The Department of Biochemistry and Molecular Microbiology

George S. Wise Faculty of Life Sciences

Tel Aviv University

Characterizing the dynamics and mobility of Fat4 and Dachsous1 (Ds1) planar cell polarity proteins

Thesis Presentation for the degree:
Master of Science

By

Nadav Gordon Bar

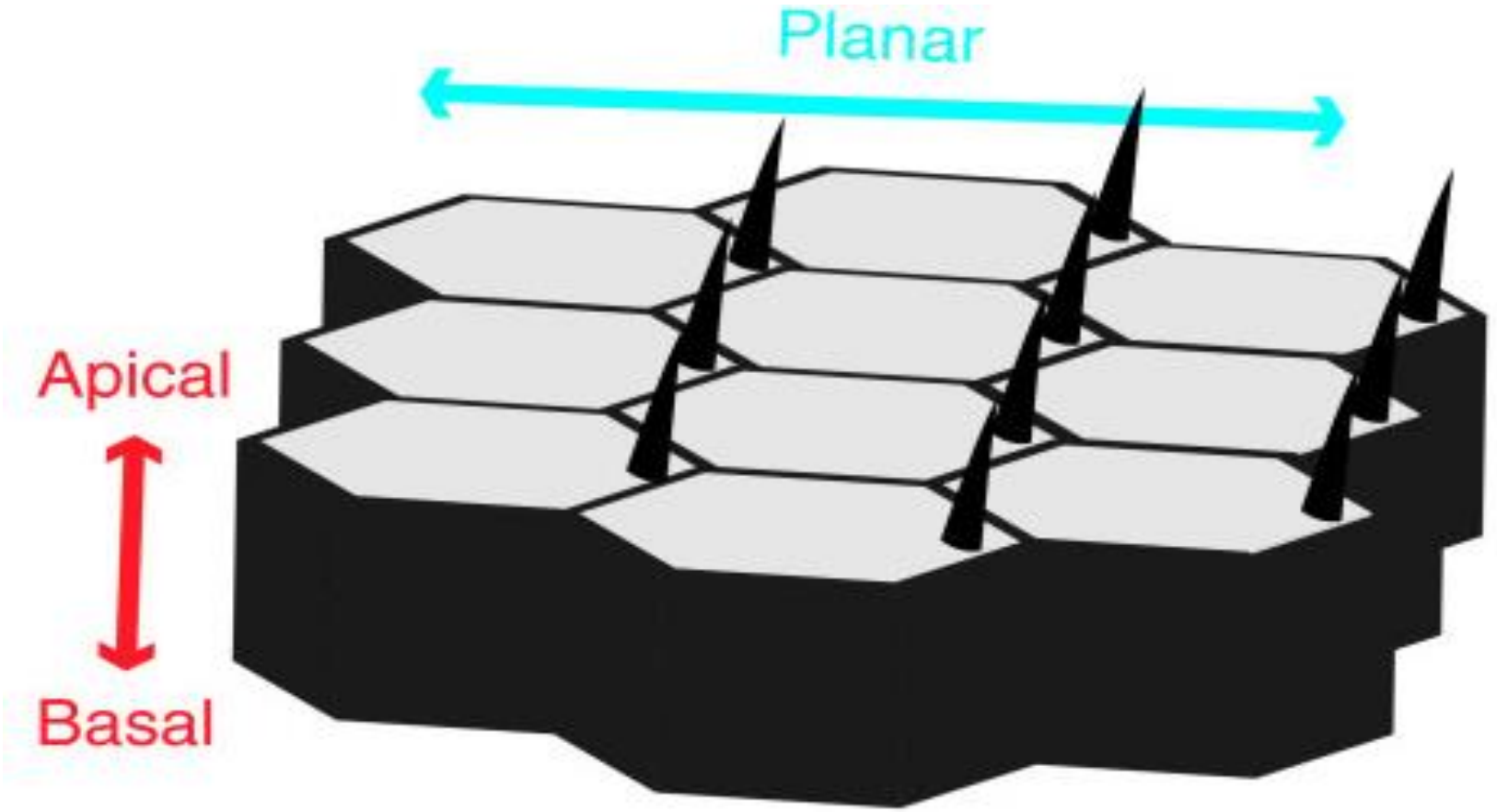
Supervision: Dr David Sprinzak

Introduction

Introduction to Planar Polarity

PCP defines **direction** in the **plane** of the cell sheet

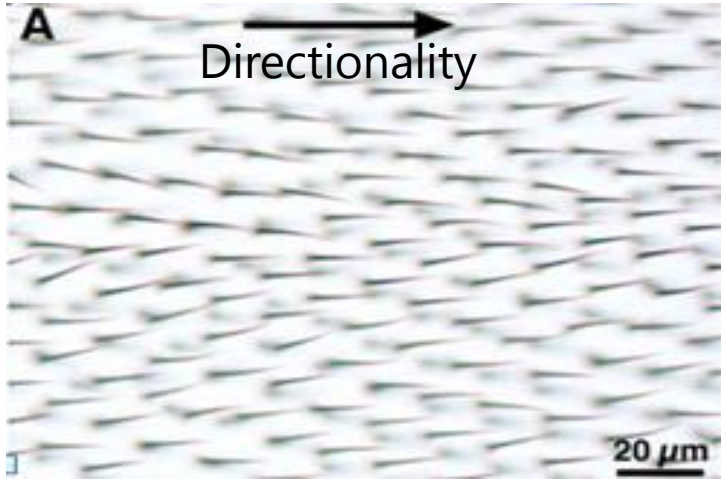
- **Two types** of **polarity**:
  - **Apical-basal**
  - **Planar polarity**



PCP **regulates directionality and growth** during **morphogenesis**

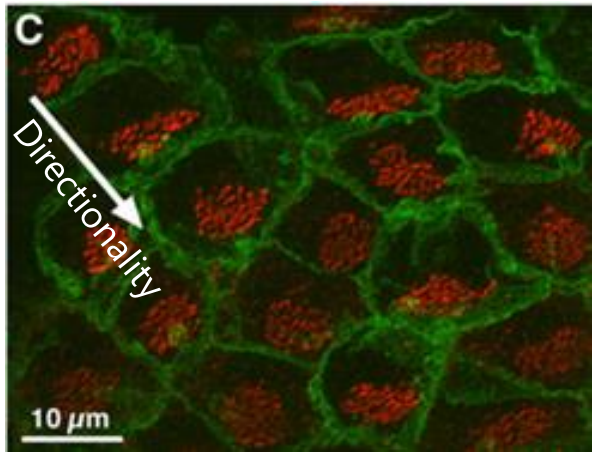
PCP is an essential aspect of developmental processes

Drosophila wing surface

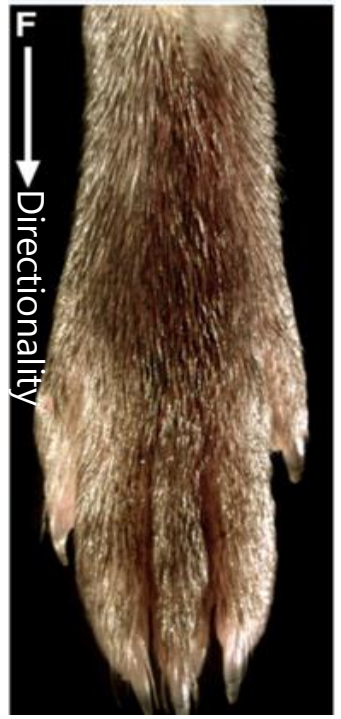


Drosophila leg

Mouse brain cells



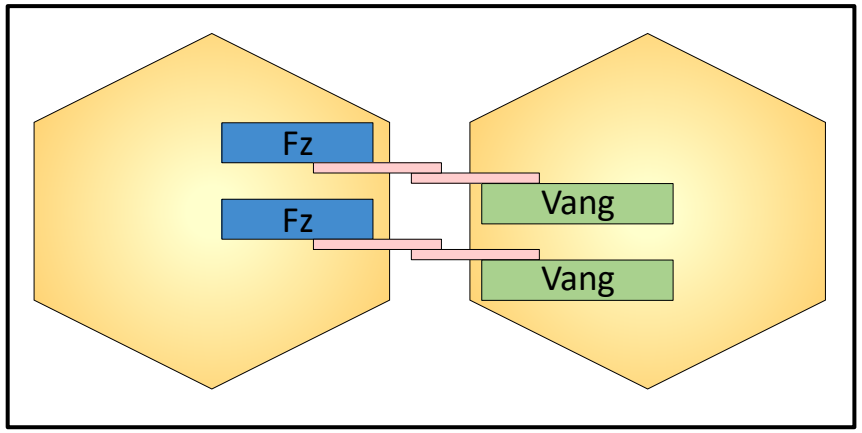
Mouse fur



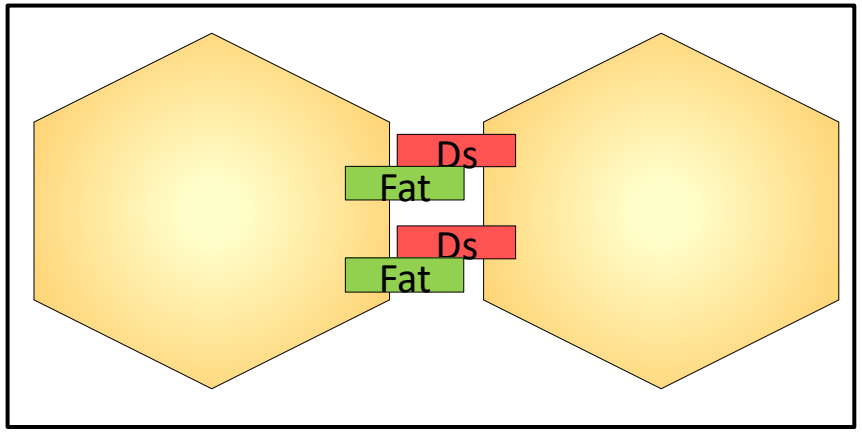
Goodrich & Strutt, 2011

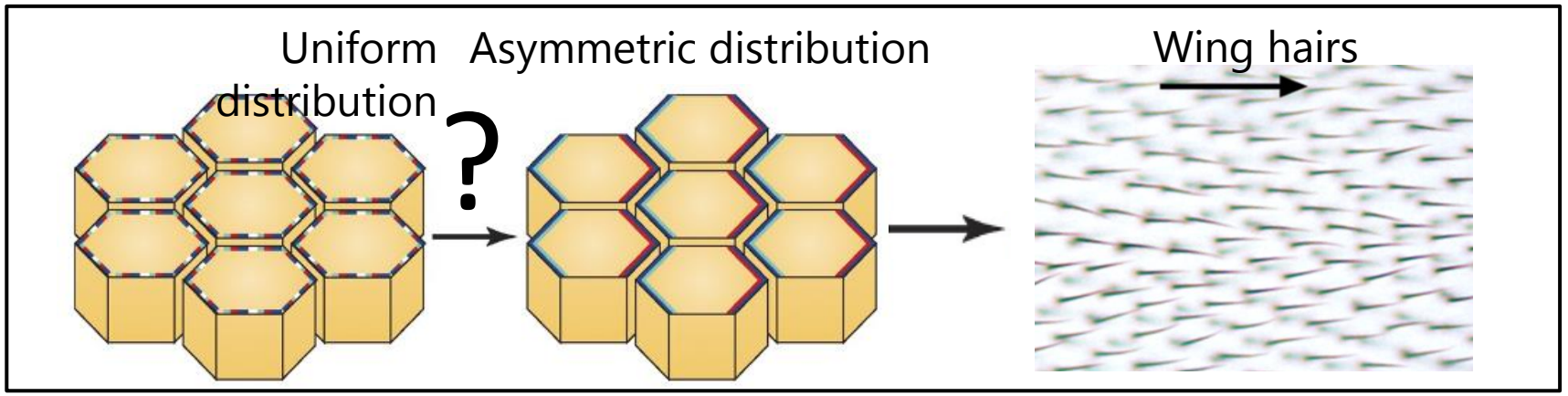
The two PCP pathways

The 'core' pathway



The Fat-Dachsous pathway



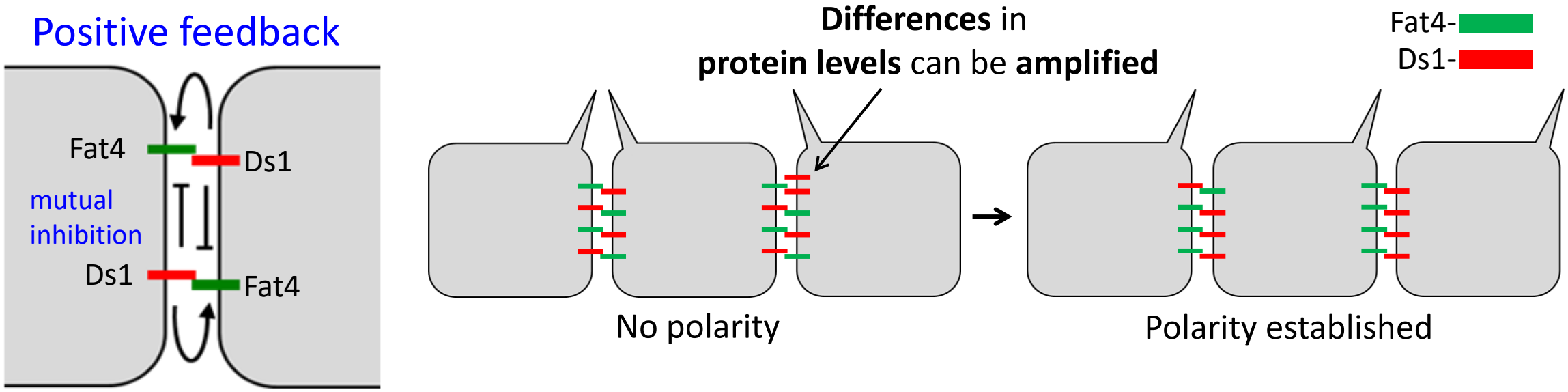


Images adopted from:

- Devenport 2014
- Goodrich & Strutt, 2011

PCP and the **Fat4-Ds1 Pathway**

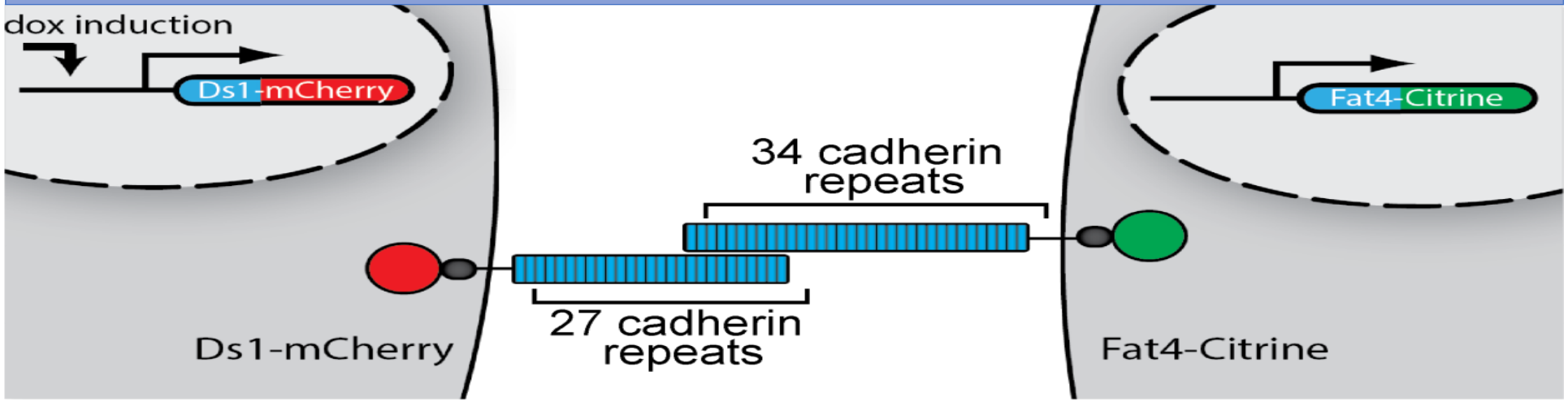
Localized feedbacks between Fat4-Ds1 complexes can give rise to PCP

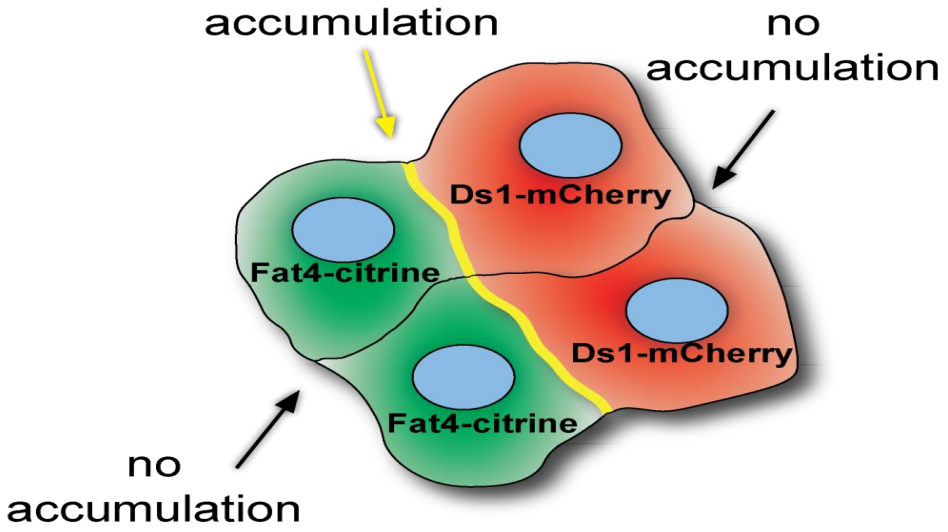


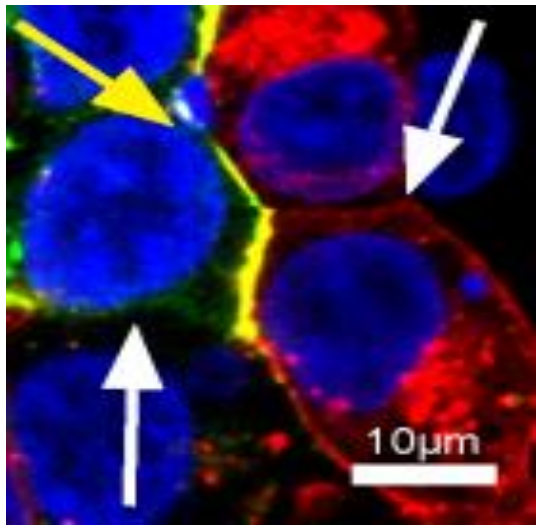
Positive feedback will lead to **spontaneous polarization** once a **critical concentration** of complexes is achieved.

Previous Work – Experimentally Test The model

A synthetic biology approach for studying Fat4-Ds1 signaling



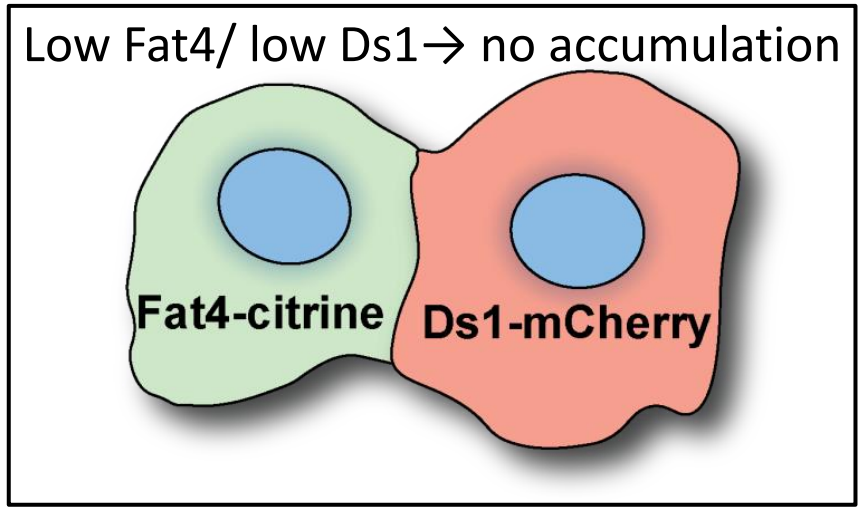


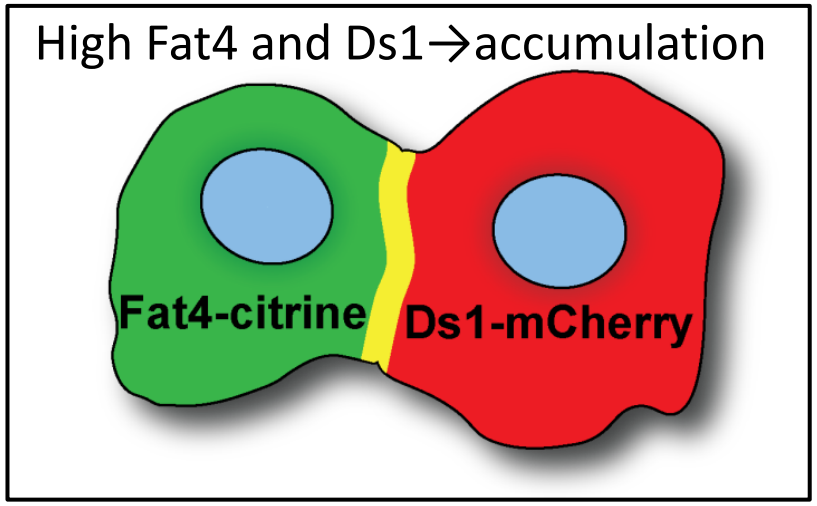


Previous Work was performed in collaboration with Dr. Olga Loza

Previous Work – **Experimentally Test The model**

Threshold accumulation response is observed

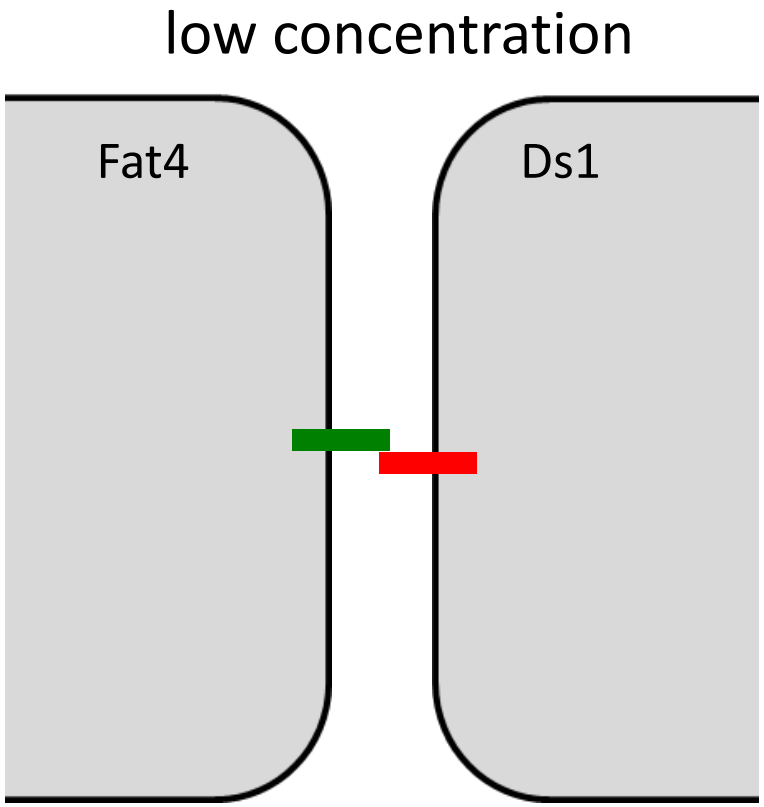




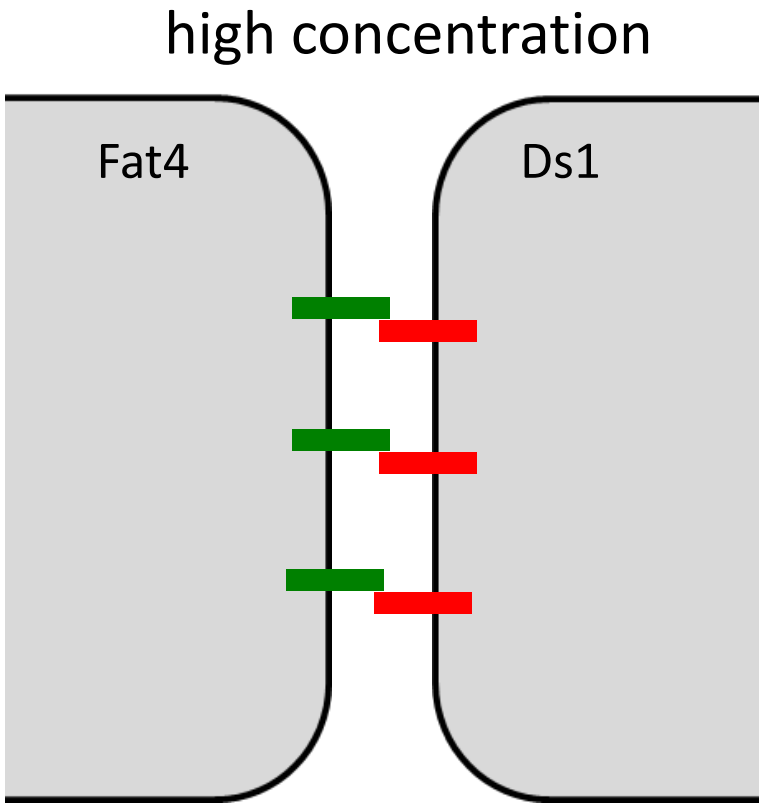
Threshold response is consistent with positive feedback model

But what is the mechanism of the Threshold response?

Potential mechanism: **Feedback by stabilization**
Based on knowledge of other Cadherins dynamics



Feedback by stabilization: **Clustering**



Stability

If our assumption is true –

We expect that as **large clusters of complexes are formed**, due to positive feedbacks by stabilization,

their **dynamics** will be **slower** than those **of unbound Fat4 and Ds1.**

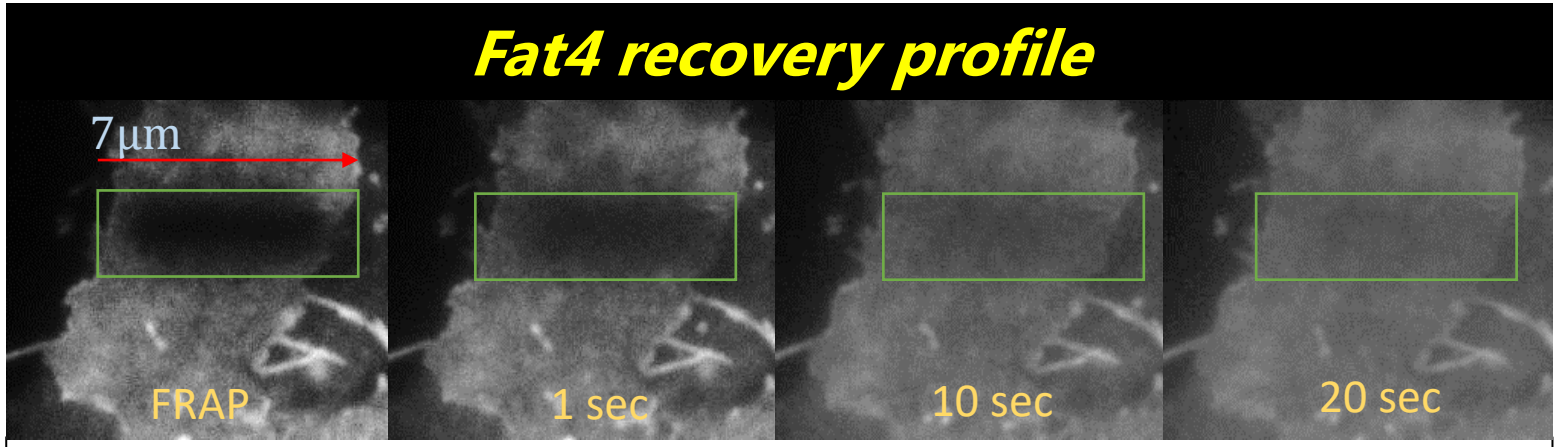
My Research goals:

a. Show that Fat4 and Ds1 **form stable complexes** at the cell boundary,
   and show **lower mobility** of the complex **compared to the unbound proteins**.

b. To search for **factors that affect** Fat4 or Ds1 **mobility**.

Fluorescence Recovery After Photobleaching (FRAP)

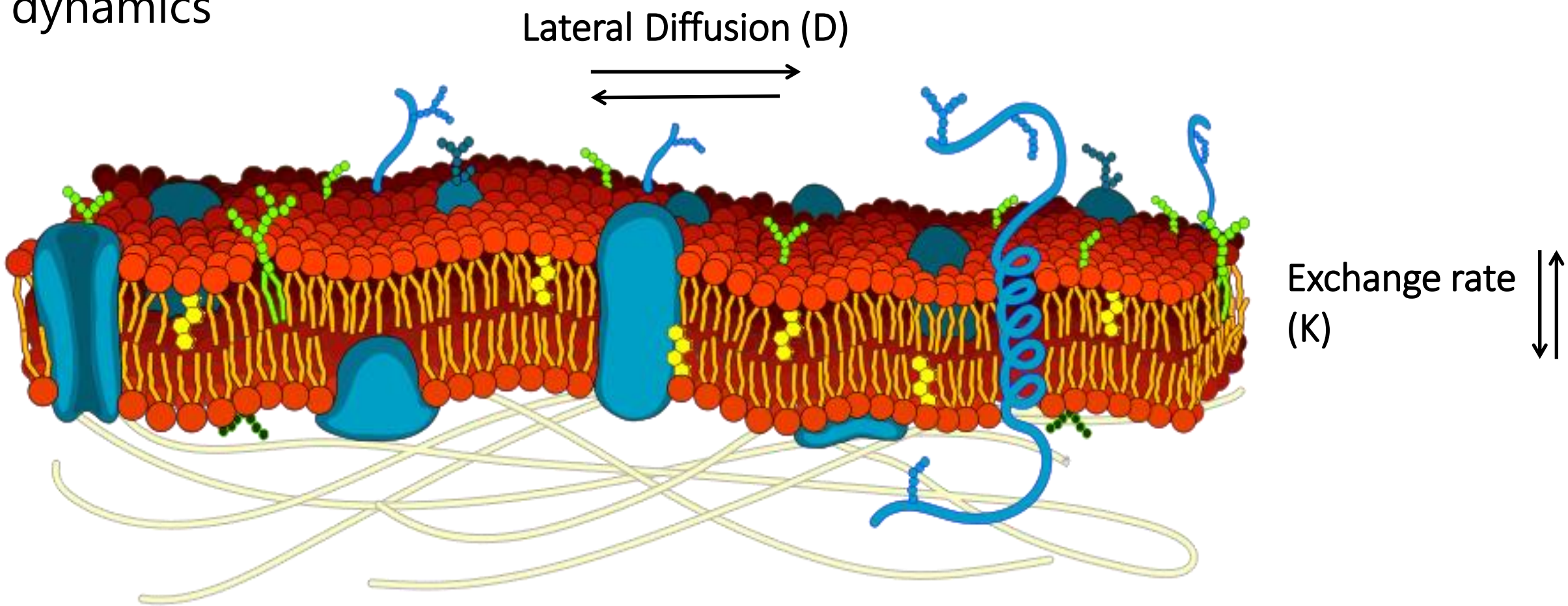
- **Photobleaching** an area.
- Follow the **recovery profile** of the fluorescence back into the bleached area.
- FRAP method is used to **study particle dynamics.**



➢ *HEK cells bleached on their cell surface, Fat4 is labeled by the citrine fluorescence marker. We see recovery in under 20 seconds.*

Protein **Dynamics** on the Membrane

The factors that we need to measure in order to study the protein dynamics

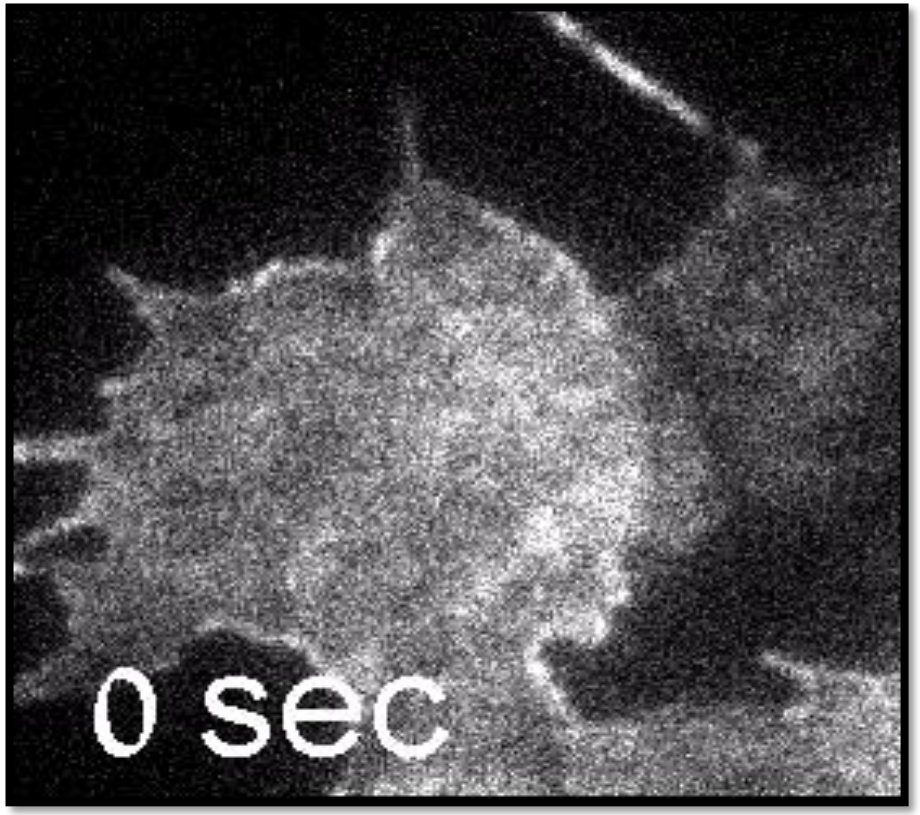


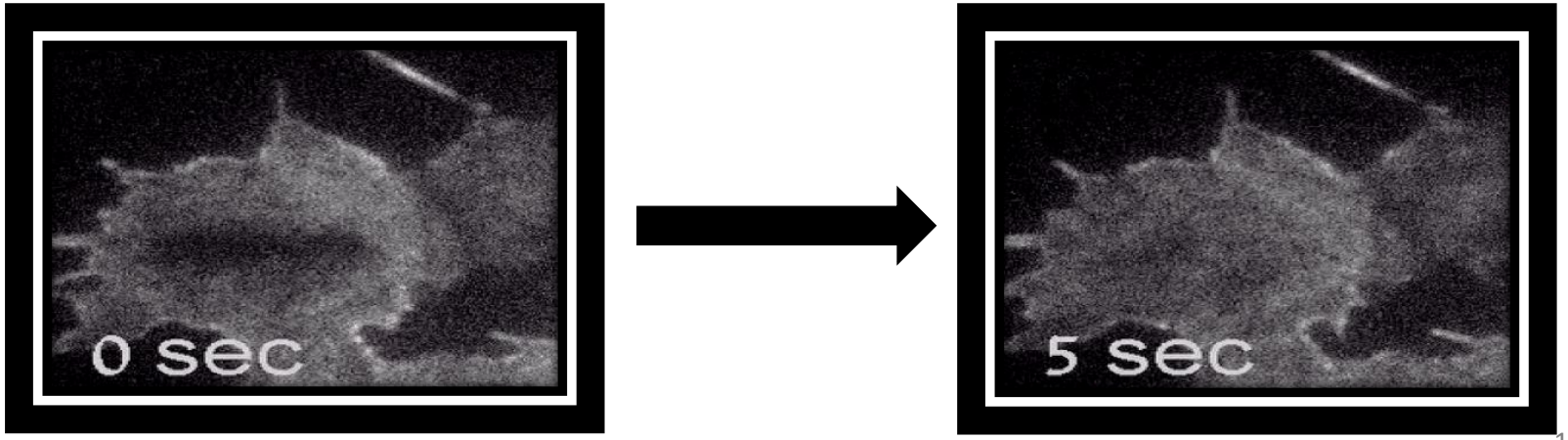
Research METHODS - **Imaging**

FRAP experiments can be performed using several imaging methods

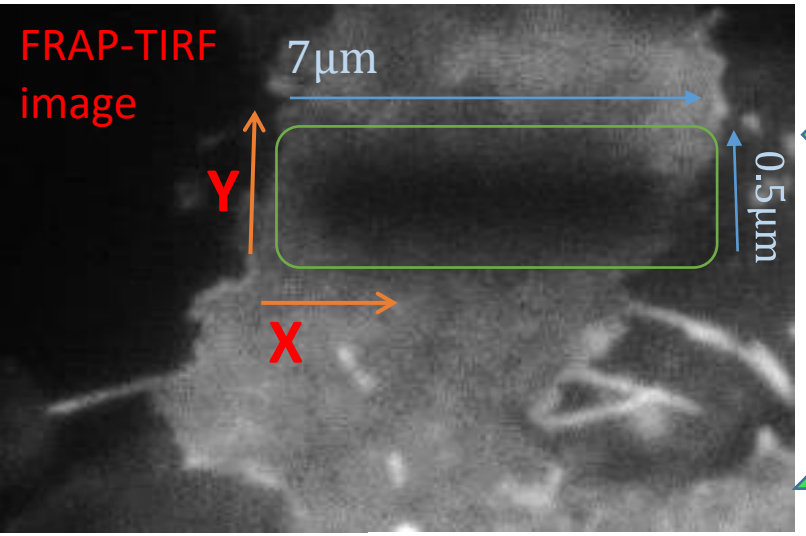
- We use two imaging methods, for **two different purposes:**
  - **<u>Confocal microscope</u> –** is suitable for looking at boundaries that are **deeper in the sample**. For **variant in z sections**.
  - **<u>TIRF microscopy</u> –** is suitable for looking **at the first 200nm of the cell boundary**. Best for analysis of **free membrane proteins**.

An example of FRAP-TIRF experiment MOVIE:

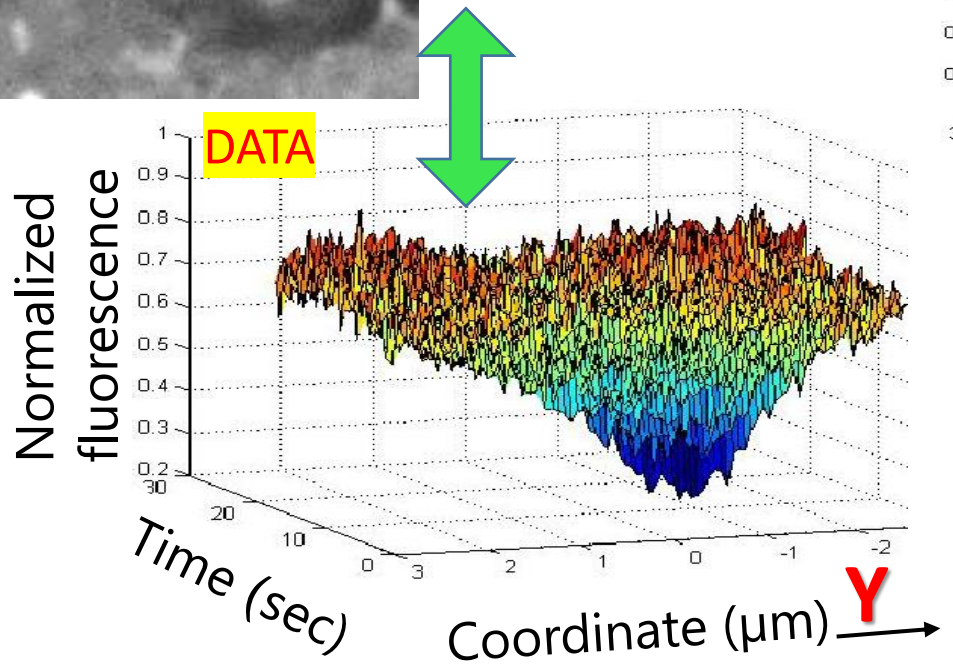


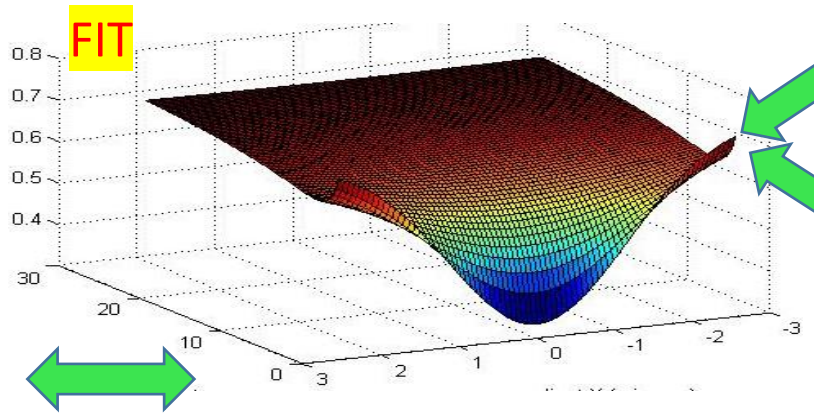


Analysis: Quantitatively measure diffusion coefficients and endocytosis rates of the membrane proteins.



fitting data to an **analytic model** that contains D&K





Diffusion Coefficients, **D** $\mu m^2/sec$

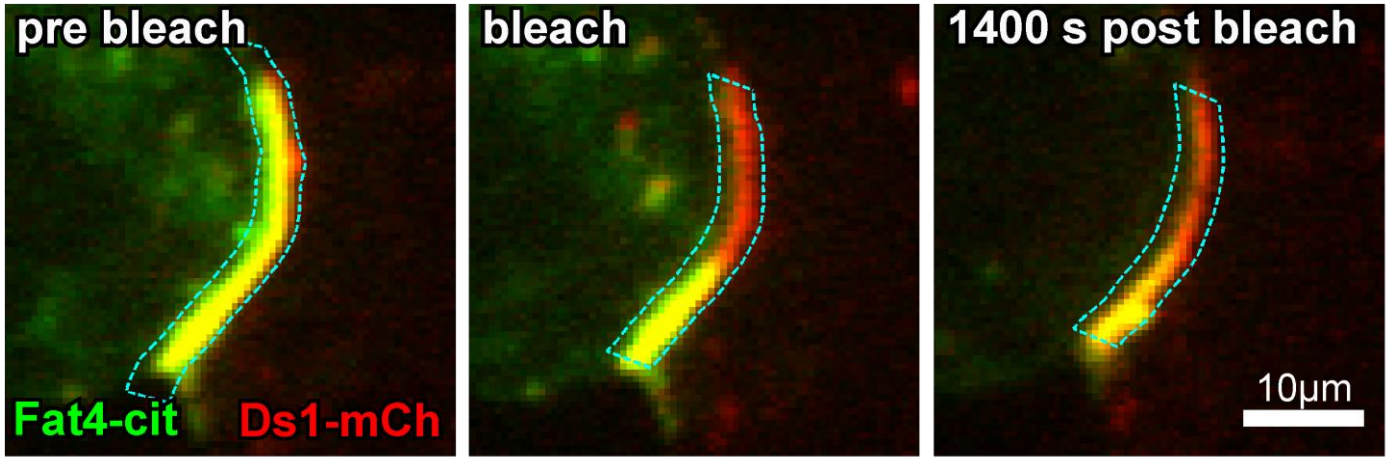
Exchange Rates, $\mathbf{K_{endo}}$ $1/s$

The FRAP analysis and fitting was taken from: Khait, I. et al, 2015

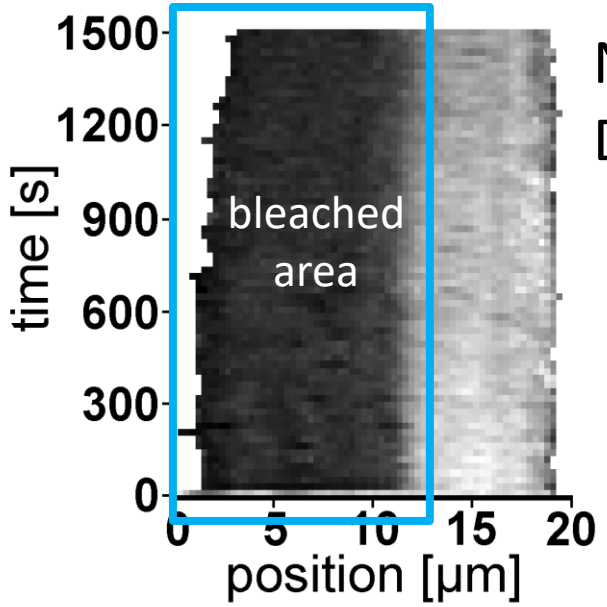
PART A

Complex Dynamics Results

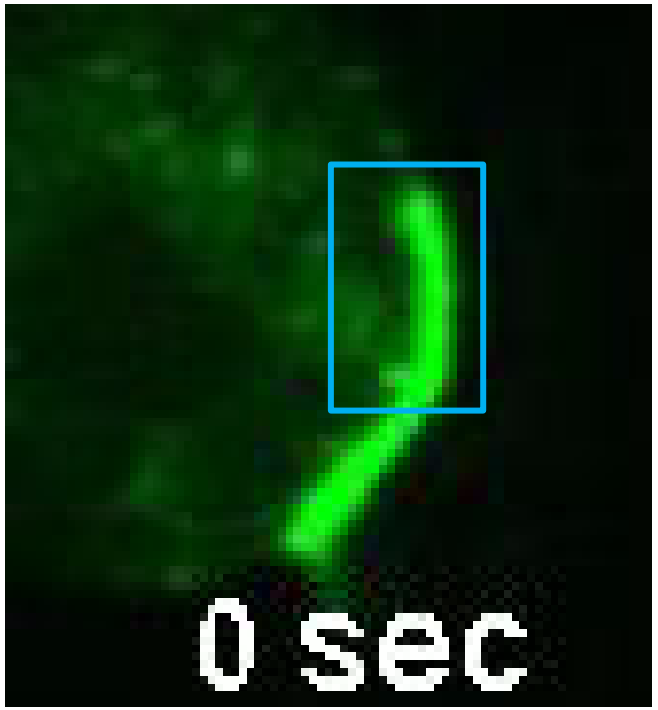
The recovery of Fat4-Ds1 complexes is extremely slow



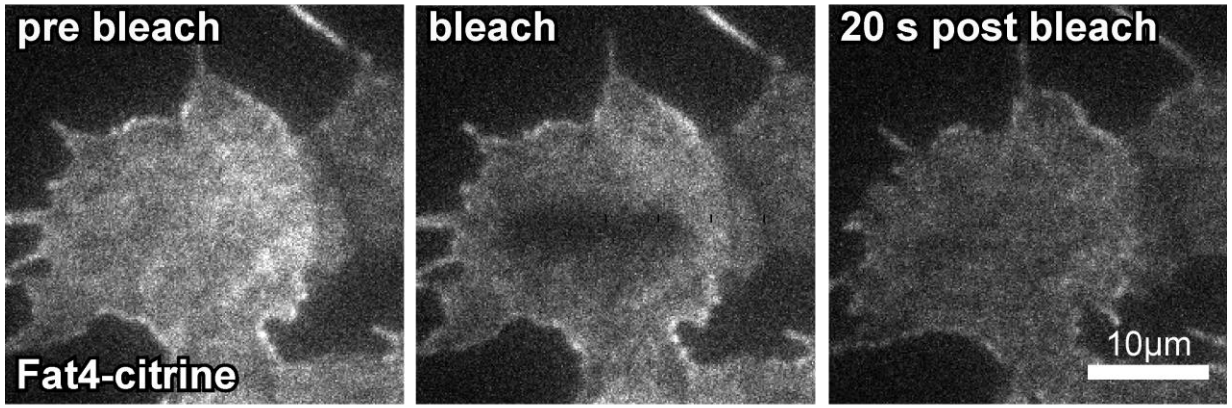
Kymograph of Fat4-Ds1 complex



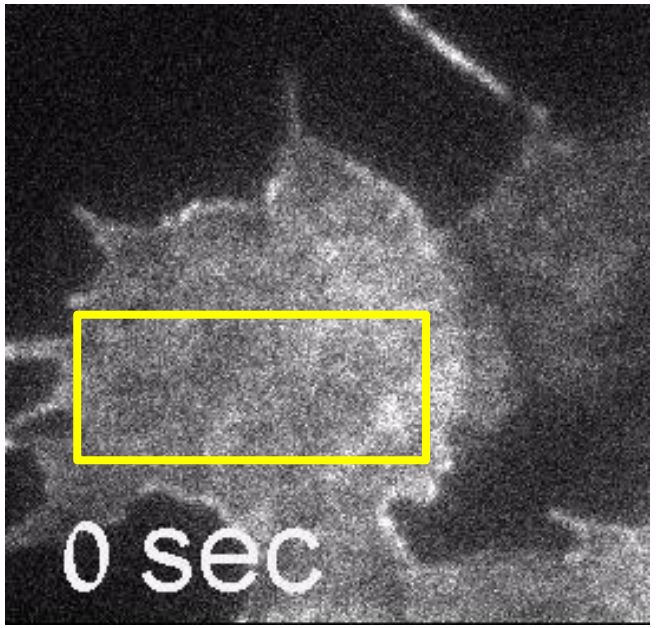
No recovery of Fat4 Ds1 complexes!



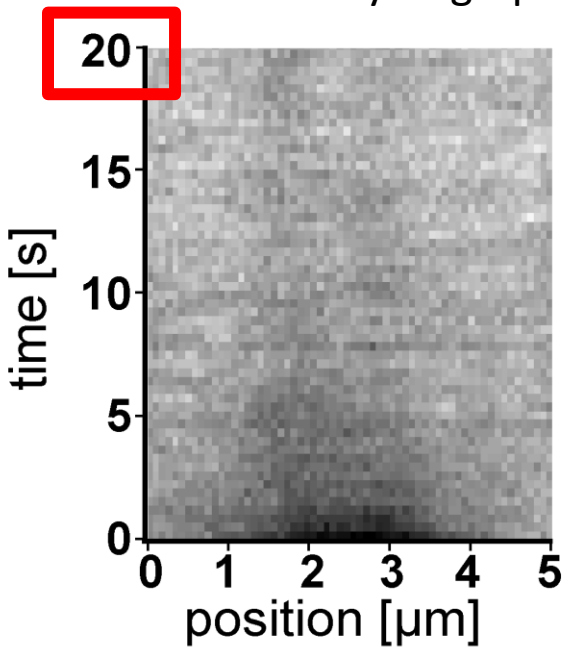
Unbound Proteins Dynamics Results



Unbound Fat4-citrine and Ds1-mCherry exhibit fast membrane dynamics

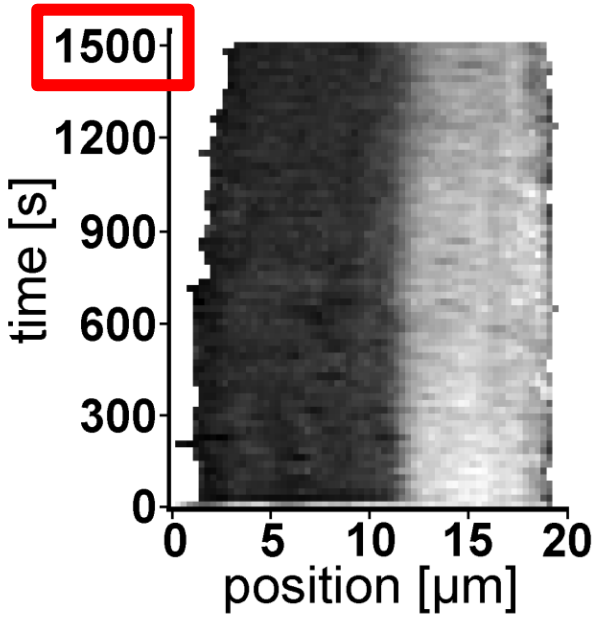


Fat4-citrine kymograph

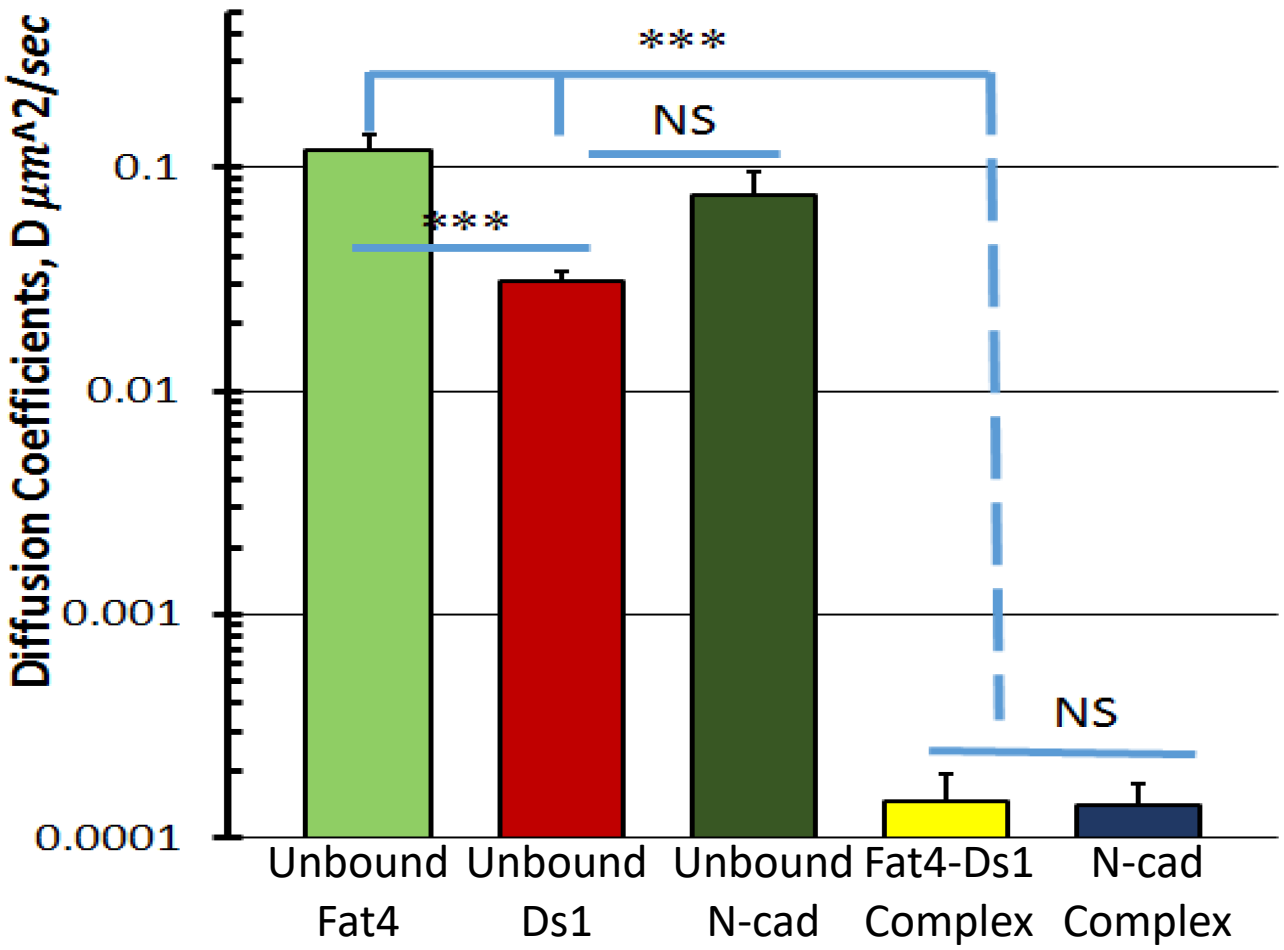


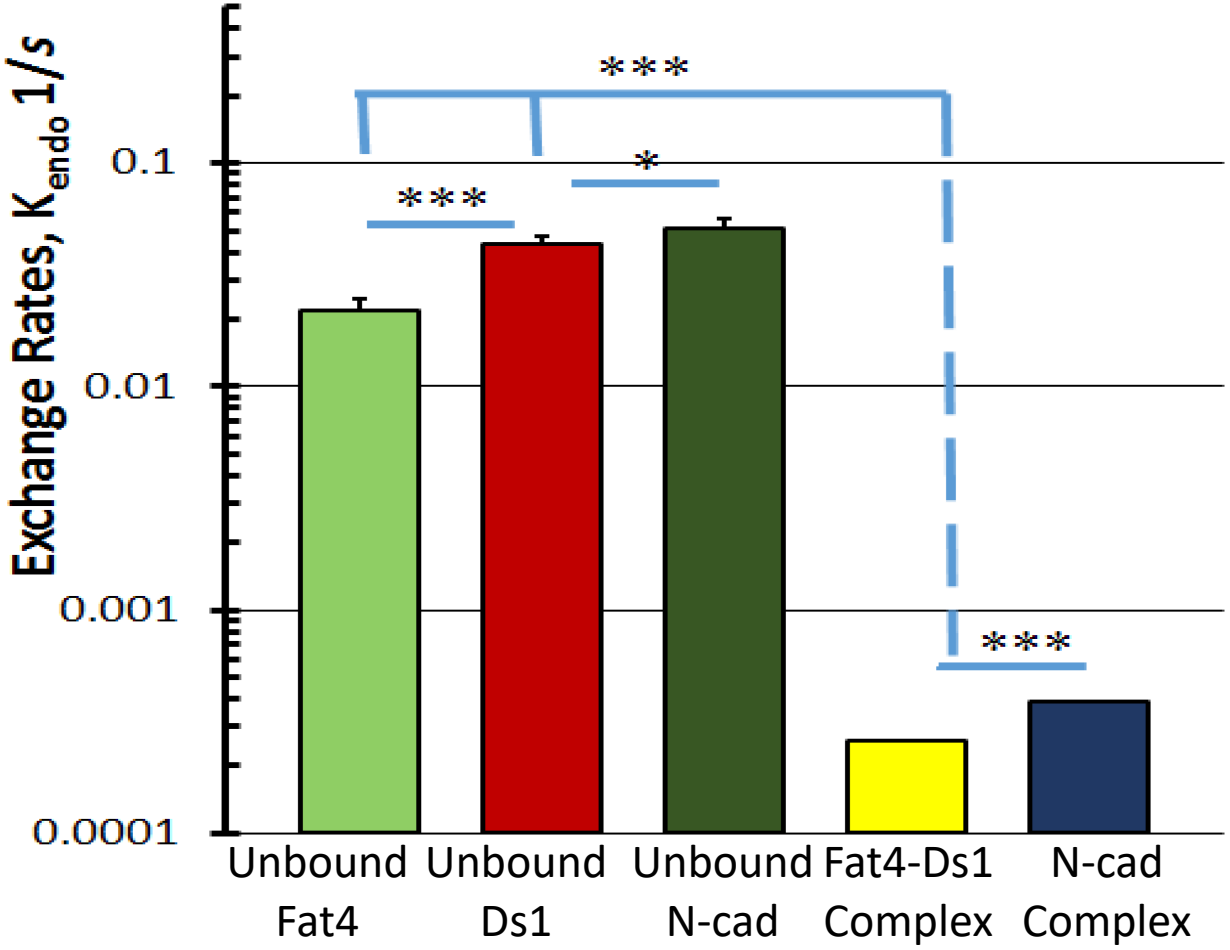
Vs

Kymograph of Fat4-Ds1 complex



Results summary – **Statistical analysis shows significant difference** between complexes and free proteins dynamics and mobility factors





The dynamics of the unbound Fat4 and Ds1 are faster than their complex, consistent with feedback by stabilization

Conclusion – Part A

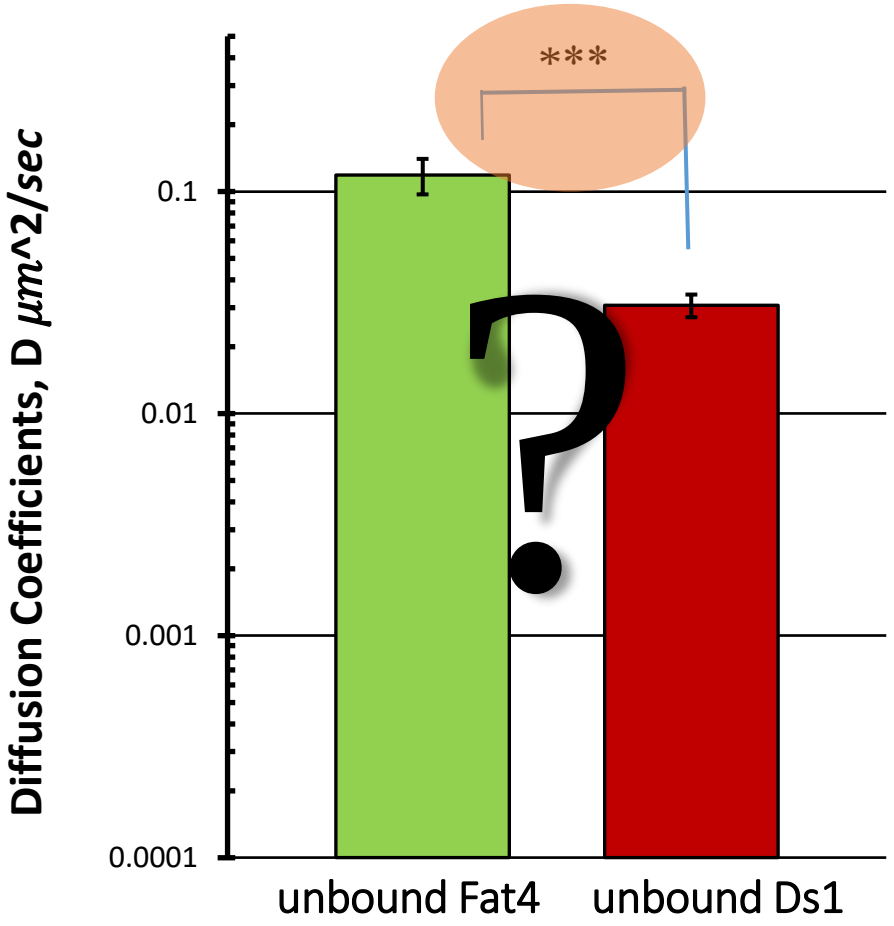
Fat4-Ds1 complexes are **extremely stable** compared to unbound Fat4 and Ds1

This is consistent with the model of **localized feedback by stabilization**

We suggest that this happens **due to clustering**

PART B

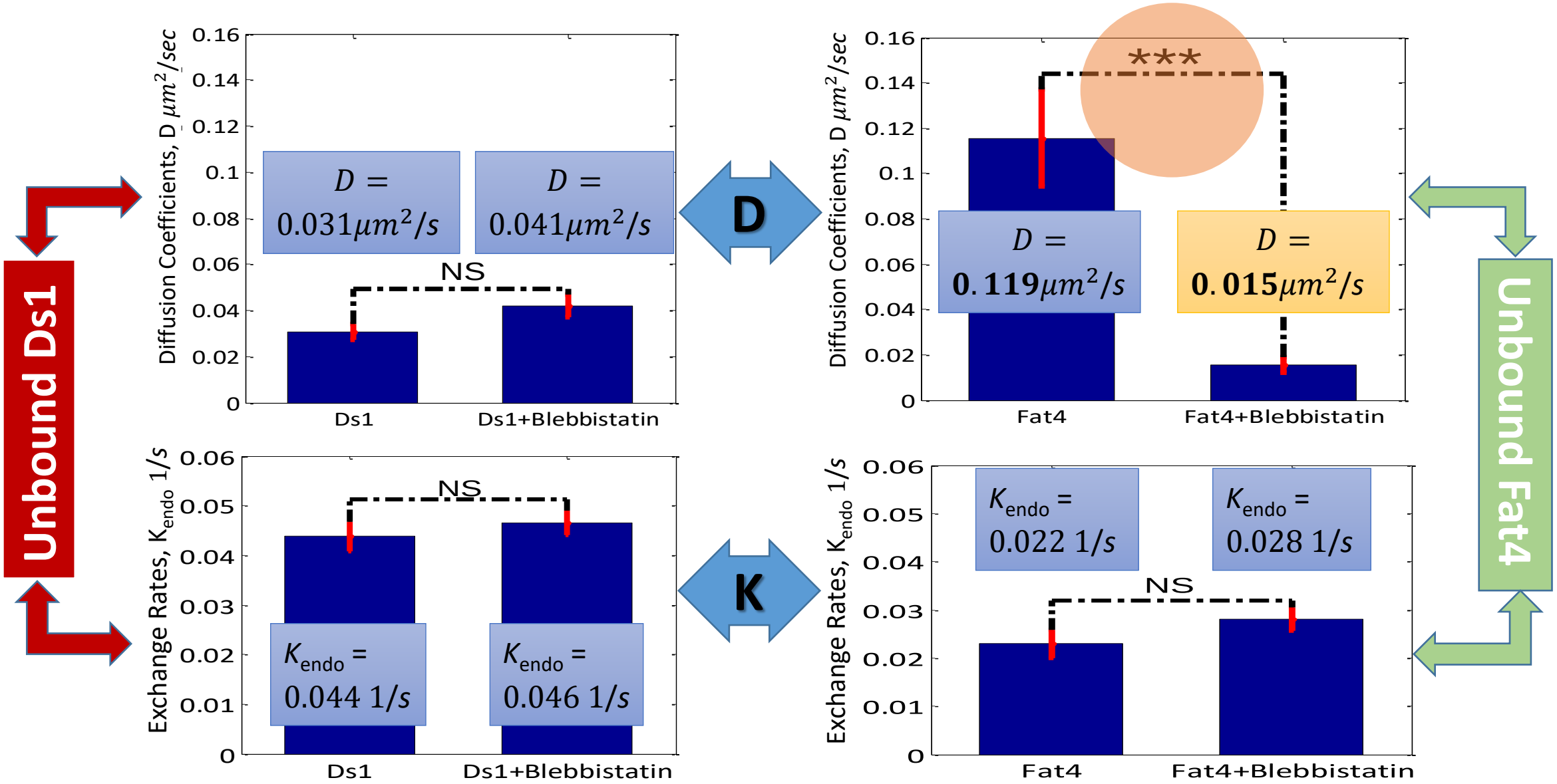
Diffusion of unbound Fat4 is **higher** than that of unbound Ds1



- **What makes unbound Fat4 Diffusion Coefficients almost <u>four times higher</u> than that of unbound Ds1?**
  - This is surprising since Fat4 is larger than Ds1 (560kD vs 380kD)
  - Typical adhesion molecule diffusion rates: orders of 0.01-0.05μm^2/sec.

Can the answer be active trafficking?

Inhibition of Myosin II by Blebbistatin affect Fat4 but not Ds1 diffusion



Conclusion – Part B

Fat4 diffusion is higher than that of Ds1

Fat4 diffusion is affected by the myosinII motor protein inhibitor Blebbistatin, while Ds1 is not

Suggests that Fat4 goes through active trafficking on the cell membrane

It is unclear what role this has in PCP

ACKNOWLEDGMENTS

I would like to thank:

Dr. David Sprinzak

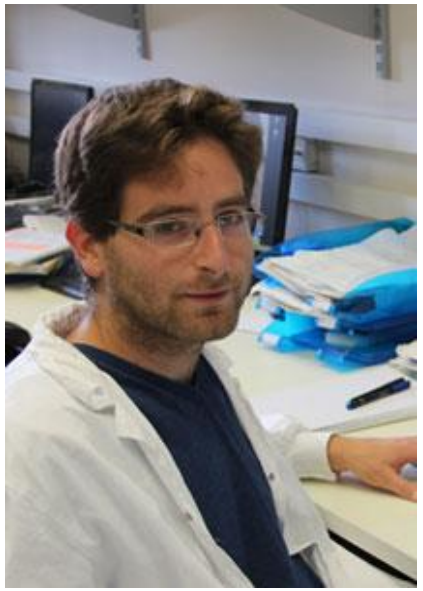
PI

Dr. Liat Amir-Zilberstein

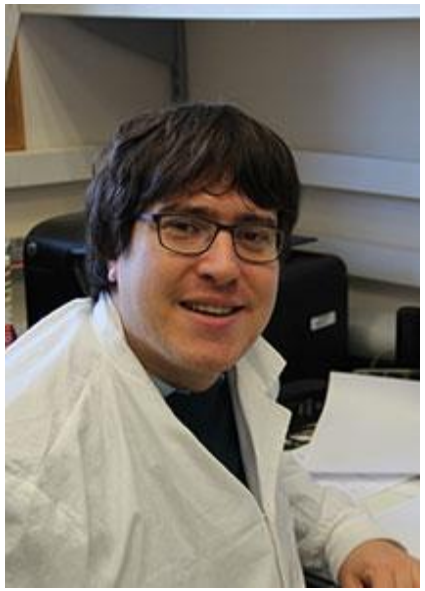
Lab manger

Dr. Olga Loza

Project manger



Yuval Orsher



Amitai Menuchin

Also…

All members of the Sprinzak lab, and all that helped as collaborators or as friends.

Thank you for listening