Dynamic DNA-Encoded Library Technology: Discovery of Kinesin 1 Activators and Inhibitors

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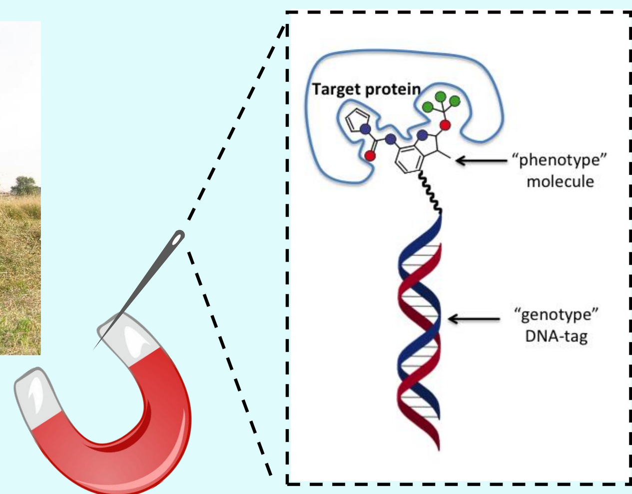
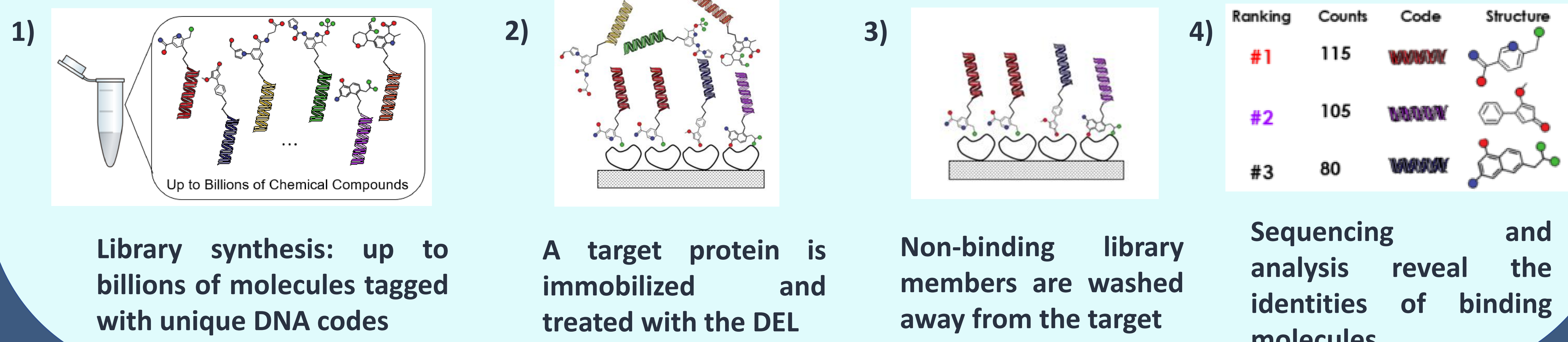
DNA-Encoded Library Discovery
Finding the Needle in the Haystack



DNA-Encoded Library (DEL) technology is an elegant approach to the needle-in-a-haystack problem of drug discovery. Running a DEL selection is like bringing a magnet to your haystack: up to billions of different molecules can be simultaneously interrogated against a protein target of interest. By tagging each molecule in the collection with a unique DNA barcode, their identities can be revealed after sequencing the DNA tags of binding molecules eluted from a binding assay. Due to this massive throughput and no need for a target-specific activity or functional assay, a DEL campaign can be completed at 10 times the speed of a traditional high-throughput screen.



1) Library synthesis: up to billions of molecules tagged with unique DNA codes

2) A target protein is immobilized and treated with the DEL

3) Non-binding library members are washed away from the target

4) Sequencing and analysis reveal the identities of binding molecules

About DyNAbind

DyNAbind GmbH is a privately held company dedicated to increasing the throughput, reliability and scope of DEL drug discovery. Our team of chemists, biologists and biotechnologists has built a discovery pipeline that's effective not only for more reliable discovery of medicinal chemistry starting points, but also for optimization of existing binders and affinity or specificity maturation.

We offer risk-minimized partnership opportunities for both industry and academia-based groups, where the majority of payments are based entirely on the success of our drug discovery programs and future development of hit-derived compounds. To date, we've worked on a number of target classes – from classical enzymes to more traditionally challenging protein-protein interactions, GPCRs and even motor proteins. DyNAbind's library collection is always growing, and currently contains over 125 million different structures ready to deploy against your target.

From April 2019, DyNAbind is proud to offer a portion of our Dynamic Fragment DEL as a kit, available exclusively through MilliporeSigma. Containing ~400,000 fragment pairs, this kit is an ideal way to get early information on potential target tractability and generate supporting data for grant or other funding applications. For groups lacking the funding for a traditional DEL discovery partnership, this kit offers a novel entry into the world of DEL drug discovery!

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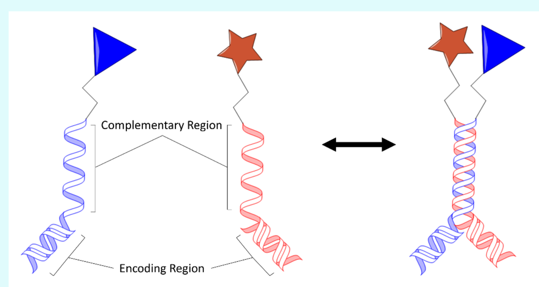
References

Reddavide, F. V. et al. (2015). Angew. Chem. Int. Ed., 54: 7924-7928.
Reddavide, F.V. et al. (2019). Chem. Commun. Advance Article
Korten et al. (2018). Lab Chip, 18, 3196-3206

Dynamic DEL Technologies and Development-Minded Library Design

Increasing DEL Reliability

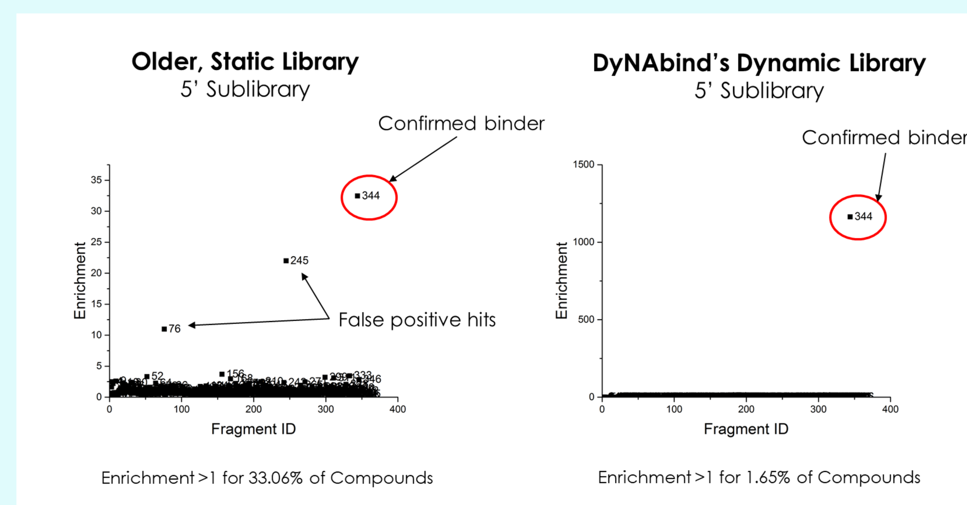


DyNAbind's Dynamic Library technology was designed to increase the reliability of DEL by improving signal-to-noise ratio and allowing a higher standard of library quality control. The technology is based on combining sublibraries of DNA-tagged molecular moieties. Via a universally complementary fragment pairs randomly assemble. Combining fragments in this way offers new chemical diversity while still maintaining quality control, as each fragment-DNA conjugate is individually purified and validated.

This universal binding region is, however, engineered to be intrinsically unstable, so that fragment pairs are constantly reshuffling themselves, until stabilized by binding to the target protein. In this way, the most strongly binding molecules are enriched, improving signal-to-noise ratio.

Dynamic Fragment Pair Reshuffling

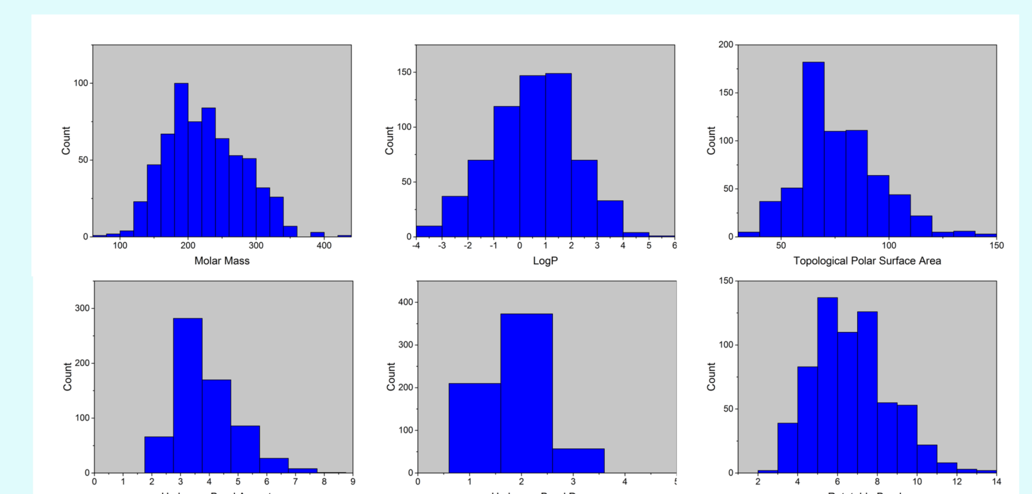
The advantages of the Dynamic Library architecture are clear; in head-to-head comparisons against older library designs that don't allow dynamic reshuffling, the Dynamic Library shows over 30-fold improved signal-to-noise ratio with less instances of false positive hits.



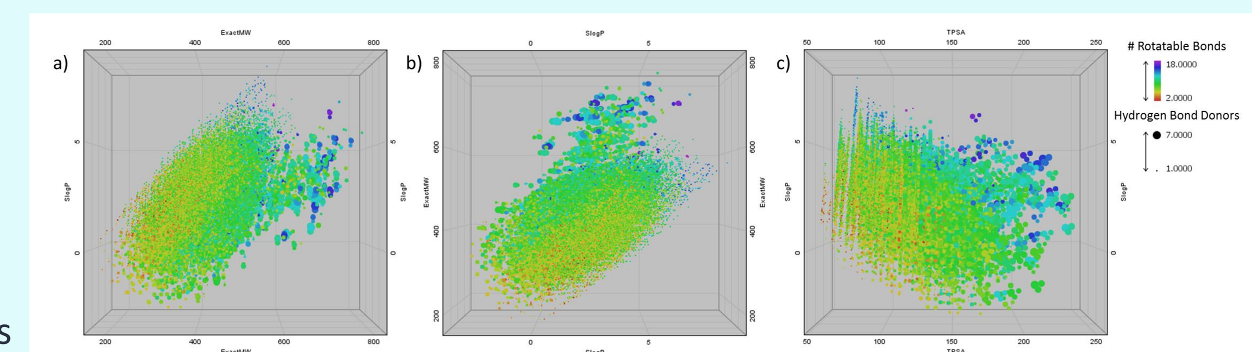
In selection experiments against human carbonic anhydrase 2, the Dynamic Library (right) enriched the signal of a known binding molecule (#344) over 30 times more than the older, static library design.

Med-Chem Focused Library Design

Taking full advantage of improvements in DNA architecture requires the selection of library members which offer maximized chemical diversity while avoiding promiscuous, toxic or reactive substructures. At the same time, care must be taken to avoid 'molecular obesity' and remain in credible chemical space that actually represents good starting points for medicinal chemistry work.



The histograms above represent physicochemical properties for the fragments we've used to build our Dynamic Libraries. In addition to being in favorable med chem space, our collection is highly diverse, with the average Tanimoto similarity between any two library members less than 0.2.

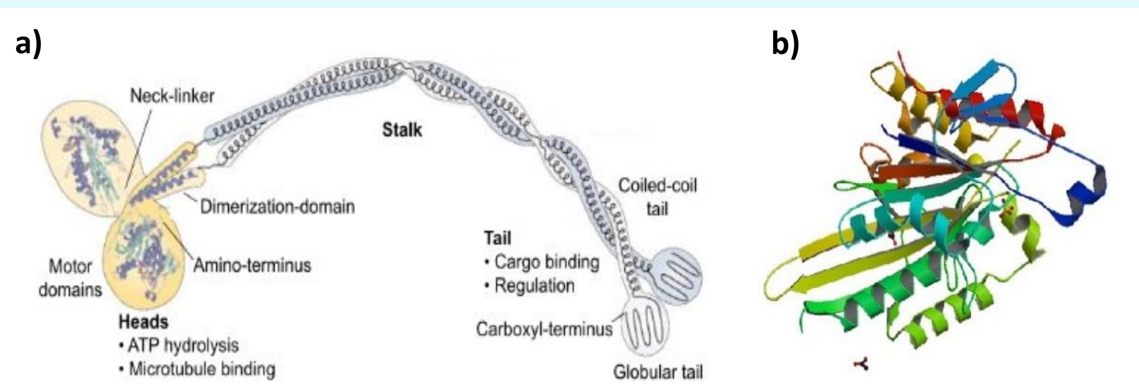


5D plots showing properties for 50,000 randomly selected small molecules from DyNAbind's collection. a, b and c respectively are aligned on the mass, logP and PSA axes. Dot color representes number of rotatable bonds and dot size represents number of hydrogen bond donors

From Selection to Initial Functional Validation on Kinesin 1

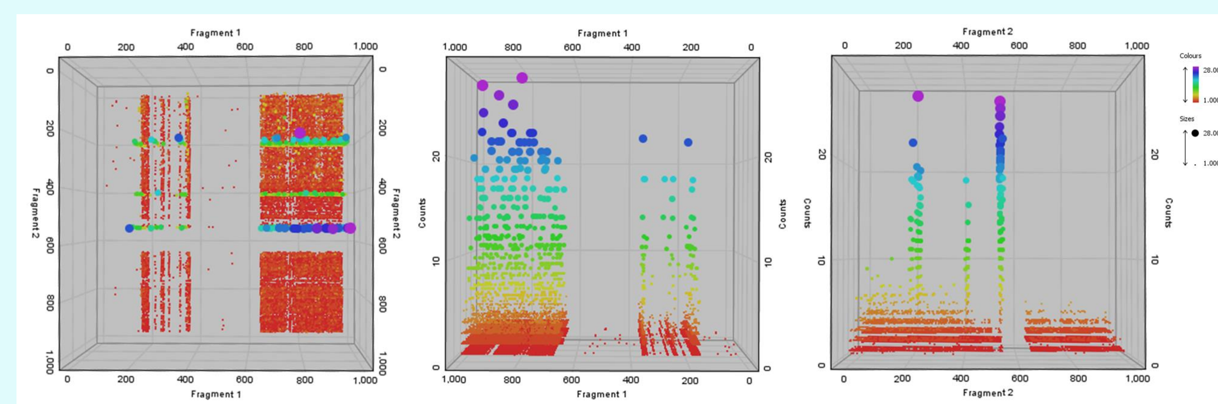
Target Identification and DEL Selection

Kinesins are motor proteins which transport cellular cargo by moving along microtubules. Among other things, they play a role in axonal transport and successful mitotic spindle function during cell division. Due to these crucial functions, kinesin activity has been implicated in a wide range of diseases, including neurodegenerative conditions such as ALS and cancer. Though these proteins are attractive targets, drugging them has been classically difficult due to the multifunctional nature of the proteins, which makes specificity crucial. As such, new chemical starting points are always in demand. Here, we focused on screening our fragment DEL collection against a truncated version kinesin 1 which contains the motor domain.



a) A schematic of the structure of kinesin-1. The motor domain on the left binds to microtubules and is the site of ATP hydrolysis which drives the transport mechanism. A long stalk region connects to the tail domain which binds to the cargo. b) Structure of the truncated motor domain region of kinesin-1 used in the selection experiments

We screened our Dynamic Dual-Fragment DEL set against the kinesin-1 motor construct in multiple conditions and were able to successfully identify multiple trending chemical structures. The plots below display the counts of paired fragments identified from the selection and sequencing. Unfortunately, due to continuing follow-up work and potential pending patent applications, no structures can be revealed at this time.

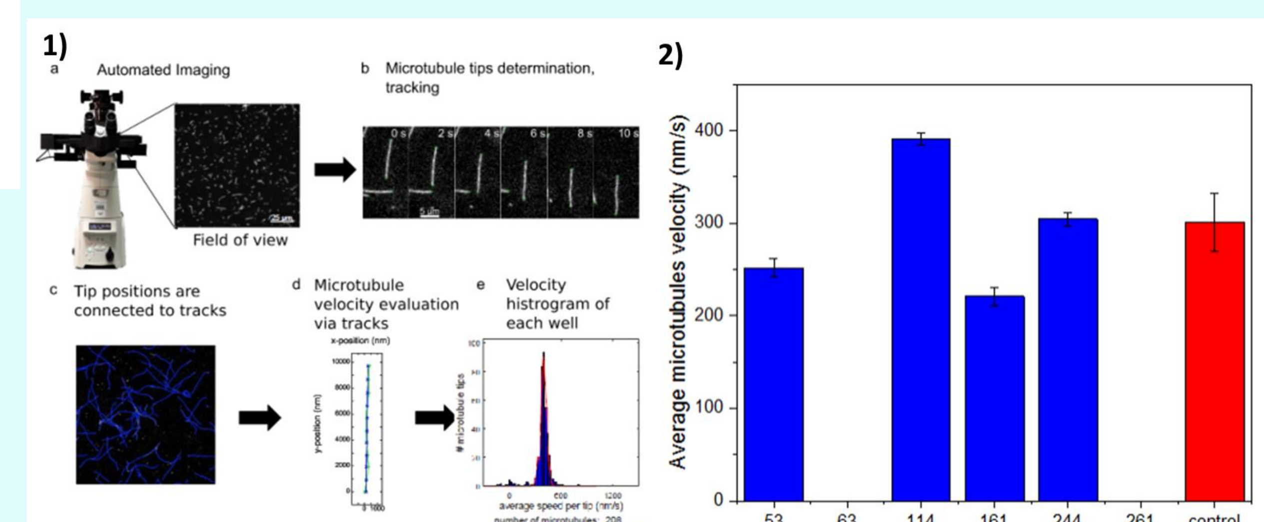


Dynamic Dual-Fragment DEL selection readout against the kinesin-1 motor domain. Each of the graphs above is rotated to align with a different axis

Hit Triage and Functional Validation

Fortunately, our collaboration partners in the Stefan Diez lab had already developed a novel functional readout for kinesin-1 activity, which allowed us to proceed directly to functional validation. With computational assistance, we selected an initial set of six structurally distinct hit compounds and prepared them off-DNA for follow up study.

The assay is based on automated tracking of microtubule transport by motor proteins. The prepared compounds were introduced into the protein solution at soluble concentrations (between ca. mM and 400 μM) and their effect on transport velocity was recorded. Two compounds, 63 and 261, completely abolished transport activity. Interestingly, compound 114 was shown to increase the transport velocity. To date, no other direct kinesin activator has been reported. An indirect activator which increases speed by preventing cargo binding has been published, but Compound 114 is completely structurally distinct and in a significantly more druglike chemical space. Current work is continuing on generating dose-response curves and optimizing structures for further development.



1) Schematic view of the automated tracking assay process. 2) Functional assay readout for selected compounds from the DEL selection at soluble concentrations,