

## Activity 4: Molecular Mechanisms of Botulinum Toxin

### Teaching Notes

This activity could be used with students who have a minimal understanding of endocytosis and exocytosis. Reading assignments review cellular structures, protein interactions, enzyme function, and protein modifications.

For non-majors, the *Essential Cell Biology* sections and websites listed in the reading assignment should be assigned. Instructors may also want to assign selections from *Life Itself*, by Boyce Rensberger. This paperback book is written for the general public and does a fabulous job of placing intricate cellular processes in context.

Intermediate level students looking for more details can review the outline, notes, and resources on the University of Washington's Neuromuscular site. More advanced science students can complete the Schiavo and Pellizari readings available on-line and listed at the end of the reading assignment. These comprehensive articles should not be assigned to non-science majors or introductory level science majors. Should the students complete these advanced readings their molecular skits should reflect a great deal more detail.

### Activity 4 at a Glance

<b>Class</b>	Medium or large classes (25-300 students) All levels: Details included in the performance will vary with scientific background
<b>Instructor Preparation</b>	Approximately 90 minutes Read <b>Assignment</b> , assigned articles, and <b>Activity 4: Molecular Skit Overview</b> . If working with advanced level science students, also read <b>Botulinum Toxin Background</b> .
<b>Useful Media</b>	Arnon, S. S., R. Schechter, et al. (2001). "Botulinum toxin as a biological weapon: medical and public health management." <i>JAMA</i> 285(8): 1059-70. <a href="http://jama.ama-assn.org/cgi/content/full/285/8/1059">http://jama.ama-assn.org/cgi/content/full/285/8/1059</a>  Allergan. Mechanism of BOTOX Action. 2002 <a href="http://www.botox.com/site/professionals/product_info/mechanism_of_action.asp">http://www.botox.com/site/professionals/product_info/mechanism_of_action.asp</a>  <i>Cell Biology Interactive</i> 3.1,3.2, 3.11, 7.2, and 13.2-13.4. See also the CD-ROM Viewing Guide. Garland Science.  Groleau R. "Making Vaccines," as seen in the NOVA Bioterror Companion Website. 2001 <a href="http://www.pbs.org/wgbh/nova/bioterror/vaccines.html#">http://www.pbs.org/wgbh/nova/bioterror/vaccines.html#</a>  Terry T. "Botulinum Toxin Mechanism of Action" as found in Biology 102: Lecture Notes: The Nervous System. <a href="http://www.microvet.arizona.edu/Courses/MIC420/lecture_notes/clost">http://www.microvet.arizona.edu/Courses/MIC420/lecture_notes/clost</a>

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	<a href="http://www.garlandscience.com/ridia/clostridia_neurotox/movie/botulinum_movie.html">ridia/clostridia_neurotox/movie/botulinum_movie.html</a>
<b>Student time</b>	In Class: Two to four hours Out of Class: Two 50-minute class sessions

### Background Reading

For the purposes of this Skit, the readings from *Essential Cell Biology*, Second Edition are most appropriate. Instructors might also wish to assign selections from *Molecular Biology of the Cell*, Fourth Edition for more advanced students.

#### *Molecular Biology of the Cell* Fourth Edition

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

- “Intracellular Vesicular Traffic.” p 711-766. This section is most relevant and contains important figures.
- “Proteins” p.129-188. Background for antibody production and function.
- “Membrane Structure” p.583-614. Background on transmembrane proteins.
- “Membrane Transport” p. 645-650. Background on nerve function

#### *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). Note that the text does not clearly define SNAREs. V-SNAREs include VAMP/synaptobrevin and syntaxin, while T-SNAREs include SNAP-25 and its homologues. For more detailed reading see MBOC.
- “Protein structure and function.”p. 117-167 (Interactive 4.1, 4.2, 4.11)
- The most relevant sections include the sections on antibody production and function (p. 144-146).
- “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.

### Implementation

#### Day 1: 15 minutes

1. Show the video clip of endocytosis and exocytosis.
2. Break the class into groups of 20 students, assign each group one botulinum serotype (there are seven total, but you could also include TeNT). Direct the students to the **Assignment**. Allow students 10 minutes in class to assign molecule roles to

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members. Because this activity requires students to interact and often touch one another, it is important to include a narrator role that might be reserved for a student who does not feel comfortable with the physical contact involved in other roles.

3. Since the assignment requires textbook, articles, and website review, allow students sufficient time to access these tools. Consider assigning this activity over a weekend. In the **Assignment**, a number of options are listed for reading and homework. Instructors must review these choices and clearly inform students of readings, activities, and evaluation.
  - For non-majors or introductory level students, assign the readings from *Essential Cell Biology* and the web sites. Be aware that this text does not define SNAREs in detail. SNAP-25, syntaxin, and VAMP/synaptobrevin are never mentioned, rather, SNAREs are described as two groups of proteins involved in secretion; V-SNAREs and T-SNAREs. Instructors may also want to direct students to the **Character Sketch** assignment. This assignment provides students with more structure and asks students to write character sketches or “Want Ads” for their molecular role using analogies or creative language. See Cordts (1999) for creative examples and discussion (links below). For students who need more guidance, a version of **Activity 4: Molecular Skit Overview** could be distributed as a structured guideline for the assignment.
  - Direct advanced level students to the websites, the *Molecular Biology of the Cell* text, and the advanced level scientific articles written by Schiavo and Pellizari. These students do not need to complete the **Character Sketch** assignment.
  - Both introductory and advanced level students will greatly benefit viewing the figures on the web-accessible article written by Arnon et al. in *JAMA*.

### Day 2: 50 minutes

4. During this class session, groups gather to develop the skit of acetylcholine (Ach) release at the neuromuscular synapse, the block induced by botulinum toxin, and the release of the block in the presence of a botulinum inhibitor or vaccine. To permit simultaneous small group discussions, instructors may choose to have students leave the classroom but require them to stay close by so progress can be monitored. Some students have difficulty keeping track of time and working in groups, so each group should have a timekeeper, note taker, etc. see **Resource One: Group Role Profiles**.

### Day 3: 50 minutes

5. During this class session, students perform their skits. Instructors should review **Activity 4: Overview of Molecular Skits** prior to this class session. This overview describes the molecules and their interactions during acetylcholine release. In small classes, the instructor can have all groups present their skits. In larger classes, the instructor may select groups to act out a small portion of the uninhibited Ach release, toxin blocked Ach release, or toxin inhibition. For instance, one group could demonstrate toxin binding, while another demonstrated toxin translocation or SNARE cleavage. Class members who are not performing could try to determine the serotype, inhibitor, or vaccine type being depicted.
6. Spend the last ten minutes of class reviewing common misconceptions and notable performances or details.

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### Assessment

Since this activity could involve individual written work and performance work, you may choose to assess the students at various points during the activity.

- If character sketches or “Want Ads” are assigned, these could be graded on an individual basis.
- If all groups have time to perform their skits in class, group presentations can be graded. The level of detail in the student skits would depend on where in the course the activity is introduced and whether the students are in an introductory or intermediate level course.
- With respect to the group presentations, audience members can also receive some credit for their ability to correctly identify which serotype of toxin or toxin inhibitor is at work in the skit.
- If the class is larger and does not allow all groups to present their skits, an instructor may opt for peer assessment of group work. You may choose to administer the **Resource One: Group Work Self-assessment**, **Resource Three: Group Work Peer-assessment**, and **Resource Four: Class Assessment of Group Work** for either version of this activity.

### Useful References

1. Rensberger B., "Pumping Protein," in *Life Itself: Exploring the Living Realm of the Living Cell*. New York, NY: Oxford University Press, 1996 (p. 189-203).
2. Washington U. "Botulism" at the University of Washington Neuromuscular Disease Center Web Site. 2001.  
<http://www.neuro.wustl.edu/neuromuscular/nother/bot.htm#protein>
3. Schiavo G, Matteoli M, Montecucco C. (2000). "Neurotoxins affecting neuroexocytosis." *Physiological Reviews* 80(2): 717-66.  
<http://physrev.physiology.org/cgi/content/full/80/2/717>
4. Pellizzari R, Rossetto O, Schiavo G, Montecucco C. (1999). "Tetanus and botulinum neurotoxins: mechanism of action and therapeutic uses." *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 354(1381): 259-68.  
<http://barbarina.catchword.com/vl=56882652/cl=32/nw=1/fm=docpdf/rpsv/catchword/rs1/09628436/v354n1381/s3/p259> or  
[http://www.cnl.salk.edu/~neubig/PDF\\_PAPERS/LINKS/Pellizzari\\_1999\\_259.pdf](http://www.cnl.salk.edu/~neubig/PDF_PAPERS/LINKS/Pellizzari_1999_259.pdf)
5. Walter P, Theriot J. (2002) *Cell Biology Interactive*.
6. Arnon SS, Schechter R, Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, et al. (2001). "Botulinum toxin as a biological weapon: medical and public health management." *JAMA*. 285(8): 1059-70.  
<http://jama.ama-assn.org/cgi/content/full/285/8/1059>
7. Cordts M. (1999) "Using "Help Wanted" Ads as a Device to Teach about Function."  
<http://www.microbelibrary.org/FactSheet.asp?SubmissionID=445&LS=> and  
<http://www.microbelibrary.org/curriculum/Toolbox/wantad.pdf>