Supporting Information

Synthetic Allergen Design Reveals The Significance of Moderate Affinity Epitopes in Mast Cell Degranulation

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Supplementary Results and Discussion

The proposed structure formed by a solution of IgE^{DNP} and HTA in solution is a bicyclic tetramer (Figure S1A; IgE_4HTA_2). The hydrodynamic radius of monomeric IgE was experimentally found to be 10.7 nm using dynamic light scattering (DLS). Using the same technique, the size of the IgE/HTA complexes was measured to be ~20 nm. These experiments were carried out using a stoichiometric ratio of tetravalent allergen to IgE (1 : 2). This is a reflection of the 4 antibody binding moieties in each tetravalent molecule and the two antigen binding domains on an IgE. The size of the IgE/HTA complex formed in solution was double the radius of monomeric IgE and no larger particles were detected. The lack of any particles larger than ~20 nm eliminated the possibility of the formation of large polymers of IgE/HTA. As DLS measures the hydrodynamic radius of particles in solution, it is not possible to distinguish between different IgE/HTA complexes with similar hydrodynamic radii. The possible complexes are described in Figure S1A: 2 IgEs binding to a single HTA (IgE_2HTA), 2 IgEs binding to 2 HTAs (IgE_2HTA_2), 3 IgEs binding to 2 HTAs (IgE_3HTA_2) and finally a bicyclic tetramer with 4 IgEs binding to 2 HTAs (IgE_4HTA_2). Out of these possible complexes, the tetravalent design of HTA prevents the formation of IgE_2HTA complex. The optimal separation distance for bivalent binding to a single IgE is 10 nm.[1] Since HTA can only span a distance of 6.2 nm when the linkers are fully extended, HTA will not be able to simultaneously reach the two antigen binding sites that exist on the two Fab arms of an IgE. The possibility of formation of IgE_2HTA_2 or IgE_3HTA_2 complexes is also eliminated based on the analysis of the DLS results. DLS experiments were carried out with exact stoichiometric amounts of HTA to IgE at 1:2 ratio. If only 2 or 3 antibodies are binding to 2 HTAs then there would still be monomeric IgE left in the sample, which would have been detected as a separate peak at 10.7 nm during DLS measurements. Taken together these results demonstrate the bicyclic tetramer (IgE_4HTA_2) as the only possible complex formed with

IgE/HTA at 2:1 ratio. The formation of this complex demonstrates the utility of the tetravalent scaffold for the efficient presentation of all 4 haptens for IgE binding.

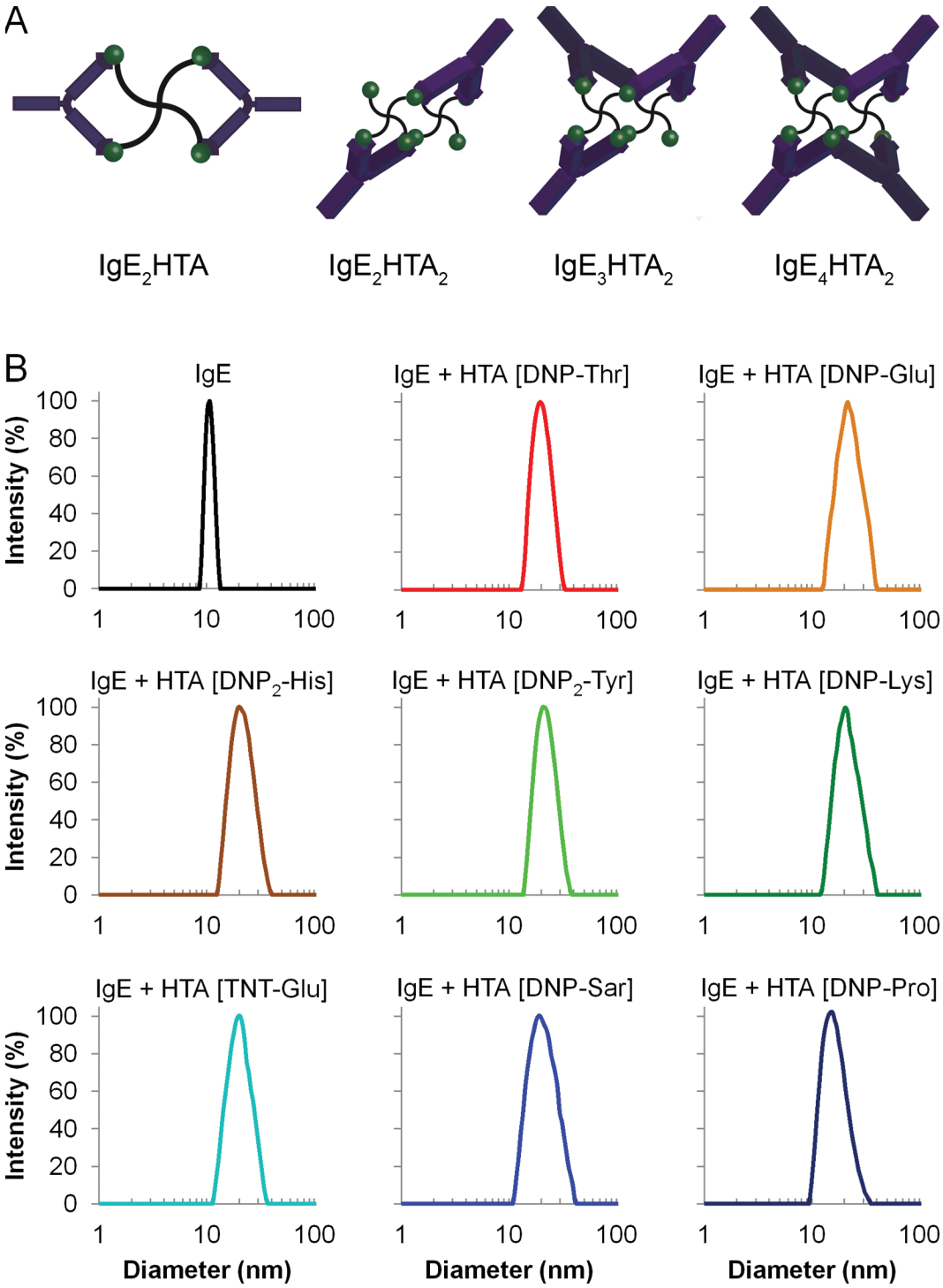

Figure S1. A) Possible IgE/HTA complexes with a hydrodynamic radius of 20 nm. **B)** IgE^{DNP} antibody formed cyclic complexes upon reaction with HTA molecules in solution. IgE^{DNP} was mixed with a stoichiometric amount of HTA and dynamic light scattering was used to determine

complex formation. The hydrodynamic radius of the monomeric IgE antibody was determined to be 10.7 nm. Upon mixing of stoichiometric amount of each HTA, the observed hydrodynamic radius increased to ~20 nm, indicating formation of bicyclic tetramer complexes.

Table S1. The hydrodynamic diameter and polydispersity index (PDI) for the IgE/HTA complexes.

Sample	Diameter (nm)	PDI
IgE^{DNP}	10.74	0.061
IgE^{DNP} + HTA [DNP-Thr]	19.95	0.101
IgE^{DNP} + HTA [DNP-Glu]	21.38	0.149
IgE^{DNP} + HTA [DNP2-His]	20.26	0.129
IgE^{DNP} + HTA [DNP2-Tyr]	21.78	0.112
IgE^{DNP} + HTA [DNP-Lys]	20.50	0.121
IgE^{DNP} + HTA [TNT-Glu]	19.23	0.146
IgE^{DNP} + HTA [DNP-Sar]	19.10	0.154
IgE^{DNP} + HTA [DNP-Pro]	16.70	0.118

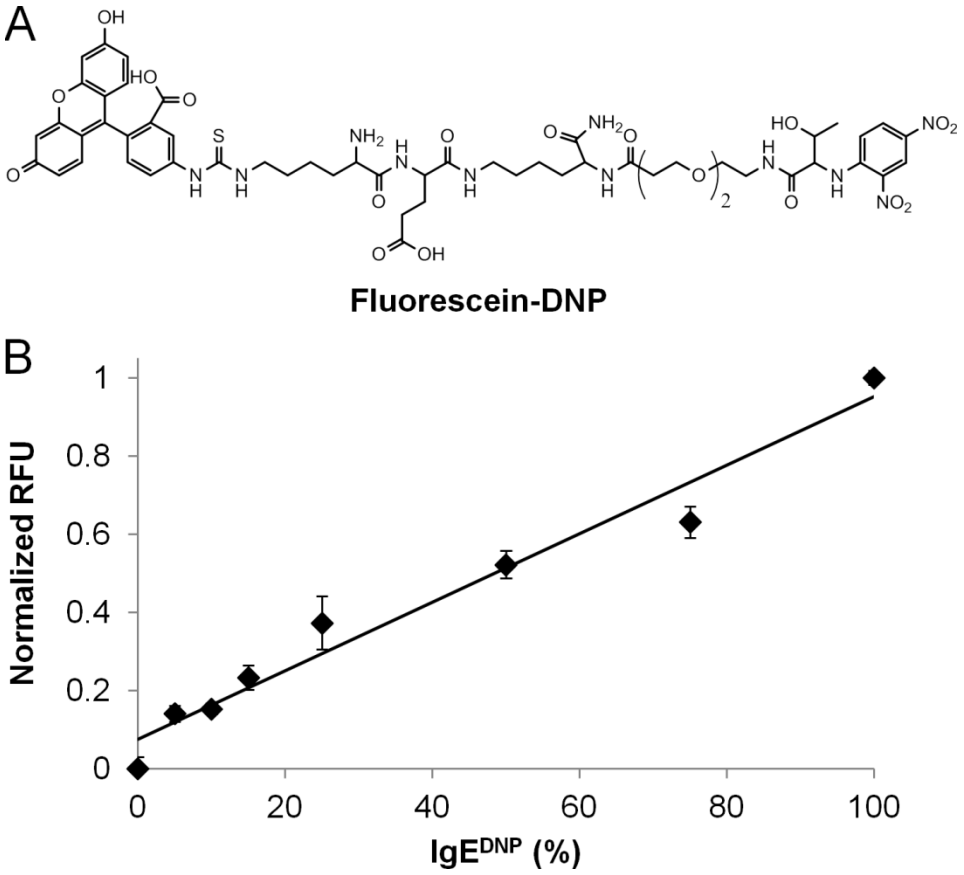

Figure S2. We determined the RBL surface bound IgE^{DNP} : IgE^{dansyl} ratio by first allowing the fluorescently labeled DNP hapten (fluorescein-DNP in **A**) to bind to IgE^{DNP} on cells, and observing the relative fluorescence intensity of the cells at different antibody ratios using flow cytometry (results shown in **B**). Briefly, mast cells were plated at 0.5 x 10^6 cells/mL in a 24 well plate and incubated overnight. IgE^{DNP} and IgE^{dansyl} antibody mixtures at varying ratios were introduced to the wells, while keeping the total antibody concentration constant at 1 µg/mL. Unbound IgE was washed away and cells were incubated with 500 nM of the fluorescein-DNP molecule. The cells were scraped then analyzed for DNP binding with a Guava easyCyte 8HT flow cytometer. The error bars represent the standard deviation of triplicate measurements. These results demonstrate that ratio of the IgE antibodies on RBL cell surface can be adjusted by controlling the ratio of the IgEs introduced into the wells.

Supplementary References

(1) Baird, B., Zheng, Y., and Holowka, D. (1993) Structural mapping of IgE-FcεR1, an immunoreceptor complex. *Acc. Chem. Res.* 26, 428-434.