Introduction
Adsorption of proteins at a solid-liquid interface is an important phenomenon in biology and highly relevant for the design of biocompatible materials. It is well-known that protein adsorption is one of the first events which take place when a foreign material comes into contact with a living body and is believed to be a key factor in controlling subsequent cellular adhesion. In the last years several studies were performed, in which protein adsorption on nano-patterned adsorbents was investigated and a dependence on the topography was determined. For example an increased adsorption of F-Actin at stripes and edges was observed on nanostructured titanium with structure heights of 1-2 nm and proteins were aligned parallel to the nanostructure [1]. Moreover, the range in which proteins are “sensitive” to structures of a certain size is usually very small and hence, a detailed knowledge of their effect on the adsorption is required.

In this article the influence of a topographical nanostructure with sharp edges and spikes is investigated by a theoretical model combining Brownian Dynamics (BD) and the Finite Differences (FD) method. The theoretical description of an adsorption process on three-dimensional rough or porous nanostructures is more complex than for a planar surface: The influence of edges and spikes on the local electric field has to be considered and it may be assumed that preferred sites of adsorption are formed. An increased adsorption rate at certain places will also affect the local neighborhood in the adsorption steps following at later times, due to protein-protein interactions. Thus, it stands to reason that a theoretical prediction is required to include protein-protein and protein-nanostructure interactions as well as a calculation of the individual motion trajectory of each single protein.

Materials and Methods
In a BD-Simulation, the displacement of each particle $i$ is derived from the forces acting on it. The new particle position after a small time step $\Delta t$ will be considered as initial position for the next step. Thus, the gradual computation of many time steps results in the motion trajectory of a protein. The algorithm for updating particle positions [2] is given by

$$\xi_i(t + \Delta t) = \xi_i(t) + \frac{D_0}{k_BT} F_i(t) \Delta t + \chi_i(\Delta t),$$

where $\xi_i(t)$ is the position of particle $i$ at time $t$, $D_0$ is the lateral diffusion coefficient for free diffusion, $k_B$ the Boltzmann constant and $T$ the temperature of the system. Hydrodynamic interactions are neglected in this model. The shift of the protein
positions is primarily determined by two factors: a systematic net force $\mathbf{F}$, which is calculated from the mutual interactions of the proteins along with the particle-nanostructure interactions, and a stochastic displacement $\chi_i$. The latter has a Gaussian distribution, a variance-covariance according to the lateral diffusion coefficient and no correlations to the systematic force.

Interactions are based on the DLVO (Derjaguin, Landau, Verwey, Overbeek) theory and account for electrostatic and dispersion forces. While protein-protein interactions can be expressed analytically by the classical DLVO theory [5], protein-nanostructure interactions need to be described numerically, due to the arbitrary shape of the adsorbent. The electrostatic interactions of the numerical part were calculated from a solution of the Poisson-Boltzmann equation by an FD multigrid solution [3]

$$\nabla (\varepsilon_0 \varepsilon_r (x) \nabla \phi) = -\phi - \sum_{\nu} q_{\nu} e_0 \cdot c_{\nu} N_A \cdot \exp \left( - \frac{q_{\nu} e_0 \cdot \phi}{k_B T} \right)$$  \hspace{1cm} (2)

and the dispersion interactions were obtained from a numerical integration of Hamaker’s equation [4]

$$U = - \frac{A}{\pi^2} \int_{V_1} dv_1 \int_{V_2} dv_2 \frac{1}{r^6}$$  \hspace{1cm} (3)

on a fine sub-grid. In the equations $\phi$ is the electrostatic potential (in Volt), $\varphi$ the partial charge distribution belonging to the nanostructure, $N_A$ the Avogadro’s number, $k_B$ the Boltzmann constant and $T$ the temperature of the system. Moreover, $dv_1$ and $dv_2$ designate the volume elements from the integrals over the total particle volume $V_1$ and $V_2$, respectively. $r$ denotes the distance between $dv_1$ and $dv_2$ and $A$ is the Hamaker constant. The analytic solution for the electrostatic part of the protein-protein interactions is given by [5]

$$U_{i,j}^{EL}(r) = \frac{q_{\nu}^2 e_0^2}{4 \pi \varepsilon_0 \varepsilon_r} \left[ \frac{\exp(\kappa \cdot a)}{1 + \kappa \cdot a} \right]^2 \frac{\exp(-\kappa \cdot r)}{r}$$  \hspace{1cm} (4)

where $\kappa^2 = \left( e_0^2 N_A \sum_{\nu} c_{\nu} q_{\nu}^2 \right) / \left( \varepsilon_0 \varepsilon_r \cdot k_B T \right)$ is the inverse Debye-Length, $a$ the radius of the protein sphere, $r > 2a$ the distance between two proteins, $\varepsilon_0$ the permittivity of free space, $\varepsilon_r$ the relative dielectric constant, $e_0$ the electron charge and $q_{\nu}$ the net charge of a protein. Moreover, each ion sort $\nu$ of the electrolyte is parameterized by its concentration $c_{\nu}$ and its charge $q_{\nu}$. The Hamaker equation for the dispersion part can be explicitly integrated for two spheres [4]

$$U_{i,j}^{DISP} = - \frac{A}{6} \left\{ \frac{2a^2}{r_{i,j}^2 - (2a)^2} + \frac{2a^2}{r_{i,j}^2} + \ln \frac{r_{i,j}^2 - (2a)^2}{r_{i,j}^2} \right\}.$$

(5)

Short-range repulsion as known from the Lennard-Jones potential is implicitly defined by the algorithm: each time a particular displacement leads to an overlap with
another protein or the nanostructure, it is reduced until the overlap disappears. The sequence, in which this is examined, is changed every time step, in order to reduce the effect of unwanted correlations. Additionally a protein is flagged as immobilized, as soon as it grazes the surface of the nanostructure (no surface diffusion).

The following model parameters are used for the calculation:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
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<tr>
<td>Protein</td>
<td>Lysozyme</td>
</tr>
<tr>
<td>Type</td>
<td>Globular / sphere</td>
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<tr>
<td>Radius</td>
<td>1.5 nm</td>
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<tr>
<td>Net charge</td>
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<tr>
<td>pH</td>
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</tr>
<tr>
<td>A(Protein-Protein)</td>
<td>$2.0 \times 10^{-20}$ J [7]</td>
</tr>
<tr>
<td>A(Protein-Surface)</td>
<td>$1.0 \times 10^{-20}$ J [7]</td>
</tr>
<tr>
<td>Protein concentration</td>
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</tr>
<tr>
<td>Surface Potential</td>
<td>$\phi_v = -0.1$ V (Mica) [8]</td>
</tr>
<tr>
<td>Electrolyte</td>
<td>NaCl</td>
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<td>$\varepsilon_r$</td>
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<td>FD-Grid</td>
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</tr>
<tr>
<td>Integration sub-grid</td>
<td>729 points / FD-Element</td>
</tr>
</tbody>
</table>

Table 1: Model parameters

Results and Discussion
An oppositely charged nano-cube with an edge-length of 16 nm was chosen as model system, because it was expected that the adsorption at edges plays a major role for such small structures. In this setup all protein-nanostructure interactions are attractive while the protein-protein interaction consists of an attractive dispersion and a repulsive electrostatic part. The resulting pair potentials (without short-range repulsion) for the Lysozyme-Lysozyme interaction are shown in figure 1. The short distances are dominated by the attractive dispersion interactions while the farther regions are more influenced by the electrostatic repulsion. The latter form a kind of barrier, whose height depends on the electrolyte concentration. At high sodium chloride concentrations, the height of the barrier is low, due to the screening effect of the electrolyte, while to lower salt concentration this barrier increases.

Figure 2 displays the adsorbed proteins at the nano-cube in the final state for three different electrolyte concentrations. The proteins are preferentially adsorbed near the edges of the cube at low salt concentrations, which is mainly due to two different effects: first, the local electric field is higher near the edges and corners than in the center of a surface. Moreover, a point that is located at a corner or an edge can be reached by more proteins of the surrounding neighborhood than a point on a surface and already possesses a higher probability of adsorption due to geometry. Since the charged protein spheres repel each other, a higher adsorption rate at the edges leads to a lower surface coverage of the nearby planar surfaces at low salt concentrations. Only a few proteins manage to cross the repulsive barrier of the previously adsorbed proteins and reach regions where the attractive interaction of the
nanostructure outweighs. At higher salt concentrations the adsorption process begins in exactly the same way. However, adsorption can be continued at later time steps, due to the higher screening effect of the electrolyte and also the surfaces are covered. At an NaCl concentration of 0.1 mol/l almost a closest packing is reached as already assumed on the basis of the surface coverage graph. A slight increase of the protein density at the corners of the cube is still determined. With all accomplished calculations the surface coverage never exceeds a monolayer, but proteins may extend into space at the edges and corners of the cube. Hence, the mechanism of protein adsorption is governed by two different stages: in the first part preferential places are occupied, which are determined by the electrical field near the surface and the size of the surrounding liquid volume. The dispersion forces have only a small influence on the selection of the adsorption sites in this model, due to their short range and fast raising character. If a protein reaches the regions near the surface of the nano-cube, where dispersion forces play a role, it is usually adsorbed in the immediate vicinity. In the second part of the mechanism, also more unfavorable sites are occupied. The significance of this part is determined by the electrolyte concentration and the prevailing charge ratios.

**Conclusion**

In this study a model for the prediction of preferred protein adsorption sites was developed by a combination of the Finite Differences Method and Brownian Dynamics. An increased adsorption rate along the edges of a (16 nm)³ model cube is reached under consideration of electrostatic and dispersion interactions. If the cube comes into contact with a protein/electrolyte solution, obviously first the favourable positions at the edges are occupied. At later times also the surfaces of the cube are covered, if the salt concentration is sufficient to screen the repelling protein-protein electrostatic interaction.

**References**


**Figure Captions**

Fig. 1 Pair potential of the Lysozyme-Lysozyme interaction for different salt concentrations

Fig.2 Final state of the adsorption process on a 16 nm$^3$ nano cube for different salt concentrations