Engineering Polymeric Aptamers for Selective Cytotoxicity

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Supporting Information

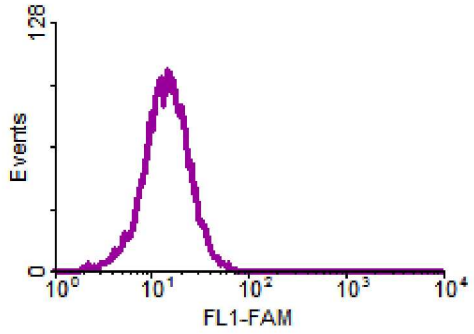


Figure S1. Competition binding test. Polyacrymide shows no significant binding to CEM cells, which specifically can be recognized by Sgc8c (blue and purple curves).

Since the autocorrelation function depends on the rate of diffusion, it seems natural to use FCS to determine molecular weights. It is known that the translational diffusion coefficient of a molecule is related to its size, and that substantial changes in molecular weight are needed to result in detectable changes in the diffusion time. By measuring and comparing the diffusion time of different molecules in a certain detection volume, the molecular weight of the polymeric aptamer can be calculated.

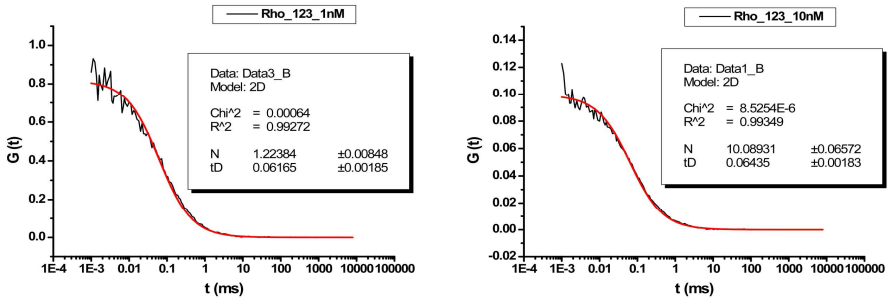


Figure S2 a. Free dye calibration to determine the detection volume.

$$\tau_D = \frac{\omega_{xy}^2}{4D}$$

$$\Rightarrow \omega_{xy} = \sqrt{4D \cdot \tau_D} = \sqrt{4 \times 3 \times 10^{-10} \frac{m^2}{s} \times (0.06435 \times 10^{-3})s}$$

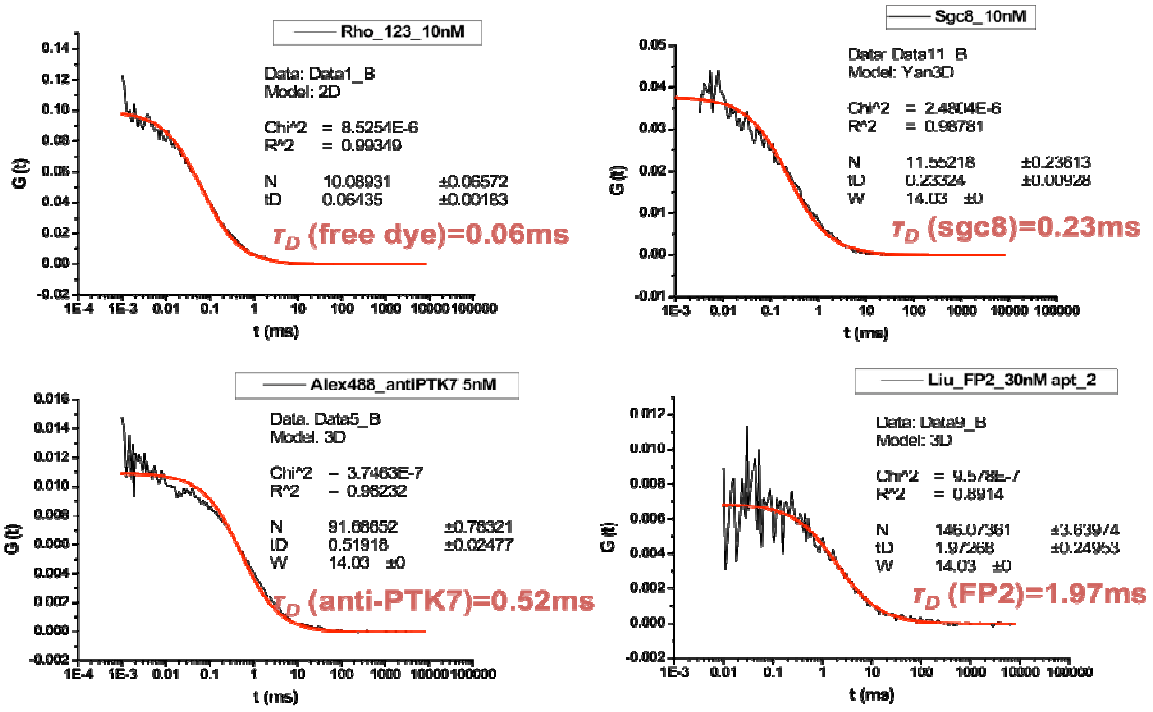
$$\Rightarrow \omega_{xy} = 0.278 \mu m$$

$$V_{eff} = \pi^{3/2} \cdot \omega_{xy}^2 \cdot \omega_Z$$

$$\Rightarrow \omega_Z = \frac{V_{eff}}{\pi^{3/2} \cdot \omega_{xy}^2} = \frac{1.68 \times 10^{-15} \times 10^{-3} m^3}{\pi^{3/2} \times \left(0.278 \times 10^{-6} m\right)^2} = 3.90 \mu m$$

$$V_{eff} = \frac{N}{N_A \cdot C} = \frac{10.08931}{6.023 \times 10^{23} \times \left(10 \times 10^{-9}\right) mol / L} = 1.68 fL$$

$$W = \frac{\omega_Z}{\omega_{xy}} = \frac{3.90 \mu m}{0.278 \mu m} = 14.03$$



$$\frac{\tau_D(antiPTK7)}{\tau_D(Rho\_123)} = \frac{0.52}{0.06} = 8.7$$

$$\left(\frac{MW(antiPTK7)}{MW(Rho\_123)}\right)^{\frac{1}{3}} = \left(\frac{150000}{380}\right) = 7.3$$

$$\frac{\tau_D(antiPTK7)}{\tau_D(sgc8)} = \frac{0.52}{0.2} = 2.6$$

$$\left(\frac{MW(antiPTK7)}{MW(sgc8)}\right)^{\frac{1}{3}} = \left(\frac{150000}{13172}\right)^{\frac{1}{3}} = 2.25$$

$$\frac{\tau_D(FP2)}{\tau_D(sgc8)} = \left(\frac{MW(FP2)}{MW(sgc8)}\right)^{\frac{1}{3}}$$

$$\Rightarrow \left(\frac{\tau_D(FP2)}{\tau_D(sgc8)}\right)^3 = \frac{MW(FP2)}{MW(sgc8)}$$

$$\Rightarrow MW(FP2) = MW(sgc8) \times \left(\frac{\tau_D(FP2)}{\tau_D(sgc8)}\right)^3$$

$$\Rightarrow MW(FP2) = 13172\,\frac{g}{mol} \times \left(\frac{1.97}{0.23}\right)^3 = 8.3 \times 10^6\,\frac{g}{mol}$$

Figure S2 b. Diffusion time τ_D for different molecular weights. Specifically, FP2 is the abbreviation for FAM-polymeric aptamer. Since it has the longest diffusion time, it also has the the largest MW.

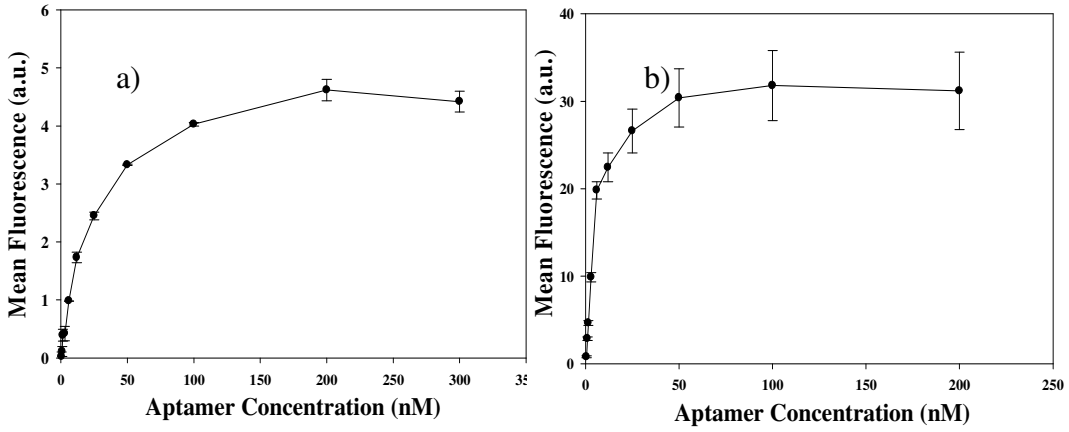


Figure S3. Binding affinity of fluorescence-labeled free T2-KK1B10 a) and **PB10** b) to K562/D cells. The mean fluorescence intensity of target cells was obtained by subtracting the mean fluorescence intensity of nonspecific binding of each probe with Ramos cells.

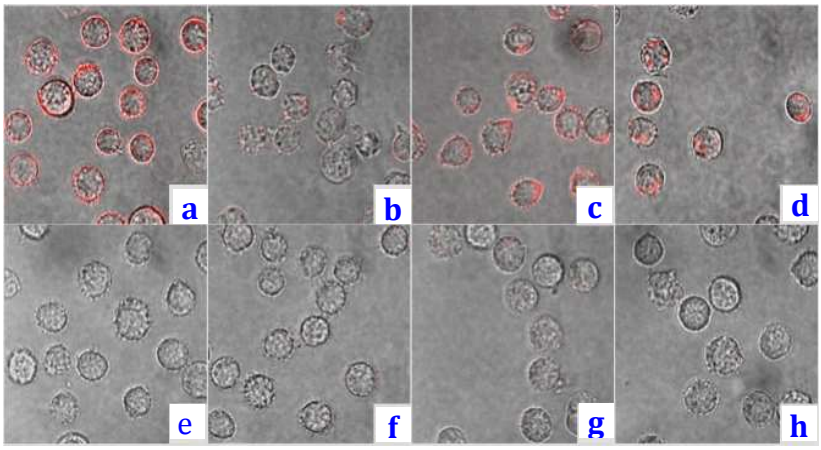


Figure S4. Internalization of **PSgc8**. All the images are overlays of optical and TMR fluorescence channels. The images in the upper panel are from CEM cells, and those in the lower panel are from Ramos cells. The images reflect **PSgc8** binding at 4 °C (a, e), washing (b, f), incubation at 37 °C (c, g) and washing (d, h).

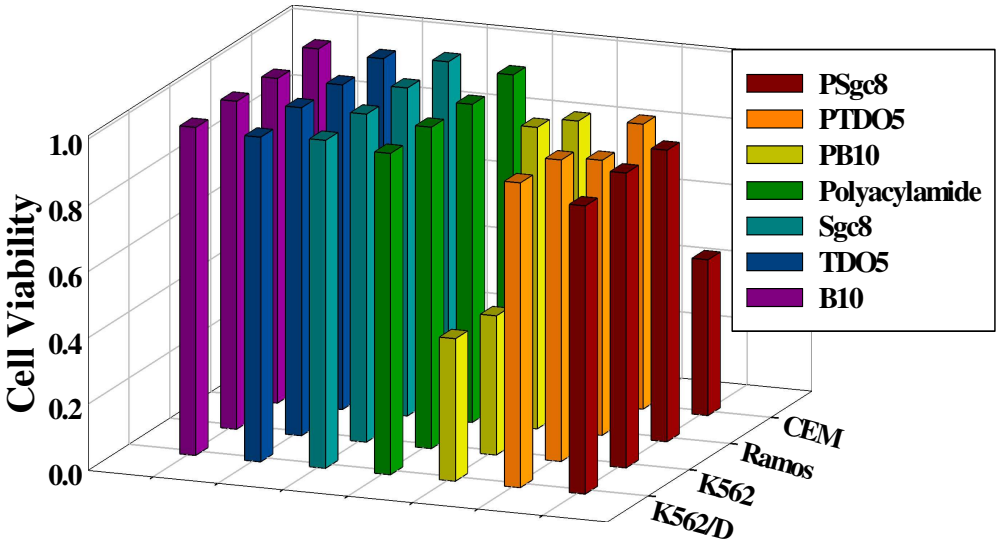


Figure S5. Overview of cell viability after exposure to 150 nM polymeric aptamers (**PSgc8**, **PTDO5** and **PB10**), polyacrylamide (0.03 x 1% w/w), and 5 µM free aptamers (Sgc8, TDO5 and T2-KK1B10).