

## Supporting Information

### Nasopharyngeal Carcinoma Related MiRNA Detection Through DSN Enzyme Assisted Tetrahedral Probe for More Accurate Prognosis

Li Zhang<sup>1</sup>, Zhiping Lou<sup>2\*</sup>

#### Author affiliation:

1. Department of otolaryngology, Zhuji Affiliated Hospital of Shaoxing University, Zhuji City, Zhejiang Province, China. (311800)
2. Department of otolaryngology, Zhuji Affiliated Hospital of Shaoxing University, Zhuji City, Zhejiang Province, China. (311800)

#### \*Corresponding author:

Zhiping Lou, Zhuji People's Hospital, 9 Jianmin Road, Taozhu Street, Zhuji City, Zhejiang Province, China. E-mail: xushayue@163.com, Fax: +86-057581782153.

## **Experimental section**

### **Reagents and materials.**

All the essential nucleic acids used in the article is listed in **Table S1** and synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The obtained purified nucleic acids were then diluted into 10  $\mu\text{M}$  with HPLC. All the related enzymes, such as the DSN enzymes, were purchased from the Sigma-Aldrich (Shanghai, China). Other reagents were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). All solutions were prepared with ultrapure water from Millipore Milli-Q water purification system (Millipore, Bedford, MA). Serum samples were obtained from Zhuji Affiliated Hospital of Shaoxing University. The assemble of the tetrahedral probe was conducted in the a BioRad T100 thermal cycler (Bio-Rad, USA). the florescence spectrum was detected through HITACHI fluorescence spectrophotometer F-4700 (Beijing, China). The polyacrylamide gel electrophoresis (PAGE) analysis was carried out on a Bio-Rad electrophoresis analyzer (Bio-Rad, USA) and imaged on Bio-Rad ChemDoc XRS (Bio-Rad, USA).

### **Assemble of the tetrahedral probe**

The four designed ssDNA probe were firstly diluted into 10  $\mu\text{M}$  and stored for the following usage. We firstly mixed 5  $\mu\text{l}$  S1 probe with 5  $\mu\text{l}$  S2 probe together and incubated at 90°C for about 10 min. Afterwards, the mixture was then cooled to room temperature gradually. The assemble of the tetrahedral probe was carried with the same procedure mixing the four ssDNA probes. The characterization of the assemble of tetrahedral probe was carried through florescence assay and PAGE analysis.

### **Cytotoxicity measurement**

In order to study the cytotoxicity of the tetrahedral probe, we have firstly cultured the MCF-7 cells for about 24 h in a 96-well plate ( $1.0 \times 10^5$  cells per well), which contained DMEM with 10% fetal bovine serum (FBS). After the incubation with designed tetrahedral probe for about 2 h, we then replaced the medium with fresh DMEM containing 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) and incubated for another 4 h. Afterwards, we have added 5  $\mu\text{l}$  dimethyl sulfoxide and measured the absorbance of each well at 492 nm.

### **Cell Transfection.**

To study the transmembrane ability of the designed tetrahedral probe and normal hairpin probe, we have mixed 5  $\mu\text{l}$  tetrahedral probe and 5  $\mu\text{l}$  hairpin probe with MCF-7 cells and incubated for different times (from 0 to 150 min). Eventually the fluorescent images were taken with a confocal laser scanning fluorescence microscope.

### **Optimization of experimental conditions**

In order to optimize the concentrations of the DSN enzymes used in the whole sensing system, we have firstly applied the method for target miRNA (100 nM) detection with different DSN enzyme concentrations and detected the fluorescence intensity. The incubation time of DSN enzyme and tetrahedral probe is also studied with the same procedure. And the optimized incubation time was determined according to the longer one.

### Feasibility of the method for in vitro miRNA detection

5  $\mu$ l assembled tetrahedral probe was firstly incubated with 5  $\mu$ l DSN enzymes, 5  $\mu$ l related buffer and miRNAs with different concentrations ranging from fM to nM. After incubation for about 1 h, we have then monitored the fluorescence signals by HITACHI fluorescence spectrophotometer F-4700 (Beijing, China) and studied the relationships between the amounts of miRNAs and obtained fluorescence signals. Specificity of the method was also investigated through detecting different miRNAs (final concentration 2  $\mu$ M) with the same procedure.

### In situ miRNA imaging

CNE-1 cells were first cultured in 75 cm<sup>2</sup> flask for 1 week with a cell density of  $4.0 \times 10^4$  cells/mL. To monitor the endogenous miRNA-3188 expression in CNE-1 cells, the cells were seeded into confocal dishes (35 mm) and cultured for 24 h at 37 °C in a 5% CO<sub>2</sub> environment. Then, 10  $\mu$ l DNA tetrahedral probe and 2  $\mu$ l DSN enzymes were added in the dishes. After 1.5 h incubation at 37 °C and 5% CO<sub>2</sub> environment, the cells were washed with PBS gently for confocal imaging with a Leica TCS SPE confocal microscope system.

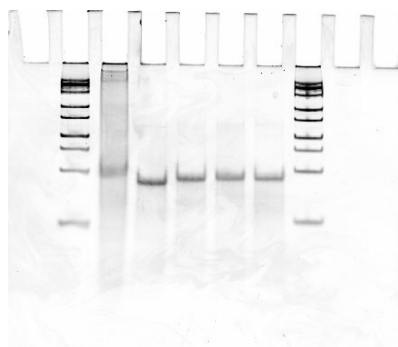
**Table S1**

Title	Sequence (5'-3')
S1	FAM-TATCACCAGGCAGTTGACAAGAGGCUUGUGCGAATAG ATGCGAGGGTCCAATAC-BHQ
S2	TACTATGGCGGCTCTTCTT ACGACACTACGTGGGAATC TCAACTGCCTGGTGATAAA
S3	TTCAGACTTAGGAATGTGC TTCCACGTAGTGTGTT GTATTGGACCCTCGATT
S4	AGAAGAGCCGCATAGTA TTACATTCCCTAAGTCTGAA ACATTCTCCGAAACACGC
miRNA-21	UAGCUUAUCAGACUGAUGUUGA

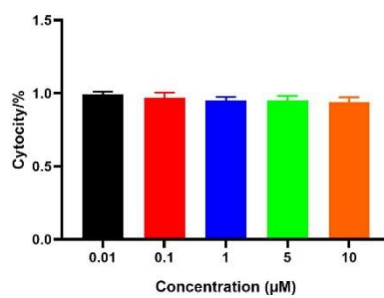
---

miRNA-155	UUAAUGC UAAUCGUGAUAGGGG
miRNA-205	UCCUUC AUUCCACCGGAGUCUG
miRNA-3188	AGAGGC UUUGUGCGGAUACGGG
miRNA-3188 inhibitor	CCCCGU AUCCGCACAAAGCCUCU

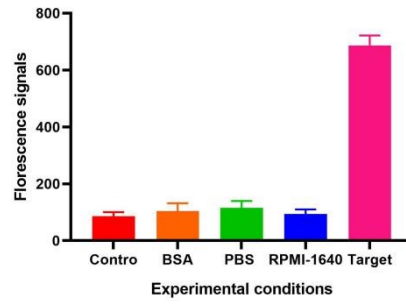
---



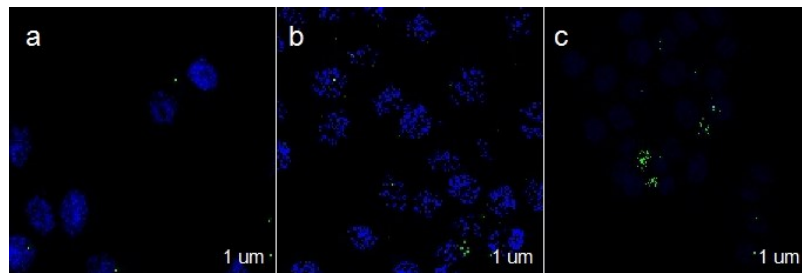
**Figure S1.** The 12% PAGE result of the assemble of tetrahedral.



**Figure S2.** Cytotoxicity percentage of the of tetrahedral probe on cells through cytotoxicity test.



**Figure S3.** florescence intensity of the designed tetrahedral probe when incubated under different experimental conditions.



**Figure S4.** Confocal result of the *in sit* miRNA imaging with lower magnification. from left to right (a to c): inhibitor, normal, mimics. The green florescence dots are the intracellular florescence generated by the proposed method when target miRNA exited. Scale bar=1 $\mu$ m.