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Title of the doctoral dissertation: **"Single-molecule fluorescence on multilayer graphene and graphene boron nitride hybrids"**

Abstract

Graphene, renowned for its exceptional optical and electronic properties, has emerged as a pivotal material for understanding and controlling molecular interactions at the nanoscale. Its unique characteristics are particularly significant for advancements in biosensing, super-resolution microscopy, and quantum technologies. Graphene energy transfer (GET), a highly distance-dependent phenomenon with an active range of up to 40 nm, provides precise control over molecular interactions. DNA origami nanostructures serve as scaffolds in this context, enabling the precise placement of fluorophores relative to graphene with nanometric axial resolution below 3 nm. These constructs underpin the development of biosensors for single-molecule detection and real-time studies of biomolecular dynamics and interactions.

This thesis advances the field by introducing complex platforms composed of two-dimensional materials, extending the capabilities of graphene monolayers. An optimized protocol for fabricating high-quality graphene monolayer on glass substrates enabled the development of multilayer graphene and heterostructures with tunable properties. Systematic investigations of mono-, bi-, and trilayer graphene revealed additive effects of multiple layers on fluorescence quenching efficiency, thereby extending the dynamic range of GET. Experimental findings were validated by a theoretical model describing the influence of graphene layer numbers on energy transfer dynamics.

The integration of graphene with hexagonal boron nitride (hBN) further enhanced the performance of these platforms. hBN was selected for its exceptional compatibility with graphene, offering chemical inertness and atomic-level flatness. DNA origami nanostructures were successfully immobilized on hBN surfaces, with hBN functioning as an ultra-thin spacer that preserved the photophysical properties of dye molecules without affecting graphene's energy transfer efficiency. These heterostructures exhibited exceptional stability and reproducibility, enabling atomic-distance sensitivity and precise nanoscale control. Using hBN as an inert spacer on graphene, the recently discovered vertical alignment of DNA molecules on graphene was successfully

demonstrated on hBN. The thickness of the hBN spacer with a resolution of 0.1 nm was measured using GET, highlighting the platform's potential for precision applications at the nanoscale.

By integrating multilayer graphene and graphene-hBN heterostructures, this thesis significantly advances the application of graphene-based systems in biosensing, single-molecule imaging, and hybrid quantum sensing technologies. The results demonstrate the potential of these platforms to detect weak biomolecular interactions, fine-tune energy transfer processes, and achieve high spatial and temporal resolution. Collectively, these findings pave the way for the next-generation fluorescence-based studies and open new horizons in nanotechnology and molecular biology.