# Two-dimensional protein arrays: *de novo* design and applications

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## INTRODUCTION

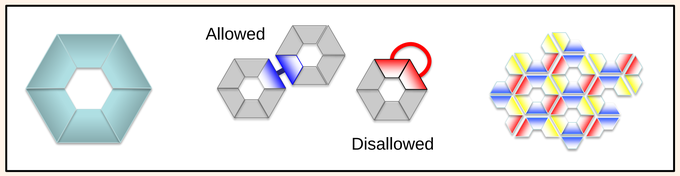
Biological building blocks that self-assemble into predetermined supramolecular structures hold promise for the production of advanced materials, devices and systems. However, our ability to predict, engineer and control short- and long-range interactions in proteins is lagging and nature's most versatile building block remains underused. Two dimensional (2D) protein arrays are of considerable interest in bionanotechnology.[1] For instance, purple membrane patches have been exploited for optical information storage and processing,[2] and archeal and bacterial S-layers have been patterned on surfaces and used as templates and display scaffolds.[3] Yet, neither the geometry, nor the chemistry or assembly of these systems can be precisely controlled from the nano- to the mesoscale. Here, we describe the computational design and construction of proteins that self-assemble into 2D hexagonal arrays with 7.25 nm periodicity and 5 nm height upon addition of calcium ions.[4] We further show that these lattices – which may exceed 100 µm in characteristic length – can incorporate modified protomers engineered with a biotinylation tag. Increasing the molar ratio of mutant to wild type protein leads to a concomitant increase in the amount of Avidin that an array can bind. Our results pave the way for using computationally designed protein arrays in a broad range of bionanotechnology applications.

## STRATEGY

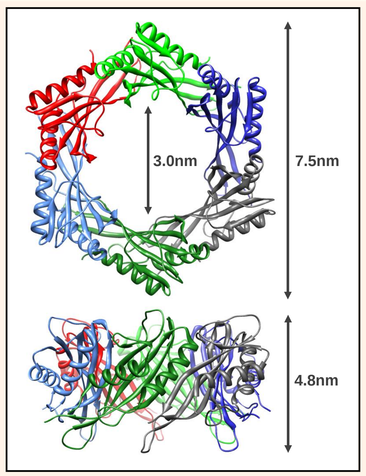
**Step 1:** Screen the PDB for all complexes with $C_3$, $C_4$ or $C_6$ symmetry

**Step 2:** Filter for ~3nm pore and allowable position of N- and C-termini

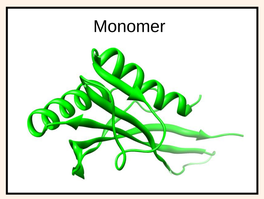
**Step 3:** Run Rosetta's symmetric docking, redesign the packing interface and optimize linker to join the C-terminus of one subunit in an oligomer to the N-terminus of a subunit in a neighboring oligomer



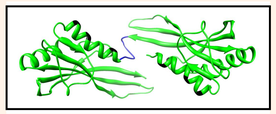

## CANDIDATE: STM4215



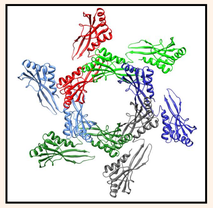

STM4215 is a *S. typhimurium* protein of unknown function whose structure was solved by the PSI. The protein assembles into a homo-hexamer formed by 175 residues protomers. It meets our requirements for packing, pore size, and the creation of a planar 2D surface.



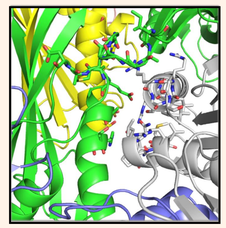

## RESULTS: Modeling



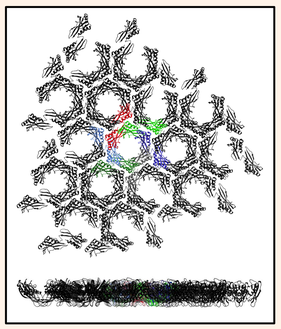
TTM consists of 2 truncated STM4215 monomers bearing 4 interface mutations and linked to one another by a hexaglycine linker



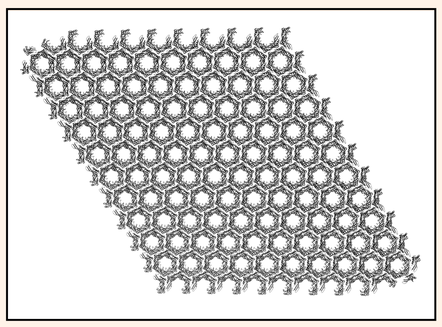
A TTM homo-hexamer with six nucleation sites defined by the fused subunits



Close up view of the mutations introduced at the dimer interface

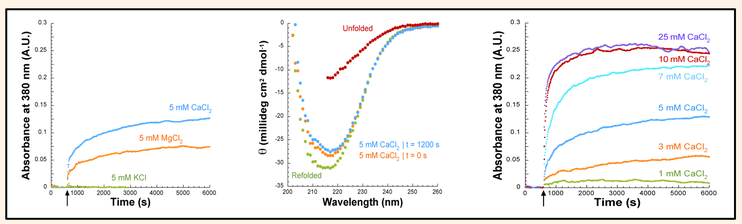


Top and side views of a growing array



Predicted structure of the crystalline array

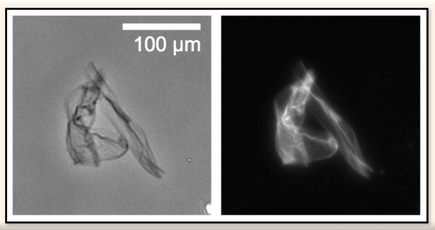
## Refolding and initiation of assembly




Addition of a divalent cation is required to initiate polymerization of refolded TTM

The rate and extent of polymerization can be tuned by varying protein and calcium concentration (not shown). Initial kinetics exhibit apparent 2nd order with respect to both TTM and $CaCl_2$ concentration.

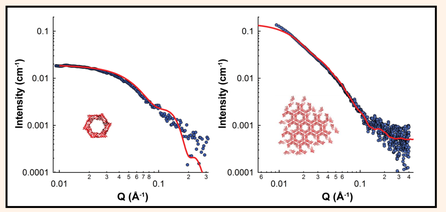
## Production of large structures



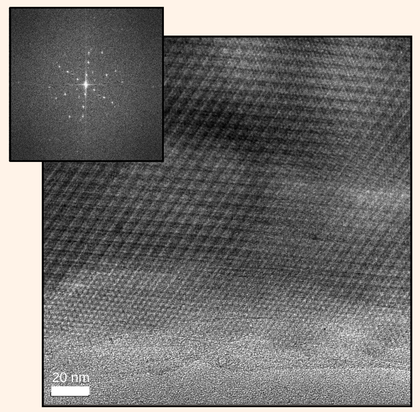

TTM structures produced upon calcium addition were visualized by phase contrast (left) and fluorescence microscopy (right) following Nile Red staining. Low protein and calcium concentration and low temperatures increase the formation of large structures.

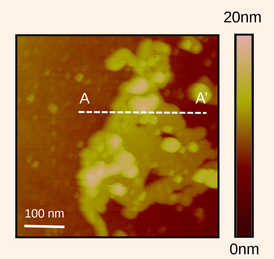
## SAXS analysis

SAXS analysis of wild type STM415 (left) and assembled TTM (right). CRYSOL models (red lines) and shape and form factors deconvolution (not shown) indicate the formation of hexameric and 2D objects, respectively.




## TEM and AFM imaging



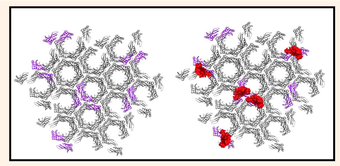




TEM imaging reveals a periodic hexagonal pattern and fringes corresponding to the stacking of multiple sheets. FFT analysis indicates that the unit cell is 7.25 nm.

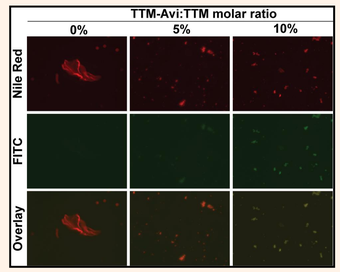
AFM imaging confirms that self-assembled TTM is nearly flat with kinked edges and a height of 4.9 nm.

## Synthesis of functional mixed arrays

Mixed arrays can be used to control the presentation density of functional domains. TTM (gray) arrays become competent for Avidin (red) binding when they incorporate TTM protomers modified with an Avi tag (purple).



Biotinylated TTM-Avi was mixed with unmodified TTM at increasing molar ratios and 2D lattice formation was allowed to proceed. The structures were incubated with FITC-labeled Avidin, washed and labeled with Nile Red. Fluorescence image overlays show that arrays incorporating TTM-Avi become functional for Avidin binding in a concentration-dependent manner.




## CONCLUSIONS

- We have used TEM, AFM, SAXS and fluorescence microscopy to demonstrate that an artificial dimer computationally designed to self-assemble into hexagonal arrays of 7.25 nm periodicity and 5 nm thickness fully meets thr design specifications.
- Polymerization is chemically triggered by calcium addition and it is possible to produce structures that exceed 100 µm in characteristic length.
- Mixed arrays incorporating dimers genetically modified to bind Avidin do so in a concentration-dependent manner.

## REFERENCES

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2. Hampp, N. (2000) Bacteriorhodopsin as a photochromic retinal protein for optical memories. *Chem. Rev.* **100**:1755-76.
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4. Matthaei, F., DiMaio, F., Richards, J.J., Pozzo, L.D., Baker, D. & Baneyx, F. (2015) Designing two-dimensional protein arrays through fusion of multimers and interface mutations. *Nano Lett.* **15**:5235-39.

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