Modeling Tubulin at Interfaces. Immobilization of Microtubules on Self-Assembled Monolayers

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A theoretical study of protein docking to self-assembled monolayers using a new approach is presented. Docking experiments based on space complementarity implemented in FTDock software were performed for three different proteins: tubulin dimer, cytochrome *c*, and lysozyme. The proteins were adsorbed on alkanethiol surfaces with different terminating groups and 50 000 best orientations of each protein were analyzed. For all systems three filters based on different chemical and biological approaches were applied. Correctly docked proteins for the cytochrome *c* and lysozyme systems were found in a list of the first 12 results after applying the geometrical and grouping filter and in a list of the first 3 results after applying the biological filter. We have found that alkanethiol monolayers with odd and even numbers of $-CH_2-$ groups have similar properties in terms of interactions with the two proteins. Docking of the tubulin dimer revealed that the orientation favored from the applicational point of view can be found in a list of the first 14 results for monolayers with different terminating groups and that there may be a noticeable difference in tubulin dimer interactions with alkanethiol chains of various length. The results for tubulin dimer docking combined with microtubules ability of reversible assembly suggest that these biological structures may become good candidates to serve as templates for fabrication of nanowires and other nanoscale electronic devices. The new method of theoretical docking presented may be used as a fast and reliable tool complementing other theoretical and experimental techniques of exploring other protein−surface interfaces.

1. Introduction

Microtubules (MTs) are filamentous intracellular structures responsible for different types of movements in all eukaryotic cells. In cooperation with other components of the cytoskeleton, microtubules are involved in several basic cellular processes such as segregation of genetic material, intracellular transport, maintenance of cell shape, positioning of cell organelles, extracellular transport by means of cilia, and movement of cells by means of flagella and cilia. They form long (0.2−20 μm), cylindrical structures which are 25 nm in diameter with a 15 nm hollow core and are composed of 13 protofilaments. The building blocks of protofilaments are the tubulin dimers composed of α- and β-tubulin. The general structure of microtubules[1] as well as the tubulin dimer[2,3] has been established recently using using cryo-electron microscopy and crystallography.

The kinetics of microtubules formation and degradation has been extensively explored. In the presence of guanosine 5′-triphosphate molecules two tubulin monomers form a heterodimer which may self-assemble into the microtubule structure. Self-assembly begins with a nucleation event involving both tubulin monomers (and in the cell, a third monomer called γ-tubulin) followed by the sequential addition of new heterodimers to the initial complex. Because of the geometry of self-assembly and differences in addition rates, MTs are polarized and contain minus and plus ends. The minus end contains an exposed α-tubulin and undergoes slower heterodimer addition rates than the plus end, which consists of an exposed β-tubulin. Therefore, net microtubule polymerization occurs from the plus end of the growing polymer or nucleation complex and MTs generated from pure tubulins exist in a dynamic state with net addition of monomers to the plus end and net removal of monomers from the minus end.[4] This "treadmilling" effect can be controlled via interaction of the MT with various chemical agents (i.e., microtubule-associated proteins, taxol), resulting in stable MTs of fixed length. In the absence of these agents and with a shortage of monomers, MTs will disassemble. Dynamic instability which depends on tubulin concentrations is an intrinsic property of MTs.[5]

Microtubule polarity and the high chemical specificity inherent in its differing ends provide the advantage of directing MTs attachment to surfaces. Immobilization of MTs on surfaces offers a huge number of possible applications in chemical and biological science, including the construction of nanoscale biosensors and functional nanoscale electronic devices. Already several different self-assembled organic materials have been used for this purpose with limited success. The use of absorbed organic molecules and DNA for self-assembled nanoscale metal rods made by electrochemical replication of porous membranes has been reported.[6] DNA has also been used as conductive wire between two electrodes after coating it with metal.[7] Other studies reported polypeptides and proteins as templates for nanowires and it has been shown that several surfactant-like peptides undergo self-assembly forming nanotubes.[8] These can be further coated with proteins such as avidin to enable the use of biological interactions based on molecular recognition.[9] First experiments on MTs show that these biological structures seem

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to be a perfect system as templates for fabricating metallic nanowires due to their ability to self-assembly and natural high specificity in biological interactions with other species.[10] Self-assembled monolayers (SAMs) of alkanethiols on gold seem to be a natural choice as a surface of MTs immobilization.[11]

Several protein−surface interfaces (i.e., cytochrome *c* on SAMs) have been widely studied using a number experimental techniques. Particularly useful data about the orientation of the protein at a surface can be obtained from surface-enhanced resonance Raman scattering (SERRS) experiments[12,13] and polarized X-ray absorption fine structure spectroscopy (XAFS).[14] Electrochemical methods,[15] time-of-flight secondary ion mass spectroscopy (TOF−SIMS),[16] differential scanning calorimetry (DSC),[17] Fourier transform infrared-attenuated total reflection (FTIR-ATR),[18] and other techniques have also been used in this field to provide valuable information about the studied systems. These techniques allow to point out the amino acids which are responsible for binding of the protein to the surface as well as investigate the conformational changes of adsorbed proteins.

The protein−SAM interfaces have also been studied using theoretical methods. Several groups performed theoretical calculations on molecular surface systems at different levels of sophistication.[19−24] The most complete approach has been proposed by Jiang's group,[25,26] which uses the Monte Carlo (MC) technique in the canonical ensemble to determine the orientation of the protein at different SAM interfaces. MC calculations provide the most probable orientation of the protein at a given surface, which is the starting point for molecular dynamics (MD) simulations. This methodology allows one not only to determine the orientation of the protein at a surface, but also find out if the protein remains in its native state. One of the disadvantages of the proposed method is the high computational cost of performing the MC step since a big number of configurations has to be sampled.

When the intereaction of proteins with modified surfaces is simulated, there are usually no experimental data which can be used to indicate the possible binding sites within the protein. Even if such data are present, this does not guarantee obtaining a 3D model of the protein−surface system, and this model is vital for performing theoretical dynamics simulation of the system. Because of this fact there is a great need of a fast algorithm that can be used for providing an approximate but reliable model of protein−surface interactions when no or limited experimental data are available.

In this paper, a fast and reliable theoretical method of determining the orientation of proteins on SAMs and other modified surfaces is presented. This method is based on docking algorithm implemented in FTDock v2.0 software used earlier in the protein−inhibitor docking experiments.[27] Here the general idea of FTDock was adapted for biomolecule−surface docking. The all-atom representation of the docking entities is strongly reduced by a suitable mapping onto the 3-D lattice. The docking is perfomed assuming rigid geometries of the biomolecule and the surface and employs a discretization of all the molecules followed by a simulation of docking the protein to the surface. To validate the proposed method four different, well described systems were tested: cytochrome *c* on COOH-terminated SAM, cytochrome *c* on OH-terminated SAM, lysozyme on OH-terminated SAM, and lysozyme on CH_3-terminated SAM. In the next step we applied our approach to two problems: the comparison of docking a protein to surfaces with odd and even number of carbon atoms in thiol chains and theoretical docking of tubulin dimer to modified surfaces.

2. Theory and Computational Details

2.1. Theory. For the docking of proteins to SAMs we use a variation of the Fourier correlation algorithm first introduced by Katchalski-Kaztir[28] as implemented in the FTDock software.[27] Since the algorithm is very well described in both works, we will give only a brief summary of it.

The algorithm starts with projection of two molecules (e.g., the protein and the surface) onto a three-dimensional grid of N^3 points. Every node (l, m, $n = \{1, 2, ..., N\}$) is assigned a discrete value:

$$f_{\mathrm{A}_{l,m,n}} = \begin{cases} 1: & \text{surface of molecule} \\ \rho: & \text{core of molecule} \\ 0: & \text{open space} \end{cases}$$

and

$$f_{\mathrm{B}_{l,m,n}} = \begin{cases} 1: & \text{inside of molecule} \\ 0: & \text{open space} \end{cases}$$

where the parameter ρ is related to the degree of surface overlap tolerated during docking.

The docking requires evaluating of the correlation function f_C:

$$f_{\mathrm{C}}\alpha_{,}\beta_{,}\gamma = \sum_{l=1}^{N}\sum_{m=1}^{N}\sum_{n=1}^{N} f_{\mathrm{A}_{l,m,n}} \times f_{\mathrm{B}_{l+}}\alpha_{,m+}\beta_{,n+}\gamma$$

where N^3 is the number of grid points and α, β, and γ represent the translation vector of molecule B relative to A. By convention, A denotes the larger of the two molecules and B denotes the smaller one. In the case of protein docking to SAMs, we have always chosen the protein as the molecule B, which is rotated and translated over the grid with the surface fixed in the center of the grid. A high value of the correlation function denotes a system which has a high surface complementarity; if molecules overlap the correlation is negative. Correlation close to zero usually means that the two molecules are not in contact.

Calculation of the correlation function uses the fast Fourier transformation (FFT), which allows one to transform the discrete functions f_A and f_B and evaluate the desired correlation function using the inverse Fourier transform. The use of FFT allows one to reduce the computational effort to the order of $N^3 \ln(N^3)$. After each translational scan the protein is rotated about one of its Euler angles until rotational space has been completely scanned. If the Euler angles are varied at fixed intervals of Δ the algorithm results in $360 \times 360 \times 180/\Delta^3$ orientations overall for which the correlation function f_C must be calculated.

Apart from shape complementarity there are also other factors involved in molecular binding, electrostatic interactions being one of the most important. FTDock uses a simple Coulombic model of electrostatic interactions to calculate electrostatic complementarity using also the Fourier transform. For the SAM charges are assigned to the atoms of the surface model and the molecule is placed in a grid. The electric field is calculated for each atom from the surface of the molecule using the equation:

$$\phi_i = \sum_j \frac{q_j}{\epsilon(r_{ij})r_{ij}}$$

where ϕ_i is the field strength at node i, q_j is the charge on atom j, r_{ij} is the distance between i and j and $\epsilon(r_{ij})$ is a distance-dependent dielectric function. In this case a pseudo-sigmoidal function based on sigmoidal function of Hingerty is used.[29] The

treatment of the protein is simpler because the charges assigned to the atoms are discretized in a grid by trilinear weighting.[30] Calculations of electrostatic interactions proceeds as described for surface correlation calculations.

2.2. Computational Details. The models studied in this work were: cytochrome *c* (pdb code: 1HRC)[31] on the 10 × 5 carboxyl-terminated alkanethiol surface (the overall size of the system of ∼2600 atoms), cytochrome *c* on the 10 × 5 methyl-terminated alkanethiol surface, lysozyme (pdb code: 7LYZ)[32] on the 20 × 10 hydroxyl-terminated alkanethiol surface (∼8200 atoms), lysozyme on the 20 × 10 methyl-terminated alkanethiol surface and tubulin dimers (pdb codes 1JFF[2] and 1TUB[3]) on 40 × 20 SAMs with different terminating moieties (∼17000 atoms). The lysozyme on OH-terminated SAM was a model of a oligo (ethylene glycol)-terminated alkanethiol SAM used in the original research.[26] The crystal structures of the proteins were obtained from the Protein Data Bank.[33] The size of the surface was chosen to be similar to the size of the protein. We have chosen a $(\sqrt{3} \times \sqrt{3})$R30° structure[34−38] of $HS(CH_2)_9R$ or $HS(CH_2)_8R$ SAM on Au(111), where R was one of the following groups: CH_3, OH or COOH. The geometries of the alkanethiols were first calculated using B3LYP/6-31G* method[39] on single thiol chains and the optimized geometries were used to construct the SAMs. We have used a simple, three-dimensional surface generator written by us in Python to obtain a full-atom SAM model.

The docking was performed using the FTDock v2.0 software with default parameters apart from the electrostatic interactions calculations.[27] The cytochrome *c* system was digitized onto a 168 × 168 × 168 grid which resulted in a grid resolution of 0.68 Å. The lysozyme system was digitized onto a 240 × 240 × 240 grid with the resolution of 0.67 Å. For the tubulin dimer we have used a 300 × 300 × 300 grid with the resolution of 1.05 Å. Since FTDock authors argue that the grid spacing of more than 1 Å is unlikely to be useful we have performed some of the calculations for tubulin also on a 340 × 340 × 340 grid (grid resolution of 0.94 Å) and we have found that the results were identical. The surface thickness of the SAM was set to 1.3 Å for all systems and each of the proteins was rotated in 15° increments. These settings resulted in over 50 000 different geometries of the docked proteins for each system studied.

All atoms of the proteins have been assigned partial charges using the original FTDock set of charges or the Amber ff03 parametrization.[40] Partial charges for the heme were taken from Giammona's Ph.D. thesis.[41] Partial charges for the surface models were calculated consistently using the same approach as used for Amber ff03 parametrization with the help of ab initio techniques and restrained electrostatic potential ESP.[42] First full quantum-mechanical optimizations were performed for single alkanethiols using HF/6-31G* method. In the second step single-point calculations on optimized systems were performed using the B3LYP functional[39] in the ccpVTZ[43] basis set with the IEFPCM continuum solvent model.[44] Effective charges were obtained by fitting the electrostatic potential using RESP method. The quantum-chemical calculations were performed in the Gaussian 03 package.[45]

The calculations were performed on a HP/Compaq Alpha GS1280 located at University of Arizona and on a Cray SV1ex-1-32 and IBM eServer 325 with 98 nodes located at the Interdisciplinary Centre for Mathematical and Computational Modeling at Warsaw University.

3. Results and Discussion

3.1. Cytochrome *c* and Lyzosyme Docking. Cytochrome *c* and lysozyme are two of the best described systems at surfaces using both experimental data and theoretical calculations, and they were chosen in our research to validate the proposed method of theoretical docking. It is generally believed that cytochrome *c* is attached to the negatively charged surfaces using the lysine patches and adopts an orientation in which the heme group is perpendicular to SAM.[13] The orientation of cytochrome *c* on CH_3-terminated SAMs is different, as the protein interacts with the surface via a peptide segment 81−85.[13] In the case of lysozyme at SAM with hydrophobic ending groups, the optimal conformation requires gly67, pro79, and ile88 in the vicinity of the surface, whereas for lysozyme at OH-terminated SAM asn77 and arg88 are close to the surface.[26]

The final conclusion from docking of 10 biological systems (6 enzyme−inhibitor systems and 4 antibody−antigen systems) performed by the FTDock authors[27] was the inability of this software to reliably dock complexes when taking into account the geometrical complementarity alone. For 3 out of 10 test systems no correctly docked complex was found in the first 4000 predictions and for the other 7 systems, the highest ranked correct answer was in a list of hundreds of alternatives. From the obtained data, it was concluded that a high surface correlation score does not necessarily indicate a correctly bound complex. The addition of electrostatics improved the results in the original research, as it allowed one to exclude the complexes with highly unfavorable electrostatic interactions. With electrostatics, a good solution was found in the top 4000 structures in 9 out of 10 systems, and the number of geometries to be evaluated was reduced by approximately 50%.[27]

Better results were obtained when different biological filters were introduced to the system. In many cases, there are some experimental data available which allow us to choose the most probable binding sites within the proteins. Even with no experimental data available, it is often possible to predict the correct binding sites by examining potential hydrogen bonding groups, clefts or charged sites on a protein surface. We can also improve the results by taking the complexes with the best score and performing a finer rotational scan in a limited space close to the original system. Combining together all these methods and filters resulted in obtaining correct results in the top 15 complexes for 9 out of 10 systems.[27] Similarly good results were obtained after using empirical scoring of amino acid pairings, as implemented in RPScore program.[46] The use of this method was shown to be successful in removing false solutions and allowed further reduction of the number of structures to be examined. Unfortunately, current implementation of the algorithm in FTDock is of no use for protein−surface interface research since there are no pair potential matrices for other moieties than amino acids and nucleotides.

We have performed theoretical docking of the proteins on the SAMs, and three different filters were applied to obtained results. In the first step, no filter was applied. In the second step (the geometrical filter), we have filtered out the complexes in which the protein was located below the SAM surface or next to it so only complexes with the protein located above the surface were taken into account. The second filter (the grouping filter) grouped the complexes which have the same or very similar orientations but different translation vectors. The use of the third filter (the biological filter) narrowed the results even further using the biological data taken from the experimental and theoretical results available.[13,25,26] For the cytochrome *c* system on COOH-terminated SAM, the last filter excluded all the docked complexes with gln16 and lys79 further than 9.0 Å away form the surface. For the same protein on hydrophobic surface we ensured that the peptide segment 81−85 is within

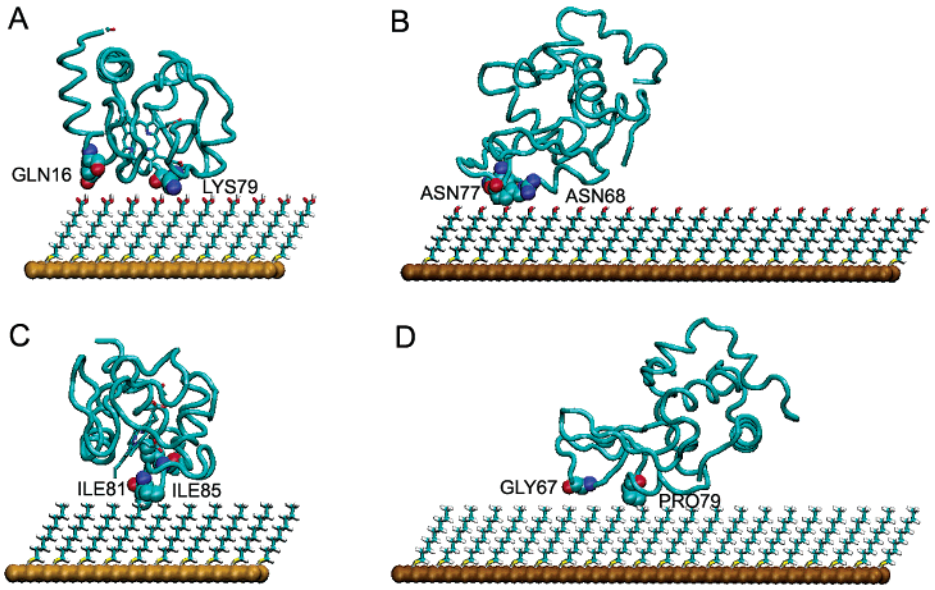

Figure 1. Snapshots of optimal orientations of protein on SAM of four studied systems found by FTDock: (A) cytochrome *c* on COOH-modified SAM; (B) lysozyme on OH-modified SAM; (C) cytochrome *c* on CH_3-modified SAM; (D) lysozyme on CH_3-modified SAM. The orientation of the proteins is almost identical to the orientation obtained using other theoretical[26] and experimental techniques.[13] Amino acids interacting with the surface are in spacefill representation.

TABLE 1: Docking of Proteins to SAMs with Varying Degrees of Filtering

	rank[a]				
system	no filter	geometrical filter	grouping filter	biological filter	top 50[b]
1HRC on COOH	1	1	1	1	6
1HRC on CH_3	252	41	8	1	1
7LYS on OH	220	25	6	2	2
7LYS on CH_3	3926	61	12	2	0

[a] Rank refers to the position of the first correct answer in the list of predictions; the grouping, geometrical and biological filters are described in the text. [b] Number of occurrences of the orientation in the top 50 results after applying the geometrical filter only.

9.0 Å of the SAM. For the lysozyme system on CH_3-modified surface the filter was defined as follows: gly67, pro79, and ile88 within 9.0 Å of the surface. The lysozyme system on OH-modified SAM included a filter which excluded all the complexes with asn77 and arg68 further than 9.0 Å away form the surface. The results of the docking are presented in Table 1 and in Figure 1.

It should be of no surprise that applying the biological filter gives very good results for the systems studied and allows us to find the correct geometry of the complex in the two best results. The more interesting results come from the use of the geometrical and grouping filter only. Applying these very obvious search criteria gives also very good results and allows us to find the correct geometry of the complex in the first 12 scores. Only limited data can be obtained from a different quantity—the number of occurrences of the orientation in the best 50 results after applying the geometrical filter only (Table 1). For our systems, the correct orientations, beside having high rank, had sometimes also a high number (≥ 5) of occurrences in the top 50 results. On the other hand for some of our systems the correct orientation did not have a single occurrence in the top 50 results.

We have also found that the use of electrostatic filter implemented in FTDock has very limited applicability in the description of protein−surface interactions. We have used the electrostatic filter with original FTDock charges and partial charges from Amber ff03 parametrization for both cytochrome *c* and lysozyme. It appears that in many cases the correctly docked systems had a very low electrostatic score and should be eliminated from the final results. Since we have obtained poor results for two different set of partial charges, this problem probably cannot be connected to an improper parametrization of the charges. Apparently, the very simple description of electrostatic forces implemented in FTDock is not sufficient to describe these interactions in a correct manner for proteins interacting with the surface.

The results for the two systems show that, contrary to the protein−inhibitor interactions, the space complementarity may be sufficient to distinguish a correctly bound system for the protein interacting with a surface. This technique may give very good results when combined with limited experimental data, since it can rapidly produce a model of the protein−surface system. It may be also possible to combine it with MC interaction energy calculations to quickly improve the score of the correct complex. The latter approach should be very rapid, because after docking only the energy of ∼20 different orientation have to be sampled in order to choose the best starting point for the MD simulations.

3.2. Docking to SAMs with Thiol Chains of Odd or Even Numbers of Carbon Atoms. One of the interesting aspects of SAMs is the comparison between alkanethiols with odd (odd SAMs) and even (even SAMs) number of carbon atoms.[47] Most experimental and theoretical data are available for the odd systems, but SAMs with even number of $-CH_2-$ groups have not been completely neglected. Friction force microscopy (FFM) experiments showed the orientation differences of the terminal methyl groups of gold-bound *n*-alkanethiolates.[48] From these experiments, it was concluded that there is a difference in the surface free energy due to different spatial dispositions of the terminal methyl groups dipole moment for SAMs with odd or even number of carbon atoms. Others have reported differences in orientation of nematic liquid crystals anchored on SAMs caused by the odd−even effect.[49] There is also a noticeable difference between odd and even SAMs when performing low-energy ion−surface reactions of small organic ions.[50]

TABLE 2: Docking of Proteins to SAMs Composed of Alkanethiols with Odd or Even Number of Carbon Atoms

	rank[a]				
system	no filter	geometrical filter	grouping filter	biological filter	top 50[b]
1HRC on COOH odd	1	1	1	1	6
1HRC on COOH even	6875	67	9	2	0
1HRC on CH_3 odd	252	41	8	1	2
1HRC on CH_3 even	238	87	9	1	0
7LYS on OH odd	220	25	6	2	1
7LYS on OH even	75	8	4	3	5
7LYS on CH_3 odd	3926	61	12	2	0
7LYS on CH_3 even	143	33	8	1	7

[a] Rank refers to the position of the first correct answer in the list of predictions. [b] Number of occurrences of the orientation in the top 50 results after applying the geometrical filter only.

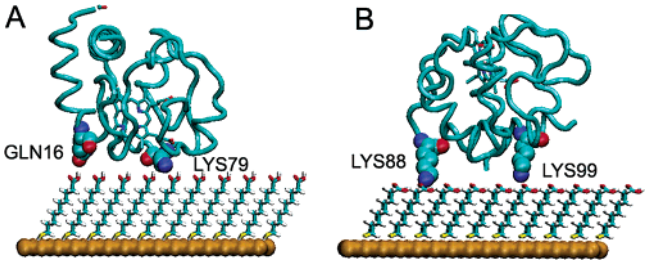

Figure 2. Snapshot of orientations of cytochrome *c* on COOH-modified SAM: (A) optimal orientation on odd SAM; (B) orientation with lys88, lys99, glu61, glu62, and glu92 close to the surface. Amino acids interacting with the surface are in spacefill representation.

The aim of this part of our research is to answer the question whether the odd−even effect plays an important role in the orientation of a protein attached to SAM. The interaction between protein and the surface is, in most cases, a combination of geometrical and electrostatic effects; therefore, our approach should well describe the adsorption phenomenon. The odd−even effect for adsorbed proteins was inspected using FTDock calculations of cytochrome *c* and lysozyme on SAMs with either odd or even number of $-CH_2-$ groups in the alkanethiol chain and different end groups (Table 2).

Comparison of the results for even and odd systems reveals several interesting observations. One of the orientations of cytochrome *c* on odd COOH-modified SAM occurs only four times in the top 15 scores, but it is a dominant geometry for the even SAM with 15 complexes in the top 20 scores. This complex of cytochrome *c* on SAM uses similar moieties to attach to the surface as the odd system, but instead of gln16 and lys79, it employs lys88, lys99, glu61, glu62, and glu92 (Figure 2). The large number of occurrences and high scores of this complex in the theoretical docking experiment may suggest that cytochrome *c* may assume such an orientation on the even SAM surface under some specific conditions.

Theoretical docking of cytochrome *c* to a CH_3-terminated SAM allows us to find the correct answer in top 9 scores after applying the grouping filter (Table 2). There are however three different orientations of the protein on the surface which have also high scores and a large number of occurrences for both the odd and even systems. One of this systems uses peptide segment 21−25 (glu-lys-gly-gly-lys) to attach to the SAM, the second one has two peptide fragments: 1−4 and 99−104 close to the surface. Third of these complexes interacts with the surface via the same combination of amino acids as for the COOH-terminated case: lys88, lys99, glu61, glu62, and glu92 (Figure 3).

Regnier et al. reported recently another study of cytochrome *c* adsorption on negatively charged surface.[51] They found that this protein may have multiple binding sites and may adopt different orientations on the surface depending on the mode of the chromatography. Nevertheless, in the experiments of Regnier et al., cytochrome *c* was usually interacting with the surface via two structural domains on a single face of the protein. The authors of that work concluded also that during the course of adsorption only small conformational change occur in the protein.

These results and the hypothesis that only one portion of the external surface of a protein can dominate its chromatographic behavior[52] are in agreement with our theoretical experiments presented in this work. In most cases, we are able to point out the orientation of the protein on the surface which is close to

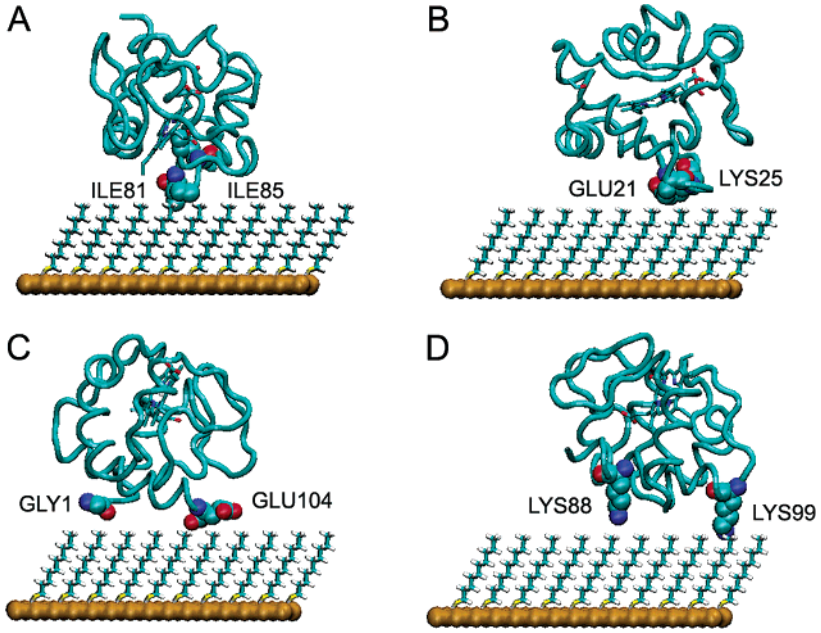

Figure 3. Snapshot of orientations of cytochrome *c* on CH_3-modified SAM: (A) optimal orientation; (B) interaction via the 21−25 protein segment; (C) interaction via 1−4 and 99−104 protein segments; (D) orientation with lys88, lys99, glu61, glu62, and glu92 close to the surface. Amino acids interacting with the surface are in spacefill representation.

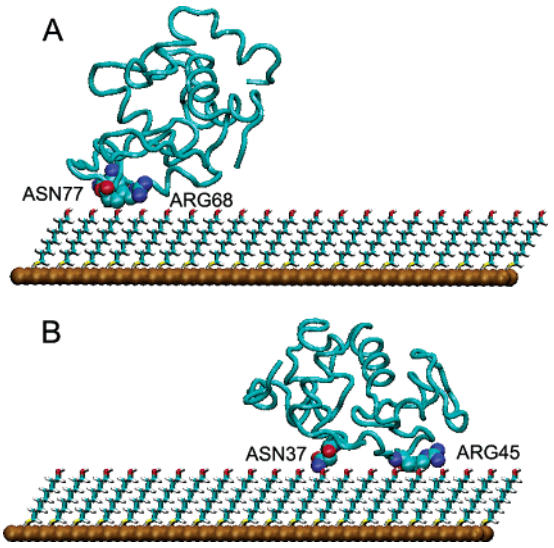

Figure 4. Snapshot of orientations of lysozyme on OH-modified SAM: (A) optimal orientation; (B) interaction via the 37−45 protein segment. Amino acids responsible for interactions with the surface are in spacefill representation.

the "native" orientation. On the other hand by analyzing not only the best orientation from the docking experiment but also other orientations with high scores and/or high number of occurrences we may get a hint of other possible conformation of a given protein at a given surface. The legitimacy of those orientations should of course be further validated by other theoretical or experimental methods.

Similar results were obtained for the lysozyme on an OH-terminated SAM. Theoretical docking of the protein to odd and even SAM reveals one orientation of lysozyme which is strongly preferred for both types of surface over the experimentally obtained orientation. In this case the protein is attached to the surface not through asn77 and arg68, but rather through a long peptide fragment 37−45 (Figure 4). It is interesting to notice that this fragment also starts with an asparagine (asn37) residue and ends with an arginine (arg45) residue. For the case of lysozyme adsorbed on CH_3-terminated SAM we have obtained an almost identical orientation as the most occurring in the top 50 scores.

Since the outer layer of odd and even SAMs is similar both in terms of electrostatic and geometrical properties we did not expect major differences in the docking of proteins. The results allow us to state that the differences are indeed minor (Table 2). When switching from odd systems to systems with even number of carbon atoms we get the same, correct results of theoretical docking in the first 12 hits after applying the grouping filter and in the first 3 hits when the biological filter was applied. There is, however, a noticeable change in the rank of some orientations as well as in their number of occurrences in the top 50 results. It may suggest small adjustments in the protein−surface interactions, which may have an nonnegligible impact on the protein orientation.

3.3. Tubulin Dimers Docking to Modified SAMs. In the third step of this research, we have applied the docking method to investigate microtubule-SAM interface. Since the size of even one microtubule system is enormous (~1 000 000 atoms) it is usually impossible to perform even simple calculations on such a system (although some effort has been made in this direction, see ref 53). On the other hand a much smaller system like the tubulin dimer, which is within present computational power, may still provide interesting results.[54] In our research, we have

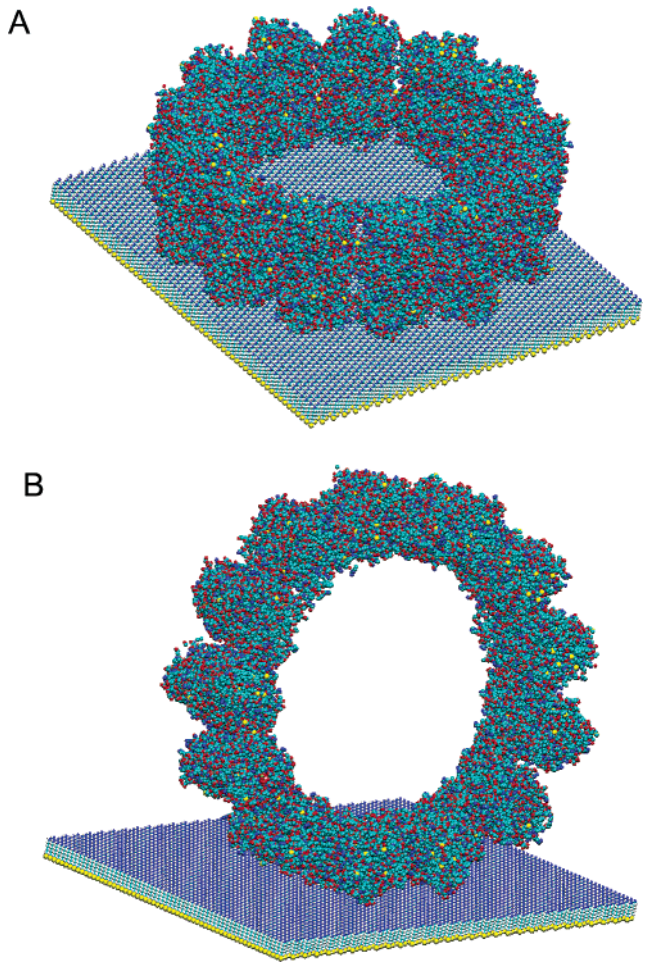

Figure 5. Models of possible orientations of a MT on SAM: (A) MT attached to the surface via the minus or the plus end; (B) attachment via both α-monomer and β-monomer.

used the tubulin dimer as the model of the microtubule. Most of the calculations were performed on the 3.5 Å resolution model of the α,β-dimer (PDB code: 1JFF),[2] although in several cases the 3.7 Å resolution model (PDB code: 1TUB) was also used. We have found that the results from the FTDock experiments were identical for these two models of tubulin dimer so we present the results for only one system. In this part of the research, we have used three different SAMs: NH_2-terminated, CH_3-terminated, and COOH-terminated. The calculations were performed using both even and odd SAMs for all of these systems.

The goal of immobilization of MTs on surfaces is to retain both their biological activity and the ability to self-assemble. This feature can be achieved only when MTs are attached to the surface via the minus end (α-tubulin) or the plus end (β-tubulin) with the MTs core perpendicular to the surface (Figure 5). A different arrangement with both α-tubulin and β-tubulin interacting with the surface and the MT core laying parallel to the surface is not favored from the applicational point of view. The theoretical docking has been performed in order to find out whether it is possible to obtain such a favorable orientations for MTs on SAM system. We have also applied a biological filter which removed all the orientations with both α-tubulin and β-tubulin relatively close to the surface (Table 3).

The analysis of the results for the odd NH_2-terminated SAM reveals three different, most-occurring orientations of the protein. All the three systems have very high scores (rank 1, 3, and 4 respectively after applying the geometrical and grouping filters)

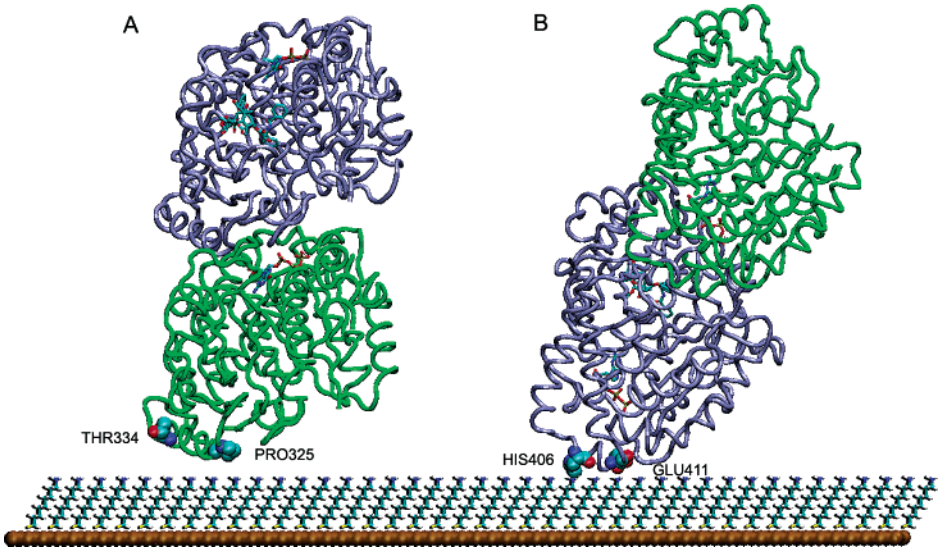

Figure 6. Snapshot of optimal orientations of tubulin dimer on NH_2-modified SAM: (A) interaction via the minus end; (B) interaction via the plus end. Amino acids interacting with the surface are in spacefill representation.

TABLE 3: Docking of Tubulin Dimer to Different SAMs

system	rank[a] no filter	geometrical filter	grouping filter	biological filter	top 50[b]
Attachment via the Minus End					
1JFF on NH_2 odd	711	13	5	3	4
1JFF on NH_2 even	938	44	14	6	1
1JFF on CH_3 odd	544	19	7	3	3
1JFF on CH_3 even	26	1	1	1	6
1JFF on COOH odd	59	12	6	2	5
1JFF on COOH even	130	13	8	2	5
Attachment via the Plus End					
1JFF on NH_2 odd	3519	70	15	5	0
1JFF on NH_2 even	147	7	3	1	6
1JFF on CH_3 odd	749	20	7	2	5
1JFF on CH_3 even	41	2	2	1	4
1JFF on COOH odd	312	67	17	7	0
1JFF on COOH even	15	5	3	1	6

[a] Rank refers to the position of the first correct answer in the list of predictions. The correct answer refers to the system with only α-tubulin or only *β*-tubulin interacting with the surface. [b] Number of occurrences of the orientation in the top 50 results after applying the geometrical filter only.

and have at least five occurrences in the top 50 results (after applying the geometrical filter only). Unfortunately, in all three orientations, both the α-monomer and the *β*-monomer are close to the SAM surface, and such an orientation is not favored from the applicational point of view.

The situation is different for the even NH_2-terminated SAMs. There are two dominating orientations of the tubulin dimer on the surface. One of these (rank 2 after geometrical and grouping filters with 9 occurrences in the top 50 results) is similar to the odd case, although different amino acids are involved in the surface binding. The second system (rank 3, 6 times in top 50) introduces the tubulin dimer attached to the surface via only one monomer—the *β*-tubulin (the plus end of MT). In this orientation the protein interacts with the surface via the peptide fragment 406−411 (his-trp-tyr-thr-gly-glu) (Figure 6).

We were also able to locate the system in which the tubulin interacts with the surface via the minus end and has a plus end exposed to the environment. For the odd NH_2-terminated system such an orientation has a relatively high score: it ranks 3-rd after applying all the filters with 4 occurrences in the top 50 results and has 5 amino acids involved in binding to the surface: pro325, asp327, ala330, ala333, and thr334 (Figure 6). The outcome for the even system is different, because such an oriented complex has only one occurrence in the top 50 and is ranked at 14th place.

For SAMs with different terminating groups, the desired orientation of the tubulin dimer has also been found. The best result was obtained for the even CH_3-terminated SAM, where the minus-end orientation was ranked first after applying the geometrical filter with 6 occurrences in the top 50 results. For all the proposed systems, we were able to get only the α-tubulin interacting with the surface within the best 14 results after applying geometrical and grouping filters. As usual, applying the biological filter gave even better results—the correct answer was now in the top 6 results. It is, however, interesting to notice that the results are completely different for the attachment of the protein via the plus end SAMs. For these systems, there is a clear difference between optimal orientations for odd and even SAMs. The orientation with the protein interacting with the surface via only *β*-tubulin has also relatively high scores for even SAMs but rather low scores for the odd systems. This result, if confirmed by other studies, would suggest that the minus end has a similar affinity to both odd and even SAMs, while the plus end is sensitive to the length of alkanethiol chains forming the SAM.

4. Conclusions

In this work, a theoretical technique devised for protein docking and implemented in FTDock software was used for examining protein−surface interfaces. We have found that the theoretical method based solely on surface compatibility reproduced well the orientations of proteins on self-assembled monolayers known from experimental data or other theoretical research. For cytochrome *c* and lysozyme the correct orientation of the protein on the surface was found in the 12 best scores after applying the geometrical and grouping filter and in the 3 best scores after applying the biological filter.

Good results achieved in docking of the two systems allowed us to perform two interesting theoretical experiments. We have examined the differences in proteins interactions with SAMs built of alkanethiols with odd and even numbers of $-(CH_2)-$ groups. The results suggest that these surfaces are similar in terms of the orientation of adsorbed proteins. Small differences

in the orientation may, however, occur for some systems and under specific conditions may probably alter the protein−surface interactions.

We have also performed theoretical experiments on the tubulin−SAM interface. The orientation of tubulin which simulates MT attached to the surface via the minus or the plus end was found for all six different SAMs with the best score for the CH_3-terminated alkanethiol surface with an even number of $-(CH_2)-$ groups. We have found an observable difference between tubulin dimer interacting with either odd or even SAMs, which suggests that the tubulin affinity toward SAMs may differ depending on the length of alkanethiols. This result may motivate other experimental and theoretical studies of MT systems on SAMs as a good candidate for nanoscale interconnectors, nanowires and other electronic devices, although the question of stability of such systems remains an issue. Application of MT systems to nanoelectronics would presumably require chemical modifications of both MTs and SAMs surface with various agents to obtain strong interactions in a stable system.

The theoretical docking algorithm may become a powerful tool, particularly if combined with more sophisticated theoretical techniques (i.e., Monte Carlo simulations, molecular dynamics) and experimental methods of surface science. The results of this work should provide useful information in the field of protein−surface interfaces, developing biosensors and other bioelectronic nanodevices. It is however important to notice the lack of a reliable description of electrostatic interactions, which also may play an important role in the protein−surface interactions. Only a combination of a reliable electrostatic model and surface complementarity (i.e., MD or applying the solution Poisson−Boltzmann equation[53] to FTDock results) would allow one to fully describe the protein−surface interface.

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