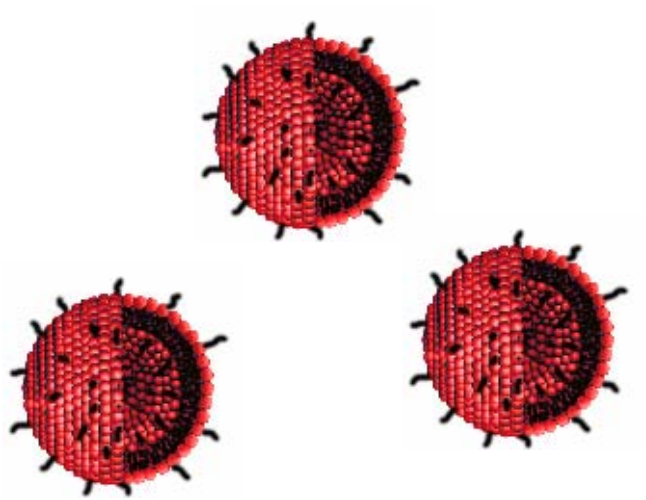
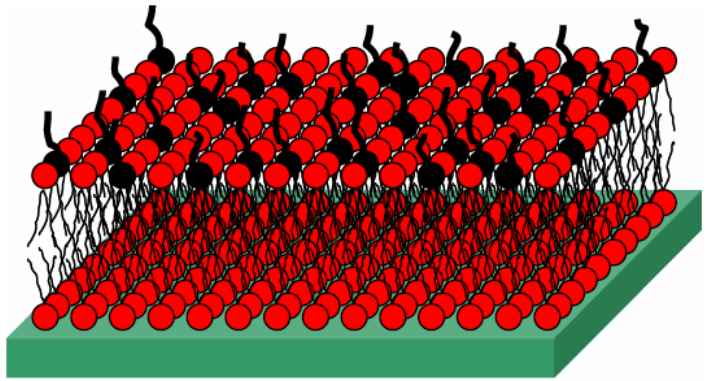
Development of Biomimetic Surfaces by Vesicle Fusion

Dimitris Stroumpoulis





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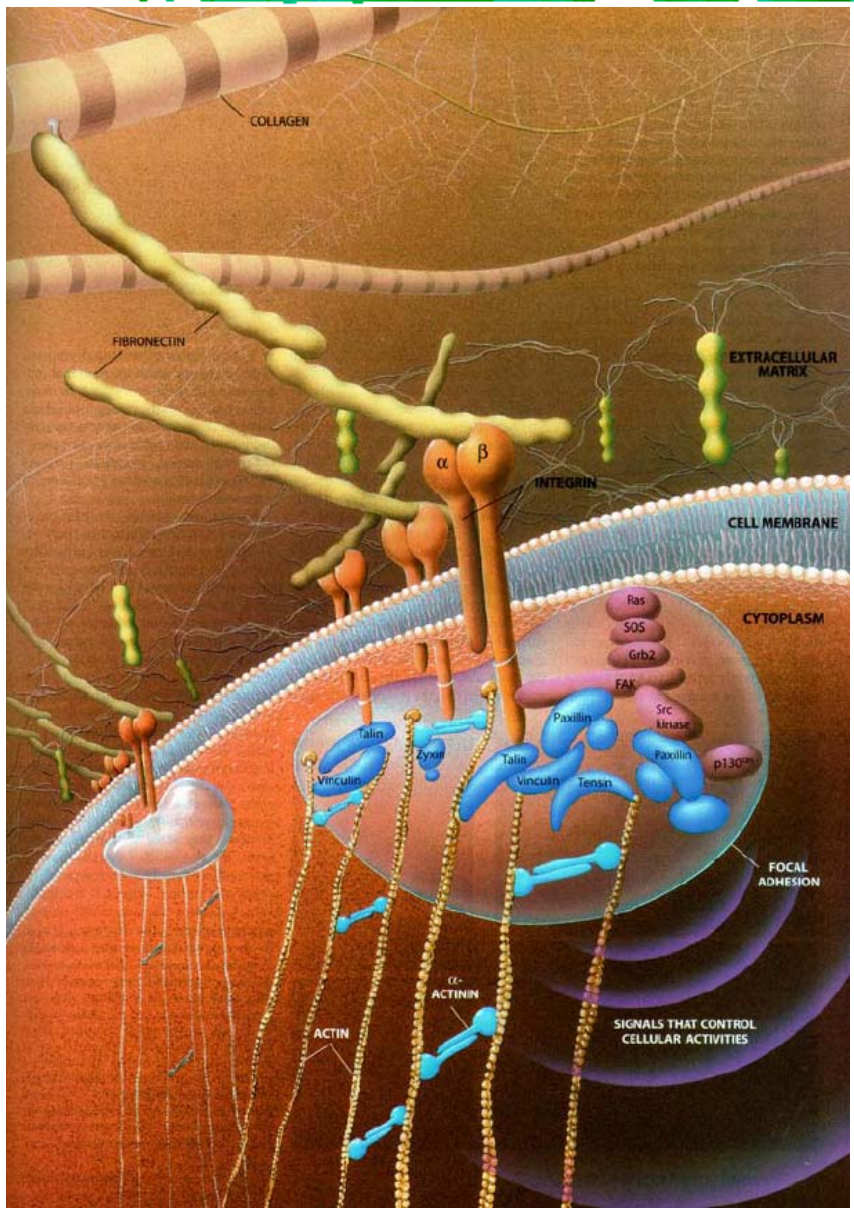
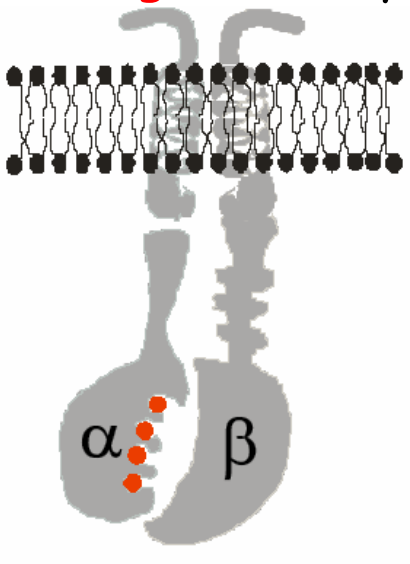
Introduction



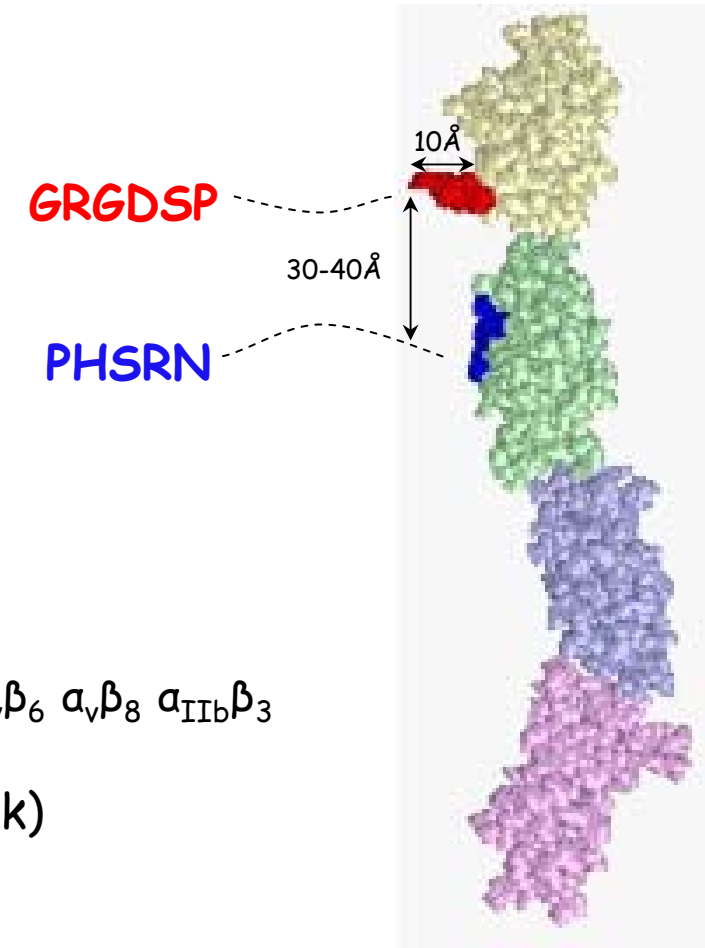
A.F. Horwitz, Scientific American, May 1997.

Fibronectin – Integrin System

Cell Surface Receptor
Integrin family:



Adhesive Ligand:



Integrins Binding RGD

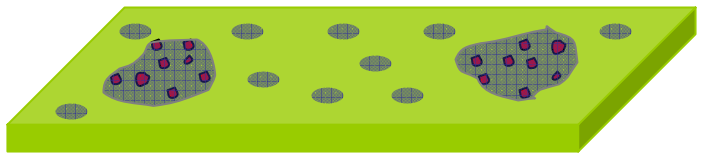
- $\alpha_5\beta_1$ $\alpha_8\beta_1$ $\alpha_v\beta_1$ $\alpha_v\beta_3$ $\alpha_v\beta_5$ $\alpha_v\beta_6$ $\alpha_v\beta_8$ $\alpha_{IIb}\beta_3$
- $\alpha_2\beta_1$ $\alpha_3\beta_1$ $\alpha_4\beta_1$ $\alpha_7\beta_1$ (weak)

Image adapted from Leahy, D.L. et al., 1996, *Cell* 84:155

Motivation

Intelligent Biomaterials:

- Minimize non-specific interactions with ECM
- Allow selective interaction with cells
  - Mimic ECM (Biomimetics)



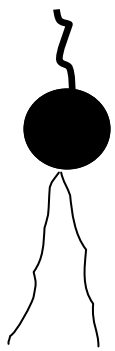
Design Goals:

- Control Microenvironment of Cells
- Direct Cell Behavior

Surface Biofunctionalization

Arrange Ligands on Surface:

- Accessibility
- Conformation
- Concentration
- Lateral Motion

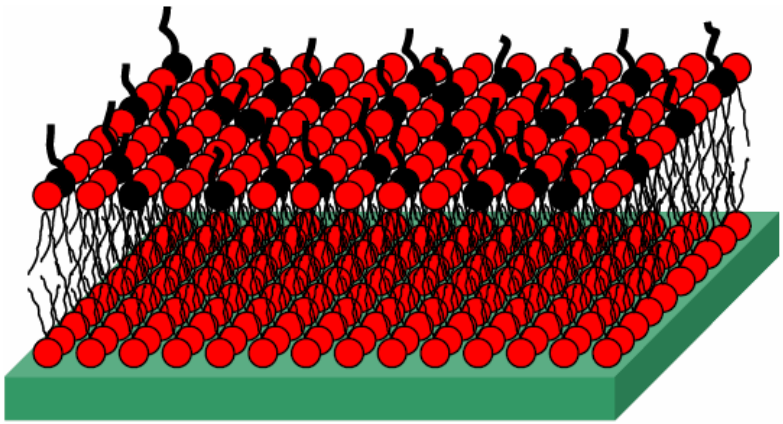


Peptide Ligand

Polar Headgroup (small)

H/C Tails (bulky)

Planar Bilayer

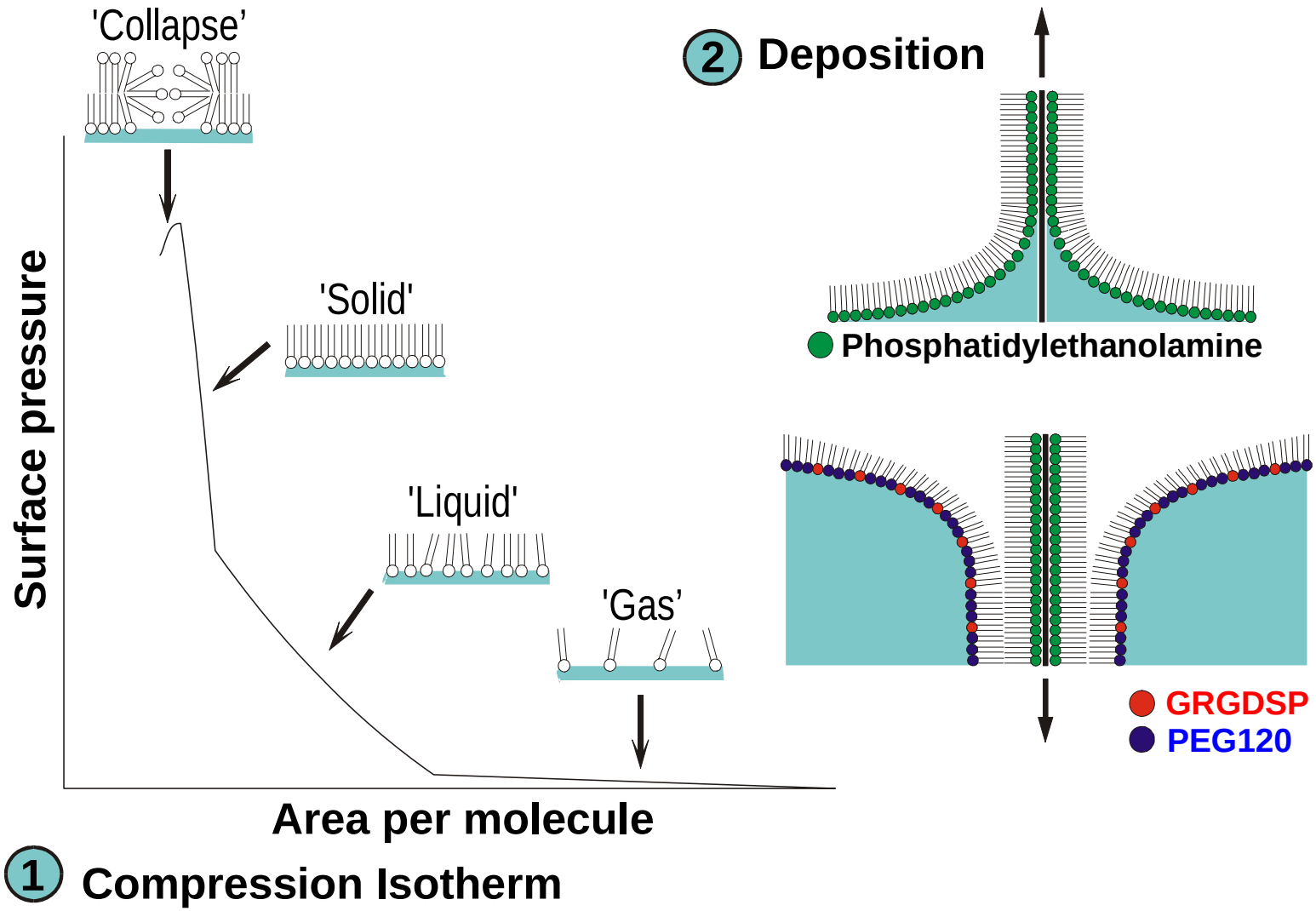


Properties of Lipid Bilayers:

- Bending easier than stretching
- Flip flop times are high (10^2-10^5 s)
- Residence time (10^4 s)

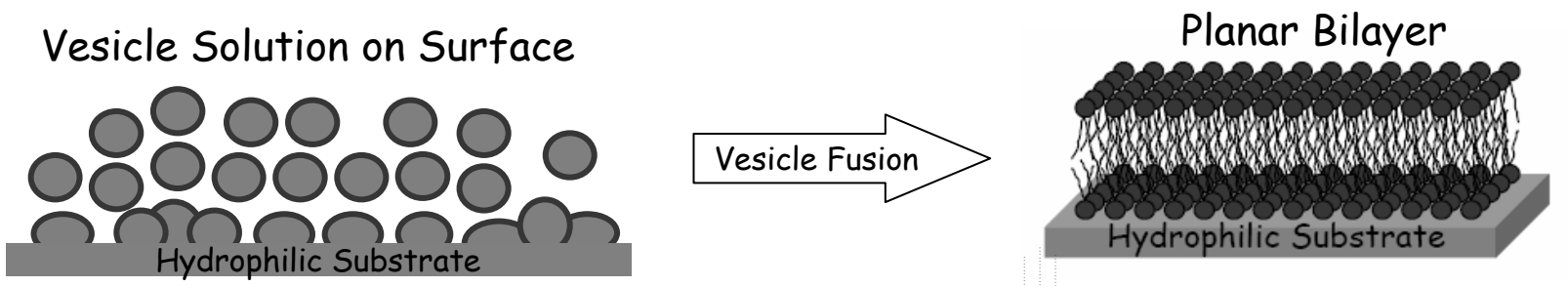
Langmuir Blodgett-Deposition

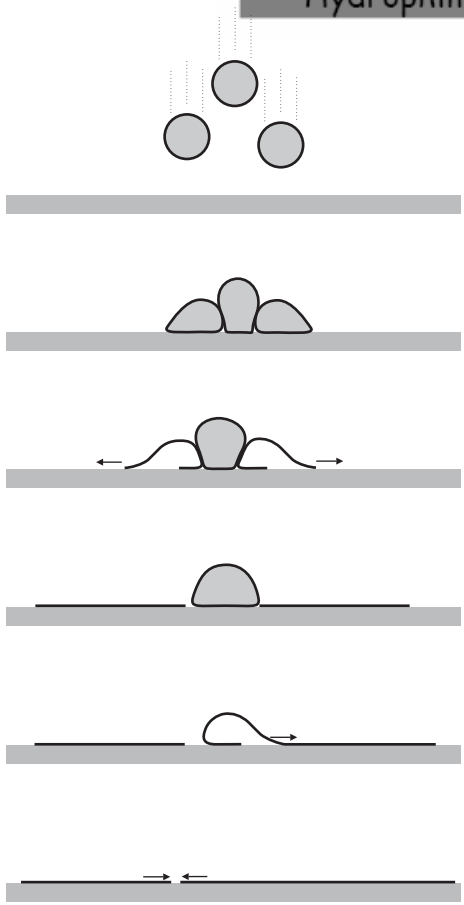
- Compress amphiphiles on the air-water interface
- Transfer 'solid' monolayer onto substrate



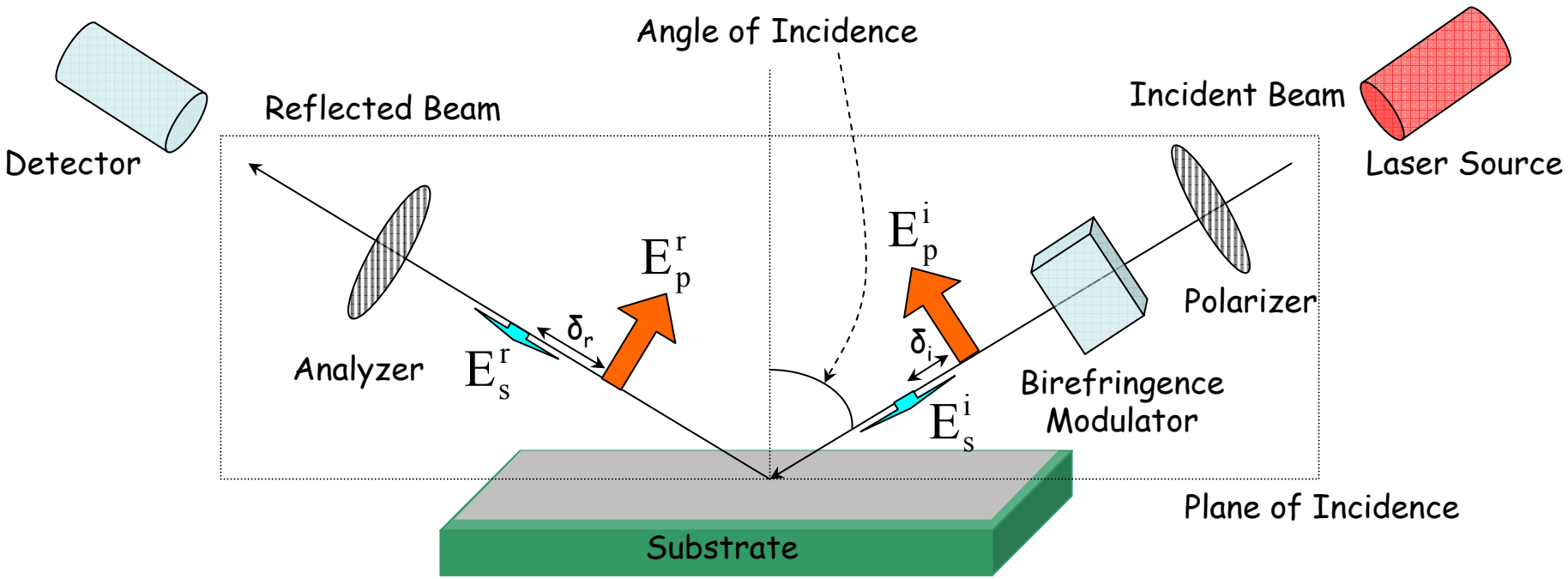
DS1

Vesicle Fusion





Ellipsometry

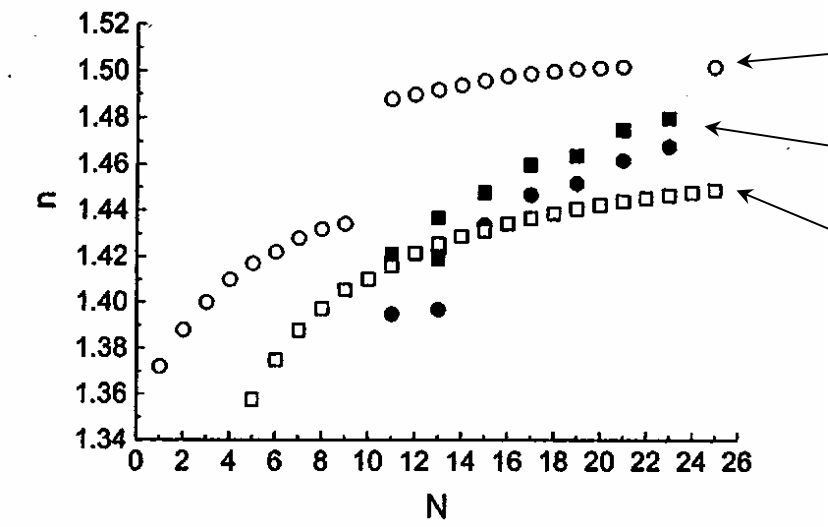


$$y = \mathrm{Im}(r)\frac{2}{1+\mathrm{Re}(r)^2+\mathrm{Im}(r)^2} \approx 2\cdot\mathrm{Im}(r)$$

$$\Delta y = \frac{2\pi\sqrt{n_1^2+n_2^2}}{\lambda\left(n_1^2-n_2^2\right)}\cdot\frac{\left(n^2-n_1^2\right)\left(n^2-n_2^2\right)}{n^2}\cdot\Delta z$$

$$r = \frac{r_p}{r_s} = \frac{\dfrac{\left|E_p^r\right|}{\left|E_p^i\right|}}{\dfrac{\left|E_s^r\right|}{\left|E_s^i\right|}} e^{i(\delta_r-\delta_i)}$$

Refractive Index of a Single Bilayer



(From Petrov et al, J. Phys. Chem. 103 (1999) 3417-3424)

Assuming n of one DMPC Bilayer ≅ n of one Monolayer (N=28)

$\Delta z = 40\text{Å}$ (for $\Delta y_{max} = 0.035$)

⇩

Good Coverage

Headgroup Area of DMPC 59Å^2 ⇨ $\Gamma_M = 3.8$ mg/m^2

The Model

(From "Self Assembly Driven by Hydrophobic Interactions at Alkanethiol Monolayers: Mechanism of Formation of Hybrid Bilayer Membranes", J. B. Hubbard, V. Silin, A. L. Plant, Biophysical Chemistry 75 (1998) 163-176)

1-D Mass Equation:

$$\frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial x^2}$$

Initial Condition:

$$C(x, t = 0) = C_o$$

Mixed Boundary Condition:

$$D \cdot \left.\frac{\partial C}{\partial x}\right|_{Surface} = K \cdot C\big|_{Surface}$$

Where K: adsorption rate constant of mass (cm/s)

C_o: bulk concentration of lipids (mg/ml)

D: diffusion coefficient of mass close to surface (cm^2/s)

$$J\big|_{Surface} = K \cdot C_o \cdot e^{\frac{K^2 \cdot t}{D}} \cdot erfc\left(\frac{K}{D^{1/2}} \cdot t^{1/2}\right)$$

General and Limiting Cases

$$\frac{d\Gamma}{dt} = \beta \cdot J\Big|_{surface} = \left[1 - \frac{\Gamma(t)}{\Gamma_M}\right] \cdot J\Big|_{surface}$$

IC: $\Gamma(t=0) = 0$

General Solution:

$$\Gamma(t) = \Gamma_M \cdot \left\{1 - e^{-\frac{K \cdot C_o}{\Gamma_M}\left[\frac{D}{K^2}\left(e^{\frac{K^2 t}{D}} \cdot \operatorname{erfc}\sqrt{\frac{K^2 t}{D}} - 1\right) + \frac{2}{K}\cdot\sqrt{\frac{Dt}{\pi}}\right]}\right\}$$

Characteristic Time of Adsorption: $\tau = D/K^2$

For $t/\tau >> 1$ (Diffusion Limited Case):

$$\Gamma(t) = \Gamma_M \cdot \left(1 - e^{-\frac{C_o}{\Gamma_M}\left(2\sqrt{\frac{D \cdot t}{\pi}} - \frac{D}{K}\right)}\right)$$

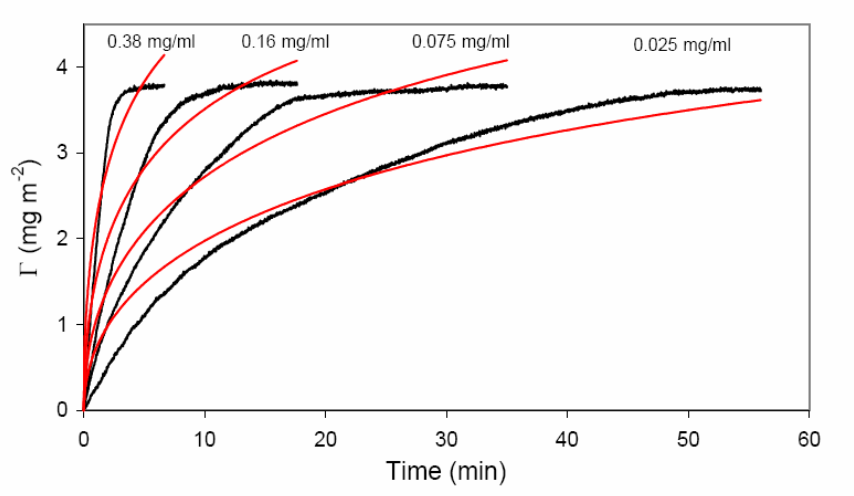
For $t/\tau << 1$ (Adsorption Limited Case):

$$\Gamma(t) = \Gamma_M \cdot \left(1 - e^{-\frac{K \cdot C_o \cdot t}{\Gamma_M}}\right)$$

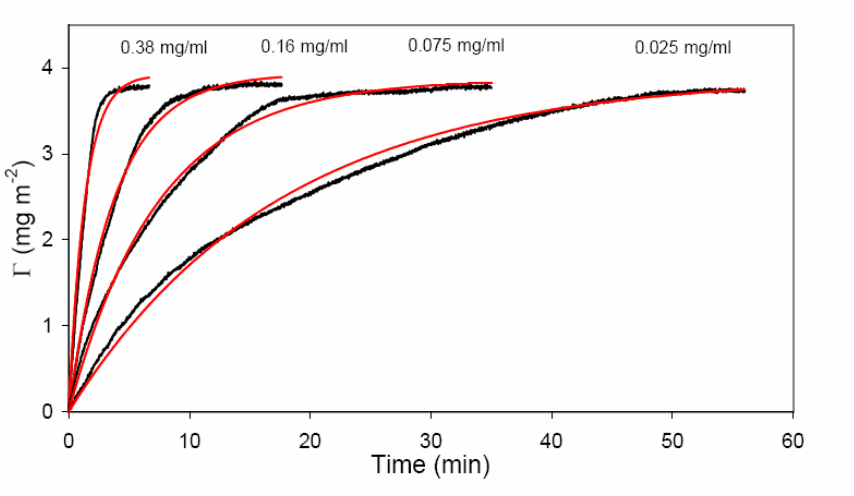
Results

(Limiting Cases Fittings)

Diffusion Limited Case



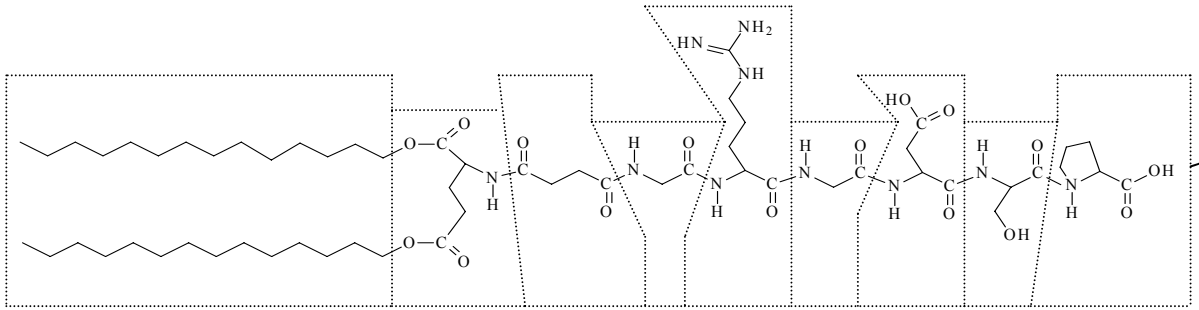
Adsorption Limited Case

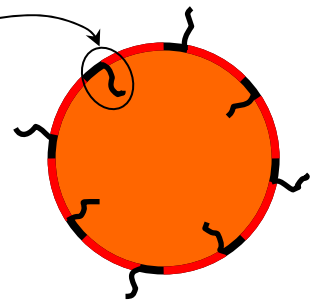


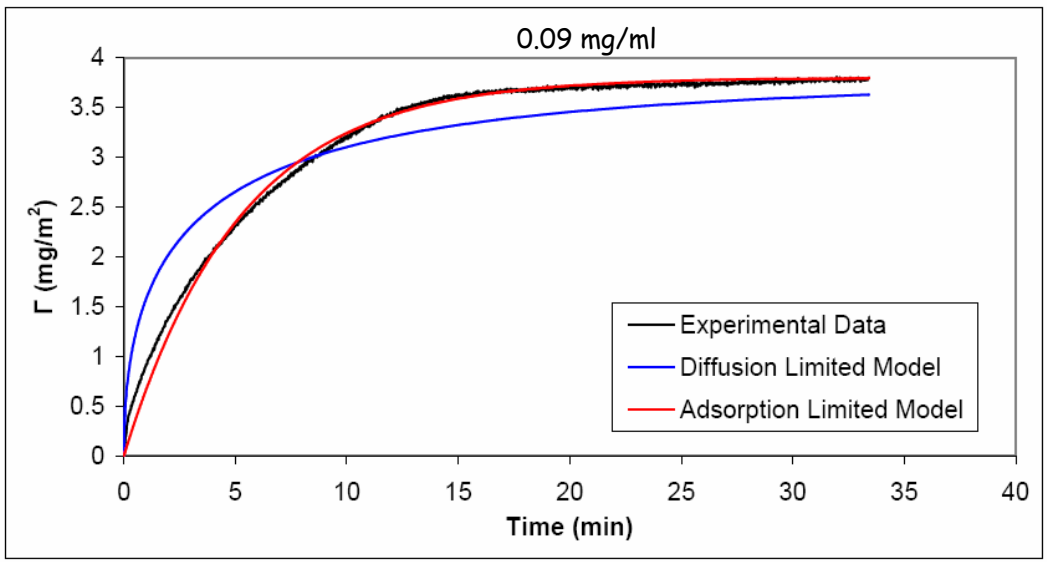
Lipid concentration C_0, mg ml^{-1}	Maximum Surface coverage Γ_m, mg cm^{-2}	Diffusion coefficient D, cm^2 s^{-1}
0.4	6.0×10^{-4}	6.6×10^{-9}
0.2	5.5×10^{-4}	1.6×10^{-8}
0.08	6.1×10^{-4}	3.0×10^{-8}
0.02	5.7×10^{-4}	1.2×10^{-7}

Lipid concentration C_0, mg ml^{-1}	Maximum Surface coverage Γ_m, mg cm^{-2}	Adsorption rate constant K, cm s^{-1}
0.4	3.8×10^{-4}	$\mathbf{1.3\times10^{-5}}$
0.2	3.8×10^{-4}	$\mathbf{1.1\times10^{-5}}$
0.08	3.8×10^{-4}	$\mathbf{1.2\times10^{-5}}$
0.02	3.8×10^{-4}	$\mathbf{1.5\times10^{-5}}$

Effect of Peptide Amphiphile Presence on the Kinetics





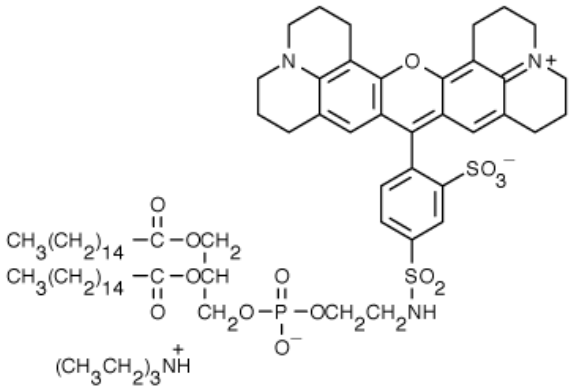


10% mole PA DMPC

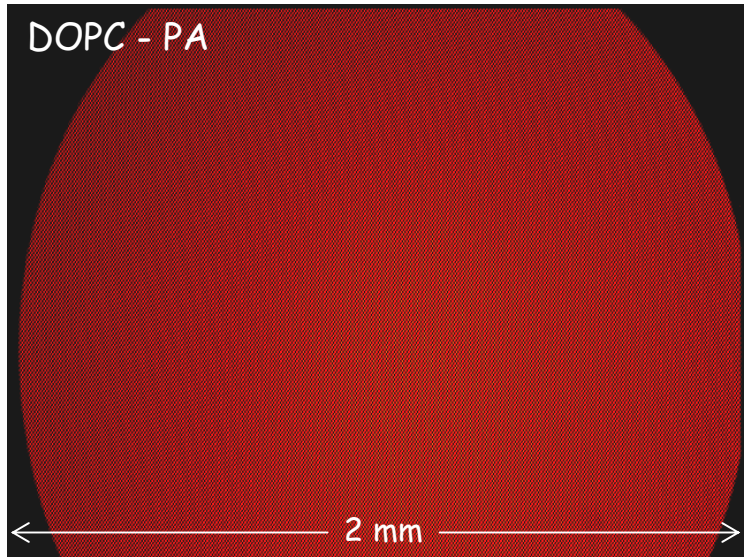
Case	Adsorption rate constant K, cm s^{-1}	Diffusion coefficient D, cm^2 s^{-1}
Diffusion Limited	--------	7.4×10^{-8}
Adsorption Limited	**1.4×10^{-5}**	--------

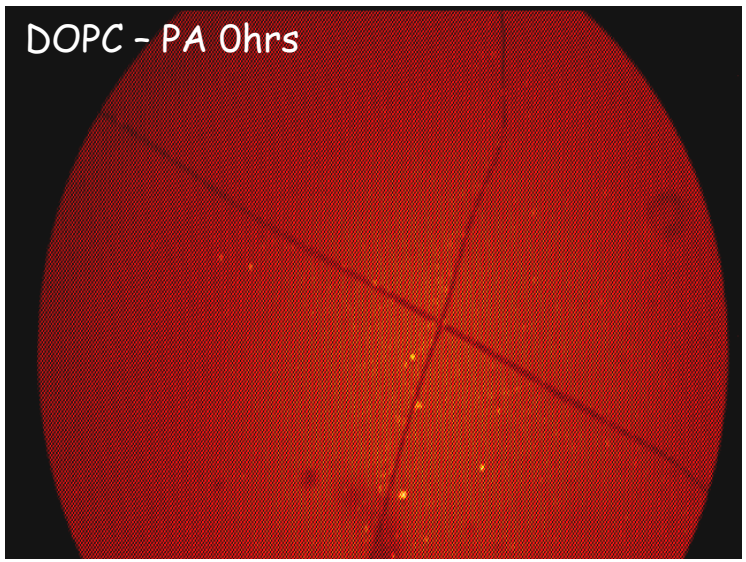
Kinetics not Interrupted by PA presence

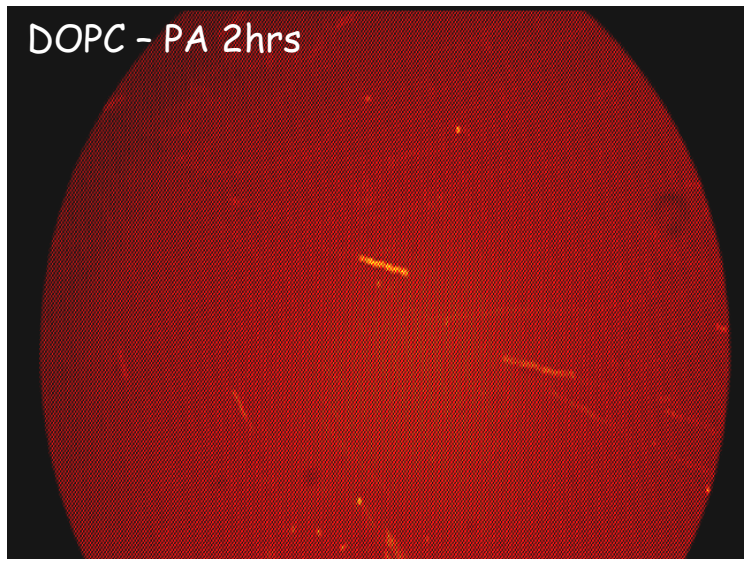
Fluorescence Visualization



1,2-dihexadecanoyl-*sn*-glycero-3- phosphoethanolamine, triethylammonium salt
(Texas Red® DHPE - Molecular Probes)

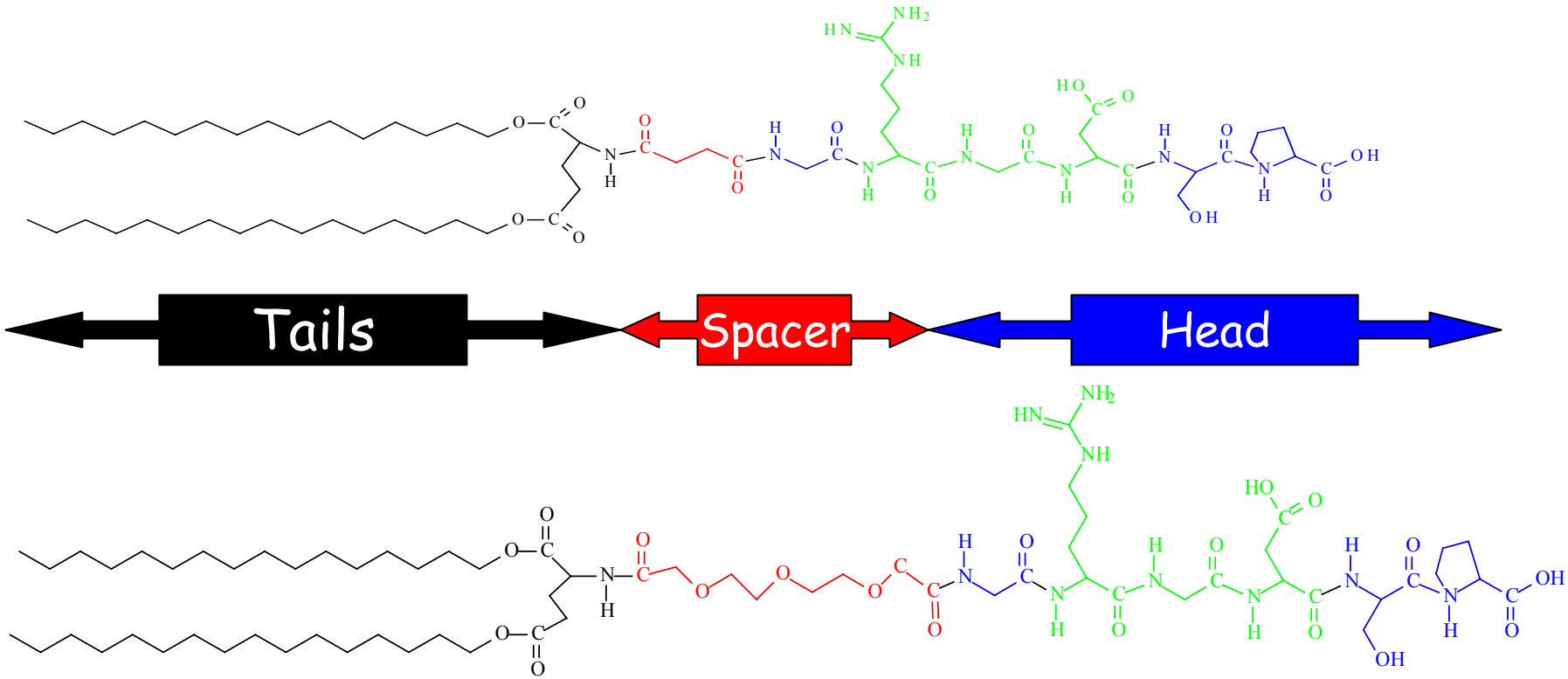






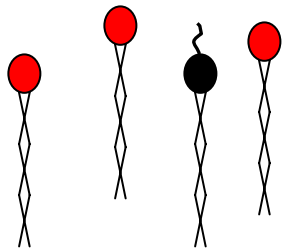
PA for Cell Adhesion

- Controls the presentation of RGD, a peptide sequence important for cell adhesion, i.e vesicles, bilayers, etc.
- Focused on two peptide amphiphiles with different spacer groups, C_2 and polyethylene oxide (PEO).

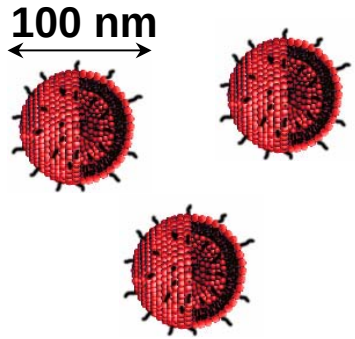


Experimental Procedure

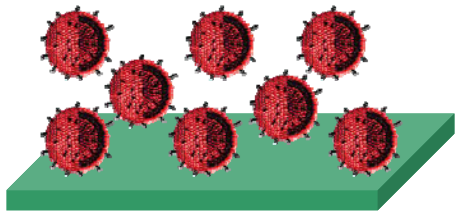
- Vesicle formation



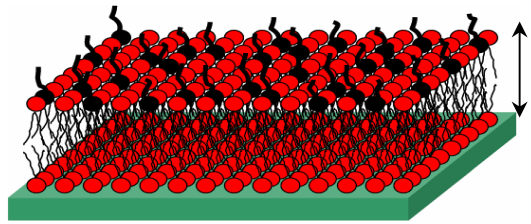
Hydration - Extrusion



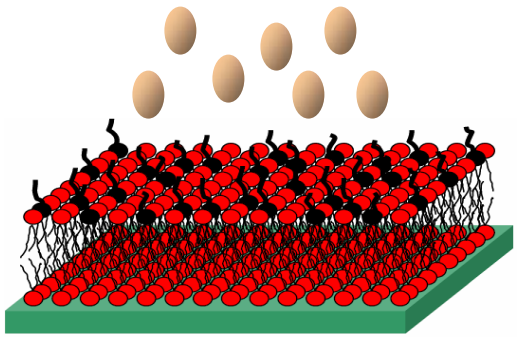
- Bilayer formation



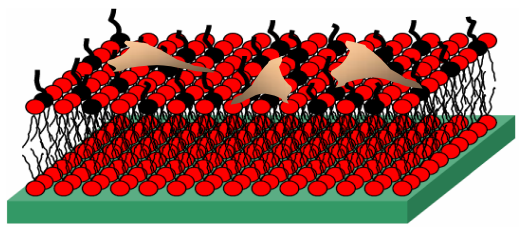
Fusion



- Addition of cells

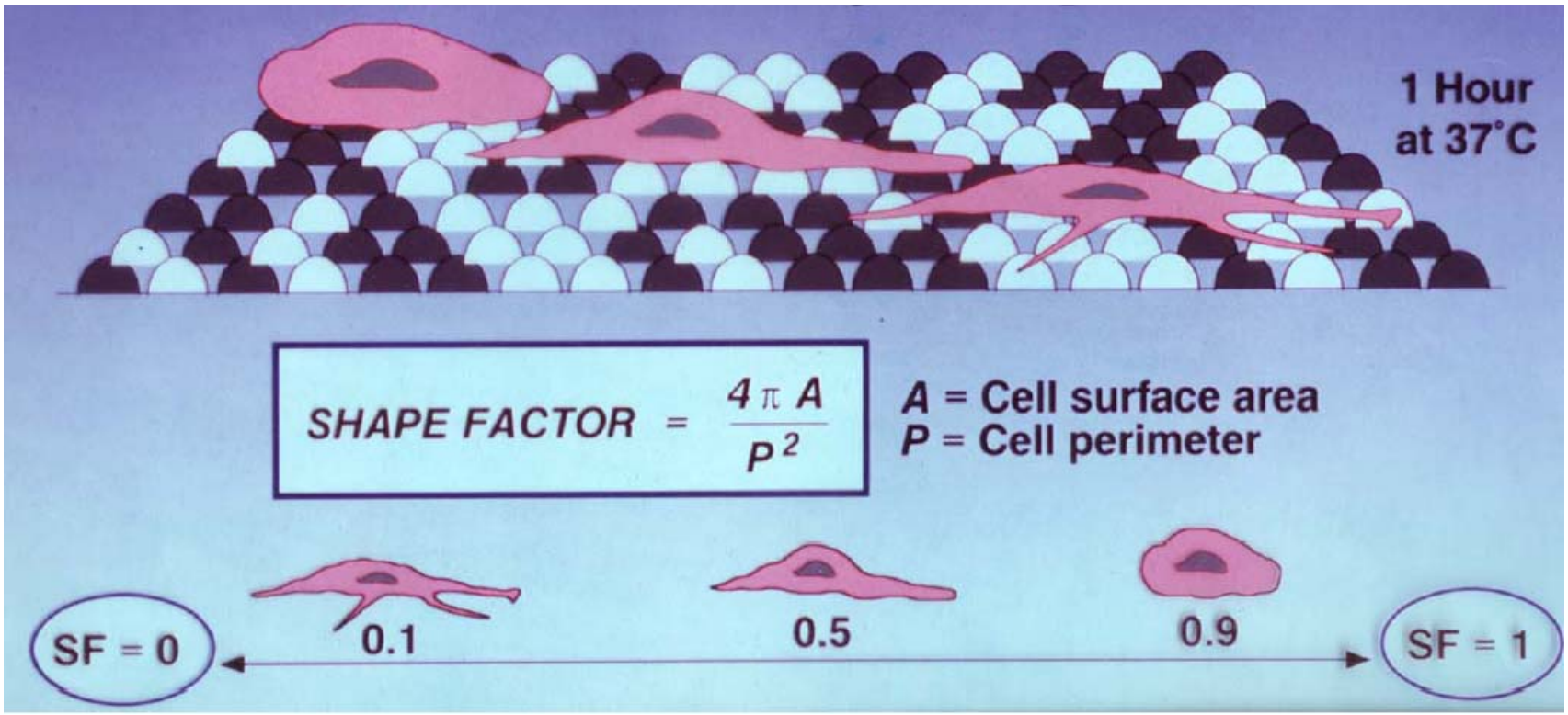


Incubation

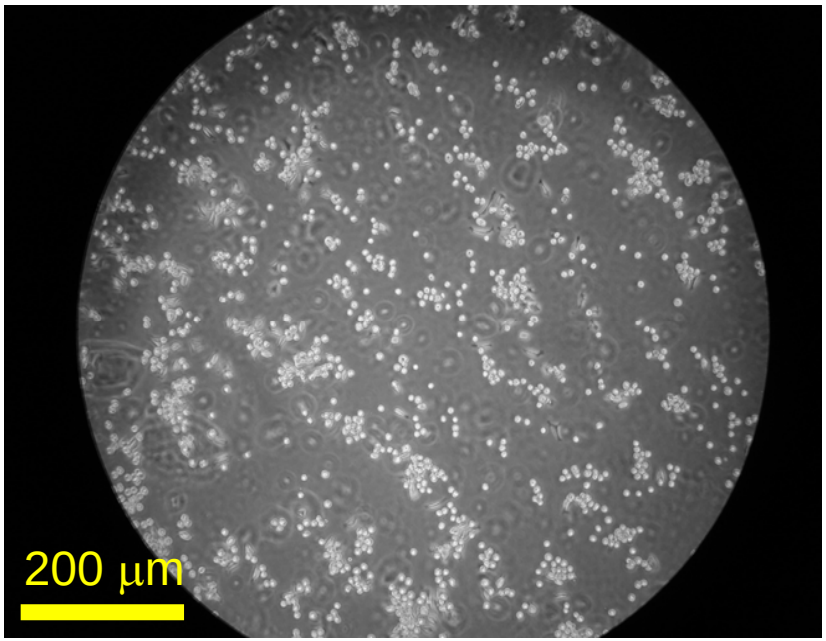


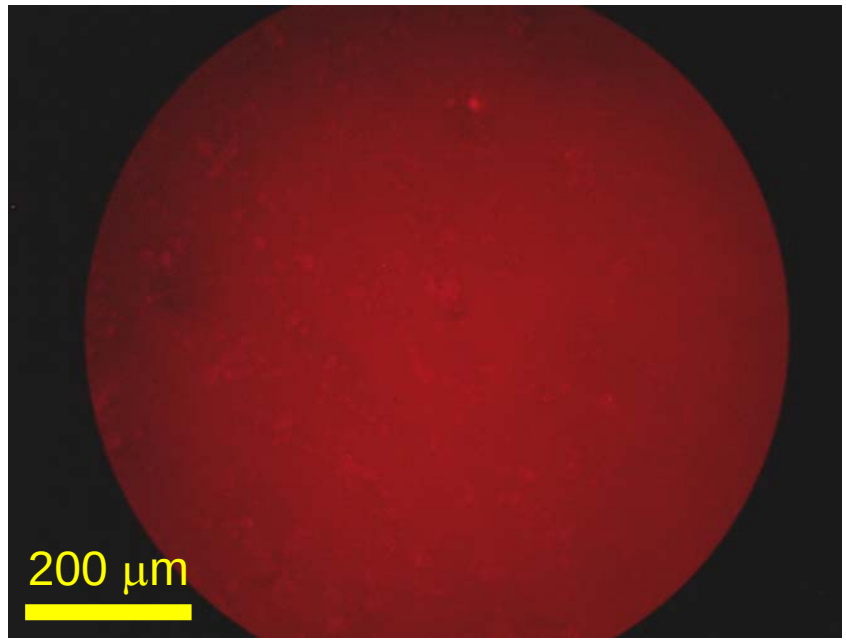
Adhesion & Spreading Evaluation

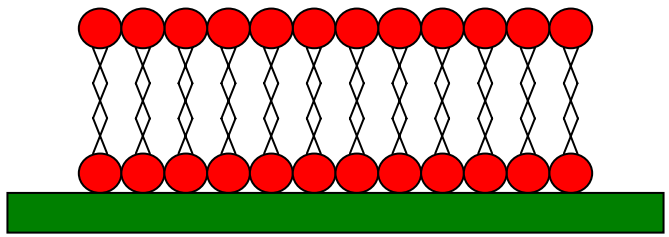
- Inertia motion caused by mild microscope stage shaking determines adhesion.
- Scion Imaging software is used to determine the "shape factor."



Results EggPC Bilayer



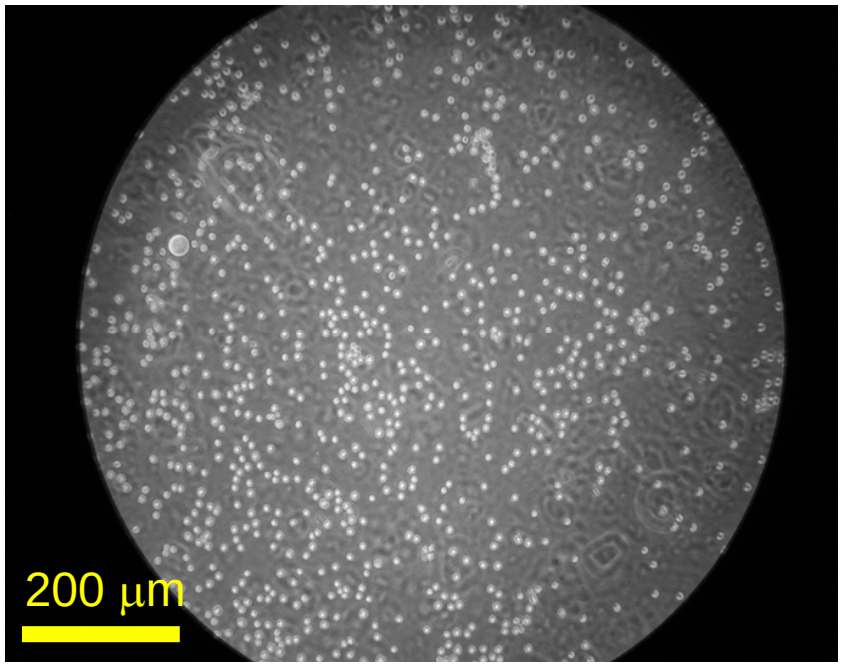


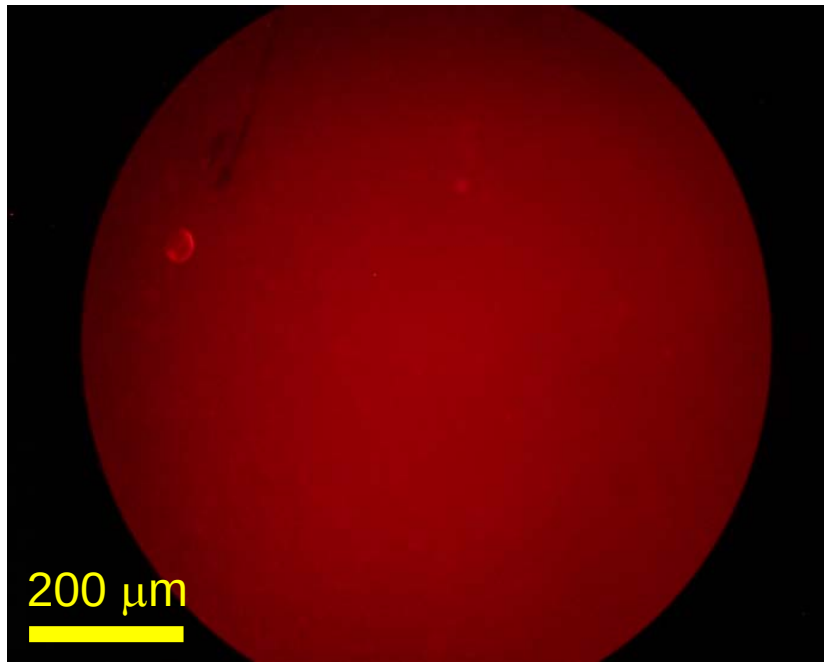


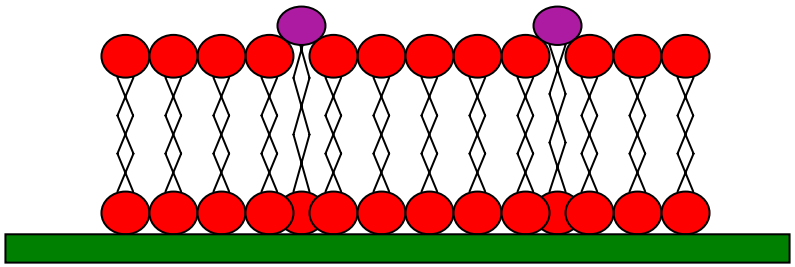
Average Shape Factor- 0.86 (4 hours)

No Adhesion

Results 10% C_2-RGD Bilayer



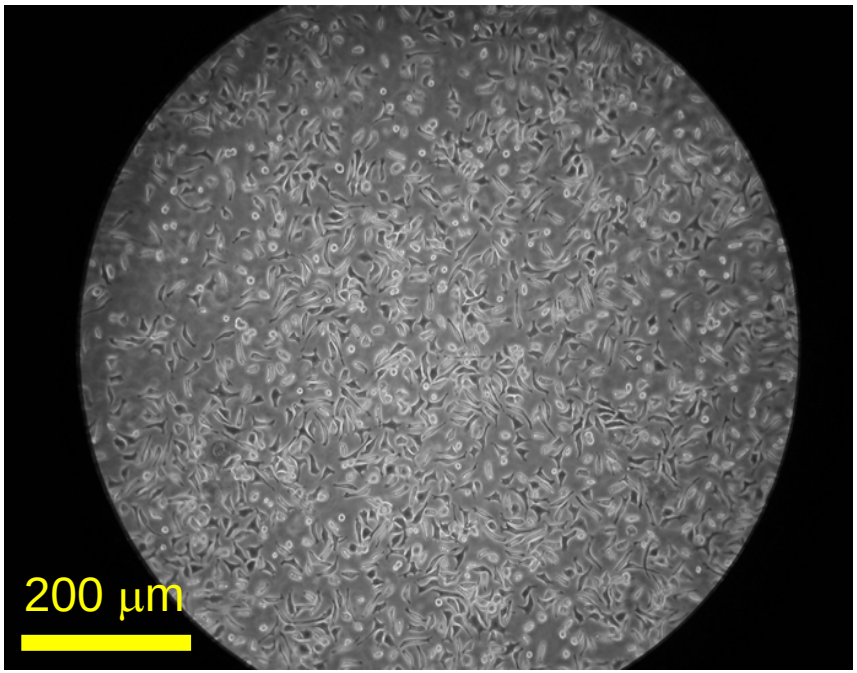


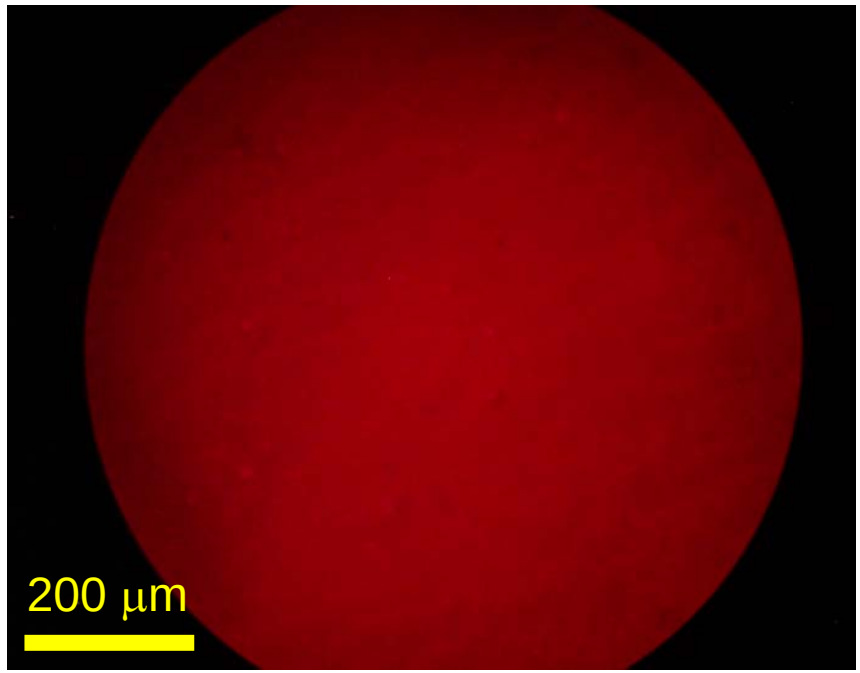


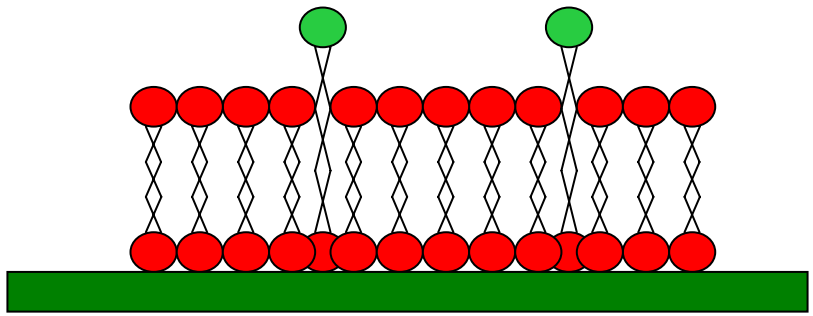
Average Shape Factor- 0.84 (3 hours)

No Adhesion

Results 10 % PEO-RGD Bilayer



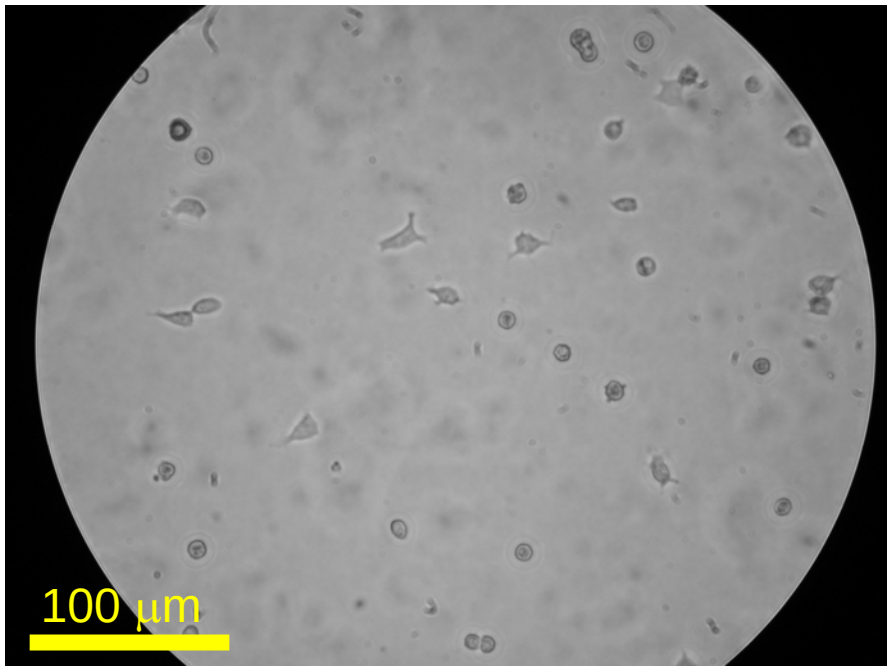




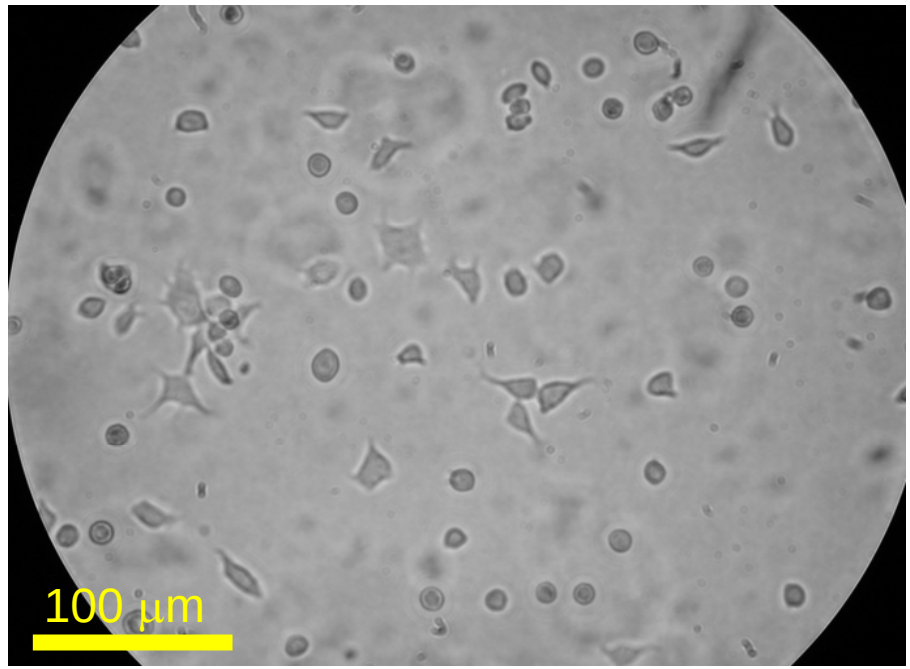
Average Shape Factor- 0.52 (4 hours)

Adhesion

Concentration Effect



5% PEO-RGD



10% PEO-RGD

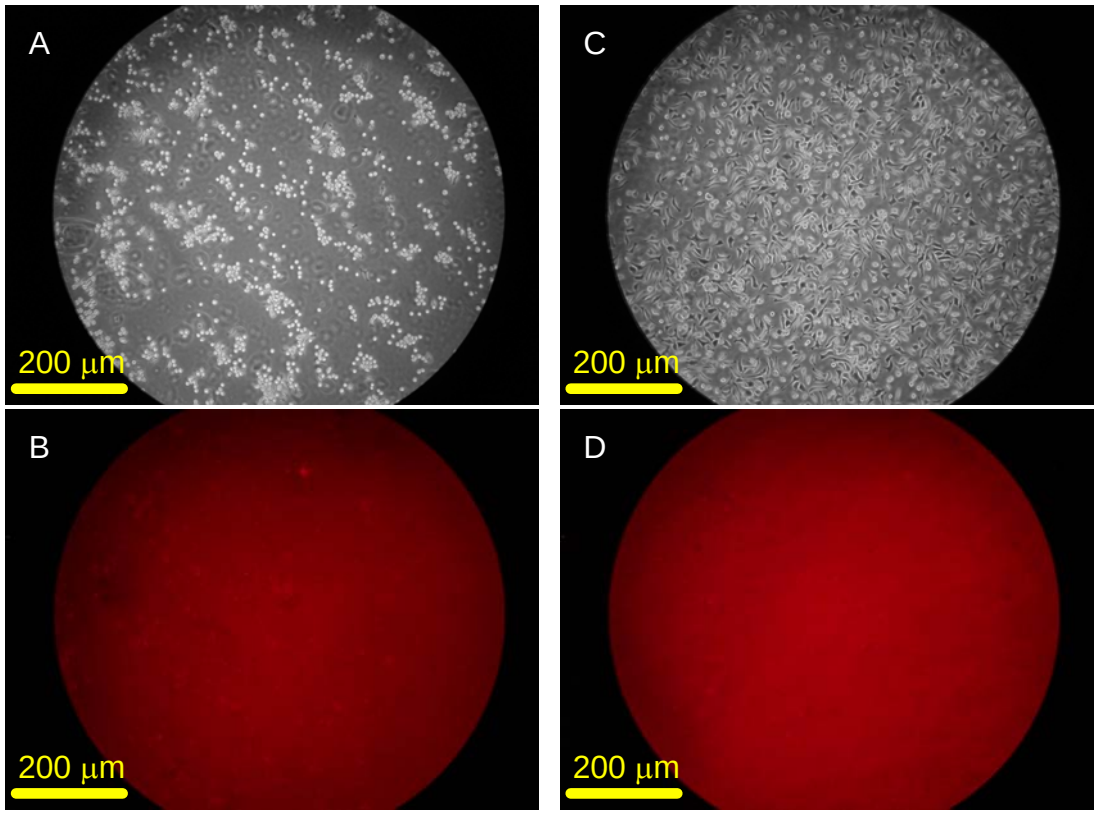
(2 hours)

Adhesion

Surface Concentration Gradients

- Supported bilayers present a physically relevant substrate to study cell-ECM protein interactions
- Active protein sequences can be presented in a control manner using peptide amphiphiles
- There is a drive for the development of multifunctional-multicomponent surfaces
- Further understanding of how specific ligand-integrin interactions modulate cell function is required
- Gradients of membrane bounded ligands can provide a useful tool to study this problem
- Vesicle fusion is the most efficient method to make surface gradients in a membrane environment

Ligand Concentration – Cell Spreading



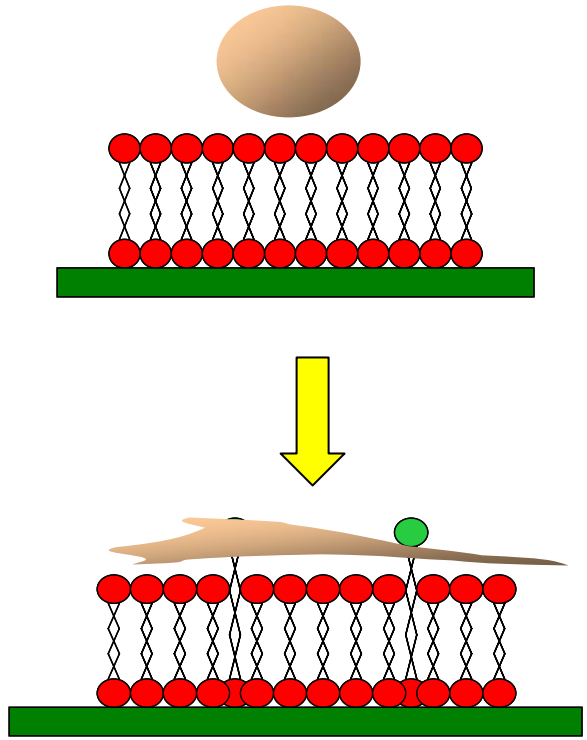
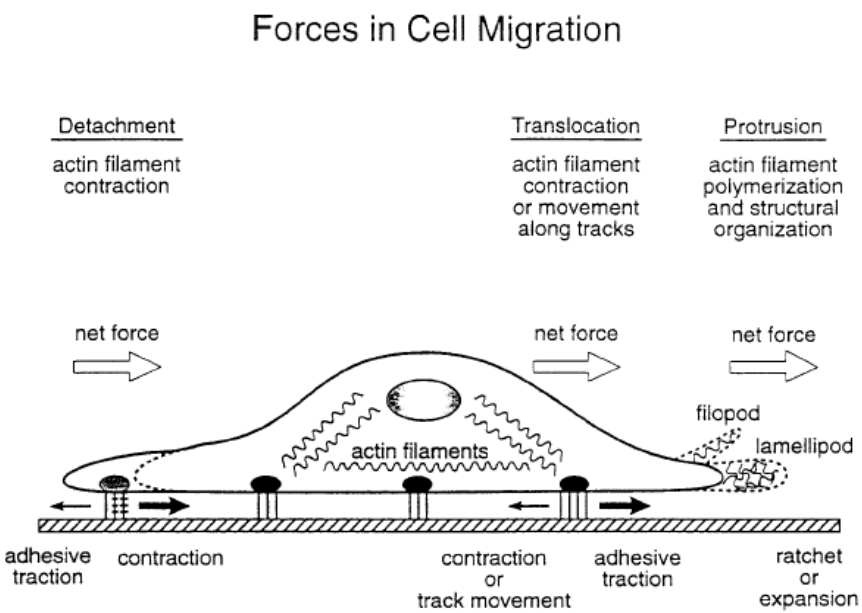
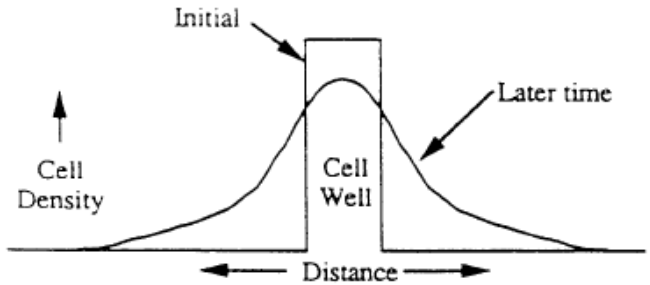


Figure: NIH3T3 cells cultured on supported membranes after 4 hours. Membranes in (A) & (B) are 99% EggPC and 1% Texas Red DHPE while in (C) & (D) 94% EggPC, 5% (C16)2-Glu-PEO-GRGDSP and 1% Texas Red DHPE. Pictures (A) and (C) are in optical mode, while (B) and (D) in fluorescent mode on the same surface spot respectively.

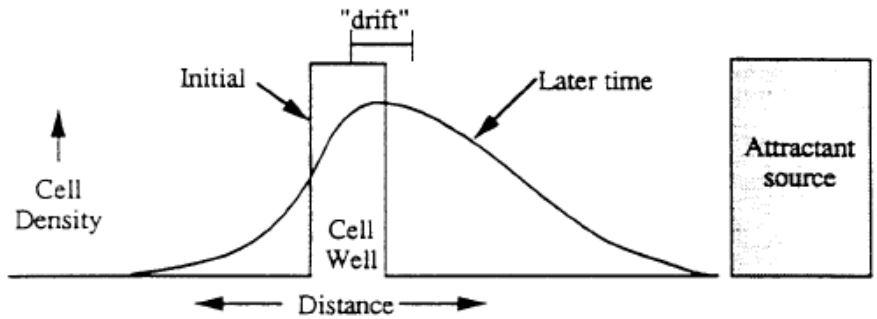
Ligand Concentration – Cell Migration



a. Uniform chemical concentration: Random motility only (μ)



b. Migration in a chemical attractant gradient:
Random motility (μ), chemokinesis ($\frac{d\mu}{da}$) and chemotaxis (χ)

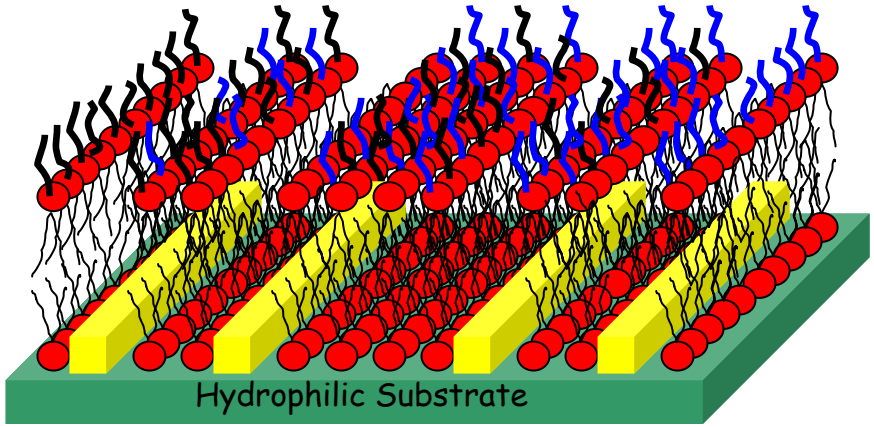


MICROSCOPY RESEARCH AND TECHNIQUE 43:358–368 (1998)

Steps to a Concentration Gradient

Barriers

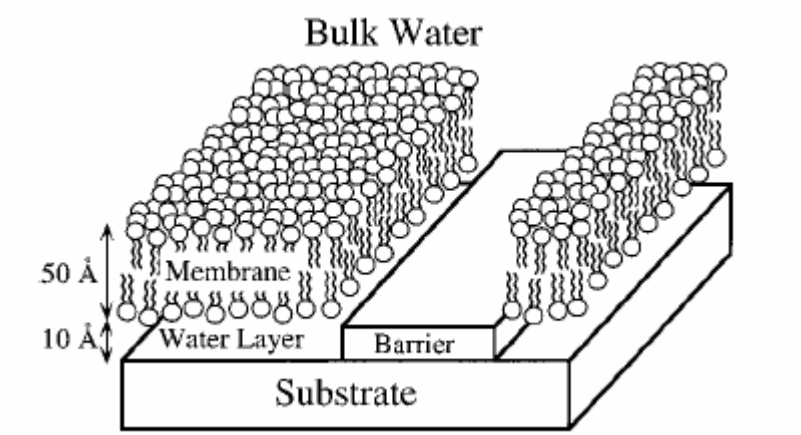
Patterning



Different ways to make barriers

Materials:

- Au, Al, Cr, Ti
- Al_2O_3, TiO_2
- Fibronectin, BSA
- Polymerized Bilayers



Chemical Nature of Barrier – not topography – Blocks Diffusion

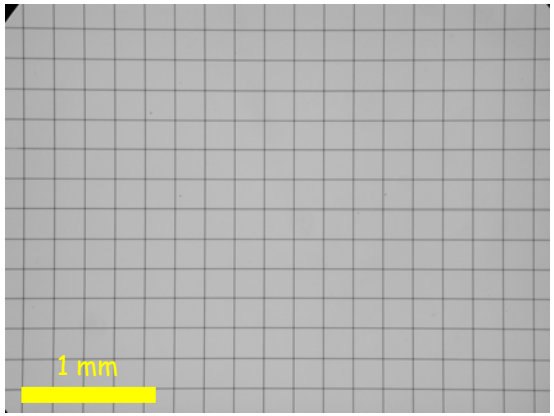
Techniques:

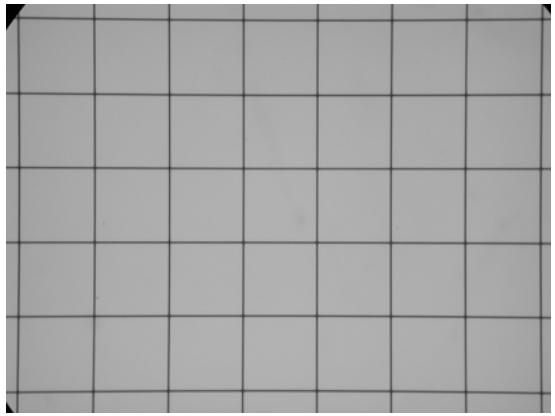
✓ Standard Photolithography (metals)

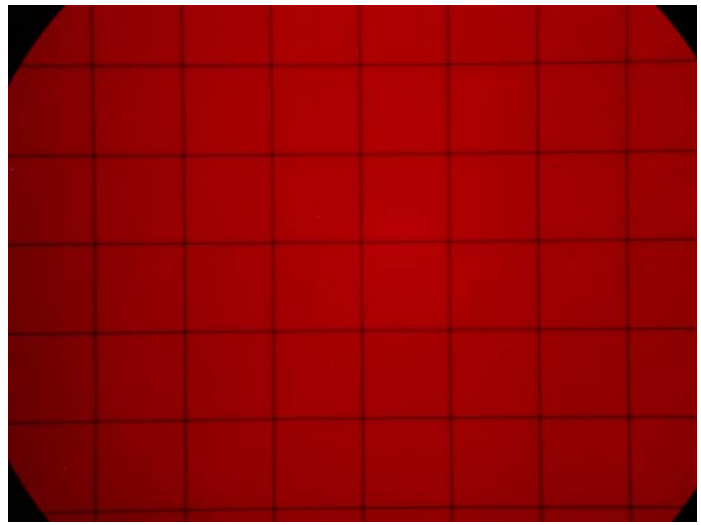
- Polymerization
- Microcontact Printing → Spread - Neutral and Low PH
- Deep Abrasion (R<<10 nm) → Stable - High PH

"Micropattern Formation in Supported Lipid Membranes", Jay T. Groves and Steven G. Boxer, Acc. of Chem. Res., 35, 149-157 (2002).

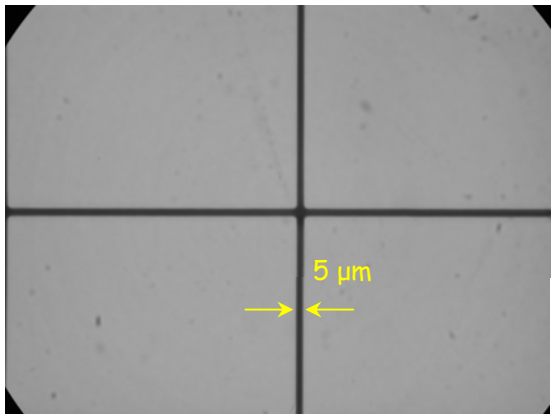
Chrome Grid

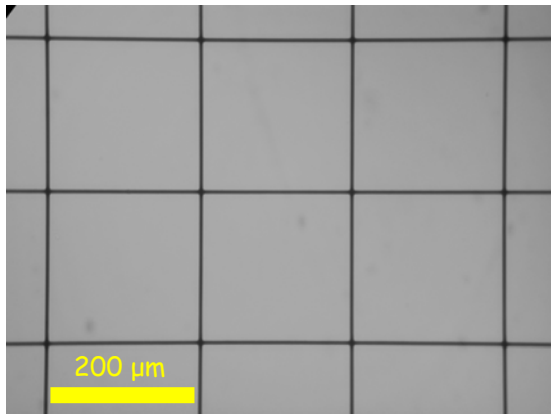




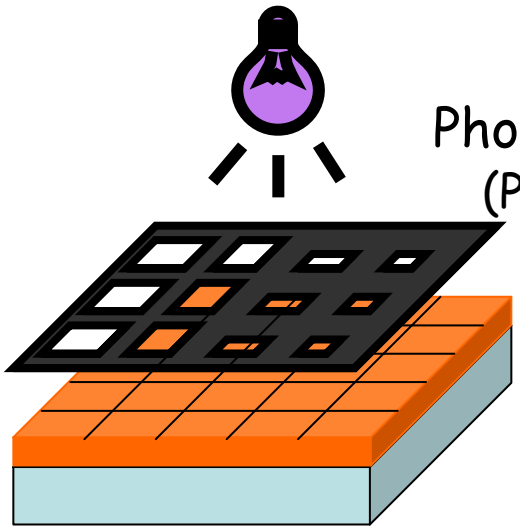


EggPC Bilayer

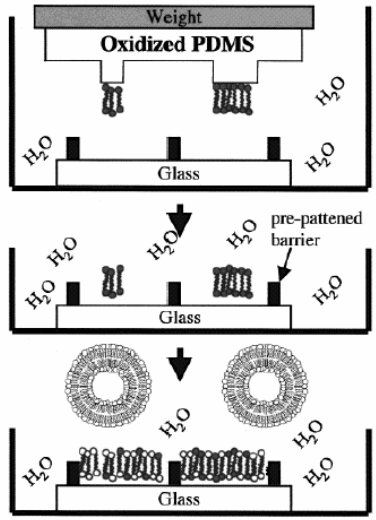




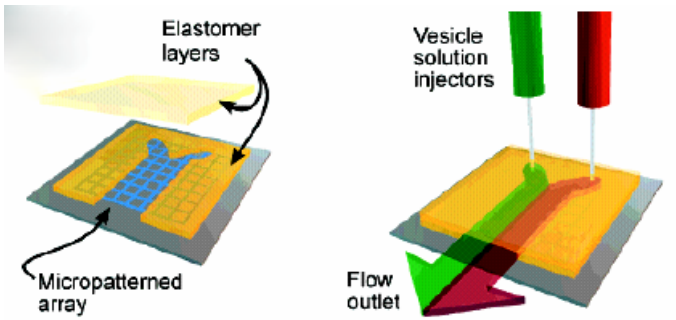
Different ways to pattern



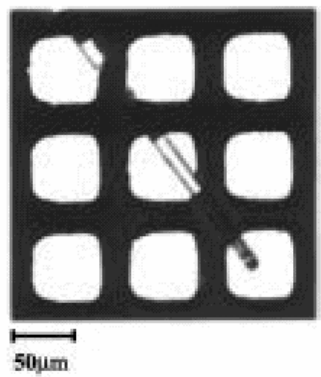
Photolithographic Patterning
(Photoactivatable peptides)

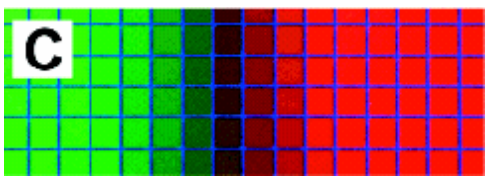


Microprinting



Direct Pipetting





Microfluidic Flow

"Micropattern Formation in Supported Lipid Membranes", Jay T. Groves and Steven G. Boxer, Acc. of Chem. Res., 35, 149-157 (2002).

Microfluidic Networks

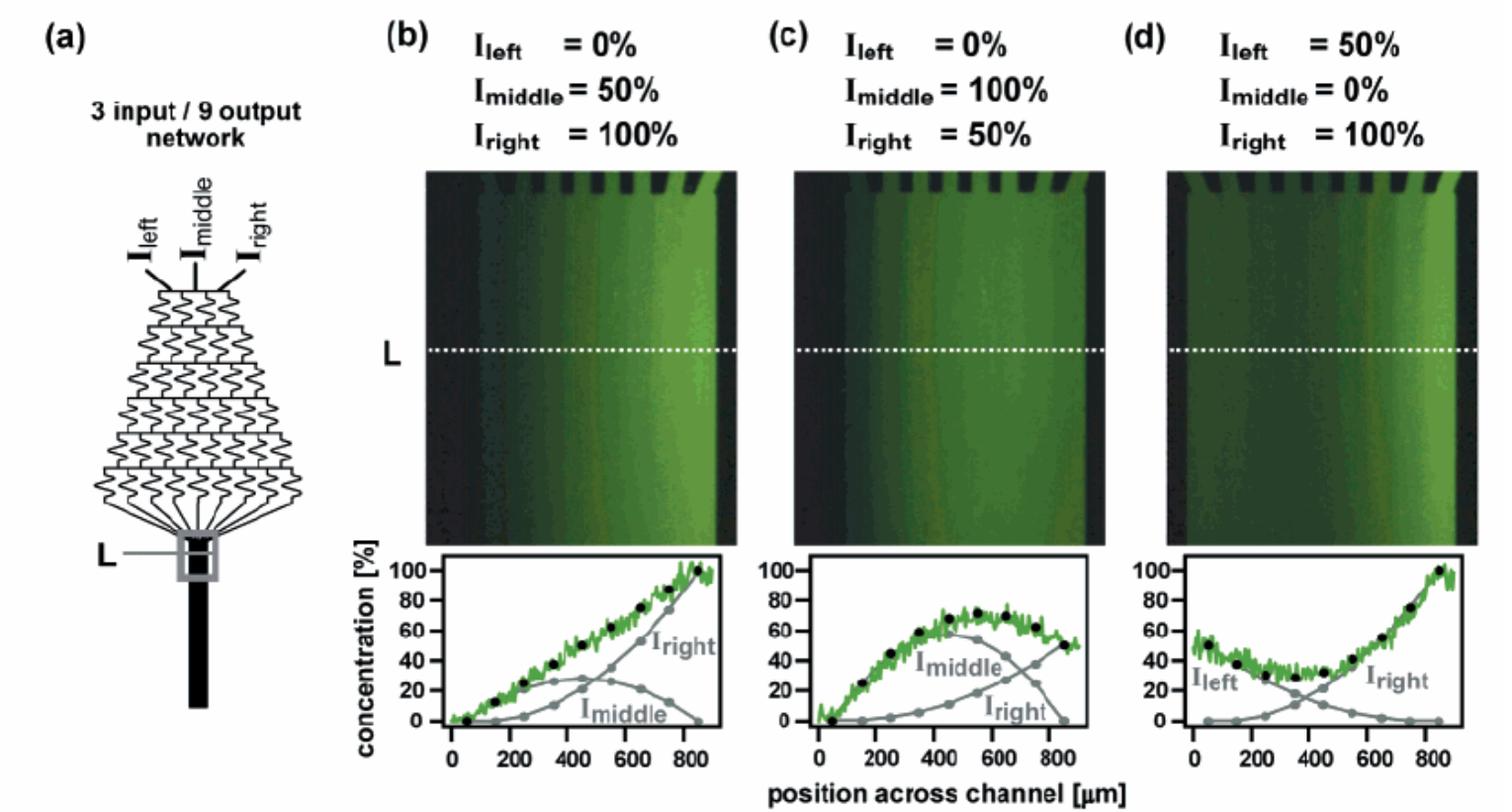
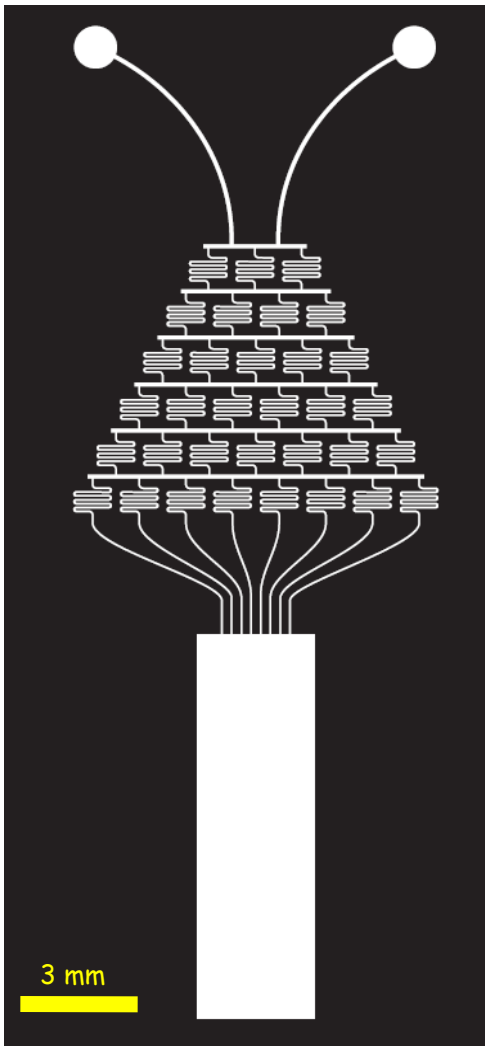
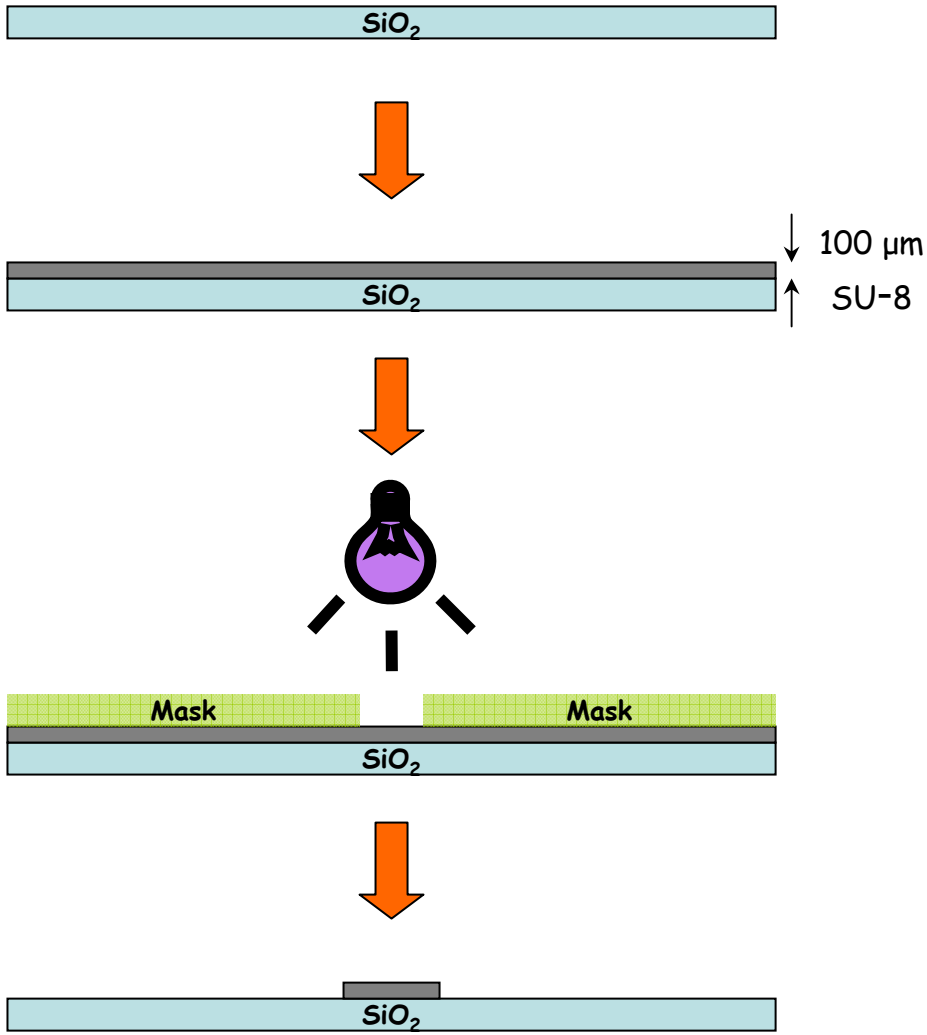


Figure 2. Fluorescence micrographs showing (b) linear and (c, d) parabolic gradients of fluorescein in solution. The microfluidic network we used for generating these gradients had 3 inlets and 9 outlets (a). The concentration of the solutions we introduced into each inlet of our microfluidic network is indicated above the micrographs. The plots below the micrographs show the corresponding fluorescence intensity profile (green line) across the broad channel (900 μm wide) 500 μm downstream (L, white dotted line) from the junction. The theoretically calculated concentration profiles of fluorescein are shown as black, round dots. The gray lines and dots in the graphs show the calculated contribution of the individual inputs to the overall profile. The flow rate in the broad channel is 1 mm/s.

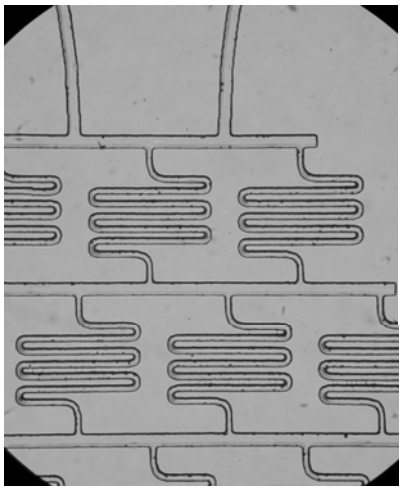
Anal. Chem. **2001,** *73,* 1240−1246

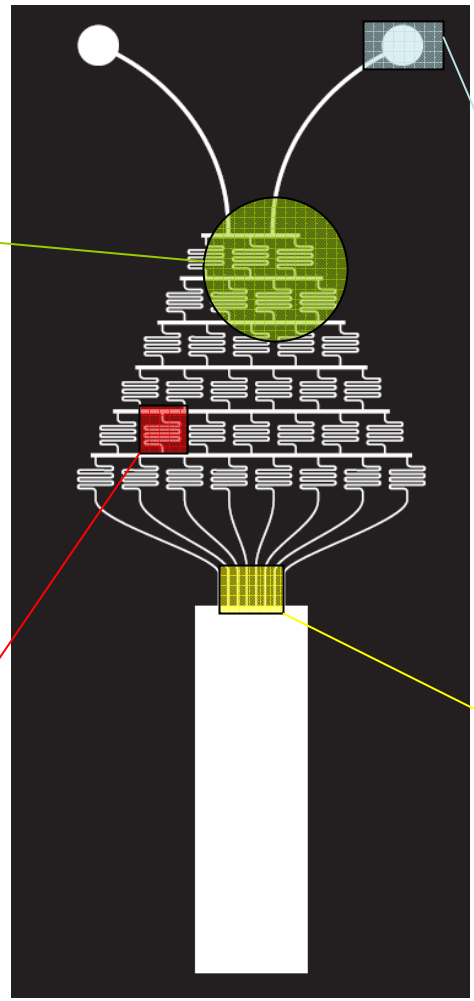
Construction

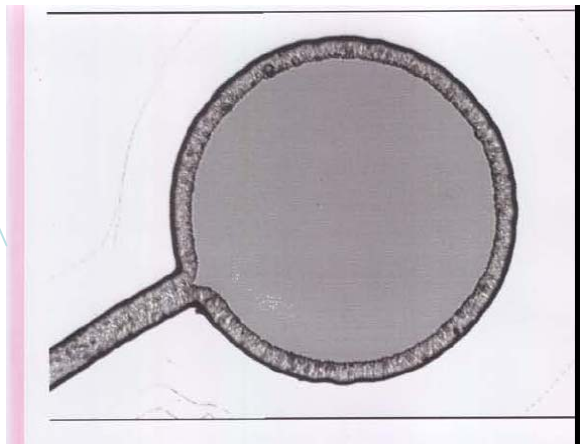


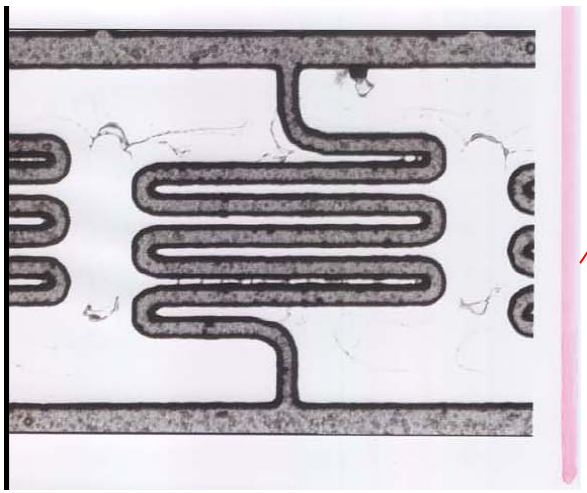


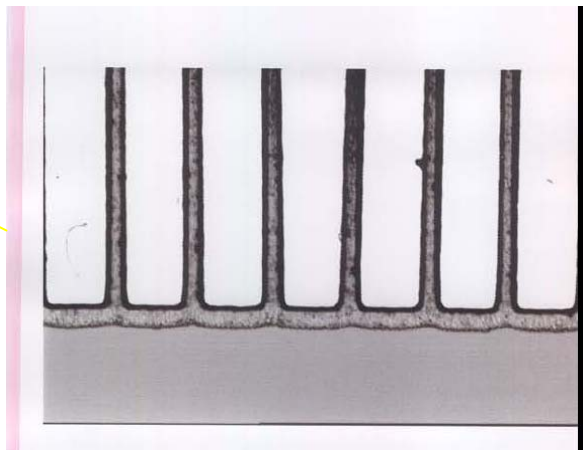
Evaluation



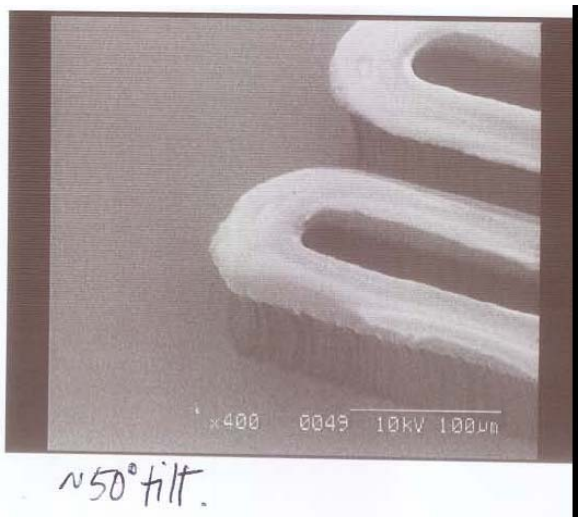


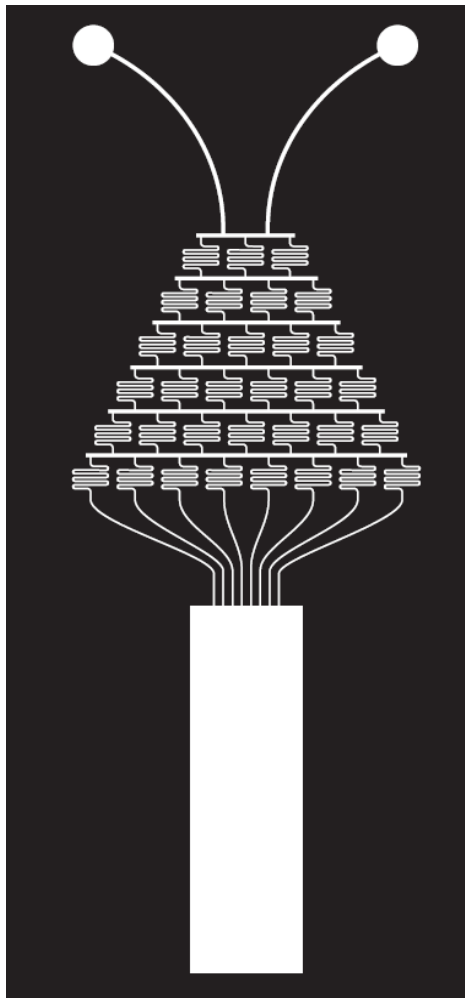




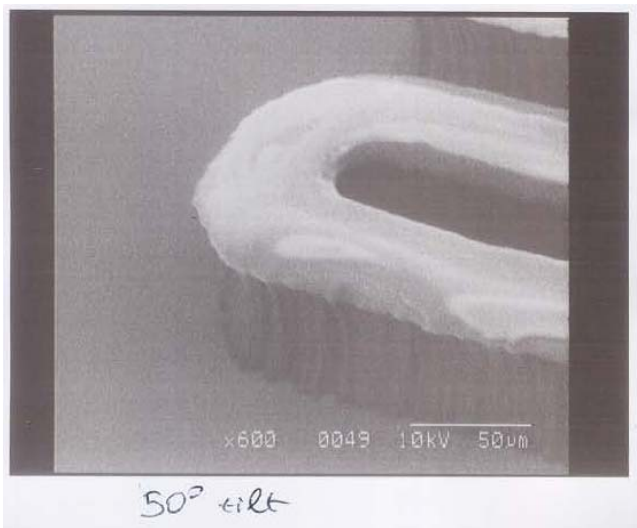


SEM

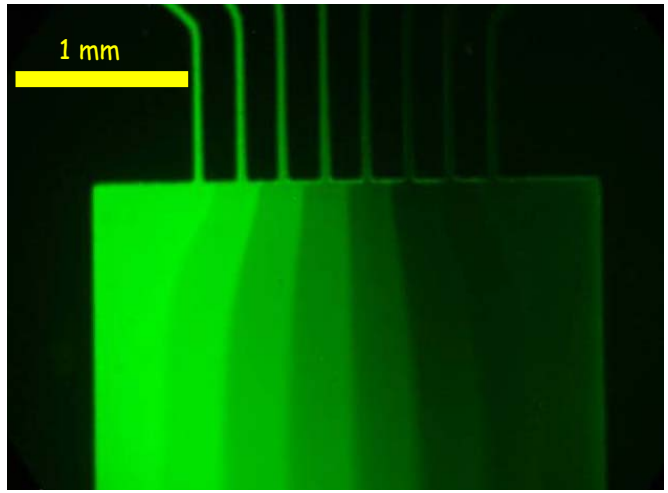

x400 0049 10kV 100µm
~50° tilt.

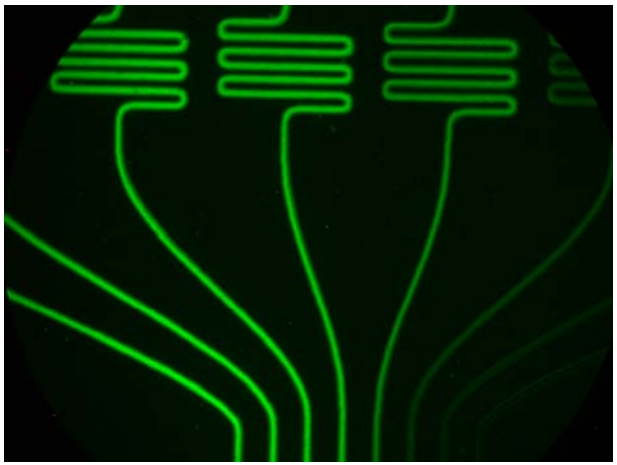


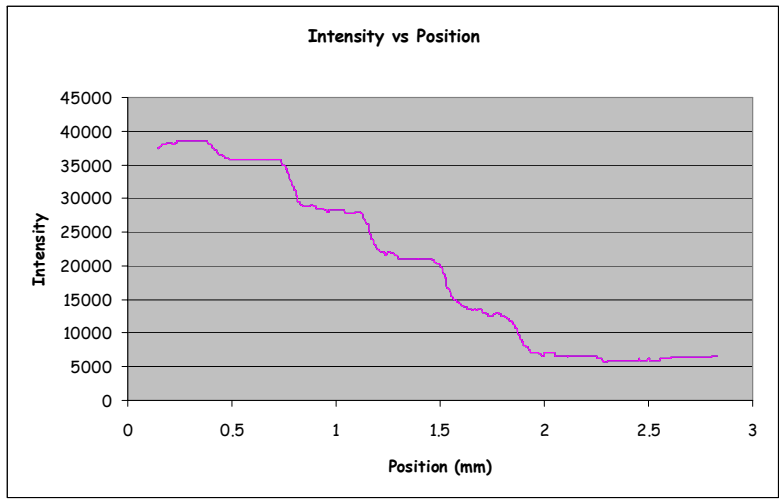

FREEZE
x600 0051 10kV 50µm


x600 0049 10kV 50µm
50° tilt

Testing

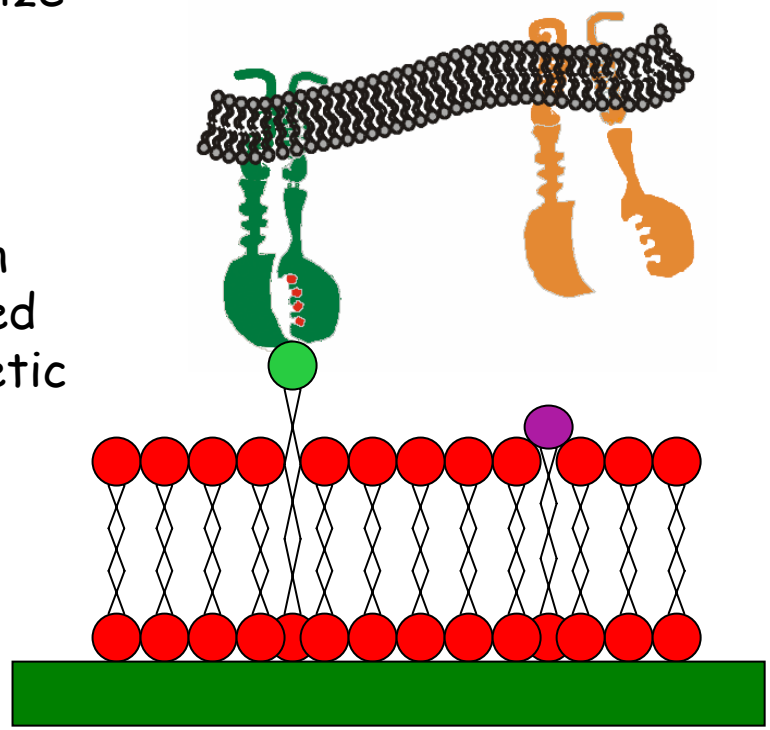

1 mm




Intensity vs Position
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Intensity
0
0.5
1
1.5
2
2.5
3
Position (mm)

Conclusions

- Ellipsometry was used to study the kinetics of VF on SiO_2
- Bilayer formation from vesicles is adsorption limited
- Fluorescence microscopy was used to visualize the membranes
- Peptide accessibility and density are important for cell binding
- Surface composition gradients are useful in studying cellular function modulation induced by ligand-integrin interactions on a biomimetic membrane
- A novel technique was developed to control the microenvironment of cells in 2-D



Acknowledgments

Dr. Alejandro Parra

Ellipsometry

Dr. Haining Zhang

PEO chemistry

Ning Cao

Clean-room Processing

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