Supporting Information

Label-free microRNA detection using a locked-to-unlocked transforming system assembled by microfluidics

Xuting Liu,[a] Yi Zhen,[a] Nengsheng Ye*[a] and Lu Zhang*[a]

[a]Department of Chemistry, Capital Normal University, Beijing 100048, P. R. China.
E-mail: zhangl@cnu.edu.cn

Table S1. Sequences of microRNAs

MicroRNA	Sequence (5' to 3')
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
SmiRNA-21	UAGCUUAUAAGACUGAUGUUGA

Table S2. Sequences of DNA probes

DNA	Sequence (5' to 3')
G4m	Biotin-TGGGTAGGGCGGGTTGGGGTTCAACATCAGTCTGATAAGCTA-Biotin
G4m-1	TGGGTAGGGCGGGTTGGGGTTCAACATCAGTCTGATAAGCTA-Biotin
G4m-2	Biotin-TGGGTAGGGCGGGTTGGGGTTCAACATCAGTCTGATAAGCTA
DNA1	CAAGTTAAAGGGGCTATAGGT
DNA2	Biotin-ACCTATAGCCCCTTTAACTTG
DNA3	Biotin-CAAGTTAAAGGGGCTATAGGT

Table S3. The flow rate ratio of MBs to G4m

Flow rate ratio (MBs / G4m)	Flow rate of MBs on both sides	Flow rate of G4m intermediate flow	Concentration of G4m
1	25000 μL/h	25000 μL/h	150 pM
2	25000 μL/h	12500 μL/h	300 pM
4	25000 μL/h	6250 μL/h	600 pM
8	25000 μL/h	3125 μL/h	1200 pM
16	25000 μL/h	1562.5 μL/h	2400 pM

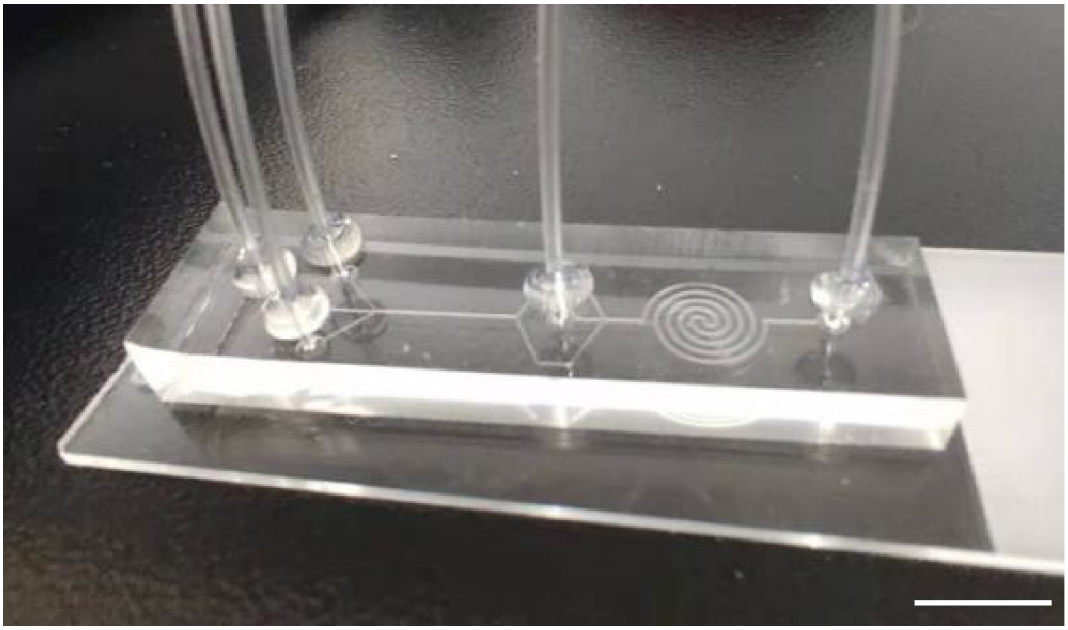

Figure S1. Photo of the microfluidic chip used to assemble the locked-to-unlocked system. There are four entrances on the left and one exit on the right. Scale bar, 1 cm.

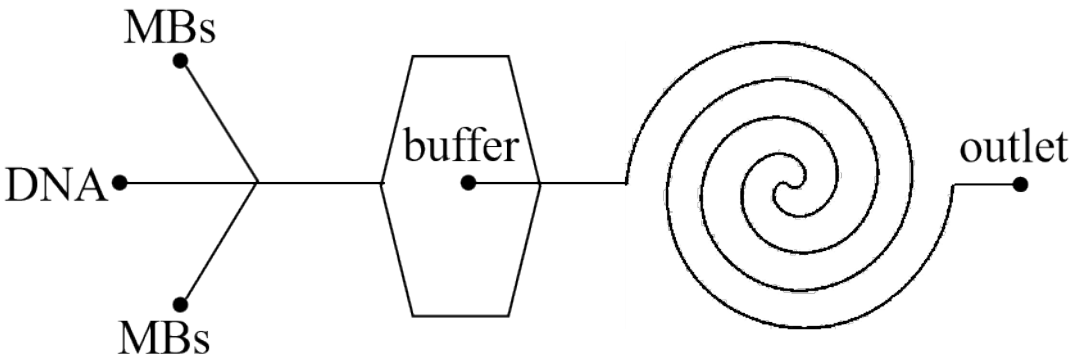

Figure S2. Schematic diagram of the microchannels in the microfluidic chip. The MBs solution is injected through a pump from both sides. The DNA and buffer are injected from the front and middle end of the chip using another pump. The MBs-DNA complexes were collected from the outlet.

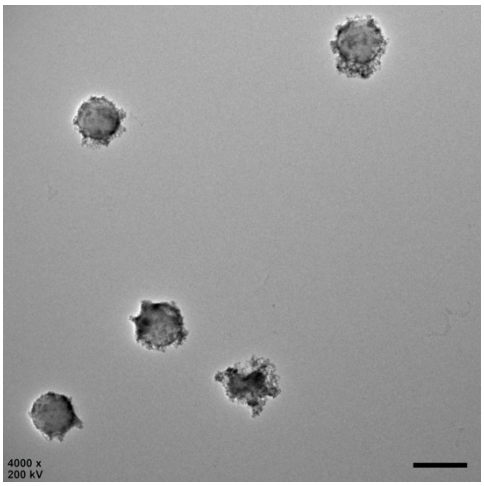

Figure S3. The TEM image of the assembled MBs-G4m complexes. Scale bar, 1 μm.

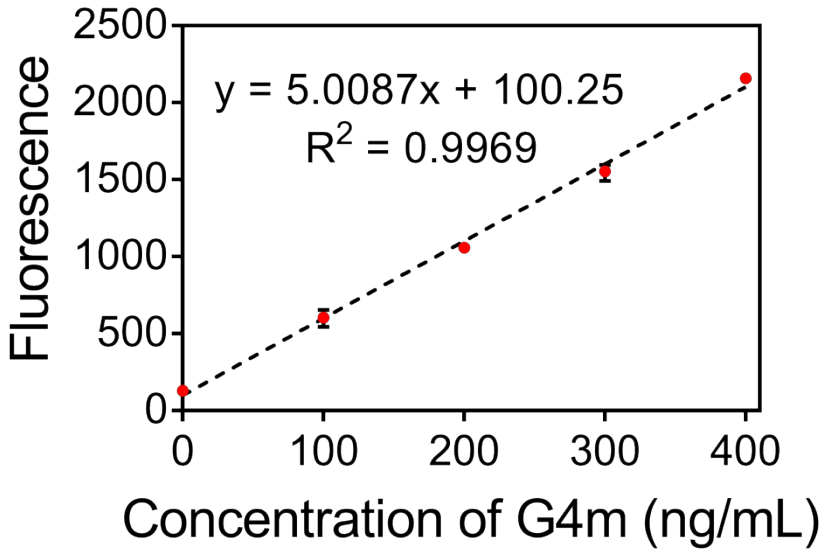

Figure S4. A standard curve used to calculate the concentration of G4m in the supernatant, which is positive correlated with the fluorescence after Oligreen staining. The amount of G4m connected to the MBs can be calculated by subtracting the amount of G4m in the supernatant from the total amount of G4m. The G4m binding efficiency equals to the ratio of the amount of G4m connected to the MBs to the total amount of G4m.

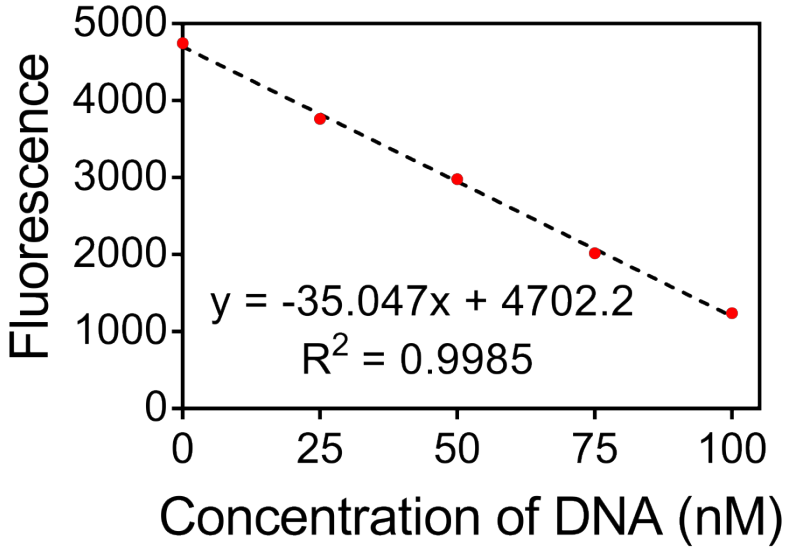

Figure S5. A standard curve used to calculate the biotin unexposed ratio. The horizontal axis is the concentration of biotin-modified DNA added to the tube to hybridize to the complementary sequences on the surface of MBs. For the complementary sequences on the surface of MBs is excessive, the concentration of biotin-modified DNA indicates the amount of biotin exposed on the MBs surface. The vertical axis is the fluorescent intensity of cy3-modified streptavidin in the supernatant, which is negatively correlated with the amount of the biotin exposed on the MBs surface. The amount of exposed biotin can be calculated by the standard curve. The amount of unexposed biotin can be calculated by subtracting the amount of exposed biotin from the total amount of biotin. The biotin unexposed ratio equals to the ratio of the amount of unexposed biotin to the total amount of biotin.

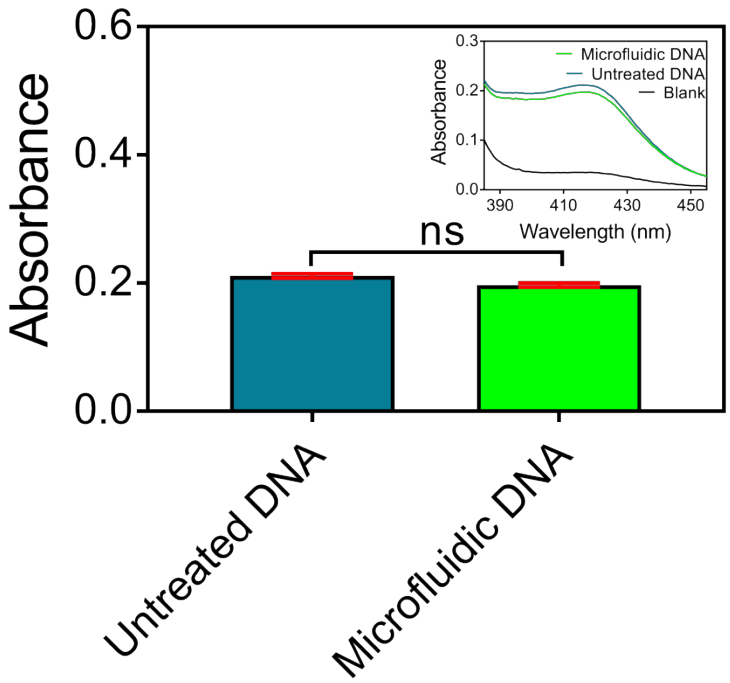

Figure S6. DNA was injected into the microfluidic chip using an injection pump to test whether DNA was broken in the flow field. The DNA used was G4m, the flow rate ratio was 4 and the temperature was 26.1 °C. The absorbance of the microfluidic-treated DNA is not significantly different from that of the untreated DNA, which proved that DNA is not broken in the microfluidic chip.

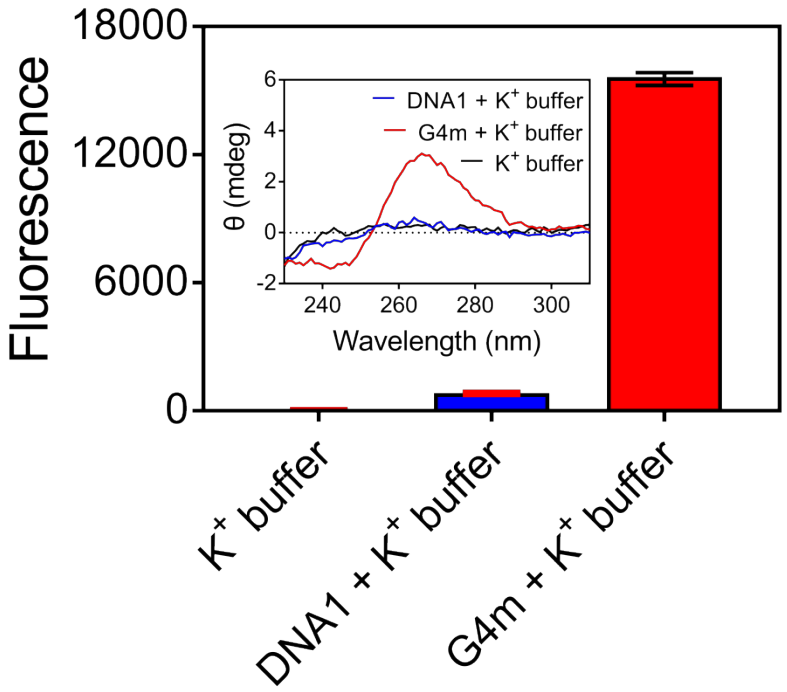

Figure S7. Fluorescence intensities and circular dichromatic spectra of G4m (G-quadruplex structure in K^+ buffer) and DNA1 (without G-quadruplex structure) combined with ThT respectively. ThT fluorescence is significantly enhanced in the presence of G-quadruplex structure.

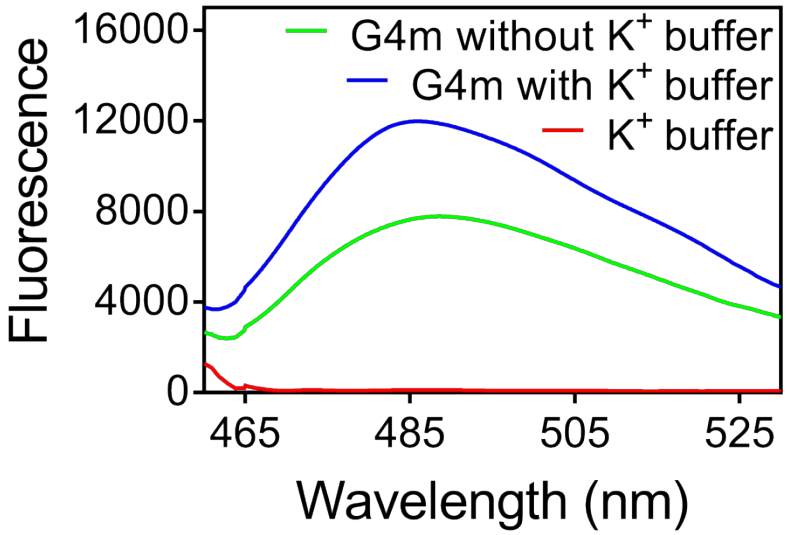

Figure S8. Fluorescence spectra of ThT bound with G4m. ThT could emit fluorescence after binding to G4m and the presence of potassium ion (K^+) could make the fluorescence stronger.

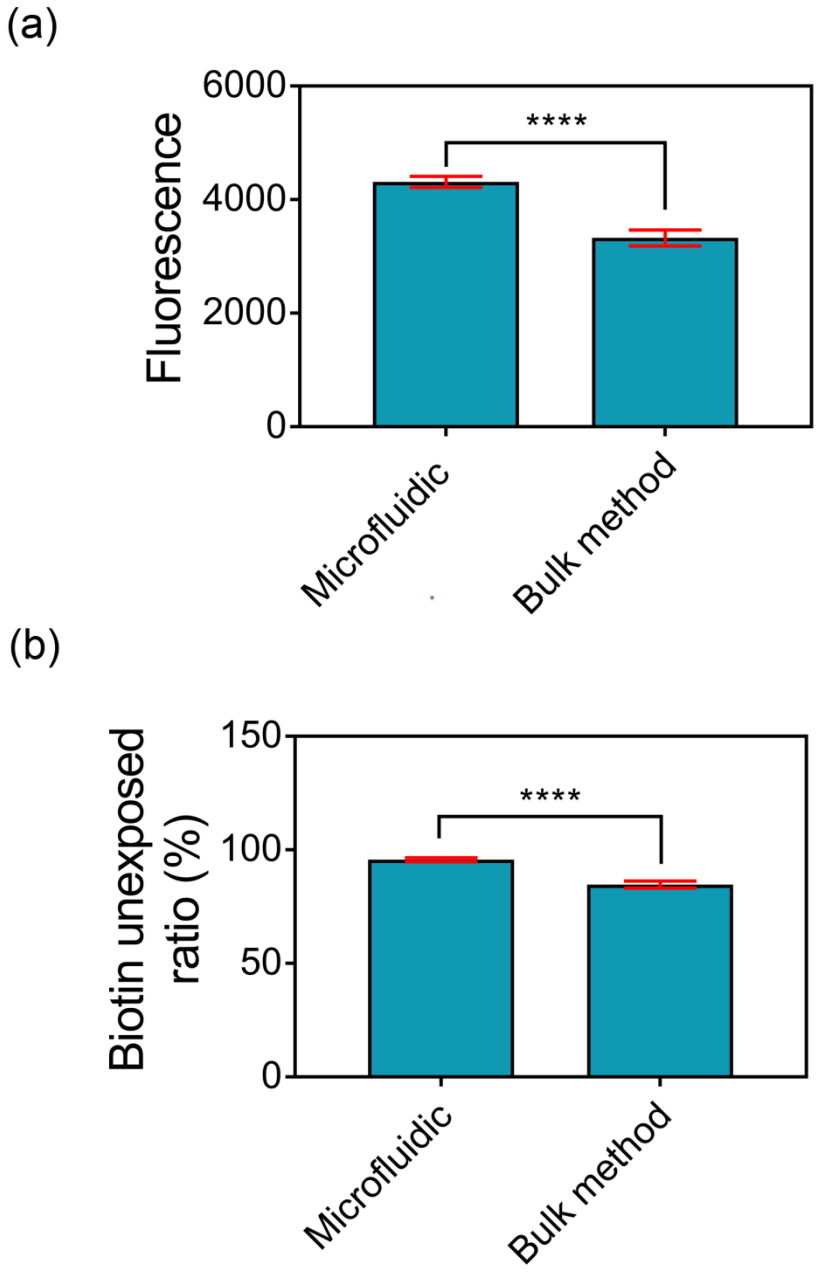

Figure S9. Comparison of microfluidic method and bulk method for assembly of MBs-G4m complexes. Fluorescence intensity of the unbound cy3-streptavidin (a) were used to calculate the biotin unexposed ratio (b). There is a significant difference between the two methods. **** P<0.0001.

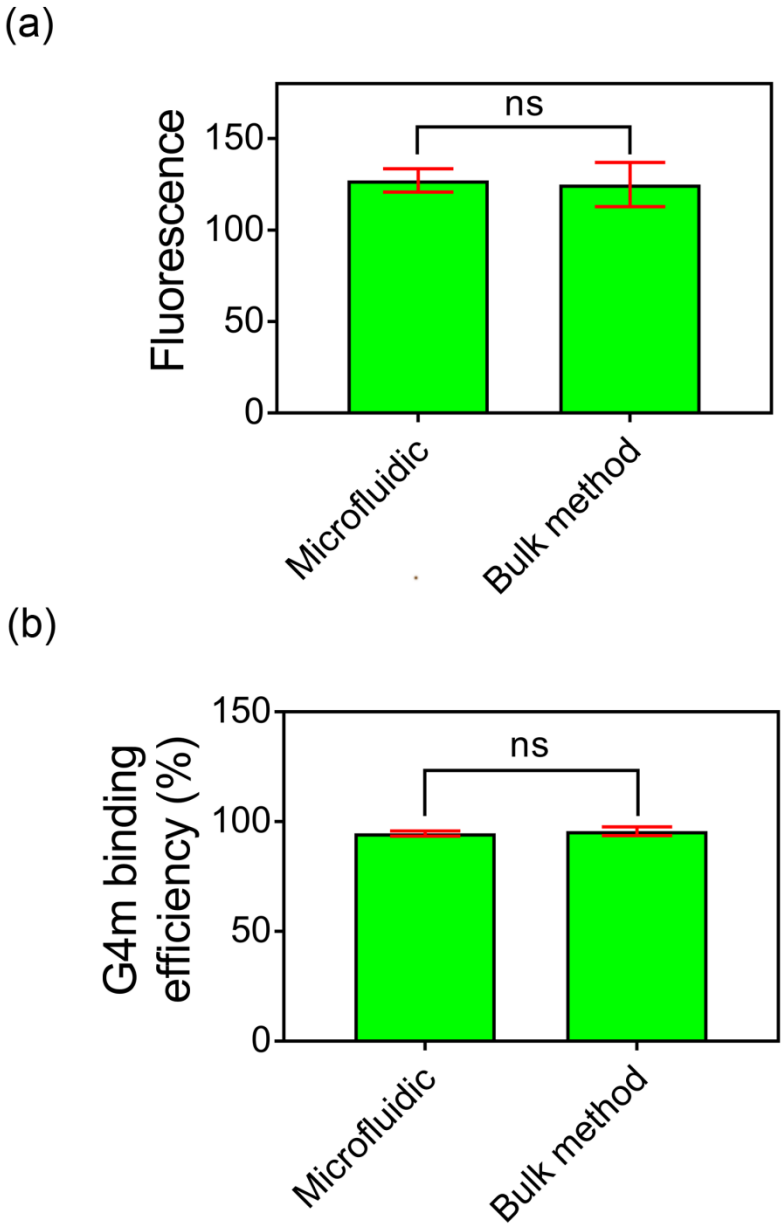

Figure S10. Comparison of microfluidic method and bulk method for synthesis of MBs-G4m complexes. Fluorescence intensity of oligreen-stained free G4m (a) were used to calculate the G4m binding efficiency (b). There is no significant difference between the two methods.

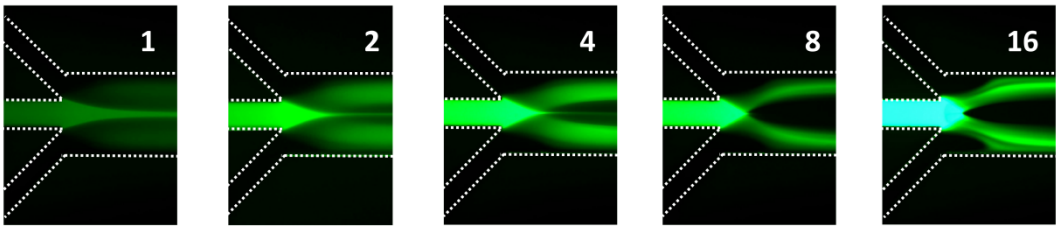

Figure S11. The photograph of the flow field at different flow rate ratios (1, 2, 4, 8, 16) in a microfluidic chip, taken with a fluorescence microscope at excitation wavelengths in the range of 460-490 nm.

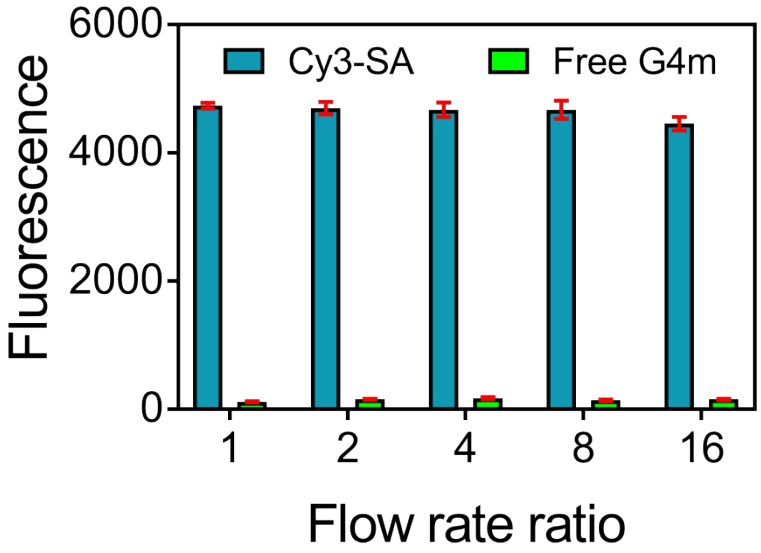

Figure S12. Fluorescence intensities of the unbound cy3-streptavidin (blue) and the oligreen-stained free G4m (green) in the supernatant, which are used to calculate the biotin unexposed ratios and the G4m binding efficiencies under different flow rate ratios (1, 2, 4, 8, 16).

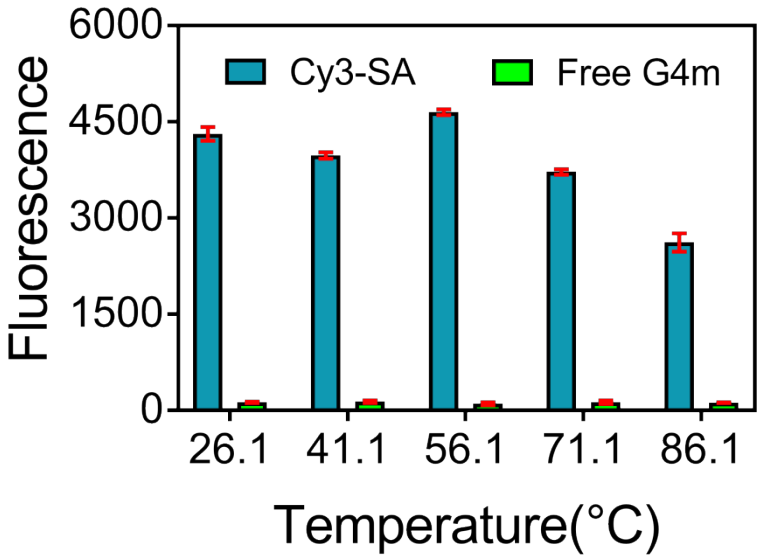

Figure S13. Fluorescence intensities of the unbound cy3-streptavidin and the oligreen-stained free G4m in the supernatant used to calculate the biotin unexposed ratios and the G4m binding efficiencies at different temperatures (26.1, 41.1, 56.1, 71.1, 86.1°C).

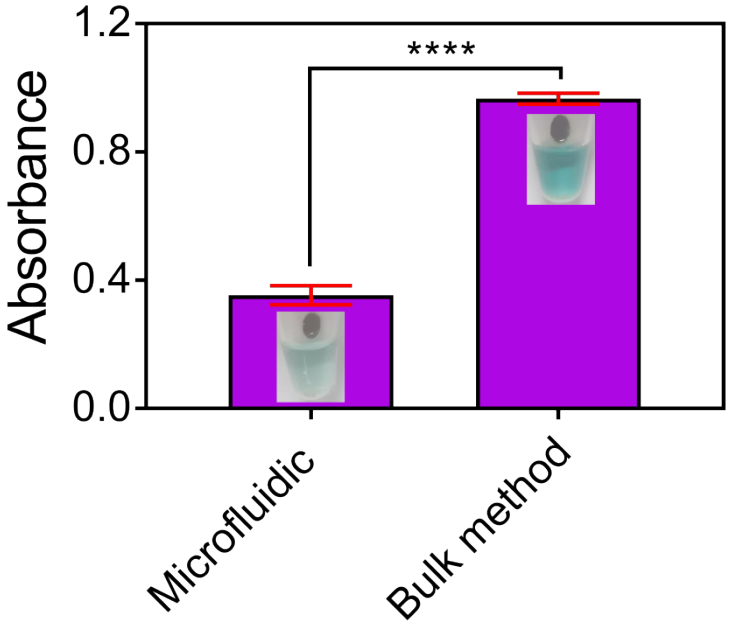

Figure S14. The absorbances and solution color after ABTS oxidation catalyzed by MBs-G4m complexes assembled in a microfluidic chip (left) and in a tube (right). There is a significant difference in the catalytic activity of the MBs-G4m complexes assembled by the two methods. **** P<0.0001.

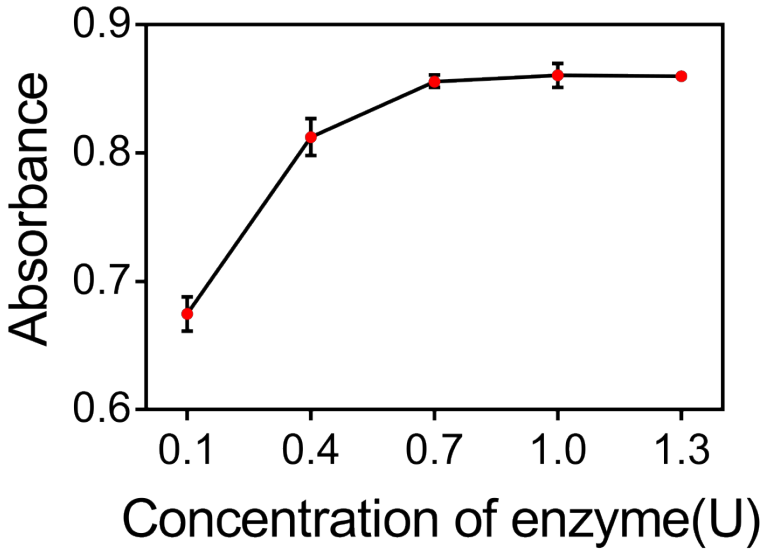

Figure S15. Optimization of the concentration of DSN enzyme.

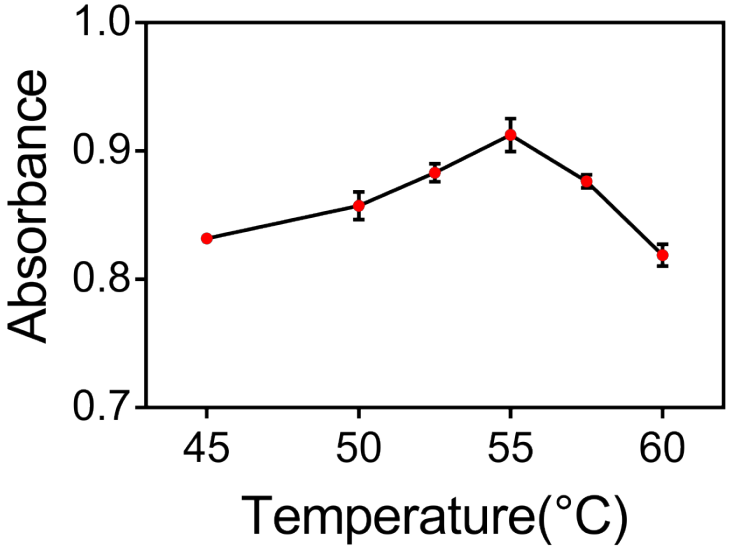

Figure S16. Optimization of the temperature at which the MBs-G4m complexes are incubated with DSN enzyme.