

## **AM Dye Loading and Imaging Protocol**

- Make stock solution of DMSO with 6% pluronic F-127 (Invitrogen). It is EXTREMELY important to create a solution that is clear as a mountain stream. Be patient on this step, use heating or buy it already in solution.
- Make stock solution of 1 mM dye in the DMSO/pluronic solution.
- Sonicate for 5 min.
- **Loading buffer:** Dilute to stock to ~2 uM dye (500x) in cell buffer with 200 uM Sulfinpyrazone (anionic transport inhibitor) and load for 20-30 min. at 37°C.
- **Imaging buffer:** Image in buffer with Sulfinpyrazone (to prevent cytosolic dye loss).

### Notes:

To make 10mM stock solution of Sulfinpyrazone (Sigma S-9509) you need to bring the pH up to ~11 and stir. Once it has dissolved bring it back down to ~8.5.

For loading tissue it works best to load cold for a couple of hours enabling the dye to penetrate. Then bring it up to 37°C for 20ish min for ester hydrolysis.