

SINGLE-MOLECULE IMMUNOASSAY OF TUMOR MARKERS IN T-CELL APOPTOSIS

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From an immunology perspective, cancer cells (e.g., melanoma cells) are viewed as altered self-cells that have escaped normal growth-regulating mechanisms. Two questions which immediately present themselves are *why* and *how* such tumor cells can escape the sufferer's immune system. Recent research suggests that tumor cells kill the immune cells (T cells) by expressing Fas ligand (FasL), a cell surface protein, and using FasL as *an antigen* to attack the immune cells. When FasL binds with Fas (receptors on a T cell), the Fas protein triggers a series of events within the T cell that *induce* the T cell to *self-destruct* through a process known as *apoptosis*. The detailed mechanisms for this self-destruction are not yet clear.

Biochemical analyses at the *single-molecule* level present unique opportunities to study and characterize the chemical and physical properties of *individual* molecules. Potential applications in biomedicine that are *unique* to *single-molecule* research include tracking of *individual* steps in a sequence of biological events, *early* diagnosis of diseases and manipulating *individual* biological reactions. These same capabilities are capitalized on to lay bare the mechanism for T cell apoptosis.

For the first time, the T-cell surface proteins (e.g., Fas, FasL) are continuously monitored in real-time at single-molecule level using single-molecule laser-induced fluorescence microscopy. Binding constants and binding ratio of Fas with FasL are studied using electrogenerated chemiluminescence. Our experimental results demonstrate the promising of real-time monitoring of T cell apoptosis *one-molecule at a time* and imply to depict of such a critical biological event at *single-molecule limit*. The detailed experimental configurations and the prospective applications of these studies will be discussed.