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Host Lab: Dr. Richard Grosberg

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Title: Immunohistochemistry for cnidocytes and sensory neuron battery complexes in *Hydractinia symbiolongicarpus*.

Rationale: To visualize battery complexes containing cnidocytes and apposed sensory neurons using confocal microscopy.

### **Preparation of animals:**

Four microscope slides containing *H. symbiolongicarpus* were prepared prior to the fixing and staining steps. Two of the slides contained two rejecting colonies each and the other two contained fusing colonies. Animals were explanted from larger stock colonies and contained 4 feeding zooids each. The two colonies were attached at equal distances in all four slides to achieve simultaneous fusion/rejection reactions.

The rest of the steps were conducted as soon as two colonies made contact.

### **Fixing:**

1. Relax the animals in 2% Urethane for 2 minutes.
2. Fix animals overnight in 4% Paraformaldehyde at 4° C.

### **Staining:**

3. Remove animals from paraformaldehyde. Wash with PBST\* five times for 5 minutes each.
4. Immerse animals in blocking solution – 20% Normal Goat Serum (NGS) in PBST for 2 hours inside a humidifying chamber.
5. Incubate overnight with primary antibodies: monoclonal anti-acetylated  $\alpha$  tubulin (1:500) and polyclonal anti-FMRamide.
6. Wash five times for 5 minutes in PBST and immerse in blocking solution - 20% Normal Goat Serum (NGS) in PBST for 2 hours inside a humidifying chamber.
7. Incubate overnight with secondary antibodies: Cy5-conjugated goat anti-rabbit IgG and Cy3-conjugated goat anti-mouse IgG.
8. Wash five times for 5 minutes in PBST and immerse in DAPI (1:1000) and ProLong antifade reagent (Invitrogen) for 1 hour.
9. Wash five times for 5 minutes with PBST and mount the samples in glycerol for microscopy.

\*PBST - 3.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 1.3mM KCl, 135mM NaCl, 0.1% Tween 20, pH 7.4.

### **References:**

Plachetzki et al.: Cnidocyte discharge is regulated by light and opsin-mediated phototransduction. BMC Biology 2012 10:17.