Surface Effects Mediate Self-Assembly of Amyloid-β Peptides

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Supporting Information:

Quantitative analysis

Surface Coverage and Number Density. All topographic images were flattened by removing the third-order polynomial background in Gwyddion software.[1] After flattening the AFM image, the surface coverage of nanostructures was measured by setting an appropriate height threshold in the Gwyddion software (an example is shown in Figure S3). Then, the total volume of peptide in the selected area was calculated. In order to obtain the total number of peptides, the total volume was divided by a single peptide's volume, 2366 $Å^3$. Here, the volume of a single peptide was calculated using the web-based Peptide Property Calculator (Northwestern university: http://www.basic.northwestern.edu/biotools/proteincalc.html). The number density is equal to the total number of peptides divided by the area of the AFM image.

Height Histogram and Height Profile. The height histogram and height profile were analyzed and extracted by Gwyddion software after flattening, then plotted in SigmaPlot (Systat Software, Inc).

Cumulative Length Distribution. Using the height threshold set up before, a binary map in Gwyddion can be created, as shown in Figure S3C. ImageJ software[2] was used to manually measure the fibril's length (end-to-end distance) in the binary map (Figure S3C). In some cases, it was difficult to judge the fibril's length due to the small signal-to-noise ratio. As a result, the topography (Figure S3A), phase (Figure S3D) and amplitude (Figure S3E) images were also used to help determine the fibril's length. Each normalized length distribution in Figure 4D included five AFM images, as shown in Figure S4, taken from different areas of the same sample.

Other sample drying methods

Kimwipe and water-rinsed samples. Three droplets of the sample solution (10 μM) were deposited on the mica substrate and incubated for 30 minutes. The excess sample solution on the surface was then removed by using a Kimwipe or by rinsing with 0.2 mL Milli-Q water, and then gently drying with a weak nitrogen stream. To prevent water from flowing backwards, which may change the surface morphology, the mica substrate was tilted ~45° during these procedures. The drying times for Kimwipe and water-rinsed procedures were 5 and 8 minutes, respectively. AFM images of the results are shown in Figure S5.

Comparison between different sample preparation methods. The Kimwipe procedure is typically used to wick away excess liquid from substrate for preparing TEM samples[3, 4] and the water-rinsed procedure can remove excess salts and unbound peptides. The water-rinsed method of drying is usually used during sample preparation in ex-situ AFM

measurements.[5-7] As shown in Figure S5B, some areas of Kimwipe-prepared sample have similar patterns and morphologies to samples prepared by slow-spinning after the same incubation time (Figure 2). The fibrils' heights and average densities are also similar (Figure S5A). However, the surface morphology of the Kimwipe-prepared sample is not homogeneous and the fibrils are not distributed evenly on the surface. In some areas, the fibrils are much longer and oriented along the same direction. The water-rinsed samples are also influenced by the drying process. Here it appears that the fibrils, which were formed on the surface during the incubation period, probably could diffuse and pack with other fibrils to form well-packed platelet morphologies during rinsing as seen in Figure S5C. Additionally, some of the spherical aggregates present in the solution are adsorbed to the surface, similar to the observations of aggregates in the fast-spinning process. The inhomogeneous morphology observed within the same sample when prepared by the two typically used drying methods shows the clear advantage of our slow spinning procedure in studies of fibril morphology on a surface. More thorough comparisons of these methods are ongoing.

References

1. Nečas, D.; Klapetek, P., Gwyddion: An Open-Source Software for Spm Data Analysis. *Cent. Eur. J. Phys.* **2012**, *10*, 181-188.
2. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W., Nih Image to Imagej: 25 Years of Image Analysis. *Nat. Methods* **2012**, *9*, 671-675.
3. Jiménez, J. L.; Nettleton, E. J.; Bouchard, M.; Robinson, C. V.; Dobson, C. M.; Saibil, H. R., The Protofilament Structure of Insulin Amyloid Fibrils. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 9196-9201.
4. Paulite, M.; Fakhraai, Z.; Li, I. T.; Gunari, N.; Tanur, A. E.; Walker, G. C., Imaging Secondary Structure of Individual Amyloid Fibrils of a Beta2-Microglobulin Fragment Using near-Field Infrared Spectroscopy. *J. Am. Chem. Soc.* **2011**, *133*, 7376-7383.
5. Shen, L.; Adachi, T.; Vanden Bout, D.; Zhu, X. Y., A Mobile Precursor Determines Amyloid-Beta Peptide Fibril Formation at Interfaces. *J. Am. Chem. Soc.* **2012**, *134*, 14172-14178.
6. Yates, E. A.; Owens, S. L.; Lynch, M. F.; Cucco, E. M.; Umbaugh, C. S.; Legleiter, J., Specific Domains of Abeta Facilitate Aggregation on and Association with Lipid Bilayers. *J. Mol. Biol.* **2013**, *425*, 1915-1933.
7. Charbonneau, C.; Kleijn, J. M.; Cohen Stuart, M. A., Subtle Charge Balance Controls Surface-Nucleated Self-Assembly of Designed Biopolymers. *ACS Nano* **2014**, *8*, 2328-2335.

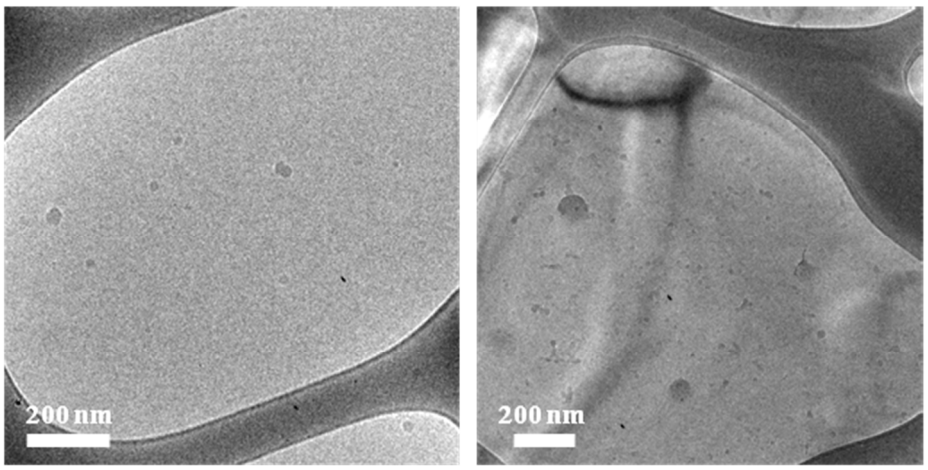

Figure S1. Cryo-TEM images of $A\beta_{12-28}$ sample solution with 10 μM concentration after incubating for three days. No fibrilar structures were observed.

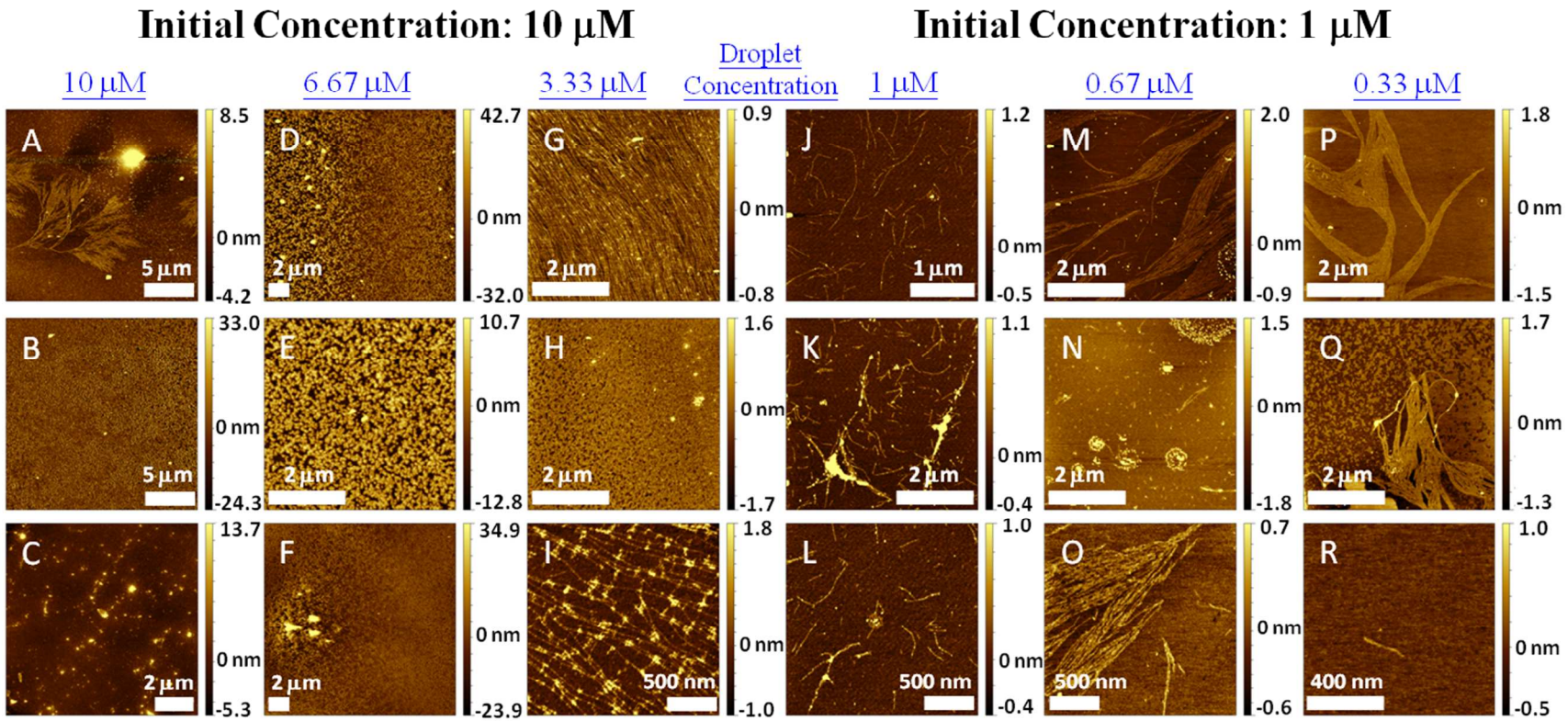

Figure S2. AFM images of slowly-dried samples. Each column represents different areas of one sample prepared using the reported concentration of the initial solution (black) and the concentration of the final solution (blue).

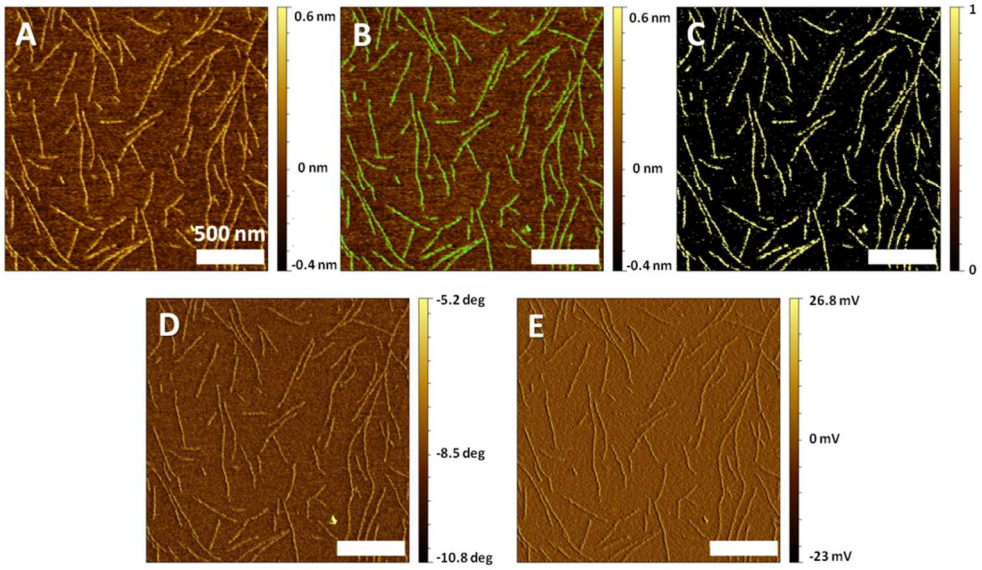

Figure S3. An example of quantitative analysis for the 60 min-1000 RPM sample. (A) The topography image after flattening. (B) The masked AFM image with appropriate height threshold. The green color highlights the selected nanostructure, which is higher than the threshold. (C) The binary map of selected nanostructures. (D) The phase image. (E) The amplitude image. The scale bar of each image is 500 nm.

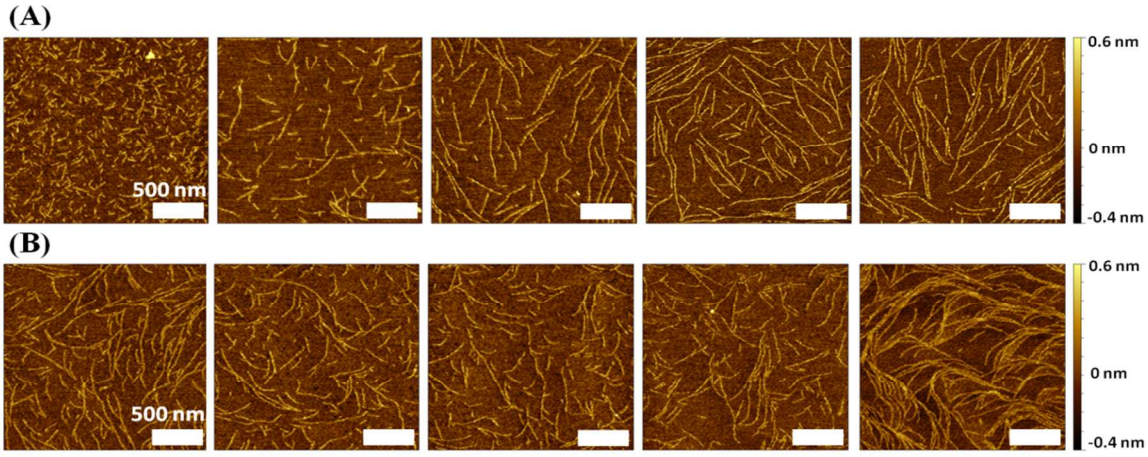

Figure S4. AFM images of five different areas of the (A) 30 min-1000 RPM sample and (B) 60 min-1000 RPM sample that were used to calculate the normalized fibril length distributions. The scale bar of each image is 500 nm.

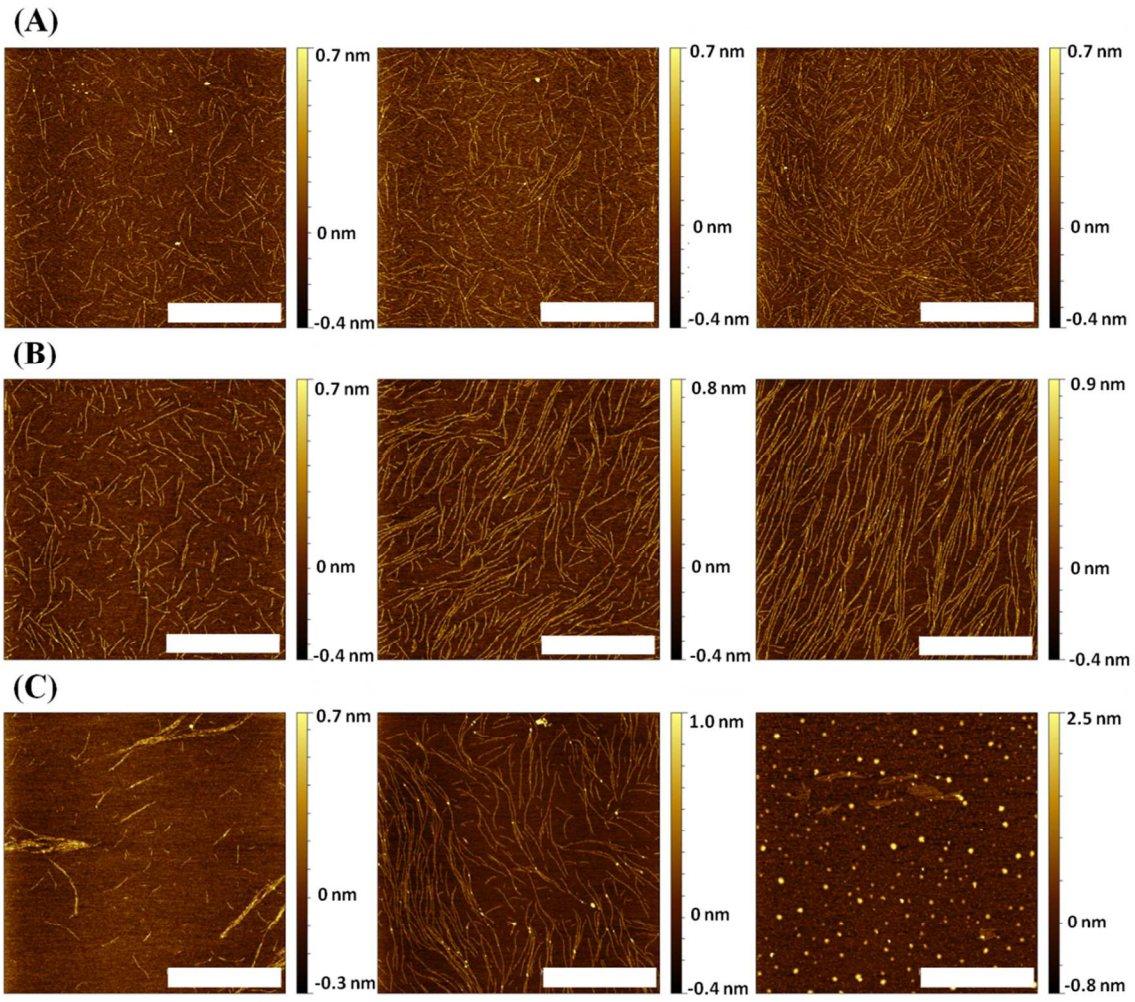

Figure S5. AFM images of different areas of the (A) 30 min-1000 RPM slow-spinning sample (B) 30 min-Kimwipe dried sample and (C) 30 min-water rinsed sample. The scale bar of each image is 2 μm.