Monitoring single antibody binding events

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Application Note

Introduction

Early detection of lung cancer is crucial for therapy and survival of patients. Since the discovery of p53 antibodies as early markers of cancer diseases it is of interest to find a reliable method of monitoring the presence of p53 auto-antibodies in human blood sera. Up to now false positive signals in the heterogeneous standard assays, such as ELISA or western blotting, circumvent a broad application of p53 auto-antibody detection in cancer prevention. The combination of recently developed sensor epitopes with ultrasensitive detection enables the visualization of individual antibody binding events and minimizes the need for washing steps.

Fluorescent sensor epitopes

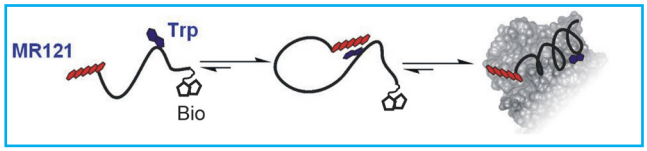

Figure 1

For the p53 assay, an immunodominant peptide epitope of p53 protein was used. The 15 amino acid residue epitope carries a tryptophan in its natural sequence that functions as a fluorescence quencher of the dye MR121.[1, 2] In the unbound form the peptide has a large conformational freedom favoring a conformation in which MR121 is quenched by tryptophan. Quenching occurs via photoinduced electron transfer (PET) from tryptophan to MR121 upon contact formation (Figure 1). Binding of the antibody separates the tryptophan from MR121 and is subsequently hidden in the binding pocket of the antibody. Concomitantly, fluorescence of the MR121 is restored and a 5-10 fold fluorescence increase is observed.[3, 4]

Heterogeneous assay for p53 detection

To exploit the sensor in a heterogenous assay format with reduced need for washing steps, epitopes were immobilized on BSA passivated coverslides with the aid of a biotin tag.[5] The signal-to-noise ratio of individual epitopes was optimized by using objective type total internal reflection microscopy in combination with detection by a back-illuminated, electron-multiplying CCD (iXon DV887 DCS-BV from Andor Technology) (Figure 2). Correctly immobilized epitopes yield dim spots in the fluorescence images (Figure 2A, red rectangle). After 20 s, a diluted solution of antibody was added. Upon binding to an antibody the fluorescence of individual epitopes is unquenched and spots become brighter (Figure 2B). Finally, the fluorescent dye is photobleached (after about 60 s for the molecule shown in Figure 2B). Figure 2C shows the number of spots as a function of time. After antibody addition, the number of spots recognized by the spot finding algorithm increases until all epitopes have bound after ~70 s. Subsequently the number of spots decreases as a result of photobleaching.

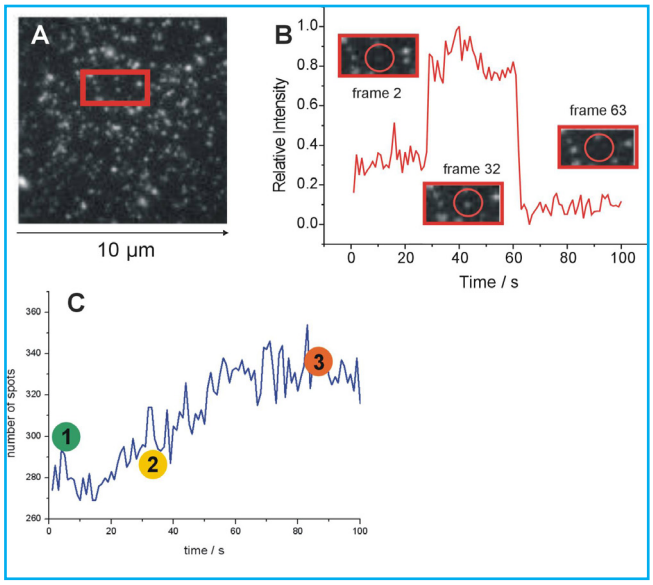

Figure 2:

A Total Internal Reflection Microscopy of immobilized epitopes.

B From sequences of images, time trajectories of individual epitopes are reconstructed and assigned to unbound epitopes, bound epitopes and photobleached epitopes.

C After addition of antibodies (frame 20) the number of strongly fluorescent spots increases until saturation is reached after ~70 s.

Conclusion

Based on the development of fluorescent sensor epitopes in combination with ultra sensitive detection, quantum yield changes of single molecules could be attributed to single antibody binding events. The research opens up exciting possibilities with respect to miniaturized antibody assays which circumvent the need for additional washing steps and calibrate themselves by exploiting the digital nature of single-molecule events.

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References

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