Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information for New Journal of Chemistry for:

Aptamer-induced thermofluorimetric protein stabilization and G-quadruplex nucleic acid staining by SYPRO Orange dye

Prashant S. Deore and Richard A. Manderville*

Departments of Chemistry and Toxicology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

Table of Contents:

1. Materials and Chemicals. SYPRO orange dye 5000x stock solution in DMSO, the HPLC purified native ODNs mentioned in Table 1, and hen-egg lysozyme were purchased from Sigma-Aldrich Ltd. (Oakville, ON). Bovine thrombin was purchased from BioPharm Laboratories LLC (Bluffdale, Utah).

2. Fluorescence Titrations and Thermal Measurements. All fluorescence spectra were recorded on a Cary Eclipse Fluorescence spectrophotometer (Agilent Technologies Inc., Santa Clara, CA) 1 × 4 multicell block equipped with a stirrer and temperature controller. All measurements were made using a Hellma Analytics ultra-micro cuvette 105.253-QS, with path length of 10 × 2 mm^2, and excitation and emission slit-widths 5 nm, with temperature control as mentioned. Fluorescence scans were taken directly after manual mixing of the solution. An intrinsic fluorescence was measured with excitation at 280 nm and emission at 340 nm. Fluorescence of SYPRO orange dye was measured with excitation at 492 nm and emission at 595 nm. For T_m measurements, samples of thrombin (5 μM), lysozyme (5 μM) and SYPRO orange dye (5X) without any DNA-aptamer and with either TBA15 (5 μM), TBA29 (5 μM) or LBA30 (5 μM) were prepared in 10 mM phosphate-buffered saline (PBS), at a pH of 7.4, with 150 mM NaCl at 25 °C. Temperature was ramped from 25 °C to 95 °C with the increment of 1 °C per minute to obtain temperature dependent fluorescence response for all T_m samples separately. The T_m values were calculated by determining the first derivative of the melting curve through the Varian Thermal software. Fluorescence emission scans were taken before and after melting of the protein for all samples.

3. CD Studies. CD spectra were recorded on a Jasco J-815 CD spectrophotometer equipped with a thermally controlled 1 x 4 multicell block. The dye and DNA were mixed manually, and CD's were measured at 15 °C in quartz cells (110-QS) with a light path of 1 mm and monitored between 200 and 350 nm at a bandwidth of 1 nm and scanning speed of 100 nm/min.

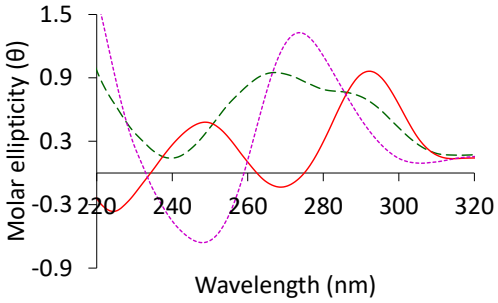

Figure S1 Overlay of CD spectra of TBA-15 (solid red trace), TBA-29 (dashed green trace) and LBA30 (dotted purple trace) measured with SYPRO Orange dye in PBS buffer (pH 7.4) and NaCl (150 mM).

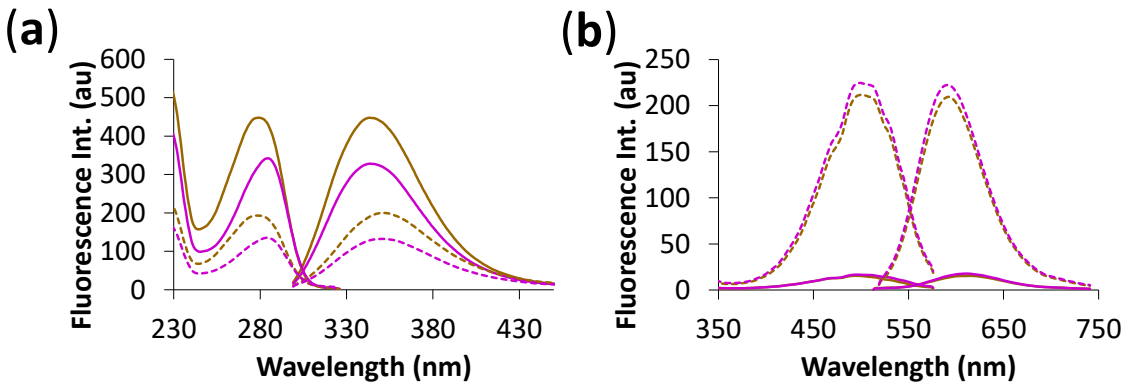

Figure S2 Fluorescence intensities obtained using (**a**) an intrinsic fluorescence of protein (5 μM, ; λ_{ex} = 280 nm, λ_{em} = 340 nm) and (**b**) fluorescence of SYPRO orange (5X; ; λ_{ex} = 492 nm, λ_{em} = 595 nm) before (solid traces) and after (dotted traces) melting of lysozyme, in absence (brown traces) and in presence of LBA30 (5 µM, purple traces) in PBS buffer pH 7.4 at 25 °C.

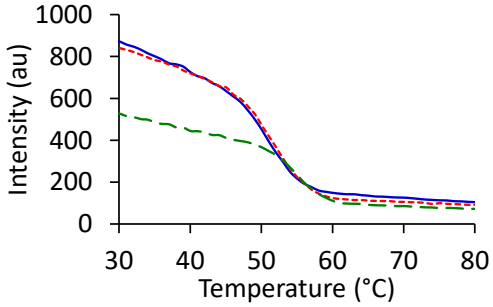

Figure S3 Melting curves obtained using intrinsic fluorescence of thrombin (5 µM; λ_{ex} = 280 nm, λ_{em} = 340 nm) in absence (solid-blue trace) and in presence of TBA15 (5 µM, dotted-red trace) and TBA29 (5 µM, dashed-green trace) in PBS buffer (pH 7.4) with NaCl (150 mM). The temperature was ramped with an increment of 1 °C per minute.