## Gram-Scale Fractionation of Nanodiamonds by

# Density Gradient Ultracentrifugation

# Supporting Information

## Comparison of different centrifugal fractionation techniques

Table S1. Compar	son of different	t centrifugal fract	tionation techniques
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	IPDGU (carbon nanotubes) <sup>1,2</sup>	Step-wise gradient RZDGU (FeCo@C) <sup>3</sup>	Multi-step centrifugation (DNDs) <sup>4</sup>	Continuous gradient RZDGU (DNDs) in this paper
Processing capacity	~0.1-1 mg (Can be increased by concentrating the raw solution and employing large-volume, industrial centrifuges)	≤ 2 mg	~20 mg	~400 mg
Operation time <sup>a</sup>	≥12 h (long centrifugation time)	≥3 h	≥8 h for 5 fractions (the time increases proportionally to the number of fractions)	≤2 h
Size- separation resolution <sup>b</sup>	High (<1nm depending on density differences)	Intermediate (~2 nm for nanoparticles with a size range of 2-12 nm)	Low (large overlap occurs between multiples fractions; resolution not reported)	Intermediate (≤10 nm for starting materials with a size rage of over 100 nm; improved to <2 nm while at a small starting size range ~40 nm)
Applicability	colloidal materials with low density	any colloidal materials	any colloidal materials	any colloidal materials
Scalability	difficult to scale in lab	difficult to scale due to the tedious preparation and little reproducibility of gradients	scalability inversely proportional to the number of fractions	Large scalability

<sup>a</sup> operation time includes preparing gradients, centrifugation time and fractionation.

#### <sup>b</sup> the size-separation resolution here is qualitatively defined as the difference between the mean sizes of adjacent fractions

In this table, operation time cannot be used to compare the efficiency of different techniques in different nanoparticle systems because the centrifugation time, included in the operation time, varies with the sedimentation coefficients of nanoparticles in solution. But from the table, we can see that for DND systems, RZDGU is a more efficient method than multi-step centrifugation.

#### Size determination by DLS

DLS is a widely used size determination technique that is quite sensitive to the concentration of sample solutions.<sup>5</sup> We confirmed this by measuring the mpDNDs in aqueous solutions at a high and a low concentration comparably (the one with the lower concentration was obtained through diluting the higher one with HCl solution, pH~3.8), as shown in Figure S1. In this work, controlling the concentration became quite important to enable us to compare the size distributions of the particles fairly in different samples and fractions. Considering the direct relationship between the concentration and the optical absorbance, we tuned the optical absorbance of the sample solution (loaded in a quartz vile) before each DLS measurement, as shown in Figure S2. This pre-controlling procedure also enabled us to compare the solution of our mpDNDs with other commercial products of DND suspensions. Figure S3 is the DLS graph of our mpDNDs and a commercial product named NanoAmando (NanoCarbon Research Institute, Ltd., Japan), which shows that the mpDNDs also have a good dispersion state.



Figure S1. Size distribution of DNDs at different concentrations by DLS



Figure S2. UV-vis spectrum of the solution of DNDs at a controlled concentration



**Figure S3.** Size distribution of the mpDNDs and NanoAmando particles at a controlled concentration by DLS.

#### **Optimization of RZDGU conditions**

We chose 20-60 wt% sucrose aqueous solutions to prepare the continuous density gradient through tilt tube rotation using a gradient station. A 20 wt% sucrose solution was first laid in the bottom of the centrifuge tube up to the 45% level, with a 12 mL Norm-Ject syringe (Henke Sass Wolf) and a Vita 14 steel needle, and then a 60 wt% sucrose solution of the same volume was injected to the bottom slowly to ensure a sharp interface between the two solutions. Continuous density gradients were obtained through tilted tube rotation using a gradient station with built-in

programs. Then, 1.6 mL of previously prepared DND solution was laid on the top of the asprepared gradient solution with a 1.0 mL BD syringe (Becton, Dickinson and Company) and a disposable Pasteur pipette (Fisher Scientific) and balanced before being placed in the ultracentrifuge. The centrifugation conditions for two iteration fractionations varied a little bit: 20,000 rpm / 50 min for the first iteration and 30,000 rpm / 75 min for the second one. Fractions in the gradient containing nanodiamonds were then collected with a 6-piston gradient fractionator. The obtained fractions were further rinsed by dialysis with an HCl aqueous solution (pH~3.8) using molecular centrifugal filters (15 mL, Amicon<sup>®</sup> Ultra – 15). By using centrifugal filters instead of ultracentrifugation to rinse the sample, the re-aggregation phenomenon could largely be avoided.

A normal program of making a continuous density gradient within this machine includes two steps: the first step is a small-angle rotation for a certain time and the second step is a largerangle rotation for a shorter time. Both the rotation time and the angle affect the slope of the gradient (either steep or flat), and they also further affect the sedimentation behavior of the nanoparticles. To improve the resolution of the fractionation, we did a detailed study on the exact influence of the gradient slope on the behaviors of different particles and on the resulting bands of nanoparticles.

Two gradient-making programs were studied: Program 1: first step: rotation time 9 min 30 s and tilt angle 50°; second step: rotation time 40 s and tilt angle 80°; Program 2: first step: rotation time 9 min 30 s and tilt angle 60°; second step: rotation time 40 s and tilt angle 80°. The gradients made by these two programs were called Gradient 1 and Gradient 2, respectively. To discover the profiles of the gradients, we separated the gradients into 25 layers of 2 mm each along the tube, and determined their densities by a density meter (Density Meter, DMA 35). The gradients made with these two programs had unique characteristics in regard to their profiles but they were typical enough for us to study the influence of the gradient slope, as is shown in Figure S4. These two gradients were then used to fractionate the mpDNDs. In our first experiment, the centrifugation condition was 20,000 rpm for 50 minutes. The photos of the resulting bands in the gradients are shown in Figure S5. It is easy to see that the bands in Gradient 1 are broader than those in Gradient 2. This is because a larger tilt angle would result in a flat gradient but with a higher density and viscosity near the top of the gradient, which would slow down the sedimentation speed of the nanoparticles and therefore narrow the bands. Considering that an increase in the length of the band in the gradient would result in an increase in the resolution of the fractionation, we chose gradient 1 for our first fractionation. Of course, we could also broaden the bands by using sucrose solutions with lower concentrations, e.g., 10-50 wt%, but this would also decrease the ability of the gradient to prevent diffusion behavior and vortex motion due to a decrease in the viscosity and density and density and density and density and density and density and behavior to be the concentration.



Figure S4. The distribution of the sucrose concentration along the tubes of two gradients.



**Figure S5.** Photos of the mpDNDs in the two gradients after centrifugation at the speed of 20,000 rpm for 50 min. Left: Gradient 1; Right: Gradient 2.

For the second iteration of fractionation, the case was different. The mixtures obtained in the first experiment were centrifuged at 30,000 rpm for 1 h in a subsequent step. Photos taken after the subsequent centrifugation are shown in Figure S6. As expected, the smallest nanoparticles at the top of the gradient moved slower in Gradient 2 than in Gradient 1. Since our aim was to deal with smaller-sized and more narrowly distributed nanodiamonds in the second iteration of the fractionation procedure, we surmised that Gradient 2 would be better to maintain the position of the primary particles at the top of the gradient but, at the same time, separate the aggregates from the smallest particles.



**Figure S6** Photos of the mpDNDs in the two gradients after a subsequent centrifugation at 30,000 rpm for 1 h following the centrifugation cycle at the speed of 20,000 rpm for 50 min. Left: Gradient 1; right: Gradient 2.

In our following experiment, we optimized the centrifugation conditions for the second iteration of fractionation to stretch the bands and therefore increase the resolution of the fractionation. Different centrifugation times of 1 h, 1.25 h, 1.5 h and 1.75 h at the speed 30,000 rpm were applied to the fractionation of the first fraction from the first fractionation. The photos are shown in Figure S7. It is easy to see that the top of the bands moved downward as the centrifugation time increased, whereas the broadness of the bands first increased when the centrifugation time increased from 1 h to 1.25 h and then remained mostly the same, which can be interpreted as increasing hindrance from the gradient compensating for the increased centrifugal force with nanoparticles moving deeper in the gradient.



**Figure S7.** Photos of DNDs from F' 1 in Gradient 2 after centrifugation at 30,000 rpm for different times, from left to right: 1 h, 1.25 h, 1.5 h, 1.75 h.

In conclusion, various factors such as the slope of the gradient, the viscosity and density at the starting end of the gradient, the centrifugation speed and the duration of centrifugation influence the sedimentation behavior of nanoparticles in the gradient. After well-designed contrast experiments and detailed discussions, we chose Gradient 1 with centrifugation conditions of 20,000 rpm for 50 min for the first fractionation procedure and Gradient 2 with centrifugation conditions of 30,000 rpm for 1.25 h for the second iteration.

## TEM

TEM images of the 7 fractions collected in the first iteration of the fractionation procedure are shown in Figure S8.



**Figure S8.** TEM images of the 7 fractions collected in the first iteration of the fractionation procedure, **a)-g**) F' 1-7 correspondingly. The scale bars in the images are all 20 nm.

## Zeta potentials



Figure S9. Zeta potentials of F' 1-7; the blue error bars indicate the standard deviations of each value.

### **AUC Analysis**

Figure S10 shows an example of how the sedimentation coefficient distributions (Figure S11) were obtained from AUC by analyzing the sedimentation boundaries with Ultrascan III.<sup>6</sup> The noise subtraction was performed by two-dimensional spectrum analysis (2DSA)<sup>7</sup> with meniscus optimization.<sup>8</sup>

The experimental (yellow) and simulated (red) sedimentation boundaries of F" 1 and the corresponding residuals are presented to demonstrate the good match between the simulated model and the raw sedimentation data in Figure S10.



Figure S10. a) .Raw experimental and simulated data, b) residuals of sedimentation coefficients of the AUC analysis of F" 1.

The sedimentation distributions (Figure S11) were obtained after optimization with Monte Carlo analysis<sup>9</sup> with 100 runs. The size distributions shown in Figure 7 were obtained from Figure S11 using the Svedberg relation:<sup>10</sup>

$$d_{H} = \sqrt{\frac{18 \eta_{s} s}{(\bar{\nu}_{p}^{-1} - \rho_{s})}},$$
 (Eq. S1)

where  $d_H$  is the hydrodynamic diameter or size; *s* is the sedimentation coefficient;  $\rho_s$ and  $\eta_s$  are the solvent density and viscosity, respectively;  $\overline{v}_p$  is the partial specific



volume, which was experimentally measured (see next section and Figure S12).

Figure S11. Sedimentation coefficient distribution of a) F" 1., b) F" 2 and c) F" 3.

#### Determination of the partial specific volume of mpDNDs

The average density of mpDNDs was taken as the inverse of the partial specific volume and was measured by obtaining the linear relation between the density and the concentration of the clear mpDND solution (without precipitation) by linear fitting four sets of raw data (see Figure S12) from which the slope could be then used in the Kratky relation<sup>11</sup> to calculate the partial specific volume. The partial specific volume of mpDNDs was calculated to be 0.326 cm<sup>3</sup>/g, and thus the particle density, which is the inverse of the partial specific volume, was 3.06 g/cm<sup>3</sup>. This density value is in good agreement with the literature for DNDs.<sup>12</sup>



Figure S12. Linear fitting of the relation between the densities and concentrations of mpDND solutions.

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