Supporting Information

Rapid and sensitive immunoassay for alpha-fetoprotein in serum by fabricating primary antibody-enzyme complexes using protein self-assembly

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Table of contents

Table S1……s-3
Table S2……s-4
Table S3……s-5
Figure S1……s-6
Figure S2……s-7

Table S1. The gene sequence of self-assembling peptides (C4bpα C-terminal fragment residues 541-597, C4bpα) and the primers for the construction of pET-22b-A1-C4bpα-ALP.

Name	Sequence
C4bpα gene	GAAACCCCGGAAGGTTGCGAACAGGTTCTGACCGGCAAACGTCT GATGCAGTGCCTGCCGAATCCGGAAGATGTGAAAATGGCCCTGG AAGTTTATAAACTGAGTCTGGAAATCGAGCAGCTGGAACTGCAA CGCGATAGCGCACGCCAGAGTACCCTGGATAAAGAACTG
A1-F	cccagccggcgatggccatggCCCAGGTGCAGCTCGTGG
A1-R	gccTTGTGGTTTTGGTGTCTTGGG
ALP-F	ataaagaactgGGAGGAGGCGGTTCAGGC
ALP-R	ctcgagtgcggccgcaagcttTTTCAGCCCCAGAGCGGC
C4-F	agacaccaaaaccacaaGGCGGCGGTGGCAGCGGT
C4-R	gcctcctccCAGTTCTTTATCCAGGGTACTCTGG

Table S2. The amino acid sequence of A1-ALP and A1-C4bpα-ALP fusion protein.

Name	Sequence
A1-ALP fusion protein: A1 labeled in blue, ALP labeled in yellow and SBP labeled in red	QVQLVESGGGLVQSGGSLRLSCAASMRGFRIVAGGWYRQSPGKQRELVADINYQDDTNYGDF VKGRFTISRDSAKNTLYLQMNSLKPEDTAVYYCSALSVIGNEFWGQGTQVTVSSEPKTPKPQGG GGSGGGGSGGGGSTPEMPVLENRAAQGDITAPGGARRLTGDQTAALRDSLSDKPAKNIILLIGD GMGDSEITAARNYAEGAGGFFKGIDALPLTGQYTHYALNKKTGKPDYVTDSAASATAWSTGV KTYNGALGVDIHEKDHPTILEMAKAAGLATGNVSTAELQGATPAALVAHVTSRKCYGPSATSE KCPGNALEKGGKGSITEQLLNARADVTLGGGAKTFAETATAGEWQGKTLREQAQARGYQLVS DAASLNSVTEANQQKPLLGLFADGNMPVRWLGPKATYHGNIDKPAVTCTPNPQRNDSVPTLA QMTDKAIELLSKNEKGFFLQVEGASIDKQNHAANPCGQIGETVDLDEAVQRALEFAKKEGNTL VIVTADHAHASQIVAPDTKAPGLTQALNTKDGAVMVMSYGNSEEDSQEHTGSQLRIAAYGPHA ANVVGLTDQTDLFYTMKAALGLKKLAAALEIKRASQPELAPEDPEDVEHHHHHH
A1-C4bpα-ALP fusion protein: A1 sequence labeled in blue, C4bpαlabeled in red and ALP labeled in yellow	QVQLVESGGGLVQSGGSLRLSCAASMRGFRIVAGGWYRQSPGKQRELVADINYQDDTNYGDF VKGRFTISRDSAKNTLYLQMNSLKPEDTAVYYCSALSVIGNEFWGQGTQVTVSSEPKTPKPQGG GGSGGGGSETPEGCEQVLTGKRLMQCLPNPEDVKMALEVYKLSLEIEQLELQRDSARQSTLDK ELGGGGSGGGGSGGGGSTPEMPVLENRAAQGDITAPGGARRLTGDQTAALRDSLSDKPAKNIIL LIGDGMGDSEITAARNYAEGAGGFFKGIDALPLTGQYTHYALNKKTGKPDYVTDSAASATAWS TGVKTYNGALGVDIHEKDHPTILEMAKAAGLATGNVSTAELQGATPAALVAHVTSRKCYGPS ATSEKCPGNALEKGGKGSITEQLLNARADVTLGGGAKTFAETATAGEWQGKTLREQAQARGY QLVSDAASLNSVTEANQQKPLLGLFADGNMPVRWLGPKATYHGNIDKPAVTCTPNPQRNDSVP TLAQMTDKAIELLSKNEKGFFLQVEGASIDKQNHAANPCGQIGETVDLDEAVQRALEFAKKEG NTLVIVTADHAHASQIVAPDTKAPGLTQALNTKDGAVMVMSYGNSEEDSQEHTGSQLRIAAYG PHAANVVGLTDQTDLFYTMKAALGLKKLAAALEIKRASQPELAPEDPEDVEHHHHHH

Table S3. Optimization of capture antibody (5H7 monoclonal antibody) and heptavalent PACE (A1-C4bpα-ALP) concentration by checkerboard titration.

A1-C4-AP (μg/mL)	5H7 (μg/mL)				
	4	2	1	0.5	0
8	1.684	0.681	0.414	0.376	0.342
4	1.648	0.506	0.324	0.268	0.294
2	1.384	0.533	0.296	0.252	0.223
1	0.955	0.474	0.322	0.264	0.261
0.5	0.385	0.281	0.251	0.237	0.234
0	0.23	0.218	0.221	0.215	0.23

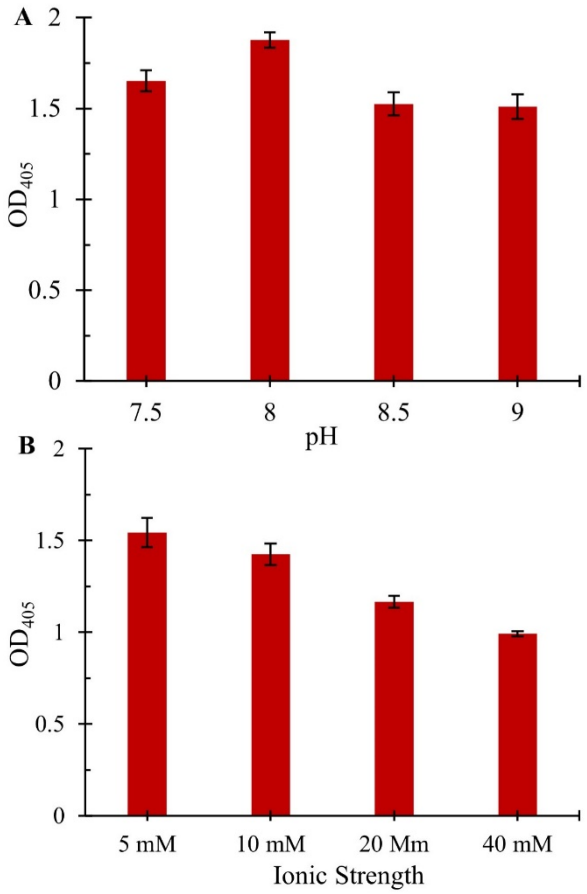

Fig.S1. Optimization of the proposed heptavalent PACE-based ELISA experimental parameters. (A): pH value; (B): Ionic strength. The error bars represent the standard deviation of three independent tests.

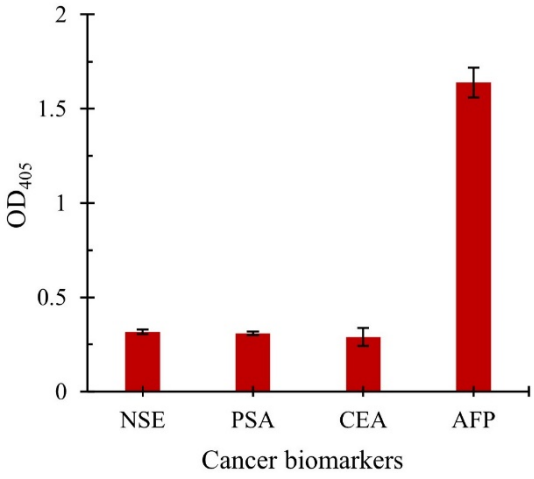

Fig.S2. Selectivity of the proposed heptavalent PACE-based ELISA for AFP. The error bars represent the standard deviation of three independent tests.