Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for:

Quantifying Aptamer-Protein Binding via Thermofluorimetric Analysis

Juan Hu, Joonyul Kim, and Christopher J. Easley*

Department of Chemistry and Biochemistry, Auburn University, Auburn, AL 36849, USA

chris.easley@auburn.edu

Supporting Information (SI) Contents:

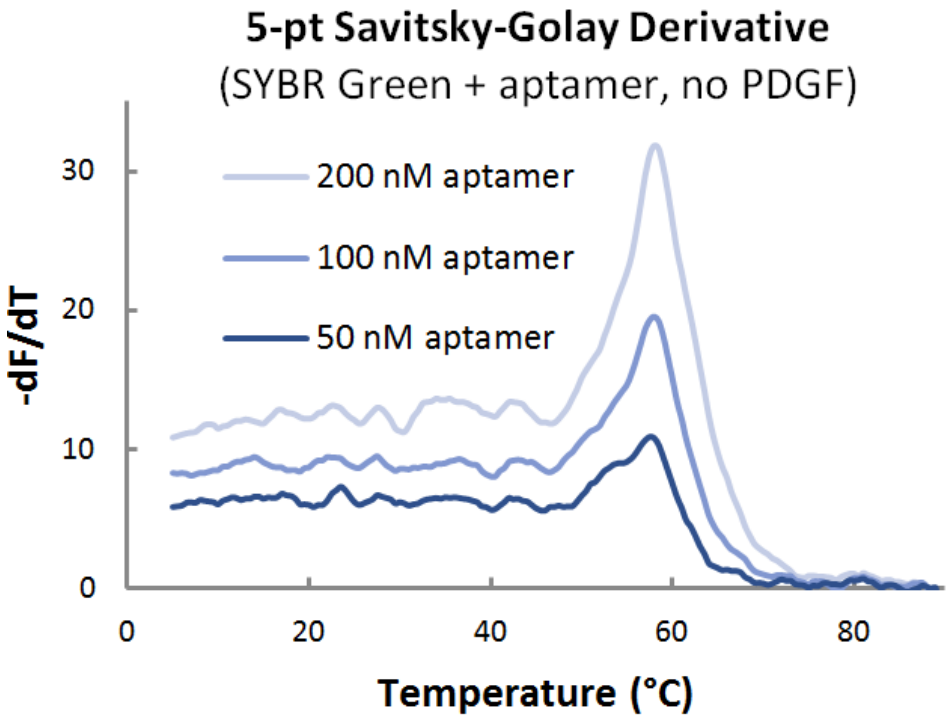

Figure S-1. Control experiments to test SYBR Green binding to varying concentrations of aptamers in the absence of PDGF confirmed that aptamer quantities could be determined using SYBR Green and TFA. -dF/dT signal was shown to be directly proportional to aptamer quantity.

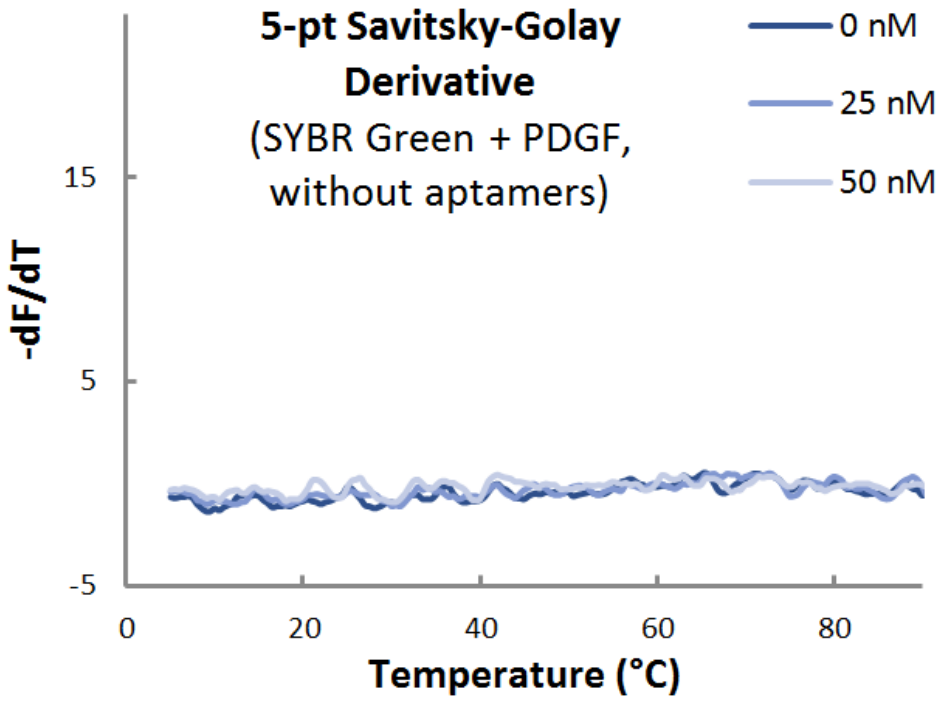

Figure S-2. Control experiments to test SYBR Green binding to varying concentrations of PDGF in the absence of aptamers suggested that there was no appreciable binding. This data confirms negligible protein-SYBR Green interactions, allowing confidence in aptamer-related peak assignments.

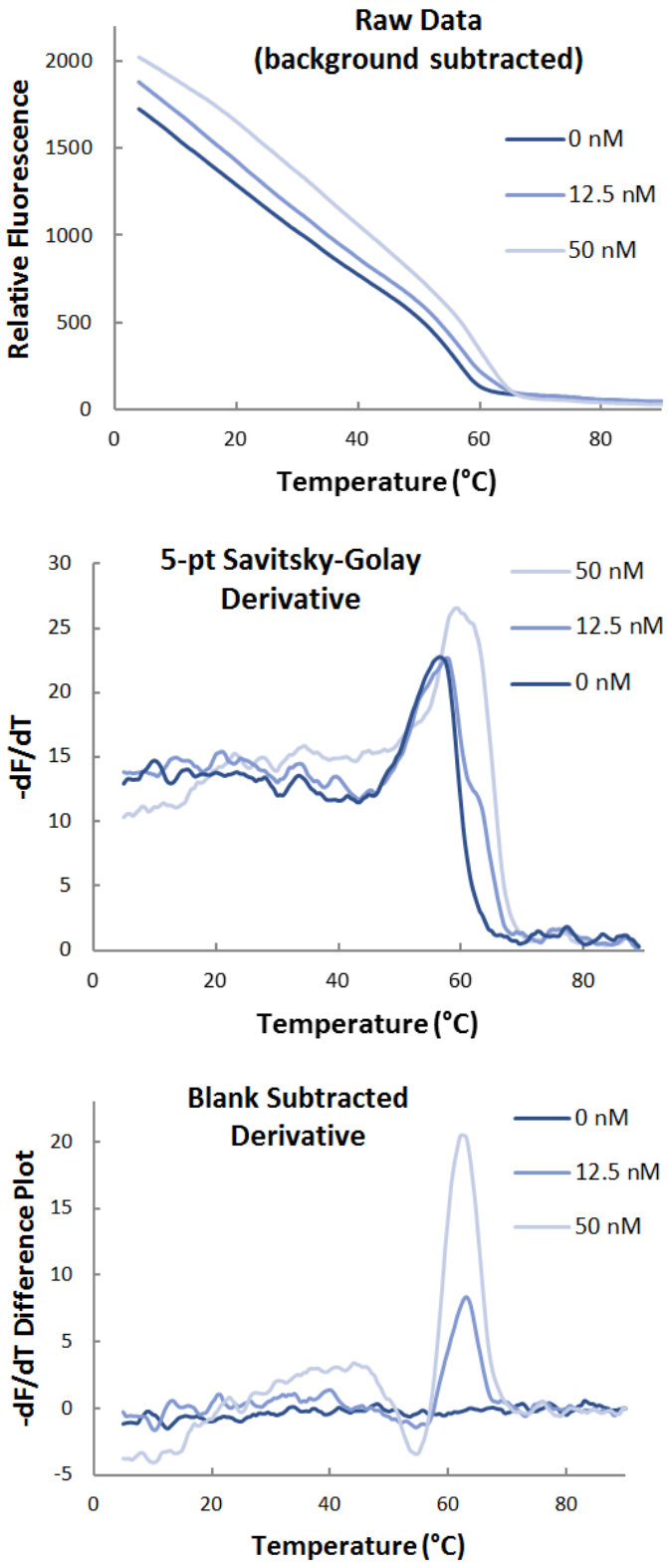

Figure S-3. Data processing workflow for -dF/dT difference plots. The background trace (SYBR Green in buffer; no aptamer or protein) was first subtracted from raw thermofluorimetric data to give the upper plots. A 5-point Savitsky-Golay derivative was calculated for DNA melting peak analysis (middle plots), and -dF/dT difference plots (lower plots) were calculated by subtraction of the average 0 nM derivative plot (blank).

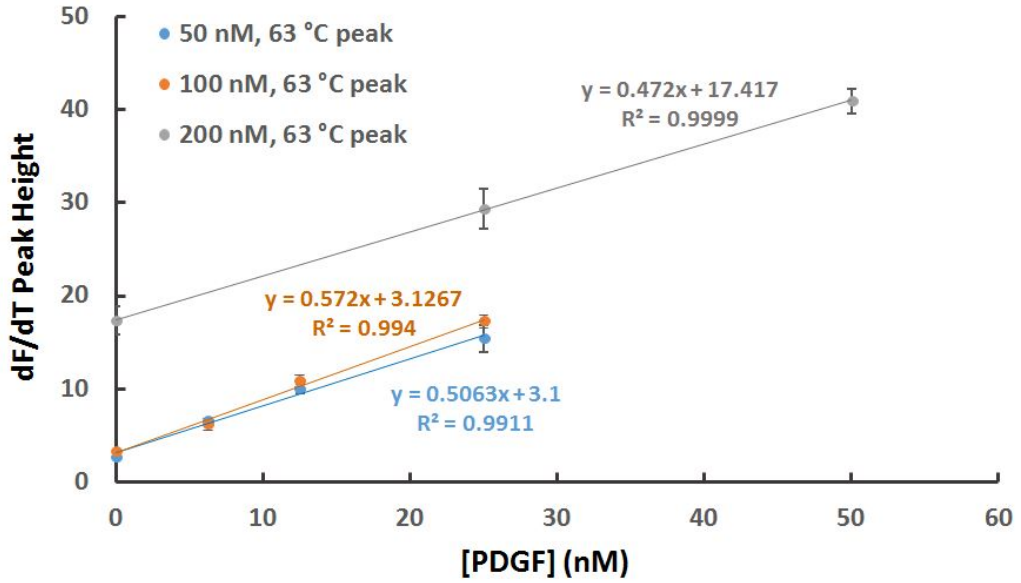

Figure S-4. TFA for PDGF quantification was evaluated with different probe concentrations (50, 100, and 200 nM). The highest sensitivity and lowest background was obtained with 100 nM probe, thus most measurements shown in this manuscript were carried out with 100 nM probe concentration. The 50 nM probe showed similarly low background but slightly lower sensitivity, while the 200 nM probe concentration exhibited much higher background and lower sensitivity toward protein.

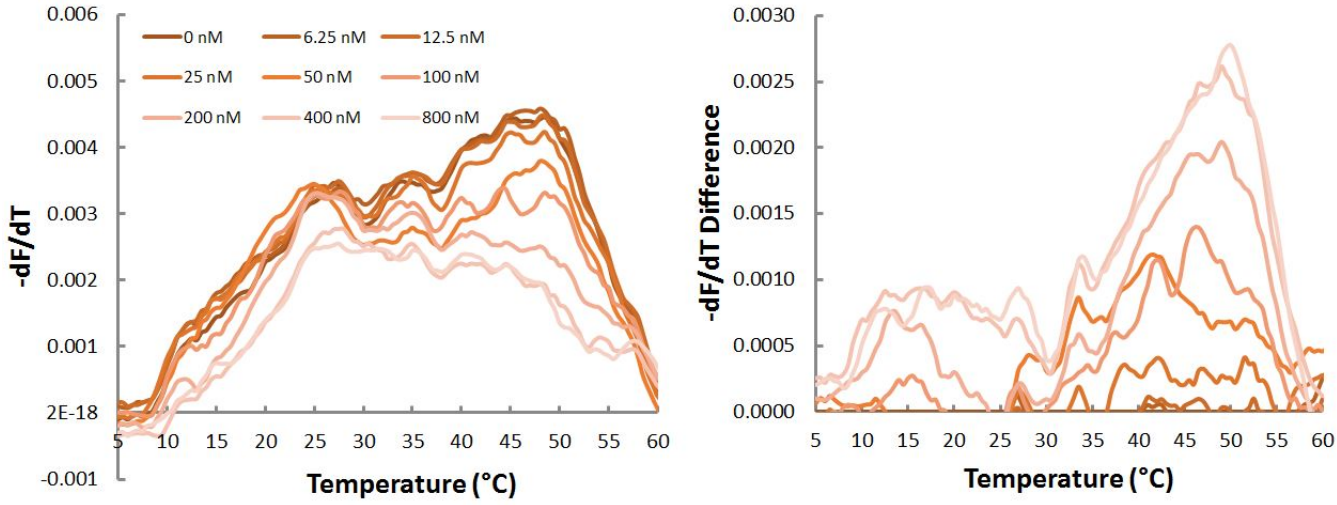

Figure S-5. Validation of thrombing TFA using thiazole orange (TO) staining dye. Although other tested dyes (SYBR Green, SYPRO Orange) were ineffective for TFA with thrombin aptamers, the G-quartet binding dye, TO, shows promise for detection of additional proteins. As noted in the text, these types of issues highlight that binding mechanism remains important, such that TFA must first be evaluated for each protein target of interest.