

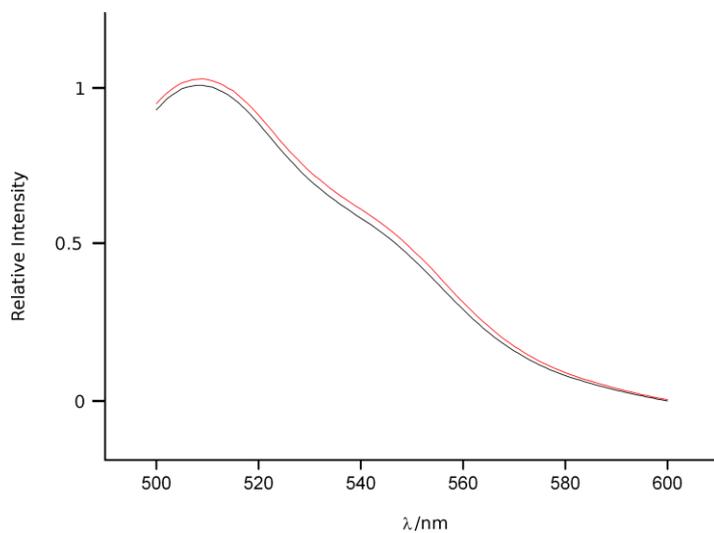
Supporting Information for:

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 |