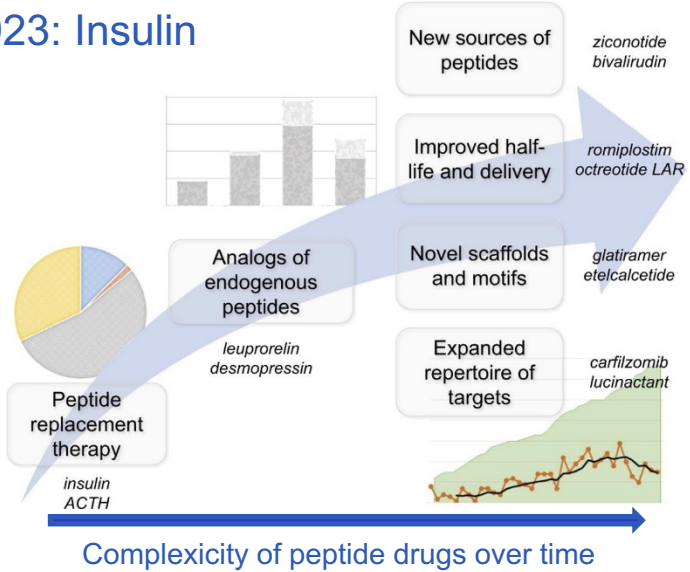
Peptides aren't neither proteins or small molecules…

Anna Laurén, on behalf of the EBF

Peptides drug are not new – but gains increasing interest

- The number of clinical trial applications and marketing authorization applications for synthetic peptides significantly increased over the last few years.
  - First commercially available peptide drug was in 1923: Insulin
  - Including high focus on Biosimilar/Generics/ANDA
- Tradionally classified as:
  - Native and natural peptides
  - Analog
  - Heterologous
- Can be manufactured:
  - Synthetic
  - Recombinant
  - Component derived from blood and tissue

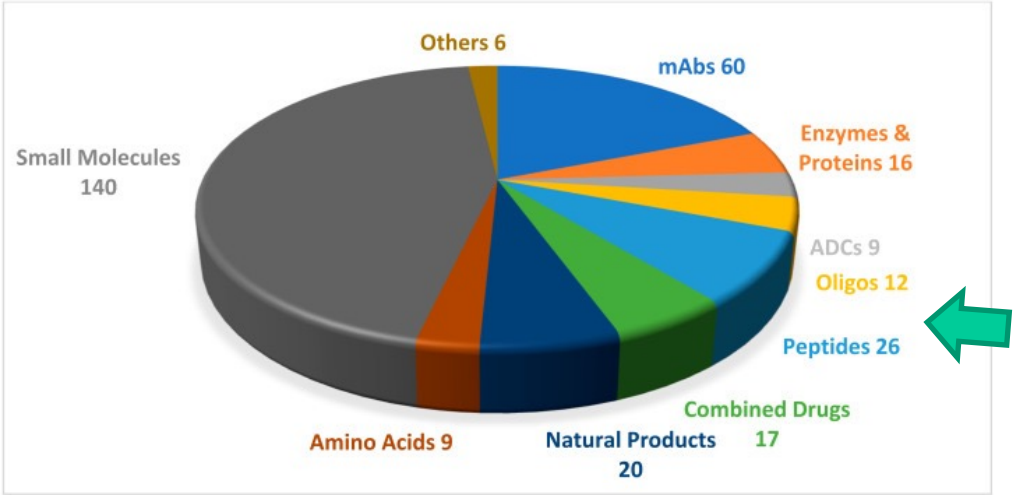


Lau J, Dunn M. 2018: Therapeutic peptides: Historical perspectives, current development trends, and future directions. Bioorganic & Medical Chemistry.

TIDES: Peptides, Oligonucleotides and ADCs whose structures contain peptides.

- Oligonucleotides and peptides (TIDES) are considered chemical entities, but occupy a delimited chemical space between biologics and small molecules.

FDA Approved drugs between 2016 and 2022

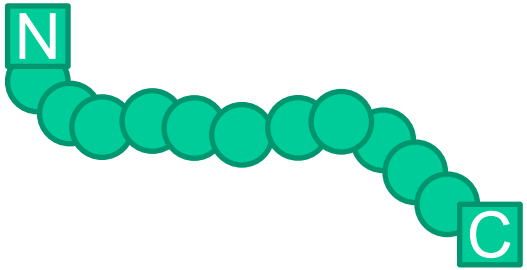


Ref: 2022 FDA TIDES (Peptides and Oligonucleotides) Harvest. Pharmaceuticals (Basel). 2023

Peptides vs proteins: The simple way

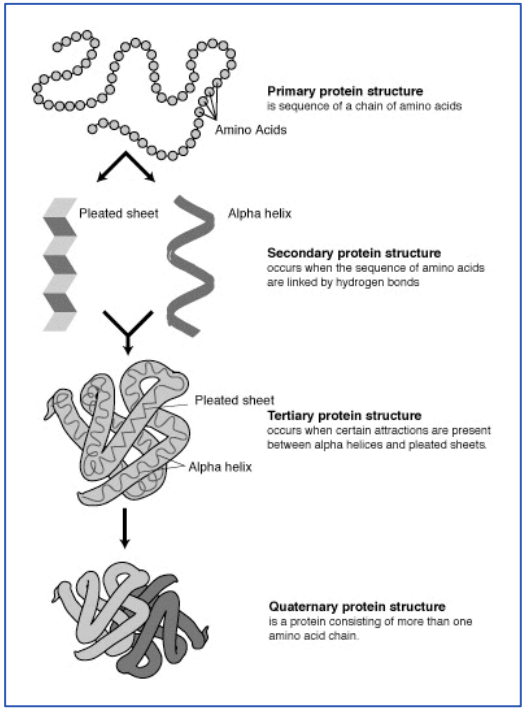
Peptides

A linear chain
of amino-acids



Building blocks for peptides and proteins are the same:

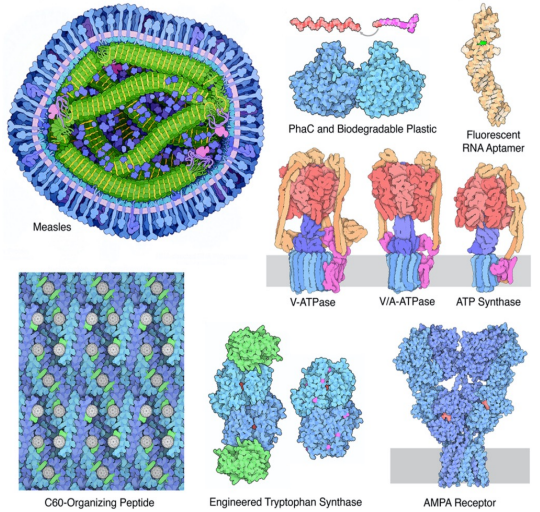
- Combination from 20 amino-acids
- N-terminus with a free amino group
- C-terminus with a free carboxyl group



Protein structure prediction - Wikipedia

Proteins

Complex multi-dimentional
structures of amino-acids



Protein Data Bank (PDB): Insights from 20 years of the Molecule of the Month. ***Biochem Molecular Bio Educ, 2020***

The issue with peptides– increase drugability

- Problem 1: The short half-life
- Problem 2: Oral bioavailability
- Problem 3: CYP inhibition and drug-drug interactions (DDIs).
- Problem 4: Side effects caused by off-target binding.

- Need to increase drugability:
  - Improve half-life.
  - Improve stability under physiological conditions.
  - Improve receptor and target selectivity.
  - Improve bioavaibility.

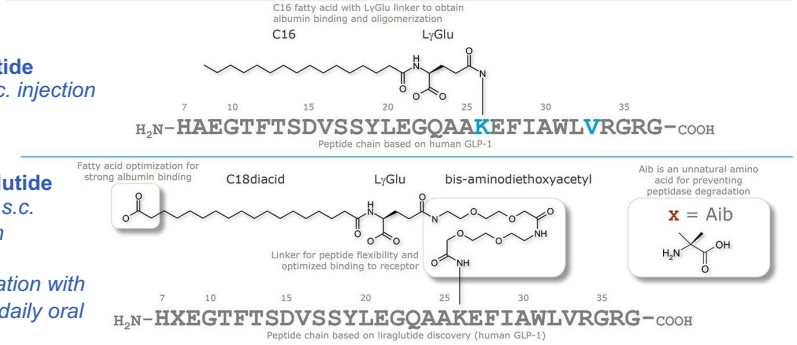
Natural peptides are less drugable due to:

- Peptidases
- Digestive cleavage
- DDI
- Off target binding

Utilisation of peptides to increase drugability

Peptide is the drug

- Increase half-life and bioavailability
- Conjugated and optimised peptides
  - Pegylation of various size and linkers
  - Fc-immunoglobulin
  - Fatty acids of various size and linkers
  - Alternative aminoacid positions
  - Chimeric peptides or cyclic peptides
  - Un-natural aminoacids



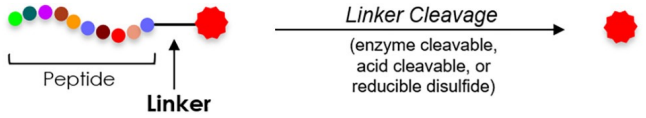
Liraglutide
Daily *s.c. injection*

Semaglutide
Weekly *s.c. injection*
Or in combination with carrier: daily oral tablets.

Picture from: The Discovery and Development of Liraglutide and Semaglutide. Front Endocrinol 2019.

Peptide–drug conjugates

- Tumor targeting peptides (TTPs):
  - Treatment
  - Diagnosis: PET/CT and PET imaging
- Linkers: Succinic acid; Disulfide; Amide; NOTA: PEG; NODAGA



Peptide-Drug Conjugate (PDC)

Drug

- enhanced efficacy
- higher circulation time
- lower off-target toxicity

Picture from: Peptide–Drug Conjugates with Different Linkers for Cancer Therapy. J. Med. Chem. 2021

Different stakeholders may have different views on guidelines based on experience – FDA as an example

The offices

- CDER: Center for Drug Evaluation and Research
  - Over-the-counter and prescription therapeutic drugs
  - “Biological therapeutics”: monoclonal antibodies, growth factors, fusion proteins, cytokines, enzymes, therapeutic toxins
  - Generic drugs
- CBER: Center for Biologics Evaluation and Research
  - Allergenics
  - Blood and blood components including clotting factors
  - Vellular and gene therapies
  - Vaccines

Regulations for filing and license

- New drug application (NDA) drug approval under the Federal Food, Drug, and Cosmetic (FD&C) Act.
- Biologics license application (BLA) required for biological products under the Public Health Service (PHS) Act.

Peptides which regulation?

- Food, Drug, and Cosmetic Act (FD&C Act)?
- Public Health Service (PHS) Act?

Confused on difference between peptide and protein?

- **A peptide is regulated as a drug under the Federal Food, Drug, and Cosmetic Act (FD&C Act) Act and as NCE**
  - High focus due to “biosimilars”/”generic drug”: depends largely on its impurity profile as compared to the impurity profile for the peptide of rDNA origin.
- March 2020: Biologicals licensed under the Federal Food, Drug, and Cosmetic Act (FD&C Act/NCE changed to license the Public Health Service Act (PHS Act)/BLA.
- Updated definitions:
  - Biological Product – “…a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein *(**except any chemically synthesized polypeptide**).*
- Changes from NCE to BLA included:
  - Peptides produced by recombinant DNA technology: insulin analogues (51 AA) and lixisenatide (44 AA, a GLP-1 analogue)
  - Proteins/hormones/enzymes: aprotinin; chorionic gonadotropin; follitropin alfa: follitropin beta; hyaluronidase; pancrelipase; pegvisomant; somatropin.

What is a peptide? EMA and FDA considerations focus mainly on manufacturing and pricing

FDA

- New definitions due to the changes between “FD&C Act/NCE” & “PHS Act)/BLA”:
- Protein – “any alpha amino acid polymer with a specific defined sequence that is greater than 40 amino acids in size…:”
- Chemically Synthesized Polypeptide – “…the term chemically synthesized polypeptide mean any alpha amino acid polymer that:
  - is made entirely by chemical synthesis
  - is greater than 40 amino acids but less than 100 amino acids in size.
- Peptide – “…a polymer composed of 40 or fewer amino acids…”

Federal Food, Drug, and Cosmetic Act (FD&C) Act.
Biologics license application (BLA) required for biological products under the Public Health Service (PHS) Act.

EMA

- Synthetic peptides and proteins with defined sequences, recombinant proteins and highly purified proteins extracted from biological matrices can be described as protein substances.
- Concept paper for need to establish new guidelines for development and manufacturing (2022).

EMA Article 57(2), second subparagraph of Regulation (EC) No. 726/2004.
EMA: Concept Paper on the Establishment of a Guideline on the Development and Manufacture of Synthetic Peptides. June 2022.

When does is matter for the Bioanalytical scientist?

- Regulatory tracks for filing is different depending on peptide manufacturing, size, impuries.

- But what about the Bioanalytical Scientists?
  - Does size matter?
  - Agonistic or antagonistic drugs?
  - Endogenous compounds?
  - Peptide–drug conjugates
  - Linkers/Conjugates
  - Metabolites?
  - Impurities?

Endogenous Molecules and ICH M10 Chapter 7

EBF

Workshop

Towards harmonised implementation of the ICH M10 Guideline

<< Chapter 7, Chrom&LBA >>

Anna Laurén, Iain Love, Amanda Wilson, Stuart McDougall, Robert Nelson, Roland Staack, and Michaela Golob on behalf of the EBF

15 November 2022, Barcelona

- Only relevant for therapeutics drugs that are **identical to endogenous.**
- **Biomarkers are out of scope of ICH M10.**
- Chapter 7 is new but drugs identical to endogenous is not.
- Replacement therapy has been used since last century.
  - Insulin for Type 1 diabetes – First treatment in 1922 and Nobel price in 1923, first fully human recombinant in 1978.
  - Hormone replacement therapy (HRT) started in the 1960s – estrogen for menopause, osteoporosis, cardiovascular events.
- Modern drugs are often slightly modified versions of endogenous compounds and thus specific bioanalysis is possible: chapter 3 or chapter 4 .

EBF proposal for implementation:
Approach adopted for each program should be scientifical and driven by the bioanalytical technology.

Peptides and PD? Free and total assays?

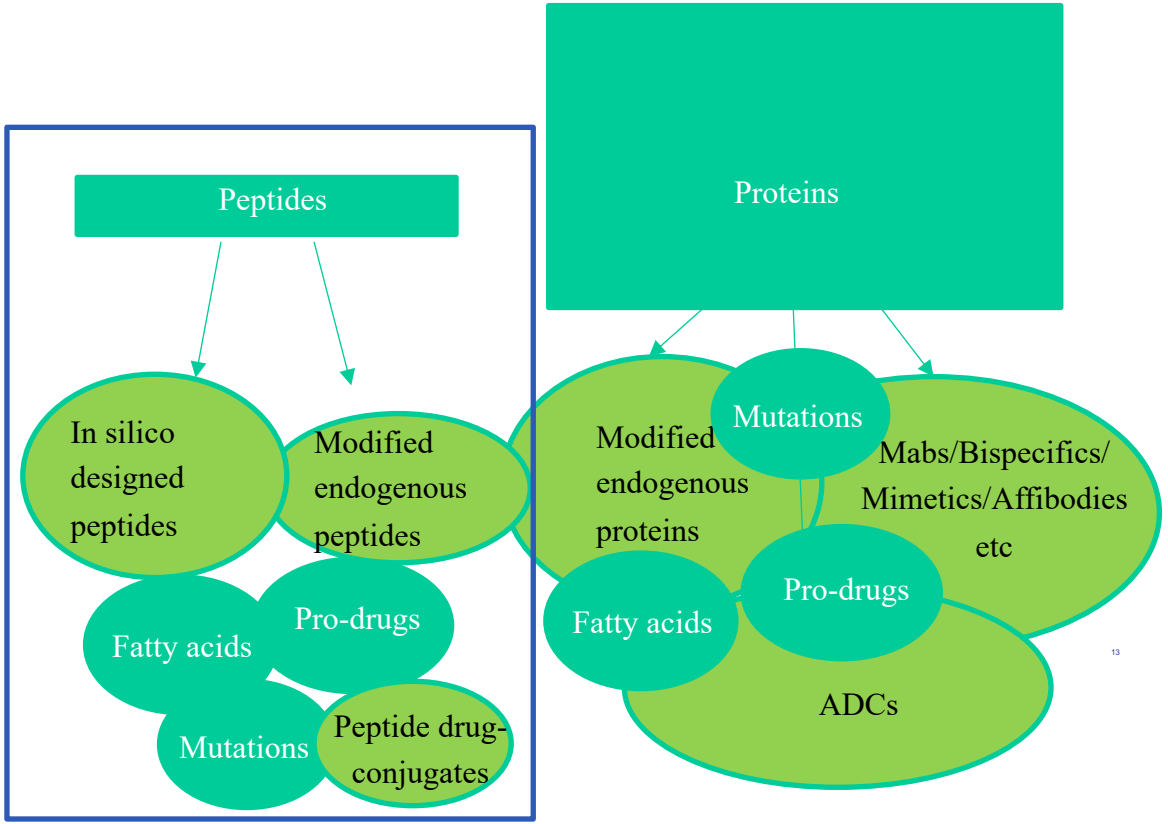
- Do we need to develop both free and target assays when peptide drug is an inhibitor?
  - Compare to NCE drugs? PK assay is always total for NCE. Target assays?
  - Compare to Mab therapeutics? History of free vs total PK and target engagement assays.

- Peptide therapeutics may have similar or different properties/Mode of Actions as Mab therapeutics and larger proteins.
  - Is drug target a cell bound receptor or a circulating target?
  - Antagonist or agonist? Ie “mini-binders” with similar properties as MAb drugs.
  - Do we need free target assays for small peptides?
  - Plasma binders?
  - Can we develop free target assays for small peptides?

- More in Session 2.

Peptide vs protein PK assays impurities/metabolites

- Size of peptide ie 5 amino-acids vs 40 aminoacids?
- Peptides and proteins are expected to be degraded in a similar way as that of endogenous peptides/proteins.
- Does selection of assay format for PK assay change your consideration for metabolite?
  - LBA vs Chrom?
- More in Session 6.



Peptides and Immunogenicity

- Is immunogenicity strategies different from larger proteins?
  - At what size of AA peptide is immunogenicity assays not needed?
  - Likelihood and consequence should drive immunogenicity strategy.
  - Relevance of nonclinical ADA same as for large proteins: Use EBF decision tree!
  - Consider replacement therapy/endogenous molecules for human subjects.

- Assay platforms for small peptides may still include RIA (radio immune assay) due to superior sensitivity using a “soluble phase assay”.

- Nab assays need to consider mode of action
  - Is drug target a cell bound receptor or a circulating target?
  - Antagonist or agonist?

- More in Session 4.

For future considerations

- Multiple bioanalytical strategies can be applied for peptides.
- Discussions involve NCE vs BLA consideration **but** should not be the driver of the BioA strategy.
- Approach adopted for each program should be scientifically driven.

Acknowledgements

EBF community and EBF teams for discussion on the topics

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www.e-b-f.eu