Supplementary Information: Cooperative aggregation of gold nanoparticles on phospholipid vesicles is electrostatically driven

Calculation of the ionic strength increase from ζ data

Fig. S1A shows the measured ζ values of the AuNPs at each KCl concentration explored in the experiments. These values have been used to calculate the number of charges per NP (**Fig. S1B**) using both the Grahame equation for planar geometries and an alternative equation (equation (2)) that includes a correction for curvature, as described in the main text. Since the relative permittivity of water significantly decreases near a strong electric field such as that of a AuNP, we have calculated the number of charges per NP using two values of relative permittivity: 80 (representing bulk water, presented as black stars in **Fig. S1B**) and 20 (reduced due to local electric field effects, as red stars). Finally, **Fig. S1C** displays the results of the subsequent calculation of the increase (%) in ionic strength in the volume between a vesicle and a NP, v_{cell}

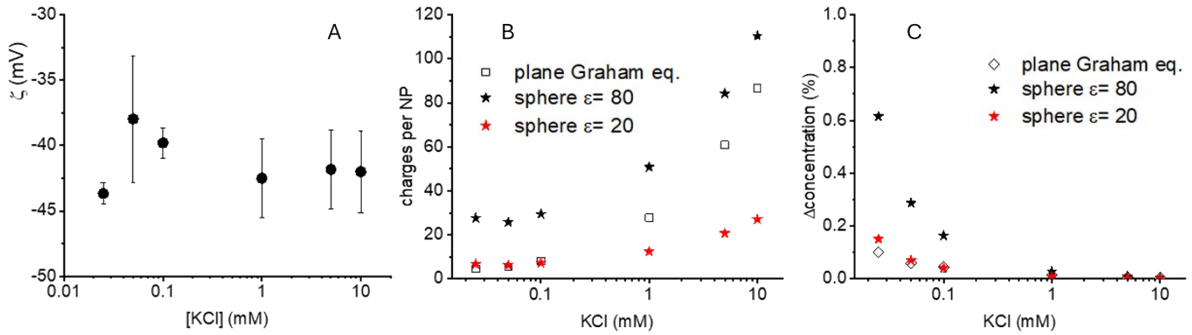

Figure S1. A) ζ values of AuNPs synthesized by PLAL at all studied KCl concentrations. B) using the Grahame equation for planar surfaces (square markers) and the curvature-corrected equation for spherical surfaces. Calculations are presented for ε= 80 (black stars), and ε=20 (red stars). C) Estimated variation in local ionic strength due to complete release of adsorbed ions upon AuNP binding to vesicles.

DLS

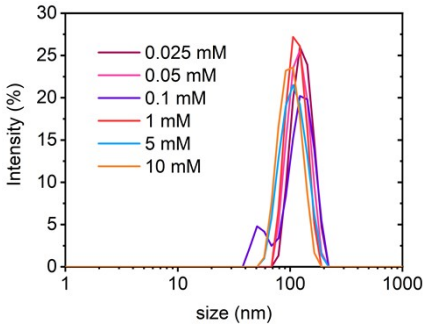

Figure S2. DLS size distributions by intensity of the mixtures of AuNPs and vesicles at a 1:2 ratio and increasing KCl concentrations.

Fig. S2 Shows the size distribution of AuNPs in presence of vesicles. Given the higher refractive index of gold compared to that of the vesicles, the DLS signal is dominated by the stronger scattering from the AuNPs. Although the solutions have different degrees of aggregation as evidenced by the extinction

spectra of Fig 2A in the main text, The hydrodynamic sizes at all the [KCl] coincide with the vesicle size. Such evidence demonstrates that, also at high ionic strength where the AuNP SPR indicates a low aggregation, all the nanoparticles are bound to the vesicles.

Derivation of the distribution of AuNP among the vesicles from the kinetic model

The temporal evolution of the different species in the kinetic schemes (a), (b) and (c) in the main text is given by differential equations that describe the rate of change of NP and vesicle concentrations:

$$\frac{d[n]}{dt} = -k[Ves_0][n] - k_c[n]\sum_{J=1}^{m}[Ves_J] \qquad (1a)$$

$$\frac{d[Ves_0]}{dt} = -k[Ves_0][n] \qquad (1b)$$

$$\frac{d[Ves_1]}{dt} = +k[Ves_0][n] - k_c[Ves_1][n] \qquad (1c)$$

$$\frac{d[Ves_j]}{dt} = +k_c[Ves_{j-1}][n] - k_c[Ves_j][n] \qquad (1d)$$

The set of recursive equations (eq. 1d) can be solved introducing the auxiliary functions $f_j = [Ves_j]e^{nk_ct}$. In terms of f_j eq. 1d becomes: $\frac{df_j}{dt} = nk_cf_{j-1}$ that is satisfied by $f_j = \frac{(nk_ct)^j}{j!}$. Accordingly, the solution of eq. 1d expresses the concentration of vesicles with j NPs as:

$$[Ves_j] = \frac{(nk_ct)^j}{j!}e^{-(nk_ct)}[Ves_{tot}] = \frac{a^j}{j!}e^{-a}[Ves_{tot}] = \frac{a}{j}[Ves_{j-1}] \qquad (2)$$

Where $[Ves_{tot}]$ denotes the total concentration of vesicles, which is constant.

At equilibrium, the total AuNP concentration ($[NP_{tot}]$) is given by:

$$[NP_{tot}] = \sum_0^\infty jV_j = [Ves_{tot}]e^{-a}\sum_0^\infty j\frac{a^j}{j!} = a[Ves_{tot}] \qquad (3)$$

Thus, the term $a = nk_ct$, represents the average number of AuNPs per vesicle $\left(a = \frac{[NP_{tot}]}{[Ves_{tot}]}\right)$, leading to a Poisson distribution under equilibrium, which is, to the best of our knowledge, the first demonstration based on chemical kinetics.

For non-cooperative cases where $k \neq k_c$, the solution above holds for j≥2. For j=1, the differential equation for [Ves1] differs and is given by:

$$[Ves_1] = \frac{(nk_c t)\,k}{1 \quad k_c}[Ves_0] = a\frac{k}{k_c}[Ves_0] \qquad (4)$$

Eq. 4 shows that the concentration of vesicles with one bound NP ($[Ves_1]$) is proportional to the initial concentration of free vesicles ($[Ves_0]$), adjusted by the rate constants and the time elapsed.

The simulations in the main text have shown that, although $k_c > k$ the two rate constants are of the same order of magnitude and eq. 9a can be approximated to

$$\frac{d[n]}{dt} \approx -\,k_c[n]\sum_{J=0}^{m}[Ves_J] \;=\; -\,k_c[n]\,[Ves_{tot}] \quad (5a)$$

Eq 5a can be easily integrated obtaining the exponential decay of free nanoparticles $[n] = [NP_{tot}]exp\big(-k_c[Ves_{tot}]t\big)$. Using this in the rate equation for unbound vesicles (eq. 1b), we can obtain the kinetics of the decay free vesicles:

$$[Ves_0] = [Ves_{tot}]exp\left\{\frac{k\;[NP]_{tot}}{k_c[Ves_{tot}]}\big[exp\big(-k_c[Ves_{tot}]t\big) - 1\big]\right\} \qquad (5b)$$

According to eq. 5b, the decay of free vesicles over time is very fast, as it is determined by the exponential of an exponential, reaching the plateau value:

$$[Ves_0(t=\infty)] = [Ves_{tot}]exp\left\{-\frac{k\;[NP]_{tot}}{k_c[Ves_{tot}]}\right\} \qquad (6)$$

Thus, the fraction of free vesicles decays exponentially based on the ratio of the rate constants k/k_c and the ratio of $[NP_{tot}]/[Ves_{tot}]$.

Starting from the equilibrium condition where no vesicles are bound to nanoparticles ($[Ves_0]$) (eq. 6), we can apply eqs. 2 & 3 to calculate the equilibrium concentration of vesicles that are decorated with 1, 2, or j nanoparticles. Since k and k_c calculated in the previous section are similar, the exponential in eq. 6 can be multiplied by $k/k_c \sim 1$, which allows us to use a recursive formula to determine the concentration of vesicles with varying number of nanoparticles, $[Ves_j]$:

$$[Ves_j] = \frac{a^j\,k}{j!\,k_c}[Ves_{tot}]exp\left\{-\frac{k\;[NP]_{tot}}{k_c[Ves_{tot}]}\right\} \qquad (7)$$

Here, a represents a scaled parameter that we need to redefine because it no longer leads to a pure Poisson distribution due to the adjustments made for the rate constants.

To maintain the total number of vesicles, we ensure that all vesicles, regardless of whether they have NPs bound or not, sum up to the total vesicle concentration. This leads us to define a based on the balance of the total number of vesicles:

$$[Ves_{tot}] = \frac{k}{k_c}[Ves_{tot}]exp\left\{-\frac{k\,[NP_{tot}]}{k_c[Ves_{tot}]}\right\}\sum_0^\infty \frac{a^j}{j!} = \frac{k}{k_c}[Ves_{tot}]e^{-\frac{k\,[NP_{tot}]}{k_c[Ves_{tot}]}}e^a \quad (8)$$

From which *a* can be calculated as eq. 9 in the main text

$$a = -\,ln\left(\frac{k}{k_c}\right) + \frac{k\,[NP_{tot}]}{k_c[Ves_{tot}]} \quad (9).$$

The parameter *a* defined in the above equation, is the expectation value of NP per vesicles if the distribution in eq. 7 holds as demonstrated below.

The expectation value for the number of bound NP per vesicle is

$$\langle J\rangle = \sum_{j=0}^{\infty} j[V_j]/[V_{tot}]$$

and according to eq. 7 it is

$$\langle J\rangle = \frac{k}{k_c}e^{-\frac{k\,[NP]_{tot}}{k_c[Ves_{tot}]}}\sum_{i=0}^{\infty}\frac{a^j}{j!} = \frac{k}{k_c}e^{-\frac{k\,[NP]_{tot}}{k_c[Ves_{tot}]}}ae^a$$

Considering eq. 9,

$$e^{-a} = \frac{k}{k_c}e^{-\frac{k\,[NP]_{tot}}{k_c[Ves_{tot}]}}$$

And therefore

$$\langle J\rangle = ae^{-a}e^a = a$$