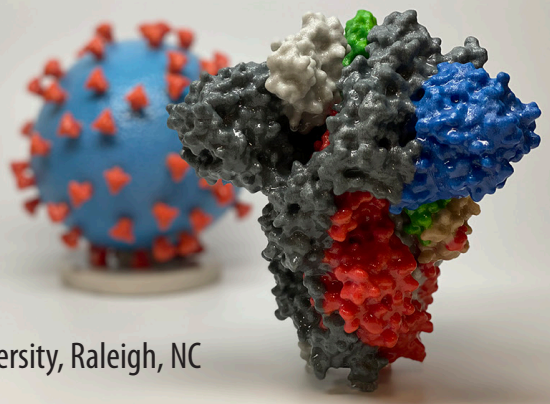
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Directed Evolution of Nanobodies for COVID-19 Prevention



by
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Introduction

The COVID-19 pandemic has led to over 190 million infections and over four million deaths worldwide as of July 2021 (WHO COVID-19 Dashboard), along with drastic changes in daily routines to limit the spread. While vaccination rates are climbing, therapeutics for prevention and treatment of COVID-19, as well as other related viruses, remain of interest.

One group recently used the process of directed evolution to develop an antibody that can block viral entry into human cells by binding the Spike protein on its surface. This process involved over 50 scientists from a range of backgrounds working together to pitch in their expertise for efficient development.

In this jigsaw case study, you will become the expert in one scientific area that is needed to complete the study. Then, you will teach your classmates and apply your knowledge to diagramming the process of drug development along with its roots in natural selection. Are you ready to become the expert for your team?

Part I – Expertise Building

Your instructor will assign you one of the following four topics at the appropriate level, either introductory (Level 1) or advanced (Level 2), to explore before your class meeting. Review the appropriate resources, answer each of the provided questions in your own words, and be prepared to discuss. You will be the expert on this topic and teach your classmates!

Topic 1 – Directed Evolution

Level 1

- Watch the following PCR introduction video:
  Polymerase Chain Reaction (PCR) Running time: 1:27 min. Produced by Cold Spring Harbor Laboratory's DNA Learning Center, 2010. <https://youtu.be/2KoLnIwoZKU>
- Read "Directed Evolution Primer" (Appendix 1 of this document, two pages).

Level 2

- Read "Introduction" and "Directed Evolution of Proteins" in the following review article:
  Cobb, R.E., R. Chao, and H. Zhao. 2013. Directed evolution: past, present and future. *AIChE Journal* 59(5): 1432–40. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4344831/>

Questions

1. What is meant here by a "library" and what are the "variants" in it?
2. What might be the goals of a scientist using directed evolution?

3. What do DNA shuffling, saturation mutagenesis, and error-prone PCR have in common other than being methods of generating variants? What are the differences among these methods? (Figure 2 from Cobb *et al.* will be particularly helpful here.)
4. What do bead pull-down and FACS have in common other than being screening methods? How do they differ?

Topic 2 – Yeast Surface Display

Level 1

- Watch the following introductory video on yeast surface display:
  Yeast Surface Display. Running time: 3:25 min. Produced by A. Bixler, 2021. <https://youtu.be/yweYD5muQw0>

Level 2

- Read "Introduction," "Yeast Surface Display Platform," and "Engineering Proteins for Increased Affinity" in the following review article:

  Cherf, G.M., and J.R. Cochran. 2015. Applications of yeast surface display for protein engineering. In: B. Liu (ed.), *Yeast Surface Display. Methods in Molecular Biology*, vol 1319. Humana Press, New York, NY. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544684/>

Questions

1. What is meant here by a "library" and what are the "variants" in it?
2. What is the purpose of the yeast surface display technique?
3. The video (and this case study) focuses on the example of antibodies as the protein being screened. What are antibodies, and what, specifically can yeast surface display do to improve antibodies? (Remember that antibiotics are different!)
4. List or diagram the steps of yeast surface display (make a bulleted list with or without sketches, or if you prefer to sketch it, just label your drawings clearly).
5. What characteristics of yeast make them a useful model for studying what happens in human cells?

Topic 3 – Nanobodies

Level 1

- Watch the following introductory video on nanobodies:
  Mini-Antibodies Discovered in Sharks and Camels Could Lead to Drugs for Cancer and Other Diseases. Running time: 2:37 min. Produced by *Science Magazine*, 2018. <https://youtu.be/p1nrzEYZ5rY>
- Read the introduction (first four paragraphs) and "Nanobody Advantages" in the following news article:
  Anon. 2020. Camelid nanobodies poised to revolutionize antibody therapeutics [webpage]. *Genetic Engineering and Biotechnology News* August 26, 2020. <https://www.genengnews.com/insights/camelid-nanobodies-poised-to-revolutionize-antibody-therapeutics/>

Level 2

- Read "Abstract," "From Heavy Chain-only Antibodies to Nanobodies," "Nb-based Phenotypic Screening," and "*In vitro* Affinity Improvement of Nbs" in the following review article:
  Hassanzadeh-Ghassabeh, G., *et al*. 2013. Nanobodies and their potential applications. *Nanomedicine* 8(6). <https://www.futuremedicine.com/doi/full/10.2217/nnm.13.86>

Questions

1. What are antibodies? What is their structure?
2. What are nanobodies (mini-antibodies or scAbs)? What is their structure?
3. Which organisms produce nanobodies naturally? What organism might be used in the future to easily produce large quantities of nanobodies?

4. What can nanobodies be used for?
5. What are the advantages of nanobodies over antibodies?

Topic 4 – Protein Binding

Level 1

- Read the "What is Binding Affinity?" and "Why Should I Measure Binding Affinity for My Application?" sections of the following webpage:
  Malvern Panalytical. *n.d.* Binding affinity [webpage]. <https://www.malvernpanalytical.com/en/products/measurement-type/binding-affinity>

Level 2

- Read "Why Measure Binding Affinity?," "When Do Researchers Measure Binding Affinity?," and "Surface Plasmon Resonance" in the following eBook:
  NanoTemper. *n.d. The Researcher's Guide to Measuring Binding Affinity and Why It Matters* [eBook]. <https://offers.the-scientist.com/hubfs/downloads/TS/TS_NanoTemper/NanoTemper_eBook_Researchers_Guide_Binding_Affinity.pdf>
- Read "What is K_D and how does it correlate to antibody affinity and sensitivity?" and the first three questions and answers of "K_D value – FAQs" at the following webpage:
  Abcam. *n.d.* K_D value: a quantitative measurement of antibody affinity [webpage]. <https://www.abcam.com/primary-antibodies/kd-value-a-quantitive-measurement-of-antibody-affinity>

Questions

1. What is binding affinity?
2. What is a ligand in this context?
3. What is meant by structure-function relationships? Why would binding affinity affect structure-function relationships?
4. Why should a scientist measure binding affinity?

Class Session I

You will be placed in a group with students who researched the *same* topic as you. This is your opportunity to ask clarifying questions and make sure everyone is equally prepared to help their teams.

A member of your group will then summarize what you have learned about the topic to a new group or the entire class. Your instructor will provide you with their preferred method for dissemination.

Part II – Application of Expertise

Now that you have some background, you will apply it to a recent topic: directed evolution in the development of COVID-19 preventative therapeutics. First, we need to review how the SARS-CoV-2 virus enters its host cell, triggering infection. The Spike protein of SARS-CoV-2, which radiates out from the surface of the viral particle, is the part that binds to a specific receptor (ACE2) on human cells, which triggers entry. You can view the graphics on the following webpage for a visual review of this process:

- Fischetti, M., V.F. Hays, and B. Glaunsinger. *n.d.* Inside the coronavirus [webpage]. *Scientific American*. <https://www.scientificamerican.com/interactive/inside-the-coronavirus/>

Our goal is the physical blockage of the Spike protein to prevent binding to ACE2 and thereby prevent viral infection. Review the appropriate resource below prior to your next class meeting.

Level 1

- Read "Summary of Schoof *et al.* (2020)" (Appendix 2 of this document, three pages).

Level 2

- Read the following article:
  Schoof, M., B. Faust, R.A. Saunders, S. Sangwan, V. Rezelj, *et al.* 2020. An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science* 370(6523): 1473–9. <https://doi.org/10.1126/science.abe3255>

Questions

1. What protein is being displayed on the yeast surface? How many variations were screened?
2. What is the ligand being used in the screen?
3. What methods were used to detect interaction?
4. After the initial rounds of selection, how many candidates were recovered?
5. After initial selection, what additional experiment was performed to narrow down the candidates?
6. What was the purpose of the affinity maturation step?
7. Compare and contrast the methods used in this study to natural selection.

Level 2 Only:

8. What is the difference between Class I and Class II candidates?
9. What feature of Nb6 led to design of a trivalent molecule?
10. What was the purpose of the second library featuring Nb6? How was the library made and how was it screened?

Class Session II

You will be placed in a group with students who researched a *different* topic than you did for the first class session. Each member will take turns articulating how their topic was integrated into the research study. Your group should also work together to clarify any remaining questions from reading the primer/paper.

After reviewing the paper and your topics, work together to create a list, diagram, or concept map connecting directed evolution to the process of natural selection. What principles are the same? What features are different? How does this process assist the researchers in reaching their goal?

It may help to think about a specific example of natural selection that was presented in class. Your product should clearly illustrate or define both natural selection and directed evolution and should include at least three similarities and three differences.

References

Articles and Webpages

Abcam. *n.d.* K_D value: a quantitative measurement of antibody affinity [webpage]. <https://www.abcam.com/primary-antibodies/kd-value-a-quantitive-measurement-of-antibody-affinity>

Anon. 2020. Camelid nanobodies poised to revolutionize antibody therapeutics [webpage]. *Genetic Engineering and Biotechnology News* August 26, 2020. <https://www.genengnews.com/insights/camelid-nanobodies-poised-to-revolutionize-antibody-therapeutics/>

Cherf, G.M., and J.R. Cochran. 2015. Applications of yeast surface display for protein engineering. In: B. Liu (ed.), *Yeast Surface Display. Methods in Molecular Biology*, vol 1319. Humana Press, New York, NY. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544684/>

Cobb, R.E., R. Chao, and H. Zhao. 2013. Directed evolution: past, present and future. *AIChE Journal* 59(5): 1432–40. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4344831/>

Fischetti, M., V.F. Hays, and B. Glaunsinger. *n.d.* Inside the coronavirus [webpage]. *Scientific American*. <https://www.scientificamerican.com/interactive/inside-the-coronavirus/>

Hassanzadeh-Ghassabeh, G., *et al*. 2013. Nanobodies and their potential applications. *Nanomedicine* 8(6). <https://www.futuremedicine.com/doi/full/10.2217/nnm.13.86>

Malvern Panalytical. *n.d.* Binding affinity [webpage]. <https://www.malvernpanalytical.com/en/products/measurement-type/binding-affinity>

NanoTemper. *n.d. The Researcher's Guide to Measuring Binding Affinity and Why It Matters* [eBook]. <https://offers.the-scientist.com/hubfs/downloads/TS/TS_NanoTemper/NanoTemper_eBook_Researchers_Guide_Binding_Affinity.pdf>

Schoof, M., B. Faust, R.A. Saunders, S. Sangwan, V. Rezelj, *et al.* 2020. An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science* 370(6523): 1473–9. <https://doi.org/10.1126/science.abe3255>

World Health Organization Coronavirus Dashboard. <https://covid19.who.int/>

Videos

Mini-Antibodies Discovered in Sharks and Camels Could Lead to Drugs for Cancer and Other Diseases. Running time: 2:37 min. Produced by *Science Magazine*, 2018. <https://youtu.be/p1nrzEYZ5rY>

Polymerase Chain Reaction (PCR) Running time: 1:27 min. Produced by Cold Spring Harbor Laboratory's DNA Learning Center, 2010. <https://youtu.be/2KoLnIwoZKU>

Yeast Surface Display. Running time: 3:25 min. Produced by A. Bixler, 2021. <https://youtu.be/yweYD5muQw0>

Internet references accessible as of August 2, 2021.

Appendix 1 – Directed Evolution Primer

Introduction

Directed evolution is "the laboratory process by which biological entities with desired traits are created through iterative rounds of genetic diversification and library screening or selection" (Cobb *et al.*, 2013). Biological entities in this case can include proteins, metabolic pathways, or entire microorganisms. Examples of desired traits include breaking down or producing particular molecules, functioning under harsh conditions (such as low pH or in the presence of solvents), increased motility or ability to form biofilms, or many other alterations to existing functions. In 2018, Dr. Frances Arnold won the Nobel Prize in Chemistry for her work in pioneering directed evolution of enzymes, which she began in the early 1990s. Today, this method is widely recognized for its ability to rapidly identify proteins displaying a property of interest.

In essence, directed evolution is an iterative (repeated over and over) two-step process (Figure 1 below):

1. Generate a library of biological variants.
2. Screen the library for a property of interest.

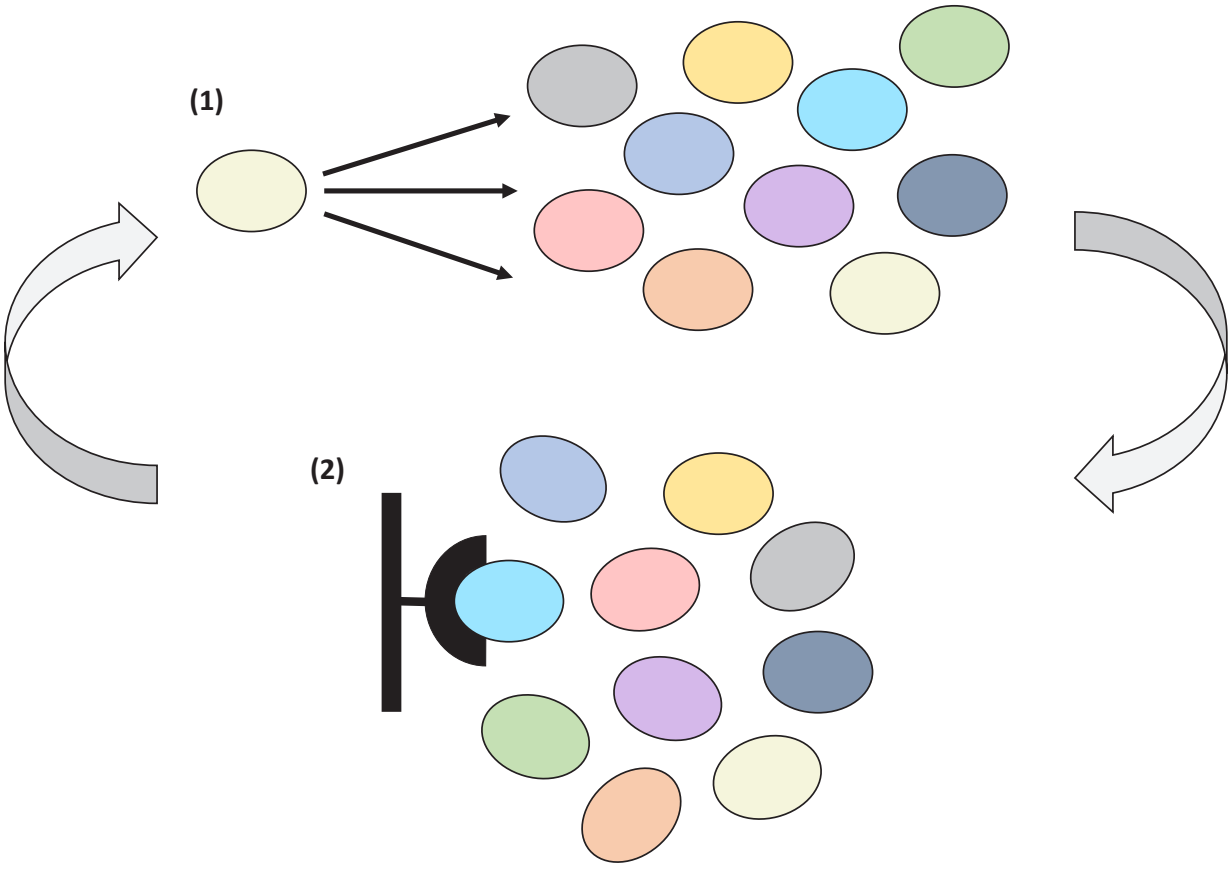

Figure 1. Two-step process of directed evolution

Following initial screening, the top hits from the screen will be modified to produce additional options for another round of screening, with the process repeating until the desired property is achieved.

Methods for Library Generation

When screening for protein properties, the library generation step relies on inducing a specific mutation rate into copies of the gene encoding the protein. Although variations of these methods have been developed, we will cover the basics here.

- Review Figure 2 in the following article:
  Cobb, R.E., R. Chao, and H. Zhao. 2013. Directed evolution: past, present and future. *AIChE Journal* 59(5): 1432–40. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4344831/>

Error-Prone PCR

In a typical PCR (polymerase chain reaction), a thermostable polymerase is added to a DNA template and a set of primers with the goal of faithfully producing many copies of the gene of interest. However, by using error-prone polymerases and an imbalanced pool of nucleotides, the reaction can be made to produce errors randomly, which results in a pool of mutant genes. This is referred to as "error-prone PCR."

DNA Shuffling

If multiple versions of the gene already exist, such as homologues from different species or previously produced mutants, they can be recombined, or "shuffled," in a test tube by cutting them into fragments with a nuclease followed by PCR to join them back together. The resulting genes will have sections from multiple starting genes, and are referred to as shuffled genes or chimeras.

Saturation Mutagenesis

If a particular amino acid position has been identified as relating to the property of interest, researchers can produce a pool of mutants that have that one amino acid swapped out for each of the twenty possible amino acids in a process called saturation mutagenesis. This is done by purchasing a special pool of primers that cover the site of interest, and then performing PCR.

Screening Methods

While a wide variety of screening methods exist, here we cover two of the most widely used, which are also employed in a journal article we will be looking at.

Bead Pull-Down

If your property of interest involves binding, the ligand of interest can be attached to tiny beads; after flowing your mutant library across the beads and washing away unattached mutants, the beads can then be recovered, along with the bound proteins. In some cases, magnetic beads are used, allowing the beads to be easily pelleted by use of a magnet.

Fluorescence Activated Cell Sorting (FACS)

Due to the ease of detection, fluorescence is a widely used screening method. If your property of interest can occur in or on a cell and triggers fluorescence, these cells may be physically separated out using fluorescence activated cell sorting, or FACS. FACS uses an instrument called a flow cytometer that flows cells one by one (but very rapidly) across a light source, which is situated across from a detector. If fluorescence is detected, the flow is averted, causing that cell to go into a separate tube. At the end of a sorting run, all of the fluorescent cells will be in one tube, while the non-fluorescent cells will be in another. The fluorescent cells can then be further analyzed to identify the mutants of interest.

- Watch the following introductory video on flow cytometry:
  Flow Cytometry Animation. Running time: 4:35 min. Produced by StarCellBio, 2015. <https://youtu.be/EQXPJ7eeesQ>

Appendix 2 – Summary of Schoof *et al.* (2020)

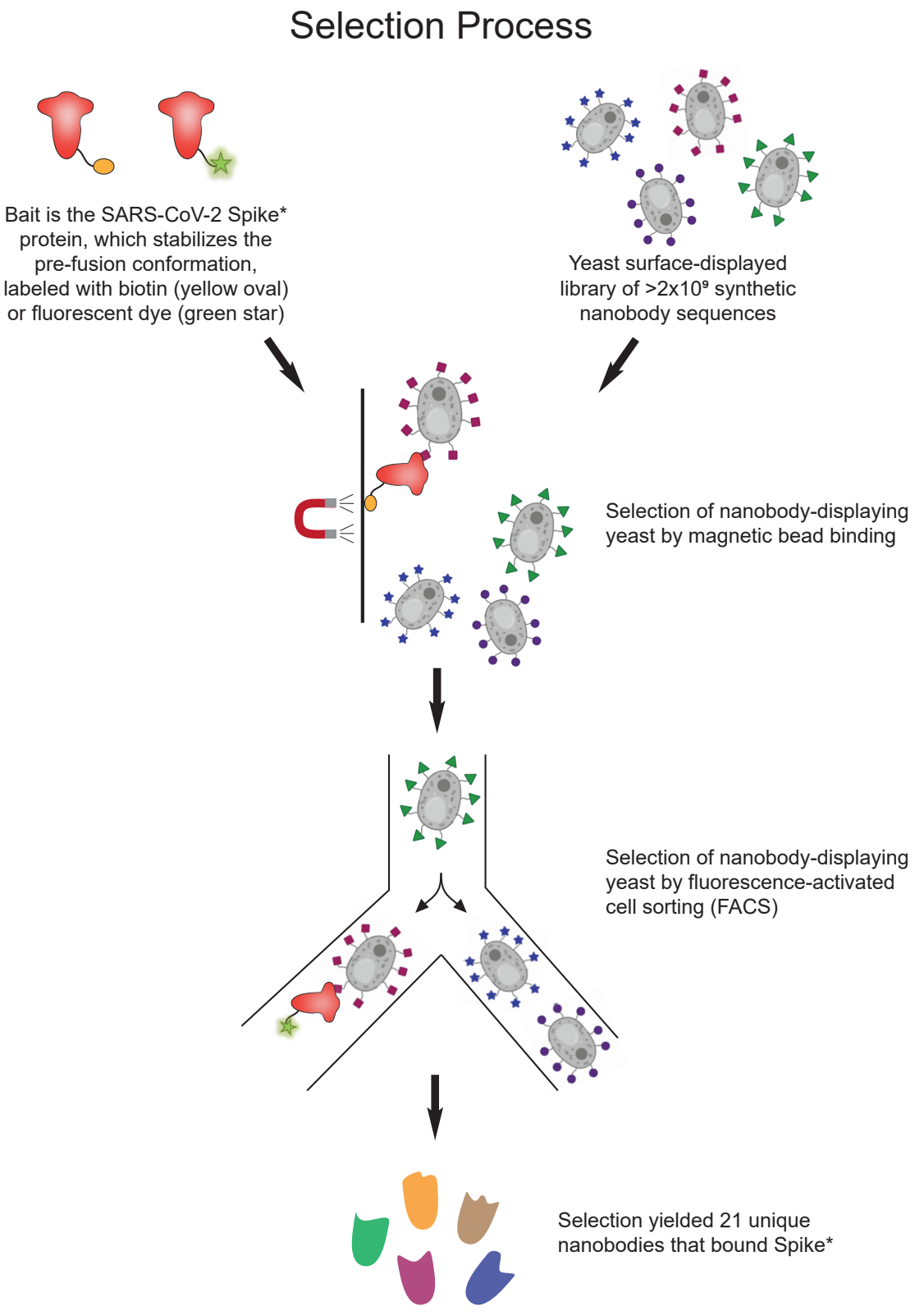

Figure 1. Selection process.

Confirmation Test

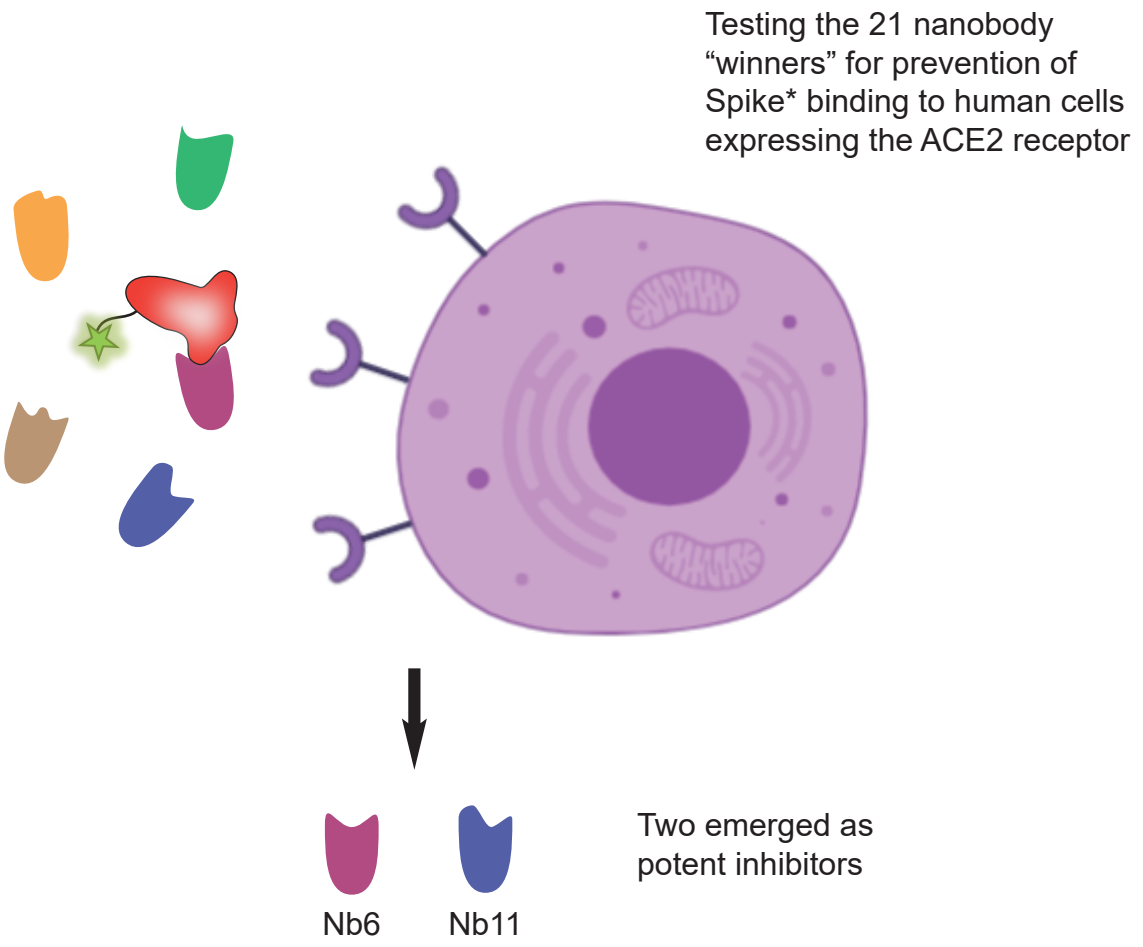

Figure 2. Confirmation test.

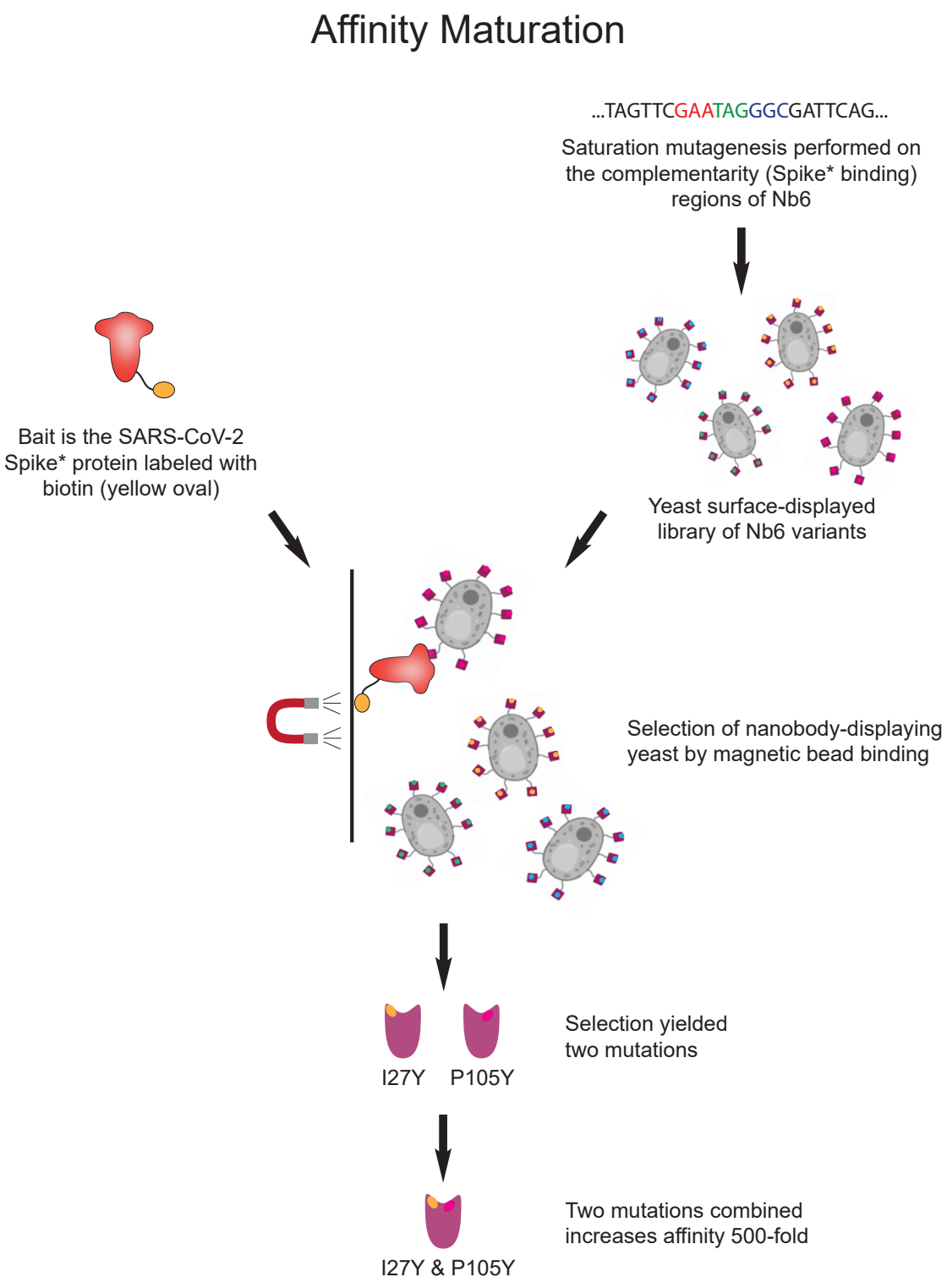

Figure 3. Affinity maturation.