The magnetosome membrane protein, MmsF, is a major regulator of magnetite biomineralization in *Magnetospirillum magneticum* AMB-1

Dorothée Murat[a], Veesta Falahati[a], Luca Bertinetti[b], Roseann Csencsits[c], André Körnig[b], Kenneth Downing[c], Damien Faivre[b] and Arash Komeili[a,1]

**Supplemental Information**

**Table S1.** List of plasmids used in this study.

**Table S2.** List of strains used in this study.

**Table S3.** List of primers used in this study.

**Table S4.** Statistical comparison of crystal length measurements. Welch's t test was used to determine the statistical significance between the mean lengths of crystals from various strains.

**Table S5.** Statistical comparison of crystal width measurements. Welch's t test was used to determine the statistical significance between the mean widths of crystals from various strains.

**Table S6.** Statistical comparison of crystal shape factor. Welch's t test was used to determine the statistical significance between the mean aspect ratios of crystals from various strains.

**Figure S1**. Biomineralization in the Δ*amb0955*, Δ*mms6* and Δ*mamFDC* mutants.

Left panels: Electron micrographs of Δ*amb0955* (A), Δ*mms6* (B) and Δ*mamFDC* (C) mutants. The insets show higher magnification of the regions boxed in black in the micrographs. Scale bars: 100 nm, inserts 50 nm. Right: crystal size distribution in the strains shown on the left.

**Figure S2**: Magnetite biomineralization defect in the ΔR1-R4 mutant of AMB-1. Electron micrographs of an AMB-1 cell (left) and the ΔR1-R4 mutant (right). The insets shower higher

magnifications of the regions boxed in black in the micrographs. Scale bar: 100 nm, inset 50 nm.

**Figure S3:** MamA and MamK are expressed in the miniMAI strain. Whole cell pellets of the wild type (lanes 1 and 5), Δ*mamAB* cluster strain (lanes 2 and 6) and two replicates of the miniMAI strain (lanes 3, 4, 7 and 8) were boiled and subjected to a Western blot analysis using either an anti-MamA (lanes 1-4) or an anti-MamK (lanes 5-8) antibody. Similar numbers of cells were loaded for each strain. The 24 kDa (red), 36 kDa (green) and 42 kDa (blue) protein standard markers are highlighted in the middle lane.

**Table S1**

| Plasmid name | Plasmid of origin | Experiment |
|---|---|---|
| | | Deletions |
| pAK249 | pAK31-derived | Deletion plasmid for *mms6*$_{cl}$ |
| pAK565 | pAK31-derived | Deletion plasmid for *amb0955* |
| pAK566 | pAK31-derived | Deletion plasmid for *mms6* |
| pAK567 | pAK31-derived | Deletion plasmid for *mmsF* |
| pAK535 | pAK31-derived | Deletion plasmid for *mamCDF*$_{cl}$ |
| pAK417 | pAK31-derived | Deletion plasmid for *amb1027* |
| pAK349 | pAK31-derived | Deletion plasmid for R2-R4 |
| pAK351 | pAK31-derived | Deletion plasmid for R6-R14 |
| | | Complementation vectors expressing : |
| pAK562 | pAK22-derived | *mms6* |
| pAK564 | pAK253-derived | *mms6*$_{cl}$ |
| pAK563 | pAK253-derived | *mmsF* |
| pAK525 | pAK253-derived | *mamCDF*$_{cl}$ |
| | | GFP fusions |
| pAK532 | pAK22-derived | N-terminal GFP fusion to *mmsF* |

**Table S2**

| Strain number | Description | Reference |
|---|---|---|
| AK30 | AMB-1 wild type | |
| AK32 | ΔR5, chloramphenicol | Murat et al. PNAS 2010 |
| AK36 | ΔR3, chloramphenicol | Murat et al. PNAS 2010 |
| AK124 | Δ*mms6cl* | This work |
| AK103 | Δ*mms6* | This work |
| AK104 | Δ*mmsF* | This work |
| AK111 | Δ*amb0955* | This work |
| AK109 | Δ$mamCDF_{cl}$ | This work |
| AK51 | ΔR1 | Murat et al. PNAS 2010 |
| AK120 | ΔR1-ΔR4 | This work |
| AK122 | miniMAI | This work |
| AK123 | miniMAI_*mmsF* | This work |

A. Genetic analysis of R3

| Primer name | Primer sequence | Used for deleting : | in plasmid |
|---|---|---|---|
| 956cl-a | GG**ACTAGT**TCCACATCCACAAGACCAAG | *mms6*$_{cl}$ | pAK249 |
| 956cl-b | CCCATCCACTAAATTTAAATACGTCAACATCCAAAGGGATT | *mms6*$_{cl}$ | pAK249 |
| LD3c | GG**ACTAGT**TGGGTCGCCCACCGGATCTGA | *mms6*$_{cl}$ | pAK249 |
| LD3d | GG**ACTAGT**CGCCGCCCTTCTCGGCCAGAG | *mms6*$_{cl}$ | pAK249 |
| 956A | GG**ACTAGT**GGTATTTTCGGTTTCGAGCA | *mms6* | pAK566 |
| 956B | CCCATCCACTAAATTTAAATAGTTGGCGATCTGAGCTGGCAC | *mms6* | pAK566 |
| 956C | TATTTAAATTTAGTGGATGGGCTGCGCGACGCGCTGGCCTGA | *mms6* | pAK566 |
| 956D | GG**ACTAGT**GTCCAGATCGCCCTTGATG | *mms6* | pAK566 |
| 957A | GG**ACTAGT**GCAAACAGAAAATGCGTTGA | *mmsF* | pAK567 |
| 957B | CCCATCCACTAAATTTAAATAGCGAAGGATAGCTTCAGTCAT | *mmsF* | pAK567 |
| 957C | TATTTAAATTTAGTGGATGGGTGGGTCGCCCACCGGATCTGA | *mmsF* | pAK567 |
| 957D | GG**ACTAGT**TGCCTTCATTGCTGTTTTCC | *mmsF* | pAK567 |
| 955A | GG**ACTAGT**GTCCAGCTTGACGATGATTG | *amb0955* | pAK565 |
| 955B | CCCATCCACTAAATTTAAATAGTGCCACAGGAAACAAGCCAC | *amb0955* | pAK565 |
| 955C | TATTTAAATTTAGTGGATGGGAAGGCGGGAAGGTGCGACTAA | *amb0955* | pAK565 |
| 955D | GG**ACTAGT**GGCTCCAGAAACGAAACATC | *amb0955* | pAK565 |
| LD3a | GG**ACTAGT**CCGGCAGCACAGGCCGCCTGA | *mamFDC* | pAK535 |
| LD3b | CCCATCCACTAAATTTAAATACCCAATTCCCCTGCGAATTTG | *mamFDC* | pAK535 |
| R3L-c | TATTTAAATTTAGTGGATGGGACGTACAGCCTGGCAAGAAT | *mamFDC* | pAK535 |
| R3L-d | GG**ACTAGT**AGAAACGAAACATCCCAACG | *mamFDC* | pAK535 |

B. miniMAI

| Primer Name | Primer sequence | Used to delete | In plasmid |
|---|---|---|---|
| LD2a | GG**ACTAGT**CGGACCGCGATAAAGTTCTAA | R2-R4 | pAK349 |
| LD2b | CCCATCCACTAAATTTAAATAGCCGAGCGGGTCTCATTGCAG | R2-R4 | pAK349 |
| LD4c | TATTTAAATTTAGTGGATGGGTTCTGGTGAAGAGAGCATCTG | R2-R4 | pAK349 |
| LD4d.1 | GG**ACTAGT**TCATGAACGCGGTATTGGACA | R2-R4 | pAK349 |
| LD6a.1 | GG**ACTAGT**TCGCCTATTTGGTTGAGG | R6-R14 | pAK351 |
| LD6b.1 | CCCATCCACTAAATTTAAATACGTCCAGGTTGTCCAAGAG | R6-R14 | pAK351 |
| LD13c | TATTTAAATTTAGTGGATGGGATGGGGATCAGCAAGCGCGG | R6-R14 | pAK351 |
| LD13d.1 | GG**ACTAGT**GAATGTCATCCGACAGCAGA | R6-R14 | pAK351 |

C. Complementation

| Primer name | Primer sequences | Expressed gene | Plasmid |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 955 3'Not1 | GG**GCGGCCGC**TTAGTCGCACCTTCCCGCC | *amb0955, mms6, mmsF* | pAK564 |
| 957 5' Spe1 | GG**ACTAGT**ATGACTGAAGCTATCCTTCG | *amb0955, mms6, mmsF* | pAK564 |
| 956 3' Spe1 | GG**ACTAGT**TCAGGCCAGCGCGTCGCG | *mms6* | pAK562 |
| 956 5'EcoR1 | GG**GAATTC**GTGCCAGCTCAGATCGC | *mms6* | pAK562 |
| 957 3'Not1 | GG**GCGGCCGC**TCAGATCCGGTGGGCGACCC | *mmsF* | pAK563 |
| 957 5' Spe1 | GG**ACTAGT**ATGACTGAAGCTATCCTTCG | *mmsF* | pAK563 |
| 5Spe-amb0954 | GG**ACTAGT**ATGGCCGCTCAGGTTGGAG | *amb0951, amb0952, amb0953, amb0954* | pAK525 |
| 3Not_mamC | GG**GCGGCCGC**TCAGGCCAGTTCGTCCCGC | *amb0951, amb0952, amb0953, amb0954* | pAK525 |

D. GFP Fusions

| Primer name | Primer sequence | Plasmid |
|---|---|---|
| 5-Bam-957 | GG**GGATCC**ACTGAAGCTATCCTTCGC | pAK532 |
| 3-Spe-957 | GG**ACTAGT**TCAGATCCGGTGGGCGACCC | pAK532 |

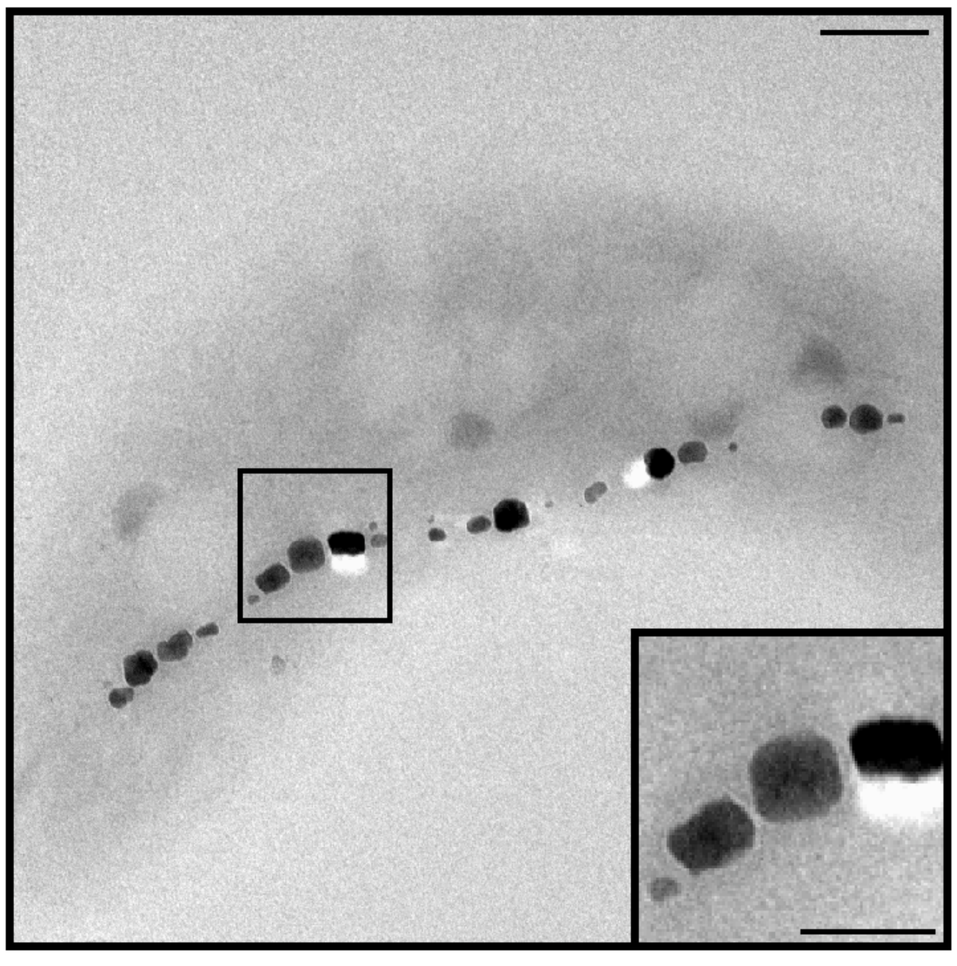
**Table S3**

| | wt | LD3 | LD3+ cluster | LD3 + mmsF | Δmms6cl | Δmms6cl+ mms6cl | Δmms6cl+ 955 | Δmms6cl+ mms6 | Δmms6cl + mmsF | Δ955 | Δmms6 | Δmms6 + mms6 | ΔmmsF | ΔmmsF+ mmsF | ΔmamFDC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **wt** | x | <.0001 | 0.269 | <.0001 | <.0001 | 0.0003 | <.0001 | <.0001 | <.0001 | 0.5513 | <.0001 | 0.0017 | <.0001 | <.0001 | 0.377 |
| **LD3** | <.0001 | x | <.0001 | <.0001 | 0.6491 | <.0001 | 0.0015 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| **LD3+ cluster** | 0.2690 | <.0001 | x | 0.0002 | <.0001 | 0.0107 | <.0001 | <.0001 | <.0001 | 0.8894 | <.0001 | 0.0254 | <.0001 | 0.0035 | 0.8713 |
| **LD3+ mmsF** | <.0001 | <.0001 | 0.0002 | x | <.0001 | 0.3023 | <.0001 | <.0001 | 0.0028 | 0.0124 | 0.1619 | 0.4585 | <.0001 | 0.3879 | 0.0002 |
| **Δmms6cl** | <.0001 | 0.0649 | <.0001 | <.0001 | x | <.0001 | 0.0028 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| **Δmms6cl + mms6cl** | 0.0003 | <.0001 | 0.0107 | 0.3023 | 0.3023 | x | <.0001 | <.0001 | <.0001 | 0.0709 | 0.0177 | 0.9109 | <.0001 | 0.8342 | 0.0085 |
| **Δmms6cl + 955** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | x | 0.0002 | <.0001 | <.0001 | <.0001 | <.0001 | 0.5735 | <.0001 | <.0001 |
| **Δmms6cl + mms6** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0002 | x | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| **Δmms6cl + mmsF** | <.0001 | <.0001 | <.0001 | 0.0028 | <.0001 | <.0001 | <.0001 | <.0001 | x | <.0001 | 0.13 | 0.0019 | <.0001 | <.0001 | <.0001 |
| **Δ955** | 0.5513 | <.0001 | 0.8894 | 0.0124 | <.0001 | 0.0709 | <.0001 | <.0001 | <.0001 | x | 0.0008 | 0.0825 | <.0001 | 0.0482 | 0.9745 |
| **Δmms6** | <.0001 | <.0001 | <.0001 | 0.1619 | <.0001 | 0.0177 | <.0001 | <.0001 | 0.13 | 0.0008 | x | 0.0628 | <.0001 | 0.0234 | <.0001 |
| **Δmms6 + mms6** | 0.0017 | <.0001 | 0.0254 | 0.4585 | <.0001 | 0.9109 | <.0001 | <.0001 | <.0001 | 0.0825 | 0.0628 | x | <.0001 | 0.9541 | 0.0209 |
| **ΔmmsF** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.5735 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | x | <.0001 | <.0001 |
| **ΔmmsF + mmsF** | <.0001 | <.0001 | 0.0035 | 0.3879 | <.0001 | 0.8342 | <.0001 | <.0001 | <.0001 | 0.0482 | 0.0234 | 0.9541 | <.0001 | x | 0.0031 |
| **ΔmamFDC** | 0.3770 | <.0001 | 0.8713 | 0.0002 | <.0001 | 0.0085 | <.0001 | <.0001 | <.0001 | 0.9745 | <.0001 | 0.0209 | <.0001 | 0.0031 | x |

| | wt | LD3 | LD3+ cluster | LD3 + mmsF | Δmms6cl | Δmms6cl+ mms6cl | Δmms6cl+ 955 | Δmms6cl+ mms6 | Δmms6cl + mmsF | Δ955 | Δmms6 | Δmms6 + mms6 | ΔmmsF | ΔmmsF+ mmsF | ΔmamFDC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **wt** | x | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | | <.0001 | 0.4666 | <.0001 | 0.004 | <.0001 | <.0001 | <.0001 |
| **LD3** | <.0001 | x | <.0001 | <.0001 | 0.0005 | <.0001 | <.0001 | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| **LD3+ cluster** | <.0001 | <.0001 | x | 0.5642 | <.0001 | 0.9354 | <.0001 | | <.0001 | 0.0070 | <.0001 | 0.2319 | <.0001 | 0.5445 | 0.3629 |
| **LD3+ mmsF** | <.0001 | <.0001 | 0.5642 | x | <.0001 | 0.6348 | <.0001 | | <.0001 | 0.0202 | <.0001 | 0.4563 | <.0001 | 0.2385 | 0.7468 |
| **Δmms6cl** | <.0001 | 0.0005 | <.0001 | <.0001 | x | <.0001 | 0.029 | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| **Δmms6cl + mms6cl** | <.0001 | <.0001 | 0.9354 | 0.6348 | <.0001 | x | <.0001 | | <.0001 | 0.0091 | <.0001 | 0.2686 | <.0001 | 0.5075 | 0.4269 |
| **Δmms6cl + 955** | <.0001 | <.0001 | <.0001 | <.0001 | 0.0290 | <.0001 | x | | <.0001 | <.0001 | <.0001 | <.0001 | 0.0929 | <.0001 | <.0001 |
| **Δmms6cl + mms6** | | | | | | | | x | | | | | | | |
| **Δmms6cl + mmsF** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | | x | <.0001 | 0.0128 | <.0001 | <.0001 | <.0001 | <.0001 |
| **Δ955** | 0.4666 | <.0001 | 0.0070 | 0.0202 | <.0001 | 0.0091 | <.0001 | | <.0001 | x | <.0001 | 0.1298 | <.0001 | 0.0021 | 0.0338 |
| **Δmms6** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | | 0.0128 | <.0001 | x | <.0001 | <.0001 | <.0001 | <.0001 |
| **Δmms6 + mms6** | 0.0040 | <.0001 | 0.2319 | 0.4563 | <.0001 | 0.2686 | <.0001 | | <.0001 | 0.1298 | <.0001 | x | <.0001 | 0.0980 | 0.6185 |
| **ΔmmsF** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0929 | | <.0001 | <.0001 | <.0001 | <.0001 | x | <.0001 | <.0001 |
| **ΔmmsF + mmsF** | <.0001 | <.0001 | 0.5445 | 0.2385 | <.0001 | 0.5075 | <.0001 | | <.0001 | 0.0021 | <.0001 | 0.0980 | <.0001 | x | 0.1282 |
| **ΔmamFDC** | <.0001 | <.0001 | 0.3629 | 0.7468 | <.0001 | 0.4269 | <.0001 | | <.0001 | 0.0338 | <.0001 | 0.6185 | <.0001 | 0.1282 | x |

| | wt | LD3 | LD3+ cluster | LD3 + mmsF | Δmms6cl | Δmms6cl+ mms6cl | Δmms6cl+ 955 | Δmms6cl+ mms6 | Δmms6cl + mmsF | Δ955 | Δmms6 | Δmms6 + mms6 | ΔmmsF | ΔmmsF+ mmsF | ΔmamFDC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **wt** | x | <.0001 | <.0001 | 0.2570 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.2478 | <.0001 | 0.6504 | <.0001 | <.0001 | <.0001 |
| **LD3** | <.0001 | x | <.0001 | <.0001 | 0.0008 | <.0001 | 0.6076 | <.0001 | 0.0023 | <.0001 | <.0001 | <.0001 | 0.0030 | <.0001 | <.0001 |
| **LD3+ cluster** | <.0001 | <.0001 | x | <.0001 | 0.1916 | <.0001 | <.0001 | 0.1409 | 0.0172 | <.0001 | 0.7983 | <.0001 | 0.0141 | <.0001 | 0.0678 |
| **LD3+ mmsF** | 0.2570 | <.0001 | <.0001 | x | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.6931 | <.0001 | 0.5927 | <.0001 | <.0001 | <.0001 |
| **Δmms6cl** | <.0001 | 0.0008 | 0.1916 | <.0001 | x | <.0001 | 0.0093 | 0.0251 | 0.4749 | <.0001 | 0.2843 | <.0001 | 0.4334 | <.0001 | 0.0052 |
| **Δmms6cl + mms6cl** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | x | <.0001 | 0.0118 | <.0001 | 0.0082 | <.0001 | <.0001 | <.0001 | 0.3873 | 0.0009 |
| **Δmms6cl + 955** | <.0001 | 0.6076 | <.0001 | <.0001 | 0.0093 | <.0001 | x | <.0001 | 0.0259 | <.0001 | 0.0002 | <.0001 | 0.0308 | <.0001 | <.0001 |
| **Δmms6cl + mms6** | <.0001 | <.0001 | 0.1409 | <.0001 | 0.0251 | 0.0118 | <.0001 | x | 0.0014 | 0.0011 | 0.1051 | <.0001 | 0.0012 | 0.0441 | 0.9531 |
| **Δmms6cl + mmsF** | <.0001 | 0.0023 | 0.0172 | <.0001 | 0.4749 | <.0001 | 0.0259 | 0.0014 | x | <.0001 | 0.0376 | <.0001 | 0.9338 | <.0001 | <.0001 |
| **Δ955** | 0.2478 | <.0001 | <.0001 | 0.6931 | <.0001 | 0.0082 | <.0001 | 0.0011 | <.0001 | x | <.0001 | 0.4656 | <.0001 | 0.0016 | <.0001 |
| **Δmms6** | <.0001 | <.0001 | 0.7983 | <.0001 | 0.2843 | <.0001 | 0.0002 | 0.1051 | 0.0376 | <.0001 | x | <.0001 | 0.0313 | <.0001 | 0.0424 |
| **Δmms6 + mms6** | 0.6504 | <.0001 | <.0001 | 0.5927 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.4656 | <.0001 | x | <.0001 | <.0001 | <.0001 |
| **ΔmmsF** | <.0001 | 0.0030 | 0.0141 | <.0001 | 0.4334 | <.0001 | 0.0308 | 0.0012 | 0.9338 | <.0001 | 0.0313 | <.0001 | x | <.0001 | <.0001 |
| **ΔmmsF + mmsF** | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.3873 | <.0001 | 0.0441 | <.0001 | 0.0016 | <.0001 | <.0001 | <.0001 | x | 0.0085 |
| **ΔmamFDC** | <.0001 | <.0001 | 0.0678 | <.0001 | 0.0052 | 0.0009 | <.0001 | 0.9531 | <.0001 | <.0001 | 0.0424 | <.0001 | <.0001 | 0.0085 | x |

A.



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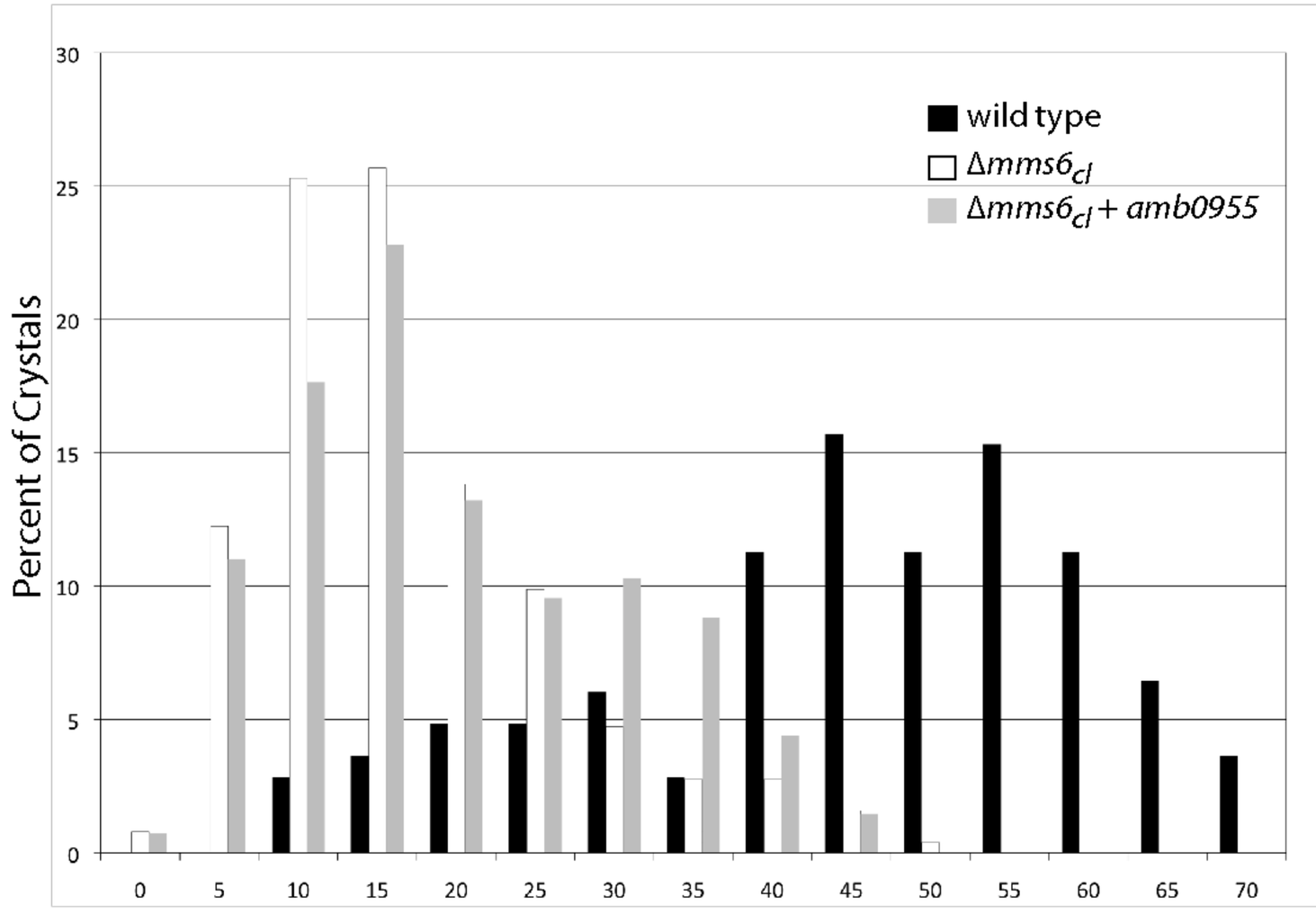


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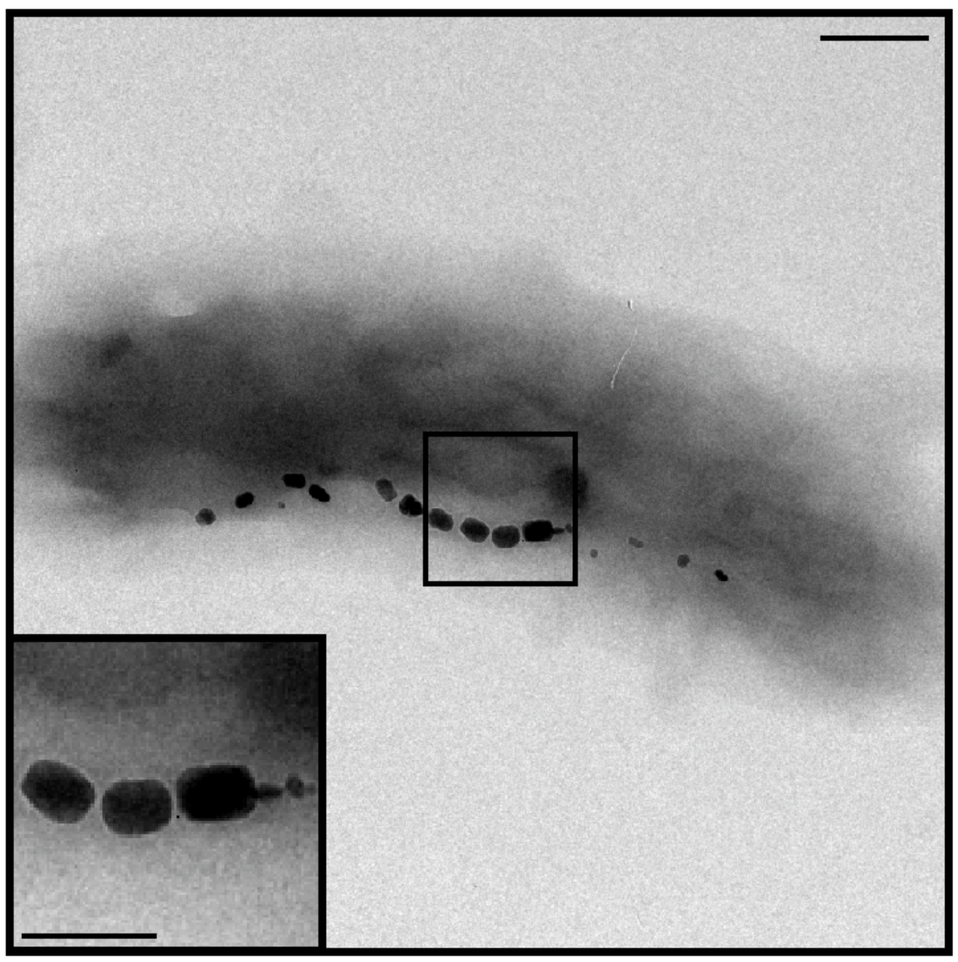

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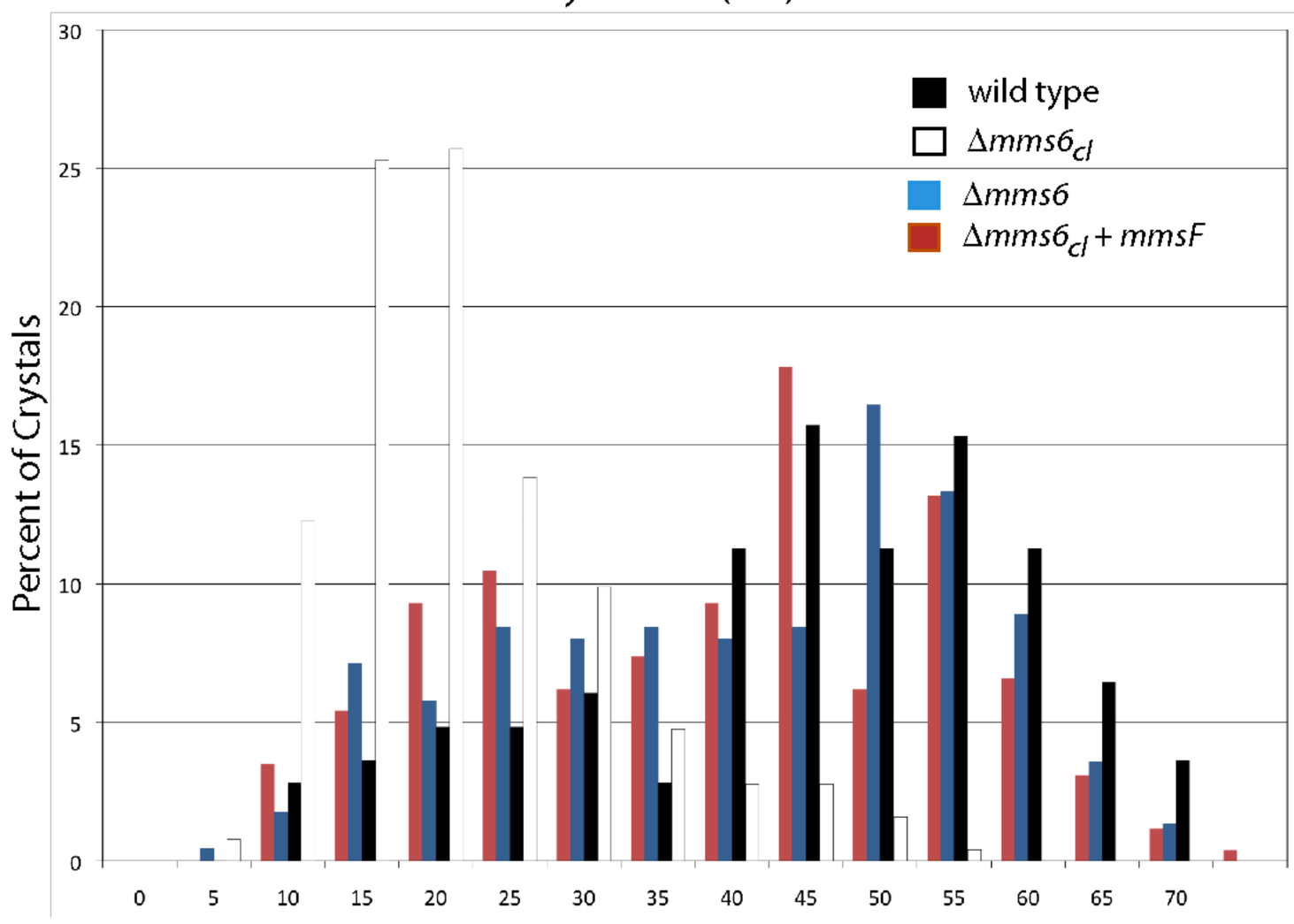


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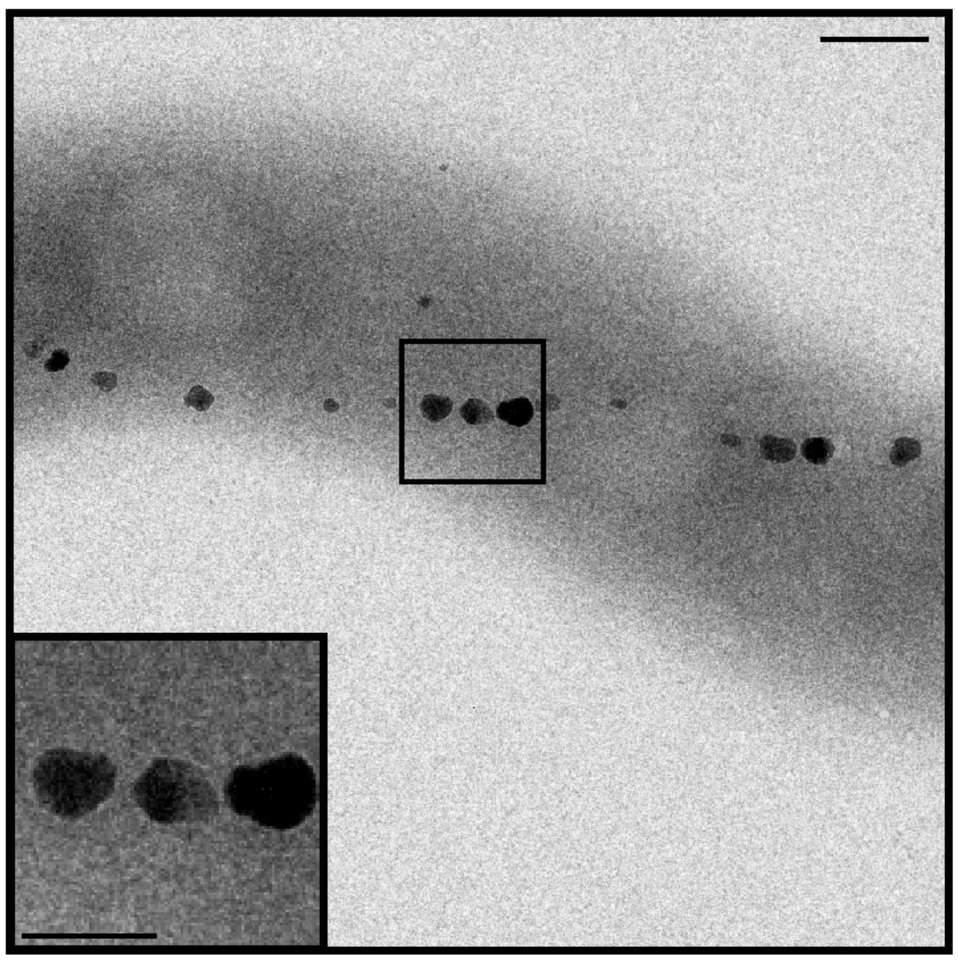

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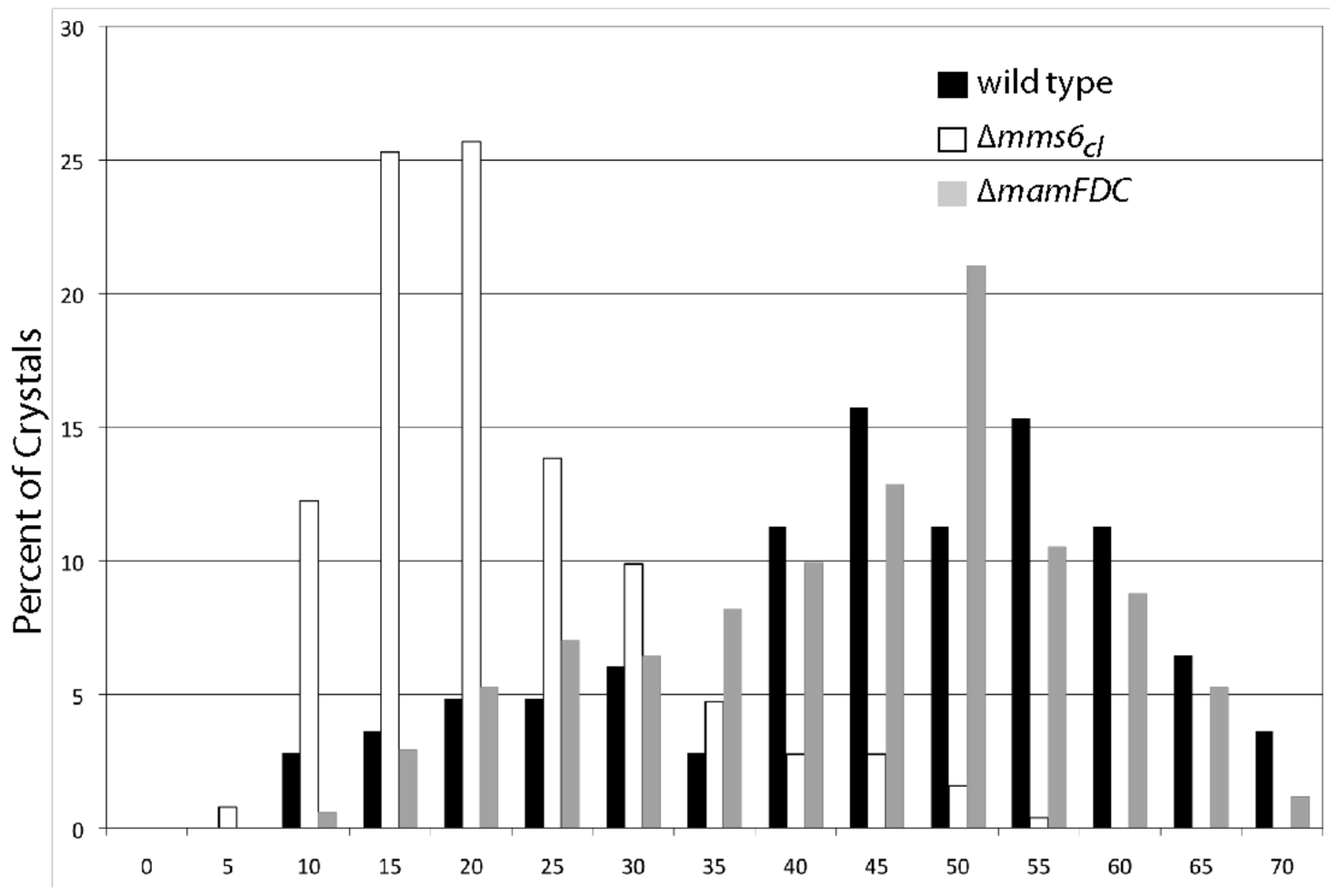
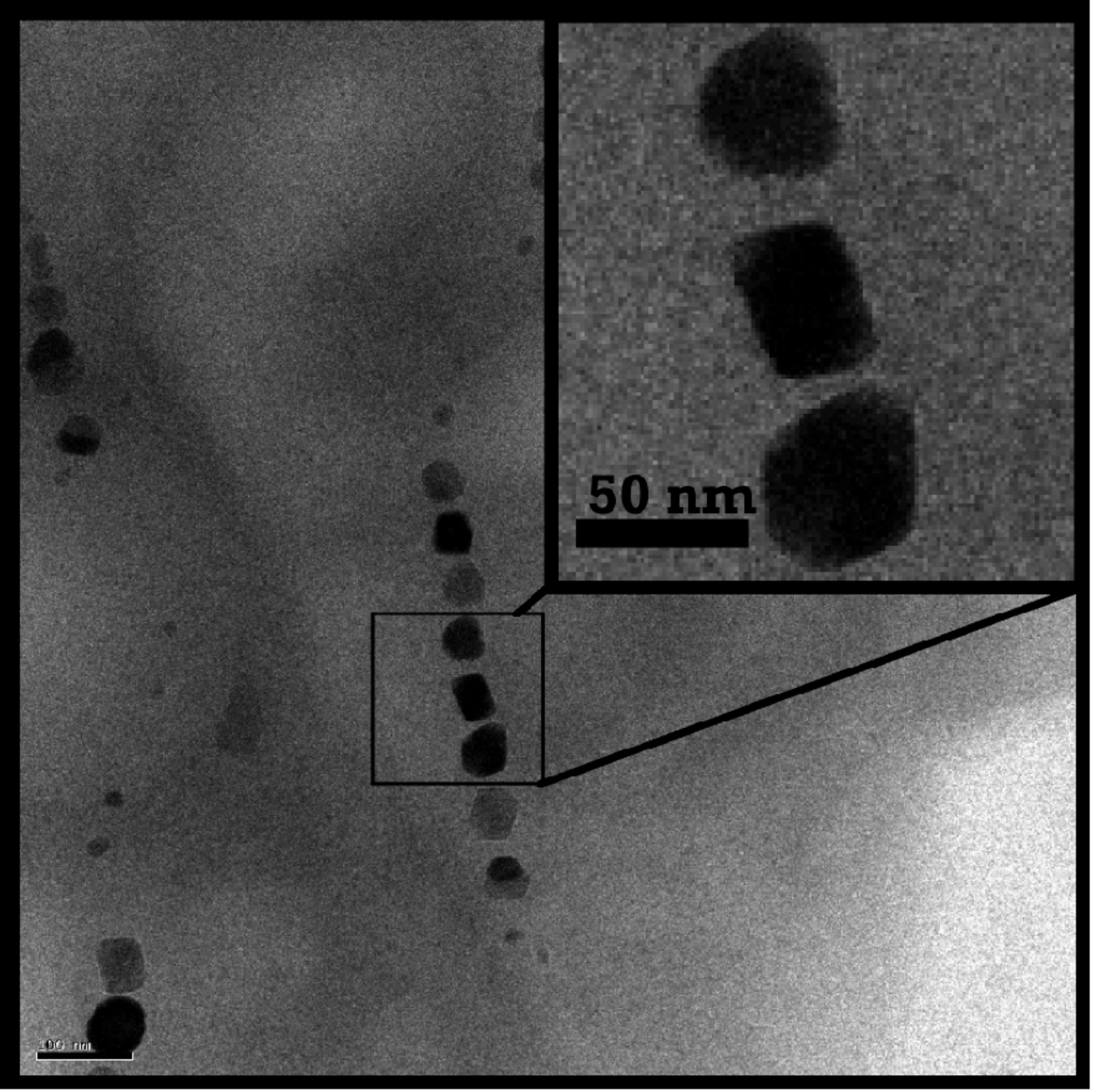
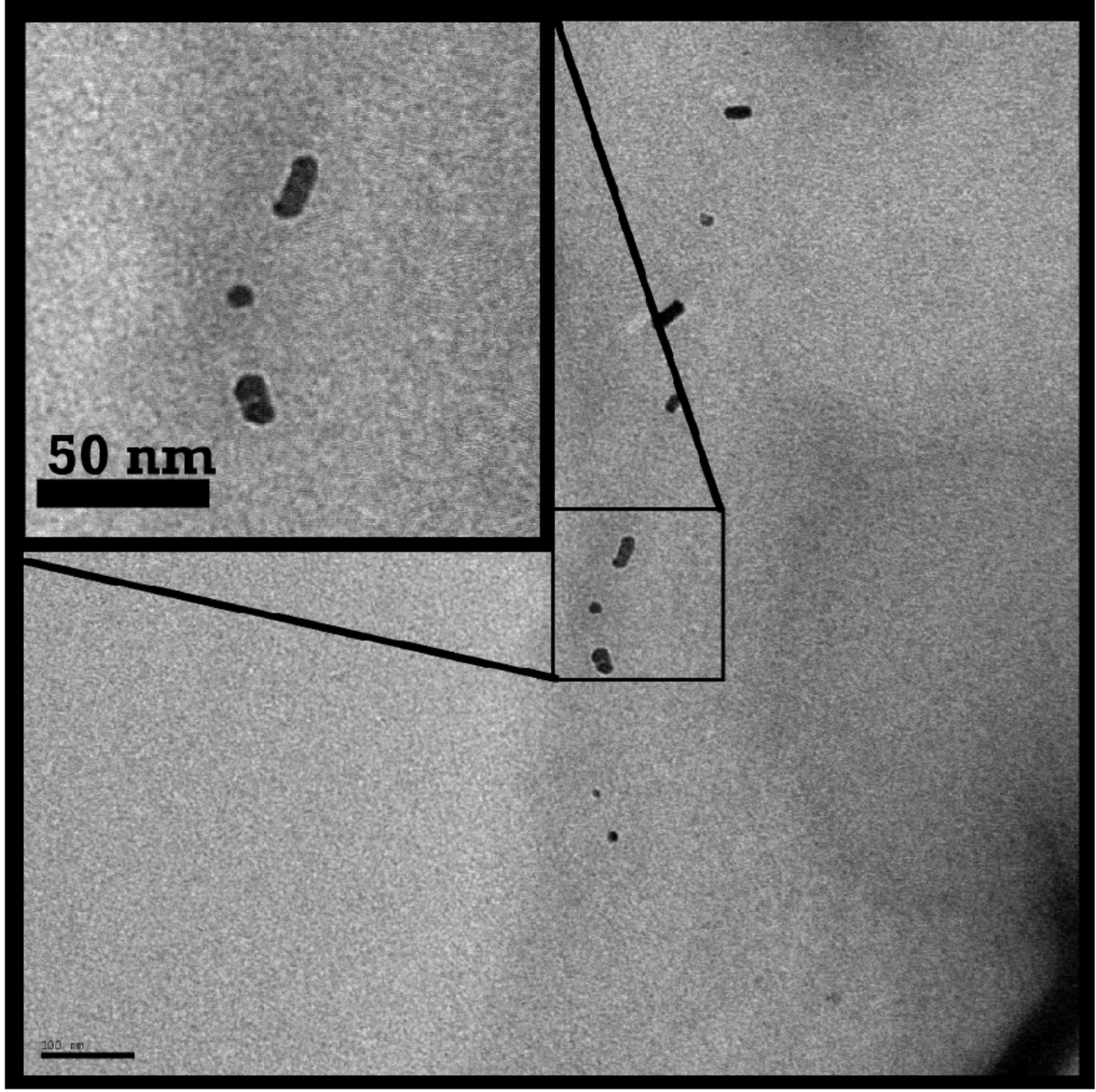





AMB-1




ΔR1-R4