

## **Supplementary Materials**

# **Evolution of Interferon-Gamma Aptamer with Good Affinity and Analytical Utility by a Rational In Silico Base Mutagenesis Post-SELEX Strategy**

**Lianhui Zhao <sup>1</sup>, Qionglin Wang <sup>2</sup>, Yingai Yin <sup>1</sup>, Yan Yang <sup>1</sup>, Huifang Cui <sup>3</sup> and Yiyang Dong <sup>1,\*</sup>**

<sup>1</sup> College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China

<sup>2</sup> Henan Key Laboratory of Children's Genetics and Metabolic Diseases, Children's Hospital Affiliated to Zhengzhou University, Zhengzhou 450018, China

<sup>3</sup> College of Life Sciences, Zhengzhou University, Zhengzhou 450001, China

\* Correspondence: yydong@mail.buct.edu.cn; Tel.: +86-010-64446260

**Table S1.** List of oligonucleotides.

DNA sequences	Sequence(5'→3')
M5-5	CCGCCCAAATCCCGAAGGGAAGAGTGTAATGACGTCAAACCAGA CACATTACACACGCA
MB-10	FAM-CGCGC <u>GTGTAATGTGG</u> CGCG-BHQ1
MB-15	FAM-CGCGC <u>GTGTAATGTGTCTGGG</u> CGCG-BHQ1
MB-20	FAM-CGCGC <u>GTGTAATGTGTCTGGTTTGAG</u> CGCG-BHQ1
MB-25	FAM-CGCGC <u>GTGTAATGTGTCTGGTTTGACGTCAG</u> CGCG-BHQ1

The underlined letters represent the complementary sequences.

**Table S2.** B1-4 and mutants with low free energy in the first round library.

Name	Sequence(5'→3')	Dot-bracket information	$\Delta G$ (kcal/mol)	ZDOCK score
B 1-4	CCGCCCAAATCCCTAAGAGAAGACTGTAATGAC ATCAAACCAGACACACTACACACGCA	.....((((.....))).	-1.03	1246.537
M1-1	CCGCCCAAATCCCTAAGAGAAGACTGTAGTGAC ATCAAACCAGACACACTACACACGCA	.....((((((.....)))))).	-4.76	1253.540
M1-2	CCGCCCAAATCCCTAAGAGAAGACTGGAATGAC ATCAAACCAGACACACTACACACGCA	.....((((..(.....)..))).	-4.38	1389.447
M1-3	CCGCCCAAATCCCTAAGAGAAGACTGTAATGAC ATCAAACCAGACACATTACACACGCA	.....((((((.....)))))).	-3.90	1387.888
M1-4	CCGCCCAAATCCCTAAGCGAAGACTGTAATGAC ATCAAACCAGACACACTACACACGCA	..((((.....)))....((((.....))).	-3.61	1307.033
M1-5	CCGCCCAAATCCCTAAGAGAAGTCTGTAATGACA TCAAACCAGACACACTACACACGCA	.....((((((.....))))).	-3.49	1438.918
M1-6	CCGCCCAAATCCCTAAGAGAAGACTGTAATGAC ATCAAACCAGTCACACTACACACGCA	.....((((((.....))))).	-3.49	1264.011
M1-7	CCGCCCAAATCCCTAAGAGAAGACTGTAATGAC ATCAAAACAGACACACTACACACGCA	.....((((..(.....)..))).	-3.46	1362.160
M1-8	CCGCCCAAATCCCTAAGGGAAGACTGTAATGAC ATCAAACCAGACACACTACACACGCA	...((((.....)))....((((.....))).	-3.38	1447.860

M1-9	CCGCCCAACTCCCTAAGAGAAGACTGTAATGAC ATCAAACCAGACACACTACACACGCA	.....(((.....)))....(((.....))).....	-3.34	1410.515
M1-10	CCGCCCAAATCCCTAAGAGAAGACTGTATTGACA TCAAACCAGACACACTACACACGCA	.....(((.....))).....	-3.18	1288.798

The matched brackets represent base pairs, and dots represent unpaired bases.

**Table S3.** Mutants with low free energy in the second round library.

Name	Sequence(5'→3')	Dot-bracket information	$\Delta G$ (kcal/mol)	ZDOCK score
M2-1	CCGCCCAAATCCCTAAGGGAAGACTGTAGTGA CATCAAACCAGACACACTACACACGCA	...(((.....))).....((((.....)))).....	-7.11	1261.302
M2-2	CCGCCCAAATCCCTAAGAGAAGACTGGAATGA CATCAAACCAGTCACACTACACACGCA	.....((((.....))).....	-6.84	1311.845
M2-3	CCGCCCAAATCCCTAAGAGAAGTCTGGAATGA CATCAAACCAGACACACTACACACGCA	.....((((.....))).....	-6.84	1297.627
M2-4	CCGCCCAAATCCCTAAGGGAAGACTGGAATGA CATCAAACCAGACACACTACACACGCA	...(((.....)))....(((.....))).....	-6.73	1229.231
M2-5	CCGCCCAACTCCCTAAGAGAAGACTGGAATGA CATCAAACCAGACACACTACACACGCA	.....(((.....)))....(((.....))).....	-6.69	1265.095
M2-6	CCGCCCAAATCCCTAAGGGAAGACTGTAATGA CATCAAACCAGACACATTACACACGCA	...(((.....))).....((((.....)))).....	-6.25	1485.757

M2-7	CCGCCCAAATCCCTAAGAGAAGACTGTAATGA CATCAAACCAGACACATTACACACGCA	.....(((.....))).....((((((.....)))))).....	-6.21	1309.004
M2-8	CCGCCCAAATCCCTAAGCGAAGTCTGTAATGAC ATCAAACCAGACACACTACACACGCA	.(((.....)))..((((.....)))).....	-6.07	1311.561
M2-9	CCGCCCAAATCCCTAAGAGAAGACTGGATTGA CATCAAACCAGACACACTACACACGCA	.....((((.....)))).....	-5.98	1310.280
M2-10	CCGCCCAAATCCCTAAGAGAAGTCTGTAATGAC ATCAAAACAGACACACTACACACGCA	.....((((.....)))).....	-5.92	1387.575

**Table S4.** Mutants with low free energy in the third round library.

Name	Sequence(5'→3')	Dot-bracket information	ΔG (kcal/mol)	ZDOCK score
M3-1	CCGCCCAAATCCCTAAGGGAAGACTGTAATG ACATCAAACCAGACACATTACAGACGCA	...(((.....))).....(((((((.....)))))).....	-9.44	1370.869
M3-2	CCGCCCAAATCCCTAAGCGAAGACTGGAATG ACATCAAACCAGTCACACTACACACGCA	.(((.....)))..((((.....)))).....	-9.42	1295
M3-3	CCGCCCAAATCCCTAAGCGAAGTCTGGAATG ACATCAAACCAGACACACTACACACGCA	.(((.....)))..((((.....)))).....	-9.42	1292.590
M3-4	CCGCCCAAATCCCTAAGGGAAGAGTGTAATG ACATCAAACCAGACACATTACACACGCA	...(((.....))).....(((((((.....)))))).....	-9.21	1385.598
M3-5	CCGCCCAAATCCCTAAGGGAAGACTGGAATG ACATCAAACCAGTCACACTACACACGCA	...(((.....)))..((((.....)))).....	-9.19	1245.219

M3-6	CCGCCCAAATCCCTAAGTGAAGACTGGAATG ACATCAAACCAGTCACACTACACACGCA	.....((((..((((..((....)..)))))).)).....	-9.16	1460.141
M3-7	CCGCCCAAATCCCTAAGAGAAGACTGGAATG ACATCAAACCAGTCACTCTACACACGCA	.....((((..((((..((....)..)))))).)).....	-8.82	1214.207
M3-8	CCGCCCAAATCCCTAAGCGAAGTCTGTAATGA CATCAAAACAGACACACTACACACGCA	.(((.....))..((((..((....)..))))).)	-8.50	1369.698
M3-9	CCGCCCAAATCCCTAAGCGAAGTCTGTAATGA CATCAAAACAGACACACTACACACGCA	.(((.....))..((((..((....)..))))).)	-8.50	1243.932
M3-10	CCGCCCAAATCCCTAAGAGAAGACTGGATTG ACATCAAACCAGTCACACTACACACGCA	.....((((..((((..((....)..)))))).)).....	-8.44	1270.633

**Table S5.** Mutants with low free energy in the fourth round library.

Name	Sequence(5'→3')	Dot-bracket information	ΔG (kcal/mol)	ZDOCK score
M4-1	CCGCCCAAATCCCTAAGGGCAGACTGTAATG ACATCAAACCAGACACATTACAGACGCA	..((((.....))..(((((((.....)))))))).	-11.42	1274.997
M4-2	CCGCCCAAATCCCTAAGGGAAGACTGTAAT GACGTCAAACCAGACACATTACAGACGCA	...(((.....))..(((((((.....)))))))).	-11.41	1293.240
M4-3	CCGCCCAAATCCCGAAGGGAAGACTGTAAT GACATCAAACCAGACACATTACAGACGCA	.....((((.....))..(((((((.....)))))))).	-11.24	1321.559
M4-4	CCGCCCAAATCCCTAAGGGAAGTGTGTAATG ACATCAAACCAGACACATTACACACGCA	...(((.....))..(((((((.....)))))))).	-11.35	1246.058

M4-5	CCGCCCAAATCCCTAAGGGCAGAGTGTAAT GACATCAAACCAGACACATTACACACGCA	..(((.....))....(((((((.....))))))....	-11.19	1235.186
M4-6	CCGCCCAAATCCCTAAGGGAAGAGTGTAAT GACGTCAAACCAGACACATTACACACGCA	...(((.....))....(((((((.....))))))....	-11.18	1341.062
M4-7	CCGCCCAAATCCCTAAGGGAAGACTGTAAT GACATCAAACCAGACACATTACAGTCGCA	...(((.....))....(((((((.....))))))....	-11.01	1313.665
M4-8	CCGCCCAAATCCCGAAGGGAAGAGTGTAAT GACATCAAACCAGACACATTACACACGCA	.....(((.....))....(((((((.....))))))....	-11.01	1296.120
M4-9	CCGCCCAAATCCCTAAGGGAAGAGTGTAAT GACATCAAACCAGACACATTACACTCGCA	...(((.....))....(((((((.....))))))....	-11.01	1321.879
M4-10	CCGCCCAAATCCCTAAGTGAAGACTGGATT GACATCAAACCAGTCACACTACACACGCA	.....((((.....))))....	-10.76	1354.180

**Table S6.** Mutants with low free energy in the fifth round library.

Name	Sequence(5'→3')	Dot-bracket information	$\Delta G$ (kcal/mol)	ZDOCK score
M5-1	CCGCCCAAATCCCTAAGTGAAGACTGGATTG ACATCAATCCAGTCACACTACACACGCA	.....((((.....))))....	-14.13	1368.866
M5-2	CCGCCCAAATCCCGAAGGGAAGACTGTAATG ACGTCAAACCAGACACATTACAGACGCA	.....(((.....))....(((((((.....))))))....	-13.21	1202.775
M5-3	CCGCCCAAATCCCTAAGGGCAGAGTGTAATG ACGTCAAACCAGACACATTACACACGCA	..(((.....))....(((((((.....))))))....	-13.16	1433.854

M5-4	CCGCCCAAATCCCTAAGGGACGAGTGTAATG ACATCAAACCAGACACATTACACTCGCA	...(((.....))).((((((((.....)))))))).	-13.01	1261.402
M5-5	CCGCCCAAATCCCGAAGGGAAGAGTGTAATG ACGTCAAACCAGACACATTACACACGCA	.....((((.....))).((((((((.....)))))))).	-12.98	1543.334
M5-6	CCGCCCAAATCCCGAAGGGAAGAGTGTAATG ACATCAAACCAGACACATTACACTCGCA	.....((((.....))).((((((((.....)))))))).	-12.78	1320.181
M5-7	CCGCCCAAATCCCGAAGGGAAGTCTGTAATG ACATCAAACCAGACACATTACAGACGCA	.....((((.....))).((((((((.....)))))))).	-12.78	1398.095
M5-8	CCGCCCAAATCCCGAAGGGAAGACTGTAATG ACATCAAACCAGACACATTACAGTCGCA	.....((((.....))).((((((((.....)))))))).	-12.78	1460.231
M5-9	CCGCCCAAATCCCTAAGGGAAGAGTGTAATG ACGTCAAACCAGACACATTACACTCGCA	...(((.....))).((((((((.....)))))))).	-12.75	1441.206
M5-10	CCGCCCAAATCCCGAAGGGATGACTGTAATG ACATCAAACCAGACACATTACAGACGCA	.....((((.....))).((((((((.....)))))))).	-12.46	1305.417

**Table S7.** Mutants with low free energy in the sixth round library.

Name	Sequence(5'→3')	Dot-bracket information	$\Delta G$ (kcal/mol)	ZDOCK score
M6-1	CCGCCCAAATCCCTAAGGGCGGAGTGTAATG ACGTCAAACCAGACACATTACACACGCA	((((((((.....)))))).((((((((.....)))))))).	-16.29	1314.663
M6-2	CCGCCCAAATCCCGAAGGGAAGTGTGTAATG ACGTCAAACCAGACACATTACACACGCA	.....((((.....))).((((((((.....)))))))).	-15.09	1406.824

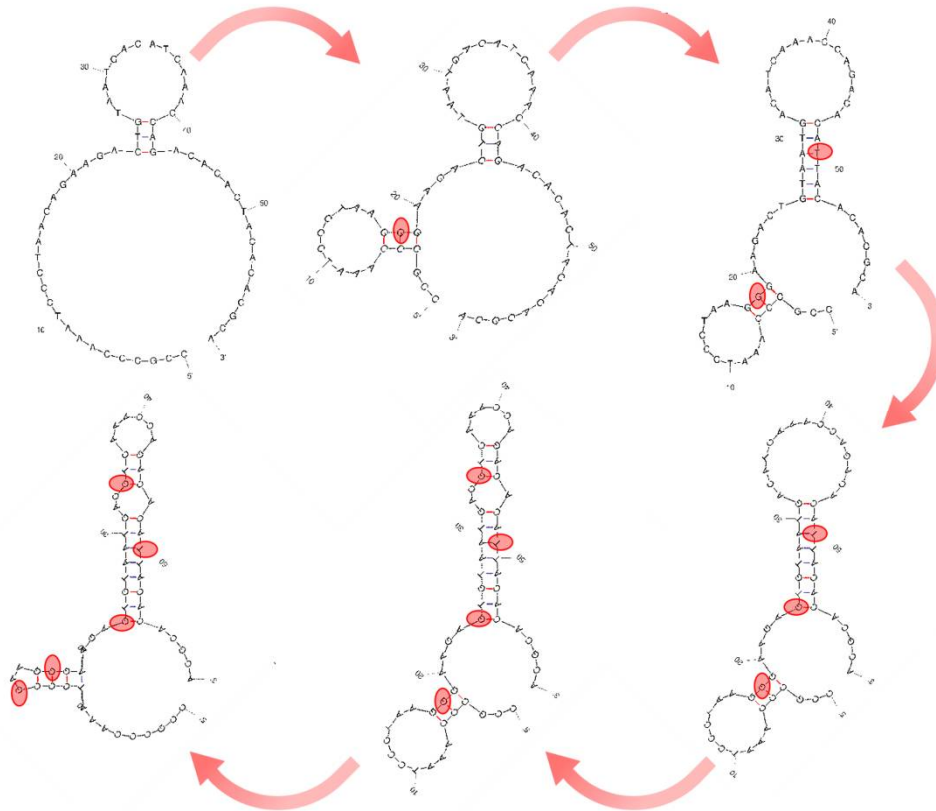
M6-3	CCGCCCAAATCCCTAAGGGCAGAGTGTAATG ACGTCAAACCAGACGCATTACACACGCA	..((((.....)))).((((((((.....))))))))......	-15.08	1343.275
M6-4	CCGCCCAAATCCCTAAGGGACGAGTGTAATG ACGTCAAACCAGACACATTACACTCGCA	...((((.....)))).((((((((.....))))))))....	-14.98	1380.029
M5-5	CTGCCCAAATCCCTAAGGGCAGAGTGTAATG ACGTCAAACCAGACACATTACACACGCA	((((((.....)))))).((((((((.....))))))))......	-14.97	1241.493
M6-6	CCGCCCAAATCCCGAAGGGAAGAGTGTAATG ACGTCAAACCAGACGCATTACACACGCA	.....((((.....)))).((((((((.....))))))))......	-14.90	1394.455
M6-7	CCGCCCAAATCCCTAAGGGAAGAGTGTAATG ACGTCAAACCAGACGCATTACACTCGCA	...((((.....)))).((((((((.....))))))))....	-14.90	1354.663
M6-8	CCGCCCAAATCCCGAAGGGAAGAGTGTAATG ACGTCAAACCAGACACATTACACTCGCA	.....((((.....)))).((((((((.....))))))))....	-14.75	1307.655
M6-9	CCGCCCAAATCCCTAAGGGCAGTGTGTAATGA CGTCAAACCAGACACATTACACACGCA	..((((.....)))).((((((((.....))))))))....	-14.70	1381.942
M6-10	CCGCCCAAATCCCGAAGGGACGACTGTAATG ACATCAAACCAGACACATTACAGTCGCA	.....((((.....)))).((((((((.....))))))))....	-14.57	1331.607

---

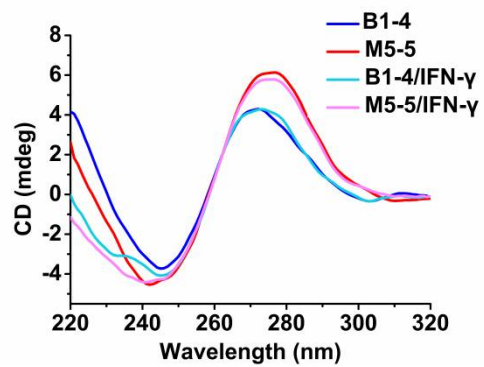
**Table S8.** The binding residues of B1-4 and M5-5 based on the Mfold prediction interacted with IFN- $\gamma$  through hydrogen bonding.

Sequence	Binding residues
B 1-4	<sup>I</sup> Tyr14-C54, <sup>II</sup> Asn25-A34, <sup>I</sup> Lys58-A32, <sup>I</sup> Lys61-G31, <sup>I</sup> Lys94-T35, <sup>I</sup> Asn97-C33, <sup>I</sup> Tyr98-A34, <sup>I</sup> Ser99-C33, <sup>II</sup> Thr101-C54, <sup>I</sup> Asp102-A34, <sup>II</sup> Gln106-C54
M 5-5	<sup>I</sup> Tyr14-C12, <sup>I</sup> Tyr14-A20, <sup>I</sup> Asn59-A9, <sup>II</sup> Asn59-C2, <sup>II</sup> Asn59-G3, <sup>I</sup> Asp63-C11, <sup>I</sup> Ser65-G14, <sup>II</sup> Thr101-A9, <sup>I</sup> Gln106-C2, <sup>II</sup> Gln106-T10

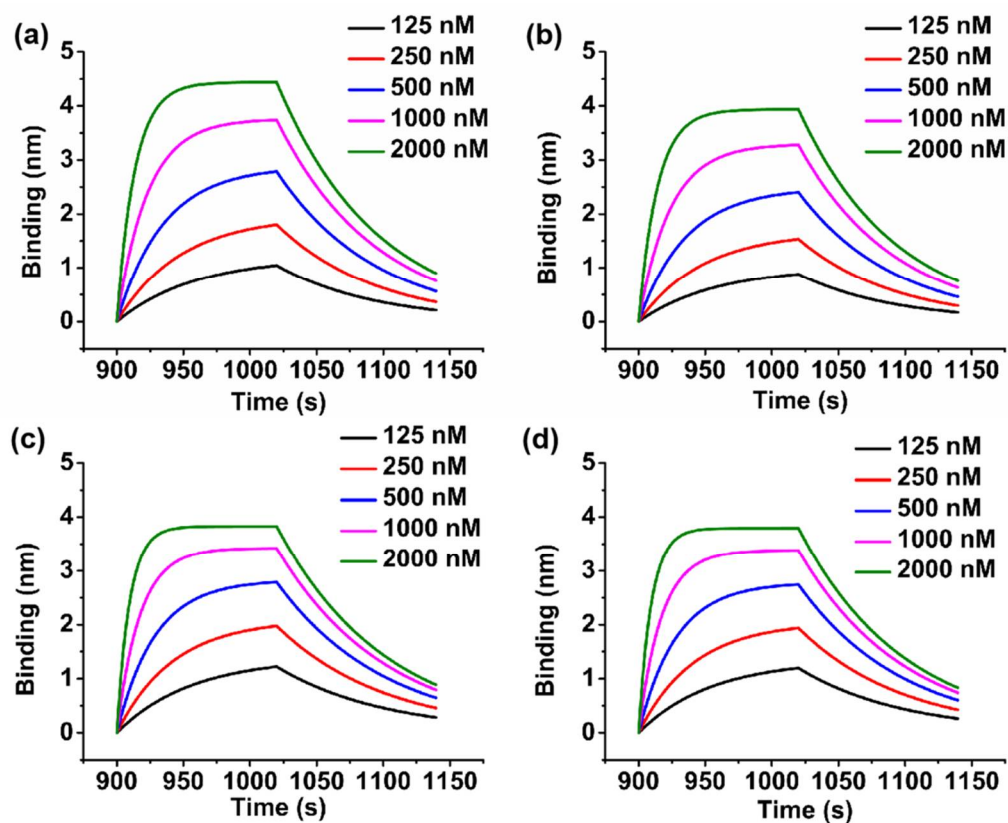
I, II representative the two monomers of IFN- $\gamma$ , respectively.



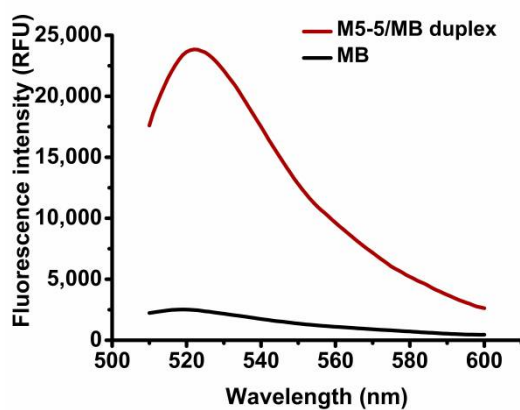
**Figure S1.** The phylogeny of the M5-5 aptamer predicted by the Mfold. The mutation sites are shaded in red.



**Figure S2.** Circular dichroism (CD) analysis of B1-4 and M5-5 with or without IFN- $\gamma$ .



**Figure S3.** BLI characterization of the affinity of (a) M1-8, (b) M2-6, (c) M3-6 and (d) M4-10.



**Figure S4.** Fluorescence emission spectra of the M5-5/MB duplex and MB.