Supplementary information

The Interaction of Proteins Associated to the Magnetosome Assembly in Magnetotactic Bacteria as Revealed by Two-Hybrid Two-Photon Excitation Fluorescence Lifetime Imaging Microscopy Förster Resonance Energy Transfer

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Figure S1: SDS-PAGE of MamK_mCherry and eGFP

Figure S2: Western Blot of a strain of E. coli (a) taken at 0, 1, 2, 3, 4, 19, 21, 24 hours after induction with IPTG. Magnification of the Western Blot showing the level of co- expression of both EGFP_MamJ ((b)1) and MamK_mCherry ((b)2).

Figure S3: FLIM image of E. coli expressing eGFP_MamJ and mCherry

Figure S4: Images of E. coli expressing MamK_mCherry

Note S1: effect of FRET on the fluorescence intensity and localization of the fluorescence proteins

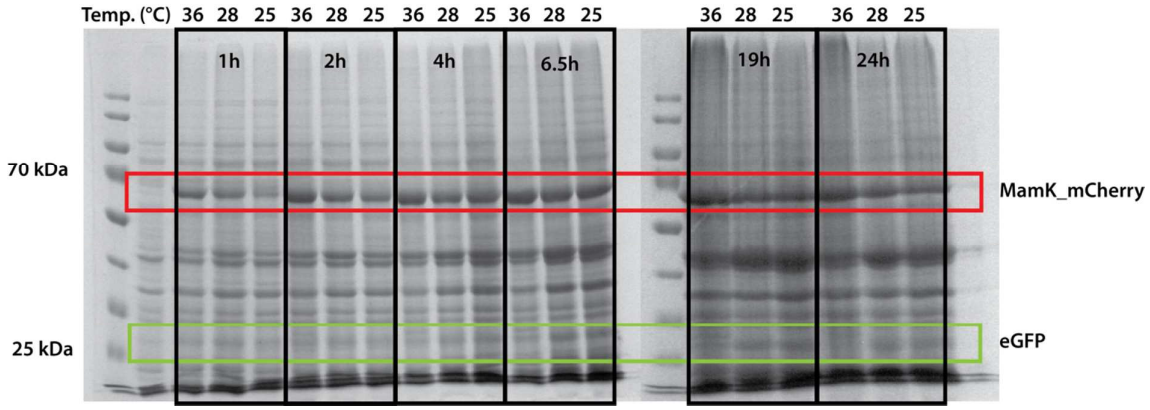

Figure S1: SDS-PAGE of the proteins coexpressed in E. coli: MamK_mCherry (ca. 68 kDa, red rectangle) and eGFP (ca. 27 kDa, green rectangle). The second column corresponds to the level of protein expression before induction with IPTG. The level of expression 1h, 2h, 4h, 6.5h, 19h and 24h after induction are shown. The expression was performed at 25, 28 and 36°C.

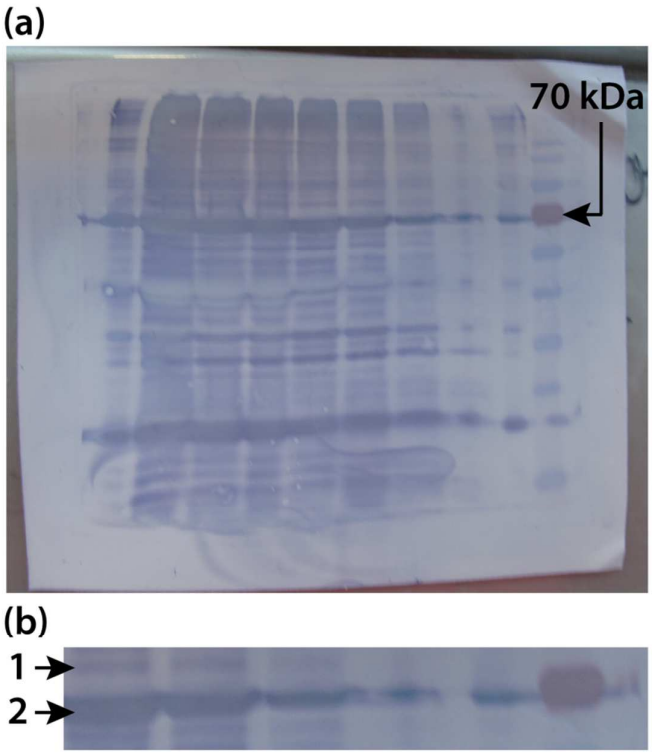

Figure S2: Western Blot of a strain of E. coli (a) taken at 0, 1, 2, 3, 4, 19, 21, 24 hours after induction with IPTG. Magnification of the Western Blot showing the level of co- expression of both EGFP_MamJ ((b)1) and MamK_mCherry ((b)2).

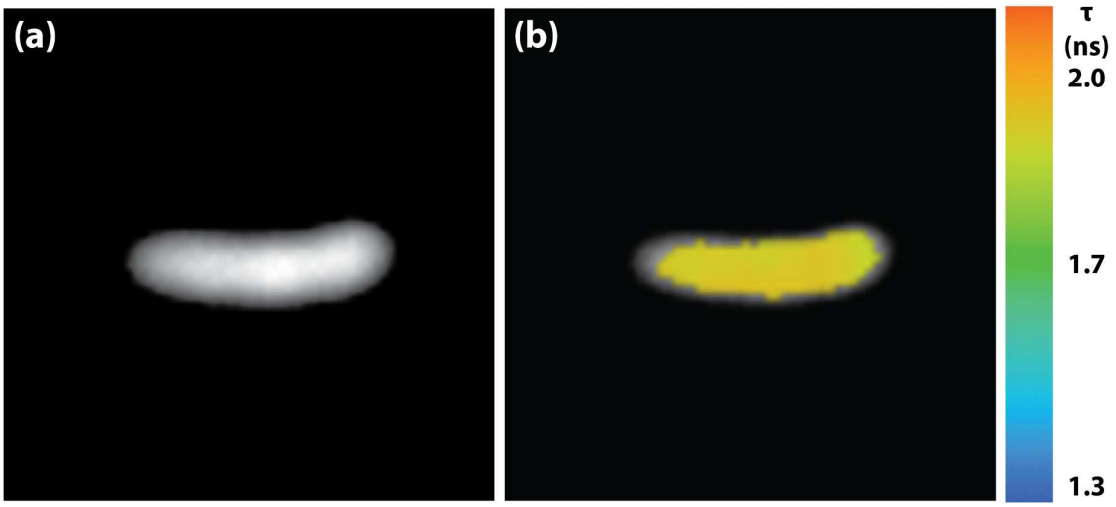

Figure S3: Fluorescence intensity (a) and fluorescence lifetime (b).images of E. coli expressing eGFP_mamJ and mCherry

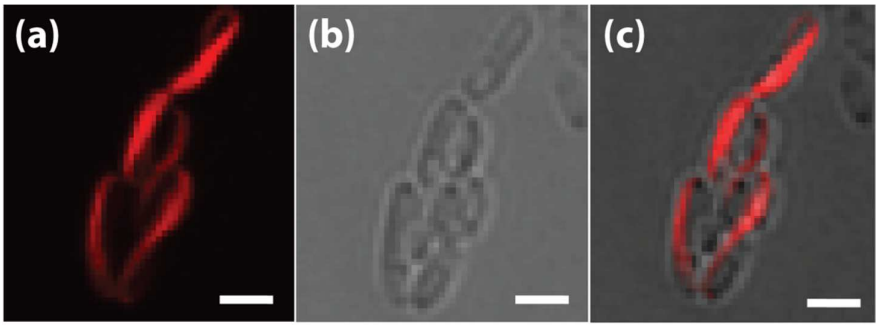

Figure S4: Fluorescence images (a) and transmission image (b) of E. coli expressing mamK_mCherry (Scale bar 2 µm) and superposition (c) of (a) and (b).

Note S1

On the effect of FRET on the fluorescence intensity and localization of the fluorescence proteins:

If FRET occurs, the quantum yield for fluorescence of eGFP decreases, which results in a lower signal intensity per fluorophore. This phenomenon would be observed if eGFP_MamJ in the presence of MamK_mCherry was homogeneously distributed within the cell. In that case, only the eGFP_MamJ that would interact with MamK_mCherry would exhibit lower fluorescence intensity. This would result in a fluorescence image in which the filament appears dimmer than the rest of the cell in the green channel. The co-expression of MamK_mCherry and eGFP_MamJ in the experiments presented here appear to have favored the expression of the former (See Figure S2). This "excess" of MamK proteins compare to MamJ proteins result in seemingly no MamJ proteins being free in the cell. Hence, the eGFP signal is almost exclusively collected from the MamK filament region and the decrease of fluorescence efficiency due to FRET is not observed in the fluorescence intensity images.