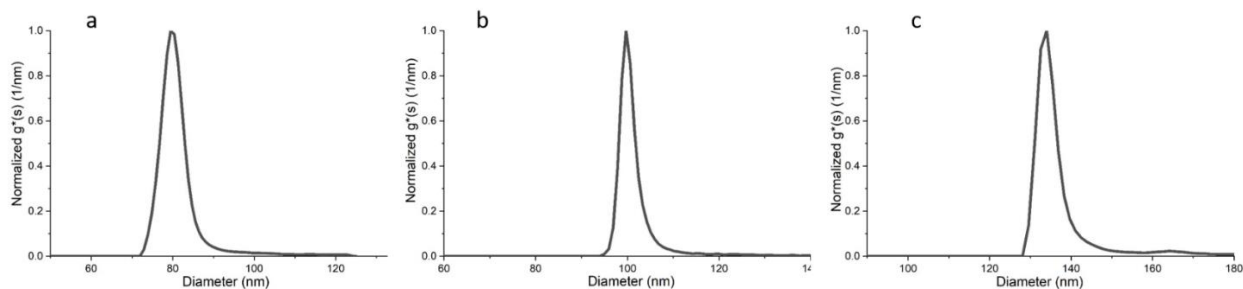
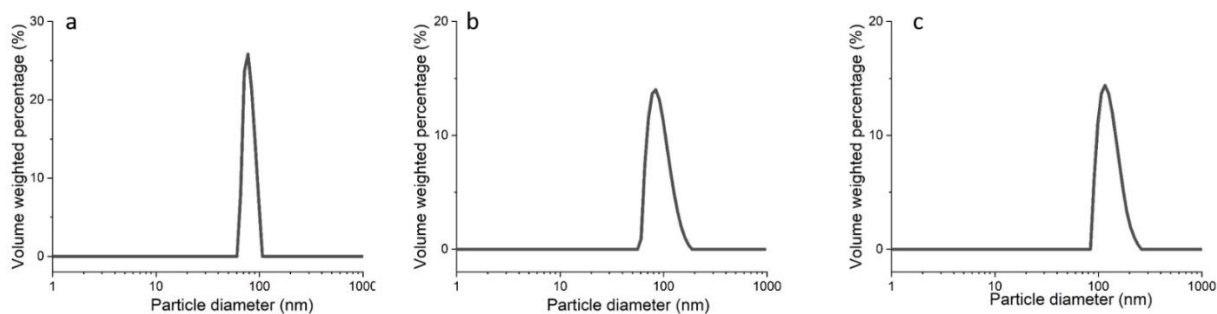


## Supporting information for manuscript “Layering of bidisperse charged nanoparticles in sedimentation”

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**Figure S1:** Particle size distributions of the three different types of silica nanoparticles (SNPs), used in the study, including a) 80 nm Rhodamine (RITC)-labeled SNPs; b) 100 nm RITC-labeled SNPs and c) 130 nm Fluorescein (FITC)-labeled SNPs, converted from the sedimentation coefficient distribution<sup>1</sup> and measured by analytical ultracentrifugation (AUC).



**Figure S2:** Particle size distributions of the three different types of silica nanoparticles (SNPs), used in the study, including a) 80 nm RITC-labeled SNPs; b) 100 nm RITC-labeled SNPs and c) 130 nm FITC-labeled SNPs, measured by Dynamic Light Scattering (Litesizer™ 500, Anton-Paar). The polydispersity index (PDI) of the three types of SNPs were 3.5%, 1.5% and 3.1% separately which indicated the high monodispersity of the samples.

**S11:** detailed AUC experiment set-up:

In a typical sedimentation–diffusion equilibrium (SDE) experiment, a 10  $\mu\text{l}$  sample of the binary nanoparticle dispersion at a typical initial concentration of 20 vol% was mixed well and injected into the sample channel of an AUC cell of 1.5 mm pathlength. A 20  $\mu\text{l}$  solvent of 80 vol% glycerol + 20 vol% water was injected into the reference cell. The filled AUC cell was then placed into a custom-made Multi-Wavelength Analytical Ultracentrifuge<sup>2-4</sup> (MWL-AUC). The measurement settings were: Temperature 25°C; Wavelength range 250–700 nm; Radial step size: 2  $\mu\text{m}$ . After a time scale of around 4 days, the sedimentation–diffusion equilibrium was normally reached by checking if the sedimentation profiles of the last 10 hours overlapped.

**S12:** detailed theoretical calculation steps:

To calculate sedimentation-diffusion equilibrium (SDE) profiles in a mixture of charged, spherical particles at high volume fractions, we follow the general approach outlined in the literature<sup>5</sup>. Briefly, we consider a mixture of particles  $i = 1:M$  with hard cores diameters  $\sigma_i$ , mass densities  $\rho_i$  and total charge  $Q_i = Z_i e$ , with a (local) volume fraction  $\phi_i(r)$  in a continuum fluid a mass density  $\rho_F$ . For every type of particle, the total potential can be written as the sum of its electrochemical potential, contribution due to the external (centrifugal) field, and the insertion potential against the total pressure:

$$\begin{aligned}\mu_i^{tot} &= \mu_i^{id} + \mu_i^{exc} + \mu_i^{el} + \mu_i^{ext} + v_i P^{tot} \\ &= \ln \phi_i + \mu_i^{exc} + Q_i \psi + \frac{m_i \omega^2 r^2}{2} + v_i (P^h - \Pi)\end{aligned}\quad (\text{S1})$$

where  $\mu_i^{id} + \mu_i^{exc}$  is the chemical potential split into an ideal contribution  $\mu_i^{id} = \ln \phi_i$  and an excess contribution (for which very good empirical expressions exist, such as BMCSL for mixtures of hard spheres).  $\mu_i^{el} = Q_i \psi = k_B T Z_i y$  is the contribution due to the electrostatic potential of charged particles in a mean-field approximation ( $Q_i = Z_i e$  is the particle charge, and  $y = e\psi/k_B T$  is the dimensionless electrostatic potential).  $\mu_i^{ext}$  is the contribution due to an external field; in this section we will specifically consider a centrifugal field, hence,  $\mu_i^{ext} = m_i \omega^2 r^2 / 2$ . Finally,  $v_i P^{tot}$  is the insertion energy, required to insert a particle of type  $i$  against the total pressure.

The expression for the total potential in **Equation S1** can be written in terms of forces, by taking the derivative with respect to  $z$  ( $F = -\partial \mu^{tot} / \partial r$ ). The SDE profile of a particle can be found by requiring that the net force acting on the particle is 0 (which is an optimization of the total potential  $\mu_i^{tot}$ ). The gradient of the hydrostatic pressure,  $\frac{\partial P^h}{\partial r} = -\rho_{susp} \omega^2 r$ , and in SDE,  $\frac{\partial \Pi}{\partial r} = -\omega^2 r (\rho_{susp} - \rho_F)$ . Hence, in SDE, the density difference between the particle and the fluid can be used to calculate the SDE profiles.

After some rewriting, this gives for mixture of particles in a centrifugal field:

$$\frac{r}{L_{\omega,i}^2} = -\frac{1}{\phi_i} \frac{\partial \phi_i}{\partial r} - \beta \sum_j \frac{\partial \mu_i^{exc}}{\partial \phi_j} \frac{\partial \phi_j}{\partial r} + \frac{\partial Z_i}{\partial r} y - Z_i \frac{\partial y}{\partial r}\quad (\text{S2})$$

where  $\beta = 1/k_B T$  and  $L_{\omega,i} = \sqrt{\frac{k_B T}{v_i(\rho_i - \rho_F)\omega^2}}$  is the centrifugal length. For particles with a fixed charge  $Z_i$ , the term  $(\partial Z_i / \partial r)$  equals 0.

The mean-field electrostatic potential is related to the particle volume fraction via the condition of local electro-neutrality<sup>5</sup>:

$$\sum_j \frac{Z_j \phi_j}{v_j} - 2n_b(1 - \tilde{\phi}) \sinh y = 0 \quad (\text{S3})$$

where  $n_b$  is the number density of monovalent ions in the bulk, and  $\tilde{\phi} = \sum_j \phi_j$  is the total fraction of the volume occupied by particles at a particular position  $r$ . Note that we do not explicitly calculate the counterion concentration profiles across the SDE profiles, and thus, we do not account for the locally enhanced number densities of counterions in the sediments with very high volume fractions of particles. To do so, one would have to include an additional force balance for the ions and solve their concentration profile self-consistently with the SDE profiles of the colloids. We plan to address the contribution of this in a future contribution.

Here, we assume that the all particles have a fixed charge  $Z_i$ . For an explanation how to take into account charge regulation of the particle charge, we refer to the literature<sup>6</sup>. For the experimental profiles shown in the main text, we found that including a regulated surface charge does not substantially change the SDE profiles for the experimentally determined particle charges and fitted ionic strength.

If all particles have a fixed charge, **Equation S3** can be rewritten, leading to the following expression for the electrostatic potential  $y$ :

$$y = \operatorname{arcsinh} \left( \sum_i \frac{Z_i \phi_i}{2n_b v_i (1 - \tilde{\phi})} \right) \quad (\text{S4})$$

The derivative of the electrostatic potential that is included in **Equation S2**, can then be written as follows:

$$\frac{\partial y}{\partial r} = \frac{1}{\sqrt{\alpha^2 + (1 - \tilde{\phi})^2}} \sum_i \frac{\partial \phi_i}{\partial r} \left( \frac{Z_i}{2n_b v_i} + \frac{\alpha}{(1 - \tilde{\phi})} \right) \quad (\text{S5})$$

with  $\alpha = \sum_j \frac{Z_j \phi_j}{2n_b v_j}$ . To find the SDE profiles of all charged particles in a mixture, we insert **Equation S5** in the force balance of **Equation S2** and collect all derivatives of the concentration profiles, and write the set of equations in matrix form<sup>5</sup>:

$$-\begin{pmatrix} 1/L_{\omega,1}^2 \\ 1/L_{\omega,2}^2 \\ \dots \end{pmatrix} = \begin{pmatrix} \mu_{11} & \mu_{12} & \dots \\ \mu_{21} & \mu_{22} & \dots \\ \dots & \dots & \dots \end{pmatrix} \begin{pmatrix} \partial\phi_1/r\partial r \\ \partial\phi_2/r\partial r \\ \dots \end{pmatrix} \quad (S6)$$

where:

$$\mu_{ij} = \mu_{ij}^{id} + \mu_{ij}^{exc} + \mu_{ij}^{el}$$

and:

$$\begin{aligned} \mu_{ii}^{id} &= \frac{1}{\Phi_i} & \mu_{ii}^{exc} &= \frac{\partial\mu_i^{exc}}{\partial\Phi_i} & \mu_{ii}^{el} &= \frac{Z_i^2}{2n_b\nu_i\sqrt{\alpha^2+(1-\Phi)^2}} + \frac{Z_i\alpha}{\sqrt{\alpha^2(1-\Phi)^2+(1-\Phi)^4}} \\ \mu_{ij}^{id} &= 0 & \mu_{ij}^{exc} &= \frac{\partial\mu_i^{exc}}{\partial\Phi_j} & \mu_{ij}^{el} &= \frac{Z_i Z_j}{2n_b\nu_j\sqrt{\alpha^2+(1-\Phi)^2}} + \frac{Z_i\alpha}{\sqrt{\alpha^2(1-\Phi)^2+(1-\Phi)^4}} \end{aligned}$$

Hard-sphere excluded volume interactions can be included using the empirical BMCSL equation of state. The expression for  $\mu_i^{exc}$  and the analytical expression for the partial derivative ( $\mu_{ij}^{exc} = \partial\mu_i^{exc}/\partial\Phi_j$ ) can be found elsewhere<sup>5</sup>.

For a two-component mixture, the expressions for  $\partial\phi_1/\partial r$  and  $\partial\phi_2/\partial r$  can be written explicitly:

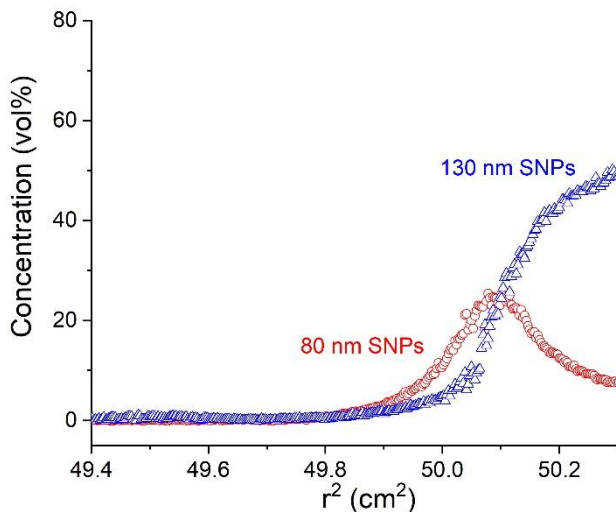
$$\frac{\partial\phi_1}{r\partial r} = \frac{\mu_{12}/L_{\omega,2}^2 - \mu_{22}/L_{\omega,1}^2}{\mu_{11}\mu_{22} - \mu_{12}\mu_{21}} \quad \frac{\partial\phi_2}{r\partial r} = \frac{\mu_{21}/L_{\omega,1}^2 - \mu_{11}/L_{\omega,2}^2}{\mu_{11}\mu_{22} - \mu_{12}\mu_{21}}$$

We solve this set of equations numerically in Matlab using a finite difference scheme. The resulting profiles are shown in the main text as solid lines.

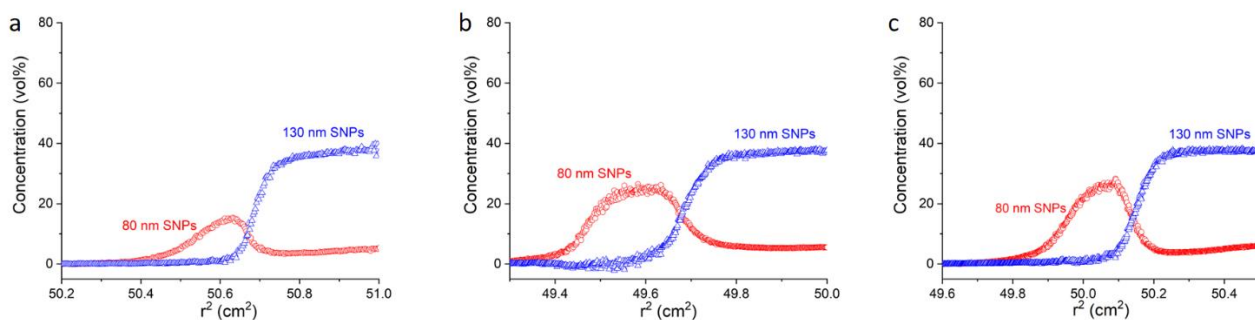
In the theoretical calculations, the surface charges used for different SNPs are listed in **Table S1**. They can be experimentally estimated by using the Debye-Hückel approximation<sup>7</sup>. From the theoretical calculations, the surface particle charge cannot be neutralized completely and there is always a small amount of charge left even after reacting with PEG-silane and being dispersed in acidic environment. The ionic strength used in the calculations (3 mM) is slightly higher than the experimental value (0.5 mM). The deviation may be due to the counter-ion accumulation with a higher particle concentration in the sedimentation.

Particles	Surface charge number (Z)
80 nm SNPs	110
100 nm SNPs	130
130 nm SNPs	200
80 nm s-SNPs	12
130 nm APTS-s-SNPs (in 0.001M HCl)	80
130 nm APTS-s-SNPs (in 0.001M HCl)	90

**Table S1:** The surface charge number Z used in the theoretical calculations.



**Figure S3:** Experimental SDE profiles for the binary mixture of 80 nm Polyethylene glycol (PEG) stabilized RITC-SNPs and 130 nm PEG stabilized FITC-SNPs at an ionic strength of 0.1M at 1100 rpm and 25 °C (the molecular weight of PEG is 1000 Da). At this high ionic strength, particles behave nearly like hard spheres. The maximum concentration reaches 54 vol% (130 nm SNPs) + 8 vol% (80 nm SNPs) at the very bottom.



**Figure S4:** Experimental sedimentation-diffusion equilibrium (SDE) profiles for the binary mixture of 80 nm RITC-SNPs and 130 nm FITC-SNPs at 1100 rpm and 25 °C of a) a varied number ratio:  $n(80 \text{ nm}) : n(130 \text{ nm}) = 1:2$ ; b) a larger sample volume: 20 ul and c) a slightly higher ionic strength 5mM.

Plot	$r_L^2$ (cm <sup>2</sup> )	$r_S^2$ (cm <sup>2</sup> )	$P$ (cm <sup>2</sup> )
a	50.69	50.54	0.15
b	49.67	49.47	0.20
c	49.96	50.15	0.19

**Table S2:** The calculated values of  $r_L^2$ ,  $r_S^2$  and  $p$  for **Figure S4 a, b and c.**

Plot	$r_L^2$ (cm <sup>2</sup> )	$r_S^2$ (cm <sup>2</sup> )	$P$ (cm <sup>2</sup> )
experimental (80 nm+130 nm)	50.21	50.19	0.02
theoretical (80 nm +130 nm)	50.21	50.18	0.03
experimental (100 nm+130 nm)	50.30	50.23	0.07
theoretical (100 nm +130 nm)	50.29	50.22	0.07

**Table S3:** The calculated values of  $r_L^2$ ,  $r_S^2$  and  $p$  for **Figure 3.**

Plot	$r_L^2$ (cm <sup>2</sup> )	$r_S^2$ (cm <sup>2</sup> )	$P$ (cm <sup>2</sup> )
experimental (0.001M HCl)	50.30	50.19	0.11
theoretical (0.001M HCl)	50.29	50.15	0.14
experimental (0.01M HCl)	50.32	50.24	0.08
theoretical (0.01M HCl)	50.25	50.16	0.09

**Table S4:** The calculated values of  $r_L^2$ ,  $r_S^2$  and  $p$  for **Figure 4 a and b.**

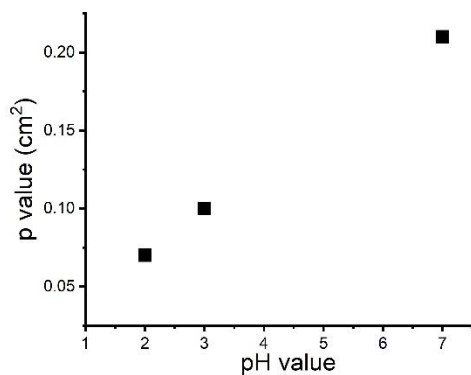
**S13:** Experimental steps for the introduction of amino groups for 130 nm SNPs:

In a typical reaction, 5  $\mu\text{l}$  (3-Aminopropyl)triethoxysilane (APTS) was diluted with 100  $\mu\text{l}$  Tetrahydrofuran (THF). The as-prepared APTS solution was added to 360  $\mu\text{l}$  PEG-stabilized 130 nm SNPs of ca. 6 mg/ml. The mixture was kept under 500 rpm stirring for 1.5 hours. Then the dispersion was purified by the centrifugation for 3 times and re-dispersed in the solvents of varied acidities.

Aminopropyltriethoxysilane (purity  $\geq 99\%$ ) and Tetrahydrofuran were purchased from Sigma-Aldrich. All chemicals were used without any further purification.

Particles	Acidity (M HCl)	Zeta potential (mV)
80 nm s-SNPs	0.001	0
130 nm APTS-s-SNPs	0.001	+ 16
130 nm APTS-s-SNPs	0.01	+ 18

**Table S5:** The zeta potential measurements (by Litesizer<sup>TM</sup> 500) for 80 nm sterically stabilized silica nanoparticles (s-SNPs) and 130 nm APTS functionalized, sterically stabilized silica nanoparticles (APTS-s-SNPs) in different acidities, namely 0.01 M and 0.001 M HCl.



**Figure S5:** The change of the  $p$  values with the pH value of the suspension.

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