Examining the components of your peptide sample with AccuPep QC

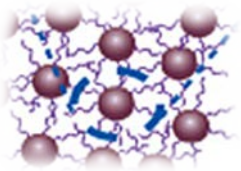
Yu Lu, Ph.D.

October 29, 2015, 9:00-10:00 AM EST

GenScript
Make Research Easy

When do I need custom peptides?

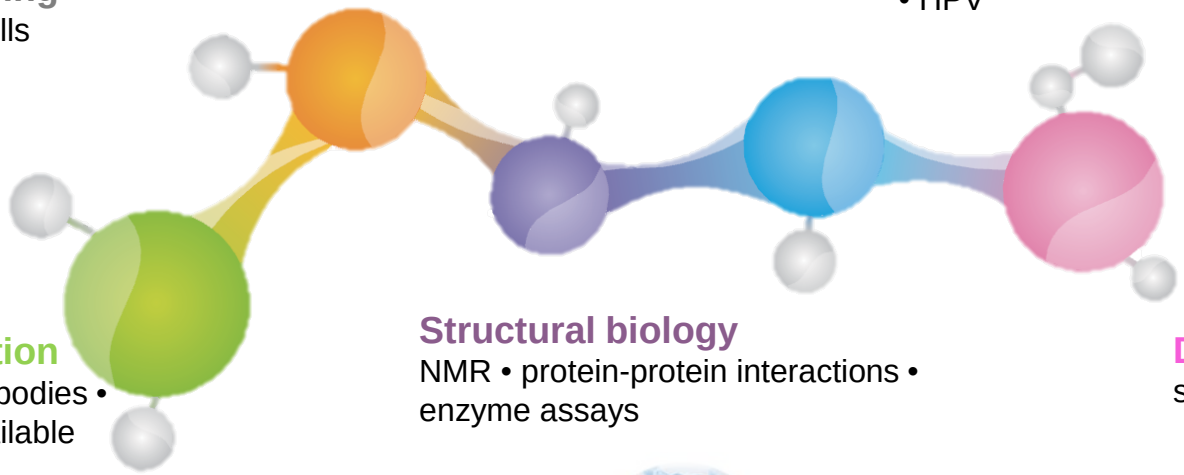
Custom peptides play an important role in many research applications



Tissue Engineering
Hydrogels • stem cells • wound healing

Drug Discovery
Antimicrobials• cancer • diabetes • neurodegenerative diseases • immunotherapy

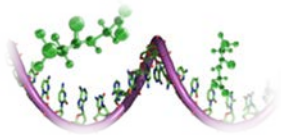
Vaccine Development
HIV • cancer • influenza • HPV



Antibody Generation
Phospho-specific antibodies • non-commercially available antibodies

Structural biology
NMR • protein-protein interactions • enzyme assays

Drug Discovery
siRNA



Common challenges with custom peptides

- Common issues that you might face

  - Unable to dissolve peptides with poor solubility
  - Improper dissolving way ruining peptide sample
  - Unknown contamination ruining your assay
  - Low experiment reproducibility from batch to batch
  - Non-reproducible results for quantitative experiments

How can these issues be addressed?

- For certain types of assays, additional testing is required to learn more about the contents of your peptide sample:
  - Appropriate solvents
  - Removal of TFA
  - The precise amount of net peptide
  - The presence of endotoxin
  - The water %
  - The pH value
  - The residual solvents
  - The identification of peptide impurities
  - …

Benefits of analyzing your peptide content

GenScript offers a comprehensive QC service, AccuPep+, to help you get the most out of your custom peptides

- Reduces experimental troubleshooting
- Increases experiment reliability
- Ensure reproducible results

Features of the AccuPep+ Service

Quantification tests

- Amino Acid Analysis
- Peptide Content Analysis
- Counter-ion Quantification Analysis
- Moisture Content Analysis

Toxicity Tests

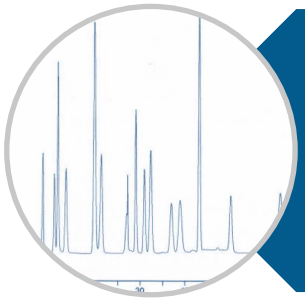
- TFA Removal and Analysis
- Endotoxin Analysis

Other tests

- Solubility Tests
- pH Test

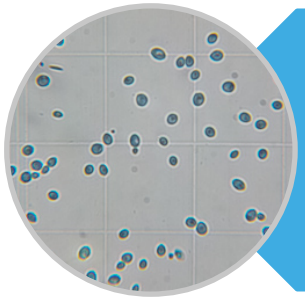
www.genscript.com/accupep_quality.html

AccuPep+ service test options



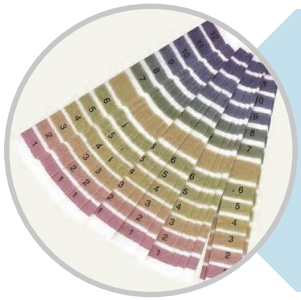
Quantification Tests:

- Do I really know all the possible components in my peptide sample?



Toxicity Tests:

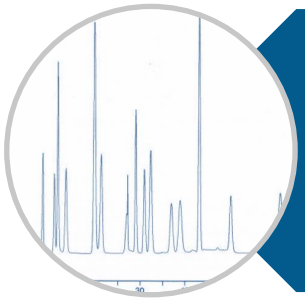
- What could make my experiment fail?



Other Tests:

- What else can I do to accelerate my experiments?

AccuPep+ service test options



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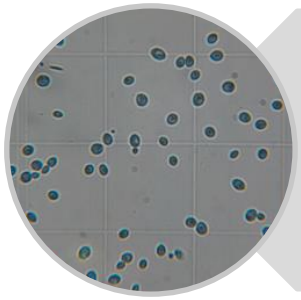

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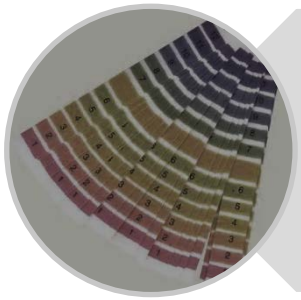


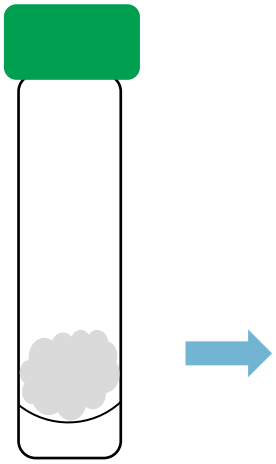
Other Tests:

- What else can I do to accelerate my experiments?

Quantification Tests: Methods

Components	Quantification Methods
Target Peptide Peptide Impurities	Amino Acid Analysis; Peptide Content Analysis (Nitrogen Element Analysis); HPLC Analysis
Counter-ion (Trifluoroacetate, Acetate & hydrochloride)	Counter Ion Quantification Analysis (Ion chromatography)
Water	Moisture Content Analysis (Karl Fischer coulometric titration)

How much peptide do I have?



Target Peptide (**Purity**)

Peptidic Impurities

The net peptide content

Counter-ion
(Trifluoroacetate, Acetate & hydrochloride)

TFA^- OAc^- Cl^-

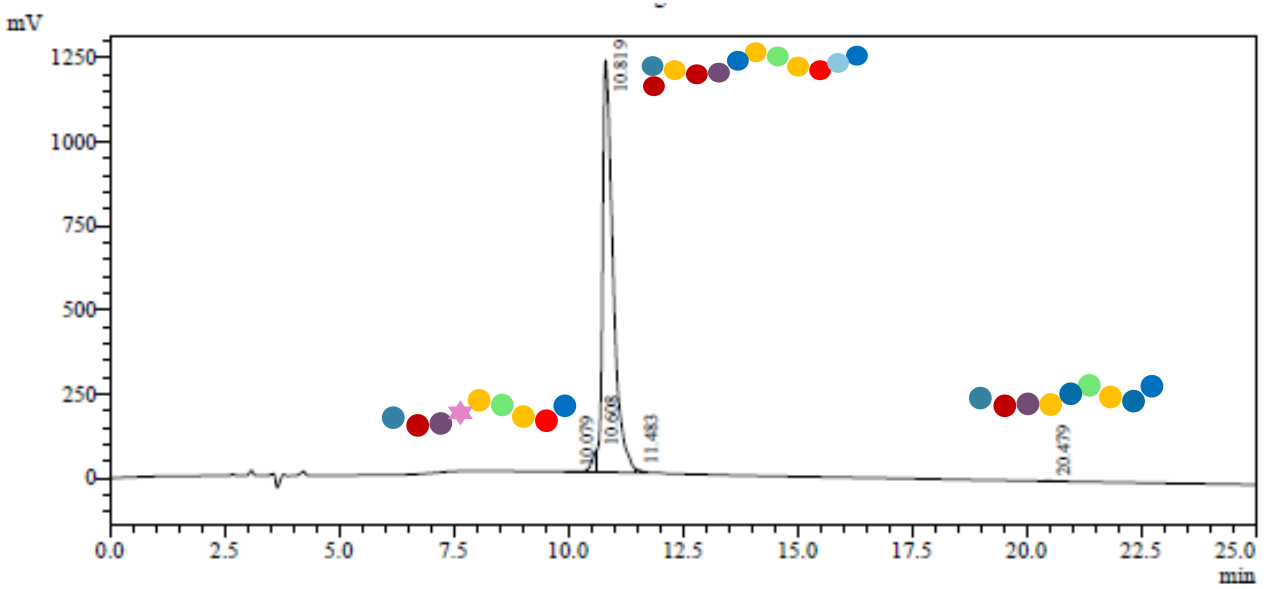
Water

ca.100%

How is peptide purity measured?

- High performance liquid chromatography (HPLC) Purity

The ratio of peak area of target peptide in relation to all detected peak area



Detector A Ch1 220nm

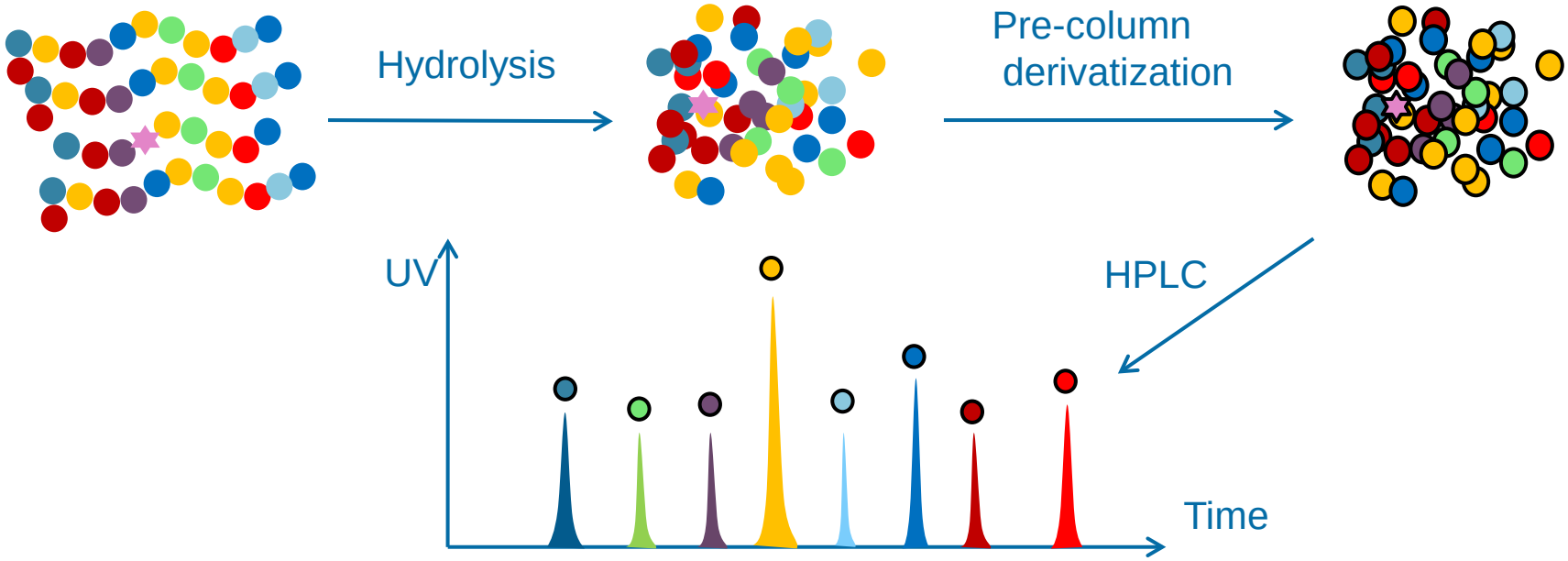
Peak#	Ret. Time	Area	Height	Area %
1	10.079	12200	1255	0.061
2	10.608	441127	63265	2.201
3	10.819	19493777	1224629	97.257
4	11.483	83012	11085	0.414
5	20.479	13507	1763	0.067
Total		20043623	1301997	100.000

Peptide Purity: 97%

What is the amino acid composition of my peptide?

- Amino Acid Analysis (AAA) is ideal for:
  - Determining the amino acid composition
  - Determining the precise amount of net peptide in your sample

- Protocol:



Example AAA report

Peptide Sequence: VFNTRA

Amino acid (a.a.) residues	Theoretical a.a. number	The measured a.a. residues concentration (μmol/ml)	Measured a.a. number
Asp/Asn	1	0.1323	1.00
Glu/Gln			
Ser			
Gly			
His			
Arg	1	0.1308	1.00
Thr	1	0.1142	0.90
Ala	1	0.1245	1.00
Pro			
Tyr			
Val	1	0.1431	1.10
Met			
Cys			
lle			
Leu			
Phe	1	0.1269	1.00
Trp			
Lys			

Tips:

1. Asn and Gln are de-aminated during hydrolysis to Asp and Glu.

2. Only highlighted a.a. residue is stable enough during hydrolysis to be used as for peptide content calculation.

Alternative way to determine the net peptide content

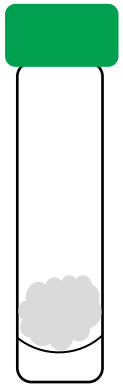
- Peptide Content Analysis: determines the precise amount of net peptide in the gross peptide sample

- Method
  - Nitrogen Element Analysis

Nitrogen % ⟶ $(C_x H_y N_Z O_n S_m)$ %

Note: Given that the counter-ions (acetate, trifluoroacetate or hydrochloride) and the adsorbed water do not contain nitrogen, Nitrogen element analysis can be use for the net peptide content measurement.

How to calculate the net peptide



Delivered peptide

Delivered (Gross) Weight:10 mg

Purity: 97%

Peptide content: 40%
(AAA)

Net peptide weight:

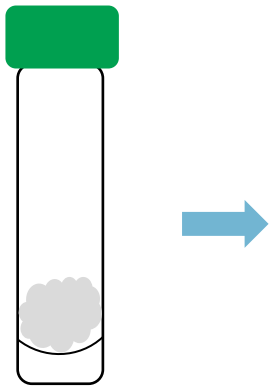
10 mg * 40% = 4.0 mg

Exact amount of target peptide:

4.0 * 97% = 3.88 mg

With the purity and peptide content as determined by AAA, you can calculate the net peptide weight and exact amount of your peptide

Other constituents in your sample



Target Peptide

Peptidic Impurities

The net peptide content

Counter-ion
(Trifluoroacetate, Acetate & hydrochloride)

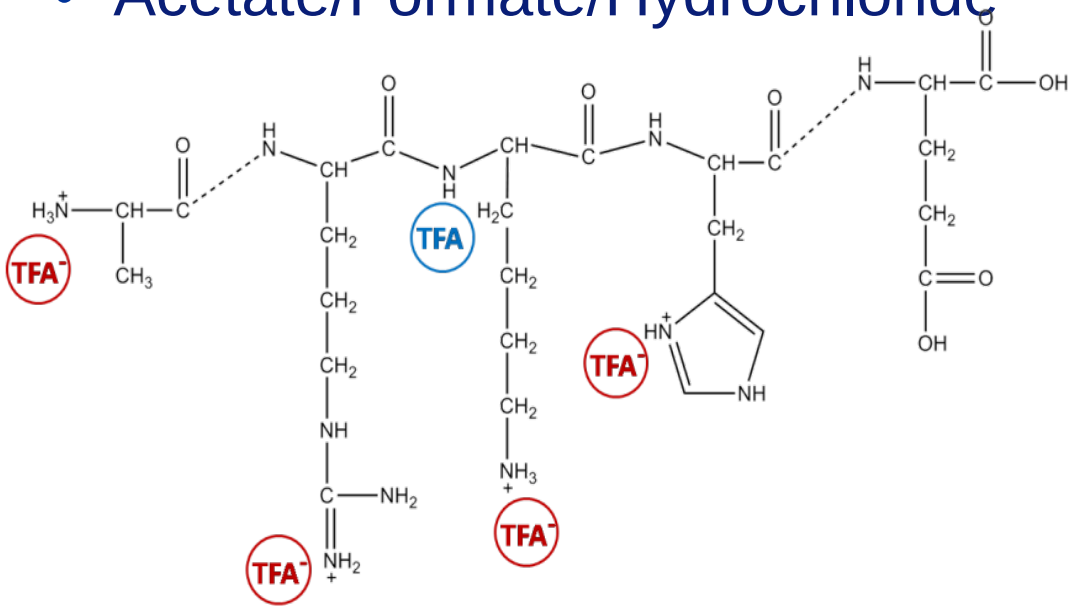
TFA^- OAc^- Cl^-

Water

ca.100%

Counter-Ion Types and Sources

- Common counter-ion types
  - Trifluoroacetate (**main type** for most delivered peptides)
  - Acetate/Formate/Hydrochloride



TFA **Free acid**

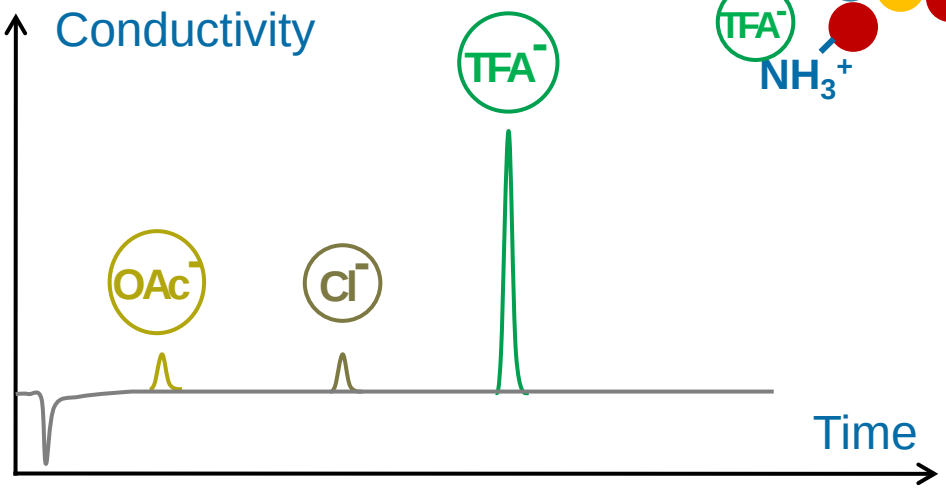
TFA⁻ **Salt**

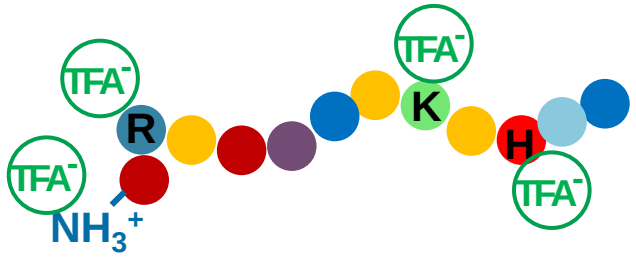
- Counter-ion sources
  - Peptide cleavage and purification
  - Counter-ion exchange

Counter Ion Quantification Analysis

- Counter Ion Quantification Analysis is useful for:
  - Cellular assays
  - Active pharmaceutical ingredients (APIs)
  - Manufactured products

- Method
  - Ion chromatography





TFA- Trifluoroacetate

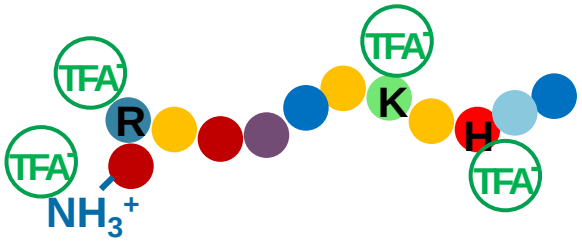
OAc- Acetate

Cl- Hydrochloride

How can I estimate the theoretical TFA amount?

Peptide molecular weight: 1500 Da

TFA molecular weight: 114 Da



Peptide (adducted with counter-ion) molecular weight: 1500+114*4 = 1956 Da

The estimated TFA % = 114*4/1956 = 23.3 %

Moisture Content Analysis

- Moisture Content Analysis is useful for hydrophilic peptides that will retain the most water.

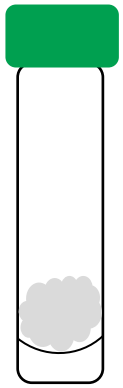
- Method
  - Karl Fischer Coulometric Titration
  - The basis:

$$H_2O + I_2 + [RNH]SO_3CH_3 + 2\,RN \Leftrightarrow [RNH]SO_4CH_3 + 2\,[RNH]I$$

I_2 reacts quantitatively with H_2O while the iodine is generated directly in the electrolyte by electrochemical means.

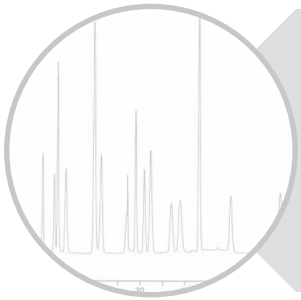
Based on the rigorously quantitative relationship between the electric charge and the amount of electrochemically generated Iodine, the amount of water eventually can be quantified.

Summary



Components	Quantification Methods
Target Peptide Peptide Impurities	Usually, 50-80%
Counter-ion (Trifluoroacetate, Acetate & hydrochloride)	Usually, 30-10% Dependent on basic a.a. residue number
Water	Usually < 10%, but could be particularly high for hydrophilic peptide

AccuPep+ service test options



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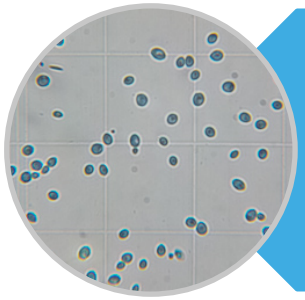
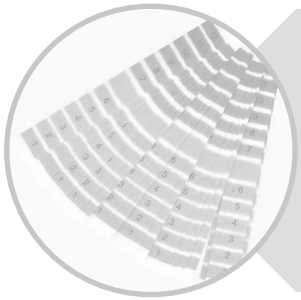


Toxicity Tests:

- What could make my experiment fail?



Other Tests:

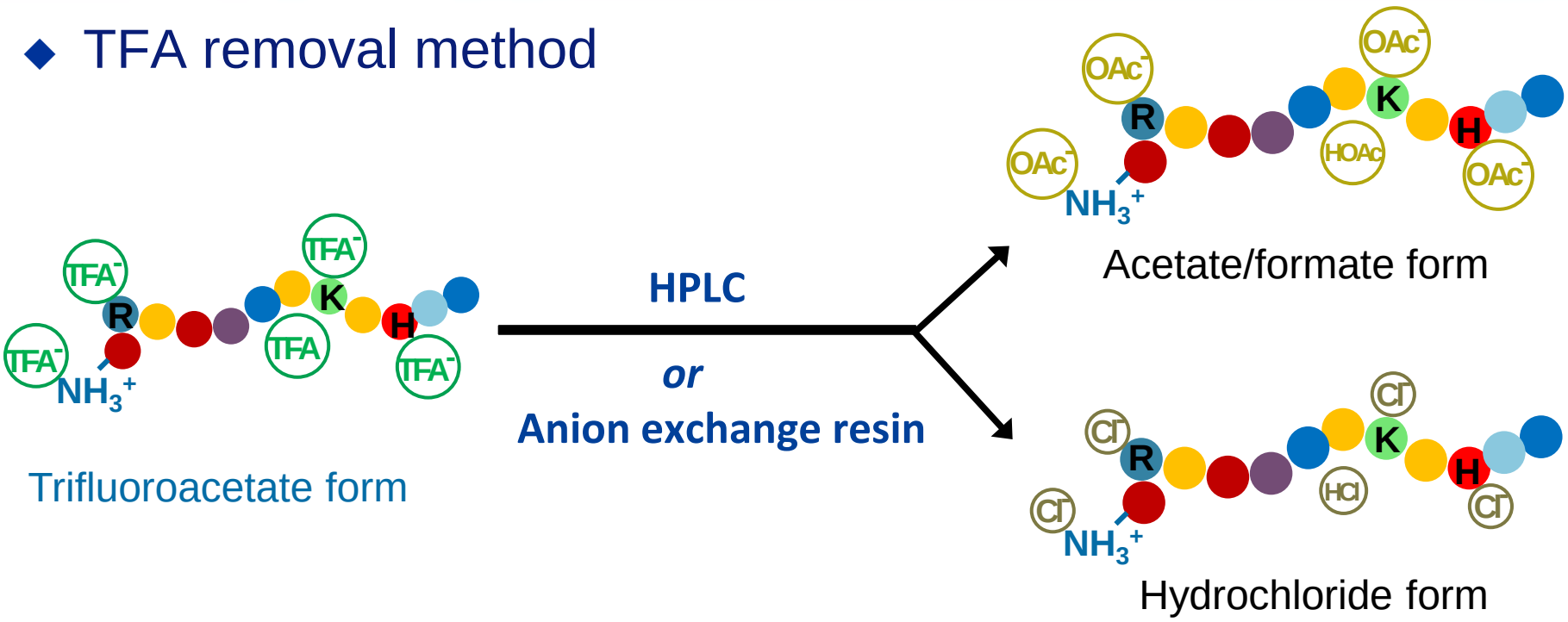
- What else can I do to accelerate my experiments?

Toxicity test options

- **TFA removal and analysis**
  - TFA is a counter anion for normal peptides
  - Trace amount of TFA can cause cytotoxicity in cell culture assays
- **Endotoxin Analysis**
  - Endotoxins are easily introduced into peptides during any process of peptide production.
  - Small concentrations of endotoxin can decrease cell viability or cause immune response in cellular assays.

TFA Removal and Analysis

- TFA removal method



Trifluoroacetate form

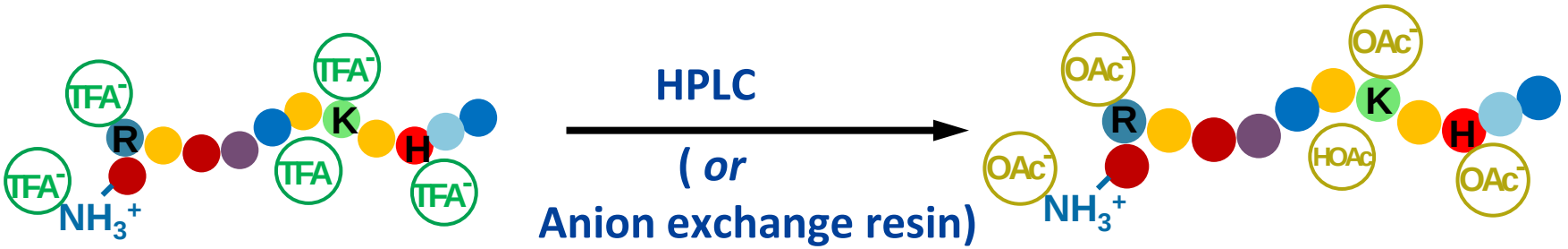
Acetate/formate form

Hydrochloride form

- How to choose counter-ion form (in the point view of production)
  - OAc⁻ **Suitable for unstable amino acids, such as Cys, Met, Gln at N-terminus**
  - Cl⁻ **Suitable for peptides with low solubility**

Two Types of TFA Removal Services

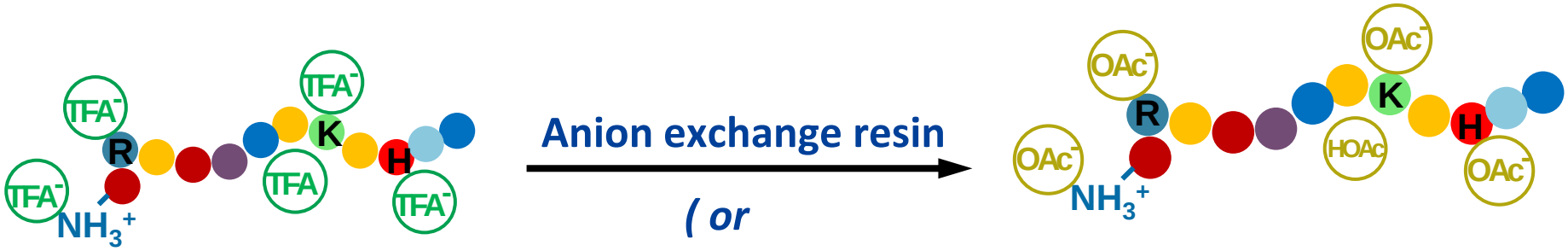
- Standard TFA Removal Service



Trifluoroacetate form

TFA Content: Undetermined, usually quater of the estimated - 1%

- Guaranteed TFA Removal Service



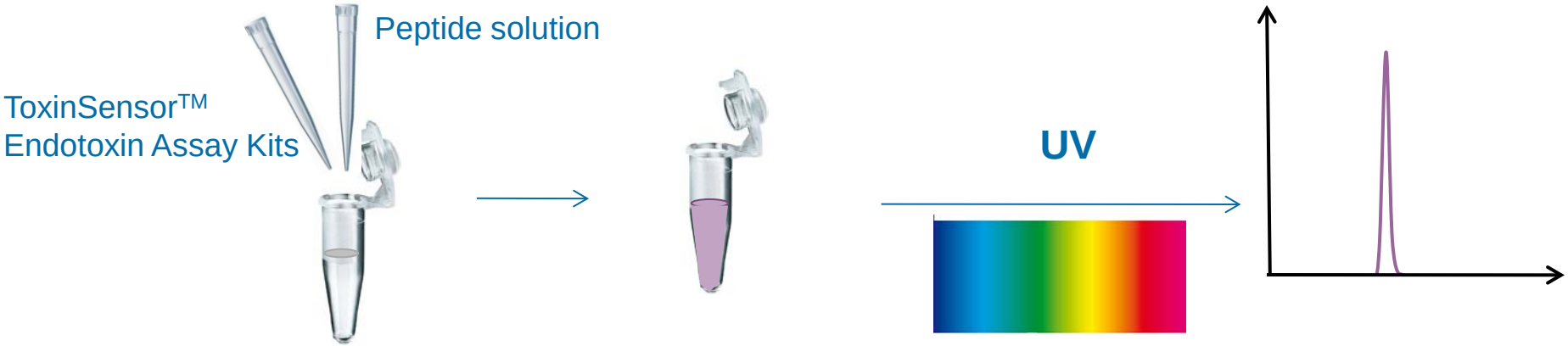
Trifluoroacetate form

TFA Content: < 1%

Endotoxin Analysis

- Endotoxins (lipopolysaccharides)
  - Major components of the cell walls of gram-negative bacteria
  - Introduced into custom peptides during peptide production

- Method
  - Chromogenic *Tachypleusamebocyte* lysate or *Limulus amebocyte* lysate test
  - Guaranteed high-sensitivity: 0.005 EU/ml



Which service is best for me?

- A variety of applications benefit from TFA removal or endotoxin analysis:

Application	Method
Analytical analyses that are influenced by TFA ions: - Infrared (IR) spectroscopy - Circular dichroism (CD) spectroscopy	TFA removal and analysis
Cell culture assays	TFA removal and analysis
Cosmetics and pharmaceutical applications	TFA removal and analysis
Cell culture assays sensitive to endotoxin or prone to immune responses	Endotoxin analysis

Case study: effect of TFA on cell culture

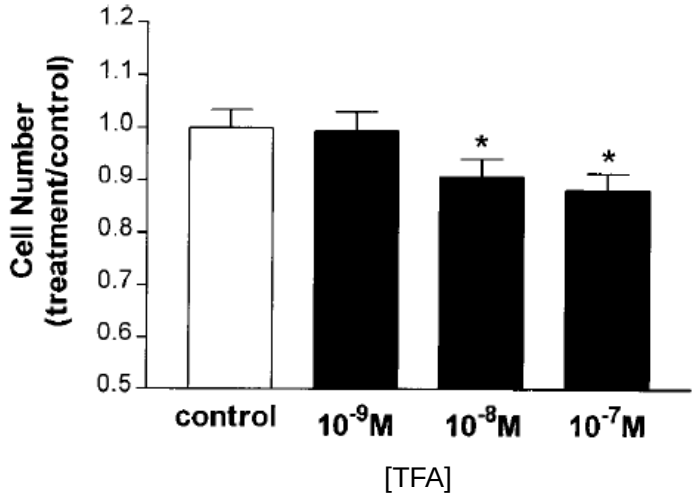
- Even trace amounts of TFA can cause cytotoxicity in cell culture assays

Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes

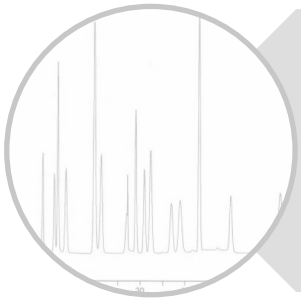
J. Cornish, K. E. Callon, C. Q.-X. Lin, C. L. Xiao, T. B. Mulvey, G. J. S. Cooper, I. R. Reid

American Journal of Physiology - Endocrinology and Metabolism Published 1 November 1999 **Vol.** 277 **no.** 5, E779-E783 **DOI:**

- Peptides containing TFA at concentrations ranging from 10^{-9} to 10^{-7}M were supplemented to osteocyte and bone cultures.
- Viability was assessed by [^{3}H]thymidine incorporation after 24 hours



AccuPep+ service test options



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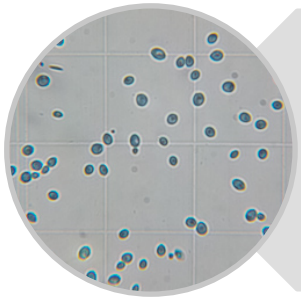

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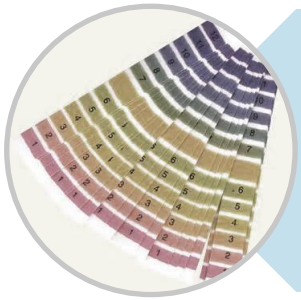


Other Tests:

- What else can I do to accelerate my experiments?

Solubility Test

- Hydrophobic peptides
  - Containing > 50% hydrophobic amino acid
  - Length > 5 a.a.

- Hydrophobic :

  Ala (A),Trp (W), Leu (L), Ile (I), Phe (F), Met (M), Val (V), Pro (P)
- Basic:

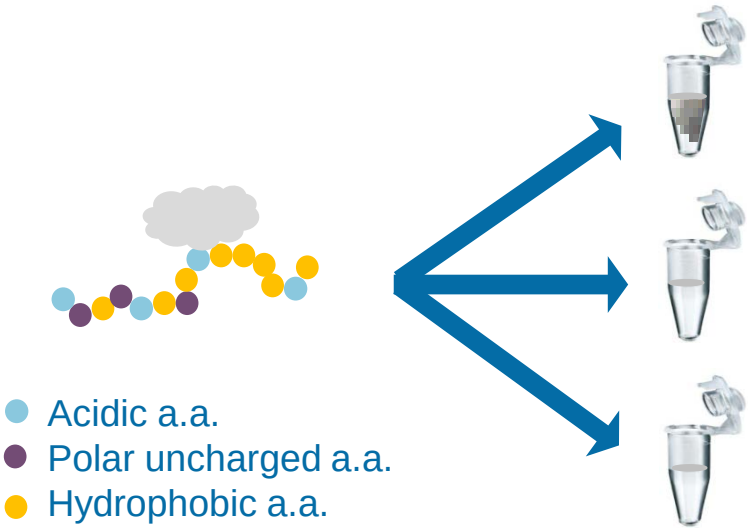
  Arg (R), His (H), Lys (K)
- Polar uncharged:

  Asn (N), Cys (C), Gly (G), Gln (Q), Ser (S), Thr (T), Tyr (Y)
- Acidic:

  Asp (D), Glu (E)

Components of the solubility report

- What is included in your solubility report?



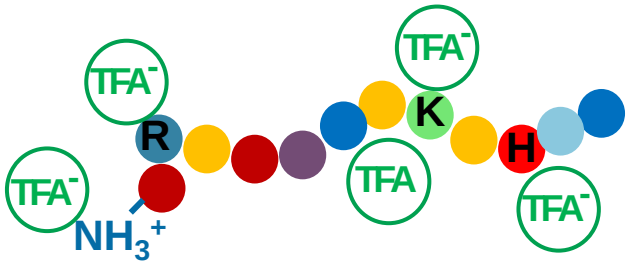
Benefits:

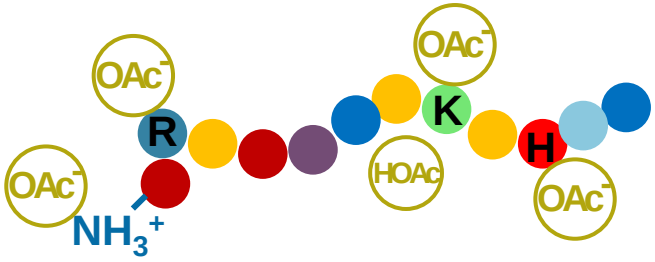
Reduces troubleshooting
Saves time and peptide products
Particularly useful for peptide libraries

Solvent	Results (Dissolved or Undissolved)	Gross Peptide Concentration
Ultrapure water	**Undissolved**	**N/A**
PBS(pH 7.1±0.1)	**Dissolved**	≤ 1mg/ml
DMSO	**Dissolved**	≤ 10mg/ml
Others*	**N/A**	**N/A**

pH Testing

- Why different pH value
  - Free TFA acid may be present
  - Different counter ions may result in various pH values of peptide solutions





Low pH

High pH

Conclusions

Quantifying each component

Amino Acid Analysis
Peptide Content Analysis
Counter Ion Quantification Analysis
Moisture Content Analysis

Removing toxins

TFA Removal and Analysis
Endotoxin Analysis

Making sample prep easier

Solubility Test
pH Test

How can I request these services?

Click on the quote button

Input your desired peptide sequence, purity, quantity and modification

Check the box to receive a free solubility test

Select a TFA Removal option (guaranteed or standard)

In the comments section, request any of the optional QC services

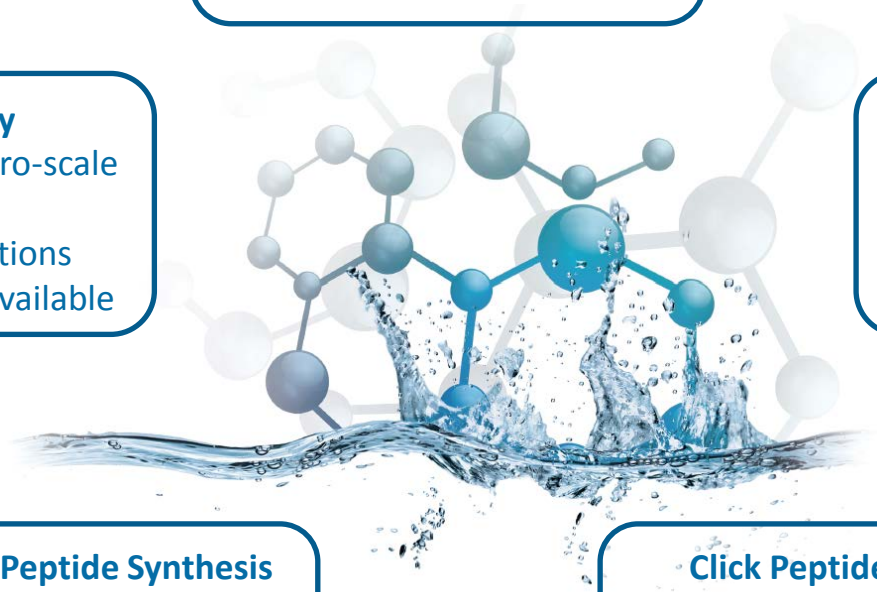
To learn more, visit

www.genscript.com/accuprep_quality.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



Peptoid Synthesis
- Proteolytic resistant peptidomimetics
- Cost-effective, fast turnaround

Cosmetic Peptide Synthesis
- High batch-to-batch reproducibility
- High capacity

Click Peptide Synthesis
- O-acyl bond incorporation technology
- Increased peptide stability

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We wish you success with your research

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Large scale genome editing for metabolic engineering of *E. coli*– *Yifan Li, Ph.D.*

November 5, 2015, 9:00 AM or 2:00 PM EST

Optimizing soluble protein expression: codon optimization, RBS design and expression vector design– *Rachel Speer, Ph.D.*

November 11, 2015, 9:00 AM or 2:00 PM EST

Antibody Drug Development: challenges & solutions – *Liusong Yin, Ph.D.*

November 18, 2015, 9:00 AM EST

Q&A

If you have any other questions, visit www.genscript.com/faq_for_peptide
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