Sensitive discrimination of single nucleotide variant using PDA microtube waveguide system with heterogeneous CHA amplification and competitive inhibition strategy

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Single-nucleotide difference in DNA or RNA sequences can provide important biological information, therefore reliably distinguishing such single nucleotide variants is important for biomedical and clinical applications. Here, we present a novel PDA microtube waveguide system for distinguishing single nucleotide variants by combining heterogeneous CHA amplification and competitive inhibition strategy.[1-2] PDA microtube waveguide system with heterogeneous CHA amplification designs can facilitate target-triggered CHA amplification and strand displacement reactions, while suppress the background leakage simultaneously.[3] By combination with competitive inhibition strategy, the single nucleotide selectivity can be enhanced nearly 10-fold compared to standard hybridization probes only with heterogeneous CHA amplification design. This discrimination strategy have been successfully applied to distinguish only a single base position difference for different members of the let-7 microRNA family. The discrimination factor of the probes still maintained about 10 even in ultralow concentration, e.g. 50fM. More importantly, the proposed probes can be applied directly in clinical serum of cancer patients, favoring the cancer diagnosis of lung, ovarian, pancreatic and gastric cancer patients from those of healthy human beings, by distinguishing the actual expression level of let-7a, let-7c, let-7e and let-7f in clinical serum simultaneously.

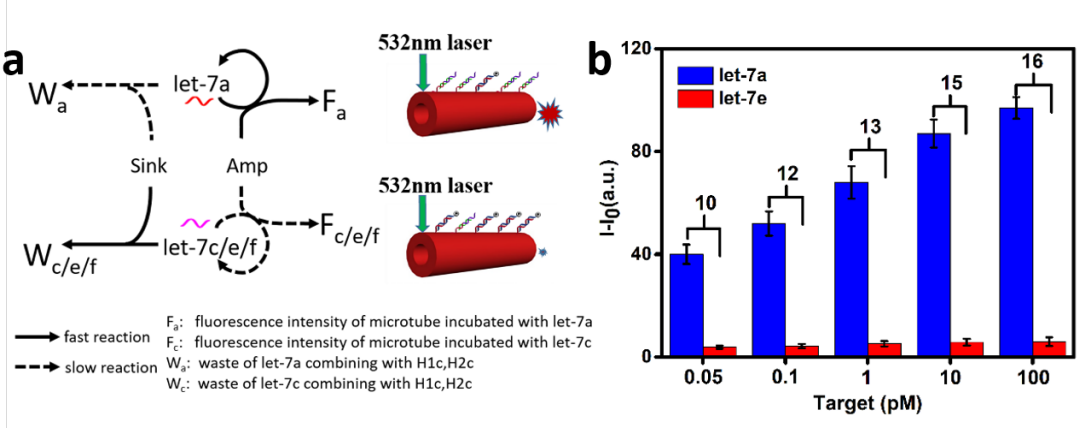

Figure 1: (a) Scheme of heterogeneous CHA amplification and competitive inhibition strategy and (b) the discrimination factor at various concentrations.

References

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