3d molecular designs

Transforming Science Learning…

Engineering Biology: *…an mRNA Vaccine Design Activity*

Faced with a global COVID-19 pandemic in 2019, researchers created an effective mRNA vaccine directed against the CoV-2 virus -- in less than one year. This remarkable achievement required a knowledge of both *Science* and *Engineering*. The necessary *Science* had evolved over the past seven decades as we discovered (i) the central dogma of molecular biology, …and (ii) how our immune system works. The *Engineering* that was applied came from a new branch of biological engineering in which the traditional materials of engineers --- lumber, steel and plastics --- were replaced with nucleic acids, proteins and cells.

In this activity, your students will be challenged to apply what they know about how life works (i.e., DNA/RNA/Proteins) to design an mRNA vaccine that can activate our immune systems to protect us from virus infection.

As your students explore the mRNA Vaccine Story, they will learn about

- ***Katalin Kariko***… a researcher who persisted in her efforts to prove the effectiveness of mRNA vaccines
- How ***pseudouridine*** -- discovered in tRNA in the 1960's -- plays a critical role in the
- The clever mechanism whereby our immune systems distinguish between our *endogenous mRNA* and *incoming viral RNA*.
- A clever application of ***codon degeneracy*** to design an optimized mRNA vaccine.

See Notes at the end of this document for links to resources describing the Katalin Kariko story, the structure of pseudouridine and the role of Toll-like Receptor 7 (TLR7) in detecting viral mRNA.

In this ***mRNA Vaccine Design Challenge,*** students will

- examine the nucleotide sequence of the beginning of the mRNA that encodes the first 10 amino acids of the CoV-2 spike protein
- compare the nucleotide sequence of the CoV-2 mRNA vaccine to the spike mRNA sequence, and ….

- use a codon preference chart to understand how the spike mRNA sequence was optimized.

What will your students need to know before they start this activity?

- That the sequence of amino acids in a protein is encoded by the sequence of nucleotides in messenger RNA….that is in turn encoded by a sequence of nucleotides in DNA
- Codons consisting of 3 nucleotides can be translated into one of the 20 amino acids that make up proteins.
- Our genetic code is degenerate…meaning that more than one codon can encode the same amino acid. (We have 4 nucleotides (A,T,G,C)… and can therefore make 64 different triplet codons)

Begin by providing your students with a model of the nucleotide sequence of the first 10 amino acids of the mRNA encoding the CoV-2 Spike protein. (The first 3 amino acids of the spike protein have already been translated on this model into the first 3 amino acids of the protein.)

Do It… 1.

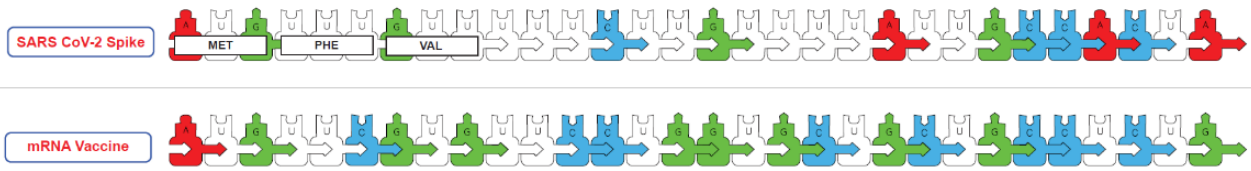
Use the Table of Genetic Codons to translate the next 7 codons into amino acids. (Pin the amino acid names on their corresponding codons)

Do It… 2.

Provide your students with the printed copy of the CoV-2 mRNA vaccine sequence and the physical model of the first the first 9 nucleotides of the mRNA vaccine. Ask your students to assemble the rest of the vaccine mRNA sequence … *using the printed sequence as a guide*.

Do It… 3.

Next, compare the physical models of the nucleotide sequences of the CoV-2 spike mRNA with the model of the sequence of the mRNA vaccine.



QUESTION: Why are these two sequences different?

Do It… 4.

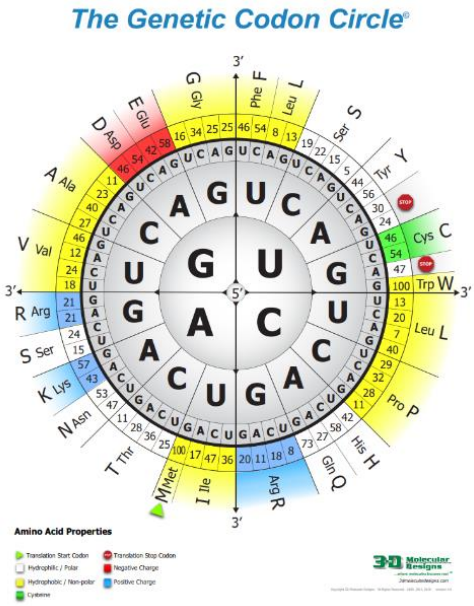
Using the Table of Genetic Codons – translate the nucleotide sequence of the mRNA vaccine into protein.

QUESTION: What did you notice?

Do It… 5.

Examine the circular Table of Genetic Codons which includes the human codon preference for all 64 codons. For example -- the third codon of the spike sequence (GUU/VAL) is used only 18% of the time whereas the third codon of the mRNA vaccine sequence (GUG/VAL) is used 46% of the time.

Compare the codon preferences of the first 10 codons of the SARS CoV-2 mRNA with the codon preferences of the engineered sequence of the mRNA vaccine.



Explain how this *"optimization"* of the sequence of the mRNA vaccine resulted in the production of more spike protein following vaccination.

DISCUSSION… The reason the two different sequences encode the same amino acid sequence is a result of ***codon degeneracy***…i.e., more than one codon can encode the same amino acid. The nucleotide sequence of the mRNA has been "codon optimized" so that the mRNA vaccine will be more efficiently translated into the spike protein once it is taken up by the dendritic cells at the vaccination site.

To understand how codon optimization works, your students will need to understand that humans (and all other species) prefer to use a subset of the 64 codons. For example, Leucine is encoded by 6 six different codons. But while CUG is used 40% of the time, UUA is only used 8% of the time.

EXTENSION -- Take away the printed sequence of the mRNA vaccine,…and ask your students to use the codon preference numbers to design a new mRNA sequence that will be more efficiently translated than the SARS CoV-2 mRNA.

But wait,…there's more… for teachers only. We have recently begun to appreciate that mRNA is not simply a single-stranded limp noodle. Instead, it can have secondary structure that is stabilized by standard Watson-Crick base pairs (A-U and G-C). In some cases, this secondary structure can influence how efficiently the mRNA is translated into protein by the ribosome. So,…vaccine engineers should keep this in mind as optimize mRNA sequences.

How might they validate their optimized designs before they commit to synthesizing and using them? For answers to this and other intriguing questions, join us at 3D Molecular Designs for stimulating conversations at one of our 5-day **Summer Courses**.

Codon Optimization was only one of several ways in which the COVID-19 mRNA vaccine was "engineered" to be a more effective vaccine. Another important modification of the mRNA was the substitution of ***pseudouridine*** for uridine. To learn more about this modification…check out the Tactile Base Pair models … coming soon from 3D Molecular Designs.

Further Resources….

This mRNA Vaccine Design activity was based on information contained in… *Modifications in an Emergency* ACS Cent. Sci. 2021, 7, 748−756 https://doi.org/10.1021/acscentsci.1c00197

The story of Katalin Kariko, pseudouridine and the 2023 Nobel Prize in Physiology and Medicine can be found in the following references:

Katalin Kariko's classic paper entitled**….** *Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability* ***Molecular Therapy*** vol. 16 no. 11, 1833–1840 Nov. 2008 1833.

The 2023 Nobel Prize in Physiology and Medicine.
https://www.nobelprize.org/prizes/medicine/2023/press-release/

mRNA: Fulfilling the Promise of Gene Therapy Drew Weissman and Katalin Karikó
doi:10.1038/mt.2015.138

A paper describing the structure of TLR7 binding to U-rich single-stranded RNA:
Structural Analysis Reveals that Toll-like Receptor 7 Is a Dual Receptor for Guanosine and Single-Stranded RNA Immunity 45, 737–748, October 18, 2016
http://dx.doi.org/10.1016/j.immuni.2016.09.011

If you want your students to understand how uridine is isomerized to pseudouridine,… check our 3DMD's new Tactile Base Pairs model….