Switch I-dependent allosteric signaling in a G-protein chaperone–B_{12} enzyme complex

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G-proteins regulate various processes ranging from DNA replication and protein synthesis to cytoskeletal dynamics and cofactor assimilation and serve as models for uncovering strategies deployed for allosteric signal transduction. MeaB is a multifunctional G-protein chaperone, which gates loading of the active 5′-deoxyadenosylcobalamin cofactor onto methylmalonyl-CoA mutase (MCM) and precludes loading of inactive cofactor forms. MeaB also safeguards MCM, which uses radical chemistry, against inactivation and recovers MCM inactivated during catalytic turnover by using the GTP-binding energy to offload inactive cofactor. The conserved switch I and II signaling motifs used by G-proteins are predicted to mediate allosteric regulation in response to nucleotide binding and hydrolysis in MeaB. Herein, we targeted conserved residues in the MeaB switch I motif to interrogate the function of this loop. Unexpectedly, the switch I mutations had only modest effects on GTP binding and on GTase activity and did not perturb stability of the MCM–MeaB complex. However, these mutations disrupted multiple MeaB chaperone functions, including cofactor editing, loading, and offloading. Hence, although residues in the switch I motif are not essential for catalysis, they are important for allosteric regulation. Furthermore, single-particle EM analysis revealed, for the first time, the overall architecture of the MCM–MeaB complex, which exhibits a 2:1 stoichiometry. These EM studies also demonstrate that the complex exhibits considerable conformational flexibility. In conclusion, the switch I element does not significantly stabilize the MCM–MeaB complex or influence the affinity of MeaB for GTP but is required for transducing signals between MeaB and MCM.

MeaB from Methylobacterium extorquens is a member of the G3E group of SIMBI P-loop G-proteins, which include several metallochaperones (1). MeaB functions as a chaperone for methylmalonyl-CoA mutase (MCM), regulating cofactor loading and offloading in a nucleotide-dependent manner (2–4). 5′-Deoxyadenosylcobalamin (AdoCbl) serves as a cofactor for MCM, which is a B_{12}-dependent isomerase (5, 6). In mammals, the tissue concentration of cobalamins is low, and formation of the active MCM holoenzyme relies on a B_{12} trafficking pathway that assimilates and inserts AdoCbl posttranslationally (7–9). Mutations in the B_{12} trafficking pathway that lead to a functional deficiency of AdoCbl or affect MCM itself result in methylmalonic aciduria, an inborn error of metabolism that is inherited as an autosomal recessive disorder (10–14).

MeaB gates the transfer of AdoCbl to MCM from adenosyltransferase, the enzyme that synthesizes this active cofactor form (Fig. 1) (15, 16). GTP hydrolysis by MeaB controls the AdoCbl transfer process and precludes loading by the inactive cob(II)alamin form (3). Once loaded with AdoCbl, MCM catalyzes the radical-based isomerization of methylmalonyl-CoA to succinyl-CoA. Inadvertent dissociation of the 5′-deoxyadenosine moiety from the active site precludes reformation of AdoCbl at the end of the catalytic cycle and results in inactivation of MCM (3). MeaB plays additional roles following cofactor loading. It protects MCM from inactivation, slowing escape from the turnover cycle ∼30-fold (2, 17), and promotes ejection of inactive cob(II)alamin when it forms during the catalytic cycle (3). Although MeaB has been studied quite extensively (2–4, 18), the human ortholog, CblA, is poorly characterized (19, 20). Nearly 40 pathogenic mutations have been identified in CblA that are associated with methylmalonic aciduria (12, 21).

MeaB exists as a homodimer and binds MCM with high affinity (K_{D} = 34–525 nM), which varies depending on the presence or absence and the identity of the ligands bound to the two proteins (4). In contrast, the metallochaperones CooC and HyPB, which catalyze the insertion of nickel into carbon monoxide dehydrogenase and [Ni-Fe]-hydrogenase, respectively, undergo NTP-dependent dimerization (22–24).

Like most G-proteins, MeaB exhibits low intrinsic GTase activity (4). In complex with MCM, the GTase activity of MeaB is enhanced ∼100-fold. Hence, MCM functions as a GTase-activating protein (GAP) for MeaB (4). Like other G-proteins, MeaB contains the signature switch I/II elements (Fig. 2A), which are important for catalysis and signaling to downstream effector proteins (25). Residues in the switch I/II

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motifs provide some of the ligands to the Mg$^{2+}$ ion and undergo conformational rearrangements in response to nucleotide binding, exchange, and hydrolysis. In the so-called “loaded spring” mechanism, direct H$_2$O-mediated or Mg$^{2+}$-mediated contacts form between the switch motif residues and the γ-phosphate of GTP and are disrupted upon hydrolysis. Besides the switch I/II elements, MeaB and its homologs have an additional mobile loop called switch III (residues 178–188), which is distant from the GTP-binding site (Fig. 2A). Switch III has been captured crystallographically in multiple conformations (17). Mutations localizing in the switch III loop have been identified in methylmalonic aciduria patients (27) and compromise the fidelity of AdoCbl loading/offloading processes and alleviate the protective effect on MCM during turnover, making it more susceptible to inactivation (17). GTP hydrolysis triggers conformational changes in the switch I/II loops that are propagated to switch III via switch II (17).

The switch II element (residues 154–162) plays an additional role in suppressing the intrinsic GTPase activity of MeaB (28). Val$^{156}$ and Val$^{158}$ in switch II are postulated to impede access of the water-activating general base (Asp$^{92}$ as discussed below). Glu$^{154}$ in switch II interacts with Arg$^{108}$ in switch I (Fig. 2B), and its mutation to alanine increases the intrinsic GTPase activity 10-fold and renders MeaB insensitive to GAP activation in the presence of MCM.

The role of the switch I sequence (residues 92–108) in the chaperone function of MeaB is not known. The switch I sequence, D$^{92}$,D$^{105}$KTR$^{108}$, is not strongly conserved and resides on an extended loop (Fig. 2A). An identical D$^{180}$,D$^{193}$KTR$^{196}$ sequence is found in the human homolog, CblA (Fig. 2C). The crystal structures of both MeaB and CblA indicate that they do not represent catalytically active conformation. Despite the presence of nucleotides in some MeaB structures, Mg$^{2+}$ is absent from all of them, and the switch I residue Asp$^{92}$, which is predicted to activate the nucleophilic water for attack on the γ-phosphorus, is displaced 11–14 Å from the active site (17, 28, 29). We have postulated that the active site of MeaB is brought into catalytic register in the MCM–MeaB complex either via donation of critical residues from MCM and/or via a conformational rearrangement of residues in the MeaB active site (28). Interestingly, most of the switch I residues are not resolved in the CblA structure (Fig.

Figure 1. Nucleotide-gated transfer of AdoCbl from ATR to MCM. AdoCbl transfer is driven by the binding of ATP to a vacant site in the ATR homotrimer and gated by GTP hydrolysis catalyzed by MeaB. AdoCbl bound to ATR is in a five-coordinate “base-off” conformation ($\lambda_{\text{max}}$ = 458 nm) and switches to a six-coordinate “base-off/His-on” ($\lambda_{\text{max}}$ = 527 nm) conformation in the MCM active site.

Figure 2. Structure of MeaB and comparison of its active site with orthologous proteins. A, structure of MeaB protomer with GMPPNP bound (Protein Data Bank code 4JYB) in which the switch I (yellow), II (magenta), and III (green) elements are highlighted. B–D, close-ups of the active site in MeaB-GMPPNP (B), CblA-GDP (Protein Data Bank code 2WWW) (C), and IcmF-GDP-AdoCbl (Protein Data Bank code 4XC6) (D). Shown in stick representation in all panels are the bound guanosine nucleotides (blue). Magnesium cations are shown as gray spheres, and switch II residues, Val$^{156}$ in MeaB and Val$^{244}$ in CblA, are shown as magenta spheres. Conserved switch I residues are labeled in boldface font.
Table 1
Dissociation constants for GDP binding to MeaB switch I mutants

| Enzyme  | Site 1 | Site 2 |
|---------|--------|--------|
| Wild type | 0.2 ± 0.01 | 2.1 ± 0.2 |
| D92A    | 0.6 ± 0.2  | 4.6 ± 1.4  |
| D92N    | 0.4 ± 0.1  | 3.5 ± 0.9  |
| D105A   | 0.07 ± 0.02 | 0.8 ± 0.2  |
| K106A   | 1.3 ± 0.6  | 7.2 ± 1.9  |
| R108A   | ND      | ND      |

Table 2
Kinetic parameters for GTP hydrolysis by switch I mutants in MeaB

| Enzyme  | kcat/M | kcat/min M | -Fold GAP activation |
|---------|--------|------------|----------------------|
| Wild type | 0.039 ± 0.003 | 4.1 ± 0.20 | 105 |
| D92A    | 0.044 ± 0.006 | 0.87 ± 0.33 | 20 |
| D92N    | 0.12 ± 0.04 | 1.92 ± 0.46 | 16 |
| D105A   | 0.16 ± 0.04 | 3.7 ± 0.3 | 23 |
| K106A   | 0.07 ± 0.0 | 2.6 ± 0.2 | 37 |

* Determined previously (2).

GTPase activity of switch I mutants

Modest changes in the intrinsic GTPase activity were observed with all the mutants compared with wild-type MeaB (Table 2). Wild-type MeaB, in the presence of MCM, exhibits an ~100-fold increase in the kcat (4.1 ± 0.2 min⁻¹) for GTP hydrolysis (4). The GAP effect of MCM ranged from 16- (D92A) to 40-fold (K106A) activation for the switch I mutants. The magnitude of GAP activation for the D105A and D92N mutants was dampened by their higher intrinsic GTPase activity.

Switch I mutations protect MCM from inactivation

Inactivation of MCM during catalysis results in the formation of OH2Cbl, which can be distinguished from the active AdoCbl cofactor by its absorption peak at 351 nm (3). MCM is inactivated to a similar extent in the presence or absence of MeaB (Fig. 3A, B, and D). However, in the presence of GMP-PNP, MeaB affords some protection to MCM against inactivation (Fig. 3, C and D). The switch I mutants D92A, D92N, D105A, and K106A also protect MCM from inactivation (Fig. 3D). Hence, the switch I loop does not appear to be important in the allosteric protection of MCM against inactivation during turnover.

Switch I mutations uncouple GTPase activity from AdoCbl loading to MCM from ATR

Two nucleotide-dependent switches, ATP binding to ATR and GTP hydrolysis by MeaB, trigger AdoCbl transfer from ATR to the MCM–MeaB complex (Fig. 1) (15, 16). GTP hydrolysis by MeaB is essential for cofactor transfer, and in the presence of GMP-PNP, ~0.06 eq of AdoCbl is transferred to MCM–MeaB, whereas the rest is released into solution (Fig. 3E). Mutations in the switch I element uncouple GTP hydrolysis from AdoCbl transfer. Thus, in the presence of GMP-PNP, ~0.4 eq of AdoCbl is transferred from ATR to the MCM–MeaB complex containing the D92N or K106A mutant with the rest of the cofactor appearing in solution. An even greater fraction of AdoCbl transfer (~0.8 eq) is seen in the presence of the D92A or D105A mutant of MeaB. Hence, the switch I loop is important for interprotein signaling during GTpase-gated AdoCbl transfer from ATR to the MCM–MeaB complex.

Reactivation of MCM by switch I mutants

Accumulation of cob(I)alamin in the active site of MCM from which the 5'-deoxyadenosine moiety has been lost triggers the repair function of MeaB (3). This rescue function of MeaB specifically evicts cob(I)alamin but not the oxidized product, OH2Cbl. Addition of GMPPNP to the wild-type
cob(II)alamin-containing MCM–MeaB complex under anaerobic conditions triggers ejection of ~97% of the cofactor (Fig. 3F). The released cofactor can be quantified as OH2Cbl (at 351 nm) following separation from the protein using a Centricon filter under aerobic conditions. Addition of GMPPNP to the MCM–MeaB complex loaded with cob(II)alamin and MeaB harboring switch I mutations led to ejection of 51–66% of the cofactor (Fig. 3F). Hence, the switch I mutations impair GTP-dependent displacement of cob(II)alamin from MCM.

**EM studies on the MCM–MeaB complex**

The stoichiometry of the MCM–MeaB complex was estimated by size exclusion chromatography coupled to mult-angle light scattering (SEC-MALS). In the presence of GMPPNP, the wild-type complex elutes as a single monodisperse species that has an average molecular mass of ~359 kDa (Fig. 4A). This matches a 2:1 stoichiometry for MCM:MeaB, which has a predicted molecular mass of 352 kDa (MCM, 142 kDa per αβ heterodimer; MeaB, 71 kDa per α2 homodimer). The SEC-MALS chromatograms for complexes containing switch I MeaB mutants were identical to the wild-type complex. Hence, the mutations in the switch I loop do not cause significant perturbations in the gross architecture or stability of the MCM–MeaB complex. Therefore, perturbations in allosteric communication between MeaB and MCM, which impair its chaperone functions, are not due to destabilization of the complex.

Next, we analyzed the MCM–MeaB complex by negative-stain EM (Fig. 4B). Reference-free 2D class averages showed three distinct globular densities that appear flexibly connected. The outside densities are elongated and larger than the central density, indicating that these domains might be MCM dimers, which flank a central MeaB dimer. To test this assignment, crystal structures of the related bacterial MCM from *Propionibacterium shermanii* (34) and of MeaB (17) were compared by manual alignment based on the 2D averages without symmetry imposed and back-projected. The resulting 2D projections of these structures indeed match the arrangement in the 2D averages of the MCM–MeaB complex, supporting the proposed architecture (Fig. 4C).

The complex is dynamic and exists in a large array of conformations ranging from an “open” conformation where each MCM heterodimer is extended out from MeaB to one that is more “closed.” Some class averages also display a mixture of states with one MCM open and the other closed. However, the effect of this complex heterogeneity is not yet understood by these data. Overall, the EM analysis is consistent with a model of MCM–MeaB complex in which each GTP-binding site in the MeaB homodimer interacts with the active α-subunit in the αβ MCM heterodimer (Fig. 5A), giving rise to the observed 2:1 stoichiometry.

**Discussion**

The crystal structure of IcmF (30, 31) provides the best molecular level model for understanding allosteric signaling between MCM and MeaB. However, the organization of the IcmF structure is inherently different from that of the stand-alone MeaB and mutase proteins, highlighting the limitations...
of using IcmF as a predictive model for understanding the interaction between MeaB and MCM. A major organizational difference is that MeaB is a homodimer and forms a hub for interaction of two MCM heterodimers as indicated by our negative-stain EM data (Fig. 4B). In contrast, the two monomeric G-domain units in the IcmF homodimer are far apart and interact with the B12-domain and substrate-binding domain of the mutase but not with each other (Fig. 5, A versus B). Also unlike the M. extorquens MCM, which is an αβ homodimer containing a single B12− and substrate-binding site in the α-subunit (Fig. 5), each subunit in the IcmF dimer binds B12 and is active for loading. Hence, although the stoichiometry of the MCM–MeaB complex is 2:1, the stoichiometry of the mutase:G-domain in IcmF is 1:1. We predict that the human MCM–CblA complex will exhibit yet another organizational structure in which the α2 homodimer of MCM is organized around an α2 CblA core (Fig. 5C).

The IcmF structure reveals an extensive network of hydrogen bonds and salt bridges between the B12− and G-domains. The switch I loop (D249,D262RIR263) in the IcmF structure is posi-
Allosteric signaling in the MeaB–MCM complex

Figure 5. Schematic representations of the interaction between G-protein chaperones and client mutases. The G-domains are in blue, mutase substrate domains are in green, and the B12-domains are in red. The GTP-binding sites are shown as yellow spheres. The organization of the stand-alone or fusion proteins is shown above the known or predicted architecture of the complexes. A, the M. extorquens MCM only has one active site per αβ heterodimer. The inactive β-subunit is in gray. MeaB is an αε homodimer with two GTP-binding sites. B, IcmF is a fusion protein with AdoCbl, MeaI (G-domain), and substrate-binding domains fused in the direction of N to C terminus as shown. The crystal structure reveals that IcmF exists as a homodimer with the G-domains split into monomers, each interacting with an active α-subunit. C, human MCM is an αε homodimer with two active subunits. The organization of the human complex is proposed to be two MCMs to one CblA and is presently unknown.

M. extorquens MCM only has one active site per αβ heterodimer. The inactive β-subunit is in gray. MeaB is an αε homodimer with two GTP-binding sites. IcmF is a fusion protein with AdoCbl, MeaI (G-domain), and substrate-binding domains fused in the direction of N to C terminus as shown. The crystal structure reveals that IcmF exists as a homodimer with the G-domains split into monomers, each interacting with an active α-subunit. Human MCM is an αε homodimer with two active subunits. The organization of the human complex is proposed to be two MCMs to one CblA and is presently unknown.

The switch I residue, Lys106 in MeaB, is equivalent to Arg263 in IcmF that forms a salt bridge with Glu76 derived from the B12-domain (Fig. 2D). The functional significance of this inter-domain interaction is not known. In the various structures of MeaB, Lys106 is either positioned on a disordered loop, solvent-exposed, or hydrogen-bonded to the carbonyl groups of Leu101 in switch I or Ile120 on the β3 sheet (17, 28, 29). The K106A mutant exhibits the largest GAP activation of all switch I mutants studied, indicating that it is the least impacted (Table 2).

In contrast to their modest effects on the GAPase activity, the switch I mutants exhibit significant effects on the following chaperone functions: GAPase-gated AdoCbl loading onto MCM from ATR and cob(II)alamin offloading from MCM. For example, although the K106A mutation has very modest effects on the intrinsic GAPase activity of MeaB and on GAP activation by MCM, it has a drastic effect on the chaperoning functions of MeaB (Fig. 3, E and F). The surface exposure of Lys106 and the adverse impact of the K106A mutation on gating AdoCbl loading and supporting cob(II)alamin off-loading suggests that this residue, like the others in the switch I domain, is important for communicating between MeaB and the B12-binding domain of MCM.

In summary, our results lead us to the unexpected conclusion that the conserved switch I residues are not essential for the GAPase activity of MeaB or for transmitting the GAP activity of MCM. They do, however, profoundly impair communication between ATR and the MCM–MeaB complex during AdoCbl loading and between MCM and MeaB during cob(II)alamin off-loading. Unlike IcmF, ATR is not required during off-loading inactive cofactor from MCM (41). Together with our previous studies, we conclude that the switch I, II, and III elements do not contribute significantly to stabilizing the MCM–MeaB complex or influence the affinity of MeaB for GTP. Furthermore, the switch elements are not significantly involved in protecting MCM against inactivation. Instead, they are collectively important for transducing signals between MeaB and MCM, and mutations in any of the switch elements impact the same chaperone functions that ensure the fidelity of loading active cofactor and allow off-loading of inactive cofactor.

G-proteins and ATPases are evolutionarily diverse regulators of varied functions ranging from DNA replication and protein synthesis and localization to cytoskeletal dynamics and coenzyme trafficking and assimilation (35, 42, 43). Studies on these proteins are uncovering multiple strategies by which they transduce allosteric signals. MeaB, a prototype of the G3E metallochaperone subfamily, with its atypical active site and an auxiliary switch III element amplifies the diversity of the catalytic and signaling strategies used by G-proteins. A high-resolution structure of MeaB in complex with MCM will provide needed insights into how a catalytically competent MeaB active site is constructed.

Experimental procedures
Materials
AdoCbl, GMPPNP, ATP, GTP, GDP, methylmalonic acid, coenzyme A, and other reagent grade chemicals were purchased from Sigma. Trifluoroacetic acid was purchased from...
Purification of MeaB, ATR, and MCM

Recombinant M. extorquens MeaB, MCM, and ATR were expressed and purified from E. coli BL-21(DE3) as described previously (3, 16). The purified enzymes were flash frozen in liquid nitrogen and stored at −80 °C in 50 mM HEPES buffer, pH 8.0, containing 0.3 M KCl, 10 mM MgCl₂, and 10% glycerol (Buffer A).

Thermodynamic analysis of GDP binding

Isothermal titration calorimetry experiments were performed at 10 °C in Buffer A using a 300-μl injection syringe and a 1.43-ml injection cell. Samples were prepared by filtration through a 0.2-μm filter and then degassed under vacuum at 4 °C using a ThermoVac sample degasser. Each titration was performed at least in duplicate. GDP (10-μl injections of 150–400 μM) was added to 15–20 μM MeaB. The data were analyzed for a two-site binding model using the MicroCal Origin program.

Protection of MCM from inactivation by MeaB

Inactivation of MCM under steady-state turnover conditions was examined by enzyme-monitored conversion of AdoCbl to OH₂Cbl (formed via oxidation of the cob(II)alamin intermediate) at 20 °C in 0.1 M potassium phosphate, pH 7.5, containing 10 mM MgCl₂. The reactions and sample preparations were performed in the dark to avert spurious formation of OH₂Cbl by photolysis of AdoCbl. Samples were prepared by the addition of reaction components in the order described below. MCM (25–30 μM) was reconstituted using an equimolar concentration of AdoCbl. A molar excess (35–40 μM) of MeaB was added to the MCM holoenzyme to obtain the MCM–MeaB complex. GMPPNP was then added to the reaction mixture to a final concentration of 1–2 mM, and the reaction was initiated by the addition of methylmalonyl-CoA to a final concentration of 4.5–5 mM. The change in absorbance at 351 nm was determined between t = 70 and 0 min and was used as a measure of the degree of cofactor inactivation.

Allosteric signaling in the MeaB–MCM complex

Transfer of AdoCbl from ATR to MCM in complex with MeaB

The ATP-dependent transfer of AdoCbl from ATR to the MCM–MeaB–GMPPNP complex was performed in the dark at 20 °C and monitored by UV/visible spectroscopy. Two equivalents of AdoCbl were added to 1 eq of ATR to generate holo-ATR in Buffer A. The apo-MCM–MeaB–GMPPNP complex was reconstituted in Buffer A by mixing 40–50 μM MCM with 50–60 μM MeaB. GMPPNP was added to a final concentration of 1 mM. Holo-ATR (2:1 AdoCbl:ATR trimer) and the MCM–MeaB–GMPPNP complex (40–50 μM) were mixed and incubated for 10 min at 20 °C before addition of ATP to a final concentration of 5 mM. Release/transfer of AdoCbl from ATR was calculated using Δε₅₂₅ = 6.69 mM⁻¹ cm⁻¹. Bound versus free cofactor was separated using an Amicon centrifuge filter (10-kDa cutoff; 20 min at 4 °C at 16,000 × g). The concentration of free AdoCbl in the filtrate was calculated using ε₅₂₅ = 8.0 mM⁻¹ cm⁻¹.

Ejection of Cob(II)alamin by switch I mutants

MCM (30–40 μM) was mixed with 45–60 μM MeaB (wild type, D92A, D92N, D105A, or K106A) in Buffer A at 20 °C under strictly anaerobic conditions such that the MCM:MeaB ratio was 1:1.5. Cob(II)alamin was generated by reduction of OH₂Cbl with tris(2-carboxyethyl)phosphine hydrochloride and was added to a final concentration equal to that of MCM. The reaction mixture was incubated for 10 min at 20 °C. GMPPNP in anaerobic Buffer A was added to a final concentration of 2 mM. The mixture was then incubated for 20 min at 20 °C. Subsequently, the sample was air-oxidized for 2 h and then applied to a Centricon YM10 filter (10-kDa cutoff) to separate free from bound OH₂Cbl. Cob(II)alamin (but not OH₂Cbl) is released from MCM in the presence of MeaB-GTP and is subsequently oxidized to OH₂Cbl, which was quantified using ε₅₃₅ = 9.3 mM⁻¹ cm⁻¹.

GTPase activity of MeaB

The GTPase activity of MeaB was determined using an HPLC assay as described previously (2). The activity assays were performed at two GTP concentrations (2.5 and 5 mM), which represent a saturating concentration. The kₘ values were determined using 20 μM wild-type or mutant MeaB. The kₐ values for GTPase activity in MCM–MeaB complexes were determined using 2.5 μM MeaB (wild type or mutant) and 10 μM MCM.

SEC-MALS analysis of MCM–MeaB complexes

Purified MeaB (25 μM; mutant or wild type) and MCM (25 μM) proteins in Buffer A were mixed with GMPPNP (250 μM) and allowed to equilibrate for 15 min on ice. Next, 50 μl of this sample was injected onto a silica-based size exclusion column (model 0505S, Wyatt Technology, Santa Barbara, CA) pre-equilibrated with 50 mM HEPES, 0.3 M KCl, 3 mM MgCl₂, pH 8.0, connected to an FPLC system and coupled to a MALS detector (Dawn Heleos II, Wyatt Technology).

Negative-stain electron microscopy

The MCM–MeaB complex sample was taken directly from the SEC-MALS column, diluted 1:30 with the column buffer
onto carbon-coated grids, and stained with uranyl formate (1%, w/v). Negative-stain micrographs were taken at 52,000× magnification with 2.16 Å per pixel using a 4000 × 4000 charge-coupled device camera (Gatan, Pleasanton, CA) on a G2 Spirit transmission electron microscope (FEI, Hillsboro, OR) operated at 120 kV. From 233 micrographs, 18,747 single particles were selected manually using E2boxer (EMAN2 (33)). The total particle set was classified into reference-free 2D class averages using iterative stable alignment and classification using 20 initial iterations and three-way matching. This process generated 200 stable classes composed of 14,884 single particles, which represented ~80% of all particles. Models of 2D projections were generated using UCSF Chimera to position MCM (Protein Data Bank code 1REQ) and MeaB (Protein Data Bank code 4JYB) relative to iterative stable alignment and classification class averages.

Author contributions—G. C. C. and M. L. designed, performed, and analyzed the biochemical experiments. A. L. Y. performed negative-stain EM experiments and analyzed EM data. D. R. S. conceived EM experiments and analyzed EM data. R. B. helped conceive the experiments and analyzed the data. All authors contributed to writing the manuscript and approved the final version.

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