Activity 2: Identification of Botulinum Toxin Molecular Targets

Teaching Notes

The assignments in this activity guide students through primary and secondary literature articles describing some of the seminal work in the field of botulinum toxin research. The assignments should be used with intermediate to advanced level biology majors who have an understanding of endocytosis and exocytosis including cellular structures, protein interactions, and protein modifications. Since the focus here is on experimental methods and approaches, having students complete the relevant textbooks readings either before or during this activity is strongly recommended (see the Background Reading selections below for textbook readings). The activity should be introduced in the middle to latter half of a cell biology course. Each of the assignments can be implemented in the same way as described below.

The assignments in this activity all aim as their learning outcomes for students to be able to do the following:

- Articulate the premise, approach, value and limitations of the study.
- Analyze data critically, propose models and make predictions.
- Distinguish between *in vitro* and *in vivo* studies and identify the merits of each.
- Understand relevant experimental techniques such as affinity chromatography, mobility shift assays, voltage clamp assays and transgenic gene expression.
- Appreciate science as an ever-evolving process.

Activity 2 at a Glance

Class	Small classes (20-50) Intermediate-level Biology Majors
Instructor Preparation	Approximately two hours, excluding grading Review Assignments and corresponding articles. Review **Answers Overview** and **Answers** Decide which assignments to use. Review the **Molecular Mechanisms** section of **Botulinum Toxin Background.**
Useful Media	Arnon, S. S., R. Schechter, et al. (2001). "Botulinum toxin as a biological weapon: medical and public health management." *JAMA* 285(8): 1059-70. See particularly Figure 1. http://jama.ama-assn.org/cgi/content/full/285/8/1059 Allergan. (2002) Mechanism of BOTOX Action. http://www.botox.com/site/professionals/product_info/mechanism_of_action.asp *Cell Biology Interactive* 3.1,3.2, 3.11, 7.2, and 13.2-13.4. See the Viewing Guide (Garland Science). Terry T. "Botulinum Toxin Mechanism of Action" as found in Biology 102: Lecture Notes: The Nervous System.

	http://www.microvet.arizona.edu/Courses/MIC420/lecture_notes/clostridia/clostridia_neurotox/movie/botulinum_movie.html WEMOVE. Botulinum Toxin Type A Mechanism of Action. http://www.mdvu.org/multimedia/videoclips/btx_moa.html
Student time	Out of class: approximately four hours. Due to the amount of assigned reading, allow students two weeks to complete this assignment. In class: none required.

Background Reading

The following textbook selections are interchangeable:

Molecular Biology of the Cell
Fourth Edition

Alberts, et al. (2002). Garland Science. New York.

- "Intracellular vesicular traffic." p.711-766. This is the most relevant to the assignment. Note that V-SNAREs include synaptobrevin/ VAMP and syntaxin, while T-SNAREs include SNAP-25 and its homologues.
- "Proteins" p.129-188.
- " Membrane Structure" p.583-614.
- " Membrane Transport" p. 645-650
- "Manipulating proteins, RNA, and DNA." p. 478-494 and 508-524.
- "Visualizing cells." p. 547-580.

Essential Cell Biology
Second Edition

Alberts, et al. (2004). Garland Science. New York.

- "Intracellular compartments and transport p. 497-531. (Interactive 15.8) The most relevant sections are the sections on clathrin mediated endocytosis (p. 512-516) and receptor mediated endocytosis (p. 525-526).
- "Protein structure and function." p.117-167 (Interactive 4.1, 4.2, 4.11) The most relevant sections include the panels depicting experimental methods (160-165)
- "Membrane structure." p 365-388 (Interactive 11.2).
- "Membrane transport: Ion channels and signaling in nerve cells." p. 411-425. (Interactive 12.8)
- Cell Communications: General Principles: p. 533-543.

Additional Selections:

Washington, U. (2001). "Presynaptic Proteins, Synaptic Vesicle Docking and Membrane Fusion" at the University of Washington Neuromuscular Disease Center Web Site. This site presents an outline and resources with much more detail.
http://www.neuro.wustl.edu/neuromuscular/pathol/snare.htm.

Recommended

- Rensberger B. (1996) "Pumping Protein" in *Life Itself* by Boyce Rensberger. Oxford University Press. New York, NY: 189-203.

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Implementation

1. Assign articles to students and direct them to one of the four **Assignments (1,2,3,4)** and **Resource Seven: Worksheet for Reading Primary Literature.** Students can work as individuals or in small groups. You may choose to assign only one article or assign different articles to different students. Should you choose to assign only one set of articles for the class, the readings from **Assignment 1** are very well written and an excellent choice for undergraduates. The readings from Assignments 1 and 4 are generally simpler, while those from Assignments 2 and 3 are more complex. Assignment 2 describes the biochemical purification of three SNAREs, while the other assignments describe how a single SNARE was identified as a target of the neurotoxin.

2. Have students complete the reading and the **Assignments** outside of class over the course of at least one week.

3. Have students submit answers to the questions that appear in the **Assignments** for grading.

Alternatives

Each of the four **Assignments** can stand alone or be used together in a course. For example, **Assignment 1** could be administered to each student in a class. Alternatively, the instructor may choose to administer all four assignments to a class spreading the assignments among students (e.g., 20 students complete Assignment 1, 20 students complete Assignment 2, etc.).

- In small classes, students could work in small groups on the same assignment to complete the questions and present their answers to the class. If you choose this latter option, direct students to **Resource One: Group Role Profiles.**
- The instructor may choose to have small groups of students or individuals present the method and significance of one of the figures in these articles instead of the entire paper.
- More time could be spent working through the papers. In small classes, this activity can take the form of student mini-lectures. Students should define unknown terms and symbols and their significance in understanding the findings of the paper. They would expected to provide visual aids and handouts for the class and would be graded on their presentation.

Assessment

Assessment will depend on what types of activities are assigned. Options include:

- Assessing student learning by reviewing answers to the questions on **Resource Seven: Worksheet for Reading Primary Literature.**
- Grading the answers to the questions that appear in **Assignment 1,2,3** and **4**, using **Activity 2: Answers Overview** and specific **Activity 2: Assignment Answers.**

- If students present their work as a group, presentations of significant figures or scientific terms could be graded using **Resource Two: Self-assessment of Group Work, Resource Three: Peer-assessment of Group Work, and Resource Four: Class assessment of Group Work**.