Supporting Information for

"Gads Dimerization Promotes Antigen Receptor Sensitivity in T and Mast Cells"

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1. Experimental Information.

2. Computational Modeling of Gads Binding to LAT.

2.1. Computational model description.

To estimate the degree of cooperativity of Gads binding to 2pY-LAT, we developed a computational model that incorporated competitive Gads binding to single- and doubly-phosphorylated LAT peptide, using both sequential and simultaneous binding mechanisms to model the binding of Gads to 2pY-LAT (Fig. S5).

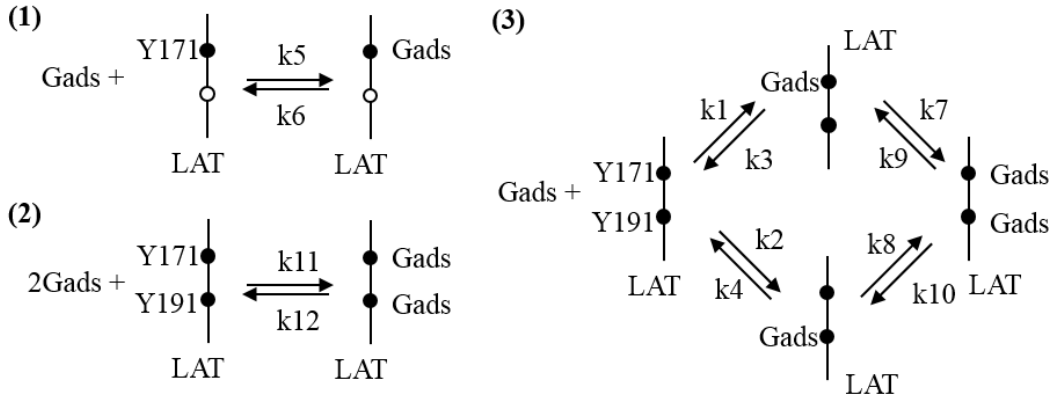

Fig. S5. Computational model of Gads binding to the singly phosphorylated LAT peptide (1) and to the doubly phosphorylated LAT peptide using simultaneous (2) and sequential (3) mechanisms.

2.2. Estimate of Gads binding cooperativity to 2pY-LAT.

To calculate the degree of cooperativity for sequential and simultaneous mechanisms, we used K_{A1} from herein experiments for the wild type Gads SH2 and F92D Gads SH2 mutant (Fig 3D). We estimated the value of $K_{A2} = k_7 / k_9$ (for sequential mechanism) and $K_A = k_{11} / k_{12}$ (for simultaneous mechanism) from the optimized k_9 and k_{12} parameters using the multivariate Ordinary Least Square (OLS) estimator (ref here (book): Hastie T., Tibshirani R. and Friedman J. "The Elements of Statistical Learning: Data Mining, Inference, and Prediction" Springer; 2nd ed. 2009). OLS minimizes the Residual Sum of Squares (RSS) between the observed responses $y_i^{\exp}(x_i)$ and the responses $f(x_i)$ predicted by solving the set of Ordinary Differential Equations (ODEs, described in the Supp. Info. section 2.3) for the reaction network shown in Fig. S5.

$$RSS = \sum_{i=1}^{n} (y_i^{\exp}(x_i) - f(x_i))^2 ,$$

where n is the number of observations, $y_i^{\exp}(x_i)$ is an experimental dependence of the percentage of Gads protein in the dimeric form with an increase of pY171-LAT concentration; $f(x_i)$ is the predicted dependence of the percentage of Gads dimer with an increase of pY171-LAT concentration.

2.3. The set of ODEs used in calculations.

We used the following set of ODEs to calculate the percentage of Gads dimer with an increase of pY171-LAT concentration. Abbreviations used in ODEs: G represents free Gads molecules; L is free doubly phosphorylated LAT peptide; L0 is free singly phosphorylated pY171 LAT peptide; L10 and L01 represent doubly phosphorylated LAT peptide bound to a single Gads molecule; L0_10 is Gads molecule bound to the singly phosphorylated LAT peptide; L11 is two Gads molecules bound to the doubly phosphorylated LAT peptide.

$$\frac{dG}{dt} = -[G][L](k_1 + k_2) - [G](k_7[L10] + k_8[L01] + k_5[L0]) + k_3[L10] + k_4[L01] + k_6[L0\_10] + \ldots$$
$$\ldots + [L11](k_9 + k_{10}) - k_{11}[G]^2[L] + k_{12}[L11]$$
(6)

$$\frac{dL}{dt} = -[G][L](k_1 + k_2) + k_3[L10] + k_4[L01] - k_{11}[G]^2[L] + k_{12}[L11] \quad (7)$$

$$\frac{dL10}{dt} = [G](k_1[L] - k_7[L10]) - k_3[L10] + k_9[L11] \quad (8)$$

$$\frac{dL01}{dt} = [G](k_2[L] - k_8[L01]) - k_4[L01] + k_{10}[L11] \quad (9)$$

$$\frac{dL11}{dt} = [G](k_7[L10] + k_8[L01]) - [L11](k_9 + k_{10}) + k_{11}[G]^2[L] - k_{12}[L11] \quad (10)$$

$$\frac{dL0}{dt} = -k_5[G][L0] + k_6[L0\_10] \quad (11)$$

$$\frac{dL0\_10}{dt} = k_5[G][L0] - k_6[L0\_10] \quad (12)$$

2.4. Kinetic parameters used in calculations.

In our calculation we use molecules/(μm)^3 as the units for concentration variables, so the rate constants are converted to the appropriate units. The following conversion table may help the reader.

Table S1. Unit Conversion Table.

1 μM = 600 molecules / μm^3
1 μM in 0.08 μm^3 volume = 600 molecules · 0.08 = 48 molecules

$(k_{on})_{3D}$ = 1 μM^{-1} s^{-1} = 0.166 · 10^{-2} μm^3 / molecules s^{-1}
1 μM^{-1} s^{-1} in 0.08 μm^3 volume = 0.166 · 10^{-2} / 0.08 molecules^{-1} s^{-1} = 0.02 molec^{-1} s^{-1}

1 μM^{-2} s^{-1} = 2.77 · 10^{-6} (μm^3 / molecules)^2 s^{-1}
1 μM^{-2} s^{-1} in 0.08 μm^3 volume = 2.77 · 10^{-6} / (0.08)^2 molecules^{-2} s^{-1} = 4.3 · 10^{-4} molec^{-2} s^{-1}
1 molecules^{-2} s^{-1} in 0.08 μm^3 volume = 2.31 · 10^3 μM^{-2} s^{-1}

Table S2. Concentration of species used in calculations (volume = 0.08 μm^3). Gads represents Gads molecules; 2pY-LAT indicates doubly phosphorylated LAT peptide; 1pY-LAT represents singly phosphorylated pY171 LAT peptide.

Species	Concentration, μM	Concentration, number of molecules in 0.08 μm^3 volume
Gads	0.7	35
2pY-LAT	5	250
1pY-LAT	0 - 20	0 - 1000

Table S3. Rate constants used in calculations.

Rate Constants	Gads allele	Experimental Units	Computer Units	Reference
k_1=k_2=k_5 (k_{on} for 1st	WT	4.52 μM^{-1} s^{-1}	0.09 molec^{-1} s^{-1}	K_D =177 nM (Fig 3D)

binding event)	F92D	1.70 μM^{-1} s^{-1}	0.03 $molec^{-1}$ s^{-1}	K_D =470 nM (Fig 3D)
$k_3=k_4=k_6$ (k_{off} for 1st binding event)	both	0.8 s^{-1}	0.8 s^{-1}	Prasad, PNAS (2009)
$k_7=k_8$ (k_{on} for 2nd binding event)	each allele was modeled separately	unknown; estimated by OLS	initial guess equals k_1	n/a
k_{11}		unknown; estimated by OLS	initial guess equals to the value of k_1 in $molec^{-2}$ s^{-1}	n/a
$k_9=k_{10}$ and k_{12}		unknown; estimated by OLS	initial guess equals k_3	n/a

2.5. Results obtained using full computational model.

We used the full computational model to estimate all kinetic parameters for the Gads binding to the LAT peptide using sequential and simultaneous mechanisms (R^2 for WT was 0.99 and for F92D was 0.95, Fig. 6F). In this model, Gads formed dimers using the sequential association constants K_{A1} and K_{A2}, and the simultaneous association constant K_A. Parameters k_9, k_{10} and k_{12} were optimized by OLS.

Table S4. Rate constants used in calculations for full computational model in computer units for WT and F92D Gads SH2.

Systems \ Rate Constants	$k_{i=1,2,5,7,8}$, $molec^{-1}$ s^{-1}	$k_{i=3,4,6}$, s^{-1}	k_{11}, $molec^{-2}$ s^{-1}	k_9 *, s^{-1}	k_{10} *, s^{-1}	k_{12} *, s^{-1}
Wild Type	0.09	0.8	0.09	0.0015	0.0015	0.009
F92D	0.03	0.8	0.03	0.002	0.002	0.045
* Estimated by OLS						

Table S5. Kinetic parameters calculated from estimated rate constants by OLS for full computational model for WT and F92D Gads SH2.

Parameters	Formulas	Units	Wild Type	F92D
simultaneous association constant	$K_A = k_{11} / k_{12}$	μM^{-2}	2.35e+04	1.55e+03
simultaneous dissociation constant	$1/ K_A$	nM^2	42.6	643
sequential association constant	$K_{A2} = k_7 / k_9$	μM^{-1}	2.8e+03	752
sequential dissociation constant	$K_{D2} = 1/ K_{A2}$	nM	0.36	1.33
cooperativity for sequential mechanism	K_{A2} / K_{A1} #	-	496	355
cooperativity for simultaneous mechanism	$K_A / (K_{A1})^2$ #	-	736	345
# K_{A1} (WT) = 5.65 μM^{-1}; K_{A1} (F92D) = 2.12 μM^{-1}				

2.6. Results obtained using simplified computational model.

Simplified computational model used sequential binding to the singly phosphorylated LAT peptide and simultaneous binding to the doubly phosphorylated LAT peptide. In this model, Gads formed dimers using only the simultaneous association constant K_A. Parameters k_{11} and k_{12} were optimized by OLS. This model was unable to recapitulate the experimental trends (R^2 for WT was 0.59 and for F92D was 0.88, Fig. 6F), and thus we did not calculate all kinetic parameters from estimated rate constants for this model.

Table S6. Rate constants used in calculations for simplified computational model in computer units.

Systems \ Rate Constants	$k_{i=1,2,7,8}$, molec^{-1} s^{-1}	$k_{i=3,4,9,10}$, s^{-1}	k_5, molec^{-1} s^{-1}	k_6, s^{-1}	k_{11} *, molec^{-2} s^{-1}	k_{12} *, s^{-1}
Wild Type	0	0	0.09	0.8	0.008	1e-06
F92D	0	0	0.03	0.8	0.004	0.005
* Estimated by OLS						