Exploring Protein Dynamics and Stability with Advanced Neutron Scattering Techniques

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Understanding the stability of protein-based therapeutics, particularly monoclonal antibodies (mAbs), is essential for ensuring their efficacy and longevity in biopharmaceutical applications. This dissertation investigates the intricate relationship between protein dynamics and thermal stability, driven by the need to develop advanced methods to assess long-term stability. Using bovine serum albumin (BSA) and the NIST monoclonal antibody (NISTmAb) as model systems, this research employs advanced neutron scattering techniques—Small-Angle Neutron Scattering (SANS) and Neutron Spin Echo (NSE) spectroscopy—to provide novel insights into protein dynamics and their relationship with the thermal stability.

One important contribution of this work is the development and validation of a technique that uses Small-Angle Neutron Scattering (SANS) to measure hydrogen-deuterium exchange (HDX) in proteins. HDX assesses protein dynamics by quantifying the exchange of solvent-accessible hydrogen atoms with deuterium, which reflects the protein's conformational stability. The application of SANS in this context, termed HDX-SANS, offers a non-invasive approach to observe the HDX of proteins in their native state, in formulation. HDX-SANS complements other HDX methods, like HDX mass spectrometry, which is destructive and can be sensitive to formulation conditions. BSA was used first to demonstrate the noninvasive and quantitative capabilities of HDX-SANS, including the measurement of temperature dependent exchange rates and the determination of an activation energy of HDX for BSA, which is found to be 81 ± 1 kJ/mol.

Building on these findings, HDX-SANS was applied to NISTmAb under various formulation conditions, using an anionic Hofmeister series of sodium salts as excipients, including sulfate (SO_4^{2-}), perchlorate (ClO_4^-), and thiocyanate (SCN^-). NISTmAb is a standard mAb widely used by industry, which is publicly accessible. Its structural similarity to many mAbs on the market ensures that these findings are broadly applicable to a wide range of therapeutics. Our experimental results show that different types of salts have a strong impact on the HDX of NISTmAb. The ranked order of HDX dynamics is observed to be: $Na_2SO_4 < NaClO_4 < NaSCN$, which is consistent with both the anticipated ranked order of stability associated with the Hofmeister series, and the effects of these anions on protein thermal stability, measured by differential scanning calorimetry. This alignment between the ranked HDX dynamics of different NISTmAb formulations and their corresponding melting temperatures suggests that the HDX dynamics observed in this study are consistent with the thermal stability of NISTmAb across various formulation conditions.

While HDX in proteins provides an indirect measurement of intraprotein domain dynamics, to further understand mAb stability in formulation, the internal domain dynamics of NISTmAb are directly measured using NSE spectroscopy. NSE is a powerful technique, uniquely capable of probing nanometer and nanosecond-scale dynamics—precisely the relevant length and time scales for capturing the individual domain motions of an antibody. The analysis of the NSE results indicate that internal domain motions increase as the NISTmAb formulations approach their thermal transition temperature. This finding suggests that internal domain dynamics likely play an important role in the thermal stability of mAbs. In summary, the observations discovered in this dissertation advance our understanding of how protein dynamics are linked to thermal stability. The novel techniques and detailed findings presented offer a robust foundation for future research that could help the development of more stable and effective protein-based therapeutics.