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Mini Review

Investigating Structure and Dynamics of Proteins in Amorphous Phases Using Neutron Scattering

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ABSTRACT

In order to increase shelf life and minimize aggregation during storage, many biotherapeutic drugs are formulated and stored as either frozen solutions or lyophilized powders. However, characterizing amorphous solids can be challenging with the commonly available set of biophysical measurements used for proteins in liquid solutions. Therefore, some questions remain regarding how the structure of the active pharmaceutical ingredient is affected during freezing and drying of the drug product and the molecular role of excipients used in formulations. Neutron scattering is a powerful technique to study both structure and dynamics of a variety of systems in both solid and liquid phases. Moreover, neutron scattering experiments can generally be correlated with theory and molecular simulations to analyze experimental data. In this article, we focus on the use of neutron techniques to address problems of biotechnological interest. We describe the use of small-angle neutron scattering to study the solution structure of biological molecules and the packing arrangement in amorphous phases, that is, frozen glasses and freeze-dried protein powders. In addition, we discuss the use of neutron spectroscopy to measure the dynamics of glassy systems at different time and length scales. Overall, we expect that the present article will guide and prompt the use of neutron scattering to provide unique insights on many of the outstanding questions in biotechnology.

Contents

1. Introduction

The structure and dynamics of biological systems in solid and liquid environments is of significant importance for applications in protein engineering and to investigate both biological function and biochemical mechanisms. Structural changes could lead to protein misfolding and aggregation, which have been correlated with loss in functionality and pathogenic mechanisms [1–3]. Additionally, proteins are not static entities, as they adopt different conformations at various time and length scales, which can directly affect biological function and stability. Because the solvent plays a direct role in dynamic processes of liquid and dried proteins [4–6], it is of interest to explore the effect of the solvent on protein dynamics and its effects on protein function and long-term chemical and thermodynamic stability.

Neutron scattering can distinctively probe the structure and dynamics of almost all materials, including biological systems, as it is sensitive to the position and motions of atoms. The first neutron

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scattering experiments were performed in the 1940s, as part of the Manhattan Project, where the feasibility of using neutrons for determining the structure of materials was demonstrated [7]. Further advances in instrumentation have led to the development of neutron scattering as an important characterization tool in science. Clifford G. Shull and Bertram N. Brockhouse received the Nobel prize in 1994 for their "pioneering contributions to the development of neutron scattering techniques for studies of condensed matter" [8]. Currently, there are more than twenty neutron sources around the world [9], where thousands of researchers in various fields are using neutron scattering to get further insights into our understanding of the structure and dynamics of condensed matter.

Some of the properties of neutrons that make them useful for a wide range of applications in biotechnology include [10]:

1. Wavelengths ranging from 0.1 Åto 15 Åthat allow the study of structures as small as atoms to biological cells.
2. A wide range of energy differences (nanoelectronvolts to electronvolts) can be probed when neutrons interact with matter; thus, neutrons are sensitive to processes such as folding and diffusion.
3. The scattering power varies randomly for different nuclei, and it can be significantly different for isotopes of the same element. This feature is clearly observed with hydrogen and deuterium; therefore, contrast techniques can be used to study different components of a multicomponent system.
4. Because neutrons do not have charge, they can easily penetrate ordinary matter. Therefore, diverse sample environments can be used without affecting the measurement. Samples are not perturbed or destroyed and can be recovered for additional analysis.
5. Neutron scattering data contain information on both the distribution of atoms and motions that are not readily accessible with most other characterization techniques.

One of the main advantages of neutrons is their selectivity to specific isotopes, which are not differentiated by photons (X-rays). This selectivity depends on the cross section of the atom σ, which represents the ratio of the outgoing current of scattered neutrons and the incident flux [14]. σ is related to the scattering length b as:

$$\sigma = 4\pi b^2, \tag{1}$$

where b represents the apparent "size" of an element during a scattering event. For X-rays, b is often referred to as $f(0)$. Biologically

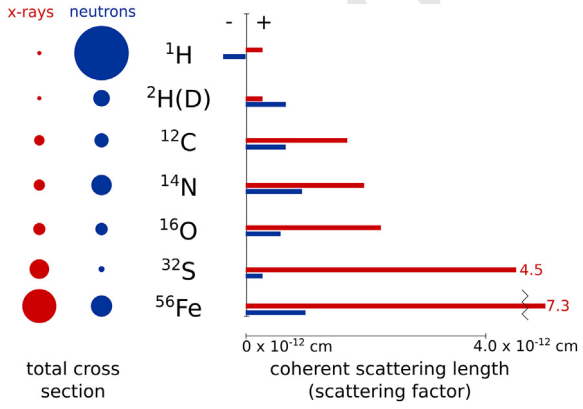

Fig. 1. X-ray and neutron scattering cross sections and coherent scattering lengths (scattering factors) for different elements. Circles and bars are drawn to scale.
Source: X-ray cross sections at 100 keV are according to the values reported in reference [11] . Neutron cross sections are taken from reference [12] . Coherent scattering length and scattering factors are taken from reference [13].

Table 1
Neutron scattering cross sections for some biologically relevant atoms [12] .

Nucleus	Cross section (10^{-24} cm^2)		
	Coherent	Incoherent	Total
^{1}H	1.7583	80.27	82.03
^{2}H	5.592	2.05	7.64
^{12}C	5.559	0	5.559
^{14}N	11.03	0.5	11.53
^{16}O	4.232	0	4.232
^{32}S	0.988	0	0.988
^{56}Fe	12.42	0	12.42

relevant atoms have distinct neutron cross sections and coherent scattering lengths as shown in Fig. 1 and Table 1. Cross sections and coherent scattering lengths for X-rays are shown in Fig. 1.

As depicted in Table 1, each element has a coherent and incoherent neutron cross section, both of which determine the total scattering intensity of the system. The coherent contribution contains information on how the atoms are distributed in the sample; thus, the coherent scattering provides information about molecular shape and morphology. The incoherent cross section originates from the existence of the neutron spin. This incoherent contribution does not exist for atoms like ^{12}C or ^{16}O, but it is significant for hydrogen ^{1}H. As depicted in Fig. 1 and Table 1, hydrogen has a large neutron incoherent cross section compared to its isotope deuterium (^{2}H(D)) and other elements; thus, hydrogen contributes significantly to the incoherent and total scattering. Consequently, changing ^{1}H by ^{2}H(D) leads to a major effect on the measured scattering. On the other hand, hydrogen and deuterium scatter X-rays equally. Depending on the information that one wishes to extract, X-ray scattering can be useful as a complementary technique to neutron scattering. Moreover, because atoms are not rigidly bound, scattering events may induce a change in the energy of the neutron, which results in inelastic scattering events. This inelastic scattering contribution contains information on the motion of atoms, allowing the use of neutrons for dynamics measurements.

In this article, we focus on a set of biotechnological relevant problems that have been studied with the following neutron scattering techniques: small-angle neutron scattering (SANS) for structure, and quasielastic neutron scattering (QENS) for dynamics. These techniques are useful for gaining information on the structural arrangement and dynamics of proteins, as well as establishing correlations with stability in amorphous phases. Readers interested in other neutron techniques, or other applications of neutron scattering in biology, are referred to general reviews discussing the fundamentals of neutron scattering [15–19], neutron scattering for structural biology studies [13, 14, 20, 21], and contrast matching techniques for biological samples [22–25].

2. Overview of Small-Angle Neutron Scattering

Structural information from biological samples can be obtained using small-angle scattering, because the direction of the scattered neutrons depends on the relative position of atoms. A schematic of a SANS experiment and its notation are presented in Fig. 2. In SANS, neutrons with wavelength λ and wave vector $\hat{\mathbf{k}}_o$, with magnitude $\frac{2\pi}{\lambda}$, interact with the sample, which results in a scattered neutron wave vector $\hat{\mathbf{k}}_f$ with scattering angle 2θ. As shown in Fig. 2, the momentum transfer $\hat{\mathbf{q}}$ can be defined as $\hat{\mathbf{q}} = \hat{\mathbf{k}}_f - \hat{\mathbf{k}}_o$, and its magnitude $|\hat{\mathbf{q}}|$, or simply q, corresponds to:

$$q = \frac{4\pi \sin\theta}{\lambda}, \tag{2}$$