DOCTORAL THESIS

Analysis of biomolecules by total internal reflection fluorescence microscopy

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Analysis of Biomolecules by

Total Internal Reflection Fluorescence Microscopy

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ABSTRACT

Total internal reflection fluorescence microscopy (TIRFM) has been widely applied in the study of biological samples. Unlike the costly, sample-intensive and pretreatment-required conventional bioanalytical assays, TIRFM offers a simple but ultrasensitive detection platform for the study of biomolecules in single-molecule level. This thesis presented the principle and application of TIRFM systems for the analysis of single biomolecules. Four research works on the detection, quantification, observation and manipulation of biomolecules with the aid of TIRFM were described herein respectively.

In Chapter 2, a pretreatment-free miRNA detection assay in single-molecule level with TIRFM was presented. MicroRNAs express differently in normal and cancerous tissues and thus are regarded as potent cancer biomarkers for early diagnosis. However, the short length and low abundance of miRNAs have brought challenges to the established detection assay in terms of sensitivity and selectivity. The established TIRFM-based assay was applied in the quantification of miRNAs among cancerous and normal cell lines respectively. The results agreed very well with those from the prevalent real-time polymerase chain reaction analysis, showing that the assay is of high potential for applications in miRNA expression profiling and early cancer diagnosis.

In Chapter 3, the first application of Group 9 metal complexes (*i.e.* iridium (III) and rhodium (III)) as inhibitors of amyloid fibrillogenesis and as luminescent probes for beta-amyloid (1–40) ($A\beta_{1-40}$) peptide was reported. These complexes contained aromatic co-ligands to interact with the hydrophobic residues around the *N*-terminal domain of the $A\beta_{1-40}$ peptide, as well as solvato co-ligands to allow coordinative bond formation with histidine residues. TIRFM was applied to monitor and estimate the growth of Aβ fibrils in the presence of metal complexes. We demonstrated that these complexes could inhibit $A\beta_{1-40}$ peptide aggregation *in vitro*, with potency superior to previous metal-based inhibitors reported. Furthermore, we have demonstrated the first example of luminescent detection of $A\beta_{1-40}$ peptides by transition metal complexes.

In Chapter 4, the effect of nanoparticles (NPs) of various sizes and surface functionalities on the self-assembling fibrillation of $A\beta_{1-40}$ was investigated with TIRFM. Dramatically, slight change in sizes of functionalized quantum dots (QD) showed obvious contrary effect on beta-amyloid fibrillation even though the concentration of QD was $1 / 5000$ of the peptide. We proposed that the dramatic effect was induced by the critical alteration of the charge-to-surface area ratio of the NPs and the interaction between QDs and Aβ of approximate sizes. On the other hand, gold nanoparticles (AuNPs) with different functionality and surface charges further extended the current study. There was no significant variation in the fibrillation rate and fibril lengths for Aβ growing both in the presence and absence AuNP and hence we attributed that significant increase in colloidal surface area of AuNP abated the charge effect and thus reduces the interactions between Aβ and nanoparticles.

In Chapter 5, a one-dimensional nanofibrillar array formed by the co-assembly of native and biotin-functionalized beta-amyloid (Aβ) peptide was developed for biomolecule sensing. With the presence of biotin moiety, a variety of biomolecular probes can be conjugated onto the nanofibrils and thus converting the protein assembly into a miniature biosensor. In this work, DNA probes were immobilized onto the fibril for the detection of complementary DNA sequences. The as-developed 'DNA-nanoarray' achieved a detection limit at sub-attomole level. This highly sensitive yet simple assay required trace amount of sample consumption (< 10 μL) and was pretreatment-free. In addition, we reported the preparation of alternate-segmented amyloid nanofibrils with multifunctionality. The fibrils hereby served as an encoded template that can be visualized with various fluorescence labeling dyes for barcode recognition purpose, and hence multiplex detection of biomolecules was achieved. Regarding that each protein nanofibril represented a single detection platform, a large number of single fibrils were simultaneously monitored with the dual-colour TIRFM in a high-throughput manner.

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