Increasing DNA Encoded Library Screening Resolution Using HT-SPR

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INTRODUCTION

DNA encoded library (DEL) technology has permitted substantial leaps in compound screening by enabling a more facile way to interrogate libraries and develop lead compounds. While a huge benefit to DEL technology is its reduction in the sheer number and tracking of discrete compounds through the screening process, it does come with a limitation in binding properties that can be gleaned for potential hits. In practice, from an initial screen numbering millions, the resulting thousands of hits are then reduced to around 50 compounds carried forward into downstream assays without any detailed characterization of their binding properties. These fundamental binding properties include kinetics as well as the target binding site. To address this resolution gap in DEL screening, demonstrated here is a technique to further characterize hits with improved resolution using high-throughput surface plasmon resonance (HT-SPR). HT-SPR characterizes the full kinetic profile for both weak and strong binders. Assay resolution can be further increased by inclusion of high-homology family members, truncated or mutated forms, binding partner disruption, etc., yielding a richer profile of lead candidates. With a screening capacity of thousands per week, HT-SPR affords a high-resolution technique that matches the throughput needs in this phase of discovery between the full library and a handful of leads. Additionally, this approach can work with any moiety having DNA attachment, including screening for targeted protein degraders (TPDs) and macrocycles.

Kinetic Screening Thousands of DEL Compounds

The Carterra LSA (**Figure 1**) is a unique technology for HT-SPR screening of binding interactions in real time. Novel microfluidics allow up to 384 samples to be arrayed on the biosensor surface, followed by screening in parallel against hundreds of injected samples. Up to 150,000 interactions can be assessed in a single run depending on the assay design. Data analysis is highly flexible using either from use of dedicated software or else by interfacing with third-party data analysis packages.

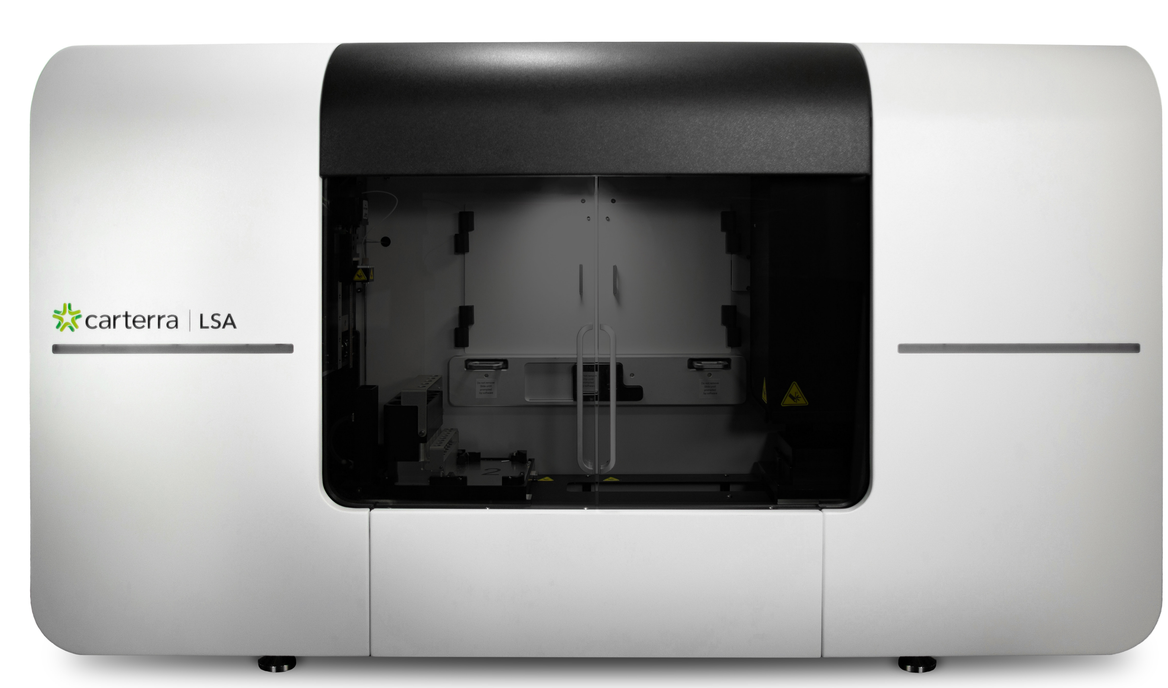

Figure 1. The Carterra LSA

Figure 2 highlights the basic steps involved in creating an array of up to 384 DEL compounds. Key to enabling this approach is the use of a DNA tag that encompasses both a region devoted to identification of the attached compound as well as a region capable of hybridizing with the corresponding LNA surface. The surface can easily be stripped of the DELs using a simple mixture of salt and base, and a new array then captured. The LSA instrument has plate capacity for up to 1,152 DEL compounds in a single run, allowing for comprehensive kinetic screening of >5,000 compounds per week.

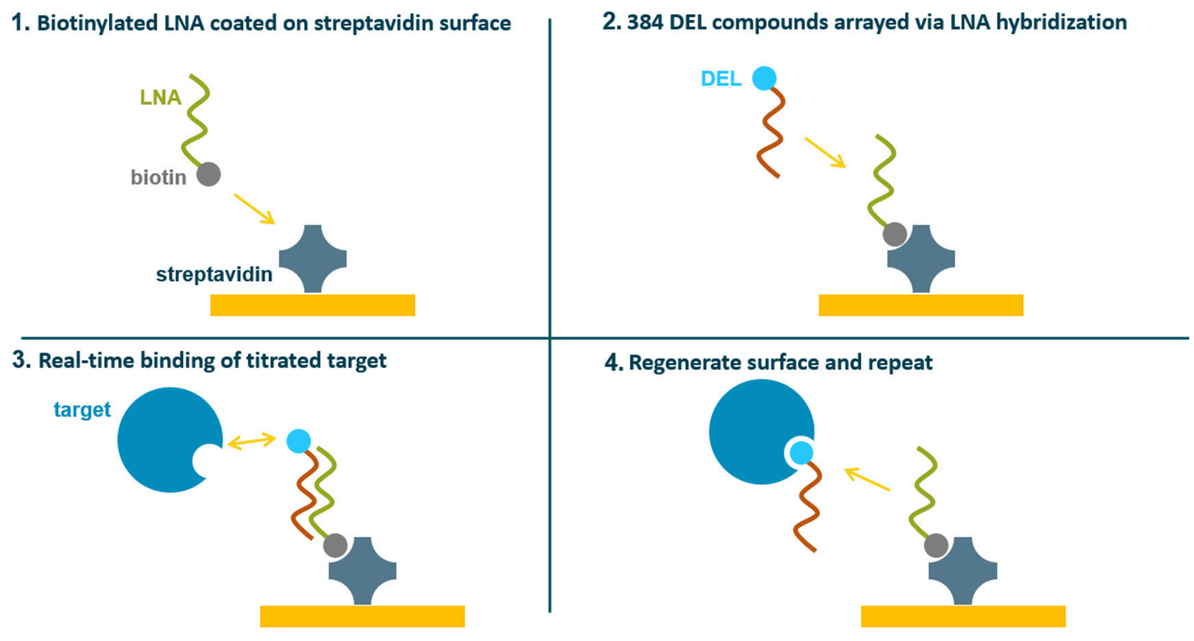

Figure 2. Approach to screening DEL compounds via HT-SPR

KINETIC SCREENING RESULTS

Representative data for a detailed kinetics screen of 96 DEL compounds captured at four densities binding a 25 kDa protein target are shown in **Figure 3**. Colored highlighting of select interaction tiles is done by automatic data flagging features in the Kinetics analysis software which indicate low binding responses or deviations from a 1:1 Langmuir model. This approach not only stratifies binders versus non-binders, but also gives highly reliable measures of association and dissociation kinetics for thousands of compounds. **Figure 4** illustrates the quality of data and level of detail ascertained for each compound. In terms of uniformity across the array, typical rate constant variation for replicates is less than 10%, allowing for confident comparison of the thousands of compounds that can be screened using this approach, **Figure 5**.

Figure 3. Array view of high-resolution DEL compound kinetics.

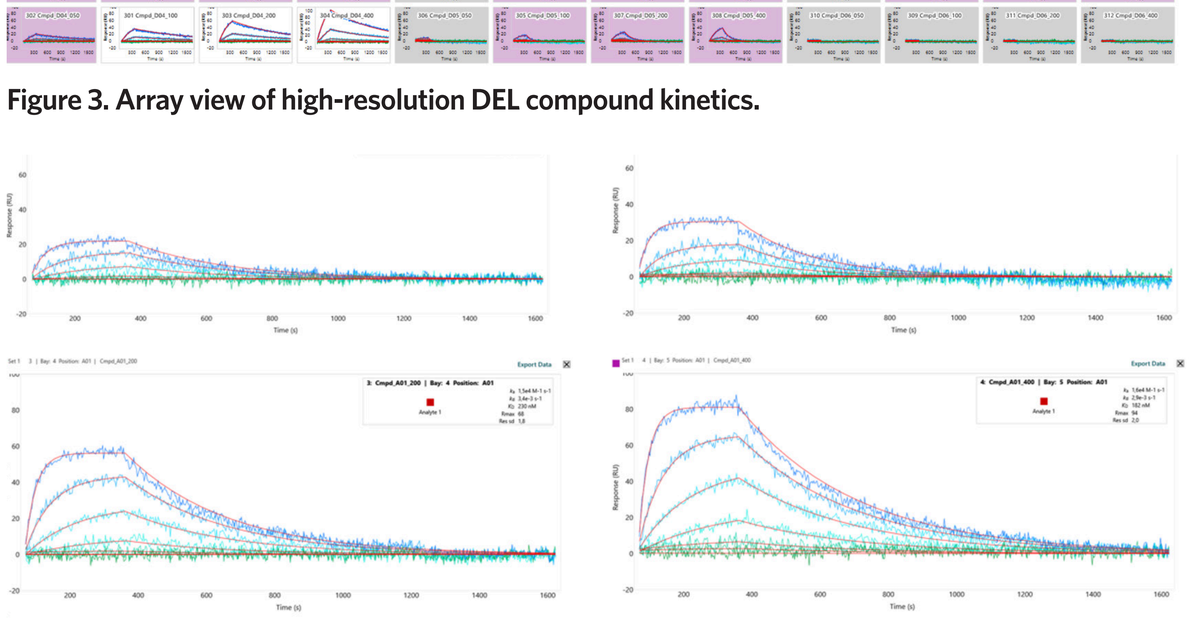

Figure 4. Zoomed view of select DEL compounds binding a 25 kDa target

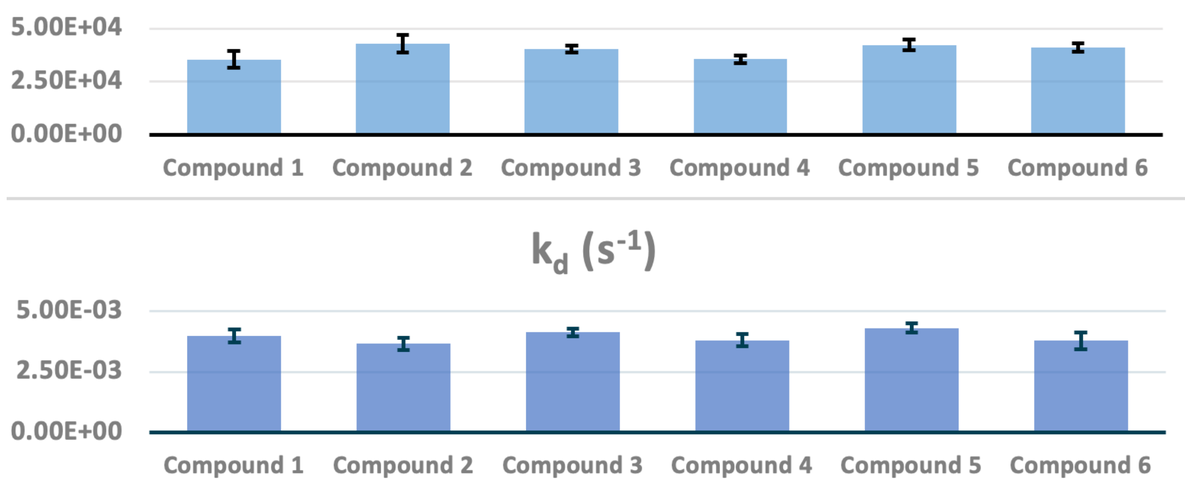

Figure 5. Rate constant mean and standard deviation for select DEL compounds tested as 8 replicates on the sensor chip surface. Standard deviation was less than 10% across all compounds.

HIGHLIGHTS

Demonstrated here is an approach to DEL compound screening that seeks to bridge the gap between initial hit screening and off-DNA lead compound scale up. By increasing the data resolution prior to off-DNA screening, this approach affords a better chance of finding drug candidates with optimal properties from thousands of potential hits. While fragments and small molecules are typically the formats screened using DEL technology, this same assay can be applied to any molecule attached to DNA, including TPDs and macrocycles. Additionally, the assay has versatility beyond kinetic analysis, where for example target isoforms and off-target reagents can be readily screened. Competitive assay formats are also adaptable to this approach and allow additional mechanistic questions to be addressed.