**Screening and Isolation of DNA aptamers against Agrochemicals by using PS-SELEX chip**

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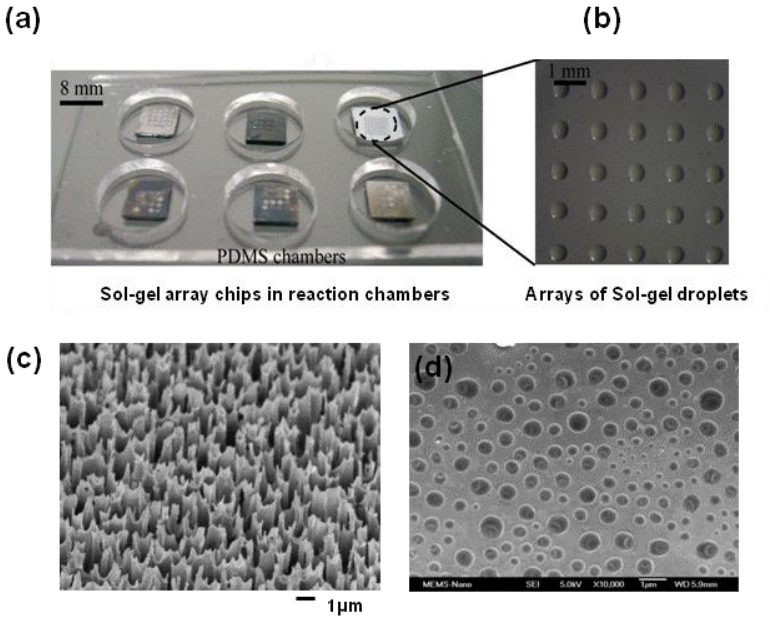
## Abstract

We have described the development of **P**orous **S**ubstrate mediated-**S**ystematic **E**volution of **L**igands by **Ex**ponential Enrichment (referred to as "PS-SELEX") technique. This method allowed us to screen and isolate high specific aptamers against chemical compounds. Azoxystrobin as a target chemical is a fungicide commonly used in agriculture and has water pollution potential. This is generally used as an active agent protecting plants and fruit/vegetables from fungicidal diseases (1). In PS-SELEX, azoxystrobin was immobilized on sol-gel networks. Especially, sol-gel immobilization is not necessary for linkage/coupling agent (2). Moreover, interacting binding materials can enter into along the complicate internal channels of sol-gels and release to outside. For improving an adhesiveness of sol-gel microdroplets on the substrate, the porous silicon substrate was newly modified in this study (Figure 1). The aptamer pools eluted from the $5^{th}$ selection rounds were cloned and individual clones were sequenced. Identical DNA aptamer pairs were classified and we finally choose the two aptamer species, Azo 5-3 and Azo 5-6 (Table 1), and analysis of the secondary structure of the isolated aptamers was performed with a free energy minimization algorithm using the *Mfold* program (3). In contrast to traditional chemical SELEX, our strategy provides the following advantages: Simple immobilization of chemical compounds, Easy to handle of aptamers in SELEX process, Decrease the non-specific bind to chip surface. Our data demonstrate that the sol-gel is a convenient partitioning and simplified retrieval method in PS-SELEX process, and isolated aptamer hold great promise for capturing pesticides as a high sensitive biosensing probe.

## References

[1] Ji-Young Ahn, Analytical Chemistry, **84** (2012) 2647-2653
[2] Ji-Young Ahn, Oligonucleotides, **21** (2011) 93-100
[3] http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/dna-form1.cgi

**Figure 1.**




PS-SELEX was performed on the small dice of porous silicon substrate. Azoxystrobin contained sol-gels were spotted on the porous chip surface. Considering the 10 to 1 ratio between the number of random ssDNA pools and target chemicals, totally 12 pmole of azoxystrobin were participated in a single round. After stable gelation of sol-gels, 120 pmole of random ssDNA pools were applied to sol-gel integrated chip. Aptamer binders were collected by heat, amplified and regenerated to ssDNA for next round SELEX. (a) assay chamber, (b) sol-gel droplets, (c) SEM image of the PS chip surface, (d) SEM image of sol-gels.

**Table 1. Sequence of isolated DNA aptamers**

| Identity | Sequence | Homology |
|---|---|---|
| Azo4-18 | -GCCAATCGGCCAAGTCCTGTCTATGCAGCCTGCATCCCT- | |
| Azo4-9 | GTTCGATCGGGTTAATGCT-CCTATGAAGGTGCCAACGCTG | 55% |
| | * ***** * * ** ***** ** ** * ** | |
| Azo4-31 | CTAAGTAGGGGAC-GTCGGACATCACC--TTTCAAATTACCC | |
| Azo4-23 | CGAATCATCGATTTGTTCGTTCTCTTCCGTTTCAAATTAC-- | 55% |
| | * ** * * ** * ** * ********** | |
| Azo4-18 | -GCCAATCGGCCAAGTCCTGTCTATGAAGCCTGCATCCCT- | |
| Azo4-9 | GTTCGATCGGGTTAATGCT-CCTATGAAGGTGCCAACGCTG | 58% |
| | * ***** * * ** ******** ** * ** | |
| Azo4-11 | TGTTATGATGCACTAGCACATCACACGAC-ACGAGCTAATG | |
| Azo4-24 | -ATAATGCGATATTAGCTCATGGGATCACCACGAGCATGTG | 58% |
| | * *** * **** *** * ** ****** ** | |
| Azo4-8 | -----GGCCAATCTGTCATTGCGTCGCGAGTCGAAGGTGAGGGGG | |
| Azo4-1 | TCCAAGGCCA--CTGTCATTGCGTCCCGAGTCGAAGGTGAGG--- | 85% |
| | ***** ********** ********** | |
| Azo5-30 | TCGGTTAGGGGGCTTCGGTTAGGGGGCTCAATCTAATCGA | |
| Azo5-38 | TCGGTTAGGGGGCTTAG---AAGCCGCGCGGTGTAGCCTGCGG | 63% |
| | ********** * * * ** * * ** * | |
| Azo5-3 | GGCTTTATTTCGCCCACACGCAGCTTTTGTAACGGGTCGC- | |
| Azo5-29 | GCTTTATTTCGCCCACACGCAGCTTTTGTAACGGGTCGCT | 98% |
| | ************************************** | |
| Azo5-6 | TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG | |
| Azo5-7 | TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG | |
| Azo5-33 | TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG | 100% |
| Azo5-34 | TGTTTGTCGGCTTCTACTCTAATTTAAAGGCCCATCATCG | |
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