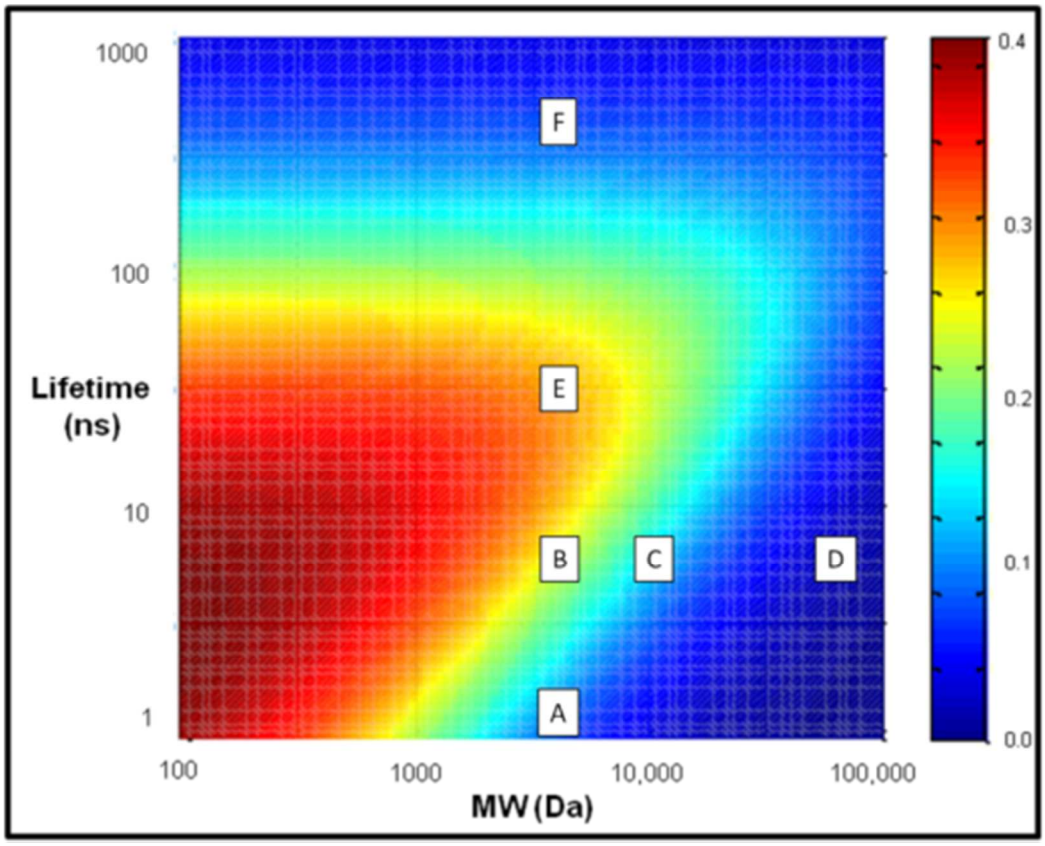
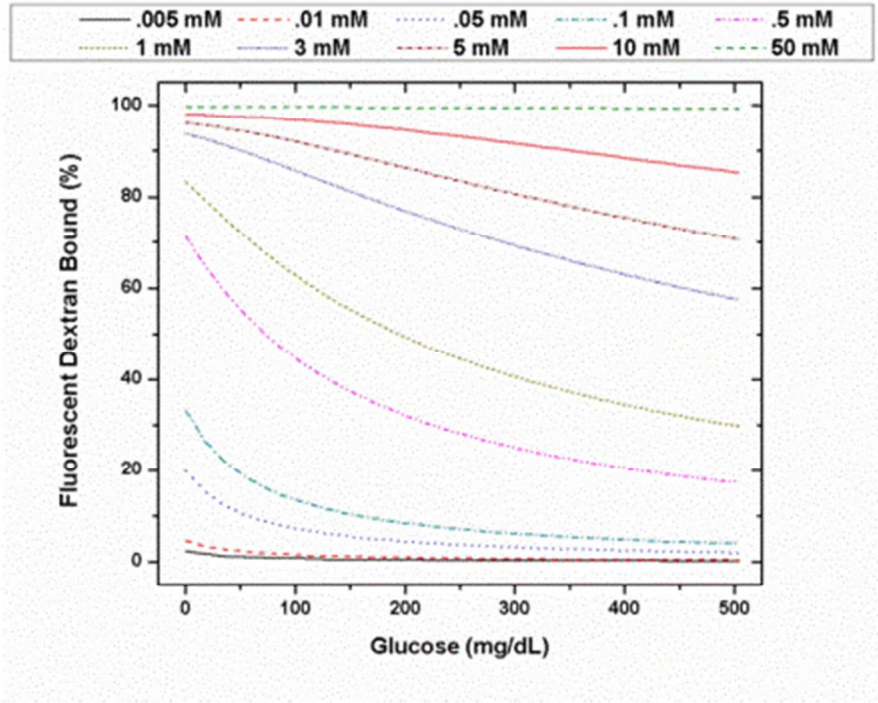
Optimization of a Concanavalin A-based glucose sensor using fluorescence anisotropy

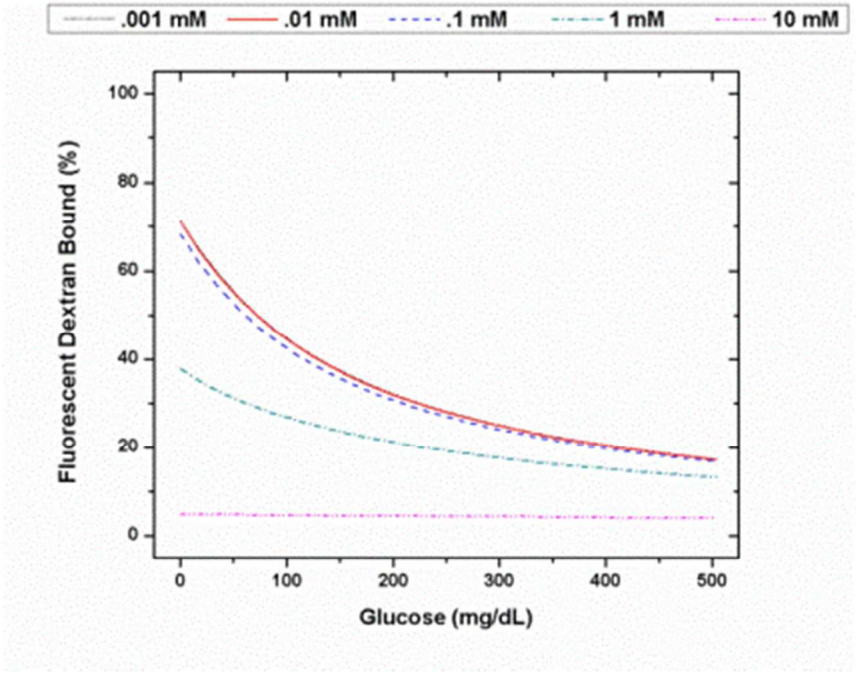
- Supplemental Information -



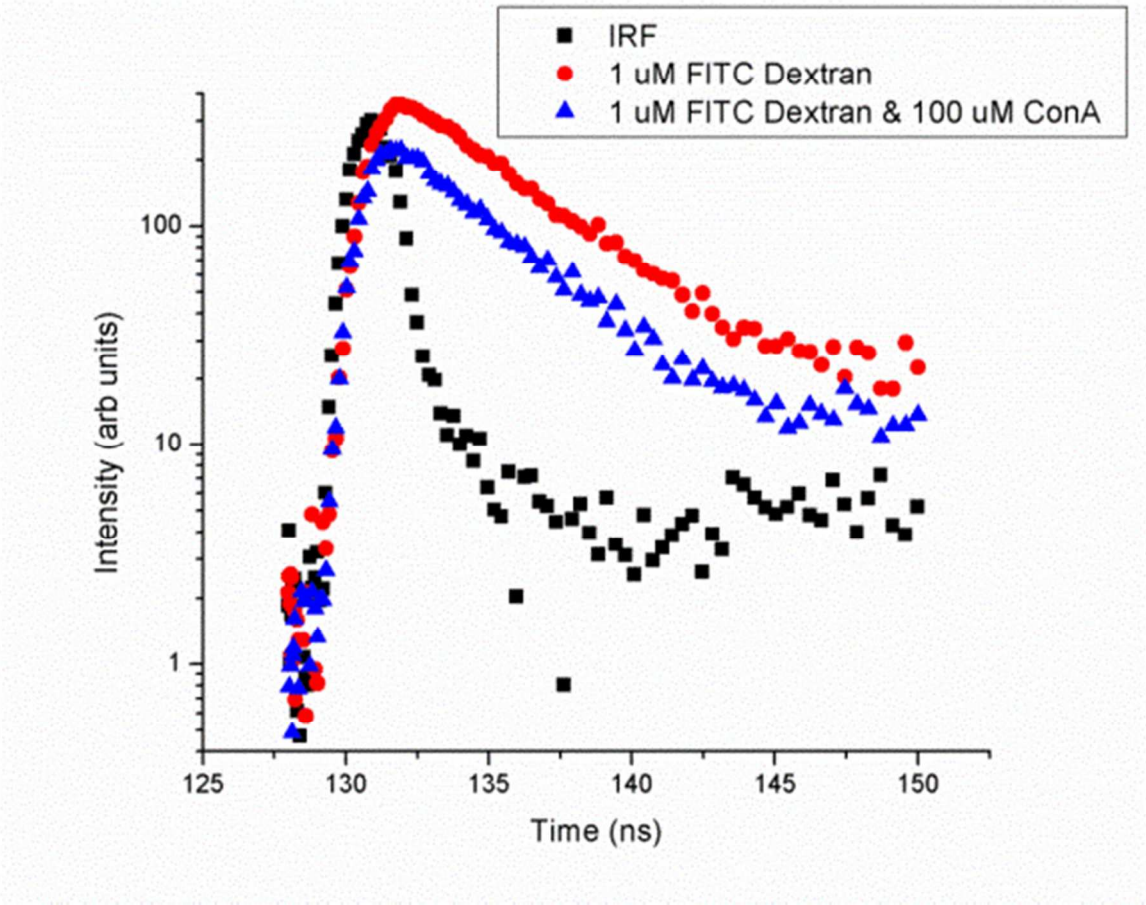
SI-1: The approximate change in anisotropy between free fluorescent competing ligand and ConA-bound fluorescent competing ligand as a function of the fluorescence lifetime and molecular weight (MW) of the fluorescent competing ligand (r_o = 0.4, T = 293 K, η = 0.01 cP, ν = 0.73 cm^3/g, h = 1.17 ml/mg, and R = 8.321*10^7 erg/K·mol). Tetrameric ConA is ~104 kDa. A: 4 kDa AF 647-dextran, B: 4 kDa FITC-dextran, C: 10 kDa FITC-dextran, D: 70 kDa FITC-dextran, E: 4 kDa Dansyl Chloride-dextran, F: 4 kDA Ruthenium-dextran. Note that 4 kDa is the smallest commercially available dextran. (from the model)



SI-2: % Fluorescent dextran bound versus glucose concentrations for competitive binding assays based on 1 μM dextran and varying ConA concentrations (from the model)



SI-3: % Fluorescent dextran bound versus glucose concentrations for competitive binding assays based on 500 μM ConA and varying dextran concentrations (from the model)



SI-4: Fluorescence lifetime studies of 1 uM FITC-dextran (red circle) and 1 uM FITC-dextran with 100 uM ConA (blue triangle). The impulse response function (IRF) (black square) is from scattered light from the pulsed LED. This was performed using a stroboscopic technique on a TM-200 PTI spectrofluormeter equipped with a 460 nm LED and a R928 photomultiplier tube collecting emission at 520 nm. The fits generated a single 3.897 ns lifetime for the 1 uM FITC-dextran with a chi-squared value of 1.297. For the combined assay, it generated a 3.897 ns lifetime and a 1.73 ns lifetime for the free and bound populations with a chi-squared value of 1.097. The fit for the combined assay showed at t=0 showed ~66% with the free lifetime and 34% with the bound lifetime, which agreed with the predicted competitive binding modeling for this assay at 0 mg/dL glucose (SI-2)

SI-5: This table shows the predicted anisotropy of 4 kDa FITC-dextran bound to ConA using the initial model that assumes the lifetime is unchanged upon binding to ConA (~4 ns) and the adjusted model that accounts for the change in lifetime (1.7 ns) using Equation 2.

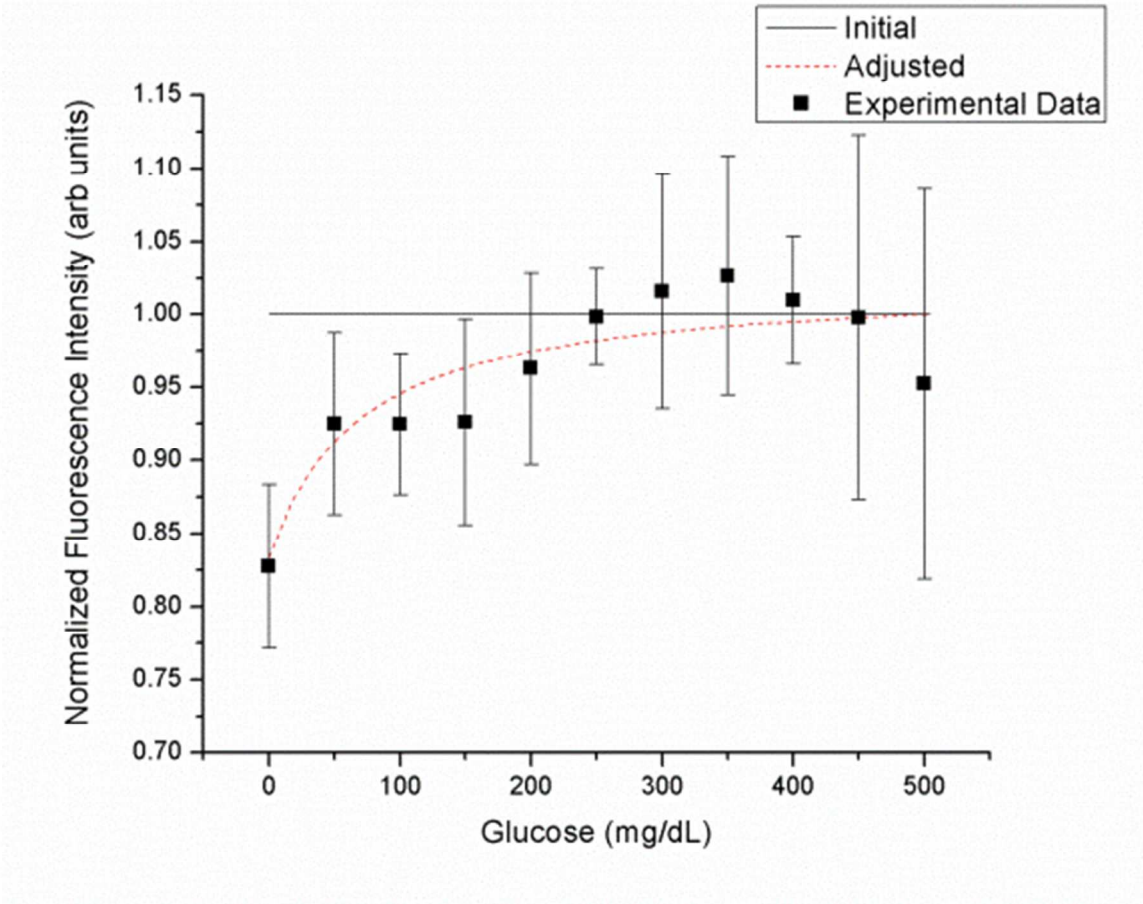
Competitive Ligand (CL)	**Initial FA $_{\text{bound to ConA}}$**	**Adjusted FA $_{\text{bound to ConA}}$**
4 kDa FITC-dextran	0.382	0.392

SI-6: This equation describes the total fluorescence intensity (F_{tot}) of the 1 uM FITC-dextran and 100 uM ConA assay after adjusting for the change to lifetime upon binding. (RQY = relative quantum yield)

$$F_{tot} = RQY_{bound} * \%CLB(glu) + RQY_{free} * (1 - \%CLB\ (glu))$$

Where RQY_{bound} and RQY_{free} equal:

$$RQY_{bound} = \frac{1.7\ ns}{3.9\ ns} = 0.436 \qquad RQY_{free} = \frac{3.9\ ns}{3.9\ ns} = 1.0$$



SI-7: Normalized fluorescence intensity, glucose-response of 1 uM FITC-dextran and 100 uM ConA assay for the initial model (solid black line), adjusted model (dashed red line) and experimental data (black squares). The initial model assumes that the fluorescent intensity is independent of binding (normalized intensity =1). The adjusted model accounts for the relative change in quantum yield to generate the normalized fluorescence intensity (SI-6). The experimental data more accurately follows the adjusted model when compared to the initial model. Each data point is a uniquely loaded well, and the large standard deviation can be attributed to pipetting error.

SI-8: This equation is the adjusted model for the anisotropy in terms of the adjusted fluorescence contributions from the free and bound populations and the adjusted anisotropy of the bound populations.

$$r_{adjusted}(glu) = \frac{RQY_{bound} * \%CLB(glu)}{F_{tot}} * r_{b-adj} + \frac{RQY_{free} * (1 - \%CLB(glu))}{F_{tot}} * r_f$$