

Zagotta et al., <http://www.jgp.org/cgi/content/full/jgp.201511531/DC1>

Amber codon (TAG) suppression strategy

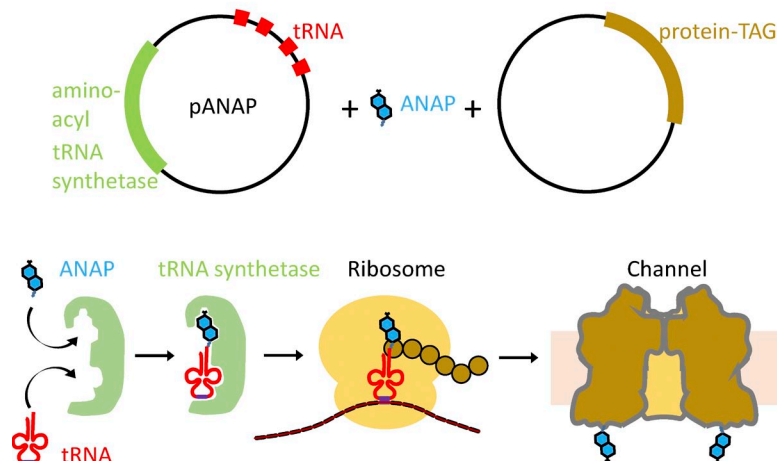


Figure S1. Cartoon representation of amber codon suppression strategy for site-specific incorporation of L-ANAP into TRPV1.

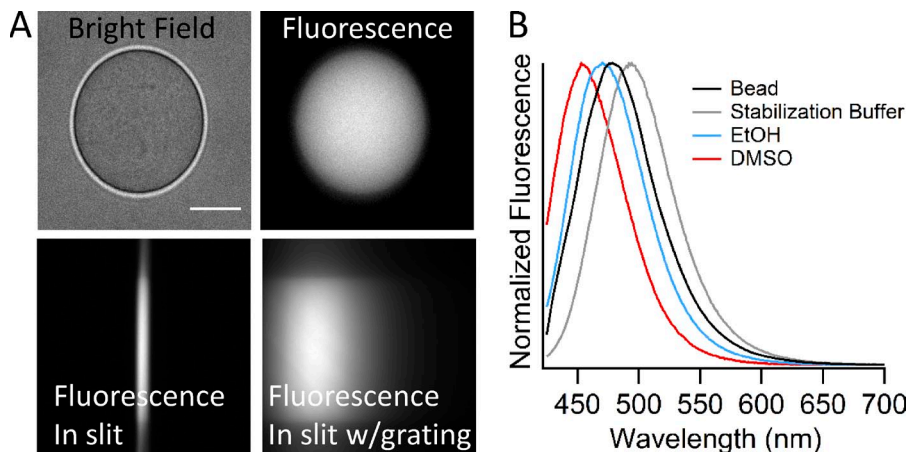


Figure S2. Emission spectra of L-ANAP-ME bound to agarose beads. Amine-reactive agarose beads were reacted with L-ANAP-ME to determine its emission spectrum on a surface. (A) The bead imaged in bright field (top left) and with the ANAP-appropriate fluorescence cube (top right). We measured the spectrum by moving a slit into the image plane (bottom left), directing the light that passed through the slit onto a diffraction grating and then directing the light onto a camera to yield an image of the emission spectrum (bottom right). The scale bar at top left is 20 μ m and applies to the first three images described. (B) A line scan through the spectral image was background subtracted, and the wavelength was calibrated with known laser lines, revealing the emission spectrum of L-ANAP-ME on a surface (black trace). The peak of the emission spectrum of L-ANAP-ME on a surface differed slightly from the spectrum measured in a fluorometer of free L-ANAP-ME in our experimental buffer (Fig. 8 B, gray trace), in ethanol (blue trace), and in dimethyl sulfoxide (red trace).

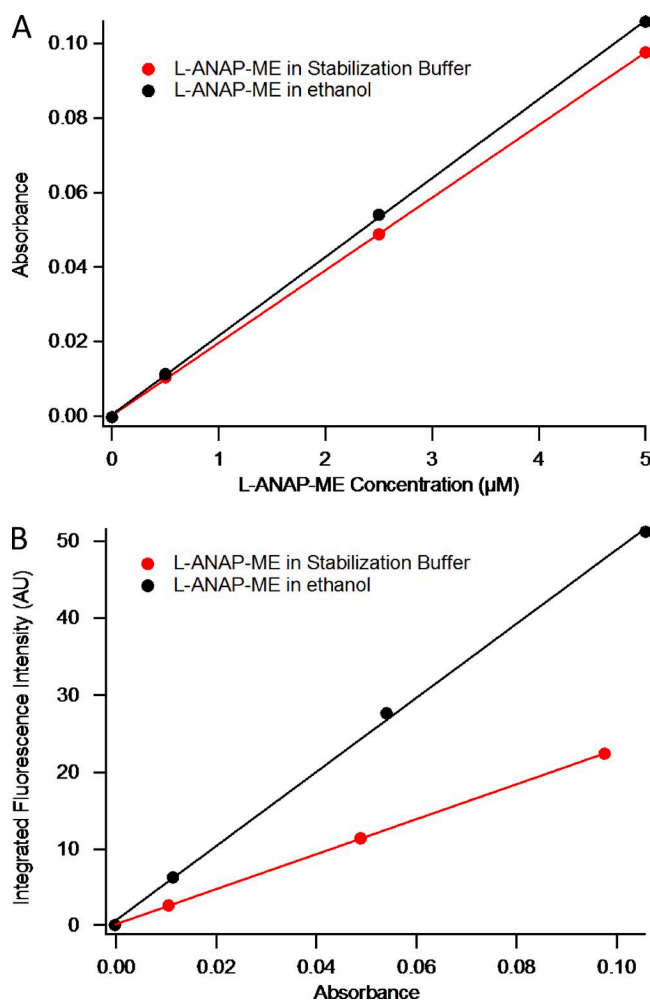


Figure S3. Determination of L-ANAP-ME extinction coefficient and quantum yield in stabilization buffer. Fluorescence and absorption were measured in stabilization buffer (red circles) and ethanol (black circles) with excitation at 360 nm. (A) The extinction coefficients of L-ANAP-ME, as measured from the slopes of the fits to the data, yielded values of $19,500 \text{ M}^{-1}\text{cm}^{-1}$ in stabilization buffer and $21,200 \text{ M}^{-1}\text{cm}^{-1}$ in ethanol. (B) The quantum yield of L-ANAP-ME in stabilization buffer (0.22) was calculated relative to the reference quantum yield of L-ANAP in ethanol (0.48; Chatterjee et al., 2013) using the following equation: $QY_{SB} = QY_{EtOH}(\text{slope}_{SB}/\text{slope}_{EtOH})(\eta_{SB})^{0.5}(\eta_{EtOH})^{0.5}$, where QY is the quantum yield of L-ANAP, slope refers to the slope of the linear fits to the data, and η is the refractive index.

REFERENCE

Chatterjee, A., J. Guo, H.S. Lee, and P.G. Schultz. 2013. A genetically encoded fluorescent probe in mammalian cells. *J. Am. Chem. Soc.* 135:12540–12543. <http://dx.doi.org/10.1021/ja4059553>