In cellulo FRET-FLIM and single molecule tracking reveal the supra-molecular organisation of the pyoverdine bio-synthetic enzymes in *Pseudomonas aeruginosa.*

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Supplementary materials

Table S1: *P. aeruginosa* strains used in this study.

Strains	Collection ID	Relevant characteristics	Source
Pseudomonas aeruginosa			
PAO1	**PAO1**	Wild-type strain	Stover *et al.*[1]
PvdA-mCherry	**PAS159**	Derived from PAO1 - mcherry chromosomally integrated	Gasser et al.[2]
PvdI-mCherry	**PAS178**	Derived from PAO1 - mcherry chromosomally integrated	This work
PvdA-eGFP	**PAS180**	Derived from PAO1 - egfp chromosomally integrated	Gasser et al.[2]
PvdA-eGFP mCherry-PvdL	**PAS181**	Derived from PAO1 - egfp and mcherry chromosomally integrated	Gasser et al.[2]
PvdA-eGFP mCherry-PvdD	**PAS186**	Derived from PAO1 - egfp and mcherry chromosomally integrated	Gasser et al.[2]
eGFP-PvdD	**PAS214**	Derived from PAO1 - egfp chromosomally integrated	This work
eGFP-PvdL	**PAS215**	Derived from PAO1 - egfp chromosomally integrated	This work
PvdI-eGFP	**PAS216**	Derived from PAO1 - egfp chromosomally integrated	This work
eGFP-PvdD PvdA-mCherry	**PAS229**	Derived from PAO1 - egfp and mcherry chromosomally integrated	This work
eGFP-PvdL PvdA-mCherry	**PAS230**	Derived from PAO1 - egfp and mcherry chromosomally integrated	This work
PvdI- eGFP PvdA-mCherry	**PAS231**	Derived from PAO1 - egfp and mcherry chromosomally integrated	This work
PvdA-eGFP PvdJ-mCherry	**PAS247**	Derived from PAO1 - egfp and mcherry chromosomally integrated	Gasser et al.[2]
PvdA-PAmCherry	**PAS405**	Derived from PAO1 - PA mcherry chromosomally integrated	This work
PvdA-eGFP PvdI-mCherry	**PAS446**	Derived from PAO1 - egfp and mcherry chromosomally integrated	This work
PvdJ-eGFP	**PAS471**	Derived from PAO1 - chromosomally integrated	This work
PvdJ-eGFP PvdA-mCherry	**PAS472**	Derived from PAO1 - egfp and mcherry chromosomally integrated	This work
Escherichia coli			
TOP10		F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(Str^R) endA1 λ^-	Invitrogen

Table S2: Plasmids used in this study.

Plasmids	Collection ID	Relevant characteristics	Source
pEXG2 PvdA-PAmCherry	**pAF10**	pEXG2 carrying the sequence to insert a PA-mCherry tag in Cter of *pvdA*	This work
pEXG2	**pEXG2**	Allelic exchange vector with pBR origin, gentamicin resistance, *sacB*	Rietsch *et al.*[3]
pME3088	**pME3088**	Allelic exchange vector with ColE1 origin, tetracyclin resistance	Voisard *et al.*[4]
pME3088 PvdI-mCherry	**pLG42**	pME3088 carrying the sequence to insert a mCherry tag in Cter of *pvdI*	This work
pME3088 eGFP-PvdD	**pVEGA15**	pME3088 carrying the sequence to insert a eGFP tag in Nter of *pvdD*	This work
pME3088 eGFP-PvdL	**pVEGA16**	pME3088 carrying the sequence to insert a eGFP tag in Nter of *pvdL*	This work
pME3088 PvdI-eGFP	**pVEGA17**	pME3088 carrying the sequence to insert a eGFP tag in Cter of *pvdI*	This work
pEXG2 PvdJ-eGFP	**pVEGA30**	pEXG2 carrying the sequence to insert a eGFP tag in Cter of *pvdJ*	This work
pEXG2 PvdA-eGFP	**pAF8**	pEXG2 carrying the sequence to insert a eGFP tag in Cter of *pvdA*	This work

Table S3 Primers used in this study.

Oligonucleotides	Sequence (5’ to 3’)	Used to construct the following plasmids
PvdI-XhoIFC	AAACTCGAGTTCGTGCCGGATCCCTTTG	pLG42
PvdI-XbaIRC	TTTTCTAGAGATCGCCTCTAGTTCGCTC	pLG42
PvdI-ClaIFC	AAAATCGATTGACCCATGCTTTCCAATCCA	pLG42
PvdI-HindIIIRC	TTTAAGCTTGCCGGTCCAGTACGCCAACTG	pLG42
mCHE-XBAF	AAATCTAGAGTGAGCAAGGGCGAGGAG	pLG42, pVEGA15, pVEGA16, pVEGA17
mCHE-CLAR	AAAATCGATCTTGTACAGCTCGTCCAT	pLG42, pVEGA15, pVEGA16, pVEGA17
PvdD-EcoRIFN	AAAGAATTCGGATGGGGTGGTGGACTACCTC	pVEGA15
PvdD-XbaIRN	TTTTCTAGACACGCTACCGCCTCTTAGGAAATC	pVEGA15
PvdD-ClaIFN	AAAATCGATCAAGCACTCATAGAGAAGGTG	pVEGA15
PvdD-HindIIIRN	TTTAAGCTTGCCCAGCAGGCCGGTCCAG	pVEGA15
PvdL-HindIIIFN	AAAAAGCTTTTCGGCGAGGCCCTGCATACCG	pVEGA16
PvdL-XbaIRN	TTTTCTAGACATCATGTGTTTTCCTGCCTG	pVEGA16
PvdL-ClaIFN	AAAATCGATGACGCCTTCGAACTTCCCACC	pVEGA16
PvdL-XhoIRN	TTTCTCGAGTACGCCGCTGAAGATCGGTTG	pVEGA16
PvdI-XhoIFC	AAACTCGAGTTCGTGCCGGATCCCTTTG	pVEGA17
PvdI-XbaIRC	TTTTCTAGAGATCGCCTCTAGTTCGCTC	pVEGA17
PvdI-ClaIFC	AAAATCGATTGACCCATGCTTTCCAATCCA	pVEGA17
PvdI-HindIIIRC	TTTAAGCTTGCCGGTCCAGTACGCCAACTG	pVEGA17
egfpF	gtgagcaagggcgaggagctgttcaccgggg	pVEGA30
egfpR	cttgtacagctcgtccatgccgagagtgatcccgg	pVEGA30
pvdJstop-832F	GTACCTGGGCGGGGAAGGGGTGGCGCGT	pVEGA30
pvdJstop+852R	GGGCCACGCGCCTGAGCGCCTGGGAC	pVEGA30
egfppvdJoverlapF	ccgggatcactctcggcatggacgagctgtacaagTAAGAGGCGGTAGCGTGCAAGCACTCATAGAGAAGGTGG	pVEGA30
pvdJegfpoverlapR	ccccggtgaacagctcctcgcccttgctcacGGAAATCAGTTTTTCAAGTTCATCGGCAGATAGACGTTTGAGCGCCTC	pVEGA30
pEXG2HindIIIR	AAGCTTGCTTTACATTTATGCTTCCGGCTC	pVEGA30
pEXG2EcoRIF	GAATTCGGTACCTTAATTAATTTCCACGGG	pVEGA30
PvdA stop-700 EcoRI For	ATCGGAATTCGATGAAGATCGCCATTATCGG	pAF8, pAF10
PvdA stop-700 Rev	GCTGGCCAGGGCGTGCT	pAF8, pAF10
eGFP For overlap PvdA	AGCACGCCCTGGCCAGCGTGAGCAAGGGCGAGGA	pAF8, pAF10
eGFP Rev	CTTGTACAGCTCGTCCATGC	pAF8, pAF10
PvdA Stop+700 For over GFP	GCATGGACGAGCTGTACAAGTGATCGGCGCCACGCCG	pAF8, pAF10
PvdA Stop+700 Hind Rev	ATCGAAGCTTCAAGGCGACCTTCTCCGC	pAF8, pAF10

Figure S1

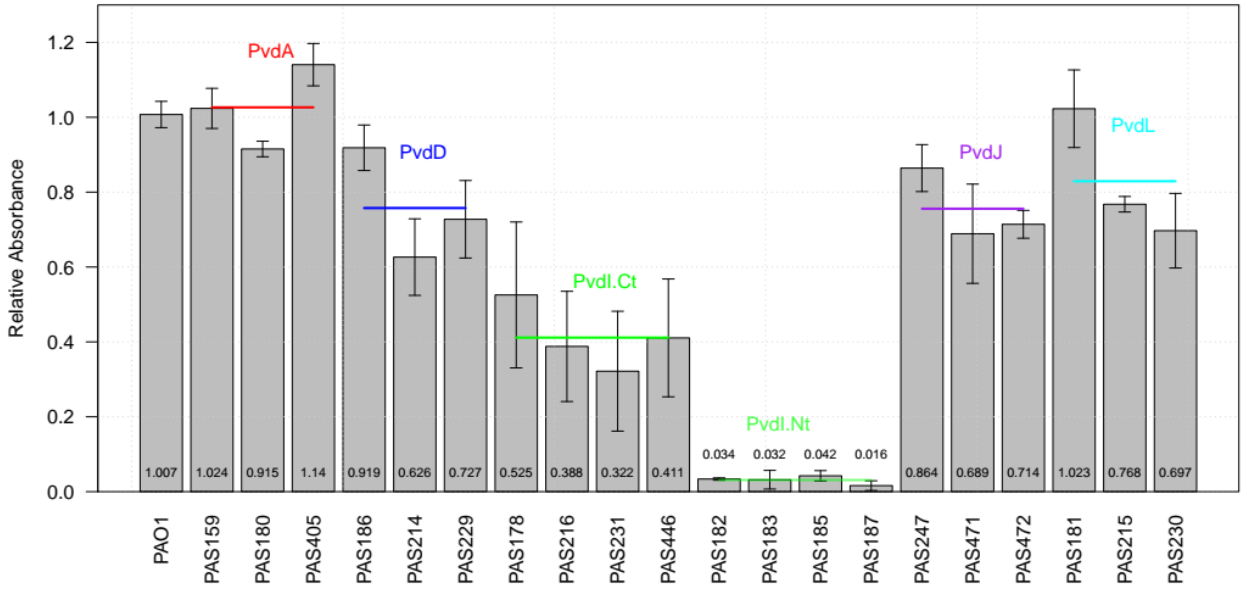

Figure S1

PVD production of the different strains used in this study.

The quantity of PVD excreted outside the bacteria cells was followed by measuring the corrected relative absorbance at 405 nm (compared to PAO1) of the filtered supernatant of the succinate growing media (SM). The absorbance was measured on cultures grown at 30°C for 48h in SM. Strains are organized on the graphic according to the labelled protein (see supplementary table S1 for details) – doubly-labelled strains (PvdA and NRPS labelling) were assigned to NRPS groups. Note that strains with PvdI modified at their N termini were not used in this study as these modifications were interfering with PVD production.

Figure S2

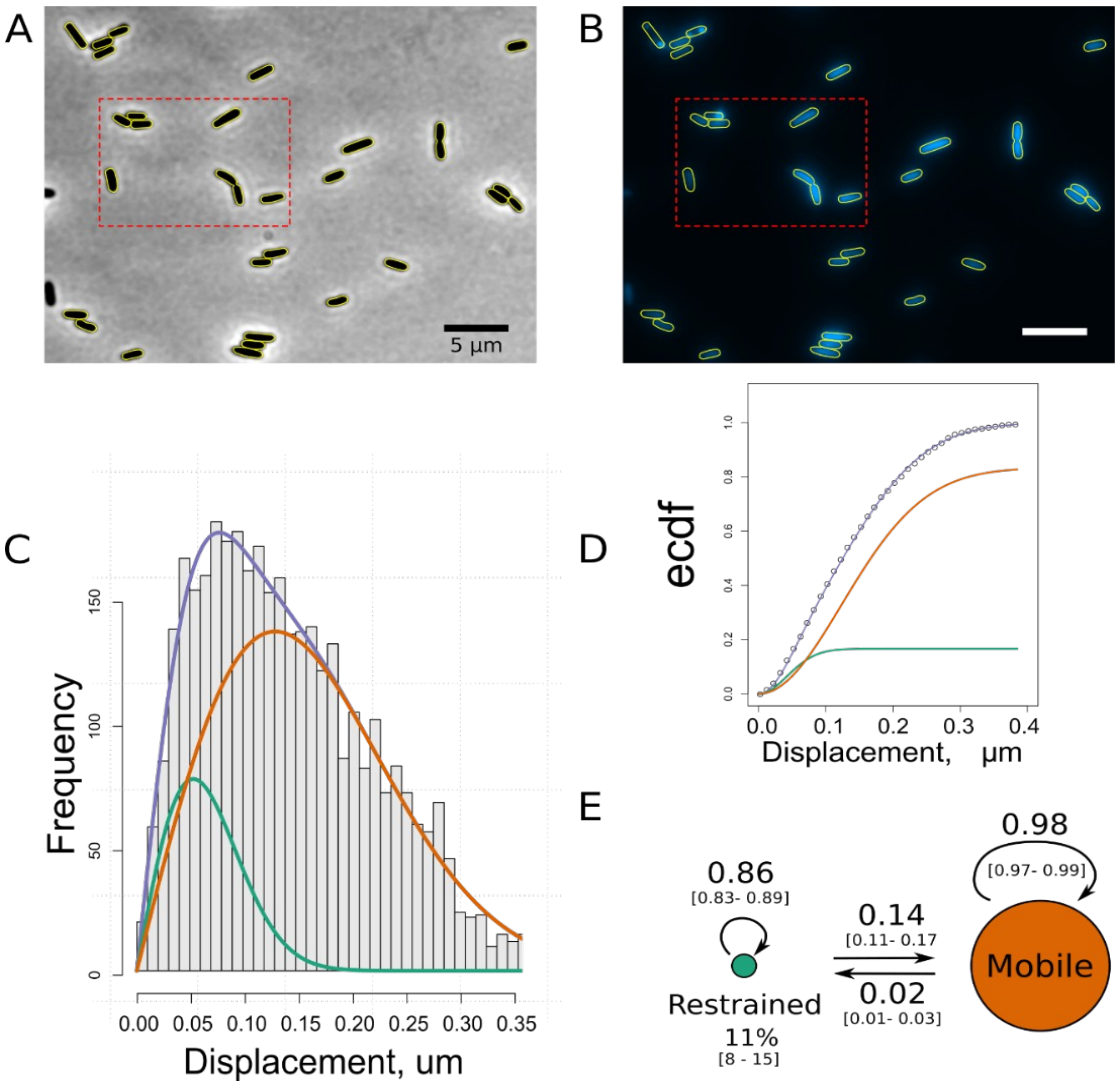

Figure S2

Single molecule tracking of PvdA-eYFP in live *P. aeruginosa*

(A) Phase-contrast (left) and (B) fluorescence (right) images of PAO1 PvdA-eGFP grown in Succinate Media at 30°C for 48 h. These images correspond to a larger field of view of the images presented in Figure 2 (red selections). Scale bars = 2 µm.

(C) Jump-distance distribution (JD) representing the Euclidean distance travelled by ~5,500 PvdA-eYFP during a 16ms time interval. These data correspond to the JD observed in 11 cells measured in two independent experiments. (E) The corresponding empirical cumulative distribution function (ecdf) was fitted assuming a two-population diffusion model to retrieve diffusion coefficients of 0.06 [0.03 – 0.09] µm^2/s and 0.48[0.46 – 0.51] µm^2/s (median [IQR]) determined at 20°C for the restrained or bound (green) and mobile (orange) species, respectively – in very good agreement with observations made with PvdA-PamCherry.

Figure S3

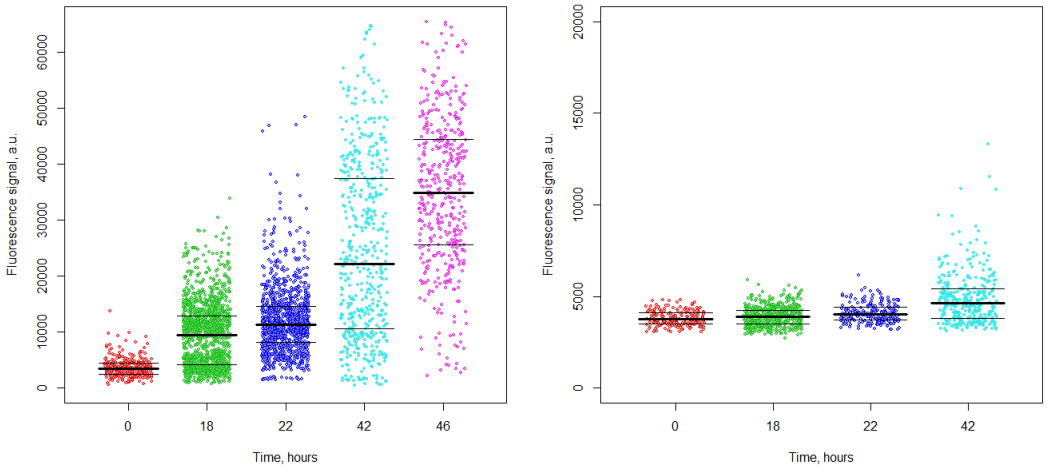

Figure S3

Single-cell fluorescence signals of PvdA-eGFP (left) and PvdI-eGFP (right) measured at different cell growth time points after culture media was changed to succinate media. Excitation wavelength was 488 nm. The fluorescence signal was filtered using a 488 nm long pass-filter. Each individual dot corresponds to the averaged fluorescence signal of one individual cell. Median and IQR intensity values of the cell signals are represented as horizontal lines. The level of expression of PvdA-eGFP was much higher than that of PvdI-eGFP in these conditions.

Figure S4

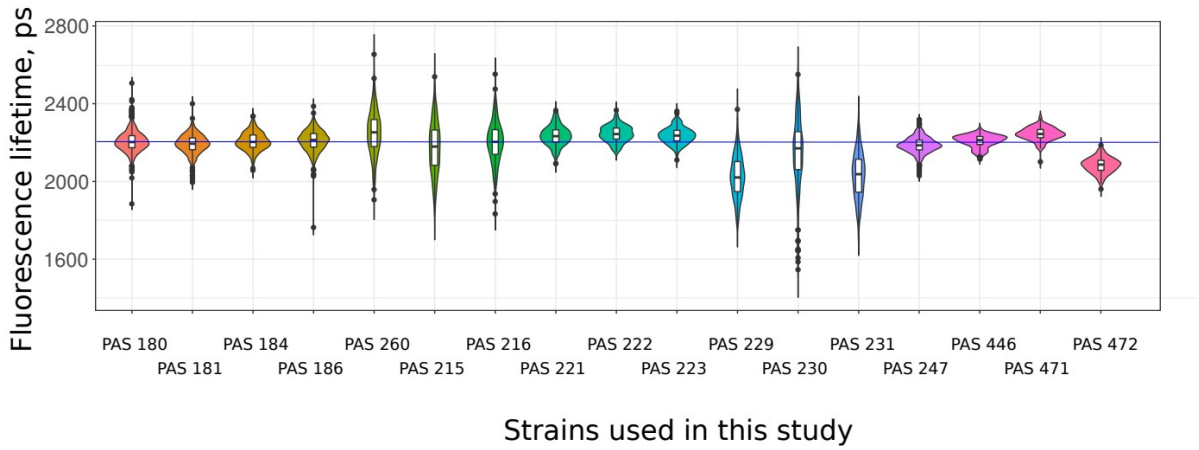

Figure S4

Fluorescence lifetime distribution of the different strains used in this study.
Violin plot of the fluorescence lifetimes (one exponential model) for all the different strains used in this study.

Figure S5

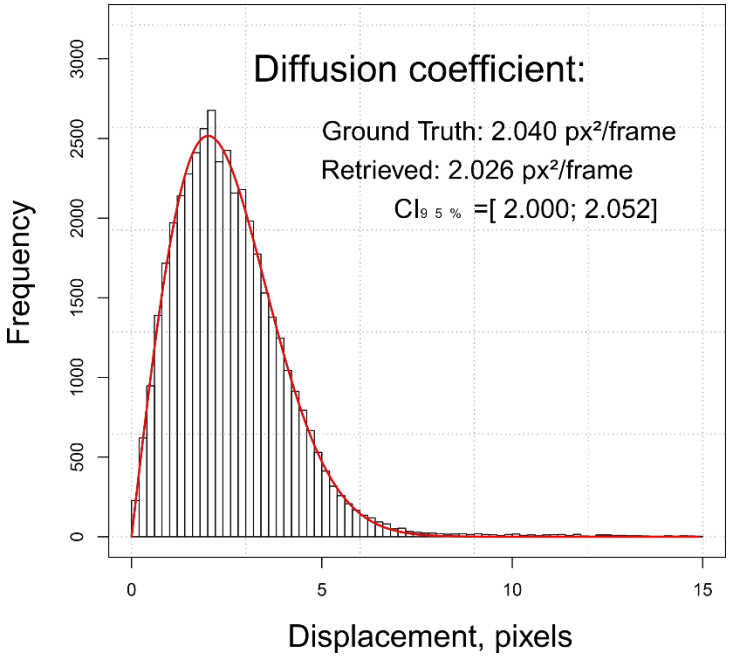

Figure S5

Jump-distance distribution (JD) analysis of a simulated image data of Chenouard, N. et al. [5]

Data were extracted from the supplementary video 1 *corresponding to simulated vesicles diffusing according to a Brownian motion with a ground truth diffusion coefficient of 2.040px²/frame. The data were simulated at medium particle density and a signal-to-noise ratio of 4. To challenge the analysis pipeline fluorescent spots were tracked and analysed. The red line corresponds to the fit of the jump distances distribution. The estimation of the diffusion coefficient inferred from this data was 2.026 [2.000; 2.052] px²/frame, in excellent agreement with the ground truth.*

Supplementary references:

(1) Stover, C. K.; Pham, X. Q.; Erwin, A. L.; Mizoguchi, S. D.; Warrener, P.; Hickey, M. J.; Brinkman, F. S.; Hufnagle, W. O.; Kowalik, D. J.; Lagrou, M.; et al. Complete Genome Sequence of Pseudomonas Aeruginosa PAO1, an Opportunistic Pathogen. *Nature* **2000**, *406* (6799), 959–964.

(2) Gasser, V.; Guillon, L.; Cunrath, O.; Schalk, I. J. Cellular Organization of Siderophore Biosynthesis in Pseudomonas Aeruginosa: Evidence for Siderosomes. *J. Inorg. Biochem.* **2015**, *148*, 27–34.

(3) Rietsch, A.; Mekalanos, J. J. Metabolic Regulation of Type III Secretion Gene Expression in Pseudomonas Aeruginosa. *Mol. Microbiol.* **2006**, *59* (3), 807–820.

(4) Voisard, C.; Bull, C. T.; Keel, C.; Laville, J.; Maurhofer, M.; Schnider, U.; Dfago, G.; Haas, D. Biocontrol of Root Diseases ByPseudomonas Fluorescens CHA0: Current Concepts and Experimental Approaches. In *Molecular Ecology of Rhizosphere Microorganisms*; Wiley-VCH Verlag GmbH: Weinheim, Germany; pp 67–89.

(5) Chenouard, N.; Smal, I.; de Chaumont, F.; Maška, M.; Sbalzarini, I. F.; Gong, Y.; Cardinale, J.; Carthel, C.; Coraluppi, S.; Winter, M.; et al. Objective Comparison of Particle Tracking Methods. *Nat. Methods* **2014**, *11* (3), 281–289.