Analysis of Protein Adsorption Force Generated at Polymer Surfaces for Designing Non-biofouling Materials

THE UNIVERSITY OF TOKYO

Sho SAKATA, Yuuki INOUE, and Kazuhiko ISHIHARA

Department of Materials Engineering, School of Engineering, The University of Tokyo

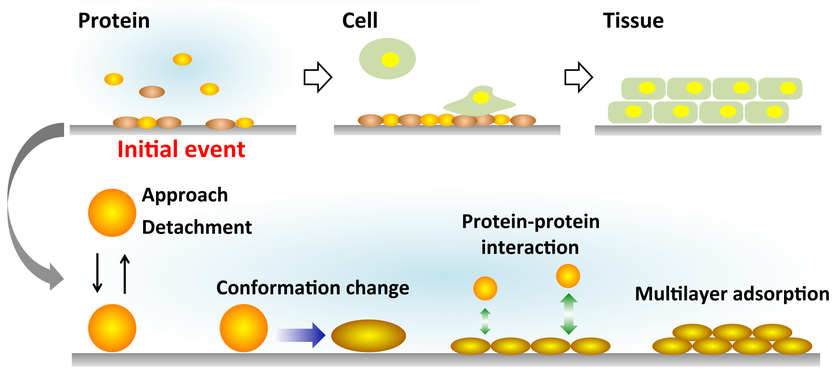
e-mail: sakata@mpc.t.u-tokyo.ac.jp http://www.mpc.t.u-tokyo.ac.jp

DESIGN OF FOR WITH MATERIALS

1. Introduction

Development of regenerative medicine, cell engineering, and biomaterials engineering
→ Biological responses at interfaces should be precisely understood and controlled.

Biological responses on materials surface



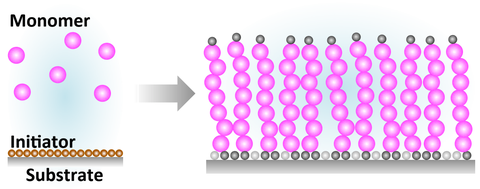
It is important to understand each process of protein adsorption phenomena based on interaction forces.

2. Objective

Understanding protein adsorption based on analysis of molecular interaction operating on the surfaces

Polymer brush surfaces

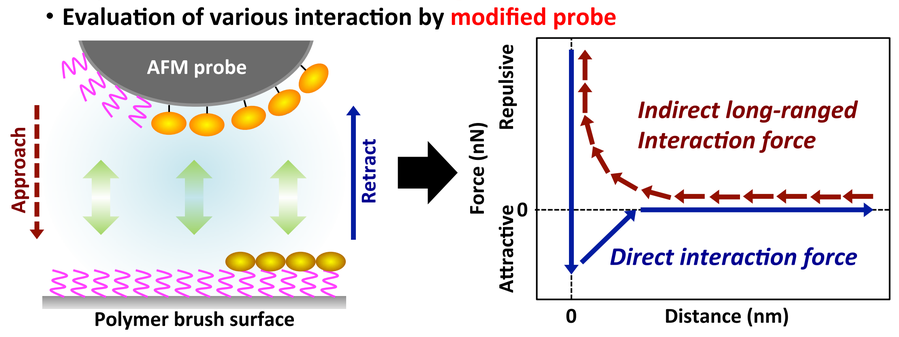
Surface-initiated atom transfer radical polymerization
→ Uniform extension of polymer chains from surface-immobilized initiators



- Polymer chains composed of single monomer unit
- Controlled surface property by chemical structure
  → Clarification of surface interaction force

Atomic force microscopy (AFM)

- Acquisition of force-versus-distance (*f-d*) curve
- Evaluation of various interaction by modified probe

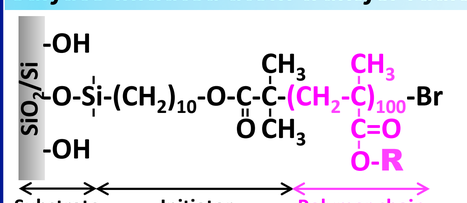


- Polymer brush layer probe → Forces on each surface
- Protein-immobilized probe → Direct interaction force with protein

Refined understanding of various molecular interaction by combination of polymer brush surfaces and AFM

3. Polymer Brush Surfaces

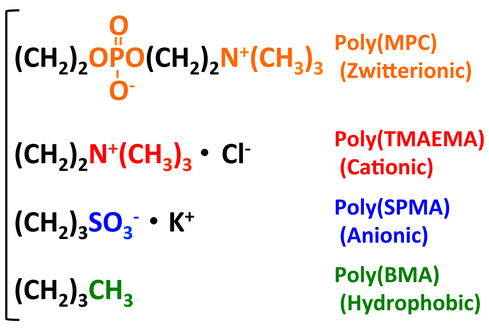
Surface-initiated atom transfer radical polymerization [1]



MPC : 2-Methacryloyloxyethyl phosphorylcholine
TMAEMA: 2-Trimethylammoniumethyl methacrylate
SPMA : 3-Sulfopropyl methacrylate
BMA : *n*-Butyl methacrylate

Degree of polymerization (DP) was controlled by the ratio of monomer and free initiator in feed.

R : Side chains with various chemical structures



→ Various interaction forces on surfaces

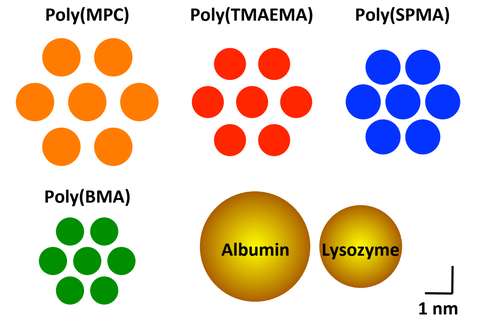
[1] S. Sakata *et al.*, *Langmuir* 30, 2745-2751, 2014.

Physicochemical surface properties

Polymer	Graft density (chains/nm^2)テキスト	Contact angle (°) in water	Contact angle (°) in PBS	ζ-Potential (mV)
Poly(MPC)	0.33	9	9	-5.9
Poly(TMAEMA)	0.45	17	14	64.9
Poly(SPMA)	0.55	13	11	-74.0
Poly(BMA)	0.75	73	73	-37.2

- Graft density: High enough to form the high-density polymer brush surface [2]
- Hydrophilicity: High in wet condition [except for poly(BMA)]
  Constant regardless of ionic strength in medium
- Surface potential: Different by charge properties of monomer units

[2] Y. Tsujii *et al.*, *Adv. Polym. Sci.*, 197, 1-45, 2006.

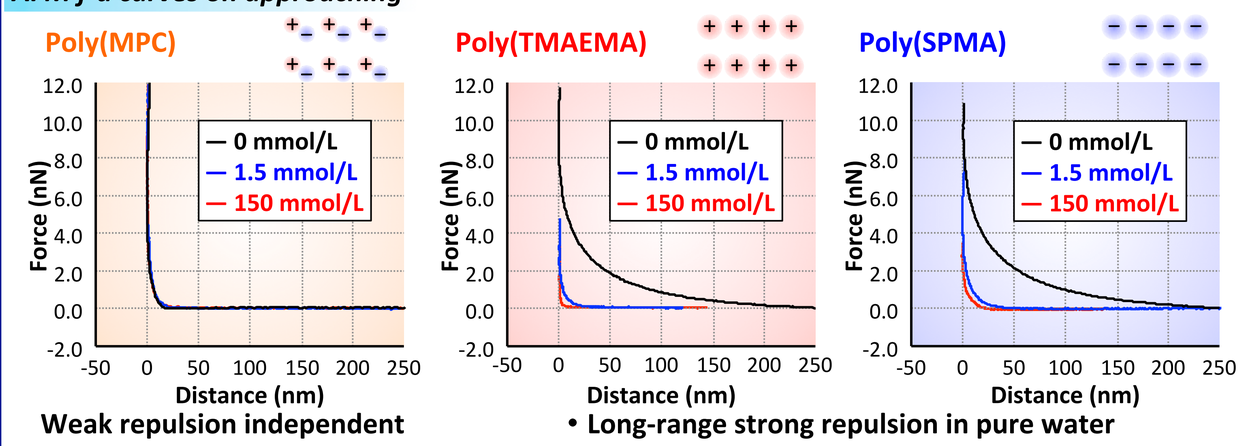


Penetration of proteins into polymer brush layers is negligible.

High-density polymer brush surfaces with controlled physicochemical properties were prepared, as model surfaces to understand the relationship between protein adsorption and interaction force.

4. Surface Interaction Forces

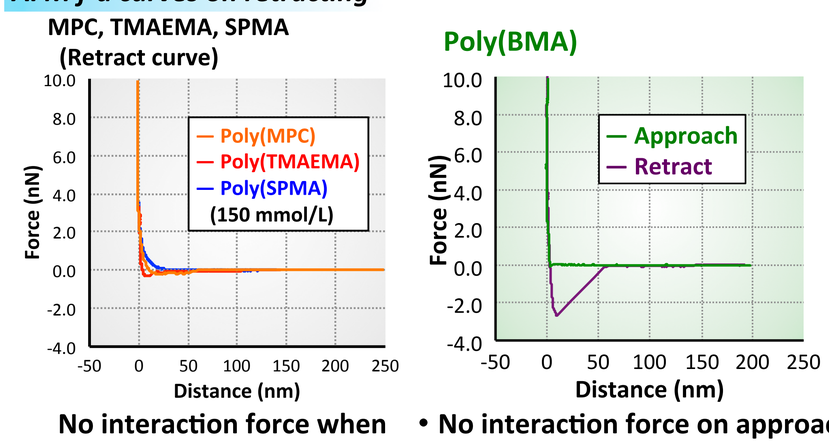
AFM f-d curves on approaching



Weak repulsion independent of ionic strength
→ No specific interaction

- Long-range strong repulsion in pure water
- Weakening with increase of ionic strength
  → Electrostatic interaction

AFM f-d curves on retracting



No interaction force when retracting after contact

- No interaction force on approach
- Strong attraction on retraction
  → Hydrophobic interaction

Zwitterionic surface
→ No specific interaction

Cationic Surface
Anionic Surface
→ Electrostatic interaction

Hydrophobic Surface
→ Hydrophobic interaction

Single or No interaction force operated on the surfaces.

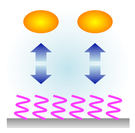
Electrostatic interaction forces and hydrophobic interaction forces generating on surfaces were clearly separated using systematically prepared polymer brush surfaces.

5. Protein Adsorption Behavior

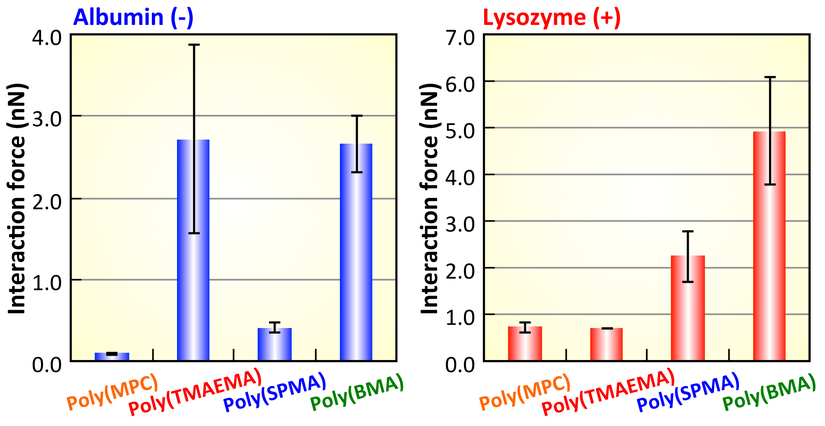
- Albumin from bovine serum (pI 4.8) → Negative net charge at pH 7.4
- Lysozyme from chicken egg white (pI 11.1) → Positive net charge at pH 7.4

Surface-protein interaction

AFM *f-d* curve measurement [3]
- Polymer brush surface
- PBS (*I* = 150 mmol/L, pH 7.4)



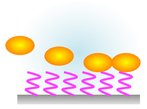
[3] Y. Inoue *et al.*, *React. Funct. Polym.*, 71, 350-355, 2011.

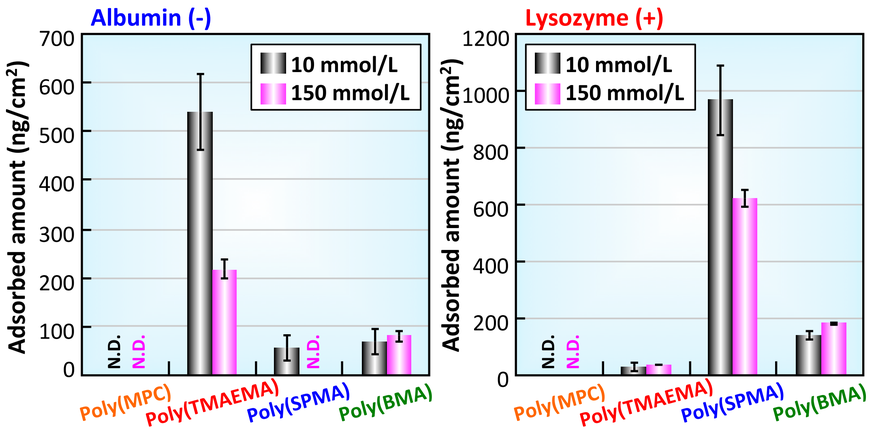


Poly(MPC) → Little interaction
Poly(TMAEMA) → Strong interaction with albumin
Poly(SPMA) → Strong interaction with lysozyme
Poly(BMA) → Strong Interaction with both proteins

Amount of adsorbed proteins

Surface plasmon resonance measurement
- 1.0 mg/mL (PBS; *I* = 10, 150 mmol/L, pH 7.4)
- 37°C, 500 µL/min, 30 min

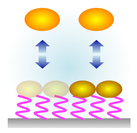


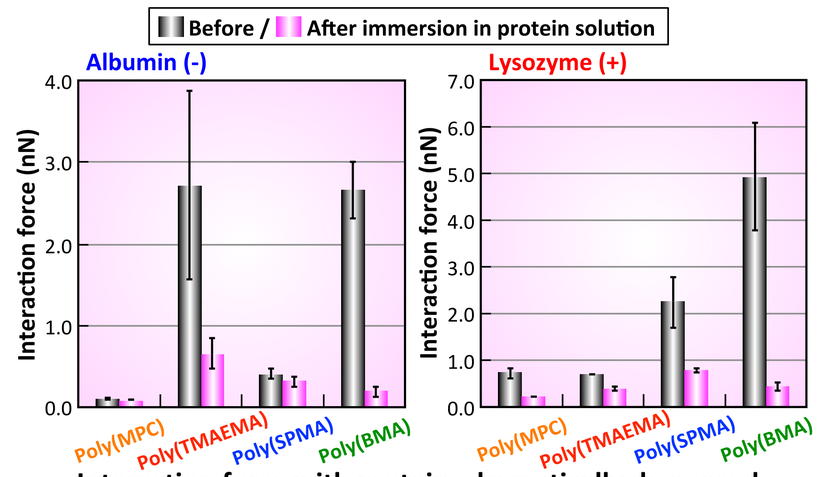


Poly(MPC) → Little adsorption (< 5.0 ng/cm^2)
Poly(TMAEMA) → High adsorption of albumin
Poly(SPMA) → High adsorption of lysozyme
Poly(BMA) → Independent of charge properties

Protein-protein interaction

AFM *f-d* curve measurement
- Protein pre-adsorbed surface
- PBS (*I* = 150 mmol/L, pH 7.4)

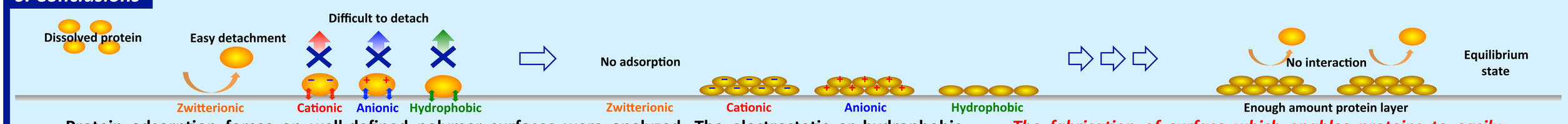




Interaction force with proteins dramatically decreased on the high protein adsorption surfaces.
→ Protein would not adsorb on the surfaces with enough amount protein layer.

Electrostatic and hydrophobic interaction forces hinder the reversible detachment of proteins from the surfaces, which would lead to high protein adsorption.

6. Conclusions



Protein adsorption forces on well-defined polymer surfaces were analyzed. The electrostatic or hydrophobic interaction generating on the vicinity of protein adsorptive surfaces plays a role as the force which inhibit the detachment of proteins from the surface.

The fabrication of surface which enables proteins to easily detach from the surface would be important to suppress protein adsorption on biomaterials surfaces.