Supporting Information

Assemble gold nanoparticles into flower by complementary base pairing of DNAs with mediation of apoferritins

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1. Experiments

1.1 Materials

Oligonucleotides (purified by HPLC), Coomassie blue R-250, Tris-HCl (1.5M, pH=8.8), Tris-HCl (1M, pH=6.8), protein marker and Acryl/Bis (29:1) solution (30% (w/v)) were purchased from Sangon Biotech Co., Ltd (Shanghai, China). Thiolated DNA strands A to F (Table 1), hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \bullet 3H_2O$, 99.9%), trisodium citrate ($Na_3C_6H_5O_7$, 99%), sodium borohydride ($NaBH_4$, 99.995%), hydrochloric acid (HCl, 1M), sodium hydroxide (NaOH, 99.998%), phosphotungstic acid ($H_3O_{40}PW_{12} \bullet xH_2O$, 99.995%), phosphate-buffered saline (PBS), sodium chloride (NaCl ≥ 99.5%), apoferritin (from equine spleen, 37mg/mL), N,N,N',N'-tetramethyl ethylene diamine (TEMED), ammonium persulfate (AR) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich Corp. (Saint Louis, US).

Table 1. Thiol modified DNA sequences of Strands A to F

Name	Sequence from 5' to 3'
Strand A	HS-$(CH_2)_6$-PO_4ACATCCGCTACTTGATGACTTAAGTGAGCCACTGAA
Strand B	HS-$(CH_2)_6$-PO_4TTCAGTGGCTCACTTAAGTCATCAAGTAGCGGATGT
Strand C	HS-$(CH_2)_6$-PO_4TTGGGAAGTAGCGACAGC
Strand D	HS-$(CH_2)_6$-PO_4GCTGTCGCTACTTCCCAA
Strand E	HS-$(CH_2)_6$- PO_4AGGCACCATCGTAGGTTTTTCTTGCCAGGCACCATCGTAGGTTTT TCTTGCCAGGCACCATCGTAGGTTTTT
Strand F	HS-$(CH_2)_6$- PO_4TCCGTGGTAGCATCCAAAAAGAACGGTCCGTGGTAGCATCCAA AAAGAACGGTCCGTGGTAGCATCCAAAAA

1.2 Methods

Ultraviolet-visible (UV-Vis) absorption spectra were recorded using a UV-2450 (Shimadzu Corp., Japan) at room temperature. Transmission electron microscopy (TEM) images were recorded on a JEM-2100 TEM (JEOL Ltd., Japan). An excessive amount of the sample solution was loaded on a carbon grid until the solution thoroughly spread on the grid. The grid was allowed to dry in air for at least 20 min. Then, a drop of 2 wt% phosphotungstic acid was loaded on the grid and spread thoroughly. The grid was again dried and stored in air before tests. The scanning electron microscopy (SEM) was conducted with an Ultra Plus microscope (Zeiss GmbH, Germany). Composition analysis was performed by using a scanning electron microscopy (Zeiss GmbH, Germany) equipped with energy dispersive X-ray spectroscopy (EDX) facility. The calcination of the flowers was performed in a SX2-12-10N muffle furnace (Shanghai Shengke Company, China). The thermal stabilities of assembled flowers were investigated on a TA Instruments (Netzsch, German) thermogravimetric analyzer under a nitrogen atmosphere. The sample was placed in an aluminum crucible and then was ramped from room temperature up to about 700 ºC at a heating rate of 10 ºC/min. The atomic force microscope (AFM) was conducted on Bruker Dimension ICON AFM using tapping mode. 20μL of the sample solution was dropped on a freshly cleaved mica surface and dried in air for at least 50min before AFM scanning. Native Polyacrylamide gel electrophoresis (native PAGE) was performed on DYY-6C (Beijing Six One instrument plant, China). The gel was prepared with 4% stacking gel and 7% resolving gel. The electrophoresis was performed at 80 Volts (about 14 mA) for 40 minutes at stacking gel and 120 Volts (about 25 mA) for 1.5 hours at 4 °C. After electrophoresis the gel was imaged by digital camera before and after staining with Coomassie blue R-250. The buffer system of the gel was Tris-glycine (0.025 M, pH 8.8).

1.3 Synthesizing AuNPs (diameter 5.1±0.7 nm)

First, the glasswares were soaked in aqua regia (3:1 v/v of concentrated hydrochloric acid and concentrated nitric acid,) for ca. 24 hours and then rinsed with distilled water.

Gold nanoparticles were prepared by adding $NaBH_4$ (17 μL, 20 mM) to a solution containing $HAuCl_4$ (0.25 mL, 10 mM), sodium citrate (0.25 mL, 20 mM), and freshly filtered water (9.5 mL). The mixture was vigorously stirred for five minutes, then stood for 2-4 hours in order to allow the hydrolysis of unreacted $NaBH_4$. After addition of $NaBH_4$, the color of the dispersion was reddish. The diameter of the obtained gold nanoparticles was about 5.1±0.7 nm.

1.4 Trapping AuNPs in apoferritin cages

Apoferritin solution (1ml, 1mg/mL) was incubated under acidic condition (pH = 2, adjusted with HCl at 1.0 M) at room temperature for 12 hours in order to break down the nanostructure of the protein into discrete subunits. Then the subunits solution was dript into AuNPs dispersion (c=1.8×10^{-8} M, 12.5 mL) slowly with magnetic stirring at low speed ([apoferritin]:[AuNPs] = 10:1). After the mixture was incubated at room temperature for at least 30 minutes, the pH of the mixture was adjusted back to pH=7-8 with NaOH (0.15 M). The resulting dispersion was incubated at least for 30 minutes. Subsequently, the excessive AuNPs was removed by ultracentrifugation at 13000 rpm for 20 minutes and the obtained product was redispersed in the buffer (pH 7.4) containing PBS (100 mM) and NaCl (100 mM). This step was repeated three times in order to remove the excessive AuNPs thoroughly.

1.5 Binding thiolated DNA strands onto apoferritin caged AuNPs

The dispersion of apoferritin caged AuNPs was divided into two equal parts. One part was mixed with an excessive thiolated DNA strand A (36 bases, 10 μM) at a molecular ratio of [AuNPs]:[DNA] = 1:20, while the other part was mixed with an excessive thiolated DNA strand B (36 bases, 10 μM) at the same molecular ratio. The bases on strands A and B were complementary to each other. A solution of phosphate buffer (pH 7.4, 1.0 M) and NaCl solution (1.0 M) was added to the two dispersions and the final concentration of the phosphate salts and NaCl were adjusted to 0.01 M and 0.1 M, respectively. The mixtures were shacked at room temperature overnight. In order to remove the excessive DNA, the dispersions were centrifuged at 13,200 rpm for 20 minutes, followed by the redispersion of the pellets in the reaction buffer (pH 7.4) containing PBS (100 mM) and NaCl (100 mM). The last step was repeated three times in order to remove the excessive DNA completely. Finally, the apoferritin caged AuNPs bound with DNA strands A and B were obtained and stored at +4 °C in Eppendorf tubes for next steps.

1.6 Self-assembling of apoferritin caged AuNPs bound with DNA into flower by complementary DNA base pairing

The apoferritin caged AuNPs bound with DNA strands A and B were mixed together. The mixture was heated to 10 °C above their melting points and cooling to room temperature slowly. The cooling process was conducted in a PCR instrument at a cooling rate of 1 °C per 10 min. After hybridization, one drop of the mixture was coated on a substrate (carbon support touch) and dried naturally at room temperature. Salts were removed using deionized water and the desalting process was repeated three times.

1.7 The melting point and content of AuNP caged in ferritin

The melting temperature of AuNPs was calculated using the equation: $T_m/T_{mb}=1-(\beta/d)$. [1] Where, $T_{mb}=1338$ K is the melting temperature of bulk gold, $\beta=1.1281$ nm. When the diameter of AuNPs is d=5 nm, the melting point of AuNP $T_m=1036$ K, i.e. 763 ºC. ([1] Nanda, K.K., S.N. Sahu, and S.N. Behera, Liquid-drop model for the size-dependent melting of low-dimensional systems. Physical Review A, 2002. 66, 013208:1-8.)

The mass of an apoferritin molecule was $481200/ (6.02\times10^{23}) =7.72\times10^{-19}$ g. The mass of an 5nm AuNP: $4/3\pi(2.5\times10^{-9})^3 \times19.32\times10^{6}=1.26\times10^{-18}$ g. The mass of an HS-DNA: $11224.4/ (6.02\times10^{23}) =1.86\times10^{-20}$ g. If each apoferritin molecule was grafted with five DNA molecules, the content of AuNPs in the flowers: $1.26\times10^{-18}/ (1.26\times10^{-18}+7.72\times10^{-19}+1.86\times10^{-20} \times5) =59.2\%$.

2. The additional supporting images

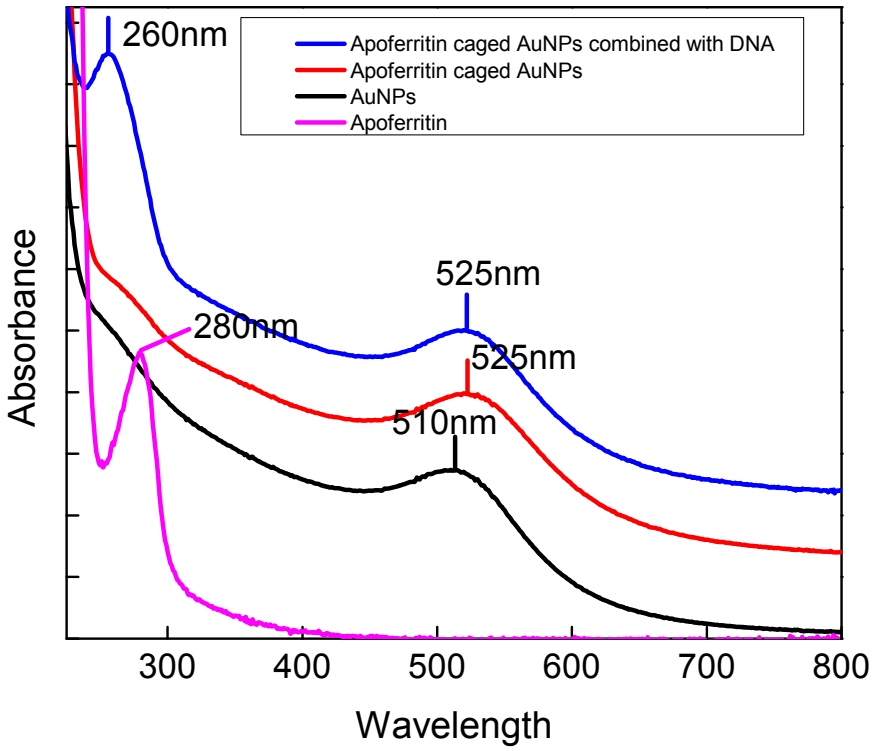

Figure S1 UV-Vis spectra of apoferritin (magenta), AuNPs (black), apoferritin caged AuNPs (red), and apoferritin caged AuNPs bound with DNA (blue). The characteristic peak of apoferritin was at 280 nm (magenta curve). The maximum peak of the AuNPs (diameter of 5.1±0.7 nm) appeared at 510 nm (black curve).The characteristic peak of apoferritin at 280 nm disappeared in the spectrum of the apoferritin caged AuNPs and the maximum peak of the AuNPs red shifted to 525 nm. In the case of the apoferritin caged AuNPs bound with DNA, the characteristic peak of DNA appeared at 260 nm and the maximum absorption peak of the apoferritin caged AuNPs remained at 525 nm.

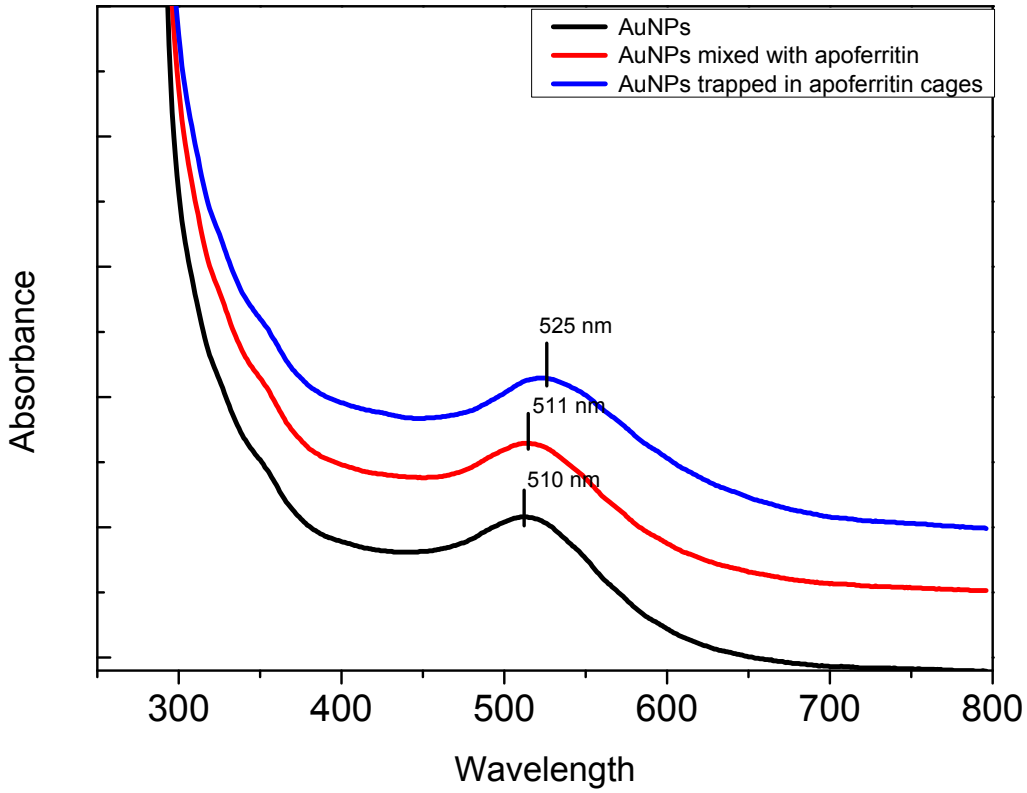

Figure S2 UV-Vis spectra of AuNPs (black), AuNPs mixed with apoferritin (red) and apoferritin caged AuNPs (blue). The maximum peak of the AuNPs appeared at 510 nm (black curve). After AuNPs and apoferritin was mixed and stood for several hours, the maximum peak of AuNPs (red curve) only red shift 1 nm. But when AuNPs was trapped in the apoferritin cages and the red shift value of the AuNP peak was 15 nm. It indicated that the AuNPs were trapped in apoferritin cages successfully instead of simply adsorption on surface of apoferritin.

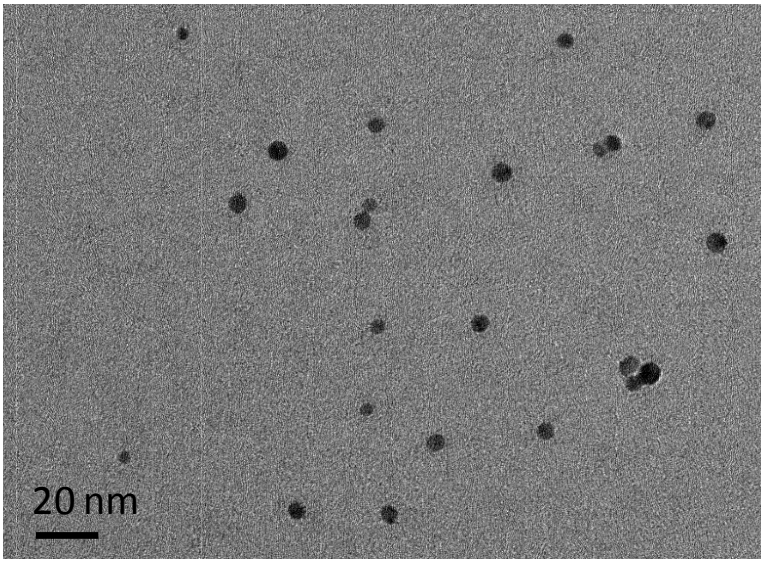

Figure S3. TEM image of AuNPs. The size of AuNP is 5.1±0.7 nm and the scale bar is 20 nm.

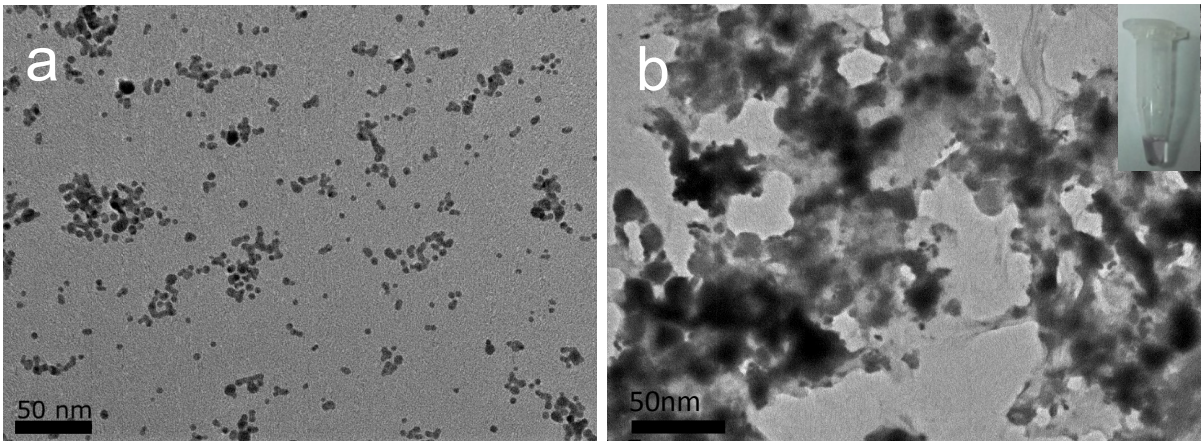

Figure S4. (a) AuNPs bound with DNAs in the absence of apoferritin as a control experiment. (b) When AuNPs (without apoferritin) bound with complementary DNA strands A and B were mixed and hybridized together, the color of the mixed dispersion turned from clear red to purple-black and some cloudy precipitate appeared at the bottom of the tube (inset of Figure S4b). The TEM image (Figure S4b) showed a random aggregation and no higher order assembling, indicating that the apoferritin cages played a crucial role during the self-assembling of AuNPs bound with DNA.

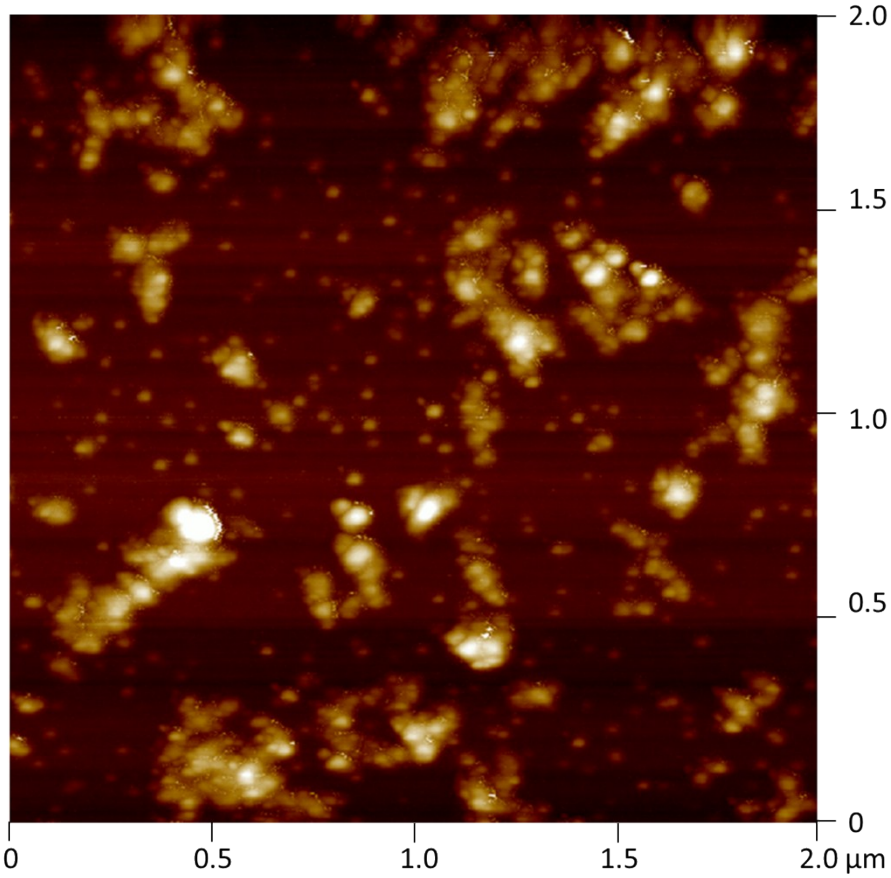

Figure S5. AFM image of apoferritin caged AuNPs bound with DNA. Several AuNPs are connected to each other through complementary base pairing of DNA.

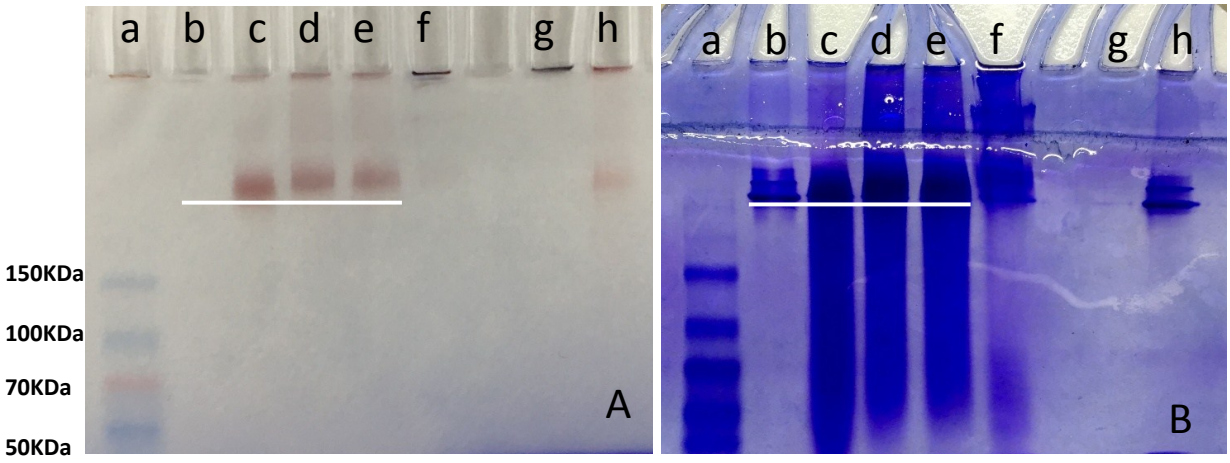

Figure S6. Native polyacrylamide gel (native-PAGE) image prior to (A) and after (B) staining with Coomassie blue. Lane **a** represents protein markers (Double color pre dyeing protein marker) and their corresponding molecular masses. Lane **b** is apoferritin. Lane **c** represents AuNPs trapped in apoferritin cage. Lane **d** and lane **e** represent apoferritin caged AuNPs combined with DNA strands A and B, respectively. **L**ane **f** represents apoferritin caged AuNPs assembled though DNA base paring. Lane **g** represents AuNPs. Lane **h** is AuNPs mixed with apoferritin without dissociation and recombination process.

Both apoferritin (lane **b**) and apoferritin caged AuNPs (lane **c**) exhibited same electrophoretic mobility in Figure S6B, suggesting that the overall charge of the apoferritin was unchanged and the protein cage remained intact during the trapping process. In addition, Lane **c** in Figure S6A was visibly red (indicating association between the protein and AuNPs) prior to staining. Lane **d** and lane **e** also showed the similar phenomenon to lane c from image Figure S6A. In addition, lane **d** and lane **e** lagged behind lane **c**, which indicated that caged AuNPs combined with DNAs successfully. The assembled structure did not run out of the well (lane **f**) because of aggregation. AuNPs did not run out of the well (lane **g**), it suggests that AuNPs were aggregated in the gels. From Figure S6A lane **h**, a small number AuNPs run out of the well which means only small part of AuNPs was adsorbed on apoferritin.

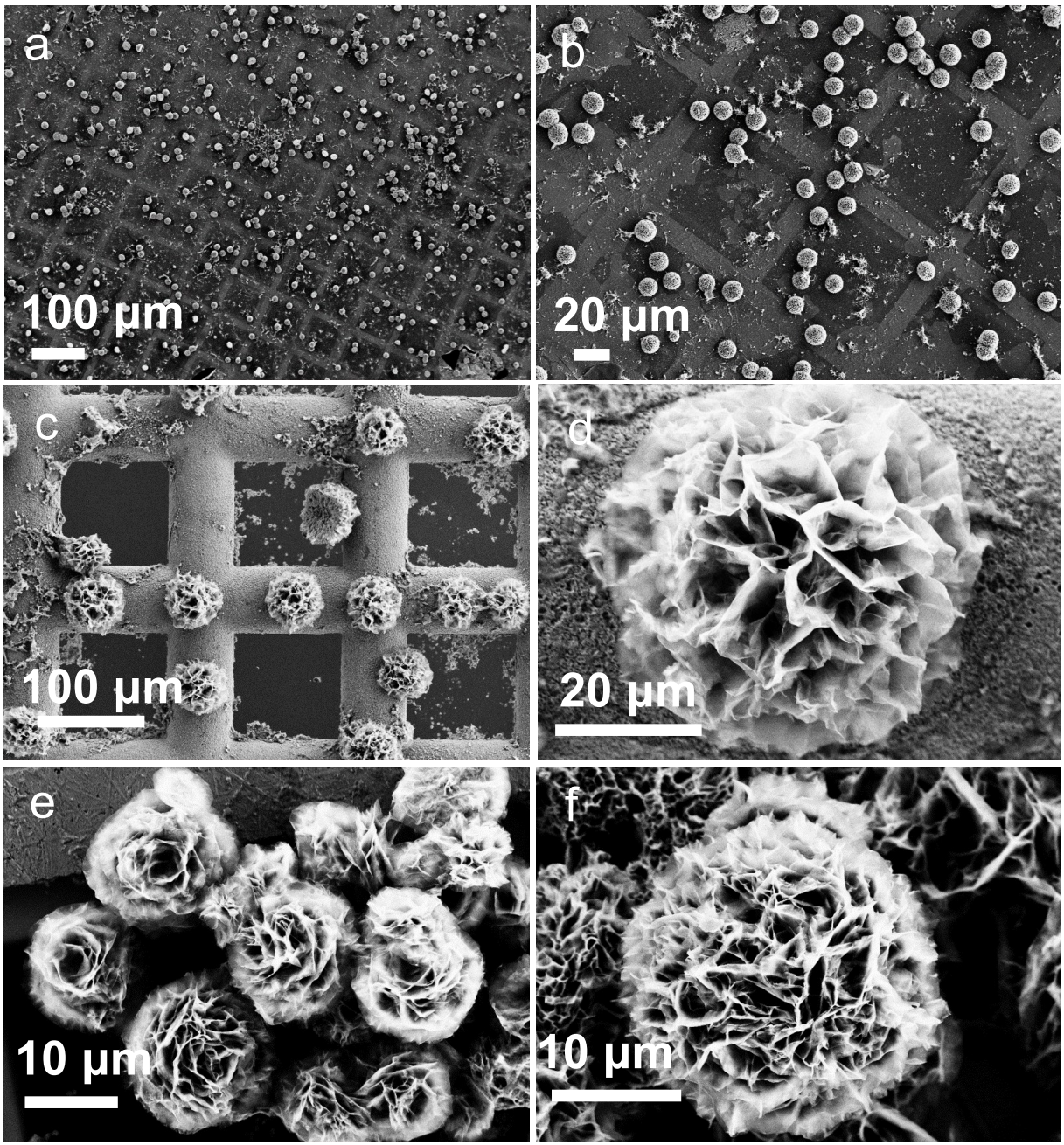

Figure S7. Additional SEM images show the obtained flowers by complementary pairing of DNA with 36 bases.

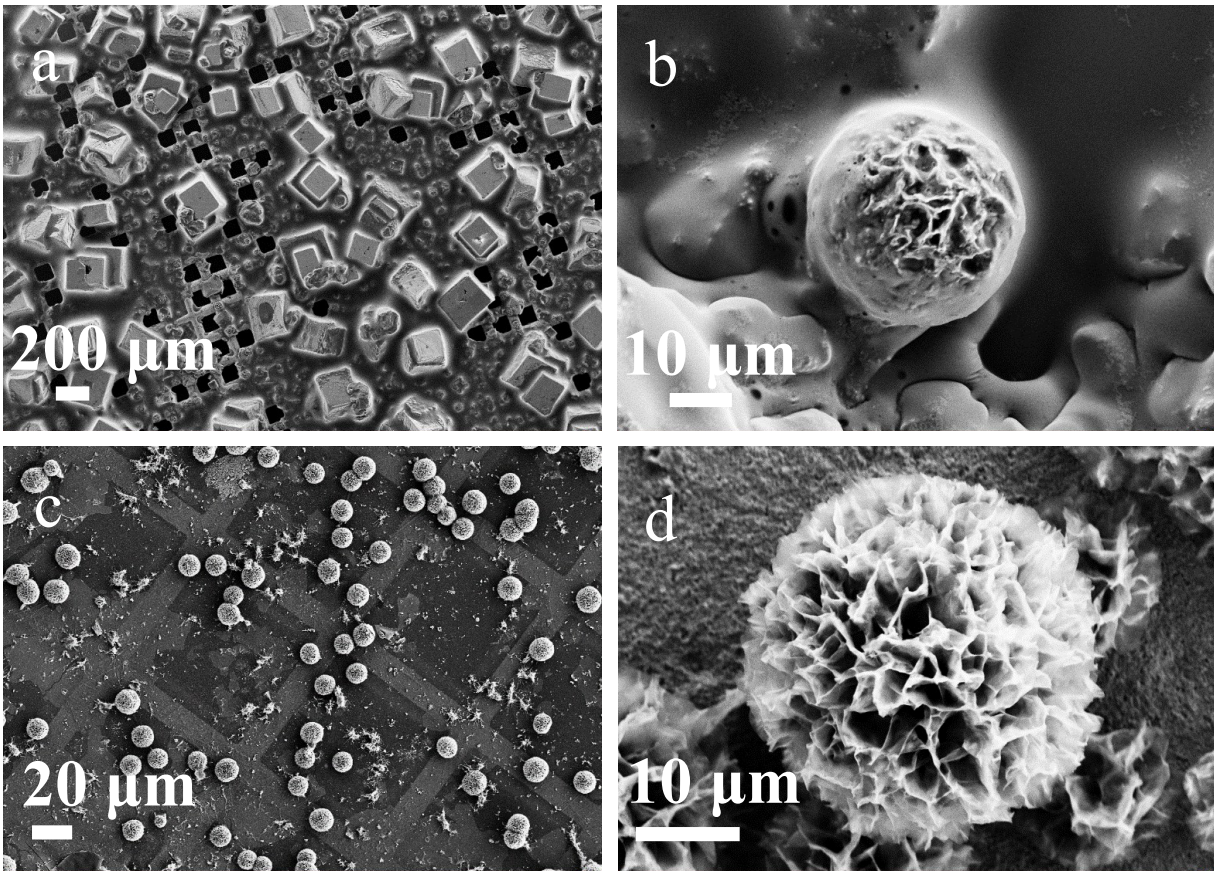

Figure S8. SEM images show the assembled flowers before (a-b) and after (c-d) salt was removed by water rinsing.

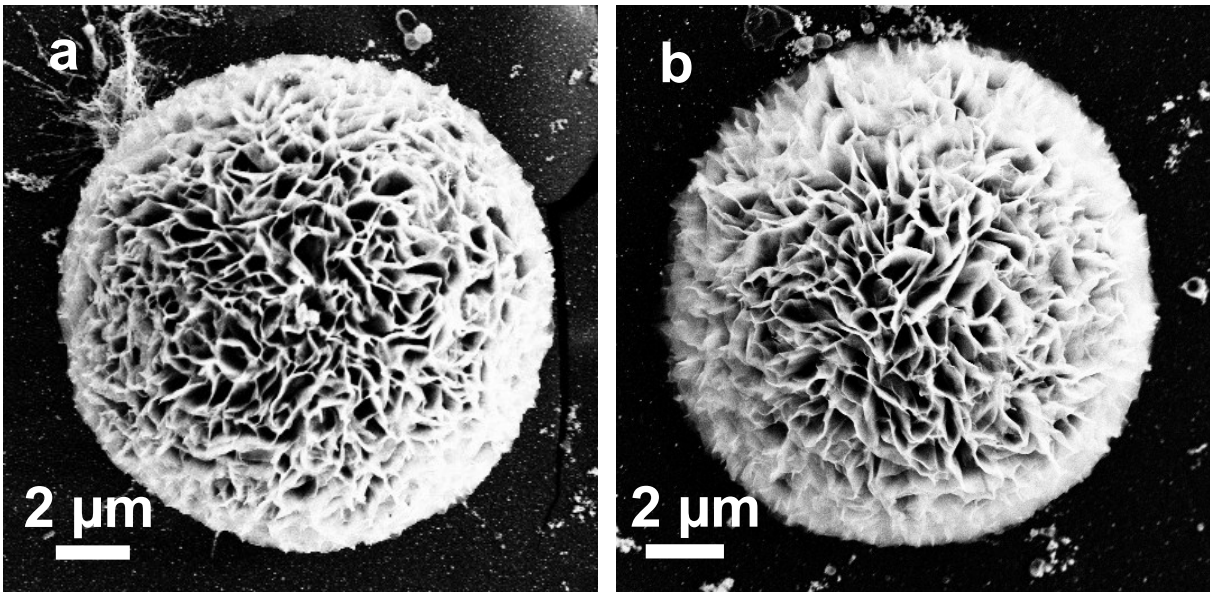

Figure S9. SEM images show the newly assembled flower (a) and the flower prepared 2 months ago (b).

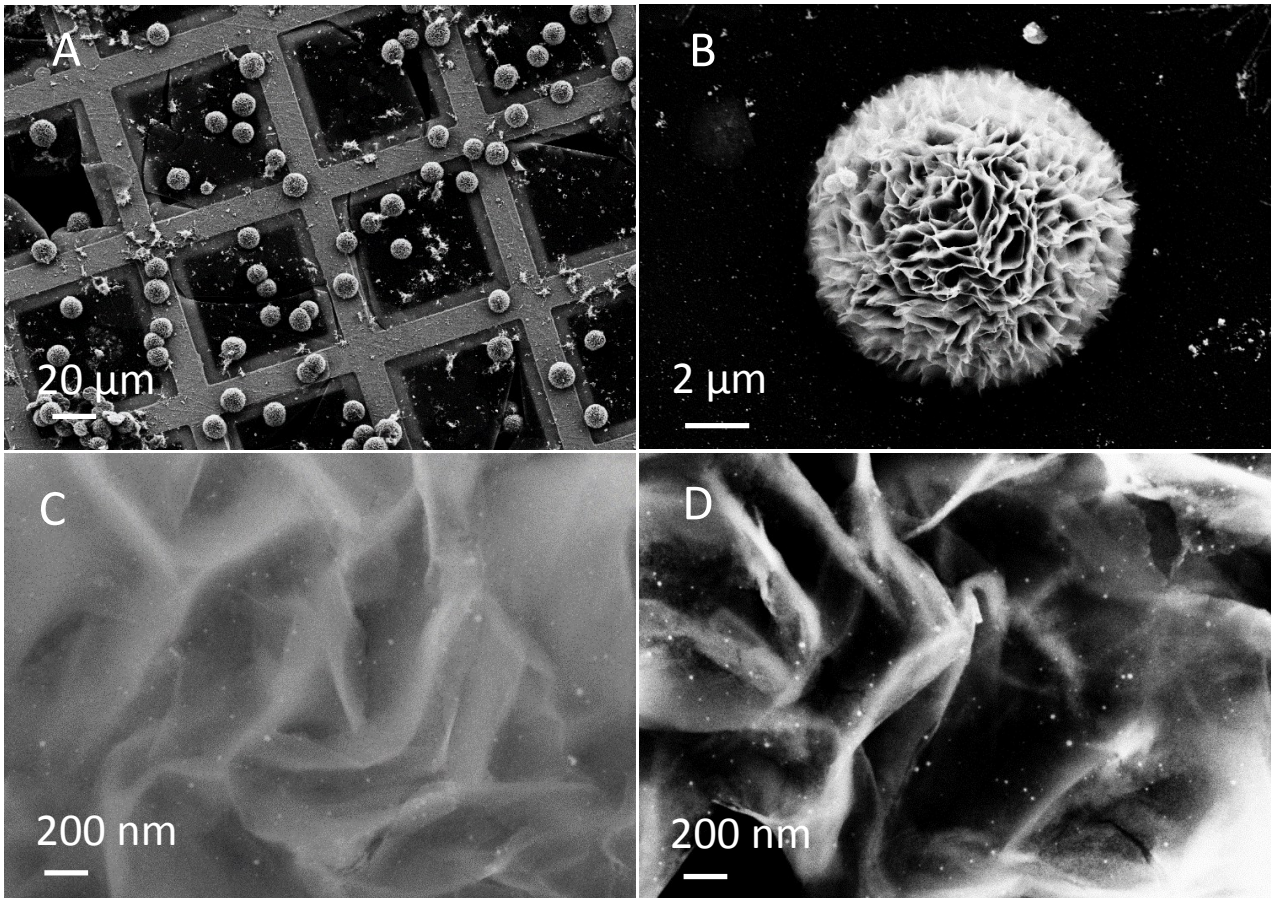

Figure S10. The SEM images of the flowers that were calcinated at 350 °C for 2 hours. From Figure S10A and B, the morphology of the flowers showed no changes after calcination. Figure S10 C and D are partial enlarged images of the flowers. From them, some of the gold nanoparticles (white dots) were observed.

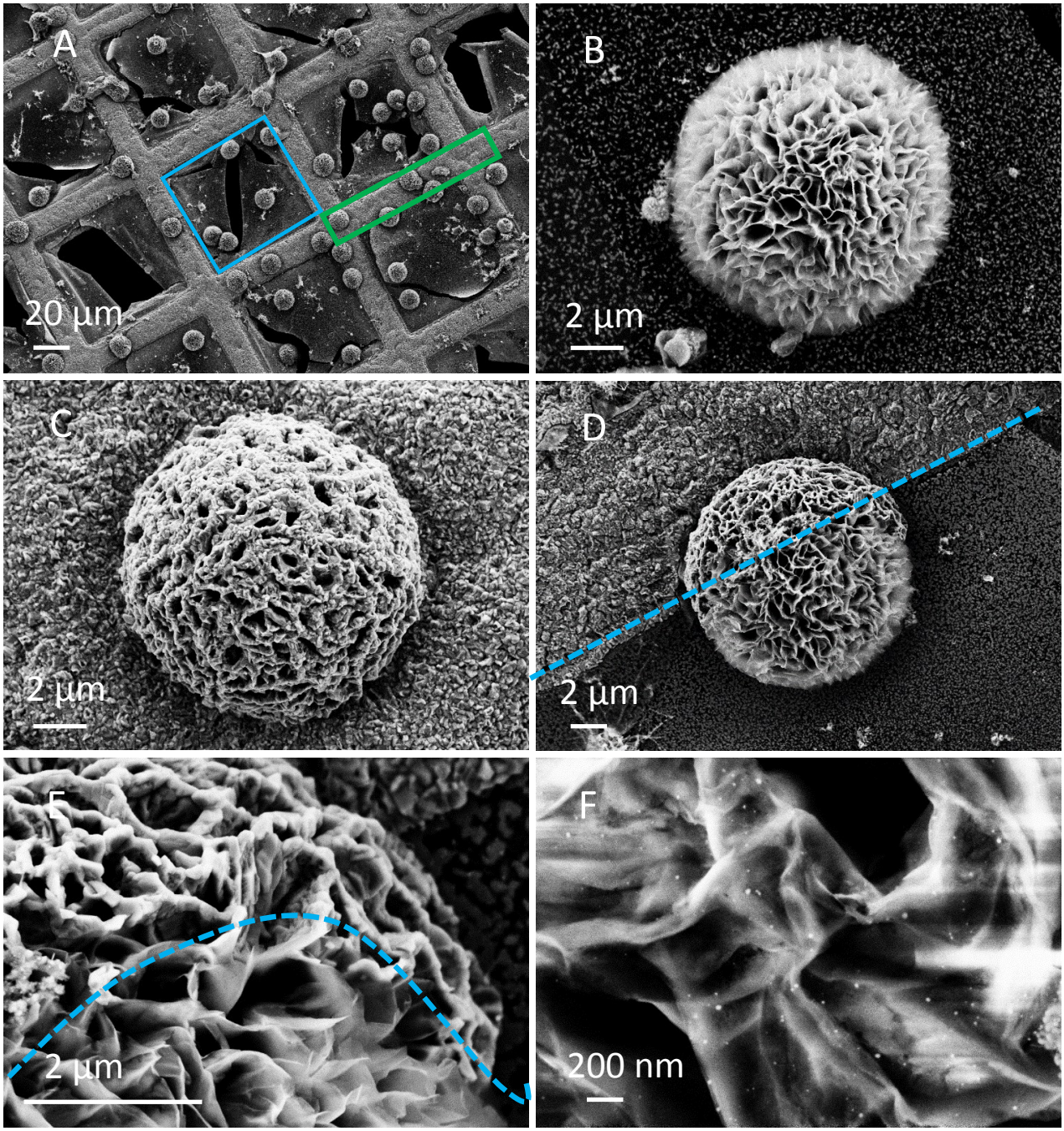

Figure S11. The SEM images of the flowers were calcinated at 500 °C for 2 hours. The flower on the carbon film (the blue square in Figure S11A) was shown in Figure S11B, which nearly didn't change. And the flower on the copper grid (the green rectangular area in Figure S11A) was shown in Figure S11C, which was covered by copper. The follower in Figure S11D and E were on the border between carbon and copper grid, which indicated the difference clearly. Figure S11E is the amplification of Figure S11D. Figure S11F is the amplification of Figure S11B, some Au NPs were found on the petal of the flower.

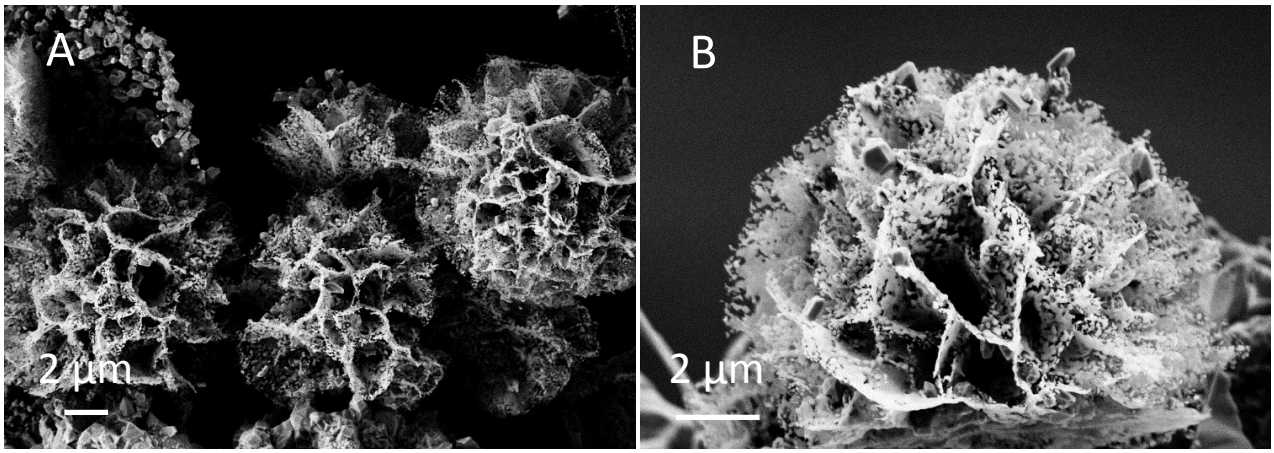

Figure S12. The SEM images of the flowers was calcinated at 600 °C for 2 hours. The petals of the flowers were still there but became porous because the organic materials were evaporated.

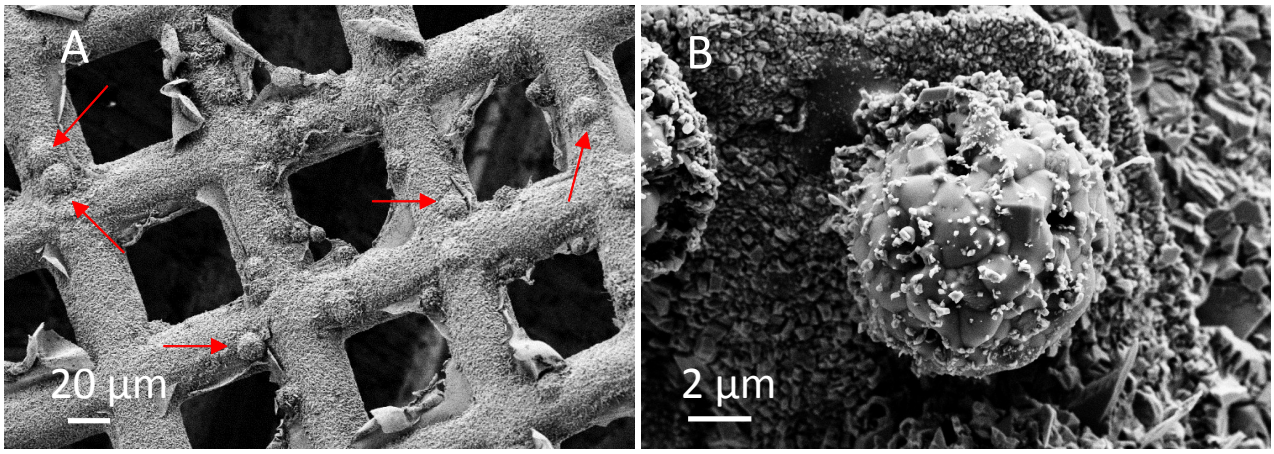

Figure S13. The SEM images of the flowers were calcinated at 700 °C for 2 hours. From Figure S13A, the carbon film were burned down but the flowers. But the flowers on the copper grid were still there and marked by red arrows. Figure S13B is a flower on the copper after calcinating. The EDX results (The results were not shown here.) showed that it was completely covered by copper.

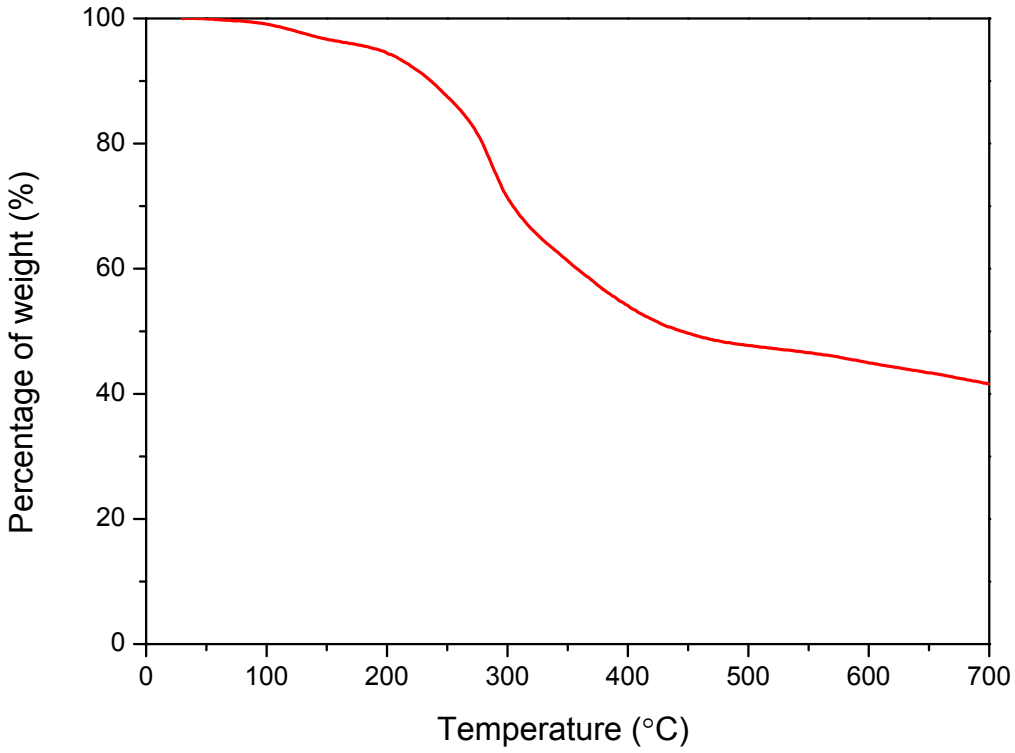

Figure S14. Thermogravimetric curves of the assembled flowers. The flowers underwent about 50 wt% of weight loss in the temperature below 500 °C, which was due to the evaporation of organic materials in the flowers. The flowers still got more than 40% of weight even when the temperature reached 700 °C, which should attribute to AuNPs.

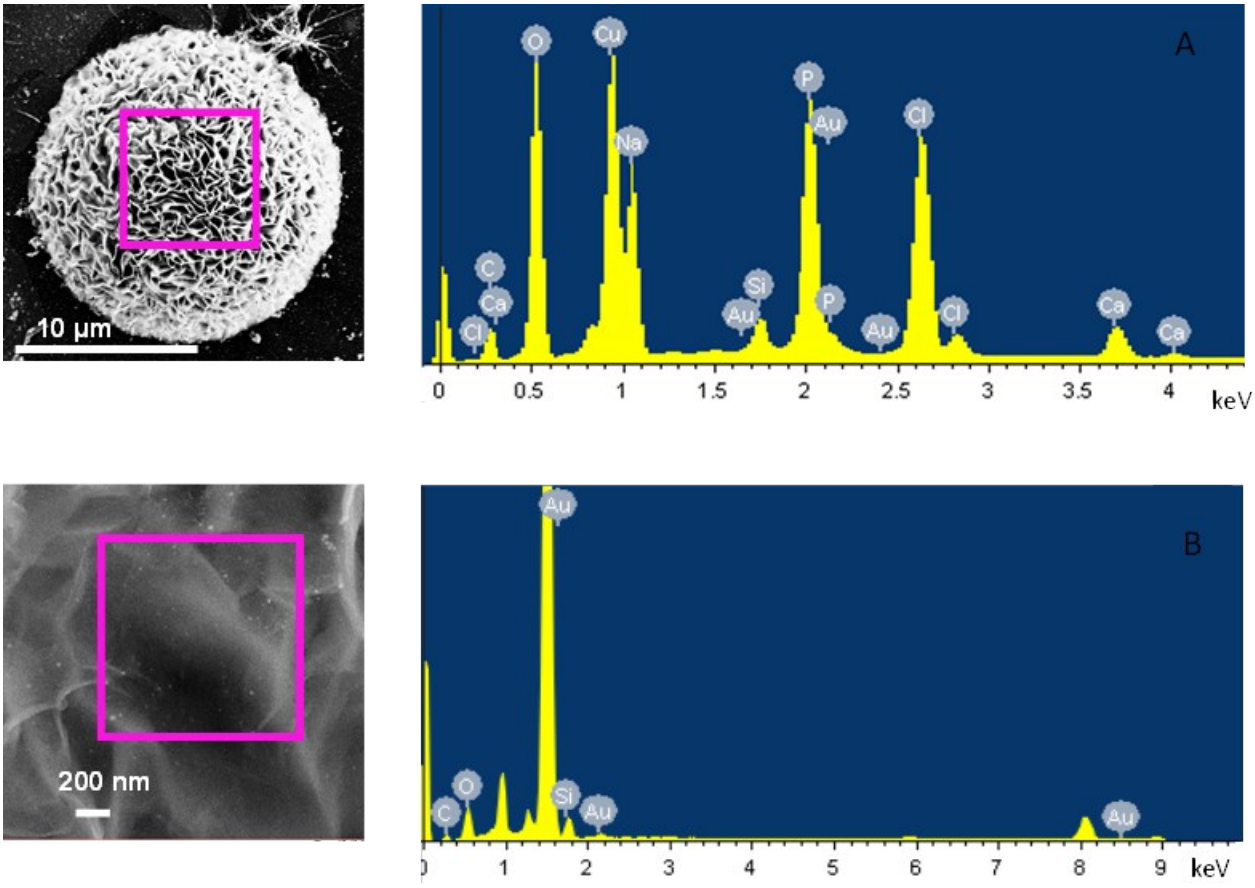

Figure S15. The EDX spectrum of the AuNPs self-assembly flower before (A) and after calcination (B) at 500 °C. The purple section is tested for the selected region.

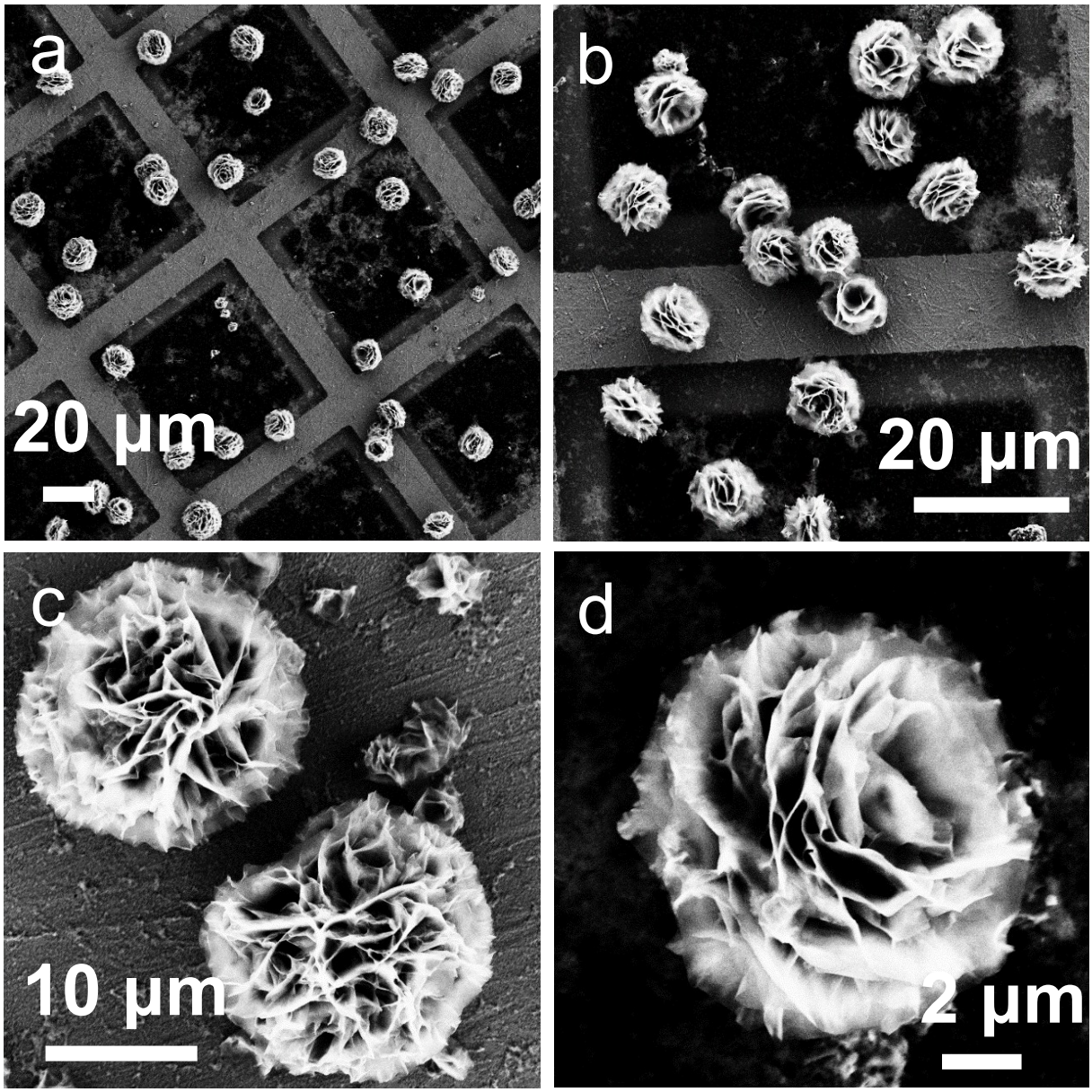

Figure S16. Additional SEM images show the obtained flowers using 72-base DNA. Scale bars are 20 μm for (a) and (b), 10 μm for (c) and 2 μm for (d).

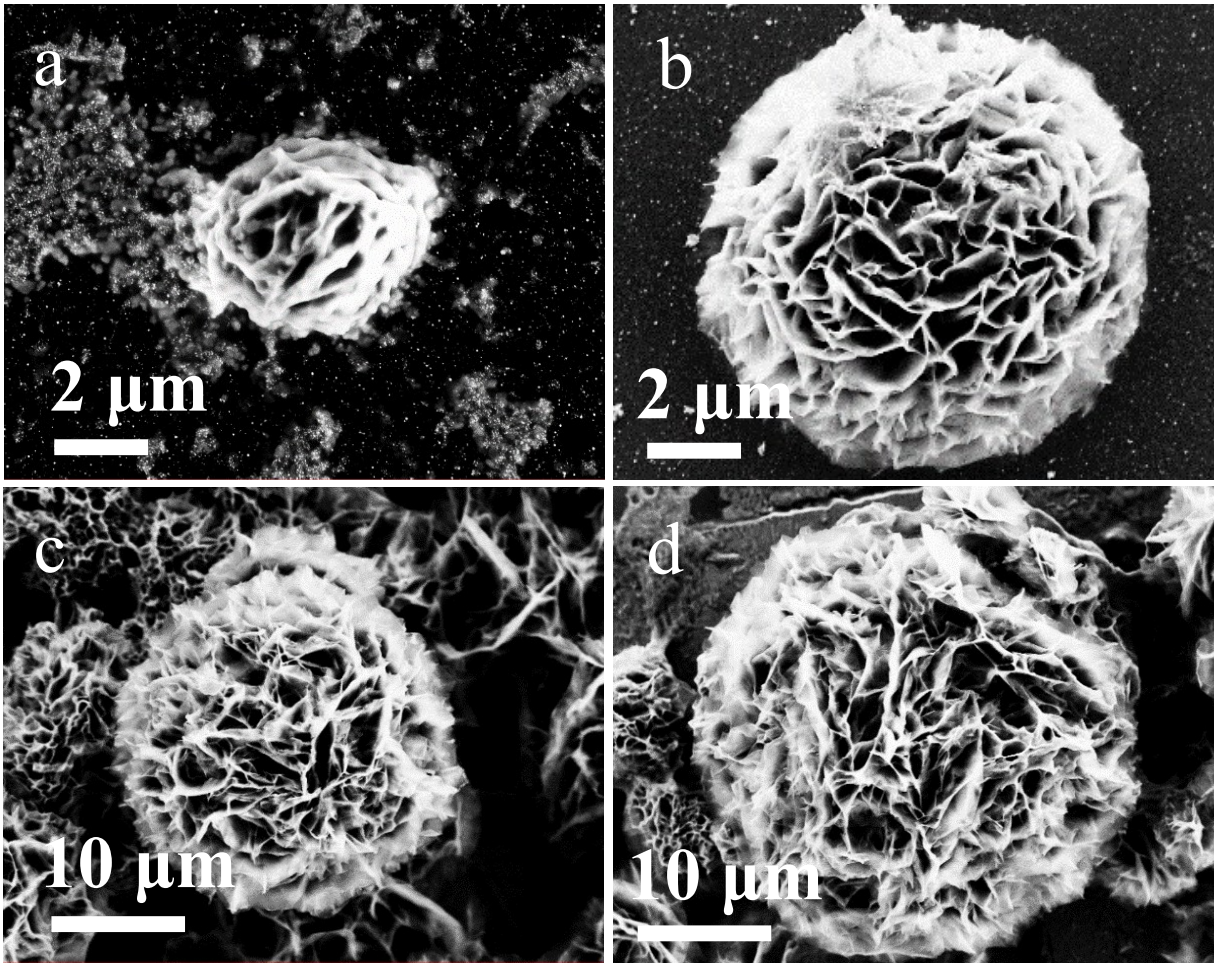

Figure S17. SEM images show the growing process of flowers. Flowers with different sizes were observed at the different growing stages. Images a-d show the diameter of flower increased gradually from 2.9 µm to 7.0 µm, 17 µm and 34 µm. Scale bars are 2 µm for (a) and (b), 10 µm for (c) and (d).