SAND2013-9244C

Pulsed Stimulation Reveals Short and Long-Term Memories in Mast Cells

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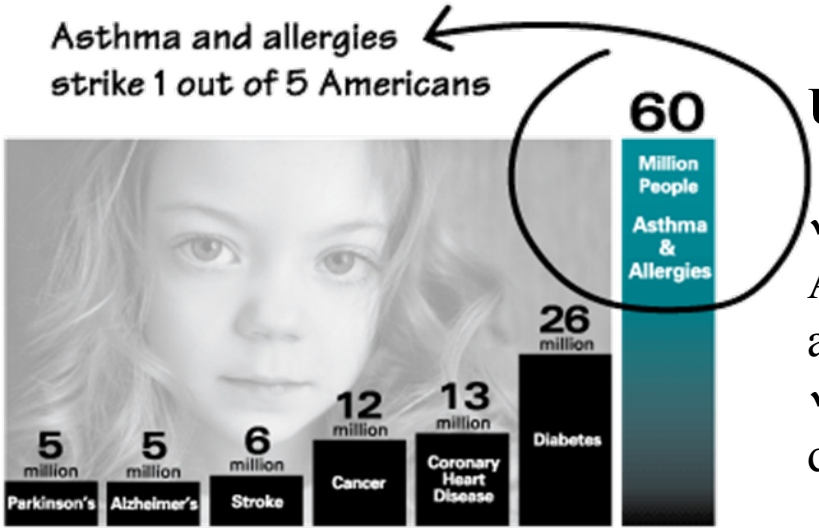
Allergic disorders afflict roughly 25% of people in the developed world.



United States:

✓ An estimated 60 million Americans suffer from all types of allergies.
✓ Allergy is the 5th leading chronic disease in the U.S. among all ages.

Worldwide:

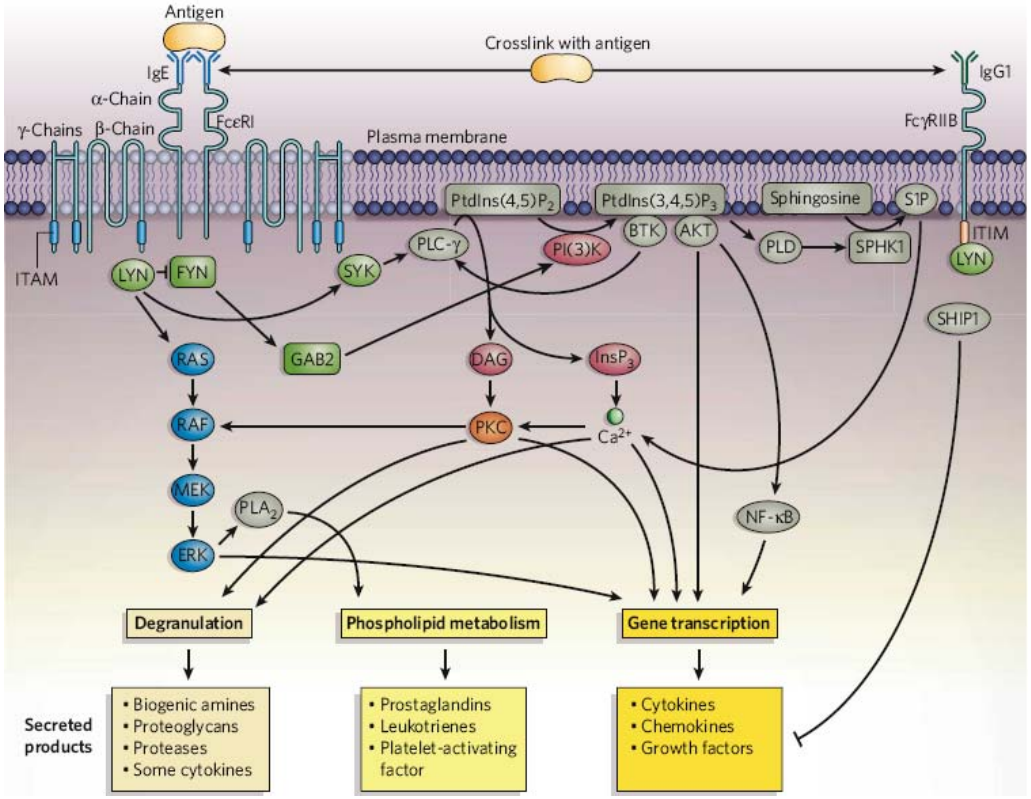
✓An estimated 300 million people suffer from asthma.

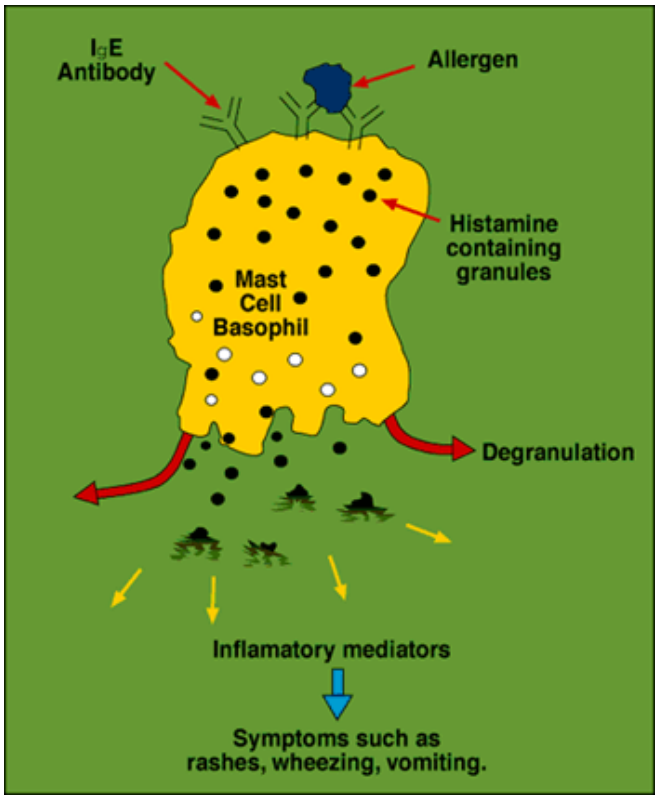
✓ The number of people with asthma will grow by more than 100 million by 2025.

✓ Approximately 250,000 people die prematurely each year from asthma.

• *Nature, 2008, 454, 445*
•*Annual U.S. Prevalence Statistics for Chronic Diseases*
• *World Health Organization. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach, 2007*

FcεRI signaling in mast cells



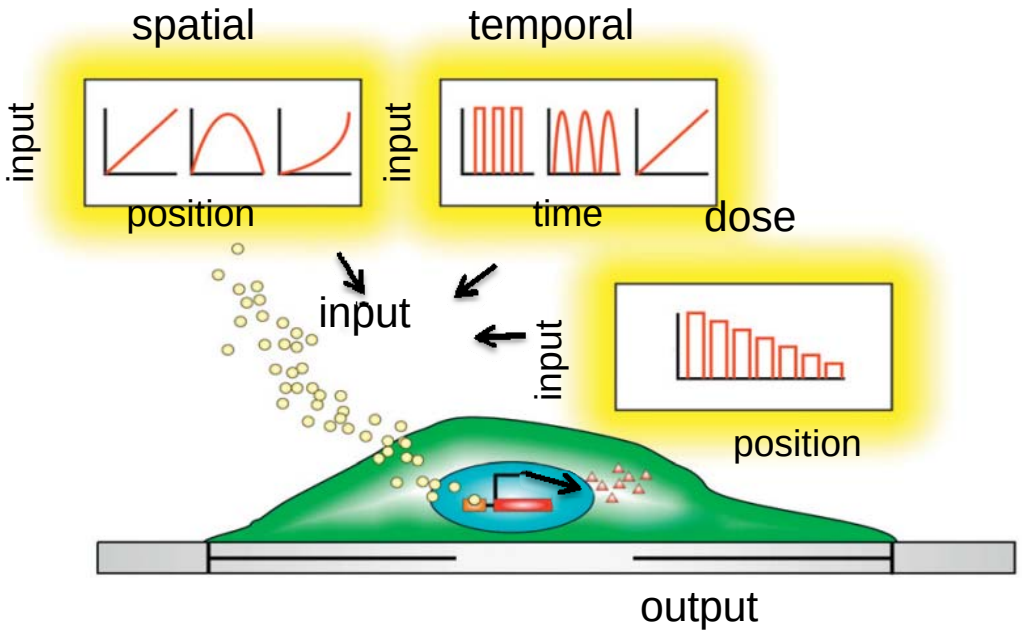


The practice in most biological labs:
SINGLE and PERSISTENT
exposure of antigens in dishes

The reality *in vivo*:
REPETIVE and PULSATILE
inflammatory signals

Nature, 2008, 454, 445

Microfluidics: precise control of fluidics in space, time and magnitude



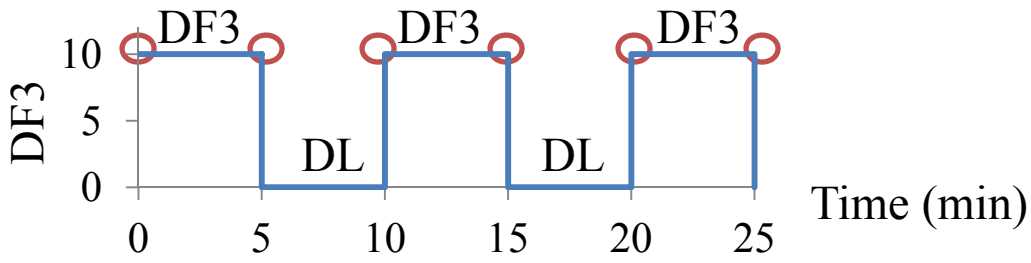
To mimic pulsatile inflammatory signals *in vivo* ,
we use **pulsed stimulation via microfluidics** to challenge mast cells.

Y. Liu, A.K. Singh, JALA, 2013, critical review
B. Lin, A.Levchenko, Curr. Opin. Chem. Biol. 2012, 16:307–317

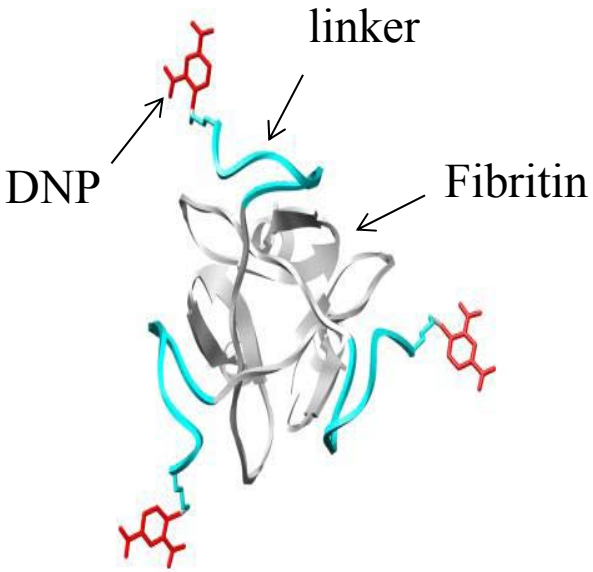
Synthetic allergen (DF3) & Allergen remover (DL)

Pulsed stimulation

Assay point

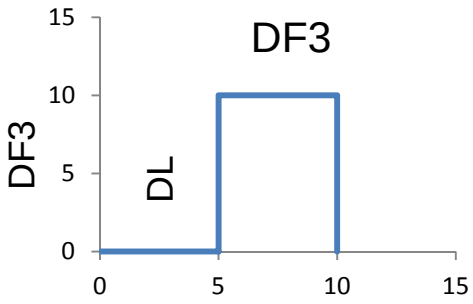


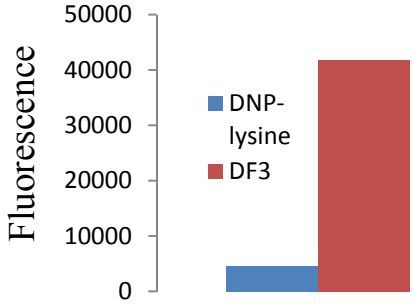
Trivalent DNP_3-Fibritin (DF3)



(DNP: 2,4 – dinitrophenol)

Monovalent DNP-lysine (DL)

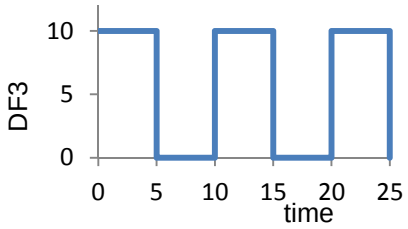




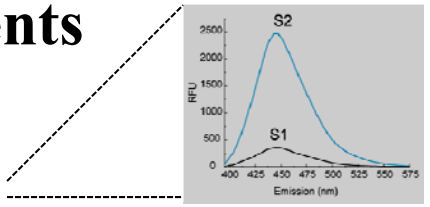
DL: 1) Not to activate cells,
2) Not to suppress the secretion,
3) To remove DF3 from IgE-FcεRI complex.

Integrated microfluidic platform

Pulsed stimulation



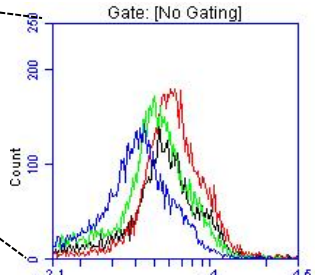
Response measurements



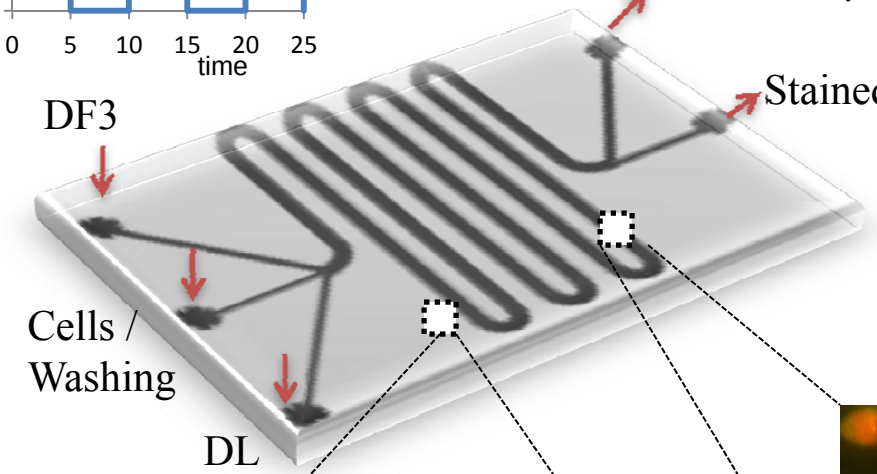
Secrected β-hexosaminidase

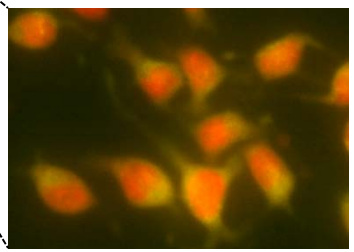
3).Fluorescence spectrometry

Stained cells

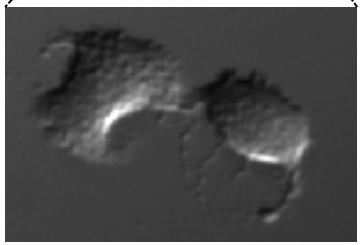


4). flow cytometry





2). Fluorescence microscopy

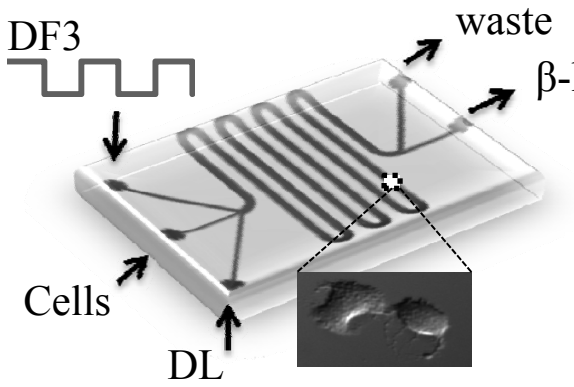


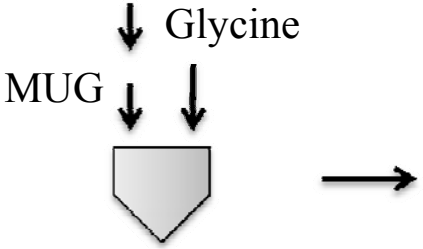
1). Live cell imaging

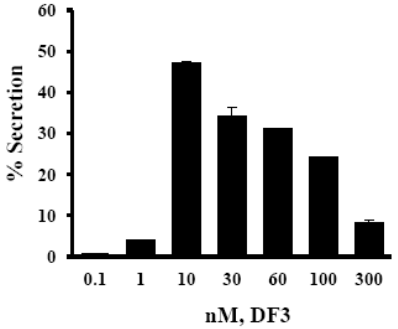
PDMS @ coverslip: 180μm (w), 50μm (d)

Y. Liu et al PLoS ONE, 2013, 8, e60159

Negative memory present in mast cells!



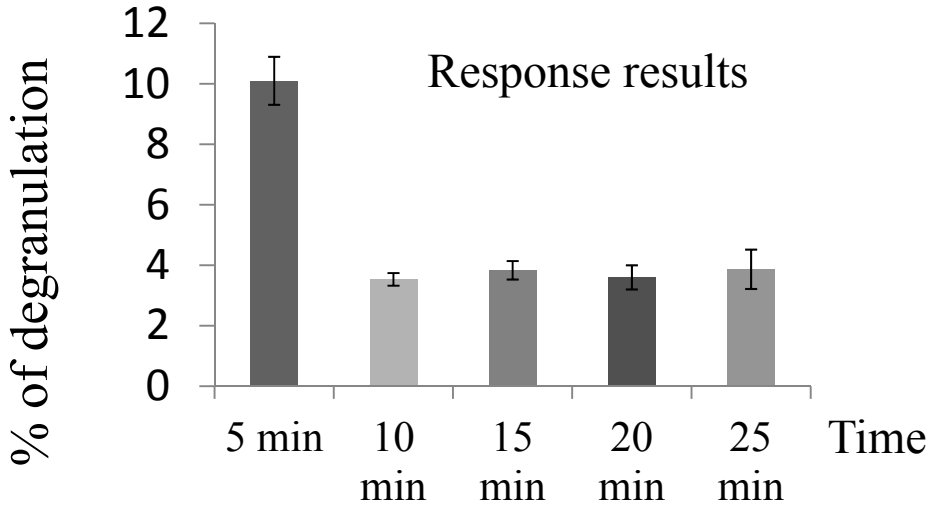




1). On-chip culture & Pulsed stimulation

2). Fluorgenic enzymatic assay

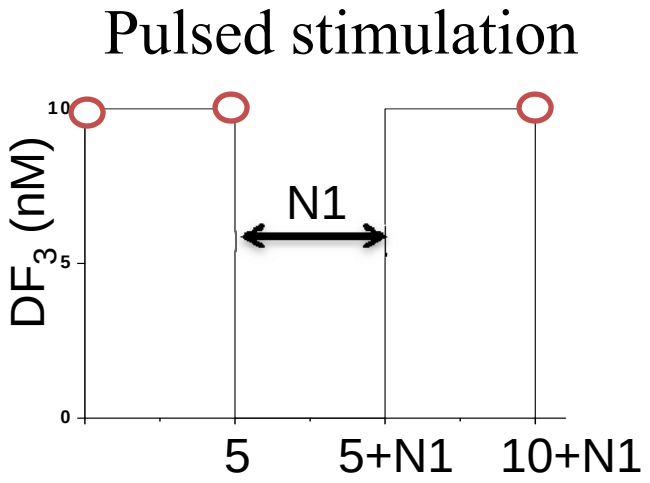
3). Degranulation response (% total β-hexosaminidase released)



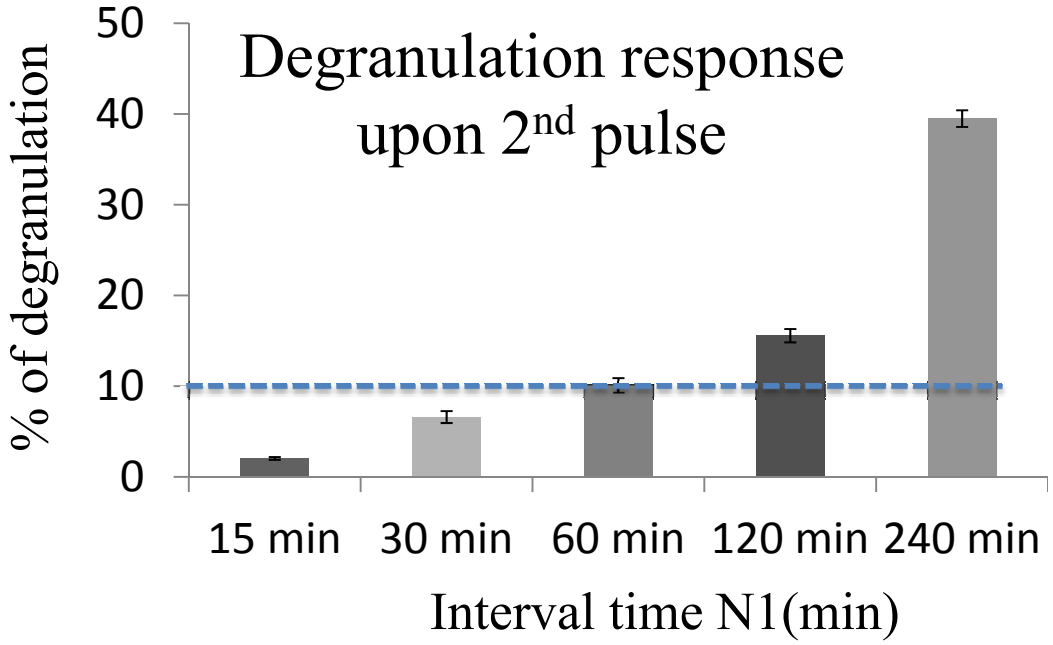
Subsequent pulses observe "de-sensitization" after the 1st pulse.

" negative memory"

Positive memory present in mast cells, too!

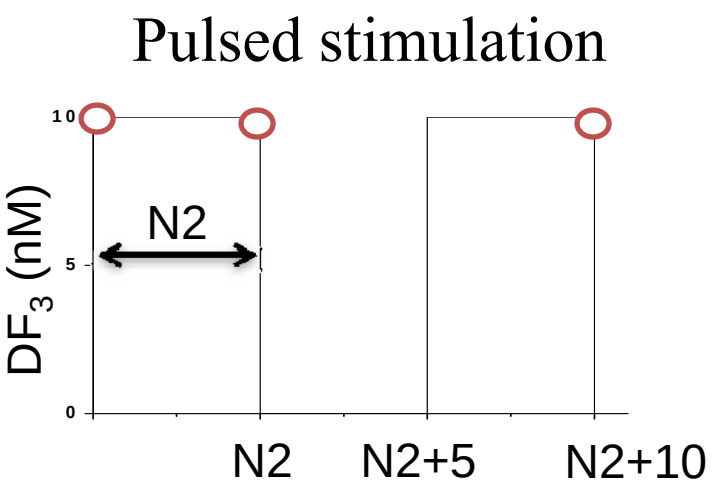


Interval time (min),
N1 = 5, 30, 60, 120, 240

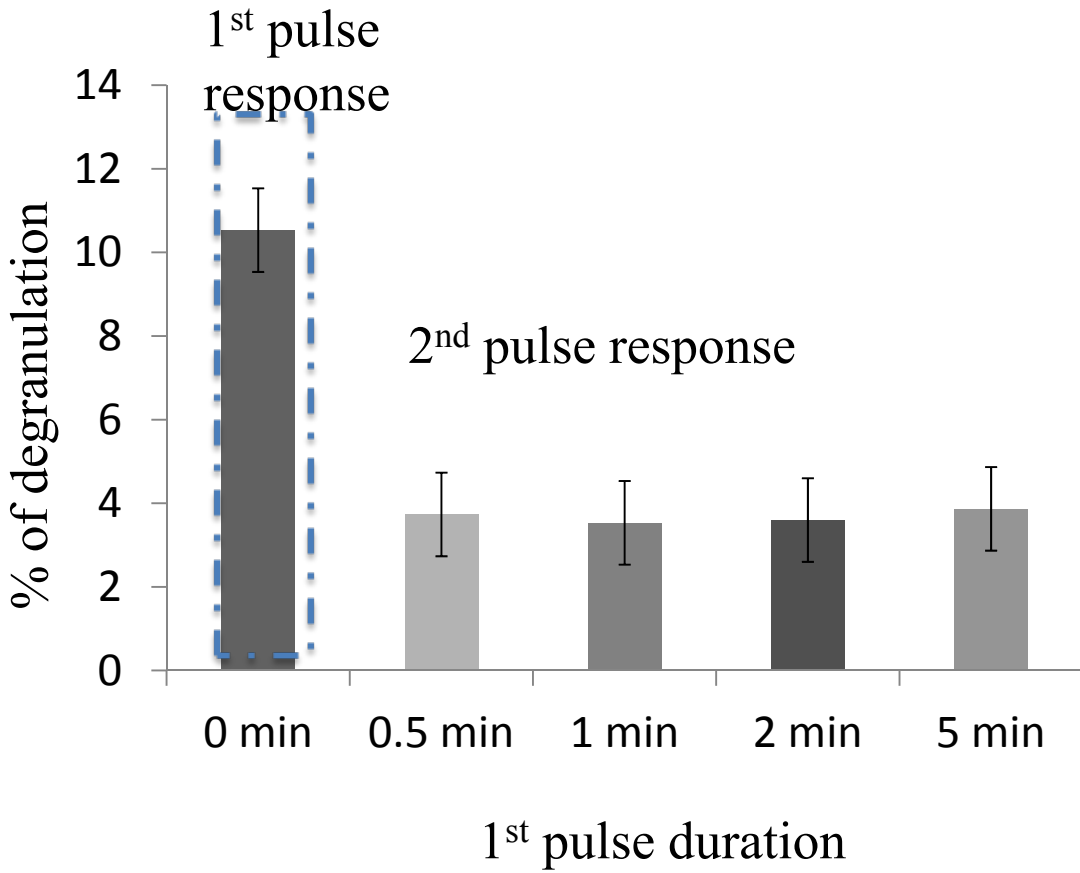


- **Short-term memory (Negative memory)**
  – suppressed response within 60 min of recovery time
- **Long-term memory (Positive memory)**
  – hyper response after 120 min of recovery period

Duration of 1st pulse matters



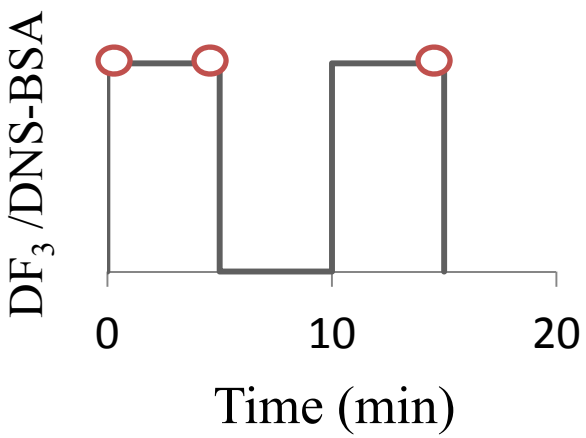
Time (min), N2 = 0, 0.5, 1, 2, 5

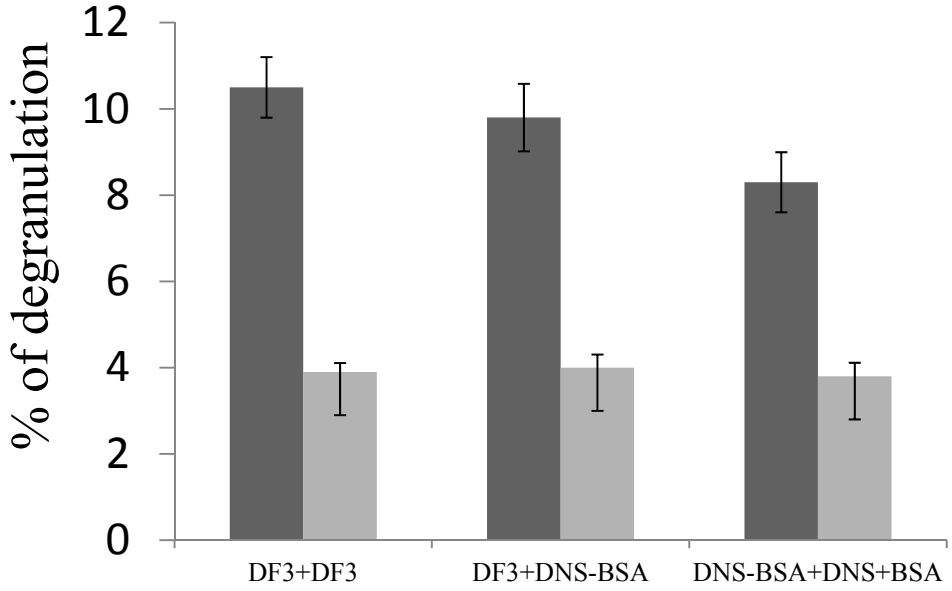


As short as ~30s pulse leads to the short memory.

Memories are not specific to DF3

- Other synthetic allergen other:
  Dansyl conjugated BSA (DNS-BSA)
- 50-50 "priming" with anti-DNP and anti-dansyl IgE

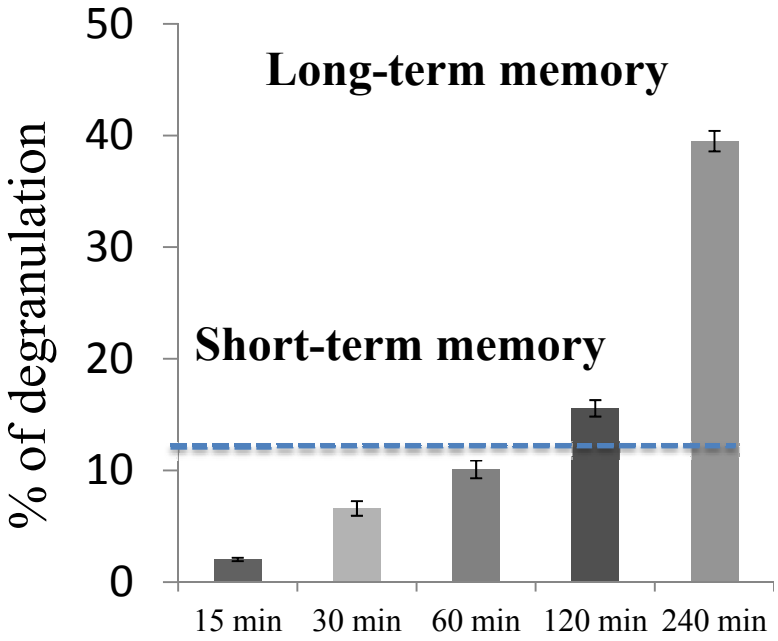




- "Memory" stimulated by Dansyl-BSA observed.
- Cells are globally activated and dampening responses to subsequent stimuli.

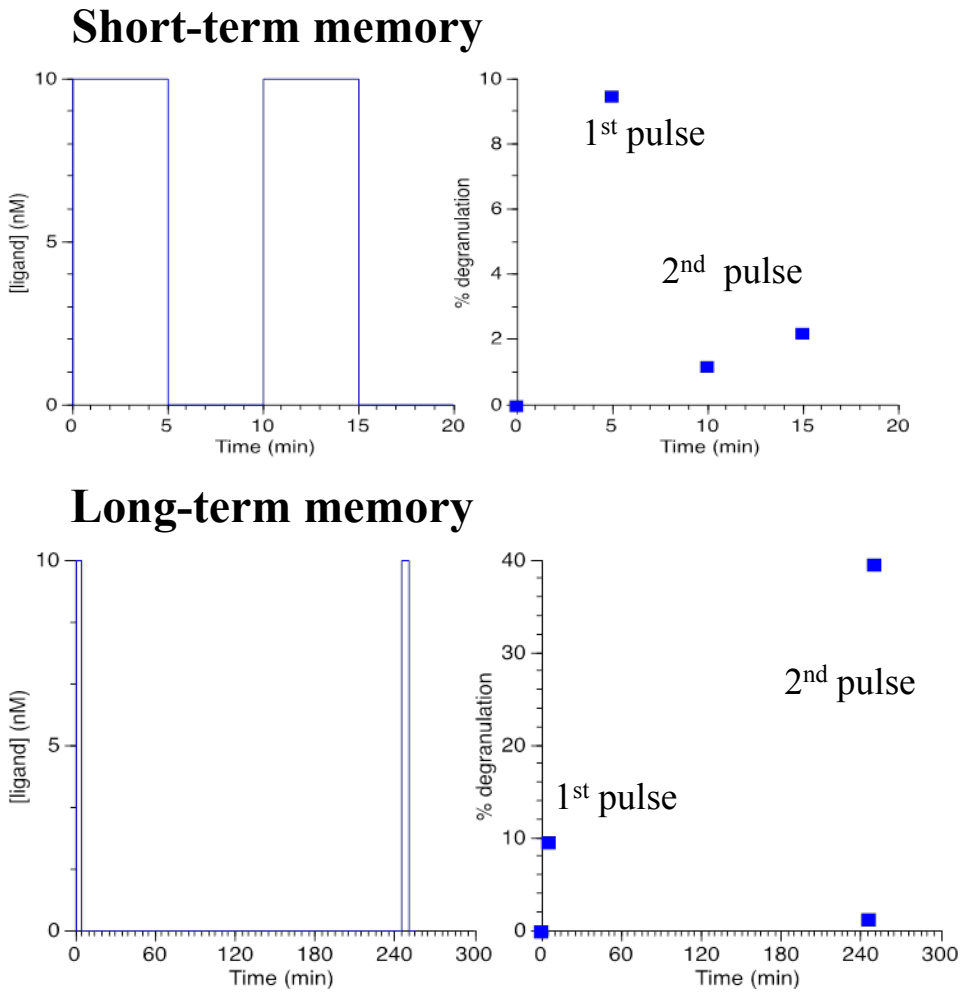
We have a match!

Experimental data:



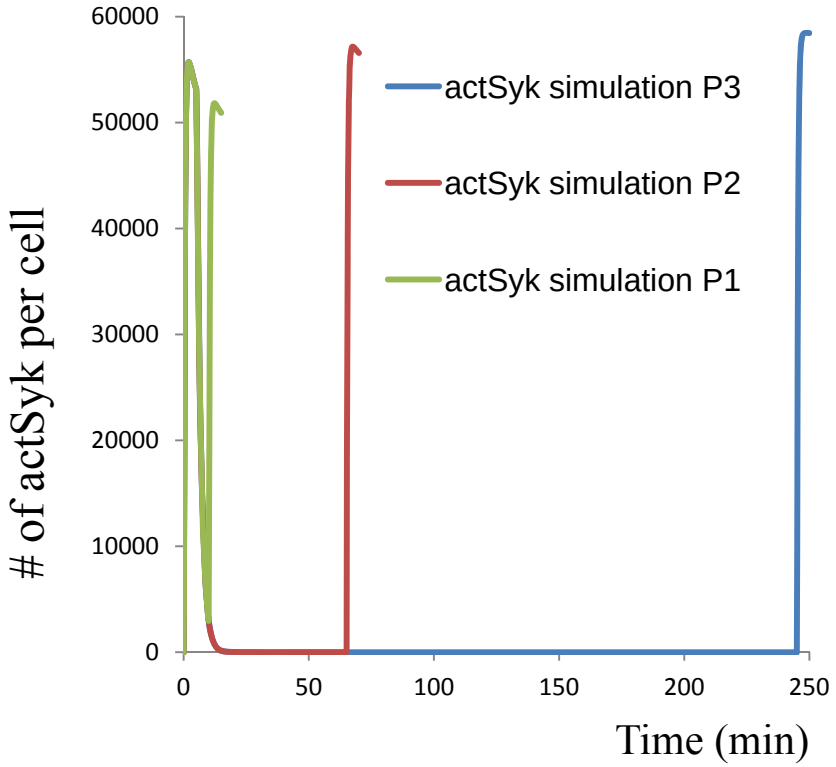
Experimental degranulation response

Computational model results for IgE receptor signaling:

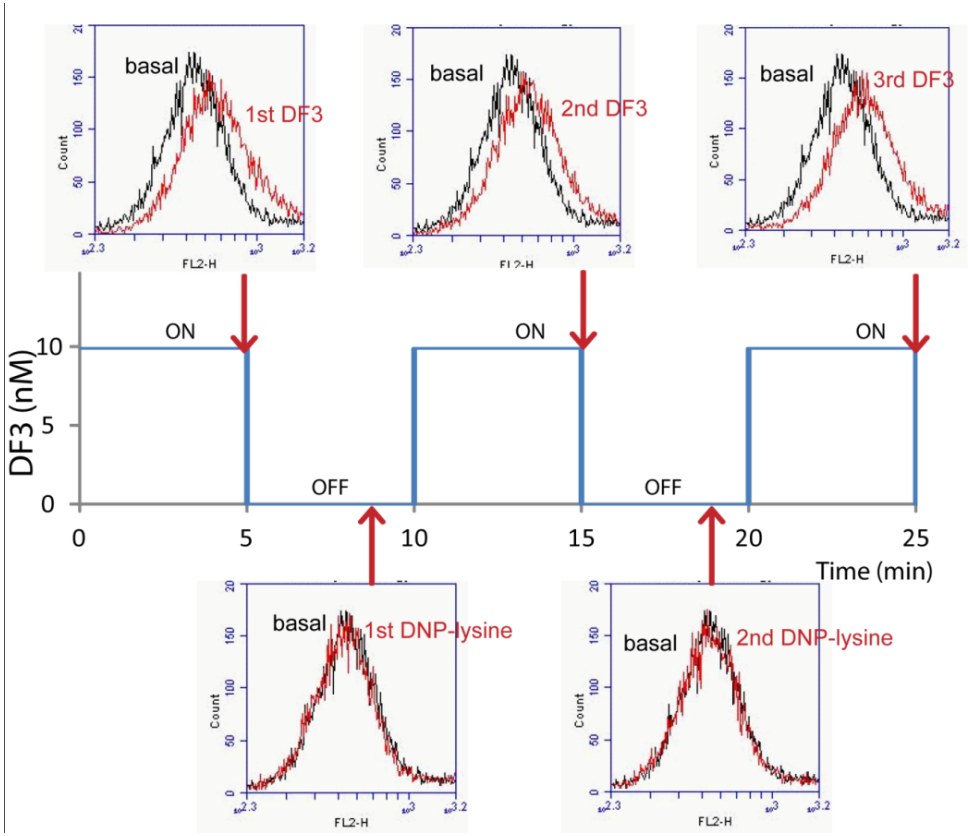


Theoretical degranulation response

Short-term memory:
Syk activates upon each pulsed stimulation



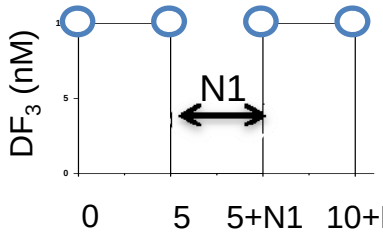
Model prediction



Flow cytometry measurement

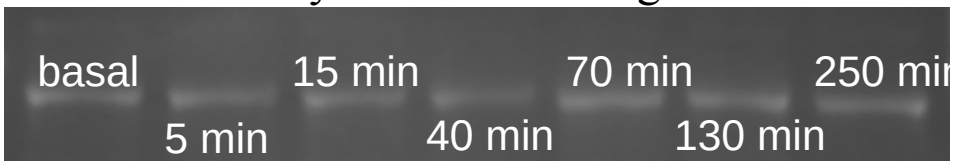
Syk phosphorylation pattern does not change from pulse to pulse as predicted by the model

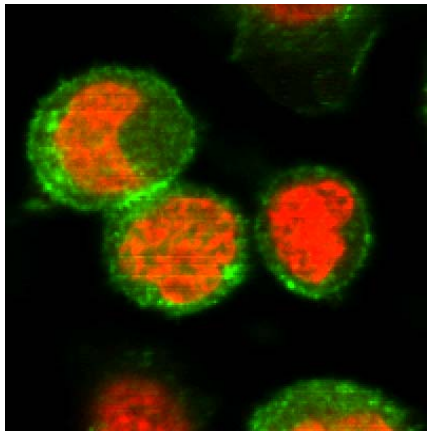
Short-term memory:
SHIP1 activity decreases over time



Interval time N1 (min), N1 = 5, 30, 60, 120, 240
Assay time points (min): T= 0, 5, 5+N1, 10+N1

Total SHIP1 by Western blotting

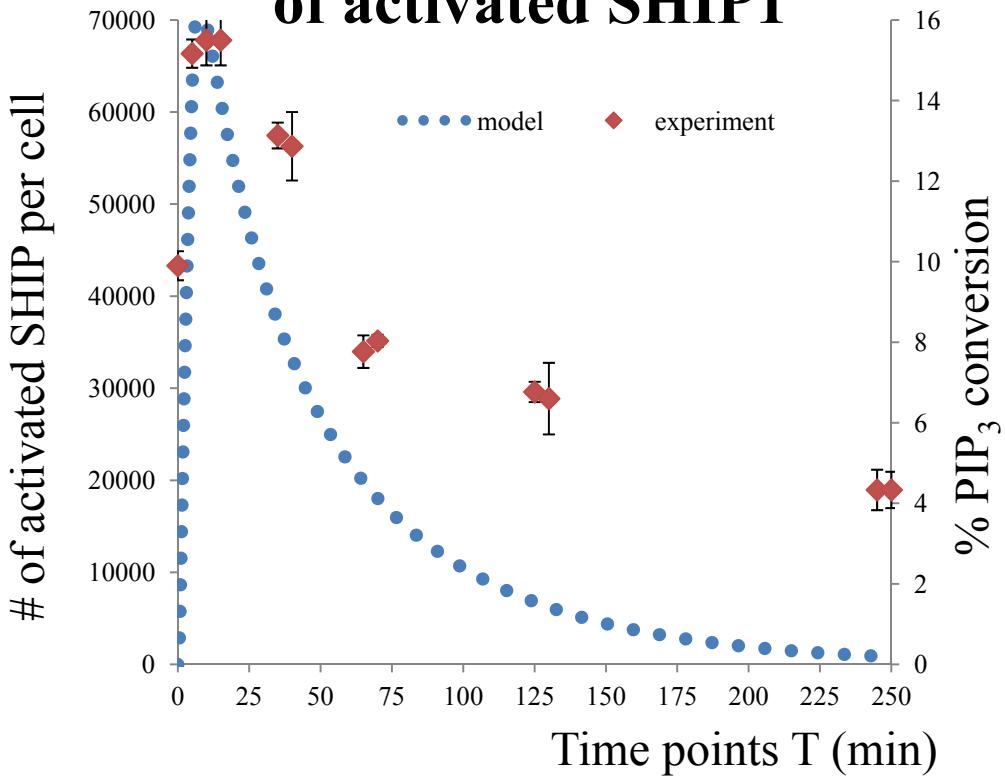




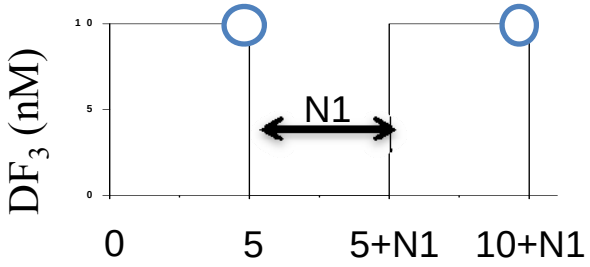
aggregation of SHIP1
@ membrane

Experiment & simulation show
SHIP1 activity decreases over time

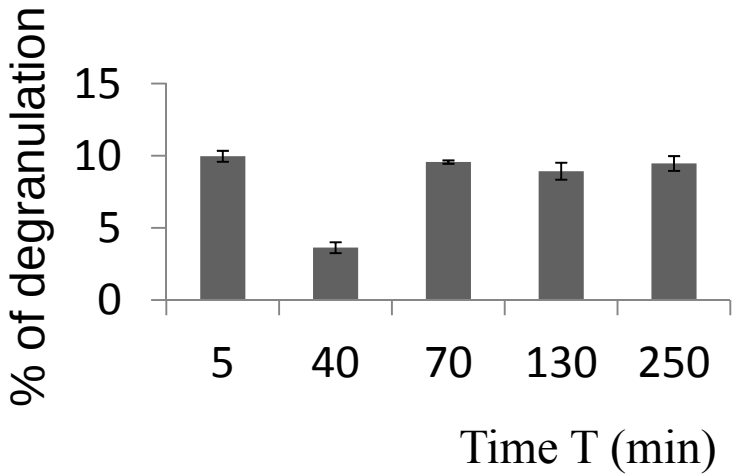
Biochemical analysis of activated SHIP1



Long-term memory:
Degradation via proteasome

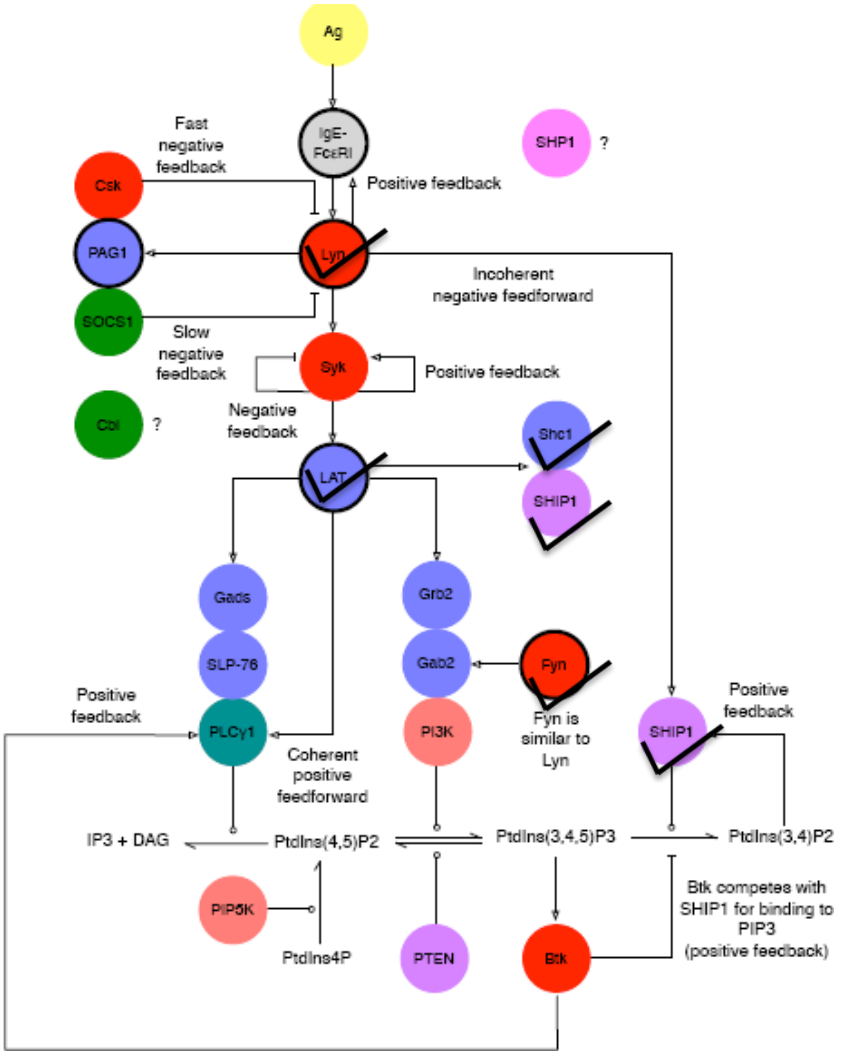


Interval time N1 (min), N1 = 5, 30, 60, 120, 240
Assay time points (min): T= 0, 5, 5+N1, 10+N1



Proteasome inhibitor MG132 removes hyper-degranulation!

The myth: who being degraded?



Summary

✓ **Integrated microfluidic platform allows pulsed stimulation.**

✓ **Two types of memories revealed via pulsed stimulation:**

1) Short-term (Negative) memory
2) Long-term (Positive) memory

✓ **Computational simulation matches experimental data, predicts the involvement of certain proteins.**

✓ **Potential mechanism: Interplay among p-Syk, actSHIP1, Lyn, LAT…**

Short-term memory : both Syk and membrane SHIP1 remains active

Long-term: a hyper-degranulation response due to the degradation of certain proteins (negative signaling) and p-Syk dominates the signaling

Acknowledgement

Sandia Nations Labs

Anup Singh
Carl Hayden
Benjamin Schudel
Pam Lane
Mais Jebrail
Junyu Mai
Brook Harmon
Oscar Negrete

Los Alamos National Lab

William S. Hlavacek

STMC
SpatioTemporal Modeling Center

University of New Mexico

Bridget Wilson
Avanika Mahajan
Diane Lidke
Anna Holmes

UNM

Sandia National Laboratories

NIGMS