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 but by design remains informationally limited.
- To address this limitation in DEL screening, demonstrated here is a technique to characterize DNA-linked molecules as well as follow up on hits off-DNA with improved granularity using high-throughput surface plasmon resonance (HT-SPR).
- With a screening capacity of thousands per week, HT-SPR affords a high-resolution technique that matches the throughput needs of early discovery.
- Additionally, this approach can work with any moiety having DNA attachment, such as targeted protein degraders (TPDs) and macrocycles.

UNIQUE FEATURES OF THE CARTERRA LSA^{XT}

The novel microfluidics of the Carterra LSA^{XT} (**Figure 1**) allow up to 384 samples to be arrayed on the biosensor surface simultaneously, 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, achieving levels of throughput not available on other platforms. Data analysis is highly flexible using either Carterra's dedicated software or else by interfacing with third-party data analysis packages.

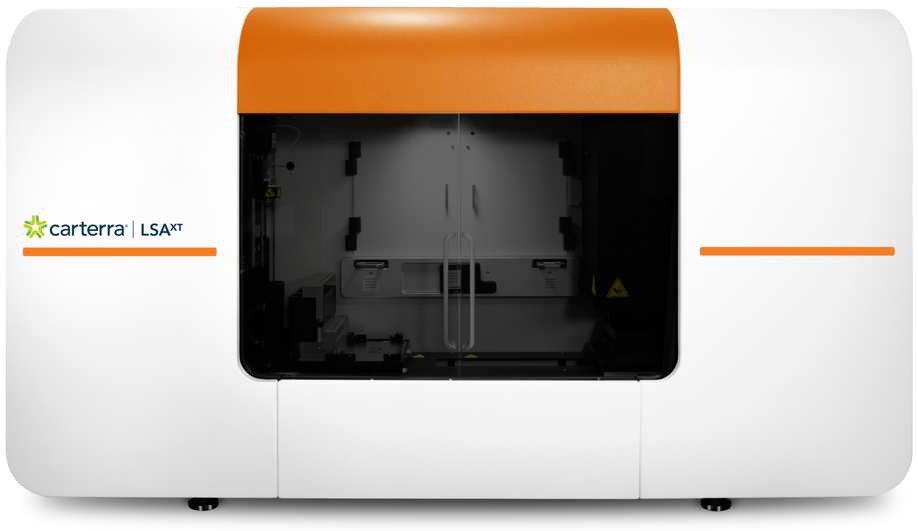

Figure 1. The Carterra LSA^{XT}

DEL COMPOUND KINETICS

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 complimentary oligonucleotide strand attached to the compound which is capable of hybridizing with the corresponding LNA oligonucleotide surface. The surface can easily be stripped of the DEL compounds using a simple mixture of salt and base, and a new array then captured. The LSA^{XT} 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.

For on-DNA experiments, the biotinylated LNA was coated on an SAP (planar streptavidin) sensor surface at 100ng/ml. DEL compounds, 192 in total, were captured at 100nM. Target was prepared in HBSTE buffer and injected as a 2-fold dilution series starting at 25uM.

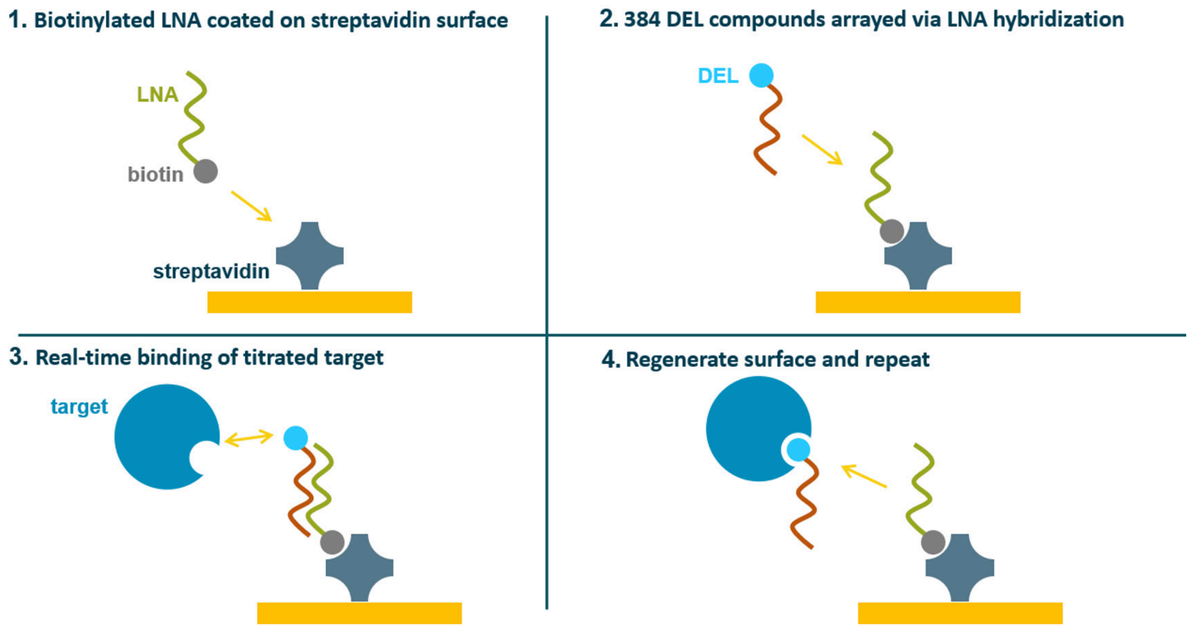

Figure 2. Creating array of DEL compounds on the LSA^{XT} sensor chip followed by injection of target to determine binding kinetics.

OFF-DNA KINETICS

Following screening of the on-DNA compounds, select compounds were synthesized off-DNA and injected as a 3-fold titration starting at 10uM against biotinylated target captured on an SAP sensor chip. See **Figure 3** for a illustration of the off-DNA format.

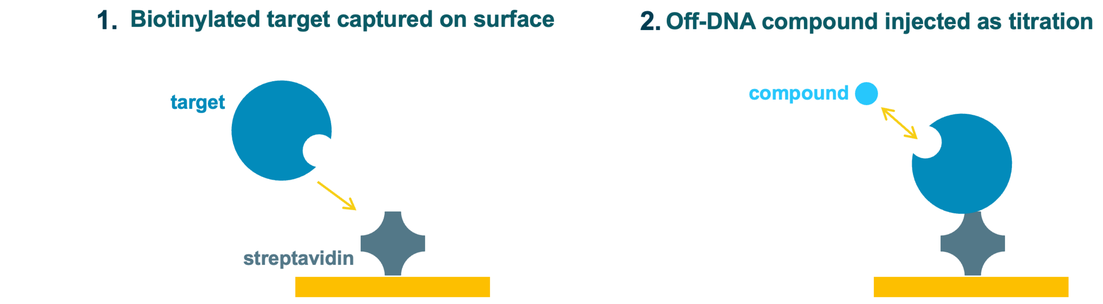

Figure 3. Assay format for screening kinetics of off-DNA compounds.

RESULTS

Full kinetic profiles for each of 192 DEL compounds are shown in **Figure 4**. Colored highlighting of select interaction tiles is done by automatic data flagging features in the Kinetics analysis software which indicate low binding responses. The LSA^{XT} not only stratifies binders versus non-binders, but also gives highly reliable measures of association and dissociation kinetics for up to thousands of compounds.

Following kinetic characterization of the DEL compounds, select molecules were synthesized off-DNA and tested against the same 30 kDa target. In **Figure 5**, an example of 290 Da compound is shown binding to the target immobilized on the chip surface with good agreement for K_D values among the replicates tested at two different densities..

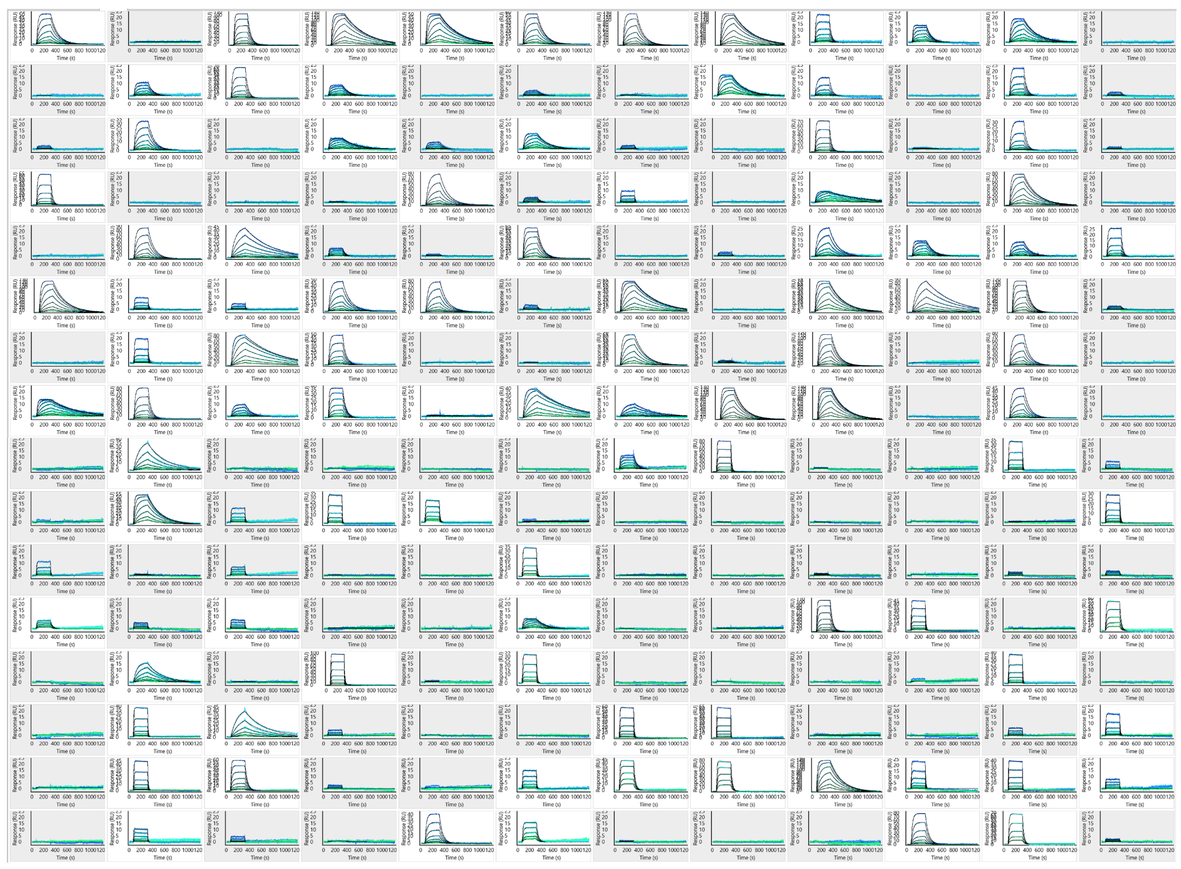

Figure 4. Array view of detailed kinetics for an injected target binding 192 captured DEL compounds. Highlighted in gray are non-binders. 1:1 Langmuir fit lines are shown in black.

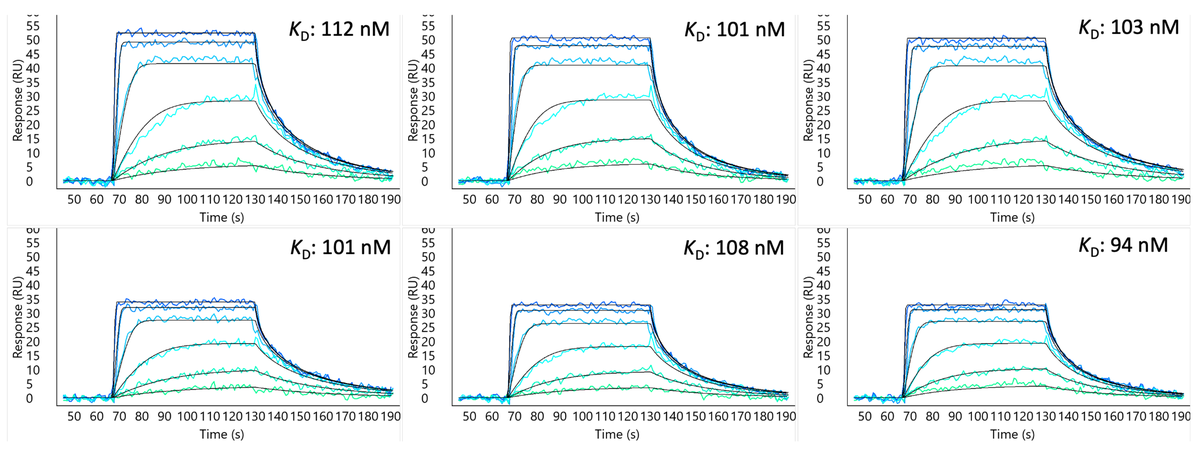

Figure 5. Kinetic profiles for a single off-DNA compound (290 Da) binding in triplicate against target immobilized at higher and lower densities.

HIGHLIGHTS

- The poor resolution of traditional DEL screening approaches can be eliminated by the enhanced sensitivity and unmatched throughput of the LSA^{XT}.
- These workflows are readily adaptable to other DNA-tethered chemistries such as TPDs and macrocycles.
- Additionally, the unique configuration of the LSA^{XT} allows for assay versatility beyond kinetic anaylsis alone, including iso-form, off-target, and competition-based approaches.