Supporting material

Engineering a Nanobody-Alkaline Phosphatase Bifunctional Probe for Enhanced Immunoassay Performance toward Microcystin-LR

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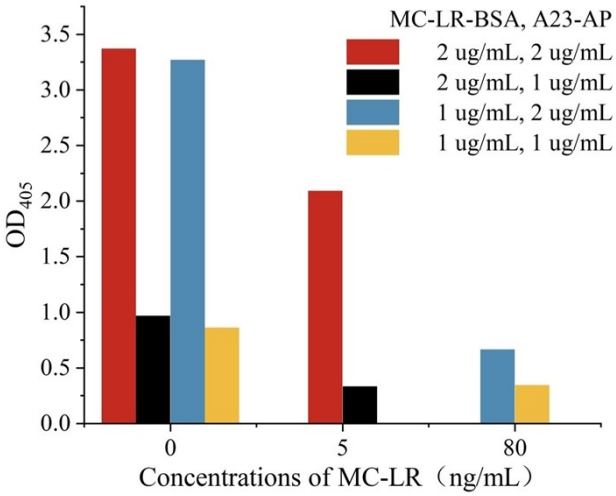

Figure.S1 The identification of the coating antigen (MC-LR-BSA)

Table S1. The gene sequences encoding the anti-MC-LR nanobody (A2.3) and alkaline phosphatase (AP)

Name	Gene Sequence
A2.3 gene	CAGGTGACCCTGAAAGAGAGCGGCGGTGGCTTAGTGCAACCGGGCGGCAGCTTACGCCTGAGC TGCGCGGCGAGCGGCGGCATCAGCCGCGTTAATGTGGCCGGCTGGTATCGCCAAGCGCCGGGT CAGCAGCGCGAAATGGTGGCGGTGATTCGCAGCGGCGGCCGCATTAACTATGCGGATTTTGTG AAAGGCCGCTTTACCTTTAGCCGCGATGATGCGAAACAGACCATTTATCTGCAGATGGATAACC TGAAAAGCGAAGATACCGCGGTGTATTATTGCTATGGCAGCCTGCTGGAAACCGGCACCTTTCA GTATCGCGAATATTGGGGTCAGGGGACCCAGGTCACCGTCTCCTCA
AP gene	ACACCAGAAATGCCTGTTCTGGAAAACCGGGCTGCTCAGGGCGATATTACTGCACCCGGCGGT GCTCGCCGTTTAACGGGTGATCAGACTGCCGCTCTGCGTGATTCTCTTAGCGATAAACCTGCAA AAAATATTATTTTGCTGATTGGCGATGGGATGGGGGACTCGGAAATTACTGCCGCACGTAATTA TGCCGAAGGTGCGGGCGGCTTTTTTAAAGGTATAGATGCCTTACCGCTTACCGGGCAATACACT CACTATGCGCTGAATAAAAAAACCGGCAAACCGGACTACGTCACCGACTCGGCTGCATCAGCA ACCGCCTGGTCAACCGGTGTCAAAACCTATAACGGCGCGCTGGGCGTCGATATTCACGAAAAA GATCACCCAACGATTCTGGAAATGGCAAAAGCCGCAGGTCTGGCGACCGGTAACGTTTCTACC GCAGAGTTGCAGGGCGCCACGCCCGCTGCGCTGGTGGCACATGTGACCTCGCGCAAATGCTAC GGTCCGAGCGCGACCAGTGAAAAATGTCCGGGTAACGCTCTGGAAAAAGGCGGAAAAGGATC GATTACCGAACAGCTGCTTAACGCTCGTGCCGACGTTACGCTTGGCGGCGGCGCAAAAACCTTT GCTGAAACGGCAACCGCTGGTGAATGGCAGGGAAAAACGCTGCGTGAACAGGCACAGGCGCG TGGTTATCAGTTGGTGAGCGATGCTGCCTCACTGAATTCGGTGACGGAAGCGAATCAGCAAAA ACCCCTGCTTGGCCTGTTTGCTGACGGCAATATGCCAGTGCGCTGGCTAGGACCGAAAGCAACG TACCATGGCAATATCGATAAGCCCGCAGTCACCTGTACGCCAAATCCGCAACGTAATGACAGTG TACCAACCCTGGCGCAGATGACCGACAAAGCCATTGAATTGTTGAGTAAAAATGAGAAAGGCT TTTTCCTGCAAGTTGAAGGTGCGTCAATCGATAAACAGAATCATGCTGCGAATCCTTGTGGGCA AATTGGCGAGACGGTCGATCTCGATGAAGCCGTACAACGGGCGCTGGAATTCGCTAAAAAGGA GGGTAACACGCTGGTCATAGTCACCGCTGATCACGCCCACGCCAGCCAGATTGTTGCGCCGGAT ACCAAAGCTCCGGGCCTCACCCAGGCGCTAAATACCAAAGATGGCGCAGTGATGGTGATGAGT TACGGGAACTCCGAAGAGGATTCACAAGAACATACCGGCAGTCAGTTGCGTATTGCGGCGTAT GGCCCGCATGCCGCCAATGTTGTTGGACTGACCGACCAGACCGATCTCTTCTACACCATGAAAG CCGCTCTGGGGCTGAAA

Table S2. The amino acid sequence of the A2.3-AP bifunctional probe

Name	Sequence
A2.3-AP bifunctional probe: A2.3 sequence labeled in blue and AP labeled in red	QVTLKESGGGLVQPGGSLRLSCAASGGISRVNVAGWYRQAPGQQREMVAVIR SGGRINYADFVKGRFTFSRDDAKQTIYLQMDNLKSEDTAVYYCYGSLLETGTF QYREYWGQGTQVTVSSKLAAALEGGGGSGGGGSGGGGSTPEMPVLENRAAQ GDITAPGGARRLTGDQTAALRDSLSDKPAKNIILLIGDGMGDSEITAARNYAEG AGGFFKGIDALPLTGQYTHYALNKKTGKPDVTDSAASATAWSTGVKTYNGAL GVDIHEKDHPTILEMAKAAGLATGNVSTAELQGATPAALVAHVTSRKCYGPSA TSEKCPGNALEKGGKGSITEQLLNARADVTLGGGAKTFAETATAGEWQGKTLR EQAQARGYQLVSDAASLNSVTEANQQKPLLGLFADGNMPVRWLGPKATYHGN IDKPAVTCTPNPQRNDSVPTLAQMTDKAIELLSKNEKGFFLQVEGASIDKQNHAA NPCGQIGETVDLDEAVQRALEFAKKEGNTLVIVTADHAHASQIVAPDTKAPGLTQ ALNTKDGAVMVMSYGNSEEDSQEHTGSQLRIAAYGPHAANVVGLTDQTDLFYT MKAALGLKLEIKRASQPELAPEDPEDVEHHHHHH*

Table S3. The optimization of pNPP concentration

pNPP (mg/ml)	A2.3-AP(μg/mL)										Control Group
	5		2.5		1.25		0.6125		0.30625		
	Experimental Group	Ratio	Experimental Group	Ratio	Experimental Group	Ratio	Experimental Group	Ratio	Experimental Group	Ratio	
8	3.0035	12	3.7072	15	3.5764	14	3.0034	12	3.544	14	0.2508
4	3.6912	23	3.7287	23	3.6976	23	3.5722	22	3.1983	20	0.1624
2	3.6612	33	3.7093	33	3.6710	33	3.4793	31	2.9709	27	0.1121
1	3.5412	38	3.3516	36	3.6013	39	3.1876	34	2.5287	27	0.0934
0.5	3.3533	36	2.3576	25	2.6631	29	2.4046	26	1.7659	19	0.0931

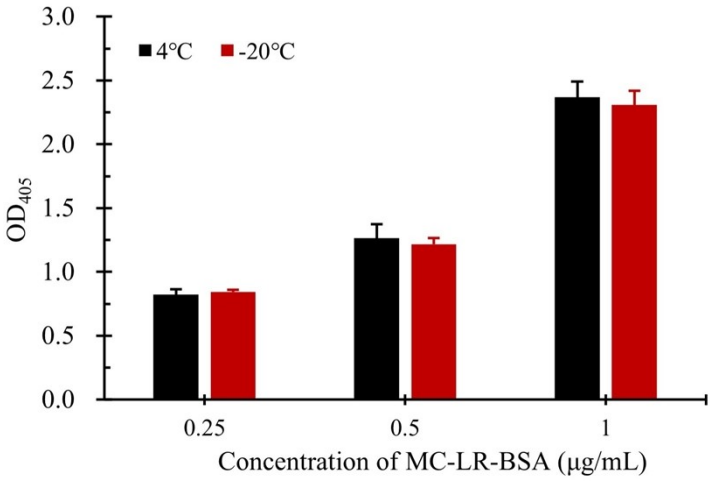

Figure.S2 The storage stability analysis of the developed A2.3-AP bifunctional probe

Table S4. The optimization of concentrations of the coating antigen (MC-LR-BSA) and A2.3-AP bifunctional probe

MC-LR-BSA	A2.3-AP (μg/mL)			
(μg/mL)	4	2	1	0.5
4	3.679	3.685	1.168	0.355
2	2.398	1.606	0.744	0.313
1	2.371	1.513	0.793	0.289
0.5	1.217	1.263	0.698	0.287

Table S5. The cross-reactivity rate of common microcystins

Analyte	IC_{50} (ng/mL)	CR (%)
MC-LR	20.86	100.00%
MC-RR	16.09	129.65%
MC-WR	22.52	92.63%
MC-LY	80.89	25.79%
MC-LW	178.61	11.68%