UNESCO/IUPAC Postgraduate Course in Polymer Science

Lecture:

Interfaces between biological and synthetic entities

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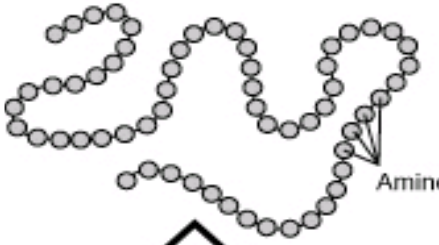
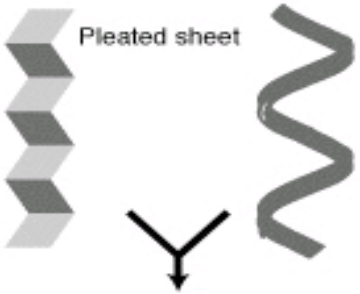
The contact an artificial object with a biological medium starts usually with adsorption of biological macromolecules, particularly proteins, from aqueous phase contained in the medium onto the object surface.

Subsequent biological processes, such as, blood coagulation, complement activation, or cell adhesion and proliferation are mediated by the adsorbed protein assembly.

Protein structure



Primary protein structure
is sequence of a chain of amino acids

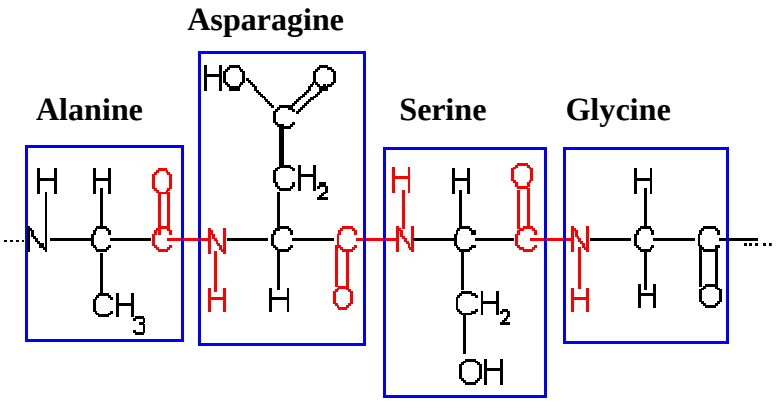


Secondary protein structure
occurs when the sequence of amino acids are linked by hydrogen bonds

Tertiary protein structure
occurs when certain attractions are present between alpha helices and pleated sheets.

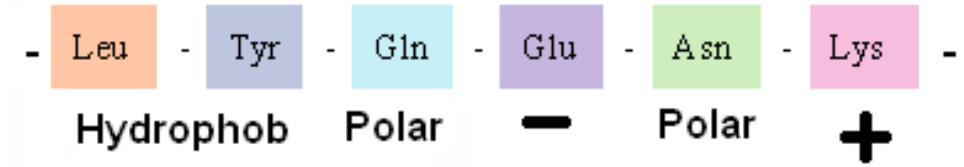
Quaternary protein structure
is a protein consisting of more than one amino acid chain.

Primary Structure



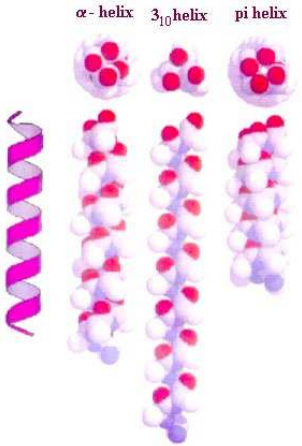
Amino acid residues

Leucine Tyrosine Glutamine Glutamate Aspargine Lysine

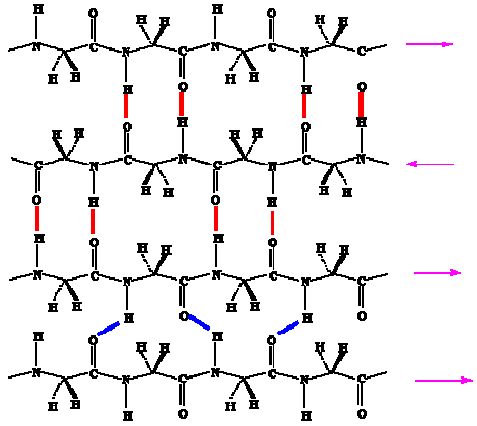


Secondary structures

Helix



β-sheet

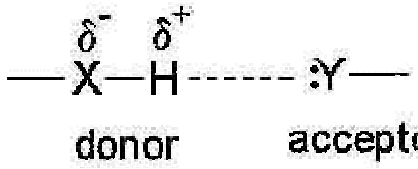


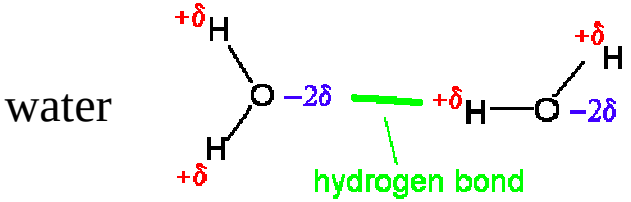
Why can proteins adsorb?

A variety of aminoacid residues on the peptide backbone can mediated the adsorption and also interaction with other molecules via hydrophobic, electrostatic, and other polar interactions. Conformation changes of protein structure can be induced by the adsorption so as the respective aminoacid residues could interact with the contacting surface.

Non covalent physical interactions stabilize secondary structures in proteins

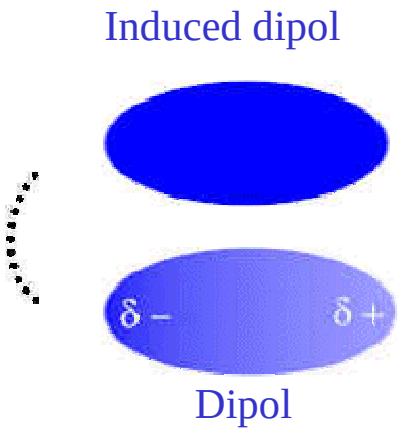
Hydrogen bonds (D-H.....A) between donor (D) and acceptor (A) atoms





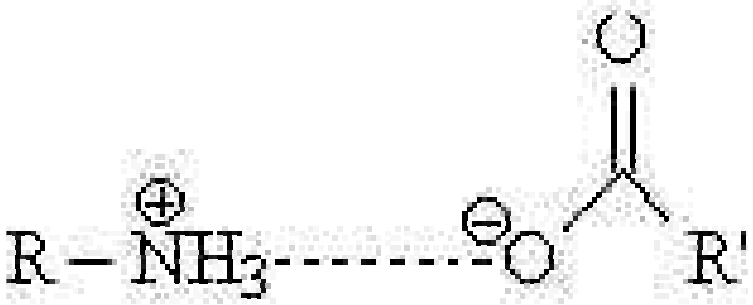
Van der Waals interactions are non covalent interactions between permanent and induced dipols

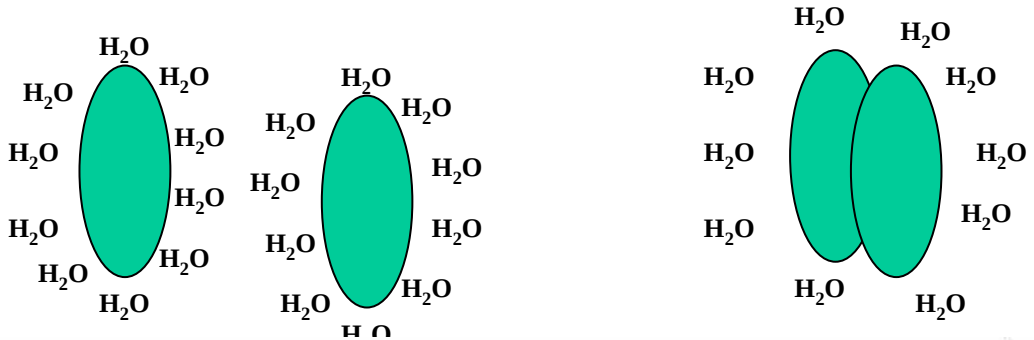
London dispersion forces between non polar groups



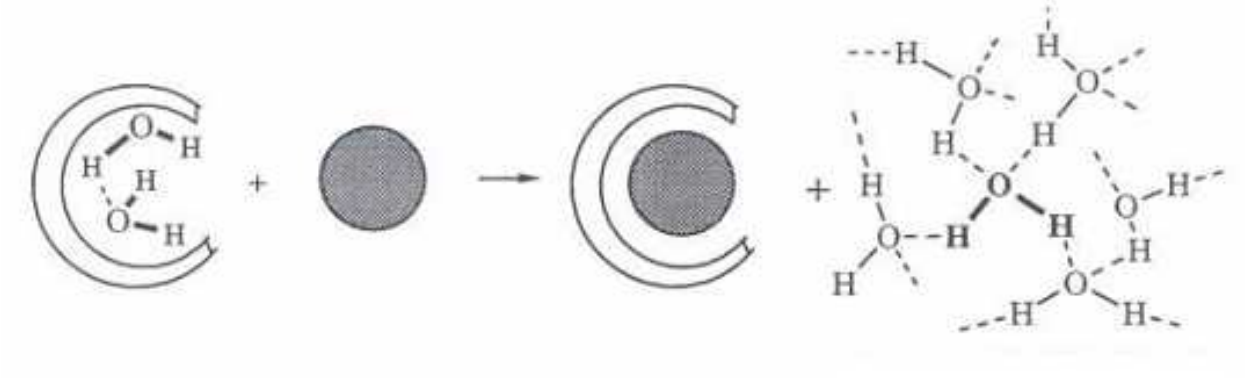
Elektrostatic interactions

Ionic bonds





Hydrophobic interactions



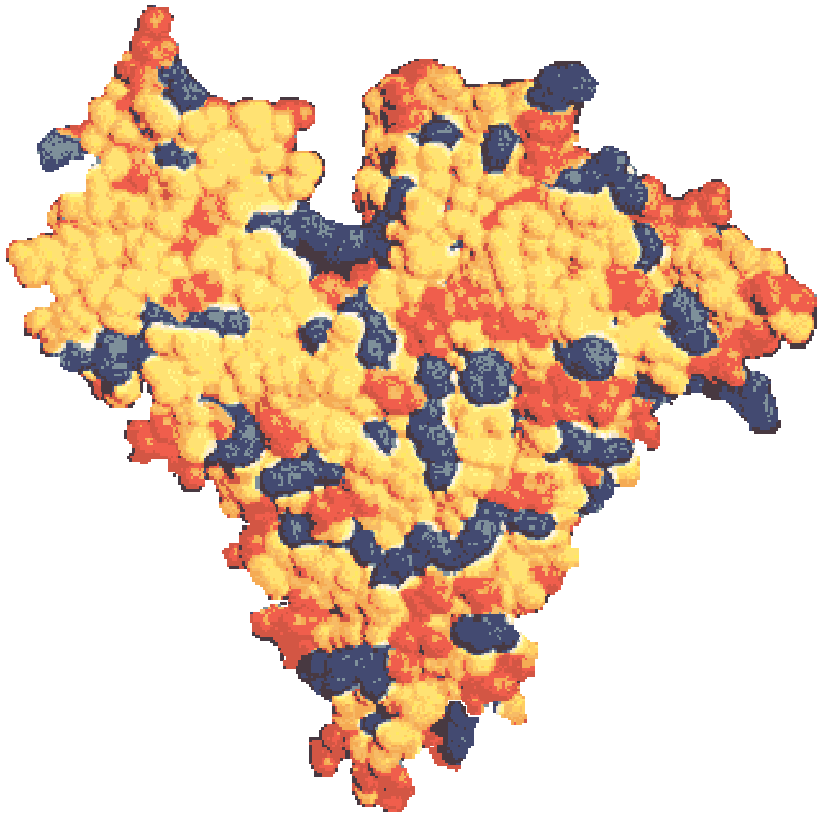
BSA bovine serum albumin

residues:

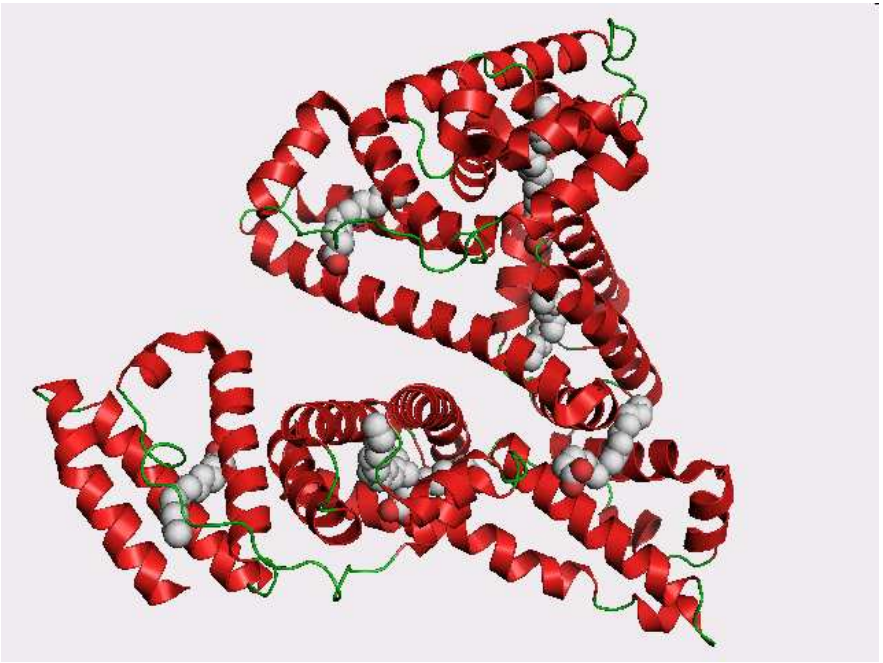
basic^{+} - blue

acid^{-} - red

neutral - yellow



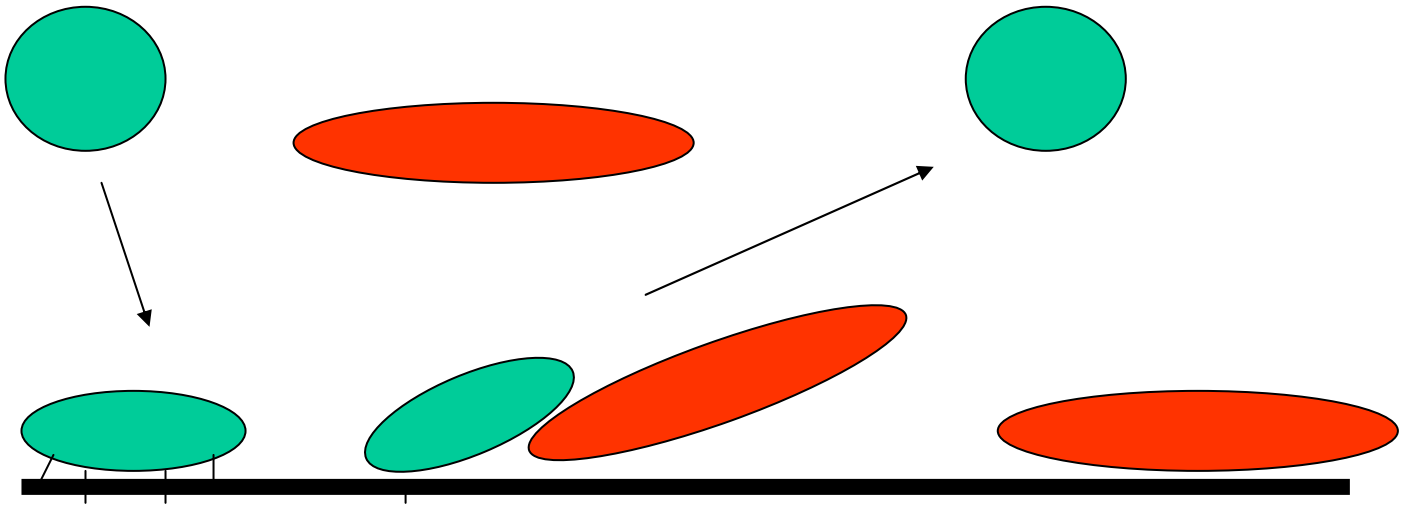
6 fatty acids in hydrophobic pockets



Proteins adsorb on nearly all artificial surfaces.

The adsorption is reduced considerably on anti-fouling surfaces, that minimize hydrophobic interaction (hydrophilic) and electrostatic interactions (uncharged).

The adsorption of proteins from biological media is a complex and dynamic process in which proteins can change their tertiary structure, first adsorbed proteins can be subsequently replaced with proteins of higher affinity to the surface, and other proteins can be specifically attached to the previously adsorbed protein layer.



Composition of the adsorbed protein layer changes with time of incubation.

Blood is composed of plasma containing red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes).

Blood plasma is obtained by separation blood cells from blood.

Plasma is a liquid containing a variety of proteins, lipoproteins, and small ions.

Main plasma proteins	
Total protein:	75–130 mg/ml
Albumin:	35–50 mg/ml
Globulin:	20–35 mg/ml
Fibrinogen	2.0–4.0 mg/ml

Blood serum is obtained from plasma by the depletion of fibrinogen

Preparation of functional synthetic interfaces

Contact of synthetic materials with biological media usually starts with adsorption of proteins mediated by physical interactions of aminoacid residues with the material surface. An undesirable protein fouling makes often troubles at applications of various devices, e.g., separation columns and membranes, biosensors, drug carriers, etc. The fouling is particularly critical for in real time detection of analytes in complex biological fluids, such as, blood, plasma, or serum, by affinity biosensors which cannot differentiate between responses to specific analyte binding and to non-specific protein adsorption.

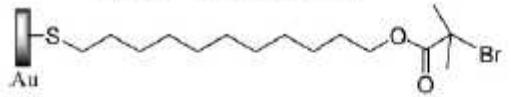
The immobilization of biologically active compounds on surfaces coated with an anti-fouling layer is a potential way in which the problem can be dealt with.

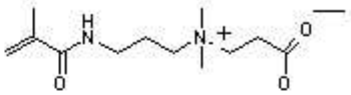
Current non-fouling surfaces

- Biacore: Carboxymethyl dextran→**100%** monolayer of proteins from blood serum (Booksh et al, Talanta 2005, 67, 918–925), (Analytical biochemistry, 205,132-136)
- Self assembled monolayers →**100%** of monolayer of proteins from blood serum (Whiteside, J.Phys. Chem. B 1998, 102, 426-436), (J. AM. CHEM. SOC. 2005, 127, 14473-14478)
- Grafted from PEG: *ca* **50%** monolayer of proteins from blood serum (Rodriguez Emmenegger, C., et al., Langmuir, 2009. **25**(11): p. 6328-6333)
- Antifouling Brushes : **Non-fouling surfaces can be achieved** (Rodriguez Emmenegger, C., et al., Langmuir, 2009. **25**(11): p. 6328-6333) (Yang, W., et al., Langmuir, 2009. **25**(19): p. 11911-11916.)

Polymer brushes prepared by surface initiated atom transfer radical polymerization (ATRP)

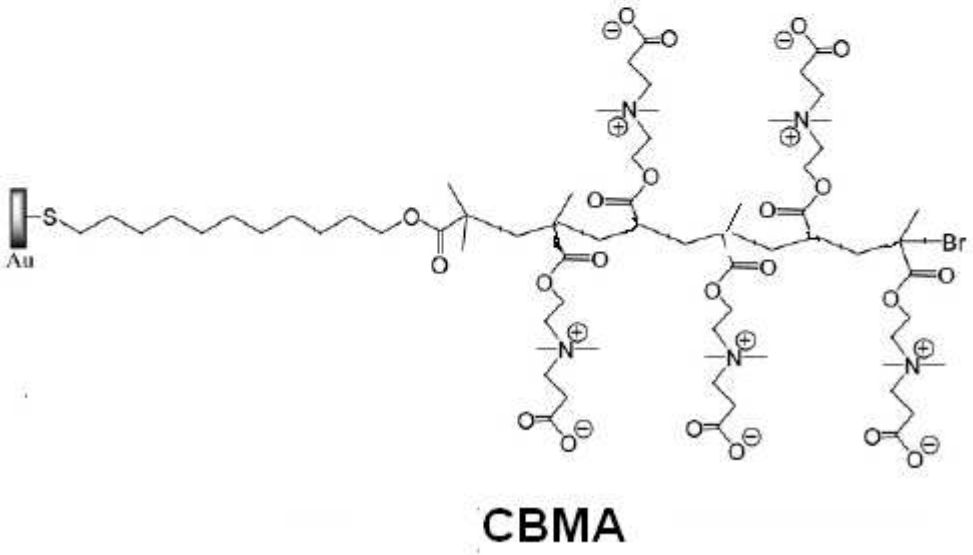
SAM of initiator





carboxybetaine methacrylate

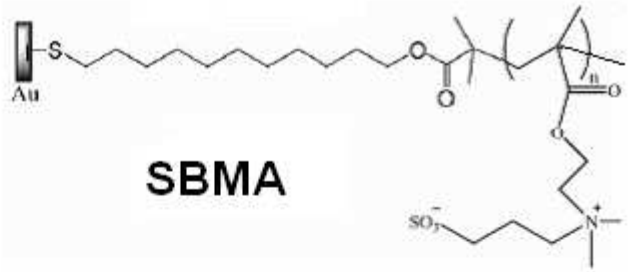
CBMA | CuBr/BPY



CBMA

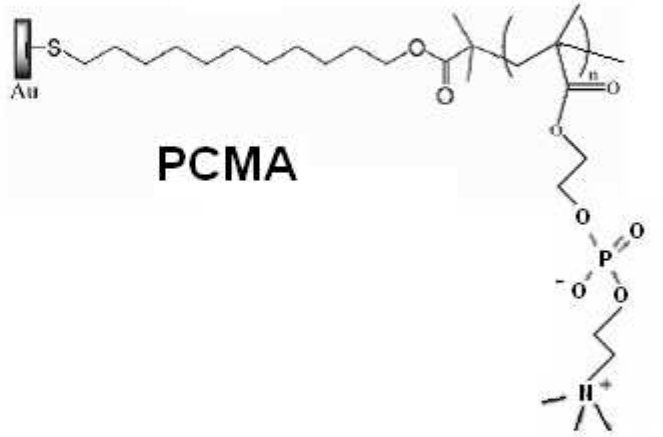
Poly(carboxybetaine methacrylate)

Poly(sulfobetaine methacrylate)



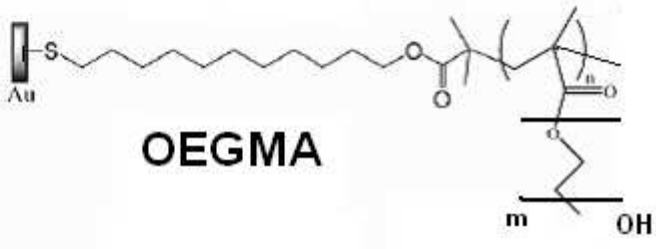
SBMA

Poly(2-Methacryloyloxyethyl phosphorylcholine)

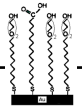
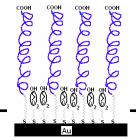
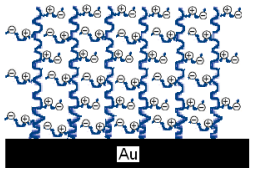


PCMA

Poly(oligoethylene glycol methacrylate)



OEGMA

surface	cont. angle [deg]		SPR response to nonspecific adsorption shift in λ_{res} [nm]				
	advancing	receding	plasma	IgG	Fbg	HSA	Lys
Gold	75	62	20	20	20	7	10
SAMs 							
$HSC_{11}(EG)_2$	30	16	11	8	2	0.7	1
$HSC_{11}(EG)_2/HSC_{15}COOH$	37	11	14	10	6	0	5
Poly(ethylene glycol) on SAMs SAM-PEG 							
$HSC_{11}(EG)_2/HSC_{15}COOH/PEG\text{-}COOH$	42	28	6	0.5	0.2	0	0.8
Polymer brushes							
PCMA 	18	7	26	0.9	0.5	0	0
SBMA	20	5.4	16	1	0	0	0
CBMA	34	11	0	0	0	0	0
CBAA	23	8	0	0	0	0	0
HOEGMA	55	27	3.5	0	0	0	0
HOEGMA- CBAA (diblock)	23	8	0	0	0	0	0

PCMA 2-Methacryloyloxyethyl phosphorylcholine
CBMA carboxybetaine methacrylate
SBMA sulfobetaine methacrylate
CBAA carboxybetaine acrylamide
HOEGMA oligo ethylene glycol methacrylate

Human plasma

IgG Human immunoglobulin G
Fbg human fibrinogen
HSA human serum albumin
Lys Lysozyme

A low plasma fouling was not directly related to a high surface wettability.

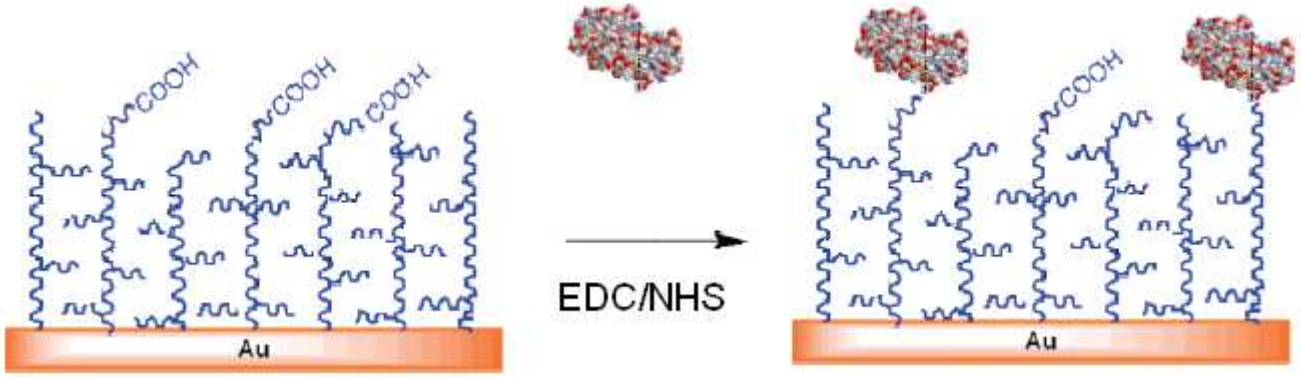
Polymer brushes suppressed completely adsorption of plasma proteins - albumin, IgG, and fibrinogen Fbg, from single protein solutions; however, only zwitterionic polycarboxybetaines (CBs) brushes, 15 nm or thicker, suppressed completely serum and plasma fouling.

A low interfacial energy with water and minor adsorption from the single protein solution are necessary, but not satisfactory characteristics of non-fouling surfaces.

Functionalization of non-fouling surface

Non-fouling surface with reactive chemical groups

Covalent attachment of bioactive compounds

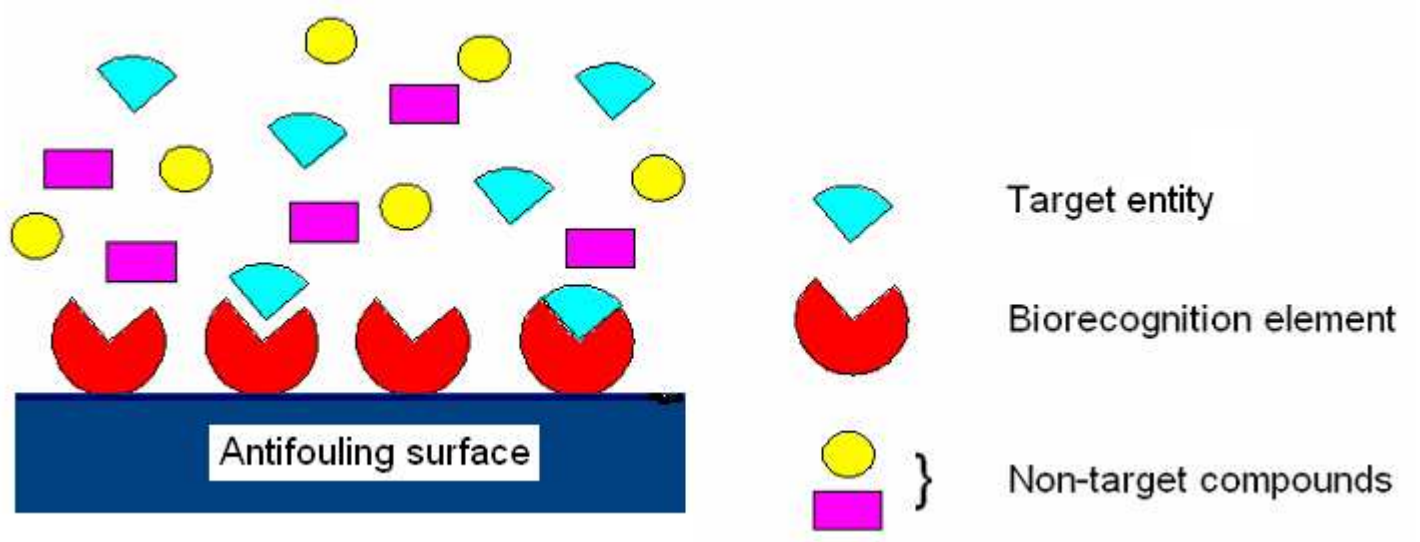


Bioactive compounds: antibodies, antigens, enzymes, recombinant proteins, aptamers, cell-interacting proteins and oligopeptides, lectins, drugs, etc.

Surfaces binding specifically biological entities

(biorecognition of molecules, viruses, bacteria, cells)

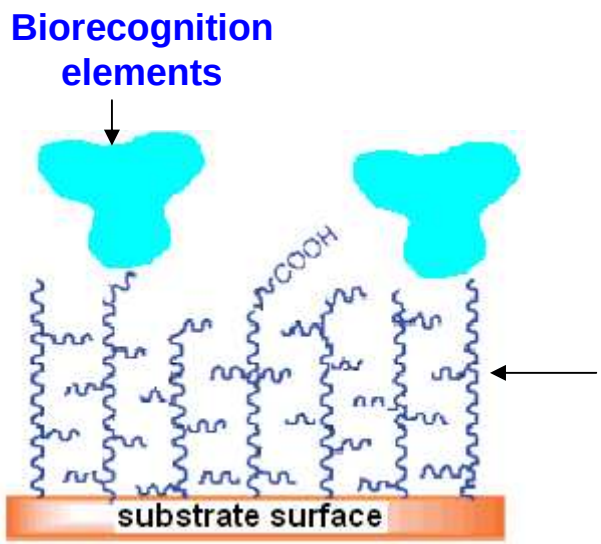
Surfaces should contain biorecognition elements capable of specific binding target entities and at the same time they should be resistant to a non specific deposition of other components from the contacting biological medium (**antifouling**).



Affinity biosensors (biorecognition layers on optical transducers)

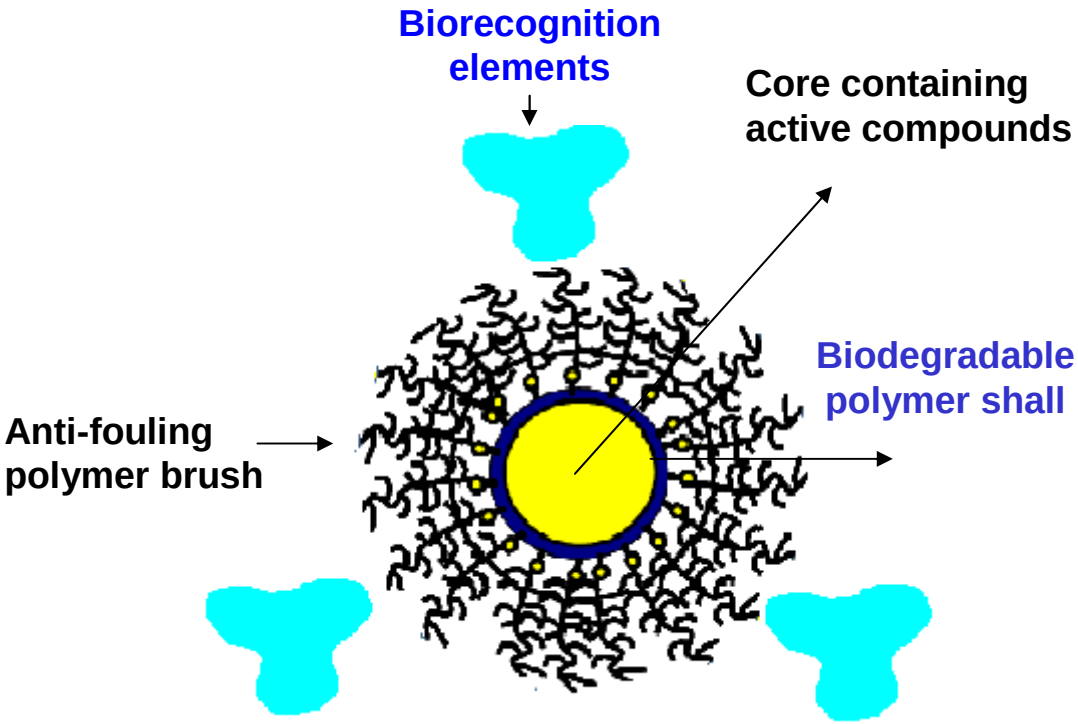
Media for affinity separation of biological compounds (biorecognition layers on magnetic micro- and nano-particles, porous membranes, hollow fibers)

Affinity surface



Affinity surfaces for biosensors (SPR) and separation media (porous membranes and particles)

Nanocapsule



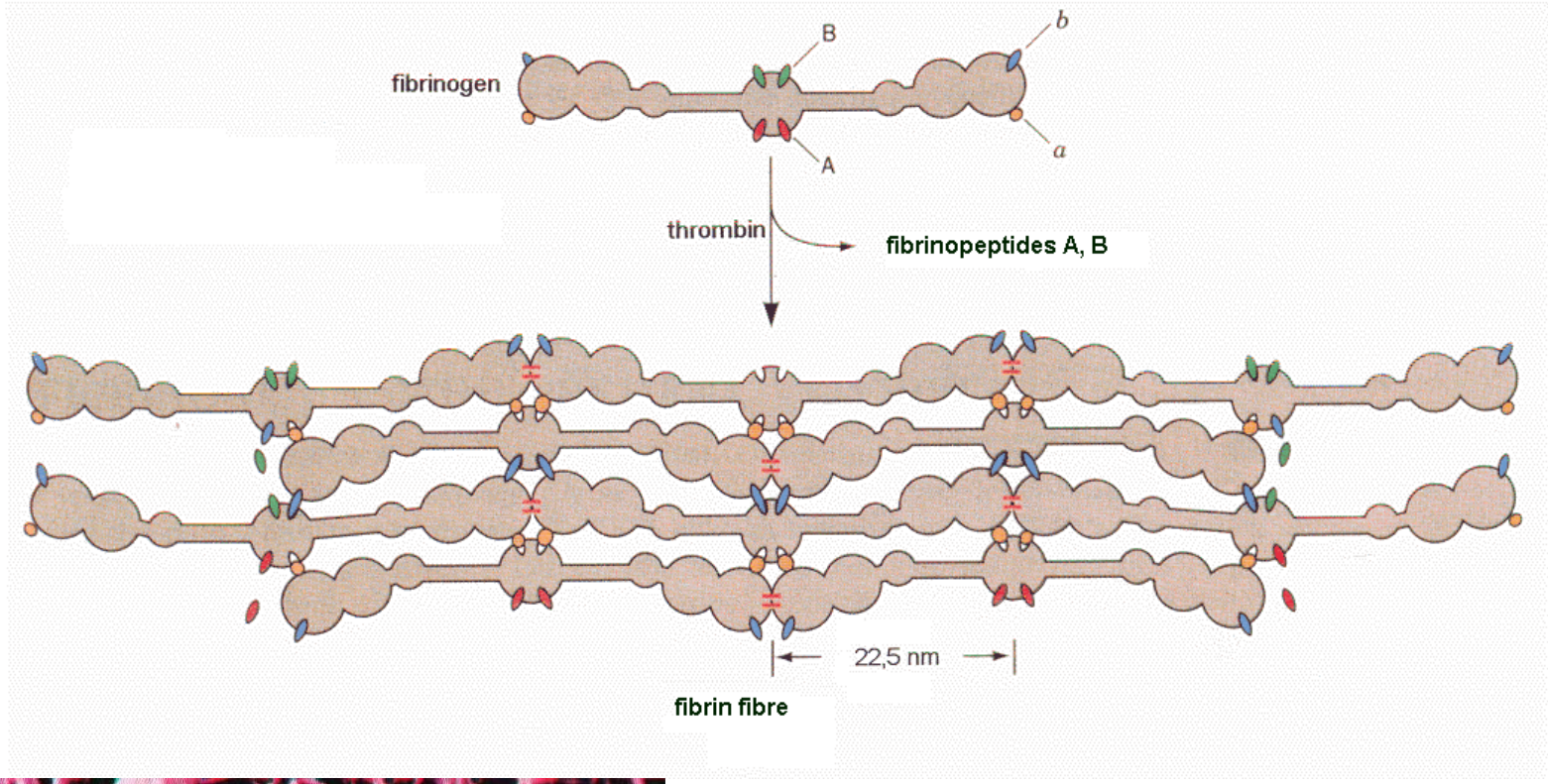
Nanoparticles for targeted drug delivery, diagnostics and separation.

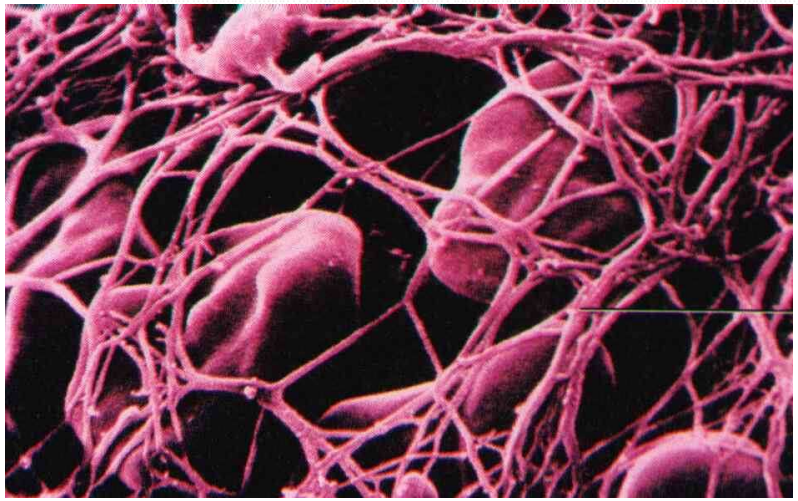
Contact with blood

When exposed to blood, any surface other than that of undamaged vascular wall endothelium induces a sequence of processes resulting in thrombus formation and complement activation. These reactions protect organism against bleeding and infection; however, they are a source of troubles for clinical application of medical devices made of artificial materials.

Immediately after the contact with blood, plasma proteins adsorb onto the material surface. Adsorbed fibrinogen and some other proteins support adhesion and activation of platelets on the surface. Activation of specific plasma proteins and the platelet activation induce a self-amplifying cascade of molecular reactions between mutually activating blood coagulation factors at the surface and at the surface of adhered platelets. Final steps in the coagulation cascade convert fibrinogen into fibrin monomers, from which a fibrin network is produced. The resulting thrombus consists of platelets and other blood cells aggregates immobilized in the fibrin network.

Formation of fibrin network





Thrombus consisting of platelets immobilized in fibrin network.

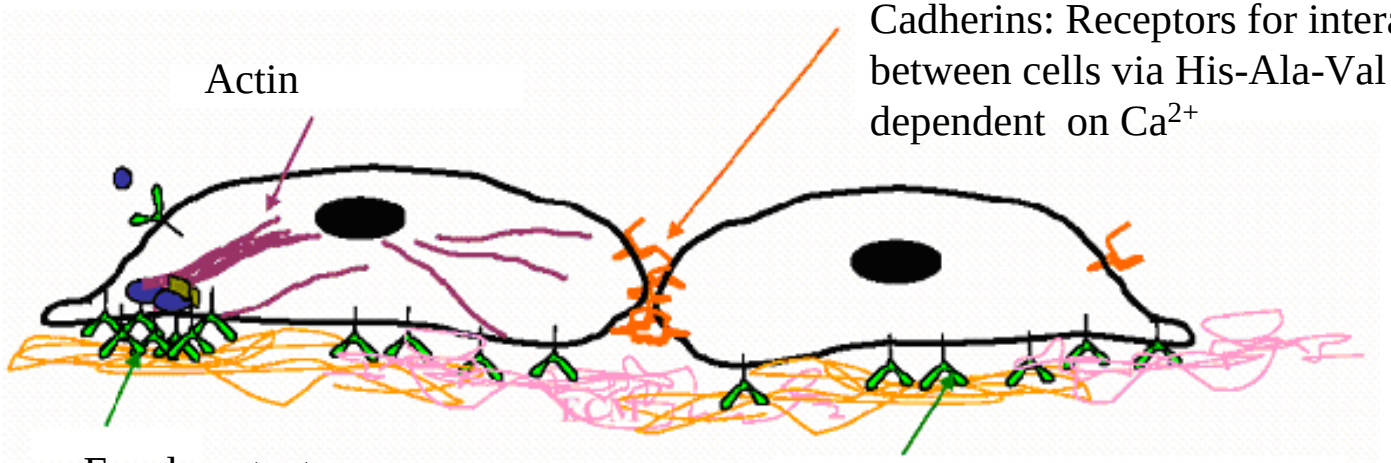
Formation of fibrin clot can be prevented by inhibiting some coagulation factors, particularly thrombin, with anticoagulants, such as heparin, or by depleting calcium ions from plasma when citrate solution is added.

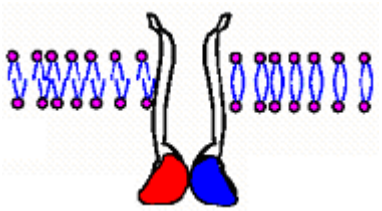
Contact with cells and tissues

Most normal vertebrate cells cannot survive unless they are anchored to a specific support.

Tissue cells are attached to the extracellular matrix (ECM) via interaction of cell transmembrane glycoprotein receptors – integrins with ECM proteins, particularly, collagens, laminin, and fibronectin.

The integrins can bind to RGD tripeptide aminoacid sequence (arginine-glycine-aspartic acid) contained both in ECM proteins and in fibrin.





Integrins: Receptors for adhesive interaction with ECM proteins by binding to Arg-Gly-Asp (RGD), Leu-Asp-Val (LDV), and other sequences dependent on Ca^{2+}/Mg^{2+}

Tissue engineering

Most normal vertebrate cells cannot survive unless they are anchored to a specific support.

Tissue cells are attached to the extracellular matrix (ECM) via interaction of cell transmembrane glycoprotein receptors – integrins with ECM proteins, particularly, collagens, laminin, and fibronectin.

Tissue engineering uses polymer scaffolds to accommodate cells and guide their growth for the repair of damaged tissues and organs.

Modification of artifficial surfaces with functional protein assemblies

We utilize hydrophobic, electrostatic, and biospecific interactions for preparation organized molecular assemblies by successive deposition of biological and synthetic macromolecules.

Antithrombogenic coatings of blood contacting devices – covalently crosslinked albumin and heparin multilayers.

Affinity assemblies for analytical and separation methods - covalently crosslinked albumin and antibody multilayers.

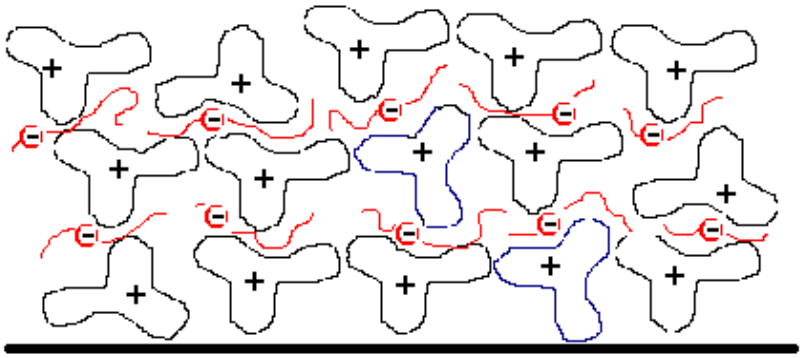
Cell-seeding coatings of scaffolds for tissue engineering – organised assebmlies composed of various types of collagen, heparin, fibronectin, and laminin mimicking extracellular matrix.

Physical interactions between proteins and other macromolecules or solid surfaces can be utilized for the controlled preparation of the assemblies by successive deposition of the molecules on a synthetic surface.

Hydrophobic interaction can be partially affected by small ions and detergents added to solutions. It takes part in protein adsorption on surfaces of all materials except of highly hydrophilic gels.

Electrostatic interactions can be controlled in a wide range by changing ionic strength and, particularly, by changing protein charge dependent on pH of the medium.

Preparation of protein assemblies



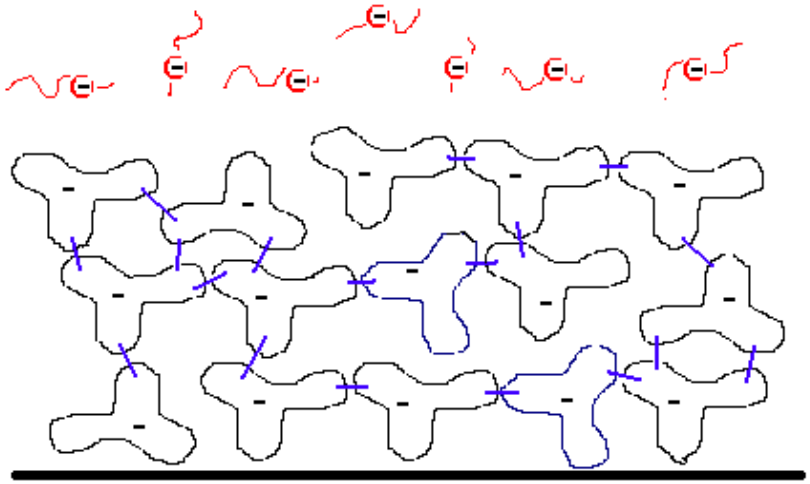
pH 4

Protein below isoeletric point is positively charged

antibody

dextrane sulphate — polyanion

covalent crosslinking



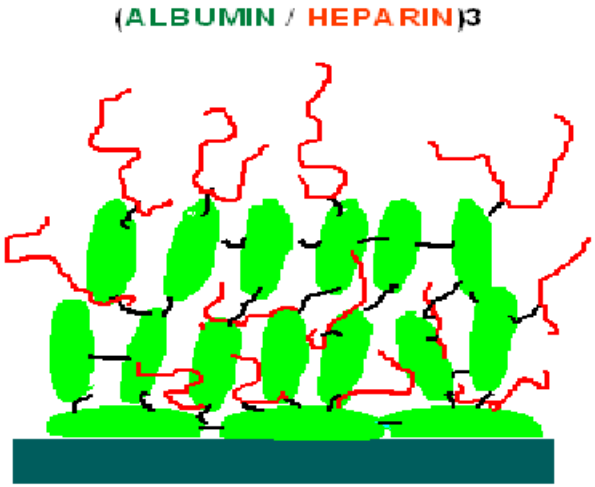
pH 7.4

Protein above isoelectric point is negatively charged

Bloodcompatible coatings of medical devices

The coating with serum albumin molecular multilayers creates a natural interface between the artificial surface and blood in which albumin is the main protein component (4-5%).

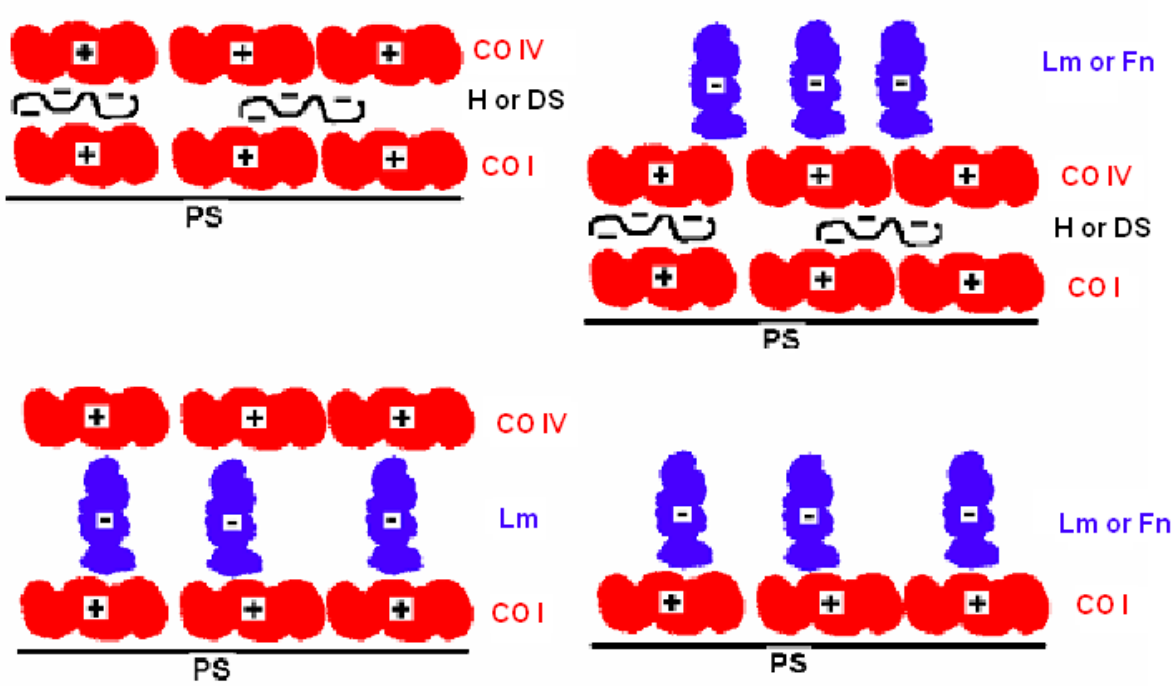
Albumin-heparin alternating multilayers utilize catalytic action of anticoagulant heparin to suppress thrombus formation.



Cell-seeding coatings

Layer-by-layer deposited molecular assemblies **mimicking extracellular matrix**

Electrostatic interaction



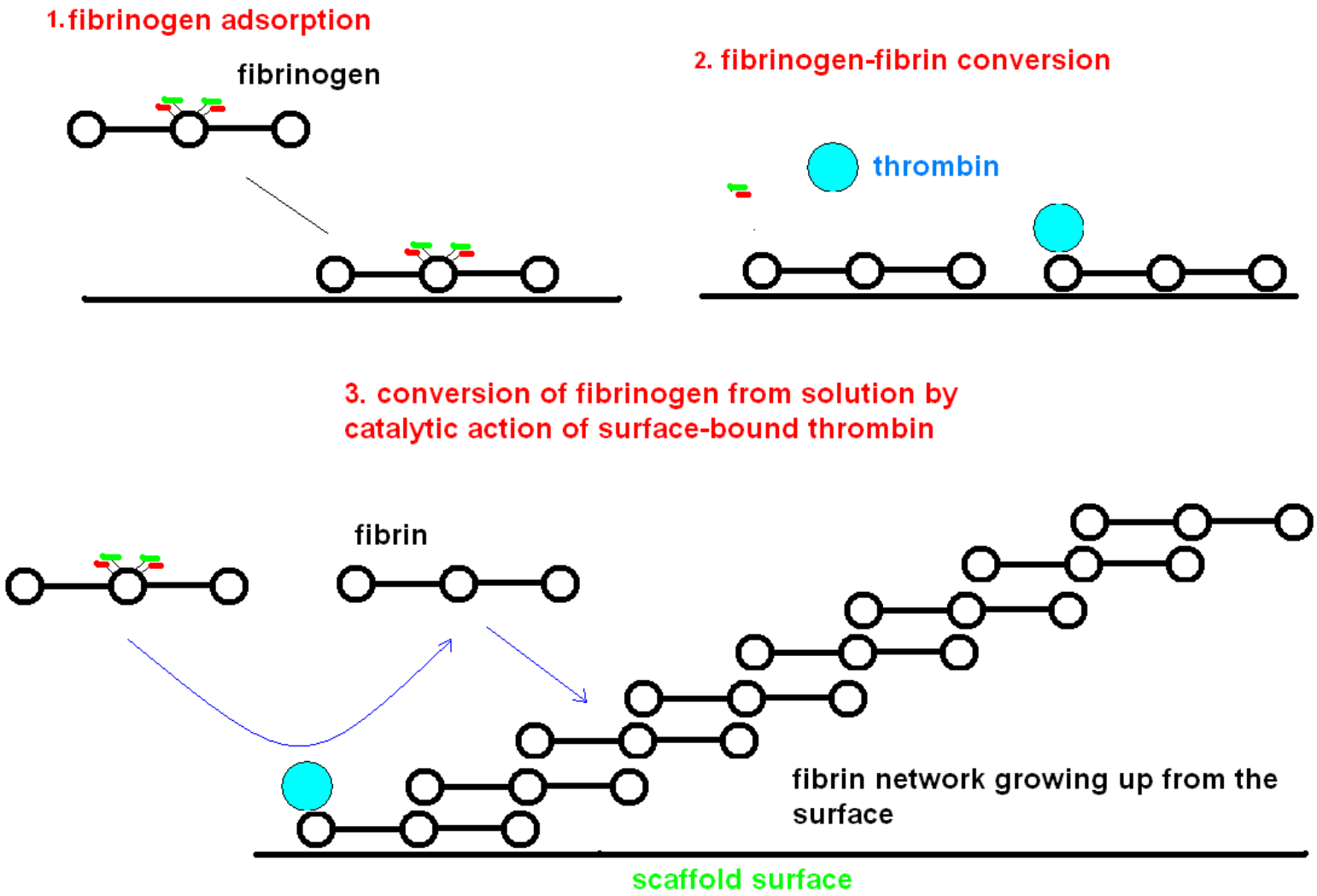
Biospecific interaction

Co I collagen I Co IV collagen IV Lm laminin Fn fibronectin

H heparin DS dextran sulfate (polyanions)

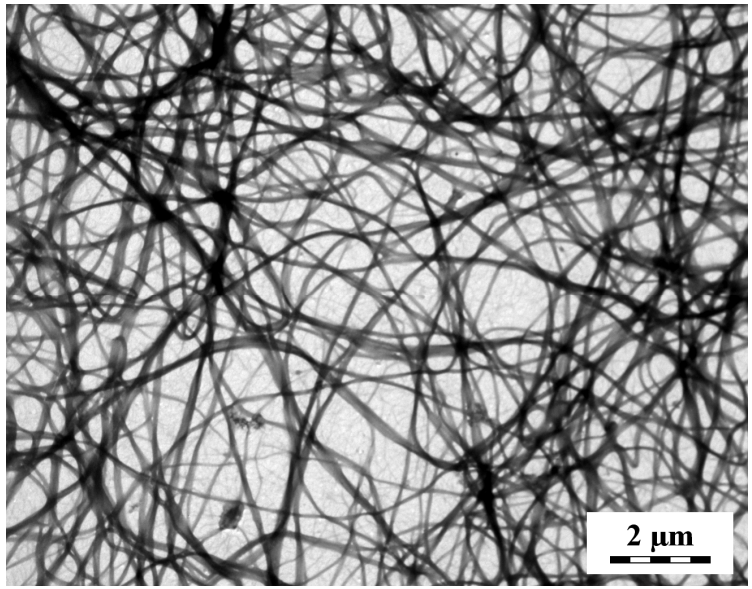
Artificial surface fibrin network

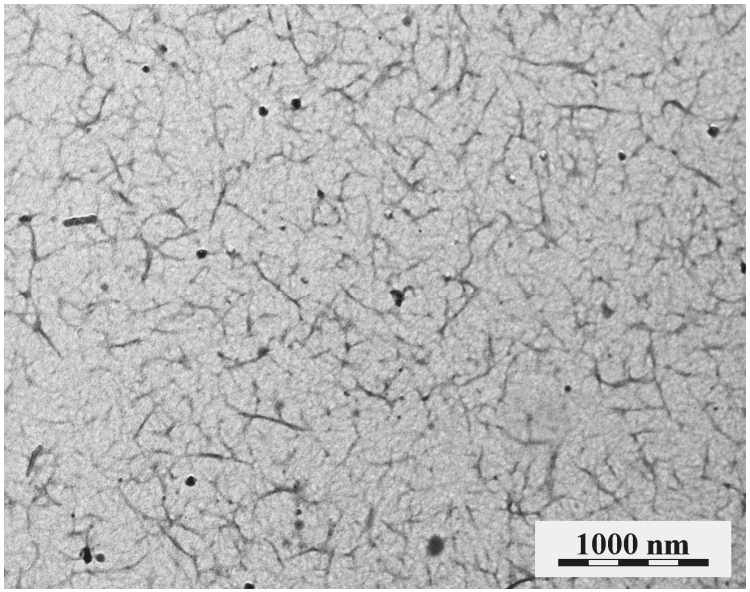
Fibrin clot formed to stop bleeding at an injury provides a temporary scaffold for subsequent migrating fibroblasts, endothelial cells, and smooth muscle cells and other cellular responses of wound and vessel repair.



Three-dimensional surface fibrin networks

TEM of dried samples on graphite





Thick three-dimensional fibrin network composed of thick fibers was produced at solid surfaces by catalytic action of thrombin bound to an adsorbed Fbg/fibrin on ambient fibrinogen solution.

Thin three-dimensional fibrin network composed of thin fibers was produced at solid surfaces by catalytic action of thrombin bound to an adsorbed Fbg/fibrin on ambient fibrinogen solution inhibited with ATIII and heparin.