SUPPLEMENTARY INFORMATION FOR "THE UNDERAPPRECIATED ROLE OF NONSPECIFIC INTERACTIONS IN THE CRYSTALLIZATION OF DNA-COATED COLLOIDS"

I. MATERIALS AND METHODS

Synthesizing DNA-coated colloids Colloidal particles are functionalized with single-stranded DNA through click chemistry and physical grafting, following a modified version of the methods described by Pine and co-workers [1]. A detailed protocol is provided in the Supporting Information of Ref. [2]. In brief, we prepare four polystyrene-block-poly(ethylene oxide) (PS-b-PEO) copolymers with roughly constant PS molecular weights and varying PEO molecular weights (6.5 kDa, 11 kDa, 34 kDa, and 67 kDa PEO) that are end-functionalized with an azide group (Polymer Source, Inc; part nos. P44891-SEO, P11118C-SEO, P1807A-SEO, and P1802-SEO)). The modified PS-b-PEO is physically adsorbed onto the surface of sulfate-modified polystyrene colloidal particles by swelling/deswelling, and a DBCO-modified ssDNA oligonucleotide is attached to the PS-b-PEO via click chemistry. Next, the colloidal particles are washed five times in aqueous buffer containing 10 mM Tris and 1 mM EDTA (1xTE) through centrifugation and resuspension. The colloidal particles are then stored in a refrigerator at 1% volume fraction in 1xTE. We study crystallization of a binary mixture of same-sized DNA-coated colloids. One particle species is coated with sequence A (5'-(T)51-GAGTTGCGGTAGAC-3') and dyed with an equal volume of a 10%-saturated solution of Pyrromethane 546 in toluene; the other particle species is coated with sequence B (5'-(T)51-AATGCCTGTCTACC-3') and dyed with an equal volume of 50%-saturated solution of nile red in toluene. Both DNA sequences are obtained from Integrated DNA Technologies (IDT) and are purified by high-performance liquid chromatography (HPLC). All crystallization experiments are performed in 1xTE buffer with 500 mM NaCl. We observed that the melting temperature decreased with increasing PEO molecular weight for both 600-nm-diameter and 430-nm-diameter colloids, which matches the observations from Pine and co-workers [3].

Crystallization Experiment The sample is prepared by mixing equal parts A and B particles. The final sample consists of 0.5% volume fraction DNA-coated colloids in 1xTE containing 500 mM NaCl. The solution is loaded into a sample chamber, which is prepared by plasma cleaning a 24 mm by 60 mm No 1. coverslip and a 22 mm by 22 mm No 1. coverslip. A box-shaped border of vacuum grease is placed on the larger cover slide. Then 4 μ L of the sample is placed inside the vacuum grease box. A 22 mm by 22 mm coverslip is placed over the vacuum grease border and excess air is pushed out of the box leaving a stable sample chamber. UV glue is added to the edges of the small coverslip and cured for 20 minutes.

We conduct bulk nucleation experiments by first holding the sample at a constant temperature just below melting and then annealing the crystals with a temperature ramp of 0.05 °C per 2 hours until few colloids in the gas phase remain. Then the sample is brought to room temperature where the crystals remain stable. The sample temperature is controlled by a thermoelectric cooler. Details of the experimental setup are provided in Refs. [2, 4]

Microscopy and particle centroiding

Bright-field microscopy images are obtained using a Nikon Ti2 microscope with a 10x-magnification, 0.45 NA objective (MRD00105, Nikon), a 1.5x-magnification tube lens, and a Pixelink M12 Monochrome camera (M12M-CYL, Pixelink) connected to a desktop computer. We image the surface of crystals with a Leica SP8 laser-scanning confocal microscope.

We measure the particle centroids using standard image analysis methods [5]. In brief, the images are split into separate channels corresponding to the two particle types in the crystal. Each image is convolved with a Gaussian to reduce the noise. A local thresholding algorithm is used to isolate the center of particles and to compensate for any large-scale variations in image intensity. Lastly, we compute the centroids to find the positions of all the particles in the facet. The positions of the particles are then used to calculate the experimental radial distribution function, g(r) [6].

II. SOLVING THE CRYSTAL STRUCTURES

We use a quantitative pipeline based on laser-scanning confocal microscopy, image analysis, and crystallography to determine the 3D crystal structures that we explore in experiment, previously described in Ref. [2]. The pipeline is as follows: (i) collect images of the crystal facets that have sedimented during growth; (ii) find particle positions for both A and B particles using image analysis and compute their radial distribution functions between A-B particle pairs, $g_{AB}(r)$, and A-A/B-B particle pairs, $g_{AA}(r)$; (iii) generate a look-up table of model $\tilde{g}(r)$ for potential crystalline facets (from here on \tilde{g} will denote model functions); and (iv) compare the experimental g(r) against all model $\tilde{g}(r)$ to find the closest match.

To create the reference $\tilde{g}(r)$ data, we identify relevant facets of proposed crystal structures that may show up in experiment. We then generate position data of A and B particles for those facets given a proposed crystal structure. In our experiments, we primarily see the (110) and (101) planes of body-centered tetragonal (BCT) structures. Once the particle positions are defined, we calculate corresponding g(r) data sets. In our lookup table we included the (100), (110), (111), and (210) facets of binary BCC (CsCl) and FCC (CuAu) structures, as well as the (110), (101) facets of BCT structures with *C*-values ranging from 0 to 1 in steps of 0.025. Other facets, such as the (100) or (111) facets of BCT, were excluded because we do not observe facets that contain only a single particle type.

Next, we compare the experimental and model g(r) data to identify the best match. In order to make this comparison, we need to introduce noise into our model data that is comparable to the noise in our experimental data. We fit the g(r) peak that is closest to $r/\sigma = 1$ (2 for $g_{AA}(r)$) with a Gaussian and then extract the mean and standard deviation; σ denotes the minimum A-B particle spacing. The mean is used to get the true lattice spacing of the crystal and to rescale the experimental g(r) to have a peak at r/σ at 1 (2 for $g_{AA}(r)$). We then convolve the model data with a Gaussian that has the same standard deviation as the experiment. Both sets of data are then normalized to set the amplitude of the first peak to one. This normalization sets a reference point from which we can compare various model facets to our experiment. For a given model $\tilde{g}(r)$, we compute the sum of the squared residuals between the model and the experimental data, $\sum_i (g(r_i) - \tilde{g}(r_i))^2$. We take the model with the smallest sum of the squared residuals as the best fit crystal structure to the experimental image.

III. FURTHER DEVELOPMENT OF THE THEORETICAL MODEL

A. Extension of the theoretical model to systems with specific A-A/B-B attraction

In systems where both A-A/B-B and A-B particle pairs interact via specific attractions, we assume that the A-B interaction is substantially stronger, so that the ordered binary harmonic crystal remains an appropriate reference system. We then treat the repulsive and attractive portions of the A-A/B-B pair potential, $u_{AA} = u_{BB}$, separately when computing the corrections to the reference crystal free energy. Specifically, we follow the Weeks–Chandler–Anderson approach [7] by splitting u_{AA} into repulsive, u_{AA+} , and attractive, u_{AA-} , parts at the minimum of the AA-particle pair potential $r = \sigma_{AA}$, where $\epsilon_{AA} = \min u_{AA} = u_{AA}(\sigma_{AA})$:

$$u_{AA+}(r) = u_{AA}(r) - \epsilon_{AA} \text{ if } r \le \sigma_{AA} \text{ otherwise } 0$$
(1)

$$u_{AA-}(r) = \epsilon_{AA}$$
 if $r \le \sigma_{AA}$ otherwise $u_{AA}(r)$. (2)

The repulsive part, u_{AA+} , substitutes for u_{AA} in Eq. (B6) of the main text, whereas the attractive part, u_{AA-} , is used to compute the corrections $\Delta \mu_{\text{fluid}}^{AA-}$ and $\Delta P_{\text{fluid}}^{AA-}$ following the λ -expansion as in App. B of the main text, while accounting for the fact that this correction only affects A-A/B-B particle pairs,

$$\beta \Delta \mu_{\text{fluid}}^{\text{AA}-} = \frac{12\eta}{\sigma^3} \int_{\sigma}^{\infty} dr \, r^2 \beta u_{\text{AA}-}(r) \tag{3}$$

$$\beta \Delta P_{\rm fluid}^{\rm AA-} v = \frac{6\eta}{\sigma^3} \int_{\sigma}^{\infty} dr \, r^2 \beta u_{\rm AA-}(r). \tag{4}$$

Since the A-A/B-B attraction is short-ranged, we use the approximate radial distribution function for A-A/B-B particle pairs, $g_{AA,ref}$, as in Eq. (B6) of the main text when applying the λ -expansion to u_{AA-} to

calculate $\Delta \mu_{\rm crystal}^{\rm AA-}$,

$$\beta \Delta \mu_{\text{crystal}}^{\text{AA}-} = z_{\text{AA}} \int_0^\infty dx \, g_{\text{AA,ref}}(x) \beta u_{\text{AA}-}(x), \tag{5}$$

where $z_{AA} = 4$ is the coordination number of A-A contacts and the integration range is extended to infinity to capture the complete range over which $g_{AA,ref}$ is nonzero. We note that, in principle, the attractive part, u_{AA-} , must be a weak perturbation relative to β^{-1} to apply the λ -expansion. Although this is generally not the case for the systems and real temperatures that we consider, we nonetheless expect that this approach will predict the correct qualitative effects of specific A-A/B-B attraction. Representative results, corresponding to Fig. 4 in the main text, are shown in Fig. S7.

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IV. ADDITIONAL FIGURES



FIG. S1. Effect of NaCl concentration on the equilibrium C value for 600 nm 6.5 kDa PEO. Violin plots of C values of crystal structures assembled by 600 nm 6.5 kDa PEO in three different salt conditions show that there is little to no change in the crystal structure through variation of the salt concentration. This observation suggests that there is little to no change in the polymer conformation at the different salt conditions and that electrostatics do not play a dominant role.



FIG. S2. Effect of NaCl concentration on the melting temperature. The melting temperatures decreases with decreasing salt concentration and approaches room temperature at the lowest salt concentrations for the systems studied in Fig. S1.



FIG. S3. Comparison of predicted and experimentally measured melting temperatures. To predict the melting temperature, we find the real temperature T at which the coexistence fluid phase packing fraction, $\eta_{\text{fluid}}(\mu_{\text{coex}})$, is equal to the total packing fraction in the experiments, 0.5%. This point on the phase diagram corresponds to the melting temperature, T_{m} , since the equilibrium volume fraction occupied by the crystal phase vanishes. In general, this occurs when the minimum of the A-B pair potential, ϵ , is approximately $-5\beta^{-1}$, although the shapes of both the A-B and A-A/B-B pair potentials affect the prediction via the harmonic and anharmonic contributions to the BCT crystal free energy. The Pearson correlation coefficient between predictions and experimental measurements is R = 0.98.



FIG. S4. Predicted free-energy landscapes for all particle sizes and PEO brushes tested. Expanded presentation of the μ versus C curves shown in Fig. 3C in the main text, showing predictions for all the cases explored in Fig. 3A,B.



FIG. S5. Theoretical prediction of the harmonic contribution to the BCT crystal entropy. The harmonic contribution to the entropy, ΔS_{harm} , is given by the second and third terms of Eq. (B2) in the main text. Although high C BCT crystals are favored by the harmonic contribution, the difference between CuAu and CsCl crystals of the phonon entropies is less than $0.1k_{\text{B}}$ per particle.



FIG. S6. Nonspecific vdW attraction is not required to observe high C values. Simulations predict the equilibrium BCT C parameter in the presence (filled circles) and absence (filled triangles) of nonspecific vdW attraction. For larger particles and shorter polymers, vdW attraction significantly shifts the equilibrium C parameter to larger values. However, vdW attraction is not required to observe C parameters greater than ~ 0.2 , as it is not the only contribution to the binary crystal free energy that tends to stabilize BCT crystal structures with larger Cvalues.



FIG. S7. Theoretical predictions of the effect of specific A-A/B-B attraction. (A) The theory predicts a continuous shift of the equilibrium BCT C parameter as the mixing fraction, α , is increased. This trend is consistent with the results of simulations and experimental measurements shown in Fig. 4 of the main text, although the midpoint of the transition is shifted to smaller values of α due to the approximations invoked in the theory. Calculations are shown here for the 600 nm, $M_{\rm W} = 67$ kDa system. (B) Representative $\mu_{\rm coex}$ versus C curves below, near, and above the midpoint of the theoretically predicted transition.



FIG. S8. Example crystal with spatial variations in the crystal structure. Confocal fluorescence micrograph of the surface of a crystal assembled from 600 nm particles with a 67 kDa PEO polymer brush and a mixing fraction of $\alpha = 0.1$. The crystal packing and symmetry varies from region to region within the crystal.