Recording binding information directly into DNA-encoded libraries using terminal deoxynucleotidyl transferase

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DNA-encoded chemical libraries (DELs) are an essential tool for hit finding in drug discovery. They are routinely selected against immobilized proteins of interest (POIs) by affinity enrichment. However, this method selects mainly for binders with slow koff rate constants and leaves an incomplete picture of potential ligands for a target.

Terminal deoxynucleotidyl transferase (TdT) is an unusual DNA polymerase that extends template-independent the 3'-end of DNA with random nucleotides. A protein of interest can be expressed as TdT fusion, and by incubation with a DNA-encoded library and dATP, the induced proximity of a binding DEL member to TdT causes the extension of its DNA barcode. The added polyA tail serves as a stable record of the binding event and enables separation from non-binders by poly(dT)25 magnetic bead pull-down. In a benchmarking system, we show that ligands spanning nanomolar to double-digit micromolar binding can be cleanly identified by TdT extension, whereas only the tightest binding ligands are identified by classical affinity selection. The method is simple to implement and can function on any DEL that bears a free 3'-end.