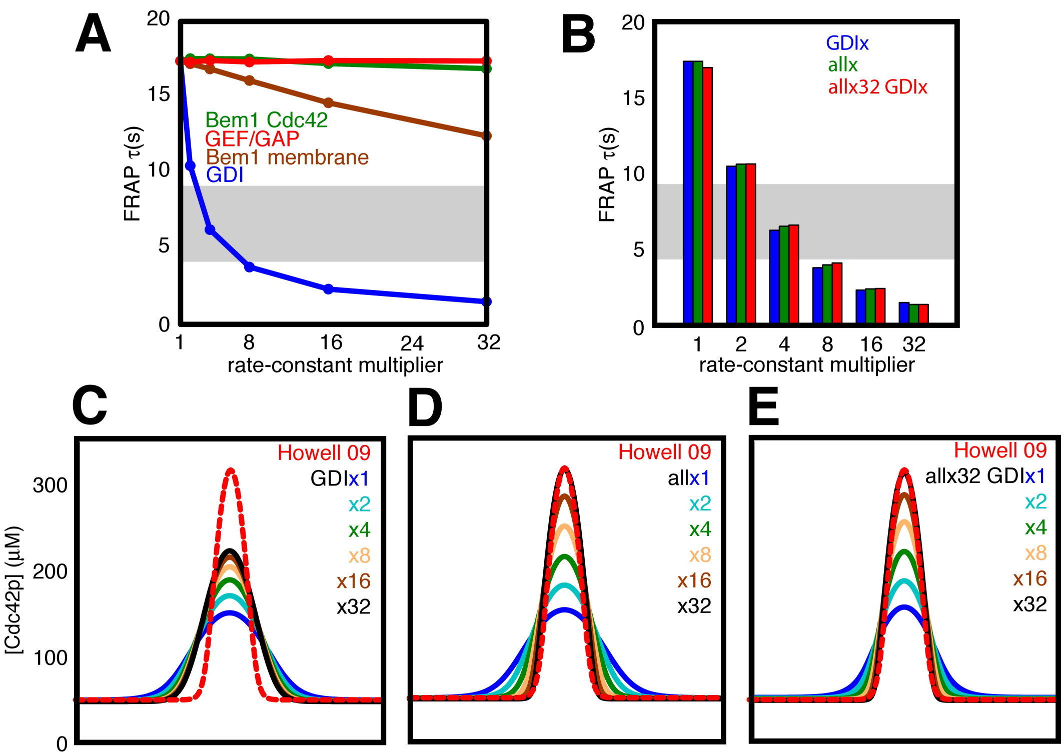
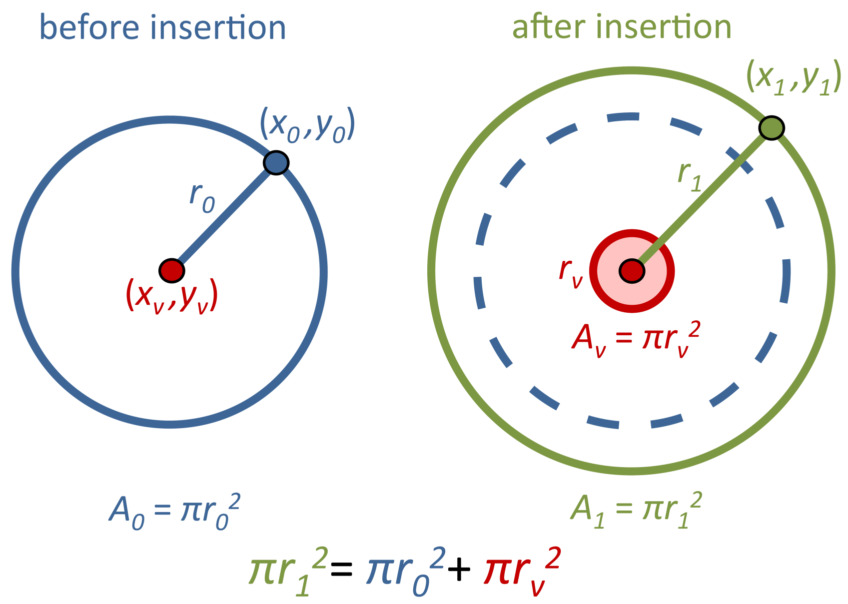


Supplementary Figure 1. Cdc42p FRAP simulations with fast diffusion.
A) Simulations of FRAP experiments varying model parameter sets as in Fig. 2C, but with D_m=0.036 $\mu m^2/s$. As before, the GDI is the dominant contributor to FRAP kinetics. **B)** FRAP simulations were conducted for the peaks shown in C (blue), D (green), or E (red) below, and the simulated recovery half-time is shown (τ). Accelerating only the GDI reaction rates (blue) yields essentially the same result as accelerating all reaction rates (green), and multiplying all reaction rates except for the GDI reactions by 32 has no further effect (red). **C)** Cdc42p profiles with with D_m=0.036 $\mu m^2/s$ and the GDI rate constants multiplied by the indicated factor. The D_m=0.0025 $\mu m^2/s$ profile (Howell et al. 2009) is shown for comparison (dashed red curve). **D)** Cdc42p profiles with with D_m=0.036 $\mu m^2/s$ and every rate constant multiplied by the same factor (indicated for each color). **E)** Cdc42p profiles with with D_m=0.036 $\mu m^2/s$ and every rate constant multiplied by 32x except for the GDI rate constants, which are multiplied by the indicated factor. These simulations show that the GDI reaction constants determine the FRAP kinetics, though other rate constants contribute to determining peak shape. Thus, with fast diffusion, the model predicts either overly rapid fluorescence recovery or a very broad Cdc42p distribution.



Supplementary Figure 2. Schematic of method used to calculate new concentrations in the membrane surrounding an inserted vesicle. The point (x_v, y_v) describes the coordinates of the center of the vesicle. The point at (x_0, y_0) before vesicle fusion moves to (x_1, y_1) after vesicle fusion such that the three points, (x_v, y_v), (x_0, y_0), (x_1, y_1), lie on a straight line. In the equation, $\pi r_1^2 = \pi r_0^2 + \pi r_v^2$, r_1 is the distance of point (x_1, y_1) from the center of the vesicle, r_0 is the distance of point (x_0, y_0) from the center of the vesicle, and r_v the radius of the vesicle. The distances r_0 can be calculated using $r_0 = \sqrt{r_1^2 - r_v^2}$ (a rearrangement of the area preserving relationship $\pi r_1^2 = \pi r_0^2 + \pi r_v^2$), and the coordinates can be calculated using, $r_0 = \sqrt{(x_v - x_0)^2 + (y_v - y_0)^2}$.