OVERVIEW

Nanopores or Ion Channels and its Applications

Types of Nanopores or Ion Channels

Nanopores or Ion Channel via Macrocycles

***t*-BLM formation and characterization of Supramolecular Nanopores via AFM**

Applications of Ion Channel

1) Cell membrane

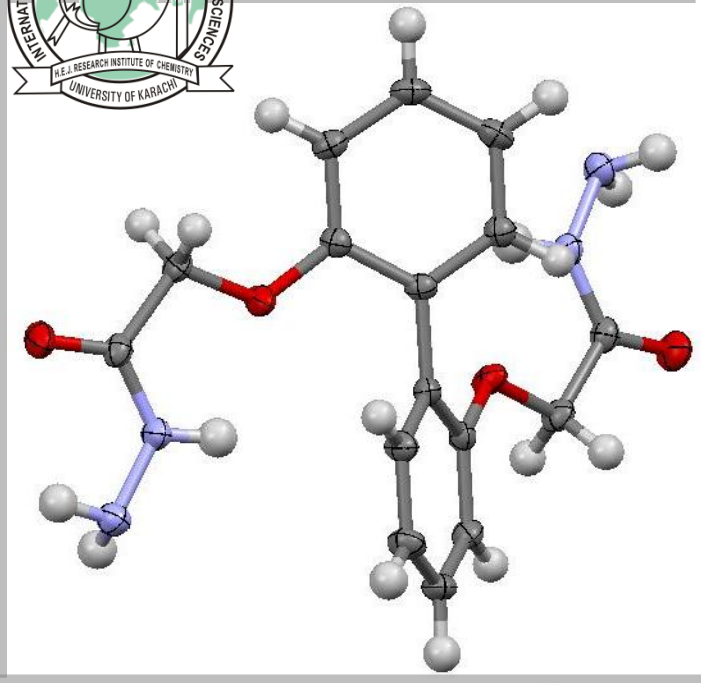
2) Main target class in drug discovery and delivery

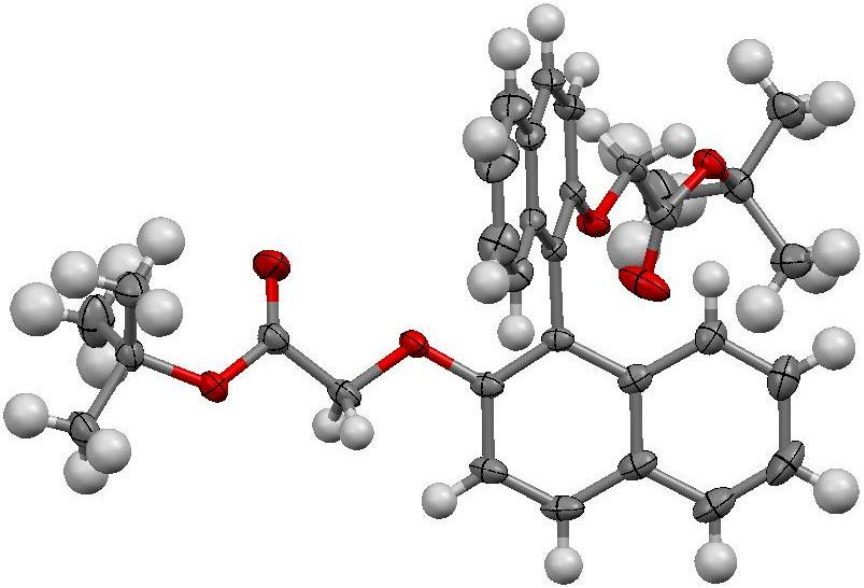
3) Protein Based Polymer in the form of Nanopores are active areas for Drug Delivery.

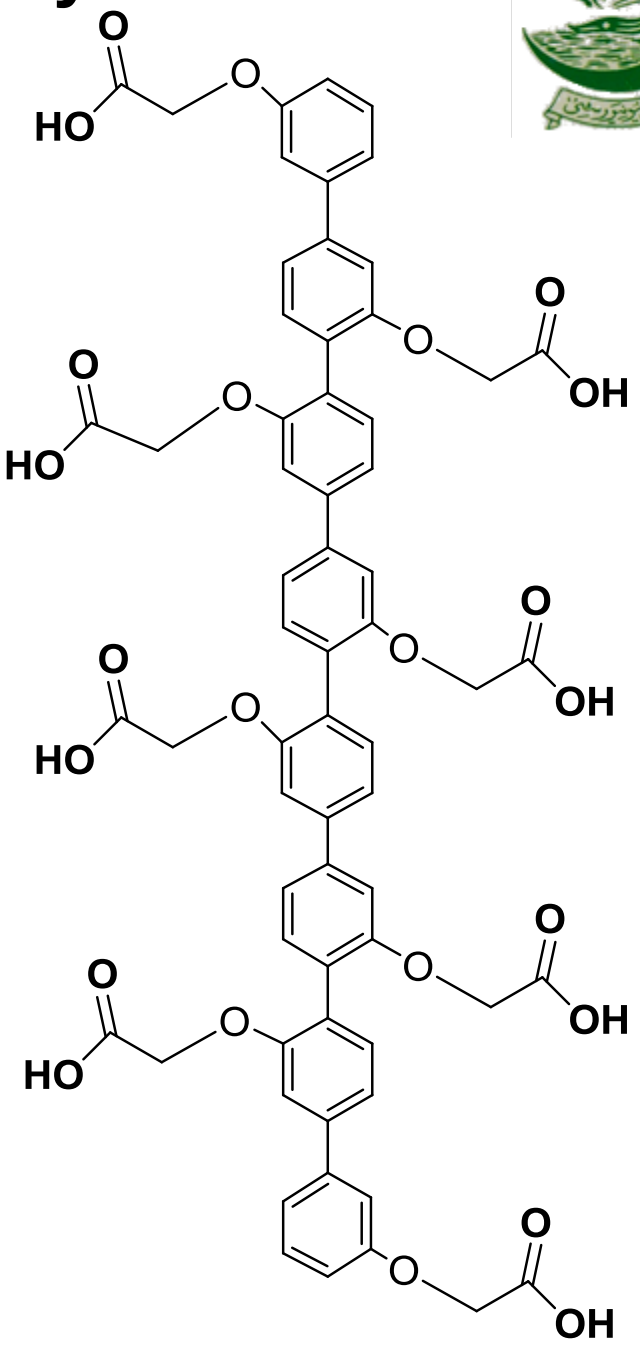
4) 50% currently available drugs interact with cell membranes through Nanopores, well know examples are Nicotine, and Inositol trisphosphate etc.

5) Despite the physiological significance and therapeutic relevance in a wide variety of biological systems, ion channels still remain under exploited as drug targets. This is to a large extent resulting from the historical lack of screening technologies to provide the throughput and quality of data required to support medicinal chemistry.

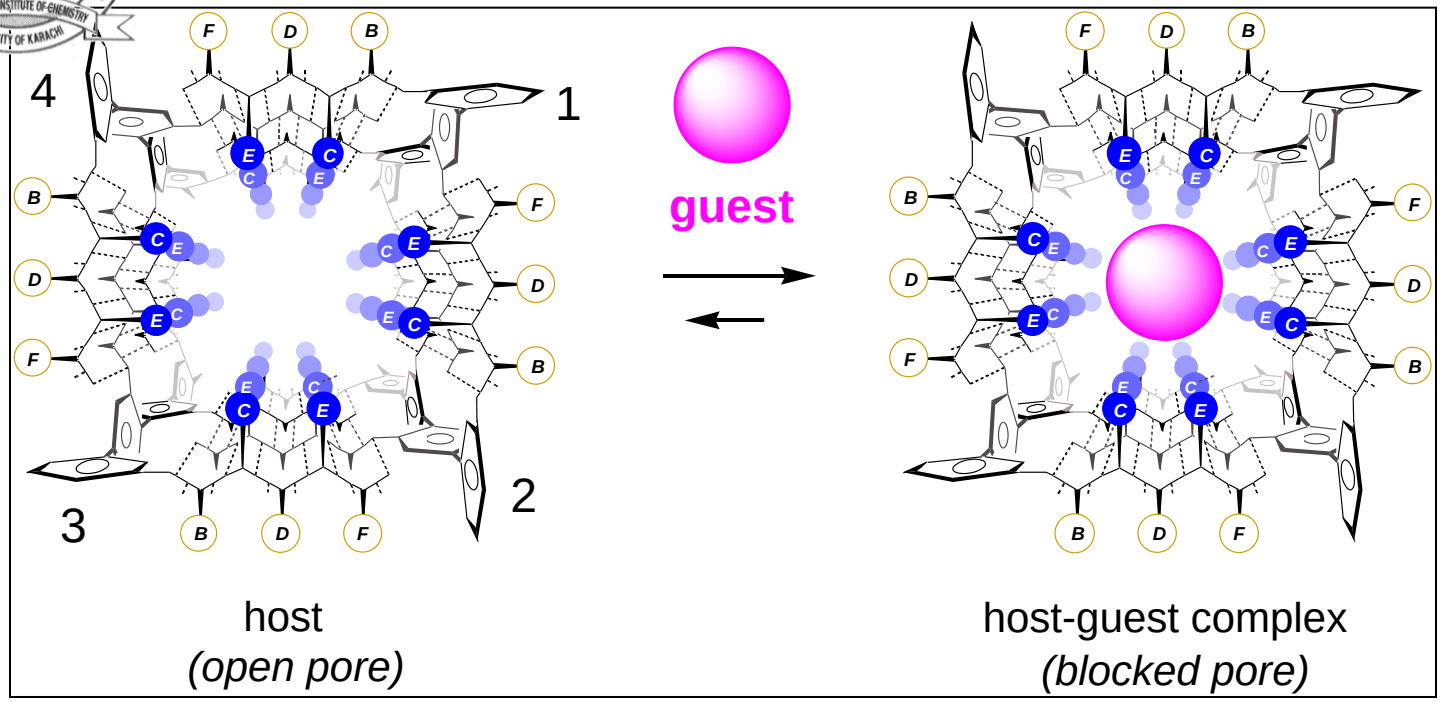
Nanopores via Macrocycles

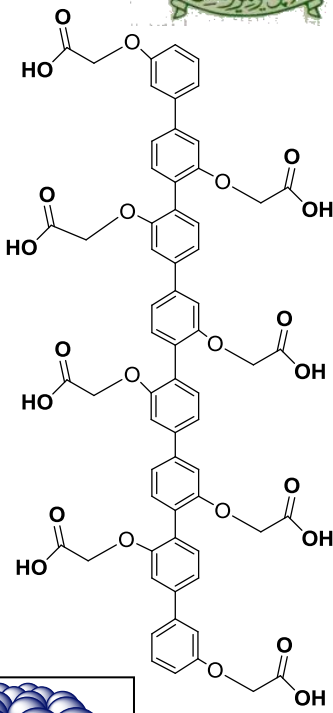


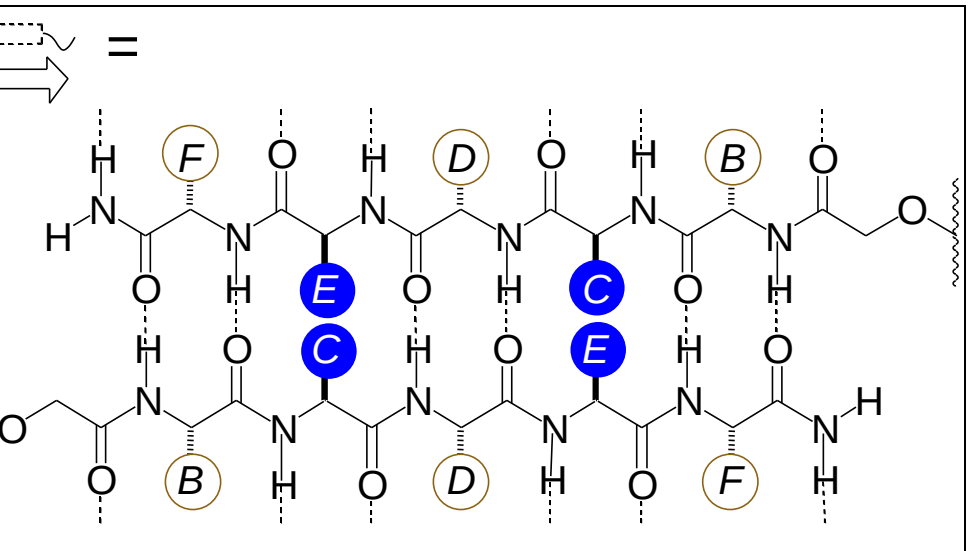


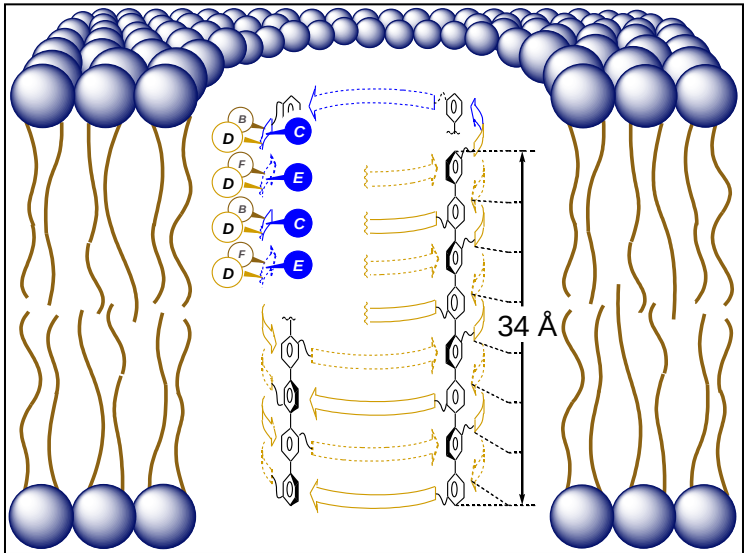


Synthetic ion channels and pores with Rigid-Rod molecules

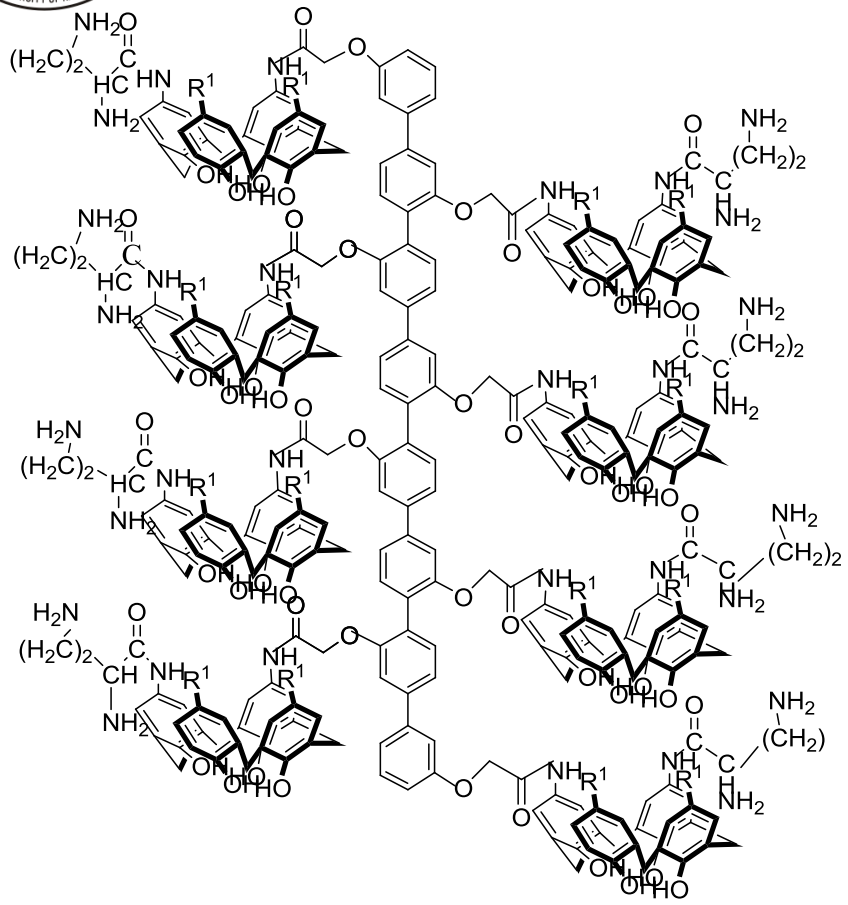


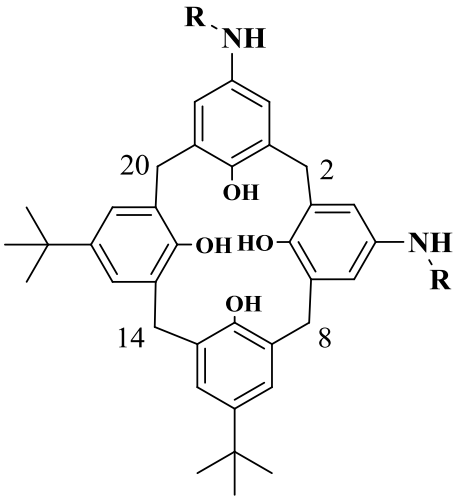


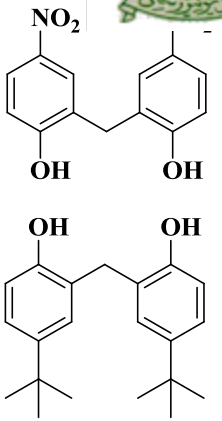


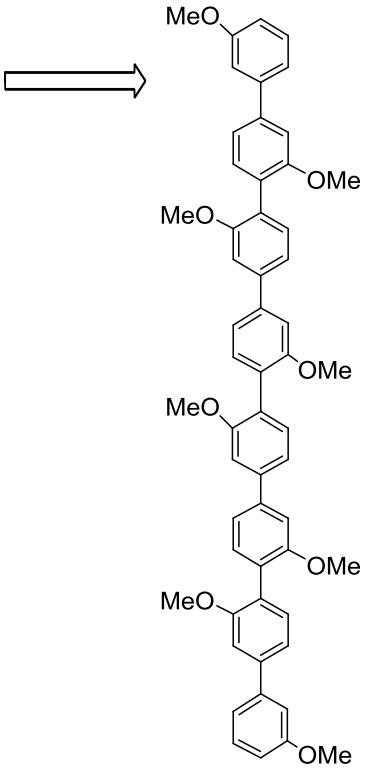


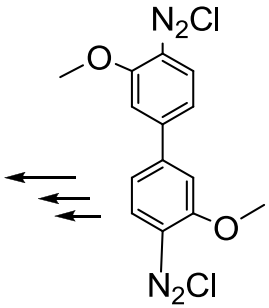
Synthesis of *p*-Octiphenyloctacalix[4]arene





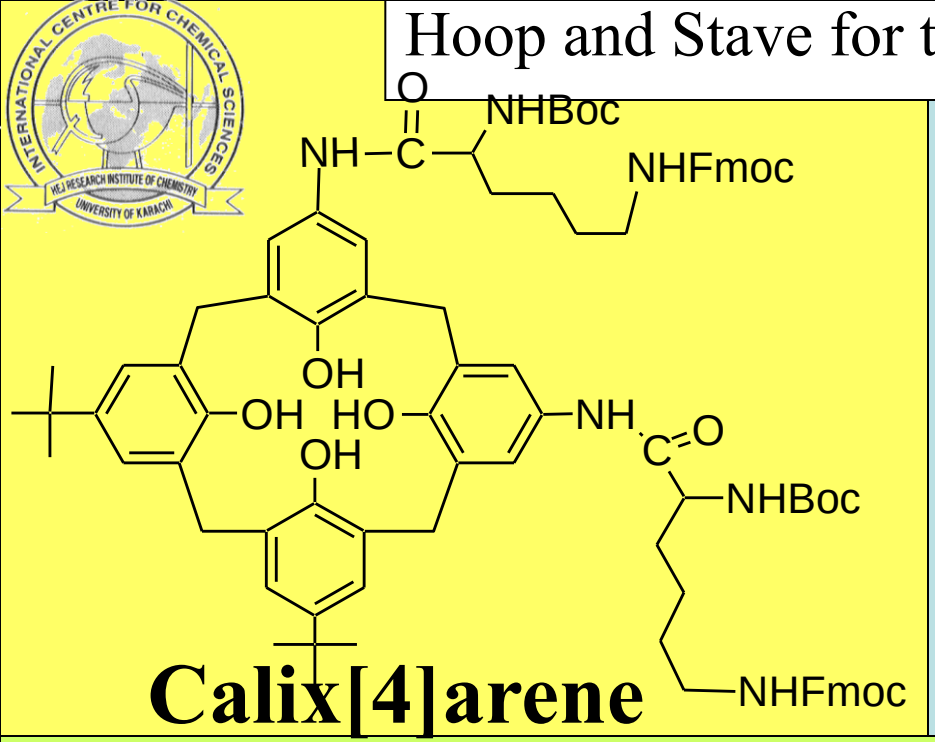




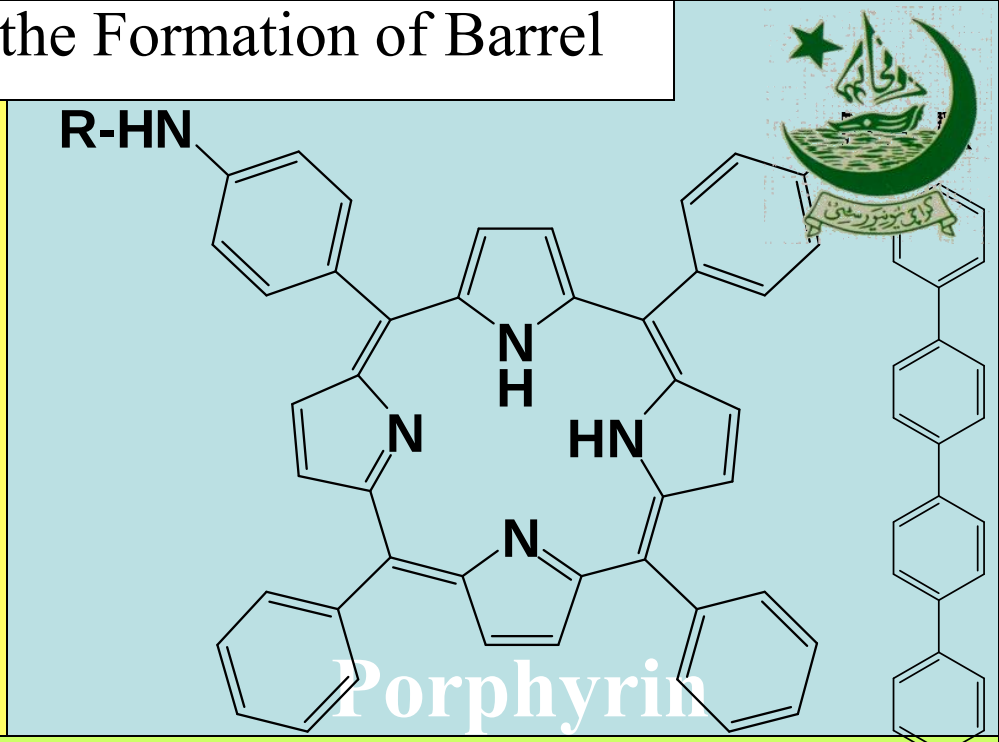


_p_-Octiphenyloctacalix[4]arene
Multifunctional Pore

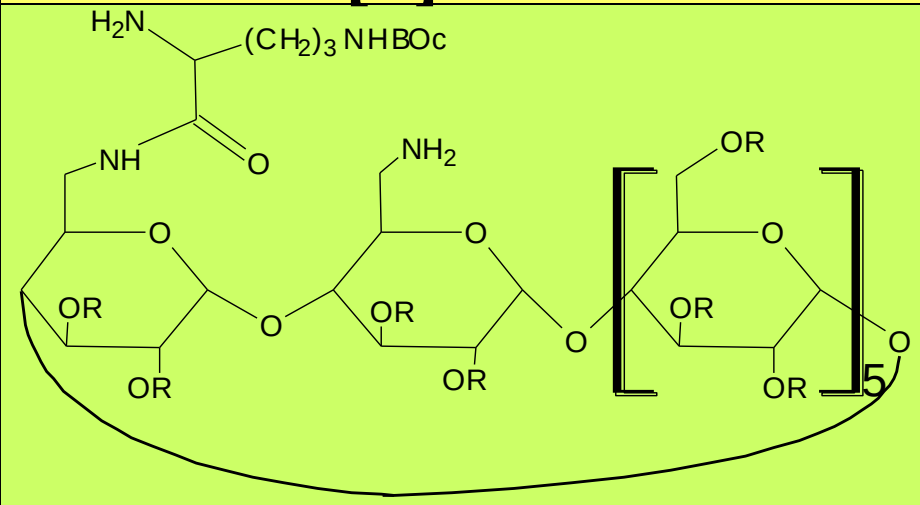
Hoop and Stave for the Formation of Barrel



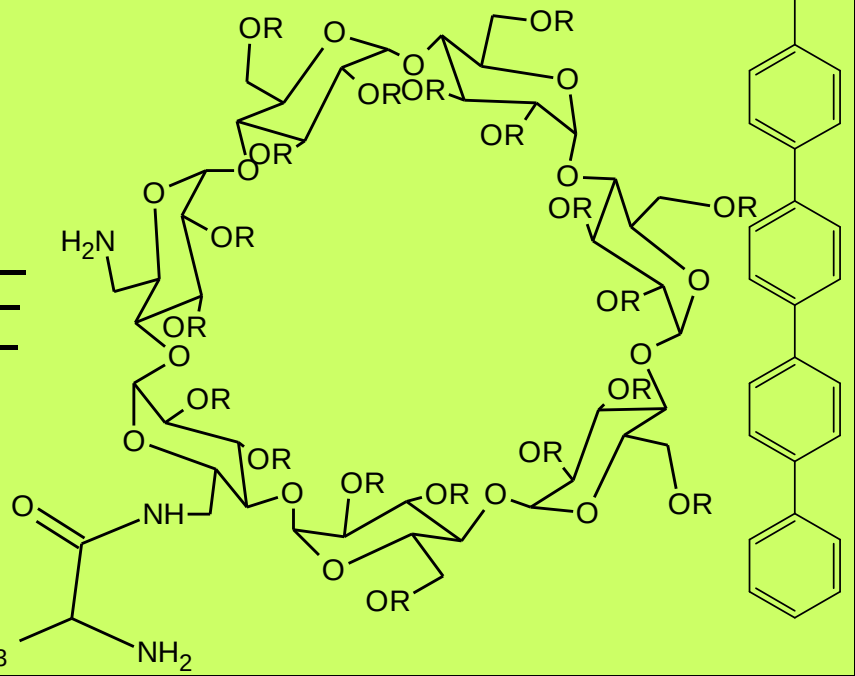
Calix[4]arene



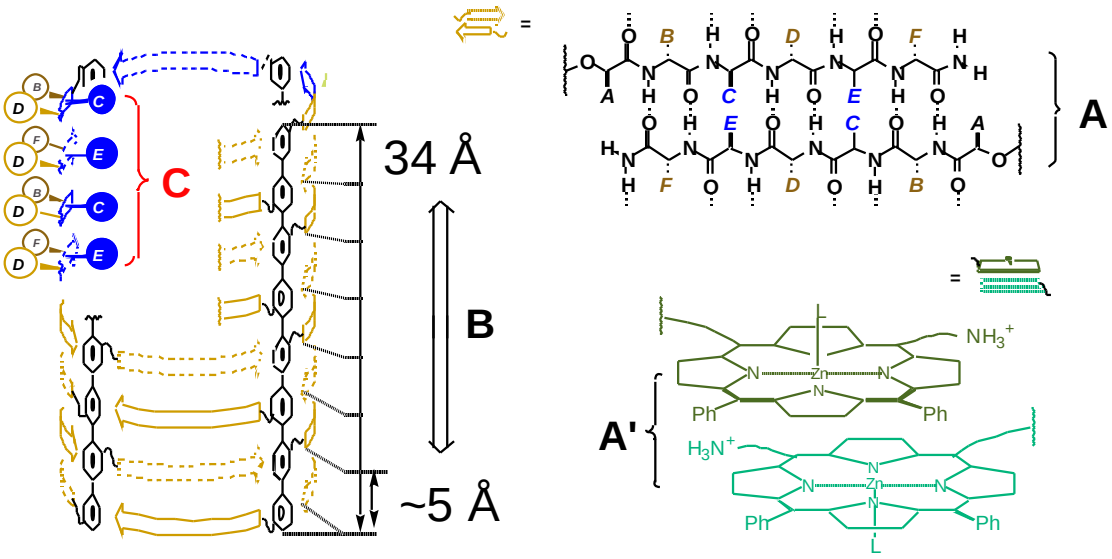
Porphyrin

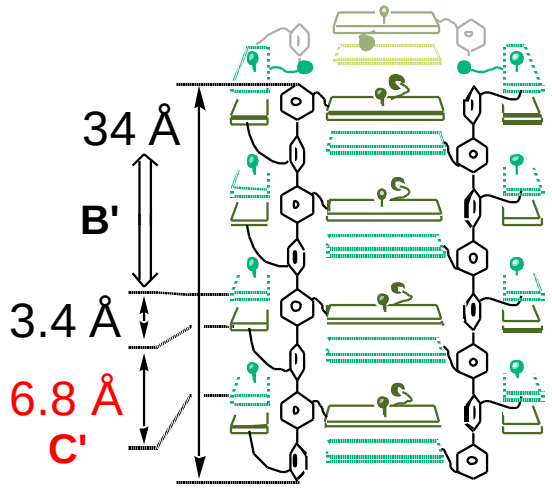


Cyclodextrin

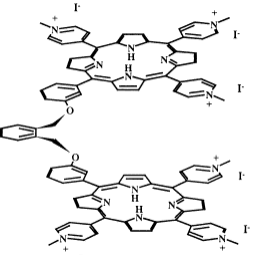


New Synthetic Multifunctional Pores: From Rigid-Rod β-Barrels Toward Rigid-Rod π-Barrels

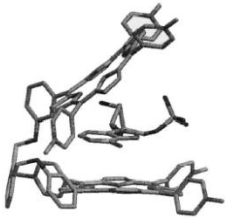




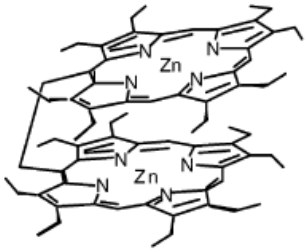
hoop-hoop π-π interaction 3.4%



Schnieder

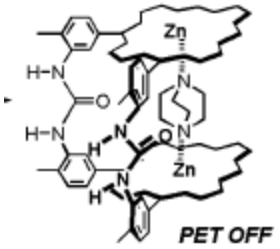


nucleotides



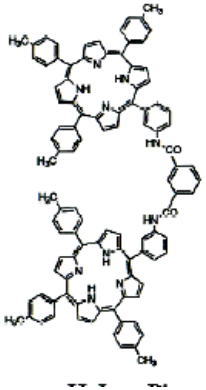
Ogoshi

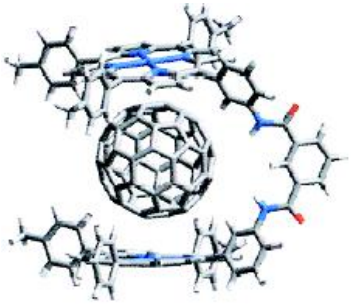
Amino acids carbohydrates



yagi

Alkaloids and amines

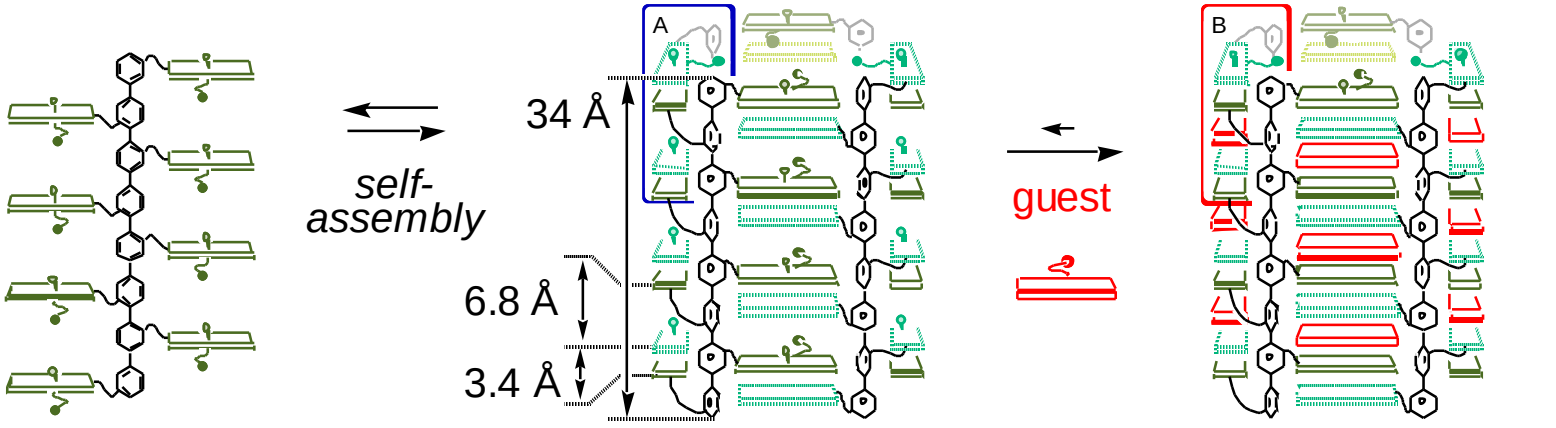




Aida

Fullerenes and amines

Design of Rigid-Rod π-Barrels as Multifunctional Pores (Specific Examples)

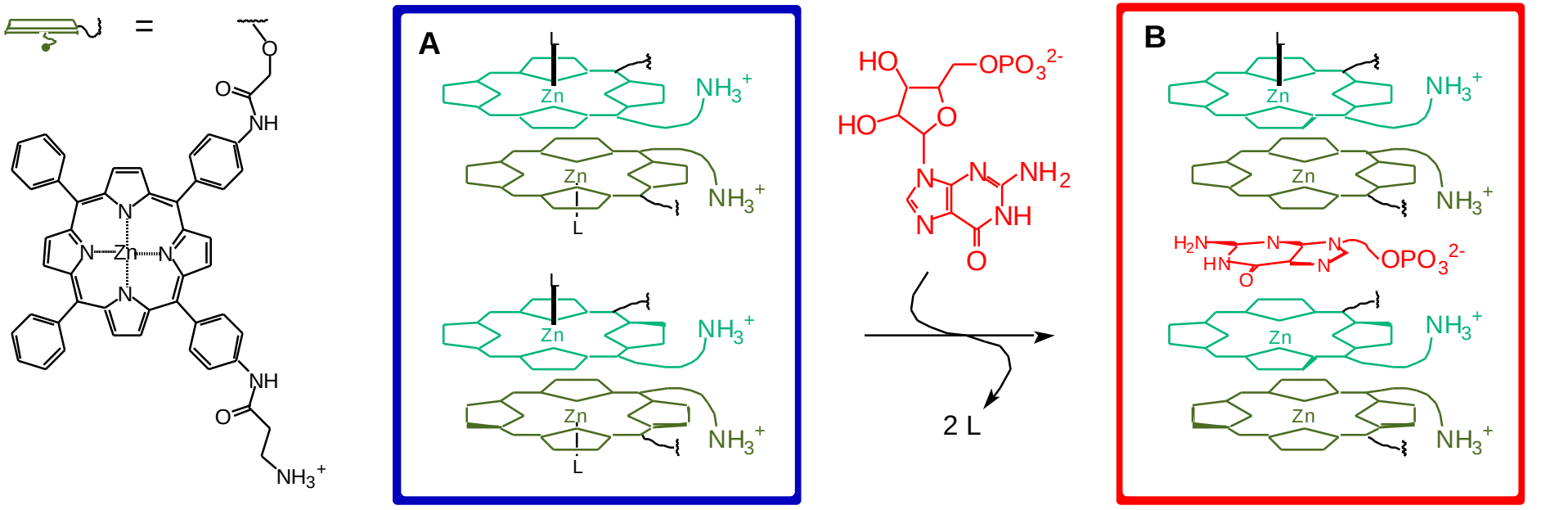


rigid-rod monomer

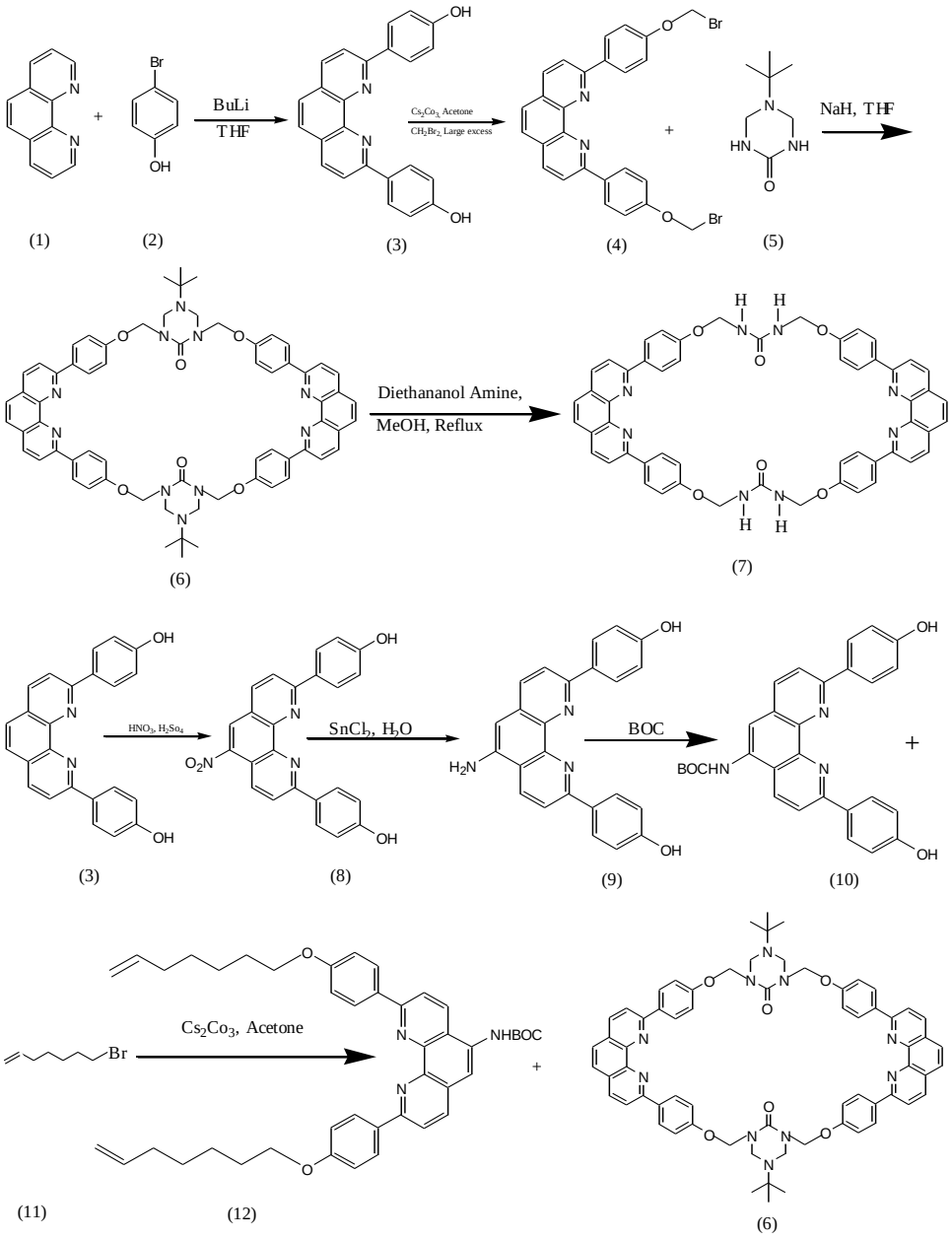
rigid-rod š-barrel

π-

host-guest complex



Pores via Urea base macrocycles

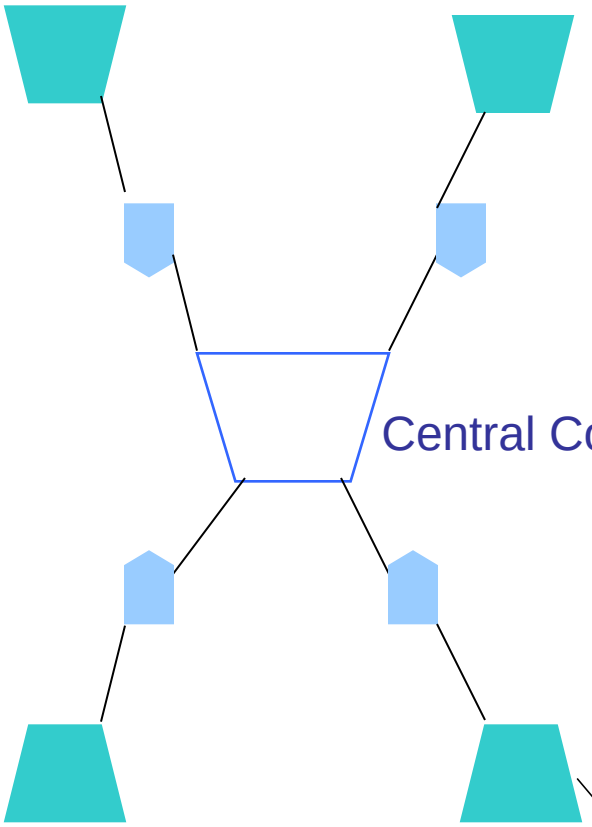


Hydrogen Bonding and Pi-pi-stacking ability can be exploited to form nanopores

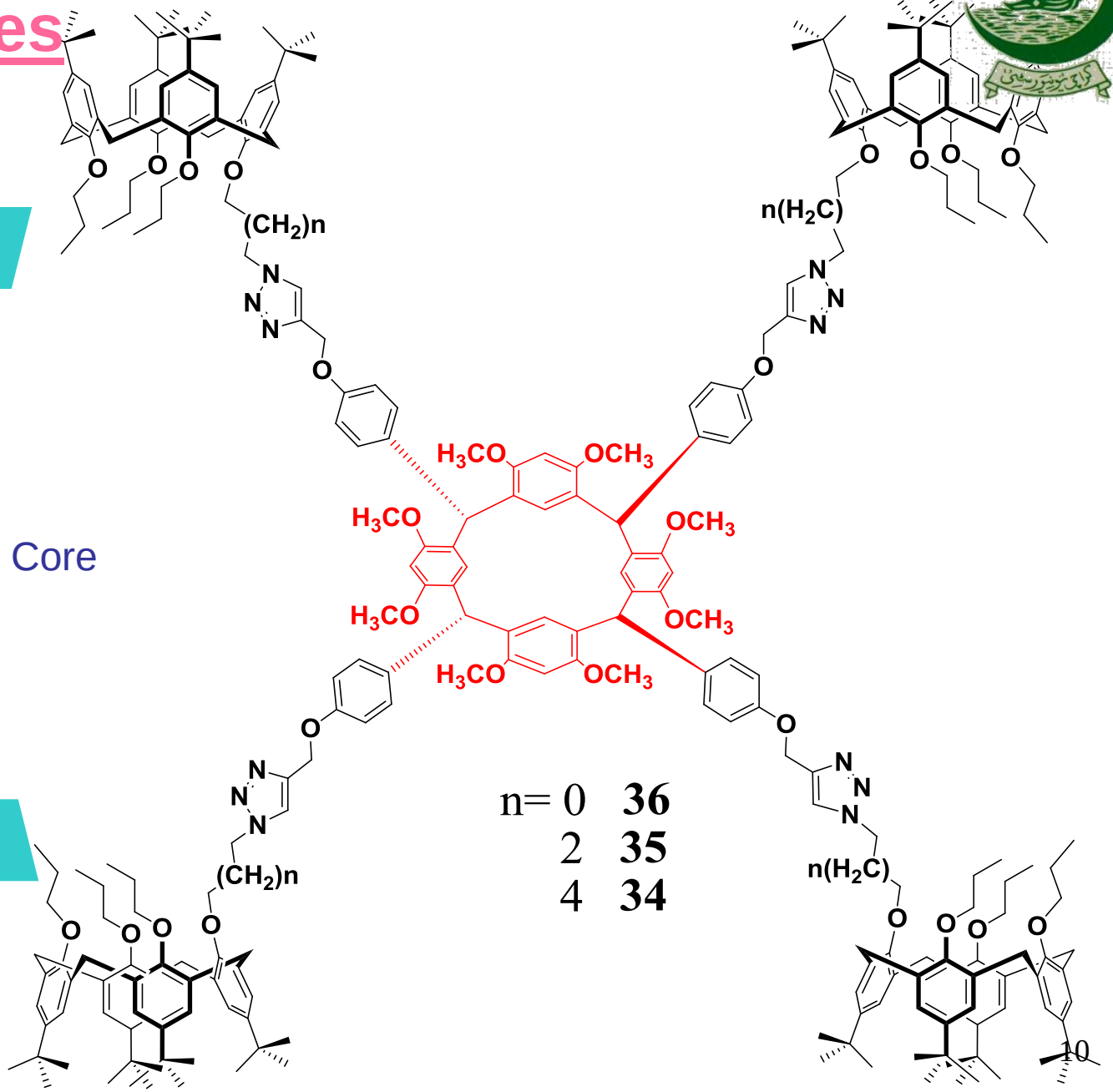
Structural Features

Pores via Hybrid Multicalixarene

Peripheral Units



Central Core



n= 0 **36**
2 **35**
4 **34**

Introduction of AFM

AFM was developed in 1986 by G. Binning and H. Rohrer

A normal AFM instrument consist of the following parts

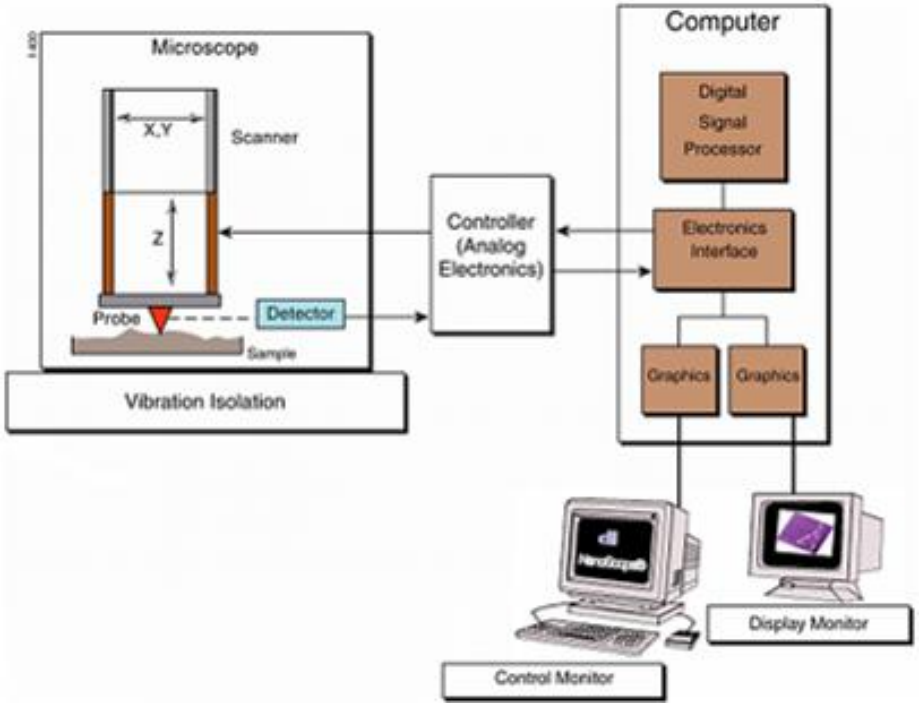
® probe (cantilvers)

® cantilever deflection monitoring system (The AFM head uses a beam deflection system to monitor the cantilever displacement.)

® scanning system (Piezoelectric scanner)

® electronics

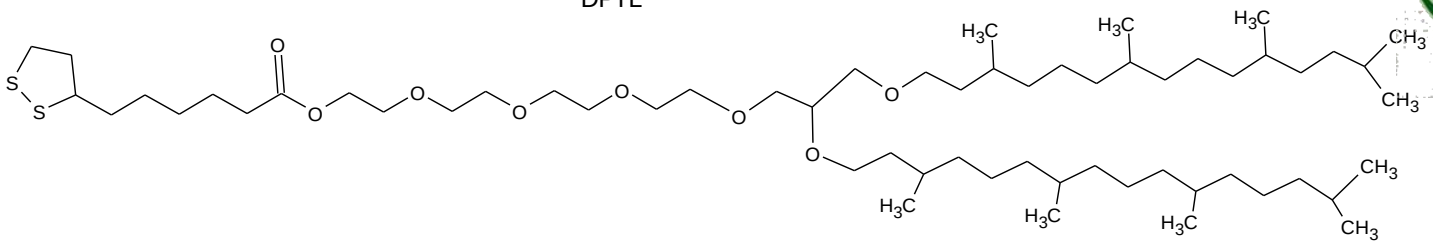
® computer



Molecules Used for SAM Formation

DPHL
DPOL
DPTDL

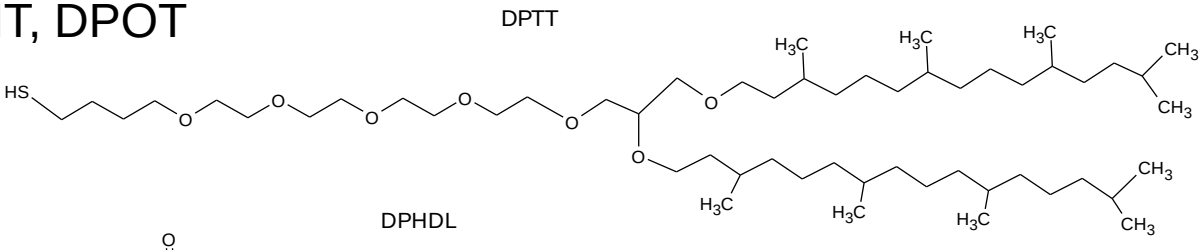
DPTL



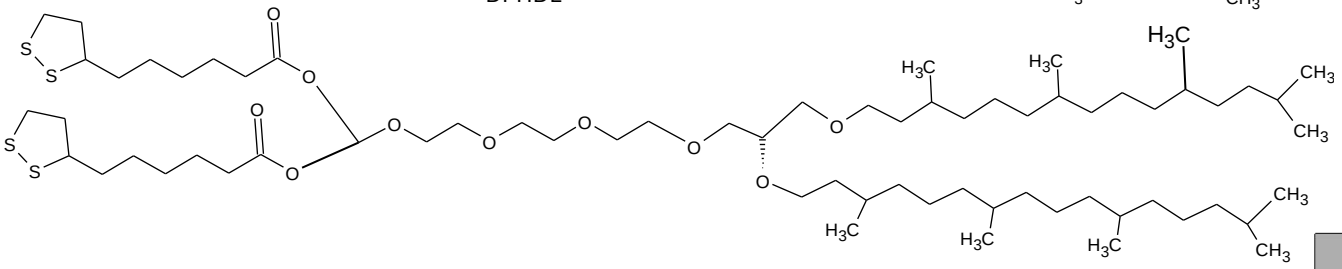
2,3-di-O-phytanyl-sn-glycerol-1-tetraethylene glycol-D,L- -lipoic acid ester lipid (DPTL).

DPHT, DPOT

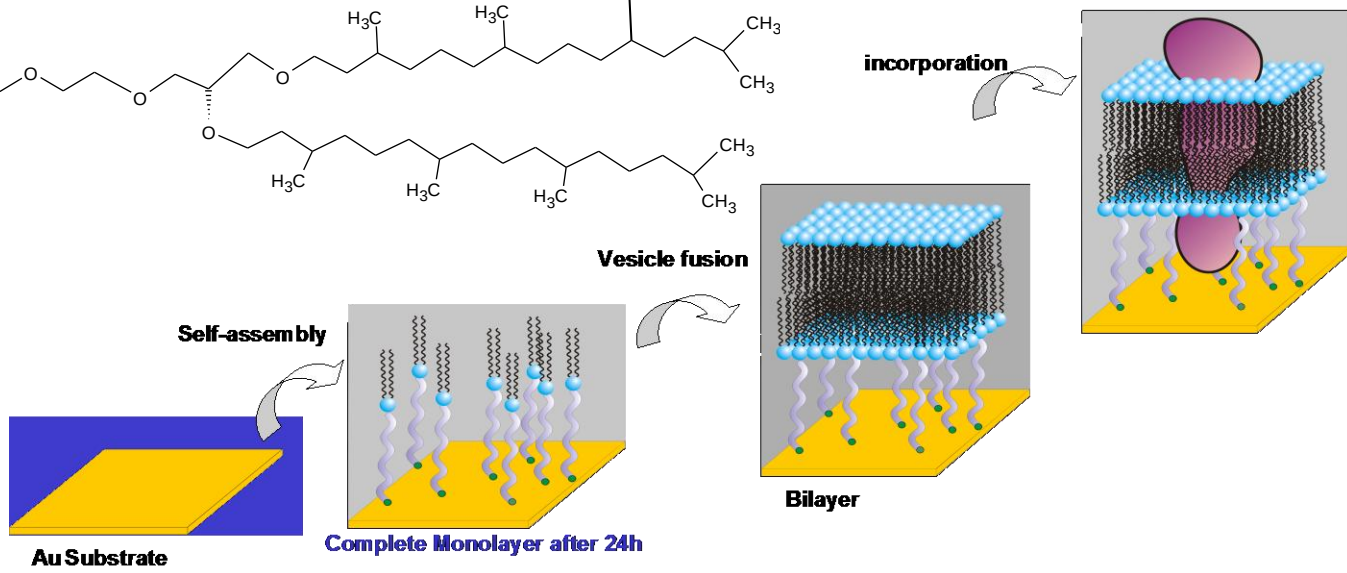
DPTT



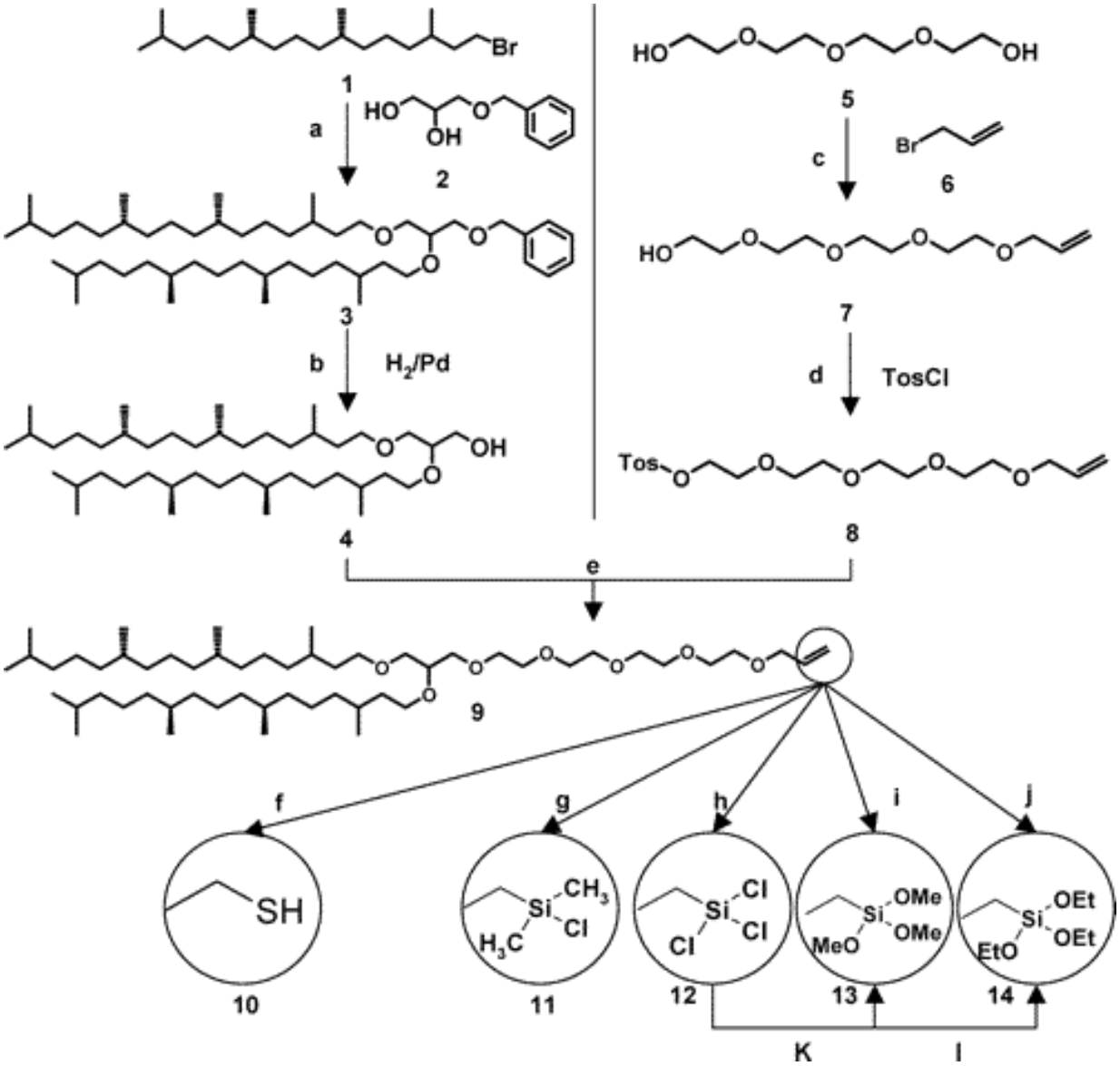
DPHDL



Investigation step by step tBLM



Synthesis of Lipids

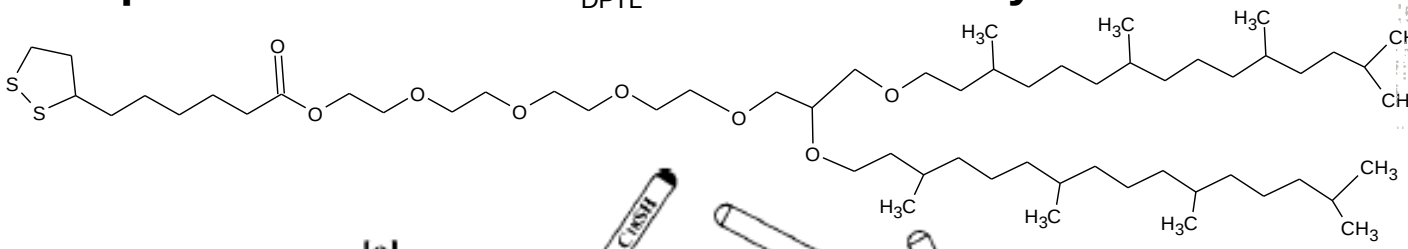


Problems in resolution and tip

1) Cantilevers silicon 70 kHz frequency (Soft, produce high resolution)
2) Cantilevers kept under vacuum for overnight
3) Cantilevers plasma cleaned prior to use
4) Liquid cell along with O-ring sonicated in detergent for 30 minutes at 40°C
5) Sonicated in ethanol for 30 minutes
6) Rinsed extensively with Milli-Q
7) Everything dried with flush of nitrogen
8) Extremely soft taping
9) Target amplitude was kept in between 2-3.
10) For higher resolution always Z-range was decreased.

Proposed Mechanism for the Self-assembly Process

DPTL



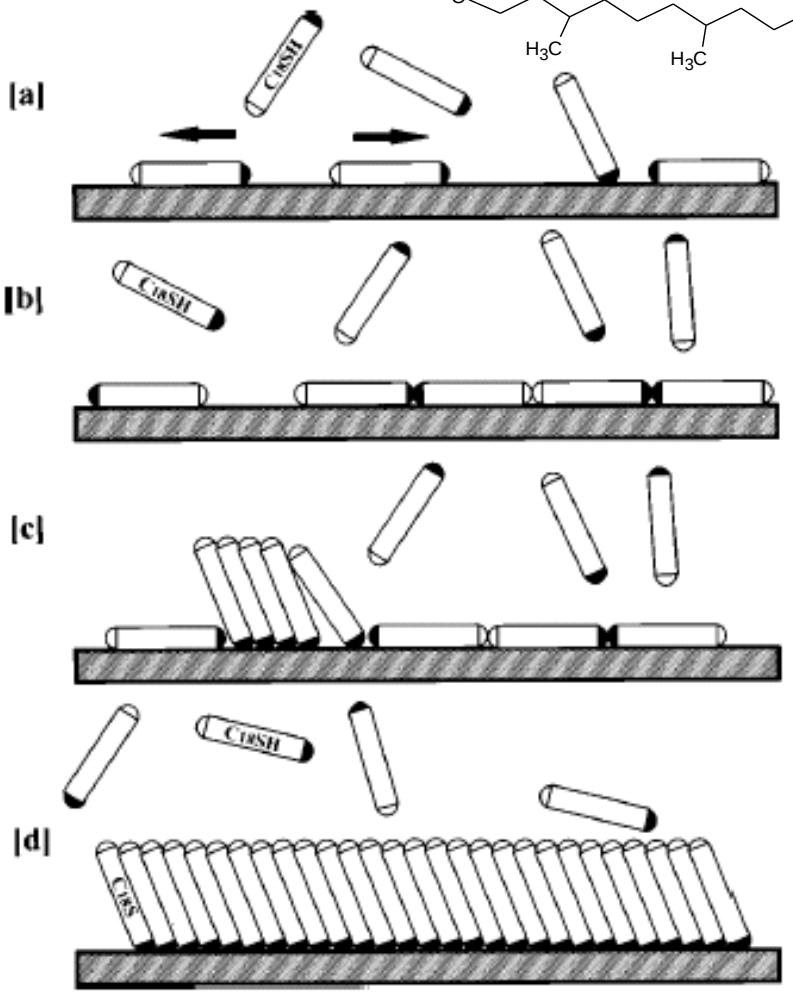
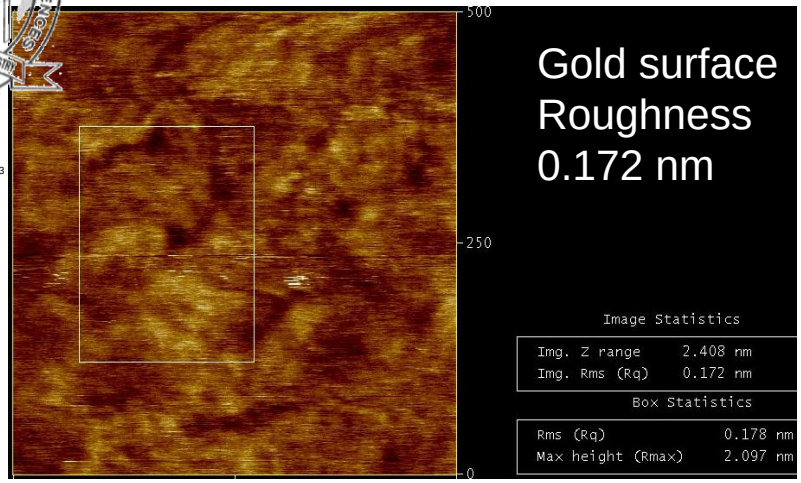
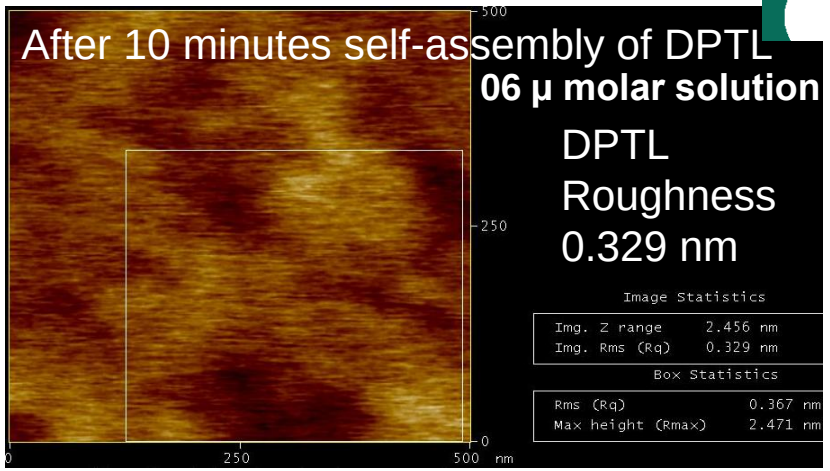
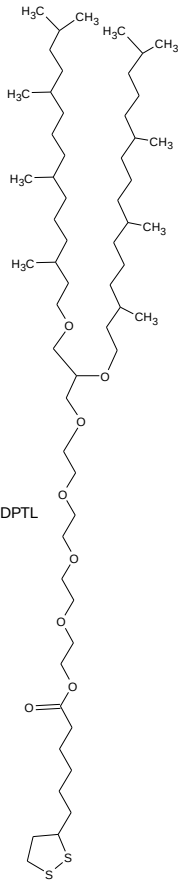


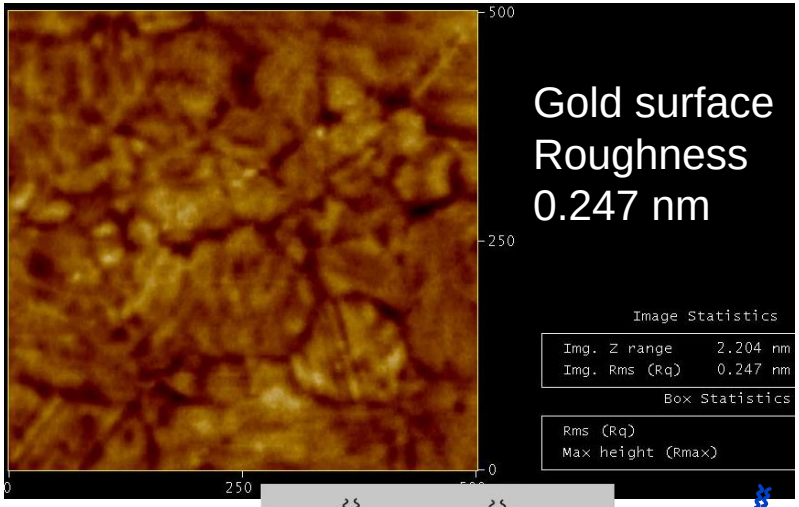
FIG. 5. Schematic mechanistic diagram for the self-assembly of $CH_3(CH_2)_{17}SH$ on Au(111). (a) Initial adsorption, (b) lying-down phase. (c) Two-dimensional phase transition from a lying-down to a standing-up configuration. (d) Formation of a complete SAM.

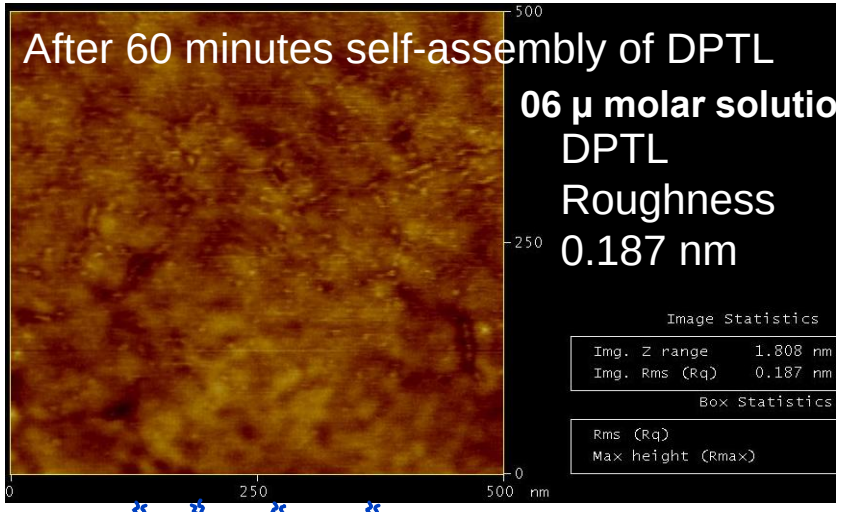
Differences in roughness indicate the self assembly process



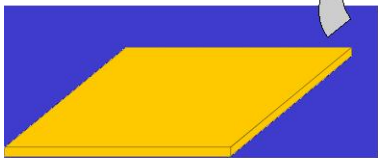




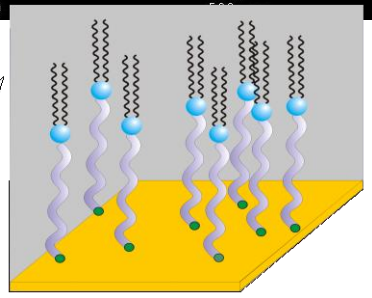




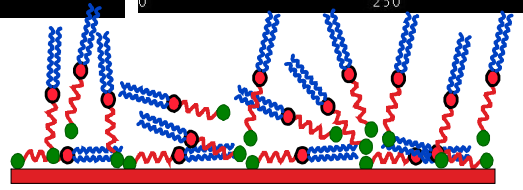
Self-assembly

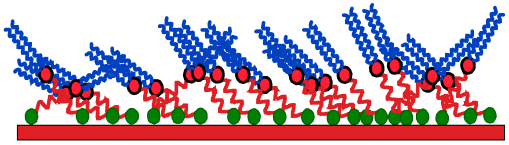


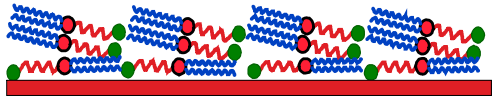
Au Substrate

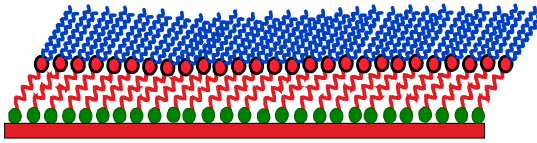


Complete Monolayer after 24h

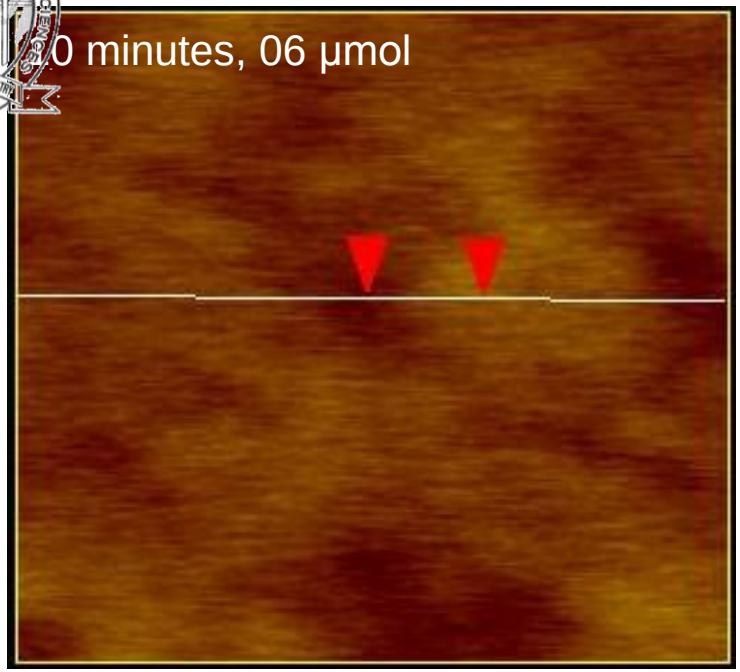


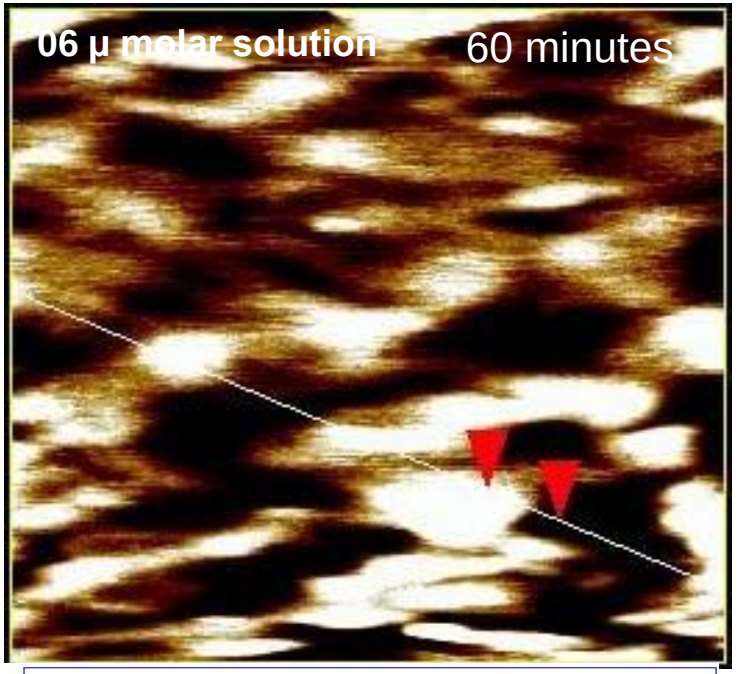


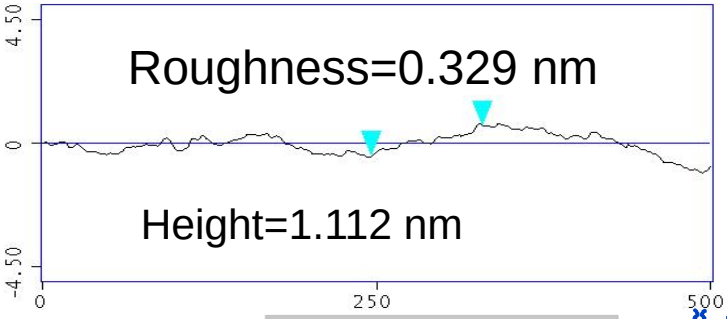


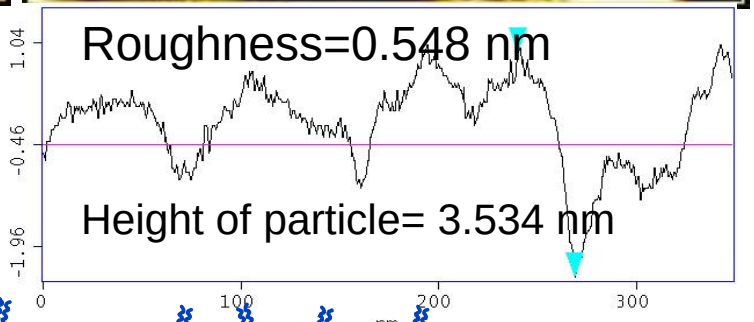


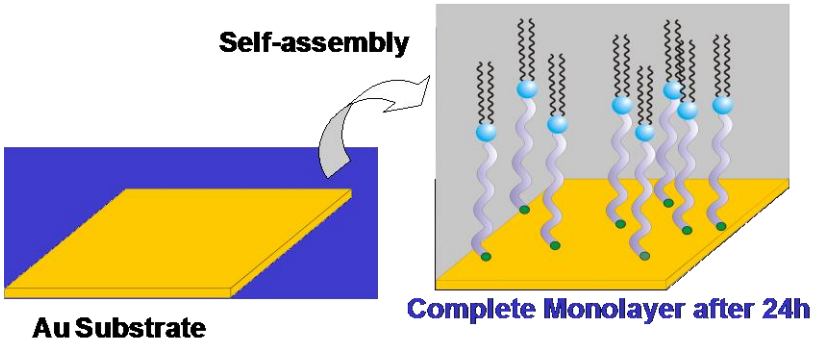
Time dependent cross-sectional analysis of DPTL after 10 and 60 minutes



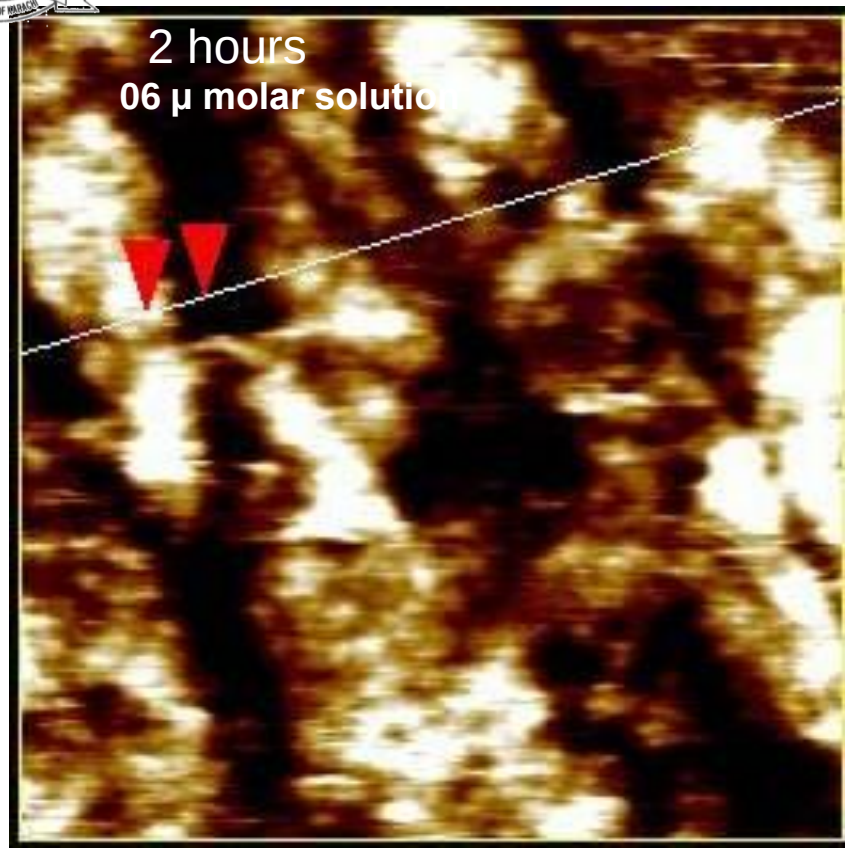


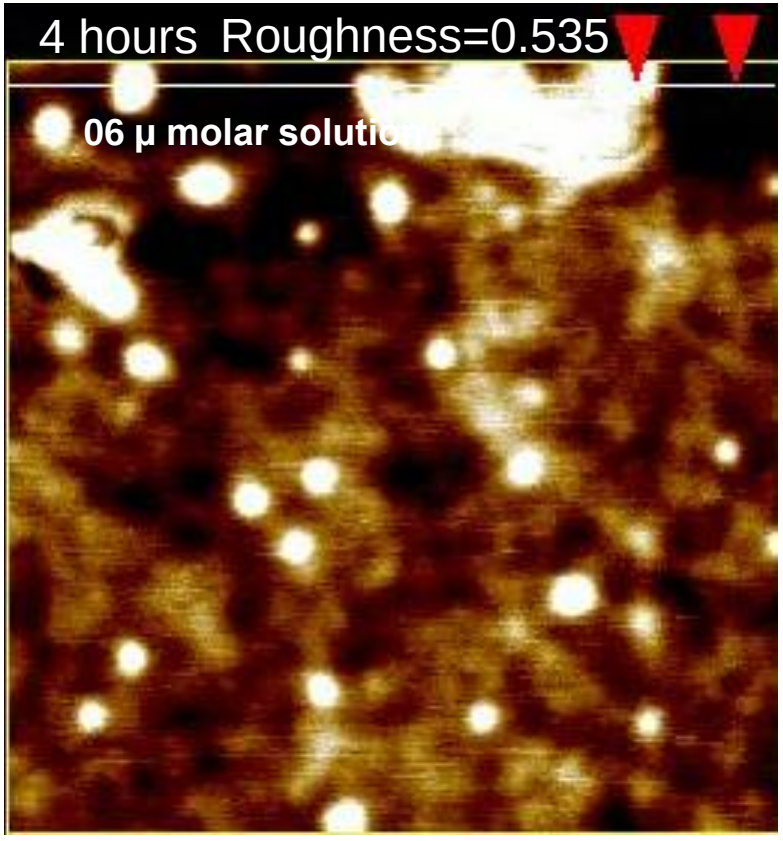


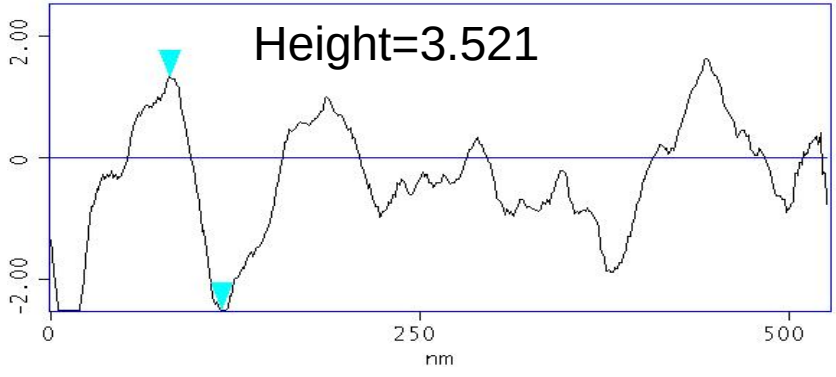


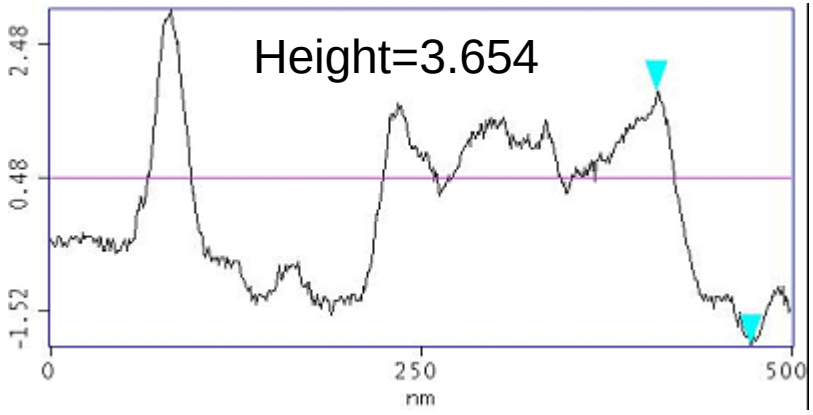


me dependent cross-sectional analysis of DPTL after 2h and 4h.

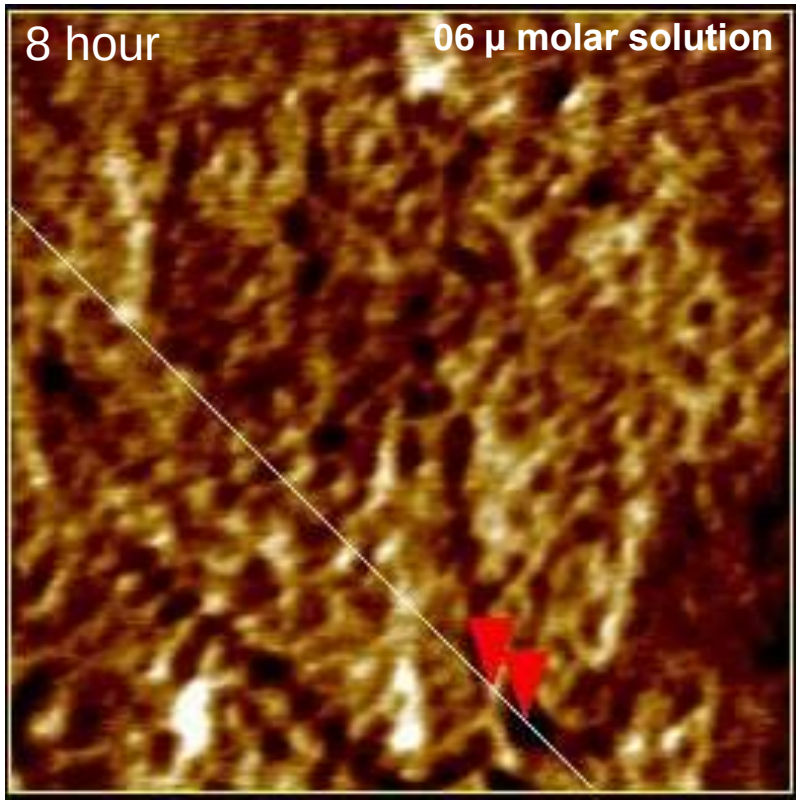


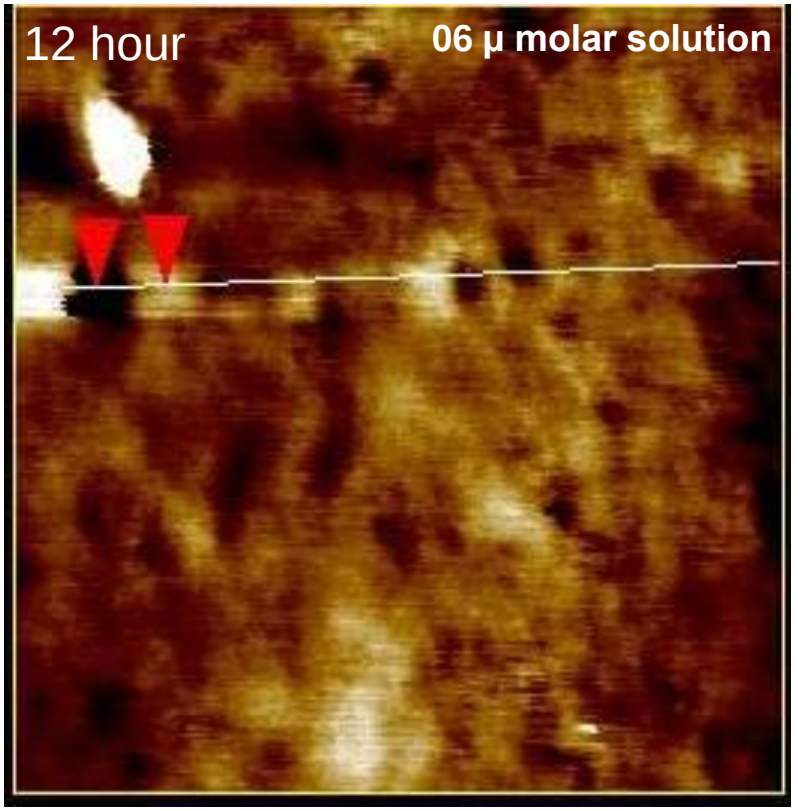


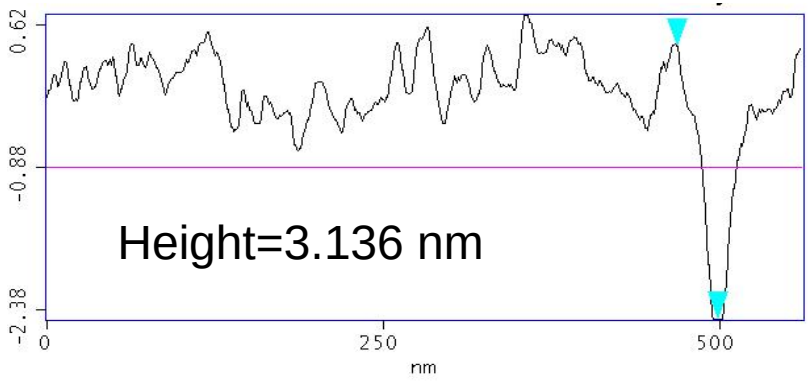


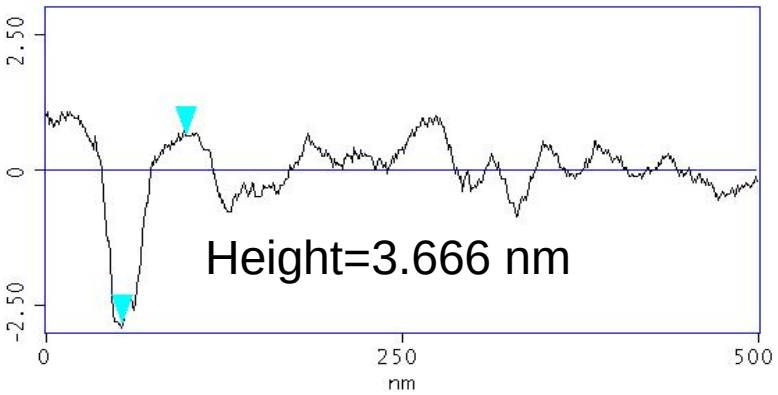


Time dependent cross-sectional analysis of DPTL after 8h and 12h

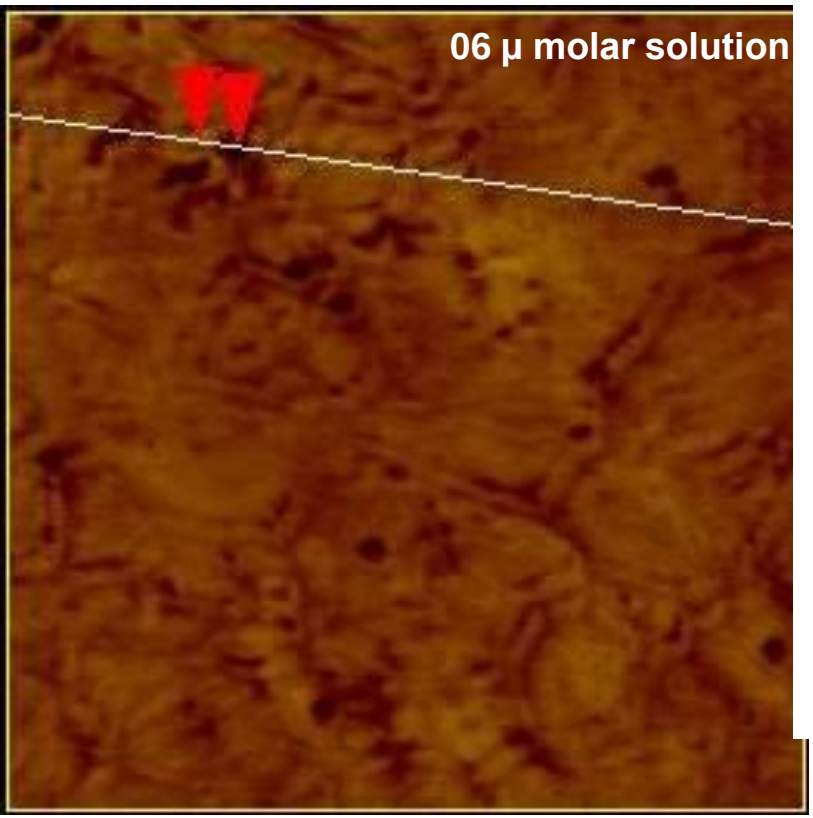


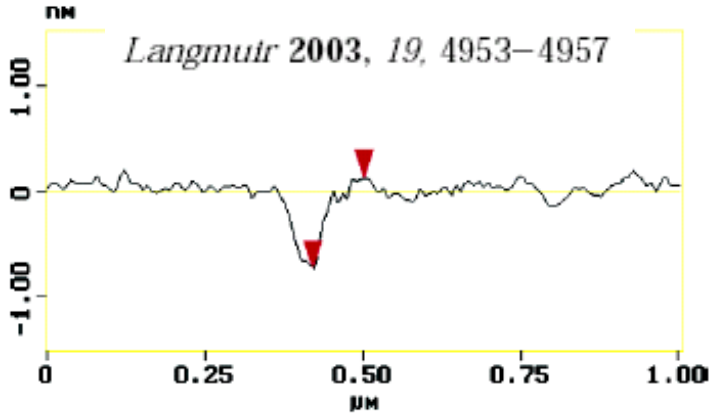






Time dependent cross-sectional analysis of DPTL after 24 hours





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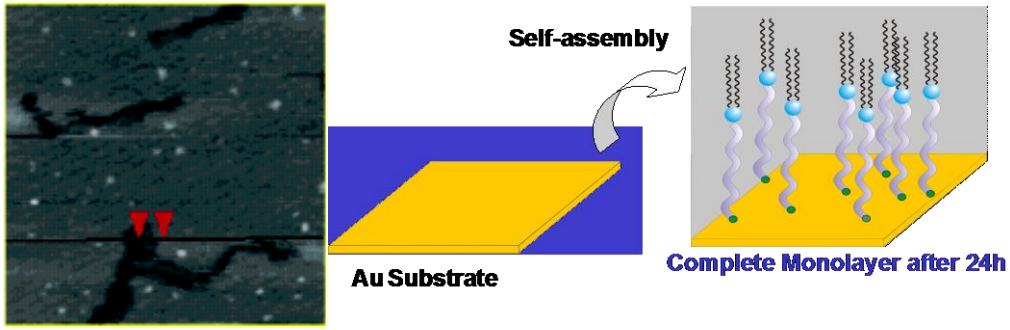
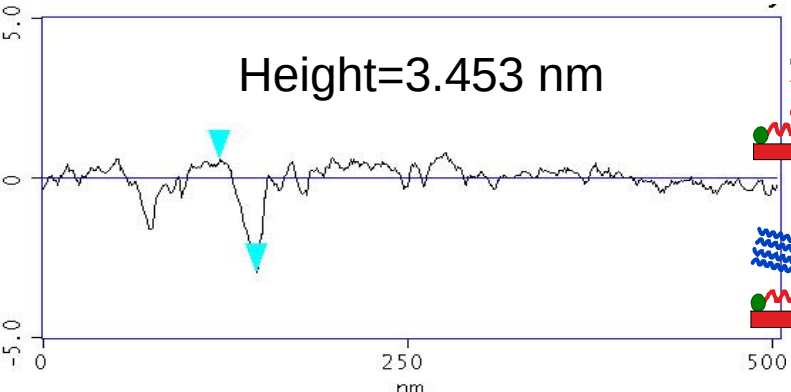
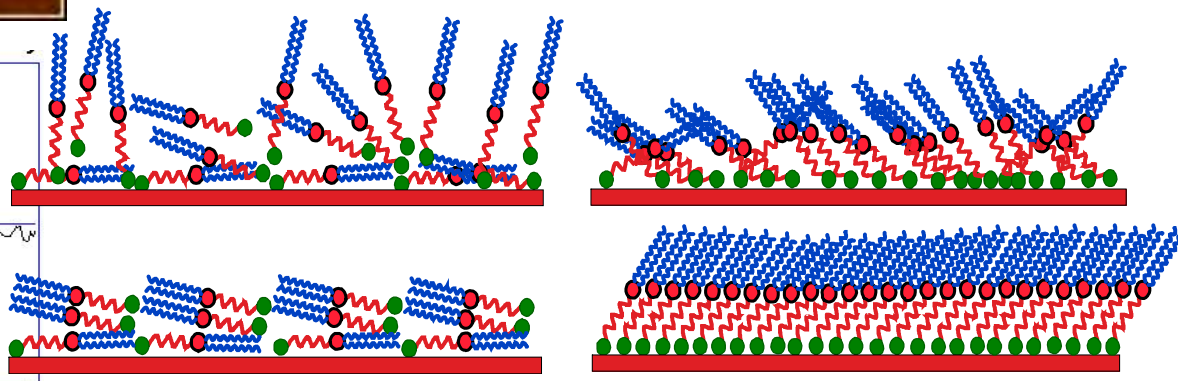


Figure 2. Section analysis of octanol film adsorbed on mica for 24 h. The depth of the two layers was estimated as 0.8 nm.





Lipid bilayer formation via vesicle fusion

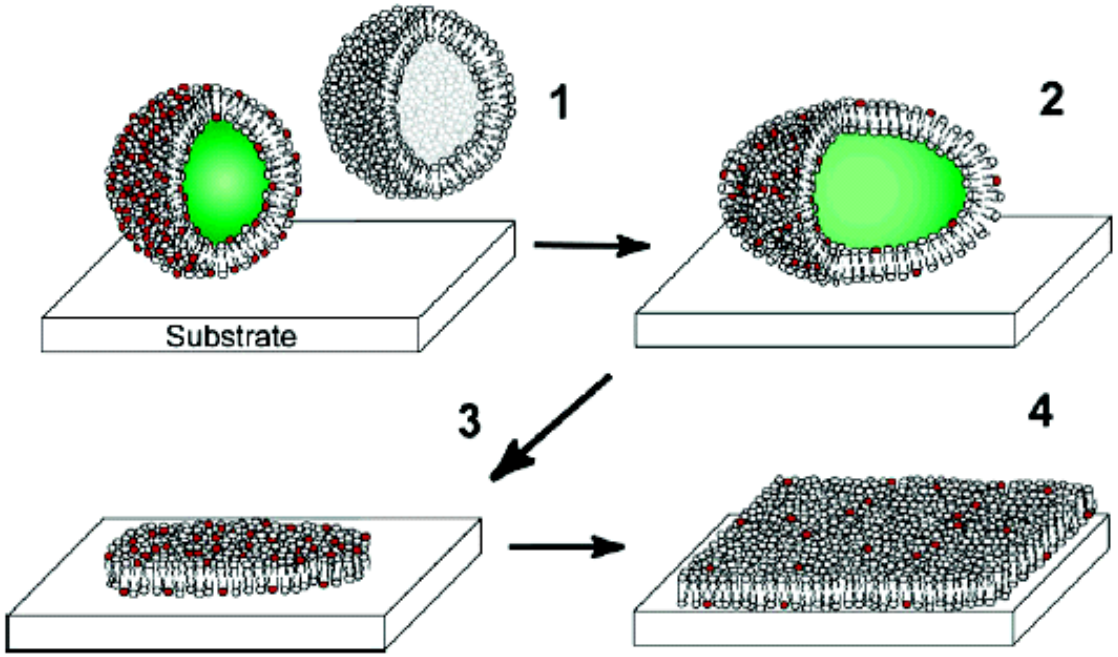


Figure 1. Four step scenario of supported bilayer formation via vesicle fusion comprising (1) vesicle adsorption, (2) fusion of vesicles at the surface to form larger vesicles, (3) rupture of the fused vesicles resulting in bilayer disks, and finally (4) merging of the disks. Here red lipids represent a leaflet fluorophore and green represents an entrapped aqueous dye (ref 1).

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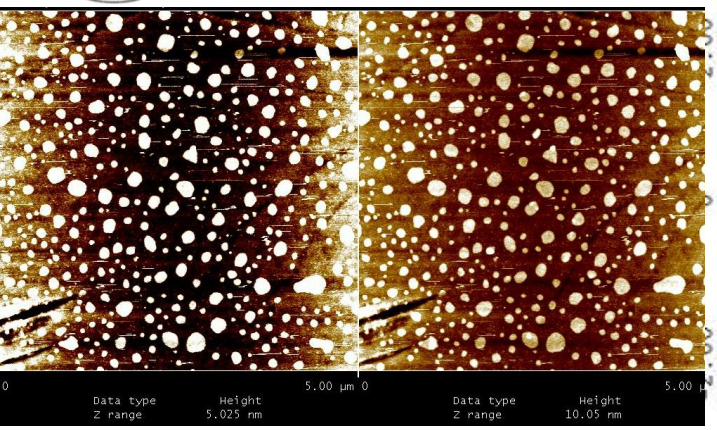
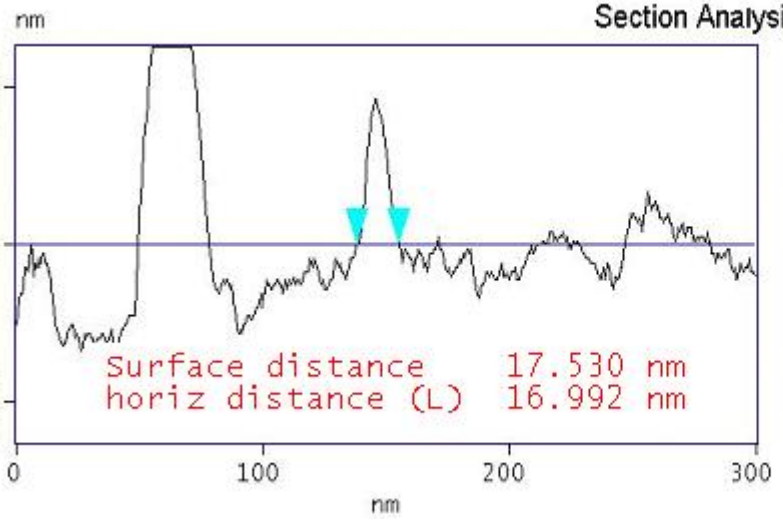
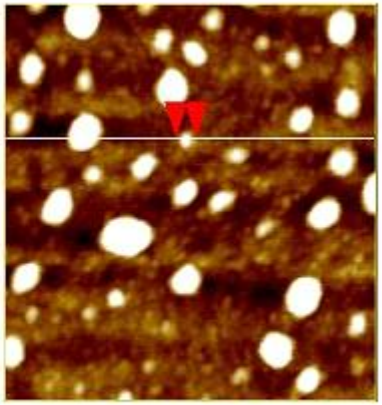
3 µg/ml solutions of DPhyPC vesicles insitu water 10 min .

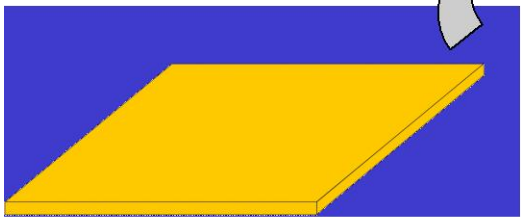




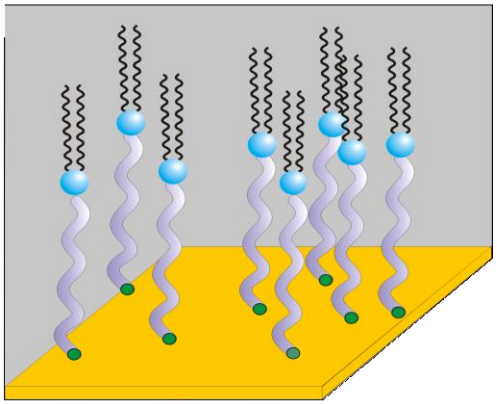


Vesicle fusion

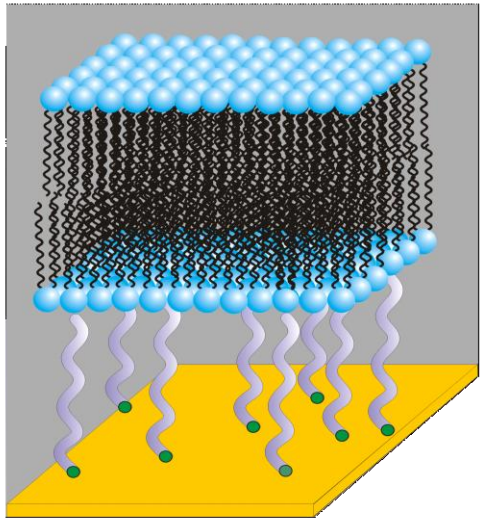
Self-assembly



Au Substrate

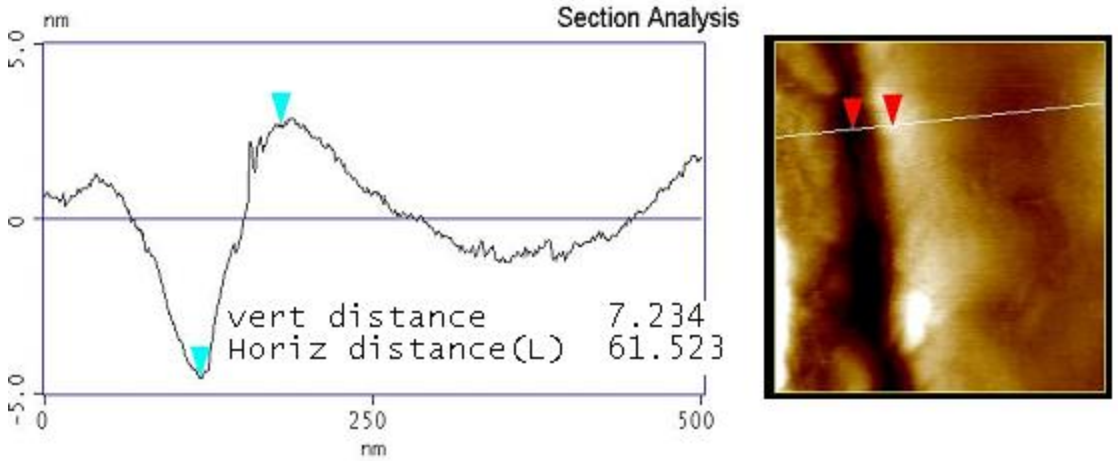


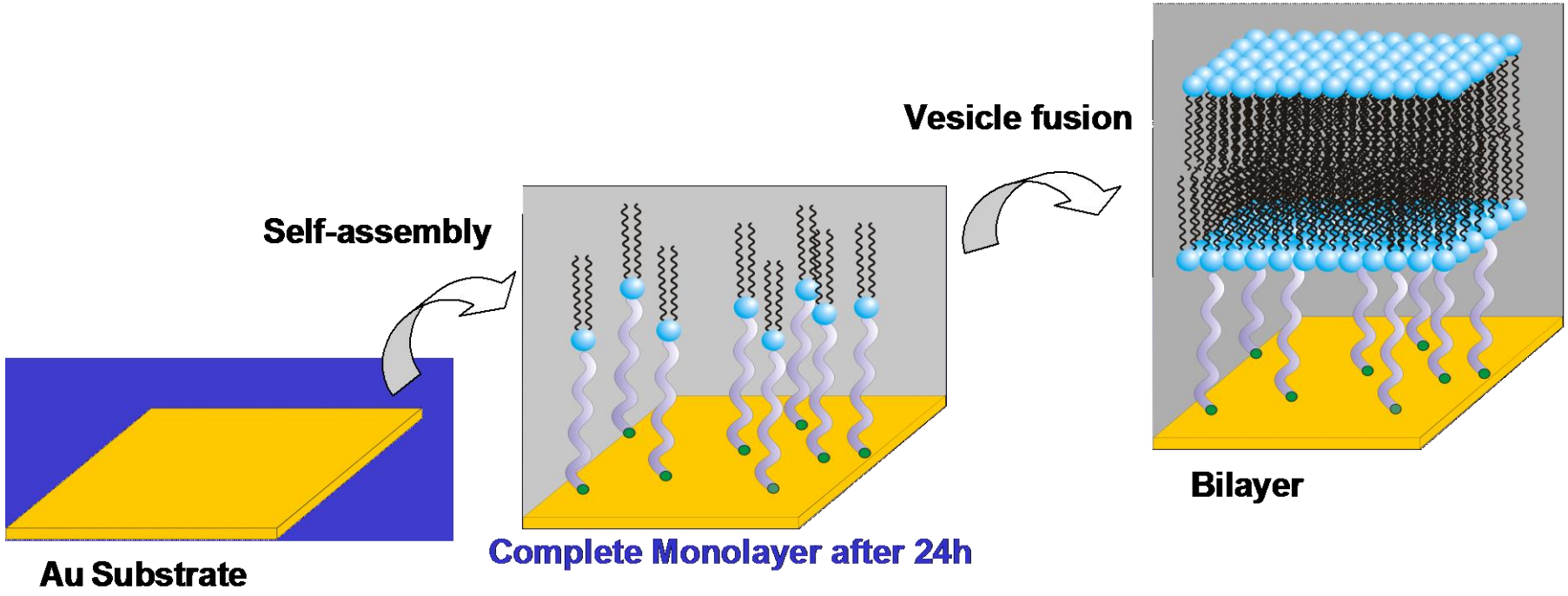
Complete Monolayer after 24h



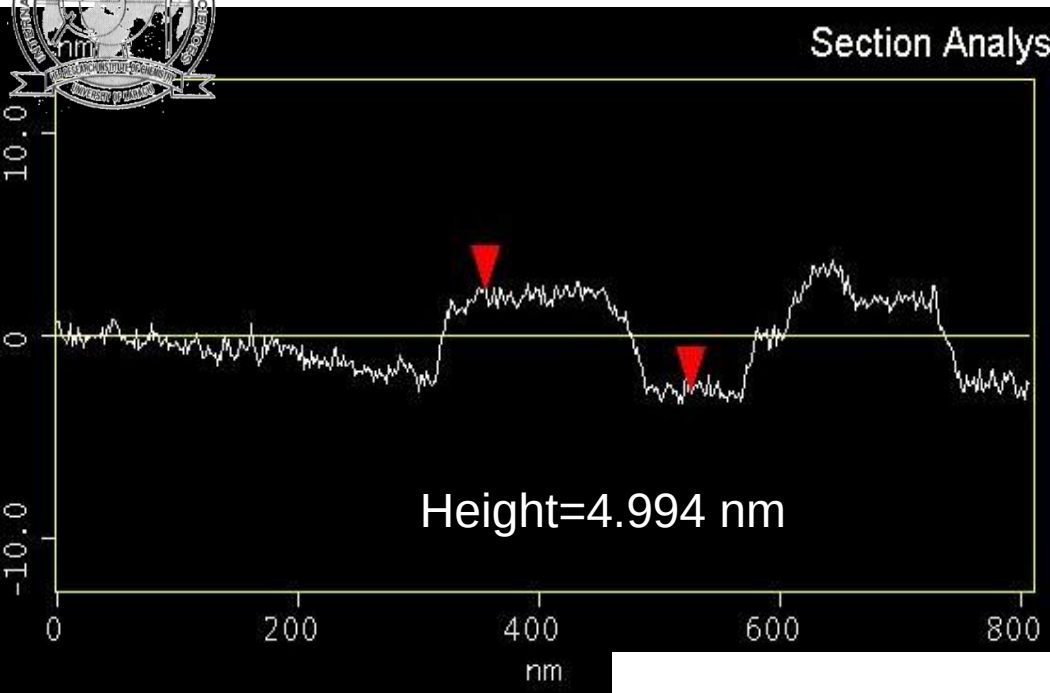
Bilayer

50 μg/ml solutions of DPhyPC vesicles. After 10 min of fusion.

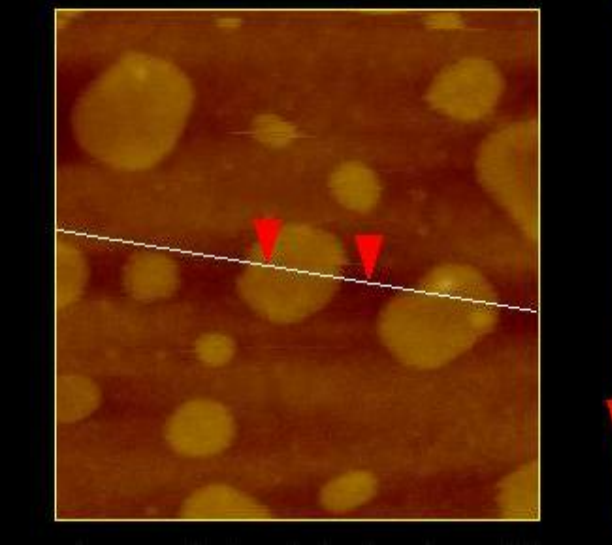


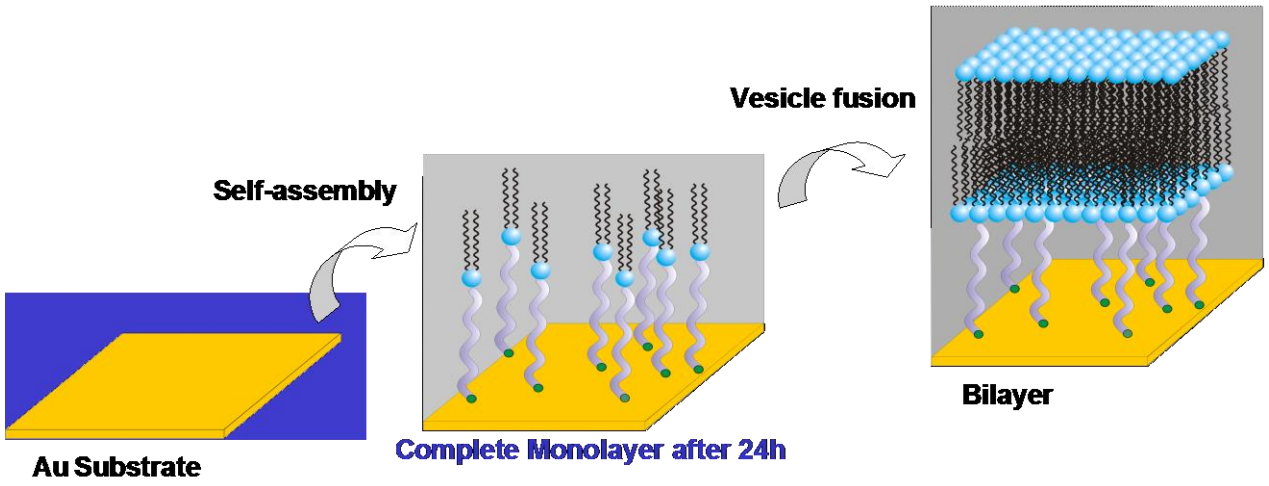


3 µg/ml solutions of DPhyPC vesicles after 10 minutes.

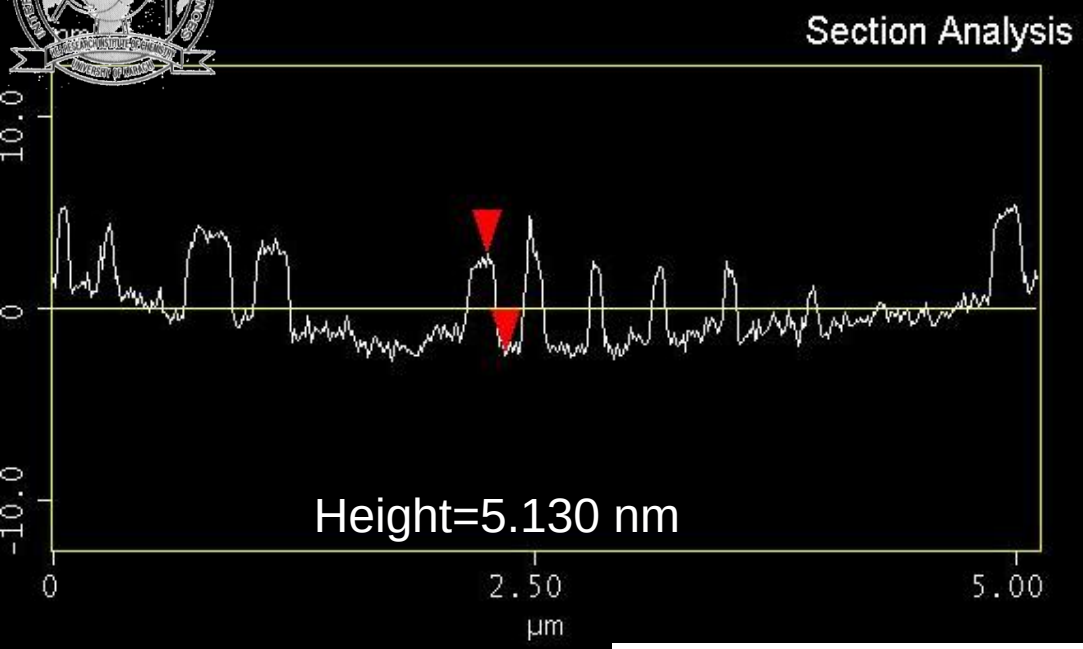


L	170.31 nm
RMS	1.899 nm
lc	DC
Ra(lc)	1.007 nm
Rmax	3.971 nm
Rz	1.625 nm
Rz Cnt	valid
Radius	771.07 nm
Sigma	0.864 nm

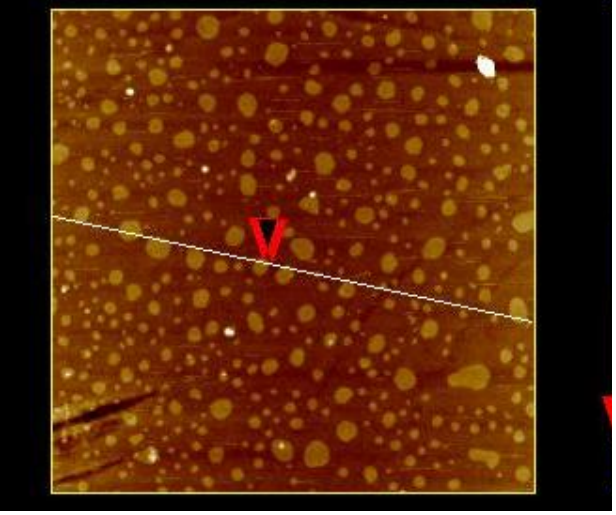


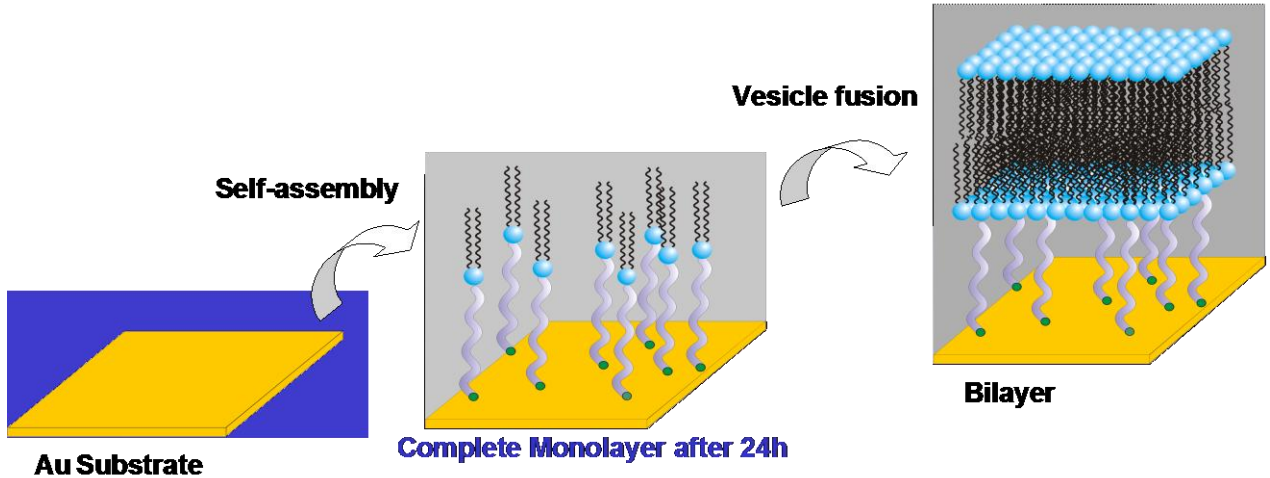


5 µm solutions of DPhyPC vesicles after 10 minutes.

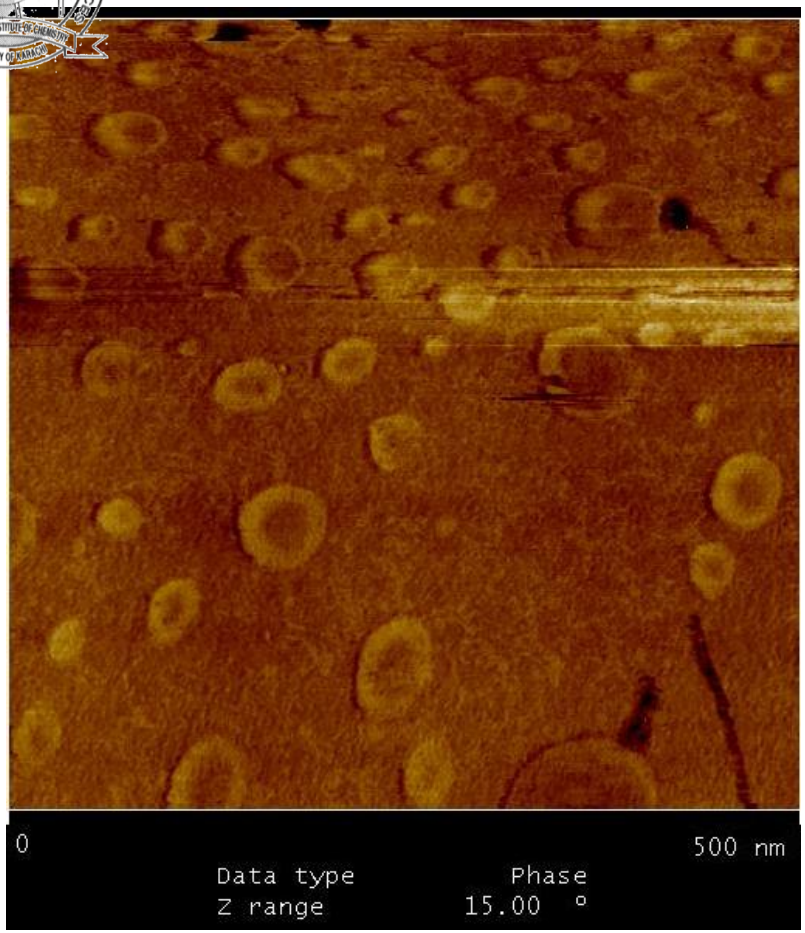


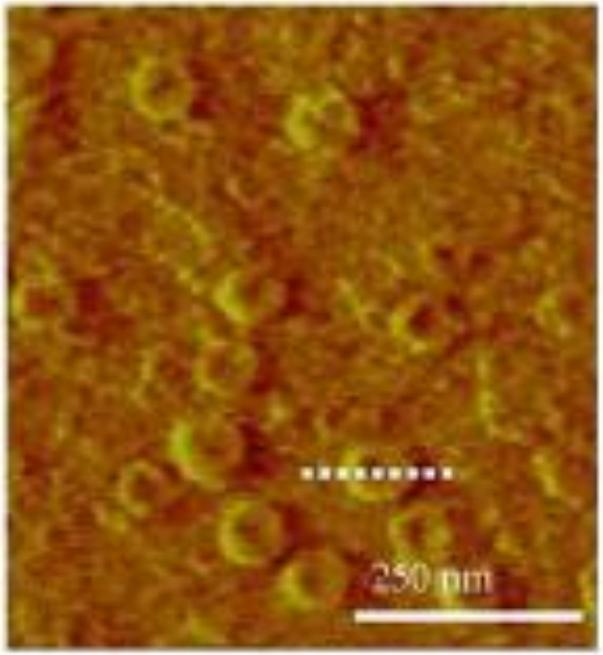
L	97.656 nm
RMS	1.909 nm
lc	DC
Ra(lc)	0.472 nm
Rmax	2.173 nm
Rz	1.147 nm
Rz Cnt	6
Radius	230.66 nm
Sigma	1.794 nm

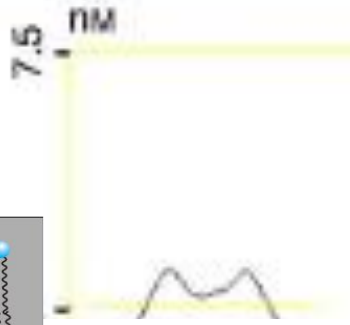




3 µg/ml solutions of DPhyPC vesicles after 10 minutes

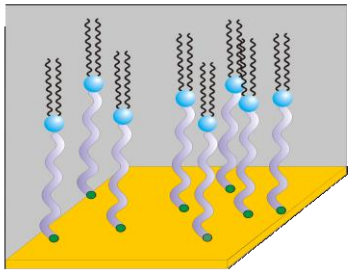


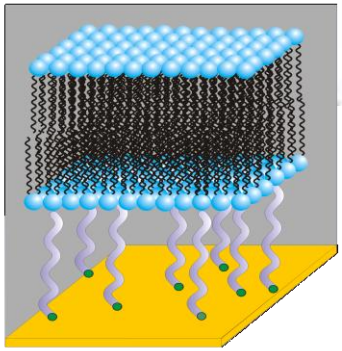




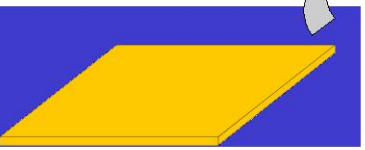
Vesicle fusion

Self-assembly





Bilayer



Au Substrate

Complete Monolayer after 24h

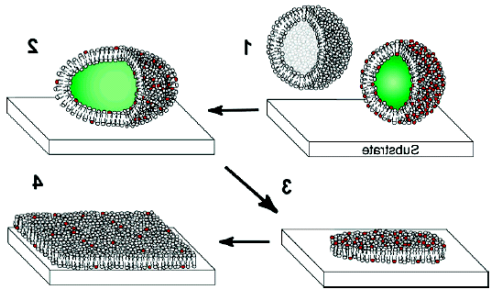
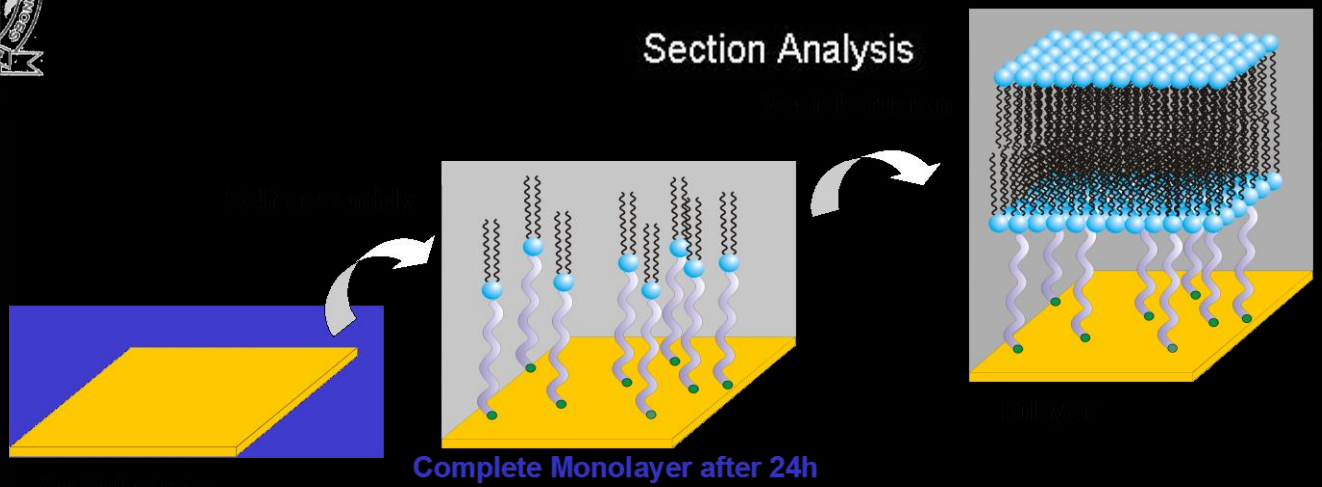
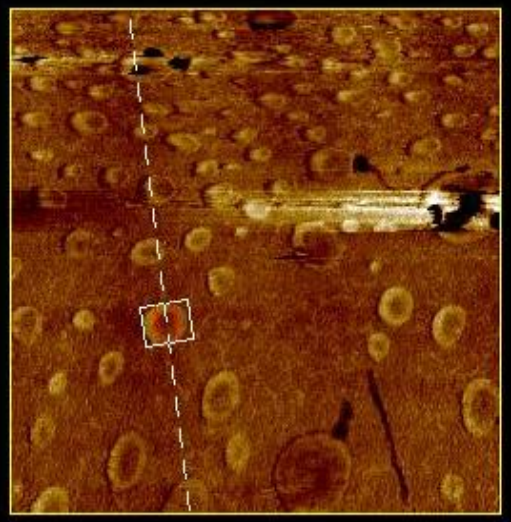


Figure 1. Four step scenario of supported bilayer formation via vesicle fusion comprising (1) vesicle adsorption, (2) fusion of vesicles at the surface to form larger vesicles, (3) rupture of the fused vesicles resulting in bilayer disks, and finally (4) merging of the disks. Here red lipids represent a lectin fluorophore and green represents an entrapped aqueous dye (ref 1).

3 µg/ml solutions of DPhyPC vesicles after 20 minutes.



L	56.02 nm
RMS	0.107 °
lc	DC
Ra(lc)	0.069 °
Rmax	0.363 °
Rz	0.253 °
Rz Cnt	6
Radius	24.065
Sigma	10.808



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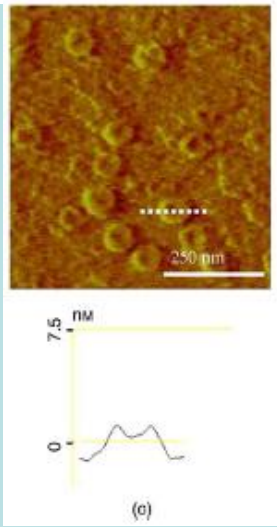
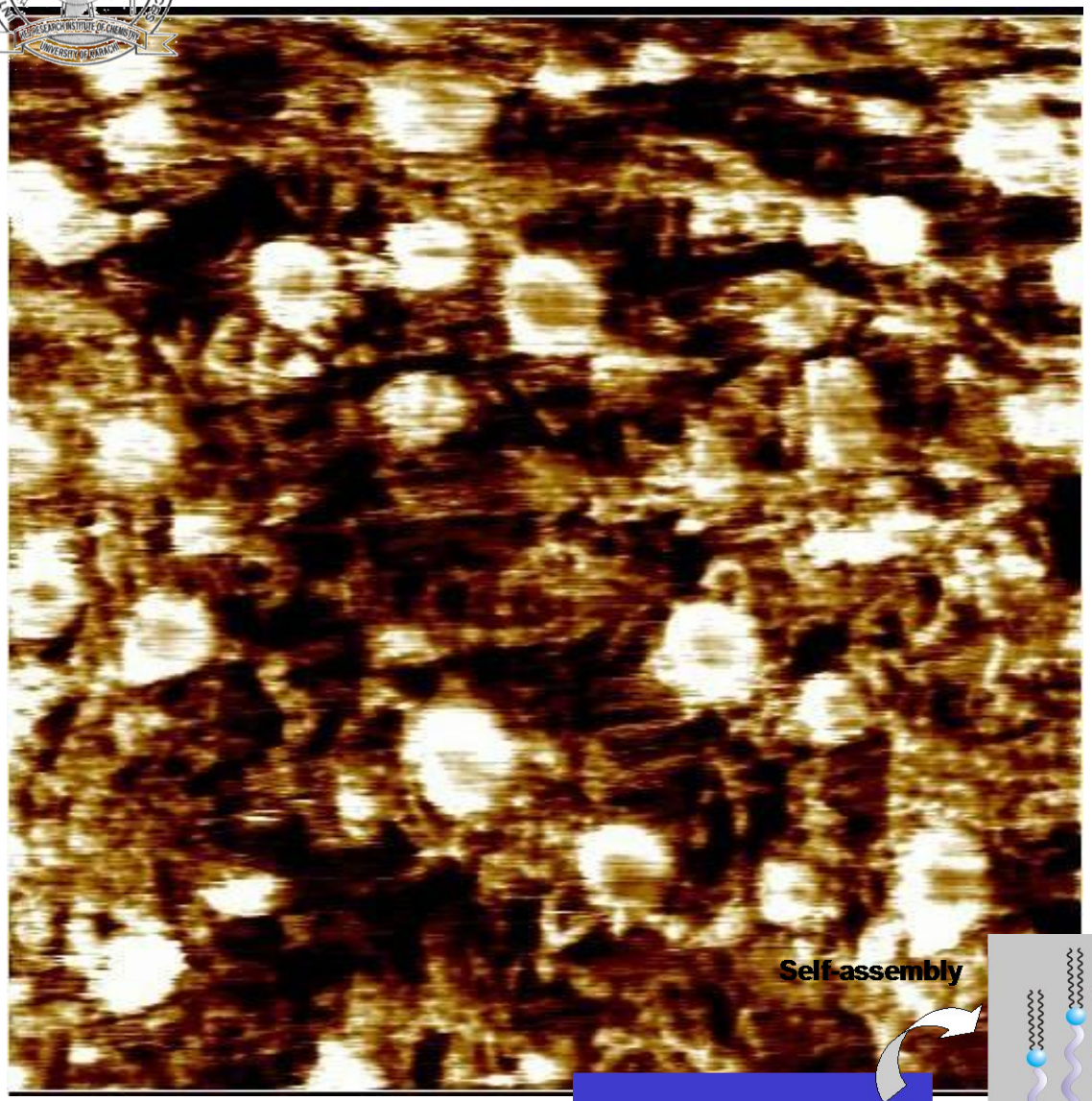
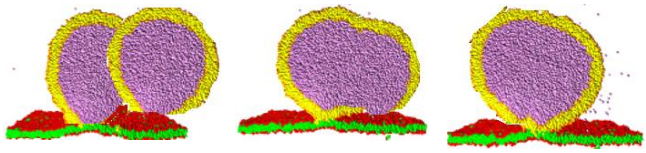


Surface distance	72.26
Horiz distance(L)	66.02 nm
Vert distance	0.126 °
Angle	
Surface distance	
Horiz distance	
Vert distance	
Angle	
Surface distance	
Horiz distance	
Vert distance	
Angle	
Spectral period	DC
Spectral freq	0 /µm

3 μg/ml solutions of DPhyPC vesicles after 1hour.



Formation of Giant Vesicle



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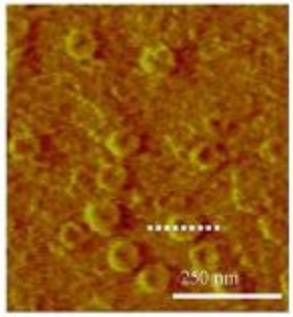
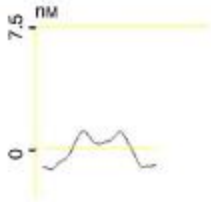
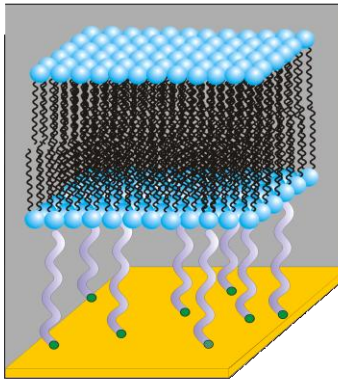
(c) of EggPC vesicles. The images were captured in pure water and with initial





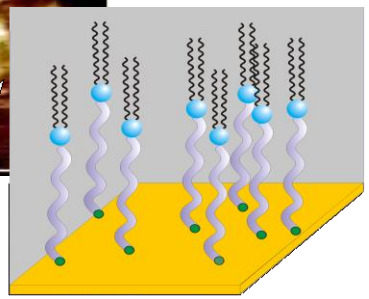
(c) **Vesicle fusion**



Bilayer

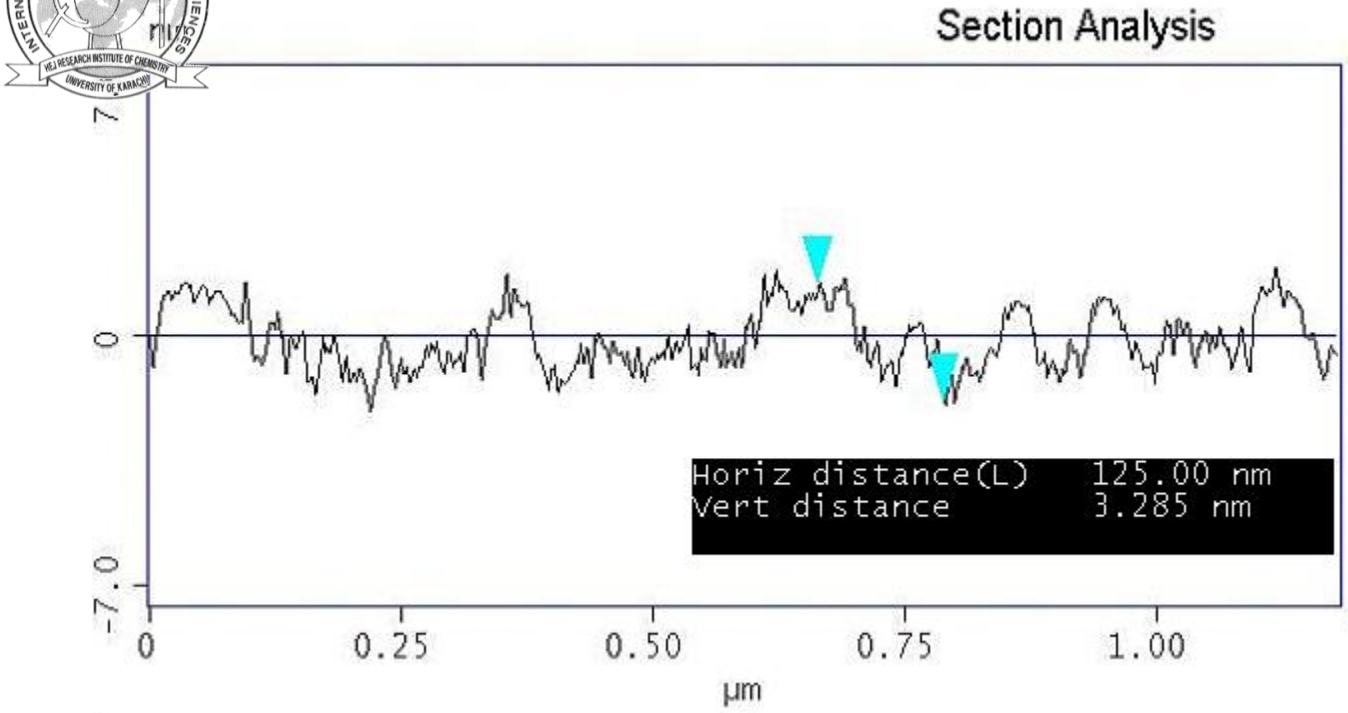
Self-assembly

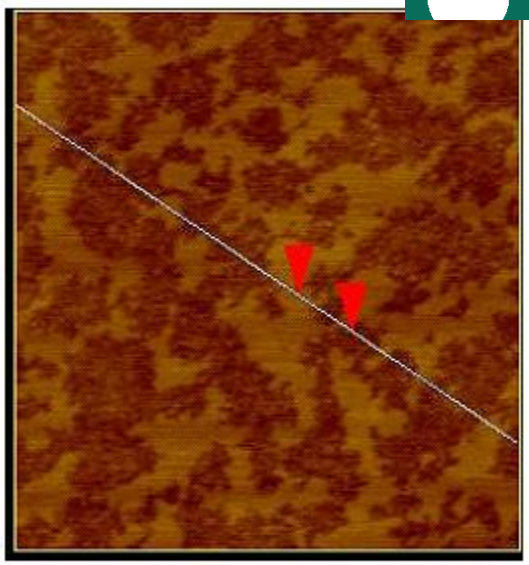
Au Substrate



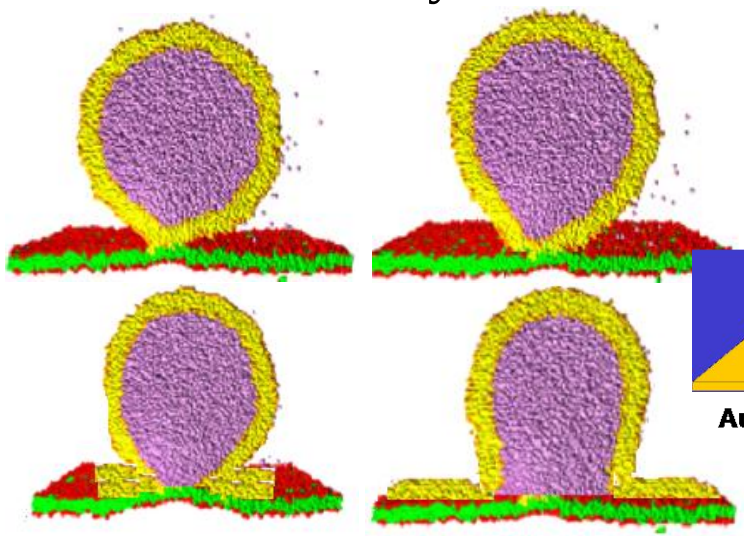
Complete Monolayer after 24h

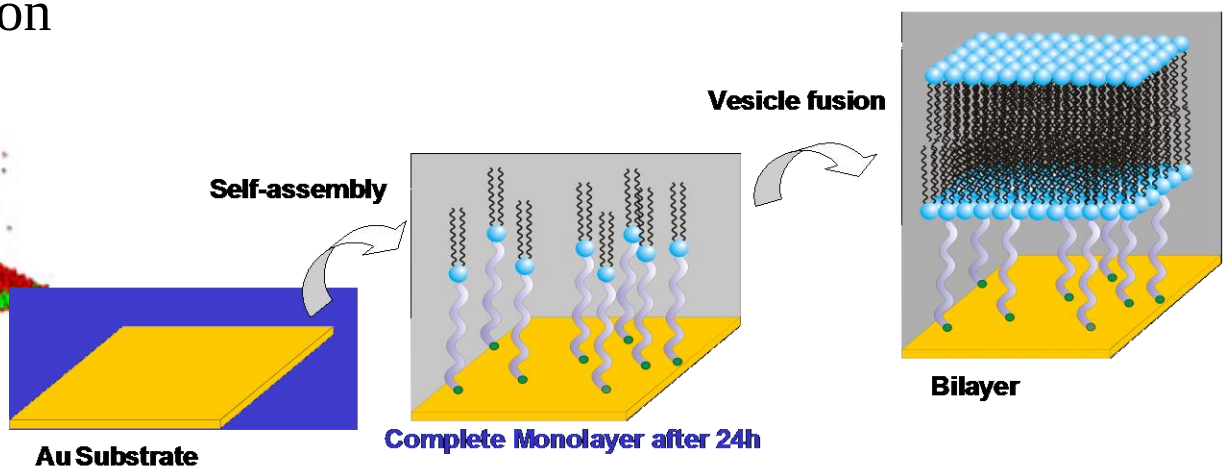
3 µg/ml solutions of DPhyPC vesicles after 2.5hours.



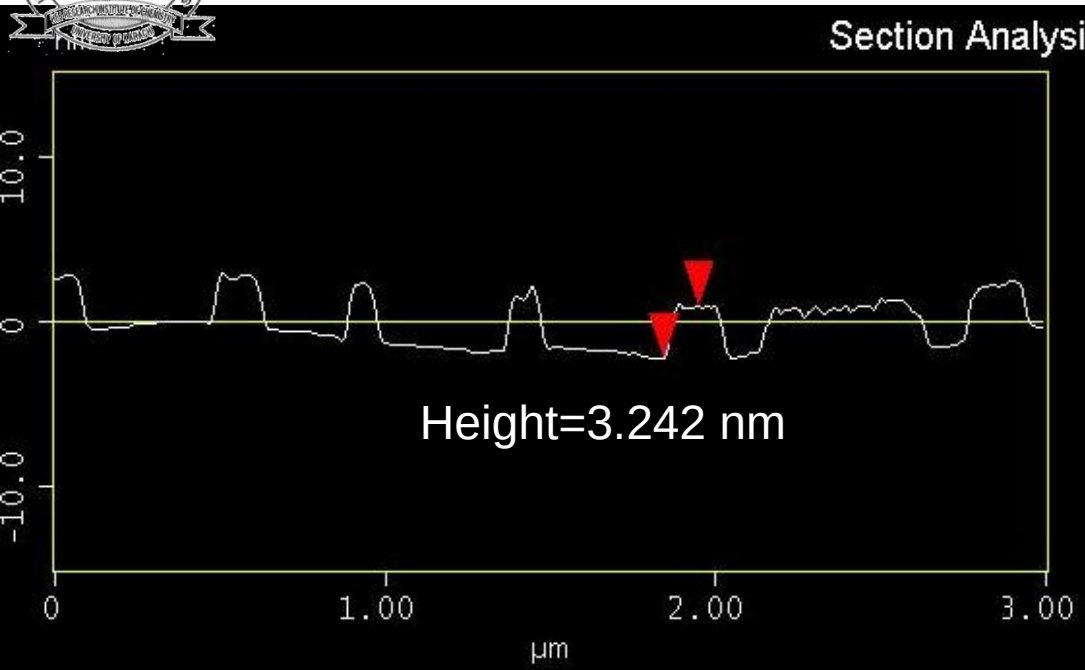


Mechanism of Bilayer formation





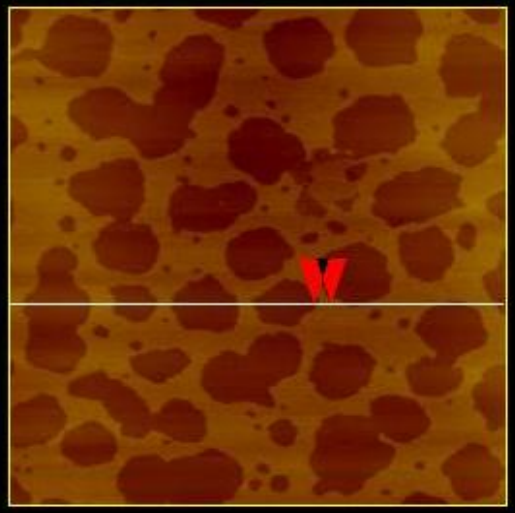
3 µg/ml solutions of DPhyPC vesicles after 3 hours.

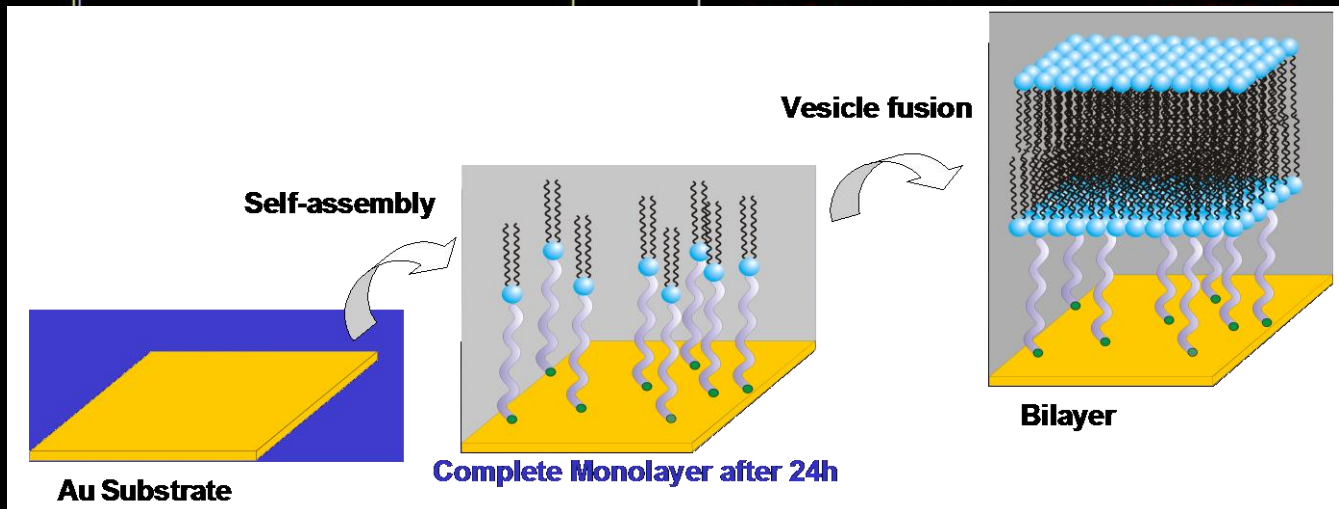


L	105.47 nm
RMS	1.075 nm
lc	DC
Ra(lc)	0.566 nm
Rmax	2.280 nm
Rz	2.280 nm
Rz Cnt	2
Radius	577.69 nm
Sigma	0.480 nm

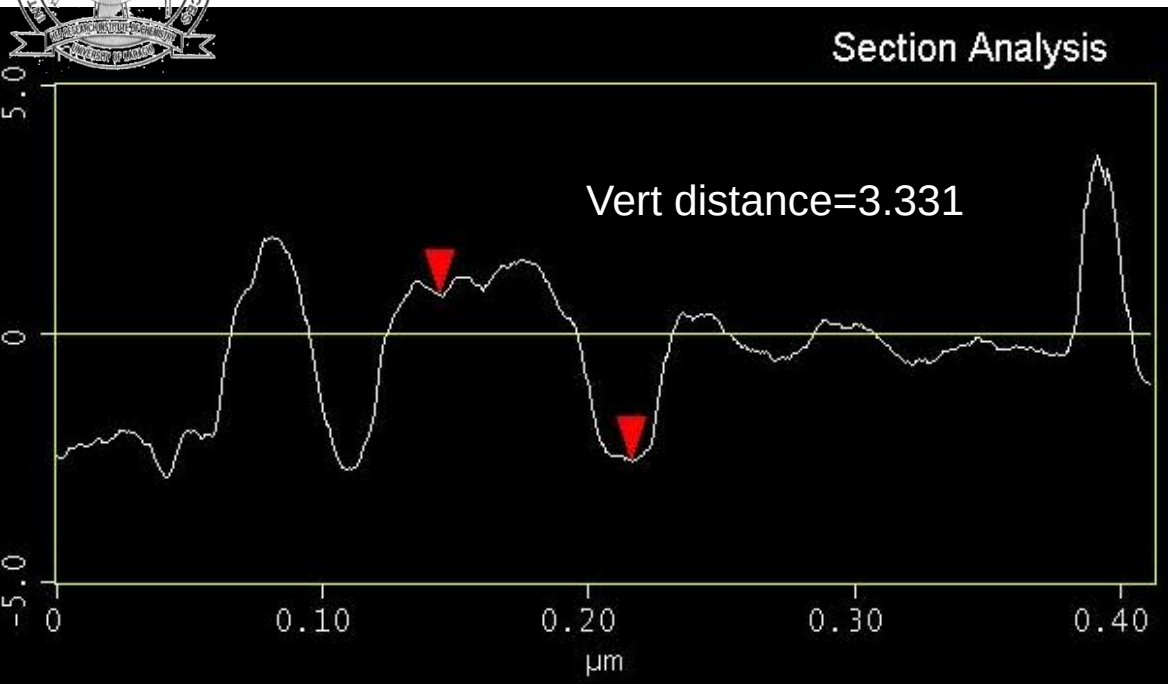
Surface distance	105.66 nm
Horiz distance(L)	105.47 nm
Vert distance	3.242 nm

Spectrum





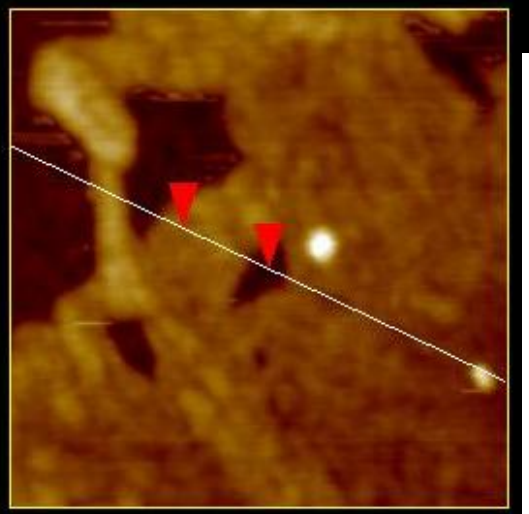
3 µg/ml solutions of DPhyPC vesicles after 6hours.

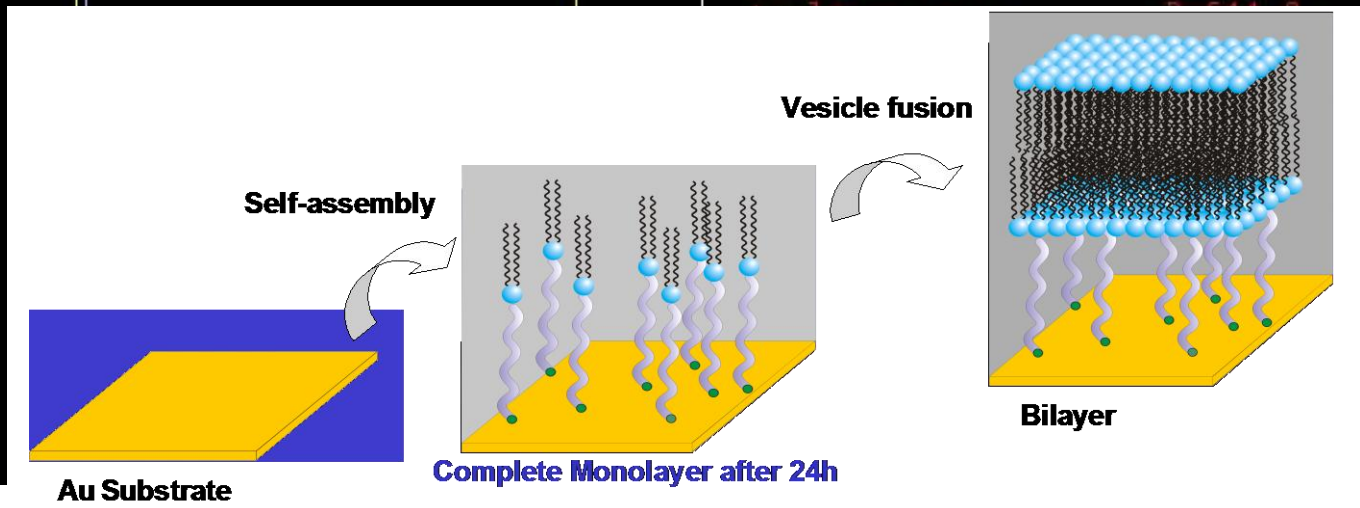


L	72.132 nm
RMS	1.425 nm
lc	DC
Ra(lc)	0.754 nm
Rmax	2.615 nm
Rz	2.615 nm
Rz Cnt	2
Radius	221.35 nm
Sigma	0.363 nm

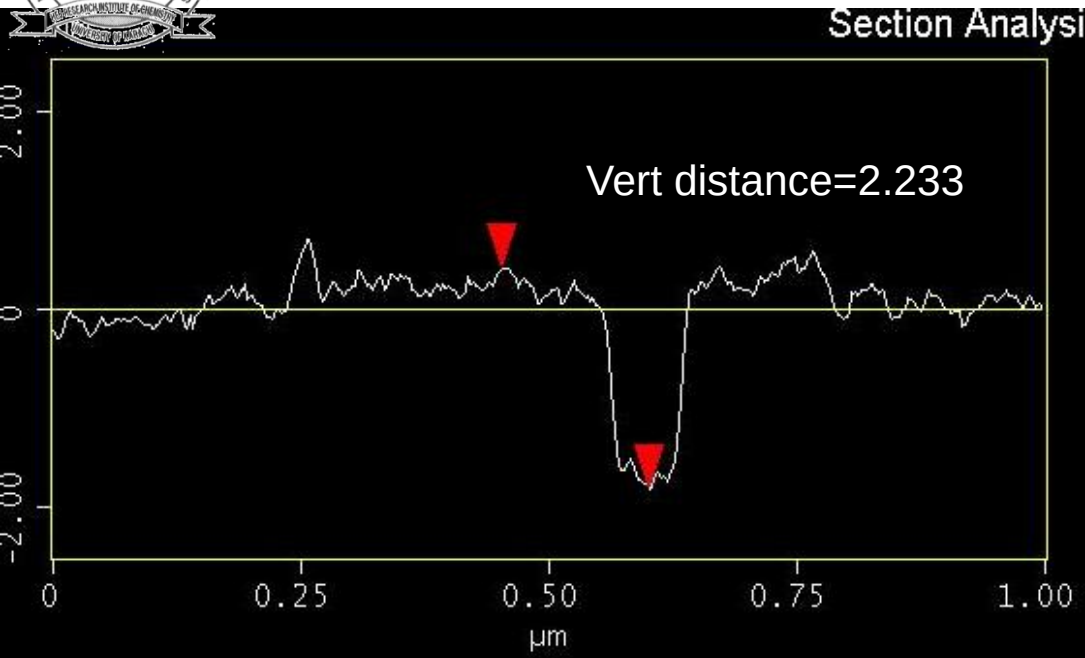
Surface distance	72.601 nm
Horiz distance(L)	72.132 nm
Vert distance	3.331 nm

Spectrum





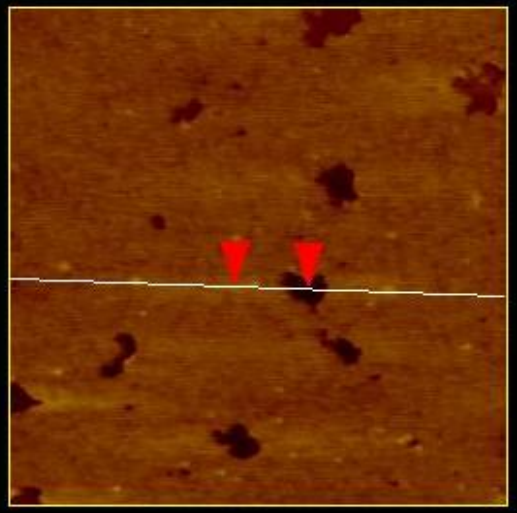
3 µg/ml solutions of DPhyPC vesicles after 8hours.

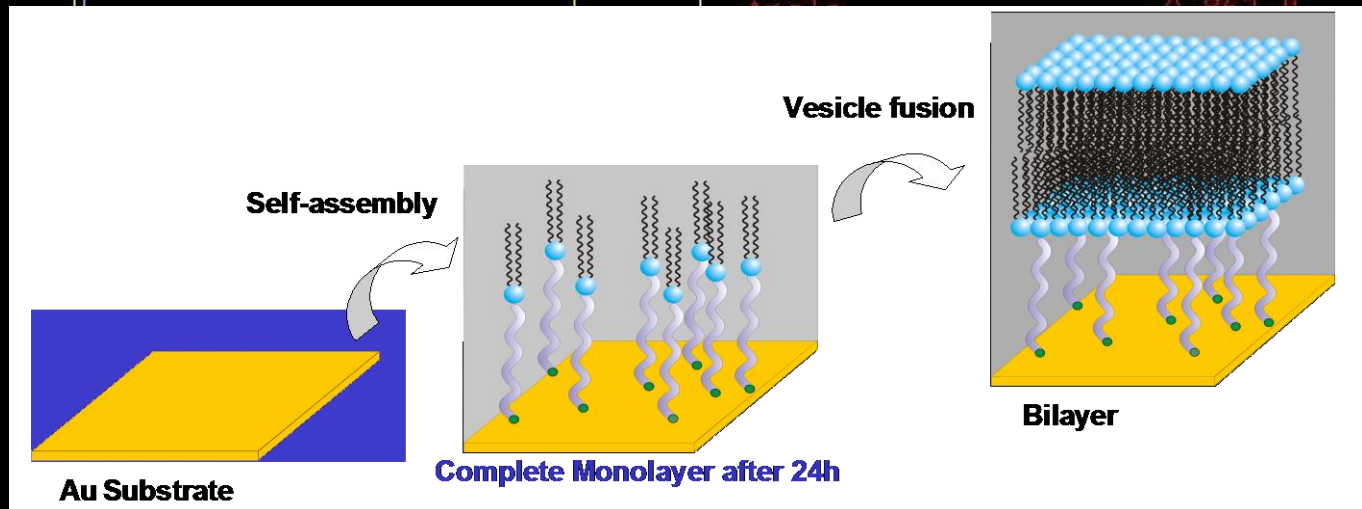


L	148.44 nm
RMS	0.775 nm
lc	DC
Ra(lc)	0.368 nm
Rmax	1.315 nm
Rz	1.315 nm
Rz Cnt	2
Radius	1.597 µm
Sigma	0.330 nm

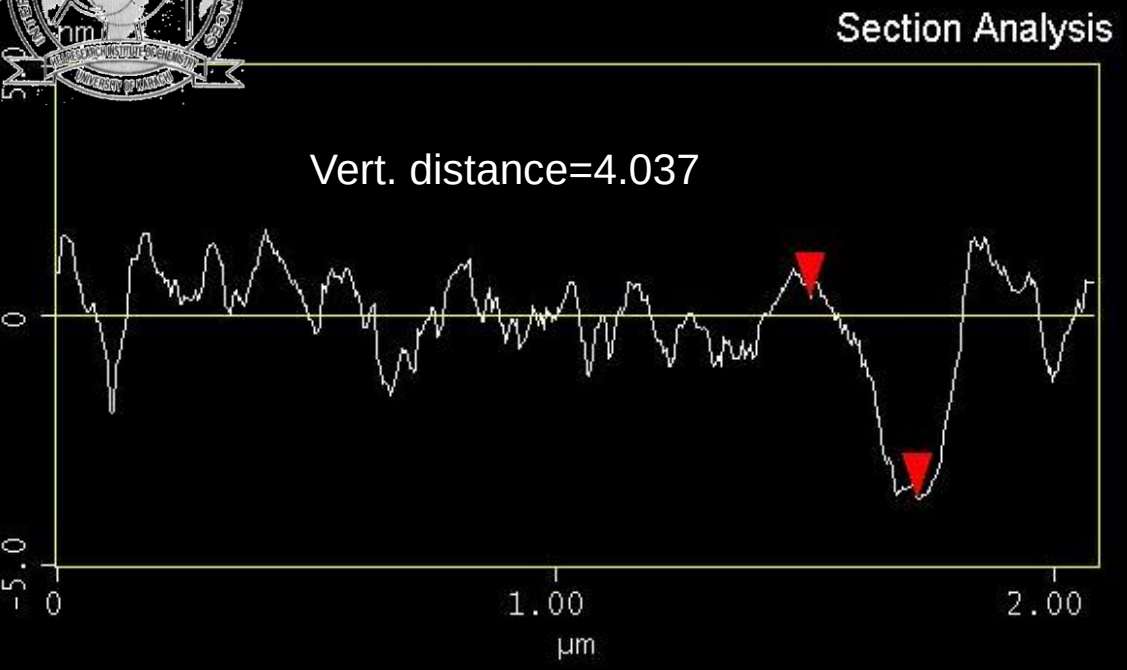
Surface distance	148.55 nm
Horiz distance(L)	148.44 nm
Vert distance	2.233 nm

Spectrum



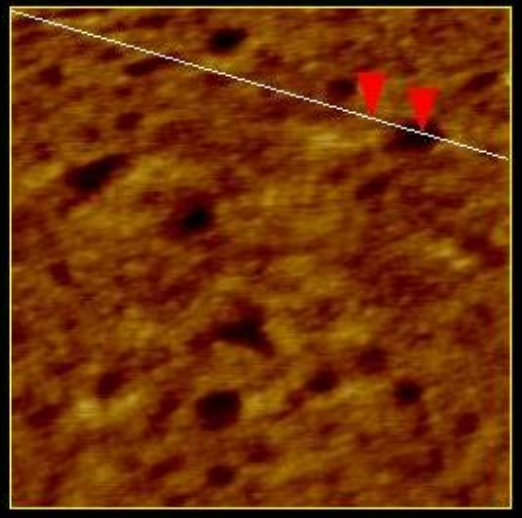


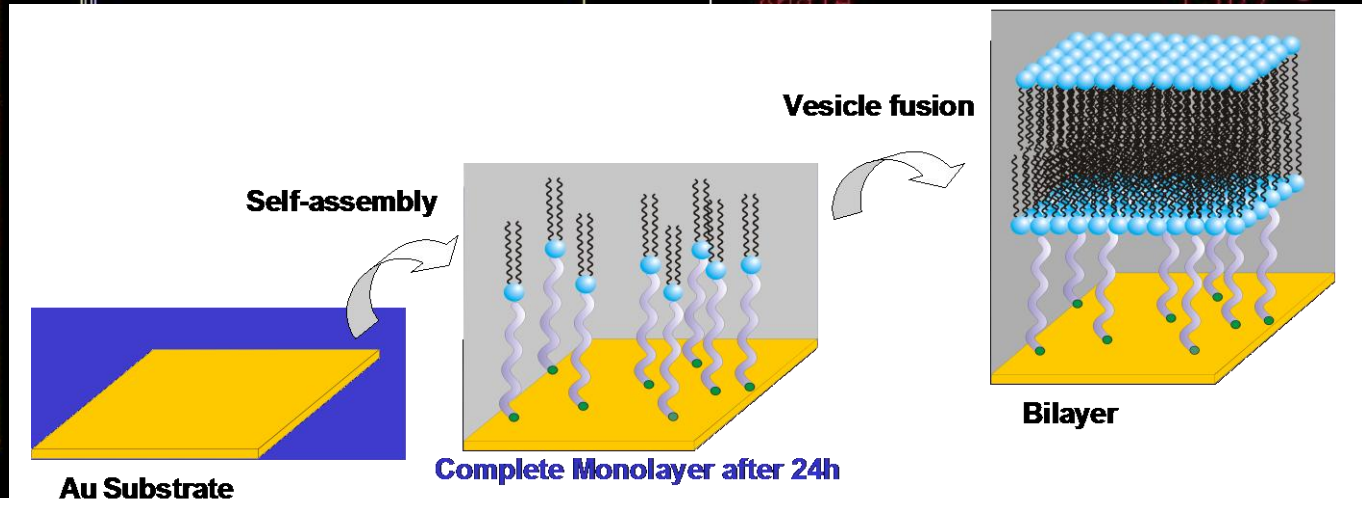
3 µg/ml solutions of DPhyPC vesicles after 9hours.



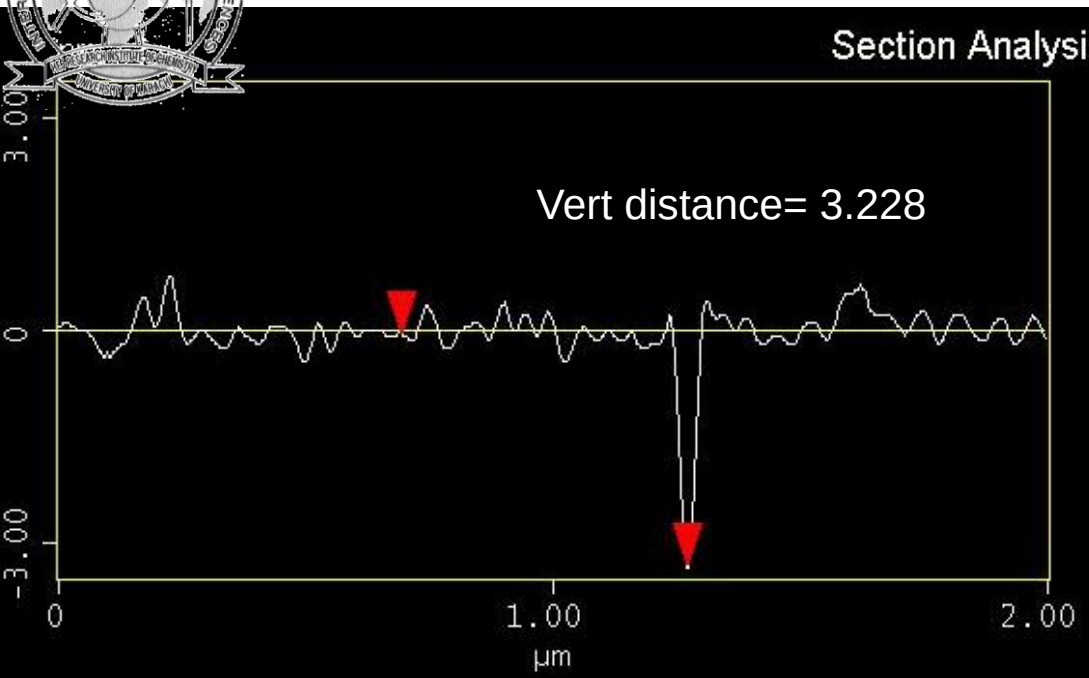
L	214.84 nm
RMS	1.475 nm
lc	DC
Ra(lc)	0.277 nm
Rmax	1.363 nm
Rz	0.577 nm
Rz Cnt	valid
Radius	1.938 µm
Sigma	0.860 nm

Surface distance	215.08 nm
Horiz distance(L)	214.84 nm
Vert distance	4.037 nm





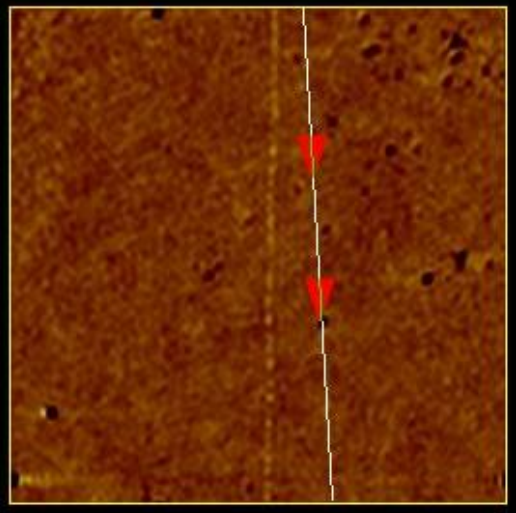
3 µg/ml solutions of DPhyPC vesicles after 12hours.

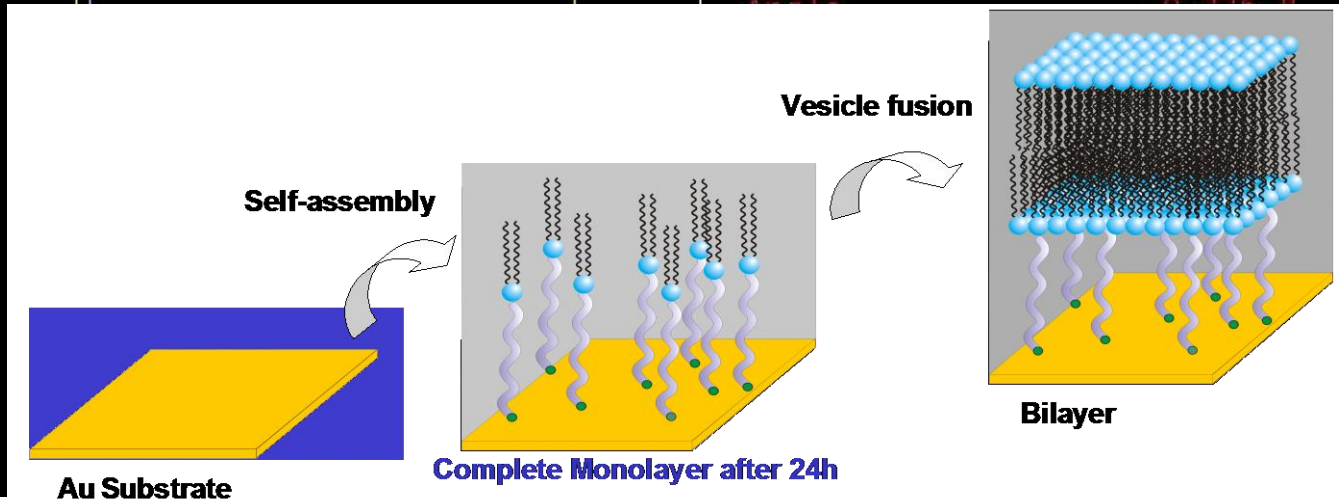


L	578.13 nm
RMS	0.524 nm
lc	DC
Ra(lc)	0.265 nm
Rmax	3.467 nm
Rz	1.255 nm
Rz Cnt	8
Radius	11.239 µm
Sigma	0.999 nm

Surface distance	578.41 nm
Horiz distance(L)	578.13 nm
Vert distance	3.288 nm

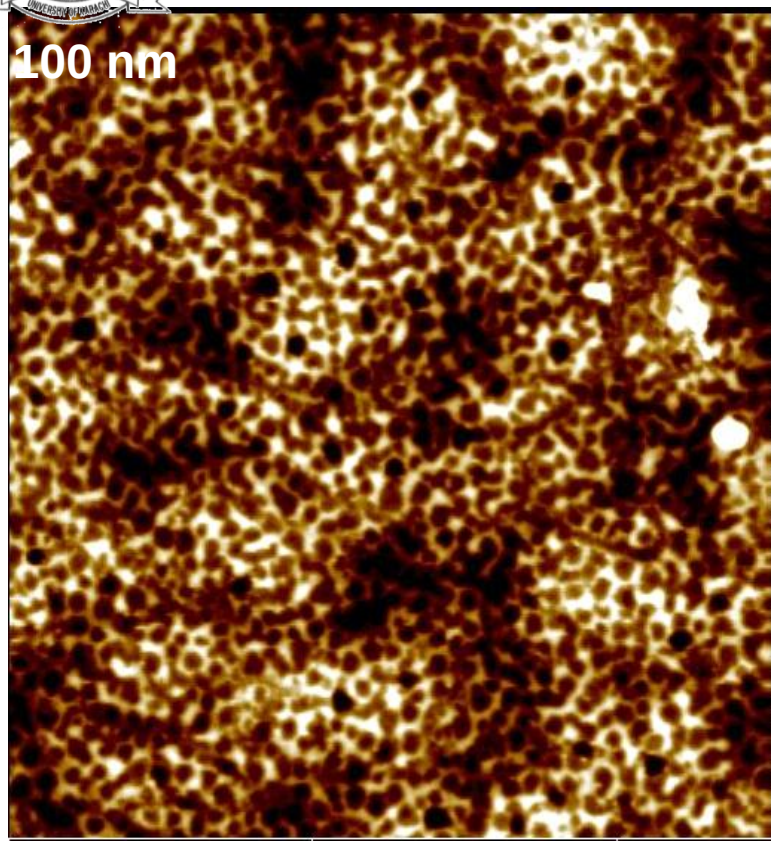
Spectrum

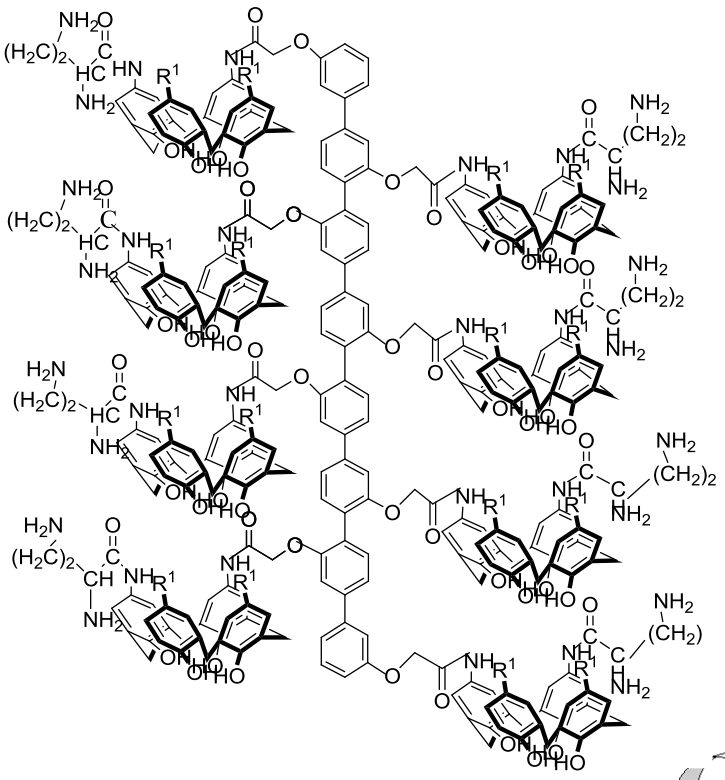


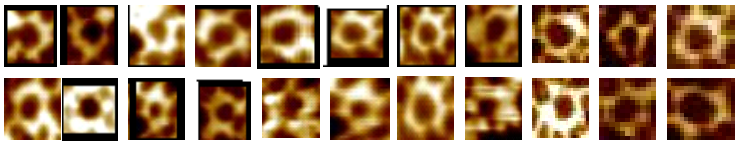


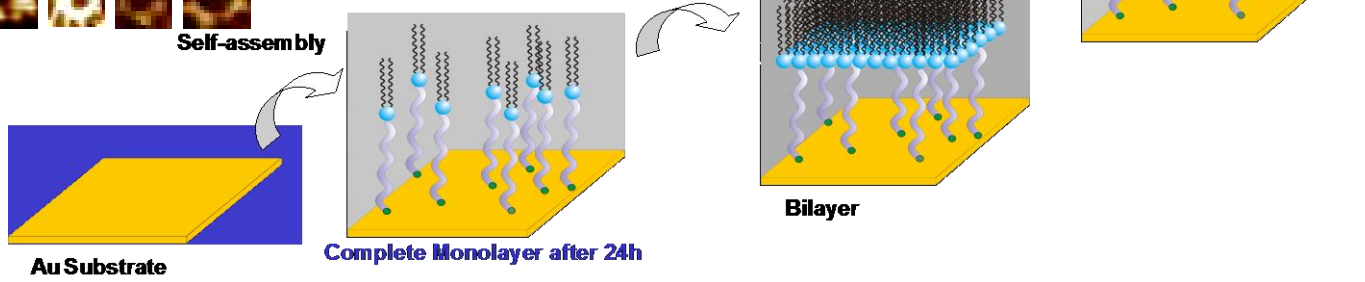
0.005µg/µl solution of Calixarene pore after 08 hours

Taking 02 µl solution from a stock of 0.5µg/µl and adding it to 400 µL

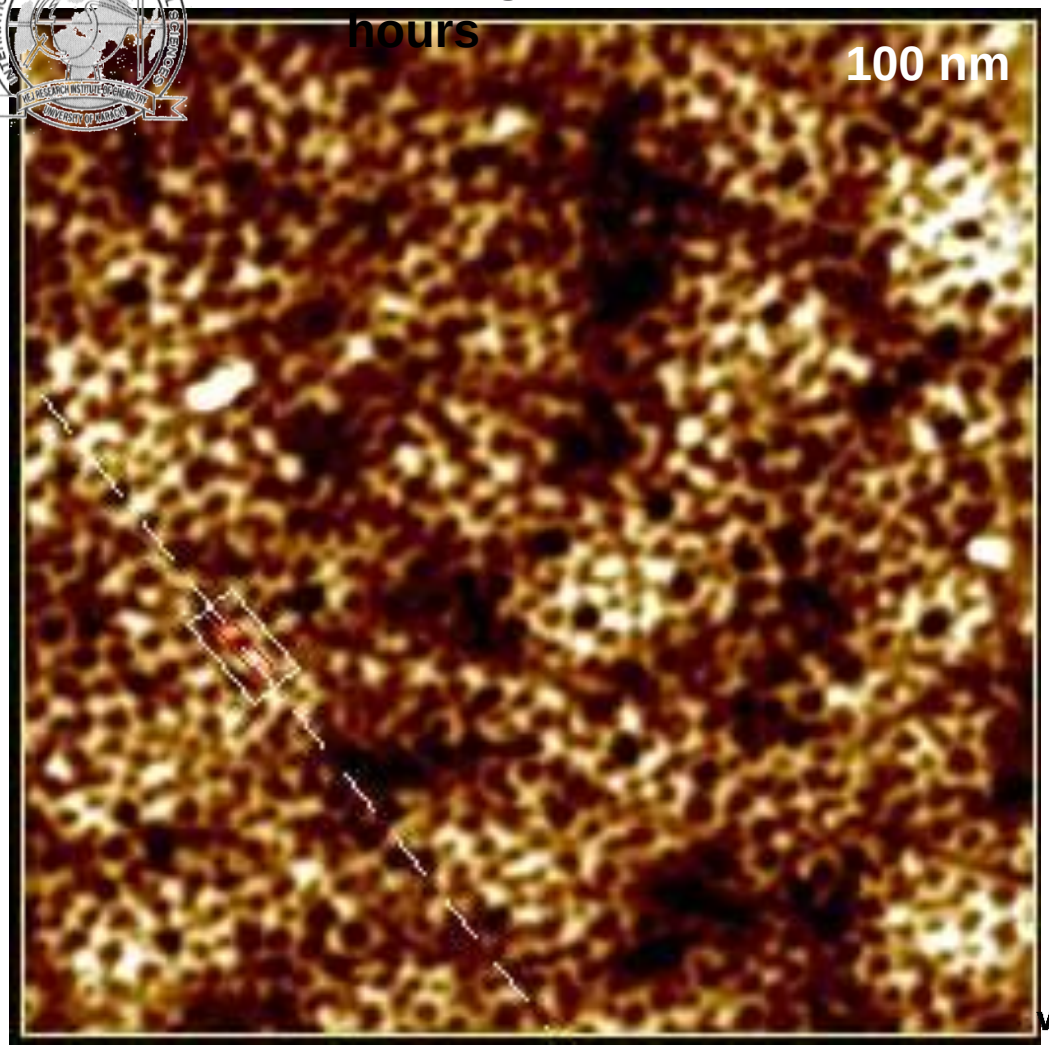


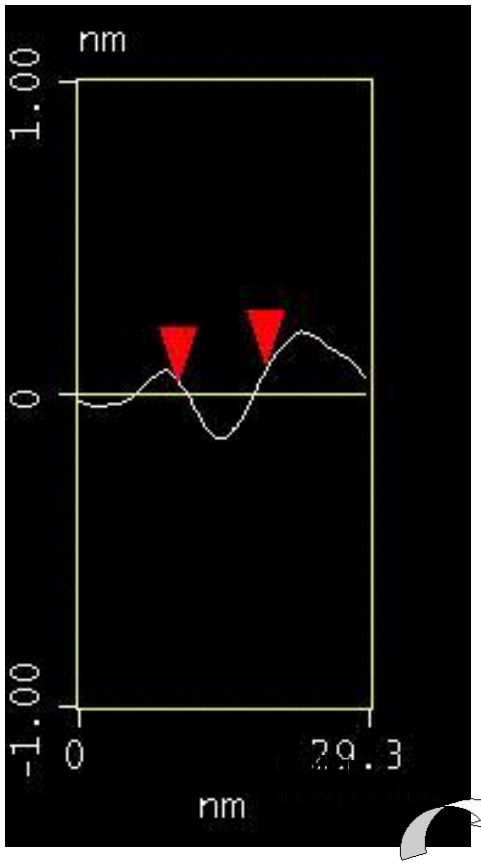




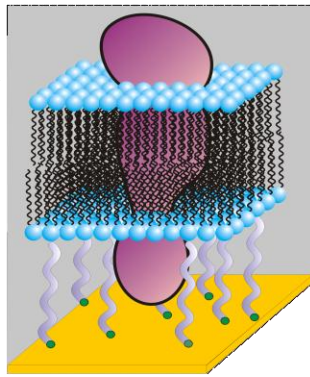


0.005µg/µl solution of Calixarene pore after 08 hours

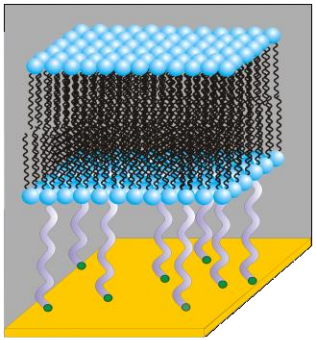




External diameter=8.858 nm

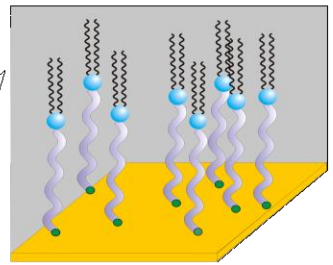


Vesicle fusion



Bilayer

Self-assembly



Complete Monolayer after 24h

Au Substrate

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