

# Protein Adsorption to Biomaterials

Radioactive Labelling Analysis & Atomic Force Microscopy

Maria Holmberg

DTU Nanotech

Technical University of Denmark

$$f(x+\Delta x) = \sum_{i=0}^{\infty} \frac{(\Delta x)^i}{i!} f^{(i)}(x)$$
$$\int_a^b \Theta^{\sqrt{17}} + \Omega \int \delta e^{i\pi} =$$
$$\infty = \{2.71828182845904523536028747135266249775724706362351902511967$$
$$\Sigma \gg !,$$

DTU Nanotech

Department of Micro- and Nanotechnology

# Background

## Biomaterials

Materials that interact with a biological system

Materials that are part of implants, drug delivery systems, biomedical equipment, disposable devices, biosensors etc.

## Protein adsorption

First step in a cascade of events that eventually can result in non-wanted responses

Influence on functionality of designed systems

## Controlling protein adsorption

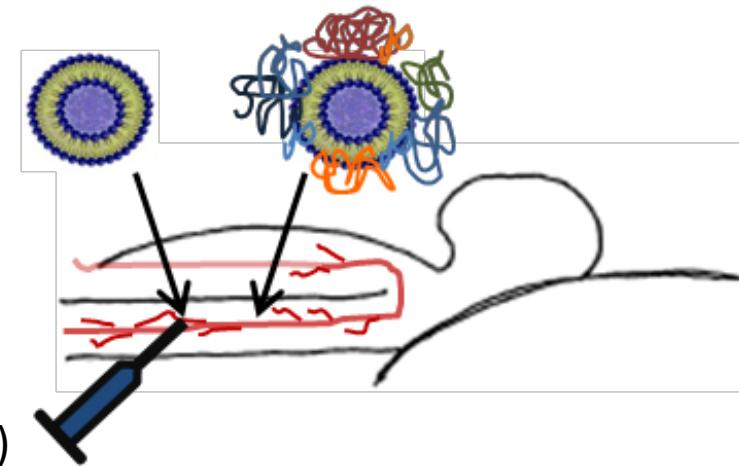
Manipulating with response

Surface characteristics

Blood protein adsorption onto polymer materials

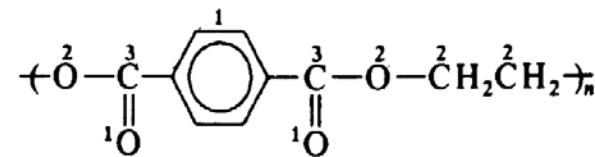
Albumin (67 kDa), IgG (150 kDa) & fibrinogen (340 kDa)

Radioactive multi-label system & AFM

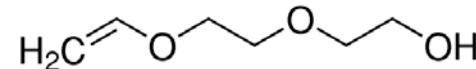


# Background

**PET (polyethylene terephthalate)**



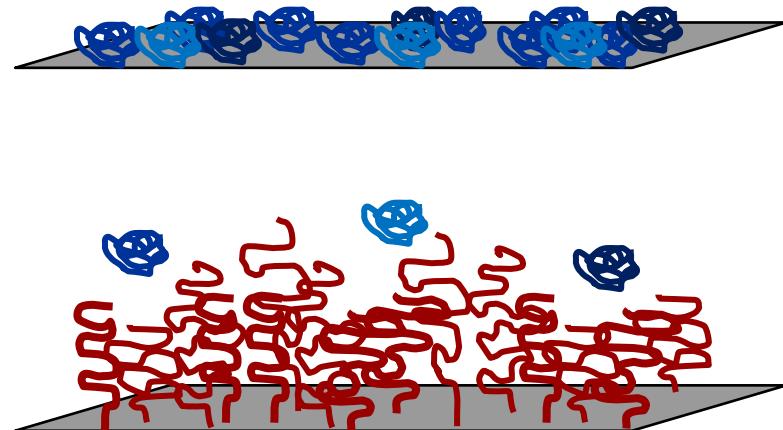
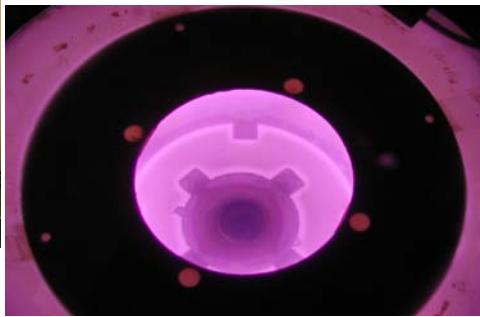
**DEGVE (diethylene glycol vinyl ether)**



Plasma polymerisation



Argon pre-treatment  
 Plasma copolymerisation  
 Working pressure 5-50 Pa  
 2 phase 50 Hz AC power supply  
 100 Hz pulsed plasma current  
 Energy ~ 1-5 Watt

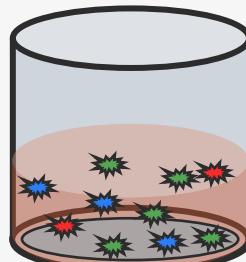


*M. Holmberg et al., J Mater Sci: Mater Med. 19 (2008), 80*

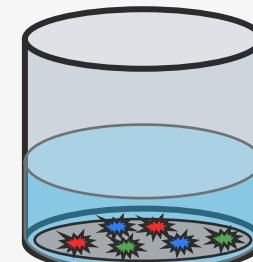
# Radioactive Labelling

## Quantitative Protein Adsorption Analysis

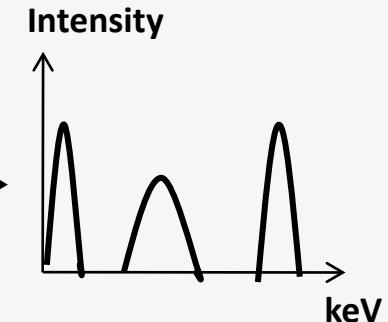
Solution with  
 $^{125}\text{I}$ -albumin  
 $^{131}\text{I}$ -IgG  
 $^{123}\text{I}$ -fibrinogen



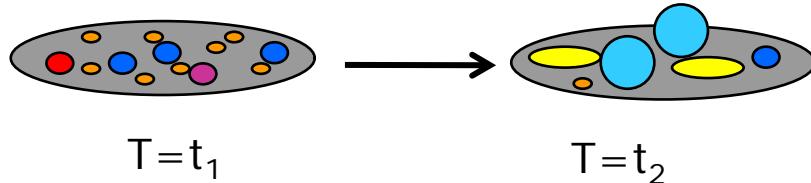
Rinsing  
Procedure



Gamma  
counting



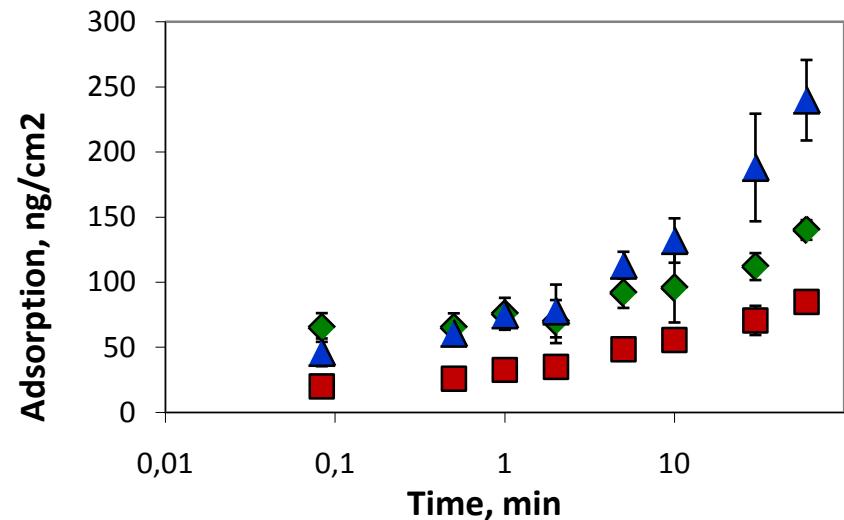
## Quantitative technique for Competitive Adsorption from Complex Solutions



$T = t_1$

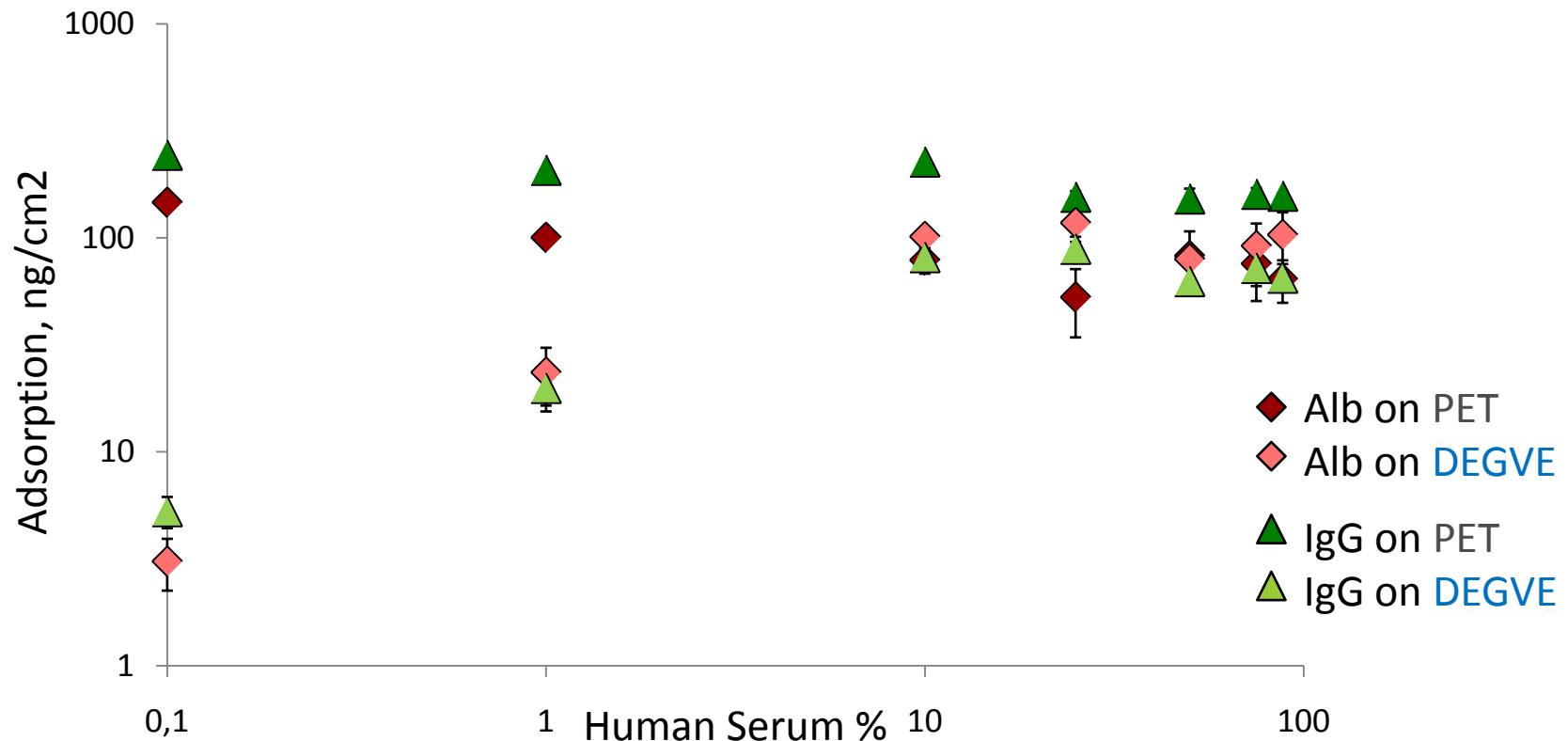
$T = t_2$

Function of time, concentration,  
surface characteristics etc.



# Competitive Protein Adsorption

Alb & IgG adsorption onto PET & DEGVE (1 h & 24 h)



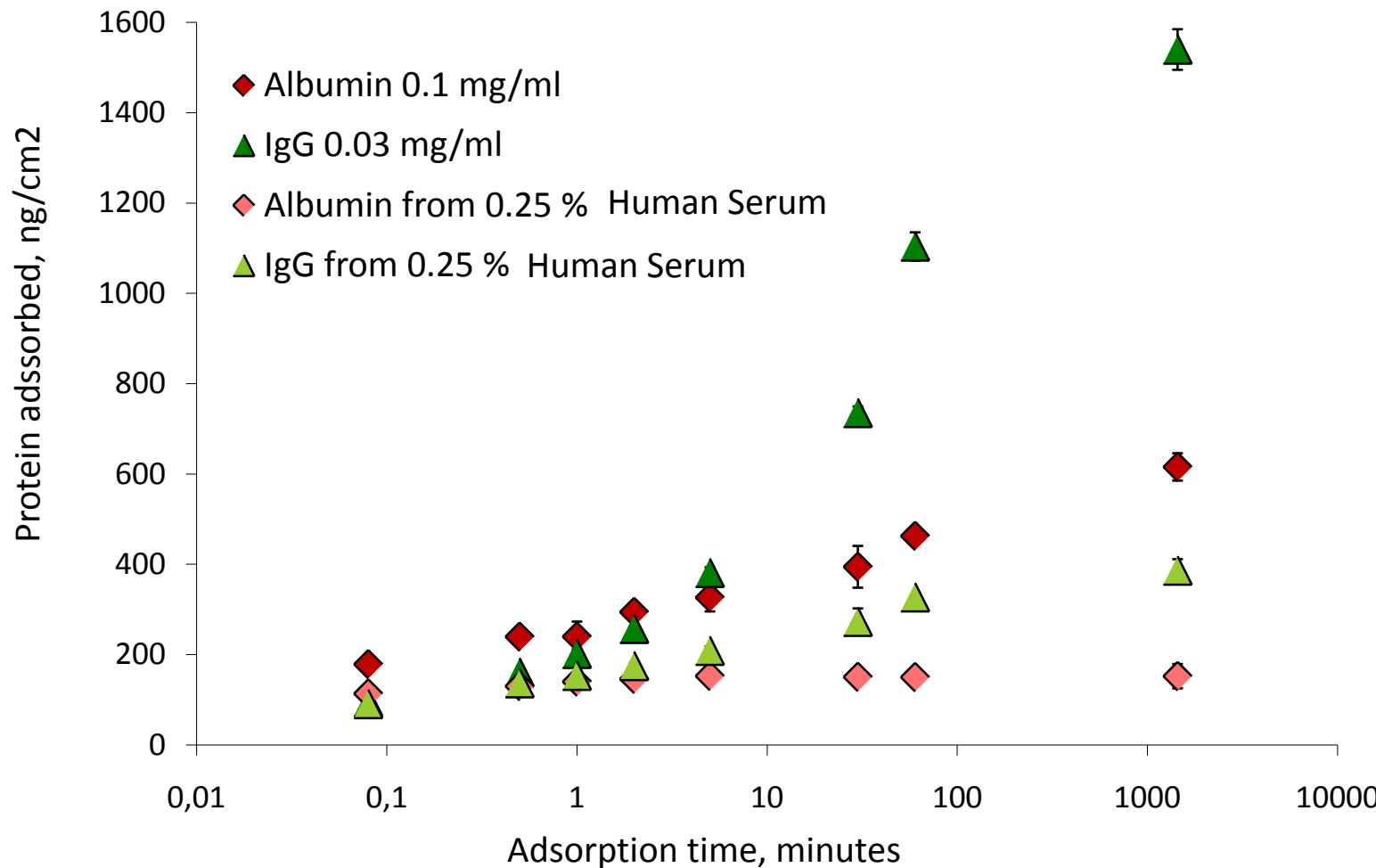
PET – decrease with increased human serum %

DEGVE – increase with human serum %

M. Holmberg et al., Langmuir 26 (2010), 938

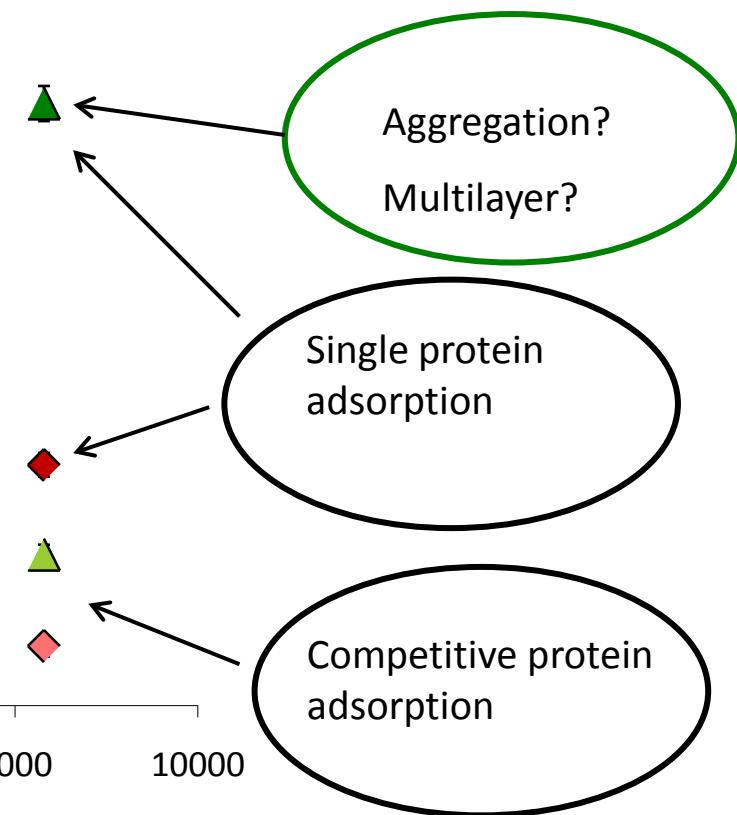
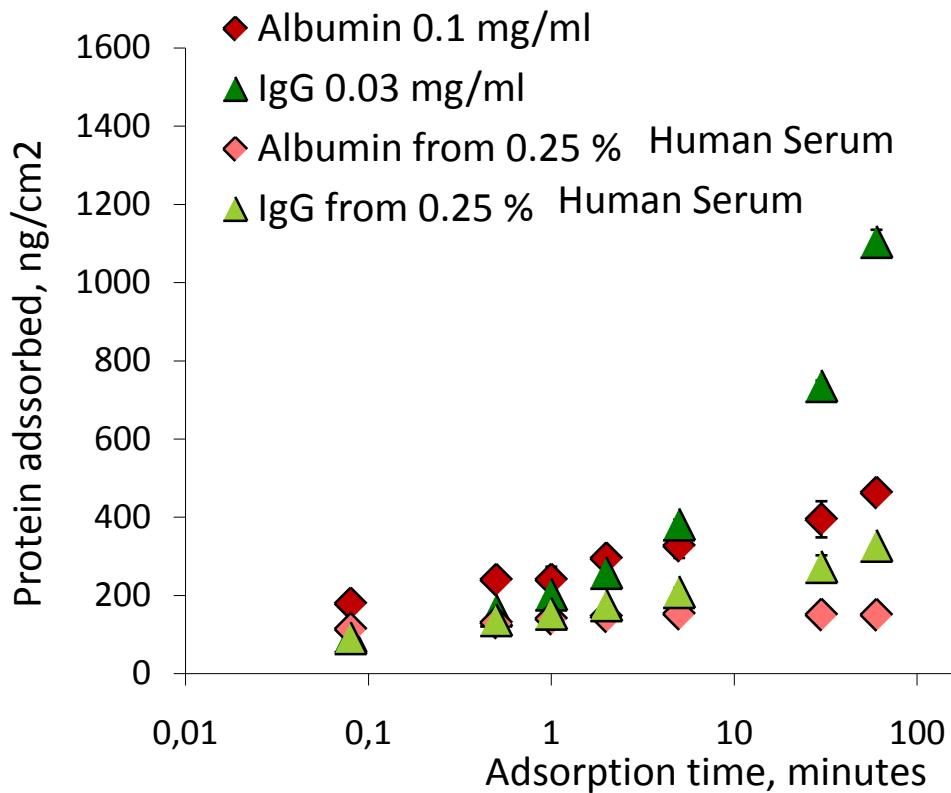
# Alb & IgG adsorption onto PET

Single protein adsorption versus Competitive protein adsorption onto PET



M. Holmberg et al., Langmuir 25 (2009), 2081

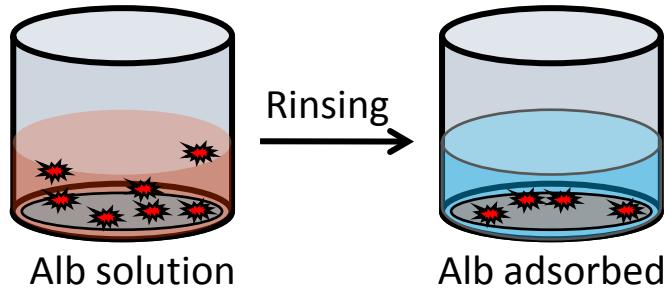
# Alb & IgG adsorption onto PET



Influence from presence of other proteins

Specific interaction between protein and surface

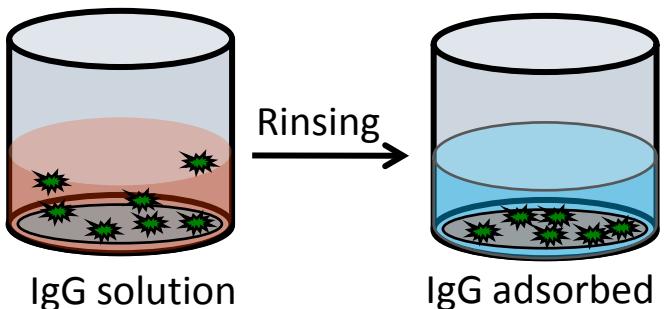
# Alb & IgG adsorption onto PET & DEGVE



Adsorption of Alb & IgG onto PET & DEGVE

10 mg/ml Alb & 3 mg/ml IgG (25 % human serum)

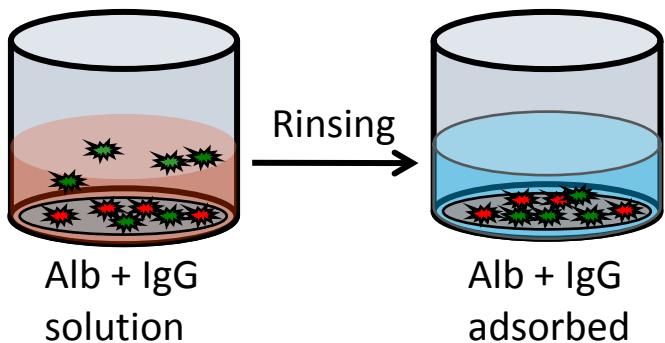
Different adsorption times (1 min versus 1 h)



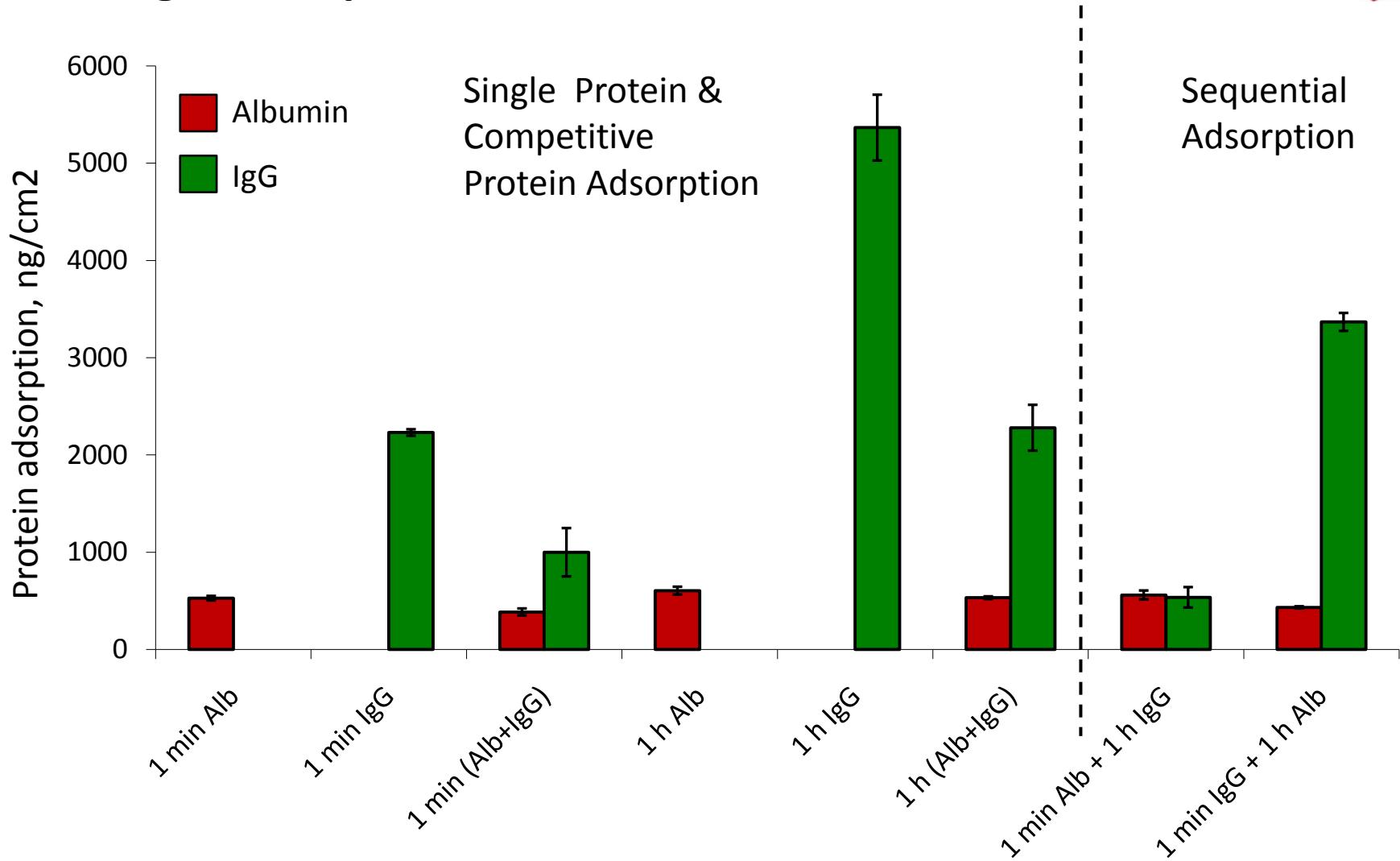
Single protein adsorption

Competitive protein adsorption

Sequential protein adsorption

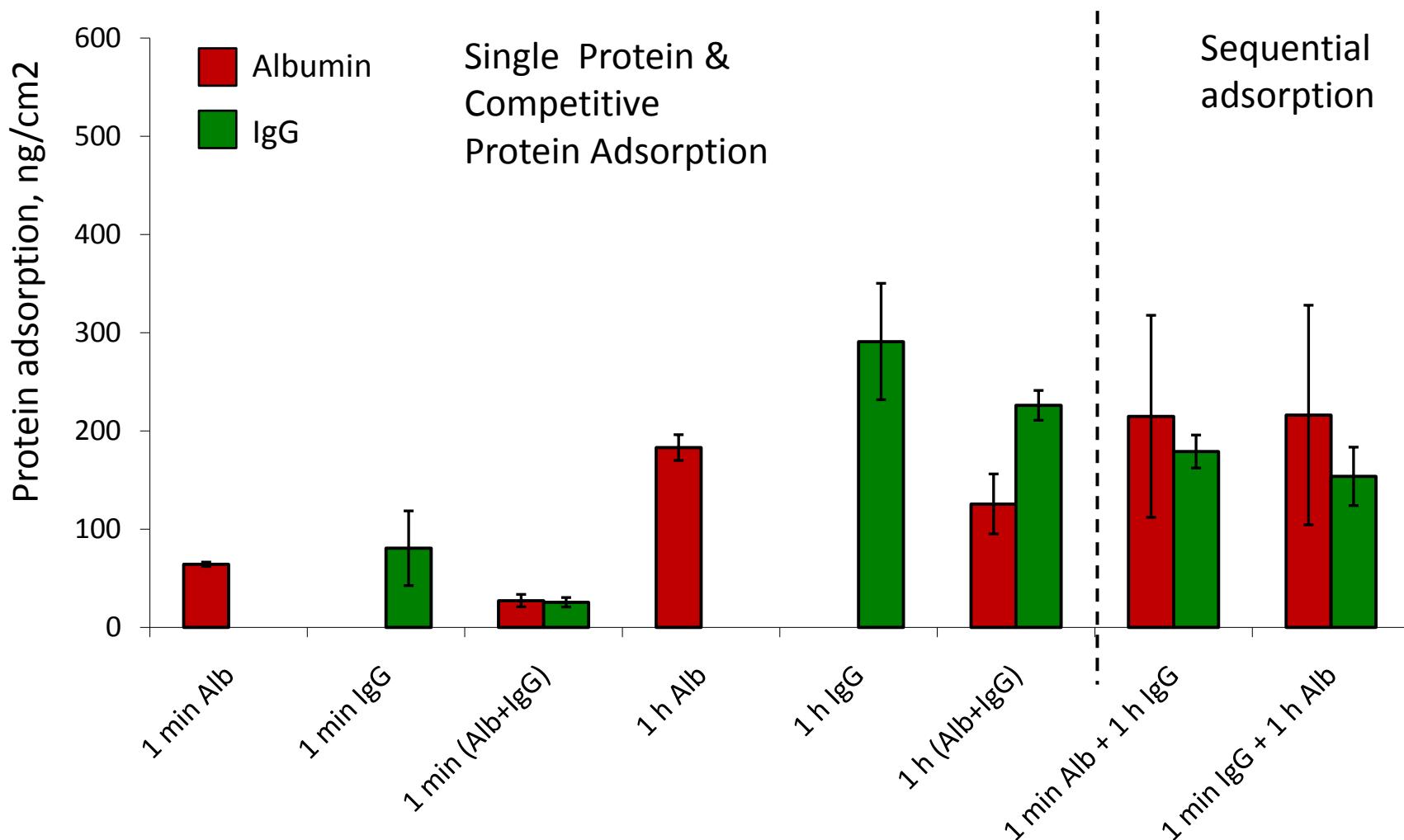


# Alb & IgG adsorption onto PET



M. Holmberg et al., Langmuir 25 (2009), 2081

# Alb & IgG adsorption onto DEGVE



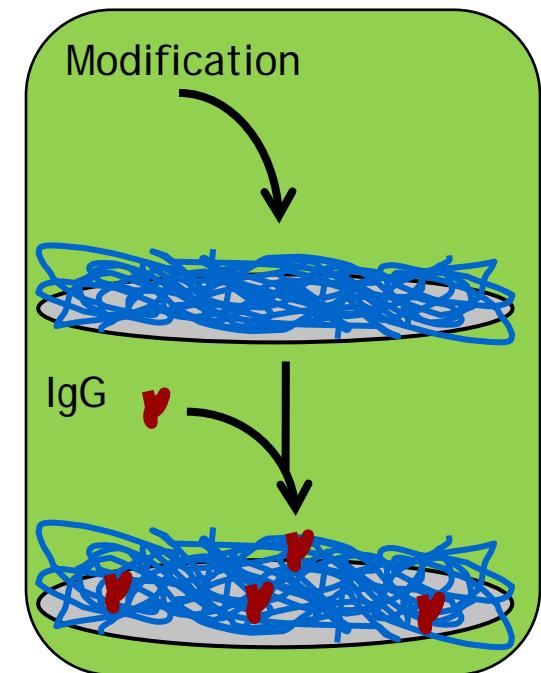
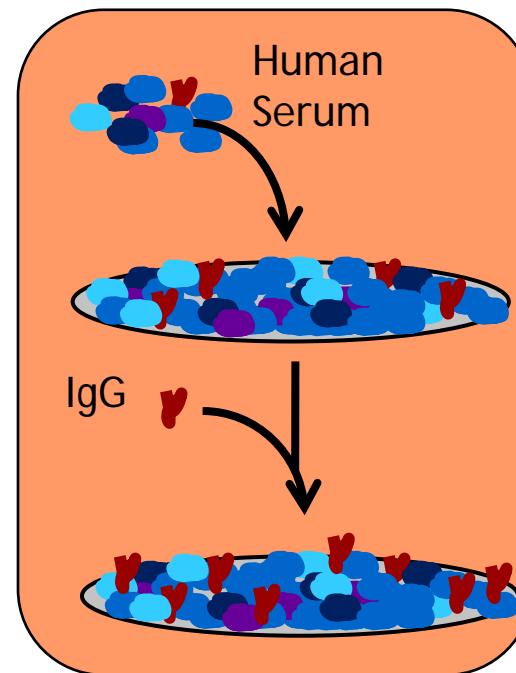
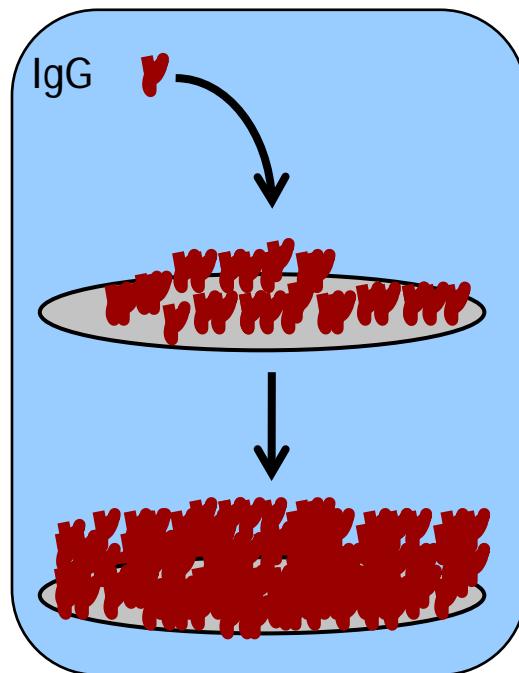
M. Holmberg et al., Langmuir 25 (2009), 2081

# Conclusions – IgG adsorption

Specific protein-surface interaction – *surface induced protein aggregation*

Influence from presence of other proteins – *competition and blocking of surfaces*

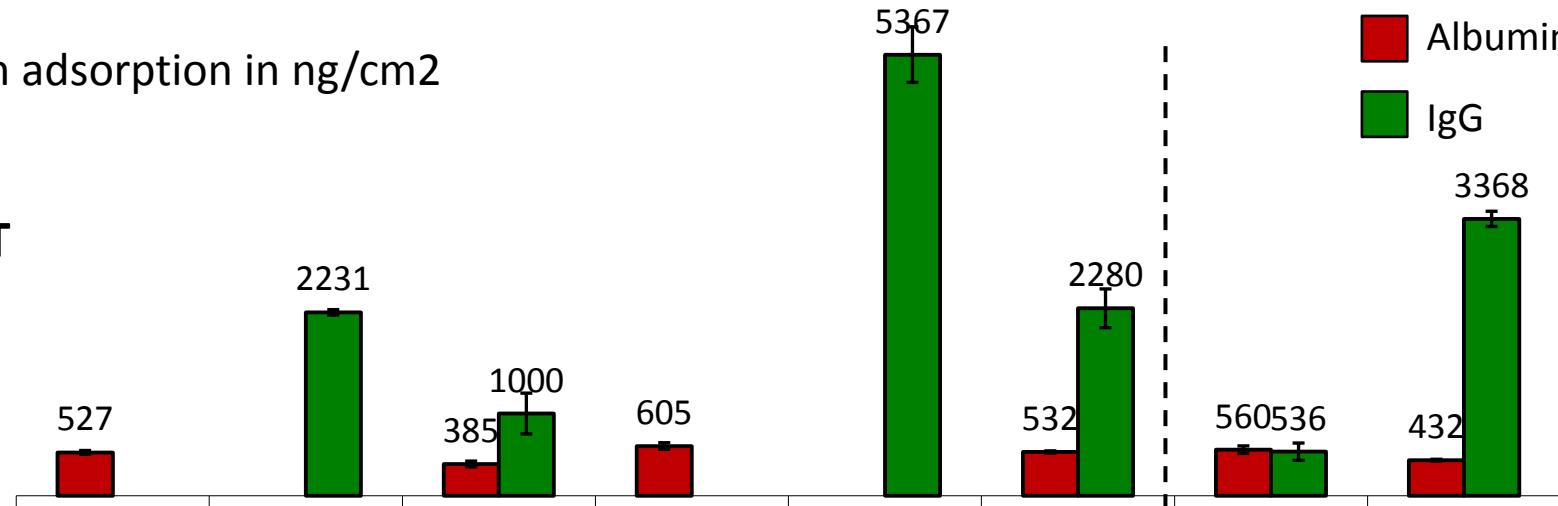
Non-fouling characteristics of DEGVE – *lower adsorption*



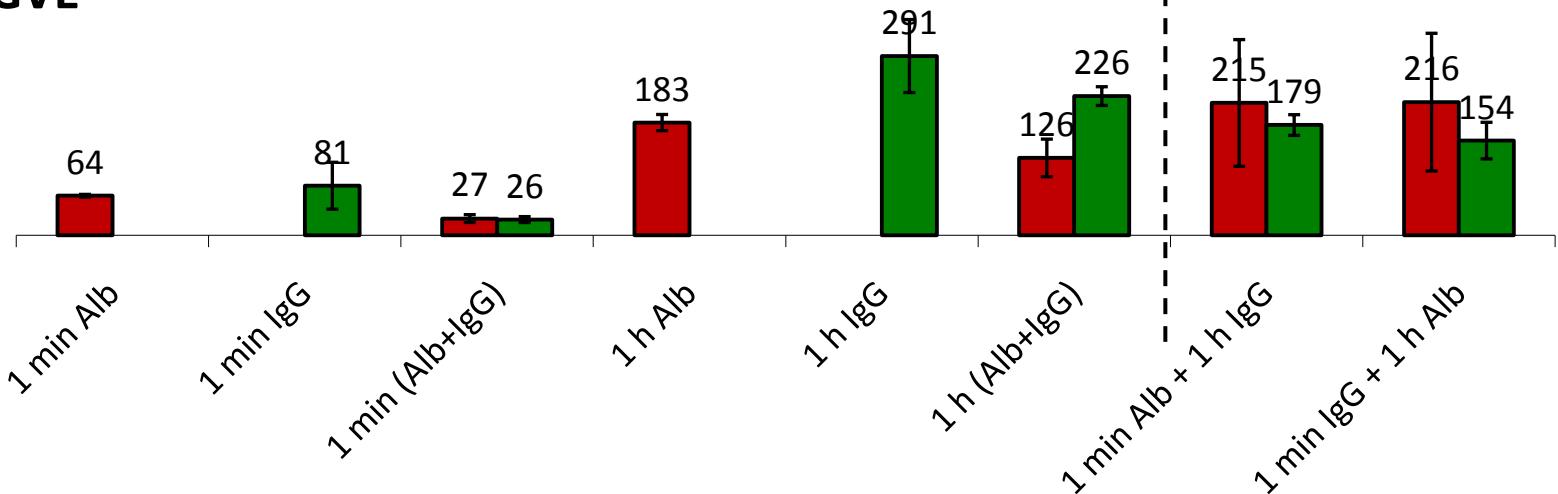
# Alb & IgG adsorption onto PET and DEGVE

Protein adsorption in ng/cm<sup>2</sup>

PET

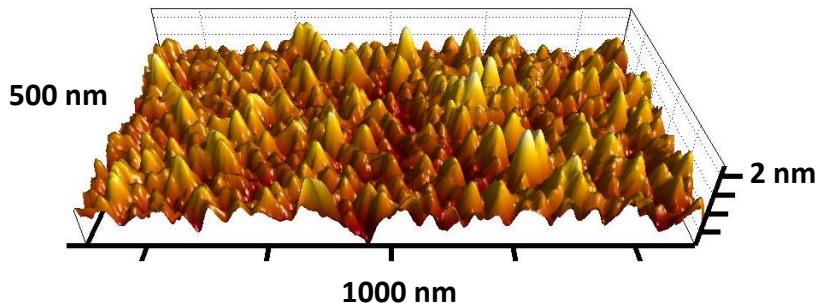


DEGVE

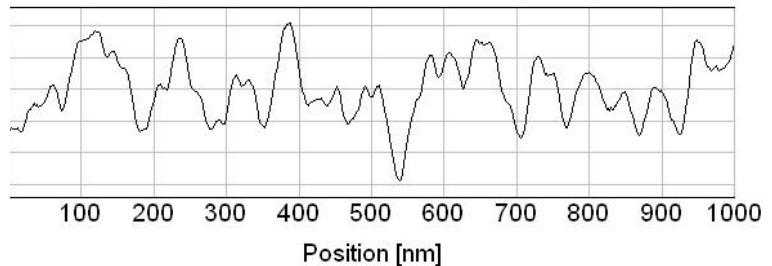


# Atomic Force Microscopy

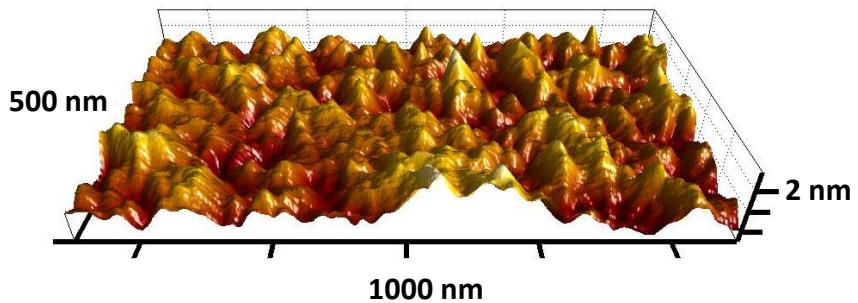
Tapping Mode in air, PET



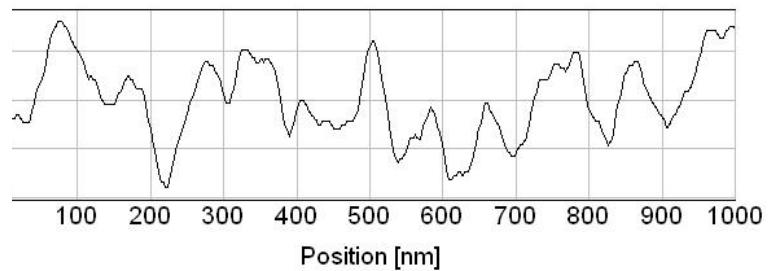
$S_q = 0.995 \text{ nm}$



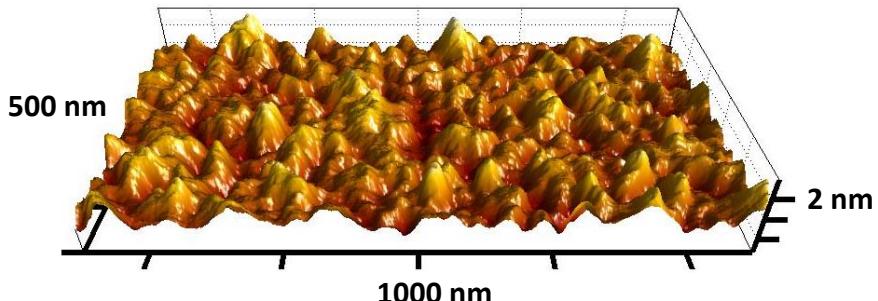
Tapping Mode in air, PET + 3 mg/ml IgG



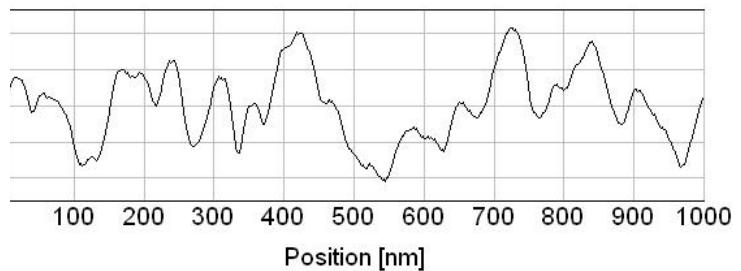
$S_q = 0.980 \text{ nm}$



Tapping Mode, PET + 10 mg/ml Alb



$S_q = 0.874 \text{ nm}$

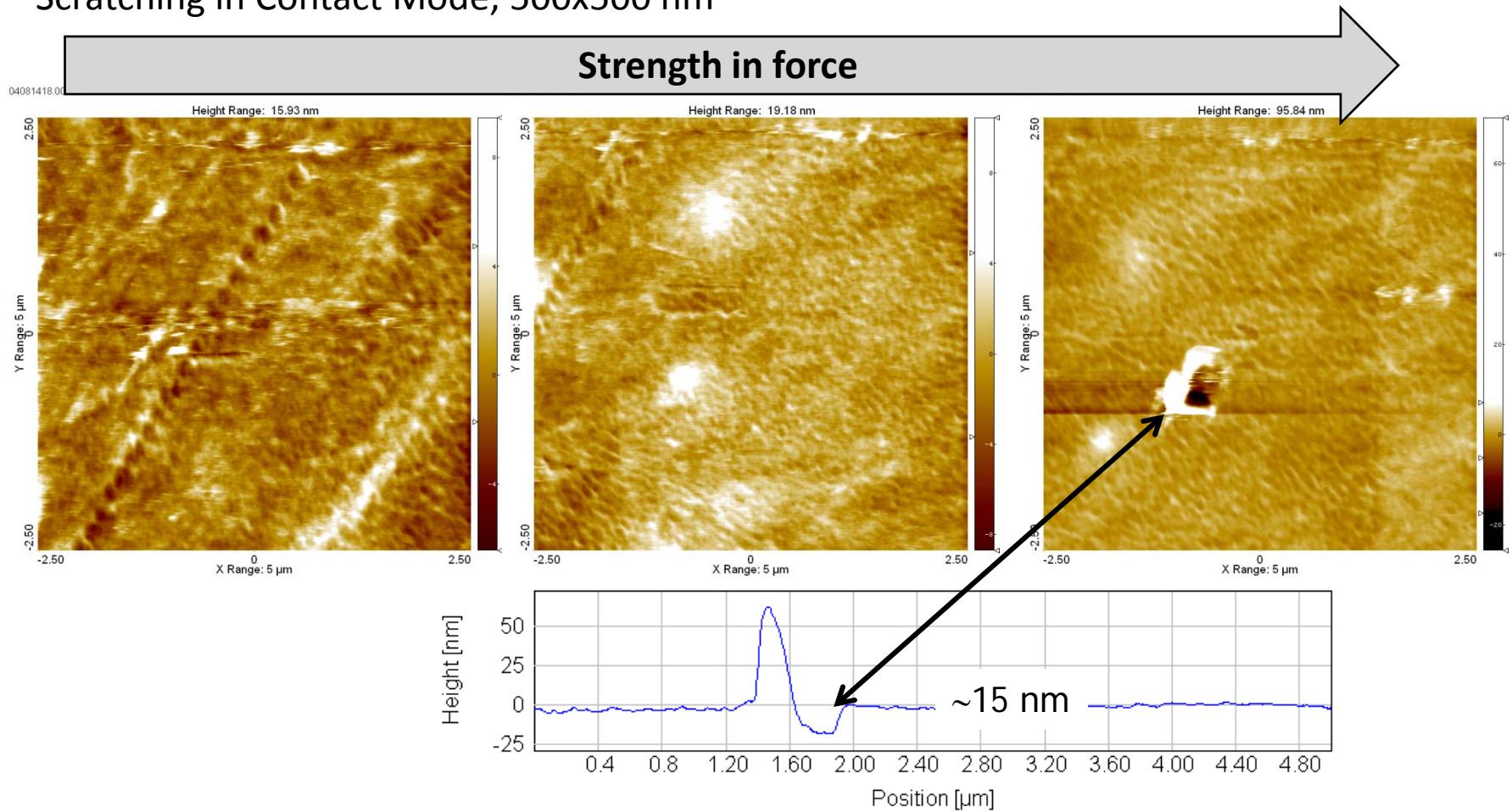


# Atomic Force Microscopy

Closed system using a liquid cell and o-ring

PET + 10 mg/ml albumin, Imaging in Tapping Mode, 5x5  $\mu\text{m}$

Scratching in Contact Mode, 500x500 nm



# Summary

Radioactive Multi-Labelling - quantitative analysis of competitive protein adsorption from complex solutions

Evaluating Biomaterials

Characteristics of protein and surface

Difference in adsorption patterns

Specific interaction between protein and surface

Presence of other proteins

Understanding the protein-surface interaction

Design of biomaterials and devices

# Acknowledgement

Xiaolin Hou

*Radiation Research Division, Risø DTU*

Lene Hubert, Lotte Nielsen & Sokol Ndoni

*DTU Nanotech, DTU*

CBIO group at DTU Nanotech

*Group leader Thomas Andresen*

Jørgen Garnæs

*DFM – Danish Fundamental Metrology*

Nils Berg Madsen

*Novo Nordisk A/S*

Hanne Everland

*Coloplast A/S*

Lydia Dahl Clausen

*Radiometer Medical Aps*