GENERAL USE

Here is how we use the Rubi compounds...

We typically reperfuse over the slice when using these, but if you are going in vivo, you will probably just either dump on the brain, or bolus inject.

We weight out the appropriate amount of the compound into a 1.5 mL eppendorf, or a 10 ml centrifuge tube. This can be done in the light, but you should be quick, especially if it is very humid. We then go to a dark room, or one with only red light, and add the ACSF to make the solution. We close it, and cover the whole thing in aluminum foil so it is protected from the light. When doing the experiment, the mouse, or rather the solution has to be kept in the dark, especially with the RuBi, because it absorbs and uncages with blue and green light. We just put a red filter on our flashlights so we can see.

For simplicity, you may want to dissolve it all in ACSF and make a strong stock solution, then just take an aliquot and dilute with ACSF to the appropriate concentration. Kept cool, like in a +4 C refrigerator, and in the dark (amber bottle, wrapped in foil), the stock should have a near indefinite shelf life. We have used it a few months after making it...

For the bath application, we use 5-7 ml recirculating, and don't do too much to control for osmolarity shifts. For a little while, we tried to monitor the overall volume of ACSF, and add some H2O to keep it constant, but now we don't usually do anything... We do most of our expts at room temp, so evaporation is less of an issue than the higher temps, but certainly one we talk about occasionally during lunch, etc. We guess we could take aliquots and test it and add H2O, but ...

We have tested re-using solution for a few days, and when we do this, we test the osmo. At the end of the day, if we see it has changed, for us, say from 307 to 280, or about 10%. we just add H2O and use it again. Not many other people in the lab reuse the solution, at least for 2-Photon expts, because they don't want even the possibility of not having good uncaging during their hard enough expts. In many cases, it was reliable for another day - but not always. However, if you have any use for it for 1 photon expts, it works very well everytime - we dilute it down to 30 uM or so, and it can be stored in the fridge in a light-tight vessel (we use glass covered a few times with Al foil) essentially indefinitely.

We have looked at the 2-P uncaging wavelength dependence of RuBi-GABA, and you can get uncaging from 725 to 900. The peak is around 800 and we'll call it 100%, and it is maybe 65-70% at 725, the same at 850, and only 30% at 900. We expect the RuBi-Glut to be similar, as the absorbtion is dominated by the RuBi portion. The Phosphine is a little different as well, but again, I would treat these numbers as reasonable estimates.

If you have any questions/comments/problems, let us know.