Engineering Biomimetic Peptides for Targeted Drug Delivery

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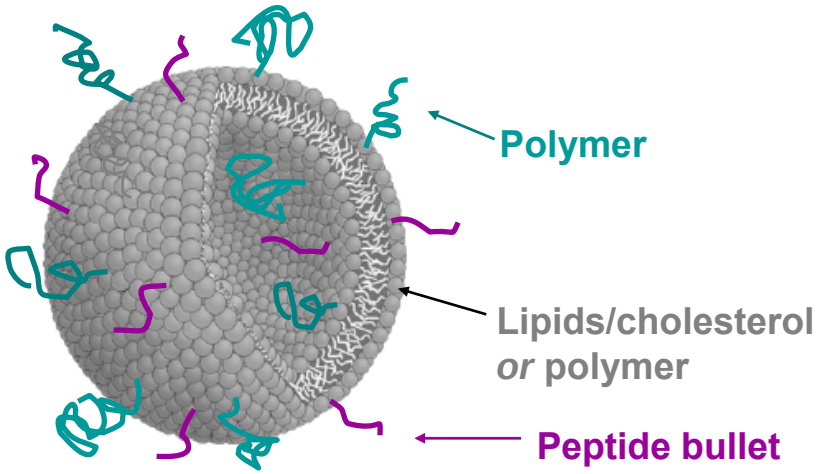
Targeted Drug Delivery

- Medical advancements have been limited by serious adverse effects associated with many of these medicines.

- The effectiveness of many drugs (genes, peptides, proteins) could be greatly enhanced or even enabled if two conditions are met:
  - the drugs are selectively *targeted* to the diseased cells
  - the drugs are *delivered* inside the cells to the site of their pharmacological activity.

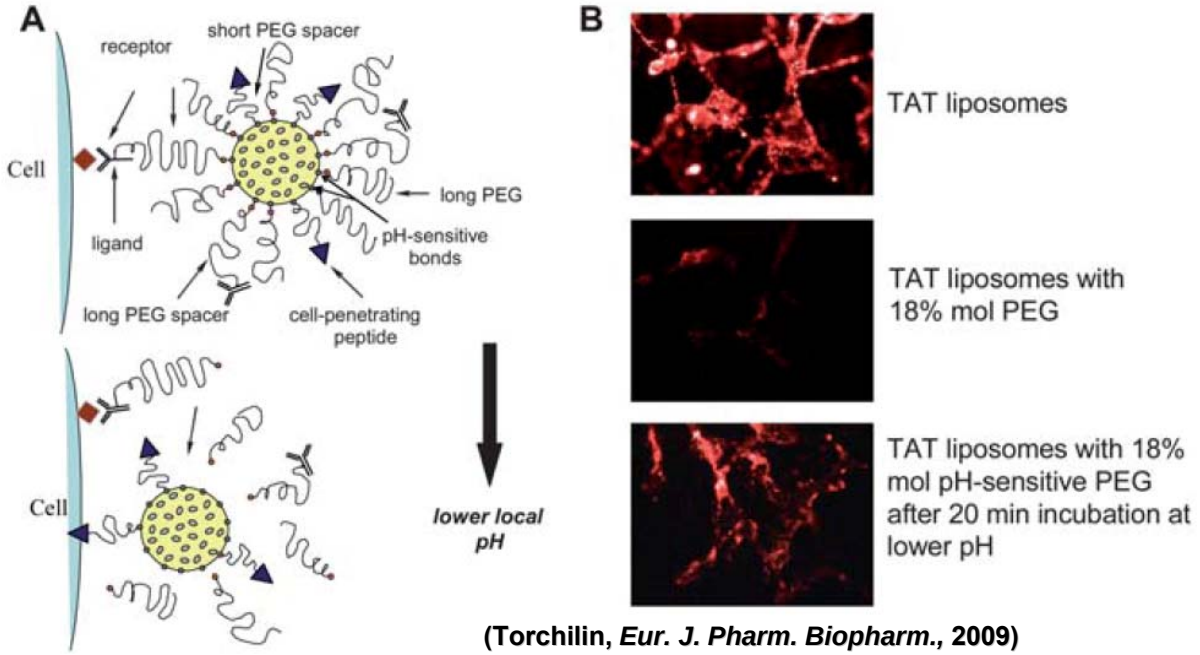


Targeted Stealth Liposomes
"inert" & pH-sensitive

Targeted Polymersomes
"inert" & biodegradable

Targeted Delivery of Nanoparticles
-Video-

Mimicking Viruses:
TAT Cell Penetrating Peptides



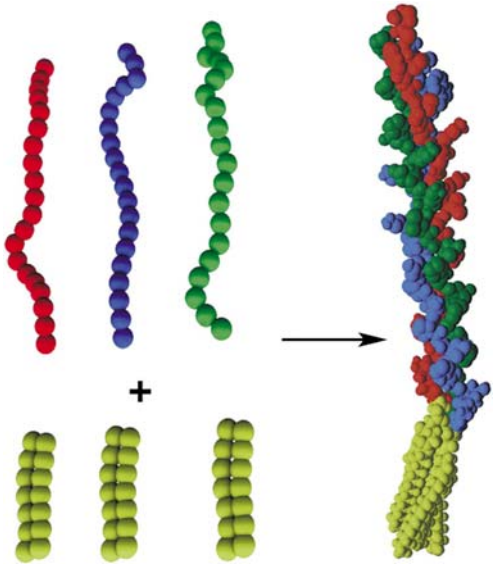
(Torchilin, *Eur. J. Pharm. Biopharm.*, 2009)

- The TAT peptide (GRKKRRQRRRPQ) is derived from the Trans-Activator of Transcription (TAT) protein of human immunodeficiency virus (HIV-1) and is a cell penetrating peptide.

- One of the major obstacles in using the TAT peptide is its lack of selectivity (it will penetrate any cell).

- Solution: Multifunctional "smart" liposomes with temporarily ''hidden'' function, for example TAT, and ''shielding'' polymeric coat with or without targeting antibody attached to it.

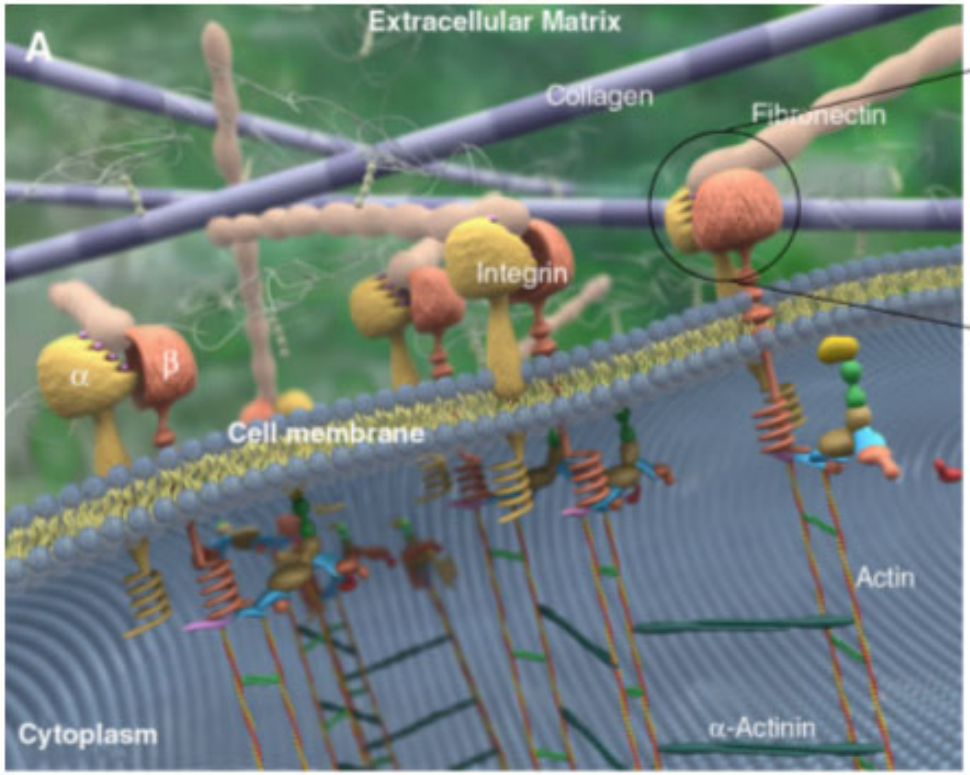
Mimicking Protein Secondary Structure: Collagen-Mimetic Peptides

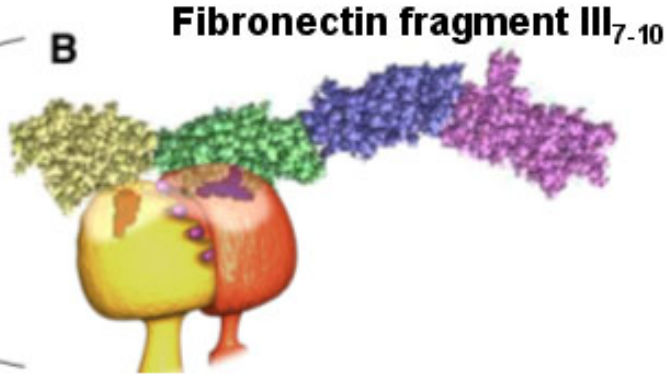


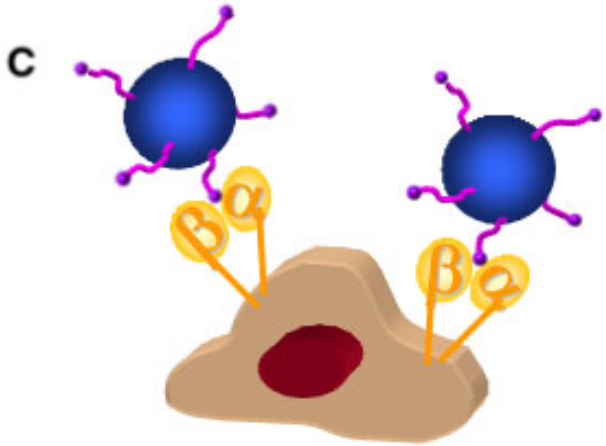
Tirrell *et al.*, *Surf. Sci.*, 2002

- Collagen-mimetic peptides have been developed to target the CD44 receptor that is over-expressed in many tumor cells.
- CD44 receptors bind to a specific amino acid sequence from type IV collagen $\alpha1(IV)_{1263\text{-}1277}$ (GVKGDKGNPGWPGAP), called IV-H1, and more importantly, binding is highly dependant on the triple helical structure of the sequence (Lauer-Fields *et al., J. Biol. Chem.,* 2003).
- IV-H1 peptide-amphiphiles were incorporated into stealth liposomes, targeted to M14#5 metastatic melanoma cells, and promoted specific ligand/receptor interactions where as non-targeted liposomes showed no binding (Rezler *et al., J. Am. Chem. Soc.,* 2007).

Mimicking Multidomain Binding: Fibronectin-Mimetic Peptides



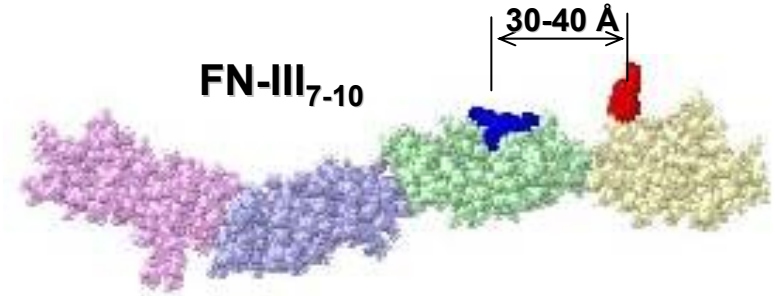




Adapted from Tirrell *et al.*, *Surf. Sci.*, 2002

- $\alpha_5\beta_1$ integrin has impact on processes such as:
  - accelerating wound healing
  - promoting angiogenesis
  - mediating adenovirus infection
  - protection mechanism against Alzheimer's disease
  - promising target for breast, colon, rectal & prostate cancer

Fibronectin-Mimetic Peptides



Leahy *et al*., *Cell*, 1996

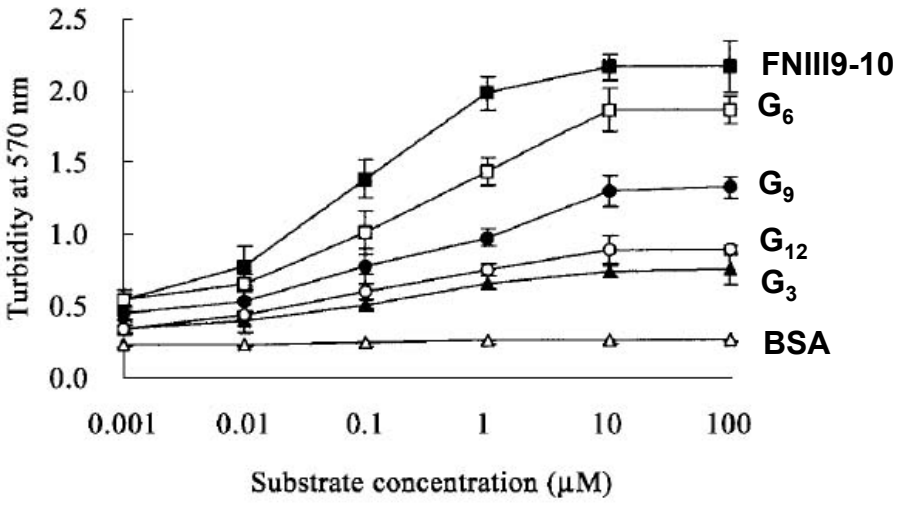
Spacer designs by others focused on the distance between PHSRN and RGD:

- G_6 – Kao *et al*., *J. Mater. Sci.-Mat. Med.*, 1999
- G_3, G_6, G_9, G_{12} – Kim *et al*., *Biotech. Let.*, 2002
- PEG hybrid – Susuki *et al*., *Chem. Pharm. Bull.*, 2002
- no linker – Aucoin *et al*., *J. Biomater. Sci. Polym. Edn.*, 2002
- G_{13} – Benoit & Anseth, *Biomaterials*, 2005

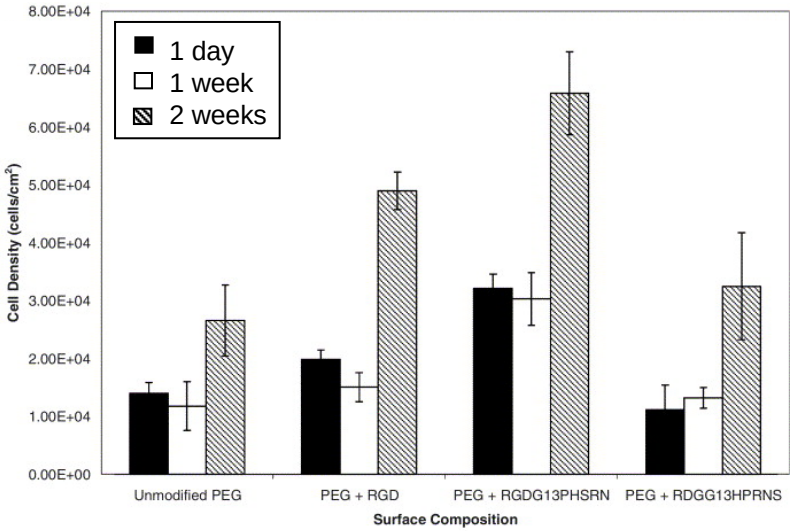
Fibronectin – Mimetic Peptides

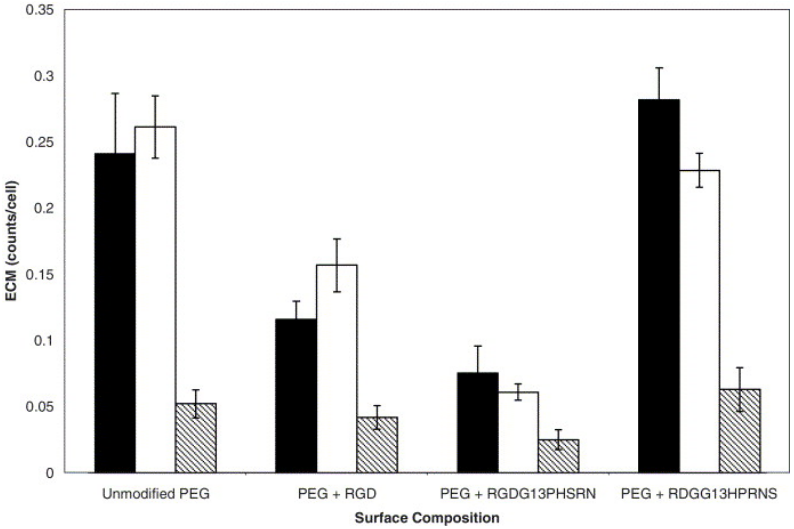
Kim *et al.*, *Biotech. Let.*, 2002

1hr incubation



Benoit & Anseth, *Biomaterials*, 2005





- Designs compared to FN showed smaller adhesion
- Surfaces used in other applications (e.g., tissue engineering) should be optimized to promote cell adhesion and ECM production

Cell Adhesion and Function: Peptides versus Proteins

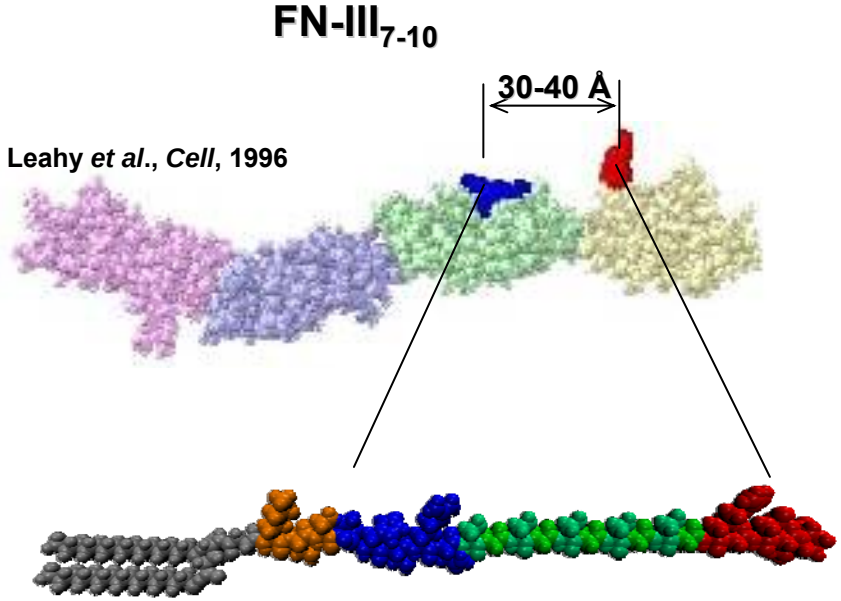
For a surface saturated with a peptide versus a surface saturated with the protein:

- More active binding sites on the peptide interface
- Easily control peptide orientation
- Prevent peptide denaturation

Importance of Hydrophobic/Hydrophilic Interactions

- Ratio of hydrophobic/hydrophilic surface sites is important for colloidal particle recognition (Kokkoli & Zukoski, *Langmuir*, 2001).

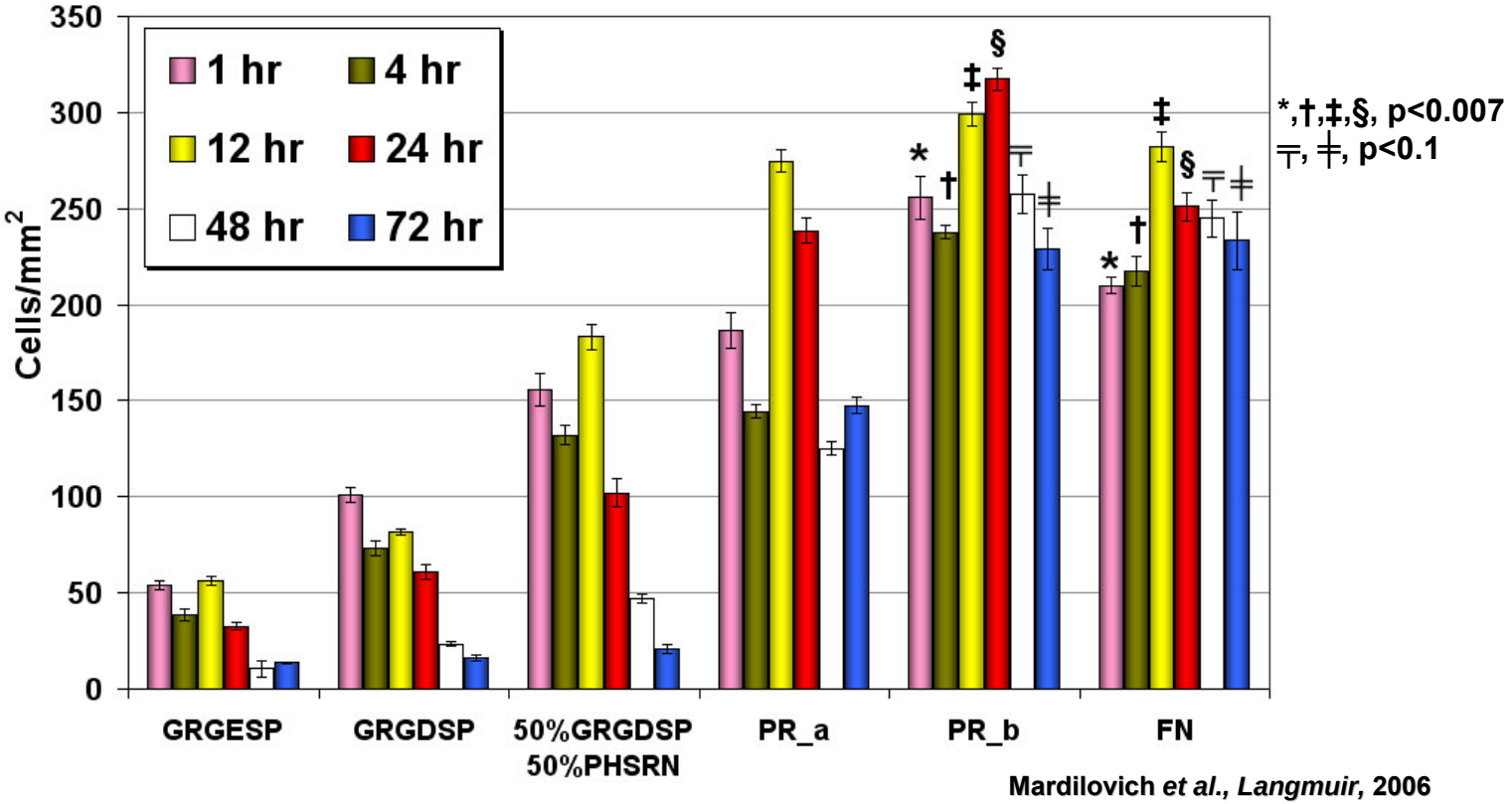
- **Our hypothesis:** Length & hydrophobicity/hydrophilicity of linker can affect integrin affinity for the biomimetic-peptide (Mardilovich & Kokkoli, *Biomacromolecules*, 2004).



$(C_{16})_2GluC_2$-KSSPHSRN$(SG)_5$RGDSP (PR_b)

37 Å

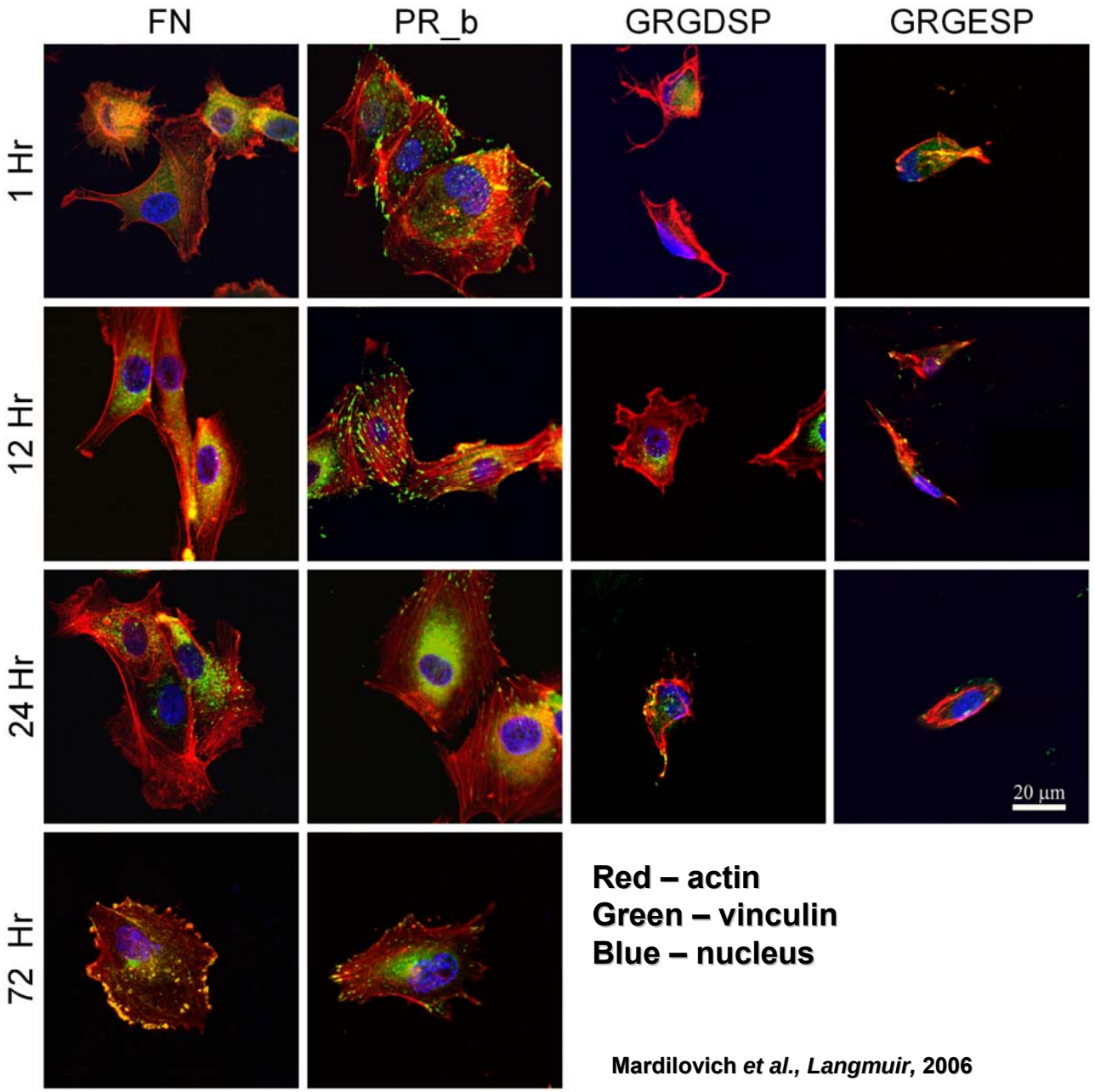
Endothelial Cell Adhesion



Mardilovich *et al.*, *Langmuir*, 2006

- Serum-free environment
- GRGDSP and 50%PHSRN-50%GRGDSP fail after 24 hr
- The PR_b peptide-amphiphile surfaces outperform all other peptide surfaces and compared to FN surfaces give higher adhesion for 1-24 hr and similar adhesion for 48-72 hr

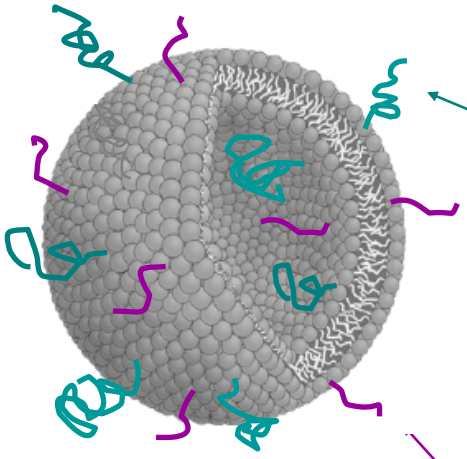
Endothelial Cell Cytoskeletal Organization



Red – actin
Green – vinculin
Blue – nucleus

Mardilovich *et al.*, *Langmuir*, 2006

- **GRGDSP and GRGESP collapse after 24 hr**
- **Highly-developed cytoskeletal structure on PR_b: elongated actin stress fibers & sharp spikes of vinculin at termination points and across actin**



$\alpha_5\beta_1$-Targeted Delivery with PR_b Functionalized Liposomes

Gene Therapy for Metastatic Colorectal Cancer (Stage 4)

Unpublished Data

Summary

- Targeted drug delivery offers many advantages, such as, specific delivery to the tumors, less side effects, and use of less drugs.

- Biomimetic peptides are promising “bullets”.

Acknowledgements