β_2-Bungarotoxin,

- a targeted phospholipase

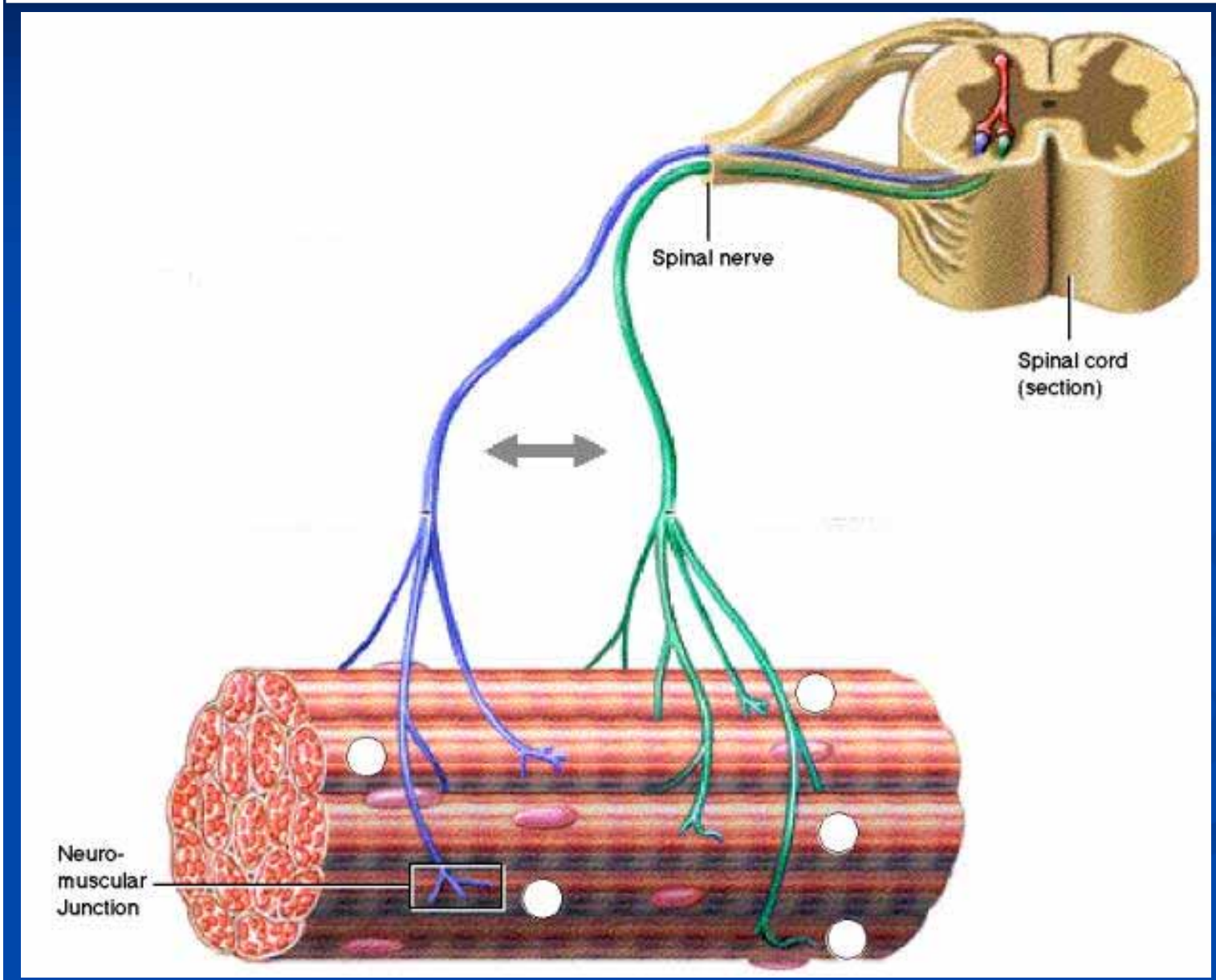
Biomolecular Structure
November 30, 2005
By Martin Schulz

Source

- Bungarus multicinctus = Taiwan (many-)banded krait

- Injects venom cocktail that works synergistically:
  - Either destroy muscle tissue
  - Or disrupt the function of the neuromuscular junction = Bungarotoxins

Excitation conduction



http://staff.fcps.net/cverdec/Adv%20A&P/Notes/Muscle%20Unit/Contration%20of%20motor/contra36.jpg

Neuromuscular junction

β-bungarotoxin acts presynaptically:
- Kunitz subunit that targets specific K^+-channels
- Phospholipase A_2

Shaker type K^+ channel (Kv 1.2)

Nerve Terminal

synaptic vesicles with acetyl choline

active zones

PRES

acetyl choline receptors

junctional fold

POSTSYNAPTIC

ion channels

α-bungarotoxin acts postsynaptically and blocks acetylcholin receptor

NEUROMUSCULAR JUNCTION

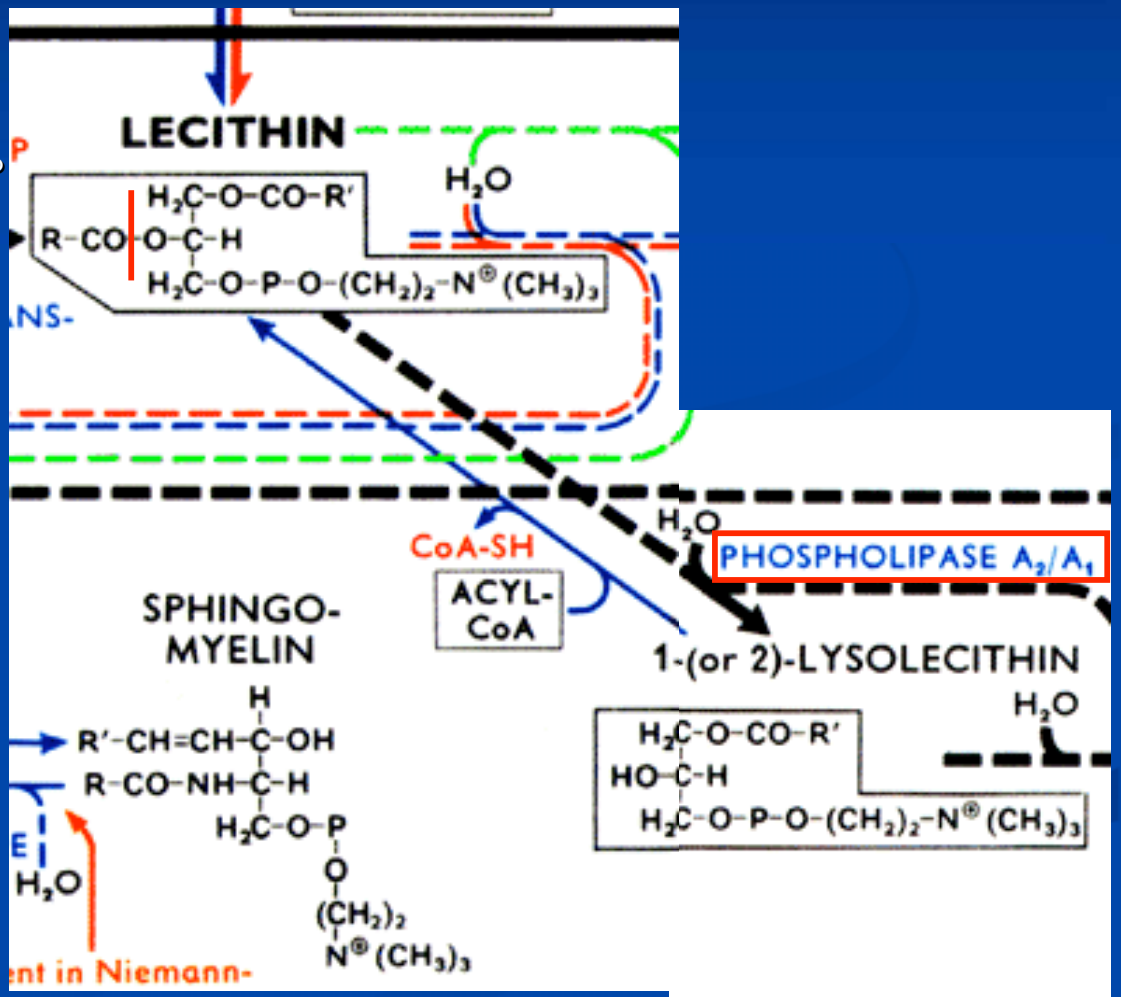
http://medlib.med.utah.edu/kw/mg/mml/mg_illus002.html

Function of Phospholipase A_2

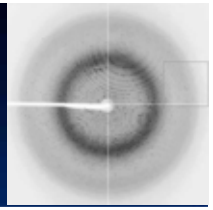
- Phospholipase A_2 = EC 3.1.1.4
  - Exclusively hydrolyses the fatty acid ester bond at the 2° position of phospholipid

⇒ Destruction of the nerve terminal

⇒ Respiratory failure



Quality of structure by Kwong



\+ Resolution: 2,45 Å

\- o R factor: 0.193
o Free R factor: 0.281

} Discrepancy, perhaps overrefinement?

\+ o Occupancy: 1 for all atoms

\+ o B-factor: 20 to 60, estimated average = 35

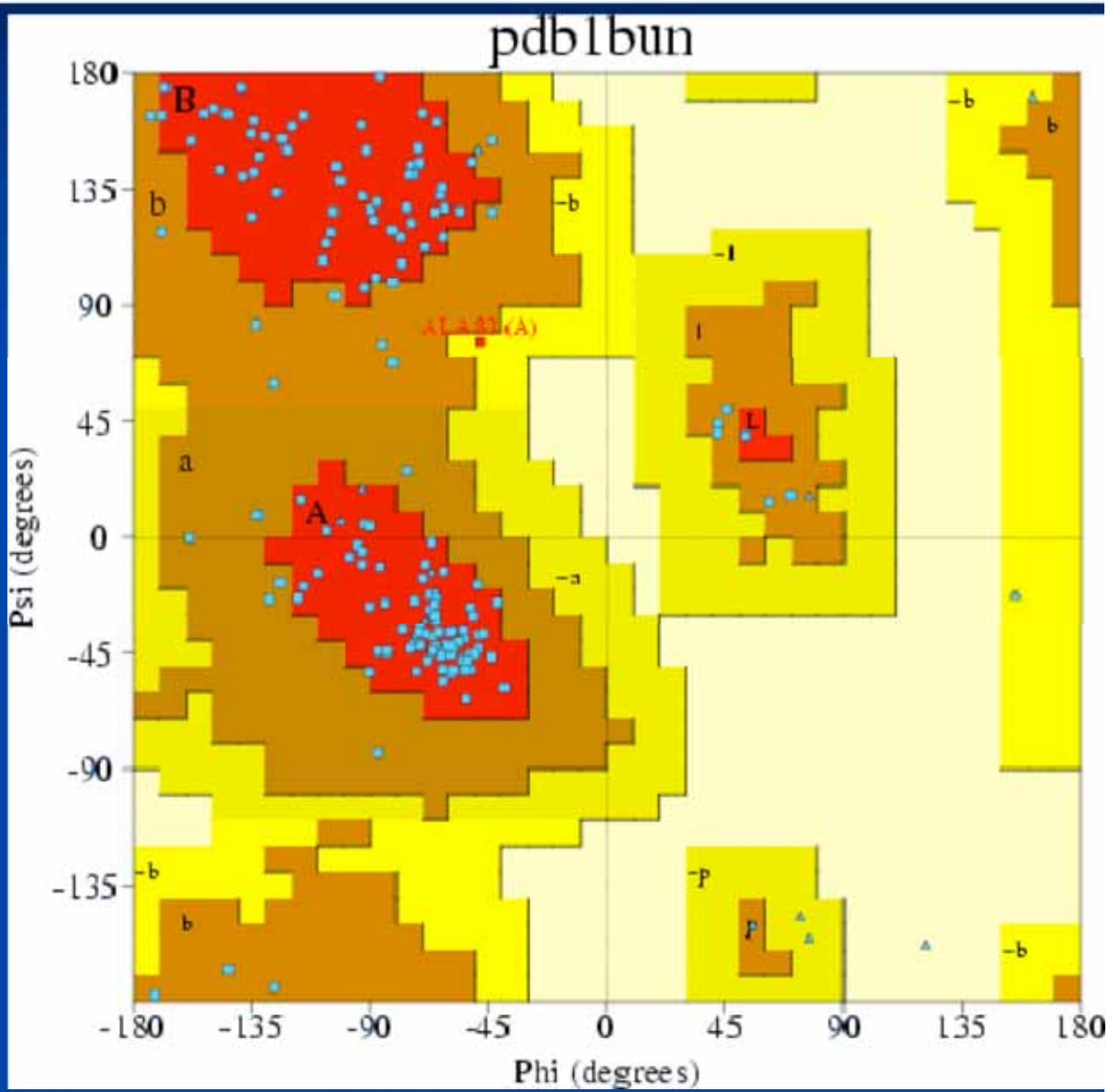
\+ o Solvent content: 81 water for 181 amino acids

o Structure solved by molecular replacement and MIR

Kwong PD et al. **Structure of 2-bungarotoxin: potassium channel binding by Kunitz modules and targeted phospholipase action**. Structure. 1995. 3:1109-1119

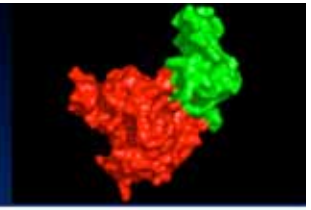
Quality of structure by Kwong

- Ramachandran plot



http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?template=main.html&o=PROCHECK&c=999&pdbcode=1bun

Structural composition of β_2-Bungarotoxin



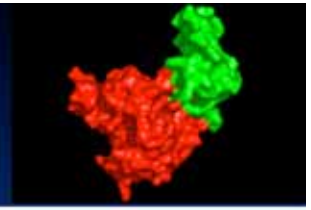
- Heterodimeric protein:
  - Phospholipase A_2 (= PLA_2) = subunit A:
  - Kunitz protease inhibitor for K^+-channel binding subunit = subunit B

- Each subunit for itself is globular *(F1,2)*

- Joining product is extended ~60 Å x 40 Å x 20 Å *(F3)*

- Separation of binding and activity is unusual for toxins

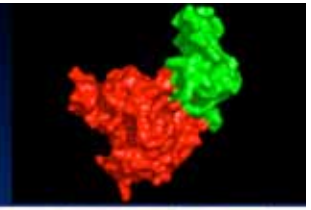
Structural composition of β_2-Bungarotoxin



- Covalently linked by one disulfide bond:
  - Cys15 of subunit PLA_2 and Cys55 of subunit Kunitz *(F3)*

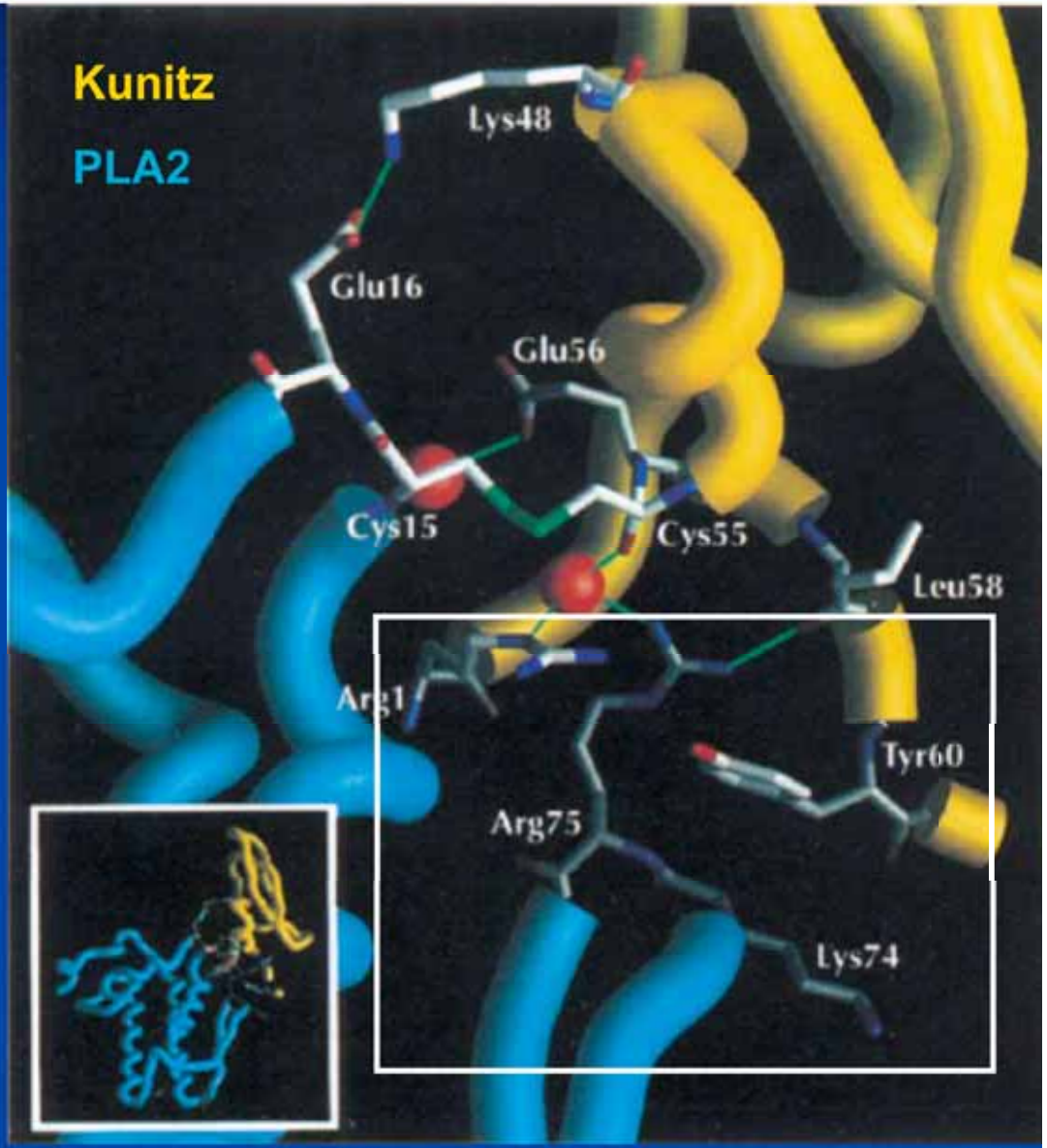
Typical for extracellular proteins

Structural composition of β_2-Bungarotoxin



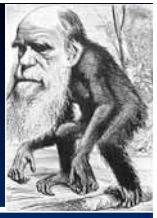
- No backbone to backbone contacts
- Only few substantial hydrophobic interactions
- majority charged, mostly water-mediated
  (94 % of surface area)

Differs from „normal“ protein-protein interaction



From [1]

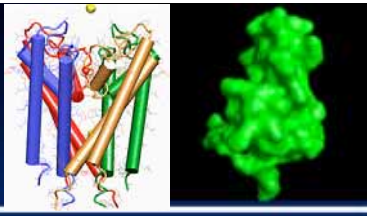
Evolution



- Toxins evolved from body proteins

  -> sequence and structure diverged, but molecular scaffold of ancestral protein is conserved

Potassium channel binding subunit

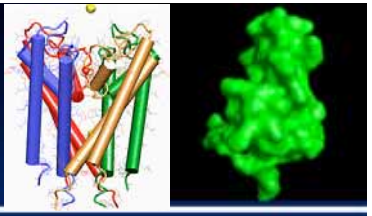


= member of Kunitz protease inhibitor superfamily

- 61 amino acids
- Stabilized by 3 disulfide bonds *(F1)*

- Homologous to bovine pancreatic trypsin inhibitor (BPTI)
- , but lost its protease inhibitory function

Potassium channel binding subunit



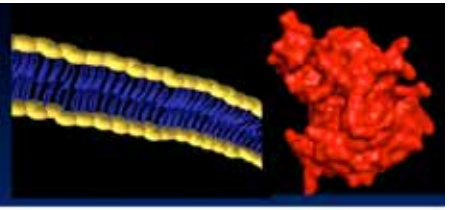
- Binds the voltage-gated Shaker type K^+ channel (Kv 1.2), which is specifically expressed in presynaptic motor nerve terminal with nanomolar affinity

- , but does not block it totally (, like most other venoms do)

  ⇒ just weak intrinsic inhibitor of this channel

=> Targeting

Phospholipase A_2 subunit



- 120 amino acids
- Stabilized by 6 disulfide bonds *(aF2)*
- Core constructed by 3 α-helices and a calcium binding loop *(aF1)*
- Phospholipase A_2 , homologous to bovine pancreatic PLA_2 *(aF3+bF1+align)*

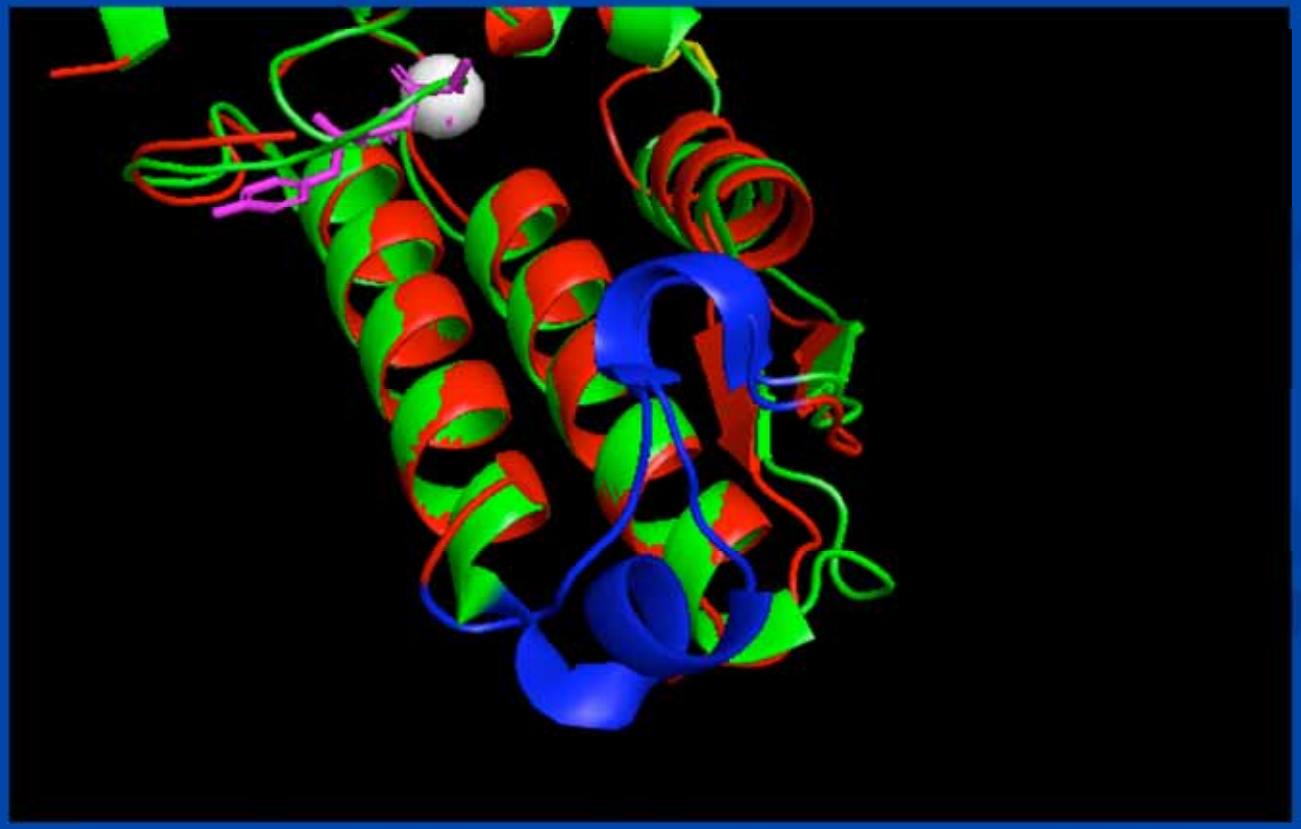
Alignment of subunit a from beta2-bungarotoxin and bovine pnacreatic PLA2:

Red = subunit a of beta2-bungarotoxin;

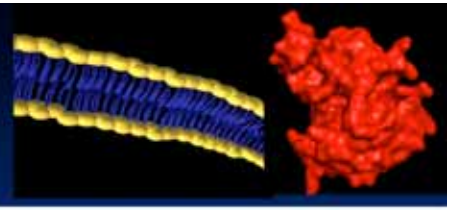
green = bovine pancreatic PLA2;

Blue = substrate binding loop, where the alpha-helix containing segment is from bovine pancreatic PLA2

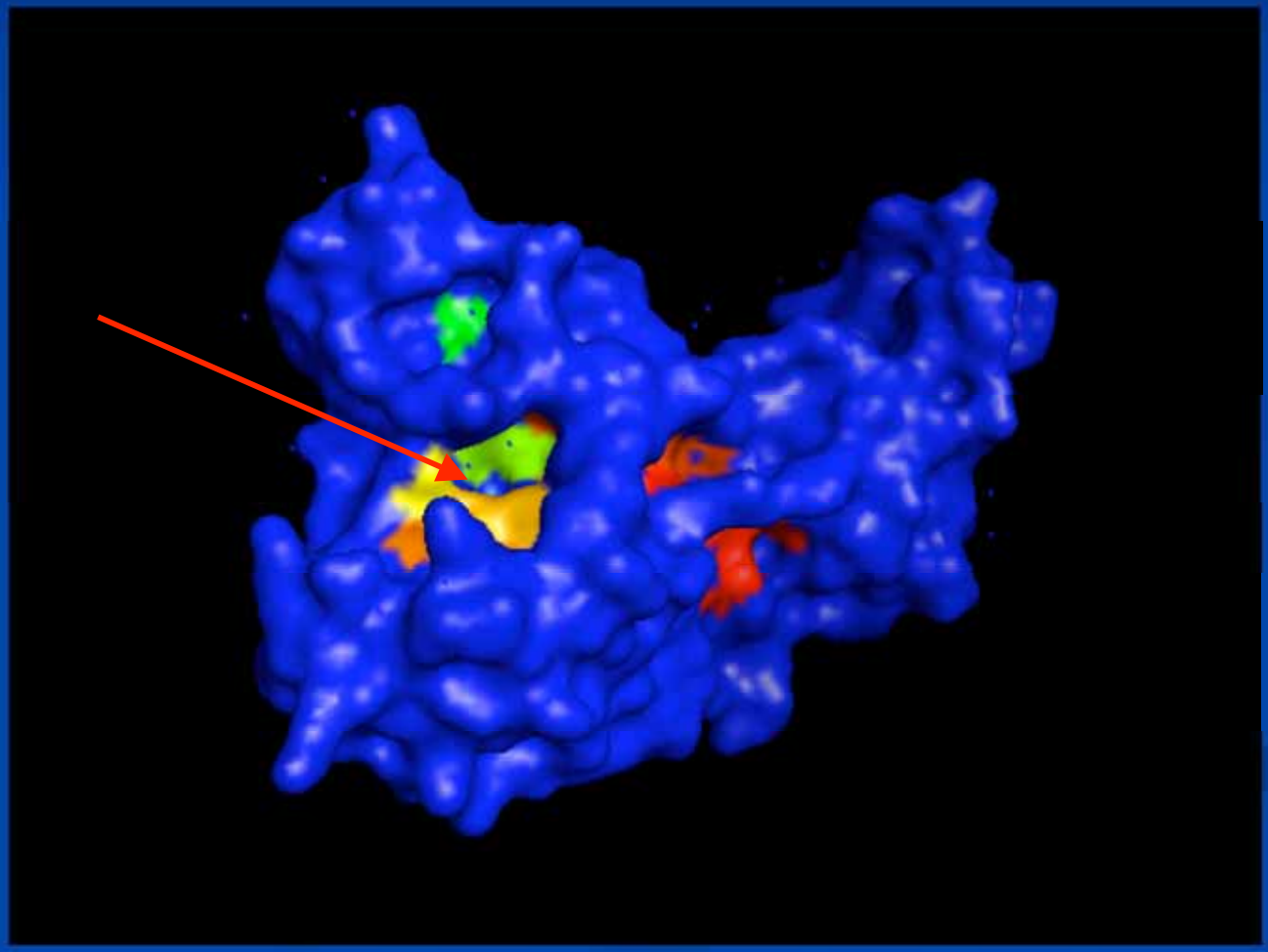
Magenta = the Ca2+ complexing residues of subunit a of beta2-bungarotoxin



Phospholipase A_2 subunit

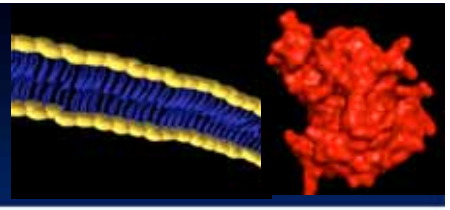


- Detecting active site location with hotpatch concavity search:



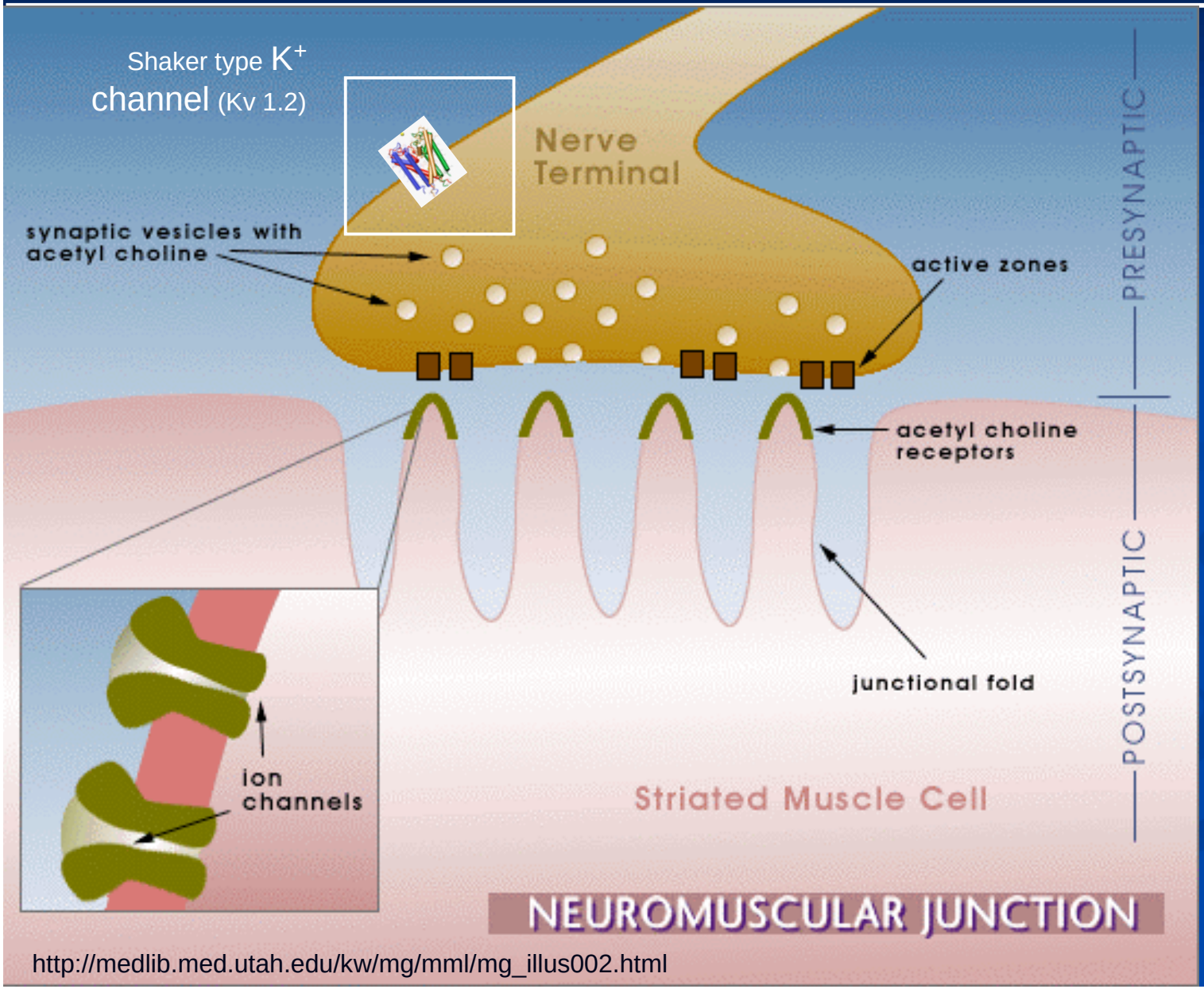
Red arrow: points on the active binding site of PLA2

Occluded active site of Phospholipase A2



- Weak enzymatic activity *(aF1)*
- , but high specificity in combination with subunit B
  => occluding Trp19 acts as lipophilic anchor *(aF2+bF1)*

Summary (1)



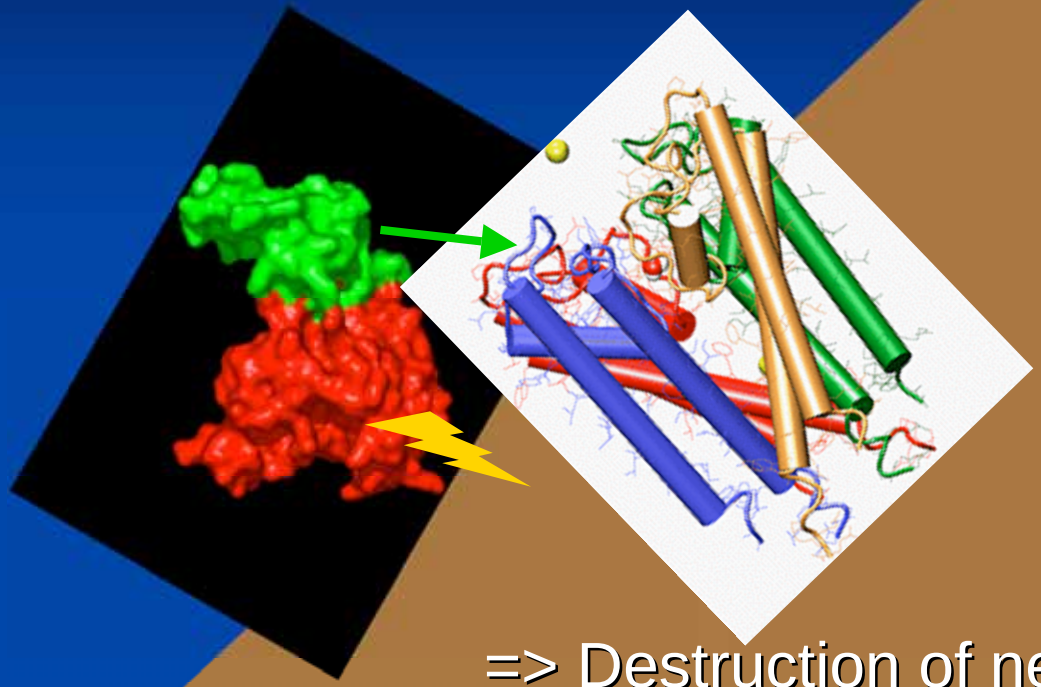
http://medlib.med.utah.edu/kw/mg/mml/mg_illus002.html

Summary (2)

Nerve terminal

Kunitz

Phospholipase A_2



=> Destruction of nerve terminus

⇒ Respiration failure

⇒ Death

Relevance

- 3 million venomous snake bites causing 100,000 deaths worldwide per year

  => Gain understanding of mechanism and develope antidots

- Research tool:
  - α-bungarotoxin helped to characterize the acetylcholine receptor
  - Ion channel probe
  - Inflammation studies by elucidating PLA_2 mechanism

- High divergence => lead compound for drug design
- Use specific molecular targeting of one subunit in complex with other therapeutical subunit

Thanks for your attention!

References

- H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne. **The Protein Data Bank**. Nucleic Acids Research. 2000. 28**:**235-242
- http://www.ebi.ac.uk/interpro/potm/2004_6/Page1.htm

[1] Kwong PD et al. **Structure of 2-bungarotoxin: potassium channel binding by Kunitz modules and targeted phospholipase action**. Structure. 1995. 3:1109-1119

[2] Rowan EG. **What does β-bungarotoxin do at the neuromuscular junction**. Toxicon. 2001. 39:107-118

Used pdb-files:

β_2-bungarotoxin = 1BUN

bovine pancreatic phospholipase A_2 = 1G4I