DETECTION OF MicroRNA USING LSPR SPECTROSCOPY FOR DIAGNOSTIC APPLICATIONS

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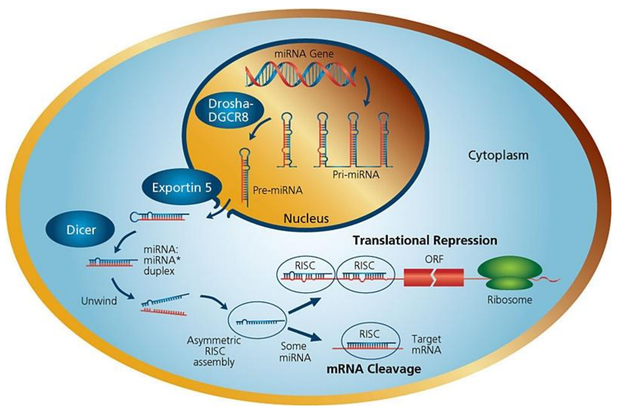
MicroRNAs (miRNAs) are an emerging class of diagnostic markers that can signify the presence of disease and be used to predict its course. MiRNAs are short strands of RNA, averaging 22 nucleotides in length (ranging from ca. 18 to 25 nucleotides), performing gene regulation either directly through cleavage of messenger RNA (mRNA), or indirectly through translation repression. MiRNAs are known to be involved in tumor metastasis, stem-cell differentiation and renewal, and viral replication. The ability to detect cancer at early, non-metastatic stages is a key factor in increasing survivability, and miRNA biomarkers may in principle identify the presence of a tumor before detection by conventional means is possible. Moreover, an ideal biomarker may allow tracking of the molecular diversity of the cancer as well as monitor the progression of the disease. A convenient, inexpensive and sensitive method for miRNAs sensing is therefore clearly needed.[1,2]

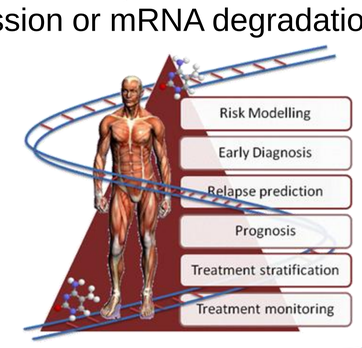
Here we propose to develop a biosensor platform based on localized surface plasmon resonance (LSPR) spectroscopy,[3] providing direct, label-free detection of miRNA. The LSPR transducers comprised gold nanoisland films, prepared by thermal evaporation on glass substrates followed by high-temperature annealing. Such transducers display a well-defined surface plasmon band in the visible range, changing in wavelength and intensity upon binding of a biological analyte. Our approach to the preparation of a miRNA recognition interface on the LSPR transducer involved immobilization of a self-assembled monolayer of a short thiolated DNA which is complementary to the target miRNA sequence. To demonstrate multiplexed detection of miRNAs, the LSPR approach was adapted to common equipment (96-well plates). This arrangement combines high-throughput screening (microarray) and label-free hybridization detection (LSPR).

microRNA

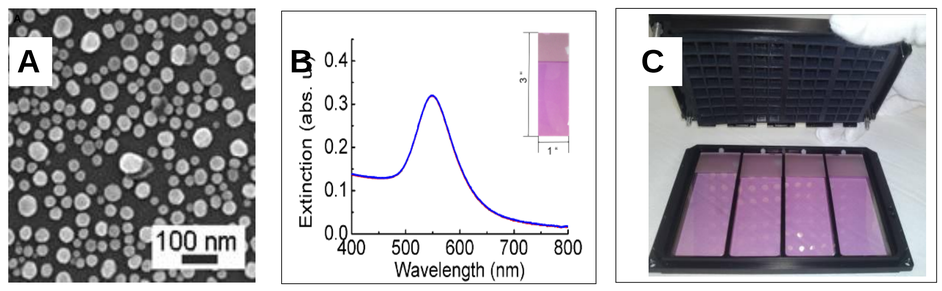


Cancer type	Overexpressed miRNA
Glioblastoma	miR-21
Tongue cancer	miR-184a
Lung cancer	miR-25, miR-223, miR-21, miR-155
Breast cancer	miR-195
Liver cancer	miR-500
Colorectal cancer	miR-92
Ovarian cancer	miR-141, miR-200
Prostate cancer	miR-141

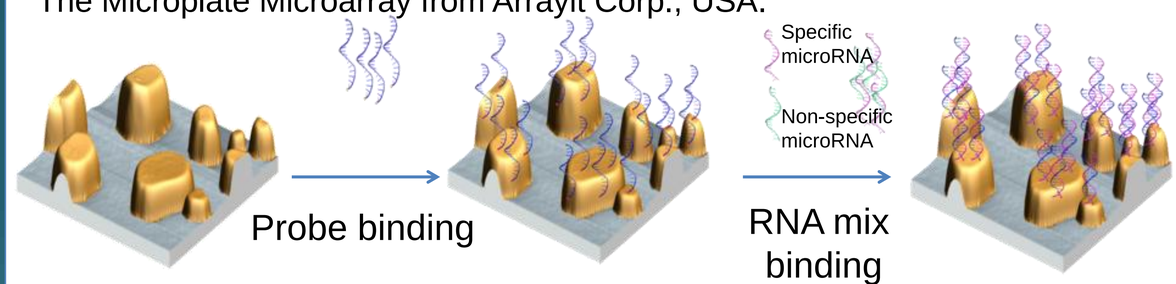
The primary transcripts of miRNAs, called pri-miRNAs, are transcribed as individual miRNA genes, from introns of proteine-coding genes, or from polycistronic transcripts. The RNAse Drosha further processes the pri-miRNA into 70- to 100-nt, hairprin-shaped precursor, called pre-miRNA, which are exported from the nucleus by Exportin 5. In the cytoplasm, the pre-miRNA is cleaved by Dicer into miRNA:miRNA* duplex. Assembled into the RISC, the mature miRNA negatively regulates gene expression by either translation repression or mRNA degradation.



Basics of the LSPR bio-sensing



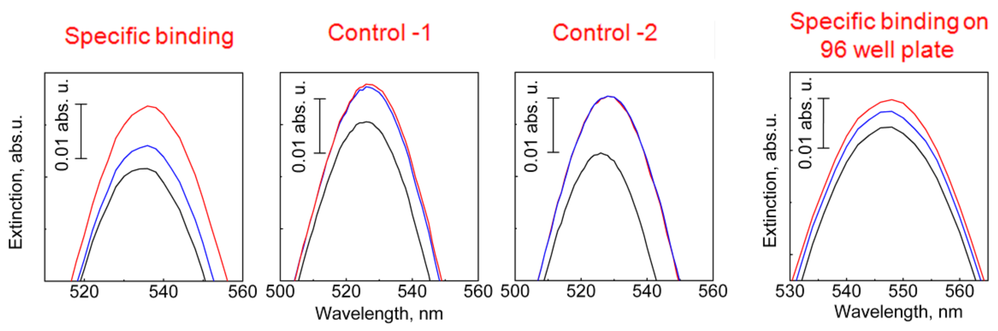
Basic LSPR transducer. (A) High-resolution scanning electron microscope image of a 5.0 nm (nominal thickness) Au island film prepared by evaporation on a glass microscope slide followed by annealing 10 h at 580 °C. (B) Transmission UV-Vis spectrum, presenting the localized plasmon absorption band of the LSPR transducer; inset: photograph of the transducer (1”×3”). (C) The Microplate Microarray from Arrayit Corp., USA.



Schematic representation of the miRNA assay. The Au island based LSPR transducer is functionalized with a SAM of covalently attached thiolated DNA having a sequence complementary to the target miRNA, followed by incubation with samples containing miRNA strands.

Proof of the concept

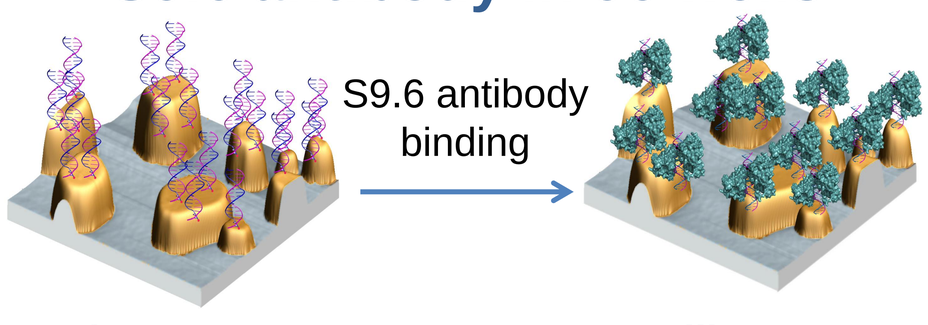
miR-145: GUCCAGUUUUCCCAGGAAUCCCU



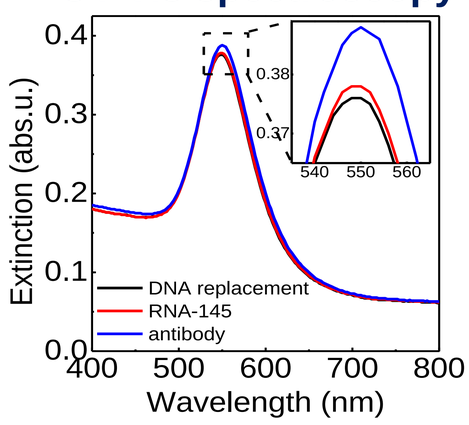
Experiment	Specific binding miR-145 on DNA-145	Control -1 G-site RNA on DNA-145	Control -2 poly-U on DNA-145	Specific binding on 96 well plate miR-145 on DNA-145
Extinction change(abs. u.)	0.008	0	0	0.003
Wavelength shift (nm)	1	0	0	0.6

Sequential transmission UV-vis spectra of a bare Au island transducer (black line), after assembly of a SAM of the thiolated DNA (blue line), and after miRNA binding (red line). Note that in the two controls the blue and red lines overlap, indicating no miRNA binding.

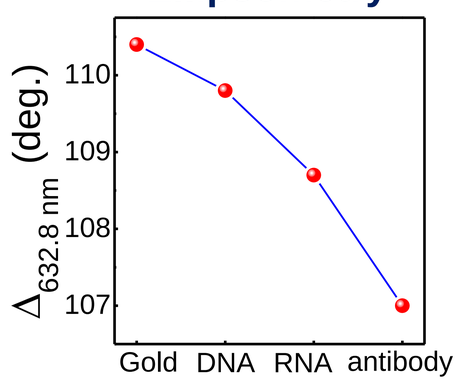
Enhancement of LSPR signal using S9.6 antibody in 96 wells



UV-vis spectroscopy



Ellipsometry



Simple transducer preparation, high sensitivity, low cost,. high throughout profiling, label-free detection.

[1] Bartel, D. P., MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* **2004,** *116*, 281-297.

[2] Croce, C. M., Causes and consequences of microRNA dysregulation in cancer. *Nat. Rev. Genet.* **2009,** *10*, 704-714.

[3] Bendikov et.al., Biological sensing and interface design in gold island film based localized plasmon transducers. *Anal. Chem.* **2008,** *80*, 7487-7498.