Protein-small molecule interaction assay based on DNA polymerase activity modulated by terminal protection

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A detection method for protein-small molecule interactions based on the regulation of DNA polymerase activity using small molecule-linked DNA polymerase aptamer is presented. In the detection method, a blocker probe (BP), whose end is terminally modified by biotin, is designed to bind a polymerase-specific aptamer probe. Upon binding of the probe to the target protein, the target protein provides protection to a BP against the nuclease-catalyzed degradation due to the steric hindrance. The protected blocker probe is designed to bind polymerase-specific aptamer probe (AP) and stops the inhibition of DNA polymerase activity. Thus, the primer extension reaction is catalyzed by the active DNA polymerase on the primer/template complex in conjugation with the TaqMan probe. The hydrolyzed TaqMan probe leads to a significantly enhanced fluorescence signal and can be easily detected by a real-time PCR instrument. Interaction of a model target protein, streptavidin, with its corresponding small molecule, biotin, was achieved using this detection method. The practical applicability of this method was also successfully verified by the reliable detection of streptavidin in human serum.