# A versatile platform for efficient affinity optimization of common light chain bispecific antibodies

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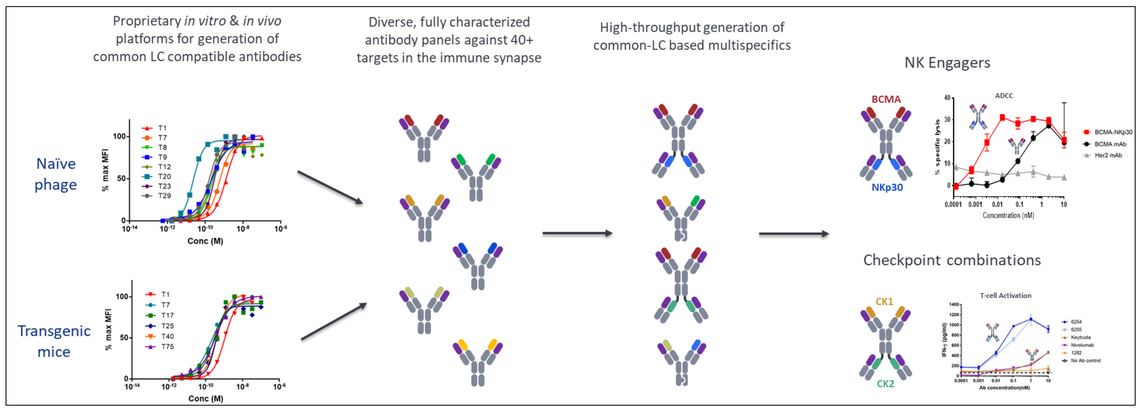
## Abstract

Antibody constructs with the ability to bind multiple targets have the potential for a broad range of clinical applications that are not possible with monospecific IgG. However, given the complexities of mispairing of VH/VL during expression and purification, development of well-behaved bispecific antibodies often requires extensive protein engineering and process optimization efforts. To circumvent these issues, Compass Therapeutics has developed a fully integrated antibody discovery and optimization platform based on common light chains (cLC). Here we present our approach to lead affinity maturation with the goal of rapidly generating high affinity bispecifics. While other cLC optimization efforts introduce beneficial mutations only in the VH, we investigate both VH and cLC mutations in parallel. This increases the ability to rapidly achieve desired optimization goals in cases where VH-only approaches fall short.

VH optimization proceeds via introduction of combinatorial diversity in all three VH CDRs and isolation of high affinity clones via phage and/or mammalian display selections. cLC optimization presents more of a challenge as introduction of mutations which lead to enhanced affinity when paired with one VH partner may be deleterious for the other VH(s) in the final construct. To tackle this challenge, we employ parallel deep mutational scanning (DMS) of the cLC CDRs in combination with each component VH. Our DMS approach couples traditional library panning/sorting schemes with NGS to quantify the enrichment or depletion of every potential single amino acid mutation in the cLC CDRs. The DMS data from desired bispecific partners is then compared to identify compatible mutations which enhance or at least maintain the affinity when paired with each VH. These parallel affinity maturation approaches enable us to routinely generate single digit nM and sub-nM binding arms that can be directly combined in stable and manufacturable bispecifics with no further engineering required.

## Compass Discovery Engine for IO Combinations

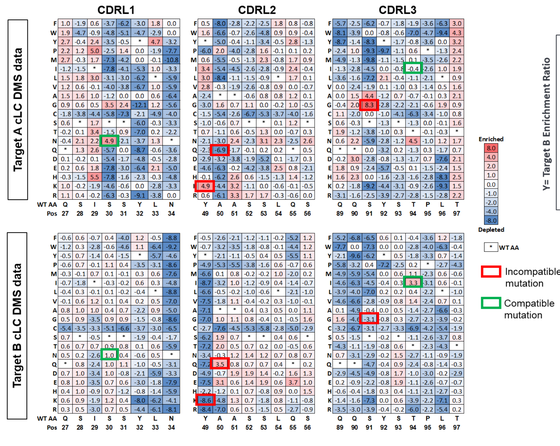
**Efficient optimization of bispecific combinations needed to support rapid progression towards INDs**



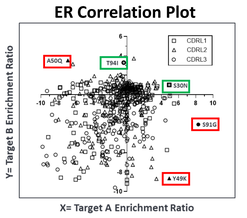

**How can we achieve affinity optimization goals in a single round of screening while maintaining cLC?**

## DMS-guided light chain design

**Enrichment Ratio data analysis used to design compatible cLCs**




**ER Correlation Plot**




**DMS data predicts binding affinity behavior**

| Heavy Chain | Light Chain | $K_D$ (M) | Fold Change |
|---|---|---|---|
| Anti-A WT VH | cLC | 4.0E-07 | WT |
| Anti-B WT VH | cLC | 1.5E-07 | WT |
| Anti-A AffMat VH | cLC | 2.0E-08 | 20 |
| Anti-B AffMat VH | cLC | 9.2E-09 | 16 |
| Anti-A AffMat VH | S91G | 7.5E-10 | 533 |
| Anti-B AffMat VH | S91G | NB | - |
| Anti-A AffMat VH | Y49K | 1.8E-09 | 222 |
| Anti-B AffMat VH | Y49K | NB | - |
| Anti-A AffMat VH | A50Q | 3.4E-07 | 1 |
| Anti-B AffMat VH | A50Q | 7.0E-10 | 216 |
| Anti-A AffMat VH | S30N | 2.4E-09 | 167 |
| Anti-B AffMat VH | S30N | 4.9E-09 | 31 |
| Anti-A AffMat VH | S30N, T941 | 1.7E-09 | 235 |
| Anti-B AffMat VH | S30N, T94I | 1.9E-09 | 81 |

VH Optimization yielded significant affinity improvement

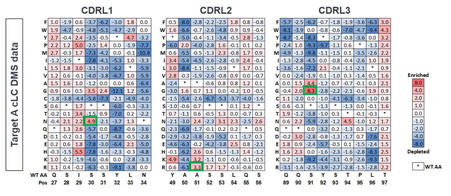
cLC DMS data predicted incompatible mutations

cLC DMS data predicted compatible mutations

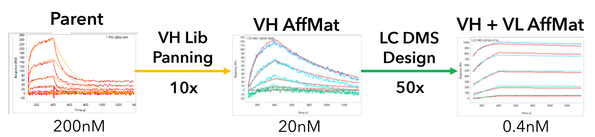
**Successful affinity optimization against both targets in a single round!**

## DMS for efficient monoclonal engineering

**Enrichment Ratio data analysis used to design improved LC**




**500-fold affinity improvement in a single round**

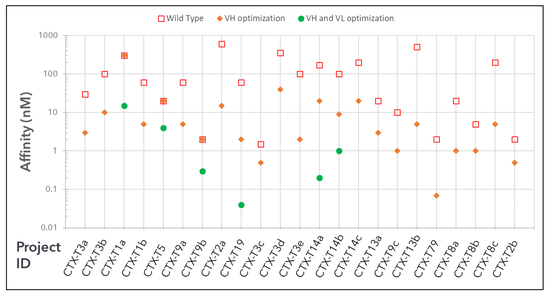



### Discussion

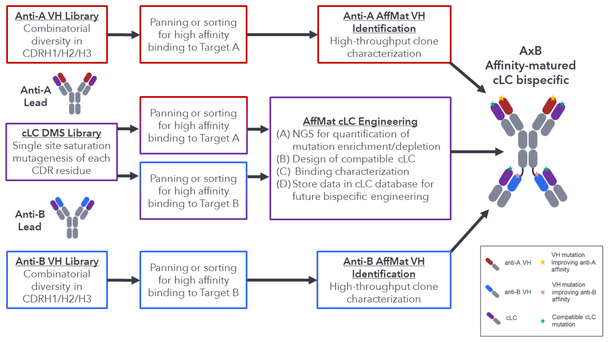
- Including cLC DMS analysis in every affinity maturation campaign allows optimization to proceed without bias to final antibody format
- DMS-guided design of optimized LCs enables rapid affinity improvements without additional rounds of library generation and sorting

## Compass Affinity Maturation Platform

**Single Round affinity maturation outputs**




**Affinity maturation workflow for common-LC bispecifics**




**Platform Highlights**

- Ability to introduce mutations into the cLC
- ≈2 months per campaign
- Library creation and screening is unbiased to final antibody format (mono-/bi-/multi-specific)
- Modular VH library components
- Ready-made cLC DMS libraries
- Phage and/or human display
- cLC Deep Mutational Scanning database under development
  - Storing, analyzing, and comparing NGS data for each cLC across campaigns
  - Anti-A can be paired/optimized with any other cLC clone
  - e.g. TAAxNKp30: Rapid optimization of novel combinations

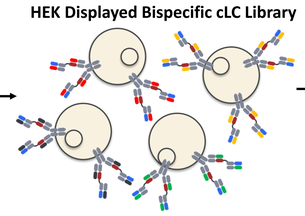
## Bispecific optimization via HEK display of cLC libraries

**cLC combinatorial library design and HEK bispecific display**

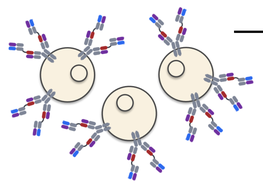
**ER cutoffs identify compatible mutations**




**HEK Displayed Bispecific cLC Library**

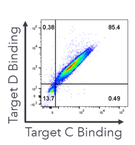


**HEK Displayed Bispecific WT cLC**



**HEK Bispecific display with WT cLC**

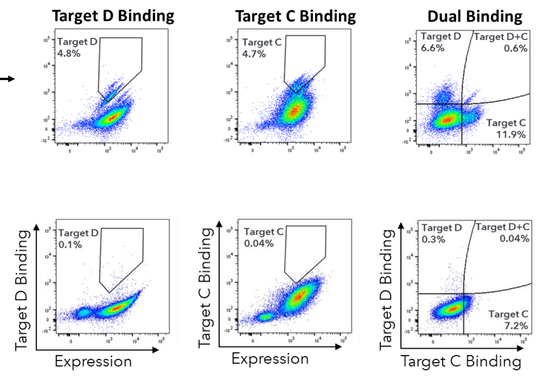
WT binding to both targets is maintained when displayed in a bispecific format




**Equilibrium Binding**

| | EC50 (nM) |
|---|---|
| Target C- mAb | 0.92 |
| Target C- bsAb | 1.00 |
| Target D- mAb | 0.62 |
| Target D- bsAb | 0.60 |

**Library sorting to isolate high affinity dual binding clones**

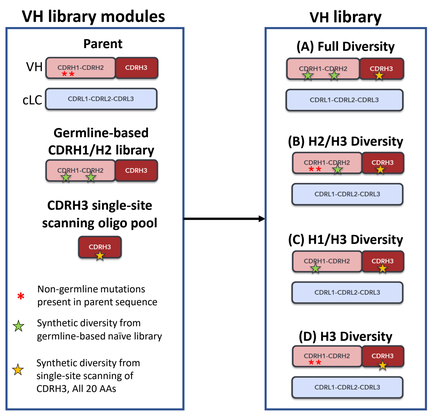



**Bispecific display library sorting and analysis**

- Analytical flow plots demonstrate the presence of high affinity dual binding clones in the library which outperform the WT cLC after off-rate competition
- The dual binding population will be sorted, sequenced, and characterized to identify dual affinity improving mutations in the cLC

## Modular VH and Ready-made cLC Libraries

**Modular VH Library Construction**



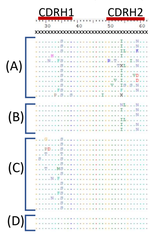




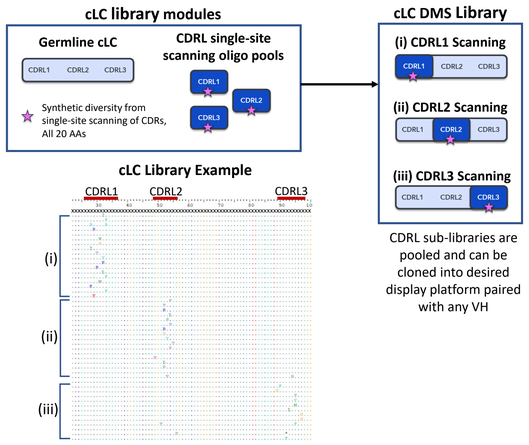
3-step Library construction

(1) PCR to generate various CDR and FW modules
(2) OEPCR to produce full-length VH sub-libraries
(3) Sub-libraries are pooled and cloned into desired display platform

**VH Library Example**




**Ready-made cLC Libraries**




**cLC Library Example**




CDRL sub-libraries are pooled and can be cloned into desired display platform paired with any VH

## Conclusions and Future Directions

- **We developed and implemented a rapid and efficient platform for affinity optimization of cLC bispecific antibodies**
- **cLC DMS analysis is included in every affinity maturation campaign:**
  - **Optimization proceeds without bias to final antibody format**
  - **cLC compatibility is maintained for any combination of lead antibodies**
  - **Affinity improvement goals achieved in a single round of mutagenesis and screening**
  - **≈2 months/campaign**
  - **Proprietary HEK bispecific display coupled with DMS-guided library design enables high throughput identification of optimal cLCs when additional fine tuning is necessary**
- **Further development and validation of cLC database to store and analyze data across campaigns:**
  - **Integration of NGS, binding, stability, structural data etc.**
  - **Implementation of lessons learned from the database for better in silico antibody design**