



Figure 1S. Trp \rightarrow α -NPG FRET measured as a result of 15 μ M α -NPG binding to LacY and displacement with 10 mM TDG in three steady-state experiments at different levels of nonspecific IFE generated by addition of α -NPGlc, which displays exactly the same IFE as α -NPG but does not bind to LacY. **A:** no α -NPGlc. **B:** 50 μ M α -NPGlc. **C:** 100 μ M α -NPGlc. Trp emission spectra (ex. 295 nm) are: 1, protein before additions; 2, after addition of α -NPG or α -NPG + α -NPGlc; 3, after displacement of α -NPG with 10 mM TDG. **D, E** and **F** demonstrate only specific fluorescence changes in experiments **A, B** and **C**, respectively, and are shown for clarity.

