Platform comparison of the binding kinetics of Nivolumab

The non-specific binding of serum proteins to a biological drug plays an important role in determining the pharmacological response of the drug (i) . Studies have suggested that interactions with plasma proteins modulate the efficacy of protein binding by effecting conformational changes in the target protein. In some extreme situations the antagonists lose their capacity to bind to the receptor. The strength with which proteins bind to their target molecule alters drug potency, dosing regimen and degree of on-target side effects. Unfortunately, the platforms typically used to measure binding kinetics such as the Surface Plasmon resonance (SPR) (ii) or Bio-Layer Interferometry (BLI) (iii), are unable to measure kinetics in a physiologically relevant system consistently and reliably.

The MagArray Protein Binding Kinetics platform that has recently been described (iv) can measure protein binding kinetics precisely, reproducibly and with great sensitivity in biological matrices. We utilized the platform to carry out a comparison study of the kinetics of Nivolumab with PD-1 on the MagArray platform in increasing concentration of serum and compared the results to those carried out on the SPR and BLI platforms.

The MagArray platform was able to provide a sensitive, reliable and rapid approach to quantify binding kinetics in a physiologically relevant buffer while the SPR platform could not measure kinetics in any buffer containing more than 0.5% serum. The BLI platform provided inconsistent and inaccurate results and could not measure binding kinetics in buffer containing serum.

Given the profound impact that cancer immunotherapy is beginning to deliver and the rapid increase in the numbers of mAb checkpoint inhibitors being investigated in cancer therapy, it is of increasing importance that we understand how immuno-oncology inhibitors are likely to behave in-vivo by examining their binding kinetics in a physiologically relevant buffer.

To learn more about how MagArray's Protein Kinetics platform can help your drug discovery efforts, reach out to us below or click here for more information.

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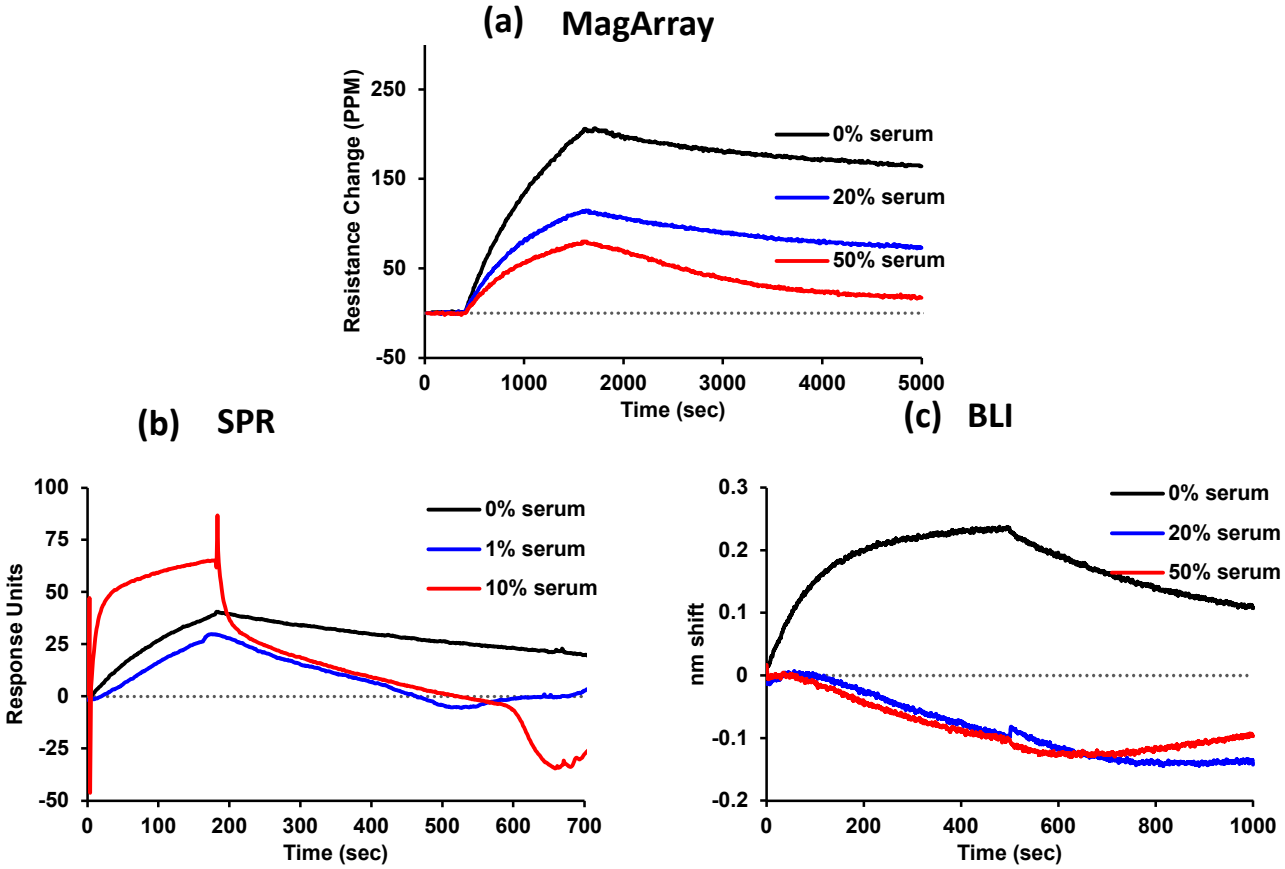

Binding of Nivolumab to PD-1 in increasing concentration of PBS-serum on (a) MagArray platform, (b) SPR and (c) BLI

Method	Buffer	k_{on} ($x10^5$ $M^{-1}s^{-1}$)	k_{off} ($x10^{-4}$ s^{-1})	K_D (nM)
Biacore	Buffer	2.5	7.7	3.1
Octet	Bufer	2.6	16	6.2
	20% serum	No signal	No signal	No signal
MagArray	0% serum	2.0	1.7	0.9
	20% serum	1.5	1.8	1.2
	50% serum	1.2	4.7	4.1

(i) Walkup, G. K. *et al.* Translating slow-binding inhibition kinetics into cellular and in vivo effects. *Nat. Chem. Biol.* **11**, 416–423 (2015).

(ii) Maillard, M. P., Centeno, C., Frostell-Karlsson, Å., Brunner, H. R. & Burnier, M. Does protein binding modulate the effect of angiotensin II receptor antagonists? *J. Renin-Angiotensin-Aldosterone Syst. JRAAS* **2**, S54–S58 (2001)
Singh, P. SPR Biosensors: Historical Perspectives and Current Challenges. *Sens. Actuators B Chem.* **229**, 110–130 (2016).

(iii) Kamat, V. & Rafique, A. Designing binding kinetic assay on the bio-layer interferometry (BLI) biosensor to characterize antibody-antigen interactions. *Anal. Biochem.* **536**, 16–31 (2017).

(iv) Saito, T. *et al.* Effects of serum matrix on molecular interactions between drugs and target proteins revealed by giant magneto-resistive bio-sensing techniques. *J. Pharm. Biomed. Anal.* **198**, 114015 (2021).