## The enhancement of enzyme cascading via tetrahedral DNA framework modification

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Fig. S1. DNA-enzyme conjugation by SPDP crosslinker.



**Fig. S2.** The structures of enzymes. (a) Sarcosine oxidase (SOX, PDB code: 3QSE). (b) Horseradish peroxidase (HRP, PDB code: 1H5A). The structures of enzymes were visualized by PYMOL.



Fig. S3. Structure of tetrahedral DNA nanostructure (TDN). The TDN structure was rendered by PYMOL.



**Fig. S4.** Characterization of TDN1 assembly by PAGE. TDN1 (A1BCD) assembly was shown as a "staircase-like" pattern by adding all four required DNA strands. Lane A1BCD-L1 confirmed the successful hybridization of TDN1 with DNA L1. Lane M: 20 bp marker.



**Fig. S5.** Characterization of TDN2 assembly by PAGE. Lane A2BCD indicated the formation of TDN2 and lane A1BCD-L2 confirmed the successful hybridization of TDN2 with DNA L2. Lane M: 20 bp marker.



Fig. S6. The AFM image of the TDNs. Scale bar: 100 nm.



**Fig. S7.** The AFM image of the TDN1-SOX. Scale bar: 200 nm. White ellipses indicate the TDN1-SOX conjugations.



**Fig. S8.** The AFM image of the TDN2-HRP. Scale bar: 50 nm. White ellipses indicate the TDN2-HRP conjugations.



**Fig. S9.** Characterization of the formation of TDN1-SOX by gel electrophoresis mobility shift assay (EMSA). Lane M: 20 bp marker. Immobilization of L1-SOX on the vertex of TDN1 decreased its migration rate significantly.



**Fig. S10.** Characterization of the formation of TDN2-HRP by gel electrophoresis mobility shift assay (EMSA). Lane M: 20 bp marker. Immobilization of L2-HRP on the vertex of TDN2 decreased its migration rate significantly.



Fig. S11. The kinetics curves of the TDN-enzymes, ssDNA-enzymes and free enzymes (pH 7.0).

Table S1 DNA sequence used in this work:

name	DNA(5'→3')
	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCC
А	GCCATAGTAAAAAAAAAAA
	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCC
A1(A-L3)	GCCATAGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCC
A2(A-L4)	GCCATAGTAAAAAAAAAAAAAAACCACCACCACCACCACCA
	SH-
	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAG
В	GGTCCAATAC
	SH-
	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGG
С	CGGCTCTTC
	SH-
	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGAC
D	CCTCGCAT
L1	TTTTTTTTTTTTTTTT-SH
L2	TTGGTGGTGGTGGTGGTGGT-SH
L3	ААААААААААААААААААА
L4	AACCACCACCACCACCA
L3'	SH-AAAAAAAAAAAAAAAAAAAAA
L4'	SH-AACCACCACCACCACCA