Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics. This journal is © the Owner Societies 2017



Fig. S1 HI-proteoid area fraction ϕ_A , osmotic pressure Π_{2D} , and equilibrium constant *K* depend the depth, *d*, measured from the top of the column. (**a**) Area fractions of monomers $(\phi_{A,M}, \text{black circles})$ and of dimers $(\phi_{A,D+}, \text{red squares})$. Solid lines are fits using eqs. S1 and S2, respectively; these lines intersect at $d^* = 101 \ \mu\text{m}$ where $\phi_{A,M} = \phi_{A,D+}$. Dashed line: fit to an exponential rise for $d \le 40 \ \mu\text{m}$, yielding the thermal-gravitational height of a monomer of $h_{g,M} \approx 10 \ \mu\text{m}$. Bars indicate \pm one standard deviation of measurements from the lines in different regions of *d*. (**b**) Total area fraction ϕ_A of proteoids (black circles; left axis) and two-dimensional osmotic pressure Π_{2D} normalized by $k_B T/A_p$ (blue squares; right axis) calculated by integrating $\phi_A(d)$. (**c**) Equilibrium constant *K* of the dimerization reaction calculated using the law of mass action: $K = \phi_{A,D+}/\phi_{A,M}^2$, where by convention we choose K = 1 at $d = d^*$. Solid line: fit of measured K(d) to $K = \exp[(d-d^*)/L_D]$, where $L_D = 9.0 \pm 0.2 \ \mu\text{m}$ is a characteristic length scale associated with dimer formation.



Fig. S2 (a) Measured two-dimensional osmotic equation of state of HI-proteoids, defined as the scaled 2D osmotic pressure $\Pi_{2D}/(k_{\rm B}T/A_{\rm p})$ versus the area fraction ϕ_A . The measurements are fit to $\Pi_{2D}(\phi_A)/(k_{\rm B}T/A_{\rm p}) = f\phi_A/[1-(\phi_A/\phi_{A,c})]$ where $f = 1.70 \pm 0.08$ is a dimensionless prefactor and $\phi_{A,c} = 0.458 \pm 0.006$ is a critical area fraction. (b) Natural logarithm of the equilibrium constant *K* for dimerization as a function of $\Pi_{2D}/(k_{\rm B}T/A_{\rm p})$; *K* increases exponentially with applied osmotic pressure. Solid line: linear least squares fit, yielding a slope of 3.5 ± 0.1 .



Fig. S3 Real-space structure and Fourier transforms of an isolated self-assembled chiral dimer crystallite (DX1) of chiral head-centered HC-proteoids. (a) Real space optical microscope image in which proteoids have been filled to a uniform black density and the space outside is uniformly white. Scale bar: 3 μ m. The HC-DX1 lattice of dimers is rectangular; average lengths of primitive vectors (red arrows) are (μ m): $|\mathbf{a}_1| = 5.1 \pm 0.1$, $|\mathbf{a}_2| = 8.4 \pm 0.1$; internal angle between \mathbf{a}_1 and \mathbf{a}_2 is $\gamma = 90^\circ \pm 2^\circ$. (b) Fourier transform of the crystallite in (a): field amplitude in reciprocal space. Complex Fourier transform of (a): (c) Real part and (d) Imaginary part. Constructive and destructive interference effects are not the same in the real and imaginary parts. Certain peaks are visible in the real pattern (c) but not in the imaginary pattern (d), and vice-versa. Inverse complex Fourier transform of (a) yields the real space image in (a). White scale bars: 3 μ m⁻¹.



Fig. S4 Structure and Fourier transforms of an isolated self-assembled chiral dimer crystallite (DX2) of chiral head centered HC-proteoids. (a) Real space optical microscope image in which proteoids have been filled to a uniform black density and the space outside is uniformly white. Scale bar: $3 \mu m$. The HC-DX2 lattice of dimers is oblique; average length of primitive vectors (red arrows) are (μm) : $|\mathbf{a}_1| = 6.5 \pm 0.1$, $|\mathbf{a}_2| = 8.3 \pm 0.2$; internal angle between \mathbf{a}_1 and \mathbf{a}_2 is $\gamma = 52^\circ \pm 2^\circ$. (b) Fourier transform of the crystallite in (a): field amplitude in reciprocal space. Complex Fourier transform of (a): (c) Real part and (d) Imaginary parts. Certain peaks are visible in the real pattern (c) but not in the imaginary pattern (d), and vice-versa. Inverse complex Fourier transform of (c) and (d) yields the real space image in (a). White scale bars: $3 \mu m^{-1}$.

Supplementary Methods

Fabrication of Chiral Proteomimetic Colloids — Solid microscale particles composed of epoxy photoresist SU-8 (Microchem Inc.) are fabricated using top-down photolithography, yielding a stable aqueous dispersion of monodisperse polymeric prismatic platelets that have customizable shapes⁴⁰. To make proteomimetic colloids, or proteoids, we modify the shape of an achiral annular sector particle (ASP)³¹ by decorating the end of only one of its arms with a superimposed circular feature, thereby causing it to be identifiably chiral. Lithographic layout software (L-Edit, Tanner Research, Inc.) is used to design an array of a particular proteoid shape in a photolithographic negative mask, suitable for 5x reduction stepper lithography. We have designed three different types of chiral ASP shapes, which we refer to as head-interior (HI), head-centered (HC), and head-exterior (HE) proteoids, based on the position of the circular head relative to the annular sector, as shown in Figure 1c. We have considered pairs of each proteoid shape at various relative positions and orientation angles, and all types sterically inhibit one chiral pathway of in-plane dimerization, so that only D+ dimers can form. As designed on the quartz-chrome photomask, HI-, HC-, and HE-proteoids have inner radii R_i of 8.9, 9.0, and 8.2 μ m, outer radii R_0 of 13.0, 13.3, and 12.3 μ m, head radii of 4.5, 6.7, and 6.7 μ m, and opening angles of $\psi = 72^{\circ}$, 86°, and 88°, respectively. Here, the opening angle ψ is defined as the interior angle between two radial lines that emanate from the mutual center of both circular arcs of the ASP. The first radial line simply intersects with the end of the arm of the ASP that does not have the head. The second radial line just barely touches the edge of the head region on the other arm of the ASP.

We have designed a single photomask that contains several different rectangular zones; each zone has a square array of transparent shapes of only one type of proteoid. For HI-, HC-, and HE-proteoids, these zones have arrays containing 1931 x 555, 1229 x 521, and 1160 x 542 replicates, respectively; each proteoid is separated with neighboring proteoids by 10 μ m. The photomask is made using an e-beam lithography system (MEBES, 50 nm feature size) loaded with a 6 in. x 6 in. x 0.25 in. quartz-chrome mask; after e-beam exposure, regions of the chrome layer are stripped away, leaving an array of transparent shapes of chiral ASPs. Dispersions of each type of proteoid are made by selectively printing corresponding regions of the photomask using an ultraviolet optical lithographic stepper (ASML, PAS 5500, i-line 365 nm ultraviolet (UV) light) that provides a 5x size reduction of features on the photomask. Thus, the spatial dimensions on the quartz-chrome photomask are 5x larger than the actual desired dimensions of the printed proteoids made of crosslinked SU-8.

To fabricate stable aqueous dispersions containing many millions of copies of identical chiral proteoids of a single head type, we spin coat a lift-off-release material, LOR-1A (Microchem Inc.), on a 4-inch diameter silicon wafer as a sacrificial layer, yielding a solid layer thickness of 120 nm after baking at a temperature of 200 °C for 120 s and then cooling to ambient temperature of 25 °C. Subsequently, a layer of SU-8-2001 negative photoresist is spin-coated on top of the LOR-1A layer, and the wafer is baked at 95 °C for 90 s, yielding a layer of SU-8 that is $\approx 1 \ \mu$ m thick. Similarly prepared wafers are loaded into the stepper, which exposes the photoresist layer at 5:1 reduction with patterned UV light (dose = 200 mJ/cm²). After a post-exposure bake at 95 °C for 60 s, the exposed SU-8 is crosslinked within the photoresist layer. Only one zone of the

photomask, corresponding to an array of only one particular chiral proteoid shape, is used by the stepper for this exposure over the surface of a single wafer. After repeated exposures that cover $\approx 90\%$ of the surface of the wafer with the array pattern (avoiding edges of the wafer), post-baking, and cooling to room temperature, the exposed wafer is immersed in a SU-8 developer solution (1-methoxy-2-propyl acetate), which dissolves the unexposed and uncrosslinked SU-8, leaving an array of proteoids attached to the sacrificial LOR layer. After rinsing with isopropyl alcohol to remove any residual SU-8 and drying with a nitrogen gas stream, the printed SU-8 proteoids are released from the wafer by dissolving the LOR layer in a basic aqueous surfactant solution containing 40 mM sodium dodecyl sulfate (SDS, MP ultrapure) and 1.8% w/v tetramethylammonium hydroxide (TMAH, Sigma-Aldrich, 25% in water). Adsorbed SDS on the surfaces of the particles inhibits particle aggregation and enables uniform dispersion in the solution. We concentrate and wash the aqueous dispersion of proteoids by gentle and brief centrifugation (2500 rpm, 20 min), remove the supernatant containing residual dissolved LOR and TMAH, and then dilute the particles with an aqueous solution having [SDS] = 5mM. To further reduce the concentrations of LOR and TMAH, we repeat this washing procedure 3 more times, yielding a stable aqueous dispersion of ≈ 200 million monodisperse proteoids produced from a single wafer in a 5 mM SDS solution.

Characterization of Chiral Proteomimetic Colloids — We characterize the printed SU-8 proteoids as shown in Figure 1d using a scanning electron microscope (JEOL, JSM-6700 FE-SEM, 5 kV). Top-down views of HI-, HC-, and HE- proteoids reveal highly uniform dimensions (averaged over at least 10 particles): outer radii, $R_0 = 2.48 \pm 0.04$ μ m, 2.51 \pm 0.02 μ m, and 2.33 \pm 0.02 μ m; inner radii, $R_i = 1.72 \pm 0.02 \ \mu$ m, 1.78 \pm 0.02

 μ m, and 1.66 ± 0.03 μ m; head radii, $R_h = 0.82 \pm 0.03 \mu$ m, 1.19 ± 0.01 μ m, and 1.16 ± 0.02 μ m; area of a face: $A_p = 8.61 \pm 0.33 \mu$ m², 10.05 ± 0.42 μ m², and 9.35 ± 0.65 μ m²; opening angle, $\psi = 78 \pm 2^{\circ}$, 99 ± 2°, and 101 ± 2°, respectively. As a consequence of lithographic exposure and development, these measured dimensions of SU-8 particles are about 5%-10% smaller than a prediction based on perfect 5x reduction of the designed dimensions; for the same reason, the actual opening angles of SU8 proteoids are larger than designed by about 10°-13°. Taking SEM edge views of released and redeposited proteoids, we find that the average thickness of proteoids is 1.0 ± 0.1 μ m. Sharp corners in the design are slightly rounded, as a consequence of the optical diffraction of UV light during exposure; the radius of curvature associated with this corner rounding is ≈ 200 nm, approximately half of the wavelength of the UV light.

Concentrating and Crystallizing Proteomimetic Colloids — We prepare and slowly concentrate a 2D Brownian monolayer of proteoids as follows. To a dilute dispersion of proteoids, we add a dispersion of a nanoscale depletion agent (Invitrogen, 42 nm diameter polystyrene spheres, carboxylate stabilized, surfactant free), yielding a post-mixing proteoid volume fraction of $\phi \approx 0.03\%$ and a post-mixing depletion-agent volume fraction of $\phi_d = 0.5\%$. This depletion agent induces a facially anisotropic roughness-controlled depletion attraction (FA-RCDA)³³. After a depletion agent is introduced, as a consequence of fabrication, each lithographic colloid has one face that is more strongly attracted to a flat smooth substrate with an energy significantly greater than thermal energy, k_BT , and an opposite face that is only weakly attracted to the substrate with an energy less than k_BT , where *T* is the temperature. This mixed dispersion containing ~10⁵ proteoids is loaded into a rectangular optical cuvette (30 mm × 2.0 mm × 0.1 mm). The

loaded cuvette is placed flat in the center of a standard glass microscope slide, and both open ends are sealed with UV optical glue (Norland, type 81); this glue also rigidly mounts the sealed cuvette to the slide without obscuring the ends of the optical cell. The cuvette is oriented horizontally, so that the earth's gravitational acceleration is perpendicular to the largest 30 mm x 2.0 mm area, corresponding to its lower and upper walls. The FA-RCDA causes a preferential attraction of only one face of each chiral ASP towards the lower flat smooth wall of the optical cuvette. After ≈ 10 hours of sedimentation while horizontal, a dilute enantiopure monolayer of mobile proteoids has formed just above the lower cuvette wall; proteoids are observed to diffuse translationally and rotationally in this dilute monolayer. Screened electrostatic repulsions, arising from the adsorbed SDS, prevent the proteoids from irreversibly adhering to the lower wall and preserve lubrication between the proteoids and the lower wall even in the presence of the depletion attraction. The diameter and post-mixing ϕ_d of the depletion agent have been optimized to induce FA-RCDA that lead to > 99.9% enantiopure monolayer formation of proteoids, as determined by examining more than 1,000 proteoids that diffuse above the flat surface in the presence of FA-RCDA.

In order to slowly concentrate the particles and enable crystal formation without substantial non-equilibrium jamming of particles that can lead to disorder, we tilt the cuvette along its long axis at a small angle of 5° and monitor the slow sedimentation towards the lower end of the cuvette. After a period of three months, during which the particles become gradually crowded towards this lower end, we then image the column from the top to the bottom using an inverted microscope (Nikon TE2000) equipped with a 40× extra-long working distance objective lens (Nikon Plan Fluor ELWD, 0.6

numerical aperture) and a Nikon D5000 camera (4288 pixels x 2848 pixels). To obtain an expanded, panoramic view that extends deep into the column while maintaining fidelity of single-particle features, we digitally combine several images that overlap, identify monomers, dimers, and crystallites, and color the particles as shown in Fig. 2b using Photoshop. In addition, using a computer-automated camera control system, we take time-lapse images of a fixed region in the dense region of a monolayer at a rate of one frame every 3 minutes over about one month using the same type of microscope that has been tilted by 5°. We combine these images into time-lapse movies that reveal particle-scale reaction events in the crowded system of proteoids. To enhance the clarity of individual proteoids in these movies, deblurring filtering has been employed in Photoshop.

Determining Area Fractions of Proteoids — To measure the area fraction of chiral proteoids as a function of depth, *d*, into the tilted 2D monolayer, bright regions inside the dark edges of proteoids in the combined micrograph are colored in green if they are in monomer (M) configurations and in red if they are in positive chiral dimer D+ configurations using light-dark edge detection in Photoshop. The area fractions of monomers, $\phi_{A,M}$, and the area fraction of D+ dimers, $\phi_{A,D+}$, are measured by counting the different colored pixels in a thin rectangular strip, which has a height of 20 μ m (*i.e.* along *d*) and a perpendicular width of 118 μ m (*i.e.* the width of the entire image). This 20 μ m strip is moved 10 μ m successively downward and the number of pixels in each strip is counted. To overcome limitations created by diffraction in the optical images, we more precisely determine the area particle fractions, ϕ_A , we convert the number of colored pixels to a bare particle area using a calibration based on SEM images. The average facial

area per proteoid for each type of proteoid is obtained from SEM images (*e.g.* see Fig. 1d). For a given proteoid shape and fixed illumination conditions, we use Photoshop's edge-detection to select and count the number of colored light pixels inside its dark edge-boundary in the optical micrograph. We determine an average number of colored pixels per proteoid from least 15 proteoids. Since this number of colored pixels per proteoid is proportional to the area of a proteoid, we count the number of colored pixels in a 20 μ m strip of the combined image and convert this number to a bare area by multiplying it with the SEM-average area per proteoid and dividing it by the average number of colored pixels proteoid is proteoid. This yields the average bare area of proteoids within the strip, and we divide this area by the entire area of the strip to determine the bare area fraction ϕ_A of proteoids in the strip.

We examine the behavior of the monomer and dimer area fractions as a function of depth in detail for HI-proteoids, shown in Supplementary Fig. S1a. As a consequence of the applied gravitational 2D osmotic pressure, Π_{2D} , the area fraction of monomers, $\phi_{A,M}$, increases with depth *d* into the column. In the dilute gas-like region, we fit $\phi_{A,M}(d)$ for $d \le 40 \,\mu$ m to a simple exponential increase expected for a barometric column, $\phi_{A,M}(d)$ $\sim \exp(d/h_{g,M})$, yielding a characteristic thermal-gravitational height of monomers of $h_{g,M} \approx$ 10 μ m. Deeper into the column, $\phi_{A,M}$ reaches a maximum value of ≈ 0.24 at $d \approx 80 \,\mu$ m. Beyond this peak, $\phi_{A,M}(d)$ decreases rapidly, as the vast majority of monomers react to form dimers in a monomer-dimer reaction zone (80 μ m < d < 120 μ m). In the bottom of the column, $\phi_{A,M}(d)$ fluctuates around 0.04; this non-zero value is likely to be a simple consequence of statistical sampling and the discrete nature of the particles (*i.e.* finding even versus odd numbers of particles in any given limited sampling area). Monomers in the dense region do not rapidly combine to form dimers because their diffusion around intervening dimers is very slow; tautomerization reactions do speed up the effective diffusion rate of monomers, but even so, the effective monomer diffusion rate that leads to dimer formation is still very low.

We perform a least-squares fit to the measured $\phi_{A,M}(d)$ of HI-proteoids using a piecewise empirical equation that captures the peak as well as the residual area fraction of monomers, $\phi_{A,R}$, deep within the column:

$$\phi_{A,M}(d) = \begin{cases} \phi *_{A,M} \left\{ 1 + \exp[-(d - d_{0,M}) / h_{g,M}] \right\}^{-1} \left\{ 1 + \exp[(d - d_{0,M}) / L] \right\}^{-1} &, d < d_{0,M} \\ (\phi *_{A,M} - 4\phi_{A,R}) \left\{ 1 + \exp[-(d - d_{0,M}) / h_{g,M}] \right\}^{-1} \left\{ 1 + \exp[(d - d_{0,M}) / L] \right\}^{-1} + \phi_{A,R} &, d \ge d_{0,M} \end{cases}$$
(S1)

where $\phi_{A,M}^*$ is a characteristic area fraction related to the amplitude of the monomer peak, $d_{0,M}$ is a reference depth near the peak, $h_{g,M}$ is a thermal-gravitational height of monomers, and *L* is a characteristic length scale associated with the decrease in the monomer peak in the monomer-dimer reaction zone. This fitting formula resembles one that has been used for achiral ASP particles³¹, except that here we have explicitly included $\phi_{A,R}$ in a continuous piecewise equation. The decrease of $\phi_{A,M}$ towards larger *d* is represented by the second inverse factor in both pieces of the equation. When fitting, we fix $h_{g,M} \approx 10 \,\mu$ m obtained most accurately from the fit to the leading edge of the peak, and allow $\phi_{A,M}^*$, $d_{0,M}$, *L*, and $\phi_{A,R}$ to vary, resulting in a good agreement (correlation coefficient = 0.97) and fit parameter values of $\phi_{A,M}^* = 0.83 \pm 0.04$, $d_{0,M} = 53 \pm 2 \,\mu$ m, $L = 29 \pm 3 \,\mu$ m, and $\phi_{A,R} =$ 0.036 ± 0.003 . Standard deviations of the measured points from the fitted line in three different regions (60 μ m < $d < 90 \,\mu$ m, 120 μ m < $d < 190 \,\mu$ m, and 260 μ m < $d < 390 \,\mu$ m) are shown as black error bars (*i.e.* total length of each bar represents \pm one standard deviation) in Supplementary Fig. S1a.

From the dilute gas-like region and into the reaction zone towards larger d, the area fraction of enantiopure chiral dimers (D+), $\phi_{AD+}(d)$, increases rapidly and then slowly continues to rise in the dense dimer region. We modify a prior semi-empirical model for the depth dependence of the dimer area fraction of achiral dimers³¹ by now including a linear increase of $\phi_{A,D+}(d)$ in the dense region. In the present system of enantiopure chiral dimers, there is a larger, non-negligible compressibility at higher depths and applied 2D osmotic pressures. This has its origin in at least two sources: there is overall a higher density of monomers in the dense region as a consequence of suppression of one reaction pathway, and also dimer crystallites are forming in the dense region, and since these dimer crystals represent a higher packing efficiency, this also contributes to local spatial density fluctuations in the system. We therefore model the compressibility at high particle densities by including a linear component in a piecewise function. Because dimer crystals have a higher area fractions that defected or disordered regions, large dimer crystals can be detected by locating the small peak-like fluctuations of $\phi_{A,D+}(d)$ shown at large d in Supplementary Fig. S1a. We fit the measured $\phi_{A,D+}(d)$ to:

$$\phi_{A,D+}(d) = \begin{cases} \phi *_{A,D+} \left\{ 1 + \exp[-(d - d_{0,D+})/h_{g,D+}] \right\}^{-1} & d < d_{0,D+} \\ \phi *_{A,D+} \left\{ 1 + \exp[-(d - d_{0,D+})/h_{g,D+}] \right\}^{-1} + \phi_{A,D+,LI}(d - d_{0,D+})/d_{0,D+} & d \ge d_{0,D+} \end{cases}$$
(S2)

where ϕ^*_{A,D^+} is a characteristic dimer area fraction associated with the degree of dimer formation in the reaction zone, d_{0,D^+} is a characteristic reference depth associated with the initial rise in $\phi_{A,D^+}(d)$, h_{g,D^+} is a length scale associated with this rise, and $\phi_{A,D^+,LI}$ is a slope describing the linear increase of $\phi_{A,D^+}(d)$ in the dense region. The fit agrees well with the measurements (correlation coefficient = 0.996), yielding parameter values of $\phi_{A,D+} = 0.34 \pm 0.01$, $d_{0,D+} = 104 \pm 1 \ \mu\text{m}$, $h_{g,D+} = 14 \pm 1 \ \mu\text{m}$, and $\phi_{A,D+,LI} = 0.024 \pm 0.004$. The red bars in Supplementary Fig. S1a represent ± 1 standard deviation of departures of the measured points from the fits in the same regions of *d* previously defined for the monomer area fraction. In the dense region, the larger bars for $\phi_{A,D+}$ as compared to $\phi_{A,M}$ are a consequence of the additional effect of dimer crystal formation. Summing $\phi_{A,M}(d)$ and $\phi_{A,D+}(d)$ gives the total area fraction $\phi_A(d)$ of proteoids, as shown in Supplementary Fig. S1b (left axis).

Gravitational 2D Osmotic Pressure — Using $\phi_A(d)$ for the system of HI-proteoids shown in Supplementary Fig. S1b (left axis), we calculate the gravitational 2D osmotic pressure Π_{2D} by summing up the effective buoyant gravitational forces of all proteoids above a given height z: $\Pi_{2D} = (k_B T / A_p) \int_z^{\infty} \phi_A(z')(dz' / h_{g,M})$, where *T* represents temperature, A_p is the facial area per proteoid, $h_{g,M} = 10 \ \mu m$ is the thermal-gravitational height of a monomer proteoid, and $z' = 390 \ \mu m - d$ is a variable of integration that points upward from the bottom of the column³¹. We present the results of this numerical integration as the scaled 2D osmotic pressure, $\Pi_{2D}/(k_B T / A_p)$, shown in Supplementary Fig. S1b (right axis).

Equilibrium Constant of the Monomer-Chiral Dimer Reaction — For chiral proteoids that have shapes that sterically suppress one chiral dimerization reaction pathway, the equilibrium reaction can be written as $2M \Rightarrow D+$. Using the law of mass action, we calculate the equilibrium constant $K = \phi_{A,D+} / \phi_{A,M}^2$ of this dimerization reaction in the reaction zone, where both $\phi_{A,M}$ and $\phi_{A,D+}$ vary systematically, as shown in

Supplementary Fig. S1c for HI-proteoids. We observe that *K* increases exponentially with *d* in this region. Here, we have set K = 1 where the area fraction of monomers equals the area fraction of dimers at $d^* \approx 101 \ \mu$ m. We fit K(d) to an exponential equation: $K = \exp[(d-d^*)/L_D]$, where L_D is the characteristic length scale associated with the rise in *K* and dimer formation in the reaction zone. This fit describes the measured K(d) well and yields $L_D = 9.0 \pm 0.2 \ \mu$ m.

2D Osmotic Equation of State — The system's 2D osmotic equation of state is determined by a scaled osmotic pressure as a function of area fraction ϕ_A , as shown in Supplementary Fig. S2a. For $\phi_A < 0.39$, the equation of state $\Pi_{2D}(\phi_A)$ can be fit accurately to a semiemprical equation $f\phi_A/[1-(\phi_A/\phi_{A,c})]$, where f is a dimensionless prefactor and $\phi_{A,c}$ is a critical area fraction, yielding the parameter values of $f = 1.70 \pm 0.08$ and $\phi_{A,c} = 0.458$ \pm 0.006. This equation captures ideal gas behavior at low ϕ_A and a non-linear divergence towards a critical area fraction $\phi_{A,c}$ associated with packing or jamming. Since the functions $\Pi_{2D}(d)$ and K(d) are both known, we can simply plot $\ln K$ versus the scaled Π_{2D} for d in the reaction zone, as shown in Supplementary Fig. S2b. The measured dependence is linear, so the equilibrium constant of the dimerization reaction increases exponentially with the applied 2D osmotic pressure. A linear least squares fit in Supplementary Fig. S2b agrees well with the measurements, yielding a slope of 3.5 ± 0.1 . Thus, the dimerization reaction of the Brownian proteoids is effectively driven by the applied 2D osmotic pressure in the gravitational column. This dependence includes the significant effective compressibility of lock-and-key dimer configurations.

Complex Fourier Transforms — 2D complex Fast Fourier Transforms (c-FFTs) are calculated using Matlab after image processing using Photoshop. Measured microscope

images in 24-bit RGB color are converted to unsigned 8-bit grayscale images. To enhance contrast in an image, the interiors of proteoids in a single dimer crystal are selected and colored black in Photoshop; after inverting this selection, regions outside the dimer crystal are colored white. This high-contrast image of a single crystallite is resized and centered so that it fits well within a white region of 2048×2048 pixels without clipping. In Matlab, this image is converted to double-precision numerical data. Using Matlab's built-in function (fft2), we calculate the 2D c-FFT of crystal image and shift the low frequency component to the center of spectrum for visualizing (fftshift). We display the real part and also the imaginary part of the 2D c-FFT in reciprocal space; values of the complex field are double precision numbers. At the centers of these real and imaginary parts of the c-FFTs, which are effectively scattering patterns, the field amplitudes are typically a few orders of magnitude higher than peaks at higher wavenumbers that reflect the self-assembled dimer crystal structures, so we mask a small region of central pixels. In a given c-FFT, non-central peaks in either the real part or the imaginary part of the c-FFT contain both large positive numbers and also large negative numbers, so we color-code the amplitudes to be black for zero field and brighter versions of two different colors for positive and negative field amplitudes. We choose to emphasize and report real and imaginary parts of the complex field in this manner, rather than the equivalent information contained in the field magnitude (i.e. amplitude) and phase, because interference effects are more directly evident in the different patterns of Bragg peaks, as shown in Fig. 3 and Supplementary Figs. S3 and S4. We have verified that the high-contrast image of a dimer crystallite can be reconstructed by performing an inverse c-FFT using the reported real and imaginary parts obtained by c-FFT.

Supplementary Note

Extended Discussion

We conjecture that HI-proteoids have formed the largest dimer crystallites under the same conditions for the following reasons. A head-interior feature does not interfere with rotations of individual monomer proteoids as much as head-centered and headexterior features. Thus, the rates of dimerization and tautomerization reactions will be correspondingly higher, since these reactions depend on the Brownian sampling of relative orientations of nearest neighboring proteoids. This Brownian sampling is related to the effective rotational diffusion coefficients of monomer proteoids in the crowded environment; protrusions that extend outside of the outer circular arc of the ASP substructure, which is the case for HC- and HE-proteoids, would tend to lower such effective rotational diffusion coefficients. Likewise, once dimers have been formed, the rate of crystal growth is also related to the rotations of proteoid dimers near the surface of the growing crystal, since these proteoids must be properly oriented in order to be incorporated into the crystal. Less compact dimer shapes that have protrusions do not rotate as readily as more compact dimer shapes that do not have protrusions, so again the kinetics of the growth of dimer crystals would be faster for HI-proteoids than for HC- and HE-proteoids.

Beyond analogies to proteins, the structures of the proteoids that we have fabricated and studied are also reminiscent of lyotropic lamellar phases of surfactant-like or lipid-like molecules that have heads and tails. For instance, the layered dimer structures of the DX1 and DX2 dimer crystals resemble to a certain extent interdigitated bilayer structures that can form of molecules that have hydrophilic head groups and hydrophobic tail groups. For both DX1 and DX2, the circular heads are positioned proximate to the heads of neighboring proteoids, and the ASP-tails are also proximate and interdigitating. These alternating regions of high densities of heads and tails lead to very strong spatial correlations of proteoid sub-structures, and this can also be seen in self-assembled lamellar phases. However, there are some differences, too, since the dimer crystals of proteoids have ordered arrangements of heads and tails within and between layers, whereas surfactant and lipid systems often have disordered liquid-like structures within individual layers.

Although we have used optical microscopy to examine microscale Brownian proteoids made using a stepper that has a minimum feature size specification of ≈ 300 nm, our methods can be directly extended to smaller proteoids using existing high-end lithographic steppers that offer minimum features sizes down to ≈ 30 nm. For such smaller proteoids, superresolution optical imaging, in-situ "wet" transmission electron microscopy, or standard TEM on fixed samples could be used instead of optical microscopy. Such smaller proeoids would potentially enable faster rates of crowding and crystallization, even if the underlying roles of core geometry and entropy maximization are the same.

The self-assembled quinary structures of hard proteoids that we have observed can be subsequently fixed to the substrate and retained by introducing a strong attractive colloidal interaction. This can be achieved in a variety of ways, such as by adding a depletion agent that has a larger size, by introducing divalent ions of the opposite sign compared to the charges on the surfaces of the proteoids (e.g. by dialyzing with a solution of $MgCl_2$ or $CaCl_2$, yielding Mg^{2+} or Ca^{2+}), and/or by modifying the pH of the aqueous continuous phase to protonate surface charges on the proteoids.

Future Directions

Exploring different histories of osmotic compression, such as varying the tilt angle in a time-dependent periodic manner, may enhance annealing, thereby reducing defects and promoting the growth of larger dimer crystallites. Likewise, turning on inplane attractions, for instance by adjusting the concentration of a second depletion agent having a larger size, could affect the resulting sizes and structures of dimer crystallites. Moreover, developing methods of fixing 2D dimer crystallites to the substrate (e.g. by dialyzing the aqueous phase with a salt solution that would sufficiently reduce the barrier provided by the Debye screened repulsion) or transferring them to other interfaces would be useful. Just as we have designed shapes of the proteoids to suppress the Ddimerization pathway and select only the other D+ pathway, we also anticipate that it may be possible to suppress the formation of one of the two competing crystal phases and selectively form only a single crystal type by further refining the shapes of the monomer proteoids. Furthermore, manipulating the process of crystallization and self-assembled crystallites by applying external electromagnetic fields could yield field-controllable structural transitions. Furthermore, predicting the distribution of sizes and types of dimer crystallites as a function of time in a slowly compressed system of proteomimetic shapes would also be useful.

Captions for Movies

Movie S1. Direct observation of a tautomerization translocation reaction (TTR) involving three HI-proteoids that occurs as a consequence of Brownian fluctuations in a system of crowded proteoids, measured using time-lapse optical photomicroscopy. Initially, a lone monomer is surrounded by lock-and-key dimers. As a consequence of Brownian fluctuations, a dimer, located adjacent to the monomer, de-dimerizes, so three monomers in proximity are observed. The initial monomer then forms a lock-and-key dimer with one of the proteoids resulting from the de-dimerization event, leaving a different monomer than the original one in a substantially different spatial position as a reaction product. Thus, looking only at the initial and final frames of the movie, a monomer appears to have diffused a large distance in a highly crowded environment of dimers. Thus, TTR effectively increases the rate of monomer diffusion in the crowded system, thereby facilitating the expulsion of monomers from growing crystallites. One frame has been recorded every three minutes in the laboratory; the movie plays at a speed of two frames per second. Scale: the outer radius of a HI-proteoid is 2.5 μ m. Average area fraction of proteoids in the field of view is $\phi_A \approx 0.41$. (AVI; 2.6 MB).

Movie S2. Direct observation of a tautomerization translocation reaction (TTR) involving four HI-proteoids in the crowded Brownian system, measured using time-lapse optical photomicroscopy. Initially, two monomers are proximate to neighboring lock-and-key dimers. Entropic fluctuations cause de-dimerization of a dimer adjacent to these monomers, yielding four monomers in proximity. The initial monomers combine with the proteoids resulting from the de-dimerization, leading to a final state that has two monomers that are significantly displaced from the location of the initial monomers. This 4-proteoid HI-proteoid TTR also increases the rate of expulsion of monomers from growing dimer crystals. One frame has been recorded every 30 minutes in the laboratory; the movie plays at a speed of two frames per second. Scale: the outer radius of a HI-proteoid is 2.5 μ m. Average area fraction of proteoids in the field of view is $\phi_A \approx 0.38$. (AVI; 5.2 MB).