# **Supporting Information**

# Self-Assembly of Mesoscale Artificial Clathrin Mimics

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### **Video S1 Caption**

ACM network budding from a butanol droplet into water. A magnet approaches from the bottom right of the video (dark region), causing an ACM network at the butanol surface to condense and collapse. Note the discrete collapse stages of the bound ACM network, which are plotted in Fig.4, representing the condensation of the open ACM network into smaller forms stabilized by the increased network rigidities at higher density. Ultimately, the magnetic force increases to the point of pull-off. Scale bar 500  $\mu$ m, 6x speed.

# Video S2 Caption

ACMs liftoff from their as-fabricated state. As the sacrificial germanium layer dissolves in a 0.3% hydrogen peroxide solution, the ACMs are individually released from the surface. Upon release, the ACMs go from their bright, flat as-fabricated state to a curved and scattering dark state. As the ACMs liftoff from the surface they aggregate in a loose and open manner. Scale bar 100 μm, 10x speed.







# Supplementary Figure 3.

Images of ACM network under 2D compression from a bar drawn over the surface. The compression of the ACM network occurs as the inertial and frictional drag of the large (3-5mm) network resists the force of the bar. Scale bar 100 µm. a) ACM network in native self-assembled state, without any compression. b) ACM network under a small amount of external force. Note the increased slip and overlapping defects in the network as tip-to-tip interactions break. c) As the network becomes increasingly compressed, a high amount of compaction and densification in the open ACM network occurs, leading to a more rigid structure. Even in the areas where tip-to-tip assembly is retained, ACMs tend to experience more curvature as evidenced by the larger dark, curved regions. This behavior is not reversible; upon relief of the compressing bar, the network retains its disrupted shape.

#### **ACM Network Compression**

A miniature trough capable of compressing the ACM network was constructed to demonstrate the effect of mechanical stress on the ACM network. As discussed further in the methods and supplementary methods, ACM islands of 3-5 mm size were initially assembled at an air-water interface in the miniature setup under microscopy. The network was then subjected to compression through the action of bar drawn along the surface of the fluid, and the results are shown in Fig. S3. A nocompression, natively assembled ACM network at low pressure is shown in Fig. S3a. Upon small levels of mechanical force, the ACM network was disrupted and additional defects began to form. The quantification of this compression was hampered as the ACM network itself possessed enough inertia and fluid drag so that as soon as the push-bar began pushing against the network, there was sufficient force to initiate compression. The primary defects are slip defects as the network's open structure collapses, and ACMs slide over top of one another. This effect helps demonstrates how open networks may collapse to space-fill, and as a result increase the opposing mechanical rigidity to further deformation.

Upon further mechanical force, the ACM network deforms surprisingly as shown in Figure S3c. Not only does the network densify with defects even further, creating a more compressed state, but the ACMs remaining in hexagonal configuration also show excess curvature, as evidenced by the greater dark regions in the image which are the result of more interfacial and ACM deformation. This directly evidences the mechanical stresses applied to the network, and suggest that not only do the tip-to-tip assembly forces and network openness play a role, but the individual ACM rigidity and resistance to bending could be used to modulate network collapse.

#### **Materials and Methods**

#### **ACM Fabrication**

The silicon wafer for ACM fabrication was initially coated with 20 nm of germanium deposited by electron beam evaporation (Innotec ES26C). Subsequently the wafer was functionalized using hexamethyldisilazane (J.T. Baker), and 700 nm of Microposit SPR 955CM-0.7 i-line photoresist was spin coated on top. The resist was exposed with an ASML PAS 5500 5:1 reducing stepper, and subsequently post exposure baked at 110°C for 90 seconds followed by development with MF-26A developer for 44 seconds, followed by a 60 second hardbake. Electron beam evaporation was subsequently used to deposit 80 nm of nickel followed by 25 nm of gold. The resist was then stripped by soaking for 1 hour in acetone, followed by sonication. The wafer with ACMs was finally cleaved into 10mm x10mm chips and residue was stripped by a 5 minute descum in a SPI Plasma Prep III plasma etcher at 100 W, 400 mTorr atmospheric plasma. To functionalize ACMs hydrophobically, they were submerged for 2 hours in a 10 mM solution of 1-Octadecanethiol (99%, Sigma Aldrich) in ethanol The ACMs were then rinsed and sonicated in isopropanol to remove excess thiols. ACMs were used for imaging immediately after functionalization.

#### ACM Imaging and Liftoff

For ACM liftoff and recruitment at an air-water interface, a droplet of either water (Milli-Q Gradient, 18.2 M $\Omega$ ) or water with 0.3% hydrogen peroxide (Sigma Aldrich) was placed on the ACM chip, so that a meniscus was formed. The droplet was then inverted and stored in a 100% humidity dish either for 20 hours with pure water or 2 hours with peroxide. The chip was imaged *via* reflected light microscopy during this period. Alternatively a 1.6 mm diameter, 25 mm length neodymium N52 magnet from K&J magnetics could be used to increase assembly speed by placing the magnet near the meniscus. To wash the assembly of excess aggregates, the droplet was inverted upright and then slowly lowered into a dish filled with water.

For ACM liftoff and recruitment at a butanol-water interface, 1-butanol was saturated with water and vice versa by vortexing the two fluids together for 5 minutes. After phase separation by gravity, the butanol phase was decanted and both phases could be used independently. In order to image budding from a butanol droplet, saturated butanol was loaded into a 100uL glass Hamilton syringe with a 1 mm OD / 0.5 mm ID borosilicate glass tube (Sutter Instruments) as the needle. The syringe was submerged into a butanol saturated water dish at approximately a 45° angle, where ACMs were allowed to liftoff in the water phase and fall *via* gravity to the dish bottom. The syringe attached droplet was then run along the bottom of the dish to pick up ACMs it came into contact with. The same neodymium magnet as used before was brought near the droplet from a 30° angle on the other side, while the budding was imaged in transmitted light microscopy.

For ACM budding imaged by fluorescence, a 1 inch glass well was attached to a petri dish using Apiezon N vacuum grease. The well was filled with butanol saturated water and ACMs were allowed to liftoff and fall to the bottom of the well *via* gravity. All but 1 mL of the water in the well was subsequently removed, and 50  $\mu$ L of 0.2 mM solution of a 24 oligomer conjugated with fluorescein (see supplementary) was added to the water phase. Following this addition, water saturated butanol was carefully layered over top of the water meniscus and the dish outside the well was filled with butanol until the well was overfilled. A neodymium magnet was then brought near the interface to promote ACM recruitment, and was lowered again until budding was achieved. The magnet was then raised but kept in the butanol phase, and moved to the petri dish and the droplet was imaged by fluorescence microscopy.

#### ACM Modelling

Simulation of the ACM mechanical properties was done in COMSOL<sup>1</sup> Multiphysics structural mechanics package. The bimetallic stress between ACM layers and total ACM strain energy was found by fitting the modelled ACM curvatures to observed values. Interfacial energy of the ACM was calculated numerically by solving the Young-Laplace equation around the ACM (see supplementary) in COMSOL. The structural energy and surface distortion energy values were iterated against one another until they were equal – this was taken to be the equilibrium ACM curvature. The equilibrium ACM curvature was then used to numerically solve for interaction energies between ACM by varying distance and orientation.

#### **DNA Fluorescein Conjugate**

Oligo sequence, 5' -> 3'

fluorescein – AAAAAAAAAAAAAAAACCCGGGCCC

## **Energetics of Budding Formation**

The forces required to achieve the transformation of a lipid membrane and a fluid-fluid interface are significantly different. The bending modulus of a typical lipid membrane is  $0.5 - 2x10^{-19}$  J,<sup>2,3</sup> and the energy to form a spherical lipid vesicle is on the order of 2.5x10-18 J. In comparison, the surface energy to form a 200 µm butanol-water droplet is 9x10-10 J, such as in Fig. 3I. This interface has a significantly lower surface tension value<sup>4</sup> of 1.8 mJ/m<sup>2</sup> compared to the surface tension of water-air, which is 72 mJ/m<sup>2</sup>, but is still substantially higher than a lipid membrane.

#### Germanium Liftoff layer

A benefit of producing ACMs *via* photolithography is our selection of germanium as a lift-off layer. Germanium is a high temperature, CMOS compatible material which can be used to lift off our ACMs in situ. This is in contrast to previous work done on lithoparticles, which use a polymer layer as a lift off layer<sup>5</sup>. The most apparent benefit is that because germanium dissolves slowly in water or rapidly in low concentrations (<0.5%) of hydrogen peroxide, we can release our particles in pure aqueous solution and avoid having to pass through an intermediate alkaline or organic solvent phase for assembly. This reduces the amount of handling necessary for ACMs, which in turn reduces the amount of nonspecific aggregation they are subject to and allows their curved structure to provide sufficient steric repulsion to avoid irreversible aggregation. ACMs can also be made at low concentrations initially and directly transferred in a dispersed manner to the water-air interface. By using different densities of ACMs, we have been able to minimize non-specific aggregates networks such as in Fig. 2f-h and Fig. 3b, though at the cost of assembly density. This can be addressed by decreasing the ratio of fluid interface area to wafer area, and concentrating particles as they reach the interface instead of as they are fabricated.

#### ACM Imaging

Recruited ACMs are easily imaged *via* reflected light microscopy; their reflectivity causes strong off-axis scattering. This scattering means that ACMs not aligned to the plane of the image appear darker than the background, as they reflect light away from the microscope objective while blocking background illumination. We can also easily determine when ACMs are bound at the interface, as bound ACMs reflect light back towards the viewer, resulting in a bright appearance. This effect is enhanced by refraction from particles submerged in the water surface compared to particles recruited to the waterair interface.

# **Electrostatic Approximation**

It interesting to note that the Laplace-Young equation  $\Delta p = -\gamma \nabla \cdot n$ , where  $\Delta p$  is the pressure difference of the boundary,  $\gamma$  is the surface tension and n is the surface normal - is very similar to the divergence equation for electrostatics  $\rho = \varepsilon \nabla \cdot E$  where  $\rho$  is the charge density,  $\varepsilon$  is the dielectric constant

and E is the electric field. We can exchange variables to make these two equations equivalent, whereby the value of surface tension can be equated to the dielectric constant, the pressure difference across the interface is equivalent to the charge density and the surface height is equivalent to the electric potential value. We can then conceptualize the surface tension mediated interactions as an equivalent electrostatic interaction. We used this Laplacian equivalence to solve the system energy in COMSOL using the electrostatics module.

#### **ACM Attachment Energy Calculation**

A simple calculation of the surface energy of ODT-water interface was referenced<sup>6</sup> as 72 mJ/m<sup>2</sup> with a 110° contact angle. The surface energy of ODT-air was therefore calculated as 96mJ/m<sup>2</sup> using Young's equation, and the energy of attachment was approximated as  $(96 \text{ mJ/m}^2 - 2* (72 \text{ mJ/m}^2))* (1 \ \mu\text{m} * 10 \ \mu\text{m} * 3) = -1.44 \times 10^{-12} \text{ J} = -3.5 \times 10^8 \text{ kT}$ . Note that this does not include the energy of interfacial distortions, as this is dependent on particle curvature.

### Supplementary References

- (1) COMSOL AB. COMSOL Multiphysics User's Guide, **2012**.
- Schneider, M. B.; Jenkins, J. T.; Webb, W. W. Thermal Fluctuations of Large Quasi-Spherical Bimolecular Phospholipid Vesicles. *J. Phys.* **1984**, *45*, 1457–1472.
- (3) Evans, E.; Rawicz, W. Entropy-Driven Tension and Bending Elasticity in Condensed-Fluid Membranes. *Phys. Rev. Lett.* **1990**, *64*, 2094–2097.
- (4) Adamson, A. W. *Physical Chemistry of Surfaces*; Wiley,: New York :, **1997**.
- (5) Hernandez, C. J.; Mason, T. G. Colloidal Alphabet Soup: Monodisperse Dispersions of Shape-Designed LithoParticles. J. Phys. Chem. C **2007**, *111*, 4477–4480.
- (6) Schwarz, J. A.; Contescu, C. I.; Putyera, K. Dekker Encyclopedia of Nanoscience and Nanotechnology, Volume 5; CRC Press: New York, 2004.