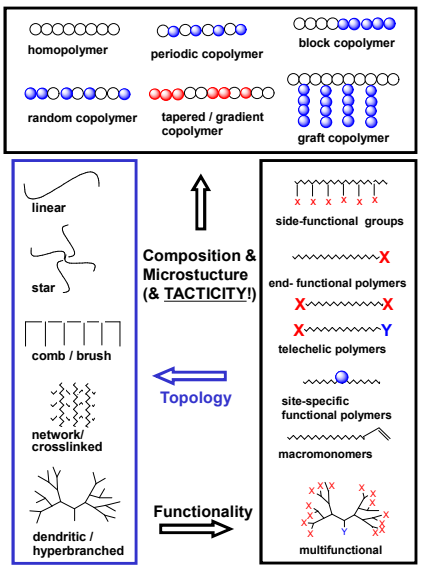
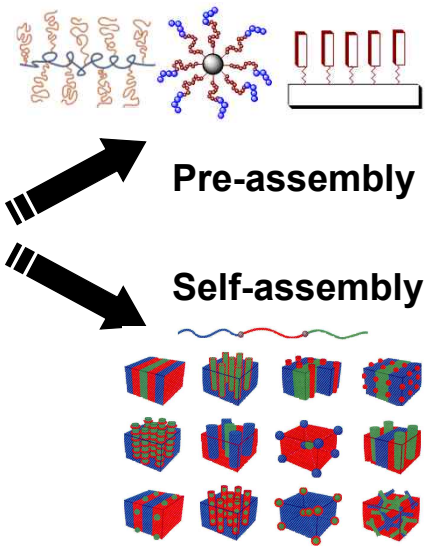
# *Surface Modification with Self-Assembled Monolayer & Polymer Brush for Biotechnology*

1 / 40

## Surface Modification



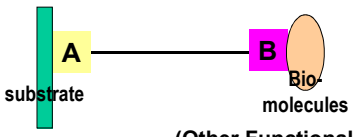




2 / 40

# Surface Modification Technique

1. Covalent Coupling
2. Adsorption
3. Affinity Linker
4. Self-Assembly Monolayer

- Interface of the Substrate & Biomolecules
  - Covalent bonding: Amide, Ester, Disulfide
  - Secondary bonding: Hydrogen Bonding, Affinity Capture, Absorption
- Surface (Gold & Glass (Silicon Wafer), Plastics) of the substrate
  - Silane (Glass)
  - Amine (Gold)
  - Thiol (Gold: SAM)



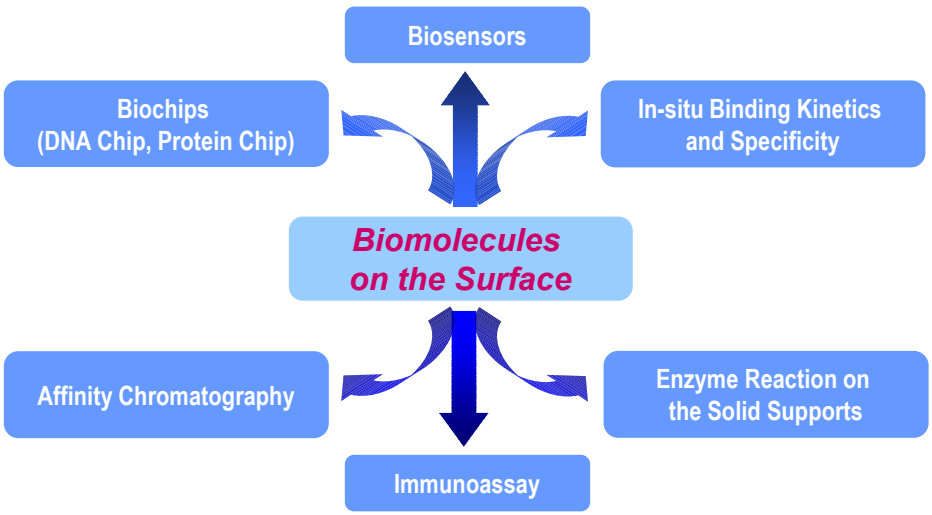

-SH, -Si(OEt)$_3$, -COOH, -NH$_2$

(Other Functional Groups) -COOH, -NH$_2$

1. *Stability*
2. *Intact Activity*
3. *Orientation*
4. *Simple Process*

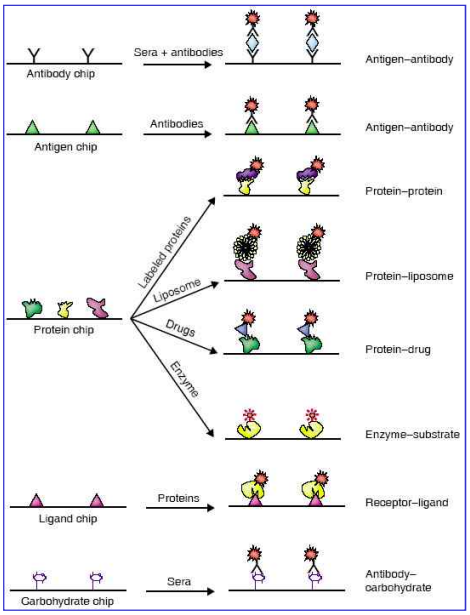
3 / 40

# Biomolecules on the Surface in Various Applications

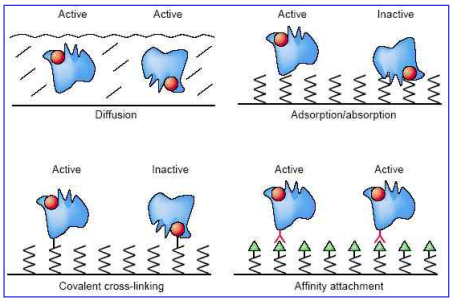



4 / 40

## Application of Protein Microarray




## Protein Attachment Method




***Curr. Opin. Chem. Biol.* 2003, *7*, 55.**

5 / 40

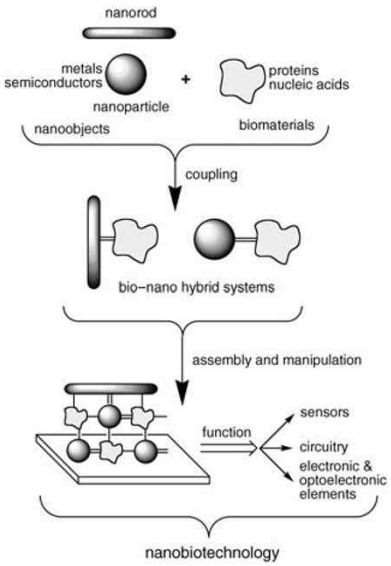
## Comparison of Current Antibody/Protein Microarray

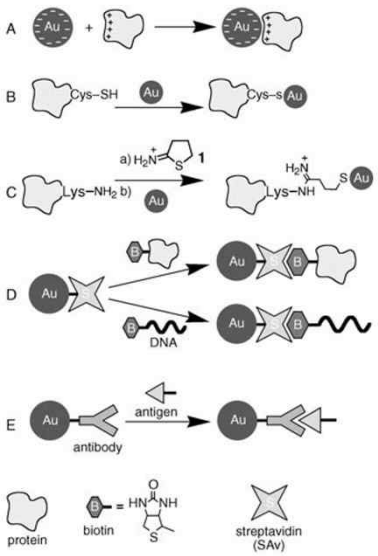
| Surface | Attachment | Advantage | Disadvantage |
|---|---|---|---|
| PVDF | Adsorption and absorption | No protein modification requirement, high protein binding capacity | Non-specific protein attachment in random orientation |
| Nitrocellulose | Adsorption and absorption | No protein modification requirement, high protein binding capacity | Non-specific binding, high background Low-density arrays |
| Poly-lysine coated | Adsorption | No protein modification requirement | Non-specific adsorption |
| Aldehyde-activated | Covalent cross-linking | High-density and strong protein attachment High-resolution detection methods available | Random orientation of surface-attached proteins |
| Epoxy-activated | Covalent cross-linking | High-density and strong protein attachment High-resolution detection methods available | Random orientation of surface-attached proteins |
| Avidin coated | Affinity binding | Strong, specific and high-density protein attachment, low-background | Proteins have to be biotinylated |
| Ni-NTA coated | Affinity binding | Strong, specific and high-density protein attachment, low-background, uniform orientation of surface attached proteins | Proteins have to be Hisx6 tagged |
| Gold-coated silicon | Covalent cross-linking | Strong and high-density protein attachment, low-background. Can be easily coupled with SPR and mass-spectrometry | Random orientation of surface attached proteins, tough to fabricate, not commercially available |
| PDMS nanowell | Covalent cross-linking | Strong and high-density protein attachment, well suited for sophisticated biochemical analyses | Random orientation of surface attached proteins |
| 3D gel pad and agarose thin film | Diffusion | High protein binding capacity, no protein modification requirement | Tough to fabricate, not commercially available |
| DNA/RNA coated | Hybridization | Strong, specific and high-density protein attachment, low-background, uniform orientation of surface attached proteins | Sophisticated *in vitro* production of labeled proteins |

***Curr. Opin. Chem. Biol.* 2003, *7*, 55.**

6 / 40

## Integrated Nanoparticle–Biomolecule Hybrid Systems

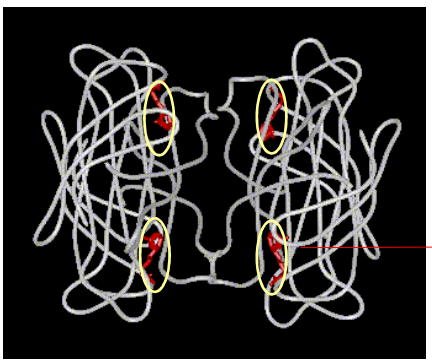


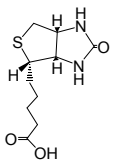




*Angew. Chem. Int. Ed.* 2004, *43*, 6042.

7 / 40

## Structure of Streptavidin & Biotin





**biotin**

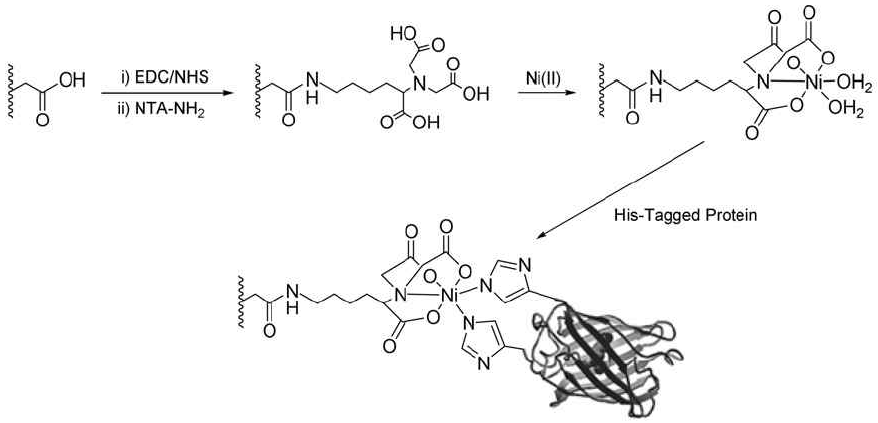
**Streptavidin**

**M.W. : ~ 60 kDa**

**association const. : $10^{13}$ $M^{-1}$**

8 / 40

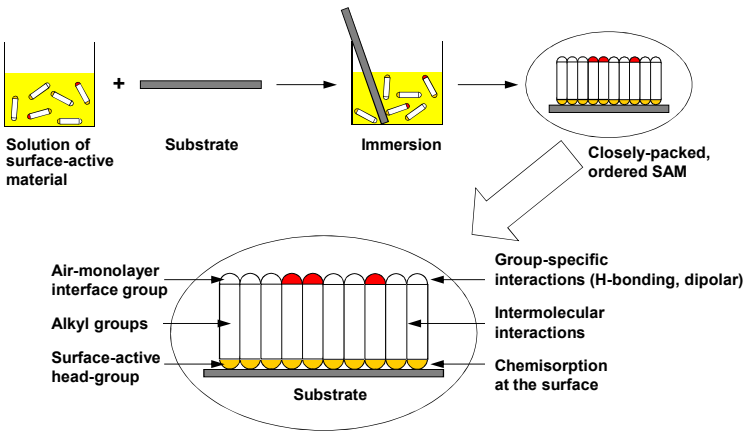
## Immobilization of histidine-tagged proteins onto NTA-presenting surfaces




*J. Phys. Chem. B* 2004, *108*, 7665.



## Self-Assembled Monolayers




**SAMs are ordered molecular assemblies formed by the adsorption of an active surfactant on a solid surface. The order in these 2D system is produced by a spontaneous chemical synthesis at the interface, as the system approaches equilibrium.**

# *Why SAMs?*

- offer unique opportunities to increase fundamental understanding of self-organization, structure-property relationships, and interfacial phenomena.
- are excellent systems for a more fundamental understanding of phenomena affected by competing intermolecular, molecular -substrates and molecules -solvent interactions like ordering and growth, wetting, adhesion, lubrication, and corrosion, thanks to the ability to tailor both head and tail groups of the constituent molecules.
- are good model systems for studies of physical chemistry and statistical physics in two dimensions, and the crossover to three dimensions because of the well-defined structure.
- provide the needed design flexibility, both at the individual molecular and at the material levels, and offer a vehicle for investigation of specific interactions at interfaces; a variety of surfaces with specific interactions can be produced with find chemical control.

→ applicable in many areas such as microarrays, molecular electronics, and sensors

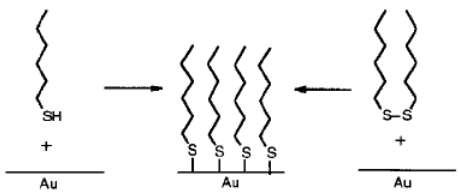
11 / 40

# Self-Assembled Monolayers on Gold

- In 1983 Nuzzo and Allara showed that SAMs of alkanethiolates on gold can be prepared by adsorption of di-*n*-alkyl disulfide from dilute solutions.

*J. Am. Chem. Soc.* 1983, *105*, 4481.

The most studied SAMs to date




*Self-assembled monolayers are formed by simply immersing a substrate into a solution of the surface-active material. The driving force for the spontaneous formation of the 2D assembly includes chemical bond formation of molecules with the surface and intermolecular interactions.*

12 / 40

## SAMs on Au(111): Mechanism

- **Proposed mechanism (*still controversial*): oxidative addition of the S-H bond to the gold surface, followed by a reductive elimination of the hydrogen**

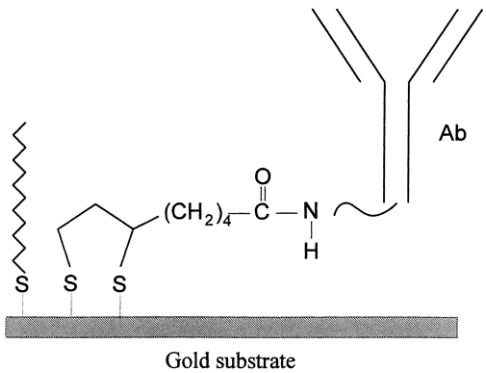
$$R\text{-}S\text{-}H + Au^{o}_{n} \rightarrow R\text{-}S^{-}Au^{+}\bullet Au^{o}_{n} + 1/2H_2$$

- **On the basis of the bond energies of RS-H, H-H, and RS-Au (87, 104, and 40 kcal/mol, respectively), the net energy for adsorption of alkanethiolates on gold would be ca. –5 kcal/mol, which is exothermic.**



## SAMs: Biosensing

**Antigen-Antibody Interaction**



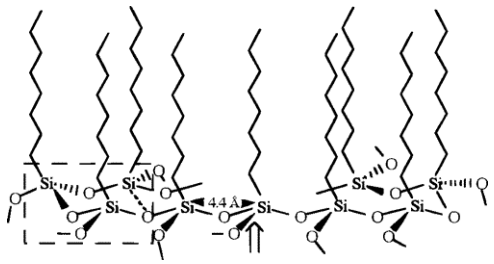

**Antibodies immobilized on the gold sensor surface via an amide bond to the self-assembled thioctic acid. After the immobilization of the antibody a long hydrocarbon thiol, 1-dodecanthiol, was introduced into the system to block any uncovered spots of the sensor surface.**

***Anal. Chem.* 1997, *69*, 3651.**

## SAMs on Silicon Oxide

- Alkylchlorosilanes and alkylalkoxysilanes

- require hydroxylated surfaces
- in situ formation of polysiloxane, which is connected to surface silanol groups (-SiOH) via Si-O-Si bonds




15 / 40

## SAMs on Silicon Oxide

- not easy to produce, mainly because of the need to carefully control the amount of water in solution

  - Incomplete monolayers are formed in the absence of water
  - Excess water results in facile polymerization in solution and polysiloxane deposition of the surface

- For the formation of closely packed monolayers, a moisture quantity of 0.15 mg/100 mL of solvent was suggested as the optimum condition

- Temperature: the threshold temperature below which an ordered monolayer is formed.
  - The threshold temperature is a function of the chain length [higher for octadecyl (18 $^{o}$C) than for tetradecyl chain (10 $^{o}$C)].

16 / 40

## SAMs on Silicon Oxide

- The chains in OTS [octadecyltrichlorosilane $CH_3(CH_2)_{17}SiCl_3$] SAMs are practically perpendicular to the substrate surface (tilt angle 0°± 5 ).
- The adsorption mechanisms of trichlorosilane and trimethoxysilane groups are different, resulting in a higher tilt angle of the chains in OTMS [octadecyltrimethoxysilane $CH_3(CH_2)_{17}Si(OCH_3)_3$] SAMs (20°± 5 ).
- The reproducibility of alkyltrichlorosilane monolayers is still a problem, since the quality of the monolayer formed is very sensitive to reaction conditions.
- Small differences in water content and in surface Si-OH group concentration may result in significant difference in monolayer quality.
- Notwithstanding, due to the unique stability of their complete monolayers, alkylsilanes are ideal materials for surface modification and functionalization applications.

17 / 40

## Self-Assembled Monolayers (SAMs)

CHARACTERISTICS

- Molecularly-Defined Surfaces
- Surfaces with Specific Chemical Functionalities
- Precise Tuning of Surface Properties

- Lubrication, Corrosion Protection, Resists, and Sensing

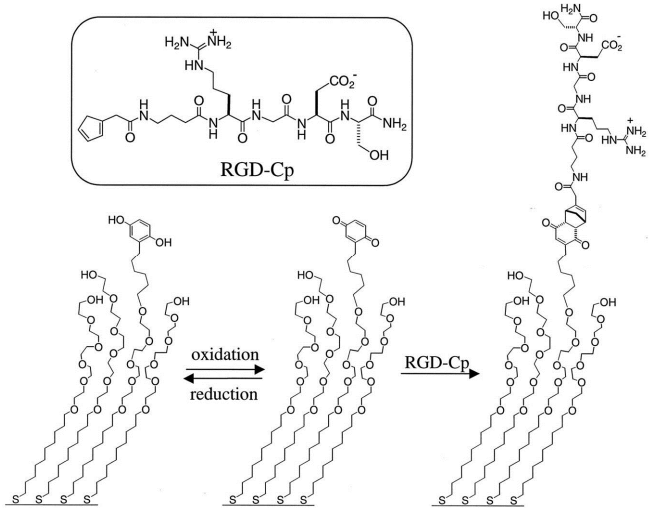
- 8.3 X $10^{-10}$ molcm$^{-2}$ (alkanethiols on gold)

CHARACTERIZATION

- Contact Angle Measurement
- Ellipsometry
- IR Spectroscopy (Grazing Angle Reflection and Transmission)
- Atomic Force Microscopy (AFM) and Scanning Tunneling Microscopy (STM)
- X-ray Photoelectron Spectroscopy (XPS) and Auger Electron Spectroscopy (AES)
- UV-vis and Fluorescence Spectroscopy
- Surface Plasmon Resonance (SPR) Spectroscopy
- Secondary Ion Mass Spectroscopy (SIMS)
- Quartz Crystal Microbalance (QCM)
- Electrochemical Characterization (CV and Impedance Spectroscopy)

18 / 40

## Dynamic Surfaces: Cell Attachment




*Angew. Chem. Int. Ed.* 2001, *40*, 1093. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 5992.

19 / 40

## Representative Surface Modified with Oligo-EG

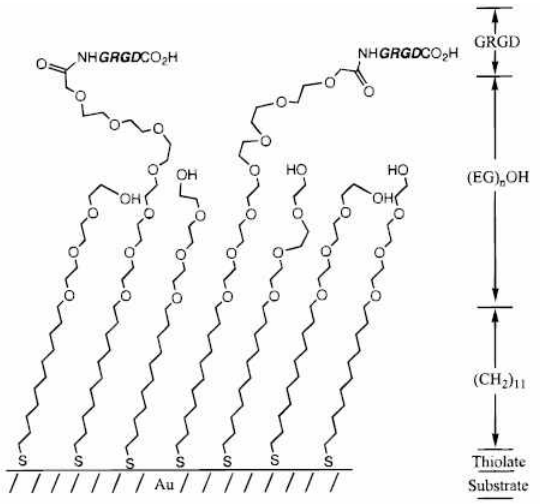
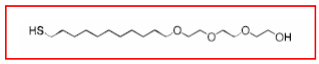



Diagram of self-assembled monolayer of alkanethiolates on gold presenting (EG)6OGRGD and (EG)3OH groups.

*J. Am. Chem. Soc.* 1998, *120*, 6548.

20 / 40

# Building Blocks for the Surface Modified with Oligo-EG




$H_2C{=}CH(CH_2)_9Br$ + $H(OCH_2CH_2)_nOH$

↓ 50% NaOH, 100 °C 24 h

$H_2C{=}CH(CH_2)_9(OCH_2)_nOH$

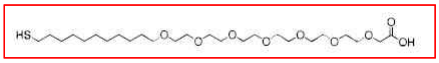
↓ $CH_3C(=O)SH$, AIBN, hv, 4h

$H_3C{-}\overset{\overset{O}{\|}}{C}{-}S{-}(CH_2)_{11}(OCH_2)_nOH$

↓ HCl/MeOH, Reflux, 4h

$HS{-}(CH_2)_{11}(OCH_2)_nOH$

*J. Am. Chem. Soc.* **1991**, *113*, 12.




$H_2C{=}CH(CH_2)_9(OCH_2)_nOH$

↓ $XCH_2COOR$

$H_2C{=}CH(CH_2)_9(OCH_2)_nOCH_2COOR$

↓ $CH_3C(=O)SH$, AIBN, hv, 4h

$H_3C{-}\overset{\overset{O}{\|}}{C}{-}S{-}(CH_2)_{11}(OCH_2)_nOCH_2COOR$

↓ Hydrolysis

$HS{-}(CH_2)_{11}(OCH_2)_nOCH_2COOH$

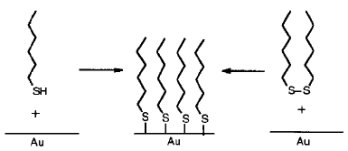
*J. Org. Chem.* **1998**, *63*, 7552.
*Anal. Chem.* **1999**, *71*, 777.
*J. Phys. Chem. B.* **2004**, *108*, 7665.

21 / 40

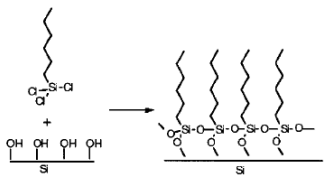
## SAMs: Reactions

**reading assignment: *Adv. Mater.* 2000, *12*, 1161.**

- **SAMs (self-assembled monolayers): excellent models for studying interfacial reactions**
- **Interfacial reactions**
  - **The control over the chemical and structural properties of surfaces finds its applications in catalysis, electronics, sensors, etc.**
  - **provides important information for fundamental studies in chemistry and biology**
    **such as cell biology on man-made surface**




**SAMs on gold**

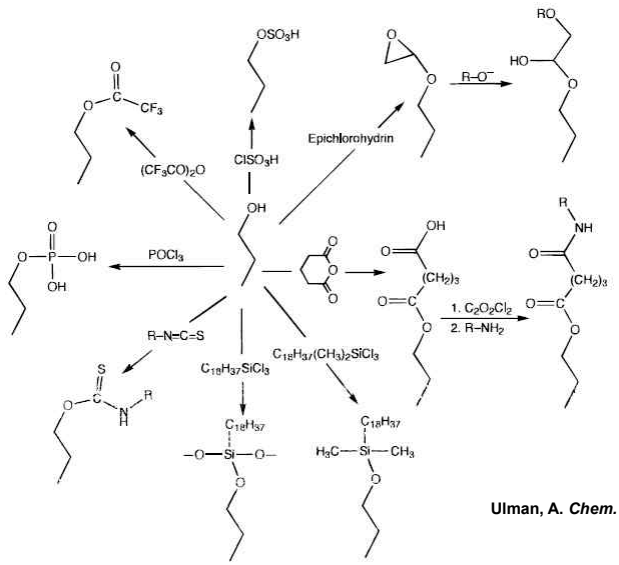



**SAMs on silicon oxide**

- **requires only highly specific reactions that result in a quantitative transformation of functional groups.**

22 / 40

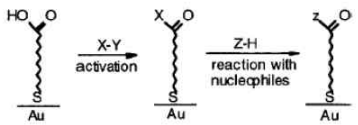
## Surface Reactions of $\omega$-Hydroxyalkanethiolate SAMs on Au(111)




Ulman, A. *Chem. Rev.* 1996, *96*, 1533.

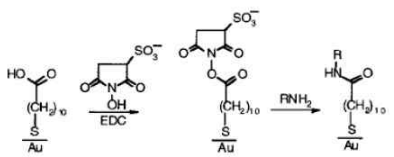


## Reactions in SAMs: Carboxyl Groups




X-Y = $SOCl_2$, ClCOOEt, DCC etc
Z-H = $RNH_2$, ROH

- activation by treatment with carbodiimides such as DCC (dicyclohexylcarbodiimide) or EDC (1-ethyl-3-(dimethylamnopropyl)carbodiimide)
- conversion to a mixed anhydride or an interchain anhydride
- conversion to a carboxyl chloride group




- EDC-mediated coupling with N-hydroxysuccinimde or N-hydroxysulfosuccinimde

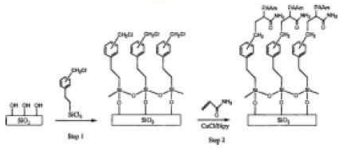
## Surface-Initiated Polymerization

• polymer (nano)brushes: applications in the areas of sensing, nonlinear optical (NLO) materials, corrosion inhibition, friction and wear, adhesion, drug-delivery, and tissue engineering
• SAMs as scaffolds: high density, few defect, and well-defined structure

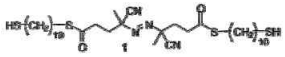
• radical polymerization
• cationic polymerization
• anionic polymerization
• ring opening metathesis polymerization (ROMP)
• etc.

25 / 40

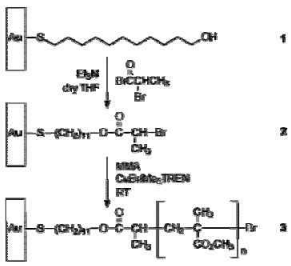
## Radical Polymerization (I)



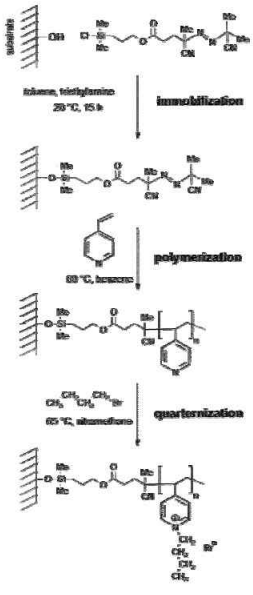
*Anal. Chem.* **1997**, *69*, 4577.



*Langmuir* **2002**, *18*, 1281.



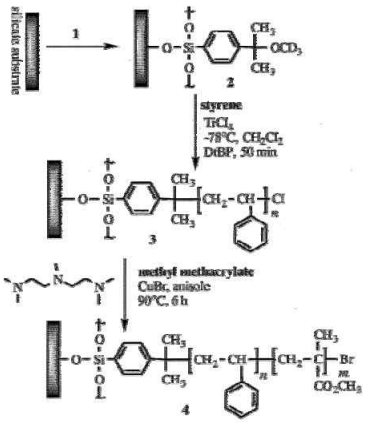
*J. Am. Chem. Soc.* **2000**, *122*, 7616.



*Macromolecules* **1999**, *32*, 2309
and references therein.

26 / 40

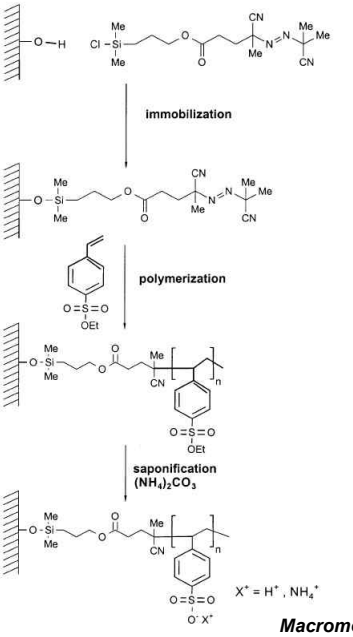
## Combination of Radical and Cationic Polymerization

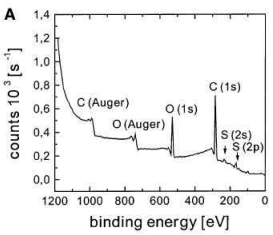


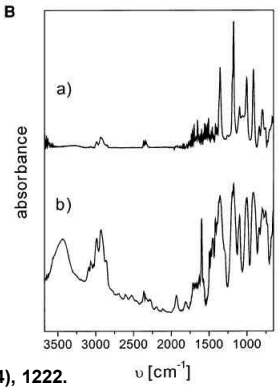

*J. Am. Chem. Soc.* **1999**, *121*, 3557.



## Poly(*p*-styrenesulfonate) Brush via Surface-Initiated Polymerization



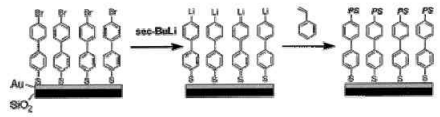




***Macromolecules* 2003, *36*(4), 1222.**

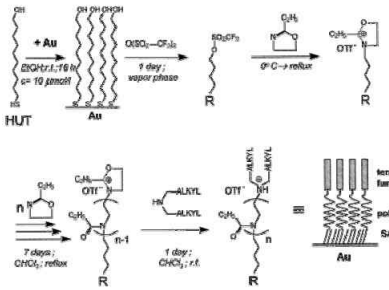
## Anionic and Cationic Polymerization

*mostly done by prof. Ulman*




- ~ 5days
- ~ 18±2 nm

*J. Am. Chem. Soc.* **1999**, *121*, 1016.

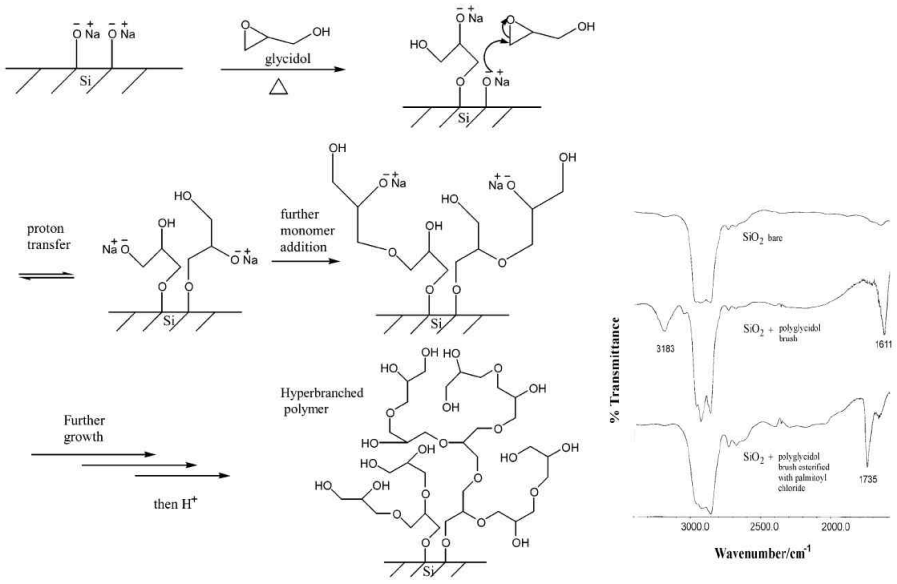



- CP of oxazolines
- ~ 8.5-nm-thick polymer film (~ 26-28 monomer units)

*J. Am. Chem. Soc.* **1998**, *120*, 243.

29 / 40

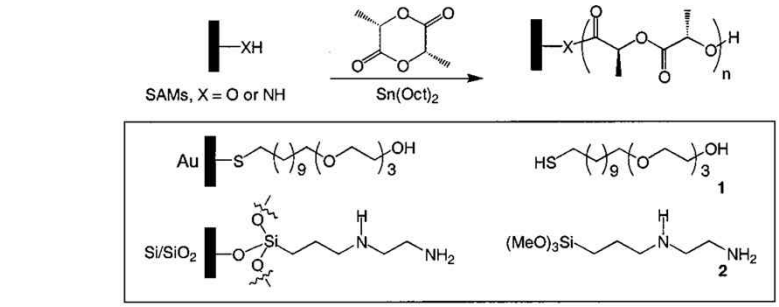
## Hyperbranched Polyglycidol on Si/SiO2 Surfaces



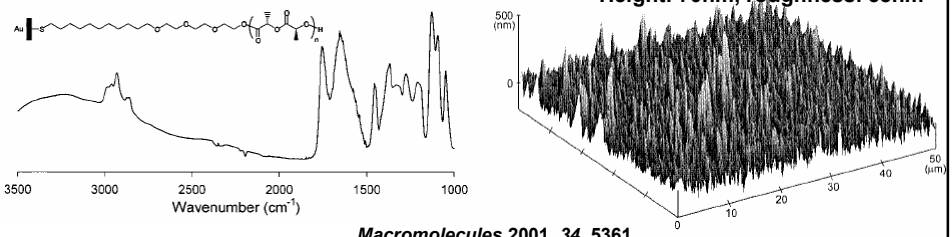

***Macromolecules* 2003, *36*, 5088.**

30 / 40

## Surface-Initiated Polymerization of L-Lactide



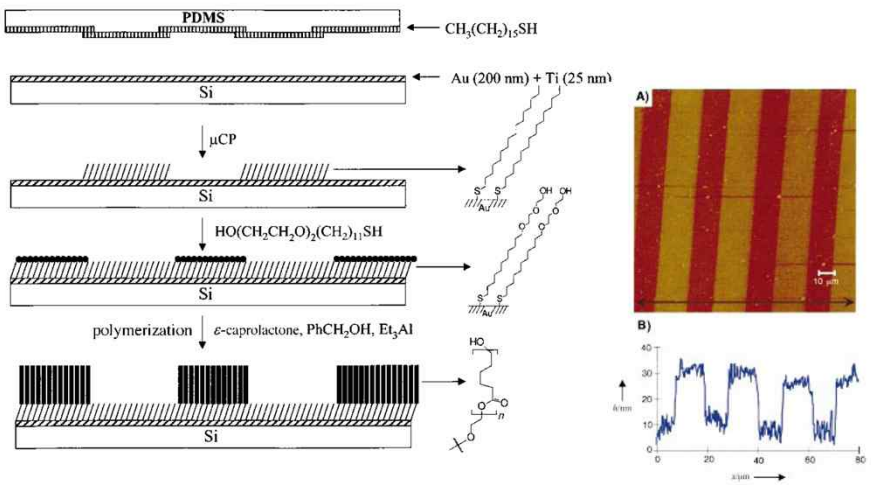

**Height: 70nm, roughness: 38nm**




***Macromolecules* 2001, *34*, 5361.**

31 / 40

## Surface-Initiated Polymerization for Amplification of Self-Assembled Monolayers Patterned by Microcontact Printing




***Angew. Chem., Int. Ed.*1999, *38*, 647.**

32 / 40

## The Issue on Non-specific Interaction

**A general problem in both heterogeneous immunoassays and immunosensors is nonspecific protein adsorption on artificial surfaces: macromolecules are not only bound to the substrate by specific antigen/antibody recognition but also adhere due to nonspecific interaction forces and hence prevent an accurate determination of molecular concentrations. For example, for the immobilization of antibodies via aminosilane and glutardialdehyde, a standard coupling procedure, it has been determined by enzyme-linked immunoassays (ELISA) that as much as 15% of the measured response must be attributed to nonspecific protein/surface interactions, even after copious washing with detergents.**

***Anal. Chem.* 1996, *68*, 176.**

**Common techniques to reduce nonspecific adsorption are based on exposing the antibody-coated substrates to other, adhesive proteins in order to block nonspecific adsorption sites. However, the efficiency of this method depends on both the substrate used and the biological system under study, and exchange processes may occur between dissolved and surface-bound species (Vroman effect). Thus, the integration of specific receptors into a matrix material which resists nonspecific protein adsorption would significantly contribute to technological progress. Candidate matrix materials with excellent protein repulsive properties are thin films of poly(ethylene glycol) (PEG) and oligo(ethylene glycol) (OEG). While PEG coatings can be immobilized on a variety of surfaces, for example, by physical or chemical grafting of copolymers, OEG is usually prepared as a self-assembled monolayer (SAM) of tailgroup-modified alkanethiolates on gold or alkyl silanes on oxidized surfaces to ensure high grafting density. *For PEG, a model was proposed that interprets protein repulsion basically as resistance to dehydration and steric confinement of the swollen polymer.* In the case of the densely packed OEG-terminated SAMs, which neither show significant swelling nor comprise high conformational freedom, this explanation appears not to be applicable. *Proposed models suggest the importance of the SAM/water interaction at the surface or relate protein resistance to repulsive electrostatic forces.***

33 / 40

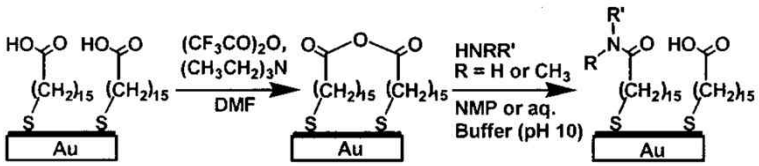
**The most common technique to integrate biospecific recognition elements into OEG-terminated SAMs is based on coadsorption from binary solutions, composed of protein resistant EG molecules and a second, functionalized molecular species suitable for receptor coupling (or containing the receptor itself). Some examples of this strategy are the formation of mixed alkanethiolate SAMs from OEG derivatives and alkanethiols functionalized with carboxy, nitrilotriacetic acid (chelated with Ni(II) for the binding of histidine-tagged receptors), biotin, benzenesulfonamide, sugar, or RGD (arginine-glycine-aspartate) groups. Another strategy for the preparation of mixed SAMs is to introduce functionalized and nonfunctionalized OEG moieties after formation of a homogeneous alkanethiolate SAM bearing carboxylic acid anhydride groups.**

**Alternatively, instead of mixing two different molecules on the surface with one providing functionality and one mediating protein repulsive properties, also direct coupling of receptors to surface-grafted end-functionalized PEG molecules has been reported. In this case, different functionalities, that is, protein resistance and the ability of receptor coupling, are provided by different parts of a specially designed copolymer. Reported biospecific or chemically active groups include biotin, peptides, and esters.**

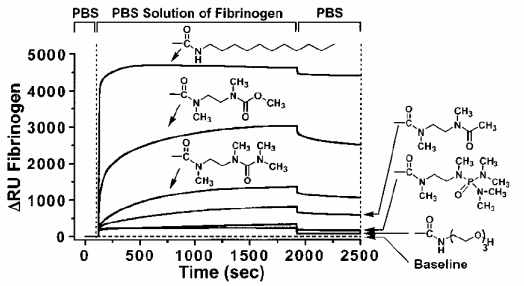
***Langmuir* 2003, *19*, 1880.**

34 / 40

## Surveying for Surfaces that Resist the Adsorption of Proteins




Synthesis of mixed SAMs that present a 1:1 mixture of –CONRR' and $CO_2H/CO_2^-$ groups using the anhydride method

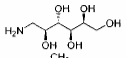
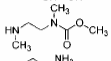
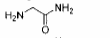
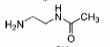
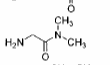
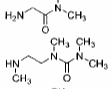
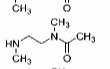
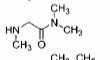
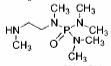
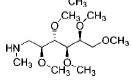



SPR data for the adsorption of fibrinogen to mixed SAMs that were prepared by the anhydride method

*J. Am. Chem. Soc.* 2000, *122,* 8303.

35 / 40

## Amount of Fibrinogen (Fib) and Lysozyme (Lys) Adsorbed to Mixed SAMS

| Entry No. | HNRR' | % ML Fib[a, b, c] | % ML Lys[a, b, c] | $\theta_a$[d] |
|---|---|---|---|---|
| 1 | $H_2N(CH_2)_{11}CH_3$ | 100 | 100 | 163[c] |
| 2 |  | 68 | 20 | 37 |
| 3 |  | 58 | 43 | 75 |
| 4 |  | 58 | 30 | 39 |
| 5 |  | 40 | 5 | 49 |
| 6 |  | 33 | 15 | 61 |
| 7 |  | 25 | 11 | 62 |
| 8 |  | 12 | 4 | 53 |
| 9 |  | 9 | 2 | 65 |
| 10 |  | 4 | < 1 | 66 |
| 11 |  | 3 | 6 | 81 |
| 12 | $H_2N(CH_2CH_2O)_3H$ | 2 | 1 | 54 |

### Protein Adsorption to Mixed SAMs that Present Unmethylated and Methylated Functional Groups

| | % ML (R = H)[a]/% ML (R = $CH_3$) | |
|---|---|---|
| –CONRR′ | fibrinogen | lysozyme |
| $-CONRCH_2(CH(OR))_4CH_2OR$ | 23 | 3 |
| $-CONRCH_2CON(R)_2$ | 6 | 15 |
| $-CONRCH_2CON(CH_3)_2$ | 4 | 6 |
| $-CONRCH_2CH_2NRCOCH_3$ | 3 | 1.5 |
| $-CONH(CH_2CH_2O)_3R^b$ | 1 | 1 |

**fibrinogen, a large (340 kD) blood plasma protein that adsorbs strongly to hydrophobic surfaces, and lysozyme, a small protein (14 kD, pI=12) that is positively charged under the conditions of our experiment (phosphate buffered saline, PBS, pH 7.4).**

36 / 40

## Typical Reversible Surface

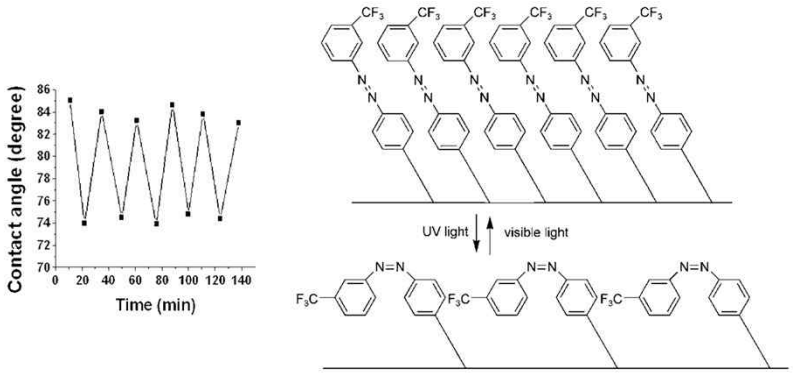
| Type | Description | Driving force | Switchable properties | Applications |
|---|---|---|---|---|
| polymer | PS-PAA, PSF-PVP (grafting to) PS-PMMA,PS-PVP (grafting from) | surrounding media | wettability, friction force | assembly of nanoparticles, proteins, charged chemical species, microfluidic devices, etc. |
| | PNIPAAm | temperature | | |
| | photochromic polymer [4′-(trifluoro methoxy-4,4′dibenzoazo)] | light | | |
| SAM | reversible association (supermolecules, oligomer, etc.) | PH, light | wettability, conductance | protein chips, patterning surfaces, smart devices |
| | reversibly conformational transition (azobenzene derivants; alkanethiolate) | light, potential | | |
| | reversible attachment (alkanethiolate) | potential | | |
| metallic oxide | ZnO, $TiO_2$ | light | wettability | self-cleaning materials |

**PS: polystyrene; PAA: poly(acrylic acid); PSF: poly(styrene-co-2,3,4,5,6-pentafluorostyrene); PVP: poly(2-vinylpyridine); PMMA:poly(methylmethacrylate); PNIPAAm: poly(N-isopropylacrylamide).**

***Chem. Eur. J.* 2005, Early View.**

37 / 40

## Dynamic Surfaces: Photoisomerization

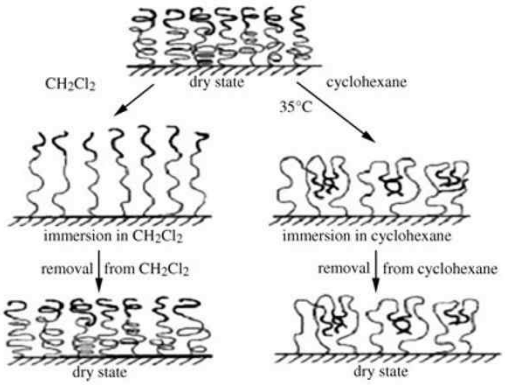



**The corresponding interfaces differ in wetting behavior.**
**Left: Reversible wettability for an 11-layer LB film on glass. Over several cycles of irradiation the value of the contact angles gradually decreased.**
**Right: Model of the structural change of a single-layer LB film of the polymer as a result of photoisomerization.**

**Langmuir 2001, *17*, 4593.**

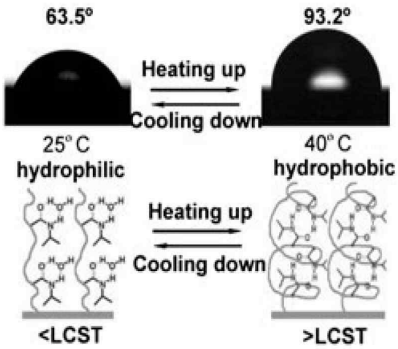
38 / 40

**Reversible responses of PS-b-PMMA brushes to different solvent treatments**




*Macromolecules* 2000, *33*, 8813.

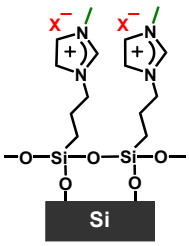
**Thermally responsive wettability for a flat PNIPAAm-modified surface**




*Angew. Chem. Int. Ed.* 2004, *43*, 357.

39 / 40

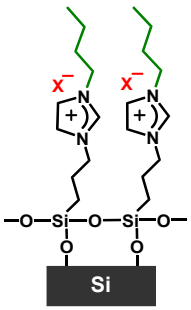
## Dynamic Surfaces: Anion Exchange




**Ellipsometry: 6 Å**

**Contact Angles:**

**X = Cl : 24°**
**$BF_4$ : 30°**
**$PF_6$ : 42°**




**Ellipsometry: 8 Å**

**Contact Angles:**

**X = Cl : 51°**
**$BF_4$ : 51°**
**$PF_6$ : 52°**

*J. Am. Chem. Soc.* 2004, *126*, 480.

40 / 40