Supporting Information

for Small, DOI: 10.1002/smll.201400470

Virus-Templated Plasmonic Nanoclusters with Icosahedral Symmetry via Directed Self-Assembly

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S1. Materials and Methods

All chemicals were Reagent Grade or better used as received from USA sources listed below.

Deionized water (18.2 MΩ-cm) was prepared by passing water through an Elix® 5 Milli-Q Plus Ultra-Pure Water System (Millipore Corp.). All buffers were filtered sterilized using a Nalgene filter 0.45 μm pore size for sterilization. For the purpose of the supporting information we denote gold nanoparticles as Au-NPs or simply nanospheres, NSs. Cowpea mosaic virus is classified as biological safety level 1 (BSL-1). Our institutional requirements include usage of protective clothing and gloves during manipulation of the samples. All solid waste is disposed as biohazard and liquid waste treated with 20% bleach and treated as hazardous waste.

S1.1. Nanoclusters (NCs) assembly and purification

S1.1.1 Synthesis of gold nanoparticles:

Preparation of 60 mM sodium citrate (SC):

0.9 g of sodium citrate tribasic dihydrate (294.1 g/mol; Sigma-Aldrich, St. Louis, MO) was mixed with 50 ml of Milli-Q water in a Falcon tube. Depending on the age of the water used the pH of the sodium citrate solution may change over time affecting the resulting size of the NS. [1]
We performed several experiments and the optimum SC solution was the one prepared one day before Au-NP synthesis with Milli-Q water pH ~ 6. (Milli-Q water stored in a sealed glass bottle at room temperature (RT) for more than a week). Upon dilution of the 60 mM SC solution (pH 8.3) to 2.2 mM with Milli-Q water, the pH of the resulting SC solution was measured as 7.3.

**Synthesis of NS, Inverted method**

Puntes method \(^2\) was slightly adapted for our application. Specifically, 5.5 ml of 60 mM sodium citrate was diluted to 2.2 mM in a final volume of 150 ml with Milli-Q water in a round bottom flask equipped with teflon stirbar and a condenser (closed system to avoid evaporation). The solution was heated for 15 min using a stirrer and upon boiling, 1 ml of 25 mM hydrogen tetrachloroaaurate (III) in water was added (HAuCl\(_4\) · 3H\(_2\)O: Sigma-Aldrich St. Louis, MO; 25 mM stock solution was aged for more than one month at RT in the dark prior to use). After 10 min boiling, the color of the solution changed from yellow to gray to pink. The temperature was decreased to 90°C after which 1 ml of the RT 60 mM sodium citrate was added and stirred for 2 min, then 1 ml of HAuCl\(_4\) 25 mM was added to the mix. The solution was stirred vigorously at 90°C for 30 min. The resulting Au NS dispersion was cooled down at RT overnight. For long term storage the NS dispersion was kept in the dark at 4°C. The NS dispersion was aged for at least one week prior to reactions with BC-CPMV. NS size was determined by DLS and TEM and the concentration in particles/ml was determined from its absorbance at 525 nm by using a calibration curve prepared with NS controls of known concentration.

**S1.1.2 BC-CPMV preparation**
BC-CPMV was supplied by John E. Johnson’s laboratory at The Scripps Research Institute. All reagents were purchased from Thermo Fisher Scientific, Waltham, MA unless otherwise specified.

BC loop cysteine mutant CPMV was cloned and inoculated into plants as described by Wang et al. 2002. [3] Mutant CPMV was propagated by grinding infected tissue with diatomaceous earth and lightly rubbing the lysate onto seven-day old plants. Infected leaves were harvested and frozen at -80°C two weeks after inoculation.

The reactive cysteine mutant particles were prone to irreversible precipitation, therefore it was important to do each step as quickly as possible and to keep the samples at 4 °C throughout the purification. Frozen tissue was crushed by hand and blended in a Warring blender (Warring, New Hartford, CT) in virus buffer, 0.1 M NaPO₄ pH 7.0 with 10 mM DTT (dithiothreitol (DTT), Akron Biotech, Boca Raton, FL.) The whole cell lysate was centrifuged for 15 minutes at 10,000 g to pellet insoluble plant tissue. The supernatant was filtered through cheese cloth and 0.7 times the volume of 1:1 chloroform:butanol was added and stirred for 30 minutes. The mixture was centrifuged at 10,000 g for 15 minutes and the top aqueous layer was collected. BC-CPMV was precipitated by adding 20% PEG 6,000/1M NaCl to the aqueous layer to reach a final concentration of 5% PEG 6,000/0.25 M NaCl in the solution. The mixture was incubated at 4°C for 30 minutes followed by centrifugation (30 min, 15,000 g, and immediately resuspended in virus buffer. The pellet containing BC-CPMV was resuspended in virus buffer and centrifuged again at 20,000 g for 15 minutes to remove insoluble material. The supernatant virus was pelleted in an ultracentrifuge at 45,000 rpm in a 50.2 Ti rotor (Beckman Coulter, Inc, Brea, CA) for 2.5 hours and immediately resuspended in the virus buffer. The BC-CPMV solution was then loaded onto a 10-40% w/v sucrose gradient and spun at 28,700 g in a SW 32 Ti rotor (Beckman Coulter,
Brea, CA) for 2 hours. Visible BC-CPMV bands were collected into sterile tubes and stored at 4 °C. The concentration of virus was kept below 1.0 mg/ml to prevent aggregation. The concentration of BC-CPMV is determined from the absorbance peak at 260 nm (absorbance at 260 nm)/8 = concentration of virus in mg/ml. \[4\]

CPMV stability and aggregation were analyzed using a superpose 6 10/300 GL chromatography column on an AKTA purifier (GE Healthcare, Piscataway, NJ.) Roughly 100 μg of each sample was diluted to 500 μL and filtered using 0.22 μm spin filters (EMD Millipore, Billerica, MA) prior to running at 1.0 ml/min with a 0.1 M sodium phosphate buffer pH 7.0 (without DTT) mobile phase.

For long term storage BC-CPMV is stored at 4°C at concentrations of 0.2-0.5 mg/ml in 10 mM DTT, 10 mM sodium phosphate buffer, 140 mM NaCl, pH 7.2, 15% sucrose as a storage buffer. Just before the reaction with NS, 100 μl of 0.25 mg/ml BC-CPMV (in the storage buffer) was diluted up to 1.5 ml with 10 mM potassium phosphate (KP), pH 6.0. The solution was filtered via a syringe filter (0.2 μm, 25 mm PES-polyether sulfone membrane, Fisher Scientific, Pittsburgh, PA). Then 1.5 ml of filtered BC-CPMV was loaded in a pre-packed Hi-Trap Desalting Column (GE Healthcare Biosciences, Piscataway, NY) pre-equilibrated in10 mM KP pH 6.0. The flow through is discarded and the BC-CPMV is found in the first 1.5 ml elution and concentration determined by its absorbance at 260 nm.

**S1.1.3 NSs and BC-CPMV reaction**

*(From this point on BC-CPMV will be simply referred as BC.)*

A 40 ml aliquot of NSs (3 x 10^{11} particles/ml) prepared by the inverted method *(section S1.1.1)* was concentrated to 600 μl using 100 k MWCO concentrators (Amicon Ultra-15, Fisher
Scientific) by centrifuging at 4,000 rpm for 2 min at RT (Eppendorf Centrifuge 5810 R equipped with a swing-bucket rotor). In this example 1171 μl of the NSs filtrate (recovered after concentration) was mixed with a solution containing 200 μl of 100 mM KP pH 6.0, 4 μl of 500 mM EDTA pH 6.0, and 25 μl of 0.02 mg/ml BC (from Hi-Trap column in 10 mM KP pH 6.0). The concentrated NSs (600 μl) dispersion was added to the solution containing BC in 50 μl increments with manual mixing by inverting the tube between additions. The reaction was incubated at RT in the dark for 36 h.

Basic water was prepared by adding μl amounts of 2 M KOH to Milli-Q water. The optimum working pH was between 8.5-9.0. To 2 ml of basic water in a 20 mL glass vial containing a Teflon stirbar, 10 μl of 20 mM thioctic acid (TA Fisher Scientific; dissolved in 8:2 v/v ethanol:water) was added. The contents were stirred vigorously using a magnetic stirrer. The reaction mixture was added slowly to the stirred basic water/TA mix in 100 μl increments and stirring was continued at RT for 4 h. The reaction mix after TA treatment was stored overnight at 4°C. The next day, 200 μl of 10 x Tris-borate-EDTA buffer (10 x TBE: 890 mM Tris, 890 mM boric acid, 20 mM EDTA (pH 8.0)) were added to the reaction mix and concentrated to 500 μl using the 100 k concentrators. A 50 μl aliquot of 50:50 v/v glycerol in water was added and 60 μl of the mixture were loaded per well into a 1% low melting agarose/ 1x TBE electrophoresis gel. The gel was run at 100 V until a clear separation between the free NSs and BC-Au bands was achieved. This can be seen visually without need of staining the gel since the NSs have a distinct red color.

**SI.1.4 Recovery of the BC-Au from agarose gels.**

Based on the method suggested by the β-agarase manufacturer, New England Biolabs
The BC-Au bands were cut from the gel (~200 mg gel/band) and transferred to a 2 ml Eppendorf tube. Gel pieces were equilibrated in 10 mM Bis-Tris, 1 mM EDTA, pH 6.5 buffer by adding 400 µl the buffer and incubating for 30 min at RT. (This was done 2x and buffer was discarded after each incubation). After buffer equilibration the agarose was melted at 70°C for 5 min. The agarose was cooled down to 42°C for 10 min, after which 2 µl of β-agarase enzyme (1000 units/ml, New England Biolabs, Ipswich, MA) were added and mixed gently. The enzymatic reaction was carried at 42°C for 4 h followed by an overnight incubation at RT. After digestion the mixture was stored at 4°C until ready to use. The NCs are stable for at least two weeks under these conditions. However, after washes with water they do aggregate more readily. The NS controls are less stable than the NCs after purification and washes since they do precipitate irreversibly while the NCs can be resuspended with gentle mixing. Prior to characterization the digested agarose was washed with Milli-Q water 2x using the 100 k centrifugation filters to remove any agarose residue and to exchange to water prior to UV-VIS, DLS, and TEM characterization. For negative controls, a similar procedure was used to recover free NSs from corresponding gel pieces. Free NSs were characterized as well via DLS, TEM, and UV-VIS spectroscopy. Number of particles per ml in NCs and free NSs was calculated from UV-VIS data using calibration curves. Calibration curves from control NSs were used as described in section S1.1.1. The contribution of the BC to the plasmon peak in NCs is minimal therefore, calibration curves were considered to be valid.

S1.2. Dynamic light scattering (DLS)

Dynamic light scattering (DLS) measurements were performed in a Brookhaven Instrument (ZetaPALS 90Plus/B1-MA). Data acquisition included five replicate measurements per sample.
for subsequent averaging of measured hydrodynamic diameters in 3 ml volume cuvettes (4 clear
sides and 1 cm path-length plastic cuvettes). Data is reported as the relative intensity of the
species of different sizes present in the solution.

S1.3. Transmission electron microscopy (TEM)

The samples were prepared by placing a 10 μl drop of the suspension onto a SPI 200 mesh
holey-carbon Cu TEM grid for 2 min. The drop was then wicked off and the sample was
allowed to dry for 2 hrs. The TEM imagery was done using a JEOL JEM-2200FS field emission
electron microscope.

S1.4. Spectroscopic Characterization

Absorbance measurements were carried out using unpolarized white light from a light source
(OceanOptics DH-2000) passing normally through a 10 mm optical path length cuvette. The
transmitted light was collected with a 400 μm core diameter fiber optic cable connected to a
spectrophotometer (OceanOptics USB4000). A Cary5000 (Agilent Technologies, Santa Clara,
CA) equipped with a NIR detector was used as well for absorbance measurements.

S1.5. Finite-element simulations

Three-dimensional finite-element simulations were undertaken using COMSOL Multiphysics
4.3a. Gold NSs, with refractive indices interpolated from the literature,[6] were placed at the
coordinates of the virus’s surface determined from crystallographic measurements of the capsid
(Table S1). The nanocluster was enclosed in a unit cell with periodic boundary conditions and
suspended in an aqueous buffer. The unit cell was probed with unpolarized light and the absorbance was calculated directly from the $S_{21}$ coefficient. If a sphere representing the virus was placed at the center of the NC we found only small differences in the resulting absorbance spectrum.

**Table S1.** The positions for the 30 nm diameter NS on the virus (nm).

| x  | y  | z   |
|----|----|-----|
| 20.15 | 15.86 | -17.65 |
| -19.59 | -16 | 16.91 |
| 24.1 | -14 | -12.96 |
| -24.4 | 13.99 | 12.9 |
| -11.32 | 25.42 | -13.35 |
| 12 | -23.82 | 14.33 |
| 27.98 | 3.79 | 11.36 |
| -27.97 | -4.14 | -12.1 |
| -4.78 | -28.32 | -10.98 |
| 6.5 | 27.11 | 11.59 |
| 1.76 | 3.15 | 30.74 |
| -1.43 | -3.65 | -30.13 |

**S2. Results and Discussion**

**S2.1. BC stability and purity assessment**

Size exclusion chromatography (SEC) was performed to analyze aggregation and virus stability (Figure S1). Wild type CPMV and two preparations of BC were made and stored at concentrations below 1.0 mg/ml. CPMV peaks in the chromatograms were identical for 22 and 99 day old samples. Retention times and peak shapes are similar for all three virus preparations indicating that the BC was stable for 99 days while kept in the storage buffer at 4°C.
Figure S1: The chromatograms of 99 day (orange) and 22 day old (blue) BC and wild type (WT) CPMV (black) are shown. Retention times and peak shapes are similar for all three virus preparations.

S2.2. Optimization experiments for Nanoclusters (NCs) fabrication:

Based on our previous experience with attaching NSs to CPMV we started by using similar reaction conditions that were successful in the past with a commercial NS source. Our first reactions with 10 nm diameter NSs and 15 nm NSs resulted in capsids half way covered (50% yield, Figure S2). There was no indication of a red shift in UV-VIS spectroscopy (Figure S2C) for these materials. For optimization purposes NS/BC ratios (120, 240, 600, and 2400 NS/virus), pH (4.5 to 7.0), temperature (RT, 37°C, and 42°C), buffer concentration (8.7, 10, 16, 27.8, 43, 50, and 100 mM) and buffer type (2-(N-morpholino)ethanesulfonic acid (MES), KP, and citrate) were varied in separate experiments using agarose electrophoresis as the tool to determine success. An example of success is shown as band 1 in Figure S2A, i.e., a new nascent band containing NSs at the known position for the BC due to the formation of the nanoclusters (NCs).
The optimum NS/virus ratio was determined to be 240 (20 x excess where 12 NSs/capsid corresponds to 1x). The best buffer conditions were 10 mM potassium phosphate pH 6.0 at RT. NS binding to the capsid was optimal when the NS dispersion was concentrated prior to reaction such that a 20 x NS level occurred in the final reaction mix. Higher concentrations of NS (> 10^{13} particles/ml) promoted massive aggregation, shown as a band that stays in the well (Figure S2E) during electrophoresis.

**Figure S2.** Nanoclusters (BC-Au or NCs) built with commercial NS. 10 nm diameter NS from Ted Pella was coupled to BC using a 20 x excess of NS in the reaction mix in concentrated condition (NS 10 x concentrated) in 10 mM 10 KP pH 6.0 1 mM EDTA and TA treatment and agarose gel purification same as described in section S1.1. A) Agarose electrophoresis (grayscale): band (1) corresponds to BC-Au nanocluster while band (2) corresponds to NSs 10 nm in diameter. B) TEM of NCs showing 3D structures with < 50% Au NS coverage. C) UV-VIS spectroscopy of purified NCs and free NSs; no plasmon band shift was observed. D) NC
built using 15 nm diameter NS from commercial source. Limited NS coverage was noted. E) Agarose gel electrophoresis of an aggregated sample, which stays very close to the well in comparison to the free NS.

The fashion in which the virus binding reaction was performed was explored as well. We tested an inverted method to mix the components (i.e., add NSs to a pre-diluted virus in 10 mM KP pH 6.0, 1 mM EDTA as opposed to adding the virus to the concentrated NS dispersion) and the inverted method gave better results than adding the virus concentrated (0.02 mg/ml) to the NS mix. After the reaction was completed the capping agent thiocytic acid (TA) was pre-diluted in basic water (pH 8.5-9.0) and stirred constantly using a magnetic stirrer during the addition of the reaction mix to the TA/basic water mix. Pre-dilution of the TA is critical for avoiding the aggregation of the assemblies. Adding TA concentrated (20 mM) to the reaction mix tends to promote aggregation.

For purification purposes, at first we attempted to use electroelution [8] to recover the NC but this gave samples that were not clean enough for our analysis. As an alternative, we used β-agarase to break down the agarose prior to the recovery of the NC. The recovery of the NC using β-agarase was more efficient, since all NCs were recovered from the gel band, and cleaner in comparison to the electroelution.

However, we were still not achieving the assemblies needed for our application since the NSs were not large enough. Based on our COMSOL simulation the best candidates were identified as BC assemblies decorated with 30 nm NS. When commercial 30 nm NS dispersions were tested at the optimum reaction conditions determined for the smaller NS, not much coverage was achieved. Therefore, we tested NSs synthesized in-house. Two methods were used: the conventional [9] and the Puntes [2] method. Both methods gave us better reactivity in comparison
to the commercial NS dispersions (SPI, TedPella, Nanopartz). Our results using the in-house conventional method gave us encouraging results (Figure S3), but the size of the NS synthesized by this method (17-20 nm) was not large enough for the target NCs. By using the Puntes method we were able to synthesize larger and near monodisperse NSs. In addition, the NSs resulting from this method were the most reactive toward the virus from the all tested NSs (Table S2). It is important to mention that even though not all the characterization data (TEM, electrophoresis, UV-VIS) is shown here for all the NCs prepared, we do present representative data throughout the SI and the main text. As shown in Table S2 not major differences were noted in the absorbance spectrum between the NCs built with 30 nm Au Ns (discussed in the main text) and the example using 34 nm Au NSs (Figure S6) therefore, we concluded it was not necessary to show all the data for both NCs (Table S2: BC-30 and BC-34) but representative data to support our claims.

Figure S3. NC built with in-house conventional NS. 17 nm diameter NS synthesized by the conventional method and coupled to BC using a 20 x excess of NSs in the reaction mix in diluted condition (NSs not concentrated) without any addition of buffer nor EDTA. TA treatment and agarose gel purification same as described in section S1.1. A) Agarose electrophoresis (grayscale): band (1) corresponds to NC while band (2) corresponds to NS 17 nm in diameter. B)
TEM of NC showing 3D structures. C) DLS of reaction mix at end point. Free NS is centered at 17 nm while the NC is centered at 75 nm.

**Table S2.** UV-VIS spectroscopy features of various NCs.

| NS source or method | Nanocluster Typea) | Plasmon Free NSs | Plasmon Nanocluster | Broad Band |
|---------------------|---------------------|------------------|---------------------|------------|
| Commercial, SPI     | BC-10               | 518 nm           | 519 nm              | No band    |
| Conventional        | BC-17               | 520 nm           | 526 nm              | No band    |
| Inverted            | BC-18               | 523 nm           | 529 nm              | Yes        |
| Inverted            | BC-24               | 523 nm           | 534 nm              | Yes        |
| Inverted            | BC-30               | 524 nm           | 536 nm              | Yes; shoulder |
| Inverted            | BC-34               | 526 nm           | 537 nm              | Yes; shoulder |

*a) BC-## denotes for a nanocluster fabricated with BC and Au-NPs of diameter ## nm (ex: BC-10 was built with 10 nm diameter Au NSs).

**S2.3. Reaction progress: Dynamic Light Scattering Study of the self-assembly**

DLS was used to monitor the reaction progress during optimization experiments. We determined that the final NS concentration and excess of NSs was critical for the improved reactivity of the BC toward the NSs. Small scale reactions in which the NS dispersion was added in a titration fashion up to 1x (NS:BC ~12:1) indicated that the NSs did not react further upon addition of more NSs. TEM data showed only incomplete clusters bearing 2-3 NSs per virus. Therefore, we discarded the possibility of using a titration to produce NCs without the addition of an excess of NSs in the reaction mix.

For the purpose of this discussion, the resultant product is evaluated based on the increase of the particle size at the maxima of the DLS. A series of experiments using various concentrations of EDTA were monitored by DLS. We found that the addition of EDTA up to 5 mM increased the reactivity of the NSs (**Figure S4**). By comparing the size of the NC without EDTA we
determined that the 34 nm NS dispersion was the least reactive of all three NS dispersions since not much change was observed for the NC size after 48 h without EDTA (Figure S4E). In contrast, the 18 nm and 24 nm NSs showed some reactivity toward the virus even without EDTA (Figure S4A and Figure S4C), though NCs having the requisite 12 NS/capsid were not fully formed. We observed this trend before with the commercial NSs, in which the reactivity of the NS decreases as its diameter increases. Reactions in which EDTA was present were more successful relative to the ones without EDTA, since larger NCs were observed after 48 h reaction (Figure 4S: B, D, F). We choose 1 mM EDTA as the final concentration for future preparations since we observed that 2 mM and 5 mM EDTA was detrimental to the NS dispersions over time, causing aggregations. TEM data indicated that reactions ran in 1 mM EDTA produced the cleanest NCs in comparison to the ones generated using 5 mM EDTA. EDTA was chosen as an additive based on previous knowledge that it prevents di-sulfide bond formation in proteins.
Figure S4. DLS of reaction mix after 48 h in presence of EDTA. Reaction of BC with various sizes of NS with and without EDTA. A, C, and E are the negative controls that do not contain any EDTA in the reaction mix, while B, D, and F contain a final 5 mM EDTA in the reaction mix. Addition of EDTA results in an improved reactivity resulting in NCs of the expected sizes. Numbers on top of maxima indicate the size of the particles of most intensity.
The fashion in which the EDTA was added was important as well. The virus tends to aggregate if stock solutions of the virus are exposed to 1 mM EDTA during Hi-Trap columns purification. Also, addition of 500 mM EDTA directly to NS dispersions promotes aggregation of NSs. Therefore, as described in section S1.1.3, the EDTA is mixed with the buffer and NS dispersion filtrate prior to the addition of BC.

In order to determine the optimum reaction time, the progress of the reaction was monitored daily for 5 days via DLS. We found out that longer reaction times (> 3 days) promoted aggregation resulting in micron size particles (Figure S5). Series of experiments indicated that reaction times > 36 h did not produce better NCs; therefore, we set the finite reaction time to 36 h.

Figure S5. DLS of reaction mix of BC and commercial NSs after 5 days. After 5 days all reactions show particles of sizes larger than the expected NCs. From left to right reaction mix with 10, 15, and 20 nm diameter NSs from a commercial source (Ted Pella, Inc. Redding, CA).

Lastly, we were able to obtain reproducible results using NSs larger than 20 nm for NC preparation. These NCs (Figure S6) show the expected band in agarose electrophoresis, band 1 (Figure S6A) while free NSs are in band 2. By comparing the intensity of bands 1 and 2 it was
concluded that 6% of the total NSs in solution were associated with BC. Imaging analysis was performed using Image J version 1.47v of electrophoresis gels scanned in color using Adobe Photoshop CS6. After purification of bands 1 and 2, samples were characterized by DLS and UV-VIS spectroscopy (Figure S6: B, C, D). DLS data agrees with expected sizes for the free NSs (major peak centered at 34 nm) and for the NCs (major peak centered at 77 nm; larger species are present too). The UV-VIS spectrum of the BC-34 (BC-Au prepared with 34 nm Au-NP) shows the plasmon peak at 537 nm, which corresponds to a red shift in comparison to the free NS peak at 526 nm (Figure S6D). A TEM image of purified BC-34 shows the corresponding nanocluster as a 3D structure with icosahedral symmetry. TEM images for negative controls (free NS after agarose gel purification) are shown in Figure S7.
**Figure S6.** BC-34 nanocluster characterization. A) 1% agarose/TBE gel of reaction mix; band 1 corresponds to BC-34 and band 2 to free NSs. After purification of bands 1 and 2 samples were analyzed by DLS: B) a major DLS peak at 34 nm for free NSs, C) a major DLS peak at 77 nm for BC-34. D) UV-VIS spectroscopy of purified samples showing a red shift of the major absorbance for the BC-34 in comparison to the free NSs. E) TEM image of a purified BC-34 nanocluster.
Figure S7. TEM images of free NSs as negative controls. A) and B) 17 nm NSs isolated from agarose gels after reactions, indicating that the sample processing does not affect the quality of the nanoparticles, B is a higher magnification of A. Some areas of minor aggregation are present that can be attributed to the drying process. However, no evidence of well-organized 3D icosahedral structures like the ones found in the NC samples (Figure S6E) is observed. Scale bar: 50 nm. C-F) free 30 nm NSs after purification from gels with no indication of 3D structures; samples were diluted prior to imaging to avoid aggregation from the drying process.

In conclusion, we were successful in the fabrication of ~80 nm diameter icosahedral NCs using BC as a scaffold. The self-assembly process is done in bulk quantities in aqueous solution (i.e., 0.5 mg of HAuCl₄ per 40 ml NS in reactions with BC, see section S1.1.3 for details) and at RT. Successful purification of intact NCs was confirmed by various techniques (UV-VIS spectroscopy, agarose electrophoresis, and TEM). Experimental data agrees well with the simulations. It is important to note that coupling Au NSs to proteins is not a generic process, but one for which meticulous optimization is required. Careful attention to the use Au NSs that
contain additives as stabilization agents is needed, since those additives may limit noticeably the reactivity of Au NSs toward proteins.

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