HIGH-THROUGHPUT ANALYSIS OF

ANTIBODY SELF-INTERACTIONS

By

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ABSTRACT

A primary challenge in the development of high concentration monoclonal antibody formulations is that of reversible antibody self-interactions that have been shown to directly influence solution properties such as viscosity and opalescence. Modulating high concentration solution properties require accurate understanding of nature of self-interactions between antibody molecules. Despite 90-95% sequence similarity between human monoclonal antibodies, different antibodies have been known to behave in a strikingly contrasting manner under the same solution condition. In addition, self-interactions between mAb molecules can be affected to varying degrees by modulation of ionic strength. Thus, there is very little scope for generalizing formulation strategies and this underscores the need for optimizing formulation conditions in a mAb specific manner. Traditional methods of biophysical characterization of antibody solutions are in general incapable of handling multiple analyses or require direct testing of the high concentration antibody solutions

The objective of this work is to adapt a high throughput gold nanoparticle based assay, self-interaction nanoparticle spectroscopy (SINS) to measure antibody self-interactions in various solution conditions. The basis of SINS is that attractive interactions between adsorbed antibodies on gold nanoparticles leads to reduction in interparticle separation distances and red-shifting of the wavelength of maximum adsorption (plasmon wavelength).

In this work, we discuss the influence of particle size in determining nanoparticle-antibody conjugate interactions in various solution conditions. We discuss methods to account for non-specific interactions between conjugates that can obscure antibody-mediated interactions. We find that facile variation of antibody loading on the surface and adsorption of thiolated polymers helps accounting for non-specific effects and enables the study of mAb-mediated interactions. We attempt to correlate the inferences of antibody interactions obtained from SINS measurements to dynamic light scattering measurements of antibody self association.