point for the obtained protein was determined and its insertion into lipid monolayers was studied.

Results: UcT I shows a typical actinoporin sequence largely identical to EQT II the most studied family member. UcT I is a strongly basic protein (I.p. 9) with potent hemolytic activity. Experiments with lipid monolayers revealed that UcT 1 requires a mixture of SM:DOPC in order to insert itself into the membrane.

Conclusion: New cytolysin from *U. crassicornis* share common functional characteristics to proteins belonging to actinoporin family and share a large degree of identity and homology to EqT II.

Keywords: sea anemone, toxin, cytolysin, actinoporin
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105. Discovery, Characterization, and Functional Implications of Conotoxins from Cone Snails Species of the Americas

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Background: Cone snails are among the most prolific and versatile peptide engineers known. Their venom is a complex concoction composed of modified peptides (conopeptides) that elicit a wide range of neurophysiological responses. The biochemical strategy developed by cone snails to target the multiplicity of neuronal receptors has provided us with a natural library of toxins with great potential therapeutic uses, including the first FDA-approved drug of marine origin, Prialt™. The expression of conopeptides is species-specific, with significant intraspecies and intraspecimen variations. Accordingly, more than 2,000 conopeptides/species can be expressed yielding an enormous library of bioactive compounds. We have concentrated our research efforts in exploring the venom of the 200+ Conus species of that inhabit the waters of the Americas (Western Atlantic and Eastern Pacific regions, respectively).

Methods: Here we described the discovery of conopeptides by using either biochemical-based approaches or efficacious bioassay-guided methods. Specifically, we have used SE and RP-HPLC to isolate nanomolar quantities of novel conopeptides, such as bru1a, bru3a, bru9a and RegIIA. Their sequences were determined by Edman degradation. Testing of these conotoxins included two-electrode voltage clamp recording on nAChRs subtypes expressed in *Xenopus laevis* oocytes. Complementary to this approach, we use *in vivo* electrophysiological measurements to evaluate the effect of conopeptides fractions on the functional outputs of a well-characterized neuronal system in *Drosophila melanogaster* known as the giant fiber circuit (GFC).

Results: These new a-conotoxins have unique selectivity profiles; i.e., RegIIA is a potent inhibitor of a3b4 nAChRs, without blocking the a4b2 subtype. This feature makes RegIIA a suitable probe to evaluate the machinery involved in nicotine addiction. While structurally related to other a-conotoxins, RegIIA has an exquisite balance of shape, charges, and polarity exposed on its structure that enable inhibition of a3b4 nAChRs. A novel a4/3 conotoxin, bru1b, was discovered using GFC approach, and it was further characterized using voltage clamp measurements on a panel of nAChRs subtypes expressed in *Xenopus* oocytes. Additionally, we will describe a new set of mini-M conotoxins of the m1-m3 subclasses. These conotoxins exhibit a remarkable level of structural diversity in their posttranslational modifications, size of intercystine loops and overall 3D structure. One of these mini-M conotoxins, bru3a, is active in the GFC indicating a potential target for these conotoxins.

Conclusions: These novel conopeptides add further molecular diversity to the *Conus* biochemical strategy for predation and are of particular interest as shown to have novel functional implications within known conotoxin superfamilies.

Keywords: conotoxins, nAChRs, oocytes, neuronal circuits, ion channels
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106. Molecular Diversity of Box Jellyfish Toxins

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Review: Box jellyfish (cubozoans) are renowned for their ability to immobilise and kill prey and inflict painful and debilitating stings to humans by injecting potent venoms from their nematocysts. *Chironex fleckeri* is the largest species of box jellyfish and its venom produces extremely rapid and potentially life-threatening effects. Advances in box jellyfish toxinology using bioactivity-guided purification methods, tandem mass spectrometry and molecular cloning techniques have revealed that *C. fleckeri* venom contains a diverse array of proteins that is dominated by a family of abundant high molecular weight venom proteins that are cytolytic, cytotoxic and cause profound cardiovascular collapse in experimental animals. Related toxins with similar biological activities are present in other jellyfish species and comparative analysis of available toxin sequences infers that this expanding family of potent cnidarian toxins forms at least two distinct protein clades. Sequence divergence among family members coupled with experimental evidence suggests there are significant structural variations between

clades that may alter their function and target specificity. In this context, an overview of this unique family of protein toxins is presented, including a brief history of their discovery and recent progress in their purification and molecular characterisation primarily from a bioinformatic perspective.

Keywords: bioinformatics, *Cnidaria*, *Cubozoa*, cytolysin, cytotoxin, nematocyst, venom, toxin
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107. The Chemical Landscape of Cnidarians as Viewed Through the Lens of Pore-Forming Proteins

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Background: Cnidarians such as hydra, sea anemones, corals and jellyfish are simple, mostly sessile animals that depend on bioactive chemicals for survival. Cnidarians utilize sophisticated stinging cells (nematocytes) to inject paralyzing venom into their prey, predators or competitors. In addition to the nematocyte venom, we show here that cnidarians produce cytolytic, "toxin-like" pore-forming proteins (PFPs) in other tissues, and suggest functional roles for these proteins.

Results and Discussion: Equinatoxins (Eqts), well-studied lethal PFPs from the sea anemone *Actinia equina*, are detected by immunohistochemistry in nematocytes and are thus probably used for prey capture. However, Eqts are also secreted into the mucous layer surrounding the anemone from gland-like cells. Eqt-2 can kill fish upon application to the water in which they swim, suggesting it may serve for defense against predators. Surprisingly, while Eqt-2 does not kill bacteria it strongly inhibits their attachment to substrates, suggesting this protein may inhibit the adhesion of pathogenic bacteria or other fouling organisms to the anemone. A second example of non-nematocystic PFPs are Hydralysins (Hlns), neurotoxic PFPs from the green hydra *Chlorohydra viridissima*: in-situ hybridization and immunohistochemistry reveal that Hlns are secreted into the gut cavity upon feeding, where they may promote osmotic disintegration of the prey during feeding. Other potential PFPs may be involved in developmental signaling and immunity in hydra.

Conclusions: We propose a model whereby, in cnidarians, bioactive compounds such as PFPs are secreted both as localized point sources (nematocyte discharges) and across extensive body surfaces, likely combining to create complex "chemical landscapes". We speculate that these cnidarian-derived chemical landscapes may affect the surrounding community on scales from microns to, in the case of coral reefs, hundreds of kilometers.

Keywords: Cnidaria, hydra, nematocyst, pore-forming protein, chemical landscape, diffusion, boundarylayer, antimicrobial
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108. Cardiovascular and Hemolytic Effects of Sp-CTx a Cytolysin Isolated from the Scorpionfish, *Scorpaena plumieri*

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Background: In a previous study, a potent hemolytic/cardiotoxin (Sp-CTx) was isolated from scorpion fish *Scorpaena plumieri* venom. In the present work we aimed to optimize the Sp-CTx purification method and to investigate the mechanisms responsible for its pharmacological effects.

Methods: Sp-CTx was purified to homogeneity from fish fin spines venom through a combination of ammonium sulfate precipitation and two chromatographic steps: hydrophobic interaction and anion exchange. Active fractions were identified by hemolytic assay. Osmotic protection assays using polyethylene glycol polymers of various sizes (30mM) were performed to test whether Sp-CTx induces hemolysis by membrane pore formation. Cardiovascular studies of Sp-CTx were evaluated on male Wistar rats both *in vivo*, and *in vitro*. The effects of Sp-CTx on L-type Ca^{2+} current ($I_{Ca,L}$) in adult rat ventricular cells were investigated using the whole cell patch clamp method.

Results: The yield of the purification procedure was 13% of the hemolytic activity from the whole venom, with a purification factor of 24 fold. Sp-CTx induced-hemolysis decreased with increasing size of osmotic protectants. *In bolus* injection of Sp-CTx in rats (70μg/Kg) produced a biphasic response which consisted of an initial systolic and diastolic pressure increase followed by a sustained decrease of both parameters. In isolated papillary muscle, Sp-CTx (80nM) produced an increase in isometric force. At 8nM concentration, this toxin increased by about 30% the $I_{Ca,L}$ in rat ventricular cells.

Discussion: In the present work, a new purification procedure of Sp-CTx was established. This method requires a smaller number of chromatographic steps and the activity recovery was improved substantially (13 fold). Hemolytic effect was prevented by the presence of osmotic protectants. At 40 nM concentration, 100% cell lysis was observed after 6 min, molecules larger than 3 nm in diameter inhibited 100% cell lysis. As we observed a biphasic response in pressure levels when Sp-CTx was injected in rats we could argue that Sp-CTx targets first calcium channels and after some time non-selective pore formation takes place leading to a decrease in both systolic and diastolic pressure levels. On the other hand, the increase in contraction force would be the result of $I_{Ca,L}$ increase.

Conclusion: The results strongly suggest that Sp-CTx may be a pore-forming protein. These data are in agreement with the significant hemolytic activity observed. Also,