Breeding smart enzymes

Educator guide

PAPER DETAILS

Original title: Directed evolution of cytochrome c for carbon–silicon bond formation: Bringing silicon to life

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DISCUSSION QUESTIONS

	LEARNING STANDARDS
1. With directed evolution, scientists now have the ability to alter the makeup and function of enzymes. What are the advantages and the hidden dangers of directed evolution?	**SP3**
2. Professor Arnold's methodology involves producing a very large library of proteins and screening several generations to identify one with the desired function. This is known as directed evolution. What are the advantages of this method over rational design of a desired enzyme?	**RST.11-12.8**
3. Directed evolution results in genetic diversity. Is this an advantage or a problem?	**RST.11-12.9**
4. Why did Prof. Arnold's group decide to mutate three specific proteins, M103, M100, and V75, to achieve greater chemoselectivity and enantiospecificity?	**SP6** **RST.11-12.6**

ACTIVITIES FOR INTERACTIVE ENGAGEMENT

	LEARNING STANDARDS
Writing an abstract Students write an abstract explaining how directed evolution was used to generate the fourth variant of Rma cyt c with desirable properties.	**RST.9-10.2** **RST.11-12.2** **NS1**
Locating this study in the larger field Students use the annotated list of references to explain how this research builds on the published work of at least one other independent group of scientists. Students will evaluate whether data from this research supports or contradicts previous conclusions and reflect on the statement that scientific knowledge is a "community effort."	**SEP5** **Patterns** **RST.9-10.8** **RST.11-12.8** **VC6** **NS1**
Science in the news Students explore news stories in the Related Resources tab and evaluate the stories for tone, accuracy, missing information, etc. They may then write their own news stories on the article.	**RST.9-10.5** **RST.11-12.5** **RST.9-10.6** **RST.11-12.6** **RST.9-10.8** **RST.11-12.8**
Reaction explanation Students will identify a reaction conducted in the lab in the presence of metal complexes to produce compounds useful to society. Explain how directed evolution can be used to conduct this reaction with better yields and selectivities.	**SEP4** **SP3**
Results and conclusions Students diagram each of the experiments presented in the study (divided up by figure, if appropriate). They then consider the results depicted in each figure, and how these results support the conclusions of the study.	**SEP4** **Cause and effect** **SP1** **VC2**
The next steps Students design a follow-on experiment to this study that either addresses flaws or unanswered questions in the research at hand or builds on it to explore a new question.	**SEP4** **Cause and effect** **SP5**

ARTICLE OVERVIEW

Article summary (recommended for educator use only)

Directed evolution was used to increase the catalytic activity of cyt c from *Rhodothermus marinus*. Mutations at M100 were introduced in the wild-type (WT) protein. Increased catalytic activity and enantioselectivity were observed in carbon–silicon bond formation. The fourth-generation variant, V75T M100D M103E, was shown to produce the desired product with 99% ee and high chemoselectivity to C–Si bond formation. This is a striking example of how the WT enzyme can be used as a starting point for directed evolution until a desired activity and selectivity is reached for reactions not observed in nature.

Importance of this research

Carbon–silicon bond is unknown in biology. However, organosilanes are important compounds that have many practical applications. Traditionally, synthesis of organosilanes involves the use of metal catalysts. Several of these metals are expensive, rare, or toxic. Professor Arnold's group uses directed evolution to synthesize organosilicon compounds whereby engineered enzyme variants of the wild-type protein catalyze the reaction with very high chemoselectivity and enantioselectivity. High selectivity of the enzyme variant circumvents the need for separating unwanted side products. Thus, directed evolution of enzymes provides an efficient, green, and sustainable route to carbon–silicon bond formation. The study can be further extended to reactions that are not typically catalyzed by enzymes. In other words, enzymes can be engineered to promote chemical reactions not found in nature.

Experimental methods

- Site-saturation mutagenesis: a method by which an amino acid residue is replaced in the wild-type protein sequence with any of the other 19 possible amino acid residues to generate a library of enzyme variants. This is a rational mutagenesis technique often used in protein engineering.
- Protein (^{1}H), carbon (^{13}C), and silicon (^{29}Si) NMR spectroscopy: commonly used techniques for elucidating the structure of molecules containing hydrogen, carbon, and/or silicon atoms.
- High-resolution mass spectrometry: a characterization technique that is used to determine the accurate mass of a molecule. It is often used to distinguish between compounds with the same nominal mass and to determine elemental compositions.
- Gas chromatography: a separation technique that can be used to separate components in a mixture based on differences in volatility and polarity.
- Thin-layer chromatography: an analytical technique used commonly for identification of components in a mixture or to follow the progress of a reaction. The adsorbent is a solid phase (example: silica gel), and the mobile phase is a solvent of suitable polarity.
- Column chromatography: a method of separation of mixtures or purification of a compound in which the adsorbent is usually a solid (example: silica gel) and the mobile phase is a solvent of suitable polarity.
- Supercritical fluid chromatography: a separation technique in which the mobile phase is usually carbon dioxide.
- Sonication: a process in which sound waves are used to break open (lyse) *Escherichia coli* cells.
- Protein expression: a process by which proteins are generated (expressed) in a suitable host organism such as *E. coli*.
- Hemochrome assay: assay that is used to determine the heme concentration in a solution.
- Enzyme library screening: screening of enzyme variants for a specific trait (better activity, stability, etc.).

- Biocatalytic reaction: use of enzymes or whole cells expressing these enzymes to perform specific reactions.
- Circular dichroism: the unequal absorption of right- and left-handed circularly polarized light. Often used to calculate enantiomeric excess.

Conclusions

- Preliminary experiments with various heme proteins showed that wild-type *R. marinus* cyt c catalyzed carbon–silicon bond formation with 97% ee and a total turnover number (TTN) of 34. Conclusion: wild-type *Rma* cyt c was chosen as the biocatalyst for mutagenesis.
- Mutation at residue M100 created the *Rma* cyt c M100D variant. This variant catalyzed C–Si bond formation with a TTN of 550 and enantiomeric excess of >99%. Conclusion: The M100D mutation resulted in a variant that was able to catalyze C–Si bond formation yielding a single product and with great improvement in the TTN over the parent.
- Mutation at amino acid V75 gave the variant *Rma* cyt c V75T M100D. This variant catalyzed C–Si bond formation with a TTN of 890 and >99% ee. Conclusion: The TTN increased with each generation of the enzyme, thereby providing encouragement to continue further along this path to achieve even greater activities.
- Mutation at M103 gave the third-generation enzyme, *Rma* cyt c V75T M100D M103E. This enzyme catalyzed the same reaction with a TTN of 1520 and >99% ee. Turnover frequency also improved with each additional mutation. The third-generation variant had a turnover frequency (TOF) 7.1 times greater than the WT. Conclusion: Directed evolution of the WT enzyme resulted in an enzyme variant that catalyzes C–Si bond formation with 45-fold higher activity than the WT. No side products were observed.
- The third-generation enzyme, *Rma* cyt c V75T M100D M103E, favored the C–Si bond formation 29 times more than the carbon–nitrogen bond formation. The WT enzyme had a slight preference for forming the amination product. Conclusion: Chemoselectivity is greatly enhanced by directed evolution of the WT enzyme.

LEARNING STANDARDS ALIGNMENT

The following tables provide an overview of the learning standards covered by this article, including A Framework for K-12 Science Education (Framework), Common Core State Standards English Language Arts & Literacy (CCSS ELA), Common Core State Standards Statistics and Probability (CCSS HSS), AP Science Practices, and Vision and Change in Undergraduate Biology Education. Where applicable, activities and information will be marked with specific standards to which they are linked.

A Framework for K-12 Science Education		
Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Analyzing and interpreting data (SEP4) Evaluate the impact of new data on a working explanation and/or model of a proposed process or system. **Using mathematics and computational thinking (SEP5)** Use mathematical, computational, and/or algorithmic representations of phenomena or design solutions to describe and/or support claims and/or explanations. **Constructing explanations and designing solutions (SEP6)** Design, evaluate, and/or refine a solution to a complex real-world problem, based on scientific knowledge, student-generated sources of evidence, prioritized criteria, and tradeoff considerations. **Engaging in Argument from Evidence (SEP7)** Evaluate competing design solutions to a real-world problem based on scientific ideas and principles, empirical evidence, and/or logical arguments regarding relevant factors (e.g., economic, societal, environmental, ethical considerations). **Obtaining, Evaluating, and Communicating Information (SEP8)** Communicate scientific and technical information (e.g., about the process of development or the design and performance of a proposed process or system) in multiple formats (including orally, graphically, textually, and mathematically).	**ESS3.C Human Impacts on Earth Systems** Scientists and engineers can make major contributions by developing technologies that produce less pollution and waste and that preclude ecosystem degradation. **ETS1.B Developing Possible Solutions** When evaluating solutions, it is important to take into account a range of constraints, including cost, safety, reliability, and aesthetics, and to consider social, cultural, and environmental impacts	**Patterns** Observed patterns in nature guide organization and classification and prompt questions about relationships and causes underlying them. **Cause and Effect** Events have causes, sometimes simple, sometimes multifaceted. A major activity of science is investigating and explaining causal relationships and the mechanisms by which they are mediated. Such mechanisms can then be tested across given contexts and used to predict and explain events in new contexts.

Common Core State Standards English Language Arts-Literacy		
Key Ideas and Details	Craft and Structure	Integration of Knowledge and Ideas
RST.9-10.1 Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions. **RST.9-10.2** Determine the central ideas or conclusions of a text; trace the text's explanation or depiction of a complex process, phenomenon, or concept; provide an accurate summary of the text. **RST.11-12.1** Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account. **RST.11-12.2** Determine the central ideas or conclusions of a text; summarize complex concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.	**RST.9-10.4** Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 9-10 texts and topics. **RST.9-10.5** Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy). **RST.9-10.6** Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, defining the question the author seeks to address. **RST.11-12.4** Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11-12 texts and topics. **RST.11-12.5** Analyze how the text structures information or ideas into categories or hierarchies, demonstrating understanding of the information or ideas. **RST.11-12.6** Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, identifying important issues that remain unresolved.	**RST.9-10.8** Assess the extent to which the reasoning and evidence in a text support the author's claim or a recommendation for solving a scientific or technical problem. **RST.9-10.9** Compare and contrast findings presented in a text to those from other sources (including their own experiments), noting when the findings support or contradict previous explanations or accounts. **RST.11-12.8** Evaluate the hypotheses, data, analyses, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information. **RST.11-12.9** Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.

AP Science Standards	
AP Science Practices	AP Biology Content Standards
Science Practice (SP3) The student can engage in scientific questioning to extend thinking or to guide investigations within the context of the course. **Science Practice (SP5)** The student can perform data analysis and evaluation of evidence.	**Essential knowledge 2.D.3 (EK2.D.3)** Biological systems are affected by disruptions to their dynamic homeostasis. **Essential knowledge 4.A.6 (EK4.A.6)** Interactions among living systems and with their environment result in the movement of matter and energy.

Connections to the Nature of Science	
Vision and Change in Undergraduate Biology Education Core Competencies and Disciplinary Practices	A Framework for K-12 Science Education Understandings About the Nature of Science
Ability to use modeling and simulation (VC3) All students should understand how mathematical and computational tools describe living systems. **Ability to understand the relationship between science and society (VC6)** Biologists have an increasing opportunity to address critical issues affecting human society by advocating for the growing value of science in society, by educating all students about the need for biology to address pressing global problems, and by preparing the future workforce. Biologists need to evaluate the impact of scientific discoveries on society, as well as the ethical implications of biological research.	**Scientific investigations use a variety of methods (NS1)** Scientific inquiry is characterized by a common set of values that include logical thinking, precision, open-mindedness, objectivity, skepticism, replicability of results, and honest and ethical reporting of findings. New technologies advance scientific knowledge. **Science models, laws, mechanisms, and theories explain natural phenomena (NS4)** Models, mechanisms, and explanations collectively serve as tools in the development of a scientific theory.