Supporting Information for:

NIR-activated content release from plasmon resonant liposomes for probing single-cell responses

Sarah J. Leung and Marek Romanowski*

Department of Biomedical Engineering, University of Arizona, Tucson, AZ

marekrom@email.arizona.edu

Table of Content

This material is available free of charge *via* the Internet at http://pubs.acs.org.

1. Supplemental Figures

1.1. Intensity weighted sizing

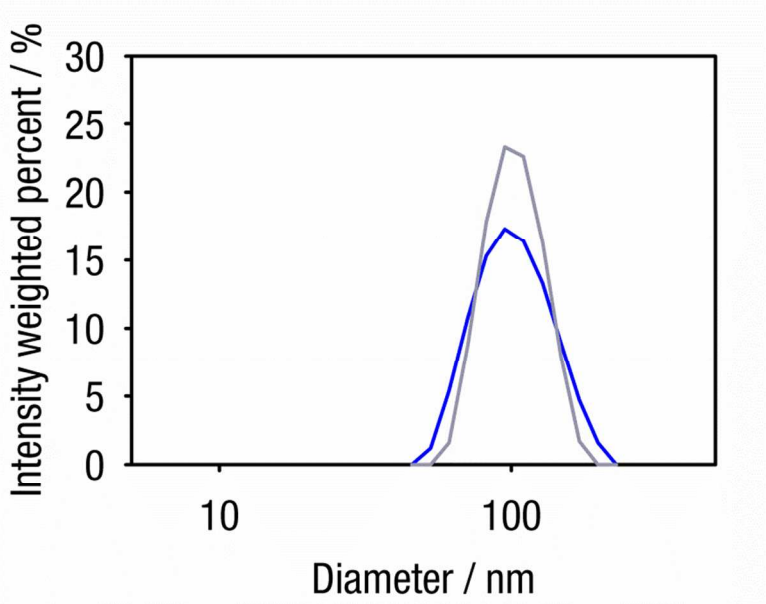

Figure S1. Intensity weighted sizing data for uncoated (grey) and 680 nm gold-coated (blue) liposomes encapsulating CCK8. Uncoated and gold-coated liposomes have average diameters around 100 nm.

1.2. HEK293/CCK2R calcium response in the laser path

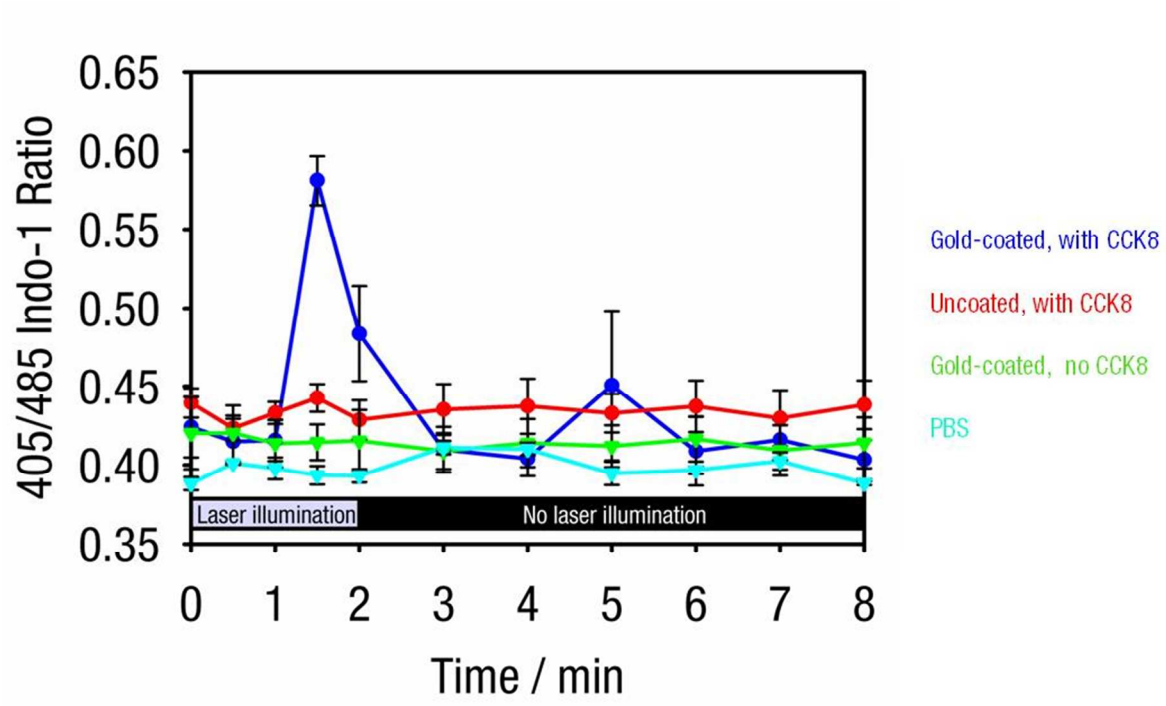

Figure S2. Time dependence of the fluorescence emission intensity ratio (405 nm to 485 nm) of HEK293/CCK2R cells in the path of the 760 nm laser. While otherwise similar to Figure 5, only cells in the laser path are reported here; this accounts for a total of two cells for each experimental condition, two trials with one cell in the beam path in each trial. Intensity counts were obtained with HEK293/CCK2R cells incubated with: gold-coated liposomes containing CCK8 (blue), uncoated liposomes containing CCK8 (red), gold-coated blank liposomes (green), and PBS (cyan). Time 0 indicates the initiation of 760 nm laser illumination and time 2 indicates the end of laser illumination. For all samples, fluorescence ratios were collected from the single cell in the laser beam path; averages and standard deviations are derived from two trials. Increases in the fluorescence ratio are seen when cells incubated with gold-coated liposomes loaded with CCK8 are illuminated with 760 nm laser light For other preparations, no significant changes in intracellular calcium levels are detected in cells illuminated with laser light.

1.3. HEK293/CCK2R calcium response to free CCK8 at different temps

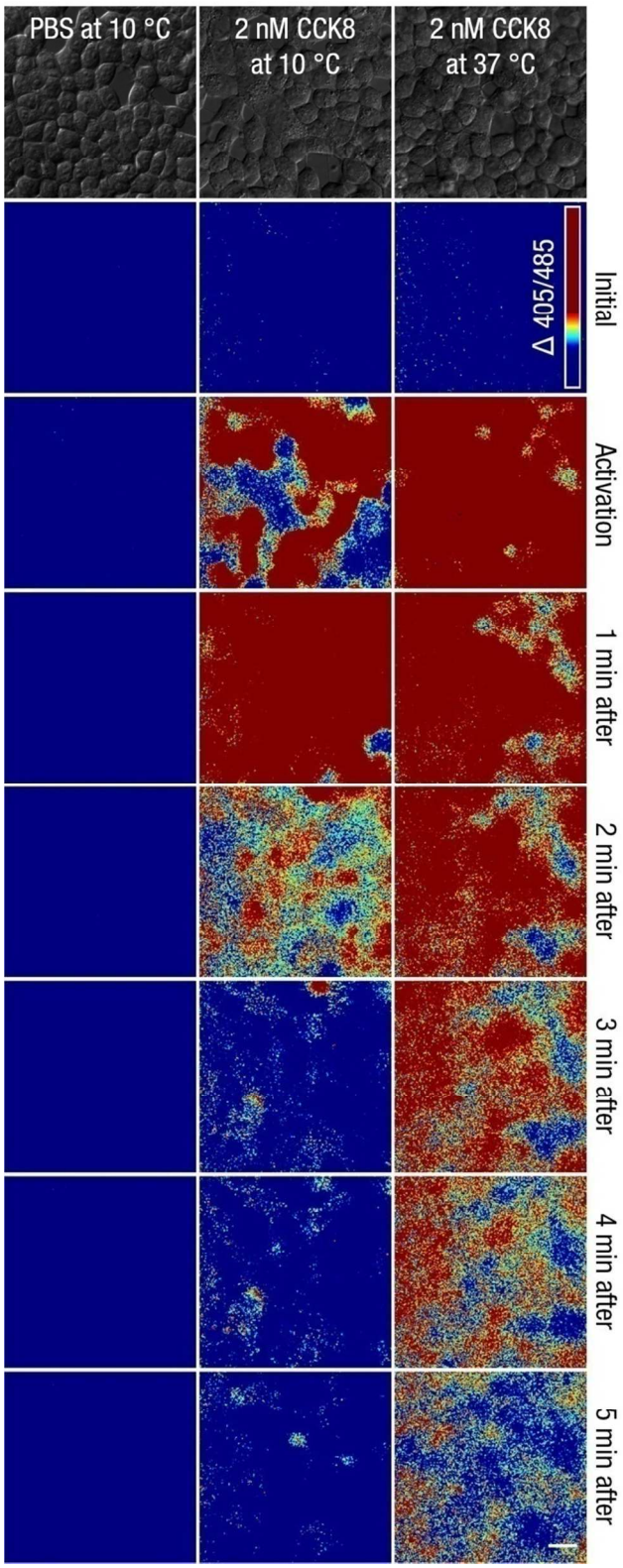

Figure S3. DIC images and time-lapse intracellular calcium concentration changes within HEK293/CCK2R cells following incubation with PBS at 10°C (left column), 2 nM free CCK8 at 10°C (middle column), and 2 nM free CCK8 at 37°C (right column). The "activation" time point signifies the

addition of PBS or free ligand. Images are derived from subtracting a baseline 405/485 ratiometric image (taken directly prior to PBS or CCK8 addition) from those of each represented time point. Addition of PBS at 10°C shows no marked increases in intracellular calcium. Addition of free CCK8 at both 10°C and 37°C results in activation of cells in the entire field of view. However, the response at 10°C requires longer to affect all cells in the field of view and the intracellular calcium response does not last as long as that of cells at 37°C. The false color scale at the top right corner applies to all panels and extends over a range of 0 to 0.1. The scale bar at the lower right corner corresponds to 20 μm.

1.4. HEK293/CCK2R calcium response to PBS at 10ºC

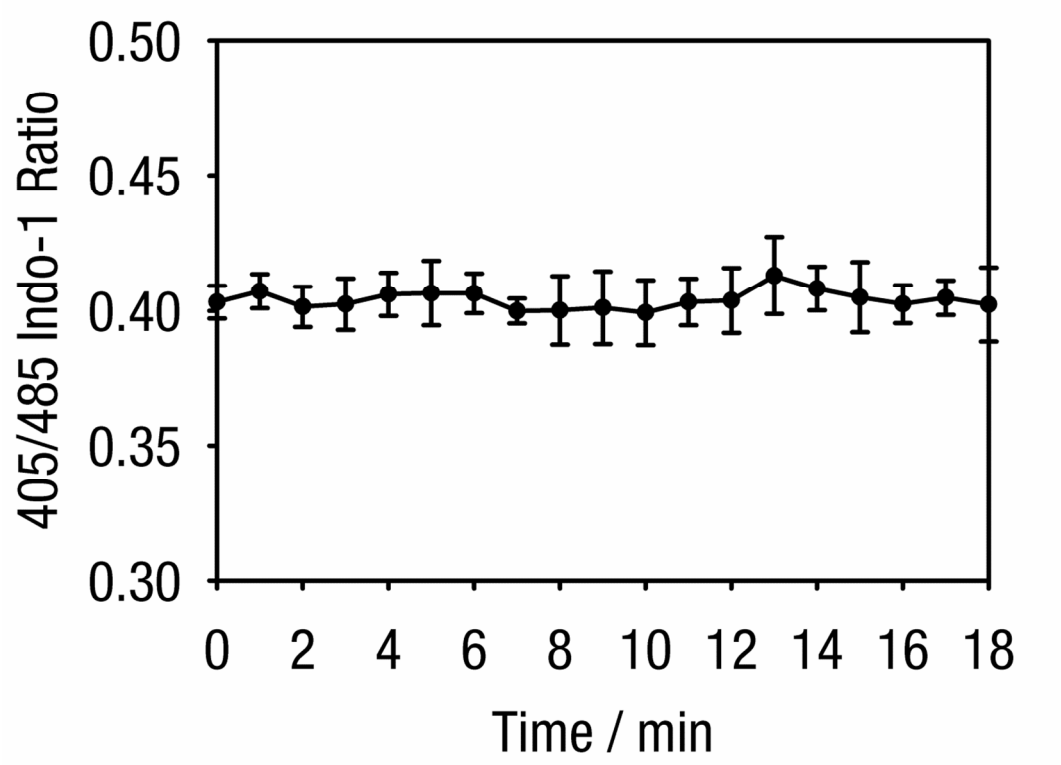

Figure S4. HEK293/CCK2R fluorescence emission intensity ratio (405/485 nm) over time in response to PBS at 10°C. Measurements were collected from five randomly selected cells in each trial for two trials; averages and standard deviations are calculated from measurements of ten cells. No significant changes in intracellular calcium levels were observed over an 18 minute time span, suggesting an absence of nonspecific calcium transients.