# Extraction and Analysis of Pigmented Ommochromes in Cephalopod Chromatophores

Christopher W. DiBona, Stephanie F. Jones-Labadie, Matthew A. Griswold, and Leila F. Deravi
Biomaterials Design Group, Department of Chemistry and Materials Science
University of New Hampshire Durham, NH 03824

## Background

- Adaptive coloration in cephalopods is facilitated by an optical organ known as the chromatophore
- Chromatophores contain a pigment sac anchored by radial muscle fibers. Within the sac are pigmented nanoparticles whose contents are not well known
- It is thought that the granules contain proteins and pigmented molecules known as ommochromes.[2]
- Ommochromes are heterocyclic molecules common to many insects, crustaceans, and reptiles
- We report the extraction and analysis of ommochromes as the main source pigment in squid *Loligo pealeii* chromatophores
- Determining molecular composition of pigment molecules will enable the squid dermal change to be mimicked

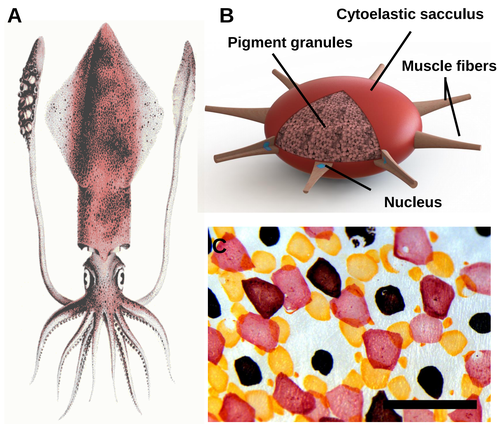



**Figure 1:** A-Illustration of a longfin inshore squid.[1] B-Illustration of a chromatophore organ and the pieces that make it up. C-Microscopic image of chromatophores from a longfin inshore squid, scale bar 1 mm.

## Methods

- Chromatophores from *L. pealeii* are dissected and pigment is extracted from the granules using the procedure illustrated in Figure 3

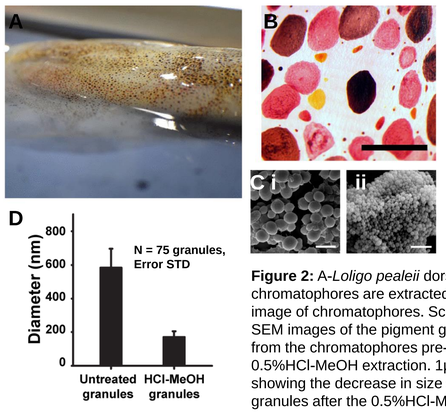



**Figure 2:** A-*Loligo pealeii* dorsal fin where chromatophores are extracted. B-Microscope image of chromatophores. Scale bar 1mm. C-SEM images of the pigment granules isolated from the chromatophores pre-and post-0.5%HCl-MeOH extraction. 1µm. D-Bar graph showing the decrease in size of the pigment granules after the 0.5%HCl-MeOH extraction.

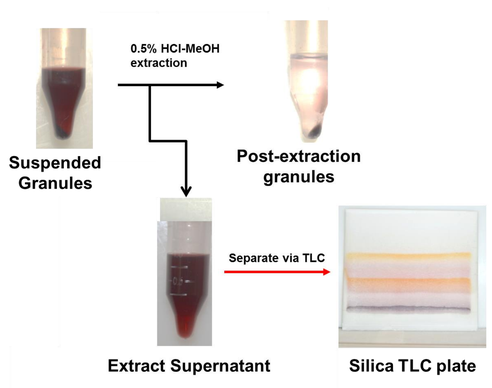



**Figure 3:** Pigment extraction from chromatophore granules

## Results: Absorbance spectra of extracted pigments

- Extracted pigment was collected and purified from silica TLC plate
- Absorbance spectra of isolated pigment suggests multiple compounds contribute to visible color

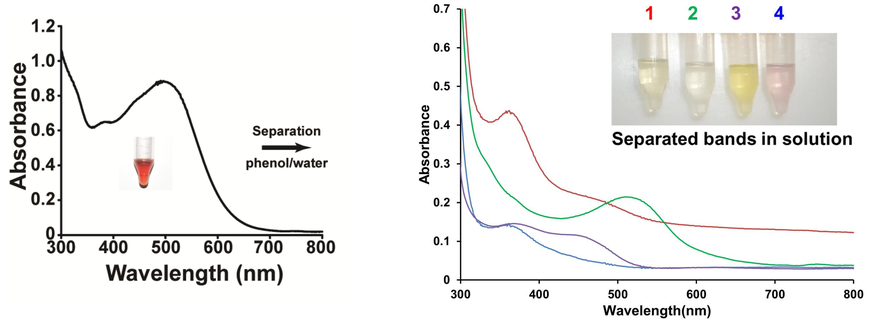
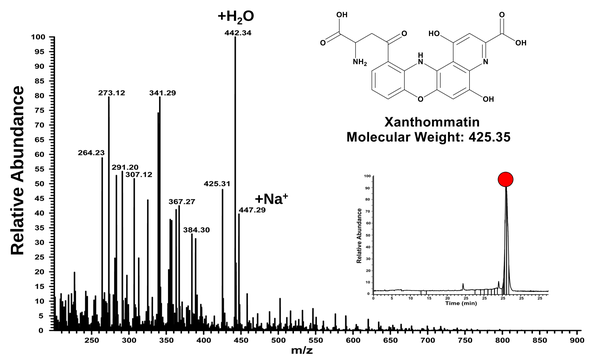
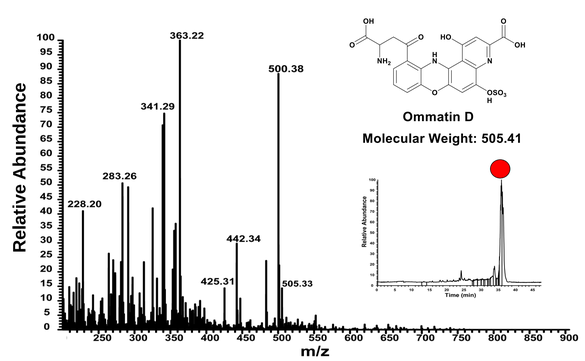


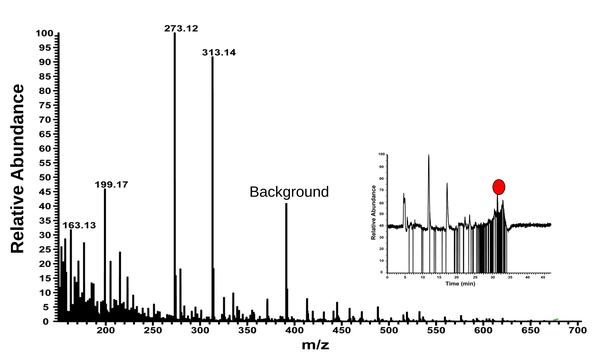

**Figure 4:** Absorbance spectra of the four bands separated (right) from pigment supernatant (left).

## Results: MS/MS Analysis

- Extracted pigments are further separated using reverse phase HPLC
- Upon separation, pigments are fragmented and analyzed using a micro-ion electrospray source of an LTQ Orbitrap XL mass spectrometer



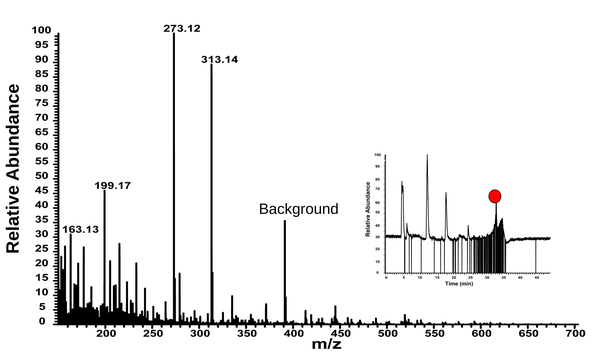




**Figure 5:** Mass spectra and their respective chromatograms. A-band 1.B-band 2. C-band-3. D-band 4.

## Summary

- Pigments were successfully extracted from chromatophore pigment granules using 0.5% HCl-MeOH
- Three different colors are separated via normal phase chromatography with 3:1 phenol:water
- MS/MS suggests that these bands contain many different compounds including a known ommochrome, Xanthommatin
- The remaining unknown compounds will need to be identified with other analyses such as further fragmentation and NMR

## References and Acknowledgments

1. C. Van Den Branden, W. D. (1976). "A Study of the Chromatophore Pigments in the Skin of the Cephalopod Sepia Officinalis L." Biol. Jb. Dodonaea 44: 345-352.
2. Deravi, L. F., et al. (2014). "The structure-function relationships of a natural nanoscale photonic device in cuttlefish chromatophores." Journal of the Royal Society Interface 11(93).
3. Nijhout, H. F. (1997). "Ommochrome pigmentation of the linea and rosa seasonal forms of Precis coenia (Lepidoptera:Nymphalidae)." Arch. Insect Biochem. Physiol. 36(3): 215-222.
4. Shamim, G., et al. (2014). "Biochemistry and biosynthesis of insect pigments." European Journal of Entomology 111(2): 149-164.

The authors gratefully acknowledge the University Instrumentation Center at the University of New Hampshire, Professor Feixia Chu, UNH Biomaterials crew and Sean Dinneen, Hamel Recreational Center of Undergraduate Research, and the University of New Hampshire Department of Chemistry