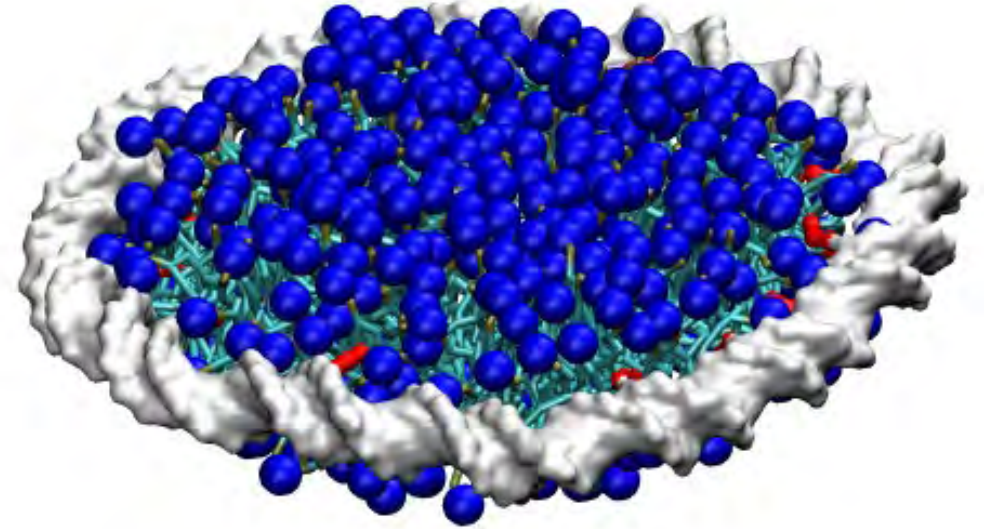


DNA-Lipid Nanodiscs

Dr. Thorsten-Lars Schmidt

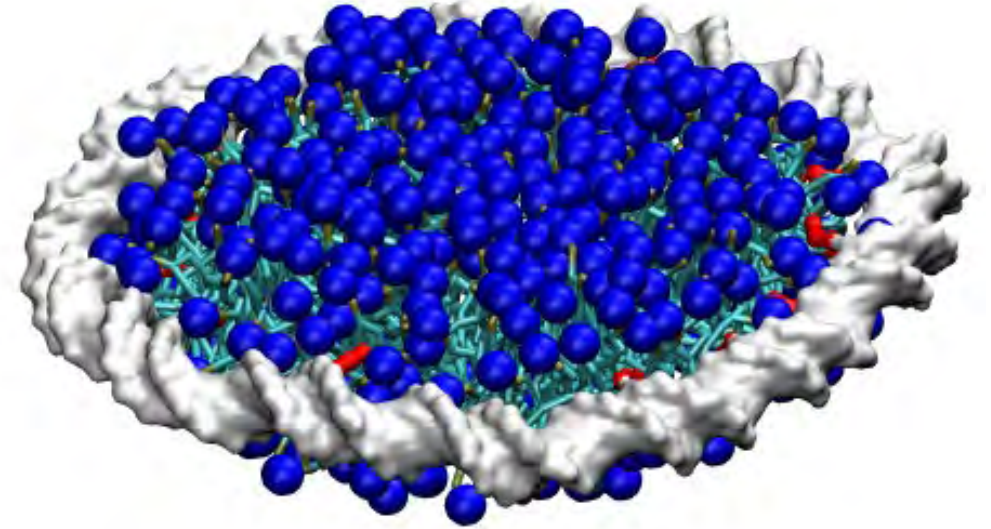
Assistant Professor for Experimental Biophysics



DNA-Lipid Nanodiscs

Dr. Thorsten-Lars Schmidt

Assistant Professor for Experimental Biophysics

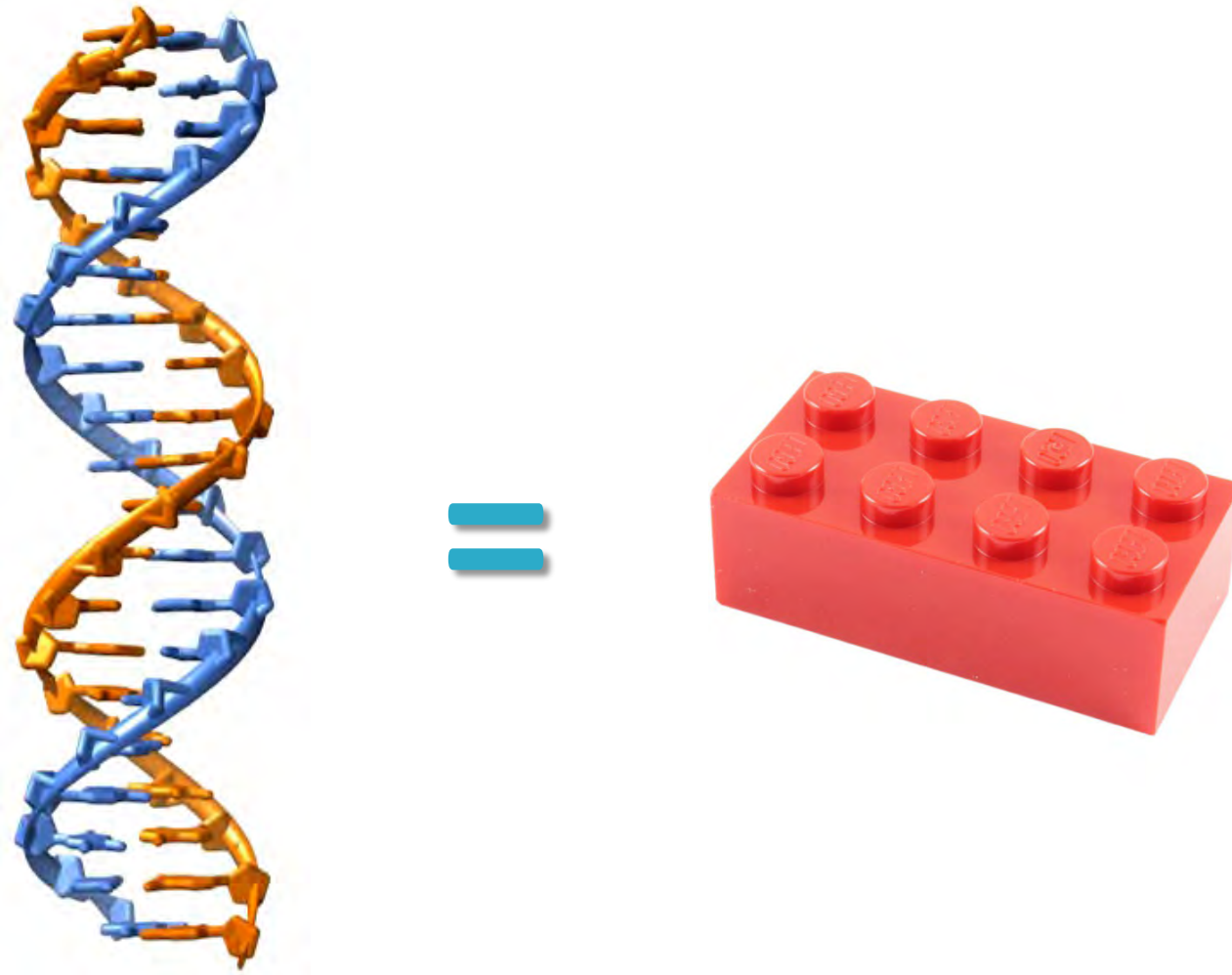


Postdoc (and PhD) position available



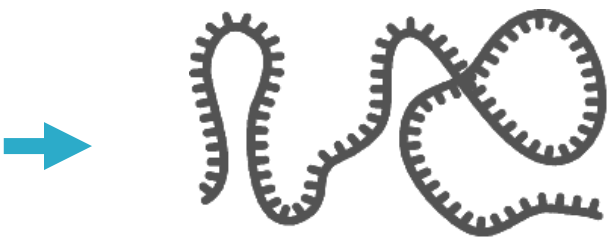
**KENT STATE.**
UNIVERSITY



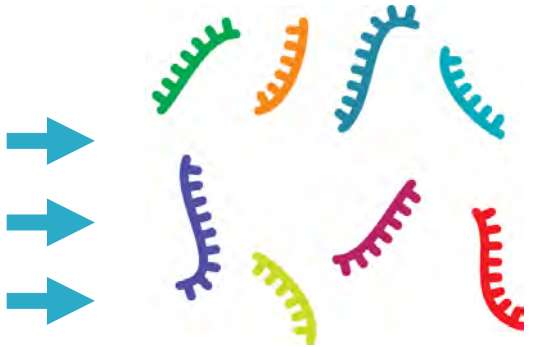
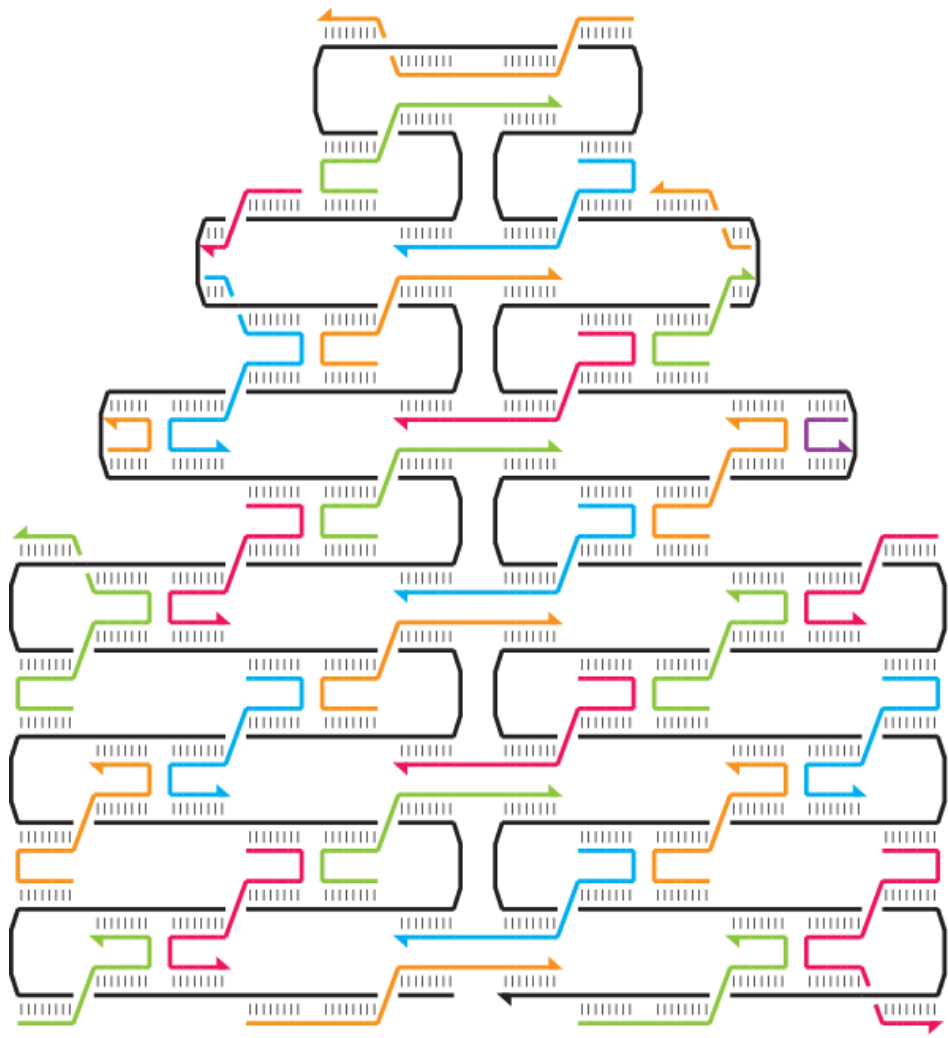


DNA nanotechnology:
DNA = material and tool

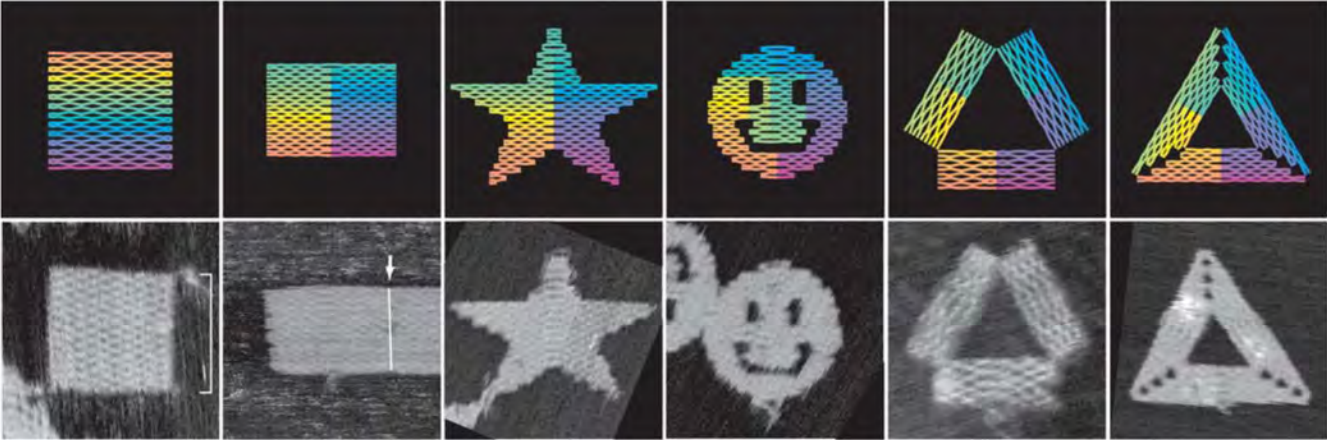
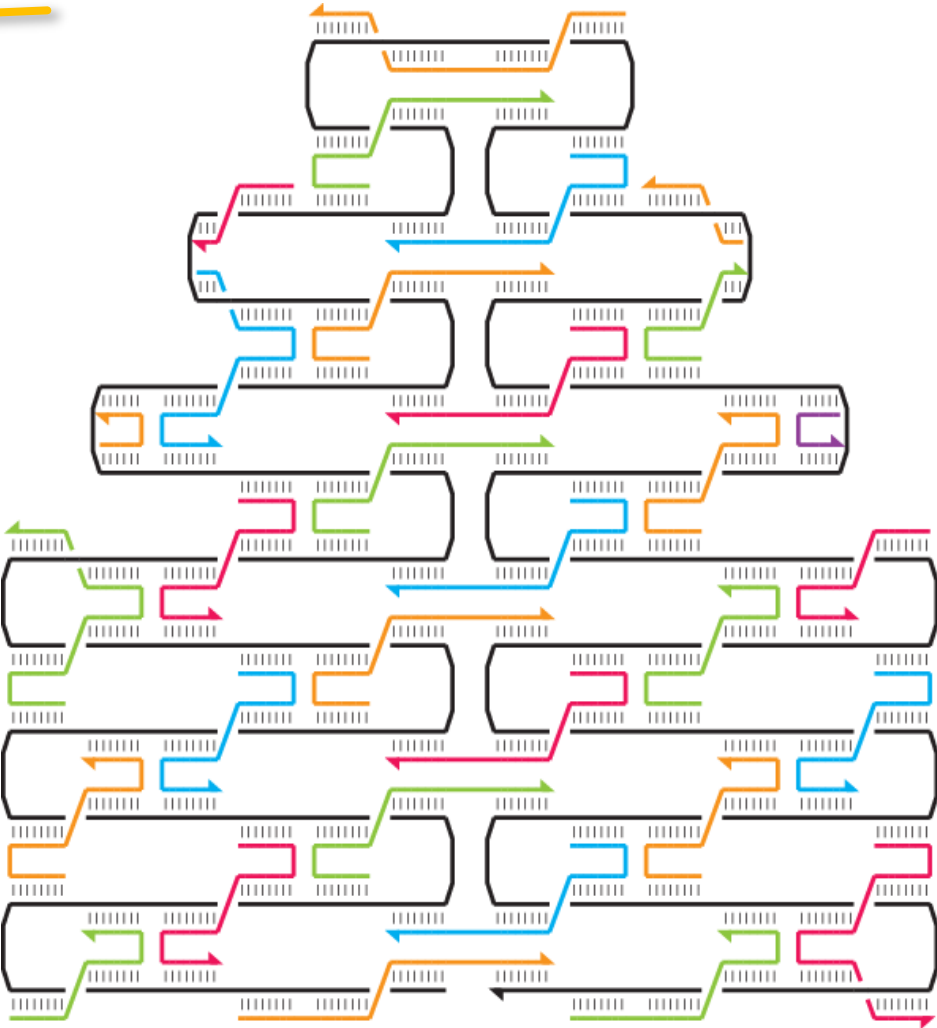
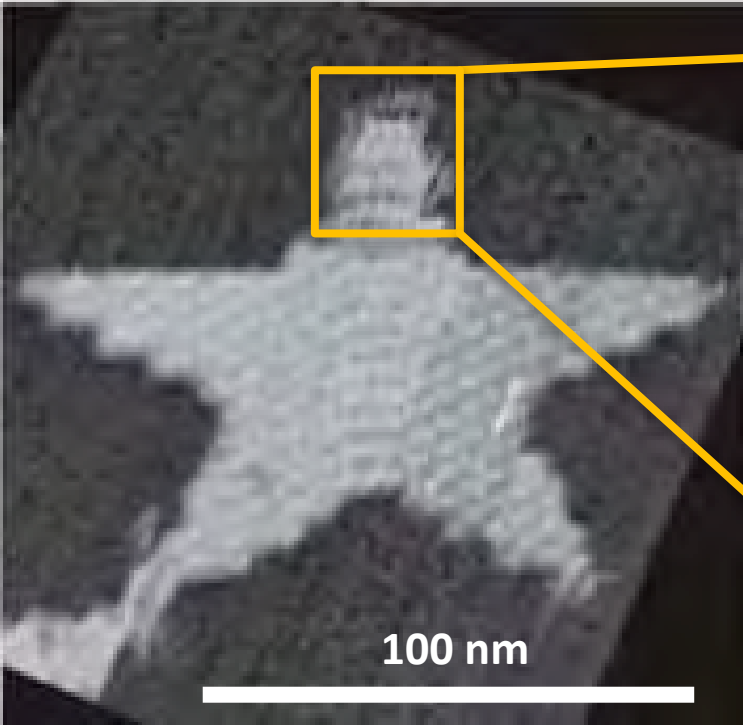
DNA origami - technique

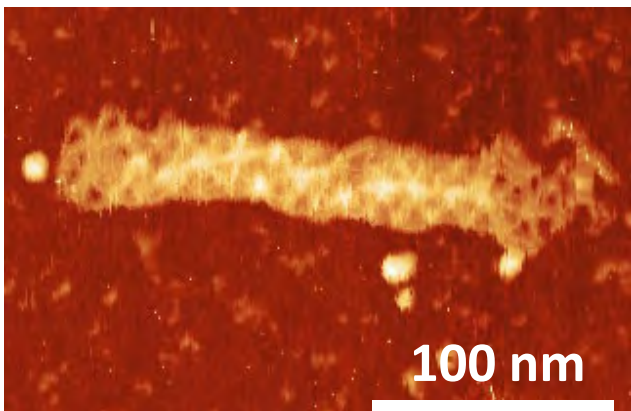
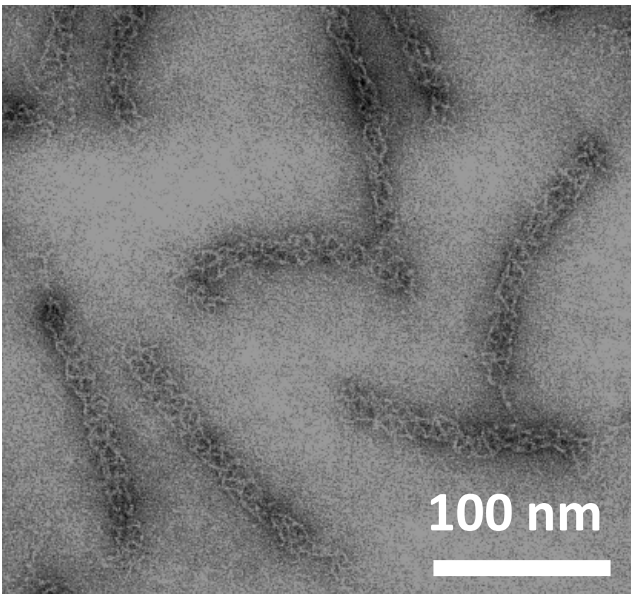
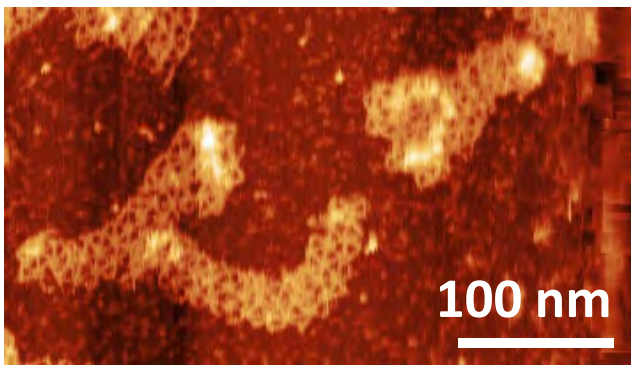


M13 bacteriophage genome
+
~200 synthetic oligonucleotides

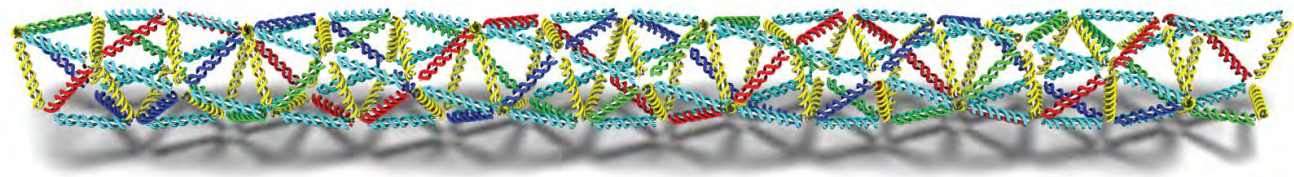
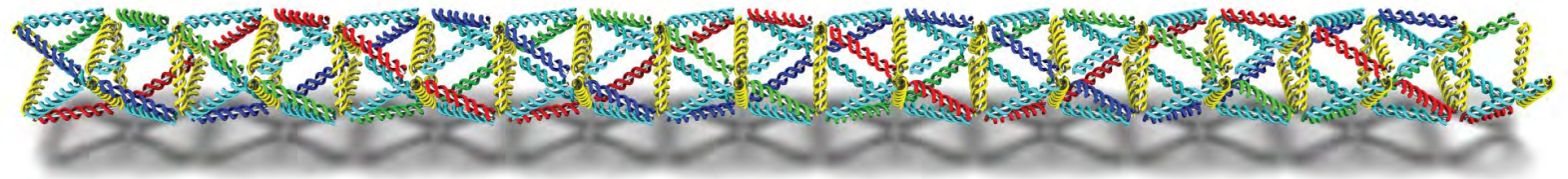
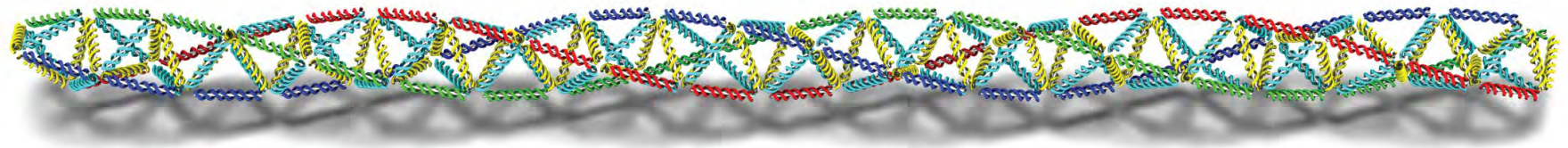
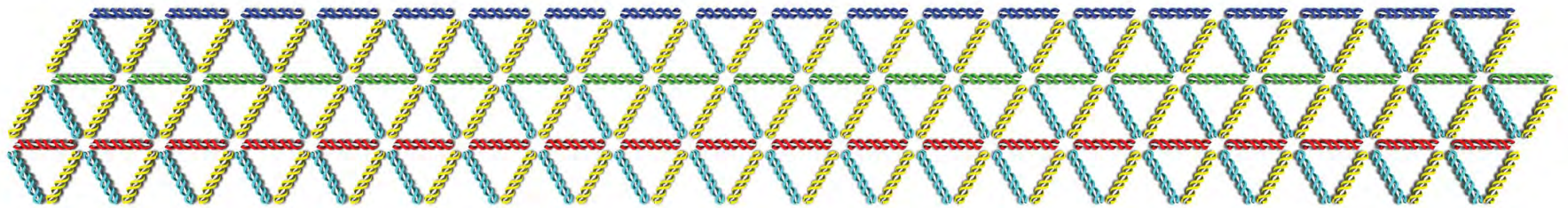


DNA origami





50 nm

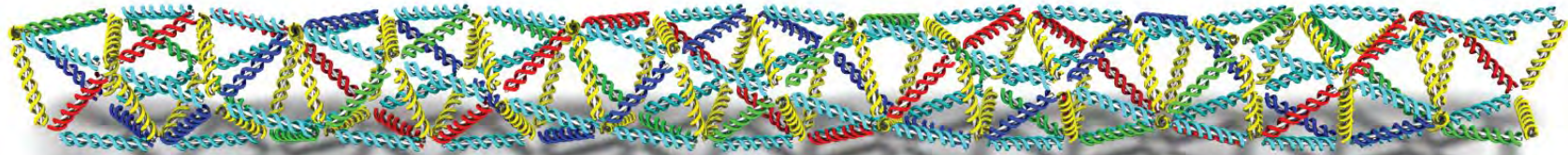
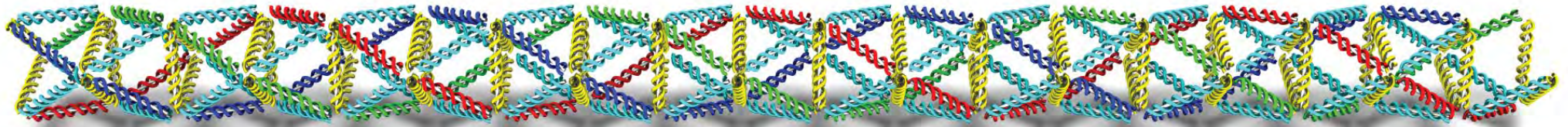
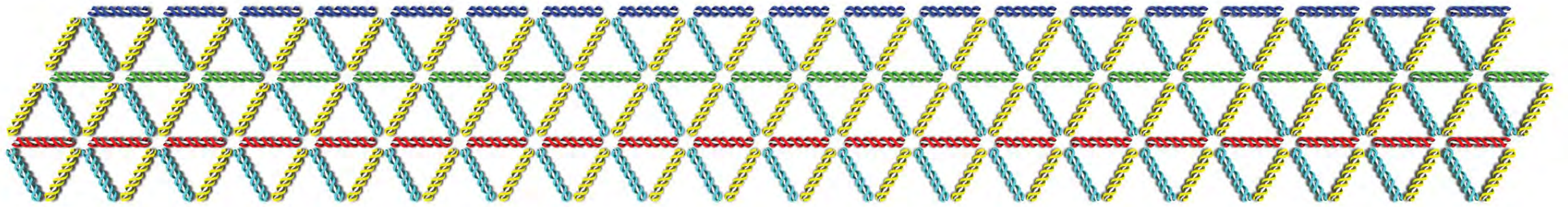


Nano Letters 2016.

ACS Nano 2018.

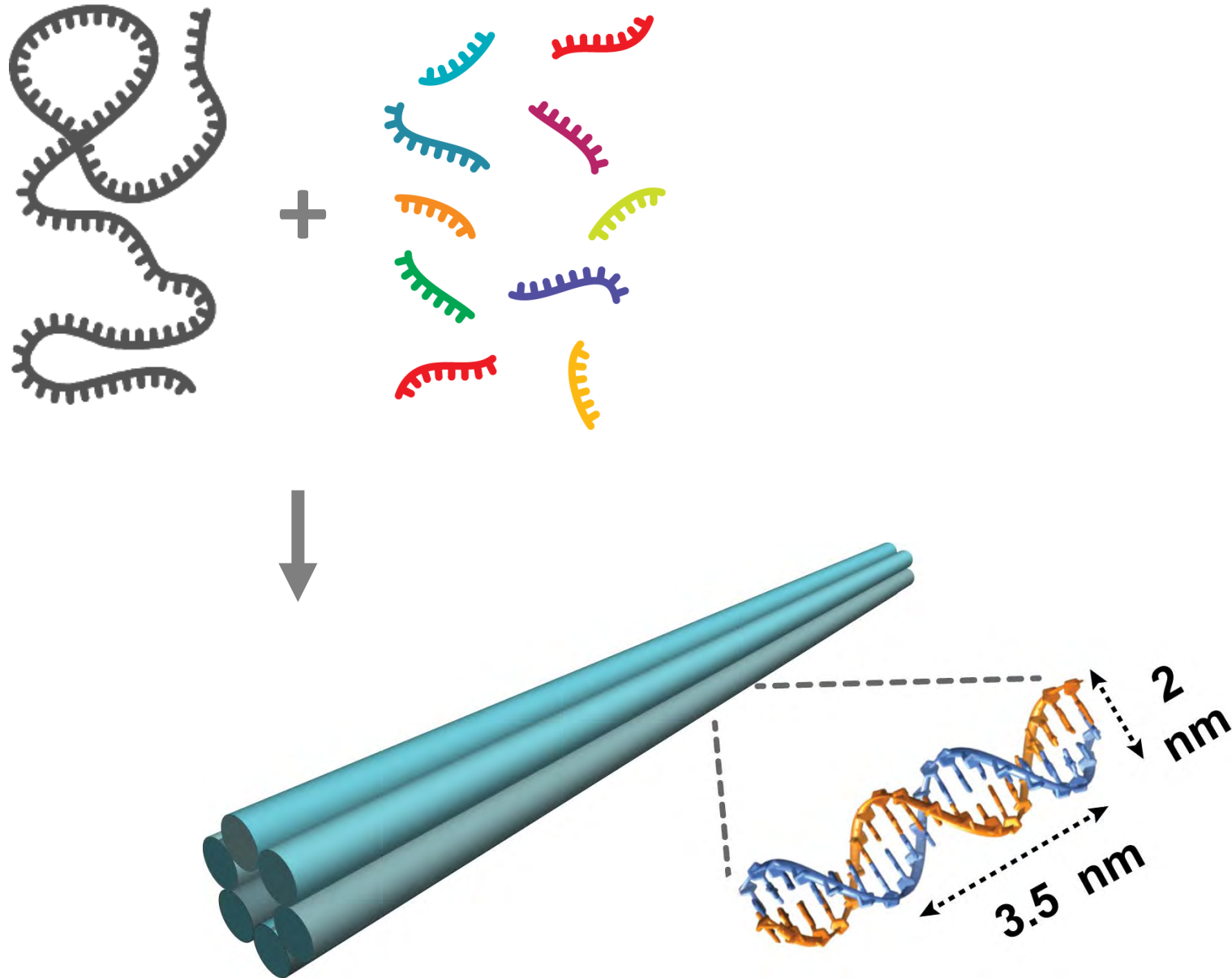
ACS Nano 2019.



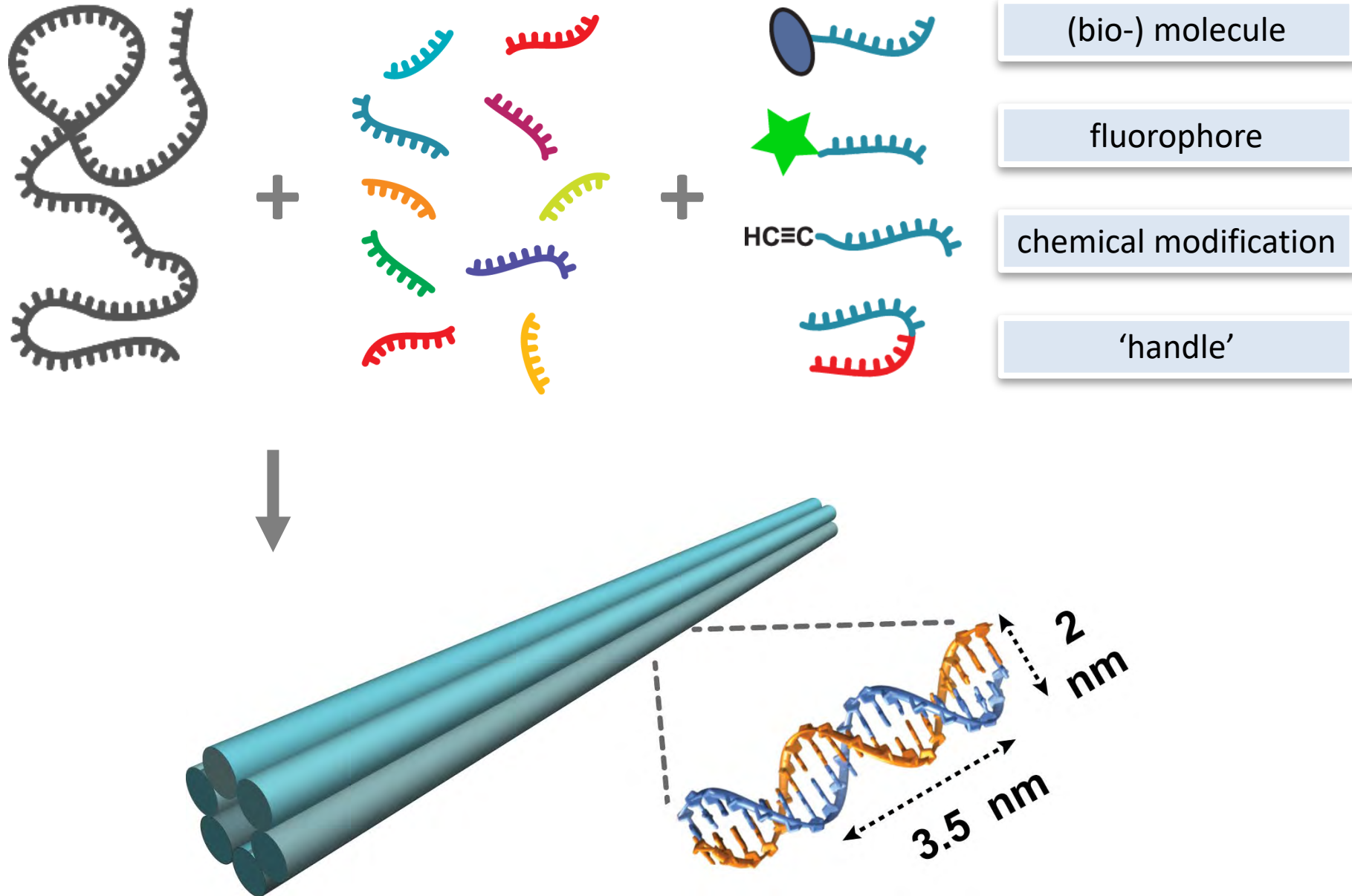


“Nice. But who cares?”

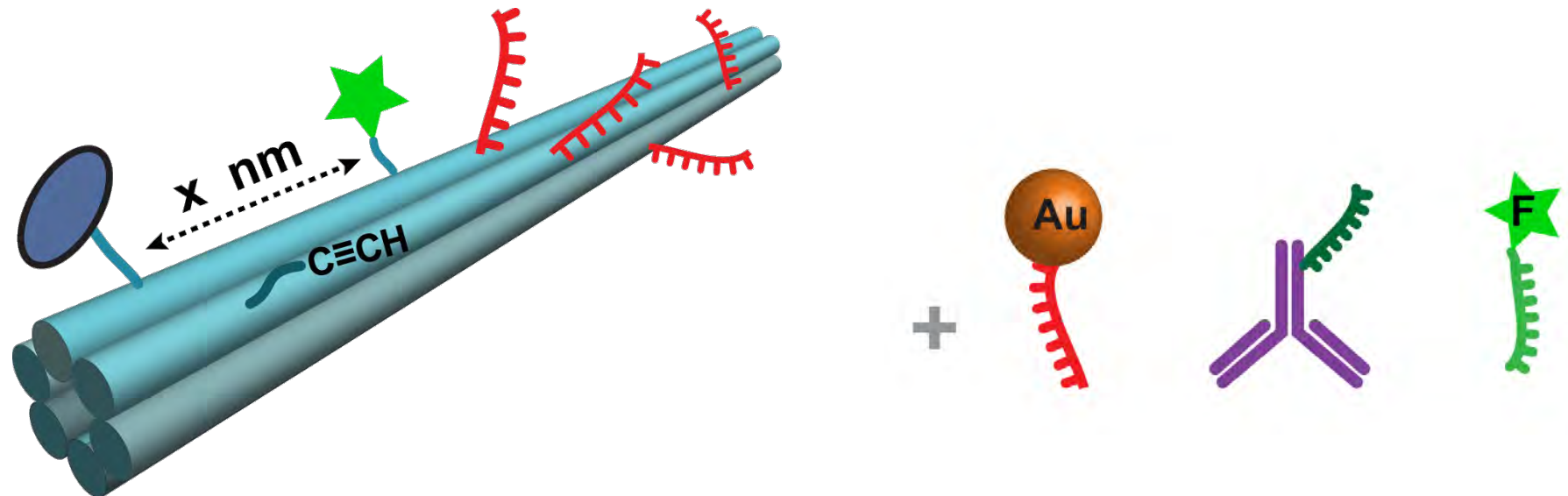
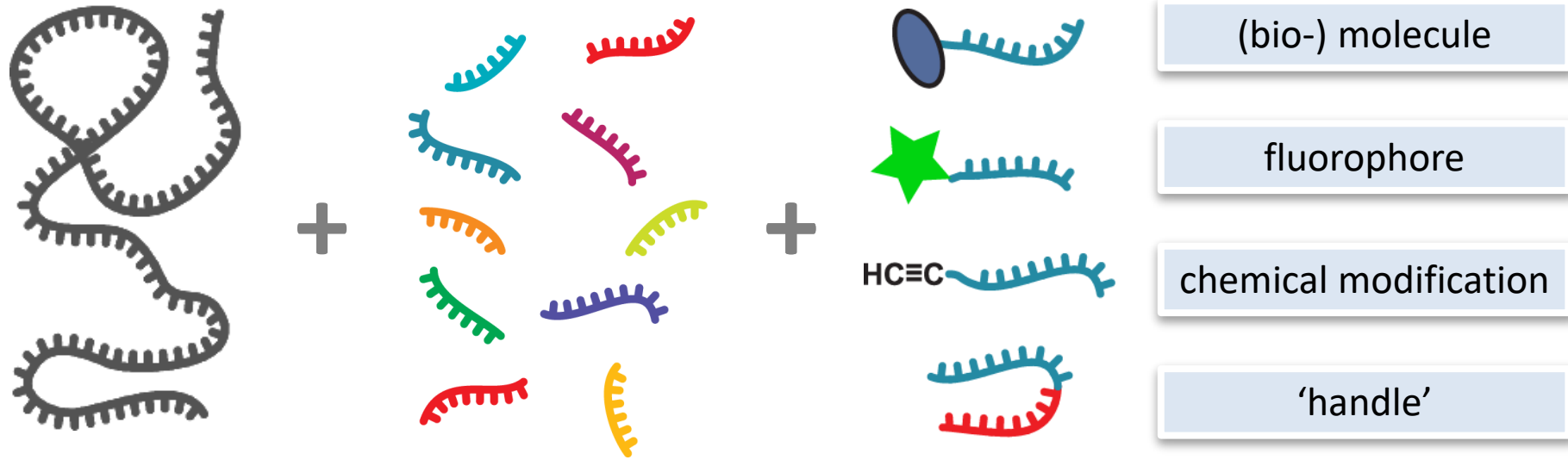
DNA Origami are fully anisotropic nanoparticles

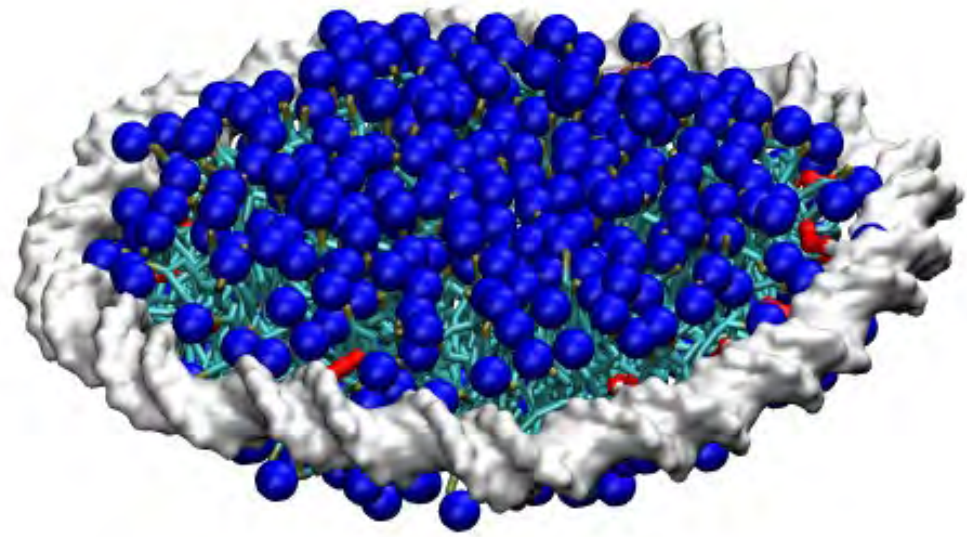


Introducing functional elements



Introducing functional elements





DNA-lipid nanodiscs

Dr. K. Iric



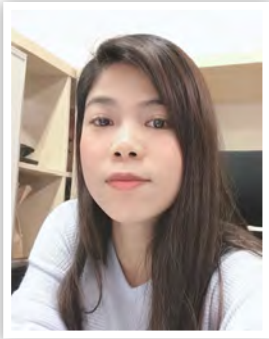
B. Weber



Prof. Y. Sato



Dr. S. Aye



P. Prakash



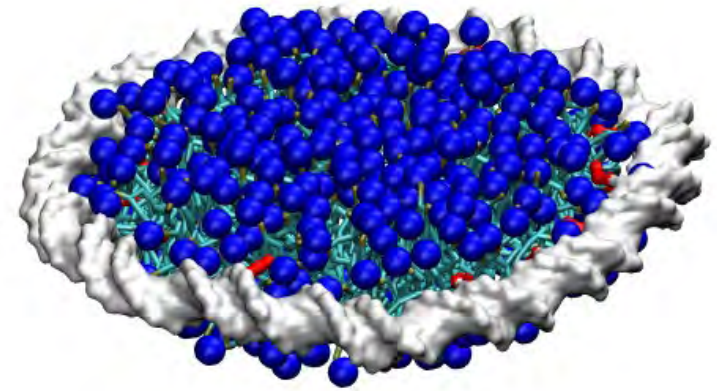
S. Chandrasekhar



Dr. S. Karanth

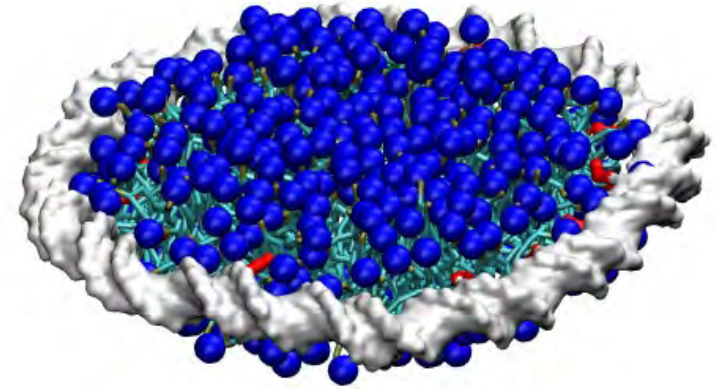
Challenges for single-molecule Cryo EM of MPs

- 1) **MPs not water soluble**
- 2) **Solve small MPs**
- 3) **MPs denature at air-water interface**
- 4) **Generate force to study
mechanosensitive MPs**



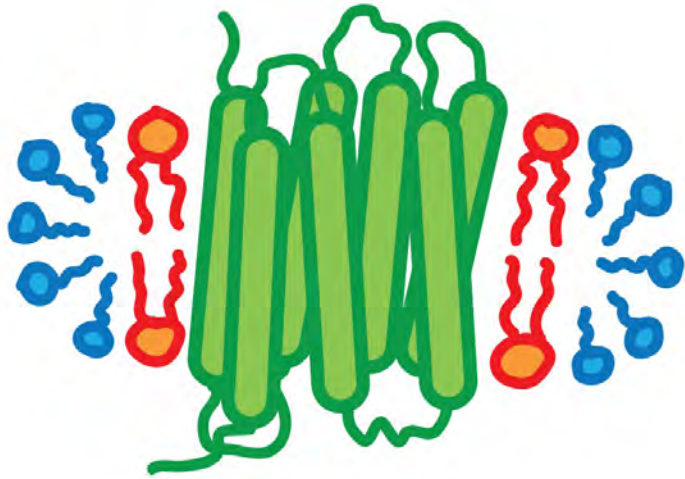
Challenges for single-molecule Cryo EM of MPs

1) MPs not water soluble



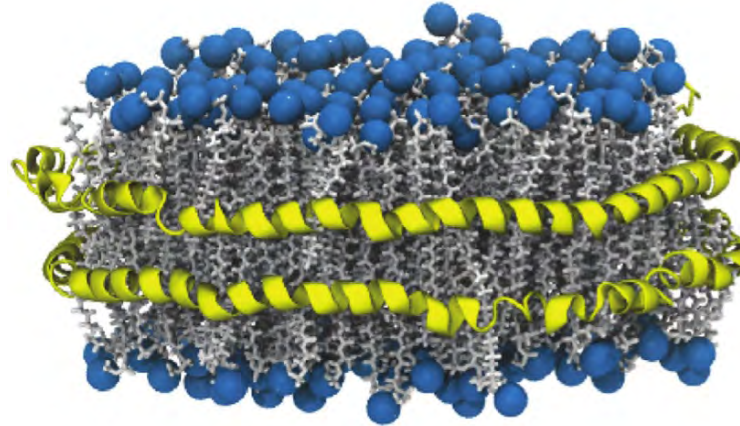
Challenge 1: Solubilize MPs

Detergents



- Prevent aggregation
- **Not native (denaturing!)**

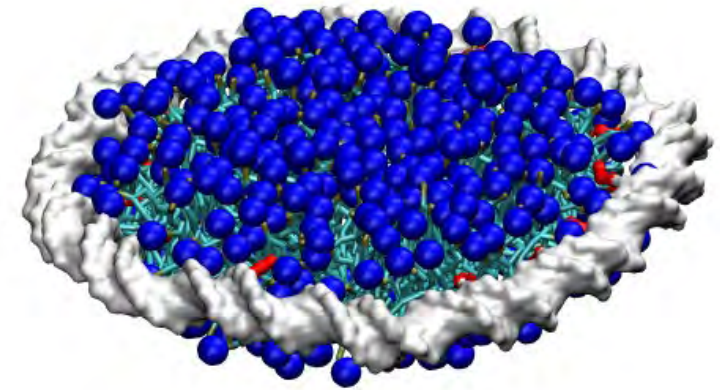
Protein- or polymer-based lipid nanodiscs



Justesen Rev. Anal Chem 2014)

- Native lipid environment
- Established in cryo EM of MPs
- **Size control difficult**
- **Difficult to functionalize**

DNA-lipid nanodiscs



- Native lipid environment
- Fully customizable (!)

Inspiration: PhD. projects

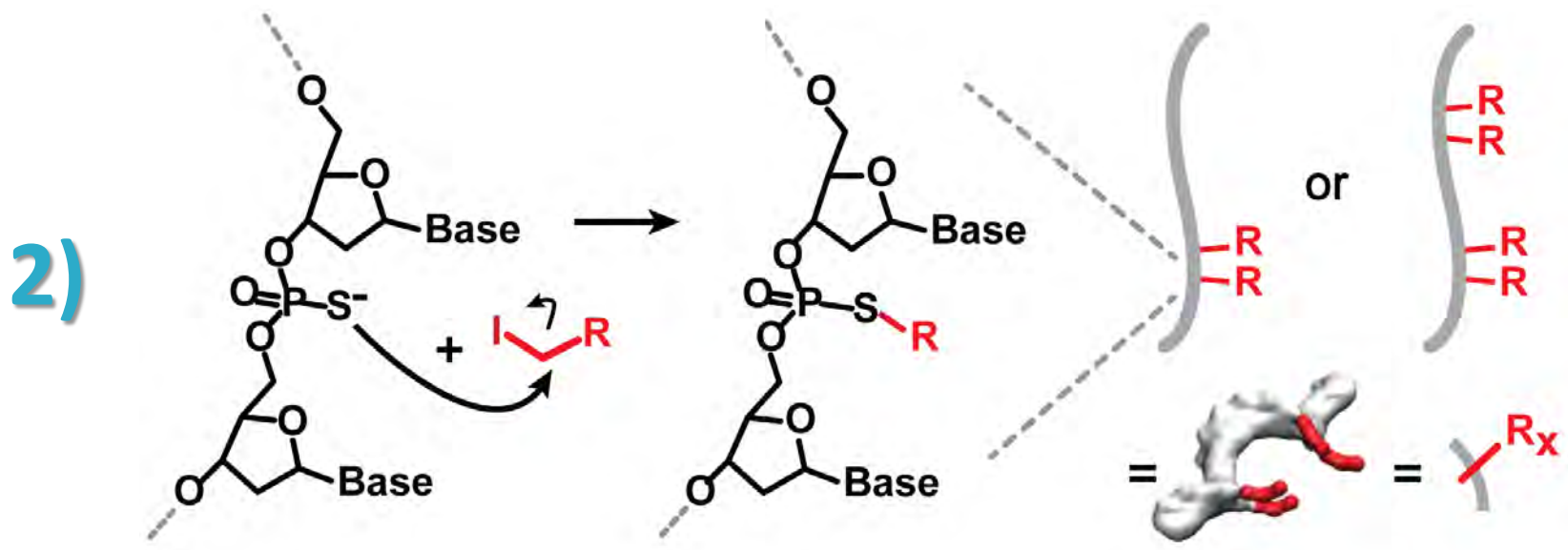
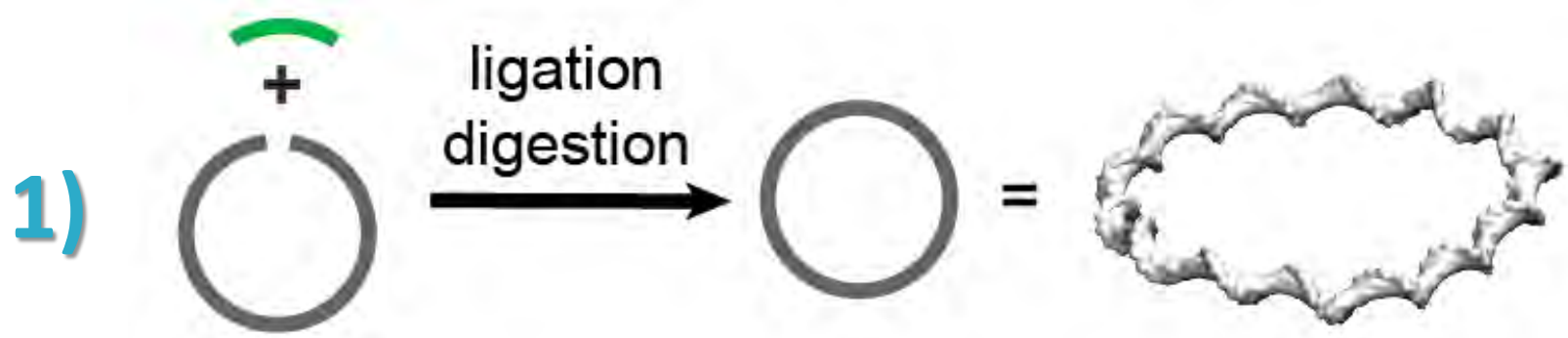


Schmidt & Heckel,
Nano Lett. 2011.

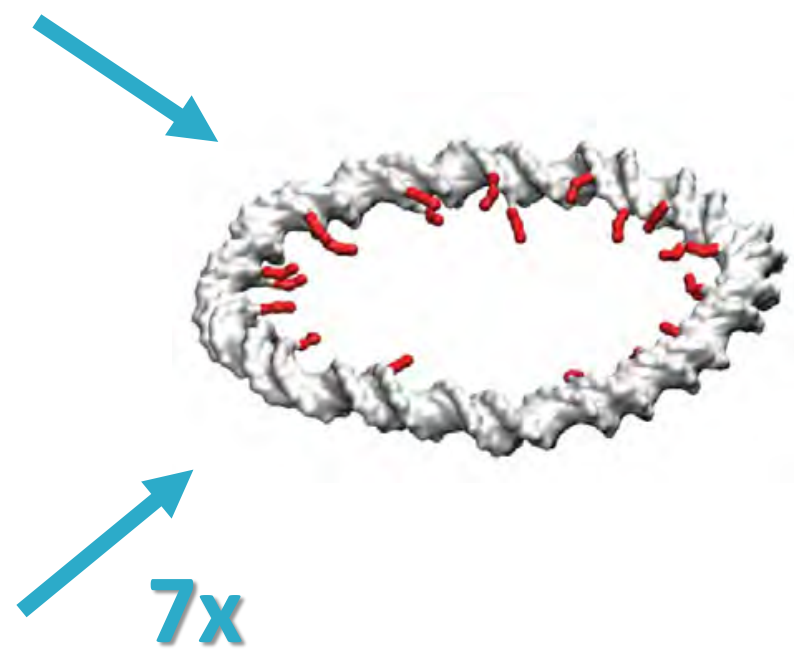


Ackermann, Schmidt *et al.*, **Nature Nanotech. 2010.**

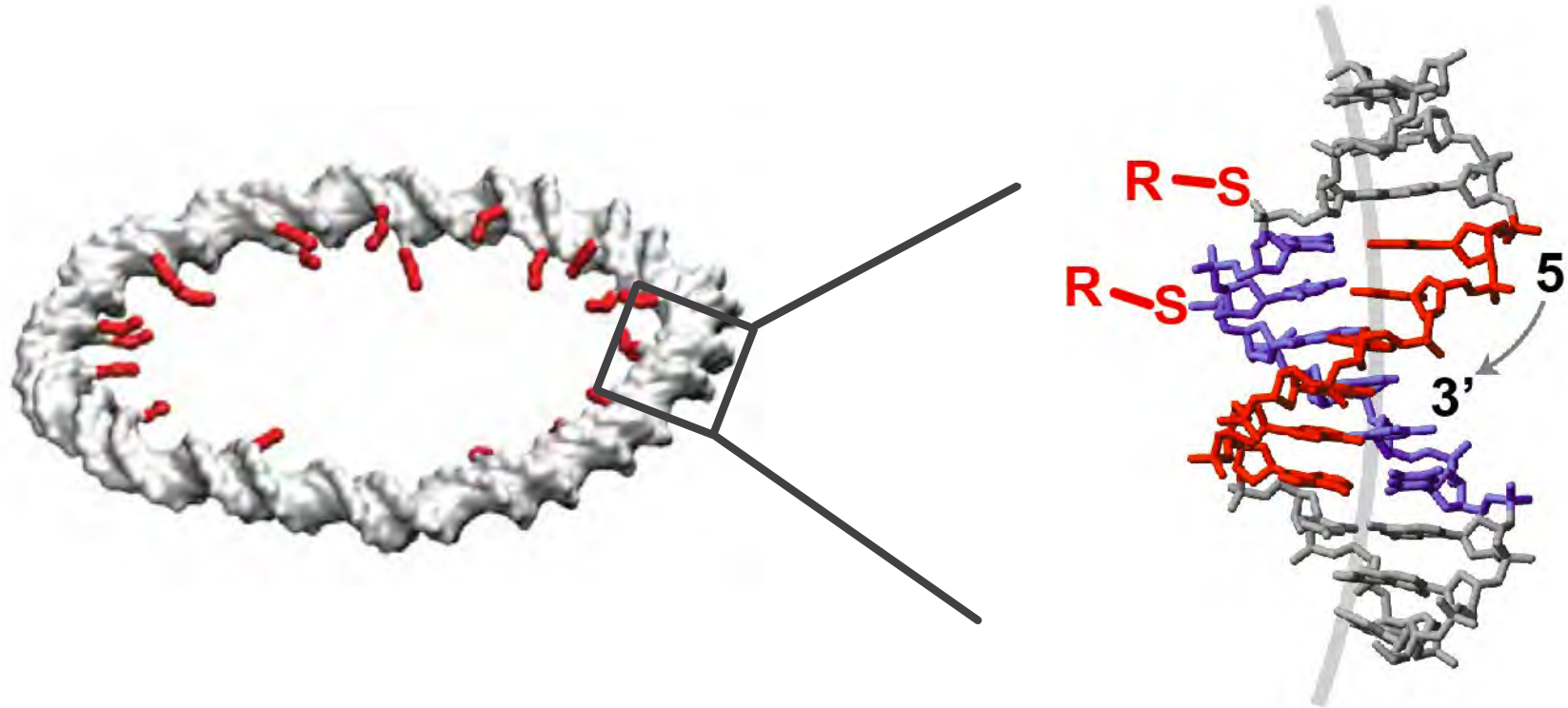
Functionalized minicircles



R = ethyl, butyl, heptyl, decyl



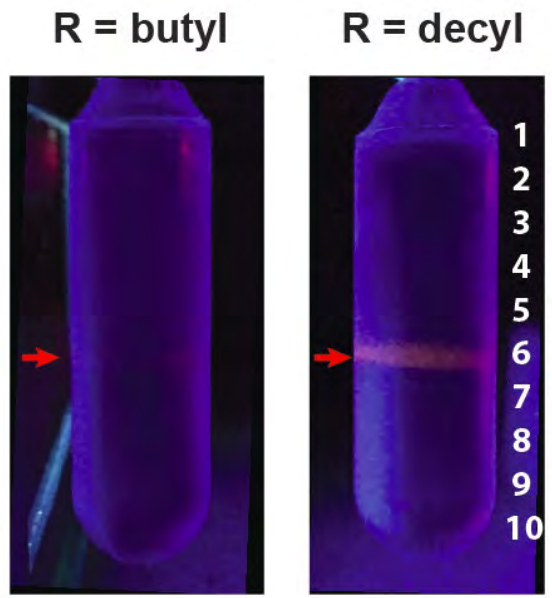
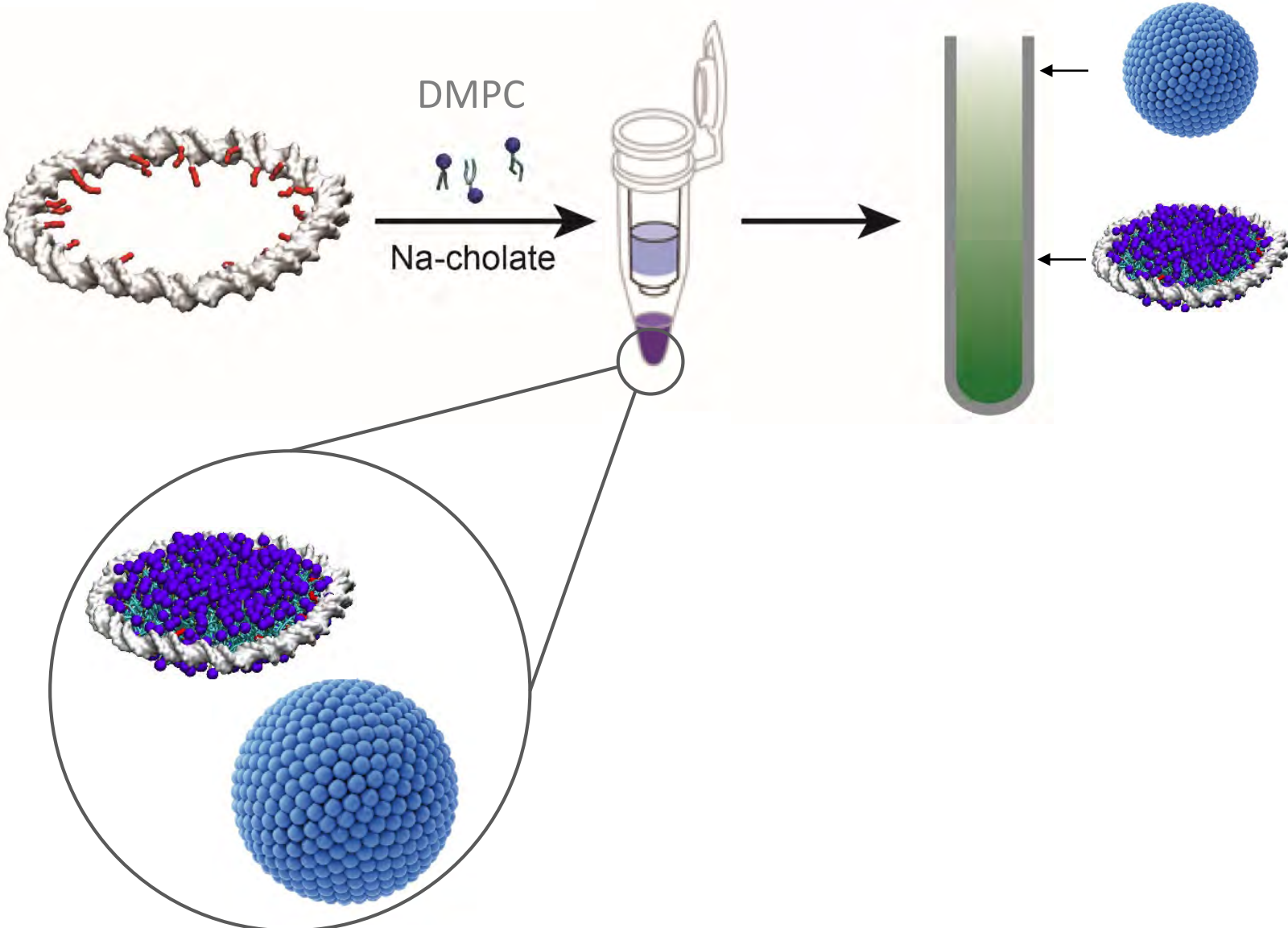
Controlling location of side chains



(A-tracts)
MacDonald,
JMB 2001, 1081.

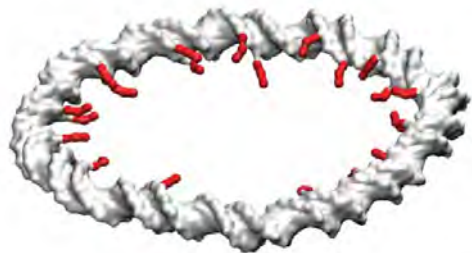
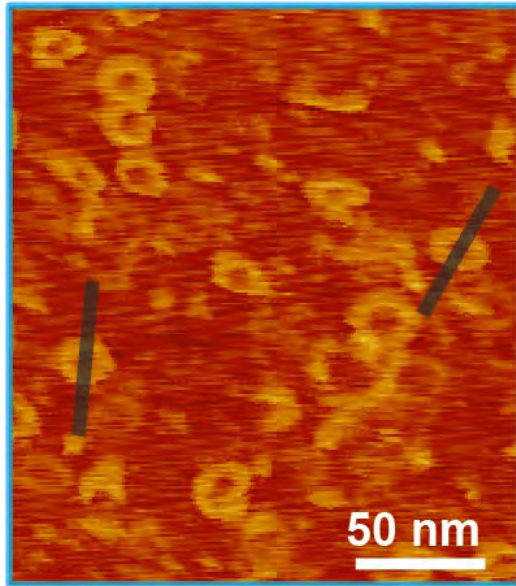
5' -TATAT**AAAAA**TCTCT**AAAAA**TATAT**AAAAA**TCTCT**AAAAA**...
3' -ATAT**TTTTT**AGAG**TTTTT**ATAT**TTTTT**AGAG**TTTTT**...

DNA-encircled lipid bilayer (DEB) assembly

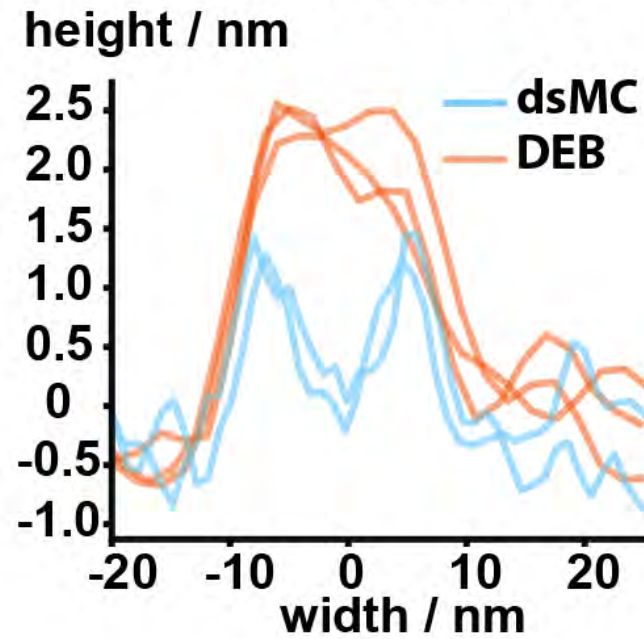


AFM characterization

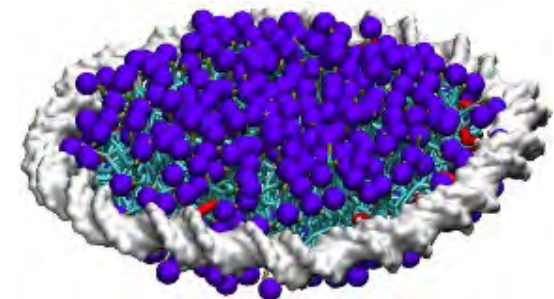
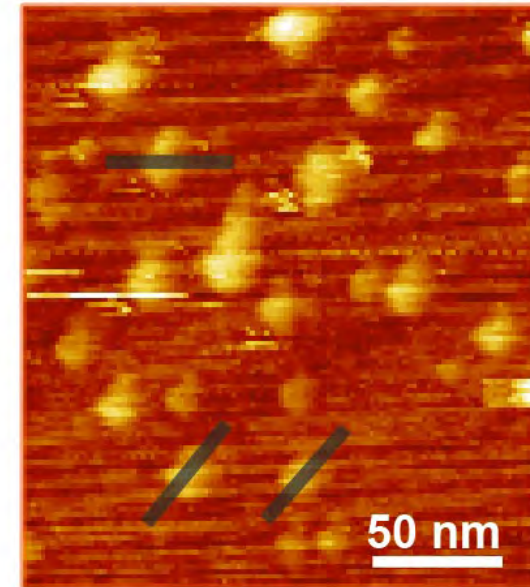
dsMC (147 bp)



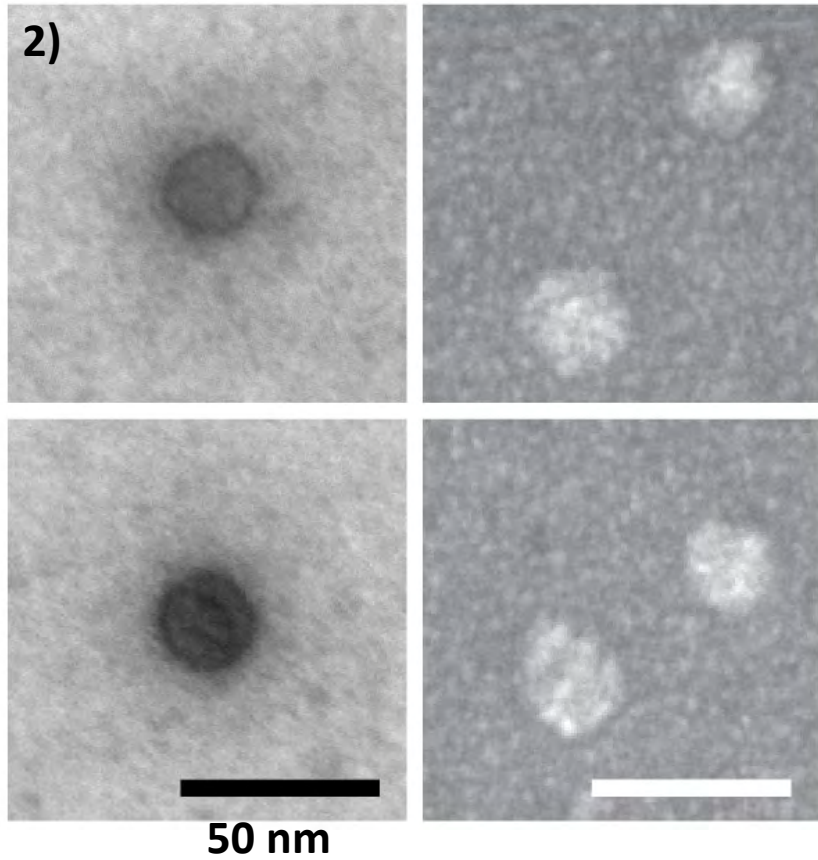
Height profile:
dsMC vs. DEBs



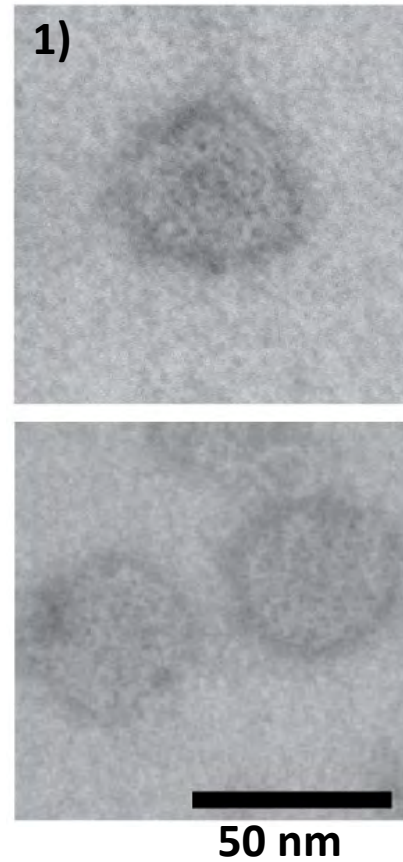
DEBs
(14 ethyl groups)



Size easily controllable



147 bp



294 bp

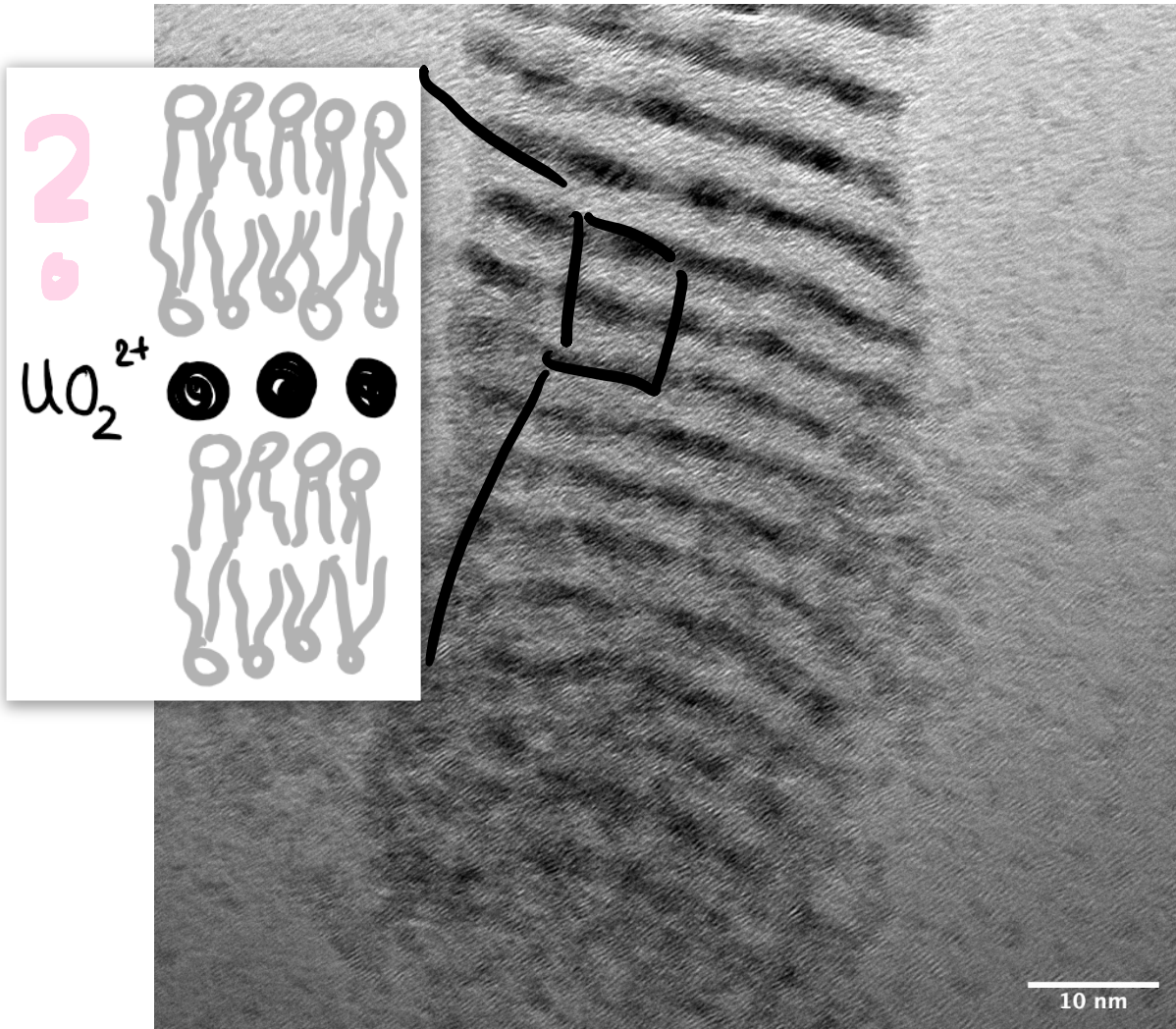
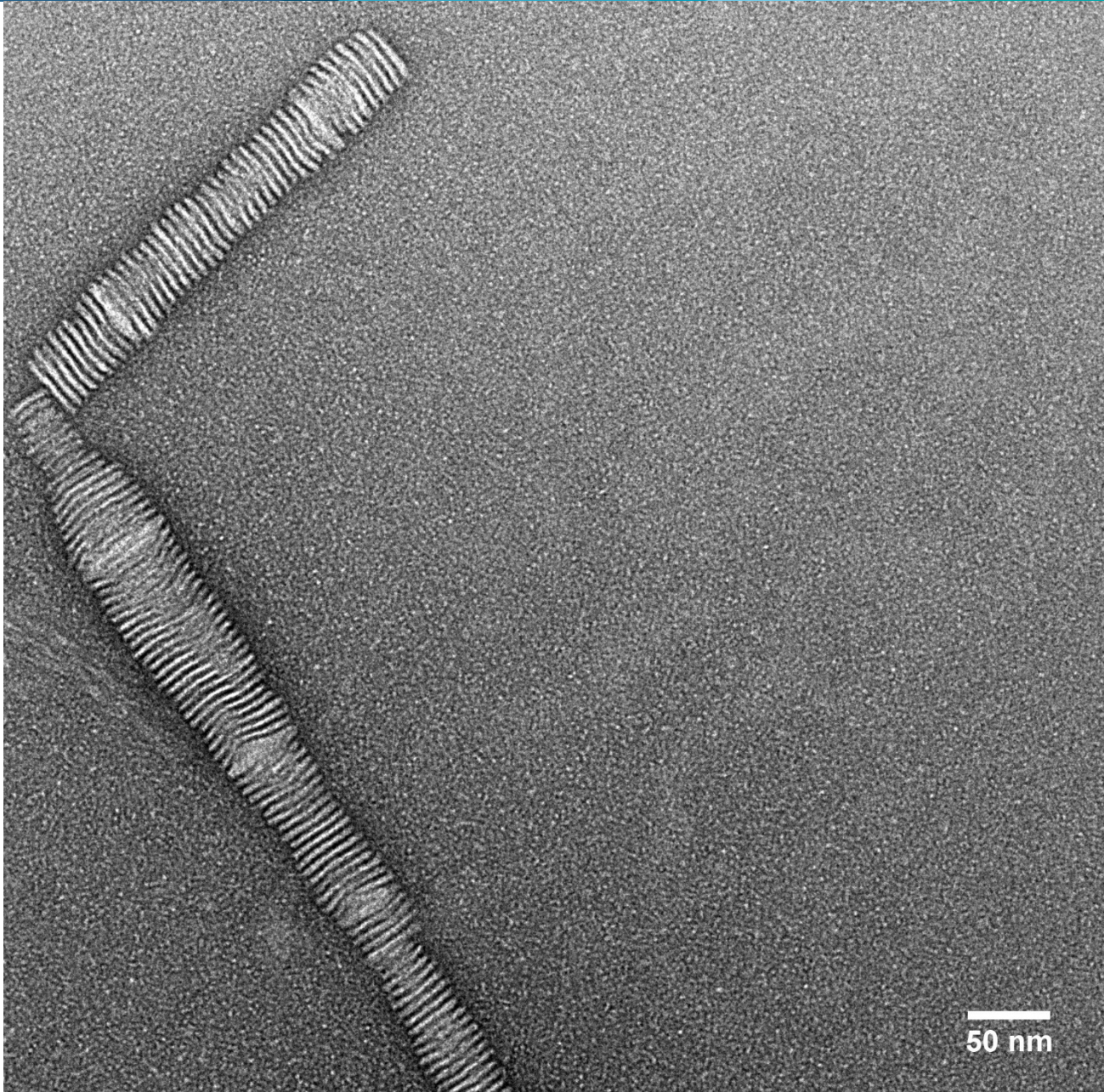
⇒ **study large membrane protein complexes?**

⇒ **study docking with viruses?**

Next steps

- **Stabilize rings against aggregation**
- **Couple to SMA polymers?**
- **Embed MPs, Cryo-EM**

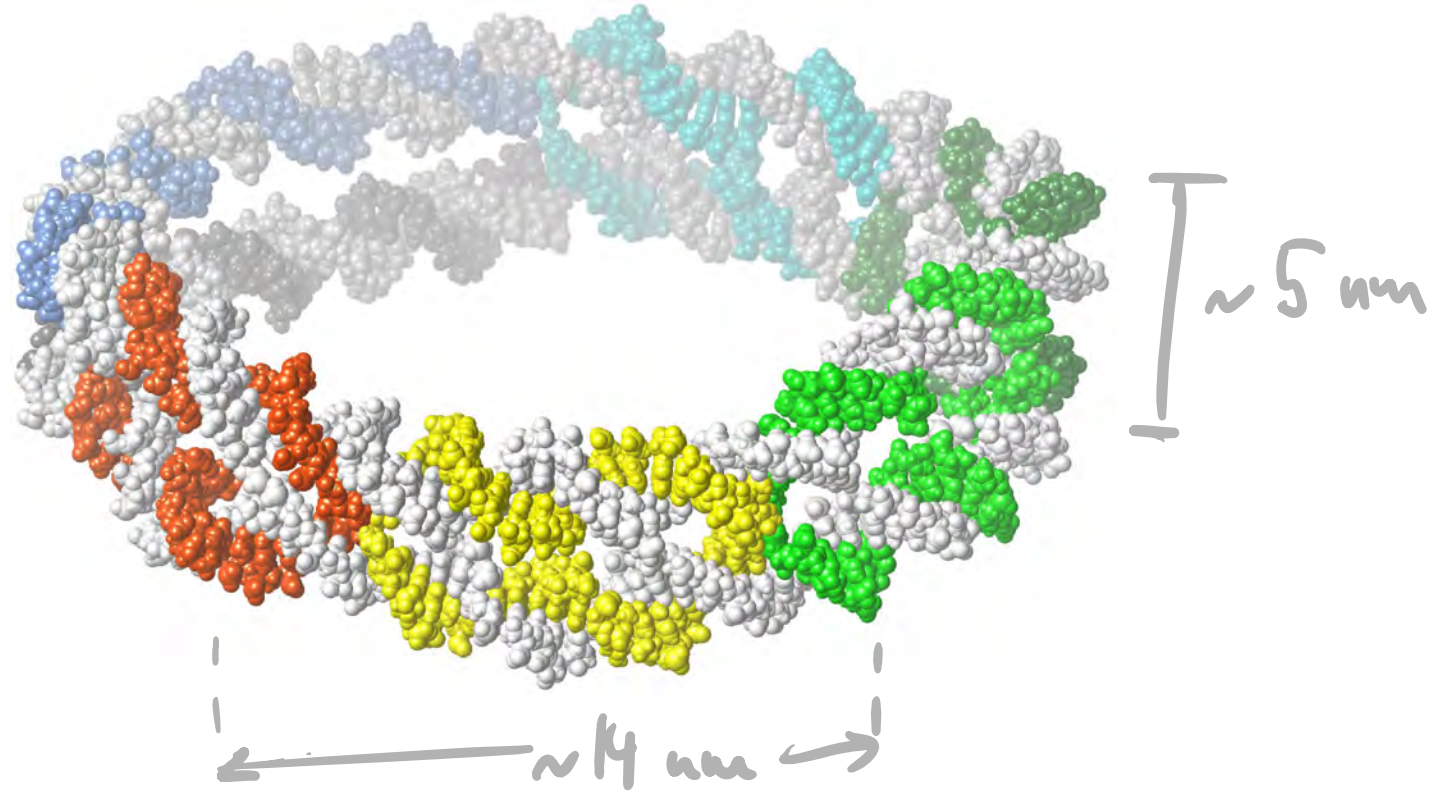
Rouleaux Effect in TEM Images?



Periodicity: 5.87 ± 0.75 nm

Two-ring nanodisc

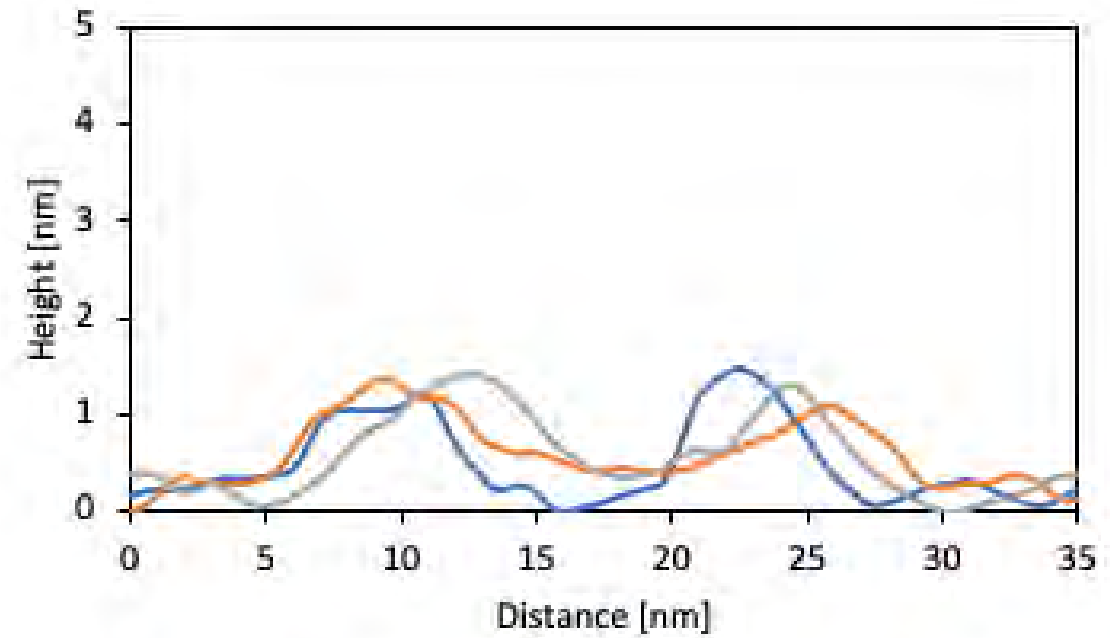
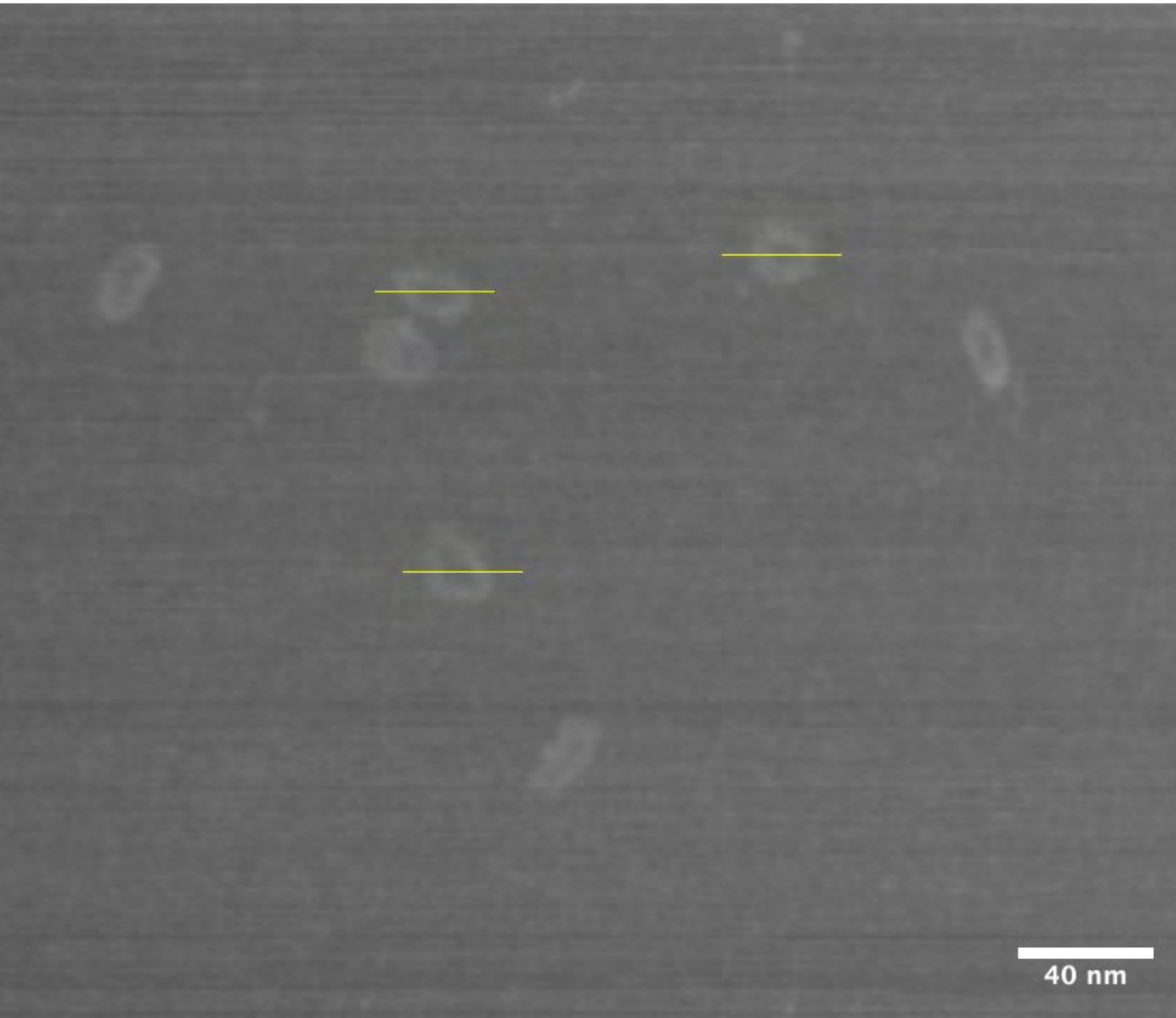
- Completely shields lipids
- More stable against aggregation?
- Larger variety of lipids?

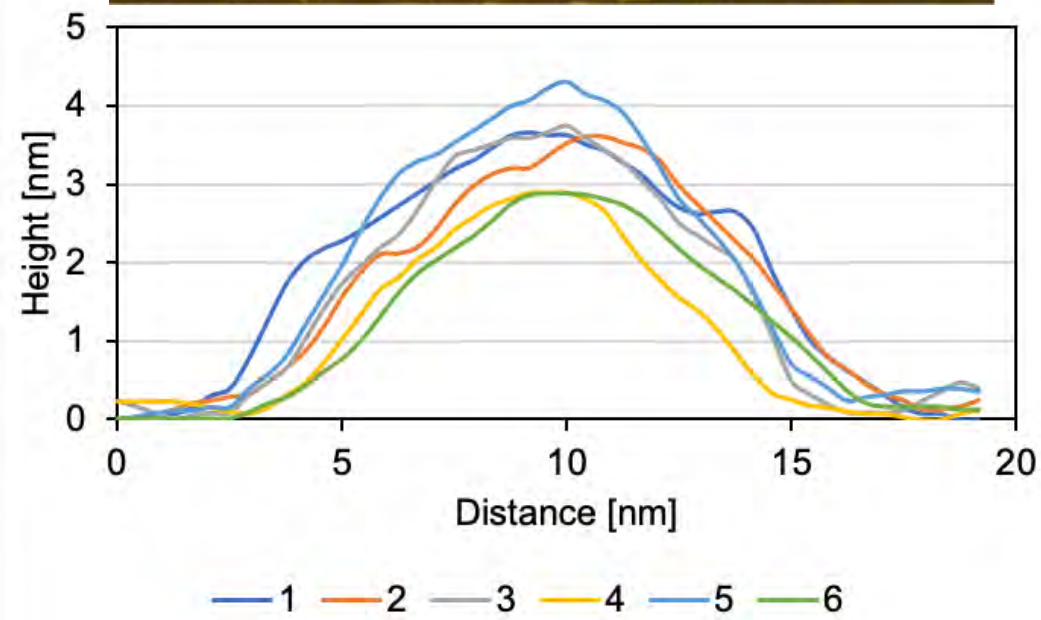
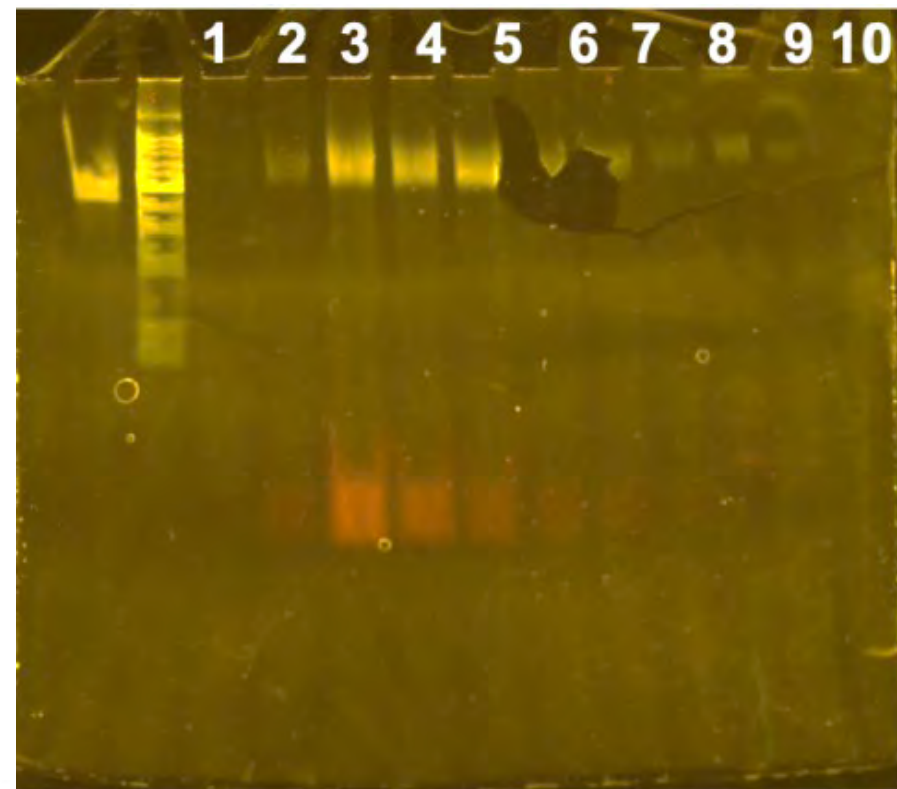
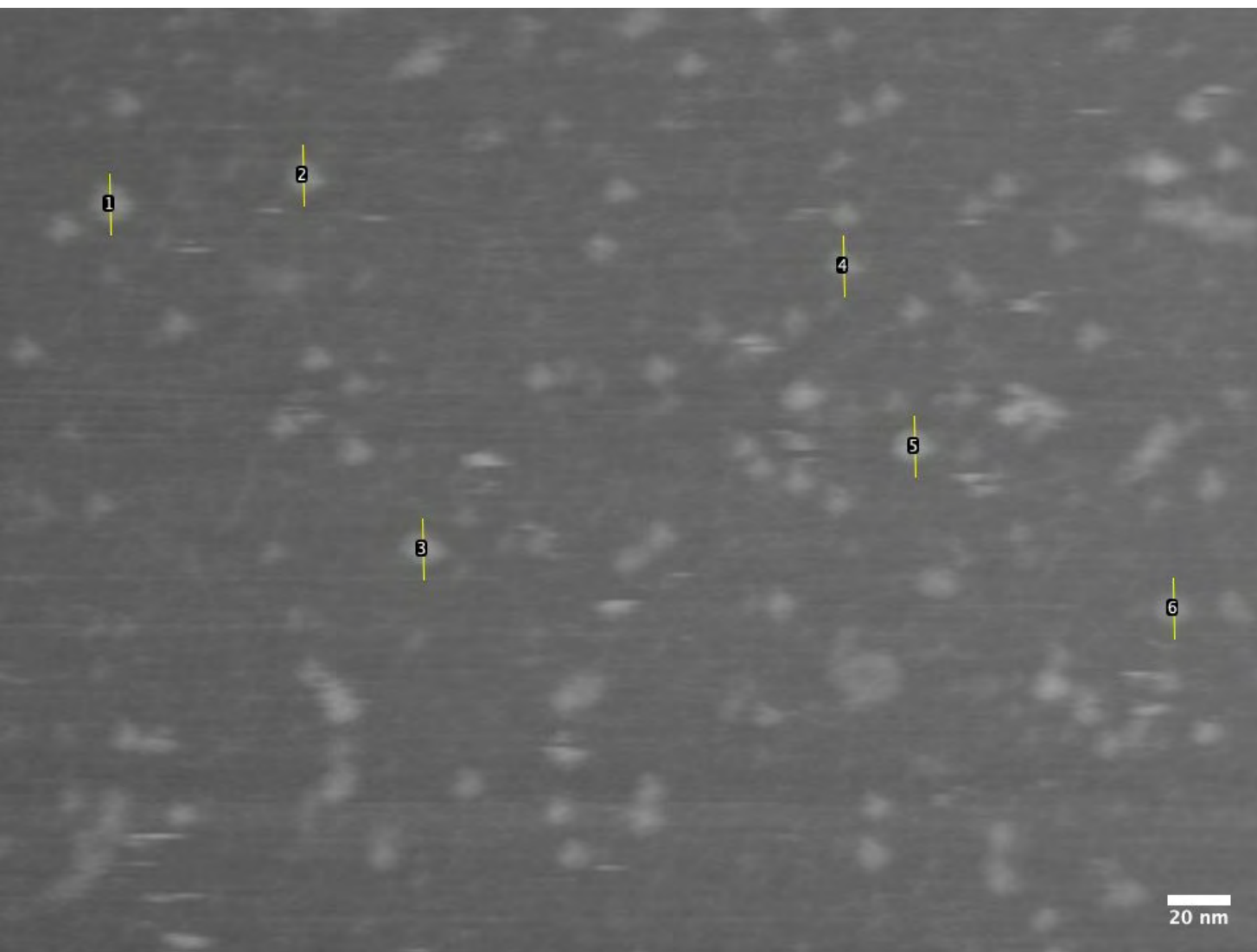


Prof. Y. Sato Dr. S. Aye



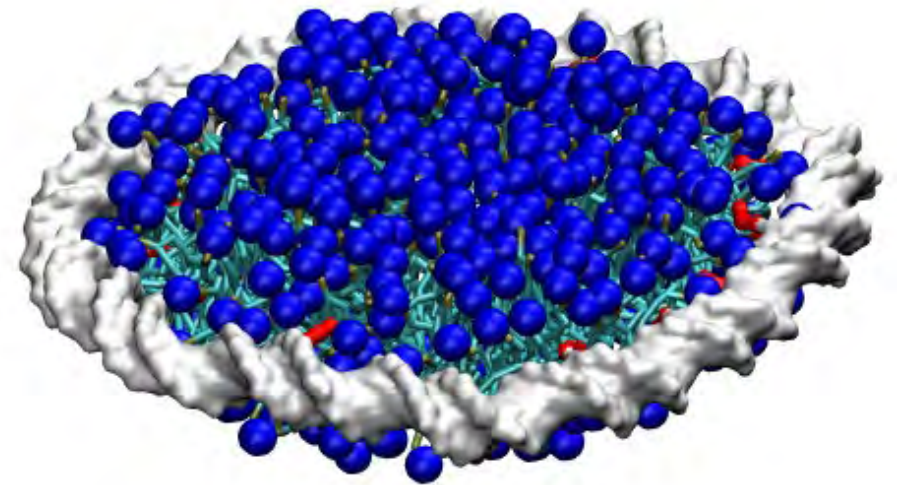
Empty 2-ring scaffold



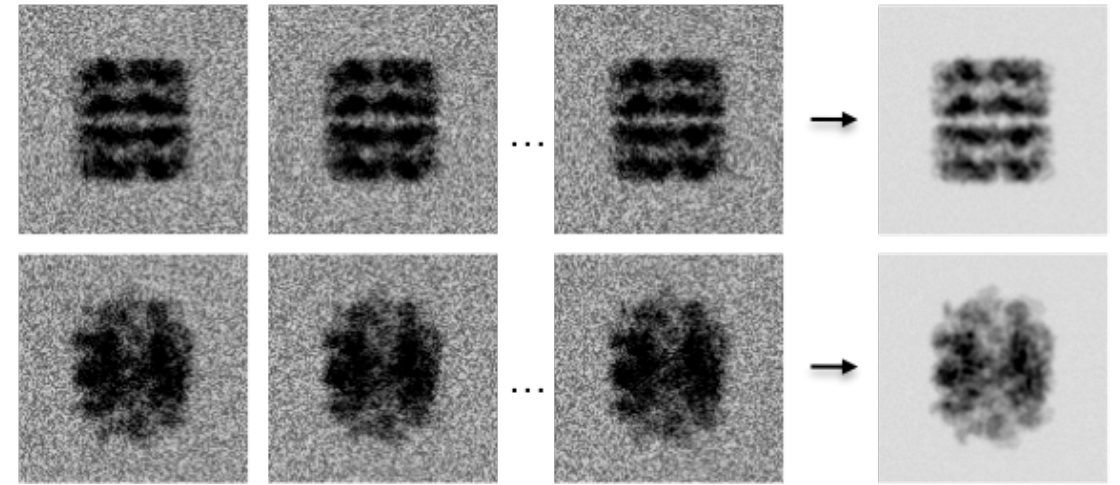
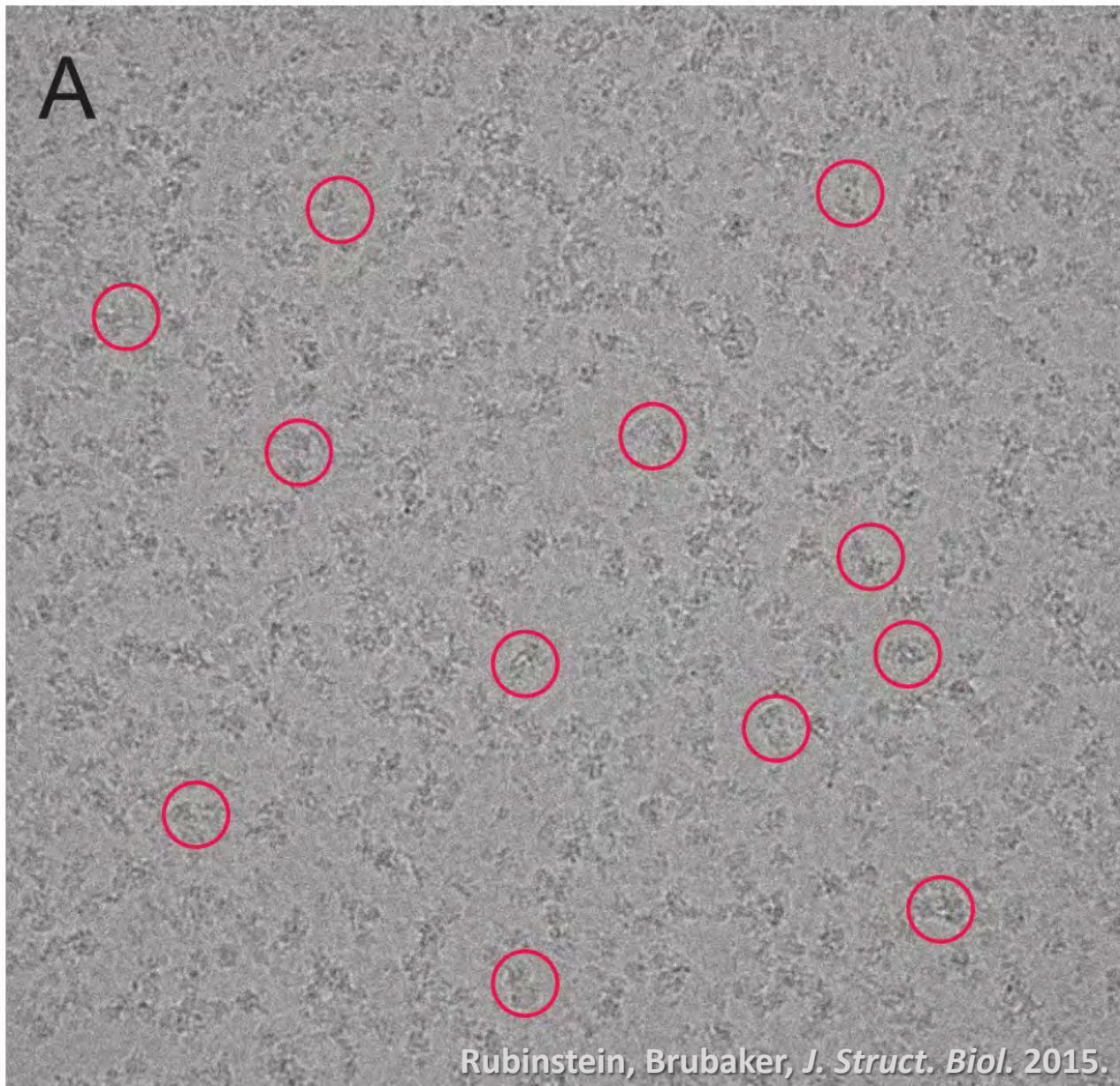


Challenges for single-molecule Cryo EM of MPs

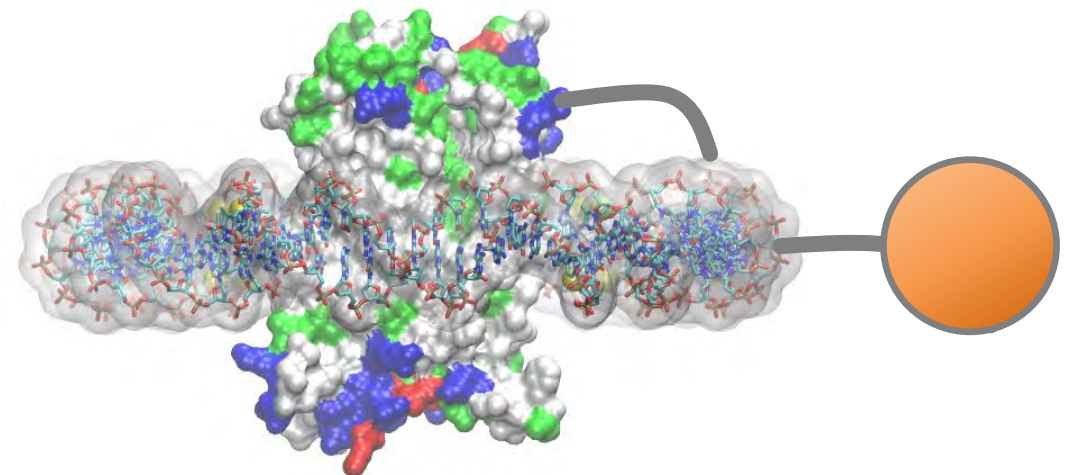
- 1) Solubilize MPs
- 2) Structures of small MPs



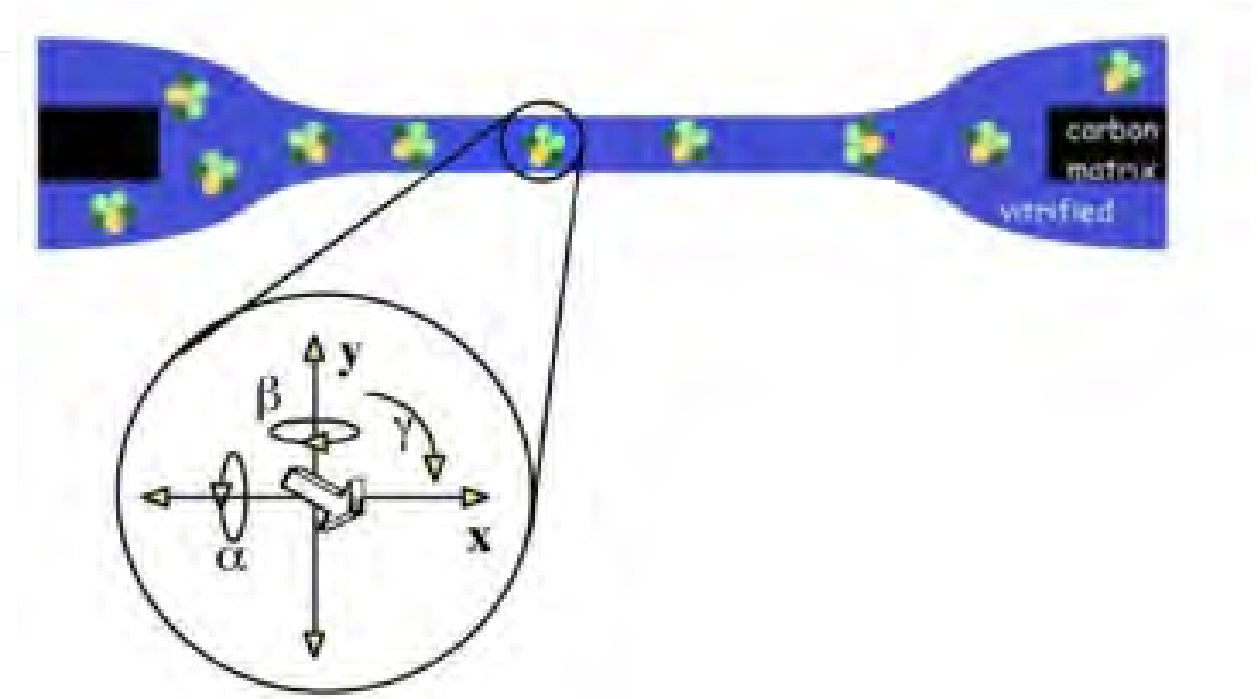
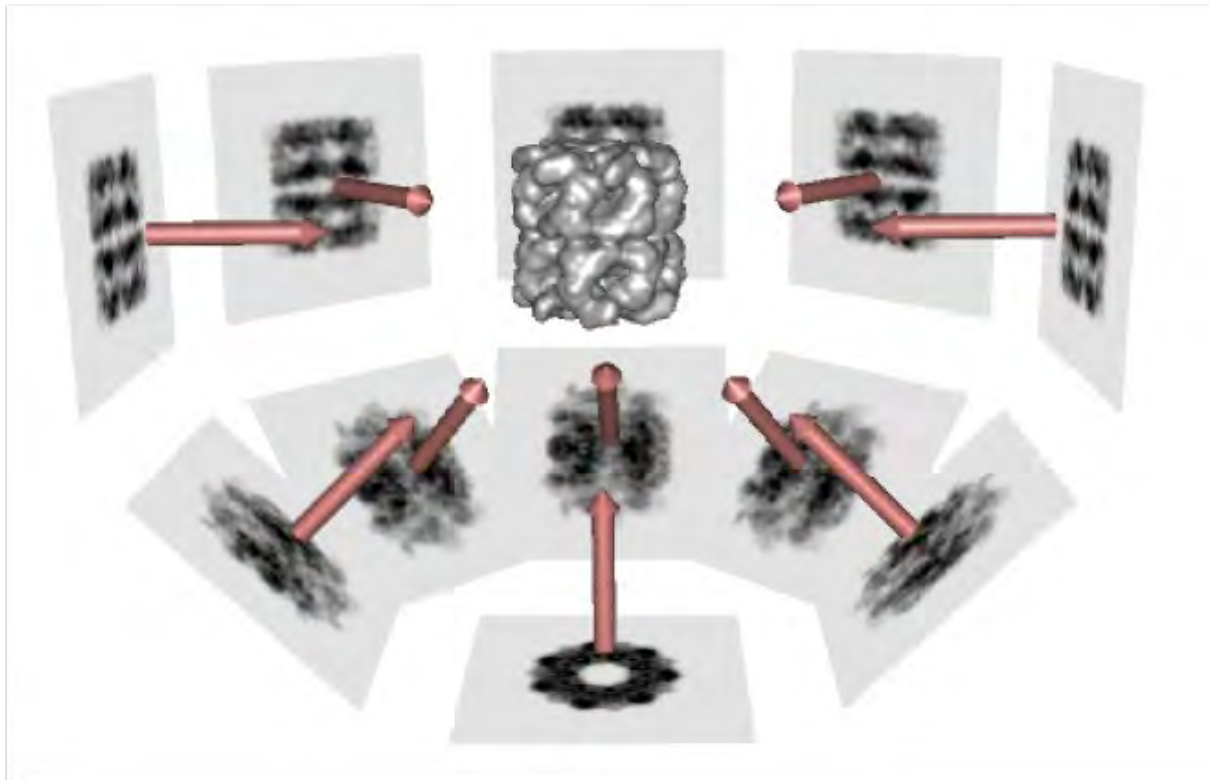
Challenge 2: Small MPs (< 100-200 kDa)



**Automated particle picking,
alignment difficult for small MPs.**

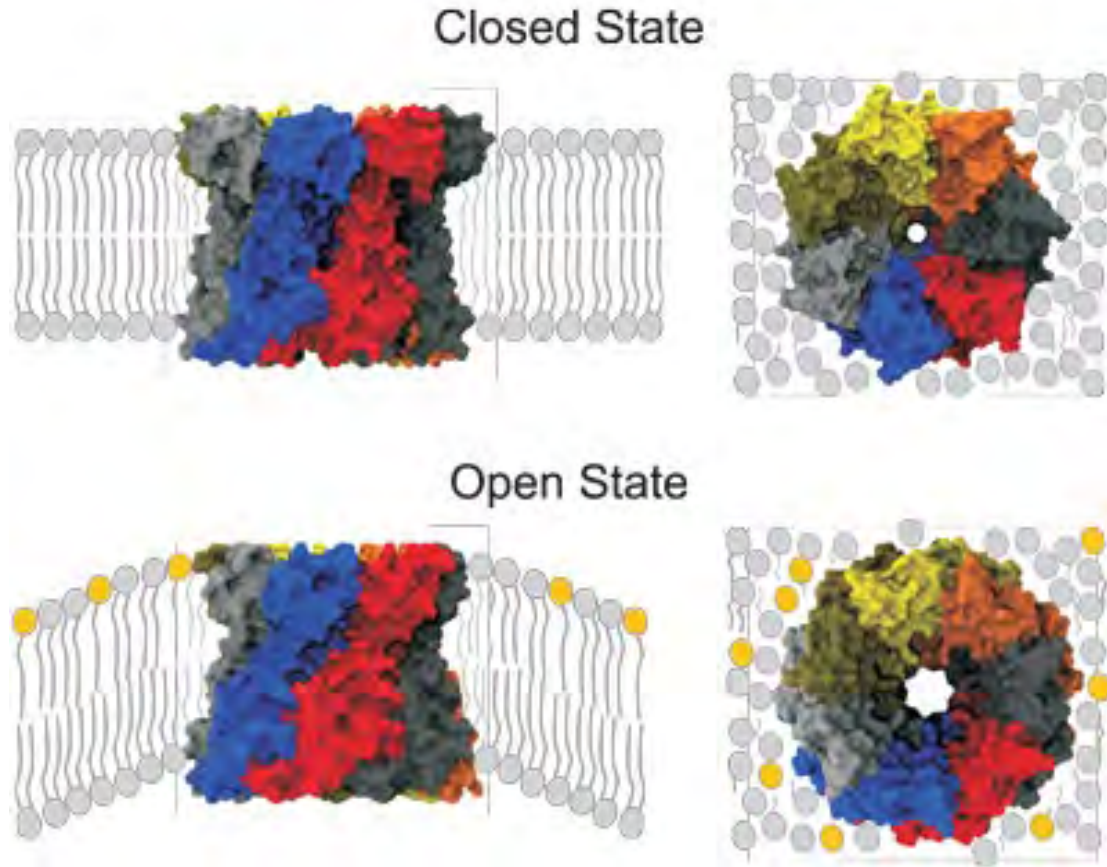


Challenge 3: Image MP in all orientations



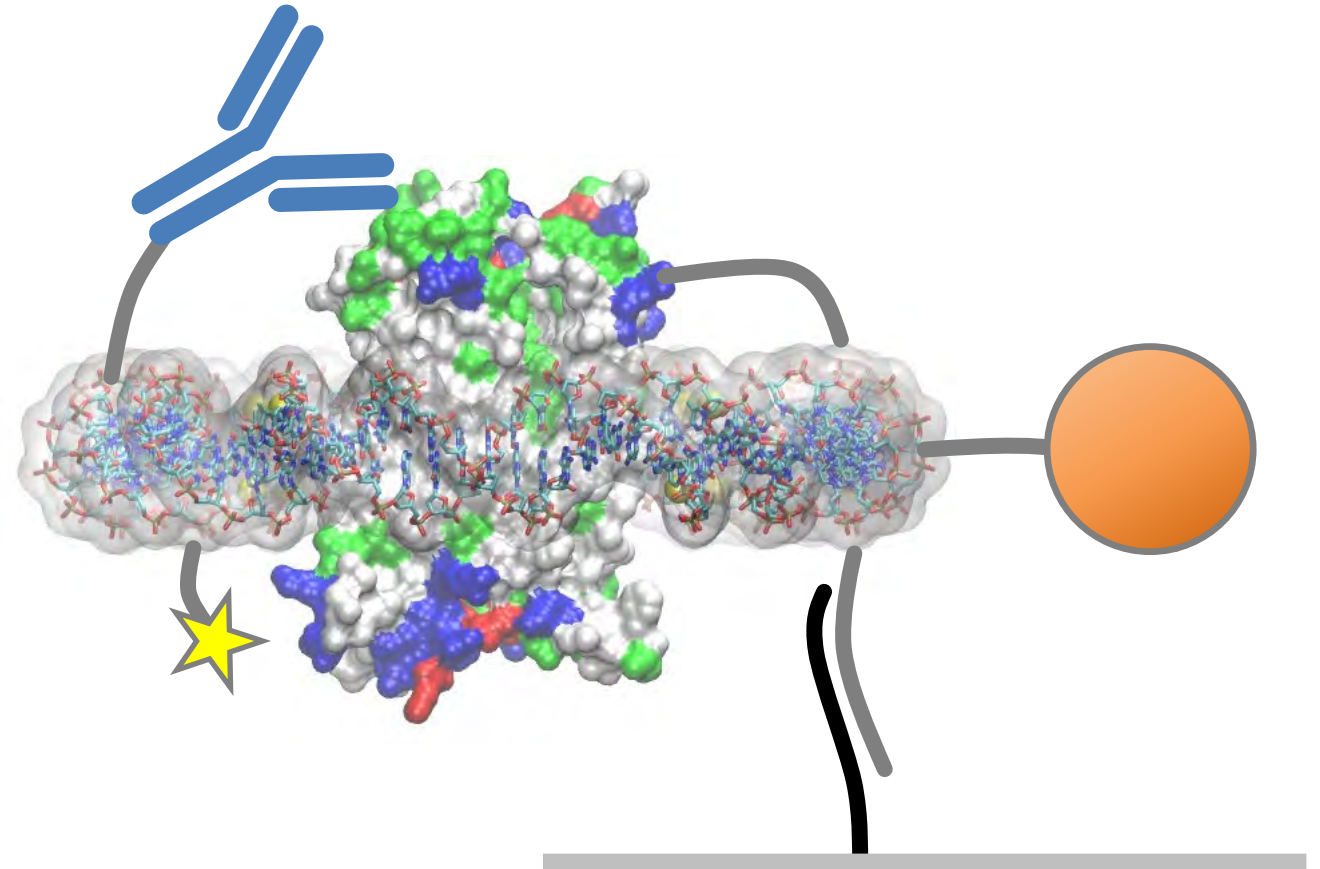
However, proteins stick to air-water interface and denature!!!

Challenge 4: Force generation / mechanosensitive MPs



Established nanoscale bilayer mimetics do not allow for generation of forces.

Swiss pocket knife for membrane proteins?



- Programmable / modular
- Fast design pipeline
- High throughput

Acknowledgements

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