# Creating Biological “Switch” by Using DNA-encoded Library Technology

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WuXi Biology

## Introduction

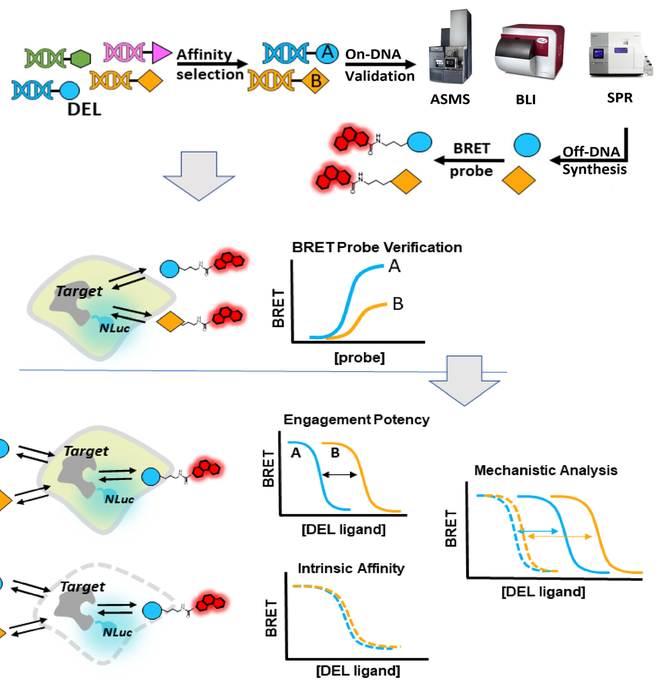
Besides identifying chemical starting points for early drug discovery process, DNA-encoded chemical library (DEL) technology has now expanded to new applications including the discovery of bifunctional affinity ligands historically difficult to develop. Herein, we describe two examples of how bifunctional affinity ligands from DEL can efficiently be applied in chemical biology studies.

One example includes the creation of biological tracers directly from DEL hits that can be applied for in-cell target engagement studies. Hits from DEL screen can be easily converted to NanoBRET-ready tracers which is crucial for NanoBRET TE assay. Using Aurora Kinase A as an example, we describe the workflow of using DEL screening to identify ligands that can be transformed into cell-active BRET tracers.

The other example includes modulating antibody specificity through generating an affinity ligand–antibody chimera. Tumor-targeting specificity and activity of T cells with a CAR consisting of an antibody with a lysine residue that catalytically forms a reversible covalent bond with a 1,3-diketone hapten can be regulated by the concentration of a small-molecule adapter identified via DNA-encoded library. The adapter controls the formation of a covalent bond between the catalytic antibody and the hapten, as well as the tethering of the CAR T cells to the tumor cells, and hence the cytotoxicity and specificity of the cytotoxic T cells.

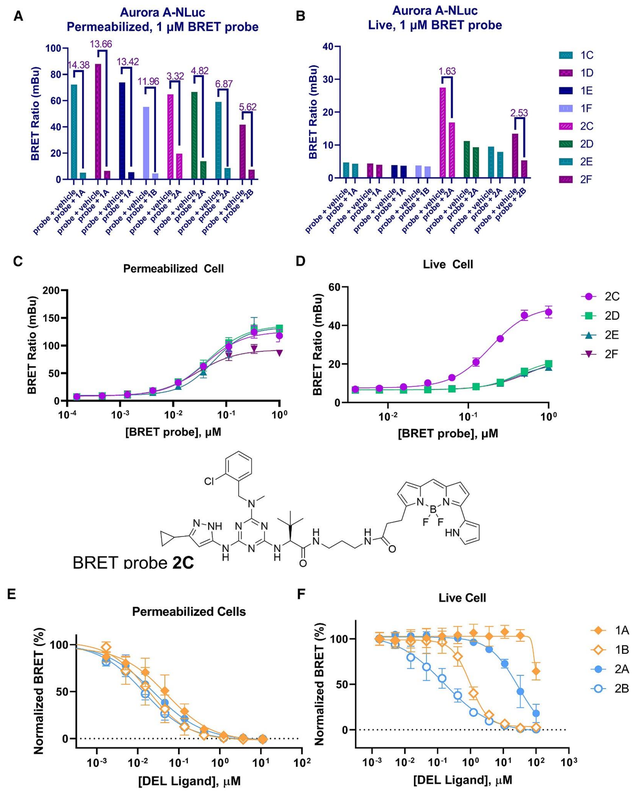
In both cases, DEL has demonstrated the power of efficiently generating affinity ligands to accelerate the downstream development process.

## Case I-1 DEL to NanoBRET™ Probe Workflow



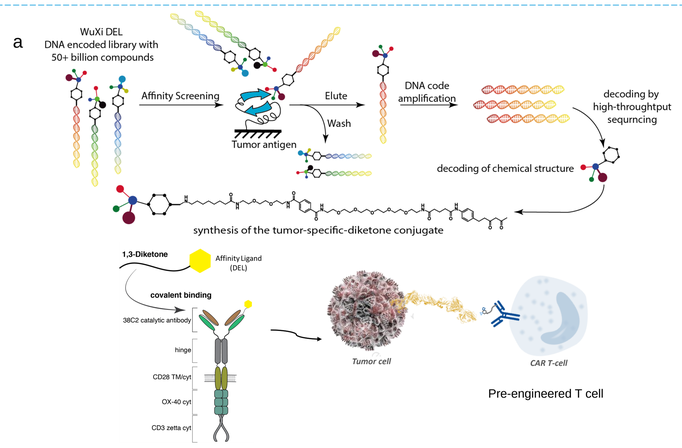

- BRET probe conversion: Bifunctional DEL derived chemical series are selected from DEL screen, validated by on-DNA assays, followed by off-DNA synthesis and functionalized with NanoBRET dyes
- Cellular assay validation: BRET probe performance is validated in cells
- Competitive displacement assay: BRET probe provides a reagent analysis of target engagement in live and permeabilized cells.

## Case I-2 Aurora A BRET Probe Verification and Target Engagement Analysis for DEL Scaffolds



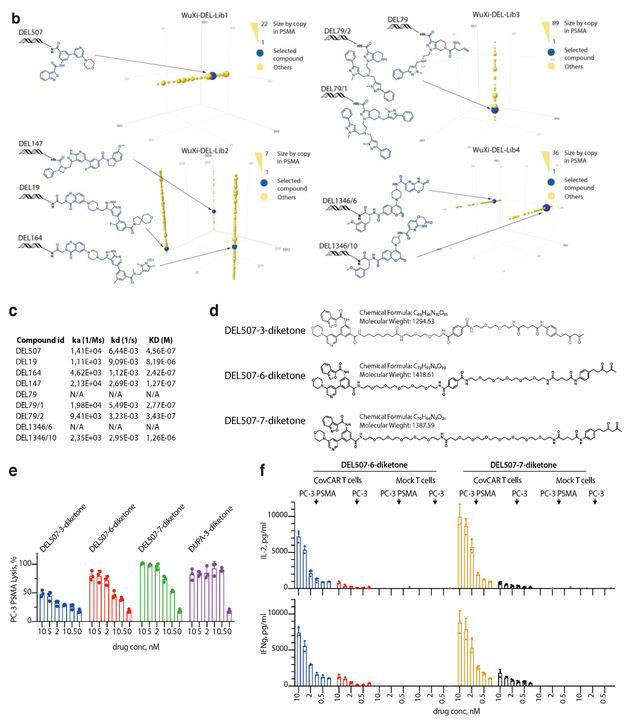

- Preliminary probe screen for aurora kinase A DEL-hit derived BRET probes (1 μM) in (A) live cells and (B) permeabilized cells either in the presence or absence of 1 or 2 parent compound.
- The affinities of probes were measured as dose-response in permeabilized (C) and live cells (D). BRET probe 2C was the best-performing BRET probe in live cells, and its chemical structure is provided.
- Using 2C as the probe, derivatives from DEL screen are tested for their cellular permeability and target affinity in both permeabilized cells (E) and in live cells (F). Despite the similar affinities in permeabilized cells, the difference in cellular permeability can be examined.

## Case II-1 DEL to Covalent CAR-T Workflow




- (a) A workflow for selection of tumor-antigen-specific ligands for covalent CAR T-cell therapy.

## Case II-2 PSMA-specific Adapter Molecules from DEL for Covalent CAR T-Cell Therapy




- DEL compounds that specifically bind to PSMA (b) Each selected synthesized as a spacer-diketone conjugate (c) Using surface plasmon resonance (SPR) measurements, affinities were measured. (e,f) DEL507-diketone conjugates to the covalent CAR T-cells and PC-3 PSMA+ cells mediated cytotoxicity and proinflammatory cytokine release in a dose-dependent manner.

## DNA-encoded Library Services at WuXi AppTec

### Services Type

| | DELpro | DELink Pro | Chemical Inducer of Proximity | DELink Lite | DELight | Peptide | RNA focused |
|---|---|---|---|---|---|---|---|
| Type | Generic | Covalent | Bivalent | Covalent | Generic | Focused | Focused |
| Conditions | 8 | 12 | 8+ | 6 | 4 | 4 | 4 |
| Service Mode | One Stop | | | Flexible Self-service | | | |
| Library Size | 40+ Billion | 1.6+ Billion | 4+ Billion | 14+ Million | 10+ Billion | 250+ Billion | 100 Million |
| Payment | Step-wise | | | | | | |
| Deliverable | Limited Exclusive Molecules | | | All Positive Data | | | |

### DEL Collections for New Modality

**Covalent DEL**

- 1.6 Billion+ diversity
- 184 Electrophiles targeting Cys, Lys, Tyr, Ser
- Electrophile reactivity and selectivity cover from low to high

**Peptide DEL**

Linear libraries — Diversity: ~95 billion

Cyclic libraries — Diversity: ~156 billion

- 500+ amino acids including β-, N-alkylated amino acids
- Backbone length 6 – 9 amino acids (23 – 32 heavy atoms)

**RNA focused DEL**

- ~100 Million diversity
- Nitrogen-containing heterocycles, multiple aromatic ring system
- Covering current published RNA chemical space

**Chemical Inducers of Proximity DEL**

- 4+ billion diversity covering different E3 ligands (CRBN, VHL, IAP)
- Various validated DNA conjugate site
- Intermediate library with handle for custom E3 ligands of interest

**On-bead DEL /OBOC**

- On-shelf library, customized library construction
- Affinity-based glue FACS On-bead DEL screening
- Biochemical & cellular assay-based activity On-bead DEL screening

## Conclusion and Publications

These studies demonstrate how DEL enables rapid discovery of novel affinity ligands for downstream biological applications, including tracers for biochemical and cellular assays and building a ‘universal‘ CAR to enhance the control and safety profile of CAR-based cellular immunotherapies.

*1. DELs enable the development of BRET probes for target engagement studies in cells, Teske et al., 2023, Cell Chemical Biology*

*2. Control of the antitumour activity and specificity of CAR T cells via organic adapters covalently tethering the CAR to tumour cells, Stepanov et al., 2023, Nature Biomedical Engineering*

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