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# New approach to molecular self-assembly through formation of dipeptide-based unique architectures by artificial supersaturation

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**Supporting Information**



**Other Supporting information for this manuscript includes the following:**

Movie S1 to S4

*1. Preparation of FF solution and gold electrode*

The as-received lyophilized powder of diphenylalanine (FF) and phenylalanine-tyrosine (FY) peptides from Bachem (Bubendorf, Switzerland) were first dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at a concentration of 100 mg/mL. The corresponding stock solution was then diluted to a final concentration of 1 mg/mL in dry methanol for the experiments. To avoid any pre-aggregation and assembly, fresh stock solutions were prepared for each experiment. Gold electrodes were fabricated on a glass substrate by e-gun deposition of chromium (10 nm) and gold (50 nm) through a mask.

*2. Fabrication and characterization of microstructures*

A drop of 4 μL FF solution was placed in between the gap of electrodes and a range of voltage ($V$ = 10 V - 100 V) had been applied between the electrodes for a time period of 25 s - 85 s at the initial stage to investigate the growth of the microstructures. When the voltage was decreased, the proportion of perfectly grown "diatom-like" structures decreased relative to the proportion of partially grown structures (Figure S5). Application of lower voltage also requires longer time for the initial growth stage. Voltage was applied and current was monitored by the instrument (Keithley, SourceMeter2400). Growth of the microstructures was recorded using a CCD camera attached with the optical microscope. Unique "diatom-like" microspheres and smooth microtubes were grown after drying the solvents (3 - 20 min). The TOF-SIMS (ULVAC-PHI, PHI TRIFT V nano TOF) positive ion spectrum for the fabricated microstructures showed a characteristic peak at 313 m/z (mass-to-charge ratio), which corresponds to the singularly charged ion of H-Phe-Phe-OH peptide (Figure S2). This indicates the structural integrity of the FF molecules under the experimental conditions used in this study.

*3. Temperature measurement*

The temperature of the sample during the application of voltage was measured by a thermograph (ViewOhre Imaging, AIR32 Micro3x). The substrate is covered by thick solution layer with a thickness of 100 – 400 μm at the initial stage, and electric current flows at the bottom of the solution. At some of red regions in Figure1c, bubbling in the solution was observed within a few seconds after application of the voltage (Movie S1), suggesting that a local temperature of the region reaches to the boiling temperature of methanol (~64.7℃). When a time to apply the voltage is short (≲ 60 s), a volume of the local region whose temperature increases largely by the Joule heating is much smaller

than that of the total solution. After their movement by the convection current, the hot solution cools down to room temperature. Therefore, red and olive regions in Figure 1c correspond to ~60 and ~24℃, respectively.

*4. Needle-manipulation to the microsphere*

In order to study internal and bottom structures of the porous microspheres, we applied needle-manipulation technique to peptide-based materials for the first time under the observation of SEM. Both sides of a sharp metal needle was removed by local suppering of FIB (Hitachi, NB5000) and a sharp "knife-like" needle was fabricated (Figures. 2a and b, Movie S2). Before the manipulation, the insulating sample was covered by thin Pt layers. Using the sharp needle, the microsphere was cut to study internal structures of the microsphere (Figures. 2a and b). The micro-hemisphere was removed using a Au-coated sharp glass needle (Figures. 2e and f). Since the cross-section and the bottom of the spheres were insulators, again Pt was deposited before the observation of SEM (Hitachi, S4800 and SU8000) (Figures. 2c, d and g-i).

*5. Nucleation and growth*

Small molecules in a solution have inherent tendency to form small aggregations due to their mutual noncovalent interaction. [S1] Molecular dynamics simulations for diphenylalanine peptide (FF) molecules estimate the reaction time of 60 - 170 ns to form small aggregations (less than 10 nm) by self-assembly in a high concentrated solution.[S2] The simulations also predict that the formation of large aggregation working as nucleation, whose size is of the order of 100 nm, needs a time of the order of 1 μs. In our experiment, the aggregations of FF molecules in the supersaturated region are thought to occur on the similar time scale. As seen in Movie S1, strong macroscopic convection current induced by the large temperature difference in the solution causes the movement of molecules from the supersaturated region near the gap to the low concentration region at a velocity of ~0.01 - 0.1 m/s. Under the convection current like as a vortex, the molecules can be placed at the supersaturated region for 10 - 1 ms. The estimated staying time is much longer than the time required to form small aggregations and nucleus, then their formation takes place at that region. The number of the small aggregations and nuclei is almost proportional to the integration of the electric power applied between the electrodes. Since the supersaturated volume is much smaller than the volume of the total solution, the microstructure grows from the nucleus in the low concentration region of the solution, keeping the balance of supply and growth.

*References:*

[S1] J. Huang, T. C. Stringfellow, L. Yu, *J. Am. Chem. Soc*. **2008**, *130*, 13973.

[S2] C. Guo, Y. Luo, R. Zhou, G.Wei, *ACS Nano* 2012, **6**, 3907.

**Figures:**

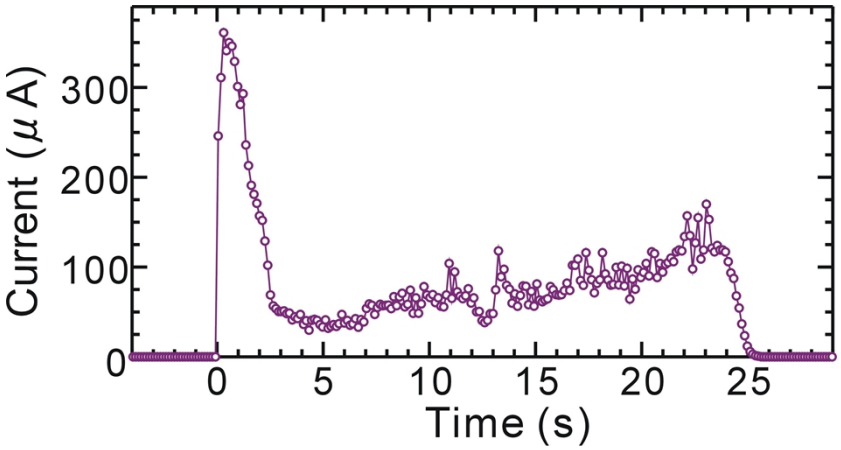


**Figure S1.** Typical plot of electric current through 1 mg/mL methanolic solution between the electrodes under the application of 100V for 25 s.

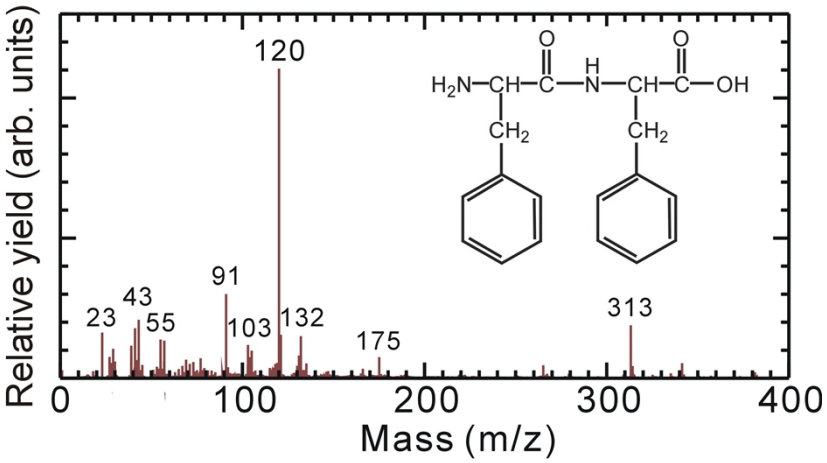


**Figure S2.** The TOF-SIMS positive ion spectrum of as-grow microstructures shows a characteristic peak of 313 m/z (mass to charge) corresponding to the singularly charged ion in linear H-Phe-Phe-OH peptide. Inset depicts a molecular structure of peptide diphenylalanine.

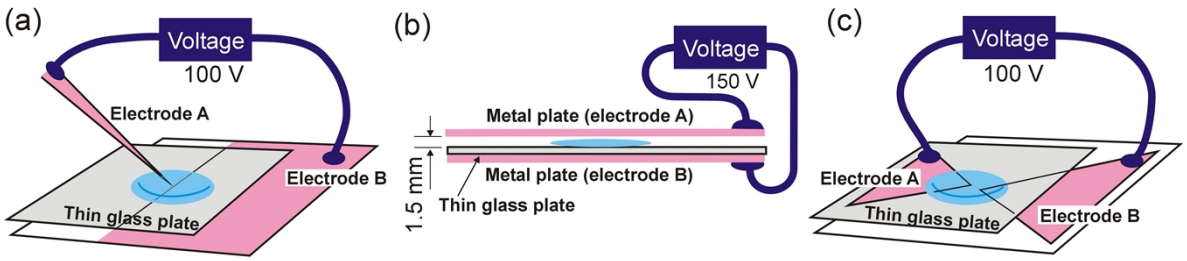


**Figure S3.** Three different configuration of electrodes to produce the same order of electric field ($10^5$ – $10^7$ V/m), which is estimated by solving Maxwell's equations numerically in the configuration with a gap separation of 120 μm, but without current flowing due to the thin glass plate with a thickness of 100 μm.

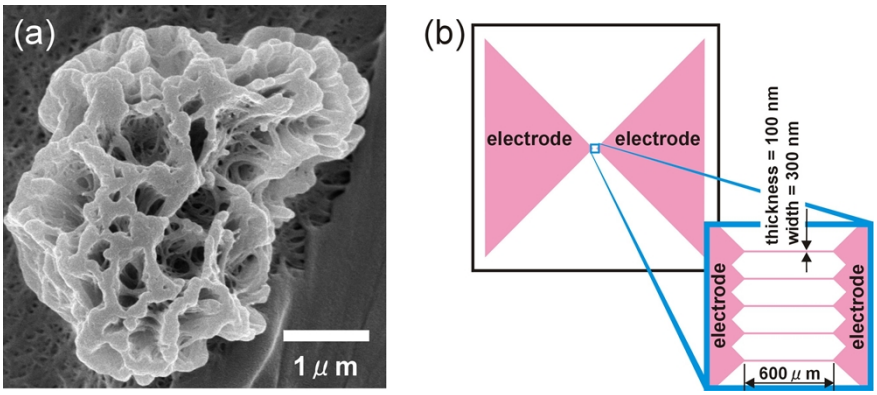


**Figure S4.** (a) SEM image of a "diatom-like" porous microstructure formed by other supersaturation induced by a Joule heating effect of the metal nanowires between the electrodes under the application of voltage ($V$ = 0.5 V) for 50 s. (b) Schematic illustration of electrodes and nanowires (heater) between the electrodes. Five gold nanowires are constructed between electrodes using conventional lithographic technique.

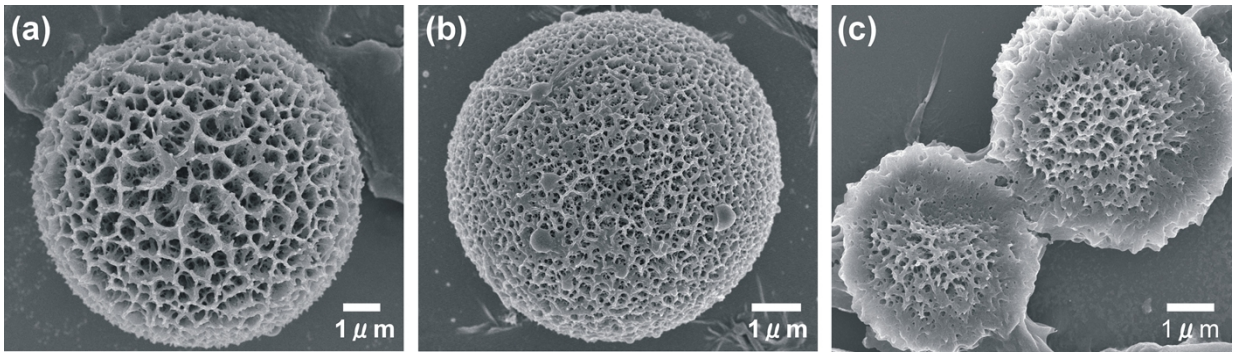


**Figure S5.** SEM images of the as grown "diatom-like" porous microspheres under the application of voltage (a) 75 V for 50 sec, (b) 50 V for 60 sec, and (c) 10 V for 85 sec from 1 mg/mL methanolic solution of FF.

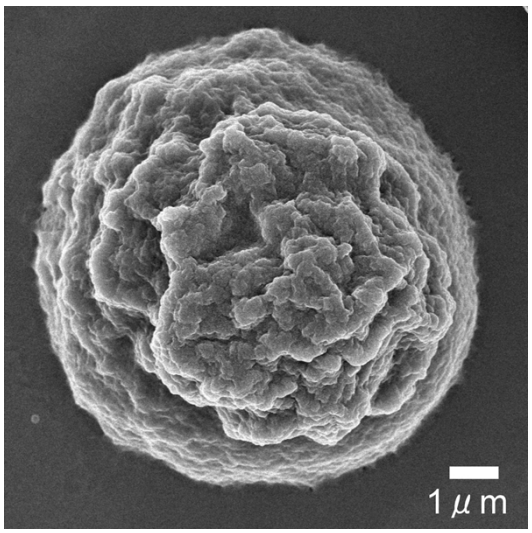


**Figure S6.** SEM image of a rough amorphous microsphere without porous shell, which was grown at the application of voltage of 5 V for 60 sec. The imperfect shell structures originate from the shortage of the electric power required for the formation of the "diatom-like" porous microsphere.

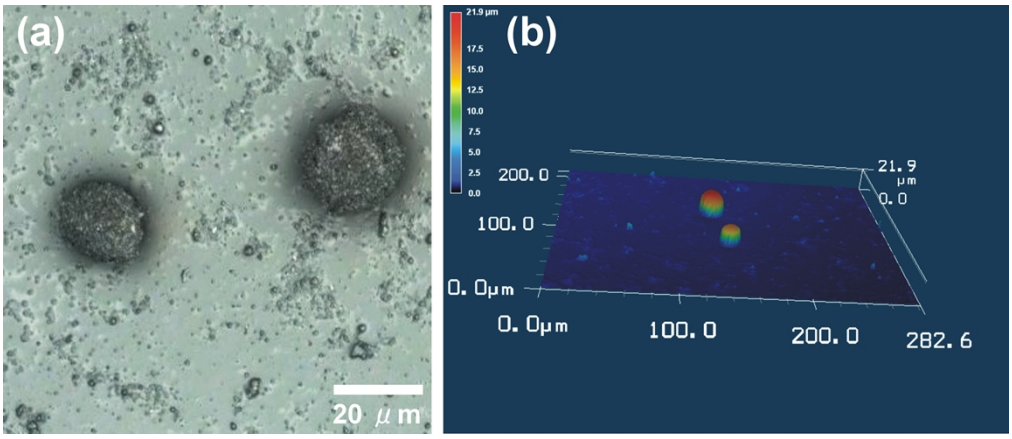


**Figure S7**. (a) Laser scanning microscope image of porous microsheres of FF peptide. (b) Corrsponding three-dimention view of the microspheres.

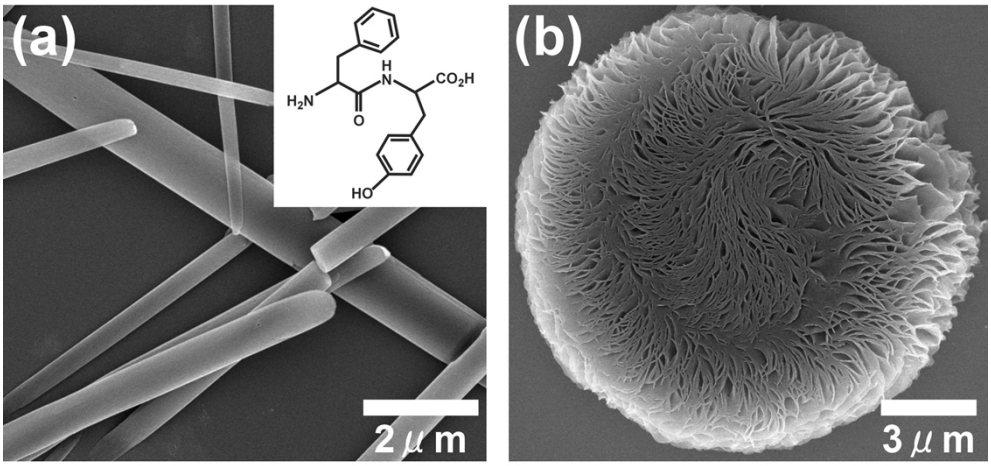


**Figure S8**. SEM images of self-assembled structures of a methanolic solution of phenylalanine-tyrosine (FY) peptide at concentration of 1 mg/mL (a) without and (b) with applying voltage of 100 V for 30 s after dropping the solution between the electrodes. Inset shows a chemical structure of FY peptide.

## Movies:

**Movie S1.** Local Joule heating, convection current, and bubbling of the solution placed in between the electrodes with a separation of 120 μm in an applied voltage of $V$ = 100 V under the observation of optical microscope. The application of voltage starts at 10 s after the dropping. The movie is recorded at real speed.

**Movie S2.** Operation of cutting the "diatom-like" porous microsphere under the observation of SEM using a sharp "knife-like" metal needle fabricated using FIB. The movie is recorded at real speed.

**Movie S3.** Real time growth of an isolated long microtube in a typical experimental condition from methanolic solution of FF under the observation of optical microscope. The vertical side of the frame corresponds to 700 μm. The playback speed of the movie is 4 times faster than the real time

**Movie S4.** Growth of microtubes near the boundary of methanolic solution of FF under the observation of optical microscope, which shows the pushing of the edge of the boundary. The playback speed of the movie is 4 times faster than the real time