Rational Drug Design lecture 6

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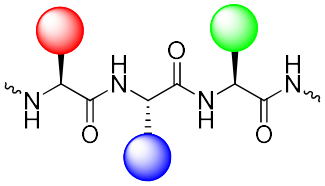
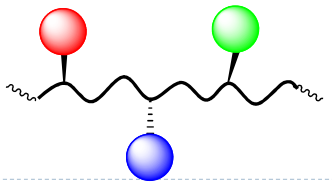
Peptides and peptidomimetics

- **Peptides** - oligomers built from amino acids linked by an amide bond.



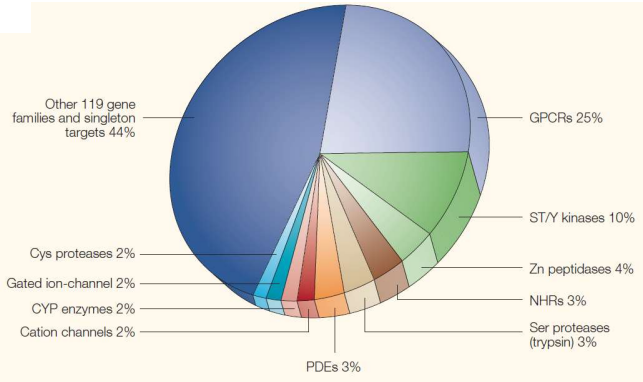
- **Peptidomimetics** - molecules that mimic the structure and function of peptides



Molecular targets

- Many molecular targets interact with peptides and their analogues, in particular they are:
  - Ligands of receptors,
  - Inhibitors of protein-protein interactions,
  - inhibitors

    enzymes.



Peptides and analogs as drugs

- Approx. 200 active substances (among commercial drugs) are peptides or analogues thereof.

Size of peptides present on the market
(number of amino acid residues)

Bioactive peptides

- Peptides may exhibit a very broad spectrum of biological activity and be used in the treatment of:
  - bacterial infections,
  - viral diseases (including HIV)
  - cardiovascular disease,
  - diabetes,
  - osteoporosis,
  - cancer,
  - …

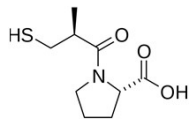
Peptides as drugs

- **Advantages:**
  - High activity and selectivity
  - Wide range of molecular targets
  - Potentially less toxic compared to low molecular weight compounds
  - Low accumulation in tissues
  - High chemical and biological diversity
  - Possible to discover at the level of genes
  - Easy synthesis of analogs

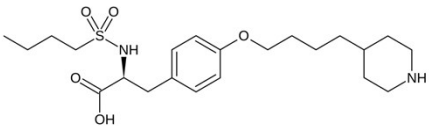
Peptides as drugs

- **Disadvantages:**
  - Low metabolic stability
  - Low permeability through biological membranes
  - Low bioavailability when administered orally
  - High production costs
  - Quickly removed from the body
  - Sometimes problems with solubility

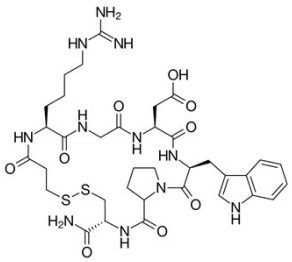
Examples of peptide drugs



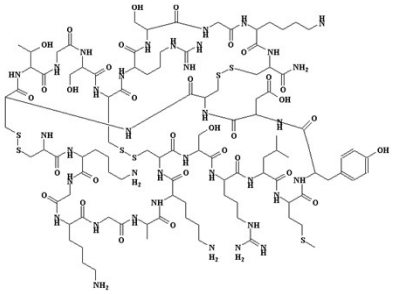
Captopril (hypertension, 1982)



Tirofibane (antithrombotic, 1998)



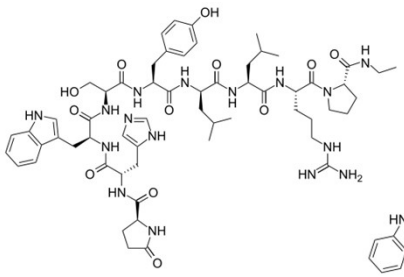
Epifibatide (antithrombotic, 1998)



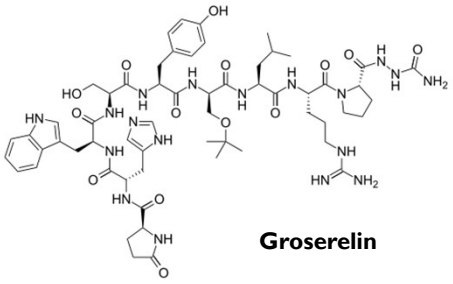
Zikonotine (painkiller, 2004)

Examples of peptide block busters

- **Leuprorelin, groserelin** - gonadotropin receptor antagonists ($ 1.5 billion and $ 1.1 billion), antineoplastic effect.



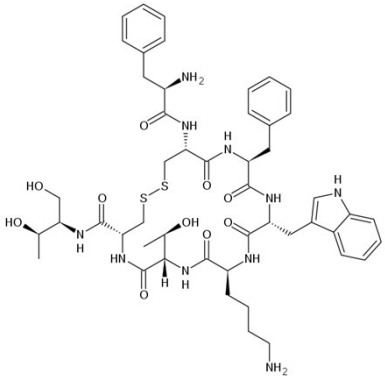
Leuprorelin



Groserelin

Examples of peptide block busters

- **Octreotide** ($ 1.3 billion) - somatostatin analog, anti-tumor.

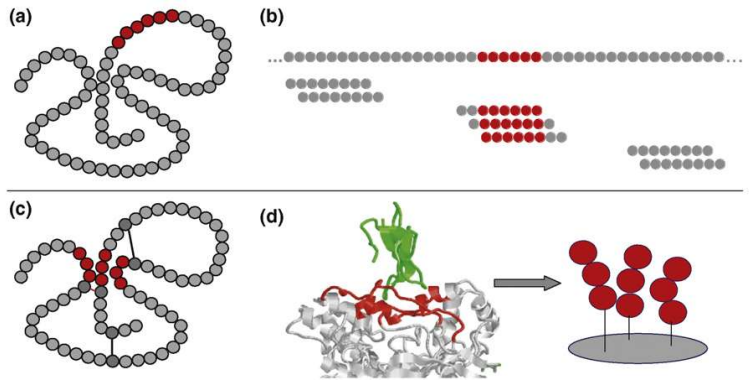


Sources of bioactive peptides

- Natural peptides produced by plants, animals and humans (peptide hormones or protein fragments)
- Peptides isolated from genetic libraries
- Chemical libraries (combinatorial chemistry)

Search for active peptides

- Frequently, protein activity is associated only with a fragment (epitope).



Methods of peptide optimization

Truncation

H_2N-XXXXXXXXXX-CO_2H
H_2N-XXXXXXXXX-CO_2H
H_2N-XXXXXXX-CO_2H
H_2N-XXXXXX-CO_2H
...

H_2N-XXXXXXXXX-CO_2H
H_2N-XXXXXXXX-CO_2H
H_2N-XXXXXXX-CO_2H
H_2N-XXXXXX-CO_2H
...

H_2N-XXXXXXXXX-CO_2H
H_2N-XXXXXXX-CO_2H
H_2N-XXXXXX-CO_2H
...

Deletion

H_2N-XXXXXXXXX-CO_2H
H_2N---XXXXXXXX-CO_2H
H_2N-X--XXXXXXX-CO_2H
H_2N-XX--XXXXXX-CO_2H
H_2N-XXX--XXXXX-CO_2H
...

H_2N-----XXXXXXX-CO_2H
H_2N-X----XXXXXX-CO_2H
H_2N-XX----XXX XXX-CO_2H
H_2N-XXX----XX XXX-CO_2H
...
.

Combinatorial Deletion

H_2N-XXXXXXXXX-CO_2H
H_2N-----XXXX XXXX-CO_2H
H_2N---X--XXX XXXX-CO_2H
H_2N---XX--XX XXXX-CO_2H
H_2N---XXX--X XXXX-CO_2H
H_2N---XXXX-- XXXX-CO_2H
...

H_2N-X----XXX XXXX-CO_2H
H_2N-X--X--XXXXX-CO_2H
H_2N-X--XX--XXXX-CO_2H
H_2N-X--XXX--XXXX-CO_2H
...

Methods of peptide optimization

Ala-scan

H_2N-XXXXXXXXX-CO_2H
H_2N-**A**XXXXXXXX-CO_2H
H_2N-X**A**XXXXXXX-CO_2H
H_2N-XX**A**XXXXXX-CO_2H
H_2N-XXX**A**XXXXX-CO_2H
H_2N-XXXX**A**XXXX-CO_2H
H_2N-XXXXX**A**XXX-CO_2H
H_2N-XXXXXX**A**XX-CO_2H
H_2N-XXXXXXX**A**X-CO_2H
H_2N-XXXXXXXX**A**-CO_2H
H_2N-XXXXXXXX**A**-CO_2H

D-scan

H_2N-XXXXXXXXX-CO_2H
H_2N-**x**XXXXXXXX-CO_2H
H_2N-X**x**XXXXXXX-CO_2H
H_2N-XX**x**XXXXXX-CO_2H
H_2N-XXX**x**XXXXX-CO_2H
H_2N-XXXX**x**XXXX-CO_2H
H_2N-XXXXX**x**XXX-CO_2H
H_2N-XXXXXX**x**XX-CO_2H
H_2N-XXXXXXX**x**X-CO_2H
H_2N-XXXXXXXX**x**-CO_2H

Disulfide-bond cyclization scan

H_2N-XXXXXXXXX-CO_2H
H_2N-**CC**XXXXXXX-CO_2H
H_2N-**C**X**C**XXXXXX-CO_2H
H_2N-**C**XX**C**XXXXX-CO_2H
H_2N-**C**XXX**C**XXXX-CO_2H
H_2N-**C**XXXX**C**XXX-CO_2H
...

H_2N-X**CC**XXXXXX-CO_2H
H_2N-X**C**X**C**XXXXX-CO_2H
H_2N-X**C**XX**C**XXXX-CO_2H
H_2N-X**C**XXX**C**XXX-CO_2H
...

Synthesis of peptides

- Peptides are typically obtained using a solid suport.
- Solid phase peptide synthesis (SPPS) was invented by R. Bruce Merrifield, 1964.
- Bruce Merrifield received the Nobel Prize in 1984.

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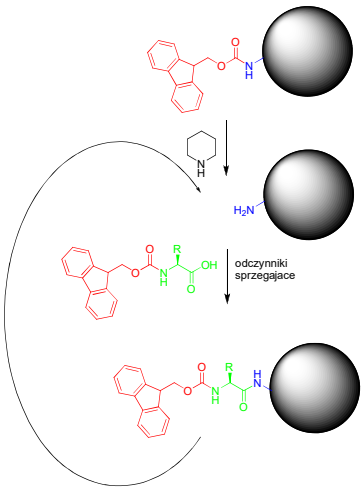
Synthesis of peptides

- Solid phase peptide synthesis involves repeated repetition of amino acid deprotection and coupling with another amino acid.
- Due to the possibility of using a large excess of reagents, the reactions proceed with very high efficiency.



Automatic peptide synthesis

Low metabolic stability

- Peptide drugs can not be used orally.

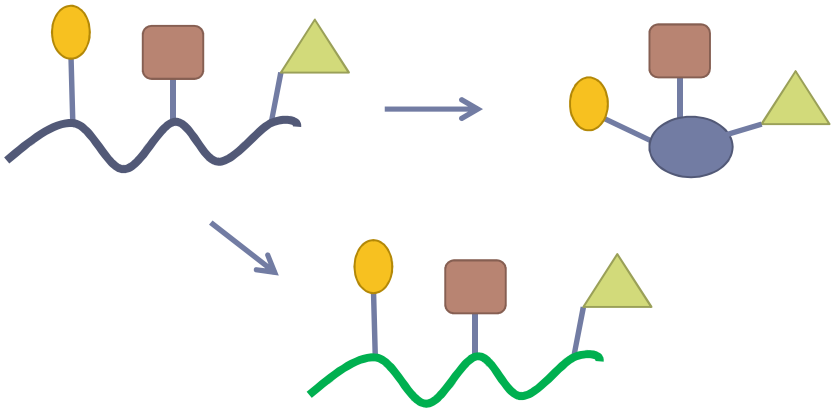
Enzymes	E.C. number	Cleavage sites
Endopeptidases		
Serine proteases	***E.C.3.4.21***	
α-Chymotrypsin	*E.C.3.4.21.1*	Tyr-|-Xaa, Trp-|-Xaa, Phe-|-Xaa, and also Leu-|-Xaa and Met-|-Xaa
Trypsin	*E.C.3.4.21.4*	Arg-|-Xaa and Lys-|-Xaa
Thrombin	*E.C.3.4.21.5*	Arg-|-Gly
Plasmin	*E.C.3.4.21.7*	Lys-|-Xaa > Arg-|-Xaa
Prolyl oligopeptidase, or prolyl endopeptidase[a]	*E.C.3.4.21.26*	Pro-|-Xaa >> Ala-|-Xaa
Plasma kallikrein	*E.C.3.4.21.34*	Arg-|-Xaa and Lys-|-Xaa, including Lys-|-Arg and Arg-|-Ser
Pancreatic elastase	*E.C.3.4.21.36*	Ala-|-Xaa, and also Gly-|-Xaa, Val-|-Xaa and Ser-|-Xaa
Leukocyte elastase, or neutrophil elastase, or lysosomal elastase	*E.C.3.4.21.37*	Val-|-Xaa and Ala-|-Xaa
Cysteine proteases	***E.C.3.4.22***	
Cathepsin B	*E.C.3.4.22.1*	Arg-Arg-|-Xaa, and also Leu-|-Xaa, Ala-|-Xaa, Phe-|-Xaa and Trp-|-Xaa
Clostripain, or endoproteinase Arg-C	*E.C.3.4.22.8*	Arg-|-Xaa including Arg-|-Pro, but not Lys-|-Xaa
Calpain-1, or μ-calpain	*E.C.3.4.22.52*	Met-|-Xaa, Tyr-|-Xaa and Arg-|-Xaa (with Leu or Val as the P2 residue)
Aspartic acid proteases	***E.C.3.4.23***	
Pepsin	*E.C.3.4.23.1*	Preferentially Phe-|-Xaa, Tyr-|-Xaa and also Leu-|-Xaa and Trp-|-Xaa, ideally with Xaa = Phe, Trp, or Tyr
Cathepsin D	*E.C.3.4.23.5*	Preferentially Phe-|-Xaa, Tyr-|-Xaa and Leu-|-Xaa, ideally with Xaa ≠ Ala or Val
Metalloproteases	***E.C.3.4.24***	
Neprilysin, or enkephalinase, or neutral endopeptidase[a]	*E.C.3.4.24.11*	Xaa-|-Tyr, Xaa-|-Phe, Xaa-|-Trp and Xaa-|-Leu
Thimet oligopeptidase, or endo-oligopeptidase A, or endopeptidase 24.15, or pz-peptidase[a]	*E.C.3.4.24.15*	Xaa-|-Arg, Xaa-|-Ser, Xaa-|-Ile, Xaa-|-Ala, Xaa-|-Gly

Low metabolic stability

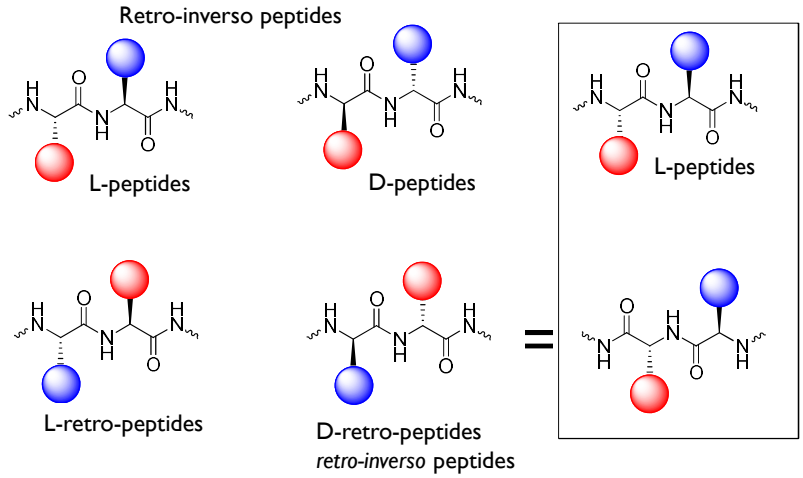
- Peptide drugs can not be used orally.

Exopeptidases		
Aminopeptidases	***E.C.3.4.11***	***N-term***
Leucyl-aminopeptidase	*E.C.3.4.11.1*	Preferentially Leu-|-Xaa, but not Arg-|-Xaa and Lys-|-Xaa
Aminopeptidase M or N, or alanyl-aminopeptidase, or membrane alanine aminopeptidase[a]	*E.C.3.4.11.2*	Preferentially Ala-|-Xaa and Tyr-|-Xaa, if Yaa-Pro-|-Xaa in N-term with Yaa = Ala, Val, Leu, Ile, Phe, Tyr or Trp then the dipeptide Yaa-Pro could be released
Aminopeptidase A, or angiotensinase, or glutamyl-aminopeptidase[a]	*E.C.3.4.11.7*	Glu-|-Xaa >> Asp-|-Xaa
Dipeptidyl-peptidases and tripeptidyl-peptidases	***E.C.3.4.14***	***N-term (di- and tripeptides)***
Dipeptidyl-peptidase I, or cathepsin C or J	*E.C.3.4.14.1*	Xaa-Yaa-|-Zaa-, if Xaa ≠ Arg or Lys, or Yaa ≠ Pro, or Zaa ≠ Pro
Dipeptidyl-peptidase IV[a]	*E.C.3.4.14.5*	Preferentially Xaa-Pro-|-Yaa- (but also Xaa-Ala-|-Yaa-) with Yaa ≠ Pro or Hyp
Prolyl tripeptyl-peptidase	*E.C.3.4.14.12*	Xaa-Yaa-Pro-|-Zaa if Zaa ≠ Pro
Peptidyl-dipeptidases	***E.C.3.4.15***	***C-term***
Peptidyl-dipeptidase A, or angiotensin-converting enzyme[a]	*E.C.3.4.15.1*	Xaa-|-Yaa-Zaa, if Yaa ≠ Pro, or Zaa ≠ Asp or Glu
Metallo-carboxypeptidases	***E.C.3.4.17***	***C-term***
Carboxypeptidase A	*E.C.3.4.17.1*	Xaa-|-Yaa if Yaa ≠ Asp, Glu, Arg, Lys or Pro
Carboxypeptidase B, or protaminase	*E.C.3.4.17.2*	Xaa-|-Arg and Xaa-|-Lys
Carboxypeptidase N, or lysine(arginine) carboxypeptidase, or kininase I[a]	*E.C.3.4.17.3*	Xaa-|-Lys >> Xaa-|-Arg
Carboxypeptidase U or R	*E.C.3.4.17.20*	Xaa-|-Arg and Xaa-|-Lys
Glutamate carboxypeptidase II, or folate hydrolase	*E.C.3.4.17.21*	Xaa-|-Glu, preferentially with Xaa = Asp or Glu

Scaffold hopping - peptidomimetics



Types of analogs



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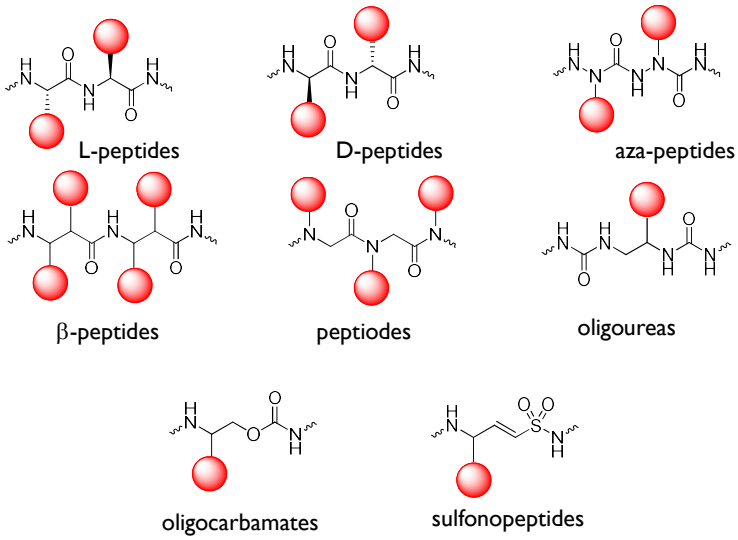
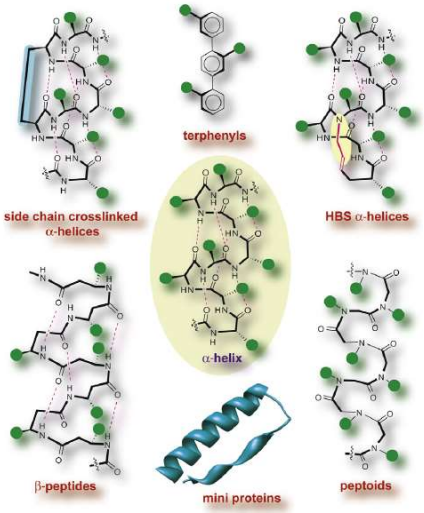


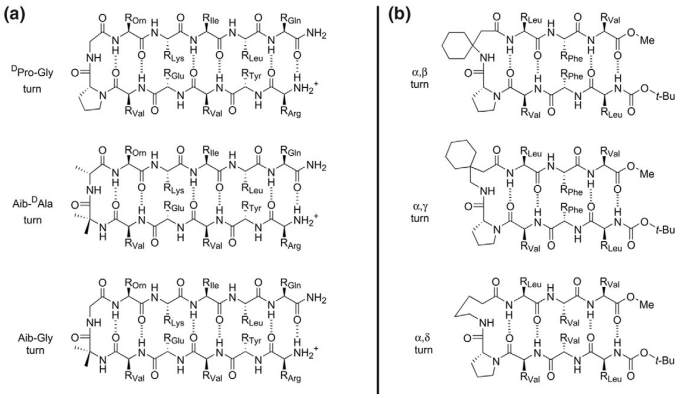
Analogs with a helix structure

- Helis are one of the key structural elements of bioactive peptides.
- Stabilization of short fragments of oligomers in helical conformation results in increased activity.

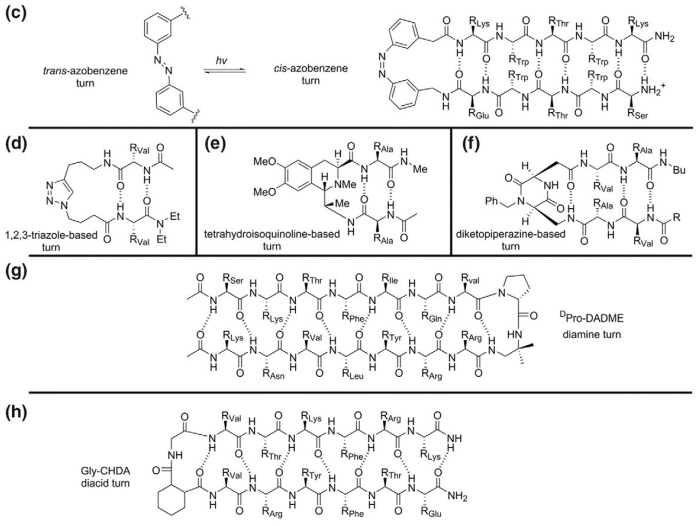


Analogs of β-turns and β-sheets

- Insertions of D-amino acid residues or β,γ,δ-amino acid residues

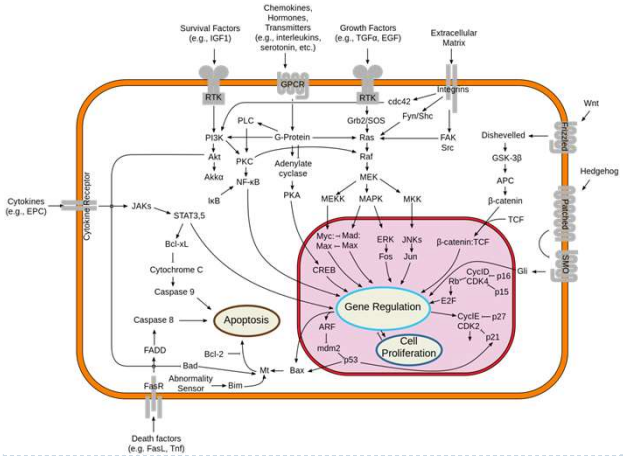


Analogs of β-turns and β-sheets



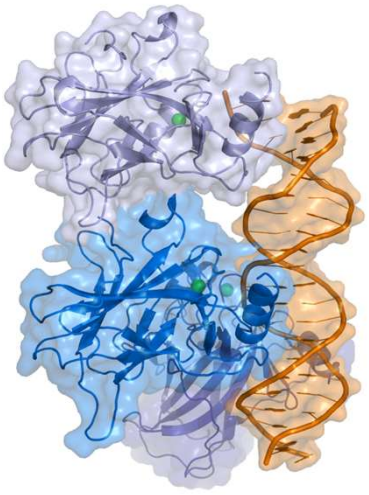
p53-MDM2

- Inhibition of the interaction of p53-MDM2 proteins is one of the more promising anti-cancer strategies.



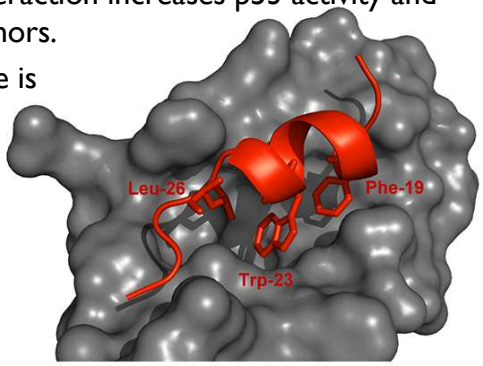
p53-MDM2

- The p53 protein has the function of tumor suppressor. It regulates the cell cycle and is involved in DNA repair and induction of apoptosis in response to damaged DNA.
- The p53 protein can be activated by a very large number of different stress conditions.



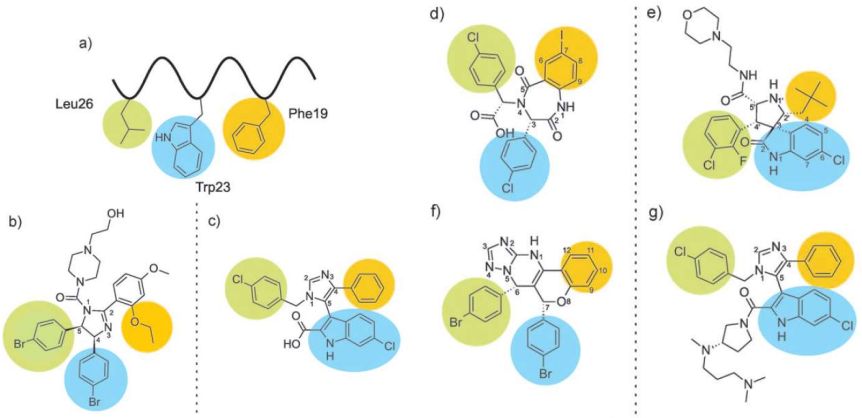
p53-MDM2

- MDM2 is a negative regulator of p53.
- MDM2 protein has ubiquitin ligase activity to p53, leading to degradation of p53.
- Inhibition of p53-MDM2 interaction increases p53 activity and inhibits development of tumors.
- Structural motif Leu, Trp, Phe is responsible for binding of p53 to MDM2



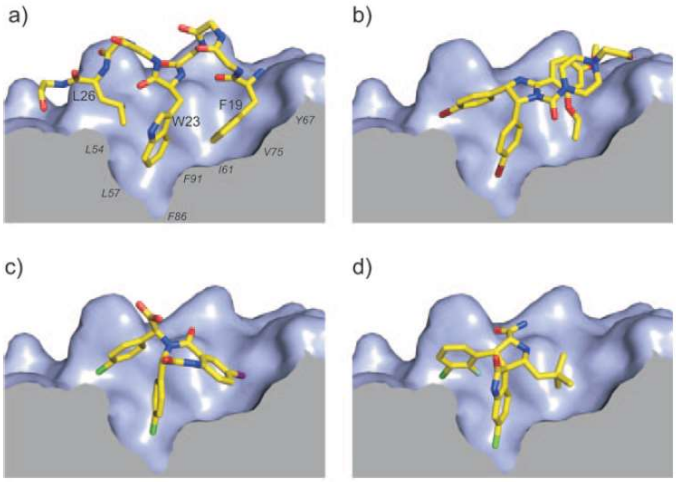
p53-MDM2

- A significant number of non-peptide analogs of Leu, Trp, Phe were designed.



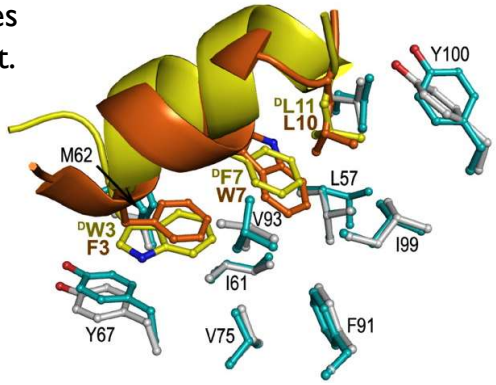
p53-MDM2

- Crystal structures showed similar binding.



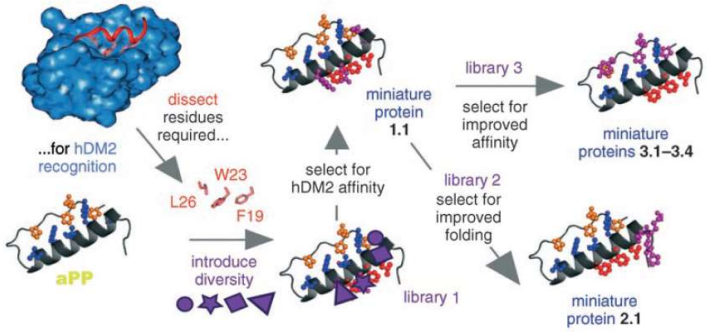
p53-MDM2 D-peptides

- D-peptides are significantly more resistant to peptidases
- D-peptides form helices with the opposite twist.



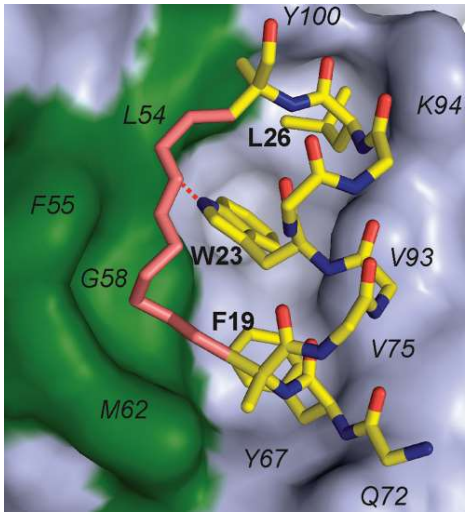
p53-MDM2, miniproteins

- Miniproteins are α-peptides with stable conformation due to the presence of disulfide bonds.
- A large range of sequence modifications is possible without changing the conformation of the molecule.



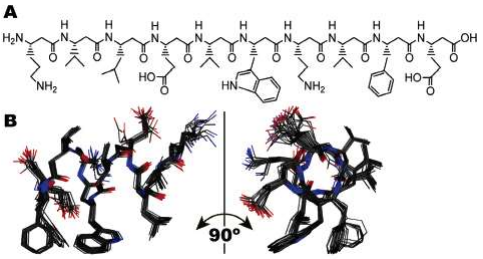
p53-MDM, stapled pepide

- '*stapled peptides*' – Fixed-conformation (mostly helical) conformation peptides by combining amino acid residues close to each other.



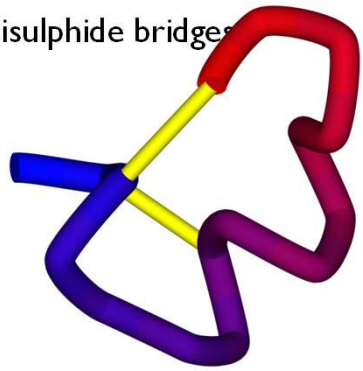
p53-MDM, beta-peptides

- β-peptides have stable secondary structures even for short sequences!
- β–peptides are completely resistant to enzymatic hydrolysis.
- The synthesis methods are analogous to the α-peptides.



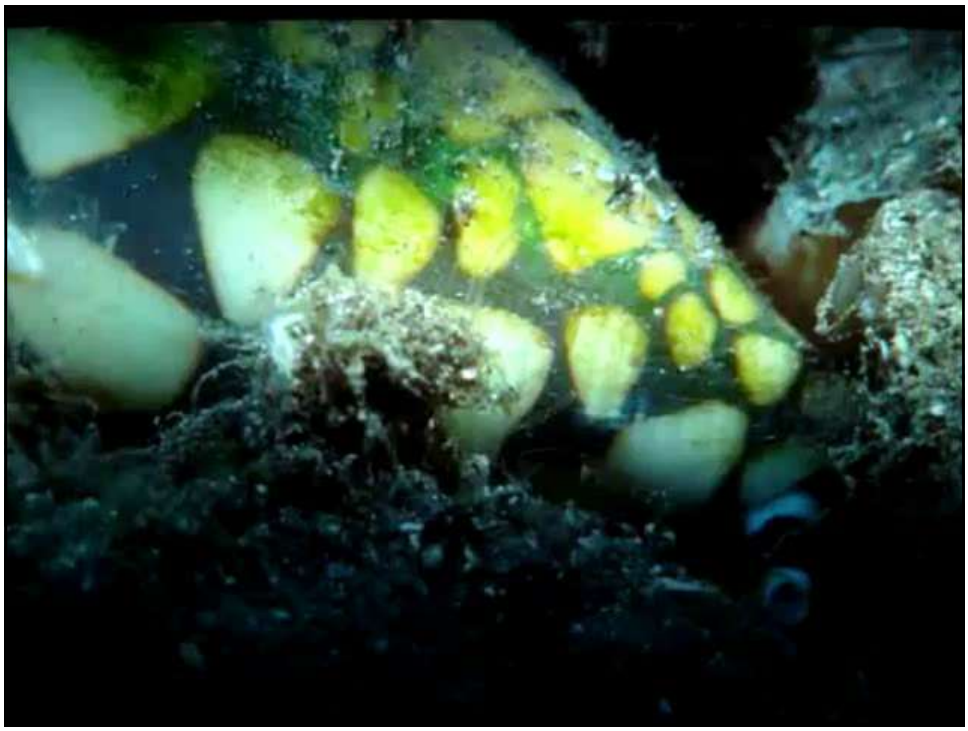
Conotoxins

- Neurotoxic peptides isolated from marine snail venom (*Conic* spp.)
- They consist of 10-30 amino acid residues
- The structure is stabilized by disulphide bridges
- Most often associated to ion channels.



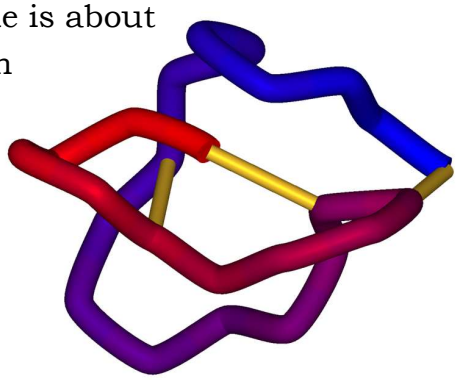
Conotoxins

- There are 500-700 species of Snails from the *Conus* family.
- They produce about 50,000 - 140,000 peptides.
- α-conotoxins inhibit acetylcholine receptors
- δ-conotoxins - sodium channels
- ω-conotoxins - calcium channels.



Conotoxins

- β-Conotoxin M VII blocks calcium channels (controlled by potential) and thereby exhibits analgesic properties.
- The effect of this peptide is about 1000 Times higher than morphine.
- ω-conotoxin has the structure of node.



Conotoxins as drugs

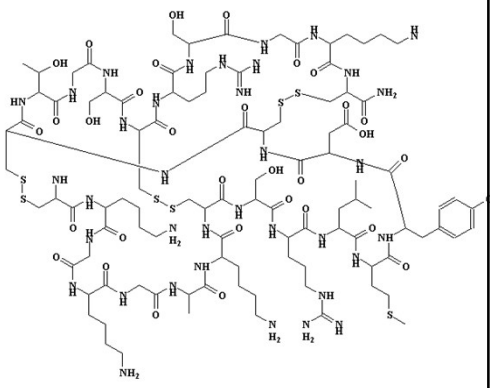
Clinical application	Conopeptide	Sequence	Target	Clinical status
Pain	ω-MVIIA (Ziconitide, Prialt®)	CKGKGAKCSRLMYDCCTGSCRSGKC*	Ca^{2+} channel ($Ca_v2.2$)	FDA approved
Pain	ω-CVID (AM336)	CKSKGAKCSKLMYDCCSGSCSGTVGRC*	Ca^{2+} channel ($Ca_v2.2$)	Phase I
Pain	Contulakin-G (CGX-1160)	ZSEEGGSNATKKPYIL	Neurotensin receptor	Phase I
Pain	α-Vc1.1 (ACV1)	GCCSDPRCNYDHPEIC*	nAChR (α9α10)	Phase I
Pain	χ-MrlA (Xen2174)	NGVCCGYKLCHOC	Norepinephrine transporter	Phase I
Pain/Neuro-protection	Conantokin-G (CGX-1007)	GEγγLQγγNQγγLIRγγKSN*	NMDA receptor (NR2B)	Preclinical
Epilepsy	Conantokin-G (CGX-1007)	GEγγLQγγNQγγLIRγγKSN*	NMDA receptor (NR2B)	Phase I
Pain	μ-conotoxins	Various	Na^+ channels	Preclinical
Myocardial infarction	κ-PVIIA (CGX-1051)	CRIONQKCFQHLDDCCSRKCNRFNKCV	K^+ channel (K_v1)	Preclinical

Ziconotide

- Peptide consisting of 25 amino acid residues and 3 disulfide bridges
- Isolated from Conus magnus (Philippine coral reef)
- Used to treat chronic pain associated with cancer.

Ziconotide

- Ziconotide blocks the calcium channels very effectively, preventing the transmission of pain signals to the brain.
- K_i = 1.1 pM (10^{-12}M)



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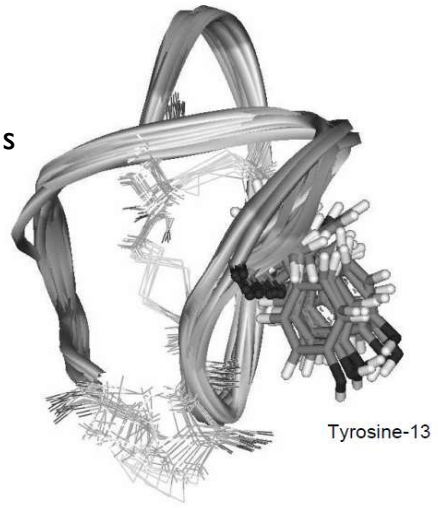
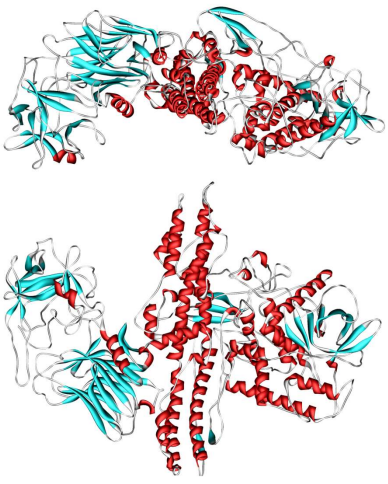


Botulinum toxin

- Protein produced by the anaerobic bacteria Clostridium botulinum.
- It is one of the most toxic substances:
- LD50 = 1.3-2 ng / kg
- Protein consists of two chains connected by a disulfide bridge (mass 147 kDa)

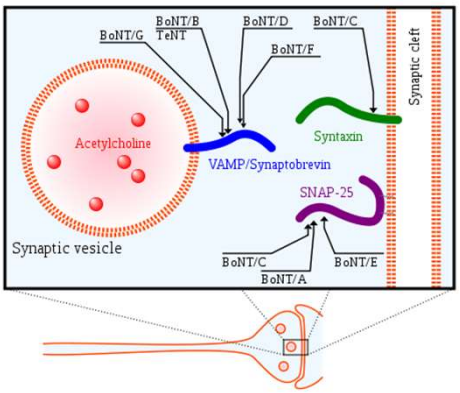


Botulinum toxin

- Present in spoiled meat (1897).
- First used to remove wrinkles in 1992 (Dermatologists, Carruthers, Canada)
- Currently, the Botox market is worth about USD 2 billion.

Botulinum toxin

- Botulinum toxin causes paralysis by blocking the secretion of acetylcholine
- The light chain has protease activity
- Botulinum A causes protein degradation SNAP-25 responsible for the secretion neurotransmitters from axon ends.



Summary

- Peptides are very good candidates for drugs with a very broad spectrum of biological activity.
- Due to rapid peptide metabolism, the method of administration and / or development of analogs is required.