Supplementary Information

Amplifying fluorescence signal contrast of aptamer-modified microsphere inspired by whispering-gallery mode lasing

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Table S1. Sequences of DNA oligomers used in this study, with the 15-meric thrombin-binding aptamer (TBA) sequence underlined.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sequence</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy5-15mer</td>
<td>Cy5-5’-AAA GGA TAA TGG CCA-3’-NH$_2$</td>
<td></td>
</tr>
<tr>
<td>ssTBA</td>
<td>NH$_2$-5’-TTC ACT GTG GTT GGT GTG GTT GG-3’</td>
<td>ssTBA bearing an amino group</td>
</tr>
<tr>
<td>NH$_2$-com</td>
<td>5’-CCA ACC ACA GTG TT-3’-NH$_2$</td>
<td>Complementary strand of ssTBA bearing an amino group</td>
</tr>
<tr>
<td>A488-com</td>
<td>5’-CCA ACC ACA GTG-3’-A488</td>
<td>Complementary strand of ssTBA bearing a green fluorophore</td>
</tr>
<tr>
<td>1</td>
<td>5’-TTC ACT GTG GTT GGT GTG GTT GG-3’</td>
<td>ssTBA</td>
</tr>
<tr>
<td>2</td>
<td>5’-CCA ACC ACA GTG-3’</td>
<td>Complementary strand of ssTBA</td>
</tr>
</tbody>
</table>
Figure S1. Representative works of WGM fluorescence emitters. The green-colored areas indicate the gain media (for example, fluorophore or fluorophore-containing solution) or pump source. (a)-(d) show the conventional WGM cavities: (a) a spherically shaped structure connected by a pump optics (for example, an optical fiber),\textsuperscript{1,2} (b) optofluidic laser that allows continuous flowing of a sample solution,\textsuperscript{3,4} (c) molecular self-assembly and crystal,\textsuperscript{5,6} and (d) fluorophore-doped microsphere (left) and fluorophore-embedded polymer bead (right).\textsuperscript{7-9} Furthermore, fluorophore-containing liquids are also recently exploited as WGM cavities such as lipid droplet and ring-shaped dried droplet.\textsuperscript{8,10}
Figure S2. FT-IR spectra of bare (a) and APTES-modified (b) glass microspheres. Whereas only Si–O–Si vibrations at 1034 cm\(^{-1}\) were observed for non-modified glass microspheres, a hypochromic shift of the Si–O–Si peak and the appearance of a C–H vibration at \(\sim 2800\) cm\(^{-1}\) were observed for the APTES-modified sample, confirming that APTES was successfully grafted on the surface of glass microspheres.
Figure S3. (a) Si 2p and (b) N 1s XPS spectra of bare (black), APTES-modified (red), and PDC-modified (blue) glass microspheres. APTES- and PDC-modified microspheres showed N 1s peaks, implying the presence of nitrogen on their surface. Peak area comparison allowed the N 1s and Si 2p ratios for APTES- and PDC-modified glass microspheres to be calculated as 0.335 and 0.392, respectively.

Figure S4. Colorimetric changes of 10 wt% ethanolic ninhydrin suspensions containing (a) bare, (b) APTES-modified, and (c) PDC-modified glass microspheres. Ninhydrin can selectively react with primary and secondary amines, resulting in yellow-to-blue color changes. Thus, primary amine-functionalized APTES-modified glass microspheres induced an instantaneous color change of the ninhydrin solution, whereas the yellow color of suspended PDC-modified glass microspheres was almost identical to that of a suspension of bare glass microspheres.
Figure S5. (a) Fluorescence microscopic image of Cy5-15mer-labeled glass microspheres ($\lambda_{ex} = 640$ nm). (b) Fluorescence spectrum obtained by excitation at the edge of the microsphere, as indicated by an arrow in Figure S5a. Almost all microspheres were well labeled with Cy5-15mer, confirming successful PDC grafting on the microsphere surface.
Figure S6. Fluorescence spectra of polystyrene beads (a) before and (b) after labeling with a SNARF dye bearing an NHS ester moiety. Upon excitation at 532 nm, both bead types exhibited intrinsic emission as well as Raman scattering, probably due to the presence of styrene groups. Although a nearly saturated reactive dye solution was used for labeling, the labeling efficiency was insufficient for amplified stimulated emission.
Figure S7. CD spectra of 5 μM ssTBA and dsTBA (e.g. double-stranded DNA composed of 1 and 2, Table S1). In the presence of potassium ions, the guanine-rich TBA oligomer forms a G-quadruplex structure capable of selective thrombin binding. The addition of KCl significantly affected the CD signal of ssTBA (red to black), with the negative and positive maxima observed at 265 and 293 nm, respectively, indicating G-quadruplex structure formation. Conversely, no such changes were observed for ds-TBA, implying that double-stranded DNA does not dissociate and spontaneously form the G-quadruplex structure in the presence of potassium ions. Thus, additional stimuli are required for the dissociation of dsTBA and the corresponding thrombin recognition.
Figure S8. Dependence of dsTBA absorbance at 260 nm on temperature in the absence (red) and presence (black) of potassium ions. Previous studies suggested that double-stranded TBA forms a G-quadruplex structure at a temperature close to its melting point. As shown in this figure, the absence/presence of KCl resulted in an approximately 17 °C difference of the above temperature. This value is reasonable, since the thermodynamic stability of double-stranded DNA increases with increasing ionic strength. As a result, all thrombin assays using dsTBA microspheres were carried out at 37 °C to induce the spontaneous dissociation of dsTBA and simulate a biologically relevant environment.
Figure S9. CD spectra of 5 μM dsTBA after 1-h incubation at 37 °C in 100 mM KCl solutions with various concentrations of thrombin. Addition of 5 μM thrombin resulted in a slight red shift of the CD signal, indicating the partial dissociation and G-quadruplex formation of dsTBA.
References


