Understanding the formation and adsorption of 2-dimensional and 3-dimensional protein aggregates on a solid surface

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ABSTRACT

Using fluorescence microscopy combined with the FRET technique we explore the formation mechanisms of protein aggregates on surfaces. We present examples for completely different scenarios including the assembly of proteins into lose, 2-dimensional protein patches, the growth of protein aggregates on the surface, and the deposition of protein clusters from the solution onto the surface.

Keywords: Protein aggregation, FRET, SAF microscopy

1 INTRODUCTION

Protein aggregation is an important topic of current protein research as it is associated with both, a crucial step for molecular machines to exhibit biological function and a source of several human diseases including Alzheimer's disease, Parkinson's disease, and Type II diabetes. Although protein aggregation mechanisms and conditions have been comprehensively investigated, a complete explanation of the formation and the fate of protein aggregates in contact with solid functionalized interfaces is lacking. To explore protein adsorption and aggregation events on solid interfaces we apply the so-called supercritical angle fluorescence (SAF) technique which selectively collects the emission of fluorophores in close proximity to the surface [1]. Due to its high sensitivity and reliability the SAF technique was successfully applied to describe protein adsorption mechanisms through kinetic analyses [2-4] or through surface imaging [5]. In our present work we combine SAF microscopy with the Förster resonance energy transfer (FRET) technique that provides distance information in the nanometer scale. In this way we created a powerful tool to investigate protein aggregation mechanisms that can be of diverse nature (Fig. 1).

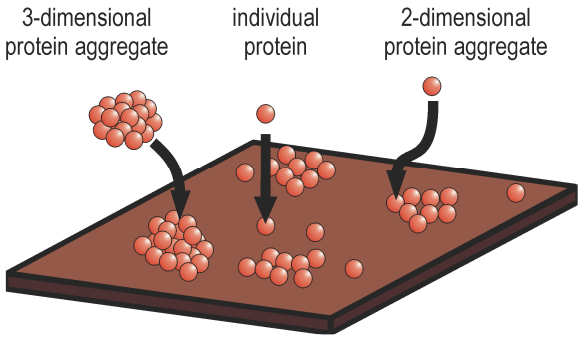

Figure 1: Schematic illustration of frequently observed surface features upon protein adsorption.

2 ASSEMBLY OF PROTEINS INTO LOSE PATCHES

If several proteins are organized in a monolayer-like, flat assembly they are typically referred to as 2-dimensional surface crystal although they do not necessarily need to be tightly packed. In our study we can show that the formation of such structures is the consequence of cooperative protein adsorption. That means proteins approaching the solid surface adsorb preferably close to other pre-adsorbed proteins as compared to unoccupied surface regions. Macroscopically these cooperative effects necessarily lead to an inhomogeneous surface distribution. Hence, when the protein occupied surface is imaged with a scanning fluorescence microscope such as the SAF microscope the formation of patches with high protein density next to regions of low protein density can be observed [5]. The microscopic surface organization of cooperatively adsorbing proteins that leads to the macroscopically observable density inhomogeneities can be explored with Monte Carlo-type simulations as demonstrated for one example in Fig. 2.

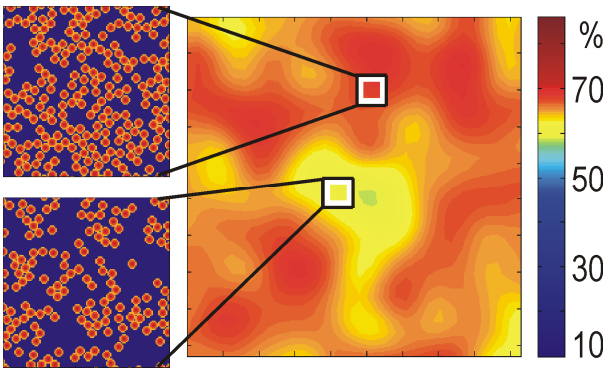

Figure 2: Representation of 2-dimensional protein assemblies (left) which necessarily lead to an inhomogeneous intensity distribution when the surface is imaged using fluorescence microscopy (right). This figure was obtained from a Monte Carlo-type simulation of cooperative protein adsorption.

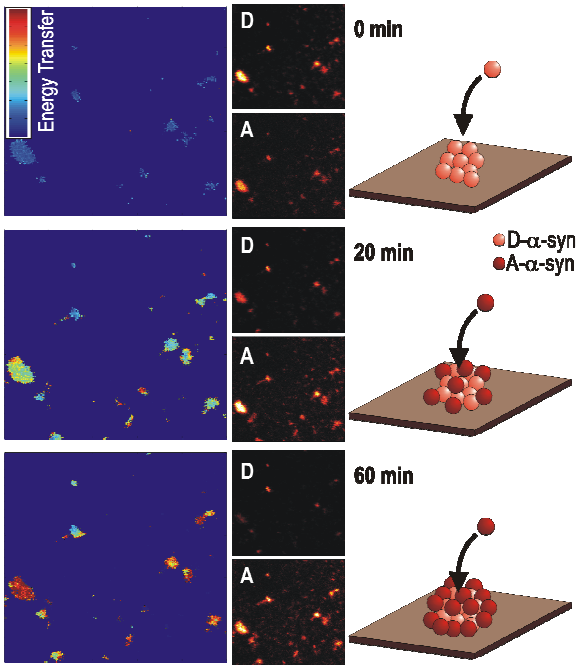

Figure 3: Experimental observation of the growth of α-synuclein aggregates on the surface. The scan images recorded with the donor channel (D), the acceptor channel (A), and the calculated FRET image (left) are presented. The scheme on the right illustrates the aggregation of donor- and acceptor labeled proteins on the surface.

3 ASSEMBLY OF PROTEINS INTO TIGHT SURFACE AGGREGATES

In contrast to lose patches proteins may also assemble into real surface aggregates in which proteins are in contact with each other. The growth of such surface clusters or surface crystals typically involves a nucleation step upon which further monomer proteins adhere. Naturally such aggregates can grow in all three dimensions which is the major difference to 2-dimensional surface assemblies. Using conventional fluorescence microscopy this form of surface aggregation can not be distinguished from the growth of lose patches of high protein density because the diffraction limit does not allow to resolve how densely proteins are packed in such a region. However, with the help of FRET measurements one can directly observe the growth of tight surface aggregates in which proteins necessarily come into contact with each other. Fig. 3 shows the continuous aggregation of the protein α-synuclein on the surface. First, donor-labeled proteins were allowed to settle and nucleate onto the surface and eventually form surface aggregates. Second, the donor-labeled proteins were replaced with acceptor labeled proteins which adhered to the donor-aggregates. Wherever donor-labeled proteins come into contact with acceptor labeled proteins the energy transfer takes place leading to a decrease of the fluorescence signal in the donor channel (D) and to an increase of the signal in the acceptor channel (A).

4 ADSORPTION OF PROTEIN CLUSTERS FROM THE SOLUTION

Another possibility how 3-dimensional aggregates evolve on surfaces consists of a deposition of clusters of several protein monomers from the solution. In a recent work we could show that in the case of BSA protein clusters composed of up to several hundreds of BSA monomers adsorb in one step onto the surface. As was also visualized by the FRET technique, these protein clusters immediately start to spread and thereby occupy a steadily increasing surface area once they are in contact with the surface (Fig. 4)[6]. Moreover, during the spreading process of the clusters pre-adsorbed protein monomers were observed to recede from the area to which the clusters expand (Fig. 5). However, a further adhesion of protein monomers to the clusters i.e., a surface growth like in the case of α-synuclein (Fig. 3), was never observed. After a sufficiently long spreading time the original shape of the protein cluster is completely disrupted. It is natural that the spreading induces a sort of flattening of the cluster such that in the end it may even resemble a 2-dimensional protein aggregate. This highlights the importance of studying the exact mechanisms of protein aggregation. The final state of a surface aggregate does not allow to draw conclusions about its evolution history.

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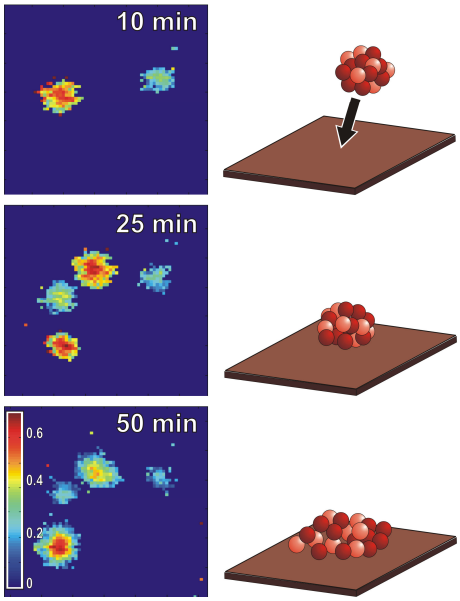

Figure 4: With the help of FRET measurements the spreading of 3-dimensional surface aggregates in time can be visualized. Protein clusters that have just been deposited from the solution onto the surface exhibit higher energy transfer rates than clusters that have been deposited earlier. The decrease of the FRET signal is a consequence of the increase of the distance between the protein monomers during the spreading process.

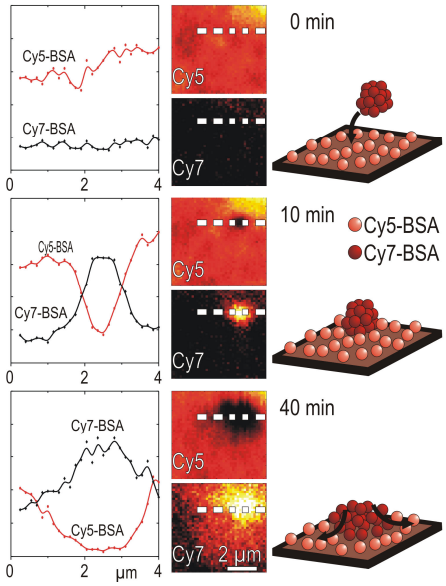

Figure 5: Experimental observation of the spreading of BSA after deposition from the solution onto the surface. Acceptor-labeled protein clusters repel pre-adsorbed donor-labeled BSA monomers without a tight contact between these two species. Left: Profile plots along the marked lines. Middle: Images of one representative protein cluster in the donor (Cy5) - and the acceptor (Cy7) - channel. Right: Schematic illustration of a cluster spreading on a monolayer of pre-adsorbed proteins.

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