Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information for "Characterization of aptamer–protein binding using fluorescence anisotropy assays in low-volume, high-efficiency plates"

Table S1. Hill Coefficient of thrombin–aptamer binding determined from FA assays in various sample volumes and incubation times. All assays performed in HE 384 well plates. Data were fit using Equation 1. Standard error for the Hill Coefficient was calculated from the regression.

Volume/well (μL)	Incubation Time (minutes)	*n* 15mer	*n* 29mer
2	20	0.97 ± 0.10	1.34 ± 0.09
2	40	1.27 ± 0.16	1.06 ± 0.09
2	60	1.58 ± 0.10	1.28 ± 0.10
5	20	1.69 ± 0.33	1.93 ± 0.18
5	40	1.94 ± 0.32	1.68 ± 0.16
5	60	1.64 ± 0.34	1.30 ± 0.10
10	20	1.55 ± 0.14	2.00 ± 0.09
10	40	1.19 ± 0.06	2.24 ± 0.13
10	60	1.37 ± 0.22	2.27 ± 0.22
18	20	1.50 ± 0.17	1.49 ± 0.07
18	40	1.69 ± 0.20	1.77 ± 0.14
18	60	1.85 ± 0.30	1.71 ± 0.11

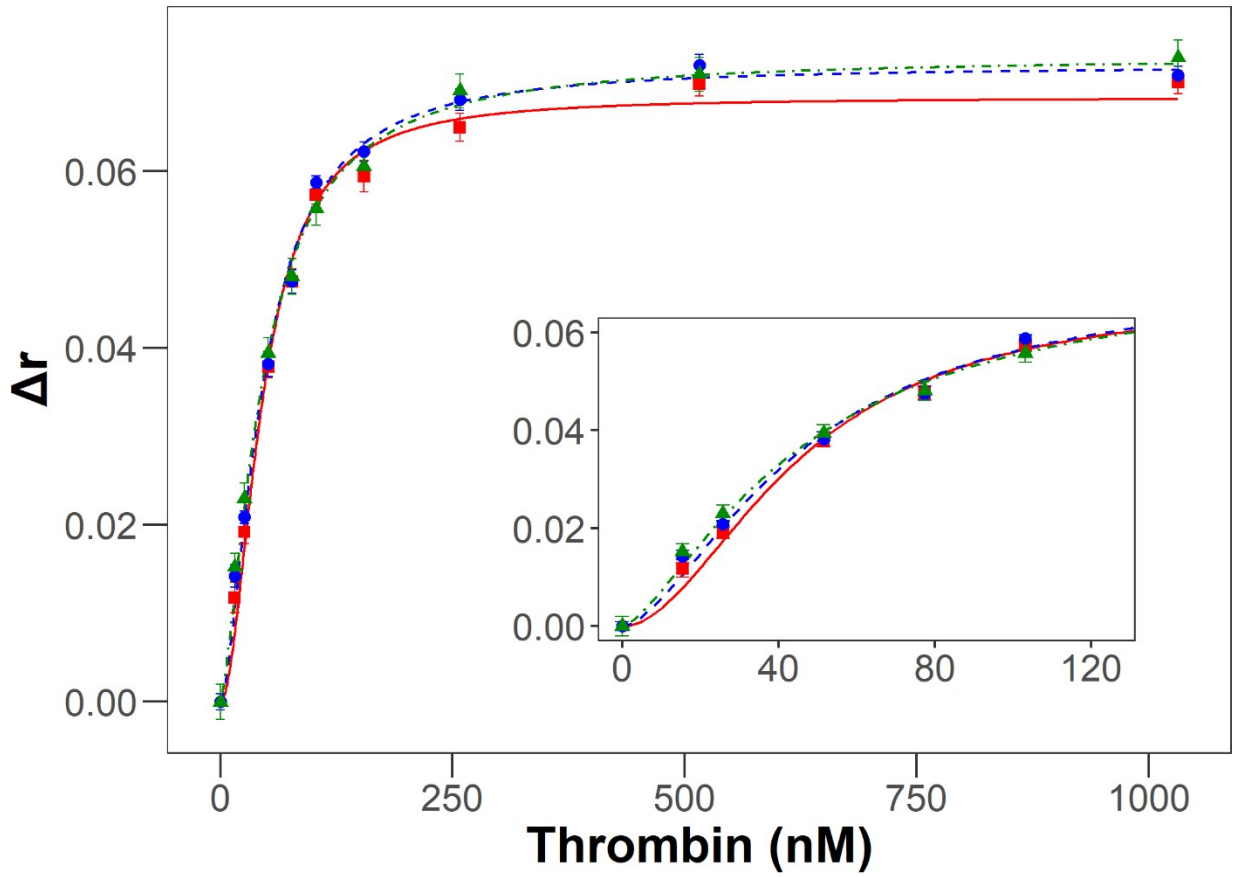

Figure S1. 29mer and thrombin binding curves with 60 µL/well at varied incubation times in normal 384-well plates. 20 minutes: red squares, solid; 40 minutes: blue circles, dashed; 60 minutes: green diamonds, dot/dash. Error bars are standard error of replicate measurements (n = 9). Each curve fit with the Hill equation. Inset shows low concentration range. Δr is change in anisotropy.

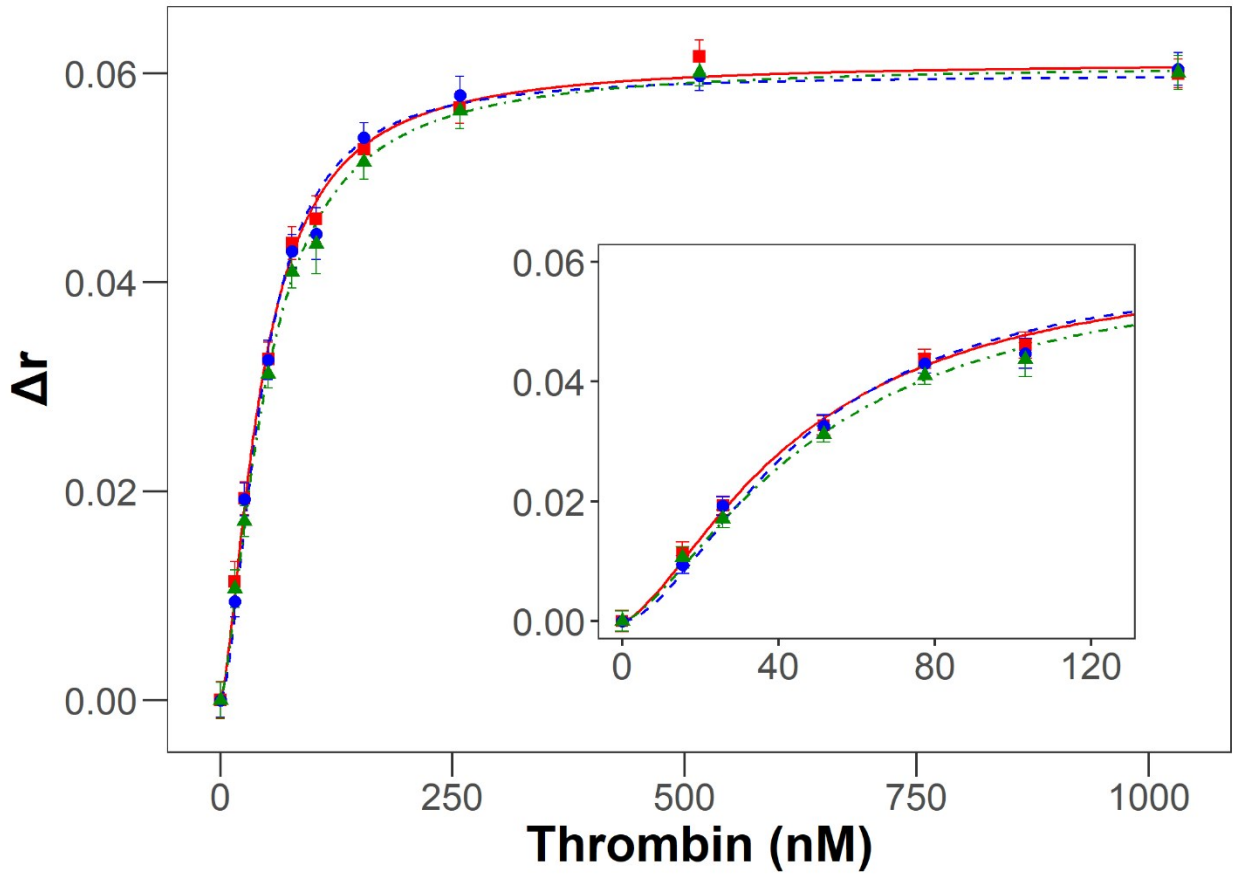

Figure S2. 29mer and thrombin binding curves with 45 µL/well at varied incubation times in HE 96-well plates. 20 minutes: red squares, solid; 40 minutes: blue circles, dashed; 60 minutes: green diamonds, dot/dash. Error bars are standard error of replicate measurements (n = 9). Each curve fit with the Hill equation. Inset shows low concentration range. Δr is change in anisotropy.

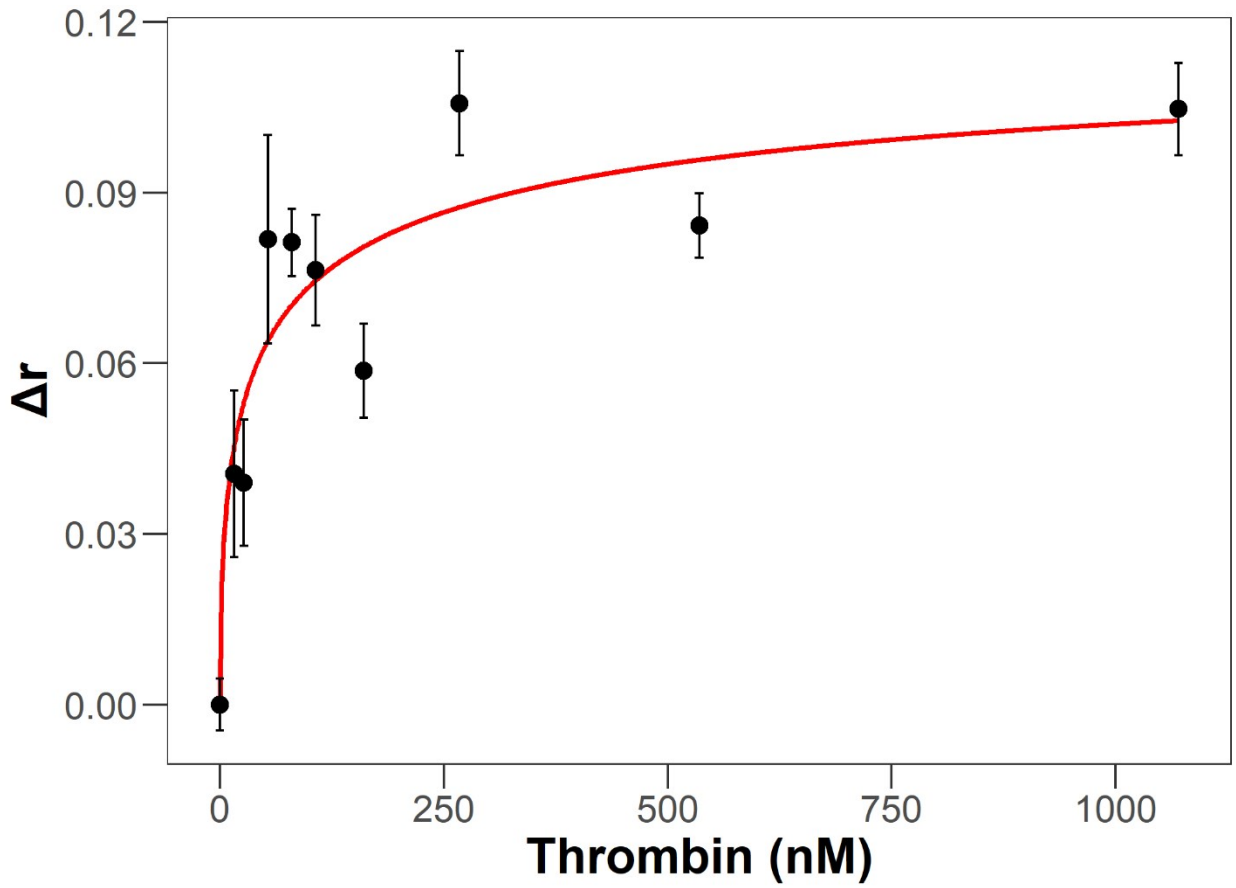

Figure S3. 29mer and thrombin binding curves after 20-minute incubation 1 µL/well in HE 384-well plates. Error bars are standard error of replicate measurements (n = 9). Fit with the Hill equation. Δr is change in anisotropy. K_A = 47 ± 45 nM.

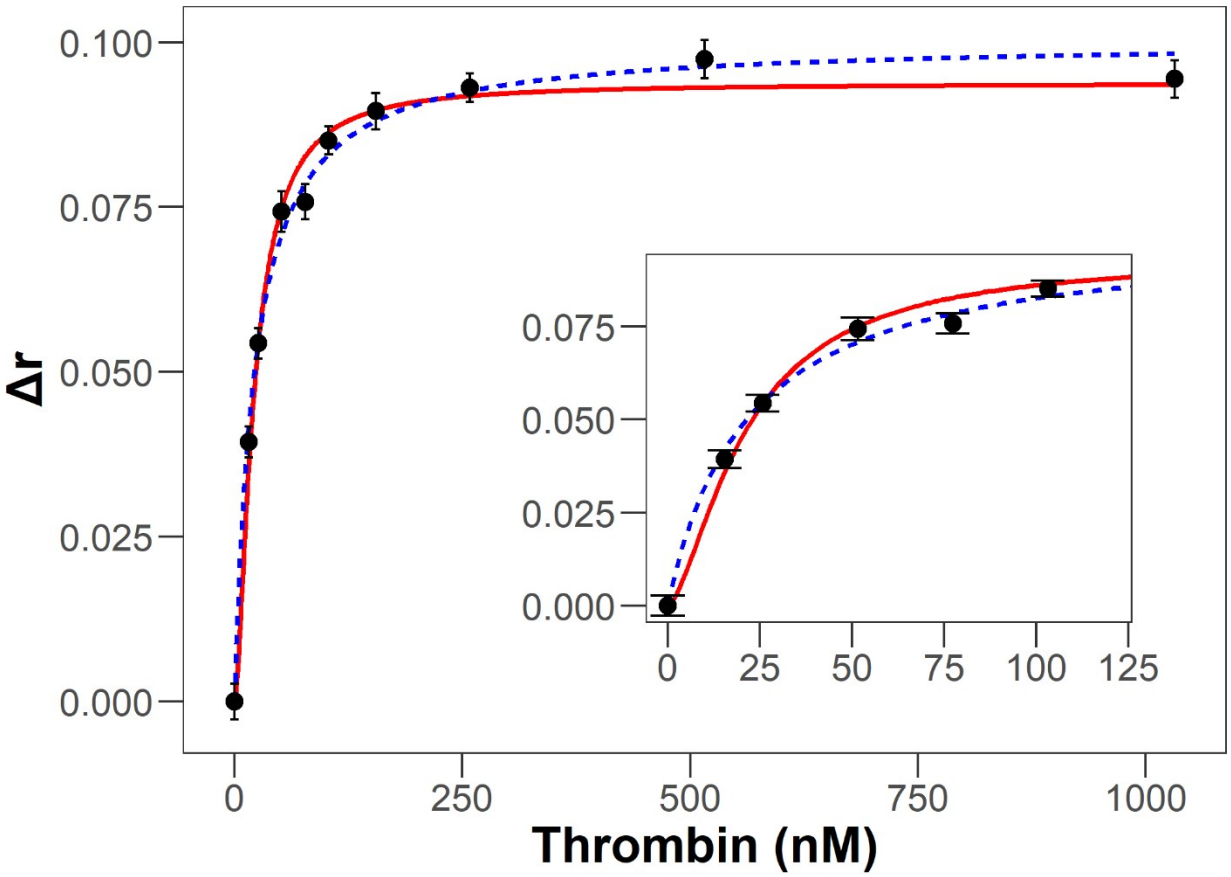

Figure S4. 15mer and thrombin binding curve, 10 µL per well, 20-minute incubation, fit with the Hill Equation (red, solid) and Square Hyperbola Equation (blue, dashed). Error bars are standard error of replicate measurements (n = 9). Inset shows low concentration range. Δr is change in anisotropy.

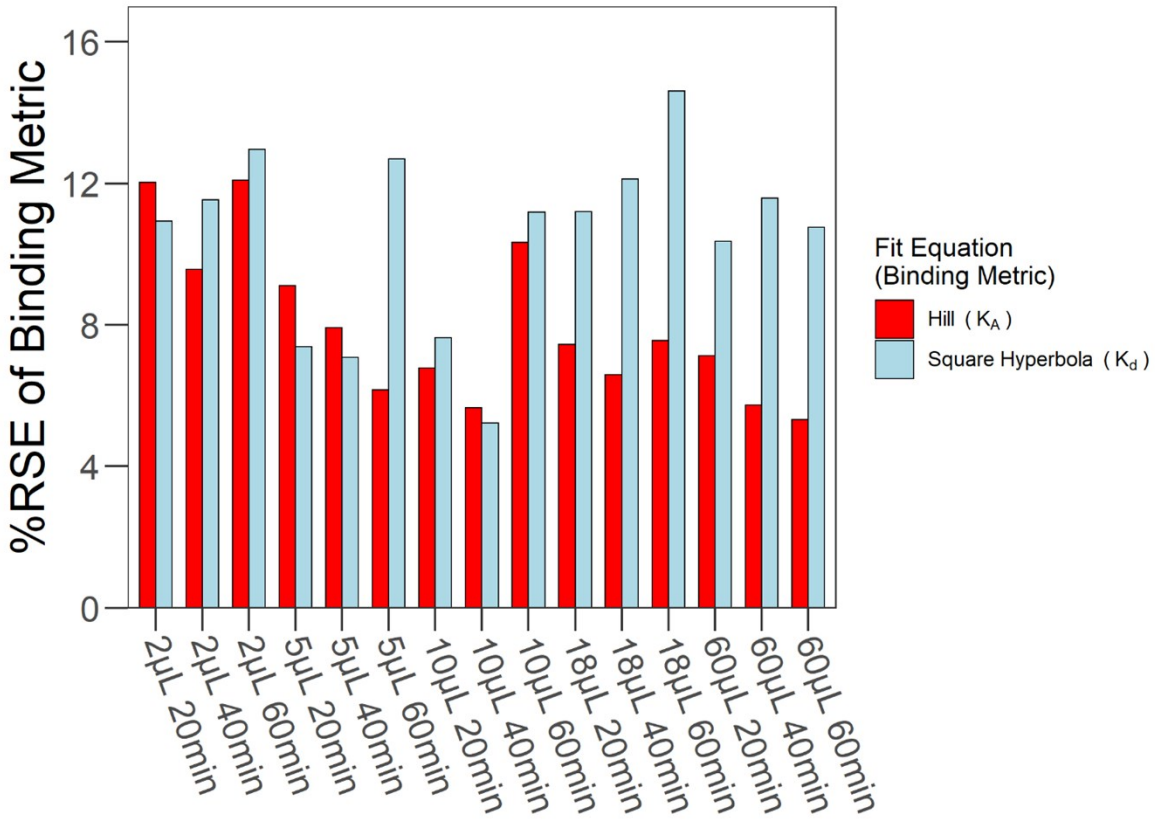

Figure S5: 15mer aptamer and thrombin binding assays. Comparison of fit quality for Hill Equation and Square Hyperbola Equation by %RSE values of K_A and K_d respectively. Hill Equation fit (K_A) - red; Square Hyperbola Equation fit (K_d) - light blue. %RSE calculated during nonlinear regression. Labels are volume/well and incubation time. For volumes less than 20 µL, assays performed in HE 384 well plates. For volumes greater than 20 µL, assays performed in normal 384 well plates.

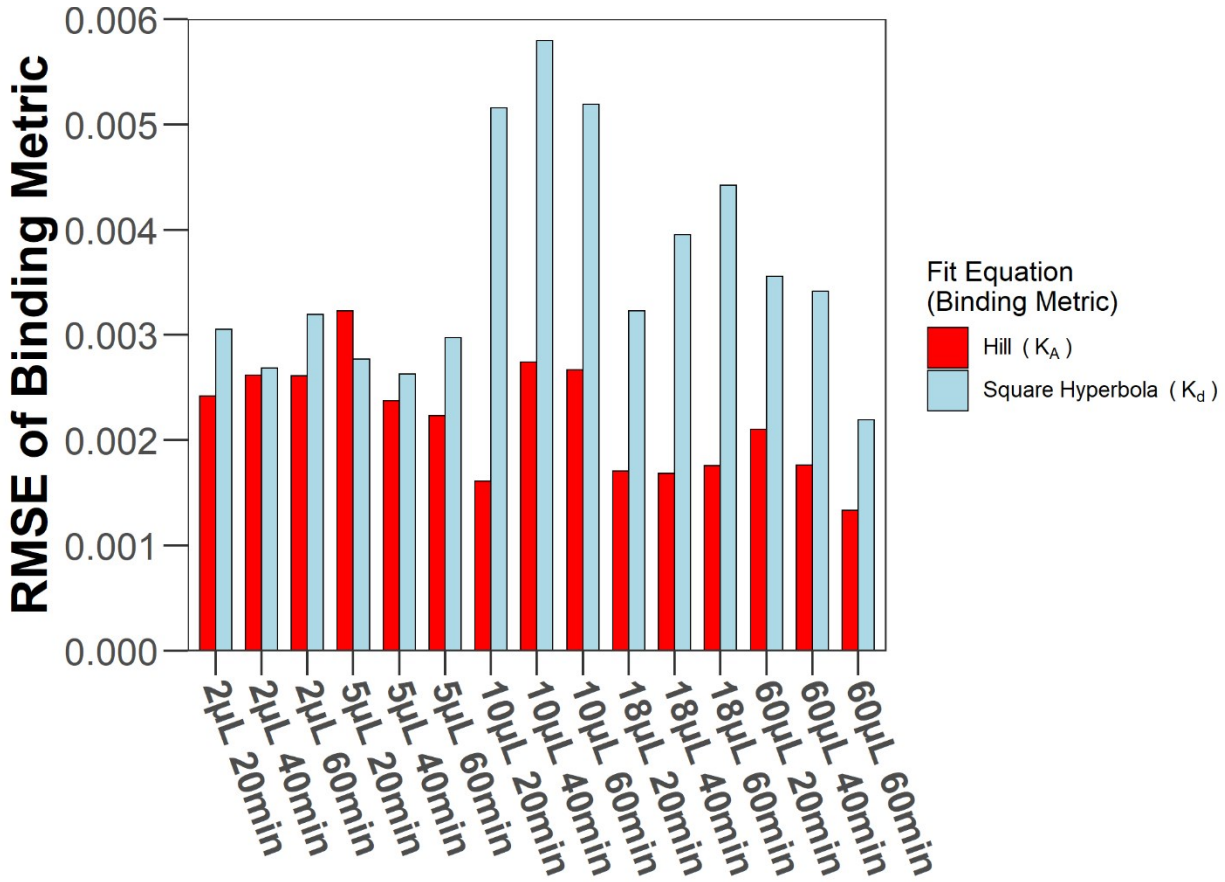

Figure S6: 29mer aptamer and thrombin binding assays. Comparison of fit quality for Hill Equation and Square Hyperbola Equation by root-mean-squared error (RMSE) values of K_A and K_d respectively. Hill Equation fit (K_A) - red; Square Hyperbola Equation fit (K_d) - light blue. RMSE calculated during nonlinear regression. Labels are volume/well and incubation time. For volumes less than 20 µL, assays performed in HE 384 well plates. For volumes greater than 20 µL, assays performed in normal 384 well plates.

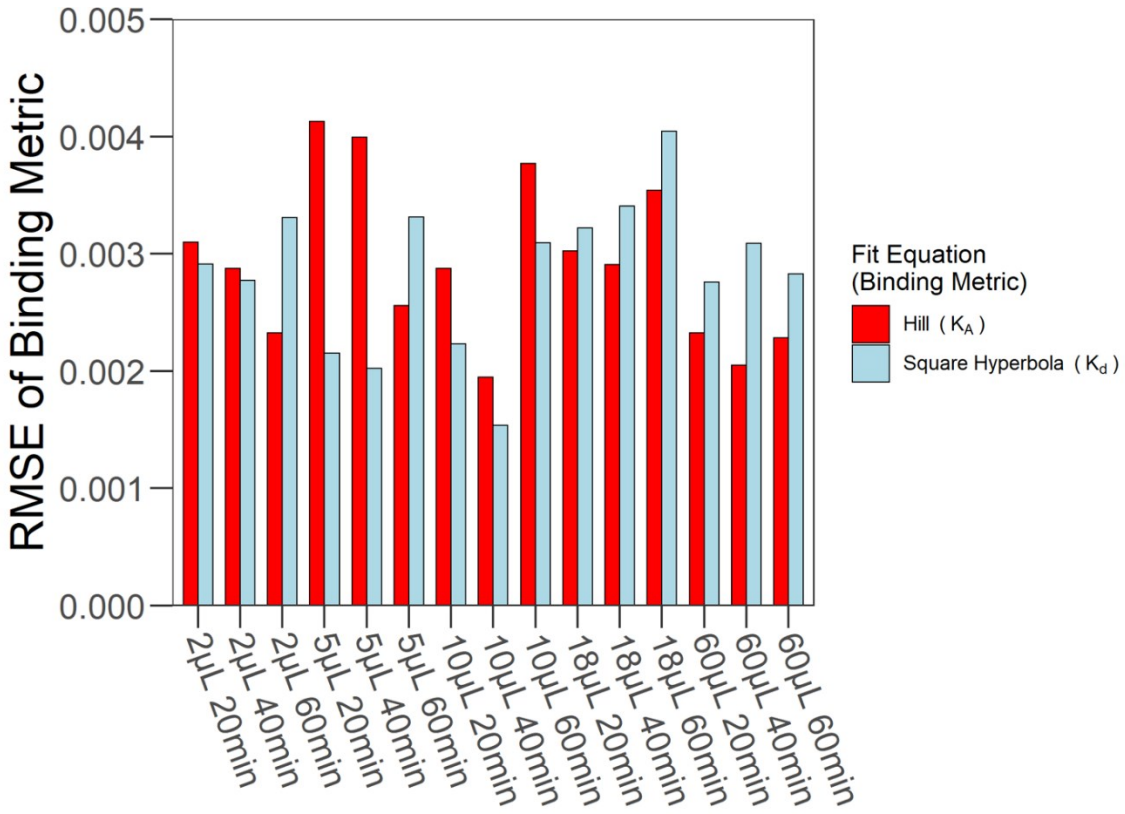

Figure S7: 15mer aptamer and thrombin binding assays. Comparison of fit quality for Hill Equation and Square Hyperbola Equation by root-mean-squared error (RMSE) values of K_A and K_d respectively. Hill Equation fit (K_A) - red; Square Hyperbola Equation fit (K_d) - light blue. RMSE calculated during nonlinear regression. Labels are volume/well and incubation time. For volumes less than 20 µL, assays performed in HE 384 well plates. For volumes greater than 20 µL, assays performed in normal 384 well plates.