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Supporting Information

Photocontrolled protein assembly for constructing programmed two-dimensional nanomaterials

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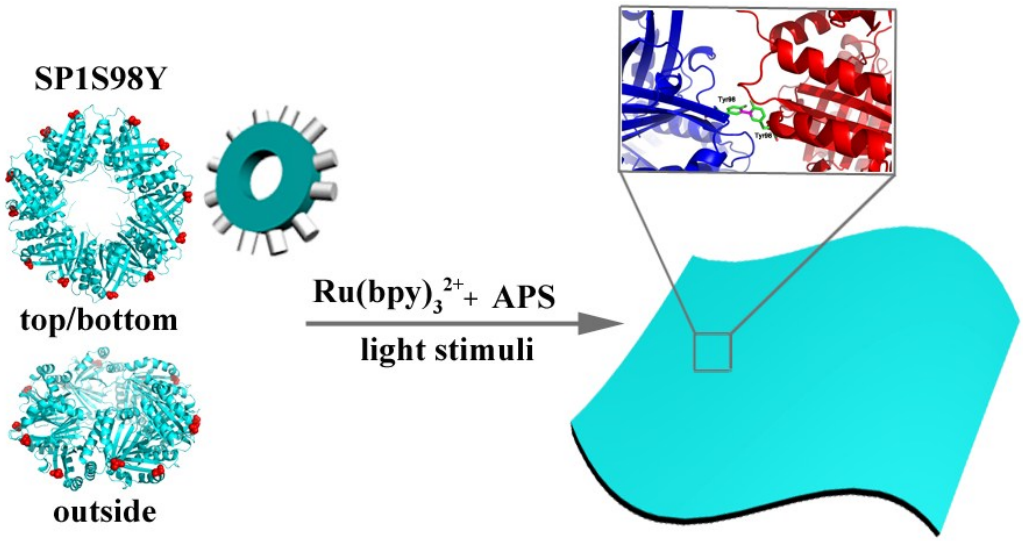

Figure S1: Schematic representation of Tyr coupling between SP1 variants.

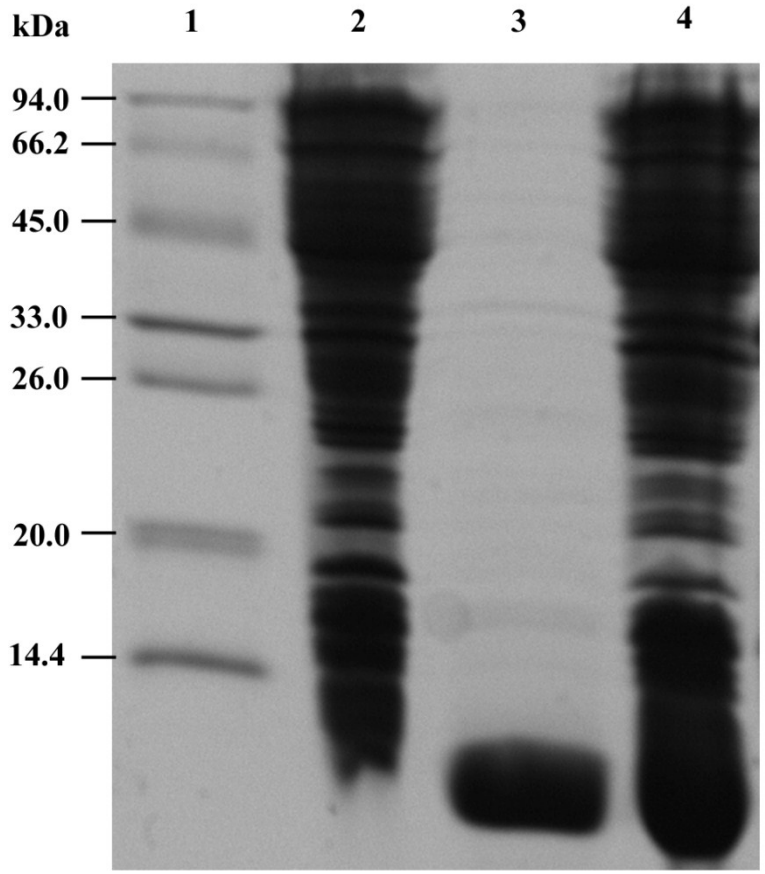

Figure S2: SDS-PAGE analysis of protein SP1 variant. The acrylamide percentage of the SDS-PAGE gels is 15%. Lane 1: Protein Marker; Lane 2: Lysates before IPTG induction; Lane 3: purified SP1S98Y; Lane 4: SP1S98Y-containing lysates after IPTG
induction.

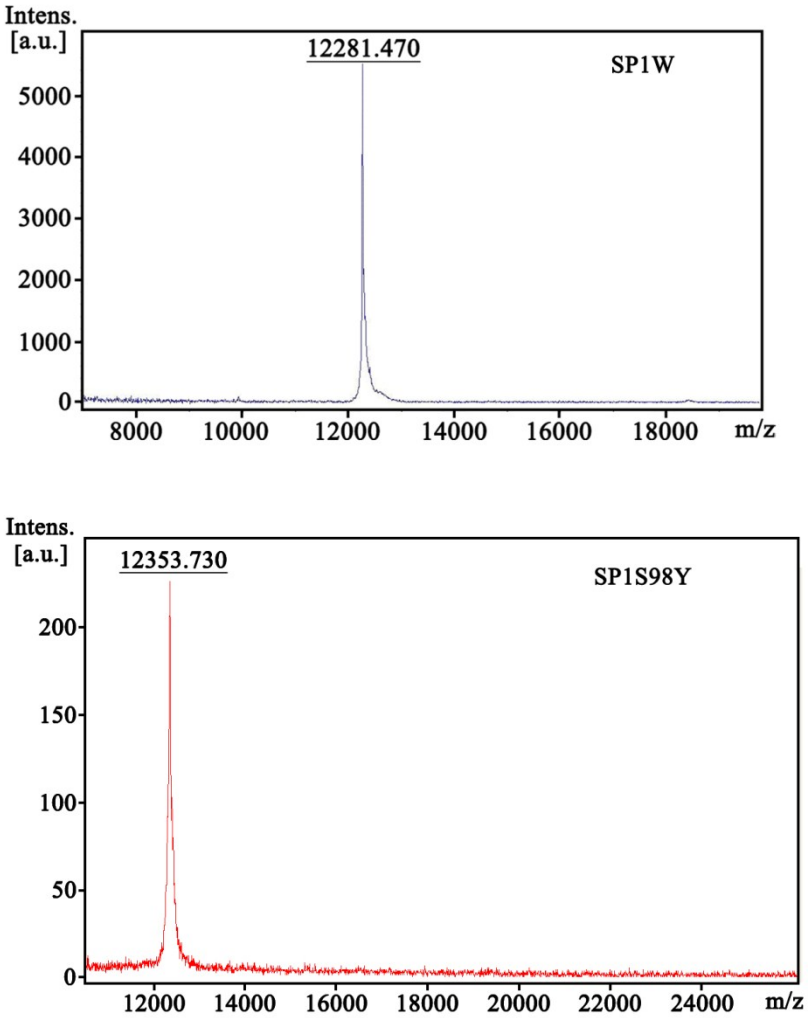

Figure S3: MALDI-TOF mass spectrometry analysis of SP1W and SP1S98Y.

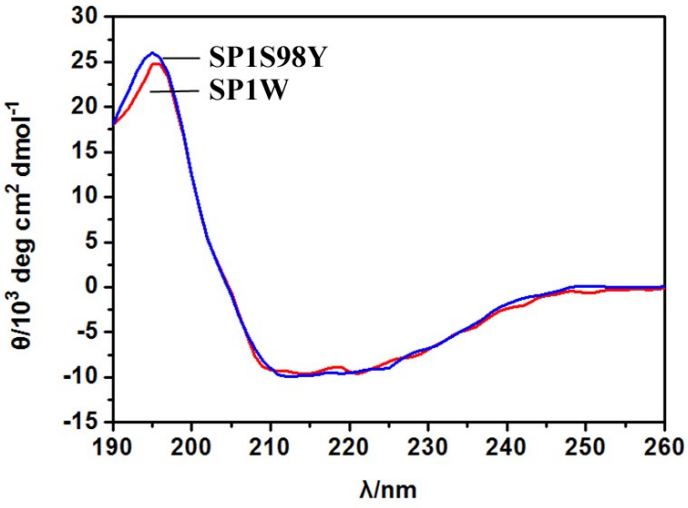

Figure S4: CD spectral data of SP1W and SP1S98Y monitored from 190 nm to 260 nm. Baseline in the absence of protein were measured and subtracted.

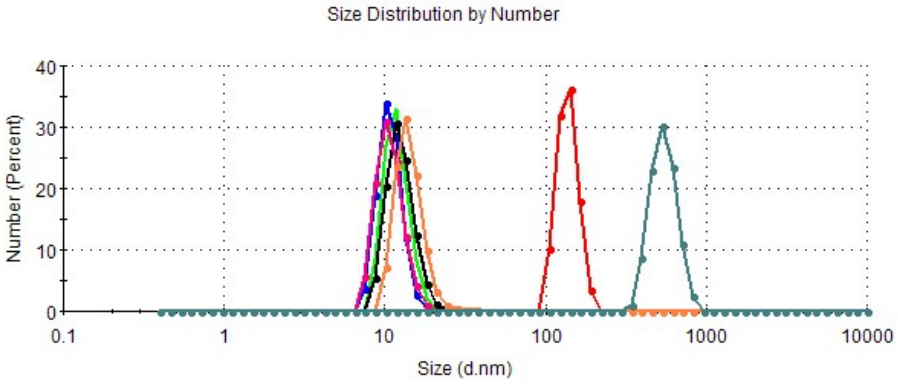

Figure S5: The dynamic light scattering (DLS) analysis of the hydrodynamic diameters of SP1W (blue), SP1S98Y (magenta), SP1S98Y+ $Ru(bpy)_3^{2+}$+APS without light illumination (green), SP1S98Y+APS with white light illumination for 30 min (black), SP1W+$Ru(bpy)_3^{2+}$+APS with white light illumination for 30 min (orange), SP1S98Y+ $Ru(bpy)_3^{2+}$ with white light illumination for 30 min (red), SP1S98Y+ $Ru(bpy)_3^{2+}$+APS with white light illumination for 30 min (dark cyan).

Investigation of assembly behavior based on SP1W

We tried to utilize SP1W as building block model and the condition for photo-induced protein assembly was the same as SP1S98Y. As shown in AFM and TEM, no matter when the system was added $Ru(bpy)_3^{2+}$ alone or both $Ru(bpy)_3^{2+}$ and APS with white light illumination for 30 min, the morphology for this assembly system kept the original random packing, which was belonged to cricoid SP1W. These control groups illustrated that the effective assembly based on SP1S98Y was attributed to the introduction of target Tyr residues at the periphery.

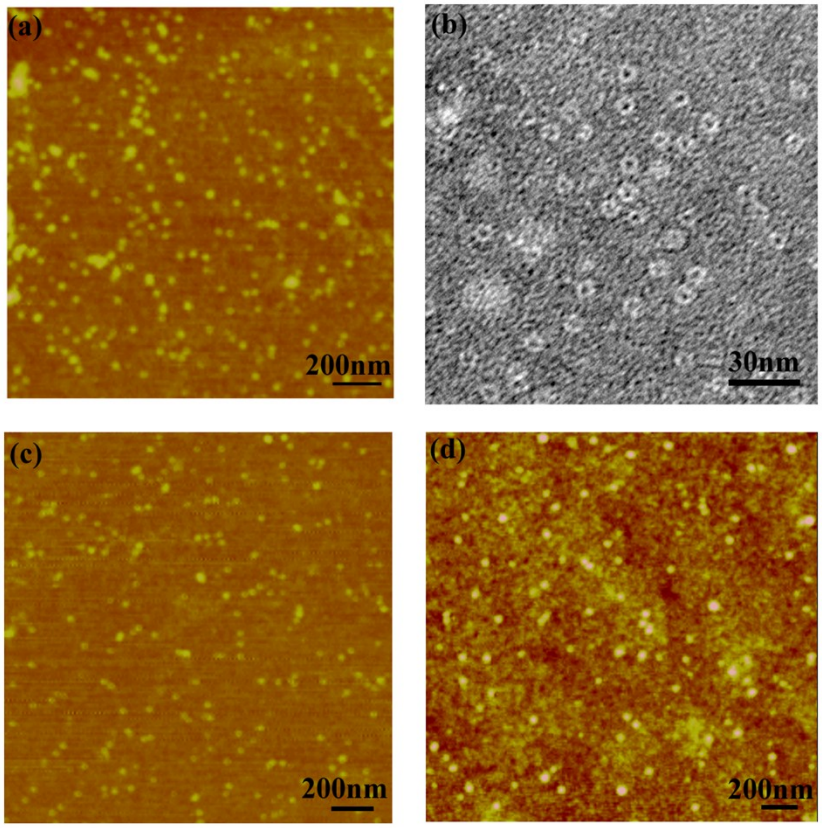

Figure S6: (a) AFM image of SP1W; (b) TEM image of SP1W; (c) AFM image of the system of SP1W+ $Ru(bpy)_3^{2+}$ with white light illumination for 30 min; (d) AFM image of the system of SP1W+ $Ru(bpy)_3^{2+}$+APS with white light illumination for 30 min.

Studies of the influence of the conditions ($Ru(bpy)_3^{2+}$, APS, light stimuli) on the SP1S98Y-based assembly system

We carried out a series of control groups to study the influence of the conditions to the assembly process. When high concentration of $Ru(bpy)_3^{2+}$ and APS were added into the assembly system, random aggregates with no regular morphology were obtained, whose height was about 20 nm, much higher than the width of SP1S98Y. In addition, there existed no obvious assemblies without light illumination, indicating the significance of light to this photo-induced assembly system. Interestingly, when simply utilizing $Ru(bpy)_3^{2+}$ to catalyze protein assembly, we can obtain relatively small sheet nanostructures compared to the synergetic catalysis of $Ru(bpy)_3^{2+}$ and APS. As far as we know, in this assembly mode, it underwent another different assembly mechanism. Photoexcited $Ru(bpy)_3^{2+}$ was known to be an efficient generator of singlet oxygen,[1, 2] which was expected to be the dominant reaction in the absence of APS. Not surprisingly, this pathway also yielded cross-linking products, but of a different nature and only after much longer irradiation.

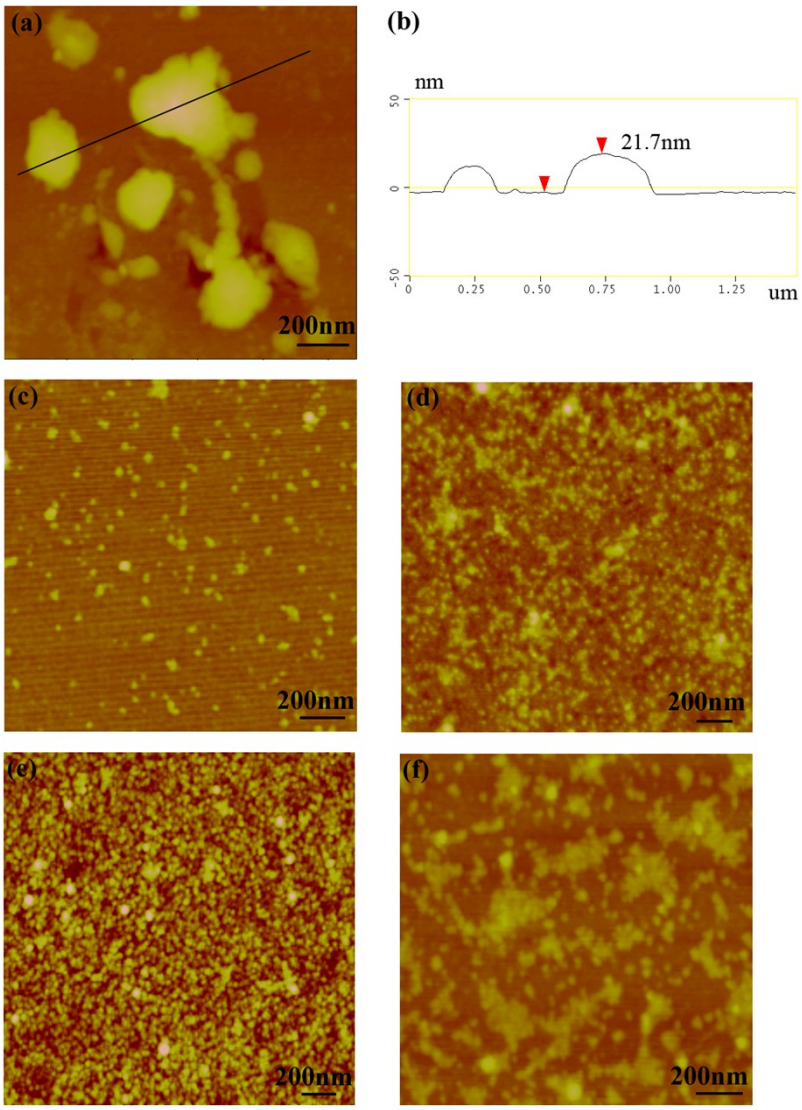

Figure S7: (a) Coassembly system of SP1S98Y with high concentration of $Ru(bpy)_3^{2+}$ and APS under white light illumination for 30 min; (b) Associated height profile along the black line in panel (a); (c) AFM image of free SP1S98Y; (d) SP1S98Y+ $Ru(bpy)_3^{2+}$+APS without light illumination; (e) SP1S98Y+APS with white light illumination for 30 min; (f) SP1S98Y+ $Ru(bpy)_3^{2+}$ with white light illumination for 45 min.

Studying the effects of the concentration of protein SP1S98Y on self-assembly size.

In order to investigate the effects of the concentration of protein SP1S98Y on self-assembly size, dynamic light scattering (DLS) analysis was utilized to characterize the hydrodynamic diameters of the assemblies. According to our knowledge, assembly system with the concentration of protein to a much high degree would be more likely to form random aggregates. So in order to obtain hierarchical nanostructures, we intended to study the concentration of protein SP1S98Y in a rational scope from 5×10^{-7} M to 10^{-5} M. As shown in DLS, when adding $Ru(bpy)_3^{2+}$ (1 uM) and APS (0.1 mM) to the assembly system with SP1S98Y in low concentration (5×10^{-7} M), only dimmers can be found after light illumination for 30 min. Under the same condition, increasing the concentration of model SP1S98Y can remarkably enhance the assembly size to about 500 nm with protein concentration at 10^{-5} M (Figure S8).

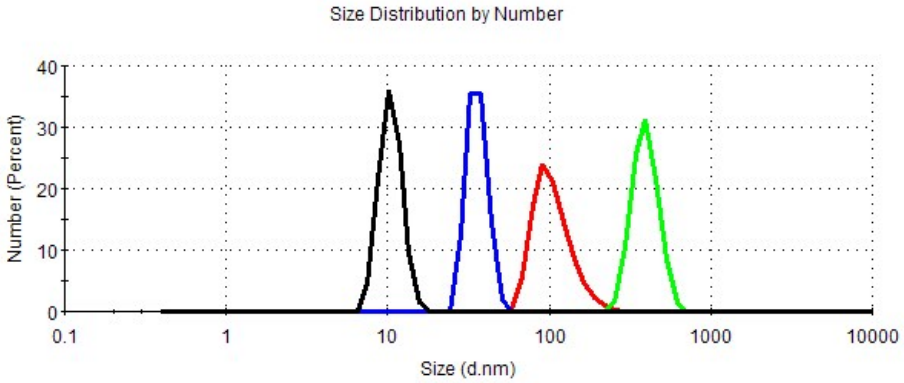

Figure S8: The dynamic light scattering (DLS) analysis of the hydrodynamic diameters of assembly system with different concentrations of protein SP1S98Y. The black line refers to free SP1S98Y and the other three lines respectively are assemblies of the concentration of SP1S98Y at 5×10^{-7} M (blue), 2×10^{-6} M (red) and 10^{-5} M (green) with white light illumination for 30 min under $Ru(bpy)_3^{2+}$ catalysis.

Investigation of the photo-induced reaction mechanism

In order to verify the coupling mechanism of photo-induced protein assembly, we chose tyrosine (Tyr) as model compound to develop a series of experiments. As shown in ^{1}H NMR spectra, after the addition of $Ru(bpy)_3^{2+}$ (0.05 mM) and APS (1.0 mM) to a D_2O solution of Tyr (1.0 mM) with light illumination for 10 min, the characteristic peaks (6.82 ppm, 7.15 ppm) that corresponded to the pure Tyr decreased, accompanied by the generation of three new peaks nearby, illustrating the effective Tyr-Tyr coupling upon photo-catalysis (Figure S9). Extending illumination time to the system resulted to the totally coupling and the three peaks (6.96 ppm, 7.08 ppm, 7.20 ppm) matched well with the products adopting a C-C coupling mode as predicted, which was a good proof to the mechanism described in Scheme 2.

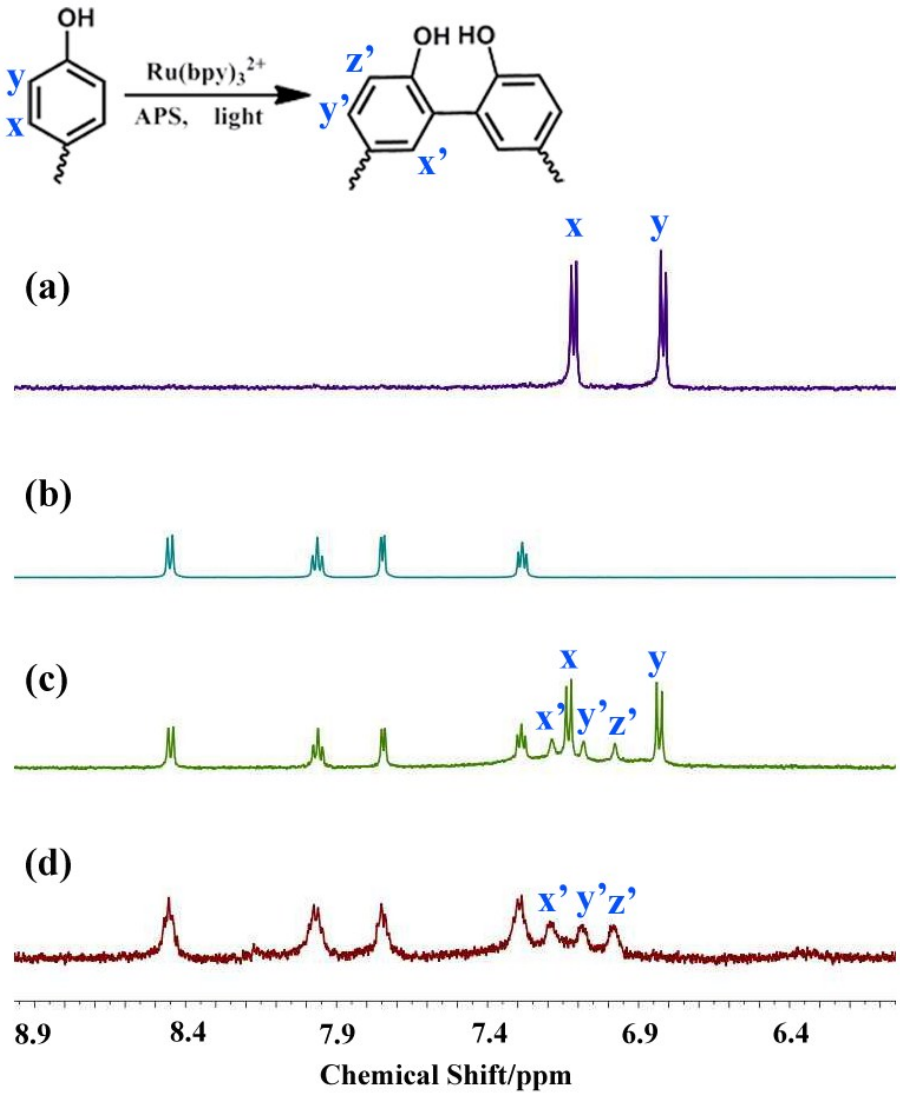

Figure S9: Partial ^{1}H NMR spectra of (a) pure tyrosine (Tyr); (b) pure $Ru(bpy)_3^{2+}$; (c) Tyr+$Ru(bpy)_3^{2+}$+APS with light illumination for 10 min; (d) Tyr+$Ru(bpy)_3^{2+}$+APS with light illumination for 30 min.

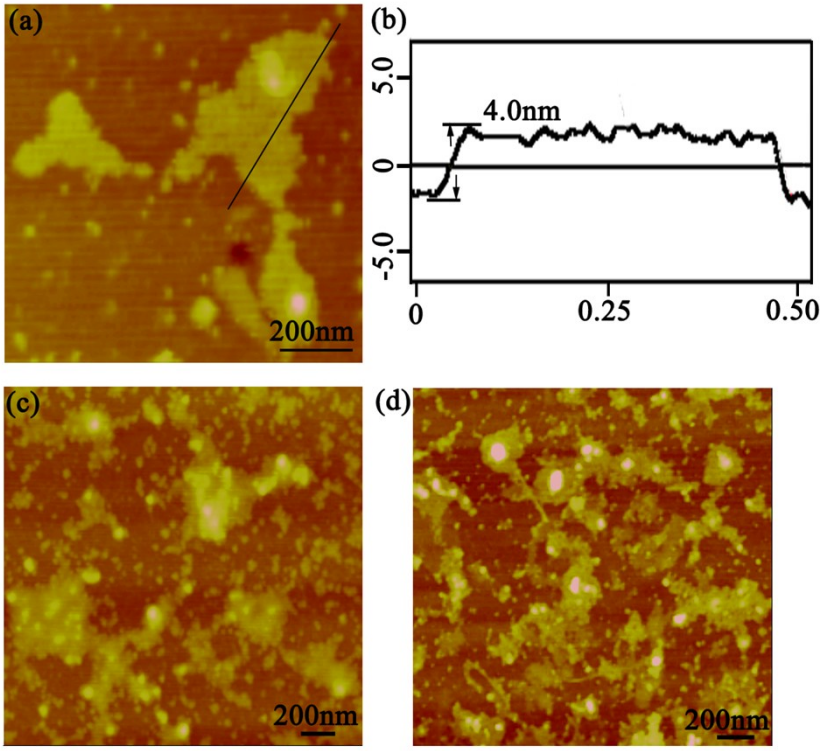

Figure S10: Thermal stability of the 2D nanoarrays. (a, c, d) AFM images of the assemblies after heat treatment at 60 °C, 70 °C, and 80 °C, respectively for 30 min; (b) associated height profile along the black line in panel (a).

References:

(1) X. Y. Zhang and M. A. J. Rodgers, *J. Phys. Chem.*, 1995, **99**, 12797–12803.

(2) C. Tanielian, C. Wolff and M. Esch, *J. Phys. Chem.*, 1996, **100**, 6555–6560.