Electronic Supplementary Material (ESI) for Analyst.

Supplementary Material

Rapid and sensitive detection of nodularin-R in water by a label-free BLI aptasensor

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1. Supporting figures

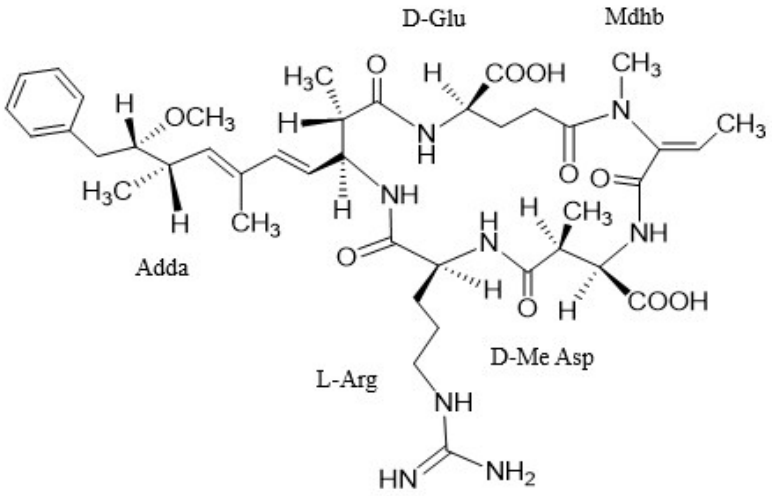
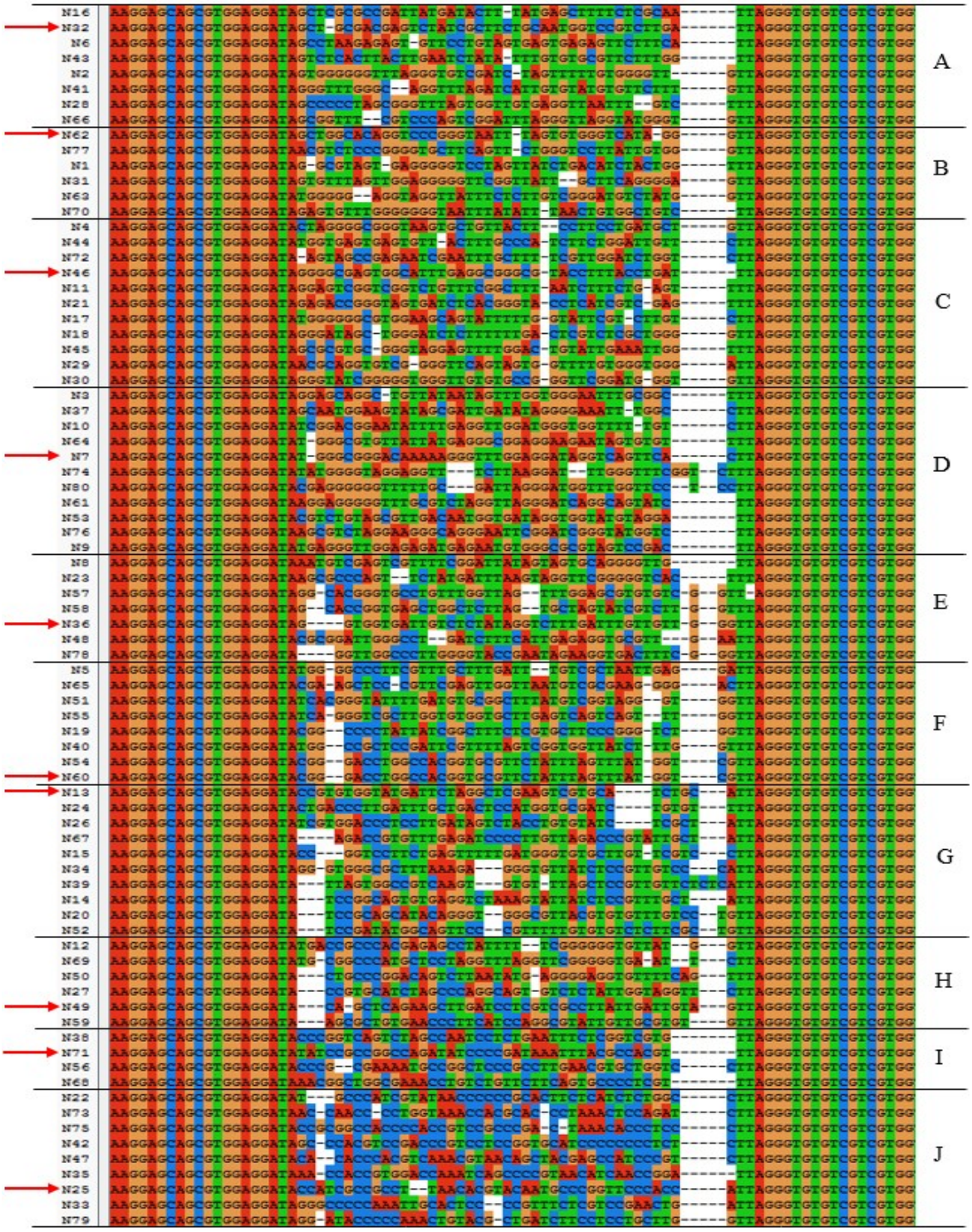


Fig. S1. Chemical structure of NOD-R. NOD-R is composed of (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda), D-glutamic acid (D-Glu), 2-methylamino-2-dehy-drobutyric acid.(Mdhb), D-erythro-b-methy-



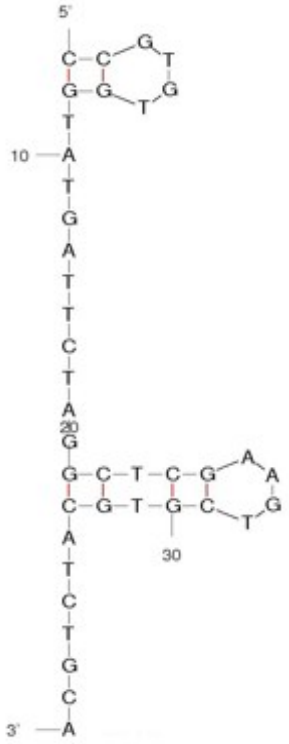
laspartic acid (D-Me Asp) and L-arginine (L-Arg).

Fig. S2. Multiple sequence alignment of sequences acquired using MB-SELEX by Clustal X 2.1 software. These sequences were grouped based on conservation into 10 families (A-J), and a representative sequence was chosen from each group for further

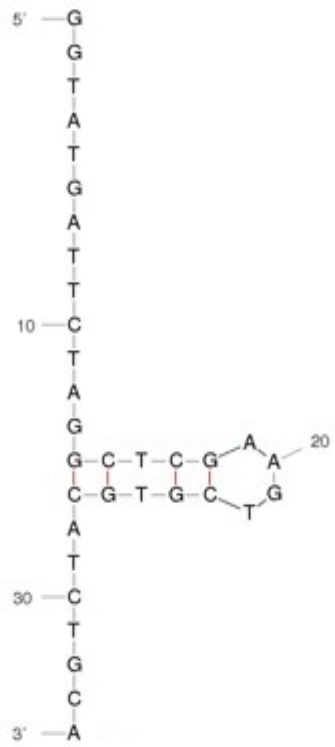
analysis (N32, N62, N46, N7, N36, N60, N13, N49, N71 and N25).

A

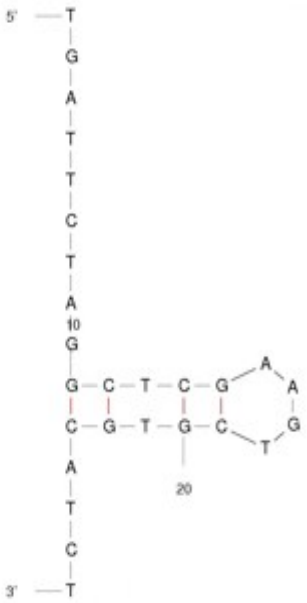
B



C



D



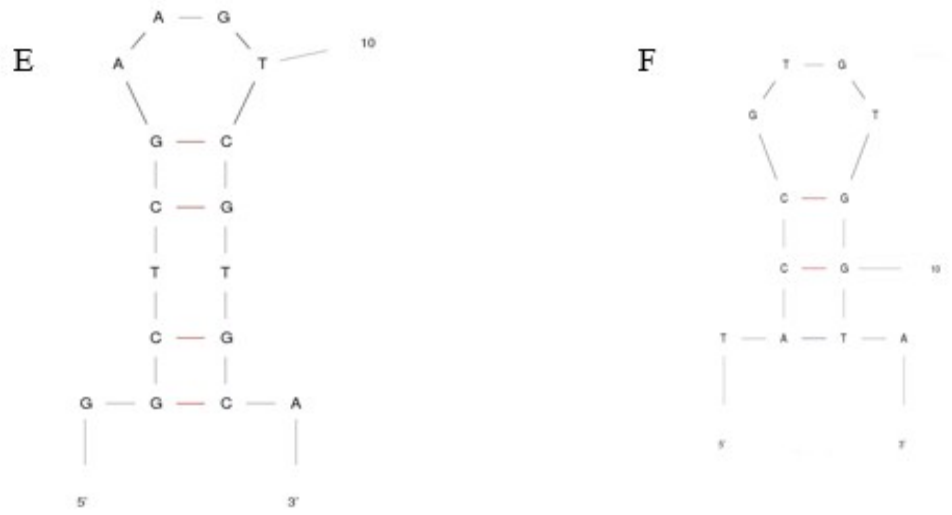


Fig. S3. Secondary structure of truncated sequences for aptamer N13 (A) N13, (B) N13-T, (C) N13-T-O1, (D) N13-T-O2, (E) N13-T-O3, (F) N13-T-O4.

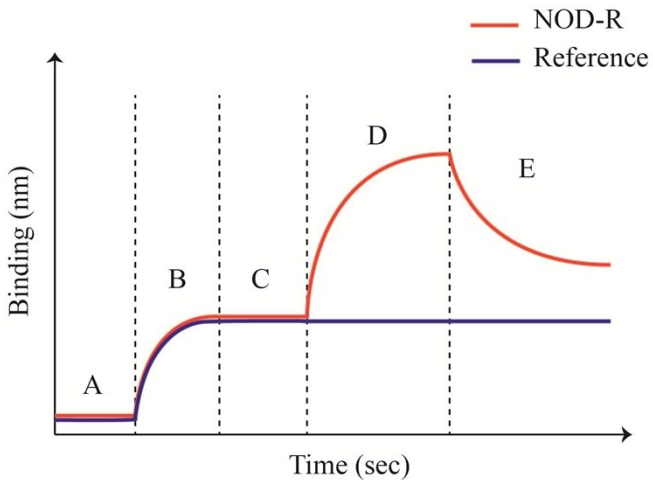


Fig. S4. BLI system assay process included five steps: A baseline; B loading; C washing; D association; E dissociation. A reference sensor is always required as a control in every assay.

2. Supporting tables

Table S1. Summary of selection protocol for the MB-SELEX processes

Selection round	Amount of ssDNA pool (pmol)	Incubation time for MB-Counter SELEX (min)	Incubation time for MB-Positive SELEX (min)	Wash times after incubation
1	3000	0	120	3
2-5	200	0	120	3
6-8	120	60	90	3
9-10	120	60	90	4
11-12	120	90	60	5

Table S2. Sequences and affinity constants between NOD-R and selected aptamers

Clone no	Family	Aptamer sequence	K_D (μM)*
N32	A	AAGGAGCAGCGTGGAGGATAGCTGCGACGAGTCTATCGCTTC TGCAATGGTCCGTCTTGATTAGGGTGTGTCGTCGTGGT	2.78
N62	B	AAGGAGCAGCGTGGAGGATAGCTGGCACAGGTCCCGGGTAAT TTAGTGTGGGTCATAGGGTTAGGGTGTGTCGTCGTGGT	14.3
N46	C	AAGGAGCAGCGTGGAGGATAGGGGCGAGTGGCATTTGAGGCG GGCGTACCTTTACCTGATTTAGGGTGTGTCGTCGTGGT	NB
N7	D	AAGGAGCAGCGTGGAGGATATGGGCGGGACAAAAAGGGTTTG GAGGATAGGTCAGTTCACTTAGGGTGTGTCGTCGTGGT	0.201
N36	E	AAGGAGCAGCGTGGAGGATAGGTGGTGATTGTCTCTATAGGT CTTTGATTTGTTGTTGGGTTAGGGTGTGTCGTCGTGGT	0.334
N60	F	AAGGAGCAGCGTGGAGGATACGGGACCTGGCCACGGTGCGTT CTATTTAGTTTATGGTCGTTAGGGTGTGTCGTCGTGGT	NB
N13	G	AAGGAGCAGCGTGGAGGATACCGTGTGGTATGATTCTAGGCT CGAAGTCGTGCATCTGCATTAGGGTGTGTCGTCGTGGT	0.138

N49	H	AAGGAGCAGCGTGGAGGATACAGCTCAGAAGCTTGATCCTCG TCGCCTTATTGATTGTAGTTAGGGTGTGTCGTCGTGGT	6.67
N71	I	AAGGAGCAGCGTGGAGGATATATCCGCCGGCCAGATATCCCC GATAAATTTACGCCACGTTTAGGGTGTGTCGTCGTGGT	0.193
N25	J	AAGGAGCAGCGTGGAGGATACCATCGCCGCCTTAACACGTAC AATGCCCGGTTCCCACCATTAGGGTGTGTCGTCGTGGT	NB

*NB: No Binding.

Table S3. Sequences and affinity constants between NOD-R and optimized aptamers

Name	Sequence	K_D (nM) *
N13	AAGGAGCAGCGTGGAGGATACCGTGTGGTATGATTCTAGGCTCGAAGTC GTGCATCTGCATTAGGGTGTGTCGTCGTGGT	138
N13-T	CCGTGTGGTATGATTCTAGGCTCGAAGTCGTGCATCTGCA	120
N13-T-O1	GGTATGATTCTAGGCTCGAAGTCGTGCATCTGCA	72.7
N13-T-O2	TGATTCTAGGCTCGAAGTCGTGCATCT	29.6
N13-T-O3	GGCTCGAAGTCGTGCA	63.9
N13-T-O4	TACCGTGTGGTA	NB

*NB: No Binding.