

a. *Thermodynamics of roGFP2.* Mathematically, the Grx-mediated exchange of electrons between glutathione and roGFP2 can be described by the Nernst equilibrium  $E_{GSH} = E_{roGFP2}$ , specifically

$$\begin{aligned} E_{GSH} &= E_{GSH}^{\circ'} - \frac{RT}{2F} \ln \left( \frac{[GSH]^2}{[GSSG]} \right) \\ &= E_{roGFP2}^{\circ'} - \frac{RT}{2F} \ln \left( \frac{[roGFP2_{red}]}{[roGFP2_{ox}]} \right) \\ &= E_{roGFP2} \end{aligned}$$

In this equation,  $R$  is the gas constant ( $8.315 \text{ J K}^{-1} \text{ mol}^{-1}$ ),  $T$  the absolute temperature (298.15 K), and  $F$  the Faraday constant ( $96,485 \text{ C mol}^{-1}$ ).  $E_{GSH}^{\circ'}$  is  $-240 \text{ mV}$  (103) and  $E_{roGFP2}^{\circ'}$  has been determined as  $-280 \text{ mV}$  (27). For practical purposes, it is ex-