



Figure S2 – Validation of Frap Routines. To correct for the finite bleaching time associated with these experiments, an increment  $dt$  equal to half the bleaching pulse length was added to the standard FRAP equations. To test the validity of this approach, FRAP experiments were simulated with COMSOL finite element software. Bleach spot size was set to  $30\mu\text{m}$  and a bleach depth resulting in 70% photobleaching over the 20s bleach interval was used. Diffusion coefficient was varied across an order of magnitude in a range typical of supported bilayers. Without the  $dt$  correction, fits resulted in large non-random residuals and large error in extracted diffusion coefficient. With the  $dt$  correction, fits were much improved as evidenced by the small residuals and  $<3\%$  error in calculated diffusion coefficient. To mitigate the effects of unwanted photobleaching during observation, recovery intensity was normalized to a reference area far from the bleach spot. This approach was also validated with finite element simulations and was found to improve fitting accuracy without adding systematic errors.