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Supplementary Materials for

Itaconyl-CoA forms a stable biradical in methylmalonyl-CoA mutase and derails its activity and repair

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Materials

All reagents used were purchased from Sigma Aldrich unless indicated otherwise.

Methods

Cloning, expression and purification of human mitochondrial B_{12} enzymes

The constructs used for expression of full-length human MCM and ATR (*25*), and CblA (*27*) and their purification have been described previously. The proteins were exchanged into 50 mM HEPES pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, and 5% glycerol. The experiments were conducted in the same buffer unless indicated otherwise.

Construction of Mtb MCM, ATR and CblA expression plasmids

The genes encoding MCM_α (Rv1492) and MCM_β (Rv1493) were amplified by PCR using genomic DNA from *Mycobacterium tuberculosis* H37Rv (ATCC 25698), which was a generous gift from Dr. James Sacchettini (Texas A&M University), using the following primers:
MCM_α forward: 5′-ATGTGTGGATCC**TGTGTCCATTGATGTACCCGAGCGTGCC**-3′
MCM_α reverse: 5′-ACACCAAAGCTT**AGGCCCCCAACCGCGTCAGCAGAT**-3′
MCM_β forward: 5′-AGTGGTCATATG**ATGACAACCAAGACACCCG**-3′
MCM_β reverse: 5′ACAACCGGATCC**CTAATCCAGCGTGTACC**-3′\

The restriction sites are underlined and the sequence in bold lettering represent the starting and terminal sequence of *Mtb* MCM. The PCR products were purified on a 1% agarose gel and extracted with agel extraction kit (Quiagen). The small (MCMα) and large subunits (MCMβ) were each cloned into TOPO 2.1 vector (Thermo Fisher) to generate TOPO-MCM_α and TOPO-MCM_β, respectively. The TOPO-MCM_β plasmid was digested with NdeI and BamHI and after gel purification, cloned into multiple cloning site 2 in the petDuet vector (Novagen) to generate the petDuet-MCM_β vector. TOPO-MCM_α was digested with BamHI and HindIII and cloned into multiple cloning site 1 in the petDuet-MCM_β vector. A TEV cleavage site was introduced between the His tag and MCMα by PCR with the following primers (the TEV site is in bold lettering):
Forward: 5′-**GAGAACCTGTACTTCCAGAGCG**TGTCCATTGATGTACCCGAGCGTG-3′
Reverse; 5′-**GCTCTGGAAGTACAGGTTCTC**AGGATCCTGGCTGTGGTGATGATGG-3′

The *Mtb* ATR gene (Rv1314c) was amplified using the following primers:
5′-GGTGGTCATATG**GCAGTCCACCGGAACCGC-**3′
5′-ACAACAGCTAG**CTTAGCTCGCCGTCCTATCACCACCG-**3′
The PCR product was purified on a 1% agarose gel and ligated into the TOPO 2.1 vector. The TOPO-ATR vector was digested with NdeI and NheI HF and the ATR insert was purified on a 1% agarose gel and cloned into the pet28b vector. This construct generated His-tagged *Mtb* ATR with a thrombin cleavable his tag.

The *Mtb* CblA gene (Rv1496) was amplified from plasmid DNA pANT7 (DNASU plasmid repository) by PCR using the following primers:

5′-ACCACCGCTAGC**TTAGCTTACCTATCCGTTAGGTTAGCTATCTCC**-3′
5′-GGTGGTCATATG**ATGGCCGCATCCC**-3′

The PCR product was purified on a1% agarose gel and ligated into the TOPO 2.1 vector. The TOPO-CblA vector was then digested with NdeI and NheI HF and cloned into the pet28b vector to generate a thrombin cleavable His tagged *Mtb* CblA construct.

The sequences of the *Mtb* MCM, ATR and CblA expression constructs were verified by nucleotide sequence analysis at the Genomics Core Facility (University of Michigan).

Purification of Mtb MCM

Mtb MCM (α and β subunits) was expressed in *Escherichia coli* BL21(DE3). The starting culture was grown overnight at 37 ˚C in Luria Bertani (LB) medium containing 100 μg/ml ampicillin. The initial culture (1 ml) was transferred to 1 L Terrific Broth (TB) medium containing 100 μg/ml ampicillin and grown at 37 °C until the OD_{600} reached 1.5 –1.8. Then, the temperature was reduced to 16 °C and 30 ml of DMSO was added (3 % v/v final concentration). Protein expression was induced with 500 μM isopropyl β-D-1-thiogalactopyranoside (IPTG) and the culture was grown for an additional 20–24 h at 16°C. The cells were harvested by centrifugation and stored at -80°C until further use.

The cell pellet was suspended (5 ml/g wet cell weight) in lysis buffer (50 mM Na_2HPO_4 pH 8.2, 300 mM NaCl, 10 mM imidazole, 10 mM β-mercaptoethanol and 5 % glycerol) containing 100 μM phenylmethylsulfonyl fluoride (PMSF) and 1 tablet protease inhibitor (Roche) and stirred for 30 min at 4°C. The cells were sonicated and the cell debris was removed by centrifugation at 38,500 × g for 45 min at 4 °C. The supernatant was loaded onto a Ni-NTA column (6 × 2.5 cm) and the column was washed with 500 ml lysis buffer. MCM was eluted from the column with 300 ml of a linear gradient containing 10 to 200 mM imidazole in the same buffer. Fractions containing MCM were pooled and concentrated to ~20 ml with an Amicon filter (50 kDa MWCO). TEV protease (0.02 mg TEV/mg protein) was added to the solution which was dialyzed against 50 mM Tris, pH 8.0, 300 mM NaCl, 1 mM DTT 0.5 mM EDTA and 5% glycerol). The cleaved His tag and uncut MCM were removed by loading the solution onto a Ni-NTA column (6 × 2.5 cm). MCM present in the flow-through was concentrated with an Amicon filter (50 kDa MWCO) to ~20 ml and dialyzed against 50 mM HEPES pH 7.0, 50 mM NaCl, 5% glycerol. MCM was purified by ion exchange chromatography on a 2.5 × 10 cm Source Q column (Omnifit). Buffer A consisted of 50 mM HEPES, pH 7.0, 50 mM NaCl and 5% glycerol while Buffer B contained 50 mM HEPES, pH 7.0, 500 mM NaCl and 5% glycerol. MCM was eluted with a linear gradient of 0–100 % Buffer B in 40 min at a flow rate of 4 ml/min. Fractions containing MCM were concentrated and dialyzed against buffer containing 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$ and 5% glycerol) and flash frozen in liquid nitrogen.

Purification of Mtb CblA

Mtb CblA was expressed in *E. coli* BL21(DE3). The starting culture was grown overnight at 37°C in LB medium containing 50 μg/ml kanamycin. Then, 1 L of TB medium was inoculated with 1 ml of starting culture and grown at 37 °C until the OD_{600} reached 1.0–1.2. Protein expression was induced by addition of 500 μM IPTG and the culture was grown for an additional 18–20 h. The cells were harvested by centrifugation and stored at -80 °C.

The cell pellet was suspended (5ml/g wet cell pellet) in lysis buffer (50 mM Tris, pH 8.0, 500 mM NaCl, 10 mM imidazole and 5 % glycerol) containing 100 μM PMSF and 1 tablet protease inhibitor and stirred for 30 min at 4 °C. The cells were sonicated and the cell debris removed by centrifugation at 38,500 × *g* for 45 min at 4 °C. The supernatant was loaded onto a Ni-NTA column (5 × 2.5 cm). The column was washed with 500 ml lysis buffer. *Mtb* CblA was eluted from the column with a 300 ml of 10 to 300 mM linear imidazole gradient in the lysis buffer. Fractions containing CblA were pooled and concentrated to ~20 ml using an Amicon

filter (30 kDa MWCO). Thrombin (5 U/mg protein) was added to the solution, which was dialyzed against 50 mM Tris, pH 8.0, 200 mM NaCl, 5% glycerol). The cleaved His tag and uncut CblA were removed by loading the solution onto a Ni-NTA column (5 × 2.5 cm). CblA present in the flow through was concentrated with an Amicon filter (30 kDa MWCO) to ~20 ml and loaded onto a benzamidine column (GE Healthscience) to remove thrombin. The column was washed with 150 ml lysis buffer. Fractions containing *Mtb* CblA were pooled and dialyzed against 50 mM HEPES, pH 8.0, 300 mM KCl, 2 mM $MgCl_2$ and 5% glycerol. Aliquots were flash frozen in liquid nitrogen.

Purification of Mtb ATR

Mtb ATR was expressed in *E. coli* BL21(DE3). The starting culture was grown overnight at 37 °C in LB medium containing 50 µg/ml kanamycin. Then, 1 L of LB medium was inoculated with 1 ml starting culture and grown at 37 °C until the OD_{600} reached 0.5–0.7. Protein production was induced by addition of 500 µM IPTG and the culture was grown for an additional 4 h at 37 °C. The cells were harvested by centrifugation and stored at -80 °C.

The cell pellet was suspended (5 ml/g wet cell pellet) in lysis buffer (50 mM Tris, pH 8.0, 300 mM NaCl, 10 mM imidazole, 1 mM TCEP and 5 % glycerol) containing 100 µM PMSF and 1 tablet protease inhibitor and stirred for 30 min at 4°C. The cells were sonicated and the cell debris removed by centrifugation at 38,500 × *g* for 45 min at 4 °C. The supernatant was loaded onto a Ni-NTA column (5 × 2.5 cm). The column was washed with 500 ml lysis buffer. *Mtb* ATR was eluted from the column with 300 ml of a 10 to 300 mM linear imidazole gradient in the lysis buffer. Fractions containing ATR were pooled and concentrated to ~20 ml with an Amicon filter (10 kDa MWCO). Thrombin (5 U/mg protein) was added to the solution which was dialyzed into 50 mM Tris, pH 8.0, 300 mM NaCl, 1 mM TCEP, 5% glycerol). The cleaved His tag and uncut ATR were removed by loading the solution onto a Ni-NTA column (5 × 2.5 cm). ATR was eluted with 30 mM imidazole in buffer containing 50 mM Tris pH 8.0, 300 mM NaCl, and 5 % glycerol and was concentrated with using an Amicon filter (10 kDa MWCO) to ~30 ml. Thrombin was removed by a benzamidine column. The column was washed with 150 ml 50 mM Tris, pH 8.0, 300 mM NaCl, 5 % glycerol followed by 100 ml 50 mM Tris pH 8.0, 500 mM NaCl, 5 % glycerol. Fractions containing *Mtb* ATR were pooled and dialysed against 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP and 5% glycerol. Aliquots were flash frozen in liquid nitrogen.

Expression and purification of succinyl thiokinase

Thiokinase (*E. coli* succinyl-CoA synthase SucCD from Addgene) was expressed in BL21(DE3) *E. coli*. A single colony was used to inoculate a 50 ml LB culture (containing 50 µg/mL kanamycin) and grown overnight at 37 °C. The next day, 10 ml of the starting culture was added per liter of LB and grown at 37 °C until OD_{600} reached 0.4. At this point, the temperature was lowered to 27 °C and thiokinase expression was induced with 1 mM IPTG when OD_{600} was 0.8. The cells were grown for an additional 3 h and harvested by centrifugation at 2880 × *g*, the cell pellet was frozen at -80 °C.

The cell pellet from a 2 lL culture was resuspended in 200 ml of 50 mM sodium phosphate, pH 8.0, 300 mM NaCl, and 10 mM imidazole, supplemented with 0.1 mM PMSF and one protease inhibitor tablet (Roche). Cells were lysed by sonication and loaded onto a 20 ml Ni(II)-NTA column. After washing with 150 ml of lysis buffer, thiokinase was eluted with a 150 ml linear gradient in lysis buffer containing 10-230 mM imidazole. Fractions containing thiokinase

(30 and 42 kDa subunits) were concentrated, dialyzed overnight against 100 mM potassium phosphate, pH 7.5, then flash frozen, and stored at -80 °C until further use.

Repair of I-CoA inactivated hMCM

First, *h*MCM (11.5 μM dimer) was loaded with 23 μM AdoCbl and mixed with 40 μM hCblA (dimer) and 800 μM GDP in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 5% glycerol (1 ml total volume) and incubated for 10 min at 30 °C. The solution was divided into three 350 μl aliquots: a "control" (no addition of I-CoA or the repair system), a "turnover" (addition of I-CoA only) and a "repair" (addition of I-CoA followed by the repair system) sample. Next, the control and turnover samples were placed in a 30 °C temperature controlled block and the repair sample in a cuvette was placed in a temperature-controlled cuvette holder (at 30 °C). Then, 10 μl of I-CoA (0.5 mM final concentration) was added to the "turnover" and "repair" samples, and an equal volume of buffer was added to the control sample. The samples were incubated for 60 min, and spectra were recorded every 2 min. Then, 40 μl of a premixed solution of *h*ATR (333 μM), ATP (50 mM), and GTP (70 mM) was added to the "repair" sample and an equal volume of buffer was added to the control and the "turnover" sample. The samples were incubated for additional 20 min. The final concentrations of reagents in the "repair" sample was: 10 μM *h*MCM dimer, 20 μM AdoCbl, 35 μM *h*CblA dimer, 33.3 μM *h*ATR trimer, 0.7 mM GDP, 7 mM GTP, 5 mM ATP and 0.5 mM I-CoA in 400 μl total volume. The samples were then assayed by the thiokinase assay described below to evaluate MCM activity. The same procedure was used to determine inactivation during turnover with 0.5 mM M-CoA replacing I-CoA.

Inactivation of *Mtb* MCM with I-CoA or M-CoA was tested in the same manner. *Mtb* MCM (25 μM) was mixed with 25 μM AdoCbl, 43.8 μM *Mtb* CblA and 0.875 mM GDP in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 5% glycerol (700 μl total volume) and incubated at 30 °C for 10 min. The solution was split into three 197 μl samples: a "control", a "turnover" and a "repair" sample. Then, 3 μl I-CoA (0.5 mM final concentration) was added to the "turnover" and "repair "sample and incubated for 60 min. A premixed solution of *Mtb* ATR (167 μM), ATP (25 mM) and GTP (35 mM) in 50 μl total volume was added to the "repair" sample whereas an equal volume of buffer was added to the "control" and "turnover" sample. The final concentration in the control sample was 20 μM *Mtb* MCM, 20 μM *Mtb* AdoCbl, 35 μM *Mtb* CblA (dimer), 33.3 μM *Mtb* ATR (trimer), 0.7 mM GDP, 7 mM GTP, 5 mM ATP, 0.5 mM I-CoA. The activity was then determined by the thiokinase assay described below.

Thiokinase assay for MCM activity

MCM activity was assessed using the thiokinase-coupled spectrophotometric assay as described previously (*36*). Briefly, the assay was conducted at 30 °C and concentrations of reagents in a final volume of 200 μl were: 2 nM *h*MCM (0.08 μg), 5 μM AdoCbl, 3 mM GDP, 100 mM potassium phosphate, pH 7.5, 0.5 mM M-CoA, 20 μg of thiokinase, and 70 μM 5,5´-dithiobis(2-nitrobenzene). First, thiokinase, GDP, and M-CoA were incubated for 5 min at 30 °C in a cuvette and the background thioesterase activity was recorded by the increase in absorbance at 412 nm. Next, the reaction was initiated by the addition of MCM and AdoCbl to the cuvette. The specific activity was calculated assuming that the amount of CoA detected is directly proportional to the concentration of succinyl-CoA formed by MCM. An extinction coefficient of 14,150 cm^{-1} M^{-1} was used for the TNB^- anion (*37*). When MCM activity in the "control", "turnover" and "repair"

samples described in the preceding section were interrogated, 2 µl of the sample was added to the thiokinase reaction mixture instead of MCM. When the thiokinase assay was used to determine the kinetic parameters, K_m and V_{max}, 10 µM MCM and 20 µM AdoCbl were pre-incubated for 10 min at 30 °C prior to an aliquot being added to the thiokinase assay.

I-CoA synthesis and purification

The final concentrations of reagents used for I-CoA synthesis were: 7.5 mM itaconate, 7.5 mM ATP, 2.5 mM CoASH (~100 mgs), 10 mM $MgCl_2$ and 40-50 mg thiokinase in a final volume of 35 ml of 50 mM HEPES, pH 7.5, 300 mM NaCl and 10 mM $MgCl_2$. The reaction mixture was stirred gently at room temperature for 30 min and stopped by precipitating thiokinase with a 1:10 volume of 14% perchloric acid (1.4% final concentration). The precipitated protein was removed by using a 0.2 µm syringe filter. Approximately 7 x 5 ml sample was injected onto a preparative C18 column (SunFire, 10 µm) equilibrated with 0.1% trifluoroacetic acid (TFA). Buffer B contained 80% acetonitrile and 0.1% TFA. The following gradient was used to elute I-CoA flowing at a flow rate of 4 ml min^{-1} (time, %B): 0 min, 0%; 10 min, 0%; 30 min, 70%; 40 min, 70%; 41 min, 0%; 45 min, 0%. I-CoA eluted with a retention time of ~23 min while unreacted CoA eluted at 21 mins. Fractions containing I-CoA were frozen, lyophilized, and stored at -80 °C. The typical yield of I-CoA was 40-50%. Before use, the I-CoA powder was resuspended in water and an $\varepsilon_{258\ nm}$ of 19.84 cm^{-1} mM^{-1} for the CoA moiety was used to estimate concentration. The stock solution is acidic due to the carryover of TFA and stabilizes the thioester. Prior to the experiment, the stock solution was diluted 1:1 with 500 mM sodium phosphate, pH 7.5, to bring the pH to a neutral range and avoid protein precipitation.

Synthesis of [$^{13}C_5$]-itaconyl-C4-CoA

In a 1.5 ml tube, 0.5 mg [$^{13}C_5$]-itaconic acid (3.8 mM), CoA (4.6 mM), ATP (20 mM), $MgCl_2$ (3 mM) were mixed in with 100 mM HEPES, pH 7.5, 150 mM NaCl in a total volume of 1 ml and warmed to 30°C. Then, 40 µM thiokinase were added and the mixture was incubated at 30 °C for 30 min. The reaction was stopped with 100 µl 10 % TFA (1 % final concentration) and centrifuged for 10 min at 21,130 × g for 10 min. [$^{13}C_5$]-itaconyl-C4- CoA was purified as described above for the unlabeled compound. [$^{13}C_5$]-itaconic acid was provided by the NIH Common Fund Metabolite Standards Synthesis Core or purchased from Santa Cruz Biotechnology (sc-495554).

Expression and purification of methylmalonyl-CoA synthase (MCS)

The expression vector for MCS was provide by Dr. Chaitan Khosla (Stanford University) The protein was expressed in BL21(DE3) *E.coli.* Following a fresh transformation, starting 200 µl LB was added to the transformation mixture and allowed to shake for 1 h at 37 °C. Next, 100 µl of this culture was added to 2 × 40 ml LB medium containing 100 µg/mL amplicillin and grown overnight at 37 °C. The next day, 10 ml of the starting culture was added per liter of LB supplemented with ampicillin and grown until $OD_{600\ nm}$ was 0.5, at which point the temperature was dropped from 37 ˚ to 20 °C. Protein expression was induced with 0.5 mM IPTG and growth was continued overnight. Cells were harvested by centrifugation and stored at -80 °C. Cell lysis and purification was performed using the same protocol as described for thiokinase. Following elution from the Ni(II)-NTA column, MCS was concentrated and exchanged into 200 mM potassium phosphate, pH 7.5. The protein was immediately used for the M-CoA synthesis since it is unstable upon storage.

M-CoA synthesis

For the synthesis of M-CoA, the reaction was performed in 200 mM potassium phosphate, pH 7.5, with the following reagent concentrations: 10.6 mM ATP, 10.6 mM methylmalonate, 12.5 mM $MgCl_2$, 5.4 mM CoASH (100 mgs), and 50-60 mg of MCS with a reaction volume of 24 ml. Following addition of MCS, the reaction was usually >95% complete after 5 min based on an HPLC analysis of remaining CoA. The reaction was quenched with a 1:10 volume of 14% perchloric acid (1.4% final concentration, usually after 45 min). M-CoA was purified in the same manner as described above for I-CoA. Under these conditions, M-CoA showed a retention time of ~27.5 min. M-CoA containing fractions were pooled, lyophilized, and treated in the same manner as described above for I-CoA. The typical yields for the enzymatic synthesis of M-CoA was ~60-70%.

Size exclusion chromatography to assess hMCM-hCblA complex formation

The repair mixture contained the following final concentrations of reagents: 37.5 μM *h*MCM, 75 μM *h*AdoCbl, 131 μM *h*CblA, 0.7 mM GDP, 7 mM GTP, 5 mM ATP, 125 μM ATR, and 1 mM M-CoA or I-CoA in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 5% glycerol. First, *h*MCM, *h*CblA, AdoCbl and GDP were mixed and incubated for 10 min at 30 °C using a temperature-controlled block. Then, M-CoA or I-CoA was added to the mixture incubation was continued for 60 min. Next, the rest of the repair system comprising ATR, ATP, and GTP were added and incubation was continued for 20 min. The reaction mixture (100 μl) was then immediately injected onto a Superdex 200 10/300 GL column (GE Life Sciences) equilibrated with 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 5% glycerol. The column was eluted with the same buffer at a flow rate of 0.4 ml min^{-1}. The absorbance of the eluate was monitored at 280, 350, and 465 nm. Fractions were also analyzed using SDS-PAGE and peaks corresponding to *h*MCM and *h*ATR were collected separately and concentrated using a 10 kDa MWCO membrane (Nanosep) before recording absorption spectra.

EPR spectroscopy and simulations

EPR samples were prepared by mixing 375 μM *h*MCM and 500 μM AdoCbl in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$ 2 mM TCEP, ~15% glycerol to which 1 mM (^{12}C or ^{13}C) I-CoA was added. EPR spectra were collected on a Bruker E500 ElexSys EPR spectrometer equipped with a Bruker ER4123 SHQE cavity and a Bruker ER4131VT liquid nitrogen flow temperature control system. Spectra were acquired at 120 K with non-saturating microwave power. Acquisition parameters: microwave frequency, 9.46 GHz; microwave power, 2.0 mW; modulation amplitude, 1.0 mT; modulation frequency, 100 kHz; time constant, 40.96 ms; conversion time, 81.92 ms; spectra represent average of 8 scans collected over a full sweep range of 129.0 – 479.0 mT. The spectra were corrected by subtraction of a buffer baseline, acquired under the same conditions.

Simulations of the cob(II)alamin-organic radical pair spectra were performed using the MATLAB (Mathworks, Natick, MA) programs that utilized the EasySpin (v. 5.2.23) tool box (*38*). The calculations were based on the following spin Hamiltonian:

$$\hat{H} = \beta_e \mathrm{B} g_1 \hat{S}_1 + \beta_e \mathrm{B} g_2 \hat{S}_2 + \beta_n \hat{S}_1 A_1 \hat{I}_1 + \beta_n \hat{S}_2 A_2 \hat{I}_2 + J \hat{S}_1 \hat{S}_2 + \hat{S} D \hat{S} \qquad \text{Eq. S1}$$

where β_e and β_n are the Bohr and nuclear magnetons, and the first two terms represent the electron Zeeman interaction of spin 1 (Co^{2+}, S=1/2) and spin 2 (radical, S=1/2), the third term represents the hyperfine interaction of spin 1 with the ^{59}Co nucleus (I=7/2), the fourth term represents the superhyperfine interaction of spin 2 with the ^{13}C nucleus (I=1/2; for the case of ^{13}C-labeled I-CoA), the fifth term represents the spin 1-spin 2 isotropic exchange interaction, and the last term represents the zero-field splitting (dipolar interaction of spins 1 and 2, in terms of the total electron spin operator). For the natural isotopic abundance radical pair, the fixed parameters included the following: g-tensor of Co^{2+} in cob(II)alamin, g_{1x}=2.30, g_{1y}=2.21, g_{1z}=2.00; g-tensor of radical, $g_{2x}=g_{2y}=g_{2z}$=2.00; $^{59}Co^{2+}$ hyperine coupling tensor, A_{1x}=28, A_{1y}=17, A_{1z}=294 MHz; line width, 1.0 mT. The $\boldsymbol{g}_1$ and $\boldsymbol{A}_1$ parameters for cob(II)alamin in MCM were as determined, previously (*12*). The principal axes of $\boldsymbol{g}_1$ and $\boldsymbol{A}_1$ were assumed to be aligned. The varied parameters included J, $\boldsymbol{D}$, the Euler angles (α, β, γ ; z-y-z rotation) that relate the principal axis systems of $\boldsymbol{g}_1$ and $\boldsymbol{D}$, and a general isotropic broadening parameter, representing unresolved superhyperfine coupling. For the ^{13}C-labeled I-CoA substrate, the superhyperfine coupling tensor, $\boldsymbol{A}_2$, was varied, and assumed to be isotropic. In the simulations, an iterative global scanning of the varied parameters, followed by Nelder-Mead simplex optimization (performed on the as-collected, derivative lineshape), was used to converge on a set of parameters, that satisfied the combination of visual and statistical (root mean square deviation, rmsd) criteria. The optimized values of the varied parameters for the natural abundance radical pair are as follows: J=6365 MHz; principal components of $\boldsymbol{D}$, D_x=363, D_y=-8, D_z= -355 MHz; Euler angles, α=13°, β=43°, γ=147°. Simulation of the radical pair formed from ^{13}C-labeled I-CoA, and in particular, the broadening of the resolved high-field septet structure, was achieved by using the parameters for the natural isotopic abundance radical pair and the ^{13}C superhyperfine coupling, $A_{2x}=A_{2y}=A_{2z}$=90 MHz.

The stability of the biradical was followed by EPR spectroscopy. For this, *h*MCM (100 μM) was mixed with 50 μM AdoCbl in the buffer described above in an anaerobic chamber (O_2 < 0.3 ppm) and incubated for 10 min at room temperature. Then, 500 μM I-CoA were added and following 2 min incubation, was transferred to an EPR tube and frozen in liquid N_2. After the EPR spectrum was recorded, the sample was thawed and incubated at room temperature for the desired time (20, 30, 45 and 60 min) before being refrozen for recording the EPR spectrum. The same procedure was used for *Mtb* MCM, except that the sample contained 200 μM *Mtb* MCM, 100 μM AdoCbl and 500 μM I-CoA. These EPR spectra were recorded on a Bruker EMX 300 equipped with a Bruker 4201 cavity and a ColdEdge cryostat. The temperature was controlled by an Oxford Instruments MercuryiTC temperature controller. EPR spectra were recorded at 80 K using the following parameters: 9.44 GHz microwave frequency, power 2 mW, modulation amplitude 10 G, modulation frequency 100 kHz, 2000 G sweep width centered at 3500 G, conversion time 164 ms, time constant 82 ms. Five scans were collected per measurement.

EPR spectroscopy of Mtb MCM crystals treated with I-CoA

Mtb MCM was crystallized at 20 mg/ml in 0.4 M sodium phosphate monobasic dihydrate, 1.6 M potassium phosphate dibasic, 0.1 M imidazole/ HCl pH 8, and 0.2 M sodium chloride. Approximately 450 crystals were harvested and placed in a solution of 2.5 mM I-CoA, 0.4 M sodium phosphate monobasic dihydrate, 1.6 M potassium phosphate dibasic, 0.1 M imidazole/HCl, pH 8, and 0.2 M NaCl and allowed to soak for 1h. Glycerol was added to the solution for cryoprotection to a final concentration of 15% glycerol, 2.5 mM I-CoA, 0.32 M sodium phosphate monobasic, 1.3 M potassium phosphate dibasic, 0.082 imidazole/HCl, pH 8,

0.16 M sodium chloride. The entire solution was then transferred to an EPR tube and frozen in liquid N_2. The EPR spectrum was recorded at 80 K with the following parameters: 9.38 GHz microwave frequency, power 20 mW, modulation amplitude 10 G, modulation frequency 100 kHz, 2000 G sweep width centered at 3500 G, conversion time 328 ms, time constant 164 ms. 25 scans were accumulated.

Product analysis of I-CoA inactivated MCM

In a 500 μL reaction volume, 110 μM human or *Mtb* MCM was mixed with 220 μM AdoCbl and the sample was allowed to equilibrate for 10 min. Next, I-CoA was added to 220 μM and the reaction was allowed to progress for 30 min at room temperature before being quenched with a 1:10 volume of 7% perchloric acid (0.7% final concentration). After centrifuging to remove precipitated protein, the sample was injected onto a preparative C18 column (SunFire C-18, 10 μm 10 × 250 mm) and purified using the following Buffer A: 75 mM Na_3PO_4 pH 4.5, and Buffer B: 50% acetonitrile, and the following gradient at a flow rate of 4 ml min^{-1} (time, %B): 0 mins, 10%; 5 min, 10%; 40 min, 45%; 41 min, 100%; 46 min, 100%; 47 min, 10%; 52 min, 10%. Fractions of interest were lyophilized, and stored at -80 °C. For MALDI MS analysis, samples were resuspended in water and zip-tipped (C_4, Millipore) to remove salts. Samples were analyzed by MALDI MS in the negative-ion mode (University of Michigan Chemistry MS Facility). Identical HPLC profiles (n=4) and MS (n=2) results were obtained with the human and *Mtb* MCM samples.

I-CoA titrations

*h*MCM (20 μM) was mixed with 40 μM AdoCbl in in 50 mM HEPES pH 7.5, 150 mM KCl 2 mM $MgCl_2$ 2 mM TCEP, 5% glycerol and the mixture was allowed to equilibrate for 10 min at 25 ˚C. An initial spectrum was recorded and after each addition of I-CoA (3.5 to 55 μM), the sample was allowed to equilibrate for 5 min before acquiring a spectrum. The change in absorbance at 530 nm was used to determine a K_d. The data were fit to a single I-CoA binding per MCM monomer using Dynafit (*39*).

Mtb MCM (30 μM) was mixed with AdoCbl (30 μM) in 50 mM HEPES pH 7.5, 150 mM KCl 2 mM $MgCl_2$ 2 mM TCEP and 5 % glycerol and incubated at 25 ˚C for 10 min. I-CoA (5 – 200 μM) was added and the formation of inactivated species was monitored. The decrease in absorbance at 525 nm was plotted against I-CoA concentration and the data were fit to a one site binding model using Dynafit.

Crystallization of Mtb MCM

AdoCbl (600 μM) was mixed with *Mtb* MCM (500 μM) in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 5% glycerol and incubated at 25 ˚C for 15 min. Unbound AdoCbl was removed by size exclusion chromatography on an S-200 column (1.6 × 100 cm) (GE Life Sciences), pre-equilibrated with the same buffer described above. Fractions containing *Mtb* MCM-AdoCbl were pooled and concentrated to 25 mg/ml with an Amicon filter (MWCO = 50 kDa).

Crystals of *Mtb* MCM-AdoCbl were obtained by the sitting-drop vapor-diffusion method by mixing 0.3 μl of protein solution (20 mg/ml) with 0.6 μl of the reservoir solution. *Mtb* MCM-AdoCbl crystals were formed at 20 °C in 100 mM sodium cacodylate, HCl pH 6.5, 800 mM sodium citrate tribasic dihydrate, 0.05% w/v casein, 0.05% w/v hemoglobin, 0.0005% w/v pepsin, 0.0005% (w/v) protease, 0.0005% (w/v) proteinase K, 0.0005% (w/v) trypsin, and 0.002

M HEPES sodium, pH 6.8. The crystals were cryoprotected in a solution consisting of 15% glycerol, 85 mM sodium cacodylate, and 680 mM sodium citrate tribasic dihydrate before being flash-cooled in liquid nitrogen. All procedures regarding crystal growth and manipulations were conducted in the dark.

Crystals of *Mtb* MCM-AdoCbl used for soaking with I-CoA were obtained by the sitting-drop vapor-diffusion method by mixing 0.45 μl of protein solution (20 mg/mL) with 0.45 μl of the reservoir solution. *Mtb* MCM-AdoCbl crystals used for soaking were formed at 20 °C in 0.4 M sodium phosphate monobasic, 1.6 M potassium phosphate dibasic, 0.1 M imidazole/HCl pH 8, and 0.2 M sodium chloride. A 28 mM freshly prepared solution of I-CoA in water was mixed at a 1:1 ratio with 1M HEPES, pH 7.0, to obtain a 14 mM solution for each soaking experiment. I-CoA was then mixed with the reservoir solution to a final concentration of 2.5 mM. *Mtb* MCM-AdoCbl crystals were transferred to a solution of 2.5 mM I-CoA, 0.32 M sodium phosphate monobasic, 1.3 M potassium phosphate dibasic, 0.082 imidazole/HCl, pH 8, 0.16 M sodium chloride, 0.1 M HEPES, pH 7 and allowed to soak for 70 min. The crystals were cryoprotected in a solution of 15% glycerol, 2.5 mM I-CoA, 0.27 M sodium phosphate monobasic, 1.1 M potassium phosphate dibasic, 0.067 imidazole/HCl, pH 8, 0.13 M sodium chloride, 0.1 M HEPES, pH 7, before being flash-cooled in liquid N_2. All procedures regarding crystal growth and manipulations were conducted in the dark.

Crystal structure determination

Diffraction data were collected at 100 K on the GM/CA-CAT 23-ID-B (for *Mtb* MCM-AdoCbl) or 23-ID-D (for *Mtb* MCM-AdoCbl + I-CoA) beamline at Advanced Photon Source, Argonne National Laboratory using a DECTRIS EIGER16M detector for MCM-AdoCbl or a DECTRIS PILATUS3-6M detector for MCM-AdoCbl + I-CoA. All data sets were processed with *XDS (40)* to 1.9 Å and 2.0 Å, respectively for the structures without and with I-CoA. Data collection and processing statistics are summarized in Table S1. The crystals of MCM-AdoCbl were indexed to space group $P2_12_12_1$ (unit-cell parameters a = 77.8, b = 103.6, c = 211.2 Å) with one molecule in the asymmetric unit (Matthews coefficient V_M = 2.77 Å^3 Da^{-1}, 55.6 % solvent content) and the crystals of MCM-AdoCbl + I-CoA were indexed to space group $P2_12_12_1$ (unit-cell parameters a = 76.6, b = 104.9, c = 194.1 Å) with one molecule in the asymmetric unit (Matthews coefficient V_M = 2.54 Å^3 Da^{-1}, 51.6 % solvent content).

Crystal structure refinement

Initial structure solutions were obtained by molecular replacement using as a search model substrate-free *P. shermanii* MCM (PDB: 2req) (*41*) for *Mtb* MCM-AdoCbl and *P. shermanii* MCM substrate complex (PDB: 4req) for *Mtb* MCM-AdoCbl + I-CoA and initial phases were calculated using *Phaser (42)*.

Both structures were initially refined with *REFMAC5* (*43*) using isotropic individual *B* factors with maximum-likelihood targets where the Babinet model for bulk-solvent scaling was utilized. *PHENIX* (*44*) was then employed for subsequent refinements. Refinement included coordinate minimization and restrained individual *B*-factor adjustment with maximum-likelihood targets. Refinement was followed by model building and modification with Coot *(45)*. Several iterative rounds of refinement followed by model building and modification were performed. In early rounds of refinement, ligands were not fitted into either structure.

Once the protein model was complete and the water molecules had been assigned, clear electron density was fitted and refined as B_{12} and dAdo ligands in both structures using *eLBOW*

(46) to generate chemical restraints. Additionally, I-CoA was modeled into residual electron density in the *Mtb* MCM-AdoCbl + I-CoA structure. Restraints for I-CoA were generated using ProDrug (*47*) and I-CoA was inserted into the model using *ReadySet* (*48*) before subsequent refinement.

The final model was used to create an unbiased refined composite $2F_O$-F_C map with *PHENIX*. The refined iterative composite omit map was calculated using the corresponding program in Phenix (*49*). Crystallographic information as well as refinement statistics are provided in Table S1. The geometric quality of the model and its agreement with the structure factors were assessed with *MolProbity (50)*. Figures displaying crystal structures were generated with *PyMOL (51)*.

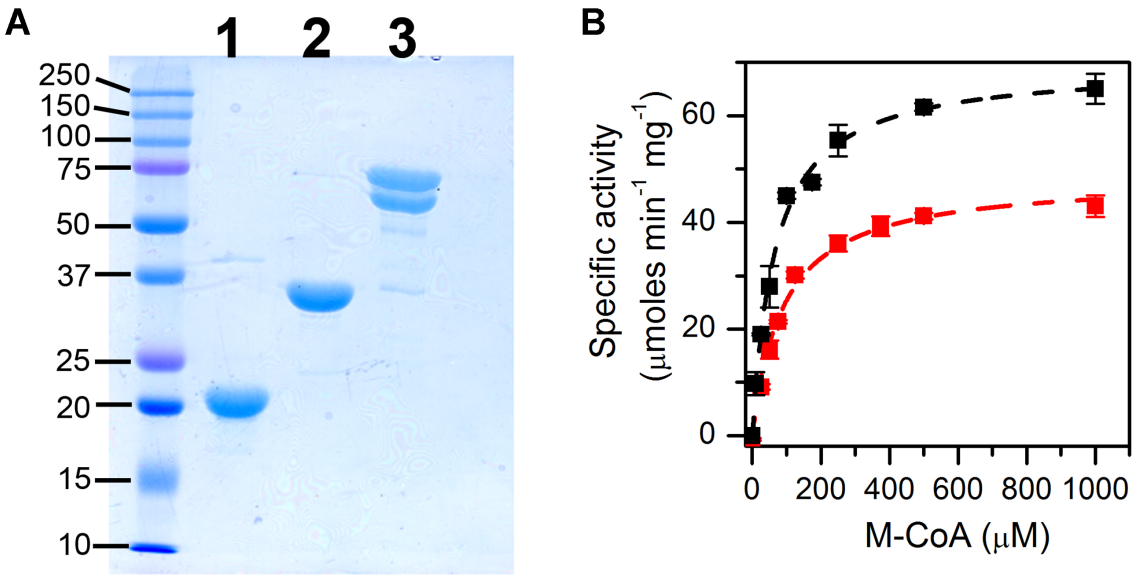
Inhibition of B_{12}-stimulated growth of Mtb on propionate

An auto-luminescent *Mtb* strain was constructed by transforming pJW276, a chromosomal-integration plasmid that allows expression of the bacterial *lux* operon in mycobacteria (a kind gift from Dr. Jeffrey Wagner, Harvard T.H. Chan School of Public Health), into wild-type *Mtb* (*52*). Wild-type *Mtb* or its luminescent derivative was cultured in sterile PETG inkwells containing 10 ml 7H9 medium with 0.2% glycerol, 0.05% Tween-80 and 10% OADC enrichment. Exponentially growing bacteria (OD_{600} ~ 0.5-0.8) were pelleted at 4,000 × g, washed once with fresh Sauton's media (*53*), then suspended in modified Sauton's media (0.05% KH_2PO_4, 0.05% $MgSO_4$, 0.2% citric acid, 0.005% ferric ammonium citrate, 0.05% $(NH_4)_2SO_4$, 1:100000 dilution of 1% $ZnSO_4$ stock solution, 1:2000 dilution of 10% Tyloxapol stock solution), supplemented with 0.2% (v/v) propionate to a final OD_{600} of 0.01. The diluted bacterial suspension was aliquoted into 96 well plates supplemented with itaconate and/or vitamin B_{12} (cyanocobalamin) at the indicated concentrations, sealed with an air-permeable membrane (Breathe-Easy, Diversified Biotech), and incubated at 37 °C with constant shaking. A multi-channel plate reader (Synergy H1 Hybrid, BioTEK) was used to monitor OD_{600} or the luminescent signal at the indicated time points. Middlebrook 7H9 medium and OADC enrichment were purchased from BD Biosciences.

Effect of Irg knockdown on itaconate, I-Coa and AdoCbl concentration

RAW 264.7 cells (TIB-71) were obtained from ATCC. The cells were cultured in DMEM supplemented with 10 % FBS, penicillin and streptomycin (both at 100 U/ml) under 5% CO_2 at 37 °C. Cells were tested for mycoplasma contamination quarterly. The LC-MS based analysis for itaconate, I-CoA and AdoCbl were performed as previously reported (*5*).

CRISPR KD RAW cells were generated by lentivirus infection as previously reported (*5*). Two sgRNAs per gene were cloned into pLentiCRISPRv2 plasmid (Addgene 52961). A lentiviral pool from the two guides was used for infection to achieve higher knockdown efficiency. The sequences of the sgRNAs are listed below:
Negative control: GCACTACCAGAGCTAACTCA
Irg1_sg1: TGAGCACGATTCTCTCGATG
Irg1_sg2: AAGCCAAAGACATACCAAAG



C

Enzyme	$K_{M(M\text{-}CoA)}$ (μM)	V_{max} (μmoles min^{-1} mg^{-1})
Human MCM	66.5 ± 6.1	69.4 ± 1.7
Mtb MCM	90.6 ± 7.5	48.4 ± 1.1

Fig. S1. Charaterization of *Mtb* MCM and its auxiliary proteins. (**A**). SDS-PAGE analysis demonstrating the purity of *Mtb* proteins: lane 1: ATR (27 kDa), lane 2: CblA (36 kDa), and land 3: MCM (α (66 kDa) and ß (80 kDa) subunits). (**B**). Michaelis-Menten kinetic analysis of *h*MCM (black trace) and *Mtb* MCM (red trace). MCM activity was determined in the thiokinase assay and performed in duplicate (*h*MCM) or triplicate (*Mtb* MCM); the data represent mean ± SD. (**C**). Summary of the kinetic parameters for human and *Mtb* MCM, which are homodimeric and heterodimeric, respectively.

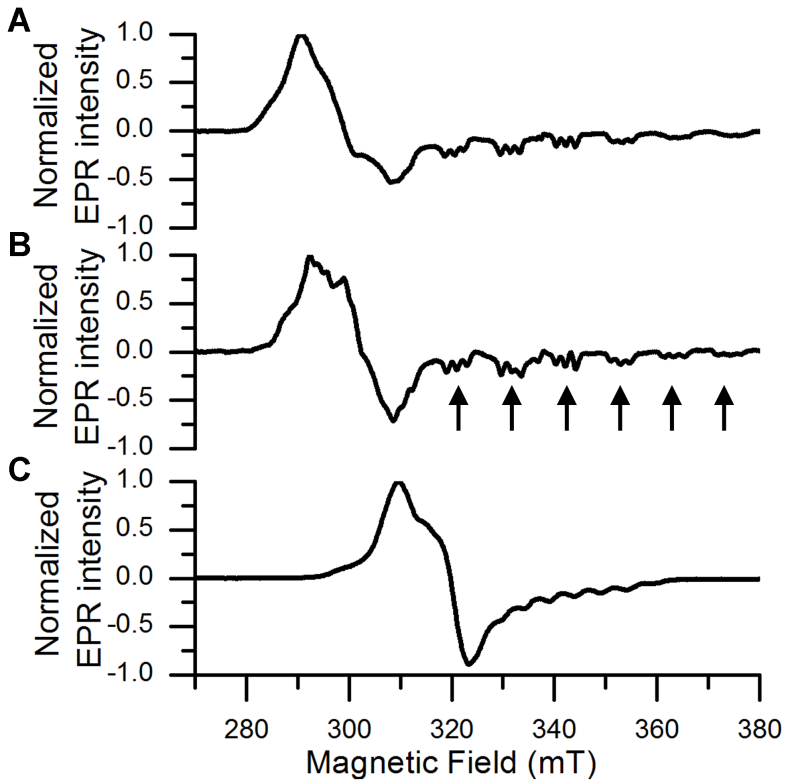


Fig. S2. EPR spectra of *Mtb* MCM. (**A**) Spectrum of free 100 μM cob(II)alamin. (**B**) Spectrum of 100 μM cob(II)alamin bound to 200 μM *Mtb* MCM. (**C**) Spectrum of sample containing 100 μM AdoCbl bound to 200 μM *Mtb* MCM and incubated aerobically with 500 μM I-CoA for 30 min. All samples were prepared in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, and 10% glycerol. The arrows highlight the cobalt hyperfine line with additional triplet splitting due to ^{14}N superhyperfine coupling.

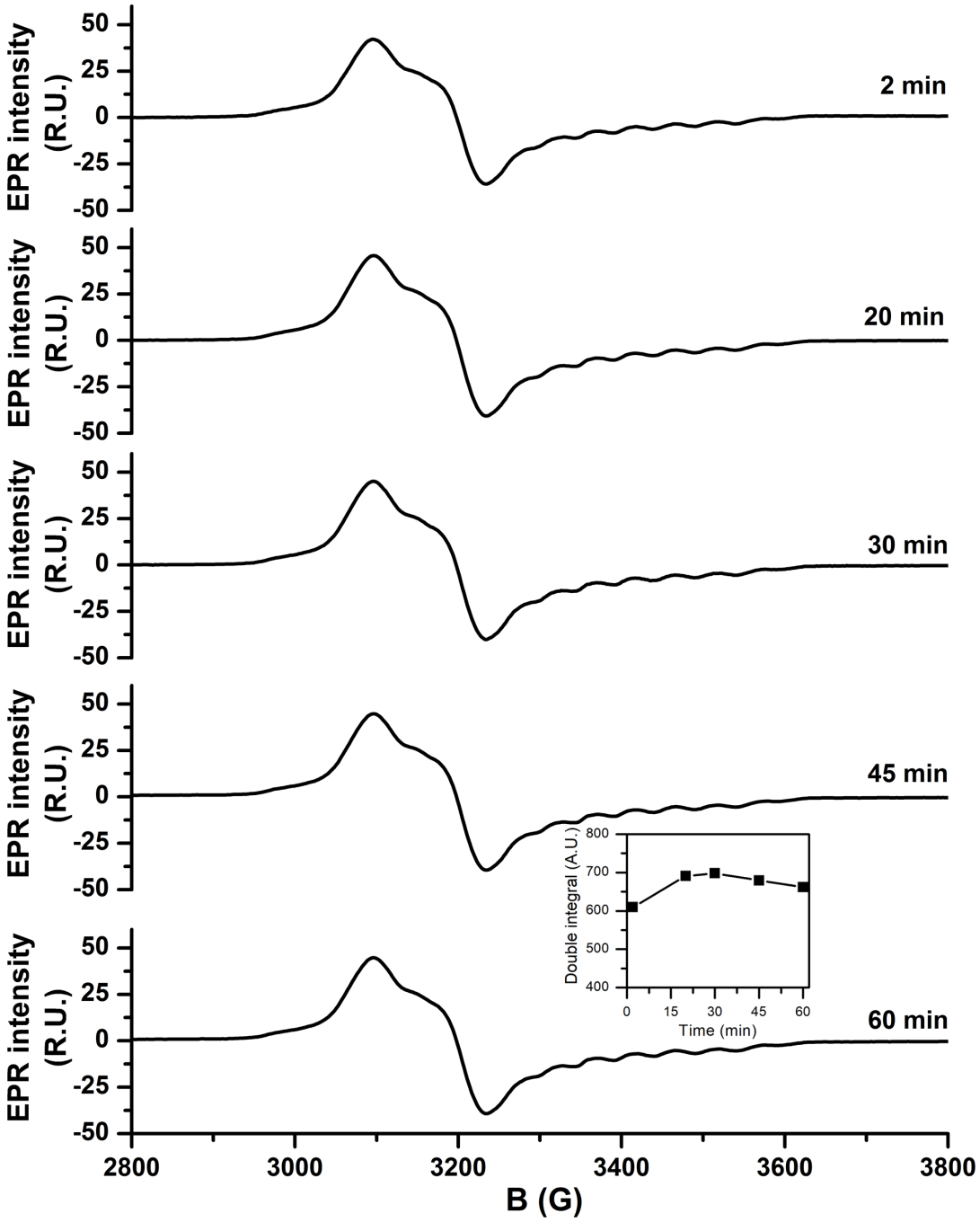


Fig. S3. Aerobic stability of the I-CoA derived biradical on *Mtb* MCM. *Mtb* MCM (200 μM) was mixed with AdoCbl (100 μM) in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP, 10% glycerol. The biradical was formed upon addition of I-CoA (500 μM). Following 2 min incubation at room temperature, the sample was frozen in liquid N_2 and an EPR spectrum was recorded. The sample was then quickly thawed and incubated for the indicated time at room temperature before being frozen in liquid N_2 to record the EPR spectrum. The inset shows the time dependence of the double integral of the EPR signal over time.

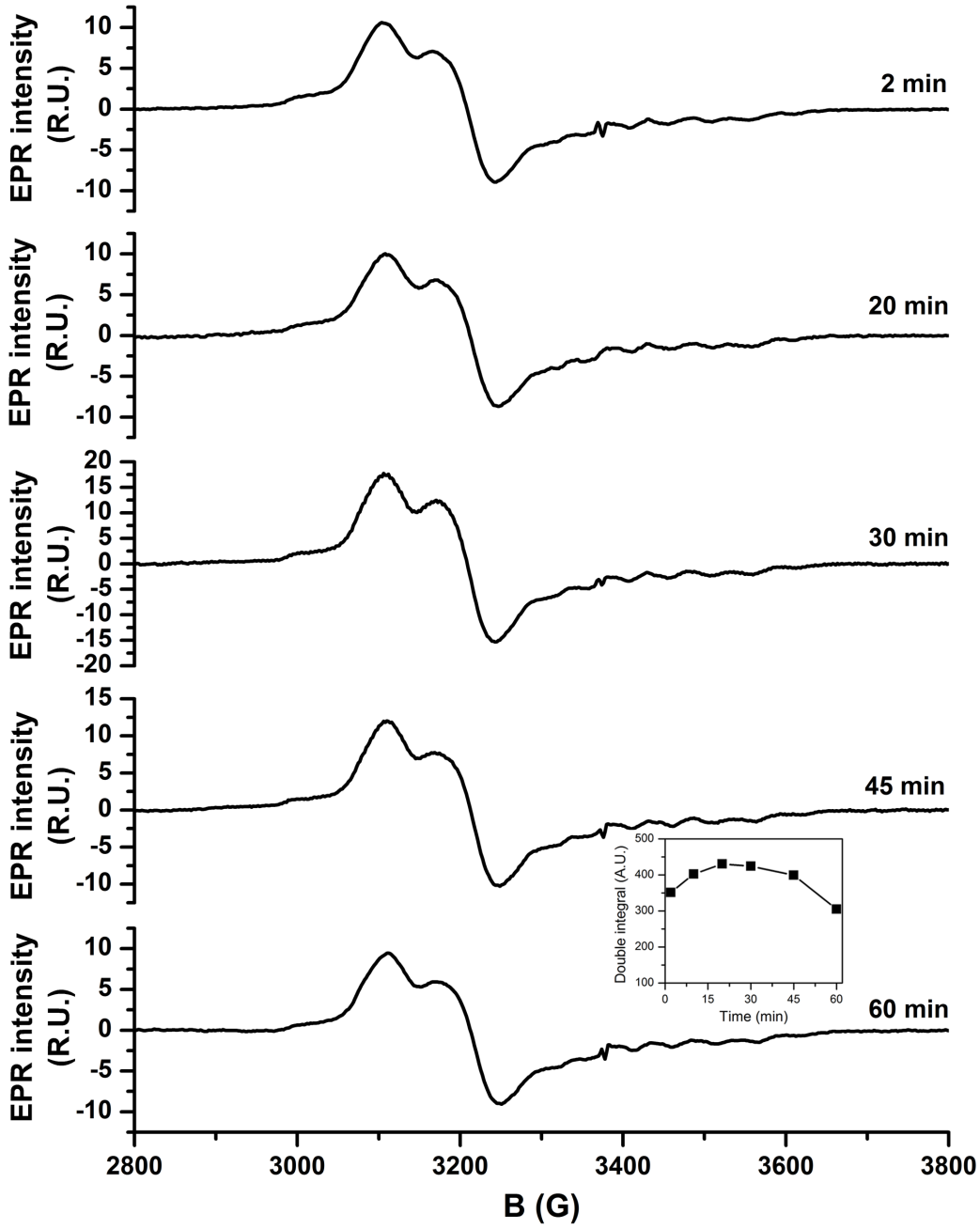


Fig. S4. Aerobic stability of the I-CoA-derived biradical on *h*MCM. *h*MCM (100 μM) was mixed with AdoCbl (50 μM) in 50 mM HEPES, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 2 mM TCEP and 10% glycerol. The biradical was formed upon addition of I-CoA (500 μM). Following incubation for 2 min at room temperature, the sample was frozen in liquid N_2 and the EPR spectrum was recorded. The sample was then quickly thawed and incubated at room temperature

for the indicated time before being frozen in liquid N_2 to record the EPR spectrum. The inset shows the time dependence of the double integral of the EPR signal over time.

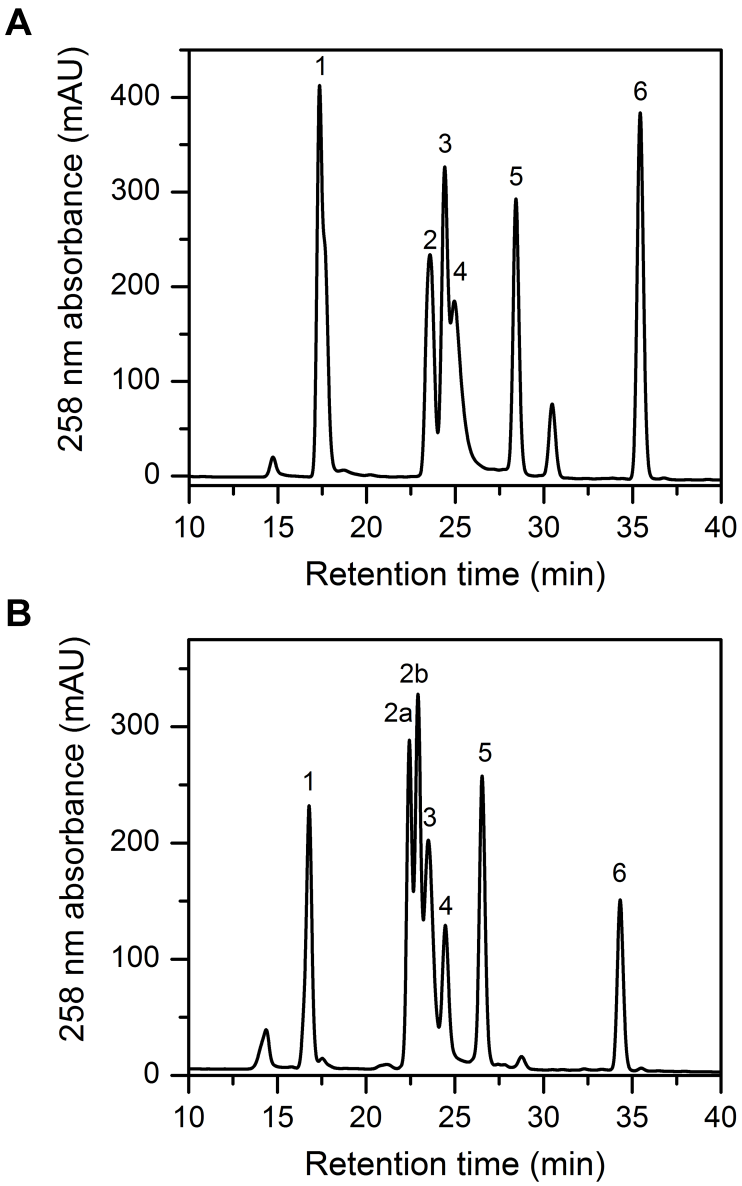


Fig. S5. Chromatograms of aerobic reaction mixtures containing I-CoA-inactivated MCM. HPLC chromatogram of products formed during the reaction of I-CoA with (**A**) *h*MCM-AdoCbl or (**B**) *Mtb* MCM-AdoCbl under aerobic conditions. Products were identified by either negative-ion MALDI/MS or co-migration with standards (OH_2Cbl and AdoCbl) with known retention times. The B_{12} peaks were also confirmed by their absorbance at 350 and 525 nm. The peaks were identified as follows: 1-CoA, 2a,b-hydroxylated adenosine adduct, 3-OH_2Cbl, 4-I-CoA, 5-decarboxylated adenosine adduct, 6-AdoCbl.

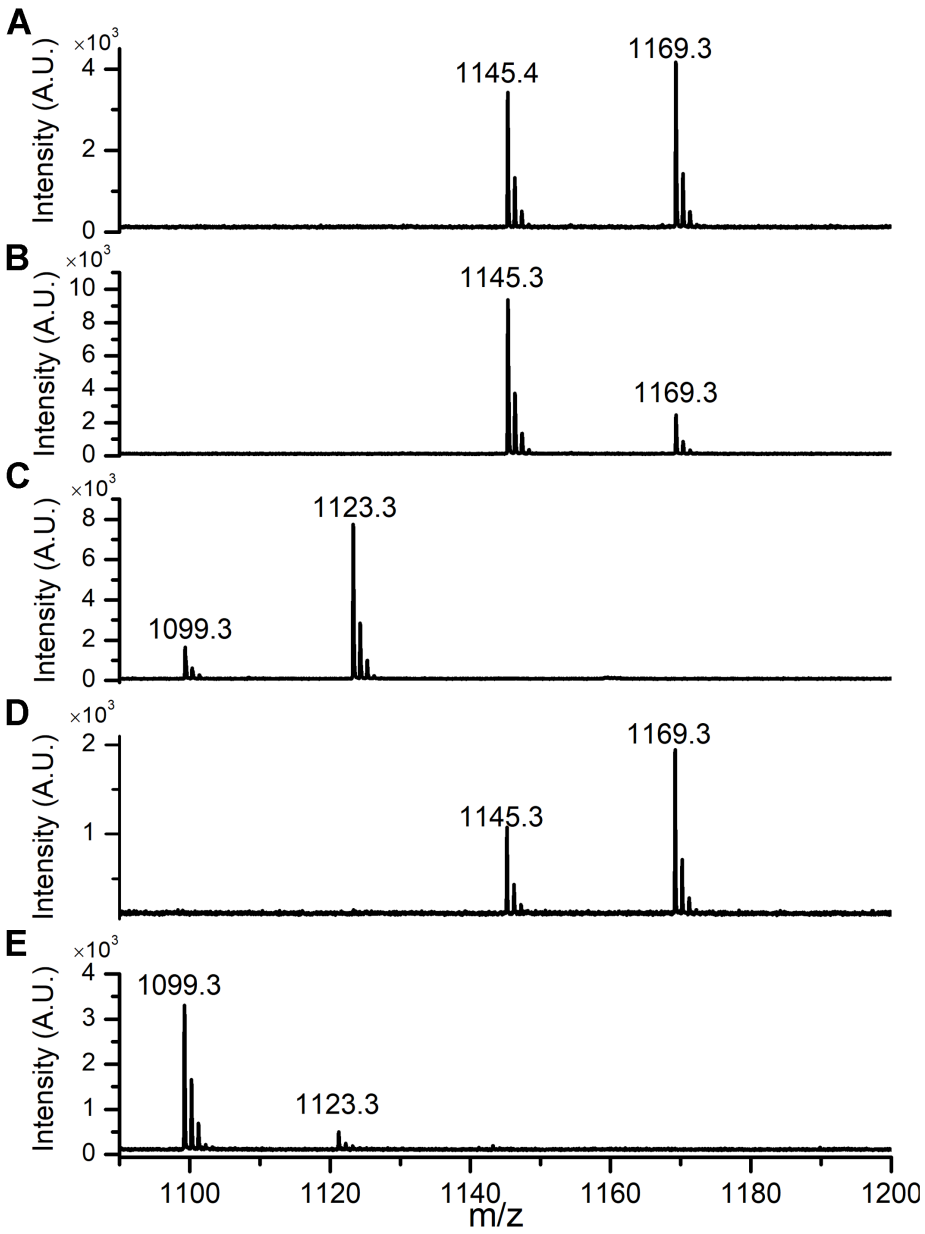


Fig. S6. MALDI MS (negative ion) analysis of products from the aerobic reaction of MCM with I-CoA. The HPLC-purified products (Fig. S5B) from fractions 2a (**A**), 2b (**B**) and 5 (**C**), were subjected to MS analysis after desalting. The identity of the +24 peaks (m/z = 1169.4, 1123.3) present in all the samples is not known.

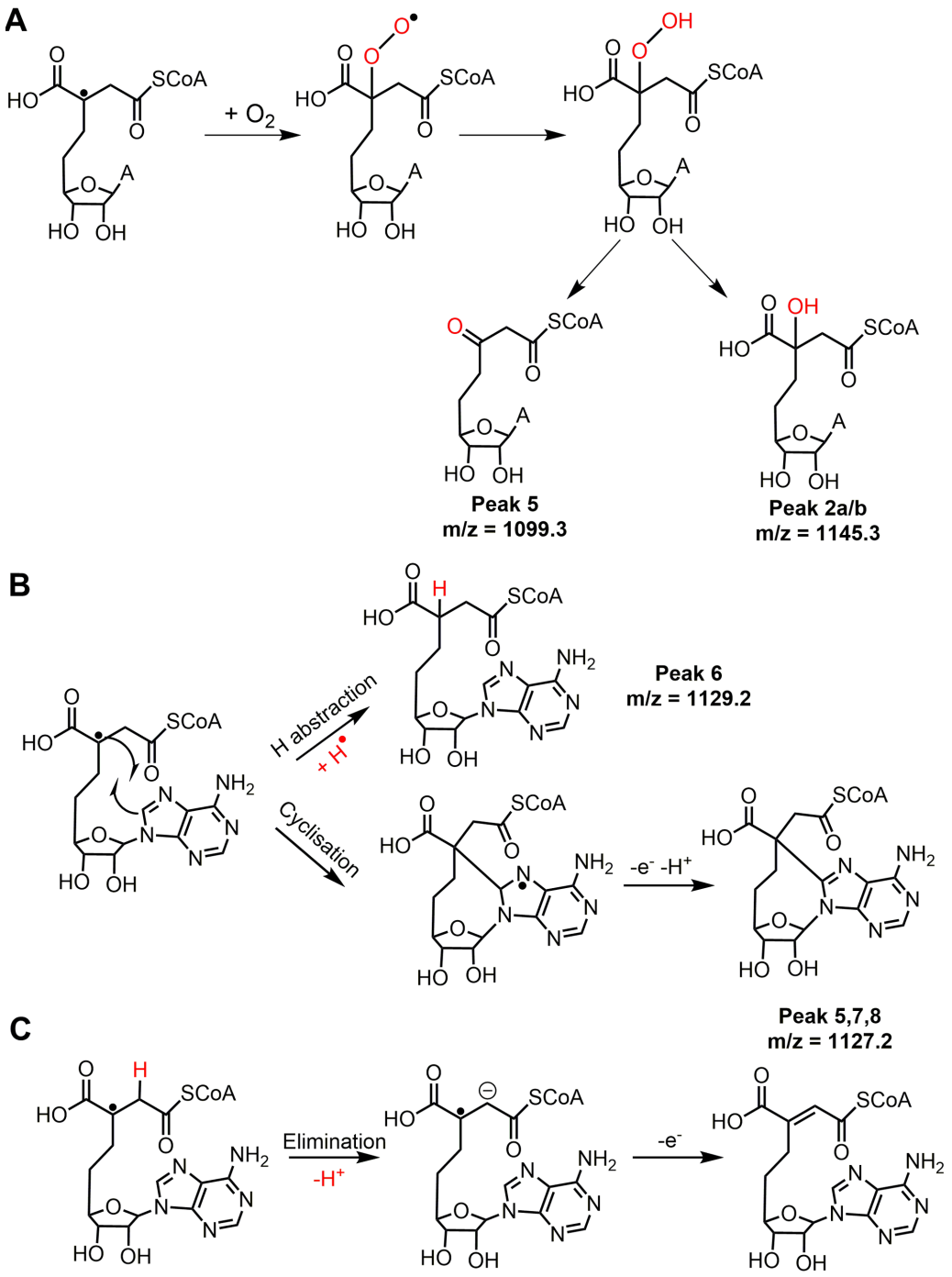


Fig S7. **Proposed mechanism for the differential reactivity of Ado• under aerobic and anaerobic conditions**. (A) Postulated mechanism for oxygen addition to the initial tertiary radical upon release into solution, forming a hydroperoxy radical intermediate, which degrades to the observed hydroxyl derivative. The second major product is proposed to form by oxidative decarboxylation of the α-hydroxy acid as described for model compounds (*54, 55*). dAdo radical addition products have been reported previously (*56, 57*). (B) Postulated mechanism for the intramolecular cyclization in the initially formed tertiary radical adduct and the 5′-dAdo moiety

under anaerobic conditions. While the loss of two mass units is assigned to an intramolecular cyclization between the tertiary radical and the adenosine moiety derived from AdoCbl (B), it could in principle, involve the 3′-phosphoadenosine group in I-CoA instead. (C) Postulated mechanism to account for Peaks 7 and 8 observed by HPLC (Fig. S8) with m/z = 1127.2 (Fig. S9). Proton loss could be facilitated by the higher acidity of the hydrogen atom adjacent to a radical center (than in a closed-shell counterpart (*58, 59*)). Deprotonation at the methylene group adjacent to the thioester moiety followed by loss of an electron (e.g. to cob(II)alamin) would also lead to a product with two mass units less than the expected addition product.

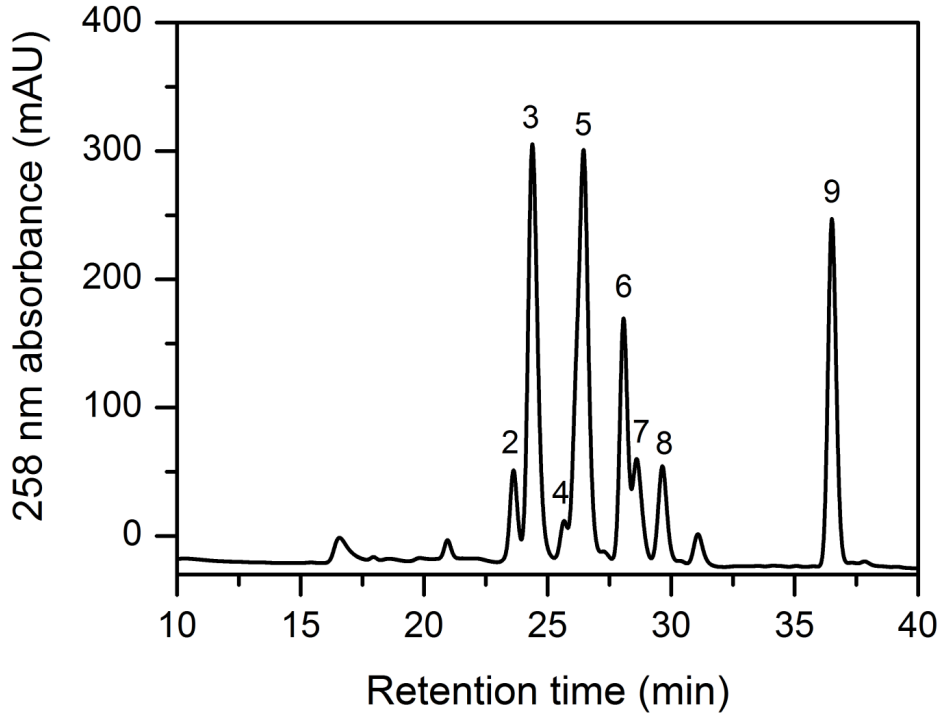


Fig. S8. Chromatograms of anaerobic reaction mixtures containing I-CoA-inactivated *Mtb* MCM. HPLC chromatogram of products formed during the reaction of I-CoA with *Mtb* MCM under anaerobic conditions. Products were identified by either negative-ion MALDI/MS or by co-migration with standards (OH_2Cbl and AdoCbl) with known retention times. The identified peaks are as follows: 2-hydroxylated-adenosine adduct, 3-OH_2Cbl, 4-I-CoA, 5 cyclic adenosine adduct, 6-I-CoA-adenosine adduct, 7,8-cyclic adducts, 9-AdoCbl. The peaks are numbered starting from 2 to show their correspondence to the peaks obtained under aerobic conditions (Fig. S5).

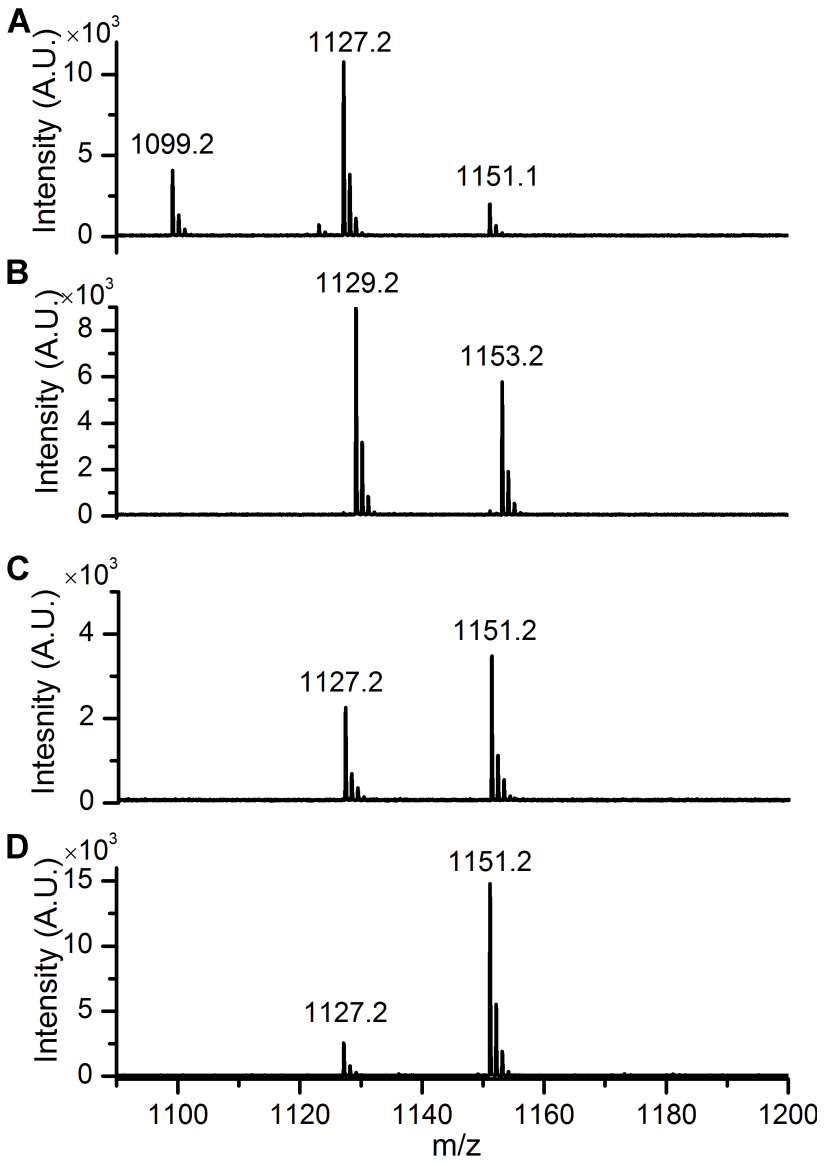


Fig. S9. MALDI MS (negative ion) analysis of products from the anaerobic reaction of *Mtb* MCM with I-CoA. The HPLC-purified products (Fig. S8) from fractions 5 (**A**), 6 (**B**), 7 (**C**) and 8 (**D**) were subjected to MS analysis.

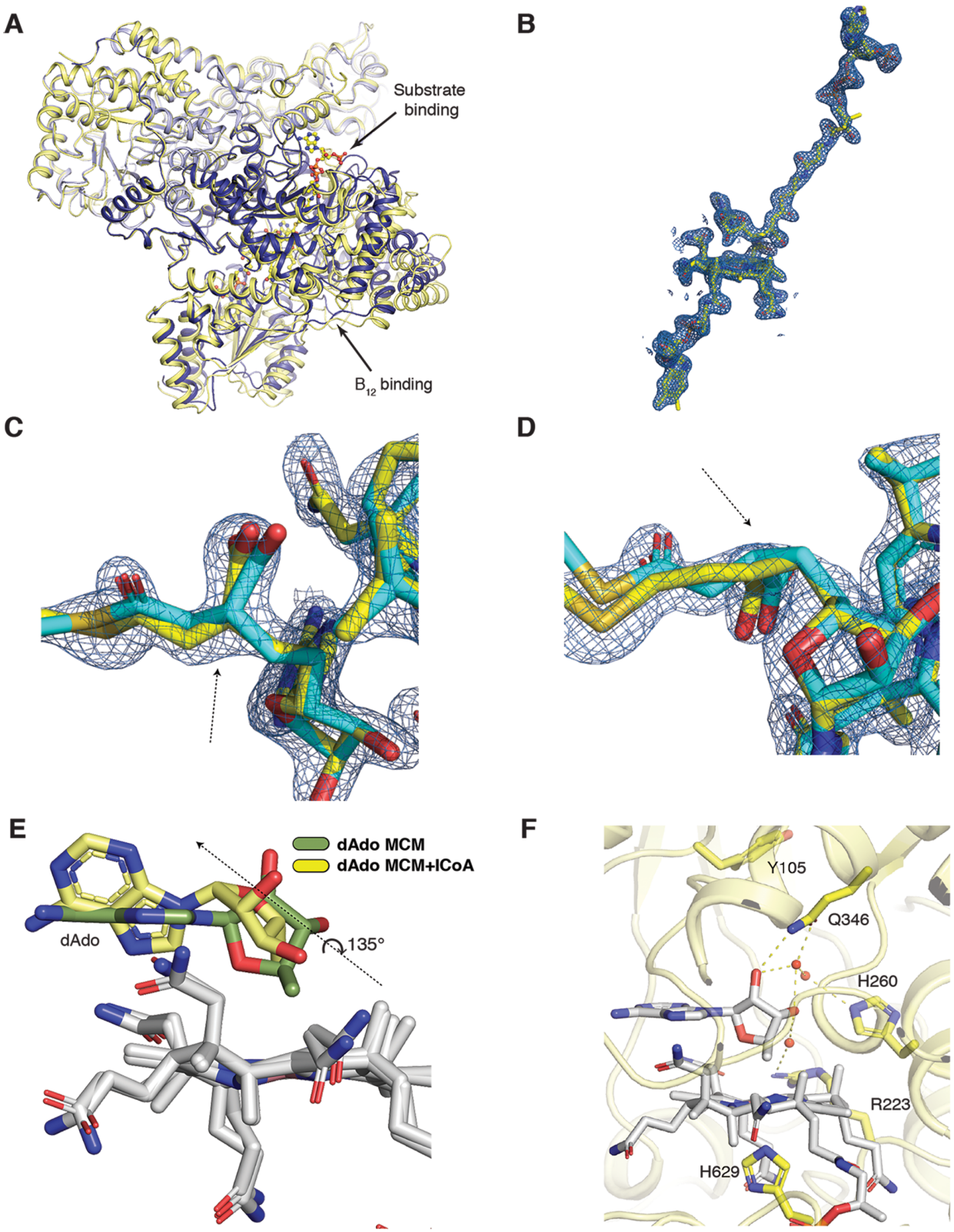


Fig. S10. Crystal structures of *Mtb* MCM with and without I-CoA. (**A**) Superposition of the I-CoA-bound (blue) and native *Mtb* MCM (yellow) structures. The inactive ß-subunit (shown in gray or light blue) shows virtually no conformational change upon binding I-CoA. The larger,

B_{12}-binding α-subunit (blue for I-CoA bound, yellow for native) shows a substantial conformational change upon I-CoA binding. B_{12} and I-CoA are shown in stick representation with carbons in yellow, oxygen in red, nitrogen in blue, and phosphorous in orange. (**B**) 2Fo-Fc omit map showing electron density (blue) around B_{12} (yellow) and I-CoA (yellow) contoured at 1.5σ. (**C** and **D**) Two views of the fit of I-CoA ligands with an sp^2- (yellow) or an sp^3- (blue) hybridized carbon geometry refined with MCM. The resulting I-CoA models were overlaid on the composite omit map (contoured at 1.5 σ). The two views clearly demonstrate the superior fit of the I-CoA model with sp^2 geometry to the omit density map. (**E**) Superposition of B_{12} (gray for both structures) and dAdo in the native (green) and I-CoA bound (yellow) structures. The corrin rings were aligned for the superposition. (**F**) Interactions between dAdo and active site residues in the native structure. The 2′-OH of dAdo engages in hydrogen-bonding interactions with Q346 and a water-mediated hydrogen-bonding network with H260 and R223.

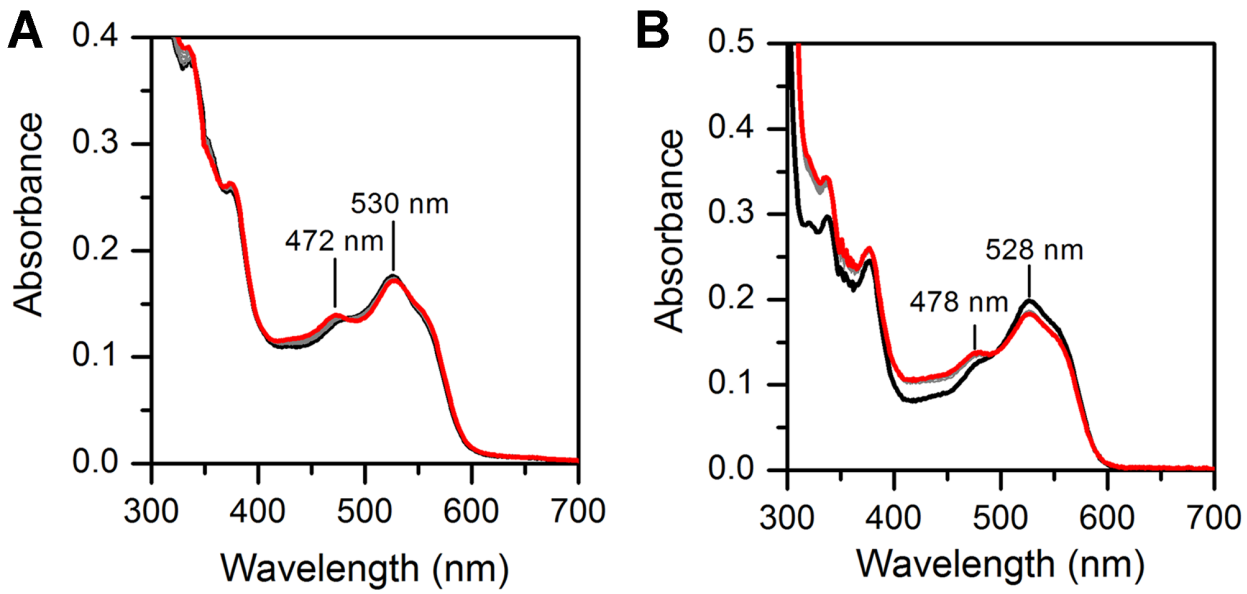


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Fig. S11. Spectra of MCM following catalytic turnover and upon addition of the repair system. Spectra of *h*MCM (**A**) or *Mtb* MCM (**B**) incubated with M-CoA for 60 min (black) to allow turnover, followed by the addition of the corresponding human and *Mtb* repair enzymes comprising CblA and ATR (gray spectra). The final spectra (red) were recorded after 20 min.

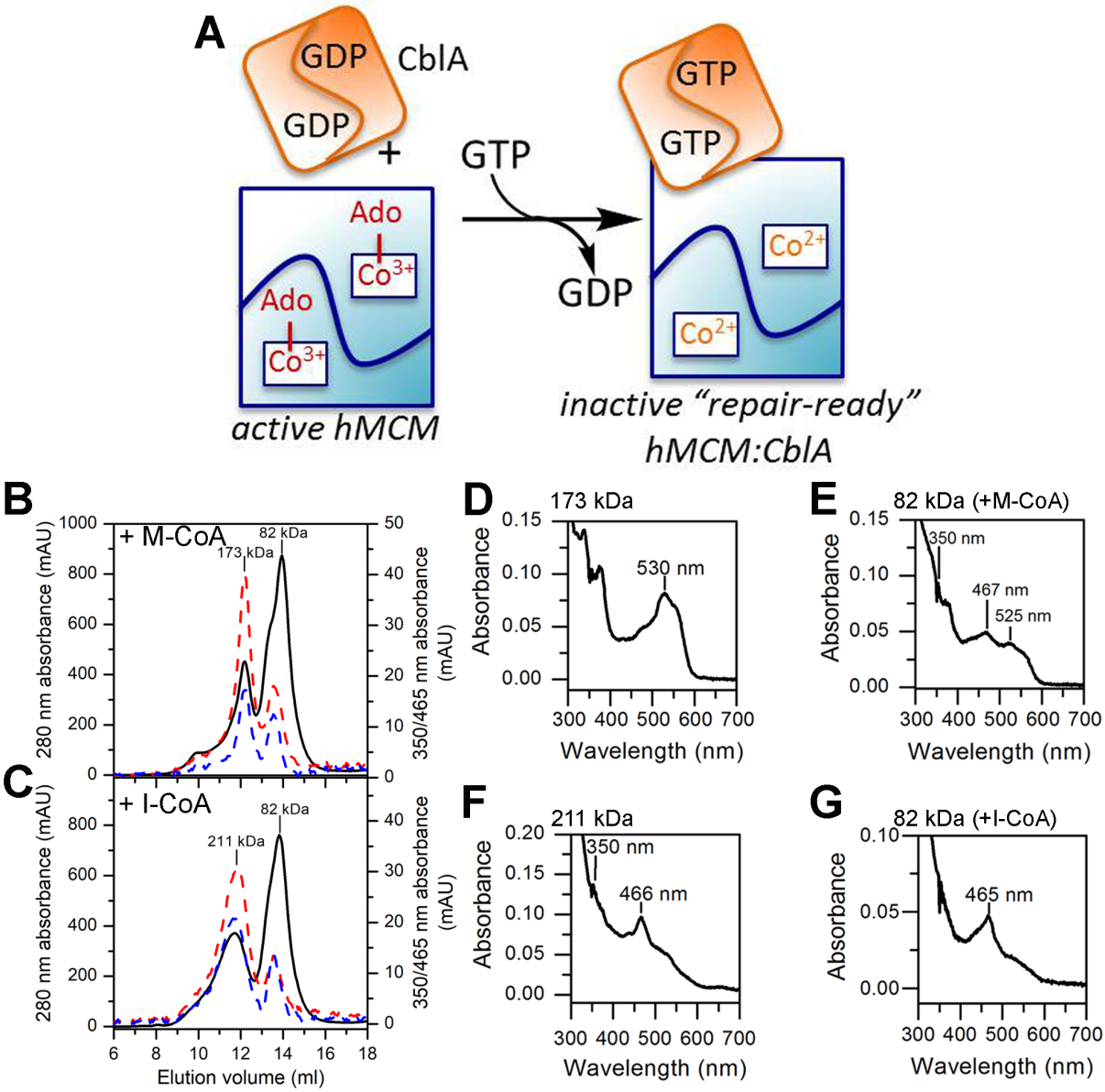

Fig. S12. **I-CoA impedes MCM repair by inhibiting CblA-MCM complex formation**. (**A**) *h*MCM interacts with *h*CblA when cob(II)alamin but not AdoCbl is bound. (**B**) Following catalysis in the presence of M-CoA (+CblA and GDP), ATR (+GTP and ATP) addition promotes off-loading of cob(II)alamin from *h*MCM to ATR. The black, blue and red lines indicate absorbances at 280, 350 and 465 nm, respectively. The MWs of *h*MCM, CblA and ATR are 160 kDa, 77 kDa and 72 kDa, respectively. (**C**) I-CoA-inactivated *h*MCM exhibits a broad peak at 211 kDa. (**D** and **E**) Spectra of the 173 and 82 kDa peaks in (B) show AdoCbl bound to MCM (530 nm (D)), and a mixture of 4-coordinate cob(II)alamin (465 nm) and OH_2Cbl (350, 525 nm) bound to ATR (E)). (**F** and **G**) Spectra of the 211 and 82 kDa peaks in (C) show cob(II)alamin bound to *h*MCM (466 nm) with a trace of OH_2Cbl (350 nm, F) and 4-coordinate cob(II)alamin (465 nm) bound to ATR (G). Analysis of the 82 kDa peak (C) thus reveals that the repair system transferred some cob(II)alamin from I-CoA-inactivated *h*MCM to ATR (465 nm).

Table S1. X-Ray Crystallography Data Collection and Refinement Statistics

	***Mtb* MCM**	***Mtb* MCM + I-CoA**
Data collection	(208)	(2111)
Beamline	APS, GMCA 23-IDB	APS, GMCA 23-IDD
Wavelength (Å)	1.0332	1.0332
Temperature (K)	100	100
Resolution (Å)	29.1-1.9 (1.93-1.90)	29.8-2.0 (2.03-2.00)
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions (Å)	a = 77.8, b = 103.6, c = 211.2	a = 76.6, b = 104.9, c = 194.1
Cell dimensions (°)	α = β = γ = 90	α = β = γ = 90
Observed reflections	537,061 (24,946)	476,084 (23,408)
Unique reflections	129,672 (6,274)	104,126 (5,172)
R_{meas} (%)	16.0 (110.4)	12.7 (124.3)
R_{merge} (%)	12.6 (88.7)	10.3 (99.7)
R_{pim} (%)	9.7 (64.9)	7.5 (73.2)
<I/σ>	7.9 (1.7)	8.9 (1.3)
CC(1/2)	0.993 (0.636)	0.996 (0.622)
Multiplicity	4.1 (4.0)	4.6 (4.5)
Completeness (%)	96.5 (95.4)	98.4 (99.8)
Overall *B* (Å^2) (Wilson plot)	24.6	28.3
Refinement		
Resolution range	29.0-1.9	29.8-2.0
Number of reflections (work/test set)	129,574/6,579	98,704/5,352
R_{work}/R_{free} (%)	16.2/20.0	16.5/20.6
No. of non-H atoms		
Protein	19,823	19,874
Water	1,390	1,127
Ligand: B_{12}	178	178
Ado	31	31
I-CoA	-	56
B-factors (Å^2)		
Protein	29.4	35.0
Water	35.5	37.8
Ligand: B_{12}	23.4	28.3
Ado	37.4	25.6
I-CoA	-	29.9
Rmsd deviations		
Bond lengths (Å)	0.011	0.008
Bond angles (°)	1.254	1.623
Ramachandran plot		
Favored/allowed/outliers	98.3/1.7/0.0	98.3/1.4/0.1
MolProbity Score	0.99 (100th percentile)	1.2 (100th percentile)
PDB	6OXC	6OXD

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