

Dispersive Raman Spectroscopy Utilization

Lonza Scientists Assess Applicability in Protein Analysis and Conformational Characterization

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Therapeutic protein characterization is a key requirement during the development and manufacturing of biopharmaceuticals. It calls for techniques that are capable of deciphering the physical, biochemical, and biological characteristics of the product. Protein conformational analysis (e.g., secondary structure) is an important characterization parameter routinely measured.

One technique that can be used is dispersive Raman spectroscopy. It is user-friendly and requires minimal training and data-interpretation skills. It has broad applicability to a variety of sciences and is invaluable as an analytical tool in many diverse areas.

Background and Principles

February 28 was the 80th anniversary of Sir Chandrasekhara Venkata Raman's discovery of frequency-shifted radiation scattered from particles. This discovery, which garnered him the 1930 Nobel Prize in Physics, was based on the fundamental principle of the inelastic scattering of photons by molecules much larger than the wavelength of the photons (Figure 1).

Large particles such as proteins have certain discrete vibrational and rotational energy states that are allowed within the rules of quantum mechanics. The state of the molecular vibrational energy determines whether it can absorb some energy from an incident photon, thereby scattering a photon of reduced frequency (Stokes scattering). If on the other hand the molecule is in a vibrationally excited state, it can add some of its vibrational energy to an incident photon, increasing the scattered photon's frequency and leaving the molecule in the vibrational ground state (anti-Stokes scattering).

The molecule does either of these by moving into a virtual vibrational state when impacted by the excitation light, then scattering a photon that is quantized with the energy difference between the initial and final states of the molecule as a result (Figure 2).

The frequency of the Stokes or anti-Stokes scattered photons is directly related to the vibrational energy of the scattering molecule (e.g., the amino acids or the amide bands of therapeutic proteins). For any molecule, the vibrational energy is related to the stiffness of its bonds and the mass of the molecule. Therefore, details of the molecule can be inferred by its Raman spectral vibrations.

Application in the Biotech Industry

The characterization of therapeutic proteins is an important analytical activity during process development and manufac-

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turing. Product characterization is a requirement for precise recognition of a therapeutic protein's biochemical structure.

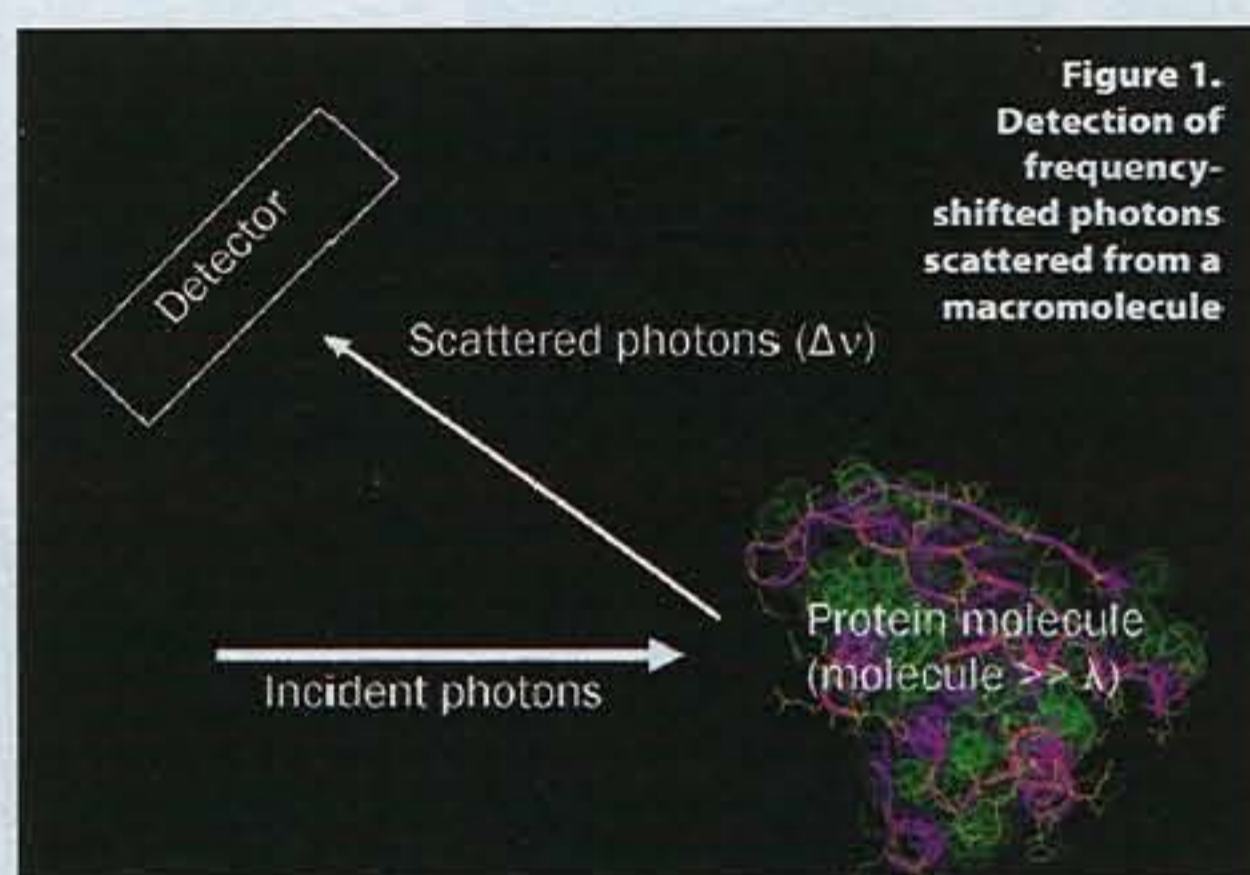


Figure 1. Detection of frequency-shifted photons scattered from a macromolecule

Biochemical characterization of therapeutic proteins is carried out using a range of techniques that fingerprint the native structure, conformation, purity, and product-related impurities and contaminants.

These techniques include peptide mapping using LC/MS for primary structure identification, MALDI-TOF-MS for molecular mass determination, and circular dichroism or FTIR for secondary structure analysis. Product-purity assays include size-exclusion chromatography and SDS-PAGE. Product isoform and immunodetection techniques such as isoelectric focusing and Western blot are also routinely used. Impurities such as host cell proteins are analyzed using ELISA and/or HCP Western blot.

At Lonza Biologics (www.lonza.com), we evaluated the application of dispersive Raman spectroscopy to determine the Raman spectral profile of a therapeutic protein. The spectra were further processed to estimate secondary structure composition of the therapeutic protein.

Specifically, we assessed dispersive Raman spectra for a therapeutic mAb (Figure 3). The spectra can be analyzed at several levels of detail and can be used to compare different therapeutic protein batches visually. The protein stability profile across time and forced degradation processes for therapeutic proteins can be analyzed in a timely manner.

The spectra can also be used for a detailed analysis to compare the structural components that have Raman vibrational modes, such as aromatic amino acids. These amino acids carry important structural details in terms of stability and integrity of the proteins. Therapeutic protein denaturation and structural perturbation can be fingerprinted in a Raman spec-

trum at the expense of changes in aromatic amino acids and compositional shifts to the secondary structures.

Tentative peak assignments constituting the amide I region of a mAb Raman spectrum are shown in Figure 3. The profile is significant in terms of the number of peaks obtained for the mAbs and the descriptive nature of the technique. The peaks are remarkable structural markers that are ideal for detailed and efficient characterization of novel therapeutic proteins.

The amide I region of a mAb was peak fitted and deconvoluted to generate underlying conformational information of the

therapeutic protein. β -sheet was the main secondary structure component of the mAb. Other secondary structures identified included α -helix, loose β -sheet, and other structures.

Dispersive Raman spectroscopy can also be used to address the increased demand for more efficient product-development and manufacturing processes. This methodology has the potential to be applied as a PAT tool for the purpose of determining protein conformation or other process-related analytes at-line or in-line during the upstream or downstream process stages.

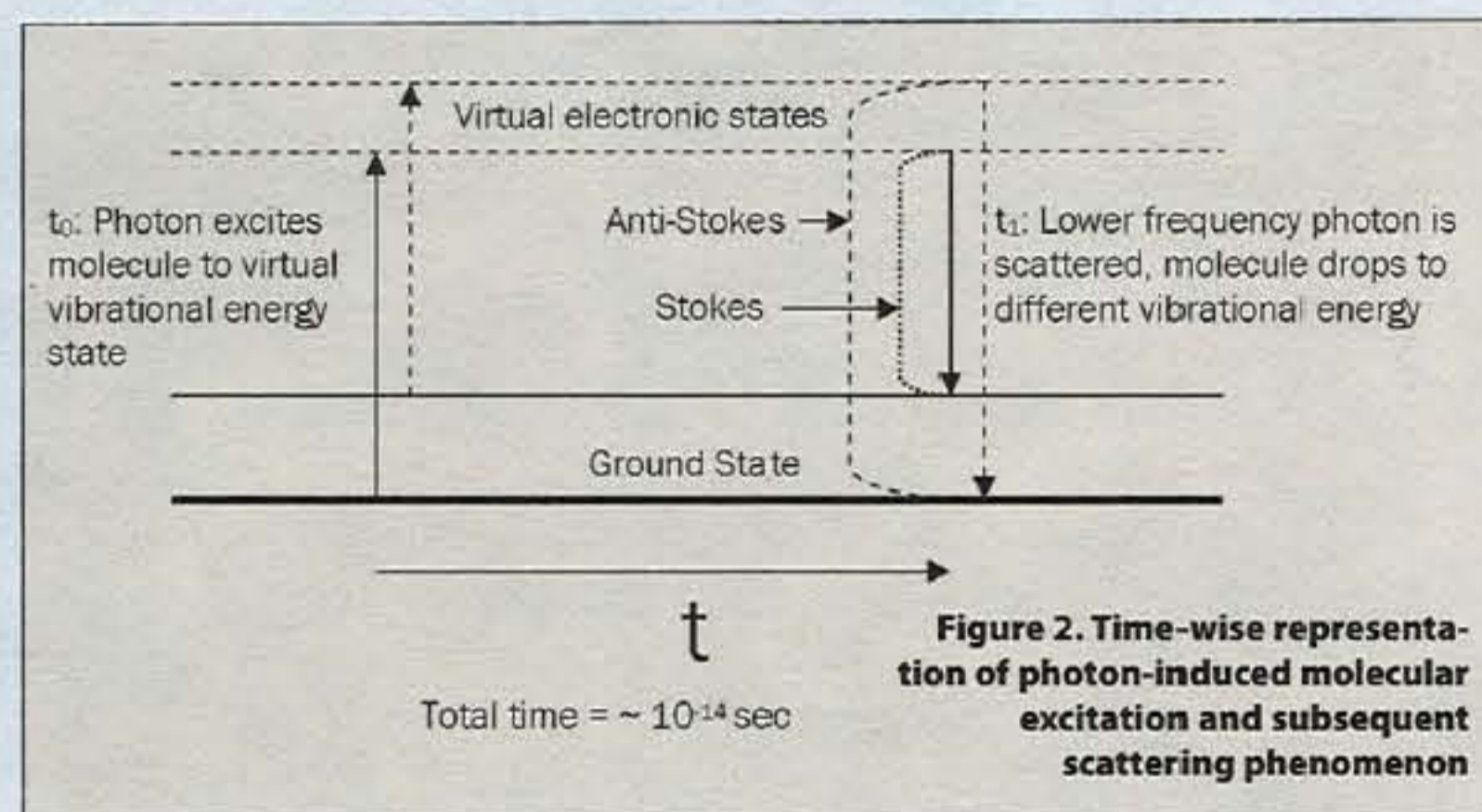


Figure 2. Time-wise representation of photon-induced molecular excitation and subsequent scattering phenomenon

Figure 3. Application of dispersive Raman spectroscopy as a therapeutic protein-characterization tool: (A) mAb, (B) Raman spectrum, (C) peak fitted amide I region

