# Preliminary application of mass spectrometry on the study of conotoxins


Chunguang WANG

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# Different poisonous animals

since 1960 1980 2000

# Different species of cone snails

*C. flavidus*

*C. imperialis*

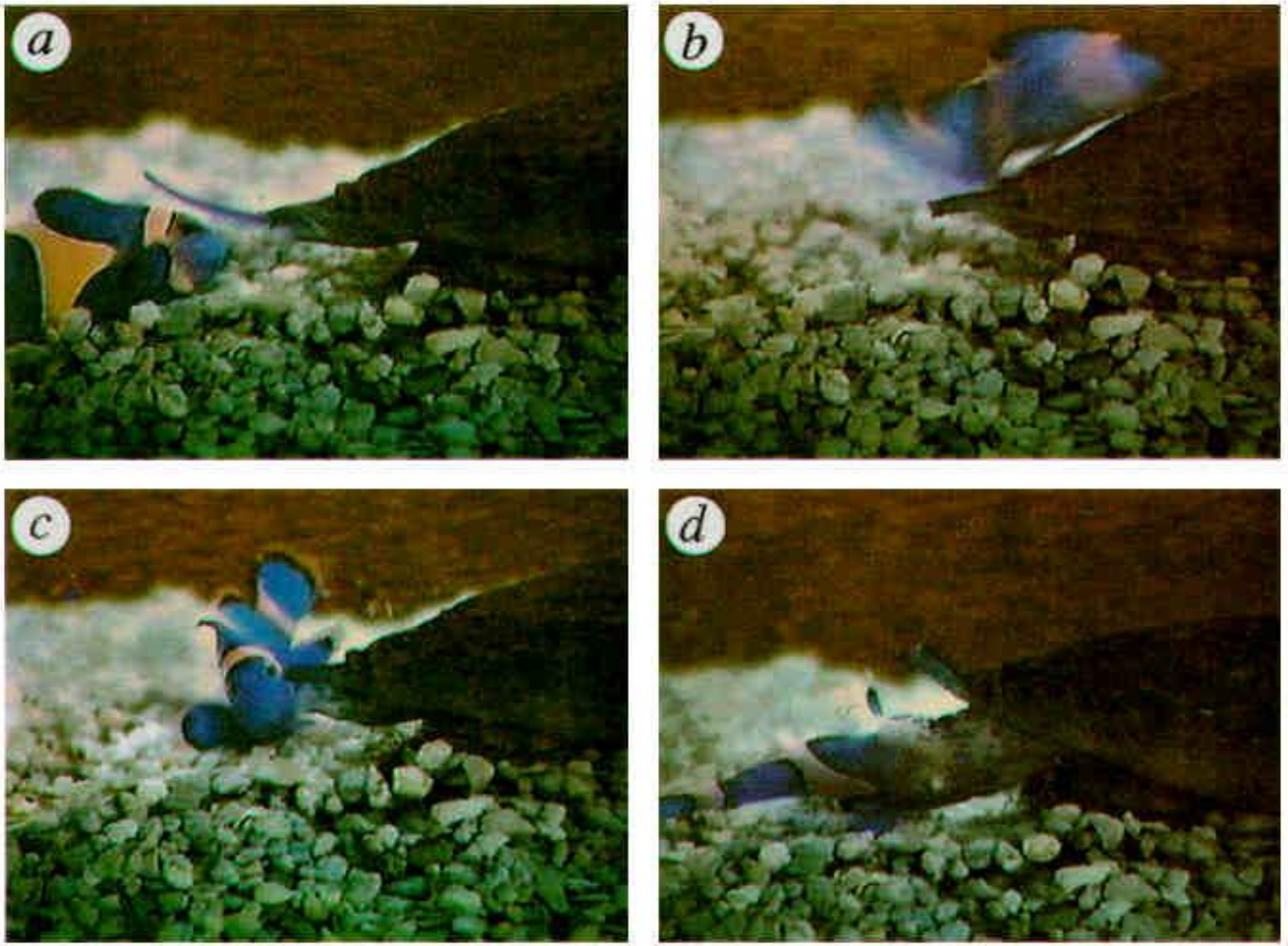
*C. caracteristicus*

*C. marmoreus*

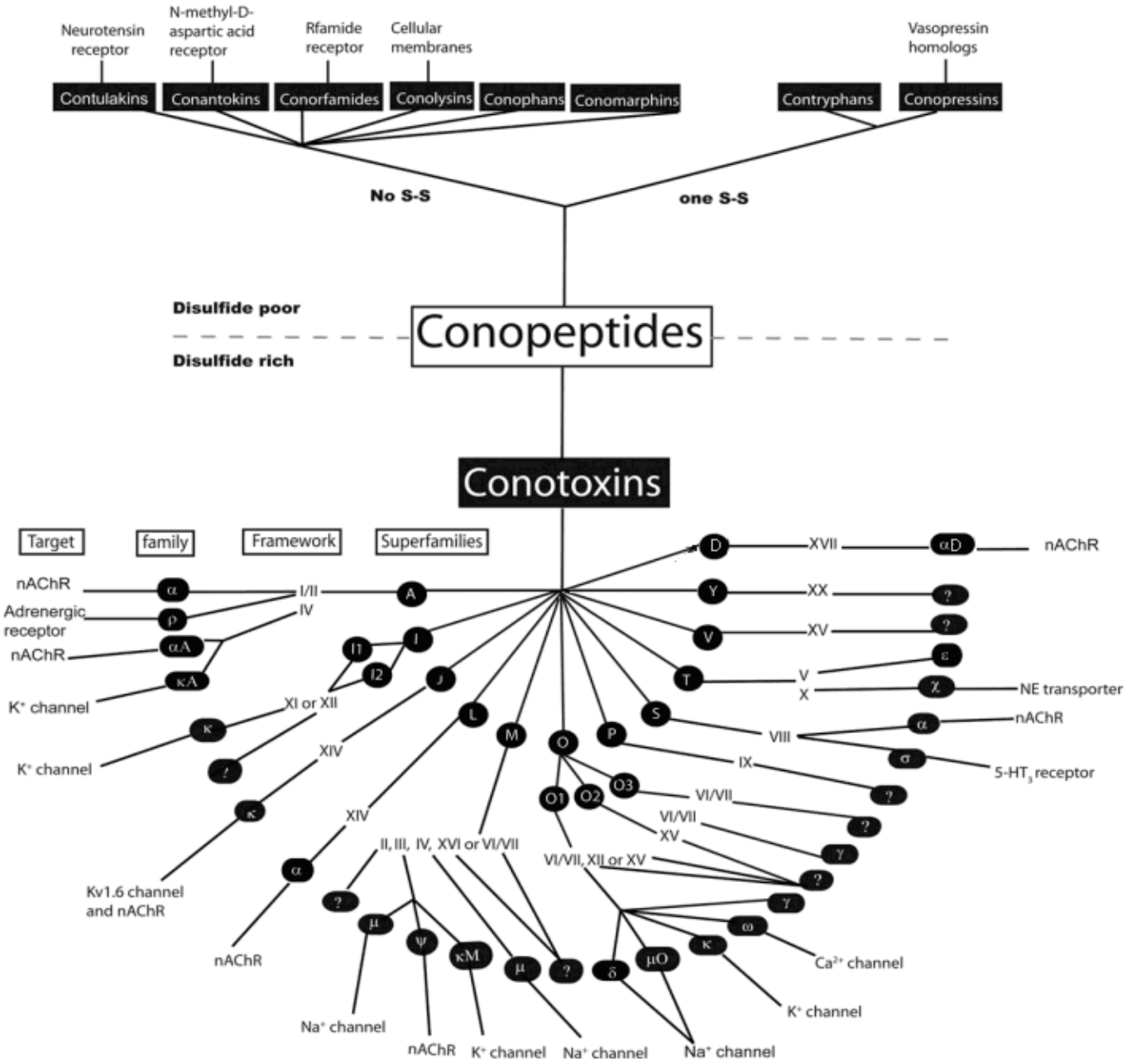
*C. generalis*

*C. vitulinus*

# *Conus purpurascens* rapidly capturing a clown fish.




*(from Nature 1996, 381:148-151)*




*(from Nat. Prod. Rep. 2009, 526-536)*

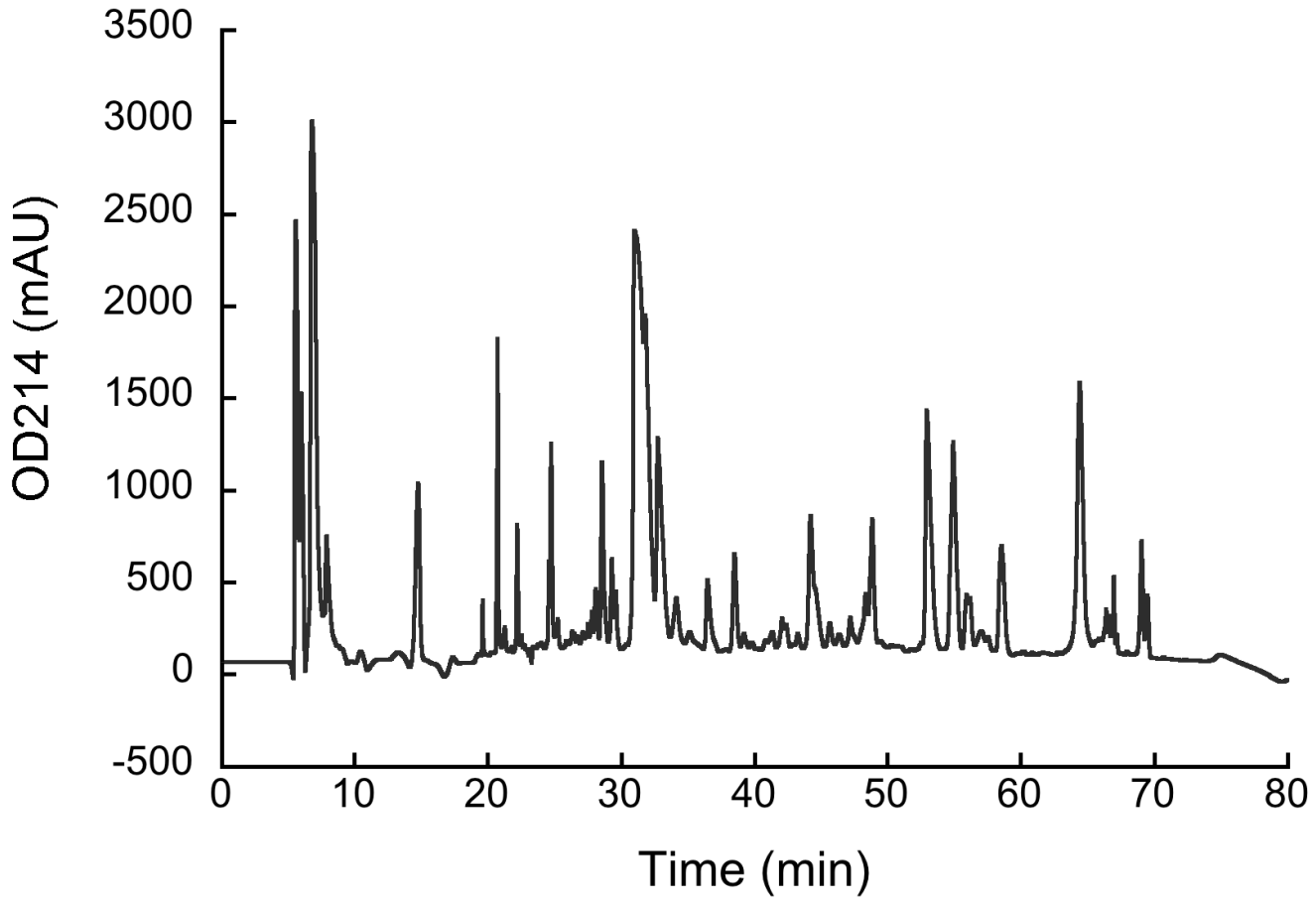
# Clinical potential of conotoxins

| Mode of Action | Name | Sequence | Clinical Potential |
|---|---|---|---|
| $Ca_v2.2$ inhibitor | MVIIA | CKGKGAKCSRLMYDCCTGSCRSGKC* | Pain (intrathecal; phase IV) |
| $Na_v$ inhibitor | SIIIA | | Pain (intravenous) |
| $Na_v1.8$ inhibitor | MrVIB | | Pain (intrathecal/intravenous) |
| $Na_v$ enhancer | EVIA | | ? |
| $K_v$ inhibitor | PVIIA | | Cardiac reperfusion |
| NET inhibitor | Xen2174 | | Pain (intrathecal; phase II) |
| nAChR inhibitor | Vc1.1 | | Pain (intravenous)[a] |
| $5HT_3$ receptor | GVIIIA | CS* | ? |
| $\alpha_1$-Adrenoceptor inhibitor | TIA | | Cardiovascular/BPH |
| NMDA-R antagonist | Con-G | | Pain/epilepsy (intrathecal)[a] |
| Vasopressin-R agonist | Cono-G | | Cardiovascular/mood |
| Neurotensin-R agonist | Cont-G | | Pain (intrathecal)[a] |


*(from Pharmacol Rev 2012, 64:259–298)*

# Venom duct of *Conus marmoreus*

# HPLC profile of crude venom of *Conus marmoreus*



# Conotoxins are highly variable.

M-superfamily conotoxin precursors:

| | |
|---|---|
| VxII | MMSKLGVLVTICLLLFPLTALPLDGDQPADHPAKRTQDHNLASPISA**WIDPSHYCCCGGGCTDDCVNC** |
| μ-SIIIA | MMSKLGVLLTVCPLLFPLTALPPDGDQPADRPAERMQDDISSDEHPLFDKR**QNCCNGGCSSKWCRDHARCC**GR |
| Conomarphin | MMSKLGVLLCIFLVLFPMATLQLDGDQTADRHADQRGQDLTEQQRNSKRVLKKR**DWEYHAHPKPNSFWT**LV |
| Vt3.1 | MLKMGVVLFIFLVLFPLATLELNADQPVERNAENIQDLNPDKRVIKIPVPRRR**GPYRRYGNCYCPI**G |

signal peptide — pro-peptide — mature peptide

A-superfamily conotoxin precursors:

| | |
|---|---|
| Pu14.1 | MGMRMMFAVFLLVVLATTVVSFNSDRASDGRNAAANVKASDLMARVLEK**DCPPHPVPGMHKCVCLKTC**R |
| κA-SIVA | MGMRMMFTVFLLVVLATTVVSTPSDRASDGRNAAVHER**QKSLVPSVITTCCGYDPGTMCPPCRCTNSC**G |
| κA-MIVA | MGMRMMFTVFLLVVLATTVVSIPSDRASDGRNAVVHER**APELVVTATTNCCGYNPMTICPPCMCTYSCPPKRKP**GRRND |
| α-Mr1.1 | MGMRMMFTVFLLVVLATTVVSFTSDRASDGRKAAAKDKASDLVALTVK**GCCSHPACSVNNPDICG** |
| α-AulB | -----MFTVFLLVVLATTVVSFTSDRASDGRKDAA----SGLIALTMK**GCCSYPPCFATNPDC**GRRR |

……

How comes this striking diversification?

# Dr. Aiping LU

2005-2009 supervised by Prof. Matthias Mann;

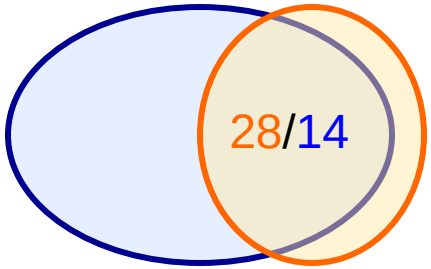
2009-2011, SIBS, CAS;

2011-now, Tongji University.

# Venomic study of *Conus flavidus*

(Lu, et al., **MCP.** 2014)

cDNA library & LC-MS/MS of crude venom




No protease digestion of crude venom;

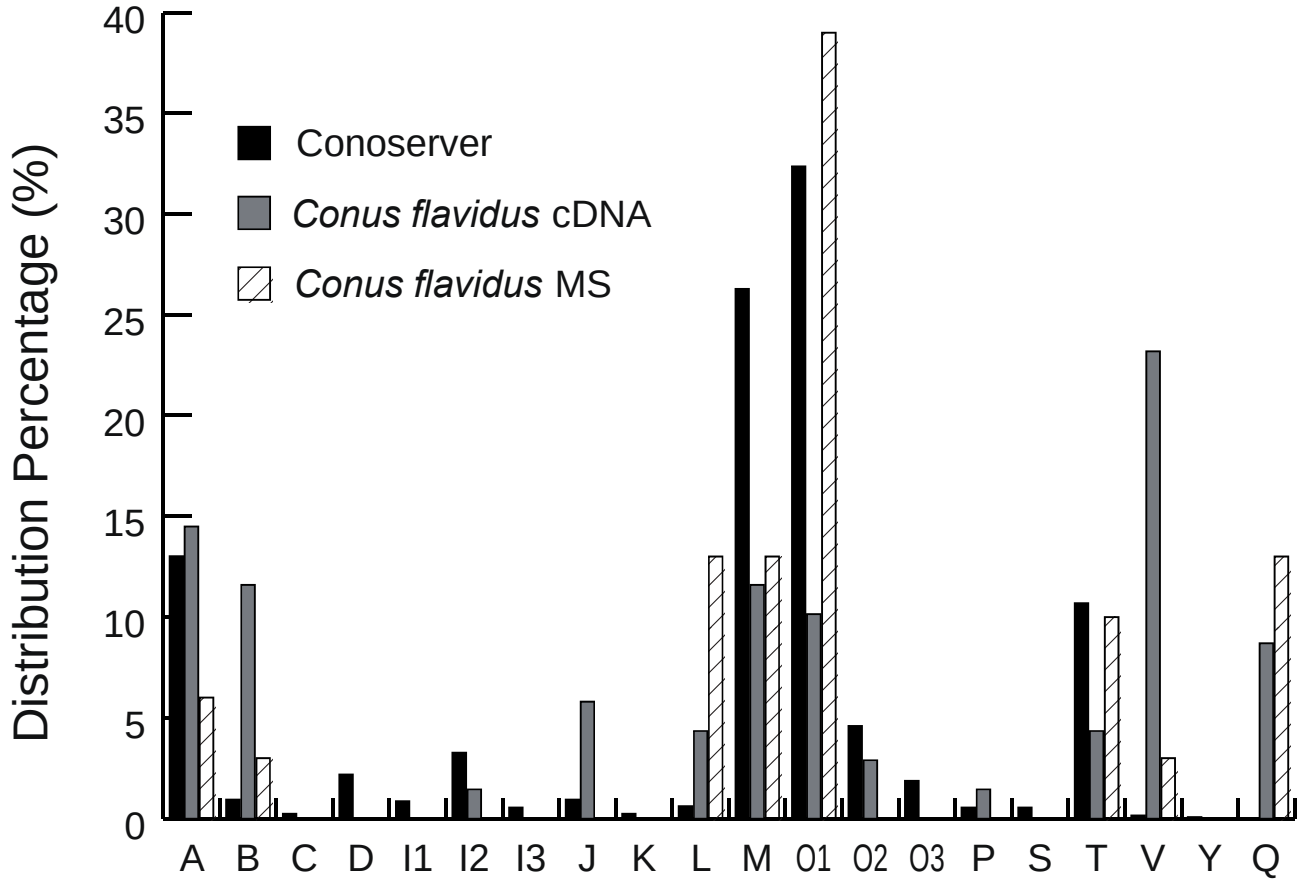
Non-specific precursor digestion during database searching;

Mass deviation < 10 ppm.

69 cDNA sequences

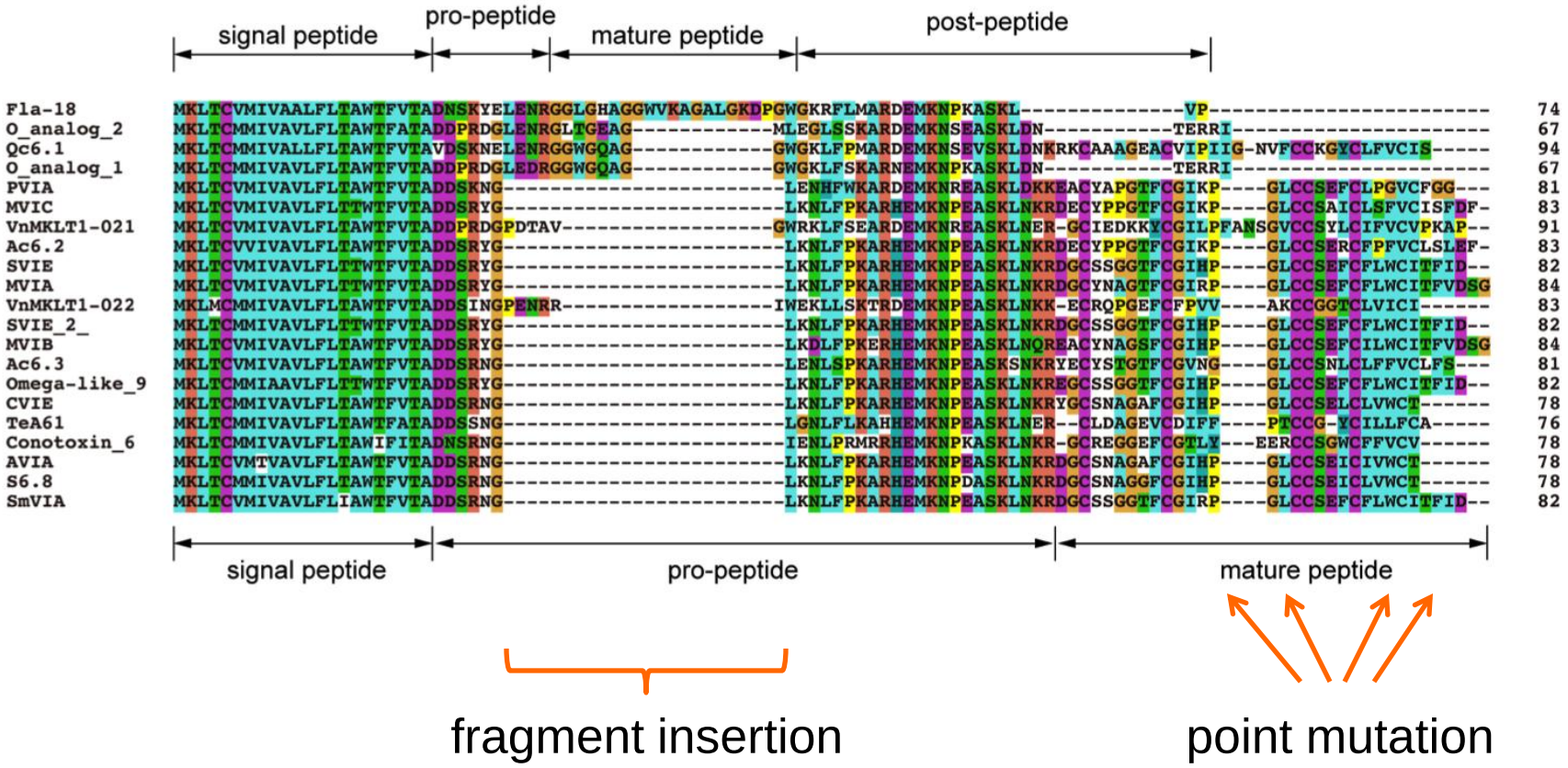
31 conotoxin sequences

# Overall superfamily distribution of *C. flavidus* conotoxins



# Conotoxin diversifications at mRNA level.

(Lu, et al., **MCP.** 2014)



# Conotoxin diversifications at mRNA level.

(Lu, et al., **MCP.** 2014)

# Conotoxin diversifications at protein level.

cDNA Fla16.2: MHTLEMLLLLLLLLPLAPGEGDGQAVAGDRNPSEARSTHE

HFLQRLIR**LIHGSDCQPCGQYVCCPPWKYAEY**RRFT

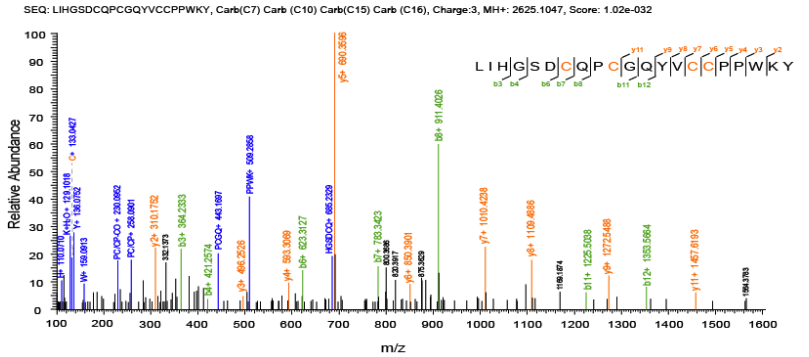
conotoxin fla16a: **LIHGSDCQPCGQYVCCPPWKY**

fla16b: **LIHGSDCQPCGQYVCCPPWKYA**

fla16c: **LIHGSDCQPCGQYVCCPPWKYAE**

fla16d: **LIHGSDCQPCGQYVCCPPWKYAEY**

A

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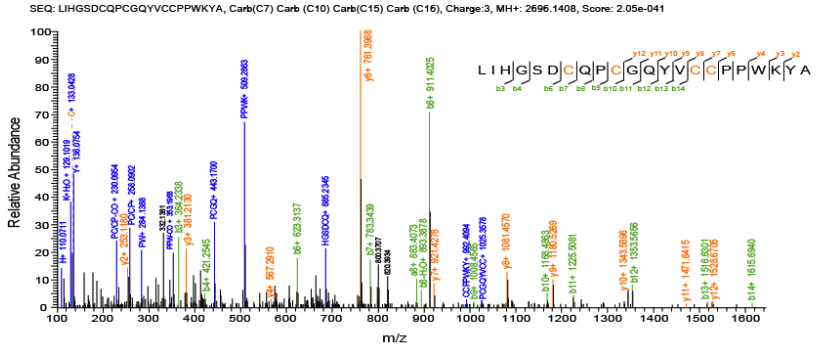


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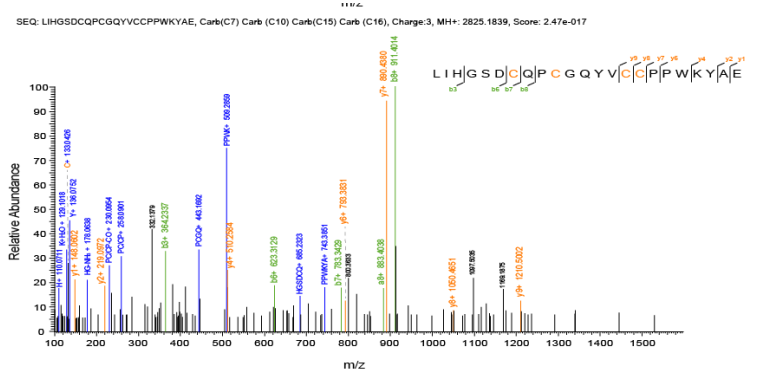
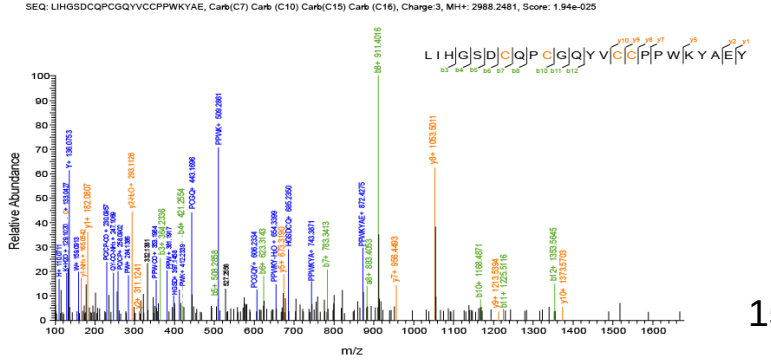



D



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cDNA Fla16.2: MHTLEMLLLLLLLLPLAPGEGDGQAVAGDRNPSEARSTHE

HFLQRLIR**LIHGSDCQPCGQYVCCPPWKYAEY**RRFT

conotoxin fla16a: **LIHGSDCQPCGQYVCCPPWKY**

fla16b: **LIHGSDCQPCGQYVCCPPWKYA**

fla16c: **LIHGSDCQPCGQYVCCPPWKYAE**

fla16d: **LIHGSDCQPCGQYVCCPPWKYAEY**

cDNA Fla14.3: MKLSVTFIVVLMLTTSLTFGFSLSSNNGERAYGSHHSYVADQ

LVRRER**ASRACNPPCSDILTCLHGTCKHLGI**G

conotoxin fla14a: **ACNPPCSDILTCLHGTCKHLGI***

fla14b: **ACNPOCSDILTCLHGTCKHLGI***

fla14c: **ACNOOCSDILTCLHGTCKHLGI***

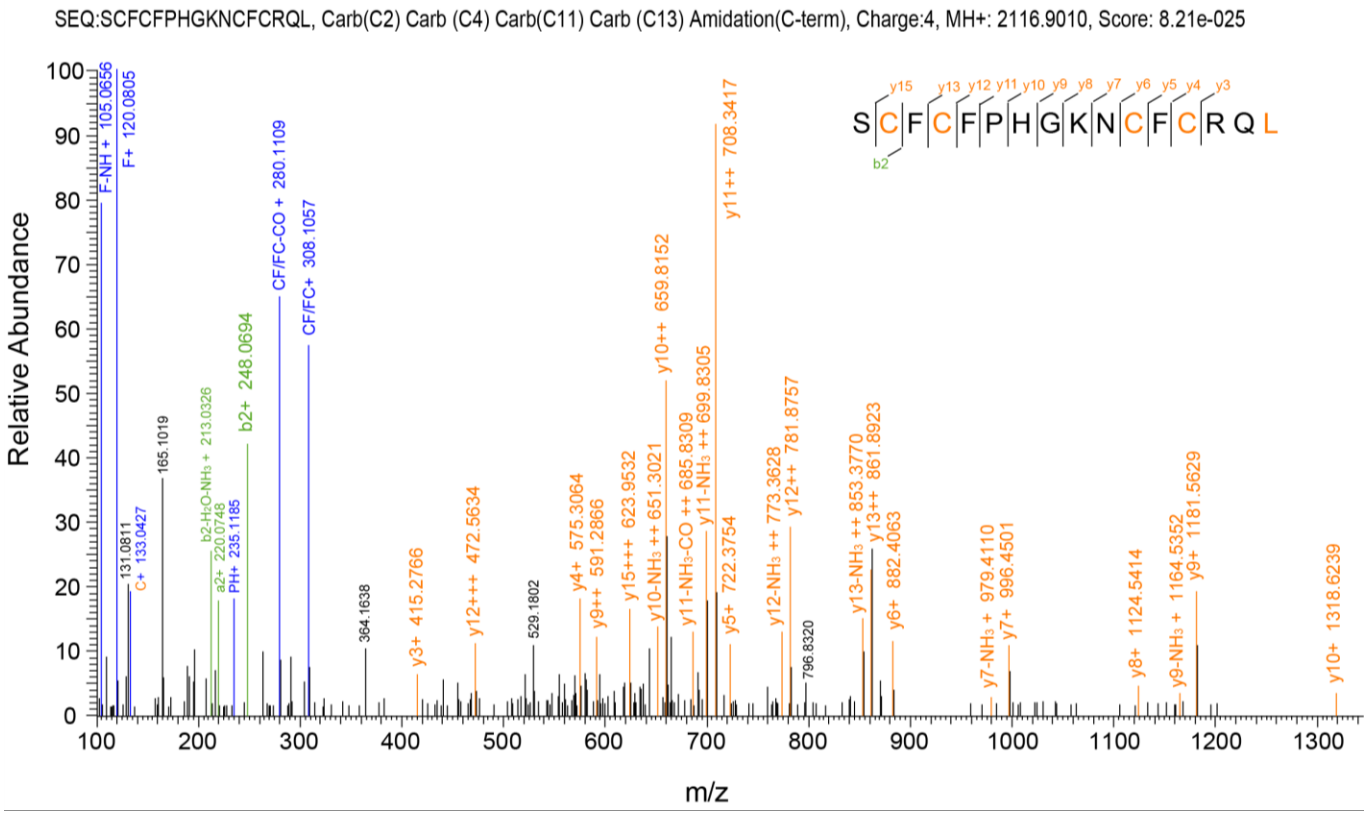
(Lu, et al., **MCP.** 2014)

# Conotoxin diversifications at protein level.

cDNA Fla15.7: MSTPKMTPFILLLLFSLTIR**C**GDGKAIQEDRDPSTRLLTGDKN

RDLSVNRR**C**S**S**C**N**G**E**I**C**C**G**S**C**F**C**F**P**H**G**K**N**C**F**C**R**Q**L**GK

conotoxin fla15a：**SCFCFPHGKNCFCRQL***




(Lu, et al., **MCP.** 2014)

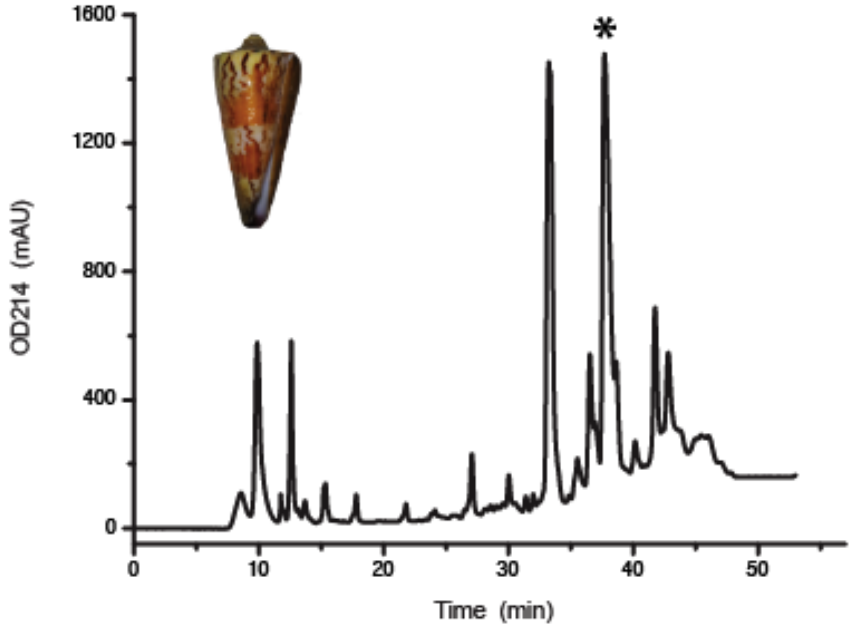
# What we expect next:

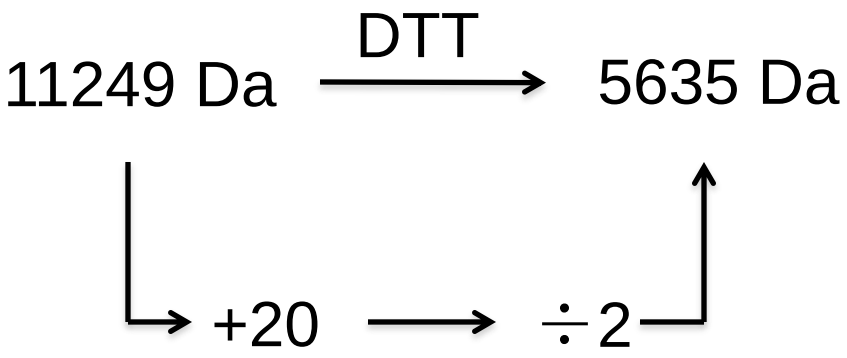
Deeper proteomics characterization;

S-S linkage identification……

Target/binding site identification……

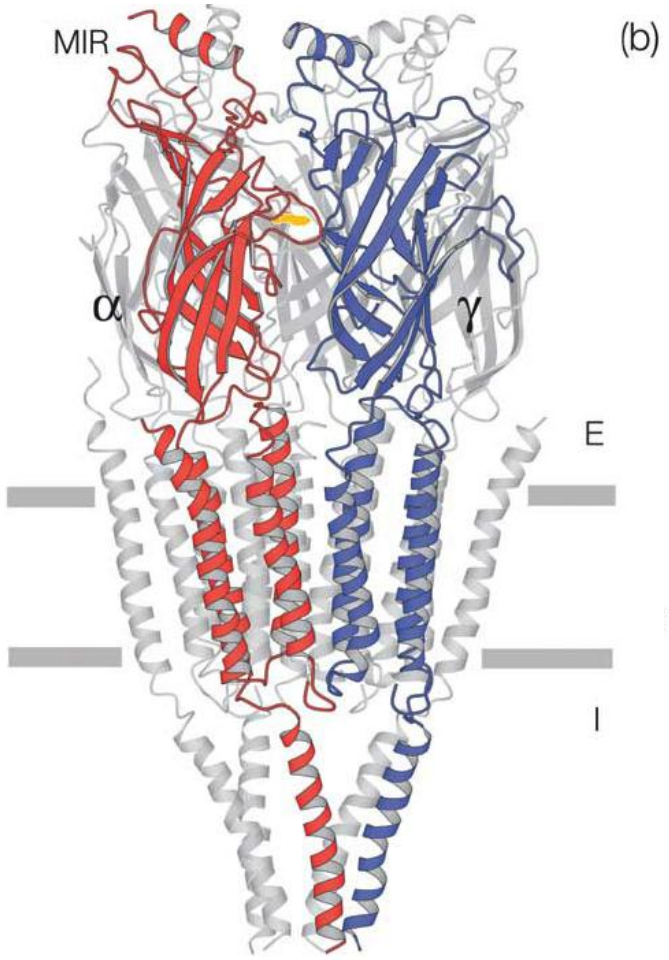
# Dimeric αD-GeXXA from *Conus generalis*




10 S-S per homodimer!

# αD-GeXXA inhibits nicotinic acetylcholine receptor (nAChR)

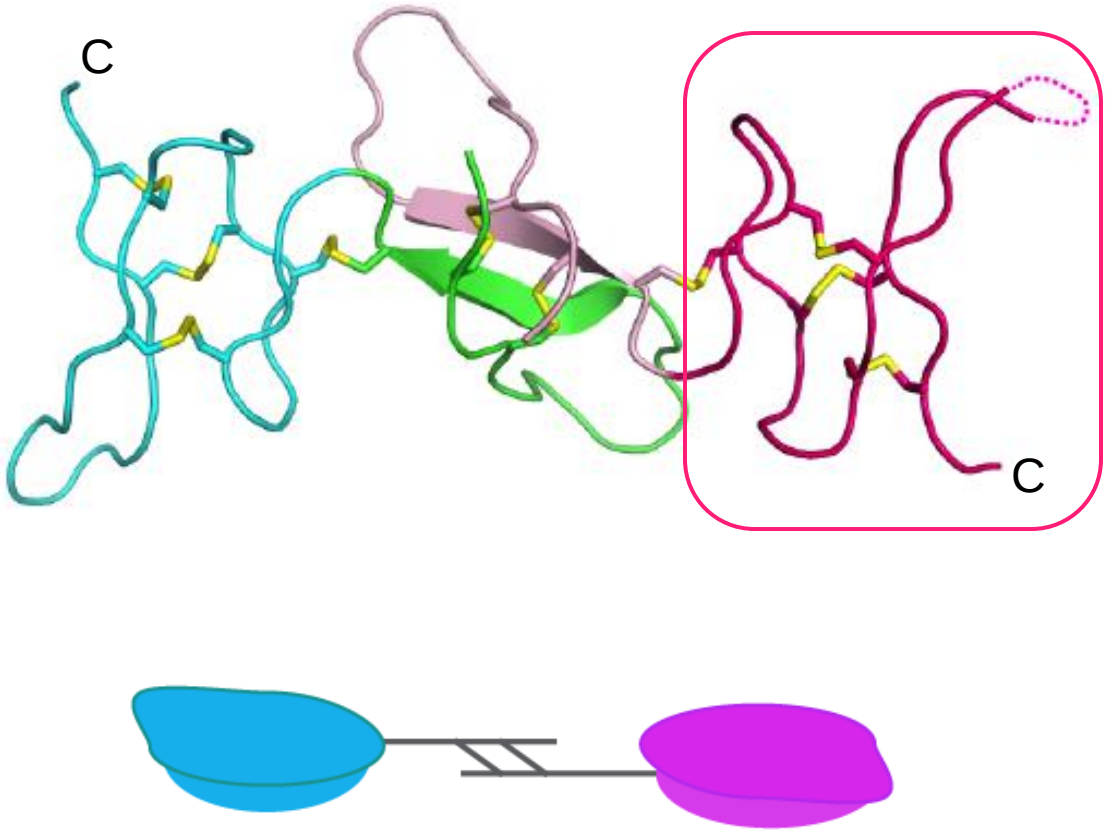



*(from JMB 2005, 346:967-989)*

| nAChR subtype | $IC_{50}$ (95% CI) |
|---|---|
| hα9α10 | 28 nM (22-35) |
| hα7 | 210 nM (174-253) |
| rα3β2 | 498 nM (407-609) |
| rα3β4 | 614 nM (491-768) |
| rα4β2 | > 3 mM |
| rα4β4 | > 3 mM |
| rα1β1δγ | 743 nM (606-911) |

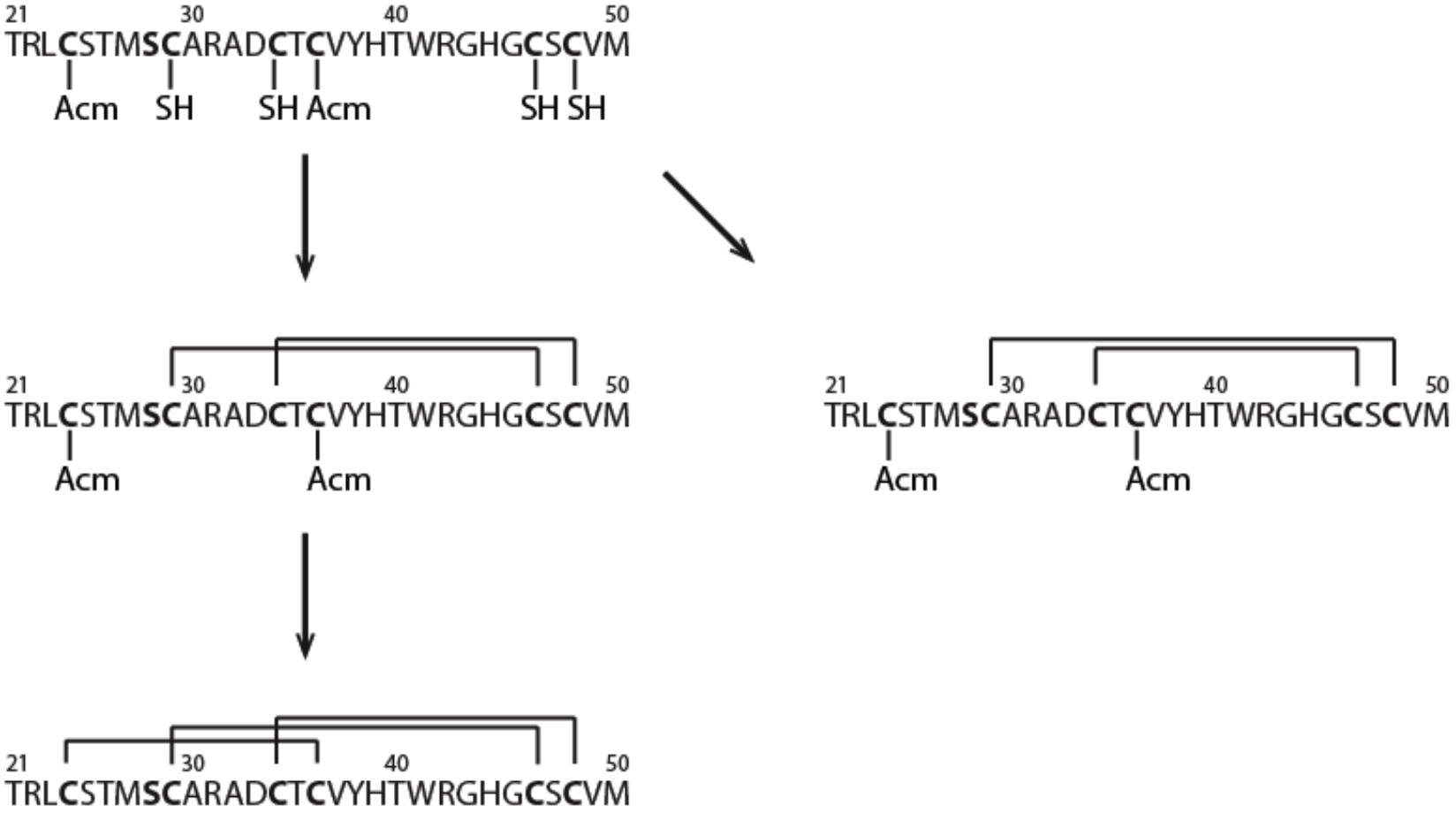
(In collaboration with Dr. D. Adams)

# Crystal structure of αD-GeXXA




(In collaboration with Dr. Jianping Ding)

# Refolding the αD-GeXXA-monomer.



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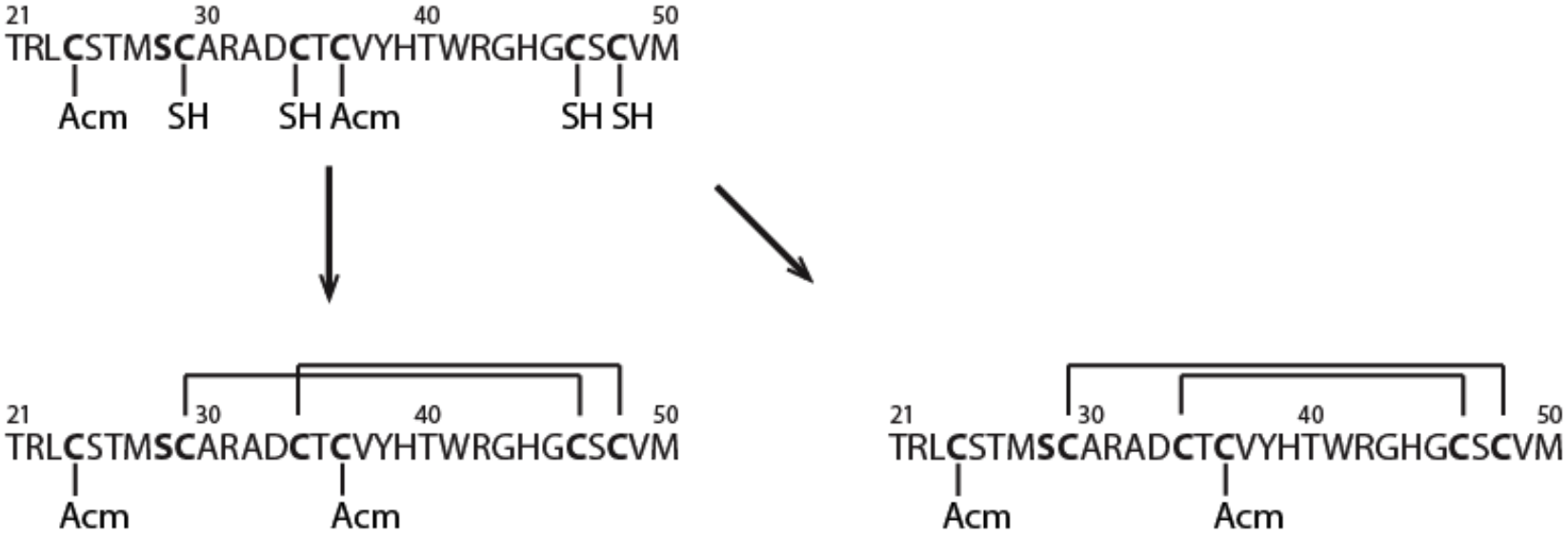


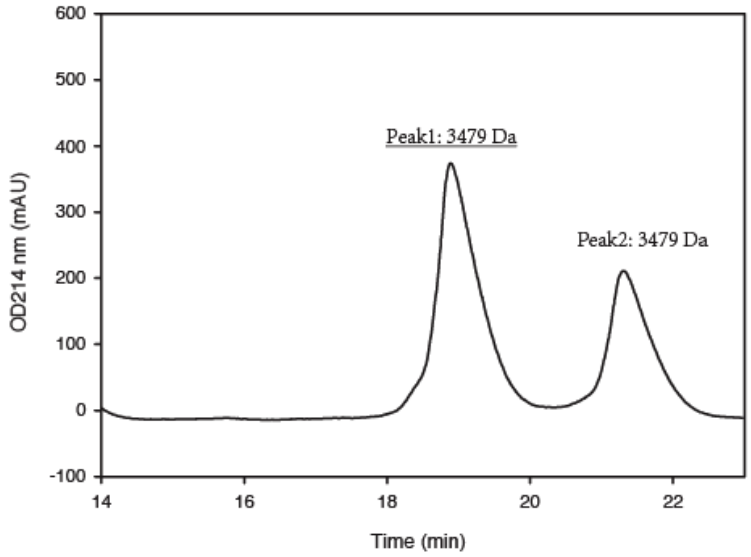

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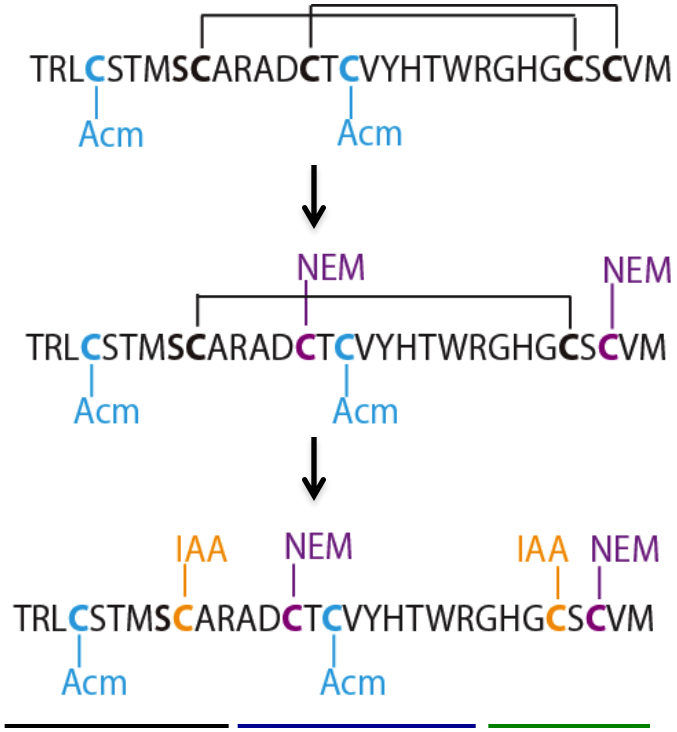


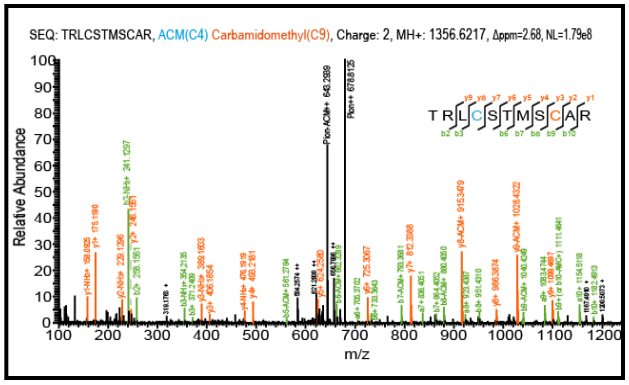


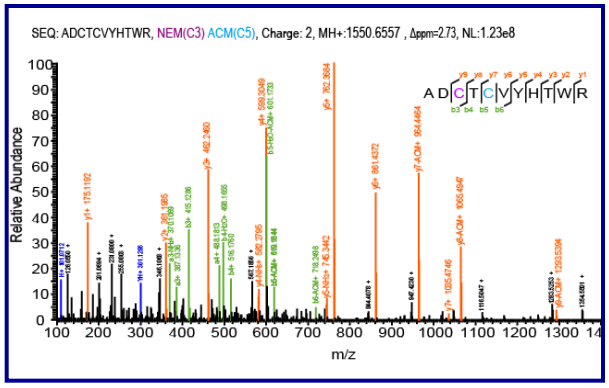


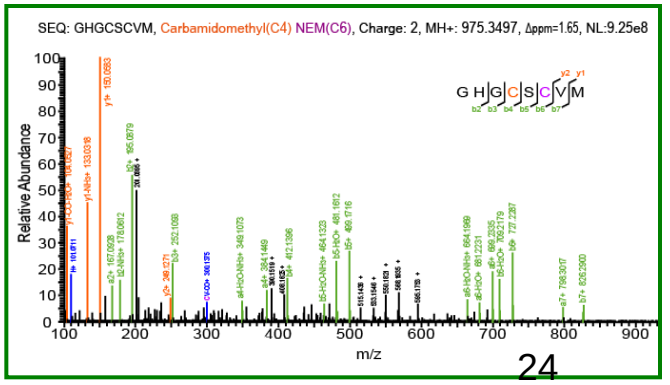

# αD-GeXXA-monomer does inhibit nAChR.

| nAChR subtype | dimer $IC_{50}$ | monomer $IC_{50}$ |
|---|---|---|
| hα9α10 | 28 nM | 2.02 μM |
| hα7 | 210 nM | / |
| rα3β2 | 498 nM | / |
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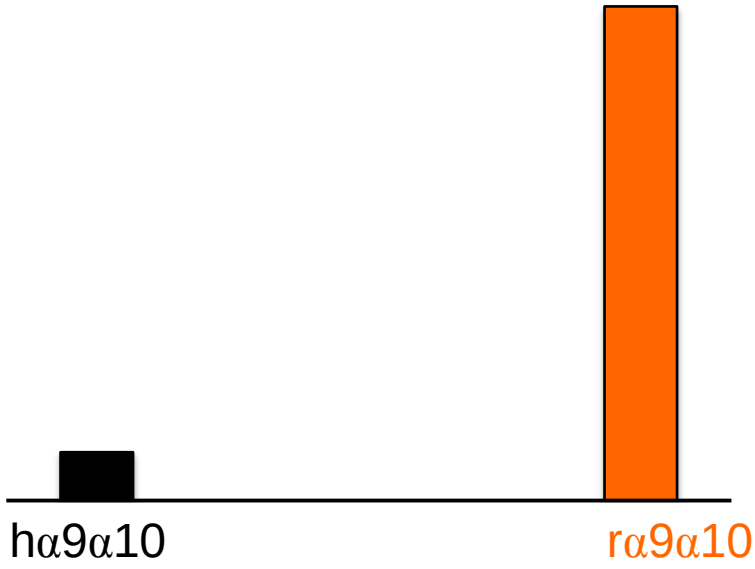

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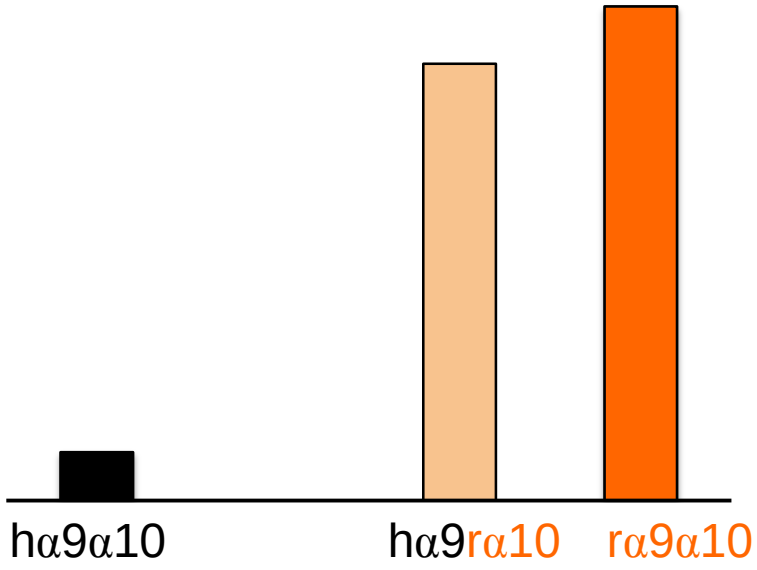

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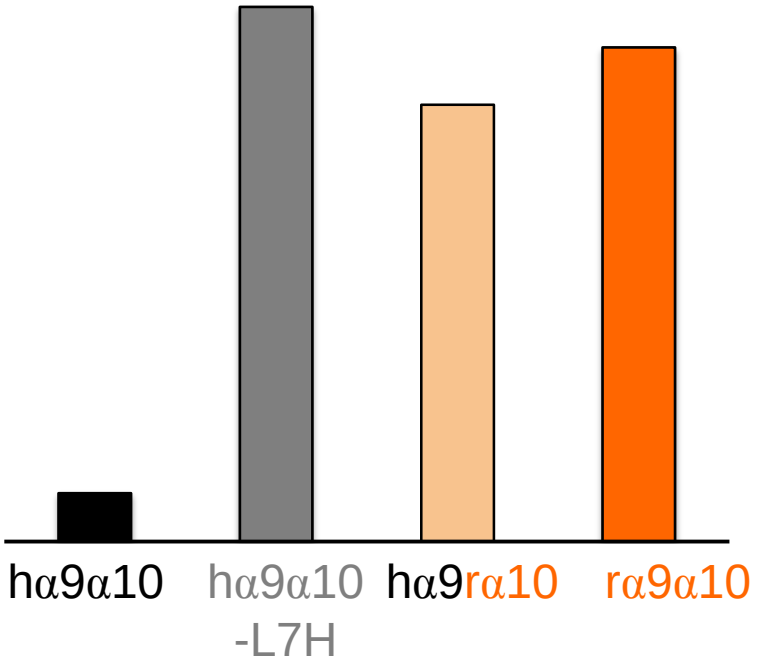
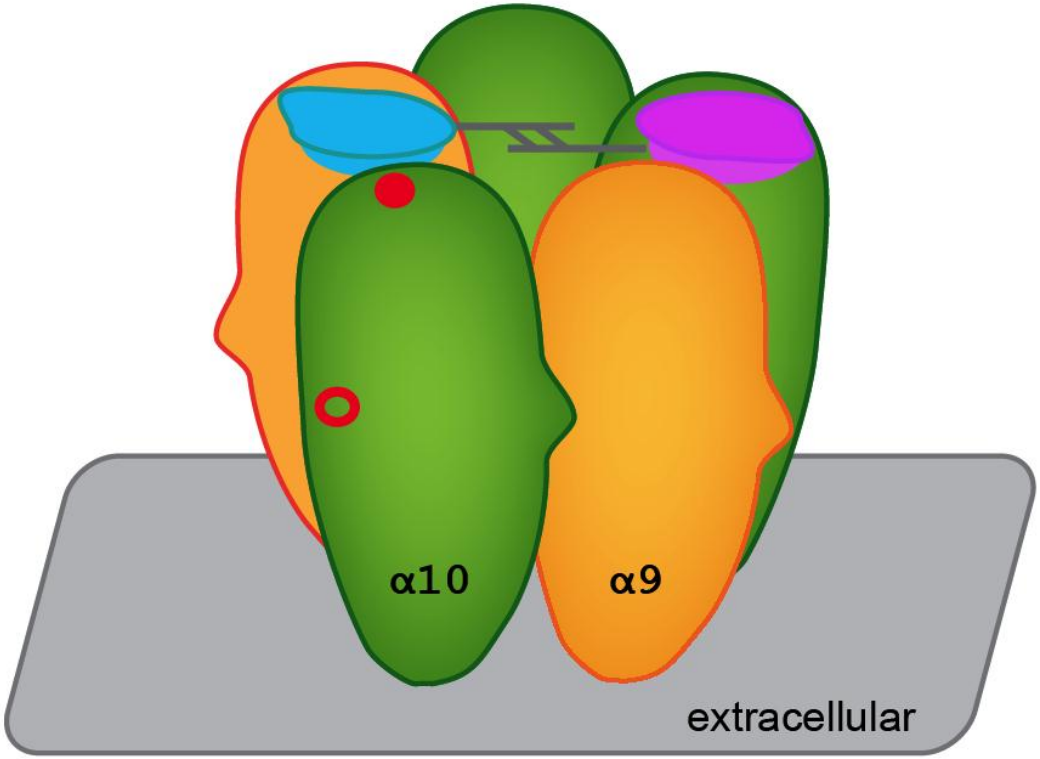


# Working model for the dimeric αD-GeXXA



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……

Collaborators:

Dr. Yong Zhang, GBI, Shenzhen;
pFind team;
Dr. David Adams, RMIT Uni., Australia;
Dr. Jianping Ding, GIBH, SIBS, CAS;
……

¥¥: MOST, NSFC, MOE…

# Thank you for your attention!