

Supplementary Figures.

Figure S1. Effect of diamide on CHO cell recovery. (a) Fluorescence micrograph showing CHO cells stably transfected with CY-RL7, imaged in the microfluidic under low flow conditions (48 μ L/min) at $t=0$, just prior to a pulse of 1.0 mM diamide. (b) A ratiometric analysis of the CY-RL7 fluorescence for four ROIs indicated in (a) are depicted in (b). The FRET parameter changes dramatically in the cytoplasm after exposure to 1 mM diamide in DMEM (solid vertical line) at $t=4$ min and recovers abruptly after a wash (dotted vertical line) at $t=8$ min. While CHO cells recover within 2 min after a wash as illustrated in (a,b), it takes about 40 min for CHO cells to recover in 1 mM diamide in DMEM without a wash. (c) Fluorescence micrograph showing CHO cells stably transfected with CY-RL7, imaged in the microfluidic under low flow conditions (48 μ L/min) at $t=0$, just prior to a step from zero to 1.0 mM diamide. (d) A ratiometric analysis of the CY-RL7 fluorescence for four ROIs indicated in (c) are depicted in (d). The FRET parameter changes dramatically after exposure to 1 mM diamide in DMEM (solid vertical line) at $t=4$ min and recovers abruptly *with no wash* only after about 40 min delay in contrast with the result in (b). As the concentration of diamide is reduced the recovery time is shortened. It takes only 16-18 min to recover in 0.5 mM diamide (no wash) and only 14-16 minutes to recover in 0.25 mM diamide with no wash. (e) Fluorescence micrograph showing CHO cells, just prior to a 0.5 mM diamide step in conditions similar to (c). (f) A ratiometric analysis of the CY-RL7 biosensor for four ROIs indicated in (e) is depicted in (f). The FRET parameter changes dramatically in the cytoplasm after exposure to 0.5 mM diamide in DMEM at $t=4$ min and recovers with no wash only after a 16-18 min delay. (g) Fluorescence micrograph showing CHO cells just prior to a step to 0.25 mM diamide in conditions similar to (c). (h) A ratiometric analysis of the CY-RL7 biosensor for four ROIs indicated in (g) and is depicted in (h). The FRET parameter changes dramatically in the cytoplasm after exposure to 0.25 mM diamide in DMEM at $t=4$ min and recovers with no wash only after a 14-16 min delay. These data are representative of 3 independent experiments using a minimum of 4 ROIs.

Figure S2. Effect of diamide concentration on change in FRET in CHO cells. We find the the change in FRET depends only weakly on diamide concentration. (a) Fluorescence micrograph showing CHO cells stably transfected with CY-RL7, imaged in the microfluidic under low flow conditions (48 μ L/min) at $t=0$, just prior to a 2.0 mM diamide pulse. (b) A ratiometric analysis of the CY-RL7 fluorescence for four ROIs indicated in (a) are depicted in (b). The FRET parameter changes dramatically in the cytoplasm after exposure to 2 mM diamide in DMEM (solid vertical line) at $t=4$ min. (c) Fluorescence micrograph showing CHO cells, just prior to a 1.0 mM diamide pulse in conditions similar to (a). (d) A ratiometric analysis of the CY-RL7 biosensor for four ROIs indicated in (c) are depicted in (d). The FRET parameter changes dramatically in the cytoplasm after exposure to 1 mM diamide in DMEM at $t=4$ min. (e) Fluorescence micrograph showing CHO cells just prior to a 0.5 mM diamide pulse in conditions similar to (a). (f) A ratiometric analysis of the CY-RL7 biosensor for four ROIs indicated in (e) and is depicted in (f). The FRET parameter changes dramatically in the cytoplasm after exposure to 0.5 mM diamide in DMEM at $t=4$ min. (g) Fluorescence micrograph showing CHO cells just prior to a 0.25 mM diamide pulse in conditions similar to (a). (h) A ratiometric analysis of the CY-RL7 biosensor for four ROIs indicated in (g) and is depicted in (h). The FRET parameter changes dramatically in the cytoplasm after exposure to 0.25 mM diamide in DMEM at $t=4$ min. These data are representative of 3 independent experiments using a minimum of 4 ROIs.

Figure S1.

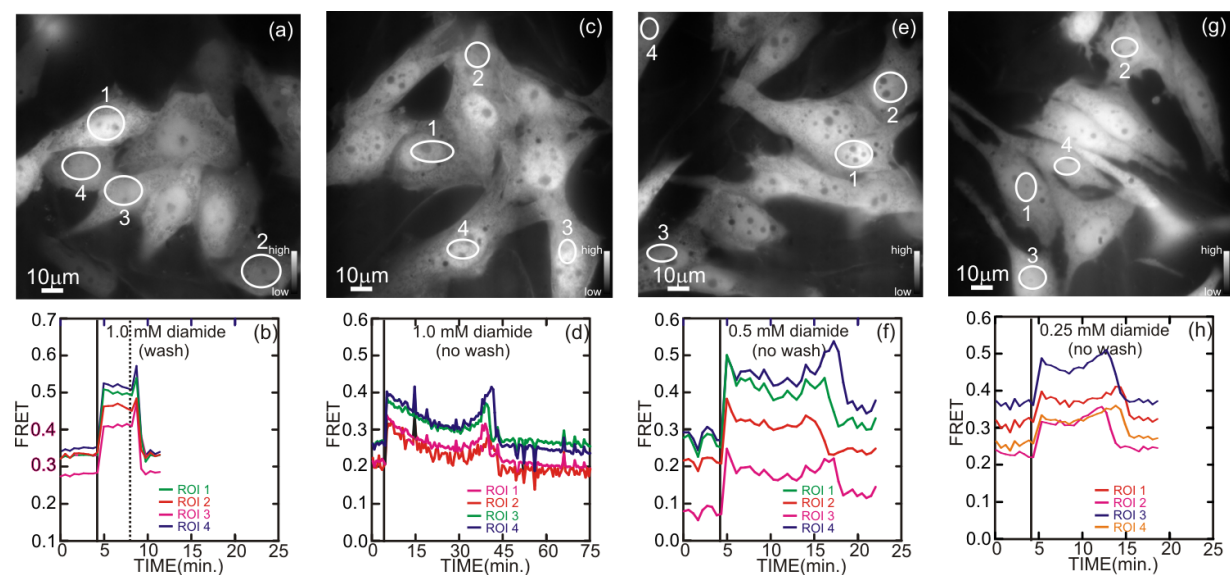


Figure S2.

