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A THEORETICAL ANALYSIS OF THE POTENTIAL INFLUENCE OF ITAM PAIRS ON SYK RECRUITMENT DYNAMICS

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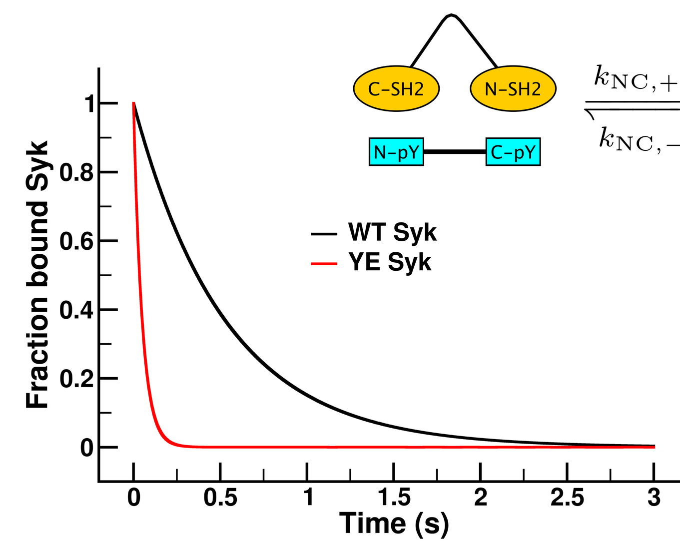
INTRODUCTION

Mast cell degranulation requires trans-autophosphorylation of spleen tyrosine kinase (Syk), which occurs in antigen-induced IgE receptor aggregates. Syk binds the receptor's γ-chain on its immunoreceptor tyrosine-based activation motif (ITAM), which contains two conserved tyrosine residues that bind to Syk's tandem SH2 domains. Recently, a mutant form of Syk (Y130E, or YE), has been shown to have distinct ITAM-binding kinetics from wild-type Syk, allowing direct investigation of the impact of Syk-ITAM association lifetime on downstream signaling (Schwartz, *et al.*, in prep). Here, we analyze a series of models to investigate physically feasible mechanisms consistent with existing data, and posit that the pairing of ITAMs in immune cell receptors may play a key role in the kinetics of Syk phosphorylation.

KINETIC PROOFREADING

Model A

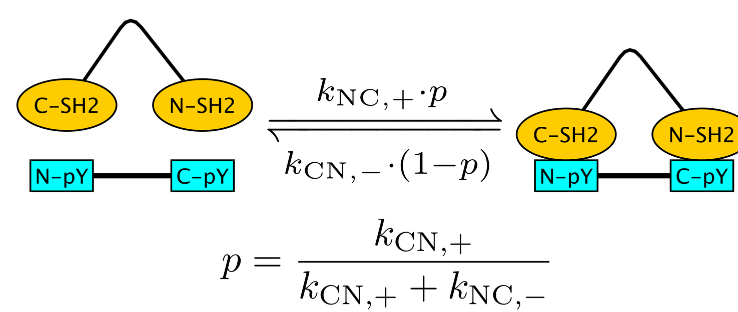


Feng & Post measured the kinetics of two-point attachment between Syk and an ITAM peptide. We used this information (model A) to calculate lifetimes of association (τ, see below table)

Model B

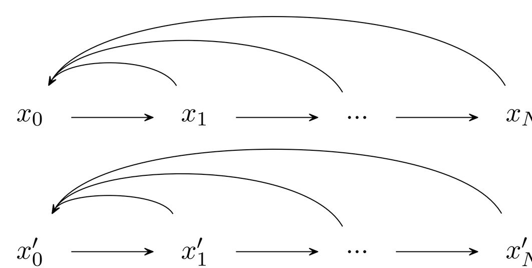
Simplification to a one-step binding mechanism (model B) does not significantly change the lifetime

Model	τ (WT)	τ (YE)
two-step	0.52 s	0.052 s
one-step	0.53 s	0.048 s

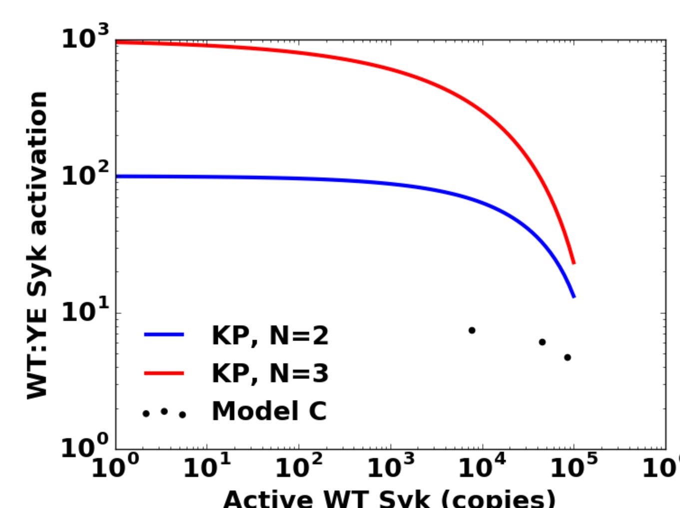


$$p = \frac{k_{CN,+}}{k_{CN,+} + k_{NC,-}}$$

We can estimate the ratio of WT to YE Syk phosphorylation with a simple kinetic proofreading model (McKeithan, 1995):



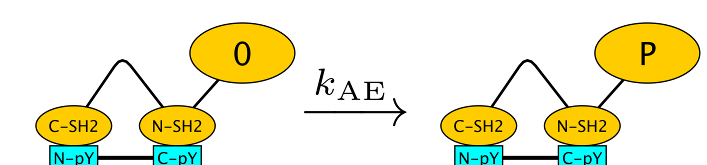
N is the number of proofreading steps, x_i is some chemical species that can be modified, and x_N is the active form (right).



The ratio of x_N to x'_N, where the x' species have faster conversion to x'_0 (shorter lifetime), decreases with the total number of active x_N or x'_N (left, red and blue).

Model C is Model B with Syk activation via a pseudo-first order rate parameter: the **autophosphorylation efficiency (AE)**

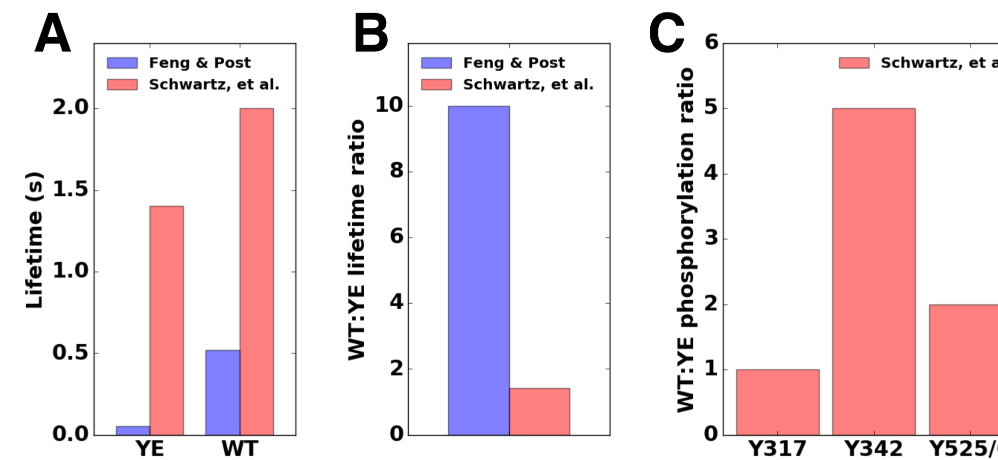
Model C

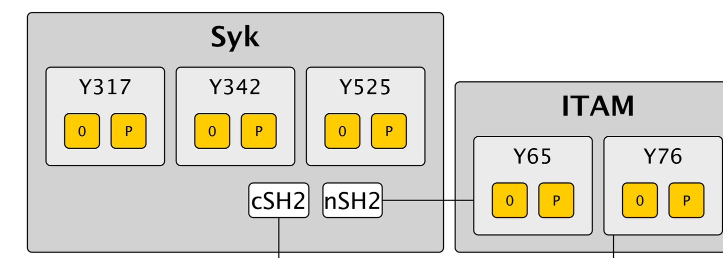


IN VIVO VS. IN VITRO KINETICS

Membrane-localization lifetimes in mast cells for WT and YE Syk are longer (A) and the ratio is smaller (B) *in vivo* than predicted by Feng & Post.

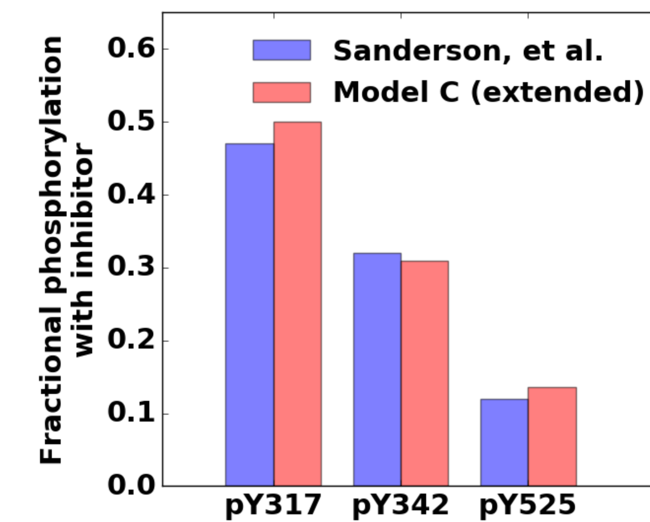
Key tyrosine residues are differentially phosphorylated on these two forms of Syk (C)





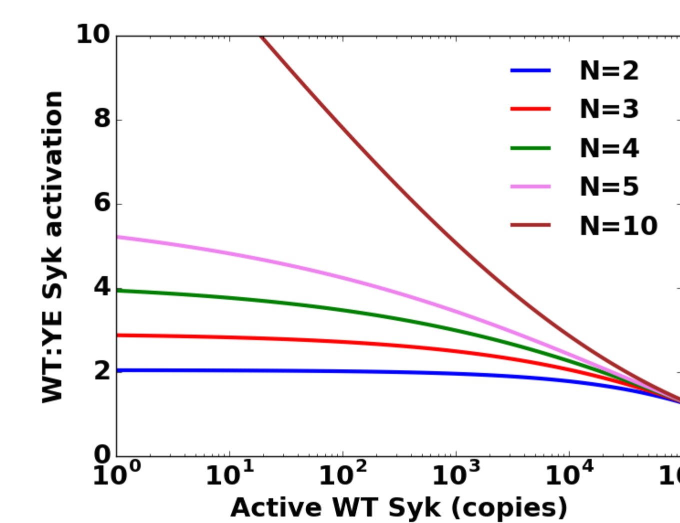
We added more mechanistic detail to Model C using the BioNetGen rule-based modeling language (Chylek, *et al.* 2014) to include Y317 and Y525/6 (left).

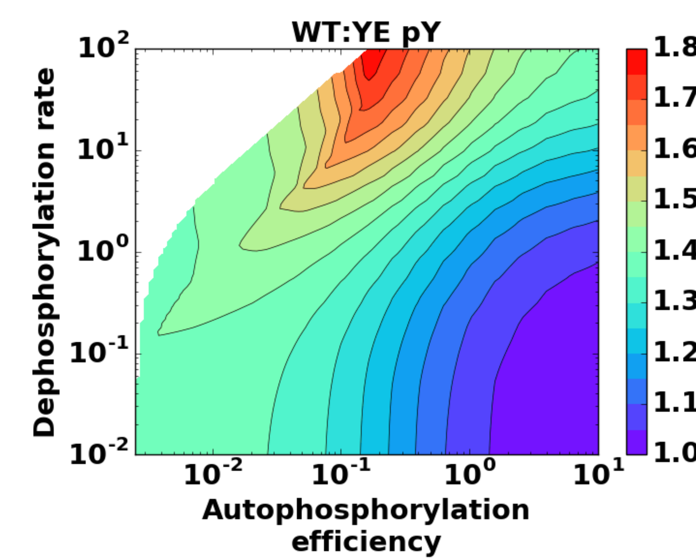
Sanderson, *et al.* (2010) observed differential fractional phosphorylation of these residues upon addition of Syk kinase inhibitor. Our extended Model C is capable of reproducing these results (right).



LIMITS OF PROOFREADING

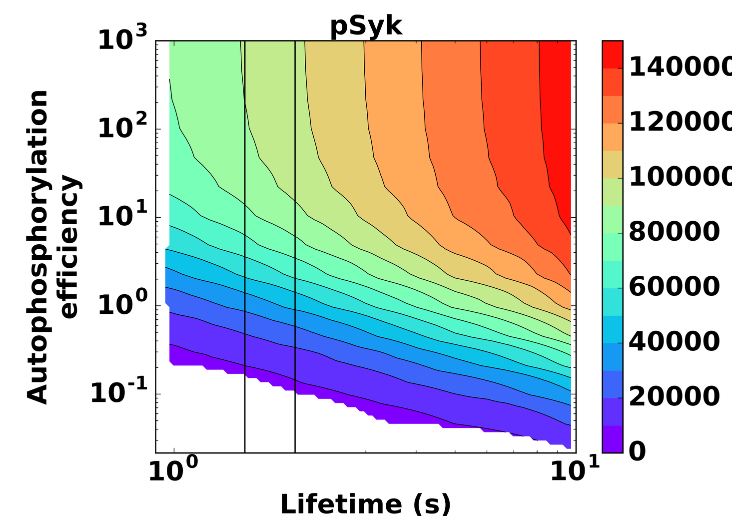
A 5-fold change with at least 0.1% Syk activation using the mast cell lifetimes in a kinetic proofreading model requires at least 5 modification steps (left). It is unclear what these 5 steps would be in a physical context





Model C, analogous to a 3-step proofreading model and modified to use parameters consistent with lifetimes from Schwartz, *et al.* (in prep), cannot produce a 5-fold change in phosphorylation.

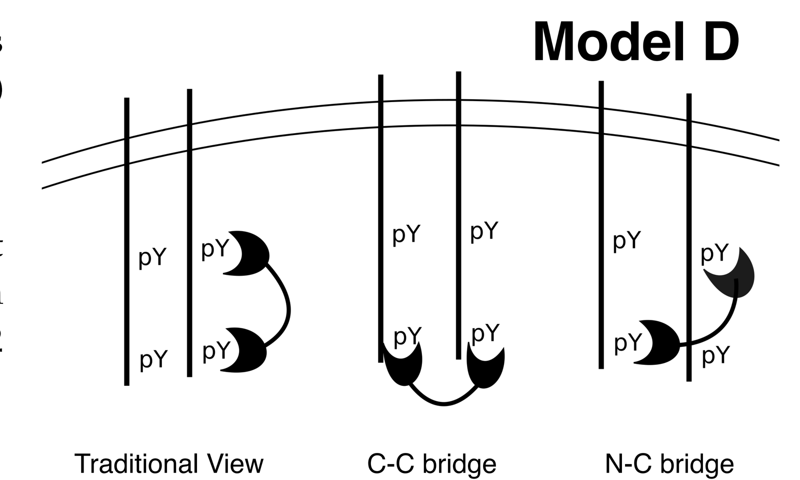
The vertical lines are the mast cell lifetimes of 1.4 s (YE) and 2.0 s (WT). Comparison with the above contour plot shows how lower absolute amounts of phosphorylation can correspond to a greater fold-change between WT and YE Syk.

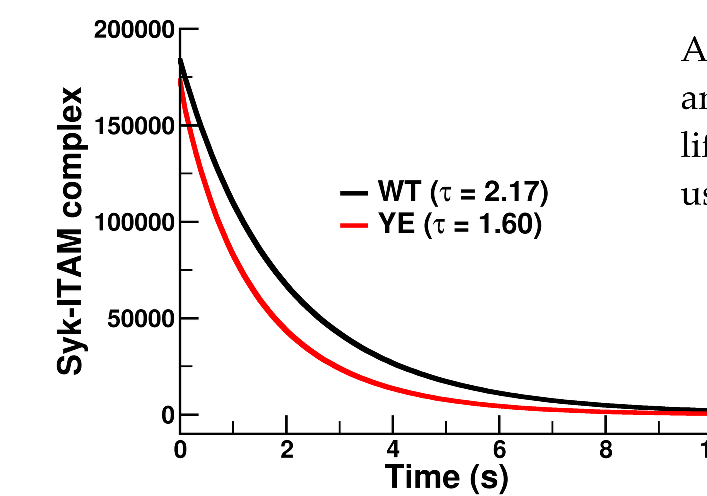


BRIDGING THE ITAM PAIRS

The presence of pairs of ITAMs (common in immune receptors) could also influence lifetimes

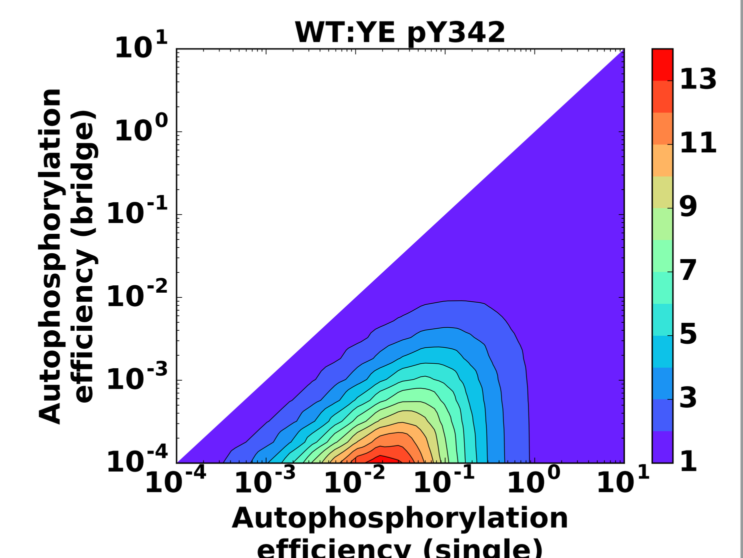
YE Syk is presumed to be better at bridging due to an increase in uncoupled motion of its SH2 domains (Feng & Post, 2015)





A model with bridging (above), can generate an ensemble of models whose mast cell lifetimes are within 20% of the *in vivo* values using the Feng & Post kinetic parameters (left).

With distinct autophosphorylation efficiencies for bridged and single-ITAM bound Syk, the bridging model achieves a 5-fold change in phosphorylation with at least 0.1% active Syk.



CONCLUSION

We present an alternative hypothesis explaining how a 30% reduction in lifetime might result in a 5-fold change in downstream phosphorylation events. Kinetic proofreading models show a greater capacity for distinguishing between WT and YE Syk lifetimes than model C. However, model C can reproduce qualitative phosphorylation dynamics in the presence of kinase inhibitor and WT:YE phosphorylation ratios for Y317 and Y525/6. The 5-fold decrease in YE Syk phosphorylation on Y342 cannot be explained with our existing understanding of the system. However, if Syk can bridge ITAM pairs, as in model D, we can reconcile all data (including the apparent discrepancy in lifetime measurements from Schwartz, *et al* and Feng & Post). Since our argument depends on the proximity of ITAMs, we expect that quantitative experiments examining how Syk phosphorylation varies with phospho-ITAM density will provide evidence for or against our predictions.

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