Quantitative live-cell imaging reveals spatio-temporal dynamics and cytoplasmic assembly of the 26S proteasome

Pack, C.-G. *et al*. *Nat. Commun.* 5:3396 (2014)

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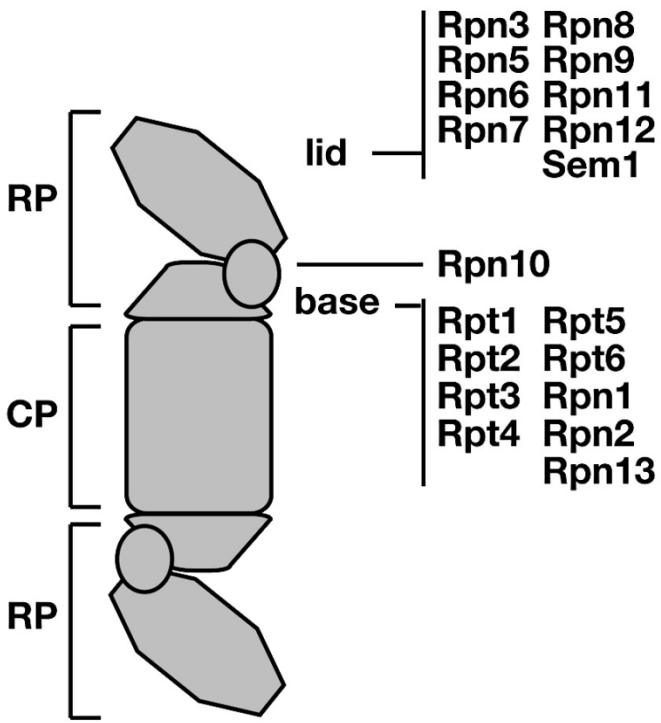
Highlights

- A method for quantitative imaging is introduced to observe protein dynamics in living cells
- The 26S proteasome is a stable complex with high mobility in yeast cells
- The 26S proteasome could be assembled in cytoplasm and imported into the nucleus as a holo-complex

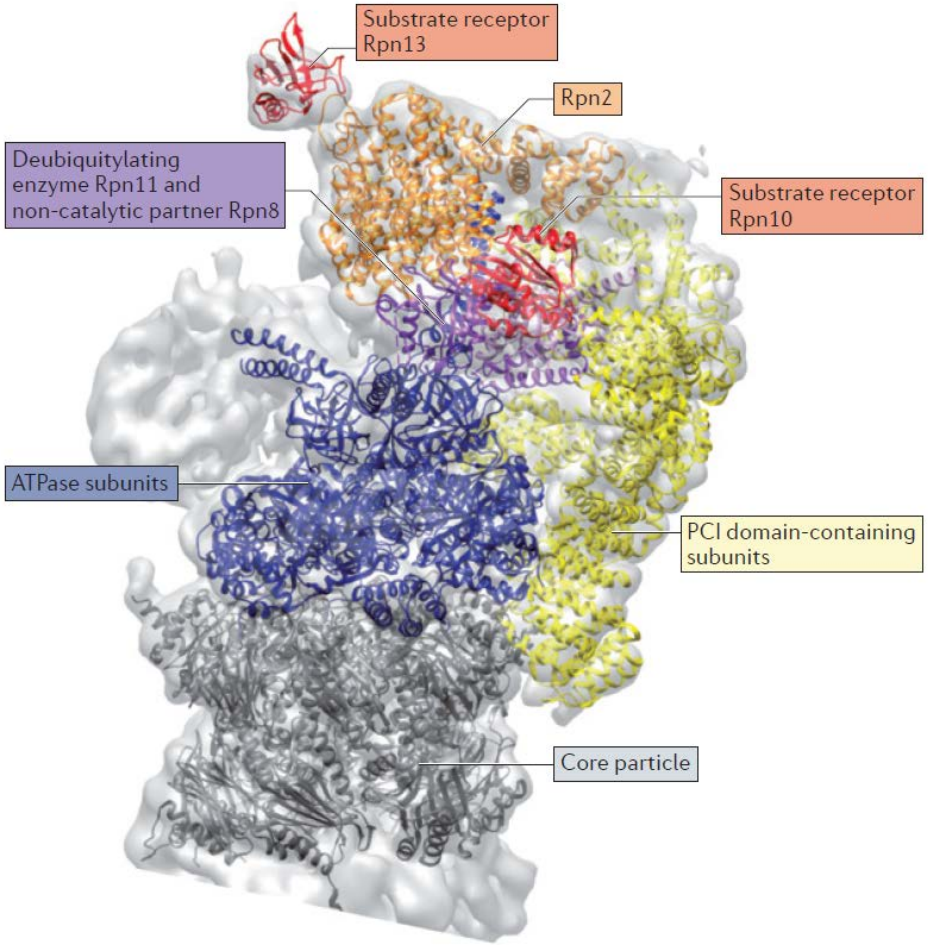
Why I am Interested in This Work

- The dynamics and assembly of the 26S proteasome is studied in *in vivo*
- A new perspective facilitating development of novel proteasome inhibitors

Background I – the structure of the 26S

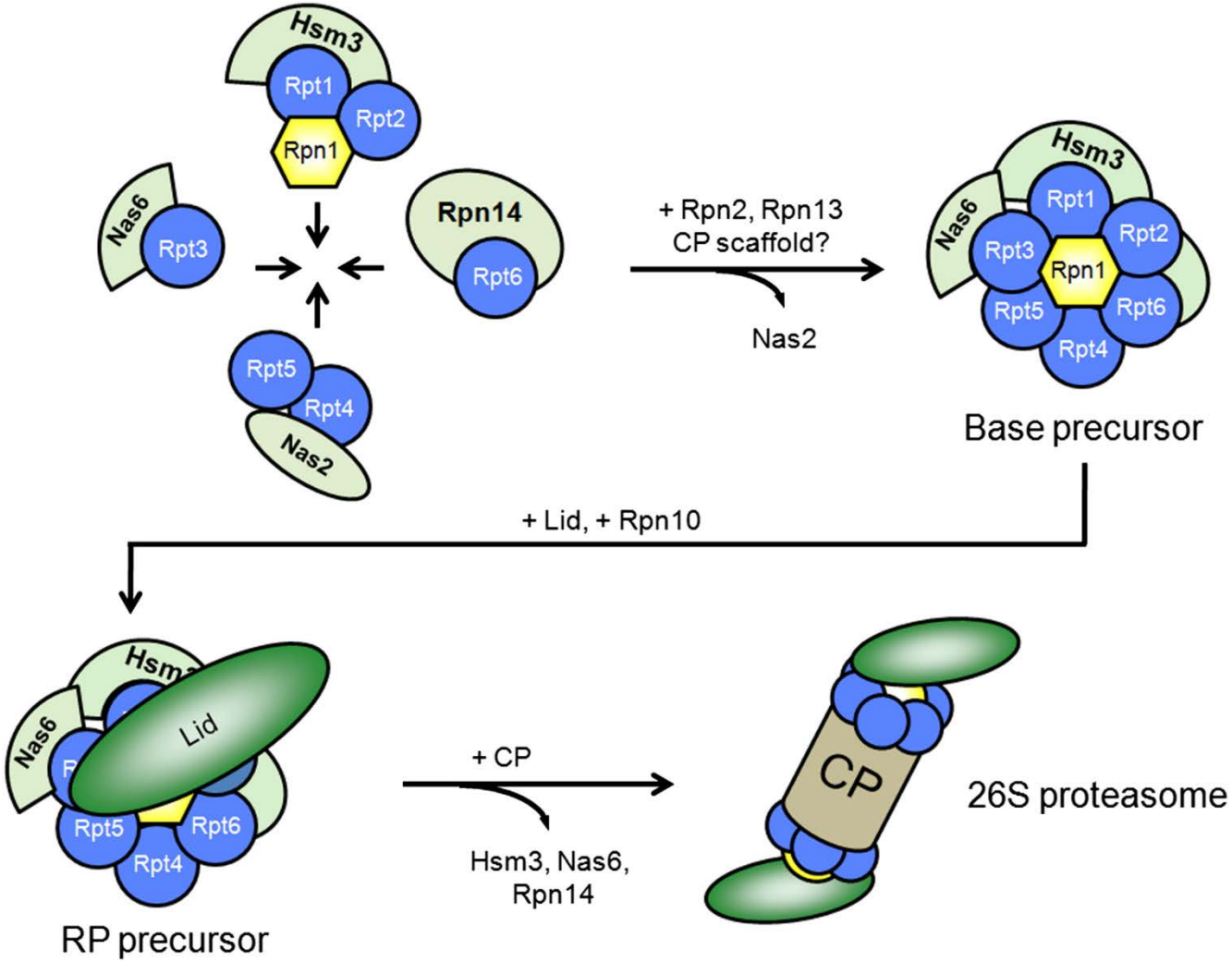


Cell (2009) Funakoshi, M. *et al*



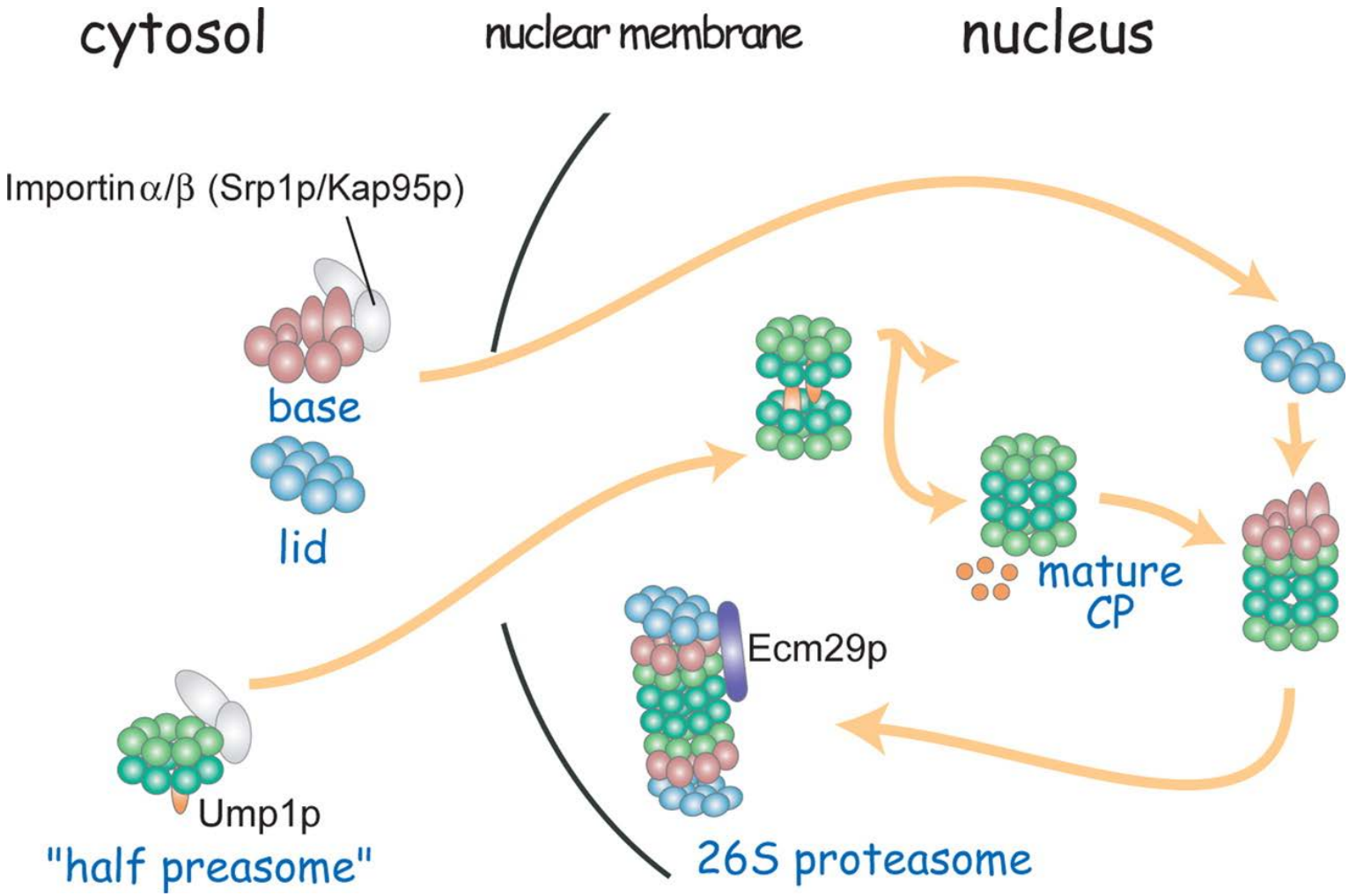
Nat. Rev. Mol. Cell Biol. (2014) Bhattacharyya, S. *et al*

Background II – the assembly of the 26S



Cell (2009) Funakoshi, M. *et al*

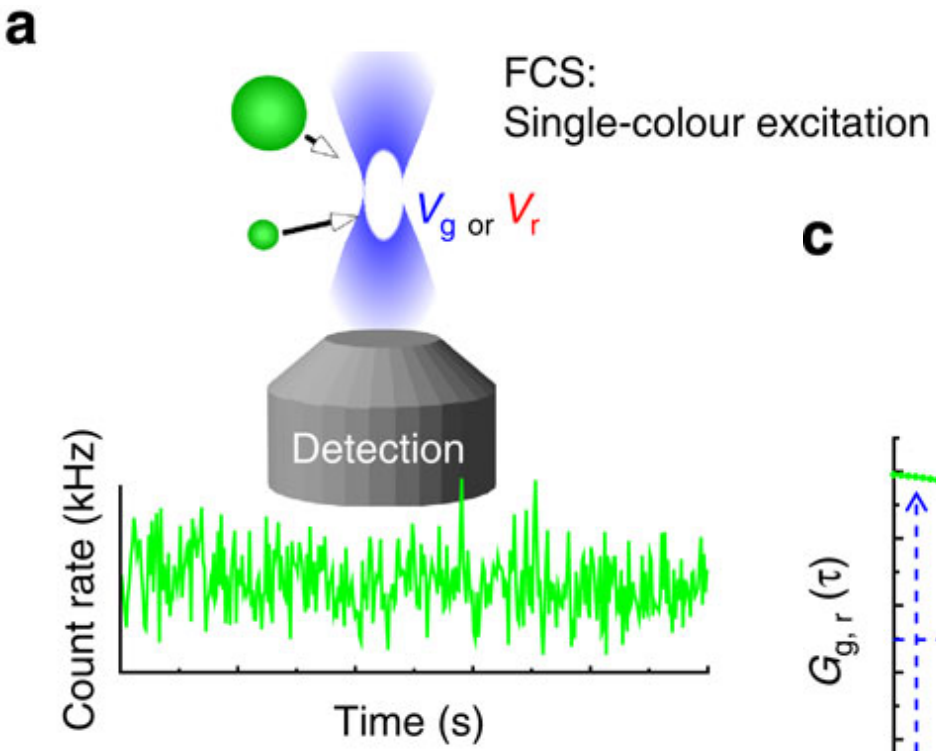
Background III – the translocation of the 26S

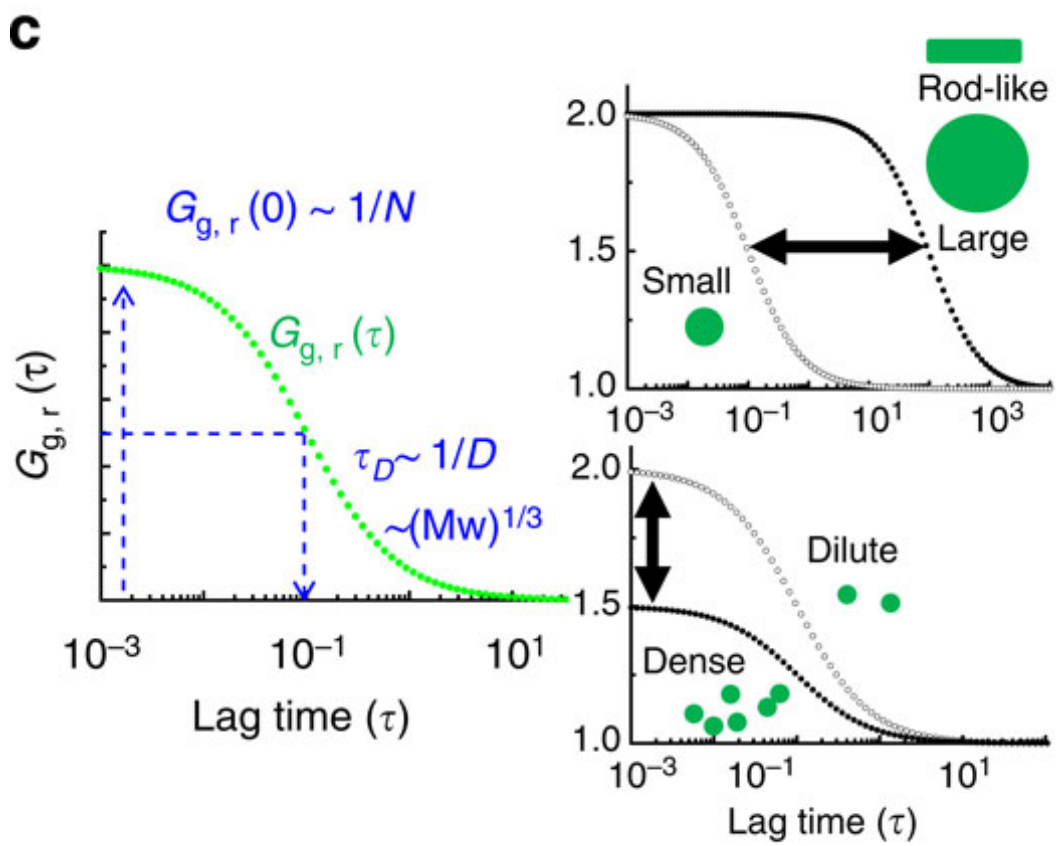


Mol. Biol. Cell (2007) Isono, E. *et al*

Fluorescence Correlation Spectroscopy

Single fluorescent molecule

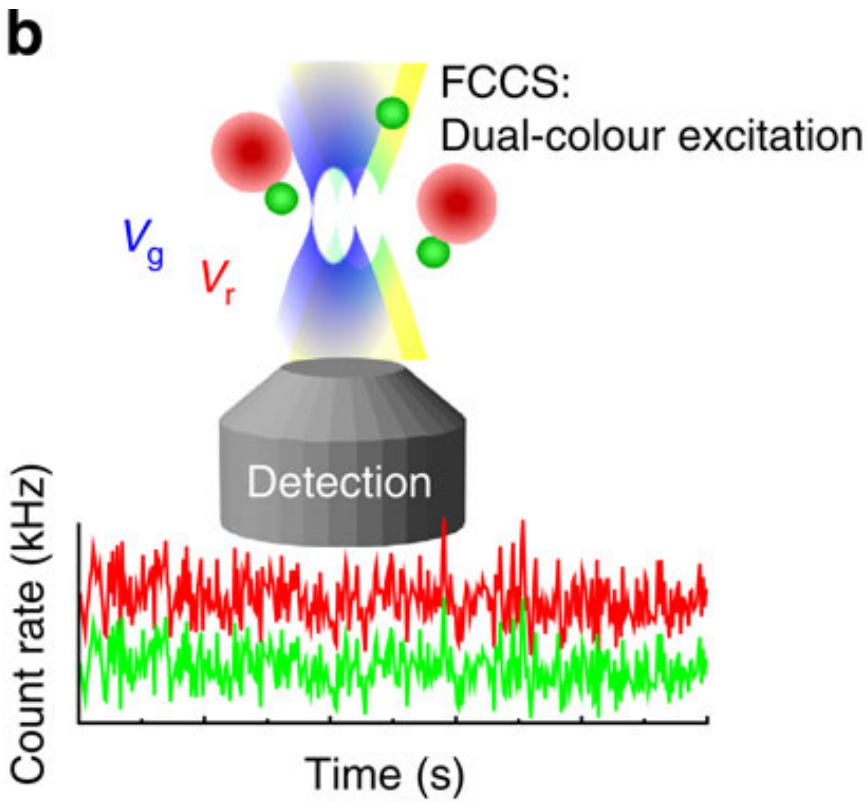


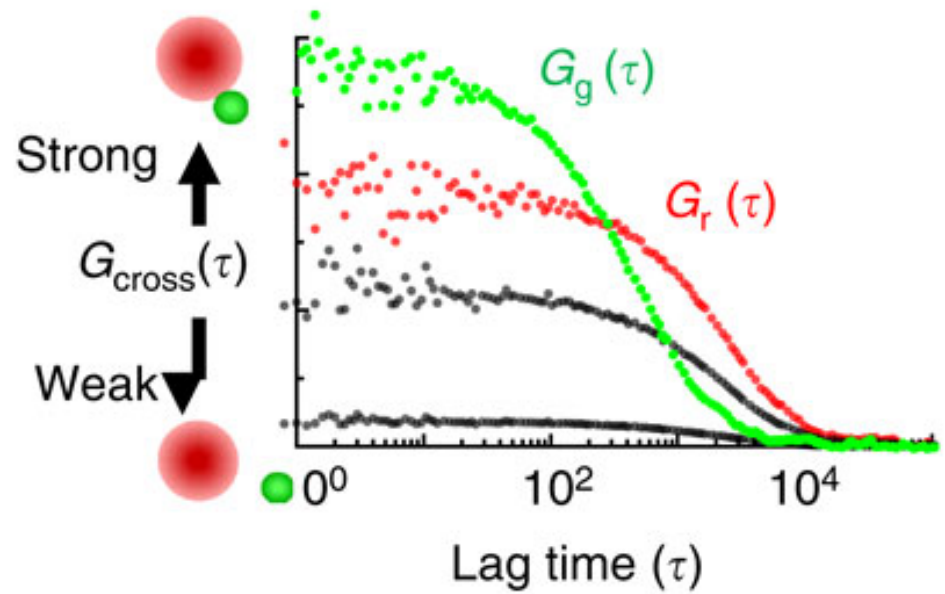


Fluorescence auto-correlation

Fluorescence Correlation Spectroscopy

Two fluorescent molecules

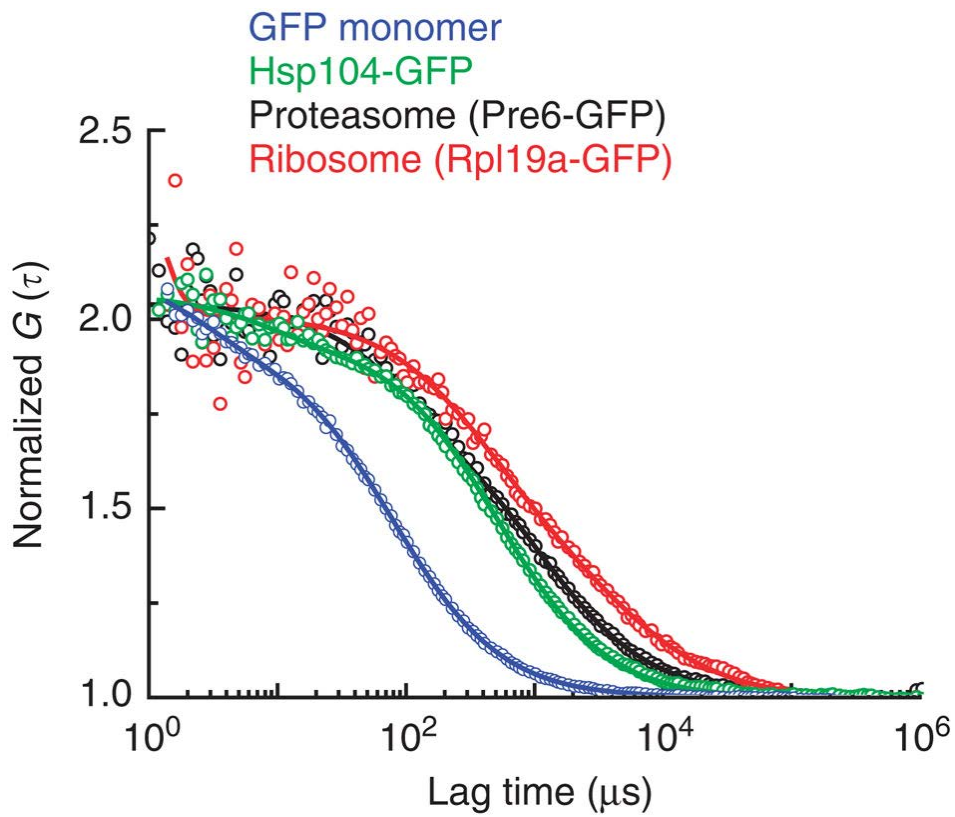




Fluorescence cross-correlation

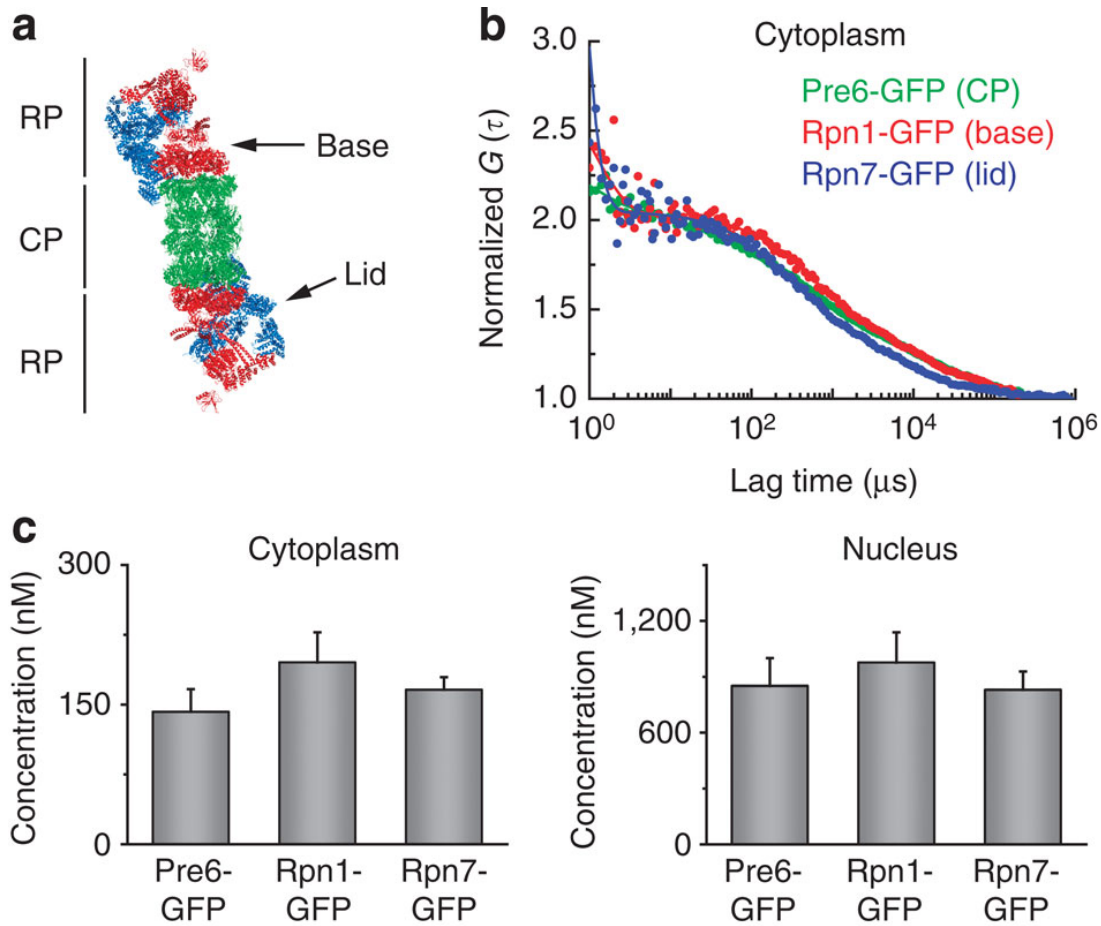
Fluorescence Correlation Spectroscopy

One example



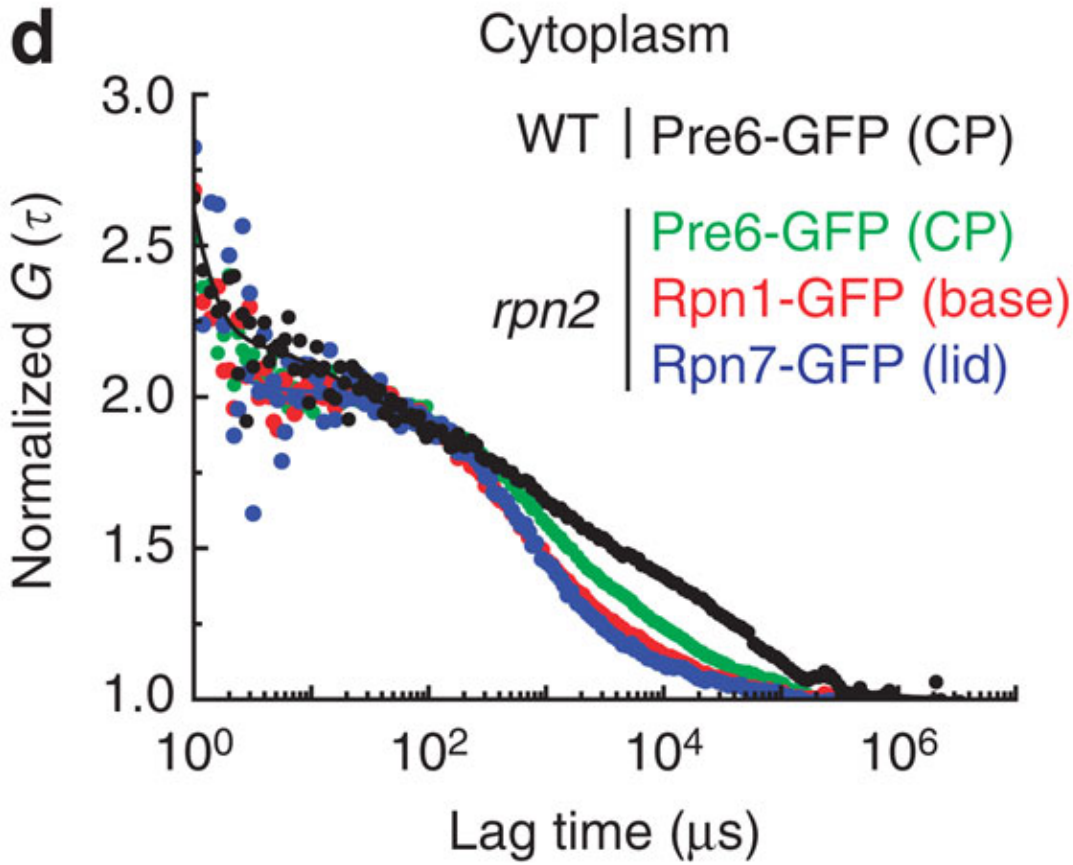
Proteins	GFP-tagged proteins	Theoretical MW	D_{fcs} ($\mu m^2 s^{-1}$)*	MW_{fcs}
Fluorescent proteins	GFP monomer, mCherry	28×10^3	78 ± 1.8	25×10^3
	Tandem 3XGFP (rod-like shape)	85×10^3	43.2 ± 1.4	164×10^3
Hsp104	Hsp104-GFP (6 nm cavity)	132×10^3 (792×10^3)†	12 ± 1.0	8.6×10^6
26S proteasome	Pre6-GFP (rod-like and small cavity)	2.56×10^6 (26S + two GFP)‡	10 ± 1.5	10×10^6
Ribosome	Rpl19a-GFP (diameter ~28 nm)	Unknown	7.5 ± 1.0	28×10^6

Dynamics of the 26S proteasome revealed by FCS



	Wild type	
GFP-tagged proteins	D_{fast} ($\mu m^2 s^{-1}$)* (%)†	D_{slow} ($\mu m^2 s^{-1}$)* (%)
Pre6 (CP)	6.0 ± 0.35 (55)	0.25 ± 0.01 (45)
Rpn1 (base)	6.0 ± 0.51 (53)	0.31 ± 0.03 (47)
Rpn7 (lid)	5.3 ± 0.48 (59)	0.31 ± 0.03 (41)
GFP monomer (control)‡	21.6 ± 0.7 (93)	0.2 ± 0.1 (7)

Dynamics of the 26S proteasome revealed by FCS

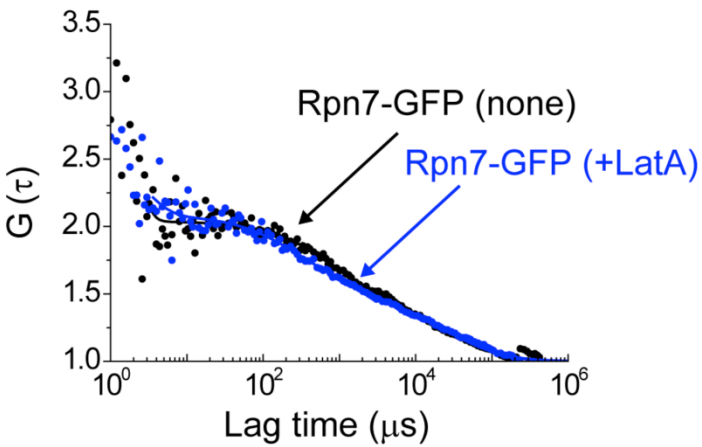


GFP-tagged proteins	Wild type		rpn2	
	D_{fast} ($\mu m^2 s^{-1}$)* (%)†	D_{slow} ($\mu m^2 s^{-1}$)* (%)	D_{fast} ($\mu m^2 s^{-1}$)* (%)†	D_{slow} ($\mu m^2 s^{-1}$)* (%)
Pre6 (CP)	6.0 ± 0.35 (55)	0.25 ± 0.01 (45)	11.3 ± 0.30 (73)	0.7 ± 0.10 (27)
Rpn1 (base)	6.0 ± 0.51 (53)	0.31 ± 0.03 (47)	12.1 ± 0.34 (80)	1.0 ± 0.14 (20)
Rpn7 (lid)	5.3 ± 0.48 (59)	0.31 ± 0.03 (41)	12.0 ± 0.50 (81)	1.3 ± 0.20 (19)
GFP monomer (control)‡	21.6 ± 0.7 (93)	0.2 ± 0.1 (7)		

Characterization of the slower-mobility species

Cytoplasm

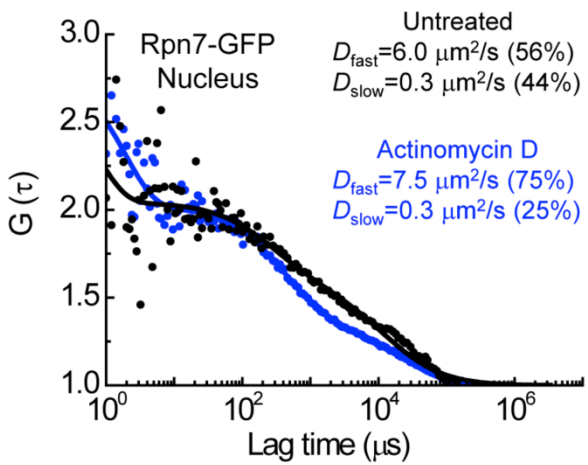
LatA disrupts actin fibres



No association with Actin

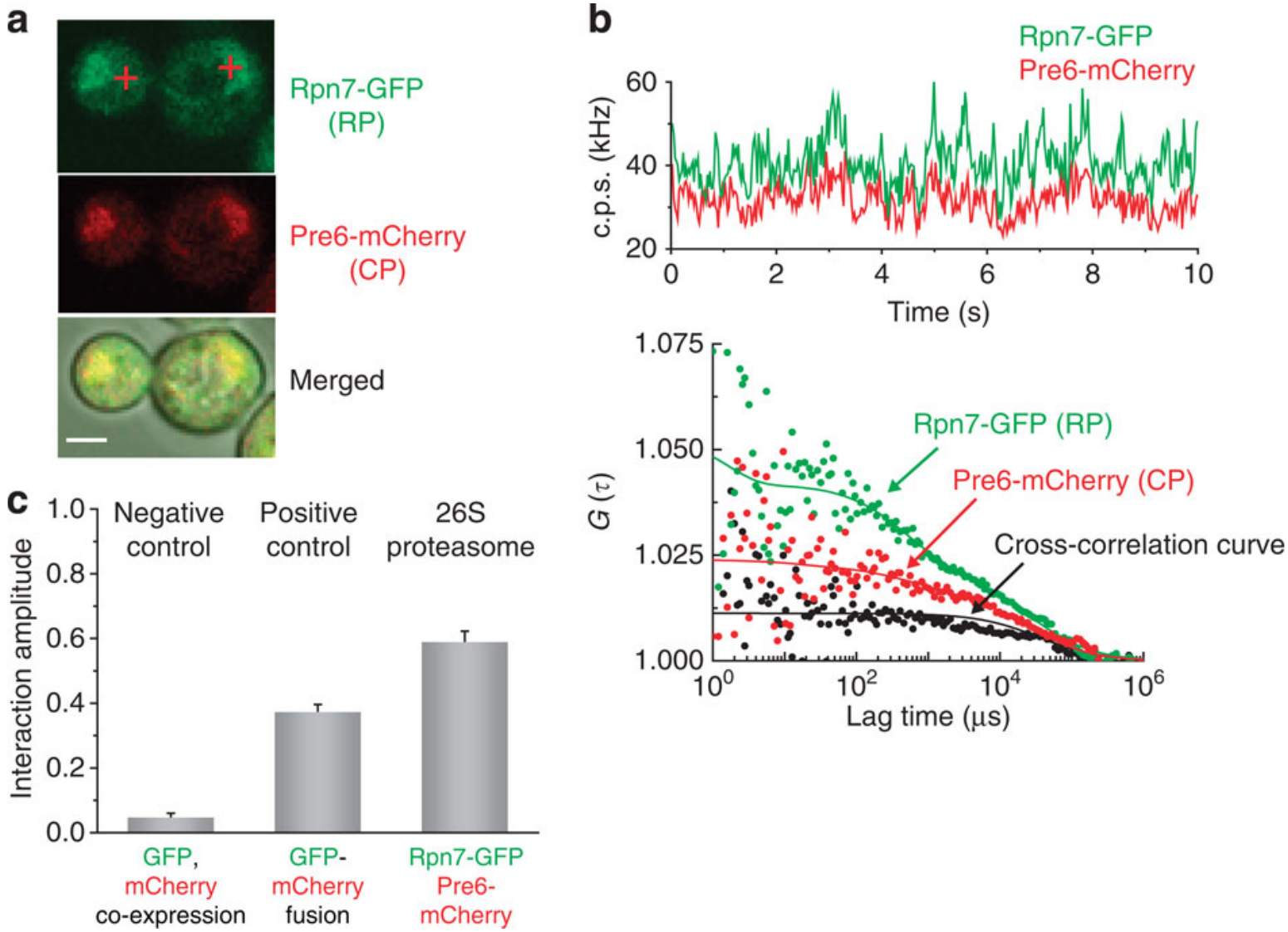
Nucleus

RNA Pol II inhibition

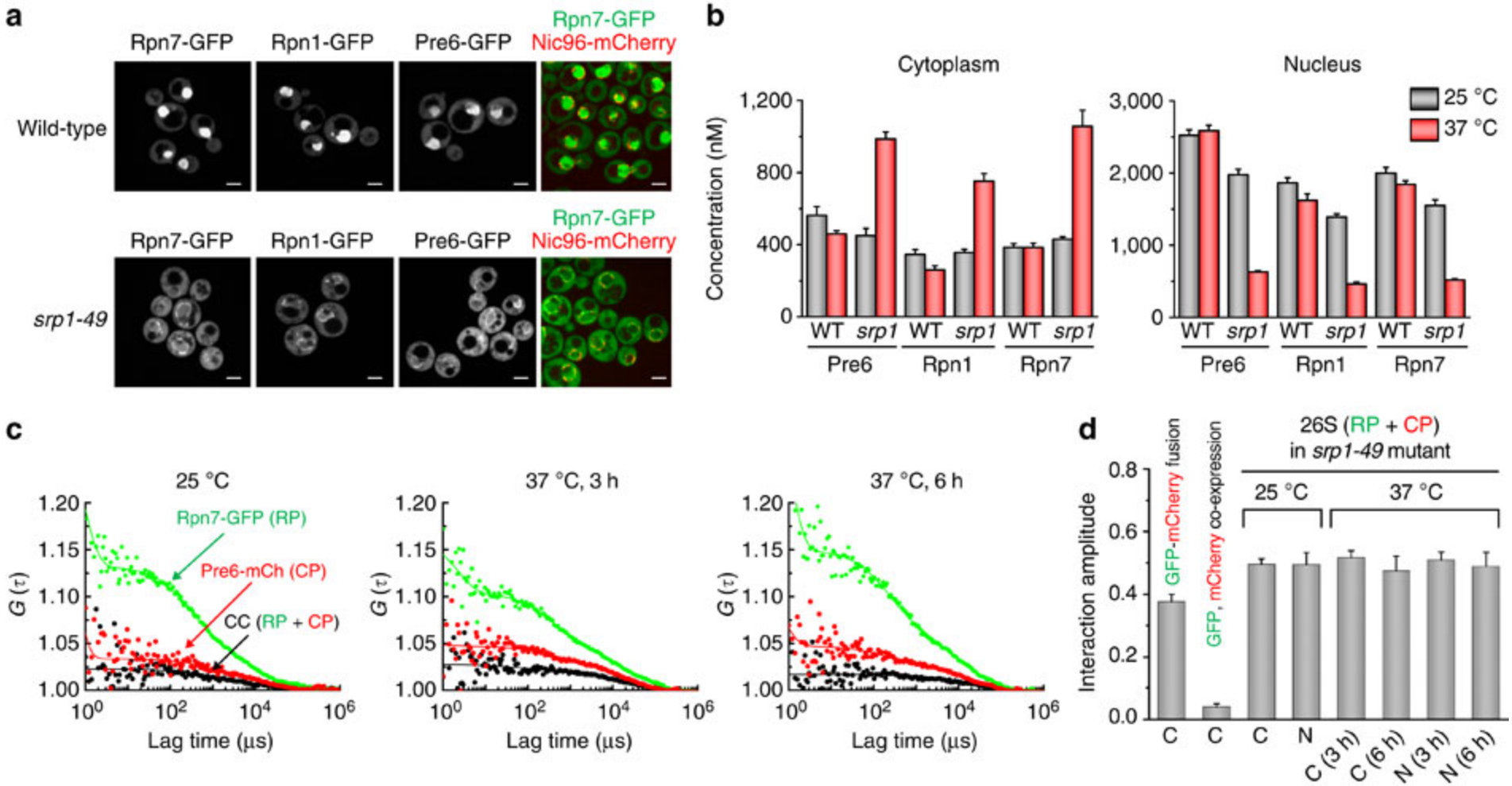


Engaged in transcription

Stable complex of the 26S in living cells

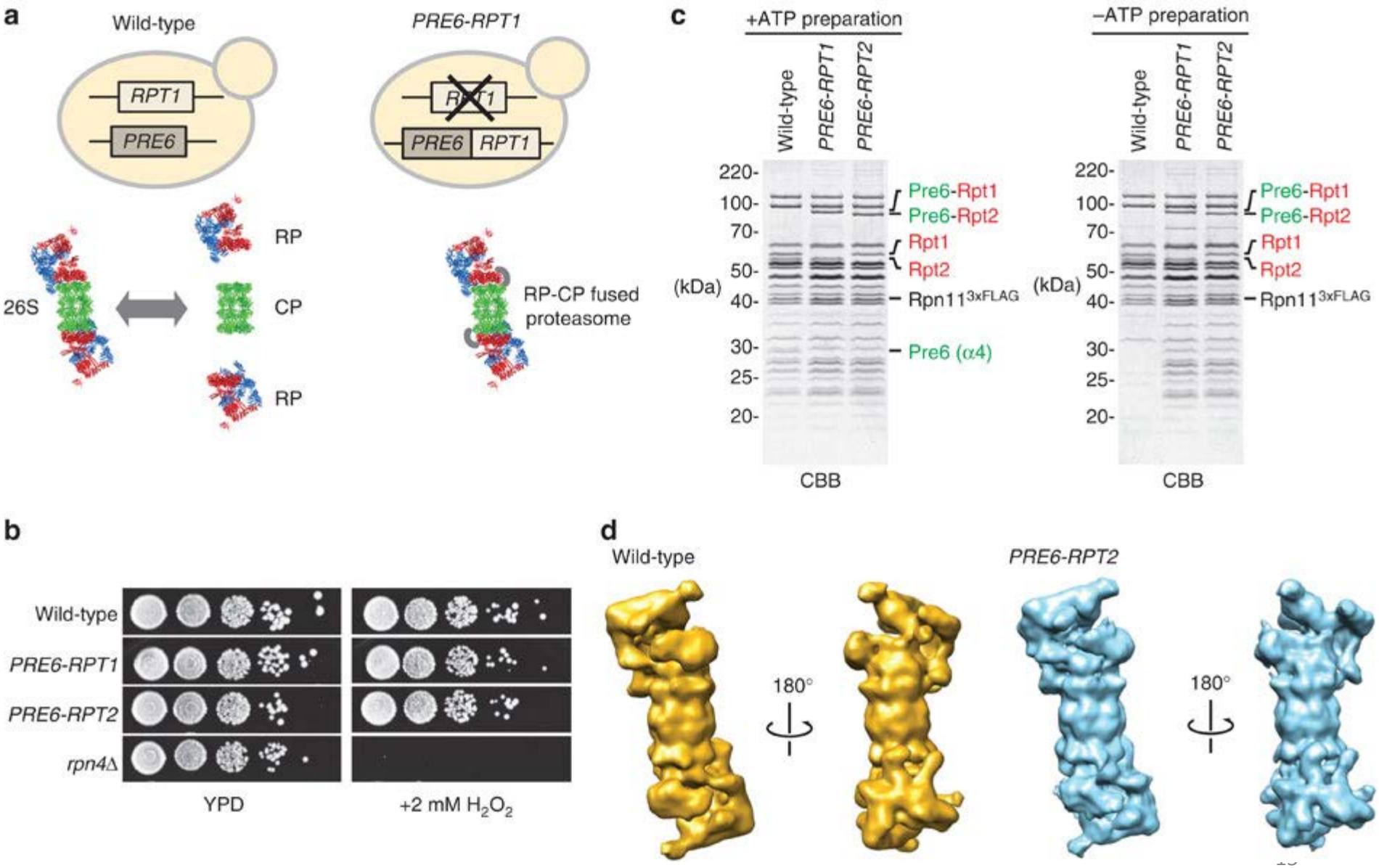


Assembly of the 26S proteasome in the cytoplasm

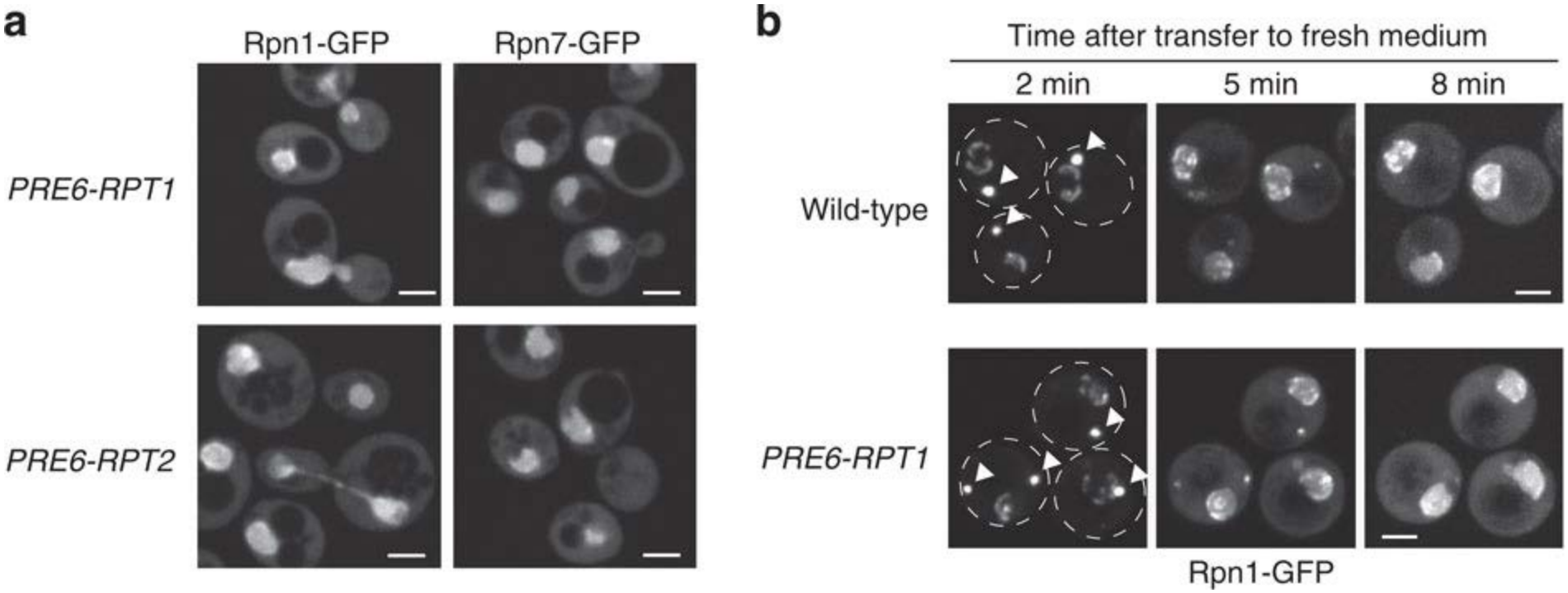


The 26S proteasome could form in the cytoplasm when nuclear import is impaired.

Localization of stabilized holo-enzyme in the nucleus



Localization of stabilized holo-enzyme in the nucleus



	Wild-type		*PRE6-RPT2*	
GFP-tagged proteins	D_{fast} (μm²/s) / (%)[a]	D_{slow} (μm²/s) / (%)	D_{fast} (μm²/s) / (%)	D_{slow} (μm²/s) / (%)
Rpn1 (base)	6.6 ± 0.51 (60%)	0.31 ± 0.03 (40%)	6.5 ± 0.06 (53%)	0.3 ± 0.03 (47%)
Rpn7 (lid)	6.9 ± 0.48 (62%)	0.31 ± 0.03 (38%)	7.0 ± 0.02 (54%)	0.3 ± 0.02 (46%)

Conclusions

- The 26S proteasome is a stable complex with no free subcomplexes or subunits at detectable levels in the cell

- The 26S proteasome is able to assembly in the cytoplasm and be imported as a holoenzyme into the nucleus

Take-Home Message

- Fluorescence correlation spectroscopy is a powerful tool to quantitatively detect dynamics and assembly of target protein in living cells

- This paper turned over several conclusions from previous studies on dynamics and assembly of the 26S proteasome in living yeast cells

Thanks