Modular transient protein-protein interaction
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 diagram showing K42A and I649K mutations in the GTPase and Middle domains.]

GTPase activity

Oligomerization
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

\[
K_d = 3.63 \times 10^{-7} \text{M}
\]
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.
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