Project Notes:

Project Title: Codon Optimization for Therapeutic Genes to Potentially Increase Transgene Expression in Mammalian Cell Lines
Name: Rathi, Svasti

Contents:

Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
What are AAVs?	Asking my mentors and parents as they both have extensive knowledge on the topic	Umass logbook and home notebook from summer	Over the summer
What is GM3 synthase deficiency and what are its symptoms?	Research over the summer and refreshed knowledge by reading part of article 5	Summary/notes of article 5 and home notebook from summer	Over the summer and 9/10-9/11
Have there been any prior experiments or treatments for GM3 Synthase deficiency?	Read article 5 for an example	Notes of article 5	9/10 -9/11
How do different promoters work in different cell types and organs throughout the body?	Asking/learning from mentors, refreshed by reading a couple articles that use different promoters. Was also refreshed	Notes for article 3 and 6, Umass logbook, google docs (elevator pitch)	Summer, 9/25 - 9/30

	with the elevator pitches		

Literature Search Parameters:

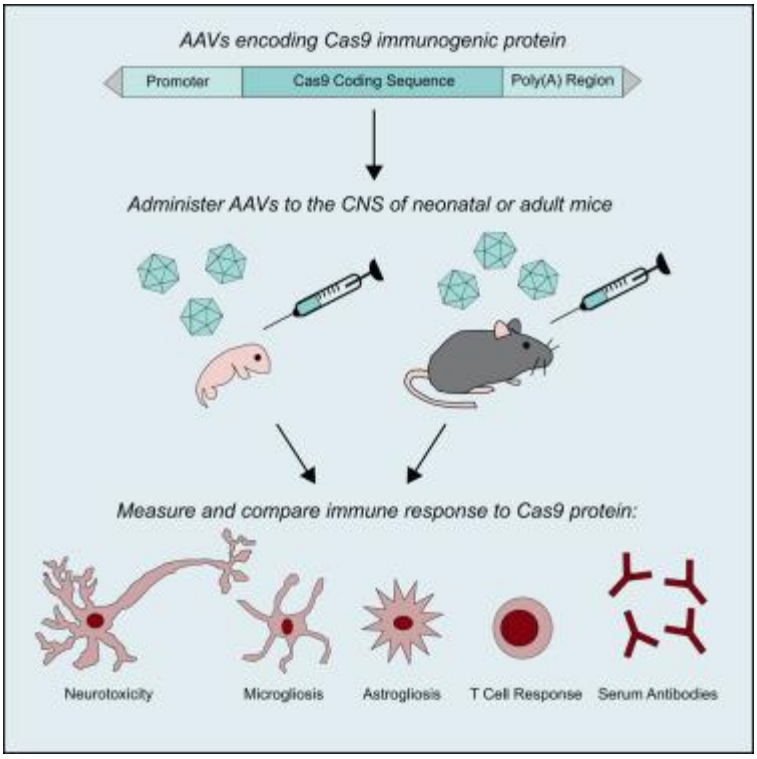
These searches were performed between 8/24/2025 and XX/XX/2025.
List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
Google Scholar	AAV, vectors, Gene editing	Found first article, showed experiment relating to key terms and also gave progress into finding future articles under similar topics as it greatly relates to my project
Google Scholar	GM3 Synthase deficiency, treatments, ST3GAL5	Found article 5, showed a previous experiment that was done to find a treatment for GM3 synthase deficiency, which relates to my project
Google Scholar	Promoters, gene editing	Found next article, showed experiments done using neuron specific vs ubiquitous promoters, and allowed me to gain more information on the different types of promoters and their effects
Google Scholar	AAV, promoters, treatments	Found multiple articles that give more information on differnet types of promoters and situations in which they can be used for.

Article #1 Notes: Early postnatal expression mitigates immune responses to Cas9 in the murine central nervous system

Article notes should be on separate sheets

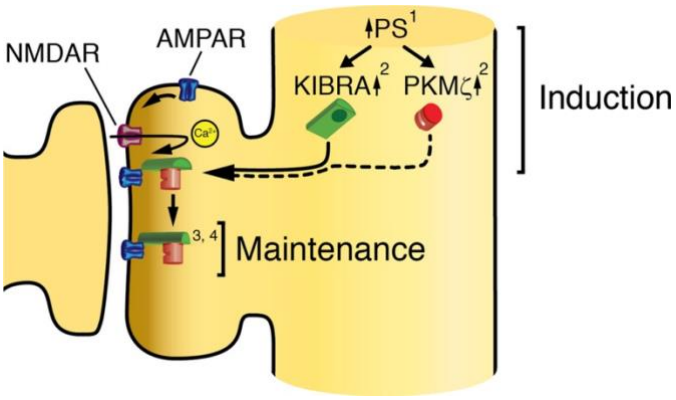
Source Title	Early postnatal expression mitigates immune responses to Cas9 in the murine central nervous system
Source citation (APA Format)	Duba-Kiss, R., & Hampson, D. R. (2025). Early postnatal expression mitigates immune responses to Cas9 in the murine central nervous system. *Molecular Therapy Methods & Clinical Development*, *33*(3), 101536. https://doi.org/10.1016/j.omtm.2025.101536
Original URL	https://www.cell.com/molecular-therapy-family/methods/fulltext/S2329-0501(25)00131-7?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2329050125001317%3Fshowall%3Dtrue#sec-3
Source type	Journal Article
Keywords	Adeno-associated virus, gene editing, green fluorescent protein, microglia, neonatal, neurodevelopmental disorder, neuroinflammation, prokaryotic, t-cell
#Tags	
Summary of key points + notes (include methodology)	In AAV gene therapy, the use of external proteins such as Cas9 can cause higher risks to immune responses, however, the age the AAV injections are administered may alter the risk of immune reactions. In this study, AAV-Cas9s were injected into neonatal and adult mice, results showing that Cas9 expression was maintained while immune response decreased in neonatal mice, comparative to adult mice where Cas9 was not detected, indicating an immune response occurring. This suggests that postnatal administration of the injection could improve safety and efficacy of treatments using Cas9 gene editing techniques. Two promoters were used for the AAV constructs. Synapsin-1, which is a neuron specific promoter, and Cytomegalovirus (CMV), which is a ubiquitous promoter, which should be expressed in multiple tissues. However, when tested, after a couple of months, the neonatal mice did have strong expression, which is expected, while the adult mice had no signs of detectable proteins, which helps prove the hypothesis.
Research Question/Problem/ Need	How can the immunogenicity risk from AAV gene editing be decreased?

Important Figures	 This image is a graphical abstract that was provided from the article
VOCAB: (w/definition)	Murine: Relating to mice or rodents Immunogenicity: The ability of a foreign substance to trigger an immune response Ubiquitous: Found everywhere
Cited references to follow up on	**The Potential of CRISPR/Cas9 Gene Editing as a Treatment Strategy for Inherited Diseases** **Prime Editing for Human Gene Therapy: Where Are We Now?** **Identification of preexisting adaptive immunity to Cas9 proteins in humans**
Follow up Questions	Can this be tested further with other proteins or Cas variants? How precise is the window for administration and how would closer ages affect the immune reactions? Would different promoters besides the synapsin and CMV cause different reactions?

Article #2 Notes: The Molecular Bond That Helps Secure Your Memories

Article notes should be on separate sheets

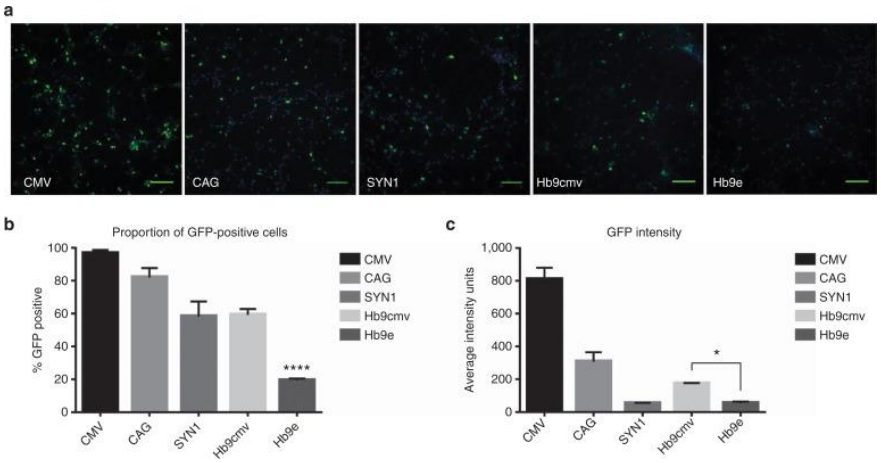
Source Title	The Molecular Bond That Helps Secure Your Memories
Source citation (APA Format)	Halilovic, A. (2025, May 12). *The molecular bond that helps secure your memories*. Quanta Magazine. https://www.quantamagazine.org/the-molecular-bond-that-helps-secure-your-memories-20250507/
Original URL	https://www.quantamagazine.org/the-molecular-bond-that-helps-secure-your-memories-20250507/
Source type	Online Journal Article
Keywords	Molecular bond, memory, proteins, KIBRA, synapse, neuron
#Tags	
Summary of key points + notes (include methodology)	The article, "The Molecule Bond that Helps Secure Your Memories," by Ajdina Halilovic explains a theory of how memories are stored over a lifetime, despite the molecules that form them degrading within a couple of days. Dr. Todd Sacktor, a professor in neurology at the SUNY Downstate Medical Center, decided to research this topic. He originally found that a protein called Protein Kinase M Zeta (PKMζ), located on the synapses of neurons, helps maintain memories for long periods. In his experiment, as the neurons fired their signals, PKMζ would strengthen, and when the protein activity was blocked, animals would lose their memories. After more research and collaborations, the importance of the protein KIBRA working with PKMζ was also found. KIBRA is a protein that holds other proteins in place within a synapse. In the specific case of long-term memory, PKMζ and KIBRA bind to each other in the synapse. Over time, as one protein within the bond degrades, the other will stay in place, and a replacement for the degraded protein will find its way to the bond and fill in the spot. The specific location of the proteins within the synapse is maintained by at least one of the proteins, which is what is believed to allow memories to stay for a long time, even as proteins and molecules continuously get replaced.
Research Question/Problem/ Need	How do memories last a lifetime when the molecules that form them turn over within days, weeks, or months?

Important Figures	 Not from article, but helps explain KIBRA and PKMζ interactions https://www.science.org/doi/10.1126/sciadv.adl0030
VOCAB: (w/definition)	Synaptic: anything of or relating to a synapse, which is the junction where nerve cells (neurons) connect and communicate with each other to transmit signals Molecular Turnover: the continuous process of a molecule's or a component's replacement
Cited references to follow up on	*no references are cited, but article includes links to other similar articles and some articles where data was collected from* KIBRA anchoring the action of PKMζ maintains the persistence of memory How 'Event Scripts' Structure Our Personal Memories
Follow up Questions	Could this data help find a cure or treatments for memory loss diseases like Alzheimer's? It this type of interaction similar throughout multiple parts of the brain or is it unique to the hippocampus?

Article #3 Notes: AAV9-mediated central nervous system–targeted gene delivery via cisterna magna route in mice

Article notes should be on separate sheets

Source Title	AAV9-mediated central nervous system–targeted gene delivery via cisterna magna route in mice
Source citation (APA Format)	Lukashchuk, V., Lewis, K. E., Coldicott, I., Grierson, A. J., & Azzouz, M. (2016). AAV9-mediated central nervous system–targeted gene delivery via Cisterna Magna route in mice. *Molecular Therapy - Methods & Clinical Development*, *3*, 15055. https://doi.org/10.1038/mtm.2015.55
Original URL	https://www.cell.com/molecular-therapy-family/methods/fulltext/S2329-0501%2816%2930145-0?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2329050116301450%3Fshowall%3Dtrue
Source type	Journal Article
Keywords	AAV9, Cisterna Magna, mediated, vector, transduction
#Tags	
Summary of key points + notes (include methodology)	The paper, “AAV9-mediated central nervous system–targeted gene delivery via cisterna magna route in mice” by Vera Lukashchuk et al. explains how certain promoters will express the Green Fluorescent Protein (GFP) differently based on being ubiquitous or neuronal. The researchers used the self complementary AAV9 vector with five different promoters (CMV, CAG, SYN1, Hb9cmv, and Hb9e) to examine the amounts of expression of the GFP in vitro and in vivo. The CMV and CAG promoters are known to be ubiquitous, while the SYN1, Hb9cmv, and Hb9e are all neuron-specific. The expression was measured by the proportion of GFP positive cells along with the intensity of the GFP. When tested in vitro by adding the virus into spinal cord cultures, all five promoters did show expression, however, the SYN1 and both Hb9 versions had significantly less expression than the two ubiquitous promoters. The CMV promoter showed both the highest proportion and the highest intensity, followed by the CAG promoter in both categories. For SYN1, a higher proportion of cells were transduced, but the GFP intensity was low. For Hb9cmv, the proportion was less, but the intensity was higher compared to SYN1. For Hb9e, both the proportion and the GFP intensity were found to be the least. The cell specificity of the SYN1 and Hb9 promoters for neuronal cells was also confirmed by immunocytochemical markers. When tested in vivo, the virus was injected into the spinal cord of the mouse through the cisterna magna, which is a standard practice to bypass the blood-brain barrier and enter the Central Nervous System (CNS). The CMV promoter was seen to once again have the highest proportion of transduced cells along with

	the most intense fluorescence. CAG and SYN1 were shown to have expression as well, but comparatively less than CMV. The SYN1 was expressed evenly throughout all neurons in the examined section, while both Hb9 promoters had specific expression only within the brainstem. Overall, data shows that the CMV promoter will have the highest expression, but will be shown across all tissues, while the SYN1 promoter will have the highest cell-specificity, but may not be enough to provide the desired outcome.
Research Question/Problem/ Need	How well can AAV9 vectors with neuron specific promoters carry transgenes into the CNS when administered through the Cisterna Magna compared to ubiquitous promoters?
Important Figures	 Shows the amount of expression from each tested promoter and helps visualize which ones are stronger.
VOCAB: (w/definition)	Cisterna Magna: a fluid-filled space located in the posterior fossa of the brain, between the cerebellum and the medulla oblongata Transduction: the transfer of genetic material from one cell to another by a virus Tropism: the specificity of a pathogen for certain cells during an infection
Cited references to follow up on	The advent of AAV9 expands applications for brain and spinal cord gene delivery Therapeutic AAV9-mediated suppression of mutant SOD1 slows disease progression and extends survival in models of inherited ALS Widespread and efficient transduction of spinal cord and brain following neonatal AAV injection and potential disease modifying effect in ALS mice
Follow up Questions	Can we use this technique to aid neurological disorders? Can this also be used to get to other cell types with different promoters? What are the major risks to using AAV9, especially regarding long term effects and expression?

Article #4 Notes: Some people are 'wired to connect with music on a deeper level,' study of 9,000 twins finds

Article notes should be on separate sheets

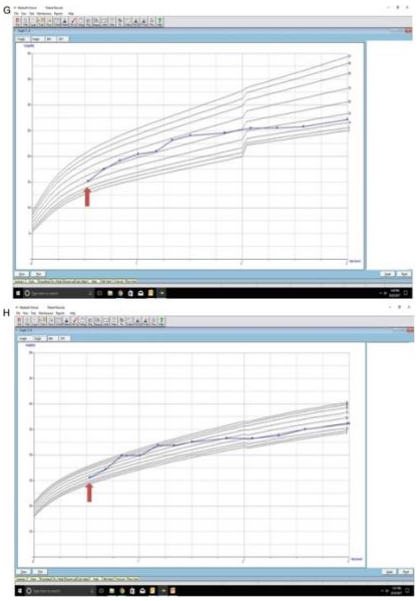
Source Title	Some people are 'wired to connect with music on a deeper level,' study of 9,000 twins finds
Source citation (APA Format)	Brincat, C. (2025, April 16). *Some people are "wired to connect with music on a deeper level," study of 9,000 Twins finds*. LiveScience. https://www.livescience.com/health/genetics/some-people-are-wired-to-connect-with-music-on-a-deeper-level-study-of-9-000-twins-finds
Original URL	https://www.livescience.com/health/genetics/some-people-are-wired-to-connect-with-music-on-a-deeper-level-study-of-9-000-twins-finds
Source type	News Article
Keywords	Genetics, Twins, Music, Reward Sensitivity, Perception
#Tags	
Summary of key points + notes (include methodology)	The article, "Some people are 'wired to connect with music on a deeper level,' study of 9,000 twins finds", describes an experiment that was done to test whether genetics play a part in how much people enjoy and how they perceive music. A total of 9000 Swedish twins were examined, both identical and fraternal pairings, using the Barcelona Music Reward Questionnaire, which asks questions regarding mood when listening to music, reactions to music, how much a person listens to music, etc. Identical twins share basically 100% of their DNA, while fraternal twins only share about 50%, and it was found that the music tastes and the effects of music for identical twins are very similar, with more than twice the similarity compared to fraternal twins. This indicated that there is a likely correlation between genetics and music perception.
Research Question/Problem/ Need	How much does genetics play a factor into the enjoyment and perception of music?
Important Figures	
VOCAB: (w/definition)	Reward Sensitivity: an individual's degree of responsiveness to positive stimuli, influencing their motivation to seek out and approach rewards, and their pleasure derived from them Homogenous Population: a group of people who share a uniform set of characteristics
Cited references to follow up	Music and Genetics https://doi.org/10.1016/j.neubiorev.2023.105302

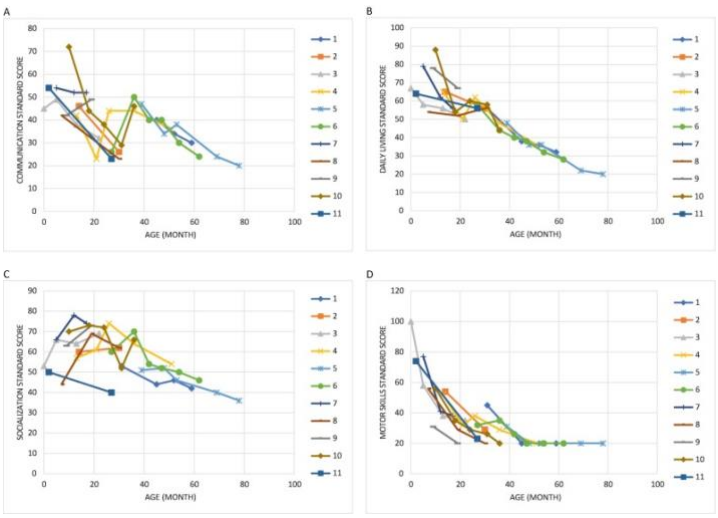
on	Identical twins don't share 100% of their DNA
Follow up Questions	Could these results possibly be used in therapy sort of situations? Can seeing how genetics effect music enjoyment gives a reason for why some people are more affected by music than others? Can other enjoyment/perception traits be studied in a similar format (with twins?

Article #5 Notes: Oral Ganglioside Supplement Improves Growth and Development in Patients with Ganglioside GM3 Synthase Deficiency

Article notes should be on separate sheets

Source Title	Oral Ganglioside Supplement Improves Growth and Development in Patients with Ganglioside GM3 Synthase Deficiency
Source citation (APA Format)	Wang, H., Sency, V., McJarrow, P., Bright, A., Huang, Q., Cechner, K., Szekely, J., Brace, J., Wang, A., Liu, D., Rowan, A., Wiznitzer, M., Zhou, A., & Xin, B. (2018). Oral ganglioside supplement improves growth and development in patients with ganglioside GM3 synthase deficiency. *JIMD Reports*, 9–20. https://doi.org/10.1007/8904_2018_134
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC6336560/
Source type	Journal Article
Keywords	Ganglioside GM3 synthase deficiency, ST3GAL5, Treatment
#Tags	
Summary of key points + notes (include methodology)	- GM3 synthase is needed to create gangliosides - Gangliosides are required for proper brain development - GM3 synthase deficiency – lack of gm3 synthase, results in lack of needed gangliosides - In infants, symptoms include intellectual disability, developmental delays, slower social development, etc - 13 kids, 11 under 40 months and 2 older kids, with gm3sd were given oral supplements of the missing gangliosides through dairy products - Ganglioside 500 was given, which is a food product made from cows milk that is made to be very rich in gangliosides - Kids were monitored over on average 34 months - Growth was measured on multiple scales: weight, height, occipitofrontal circumference, developmental and cognitive evaluation (Vineland), plasma ganglioside levels - Summary of results: general growth was improved after a couple months on the supplement, some kids with microcephaly also became normocephalic - Developmental improvements also seen, mostly in communication and social factors - However, many results faded over time, so rather than continuing on the improved path, they went back to what was expected because of the disease

	In the paper, Oral Ganglioside Supplement Improves Growth and Development in Patients with Ganglioside GM3 Synthase Deficiency, the researchers (Wang. H, et al.) wanted to see if there was a way to possibly cure a devastating neurological disorder, GM3 Synthase Deficiency (GM3SD). GM3SD is caused by a lack of essential gangliosides in infants, causing developmental delays, issues in growth, seizures, etc. In this experiment, the missing gangliosides were given as a supplement to multiple kids, mostly under 40 months) who were diagnosed with GM3SD. They improvements and growth was tracked over a span of an average of 34 months. Throughout many of the kids, overall growth was seen. This includes weight, height, and head circumference (microcephaly to normocephalic). Developmental growth was also seem, mostly within the communication and social aspects. However, these improvements seemed to fade away after some time in the patients Ratehrt han ocntinuing on the corrected path of growth, the kids went back to what was expected due to the disease. There also was no increase in ganglioside levels in blood plasma, which will need further research to see the cause.
Research Question/Problem/ Need	Can giving oral supplements of gangliosides such as GM3 improve growth and development in children with GM3 Synthase Deficiency?
Important Figures	 Shows the height and weight growth of patient 10 after being given the supplement, earlier figures used to compare to expected growth wihtout supplement

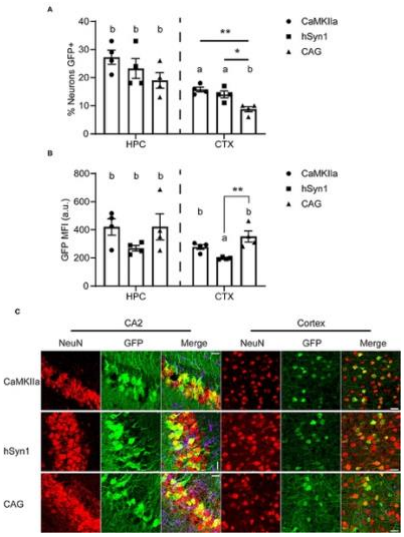
	 Shows effects of supplement on developmental behaviors, showing how slight improvement occurred but did eventually go back down
VOCAB: (w/definition)	Ganglioside: any of a group of complex lipids which are present in the gray matter of the human brain. Microcephaly: a medical condition characterized by an abnormally small head circumference Normocephalic: a medical term used to describe a person's head that is of normal size and shape Blood Serum: the clear, yellowish liquid component of blood that remains after the blood has clotted
Cited references to follow up on	Early growth and development impairment in patients with ganglioside GM3 synthase deficiency Brain ganglioside and glycoprotein sialic acid in breastfed compared with formula-fed infants Gangliosides: glycosphingolipids essential for normal neural development and function
Follow up Questions	Would gene therapy be effective in possibly improving the effects of this disorder? Could another form of delivery of the supplement be more effective? What is causing the improvements in development to stop and go back to what is expected without treatment?

Article #6 Notes: Toward Development of Neuron Specific Transduction After Systemic Delivery of Viral Vectors

Article notes should be on separate sheets

Source Title	Toward Development of Neuron Specific Transduction After Systemic Delivery of Viral Vectors
Source citation (APA Format)	Finneran, D. J., Njoku, I. P., Flores-Pazarin, D., Ranabothu, M. R., Nash, K. R., Morgan, D., & Gordon, M. N. (2021). Toward development of neuron specific transduction after systemic delivery of viral vectors. *Frontiers in Neurology*, *12*. https://doi.org/10.3389/fneur.2021.685802
Original URL	https://www.frontiersin.org/journals/neurology/articles/10.3389/fneur.2021.685802/full
Source type	Journal Article
Keywords	adeno-associated viral vectors, AAV, CaMKIIα promoter, Syn1 promoter, intravenous delivery
#Tags	

Summary of key points + notes (include methodology)	- Previously, only portions of the brain are transduced because the therapy gene can not disperse across the whole brain
	- AAVs can cross the blood brain barrier, and the current promoters that have better transduction generally leak into surrounding tissue rather than just neurons
	- 3 promoters are tested to see their transduction efficiency and transgene expression strength
	  o CAG – ubiquitous
	  o hSyn1 – human synapsin 1, neuron specific
	  o CaMKlla – excitatory neuron specific
	- Was injected intravenously into mice
	- They analyzed GFP expression in the central nervous system and nearby organs like the liver, heart, and skeletal muscles
	- All three promoters had expression in the brain, with highest transduction in the hippocampus and lower in the cerebellum
	- The neuron specific promoters had stron expression in the neuron, and very very little in the surrounding organs, which is good
	- The ubiquitous had highest expression in brain, but had expression in organs
	- Overall, all of the promoters worked and had very good expression in neurons and the neuron specific promoters were helpful in not expressing in organ
	- Can possibly be used now with gene therapy techniques to help neurological disoders

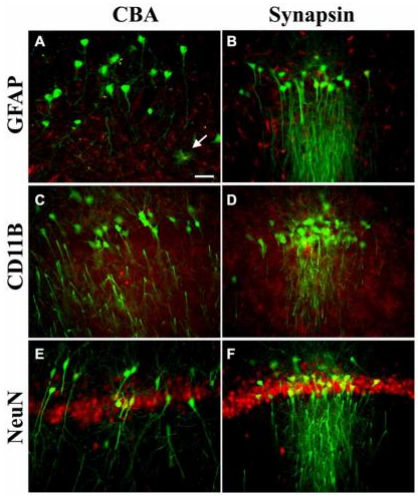
Research Question/Problem / Need	By using different AAV caspids and promoters, is there a possibility to increase transduction in the brain and distribute a therapy gene to more parts in the brain?
Important Figures	 Shows the expression from each promoter and shows that they are very close in expression rates
VOCAB: (w/definition)	Genome: all the genetic information of an organism or cell GFP: Green Fluorescent Protein, a protein discovered in jellyfish that emits a green glow when exposed to blue or ultraviolet light, allowing scientists to visualize cellular processes, track protein movement, and study gene expression by tagging them with the GFP gene Transgenic models: a living organism whose genome has been altered by the insertion of foreign or modified genetic material through genetic engineering techniques
Cited references to follow up on	CNS transduction benefits of AAV-PHP.eB over AAV9 are dependent on administration route and mouse strain Gene therapy for neurodegenerative diseases: slowing down the ticking clock AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems Better targeting, better efficiency for wide-scale neuronal transduction with the synapsin promoter AAV-PHP.B
Follow up Questions	Could this be tested with different AAV caspid types and would those be even better than what was tested? Is expression consistent over time or does it change after age or some sort of immune response? Can this be used to help provide treatment for neuro diseases that might need overexpression of proteins?

Article #7 Notes: Better Targeting, Better Efficiency for Wide-Scale Neuronal Transduction with the Synapsin Promoter and AAV-PHP.B

Article notes should be on separate sheets

Source Title	Better Targeting, Better Efficiency for Wide-Scale Neuronal Transduction with the Synapsin Promoter and AAV-PHP.B
Source citation (APA Format)	Jackson, K. L., Dayton, R. D., Deverman, B. E., & Klein, R. L. (2016). Better targeting, better efficiency for wide-scale neuronal transduction with the synapsin promoter and Aav-PHP.B. *Frontiers in Molecular Neuroscience*, *9*. https://doi.org/10.3389/fnmol.2016.00116
Original URL	https://www.frontiersin.org/journals/molecular-neuroscience/articles/10.3389/fnmol.2016.00154/full
Source type	Journal Article
Keywords	TDP-43; adeno-associated virus; amyotrophic lateral sclerosis; gene therapy; gene transfer; promoter; synapsin promoter; targeting
#Tags	

Summary of key points + notes (include methodology)	- Want to improve neuron-specific gene delivery throughout whole brain and spinal cord
	- Prior methods (AAV9 + CBA promoter)
	  o Caused expression in non-neuronal organs (heart, liver)
	- Researchers tested whether neuron specific promoter (synapsin) and aav capsid could increase accuracy and efficiency
	- They compared two promoters, CBA (ubiquitous) vs Synapsin (neuron specific)
	- Compared two AAV capsids (AAV9 and AAV-PHP.B -> stronger delivery in CNS)
	- Used intravenous and intracerebroventricular injections to give the viruses to both neonatal and adult rats
	  o ICV for adult and intravenous for neonatal
	- Used GFP or YFP as the reporter genes since they are fluorescent proteins
	  o Used an antibody which recognizes both
	- Animals were evaluated over time for motor function
	  o Rotarod, escape reflex, and survival
	- Found the synapsin promoter had strong neuronal targeting
	  o Very little heart expression
	  o Some expression in liver
	  o Generally, very limited effects not in the CNS

	○ Expression weakened over time, less stable - CBA promoter had very strong expression ○ However, lots of expression in other organs ○ Not specific expression - Second capsid had wider and stronger expression throughout brain compared to AAV9 ○ Less liver expression ○ More helpful in adults compared to neonatal - ICV injections also had broader expression and less exposure in the surrounding area - Basically: ○ Syn + AAV9 = neuron specific but too weak ○ CBA + AAV or AAV-PHP.B = strong expression but also a little too strong, can cause toxicity ○ Syn +. AAV-PHP.B = good balance, neuron specific and efficient - Can be useful in gene therapy and neurodegenterative disease models - However, promoter weakens over time, a bit of expression in other organs, and only a small sample size was used
Research Question/Problem/ Need	Can using a neuron specific promoter with a more efficient AAV capsid improve specificity in expression and gene delivery throughout the brain, while also decreasing expression outside of the CNS?
Important Figures	 Shows the expression in different cells throughout the CNS. Shows pretty equal expression, which is good.
VOCAB: (w/definition)	Capsid: the protein shell of a virus that protects its genetic material and plays crucial role in delivering it to a host cell Astrocyte: a star-shaped glial cell of the central nervous system Microglia: the immune cells of the central nervous system (CNS), acting as its "trash collectors" to patrol for and remove debris, dead cells, and pathogens Immunohistochemistry: the immune cells of the central nervous system (CNS), acting as its "trash collectors" to patrol for and remove debris, dead

	cells, and pathogens Attenuation: Weakening or decline of gene expression over time
Cited references to follow up on	In vivo selection yields AAV-B1 capsid for central nervous system and muscle gene therapy Intracerebroventricular delivery of self-complimentary adeno-associated virus serotype 9 to the adult rat brain
Follow up Questions	How can the syn+ AAV-PHP.B grouping be used in gene therapy for neurological disorders? Are there any potential immune risks in using this AAV in humans? Can other engineered AAVs be used with this same/similar promoters to target different cell types/neurons?

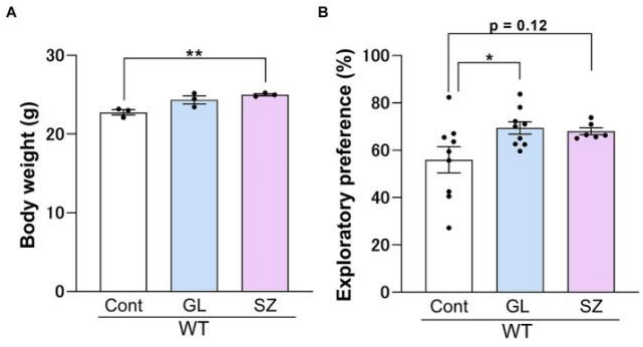
Article #8 Notes: Dietary gangliosides rescue GM3 synthase deficiency outcomes in mice accompanied by neurogenesis in the hippocampus

Article notes should be on separate sheets

Source Title	Dietary gangliosides rescue GM3 synthase deficiency outcomes in mice accompanied by neurogenesis in the hippocampus
Source citation (APA Format)	Inokuchi, J., Go, S., Suzuki, A., Nakagawasai, O., Odaira-Satoh, T., Veillon, L., Nitta, T., McJarrow, P., Kanoh, H., Inamori, K., Tan-No, K., & Collett, M. (2024). Dietary gangliosides rescue GM3 synthase deficiency outcomes in mice accompanied by neurogenesis in the hippocampus. *Frontiers in Neuroscience*, *18*. https://doi.org/10.3389/fnins.2024.1387221
Original URL	https://www.frontiersin.org/journals/neuroscience/articles/10.3389/fnins.2024.1387221/full
Source type	Journal Article
Keywords	GM3 synthase deficiency, milk gangliosides, GM3, GD3, oral supplementation, neurogenesis, hippocampus, cognitive function
#Tags	

Summary of key points + notes (include methodology)	- Want to see if oral supplements of gangliosides through milk can improve cognitive function in mice with GM3 Synthase Deficiency
	- Maybe see if the supplements can improve memory and learning deficits that were caused by the disease
	- Also does it restore the ganglioside levels or no
	- They used mice with GM3 Synthase Deficiency
	  o Mice with mutated ST3GAL5 gene, don't have required enzyme, can't make proper gangliosides, leads to the cognitive deficits
	- Gave the mice the milk supplements every day for a set period
	  o Control got normal milk without the ganglioside supplement
	- Teste behavior and cognitive ability through two tests
	  o Morris Water Maze: testing spatial learning and memory
	  o Novel Object Recognition: tests recognition memory
	- For brain analysis
	  o Used immunohistochemistry to measure neurogenesis markers
	  o Used biochem assays to quantify the ganglioside levels in the

	brain
	- For data analysis, compared the control mice vs the mice that were given the supplement for behavior, ganglioside levels, and neurogenesis markers
	- Mice with supplement had improved spatial learning and memory
	  o Enhanced performance in both tests
	- There was a higher ganglioside level in the mice after the supplement
	- There was an increase in progenitor cells in the hippocampus, which is good
	- Overall, shows supplements can be a good way to improve cognitive deficits in conditions that are linked to ganglioside synthesis disorders
	  o The cognitive improvement is most likely due to better neurogenesis and higher ganglioside levels

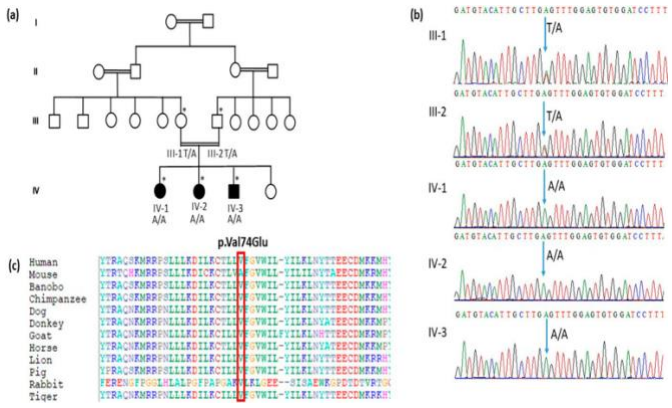
Research Question/Problem/ Need	Can oral supplements from that are enriched with gangliosides help imporve cognitive function and promote neurogenesis in the brain in mice with GM3 Synthase?
Important Figures	 Shows how GL500/the supplement affected body growth and cognitive function of the mice. Shows increase in body weight and shows enhancement of cognitive function
VOCAB: (w/definition)	Neurogenesis: the growth and development of nervous tissue. Progenitor: a "parent" cell that divides and gives rise to other distinct cells, serving as a precursor or ancestor in a cell lineage Oral Administration: route of administration where a substance is taken through the mouth and swallowed
Cited references to follow up on	Whole exome sequencing reveals a novel homozygous variant in the ganglioside biosynthetic enzyme, ST3GAL5 gene in a Saudi family causing salt and pepper syndrome. Recessive GM3 synthase deficiency: natural history, biochemistry, and therapeutic frontier. Altered expression of ganglioside GM3 molecular species and a potential regulatory role during myoblast differentiation.

Follow up Questions	Can the gangliosides play a role in preventing cognitive decline rather than improving it? How can these findings be useful in influencing the diet of babies, since human milk has a lot of gangliosides?

Article #9 Notes: Whole Exome Sequencing Reveals a Novel Homozygous Variant in the Ganglioside Biosynthetic Enzyme, ST3GAL5 Gene in a Saudi Family Causing Salt and Pepper Syndrome

Source Title	Whole Exome Sequencing Reveals a Novel Homozygous Variant in the Ganglioside Biosynthetic Enzyme, ST3GAL5 Gene in a Saudi Family Causing Salt and Pepper Syndrome
Source citation (APA Format)	Abdulkareem, A. A., Shirah, B. H., & Naseer, M. I. (2023). Whole exome sequencing reveals a novel homozygous variant in the ganglioside biosynthetic enzyme, ST3GAL5 gene in a Saudi family causing salt and pepper syndrome. *Genes*, *14*(2), 354. https://doi.org/10.3390/genes14020354
Original URL	https://www.mdpi.com/2073-4425/14/2/354
Source type	Journal Article/Case Study
Keywords	Salt and pepper developmental regression syndrome, epilepsy, developmental delay, GM3 synthase, short stature, Saudi Arabia
#Tags	

Summary of key points + notes (include methodology)	- Salt and Pepper Syndrome
	  o A rare, genetic disorder
	  o Symptoms include seizures, severe intellectual disabilities, movement disorders, changes in skin color/pigmentation (“salt and pepper skin”), and facial dysmorphism
	- ST3GAL5 is a gene, codes for GM3 Synthase
	- Mutations in ST3GAL5 cause deficiency in GM3 Synthase
	- This specific study, they wanted to find what gene was causing this SPDRS in the specific family
	- They studied a Saudi Arabian family that has 3 affected siblings, parents, and one unaffected child
	- They wrote descriptions on the developmental delays, seizures, failure to thrive, movement disorders, speech impairments, and growth failures of the patients
	- To do the diagnosis, EEGs, MRI’s, and physical examinations were

	done - They got DNA from blood samples, and did Whole Exome Sequencing (WES) on one of the affected patients - Also did Sangar Sequencing for all of the family to check how certain genes were separated - They would a homozygous missense variant in ST3GAL5, which causes a change in amino acids - This variant wasn't previously reported in literature, so the researchers basically found something new - All three of the siblings were homozygous for the variant - The parents are heterozygous carriers - The unaffected child had no homozygous variant - The variant supports that the loss of GM3 Synthase can cause severe neurological disorders - Shows that there is a larger field affected by mutations in ST3GAL5, rather than the originally though, older amish populations -
Research Question/Problem/ Need	Can exome sequencing help in identifying a genetic mutation causing Salt and Pepper Syndrome? Does this reveal anything about the ST3GAL5 gene in neurodevelopmental disorders?
Important Figures	 Pedigree shows family members with recessive traits and who are affected; Sangar sequence shows all affected family members having same homozygous allele causing disorder; protein alignment shows amino acid being conserved throughout multiple species, specfically the one that is the variant
VOCAB: (w/definition)	Whole Exome Sequencing (WES): a genetic test that analyzes the protein-coding regions (exons) of the genome to identify genetic variants that cause diseases, particularly rare genetic disorders Homozygous: having two identical alleles of a particular gene or genes. Heterozygous: having two different alleles for a specific gene, meaning one allele was inherited from each parent and they are not identical Variant: any deviation from a typical or common form, often a change in the DNA sequence of an organism or a virus

Cited references to follow up on	Recessive GM3 synthase deficiency: Natural history, biochemistry, and therapeutic frontier Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase Early growth and development impairments in patients with ganglioside GM3 synthase deficiency
Follow up Questions	Could this lead to other discoveries with neurological or developmental disorders? Can this help in collecting a lot more data on the ST3GAL5 gene and its effects/disorders? Why are gangliosides so important for brain development/neurological function?

Article #10 Notes: Altered expression of ganglioside GM3 molecular species and a potential regulatory role during myoblast differentiation

Article notes should be on separate sheets

Source Title	Altered expression of ganglioside GM3 molecular species and a potential regulatory role during myoblast differentiation
Source citation (APA Format)	Go, S., Go, S., Veillon, L., Ciampa, M. G., Mauri, L., Sato, C., Kitajima, K., Prinetti, A., Sonnino, S., & Inokuchi, J. (2017). Altered expression of ganglioside GM3 molecular species and a potential regulatory role during myoblast differentiation. *Journal of Biological Chemistry*, *292*(17), 7040–7051. https://doi.org/10.1074/jbc.m116.771253
Original URL	https://www.jbc.org/article/S0021-9258(20)42893-5/fulltext
Source type	Journal Article
Keywords	Ceramide, differentiation, ganglioside, sialic acid, skeletal muscle
#Tags	
Summary of key points + notes (include methodology)	- Gangliosides are lipids in cell membranes and are involved in cell signaling and growth - GM3 is a pretty simple ganglioside - But it has a lot of different species with different lengths and saturations - Previous studies show that gangliosides can affect cell fate and differentiation in a lot of cell types - Being able to see the muscle/myoblast differentiation is important so we can see how lipids can regulate cellular transitions - For this project, myoblast cells were grown as a cell culture and then induces to differentiate - GM3 species were isolated and quantifies - Chromatography and mass spectrometry were done to quantify the different species - Gene and protein expression was measured as markers so they could see if any correlation between ganglioside level changes existed - Showed very distinct patterns of expression during the stages of myoblast differentiation

	- Some had increased fatty acid chains, others had decreased, etc - The changes also correlated with the markers of differentiation - Concludes that GM3 species may actively influence the signaling pathways during differentiation - However, not all of the species/ types of GM3 act the same - GM3 can possibly affect the membranes, receptors, or growth factor signaling related to myoblast functions
Research Question/Problem/ Need	How do different types of GM3 change expression during myoblast differentiation, and do they play a specific role in muscle cell differentiation?
Important Figures	
VOCAB: (w/definition)	Molecular Species: defines species based on similarities and differences in their DNA or other biochemical molecules, rather than morphology or reproductive compatibility Myoblast: undifferentiated, single-nucleated precursor cells that are destined to become skeletal muscle cells Myogenesis: the fundamental biological process responsible for forming and developing muscle tissue in an organism, from the embryonic stage through postnatal growth Differentiation markers: molecules on the surface of a cell, such as glycoproteins or proteins, that act as specific identification tags, allowing scientists to identify and classify different cell types and their stages of development
Cited references to follow up on	Identification of ganglioside GM3 molecular species in human serum associated with risk factors of metabolic syndrome Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains
Follow up Questions	Could changing the amount and make of GM3 help muscle regeneration or growth in certain diseases like muscular dystrophy? How do different ages change the regulation of GM3 in muscle cells? What are some actual applications of this finding? Can it be used for therapy or muscle loss/injury, etc?

Article #11 Notes: Codon optimization of genes for efficient protein expression in mammalian cells by selection of only preferred human codons

Article notes should be on separate sheets

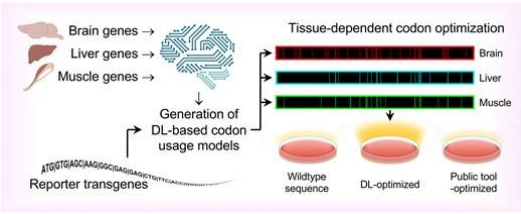
Source Title	Codon optimization of genes for efficient protein expression in mammalian cells by selection of only preferred human codons
Source citation (APA Format)	Inouye, S., Sahara-Miura, Y., Sato, J., & Suzuki, T. (2015a). Codon optimization of genes for efficient protein expression in mammalian cells by selection of only preferred human codons. *Protein Expression and Purification*, *109*, 47–54. https://doi.org/10.1016/j.pep.2015.02.002
Original URL	https://www.sciencedirect.com/science/article/abs/pii/S1046592815000133
Source type	Journal Article
Keywords	Codon optimization, Synthetic gene, Luciferase, Photoprotein, Reporter gene
#Tags	
Summary of key points + notes (include methodology)	- This research work targeted improving protein expression in mammalian cells by codon optimization of genes. - Only the most frequently occurring human codons were used, with a preference for a high GC content of more than 60% - Six bioluminescent proteins were selected as models because their activity is easy to measure - Synthetic genes were compared to wild-type sequences in CHO-K1 mammalian cells - All the preferred human codon-optimized genes demonstrated enhanced protein expression levels compared to their wild type genes. - This implies that the use of codons by itself is enough to produce a significant enhancement of protein production. - It is easier to implement than some of the other approaches for optimizing codons. - Codon optimization does not alter the amino acid sequence, but it helps in improving translation efficiency - It can be used in research on proteins, biotechnology, or production of therapeutic proteins - Preferred human codons likely increase translation efficiency by matching abundant tRNAs - Content of GC nucleotides could also affect stability of mRNA transcripts and their translation

	- This study shows that careful gene design can improve expression without altering protein function
Research Question/Problem/ Need	How does using preferred human codons in synthetic genes affect the efficiency of protein expression in mammalian cells compared to wild-type sequences?
Important Figures	
VOCAB: (w/definition)	Codon: a sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecule. GC content: the percentage of guanine (G) and cytosine (C) bases in a DNA or RNA sequence Transient: lasting only for a short time; impermanent.
Cited references to follow up on	Multiparameter RNA and codon optimization: a standardized tool to assess and enhance autologous mammalian gene expression Codon optimization can improve expression of human genes in Escherichia coli: a multi-gene study
Follow up Questions	What are the molecular mechanisms involved in the augmentation of gene expression by higher GC content in mammalian cells? How does this “preferred human codon” approach compare with other codon optimization algorithms or software regarding efficiency and robustness? How might the optimized human codon approach be generalized for application with other classes of proteins, such as larger or membrane-bound protein

Article #12 Notes: A deep learning model trained on expressed transcripts across different tissue types reveals cell-type codon-optimization preferences

Article notes should be on separate sheets

Source Title	A deep learning model trained on expressed transcripts across different tissue types reveals cell-type codon-optimization preferences
Source citation (APA Format)	Ravi, S., Sharma, T., Yip, M., Yang, H., Xie, J., Gao, G., & Tai, P. W. L. (2025). A deep learning model trained on expressed transcripts across different tissue types reveals cell-type codon-optimization preferences. *Nucleic Acids Research*, *53*(6). https://doi.org/10.1093/nar/gkaf233
Original URL	https://academic.oup.com/nar/article/53/6/gkaf233/8099988
Source type	Journal article
Keywords	Deep learning, Codon optimization, Tissue specific expression, coding sequence, GC content
#Tags	
Summary of key points + notes (include methodology)	- This research work proposes developing a more effective codon optimization algorithm using deep learning techniques to overcome drawbacks of existing codon optimization algorithms. - Codon optimization increases protein production, but often software can't work effectively to avoid protein misfolding or low expression. - Brain, liver, and muscle gene expression data were employed for training tissue-specific models using DL. - The DL model (Architecture: RNN/BiLSTM) learned patterns of codon utilization from highly expressed transcripts. - Sequences optimized for DL were found to produce more protein expression than the original sequences as well as the commercially optimized sequences, as evaluated in cells. - Liver-trained models of DL showed the highest expression even in non-liver cells. - The DL models preferred G/C-ending codons and reduced rare codons, which helped in improving CAI values. - Optimized sequences also had a lower number of CpG motifs, which would reduce the risk of immune activation, an important aspect for gene therapy applications. - It can be applied for designing more effective vaccine candidates, therapeutic proteins, or gene therapy vectors.

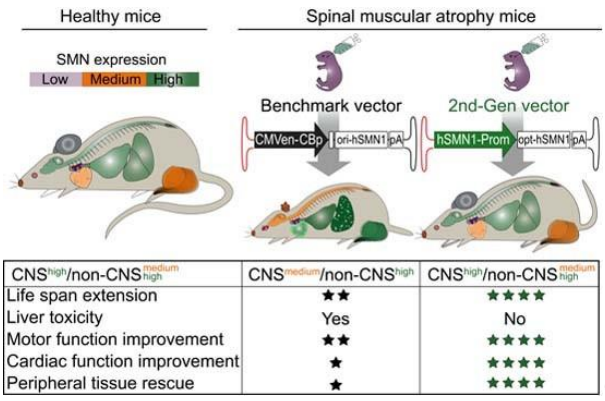
	- This reveals tissue-specific patterns of codon usage, which indicate biologically distinct patterns of preference.
Research Question/Problem/ Need	How could a deep learning network that is trained using strongly expressed gene transcripts of various tissue types be utilized for predicting cell type-specific codon preference and the optimization of codon sequences for better protein expression?
Important Figures	 Graphical Abstract
VOCAB: (w/definition)	Codon: a sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecule RNN: a type of deep learning model designed for processing sequential data Transcriptome: the complete set of all RNA molecules (transcripts) produced by a genome in a cell, tissue, or organism at a specific time, reflecting which genes are actively "turned on" or expressed, including protein-coding (mRNA) and non-coding RNAs, providing a dynamic snapshot of cellular function and response to conditions
Cited references to follow up on	Codon Bias as a Means to Fine-Tune Gene Expression Enhancing the Translational Capacity of E. coli by Resolving the Codon Bias Engineering genes for predictable protein expression
Follow up Questions	In what ways could models trained on the liver data set potentially perform better relative to other models? How could this DL codon optimization be utilized for designing gene therapy? What are the drawbacks of using mRNA expression information (transcriptomics) over direct protein expression information for training? How do G/C-ending codons affect protein folding and stability

Article #13 Notes: Improved gene therapy for spinal muscular atrophy in mice using codon-optimized hSMN1 transgene and hSMN1 gene-derived promotor

Article notes should be on separate sheets

Source Title	Improved gene therapy for spinal muscular atrophy in mice using codon-optimized hSMN1 transgene and hSMN1 gene-derived promotor
Source citation (APA Format)	
Original URL	https://link.springer.com/article/10.1038/s44321-024-00037-x
Source type	Journal Article
Keywords	AAV9, Gene therapy, hSMN1, Codon Optimization
#Tags	

Summary of key points + notes (include methodology)	- SMA is caused by the loss of function of SMN1; the current gene therapy employs AAV9 to deliver the native SMN1 gene driven by a strong viral promoter.
	- Highly expressed and ubiquitous SMN can cause adverse events, like liver toxicity, and raise safety concerns.
	- They want to create an enhanced gene therapy vector, AAV9-Improved, which reestablishes SMN expression at closer-to-normal (physiological) levels throughout the tissues in hopes of improving safety and efficacy.
	- The second-generation vector was engineered with:
	  o Codon-optimized hSMN1 transgene for more efficient expression regulation.
	  o Endogenous SMN1 promoter instead of a strong CMVen/CB viral promoter.
	- Benchmarked this new vector against a control vector identical to Onasemnogene abeparvovec enriched with the benchmark promoter.
	- Increased life span: The second generation vector significantly increased survival in severe SMA mice at various doses when compared to benchmark.
	- Motor & physiological improvements: Better motor function, gain in body weight, and decrease in disease signs were noted in treated

	mice - Tissue-specific expression: SMN in the brain, spinal cord, heart, and muscles were closer to healthy levels with the new vector versus the benchmark. - Reduced toxicity: The new promoter reduced supraphysiological SMN expression in peripheral organs like the liver and also minimized associated toxicity. - his modified vector has the advantage of safer and more efficient gene therapy, which is achieved by mimicking physiological expression patterns rather than overexpressing the therapeutic gene. -
Research Question/Problem/ Need	Does a gene therapy vector with a codon-optimized hSMN1 transgene under the control of an endogenous SMN1 promoter improve safety and therapeutic efficacy compared to current AAV9 gene therapy designs in a mouse model of SMA?
Important Figures	 Graphical Summary
VOCAB: (w/definition)	Promoter: a specific DNA sequence located upstream of a gene that acts as the binding site for RNA polymerase and transcription factors Endogenous: originating or developing from within an organism, cell, or system Benchmark Vector: a numerical representation of a biological entity (like a gene or protein) used in a standardized test (benchmark) to evaluate the performance of computational methods
Cited references to follow up on	The neurobiology of childhood spinal muscular atrophy. Intravenous scAAV9 delivery of a codon-optimized SMN1 sequence rescues SMA mice.
Follow up Questions	Why would controlling expression with an endogenous promoter improve safety compared with a strong viral promoter? What are the implications in systemic gene delivery for reduced liver toxicity? How might codon optimization independently contribute to improved expression in different tissues?

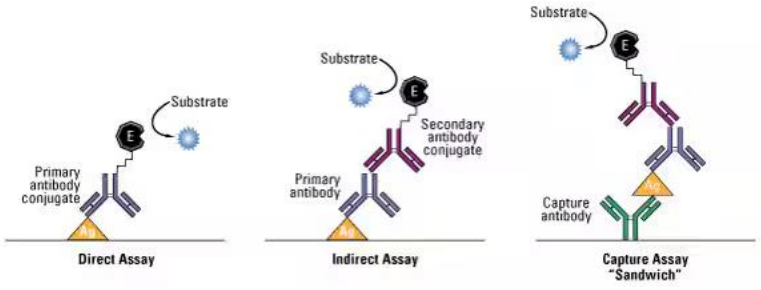
CNS^{high}/non-CNS^{medium}_{high}	CNS^{medium}/non-CNS^{high}	CNS^{high}/non-CNS^{medium}_{high}
Life span extension	★★	★★★★
Liver toxicity	Yes	No
Motor function improvement	★★	★★★★
Cardiac function improvement	★	★★★★
Peripheral tissue rescue	★	★★★★

	What are some of the remaining challenges in translating this improved vector into human clinical trials?

Article #14 Notes: ELISA Assay: Principles, Types, & Applications

Article notes should be on separate sheets

Source Title	ELISA Assay: Principles, Types, & Applications
Source citation (APA Format)	*Elisa Assay Technique: Thermo Fisher Scientific - US*. ELISA Assay Technique | Thermo Fisher Scientific - US. (2025). https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html
Original URL	https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html
Source type	Educational Review
Keywords	ELISA, Antigen, Antibody, Enzyme conjugate, Microplate, Colorimetric signal
#Tags	
Summary of key points + notes (include methodology)	- ELISA is an immunoassay technique that couples selective binding between antigens and antibodies with an enzymatic detection system to quantify the presence or amount of a given material (antigen or antibody) in a biological sample. - This is based on the immobilization of one of the partners (antigen or antibody) on a microplate with the subsequent production of a signal following an enzymatic reaction with the substrate. - The key concept is the specific binding of antigens to antibodies, with the degree of binding of the enzyme conjugate related to the amount of analyte. - A substrate reacts with the enzyme (often HRP or AP) to produce a signal that can be quantitatively measured. - Direct ELISA: Antigen fixed to plate + Primary antibody linked to enzyme → Signal - Indirect ELISA: Antigen fixed, followed by primary antibody + enzyme-linked secondary antibody → amplified signal. - Sandwich ELISA: HePTP capture antibody binds to antigens, while HePTP detection antibody-enzyme conjugates - Competitive ELISA: Both labeled antigen and antigen in the sample compete for the binding of the antibody, with a proportional reduction of the signal with the increase of the sample antigen - Can be used for:

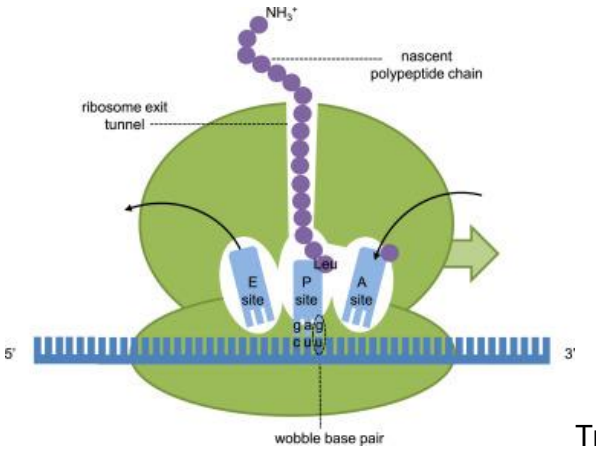
	○ Clinical Diagnostics: Identifying disease markers such as viral antibodies or hormones. ○ Research: Quantify proteins, cytokines, or biomarkers in research ○ Vaccine monitoring: Assess immune system response (e.g., post vaccination). ○ Food Safety: Find allergens or toxins. ○ Drug development: Drug or biomarker measurement for pharmacology. - High sensitivity and specificity for target detection. - Capable of processing multiple samples concurrently by employing microplates - Risk of cross-reactivity or non-specific binding - Highly sensitive to optimization of dynamic range
Research Question/Problem/ Need	How does the ELISA technique utilize the specificity of the antigen-antibody reaction and enzyme labeling for the measurement of the presence or concentration of biological molecules such as proteins, antibodies, or hormones?
Important Figures	 Diagram of common ELISA formats (direct vs. sandwich assays)
VOCAB: (w/definition)	Immunoassay: a lab test that uses the specific binding of antibodies to antigens (like proteins, hormones, or pathogens) to detect and measure substances in a sample Analyte: a substance whose chemical constituents are being identified and measured Spectrophotometer: a scientific instrument that measures the intensity of light as a function of its color (wavelength), determining how much light a substance absorbs or transmits, which helps quantify its concentration, purity, and properties Capture Antibody: an antibody fixed to a solid surface (like a microplate) to "catch" or bind a specific target molecule (antigen) from a sample
Cited references to follow up on	N/A
Follow up Questions	Why would one opt for the sandwich ELISA technique as opposed to direct or indirect methods for certain applications? How do enzyme selection and substrate influence the degree of sensitivity

	and type of signal in ELISA? When could ELISA be more applicable with respect to outbreak response, testing, or personalized medicine relative to other techniques such as PCR?

Article #15 Notes: Codon Bias as a Means to Fine-Tune Gene Expression

Article notes should be on separate sheets

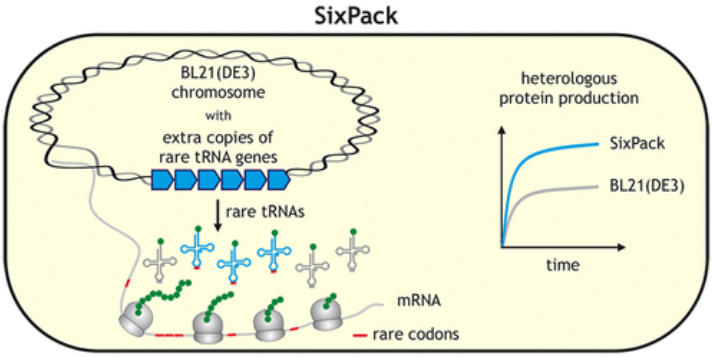
Source Title	Codon Bias as a Means to Fine-Tune Gene Expression
Source citation (APA Format)	Quax, T. E. F., Claassens, N. J., Söll, D., & van der Oost, J. (2015). Codon bias as a means to fine-tune gene expression. *Molecular Cell*, *59*(2), 149–161. https://doi.org/10.1016/j.molcel.2015.05.035
Original URL	https://www.cell.com/molecular-cell/fulltext/S1097-2765(15)00402-5?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS1097276515004025%3Fshowall%3Dtrue
Source type	Review Article
Keywords	Codon bias, synonymous codons, tRNA pools, Translation elongation rate
#Tags	
Summary of key points + notes (include methodology)	- Codon bias is the preferential usage of a set of codons over another set of codons in a species' genome - Although translation initiation is a major control point in translation, codon bias is presently identified as a critical determinant governing translation elongation rates, leading to modulation in protein production and structure - In this report, different known forms of codon bias and their effects in translation are discussed - Frequency bias: Optimal codons can correspond to abundant species of tRNA, leading to increased efficiency. - Intragenic landscapes: A gene contains non-uniform codon usage, with regions called 'ramps' where low-demand codons cause pauses in elongation to avoid congestion on ribosomes during translation. - The codon usage can affect co-translational folding of domains in proteins depending on translation rates. - The influence of codon bias is noted in differential expression of genes, including in operon genes, under different situations such as starvation. - Selection pressure acts as a force creating codon biases among organisms. The pressure favors efficient translation, which improves fitness. - Knowledge gained concerning codon biasing has also been used in biotechnology in order to improve protein expression, such as changing the level of tRNA in host cells or designing codon usage in genes according to host preference
Research Question/Prob	In what ways does codon bias affect translation efficiency, protein folding, and gene regulation in living organisms, and how can a comprehension of these principles be applied in

lem/ Need	order to hone gene regulation in both natural and man-made environments?
Important Figures	 Translation in the ribosome and tRNA
VOCAB: (w/definition)	Synonymous codon: different codons that code for the same amino acid Translation elongation: the central stage of protein synthesis where ribosomes read the mRNA sequence and build a growing polypeptide chain Operon: a functional unit of DNA in prokaryotes (like bacteria) where related genes are clustered together and controlled by a single promoter Heterologous expression: the process of inserting a gene from one organism into a different, often simpler or faster-growing, host organism (like bacteria or yeast) to produce large quantities of the encoded protein, study its function, or even create novel molecules
Cited references to follow up on	Heterologous protein expression is enhanced by harmonizing the codon usage frequencies of the target gene with those of the expression host Efficient translation initiation dictates codon usage at gene start A role for codon order in translation dynamics
Follow up Questions	What is the impact of rare codons compared to frequent codons in a given gene on protein folding and function? In what way can codon bias be harnessed in synthetic biology for enhanced production of a therapeutic protein in a given host such as E. coli or yeast? What are the current limitations in codon optimization when dealing with complex eukaryotic genes?

Article #16 Notes: Enhancing the Translational Capacity of E. coli by Resolving the Codon Bias

Article notes should be on separate sheets

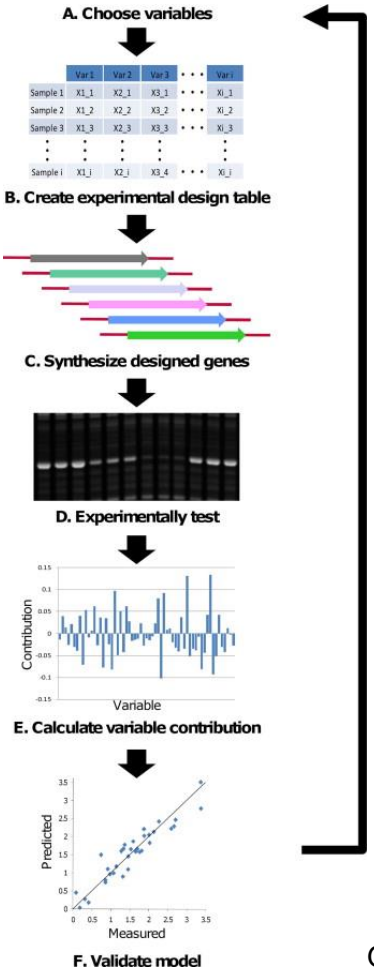
Source Title	Enhancing the Translational Capacity of E. coli by Resolving the Codon Bias
Source citation (APA Format)	Lipinszki, Z., Vernyik, V., Farago, N., Sari, T., Puskas, L. G., Blattner, F. R., Posfai, G., & Gyorfy, Z. (2018). Enhancing the translational capacity of E. coli by resolving the codon bias. *ACS Synthetic Biology*, *7*(11), 2656–2664. https://doi.org/10.1021/acssynbio.8b00332
Original URL	https://pubs.acs.org/doi/10.1021/acssynbio.8b00332
Source type	Journal Article
Keywords	Codon bias, translational capacity, E. coli, Rare codons, protein yield
#Tags	
Summary of key points + notes (include methodology)	- E. coli is a widely used vector for expressing other proteins; however, codon usage differences between E. coli and other genes can affect the production level of proteins. - Highly rare codon-containing genes can slow down translation, lower translation efficiency, and lead to improperly translated or improperly folded proteins. - To make E. coli more translationally efficient by taking into consideration codon bias rather than re-optimizing the sequence of the gene. - The work concentrated on solving codon bias at a host level primarily through an increased availability of those tRNAs that correspond to rare codons. - Engineered E. coli strains were added additional genes for tRNA with rare codons. - Proteins produced from heterologous genes were tested in both normal and mutant strains. - The addition of rare tRNAs led to a substantial improvement in the production of proteins from codon - Translation increased in accuracy with fewer ribosomal pauses during elongation. - The quality and production of proteins were enhanced with no effect on the original sequence of the gene. - Shows that host engineering is a successful alternative to codon

	optimization of the coding sequence of the gene. - Emphasizes codon usage bias as a critical factor in translational efficiency in prokaryotes. - Has major implications in biotechnology, synthetic biology, and industrial production of proteins. -
Research Question/Problem/ Need	Can translational capabilities in E. coli improve by overcoming codon bias with the addition of host tRNA to better express codon-biased heterologous genes?
Important Figures	 Graphical abstract
VOCAB: (w/definition)	Codon bias: the non-random preference for certain synonymous codons (different codons that code for the same amino acid) over others in a genome Rare codon: a specific three-nucleotide sequence (codon) that codes for an amino acid but is used infrequently by an organism, often due to a lower abundance of its corresponding transfer RNA (tRNA) tRNA: a crucial RNA molecule that acts as an adapter, linking specific mRNA codons (code) to their corresponding amino acids (ingredients) during protein synthesis (translation) at the ribosome Recombinant protein: a man-made protein, created in a lab by combining DNA from different sources (like inserting a human gene into bacteria or yeast) to produce large quantities for medicine (like insulin, growth factors) and research (studying cell functions) Protein yield: the quantity of protein produced from a biological system
Cited references to follow up on	Overcoming the codon bias of E. coli for enhanced protein expression Codon influence on protein expression in E. coli correlates with mRNA levels
Follow up Questions	What is the cost and scalability implications of this tRNA supplementation relative to codon optimization? Could overexpressing rare tRNAs have any deleterious consequences for endogenous E. coli transcription? How well might this strategy work for very large or complicated eukaryotic genes? Can such methodologies be adopted in yeast or mammalian systems?

Article #17 Notes: Engineering genes for predictable protein expression

Article notes should be on separate sheets

Source Title	Engineering genes for predictable protein expression
Source citation (APA Format)	Gustafsson, C., Minshull, J., Govindarajan, S., Ness, J., Villalobos, A., & Welch, M. (2012). Engineering genes for predictable protein expression. *Protein Expression and Purification*, *83*(1), 37–46. https://doi.org/10.1016/j.pep.2012.02.013
Original URL	https://www.sciencedirect.com/science/article/pii/S1046592812000629?via%3Dihub
Source type	Review Article
Keywords	Predictable protein expression, gene engineering, synthetic biology, codon usage
#Tags	
Summary of key points + notes (include methodology)	- One of the most important tasks in biotechnology and synthetic biology is reliable and adjustable protein expression. - Traditionally, gene design can be unpredictable because a variety of factors influence expression. - Expression of proteins can be optimized in a more predictable manner by designing elements of genes rather than emphasizing a single parameter. - The article underlines that expression is a system-level phenomenon because it is determined by transcription, translation, and mRNA dynamics. - The rate of transcription is controlled by promoter strength but not final protein concentration. - Features of mRNA sequence, such as secondary structure, codon usage, and UTR regions, can influence translation efficiency and stability. - "The choice of codon affects elongation rate, transport of ribosomes, and protein folding." - Gene context may affect expression by neighboring DNA, vector backbone, or host cell. - Development of standard genetic components with minimal variability (promoters, RBS, Terminators). - Prediction of translation speed based on sequence characteristics using computer models. - Repeating sequence elements that form high mRNA secondary structures close to the start codon. - Use of a combination of design principles rather than relying on a single principle such as codon optimization. - Predictable expression makes possible reliable biological circuits, large-scale

	production of proteins, and repeatable experiments. - Principles described above serve as a guideline in designing genes for bacteria, yeast, and other organisms.
Research Question/Problem/ Need	In what ways can genes be rationally engineered to allow for predictable and reproducible control of protein levels?
Important Figures	 Gene engineering steps by systematic sampling and machine learning
VOCAB: (w/definition)	Gene expression: the fundamental biological process where the instructions in DNA are used to create functional products, usually proteins or functional RNA, allowing cells to perform their jobs and adapt to their environment Translation initiation: the crucial first step of protein synthesis where the ribosome, mRNA, and the first transfer RNA (tRNA) assemble at the start codon (AUG) on the messenger RNA (mRNA) to form a functional complex, signaling the beginning of polypeptide chain construction Ribosome binding site: a specific RNA sequence on messenger RNA (mRNA) that ribosomes recognize to start protein synthesis (translation) Expression noise: the inherent, random fluctuation in the amount of protein or

	mRNA produced by a gene, even in genetically identical cells under the same conditions, due to the probabilistic nature of biochemical reactions like transcription and translation
Cited references to follow up on	RNA interference technology to improve recombinant protein production in Chinese hamster ovary cells Designing genes for successful protein expression
Follow up Questions	Why can a strong transcription event not translate to high-level protein production? What role does mRNA structure have in translation initiation? What role can computer models have in increasing the reliability of gene design? What role does predictability play in synthetic biology and gene circuits?

Article #18 Notes: Alpha-1-antitrypsin deficiency: Genetic variations, clinical manifestations and therapeutic interventions

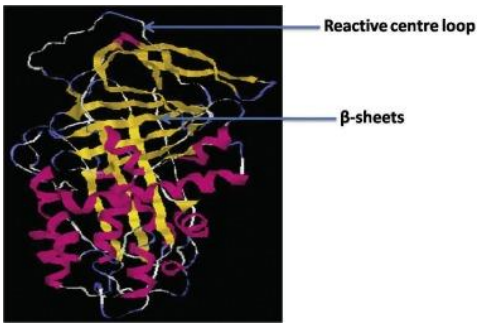
Article notes should be on separate sheets

Source Title	Alpha-1-antitrypsin deficiency: Genetic variations, clinical manifestations and therapeutic interventions
Source citation (APA Format)	Hazari, Y. M., Bashir, A., Habib, M., Bashir, S., Habib, H., Qasim, M. A., Shah, N. N., Haq, E., Teckman, J., & Fazili, K. M. (2017). Alpha-1-antitrypsin deficiency: Genetic variations, clinical manifestations and therapeutic interventions. *Mutation Research/Reviews in Mutation Research*, *773*, 14–25. https://doi.org/10.1016/j.mrrev.2017.03.001
Original URL	https://www.sciencedirect.com/science/article/pii/S1383574216300771?via%3Dihub

Source type	Review Article
Keywords	Alpha-1-antitrypsin, Liver disease, COPD, Serine-protease inhibitor, Panniculitis, Vasculitis, Gain of function, Loss of function
#Tags	

Summary of key points + notes (include methodology)	- Alpha-1-antitrypsin deficiency is an inherited disorder of the lung and liver
	- It is caused by mutations in the SERPINA1 gene, leading to reduced levels or malfunctioning AAT protein.
	- The most common normal allele is Pi*M.
	- Examples of disease-associated variants include;
	  o Pi*Z-severe deficiency because of protein misfolding and retention in the liver
	  o Pi*S – causes moderate reduction in AAT levels
	- The disease severity and age of onset vary with different genotypes.
	- This allows neutrophil elastase to destroy alveolar tissue in the lungs, resulting in emphysema due to the lack of AAT.
	- In the liver, the misfolded AAT accumulates in hepatocytes, inducing an inflammatory response, fibrosis, and ultimately cirrhosis.
	- Pulmonary symptoms include the early development of emphysema, dyspnea, and chronic cough.
	- Hepatic symptoms include neonatal jaundice, hepatitis, cirrhosis, and hepatic failure.
	- Other effects: panniculitis inflammation of skin, rarely
	- Tobacco smoking severely accelerates the development of lung disease.
	- Therapeutic Interventions:
	  o Augmentation therapy-intravenous infusion of purified AAT to slow lung damage
	  o Supportive care: bronchodilators, supplemental oxygen, and smoking cessation
	  o Liver transplantation: resorted to in cases of serious liver diseases
	- Other emerging therapies include gene therapies, RNA-based therapies, and protein-folding correctors.
	- Early diagnosis allows better management of the disease and, consequently, prevention of complications.
	- Knowledge of genetic variation helps to guide personalized treatment strategies.
	- Investigation of this topic has shown that while most disordered conditions undergo significant improvement, less than 5% of subjects with these conditions still have a persistent remnant.

Research Question/Problem/ Need	How do genetic variations in the SERPINA1 gene lead to alpha-1-antitrypsin deficiency, what clinical manifestations result from this deficiency, and what current and emerging therapies can effectively manage or treat the disease?

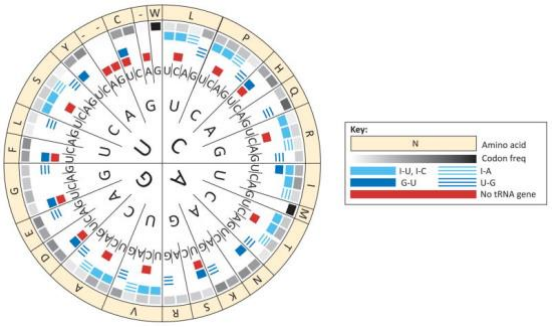
Important Figures	 3-D protein structure of AAT
VOCAB: (w/definition)	Protease inhibitor: drugs or molecules that block proteases, enzymes that cut proteins, essential for viruses like HIV to mature and spread, and for normal cell functions like blood clotting Emphysema: a chronic lung disease, part of COPD, where damage to the tiny air sacs (alveoli) makes it hard to breathe by trapping air and reducing oxygen exchange Hepatocyte: a liver cell Misfolded protein: a protein that fails to achieve its correct 3D shape, losing function and becoming toxic Allele: one of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome
Cited references to follow up on	Cell-specific expression of alpha 1-antitrypsin in human intestinal epithelium Biosynthesis of alpha1-proteinase inhibitor by human lung-derived epithelial cells
Follow up Questions	Why does the same genetic mutation cause lung disease in some patients and liver disease in others? How does smoking accelerate the lung damage process in AAT-deficient individuals? How could gene therapy potentially effect a long-term cure for AATD?

Article #19 Notes: A critical analysis of codon optimization in human therapeutics

Article notes should be on separate sheets

Source Title	A critical analysis of codon optimization in human therapeutics
Source citation (APA Format)	Mauro, V. P., & Chappell, S. A. (2014). A critical analysis of codon optimization in human therapeutics. *Trends in Molecular Medicine*, *20*(11), 604–613. https://doi.org/10.1016/j.molmed.2014.09.003
Original URL	https://www.cell.com/trends/molecular-medicine/abstract/S1471-4914(14)00140-3?mobileUi=0
Source type	Review Article
Keywords	Codon optimization, gene therapy, mRNA therapy, vaccine, A-to-I editing, tRNA wobble
#Tags	

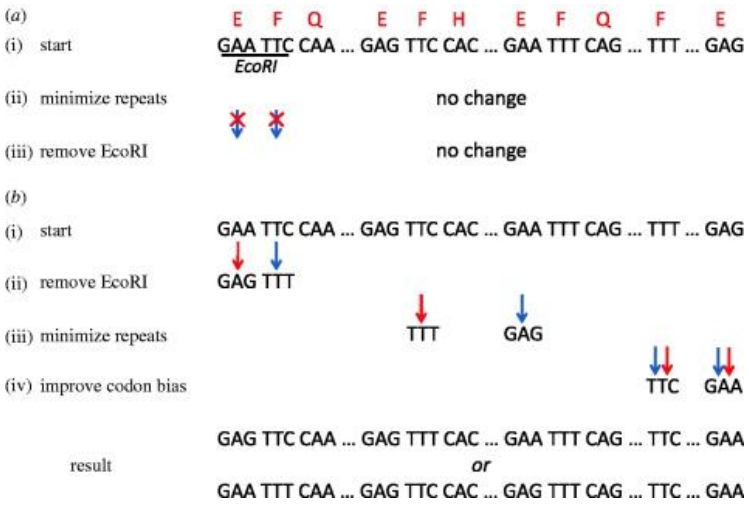
Summary of key points + notes (include methodology)	- Codon optimization is often employed to enhance the production of proteins for human therapeutics.
	- This article critiques whether or not codon optimization is an effective step in achieving better results.
	- It explains: ‘‘It has been reported that several optimization strategies primarily aim at exploiting the bias for frequent host codons
	- Protein synthesis gets affected not only by the above-mentioned codon frequencies
	- mRNA structure, stability, and folding are prominent contributors to the efficiency of translation
	- Over-optimization sometimes causes unexpected issues
	  o Unfolded or abnormal proteins
	  o Abnormal protein functions
	- Codon Usage may influence Translation Speed, which affects Protein Folding
	- The article has mentioned instances where optimized genes resulted in the production of less functional protein.
	- It underlines that in silent mutations, the mutations are not necessarily ‘silent’ at a biological level
	- The codon optimization process has the potential to affect the regulatory regions of the RNA strands.
	- This is particularly important in the case of therapeutic proteins for the treatment of humans
	- Codon optimization has to be used discriminatively, without being

	indiscriminate - A balanced approach considering codon usage, mRNA structure, and protein folding is recommended - codon optimization can be a powerful tool, but this is dependent on the therapeutic being utilized
Research Question/Problem/ Need	When and how does codon optimization enhance or suppress the expression and biological function of proteins in human therapeutic contexts?
Important Figures	 The degenerate codon code, or where multiple codons can code for the same amino acid
VOCAB: (w/definition)	Codon bias: the non-random preference for certain synonymous codons (different codons that code for the same amino acid) over others in a genome Silent Mutation: a change in a DNA sequence that doesn't alter the resulting amino acid sequence of a protein Protein folding: process where a linear chain of amino acids, made by ribosomes, spontaneously twists and folds into a precise 3D shape (its "native state") that enables it to perform its specific biological function, driven by interactions between amino acids Over-optimization: the practice of excessively applying specific techniques to maximize performance on a narrow metric, which ultimately harms the overall quality, functionality, or user experience of a system Recombinant protein:
Cited references to follow up on	You're one in a googol: optimizing genes for protein expression Silent substitutions predictably alter translation elongation rates and protein folding efficiencies The effects of the synonymous codon usage and tRNA abundance on protein folding of the 3C protease of foot-and-mouth disease virus
Follow up Questions	What role may translation speed affect protein function? Should codon optimization strategy vary for therapeutic versus research proteins? What are the risks associated with codon optimization for clinical therapeutics?

Article #20 Notes: You're one in a googol: optimizing genes for protein expression

Article notes should be on separate sheets

Source Title	You're one in a googol: optimizing genes for protein expression
Source citation (APA Format)	Welch, M., Villalobos, A., Gustafsson, C., & Minshull, J. (2009). You're one in a Googol: Optimizing genes for protein expression. *Journal of The Royal Society Interface*, *6*(suppl_4). https://doi.org/10.1098/rsif.2008.0520.focus
Original URL	https://royalsocietypublishing.org/rsif/article-abstract/6/suppl_4/S467/1142/You-re-one-in-a-googol-optimizing-genes-for?redirectedFrom=fulltext
Source type	Review Article
Keywords	heterologous expression, synthetic biology, codon bias, gene optimization, gene design algorithms
#Tags	
Summary of key points + notes (include methodology)	- This report highlights the reasons for the inefficiency in protein expression that occurs in the absence of gene optimization. - Many different DNA sequences can encode the same protein - A much smaller percentage of sequences are likely to express high levels of protein. - Codon choice appears to be an important factor in determining expression efficiency - frequency distributions for codons differ between species - Using non-optimal codons can lead to slow translation and decreased protein production - The article describes how tRNA supply impacts the translation rate - Gene optimization can increase crop yield without altering the protein's amino acid sequence - Oversimplified optimization will cause problems with translation timing - optimization requires a trade-off between speed and accuracy - Gene optimization plays an important role in recombinant protein production - The paper concludes that genetic design for improved function through rational design is highly effective in increasing the probability of expression success

Research Question/Problem/ Need	Why are just a small fraction of the possible gene sequences able to produce high levels of protein, and how can gene optimization help with protein expression?
Important Figures	 Example of different algorithms for optimization and how they are affected by the sequence constraints
VOCAB: (w/definition)	Codon: a sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecule. Codon Bias: the non-random preference for certain synonymous codons (different codons that code for the same amino acid) over others in a genome Host organism: any living being (animal, plant, human, or even a single cell) that provides a habitat, nutrients, and resources for another organism Translation rate: the speed at which messenger RNA (mRNA) is converted into protein Genetic code degeneracy: multiple codons (three-nucleotide sequences) can specify the same amino acid, creating redundancy, but no single codon codes for more than one amino acid
Cited references to follow up on	Heterologous protein expression is enhanced by harmonizing the codon usage frequencies of the target gene with those of the expression host Recombinant protein expression in *Escherichia coli*
Follow up Questions	Why does a high number of potential DNA sequences encoding the same protein lead to poor expression? What is the difference in codon usage between living creatures, and what is the importance of this difference? When does codon optimization have the greatest effect on protein production? Can over-optimization deteriorate protein quality or activity?

Patent #1 Notes: Codon optimization

Article notes should be on separate sheets

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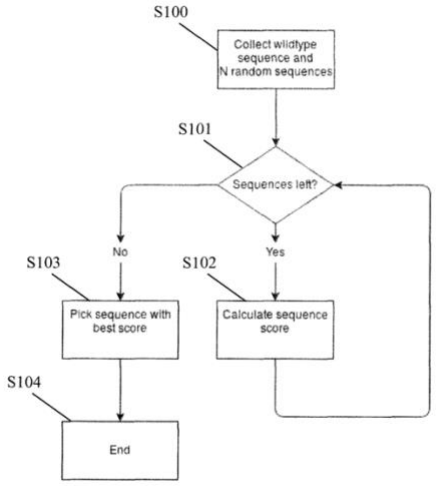
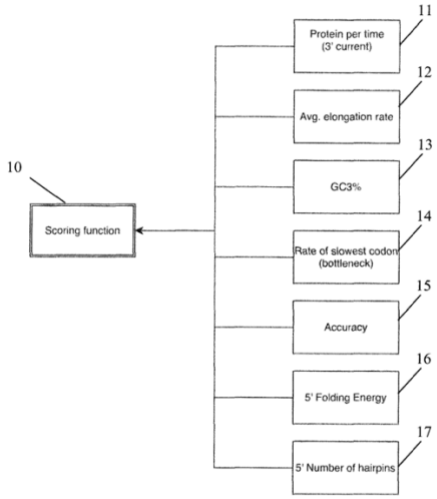
Source Title	Codon optimization
Source citation (APA Format)	Lipowsky, R., Rudorf, S., Lössner, H., Trösemeier, J.-H., Koch, I., & Kamp, C. (2023, December 19). Codon optimization.
Original URL	https://patents.google.com/patent/US20190325989A1/en
Source type	Patent
Keywords	Codon optimization, codon bias, COSEM, protein expression score, GC3 content, elongation rate, translational accuracy
#Tags	
Summary of key points + notes (include methodology)	- It describes a way and system to optimize nucleotide sequences in the field of protein expression in host cells. - Explains that merely substituting with "preferable" codons is not always the best strategy - Describes a codon-specific elongation model, called COSEM, which provides an improved prediction for the effect of codon choice on translation - COSEM takes into consideration the speed of translation, accuracy, and other characteristics of the sequence for the creation of a score of protein expression - It makes use of several candidate nucleotide sequences encoding the identical protein in order to find the best scored sequence - All features can include sequence features such as GC3 content, elongation rate, accuracy, and mRNA folding energy - The method can be used to increase or decrease expression depending on goals. For example, higher yield or attenuated viruses - It's parameterized with data from bacteria, yeast, and human cells to make predictions more accurate. - The optimized sequence is then introduced into a host cell via vectors or expression cassettes to produce the desired protein. - The model is maybe mechanistic, whereas traditional heuristic codon optimization is based on frequency, and therefore the model could be better
Research Question/Problem/ Need	How can codon optimization be improved beyond simple codon frequency bias to predict and tailor protein expression levels in different host organisms?

Important Figures	 Fig. 1 Graphical process for optimization  Fig. 3 Process for scoring the sequence
VOCAB: (w/definition)	Codon: a sequence of three nucleotides which together form a unit of genetic code in a DNA or RNA molecule Codon Bias: the non-random preference for certain synonymous codons (different codons that code for the same amino acid) over others in a genome COSEM: The Codon-Specific Elongation Model (COSEM) is a computational model used in molecular biology and bioinformatics to simulate the dynamics of mRNA translation and predict protein synthesis rates Protein expression score: quantifies how much of a specific protein is made in cells or tissues

	Elongation rate: the speed at which a nucleic acid (DNA/RNA) or polypeptide chain grows during synthesis, measured by the number of nucleotides or amino acids added per unit of time
Cited references to follow up on	Codon influence on protein expression in E. coli correlates with mrna level Codon optimization online (cool) : a web based multi -objective optimization platform for synthetic gene design
Follow up Questions	How does the COSEM model improve the traditional codon adaptation index methods? How will the model distinguish between the prokaryotic and eukaryotic translation systems? What real-world applications could take advantage of this optimization approach, such as vaccines or biologics?

Patent #2 Notes: Engineering and optimization of systems, methods and compositions for sequence manipulation with functional domains

Article notes should be on separate sheets

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Source Title	Engineering and optimization of systems, methods and compositions for sequence manipulation with functional domains
Source citation (APA Format)	Zhang, F., Cong, L., Platt, R. J., Sanjana, N. E., & Ran, F. (2025, August 7). Engineering and optimization of systems, methods and compositions for sequence manipulation with functional domains.
Original URL	https://patents.google.com/patent/US20250250577A1/en?q=(codon+optimization)&oq=codon+optimization
Source type	Patent
Keywords	CRISPR, Sequence manipulation, optimization, functional domains, vector, gene editing, host cell
#Tags	
Summary of key points + notes (include methodology)	- The invention focuses on designing vectors, methods, and compositions that improve precision and performance of sequence editing. - It includes ways to control gene expression and enhance sequence targeting efficiency in cells. - Functional domains are integrated into editing systems to expand capabilities beyond standard CRISPR components. - The patent builds on earlier inventions in the same family that focus on optimized CRISPR complex components. - Applications may include genome editing, therapeutic gene modulation, and functional genomics research. - The methods can be applied in both prokaryotic and eukaryotic host cells to manipulate target DNA sequences. - The invention claims priority from earlier patents on CRISPR system engineering from The Broad Institute.
Research Question/Problem/ Need	How can systems, methods, and compositions be engineered and optimized to improve efficiency and functionality of sequence manipulation technologies like CRISPR?
Important Figures	N/A

VOCAB: (w/definition)	CRISPR: a segment of DNA containing short repetitions of base sequences, involved in the defense mechanisms of prokaryotic organisms to viruses Sequence manipulation: the computational processing and transformation of biological (DNA, RNA, protein) or other ordered data to extract information, analyze patterns, or prepare it for further study Vector: a disease vector, which is an organism that carries and transmits pathogens to a host Optimization: the principle that natural systems, from genes to behaviors, evolve to be the most efficient or "best" possible under given constraints, balancing costs (like energy) against benefits (like survival/reproduction) to find the ideal strategy, function, or design Functional Domain: a distinct, stable, and independently folding region within a protein (or sometimes DNA/RNA) that performs a specific job, like binding molecules, catalyzing reactions, or mediating interactions
Cited references to follow up on	Small CRISPR RNAs guide antiviral defense in Prokaryotes Engineering RNA 5 sequence specificity of Pumilio repeats Essential features and rational design of CRISPR RNAs that funtion with the Cas RAMP module complex to cleave RNAs
Follow up Questions	How do these optimized systems improve target specificity compared with standard CRISPR tools? Can the methods be used for therapeutic genome editing in humans? What challenges in sequence manipulation are these optimizations designed to solve? What are the key differences between this patent and other sequence optimization systems