Visualizing membrane binding/unbinding event of Src homology 2 (SH2) modules

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# Epidermoid Carcinoma A431 Cell

What is happening inside the cell after extracellular stimulation is applied ?



EGF added at 8 min, cell immediately develops lamellipodia, at 28 min cell shrinks and begins to move.

### Phosphotyrosine (pTyr) signaling pathways inside cell



#### The phosphotyrosine tool box



Lim, W. A., and Pawson, T. (2010) Cell 142, 661-667

-Tyrosine phosphorylation, discovered by Tony Hunter in 1979, is the basis of almost all cellular signaling pathways.

-SH2 protein domain, first identified on Src by Tony Pawson in 1986, connects phosphorylated signal receptors with the rest of their pathway.

### In vivo membrane recruitment of SH2 domains

TIRF microscopy (at lower video rate for longer imaging time) Real time imaging of membrane recruitment of  $PLC\gamma_{SH2(C)}$ PLC $\gamma_{SH2(N)}$  domains following 25 ng/ml of EGF stimulation



### Dynamic membrane recruitments of SH2 modules



Related SH2 modules show different EGF response curves,

implying the specific tyrosine phosphorylation rate for different SH2 modules or unknown binding mechanism. ?? In vivo kinetic parameter measurements

Single molecule TIRF microscopy (at fast video rate for shorter imaging time)

apparent dissociation rate ( $\lambda_{off}(t)$ )

# Tracking molecule using standard cross-correlation method Gelles, J. et al. Nature 331, 405-453 (1988)





## Hazard rate $\lambda(t)$ of Grb2<sub>SH2</sub>



$$\lambda(t)_{SH2} = \lambda(t) - \lambda(t)_{pb}$$

additive for independent processes



## Correlation between binding time and mobility



Observed correlation is fitted with the equation derived from the theory of diffusion-reaction model: Otto G. Berg. Che. Phy. 31 (1978)

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## Rebinding model of SH2 to the p-Tyr of EGFR



~20 time rebinding for Grb2 (SH2)

Fast rebinding event depends on the concentration of p-Tyr in the vicinity of the first binding





# Conclusion

SH2 modules stay longer in the clustered-pTyr regions due to the fast rebinding (positive correlation between D(t) and  $\lambda_{off}(t)$ ).

The binding rate of SH2 modules to pTyr was prolonged in a clustered-pTyr regions presumably due to protection of pTyr site from phosphatases through multiple rebinding of SH2.