Using Computational Simulation to Guide DPAC Sensor Design for Viral Detection

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Introduction

The COVID-19 pandemic has caused a massive shift in the way we go about our daily lives. The prevalence and importance of reliable testing initiatives for viral screening has evolved rapidly in response to the pandemic. Current and frequently used methods of testing include PCR Amplification and Antibody Screenings, both having some significant drawbacks.

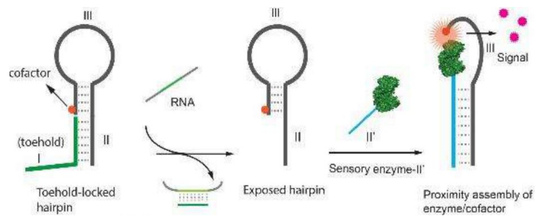
- PCR Amplification requires expensive equipment, trained personnel, is time consuming, and is not isothermal. Results may take more than a day.
- Despite being cheaper and more rapid than PCR, antibody testing does not screen for the presence of viral material and can provide a false negative result in patients.

SARS-CoV-2, the causative agent of the COVID-19 pandemic, has nucleic acid sequences that can be detected using a variety of different methods. Additionally HCV, the causative agent of Hepatitis C, is a commonly encountered virus that causes inflammation of the liver. Utilizing the novel nucleic acid detection technology, the DNA-mediated Proximity Assembly Circuit (DPAC) and NUPACK, a nucleic acid reaction simulation suite, we demonstrate two potential designs for the detection of the nucleic acid sequences of the RNA viruses.

Background

The DPAC Sensor is a DNA nanotechnology that utilizes strand displacement reactions in order to detect a target nucleic acid sequence. Comprised of a toehold-locked hairpin structure with an associated cofactor, as well as an assisting enzyme, the DPAC sensor utilizes strand displacement reactions in order to detect SARS-CoV-2 and Hepatitis C in our experiment.

Upon target introduction, the toehold is displaced from the hairpin, and the now exposed hairpin cofactor can interact with the sensory enzyme to produce a positive result in the form of fluorescence.



Hypothesis: Using computational aided design, a promising DPAC sensor design for the detection of SARS-CoV-2 and HCV can be created.

References

Oh, S.W., Pereira, A., Zhang, T., Li, T., Lane, A., and Fu, J. (2018). DNA-Mediated Proximity-Based Assembly Circuit for Actuation of Biochemical Reactions. Angewandte Chemie International Edition 57, 13086–13090.

Zadeh, J.N., Steenberg, C.D., Bois, J.S., Wolfe, B.R., Pierce, M.B., Khan, A.R., Dirks, R.M., and Pierce, N.A. (2011). NUPACK: Analysis and design of nucleic acid systems. Journal of Computational Chemistry 32, 170–173.

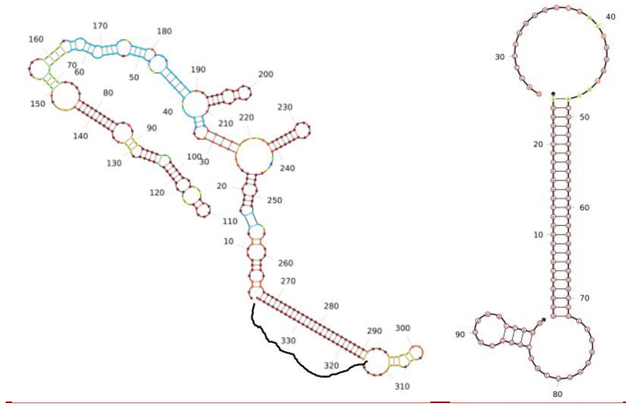
Methodology and Results

A general protocol was established in order to procedurally generate designs for the DPAC sensor:

1. Obtain established PCR primer and probe sets for desired target nucleic acid.
2. Establish a target region on the secondary structure of the target nucleic acid by assessment through NUPACK. Targeting the most favorable region on the amplicon that can avoid self-folding areas.
3. Generate an antisense toehold complementing the most exposed region of the target secondary structure.
4. Generate a sense region to toehold in order to stabilize the hairpin to prevent premature opening, leaving a 10nt toehold binding region exposed in order to initiate the strand displacement reaction.
5. Generate an antisense enzyme region for binding to the opened hairpin following strand displacement to produce signal. The enzyme strand should be complementary only to the newly exposed hairpin region following strand displacement.
6. Utilize NUPACK in order to test several criteria with respect to the new design:
   a) Toehold-Hairpin Sensor Stability (testing stability of the design)
   b) Toehold Displacement Efficiency (testing if the toehold can be displaced from the hairpin)
   c) One-Pot Assay (testing of hairpin to enzyme strand hybridization when all components are present)
   d) Background Signal Assessment (testing the relative noise generated without trigger strand)
   e) Positive and Negative Controls (to assess the effect on signal when toehold is absent or present with no target present in both tests)
   f) Target Titration (to assess the sensitivity of the design)

Test Name	HCV	SARS-CoV-2
Toehold-Hairpin Stability	100%	98%
Toehold Displacement Efficiency	100%	97%
One-Pot Assay	100%	100%
Background Signal	36%	7%
Positive Control Yield	100%	94%
Negative Control Yield	16%	15%

Signal Titration (Trigger Gain)	HCV	SARS-CoV-2
0.1% Target Concentration	0%	47.71%
1% Target Concentration	0%	49.27%
10% Target Concentration	4%	63.59%
100% Target Concentration	64%	93.23%



The structure of the amplicon bound to the experimentally designed Toehold. HCV (Left) and SAR-CoV-2 (right).

Discussion

The results shown previously demonstrate the following takeaways:

a) Simulated DPAC sensor stability for both viruses demonstrate stable sensor designs.
b) Toehold displacement efficiency demonstrates that designed toeholds have a high binding affinity to the target and can be displaced from their respective hairpin structures.
c) One Pot Assay yielded 100% desired product. Demonstrating that both reactions are likely to produce signal when the target is introduced.
d) Background signal in the Hepatitis C design is significantly higher than in the SARS-CoV-2 design.
e) Positive control shows the relative hybridization of the Hairpin and Enzyme when the toehold is not present in the sensor at all.
   1. Thermal equilibrium assessment reports that both reactions will proceed to completion and generate a strong amount of signal.

   Negative Control simulation reports the relative shelf life of the sensor by testing the hybridization of the hairpin-enzyme complex when target is not introduced.
   2. Thermal equilibrium assessment reports that both sensors are unlikely to spontaneously react without any target present to displace the toehold.
f) Due to background noise, the Hepatitis C design shows extremely poor sensitivity which may warrant a redesign of the hairpin and enzyme strand sequences. The SARS-CoV-2 design shows promising sensitivity, and warrants further optimization of the design.

Conclusions and Directions for Future Research

Proposed sensor designs show a potential to detect the presence of target nucleic acids. These results demonstrate that computational simulation can prove useful while obtaining preliminary data on designs prior to performing actual tests in the wet lab.

By utilizing this computational simulation method, a few benefits are provided:

- Any unfavorable designs for the DPAC components can be ruled out before experimental validation.
- Multiple designs can be tested simultaneously, which can accelerate the design process.
- Computational testing can be performed from anywhere.
- Computational testing costs far less than experimentally testing the same quantity of proposed designs.

However, there are drawbacks as well:

- Reaction kinetics cannot be directly determined, as we can only speculate upon kinetics utilizing the equilibrium concentration of certain components and their associated $\Delta\Delta G$.
- Computational yield is likely higher than what should be expected in real testing, as there are real world factors that are not accounted for in NUPACK.

In the future, despite the theoretical promise of the designs, there is still a need for physical experimental validation to verify the accuracy of the computational analysis.

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