**Figure captions**

**Figure 1**. **The effect of crowding agent PEG-8000 on the spectral properties of iRFP713 in the holoform.** (A) Absorption spectra, tryptophan fluorescence spectra (ex = 295 nm) and the chromophore fluorescence spectra (ex  = 690 nm). (B) CD spectra in the far-UV, near-UV and visible region of the spectrum. The color of the curves corresponds to different concentration of PEG-8000: 0 mg/ml (black line), 80 mg/ml (red line), 120 mg/ml (gray line) and 300 mg/ml (blue line).

**Figure 2**. **The effect of crowding agents Dextran-40 and Dextran-70 on the spectral properties of iRFP713 in the holoform.** The designations on panels A–B are the same as in the caption to figure 1. The color of the curves symbolizes the different experimental conditions: 0 mg/ml crowding agent (black line), 240 mg/ml of Dextran-40 (red line) and 240 mg/ml of Dextran-70 (green line).

**Figure 3. Unfolding of iRFP713 in the holoform induced by guanidine hydrochloride (GdnHCl) in the presence of PEG-8000.** (A and B) Changes in the tryptophan fluorescence intensity at registration wavelengths of 320 and 365 nm. The fluorescence was excited at wavelength of 295 nm. The values of fluorescence intensity *I*320 and *I*365 were normalized to unity at zero denaturant concentration. (C) Changes in optical density of the solution;  = 690 nm. (D) Changes in the chromophore fluorescence intensity at an excitation wavelength of 690 nm, corrected for the primary inner filter effect taking into account changes in the absorbance of the solution at the excitation wavelength (see Materials and Methods). (E) Changes in the parameter *A* = *I*320/*I*365 at an excitation wavelength of 295 nm. (F) Changes in fluorescence anisotropy at excitation and emission wavelengths of 295 and 365 nm. (G) Changes in the ellipticity at 222 nm. The color of the curves corresponds to different concentration of PEG-8000: 0 mg/ml (blue circles), 80 mg/ml (cyan circles), 120 mg/ml (gray line) and 300 mg/ml (orange circles). The measurements were performed after 24 h incubation of the native protein in the presence of GdnHCl.

**Figure 4. The change in the recorded absorption spectra of iRFP713 in the holoform at GdnHCl-induced unfolding in the presence of PEG-8000 at a concentration of 80 mg/ml.** Numerals at the curves are the final concentration of the denaturant in the protein solutions.

**Figure 5. Unfolding of iRFP713 and its mutant variants in their holoforms induced by guanidine hydrochloride (GdnHCl).** The designations on panels A–F are the same as in the caption to figure 3. Symbols of different color correspond to different proteins: iRFP713 (black symbols), iRFP713-W109 (red symbols), iRFP713-W281 (green symbols) and iRFP713-W311 (blue symbols). The measurements were performed after 24 h incubation of proteins in the presence of GdnHCl.

**Figure 6. Unfolding of iRFP713 in the apo- and holoform induced by guanidine thiocyanate (GTC) in the presence of PEG-8000 at a concentration of 300 mg/ml.** The designations on panels A–F are the same as in the caption to figure 3. The values of fluorescence intensity *I*320 and *I*365 of the holoprotein were normalized to unity at zero denaturant concentration. . The values of fluorescence intensity *I*320 and *I*365 of the apoprotein were normalized to a value that is equal to the ratio of the fluorescence intensity at the corresponding registration wavelength of apo- and holoprotein at zero denaturant concentration. The color of the symbols indicates the concentration of PEG-8000 in solutions of apo- and holoprotein: 0 mg/ml (red and blue circles, respectively), 300 mg/ml (pink and blue circles, respectively). The measurements were performed after 24 h incubation of apo- or holoprotein in the presence of GdnHCl.

**Figure 7. Unfolding of iRFP713 in the apo- and holoform induced by guanidine hydrochloride (GdnHCl) in the presence of Dextran-70.** The designations on panels A–G are the same as in the caption to figure 3. The color of the symbols denotes the concentration of dextran-70 in solutions of apo- and holoprotein: 0 mg/ml (red and blue circles, respectively), 240 mg/ml (pink and blue circles, respectively). The measurements were performed after 24 h incubation of apo- or holoprotein in the presence of GdnHCl.

**Figure 8. Unfolding of iRFP713 in the apo- and holoform induced by guanidine hydrochloride (GdnHCl) in the presence of Dextran-40.** The designations on panels A–G are the same as in the caption to figure 3. The color of the symbols denotes the concentration of dextran-40 in solutions of apo- and holoprotein: 0 mg/ml (red and blue circles, respectively), 240 mg/ml (pink and blue circles, respectively). The measurements were performed after 24 h incubation of apo- or holoprotein in the presence of GdnHCl.

**Figure 9. The change in the recorded absorption spectra of iRFP713 in the holoform at GdnHCl-induced unfolding in the presence of Dextran-40 at a concentration of 240 mg/ml.** Numerals at the curves show the final concentration of the denaturant in the protein solutions.