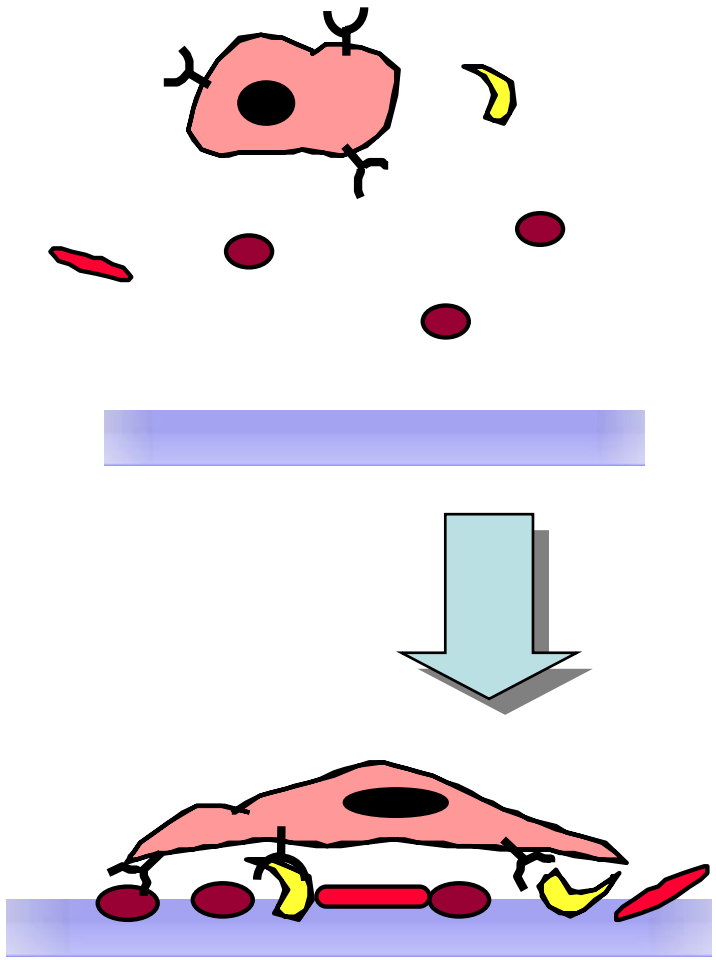
Materials with Biological Recognition (continued)

Last time:	Biological recognition *in vivo* Engineering biological recognition of biomaterials: adhesion/migration peptides
˙oday:	Engineering biological recognition of biomaterials: enzymatic recognition and cytokine signaling
Reading:	J.C. Schense et al., 'Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension,' *Nat. Biotech*. **18**, 415-419 (2000)
Supplementary Reading:	-

ANNOUNCEMENTS:

Cell adhesion on biomaterials:

Cell responses to non-biological, synthetic biomaterials



1. Protein adsorption
2. Denaturation (unfolding)?
3. Cell responses to expected and unexpected epitopes
4. Reorganization?
   - Vroman effect: protein exchange

≥ CRITICAL FACTORS CONTROLLING ADHESION ON BIOMTLS:

(1) PROTEIN ADSORPTION/PRESENTATION

(2) SUBSTRATE STIFFNESS

Control of cell attachment by mechanical properties of substrate

Polyelectrolyte multilayers (Rubner lab MIT):

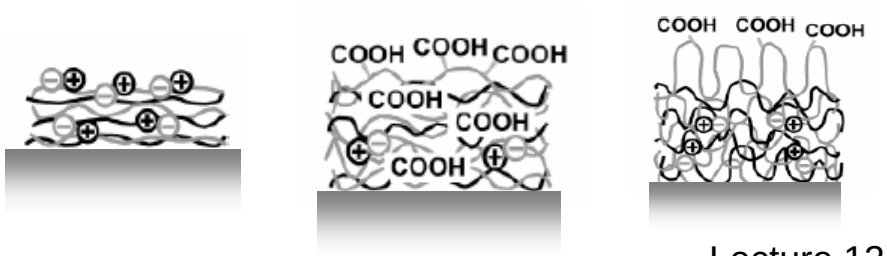
Images removed due to copyright reasons.
Please see:
Mendelsohn, Jonas D., Sung Yun Yang, Jeri'Ann Hiller, Allon I. Hochbaum, and Michael F. Rubner. "Rational Design of Cytophilic and Cytophobic Polyelectrolyte Multilayer Thin Films." *Biomacromolecules* 4 (2003): 96-106.

Control of cell attachment by mechanical properties of substrate

(Van Vliet and Rubner labs):

Graph removed due to copyright reasons.
Please see:
Figure 3 in Thompson, M. T., et al. *Biomaterials* 26 (2005): 6836–6845.

Graph removed due to copyright reasons.
Please see:
Figure 4 in Thompson, M. T., et al. *Biomaterials* 26 (2005): 6836–6845.

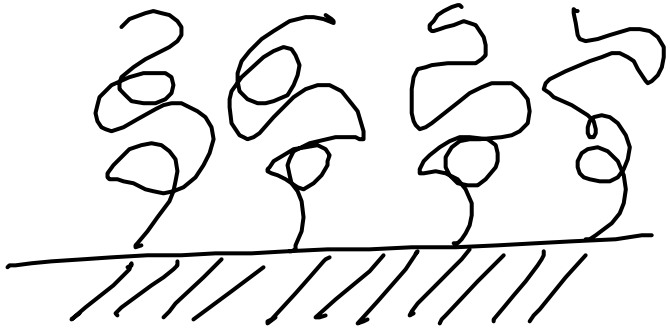


Controlling cell response to biomaterials by building in ECM cues on a 'blank slate' background

MAKING A PROTEIN-RESISTANT SURFACE: POLYMER BRUSH

GRAFTED HYDROPHILIC POLYMER CHAINS:

e.g., PEO
DEXTRAN
PVA → $+CH_2-CH+_n$ (OH)



$\sigma \equiv$ GRAFTING DENSITY $= \frac{\#\ \text{CHAINS}}{\text{AREA}}$

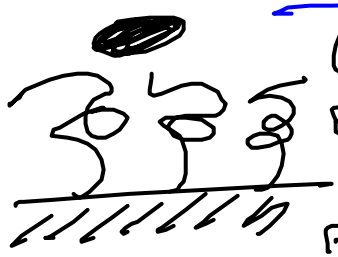
2 APPROACHES TO PROTEIN RESISTANCE:

KINETIC

← ENTHALPIC GAIN OUTWEIGHS ENTROPIC PENALTY

EQUILIBRIUM STATE TAKES MUCH LONGER THAN EXPERIMENTAL TIME SCALES TO REACH

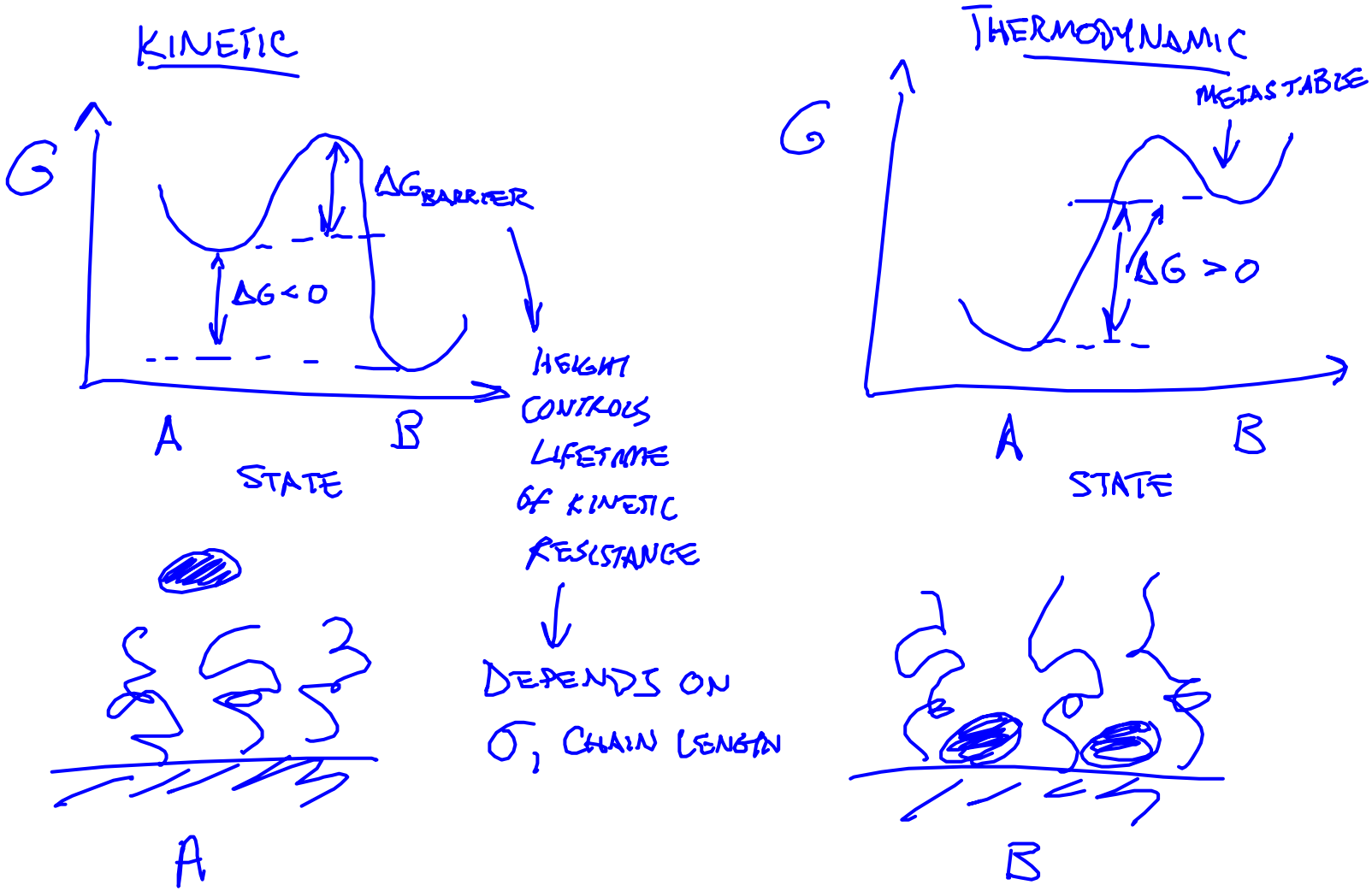
THERMODYNAMIC



LOWEST FREE ENERGY STATE EXCLUDES PROTEIN FROM SURFACE: ENTROPIC PENALTY FOR STRETCHING CHAINS TOO LARGE.

↳ REQUIRES HIGH σ $\frac{\text{AND}}{\text{OR}}$ LONG CHAINS

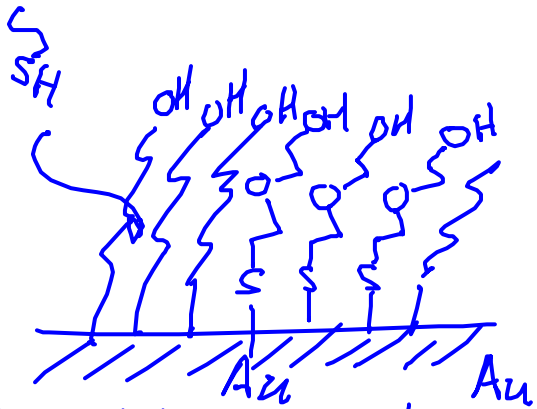
Design of protein adsorption-resistant surfaces



Design of protein adsorption-resistant surfaces

Surface modification strategies:

Self-assembled monolayers (SAMs):

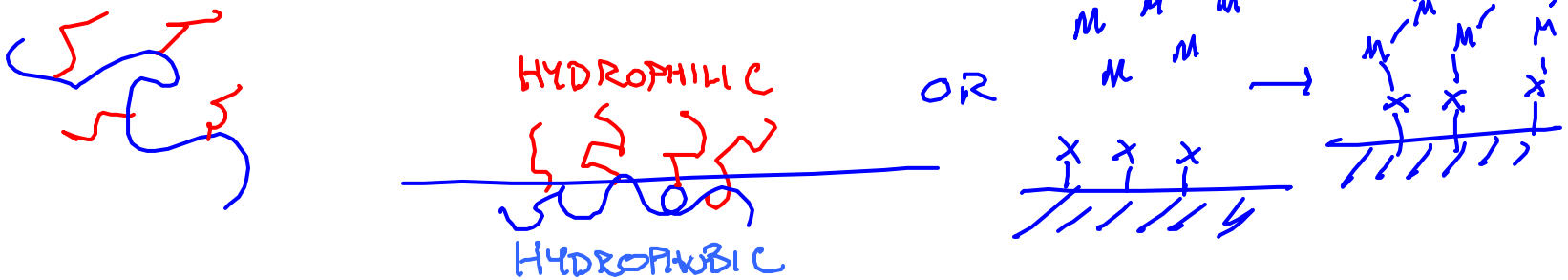


VERY HIGH Θ ACHIEVED BY USING VERY SHORT CHAINS:

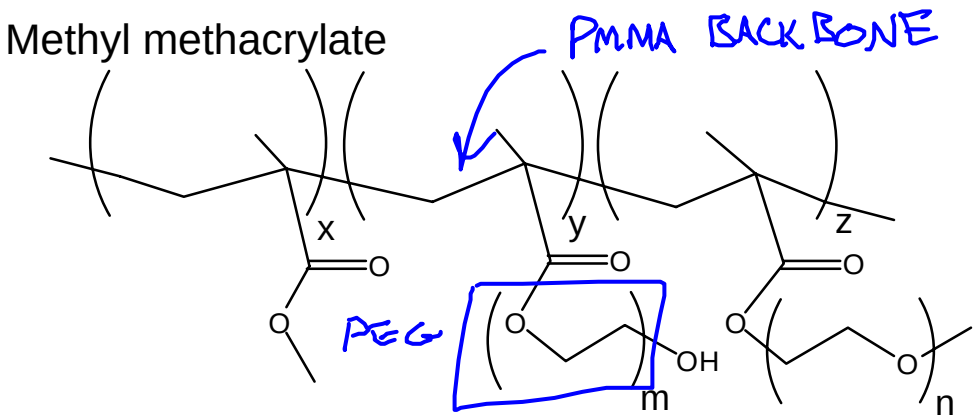
EVEN 3 "EO" REPEAT UNITS CAN GIVE PROTEIN RESISTANCE!

Surface grafting:

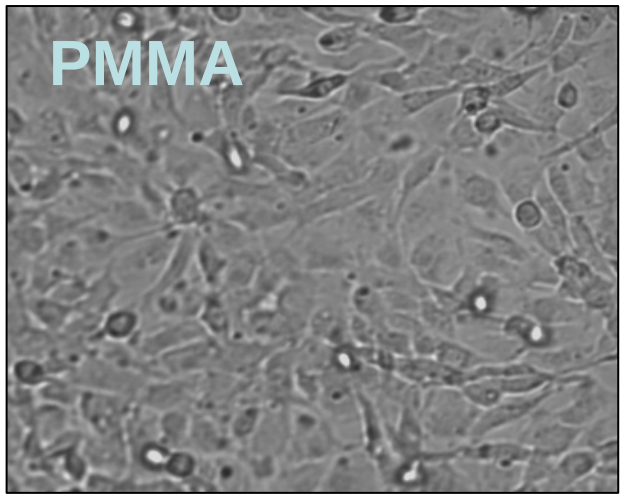
Graft copolymers or surface polymerization:

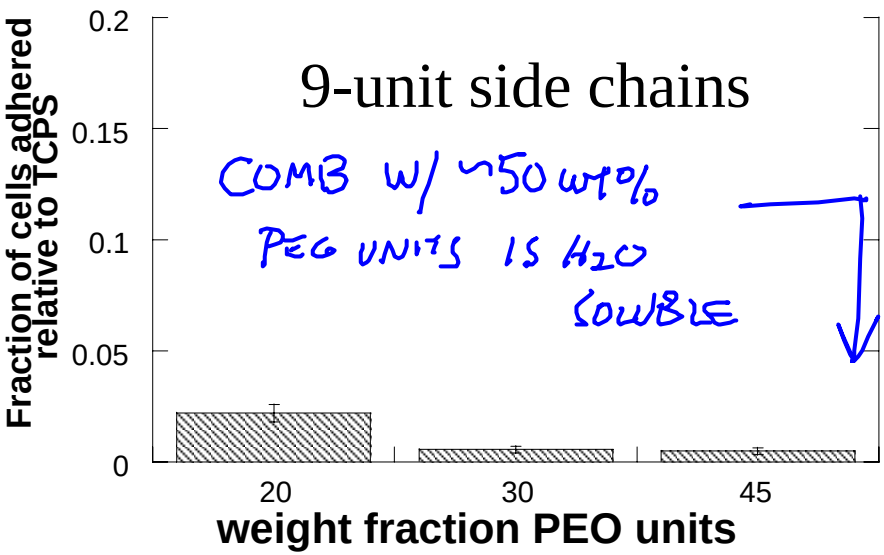


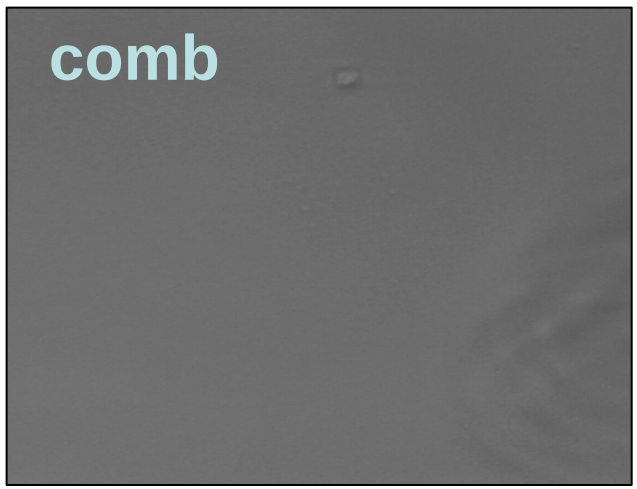
Limiting nonspecific cell adhesion



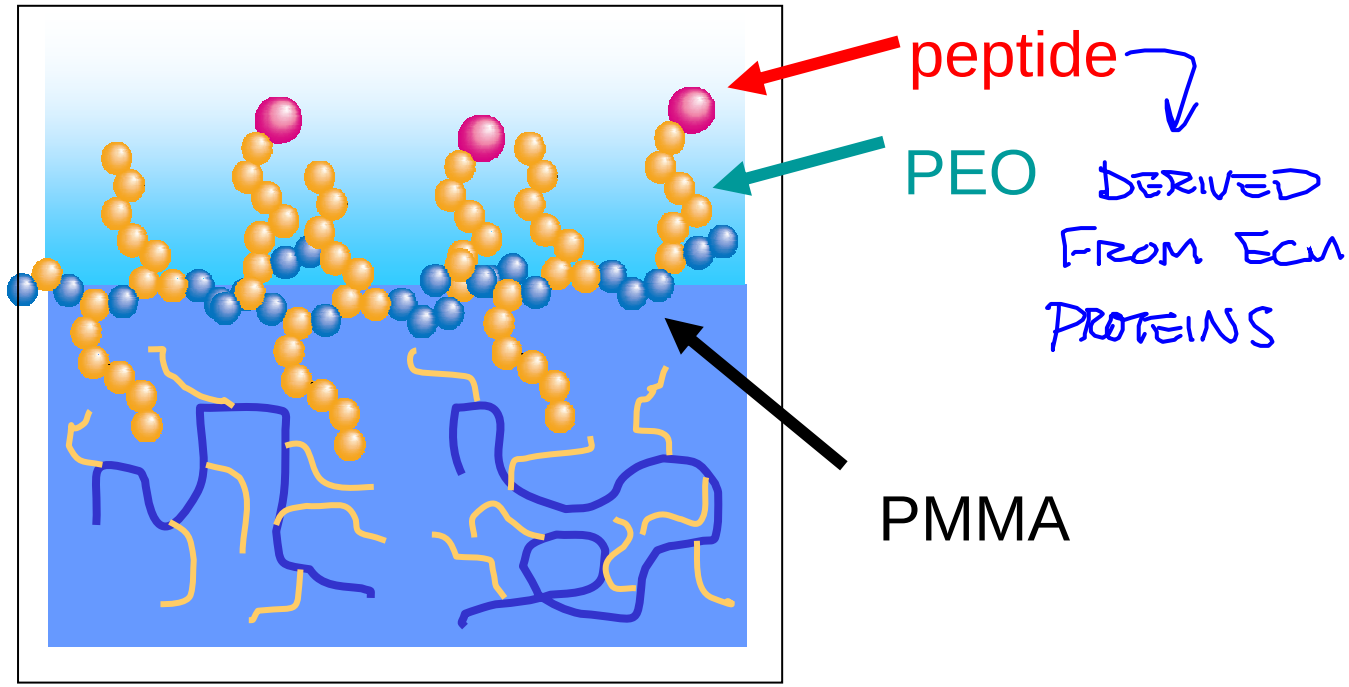
Poly(ethylene glycol) methacrylates







Tailoring cell adhesion on biomaterials via immobilized ligands



Peptide integrin-binding GRGDSP sequence

PEO short 6-9 unit side chains for protein resistance

PMMA backbone anchors hydrophilic side chains

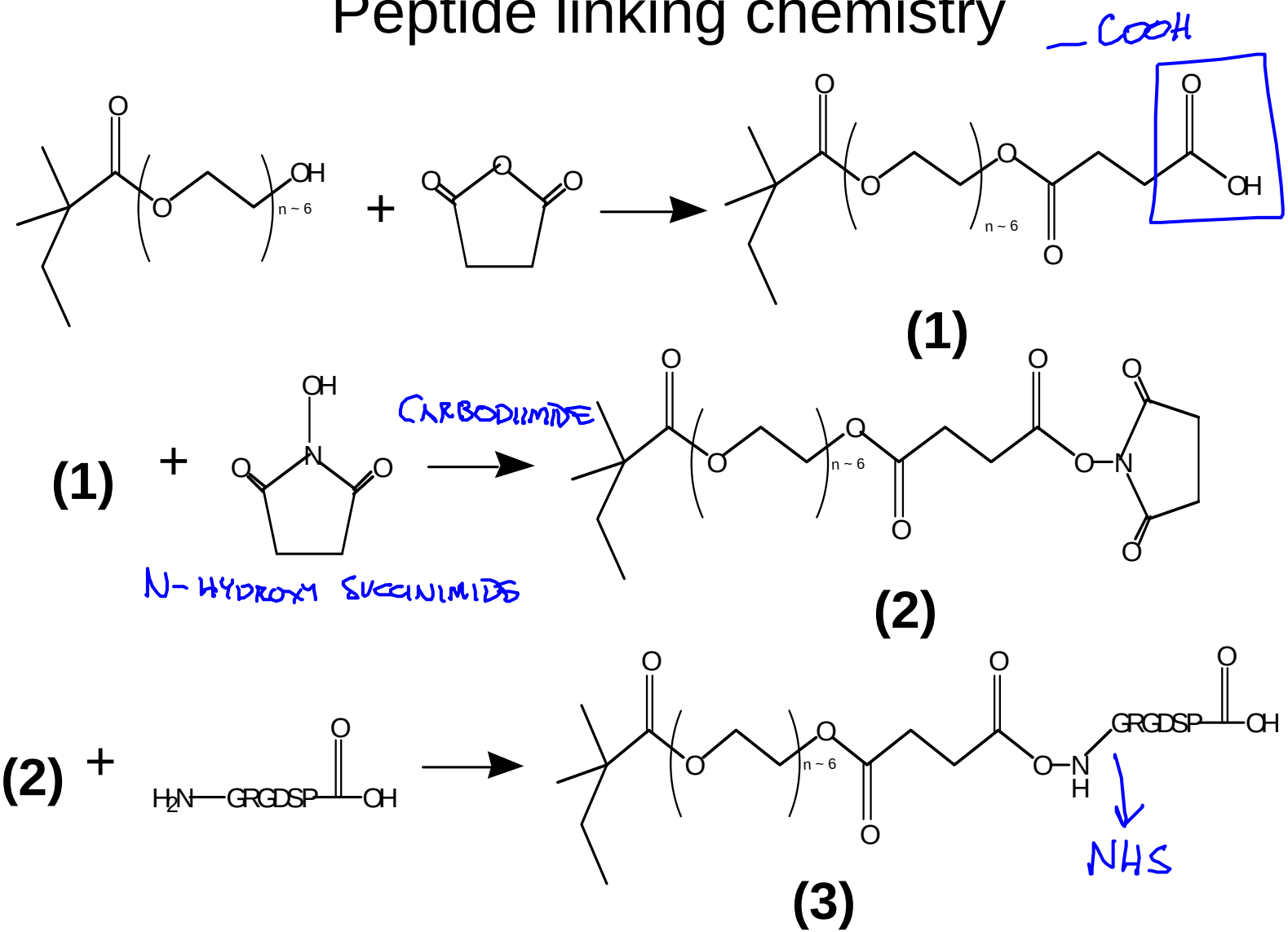
Peptides used to modulate cell adhesion on biomaterials

Peptide sequence	Derived from	Conjugate receptor	Role
IKVAV	Laminin α-chain	LBP110 (110 KDa laminin binding protein)	Cell-ECM adhesion
RGD	Laminin α-chain, fibronectin, collagen	Multiple integrins	Cell-ECM adhesion
YIGSR	Laminin β1-chain	$\alpha_1\beta_1$ and $\alpha_3\beta_1$ integrins	Cell-ECM adhesion
RNIAEIIKDI	Laminin γ-chain	unknown	Cell-ECM adhesion
HAV	N-cadherin	N-cadherin	Cell-cell adhesion
DGEA	Type I collagen	$\alpha_2\beta_1$ integrin	Cell-ECM adhesion
VAPG	Elastase	Elastase receptor	Cell-ECM adhesion
KQAGDV	Fibrinogen γ-chain	β_3 integrins	Cell-ECM adhesion

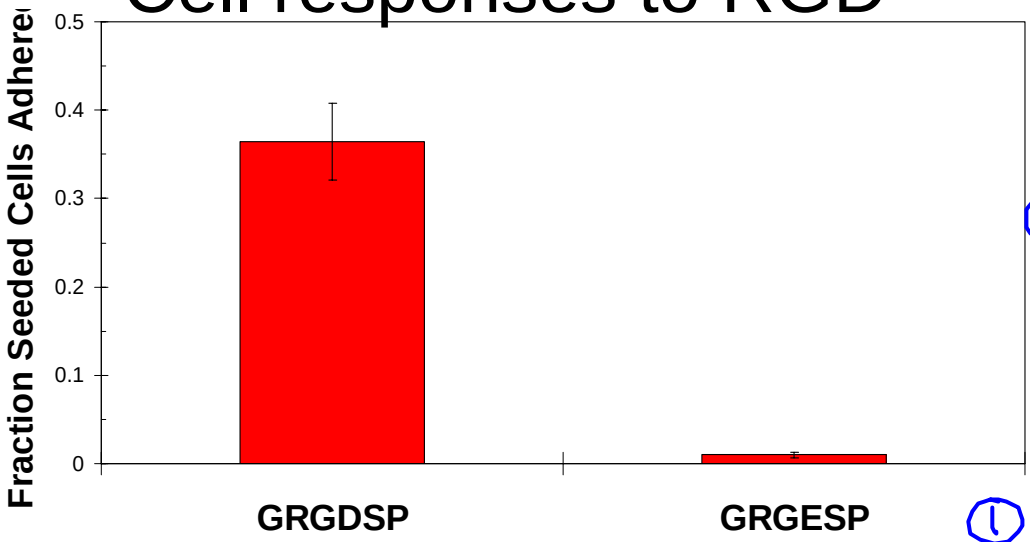
RGD BINDS INTEGRINS W/1000-FOLD LOWER K_D

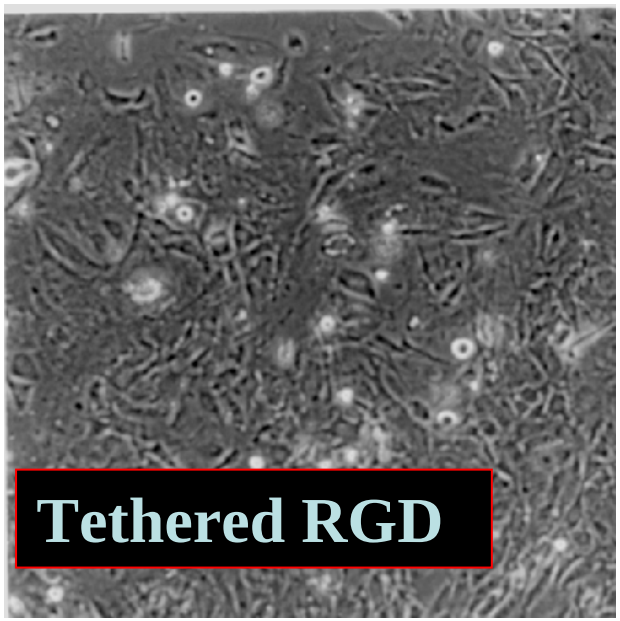
- PEPTIDES MORE ROBUT THAN INTACT PROTEIN
- EASY TO SYNTHESIZE IN HIGH PURITY
- K_D (BINDING AFFINITY) OR RECEPTOR BINDING TO MINIMAL PEPTIDES TYPICALLY MUCH WEAKER THAN NATIVE PROTEIN

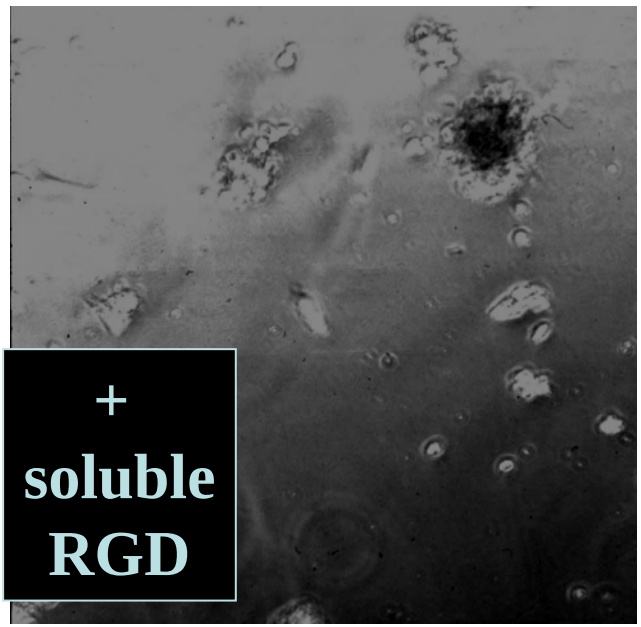
Peptide linking chemistry

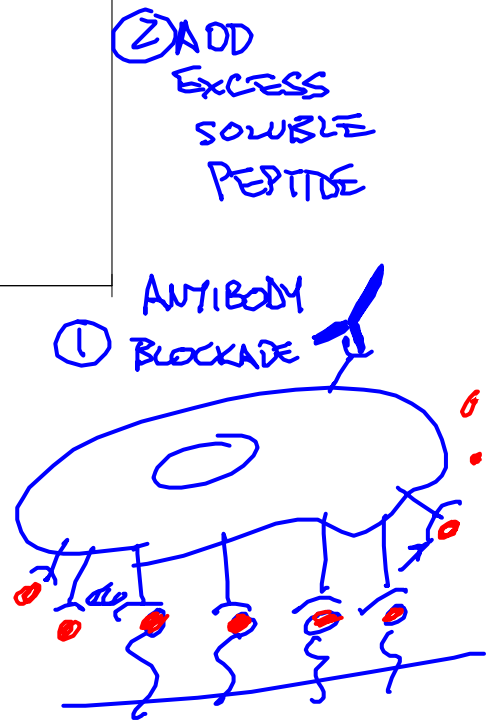


Cell responses to RGD

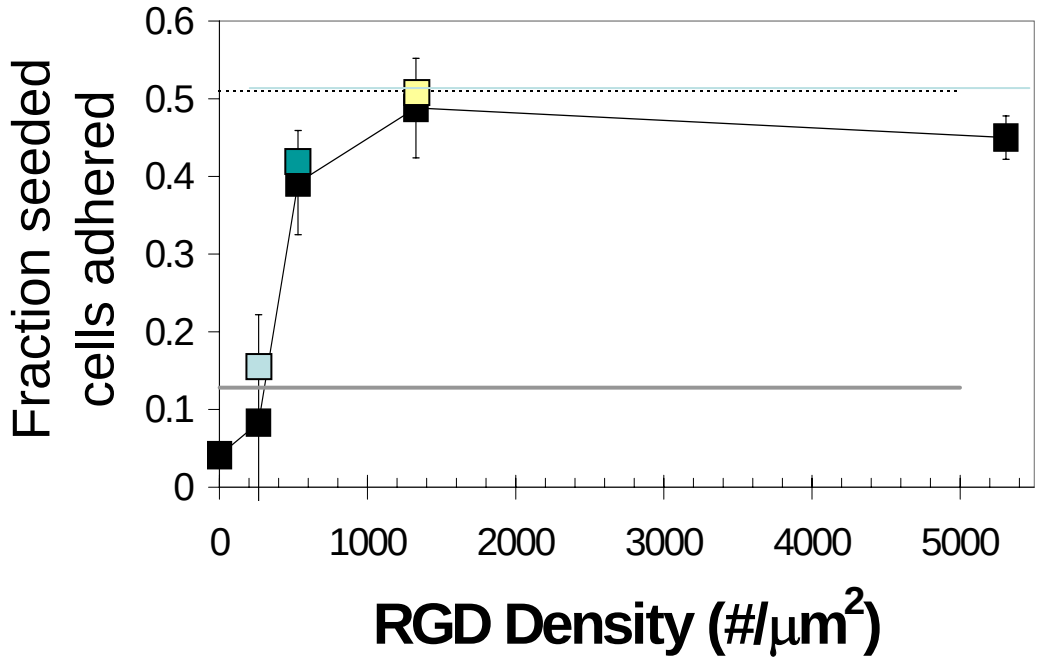


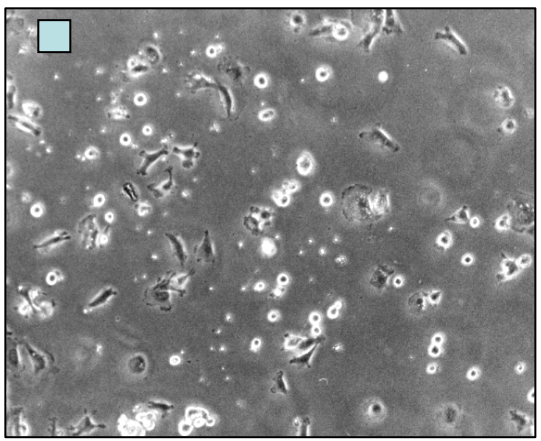


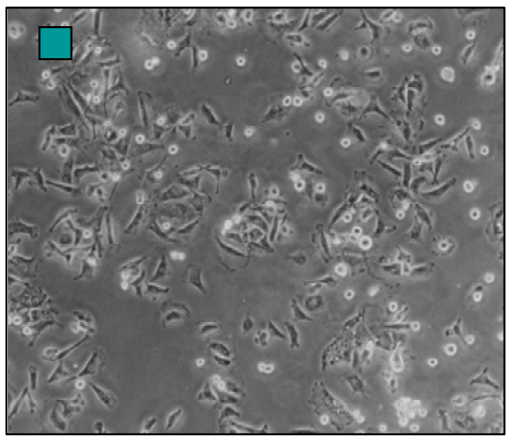


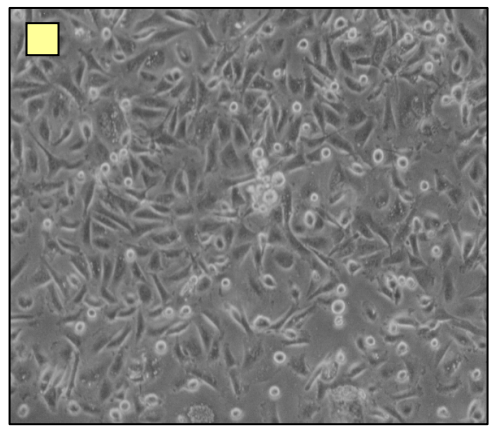


Cells respond to control of ligand density at the surface









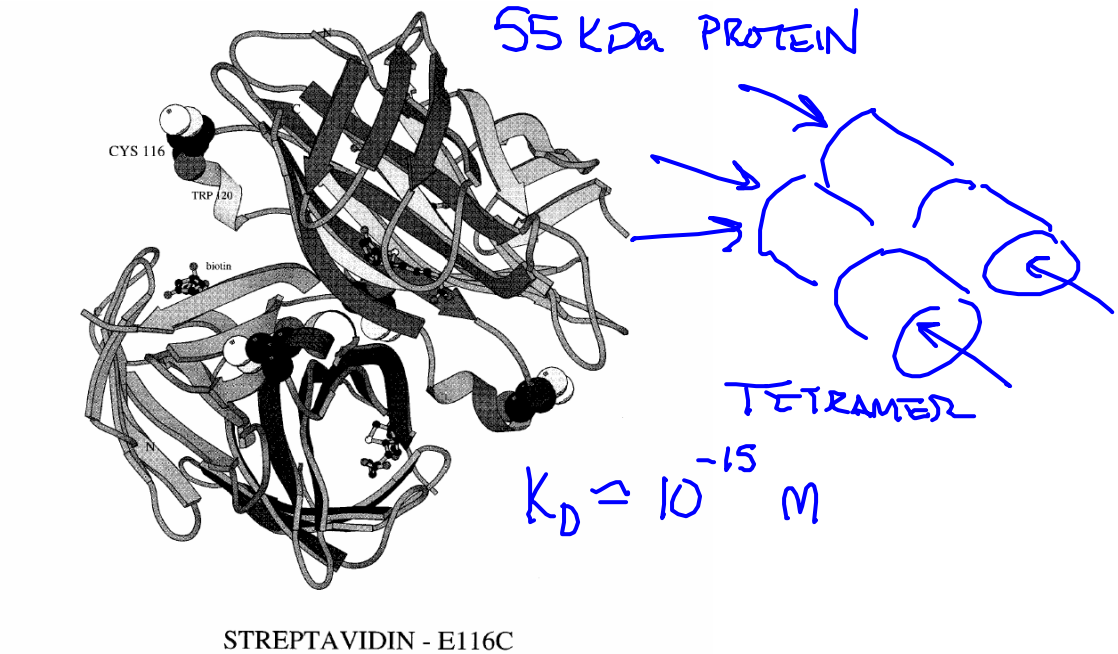
Cells respond to control of ligand density at the surface

Cell migration on fibronectin-coated substrates:

Graph removed due to copyright reasons.
Please see:
Figure 1b in Palecek, S. et al. "Integrin-ligand Binding Properties Govern Cell Migration Speed Through Cell-substratum Adhesiveness." *Nature* 385 (6 February, 1997): 537 - 540.

Graphs removed due to copyright reasons.
Please see:
Figure 2b in Palecek, S., et al. "Integrin-ligand Binding Properties Govern Cell Migration Speed Through Cell-substratum Adhesiveness." *Nature* 385 (6 February, 1997): 537 - 540.

Alternative functionalization approaches: avidin-biotin chemistry



STREPTAVIDIN - E116C

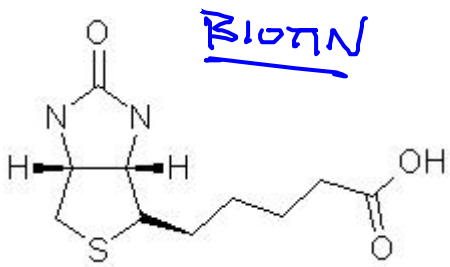


Image removed due to copyright reasons.
Please see:
Patel, et al. *FASEB Journal* 12 (1998): 1447-454.

Controlling gross physical distribution of cells

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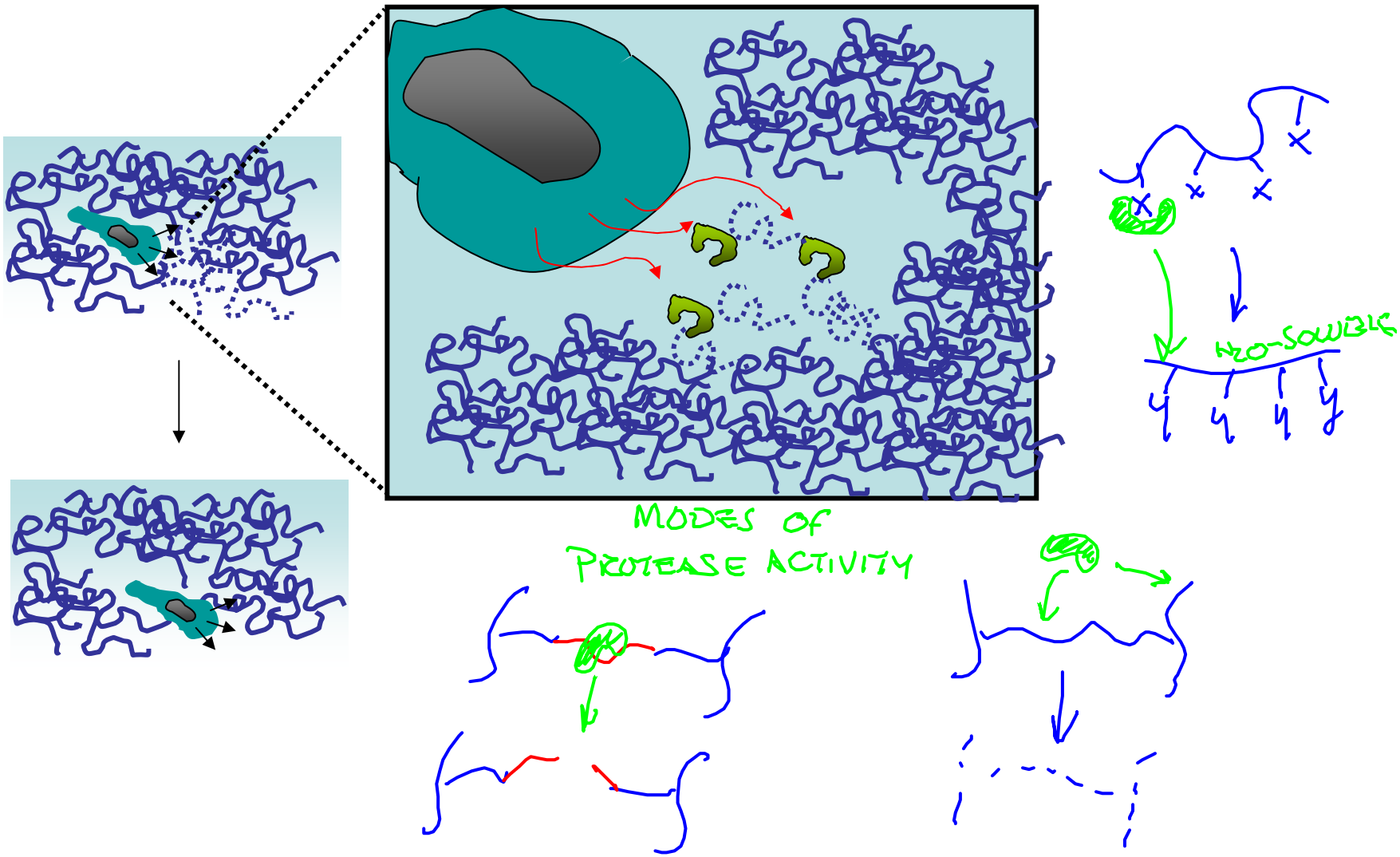
Please see:
Patel, et al. *FASEB Journal* 12 (1998): 1447-454.

Cellular responses to physically patterned ligand- with nonadhesive background

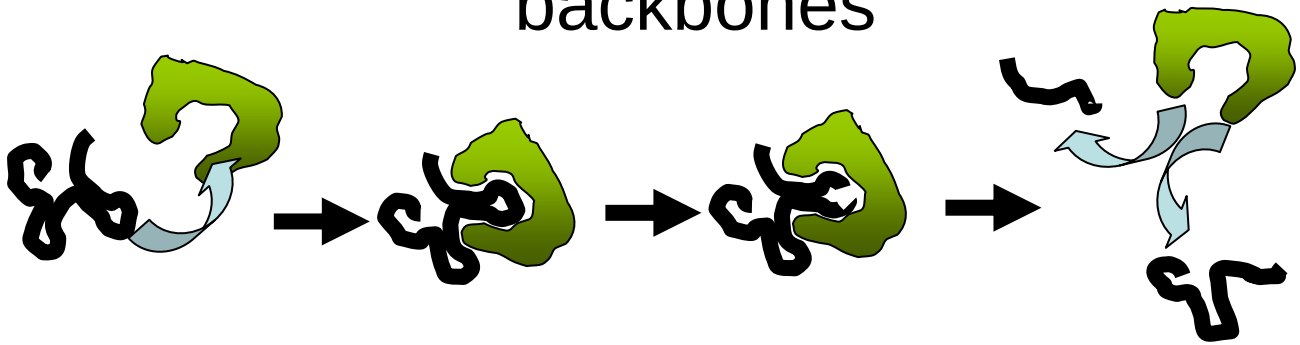
Images removed due to copyright reasons.
Please see:
Patel, et al. *FASEB Journal* 12 (1998): 1447-454.

Biomaterials recognized by cell-secreted enzymes:
synthetic ECMs

Enzymatic remodeling of synthetic ECMs



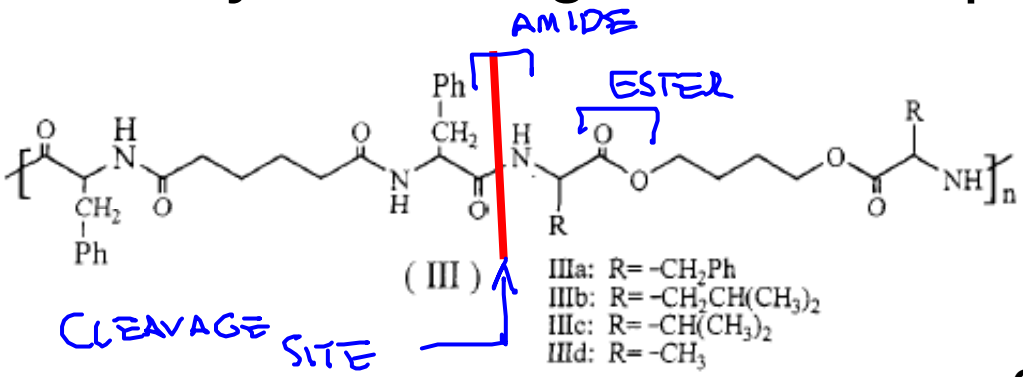
Enzymatic recognition of synthetic polymer backbones



Cleavage of synthetic polymers by enzymes

Cell source	Enzyme	Native function	Acts on	Degradation Mechanism	Result
Various bacteria	lipases	protease	Polyesters, polyesteramides	III	Monomers or dimers
Tritirachium album (mold)	Proteinase K	Protease	Poly(lactide)	III	Monomers or dimers
Mammalian cells	esterases	protease	Poly(alkyl cyanoacrylates)	II	Water-soluble polymers
Mammalian cells	Papain, pepsin	proteases	polyesteramides[2]	III	Untested
Mammalian cells	α-chymotrypsin	Serine protease	Aromatic peptides in polyesteramides[3] (e.g. Ala, Val, Leu)	III	Untested
Mammalian cells	elastase	protease	Polyesteramides	III	untested

Enzymatic degradation of polyesteramides



Enzymatic breakdown by papain:

N. Paredes et al. *J. Polym. Sci. A* **36**, 1271 (1998)

Compare with hydrolysis: (poly(ortho ester))

Graph removed due to copyright reasons.
Please see:
Figure 10 in Paredes, N., et al. *J. Polym. Sci. A* 36, no. 1271 (1998).

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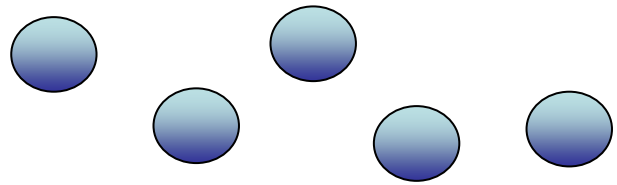
Please see:
Figure 12 in Paredes, N., et al. *J. Polym. Sci. A* 36, no. 1271 (1998).

Esterase attack on poly(alkyl cyanoacrylates)

Degradation of 250 nm-diam. porous particles:



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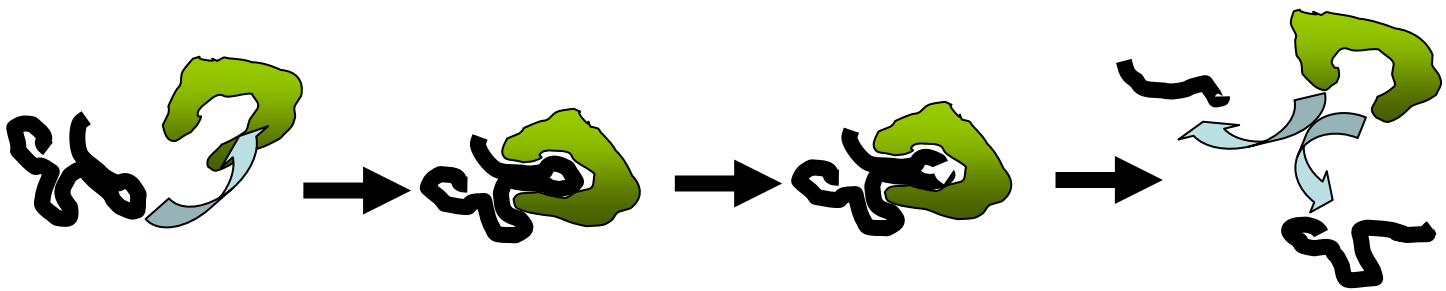
Please see:
Figure 11 in Paredes, N., et al. *J. Polym. Sci. A* 36, no. 1271 (1998).

Graph removed due to copyright reasons.
Please see:
Figure 2 in Paredes, N., et al. *J. Polym. Sci. A* 36, no. 1271 (1998).

Engineering enzymatic recognition of hydrogel biomaterials: recognition of peptide motifs

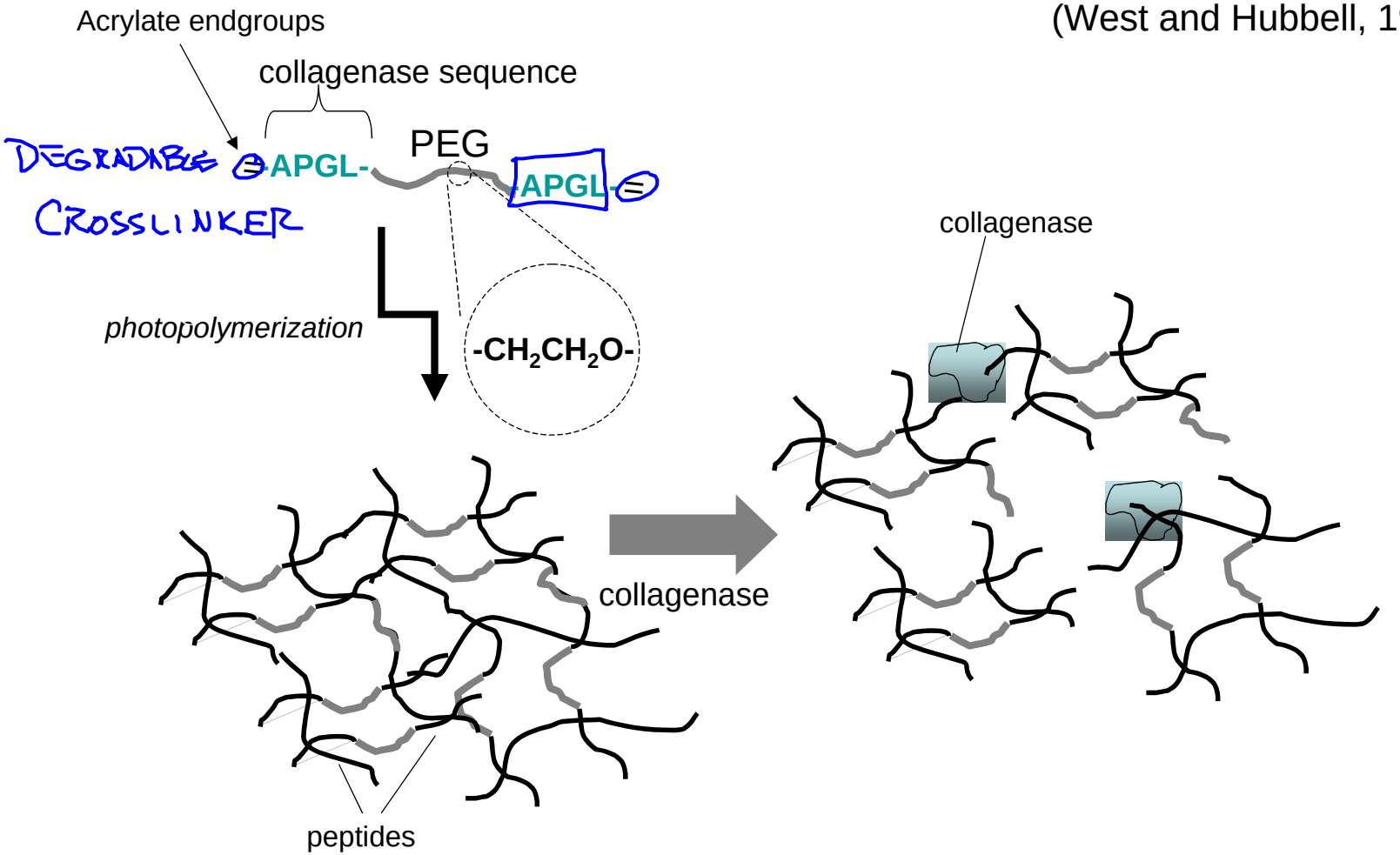
Enzymatic activity in vivo on peptide sequences: [5,6]

Cleavage Enzyme	**Functions *in vivo***	**Target amino acid sequences**
Plasminogen activator (urokinase or tissue-type plasminogen activator) / plasminogen → plasmin	Degradation of fibrin matrices, angiogenesis, tumor progression; urokinase can bind to cell surface receptor	on fibrinogen: Arg_{104}-Asp_{105}, Arg_{110}-Val_{111}, Lys_{206}-Met207, Arg_{42}-Ala_{43}, Lys_{130}-Glu_{131}, Lys_{84}-Ser_{85}, Lys_{87}-Met_{88}
Matrix metalloproteinases (soluble and cell-surface): e.g. Fibroblast Collagenase (MMP I)	Facilitate cell migration	Type I collagen: Gly_{775}-Ile_{776} In smaller peptides: Gly-Leu or Gly Ile bonds —G—I— —G—L—
Elastase	Elastin remodeling	Poly(Ala) sequences —A—A—A—A—



Enzyme-sensitive crosslinks in hydrogel biomaterials

(West and Hubbell, 1999)



Effect of enzyme concentration

Gel containing collagenase sequence

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Please see:
Figure 1 in West, J.L. and J. A. Hubbell. "Polymeric Biomaterials with Degradation Sites for Proteases Involved in Cell Migration." *Macromolecules* 32 (1999): 241-244.

Gel containing elastase sequence

Graph removed due to copyright reasons.
Please see:
Figure 2 in West, J.L. and J. A. Hubbell. "Polymeric Biomaterials with Degradation Sites for Proteases Involved in Cell Migration." *Macromolecules* 32 (1999): 241-244.

Cellular migration through enzymatically-recognized hydrogels

Biphasic migration response in 3D matrix:

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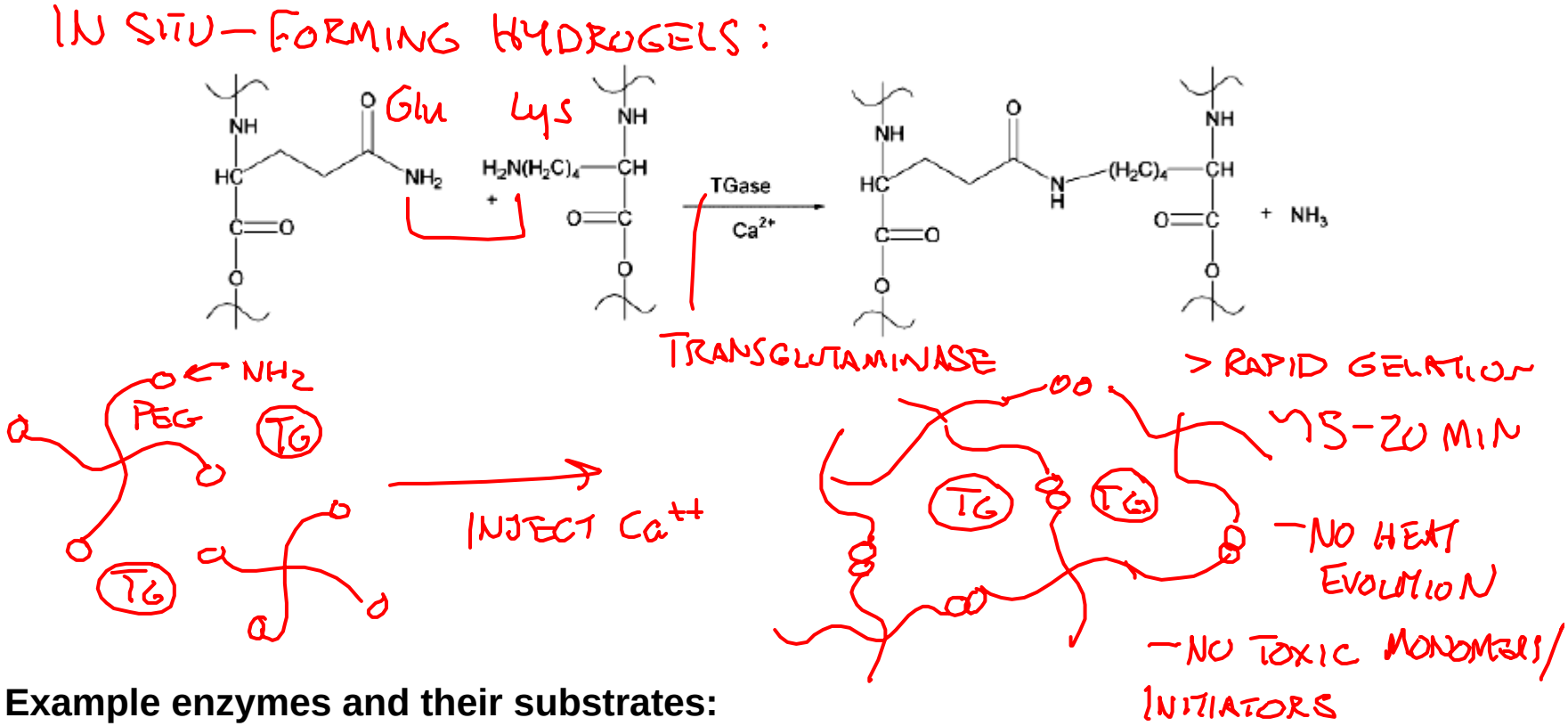
Please see:
Figure 4 in Gobin, A.S. and J. L. West. “Cell Migration Through Defined, Synthetic ECM Analogs.” *Faseb J* 16 (2002): 751-3.

Image removed due to copyright reasons.
Please see:
Figure 6 in Gobin, A.S. and J. L. West. “Cell Migration Through Defined, Synthetic ECM Analogs.” *Faseb J* 16 (2002): 751-3.

Enzymatic recognition of biomaterials II: Enzymatic cross-linking/modification of biomaterials



Example enzymes and their substrates:

Enzyme	Substrate *in vivo*	Synthetic substrates	Result
Transglutaminase	Glutamines	Glu-containing peptides	Amide bond formation
Factor XIII	Fibrin γ-chain	Peptides derived from γ-chain FXIII binding site	Amide bond formation

(Zhang et al. 2002)

Biomaterials that mimic signals from soluble factors or other cells

Cytokine receptor-based recognition of biomaterials

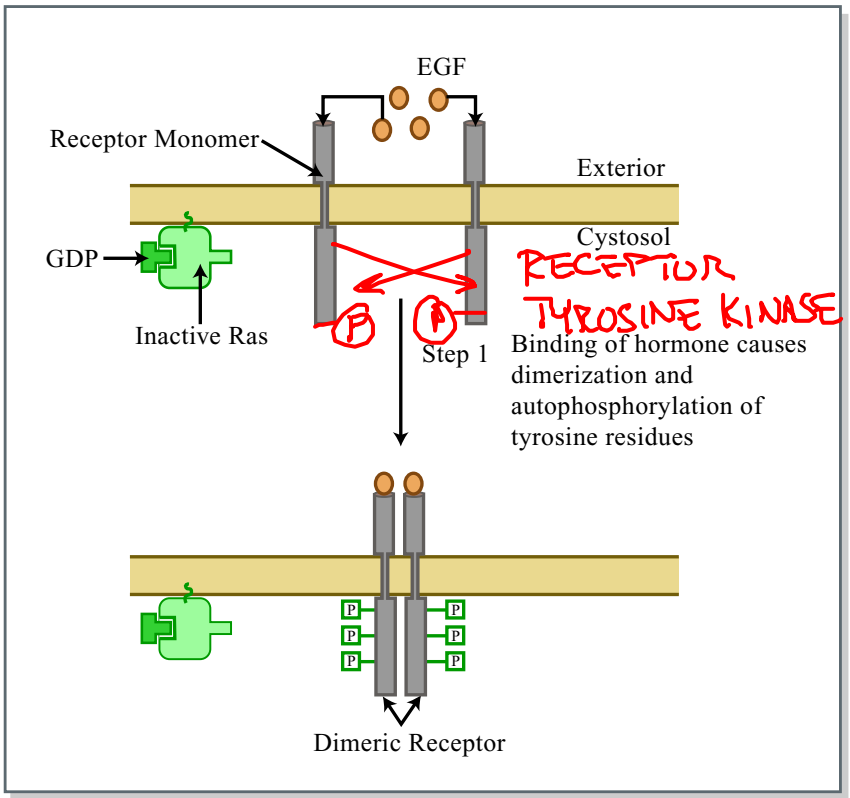


Figure by MIT OCW.

Diverse functions of cytokines:

- Induce cell migration/stop cell migration
- Induce cell growth
- Induce differentiation
  - Upregulate tissue-specific functions

Characteristics:

↳ BIND RECEPTORS W/HIGH AFFINITY

- Typically potent, act at pmol concentrations
- Synergize with other receptor signals
  - e.g. integrins

Changes in signaling achieved by cytokine immobilization on surfaces

Image removed due to copyright reasons.
Please see:
Figure 1 in Ito, Y., et al. "Tissue Engineering by Immobilized Growth Factors." *Materials Science and Engineering C6* (1998): 267-274.

Immobilized insulin:

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Please see:
Figure 1 in Ito, Y., et al. "Tissue Engineering by Immobilized Growth Factors.“ *Materials Science and Engineering C6* (1998): 267-274.

Local control of gene expression by non-diffusable cytokines:

Patterned immobilization of EGF:

Image removed due to copyright reasons.
Please see:

Figure 4 in Ito, Y. "Regulation of Cell Functions by Micropattern Immobilized Biosignal Molecules." *Nanotechnology* 9 (1998): 200-204.

Surface immobilization can induce new function in cytokines: case of tethered EGF-triggered neuronal cell differentiation

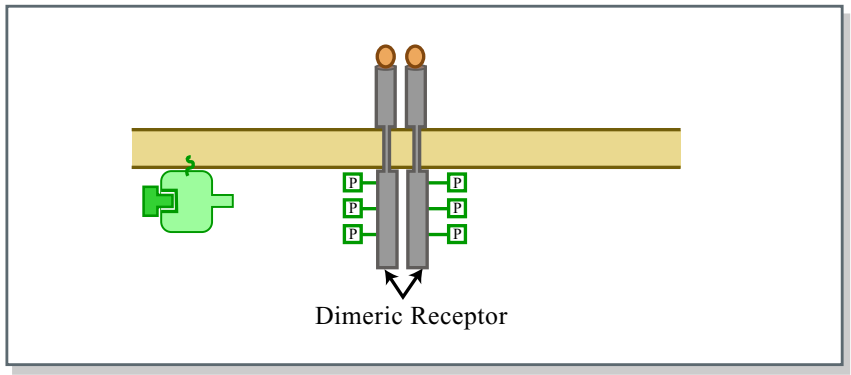


Figure by MIT OCW.

PC12 cell line:

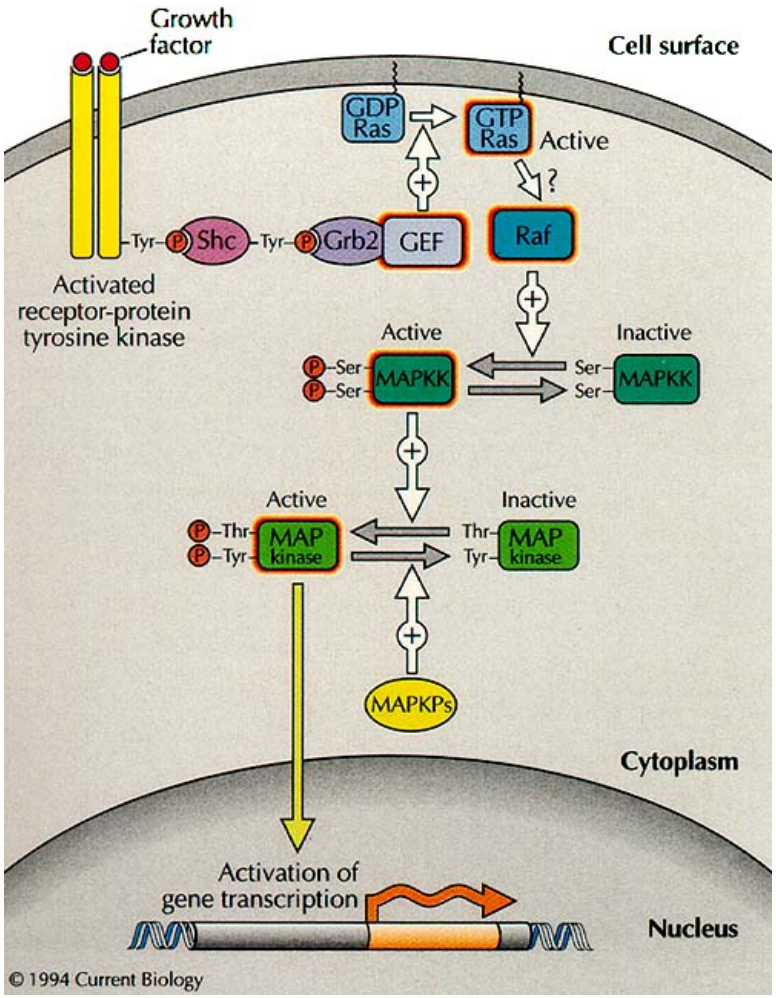
- induced to differentiate and extend axons under stimulation of **NGF** (nerve growth factor)

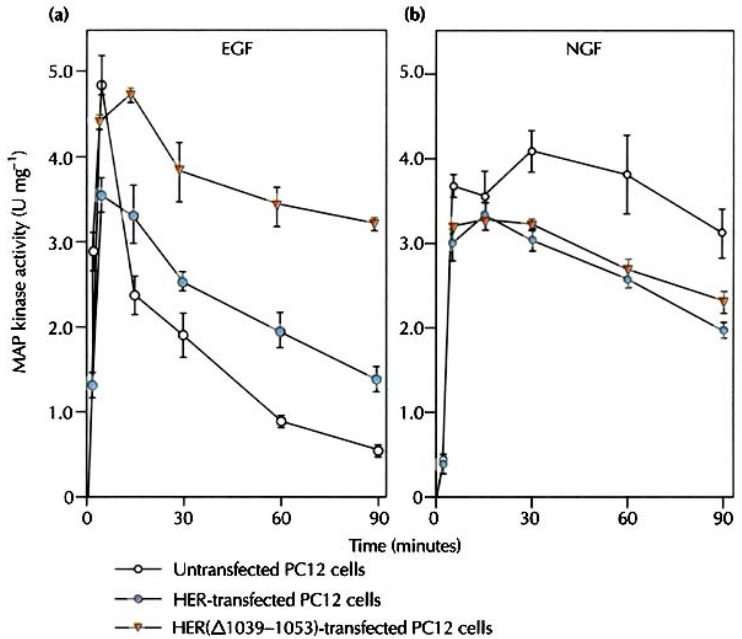
Signal doesn't trigger internalization of receptor; thus signal lasts longer and triggers differentiation

- induced to proliferate by **EGF**

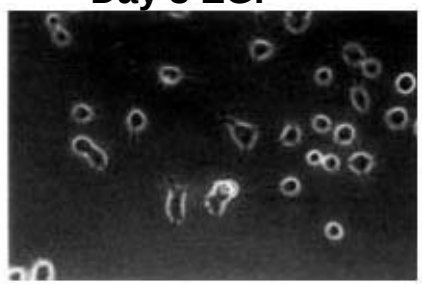
Signal triggers internalization of receptor; short signal triggers proliferation

NGF vs. EGF signaling in PC12 neuronal cells

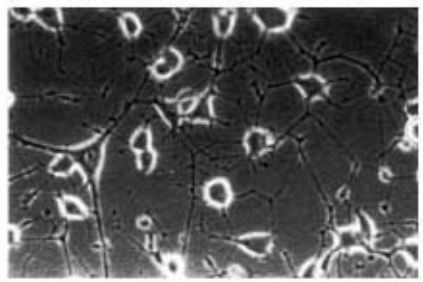




Day 3 EGF



Day 3 NGF



(Traverse et al. 1994)

Further Reading

1. Voet & Voet. in *Biochemistry*.
2. Paredes, N., Rodriguez, G. A. & Puiggali, J. Synthesis and characterization of a family of biodegradable poly(ester amide)s derived from glycine. *Journal of Polymer Science, Part A: Polymer Chemistry* **36**, 1271-1282 (1998).
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