Surface display using self-assembly for screening cell-adhesive peptides

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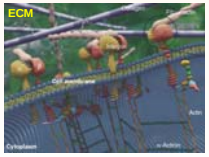
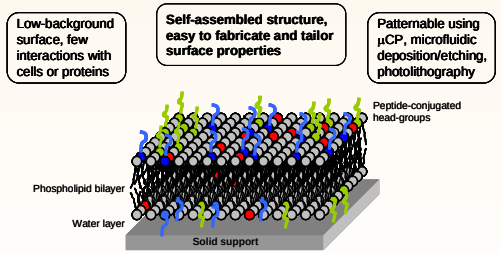
Introduction

The development of biomaterials for regenerative medical constructs such as engineered tissue, as well as advanced biomedical devices such as sensors, will require exquisite control over material structure and biological function. Natural biological materials, such as the *extra-cellular matrix (*ECM) that surrounds cells in tissue, achieve such control by directing the assembly and function of *proteins*. It is often the case that the activity of the protein can be replicated by a short *peptide*, with potential advantages in terms of specificity and cost. It is therefore important to develop techniques to decorate biomaterial surfaces with peptides in a way that retains the peptide's conformation and its activity. It is also a challenge to functionalize surfaces with *multiple* peptides with control over their micro- and nano-scale spatial organization, which is often essential for biological activity.



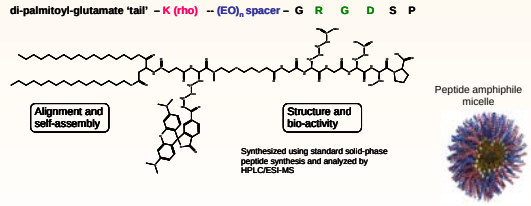
Supported lipid bilayers for surface functionalization



Solid-supported lipid bilayers have been studied extensively as model systems that mimic biological membranes. *Self-assembly* has emerged as a powerful way to create bilayers on surfaces that can be *patterned* on the micro- and nano-scale.

[Groves *et al., Acc. Chem. Res.*, **35**, 149 (2002), Stroumpoulis *et al.*, *Langmuir*, **23**, 3849 (2007)]

Peptide amphiphiles



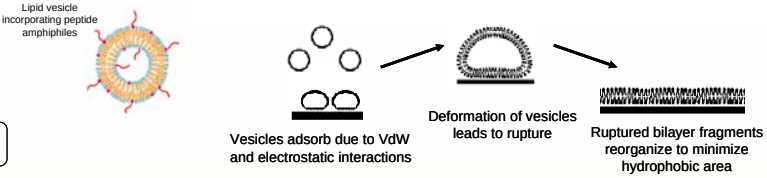
Peptide amphiphiles are synthetic surfactant-like molecules with a *modular* structure consisting of a peptide head-group, PEG-like spacer, and alkyl tail. Their self-assembly allows us to create peptide-functionalized interfaces *in 2D and 3D* in a controlled yet versatile way.

[Berndt *et al.*, *JACS*, **117**, 9515 (1995), Tirrell *et al.*, *Surf. Sci.*, **500**, 61 (2002)]

Supported bilayers displaying peptides formed by vesicle fusion

Vesicle fusion is a facile way to create supported bilayers on solid surfaces using self-assembly. This method is scaleable and fairly robust, further, it can be used to create multi-component mixtures and gradients in membranes by simple physical means.

[Groves *et al., Acc. Chem. Res.*, **35**, 149 (2002), Keller *et al*., *PRL*, **84**, 5443 (2000)]



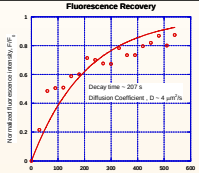
Bilayer Fluorescence Microscopy

100 nm EggPC vesicles: 1 %mol. DiD
5 %mol. di(C16)-K(Rho)-EO2-RGD

Fluorescence imaging of the surface showed smooth topography with some defects.

Fluorescence Recovery After Photobleaching (FRAP)

Measures the recovery of fluorescence due to diffusion of unbleached molecules into a photo-bleached spot

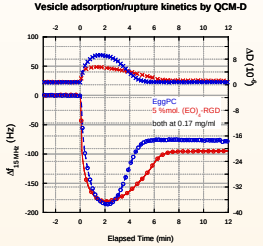


Scaling analysis: $F/F_0 = 1 - \exp(-t/\tau)$; ($\tau$ from fit)
$D = L^2/4\tau$ (L = spot dia)

Fluorescence recovery (FRAP) verified the fluidity (due to lipid mobility) of the bilayer formed. Estimated *D* was of the order reported for fluid lipid bilayers [Ratto *et al.*, *Biophys J.*, **83**, 3380 (2002)]

Quartz-Crystal Microbalance with Dissipation measurement

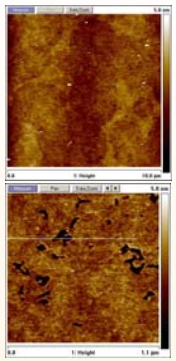
Measures the change in frequency (deposited mass) and dissipation rate (viscosity) of an oscillating quartz crystal



QCM-D data showed that an elastic surface layer was formed. Δf was higher for the more massive peptide amphiphile-containing bilayer; further, the fusion process was slower due to the larger head-groups.

AFM

100 nm EggPC vesicles: 1 %mol. DiD
5 %mol. di(C_{16})-K(Rho)-EO_2-RGD

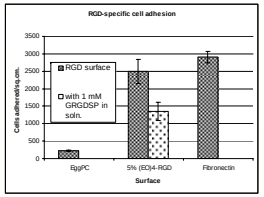


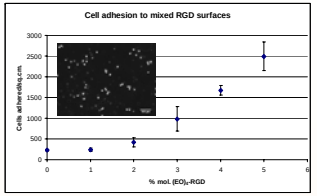
AFM of fully hydrated peptide-displaying bilayers showed a heterogeneous surface with defects, but no clearly observable domains.

Cell adhesion and growth

Cell adhesion to RGD bilayers

Fibroblast cells (NIH 3T3) were incubated on surfaces at 5000 cells/sq. cm. for 1 h. in *serum-free* media. Cells remaining after removal of non-adherent cells were fixed, stained with Hoechst dye and counted.





3T3 cells adhered only on supported bilayers displaying GRGDSP peptides; further, the binding was due to specific recognition of the RGD sequence.

Vesicles displaying GRGDSP peptide were mixed with EggPC vesicles to produce intermediate surface densities. Cell adhesion to these surfaces increased with increasing RGD density.

Current work

Optimal presentation of GRGDSP peptide:
We are currently optimizing the length of the PEG spacer for GRGDSP binding, and exploring the effect of including the PHSRN peptide, which serves as a synergy binding sequence.

Multi-component peptide surfaces for stem cell bioengineering:
The culture of stem cells, especially human Embryonic Stem Cells (hESCs), in fully defined conditions free of animal-derived cells or growth factors, requires development of substrates that can support hESC adhesion and self-renewal. We are using our surface display method to screen combinations of RGD peptides with other adhesion-promoting peptides to develop substrates for stem cell bioengineering.