Supporting Information

Control of methylglyoxal synthesis in *Bacillus subtilis*: Structural basis for the regulatory interaction of the methylglyoxal synthase MgsA with the carbon flux regulator Crh

Achim Dickmanns[1]*[§], Christopher P. Zschiedrich[2§], Johannes Arens[1], Iwan Parfentev[3, 4], Jan Gundlach[2], Romina Hofele[3, 4], Piotr Neumann[1], Henning Urlaub[3, 4], Boris Görke[2$], Ralf Ficner[1], and Jörg Stülke[2*]

[1] Department of Molecular Structural Biology, GZMB, Georg-August-University Göttingen, Germany
[2] Department of General Microbiology, GZMB, Georg-August-University Göttingen, Germany
[3] Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
[4] Bioanalytics Group, Institute for Clinical Chemistry, University Medical Center Göttingen, Germany

[§] These authors contributed equally

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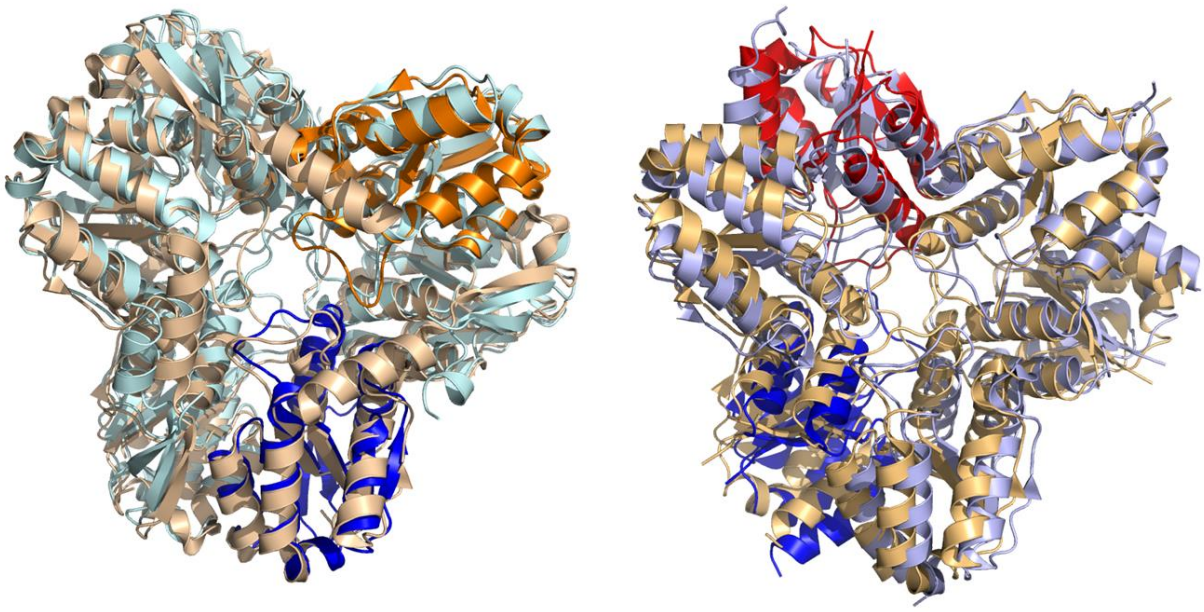

Figure S1. The Methylglyoxal synthases form hexamers of identical arrangement in crystal structures. Shown are the hexamers with one monomer depicted in a more prominent color. Left panel: *E. coli* Mgs (PDBid: 1B93; pale cyan/ blue), *Thermotoga maritima* (PDBid: 1VMD; wheat), and right panel: *Thermus thermophilus* (PDBid: 1WO8; light orange/ red) and *Thermus spec.* (PDBid: 2X6W; light blue/ blue).

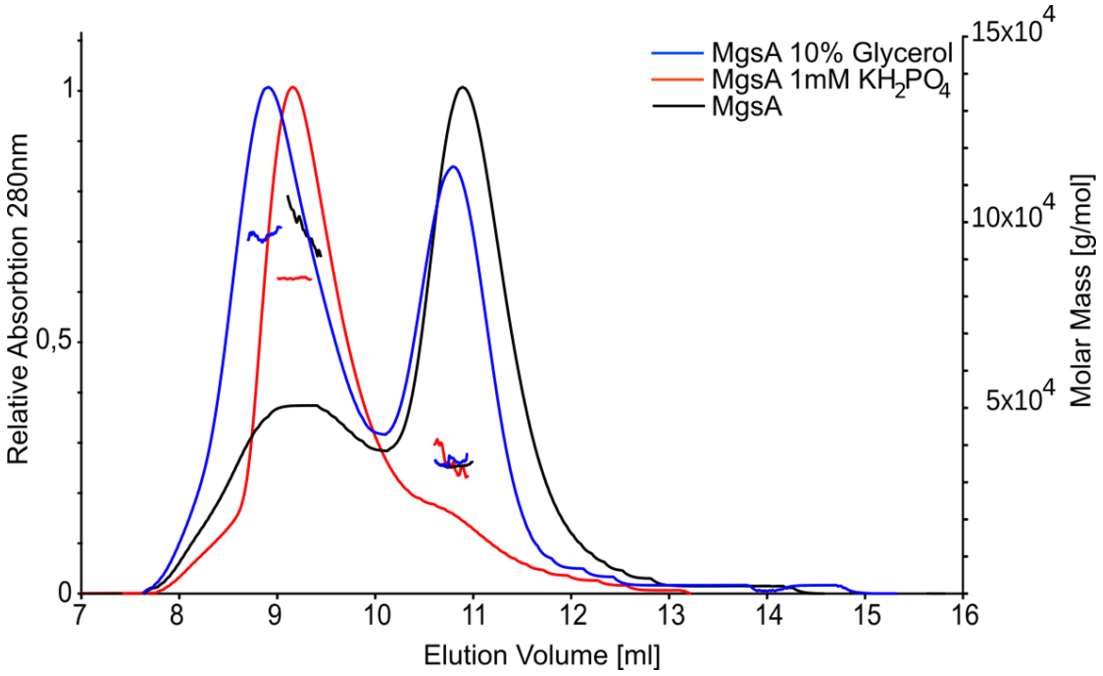

Figure S2. The formation and stability of the MgsA hexamer depends on their environment. Thawed MgsA was analyzed by SEC-MALS using different buffers as indicated. The addition of Pi or Glycerol shifts the hexamer:dimer ratio of MgsA from about roughly 30:70 (buffer only) to 70:30 or 90:10, respectively.

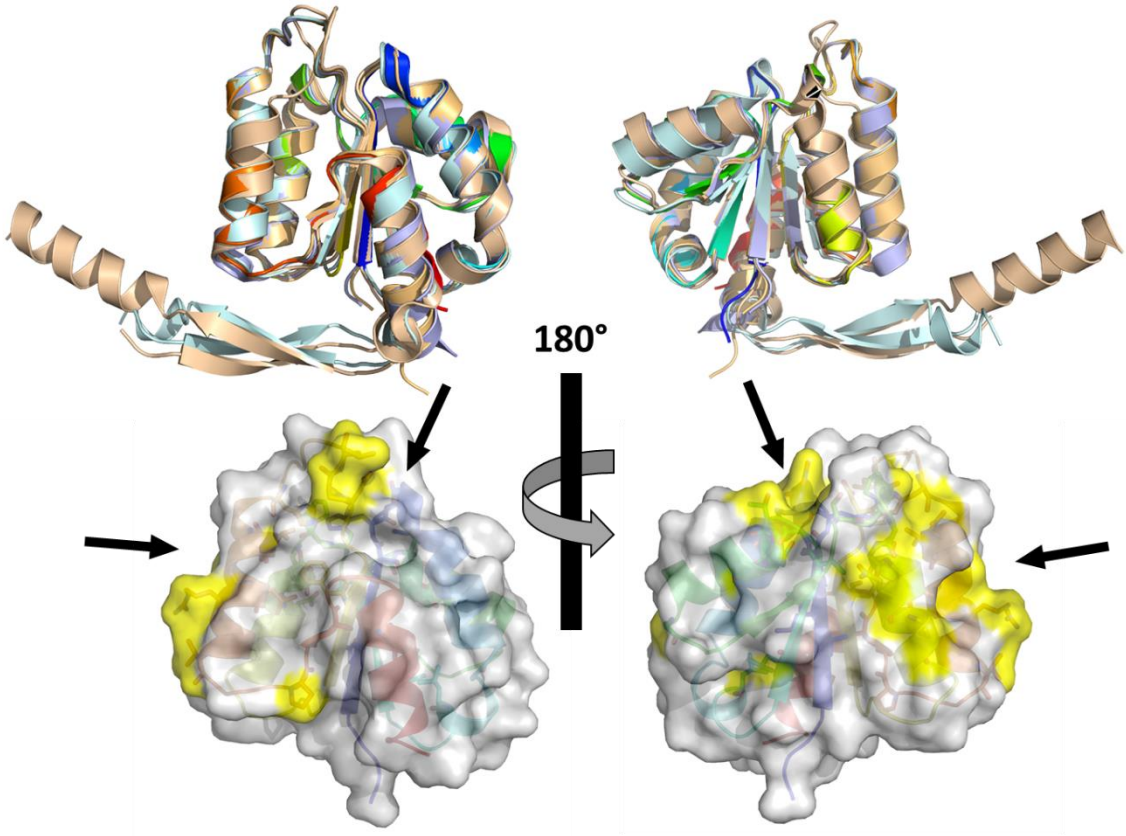

Figure S3. The MgsA overall-fold is identical to other Methylglyoxal synthases (Mgs). This allows to deduce identical residues and the active site region. Top panels. The superimposition reveals an identical arrangement of the secondary structure elements for MgsA from *B. subtilis* (rainbow coloring from blue to red (N- and C-terminus, respectively)) and the methylglyoxal synthases from *E. coli* (PDBid: 1B93; pale cyan), *Thermotoga maritima* (PDBid:1VMD; wheat), *Thermus thermophilus* (PDBid: 1WO8; light orange) and *Thermus spec.* (PDBid: 2X8W; light blue). Bottom panels. bsMgsA oriented and colored as in top panel with surface representation added. Identical residues in all four structures known reveal two patches of high identity one on the side and one on top of the molecule (indicated by arrows). See text for details.

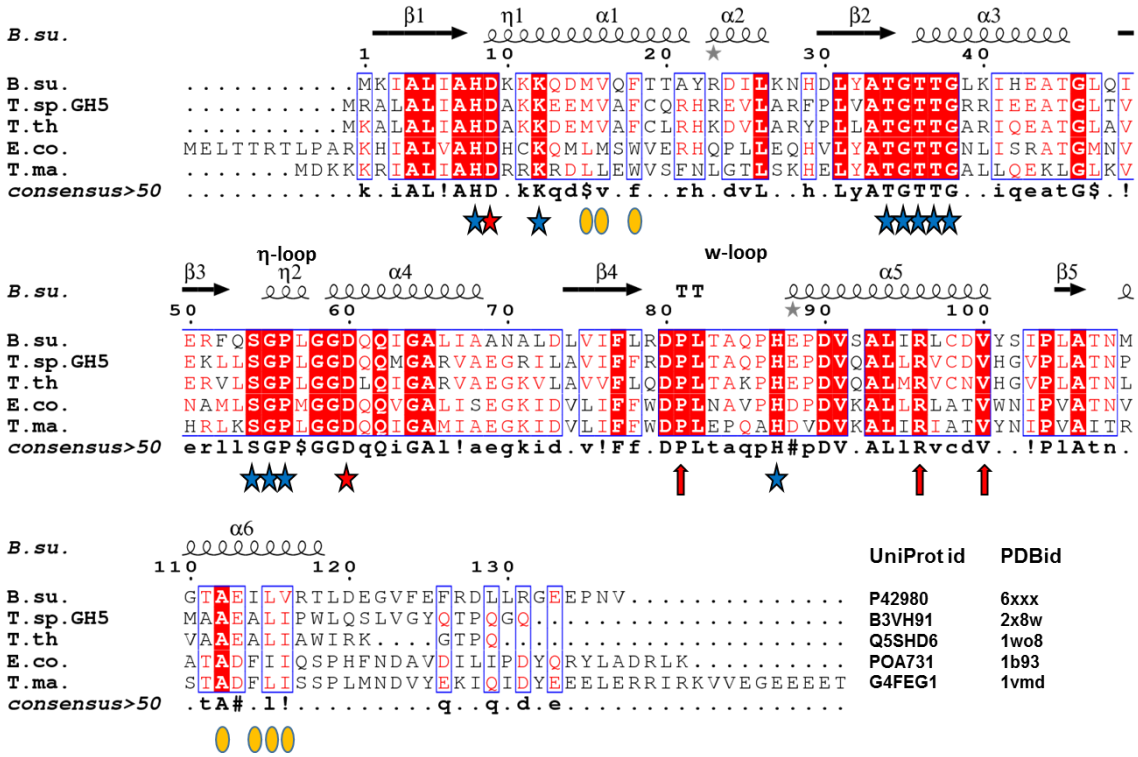

Figure S4. Structure based sequence alignment of MgsA reveals conserved residues. MgsA from *B. subtilis* was aligned with Mgs from *E. coli* (PDBid:1B93) *Thermotoga maritima* (PDBid:1VMD), *Thermus thermophilus* (PDBid:1WO8) and *Thermus spec.* (PDBid: 2X8W). The secondary structure element and their numbering assignment is according to *B. subtilis* MgsA. Active site residues deduced from *E. coli* MgsA are highly conserved (marked by blue stars) and additionally involved in catalysis (marked by red stars) are indicated. Light orange ellipsoids indicate conserved hydrophobic residues found in Crh docking simulations. Red arrows indicate highly conserved residues involved in an allosteric pathway (See main text for details).

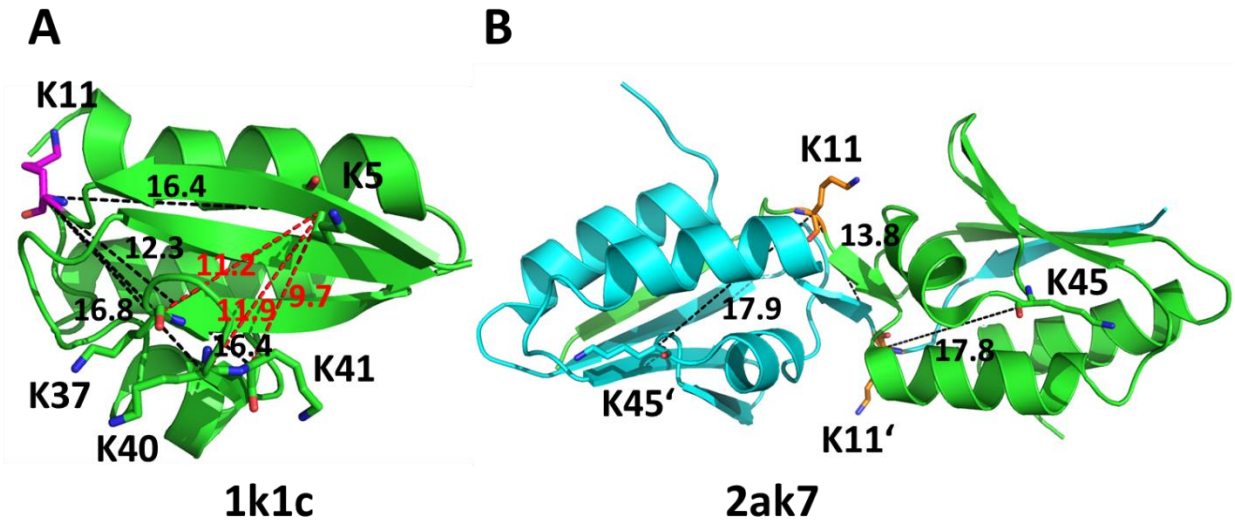

Figure S5. Intra- and intermolecular crosslinks found in Crh and their respective distances. The structures show either (**A.**) a monomer (PDBid: 1K1C) or (**B.**) a homodimer (PDBid: 2AK7) with the lysines labelled and represented in stick mode. The connections are indicated as red or blue (for K11-K40) dashed lines and the corresponding distances between the Cα atoms are shown.

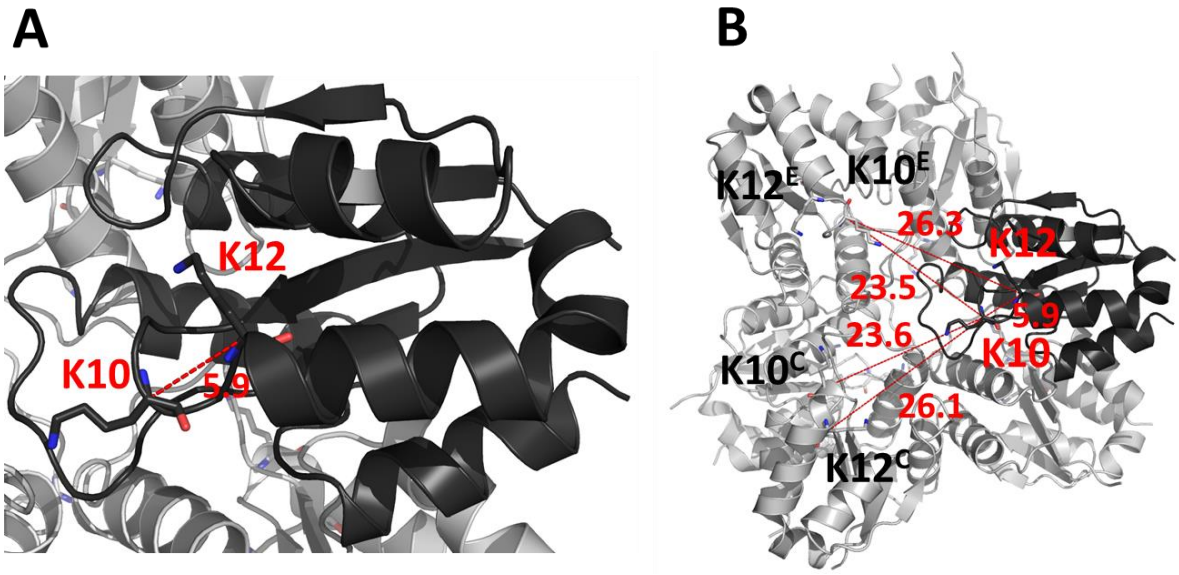

Figure S6. Intra- and intermolecular crosslinks found in MgsA and their respective distances. The structures show the hexamer with the lysines represented in stick mode and labelled accordingly. The connections are indicated as red dashed lines and the corresponding distances are shown. **A.** Intramolecular crosslinks with the Lysines labelled and colored in orange. **B.** overall view of the hexamer displaying the longer distance intra and intermolecular crosslink between Lys-2 (colored in red) and Lys-12.

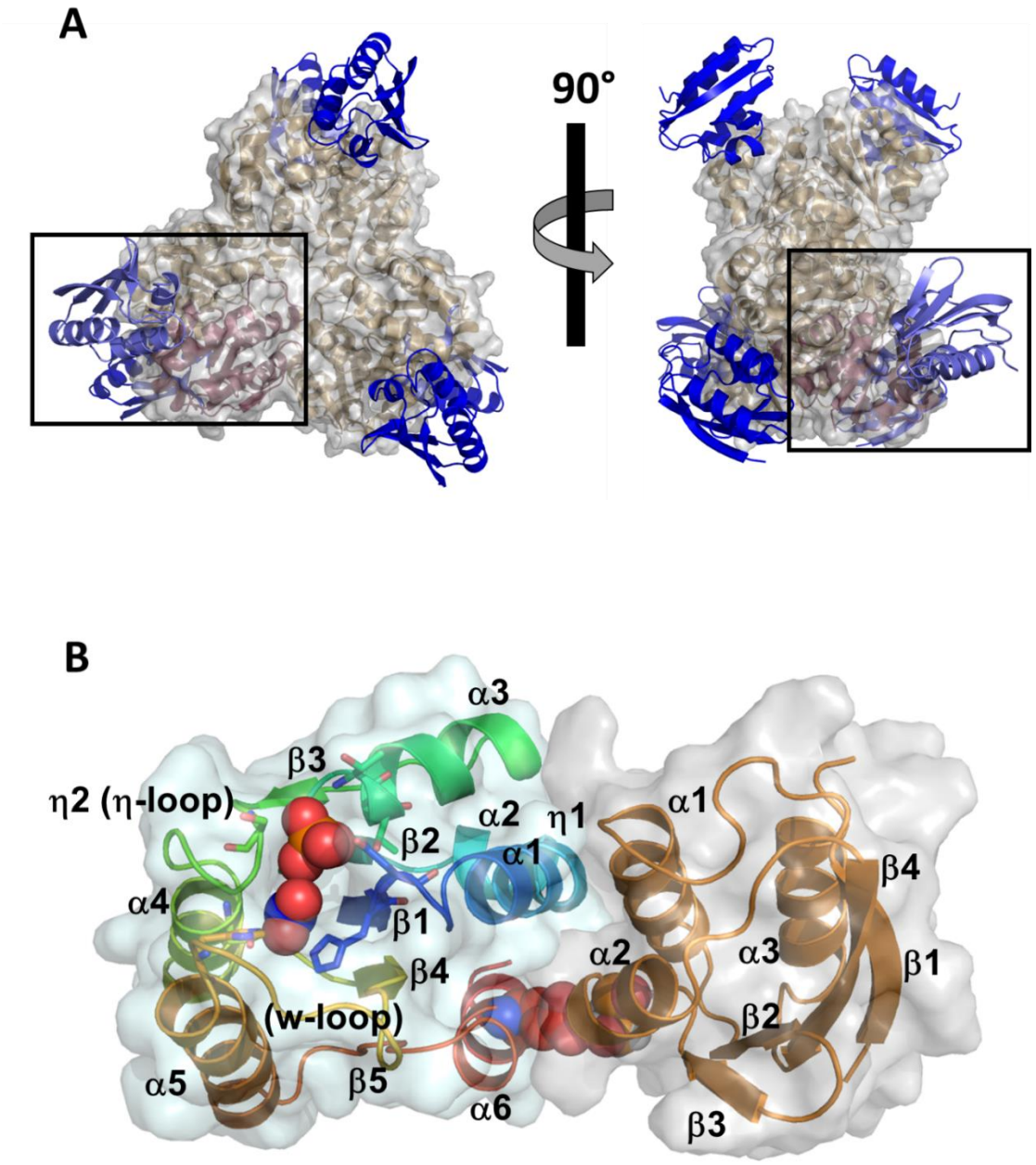

Figure S7. Interaction of Crh and MgsA as obtained by blind docking experiments. A. Dimer formation of MgsA and Crh as obtained does not interfere with hexamer formation. Two views (top and side) of the MgsA hexamer with six Crh molecules bound are shown. The individual MgsA molecules and their bound Crh molecules are colored identically. **B.** Detailed presentation of the MgsA-Crh interaction. MgsA is depicted in rainbow coloring with a cyan surface, whereas Crh is presented in orange with the surface in grey. The individual secondary structure elements are indicated, as well as the active site residues (in stick mode) and ions (formate and phosphate) in the active site cavity in sphere mode (overlaid from PDBid: 1B93).

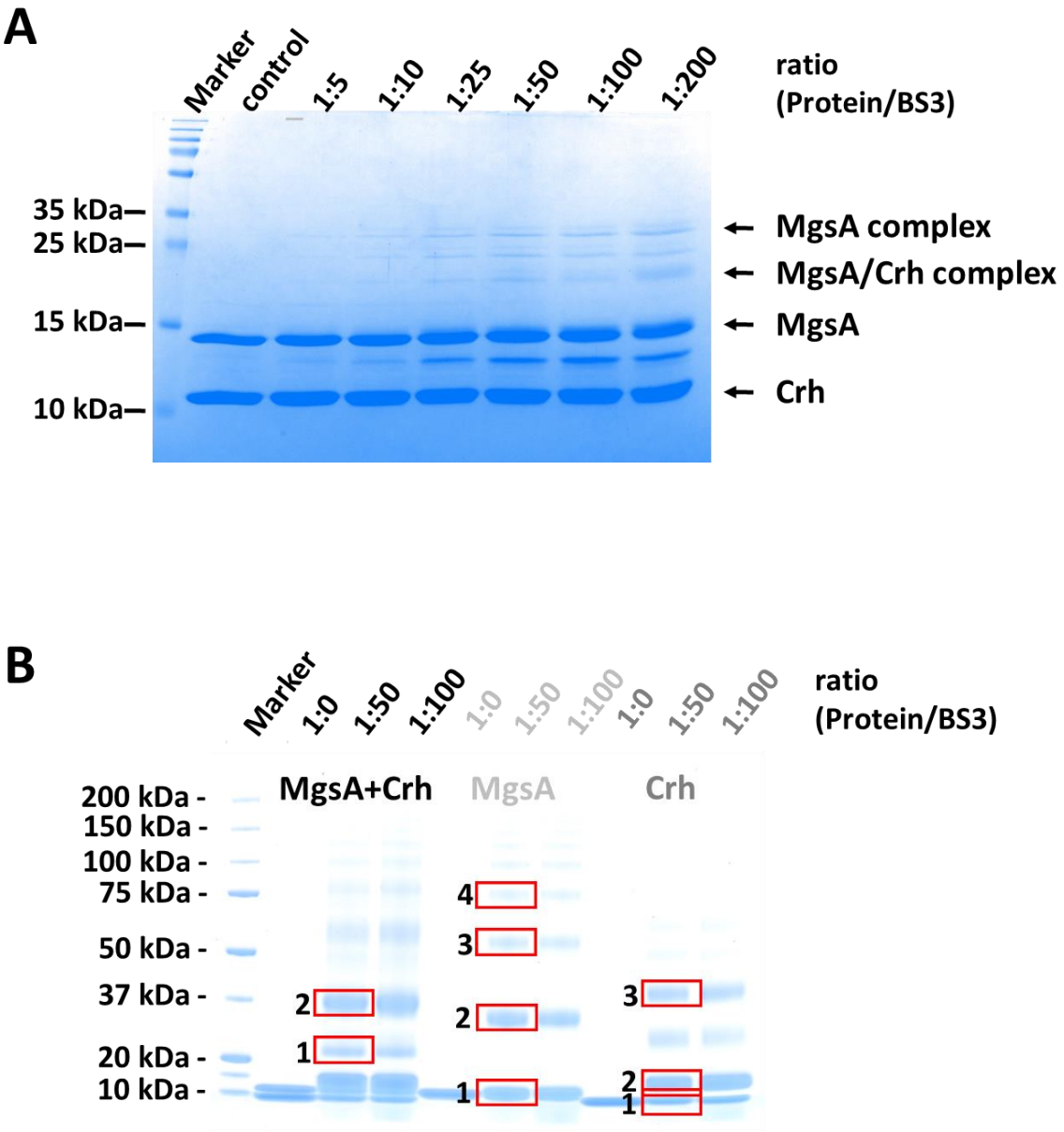

Figure S8. Determination of cross-linking conditions. A *In vitro* chemical cross-linking of MgsA and Crh. The complex of MgsA and Crh was treated with increasing amounts of BS3 to determine the optimal protein:cross-linker ratio. According to the size and a Western blot, bands that represent the complex of MgsA and/ or MgsA-Crh were analyzed by LC-MS. **B**. *In vitro* chemical cross-linking of MgsA and Crh either alone or in a complex formed in a 1:1 ratio. The samples were treated with the optimal amounts of BS3 using a 1:50 and 1:100 protein:cross-linker ratio. The bands analyzed by MS are indicated by red boxes.