

Supporting Information for:

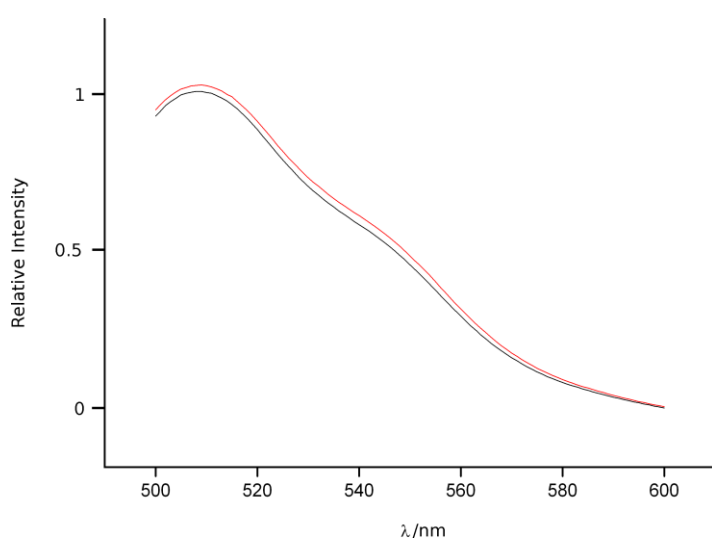
Heavy water reduces GFP expression in prokaryotic cell-free assays at the translation level while stimulating its transcription

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Supporting Figures:



Supporting Figure 1: Emission spectra of GFP diluted in H₂O or D₂O. Black line: GFP fluorescence emission spectra for GFP diluted in H₂O. Red line: GFP fluorescence emission spectra for GFP diluted in D₂O. Mature/purified GFP was diluted one to ten in H₂O or D₂O respectively and the fluorescence emission spectra were recorded. As can be seen, the GFP fluorescence spectra are quantitatively and qualitatively identical for GFP diluted in H₂O and D₂O. This result is in accordance with previous findings for e.g. the GFP variant S65T. Here, again the observed absorbance spectra of the GFP in H₂O or D₂O are identical [24, 25]. We are therefore confident, that the increase of the relative GFP level in **Fig. 1** is mainly due to a direct effect of D₂O on GFP expression.

Supporting Tables:

Table 1: pH values of the D₂O-H₂O mixtures used in this study

D ₂ O concentration	pH value
0%	6.98 ± 0.06
10%	6.99 ± 0.07
20%	7.08 ± 0.17
30%	7.08 ± 0.12
40%	7.25 ± 0.20
50%	7.28 ± 0.19
60%	7.53 ± 0.08