Bioconjugate Materials: Nanopatterns of Biomolecules on Surfaces

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University of California, Los Angeles, USA

Topic of Today's Lecture

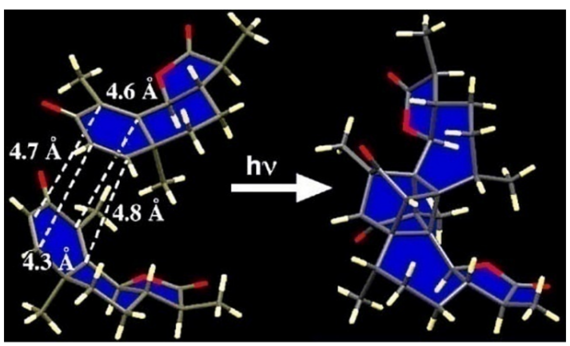
This lecture will focus on nanomaterials research, specifically combining NANO and BIO on surfaces

UCLA

UCLA

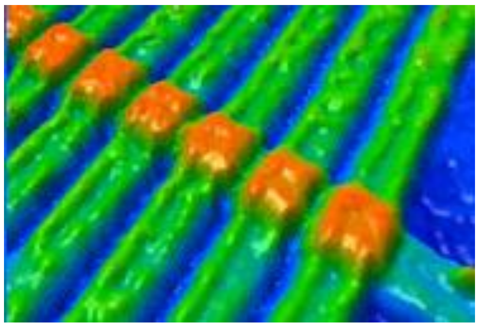
UCLA

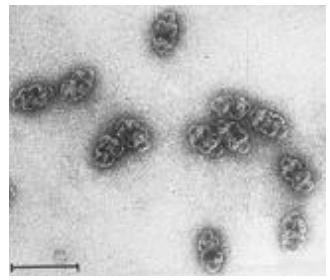
Department of Chemistry and Biochemistry provides exciting opportunities for graduates and postdocs for collaborative research at the interface of chemistry and materials

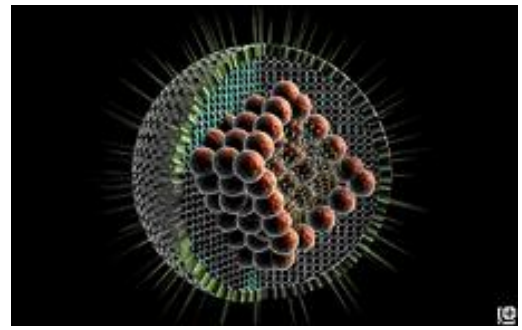


California NanoSystems Institute

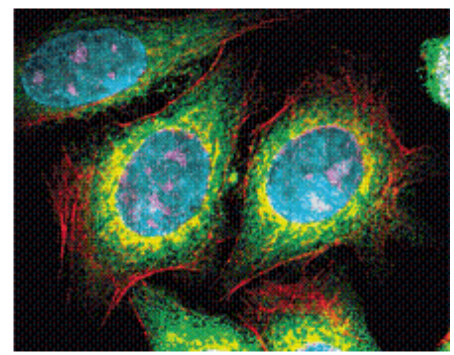
Established by the State of California in 2000

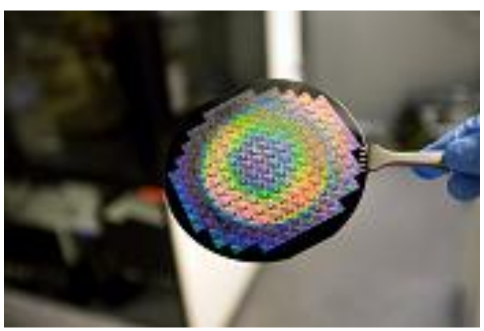






Interdisciplinary research and education focused on nanotechnology





Joint Institute between UCLA and UCSB

Topic of Today's Lecture

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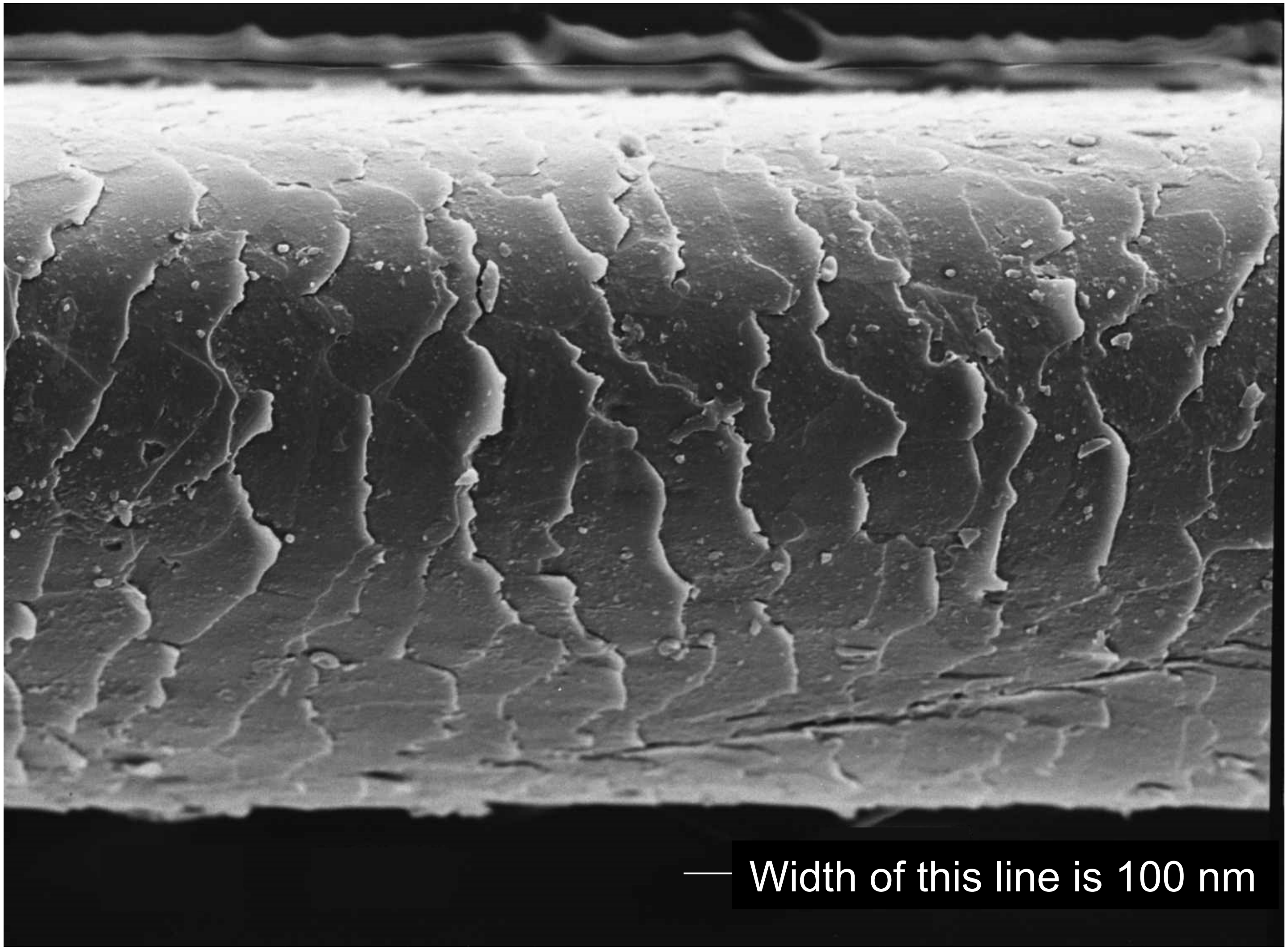
What is Nano?

- Nanoscience is the study of objects measured in nanometers
  - 1-billionth of a meter
  - ~80,000 times smaller than the diameter of a single human hair

Closer Look at a Human Hair

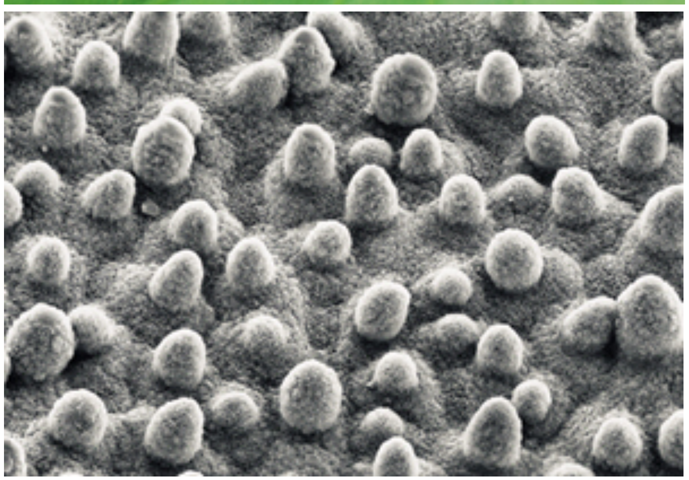


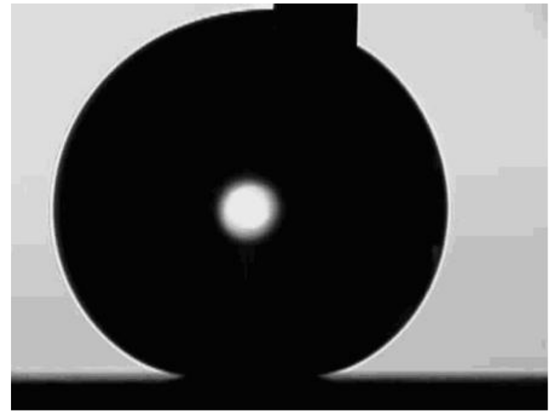
http://www.aber.ac.uk/bioimage/image/uwbl-0411-w.jpg

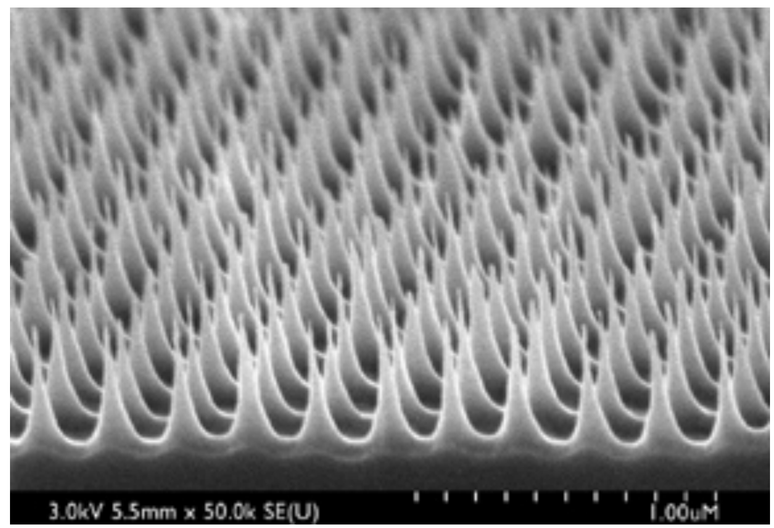
What is Nano?

- Nanoscience is the study of objects measured in nanometers
  - – 1-billionth of a meter
  - – ~80,000 times smaller than the diameter of a single human hair
  - – New properties emerge at the nanoscale
    - Size and shape matter

Super-Repellent Nano-Materials







http://cjmems.seas.ucla.edu/members/changhwan/main.html
http://www.engineer.ucla.edu/magazine/fall06/noslip.html

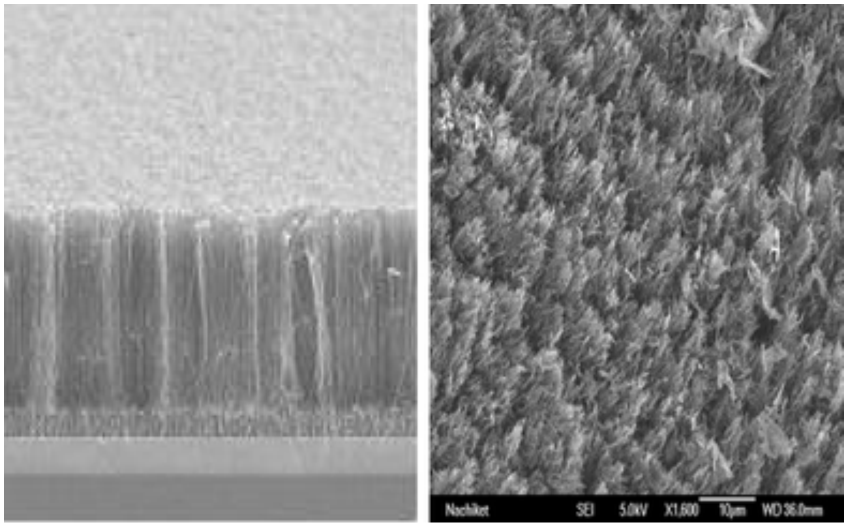
Geckos Walk on Walls

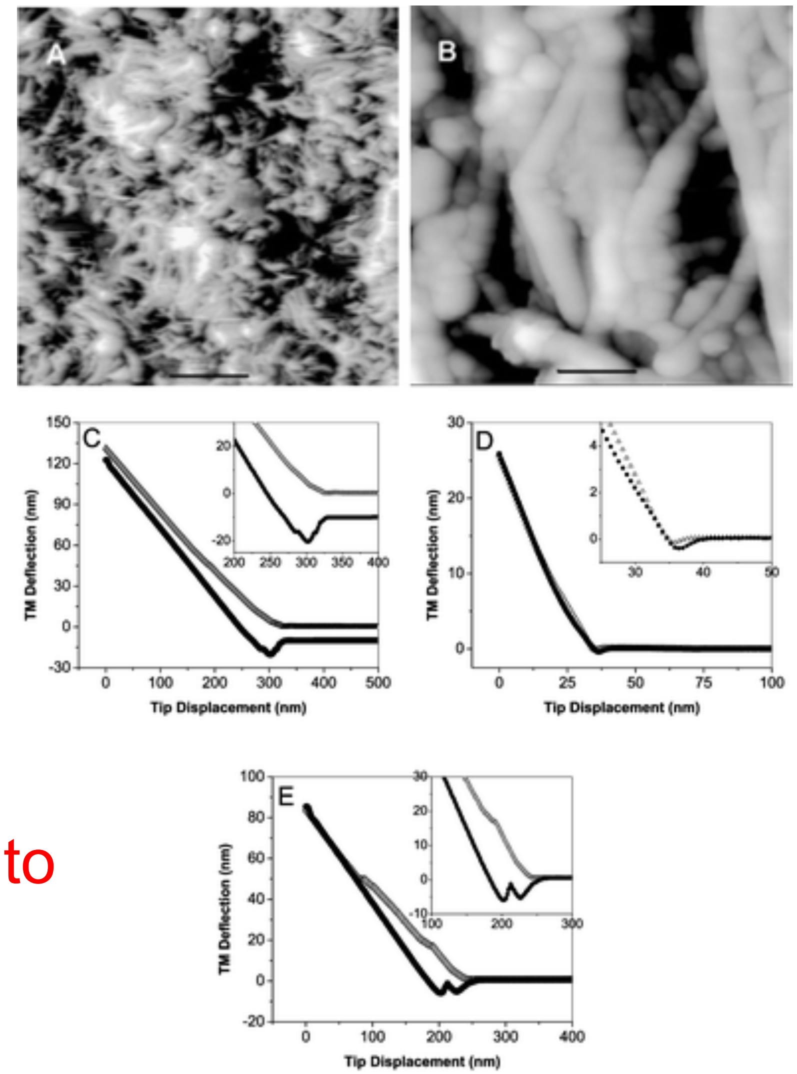
Nano-Finger Tips Allow Geckos to Stick

http://robotics.eecs.berkeley.edu/~ronf/Gecko/index.html

Man-Made Geckos

Super Adhesive Nano-Materials





Synthetic nano-materials can exhibit strong adhesion similar to gecko fingers

Yurdumarkan et al, Chem. Commun. 2005, 3799-3801

Topic of Today's Lecture

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Protein

Protein

Protein comes from Greek word proteios meaning primary

Proteins serve many different functions

Structure of protein called myoglobin which delivers oxygen to muscle tissues

Examples

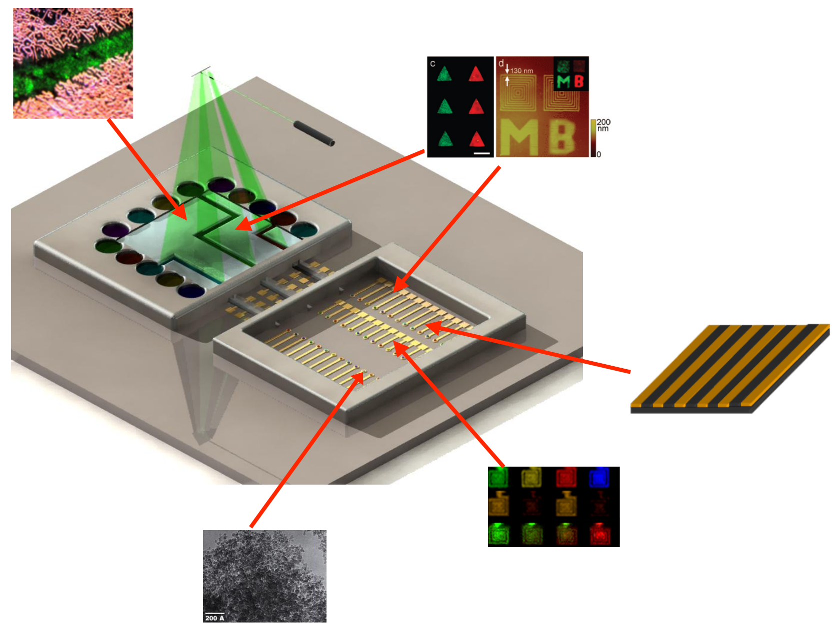
Hemoglobin carries oxygen through the body.
Melanin gives skin pigmentation and the iris color.
Keratin provides structure of hair and nails.
Serum Albumin maintains blood pressure.
Alcohol Dehydrogenase breaks down alcohol in the liver.

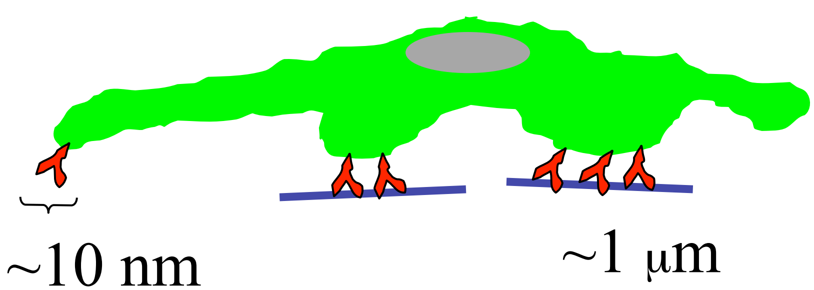
http://en.wikipedia.org/wiki/Protein

Why Nano and Bio on Surfaces

Diagnostics

- Achieve greater sensitivity
- Simultaneous detection of multiple disease markers



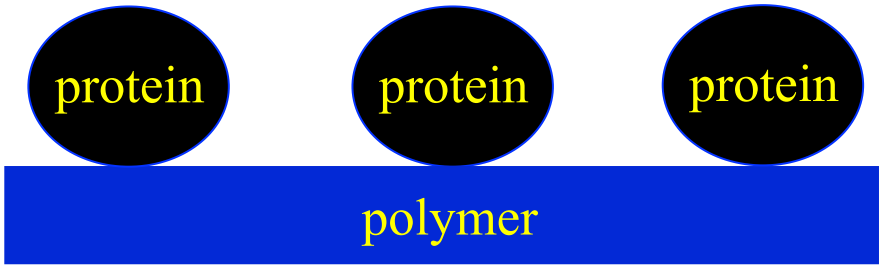


Biomaterials

–Better mimicry of extracellular matrix

(control of cell differentiation and behavior)

How to Pattern and Critical Features



Christman, Enriquez-Rios & Maynard, *Soft Matter*, 2006, *2*, 928

Many Techniques to pattern:
-scanning probe techniques
-stamping
-self assembly
-lithography:
e-beam, photolithography

Diagnostics, biomaterials, tissue engineering and most applications require bioactive proteins on the surface

Fully active proteins are especially important for nanoscale patterns of proteins

Chemoselective reactions that occur under mild, aqueous conditions with out the addition of reagents are important

Outline

- Overview of techniques to pattern biomolecules at the nanoscale
- Example 1: Multiprotein patterns by e-beam lithography
- Example 2: Cell adhesive materials

Outline

- **Overview of techniques to pattern biomolecules at the nanoscale**
- Example 1: Multiprotein patterns by e-beam lithography
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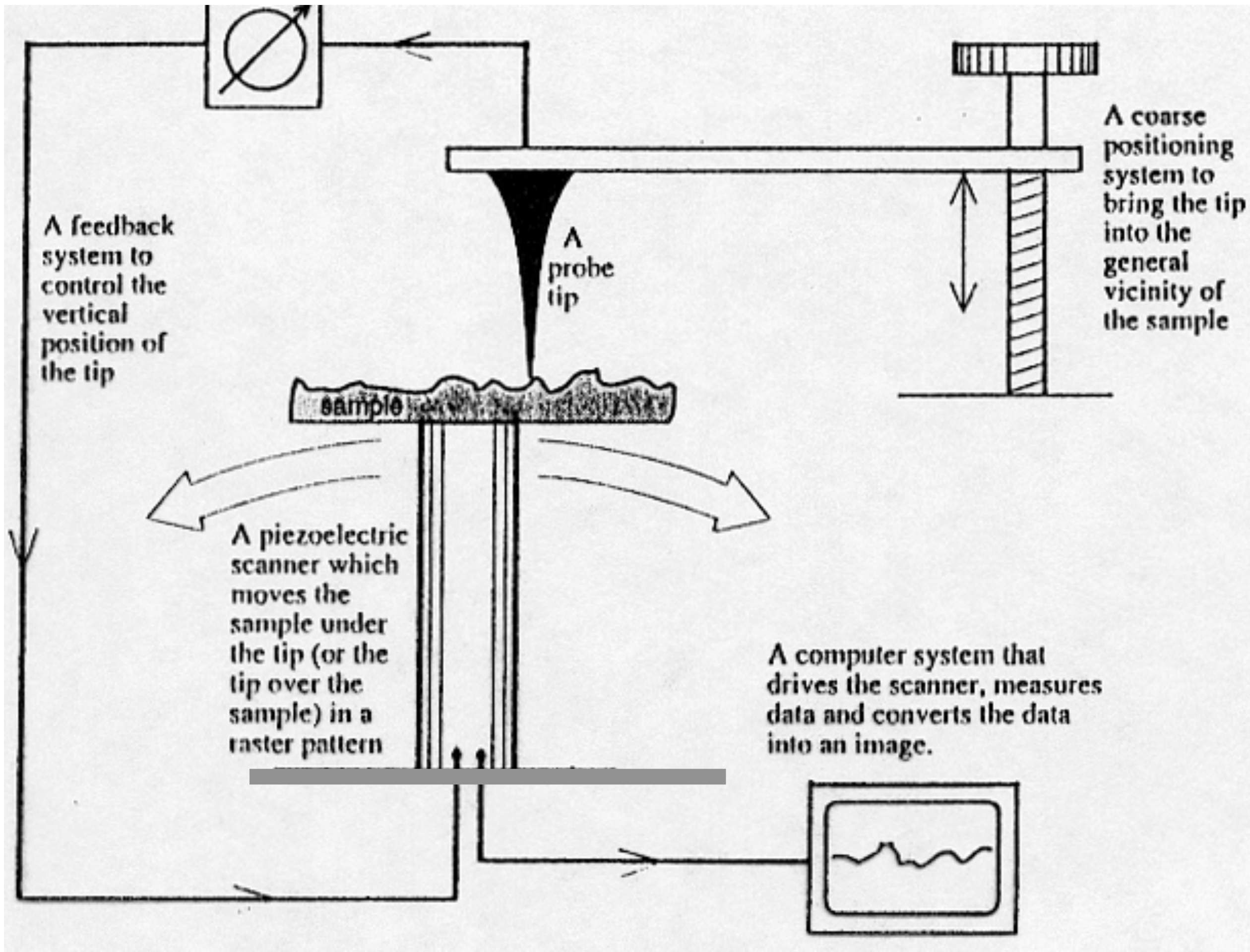
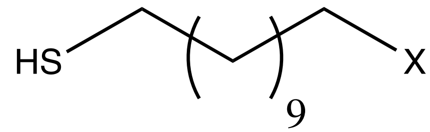
Introduction to AFM

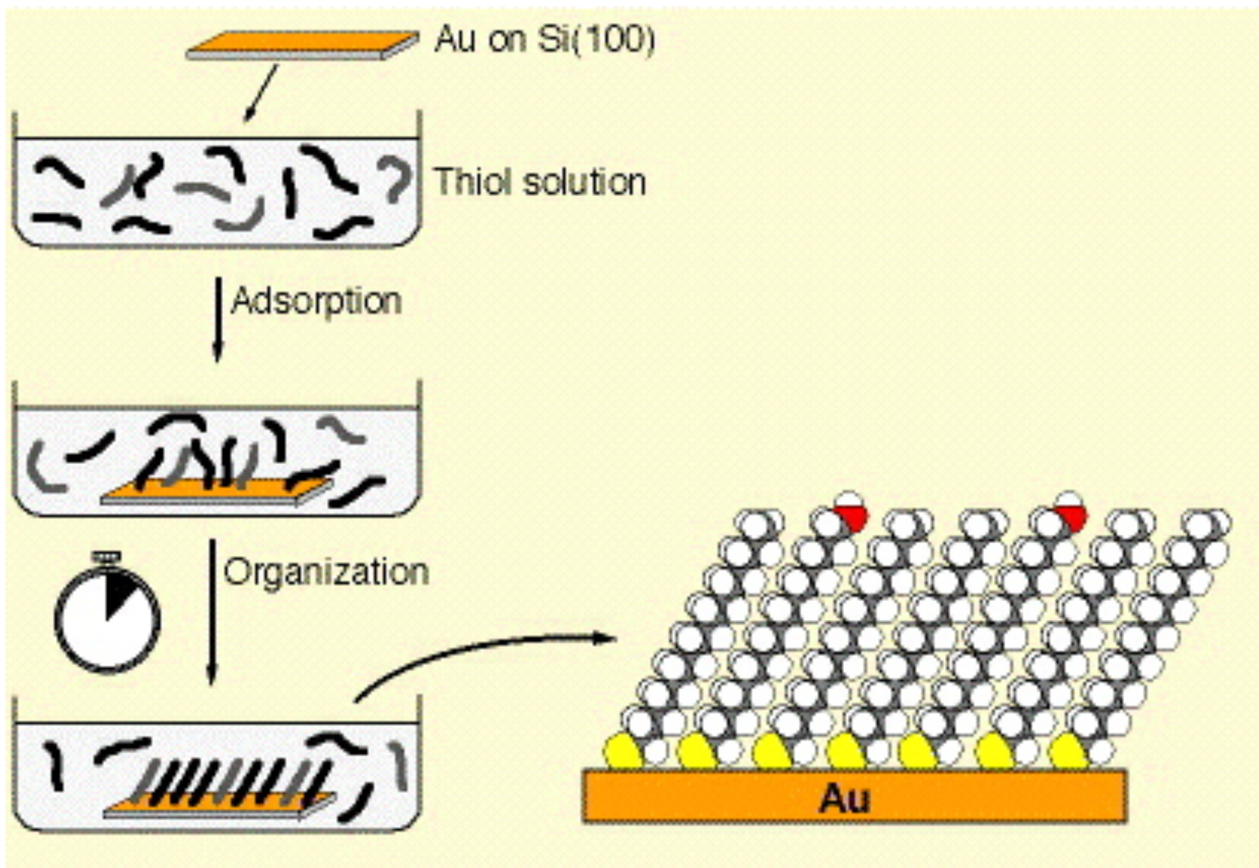


Slide from Lía Pietrasanta

SAMS (Self Assembled Monolayers)

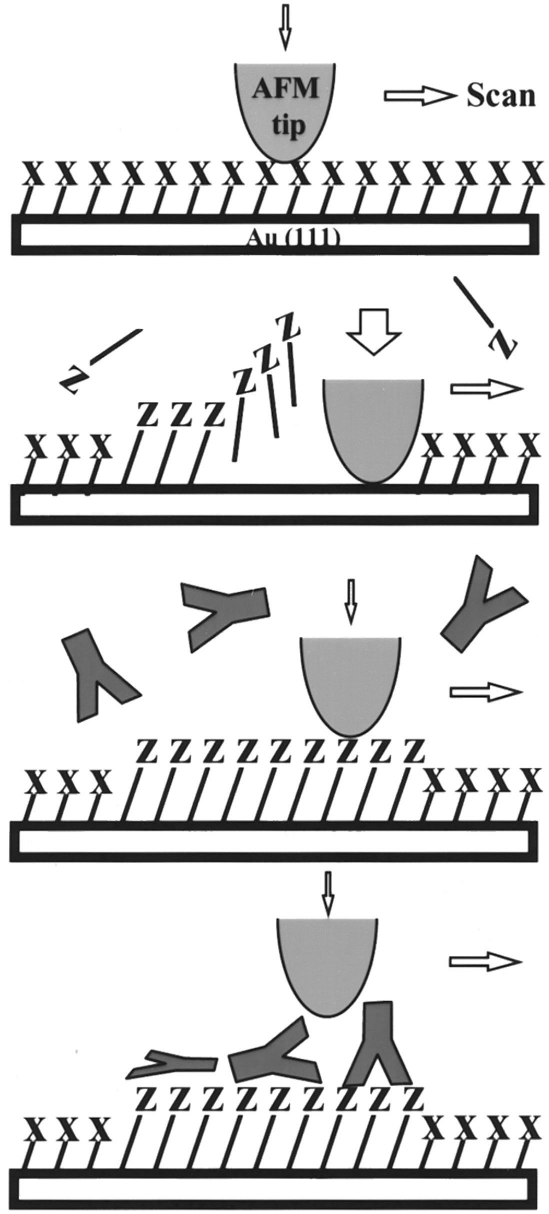
Alkane thiol:



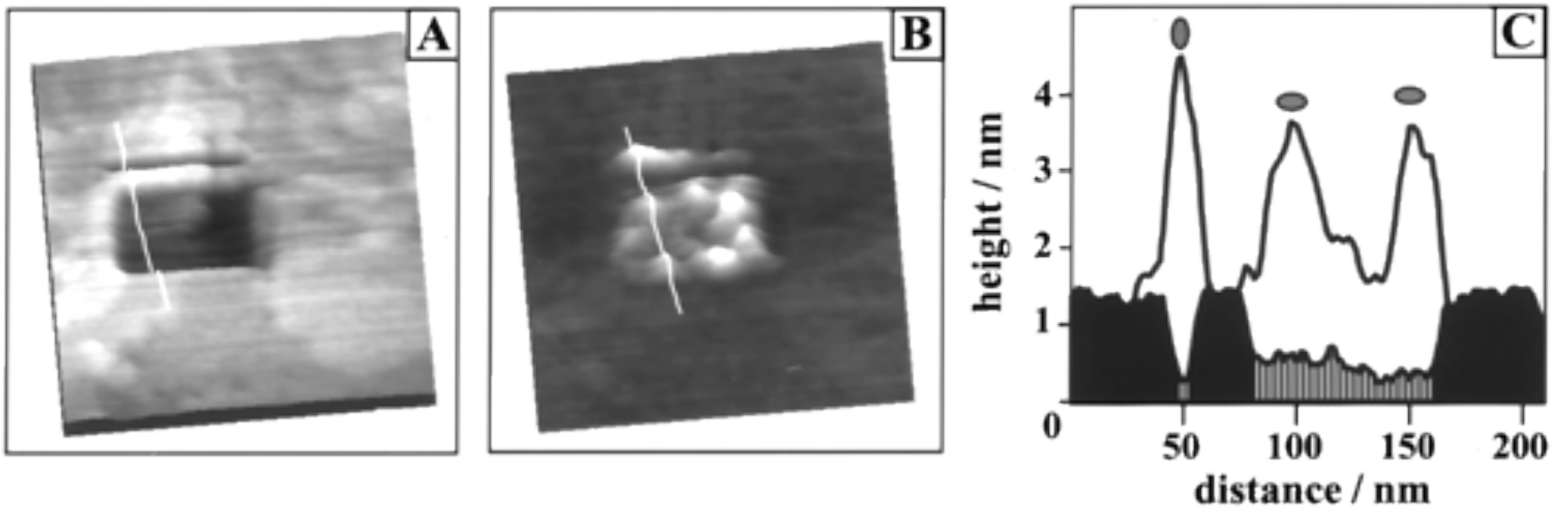


http://www.ifm.liu.se/Applphys/ftir/sams.html

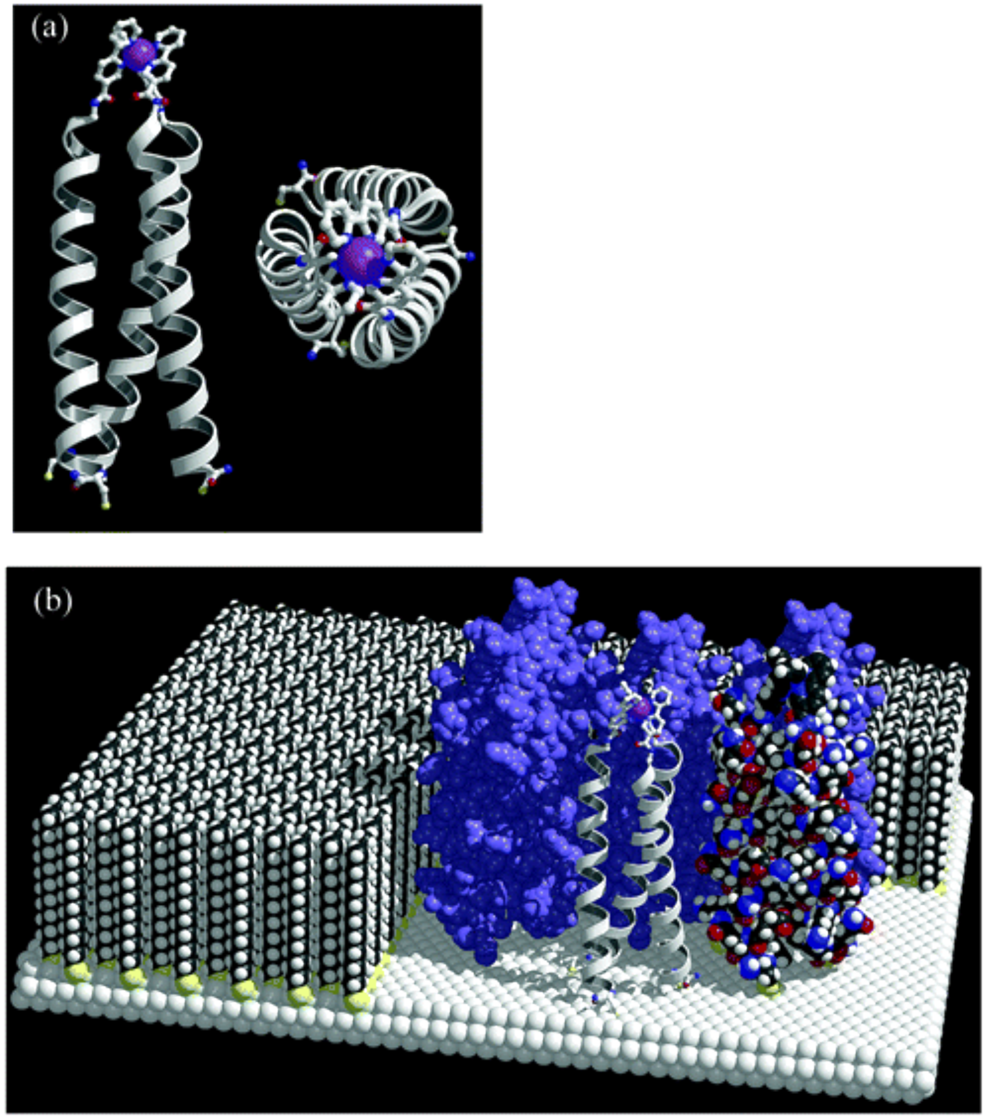
AFM, Nanografting



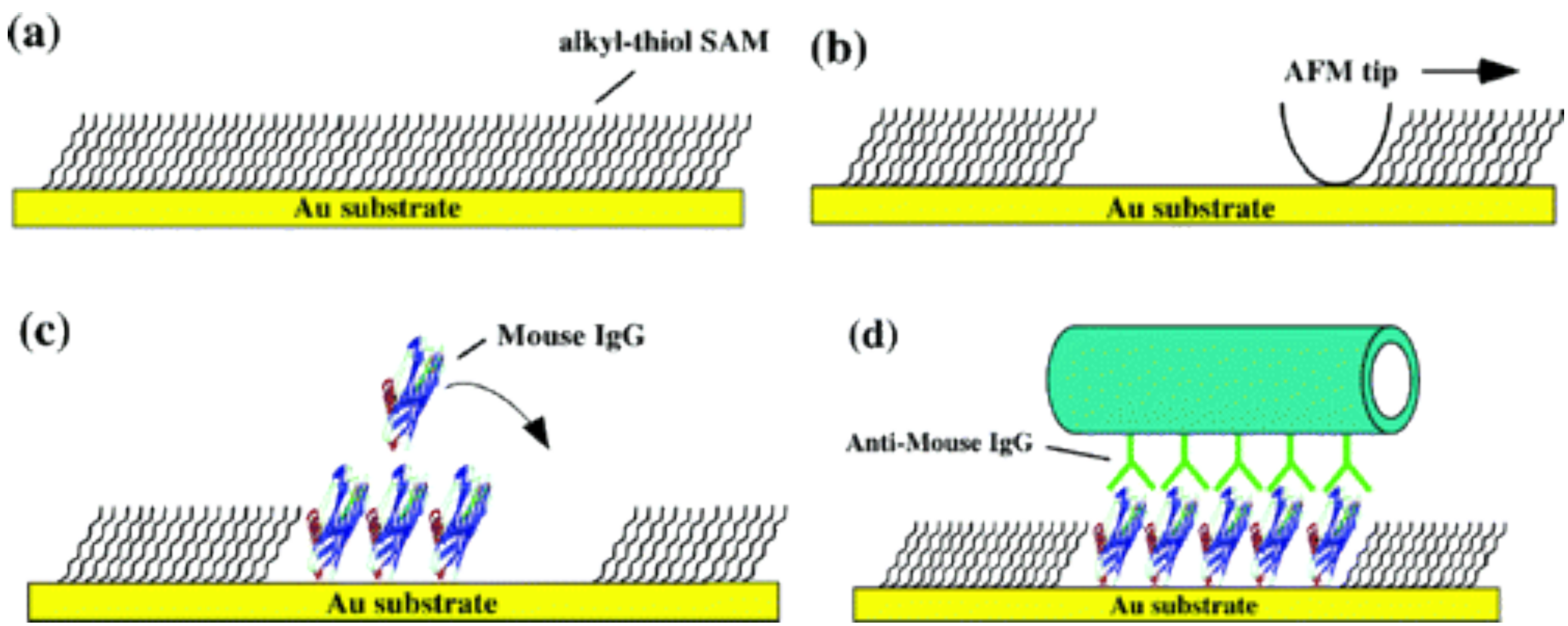
Wadu-Mesthrige, et al. *Langmuir*, **1999**, *15*, 8580-8583



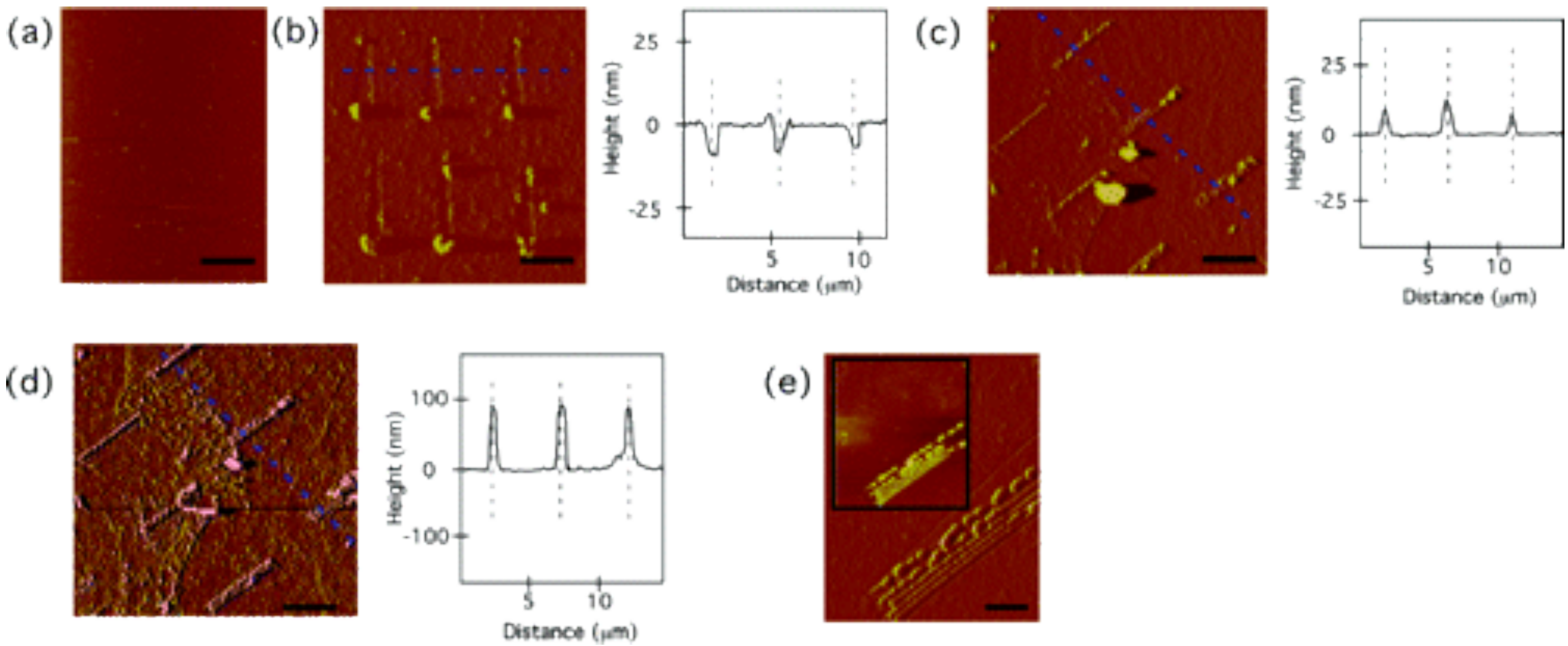
Wadu-Mesthrige, et al. *Langmuir*, **1999**, *15*, 8580-8583



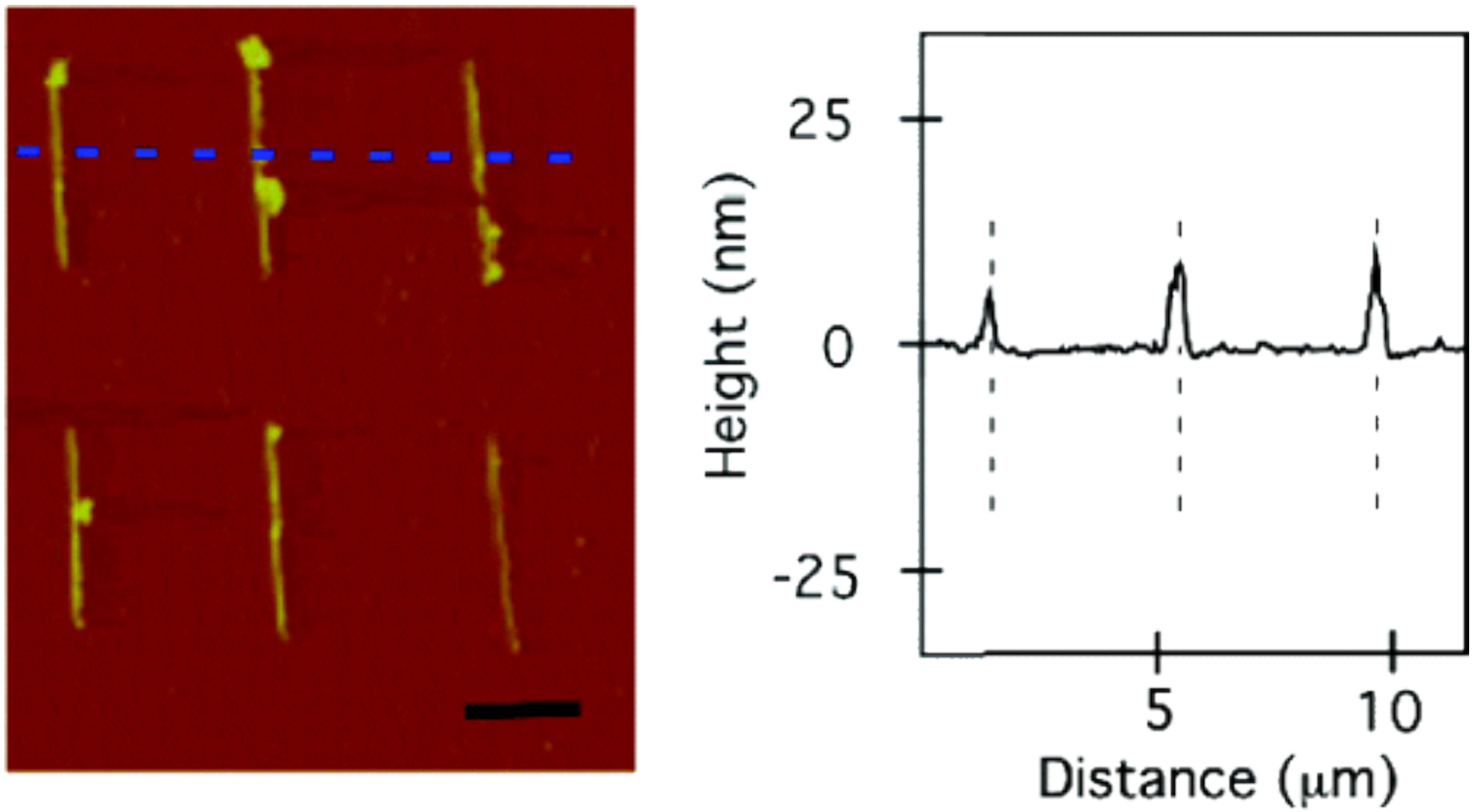
Case, et al. *Nano Lett.*, **2003**, *3*, 425-429



Nuraje, et al. *JACS*, **2004**, *126*, 8088-8089

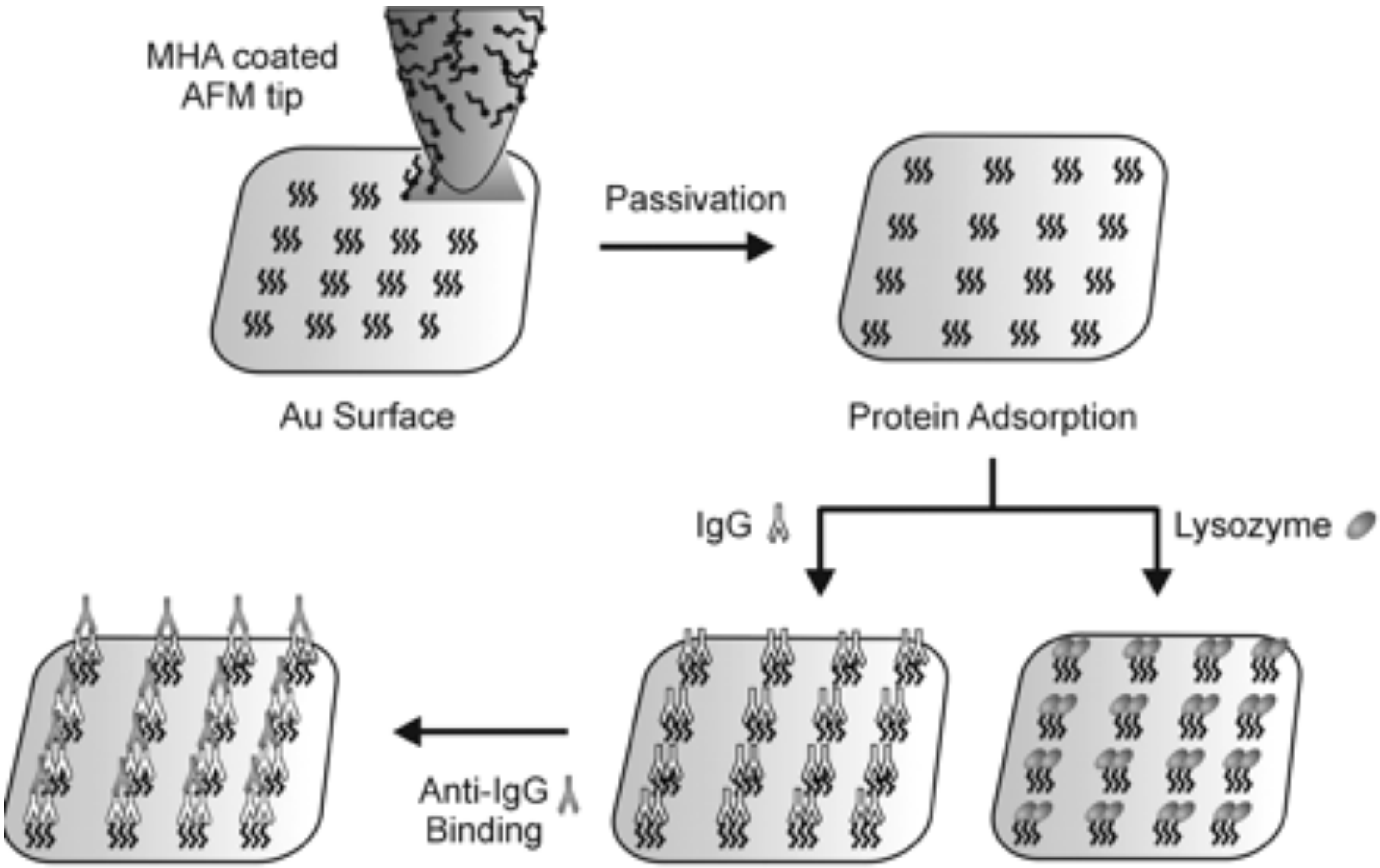


Nuraje, et al. *JACS*, **2004**, *126*, 8088-8089

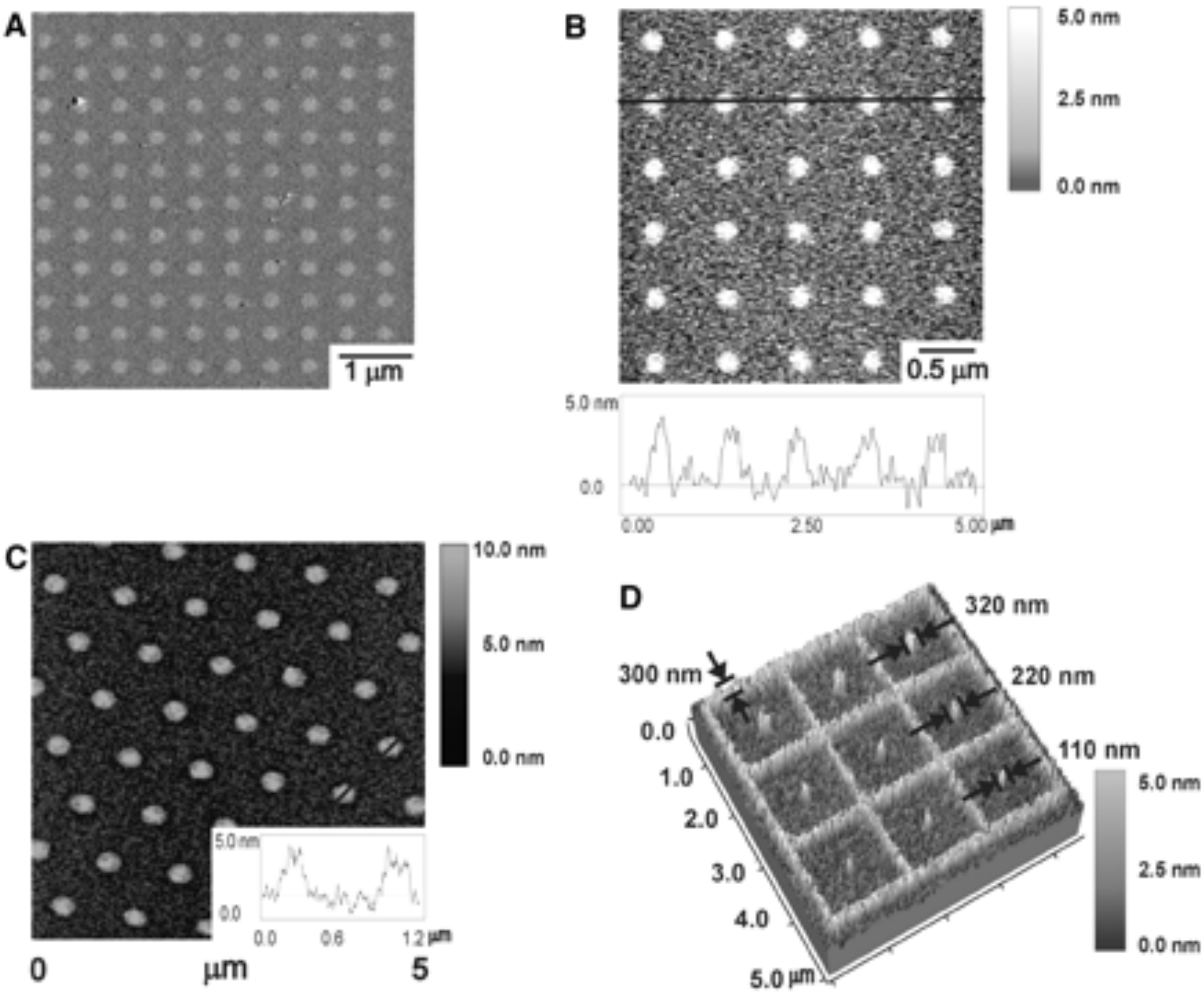


Nuraje, et al. *JACS*, **2004**, *126*, 8088-8089

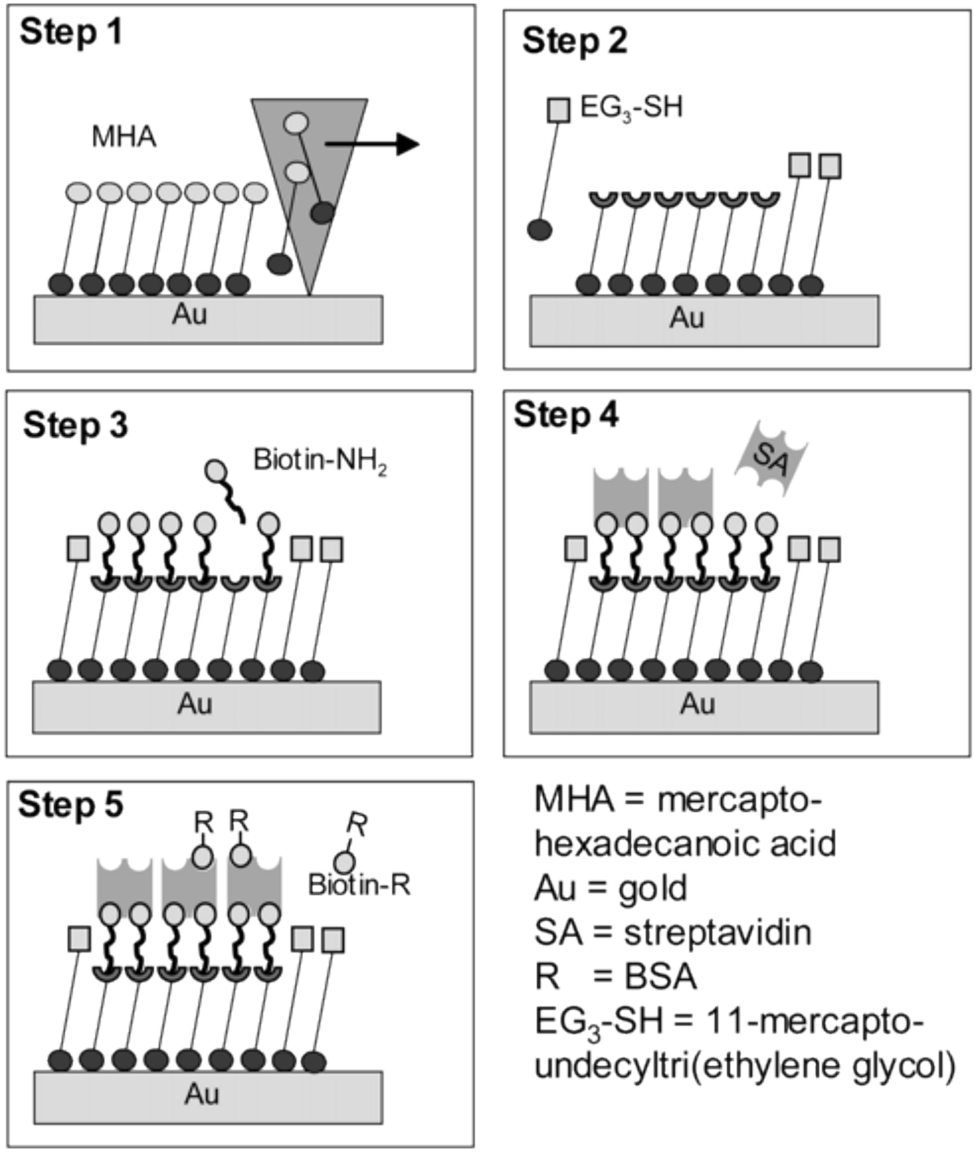
AFM, Dip Pen Lithography



Lee, et al. *Science*, **2002**, *295*, 1702-1705

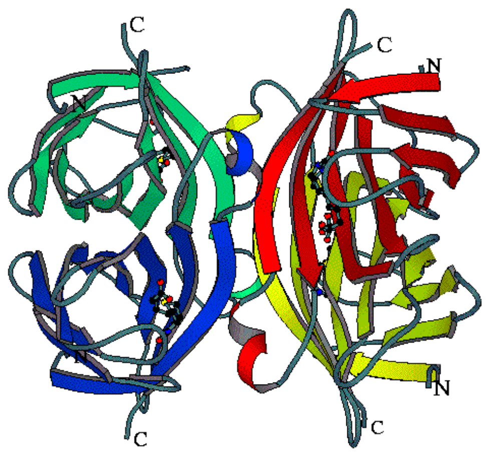


Lee, et al. *Science*, **2002**, *295*, 1702-1705

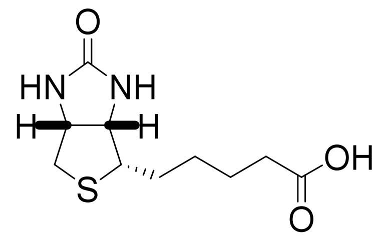


Hyun, et al. *Nano Lett.*, **2002**, *2*, 1203-1207

Why Patterns of Streptavidin



Biotin-streptavidin complex
Freitag, S. et al., *Protein Science* 1997, *6*, 1157



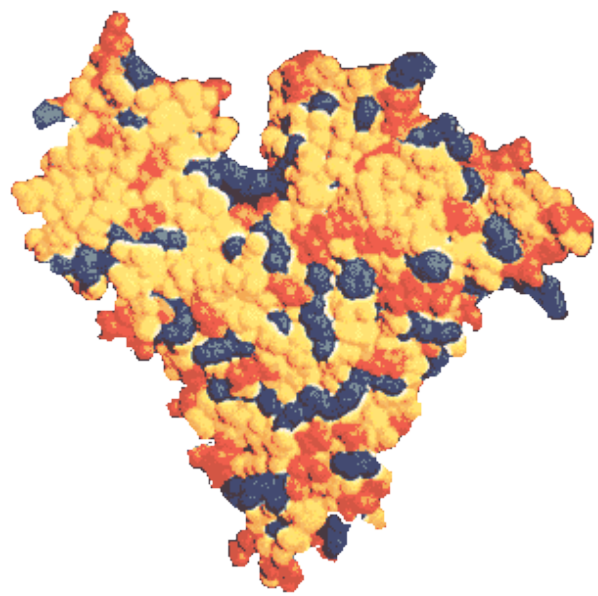
Biotin

- Streptavidin binds four biotins with high affinity ($K_a = 10^{15}$
- Used as adapter molecule for many applications

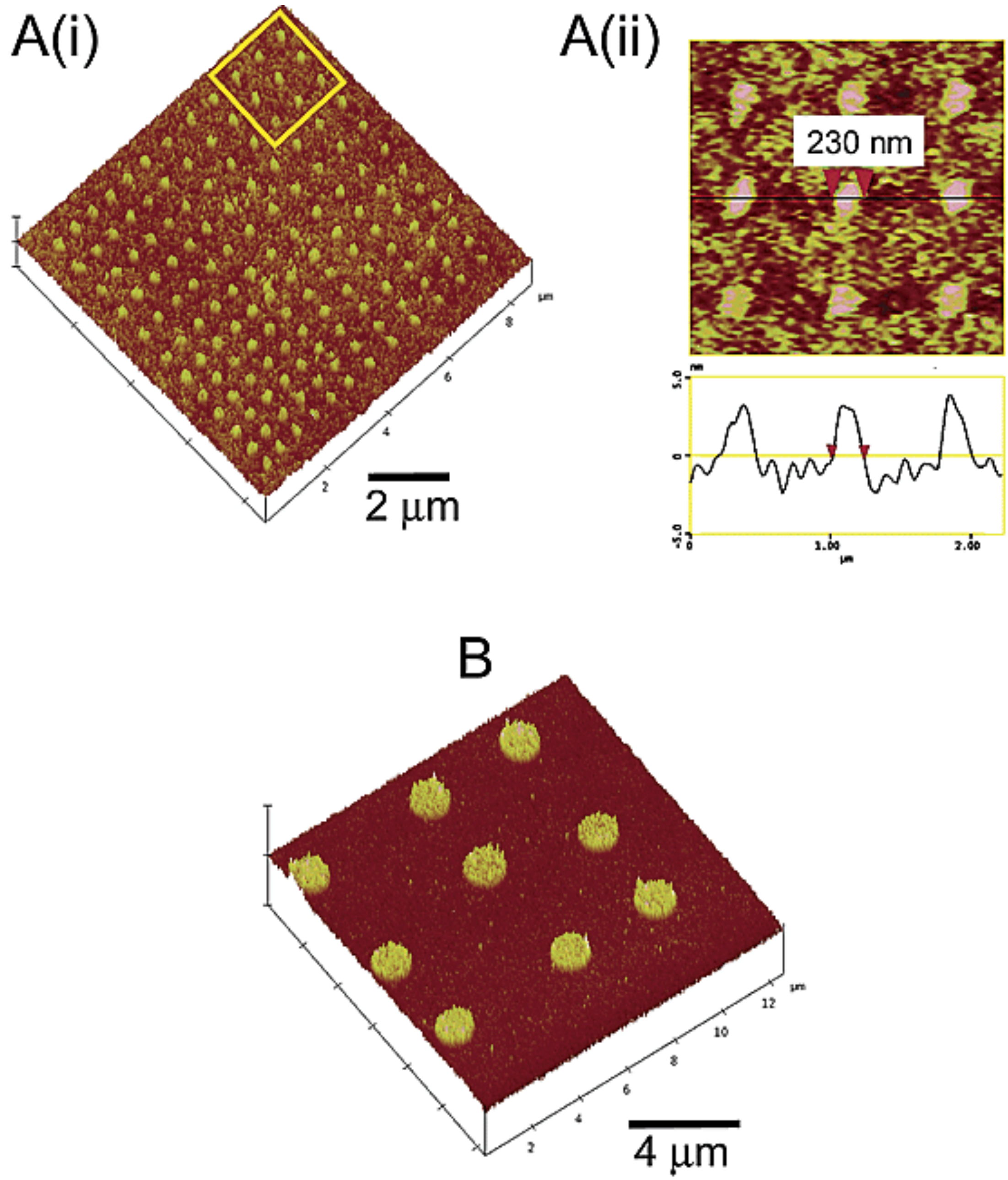
Patterns of streptavidin are an excellent platform for further elaboration because many biotinylated molecules are available

Bovine Serum Albumin (BSA) as a Model Protein

- Conjugation to BSA
  - Most common protein in blood
  - One free cysteine



Carter & Ho, *Protein Chem.* **1994**, *45*, 153-204



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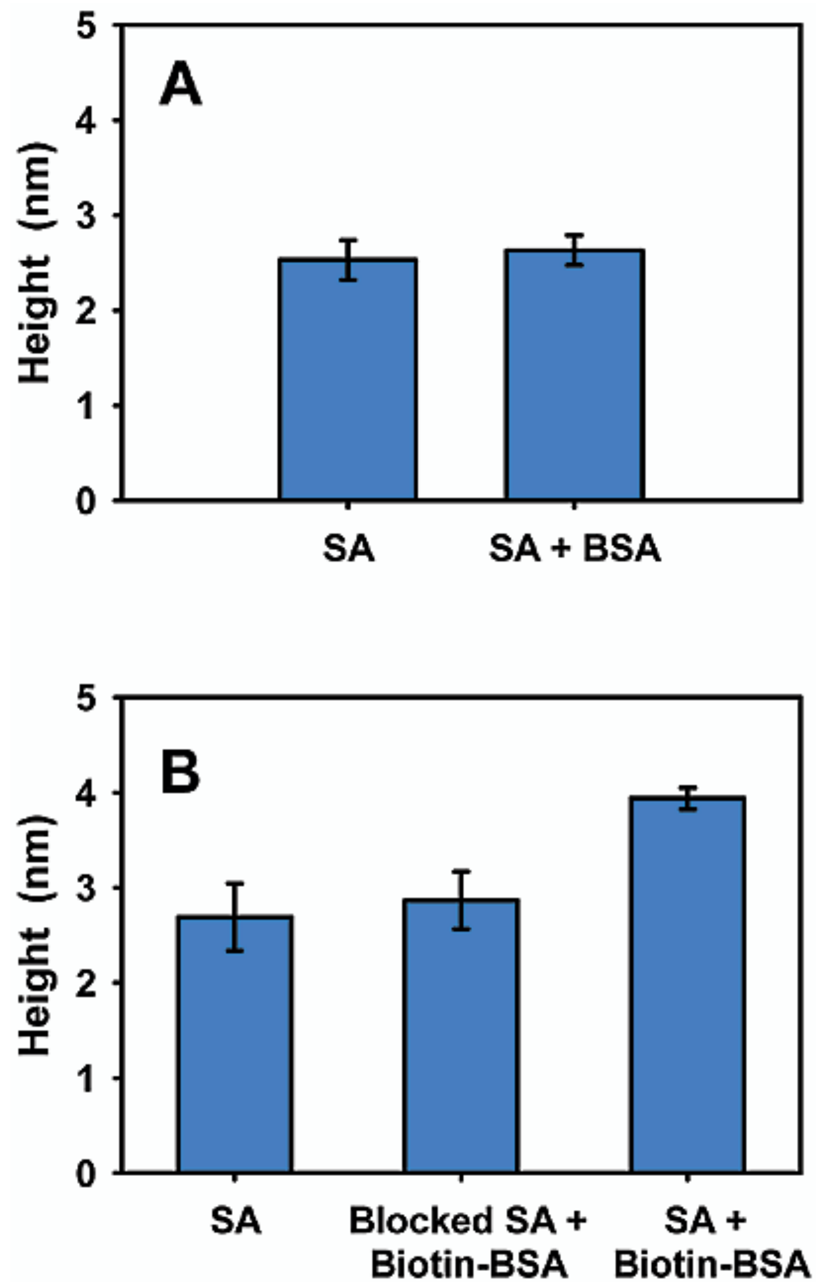
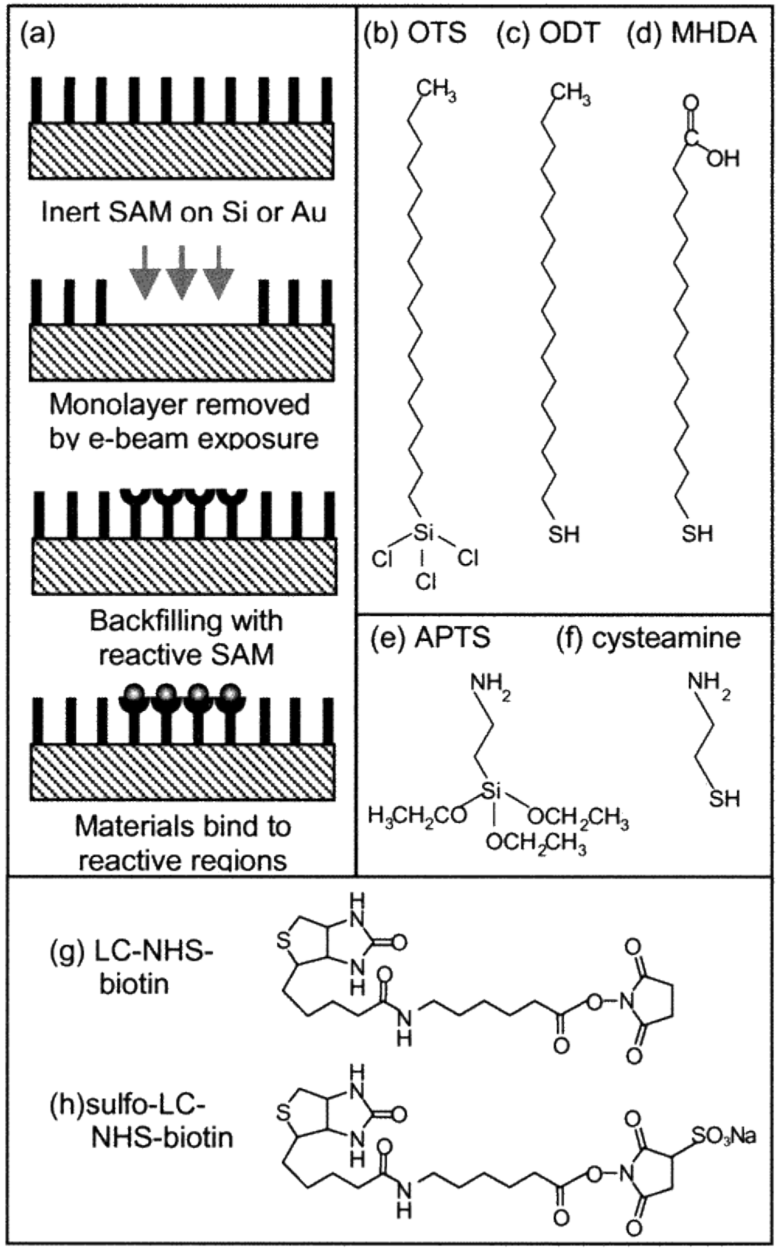
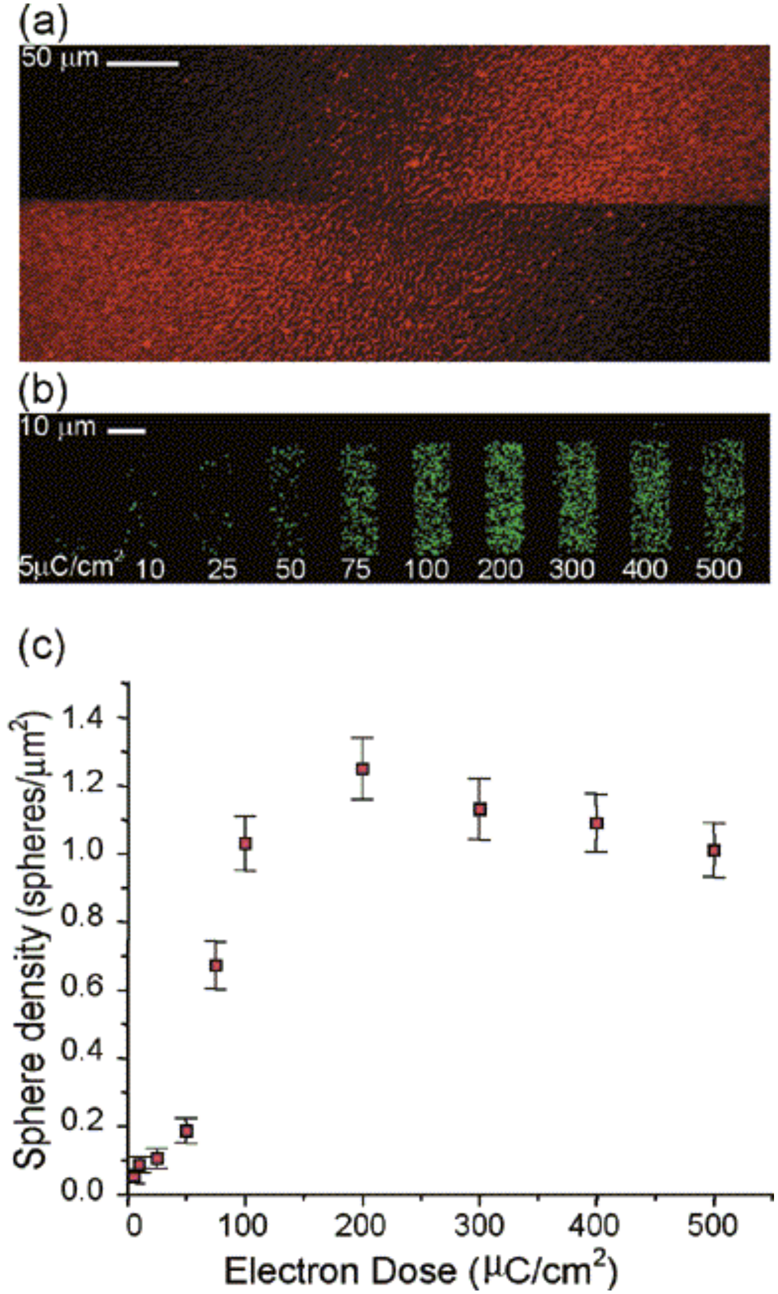


Hyun, et al. *Nano Lett.*, **2002**, *2*, 1203-1207

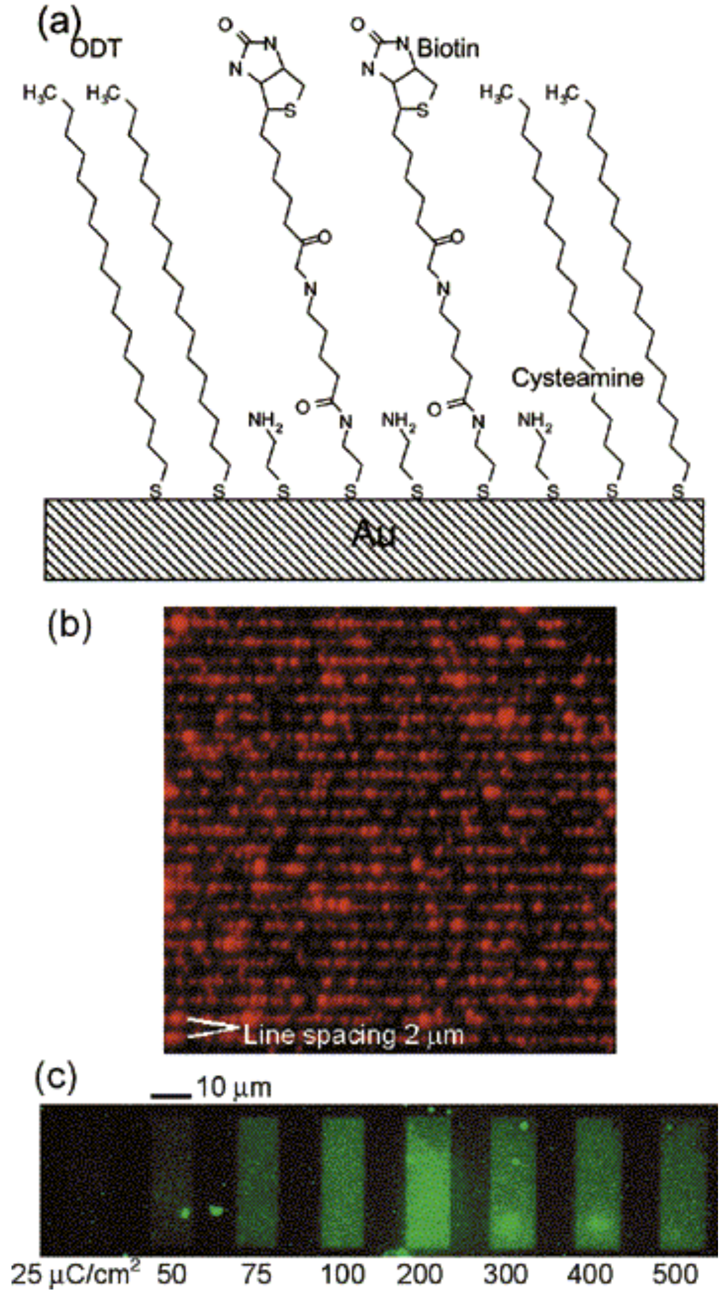
Electron Beam (E-beam) Lithography



Harnett, et al. *Langmuir*, **2001**, *17*, 178-182

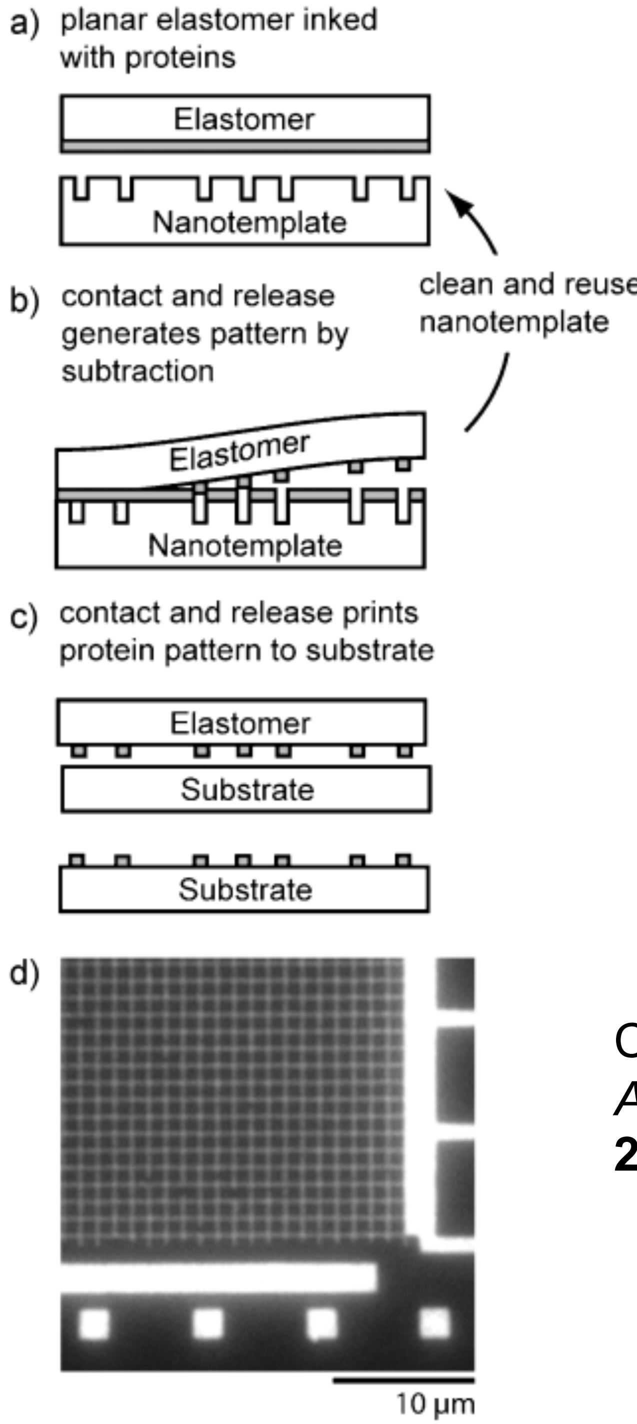


Harnett, et al. *Langmuir*, **2001**, *17*, 178-182

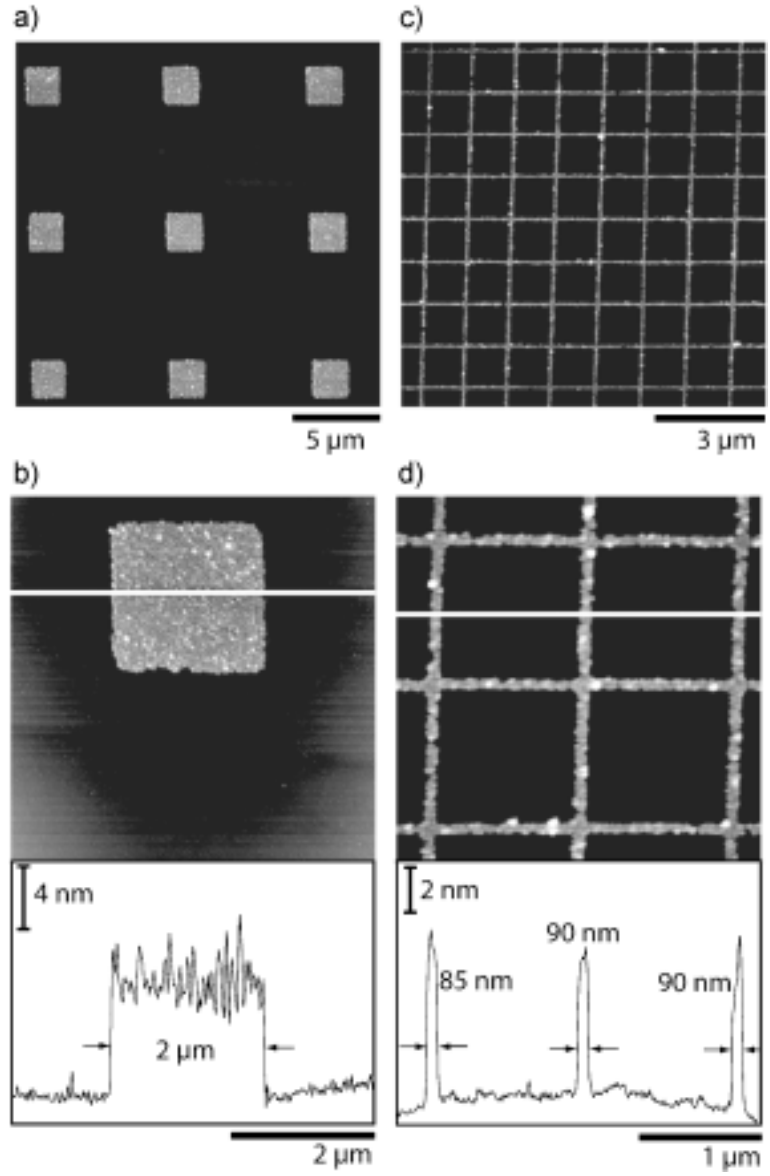


Harnett, et al. *Langmuir*, **2001**, *17*, 178-182

NanoStamping

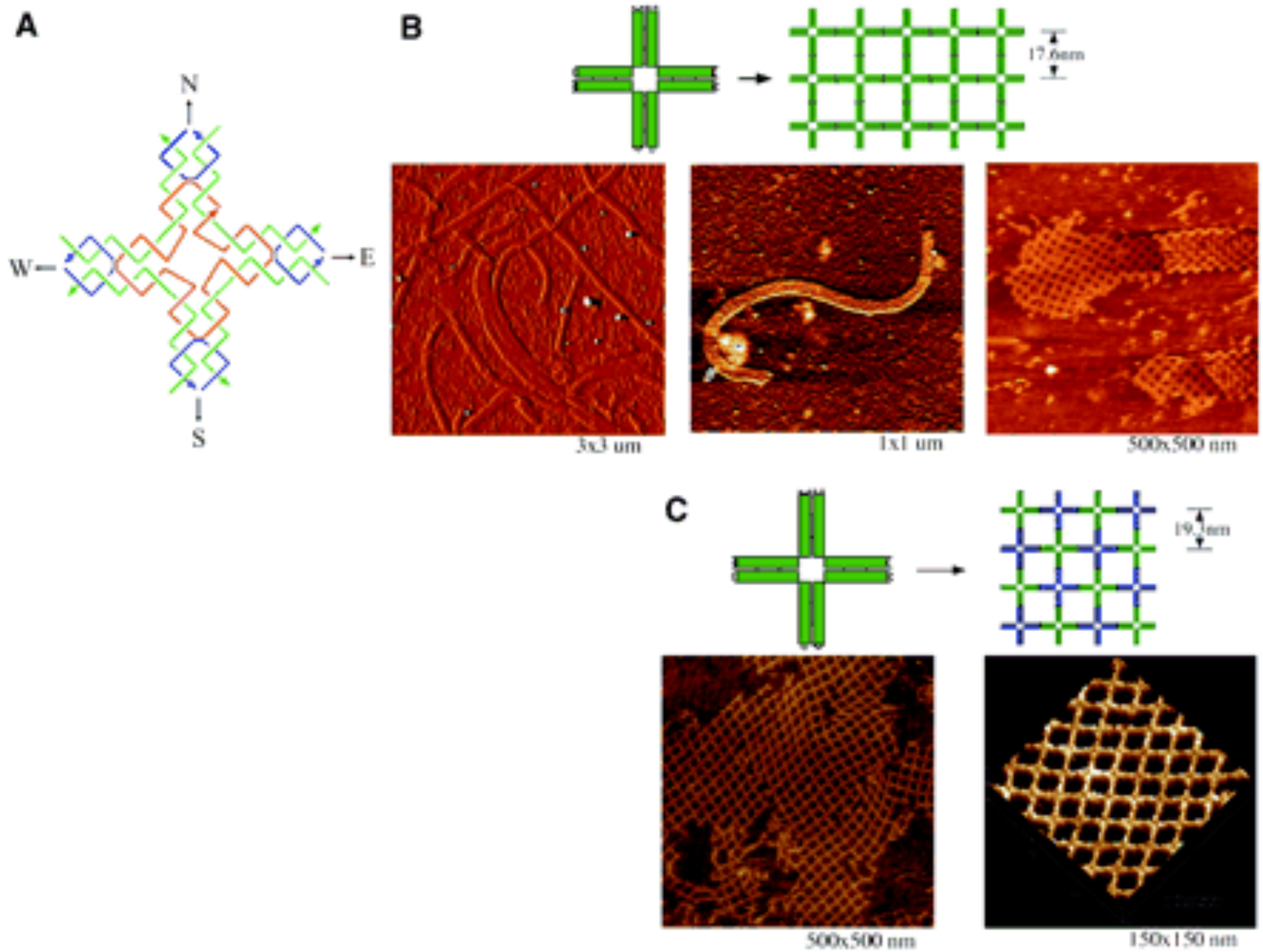


Coyer, S. R. et al.
Angew. Chem. Int. Ed.
2007 *46*, 6837-6840

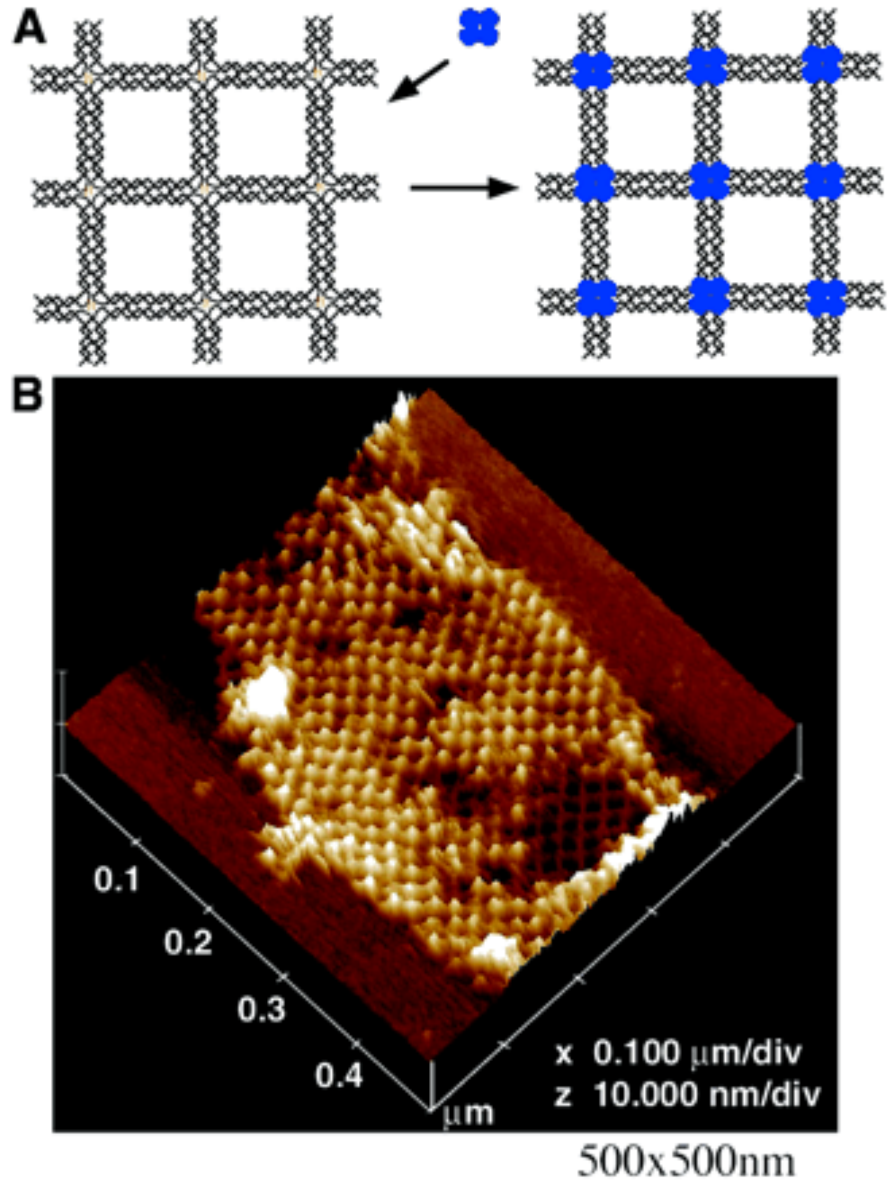


Coyer, S. R. et al. *Angew. Chem. Int. Ed.* **2007** *46*, 6837-6840

Self Assembly - DNA

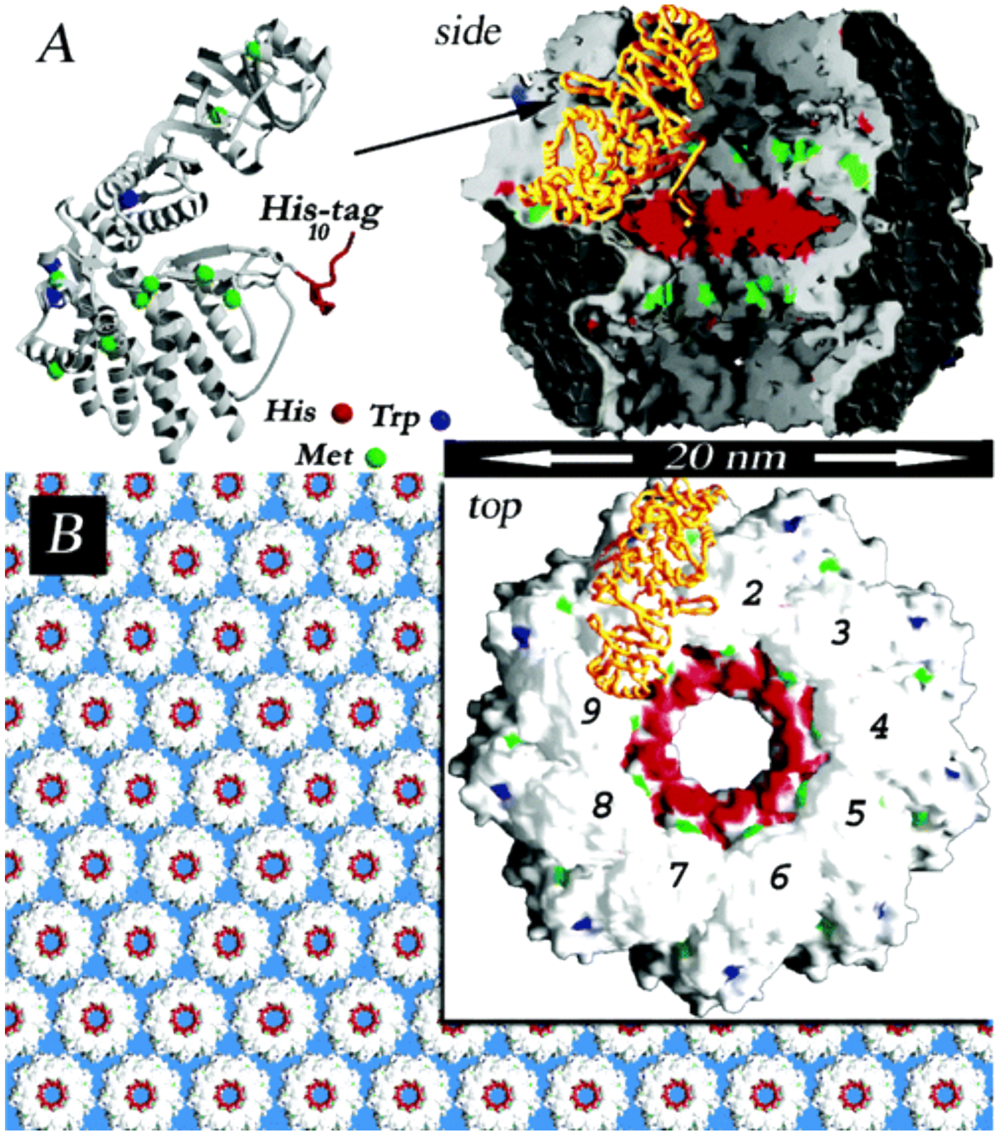


Yan, et al. *Science* **2003**, *301*, 1882-1884

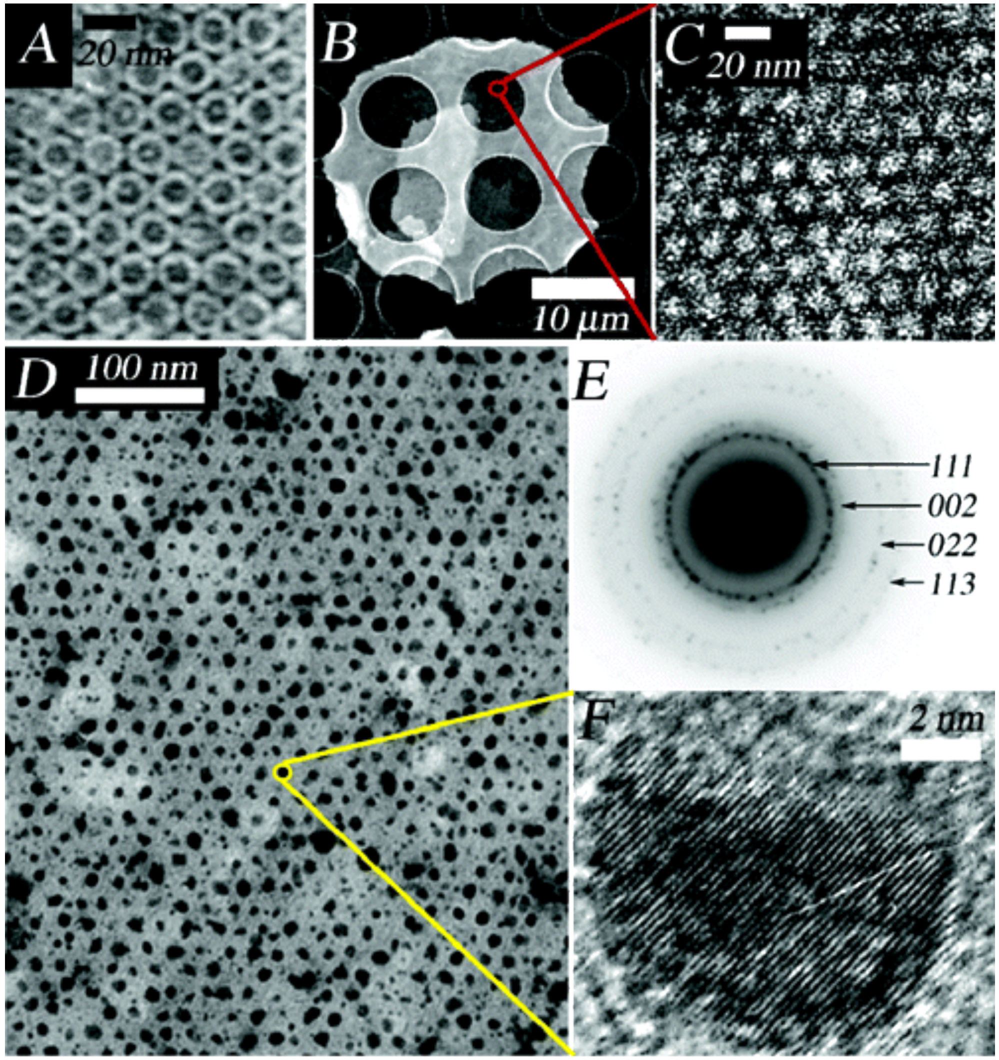


Yan, et al. *Science* **2003**, *301*, 1882-1884

Self Assembly - Proteins



McMillan, et al. *JACS* **2005**, *127*, 2800-2801



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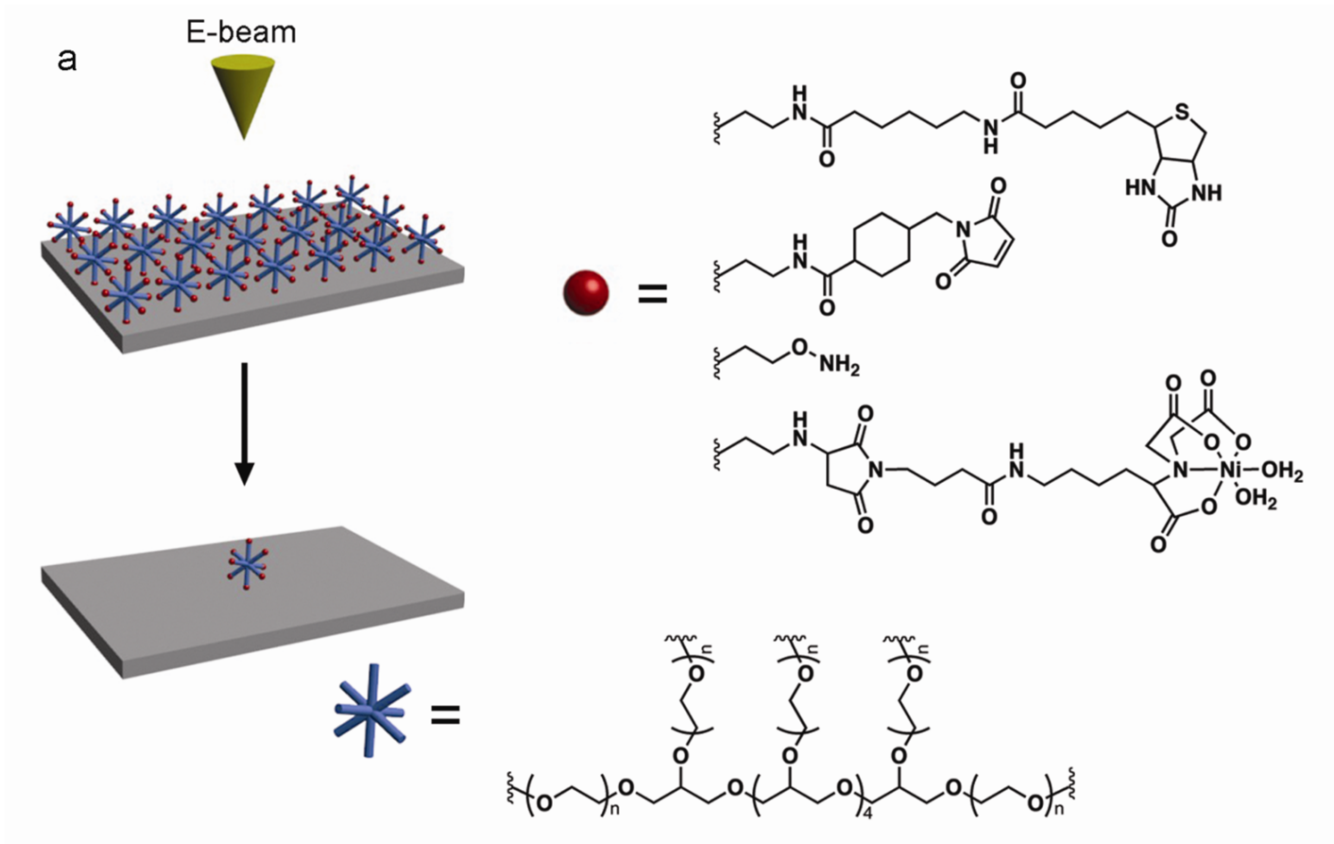
Outline

- Overview of techniques to pattern biomolecules at the nanoscale
- **Example 1: Multiprotein patterns by e-beam lithography**
- Example 2: Cell adhesive materials

Experimental Approach

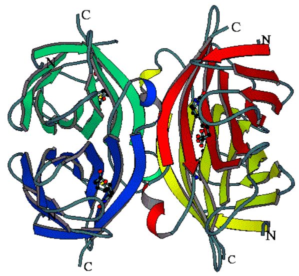
PEGs cross-link to surfaces (Merrill *J. Biomed. Mater. Res.* **1998**; Libera, *Langmuir*, **2004**; Brough et al. *Soft Matter*, **2007**)

Our approach:
-Synthesize 8-arm star PEGs with groups that can bind to specific sites on proteins and cross-link to the surface using electron beams

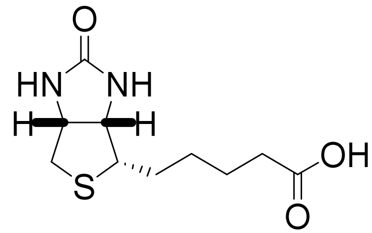


Site Specific Conjugations

Biotin – Streptavidin:



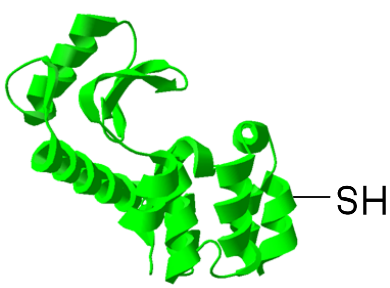
(Freitag, S. et al., *Protein Science* 1997, *6*, 1157)

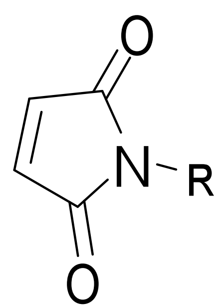


Biotin

- Streptavidin binds four biotins with high affinity

Maleimide – Free Cysteines



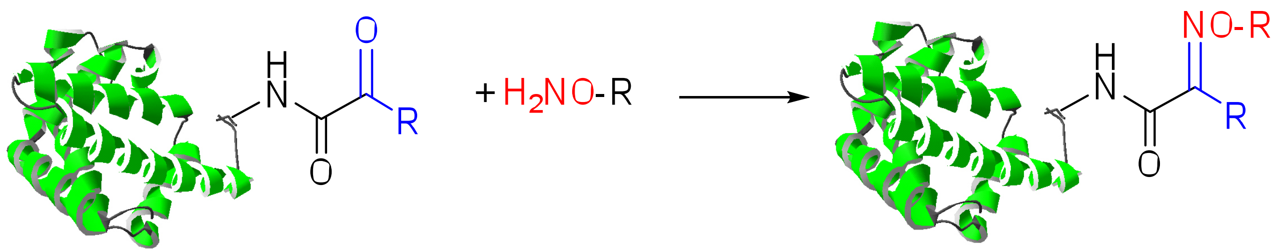


Maleimide

- Maleimide reacts selectively with cysteines not in disulfide bonds

More Site-Specific Conjugations

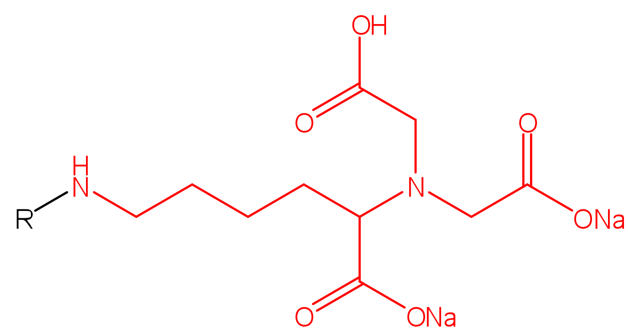
Ketone - Aminooxy



- N-terminal α-oxoamide protein binds to aminooxy to form oxime bond

NTA-Ni^{2+} - Histidine

Proteins modified with His-Tags

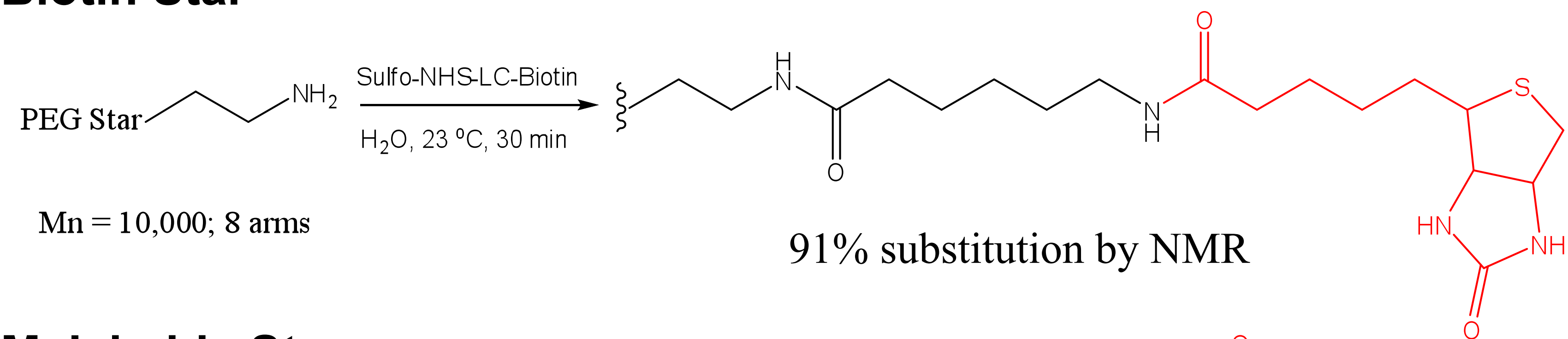


NTA group

- Histidine tagged proteins bind to Ni^{2+} - NTA

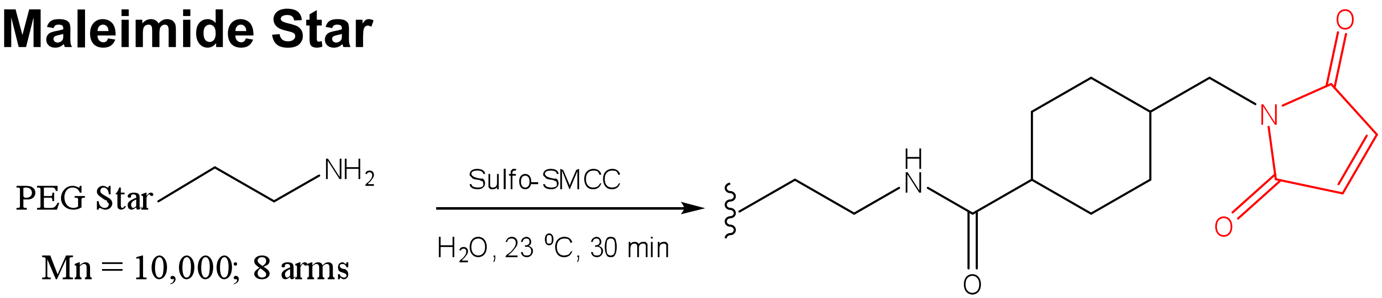
Polymers for Site Specific Protein Conjugation

Biotin Star



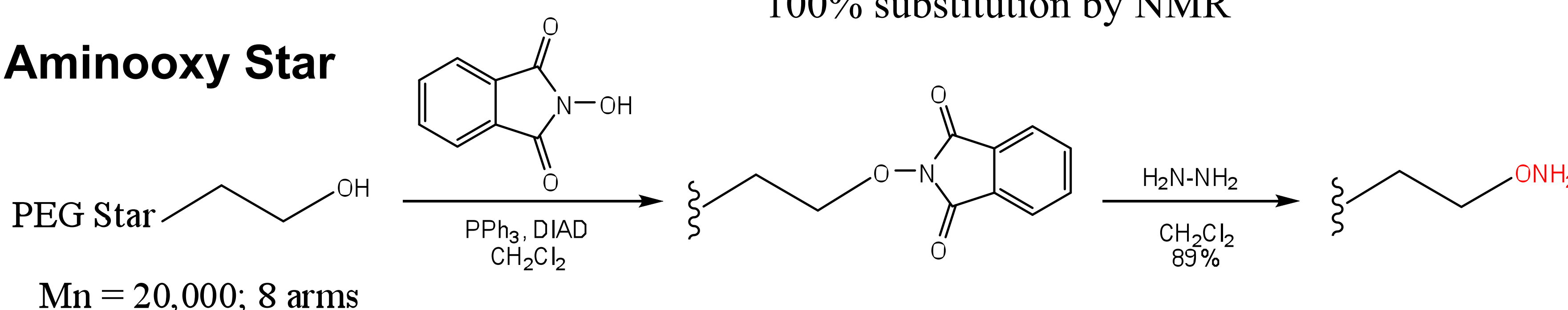
91% substitution by NMR

Maleimide Star



100% substitution by NMR

Aminooxy Star

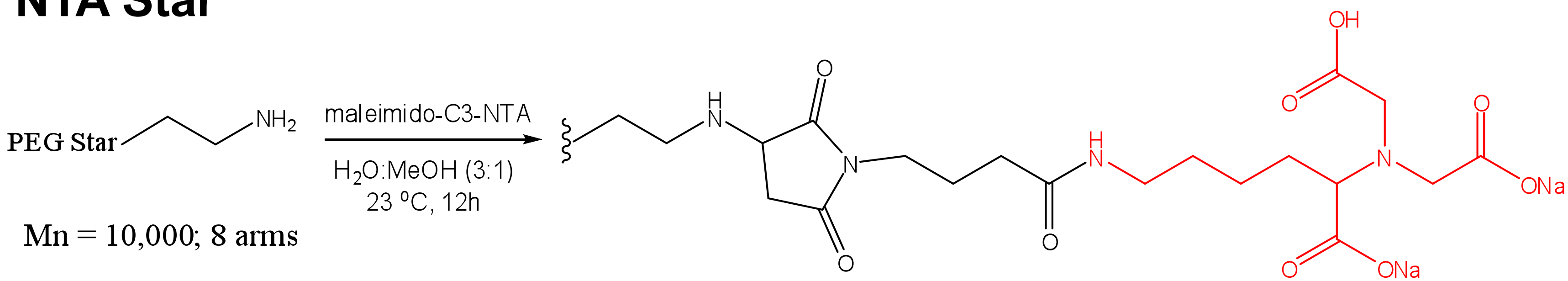


97% substitution by NMR

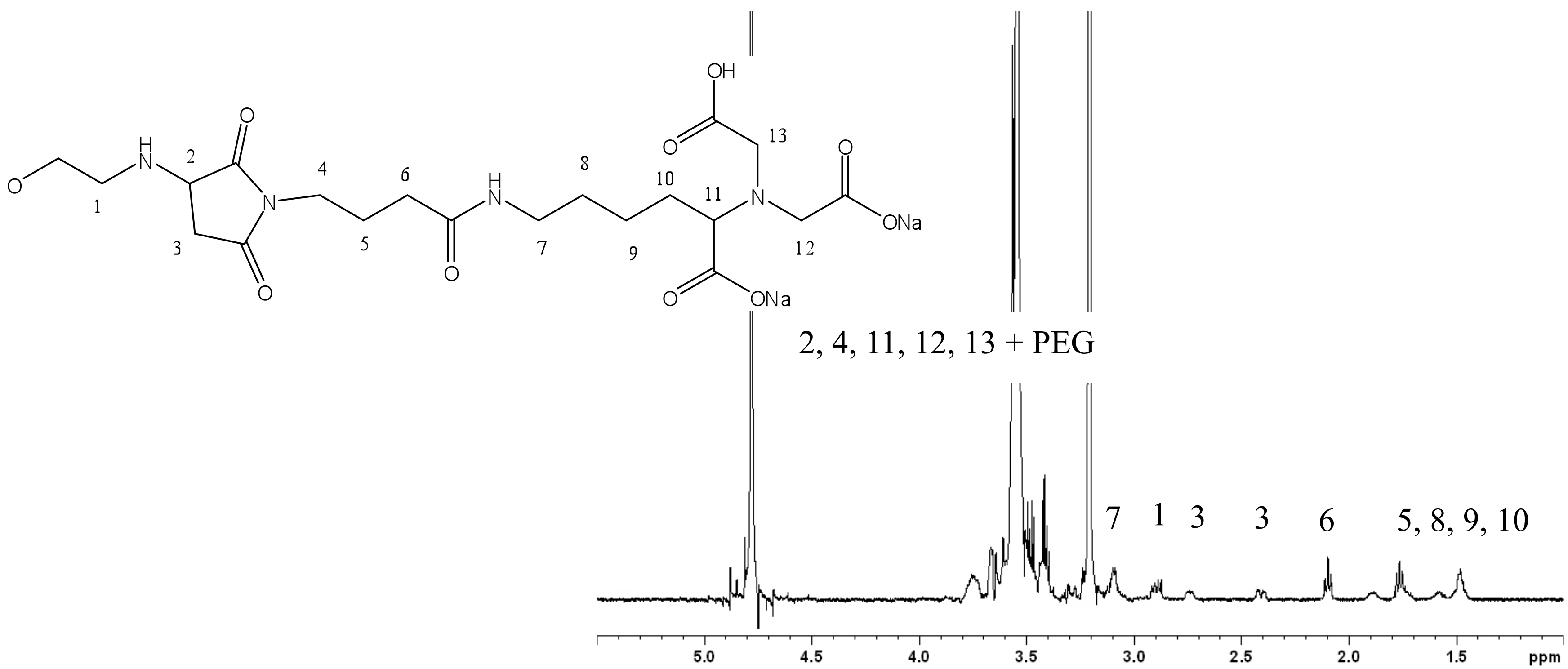
(Schlick, T.L., et al. *JACS*, **2005**)

Polymers for Site Specific Protein Conjugation

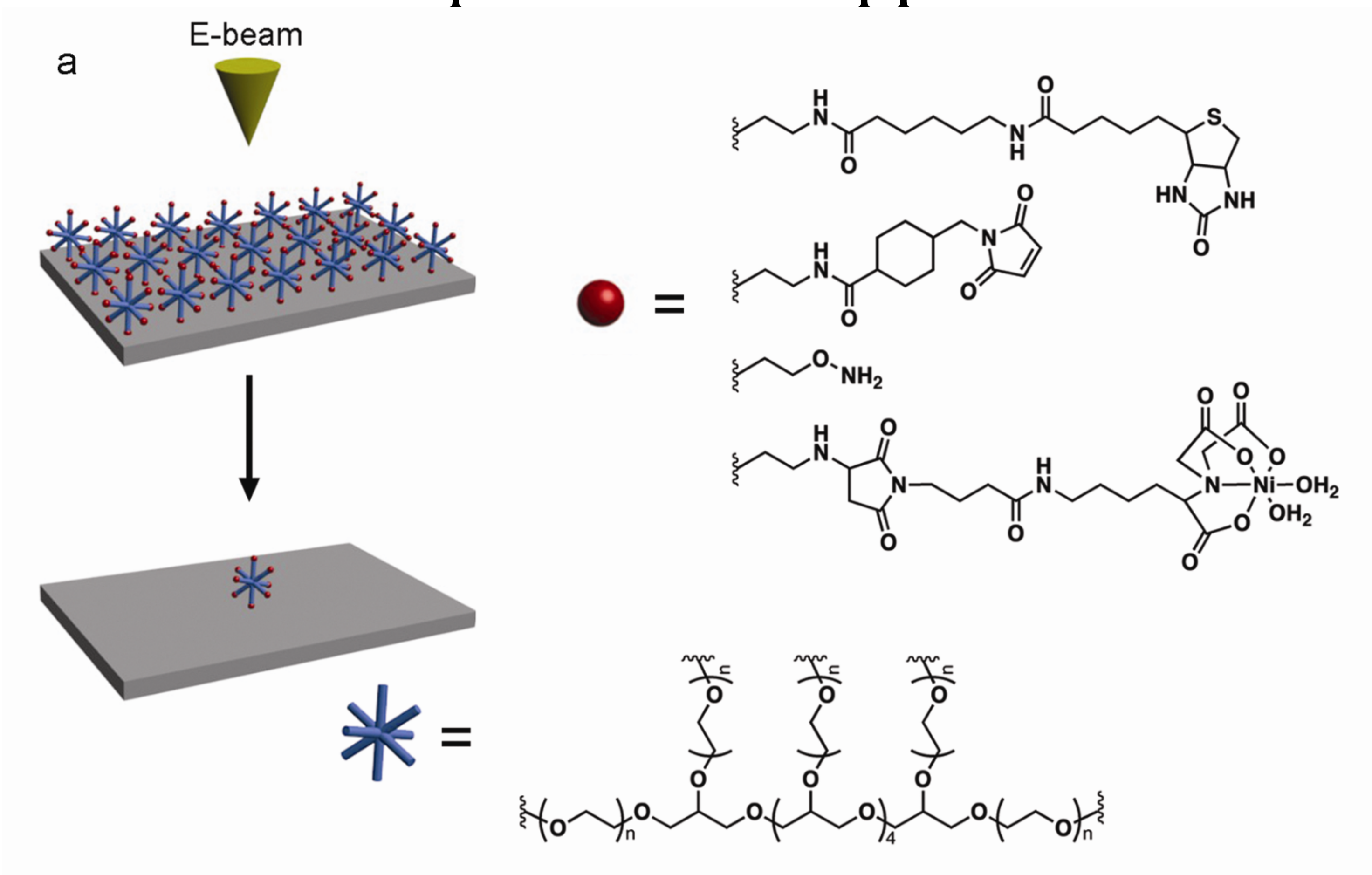
NTA Star



100% substitution by NMR



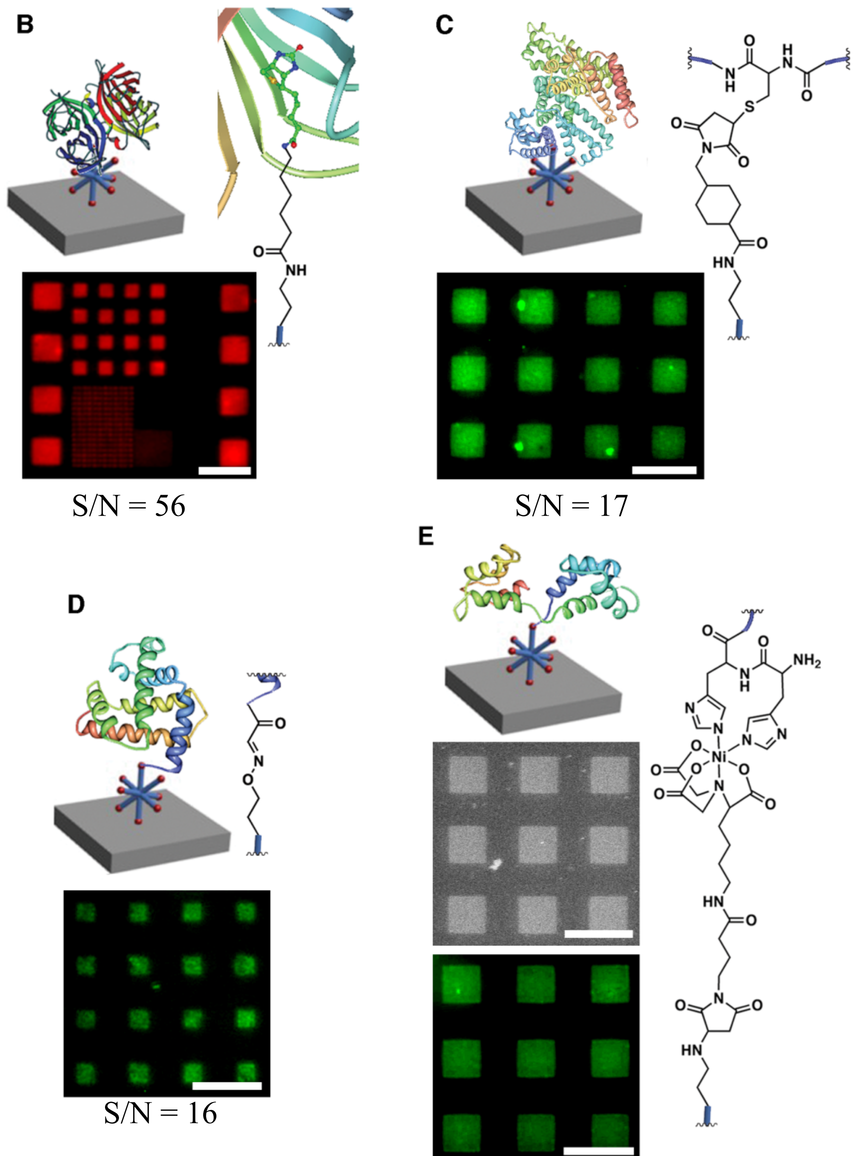
Experimental Approach



E-beam: accelerating voltage 30kV, current 4.5 pA, dose 1.1 - 140 μC/cm^2

-E-beam induced cross-linking produces patterns of functional groups

Micron-Sized Arrays of Single Proteins



Scale bar = 20 μm

JEOL JSM-6700F FE-SEM 10 kV, 10 pA; 8 mm; S/N = 13

-SAv bound by ligand binding sites (biotin)

-BSA Michael addition of free thiol to maleimide

-α-glyoxylamide-modified myoglobin binds via oxime bond formation

-Histidine-tagged calmodulin binds to nickel (II) surface (top - SEM before protein adsorption)

All reactions are under mild aqueous solutions and do no require additional reagents that can lead to protein denaturation or reduced activity

Multicomponent Nanopatterns

For many desired applications, multiple proteins are required

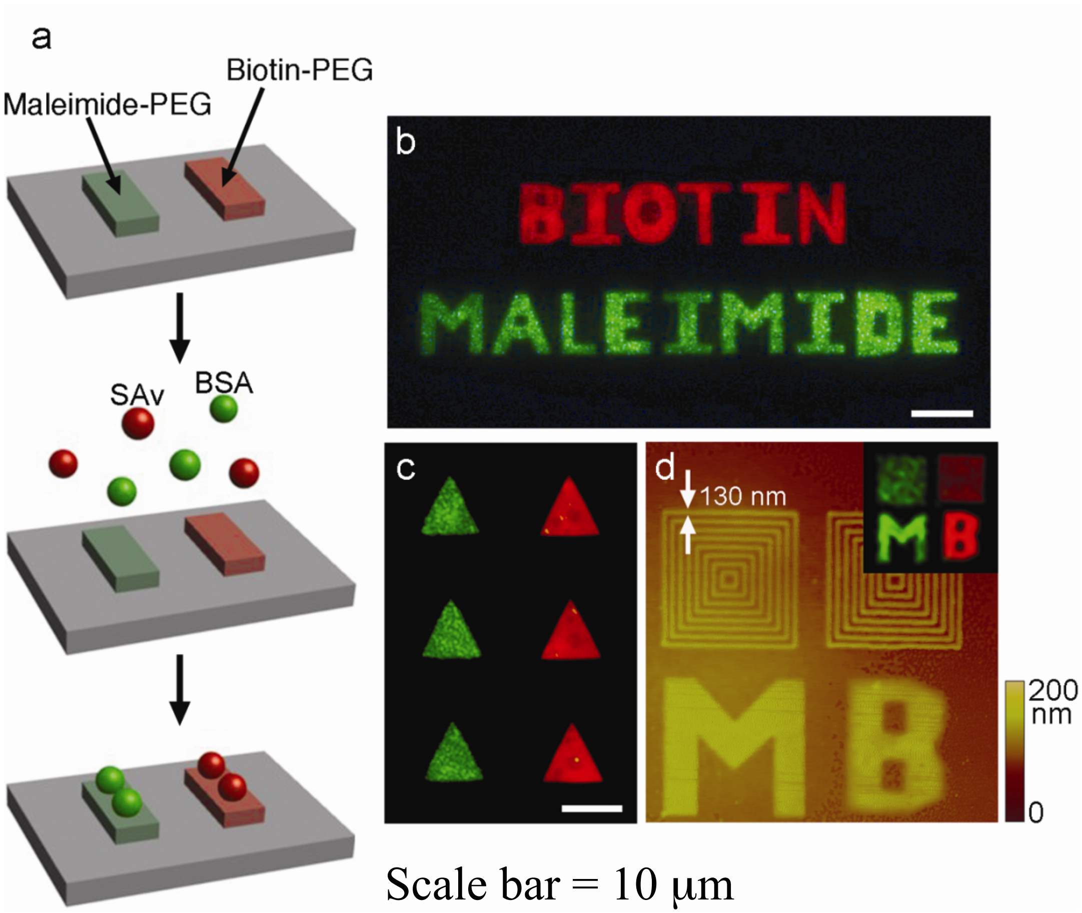
Yet this is difficult to achieve

See examples by: Mirkin and coworkers, *JACS* 2003; *Angew. Chem. Int. Ed.* 2003; Zhao, Banerjee, Matsui, *JACS* 2005; Coyer, S. R. et al. *Angew. Chem. Int. Ed.* **2007** *46*, 6837-6840; Tinazli, et al. *Nature Nanotech.* **2**, 220-225 (2007).

Can we utilize e-beam lithography to achieve this? With e-beams, nanoscale spacings are possible.

Pattern PEGs with orthogonal reactivity side-by-side

Multiple Proteins by E-beam Lithography



Scale bar = 10 μm

E-beam-induced cross-linking of biotin-PEG and maleimide-PEG, followed by modification with SAv and BSA proteins

Dimension 3100 (Digital Instruments) AFM in tapping mode: silicon cantilever, spring constant = ~ 40 N/m, tip radius = < 10 nm, scan rate = 1.5 Hz

Simultaneous immobilization of multiple proteins from mixtures at the micron and nanometer scale

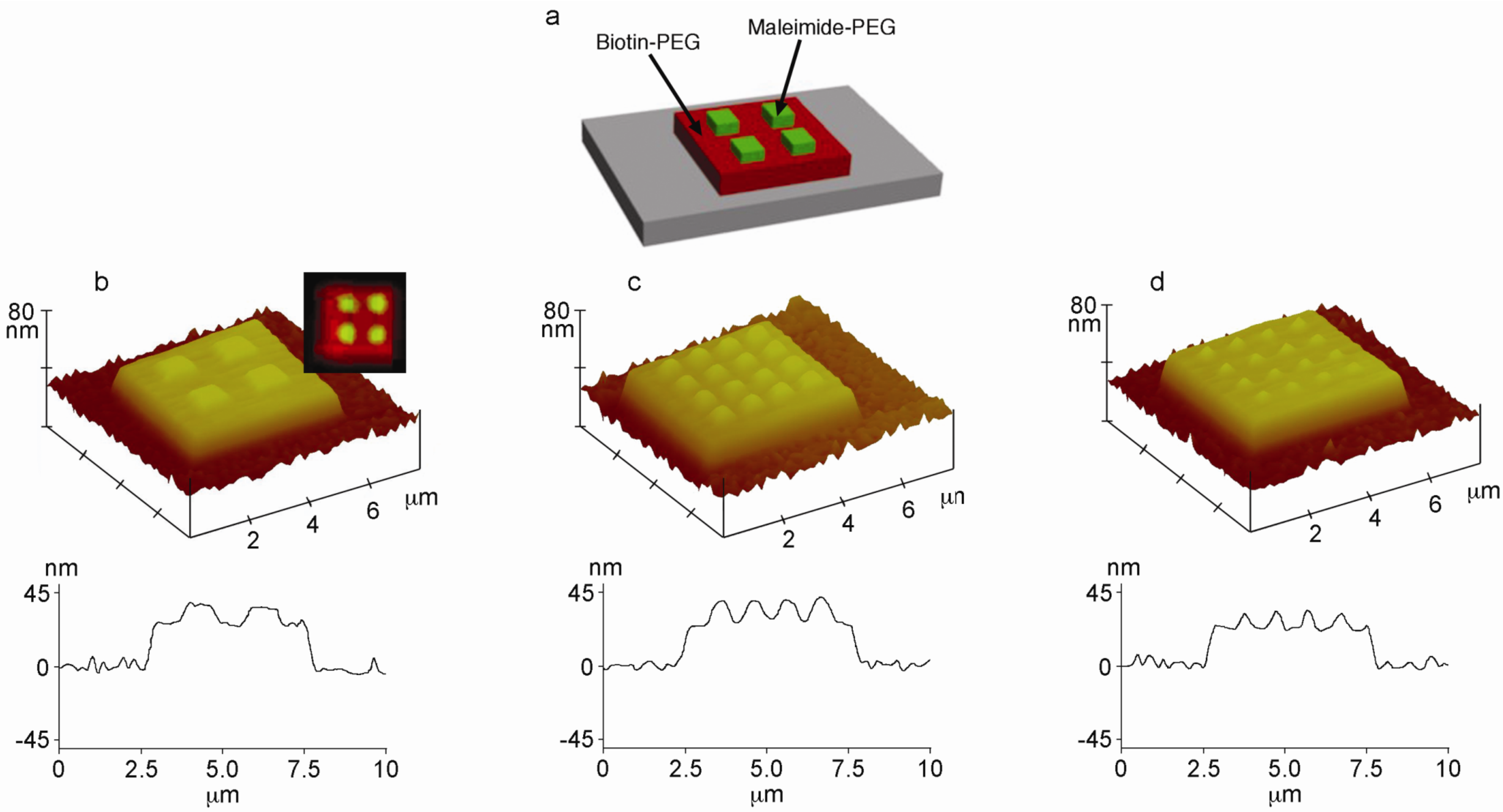
Multilayer Three-Dimensional Patterning

PEG can be cross-linked to itself

Can we use this strategy to prepare 3D multilayer patterns of multiple proteins?

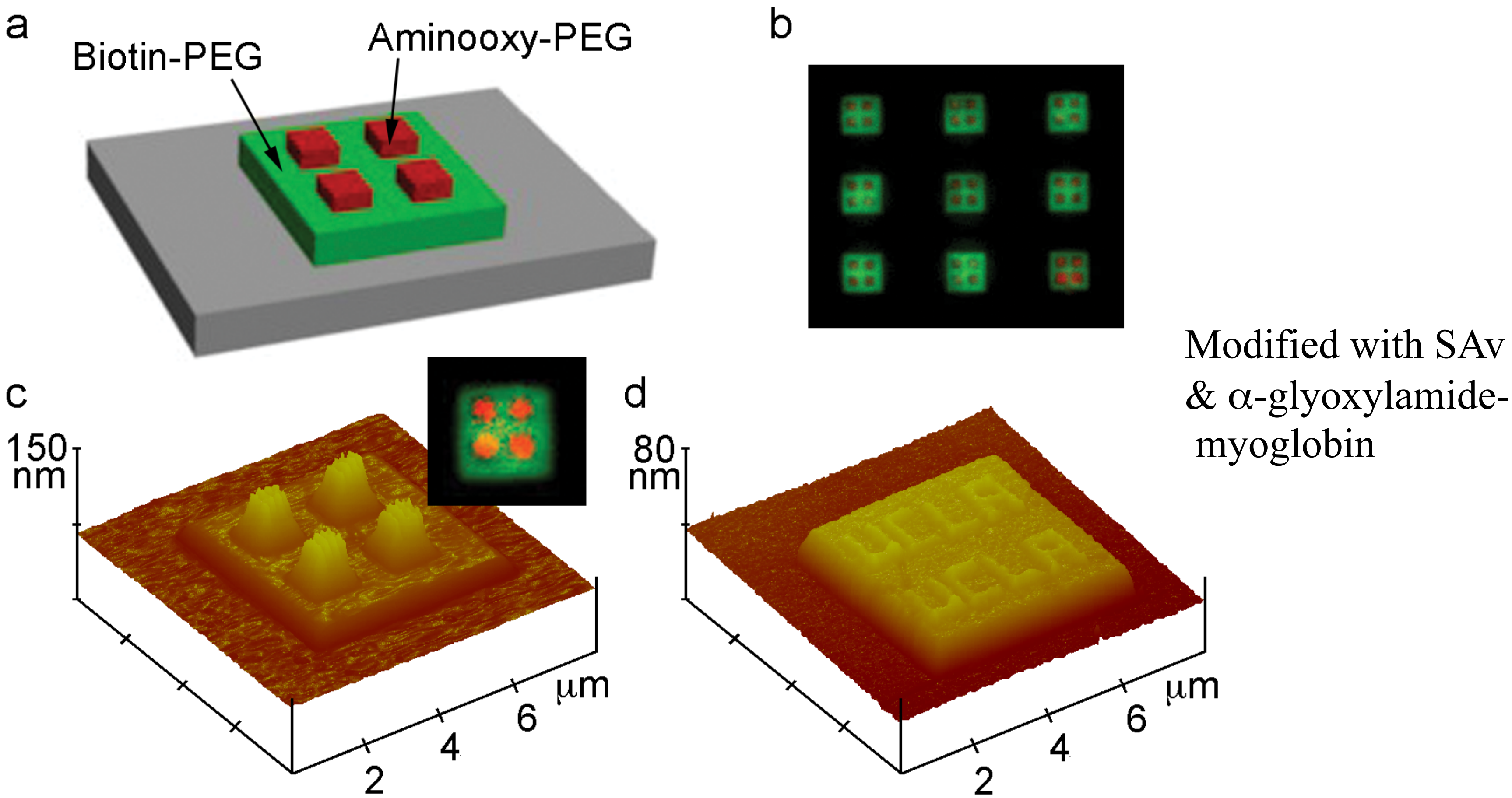
This would be interesting to produce multiplexed biomolecules in three-dimensional multilayer formats for a wide variety of applications such as site-isolation enzyme cascades, "nanoscale factories," mimic natural complex structures such as protein-signaling assembles and viral capsids, present chemical and topographical cues to study and control cell adhesion

SAv-BSA Multilayer Protein Patterns



Simultaneous immobilization of SAv and BSA from a mixture

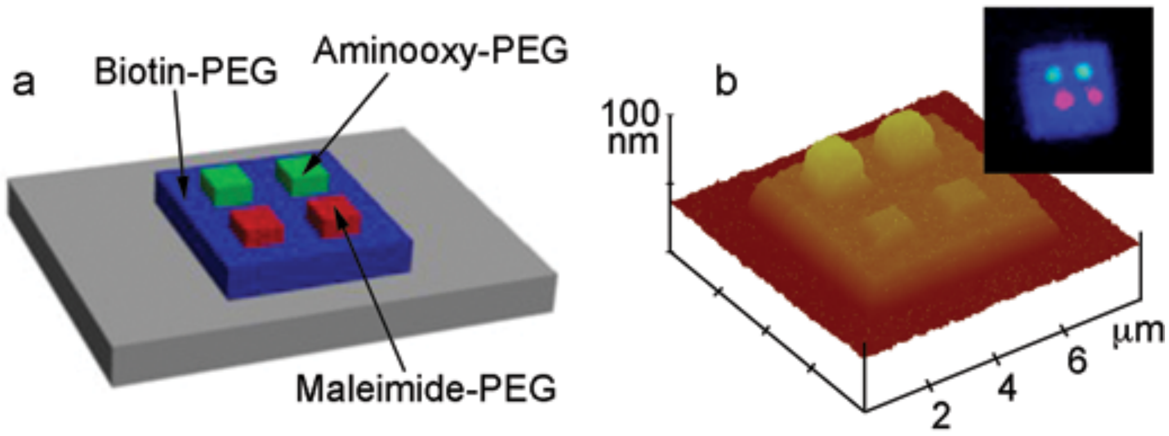
Other Proteins



Modified with SAv & α-glyoxylamide-myoglobin

Range of multicomponent, multilayer nanoscale patterns are possible

Three-Component Structures



Modified with Sav, BSA, & α-glyoxylamide- myoglobin

Complex patterns with multiple proteins and different topographies are readily prepared

Start to explore biological questions utilizing these strategies

Christman, Schopf, Broyer, Li, Chen, Maynard, *JACS*, **2009**, *131*, 521

Outline

- Overview of techniques to pattern biomolecules at the nanoscale
- Example 1: Multiprotein patterns by e-beam lithography
- **Example 2: Cell adhesive materials**

Protein adsorption

- Results in bioactive surfaces that mediate cell attachment
- Causes attachment of cells
  - Sometimes advantageous: osteoblasts in a bone implant
  - Sometimes disadvantageous: platelets on the lining of an vascular graft
- How and why do cells attach to these surfaces?

Cell Attachment

- Adsorbed adhesion proteins such as fibronectin, fibrinogen, and vitronectin
- Cells attach to adsorbed proteins as they do to native extracellular matrix (ECM) proteins

There are five principal classes of cell-adhesion molecules (CAMs)

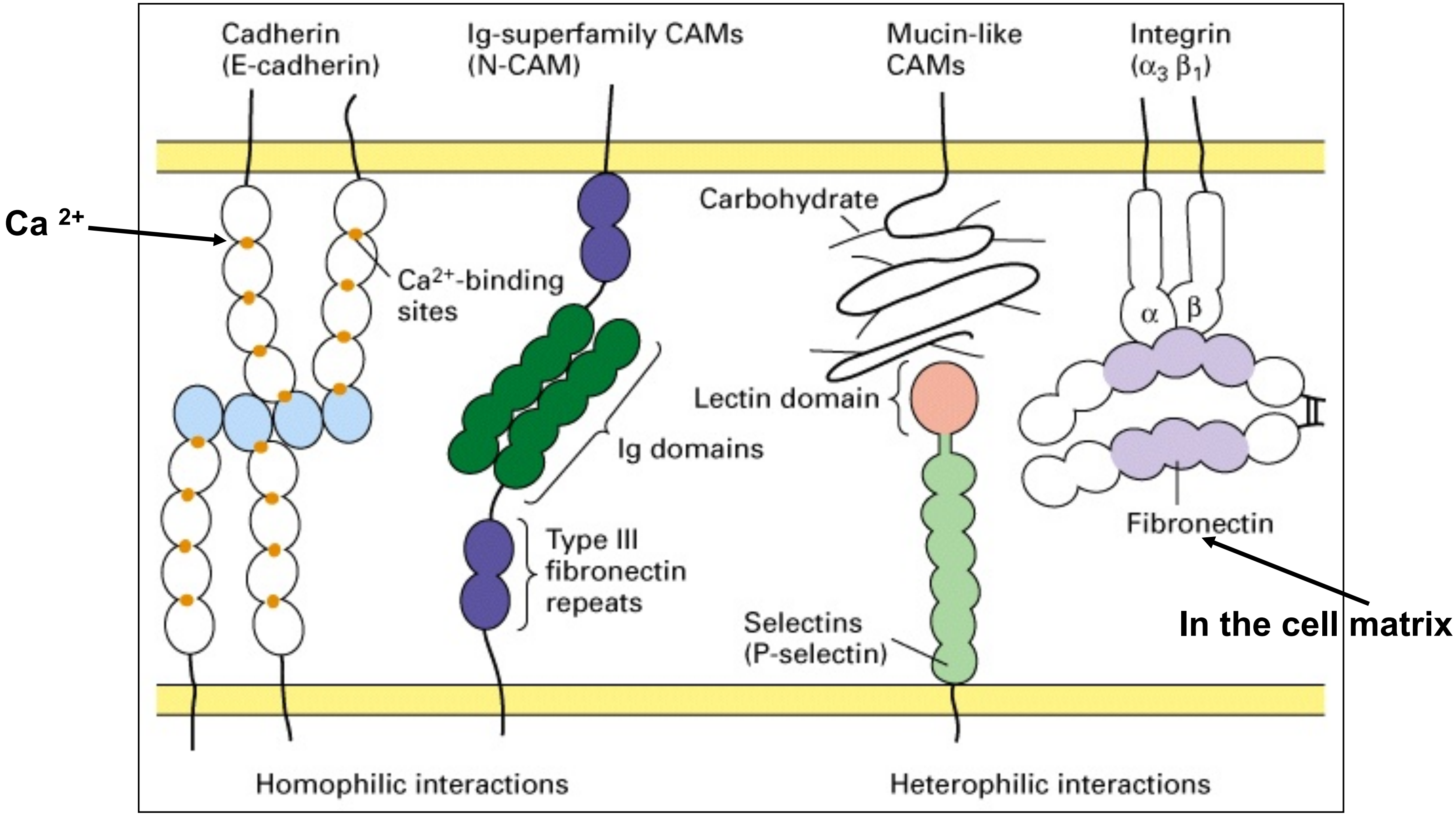


Figure 22-2

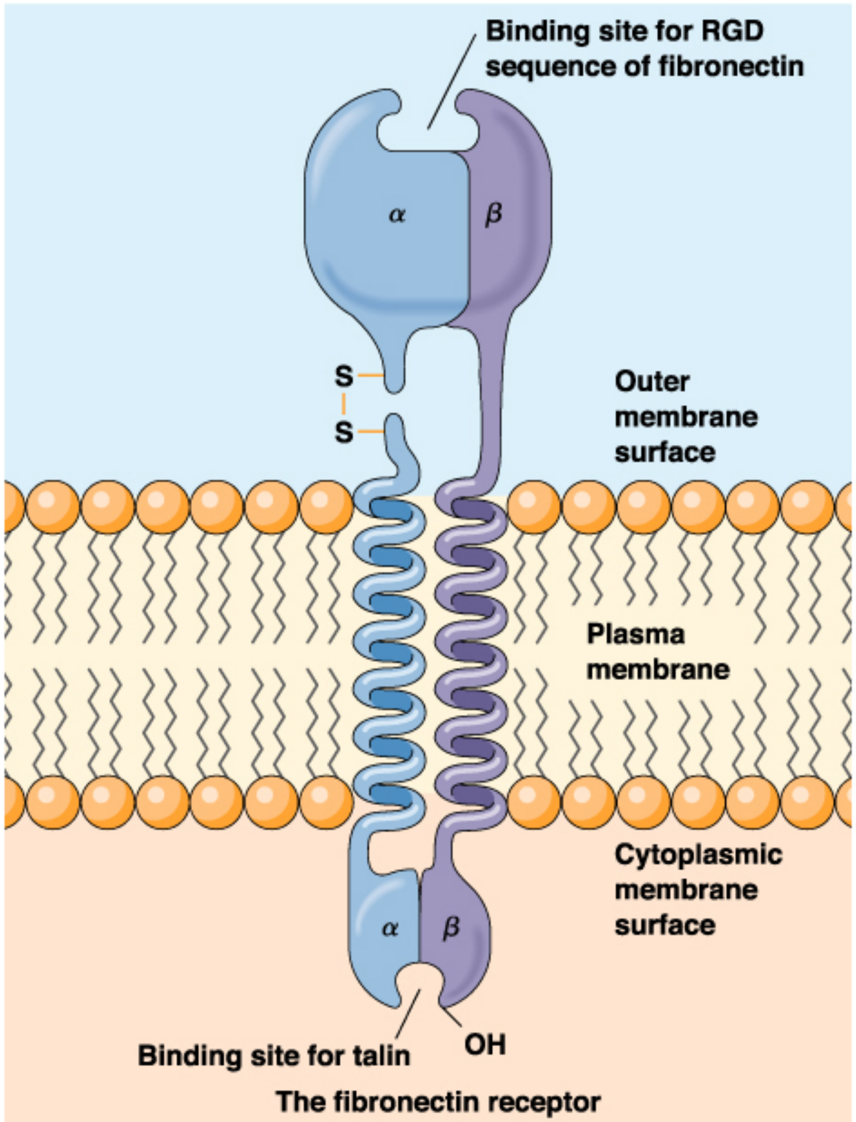
Integrins

A family of membrane glycoproteins that bind to collagen, laminin, fibronectin and other ECM components.

Cell Surface Receptors for ECM Constituents

Involved in cell adhesion, migration, survival, growth, differentiation, and gene expression

Structure of Integrins



Each consists of two different transmembrane polypeptides, α and β subunits.

Extracellular binding sites recognize RGD and other parts of glycoproteins.

The intracellular portions of integrins have the binding sites for cytoskeleton molecules.

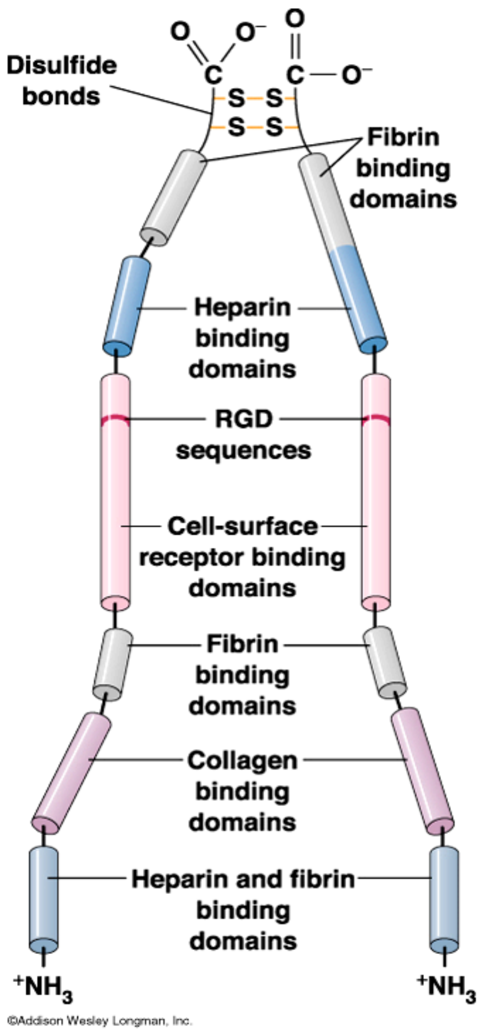
Intracellular cytoskeleton and extracellular matrix are integrated by integrins.

Receptors

- Specific amino acid sequences in ECM molecules bind to cell surface receptors (integrins)

  – arginine-glycine-aspartic acid (RGD) tripeptide: 1st discovered in fibronectin (Pierschbacher and Ruoslahti, 1984)

Fibronectin



Soluble plasma and fibrillar ECM protein

Fibrin – blood clotting protein

Heparin – anti-clotting protein

RGD binding sequence

There are separate domains for Type I, II, and III collagen

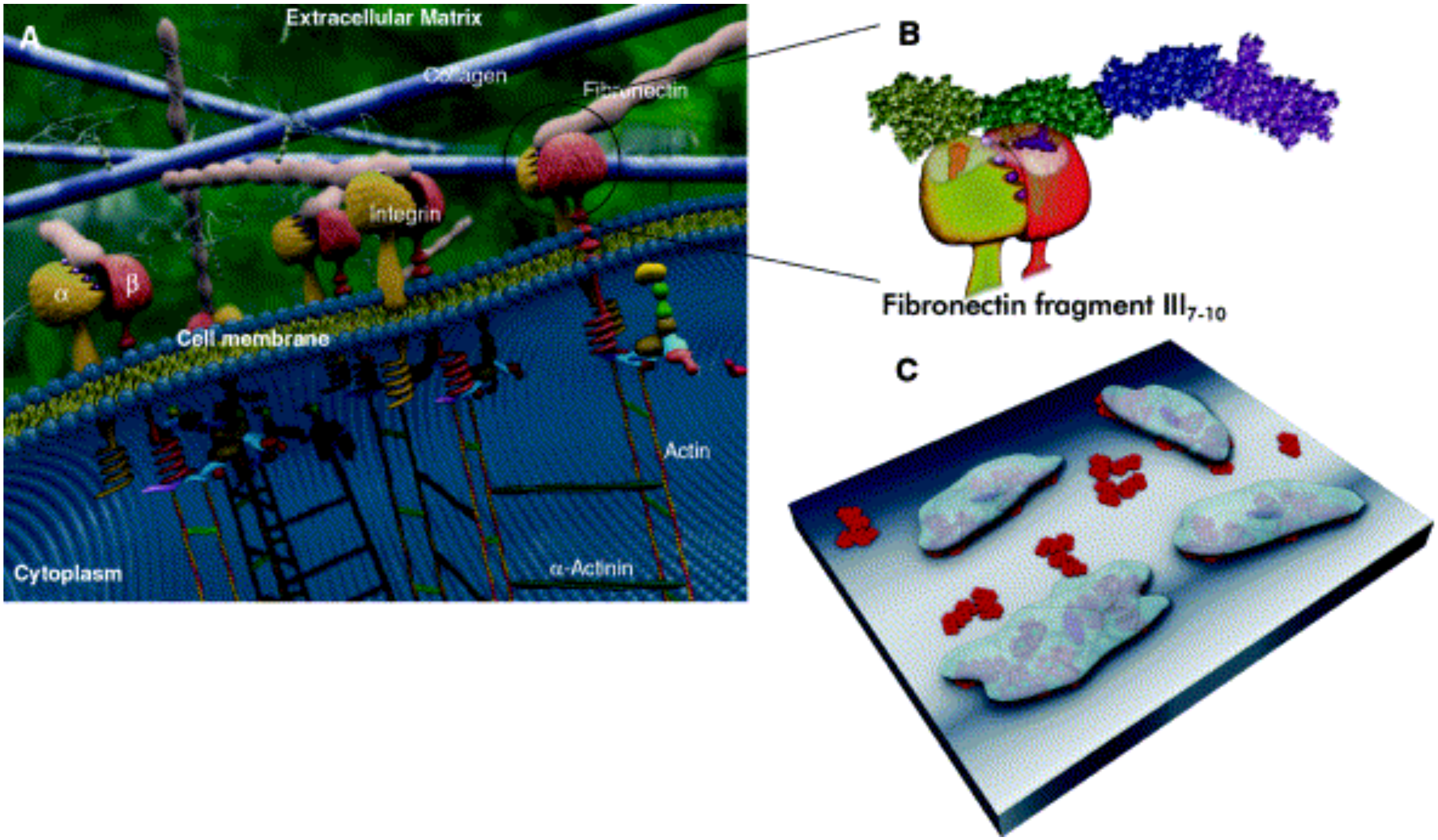
Cytoskeleton-ECM

The influence between cytoskeleton and ECM is mutual.

By binding to integrin, fibronectin can trigger the reorganization of cytoskeleton inside the cell, which affects cell shape and motility.

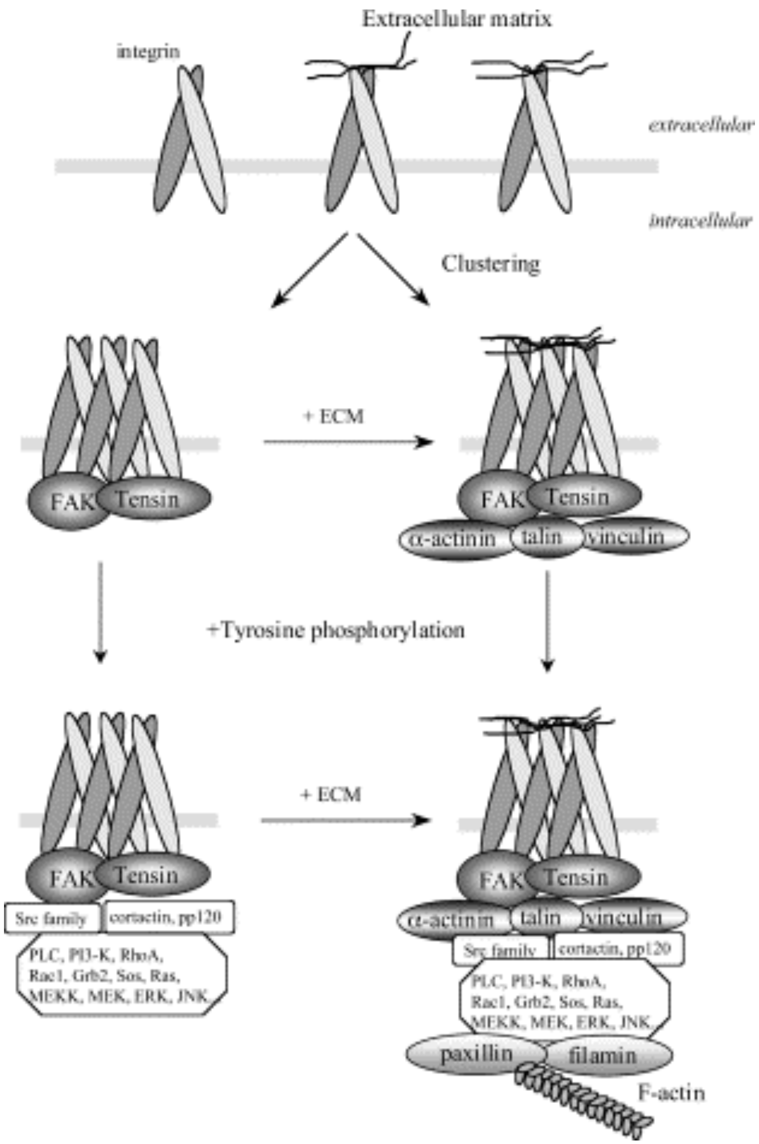
Intracellular cytoskeleton can also influence the attachment and orientation of ECM.

Cell Adhesion to Surfaces via Integrins



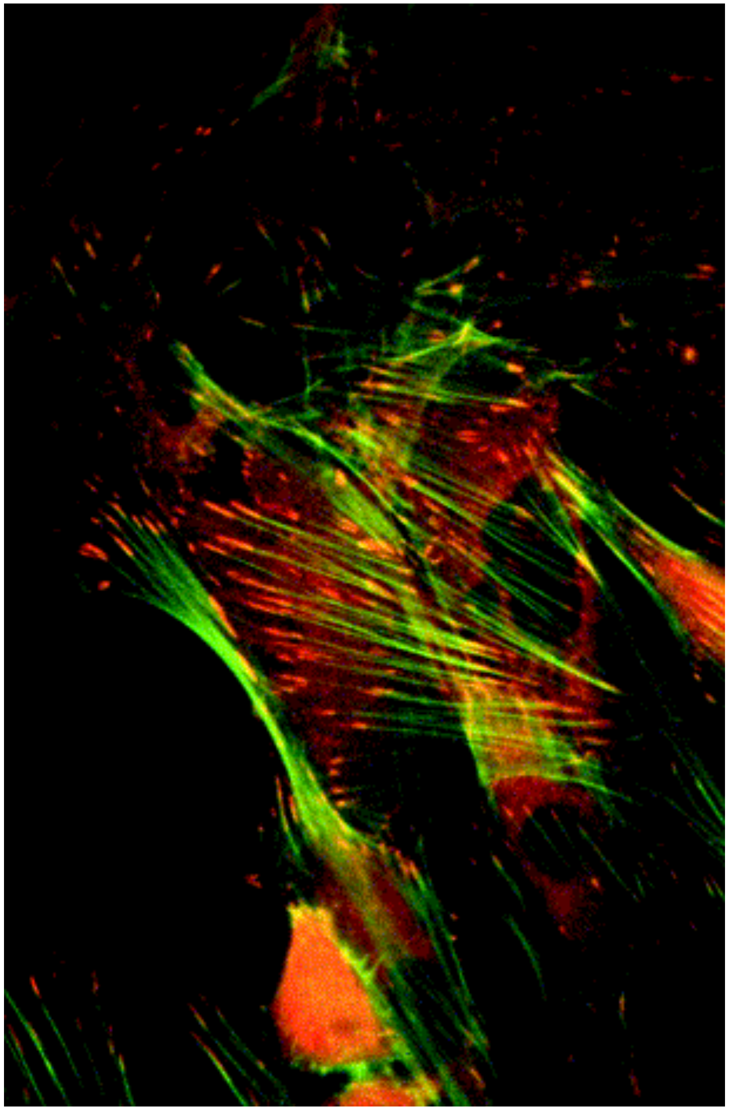
(Tirrell, *et al.* Surface Science, 500 (2002) 61-83

Focal Adhesion Formation – Integrin Clustering



Petit & Thiery, Biology of the Cell, 92 (2000), 477-494

Focal Adhesions & Stress Fibers

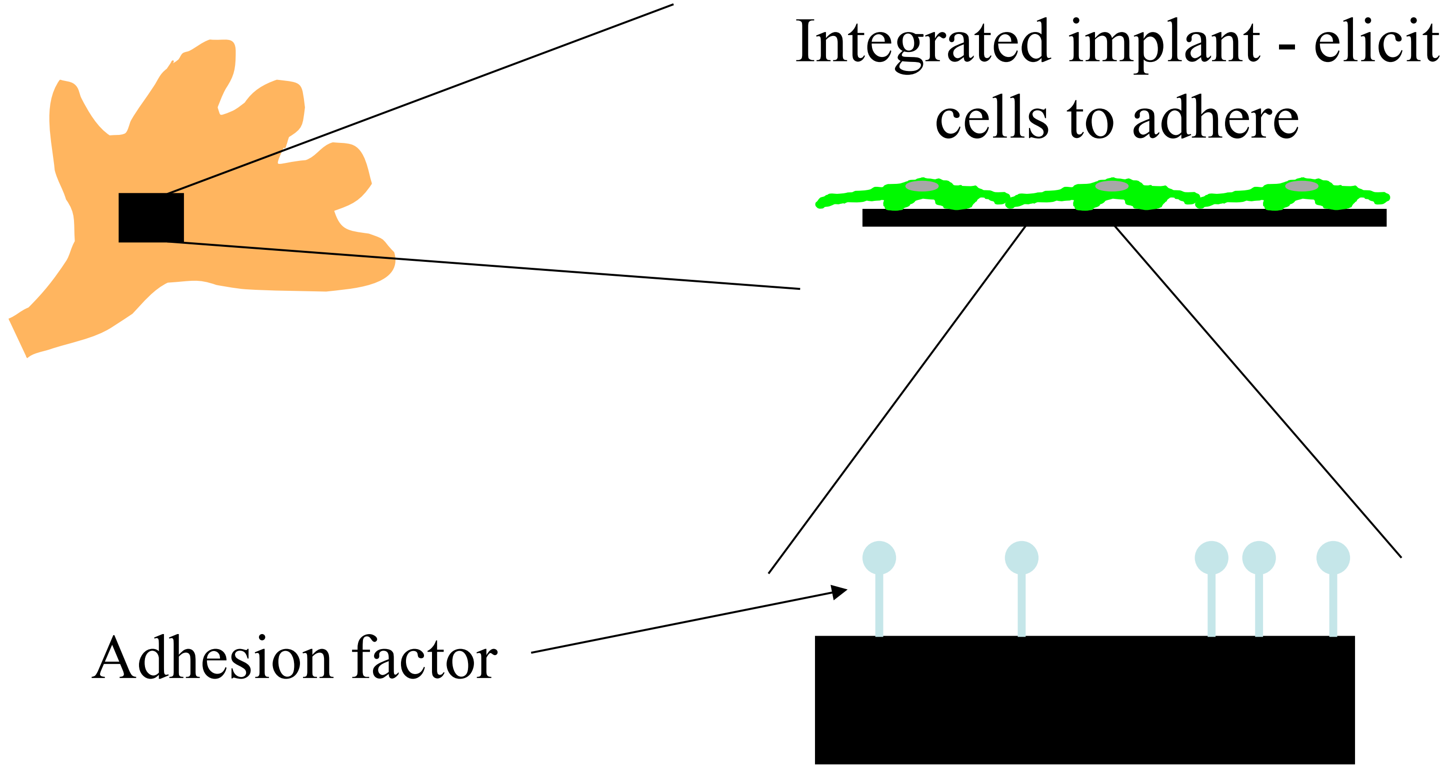


Petit & Thiery, Biology of the Cell, 92 (2000), 477-494

Bioengineering Surfaces

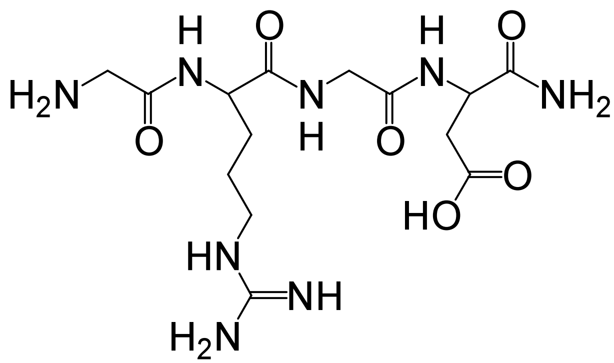
- Coat surfaces with ECM molecules (for example, fibronectin)
- Design ligands and ligand-bearing surfaces to optimize attachment (and/or cell function) by mimicking the ECM

Integrated Implant Materials



An inert surface allows one to control the biological response

RGD-Promotes Cell Adhesion

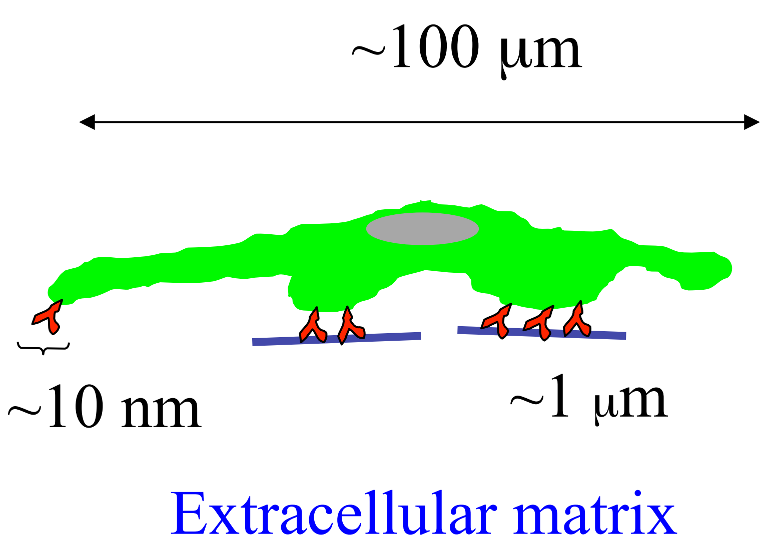


Gly-Arg-Gly-Asp

GRGD

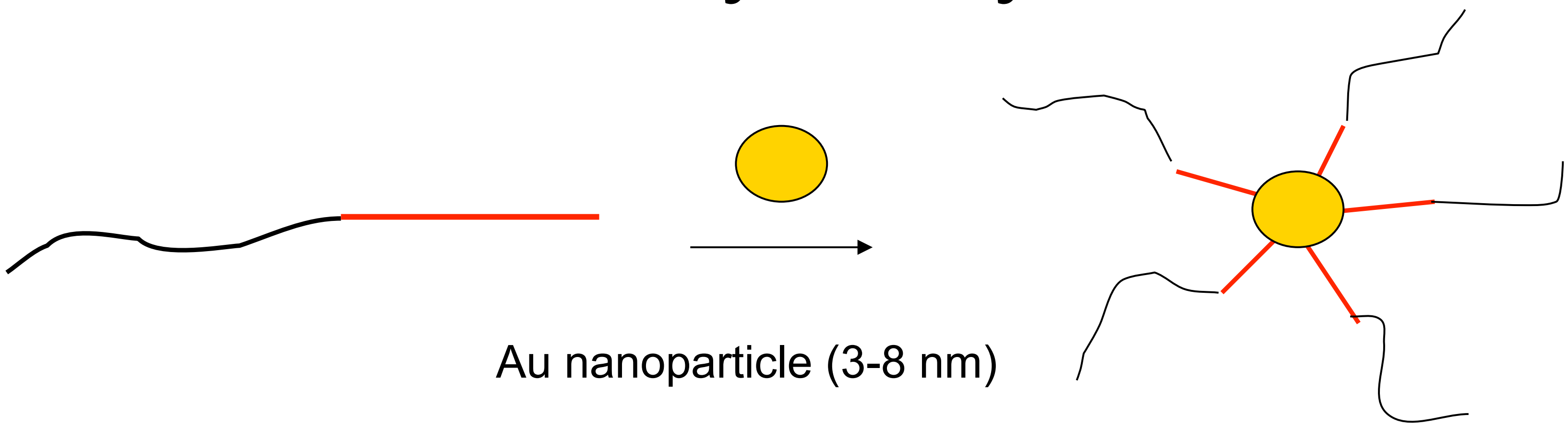
- Soluble peptide inhibits cell adhesion to fibronectin
- Surfaces coated with RGD peptide promote cell attachment and spreading
  - Utilized in numerous biomaterials

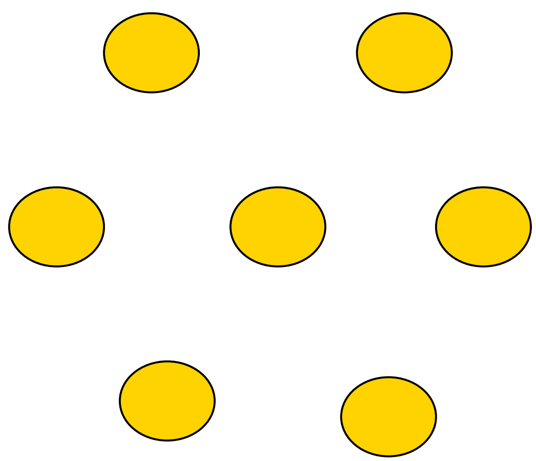
Relevant Scales of Cell Adhesion

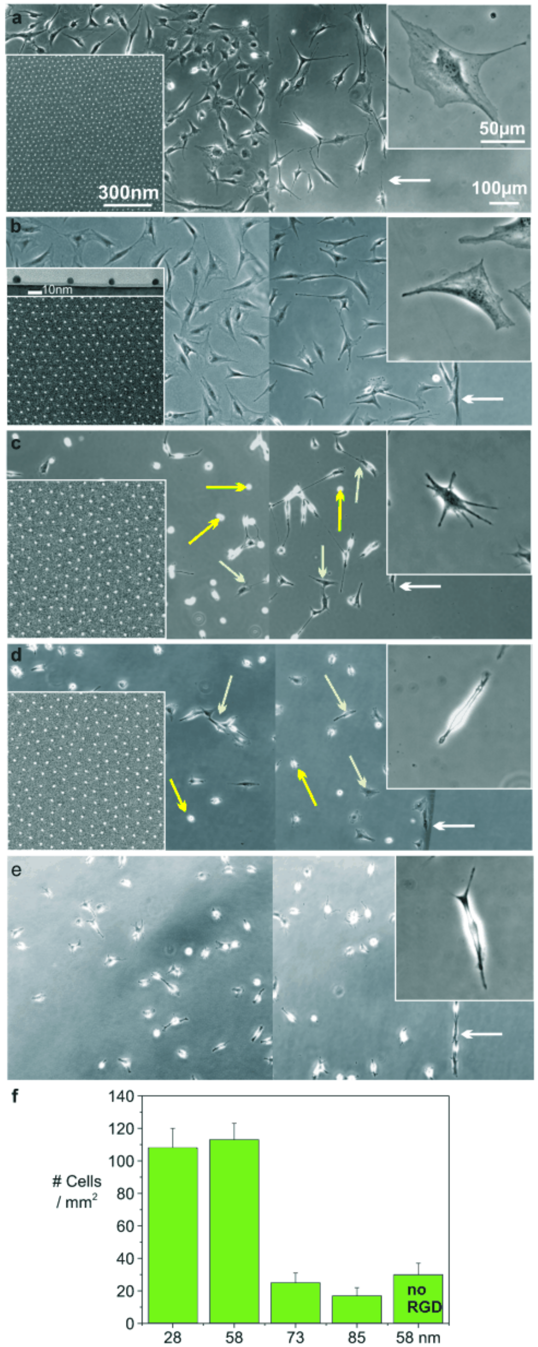


Nanoscale presentation of ligands is critical for cell adhesion – yet few examples

Self Assembly - Polymers







28 nm - RGD

58 nm - RGD

73 nm - RGD

85 nm - RGD

58 nm – no RGD

Arnold et al. *ChemPhysChem* **2004**, *5*, 383-388

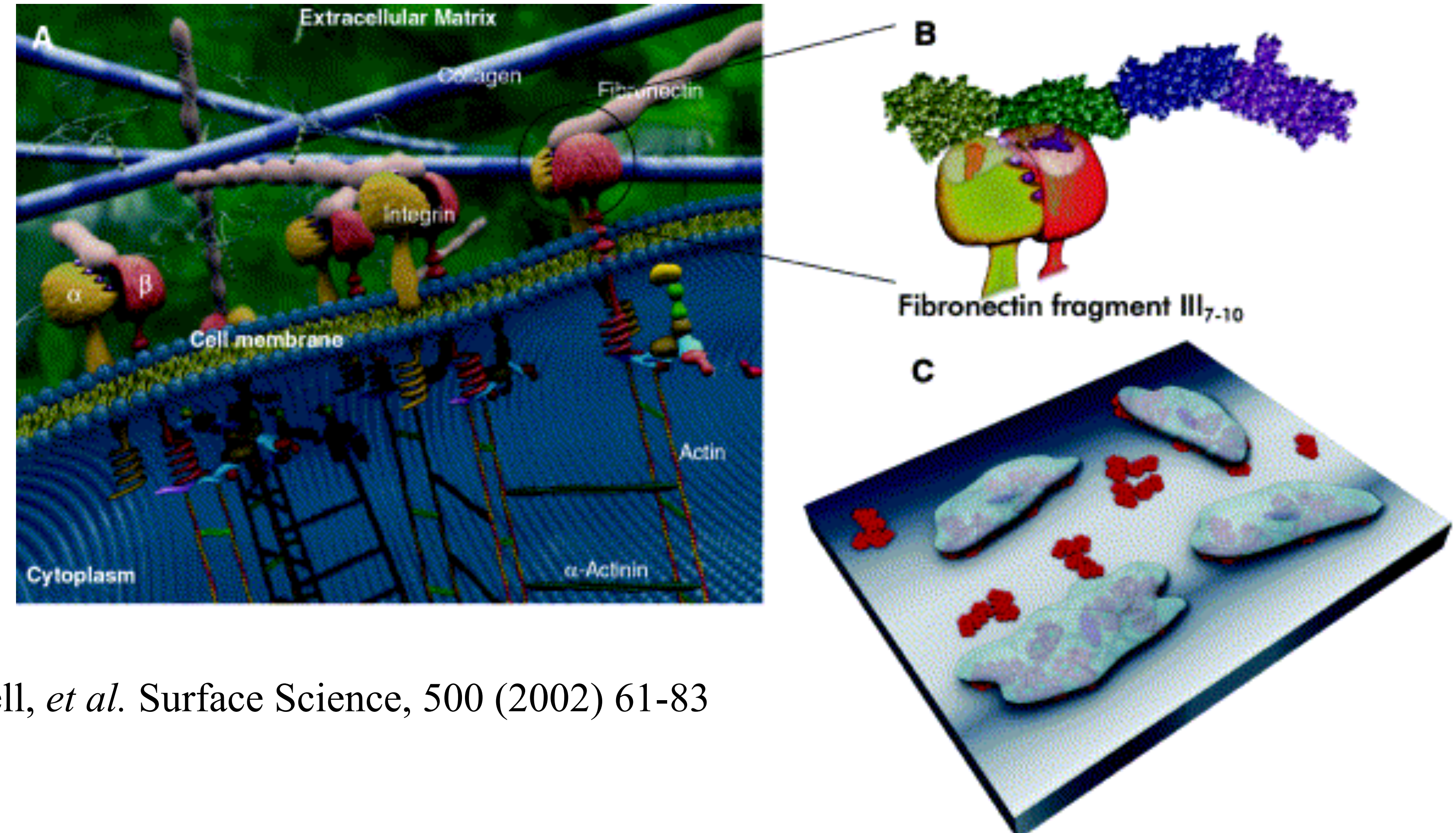
Table 1. ***Surface-pattern and cell-adhesion characteristics.***

PS(x)-*b*-P2 VP(y)[a]	Au dot diameter [b][nm]	Au dot separation [c][nm]	Au dot density [dots/ m^2]	Cell spreading[d]	Focal adhesion formation[e]	Actin fiber formation [e]
190-*b*-190	3±1	28±5	1100	yes	yes	yes
500-*b*-270	5±1	58±7	280	yes	yes	yes
990-*b*-385	6±1	73±8	190	no	no	no
1350-*b*-400	8±1	85±9	100	no	no	no
micro-nanostructures with 500-*b*-270	5±1	58±7[f]	90[f]	yes	yes	yes

Arnold et al. *ChemPhysChem* **2004**, *5*, 383-388

Self Assembly or **E-beam** lithography – Polymers to probe cell adhesion at the nanoscale

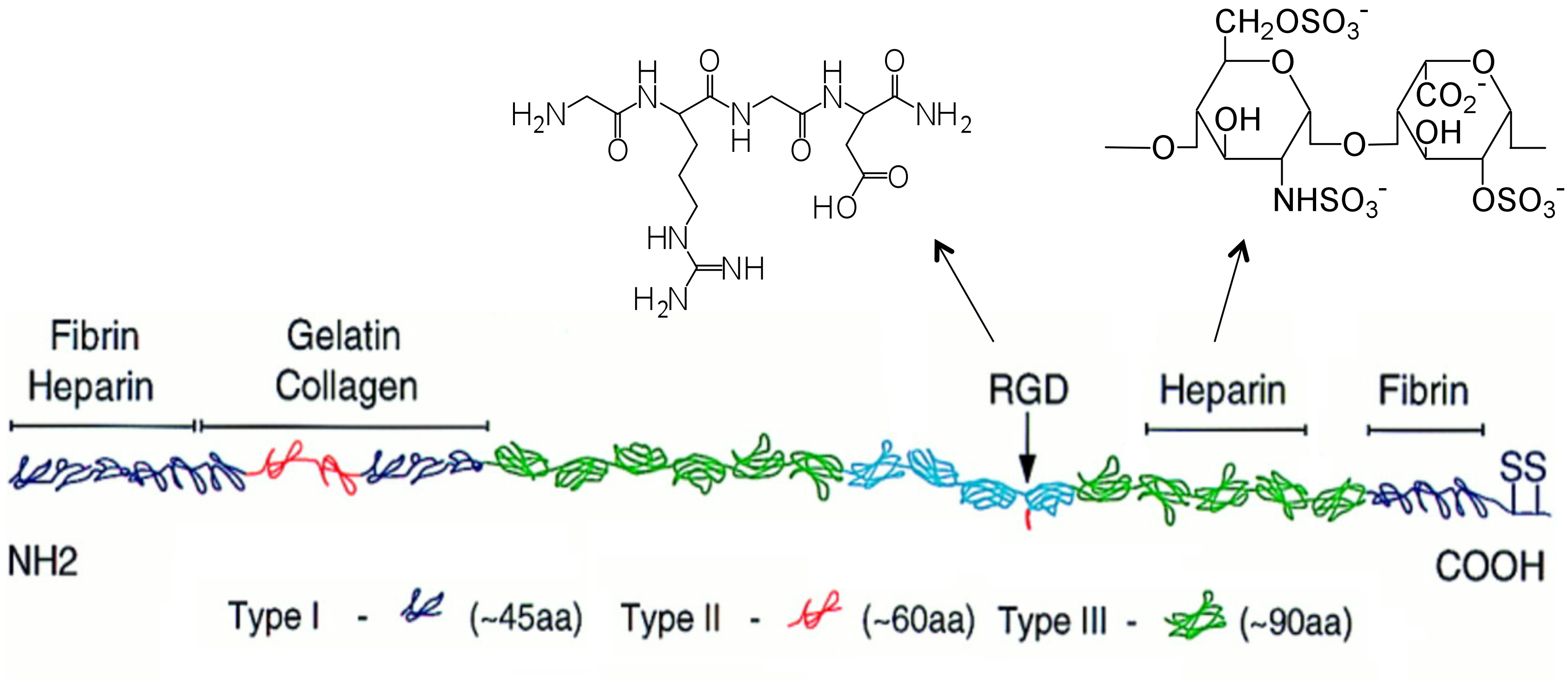
Cell Adhesion to Surfaces via Integrins



Tirrell, *et al.* Surface Science, 500 (2002) 61-83

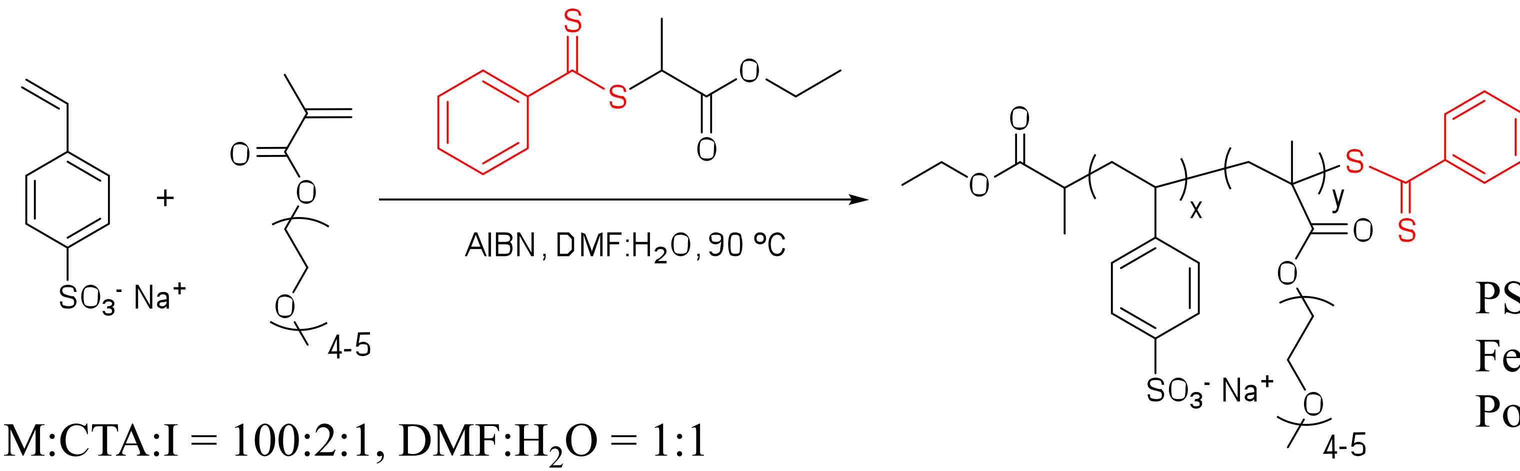
Pattern components of the extra cellular matrix (ECM): peptide RGD and polysaccharide heparin

Fibronectin-Primary Component of ECM



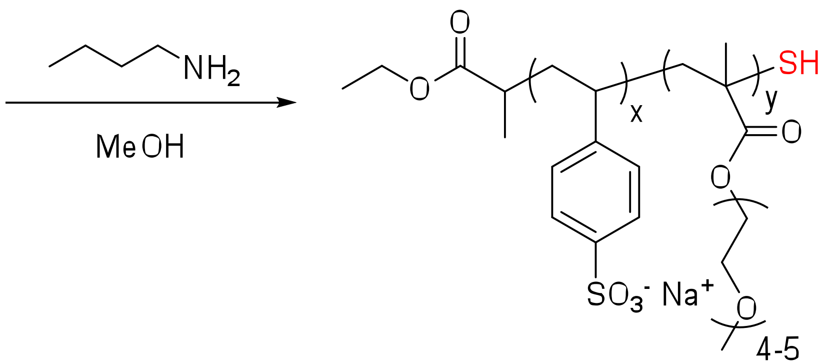
- RGD peptide binds to cells via cellular integrins
- Heparin is polysaccharide found in the ECM and on cell surfaces that binds to growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)

Stable Heparin Mimics by RAFT

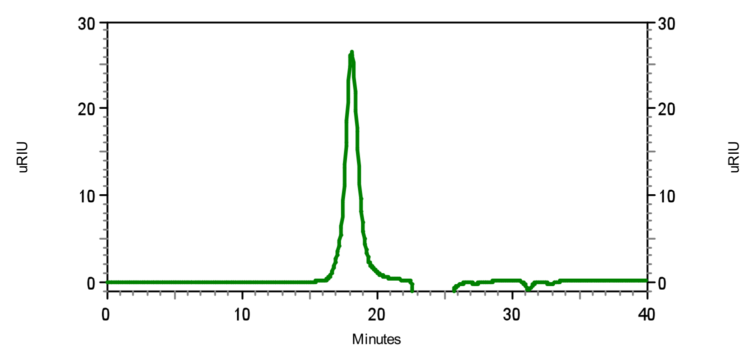


M:CTA:I = 100:2:1, DMF:H_2O = 1:1

PSS:PEGMA
Feed: 2:1
Polymer: 2.2:1

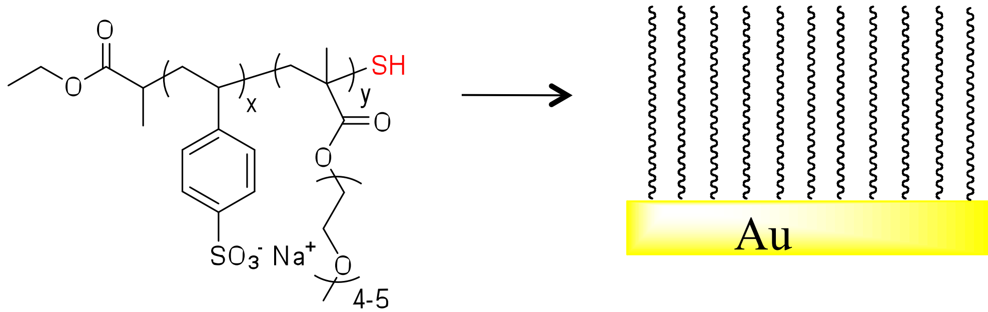


M_n (GPC) = 24,000
PDI = 1.17

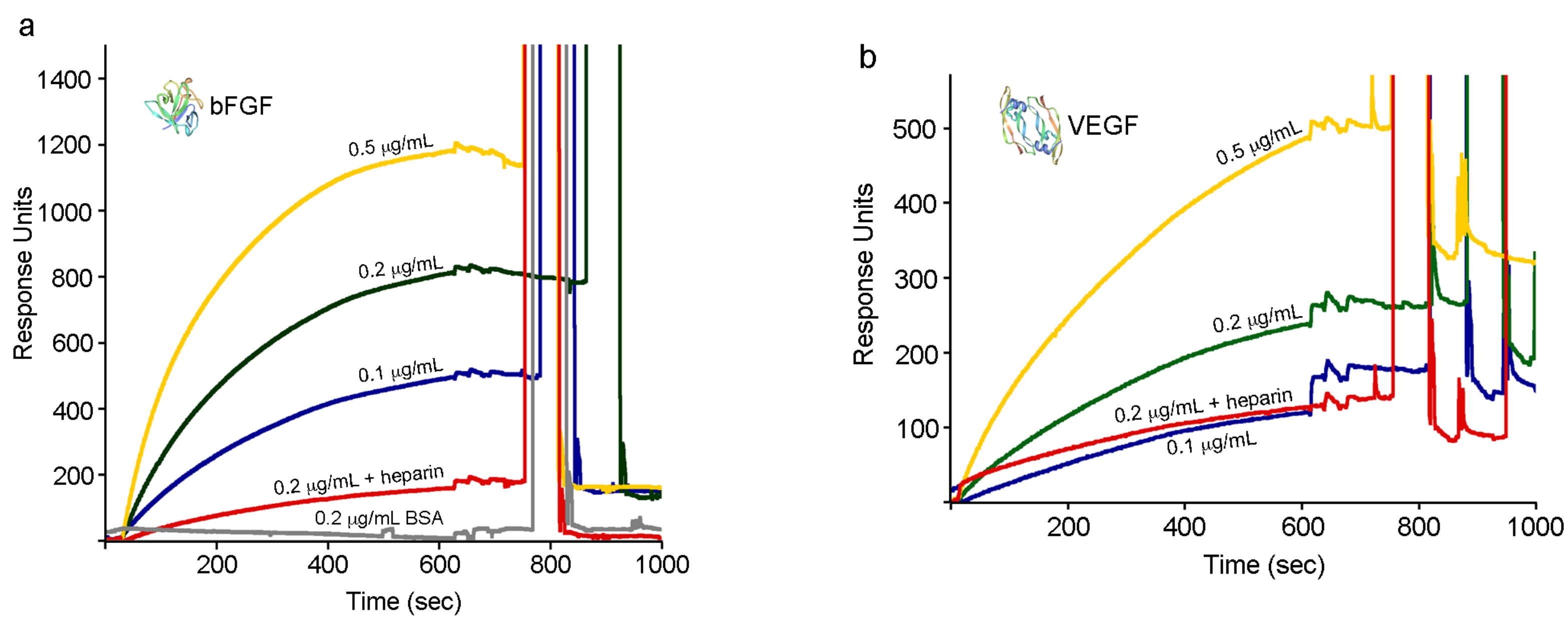


Polymer readily synthesized and reduced to free thiol

Sulfonated Polymer Binds bFGF & VEGF



Modify gold surfaces with polymer for surface plasmon resonance (SPR) studies

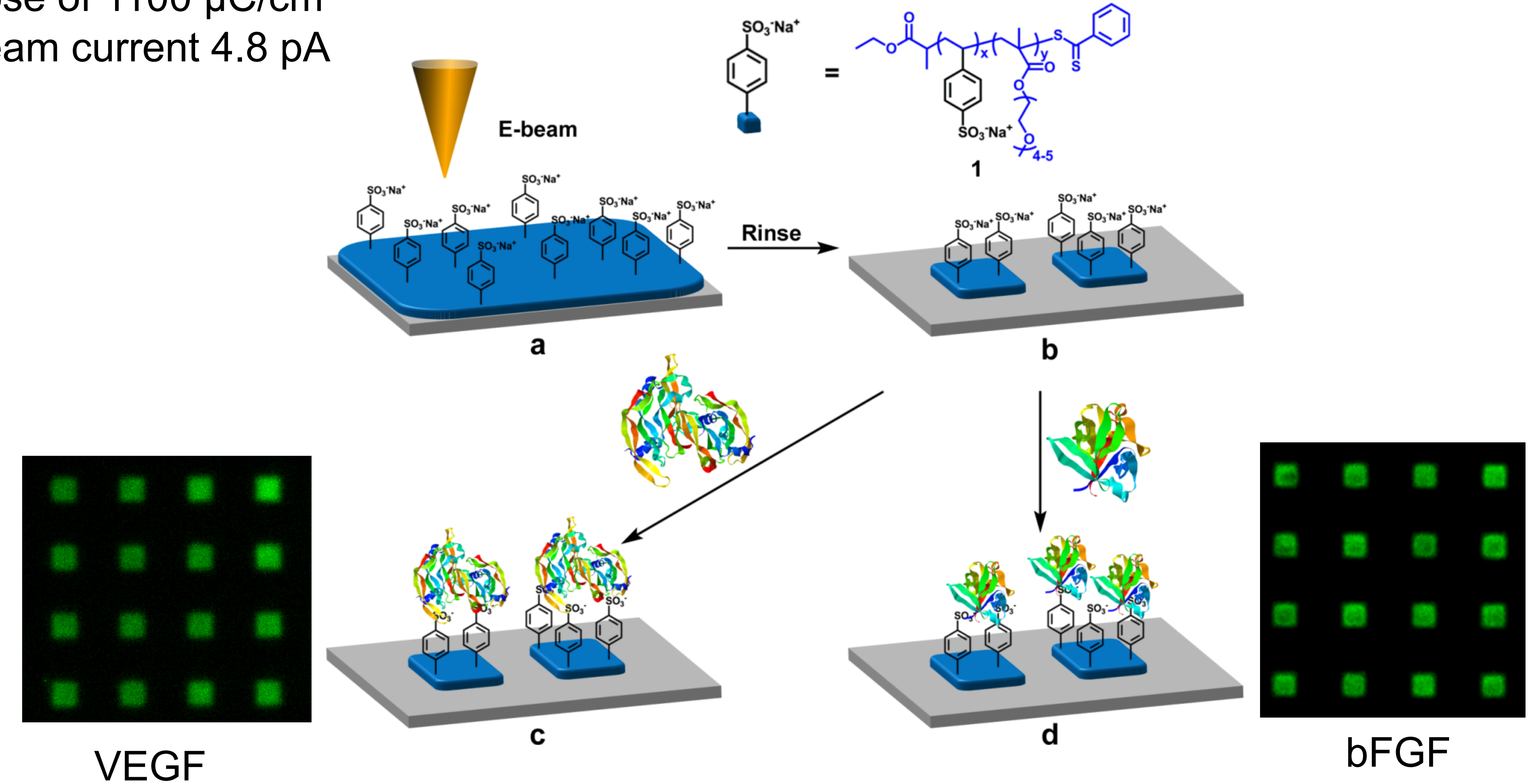


SPR results demonstrate that both bFGF and VEGF bind to polystyrene sulfonate via the heparin binding domain

Pattern Heparin Mimic Polymer

Utilize e-beam radiation to cross-link heparin mimic polymer to surface at the micron scale:

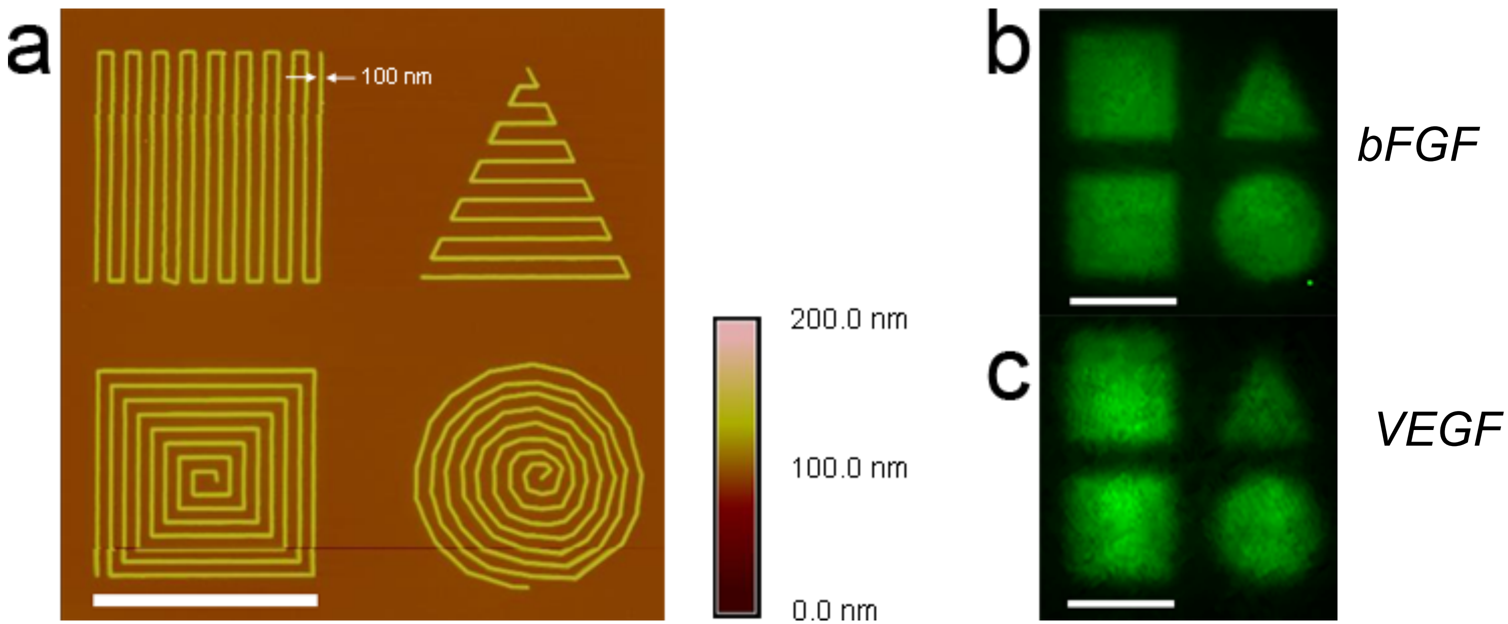
dose of 1100 μC/cm^2
beam current 4.8 pA



Features = 5 x 5 μm^2

Nanoscale growth factor patterns

dose of 60 nC/cm
beam current 4.8 pA



Scale bar = 5 μm

VEGF and bFGF can be patterned at the micron and nanometer scale using e-beam lithography on a heparin mimicking polymer

Christman, Vazquez, Schopf, Kolodziej, Li, Broyer, Chen, Maynard, *JACS*, 2008, *130*, 16585

Topic of Today's Lecture

Combining NANO and BIO on surfaces provides exciting opportunities for engineering development, as well as application

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