Supporting Information for:

Electrostatic co-assembly of nanoparticles with oppositely charged small molecules into static and dynamic superstructures

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Gold nanoparticles (NPs) were synthesized and functionalized following a previously published procedure \(^1\).

1. Synthesis of TMA-functionalized gold nanoparticles (Au·TMA)

1.1. Nanoparticle synthesis

**Synthesis of 2.8 nm gold nanoparticles:** Gold NPs with an average diameter of 2.8 nm were synthesized by reducing HAuCl\(_4\) with tetrabutylammonium borohydride (TBAB) in toluene in the presence of dodecylamine (DDA) and didodecyl(dimethyl)ammonium bromide (DDAB) as surfactants. Specifically, a DDAB stock solution was prepared by dissolving DDAB (925 mg; 2.00 mmol) in toluene (20 mL) with ultrasonication. DDA (450 mg; 2.43 mmol) and HAuCl\(_4\)·3H\(_2\)O (50 mg; 127 µmol) were added to 12.5 mL of the stock solution and sonicated until completely dissolved. Gold(III) was then reduced by rapidly adding tetrabutylammonium borohydride (TBAB) (125 mg; 486 µmol) dissolved in 5 mL of the DDAB stock solution under vigorous stirring. The resulting NPs (diameter = 2.8±0.4 nm) were aged for 24 h before functionalization with TMA or the growth of larger NPs (examples described below).

**Synthesis of 5.3 nm gold NPs:** Gold NPs with an average diameter of 5.3 (±0.4) nm were synthesized by seeded growth using 2.8 nm NPs as the seeds. First, the growth solution was prepared by adding to toluene (50 mL) in the following order: DDAB (1.00 g), DDA (1.85 g), HAuCl\(_4\)·3H\(_2\)O (200 mg), and the seed solution (7.0 mL). Then, 131 µL of hydrazine monohydrate dissolved in 20 mL of the DDAB stock solution (with ultrasonication) were added dropwise (~1 drop/sec) to the growth solution under vigorous stirring, and the resulting solution was stirred overnight.

**Synthesis of 7.4 nm gold NPs:** Gold NPs with an average diameter of 7.4 nm were synthesized by a procedure analogous to 5.3 nm NPs, except that 4.0 mL of the 2.8 nm seed solution were used. The resulting NPs had a diameter of 7.4 (±0.6) nm.

**Synthesis of 11.4 nm gold NPs:** Gold NPs with an average diameter of 11.4 (±0.8) nm were synthesized by a procedure analogous to 5.3 nm NPs, except that 5.3 nm NPs (13.9 mL) were used as the seeds.

1.2. Nanoparticle functionalization

All the NPs were functionalized with a single-component monolayer of TMA ((11-mercaptoundecyl)-N,N,N-trimethylammonium bromide; see Fig. 1b in the main text). TMA was synthesized based on a previously reported literature procedure \(^2\).

**Functionalization of 2.8 nm gold NPs:** To 5 mL of 2.8 nm DDA-capped gold NPs dispersed in toluene (~1.43 mg/mL), 20.1 mg of TMA (10 equivalents with respect to the number of binding sites on gold, assuming that a single thiolate moiety occupies an area of 0.214 nm\(^2\)) dissolved in 5 mL of ethanol were added and shaken for 4 h. The resulting precipitate was collected by centrifugation and redispersed in 100 µL of methanol. The NPs were precipitated with 5 mL of ethyl acetate and collected by centrifugation. The resulting solids were redispersed in 100 µL of methanol and the purification process was repeated twice. Finally, the NPs were dispersed in 5 mL of deionized water.

**Functionalization of 5.3 nm gold NPs:** Prior to functionalization, 5.3 nm gold NPs were purified from an excess of surfactants (DDA and DDAB). Specifically, the toluene solution of NPs (5 mL, ~1.25 mg/mL) was mixed with 5 mL of ethanol and left undisturbed for 4 h. The resulting black precipitate was collected by decantation, washed with ethanol (10 mL), and then dissolved in toluene (5 mL). Next, 9.3 mg (10 eq) of TMA in 5 mL ethanol solution was added and the mixture was shaken for 4 h. The resulting precipitate was collected by centrifugation and dispersed in 100 µL of methanol. The NPs were precipitated with 5 mL of ethyl acetate and
collected by centrifugation. The resulting solids were redispersed in 100 μL of methanol and the purification process was repeated twice. Finally, the NPs were dispersed in 5 mL of deionized water.

*Functionalization of 7.4 nm gold NPs:* Analogously to 5.3 nm NPs, except that 6.7 mg of TMA was used (10 eq).

*Functionalization of 11.4 nm gold NPs:* Analogously to 5.3 nm NPs, except that 4.3 mg of TMA was used (10 eq).

*Functionalization of 12.4 nm gold NPs:* Analogously to 5.3 nm NPs, except that 4.0 mg of TMA was used (10 eq).

Supplementary Fig. 1 | Representative TEM images of differently sized TMA-functionalized Au NPs; from the left: 2.8 (±0.4) nm, 7.4 (±0.6) nm, and 12.4 (±0.9) nm.

To determine the surface density of TMA on Au NPs, we adapted a previously published procedure\(^1\text{,}^3\). The procedure was carried out on medium-sized 5.3 nm NPs. To 5 mL of 5.3 nm DDA-capped gold NPs dispersed in toluene (~1.25 mg/mL) were added 9.3 mg TMA (10 equivalents) in 5 mL of ethanol and the mixture was shaken for 4 h. The precipitated NPs were collected by centrifugation, and redispersed in 100 μL of methanol. The NPs were reprecipitated using 5 mL of ethyl acetate, collected by centrifugation, and redispersed in 100 μL of methanol. Reprecipitation and redispersion were repeated two more times to ensure the removal of unbound TMA. The solvent was removed under a stream of nitrogen, resulting in 7.2 mg of solid TMA-functionalized NPs. The NPs were redispersed in 0.75 mL of CD\(_3\)OD with 1.0 mg benzyl ether added as the standard. Then, 5 mg (excess) I\(_2\) was added and the solution was sonicated for 3 min to ensure complete dissolution of the NPs (note: Au-bound TMA thiolate ligands are liberated as the corresponding disulfides). The resulting solution was transferred to an NMR tube and a \(^1\)H NMR spectrum was recorded. A representative \(^1\)H NMR spectrum is shown in Supplementary Fig. 2. The amount of TMA on the NPs prior to iodine etching was determined by integrating the signals due to the TMA’s methyl protons (~3.15 ppm) vs. the aromatic protons of (C\(_6\)H\(_5\)CH\(_2\))\(_2\)O (~7.38 ppm) (Supplementary Fig. 2). Based on this analysis, the number of TMA ligands per 5.3 nm Au NP was found to be 388, which corresponds to a 0.227 nm\(^2\) footprint, in close agreement with the previously reported value of 0.214 nm\(^2\) for thiolates on a planar gold surface\(^4\).
Supplementary Fig. 2 | $^1$H NMR spectrum of a solution of TMA-functionalized 5.3 nm Au NPs after etching with iodine in the presence of benzyl ether as the standard (400 MHz, CD$_3$OD). The signals at ~7.38 ppm correspond to the aromatic protons of dibenzyl ether (standard) (m, 10H). The signal at 3.15 ppm corresponds to the methyl groups of TMA’s quaternary ammonium group (s, 9H).

2. Synthesis of MUS-functionalized gold nanoparticles (Au·MUS)

2.1. Nanoparticle synthesis

Monodisperse 4.7 (±0.4) nm gold NPs were synthesized by reducing HAuCl$_4$ with borane–$^{\text{tert}}$-butylamine (BTBA) complex in toluene in the presence of oleylamine, according to a modified literature procedure$^5$. Specifically, the precursor solution was prepared by dissolving HAuCl$_4$·3H$_2$O (49.5 mg, 0.126 mmol) in a mixture of toluene (2 mL) and oleylamine (2 mL). Then, a solution of borane–$^{\text{tert}}$-butylamine (21.7 mg, 0.25 mmol) in a mixture of toluene (0.5 mL) and oleylamine (0.5 mL) was quickly injected into the precursor solution at 0 °C under nitrogen atmosphere with vigorous stirring. The color of the mixture changed from orange to dark-red within 10 s. After 5 min, the reaction was slowly heated to 25 °C and kept at this temperature for 2 h. Thereafter, gold NPs were precipitated by pouring ethanol (10 mL) and then collected by centrifugation. The resulting solids were redispersed in toluene (2 mL) and precipitated again with ethanol (10 mL). After the redispersion–precipitation cycle was repeated twice, the NPs were finally redispersed in toluene (25 mL).

2.2. Nanoparticle functionalization

The NPs were functionalized with a single-component monolayer of MUS (sodium 11-mercaptoundecanesulfonate; Fig. 5a in the main text). First, MUS was synthesized based on a previously reported literature procedure$^6$. MUS (7.5 mg, 25.8 mmol) dissolved in ethanol (5 mL) was mixed with a toluene solution of 4.7 nm oleylamine-
capped gold NPs (5 mL, 1 mg/mL) and the mixture was shaken for 4 h. The resulting precipitates were collected by centrifugation and dispersed in 1 mL of methanol. Then, the NPs were precipitated with ethyl acetate (10 mL) and collected by centrifugation. The resulting solids were redispersed in methanol (1 mL) and the precipitation–redispersion cycle was repeated twice. Finally, the NPs were dispersed in 5 mL of deionized water.

Supplementary Fig. 3 | Representative TEM image of 4.7 (±0.4) nm MUS-functionalized Au NPs.

3. Studying the interactions between Au·TMA and negatively charged molecules

Supplementary Fig. 4 lists the structural formulas of multiply charged anions used to mediate attractive interactions between TMA-functionalized Au NPs. In a typical titration experiment, the pH values of the anion solution and of the Au·TMA solution were adjusted to pH 9 using NaOH. Then, 5-μL aliquots of 0.2 mM anion solution were added to a solution of Au·TMA (1 mL; 20 nmol of the TMA groups; each aliquot of the titrant corresponded to 1 nmol of the anion) and the solution was shaken for 30 s (we verified that no further changes were observed beyond 30 s, except for NPs larger than 10 nm, which gradually precipitated from the solution if attractive interactions between the anions and the NPs existed). A UV/Vis absorption spectrum was recorded after each aliquot of the titrant was added. For the reverse titration experiment, 5-μL aliquots of Au·TMA (each aliquot containing 1 nmol of TMA) were added to a solution of 6 nmol of a multiply charged salt in 1 mL of deionized water and shaken for 30 s. A UV/Vis absorption spectrum was recorded after each aliquot of the titrant was added. Typical titration curves are shown in Fig. 1d, e of the main text. As an additional example, Supplementary Fig. 5 shows typical titration curves for the titration of 5.3 nm Au·TMA with the ATP tetraanion and vice versa.

Supplementary Fig. 4 | Structural formulas of anions used to mediate the self-assembly of TMA-functionalized Au NPs. First row: Citrate, ethylenediaminetetraacetate (EDTA; monoprotonated7), trimetaphosphate, pyrophosphate, and triphosphate. Second row: Hexametaphosphate, adenosine triphosphate (ATP), and adenosine [β,γ-imido]triphosphate (APPNP). Third row: tricarballylic acid (propane-1,2,3-tricarboxylic acid), butane-1,2,4-tricarboxylic acid, pentane-1,3,5-tricarboxylic acid, trimesic acid (benzene-1,3,5-tricarboxylic acid).
Supplementary Fig. 5 | Additional titration experiments. **a**, Representative titration curve for the titration of Au·TMA (here, 5.3 nm; overall 66 nmol of TMA groups) with ATP. The dashed red line denotes the point of electroneutrality (16.5 nmol of quadruply charged ATP). **b**, Representative titration curve for the titration of ATP (24 nmol), with 5.3 nm Au·TMA. The dashed red line denotes the point of electroneutrality (96 nmol TMA on Au NPs).

Supplementary Fig. 6 | TEM images of aggregated NPs. **a**, Representative TEM images of aggregates of TMA-decorated 5.3 nm Au NPs (Au·TMA) and EDTA obtained by titrating Au·TMA with EDTA$^{3-}$. **b**, Representative TEM images of aggregates of TMA-decorated 5.3 nm Au NPs (Au·TMA) and EDTA obtained by titrating EDTA$^{3-}$ with Au·TMA.
4. Molecular dynamics simulations

Molecular dynamics (MD) simulations were performed at two different molecular resolutions: all-atom (AA) and coarse-grained (CG). The corresponding models of TMA-functionalized Au NPs and di-/trianions are shown in Supplementary Fig. 7.

**Supplementary Fig. 7** | Atomistic (a) and coarse-grained (b) models of TMA-coated Au NPs (blue surface ammonium groups bearing +1 charge each) and anions bearing 2 or 3 negative charges.

4.1. All-atom (AA) simulations

For gold atoms of the NP core, we used the parameters described in Ref. 8. For the surface ligands, the model was parametrized using the general AMBER force field (GAFF). Water molecules were treated explicitly in the AA models, using an explicit TIP3P water model. Cl\(^{-}\), HPO\(_4\)^{2-}, and citrate\(^{3-}\) (all with Na\(^{+}\) as the counterion) were also treated explicitly in the AA models and parametrized consistently. The diameter of the metallic core of the NPs in our simulations was \(\sim 7.4\) nm, in agreement with a typical experimental size for the NPs. First, we built an AA model of one NP hemisphere, where the NP surface is coated with 347 TMA ligands, each bearing a charge of 1\(^+\). The same number of counterions (Cl\(^{-}\)) was added to a simulation box filled with explicit water molecules in order to guarantee the system’s neutrality. We then added an extra single Cl\(^{-}\), HPO\(_4\)^{2-}, or citrate\(^{3-}\) anion to the system (together with 1, 2, or 3 associated Na\(^{+}\) counterions; see Fig. 2a in the main text) and ran AA metadynamics (MetaD) simulations\(^{11}\) to explore the binding/unbinding of the anions to the NP surface in the presence of competing ions (we employed well-tempered metadynamics; WT-MetaD\(^{12}\)). Based on these AA WT-MetaD simulations, we reconstructed the free-energy surface (FES) for Cl\(^{-}\) / HPO\(_4\)^{2-} / citrate\(^{3-}\) binding to the NP surface (see Fig. 2b in the main text). The FESs show a minimum value close to the charged NP surface, indicating that the HPO\(_4\)^{2-} / citrate\(^{3-}\) ions bind to the NPs, thus winning the competition with the Cl\(^{-}\) ions. With an additional Cl\(^{-}\) anion, the minimum was shallower, as expected (Fig. 2b in the main text). These AA WT-MetaD simulations were conducted in an isothermal–isobaric (NPT) ensemble (the number of particles \(N\), pressure \(P\), and temperature \(T\) were all constant), under periodic boundary conditions; a timestep of 2 fs was used. The temperature was maintained at \(T = 300\) K using the V-rescale thermostat\(^{13}\) and the pressure was kept at \(P = 1\) atm using the Parrinello-Rahman barostat with anisotropic pressure scaling (\(x\) and \(y\) decorrelated from \(z\), where \(z\) is the direction of ion binding/unbinding biased during the AA WT-MetaD simulations)\(^{14}\). Long-range electrostatics were treated by means of Particle Mesh Ewald\(^{15}\). For the collective variables (CVs) (descriptors of the ion binding/unbinding events) in the AA-MetaD simulations, we used the distance of the center of mass (COM) of the Cl\(^{-}\) / HPO\(_4\)^{2-} / citrate\(^{3-}\) ion from i) the charged heads of the TMA ligands (CV1), and ii) the center of the NP (CV2) with a gaussian height of 1.0 kJ·mol\(^{-1}\), \(\sigma\) values of 0.025 nm and 0.1 nm (for CV1 and CV2, respectively), and a bias factor of 8.
To complement the above simulations, we also performed additional AA-MetaD simulations, in which no extra Na\(^+\) ions were added to the system. For Cl\(^-\), we probed the interactions of one of the 347 Cl\(^-\) ions (the counterions for NP-bound TMA) with the NP. For HPO\(_4^{2-}\) and citrate\(^3-\), we replaced two and three of the 347 Cl\(^-\) with phosphate and citrate, respectively. The results of these simulations are similar to those in which extra NaCl, Na\(_2\)HPO\(_4\), and trisodium citrate were added; they are reported in Supplementary Fig. 8 below.

**Supplementary Fig. 8** | AA-MetaD simulations of TMA-coated NPs in the presence of oppositely charged ions, with no extra Na\(^+\) added. The plot shows free-energy profiles for the interaction between Au·TMA and Cl\(^-\) (yellow), HPO\(_4^{2-}\) (blue), and citrate\(^3-\) (red) (expressed as a function of the distance \(d\) between the center of mass of the ion and the center of mass of the closest TMA’s charged headgroup (CV1); errors bars are calculated as the standard error of the mean).

We then created an AA model system composed of two mirror NP hemispheres, each coated with 347 positively charged TMA ligands (neutralized by the same number of Cl\(^-\) anions), as represented in Supplementary Fig. 9a. This model system was created in order to explore the efficiency of the HPO\(_4^{2-}\) / citrate\(^3-\) ions to induce NP adhesion/self-assembly in a competing ionic environment containing an excess of Cl\(^-\) ions. To this end, we created two system variants: we inserted 10 citrate\(^3-\) or 15 HPO\(_4^{2-}\) anions (accompanied by 30 Na\(^+\) counterions). We then ran two AA-MetaD simulations, biasing the binding/unbinding of the NPs in the presence of the “gluing” citrate\(^3-\) / HPO\(_4^{2-}\) ions. During these runs, we imposed a cylindric restraint on the citrate\(^3-\) / HPO\(_4^{2-}\) ions in such a way that they remained in between the NP halves during binding/unbinding. These AA-MetaD simulations were run by using the distance between the NP centers as the CV, using a gaussian height of 0.25 kJ⋅mol\(^{-1}\) and a \(\sigma\) value of 0.1 nm, as defined above. From these AA-MetaD runs, we extracted free-energy profiles for the NP–NP binding in the presence of HPO\(_4^{2-}\) vs. citrate\(^3-\) ions in the system. The extreme complexity and large size of these systems (~230k atoms) do not allow for a satisfactory sampling of the binding/unbinding transitions. However, the obtained FES (Supplementary Fig. 9b) clearly enabled us to draw the following qualitative conclusions: (i) the citrate ions can efficiently trigger the adhesion/self-assembly of NPs in solution (Supplementary Fig. 9b, a sharp minimum in the red FES at an inter-NP distance of ~9.3 nm), and (ii) replacing citrate\(^3-\) with HPO\(_4^{2-}\) under the same ionic strength does not allow for NP self-assembly (Supplementary Fig. 9b, blue FES); the HPO\(_4^{2-}\) ions are thus inefficient in promoting inter-NP adhesion.
Supplementary Fig. 9 | All-atom molecular dynamics simulations of TMA-coated NPs in the presence of oppositely charged small anions. a, A model of two 7.4 nm NP hemispheres interacting with ten citrate anions (Na⁺, Cl⁻, and water molecules are not shown, for clarity). b, Free-energy profiles of NP–NP interaction in the presence of HPO₄²⁻ and citrate³⁻ as a function of NP–NP separation (we note that due to the high complexity of the system, the results should be treated qualitatively, e.g., the energy barriers, ΔG, are probably overestimated due to the difficult sampling).

We note that although the barrier between the assembled and the disassembled state (red FES in Supplementary Fig. 9b) is relatively high (and probably overestimated due to insufficient sampling), the free energy of the disassembled state (free NPs) is relatively low. We can attribute this result to the presence of Cl⁻ ions, which can efficiently “solvate” the free NPs. In other words, citrate-mediated self-assembly in such a competing environment is due to multivalent electrostatic interactions, which are stabilized only after the NPs come into contact with each other.

4.2. Coarse-grained (CG) simulations

4.2.1. Anion-induced self-assembly of Au·TMA

Studying the anion-driven self-assembly of NPs on a larger scale far exceeds the possibilities of explicit-solvent AA molecular simulations. Hence, we developed coarse-grained (CG) molecular models for these systems based on the widely used MARTINI CG force field. This approach allows us to obtain relatively fine CG models (resolution ~5 Å, each CG bead accounts for 3–4 heavy atoms) that, combined with MetaD simulations are useful for exploring the interactions, supramolecular structure, and dynamics of self-assembled systems. The reduction of structural detail from the AA to the CG model has intrinsic effects on the dynamics of the simulated system (e.g., enhanced sampling, acceleration of slow processes, and overall simplification of the models, which results in reduced entropy of the model system). The CG model allows to considerably extend the spatial and temporal scales that can be effectively explored during the simulations; at the same time, the information that is extracted from such approximated models should be considered qualitative. In this specific case, although the balance between enthalpy and entropy in the CG model may be approximated, the results obtained from such simulations can be still reliably used to qualitatively compare between states in the model system or between system variants.

We developed two CG model variants: one based on the standard MARTINI description with an explicit description of the solvent via CG water beads, and the other with an implicit solvent description, based on the “dry” MARTINI force field. Although the first one is more accurate in accounting for the solute–solvent interactions, it is limited (by the large number of water CG beads that need to be modeled) in terms of the size of the system that can be effectively simulated; the second one allows larger systems to be simulated more efficiently. By comparing the two CG models, we could observe that they behave qualitatively the same way. Thus, we focused on the implicit solvent CG model. To simplify the description of the system, Cl⁻ ions were not included in these simulations. In these CG models, each citrate³⁻ anion was modeled as three CG beads, each...
carrying one negative charge, whereas the HPO$_4^{2-}$ ions were modeled as a single CG bead carrying a $-2$ charge (Supplementary Fig. 7b). We built a CG model for a full NP (the diameter of the inner (Au) sphere was $\sim 7.4$ nm) covered with 804 positively (+1) charged surface ligands (TMA). We then constructed a CG model system containing two entire NPs and the corresponding number of HPO$_4^{2-}$ or citrate$^{3-}$ ions for ensuring the system’s neutrality. These CG models were then simulated by means of classical CG molecular dynamics (CG-MD) simulations. Representative snapshots taken from these CG-MD simulations in the presence of citrate$^{3-}$ and HPO$_4^{2-}$ are shown in Fig. 2c of the main text.

Supplementary Fig. 10 | Snapshot from a CG-MD simulation of the citrate-mediated self-assembly of TMA-decorated Au NPs focusing on the details of the NP–NP interface. Citrate$^{3-}$ anions are denoted in red. The corresponding movie is uploaded as Supplementary Video 1.

We have also extended the system to four TMA-functionalized NPs (in the presence of the corresponding number (1072) of citrate anions). Similar to the case of two particles, four NPs rapidly (within 100–500 ns) clustered into a stable ensemble stabilized by citrates (Supplementary Fig. 11a). The simulations revealed (Supplementary Fig. 11b) that self-assembly occurred concomitantly with the diffusion of citrate anions between the NPs (see also Supplementary Video 2). An analogous process in the presence of HPO$_4^{2-}$ could not be studied at long timescales due to the technical instabilities of the system, caused most likely by the large number of highly mobile charged ions. Nevertheless, simulations involving two NPs (Fig. 2c in the main text) clearly demonstrate that HPO$_4^{2-}$ anions are incapable of mediating the self-assembly of TMA-coated NPs.
Supplementary Fig. 11 | CG-MD dynamics of four TMA-coated NPs in the presence of citrate$^{3-}$ anions. a, Representative CG-MD snapshots before and after the aggregation of four TMA-coated NPs. b, Details of four consecutive CG-MD snapshots highlighting the diffusion of citrate$^{3-}$ onto the surfaces of the four NPs and at the interfaces between them (see also the zoom in the lower panel).

4.2.2. Dynamics of citrate on the surface of Au·TMA

To characterize the arrangement and dynamics of the citrate anions mediating the interaction between TMA-functionalized NPs, we performed another analysis as described below. We considered the CG-MD of two NPs in the presence of neutralizing citrate$^{3-}$ anions (see Fig. 2c of the main text). It was interesting to obtain an indication of the dynamics of the anions while the two NPs are aggregated. To this end, we analyzed part of the CG-MD trajectory starting at $t = 900$ ns (i.e., after the NP aggregation had stably taken place), up to $t = 8.4$ $\mu$s (the two NPs remain bound for the whole simulation time). A sampling stride of 25 ns between frames was used for the analysis.

During this CG-MD trajectory, all the citrate ions remain attached to the surfaces of the two aggregated NPs (due to the strong electrostatic attraction) for the entire duration of the simulation. Nevertheless, we observed that ions slowly diffused across the NP surfaces. The rate of diffusion depended on the location of the citrates, with those residing between the two NPs being less dynamic than the citrates interacting with only one NP.

In order to distinguish between the different states of citrate ions, we performed an unsupervised machine-learning analysis that allowed us to classify the arrangement of different ions in a rigorous and robust way. The configuration of each citrate in the CG-MD trajectory was classified according to its molecular environment by means of a Smooth Overlap of Atomic Positions (SOAP)$^{23}$, an approach that encodes the atomic/molecular environment of each citrate as a rotationally invariant representation (SOAP vectors). The SOAP vectors of each citrate were computed, considering the positions of all the surrounding citrates (to calculate the SOAP descriptors, we used the centers of the mass of the ions). This approach was previously shown to be capable of providing remarkable insights into the possible structural arrangements of self-assembling monomers in
supramolecular fibers\textsuperscript{24}. We carried out this SOAP analysis with the Python package \textit{DScribe}\textsuperscript{25}, setting the input parameters as $r_{\text{cut}} = 35$ Å, $n_{\text{max}} = 8$ Å, $l_{\text{max}} = 8$ Å, and leaving the other parameters as default.

The citrates could then be classified into different states by applying a clustering algorithm to the SOAP dataset, to obtain the most probable states in the multidimensional descriptor space. Nonetheless, we first reduced the high-dimensional SOAP features via principal component analysis (PCA) by keeping only the first 5 components. This approach allowed for a less demanding data treatment while maintaining a small loss of accuracy (more than 95\% of the information was retained). Linear PCA dimensionality reduction was performed using the Python package \textit{Scikit-Learn}\textsuperscript{26}. At this point, we performed unsupervised clustering of the dimensionally reduced dataset by applying the density-based clustering scheme Probabilistic Analysis of Molecular Motifs (PAMM)\textsuperscript{24,27,28}, which allowed us to classify all the possible arrangements of the citrate anions during the CG-MD trajectory. In Supplementary Fig. 12a, the different clusters detected by the PAMM method are represented by different colors and are projected onto the first two PCA components. These clusters were then merged into macroclusters (see Refs. 24, 27, 28 for details) in order to obtain a classification with a more direct interpretation in terms of the citrate’s arrangement within the aggregate (Supplementary Fig. 12b). The citrate anions are thus classified into three different states: the citrates at the interface between the two NPs (red), the citrates interacting with a single NP (light-gray), and an intermediate state between the first two (green), as shown in Supplementary Fig. 12c, \textit{left}.

Finally, as described in a previous work\textsuperscript{24}, our detailed analysis of citrate classification along the CG-MD trajectory allowed us to quantify the probability of transition among different citrate states (Supplementary Fig. 12d). Although these results have been extracted from a simplified CG model, they provide a clear picture of the citrate dynamics within a system of aggregated NPs (specifically, the different dynamics on the NP surface vs. at the NP–NP interface). Furthermore, this methodology allowed us to obtain transition probabilities, denoted as black arrows in Fig. 3b of the main text.

\begin{center}
\textbf{Supplementary Fig. 12 | Clustering analysis of the CG-MD trajectory of two NPs with citrate ions.} a, Projection along the first two principal components (PCA1 and PCA2) of a SOAP descriptor, computed for each citrate along the CG-MD trajectory. Different colors indicate the different clusters determined by the unsupervised PAMM algorithm. b, Same as (a), with gray, green, and red denoting the distinction between three macro-clusters associated with the three relevant states of the citrate$^{3–}$ions indicated in Fig. 3a, b of the main text. c, Two snapshots of the CG-MD of two TMA-functionalized NPs and citrate ions, immediately after the stable binding of the two NPs (t = 0.9 µs, \textit{left}) and at the end of the CG-MD simulation (t = 8.4 µs, \textit{right}). For clarity, only the
\end{center}
citrate ions are shown, with the NPs represented as solid yellow spheres. The different colors of citrates correspond to the macro-cluster to which they belong in the initial snapshot. The different distribution of the citrates in the second snapshot reveals the dynamics among states. Transition matrix describing the probability of transition between states (macro-clusters) obtained from the CG-MD trajectory. The numbers denote the transition probability within and between the clusters (the transition direction: a vertical axis into a horizontal axis; the color intensity is proportional to the transition probability).

To support the interpretation of the results provided by our unsupervised classification analysis, we also carried out a standard classification of the state of the citrate anions by defining two variables, CONT and DIST, which monitored the contacts of the anions with the NP-adsorbed ligands and their positions in the reference frame of the two NPs as indicated in Supplementary Fig. 13a and b, respectively. Specifically, we first assigned labels “A” and “B” to the two TMA-coated NPs in the system. Next, Cont(A) and Cont(B) were defined as the numbers of contacts between a given citrate ion and the ligands on nanoparticle A, and between the same citrate and ligands on nanoparticle B, respectively. Then, variable CONT was defined as the absolute value of the difference between Cont(A) and Cont(B) (see Supplementary Fig. 13a).

The number of contacts, Cont, is computed by assigning a value ranging from 1 to 0 to each of the TMA ligand tips (consisting of a positively charged CG bead) depending on its distance to the center of the mass of the citrate. The values decrease continuously from 1 to 0, following a smooth rational switching function of the form:

\[
\text{switch}(r) = \frac{1 - \left( \frac{r - d_0}{R_0} \right)^n}{1 - \left( \frac{r - d_0}{R_0} \right)^n},
\]

where \(d_0 = 0.0, n = 6, m = 12, \) and \(R_0 = 10 \, \text{Å}, \) which takes into account the fluctuation of the citrates on the charged surface of the NP and makes the number of contacts continuous.

**Supplementary Fig. 13 | Definition of variables CONT and DIST.** a, Definition of the contact variable CONT used in the calculation of the FES (see Fig. 3b in the main text). For every citrate ion, contacts with the ligands on the two NPs were computed. CONT is defined as the absolute value of the difference between the contacts with nanoparticle A and nanoparticle B. b, Definition of the distance variable DIST used in the calculation of the FES. DIST is defined as the absolute value of the difference between i) the distance of a citrate from the center of nanoparticle A and ii) the distance of a citrate from the center of nanoparticle B.

We found that the citrate ions do not detach from the NPs along the CG-MD trajectory, and that they establish a number of contacts, Cont, ranging from 5 to 9 with the NP-adsorbed ligands (they can fluctuate on the charged
surface). If a citrate ion interacts only with nanoparticle A or only with nanoparticle B, the corresponding \( CONT \) variable will lie within the same range; if, on the other hand, the citrate is at the interface between the two NPs, \( CONT \) will approach lower values (<4).

The fluctuations in the number of contacts do not guarantee that the \( CONT \) variable alone can distinguish between the different states of citrates in a system of two aggregated NPs. Therefore, we have defined a second variable, \( DIST \), as the absolute value of the difference between the distances in the citrate’s center of mass and the centers of the two NPs (Supplementary Fig. 13b). Given that all the citrates interact with the NP surfaces, \( DIST \) approaches 0 only when a citrate is located at the interface between the two NPs, acquiring larger values for citrates interacting with a single NP only. Monitoring the two variables \( CONT \) and \( DIST \) allowed us to distinguish between three different states in the dynamics of citrates (see Fig. 3b in the main text), in agreement with the classification obtained with the more general information on the SOAP descriptors.

To further characterize the variable dynamics of citrate in our system, we assessed the diffusivity of the anions along the surface of the ensemble of two aggregated NPs, taking advantage of the classification obtained with the SOAP analysis. Specifically, we aimed at estimating the diffusivities of citrates \( i \) located at the interface between the two NPs (the “red” state in Supplementary Fig. 12) and \( ii \) interacting with a single NP only (the “gray” state in Supplementary Fig. 12). To this end, we defined two ensembles, one containing citrates that remain in the “red” state for the whole length of the trajectory, and the other containing citrates that remain in the “gray” state for the whole length of the trajectory.

To determine ion diffusivities within the two citrate ensembles, we then computed the mean square displacement, MSD, of the citrates as a function of the time interval \( \Delta t \) between two frames:

\[
\text{MSD}(\Delta t) = \langle [x(t + \Delta t) - x(t)]^2 \rangle,
\]

where \( x \) is the position of the citrate, and the average is performed over all the citrates in the ensemble over time \( t \) (see Supplementary Fig. 14). Following the Einstein relation\(^2\), we then performed a linear fit of the two resulting MSD(\( \Delta t \)) curves (in the range 1 \( \mu s \leq \Delta t \leq 6 \mu s \)) and compared the angular coefficients, obtaining a diffusivity 7.8 times larger in the “gray” state (see Supplementary Fig. 12c) with respect to the “red” state (Supplementary Fig. 14). We note that it was impossible to estimate diffusivity for citrates in the “green” citrate due to undersampling (i.e., an insufficient number of citrate ions remained in the “green” state, without transitioning to either of the other two states).

We emphasize that, for each citrate, the MSD was computed in the frame of reference of the NP to which it is bound to exclude the motion of the NPs from the estimate. For the citrates in the “red” state (interacting with both NPs at once), the MSD was computed in both frames of reference and then averaged.

Supplementary Fig. 14 | Mean square displacement (MSD) of citrate\(^3^+\) ions. MSD as a function of the time interval between frames \( \Delta t \), for citrate ions remaining in the gray and red states (as defined in the text). By linear regression of the MSD (Einstein relation) for a time interval varying from 1 to 6 \( \mu s \) we estimated the diffusion constant for both ensembles as \( D_{\text{gray}} = 3.43(\pm0.23) \times 10^{-10} \text{cm}^2/\text{s} \) and \( D_{\text{red}} = 0.44(\pm0.10) \times 10^{-10} \text{cm}^2/\text{s} \).
5. Probing the effect of the flexibility of negatively charged molecules

To determine whether the conformational flexibility—in addition to charge—can affect negatively charged molecules’ propensity to mediate the assembly of positively charged NPs, we titrated TMA-functionalized 5.2 nm Au NPs in water with the four tricarboxylates shown in the bottom row of Supplementary Fig. 4. First, 100 μL of aqueous Au·TMA (100 nmol of TMA) was mixed with a 900 μL of a dilute aqueous NaOH solution (pH = 9.55) to ensure a basic pH. To the resulting solution were added 5-μL aliquots of a tricarboxylate (1 mM with pH adjusted to 9.55; each aliquot contained 5 nmol of the corresponding tricarboxylate). After each aliquot was added, the sample was shaken for 10 s, left undisturbed for 10 s, and a UV/Vis absorption spectrum was recorded.

The conformational flexibility of tricarballylic acid (orange in Supplementary Fig. 15) was gradually increased by incorporating an additional CH₂ group (butane-1,2,4-tricarboxylic acid; blue in Supplementary Fig. 15) and two CH₂ groups (pentane-1,3,5-tricarboxylic acid; red in Supplementary Fig. 15). The one or two additional methylene groups did not increase the trianion’s potency to assemble Au·TMA. Then, we considered a more drastic change in conformational flexibility by connecting the terminal methylene groups in pentane-1,3,5-tricarboxylic acid (red in Supplementary Fig. 15) with an extra CH₂ to afford trimesic acid (green in Supplementary Fig. 15). Still, no appreciable change in the titration curve was observed. From these experiments, we conclude that the conformational flexibility of multiply charged anions has little effect on their propensity to mediate the assembly of positively charged NPs.

For comparison, we also titrated 5.2 nm Au·TMA with trisodium citrate under the same conditions. Interestingly, the behavior of citrate deviates from the other four tricarboxylic acids (purple in Supplementary Fig. 15) in that it is less potent in mediating the assembly of NPs. This behavior can be explained by the fact that citrate ions tend to “curl up” in aqueous solutions to form intramolecular hydrogen bonds, which effectively decreases the negative charge.

**Supplementary Fig. 15 | Titration experiments with tricarboxylic acids.** Left: Change in the position of Au·TMA’s SPR peak as a function of the amount of the tricarboxylate anion added. The dashed red line denotes the point of electroneutrality (33.3 nmol of triply charged anions). Right: Structural formulas of the five tricarboxylates studied.
6. Preparation of crystalline assemblies of Au·TMA and multiply charged anions

Crystalline assemblies of TMA-functionalized Au NPs and multiply charged anions were prepared as described in the Methods section. Examples are shown in Fig. 4b–i, Extended Data Fig. 2, and Supplementary Figs. 16–19 below.

Supplementary Fig. 16 | Representative SEM images of colloidal crystals co-assembled from TMA-functionalized 5.2 nm Au NPs and the anions of different tricarboxylic acids (see Supplementary Fig. 4 for the structural formulas): a, tricarballylic acid; b, butane-1,2,4-tricarboxylic acid; c, pentane-1,3,5-tricarboxylic acid (here, an edge of a crystal is shown, showing a regular arrangement of NPs); d, and trimesic acid.

Supplementary Fig. 17 | Representative SEM images of colloidal crystals co-assembled from TMA-functionalized 4.7 nm Au NPs and P3O10 at different NP concentrations. We first treated Au·TMA dissolved in water with an aqueous solution of pentasodium triphosphate under vigorous shaking until the NPs’ λ_{SPR} plateaued. The resulting precipitate was dissolved in a small volume of a 2.5 M aqueous solution of (NH₄)₂CO₃ such that the concentration of the NPs amounted to 40 mg/mL. The resulting solution was split into ten portions and either not diluted or diluted with a 2.5 M solution of (NH₄)₂CO₃ 2-fold, 4-fold, 5-fold, 8-fold, 16-fold, 32-fold, 64-fold, 128-fold, and 256-fold, and the solutions were left undisturbed to allow for decomposition of (NH₄)₂CO₃ and consequently, NP crystallization. Therefore, the colloidal crystals were obtained at NP concentrations of a, 0.156 mg/mL; b, 0.312 mg/mL; c, 0.625 mg/mL; d, 1.25 mg/mL; e, 2.5 mg/mL; f, 5 mg/mL; g, 8 mg/mL; h, i, 10 mg/mL; j, 20 mg/mL; k, l, 40 mg/mL.
Representative SEM images of colloidal crystals co-assembled from TMA-functionalized 4.7 nm Au NPs and ATP. The crystallization experiments were carried out as described in Supplementary Fig. 17 for pentasodium triphosphate. The colloidal crystals were obtained at NP concentrations of a, 0.156 mg/mL; b, 0.312 mg/mL; c, 0.625 mg/mL; d, 1.25 mg/mL; e, 2.5 mg/mL; f, 5.0 mg/mL (note that all the images were acquired at the same magnification).

Representative SEM image of colloidal crystals obtained from a mixture of TMA-functionalized 4.7 nm Au NPs and \( P_3O_5^- \) (\( C_{Au} = 5 \text{ mg/mL} \)) showing a bimodal size distribution of the crystals.

7. Studying the interactions between Au·MUS and positively charged molecules

As model cationic species, we used \( N,N,N',N'',N''',N''''-\text{octamethyldiethylenetriammonium triiodide}^{31} \) (OMA\(^{3+}\)) as the trication and \( N,N,N',N'',N'''-\text{hexamethyleneaminium diiodide}^{32} \) (HMA\(^{2+}\)) as the dication. OMA\(^{3+}\) was synthesized by the dropwise addition of iodomethane (5.46 g, 38.4 mmol, 10.0 eq) to \( N,N,N',N'',N'''-\text{pentamethyldiethylenetriamine} \) (666 mg, 3.84 mmol, 1.0 eq) in a pressure tube. A white solid precipitated during the addition; after complete addition, the vessel was closed and heated to 80 °C for 2 h under stirring. After removal of volatiles, the reaction was found to be incomplete. Thus, DMF (5 mL) and iodomethane (5.46 g, 38.4 mmol, 10.0 eq) were added and the mixture was stirred in a closed pressure tube at 80 °C overnight. Subsequently, volatiles were removed under reduced pressure and the desired product was isolated as a colorless solid in a near-quantitative yield (\(^1\)H NMR (400 MHz, D\(_2\)O): \( \delta = 4.17 \) (s, 8H), 3.42 (s, 6H), 3.34 (s, 18H)).

HMA\(^{2+}\) was synthesized by dropwise addition of iodomethane (3.80 g, 26.7 mmol, 4.0 eq) to a solution of \( N,N',N''-\text{tetramethylethylene diamine} \) (777 mg, 6.69 mmol, 1.0 eq) in MeCN (10 mL) in a pressure tube, during which precipitation of a white solid was observed. Once the addition was completed, the vessel was closed and heated to 80 °C overnight under stirring. Subsequently, volatiles were removed under reduced pressure and the desired product (1.65 g, 4.12 mmol; yield = 62%) was isolated as a colorless solid (\(^1\)H NMR (400 MHz, DMSO-\( d_6 \)): \( \delta = 3.93 \) (s, 4H), 3.19 (s, 18H)).
Supplementary Fig. 20 | Structural formulas of an organic trication used to mediate the self-assembly of MUS-functionalized Au NPs (OMA$^{3+}$) and a control dication (HMA$^{2+}$); counterions, iodides.

In a typical titration experiment, to a solution of 4.7 nm Au·MUS (1 mL; 50 nmol of the MUS ligands) were added 5-μL aliquots of OMA$^{3+}$/HMA$^{2+}$ ($c = 0.4$ mM, i.e., each aliquot corresponded to 2 nmol of the d/trication). After the addition of each aliquot of the titrant, the solution was shaken for 30 s and a UV/Vis absorption spectrum was recorded. In the reverse titration experiment, to a solution of 20 nmol of OMA$^{3+}$/HMA$^{2+}$ in 1 mL of deionized water were added 5-μL aliquots of Au·MUS (each aliquot contained 2 nmol of MUS). After the addition of each aliquot of the titrant, the solution was shaken for 30 s and a UV/Vis absorption spectrum was recorded. Representative titration curves are shown in Fig. 5c, d of the main text.

To ensure that the aggregation of Au·MUS is not induced by the presence of a large excess of HMA$^{2+}$, we continued the titration until 5000 nmol was added (i.e., a 200-fold excess of positive charges with respect to the NP-absorbed MUS (50 nmol); see Supplementary Fig. 21).

Supplementary Fig. 21 | No significant changes in the wavelength of maximum absorption ($\lambda_{\text{SPR}}$) of MUS-functionalized 4.7 nm Au NPs upon the addition of a large excess of a dication (HMA$^{2+}$).

8. Dissipative self-assembly of Au·TMA induced by ATP in the presence of apyrase

Supplementary Fig. 22 schematically illustrates the dissipative self-assembly of TMA-functionalized NPs induced by an oligophosphate (here, ATP) in the presence of a phosphatase enzyme (here, apyrase). In a typical experiment, Au·TMA ($n_{\text{TMA}} = 19.2$ nmol; in terms of TMA units on NP surfaces) in the presence of 11 units/mL of potato apyrase stabilized with 100 μM of MgCl$_2$ was treated with 54 nmol of ATP. Since the rate of ATP-mediated NP aggregation was faster than that of ATP hydrolysis, the NPs aggregated. However, ATP was gradually hydrolyzed into species that do not support the aggregated state of the NPs; hence, the aggregates spontaneously disassembled. This process could be repeated for multiple cycles (Supplementary Fig. 22, right).

Supplementary Fig. 22 | Schematic illustration of dissipative self-assembly of TMA-functionalized NPs over multiple assembly–disassembly cycles.
Supplementary Fig. 23 | Representative SEM images of TMA-functionalized 7.4 nm Au NPs in the presence of 11 units/mL of apyrase before ($t = 0$) and after different times following the injection of 54 nmol of ATP. The corresponding absorption maxima are denoted in red/purple.

Supplementary Fig. 24 | Representative SEM side views of NP aggregates obtained by treating Au·TMA with 54 nmol of ATP in the presence of 11 units/mL of apyrase (the images were recorded 18 min after injecting ATP).
Supplementary Fig. 25 | Representative TEM images recorded at the same times as the SEM images shown in Supplementary Fig. 23.
Supplementary Fig. 26 | Reversible self-assembly of nanoparticles induced by ATP. a, Seven cycles of dissipative self-assembly of 7.4 nm Au-TMA in the presence of 22.2 units/mL of potato apyrase, followed by monitoring the position of the NPs’ SPR band. For each cycle, 27 nmol of ATP were used. b, Seven cycles of dissipative self-assembly of Au-TMA in the presence of 11 units/mL of potato apyrase, followed by monitoring the dynamic light scattering. For each cycle, 27 nmol of ATP were used.

Supplementary Fig. 27 | Dissipative self-assembly of 2.8 nm nanoparticles. a, Evolution of the UV/Vis absorption spectra of TMA-functionalized 2.8 nm gold NPs in the presence of apyrase subjected to ATP for five consecutive assembly–disassembly cycles. Each cycle was initiated by adding 54 nmol ATP to 0.47 nmol of NPs in the presence of 11 units/mL apyrase. b, Monitoring the reversible self-assembly process by following changes in the absorbance at 800 nm (low for non-interacting NPs; high for NP aggregates). c, Monitoring the reversible self-assembly process by following changes in the position of Au NPs’ SPR peak (a low λ_{SPR} value for non-interacting NPs; a high λ_{SPR} value for NP aggregates).
Supplementary Fig. 28 | Dissipative self-assembly of 7.4 nm nanoparticles. a, Evolution of the UV/Vis absorption spectra of TMA-functionalized 7.4 nm gold NPs in the presence of apyrase subjected to ATP for six consecutive assembly–disassembly cycles. Each cycle was initiated by adding 27 nmol ATP to 0.025 nmol of NPs in the presence of 11 units/mL apyrase. b, Monitoring the reversible self-assembly process by following changes in the absorbance at 800 nm. c, Monitoring the reversible self-assembly process by following changes in the position of the Au NPs’ SPR peak.

Supplementary Fig. 29 | Dissipative self-assembly of 12.4 nm nanoparticles. a, Evolution of the UV/Vis absorption spectra of TMA-functionalized 12.4 nm gold NPs in the presence of apyrase subjected to ATP for eight consecutive assembly–disassembly cycles. Each cycle was initiated by adding 27 nmol ATP to 0.011 nmol of NPs in the presence of 11 units/mL apyrase. b, Monitoring the reversible self-assembly process by following changes in the absorbance at 800 nm. c, Monitoring the reversible self-assembly process by following changes in the position of the Au NPs’ SPR peak.
Supplementary Fig. 30 | Following the dissipative self-assembly of nanoparticles with DLS. a, Changes in the maxima of the DLS profiles of a solution of TMA-functionalized 2.8 nm Au NPs in the presence of 11 units/mL of apyrase after injecting 27 nmol of ATP at $t = 0$. b, Changes in the maxima of the DLS profiles of a solution of TMA-functionalized 7.4 nm Au NPs in the presence of 11 units/mL of apyrase after injecting 54 nmol of ATP at $t = 0$ (replotted from Fig. 6d). c, Changes in the maxima of the DLS profiles of a solution of TMA-functionalized 12.4 nm Au NPs in the presence of 11 units/mL of apyrase after injecting 27 nmol of ATP at $t = 0$.

Supplementary Fig. 31 | Controlling the lifetime of dynamic NP aggregates by the amount of multiply charged anions (here, ATP). a, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) in the presence of 11 units/mL apyrase before ($t = 0$) and after the addition of 27 nmol of ATP. b, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) in the presence of 11 units/mL apyrase before ($t = 0$) and after the addition of 36 nmol of ATP. c, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) in the presence of 11 units/mL apyrase before ($t = 0$) and after the addition of 54 nmol of ATP (replotted from Fig. 6c). d, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) in the presence of 11 units/mL apyrase before ($t = 0$) and after the addition of 72 nmol of ATP. e, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) in the presence of 11 units/mL apyrase before ($t = 0$) and after the addition of 91 nmol of ATP. f, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) in the presence of 11 units/mL apyrase before ($t = 0$) and after the addition of 109 nmol of ATP.
Supplementary Fig. 32 | Controlling the lifetime of dynamic NP aggregates by the amount of a multiply charged anions (here, ATP). Maxima of absorption due to the NPs’ surface plasmon resonance (SPR) plotted as a function of time (based on the data in Supplementary Fig. 31). In each case, 7.4 nm Au·TMA ($n_{TMA} = 19.2$ nmol) in the presence of 11 units/mL of apyrase were used; they were treated with a, 27 nmol of ATP; b, 36 nmol of ATP; c, 54 nmol of ATP; d, 72 nmol of ATP; e, 91 nmol of ATP, and f, 109 nmol of ATP.

Supplementary Fig. 33 | Controlling the lifetime of dynamic NP aggregates by the amount of the enzyme. a, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{TMA} = 19.2$ nmol) before ($t = 0$) and after the
addition of 27 nmol of ATP in the presence of 22.2 units/mL apyrase. 

b, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) before ($t = 0$) and after the addition of 27 nmol of ATP in the presence of 11 units/mL apyrase. 

c, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) before ($t = 0$) and after the addition of 27 nmol of ATP in the presence of 5.6 units/mL apyrase. 

d, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) before ($t = 0$) and after the addition of 27 nmol of ATP in the presence of 2.8 units/mL apyrase.

9. Dissipative self-assembly of Au·TMA induced by ATP in the presence of ALPase

Dissipative self-assembly of TMA-functionalized Au NPs in the presence of alkaline phosphatase (ALPase) was carried out as described in Section 8 for potato apyrase, except that 200–1000 units/mL of ALPase were used.

Supplementary Fig. 34 | Dissipative self-assembly of 7.4 nm Au nanoparticles induced by ATP in the presence of alkaline phosphatase. 

a, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19$ nmol) in the presence of 1000 units/mL of alkaline phosphatase (ALPase) before ($t = 0$) and after the addition of 27 nmol of ATP. 

b, Following the position of the NPs’ SPR band as a function of time (replotted from a). 

c, Evolution of the UV/Vis spectra of TMA-functionalized 7.4 nm gold NPs in the presence of ALPase subjected to ATP for six consecutive assembly–disassembly cycles. Each cycle was initiated by adding 27 nmol ATP to 0.025 nmol of NPs in the presence of 1000 units/mL ALPase. 

d, Six cycles of dissipative self-assembly of Au·TMA in the presence of 1000 units/mL of ALPase, followed by monitoring the dynamic light scattering. For each cycle, 27 nmol of ATP were used.
Supplementary Fig. 35 | Dissipative self-assembly of 7.4 nm Au nanoparticles induced by P$_3$O$_{10}^-$ in the presence of alkaline phosphatase. **a**, Time-resolved UV/Vis absorption spectra of 7.4 nm Au·TMA ($n_{\text{TMA}} = 19.2$ nmol) in the presence of 1000 units/mL of alkaline phosphatase (ALPase) before ($t = 0$) and after the addition of 20 nmol of P$_3$O$_{10}^-$. **b**, Following the position of the NPs’ SPR band as a function of time (replotted from a). **c**, Evolution of the UV/Vis spectra of TMA-functionalized 7.4 nm gold NPs in the presence of ALPase subjected to P$_3$O$_{10}^-$ for four consecutive assembly–disassembly cycles. Each cycle was initiated by adding 20 nmol P$_3$O$_{10}^-$ to 0.025 nmol of NPs in the presence of 1000 units/mL ALPase. **d**, Monitoring the reversible self-assembly process for four cycles by following changes in the position of the Au NPs’ SPR peak. **e**, Monitoring the reversible self-assembly process for four cycles by following changes in the absorbance at 800 nm. For each cycle, 20 nmol of P$_3$O$_{10}^-$ were used.

11. Effect of repeated assembly–disassembly cycles on the crystallinity of NP aggregates

We have demonstrated that the gradual decomposition of (NH$_4$)$_2$CO$_3$ can result in the formation of well-defined colloidal crystals of TMA-functionalized Au NPs in the presence of multiply charged anions, including various oligophosphates (Supplementary Fig. 36a). At the same time, adding oligophosphates to Au·TMA in the presence of ß of time, after which they spontaneously disassembled, but could be reassembled upon adding a new aliquot of the oligophosphate. To verify that the repeated assembly–disassembly cycles do not hamper Au·TMA’s ability to form colloidal crystals, we first performed five dissipative self-assembly cycles in the presence of apyrase, with ATP as the oligophosphate. To this solution, i) hexametaphosphate was added to generate stable NP aggregates (note that the cyclic hexametaphosphate lacks a terminal phosphate and is not a substrate of apyrase). These aggregates were disassembled using a 2.5 M aqueous solution of (NH$_4$)$_2$CO$_3$, which gradually decomposed, resulting in the formation of co-crystals of Au·TMA and hexametaphosphate (Supplementary Fig. 36b). In a separate experiment, ii) Au·TMA in the presence of apyrase was subjected to five assembly–disassembly cycles with ATP as the oligophosphate. Then, ATP was added to induce the aggregation of Au·TMA for the
sixth time. The resulting aggregates were collected by centrifugation, washed briefly with anhydrous ethanol, and finally redispersed in a 2.5 M solution of (NH₄)₂CO₃ in water. (Note that washing with ethanol was essential to deactivate the enzyme; extensive washing of the ATP–Au·TMA aggregates with pure water was not enough to remove the enzyme, whose residual activity caused the aggregates to disassemble.) Addition of (NH₄)₂CO₃ followed by its slow decomposition afforded crystalline aggregates (Supplementary Fig. 36c). In both cases—experiment i) and ii)—the quality of the crystals was appreciably worse compared with the freshly prepared ones, which can be explained by the presence of a large amount of AMP and HPO₄²⁻ (from five ATP-induced assembly–disassembly cycles) and (experiment i) the enzyme.

Supplementary Fig. 36 | Crystallizing TMA-functionalized Au nanoparticles after multiple assembly–disassembly cycles. a, Schematic illustration of the process. b, Colloidal crystals obtained from Au·TMA and hexametaphosphate after five ATP-induced assembly–disassembly cycles in the presence of apyrase. c, Colloidal crystals obtained from Au·TMA and ATP after five ATP-induced assembly–disassembly cycles in the presence of apyrase, following deactivation of the enzyme.
Attempts to follow apyrase-induced etching of the co-crystals of Au·TMA and ATP

We were interested in determining whether the colloidal crystals obtained from Au·TMA and ATP would etch isotropically or anisotropically (i.e., with preferential etching at the faces vs. edges/corners of the colloidal crystals). First, we attempted to follow the process in-situ using environmental SEM. To this end, we immersed ATP–Au·TMA crystals resting on a silicon wafer in an aqueous solution of apyrase. Unfortunately, the results were inconclusive, since SEM imaging in the presence of the solution and under a near-atmospheric pressure failed to resolve the fine features of the crystals (results not shown).

Next, we recorded images of freshly prepared ATP–Au·TMA crystals on a silicon wafer under a low pressure typical for operating an SEM, removed the sample from the chamber, applied a drop of apyrase solution under ambient conditions, then dried and washed the sample, and finally imaged the same crystals. Here, however, we observed no changes in the structure of the colloidal crystals, which could be explained by partial coalescence of the neighboring NPs induced by the electron beam (accelerating voltage = 10 kV). In other words, the initial imaging rendered the crystals “static” (i.e., insensitive to the presence of the enzyme).

To tackle this problem, we imaged the samples under the lowest accelerating voltage that still allowed us to resolve the structure of the crystals (1.5 kV). Under these conditions, we observed changes in the structure of the colloidal crystals (Supplementary Figs. 37, 38) – we found that the crystals dissolved preferentially at the face sites, resulting in structures with pronounced edges and corners. Since beam-induced stabilization of NP assemblies most likely occurs uniformly throughout the sample (i.e., without the preference for edge/corner sites), we concluded that the dissolution of Au·TMA occurs preferentially at faces and, interestingly, not at edges or corners – i.e., the most exposed sites. Unfortunately, even under the low accelerating voltage of 1.5 kV, a pronounced stabilization by the beam was observed, resulting in only partial etching of the crystals (Supplementary Figs. 37, 38).

Supplementary Fig. 37 | SEM images of a colloidal crystal of ATP–Au·TMA before (left) and after (center and right) immersing in an aqueous solution of potato apyrase (example 1).

Supplementary Fig. 38 | SEM images of a colloidal crystal of ATP–Au·TMA before (left) and after (right) immersing in an aqueous solution of potato apyrase (example 2).
Cryo-TEM analysis of Au·TMA/ATP and Au·TMA/P₃O₅₁₀ aggregates

The experimental details for cryo-TEM analysis are described in the Methods section.

**Supplementary Fig. 39** | Representative cryo-TEM images of aggregates of TMA-functionalized Au NPs and P₃O₅₁₀.

**Supplementary Fig. 40** | Representative cryo-TEM images of aggregates of TMA-functionalized Au NPs and ATP.

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