

Supporting information

Specificity from non-specific interaction: regulation of TNF- α activity by DNA

Helena Andrade, Weilin Lin, and Yixin Zhang

Table S1. DNA sequences used in this project.

DNA sequences with rational design for Figs. 1, 2C, 3A, 3B, S3B	
ssDNA	TGGATCCGCATGACATTCGCCGTAAG
dsDNA	GACAATTCACACACGTCCGCAGTCTGACTGATCACTGGACATGAGATCGGAAGAGCGTCG CGACGCTCTTCCGATCTCATGTCCAGTGATCAGTCAGACTGCCGACGTGTGTGAATTGTC
Y-DNA	TGGATCCGCATGACATTCGCCGTAAG CTTACCGCGAATGACCGAATCAGCCT AGGCTGATTCGGTTCATGCGGATCCA
DNA sequences without potential CpG motif for Fig. 3C	
ssDNA*	TGGATCCGCATGACATTCGCCGTAAG
dsDNA*	TGGATCCGCATGACATTCGCCGTAAG CTTACGGCGAATGACCGAATCAGCCT
Y-DNA*	TGGATCCGCATGACATTCGCCGTAAG CTTACGGCGAATGACCGAATCAGCCT AGGCTGATTCGGTTCATGCGGATCCA
DNA sequences for Figs. 2A, 2B, S1, S2	
ssDNA	Cy5-TGGATCCGCATGACATTCGCCGTAAGCGACGCTCTTCCGATCTCAT
dsDNA	Cy5-TGGATCCGCATGACATTCGCCGTAAGCGACGCTCTTCCGATCTCAT ATGAGATCGGAAGAGCGTCGCTTACGGCGAATGTCATGCGGATCCA
Y-DNA	Cy5-TGGATCCGCATGACATTCGCCGTAAGCGACGCTCTTCCGATCTCAT CTTACGGCGAATGACCGAATCAGCCTCGACGCTCTTCCGATCTCAT AGGCTGATTCGGTTCATGCGGATCCACGACGCTCTTCCGATCTCAT
DNA sequences for Fig. S4B	
DNA	CTTACGGCGAATGACCGAATCAGCCT AGGCTGATTCGGTTCATGCGGATCCA
Salmon sperm DNA	
S-DNA	Undefined, 100 to 200 bp products

Table S2. His-tagged TNF- α .

MKHHHHHMKQVRSSRTPSDKPVAVHVVANPQAEGLQWLNRRANALLANGVELRDNQLVVPSEGLYLI
YSQVLFKGGQCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLE
KGDRLSAEINRPDYLDFAESGQVYFGIIAL. (M.W., 18821 g/mol)

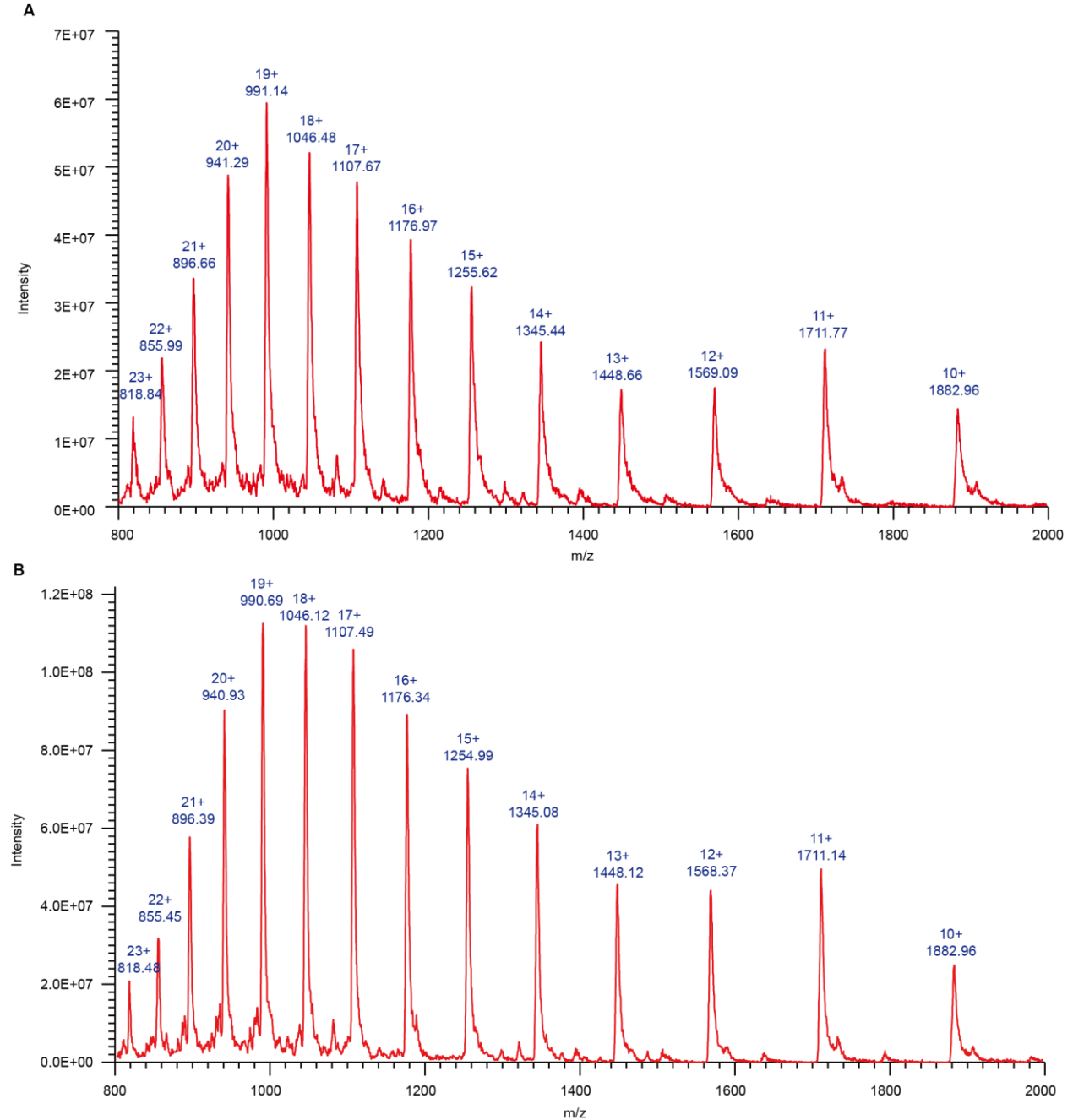


Figure S1. TNF- α mass spectrum. (A) The eluted fractions from His-column). Observed M.W. 18815 ± 5 g/mol. (B) The eluted fractions from Superdex 75. Observed M.W. 18808 ± 6 g/mol.

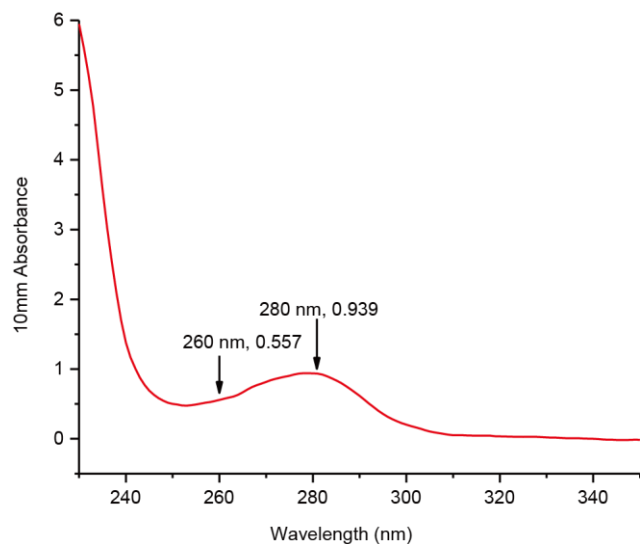


Figure S2. TNF- α UV spectrum in Nanodrop. The ratio of A260/A280 is 0.59, which is close to 0.57 (100% protein). It indicates the purified protein has only traces of nucleic acid contaminants.

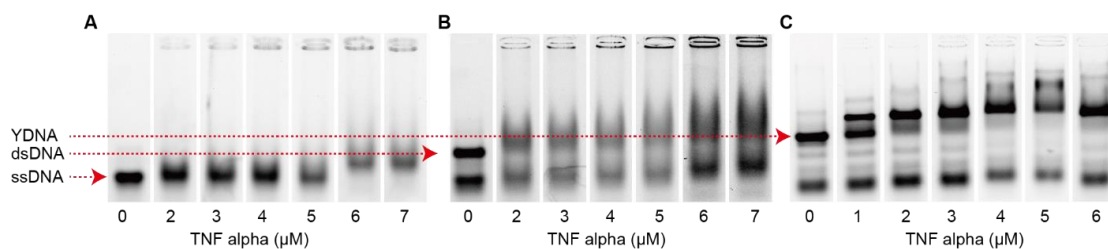


Figure S3. Band-shift assays of the co-incubation products of 100 nM Cy5-ssDNA (A), Cy5-dsDNA (B), and Cy5-Y-DNA (C) with various concentration of TNF- α . Trimeric TNF- α is in favor of binding to the branched Y-DNA.

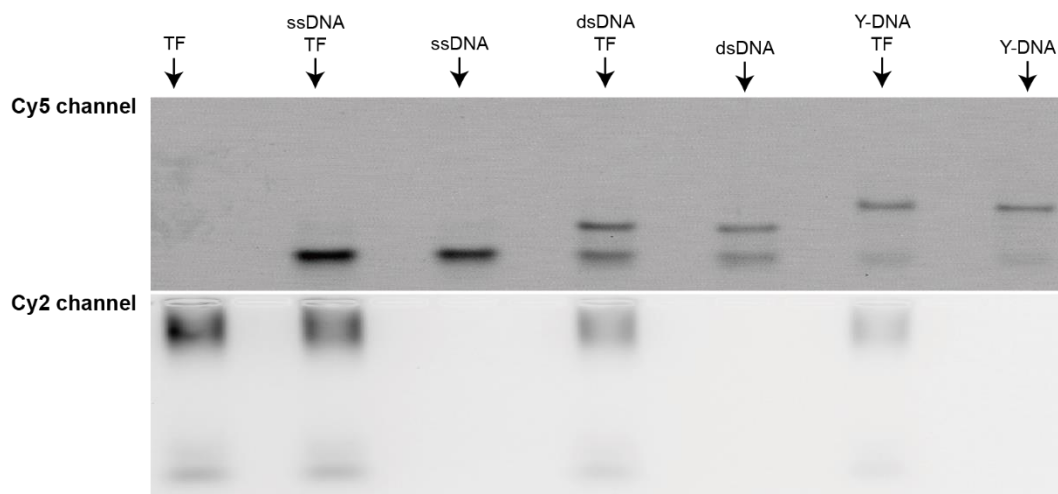


Figure S4. Band-shift assay of the co-incubation products of 10 μ M TF (TNF- α -fluorescein) with 100 nM Cy5-DNA. The addition of TF (as shown in Cy2 channel) does not change the migration of DNA (as shown in Cy5 channel).

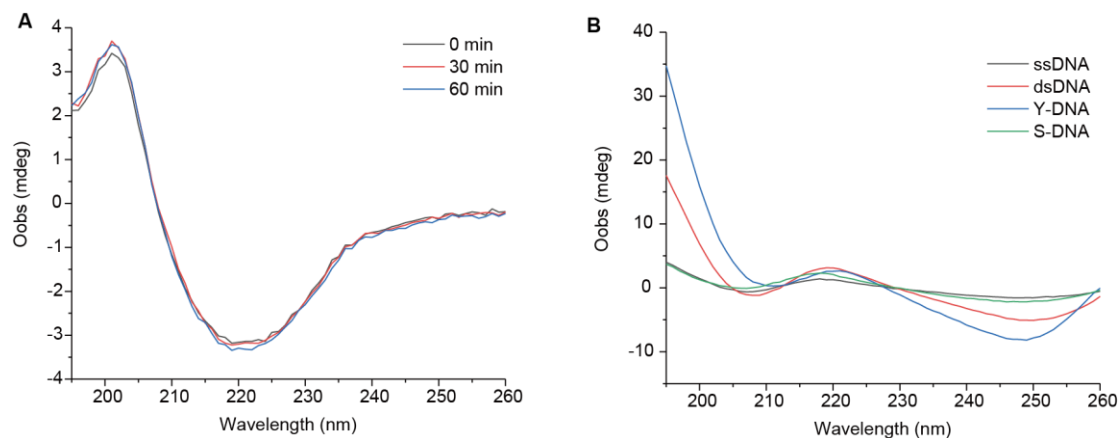


Figure S5. CD spectra of 10 μM TNF- α after various incubation time (A) and after adding 10 μM DNA of different forms (B). The spectrum of TNF- α is stable over time. DNA structures have very weak signal at 217 nm. The CD spectra of DNA have strong signal between 240 to 260 nm, while the signal in the range between 204 to 230 nm is weak. Therefore, the large change in the range between 204 to 230 nm can be mainly assigned to the protein. Because of the strong background noise of DNA in the range between 240 to 260 nm, it is difficult to calculate the structure. We tried several methods and programs to calculate the β -sheet content, and none of them had a satisfactory fitting.

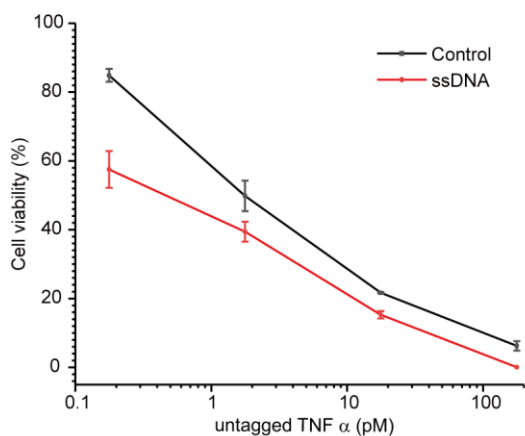


Figure S6. Untagged TNF- α cytotoxicity with and without 5 μM ssDNA.

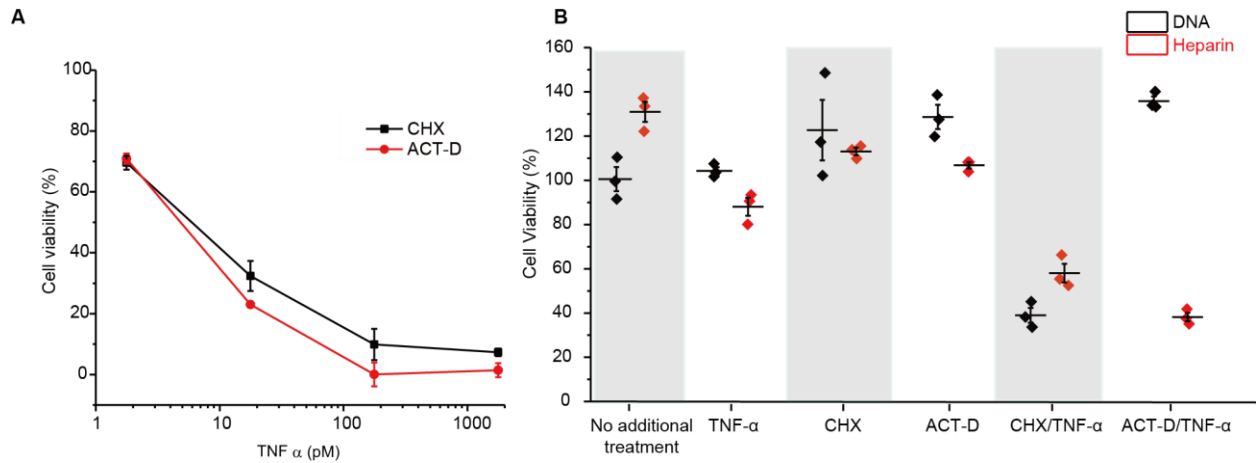


Figure S7. (A) Co-treatment of cells using TNF- α with cycloheximide (CHX, 8 μ M) or actinomycin D (ACT-D, 1.6 μ M) led to concentration dependent decrease of cell viability. (B) Treating cells using DNA (5 μ M) or heparin (5 μ M) with only TNF- α , or CHX, or ACT-D did not affect cell viability. Adding DNA or heparin to CHX/TNF- α co-treated cells reduced cell viability. Adding heparin to ACT-D/TNF- α co-treated cells reduced cell viability. Adding DNA to ACT-D/TNF- α co-treated cells did not reduce cell viability. Instead, it rescued the cells from the drug treatment.

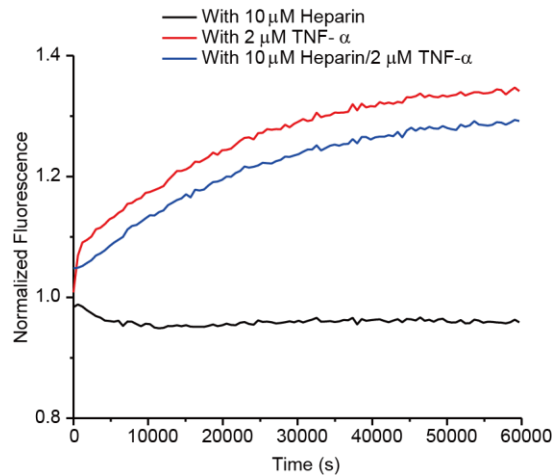


Figure S8. Homoquenched fluorescence release from the incubation of 300 nM TF with 2 μ M TNF- α and 10 μ M heparin. The fluorescence was normalized per fluorescence of TF.

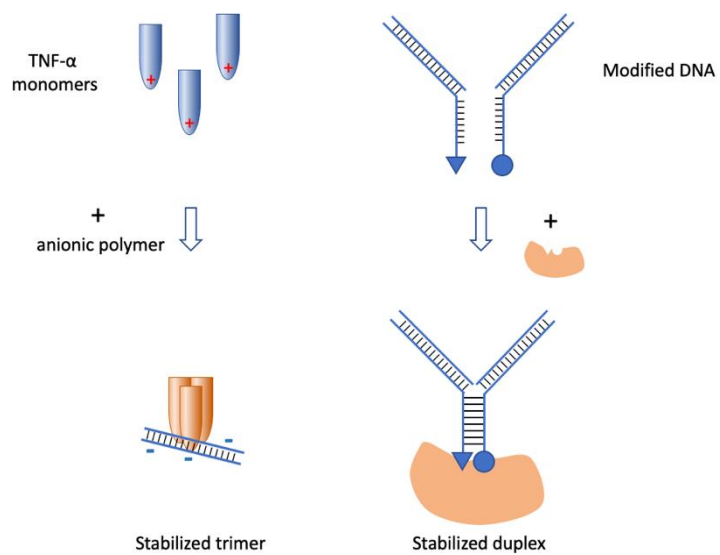


Figure S9. Comparison between a dynamic chemical library and TNF- α multi-valent interaction.