Assembly Principles in Two Dimensional Ordered Virus Arrays

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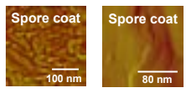
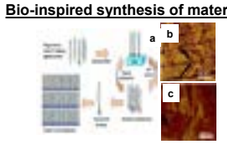
Introduction

Motivation: Scientific & technological challenges associated with macromolecular assembly

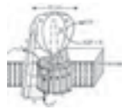
Physics of protein aggregation



Bio-inspired synthesis of materials



Formation of protein machines



Protein structure determination

Cowpea Mosaic Virus (CPMV) is an ideal system to investigate the assembly with nanoscale materials

Unique characteristics

- Robust – stable in 60 ºC water
- Icosahedron shape + well-studied crystal structure
- Multiple-charged sites to coordinate crystallization
- Can be produced in grams per harvest

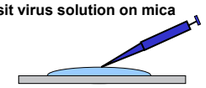
Objectives

- Study the 2-D assembly of ordered CPMV arrays induced by evaporation of electrolytes
- Identify the effect of surfactants in controlling the interaction strength and configurations between virions
- Determine assembly principles: Predict packing configurations

Experimental Method

Drop-dry experiments to determine appropriate sets of parameters for ordered packing

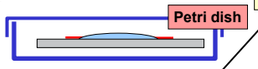
1. Deposit virus solution on mica



Virus solution:
30 µl volume, 0.50 mg/ml virus,
2 mM phosphate buffer pH = 7

Vary solution composition:
- Virus concentration
- Surfactant composition (6kDa polyethylene glycol, PEG)

2. Dry under a controlled environment



3. Image films by atomic force microscopy

Results

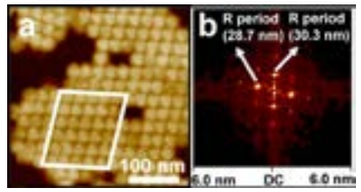
2D arrays on mica show effect of interaction potential on degree of order

Dependence of packing on % PEG

Evolution of packing | **AFM Image** | **2-D Fourier Transform**

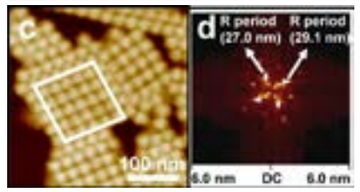
PEG: 0 %

Low order: ranging from square to rhombic



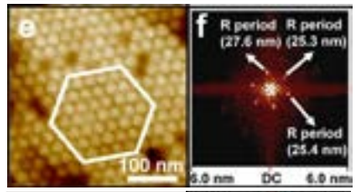
PEG: 0.0002 %

Better order: mostly rhombic, some square



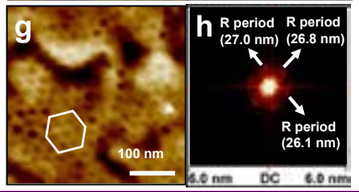
PEG: 0.001 %

Well ordered: hexagonal



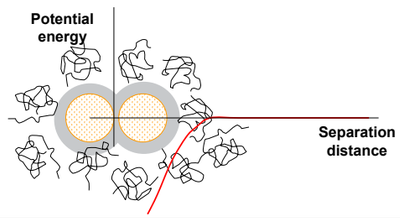
PEG: 0.04 %

PEG co-deposited prevents imaging



Discussions

Addition of polymers can dramatically change the interaction potentials on macromolecular assembly

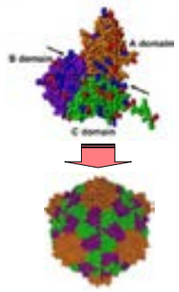


Addition of surfactant polymers (typically PEG):

1. Introduces a non-specific, depletion mediated attraction
   - When particles are close, absence of PEG between the particles creates an osmotic pressure gradient to promote aggregation of particles
2. Increase the nucleation rate of proteins
3. Size and concentration of polymer controls range of attraction

Analysis of amino acids groups on virus capsids provides links to their charge potential landscape

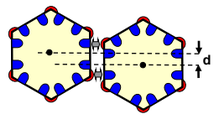
Distribution of the negatively (Asp/Glu) and positively (Arg/Lys/His) charged amino acid residues at pH 7



Steric & electrostatic complementarity of two neighboring CPMV capsids

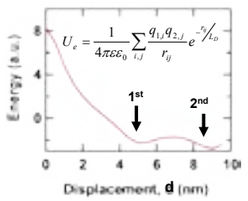
Interaction Model: Two neighboring CPMVs

Hexagons with -ve charged protrusions & +ve charged patches



d: transverse shift of the position of the symmetry axes through the center of the projected images

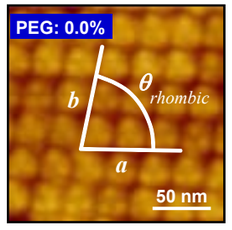
Electrostatic potential energy landscape of neighboring CPMVs



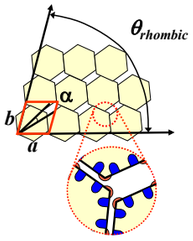
Complementary electrostatic interactions (arrowed)

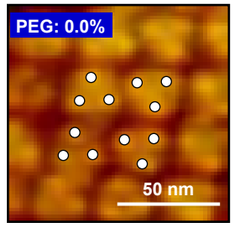
Steric & electrostatic model for 2-D CPMV assembly

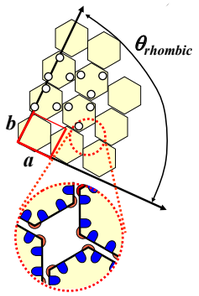
AFM image



Interaction model







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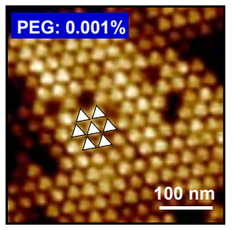
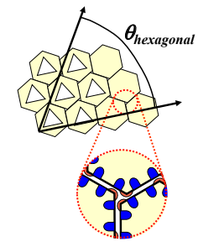
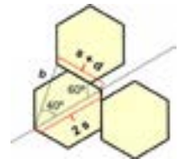


Interaction model



Deduction of the average size of a CPMV virion from the 2D array AFM data

Hexagon representation of a virus array



2s: Diameter of each virus (hexagon)
d: Relative transversal shift of two neighboring hexagons from their symmetry axes
Note: Adjacent distance between a positively charge patch and a negative charge patch is ~ 5.1 nm

Average size of a CPMV virion and the transversal shift (d) between two virions obtained from the 2D CPMV arrays data

PEG concentration in the virus solution for the array formation (wt. %)	0	0.0002	0.001
Average size of a CPMV virion in the array, 2s (nm)	30.2	30.2	28.6
Average transversal shift, d, between two adjacent virions (nm)	5.88	5.88	5.15

Conclusions

- Electrostatic potential and geometric arrangement of charge patches on CPMV capsids can be used to deduce interaction configurations between neighboring CPMVs.
- Postulated electrostatic and steric complementarity principles explain three different observed 2-D CPMV assembly configurations.
- Steric and electrostatic complementarity principles can be applied to design and assemble other complex biological building blocks.

Acknowledgements

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