Aptamers are nucleic acid molecules which are in vitro selected to specifically bind to their targets with high affinity. Aptamers are discovered through the SELEX process, which is labor-intensive and time-consuming because it requires multiple rounds (8~15 rounds) of selection and amplification. Previously, we described the advantages of using microfluidics technology to accelerate the aptamer discovery process [1]. In this work, we made significant enhancement to this technique by combining microfluidic selection with a process of kinetic challenge through volume dilution (KCM-SELEX). In this method, we exploit the microfluidic capability to concentrate small quantities of target from a dilute, large-volume sample, and the ability to perform continuous and controlled washing with minimal target loss to exert reproducible selection stringencies. To demonstrate the effectiveness of the KCM-SELEX technique, we used Streptavidin (SA) as a target molecule and report the discovery of novel aptamer sequences that specifically bind to it with low nanomolar affinities with in 3 rounds.

For the selection, a random ssDNA library consisting of ~10^{14} molecules was first incubated with SA-coated magnetic beads (Fig 1A). Then, the incubation volume was highly diluted in order to dissociate the aptamers with fast off-rates from the target (Fig 1B). This diluted mixture was immediately loaded into the MicroMagnetic Separation (MMS) chip to partition the target-bound aptamers from unbound oligos under continuous flow (Fig 1C). After the separation, the beads carrying bound aptamers were eluted from the device by removing external magnets (Fig 1D), and the selected aptamers were PCR-amplified (Fig 1E). From the double stranded amplicons, ssDNA were then generated by lambda exonuclease digestion (LED) (Fig 1F). Finally, the resulting ssDNA pool was purified (Fig 1G) and used for the next round of selection. After three rounds of selection (steps B through G), the enriched aptamer pool showed considerable affinity to the SA target (K_d = 62.5 ± 5.1 nM). Individual sequences obtained by cloning and sequencing through E. coli exhibited low nanomolar affinity (K_d = 35 nM) as well as high specificity. Compared to previous methods, we found that our microfluidic selection methodology is significantly more efficient; for example, Stoltenburg et al. reported the selection of SA binding aptamers in 13 rounds yielding K_d values of ~56-86 nM [2] whereas our method yielded higher affinity aptamers in 3 rounds.

Fig 1. Overview of the selection process. (A) Incubation of the SA-coated beads with ssDNA library in binding buffer. (B) Kinetic challenge by volume dilution to remove weakly bound aptamers. (C) Microfluidic partitioning through continuous washing in the MMS device. (D) Elution of magnetic beads carrying the target-bound aptamers. (E) PCR amplification of selected aptamers with phosphorylated reverse primers. (F) Generation of ssDNA via LED method. (G) Purification of digested ssDNA pool by phenol/chloroform extraction and ethanol precipitation.