Supplemental Information

Imaging and quantification of trans-membrane protein diffusion in living bacteria

Felix Oswald[1,2,3], Ernst Bank[3], Yves J.M. Bollen[1,3], and Erwin J.G. Peterman[1,2,*]

[1]LaserLaB Amsterdam, VU University, Amsterdam

[2]Department of Physics and Astronomy, VU University, Amsterdam

[3]Department of Molecular Cell Biology, VU University, Amsterdam

* Corresponding author. De Boelelaan 1081, 1081 HV, Amsterdam. Email: e.j.g.peterman@vu.nl

Supplemental Methods

Plasmids

In order to express an eGFP-labelled membrane protein that is monomeric and does not bind to other proteins or localize to a specific part of a cell, we created an artificial, non-natural protein using standard cloning techniques. The gene encoding enhanced green fluorescent protein (eGFP) was fused via a linker (peptide sequence TSRHGSAL) to a genetically encoded WALP peptide, consisting of an Alanine-Leucine repeat ($HWR(AL)_{11}AER$), which was then fused to the second transmembrane helix of the *E. coli* potassium channel protein KcsA. This fusion gene was cloned into the vector pBAD24, under control of the arabinose-inducible BAD promotor. The resulting plasmid was named pBAD24-GFP-WALP-KcsA. The sequence was confirmed by gene sequencing.

Sample preparation

The bacterial strain MC4100 was transformed with the plasmid pBad24 encoding for WALP-KcsA-eGFP. Cells from an overnight culture were regrown in YT growth medium till mid-log phase, then spun down and resuspended in minimal medium (M9). For immobilization, bacteria were sandwiched between a coverslip and a glass slide on which a pad of 2% agarose-M9 was deposited.

Single-molecule fluorescence imaging

Bacteria were imaged using a commercial inverted microscope (Nikon, Eclipse Ti) equipped with an apochromatic 100x 1.49 NA TIRF oil-immersion objective. Excitation light (wavelength 491 nm, intensity ~200 W/cm^2 in image plane) was provided by Cobolt Calypso laser. Fluorescence images were taken continuously with an EMCCD camera (Andor iXon3 type 897), with an integration time of 32ms per image. The total magnification was 200x, corresponding to 80 nm by 80 nm per pixel.

Single-particle tracking

For automated tracking the tracking algorithm *utrack*[1] was modified. Instead of the original version of the algorithm, the location of the particles was obtained by a Gaussian fit with variable width, in

order to account for changes in the point-spread function due to axial movement of particles along the bacterial surface (in or out of focus). In addition, background subtraction was performed differently, using a local approach allowing multi-particle localization[2]. Linked trajectories from multiple independent image-sequences were pooled. Trajectories with a length below 4 displacements were discarded.

Modelling of the bacterial shape

The bacterial shape was modelled by a cylinder with diameter 0.5 µm and length 1 µm, capped on both end with a half sphere with diameter 0.5 µm. Triangulation of this model was achieved using 3D modelling software (Google Sketchup). The cylindrical part of the model consists of 38 triangles forming 24 flat patches, while each of the half-sphere end caps is composed of 264 triangles forming 144 flat patches. The average angle between each flat patch is 15 degrees.

Brownian motion simulation

Brownian motion along the triangulated model surface was simulated using a random-walk approach using Matlab (Mathworks) software. Starting from a randomly chosen point on the surface model, first a step length was determined according to a Rayleigh distribution given a time lag *t* and diffusion constant *D*. Second, an angle was randomly picked, defining a vectorial step traced along the model surface to the coordinate of the next time point. In this way, 1000 trajectories were simulated with a length of 100 displacements for each trajectory yielding a data set of 100000 displacements in total. The projected displacements distribution was obtained by projecting the vectors between consecutive time-points on a 2D plane parallel to the long axis of the bacterium.

Supplemental references

1. K. Jaqaman, D. Loerke, M. Mettlen, H. Kuwata, S. Grinstein, S. L. Schmid and G. Danuser, *Nature Methods*, 2008, 5, 695-702.
2. M. C. Leake, N. P. Greene, R. M. Godun, T. Granjon, G. Buchanan, S. Chen, R. M. Berry, T. Palmer and B. C. Berks, *Proc Natl Acad Sci U S A*, 2008, 105, 15376-15381.