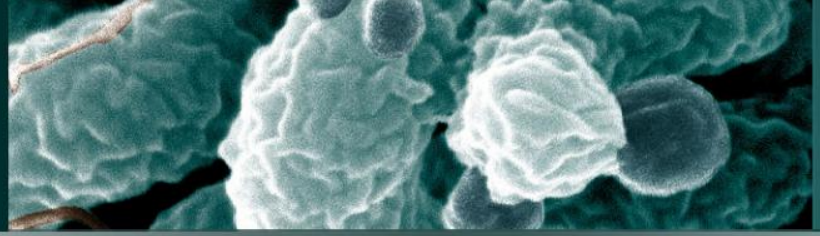

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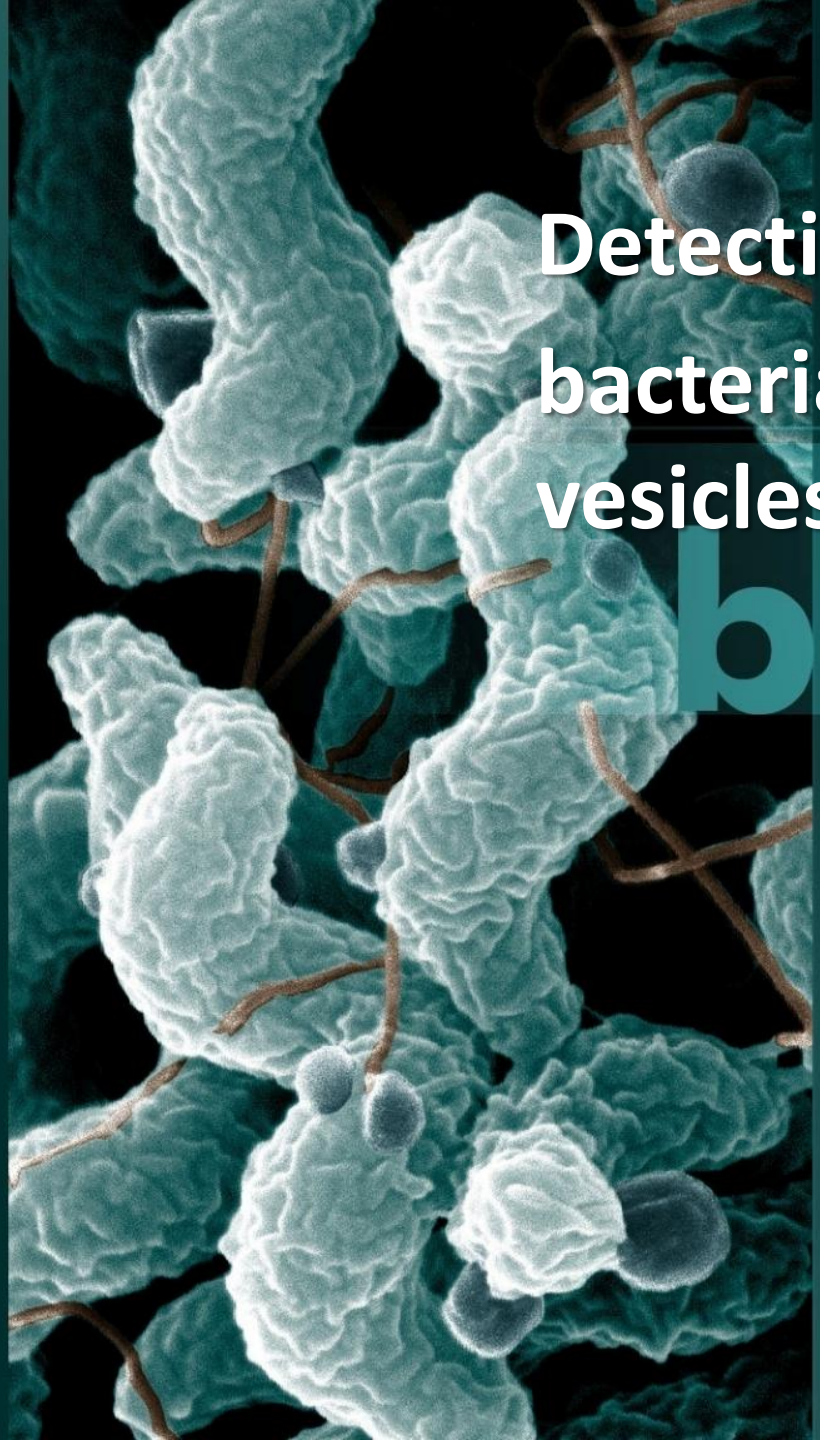

Detection of Gram-negative bacterial outer membrane vesicles, using DNA aptamers

bacteria

Presented by :
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Detection of Gram-negative bacterial outer membrane vesicles using DNA aptamers

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Hye-Su Shin, Vinayakumar Gedi, Joon-Ki Kim & Dong-ki Lee

Infection of various pathogenic bacteria causes severe illness to human beings. Despite the research

molecules

MDPI

Review

Inside the Black Box: What Makes SELEX Better?

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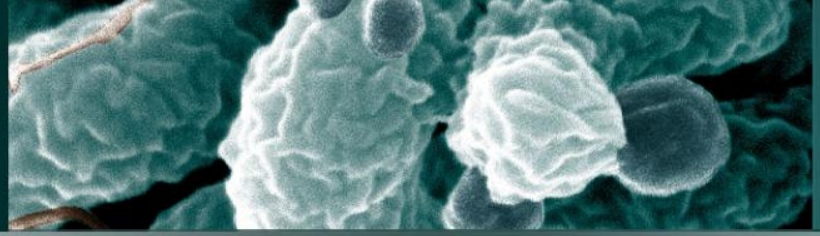
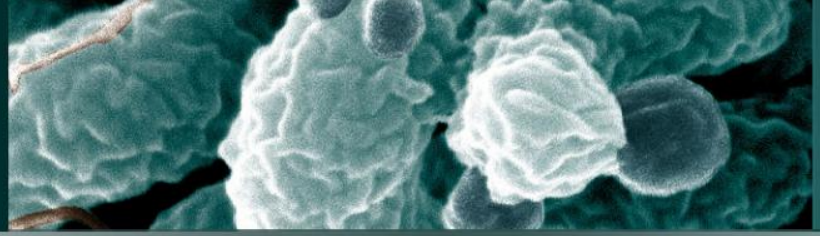
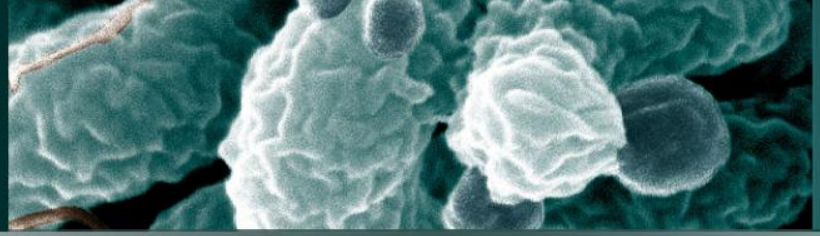


OUT LINE

- Difinitions
- Material and Methods
  - Results
  - Conclusion



❑ Difinitions

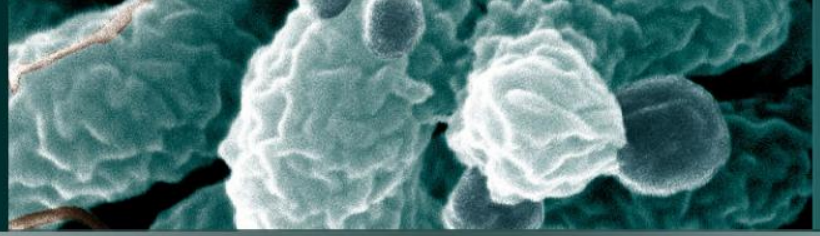


- Despite the research advances, current identification tools still exhibit limitations in detecting Gram-negative bacteria with high accuracy.

- In this study, we isolated single-stranded DNA aptamers against multiple Gram-negative bacterial species using Toggle-cell-SELEX.

- Aptamer-based detection tool towards bacterial secretory cargo released from outer membranes of Gram-negative bacteria.

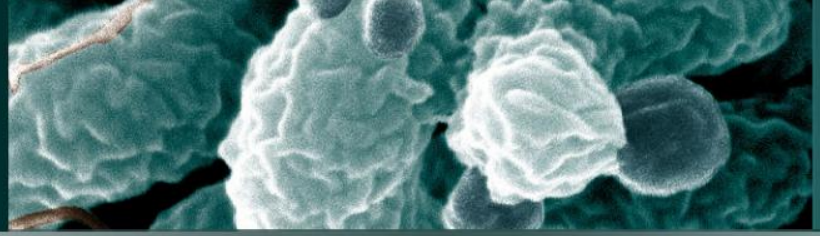
- Three Gram-negative bacteria, *Escherichia coli DH5α*, *E. coli K12*, and *Serratia marcescens*.



Aptamer

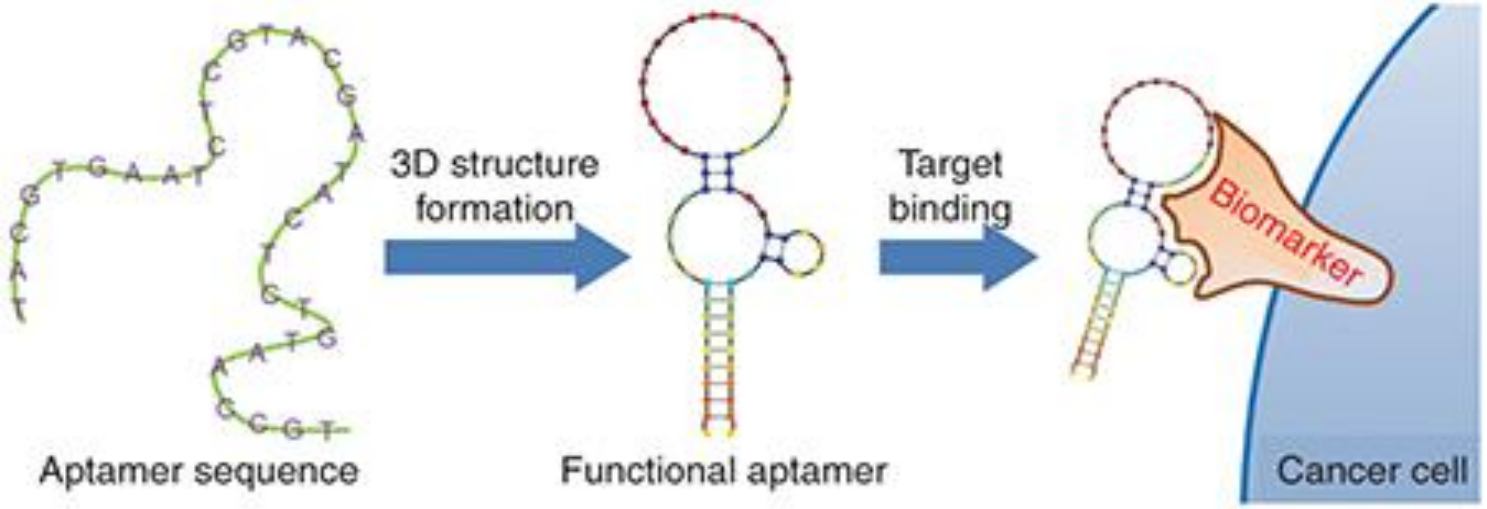
- Aptamers are small synthetic nucleic oligonucleotides that serve as ligands to target molecules. With their small size, low synthesis cost, thermal stability, ease of labeling, and ability to regenerate.

- Aptamers against a particular target can be obtained by the iterative selection of target-binding species from a large library of random oligonucleotide sequences. This procedure, called SELEX (Systematic Evolution of Ligands by Exponential Enrichment), was first proposed in 1990 simultaneously by Ellington and Szostak and Tuerk and Gold.



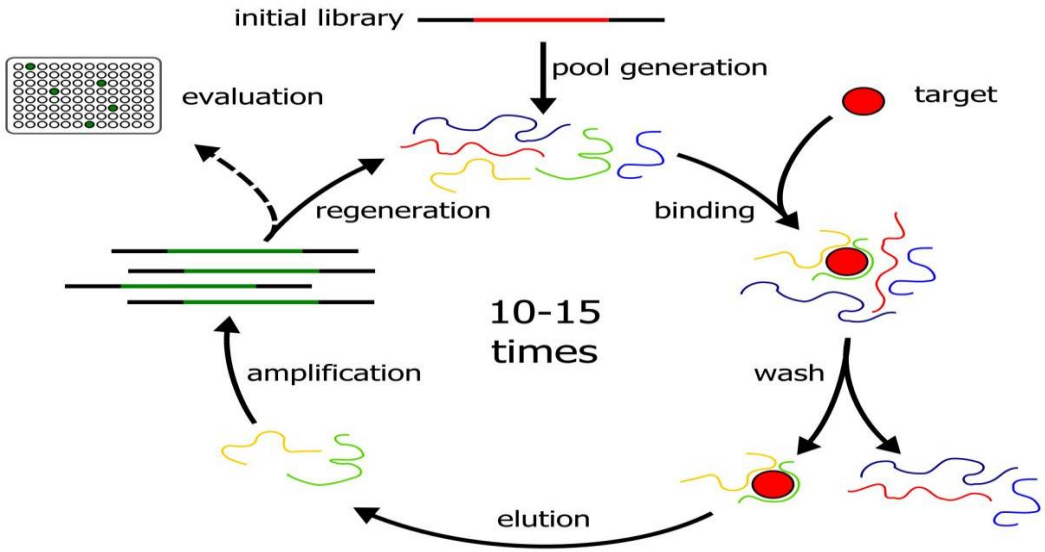
Aptamer

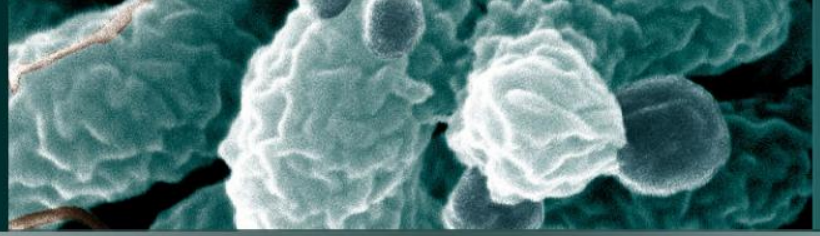
- Aptamers are a class of small nucleic acid ligands that are composed of RNA or single-stranded DNA oligonucleotides and have high specificity and affinity for their targets.



SELEX

- Systematic Evolution of ligands by Exponential Enrichment (**SELEX**), also referred to as in vitro selection or in vitro evolution, is a combinatorial chemistry technique in molecular biology for producing oligonucleotides of either single-stranded DNA or RNA that specifically bind to a target ligand or ligands, which are commonly referred to as aptamers

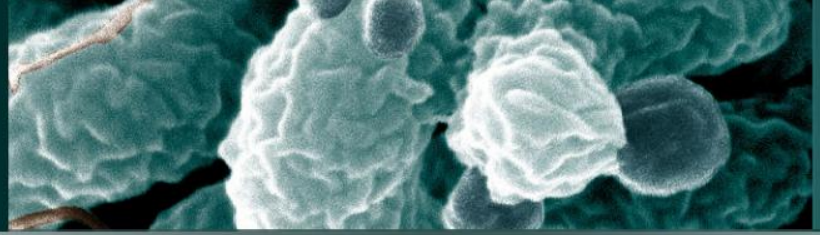




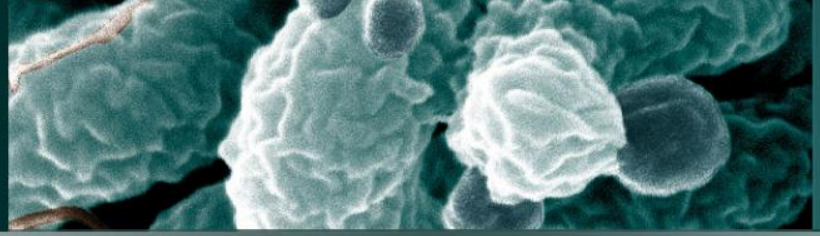
OMVs

- Outer membrane vesicles (OMVs) with size of 20 to 250 nm, which are commonly produced and secreted from outer membranes of bacteria, carry various bacterial membrane proteins and other virulence factors.

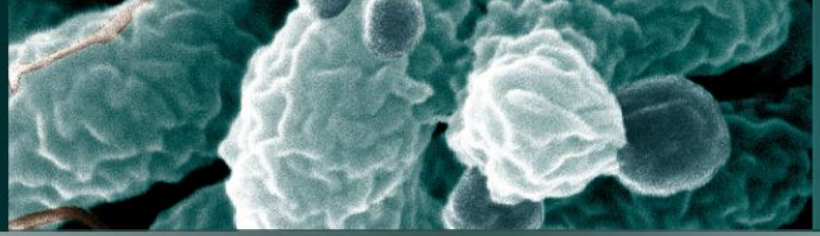
- OMVs are known to trigger severe pathogenesis, enhance bacterial survival, transfer genetic and protein components for cell-free intercellular communication, deliver toxic compounds, and trigger immune response in host cells.



❑ Material and Methods



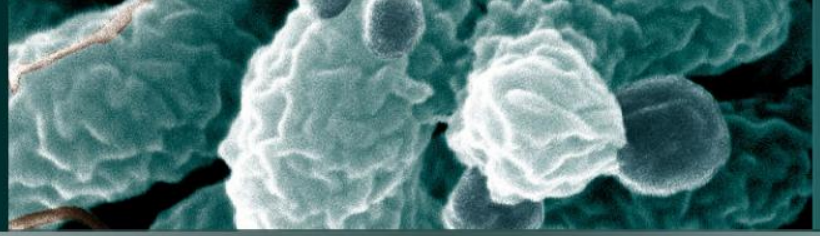
1. Bacterial strains and culture
2. Gram negative bacterial toggle-cell SELEX
3. Binding enrichment test using quantitative real-time PCR
4. Fluorescence-based binding assays for aptamers
5. Isolation and characterizations of OMVs
6. Aptamer-based direct OMVs detection



Bacterial strains and culture

- All bacteria were purchased from the Korean Collection for Type Culture (KCTC, Korea). *E. coli DH5α, E. coli K12* were cultivated at 37 °C in LB medium.
- *Serratia marcescens* were cultivated at 30 °C in NB medium.

- followed by centrifugation (10,000 rpm) for 10 min at 4 °C, and washing twice with Tris-HCl buffer and the washed bacteria were resuspended in binding buffer.

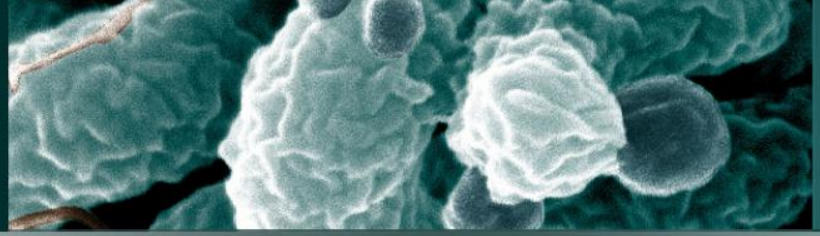


Gram negative bacterial toggle-cell SELEX

- All oligonucleotides were purchased from Integrated DNA technologies (**Coralville, USA**). The single-stranded DNA library consisted of an N40 randomized region flanked by two **18-nt** primer-binding regions for PCR (5′-ATA CCA GCT TAT TCA ATT **N40**-AGA TAG TAA GTG CAA TCT-3′).

- The following forward primer (5′-ATA CCA GCT TAT TCA ATT-3) and reverse primer (5′-AGA TTG CAC TTA CTA TCT-3′) were used for PCR.

- The reverse primer modified with poly-A tail overhang (5′-AAA AAA AAAAAAAAAAAA AA/iSp9//iSp9/AGA TTG CAC TTA CTA TCT3′) was used for PCR in SELEX process.



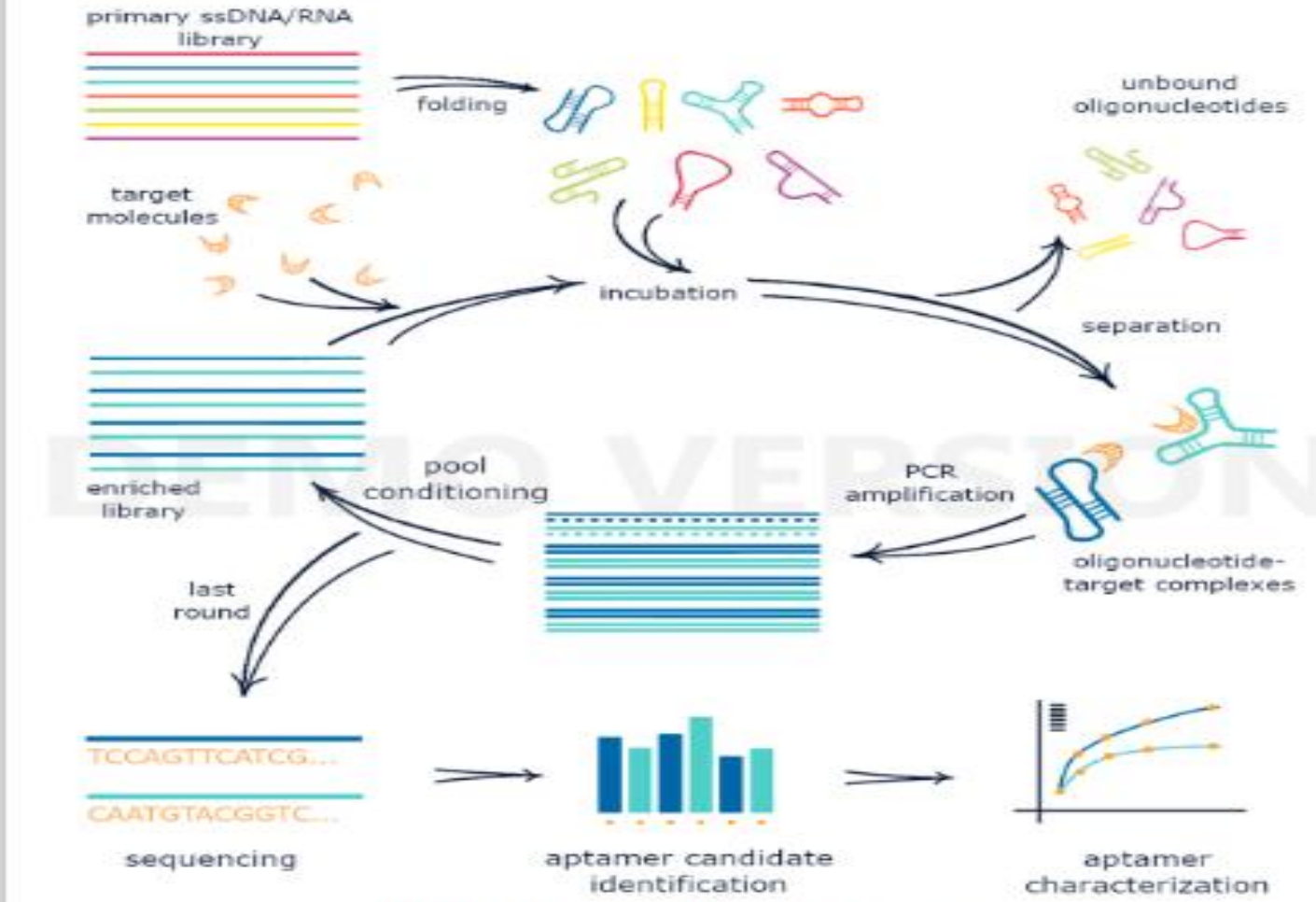
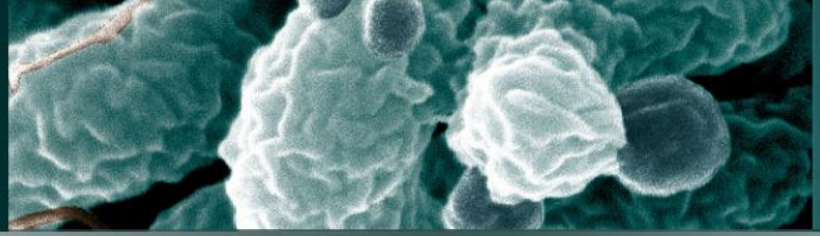


Figure 1. Illustration of the SELEX procedure.

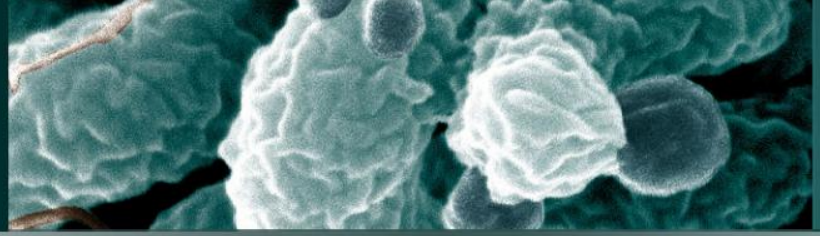


SELEX

- To separate the interested ssDNA, gel purifications in UREA-PAGE gel (10%) were performed using asymmetric poly-A tailed reverse primer.

- For the next round of selection 100 pmol of ssDNA from the firs round was mixed with 10^8 cells of *E. coli K12*. and *S. marcescen* the following procedure.

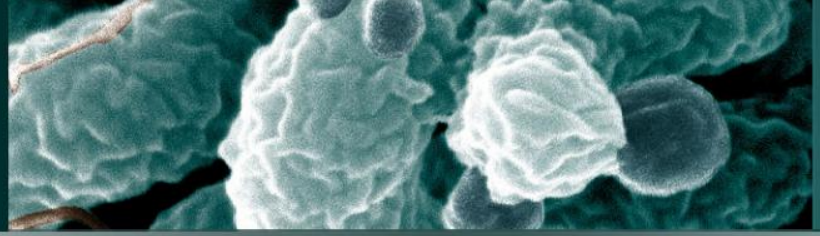
- Two aptamers selected, GN6 and GN12.



Binding enrichment test using quantitative real-time PCR

- The following forward primer (5′-ATA CCA GCT TAT TCA ATT-3) and reverse primer (5′-AGA TTG CAC TTA CTA TCT-3′) were used for quantitative real-time PCR.

- The standard controls were made by serial dilution of samples. Each ssDNA sample was mixed with 0.2 μM of forward, reverse 18-nt primer and SYBR® Premix Ex ^{TaqT} .



Fluorescence-based binding assays for aptamers

- The binding affinity and capacity to bacteria were quantified by binding 3′-FAM (carboxyfluorescein) at th labelled aptamers to the bacterial cells. For measuring the dissociation constants, 10^8 cells of bacterial species were bound to different aptamer concentrations at RT for 15 min.

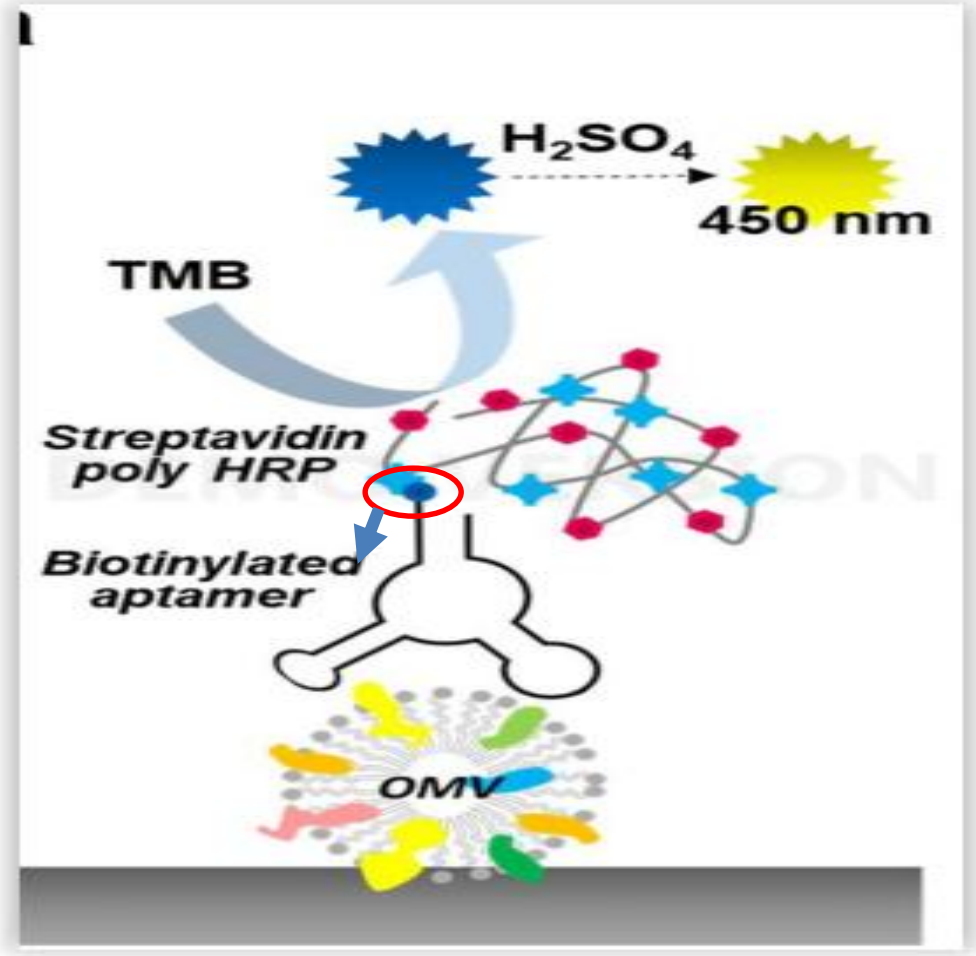
- Their fluorescence intensity was measured using VICTOR X2 Multilabel Plate Reader.

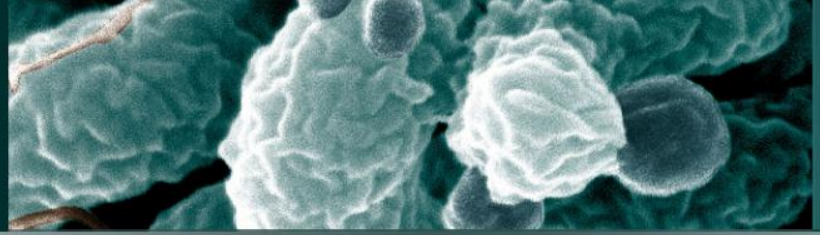
- The binding efficiency or capacity of the two selected aptamers at 250 nM concentration was measured by incubating 10^8 and 10^5 cells of bacteria. It was also compared with that of 3′-FAM-labeled N40 random ssDNA upon incubation with 10^8 and 10^5 cells of bacteria.

Aptamer-based direct OMVs detection

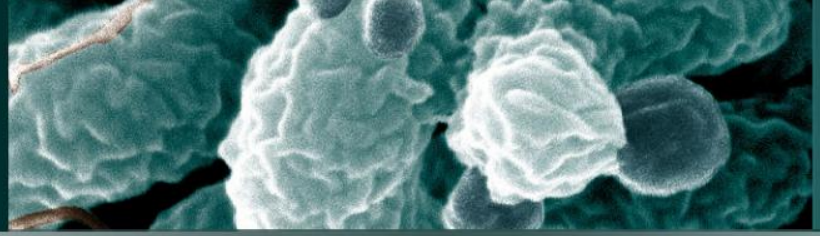
GN6 ELAA (Enzyme-linked aptamer assay)

- **TMB**: Tetramethylbenzidine
- **HRP**: Horseradish peroxidase





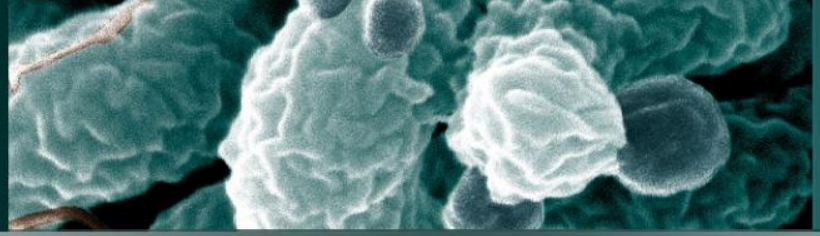
❑ Results



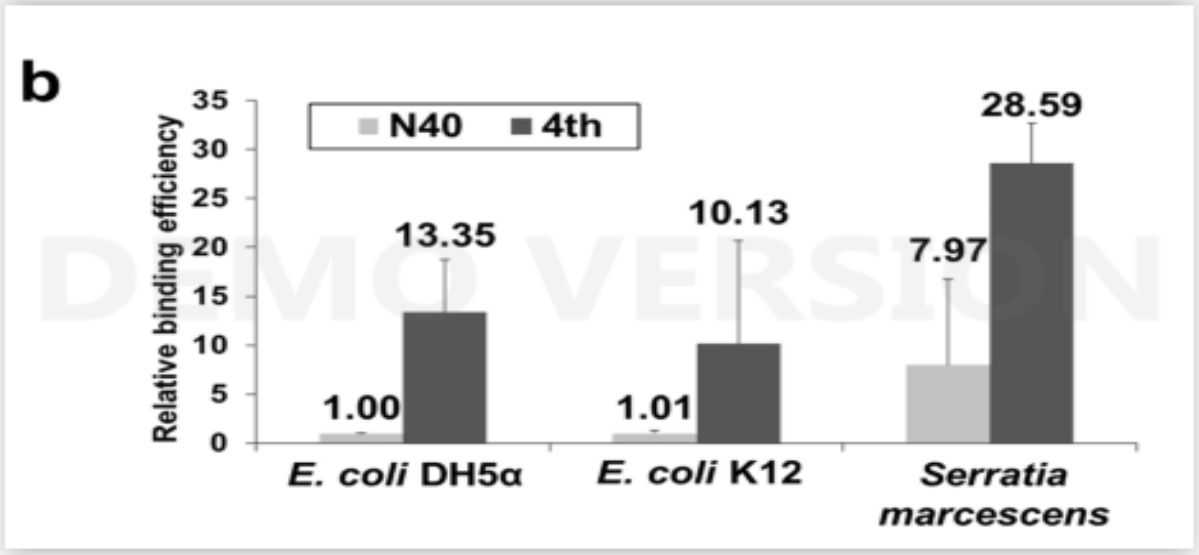
Gram-negative bacterial toggle-cell SELEX.

ID	DNA sequence (5′ to 3′)
GN6	ATA CCA GCT TAT TCA ATT GGG TGA GGG GGG GTT CAC AAC GTT AAA GAT AGA CGG GGG AAG ATA GTA AGT GCA ATC T
GN12	ATA CCA GCT TAT TCA ATT CCG AGT CCA GAC TCA CCG CCG CCT CCT CAA GAC GTG CTG GAG ATA GTA AGT GCA ATC T

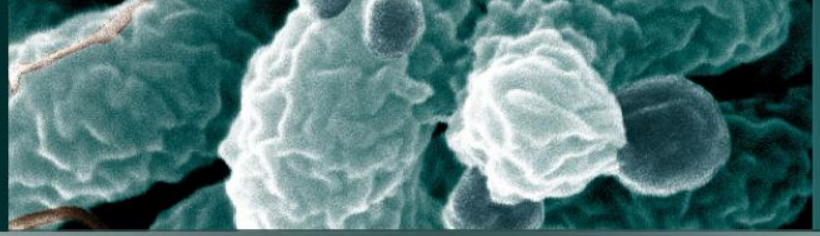
Table 1. ssDNA sequences of two isolated aptamers after Gram-negative bacterial Toggle-cell SELEX.



Binding enrichment test using quantitative real-time PCR



This toggle loop was repeated 4 times until the relative amount of binding, measured using quantitative real-time PCR, of DNA aptamers mixtures was 13.35-, 10.13- and 28.59- times **higher than** that of **the starting DNA library** to three bacteria, respectively.

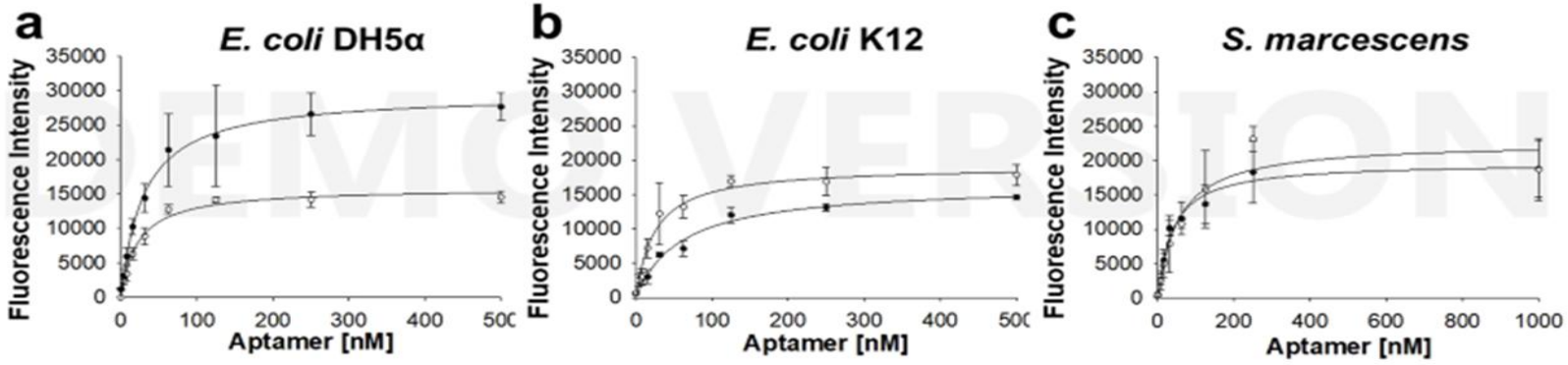


Binding affinity of isolated aptamers

- Various concentrations of aptamers labelled with FAM (carboxyfluorescein) at the 3′-end were added to 10^8 cells of bacteria: S = Bmax × C/(Kd + C)

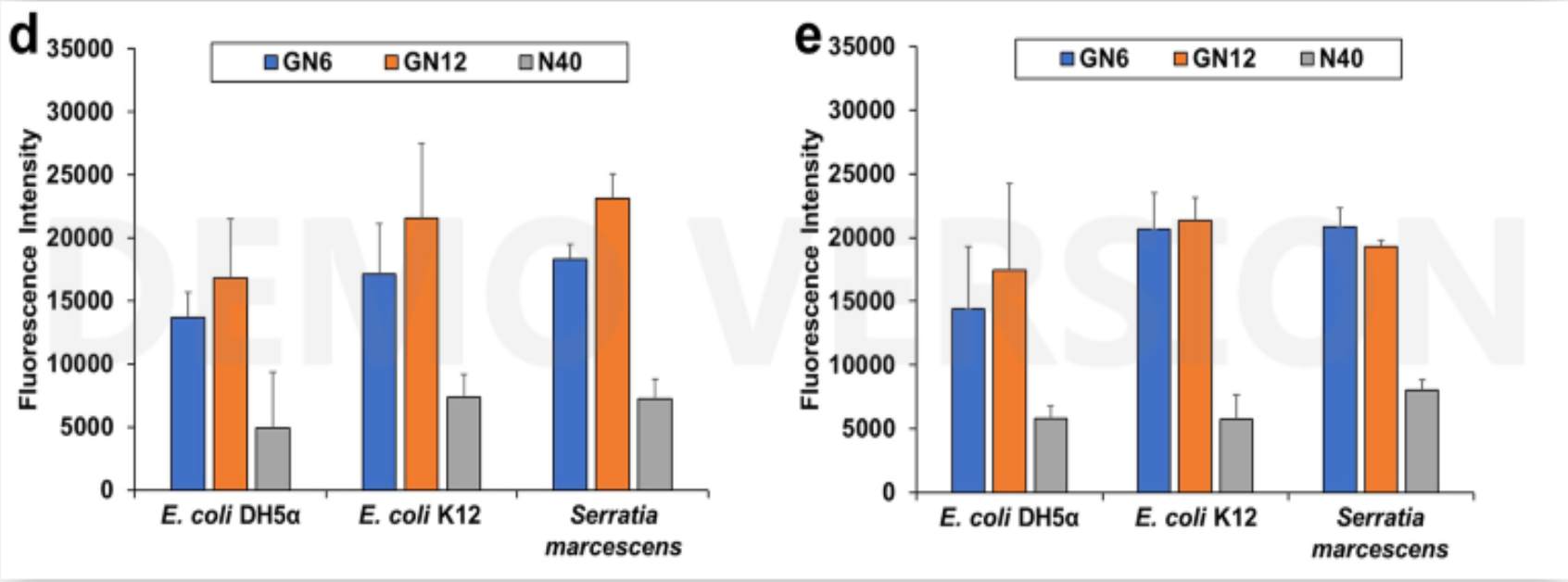
Aptamer	*E. coli* DH5α	*E. coli* K12	*S. marcescens*
GN6	29.94 ± 2.49 nM	59.70 ± 10.89 nM	38.98 ± 6.46 nM
GN12	20.36 ± 2.38 nM	24.80 ± 3.98 nM	53.83 ± 17.70 nM

Table 2. Dissociation constants (K_d) of GN6 and GN12 aptamers against three bacteria.

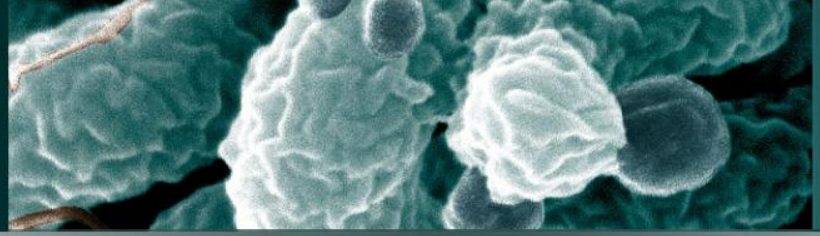


Binding affinity of isolated aptamers

- Both **GN6** and **GN12** aptamers showed average 2.55- and 3.19-times higher binding towards **10^8** cells of bacteria than N40 random sequence. Likewise, it showed average 2.89- and 3.03-times higher binding to **10^5** cells of bacteria than the same control.



(d) 10^8 cells and (e) 10^5 cells of bacteria were incubated with the two selected aptamers at 250 nM concentration and N40 library as control



Aptamer cross-reactivity towards multiple Gram-negative bacteria.

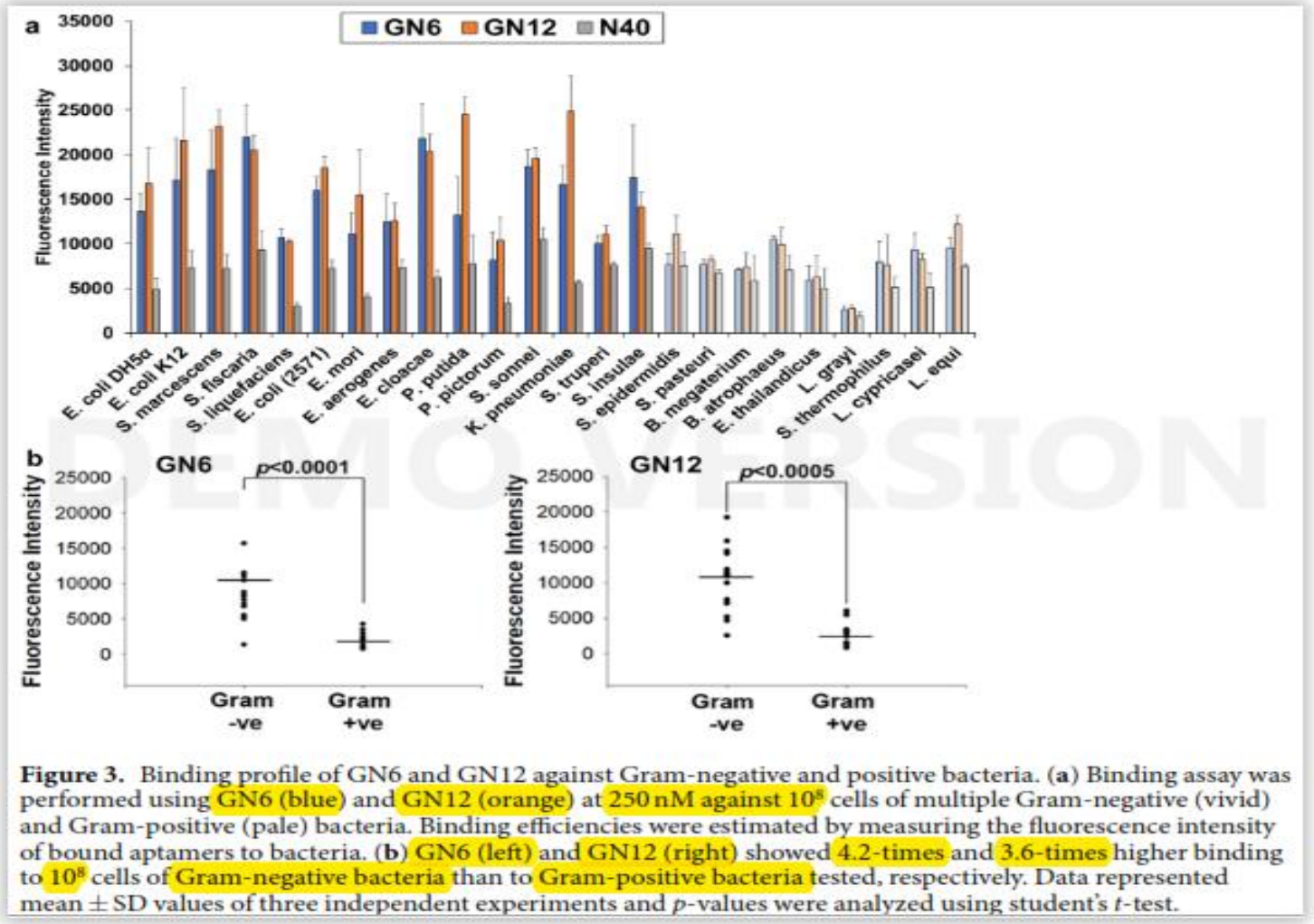
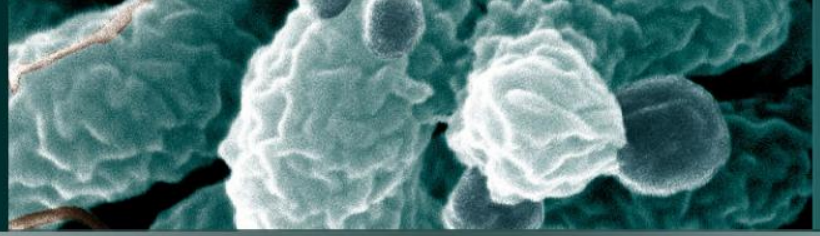


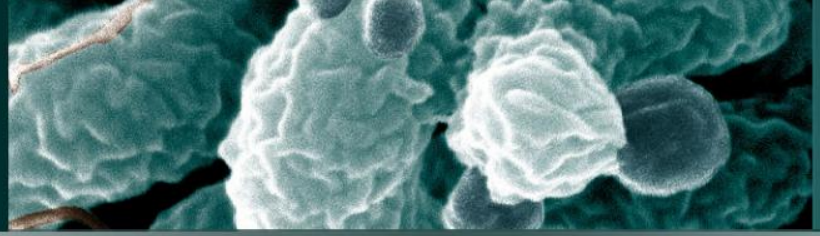
Figure 3. Binding profile of GN6 and GN12 against Gram-negative and positive bacteria. (**a**) Binding assay was performed using GN6 (blue) and GN12 (orange) at 250 nM against 10^8 cells of multiple Gram-negative (vivid) and Gram-positive (pale) bacteria. Binding efficiencies were estimated by measuring the fluorescence intensity of bound aptamers to bacteria. (**b**) GN6 (left) and GN12 (right) showed 4.2-times and 3.6-times higher binding to 10^8 cells of Gram-negative bacteria than to Gram-positive bacteria tested, respectively. Data represented mean ± SD values of three independent experiments and *p*-values were analyzed using student's *t*-test.



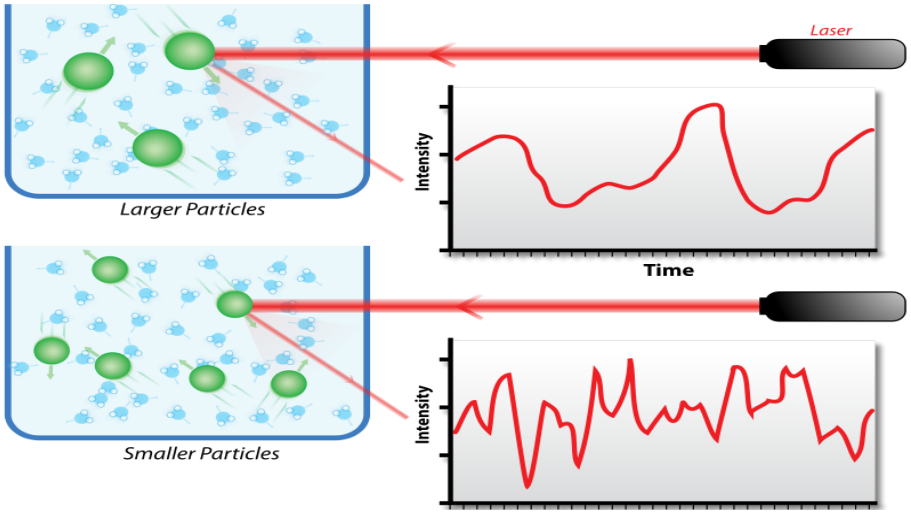
Characterizations of bacterial OMVs.

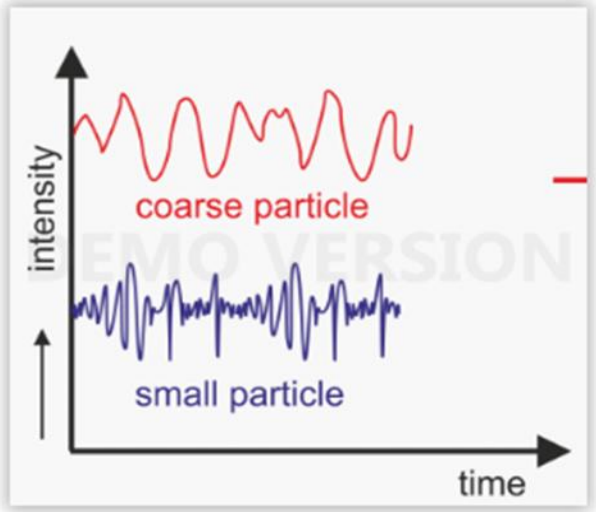
- DLS analysis exhibited the size distribution of OMVs ranging from 84.29 to 176.4 nm.

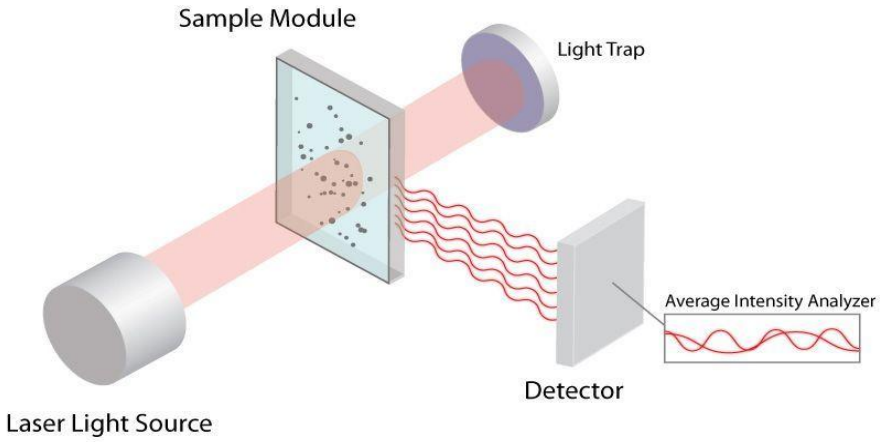
	Z-average (diameter, nm)
E. coli DH5α	105.9
E. coli K12	97.96
S. marcescens	164.4

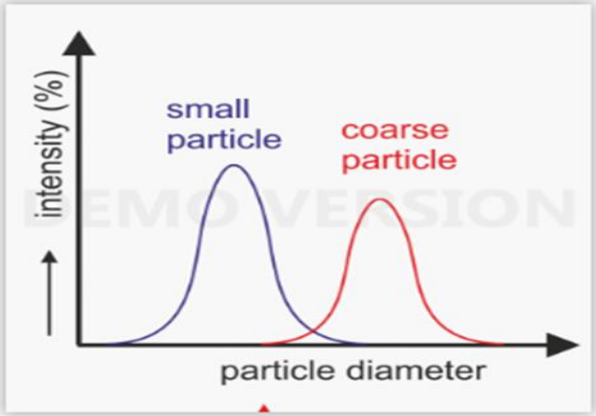


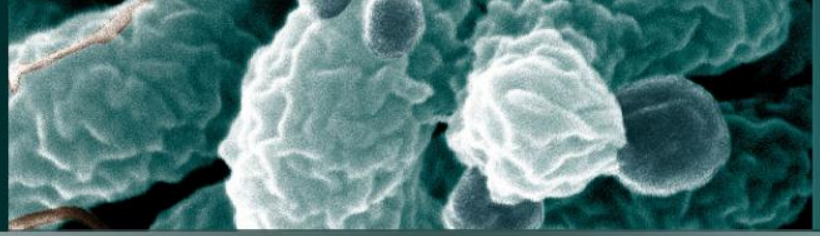
Dynamic light scattering (DLS)











ELAA platform for detecting Gram-negative bacterial OMVs

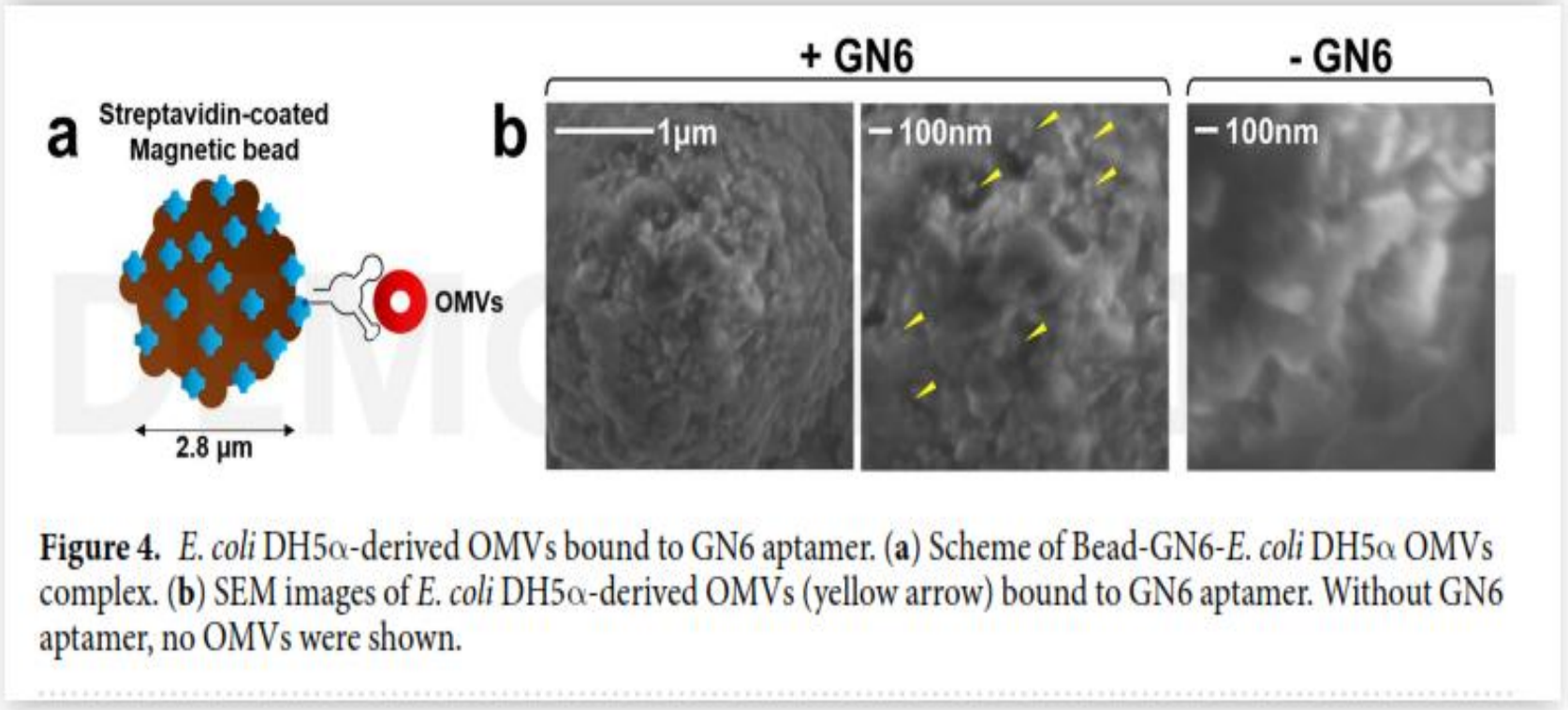
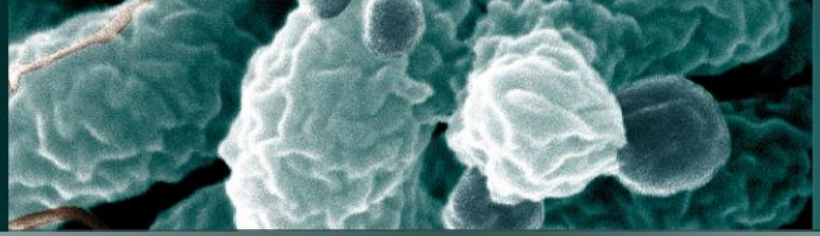
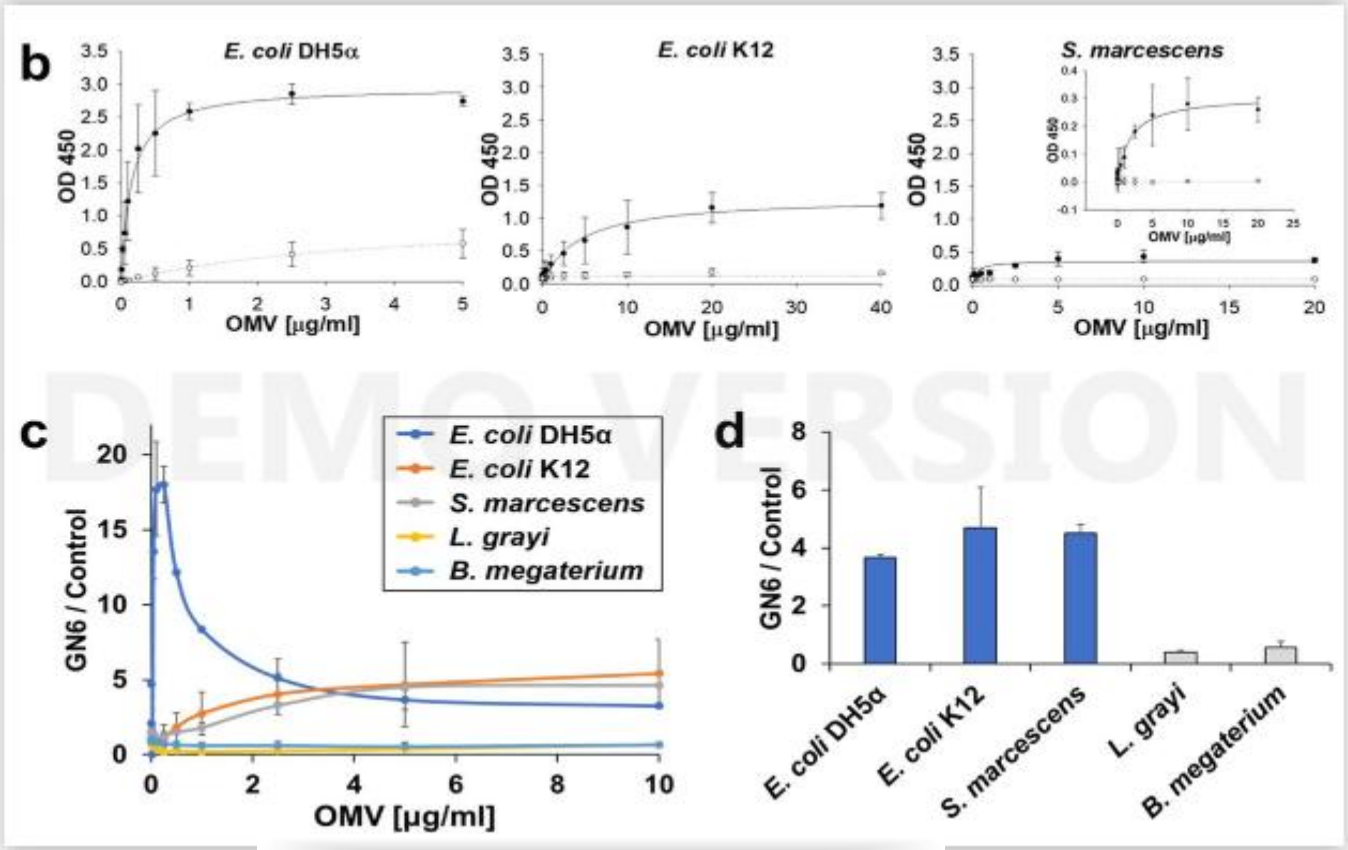


Figure 4. *E. coli* DH5α-derived OMVs bound to GN6 aptamer. (**a**) Scheme of Bead-GN6-*E. coli* DH5α OMVs complex. (**b**) SEM images of *E. coli* DH5α-derived OMVs (yellow arrow) bound to GN6 aptamer. Without GN6 aptamer, no OMVs were shown.

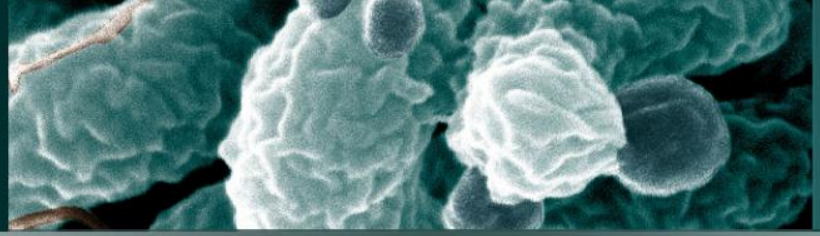
This GN6-ELAA platform showed high sensitivity to detect as low as **25 ng/Ml** bacterial OMVs.





GN6-ELAA for Gram-negative bacterial OMVs

(**b**) Three Gram-negative bacterial OMVs showed highly sensitive binding to GN6 aptamer (●) rather than N40 control (○) at 250 nM concentration. (**c**) GN6 ELAA towards both Gram-negative and Gram-positive bacterial OMVs. (**d**) GN6 ELAA showed higher specificity to 5 μg/mL of Gram-negative bacterial OMVs compared to 5 μg/mL of Gram-positive bacterial OMVs from *L. grayi* and *B. megaterium*. Data represented mean ± SD values of three independent experiments.



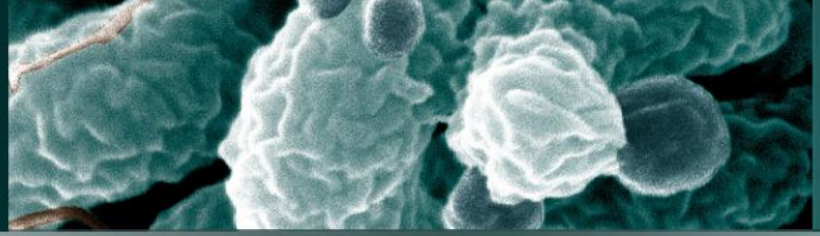
Conclusion

- Here, we developed highly specific DNA aptamers, GN6 and GN12, against many Gram-negative bacteria, including pathogenic strains.

- Using GN6 aptamer, we developed an GN6-ELAA to detect Gram-negative bacterial OMVs from cell-free supernatant.

- the GN6-ELAA had high sensitivity to low concentration of Gram-negative
- bacterial OMVs and high specificity exclusively bound to them.

- We believe that the aptamer-based Gram-negative bacterial OMV detection has a great potential to facilitate medical diagnosis and early detection of bacterial terrorism.



موفق باشید

پاسگزارم