Glucose biosensor based on DNA origami

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Abstract: In this paper, I designed a glucose biosensor that can help us measure glucose levels. Specifically, glucose can be detected by the aptamer on the biosensor, which changes conformation and results in a change in FRET. Thus, by using fluorescence spectroscopy, glucose levels can be measured. The biosensor functions as a point of care device that people can use right at home to keep track of their blood glucose levels. The biosensor is based on DNA origami, fluorescence sensing strategy, and also an aptamer to detect glucose. Specifically, a dynamic DNA origami is used to design a glucose biosensor, which is precisely decorated with arrays of fluorophores acting as donors and acceptors that can show the presence of glucose according to the conformation of the DNA origami, which changes as an aptamer detects glucose.

1. Introduction

For living beings, maintaining glucose levels is crucial. Irregular blood glucose levels can lead to serious problems. For example, hypoglycemia is an insufficient supply of energy to the brain (neuroglycopenia), with symptoms such as hunger, headaches, falling asleep, mental confusion, hallucinations, and finally convulsions and coma [4]. Therefore, it is important for people to keep track of their blood glucose levels.

In order to develop a novel type of biosensors that are easier to mass produce, more sensitive, I approached the design problem in the field of DNA nanotechnology, specifically DNA origami.

DNA origami technique, given its ease of preparation, has led to an explosive growth in structural DNA nanotechnology to address challenges in a variety of research areas. The technique is based on a long single-stranded DNA (scaffold strand) folded into desired shapes by using hundreds of short complementary oligonucleotides (staple strands), and has been used to create not only two-dimensional sheets but also complex three-dimensional objects. Such self-assembled DNA nanostructures have served as scaffolds for the arrangement of proteins and nanoparticles. In addition, structures that can respond to external stimuli have been developed[7].

For the recognition of the glucose molecules in the samples, the prospective device is functionalized with an aptamer in a position that can induce a conformational change in the device's structure. Aptamers are a special class of nucleic acid molecules that are beginning to be investigated for clinical use. These small RNA/DNA molecules can form secondary and tertiary structures capable of specifically binding proteins or other cellular targets; they are essentially a chemical equivalent of antibodies[5]. In order to study conformational change, a set of fluorescent molecules are positioned in the structure so that a FRET signal is produced in one of the possible states. Fluorescence resonance energy transfer (FRET) is a distance-dependent physical process by which energy is transferred nonradiatively from an excited molecular fluorophore (the donor) to another fluorophore (the acceptor) by means of intermolecular long-range dipole–dipole coupling[6].

2. Method

2.1. Nupack

The glucose aptamer for the biosensor is based on the design presented in the paper "Aptamer-field-effect transistors overcome Debye length limitations for small-molecule sensing"[3].

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According to the paper, the glucose aptamer sequence is "CTCTCGGGACGACCGTGTGTTGCTGTAACAGTGTCCATTGTCGTCCC". By adding the sequence to Nupack, the aptamer, the secondary structures, can be evaluated together with its suitability to be introduced into the DNA origami structure, as shown in Figure 1.

Aptamer	Strand	Sequence (5′ → 3′)	Concentration	Buffer
Dopamine	Sensor	/56-FAM/ CTC TCG GGA CGA CGC CAG TTT GAA GGT TCG TTC GCA GGT GTG GAG TGA CGT CGT CCC	50 nM	PBS
	Capture	CGT CGT CCC GAG AG/3Dab/	250 nM	
Serotonin	Sensor	/56-FAM/ CTC TCG GGA CGA CTG GTA GGC AGA TAG GGG AAG CTG ATT CGA TGC GTG GGT CGT CCC	50 nM	PBS
	Capture	GTC GTC CCG AGA G/3Dab/	500 nM	
Glucose	Sensor	/56-FAM/CTC TCG GGA CGA CCG TGT GTG TTG CTC TGT AAC AGT GTC CAT TGT CGT CCC	50 nM	HEPES
	Capture	GGT CGT CCC GAG AG/3Dab/	250 nM	
S1P	Sensor	/56-FAM/CTC TCG GGA CGA CGT GGT GTG GGA GAA AGA ATT TTC ATT GGG GTA GGG GGT CGT CCC	50 nM	HEPES
	Capture	GTC GTC CCG AGA G/3Dab/	150 nM	

Figure 1 Sequences of aptamers and complementary (capture) strands, and concentrations and buffers for fluorescence assays from Aptamer-field-effect transistors overcome Debye length limitations for small-molecule sensing.

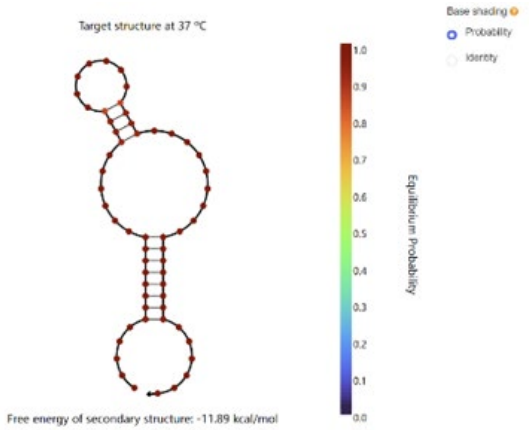

Figure 2 DNA origami structure.

2.2. Cadnano (https://cadnano.org/)

DNA origami is dynamic, and consists of two same layers, which connect to each other on one side by flexible hinges. In this way, the DNA origami is shaped like a book, which can open and close to the other side without hinges. The reason for designing a dynamic origami is that by opening and closing the structure, the FRET efficiency would change due to the change in the distance between fluorophores in both layers, so that glucose can be detected by measuring the FRET efficiency. Figure 2 and 3 show one layer of origami, which is designed in Cadnano2.

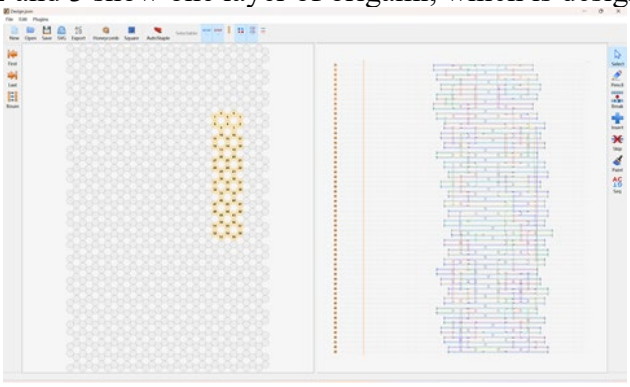

Figure 3 DNA origami design using cadnano2.

2.3. Overview

The Oxview tool of visualization (https://sulcgroup.github.io/oxdna-viewer/) allowed me to introduce the modifications necessary in the structure for it to be responsive to glucose levels. Specifically, a staple coming out of the upper layer of the biosensor had the sequence of the aptamer in figure 1 added to its 3' end. Similarly, the complementary strand of the aptamer is added to the lower layer. To ensure that the aptamer can bind to targets, a few mismatches between the aptamer and its complementary ssDNA are made.

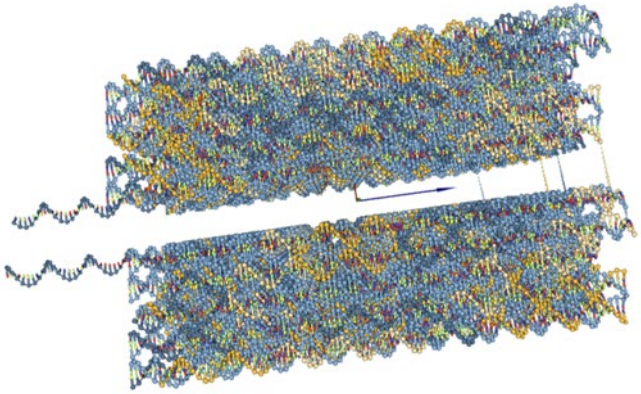

Figure 4 DNA origami design using cadnano2.The biosensor synthesized in Oxview, with an added aptamer

3. Result and Discussion

The whole structure is made by myself. The basic strategy for detecting glucose and displaying the information is done using aptamer and fluorescence. Specifically, a lock-and-key system is used in the design of aptamer-based functionalization, a method used in previous studies on DNA nanoboxes[2]. The upper layer of the origami presents the aptamer, and the lower layer presents a ssDNA with sequences complementary to those of the glucose aptamer. Therefore, the layers closely attach to each other through base complementarity. Then, if the aptamer detects glucose, it will instead bind to the target, thus breaking the hydrogen bonds between the sequences, removing the connection between the upper and lower layers of the DNA origami. This will allow the opening of the DNA origami. Thus, with the lock-and-key system, the conformational change in origami is achieved, as shown in Figure 4.

Due to the dynamic characteristics of my origami, I decided to take advantage of the change in FRET efficiency according to the distance between fluorophores. Specifically, fluorophores are placed on both layers of origami. The fluorophores, namely Cy3 and Cy5, on the upper layer act as acceptors, while the fluorophores on the lower layer act as donors[1] . Therefore, when in close proximity, an excited Cy3 will keep the FRET efficiency high, while increasing the distance between fluorophores will deactivate Cy3, keeping FRET efficiency low. The paper DNA origami book biosensor for multiplex detection of cancer-associated nucleic acids[1] provides evidence. To determine the reaction time of the DNA origami book biosensor to fully open, the structures were assembled in a closed state with a single column of FRET pairs (column 1). Plotted FRET efficiency (measured using spectrophotometer) showed a decrease over time , indicating that the structure opened within 10 min after the addition of the target was added.

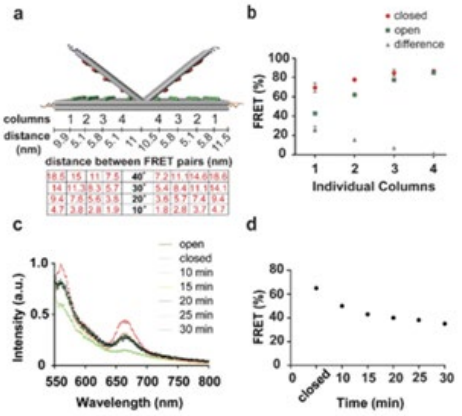

Figure 5 Determination of FRET efficiency of DNA origami book biosensor using fluorescence spectroscopy from DNA origami book biosensor for multiplex detection of cancer-associated nucleic acids

4. Conclusion

With DNA origami, lock-and-key system, and fluorescence sensing strategy, I can determine glucose levels in samples from human bodies by using a spectrophotometer to measure FRET efficiency, as shown in Figure 5. However, since the biosensor functions outside of the human body, people still need to take blood samples from their bodies to test glucose levels. Therefore, future studies should focus on delivering the biosensor into human bodies, so that people can test their blood glucose levels in a more convenient way.

References

[1] Domljanovic, I., Loretan, M., Kempter, S., Acuna, G. P., Kocabey, S., & Rüegg, C. (2022c). DNA origami book biosensor for multiplex detection of cancer-associated nucleic acids. Nanoscale, 14(41), 15432–15441. https://doi.org/10.1039/d2nr03985k

[2] Andersen, E. J., Dong, M., Nielsen, M. M., Jahn, K., Subramani, R., Mamdouh, W., Golas, M. M., Sander, B., Stark, H., Oliveira, C. L. P., Pedersen, J. S., Birkedal, V., Besenbacher, F., Gothelf, K. V., & Kjems, J. (2009b). Self-assembly of a nanoscale DNA box with a controllable lid. Nature, 459(7243), 73–76. https://doi.org/10.1038/nature07971

[3] Nakatsuka, N., Yang, K., Abendroth, J. M., Cheung, K. M., Xu, X., Yang, H., Zhao, C., Zhu, B., Rim, Y. S., Yang, Y., Weiss, P. S., Stojanovic, M. N., & Andrews, A. M. (2018b). Aptamer–field-effect transistors overcome Debye length limitations for small-molecule sensing. Science, 362(6412), 319–324. https://doi.org/10.1126/science.aao6750

[4] MEFANET, Czech and Slovak medical faculties network. (n.d.). Disorders of glucose metabolism - WikiLectures. https://www.wikilectures.eu/w/Disorders_of_glucose_metabolism

[5] Ni, X., Castanares, M., Mukherjee, A., & Lupold, S. E. (2011). Nucleic acid aptamers: clinical applications and promising new horizons. Current Medicinal Chemistry, 18(27), 4206–4214. https://doi.org/10.2174/092986711797189600

[6] Sekar, R. B., & Periasamy, A. (2003). Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. Journal of Cell Biology, 160(5), 629–633. https://doi.org/10.1083/jcb.200210140

[7] Chandrasekaran, A. R., Anderson, N., Kizer, M. E., Halvorsen, K., & Wang, X. (2016). Beyond the fold: emerging biological applications of DNA origami. ChemBioChem, 17(12), 1081–1089. https://doi.org/10.1002/cbic.201600038.