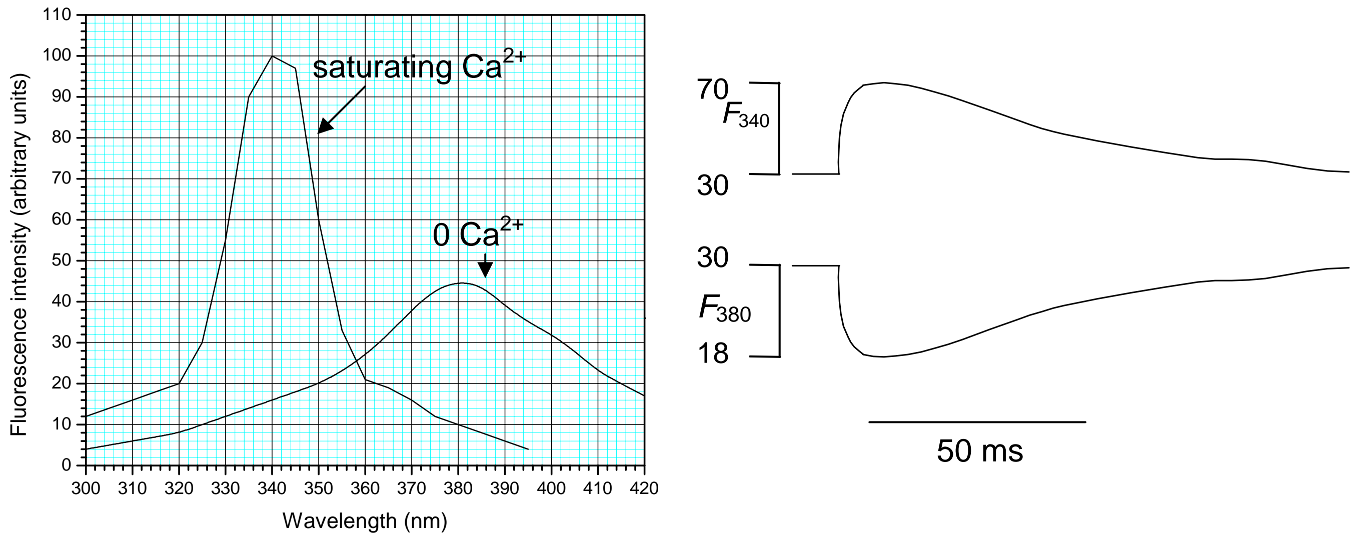
(Posted 2/16/06; due 2/24/06)

1. The graph below (*left*) shows the fluorescence excitation spectra obtained from an in vitro calibration of a synthetic fluorescence Ca^{2+} indicator at zero and saturating Ca^{2+} concentrations. The figure on the right shows fluorescence intensity signals (following excitation at 340 nm and 380 nm) recorded from a cell loaded with the fluorescent Ca^{2+} indicator.



Answer the following questions assuming that: (i) the binding of Ca^{2+} and indicator is 1:1; (ii) $k_{on} = 4 \times 10^8\ M^{-1}\ s^{-1}$; (iii) $k_{off} = 100\ s^{-1}$; (iv) the properties of the dye are identical between *in vitro* and intracellular conditions; (v) the intracellular concentration of the dye is 50 μM.

(A) Compute the resting intracellular Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$).

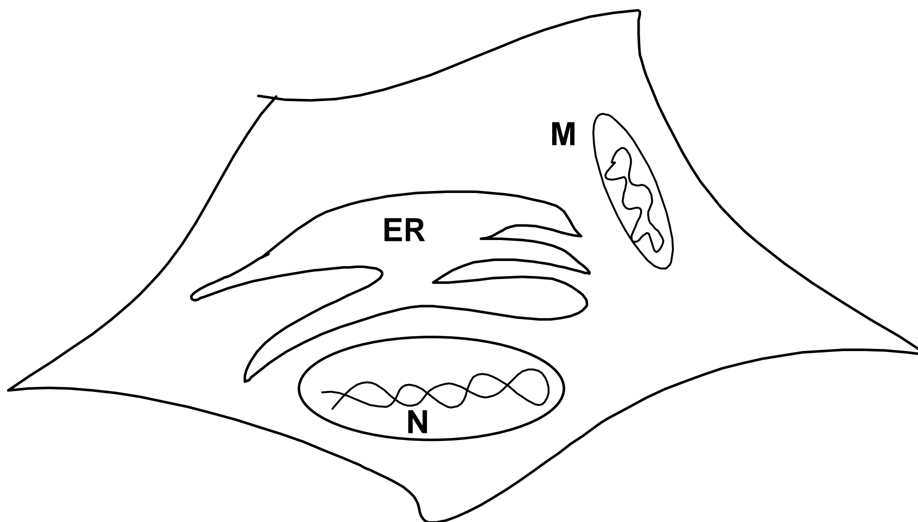
(B) Compute the $[Ca^{2+}]_{cyt}$ at the peak of the Ca^{2+} transient.

(C) How would your answers to A and B change if the intracellular concentration of dye was slightly more than 50 μM.

(D) Calculate the concentration of free indicator at rest and during the peak of the Ca^{2+} transient, assuming steady-state distributions of reactants in both these instances.

(E) Suppose that at the peak of the intracellular Ca^{2+} transient, a physiological stimulus resulted in a further increase in intracellular Ca^{2+} concentration, doubling the peak $[Ca^{2+}]_{cyt}$. Comment briefly on how well this new change in Ca^{2+} will be tracked by the dye. Briefly describe what parameters of the dye you would adjust to improve its performance in this regime.

2. The figure below shows a generic cell with the following intracellular organelles labeled— N, nucleus; ER, endoplasmic reticulum, and M, mitochondria.



A. On the figure above, sketch in and label the principal mechanisms responsible for removing Ca^{2+} from the cytoplasm, and state the energy source used to drive Ca^{2+} fluxes.

B. You have a generic cameleon (cameleon-A) construct consisting of calmodulin and M13 sandwiched between a cyan fluorescent protein (excitation peak, 440 nm; emission peak, 480 nm) and a yellow fluorescent protein (excitation peak, 500 nm; emission peak, 525 nm). The calcium sensor in the cameleon has been mutated such that Ca^{2+} binding occurs with a single apparent dissociation constant K'_d (with $k_{on} = 2 \times 10^6\ M^{-1}\ s^{-1}$, and $k_{off} = 12\ s^{-1}$).

(i) What changes would you make in cameleon-A that would permit you to monitor Ca^{2+} concentration changes in the endoplasmic reticulum (cameleon-B) and mitochondria (cameleon-C), respectively?

(ii) You have different cells expressing cameleon-A, cameleon-B and cameleon-C, respectively and are able to excite the fluorophores with 440 nm wavelength light. For each of the three cameleons listed, sketch the waveform of the emission spectrum expected under resting conditions (solid line), and after treatment with thapsigargin (dashed line). Label all axes.

(iii) Briefly outline the steps you would take to calibrate the emission spectra data in terms of Ca^{2+} concentration in the context of the transfected cells.