Exploring Protein-Protein Interactions with Fluorescence Correlation Spectroscopy

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CASSS, HIGHER ORDER STRUCTURES 2021

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- Amy Schoenhofen
- Cassie Morris
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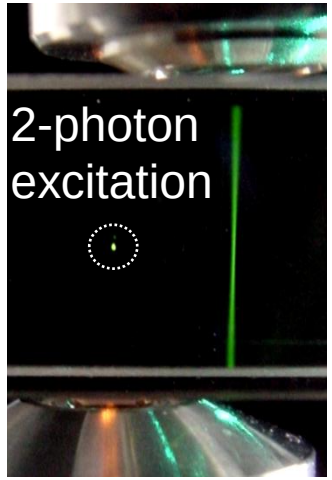
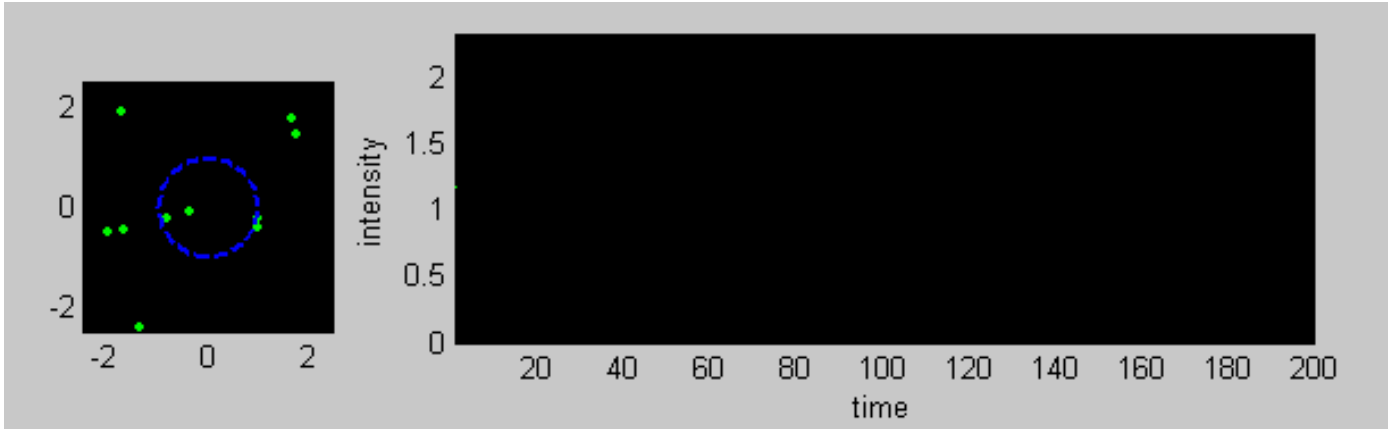
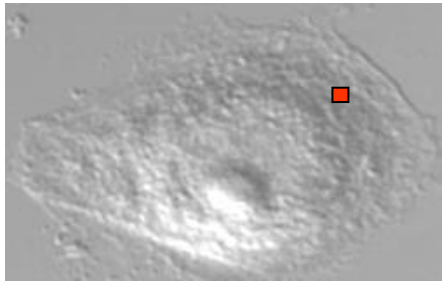
Funding: NIH

Fluorescence Correlation Spectroscopy (FCS)

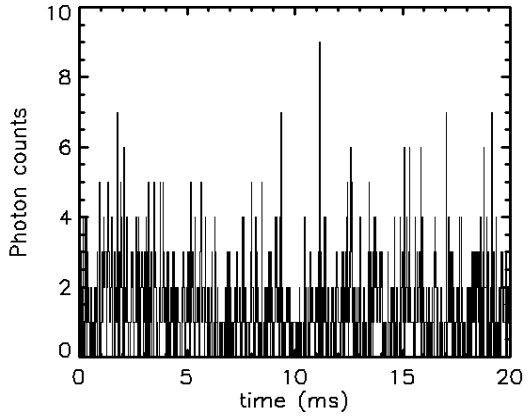




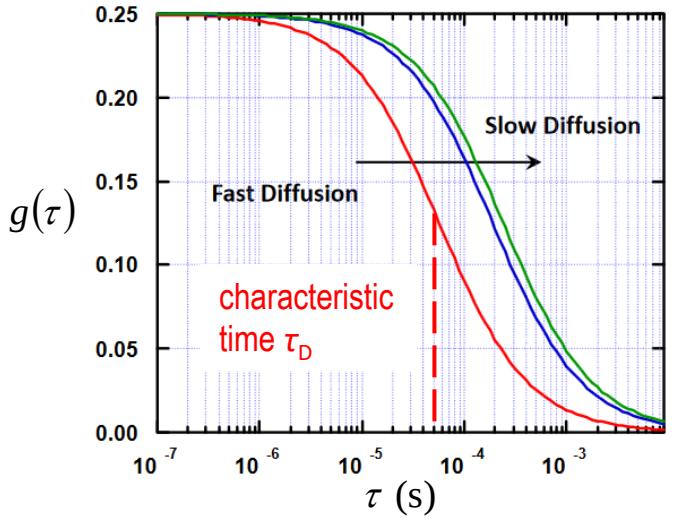
Cellular FCS



Photon count trace



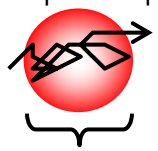
Autocorrelation Function (ACF)



Dye
Monomer
Dimer

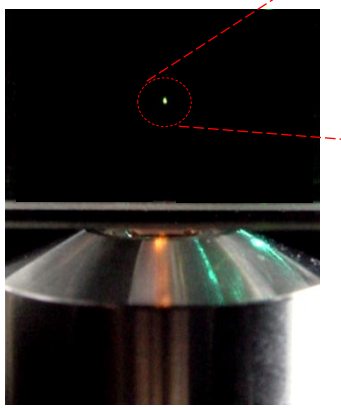
change of τ_D by only $2^{1/3} = 1.26$

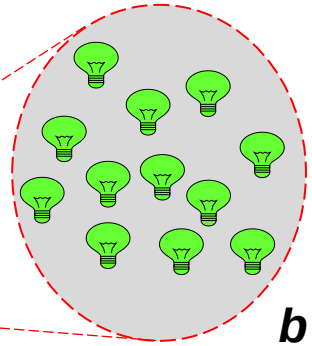
two-photon spot



$$\tau_D \propto \frac{1}{D}$$

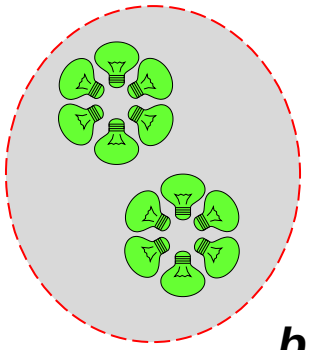
Stoichiometry & Brightness *b*



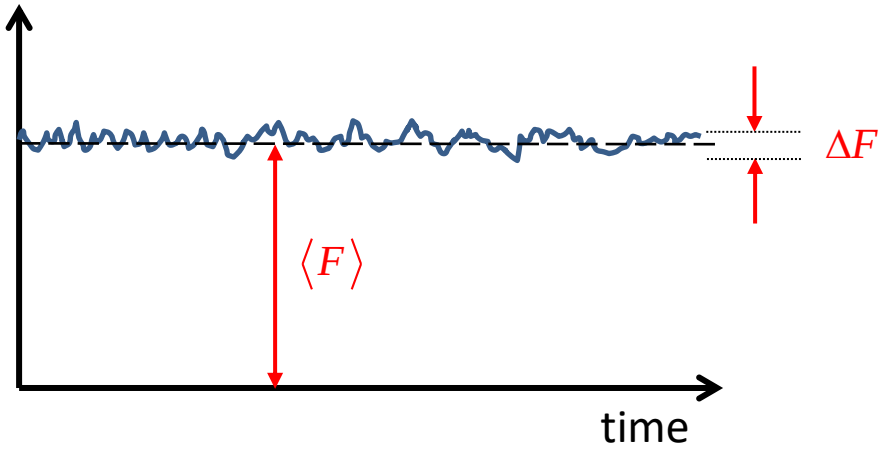


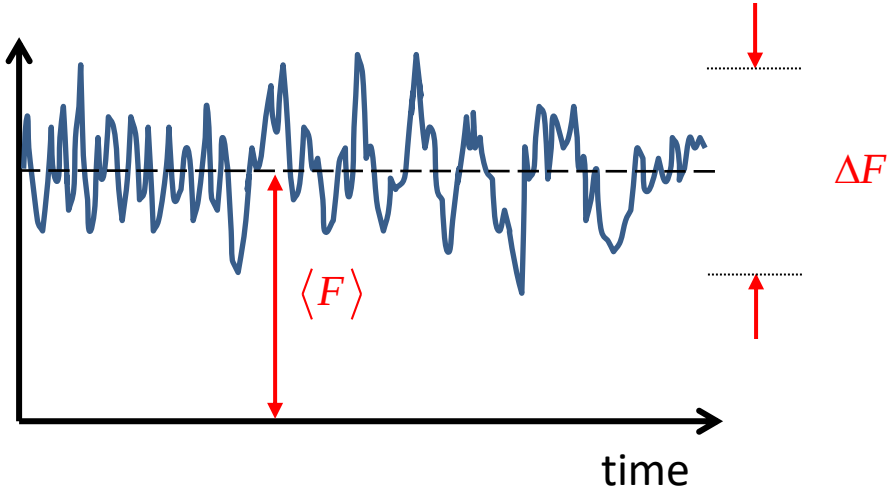
***b* = 1**

oligomerize



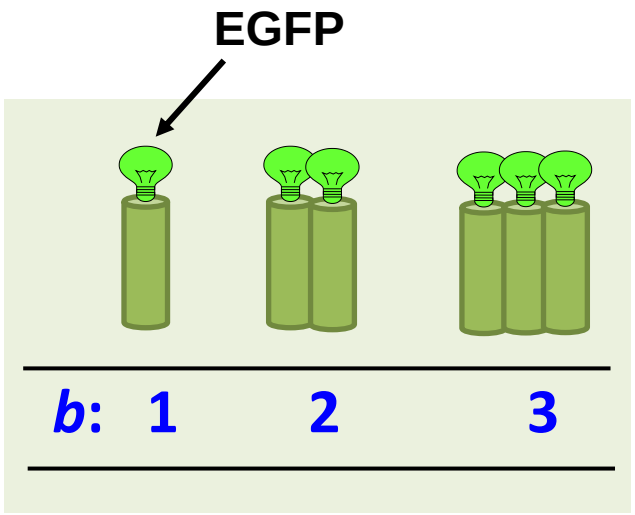
***b* = 6**

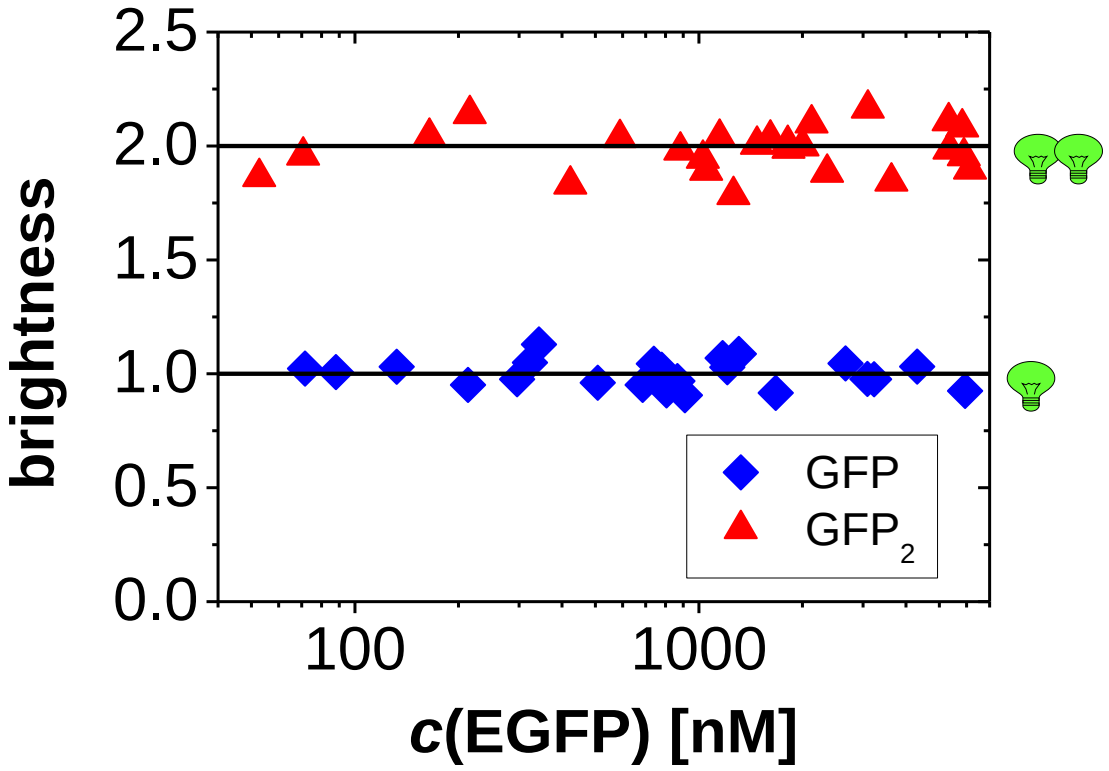




$$b \propto \frac{\langle \Delta F^2 \rangle}{\langle F \rangle}$$

Stoichiometry & brightness *b*

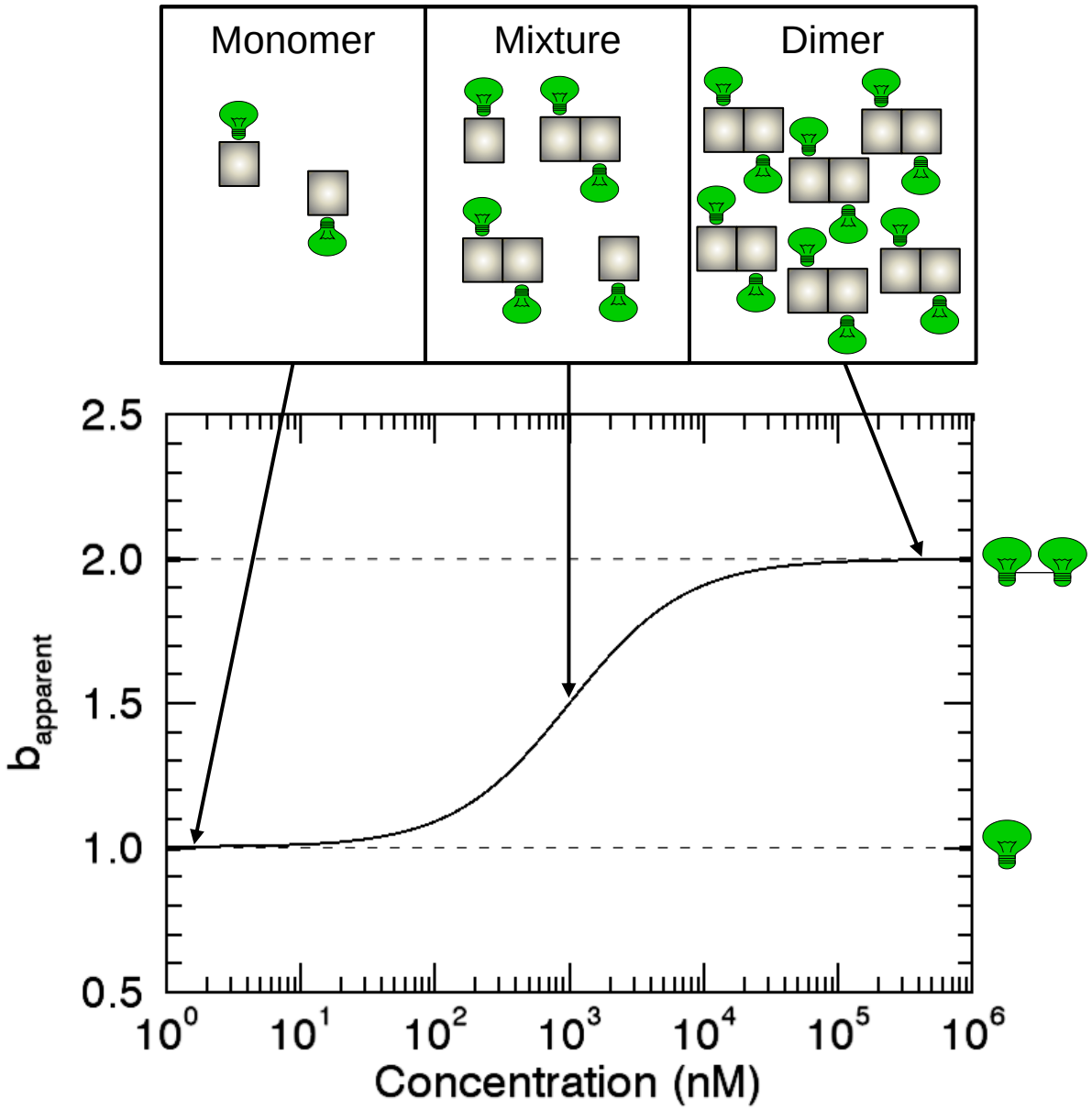




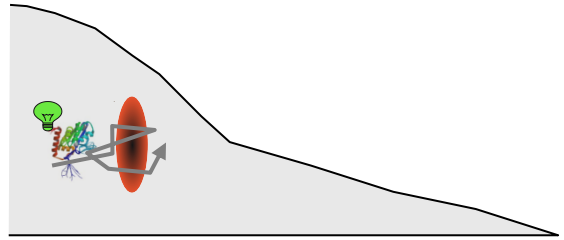
Chen et al. PNAS USA 2003

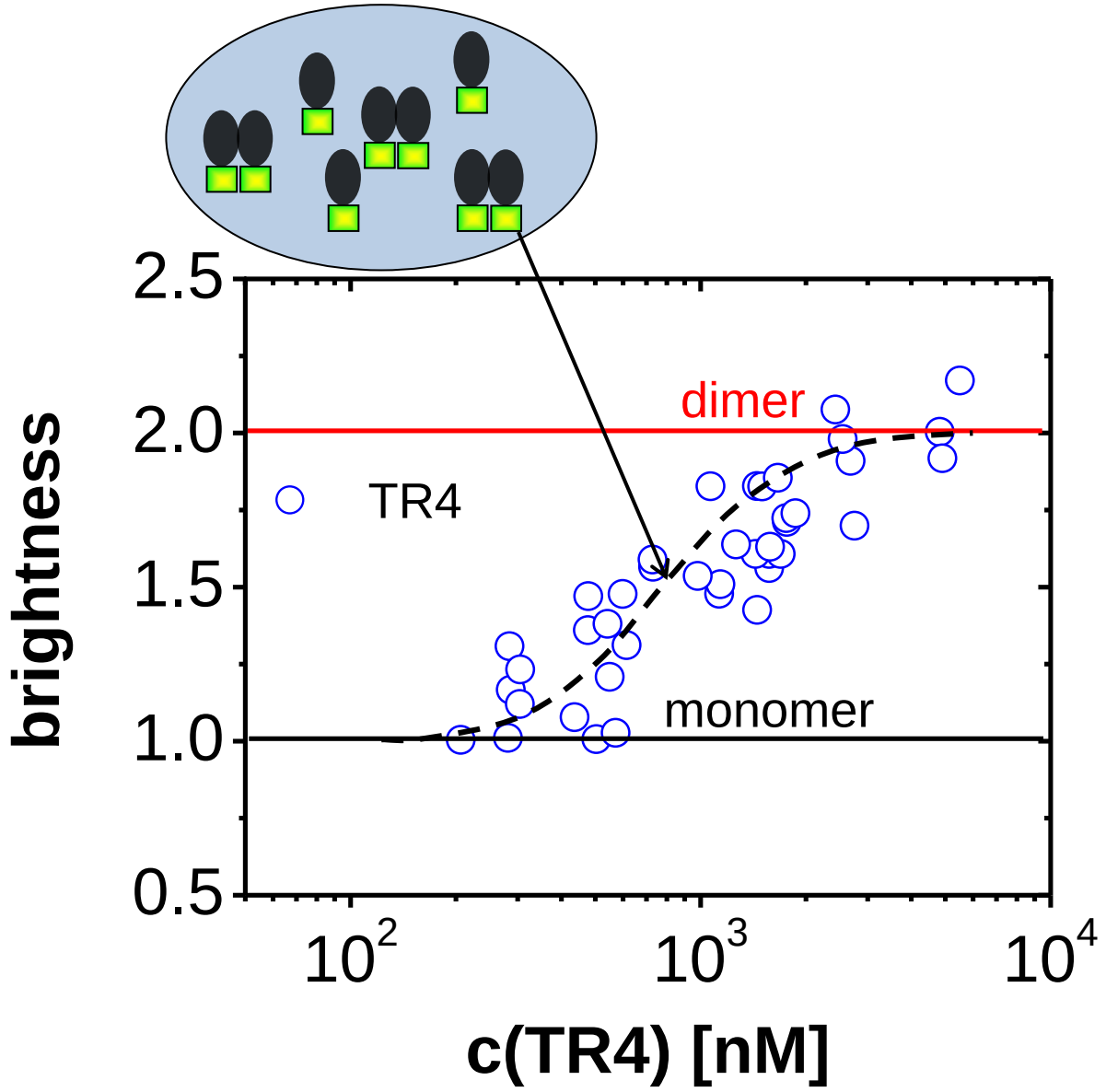
EGFP is a quantitative brightness marker in cells

Brightness Titration



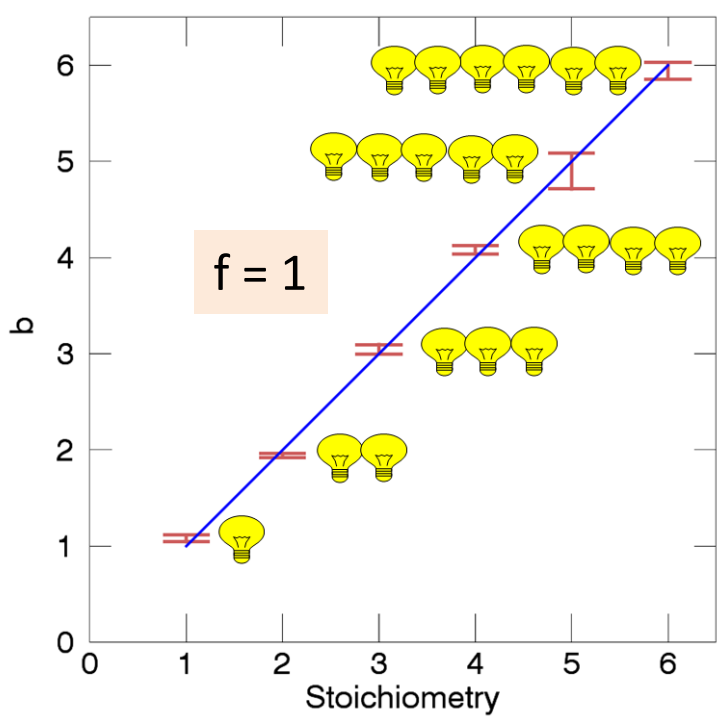
Brightness Titration in Living Cells

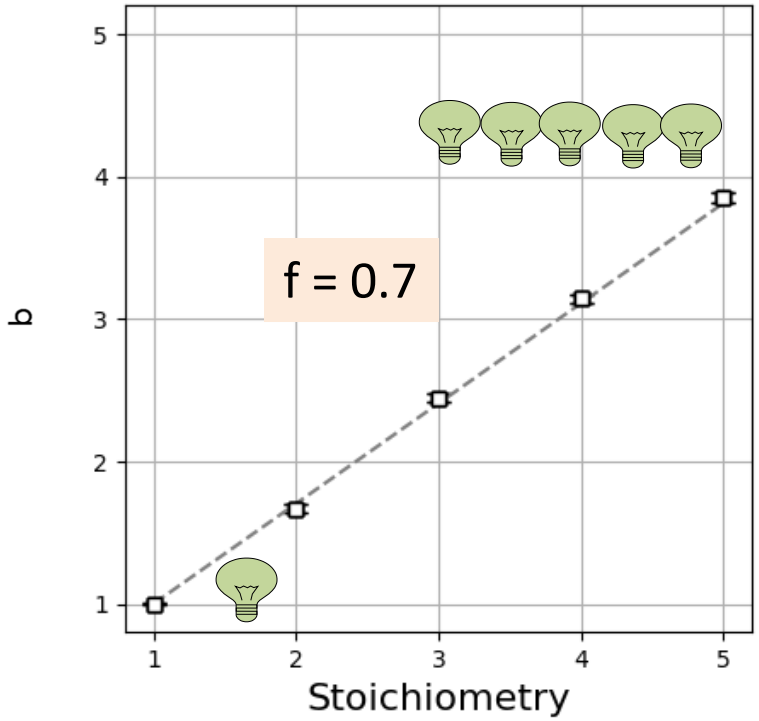




Chen et al. Proc. Natl. Acad. Sci. USA 2003 (100) 15492

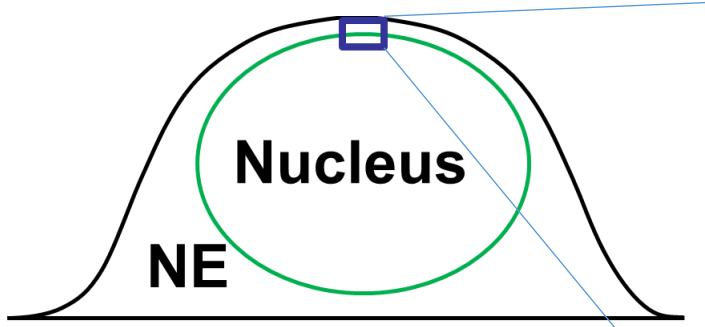
Brightness Ladder

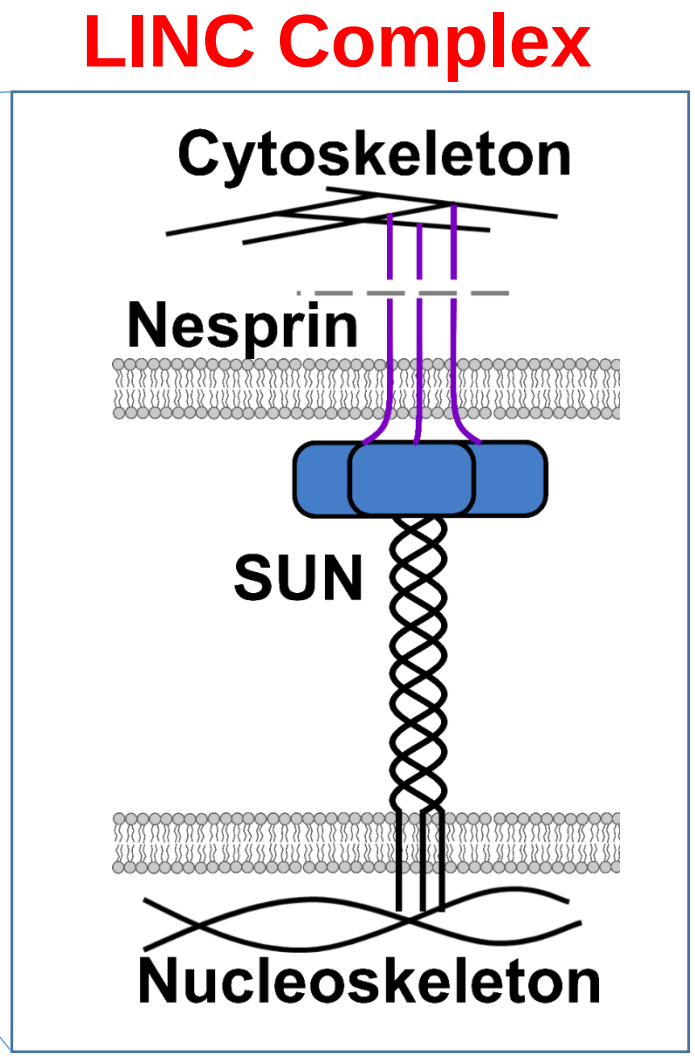




Brightness calibration required !

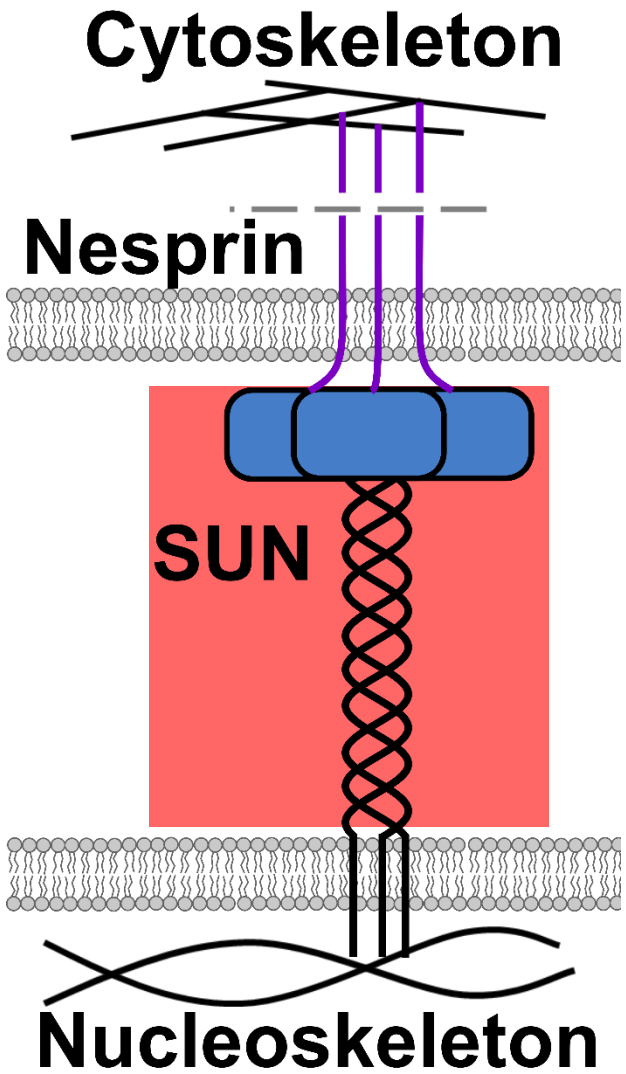
Protein Assembly at the Nuclear Envelope (NE)

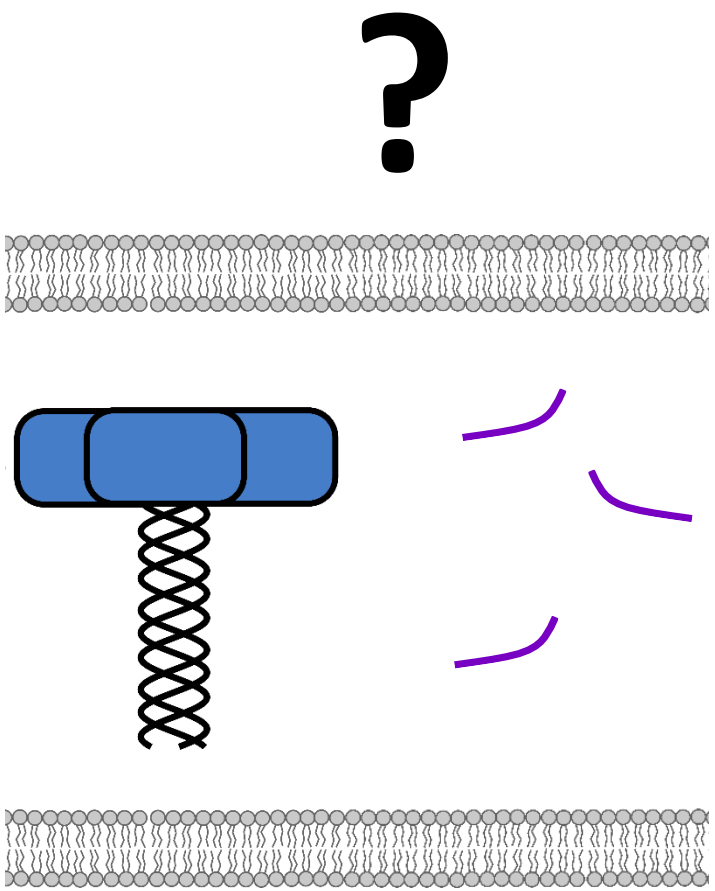


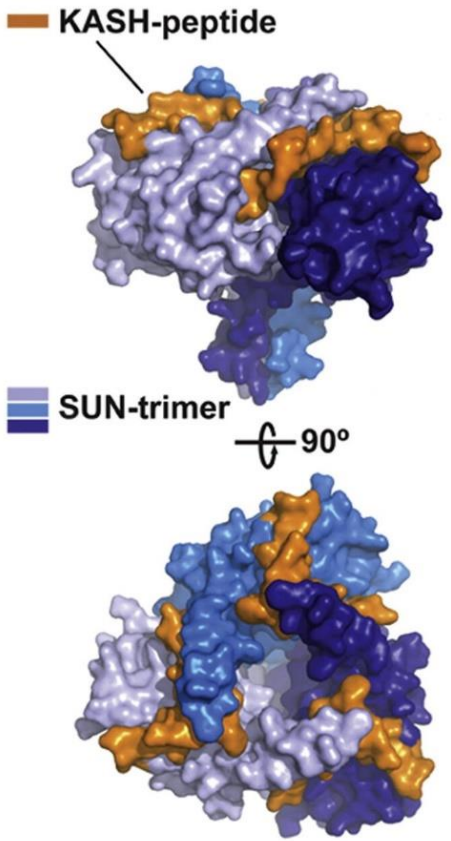


- Nuclear positioning
- Force-dependent gene expression
- DNA damage repair

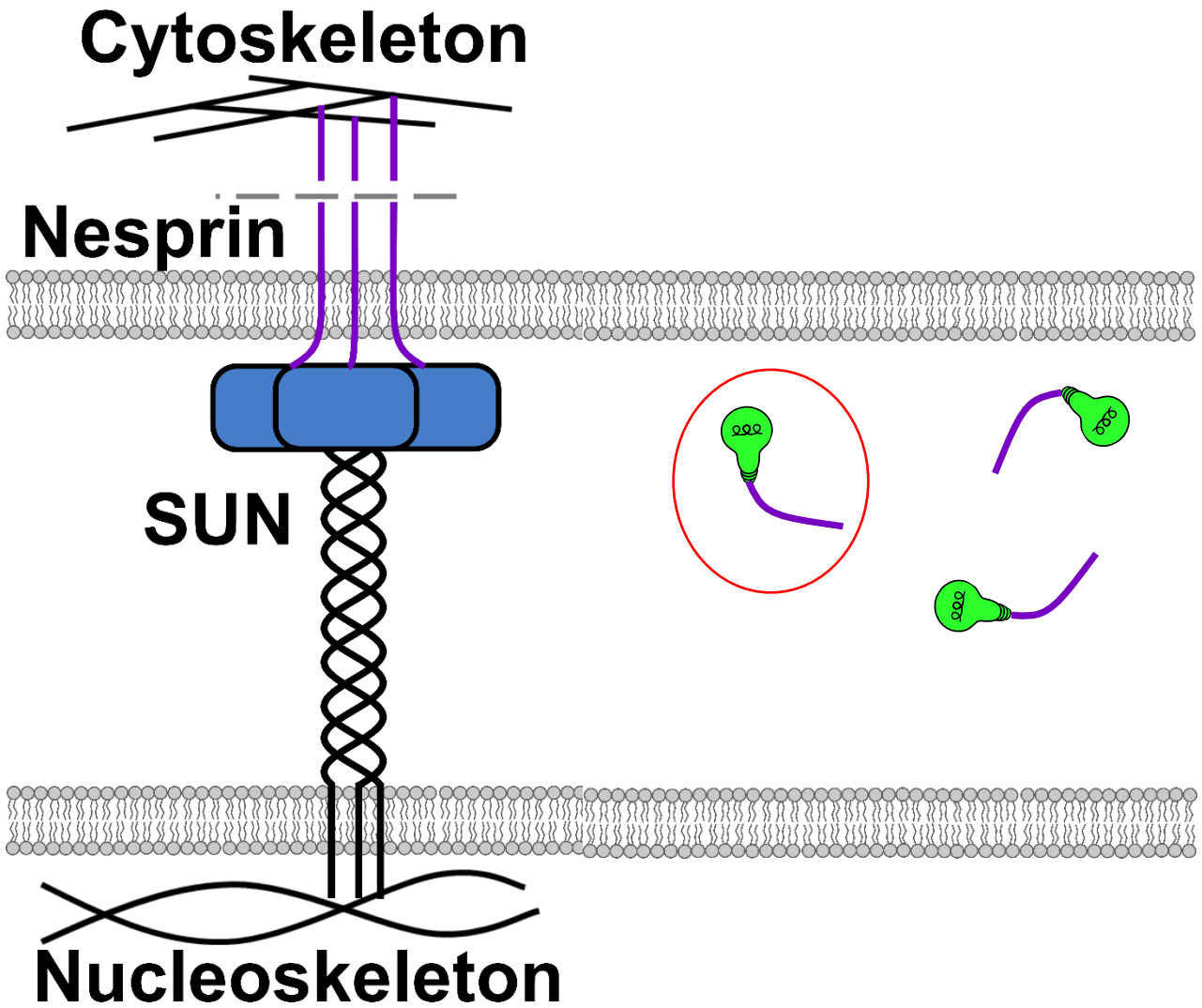
Measuring LINC Complex Assembly in Living Cells

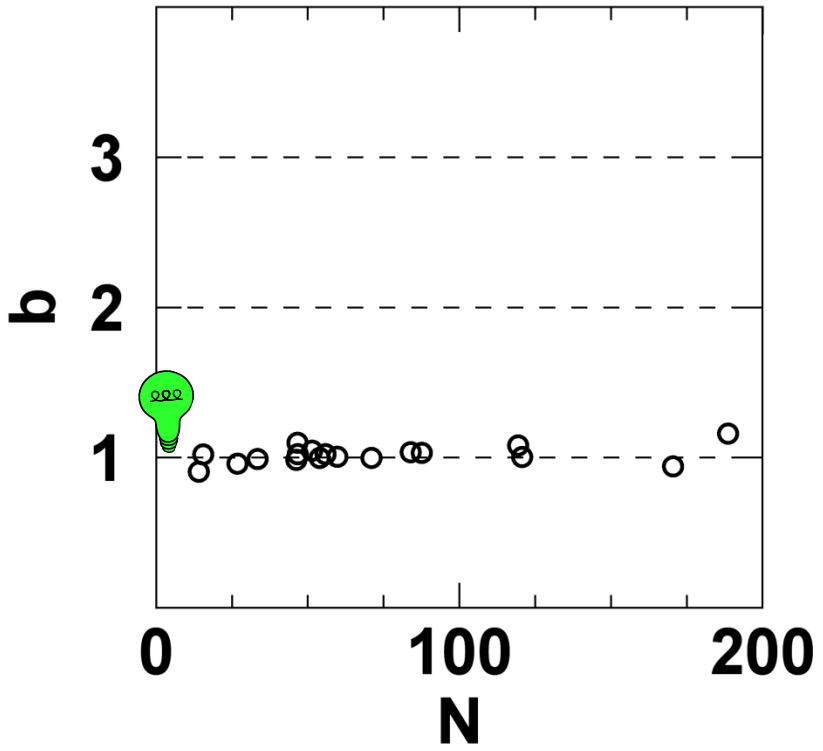






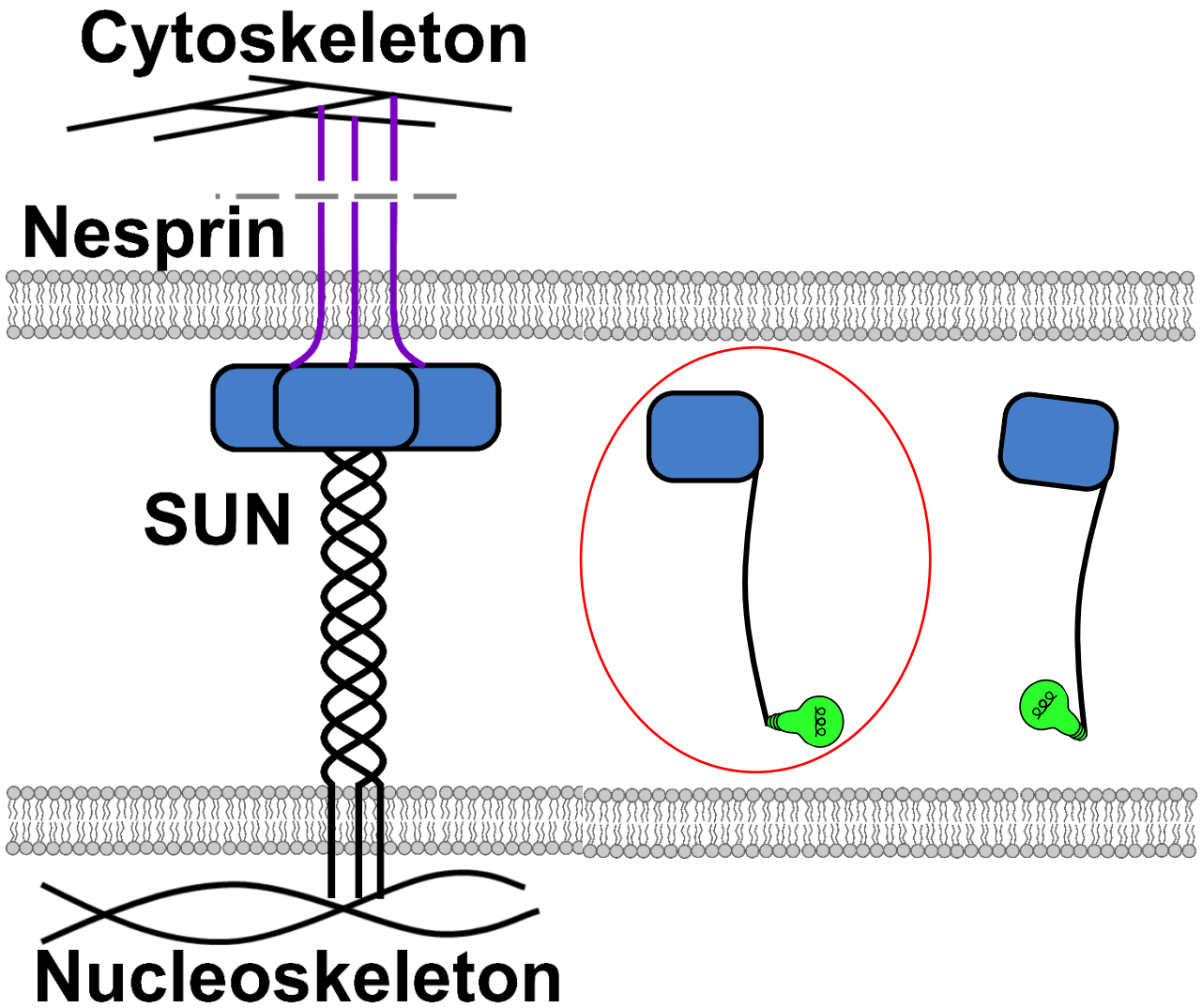
KASH Domain of Nesprin2 Remains Monomeric

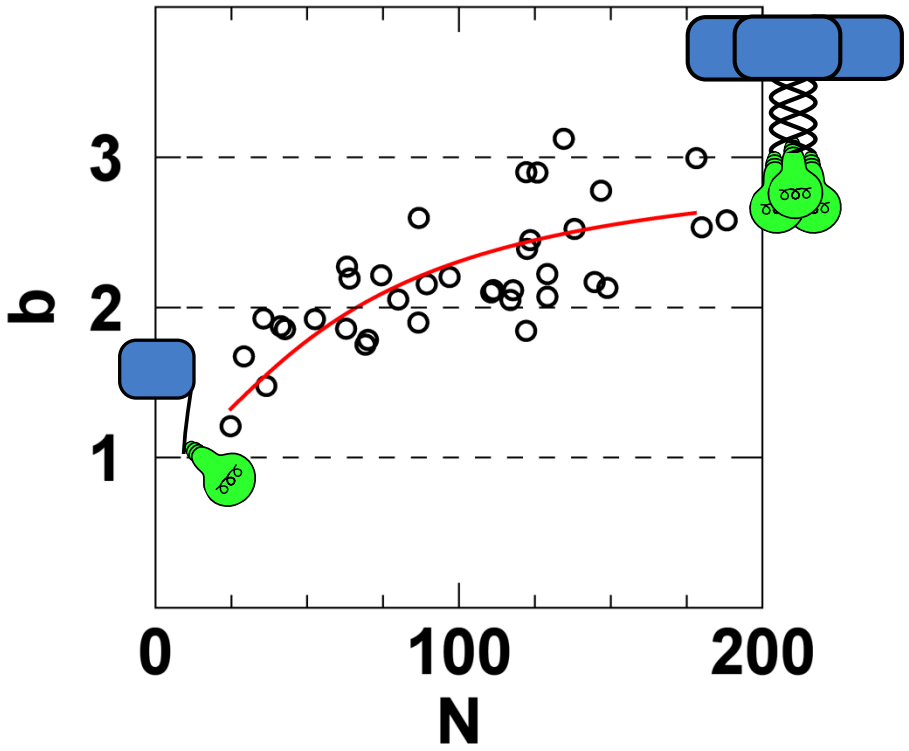




Hennen et al. Biophysical Journal (2017).
Hennen et al. Molecular biology of the cell (2018).

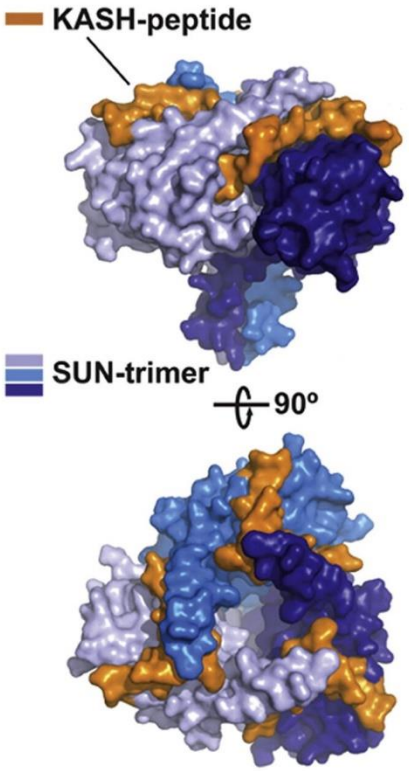
Luminal Domain of SUN2 forms Trimers

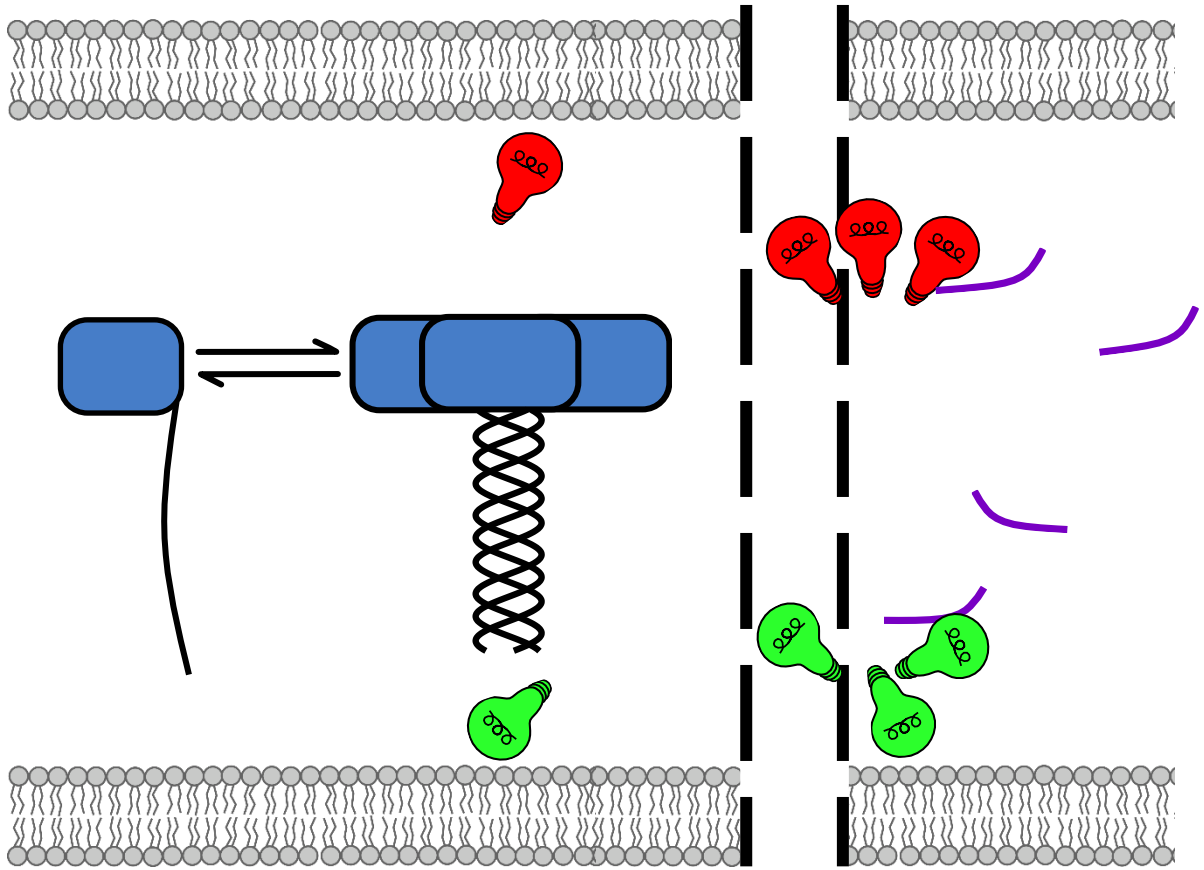




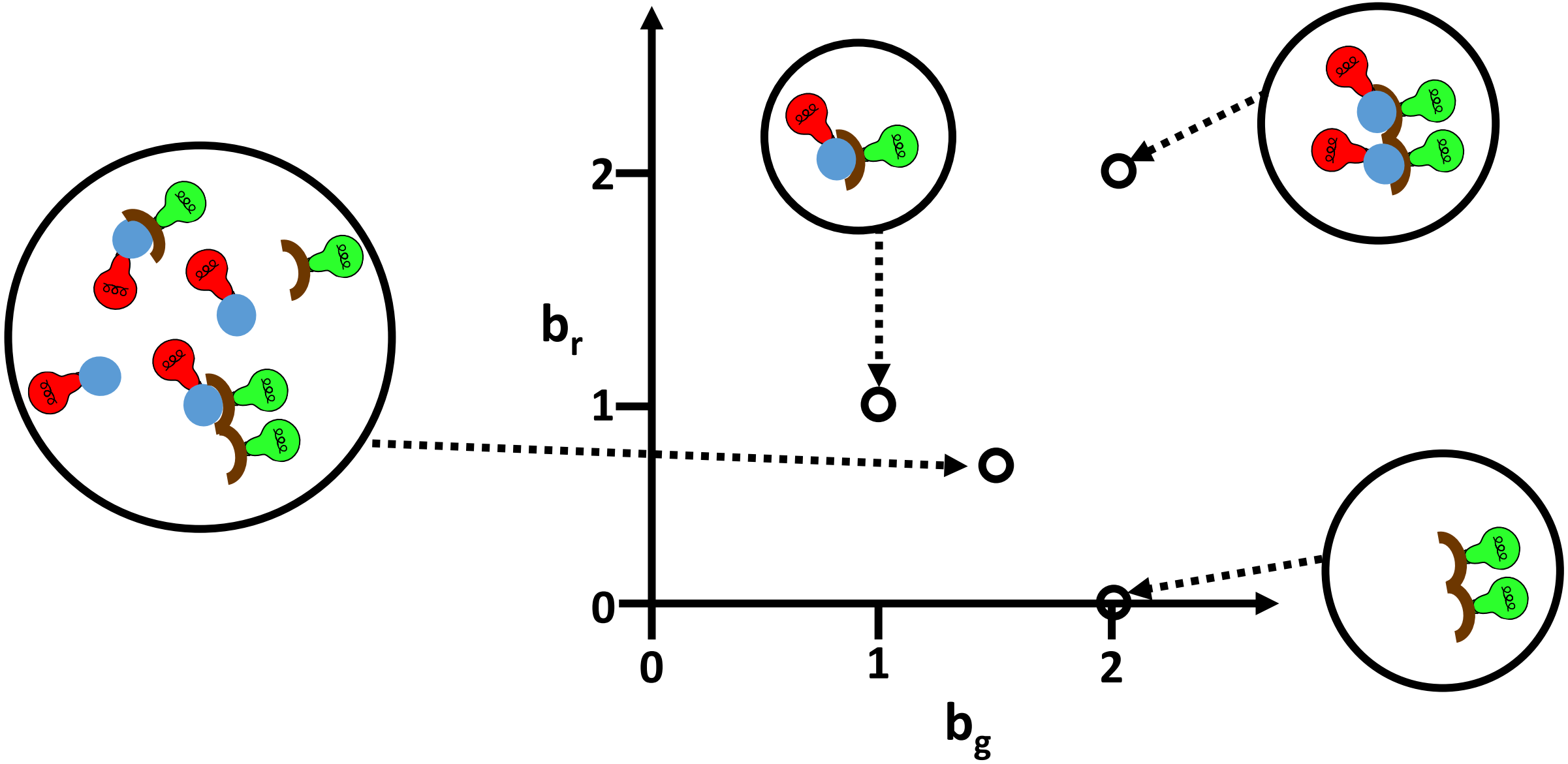
Hennen et al. Biophysical Journal (2017).
Hennen et al. Molecular biology of the cell (2018).

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





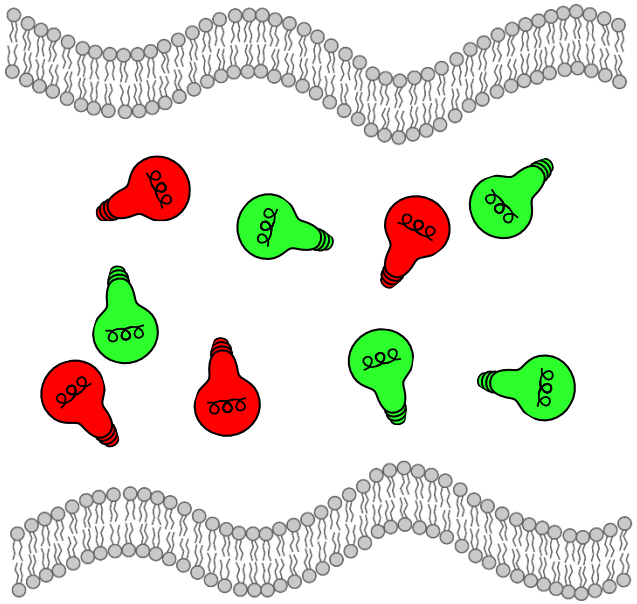
Dual-color Brightness Plot

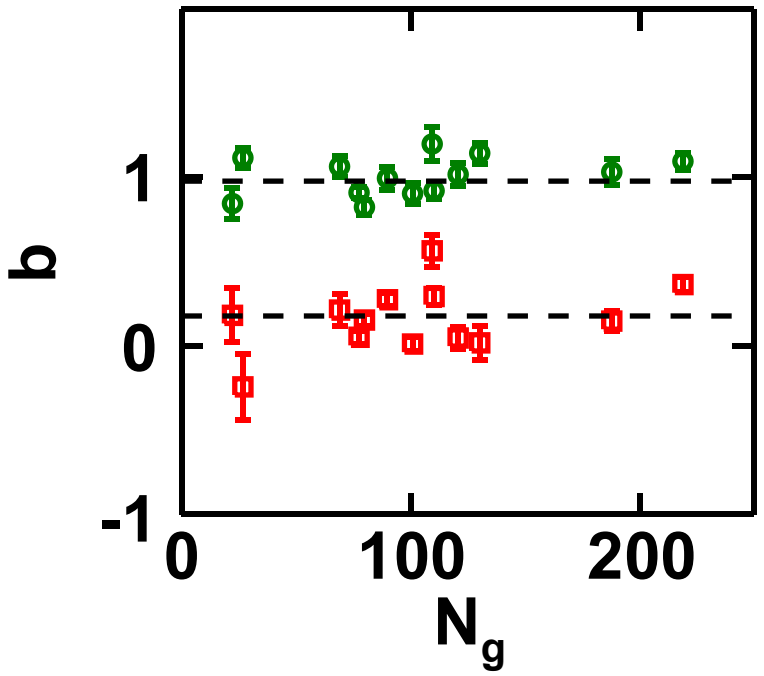


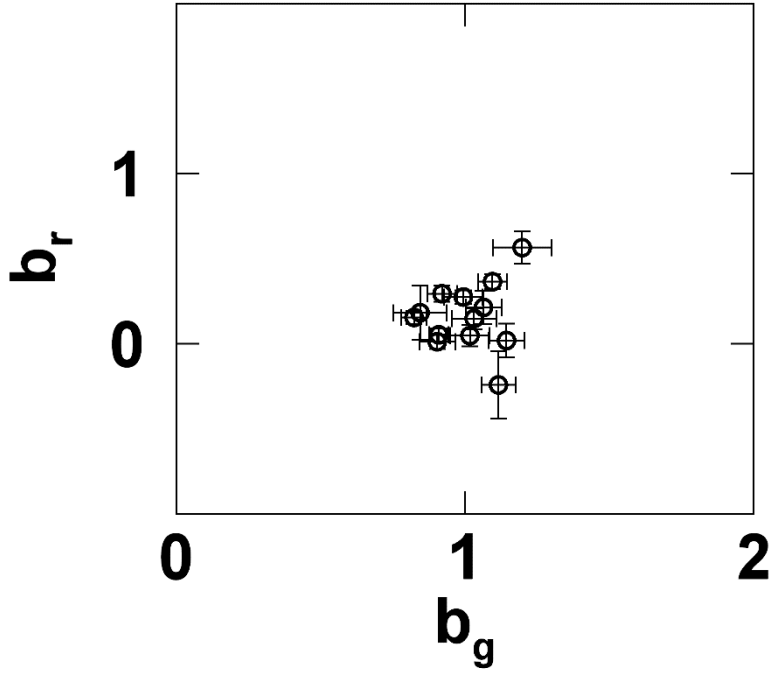
Wu, Chen, and Mueller, "**Heterospecies partition analysis** reveals binding curve and stoichiometry of protein interactions in living cells," PNAS (2010).

Negative Control: No interactions

SS-mCherry-KDEL and SS-EGFP



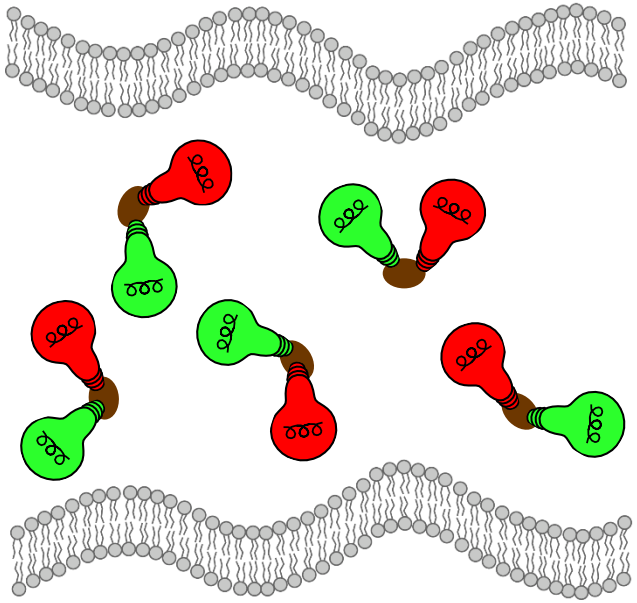


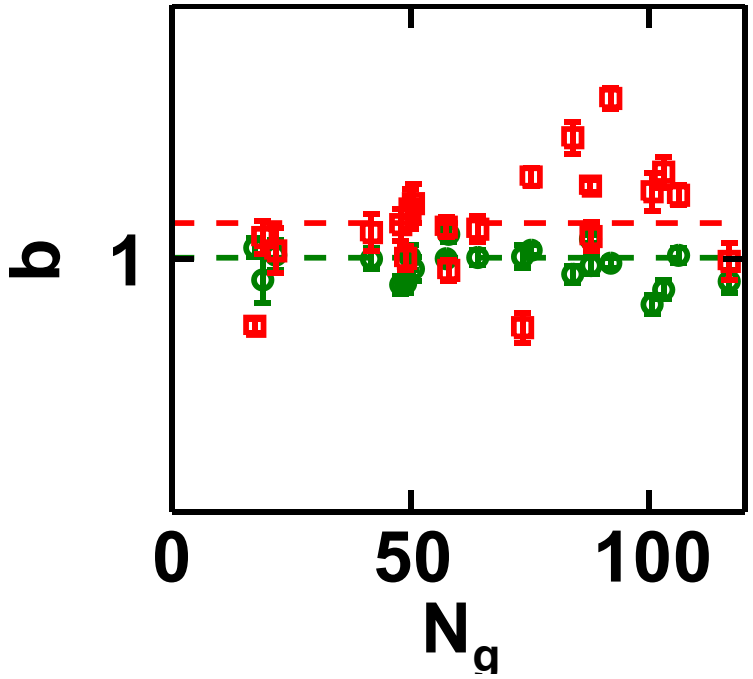


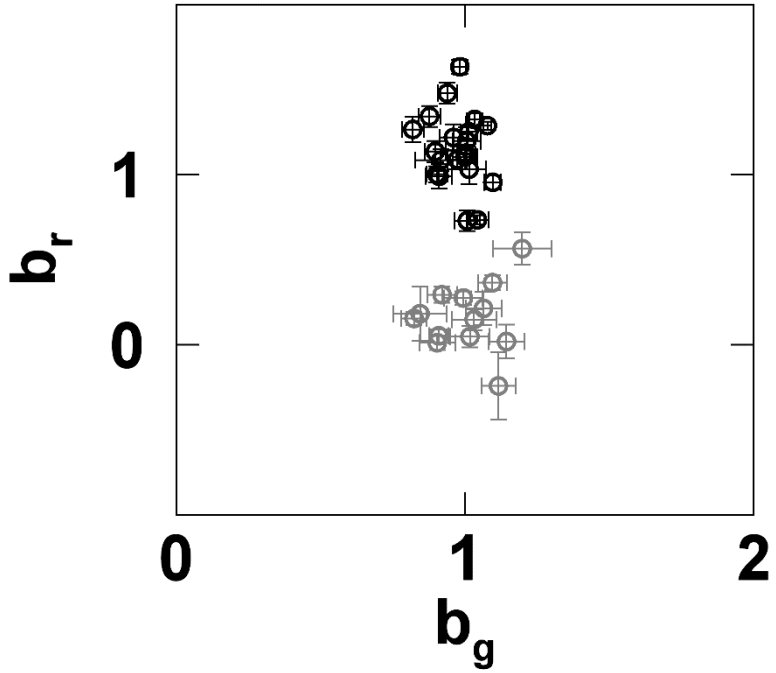
Hennen et al. "Identifying Heteroprotein Complexes in the Nuclear Envelope," Biophysical Journal (2020).

Positive Control: Hetero-Dimer

SS-mCherry-Linker-EGFP

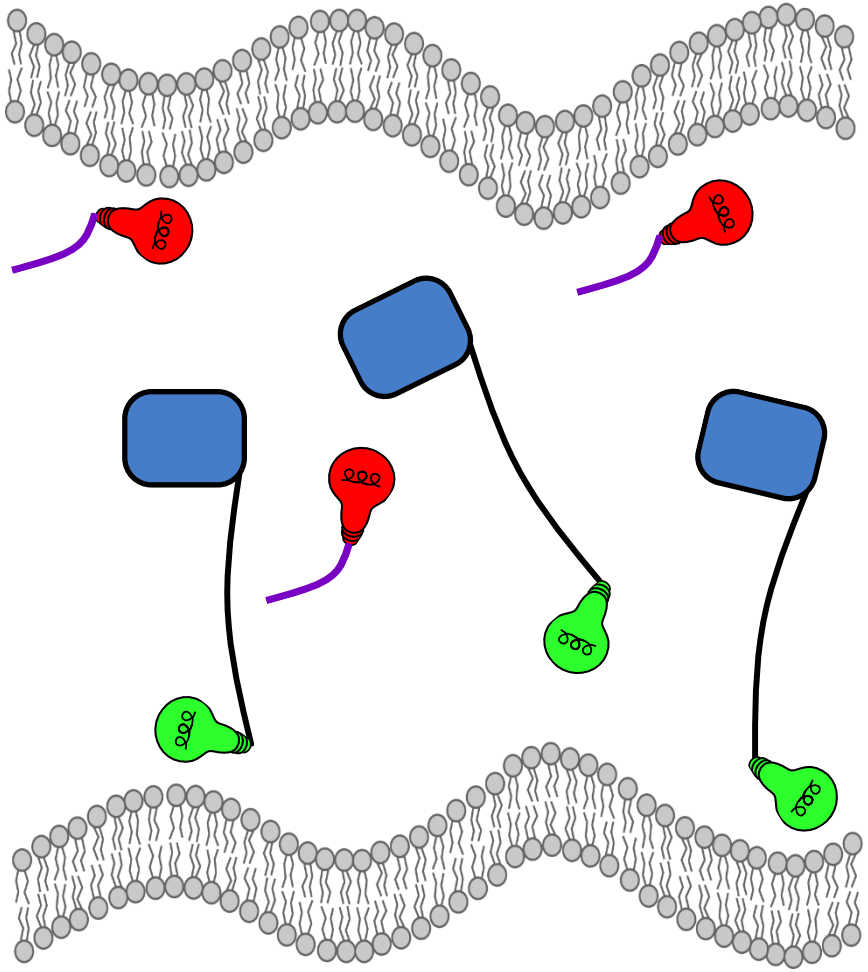


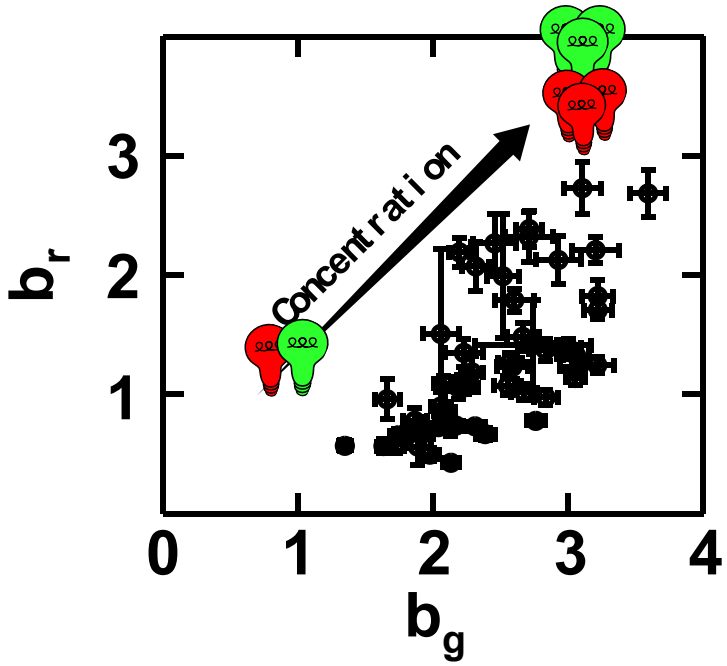


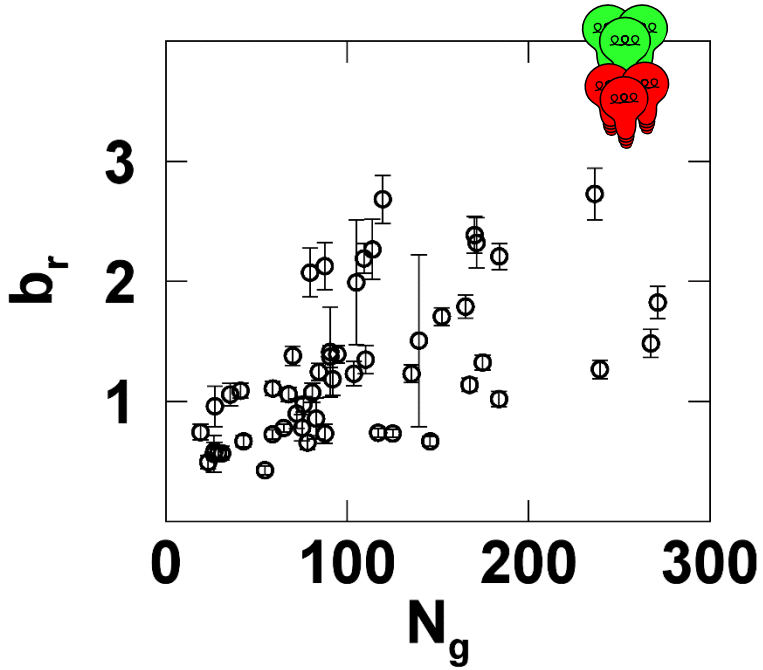


Hennen et al. "Identifying Heteroprotein Complexes in the Nuclear Envelope," Biophysical Journal (2020).

Assembly of SUN2-KASH in Lumen of Nuclear Envelope

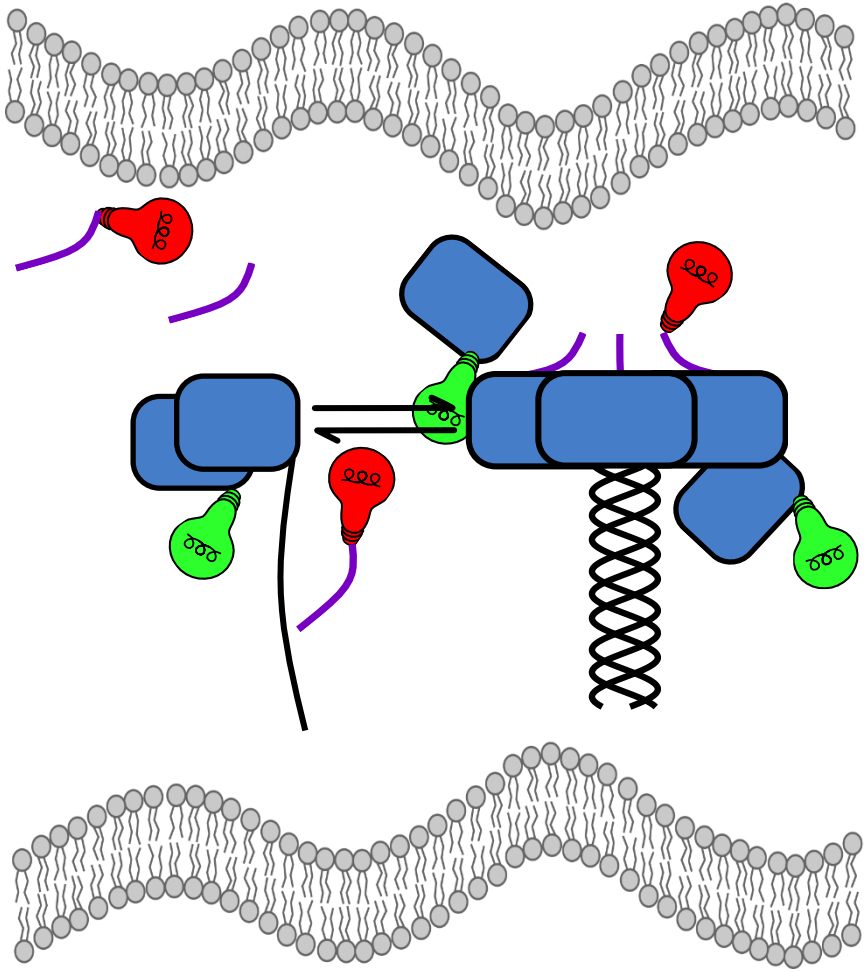


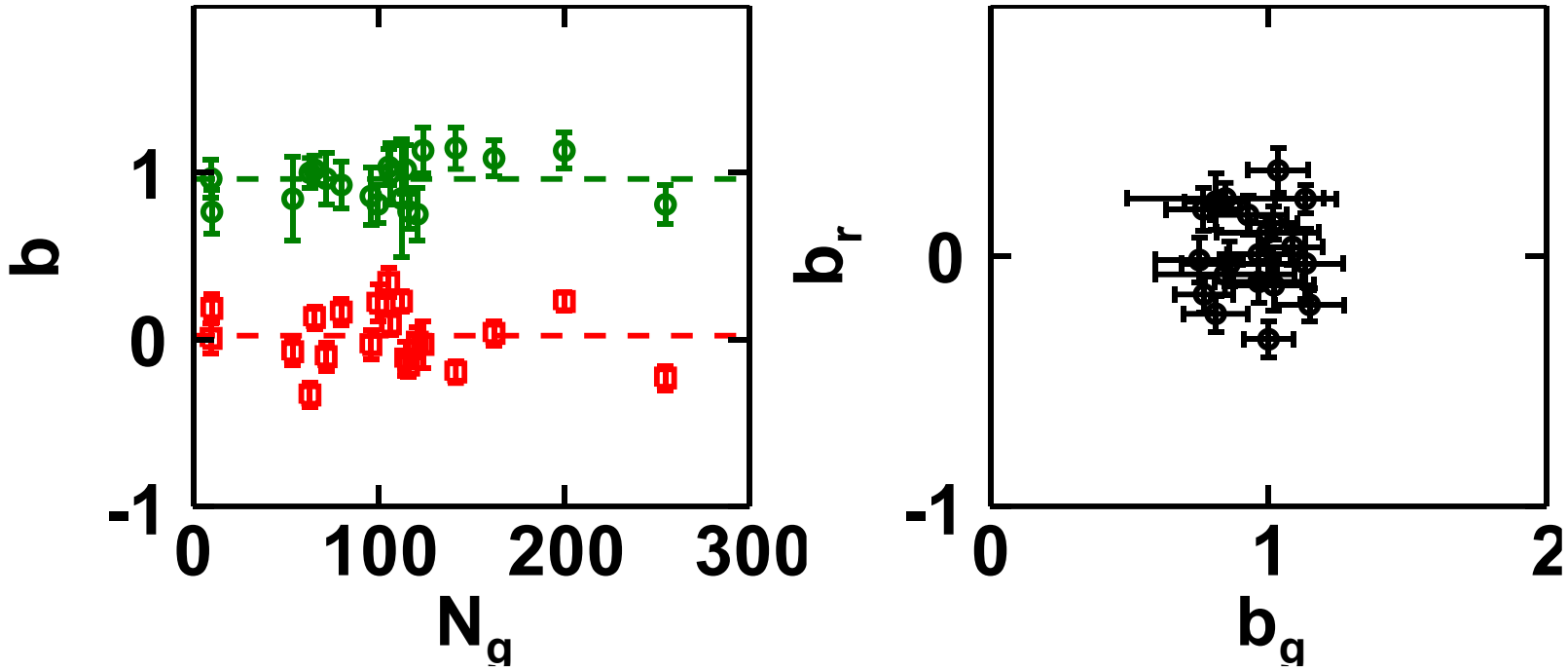




Hennen et al. "Identifying Heteroprotein Complexes in the Nuclear Envelope," Biophysical Journal (2020).

No Monomer-Monomer SUN2 domain - KASH Interactions





Hennen et al. "Identifying Heteroprotein Complexes in the Nuclear Envelope," Biophysical Journal (2020).

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