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Expanding the ProteOn XPR36 biosensor into a 36-ligand array expedites protein interaction analysis

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ABSTRACT

Here we demonstrate methods to expand the throughput of the ProteOn XPR36 biosensor allowing for the simultaneous kinetic characterization of several multiplexed formats, such as 36 disparate antibodies targeting the same antigen, and facilitating detailed epitope binning and mapping studies. The kinetic rate constants determined by these methods correlated with those obtained on Biacore 2000 and the absolute parameter values obtained on the ProteOn's alginate-based GLC chip agreed closer with those from Biacore's flat C1 chip than Biacore's dextran-based CM4 chip. Pairwise epitope binning data from the ProteOn 36-ligand array format and those generated on an orthogonal array-based biosensor, the Octet QK384, gave similar results. In an epitope mapping study using biotinylated peptides, all three biosensor platforms were similar in their ability to identify antibodies that bound to linear epitopes. We apply alternative formats of the ProteOn array that enable a significantly higher number of assays to be conducted simultaneously than previously anticipated on this platform.

Label-free real-time biosensors are commonly used to characterize the interactions between diverse biomolecules and are cited in more than 1400 peer-viewed publications annually [1]. Most biosensors employ surface plasmon resonance (SPR)[1] in the classic Kretschmann configuration [2] to detect the binding of a solution partner (analyte) to an immobilized partner (ligand) on a solid support. Traditional Biacore platforms, such as 2000, 3000, and T100 (and T200, the recent update to the T100), employ a "one-on-many" approach by delivering one analyte at a time over three serially addressed ligand-coated surfaces and a reference surface. Array-based SPR-imaging biosensors employ a one-on-many approach with up to hundreds of immobilized ligands, such as those commercialized by Biacore (Flexchip), Genoptics, Plexera, and IBIS. Emerging platforms are now routinely multiplexed in that they deliver multiple analytes over multiple ligands simultaneously, enabling "many-on-many" approaches, in an effort to meet the increasing demands of drug discovery for higher throughput [3]. For example, Biacore A100 (and Biacore 4000, the recent update to the A100) [4] analyzes 16 interactions at once via the use of four independent flow cells that each contain four ligand spots and a reference spot. ForteBio's Octet QK384 and Red384 are based on biolayer interferometry and each processes 16 interactions simultaneously on independent disposable fiber-optic sensor tips that dip into and read samples arrayed within the wells of a microplate, thereby eliminating the need for the microfluidic delivery of samples [5]. Each of these multiplexed technologies enables higher-throughput, higher-data-content assays than those previously possible.

This study describes applications of another multiplexed technology, namely BioRad's ProteOn XPR36 platform, which addresses interactions on 36 reaction spots simultaneously by combining the use of six parallel flow channels with a microfluidic pathway that can rotate between vertical and horizontal directions [6]. This creates a two-dimensional interaction array within which the intersecting and nonintersecting flow paths define the reaction and reference spots, respectively. We demonstrate how to expand the number and diversity of interactions that can be addressed on the ProteOn by treating the surface as a 36-ligand array to exploit its full ligand capacity. For example, the array can contain 36 unique ligands, or fewer ligands immobilized under various conditions. We compare the throughput that can be achieved when this platform is used in a one-on-many format, with similar assays performed on orthogonal platforms, namely the Biacore 2000 and Octet QK384. Our examples draw from three applications that are important in the drug discovery of monoclonal antibodies: kinetic characterization, epitope binning, and epitope mapping.

In 2006, Karlsson et al. described an algorithm for fitting the binding responses of an analyte concentration series that is delivered in order of increasing concentration over the same ligand surface without regenerating it between successive analyte injections

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[1] *Abbreviations used*: BSA, bovine serum albumin; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; mAbs, monoclonal antibodies; MES, 2-(*N*-morpholino)ethanesulfonic acid; SNHS, sulfo-*N*-hydroxysuccinimide; SPR, surface plasmon resonance.

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and referred to this methodology as "kinetic titration." They described its use in the context of one-on-many biosensors (Biacore 2000, 3000, S51, and T100 instruments) and concluded that, "kinetic titration has the potential to improve throughput and may be particularly suitable for the parallel analysis required by protein arrays" [7].

In the same year, Bravman et al. described a "one-shot" method for analyzing kinetic data on the ProteOn biosensor, which involved immobilizing six ligand strips along whole channels in the vertical flow paths and then delivering the members of an analyte concentration series along parallel channels in the horizontal flow paths [6]. While this generates analyte-binding data on 36 reaction spots simultaneously, it yields full kinetic profiles of only six analyte/ligand pairs because the information from six replicate reaction spots along a whole channel is combined to construct a full kinetic profile of each analyte/ligand interaction. This is a fast way of analyzing the kinetics of one analyte binding to six immobilized ligands without the need to regenerate the surfaces (Fig. 1A), but multiple sensor chips and/or multiple runs are required when there are more than six unique interactions in the test panel.

Here we combine a 36-ligand array with the kinetic titration method to study 36 unique analyte/ligand pairs simultaneously without any regeneration, by injecting the members of an analyte concentration series in succession from low to high concentration through the same flow channel, so that an entire analyte concentration series interrogates each spot. The kinetic titration method thus enables the use of various multiplexed assay formats. For example, six different analytes can be flowed perpendicularly across six ligand strips, giving a six-on-six analyte-on-ligand format (Fig. 1B). The scope of the kinetic titration method can be altered to a one-on-36 analyte-on-ligand format by arraying ligands onto individual reactions spots instead of immobilizing them along whole channels (Fig. 1C). To further diversify the amount of information that can be obtained per chip, ligands that target the same analyte can be arrayed onto the spots that lie within the same channel to enable six unrelated panels of one-on-six to be studied simultaneously (Fig. 1D); this format can also be used to study other combinations, such as three unrelated panels of one-on-12 or two unrelated panels of one-on-18.

Materials and methods

All experiments were performed at 25 °C using HBST as the running buffer [10 mM Hepes, pH 7.4, 150 mM NaCl, and 0.05% (v/v) Tween 20] unless noted otherwise. A ProteOn XPR36 biosensor equipped with GLC and GLM sensor chips and coupling reagents (10 mM sodium acetate, pH 4.5, sulfo-*N*-hydroxysuccinimide [SNHS], 1-ethyl-3-(3-dimethylaminpropyl)-carbodi-

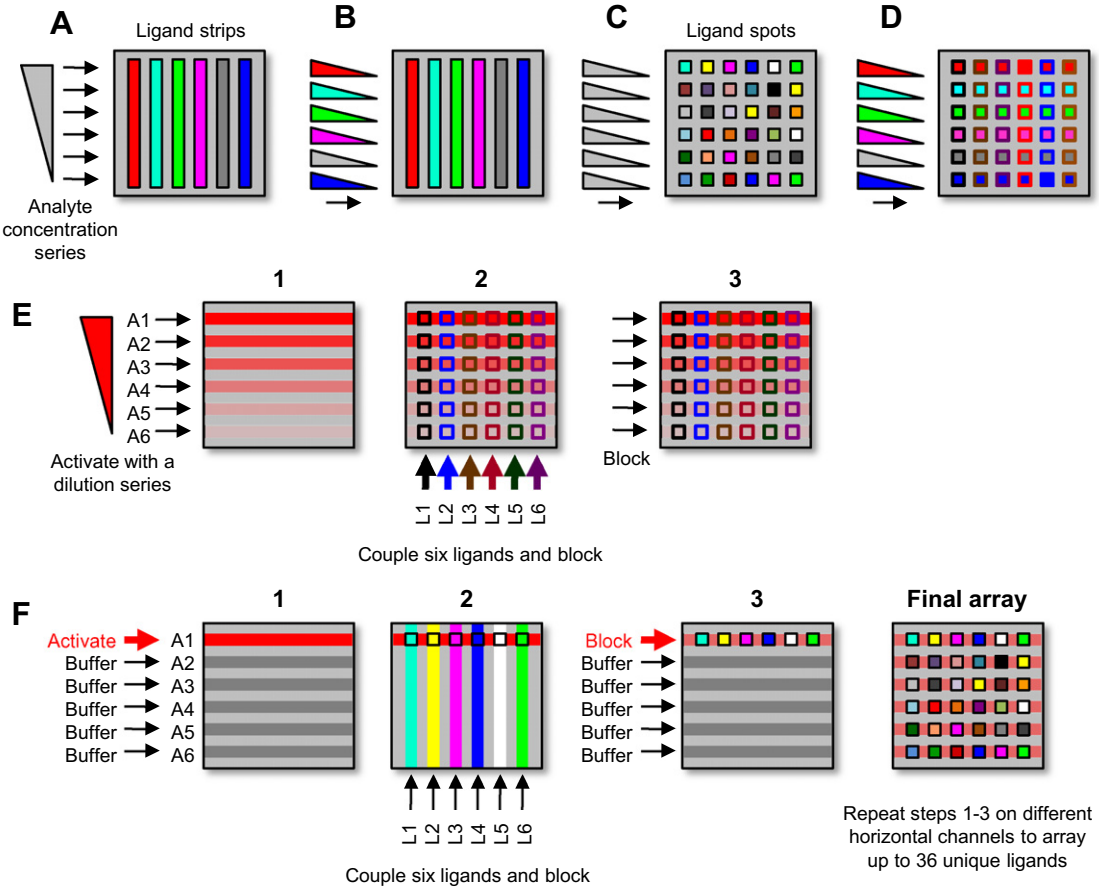

Fig.1. Single spot analysis diversifies the "analyte-on-ligand" kinetic assay formats that are available on the ProteOn. (A) "One-shot" kinetics gives a "one-on-six" format. Kinetic titration enables (B) a "six-on-six" format when six analytes (distinguished by the different colored triangles) are flowed over six ligand strips, (C) a "one-on-36" format when ligands are arrayed onto individual spots, and (D) six unrelated panels of "one-on-six" when six analytes are flowed along horizontal channels where their respective ligands are arrayed. Arraying (E) six ligands each at six capacities or (F) up to 36 unique ligands. Methods E and F involve rotating the flow path two and 12 times, respectively; fewer rotations are needed if less than 36 unique ligands are arrayed. In the ProteOn Manager control software, the six horizontal and six vertical channels are referred to as Analyte (A1–A6) and Ligand (L1–L6) channels, respectively. The reaction spots within the array can therefore be identified by their Analyte/Ligand coordinates, e.g., reaction spot A1L1 is located at the intersection of channels A1 and L1. Both methods create two types of interspot surfaces for local referencing, namely "activated and blocked" along the horizontal flow paths (represented by the orange strips) and "unmodified chip" along the vertical flow paths.