A Novel Strategy to Engineer Agonists and Antagonists to Complex Membrane Targets Utilizing V(D)J Recombination in a Mammalian Cell

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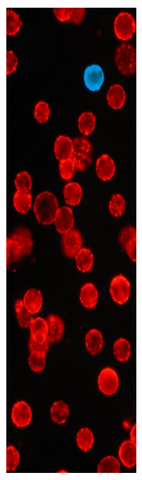
Abstract

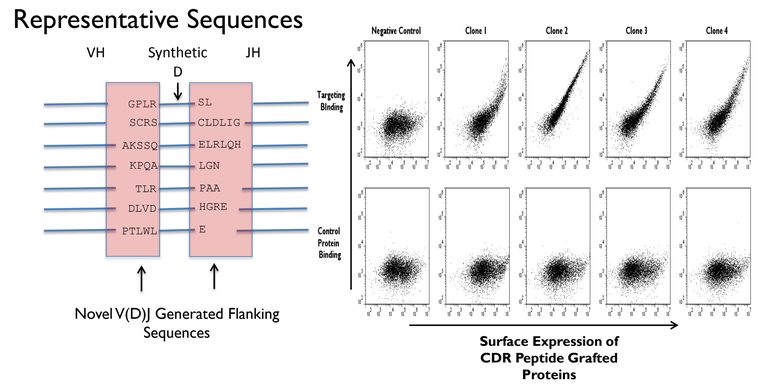
Although antibodies have been generated against numerous epitopes, there are important clinically relevant receptor classes, including GPCRs and ion channels, which have historically been difficult to target. Peptides show promise against these challenging targets but they often have limitations as therapeutics. We report the use of a mammalian display system to graft peptides with reactivity to complex membrane antigens into the CDRs of the full length human IgG scaffold. Characterization of a library of greater than 100 million peptide variants generated in the context of all the human heavy chain variable and human light chain variable gene segments is presented. The advantages of diversifying both the length and composition of peptide flanking sequences as well as strategies to isolate rare variants with the desired properties are described.

Technology Overview – HuTARG™



- Very high surface expression allows for efficient selection of antigen specific cells
- Libraries consist of all human V, (D) and J segments
- Antibodies expressed as full length IgG (no reformatting)
- Antibodies can be induced to secrete (1-5ug/ml, 3 day exhaust)
- Complex screens can be built into the cell line to allow for functional screening up front (receptor neutralization, species cross reactivity)

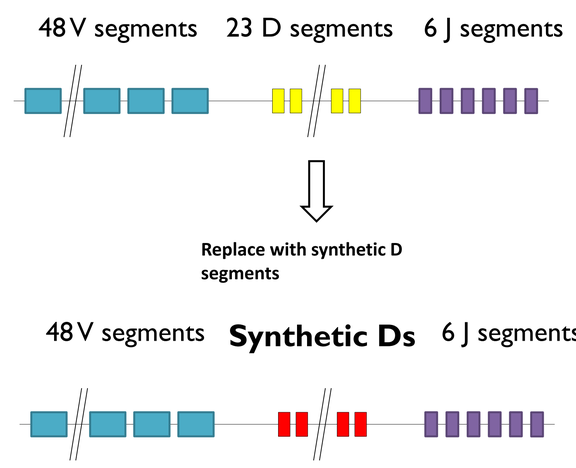
Synthetic D Example 1: (Undisclosed)



Conclusion:

Binding activity of grafted peptide is affected by conformational context determined by both length and composition of flanking sequences.

Peptide Grafting Approach



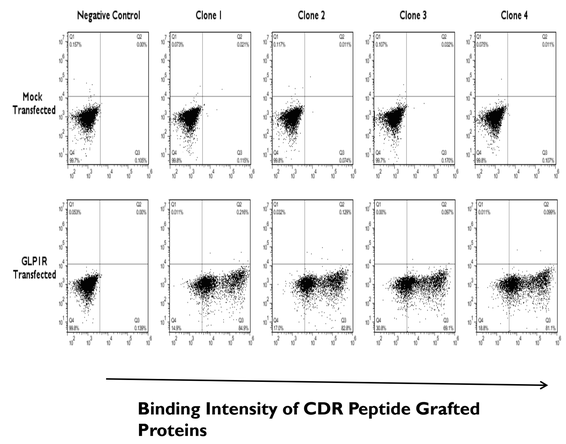
Synthetic D segments:

- Simultaneous diversification of V proximal and J proximal flanking sequences
- Simultaneous diversification of amino acid length and composition
- Optimal conformational context for function identified within the complete IgG scaffold
- Generation of very complex libraries within mammalian environment

V(D)J Recombination

Synthetic D Example 2: (GLP1R)

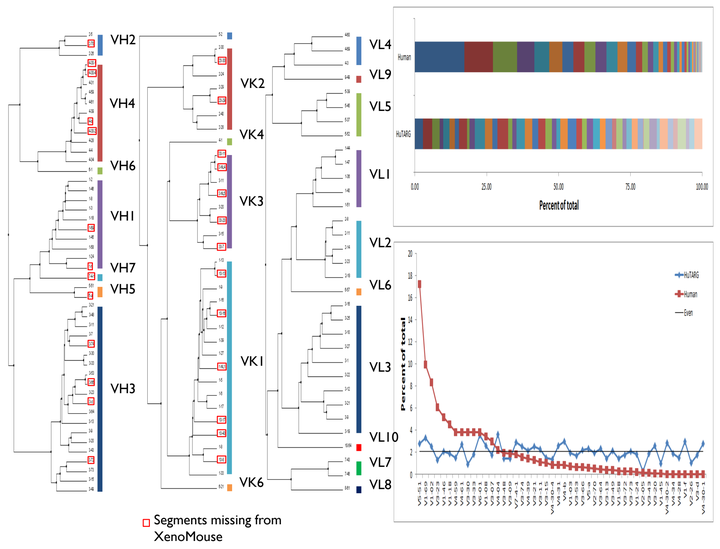
- Libraries of peptide grafted variants were generated within the context of the entire germline encoded antibody framework
- Selections were based on high mammalian expression of peptide grafted antibody and GLP1R binding
- Greater than 100 anti-GLP1R binding variants were generated
- Isolated clones exhibit strong binding to surface expressed receptor



Conclusion:

Peptide grafting within an antibody framework is an effective approach to target complex membrane targets such as GPCR's

VH Segment Utilization



- HuTARG™ repertoires have unbiased usage of VH segments
- In humans seven VH segments represent >50% of the repertoire
- Transgenic systems also have dramatic segment utilization biases
- Kappa and Lambda V gene usage is also unbiased (data not shown)

Synthetic D Options

- Anti-GPCR/Ion Channel:
  - Library consisting of synthetic D's derived from snake venom peptides. Peptides are small, with loop structure, ideal for CDR grafting
  - Functional ligands (i.e. GLP)
- Anti-Integrin: RGD containing peptides derived from integrin ligands
- Catabolic Antibodies: Incorporate synthetic antibody segments with histidine residues to increase frequency of pH sensitive binding.

Conclusion

The V(D)J reaction in vitro allows for the simultaneous diversification of both length and composition of flanking amino acids in the context of the entire VH and VK repertoire to find conformations that permit the functional grafting of peptides into CDRs of antibodies, allowing one to take advantage of peptide biology while at the same time retaining the intrinsic advantages of an antibody framework.

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