Supplementary Information

for

Single-molecule FRET and molecular dynamics simulations reveal early activation steps of MET receptor by *Listeria monocytogenes*

Short Title: Activation of the MET receptor by *Listeria monocytogenes*

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Supplemental Note 1.

The T-H and H-T FRET datasets are almost identical. The InlB T-Cy3B and H-ATTO 647N and InlB H-Cy3B and T-ATTO 647N variants were measured individually. From the E,S-histograms of T-H and H-T, FRET efficiencies of 0.525 ± 0.005 and 0.594 ± 0.009 were determined, respectively (**Figure S4**). Given that the MET:InlB dimer is not symmetric, the position of donor dye could be arbitrarily distributed across the two possible T-H positions. Intriguingly, due to the slight asymmetry of the dimer structure, the donor accessibility and lifetime is affected and leads to a broader FRET efficiency distribution relative to T-T (**Figure 3A**). For the H-T pair, the donor dye is labeled to the H variant of $InlB_{321}$, which faces the outside of the protein complex. This configuration enables a larger rotational freedom of the fluorophore, leading to a sparser FRET distribution relative to the T-H combination and a marginally larger FRET efficiency. The data sets for H-T and T-H were merged, to average out the bias of two possible configurations.

Table S1: Used $InlB_{321}$ variants and the respective mutations for fluorophore labeling. The degrees of labeling (DOL) of Cy3B- or ATTO 647N-labeled variants were determined by absorption spectroscopy.

Variant	Mutation	DOL
InlB-T-Cy3B	K280C	87%
InlB-T-ATTO 647N	K280C	69%
InlB-H-Cy3B	K64C	70%
InlB-H-ATTO 647N	K64C	103%

Table S2: Density of MET receptor cluster in different cell lines. Mean receptor densities were corrected for the background. The errors are standard deviations.

Cell line	Receptor density / µm²
23132/87	7.8 ± 2.5
HeLa	8.7 ± 2.7
Huh7.5	3.5 ± 1.1
U-2 OS	2.8 ± 1.2
U-251	10.6 ± 2.7

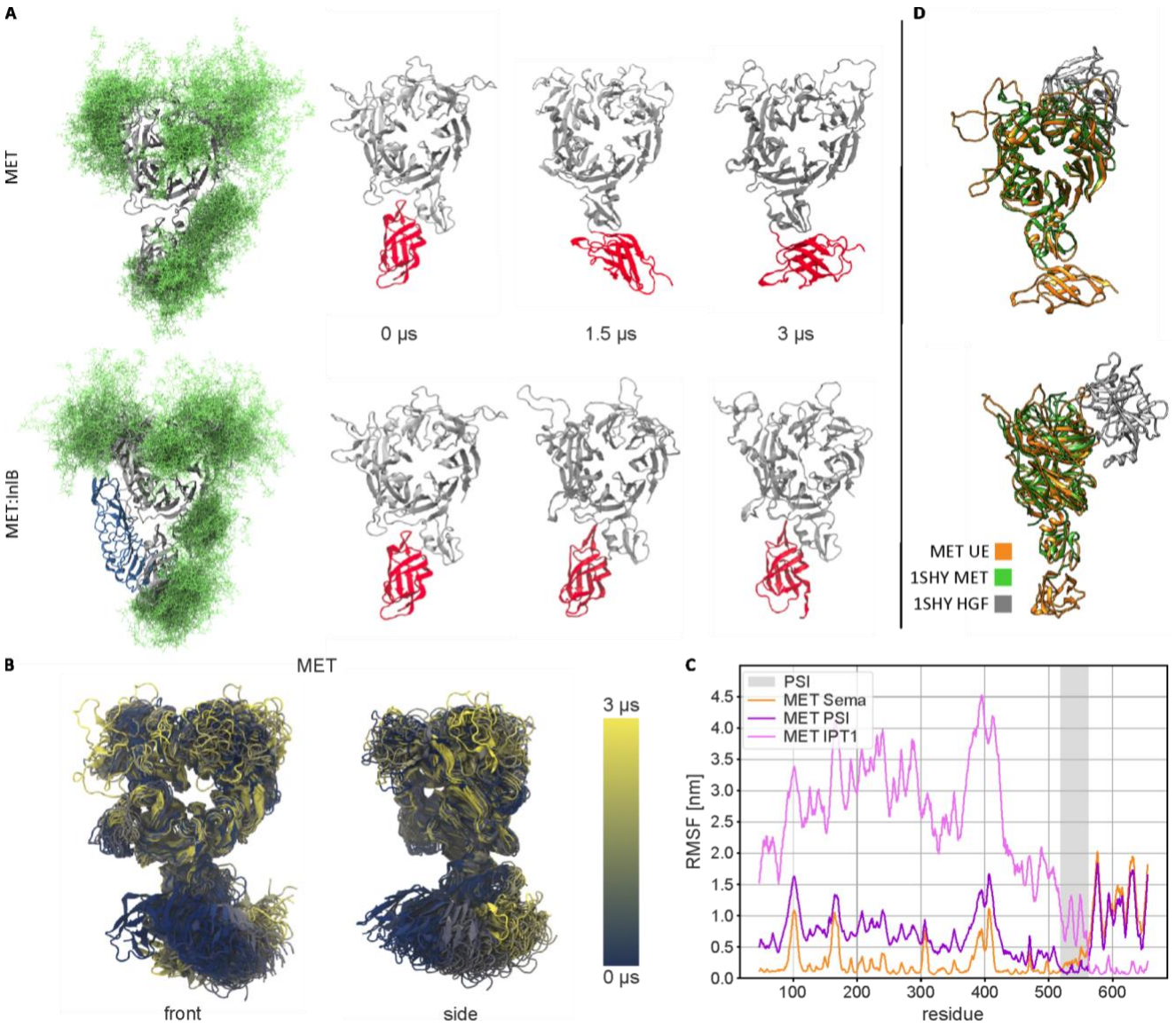

Figure S1: (**A**) Renders of the N-glycosylated MET upper ectodomain system in isolation (top row, MET upper ectodomain) and bound to InlB (bottom row, MET upper ectodomain:InlB). The renders show the MD models of the MET receptor and three snapshots of the corresponding trajectories. In the first column, the models of the glycosylated MET in isolation and glycosylated MET in complex with InlB are shown (InlB in blue, MET in silver, and in green glycan conformations from the first 200 ns of the trajectory, sampled every 2.5 ns; water and ions are not shown for clarity). In the other columns only the MET receptor is shown for both models at different time points. Sema and PSI are represented in silver, the IPT1 domain in red. Glycans, water and ions not shown for clarity. (**B**) Side and front view of the MET upper ectodomain along the model trajectory (every 50 frames). The cartoon representation is colored according to the frame index (according to the color bar). (**C**) Flexibility of the MET upper ectodomain with respect to the different domains. The root mean square fluctuation (RMSF) of the MET upper ectodomain Cα atom positions with respect to the first frame. The trajectory was aligned to the Sema (orange), PSI (dark purple), and IPT1 (light purple) domain, respectively. The PSI domain residues are highlighted by the gray area. (**D**) Alignment of the MET:HGF structure (PDB 1SHY, MET in green, HGF in silver) to the MET upper ectodomain in the last frame of the simulation (MET in orange).

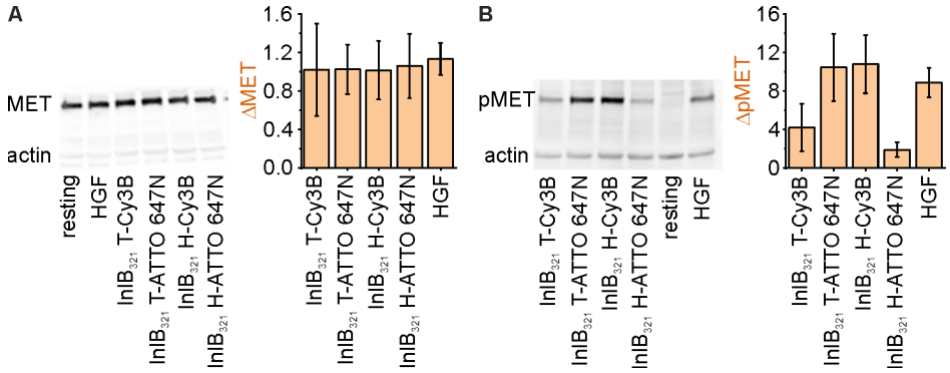

Figure S2: Western blot analysis of MET in resting and ligand-stimulated U-2 OS cells. Exemplary western blots of (A) MET and (B) phosphorylated MET (pMET) are shown (left). Cells were incubated for 15 min with ligand ($InlB_{321}$ or HGF) or with solely medium for the resting condition. Actin was co-labeled for quantification. Page ruler was used as a size marker. In the bar graphs (right) the relative difference in the amount of MET and pMET, respectively, are shown. For this purpose, the MET bands were normalized with the actin bands and the ligand-activated conditions were compared with the resting cells. 3-4 independent experiments were averaged. Errors are given as standard deviations.

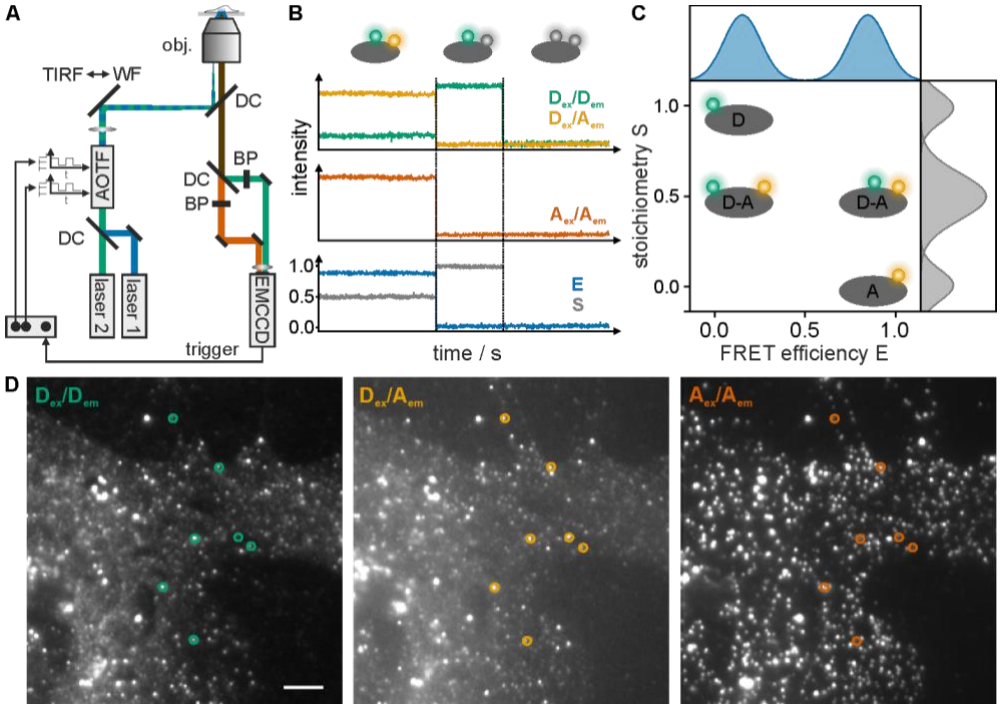

Figure S3: Single-molecule FRET with alternating laser excitation (ALEX). (A) Scheme of a microscope setup for single-molecule FRET measurements with alternating laser excitation. A donor and an acceptor excitation laser are alternated using an acousto-optical filter (AOTF). An adjustable mirror is used to adjust illumination to total internal reflection (TIRF) to solely illuminate the lower plasma membrane of the cells and reduce background fluorescence. DC: dichroic mirror, obj.: objective, BP: bandpass filter. (B) Schematic single FRET pair traces. Shown are the donor (green) and acceptor emission (light orange) upon donor excitation and the acceptor emission (dark orange) upon acceptor excitation. From these intensities the FRET efficiency E (blue) and the stoichiometry S (gray) of donor and acceptor can be calculated. On the vertical lines, the donor or acceptor photobleaches, which can be seen in the intensity traces as well as the E and S traces. (C) Two-dimensional ALEX histogram showing the expected populations for different cases. In the case of active donor and acceptor, a stoichiometry of 0.5 is expected and the FRET efficiency relates to the distance between donor and acceptor. In a scenario where only the donor is present; a stoichiometry of 1 is expected, while a molecule having only an active acceptor exhibits a stoichiometry of 0. (D) Exemplary smFRET data of $InlB_{321}$-labeled MET receptors in U-2 OS cells. D_{ex}/D_{em} shows donor emission upon donor excitation. D_{ex}/A_{em} shows acceptor emission upon donor excitation. A_{ex}/A_{em} shows acceptor emission upon acceptor excitation. The image is the average intensity of the first 100 frames. FRET pairs are highlighted by circles. Scale bar 5 µm.

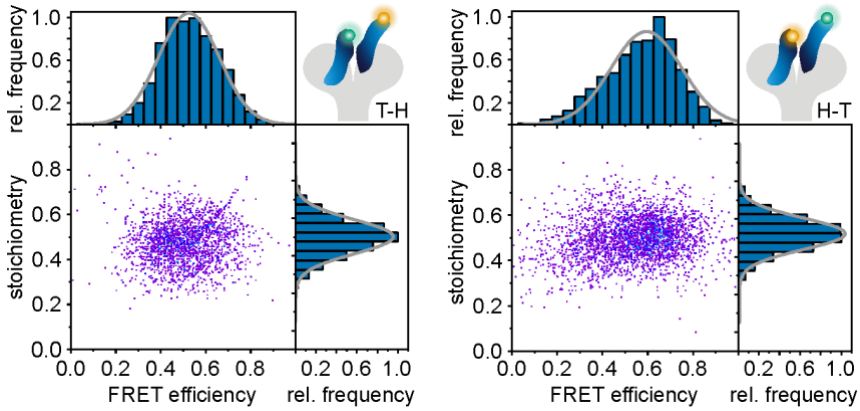

Figure S4: Single-molecule E,S-histogram obtained from smFRET experiments of $(MET:InlB)_2$ dimers in U-2 OS cells. Left: E,S-histogram for InlB T-Cy3B and H-ATTO 647N variant (N = 25 smFRET traces from 20 cells); Right: E,S-histogram for InlB H-Cy3B and T-ATTO 647N variant (N = 24 smFRET traces from 19 cells).

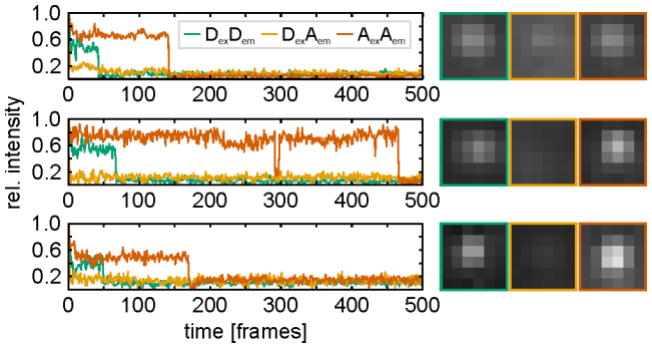

Figure S5: Exemplary intensity traces for InlB H-Cy3B and H-ATTO 647N variants. The intensity traces for the donor (green) and the acceptor (light orange) upon donor excitation as well as the acceptor intensity upon acceptor excitation (dark orange) are shown. No FRET signal is observed in the $D_{ex}A_{em}$ channel. Traces are normalized to 1.

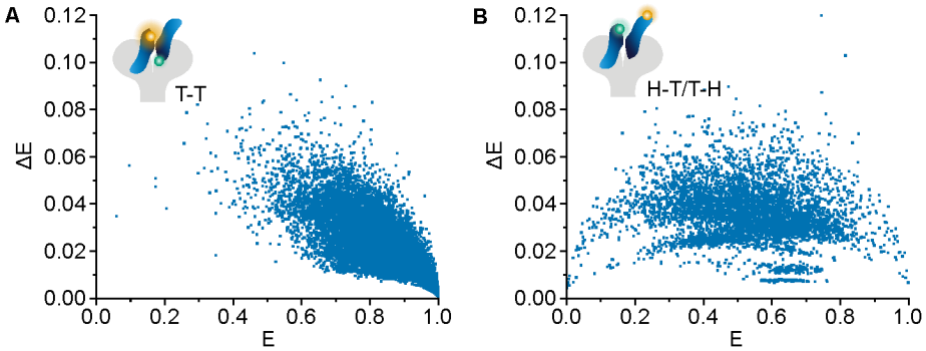

Figure S6: Shot-noise limited standard deviation (ΔE) of the detected FRET efficiency of T-T (A) and H-T/T-H FRET measurements (B). Calculated according to Gopich et al. (*52*).

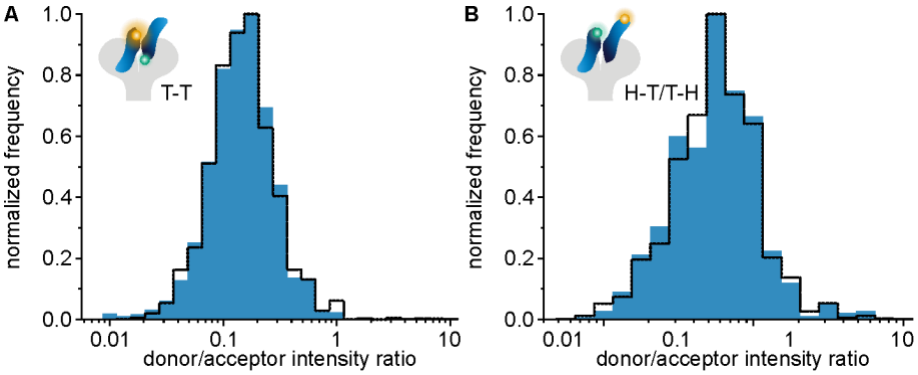

Figure S7: Photon distribution analysis (PDA) of T-T (A) and H-T/T-H FRET measurements (B). In blue the histograms of the ratio of donor emission and acceptor emission upon donor excitation are shown. The intensities are not background corrected. The dashed line represents the simulated histograms according to Antonik et al. (*38*) with a single-state model.

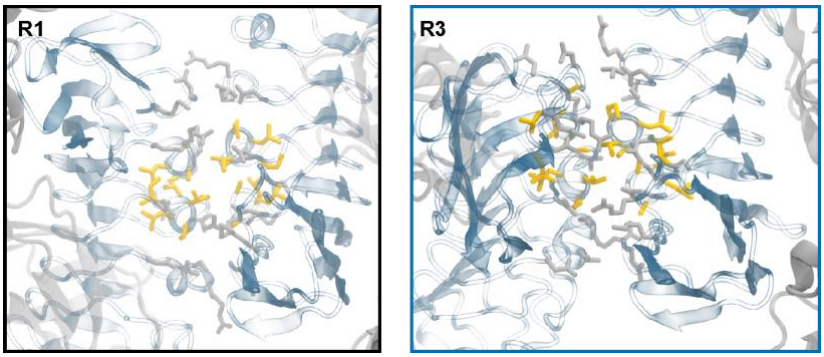

Figure S8: Hydrophobic core of the dimer interaction interface in replica 1 and 3. Hydrophobic amino acids are highlighted in yellow. InlB and MET are shown as a transparent cartoon, respectively blue and gray. The residues involved in the interfaces are shown in licorice representation, hydrophobic residues are colored in yellow and non-hydrophobic in silver.