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Interferometric Scattering Microscopy of Albumin-bound Paclitaxel Nanoparticles

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Due to their size and potential for including multiple active drug components and targeting ligands, nanoparticle-based therapeutics can have significant advantages over small molecule and protein drugs [1]. Nanoparticle drugs can carry hydrophobic drugs payloads with increased circulation time in the blood, reduced toxicity, and enhanced permeability and retention (EPR) in tumors [2]. Particles with sizes in the range of 150 – 300 nm have increased blood circulation times and are not readily cleared from the body like smaller nanoparticles with sizes < 50 nm.[3] In contrast to small molecules and proteins, nanoparticles are supramolecular structures containing millions to billions of individual molecules and each nanoparticle has a unique size, shape, and overall structure. The particle size and shape distributions of nanoparticle drugs affect how they interact with biological tissues and impact pharmacokinetics, biodistribution, and safety of the nanoparticle drug [3]. The impacts of nanoparticle size and shape dispersity on pharmaceutical properties emphasize the need for advanced characterization of these key properties. Conventional analytical tools that have been developed to characterize small molecules and protein-based drugs are not suitable to fully characterize polydisperse nanoparticle-based drugs. There is currently a gap in simple, high throughput, and robust particle characterization methods for nanoparticles in the 100 – 1000 nm range, which corresponds to the size range of many nanoparticle-based drugs.

One of the first FDA approved nanoparticle-based drugs was the cancer drug Abraxane®, which consists of paclitaxel nanoparticles bound by human serum albumin on the surface to form ~130 nm particles. Cryo electron microscopy measurements have shown significant dispersity in the size and shape of nanoparticles in Abraxane [4]. There could be associated heterogeneity in the particle-to-particle active drug ingredient concentration, but conventional ensemble methods, such as light scattering, chromatography, and nuclear magnetic resonance, are incapable of revealing the composition of a single nanoparticle. Here we demonstrate single particle scale characterization of albumin-bound paclitaxel nanoparticles in the drug Abraxane® using hyperspectral interferometric scattering microscopy (h-IFS). H-IFS is an optical scattering technique that illuminates nanoparticles on a sensor with monochromatic light of different wavelengths to detect sub-micron particles [5]. The scattering intensity of each particle in the IFS image is proportional to the particle volume or mass. A hyperspectral datacube of h-IFS images is acquired, which is processed using automated image analysis to extract the scattering intensity of each paclitaxel particle as a function of illumination wavelength. The single particle spectrum is expected to change as a function of the particle composition and physical properties, such as refractive index and particle shape.

Dynamic light scattering (DLS) and h-IFS of nanoparticles in the fully concentrated Abraxane® drug solution showed that the albumin bound nanoparticles were ~100 nm in size (Figure 1a). DLS also showed a particle type near 5 nm in diameter, which was unbound serum albumin. DLS only provides qualitative measurements of the particle size distribution (PSD), while h-IFS measures the true shape of the number-based PSD because it builds PSD from single nanoparticle measurements. Figure 1b,c show

the raw and processed IFS images of paclitaxel nanoparticles deposited onto the silicon IFS sensor surface. Quantitative analysis of the processed IFS images showed a single peak in the scattering intensity-based particle size distribution (Figure 1d). h-IFS of paclitaxel nanoparticles revealed a minority (< 5% by number) of small paclitaxel nanoparticles had red shifted and blue shifted maximum scattering wavelengths, suggesting these particles had a different composition (*e.g.*, paclitaxel:albumin ratio) or physical morphology compared to the majority. We will show scanning electron microscopy (SEM) and optical scattering simulation validation of the h-IFS based particle characteristic classification [6].

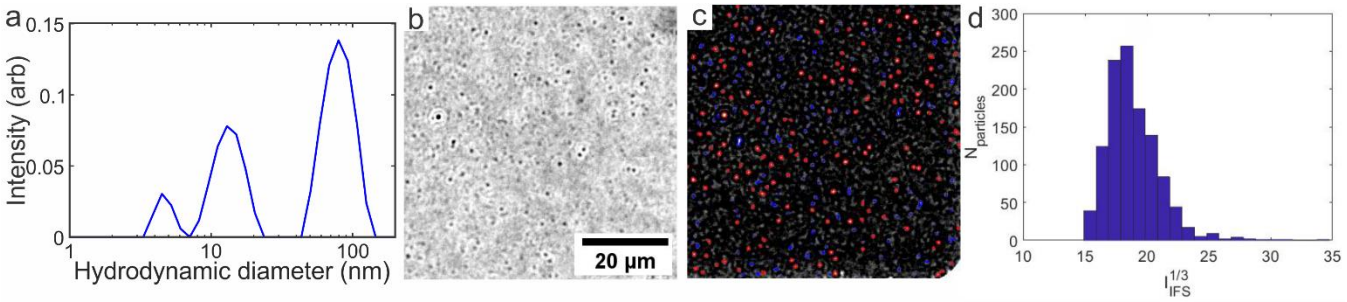

Figure 1. IFS and DLS measurement of albumin-bound paclitaxel particles. (a) DLS of paclitaxel nanoparticles at the full drug formula concentration. (b) Representative raw h-IFS image taken with 555 nm light. (c) Processed h-IFS image with image analysis overlaid showing tracked particles (in red) and background noise and contaminant particles (blue). Only the red outlined particles are considered in the quantitative analysis. (d) IFS scattered light-based particle size distribution.

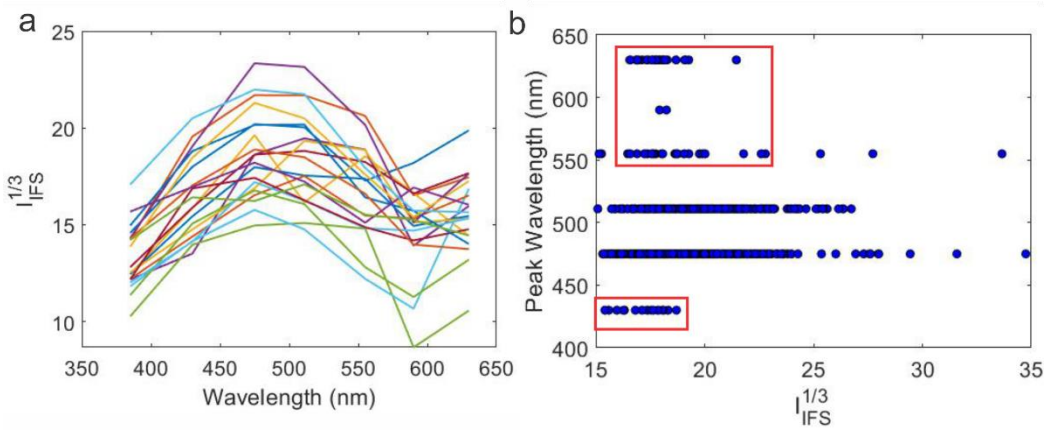

Figure 2. h-IFS analysis of paclitaxel nanoparticles. (a) IFS scattered light spectra of a few nanoparticles from the images in Figure 1b,c. (b) Peak scattering wavelength vs the max IFS intensity of all particles in Figures 1b,c. Red boxes highlight minority populations of small particles with abnormal peak wavelengths.

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