Peptide Design Strategy: Basics, Optimization, and Application

Presented by:
Tiffany Gupton Campolongo, Ph.D.

GenScript
Make Research Easy

Presentation overview

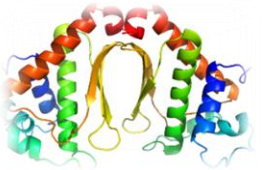
1. Introduction
2. Peptide Design Basics
3. Advanced Design Strategy
4. Strategy and Service Summary

Why design custom peptides?

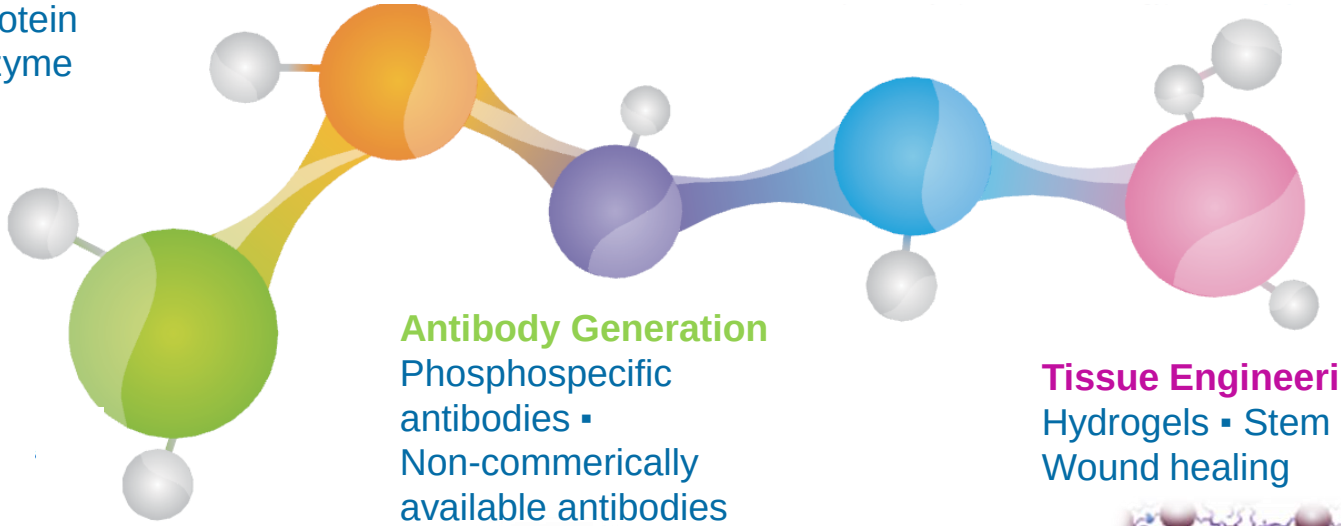
Applications of custom peptides

Drug Discovery
Antimicrobials, cancer (GPCR agonists) ▪ diabetes (GIP and GLP-1 agonists) ▪ Neurodegenerative disease (beta amyloid inhbitors)

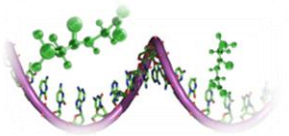
Vaccine Development
HIV ▪ Cancer ▪ Influenza ▪ HPV



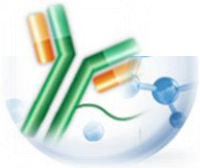
Structural Biology
NMR ▪ Protein-protein interactions ▪ Enzyme assays



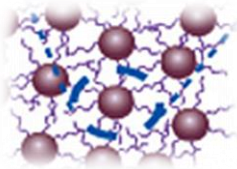
Drug Delivery
siRNA delivery



Antibody Generation
Phosphospecific antibodies ▪ Non-commerically available antibodies



Tissue Engineering
Hydrogels ▪ Stem cells ▪ Wound healing

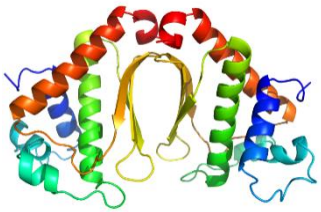


Why design custom peptides?

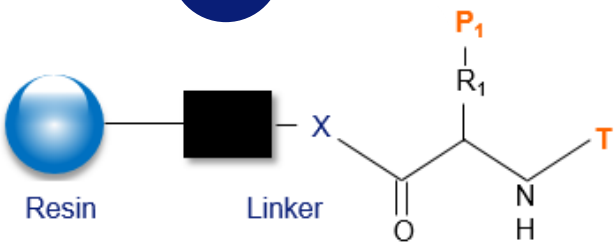
Design considerations are dependent on:

1. Biochemistry
2. Chemical process of peptide synthesis
3. Application

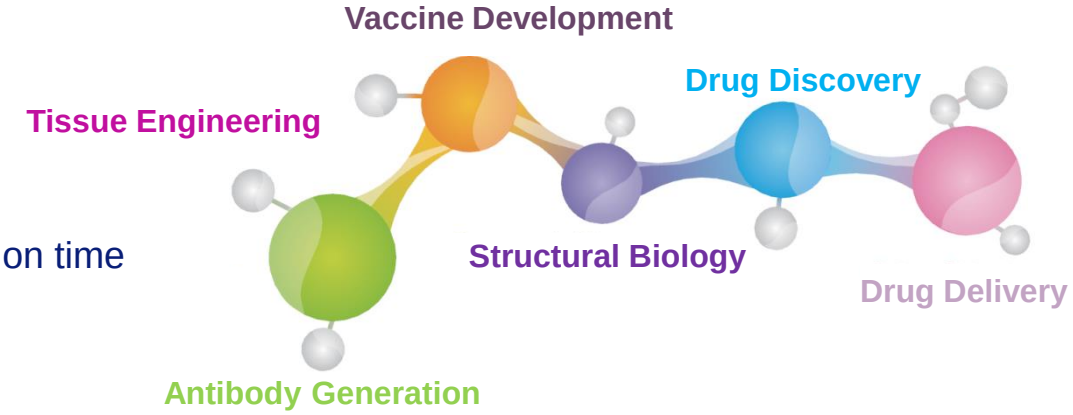
1



2



3



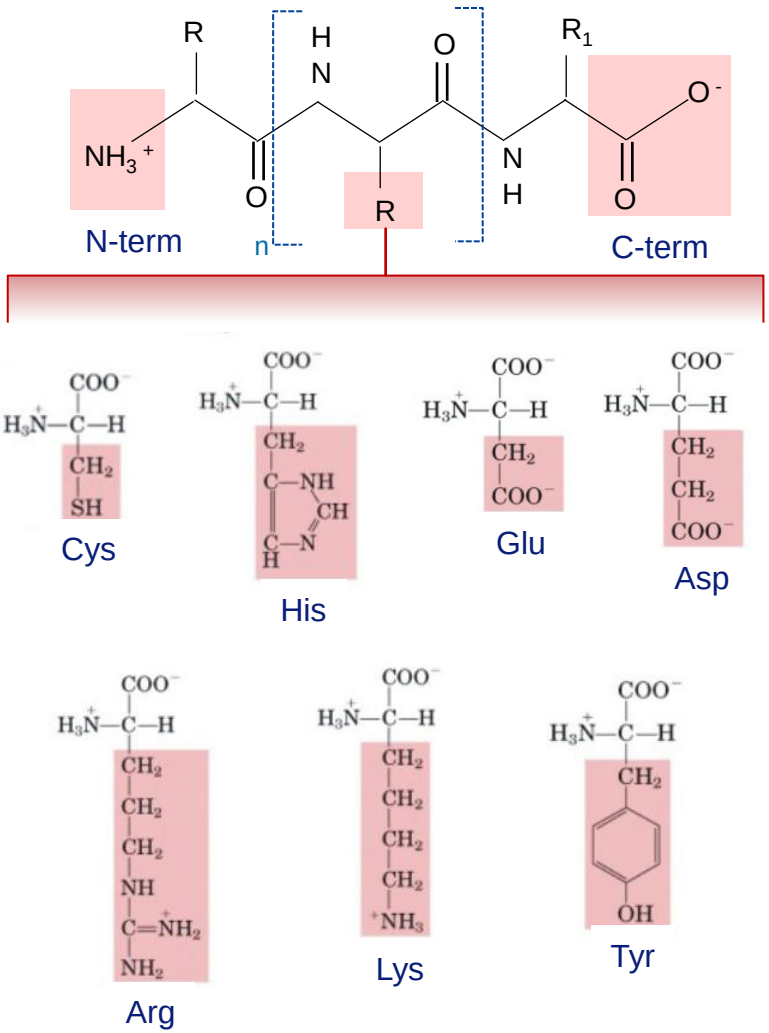
Survey Results

1. Designing novel effective sequences
2. Designing sequences that can be delivered on time
3. Avoiding hydrophobicity
4. Choosing the right modification

Peptide Design Basics

Peptide design basics: charge

- **Charge influences:**
  - Solubility
  - Peptide activity
  - Attraction to contaminants
- **Charge is dependent on ionizable groups:**
  - N-term amine, C-term carboxyl
  - R-groups: Asp, Glu, His, Cys, Tyr, Lys, Arg
- **Key solubility relationship: pH/pI**
  - pH = pI: minimal solubility, precipitation
  - pH < pI: net positive charge
  - pH > pI: net negative charge
- **Key peptide activity relationship**
  - N or C-term charges
- **Key contamination relationships**
  - Lys, His, Arg bind TFA
  - Lys, Arg bind water molecules
  - Tyr, Glu bind protective groups



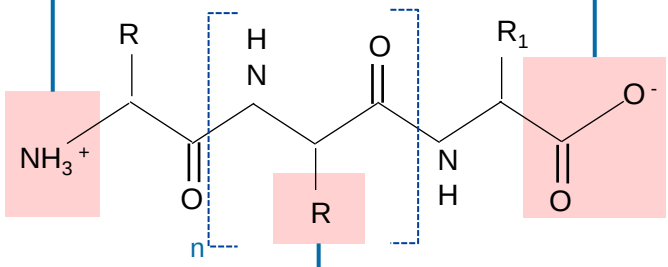
Peptide design basics: charge

Calculate net charge or pI

- By hand:
  - http://www.genscript.com/amino_acid_structure.html
- Using the net peptide calculator:
  - https://www.genscript.com/ssl-bin/site2/peptide_calculation.cgi

Modify N or C terminal to remove charges

- e.g. capping: acetylation or amidation



Make changes to peptide sequence

- Change non-essential amino acids

Peptide design basics: hydrophobicity

- Hydrophobic peptides are
  - >5 AA long
  - containing >50% hydrophobic amino acids

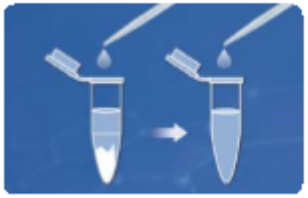
- Avoid hydrophobicity by replacing non-essential hydrophobic amino acids with charged or polar residues.

▪ Residue substitutions

Replace:	With:
▪ Tryptophan ▪ Isoleucine ▪ Leucine ▪ Phenylalanine ▪ Methionine ▪ Valine ▪ Tyrosine	▪ Aspartic acid ▪ Lysine ▪ Arginine ▪ Histidine ▪ Glutamic Acid

Solubility testing service

- **Process:** Custom peptide is tested in multiple solvents at varying pH
- **Deliverable:** Custom solubility report
- **Price:** Free upon request



Peptide Solubility Test Report

Solvent[1]		pH Value	Results[1,2] (Dissolvde or Undissolved)	Highest Gross Peptide Concentation
Type1	ultrapure water	N/A	**Dissolved**	10 mg/ml
	0.1% acetic acid solution	N/A	N/A	N/A
	3% ammonia water	N/A	N/A	N/A
	0.1 M PBS*	7.40	**Dissolved**	10 mg/ml

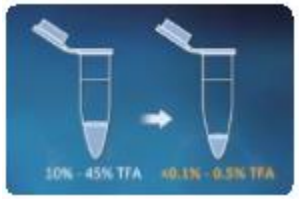
Learn more at: http://www.genscript.com/peptide_solubility_testing.html

Request your solubility test via our instant online quotation system:

Peptide Services › Peptide Synthesis › Get a Quote Now Via Secured Online Quotation

Guaranteed TFA removal service

- **Process:** TFA is exchanged for another salt via proprietary counter-ion exchange protocol
- **Deliverable:** TFA content report



TFA Removal Packages

Service type	Final TFA counterion %		
	HCl	formate	acetate
Guaranteed	< 0.1 %	< 0.5 %	< 0.5 %
Standard	TFA counterion % not guaranteed		

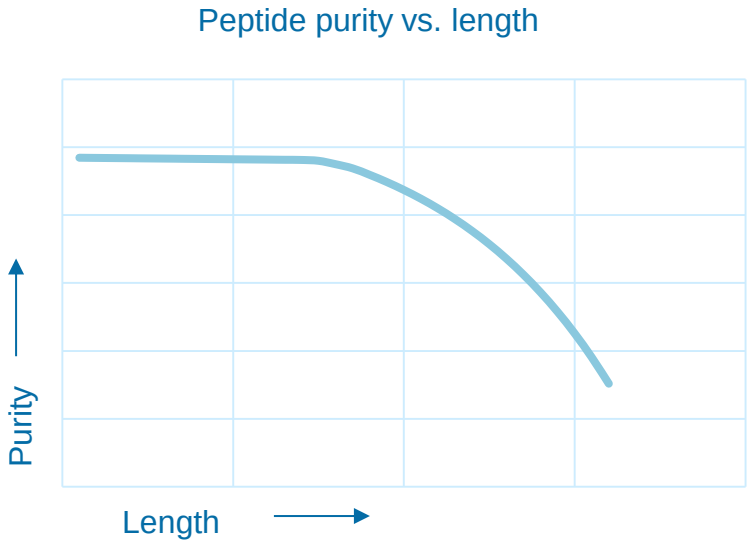
Learn more at: http://www.genscript.com/tfa_removal_service.html

- **Recommended for:**
  - Peptides that will be used in cellular assays
  - Peptides that will be used as APIs or in manufactured products
  - Hydrophilic peptides containing numerous basic residues

Request your solubility test via our instant online quotation system:

Peptide Services › Peptide Synthesis › Get a Quote Now Via Secured Online Quotation

Peptide design basics: length

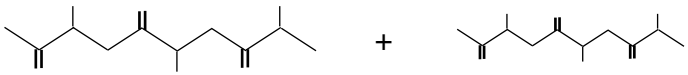


Optimal Peptide length: 15 AA

Long peptide synthesis technologies

GenScript Technologies

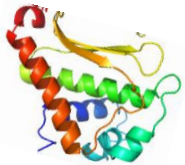
Proprietary Ligation Technology



- Couples smaller peptides together to make larger ones
- For long peptides requiring modifications, non-natural amino acids

Learn more at: http://www.genscript.com/peptide_tech.htm

Recombinant Peptide Synthesis



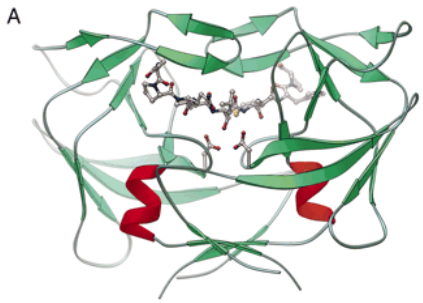
- Powered by recombinant protein expression
- For peptides longer than 150 AA

Learn more at: http://www.genscript.com/recombinant_pep.html

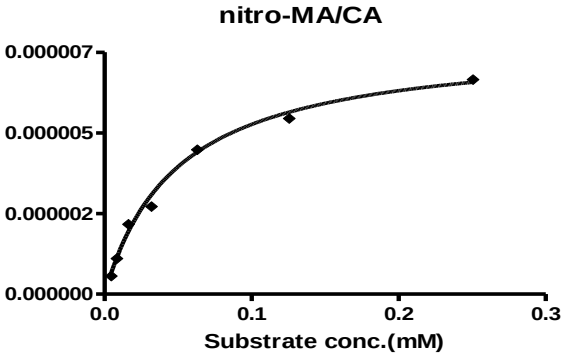
Case study: long peptide synthesis

About the sequence:

Human T-cell Leukemia Virus 1 Protease (HTLV PR), a 126 amino acid sequence having complex secondary structure.



Peptide structure



Activity assay

Kcat: 0.16 ± 0.011 s^{-1}
Km: 0.053 ± 0.01 mM
Kcat / Km: 3.02 $s^{-1}mM^{-1}$

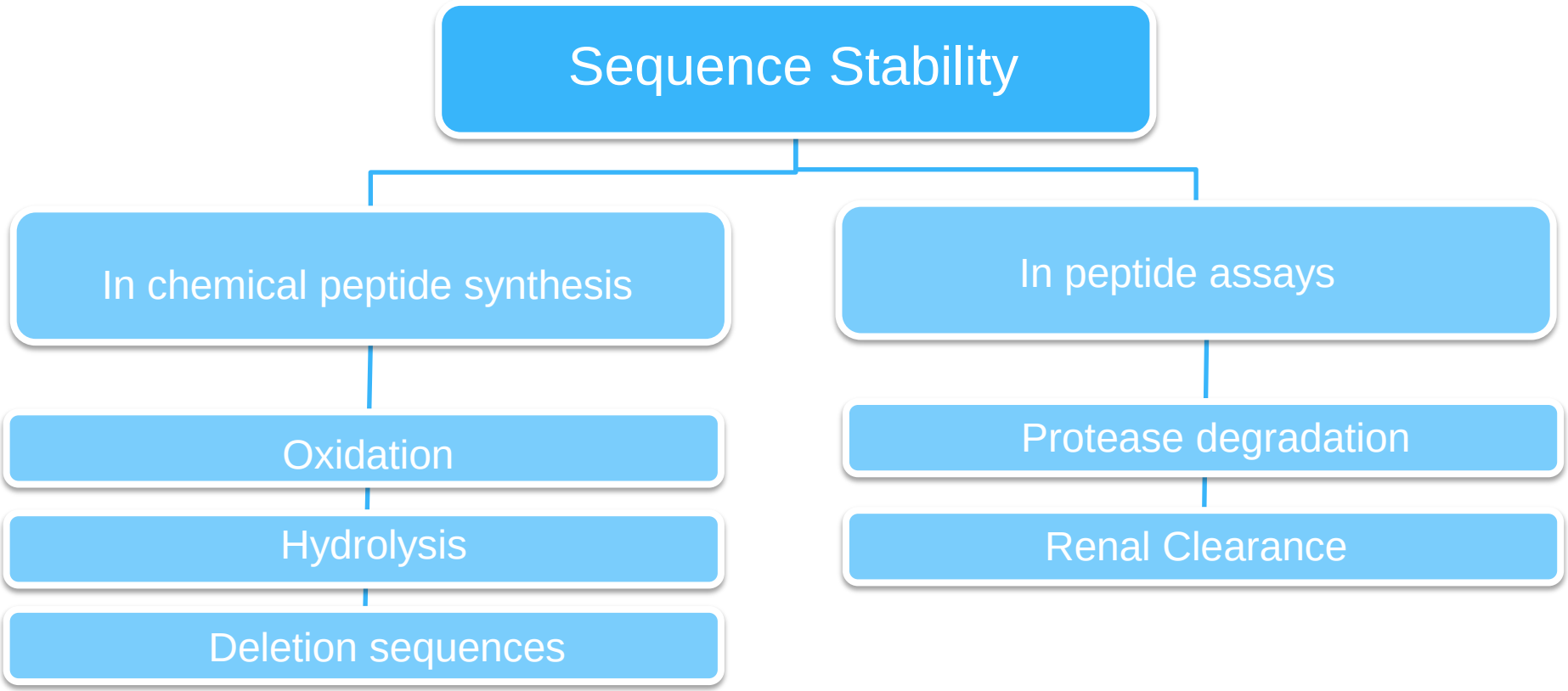
Design by application: purity selection

Peptide Grade	Purity	Application
Immunograde	70%	• ELISA testing • Peptide arrays • Antigens for polyclonal antibody production or affinity purification
Biochemistry Grade	85%	• NMR studies • Epitope mapping • Phosphorylation studies • Peptide blocking studies for Western Blot • Cell attachment studies
High Purity Grade	95%	• SAR studies • Quantitative receptor-ligand interactions studies • Quantitative blocking and competitive inhibition studies • Quantitative phosphorylation studies • Quantitative proteolysis studies • In vitro bioassays • In vitro studies
High Purity Grade	98%	• Crystallography • cGMP peptides for drug studies • Cosmetic peptides for cosmeceuticals • Clinical trials

http://www.genscript.com/recommended_peptide_purity.html

Advanced Design Strategy

Advanced design strategy: sequence stability



Advanced design strategy: sequence stability

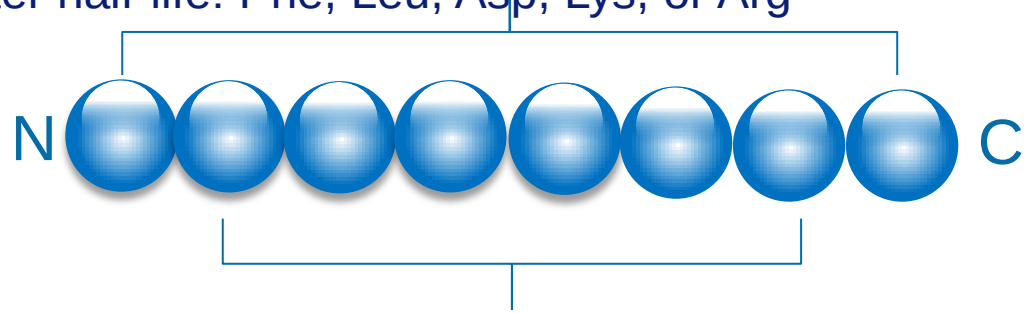
In peptide synthesis

Design Constraint	Susceptible amino acids & Sequence	Strategies to increase stability
Cyclization	▪ Asp-Gly ▪ N-terminal Glu	▪ Substitute Asp with other amino acids ▪ Acetylate N-terminal Glu
Secondary structure formation	Multiple Glu, Ile, Leu, Phe, Thr, Tyr, Val	▪ Asp for Glu ▪ Ser for Thr ▪ Pro or Gly every third residue
Oxidation	▪ Cys ▪ Met	▪ Cysteine with Serine ▪ Methionine with Norleucine
Hydrolysis	▪ Asp-Gly ▪ Asp-Pro ▪ Asp-Ser	Substitute Asp with other amino acids
Sequence deletions	Multiple adjacent Ser	Substitute Ser with other amino acids

Advanced design strategy: sequence stability

Protease/peptidase degradation

- **Types of proteases:** exopeptidases, (e.g. aminopeptidases, carboxypeptidases)
- **N-terminal residues correlation:**
  - Longer half-life: Met, Ser, Ala, Thr, Val, or Gly
  - Shorter half-life: Phe, Leu, Asp, Lys, or Arg



- **Types of proteases:** endopeptidases (e.g. trypsin, chymotrypsin, pepsin, elastase)
- **Susceptible domains:** Pro, Glu, Ser, and Thr rich

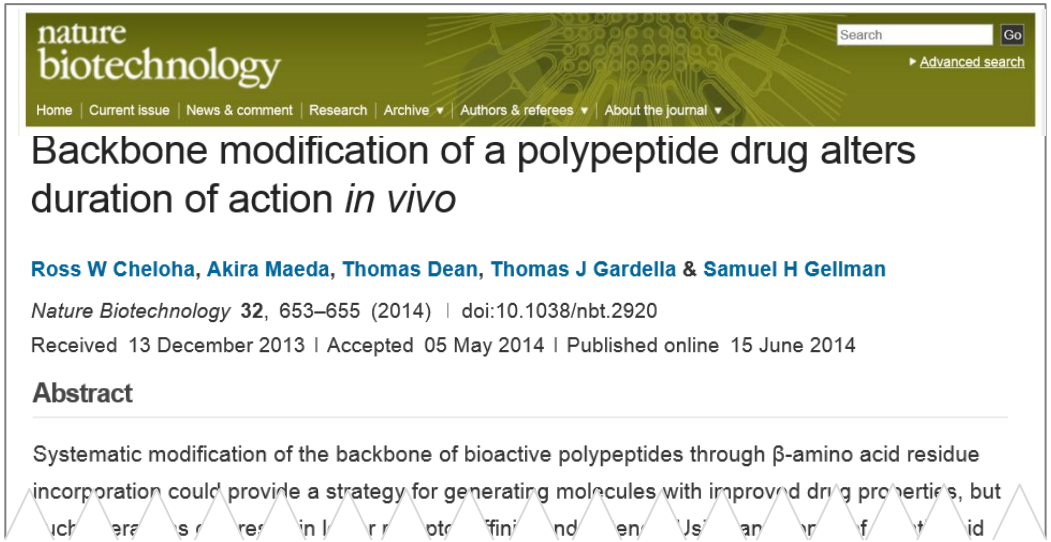
http://web.expasy.org/peptide_cutter/

Advanced design strategy: sequence stability

Protease/peptidase degradation

- Strategies to reduce degradation
  - Cyclization
  - Acetylation
  - Amidation
  - D-amino acid replacement
  - Peptoids
  - Hydrocarbon stapling

Case study: β-amino acid incorporation



nature biotechnology

Search Go

Advanced search

Home | Current issue | News & comment | Research | Archive | Authors & referees | About the journal

Backbone modification of a polypeptide drug alters duration of action *in vivo*

Ross W Cheloha, Akira Maeda, Thomas Dean, Thomas J Gardella & Samuel H Gellman

Nature Biotechnology **32**, 653–655 (2014) | doi:10.1038/nbt.2920

Received 13 December 2013 | Accepted 05 May 2014 | Published online 15 June 2014

Abstract

Systematic modification of the backbone of bioactive polypeptides through β-amino acid residue incorporation could provide a strategy for generating molecules with improved drug properties, but

The Design:

- Incorporated β-residues into parathyroid hormone receptor (PTH) - every fourth residue
  - Incorporates CH_2 residues into backbone, but maintains native sequence sidechains
  - Successfully mimics α-helix

NLGKWLNSMERVEWLRKKLQDVHNF

The Test:

- 6 PTH mimetics were compared to native PTH using a PTHR1 signaling assays
  - Tested in HEK293 cells expressing PTHR1 and rats
  - Monitored cGMP or $Ca2^+$ levels

The Result:

- PTH mimetic was considerably more potent that native PTH, presumably due to increased stability and half-life.
  - Raised calcium levels higher
  - Persisted longer *in vivo*

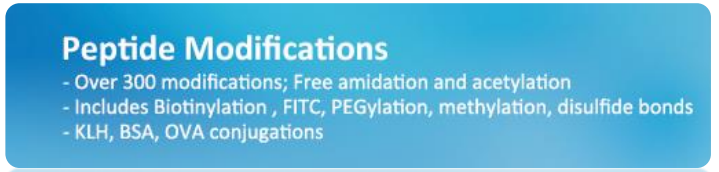
Case studies: D-amino acid replacement

Replacement of select residues

- Vasopressin:
  - Normal half-life: 10–35 min
  - Half-life with single L-Arg to D-Arg change: 3.7 hrs

Replacement of all residues (Mirror image peptides)

- Antiarrhythimic – Rotigapeptide
- HIV (PIE12-trimer) - Navigen
- Ebola D-peptide inhibitors - Navigen

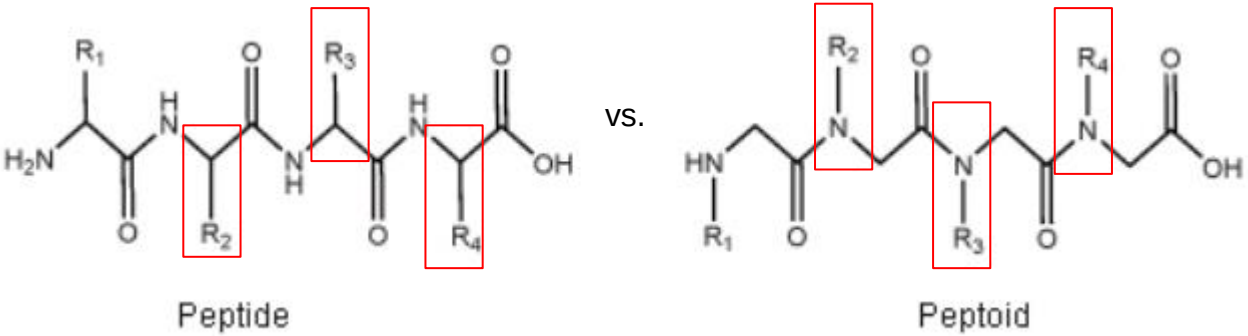


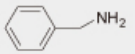
http://www.genscript.com/peptide_modification.html

Peptoid service

- Structure: R-groups are attached to nitrogen atoms instead of α-carbon (called poly-*N*-substituted glycines)

- Advantage: Increased stability
  - Protease resistant
  - Denaturation resistant



Side Chain Name	Structural Formula
Methylamine	H_3C-NH_2
Ethylamine	$H_3C-C(H_2)-NH_2$
n-propylamine	$H_3C-C(H_2)-CH_2NH_2$
Isopropylamine	$H_3C-CH(CH_3)-NH_2$
n-butylamine	$H_3C-C(H_2)-C(H_2)-C(H_2)-NH_2$
Sec-butylamine	$H_3C-C(H_2)-C(H)(NH_2)-CH_3$
Isobutylamine	$H_3C-C(H)(CH_3)-C(H_2)-NH_2$
Benzylamine	
β-phenylethylamine	

- Modifications: acetylation, amidation, Biotin, FAM, FITC, TMR labeling

- 20 different residues available

Advanced design strategy: sequence stability

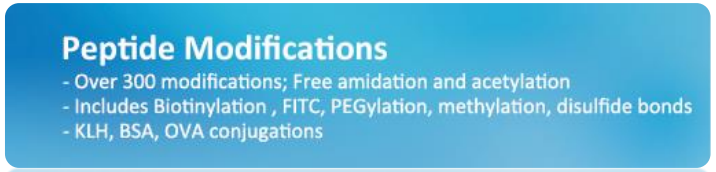
Renal Clearance

- Hydrophilic peptides <25 kDa are susceptible to rapid filtration through the glomeruli of the kidney

- Peptides not easily reabsorbed through the renal tubule

Strategy to decrease renal clearance

- Conjugation to macromolecules or polymers:
  - Polyethylene glycol (PEG)
  - Polysialic acid (PSA)
  - Hydroxyethylstarch (HES)
  - Bovine serum album (BSA)

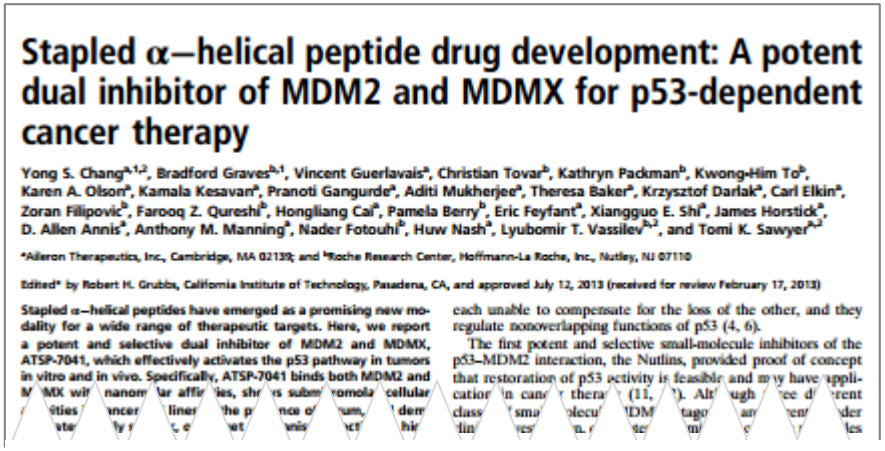


http://www.genscript.com/peptide_modification.html

Case study: stapled peptides

The Design:

- α-helix stabilized by the incorporation of a hydrocarbon
  - Called ATSP-7041
  - Designed to inhibit p53 inhibitors, MDMX and MDM2



Stapled α–helical peptide drug development: A potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy

Yong S. Chang[a,1,2], Bradford Graves[b,1], Vincent Guerlavais[a], Christian Tovar[b], Kathryn Packman[b], Kwong-Him To[b], Karen A. Olson[a], Kamala Kesavan[a], Pranoti Gangurde[a], Aditi Mukherjee[a], Theresa Baker[a], Krzysztof Darlak[a], Carl Elkin[a], Zoran Filipovic[b], Farooq Z. Qureshi[b], Hongliang Cai[a], Pamela Berry[b], Eric Feyfant[a], Xiangguo E. Shi[a], James Horstick[a], D. Allen Annis[a], Anthony M. Manning[a], Nader Fotouhi[b], Huw Nash[a], Lyubomir T. Vassilev[b,2], and Tomi K. Sawyer[a,2]

[a]Aileron Therapeutics, Inc., Cambridge, MA 02139; and [b]Roche Research Center, Hoffmann-La Roche, Inc., Nutley, NJ 07110

Edited* by Robert H. Grubbs, California Institute of Technology, Pasadena, CA, and approved July 12, 2013 (received for review February 17, 2013)

Stapled α–helical peptides have emerged as a promising new modality for a wide range of therapeutic targets. Here, we report a potent and selective dual inhibitor of MDM2 and MDMX, ATSP-7041, which effectively activates the p53 pathway in tumors in vitro and in vivo. Specifically, ATSP-7041 binds both MDM2 and M[illegible]MX with nanomolar affinities, shows submicromolar cellular activities in cancer cell lines with wild-type p53, induces dose-dependent [illegible] each unable to compensate for the loss of the other, and they regulate nonoverlapping functions of p53 (4, 6).

The first potent and selective small-molecule inhibitors of the p53–MDM2 interaction, the Nutlins, provided proof of concept that restoration of p53 activity is feasible and may have application in cancer therapy (11, [illegible]). Although three different classes of small molecule MDM2 antagonists are [illegible]

The Test:

- ATSP-7041 efficacy was compared to small molecule MDM2 inhibitors
  - Tested in cancer cells and xenograft models
  - Monitored cell cycle arrest, apoptosis and tumor size

The Result:

- ATSP-7041 inhibited MDMX and MDM2 interactions with p53 interaction, causing the re-activation of p53 to induce apoptosis

Case study: Dithiol amino acid incorporation

nature chemistry

ARTICLES

PUBLISHED ONLINE: 31 AUGUST 2014 | DOI: 10.1038/NCHEM.2043

Dithiol amino acids can structurally shape and enhance the ligand-binding properties of polypeptides

Shiyu Chen[1], Ranganath Gopalakrishnan[1], Tifany Schaer[2], Fabrice Marger[2], Ruud Hovius[1], Daniel Bertrand[2], Florence Pojer[3] and Christian Heinis[1]*

The disulfide bonds that form between two cysteine residues are important in defining and rigidifying the structures of

The Design:

- Designed a novel amino acid containing 2 sulfhydryl groups
  - Mimics cysteine
  - Capable of forming cyclic and branched secondary structures

The Test:

- Replaced adjacent cysteines in a serine protease inhibitor and nicotinic acetylcholine receptor inhibitor with dithiol amino acids

The Result:

- Dithiol amino acids enhanced peptide activity
  - Serine protease inhibitor and nicotinic acetylcholine receptor inhibitor activities increased by 40 and 7.6-fold, respectively

Design strategy summary

	Design considerations	Strategies
Basic	• Charge	▪ Capping ▪ Non-essential amino acids replacement
	• Hydrophobicity	• Residue substitutions with charged or polar residues
	• Length	• Design peptides less than 50 AA long if possible • Ligation technology or recombinant peptide synthesis
	• Purity	• Use recommended peptide purity chart
Advanced	• Stability	• Unnatural amino acid incorporation • Peptoids • Hydrocarbon stapling
	• Secondary structure	• Unnatural amino acid incorporation • Cyclization • Disulfide bridge incorporation

Service selection/resource summary

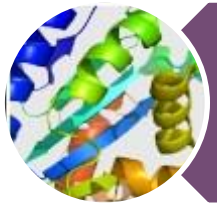
	Services
Long Peptide Synthesis	• Ligation technology (Chemical protein synthesis) • Recombinant peptide synthesis
Peptide Stability	• Capping (free acetylation and amidation) • Peptoid synthesis • Unnatural amino acid modifications • Macromolecule/polymer conjugations • Cyclization, disulfide bonds • Free argon shield packaging
Contamination	• Guaranteed TFA Removal Service
Solubility	• Free solubility testing
Resources	• Webinar: Avoiding peptide assay failure: hidden problems and solutions • Webinar: Protein or peptide antigen: choosing the optimal immunogen for antibody production • Peptide property calculator • Amino acid chart property chart • Recommended peptide purity chart • Peptide solubility guidelines

Thank you for your participation

We wish you all success in your research

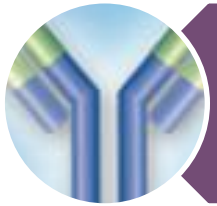
Email me: Tiffany.Campolongo@GenScript.com

Register for other webinars in the GenScript Webinar Series or download past webinars at @ http://www.genscript.com/webinars.html



Fusion tags for recombinant soluble protein production in *E. coli* – Keshav Vasanthavada
December 3, 2014/ 8:00 am & 2:00 pm EST

Avoiding peptide assay failure: hidden problems and solutions- *Tiffany Campolongo, Ph.D*
On demand



Protein or peptide antigen: choosing the optimal immunogen for antibody production- *Liyan Pang, Ph.D*
On demand