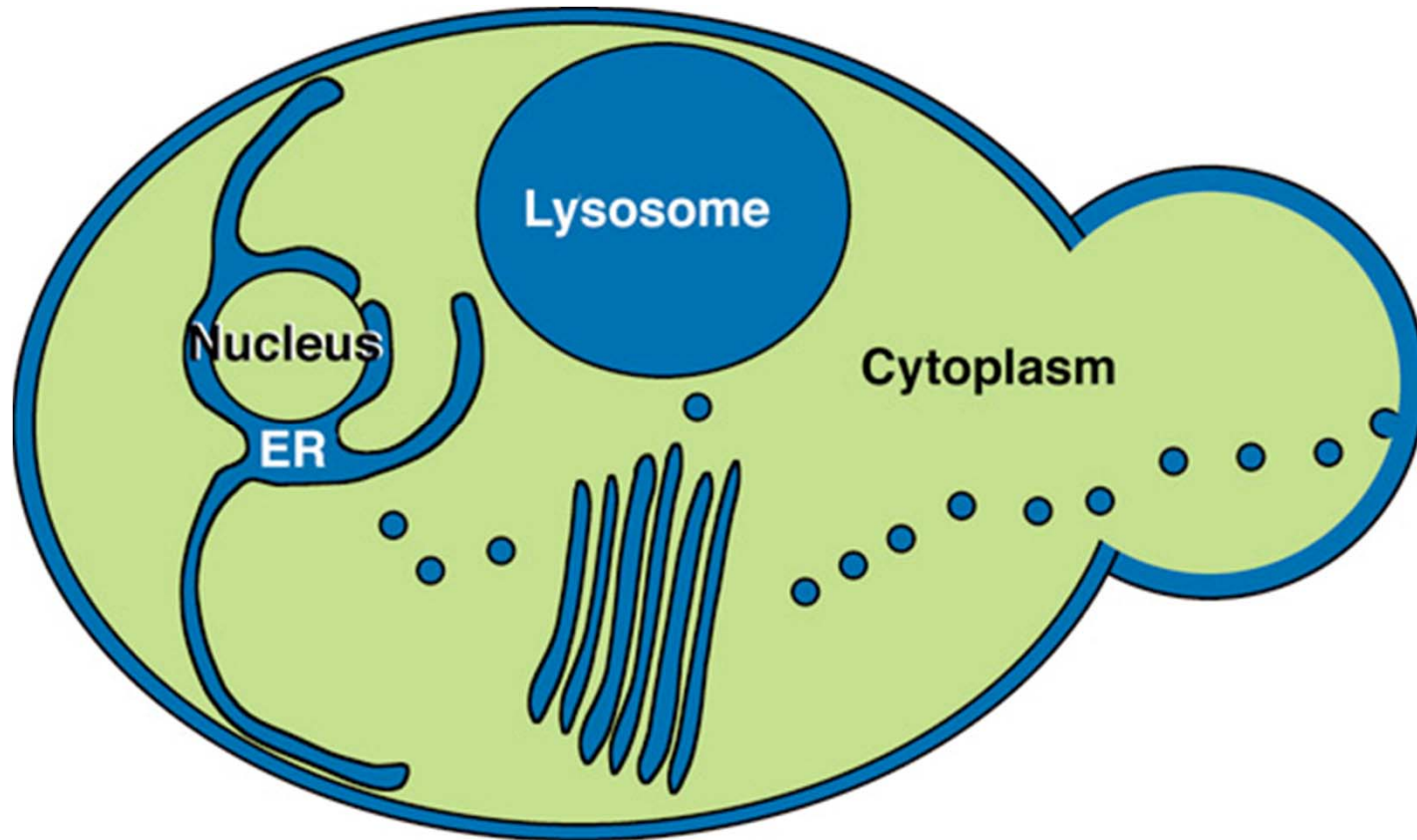


Modular transient protein- protein interaction

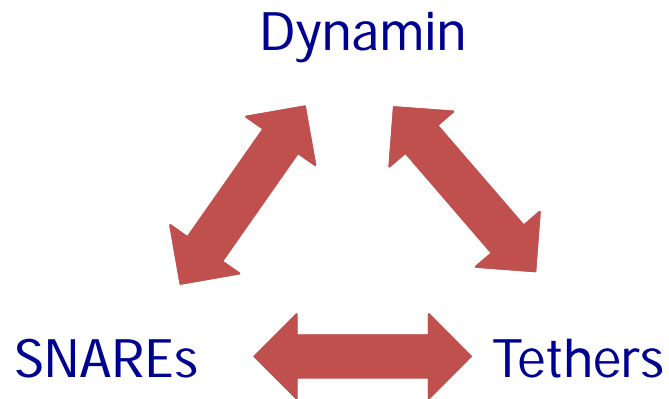
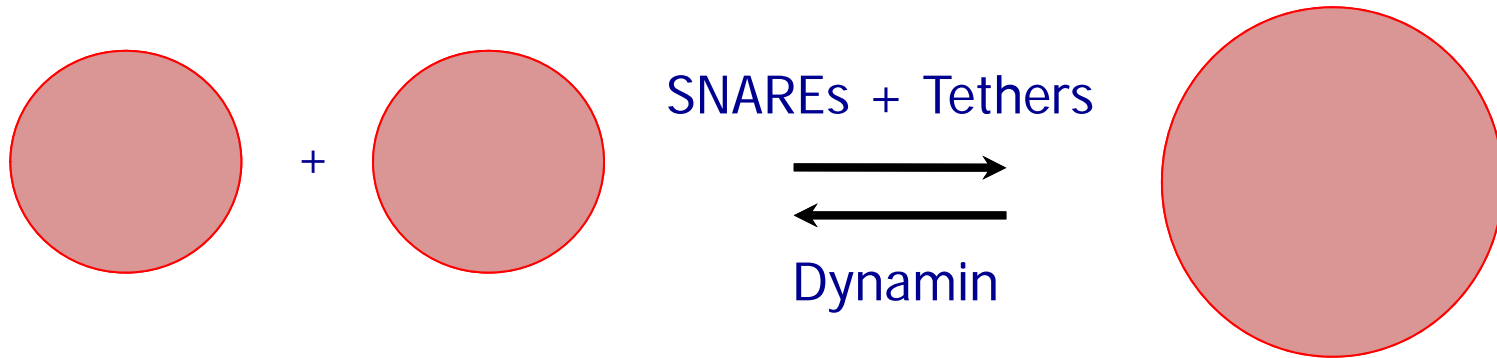
Kannan Alpadi
Department of Biochemistry
Baylor College of Medicine

Why do cells care about organelle dynamics?



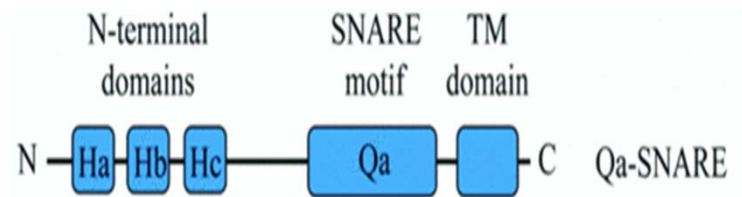
- Cellular reactions and pathways are divided among the individual organelles and cytoplasm, all of which have some size, shape and number.
- Organelle size should be suited to cellular needs

Organelle dynamics controlled by cycles of fusion and fission

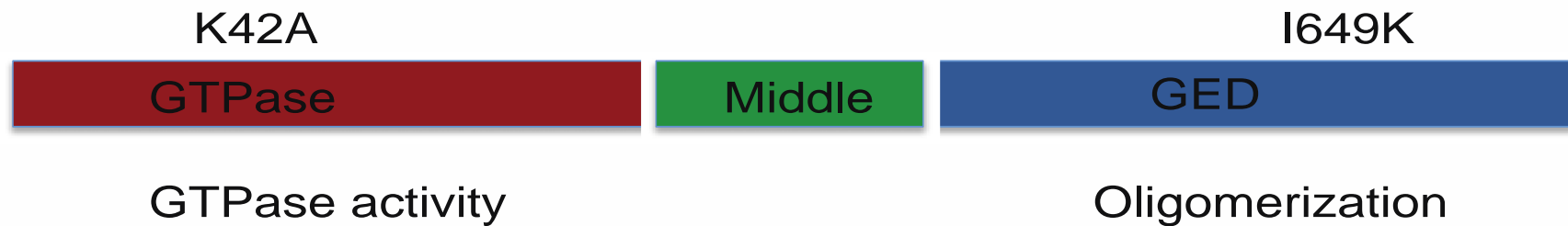


Domain structure of dynamin and syntaxin

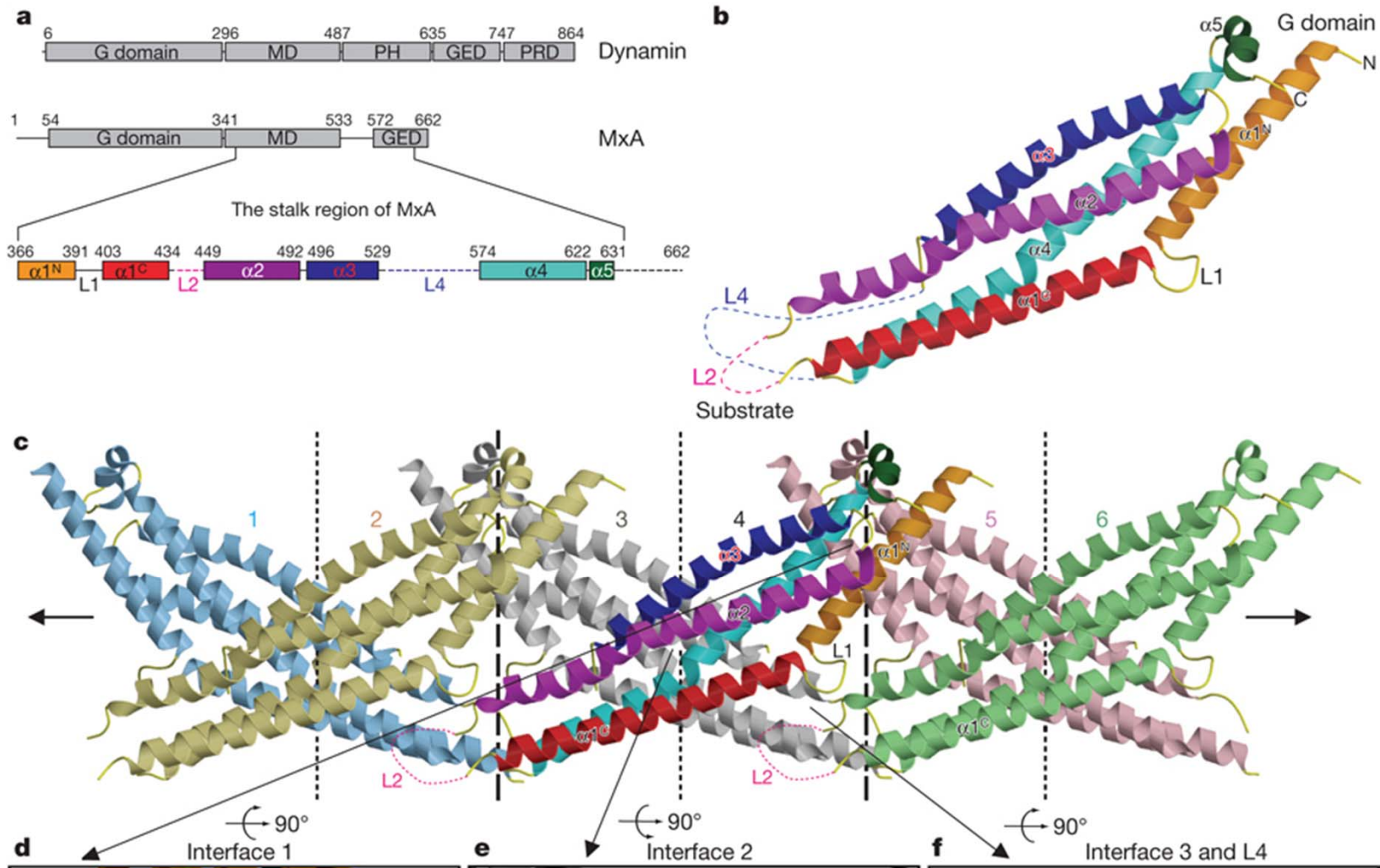
Syntaxin



Dynamin



Dimer-Dimer interaction of dynamin



Experimental approaches- unsuccessful

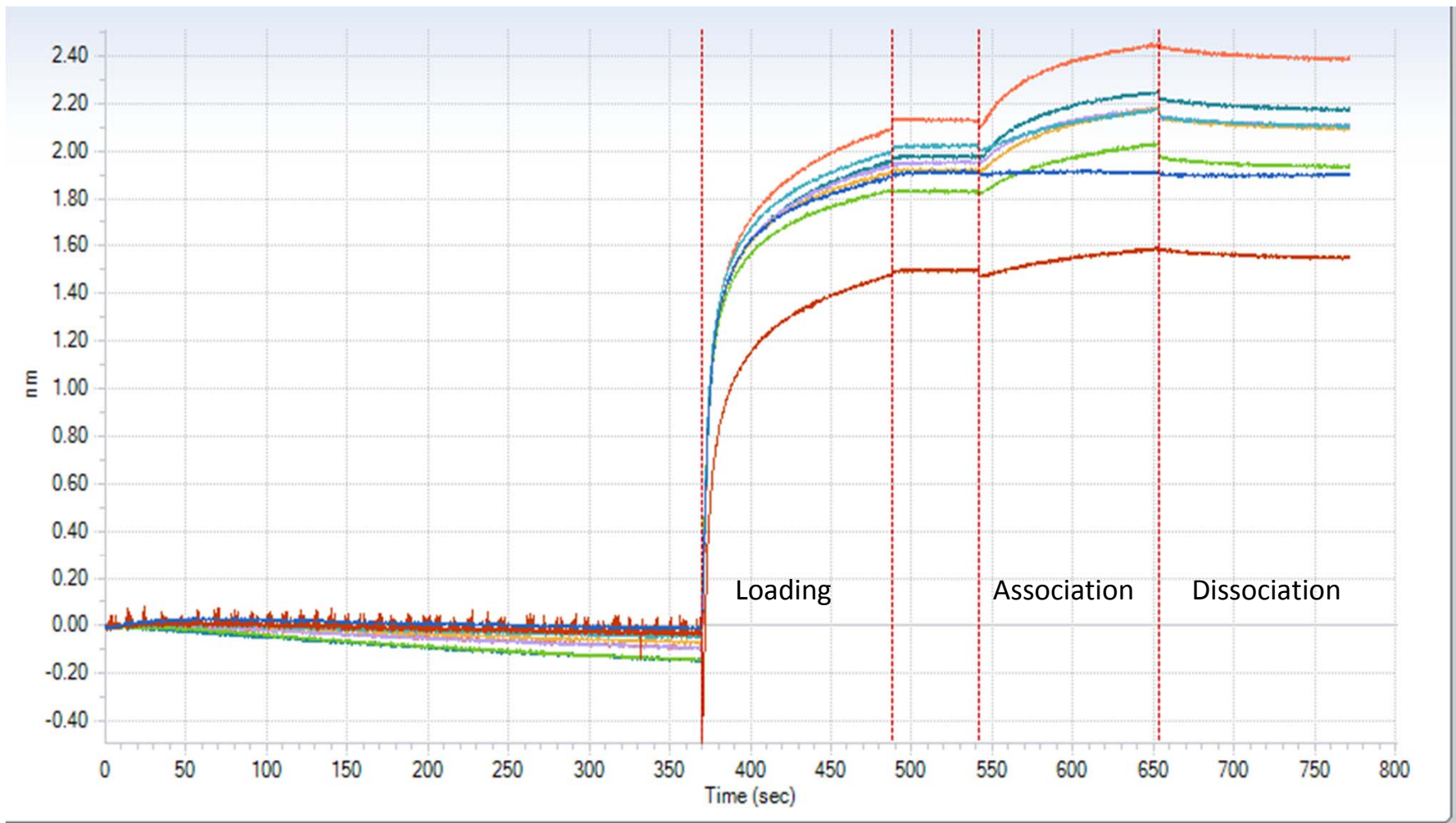
Yeast Two hybrid – tagging of protein on c- or N-terminus leads to nonfunctional

GST pull down– tagging of protein on c- or N-terminus leads to nonfunctional
low yield of protein
oligomerization during assay

Fluorescence polarization– background fluorescence
release of dye
reviewer not accepting
more protein needed

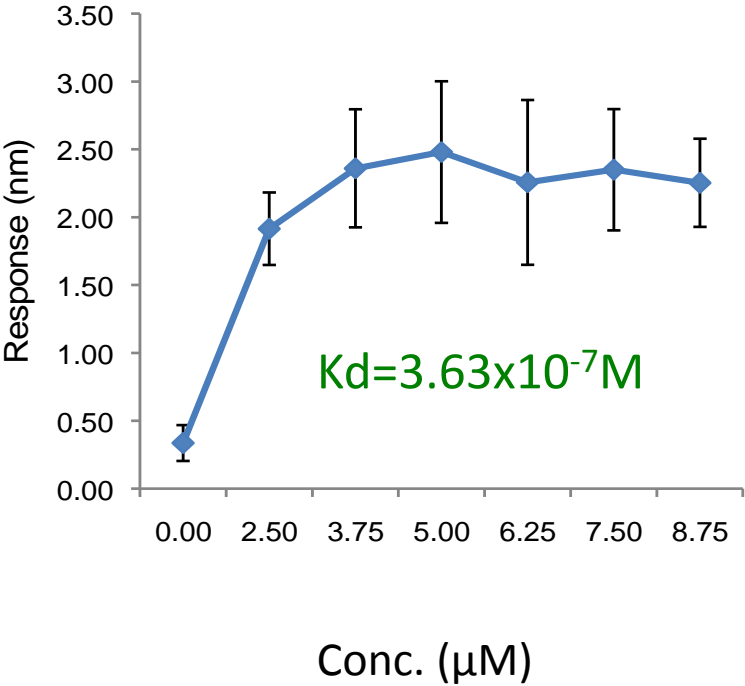
FRET- unsuccessful

Dynamin interacts with syntaxin- BLI



Finally successful demonstration

Dynamin interacts with syntaxin- BLI



Summary

Using BLI we were able to demonstrate the dynamin-SNARE interaction

Most of the protein interactions in the vesicular trafficking pathway are transient; and they are spatio-temporally regulated.

BLI helps to overcome the difficulties of showing transient protein-protein interaction;

It will be an important tool to identify the novel regulatory proteins whose interactions are mostly transient and modular

Acknowledgements

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