Detection of micro RNA-210 Using Optical Biosensing Platform Based on the Actuation of Discrete Gold Nanoparticle Dimers

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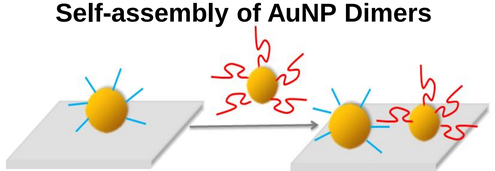
Introduction

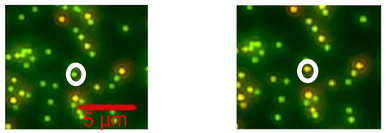
A micro RNA (miRNA) is a small non-coding RNA molecule that functions in transcriptional and post-transcriptional regulation of gene expression. MiRNAs could be potential biomarkers for diseases. MiRNA-210 is a well known sensor for hypoxia as the level of miRNA-210 typically rises in response to low oxygen tension in various cell types. It is up-regulated in hypoxia associated diseases, such as Pre-Eclampsia - a medical condition which results in a poor exchange of nutrients, wastes and gases between the mother and fetus. It affects approximately 6-8% of all pregnancies worldwide.[1] Early detection of miRNA-210 can help early diagnosis and prevent the onset of serious complications. However, the detection is challenging due to the short length and relatively low stability of miRNA. Currently, RT-PCR is used to detect miRNA-210, but it is time-consuming and costly. The goal of our project is to develop an optical biosensing platform based on dynamically linked gold nanoparticle (AuNP) dimers. Detection is achieved by monitoring a wavelength shift in the scattering spectra of Au dimers.[2]

DNA-functionalized Gold Nanoparticle Dimers for Sensing

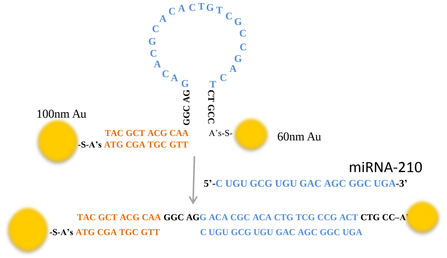
Self-assembly of AuNP Dimers



Darkfield microscope images



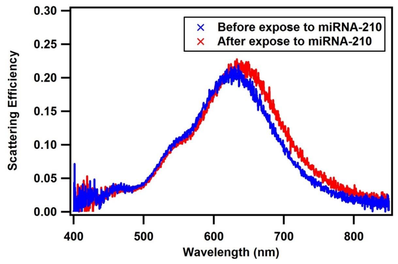
The dimers can be identified visually by the color change before (left) and after (right) Au dimer formation.



- A geometric extension is achieved when complementary single-stranded oligonucleotide (e.g. miRNA-210) binds to stem-loop DNA linker in Au dimer.

100 nm AuNP was deposited on cover slip and then functionalized with DNA sequence. The plasmonic gold dimers were formed when DNA-functionalized 60 nm AuNP was added and hybridized with DNA-functionalized 100 nm AuNP

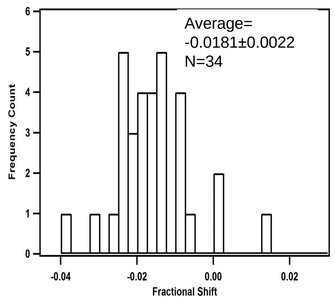
Scattering spectra



- Geometric extension weakens plasmon coupling and results in a spectral blue shift.
- The spectra shown was obtained from single-nanostructure spectroscopy.
- A blue shift of ~10nm was observed when dimer was exposed to the target, miRNA-210.
- The spectra of many dimers were acquired and fractional shifts (defined as $\Delta\lambda/\lambda = \frac{\lambda(\text{after}) - \lambda(\text{before})}{\lambda(\text{before})}$) were calculated.

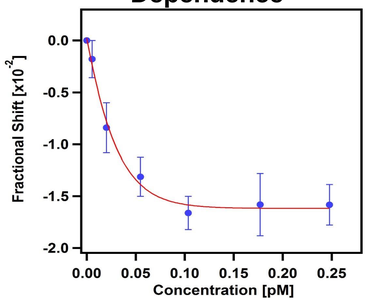
Detection Limit and Selectivity of Dimer Sensor

Detection of Synthetic miRNA-210



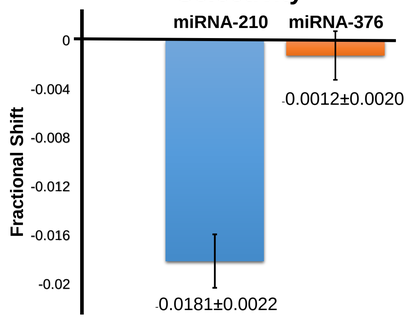
Obtained from 34 Au dimers in a sample.

miRNA-210 Concentration Dependence



Achieved ultra low detection limit, in sub-pM range (10^{-13}M).

Selectivity

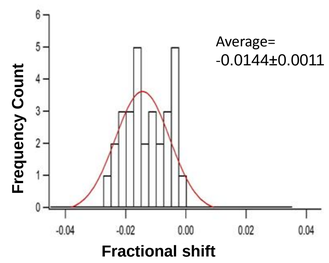


Control experiment (exposure to miRNA-376) showed negligible shift, demonstrating the selectivity of the dimer sensor.

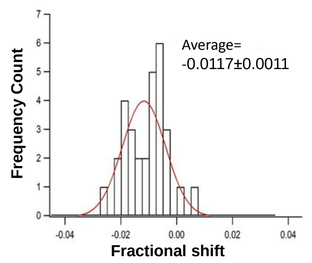
Detection of miRNA-210 in Cell Extracts

- Hypoxic cells were cultured under 3% oxygen, while normoxic cells were at normal oxygen level, 21%.
- Using the calibration curve, hypoxic cell extract shows 2-3 times higher level of miRNA-210 compared to normoxic cell extract, while PCR gives a 3-4 fold increase. The results are in good agreement.

Hypoxic Cell Extract



Normoxic Cell Extract



Cell Extract	miRNA added (ng)	# cells	miRNA extracted (ng)	Fractional Shift	Concentration (pM)	# of RNA/Cell
Normoxic	1000	50000	2878	-0.0117	0.0380	72
Hypoxic	1000	50000	3505	-0.0144	0.0653	152

Conclusions:

- MiRNA-210 was successfully detected using actuatable AuNP dimers with ultralow detection limit and good selectivity.
- This novel of optical biosensing platform can be applied for the detection of other short oligonucleotide sequences, proteins and specific ions. It may provide a promising future for the facile detection of biomarker at early stages of diseases.

Future Work:

- Detection of miRNA-210 in cell lysates
- Further study on detection specificity, such as ability to differentiate from a family of miRNA sequences

Reference

1. Fu G.; Brkic J.; Hayder H.; Peng C.; MicroRNAs in Human Placental Development and Pregnancy Complications, *International Journal of Molecular Science.* **2013**, 14(3), 5519-5544. 2. Chen. J.; Chen, Y.; Ginger. D.; Plasmonic Nanoparticle Dimers for Optical Sensing of DNA in Complex Media, *Journal of American Chemical Society*. **2010**, 132(28), 9600-9601.