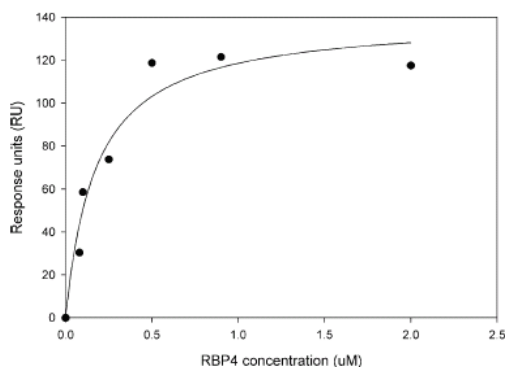


**Reference:** Su Jin Lee, Byung-Soo Youn, Ji Woo Park, Javed H. Niazi, Yeon Seok Kim, and Man Bock Gu, "ssDNA Aptamer-Based Surface Plasmon Resonance Biosensor for the Detection of Retinol Binding Protein 4 for the Early Diagnosis of Type 2 Diabetes", *Analytical Chemistry*, 2008, 80, 2867-2873.

**Bioanalysis and Sample:** A SPR biosensor based on ssDNA aptamer to specifically bind retinol binding protein 4 (RBP4) with high affinity is developed. The authors firstly employ Flu-mag SELEX to select the desired aptamer from the random oligonucleotide library ( $3 \times 10^{16}$  DNA molecules). The aptamer with high affinity and specificity is cloned in *Escherichia coli* TOP10; sequenced and secondary structure analyzed by free-energy minimization algorithm. SPR assay and  $K_d$  determination by interaction of immobilized biotinylated ssDNA aptamer on streptavidin coated gold chip and RBP4 in artificial serum sample show properties over traditional ELISA and Western blot assay.

**Importance:** Insulin resistance is correlated with a wide range of disease as cardiovascular disease, mortality and most commonly type 2 diabetes. The level of RBP4 in serum of insulin resistant human subjects increases even before the diabetes develops. Thus, RBP4 is a useful biomarker in diagnosing and monitoring type 2 diabetes. However, traditional ELISA and Western blot analysis is limited in terms of dynamic range, saturation problem, slow response, etc. The novel ssDNA aptamer-based SPR biosensor shows an improvement in sensitivity, response time, and most importantly, a non-antibody-based system.

**Technique:** In Flu-mag SELEX, magnetic beads are used to immobilize RBP4 and then incubated with DNA library. The unbound DNA sequences are eluted away while the binding ones are collected later for PCR and purification, and then the new pool of DNA sequences are used as library in next selection. Fluorescein is used to partially label DNA for further quantification. In SPR assay, the desired DNA is immobilized on the gold chip in appearance of artificial serum sample to detect RBP4. Results are collected in terms of  $K_d$ , dynamic range, sensitivity and response time, which are compared with ELISA and Western blot. The authors also employed counter selection to exclude DNAs bind to both RBP4 and interference proteins in SELEX and SPR studies. Other techniques used here include DNA cloning and purification (Qiagen), structural analysis by free-energy minimization algorithm, etc.



#### Example of Results:

The left plot is the SPR responses with different RBP4 concentrations (average of 3 for each data). This assay is carried out with fixed amount of aptamer while varied concentrations of RBP4. The data points are fitted with a nonlinear regression equation:  $y = B_{\max} (\text{free ssDNA}) / K_d + (\text{free ssDNA})$ .  $B_{\max}$  stands for the number of maximum binding sites,  $y$  is SPR response and  $K_d$  is the dissociation constant. In experiment,  $K_d$  value is determined to be  $0.201 \pm 0.029 \mu\text{M}$ .

**Opinion:** RBP4 studies in this paper are very important to early diagnose and monitor type 2 diabetes. The aptamer selected through Flu-mag SELEX and used in SPR biosensor for RBP4 shows very good specificity (RU 100 compared with 6.5 for BSA) and high affinity ( $K_d = 0.201 \pm 0.029 \mu\text{M}$ ) with a low detection limit (75 nM). Moreover, this assay exploits assay in a field with aptamer rather than antibody-based immunoassay, shows tremendous potential of application of a new category of bio-interaction.