Increasing DNA Encoded Library (DEL) Compound Screening Resolution Using HT-SPR

Key Takeaways

- Detailed kinetics for >5,000 molecules per week
- Compatible with any DNA-labeled format such as fragments, small molecules, TPDs, macrocycles, etc.
- Perform full kinetic analysis for both on- and off-DNA hits on the same instrument

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.

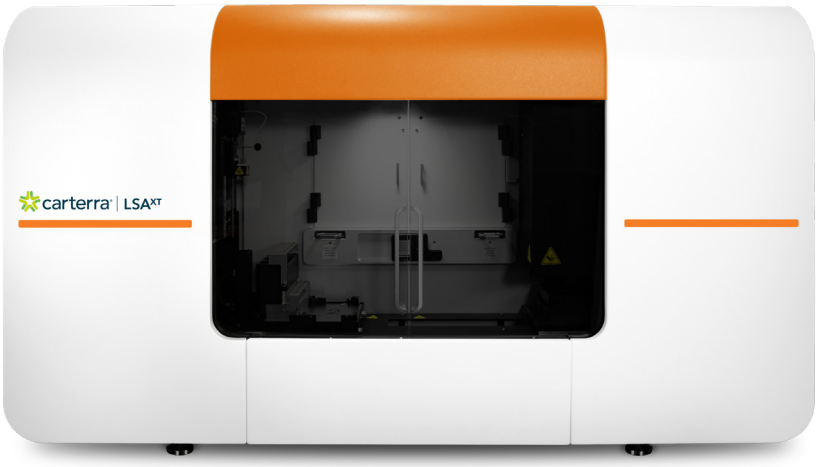

Figure 1: The Carterra LSA^{XT}

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 by interfacing with third-party data analysis packages.

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.

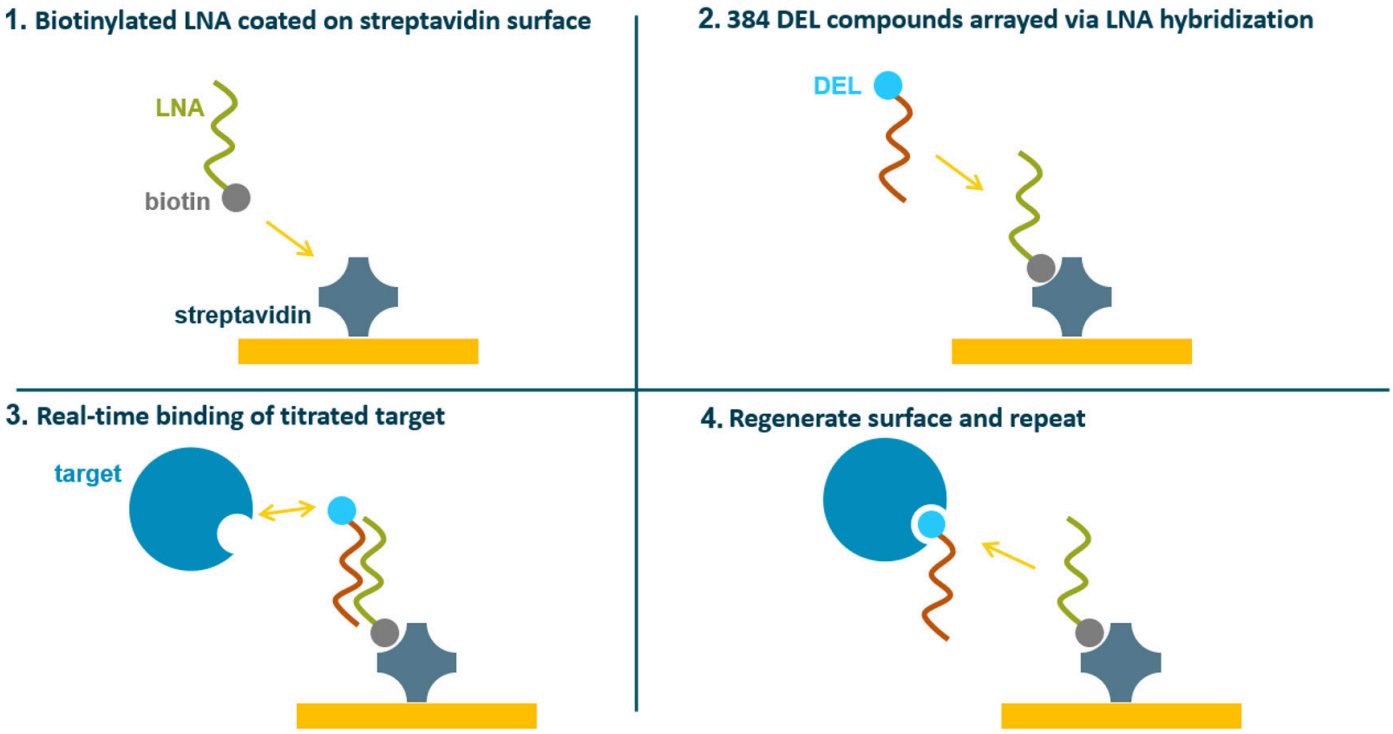

Figure 2: Creating array of DEL compounds on the LSA^{XT} sensor chip followed by injection of target to determine binding kinetics.

Off-DNA Compound 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 SAHC30M sensor chip. See **Figure 3** for an illustration of the off-DNA format. The interaction temperature was set to 15°C. Compounds were prepared in HBS + 0.005% Tween-20, 10 mM MgCl2, 3% DMSO, pH 7.4 running buffer. Data were fit to a simple 1:1 Langmuir model.

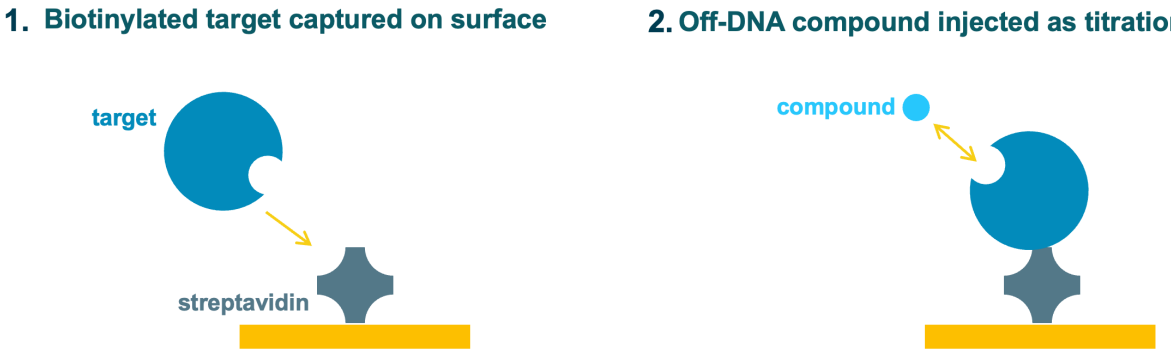

Figure 3: Assay format for screening off-DNA compound kinetics.

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 as gray. 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 301 Da compound is shown binding to the target immobilized on the chip surface with good agreement for KD values among the replicates tested at two different densities.

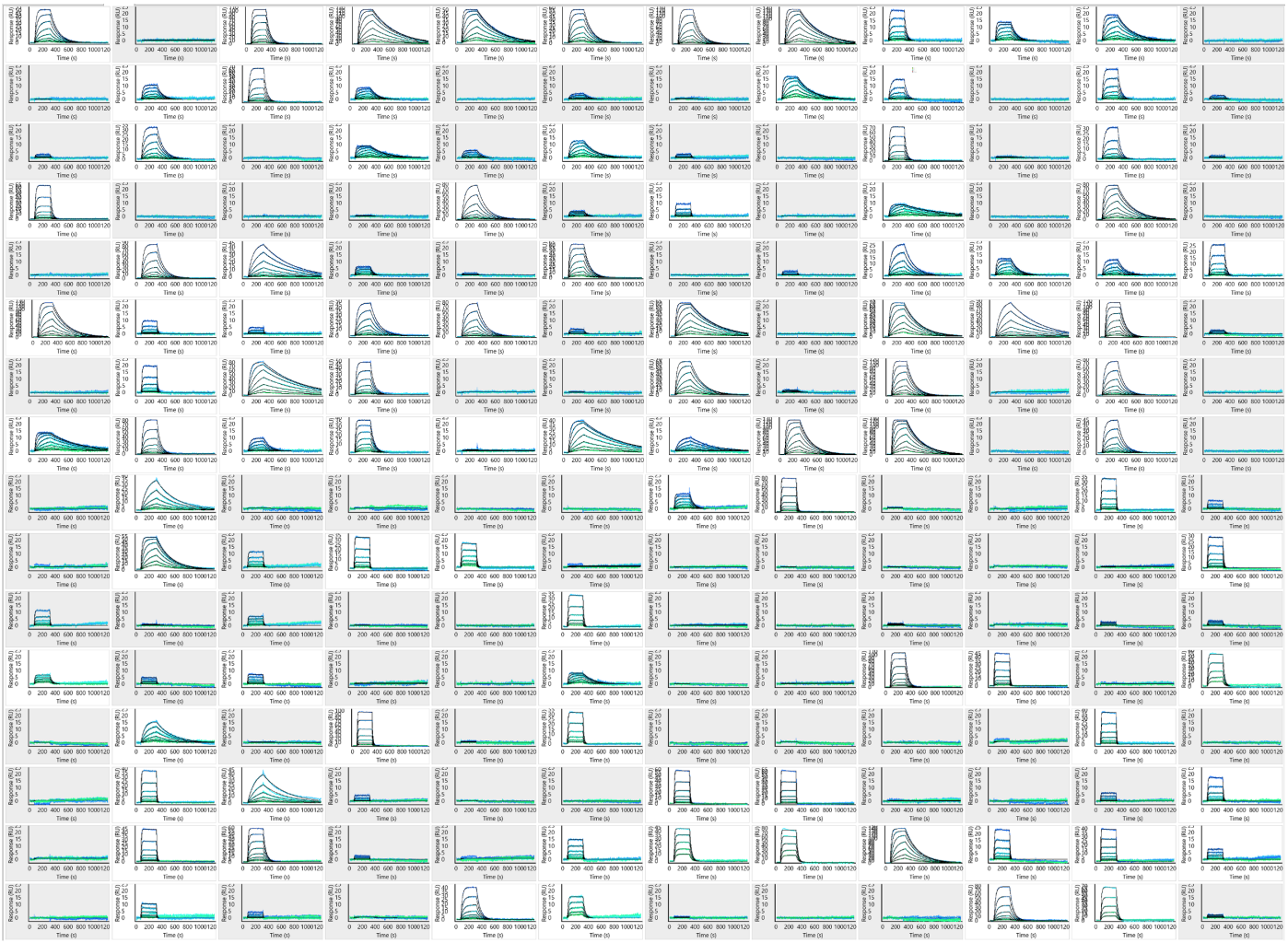

Figure 4: Array view of detailed kinetics for a titration of target injected across 192 captured DEL compounds. Highlighted in gray are compounds classified as non-binders. 1:1 Langmuir fit lines are shown in black.

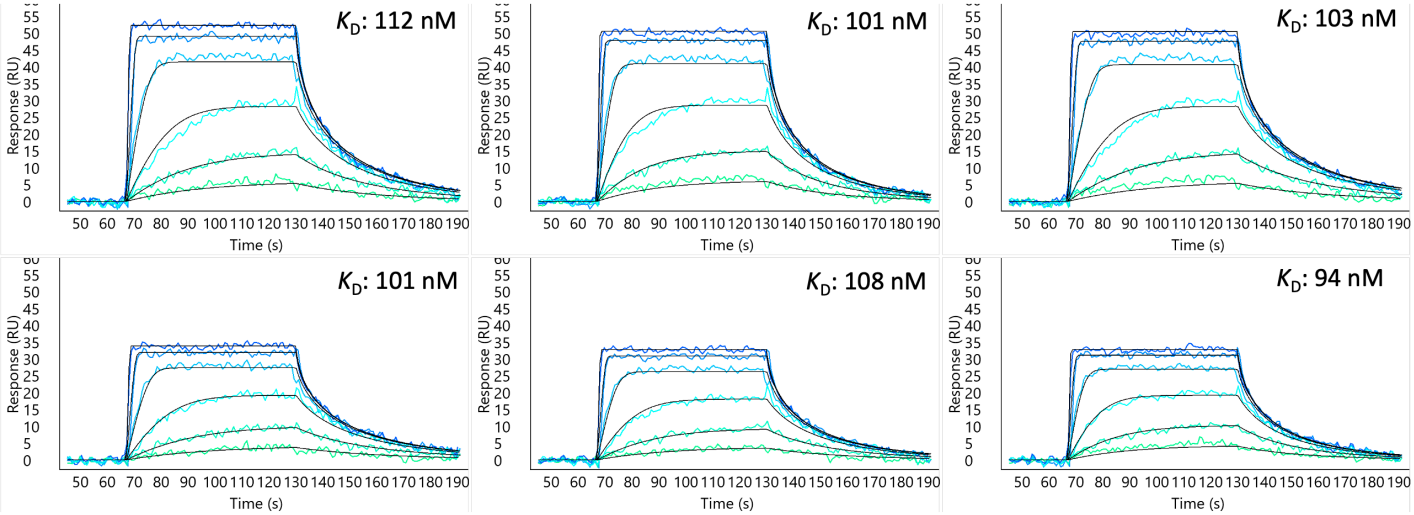

Figure 5: Kinetic profiles for a single off-DNA compound (301 Da) binding in triplicate against target immobilized at higher and lower densities on an SAHC30M sensor chip. 1:1 Langmuir fit lines are shown in black.

Summary

The poor resolution of traditional DEL screening approaches can be eliminated by the enhanced sensitivity and unmatched throughput of the LSA^{XT}. The workflow here 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. The sensitivity of the LSA^{XT} also allows for follow up characterization of off-DNA hits for molecules down to at least 300 Da. While fragments and small molecules are typically the formats screened using DEL technology, this same strategy 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.

References

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2. Plais L, Scheuermann J. Macrocyclic DNA-encoded chemical libraries: a historical perspective. *RSC Chem Biol*. 2021 Oct 29;3(1):7-17.
3. Gironda-Martínez A, Donckele EJ, Samain F, Neri D. DNA-Encoded Chemical Libraries: A Comprehensive Review with Successful Stories and Future Challenges. *ACS Pharmacol Transl Sci*. 2021 Jun 14;4(4):1265-1279.

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