INTRODUCTION

DNA origami was invented in 2006 by P. Rothemund (1) by rendering in DNA solid two-dimensional (2D) geometries including squares, triangles, stars, and a smiley face. In this first implementation, rectilinear, parallel DNA duplexes were interconnected with crossovers of antiparallel DNA strands consisting of a long scaffold strand from the M13 phage genome hybridized to hundreds of shorter, synthetic staple strands. This rectilinear or brick-like fabrication strategy is powerful because it offers straightforward scaffold strand routing and staple sequence design manually or using simple computer-aided design tools (2), rendering the technique broadly accessible to nonexperts. However, because these 2D origami objects are visualized nearly exclusively on 2D surfaces using atomic force microscopy (AFM) or transmission electron microscopy (TEM), objects typically appear flat despite their curvature, bend, or twist in 3D solution (3–5). Although 3D structure prediction tools such as CanDo (6) have been used to reduce out-of-plane deformations (7–9), experimental validation of planarity has remained elusive, together with general design rules to attain and maintain planarity for arbitrary 2D geometries. While there have been several attempts to both study and control the planarity of 2D DNA origami rendered with single-layer parallel duplexes in recent years (10, 11), resolving the 3D structure of these assemblies in solution has remained elusive, likely because of their flexibility and heterogeneity in solution, corroborated by solution scattering data (10) and AFM (11). One exception is a recent 3D cryo–electron microscopy (cryo-EM) study that revealed significant flexibility and curvature of a rectangular origami rendered with single-layer duplexes organized in parallel, as originally implemented by Rothemund (1), whereas the 3D structures of rigid and largely homogeneous multilayer brick-like origami were reconstructed to nucleotide resolution in the same report (12).

While experimentally elusive to realize and validate, planarity of 2D origami is of paramount importance to numerous applications that seek to organize secondary materials with nanometer-scale precision (13, 14), including fundamental studies of light harvesting and excitonics (15–18), single-molecule (19–21) and superresolution imaging (19, 22), molecular biophysics (23), photonics (24), cellular biophysics (25–28), and surface-based patterning and lithography (29, 30). Multilayer honeycomb (31) and square lattice (32) brick-like origami designs offer alternatives to fabricating monolayer 2D origami, but they achieve planarity while reducing the overall lateral dimension of objects that can be rendered because of the increased length of scaffold required; they may require careful sequence design with iterative feedback from structural simulations and experiment to reduce or eliminate intrinsic twist (6, 8, 9, 32), and they are largely limited geometrically to rendering rectilinear geometries that consist of parallel duplexes throughout the object, with (33) or without curvature (1, 34). Attaching 2D monolayer origami to surfaces using high-affinity ligand-receptor pairs may be used to partially flatten objects, although experimental validation is again challenging because of the perturbative nature of AFM and the low contrast of TEM, and numerous applications are not amenable to this biochemical immobilization strategy.

As an alternative to rectilinear, brick-like origami, 2D wireframe DNA origami has recently emerged as an alternative for positioning secondary materials in 2D with nanometer-scale precision and local orientational control over duplex axes (35, 36). Compared with conventional, rectilinear brick-like origami, wireframe geometries can render polyhedral geometries that are not accessible to rectilinear duplex assemblies (35, 36); they reduce the overall scaffold length required to render 2D objects of a given lateral dimension because of their open, mesh-like structure that minimizes the use of DNA, and they also offer local orientational control over duplex orientations that may be required for some applications, such as organizing chromophores to control molecular excitonics (37–39) and photonics (30).
The fully automated sequence design tool METIS (Mechanically Enhanced and Three-layered origami Structures) offers, in principle, the ability to render planar 2D wireframe objects using multilayer, six-helix bundle (6HB) edges, although, to date, experimental characterization has been limited to 2D imaging that suffers similarly from potential artifacts from 2D solid support (35). Here, we report results of 3D cryo-EM to resolve the first examples of planar 2D DNA origami structures rendered using 6HB wireframe designs up to 80 nm in lateral dimensions. To test the breadth and diversity of 2D objects that can be fabricated, we explore two distinct categories of wireframe objects, which are polygonal with and without internal structure. In addition, to establish the sizes of objects that can be rendered in this manner, we vary lateral edge lengths from 42 to 210 base pairs (bp). 3D reconstruction demonstrates that homogeneous, planar objects result from our approach, with resolutions of 10 to 18 Å that suggest minimal structural deviation from planarity up to an ~80-nm lateral dimension. This class of planar DNA origami is rendered from the top-down using the fully automatic sequence algorithm METIS (35) implemented in the graphical user interface (GUI) ATHENA (40), rendering it broadly useful for 2D nanoscale material design and patterning.

RESULTS

Computer-aided design of 2D origami

6HB edge–based 2D wireframe DNA origami structures were rendered using METIS (33) within ATHENA (40) (Fig. 1 and fig. S1). To investigate the planarity of origami objects, diverse target planar geometries were chosen including hexagons, pentagons, and triangles that are symmetric versus asymmetric. To also evaluate the effects of an internal mesh on planarity, we rendered hexagons and pentagons both with and without internal wireframe support (table S1). Last, pentagons with different edge lengths were generated as described previously (40) to explore the impact of lateral dimensions on the planarity attained.

Planarity of 2D wireframe origami

The METIS algorithm leverages the 6HB edge motif to attain structural rigidity of edges and the maximum number of vertex crossovers between each duplex in adjacent edges to also endow overall rigidity and ideally planarity across the complete object (Fig. 1).

While this multilayer design with multiway vertex connections was previously shown to enhance in-plane structural integrity compared with corresponding DX (double-crossover)-based objects composed of only two duplexes per edge, AFM and TEM characterization in that work were unable to determine out-of-plane deformations versus planarity of the fabricated structures in solution (35).

Using distinct target boundary geometries including a hexagon and pentagon, we used cryo-EM to first evaluate the planarity for these objects with and without an internal mesh (Fig. 2). The internal mesh was originally introduced to reinforce the in-plane structural fidelity of the target 2D wireframe structures, which were verified using AFM and TEM to have accurate target angles between neighboring arms (35). Cryo-EM imaging and 3D reconstruction here verified that the objects not only have accurate internal angles but also remain planar within ~6 nm across their lateral 80-nm dimension, irrespective of whether the internal mesh is present (Fig. 2).

The hexagon and pentagon with internal mesh structures were assigned a minimum edge length of 84 bp, whereas their corresponding hollow structures were assigned a minimum edge length of 106 bp for the hexagon and 122 bp for the pentagon to achieve the same diameter of 80 nm. As shown in Fig. 2A, the hexagonal structure with internal mesh is in agreement with our design when facing up during imaging, which is consistent with its AFM and TEM images (35), although, now, the structures were frozen in solution without any surface-imposed restrictions.

Individual DNA structures in different orientations are also identifiable in vitreous ice, with their planarity apparent from different orientations of the 2D objects and the most apparent case evident from 2D objects in a vertical orientation (fig. S2). Similar observations were made for the pentagonal structure with internal mesh (fig. S3), as well as for the hexagonal and pentagonal structures without internal mesh. As shown in figs. S4 and S5, both the hollow hexagon and pentagon are planar in solution, appearing as a straight line when oriented vertically in cryo-EM imaging. While the preponderance of objects appeared planar at the single-particle level, occasionally, structures also show some curvature (figs. S2 to S6). However, single-particle heterogeneity is sufficiently low that 2D class averages are easily generated (Fig. 1B and figs. S3 to S5).

Aside from achieving planarity for complex wireframe origami objects, these 3D reconstructions also offer the first reported examples

![Fig. 1. Cryo-EM characterization of 2D wireframe structures. The input used for ATHENA software can be specified as arbitrary-shaped target geometries. On the basis of the target geometry, the METIS algorithm was used to generate scaffold and staple routing of wireframe DNA origami with six-helix bundle (6HB) edges. Maximum crossovers between adjacent edges are used to ensure the rigidity of the vertex design. The pseudo-atomic model was generated to compare to the structure determined by cryo-EM 3D reconstruction.](image-url)
Fig. 2. 6HB-based wireframe DNA origami structures with and without internal mesh characterized by cryo-EM. (A) A representative METIS 2D wireframe DNA origami structure characterized by cryo-EM: a hexagonal origami with internal mesh. Different random orientations of the structure under cryo-EM imaging and 2D class averages reflected its planarity. 3D reconstruction shows that the hexagonal origami structure is 2D and planar, with a diameter of 80 nm and a thickness of only 5 nm. Scale bars, 50 nm. (B) Two different geometries (hexagon and pentagon) with the same diameter were generated with the computer-aided design tool. The predicted atomic models are compared to the reconstructed 3D structures, which show good matches for all four structures. As demonstrated by side views, the wireframe structures without internal mesh show almost perfect planarity, similar to the corresponding structures with internal mesh. No twisting was observed for most of the edges shown above, except the internal mesh edges of the pentagon structure as pointed out by the arrow (row 2). Missing density in the vertex was also observed in these areas.
of 2D cryo-EM DNA origami structures with a size of 80 nm that we are aware of. Rotation of the objects along one axis reveals not only planarity but also lack of overall twist, as observed in another study (12). While most of the 6HB edges in our structures do not show twist, the internal mesh of the pentagonal object does exhibit a left-handed twist that is apparent on the inner spoke edges. This is consistent with the correlation coefficient between the pseudo-atomic model and density map: The pentagonal structure with internal mesh has a correlation coefficient of 0.75, which is lower than the hexagonal structure with internal mesh (0.82). For the hollow hexagon and pentagon, the correlation coefficients are 0.84 and 0.83, respectively (table S2). Resolutions of the four objects are also comparable, with the hexagon and pentagon with internal mesh reconstructed to a resolution of 18 and 17 Å, respectively, and the two hollow structures reconstructed to 16-Å resolution.

Impact of edge length on planarity

In light of the planarity achieved by wireframe origami without an internal mesh, we chose the pentagon as a model geometry to investigate whether changing edge length may affect planarity. Comparison of 84- and 122-bp minimum edge length pentagonal structures showed retention of planarity despite changes in edge length, with a resolution of 16 Å based on Fourier shell correlation (FSC) curves (Fig. 3A and figs. S5 and S6). For both structures, the pseudo-atomic model prediction (Protein Databank or PDB file) generated by ATHENA fits well within the density map, with the same correlation coefficient of 0.83, and there is no obvious twisting along the edges regardless of the differing edge lengths. Because the differences in edge lengths between the two structures are not an integer number of helical turns of DNA, the staple crossover patterns in the vertices are different between the two pentagons, although the vertex angles are the same for both objects (fig. S7). The fact that the resolution, model fitting, and quality of both cryo-EM structures are the same suggests that our vertex design parameters are likely to be generally valid. A discrete advantage of using a 6HB edge compared with a single DNA duplex- or DX-based edge is its relatively large persistence length of 1 to 2 µm associated with its rigidity (41, 42), which is essential for the structural integrity of wireframe DNA origami, particularly for the hollow structures fabricated here. To further investigate the edge length variation, five pentagonal structures with edge lengths ranging from 42 to 210 bp (14 to 71 nm) were characterized by cryo-EM imaging (Fig. 3B). Smaller-sized pentagons of 42- to 126-bp edge lengths can readily adapt to different orientations in vitreous ice, with their planarity observed in cryo-EM imaging. In contrast, pentagons with 168-bp edge length appeared largely regular, although they adopted fewer distinct orientations.

**Fig. 3.** Pentagon-shaped wireframe DNA origami structures designed with different edge lengths and characterized by cryo-EM. (A) The 3D reconstruction of two pentagon structures, one designed with 84-bp minimum edge length and the other designed with 122-bp minimum edge length. Both structures are planar, and their vertex designs match well with the reconstructed structure. (B) Cryo-EM imaging of different-sized pentagon structures, from 42- to 210-bp edge length. Scale bars, 50 and 100 nm (zoom-in and zoom-out images, respectively).
during imaging because of their larger size (~100 nm in diameter). When the edge length reached 210 bp, kinking and bending were observable along the edge and vertex of the pentagonal structure, suggesting an upper limit of ~100-nm overall dimension and ~60-nm edge length for METIS to realize planar objects with accurate internal target angles based on cryo-EM imaging results (Fig. 3B).

**Rendering symmetric versus asymmetric objects**

To test whether planarity is retained with asymmetric objects, asymmetric and symmetric triangles with equal maximum edge lengths of 84 bp were examined, with reconstruction global resolutions of 11 and 13 Å, respectively, and planarity is again retained together with target internal angles (Fig. 4 and figs. S8 and S9). To investigate the structural fidelity of the triangular structures, pseudo-atomic models generated by ATHENA were used to fit the density maps from the 3D cryo-EM reconstructions. The total correlation coefficient between the atomic model and density map was 0.85 for the symmetric triangle and 0.87 for the asymmetric triangle. For the symmetric triangle, there was missing density in the vertex, as can be seen from the reconstructed density map. This is consistent with molecular dynamics simulations performed in previous work (35), in which higher local conformational flexibilities at the vertices were observed. The local resolution maps also show lower resolutions of both symmetric and asymmetric triangle in all the vertices, whereas the highest resolutions are observed in the edge, with up to 10 Å for symmetric triangle (fig. S10). For the asymmetric triangle, although the vertices show better resolved electron density, the shortest edge exhibits outward bowing that is only slightly visible in the other edges of this and the symmetric triangular object. Comparison of the cryo-EM reconstruction of symmetric and asymmetric triangles also offered the ability to test whether the resolution of the symmetric structures is limited by imposing symmetry during class-averaging and 3D reconstruction. Figure S11 shows the linear correlation between particle number and map resolution (43), with B-factors estimated to be 1954.5 and 2276.8 Å² for the symmetric and asymmetric triangle, respectively. Because it can be inferred from these plots that using the same number of particles (or even triple the number) for reconstruction will result in similar resolutions for the symmetric and asymmetric triangles, this suggests that not only the limitation in resolution does arise from symmetry but also vertex design and overall 6HB rigidity and structural fidelity are likely also factors that limit the highest resolution that can be achieved. With an 11-Å global resolution for symmetric triangles and 13 Å for asymmetric triangles, these represent the highest resolutions achieved for 6HB structures to date.

**Molecular simulation of 2D origami objects**

To gain dynamical insight into the molecular-level structure and flexibility of the designed origami objects, we performed coarse-grained molecular dynamics simulations with oxDNA2 to complement experimental cryo-EM data (Fig. 5). The oxDNA2 coarse-grained model was used because it accurately represents the thermodynamic and mechanical properties of DNA while enabling long time scale simulations at lower computational cost compared with classical
all-atom models (44, 45). As the largest and most complex wire-frame origami folded in this study, the hexagonal and pentagonal DNA origami objects with internal meshes were first chosen for oxDNA2 simulation (figs. S12 and S13). Simulations demonstrated general planarity of 2D DNA objects and varied flexibility for different objects, indicated by values of RMSF (root mean square fluctuations) relative to the mean structure (Fig. 5). Compared with the hexagon, the pentagonal structure exhibited higher flexibility in vertices, consistent with our experimental data that showed missing density and curved edges in the reconstructed pentagonal object. In addition, the two triangles with the highest structural resolutions were simulated, with the symmetric triangle in good agreement with our previous fully atomistic MD simulation (35). Moreover, there are lower RMSF values of both triangular objects compared to larger structures such as the hexagon and pentagon. However, the RMSF analysis reveals higher flexibility at the vertices, while the edges showed only minor temporary deviations from planarity leading to very flat structures on average (detailed RMSF values and structure files are provided in Supplementary Materials). At the level of individual helices, the greater spacing between crossovers and loops led to a larger flexibility at the vertices compared to the edges, as well as more pronounced splaying at the inside of sharp bends. Together, these simulations corroborate the high structural fidelity and planarity of these METIS-designed 2D DNA origami objects observed using cryo-EM.

DISCUSSION

2D DNA origami is emerging as a very widely used template for functional material fabrication, with applications to molecular biophysics (23), excitonics (15–18), photonics (24), and plasmonics (14) among others. In the present work, we investigate the planarity of 2D wireframe DNA origami designed using the algorithm METIS, as characterized by free-standing cryo-EM. Planarity was exhibited by regular shapes such as hexagonal and pentagonal objects, whether reinforced internally with an internal mesh or not, up to an overall lateral dimension of ~80 nm. In addition, an irregular, asymmetric triangle was reconstructed to 13-Å resolution by cryo-EM, which, together with up to 10-Å resolution for the symmetric triangle, represents the highest resolutions to date for DNA origami cryo-EM structures based on the 6HB motif. The enhanced vertex designs used by the METIS algorithm suggest that diverse classes of 2D objects designed will remain planar, although further experimentation

**Fig. 5. Comparison between oxDNA2 coarse-grained simulations and cryo-EM structures.** Hexagonal and pentagonal origami with internal mesh and symmetric and asymmetric triangles were chosen for oxDNA simulations. Subtle structural features predicted by the simulations are consistent with experimental observations, as highlighted by arrows. The structures from the trajectory with the lowest RMSF to the mean structure are shown (centroid structures). The RMSF (nm) values were represented as a color bar: 0.64 (blue) to 4.84 (red). Ranges of RMSF (nm) for each structure are the following (from top to bottom): 1.14 to 3.15, 1.43 to 4.84, 0.64 to 1.70, and 0.67 to 1.92.
is needed to support this hypothesis. These 2D planar DNA origami may be used in the future as a platform for the judicious placement of functional components, such as proteins (28, 46, 47), chromophores (48, 49), and nanoparticles (50, 51) with high structural fidelity in solution, which opens exciting possibilities for diverse 2D functional materials and biomolecular applications accessible to a broad community of researchers through the open-source software METIS and its GUI ATHENA.

**MATERIALS AND METHODS**

**Top-down sequence design**

ATHENA was used to design 2D DNA wireframe structures using the METIS “top-down” approach. It is provided online for use as standalone open-source software (https://github.com/lcbb/athena) for the custom design of 2D and 3D wireframe scaffolded DNA origami objects. Output files include staple strand sequences (tables S3 to S14) and PDB files for oxDNA simulations to study structural and conformational dynamics.

**Materials**

DNA origami staple strands were purchased in a 96-well plate format from Integrated DNA Technologies Inc. at 25-nmol synthesis scale, with strands purified by standard desalting and calibrated to 200 μM based on full yield. Staple strands were mixed in equal volume from the corresponding wells and used directly for DNA origami folding without further purification. DNA scaffolds of lengths 2775 and 7249 nucleotides (nt) were used. The 2775-nt DNA scaffold was produced using restriction enzyme cloning. The 2775-nt plasmid assembled using restriction enzyme cloning was transformed into *Escherichia coli* containing the M13cp helper plasmid. The 2775-nt scaffold was subsequently amplified in bacteria in 2xYT incubated for 8 hours at 37°C and then harvested and purified (52). The 7249-nt DNA scaffold (M13mp18) was purchased from Guild BioSciences at a concentration of 100 nM. TAE (Tris-acetate-EDTA) buffer (1×) with 12.5 mM Mg(OAc)₂ was prepared with 10× TAE buffer (molecular biology grade) was purchased from MilliporeSigma. Magnesium acetate tetrahydrate (molecular biology grade) was purchased from Alfa Aesar. Magnesium acetate tetrahydrate (molecular biology grade) was purchased from MilliporeSigma. TAE buffer (1×) with 12.5 mM Mg(OAc)₂ was prepared with 10× TAE buffer and magnesium acetate tetrahydrate. Agarose (molecular biology grade) was purchased from IBI Scientific.

**Origami self-assembly**

All METIS structures were folded following the same protocol. DNA scaffold (10 nM) was mixed with 15 equivalents corresponding DNA scaffold (10 nM) was mixed with 15 equivalents corresponding scaffold was subsequently amplified in bacteria in 2xYT incubated as restriction enzyme cloning was transformed into *Escherichia coli* containing the M13cp helper plasmid. The 7275-nt scaffold was subsequently amplified in bacteria in 2xYT incubated for 8 hours at 37°C and then harvested and purified (52). The 7249-nt DNA scaffold (M13mp18) was purchased from Guild BioSciences at a concentration of 100 nM. TAE (Tris-acetate-EDTA) buffer (1×) was purchased from Alfa Aesar. Magnesium acetate tetrahydrate (molecular biology grade) was purchased from MilliporeSigma. TAE buffer (1×) with 12.5 mM Mg(OAc)₂ was prepared with 10× TAE buffer and magnesium acetate tetrahydrate. Agarose (molecular biology grade) was purchased from IBI Scientific.

**Cryo-EM imaging with 3D reconstruction**

DNA origami samples (3 μl) were applied onto glow-discharged copper C-flat thick R2/1 300-mesh grids and frozen in liquid ethane using a Vitrobot Mark IV (Thermo Fisher Scientific). For hexagonal DNA origami with internal mesh (84-bp edge length), pentagonal DNA origami with internal mesh (84-bp edge length), and pentagonal DNA origami without internal mesh (84-bp edge length), the grids were imaged on a Titan Krios G3i cryo–electron microscope (Thermo Fisher Scientific) operated at 300 kV at a magnification of ×53,000 (corresponding to a calibrated sampling of 1.7 Å per pixel). Micrographs were recorded by EPU software (Thermo Fisher Scientific) with a Falcon K3 direct electron detector in counting mode, where each image is composed of 30 individual frames with an exposure time of 6 s and a total dose of ~40 electrons/Å². A total of 1594 images for the hexagonal DNA origami with internal mesh (84-bp edge length), 1048 images for the pentagonal DNA origami with internal mesh (84-bp edge length), and 1100 images for the pentagonal DNA origami without internal mesh (84-bp edge length) were collected with a defocus range of −1 to −3 μm. For hexagonal DNA origami without internal mesh (106-bp edge length) and pentagonal DNA origami without internal mesh (122-bp edge length), the grids were imaged on a Titan Krios G3i cryo–electron microscope (Thermo Fisher Scientific) operated at 300 kV at a magnification of ×37,000 (corresponding to a calibrated sampling of 2.1 Å per pixel). Micrographs were recorded by EPU software (Thermo Fisher Scientific) with a Falcon 4 direct electron detector in gain-normalized mrc mode, where each image is composed of 20 individual frames with an exposure time of 10 s and a total dose of ~20 electrons/Å². A total of 1170 images for the hexagonal DNA origami with internal mesh (106-bp edge length) and a total of 1331 images for the pentagonal DNA origami with internal mesh (122-bp edge length) were collected with a defocus range of −2 to −3.5 μm. For symmetric triangular DNA origami (84-bp edge length) and asymmetric triangular DNA origami (84-, 73-, and 63-bp edge lengths), the grids were imaged on a Talos Arctica G2 cryo–electron microscope (Thermo Fisher Scientific) operated at 200 kV at a magnification of ×64,000 (corresponding to a calibrated sampling of 1.4 Å per pixel). Micrographs were recorded by EPU software (Thermo Fisher Scientific) with a Gatan K3 direct electron detector in counting mode, where each image is composed of 40 individual frames with an exposure time of 2.5 s and a total dose of ~50 electrons/Å². A total of 4093 images for the symmetric triangular DNA origami (84-bp edge length) and a total of 4135 images for the asymmetric triangular DNA origami (84-, 73-, and 63-bp edge lengths) were collected with a defocus range of −1.5 to −3.5 μm.

Single-particle image processing and 3D reconstruction was performed as previously described (53). Briefly, all the images were motion-corrected using MotionCor2, and CTF (Contrast Transfer Function) was determined using CTFFIND4. All particles were autopicked using NeuralNet option in EMAN2 and further checked manually, yielding 14,029 particles for hexagonal DNA origami with internal mesh (84-bp edge length), 30,279 particles for pentagonal
The production simulations were run for 0.303 ms (10^8 steps; time 10^7 steps) at 300 K using the Langevin thermostat (diff_coeff of 2.5).

Coarse-grained molecular dynamics simulations using the oxDNA2 model
MD simulations of DNA nanostructures were performed using the oxDNA2 model and simulation software (44, 45, 56, 57). The oDNA model is a coarse-grained approximation to study the thermodynamic and mechanical properties of DNA, enabling longer time scales and larger system sizes to be simulated (44, 45). Fully atomistic DNA nanostructures from ATHENA were converted into oxDNA file format using tacoDNA (58). Systems were simulated at a salt concentration of 1 M [Na+] as recommended by the developers of oxDNA to represent typical experimental conditions (3). All structures were energy-minimized for 2000 steps, followed by short simulations to equilibrate the structures for 3.03 to 30.3 µs (10^6 to 10^7 steps) at 300 K using the Langevin thermostat (diff_coef 2.5). The production simulations were run for 0.303 ms (10^7 steps; time step of 0.1515 ps) at 300 K using the Anderson-like Joint thermostat (diff_coef 2.5). Initial velocities were refreshed from a Maxwellian distribution. The simulations were visualized using oXView and analyzed using oxDNA analysis tools (57, 59).

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.sciadv.aabn0039

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Acknowledgments: We thank E. Brignole for assisting cryo-EM imaging. Cryo-EM images are collected at MIT-nano on a Talos Arctica microscope, which was a gift from the Arnold and Mabel Beckman Foundation. Funding: We are grateful for funding support from National Science Foundation NSF CBET-1729397 and CCF-1956054 and Office of Naval Research N00014-21-1-4013 and N00014-20-1-2084 (to W.C.), and the research was sponsored by the U.S. Army Research Office and accomplished under cooperative agreement W911NF-19-2-0026 for the Institute for Collaborative Biotechnologies (ICB subaward KK1955). T.J. acknowledges financial support from the Alexander von Humboldt Foundation through a Feodor-Lynen Research Fellowship. H.F. is grateful to the EPSRC for support through the Centre for Doctoral Training Theory and Modelling in Chemical Sciences under grant EP/L015722/1. Author contributions: X.W., H.J., and M.B. conceived the research. X.W., H.J., and M.B. implemented the autonomous design algorithm and optimized the experimental data. K.Z. analyzed the experimental data. T.J., H.F., and J.P.K.D. implemented the theoretical approaches in molecular dynamics simulations on GPUs. J. Phys. Chem. Lett. **9**, 1606–1616 (2012).

Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Representative image data generated and/or analyzed during the current study are shown in figures and the Supplementary Materials. The 3D cryo-EM density maps reported in this manuscript have been deposited in the Electron Microscopy Data Bank (EMDB) with entry ID numbers EMDB-26323, EMDB-26324, EMDB-26325, EMDB-26326, EMDB-26327, EMDB-26328, and EMDB-26329.