

Protein-based drugs are amazingly effective, but solution instability of proteins means these therapeutics require costly refrigeration, impeding their accessibility [1,2]. Drying can increase stability, but most proteins do not withstand dehydration [3]. Protective molecules, excipients, are often added to safeguard proteins during drying [4,5]. Formulation, however, is empirical and of varying efficacy due to a paucity of high-resolution information about dry proteins; we do not fully understand the mechanism(s) of protection [4,6].

To solve this problem, we developed Liquid-Observed Vapor Exchange (LOVE) NMR, a technique that reports on dry protein structure at the residue level [7]. Using LOVE NMR, we showed that dry proteins comprise an immobile but diverse conformational ensemble [7]. We also showed that electrostatic interactions and disorder are important for protection by desiccation-tolerance proteins from a tardigrade and a midge [8].

We will describe our recent efforts on protection by sugars, including trehalose, a well-known excipient [9,10]. We will also present data on new client proteins that allow us to apply both LOVE NMR and activity assays, enabling investigation of both structure and function. Such efforts elucidate mechanisms of dehydration protection, allowing rational design of excipient formulations and making protein products more affordable and accessible [11].

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1. Frokjaer S, Otzen DE (2005) *Nat Rev Drug Discov* 4: 298-306.
2. Devi S (2021) *The Lancet* 398: 1791.
3. Klibanov AM, Schefiliti JA (2004) *Biotechnology Letters* 26: 1103-1106.
4. Piszkiwicz S, Pielak GJ (2019) *Biochemistry* 58: 3825-3833.
5. Bjelosevic M, et al. (2020) *Int J Pharm* 576: 1-12.
6. Moorthy BS, et al. (2015) *Curr Pharm Des* 21: 5845-5853.
7. Crilly CJ, et al. (2021) *Biochemistry* 60: 152-159.
8. Crilly CJ, et al. (2022) *Protein Sci* 31: 396-406.
9. Xie G, Timasheff SN (1997) *Biophys Chem* 64: 25-43.
10. Colaço C, et al. (1992) *Bio/Technology* 10: 1007-1011.
11. Hill AB, et al. (2016) *Curr Opin Biotechnol* 42: 67-73.