

## **Supporting Information for:**

# **Universal Aptameric System for Highly-Sensitive Detection of Protein Based on Structure-Switching Triggered Rolling Circle Amplification**

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### **Incubation Time for RCA Reaction**

In order to obtain high assay sensitivity, the time dependence of RCA reaction was also investigated. After ligation, the RCA reaction was allowed to proceed at 37 °C for a specific period. Then, the resulting sample was immediately incubated in a water bath at 65 °C for 10 min to inactivate the Phi29 polymerase. The reaction of RCA is thereupon terminated. After being cooled to room temperature, the resulting solution was used for subsequent experiments. The results are shown in Figure S1. As can be seen, the fluorescence intensity increased rapidly with the incubation time because longer RCA products can be gained. The maximum fluorescence intensity was observed when the RCA reaction was maintained for 60 min. However, the fluorescence signal decreases rather than increases when the RCA reaction is allowed to continue. This observation might be attributed to the fact that the RCA products got entangled with each other when the reaction time went beyond 60 min. The entangled

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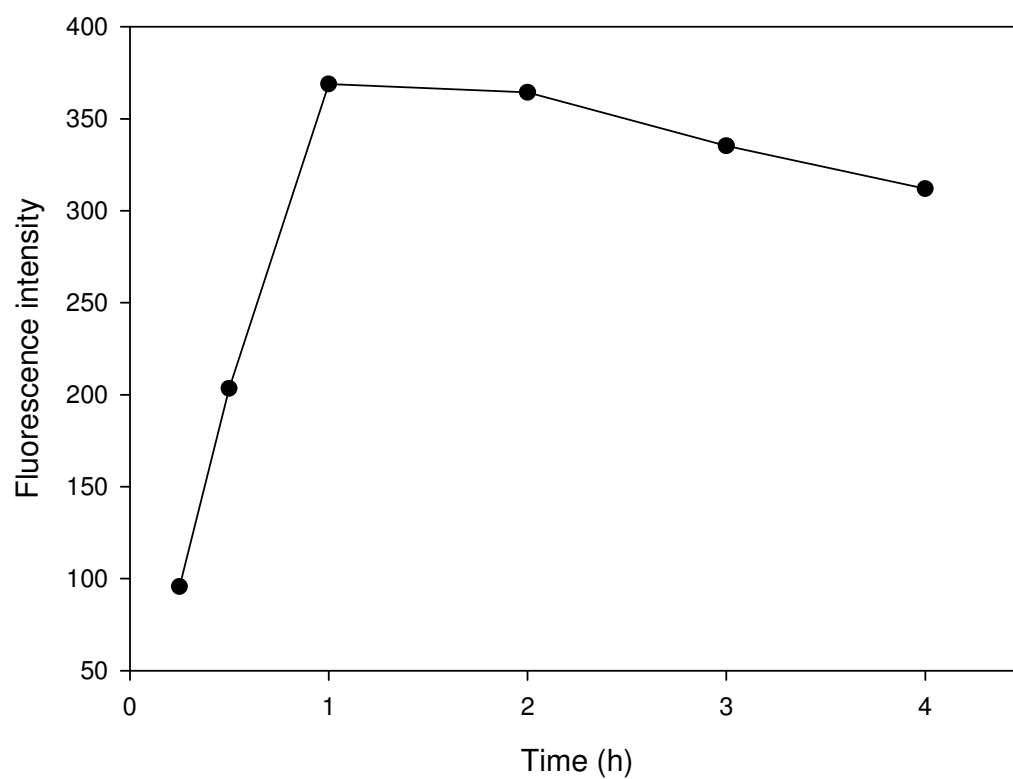
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products hindered their hybridization with the detection probes to some extent.

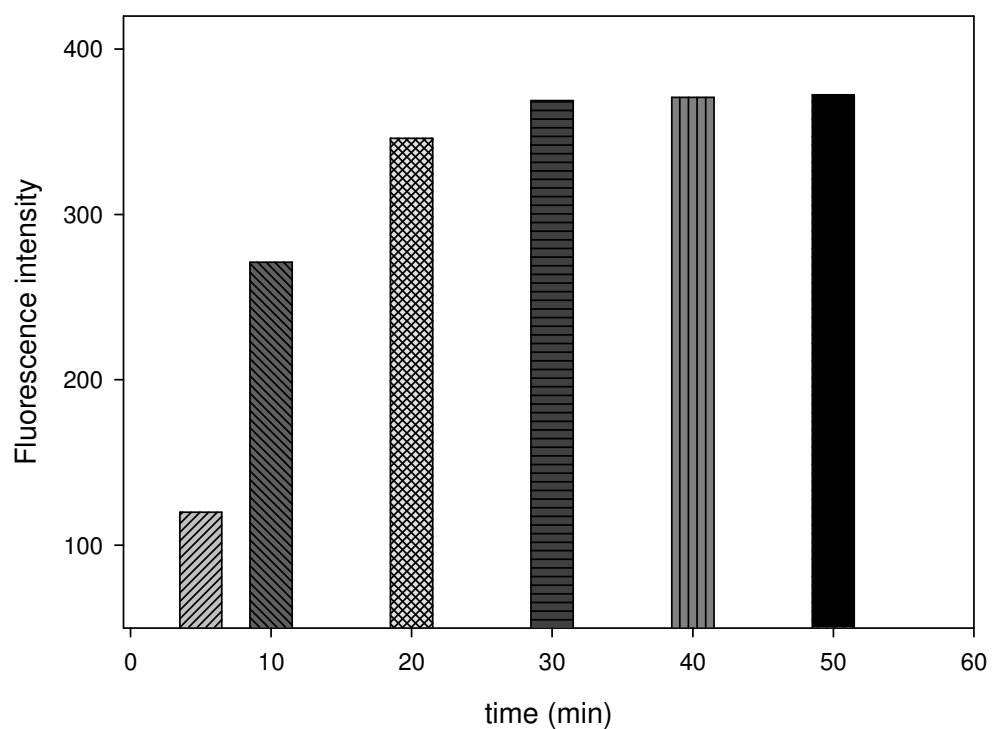
Therefore, 60 min was chosen as the reaction time for RCA.

### **Incubation Time for Target Binding**

The incubation time can influence the binding of target to aptamer, leading to the change in fluorescence signal. In order to obtain a desirable fluorescence response, the incubation time for target binding is investigated, and the results were shown in Figure S2. One can notice that the fluorescence signal increases with the increment of incubation time up to 30 min and then levels off to a constant, indicating that binding reaction was complete. Thus, the incubation time of 30 min was used for the target/aptamer binding in this work.



**Figure S1.** Influence of the incubation time for RCA reaction on the intensity of fluorescence response. The target detections were carried out according to the procedure described in Experimental section except for the RCA reaction time. The concentration of target protein was 8.4 nM.



**Figure S2.** Influence of the incubation time for analyte binding on the fluorescence signal. The target detections were carried out according to the procedure described in Experimental section except for the target/aptamer binding time. The concentration of target protein involved in this section was 8.4 nM.