HXPep Calculations

k_{ch} for every single amide was calculated by the HXPep algorithm at the applied experimental conditions. The deuterium incorporation at different time points can be calculated from equation 1 (Keppel, Howard, & Weis, 2011)

$$D(t) = \sum_{i=3}^{n}\left[1 - e^{-k_{ch}(i)\times t}\right] \qquad \text{Equation 1}$$

where *D(t)* is deuterium incorporation after the time *t* for a random coil protein, *n* is the residue number and $k_{ch}(i)$ is the chemical exchange rate for the i'th residue. The rate of Pro-residues was set to zero as they do not contain any amide hydrogens. The summation is started at the third residue, since the first is transformed to a primary amine and the second back exchange too rapidly to be retained in conventional LC-systems (Bai, Milne, Mayne, & Englander, 1993; Keppel et al., 2011).

To account for back exchange a normalisation to 90% control samples was performed by the use of equation 2.

$$\frac{D(t)}{EA} \times D_{90\%} = D(t)_{normalized} \qquad \text{Equation 2}$$

where *D(t)* is deuterium incorporation after the time *t* for a random coil protein, *EA* is exchangeable amide hydrogens, the first and second amide is considered "non-exchangeable" as described above, $D_{90\%}$ is the relative deuterium uptake of 90% control samples and $D(t)_{normalized}$ is the value that can be compared with the actual determined relative deuterium uptake.

Bai, Y., Milne, J. S., Mayne, L., & Englander, S. W. (1993). Primary Structure Effects on Peptide Group Hydrogen Exchange. *Proteins*, *17*(1), 75–86. doi:10.1002/prot.340170110.Primary

Keppel, T. R., Howard, B. A., & Weis, D. D. (2011). Mapping Unstructured Regions and Synergistic Folding in Intrinsically Disordered Proteins with Amide H/D Exchange Mass Spectrometry. *Biochemistry*, *50*, 8722–87332.

Protocol Log:

Date	Version	Comments/Changes	Responsible
2014-12-16	1.0	New Protocol	PSM