Converting Protein into a Bioactive Peptide: Discovery of IL-6 antagonist

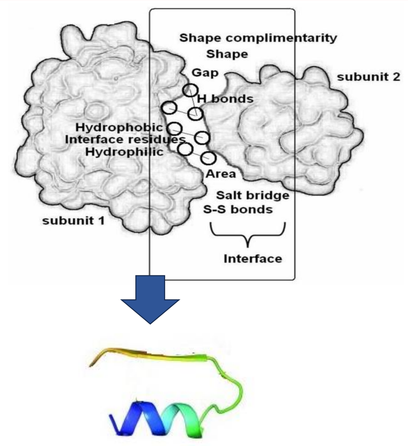
Alexander Pisarchik[1]*

1*Alcamena Stem Cell Therapeutics 1450 S. Rolling Rd., Suite 4.069, Halethorpe MD 21227
a.pisarchik@alcastem.com

ABSTRACT

We have developed a novel peptide discovery technology which we called PepFusion. It is based on simultaneous identification and optimization of the sequences critical for protein-protein interactions or ligand binding. We tested it by selecting peptide antagonists of interleukin-6 (IL-6), a key mediator of several inflammatory diseases. The PepFusion library demonstrated superiority over a random library by yielding a peptide with low micromolar affinity for IL-6, whereas the random library failed. The affinity of the lead peptide was improved through additional round of mutagenesis leading to peptide variants with low nanomolar affinity toward IL-6 as well as low nanomolar IC50 in cell-based assay.

Our Peptide Discovery Platform

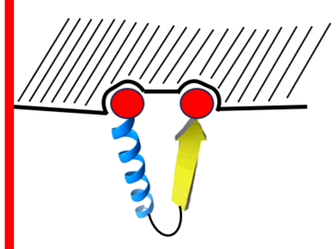


Exploit naturally occurring protein-protein interactions

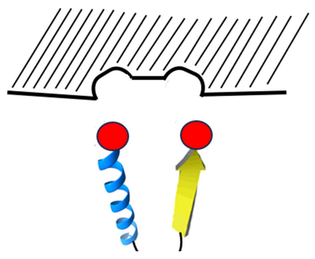
Identify and optimize of sequences involved in protein-protein interactions

Select a bioactive peptide drug candidate

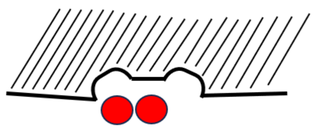
Original Interaction



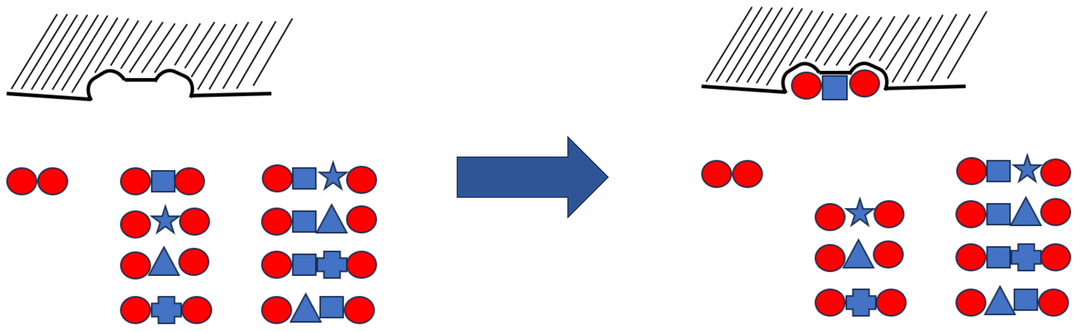
Option 1
Protein is split into peptides
NO INTERACTION



Option 2 (Rational Design)
Interacting residues are combined into a peptide
NO INTERACTION



OUR APPROACH



We build a specialized library that makes use of residues selected by nature
- Controlled degeneracy
- Insertions
- Deletions
- Substitutions

PERFECT BINDER

Why IL-6

IL-6 is proinflammatory cytokine playing a major role in many diseases:

- Arthritis (market size $74 billion)
- Crohn's disease (market size $11 billion)
- Castleman disease
- Psoriasis (market size $25 billion)
- Cytokine storm (e.g. in COVID-19) (market size $23 billion)
- Certain cancers
- Ageing

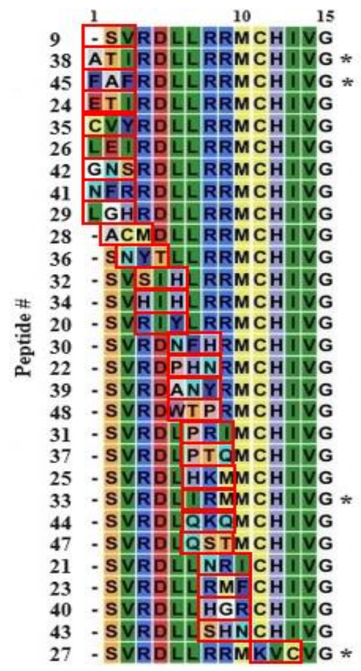
The global anti-inflammatory drugs market size is over $120 billion

RESULTS

1. Original Peptide: Peptide 9

SVRDLLRRMCHIVG KD 2.4 µM

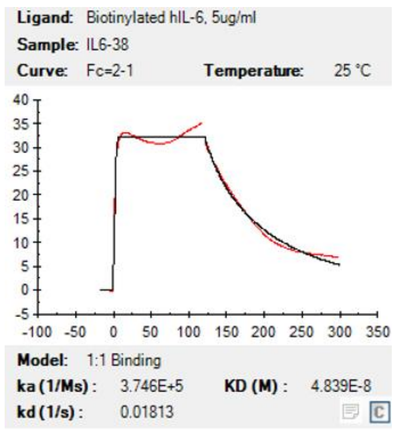
2. Peptide Optimization: Round 1

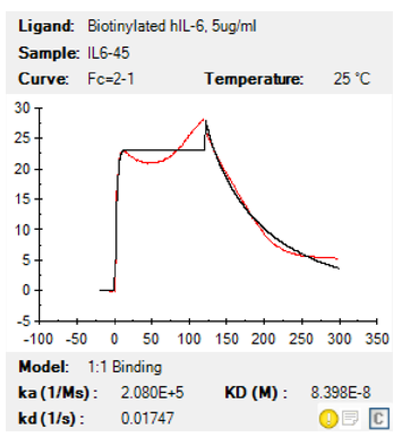


Our goal was not just to find beneficial mutation but to find synergistically interacting combinations

- A range of libraries created covering the whole peptide coding sequence
- 3 positions were mutated simultaneously
- Selection by mRNA Display

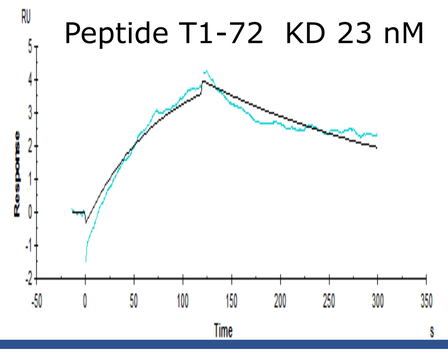
Affinity of Peptides T1-38 and T1-45





2. Peptide Optimization: Round 2 (combinatorial)

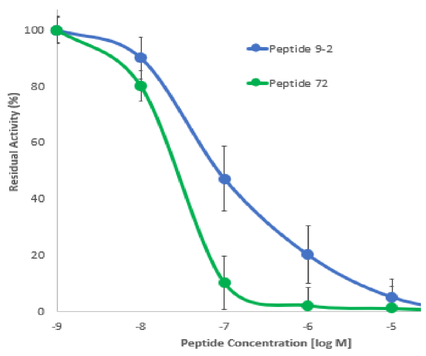
Peptide T1-72 KD 23 nM



Peptide variants were synthesized chemically

Affinity was measured by BiaCore

Inhibition of IL-6 Signaling in a Cell Culture Assay



Peptide 9 - original peptide discovered after 8 rounds of selection

Peptide 72 – optimized peptide
It inhibits IL-6 signaling in mammalian cell assay with IC50 23 nM

CONCLUSIONS

- We have developed an advanced method for identifying highly selective peptides
- We've demonstrated its capabilities by discovering and optimizing a bioactive peptide that effectively blocks the interaction of IL-6 with its receptor