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RENSSELAER POLYTECHNIC INSTITUTE :: CENTER FOR BIOTECHNOLOGY AND INTERDISCIPLINARY STUDIES

Protein Interaction Networks in Live Cells: Who, what, when, where, how and why ?

Catherine Royer

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Transcriptional control of t switch between glycolysis a gluconeogenesis in *B. subtil*

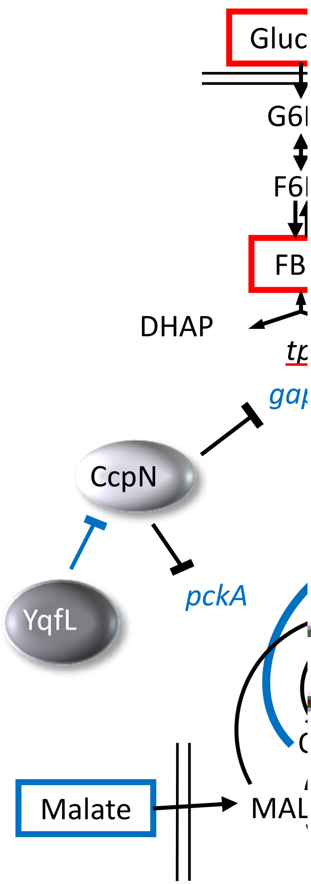
N. Declerck, S. Aymerich
CBS, INRA – Grignon
G. Rivas, S. Zorilla, Madrid
Sarah Cianferani - Strasbourg

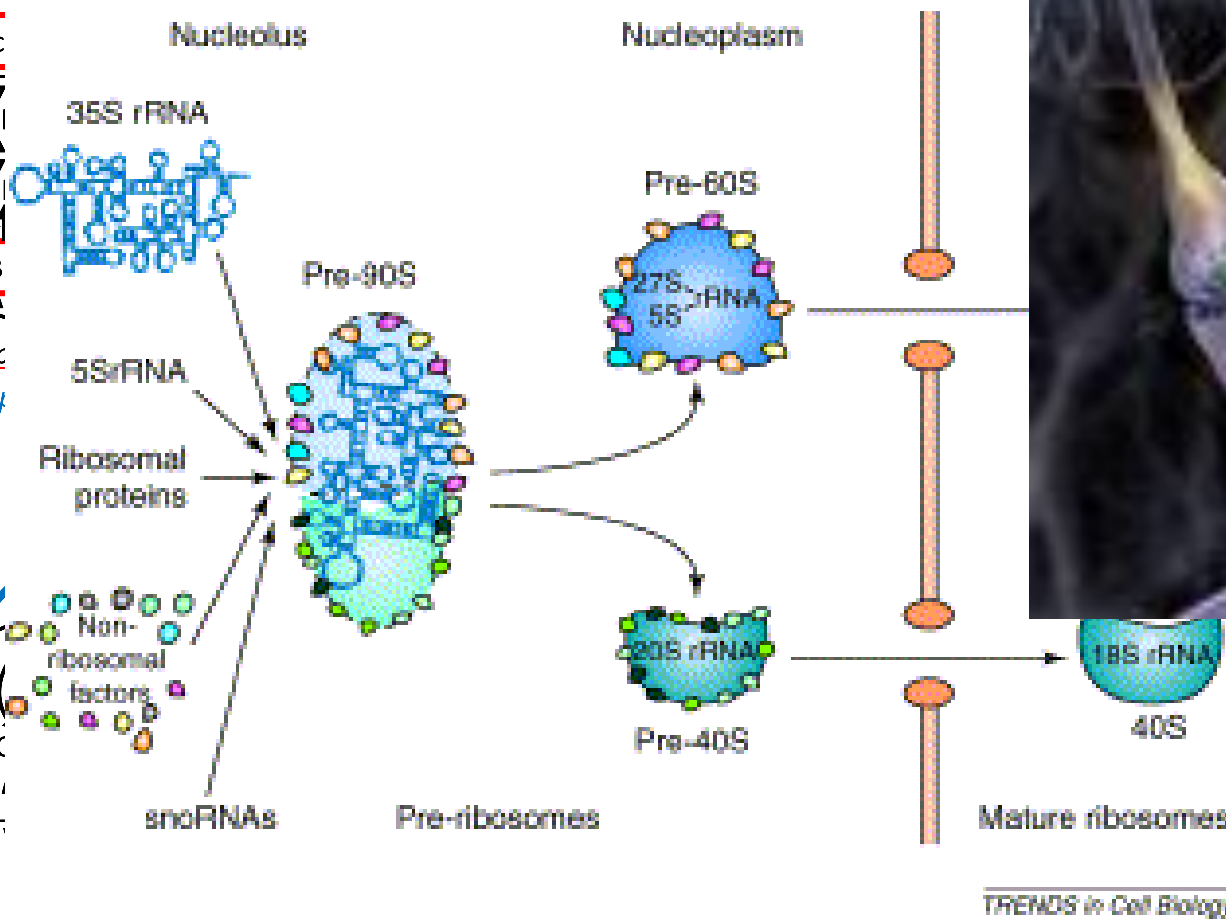
Protein interactions in ribosome biogenesis in yeast

C. Clerté - CBS
J-P Ballesta, P. Lillo
CSIC, Madrid

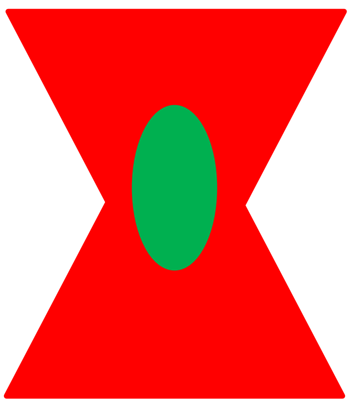
GKAP – DLC2 interactions in the PSD scaffold complex of brain synapses

J. Perroy, E. Moutin, L. Fagni
IGF, Montpellier

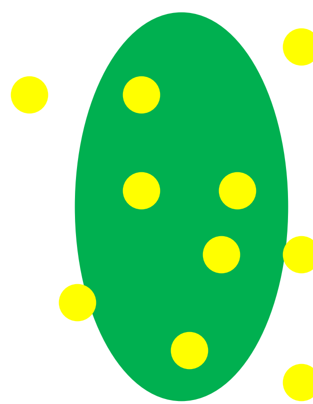




Particle Number, Interactions and Stoichiometry using Fluorescence Fluctuation Spectroscopy



Femto-second pulsed IR laser creates a very small (0.3 fL) 2-photon excitation volume in the sample

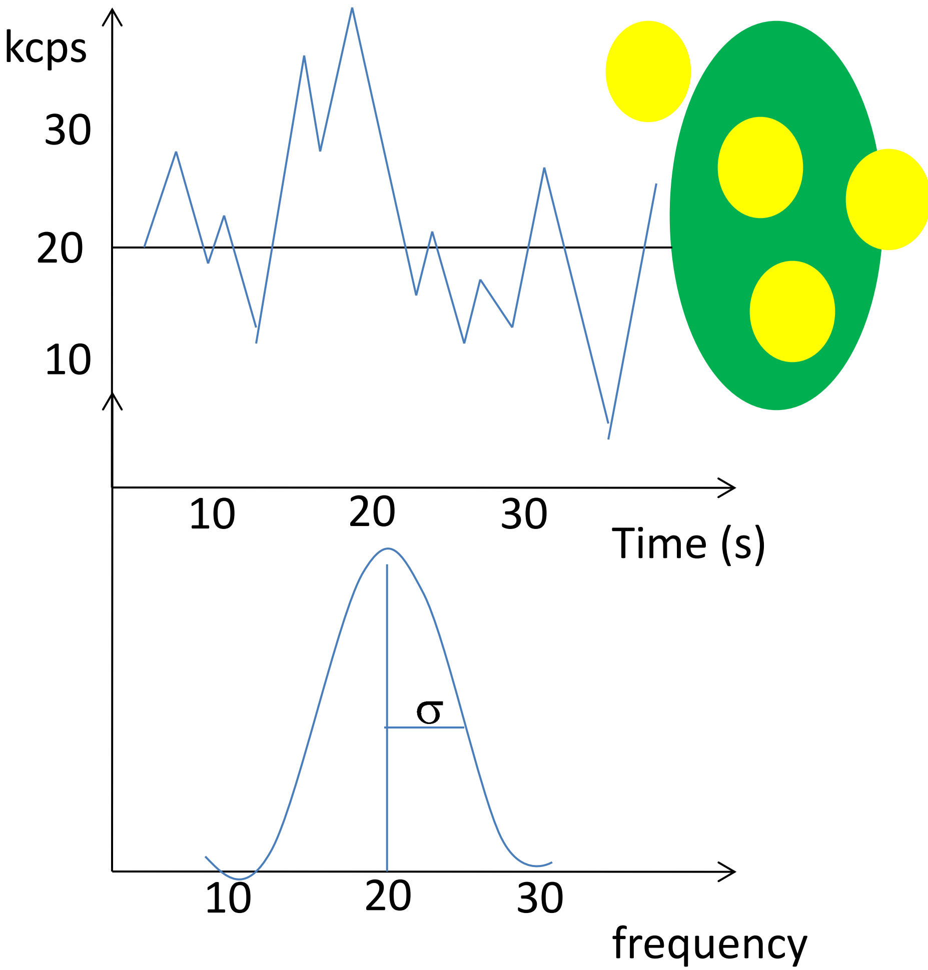


Fluorescent molecules diffusing into and out of the excitation volume cause fluctuations in the intensity that obey Poisson statistics

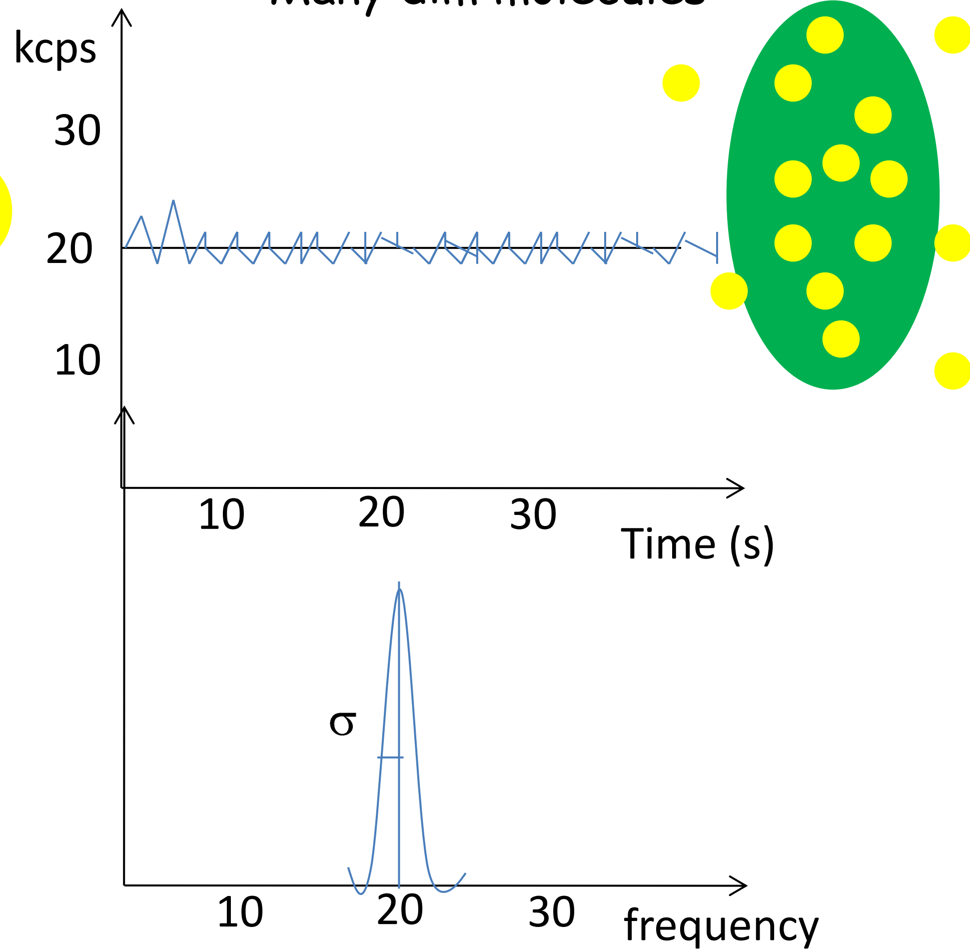
Magde, Elson, Webb, 1972

Qian and Elson, 1990

A few bright molecules



Many dim molecules



$$\langle F \rangle = \frac{\sum_{i=1}^{K} f_i}{K}$$

Average intensity for
K measurements
First moment

$$\sigma^2 = \sum_{i=1}^{K} \frac{(f_i - \langle F \rangle)^2}{K}$$

Variance
Second moment

$$N = \frac{\langle F \rangle^2}{\sigma^2}$$

Number

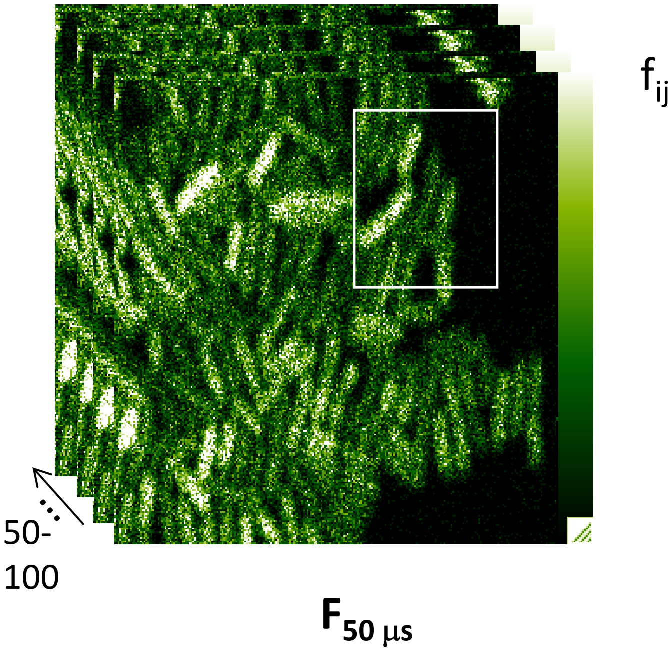
$$B = \frac{\sigma^2}{\langle F \rangle}$$

Brightness (cpspm)

Scanning Number and Brightness – sN&B

i = scan number, j = pixel number

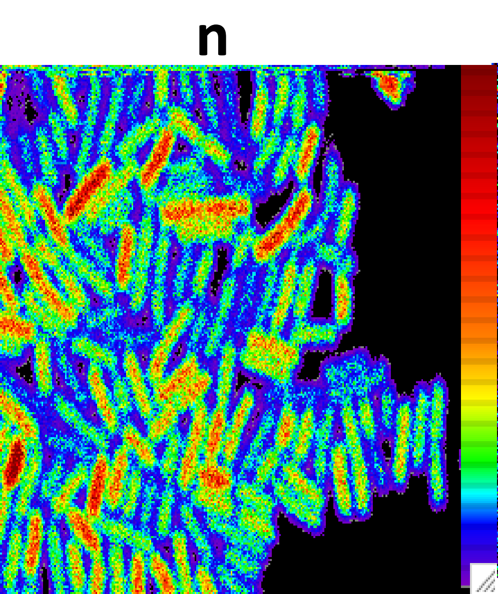
Digman and Gratton, BJ, 2008

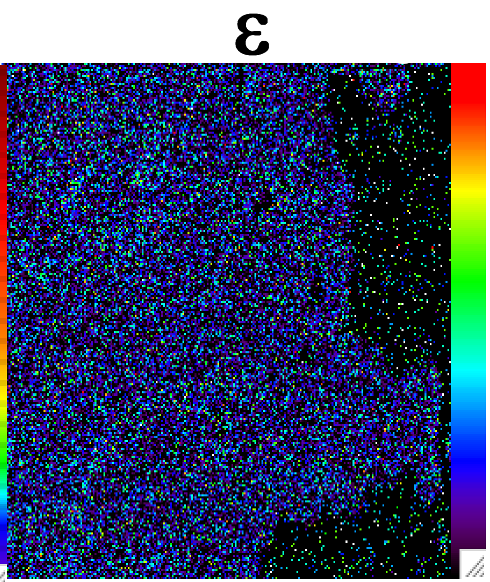


True number (n) and
True Brightness (ε)
must be shot noise corrected

$$B - 1 = \varepsilon$$

$$n = \langle F\rangle^2/(\sigma^2 - \langle F\rangle);$$
$$\varepsilon = (\sigma^2 - \langle F\rangle)/\langle F\rangle$$

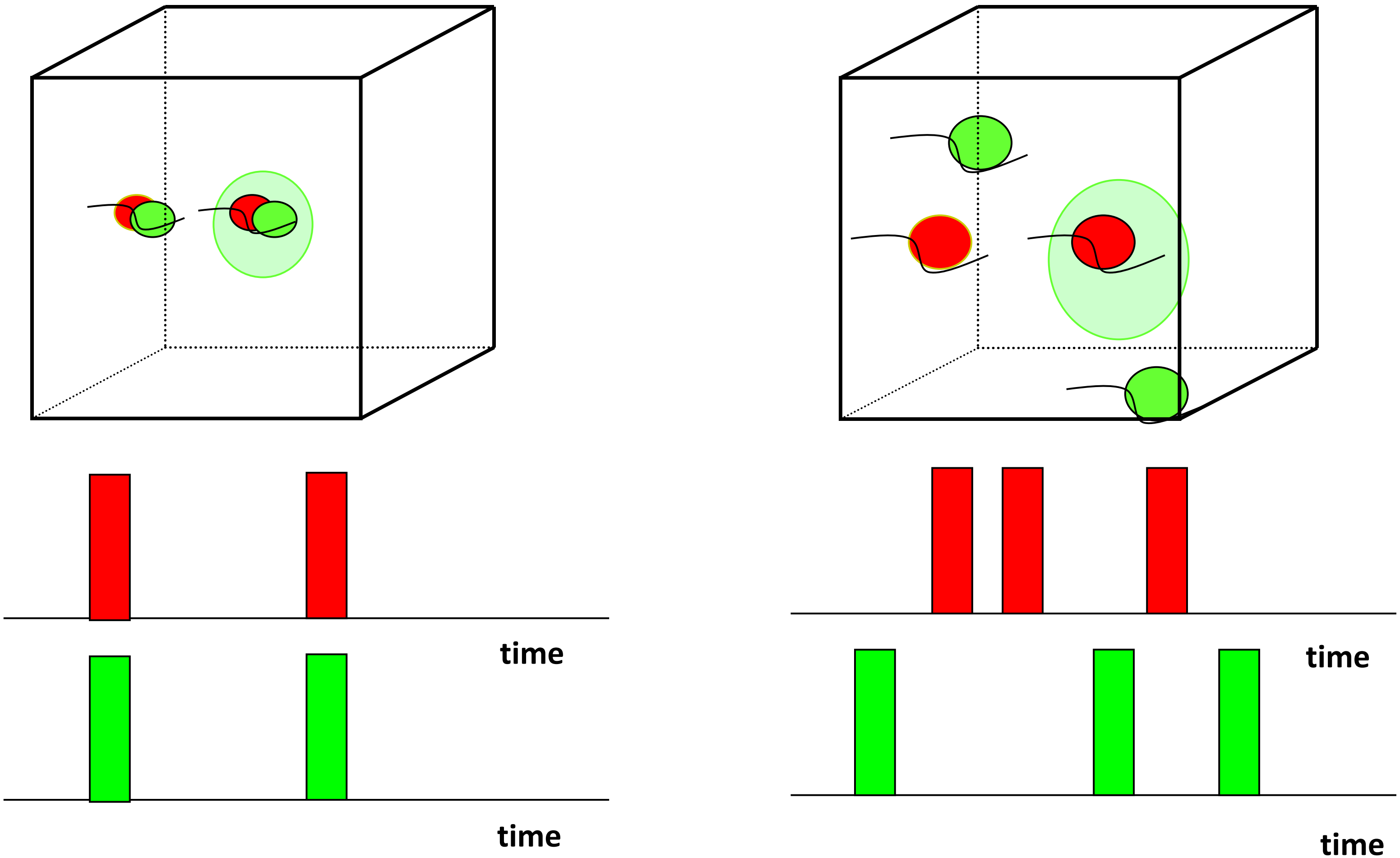




Advantages of sN&B

- spatial information on number and brightness (stoichiometry)
- greatly diminished photobleaching
- image-wide dynamic information available via RICS

Scanning Cross Brightness - (Bcc)



$$Bcc = \frac{\sigma_G \times \sigma_R}{\langle F \rangle_G \times \langle F \rangle_R}$$

Digman et al, Gratton, PNAS 2009

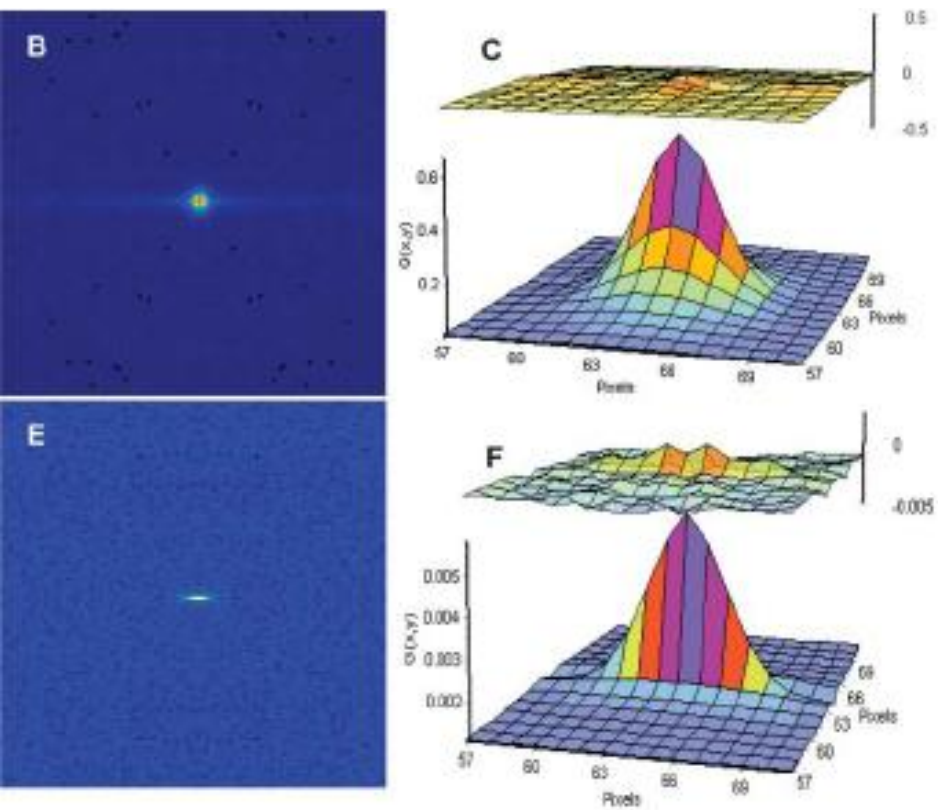
Raster Scanning Image Correlation Spectroscopy (RICS)

Digman et al, Gratton BJ 2005

Exploiting the hidden time information in the pixel to pixel and line to line scan

$$S(x,y) = \exp\left(-\frac{\frac{1}{2}\left[\left(\frac{2x\delta r}{w_0}\right)^2 + \left(\frac{2y\delta r}{w_0}\right)^2\right]}{\left(1 + \frac{4D(x+ny)\tau}{w_0^2}\right)}\right) \quad [2]$$

$$G(x,y) = \frac{\gamma}{N}\left(1 + \frac{4D(x+ny)\tau}{w_0^2}\right)^{-1}\left(1 + \frac{4D(x+ny)\tau}{w_z^2}\right)^{-1/2}. \quad [3]$$



S = Spatial correlation function
G = time correlation function

An immobile bead gives a sphere with the diameter of the PSF

A moving florescent object will spread in x (pixel) and narrow in y (line)

Protein interactions implicated in the control of the Central Carbon Metabolism in *Bacillus subtilis in vivo*

Studied using GFP-promoter fusions

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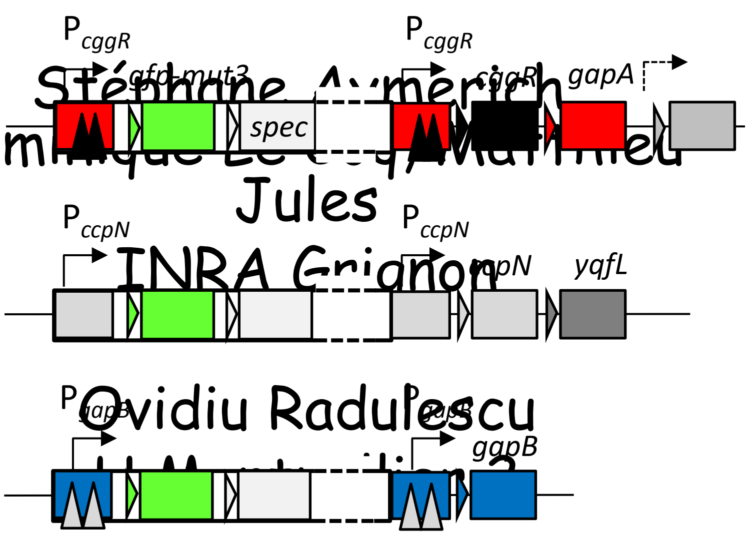
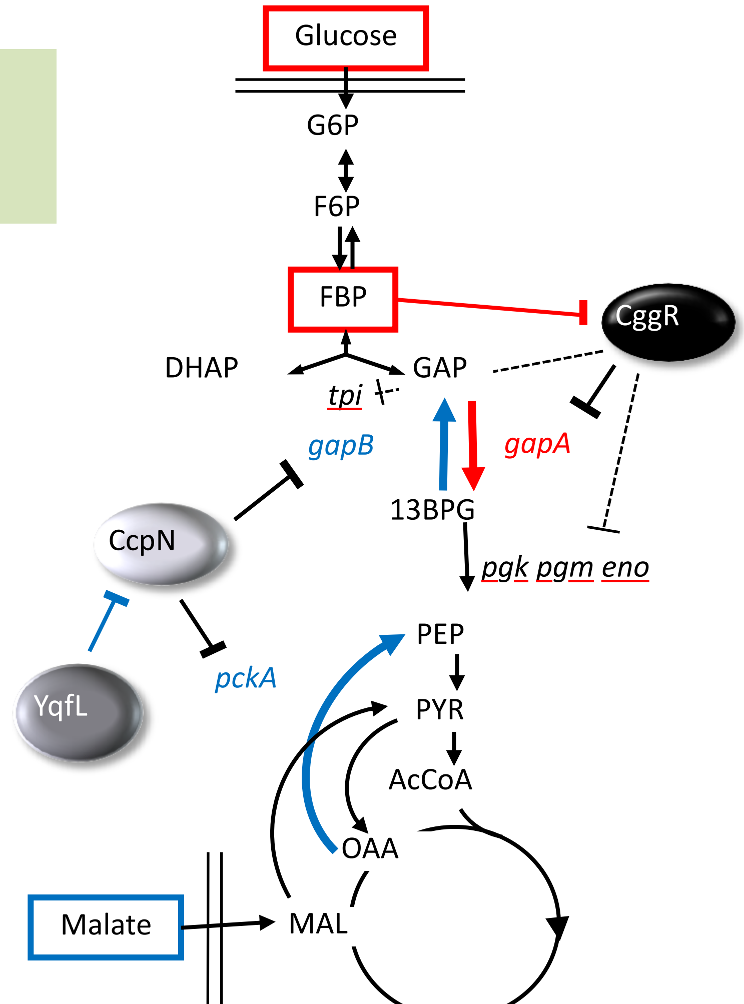
Dominique Le Coq/Matthieu Jules
INRA Grignon
Ovidiu Radulescu



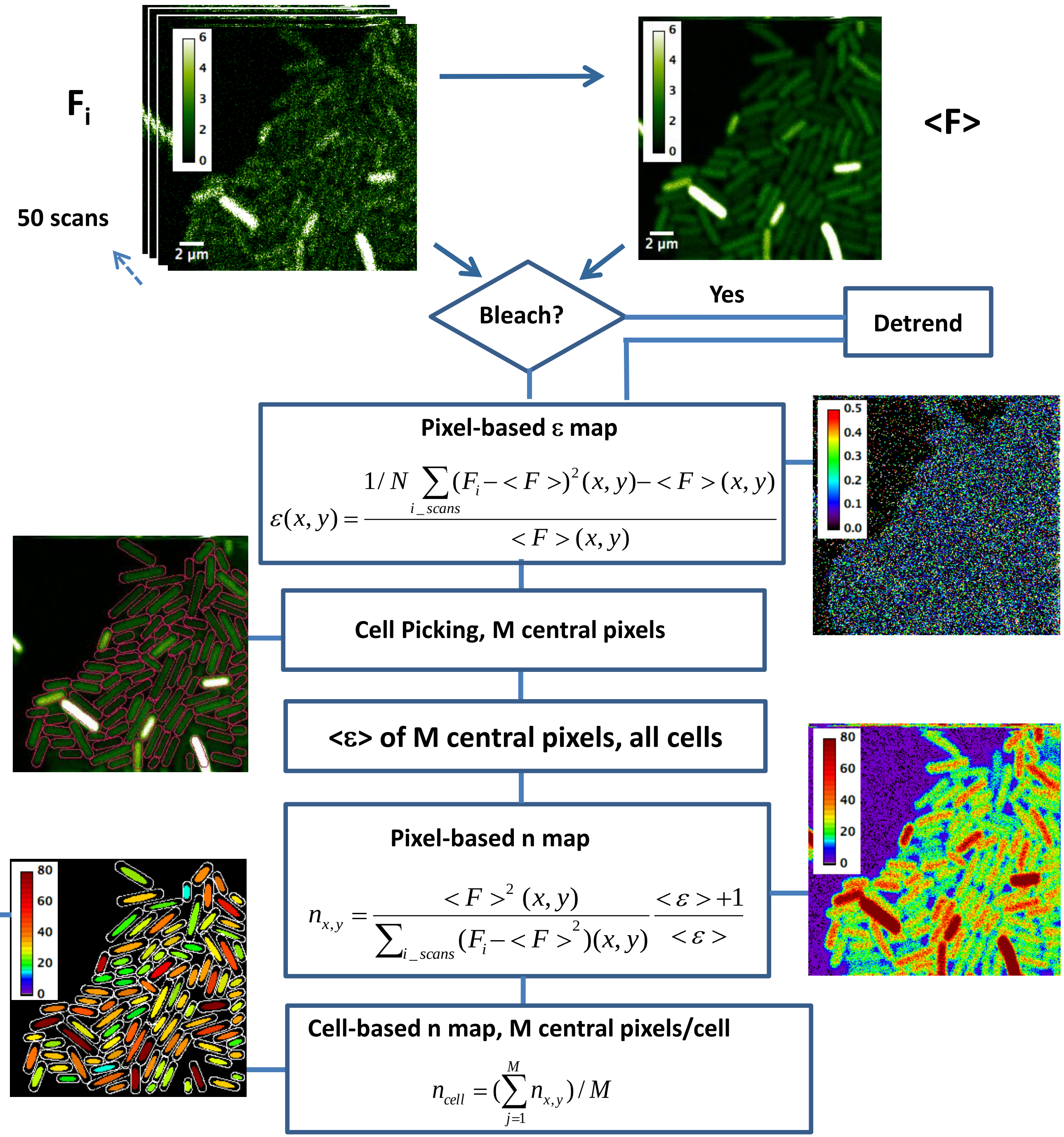


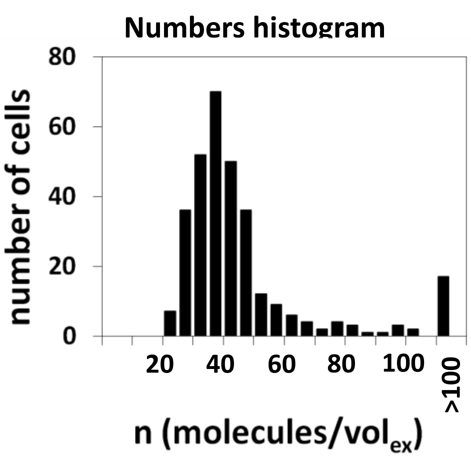
Determining absolute protein concentrations in bacteria by N&B

The PSF and the small size of the bacteria

The shot noise

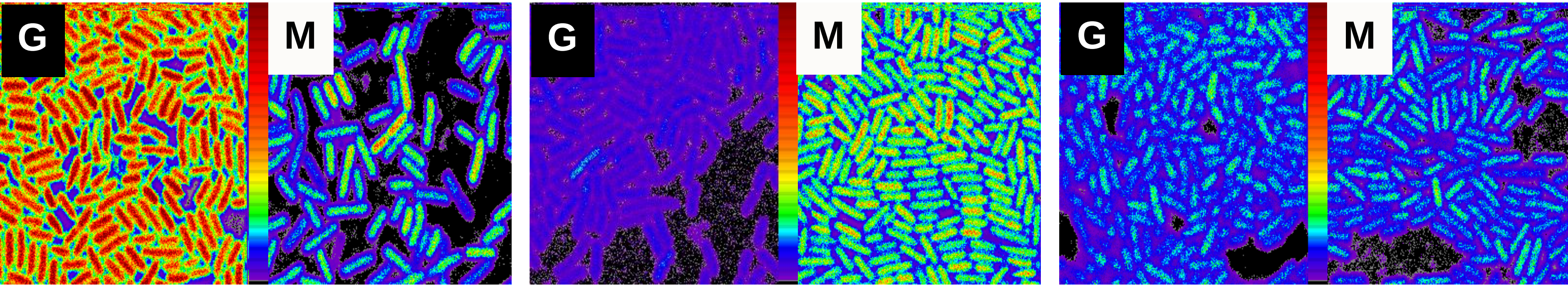
The low count rate



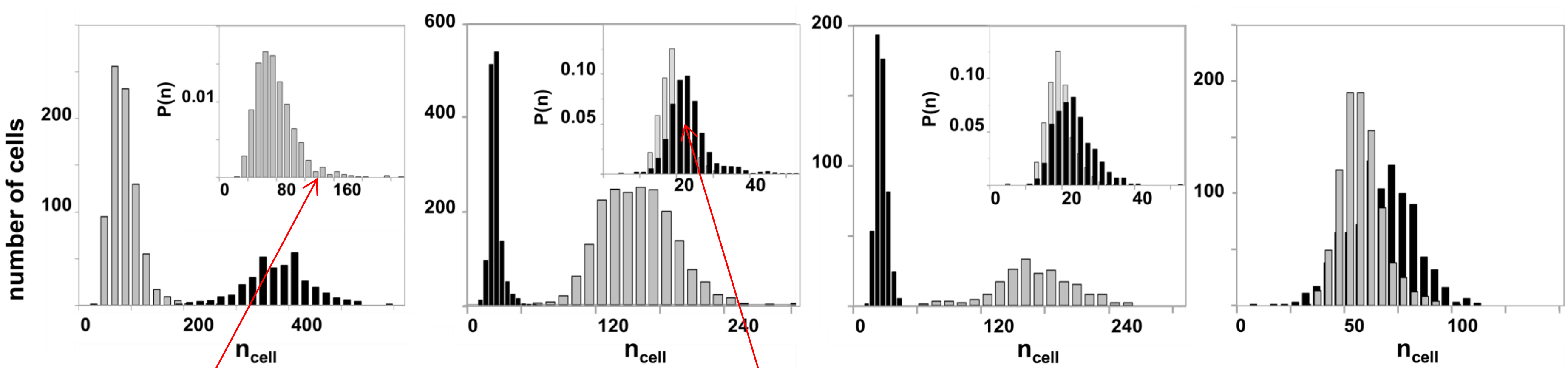


GFP expression on glycolytic (glucose) and gluconeogenic (malate) carbon sources

Absolute Numbers Maps



PcggR ***PgapB*** ***PpckA*** ***PccpN***

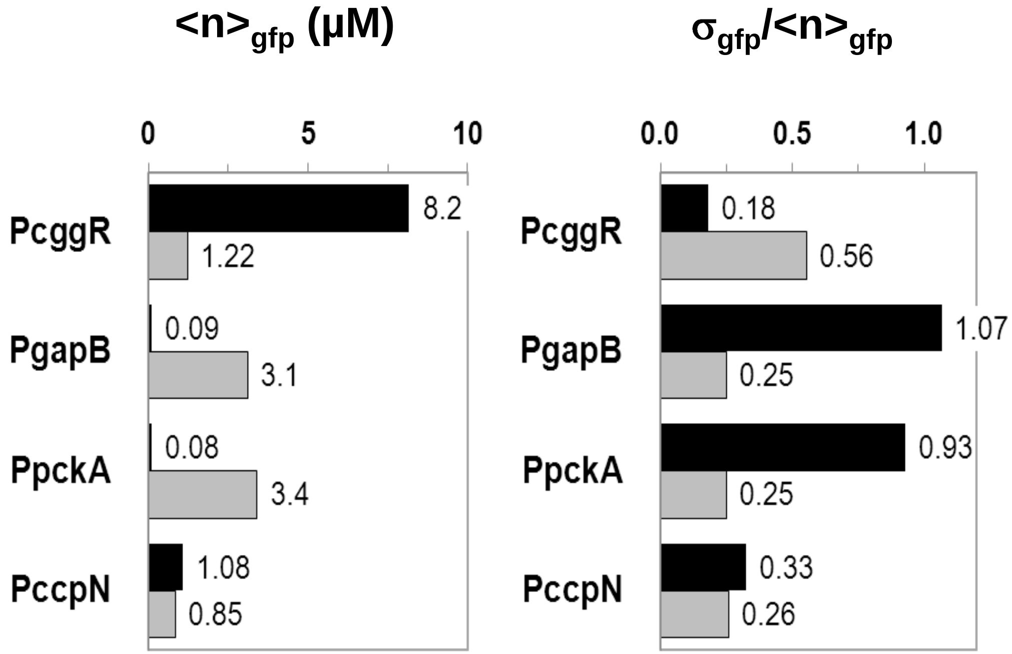


Skewed and highly Heterogeneous distribution for *PccgR* under malate (repressed)

Expression just above background for *PgapB* under glucose ($\langle n \rangle_{FP}$ = 3) Strong catabolite repression

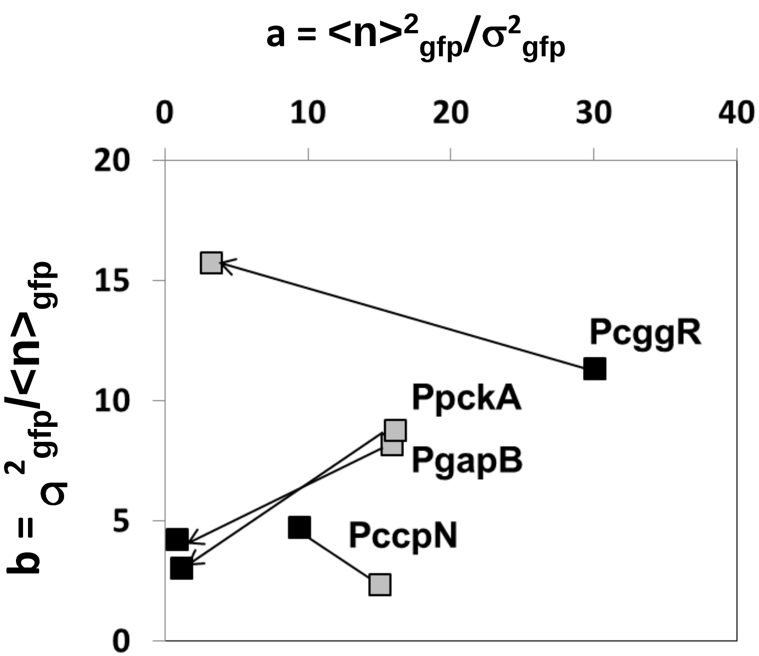
No signficant change for *PccpN*

Biological Noise Patterns



Glucose

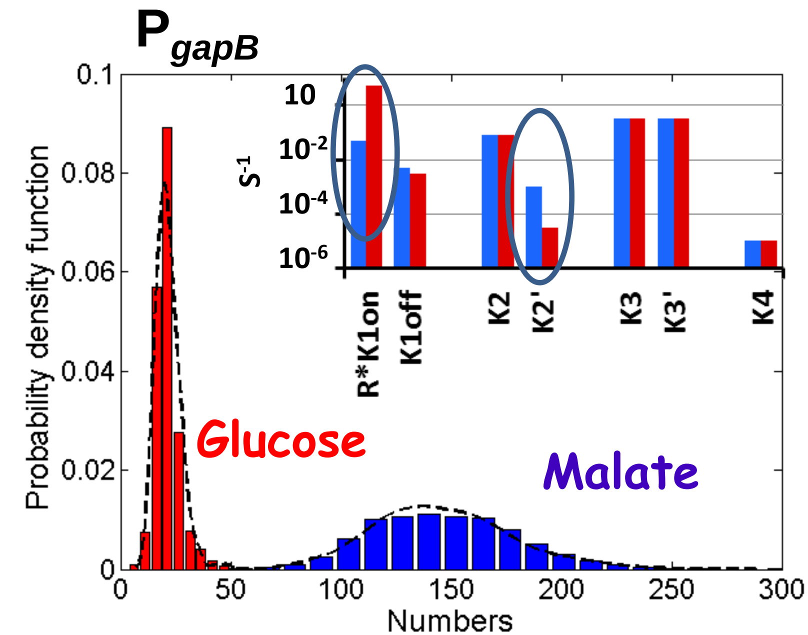
Malate



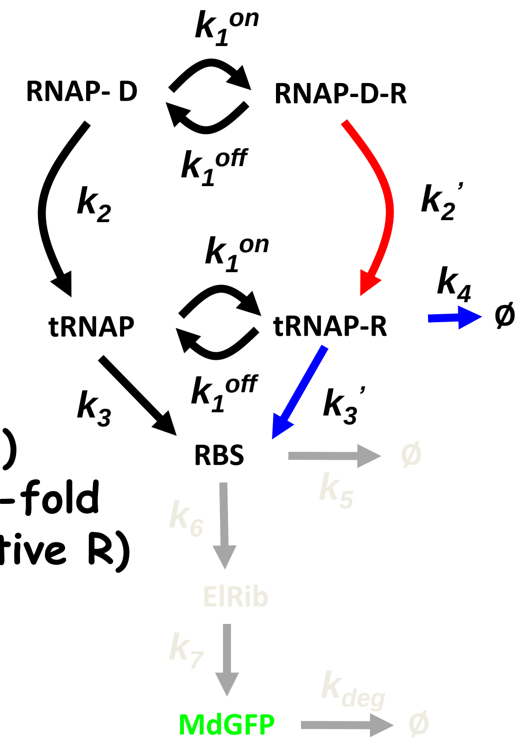
We are observing transcriptional bursting

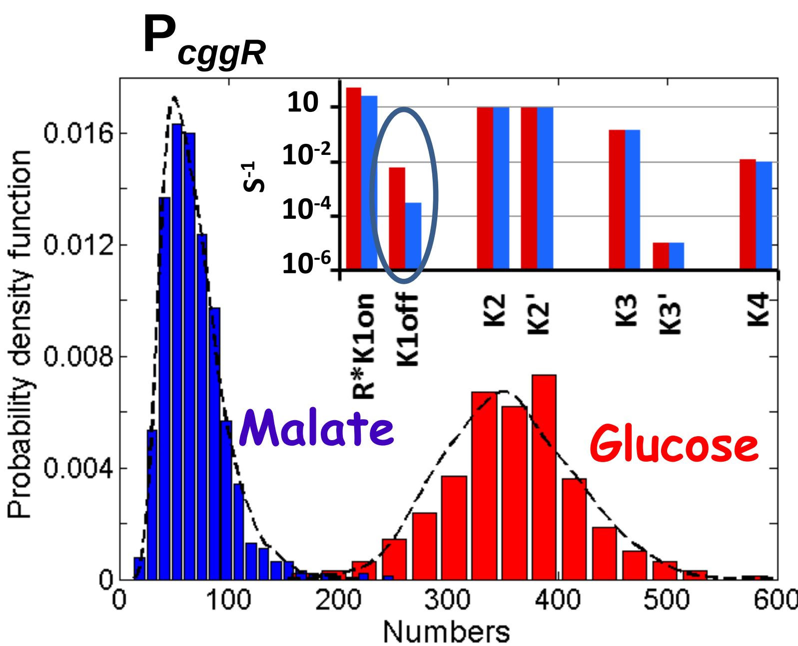
Distinct noise patterns observed for the glycolytic and gluconegenic promoters

P_{cggR}, the auto-repressed promoter exhibits strong transcriptional bursting under repression while P_{gapB} has very low burst size

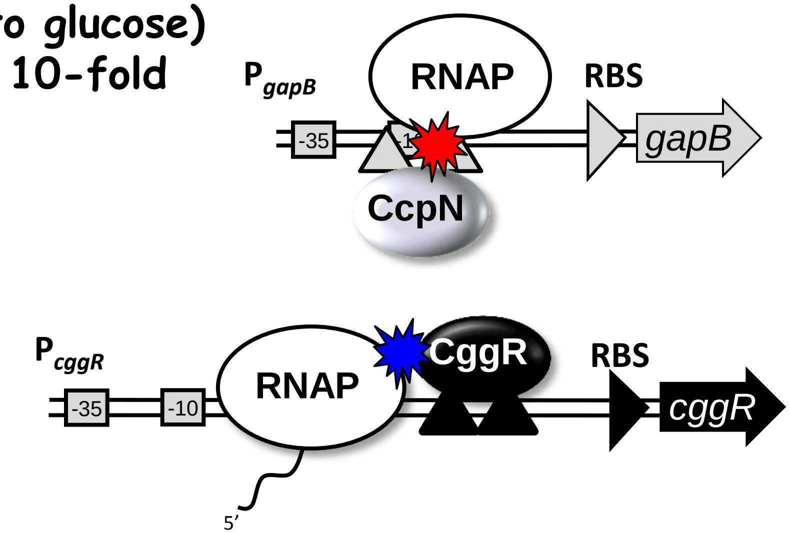


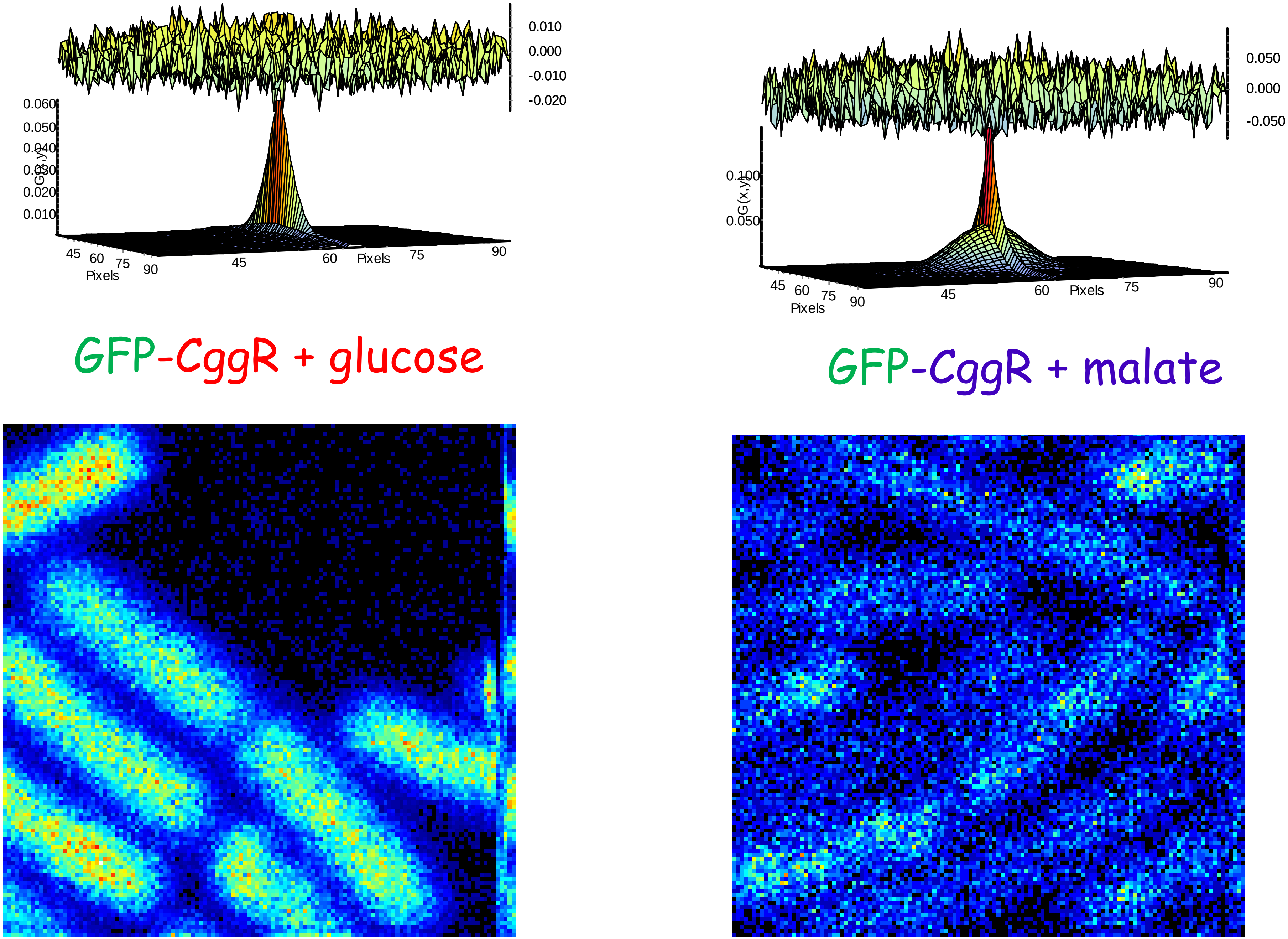
Upon induction (switch to malate) Rk_1on down 1000-fold (difference in active R) k_2' up 100-fold



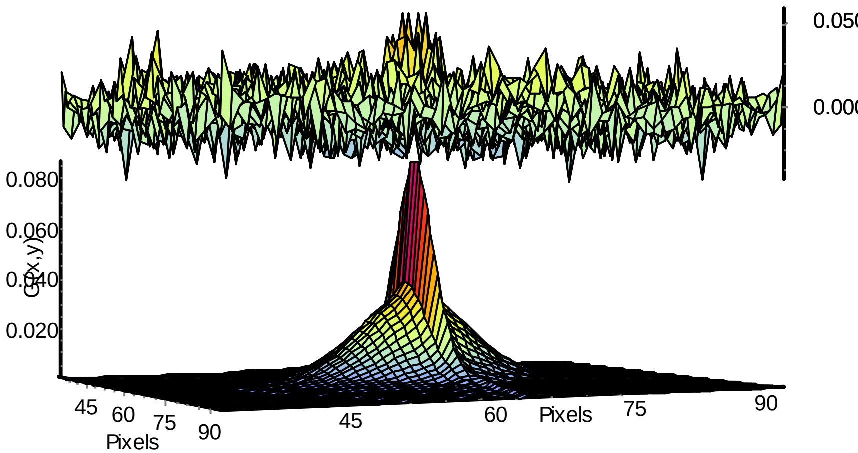


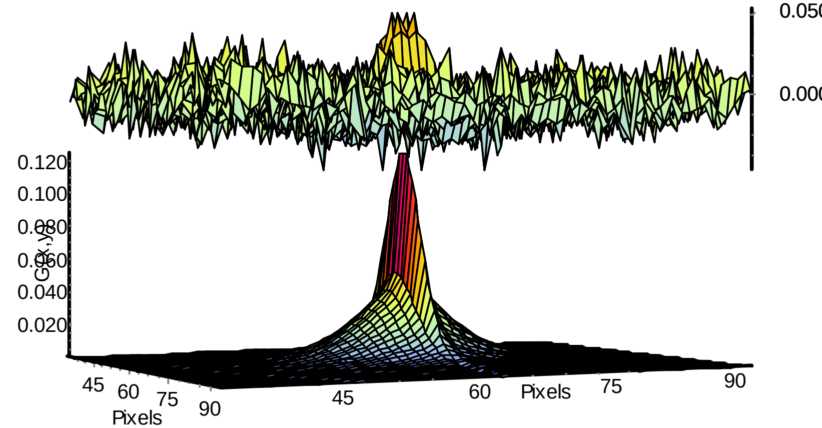
Upon induction (switch to glucose) k_1off up 10-fold





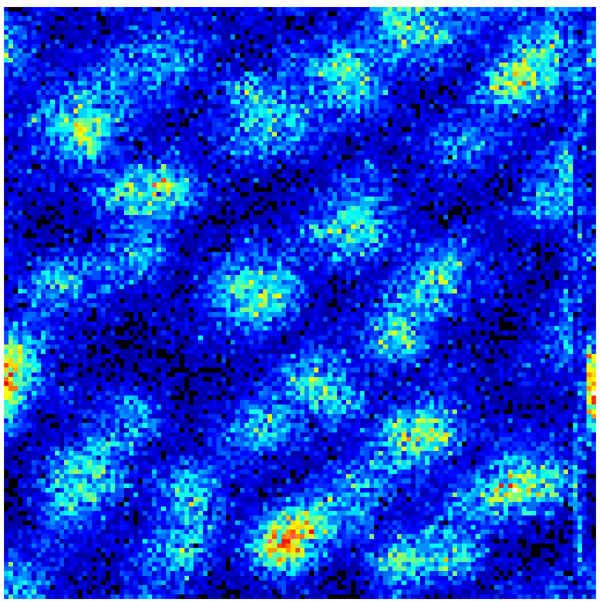
0.010
0.000
-0.010
-0.020
0.060
0.050
0.040
0.030
0.020
0.010
G(x,y)
45
60
75
90
Pixels
45
60
Pixels
75
90
0.050
0.000
-0.050
0.100
0.050
G(x,y)
45
60
75
90
Pixels
45
60
Pixels
75
90
GFP-CggR + glucose
GFP-CggR + malate

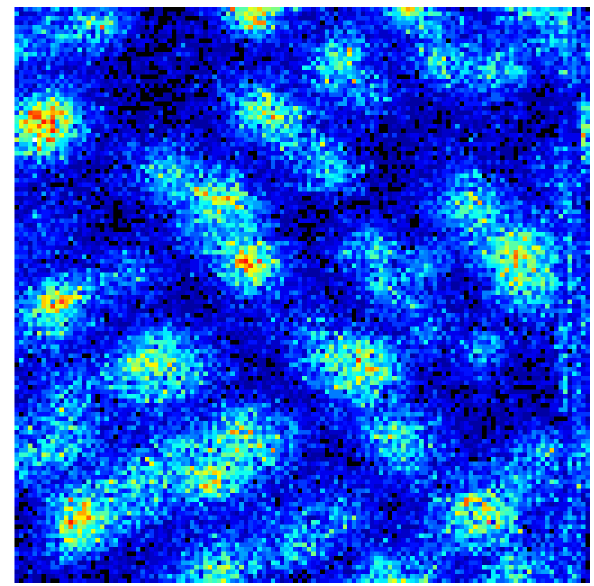


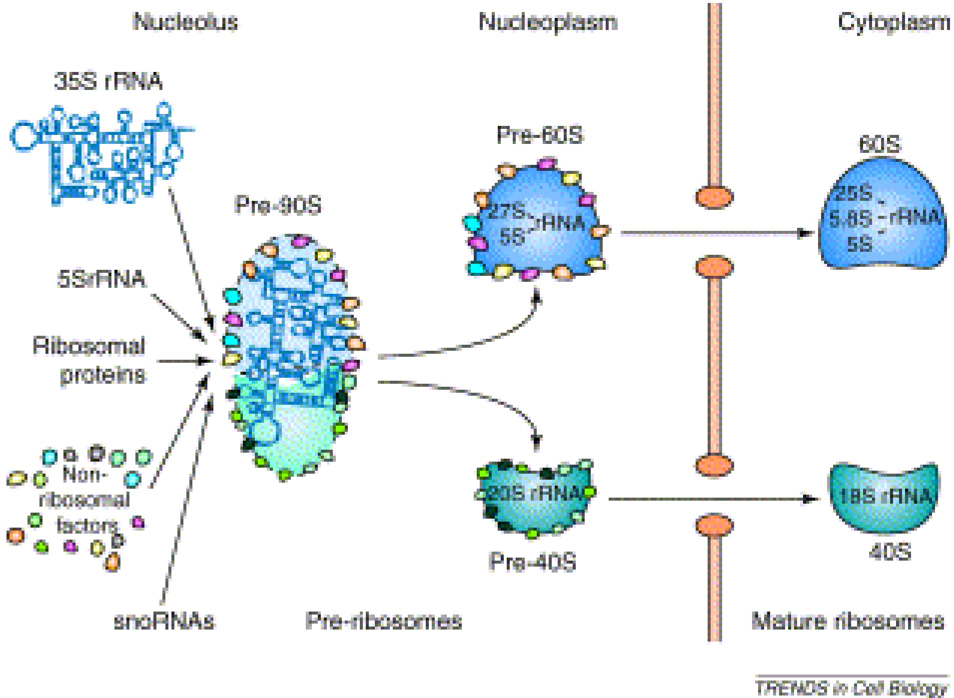


GFP-CcpN - glucose

GFP-CcpN - malate



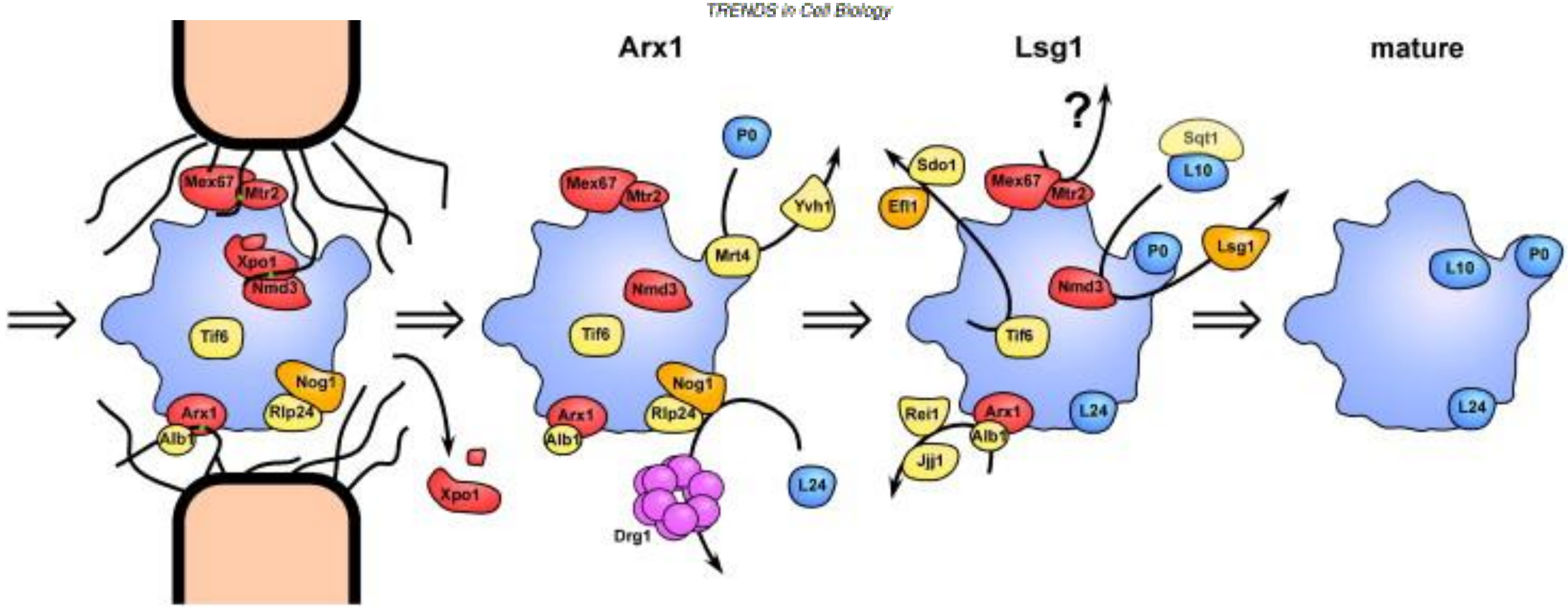




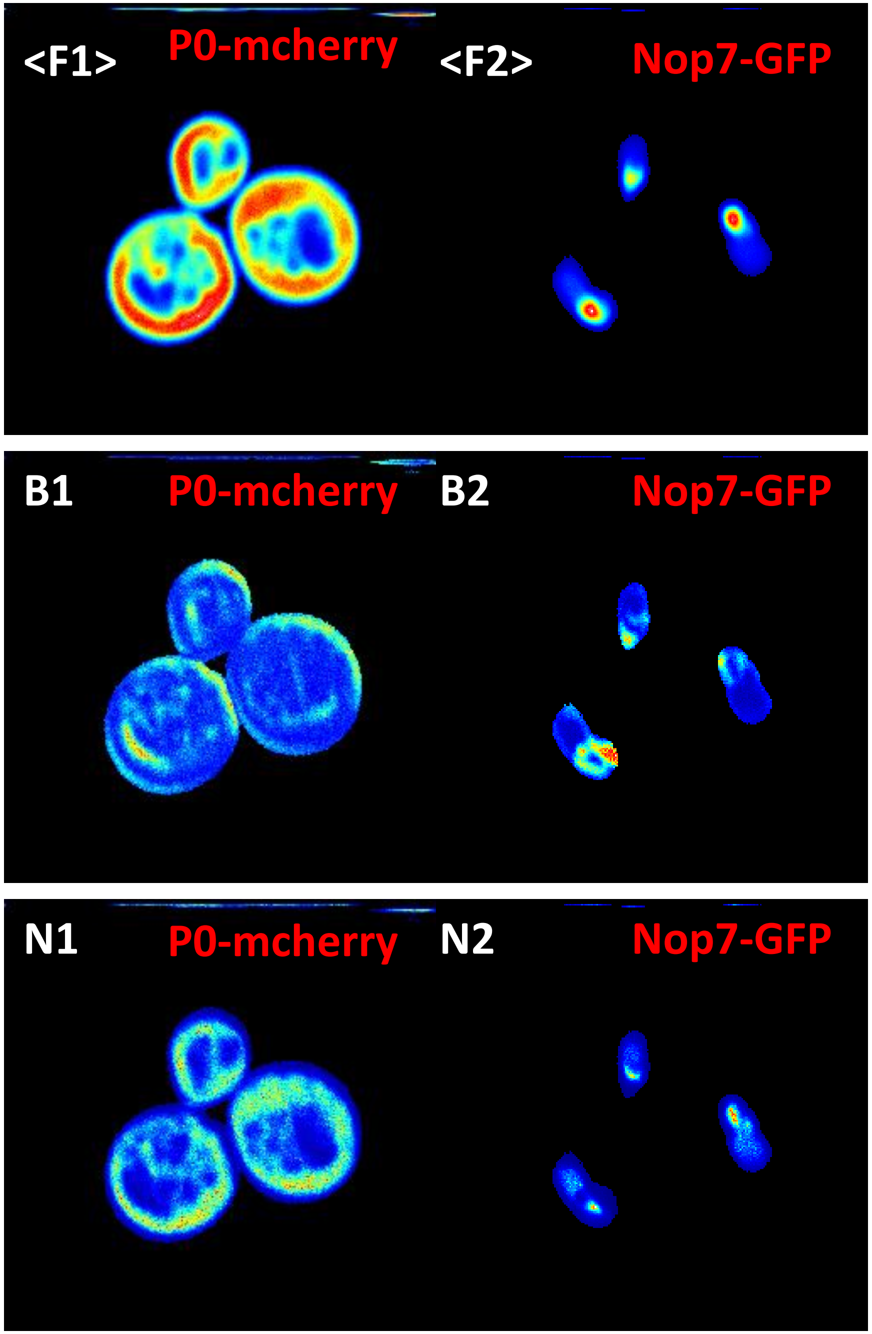
Ribosome Biogenesis In Yeast

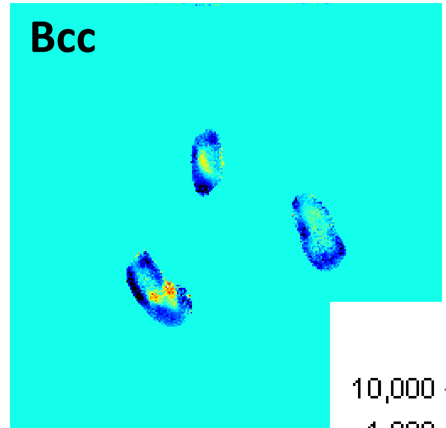
P0, Nop7, Mrt4
Where and when do the assembly factors interact?

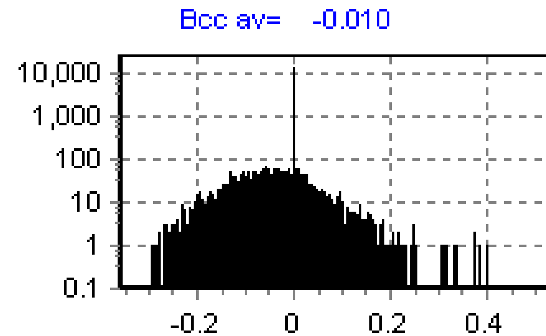
Cross-brightness on GFP and mcherry protein fusions



Juan Pedro Ballesta, Pilar Lillo, Caroline Clerté



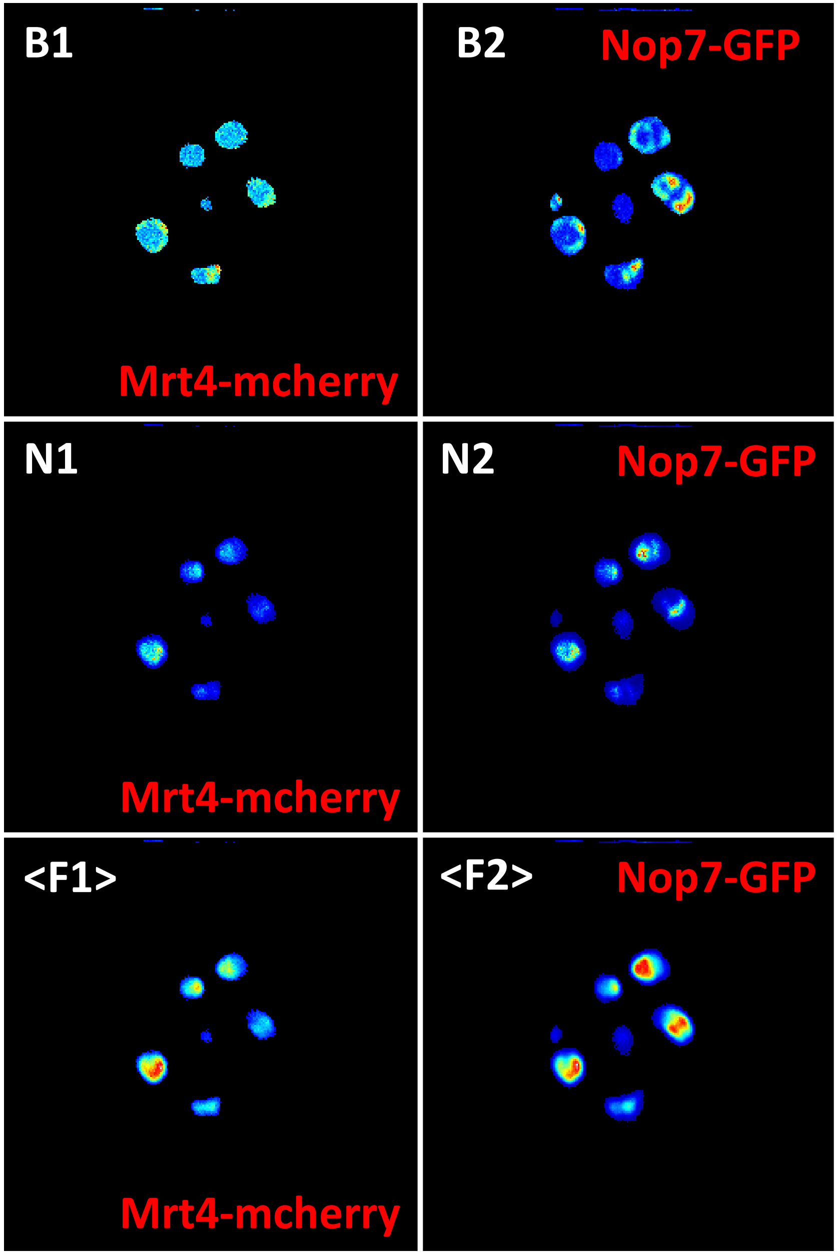


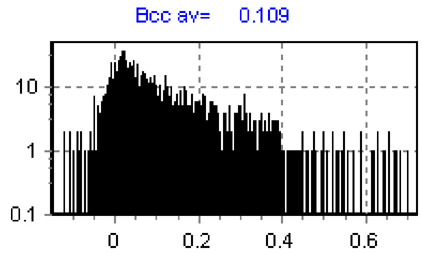


P0 interacts with Nop7 in the nucleus

Spatially and structurally distinct complexes between Nop7 and P0

Negative Bcc → FRET
Different molecular brightness
→Different stoichiometry
→ Structurally distinct complexes





Heterogeneous spatial distribution of complexes between
Mrt4 and Nop7 in the nucleus

Only positive Bcc → no FRET
Complexes at the nuclear membrane

GKAP functionally and physicial links NMDA receptors to metabotropic glutamate receptors in the PSD of brain synapses

Coll: Julie Perroy
Enora Moutain
Laurent Fagni
IGF

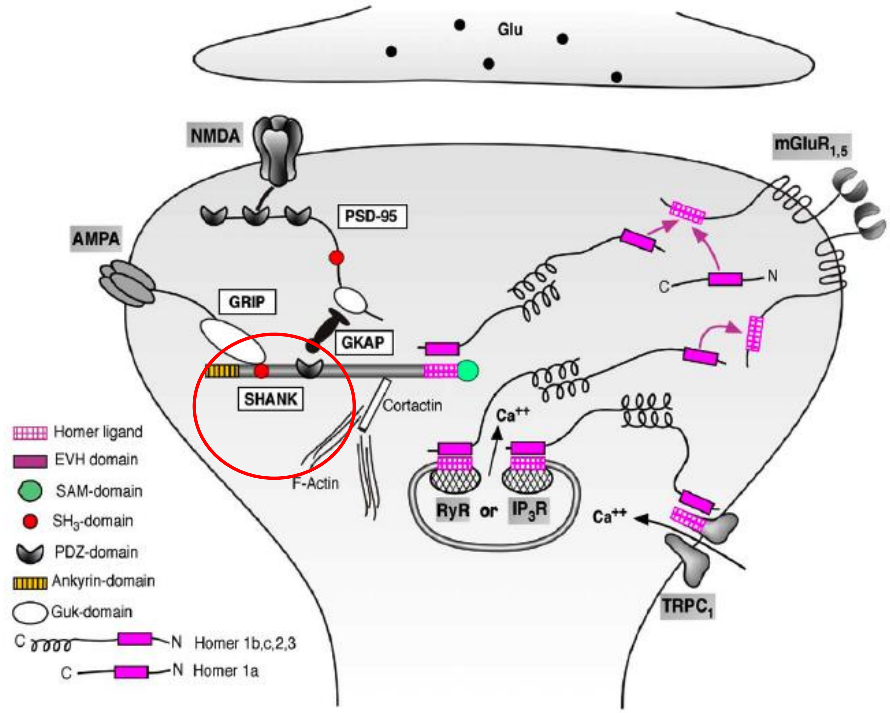


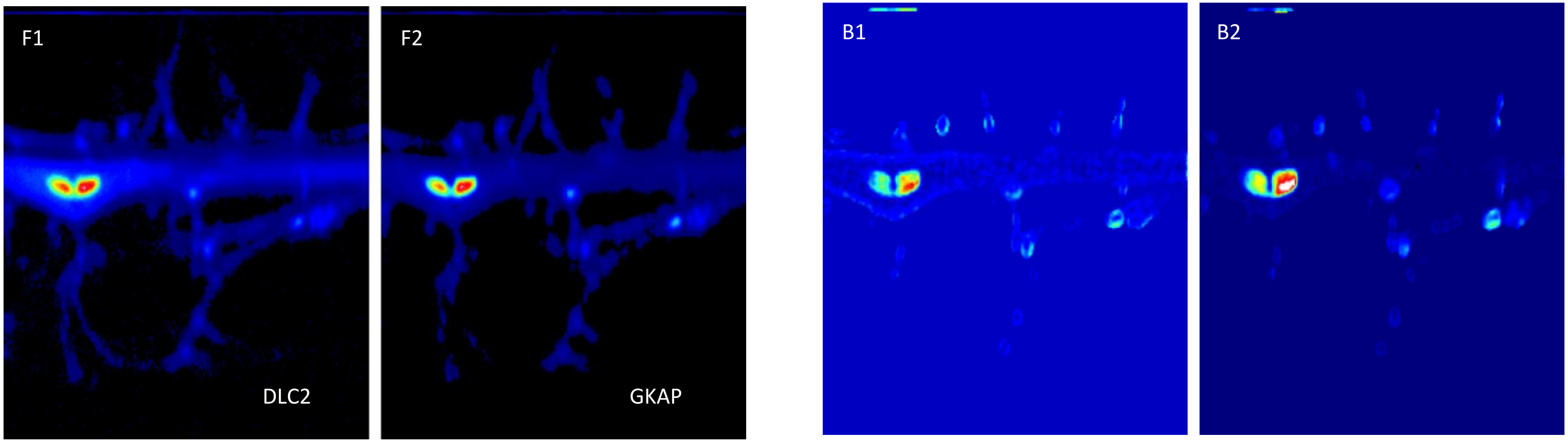
Fig. 2. The postsynaptic Homer-Shank-mGluR1a,5 "receptosome." For explanations, see Section 2.2.2 and Fagni et al. (2004).

Remodeling of the synaptic complexes implicates traficking and an interaction between GKAP and a motor protein adaptor DLC2.

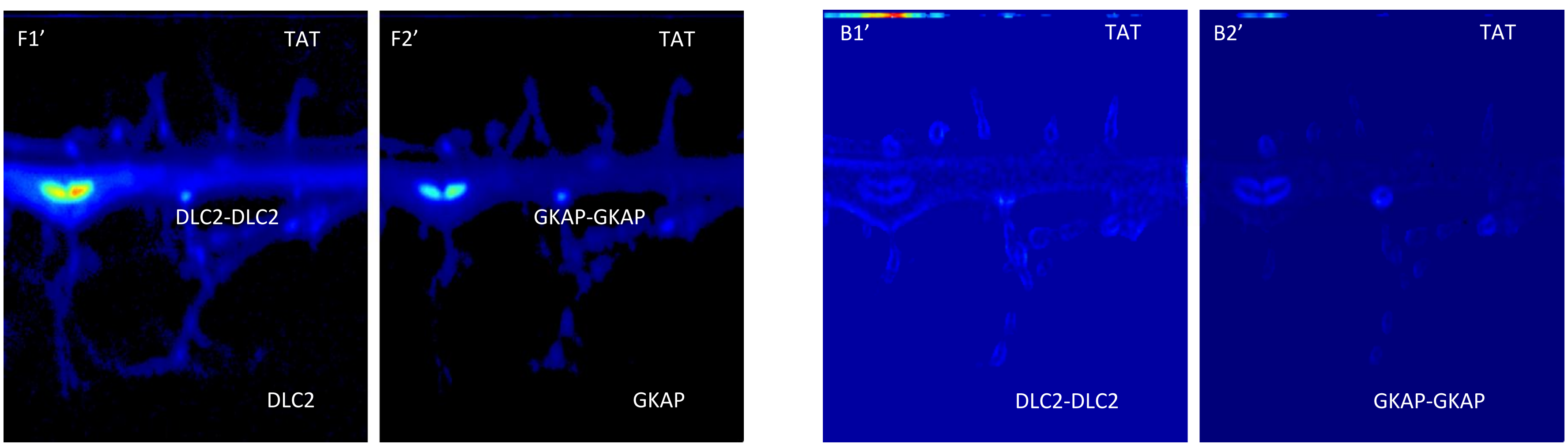
GKAP-DLC2 Interactions by Cross-N&B

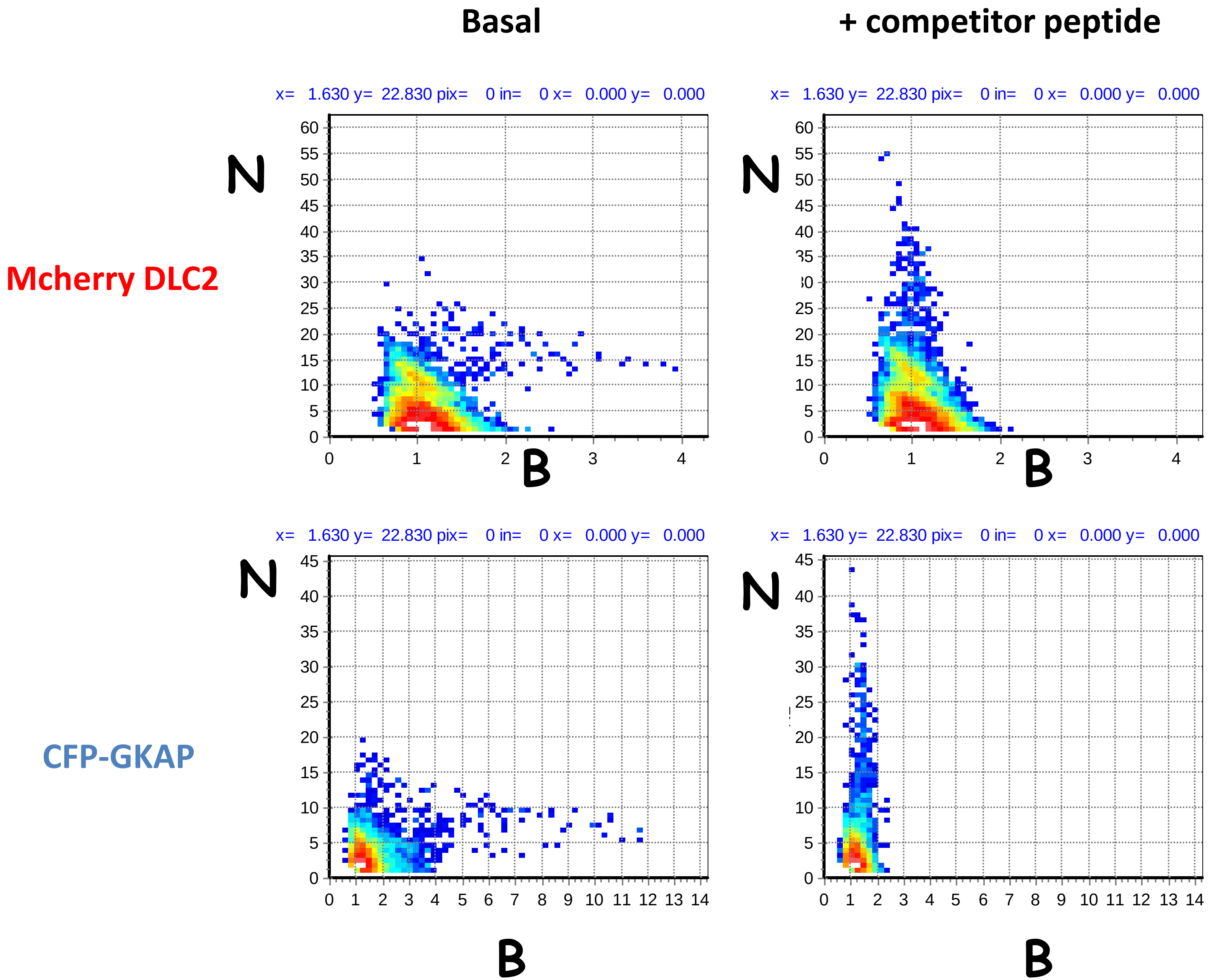
expressing mcherry-DLC2 and Cerulean GKAP in neurons

mcherry-DLC2 – CH1 and CFP-GKAP – CH2 in absence of competitor peptide

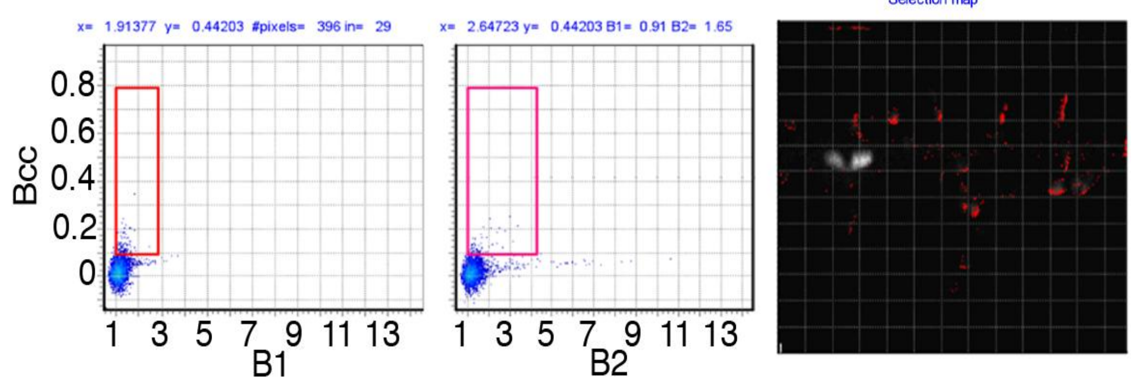


mcherry-DLC2 – CH1 and CFP-GKAP in presence of competitor peptide

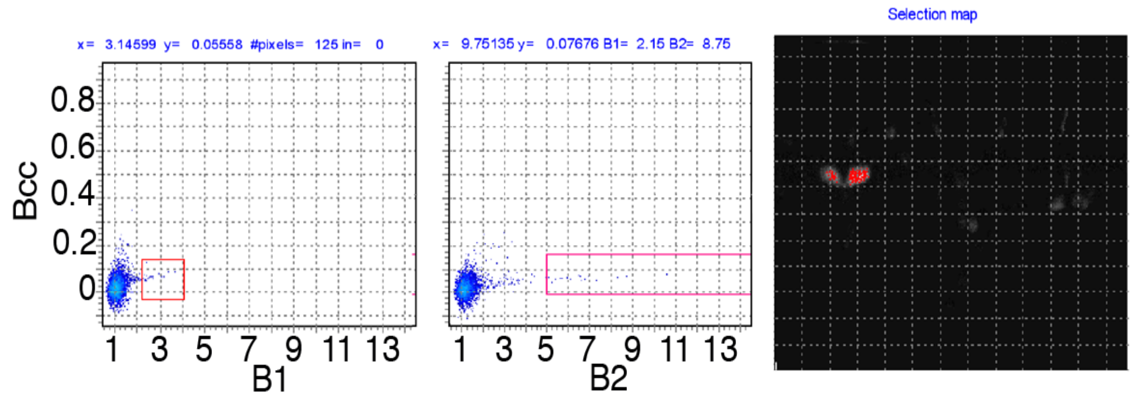




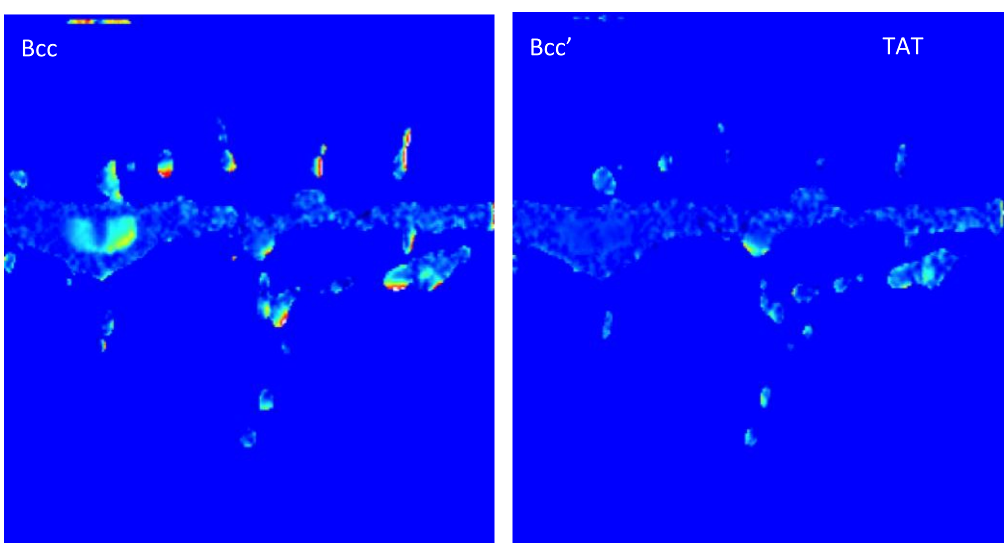
Basal
+ competitor peptide
Mcherry DLC2
CFP-GKAP
x= 1.630 y= 22.830 pix= 0 in= 0 x= 0.000 y= 0.000
N
B



High Bcc and low B1, B2 in spines



Low Bcc and low B1, B2 in CB



Highest Bcc in spines

Addition of competitor peptide
Nearly abolishes heterologous
DLC2-GKAP interactions

Quantitative measure of protein and protein complex concentrations and spatial organization is important *in vitro* and possible in live cells

The variety of applications of such approaches is quasi-unlimited

Current challenges include:

- devising physiologically relevant tests of the implications of protein complex formation (particularly in eukaryotic cells and organisms)

- scaling up to probe multi-dimensional physiological space or testing of multiple ligands

- increasing the photon budget

CCM

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Matt Ferguson
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Caroline Clerté

Stéphane Aymerich
Dominique LeCoq
Matthieu Jules
Ovidiu Radulescu

Synaptic Complexes

Julie Perroy
Enora Moutin
Laurent Fagni
Caroline Clerté

Yeast Ribosome

Pilar Lillo
Juan Pedro Ballesta
Caroline Clerté

Association pour la Recherche sur le Cancer

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Instituts thématiques

Inserm
Institut national de la santé et de la recherche médicale

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WHERE DISCOVERIES BEGIN

la Région Languedoc Roussillon