Exploring Protein-Protein Interactions with Fluorescence Correlation Spectroscopy

Joachim Mueller

School of Physics and Astronomy

CASSS, HIGHER ORDER STRUCTURES 2021
Mueller Lab

- Jared Hennen
- Yan Chen
- Kwang Ho Hur
- Isaac Angert
- John Kohler
- Siddarth Reddy Karuka
- Rayna Addabbo

Luxton Lab (UC Santa Cruz)

- Gant Luxton
- Amy Schoenhofen
- Cassie Morris
- Cosmo Saunders

Funding: NIH
Fluorescence Correlation Spectroscopy (FCS)

2-photon excitation

Cellular FCS

Photon count trace

Autocorrelation Function (ACF)

\[
\tau_D \propto \frac{1}{D}
\]

two-photon spot

change of \(\tau_D\) by only \(2^{1/3} = 1.26\)
Stoichiometry & Brightness $b$

$b = 1$

oligomerize

$b = 6$

\[ b \propto \frac{\langle \Delta F^2 \rangle}{\langle F \rangle} \]
Stoichiometry & brightness $b$

EGFP is a quantitative brightness marker in cells

Chen et al. PNAS USA 2003
Brightness Titration

- Monomer
- Mixture
- Dimer

Graph showing the apparent brightness ($b_{apparent}$) against concentration (nM) with different states represented.
Brightness Titration in Living Cells

Chen et al. Proc. Natl. Acad. Sci. USA 2003 (100) 15492
Brightness Ladder

$f = 1$

$f = 0.7$

Brightness calibration required!
Protein Assembly at the Nuclear Envelope (NE)

- Nuclear positioning
- Force-dependent gene expression
- DNA damage repair
Measuring LINC Complex Assembly in Living Cells

Cytoskeleton

Nesprin

SUN

Nucleoskeleton

KASH-peptide

SUN-trimer

90°
KASH Domain of Nesprin2 Remains Monomeric

Luminal Domain of SUN2 forms Trimers

LINC Complex formed by SUN and KASH: Dual-color FCS
Dual-color Brightness Plot

Negative Control: No interactions

SS-mCherry-KDEL and SS-EGFP

Positive Control: Hetero-Dimer

SS-mCherry-Linker-EGFP

Assembly of SUN2-KASH in Lumen of Nuclear Envelope

No Monomer-Monomer SUN2 domain - KASH Interactions

Conclusions

- Brightness = average oligomeric state of labeled protein complexes
- Brightness titration characterizes binding equilibria and stoichiometry
- Requires diffusing proteins & minimize presence of endogenous protein
- Characterized the assembly of LINC complexes in cells