Peptide Design: Application in Agriculture and Medicine

Lytic Peptides: A Historical Perspective

- Melittin
- Cecropins
- Defensins
- Magainins
- Etc……

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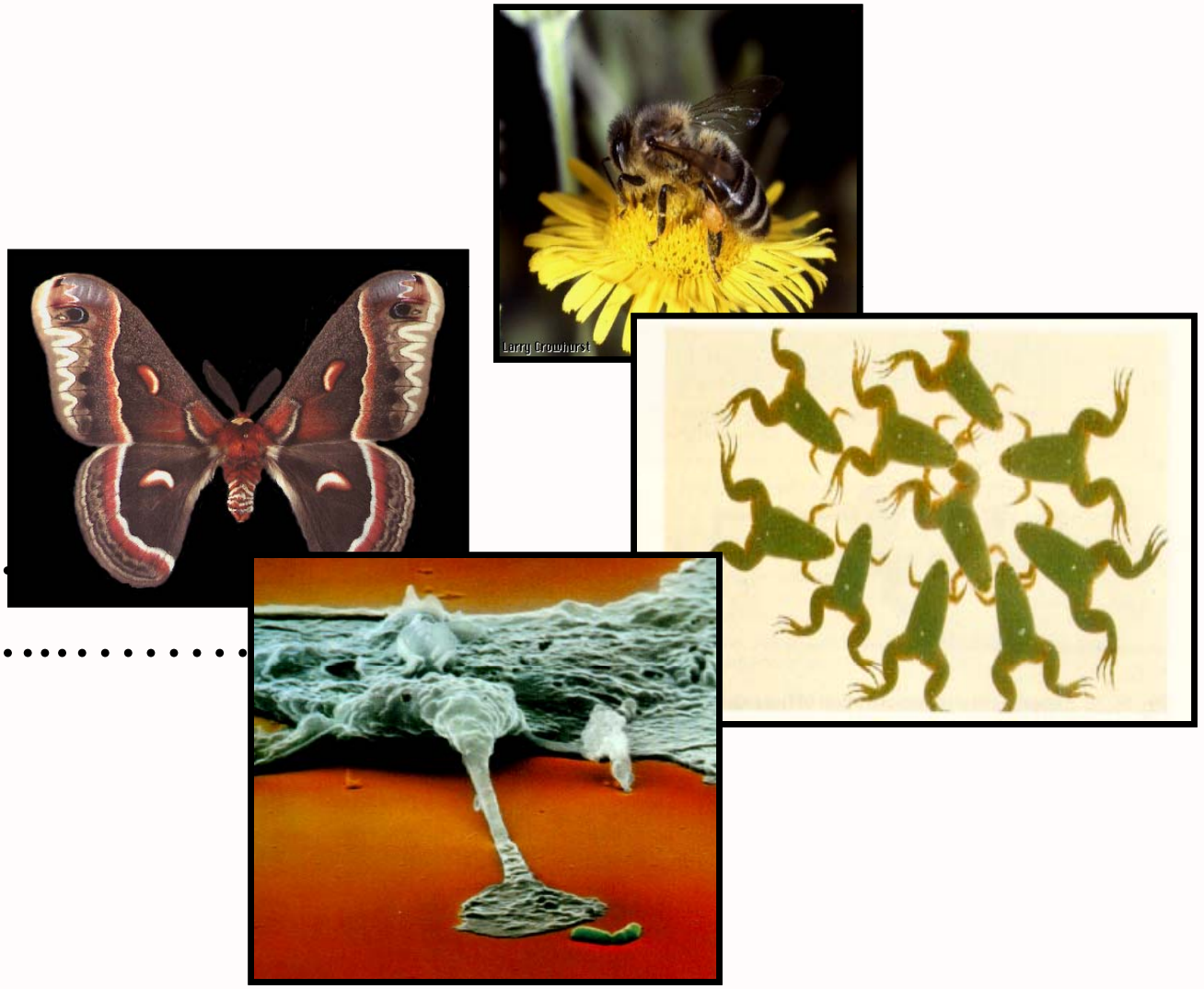

Natural Lytic Peptides

1	**temporin h**	LSPNLLKSLLGK
2	**indolicidin**	ILPWKWPWWPWRR
3	**temporin b**	LLPIVGNLLKSLLGK
4	**temporin g**	FFPVIGRILNGILGK
5	**bombinin h**	IIGPVLGLVGSALGGLLKKI
6	**bombinin h-met 8**	IIGPVLGMVGSALGGLLKKI
7	**ranalexin**	FLGGLIKIVPAMICAVTKKC
8	**melittin f**	KVLTTGLPALISWIKRKRQQ
9	**brevenin 1eb**	VIPFVASVAAEMQHVYCAASRKC
10	**brevenin 1ea**	FLPAIFRMAAKVVPTIICSITKKC
11	**brevenin 1e**	FLPLLAGLAANFLPKIFCKITRKC
12	**brevenin 1**	FLPVLAGIAAKVVPALFCKITKKC
13	**gaegurin 5**	FLGALFKVASKVLPSVFCAITKKC
14	**gaegurin 6**	FLPLLAGLAANFLPTIICKISYKC
15	**bombinin-like blp-3**	GIGAAILSAGKSALKGLAKGLAEHF
16	**bombinin-like blp-4**	GIGAAILSAGKSIIKGLANGLAEHF
17	**melittin 1**	GIGAVLKVLTTGLPALISWIKRKRQQ
18	**melittin 2**	GIGAILKVLSTGLPALISWIKRKRQE
19	**melittin 3**	GIGAILKVLATGLPTLISWIKNKRKQ
20	**bombinin-like blp-1**	GIGASILSAGKSALKGLAKGLAEHFAN
21	**bombinin-like blp-2**	GIGASILSAGKSALKGLAKGLAEHFAN
22	**bombinin-like blp**	GIGGALLSAAKVGLKGLAKGLAEHFAN
23	**melittin 4**	GIGAVLKVLTTGLPALISWISRKKRQQ
24	**antibacterial peptide**	KGVLGWLSMASSALTGPQQPNSPWLAKIKNHK
25	**myeloid antibacterial 23**	RIIDLLWRVRRPQKPKFVTVWVR
26	**cecropin-melittin hybrid**	RWKLFKKIEKVGRGIGAVLKVLTTGL
27	**dermaseptin 4**	ALWMTLLKKVLKAAAKALNAVLVGANA
28	**dermaseptin b**	DVLKKIGTVALHAGKAALGAVADTISQ

Etc.

~500	**coleoptericin**	SLQGGAPNFPQPSQQNGGWQVSPDLGRDDKGNTRGQIEIQNKGKDHDFNAGWGKVIRGPNKAKPTWHVGGTYRR

What's Possible:
Sum of a Finite Geometric Series

$$\sum_{i=2}^{200} 20^{i} = a_1 \frac{1 - r^{n}}{1 - r}$$

"a_1" is the first term, "n" is the number of terms, and "r" is the common ratio of the series increase, i.e., it goes up by a factor of 20 each time (the number of different protein amino acids). When one goes through the arithmetic, the number of possible combinations of proteins, from two amino acids in length to 200, is 8.458×10^{257}. A huge number to say the least, particularly, when one considers that the total number of atoms of matter in the universe is estimated to be less than 10^{100}! Also, it should be noted that there are many proteins far larger than 200 amino acids in length.

Possible Number of Protein Combinations from 2-->200

84,580,000,000,000,000,000,000,000,000,000,000,000,000,000,
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000,000,000,000,000,000,000,000,000,000,000,000,000,000,000'
000,000,000,000,000,000,000,000,100,100,000

# of all types of proteins ever made

# of all types of human proteins

# of atoms in the universe

Amino Acid Characteristics

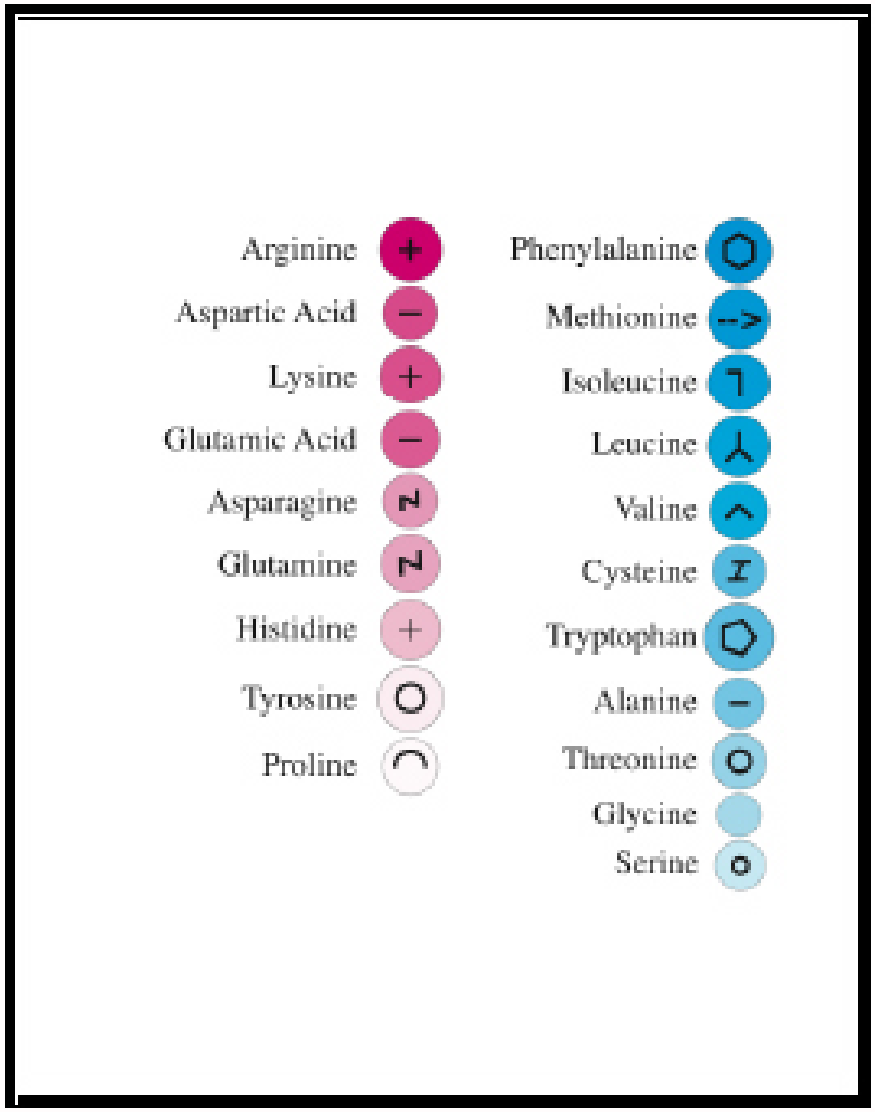
Amino Acid	Volume	Diameter	Kcal/mole	Glyph
Phenylalanine	189.9	7.13	3.7	
Methionine	162.9	6.78	3.4	
Isoleucine	166.7	6.83	3.1	
Leucine	166.7	6.83	2.8	
Valine	140.0	6.44	2.6	
Cysteine	108.5	5.92	2.0	
Tryptophan	227.8	7.58	1.9	
Alanine	88.6	5.53	1.6	
Threonine	116.1	6.05	1.2	
Glycine	60.1	4.86	1.0	
Serine	89.0	5.54	0.6	
Proline	122.7	6.16	-0.2	
Tyrosine	193.6	7.18	-0.7	
Histidine	153.2	6.64	-3.0	
Glutamine	143.9	6.50	-4.1	
Asparagine	117.7	6.08	-4.8	
Glutamic Acid	138.4	6.42	-8.2	
Lysine	168.6	6.85	-8.8	
Aspartic Acid	111.1	5.96	-9.2	
Arginine	173.4	6.92	-12.2	

Hydrophobic

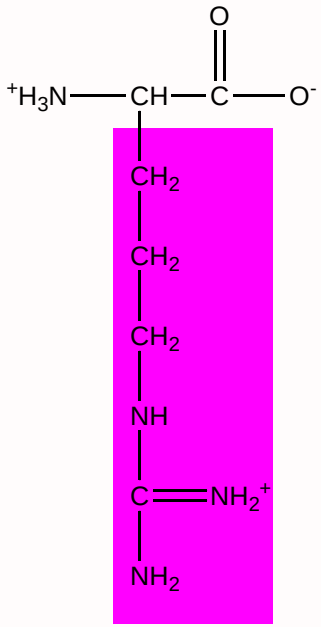
Hydrophilic

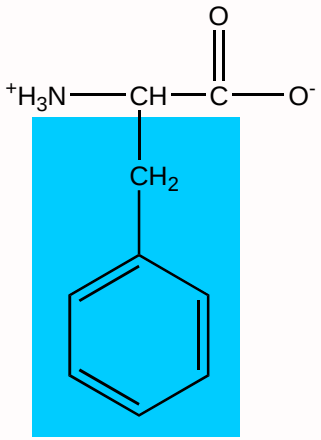
Amino Acid volumes are in cubic angstroms. Free energies are in kcal/mol, for the transfer of an amino acid residue in an α-helix from the membrane interior to water, assuming a dielectric constant of 2 (from, Engelman, D.M. Ann. Rev. Biophys. Chem. 15: 330-357. 1986.).

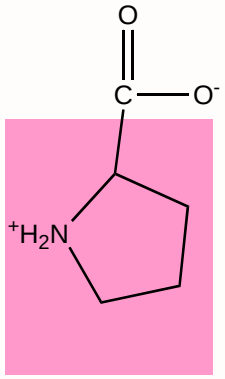
Design of Moli

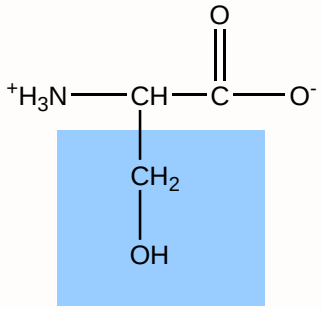


Hydrophobicity Comparison









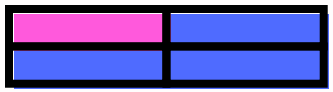
What Guides Design: Structure/Function Relationships

Thoughts on the Structure/Function Paradigm

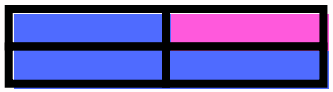
To best illustrate the physical connections between proteins and peptides (including our own), it is necessary to display their sequences in ways that make it easier to visualize structural differences and similarities. If we view our peptides, there are a number of physical features that appear to be important in modulating their activity:

1. Degree of amphipathy
2. Length of amphipathy
3. Heterogeneity of amphipathic section
4. Placement of amphipathic section (N or C terminal)
5. “+” Charge density (less or more)
6. Hydrophobicity of amphipathic section
7. Presence of hydrophobic tail
8. Length of hydrophobic tail
9. Hydrophobicity of tail
10. Placement of hydrophobic tail (N or C terminal)
11. Absence, presence, & position of “+” charged center
12. Absence or presence & position of flanking sequence
13. Predominating secondary structure
14. Termini modification (N-acetylation, C-amidation)
15. Surface area of hydrophilic and hydrophobic faces
16. Steric or volume considerations.

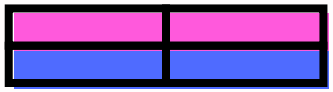
Lytic Peptide Classes



Cecropin's A, B, & D
Lepidopteran
SB-37
Shiva 1
Class-2



Melittin
Class-1

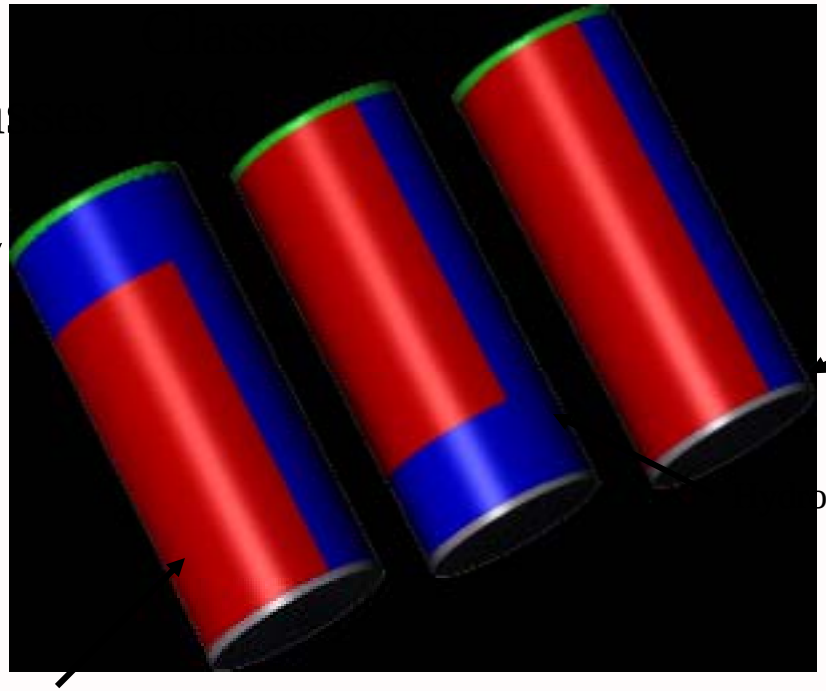


Magainin's 1 & 2
Sarcotoxin's IA, IB, & IC
Vishnu Series
Class-3

Proposed Lytic Peptide Classes

Class 3&6

Cla



Amino terminus

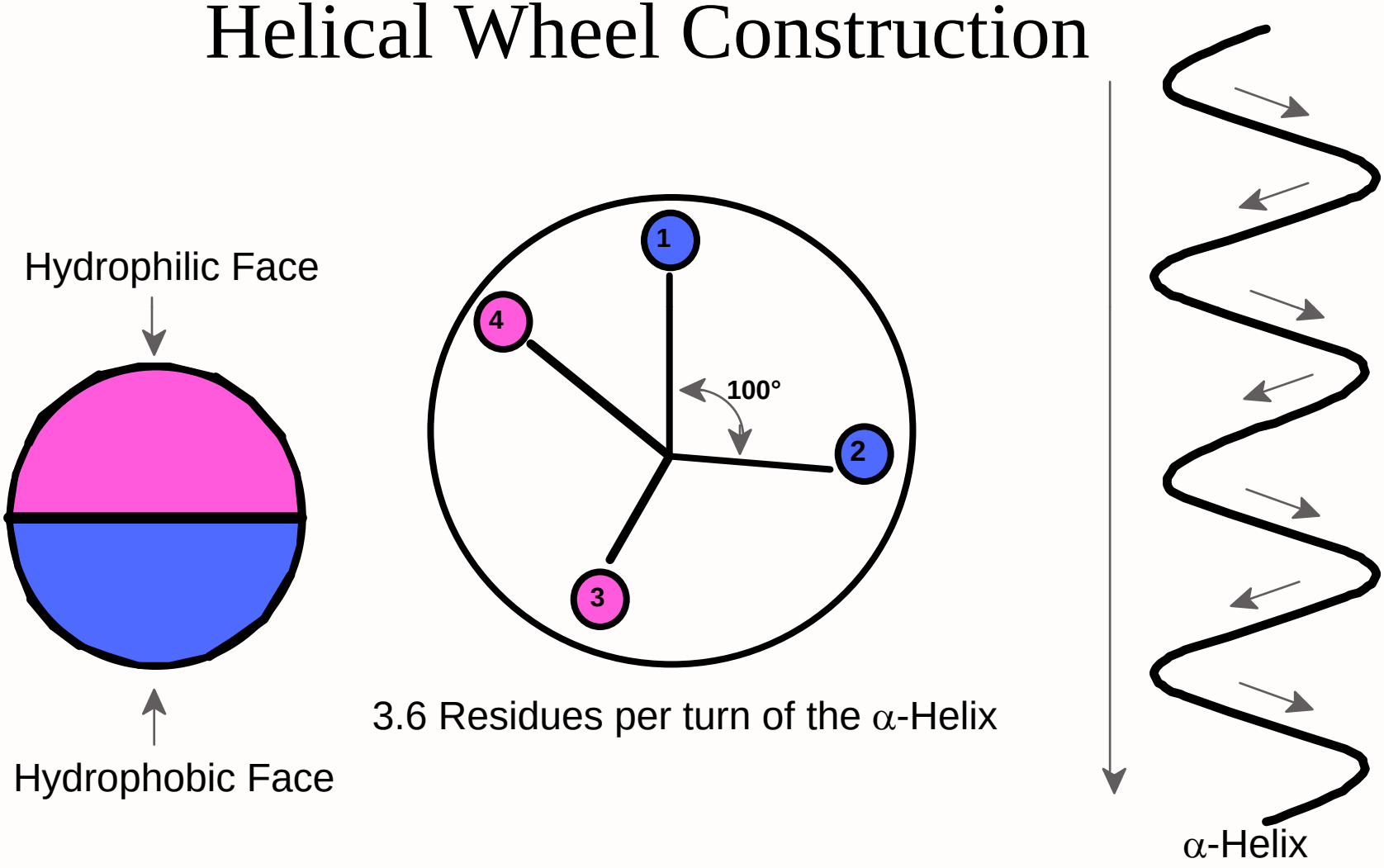
Carboxy terminus

ophobic Face

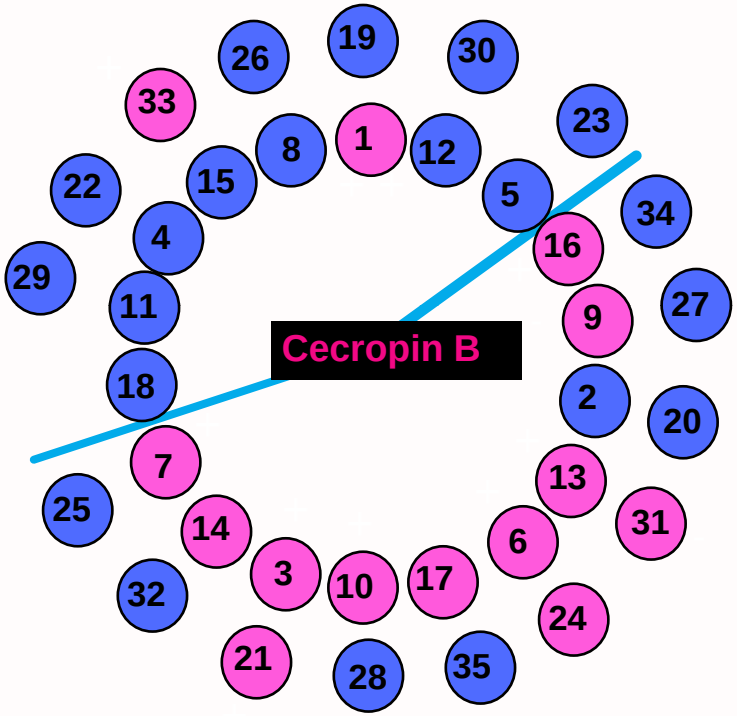
Hydrophilic Face

Classes 1, 2, & 3 are alpha helical and
Classes 4, 5, & 6 are beta sheet

Helical Wheel Construction



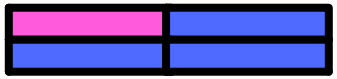
Cecropin B Amphipathy



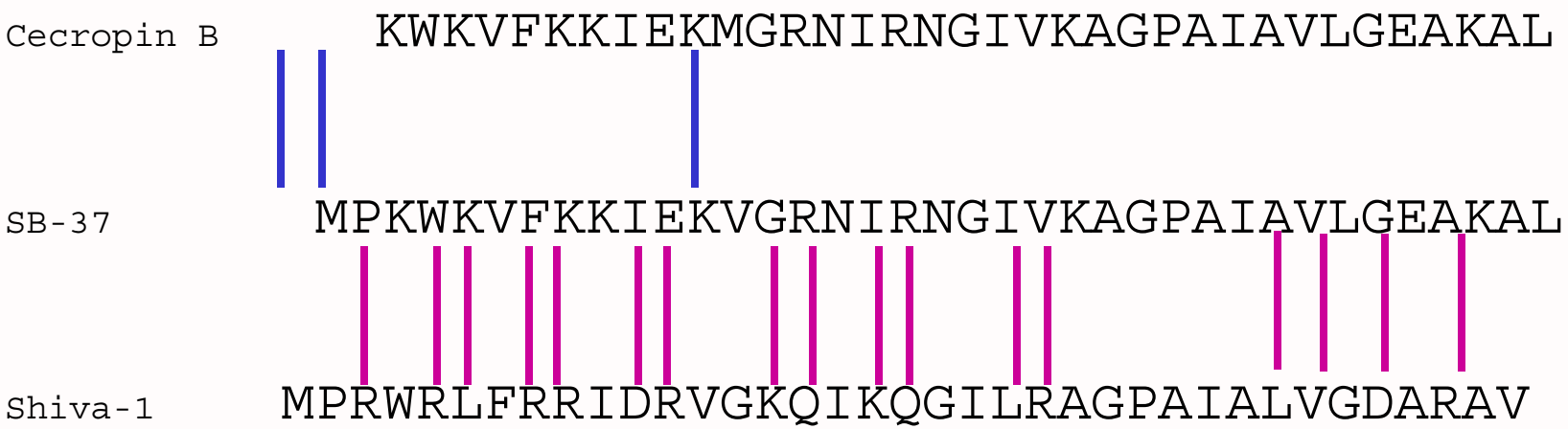
Hydrophobic

Hydrophilic

KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL



First Attempts at Design



Amino acid differences between cecropin B and SB-37

Amino acid differences between SB-37 and Shiva-1

Examples of Classes

Class 1

Pipinin 1 FLPIIAGVAAKVLFPKIFCAISKKC

Class 2

Cecropin B KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL

Class 3

Magainin 2 GIGKFLHSAKKFGKAFVGEIMNS

Representative Designs

Class 1

Pipinin 1 FLPIIAGVAAKVLFPKIFCAISKKC

D1A21 FAFAFKAFKKAFKKFKKAFKKAF

Class 2

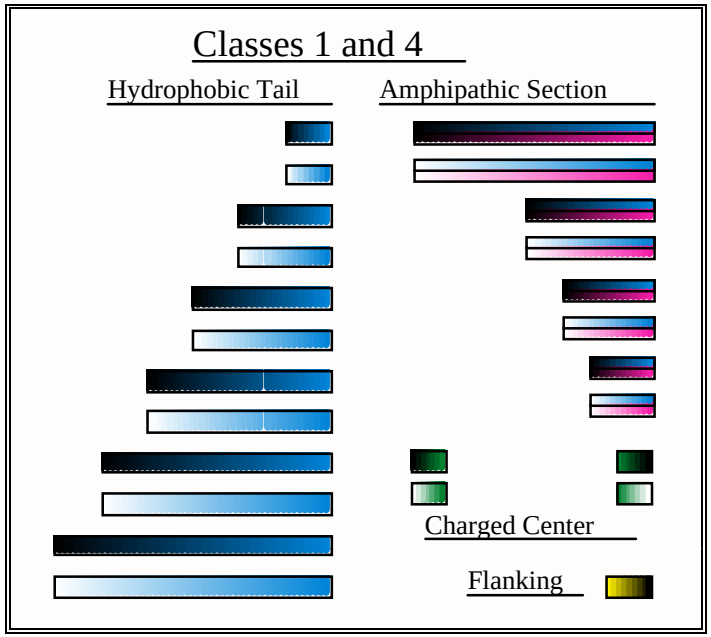
Cecropin B KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL

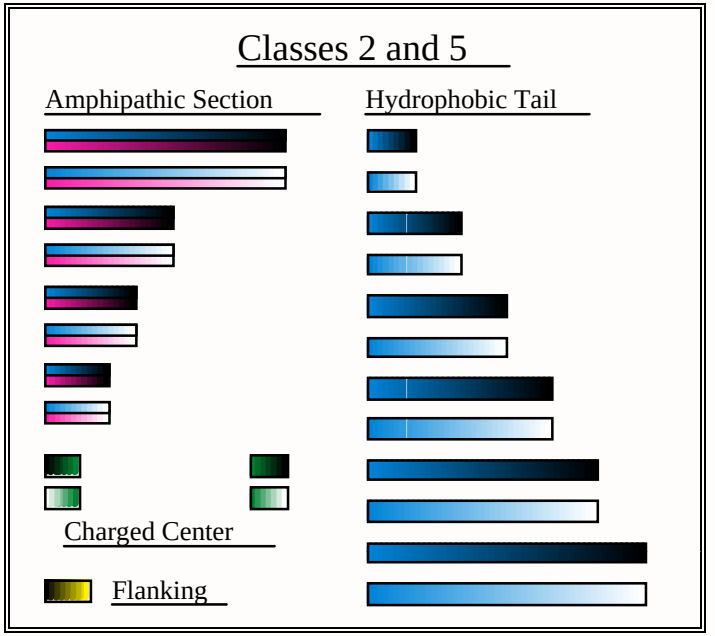
D2A21 FAKKFAKKFKKFAKKFAKFAFAF

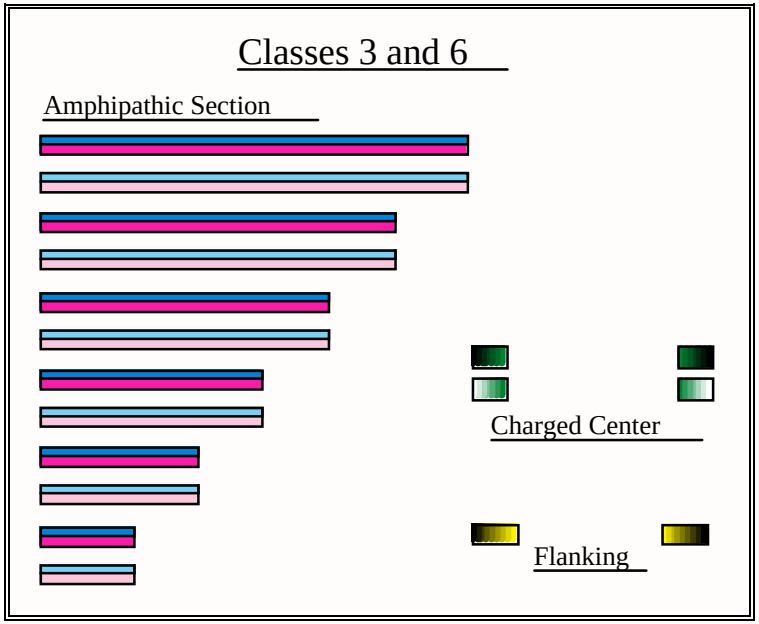
Class 3

Magainin 2 GIGKFLHSAKKFGKAFVGEIMNS

D3M1 FVKKVAKKAKKVAKKAVKVAKKV


Classes 1 and 4
Hydrophobic Tail
Amphipathic Section
Charged Center
Flanking

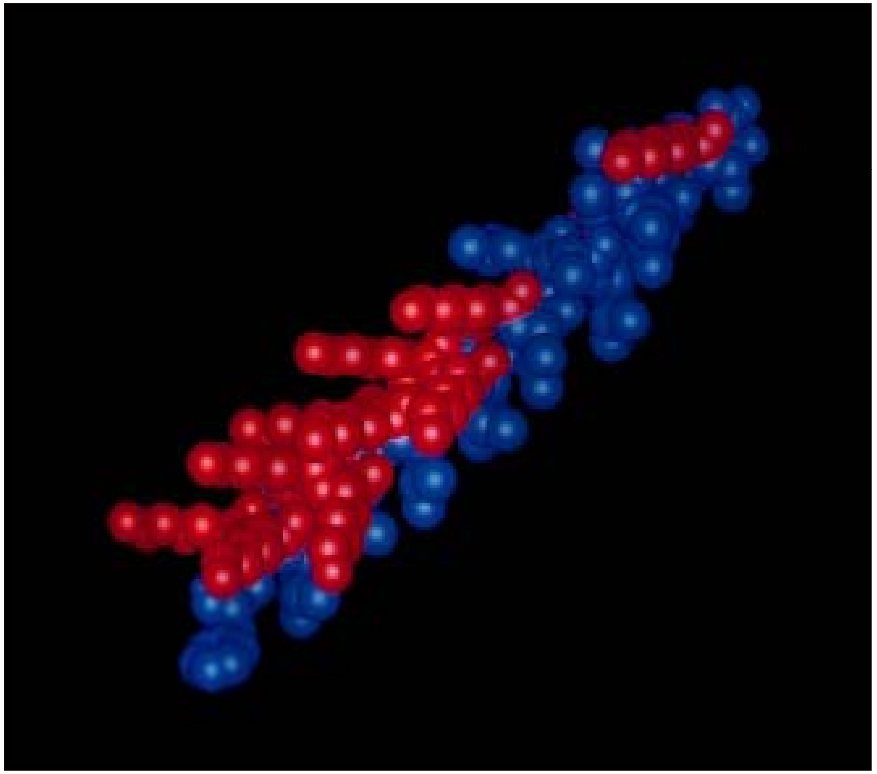

Classes 2 and 5
Amphipathic Section
Hydrophobic Tail
Charged Center
Flanking


Classes 3 and 6
Amphipathic Section
Charged Center
Flanking

Novel Peptides

- Designed for increased activity and reduced toxicity
- Designed by altering specific physical characteristics:
  - Hydrophobicity
  - Amphipathy
  - Charge density, etc…..

Potential for many new designs



We Were First in the World to Demonstrate

- Antifungal Activity
  - Cetiner S, JM Jaynes, B Blackmon. (1987) Effect of Novel Lytic Peptides on Plant Pathogenic Fungi. *Hortscience* 22(5):1057.
- Antiprotozoal Activity
  - Jaynes JM, CA Burton, SB Barr, GW Jeffers, GR Julian, KL White, FM Enright, TR Klei, and RA Laine. (1988) *In Vitro* Effect of Novel Lytic Peptides on *Plasmodium falciparum* and *Trypanosoma cruzi*. *FASEB.* 2(13): 2878-2883.
- Anticancer Activity
  - Jaynes JM, GW Jeffers, GR Julian, KL White, and FM Enright. (1989) *In Vitro* Cytocidal Effect of Lytic Peptides on Several Transformed Mammalian Cell Lines. *Peptide Research.* 2(2): 157-160.
- Enhanced Plant Disease Resistance
  - Jaynes JM, P Nagpala, L Destefano-Beltran, JH Huang, JH Kim, T Denny and S Cetiner. (1993) Expression of a Cecropin B Like Lytic Peptide in Transgenic Tobacco Confers Enhanced Resistance to Bacterial Wilt Caused By Pseudomonas Solanacearum. *Plant Science*: 89:43-53.
- Enhanced Animal Disease Resistance
  - Reed WA, PH Elzer, FM Enright, JM Jaynes, JD Morrey, KL White. (1997) Interleukin 2 Promoter/Enhancer Controlled Expression of a Synthetic Cecropin-Class Lytic Peptide in Transgenic Mice and Subsequent Resistance of Brucella Abortus. *Transgenic Research*. 6(5): 337-347.