nm with a UV-Vis spectrophotometer in the presence of $CaCl_2$ (11.4 mM) and pnpp (7 mM). Straight lines are obtained through fittings with the Hill function.

2. QCM-D measurements

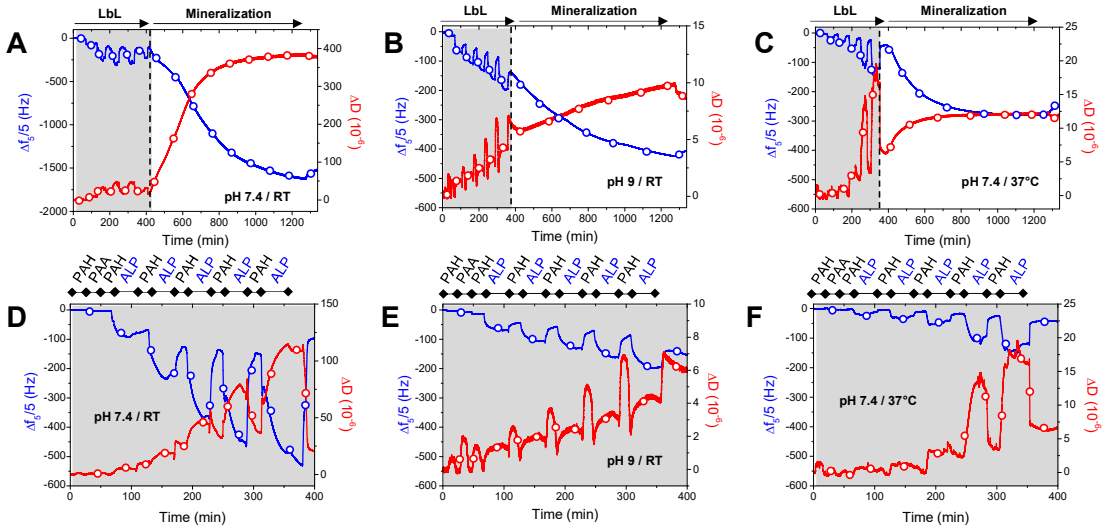

Fig. S2. QCM-D measurements showing frequency changes in the 5th overtone and the corresponding dissipation during $(PAH/ALP)_n$ LbL assembly followed by the mineralization in different conditions: (A) pH 7.4 (RT), (B) pH 9 (RT) and (C) pH 7.4 (37 °C); (D-F) the zoom for the LbL assembly of respectively (A), (B) and (C).

The formation of minerals was not observed after incubating $(PAH/ALP)_5$ multilayers in solutions containing Ca^{2+} or α-glycerol phosphate only.

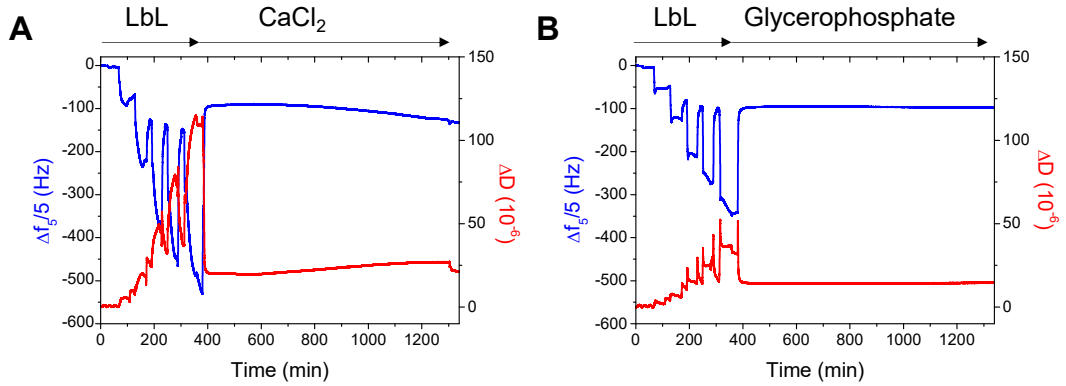

Fig. S3. QCM-D measurements showing frequency changes in the 5th overtone and the corresponding dissipation change during LbL assembly of $(PAH/ALP)_5$ multilayers followed by the injection of (A) $CaCl_2$ solution, or (B) α-glycerol phosphate solution in ultrapure water at pH 7.4 (RT).

3. Morphology of $(PAH/ALP)_n$ nanotubes

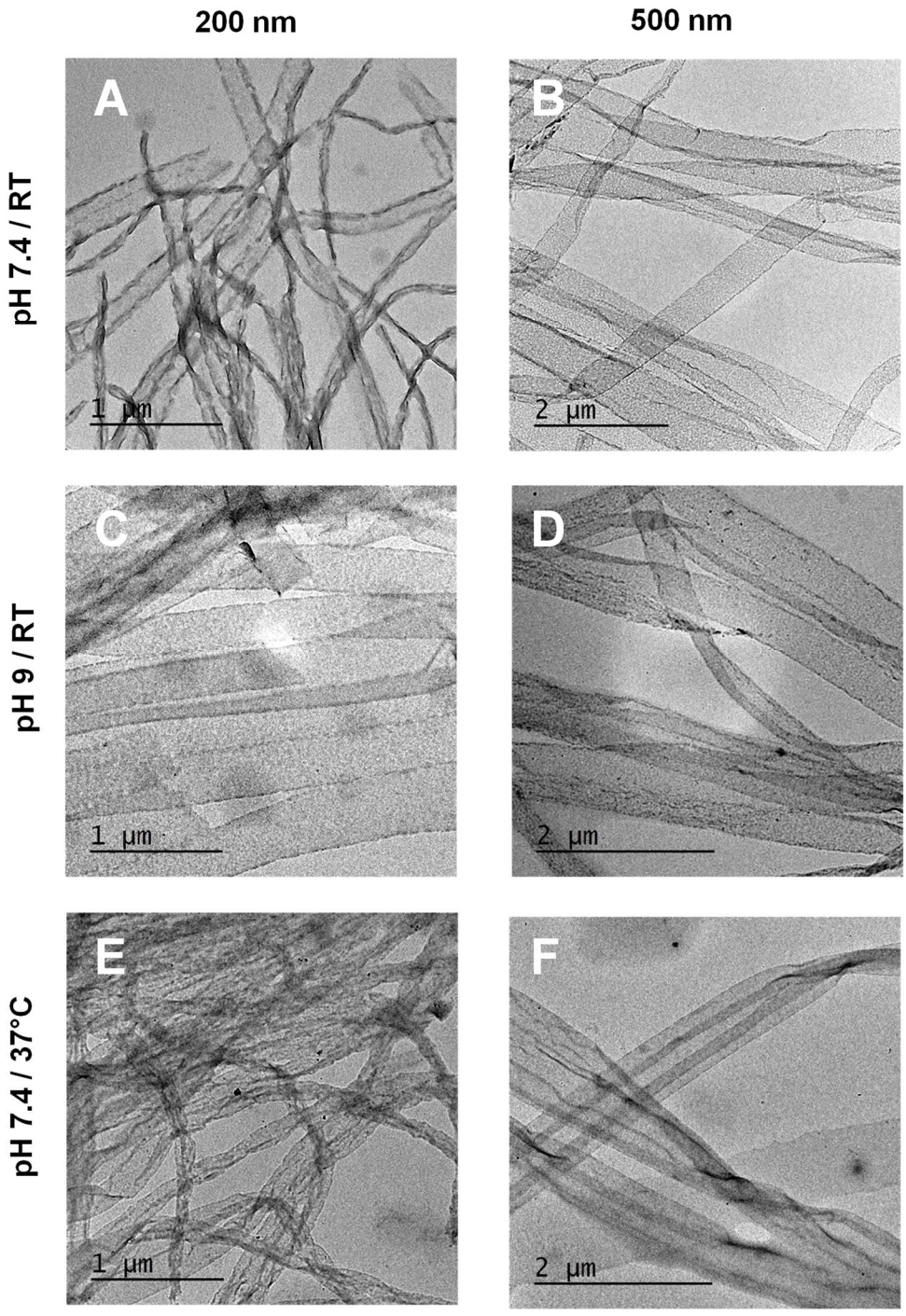

Fig. S4. $(PAH/ALP)_5$ nanotubes synthesized in 200 nm (left) or 500 nm (right) pore size template at (A-B) pH 7.4 (RT); (C-D) pH 9 (RT); (E-F) pH 7.4 (37 °C).

4. Cleaning procedure of the PC track-etched membrane

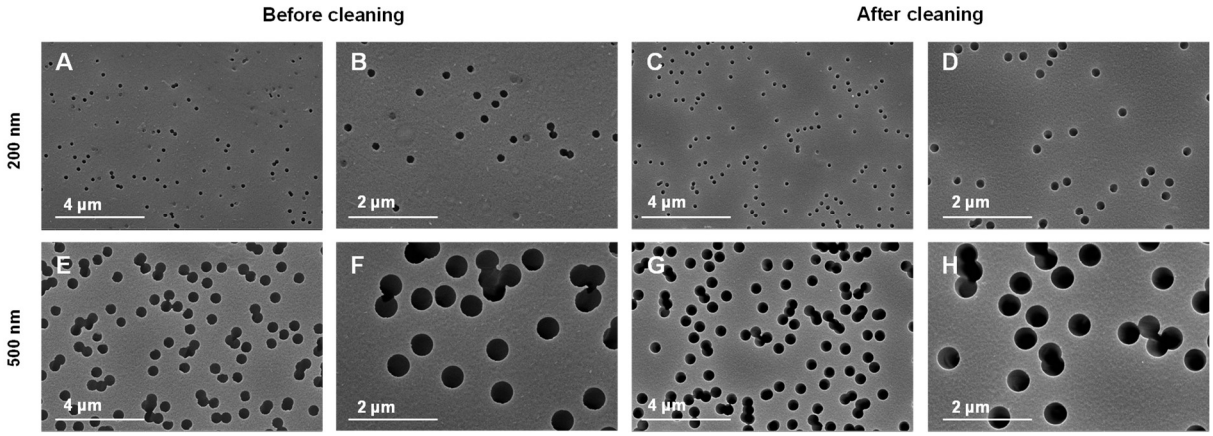

Fig. S5. SEM images of PC membranes after the construction of $(PAH/ALP)_5$ multilayers in (A-D) 200 and (E-H) 500 nm pore size template (A, B, E and F) before and (C, D, G and H) after cleaning the top and bottom surfaces of the membrane with NaCl solution (3 M, pH 12).

5. Enzymatic activity of $(PAH/ALP)_5$ multilayers within nanopores

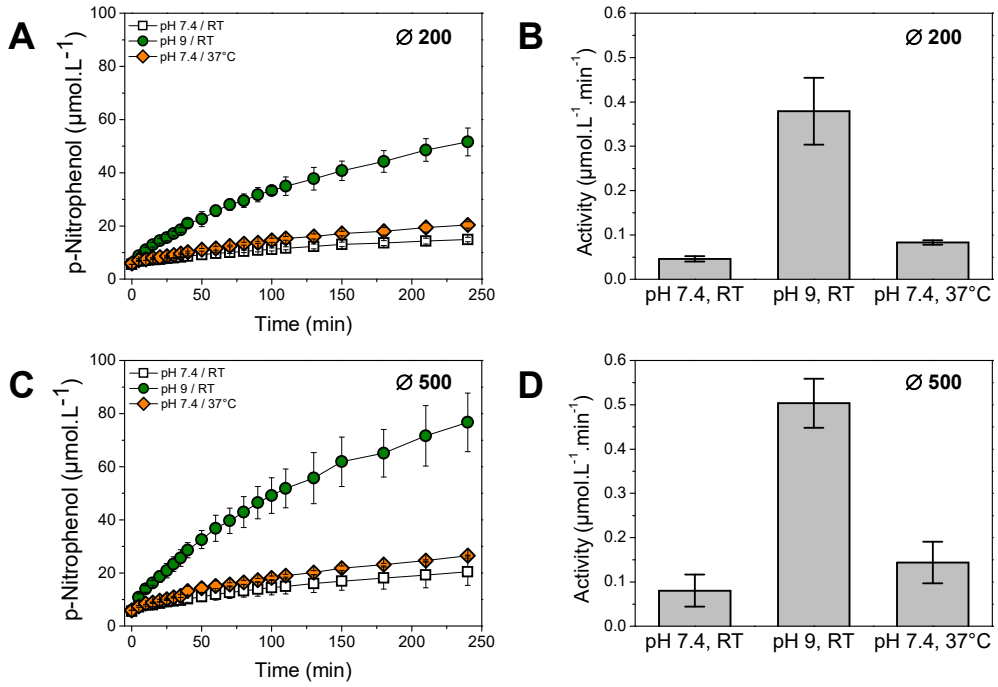

Fig. S6. Amount of p-Nitrophenol produced by the $(PAH/ALP)_5$ nanotubes synthesized in water at pH 7.4, (RT), pH 9 (RT) and pH 7.4 (37 °C) as a function of time in the (A) 200 nm and (C) 500 nm pore size template. (B-D) Enzymatic activity computed from (A) and (C) graphs respectively. The measurements were performed at 410 nm with a UV-Vis spectrophotometer in the presence of $CaCl_2$ (11.4 mM) and pnpp (7 mM).

6. Characteristics of CaP nano-objects

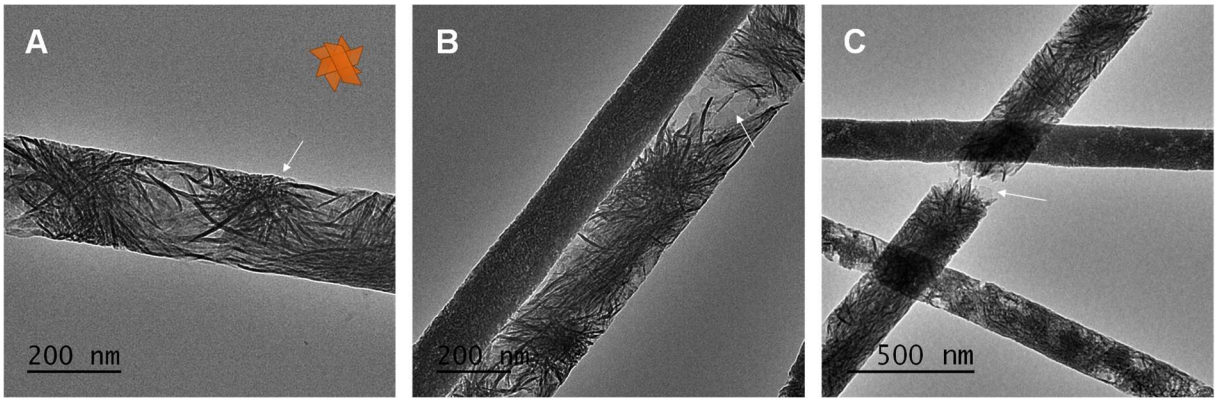

Fig. S7. TEM micrographs showing typical morphologies of nanowires synthesized at pH 7.4, 37 °C for 48 h in the 200 nm pore size template. (A) The inset depicts the expected stacking of platelet-shaped hydroxyapatite particles within the tubular structure (see arrow). The stacking of platelets can be visualized in (B) areas with relatively low density (see arrow), or (C) in some broken nanowires.

Table S1. Characteristics of calcium phosphate compounds formed in solution, on planar surface or in nanopores at room temperature (RT) or at 37 °C (HAP = hydroxyapatite).

	In solution				On planar surface		In nanopores			
	without PAH		with PAH				200 nm		500 nm	
	RT	37 °C	RT	37 °C	RT	37 °C	RT	37 °C	RT	37 °C
Morphology	platelet	platelet	globular	globular	globular	globular	none	platelet	globular	layer
Size (nm)	168 ± 23	145 ± 23	24 ± 4	80 ± 8	177 ± 23	127 ± 20	-	80 ± 9	37 ± 6	-
Crystal phase	HAP	HAP	Amorphous	Amorphous	Amorphous	Amorphous	-	HAP	Amorphous	Amorphous