Manuscript title:

TRIVALENT LIGANDS WITH RIGID DNA SPACERS REVEAL STRUCTURAL REQUIREMENTS FOR IGE RECEPTOR SIGNALING IN RBL MAST CELLS.

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Supporting Information for print:

Supplemental Table S1:

Sequence of n nucleotide bases in component ssDNA used for hybridized Y-shaped dsDNA scaffolds and Yn-DNP_3 ligands (n= 16, 26, 36, 46)

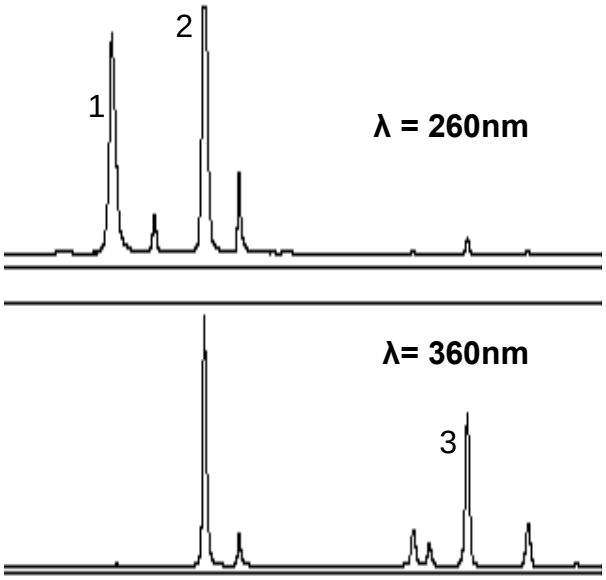
Supplemental Table S1. Single strand DNA sequence for Yn-DNP_3 ligands

Yn[1]	Sequence of strands
16	5'- /5AmMC6/CGTGGTAGGACGATGC-3' 5'- /5AmMC6/GCATCGTCACAGCTGC-3' 5'- /5AmMC6/GCAGCTGTCTACCACG- 3'
26	5'- /5AmMC6/TCGATCCGCATGACATTCGCCGTAAG-3' 5'-/5AmMC6/CTTACGGCGAATGACCGAATCAGCCT-3' 5'-/5AmMC6/AGGCTGATTCGGTTCATGCGGATCCA-3'
36	5'-/5AmMC6/ACCACTGGATCCGCATGACATTCGCCGTAAGCACAC-3' 5'-/5AmMC6/GTGTGCTTACGGCGAATGACCGAATCAGCCTGCTGA-3' 5'-/5AmMC6/TCAGCAGGCTGATTCGGTTCATGCGGATCCAGTGGT-3'
46	5'-/5AmMC6/ACCACTGGATCCGCATGAGGTAGGACGACATTCGCCGTAAGCACAC-3' 5'-/5AmMC6/GTGTGCTTACGGCGAATGTCGTCACAGCACCGAATCAGCCTGCTGA-3' 5'-/5AmMC6/TCAGCAGGCTGATTCGGTGCTGTCTACCTCATGCGGATCCAGTGGT-3'

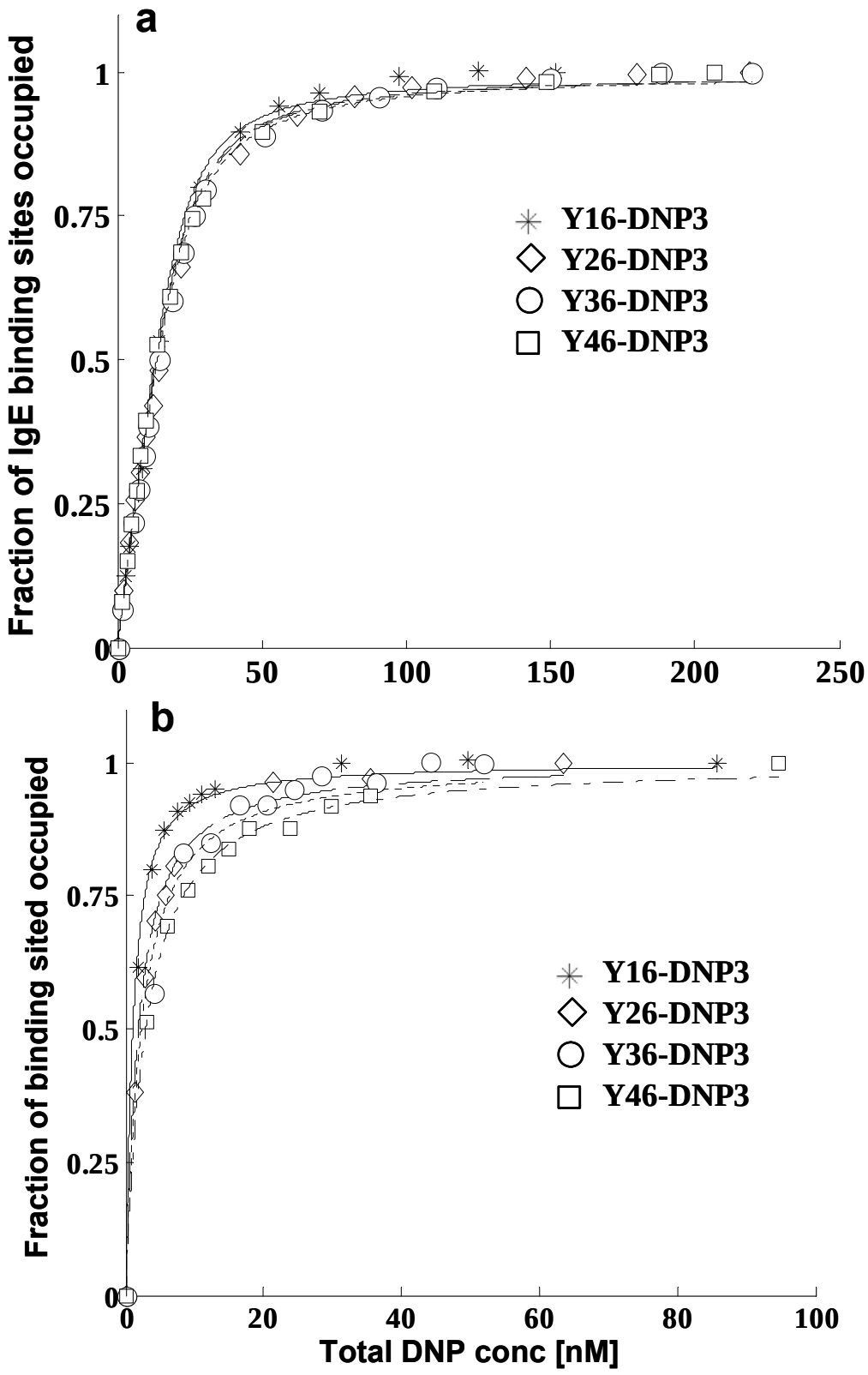
1. Y-shaped dsDNA scaffold formed from three single DNA strands of length n.

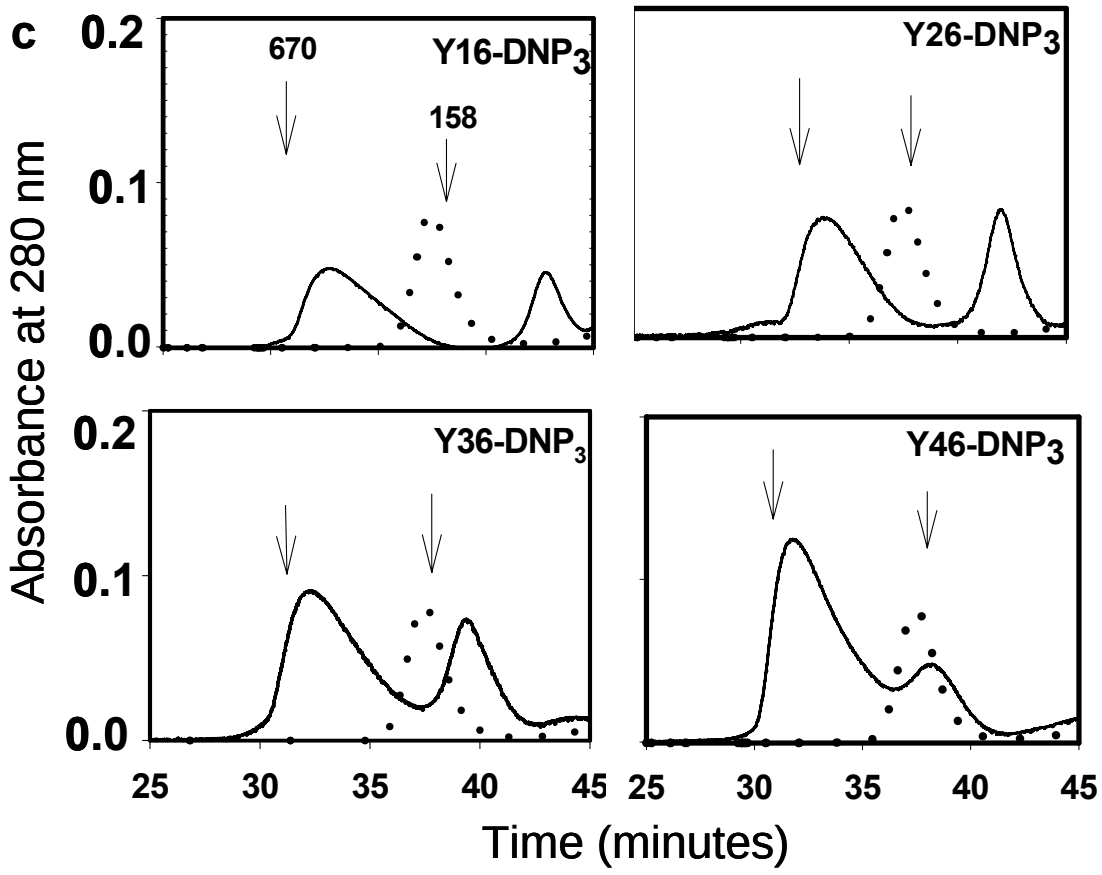
Supplemental Figure S1: Purification of ssDNA components that are conjugated with DNP

Representative HPLC chromatograms, as monitored by absorption, after reaction of ssDNA 5' amino groups with DNP-succinimidyl ester. Distinct peaks corresponding to unlabeled ssDNA (1), DNA-DNP (2), and free DNP (3) are identified according to characteristic absorption at 260nm (DNA) and 360nm (DNP).



Supplemental Fig S2: Equilibrium binding of trivalent Yn-DNP_3 ligands with IgE in solution and IgE bound to FcεRI on RBL cell surface. Equilibrium binding was evaluated with a fluorescence quenching method previously established (17). Data were fit to a simple model: $r = [L]/(K_d + [L])$ where r is the fraction of sites bound, [L] is the concentration of free Yn-DNP_3 and K_d is the apparent affinity (a measure of Yn-DNP_3 avidity). (a) Best fit curves for the four Yn-DNP_3 ligands binding to soluble IgE ($[IgE]_{total}$ = 20 nM); K_d = 4.0 nM for all. (b) Best fit curves for the four Yn-DNP_3 ligands binding to the IgE-FcεRI on cells ($[IgE]_{total}$ = 1 nM); K_d =1.2 - 1.6 nM. No trends in K_d were observed with respect to ligand length in multiple experiments, and average values are listed in Table 1. (c) Size exclusion chromatography of IgE bound to Yn-DNP_3. Y16-DNP_3, Y26-DNP_3, Y36-DNP_3, or Y46-DNP_3 (2 μM) were incubated with anti-DNP IgE (1 μM) in solution for 45 min at room temperature and eluted with borate buffered saline (pH =8.2) on a Sepharose 6 column. The eluent was monitored by absorption at 280nm: The first peak of the solid trace corresponds to Yn-DNP_3-IgE complexes, and a second peak eluting subsequent to the elution time for monomeric IgE represents unbound ligand, which is in modest excess in the mixtures chromatographed. The dotted trace shows the elution position of IgE in absence of any ligands, and arrows indicate the elution positions of Thyroglobulin (670 kDa) and IgG (158 kDa).


a
Fraction of IgE binding sites occupied
1
0.75
0.5
0.25
0
0
50
100
150
200
250
Y16-DNP3
Y26-DNP3
Y36-DNP3
Y46-DNP3
b
Fraction of binding sited occupied
1
0.75
0.5
0.25
0
0
20
40
60
80
100
Total DNP conc [nM]
Y16-DNP3
Y26-DNP3
Y36-DNP3
Y46-DNP3


c
0.2
0.1
0.0
670
158
Y16-DNP3
Y26-DNP3
Y36-DNP3
Y46-DNP3
Absorbance at 280 nm
25
30
35
40
45
Time (minutes)