**Figure 7 – source data 1. Determination of the sign of the 15N chemical shift difference between the excited state and the ground state determined by comparison of HSQC and HMQC spectra collected at 750 MHz.\***

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| --- | --- | --- | --- |
|  | ΔωHSQC-HMQC (ppm) | ΔωHSQC-HMQC (Hz) | Sign of ΔωHSQC-HMQC |
| V64 | -0.00052 ± 0.00092 | -0.039 ± 0.070 | n.d. |
| W65 | +0.00901 ± 0.00237 | +0.684 ± 0.180 | positive |
| E69 | -0.03161 ± 0.00027 | -2.402 ± 0.020 | negative |
| F70 | +0.00494 ± 0.00110 | +0.375 ± 0.084 | positive |
| Y71 | -0.00384 ± 0.00055 | -0.292 ± 0.042 | negative |
| G72 | +0.00913 ± 0.00010 | +0.693 ± 0.007 | positive |
| K73 | +0.01983 ± 0.00067 | +1.507 ± 0.051 | positive |
| S74 | -0.00083 ± 0.00140 | -0.063 ± 0.106 | n.d. |

\*Chemical shift differences were measured from three pairs of HSQC/HMQC spectra collected at 750 MHz using pulse sequences developed by Skrynnikov *et al*. (Skrynnikov, Dahlquist, and Kay 2002). For W65, E69, F70, G72 and K73, |ΔωHSQC-HMQC| is found to be > 0.3 Hz, the cut-off value proposed by Skrynnikov *et al.* for confidently reconstructing the sign information. For Y71, |ΔωHSQC-HMQC| is just below the cut-off value of 0.3 Hz; however, negative values of ΔωHSQC-HMQC are measured in all three repeats increasing the confidence in the sign information. By contrast, for V64 and S74 both positive and negative ΔωHSQC-HMQC values were measured and the sign cannot be determined with confidence. The signs determined refer to Δω = ωexcited state – ωground state. In Figure 7, the sign of Δω has been reversed because the chemical shift differences plotted are between the ground state (nDsbDox) and the excited state (Δω = ωground state – ωexcited state).