APTAMER SELECTION THROUGH MAGNETIC BEADS-BASED SELEX TECHNOLOGY FOR GLYCOPEPTIDE ANTIBIOTIC

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Introduction

Aptamers are short single-stranded DNA or RNA oligonucleotides that bind to a specific target molecule, reproducing antibodies' role while improving the functional effect. Aptamers are obtained via an *in vitro* chemical process named as systematic evolution of ligands by exponential enrichment (SELEX). The SELEX technology achieved high improvements using magnetic-beads for the aptamer-target molecule selection.

Vancomycin (Van) is a powerful glycopeptide antibiotic, which can be toxic in high doses to renal and auditive systems, but also at low doses can cause hypersensitivity reactions. It is critical to measure with as high as possible accuracy the concentration of vancomycin from biological and environmental samples, having the aim to improve the patient compliance to treatment and to overcome the multi-antibiotic resistance issue.

Strategy

Selection of aptamers for vancomycin and its detection in biological and environmental samples

Functionalization of the magnetic beads modified with carboxylic acid with vancomycin (MBsVan) → **Interaction of MBsVan with the DNA pool and PCR procedure** → **Cloning and sequencing** → **Selection of the aptamer** → **Binding constant evaluation by SPR measurement** → **Development of an aptasensor for vancomycin detection**

Magnetic-beads based SELEX technology

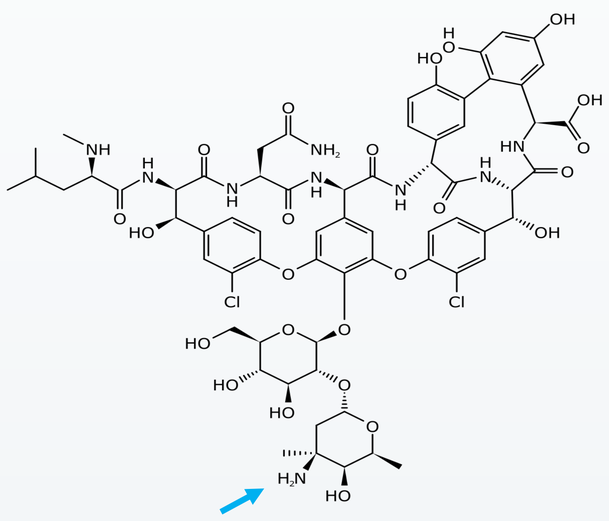


Figure 1. Chemical structure of vancomycin and covalent binding site to MBs

PCR conditions: Initial step of 120 s at 95°C; second step: 95°C, 15 s; 60°C, 30 s; 72°C, 45 s; followed by holding at 72°C for 120 s.

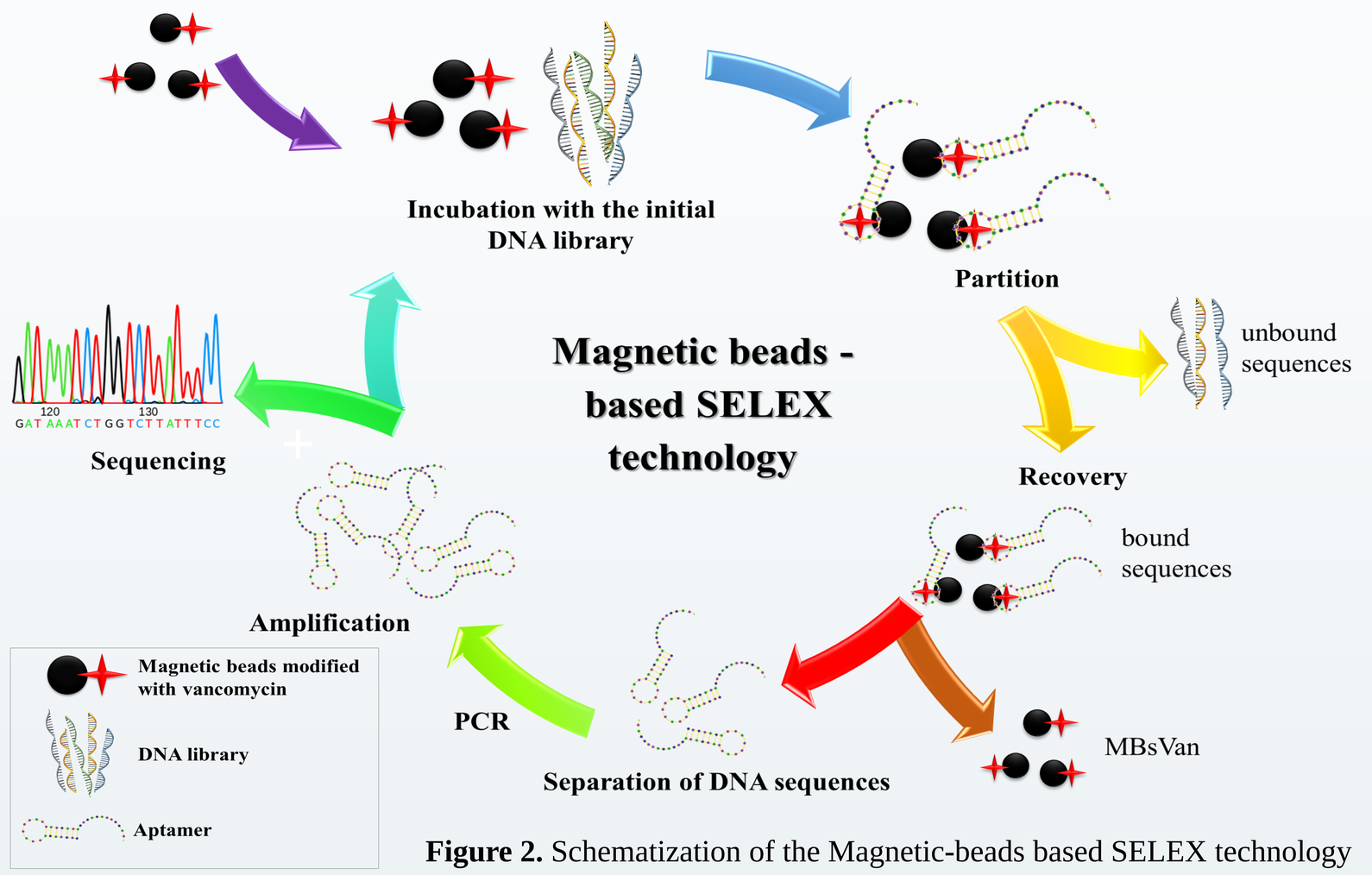
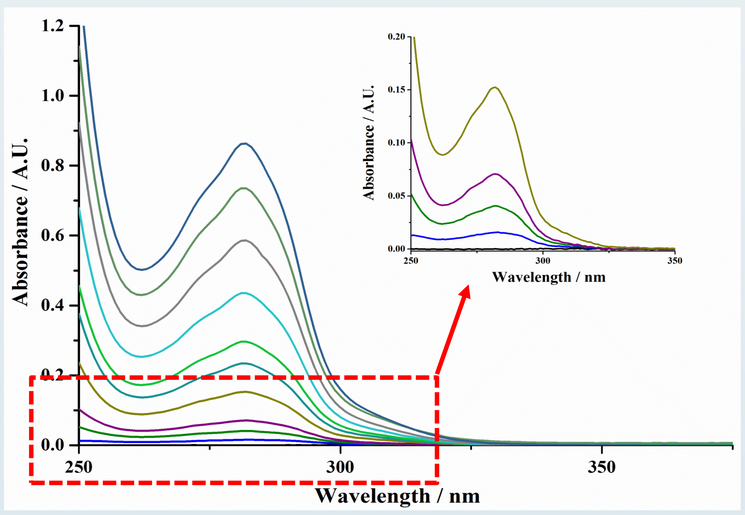


Figure 2. Schematization of the Magnetic-beads based SELEX technology

Preliminary results

Optimization of the functionalization of MBs-COOH with vancomycin

Vancomycin hydrochloride shows a maximum absorbance peak at 282 nm in HEPES buffer, pH 8.3. A regression equation was constructed in the range between 1 – 150 µM: A (u.a.) = 0.0057*[Van/µM] + 0.0108, R^2 = 0.9998.



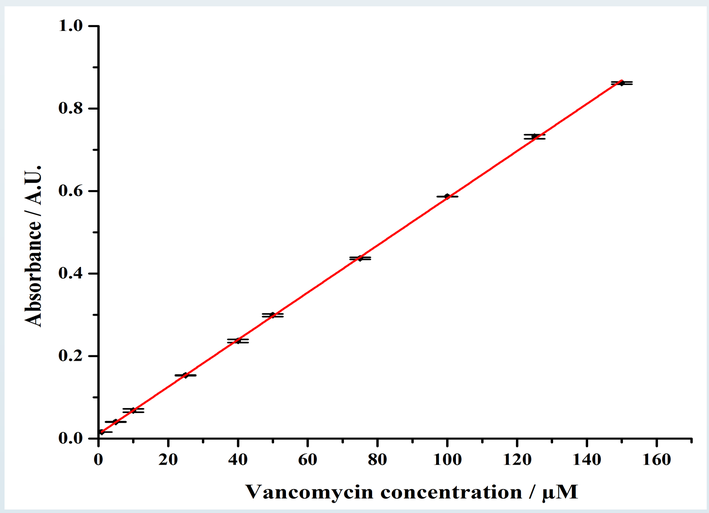


Figure 3. UV absorption spectra of vancomycin hydrochloride at different concentrations: blank (**blank**); 1µM (**blue**); 5µM (**olive**); 10µM (**purple**); 25µM (**dark yellow**); 40µM (**dark teal**); 50µM (**green**); 75µM (**turquoise**); 100µM (**gray**); 125µM (**green**); 150µM (**dark blue**). Test were performed in triplicate.

Table 1. Optimization of the vancomycin hydrochloride concentration immobilized at the MBs-COOH surface. Measurements were recorded by UV determinations at 282 nm in HEPES buffer, pH 8.3 (*$n=3$).

Added	Found (mM)	Unbound* (%)	Bound* (%)
40 mM	36.31	90.77 ± 0.54	9.00 ± 0.54
10 mM	8.86	88.60 ± 1.10	11.40 ± 1.10
2.5 mM	2.42	96.73 ± 0.06	3.27 ± 0.06

Conclusions and future perspective

- ✓ Optimization of all steps involved in the Magnetic beads - based SELEX technology is envisaged to obtain an aptamer for vancomycin with a low dissociation constant (Kd in the nM range);
- ✓ The final goal of the study is to develop an aptasensor for the sensitive detection of vancomycin in biological and environmental samples.

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