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RESEARCH ARTICLE

Simulation of gene regulatory elements for biosensing

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Abstract: Gene regulatory studies are of significant importance in many scenarios such as mental illness. 21% of U.S adults experience mental illnesses including 1 in 4 active-duty military personnel. Mental health can be identified in the body by different biomarkers. These biomarkers potentially controlled by riboswitches, which are located in mRNA and switch "ON" or "OFF" depending on the concentration of a biomarker. In this research, a known riboswitch reengineered and its response in the presence of a biomarker investigated. We changed computationally $PreQ_1$, a known riboswitch that has the smallest aptamer, and then experimentally tested against biomarkers, dehydroepiandrosterone-sulfate (DHEA-S), Serotonin, Cortisol, Dopamine, Epinephrine, and Norepinephrine. A total of 7 variant riboswitches were tested in this research, 4 created computationally discussed here and 3 experimentally not covered in this paper. The results from these variants showed that variants 1 and 2 had different responses to DHEA-S then the expected $PreQ_1$ response. A dose response showed downward trend as DHEA-S concentration increased. In conclusion of this research, riboswitches can be re-engineered to have a different response to biomarkers at the same time keeping the same structure.

Keywords: riboswitches, biomarkers, protein, regulation, $PreQ_1$, computer simulations

1 Introduction

The body physiology has self-made processes to help regulate different levels of proteins, analytes, and many other cellular metabolites. This prevents systems from deuterating and helps keep homeostasis throughout the body. An example of this system, if there was a high level of serotonin, a biomarker would recognize this surplus and transmit data to all systems that can regulate serotonin. The regulation part of this system would control serotonin production and reduce the surplus as well.

In the above example, riboswitches help regulate the serotonin through RNA. An analyte or biomarker would bind to the aptamer of the riboswitch. The riboswitch would then regulate protein expression. Depending on the purpose of the riboswitch, proteins that would be produced would help lower the serotonin surplus. If the riboswitch stops producing proteins, then serotonin production continues.

Riboswitches are RNA-based biological sensing elements that bind with metabolites without the need for protein involvement [1]. They are used to regulate levels of biomarkers through the expression of proteins [2]. There are 17 different types of riboswitches that have been discovered and more than 100 are continuing to be experimentally validated [2]. Synthetic riboswitches are being engineered to help with gene therapy.

Biomarkers are cellular, biochemical, or molecular alterations measured in biological media, such as human tissues, cells, or fluids [3]. These markers have been studied as potential medical signs leading to disease. Biomarkers can be used to explain changes in the body that lead to mental illnesses, cardiovascular disease, and specific type of cancers. Riboswitches can be used to regulate biomarkers. Identifying riboswitch and biomarker connection is key to the regulation of these biomarkers. Therefore, there is a need to understand different biomarkers and their response with riboswitch(s).

In this research, $PreQ_1$ is the riboswitch further characterized. It was chosen due to having the smallest known aptamer with 34 nucleotides [4]. The biomarkers examined in this research are dehydroepiandrosterone-sulfate (DHEA-S), Serotonin, Cortisol, Dopamine, Epinephrine, and Norepinephrine. The biomarkers are shown in Table 1 along with related health conditions they influence.

Table 1 Biomarkers and their related health conditions

Biomarker	Related health condition(s)
DHEA-S	Cognitive function [5]
Serotonin	Mood regulation, appetite, antidepressant [6]
Cortisol	Stress hormone, acute stress, low-blood glucose, obesity, depression, diabetes [6]
Dopamine	Feelings of happiness [6]
Epinephrine	Drives automatic nervous system emergency response, increases heart rate, blood pressure [6]
Norepinephrine	Stress hormone, high glucose, increased heart rate, anxiety, high blood pressure [6]

Synthetic riboswitches could be extended to work with biomarkers to help with mental illnesses and regulate diseases. People with depression have a 40% higher risk of developing cardiovascular and metabolic diseases than the control group [7]. Using riboswitches as a potential way to develop therapeutic solution is desirable because they can respond without affecting vital functions. Each riboswitch has different variants that respond in the same manner. A synthetic riboswitch would have the same design in the RNA makeup but would respond inversely. This can be done through the aptamer section of the riboswitch.

2 Literature review on riboswitches

A riboswitch is made of two parts; an aptamer and expression platform, located within the mRNA. A riboswitch operates at metabolite abundance threshold, the ligand binds to the aptamer. This leads to a downstream affect by the expression platform with a conformational change [2]. The conformational change generates the expression platform to either be in an "ON" or "OFF" state [2]. Aptamers bind to ligands with affinities and selectivity. The complexity of the aptamer and ligand separately make it challenging to engineer. Every riboswitch will be reengineered differently based on the chemical composition of that specific riboswitch. Although a significant amount of research has been done, most aptamers have not been converted to functional riboswitches [8]. There are coenzymes, amino acids, signaling molecules, ions, nucleotide derivatives, and other metabolites. Each riboswitch class can have multiple representatives, for example TPP has more than 15,000 representatives that are known. The structure of each riboswitch is made up of different nucleotides: adenine, uracil, cytosine, and guanine. The bonding of these nucleotides forms knotting and folding of the riboswitch.

Riboswitches can be placed into two large groups: pseudo-knotted and junctional riboswitches. A pseudo-knotted riboswitch is "predominantly on the basis of a single pseudo-knot, a knot-shaped conformation formed through base pairing between a loop of an RNA stem-loop structure and an outside region" [9]. Junctional riboswitches contain a multihelical junction which connects various numbers of helices [9]. Hairpins, stem loops, and kissing loops are also structural elements. These can also play a part in different bonding [10].

Bonding in riboswitches is one controlling factor for ligand affinity. Hydrogen bonding is most important for specificity of recognition to the ligand [9]. Ligands have donors and acceptors for hydrogen bonding. When reengineering riboswitches, changing a nucleotide can change the geometric shape and lead to the recognition of the ligand. Hydrogen bonding is a dipole-dipole attraction between molecules. This attraction comes from a hydrogen molecule and an electronegative molecule such as oxygen, nitrogen, or fluorine. For RNA, the nitrogen bases are helping to form temporary hydrogen bonds [9].

3 Materials and methods

In this section the selected riboswitch and biomarkers are elaborated.

3.1 $PreQ_1$

$PreQ_1$ is known from its interaction to produce the hypermodified guanine nucleotide, queuosine (Q). This is a multistep reaction that starts with GTP then going to $PreQ_0$ and $PreQ_1$ to end in Q, as shown in Figure 1.

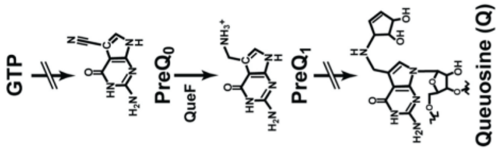

Figure 1 Schematic diagram of queuosine-precursor and $preQ_1$ riboswitch organization [4]

There different subclasses of $PreQ_1$ based on where they are found, type I and II. Type I is found in *T tengcongensis* and type II in *Bacillus subtilis*. Type I is involved in translational regulation and type II in transcriptional regulation. Both types of aptamers are H-type pseudoknots, but for type II the presence of a ligand causes conformation changes when bonded. For example, in *Bacillus subtilis* when $PreQ_1$ binds to the ligand, the riboswitch folds into an H-type pseudoknot. This folding causes a conformational change into a terminator hairpin. $PreQ_1$ bonded with the ligand is considered "ON" and gene expression is terminated in this situation. When the ligand is not bonded, the riboswitch is "OFF", and gene expression continues. The Figure 2 shows the aptamer domain of $PreQ_1$. The aptamer domain for this riboswitch has one loop shown. When re-engineering this loop, the loop has to be sustained.

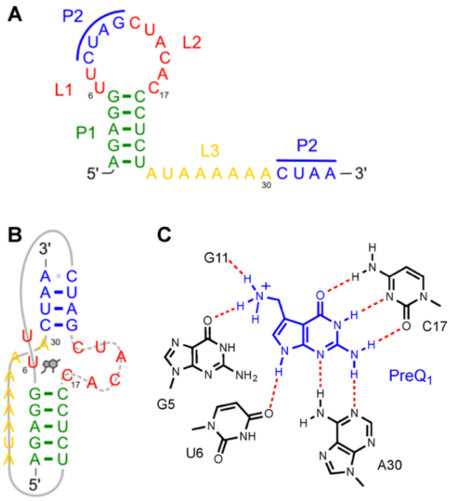

Figure 2 Structure of PreQ1 Aptamer [11]

3.2 Biomarkers

Biomarkers may have molecular, histologic, radiographic, or physiologic among other characteristics [6]. Types of biomarkers are hormones, neurotransmitters, enzymes, and metabolites. Biomarkers can range is size and weights as they are made up of molecules of different sizes [6]. Depending on these factors, riboswitch aptamers can bind based on their affinities for each biomarker. If there is a high concentration of a biomarker, a riboswitch aptamer could bind to it and a conformational change would occur. This would lead to the expression platform of the riboswitch to react. As stated above, an expression platform would then have multiple different responses based on the bonding.

3.2.1 Cortisol

Cortisol is released from adrenal glands through activation of the hypothalamic-pituitary-adrenal axis. It is a glucocorticoid steroid hormone with the primary function to increase blood sugar. It does this through the process of gluconeogenesis. The chemical structure of cortisol is shown below in Figure 3.

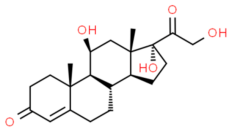

Figure 3 Chemical structure of cortisol

Cortisol is known to be a biomarker for stress. An acute high level of cortisol is used for survival. Prolonged stress or high levels of stress can deuterate the body and cause physiological problems. These situations are where cortisol is at a chronic high level. Mental issues can arise from chronic high levels of cortisol as well.

3.2.2 Serotonin

Serotonin has various roles like vascular resistance and blood pressure. It also can be related to virtually all major organ systems. Serotonin is produced in the central nervous system. The chemical structure of serotonin is shown in Figure 4.

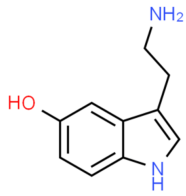

Figure 4 Chemical structure of serotonin

Serotonin regulates behavioral and neuropsychological processes such as mood, perception, reward, anger, aggression, appetite, memory, sexuality, and attention. Mental illnesses like anxiety are treated through drug that target serotonin receptors.

3.2.3 Dopamine

The dopaminergic system is when dopamine is involved as a neurotransmitter. This system plays roles in neuromodulation and influences different immune systems. For neuromodulation the following are included: movement and motor control, spatial memory function, motivation, arousal, reinforcement, reward, sleep regulation, attention, affect, cognitive function, feeding, olfaction, and hormone regulation. Immune systems that are involved are cardiovascular, gastrointestinal, and renal. The chemical structure of dopamine is shown in Figure 5.

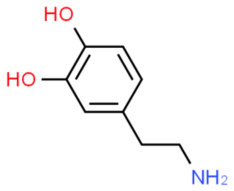

Figure 5 Chemical structure of dopamine

3.2.4 Epinephrine & norepinephrine

Epinephrine and norepinephrine are synthesized from of dopamine. They both bind to adrenergic receptors. Epinephrine has a fast and short stress-response signal. These two differ by a methyl group on the nitrogen side chain. This is shown in the Figure 6 and 7; the chemical structures for both are shown.

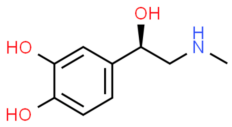

Figure 6 Chemical structure of epinephrine

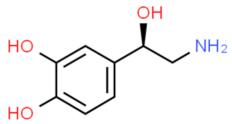

Figure 7 Chemical structure of norepinephrine

3.2.5 DHEA-S

DHEA-S is produced by the adrenal glands in women and men. It is a protective anabolic hormone with its role being maintaining and restoring the human body. The chemical structure of DHEA-S is shown below in Figure 8.

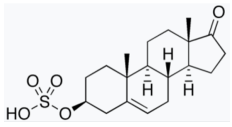

Figure 8 Chemical structure of DHEA-S

3.3 Experimental design

The experimental design for this research was to create riboswitch designs for $PreQ_1$ computationally using the programs KineFold and NUPACK. Four riboswitch strands were created,

and three riboswitch strands were from research [11] to test as well. These strands were then prepared to test against the original $PreQ_1$ strand with different biomarkers. This is further explained below. Variants investigated are summarized in Table 2.

Table 2 Sequences of DNA templates for amplification of PreQ1 riboswitch variants

Riboswitch variant	DNA template
1	AAGTGAAAAAATTGAAGAAAATCCGTGCGATATGCGGGAGAGGAAGATCGATGTGC CTCTATAAAAAACTAAGGACGAGCTGTATCCTTGGATACGGCCTTTTTTCGTTATGGC AGGAGCAAACT
2	AAGTGAAAAAATTGAAGAAAATCCGTGCGATATGCGGGAGAGGAAGATCCTACACC CTCTATAAAAAACTAAGGACGAGCTGTATCCTTGGATACGGCCTTTTTTCGTTATGGC AGGAGCAAACT
3	AAGTGAAAAAATTGAAGAAAATCCGTGCGATATGCGGGAGAGGTTCTAGGATGTGC CTCTATAAAAAACTAAGGACGAGCTGTATCCTTGGATACGGCCTTTTTTCGTTATGGC AGGAGCAAACT
4	AAGTGAAAAAATTGAAGAAAATCCGTGCGATATGCGGGAGAGGGGCTATCTACACC CTCTATAAAAAACTAAGGACGAGCTGTATCCTTGGATACGGCCTTTTTTCGTTATGGC AGGAGCAAACT
5	AAGTGAAAAAATTGAAGAAAATCCGTGCGATATGCGGGAGAGGTTCTAGCTACATCC TCTATAAAAAACTAAGGACGAGCTGTATCCTTGGATACGGCCTTTTTTCGTTATGGCA GGAGCAAACT
6	AAGTGAAAAAATTGAAGAAAATCCGTGCGATATGCGGGAGAGGTTCTAGTTACACC CTCTATAAAAAACTAAGGACGAGCTGTATCCTTGGATACGGCCTTTTTTCGTTATGGC AGGAGCAAACT
7	AAGTGAAAAAATTGAAGAAAATCCGTGCGATATGCGGGAGAGGTTCTAGTTACATCC TCTATAAAAAACTAAGGACGAGCTGTATCCTTGGATACGGCCTTTTTTCGTTATGGCA GGAGCAAACT

3.3.1 Riboswitch structure modeling

Riboswitch variants were designed, based on the aptamer layout for $PreQ_1$. Variants 1-4 were designed to have 22 nucleotides and be able to design a loop compared to the original in KineFold. By exchanging different nucleotides in the original, each variant was created. Figure 9 to 13 shows the KineFold simulations, which were compared to the original in Figure 9. Simulations show the free energy of each variant on the bottom of the figures. Variants 5-7 were taken from literature. Table 2 shows the sequences of each variant.

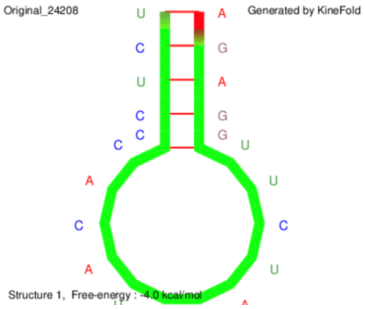

Figure 9 $PreQ_1$ riboswitch simulation made on KineFold

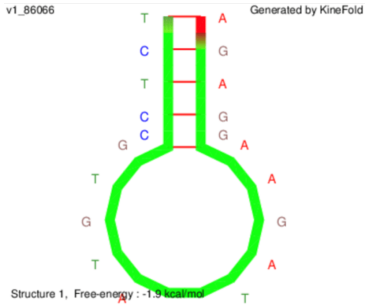

Figure 10 Variant 1 simulation made on KineFold

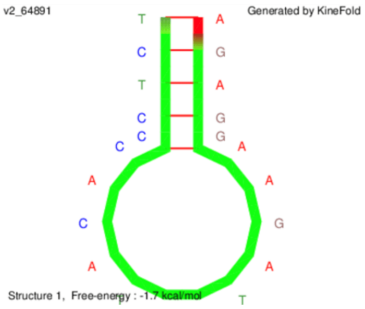

Figure 11 Variant 2 simulation made on KineFold

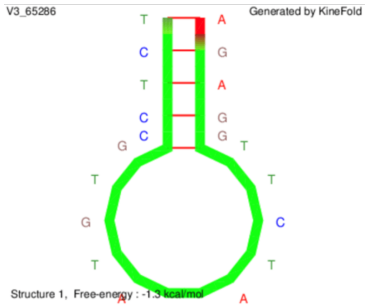

Figure 12 Variant 3 simulation made on KineFold

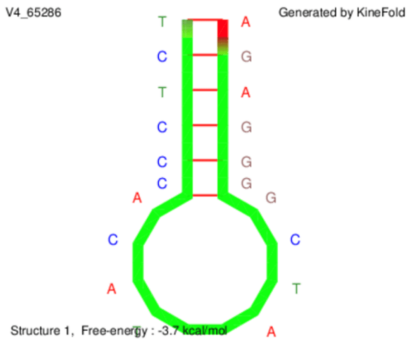

Figure 13 Variant 4 simulation made on KineFold

4 Results & Discussion

4.1 Chemical structure

The software NUPACK was used to simulate the chemical structure. The input requirements are in terms of DNA sequence to output the structure and chemical properties. Figure 14 shows the simulated results for $PreQ_1$, by the software. In Figure 14, the structure of $PreQ_1$ is shown on the left column showing the identity and the equilibrium probability for each position, whereas the right column in this figure shows the helicity of this aptamer. The different nucleotides, A, C, G, and T, form the loop shown in Figure 14 and based on their sequence determines hydrogen bonding, equilibrium probabilities, and potential ligand binding. The DNA configuration can be broken down into loops, which are scored in terms of free energy. In Figure 14 and 15, the free energy that is stated is the sum of the secondary loops. The secondary loops impact ligand bonding. The probability shown is the probability of the sequence to be in that formation. The ensemble defect is the average number of nucleotides that are incorrectly paired. For this number, 0 is the best and N would be the worst combinations. Normalized ensemble defect is the ratio of percentage of 0% being the best and 100% being the worst. $PreQ_1$ will be the structure that the variants will be compared to, as $PreQ_1$ is already an established riboswitch.

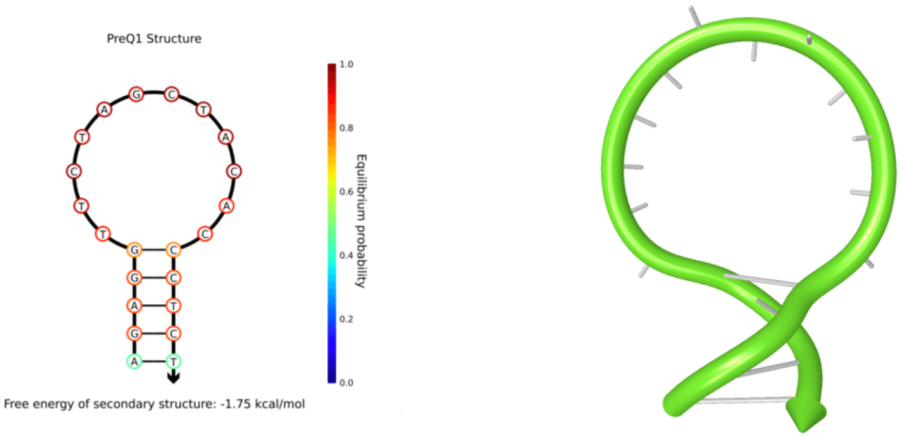

Figure 14 NUPACK simulation of $PreQ_1$ with identity and equilibrium probability shown to the left, To the right shows the helicity.

Sequence properties

Free energy:	−2.44 kcal/mol	
Base	Number	%
A	5	22.7
C	7	31.8
G	4	18.2
T	6	27.3
Other	0	0.0

Sequence/structure properties

Free energy:	−1.75 kcal/mol
Probability:	0.326
Ensemble defect:	3.4 nt
Normalized ensemble defect:	15.3 %
Nucleotides:	22 nt

Figure 15 Structure properties for $PreQ_1$ made by NUPACK

$PreQ_1$ structure has strong equilibrium probabilities throughout the loop. In the details, it shows that 22 nucleotides are being examined in the aptamer domain. The details also show that

there is a 0.326 probability that aptamer domain would present this way. A 15.3% normalized ensemble defect is also shown through this design structure. These details are then compared to the structures that were designed to loop this way versus this design where it naturally occurs.

In addition to simulating the free energy structure, NUPACK can take input on the structure. This allows for riboswitches to be designed and show the properties for the structure. In the Figure 16 to 23, each variant is shown and was designed how $PreQ_1$ exists naturally. The properties are shown for each design in Figure 17, 19, 21 and 23, respectively.

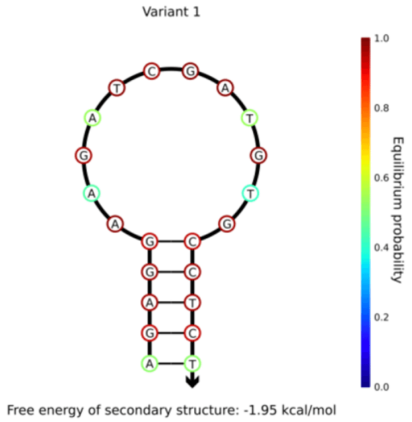

Figure 16 NUPACK design of Variant 1 with identity and equilibrium

Sequence properties

Free energy:	−3.04	kcal/mol
Base	Number	%
A	6	27.3
C	4	18.2
G	7	31.8
T	5	22.7
Other	0	0.0

Sequence/structure properties

Free energy:	−1.95	kcal/mol
Probability:	0.170	
Ensemble defect:	3.5	nt
Normalized ensemble defect:	16.0	%
Nucleotides:	22	nt

Figure 17 Structure properties for Variant 1 made by NUPACK

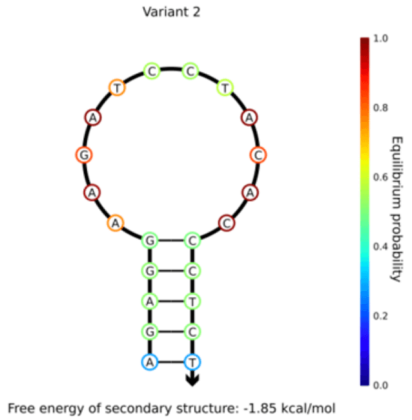

Figure 18 NUPACK design of Variant 2 with identity and equilibrium

Sequence properties

Free energy:	−2.96	kcal/mol
Base	Number	%
A	7	31.8
C	7	31.8
G	4	18.2
T	4	18.2
Other	0	0.0

Sequence/structure properties

Free energy:	−1.85	kcal/mol
Probability:	0.166	
Ensemble defect:	7.6	nt
Normalized ensemble defect:	34.3	%
Nucleotides:	22	nt

Figure 19 Structure properties for Variant 2 made by NUPACK

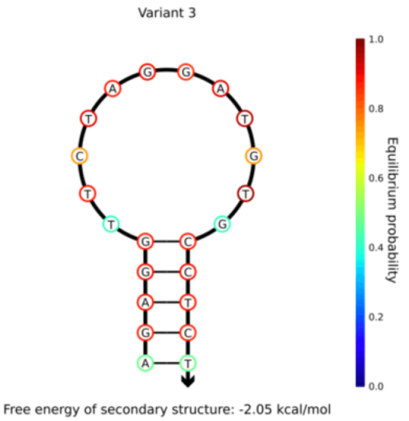

Figure 20 NUPACK design of Variant 3 with identity and equilibrium

Sequence properties

Free energy:	−3.36	kcal/mol
Base	Number	%
A	4	18.2
C	4	18.2
G	7	31.8
T	7	31.8
Other	0	0.0

Sequence/structure properties

Free energy:	−2.05	kcal/mol
Probability:	0.120	
Ensemble defect:	4.4	nt
Normalized ensemble defect:	19.9	%
Nucleotides:	22	nt

Figure 21 Structure properties for Variant 3 made by NUPACK

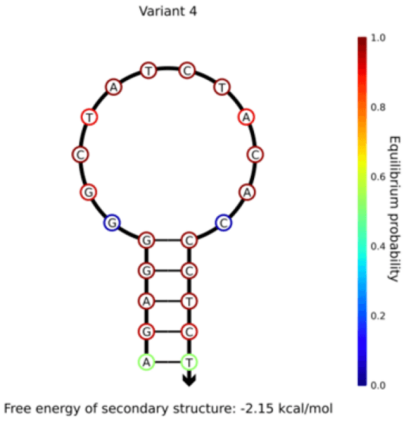

Figure 22 NUPACK design of Variant 4 with identity and equilibrium

Sequence properties

Free energy:	−4.71	kcal/mol
Base	Number	%
A	5	22.7
C	7	31.8
G	5	22.7
T	5	22.7
Other	0	0.0

Sequence/structure properties

Free energy:	−2.15	kcal/mol
Probability:	0.016	
Ensemble defect:	3.4	nt
Normalized ensemble defect:	15.3	%
Nucleotides:	22	nt

Figure 23 Structure properties for Variant 4 made by NUPACK

The results above show the four variant designs of $PreQ_1$ that were designed. The variants equilibrium probabilities show different probabilities than the original. Variants 1 and 2 are most different with multiple green and blue tones in Figure 16 and 18, indicating they have a lower probability of this structural formation. Variants 3 and 4 have differences in comparison to the original, but they keep to a closer probability as the original. From the equilibrium probability it is expected that variants 3 and 4 will respond similarly to $PreQ_1$.

The NUPACK for each structure provides the probability for the formation of each structure. For the original structure of $PreQ_1$ there is a 0.326 probability. For variant 1 the probability is 0.170, 2, 0.166, 3, 0.120, and 4, 0.016, respectively. For these details, the riboswitch aptamer is not bonded to a ligand. This means in the presence of a ligand; the

probabilities could be different than a computational model. The probability equilibrium is more accurate, due to it being based on the pairing probabilities. The closest probabilities to the original will most likely react similarly to a ligand.

4.2 Riboswitch activation time results

Results of each analyte tested against the original $PreQ_1$ and variants are shown (Figure 24 to Figure 31). In each figure a line represents riboswitch activation over time. The activation is measured by the excitation and emission fluoresce (excitation at 470nm and emission at 510nm). When the solution of the RNA mixture with either an analyte or control, the relative fluorescence units (RFU) value over time will have a linear trend. As the solution balances out and the ligand binds, the RFU results in a plateau. The following figures show this trend. The peak and plateau level depicts if the riboswitch is transcribing proteins or terminating.

A test was run for the riboswitch being OFF in water and dimethyl sulfoxide (DMSO) for comparison with each analyte. $PreQ_1$, DHEA-S, and cortisol are compared against the OFF state with DMSO. The other biomarkers are compared against the OFF state with water. These graphs show the variants response with respect to the original design. The OFF state is when the aptamer is not bonded to a ligand. When the analyte is added, the riboswitch aptamer binds. The analyte or biomarker is the ligand in this occurrence. The riboswitch is then in an ON state. When there is no ligand present, $PreQ_1$'s expression platform is transcribing, which explains the OFF state having higher RFU. This is shown with the OFF (DMSO) having a peak around 2.50×10^7 and $preQ_1$ is close to zero.

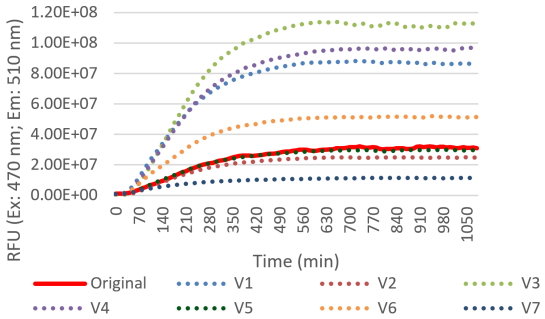

Figure 24 Riboswitch time log with OFF DMSO

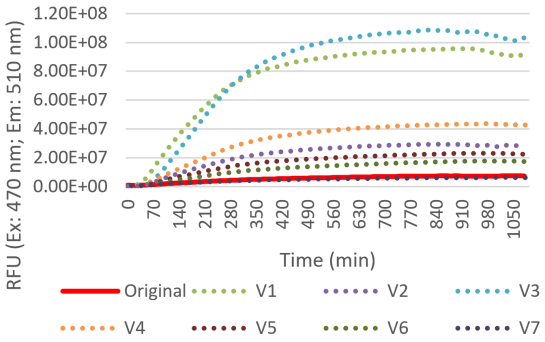

Figure 25 Riboswitch time log with $PreQ_1$

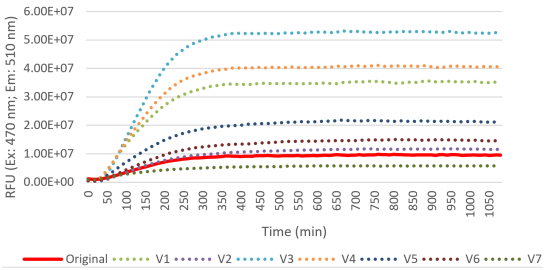

Figure 26 Riboswitch time log with DHEA-S

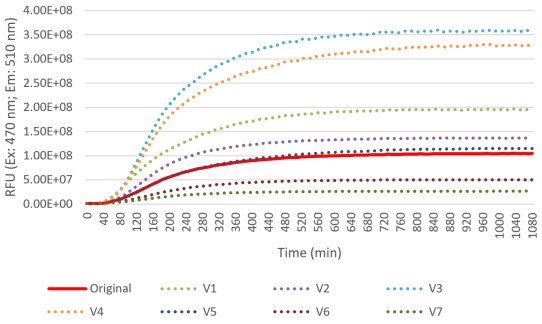

Figure 27 Riboswitch time log with Cortisol

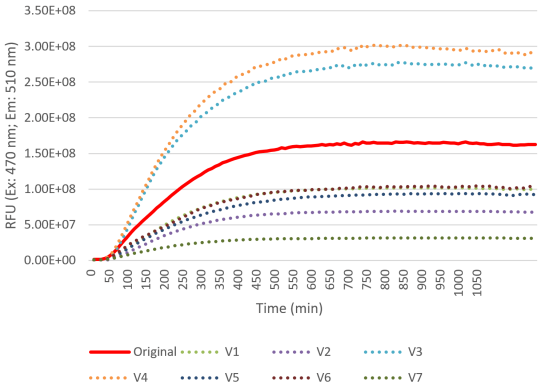

Figure 28 Riboswitch time log with OFF Water

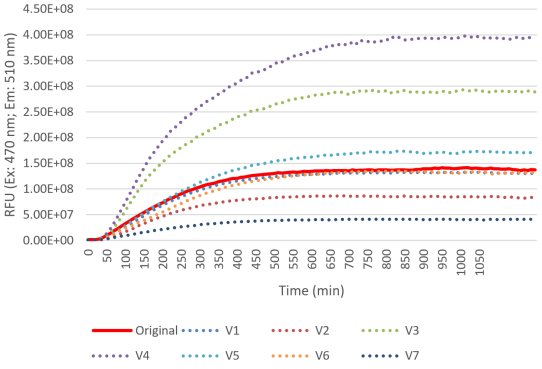

Figure 29 Riboswitch time log with Epinephrine

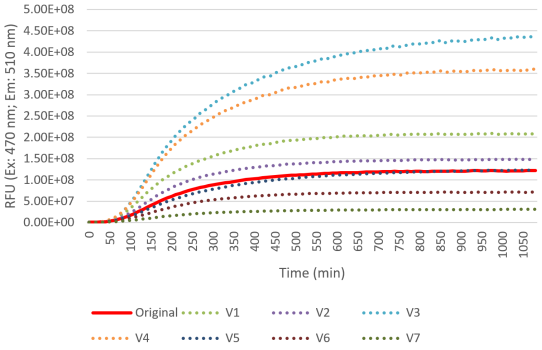

Figure 30 Riboswitch time log with Norepinephrine

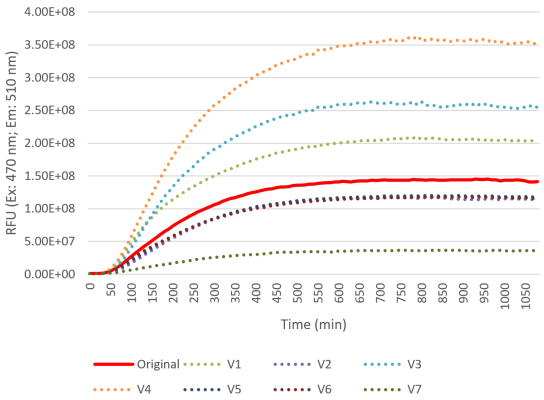

Figure 31 Riboswitch time log with Dopamine

The figures above show the variants over time with reference to the preQ$_1$, while the data shown in Biomarker Results show the peak of each variant ON to OFF.

4.3 Biomarker results

The following figures, Figure 32 to 38, show the riboswitch peak activation results from the Time Response data above. This data is extracted from the time activation graphs when the riboswitch peaks and levels off. The bar graphs are comparing PreQ$_1$ as the original riboswitch and each of the variants with the two bars showing an OFF versus ON. The difference in the bar values show how the riboswitch is transcribing or terminating. In the figures, the orange bars will represent the OFF state and the blue bars will represent the ON state. When the riboswitch is OFF, the ligand is not attached to the aptamer, and the gene expression is on. When the riboswitch is ON, the ligand is attached to the aptamer, and the expression is terminated. This is shown from the OFF being higher than the ON. This is expected due to the gene expression being on when the aptamer is not attached to the ligand. Variants 1 and 2 do not follow this by the ON, when the ligand is bonded, being higher.

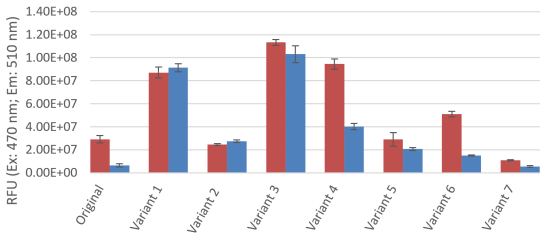

Figure 32 PreQ1 biomarker against original riboswitch and variants

Figure 32 PreQ$_1$ biomarker against original riboswitch and variants

PreQ$_1$ is an example of how the riboswitch should respond. The following biomarkers that were tested are being compared to the original riboswitch, as well as the above figure. DHEA-S shows a difference with how all the variants follow the original riboswitch response. Variants 1 and 2 did not follow the original response with the preQ$_1$ analyte, however.

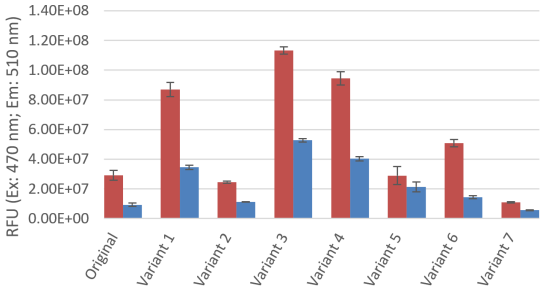

Figure 33 DHEA-S biomarker against original riboswitch and variants

The following figures differ from preQ$_1$ by the ON state being higher than the OFF state. In cases when variant 3-7 respond differently than the original, it could be due to error. This is shown in cortisol, where variant 6 is slightly higher in the OFF state than ON. The standard deviation is shown by the bars in the figures. This is also shown in serotonin with variant 4 and epinephrine with variants 3 and 4.

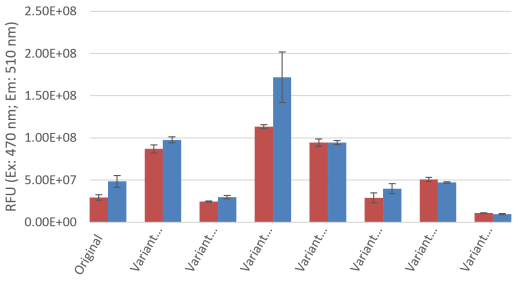

Figure 34 Cortisol biomarker against original riboswitch and variants

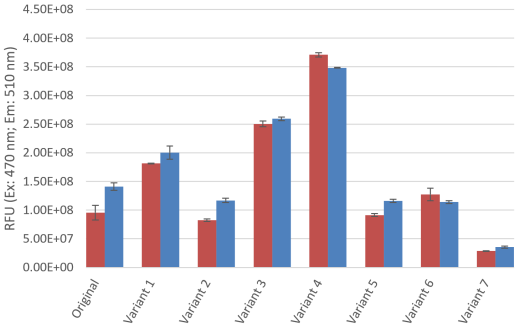

Figure 35 Serotonin biomarker against original riboswitch and variants

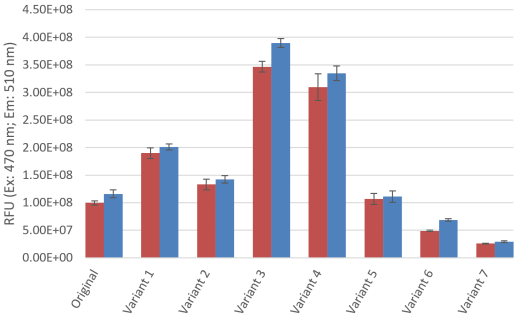

Figure 36 Dopamine biomarker against original riboswitch and variants

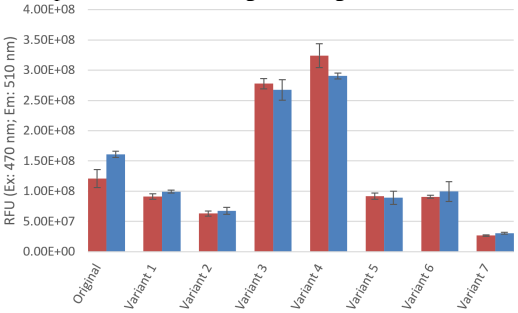

Figure 37 Epinephrine biomarker against original riboswitch and variants

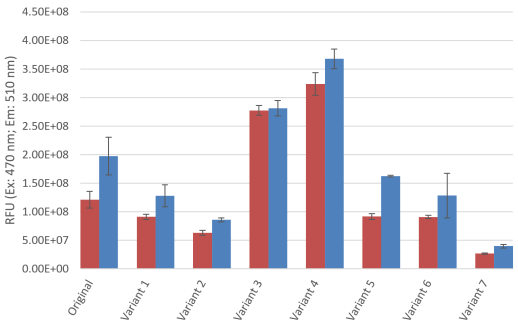

Figure 38 Norepinephrine biomarker against original riboswitch and variants

4.4 Variant results

In this section, $PreQ_1$, Variant 1, and Variant 2 are discussed further. The Figure 39 to 41, take the data that was presented above and show the riboswitches original, variant 1, and 2 response with each analyte in the same figure. The bars follow the same pattern as above with orange bar is OFF, and the blue bar is ON. Variants 1 and 2 responses are compared to $PreQ_1$ whether or not the aptamer of the riboswitch binding to the $PreQ_1$ and DHEA-S ligand. The response shows $preQ_1$ and DHEA-S respond the same with the original $PreQ_1$ riboswitch but differs in variants 1 and 2. $PreQ_1$'s response, the relationship of OFF to ON is OFF being higher with $PreQ_1$ and DHEA-S and ON higher for all others. The difference with variants 1 and 2 is that DHEA-S follows the original, but $PreQ_1$ does not. Therefore, a dosage test was conducted in the increasing concentration scale. The results are shown below.

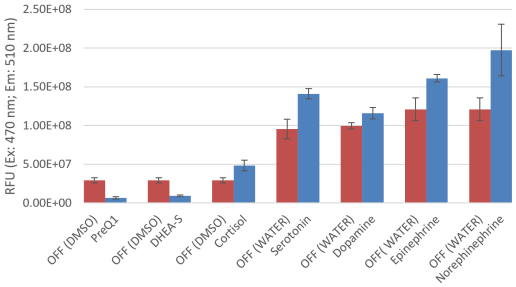

Figure 39 $PreQ_1$ riboswitch against different biomarkers

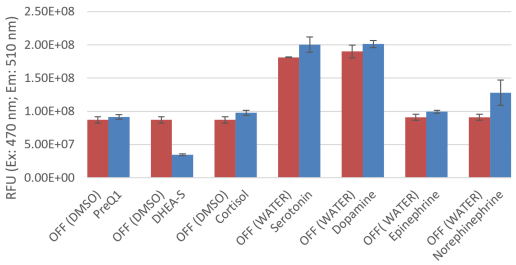

Figure 40 Variant 1 riboswitch against different biomarkers

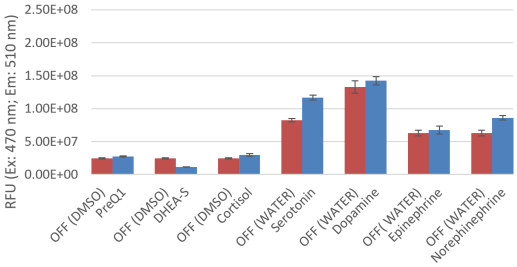

Figure 41 Variant 2 riboswitch against different biomarkers

4.5 Dosage results for variant 1 and 2

In this section, variants 1 and 2 are shown with different concentrations of DHEA-S. For both responses, there is a linear, downward trend, with higher concentration of DHEA-S. The Figure 42 and 43, show variant 1 and 2 responses to different concentrations of DHEA-S.

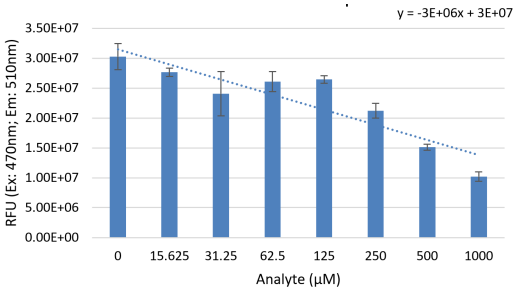

Figure 42 Variant 1 against DHEA-S dose response

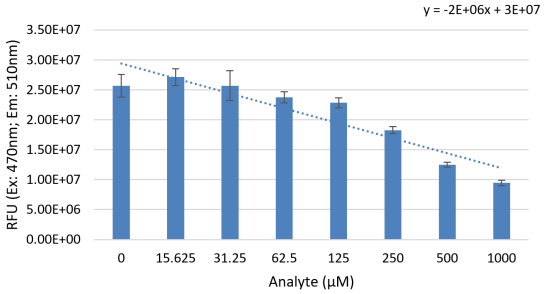

Figure 43 Variant 2 against DHEA-S dose response

Figure 42 and 43, the linear equations have a negative slope. This is due to the riboswitch having a higher RFU since there is no DHEA-S and the riboswitch being OFF and transcribing proteins. Comparing these graphs to the time activation graphs and biomarker graphs for DHEA-S and $PreQ_1$, the trendlines follow the same pattern. As the concentration of the analyte increases, the variants are showing how they terminated their protein production. If this was $PreQ_1$ as the analyte, the production would have stayed on with increased concentration.

5 Conclusion

This research describes the methods of reengineering riboswitches. In this experimentation, $PreQ_1$ was examined and engineered into four new variants. Through the computational and experimental tests were conducted, it is shown that two of these variants responded to DHEA-S differently than $PreQ_1$. Three variants, from literature were tested with the same procedures and showed to be consistent. In conclusion, riboswitches can be reengineered to respond to different biomarkers.

Based on the presented data analysis above, it can be concluded that variants 1 and 2 were reengineered to respond to DHEA-S when the original riboswitch did not. The computational data confirms this through variants 1 and 2 having equilibrium probabilities that indicate the structural formation would not be likely. Variants 3 and 4 indicated similar to $PreQ_1$ based on their equilibrium probability. This is also shown through the response of variants 1 and 2 differing when testing against $PreQ_1$. All variants had similar results when presented with the aptamer $PreQ_1$. This change did not affect the response against DHEA-S though. Due to this, a closer examination was done through a dose response test. A downward trend as the concentrations of DHEA-S increased the RFU decreased.

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