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A rapid method for determining dynamic binding capacity of resins for the purification of proteins

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ABSTRACT

Biolayer interferometry is a novel method for quantifying macromolecules, such as proteins, in solution. The presence of other, non-binding molecules does not interfere with quantification, which allows one to measure the concentration of the molecule of interest in a crude mixture. Here we apply this method to determining the dynamic binding capacity of affinity resins.

Determining the dynamic binding capacity of resins is an essential step in optimization of a purification process. Dynamic binding capacity is defined as the amount of product that will bind to the resin under typical flow conditions and must be determined under specific flow conditions and load characteristics [1,2]. It is calculated based on the amount that can be loaded before significant product levels are measured in the flowthrough (the breakthrough point). The information gained about resin performance is useful for assessing loading conditions and column lifetime [3,4].

For the first capture step in the purification process of a recombinant protein, the high background absorbance of the load (clarified cell culture supernatant) makes it impossible to use A280 to detect protein product breakthrough. SDS–PAGE or HPLC can be used to get an approximate measure of where product breaks through, but these are time-consuming [4,5]. Unless the product is tagged, it is necessary to use a product-specific ELISA to determine where the product appears, and at what concentration, in the flowthrough [3]. Here we demonstrate the use of a new technology, developed by ForteBio, Inc., that can be used to rapidly quantitate immunoglobulins, even in crude supernatants. We have used this approach to compare the dynamic binding capacity of three protein A resins for a recombinant chimeric antibody. The method can be modified for quantification of any protein for which a specific capture molecule is available.

The ForteBio technology is a novel label-free methodology for analyzing macromolecular ligand/capture interactions. While label-free methods are widely used for the analysis of biomolecular interactions, a majority of the applications are in kinetic analysis [6,7]. In contrast, the Octet can be used for kinetics or run in quantitation mode for rapid determination of protein-specific concentrations. The instrument is designed to assay up to eight samples in parallel in 2 min from a standard 96-well microplate. The ability to rapidly assay as few as one sample and as many as 96 samples in a single run provides a flexible platform to use for monitoring purification column performance as presented here.

The detection technology utilized in the Octet is based on Biolayer Interferometry (BLI).[1] BLI relies on the reflective property of white light and is implemented in the Octet via the use of fiber optic-based biosensors. The biosensors are coated with molecules chosen to capture the targeted ligands. In brief, the Octet shines white light down the biosensor and a small portion of the light is reflected back along the fiber optic and collected by the spectrometer. The reflected light originates from two interfaces: (a) the reference layer and (b) the interface between the immobilized protein and the surrounding solution. When light waves propagate back from the two reflecting surfaces they interact; some wavelengths show constructive interference, others destructive interference. This interference is captured by a spectrometer across the entire white light spectrum as a pattern of intensity variation by wavelength with a characteristic profile of peaks and troughs. When a protein in the solution binds to the end of the biosensor, this shifts the second reflective interface (b) and changes the pattern of peaks

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[1] *Abbreviations used:* BLI, biolayer interferometry; CHO, Chinese hamster ovary; CV, column volumes; DBC, dynamic binding capacity.

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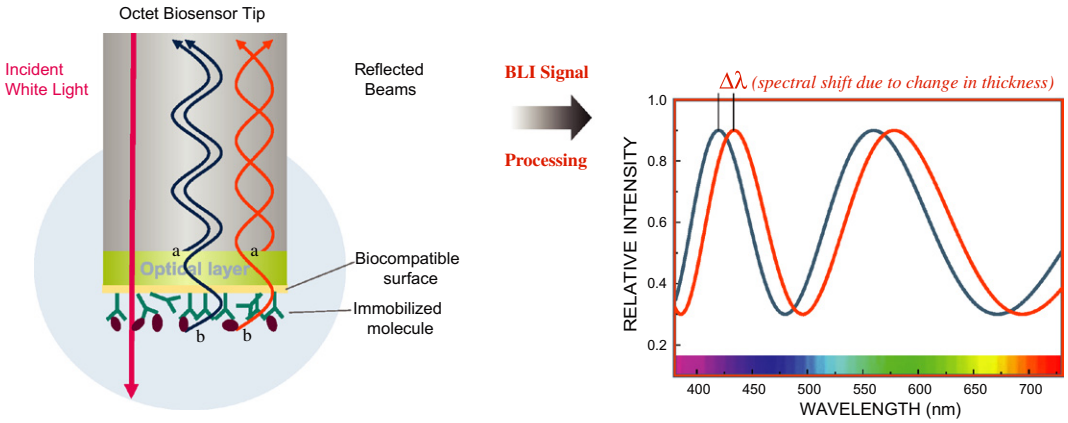

Fig. 1. Basic principles of biolayer interferometry. Light is reflected from two surfaces: a layer of immobilized capture molecules (typically, protein) and an internal reference layer. Any change in the number of molecules bound to the sensor tip causes a shift in the interference pattern that can be measured in real time. The wavelength shift is a direct measure of the change in thickness of the protein layer.

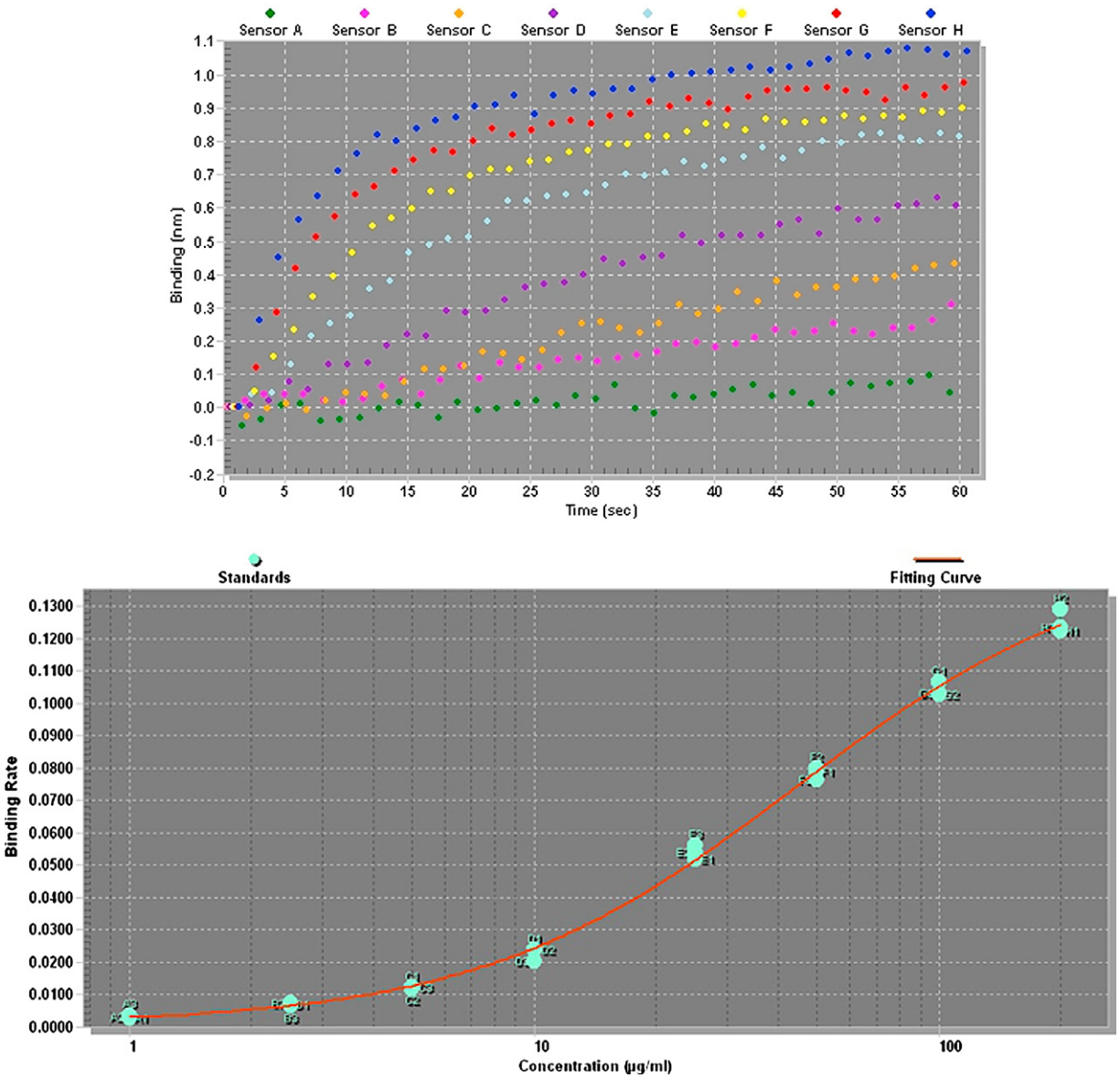

Fig. 2. Real-time binding curves of human IgG standards (top) and the resulting calibration curve (bottom). The real-time binding curves are shown as a function of nanometer (nm) optical thickness increase of protein on the biosensor surface. Using the real-time data, the Octet software calculates the binding rate for each calibration sample assayed. A calibration curve is then created by plotting the concentration of each calibration sample versus the calculated binding rate.