

Figure S1 – Fluorescence spectra in response to potassium (a) and sodium (b) and the corresponding absorbance spectra in response to potassium (c) and sodium (d). Error bars in (d) indicate one standard deviation

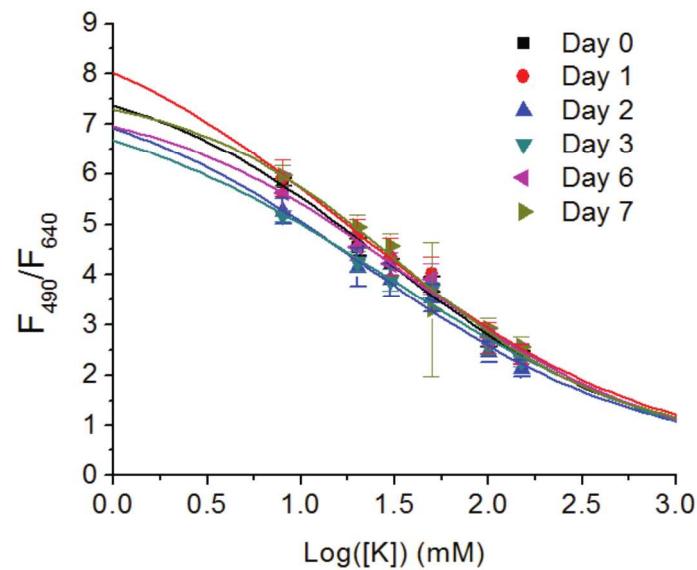


Figure S2 – Nanosensor shelf stability showing similar calibration responses on day 0 and day 7. Storage conditions were in room temperature and shielded from light. Error bars indicate one standard deviation

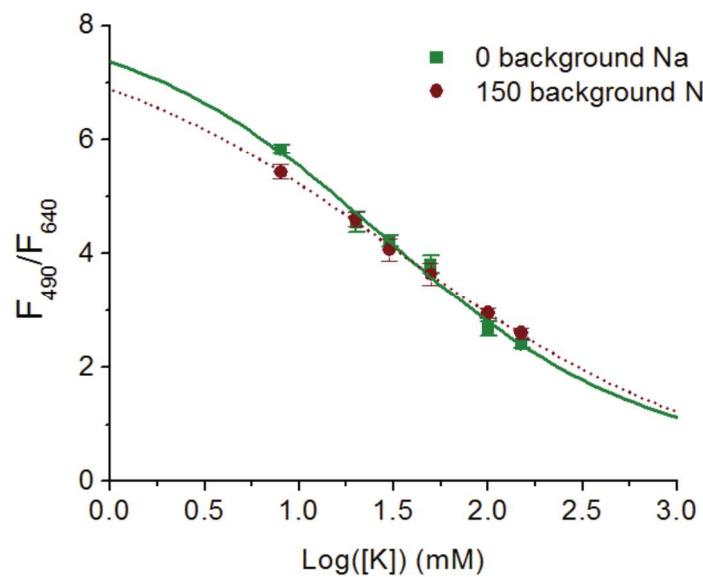


Figure S3 – Calibrations of nanosensors in 0 or 150 mM background Na. Error bars indicate one standard deviation

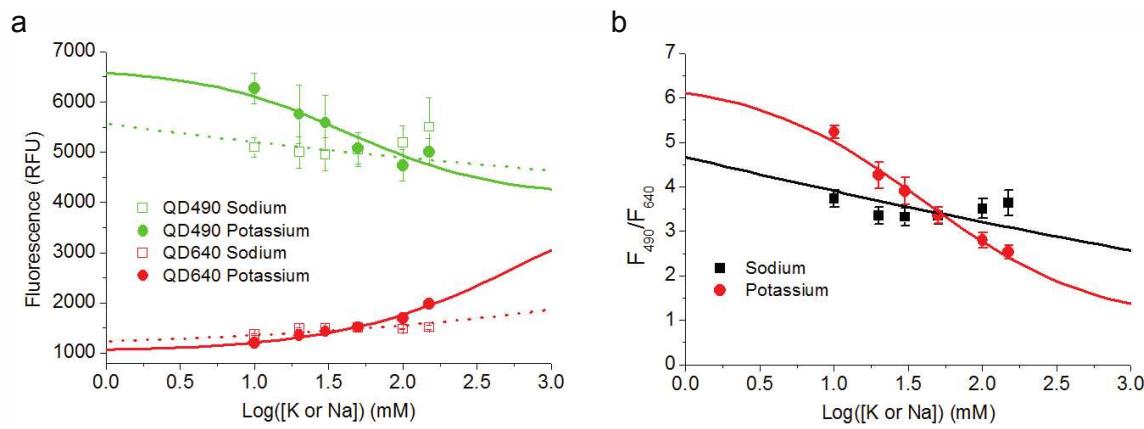


Figure S4 – Calibrations of nanosensors in 50 mM background ionic strength for responses to sodium or potassium. Individual channels (a) and the ratiometric measure (b) all show selective responses to potassium and no specific response to sodium. Error bars indicate one standard deviation

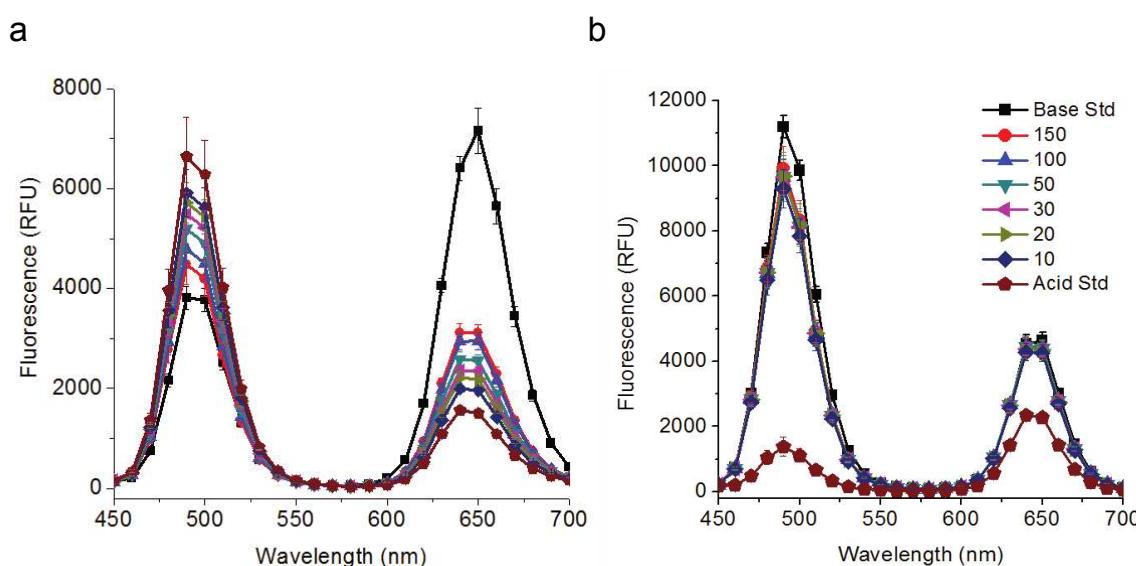


Figure S5 – Fluorescence spectra of potassium nanosensors made with (a) and without (b) chromoionophore showing that the quantum dots do not respond to potassium and the chromoionophore mediates the dynamic fluorescence response to potassium. Error bars indicate one standard deviation

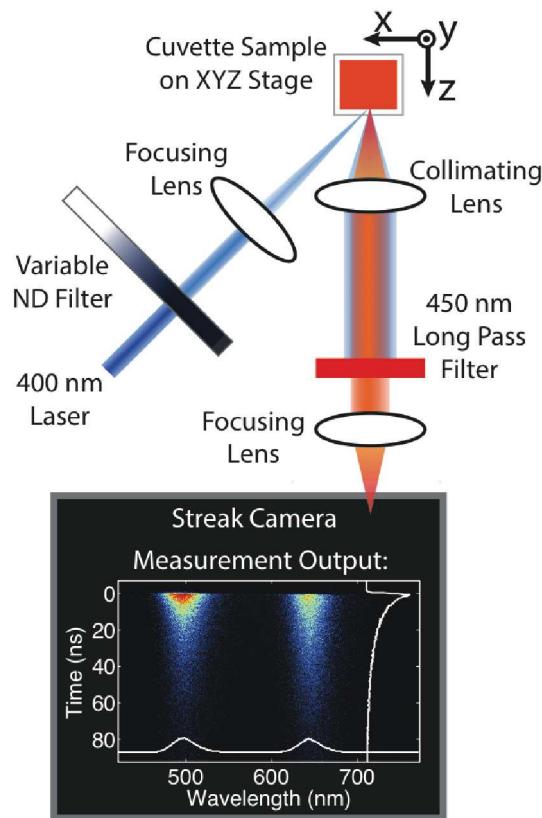


Figure S6 – Optical system for recording time-resolved spectra recorded from 440 nm to 780 nm over a 100 ns interval.

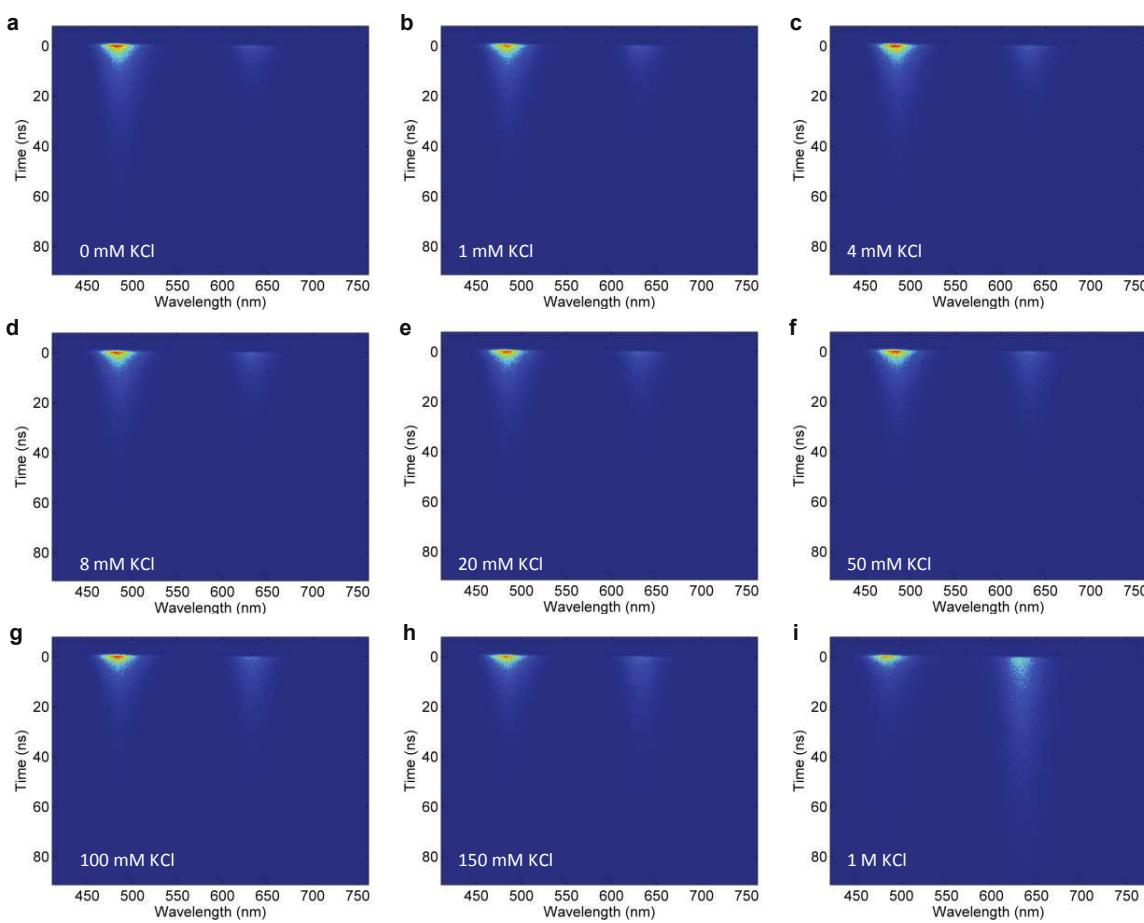


Figure S7 – Streak camera images from nanosensors in KCl solutions. Solution concentrations, in mM, of 0 (a), 1 (b), 4 (c), 8 (d), 20 (e), 50 (f), 100 (g), 150 (h), and 1000 (i) KCl.

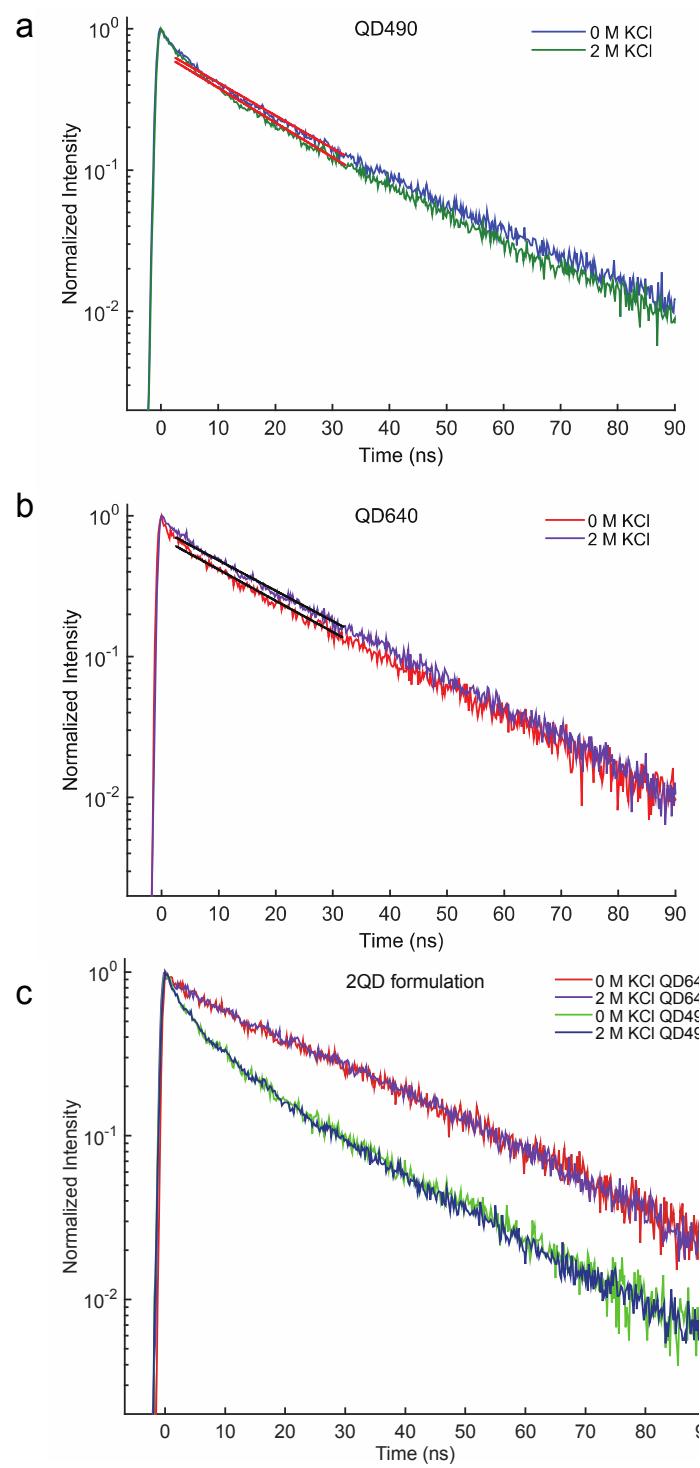


Figure S8 - Time-resolved fluorescence for nanosensors with QD490 (a), QD640 (b), and both QDs (c). None of these contained chromoionophore, and none exhibited any potassium dependent change in lifetime.

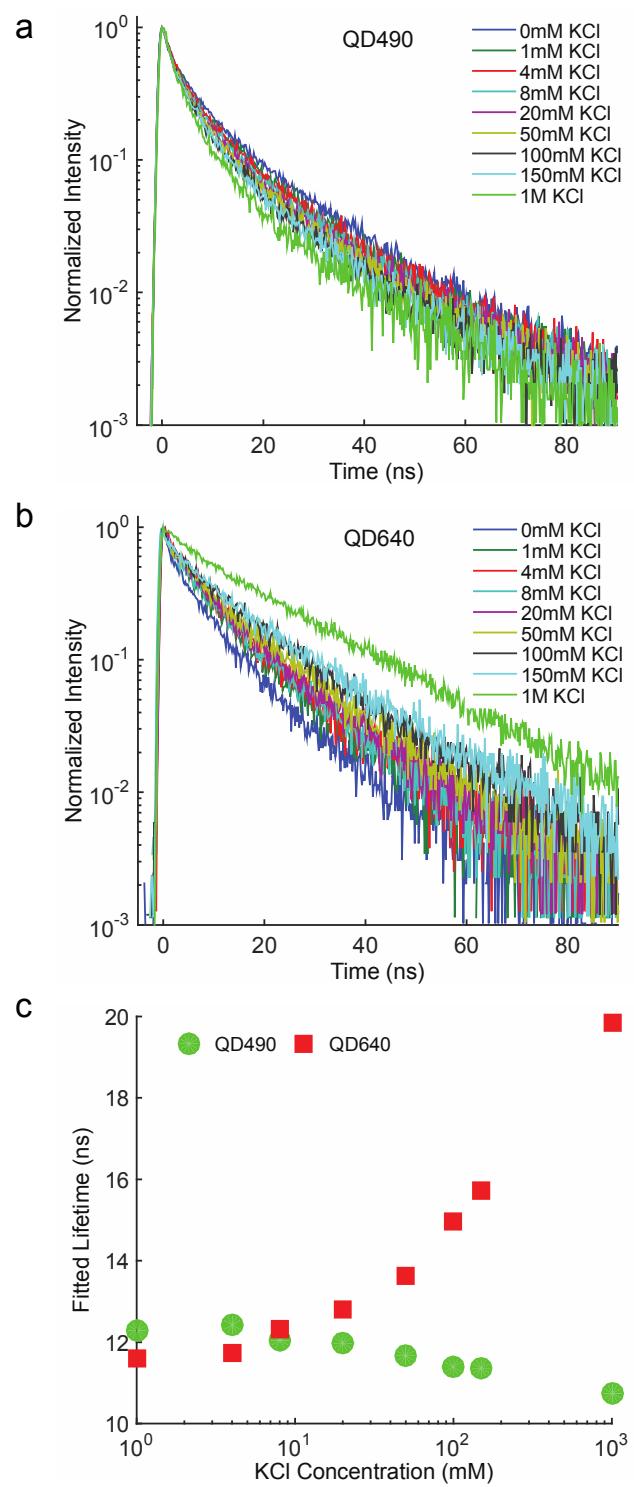


Figure S9 – Time-domain fluorescence lifetimes for QD490 (a) and QD640 (b) showing potassium-dependent fluorescence. Lifetimes are plotted against potassium concentration (c).

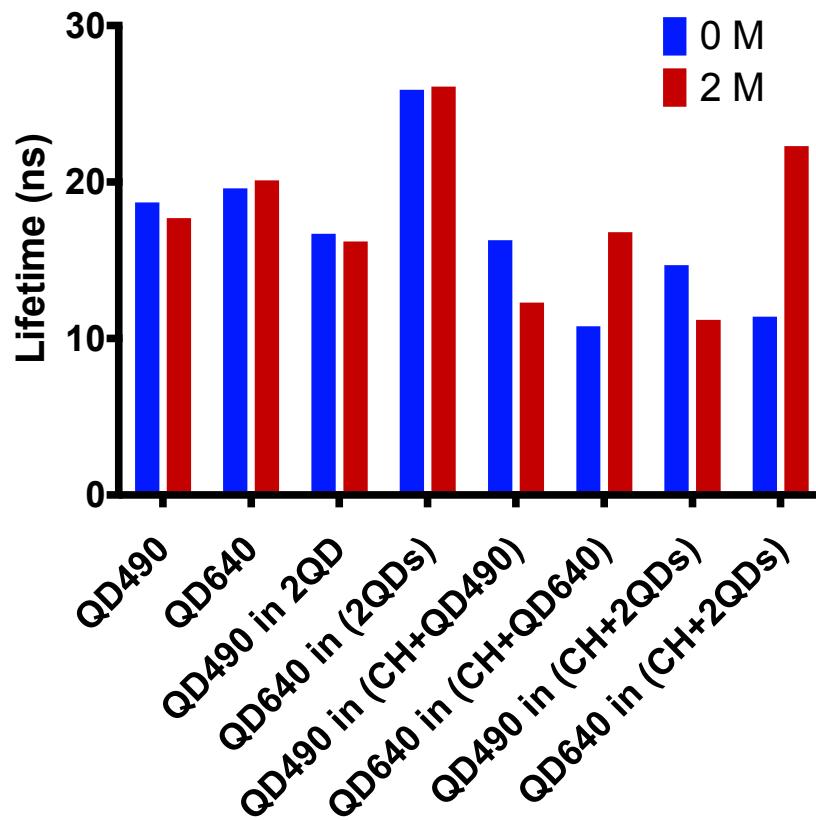


Figure S10 – Fluorescence lifetimes determined from fitting time-domain fluorescence images. X-axis indicates the component with the deconstructed nanosensor composition in parenthesis.

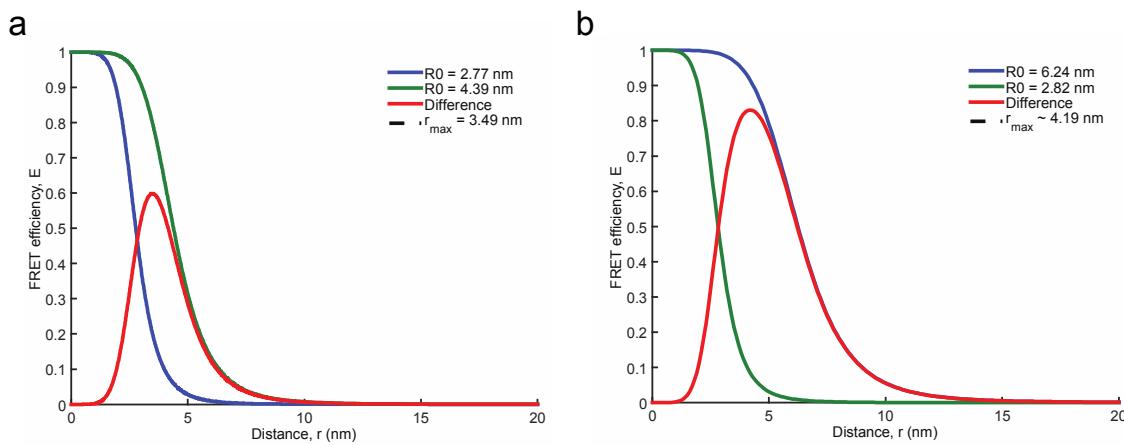


Figure S11 – FRET overlap integrals calculated from chromoionophore absorbance spectra and quantum dot emission spectra for QD490-CHII (a) and QD640-CHII (b). The difference in overlap integrals for the protonated CHII case (blue lines) and deprotonated case (green lines) is also plotted to show the QD-CHII spacing that will lead to maximal contrast between the two cases

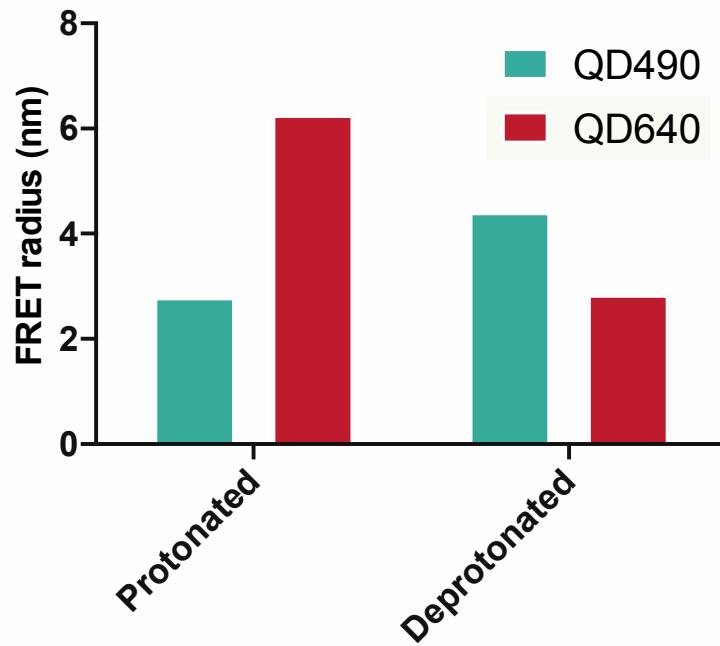


Figure S12 – FRET overlap integrals in the protonated and deprotonated chromoionophore cases predict the FRET radii from either QD to the chromoionophore in both conditions

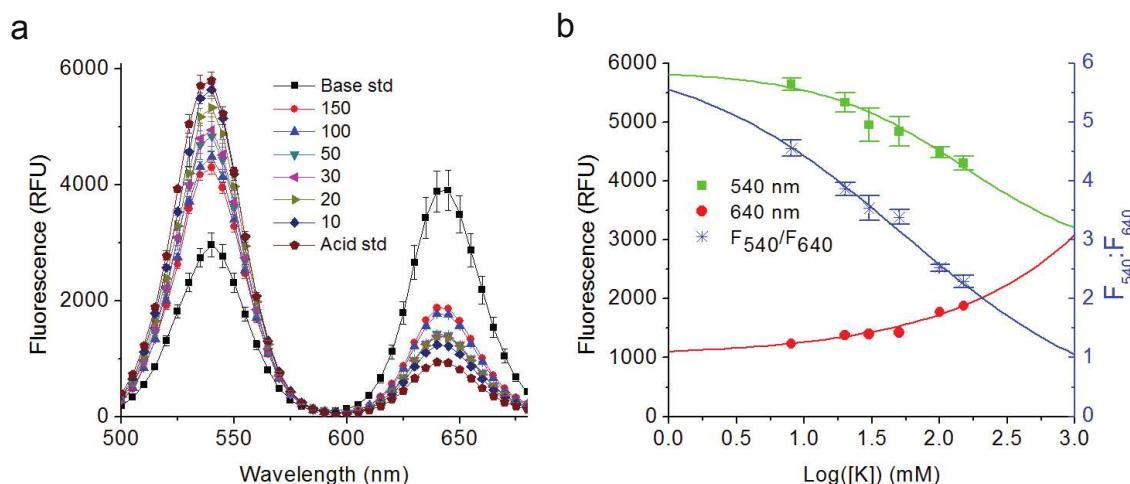


Figure S13 – Nanosensors made with QD540 and QD640. The fluorescence spectrum (a) and the resulting fitted calibration curves for both QDs and the ratiometric measure  $F_{540}/F_{640}$  (b). Error bars indicate one standard deviation

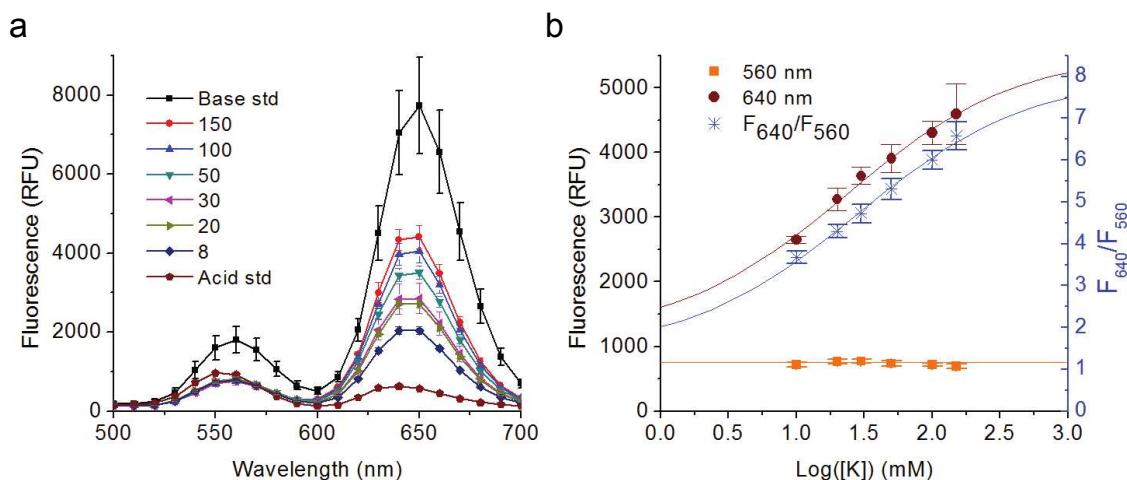


Figure S14 – Nanosensors with QD560 and QD640. The fluorescence spectrum (a) and the resulting fitted calibration curves for both QDs and the ratiometric measure  $F_{540}:F_{640}$  (b). The fabrication method was described in the methods, and the formulation consisted of 5 mg potassium ionophore II, 6 mg NaTFPB, 500 mg Chromoionophore II, 200 mL nitrophenyl octyl ether. Each batch contained 60 mg QD560 and 189 mg QD640. Error bars indicate one standard deviation

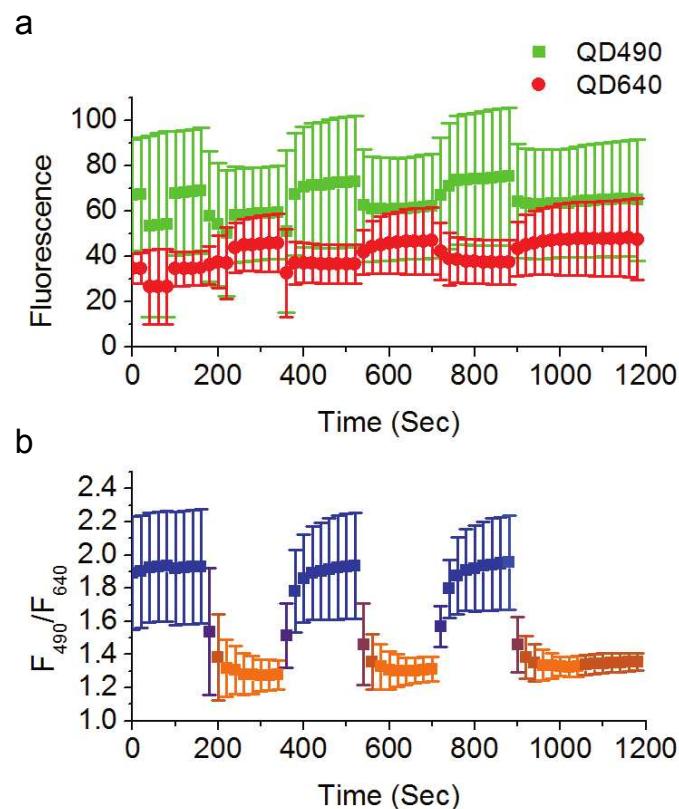


Figure S15 – Nanosensors are fully reversible for individual channels (a) and ratiometric signal (b) through over 20 minutes of imaging when exchanging solutions of 100 mM KCl

(b, blue) or 10 mM KCl (b, orange) in a perfusion chamber. Error bars indicate one standard deviation

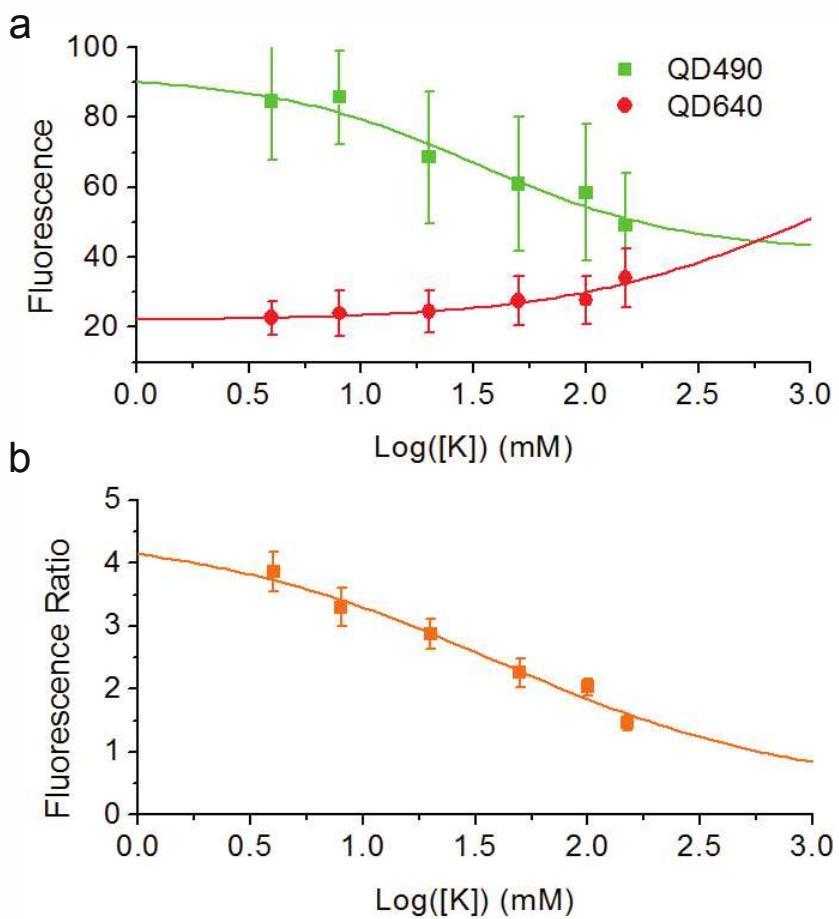


Figure S16 – Calibration curves fitted to data acquired by imaging nanosensors immobilized in dialysis tubing on a confocal microscope (Ex: 405 nm). Fluorescence from both QDs (a) and the intensity ratio (b) in response to potassium concentration. Error bars indicate one standard deviation