Protein imaging via AFM: a feasibility study.

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Motivation

Structural biology plays a prominent role in the current research, since the understanding of biological functions of proteins is tightly linked to their structure. Classic techniques for structure determination, X-Ray diffraction and NMR, are still very expensive and time consuming, even though they have been significantly improved in recent times.

On the other hand, to acquire images of protein surfaces using the Atomic Force Microscope (AFM) can in principle save time and economical resources. Unfortunately, the use of scanning probe microscopy on biological materials is limited to membrane proteins or macromolecular aggregates, since they are both immobilized on a surface.

Soluble proteins are, on the contrary, moved by the probe interaction and cannot be scanned.

ProteoGen Bio S.rl. has patented a method able to block a hydrophilic protein on a surface without significantly alter its 3D structure. The present work is aimed at exploring which experimental observation conditions can be set to make this achievement useful to obtain protein images at a resolution interesting for biologists.

Methods

The crystallographic structure of a well known hydrophilic protein, the proteolytic enzyme Trypsin, has been downloaded from the PDB databank. A lab-made algorithm for a fine calculation of the electronic density of the molecular surface has been used to generate different solid models of this protein. This algorithm, based on an 'ab initio' approach, requires the user to set an electron density threshold, and computes the isodensity molecular surface having such threshold value.

The obtained solid models are each other different with respect to electronic density, orientation, and spatial resolution. A 3D reconstruction of the AFM tip probe is generated and, by convolving its representation with the solid model of the molecule, one obtains a simulation of a topography image obtained by AFM experimental observation. The real Trypsin protein has been engineered by using ProteoGen Bio proprietary technology, and then bound to a silicon slab. AFM observations of this sample have been made, and their relative images acquired.

A lab-made algorithm computed the mutual information between the simulated and the experimentally obtained images. The simulation parameters have been refined thanks to a multiscale optimization procedure, consisting of two steps: a first search on a pre-built simulated images archive, followed by a local iterative optimization.

Results

The binding technology by ProteoGen Bio has been proved to efficiently immobilize Trypsin on the silicon slab, making it observable with the AFM.. The computational approach is able to a priori determine the size of the optimal AFM tip to be used for imaging an assigned protein, thus maximizing the quality of the experimental image.

Moreover, it is possible to quantify the decrease in the experimental image quality dependently on a given tip size. The studies performed on Trypsin with a tip with 5 nm radius of curvature showed that, by using our approach, it is possible to identify the molecule orientation (trihedral angle). Our approach is naturally extensible to compute other characteristics of the observed molecule, such as the 3D structure preservation. Finally, it is also potentially able to discriminate among different molecules, in the case a pool of proteins or a multimolecular complex is present.

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