Discovery of CLIPS™ binders to anti-TNFα mAb's using Phage Display Libraries

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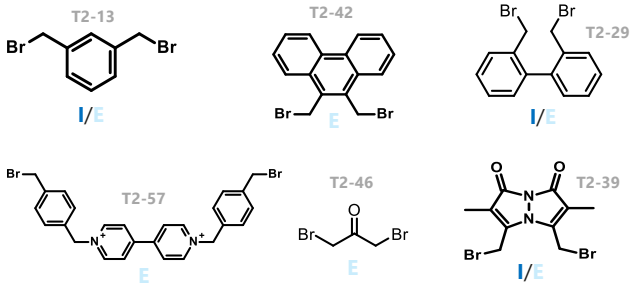
PEPSCAN

"When linear is not enough..."

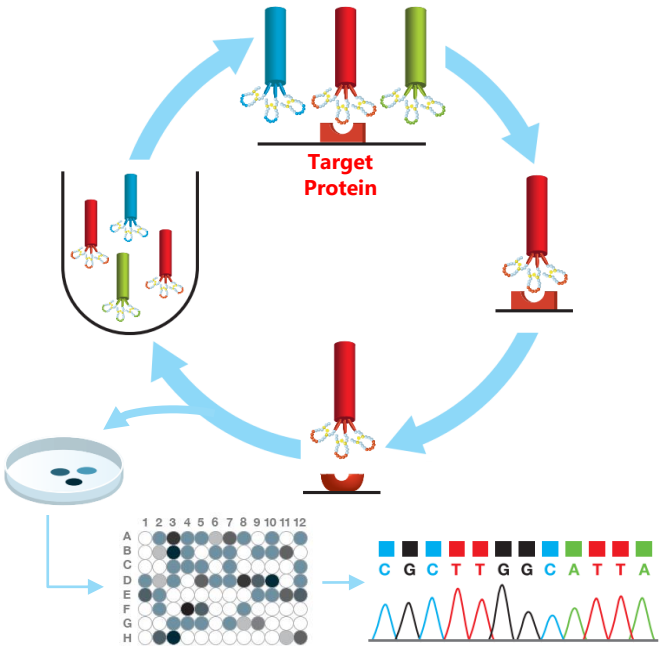
Intro

Mimotope peptides selected from combinatorial peptide libraries can be used as capture reagents for immunoassay detection of therapeutic monoclonal antibodies (mAbs).[1] Previous attempts by Abreos, independent of Pepscan, to identify peptide-binders from 7aa and 12aa linear peptides and C7C and 7C7C7 SS-looped peptide libraries did not yield any hits that showed reproducible binding in ELISA. Here we report the use of a monocyclic phage library encoding for a fully randomized 10-mer peptide sequence flanked by two cysteines that are linked via a CLIPS™ scaffold ($AC_{T2}XXXXXXXXXC_{T2}G$) to identify peptide binders (Veritopes™) that bind to Infliximab (brandname *Remicade*™), a therapeutic mAb indicated to treat autoimmune diseases like Rheumatoid Arthritis (RA) and Crohn's Disease.

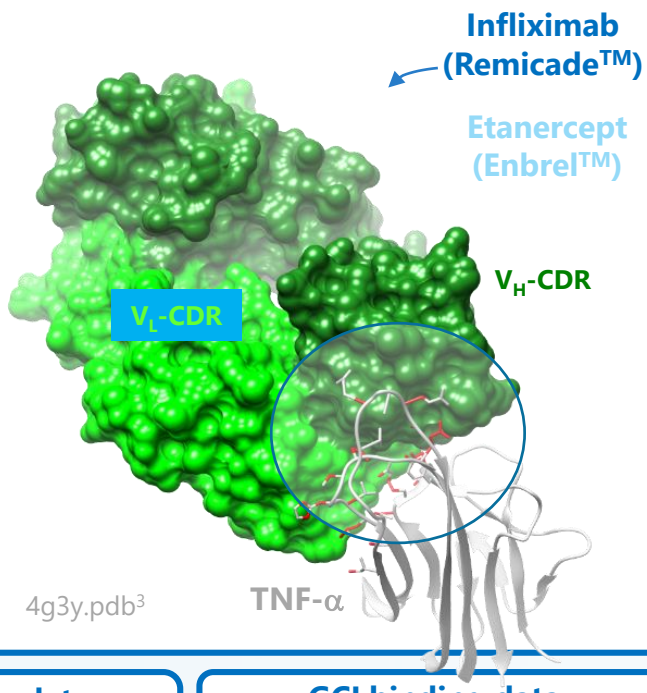
CLIPS™ scaffold Chemistry[2]



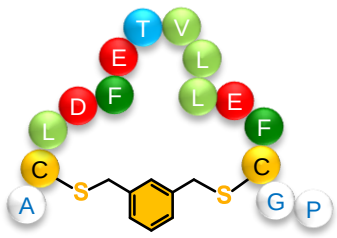
Methodology: Phage Display Screening



Target Binding Sites



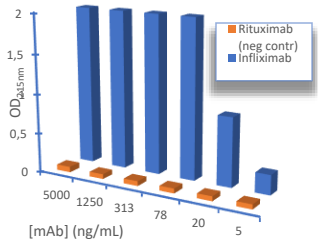
Best Lead Peptide



Sequence*	EC_{50} (ng/mL)
Lead (T2-13)	**35**
Lineair/SS-loop	>1000
Scrambled-1/2/3	>1000
Lead (T2-42/57)	>1000

**sequence was anonymized for confidentiality reasons*

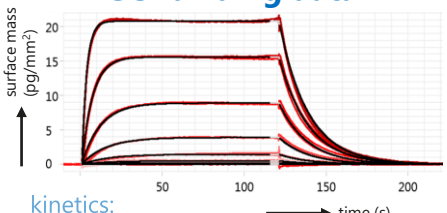
ELISA binding-data



EC_{50} **35 ng/mL**

Specificity **>10000**

GCI binding data



kinetics:

$k_a = 8.5 \times 10^4\ M^{-1}s^{-1}$

$k_d = 5.7 \times 10^{-2}\ s^{-1}$

K_d = **670 nM**

Conclusion: Successful identification of nM CLIPS-binder to Infliximab where other technologies failed sofar.

References: 1. Ruff LE *et al., Sci. Rep.* **2018**, *8*, 14473; 2. Timmerman P *et al. ChemBiochem* **2005**, *6*, 821-4; 3. Liang SY *et al. J. Biol. Chem.* **2013**, *288*, 13799-807.