Peptide libraries: applications, design options and considerations

Laura Geuss, PhD

May 5, 2015, 2:00-3:00 pm EST

GenScript
Make Research Easy

Overview

1. Introduction
2. Peptide library basics
3. Peptide library design considerations
4. Service summary and resources
5. Q&A

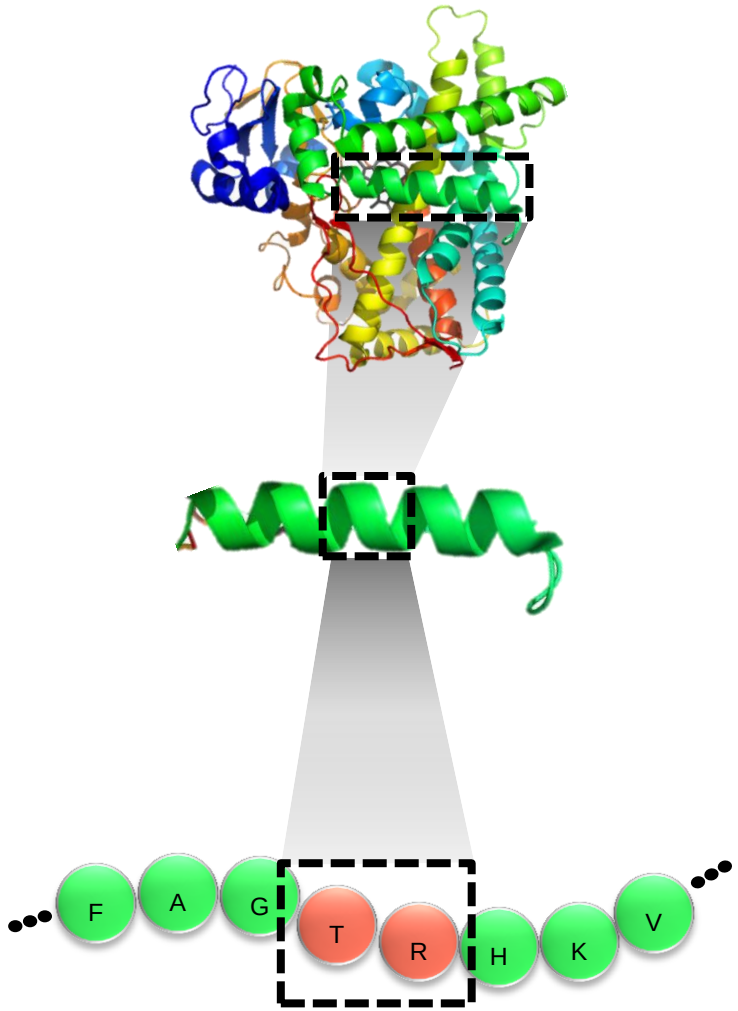
Challenges in protein research

- Problem:

  As progress continues to be made in drug discovery and development, we need higher-throughput and more accurate methods to discover important protein targets

- Solution

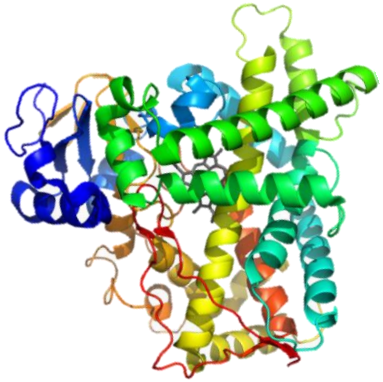
  By spanning entire sequences of these important epitopes, peptide libraries ensure no potentially important sequences are missed.



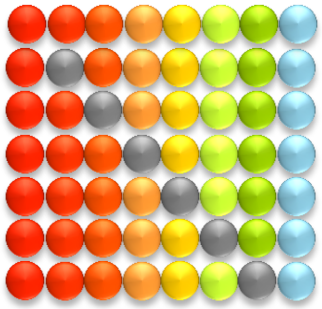
What is a peptide library?

- Peptide libraries contain a systematic combination of a large number of different peptides that represent important bioactive regions or epitopes on a protein

Determine sequence of bioactive protein region



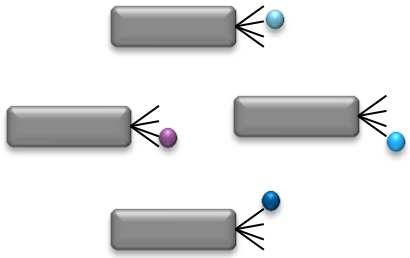
Choose a combination of amino acid sequences



Peptide sequences are aliquoted into multi-well plates

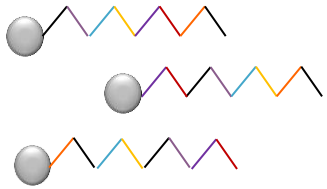
What is the difference between a phage-display and synthetic peptide library?

Phage-Display Peptide Library



- A library of phages that display peptides on their surface. Specific clones are selected based on binding affinity to a target
- Advantages: simple assay, not limited by peptide length.
- Disadvantages: labor intensive, limited to natural, L-amino acids

Synthetic Peptide Library



- Peptide sequences are synthesized by solid phase
- Allows for library design flexibility
- Advantages: can use D-amino acids, flexible design options. Less time needed to synthesize peptides in lab.

Synthetic Peptide Library options

	Library type	Features	Typical applications
	**Micro-scale peptide library**	0.2-0.5 mg, 5-20 AA	• Preliminary peptide screening • Proteomics • Mass Spectrometry
	**Purified peptide library**	1-4 mg, 5-25 AA	• Immune monitoring • Cell based assays • Drug discovery • Clinical trials
	**Crude peptide library**	1-20 mg, 5-25 AA	• Biomarker discovery • T cell binding assays

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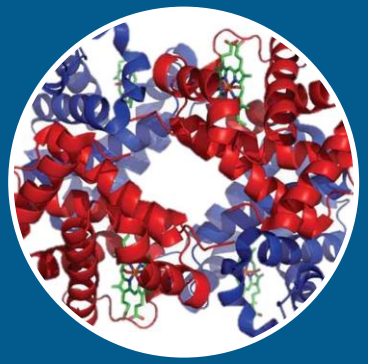
How do you design a peptide library?

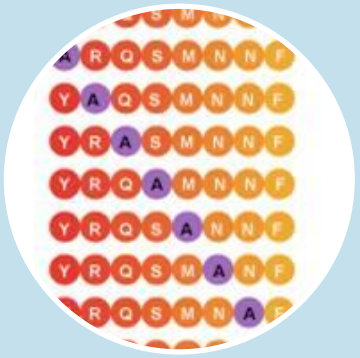


Identify the bioactive region you are interested in

- Online tools (ex: http://bioware.ucd.ie)
- Input UniProt ID, can identify most likely bioactive regions

Determine what peptide combinations to screen

- GenScript has free online tools to generate peptide library
- Input the bioactive region peptide sequence to generate peptide combinations



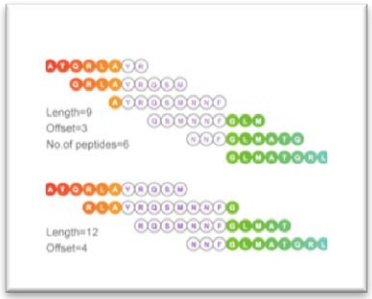
Choose the most appropriate library based on your end application

- Micro-scale peptide library or standard peptide library

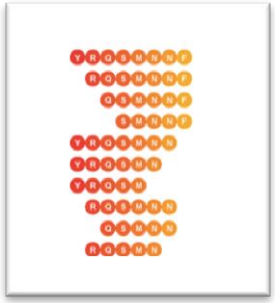
Peptide library design options

- In general, there are six common peptide library designs. The type you choose depends on the end application.

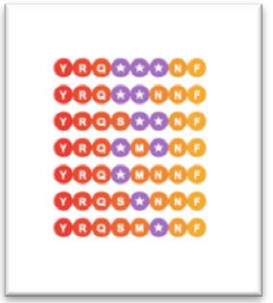
Overlapping Peptide Library



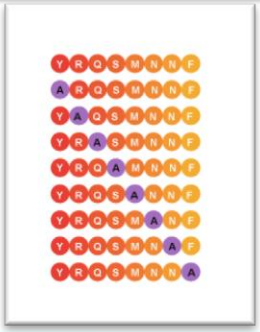
Truncation Peptide Library



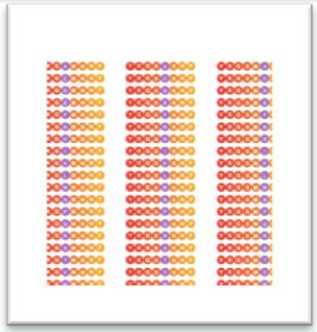
Random Peptide Library



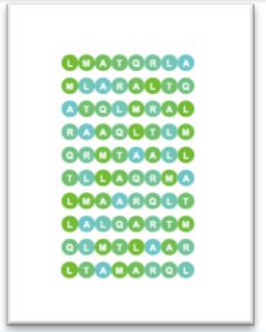
Alanine Scanning Peptide Library



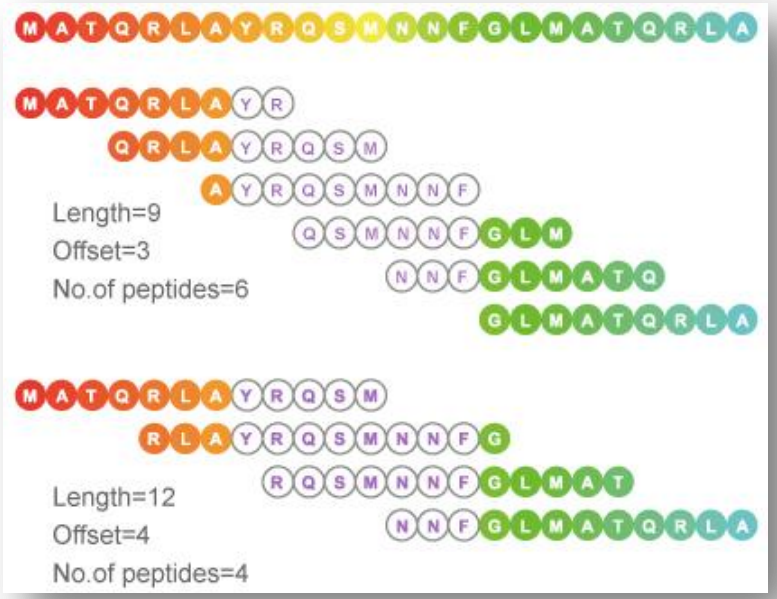
Positional Scanning Peptide Library



Scrambled Peptide Library



Option 1: Overlapping peptide library



Why it is useful

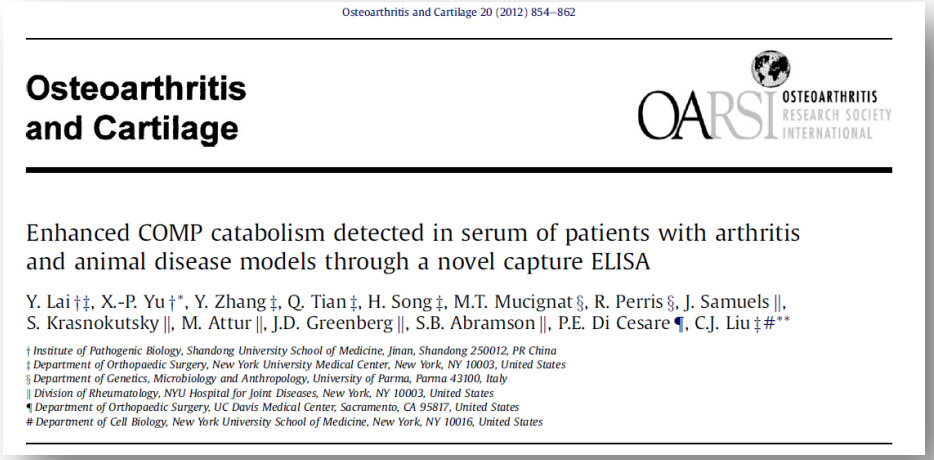
- Ideal for linear (or continuous) epitopes
- Span the entire epitope sequences

Research Applications

- Epitope Mapping: B and T cells
- Identify which sequences within the epitope are most important for activity

Case study: overlapping peptide libraries for ELISA design

- Background
  - There is no reliable biomarker to diagnose or predict the onset of osteoarthritis; however, the degradation products of cartilage oligomeric matrix protein (COMP) might serve as predictors for the disease.



Osteoarthritis and Cartilage 20 (2012) 854–862

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Enhanced COMP catabolism detected in serum of patients with arthritis and animal disease models through a novel capture ELISA

Y. Lai †‡, X.-P. Yu †*, Y. Zhang ‡, Q. Tian ‡, H. Song ‡, M.T. Mucignat §, R. Perris §, J. Samuels ‖, S. Krasnokutsky ‖, M. Attur ‖, J.D. Greenberg ‖, S.B. Abramson ‖, P.E. Di Cesare ¶, C.J. Liu ‡#**

† Institute of Pathogenic Biology, Shandong University School of Medicine, Jinan, Shandong 250012, PR China
‡ Department of Orthopaedic Surgery, New York University Medical Center, New York, NY 10003, United States
§ Department of Genetics, Microbiology and Anthropology, University of Parma, Parma 43100, Italy
‖ Division of Rheumatology, NYU Hospital for Joint Diseases, New York, NY 10003, United States
¶ Department of Orthopaedic Surgery, UC Davis Medical Center, Sacramento, CA 95817, United States
# Department of Cell Biology, New York University School of Medicine, New York, NY 10016, United States

- How was the library designed?
  - Overlapping peptides that cover the entire COMP epitope using GenScript's services
    - 51 biotinylated peptides
    - 15 AA long, 10 AA overlap

- What were the results?
  - Authors were able to identify the exact epitope of an anti-COMP mAb and consequently developed a novel ELISA to predict osteoarthritis

Lai *et al*. Osteoarthritis and Cartilage. 2012; 20(8): 854

Option 2: Alanine scanning library

Why it is useful

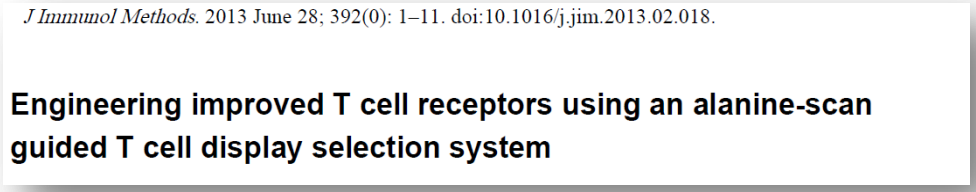
- Alanine, the smallest AA, is substituted in for each non-Ala residue in a peptide sequence
- In some cases, substitution of a key residue with Ala will cause changes in epitope binding activity
- Can quickly determine each of AA's contribution to peptide function

Research Applications

- Find protein binding sites and enzyme substrates
- Discover functional epitopes

Case study: Using alanine scanning to engineer T cells for immunotherapy

- Background
  - Naturally occurring T Cell receptors bind self (tumor) peptides with low affinity
  - Engineered T cells with higher MHC affinity may represent another immunotherapy option



- How was the library designed?
  - Each residue of the TCR region that mediates peptide specificity of T cell recognition was replaced with an Ala
  - Which residues are most important for the TCR-MHC binding interaction?

- What were the results?
  - Alanine substitutions to any region of the CDR3 region of the MHC significantly decreasing binding, identifying a key region that can be targeting for the discovery of immunotherapeutics

Malecek *et al*. Journal of immunological methods. 2013; 392(1-2): 1-11.

Option 3: Truncation peptide library

Why it is useful

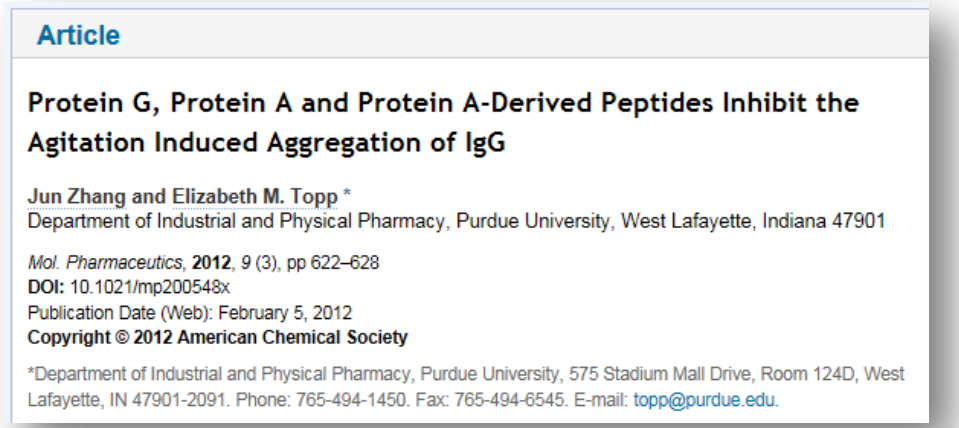
- Peptides are systematically truncated from the N and C terminus

Research Applications

- Identify the minimum peptide length for activity
- Allows you to identify the peptides that have enhanced proteolytic stability

Case study: Truncation libraries for better antibody design

- Background
  - Antibody-based drugs are widely used for drug development, but their potential to aggregate can results in life-threatening side-effects.
  - What are the mechanisms behind aggregation? How do proteins A and G contribute?



Article

Protein G, Protein A and Protein A-Derived Peptides Inhibit the Agitation Induced Aggregation of IgG

Jun Zhang and Elizabeth M. Topp *
Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, Indiana 47901

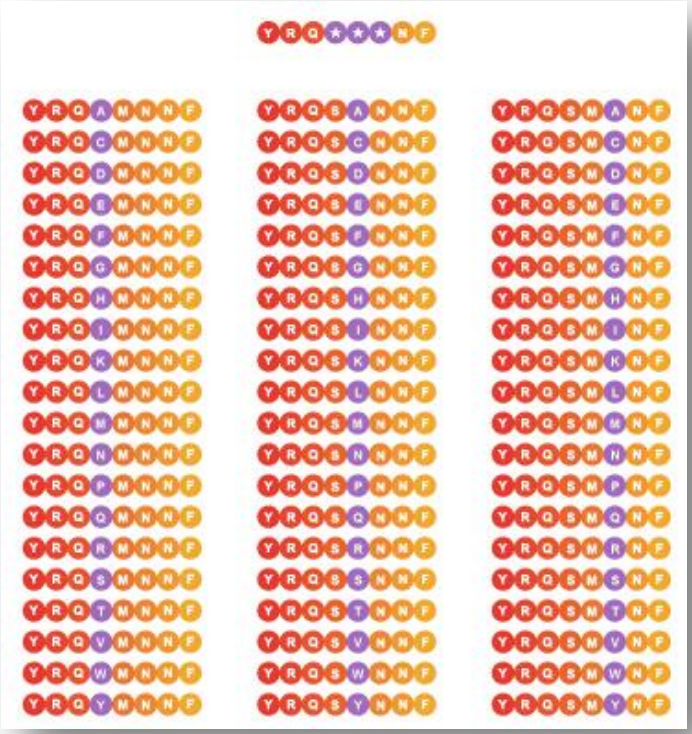
Mol. Pharmaceutics, **2012**, *9* (3), pp 622–628
DOI: 10.1021/mp200548x
Publication Date (Web): February 5, 2012

*Department of Industrial and Physical Pharmacy, Purdue University, 575 Stadium Mall Drive, Room 124D, West Lafayette, IN 47901-2091. Phone: 765-494-1450. Fax: 765-494-6545. E-mail: topp@purdue.edu.

- How was the library designed?
  - Peptides derived from protein A were sequentially truncated at GenScript to identify the peptide with the most dominant role in aggregation
- What were the results?
  - The authors identified protein A as being critical for mediating aggregation, providing a target for future antibody design

Zhang et al. Mol. Pharmaceutics. 2012; 9(3): 622-628.

Option 4: Positional Scanning library



Why it is useful

- AAs at a specific area of interest are systematically substituted with other natural AAs
- Can identify which AAs may enhance binding

Research Applications

- Peptide sequence optimization
- Identify T cell epitopes from complex mixtures of proteins

Case study: positional screening of peptides important for enzyme catalytic activity

- Background
  - What regions of an enzyme active site contribute the most to activity and specificity?
  - Sought to understand functional constraints of an enzyme, LHE, which is used as a genome-editing agent.

- How was the library designed?
  - Positional peptide library prepared at GenScript
  - AAs were substituted at specific positions in non-catalytic and catalytic regions

- What were the results?
  - The authors identified non-variant residues that are important for LHE activity

McMurrough et al. PNAS. 2014; 111(23): E2376-E2383.

Option 5: Random peptide library

Why it is useful

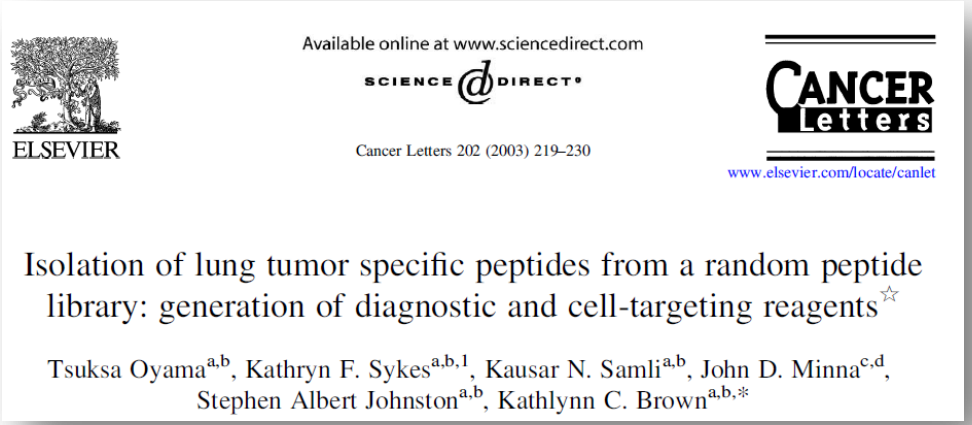
- Randomly and simultaneously generated variations with all other AA via a shotgun approach
- Can potentially generate peptides that have enhanced activity for a specific function

Research Applications

- Identify highly active and novel peptide sequences
- Peptide sequence optimization

Case study: random peptide library for tumor cell ligand screening

- Background
  - Targeted tumor treatment first requires identification of a specific, identifying ligand on the tumor cell.
  - The authors sought to isolate peptides that bound specifically to lung cancer cells.



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Cancer Letters 202 (2003) 219–230

CANCER Letters

www.elsevier.com/locate/canlet

Isolation of lung tumor specific peptides from a random peptide library: generation of diagnostic and cell-targeting reagents☆

Tsuksa Oyama[a,b], Kathryn F. Sykes[a,b,1], Kausar N. Samli[a,b], John D. Minna[c,d], Stephen Albert Johnston[a,b], Kathlynn C. Brown[a,b,*]

- How was the library designed?
  - Used a phage display approach to create a library of cell-targeting peptides
- What were the results?
  - The authors were able to identify peptide sequences with high binding affinity to specific tumor cancer cells, which presents a potential target for downstream drug design.

Oyama *et al*. Cancer Letters. 2003; 202: 219-230.

Option 6: Scrambled peptide library

Why it is useful

- Provides the highest level of variation

Research Applications

- Create the ideal scenario for peptide sequence optimization
- Probe target molecules of interest including proteins, antibodies and DNA

Case study: scrambled libraries for finding peptide mimics

- Background
  - The carbohydrate L2/HNK-1 is an important neural recognition molecule involved in many neural cell interactions.
  - Further study of its biological role is important, but limited natural availability makes this difficult: finding a peptide mimic will make this possible

Journal of Neurochemistry, 2002, **83**, 1380–1388

Identification of a peptide mimic of the L2/HNK-1 carbohydrate epitope

Maryline Simon-Haldi,* Ned Mantei,*,[1] Jens Franke,† Hans Voshol‡ and Melitta Schachner†

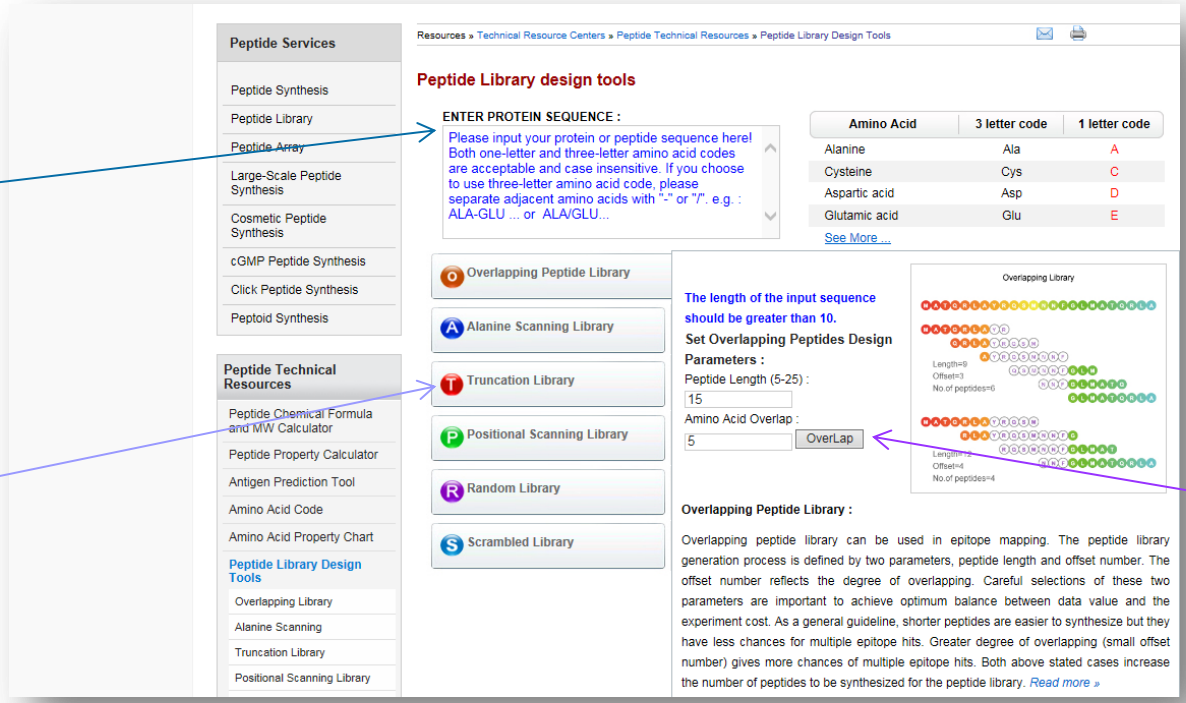
*Department of Neurobiology, Swiss Federal Institute of Technology, Hönggerberg, Zürich, Switzerland
†Zentrum für Molekulare Neurobiologie, Universität Hamburg, Hamburg, Germany
‡Novartis Pharma, Functional Genomics Area, Basel, Switzerland

- How was the library designed?
  - 15-mer candidate peptides were generated with a random, phage-display library.
- What were the results?
  - The authors isolated the peptides that bound to anti-L2/HNK-1 antibodies, opening up the open for future studies with the carbohydrate.

Simon-Haldi *et al*. J of Neurochem. 2002; 83: 1380-1388.

GenScript's free online design tool

www.genscript.com/peptide_screening_tools



Step 1

Step 2

Step 3

Input your protein sequence → Choose your library design → Input the parameters → Click the quote button to get a quote for the selected sequences

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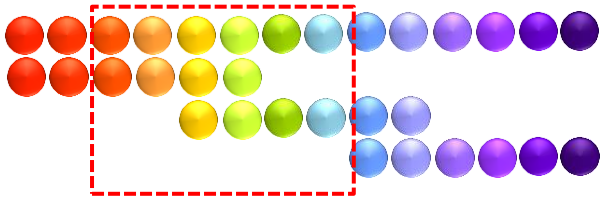
Important design considerations: offset number

- When designing overlapping peptide libraries, consider the offset number

  - Libraries with longer offset numbers will cost less, but the chance of missing important AA combinations increases

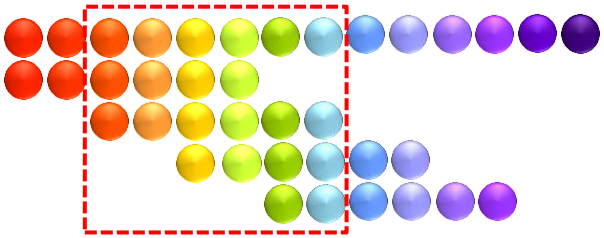
  - Choose shorter offset numbers (1/3 the peptide length) to ensure you make the most of your library

Length = 6
Offset = 4



- Fewer peptides
- Fewer possible epitopes

Length = 6
Offset = 2



- More peptides
- More possible epitopes

Important design considerations: hydrophobicity

- Highly hydrophobic residues are difficult to purify and also difficult to solubilize.

- If your library includes hydrophobic peptides, consider:
  - Choosing a lower purity;
  - Substituting the hydrophobic residues;

• Tryptophan • Isoleucine • Leucine • Phenylalanine • Methionine • Valine • Tyrosine	Replace with: →	• Glutamic Acid • Aspartic Acid • Lysine • Arginine • Histidine

  - Choose different peptide sequences.

Important design considerations: reaction monitoring with Mass Spec

- If you are designing a micro-scale peptide library to be combined with MS for reaction monitoring, choose:
  - Peptide lengths around 10 AA
  - No short hydrophilic or long hydrophobic sequences
  - No residues that are prone to oxidation (such as Methionine or Tryptophan)

Review: Picotti et al. Nature Methods. 2012. 9(6): 555.

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Peptide library summary

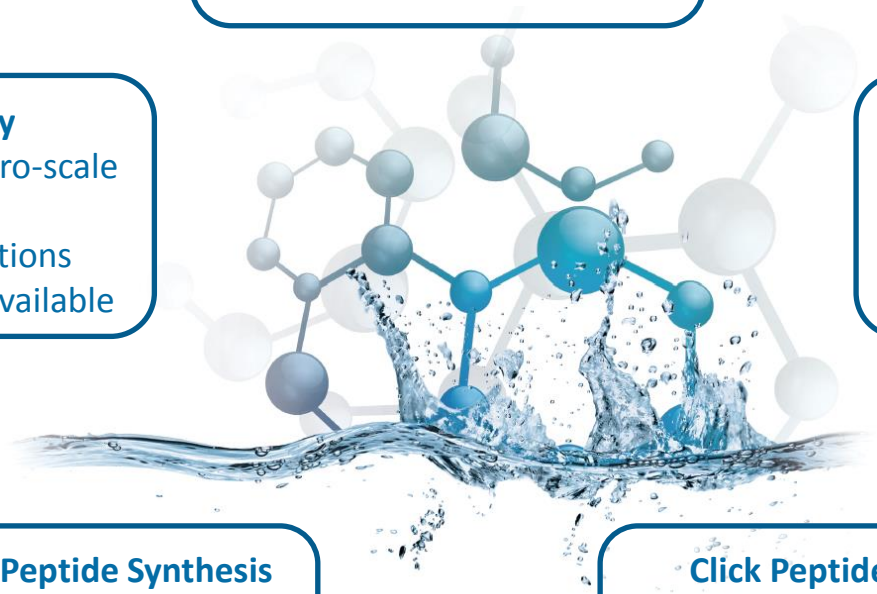
	Standard Crude Peptide Library	Standard Purified Peptide Library	Micro-scale Peptide Library
Quantity	1-20 mg	1-4 mg	0.2-0.5 mg
Peptide Length	5-25 AA	5-25 AA	5-20 AA
Purification	~ >20-30%	>70%, >75%, >80%, >85%, >90%, >95%, >98%	Crude or >70%
QC	MS only; HPLC, MS for each peptide	COA, HPLC and MS for each peptide	COA, HPLC and MS for each peptide; Analytical HPLC option
Additional options	TFA removal service Extensive modification options		
Key applications	Epitope discovery, T-cell assays	Drug development, immune monitoring, preclinical trials	Biomarker discovery Proteomics Reaction monitoring

www.genscript.com/peptide-library.html

Peptide services at GenScript

Standard peptide synthesis
- Starting at $3.2/AA
- Up to 200 AA
- Mg to kg quantity

Peptide Library
- Standard and micro-scale quantity
- Flexible purity options
- Peptide pooling available



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- Increased peptide stability

www.genscript.com/peptide-services.html

Thank you for your participation
We wish you success with your research
Email me: Laura.Geuss@genscript.com

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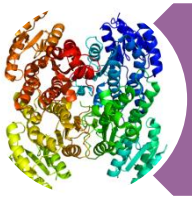

Chaperone co-expression strategies for recombinant soluble protein production in *E.coli – Bo Wu Ph.D*

May 12, 2015, 2:00 pm EST

Analyzing antibody sequences for recombinant antibody expression – *Hangxing Yu, Ph.D*

May 20, 2015, 9:00 am EST

Expression vectors: how to choose, or customize, vectors for gene & protein expression – *Rachel Speer, Ph.D*

June 3, 2015, 11:00 am EST

Q&A

If you have any other questions, visit www.genscript.com/faq_for_peptide
Or email: Laura.Geuss@genscript.com