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Atomic Force Microscopy and Particle Clustering Reveal Altered Photosystem II Organization in the Suppressor of Quenching 1 Mutant of Arabidopsis

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Plants are exposed to fluctuations in light and therefore need to balance productive photochemistry and dissipative photoprotection. This is achieved by regulation of the structure and organization of pigment-proteins throughout the thylakoid membrane. In particular, photosystem II (PSII) and its closely associated light-harvesting complex II (LHCII) form supercomplexes within the grana that undergo reversible molecular modifications and large-scale rearrangements to conserve such equilibrium. Photoprotection is achieved by triggering a series of dissipative or repairing reactions denominated non-photochemical quenching, NPQ. A variation of NPQ has been unveiled during the discovery of a new thylakoid protein, a suppressor of quenching, SOQ1.

Using atomic force microscopy, we characterized the structural attributes of grana thylakoids from plants lacking SOQ1 to correlate them with its role in NPQ. We developed a novel image analysis methodology that allowed us to interrogate each detected protein complex and assign its unique identity as part of a densely packed membrane. Our algorithms not only discriminate between crystalline and non-crystalline complexes, they also cluster crystalline particles in different categories. SOQ1 induces protein rearrangements that favor larger separations between photosynthetic complexes in the majority (disordered) phase, and reshapes the PSII crystallization landscape during photoprotection (high-light exposure). It is thought that SOQ1 suppresses quenching directly or indirectly within the LHCII complex. Our structural data indicate that removal of SOQ most likely weakens interactions among light-harvesting antenna complexes, consequently, the separation among supercomplexes increases while the complexes' density decreases. The structural light-induced rearrangements that we detect are distinct from known protein organizations associated with the typical heat dissipation mechanisms (qE) providing further support for a role of SOQ1 in a novel NPQ pathway.

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Tunable Binding Reactions on DNA Origami Nanostructures

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Two-dimensional DNA origami shows promise as scaffolds for the assembly of nanoscopic electrical or photonic devices and studies of individual molecular reactions. Maximal binding of functional materials to predefined sites on all origami is essential for robust applications and reliable measurements. However, the two-sided nature of the scaffold and its randomly adsorption onto surfaces have proved limiting in the production of identically oriented assemblies on practically or technologically useful solid substrates. We have discovered that the holes within DNA origami scaffolds are ~1nm, sufficiently large for the passage of ssDNA. Ligands attached to long ssDNA spacers initially on one side of the origami are thus able to go through the block to the other side, obviating any dependence on the substrate-binding side for subsequent binding reactions. Direct monitoring of single-molecule reactions by using atomic force microscopy, we find that the spacer lengths of 5 bases are enough for helping ligands, here biotins, from one side origami surface to the other side, consistent with expectations, and spacer lengths of10 bases are with the maximal streptavidin-biotin binding efficiency and rate.

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Antibody Adsorption Over Graphene: An Atomistic MD and MF-AFM Study

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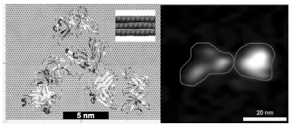
Protein-surface interaction has great technological relevance for the development of biocatalysts, implants and biosensors. Recent advances on both molecular-dynamics (MD) simulations and atomic-force-microscopy(AFM), allow studying such large systems with atomistic detail. Here we have combined MD simulations with high-resolution multi-frequency-AFM experiments to study the adsorption of the IgG antibody (150kDa) over graphene. IgG provides the majority of antibody-based immune response. Thefore studying its biocompatibility/activity over graphene is of interest to address the graphene usage as an implant material as well as to develop more sensitive immunoassays.

We have developed a protocol combining steered-MD simulations and long (>150ns) equilibration runs to address several key open questions concerning protein adsorption: the interaction mechanisms behind the adsorption, the role of the water molecules in such process, and under which conditions the protein unfolds due to the interaction with the substrate. Moreover we determine the most favorable adsorption orientation of the IgG, which in turn allows us to set up a strategy to control the IgG adsorption over graphene. Both the bioactivity and adsorption orientation statistics are in good agreement with experiments.



[1] Antibody adsorption over graphene; submitted to NanoLetters

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Molecular Mechanisms of Misfolding of Amyloid Peptides

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The current model for the development of Alzheimer's, Parkinson's, Huntington's, prion, and other neurodegenerative diseases involves protein misfolding as the early step followed by spontaneous aggregation, with specific proteins identified as the primary initiators for disease development. Therefore, elucidating the properties of the disease-prone misfolded states, understanding the mechanism of their formation, and identification of their most toxic forms will open prospects for the development of early diagnostics and specific therapeutics for these diseases. We have developed single molecule AFM force spectroscopy (SMFS) experimental approach enabling us to probe interprotein interactions and to identify those interactions that correspond to misfolded protein states. Using SMFS, we have discovered that the misfolded dimers are very stable and have a lifetime in a second time scale. Such a long lifetime of dimers suggests that the formation of dimers is the mechanism by which the protein misfolded state is stabilized. We hypothesize that the formation of highly stable misfolded dimers is a critical step in the entire process of the peptide self-assembly into aggregates. The Molecular Dynamics (MD) simulation performed at the μs timescale demonstrated that isolated non-structured monomer upon approaching to each other changed dramatically their initial conformation and formed dimers with antiparallel beta-sheet structures. Steered MD approach showed that the dimers dissociated cooperatively resulting in a sharp rupture peak corresponding to breakage of the beta-sheet structure. Altogether, the SMFS experimental study and computational analysis revealed a critical role of the interpetide interaction in the misfolding process and highlighting the key role of the dimerization in the amyloid aggregation process.

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