Effects of Biomineralization Peptide Topology on the Structure and Catalytic Activity of Pd Nanomaterials

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Electronic Supplementary Information

Materials and Methods

Chemicals. All Fmoc-protected amino acids and the rink amide resin used were obtained from Novabiochem whereas the other reagents used in the peptide synthesis such as HOBt, HBTU, piperidine, TFA and solvents were purchased from Watanabe Chem. Ind. Ltd. $NaBH_4$ and 4-nitrophenol were purchased from Nacalai-Tesque, K_2PdCl_4 and HEPES were both obtained from Sigma-Aldrich. 2,2'-furildioxime was sourced from Wako Pure Chemical Industries, Ltd.; 2-nitrophenol and 3-nitrophenol were obtained from Tokyo Chemical Industry, Co. Ltd. All purchased reagents were used as received without further purification. Milli-Q water was used throughout all experiments.

Peptide Synthesis and Characterization. The peptides (Table S1) were synthesized using an Applied Biosystems 433A automated peptide synthesizer utilizing the standard Fmoc synthetic strategy on a rink amide resin. The cleaved peptide obtained after treatment with Reagent K (9 mL trifluroacetic acid (TFA), 0.5 mL milli-Q, 0.5 mL phenol, 0.5 mL thioanisole and 0.25 mL of ethanedithiol) was purified using a Shimadzu LC-6AD HPLC equipped with a 22 x 250 mm^2 Vydac C8 column with a binary gradient of buffered CH_3CN/H_2O as the solvent system. The purified peptides were characterized using an Applied Biosystems Voyager 4379 MALDI-TOF MS. The concentrations of the Pd4 conjugated variants and the p53Tet were determined by absorption measurements at 280 nm corresponding to the absorptivity of a single tyrosine residue (ε = 1280 $M^{-1}cm^{-1}$) in a denaturing solution composed of 25 mM sodium phosphate (pH 6.5) and 7.5 M Gu•HCl. Due to the absence of a highly absorbing chromophore in the Pd4 peptide, its concentration was determined by mass. Circular dichroism spectra were obtained on a Jasco J-805S spectropolarimeter using a 1 mm quartz cuvette at room temperature. Purified peptides were dissolved at a final concentration of 10 μM in 50 mM phosphate buffer with a pH of 7.4. The secondary structural proportions were calculated using the software SSE-338W.

Biomineralization reaction. The peptides were dissolved into a 2.5 mM HEPES-NaOH buffered solution with a pH of 7.4 at a final concentration of 40 μM. Into the solution, a five-fold equivalence of 50 mM K_2PdCl_4 stock solution was added and the resulting peptide – Pd^{2+} solution was incubated for 45 minutes. After this incubation period, an excess of freshly prepared 100 mM $NaBH_4$ was added. After the introduction of the $NaBH_4$, the solution color transitioned from yellow to gray. The reduction was allowed to proceed for 90 minutes. Purification of the biomineralization product involved centrifugation at 15000 rpm for 20 minutes wherein the pellet was resuspended in 1 mL of milli-Q water. The homogenous solution was then subjected to electron microscopic analysis.

Electron microscopic analysis. Sample preparation for electron microscopy involved the introduction of 4μL of the purified, re-suspended samples onto a 100 μm copper pitch grid coated with a thin layer of carbon. The prepared copper grids were air and vacuum dried. STEM images, elemental analyses and ion maps were obtained using a Hitachi HD-2000 operating at 200 kV equipped with an EDAX Genesis energy dispersive X-ray. High resolution TEM images and selected area diffraction patterns were obtained using a JEOL JEM-2100F TEM.

Catalytic Reaction. The palladium content of the Pd4-p53Tet-based materials and the nanoparticles obtained from peptide free conditions was determined spectrophotometrically with the aid of 2,2'-furildioxime as the complexing ligand (O. Menis, T.C. Rains, *Anal. Chem.* 1955, **27**, 1932)**.** In a typical procedure, 0.1 mL of purified material was dissolved in concentrated HCl. The resulting solution was evaporated after which the Pd ions were

complexed with 5 mM of the ligand in an acidic environment. The resulting complex was bright yellow and its absorbance was immediately measured at 425 nm. From a standard curve, the palladium content of the materials was determined.

For the reduction of the nitrophenol isomers, 10 μL of 5 mM nitrophenol was added to an excess of $NaBH_4$ to give a final concentration of 100 mM (200 fold excess). Subsequently, the necessary amount of the purified Pd material to give a final concentration of 0.40 μM Pd (0.8%) was added to the solution. The progress of the reaction was monitored by UV-Vis for 5 minutes with measurements taken every second. From the absorbance readings, the pseudo-first order rate constants were calculated as well as the turnover frequency for each reaction. All quantitative analyses were conducted in triplicate.

Table S1. Sequence of synthesized peptides

Peptide	Sequence
Pd4	TSNAVHPTLRHL-amide
Pd4-p53Tet	TSNAVHPTLRHLGGDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKE-amide
Pd4-p53Mono	TSNAVHPTLRHLGGDGEYFTAQARGRERFEMFREANEALELKDAQAGKE-amide
p53Tet	DGEYFTLQIRGRERFEMFRELNEALELKDAQAGKE-amide

Table S2. Calculated distribution of the secondary structures of the different peptides based on their circular dichroism (CD) spectra

Peptide	α-helix	β-sheet	Turn	Random
Pd4	0	32.1	16.7	51.2
Pd4-p53Tet	36.9	31.7	2.7	28.7
p53Tet	38.8	28	6.7	26.5
Pd4-p53Mono	0	34.8	11.8	53.3

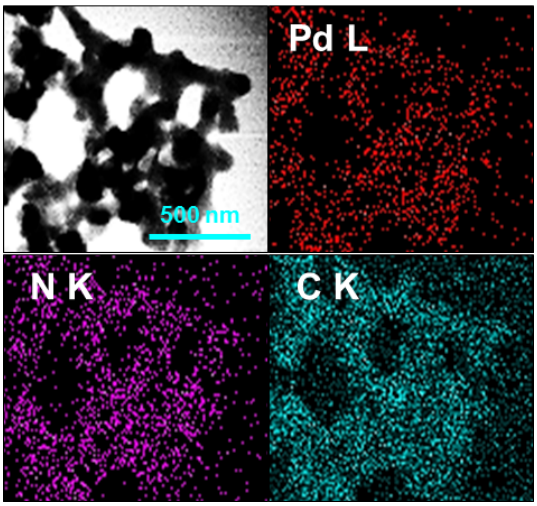

Figure S1. EDX elemental mapping of Pd nanocorals from Pd4-p53Tet. From top left clockwise: BF-STEM image of Pd nanocorals, Pd map from L line, C map from K line and N map from K line

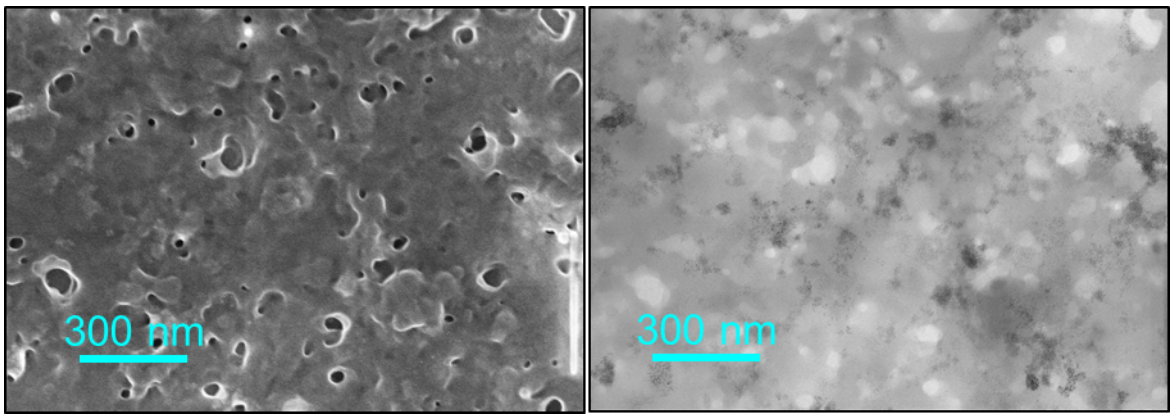

Figure S2. SE-STEM (left) and BF-STEM (right) images of Pd nanostructures from Pd4-p53Tet after omitting the pre-reduction incubation period. This control experiment suggests that the formation of the porous, coral-like materials involves a step-wise process.

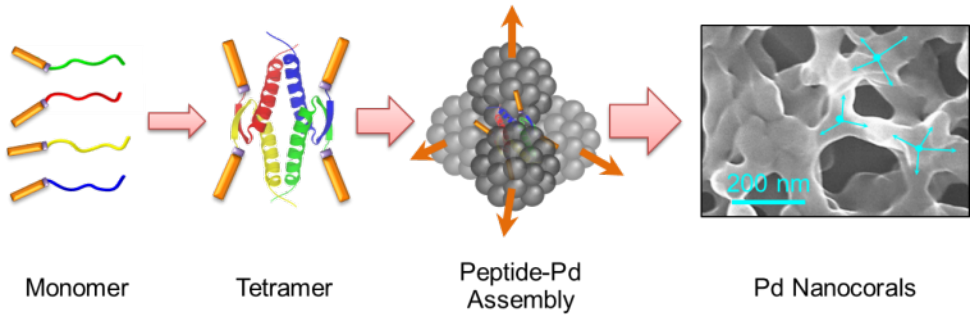

Figure S3. Proposed model of Pd nanocoral formation with Pd4-p53Tet. Starting from the monomer of Pd4-p53Tet, the four monomers tetramerize. The spatially-fixed orientation of Pd4 leads to an oriented assembly of growing Pd nanoparticles. Continuous accumulation of Pd at the four sites results to crowding which forces nanostructure growth into tetrahedral directions. Further assembly of similar units yields the hierarchical nanocorals.

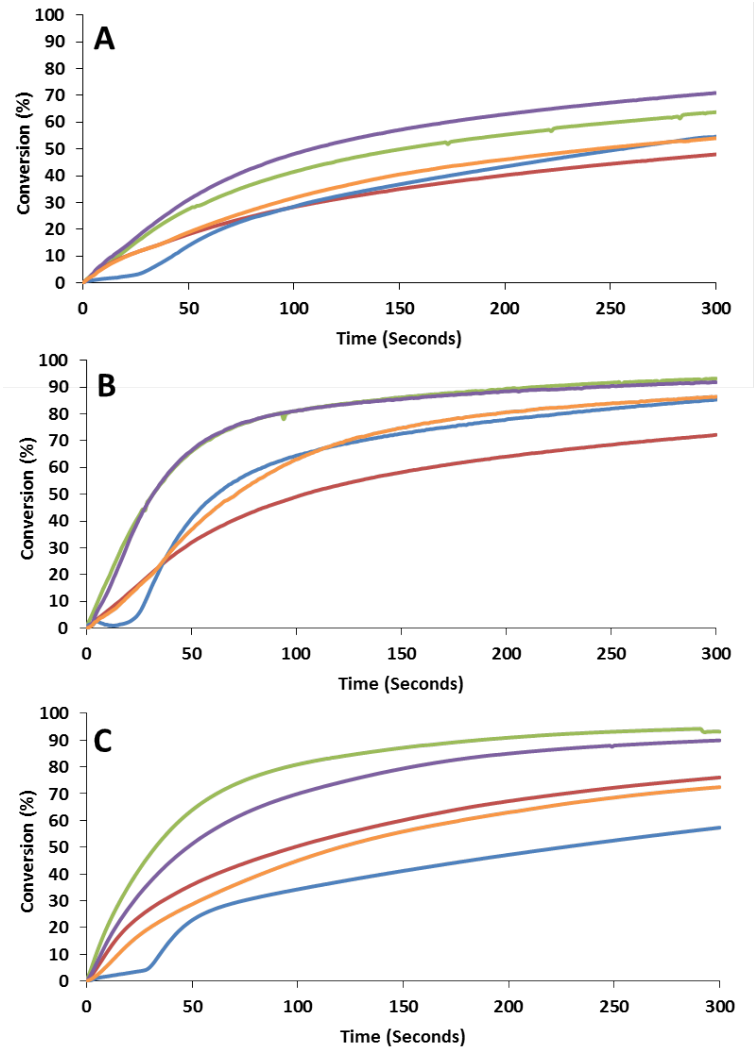

Figure S4.Time course reduction of nitrophenol isomers (Triplicate analyses, continuous measurement, 1 second interval). **A)** 2-nitrophenol, **B)** 3-nitrophenol, **C)** 4-nitrophenol. Green = Pd4-p53Tet Pd nanocorals, Red = No Peptide Pd nanoparticles, Blue = Pd4 Pd nanoparticles, Purple = p53Tet Pd nanostructures, Orange = Pd4-p53Mono Pd nanostructures