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Spatio-temporal organisation of cell wall elongation machineries in *Bacillus subtilis*

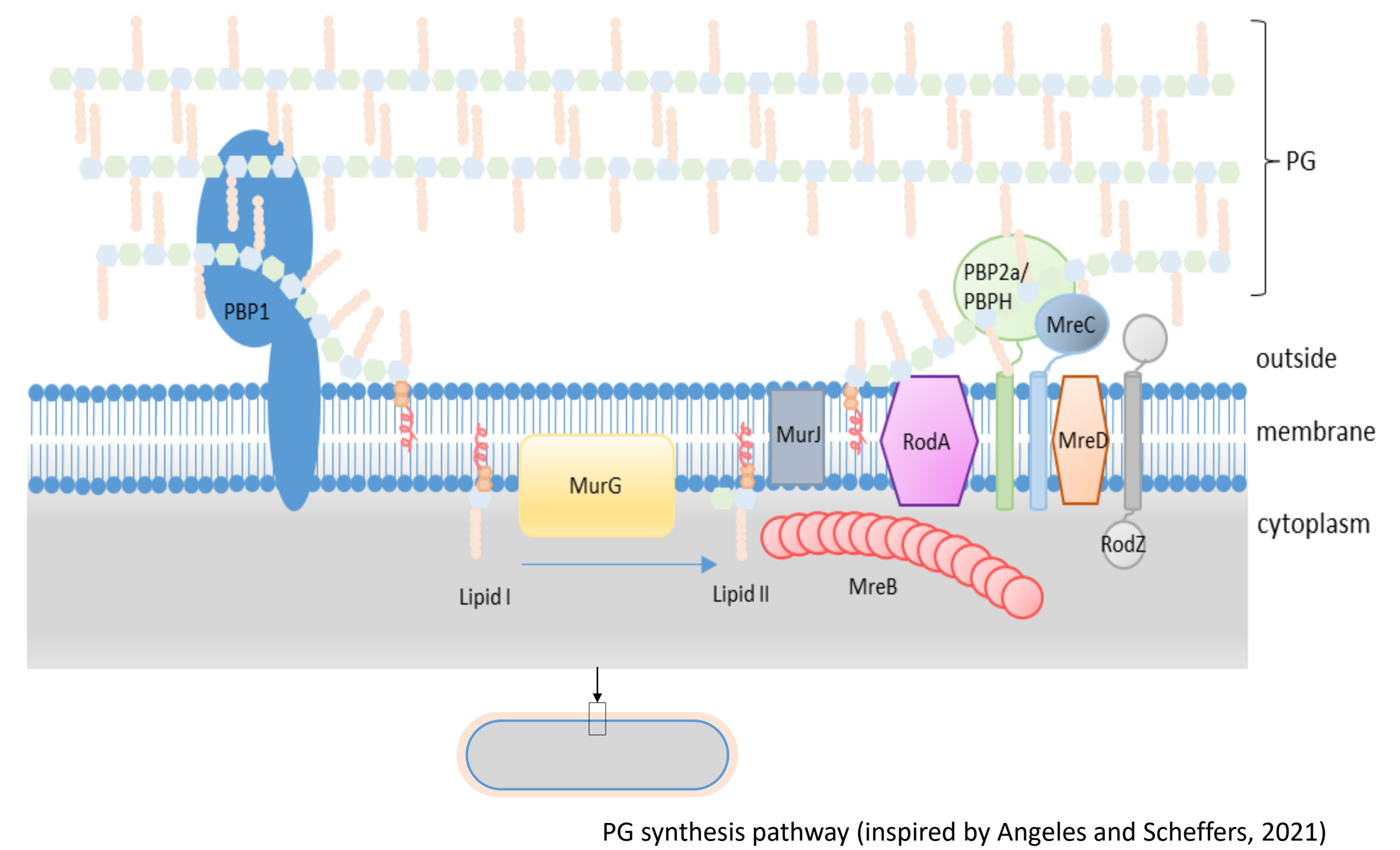
Claire-Jing Rouchet¹, Rut Carballido-Lopez¹, Charles Kervann², Cyrille Billaudeau¹

Context

The cell wall (CW) is the physical determinant of bacteria's shape. It is an external layer of the cell mostly composed of peptidoglycan (PG) creating a rigid mesh. The PG unit consists of two saccharides and five peptides. Units are assembly by transglycosylation (saccharide-saccharide bond) and transpeptidation (peptide-peptide bond). The CW's size is modulated according to the cell's cycle and must be lengthened during cell elongation. The Peptidoglycan Elongation Machinery (PGEM) synthesizes and incorporates new PG units into this structure during cell growth. It is composed of two complementary mechanisms: the Rod system and class A Penicillin Binding Proteins (PBPs). The first one is the major one for PG synthesis during cell elongation. It is composed of the central component **MreB protein**, the transglycosylase RodA, the transpeptidases PBP2a and PBP2H, two bPBPs, and MreCD (Domínguez-Escobar et al., 2011). The aPBPs are capable of transglycosylation and transpeptidation. The major one is **PBP1** (Kawai et al., 2019). Those two machineries are autonomous but should be coordinated (Cho et al., 2016). This study focuses on their spatio-temporal organization during cell elongation.

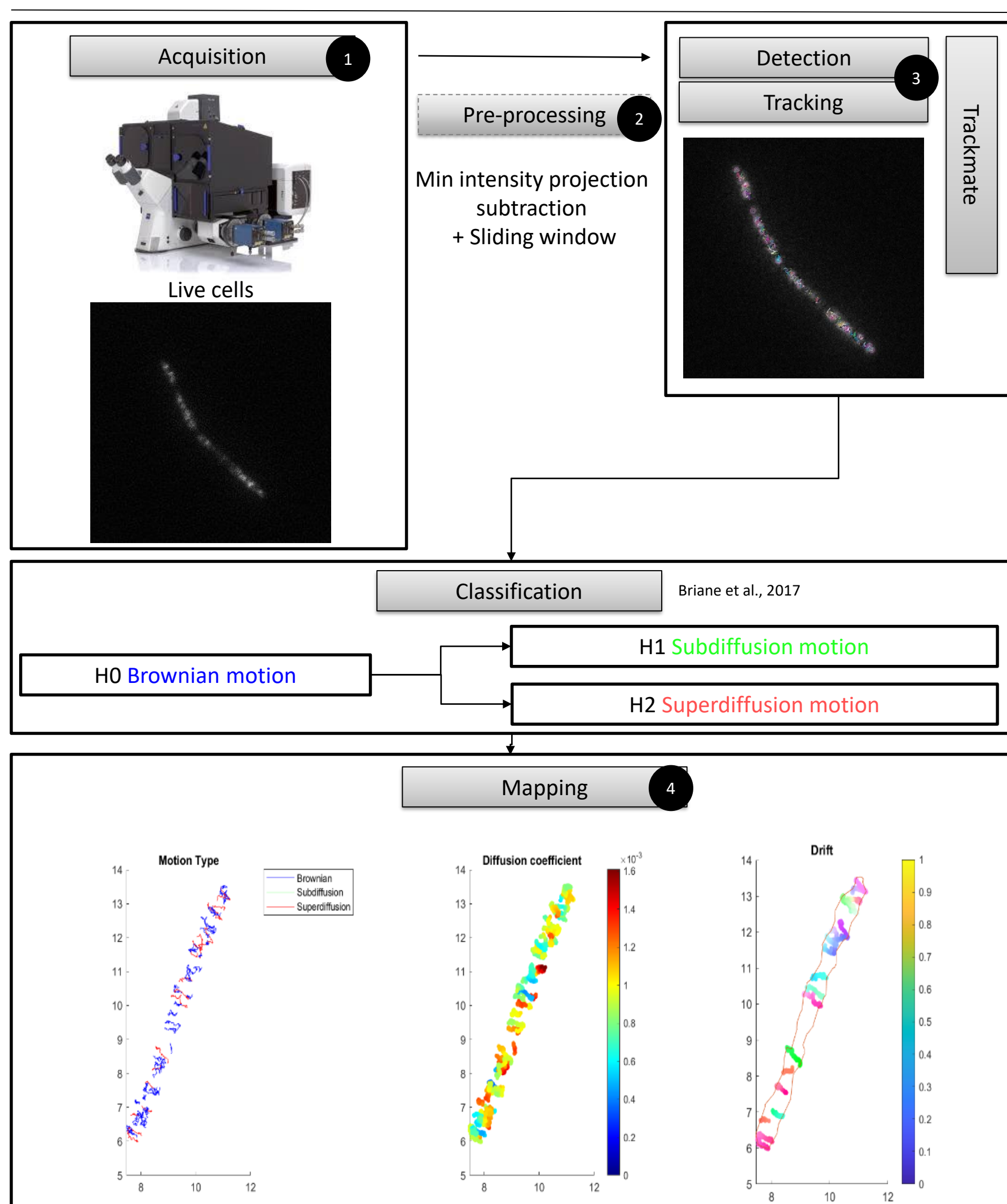
MreB proteins polymerize into membrane-associated filaments. The active form represents a third exhibiting circumferential motion around cell's periphery, the remaining show random or constrained diffusion but their role is unknown (Billaudeau et al. 2017). PBP1 has been shown to have a random diffusion along the cell but its active form has a very slow diffusion (Cho et al., 2016).

To study the localization and dynamics of those proteins, the Dense Mapping approach (Salomon et al., 2020) generates maps from (single) particle tracking ((S)PT) with a limited number of trajectories without degradation of the spatial resolution. This approach is adapted to the constraints of bacteria (*i.e.* small cell volume and limited amount of observed molecules).



Visualization of proteins' dynamics

Workflow of Dense Mapping approach: optimization for bacterial proteins studies



1 Fluorescent molecule: brightness and robustness; adapted to the protein of interest (abundance, dynamics). Ex: GFP, *mNeonGreen*, *mMaple* and *Halo w/ JFx dyes*.

Growth media: prevent auto-fluorescence of it. Ex: LB, CH, EzRM.

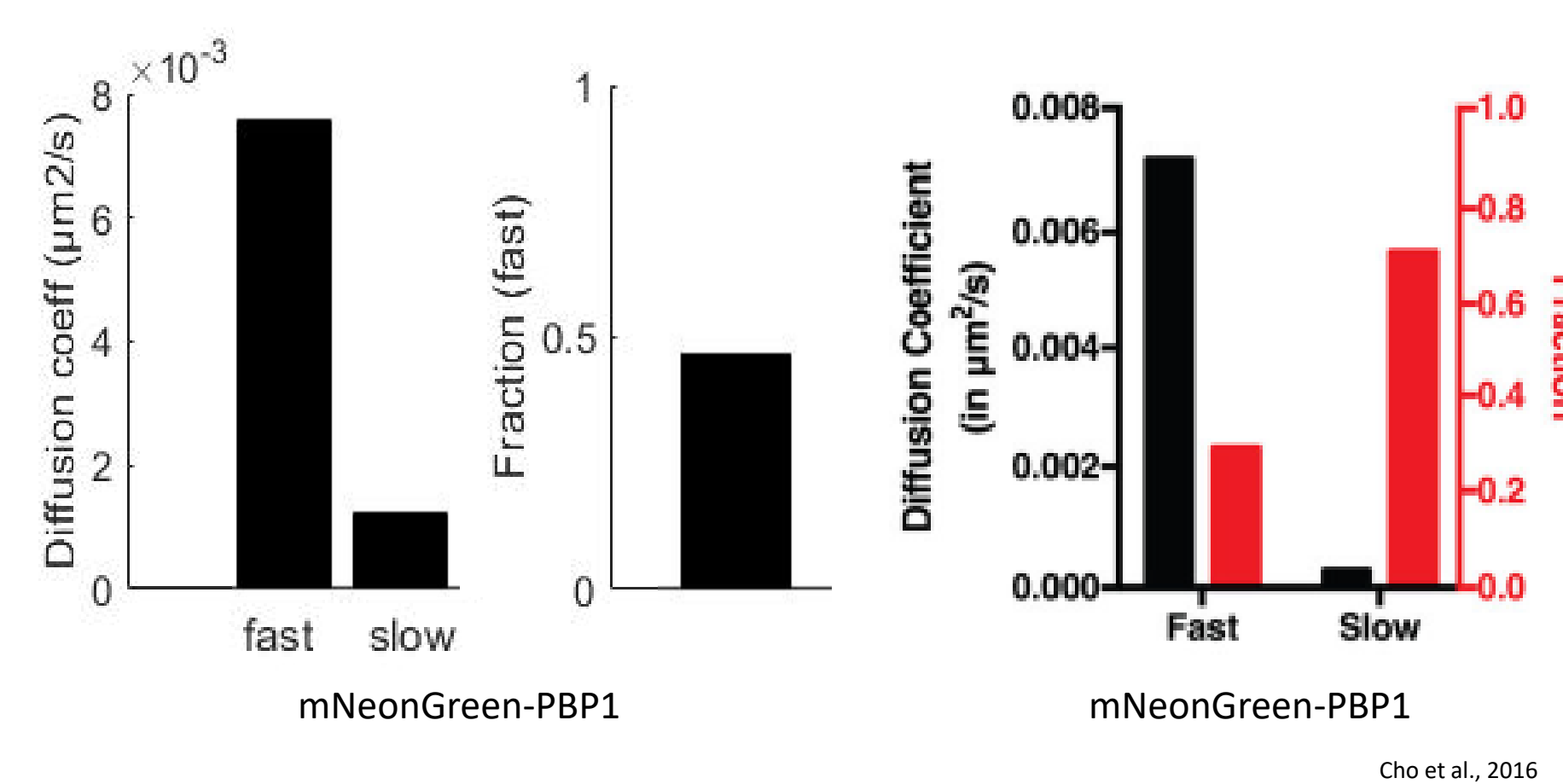
Acquisition parameters: acquisition time, number of frames per second, exposure time, laser power.

2 Optimization of Signal/Noise ratio: increase accuracy of detection and tracking → *Minimum intensity projection subtraction + 0.5s sliding window*

3 Detection parameters: size of the object, threshold
Tracking parameters: linking distance, image gap, linking distance with image gap.

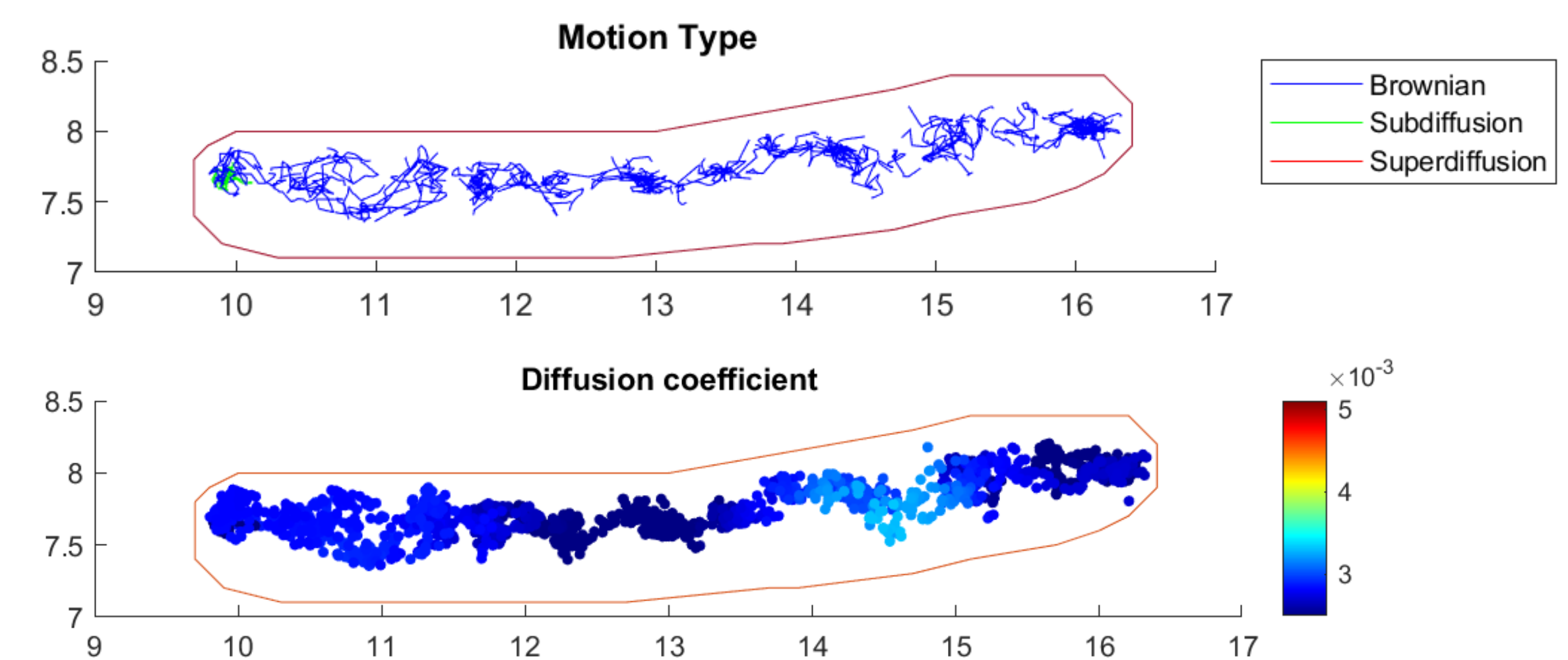
4 Maps analysis: extract biological information from tracks' localization and motion, diffusion and drift maps.

PBP1 spatial organization in *Bacillus subtilis*



We characterize in vivo *mNeonGreen*-PBP1 in *B. subtilis* at exponential phase. The strain comes from Ethan Garner's laboratory (Cho et al., 2016). By using a Cumulative Distribution Function (CDF) analysis, my experimental data has previously published: two diffusive states in the population, "fast" ($\sim 0.007 \mu\text{m}^2/\text{s}$) and "slow" ($\sim 0.0005 \mu\text{m}^2/\text{s}$). We optimized Dense Mapping (DM) approach for bacterial protein with this dataset.

Diffusion maps got extracted from the data with DM analysis. PBP1 is largely diffusive (>98% Brownian motion, data not shown). The protein appears to be located in more visited areas, evenly distributed along the cell. Its diffusion seems homogeneous along the cell with very slight variations. Further analysis of those maps is underway in order to decipher PBP1 dynamics during cell elongation.



"Fast" and "slow" diffusions, visible with CDF analysis, could not be distinguish with DM analysis. Those "slow" tracks do not seem to be cell's dependent or linked to their localization in the cell (data not shown).

The CDF analysis allows us to verify the quality of our dataset with already published results but the estimation of diffusion coefficients is different between the CDF and DM analysis. The robustness of the DM analysis compared to another method, MSD analysis, has been shown (Briane et al., 2017). The DM approach seems to be adapted to the challenge of small cell size and short trajectories of bacterial proteins but could miss some biological information such as the "fast" and "slow" diffusions. Further study of the most suitable method(s) is required for bacterial protein studies (localization, dynamics, diffusion coefficient...).

Conclusion/Perspectives

DM implementation for bacterial proteins show the feasibility if this technique with small organisms' proteins with shorter tracks. The maps extracted showed potential for understanding the organization of proteins in the cells.

Further experiments are required in order to establish a model of PBP1 spatio-temporal organization. Its role might be linked with the Rod system. An analyze of PBP1 with MreB protein is underway, in the mutant of the other and simultaneously.