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MECHANIZMY ADSORPCJI BIAŁEK FAKTY I MITY

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**Seminarium Mechaniki Płynów IPTT PAN,
Warszawa 2011**

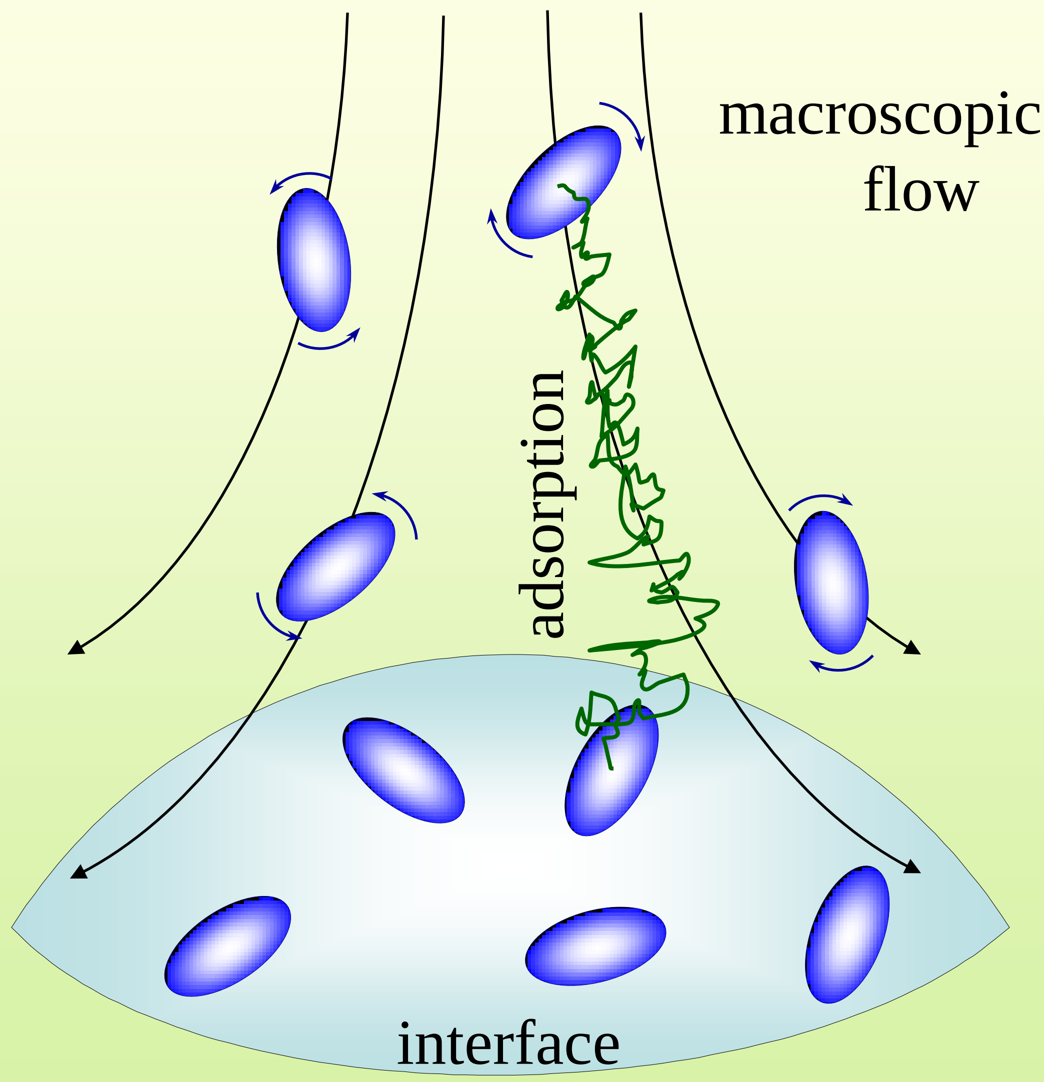
THE PROBLEM

Particles (solutes):

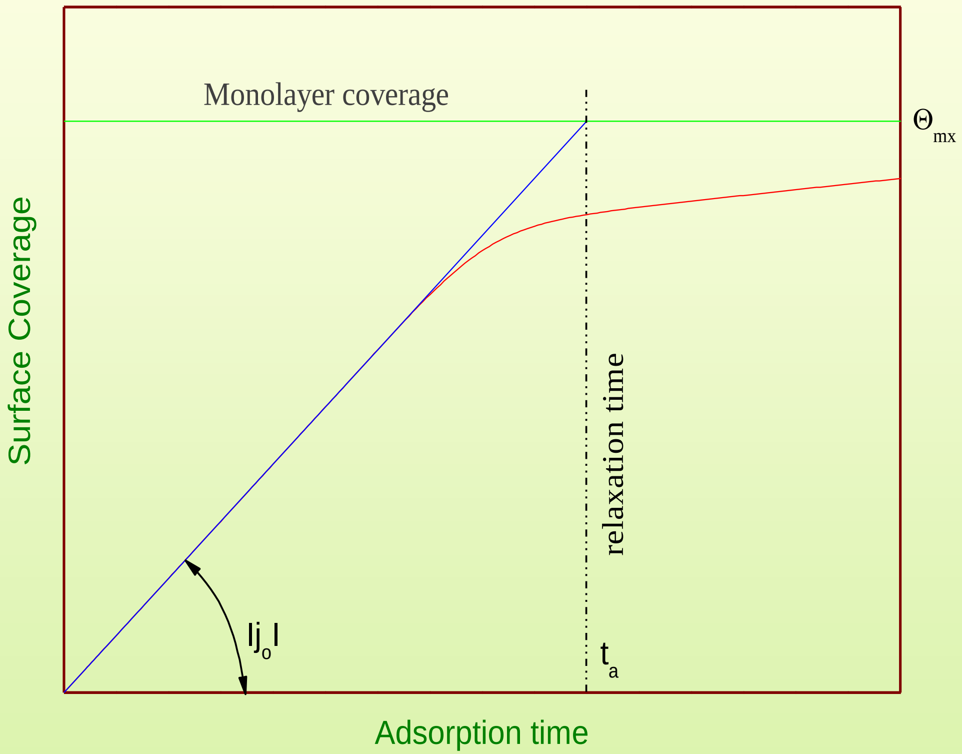
- Protein, DNA, viruses, cells
- Polyelectrolytes
- Colloids
- Surfactants

Significance/Processes:

- biosensors
- separation of DNA, proteins, viruses, cells
- immunological assays
- affinity chromatography



PREDICTING PARTICLE ATTACHEMENT KINETICS (RATE AND MONOLAYER COVERAGE)



Particle flux:

$$j_o = - D\, n_b / R$$

D – diffusion coefficient

n_b – concentration in the bulk

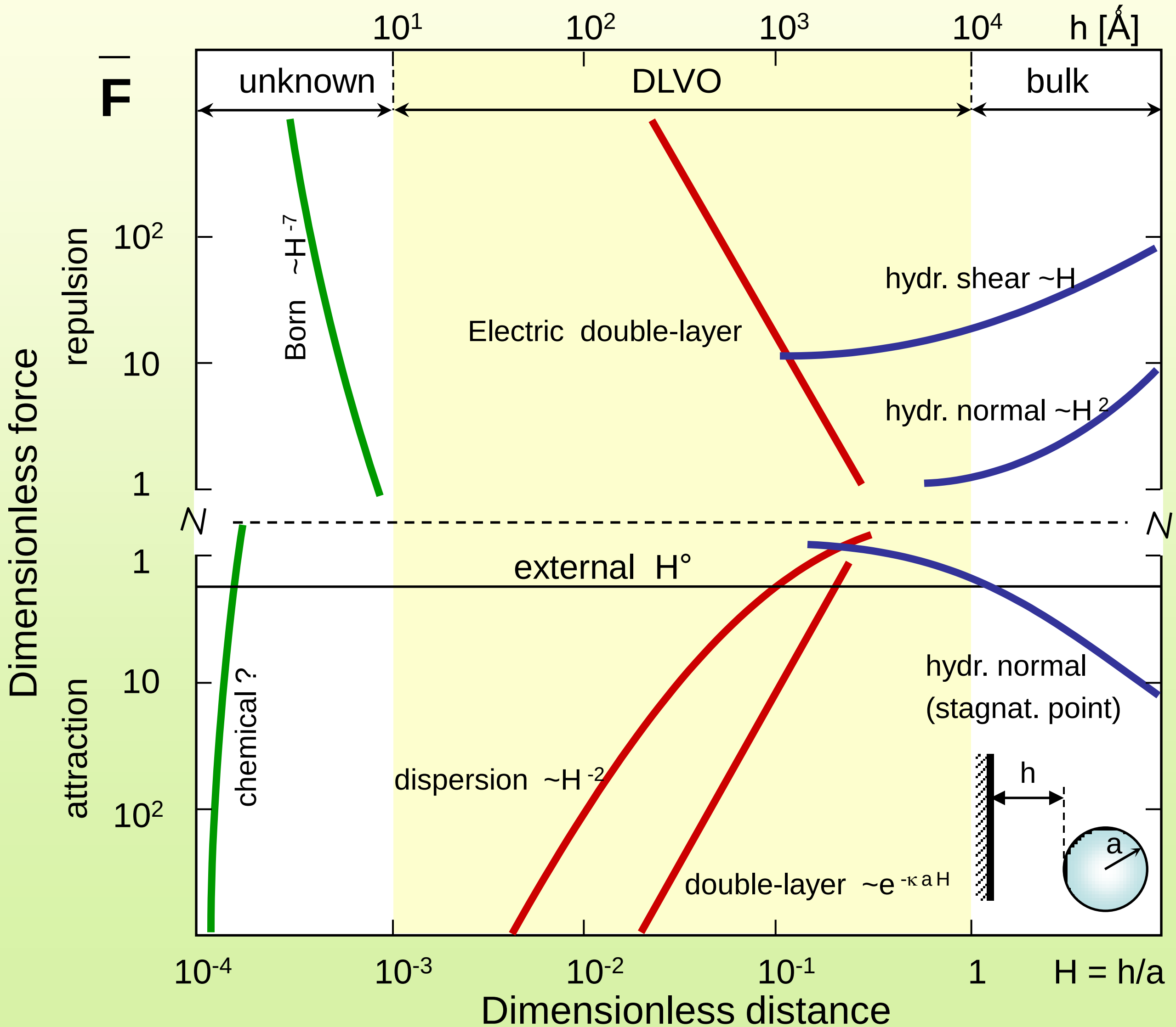
$$R = R_{bulk} + R_{surf}\,(\Theta_{mx})$$

R_{bulk} – bulk transport resistance.

R_{surf} - surface resistance

Θ_{mx} – from simulations

INTERACTIONS AFFECTING PARTICLE (PROTEIN) ATTACHEMENT



QUESTION: IS THIS VALID FOR PROTEINS ?

IN VIEW OF ANOMALOUS PHENOMENA SUCH AS:

APPARENT ATTRACTION OF LIKE CHARGES?

TRIPLE REPULSION = ATTRACTION ?

IS THERE SOMETHING WRONG

WITH COULOMB LAW ?

THESIS: BASIC PHYSICS WORKS IN PROTEIN BUSINESS

APPARENT DEVIATIONS ARE DUE TO WRONG INTERPRETATION

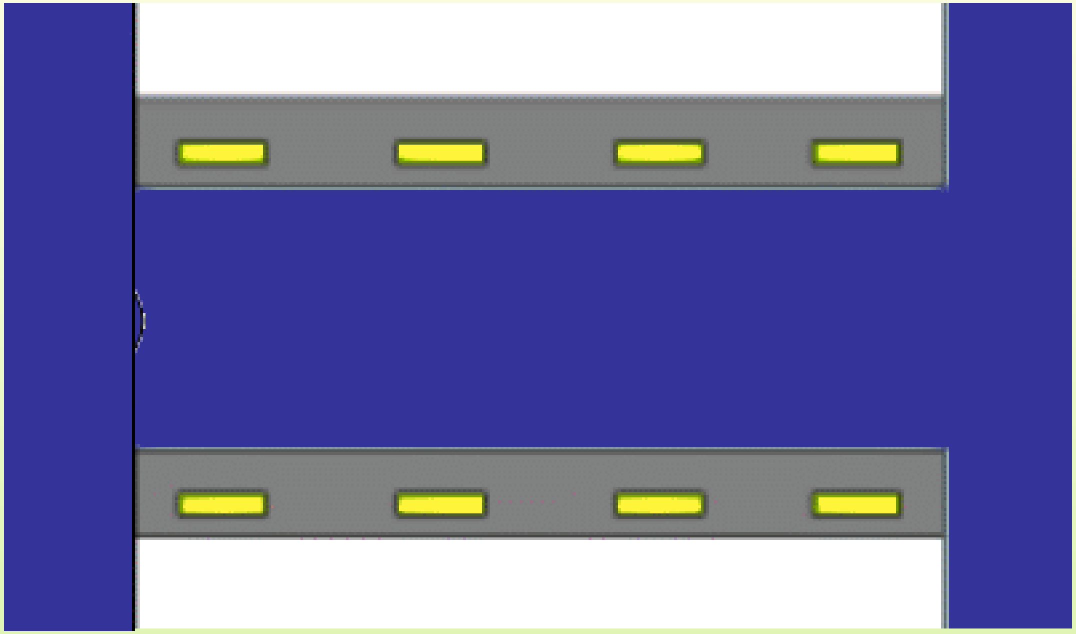
(LACK OF REFERENCE EXPERIMENTAL RESULTS FOR MODEL SYSTEMS)

MEANS OF PROVING THIS:

- ***THEORETICAL CALCULATIONS AND SIMULATIONS***

- ***EXPERIMENTAL MEASUREMENTS USING in situ ELECTROKINETIC METHODS (Streaming potential)***

ORIGIN OF STREAMING POTENTIAL



Marian von SMOLUCHOWSKI 1872 –1917

- *Statistical physics- fluctuation theory*
- *Brownian motion and diffusion*
- *Colloid statistics - coagulation theory*
- *Streaming potential-electrokinetic phenomena*

SMOLUCHOWSKI'S EXPRESSIONS FOR ELECTROPHORETIC MOBILITY AND STREAMING POTENTIAL

D'autre part, la pression électroosmotique est, d'après les expériences de Wiedemann[1]), proportionelle à $\frac{I\sigma d}{\Omega}$, [où $d =$ épaisseur, $\Omega =$ surface du diaphragme], ce qui résulte aussi de la formule (16), en considérant que la constante C (définie plus haut) doit être proportionnelle, pour des diaphragmes à structure homogène, à $\frac{d}{\Omega}$.

§ 8. Mais il y a un troisième phénomène, outre ceux-ci, qui est embrassé par notre théorie: celui du transport électrique de petites particules suspendues dans un liquide, phénomène étudié surtout par Quincke[2]).

Imaginons une sphère isolante, plongée dans un liquide, sous l'influence d'un champ électrique homogène. En acceptant la direction de celui-ci comme axe d'un système de coordonnées polaires, nous aurons l'expression suivante du potentiel extérieur Φ:

$$\Phi = -cx\left(1 + \frac{a^3}{2r^3}\right) = -c\cos\theta\left[r + \frac{a^3}{2r^2}\right] \tag{18}$$

Donc, si la sphère était fixe, elle produirait d'après (13) un mouvement potentiel du liquide environnant dans la direction des lignes de force; la vitesse à grande distance aurait la valeur constante

$$u = \frac{\varphi_i - \varphi_a}{4\pi\mu} c. \tag{19}$$

Mais si la sphère est mobile, dans un liquide sans mouvement, il est évident qu'elle sera poussée avec cette vitesse dans la direction de la cathode vers l'anode. Pour donner une idée de la valeur de cette vitesse, qui est indépendante des dimensions de la sphère, supposons:

$$\varphi_i - \varphi_a = 2 \text{ Volt}, \quad \mu = 0{\cdot}018, \quad c = 1\,\frac{\text{Volt}}{cm};$$

ce qui donne

$$u = 0{\cdot}000093\,\frac{cm}{sec}.$$

C'est justement l'ordre des vitesses des ions dans l'électrolyse, fait curieux qui pourrait suggérer des spéculations d'ailleurs hasardées.

[1]) Voir aussi Tereschin, Wied. Ann. 32, p. 333 (1887).
[2]) Wiedem. Ann. 113, p. 516 (1861).

$$4\pi\int_0^\delta v_\zeta \frac{\partial\varepsilon}{\partial\zeta}\,d\zeta = -\int_0^\delta v_\zeta \frac{\partial^3\varphi}{\partial\zeta^3}\,d\zeta = \int_0^\delta \frac{\partial^2 v_\zeta}{\partial\zeta^2}\frac{\partial\varphi}{\partial\zeta}\,d\zeta. \tag{24}$$

Considérons maintenant l'équation mécanique formée d'après (5), mais avec Φ égal à zéro:

$$\frac{\partial P}{\partial\zeta} = \mu\Delta^2 v_\zeta \tag{25}$$

où P satisfait à l'équation $\Delta^2 P = 0$ et, à la surface de la couche, se transforme d'une façon continue en la pression hydraulique ordinaire p. Par conséquent, on peut considérer P comme constant dans l'étendue de la couche δ; d'autre part, en négligeant les termes plus petits, on aura:

$$\Delta^2 v_\zeta = \frac{\partial^2 v_\zeta}{\partial\zeta^2}.$$

Donc, la valeur de l'intégrale (24) sera:

$$\frac{1}{\mu}\frac{\partial P}{\partial\zeta}\int_0^\delta \frac{\partial\varphi}{\partial\zeta}\,d\zeta = \frac{\varphi_i - \varphi_a}{\mu}\frac{\partial P}{\partial\zeta}.$$

Nous aurons:

$$V = \frac{\sigma}{4\pi}\frac{\varphi_i - \varphi_a}{4\pi\mu}\int\int\frac{\partial P}{\partial\zeta}\frac{dS}{r} \tag{26}$$

et par suite de $\Delta^2 P = 0$:

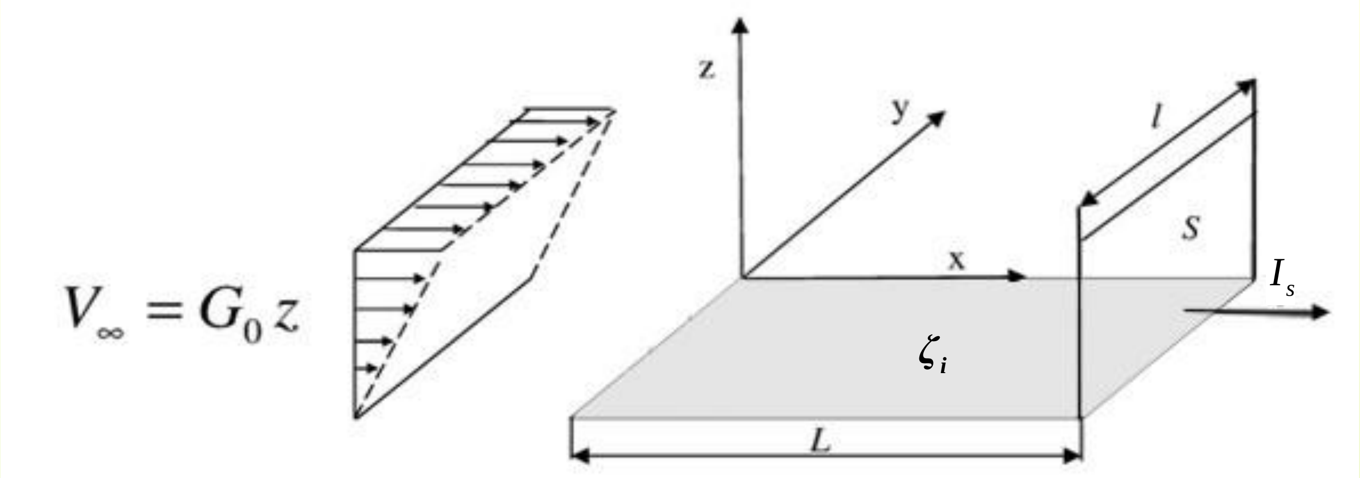
$$V = \sigma\,\frac{\varphi_i - \varphi_a}{4\pi\mu}\,P + \text{const.} \tag{27}$$

Donc, la différence de potentiel en deux points de l'intérieur du liquide sera:

$$V_2 - V_1 = \frac{\varphi_i - \varphi_a}{4\pi\mu}\,\sigma(p_2 - p_1). \tag{28}$$

§ 10. Cette formule paraît identique avec le résultat analogue de Helmholtz, à cette différence près qu'elle ne s'applique pas seulement aux tubes capillaires, mais à des vaisseaux quelconques, où le liquide est animé d'un mouvement lent. En effet, les mesures de Quincke, où la pression et les dimensions des diaphragmes variaient, ont démontré la proportionnalité de la force électromotrice à la pression active et l'indépendance des dimensions du diaphragme. La relation avec σ est indiquée par l'observation

STREAMING POTENTIAL OF BARE SURFACES



Smoluchowski's formula for homogeneous surfaces (simple shear flows)
(Bull. Acad. Sciences de Cracovie 1903, pp 182-199)

$$I_s = -G_o \varepsilon l \zeta_i$$

ε – permittivity, ζ_i – zeta potential of interfaces

$$E_s = -I_s R_e$$

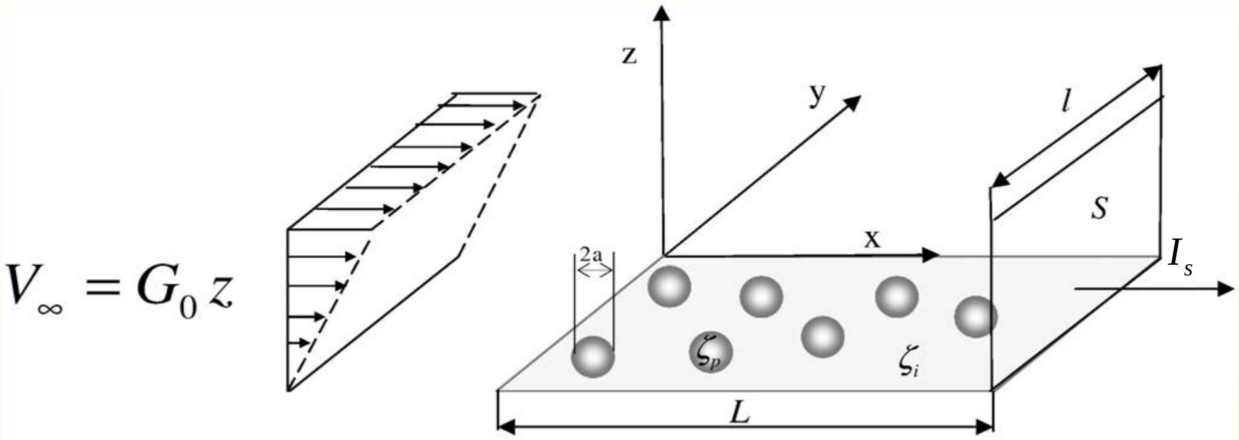
R_e – electric resistance

For channel flows:

$$E_s = \varepsilon \frac{\Delta P}{\eta} \rho \zeta_i = C_o \zeta_i$$

$\triangle P$ – hydr. pressure difference
η – dynamic viscosity
ρ – specific electric resistance

STREAMING POTENTIAL OF COVERED SURFACES



Particle coverage: $\Theta = \pi a^2 N$ (N – surface concentration)

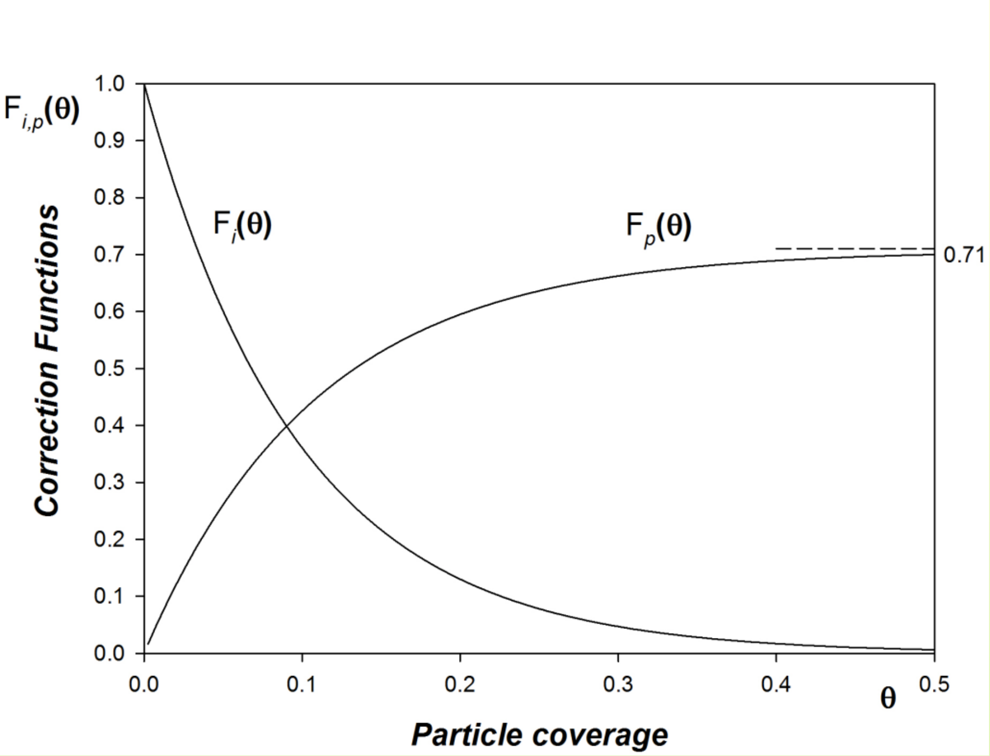
Formula for particle (protein) covered surfaces (arbitrary shearing flow)

$$E_s / C_o = F_i(\Theta)\zeta_i + F_p(\Theta)\zeta_p$$

F_i **= correction function describing flow damping**

F_p **= correction function describing particle induced curent**

CORRECTION FUNCTIONS FOR PARTICLE COVERED SURFACES



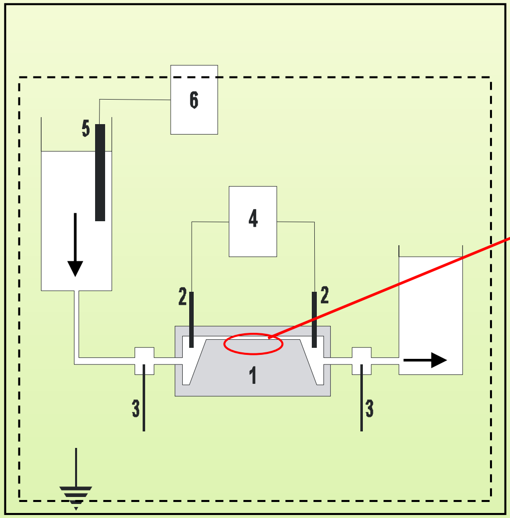
$$F_i(\Theta) = e^{-10.2\Theta}$$

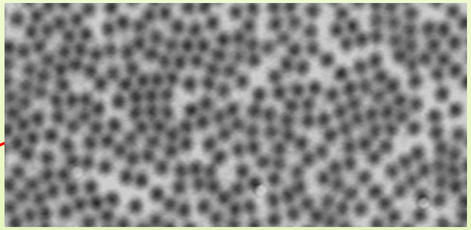
(Z. Adamczyk et al. *Bull. Pol. Ac. Chem*. 1999, *47*, 239- 258)

$$F_p(\Theta) = \frac{6.51 - 2.38\Theta}{1 + 5.46\Theta}\Theta \square \frac{1}{\sqrt{2}}\left(\frac{1 - e^{-\sqrt{2}\,6.51\Theta}}{\Theta}\right)$$

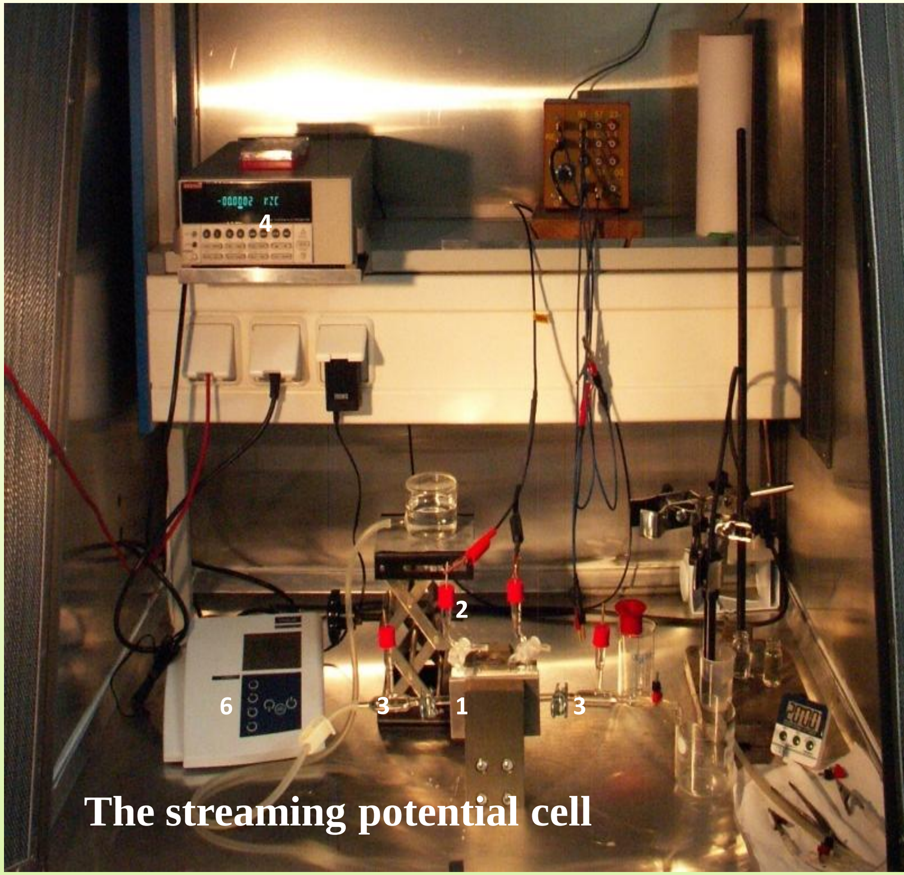
(K. Sadlej, E. Wajnryb, J. Bławdziewicz, M.L. Ekiel-Jeżewska, Z. Adamczyk, *J. Chem. Phys*. 2009, *130*, 144706)

STREAMING POTENTIAL MEASUREMENTS





Interface with particles



1 – parallel-plate channel
2 - Ag/AgCl electrodes for streaming potential measurements
3 - electrodes for cell resistance determination
4 - Keithley electrometer
5 - conductivity cell
6 - conductometer

-*the parallel plate channel of dimensions*

0.027 x 0.29 x 4 cm

- *in situ method*

OTHER MEASUREMENTS

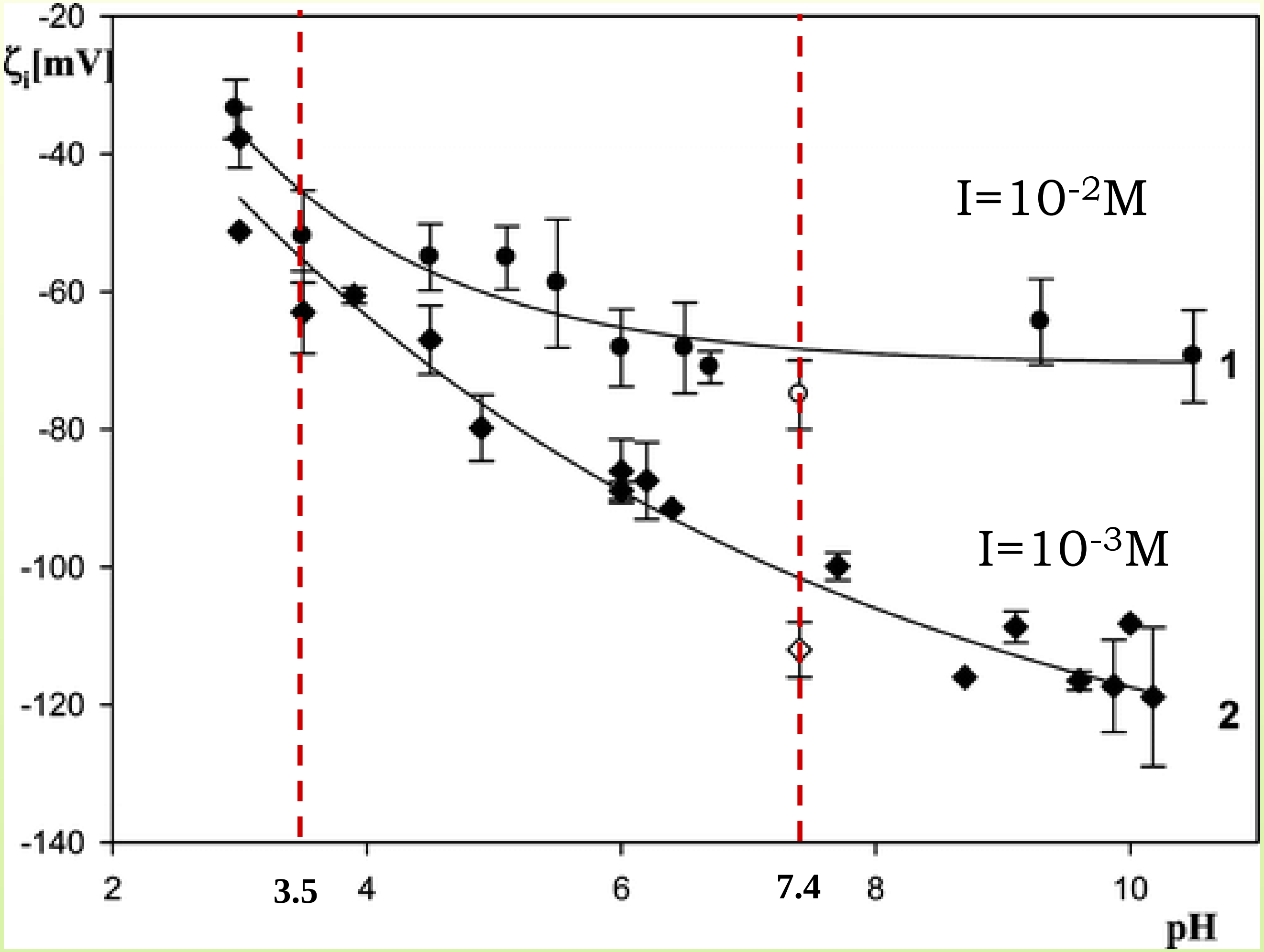
Dynamic light scattering

AFM - INTEGRA

Dynamic viscosity measurements

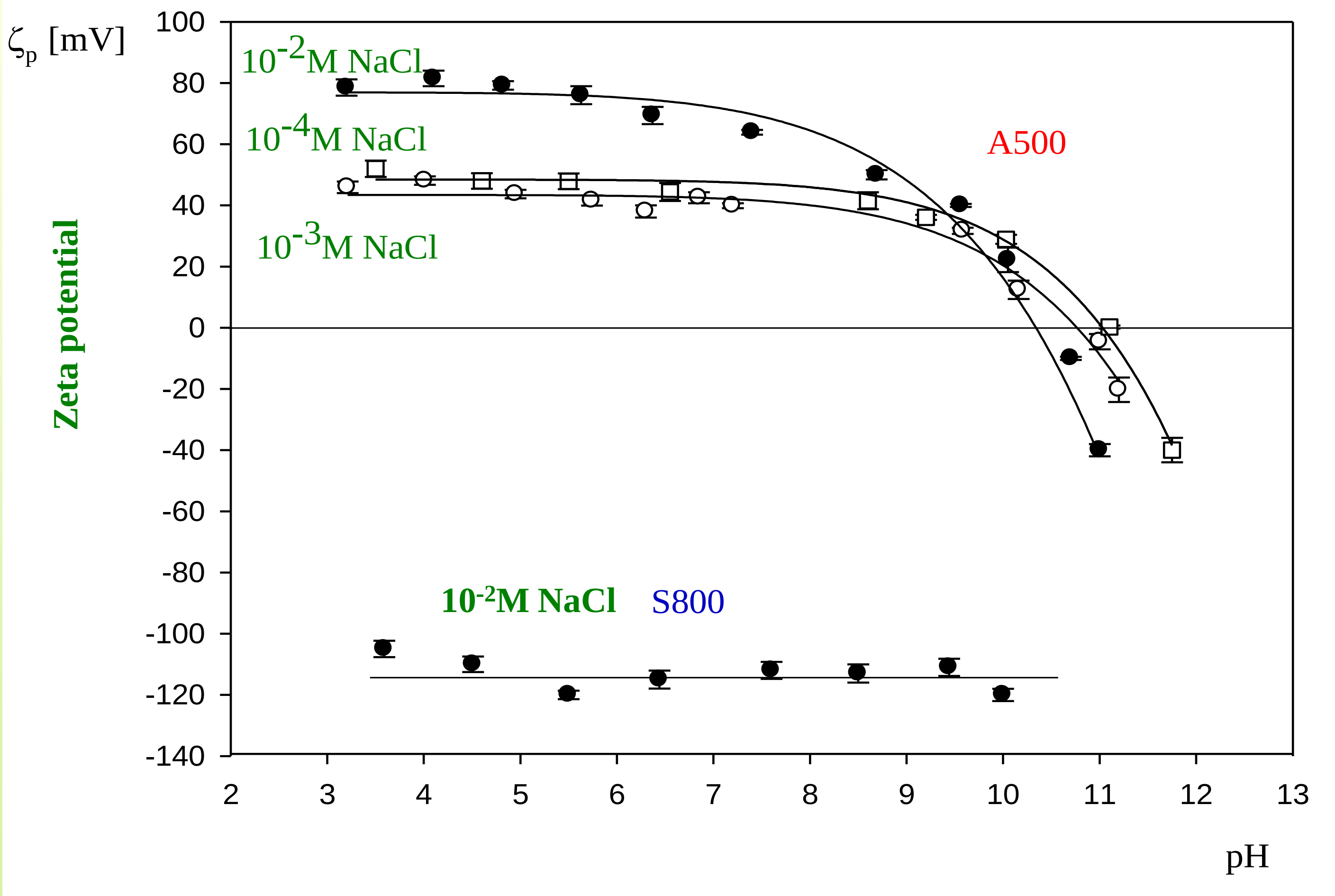
SUBSTRATE SURFACE CHARACTERISTICS

Zeta potential of mica vs. pH



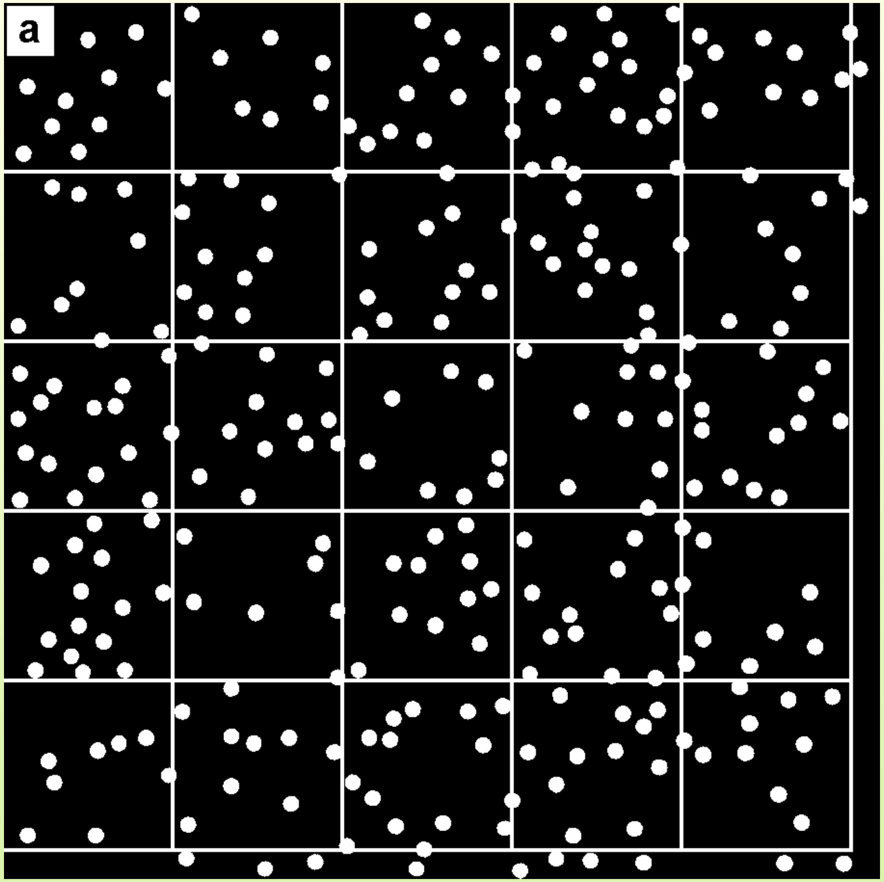
M. Wasilewska, Z. Adamczyk, ***Langmuir*****, 2011,** *27*, 689-696

CHARACTERISTICS OF MODEL COLLOID PARTICLES (POSITIVE - A500 AND NEGATIVE S800 LATEX)

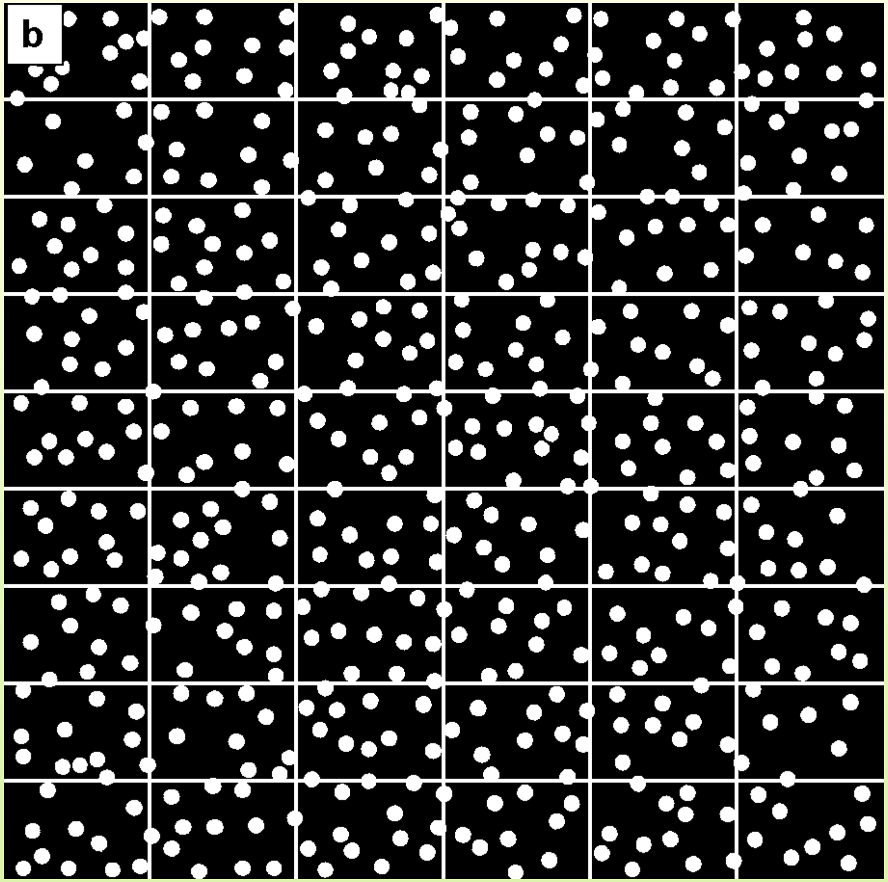


COLLOID PARTICLES AT INTERFACES (MICA)

$\theta = 0.1$



$\theta = 0.3$

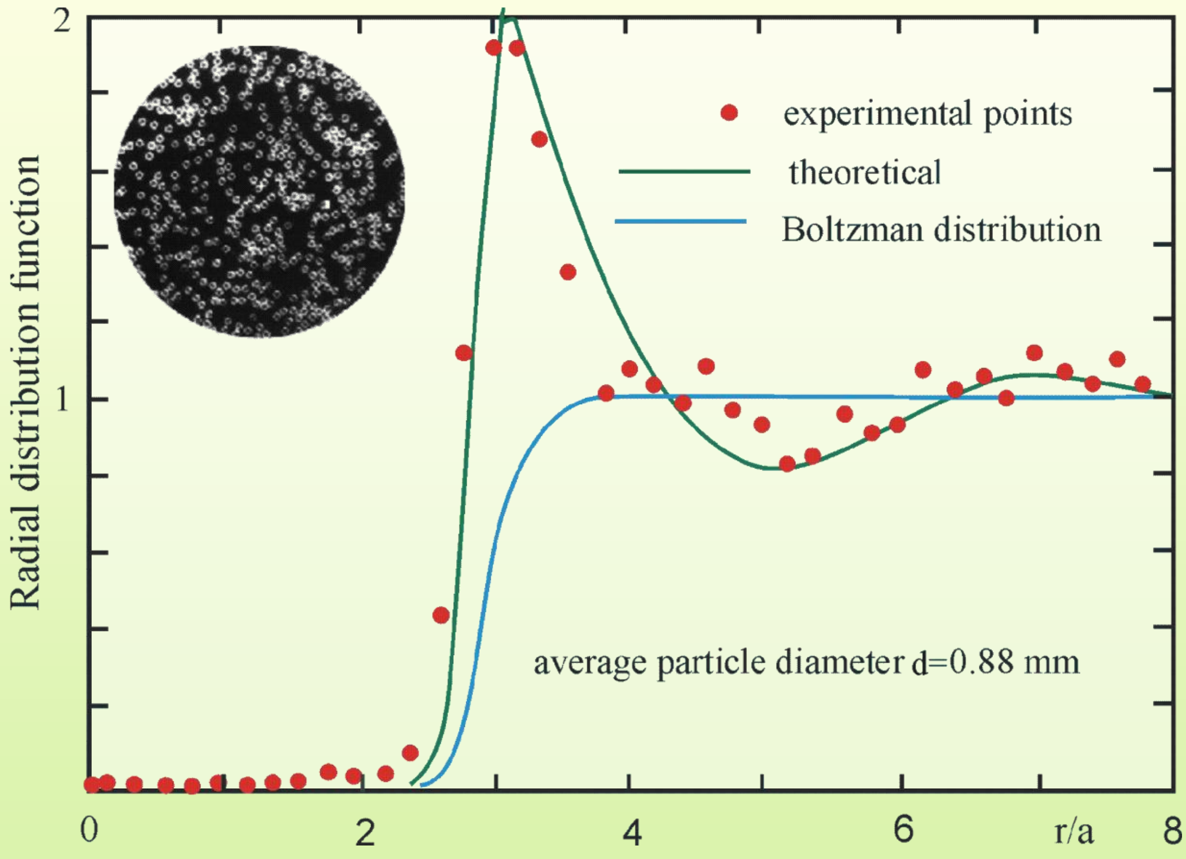


Irreversibly adsorbed particles- Fluctuations

FLUCTUATIONS IN THE SKY

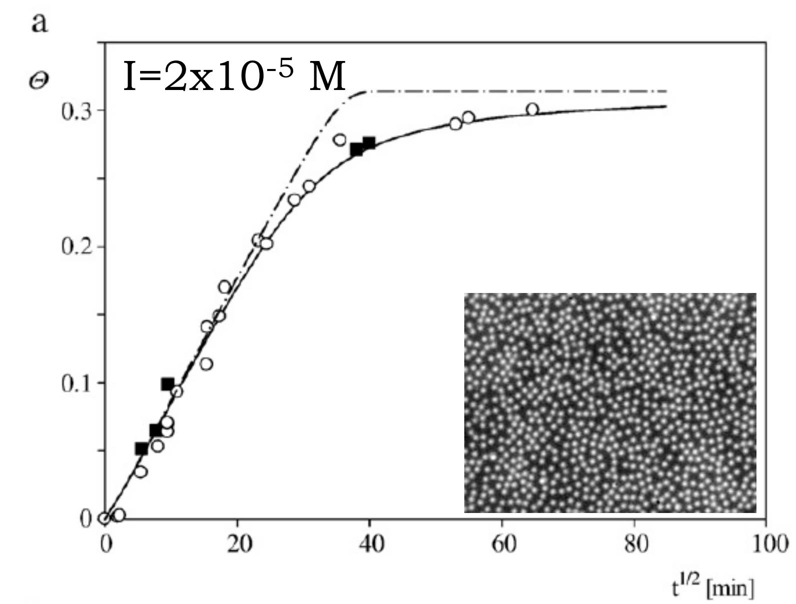
V. van Gogh "The Starry Night"

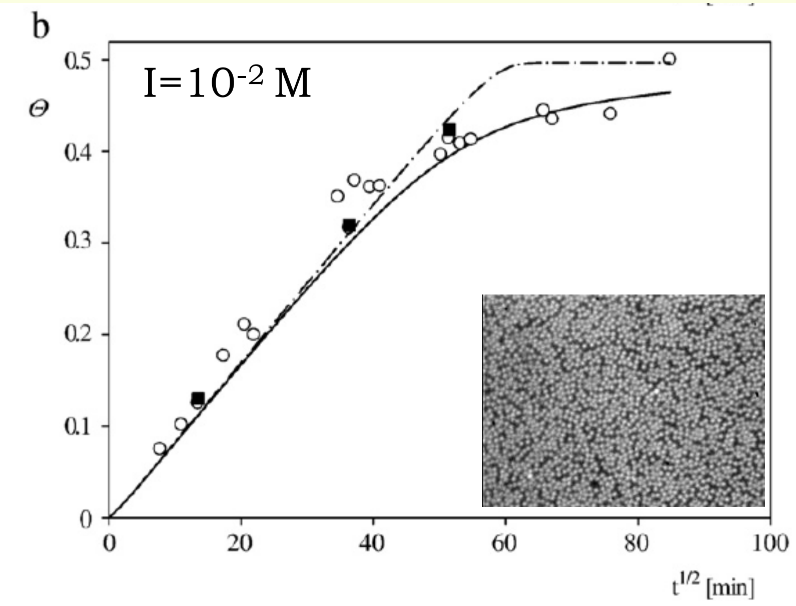
STRUCTURE OF COLLOID PARTICLE MONOLAYERS
(LATEX ON MICA)



Pair correlation function for latex particles adsorbed on mica
(under the quasi-liquid state)

KINETICS OF COLLOID PARTICLE DEPOSITION IN MODEL SYSTEMS (latex /mica)



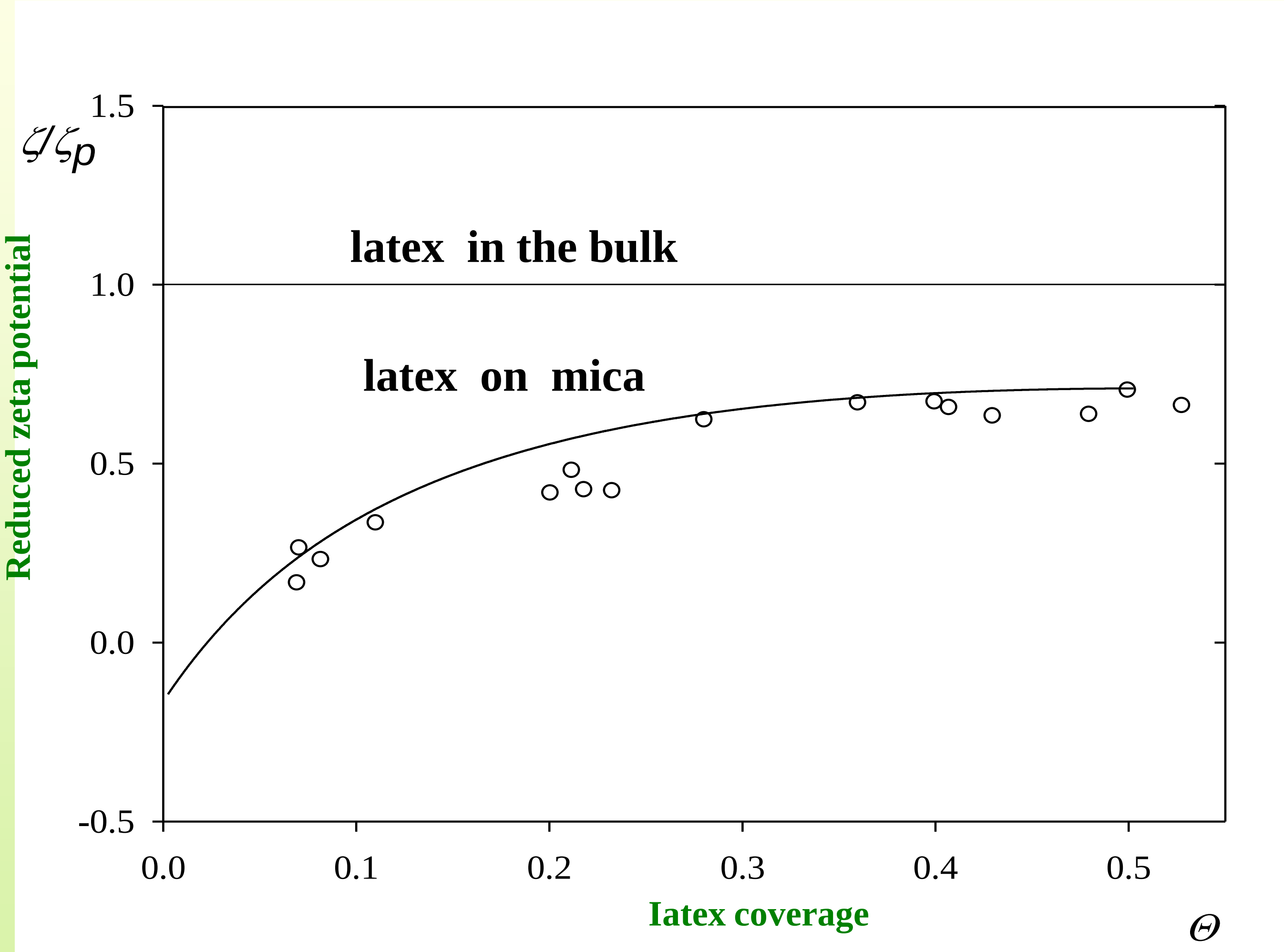


Diffusion cell, Points - AFM and optical microscopy the solid lines denote the RSA model, the dashed line-Langmuir model

Z. Adamczyk et al., ***Advances CIS*****, 2010,** *153*, 1-29

REFERENCE DATA FOR COLLOIDAL PARTICLES

ZETA POTENTIAL OF MICA COVERED BY POSITIVE LATEX

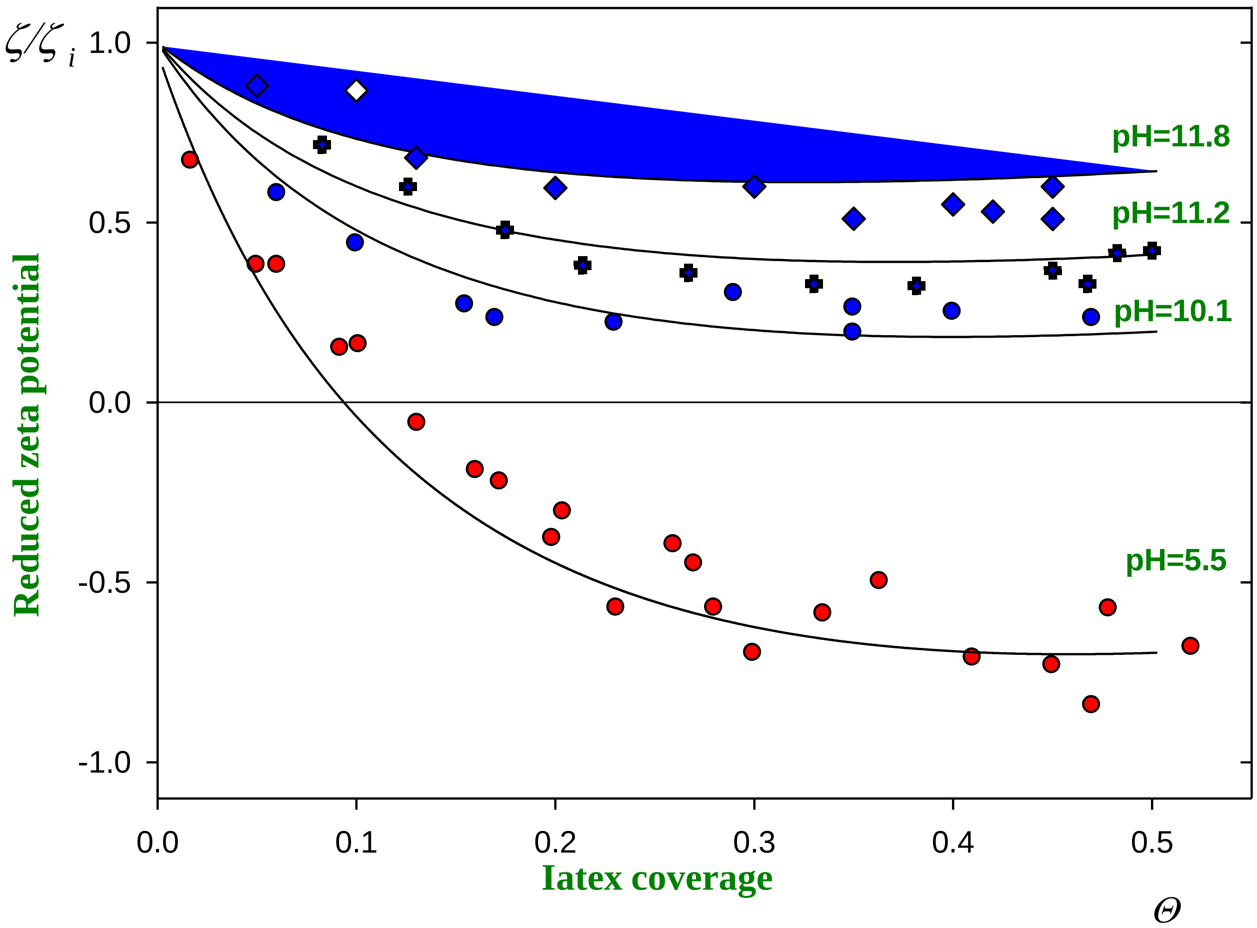


Points- experimental results: Z. Adamczyk et al., *Langmuir* , 2010, *26*, 9368

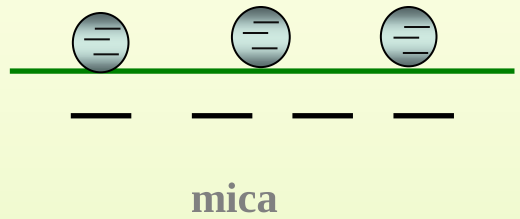
lines - exact theoretical results K. Sadlej et al, *J. Chem. Phys.* 2009, *130*, 144706–144711

REFERENCE DATA FOR COLLOIDAL PARTICLES

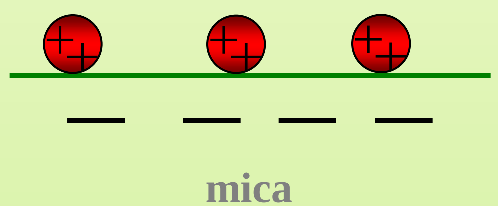
ZETA POTENTIAL OF MICA COVERED BY NEGATIVE AND POSITIVE LATEX



Latex A500
(negative for pH > 10)

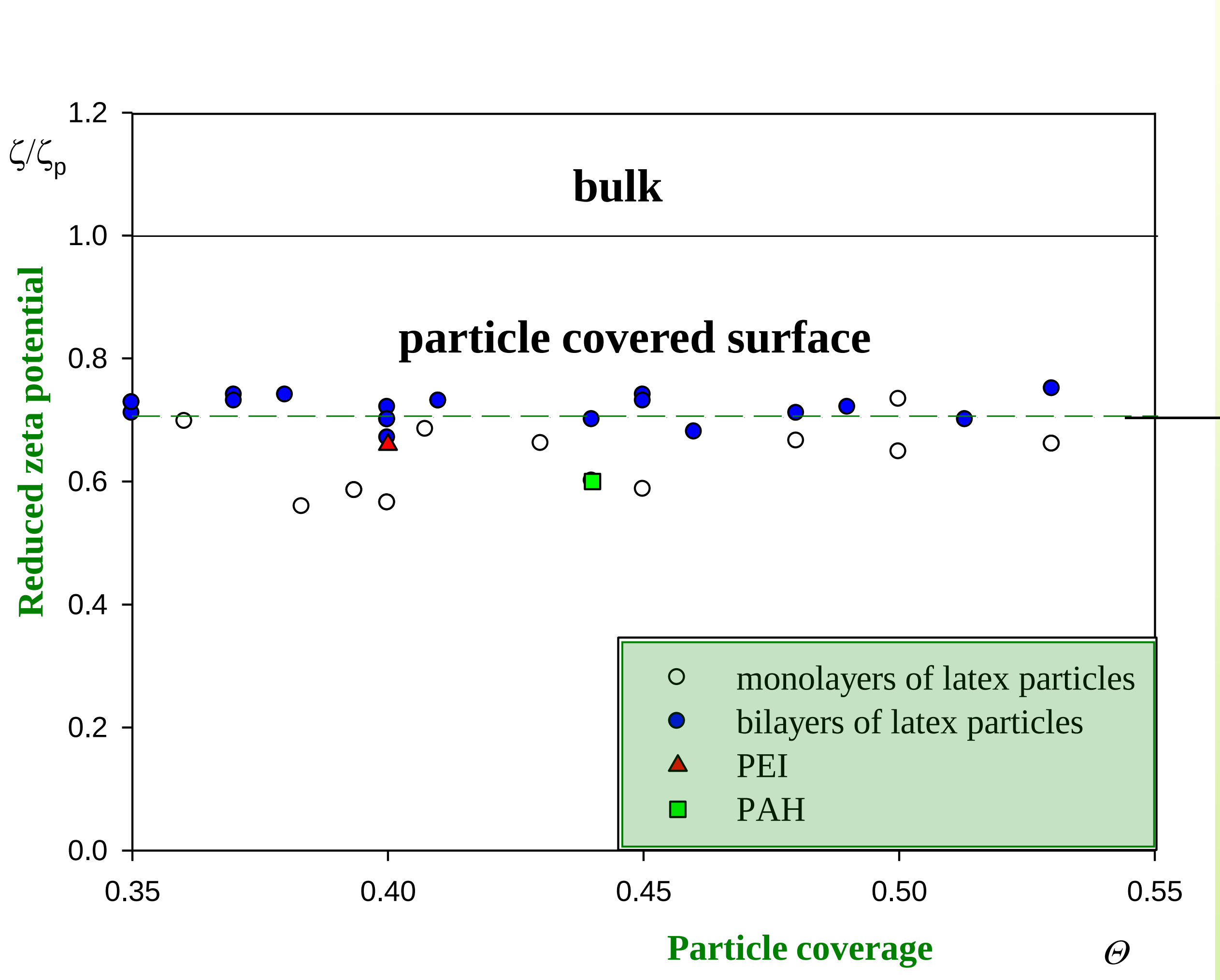


Latex A500
(positive for pH < 10)



Points- experimental results: Z. Adamczyk et al., *Langmuir* , 2010, *26*, 9368
lines - exact theoretical results K. Sadlej et al, *J. Chem. Phys.* 2009, *130*, 144706–144711

ZETA POTENTIAL OF MICA COVERED BY POSITIVE LATEX PARTICLES



Theoretical

$$\boxed{\frac{1}{\sqrt{2}}\zeta_{\mathrm{bulk}}}$$

Points- experimental results: Z. Adamczyk et al., *Langmuir* , 2010, *26*, 9368

lines - exact theoretical results K. Sadlej et al, *J. Chem. Phys.* 2009, *130*, 144706–144711

BULK VS. SURFACE ZETA POTENTIAL OF MICA COVERED BY LATEX PARTICLES

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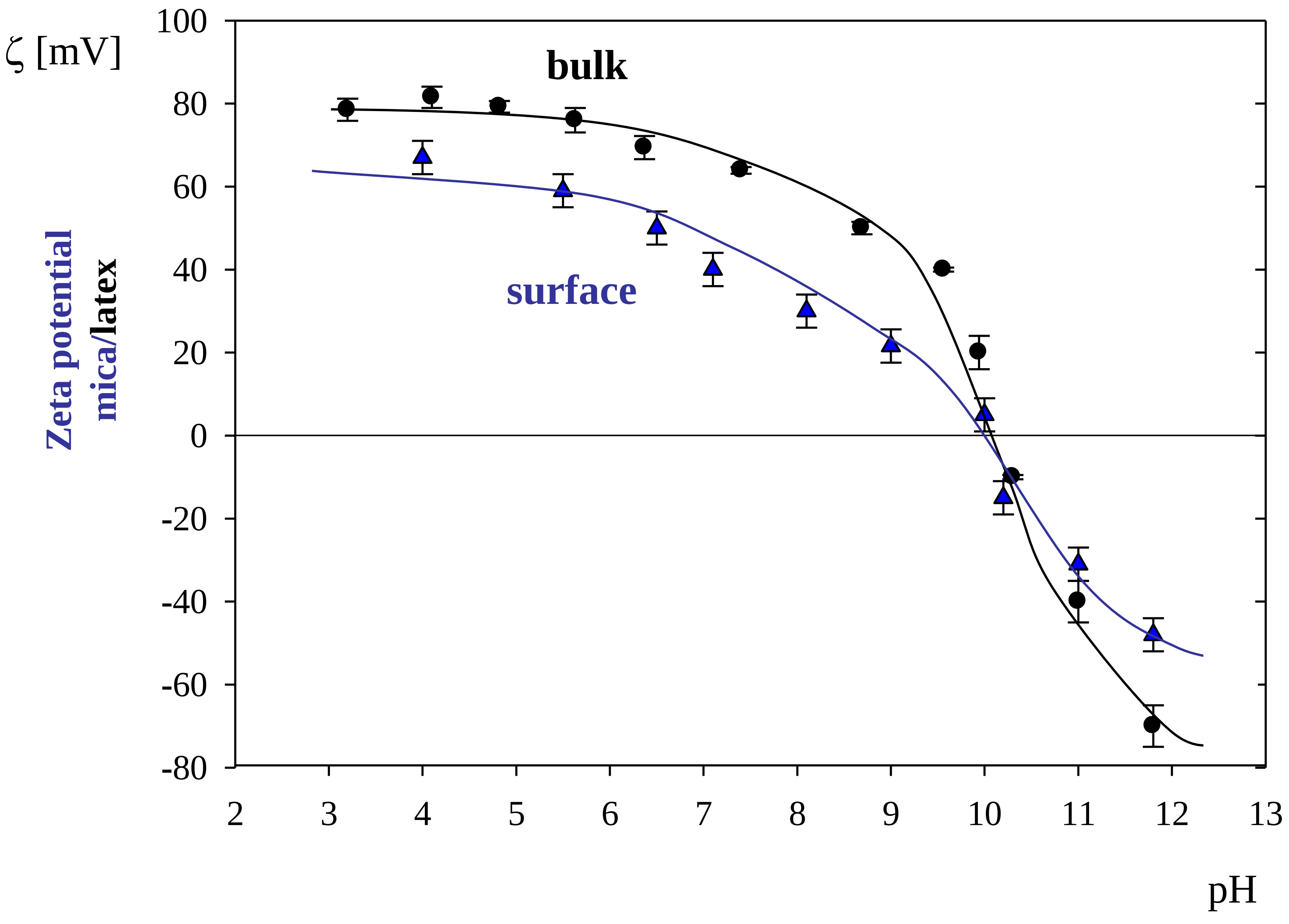
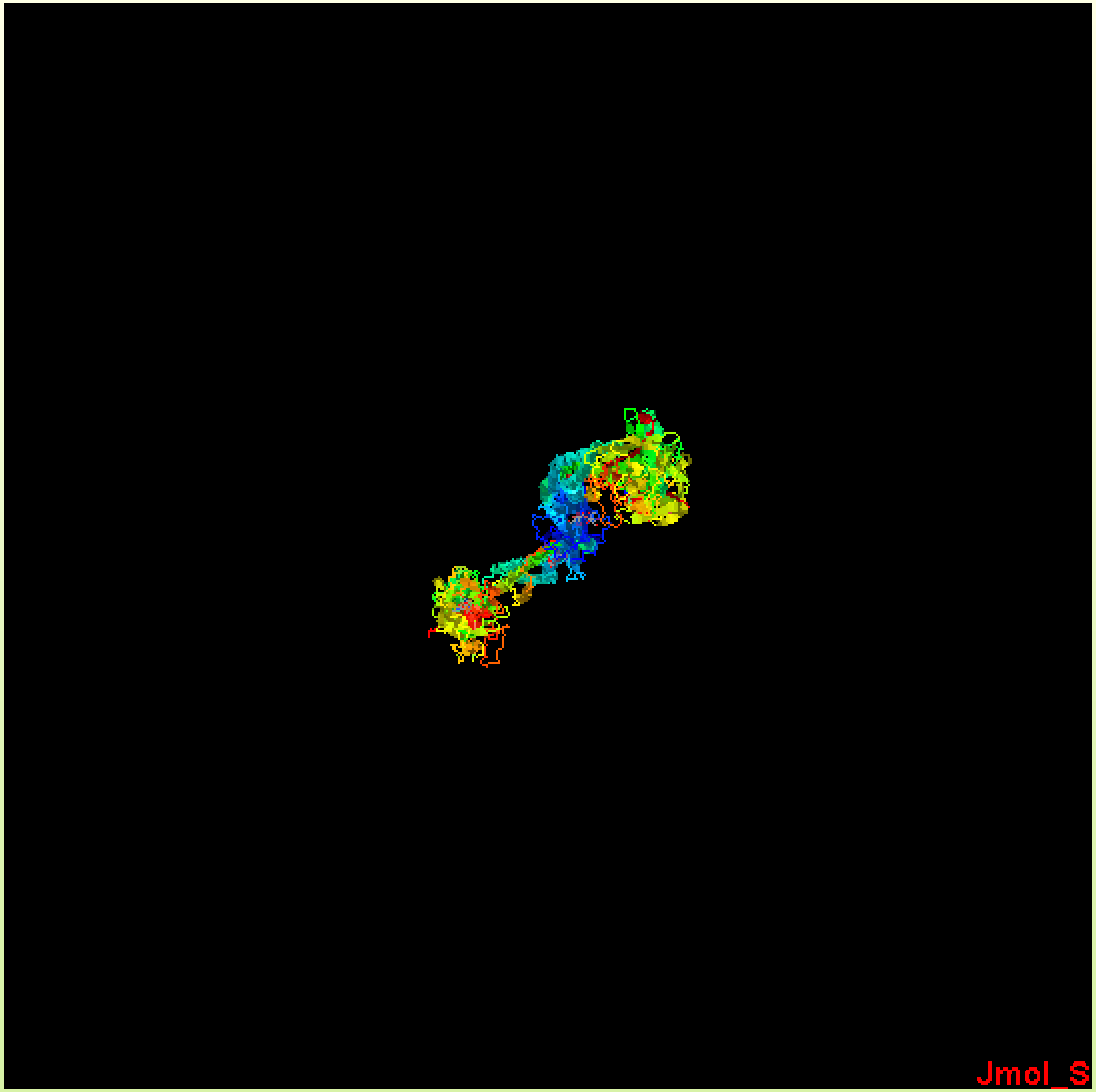


HOW ABOUT REAL LIFE?

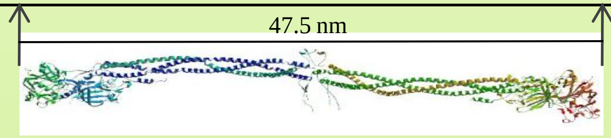
THE FIBRINOGEN STORY BEGINS

THE FIBRINOGEN MOLECULE



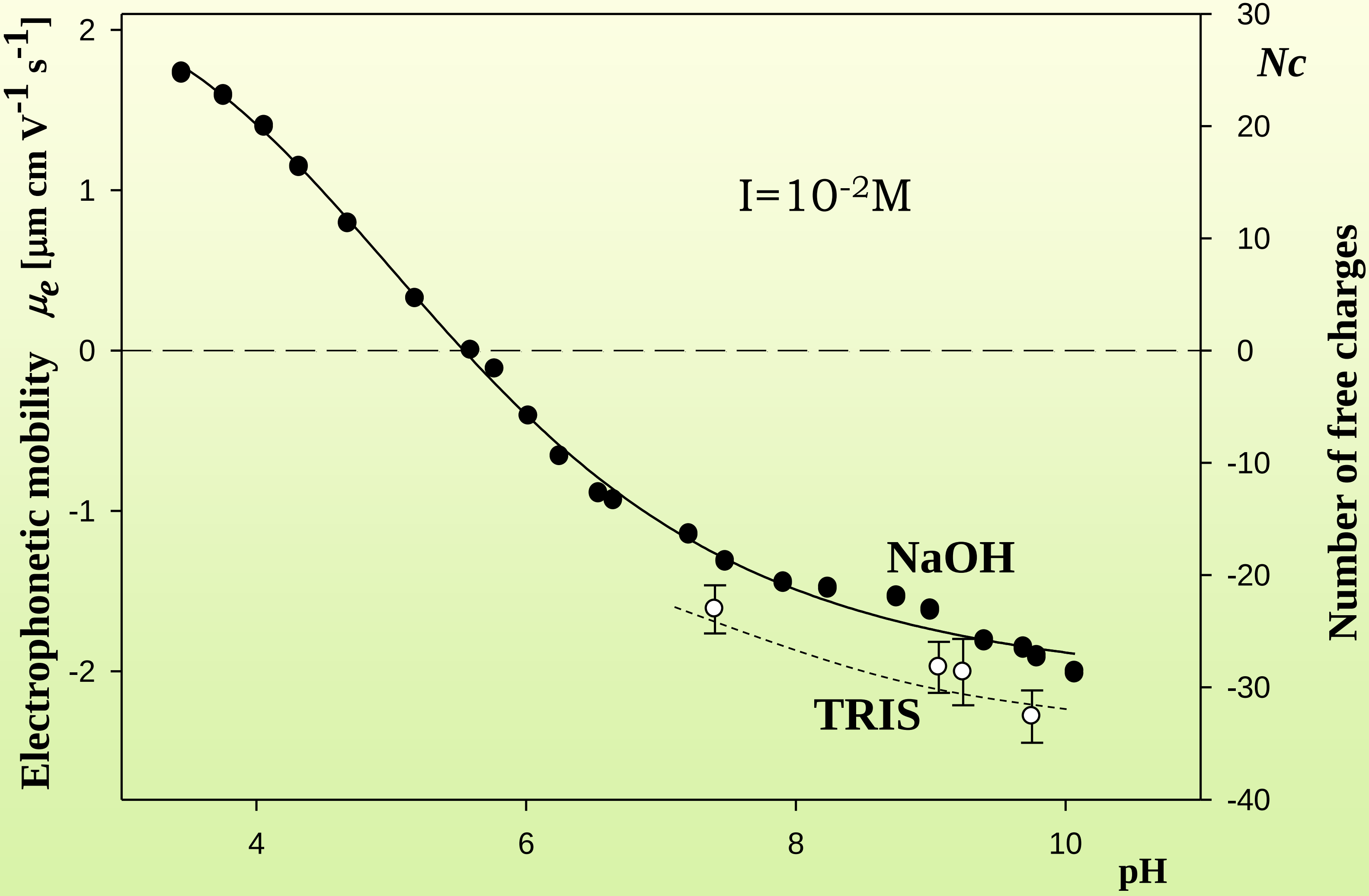
THE FIBRINOGEN STORY

1. Physicochemical characteristics

Molecular weight [Da]	340 000
Specific density [g·cm^{-3}]	1.38
Specific volume [nm^3] **Crystalline state**	372
Hydrated volume [nm^3]	440
Equivalent sphere radius [nm]	4.5
Diffusion coefficient cm^2 s^{-1}	2.1x10^{-7}
Hydrodynamic radius [nm]	12 (pH = 3.5, I = 10^{-2} M)
Molecular shape **(crystalline state)**	
Approximated shape **bead model A**	

THE FIBRINOGEN STORY

2. Electrophoretic mobility and free charge vs. pH



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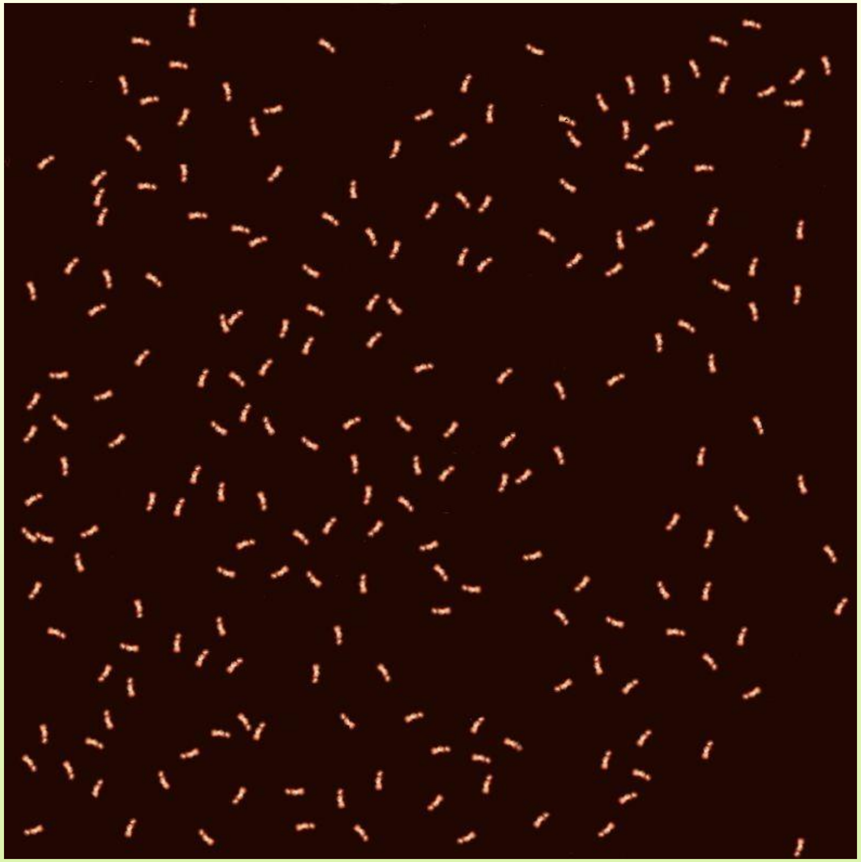
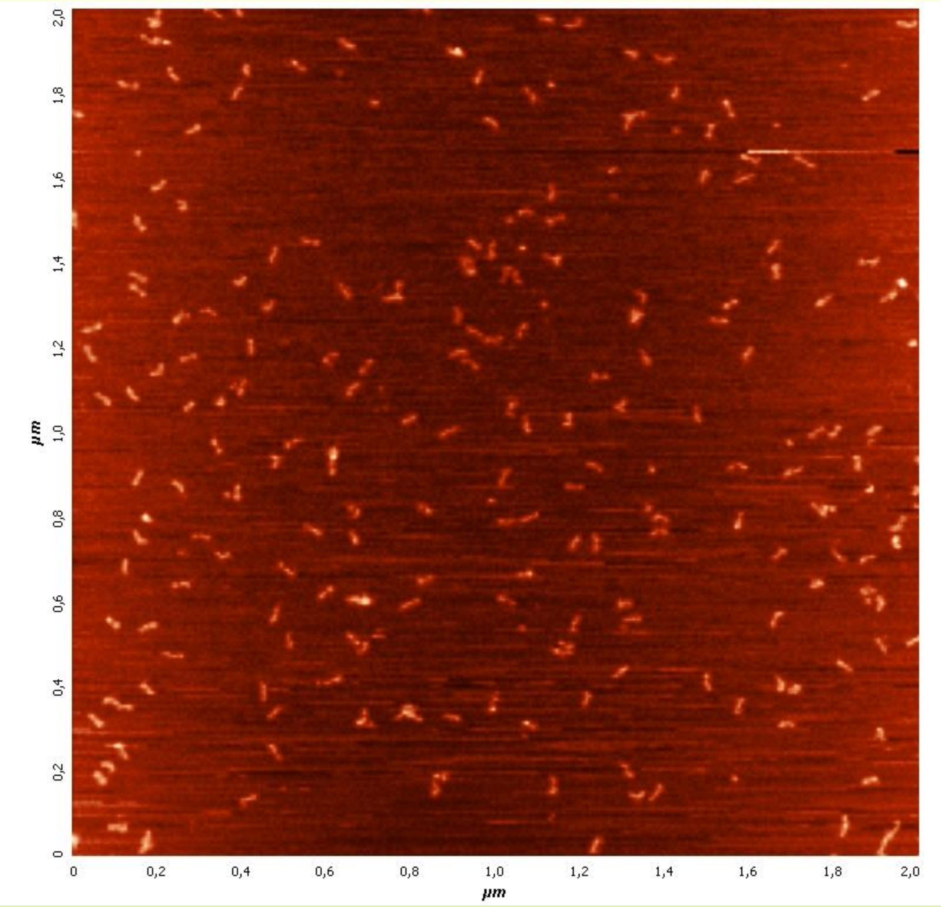
THE FIBRINOGEN STORY

3. Fibrinogen monolayers on mica



Simulations, RSA, model A

Z. Adamczyk et al., ***Langmuir*,**
2010, *26,* 11934



Experimental

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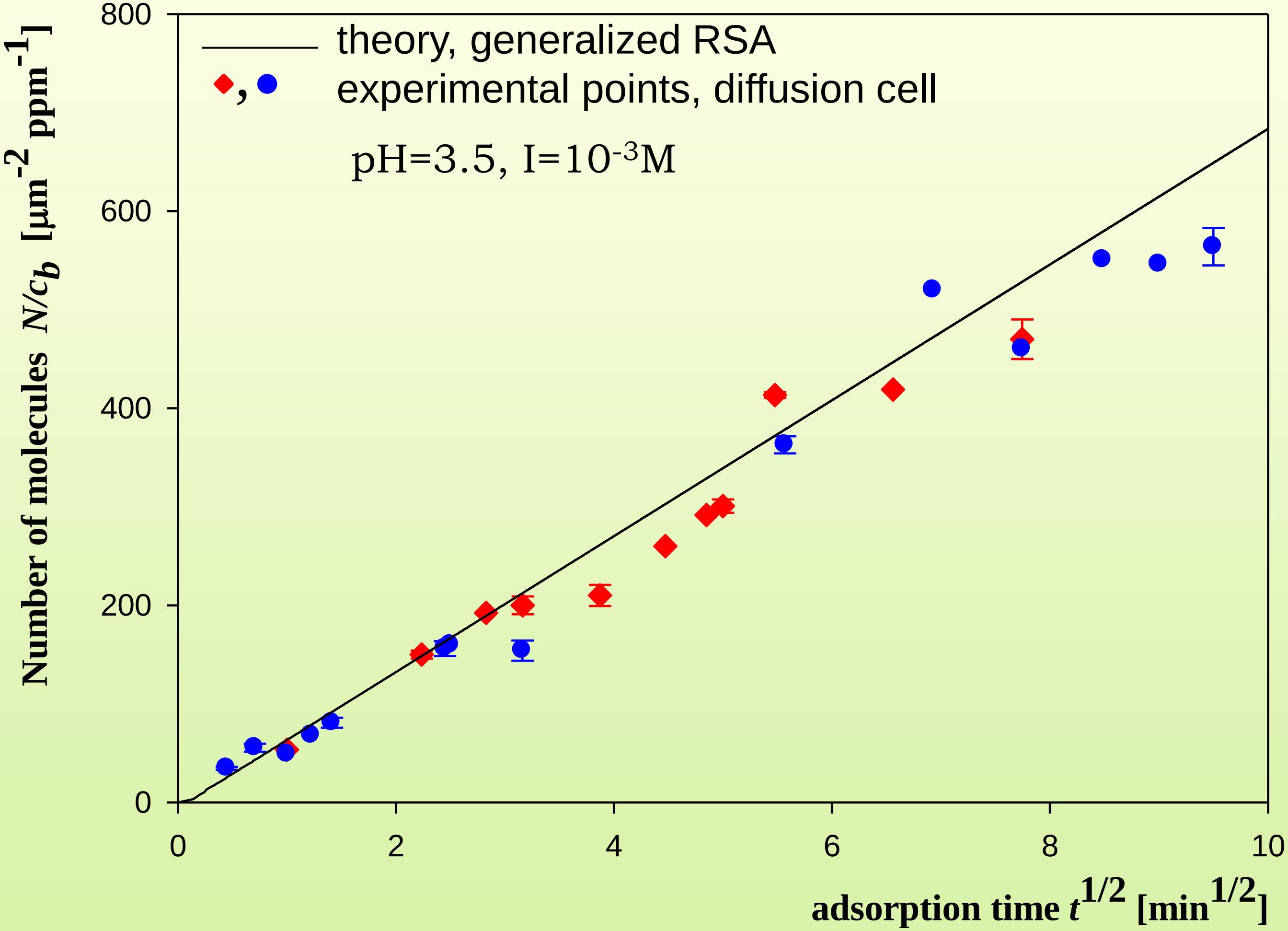
THE FIBRINOGEN STORY

4. Kinetics of fibrinogen adsorption on mica diffusion transport, AFM



Bulk transport controlled adsorption !

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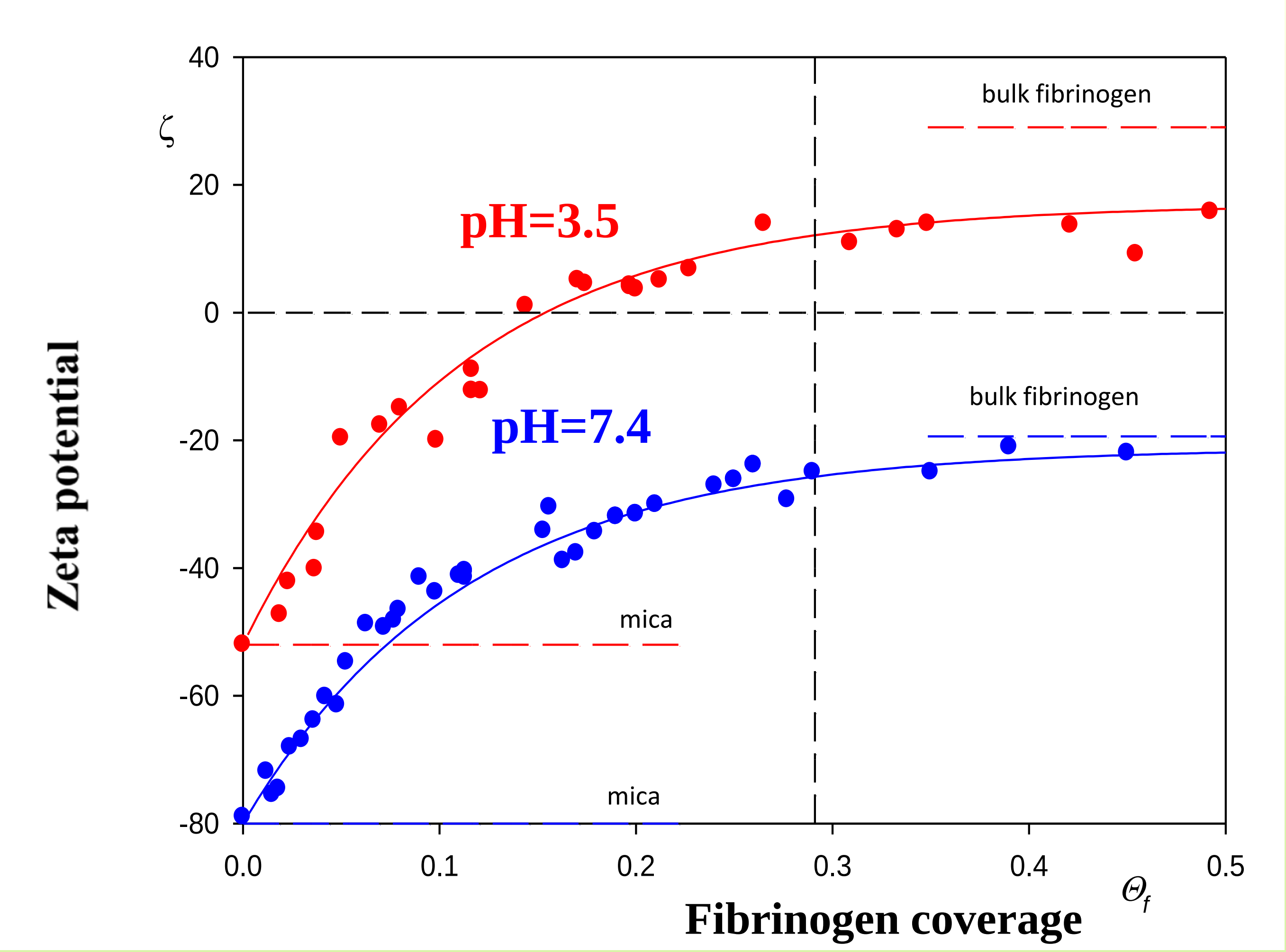
THE FIBRINOGEN STORY

5. Zeta potential changes of mica substrate upon fibrinogen adsorption



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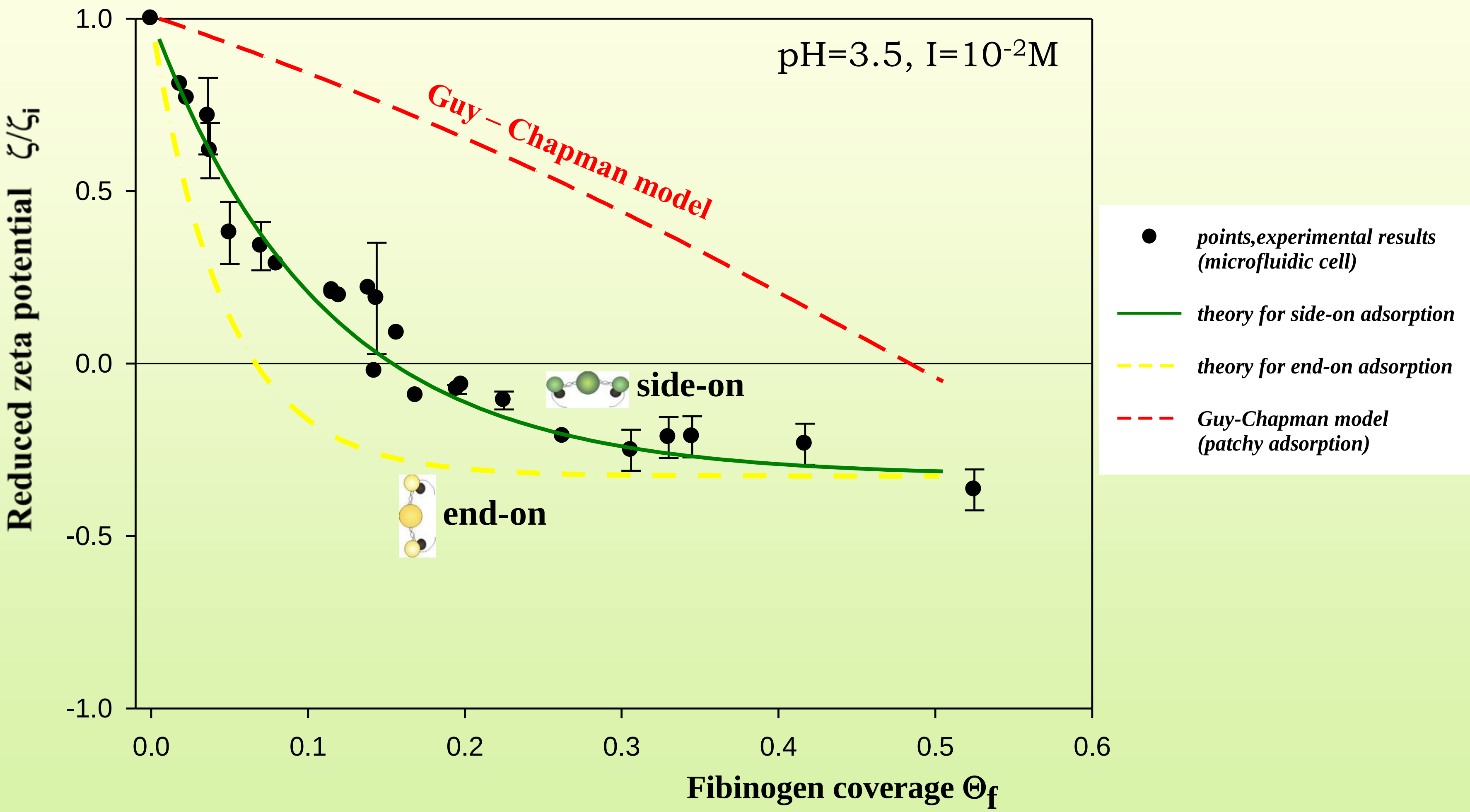
MECHANISM OF FIBRINOGEN ADSORPTION ON MICA DETERMINED BY STREAMING POTENTIAL



Side-on adsorption of fibrinogen !

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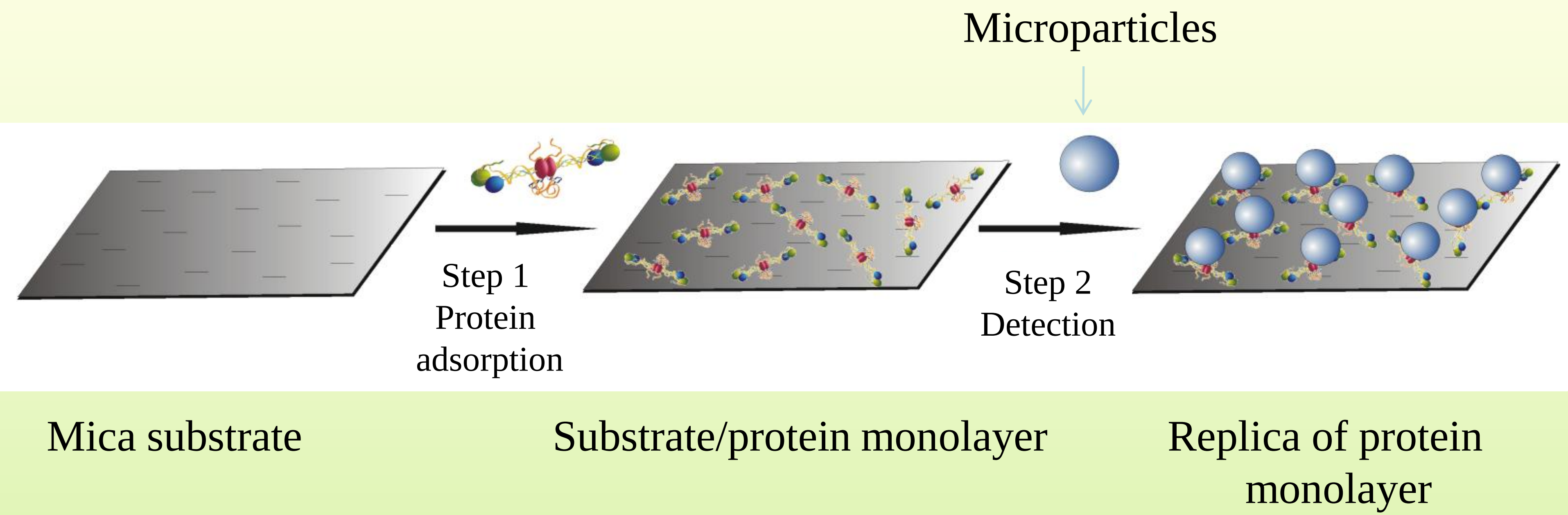
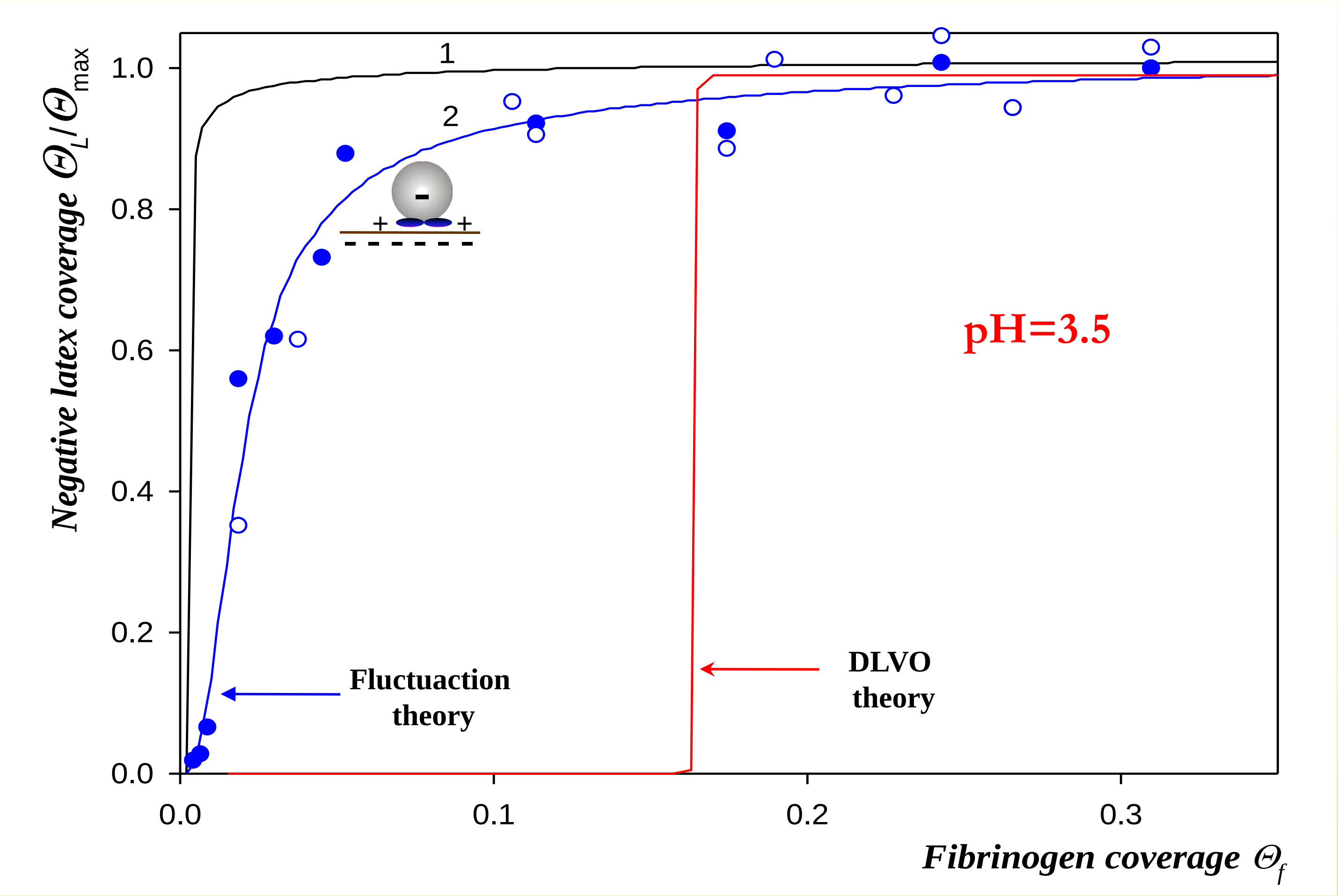
COLLOID ENHANCEMENT OF FIBRINOGEN MONOLAYERS



Microparticles: Negative Latex L800 (diameter 800nm)
Positive Latex A800 (diameter 810 nm)

LATEX PARTICLE DEPOSITION ON SITES FORMED BY FIBRINOGEN ON MICA



Points: experimental results for negative latex L800, (●) optical microscopy, (○) *AFM*

Lines: theortical results for adsorption site composed of 1 and 2 fibrinogen molecules

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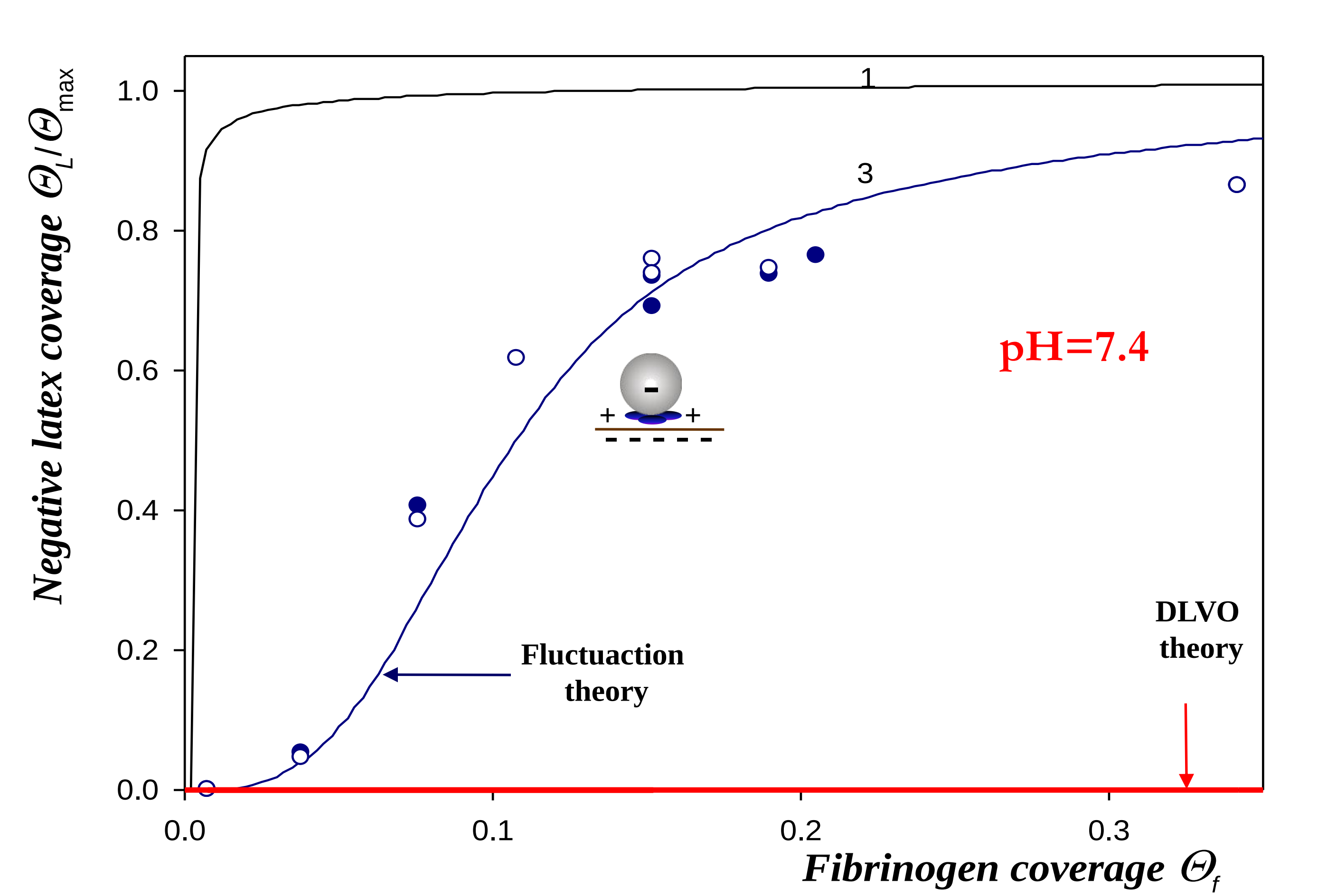
LATEX PARTICLE DEPOSITION ON SITES FORMED BY FIBRINOGEN ON MICA



*Points: experimental results for negative latex L800, (●) **optical microscopy**, (○) AFM*
Lines: theortical results for adsorption site composed of 1 and 3 fibrinogen molecules

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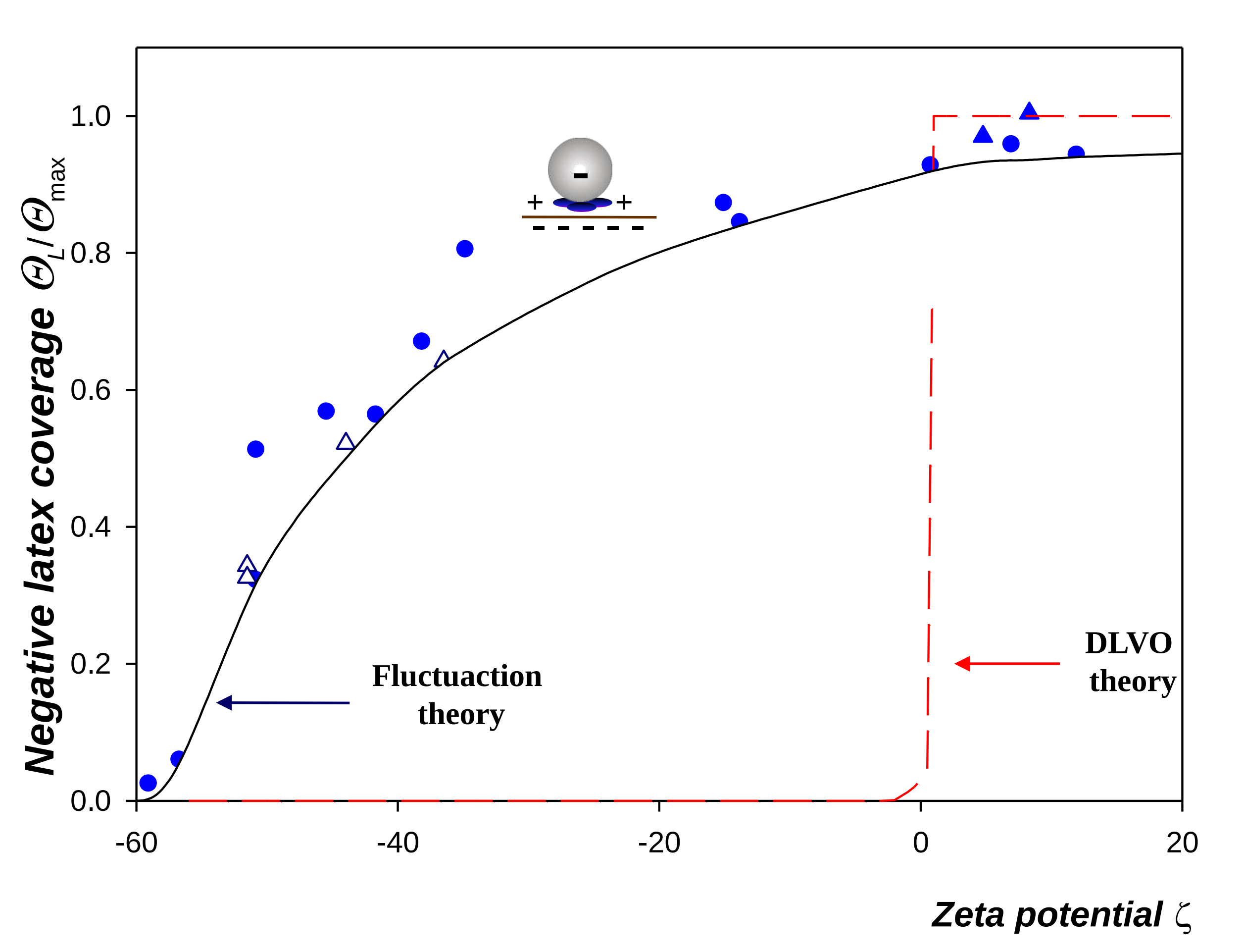
LATEX PARTICLE DEPOSITION ON FIBRINOGEN OVERED MICA



Points: experimental results for negative latex L800, by **optical microscopy and** *AFM.*
Line: theortical results derived from fluctuation theory.

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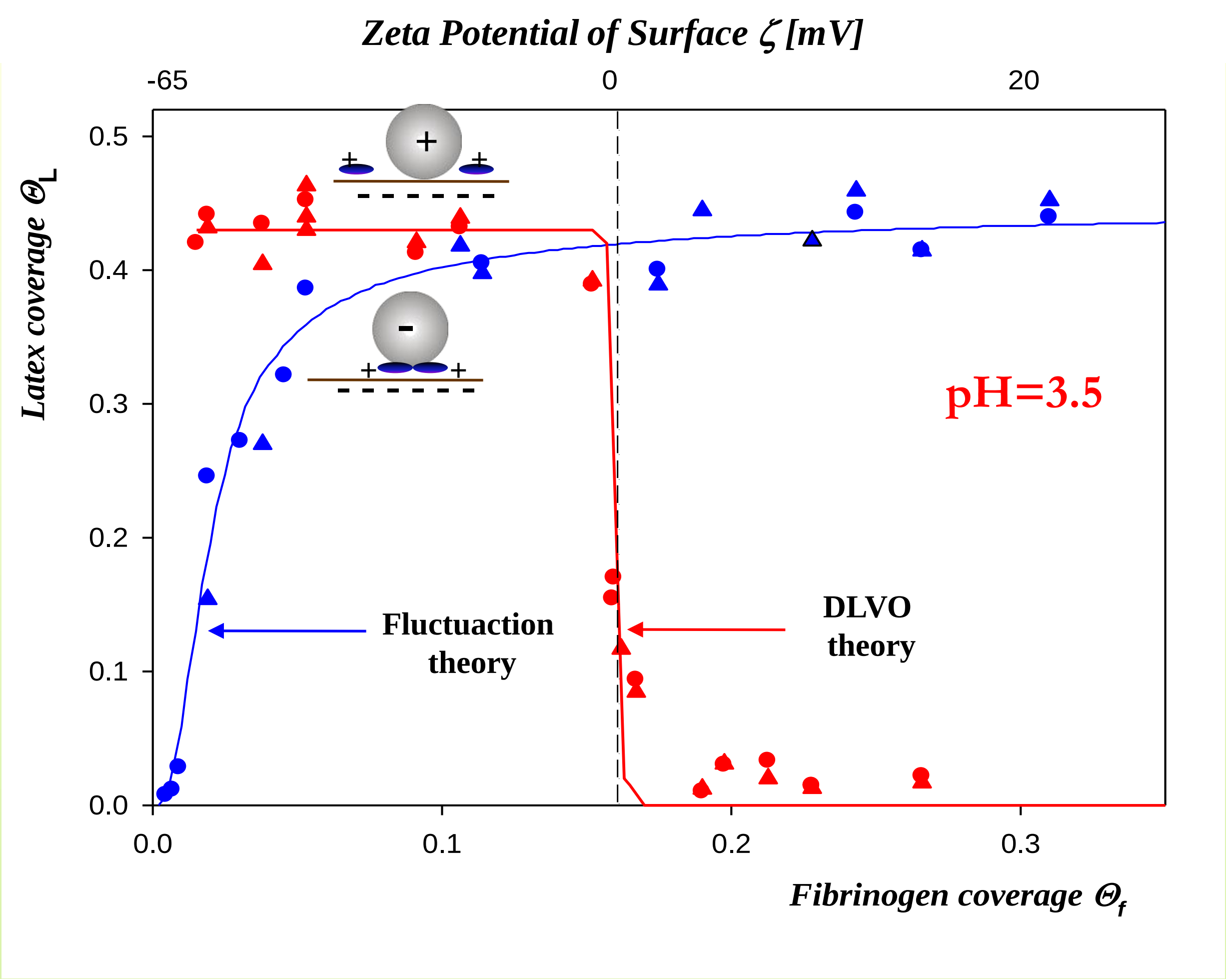
COLLOID ENHANCEMENT OF FIBRINOGEN LAYERS ON MICA
(FORMATION OF SUPER-ADSORBING SURFACES !)



Points: experimental results for negative latex L800, by **optical microscopy and** *AFM.*

Blue Line: theortical results derived from fluctuation theory.

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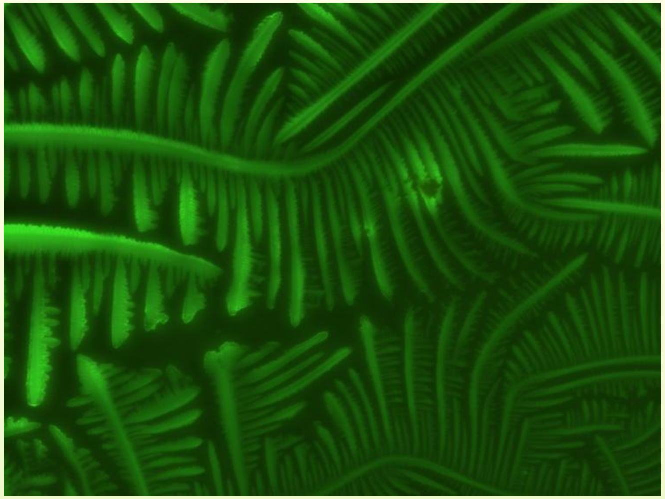
COLLOIDAL ART

AND REAL…



L. Szyk-Warszyńska, 2005.
Fluorescein on mica

Henri Rousseau, 1910.
Oil on canvas

CONCLUSIONS

- Streaming potential measurements combined with colloid deposition proved heterogeneous charge distribution over protein molecules.

- This explains anomalous adsorption of fibrinogen at pH = 7.4 and deviations from predictions of the DLVO theory.

- The Coulomb law is correct, but the application of the continuous DLVO theory to protein adsorption is wrong.

- Protein adsorption phenomena are governed by ordinary physical laws, there is no need for introducing additional interactions, especially the hydrophobic forces

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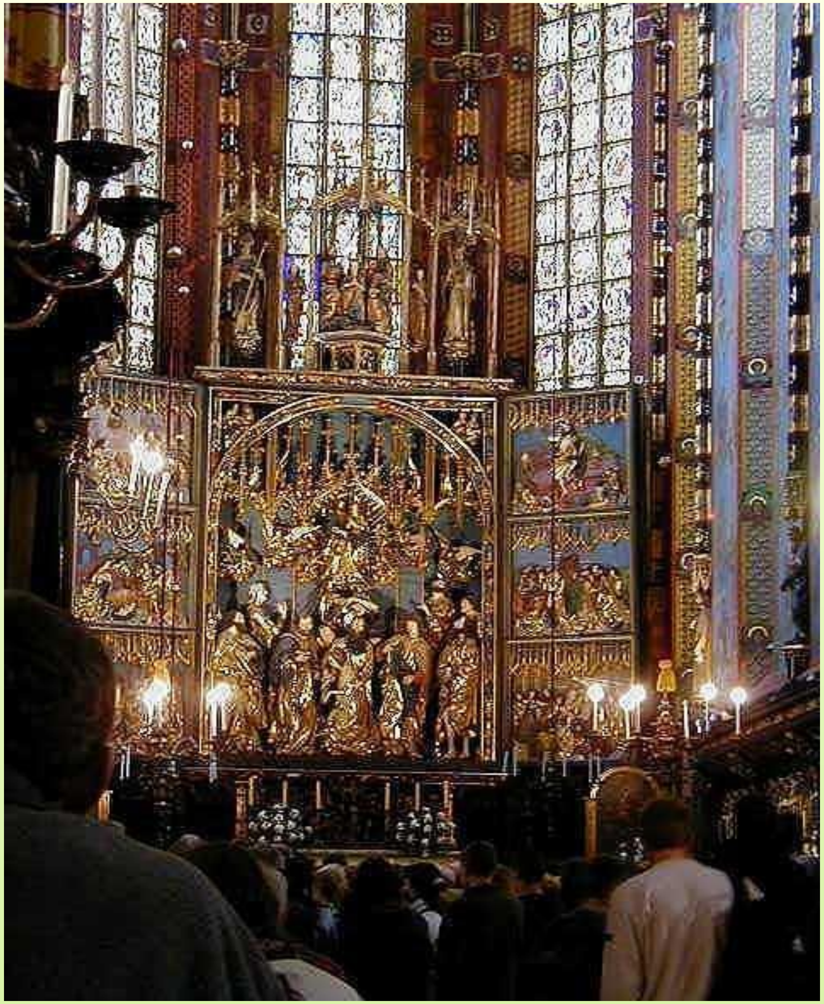
Photo by J.Barbasz

Cracow, Main Market Square

CRACOW, ART

Beauty at large...



Veit Stoss Altar in Mariacki Church,
1477-1489

Lady with the Ermine
by Leonardo da Vinci
in Czartoryski's Museum

Thank you for your attention

OUTLINE

- ***Significance of Adsorption (Nanoarchitecture)***
- ***Defining Driving Forces***
- ***Theoretical Methods & Results***
- ***Illustrative Experimental Results***
- ***Conclusions***

Theoretical result for particles covered surfaces

General expression for the reduced zeta potential for particle covered surfaces

$$\bar{\zeta} = 1 - A_i(\theta)\theta + \frac{\zeta_p}{\zeta_i} A_p(\theta)\theta$$

$\bar{\zeta}$ the reduced zeta potential of interface with adsorbed particles
ζ_i the zeta potential of the bare interface
ζ_p the zeta potential of particles

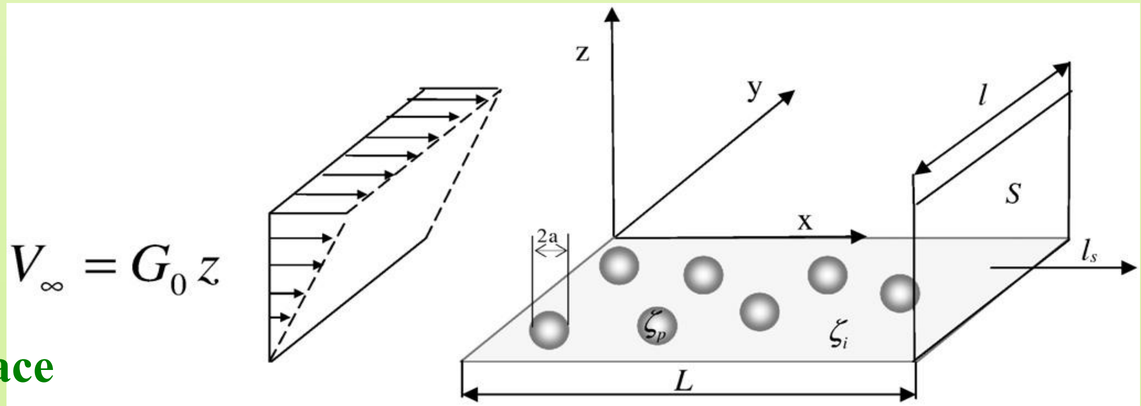
$$\theta = S_g N$$

Particle coverage

$$S_g = \pi a^2$$

N the number of particles per unit area

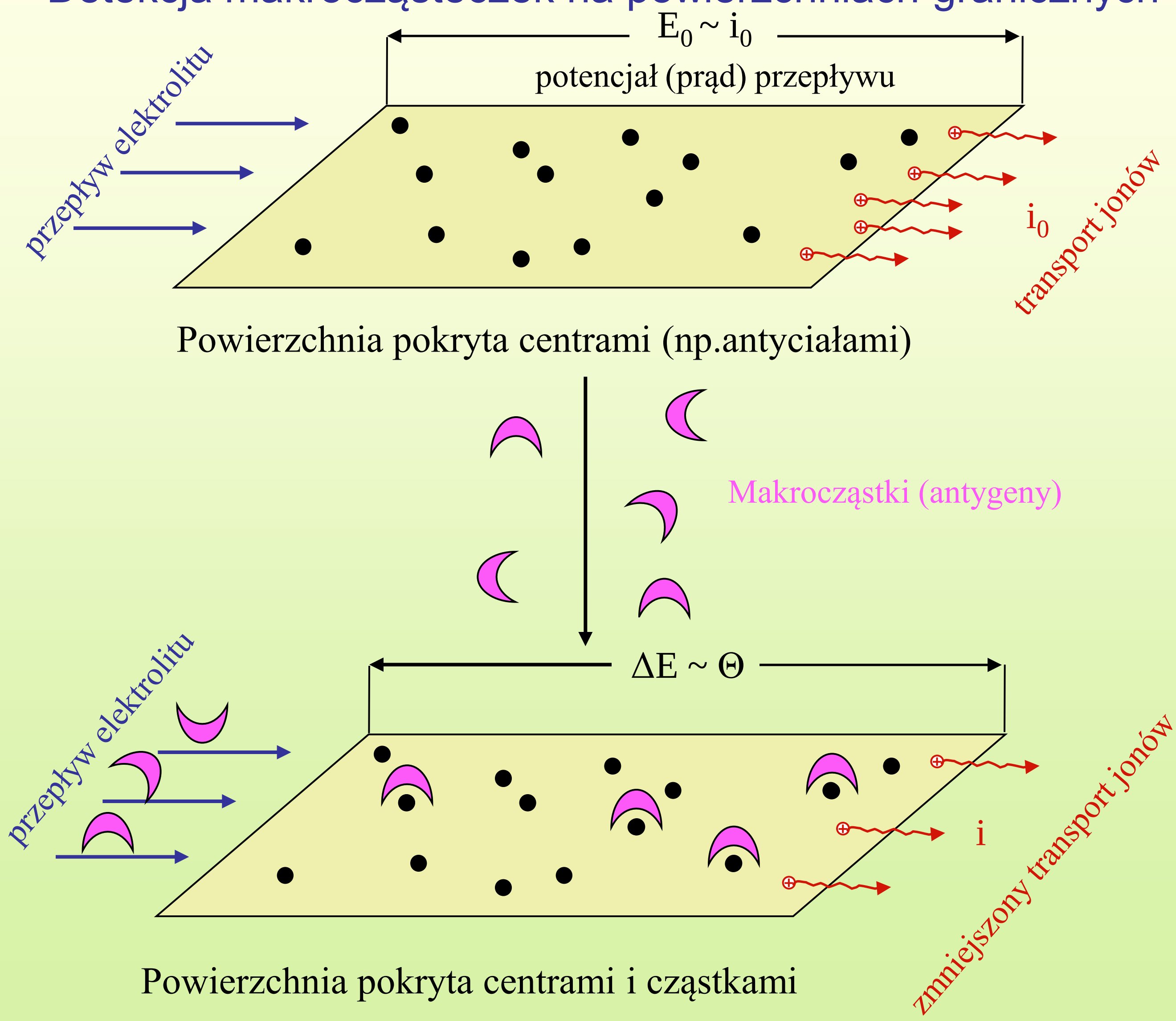
Schematic view of the shear flow past colloid particles adsorbed at a solid/liquid interface.



shear flow near interface

ZAGADNIENIE

Detekcja makrocząsteczek na powierzchniach granicznych



MONODYSPERSYJNE SUSPENSJE KOLOIDALNE