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Is seeing believing? An assessment of the impact of fluorescent labelling on protein structure and interaction potential[1] MICHELLE K. QUINN, SUSAN JAMES, RUTH MCNAMARA, JENNIFER J. MCMANUS, National University of Ireland Maynooth — Fluorescent labelling is extensively used in conjunction with spectroscopy and microscopy for the in-vivo and in-vitro study of proteins. However, there is little data quantifying how this impacts on the protein in terms of its net interaction potential and its structure. Human ?D-crystallin (HGD), a protein found in the eye lens at high concentrations, undergoes liquid-liquid phase separation (LLPS) and has a well-studied phase diagram. LLPS is indicative of short-ranged attractive interactions between the proteins and the conditions this occurs under are sensitive to changes in the protein itself (e.g. mutations, dimer formation) and its environmental conditions (e.g. pH, salt concentration). HGD is produced recombinantly in E. coli and fluorescently labelled via covalent attachment after purification. Comparison of the coexistence curves for labelled and unlabelled protein indicates if there has been a change in the net interaction potential and various spectroscopic techniques are used to elucidate structural changes between the labelled and unlabelled protein. These studies are important for understanding the relationship between in-vitro phase diagram experiments and those conducted in complex biological fluids, such as plasma or cells where fluorescent tagging is required.

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