TIPS, TRICKS & TECHNIQUES

Doing the Molecular Splits: Hands-On Demonstration Tips to Promote Student Engagement Using Split Inteins in Molecular Biology

NABT BioClub RECOMMENDATION

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Abstract

Engaging undergraduates in the mechanisms of post-translational modifications lies at the heart of molecular and cell biology education. An important challenge for science educators is developing inclusive and equitable approaches and hands-on demonstrations to clarify post-translational modification mechanisms, such as the protein-splicing mechanism called split inteins. Here, we describe step-by-step assembly of recycled materials to help clarify the molecular action of split inteins in inclusive classroom teaching settings.

Key Words: *split intein; inclusion; equity; three-dimensional learning framework; hands-on demonstration; synaptojanin;* Caenorhabditis elegans.

Introduction

Post-translational modification mechanisms are among the core essentials of molecular cell biology research (Shah & Muir, 2010; Wong et al., 2012; Alberts et al., 2014; Dong et al., 2015). For example, the post-translational protein-splicing mechanism called *in*tervening pro*tein*, or *intein*, is used by diverse sets of organisms in the tree of life – including the domains Bacteria, Archaea, and Eukarya (Shah & Muir, 2010). Inteins have also been found in viral genomes (Shah & Muir, 2010). One reason to engage students in intein function is to focus on pathogenic bacteria, such as *Mycobacterium tuberculosis*, that use intein catalysis and that may serve as molecular targets for developing antimicrobial therapies (Zhang et al., 2011). Furthermore, there are many proteins that use intein catalysis for cellular and genetic mechanisms such as DNA replication, recombination, and repair (Lennon & Belfort, 2017). As science educators, how do we engage undergraduates with cutting-edge research that utilizes protein-splicing mechanisms to address important research questions in molecular cell biology research? Inclusive and equitable teaching strategies, hands-on demonstrations, and original research papers are all valuable ways to clarify molecular cell biology principles (Crowe et al., 2008; AAAS, 2011; Brownell et al., 2014), and course-based undergraduate research experiences (CUREs) provide inclusive research experiences for undergraduates (Auchincloss et al., 2014; Bangera & Brownell, 2017).

Our lesson plan to engage undergraduates in the functioning of split inteins includes five learning objectives (Table 1), all of which are linked to both *Vision and Change* (AAAS, 2011) and the three-dimensional learning framework (National Research Council, 2012). In order to establish inclusive and equitable approaches, we used the culturally responsive SOAR framework and began each course with three themes: (1) Health and Environment; (2) Communities; and (3) Molecules, Cells, Organs and Organisms (Figure 1). SOAR stands for the following: Spiral curricula and process of inquiry ("spiralquiry"); Observations from experiments to evaluation of data; Affective learning in active-learning settings; and Research year-round across the undergraduate curricula (Kao, 2018).

We incorporated the stories of Guillermina Lozano and Mina Bissell to illustrate the importance of thinking outside-the-box when designing experiments, encouraging student reflection on how their lived experiences connect with these two excellent scientist role models. Drs. Lozano and Bissell won the E.E. Just Award and the E.B. Wilson Medal, respectively, from the American Society for Cell Biology (Fleischman, 2016; Spiro, 2018). The students' reflections on the lives of Lozano and Bissell throughout the semester are similar to the *Scientist Spotlight* described by Schinske et al. (2016), helping establish a sense of belonging, inclusive learning, and diversity in STEM.

Furthermore, we linked the concept of inteins to community-based health care and how environment affects human health (e.g., Parkinson's disease). Finally, as a step to address important questions in neuroscience, we linked inteins, which can be used to address important neuroscience research questions, to scientific inquiry into the molecular mechanisms of neurotransmission. By using culturally responsive teaching and mentoring for all undergraduates, we were able to scaffold molecular cell biology of split inteins around multiple dimensions of learning in both class and lab settings.

The American Biology Teacher, Vol. 82, No. 7, pp. 499–502, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2020.82.7.499.

Table 1. Split-intein learning objectives linked to *Vision and Change* (AAAS, 2011) and the three-dimensional learning framework (National Research Council, 2012).

Vision and Change	Three-Dimensional Learning	Learning Objectives
Competencies • Ability to Apply the Process of Science • Ability to Use Modeling and Simulation	*Dimension 1: Practices* • Asking Questions • Developing and Using Models • Planning and Carrying out Investigations	1. Clarify and define the mechanism of split inteins in context of neuroscience research. 2. Define the role of split inteins across domains Eukarya, Bacteria, and Archaea, as well as viruses. 3. Apply the concept of split inteins to addressing important questions in neuroscience research. 4. Predict the effect of a loss-of-function mutation on the molecular mechanism of split-intein function. 5. Construct a molecular model of split-intein molecular action.
Concepts • Pathways and Transformations of Energy and Matter • Structure and Function • Evolution	*Dimension 2: Crosscutting Concepts* • Structure and Function • Cause and Effect • Energy and Matter: Flows, Cycles, and Conservation	
	Dimension 3: Disciplinary Core Ideas • LS1: From Molecules to Organisms: Structures and Processes • LS4: Biological Evolution: Unity and Diversity	

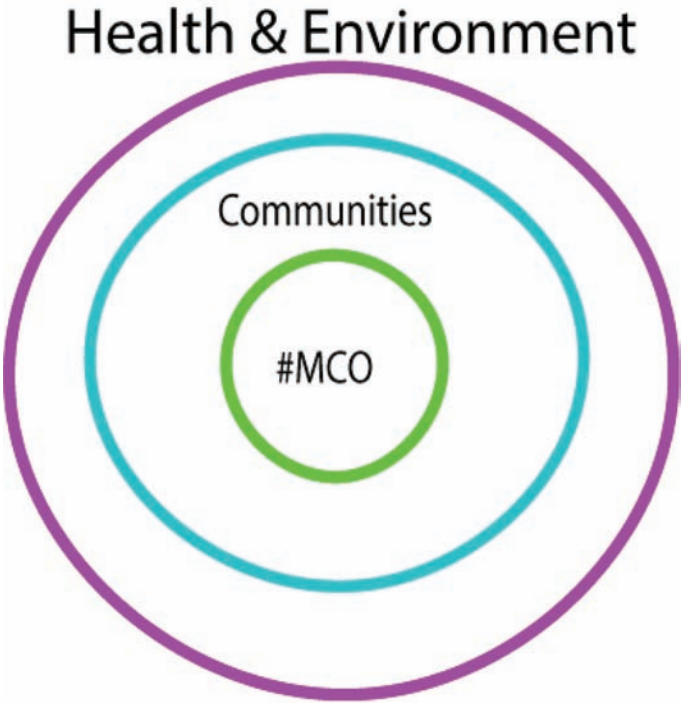

Figure 1. Adaptive multidimensional framework for molecular cell biology, with three concentric circles: Health and Environment (magenta); Communities (cyan); and Molecules, Cells, Organs and Organisms (#MCO, green).

Over the past three years, we have developed, in a partnership between Heritage University and the Fred Hutchinson Cancer Research Center, a molecular cell biology CURE. All undergraduates in our molecular cell biology course at Heritage University read about research from Dr. Jihong Bai's research group (at "Fred Hutch") using split inteins to test the molecular actions and protein folding of a key protein involved in synaptic transmission, called synaptojanin (Dong et al., 2015). Dong and colleagues used a special form of split DnaE intein derived from the filamentous cyanobacterium *Nostoc punctiforme* in their study. The following Tips, Tricks, and Techniques provide two sets of step-by-step directions for assembly of split-intein hands-on demonstrations based on this collaboration.

○ Materials for the Hands-On Demonstrations

- Dollar-store pipe cleaners
- Recycled easel pens
- Velcro-brand One-Wrap tie straps, two different colors (self-gripping cable ties, 8 × 0.5 inches, available from Amazon)

○ How to Assemble the Demonstration of Split-Intein Molecular Action

1. Place two easel pens of different colors side-by-side (Figure 2A). The knob end of each pen symbolizes the N-terminus of the polypeptide, while the blunt end represents the C-terminus of the polypeptide. The two pens symbolize the two parts of the synaptojanin protein involved in synaptic transmission in *Caenorhabditis elegans*.
2. Next, tie a knot at the C-terminus end of the first half of the polypeptide using the pipe cleaners, and do the same at the N-terminus of the second half of the polypeptide. The pipe cleaner tied to the first half of the polypeptide represents the N-intein, while the pipe cleaner tied to the second half of the polypeptide represents the C-intein (Figures 2B and 1C).

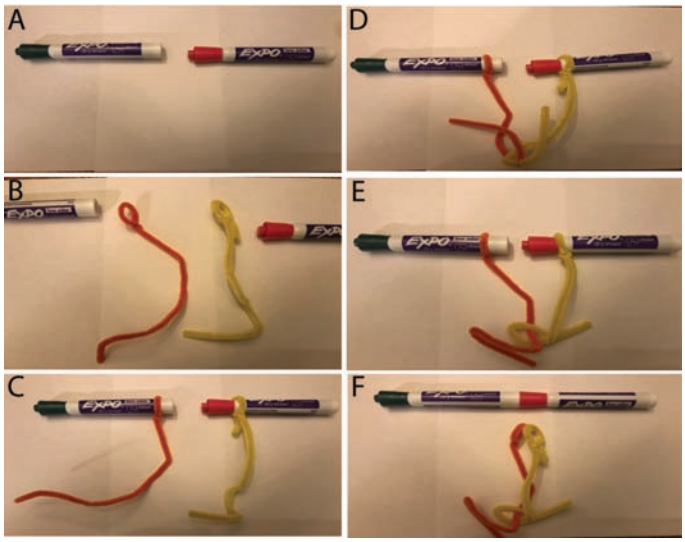

Figure 2. Step-by-step assembly for demonstration of split-intein molecular action (**A**–**F**). See text for detailed directions and materials.

3. To demonstrate the action of protein splicing, link the N-intein and C-intein pipe cleaners together (Figure 2D, E).
4. As the intervening protein (intein) is spliced out, the two polypeptide proteins are now joined or ligated together as one intact polypeptide (Figure 2F).

○ How to Assemble the Demonstration of Wild-Type & Loss-of-Function Split Inteins

1. This demonstration will allow students to compare wild-type and loss-of-function split-intein function. First, make a loop in two One-Wrap tie straps of different colors (Figure 3A). To model wild-type split-intein function, ensure that the fuzzy side of the Velcro is facing up.
2. Fasten one of the looped tie straps to the C-terminus end of the easel pen that corresponds to the carboxyl tail of the polypeptide.
3. Fasten the other looped tie strap to the other (N-terminus) end of the other pen that corresponds to the amino terminal of the polypeptide (Figure 3A, B).
4. To demonstrate intein catalysis, press the two ends of the tie straps to make them stick together (Figure 3C).
5. Make a loop between the N-intein and the C-intein (Figure 3D, magenta arrow).
6. Finally, join the carboxyl and amino terminal ends of the two polypeptide easel pens and stick them together to represent polypeptide ligation (Figure 3D).
7. Next, to demonstrate the loss-of-function intein, prepare two tie straps as in steps 1 and 2.
8. To model the loss-of-function N-intein, place one tie strap upside-down, such that the Velcro is teeth-side-up and the fuzzy side is facing the easel pen (Figure 3E, F).

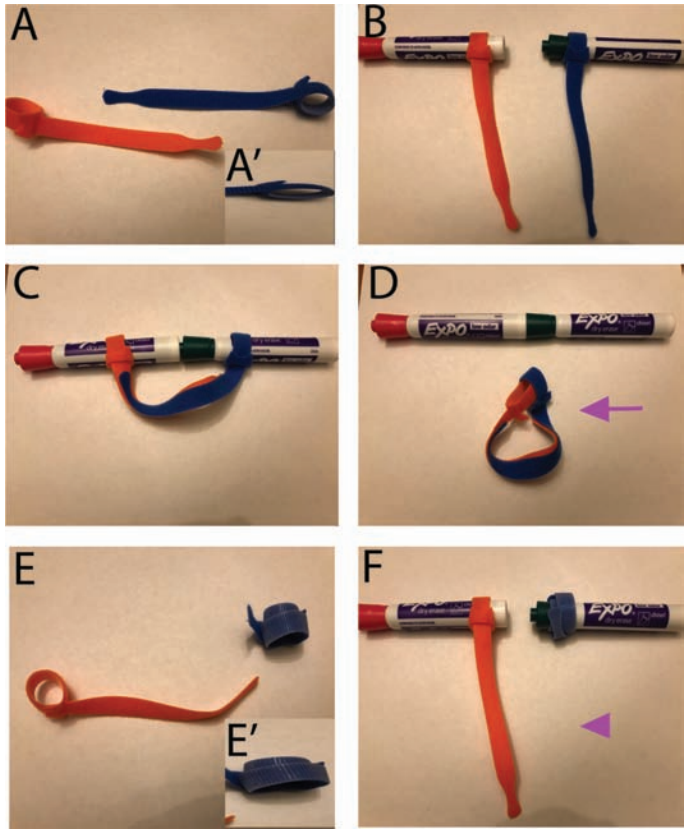

Figure 3. Step-by-step assembly for demonstrations of wild-type (**A**–**D**) and loss-of-function (**E**–**F**) split-intein catalysis. See text for detailed directions and materials.

9. Place the N-intein from step 8 at the amino terminal end of one easel pen (Figure 3E).
10. Similar to steps 1 and 2, fasten the C-intein to the carboxyl terminal of the other easel pen (Figure 3F). Due to loss of function in the N-intein, there is no functional split-intein catalysis (Figure 3F, magenta arrowhead).

○ Integrating These Hands-On Demonstrations into the Classroom

We use inclusive and equitable approaches to help promote metacognition in the classroom (Michael, 2003; Modell, 2005; Tanner, 2007, 2012, 2013). At the beginning of class discussions, we ask students: *What terms or concepts were unclear or confusing?* The theme of split inteins has come up repeatedly over the past three years when our students have reflected in their community-of-scholars teams. With student needs in mind, we offer teachers the following tips on using these demonstrations to better promote student comprehension of split insteins.

- After split inteins have been visualized for the class using PowerPoint (see Supplemental Material with the online version of this article), we use the hands-on demonstration of split-intein molecular action to complement and clarify the molecular process.
- We record class discussions to make a podcast that the more audio-based learners can use for review.

- We incorporate the "view from the inside" described by Modell (2007). All the students observe, reflect, and analyze the molecular action of split inteins as a community of scholars, while we act out the split intein's molecular catalysis reaction.
- After clarifying the molecular action of protein splicing, we ask students in their research teams to make a prediction: if one of the intein sequences had a loss-of-function mutation, how would that affect the protein splicing? These prediction questions are used to help develop students' critical thinking skills in community-of-scholars settings.
- In addition, the Velcro-based approach to split-intein modeling may also be used for discussions.
- While the context described here was synaptojanin molecular function in *C. elegans* neurotransmission research (Dong et al., 2015), these hands-on demonstrations can easily be adapted for many other topics in biology.
- Finally, this hands-on demonstration may be an excellent way for students who are more tactile to independently study protein-splicing mechanisms.

○ Supplemental Material

- PowerPoint guide on split-intein mechanism for class discussions

○ Acknowledgments

We wish to thank the anonymous reviewers for their input and comments. This work was supported by the Society for Developmental Biology through a science education grant (2018–2019). We also thank Dr. Jihong Bai and Ms. Yan Liu for their continuous support in our partnership between Heritage University and the Fred Hutchinson Cancer Research Center, as well as Drs. Emily Wiley, Lina Dahlberg, and Suzanne Lee for discussions on developing CUREs.

References

AAAS (2011). *Vision and Change in Undergraduate Biology Education: A Call to Action.* Washington, DC: American Association for the Advancement of Science.

Alberts, B., Johnson, A.D., Lewis, J., Morgan, D.M., Raff, M., Roberts, K. & Walter, P. (2014). *Molecular Biology of the Cell, 6th ed*. New York, NY: W.W. Norton.

Auchincloss, L.C., Laursen, S.L., Branchaw, J.L., Eagan, K., Graham, M., Hanauer, D.I., et al. (2014). Assessment of course-based undergraduate research experiences: a meeting report. *CBE–Life Sciences Education*, *13*, 29–40.

Bangera, G. & Brownell, S.E. (2017). Course-based undergraduate research experiences can make scientific research more inclusive. *CBE–Life Sciences Education*, *13*, 602–606.

Brownell, S.E., Freeman, S., Wenderoth, M.P. & Crow, A.J. (2014). BioCore Guide: a tool for interpreting the core concepts of vision and change for biology majors. *CBE–Life Sciences Education*, *13*, 200–211.

Crowe, A., Dirks, C. & Wenderoth, M.P. (2008). Biology in bloom: implementing Bloom's Taxonomy to enhance student learning in biology. *CBE–Life Sciences Education*, *7*, 368–381.

Dong, Y., Gou, Y., Li, Y., Liu, Y. & Bai, J. (2015). Synaptojanin cooperates in vivo with endophilin through an unexpected mechanism. *Elife*, *4*.

Fleischman, J. (2016). E.B. Wilson Medal – Mina Bissell. American Society for Cell Biology. https://www.ascb.org/science-news/e-b-wilson-medal-mina-bissell/ (accessed January 1, 2017).

Kao, R.M. (2018). Helping students SOAR: Quizfolio tips to engage first-generation underrepresented minority undergraduates in scientific inquiry. *American Biology Teacher*, *80*, 228–234.

Lennon, C.W. & Belfort, M. (2017). Inteins. *Current Biology*, *27*, R204–R206.

Michael, J.A. & Modell, H.I. (2003). *Active Learning in Secondary and College Science Classrooms: A Working Model for Helping the Learner to Learn*. Mahwah, NJ: Lawrence Erlbaum Associates.

Modell, H. (2007). Helping students make sense of physiological mechanisms: the "view from the inside. *Advances in Physiology Education*, *31*, 186–192.

Modell, H., Michael, J. & Wenderoth, M.P. (2005). Helping the learner to learn: the role of uncovering misconceptions. *American Biology Teacher*, *67*, 20–26.

National Research Council (2012). *A Framework for K–12 Science Education*. Washington, DC: National Academies Press.

Schinske, J.N., Perkins, H., Snyder, A. & Wyer, M. (2016). Scientist spotlight homework assignments shift students' stereotypes of scientists and enhance science identity in a diverse introductory science class. *CBE–Life Sciences Education*, *15*(3).

Shah, N.H. & Muir, T.W. (2010). Inteins: nature's gift to protein chemists. *Chemical Science*, *5*, 446–461.

Spiro, M. (2018). Lozano named 2018 E.E. Just Award winner. American Society for Cell Biology. https://www.ascb.org/member-news/lozano-named-2018-e-e-just-award-winner/ (accessed January 1, 2019).

Tanner, K. (2012). Promoting student metacognition. *CBE–Life Sciences Education*, *11*, 113–120.

Tanner, K. & Allen, D. (2007). Cultural competence in the college biology classroom. *CBE–Life Sciences Education*, *6*, 251–258.

Tanner, K. (2013). Structure matters: twenty-one teaching strategies to promote student engagement and cultivate classroom equity. *CBE–Life Sciences Education*, *12*, 322–331.

Wong, S.S., Kotera, I., Mills, E., Suzuki, H. & Truong, K. (2012). Split-intein mediated re-assembly of genetically encoded Ca(2+) indicators. *Cell Calcium*, *51*, 57–64.

Zhang, L., Zheng, Y., Callahan, B., Belfort, M. & Liu, Y. (2011). Cisplatin inhibits protein splicing, suggesting inteins as therapeutic targets in mycobacteria. *Journal of Biological Chemistry*, *286*, 1277–1282.

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