Supporting Information

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**Bismuth Complexes Inhibit the SARS Coronavirus**

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**Expression and purification of the SCV helicase protein.** The SCV helicase domain (nsp13-pp1ab, accession number NP_828870, originally denoted as nsp10) was cloned and purified as previously described.[12]

**Expression and purification of metal binding domain (MBD) from the SCV helicase** (E. J. Snijder et al., J. Mol. Biol. 2003, 331, 991-1004). The MBD (defined as comprising residues Ala1-Ser100 (inclusive) of the identified nsp13 protein) was amplified by PCR using Taq polymerase, using the plasmid pHelA12[11] as a template, with the primers MBD1for (TATACCATGGCTGAGGTTTTTGTA) and MBDrev1 (TTAACTCGAGTTAACTGCTACACATGGTTTTTGTA). The PCR product was digested with NcoI/XhoI (sites underlined in primer sequences) and ligated into a similarly digested pET32a vector (Novagen) to create plasmid MB32-2. This plasmid was digested with NdeI then self-ligated to remove the encoded N-terminal Trx fusion gene, creating plasmid (MB32-2∆NdeI). Two liters of LB broth supplemented with ampicillin (50 µg/ml) were inoculated with saturated MB32-2∆NdeI/BL21 (DE3) culture (1/200 dilution) and grown at 37 °C until A600 = 0.6. Protein expression was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside (0.25 mM), and cultures were incubated at 37 °C for 4 h. The cells were harvested by centrifugation and lyzed by sonication. The protein pellets were unfolded in 6 M Guanidine-HCl and then refolded in a similarly digested pET32a vector (Novagen) to create plasmid MB32-2. This plasmid was digested with NdeI then self-ligated to remove the encoded N-terminal Trx fusion gene, creating plasmid (MB32-2∆NdeI). Two liters of LB broth supplemented with ampicillin (50 µg/ml) were inoculated with saturated MB32-2∆NdeI/BL21 (DE3) culture (1/200 dilution) and grown at 37 °C until A600 = 0.6. Protein expression was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside (0.25 mM), and cultures were incubated at 37 °C for 4 h. The cells were harvested by centrifugation and lyzed by sonication. The protein pellets were unfolded in 6 M Guanidine-HCl and then refolded in buffer containing Zn2+ (20 mM Tris-HCl pH 8.5, 200 mM NaCl, 50 µM ZnCl2, 100 µM TCEP, 10% glycerol). The refolded MBD was purified on a nickel-charged 5ml HiTrap chelating column. After removal of the short N-terminal fusion by thrombin cleavage, the MBD was further purified by gel filtration chromatography on a HiLoadTM 26/60 Superdex 200 column on an FPLC system.

**ATPase assays.** ATPase assays were performed using the phosphomolybdate-malachite green assay described previously[12]. Titration of ATPase activity with RBC in the presence of fixed concentrations of polynucleotide and ATP was described by a modified logistic equation: \( A([L]) = 1-\frac{(A_\infty([L]))/(EC_{50} + [L])}. \) Assay conditions were 200 nM (dT)24, 0.5 mM ATP, 5 mM MgCl2 in 50 mM Tris-HCl pH 8.5, 200 mM NaCl, 50 µM ZnCl2, 100 µM TCEP, 10% glycerol.

**DNA unwinding (helicase) assays.** Assays were performed as described previously,[12] using the 32P-O4-radio labelled 5T-duplex DNA substrate described therein, which contains a 5' single stranded overhang.

**FRET-based DNA unwinding (helicase) assays.** The principal behind the FRET-based assay is thus: two nucleotide oligomers are designed so that they anneal with a 5’ overhang. At the 3’ end of the longer oligomer (that has the 5’ overhang) there is a Cy3 fluorophore. At the 5’ end of the shorter oligomer there is a BHQ-2 quencher. When the two oligomers are annealed, the Cy3 fluorescence is quenched by a FRET effect, due to the close proximity of the BHQ-2 moiety. If the oligomers are dissociated via the actions of the helicase protein, then the strong fluorescent emission from Cy3 (maximal absorption 550 nm, maximal emission 570 nm) is restored. For the FRET-based assays, we used a protocol modified from that described[23], using oligomers suitable for monitoring the activities of a helicase with 5’ to 3’ polarity. Two DNA oligomers were synthesized and purified by HPLC: DT20Cy3 (5’-TTTTTTTTTTTTTTTTTTTTGACCGACCCGCTGCGCATCC(Cy3)-3’), and ReleaseBHQ (5’-(BHQ2)GGTACAAGGCGAGCGGTGCTG-3’) (Proligo). The two oligomers were annealed by mixing a 1:1.2 ratio of DT20Cy3:ReleaseBHQ at a concentration of 8.2 µM (of DT20Cy3) in 10 mM Tris-HCl pH 8.5, heating to
90°C, then cooling slowly to 40°C over one hour. The FRET assay was performed in a fluorescence cuvette, with a reaction volume of 1 ml containing: 5 nM DT20Cy3:ReleaseBHQ, 10 nM Release oligomer (5'-GGTGCGACGGCAGCGGTCGCTG-3'), 0.5 mM ATP, 0.1 mg/ml BSA, 2 nM SCV helicase, 5 mM MgCl₂, 50 mM Tris-HCl pH 6.8. Reactions were incubated at 25°C for 1 minute, and then the change in fluorescence (λ_ex = 550 nm, λ_em = 570 nm) was measured to determine the extent of DNA duplex unwinding.

**Cytopathic effects.** Fetal rhesus kidney (FRhK-4) cells were plated onto 96-well plates (200 cells per well, in minimum essential medium). To determine the anti-SCV activities of the bismuth complexes, the FRhK-4 cultures were treated with a range of different concentrations 10 minutes before infection with SCV (strain GZ50). Thirty-six hours post-infection, cytopathic effects (CPEs) were observed by phase-contrast microscopy.

**Viral viability assays.** FRhK-4 cell cultures were infected with SCV either one hour before or one hour before addition of RBC. Following incubation for 24, 36, or 48 hours, viable SCV production was measured by back titration of the culture media supernatant using a TCID₅₀ (50% tissue culture infectious dose) protocol. The TCID₅₀ was determined by observation of cytopathic effects in FRhK-4 cells after 3 days of culture.

**Drug cytotoxicity.** Drug cytotoxic concentrations were determined using a standard methylthiazolyl diphenyl-tetrazolium bromide (MTT) assay using amanitin (30 µg/ml) as a toxic (positive) control.

**Quantitative Real-time PCR (Q-RT-PCR).** FRhK-4 cells (from control cultures, as well as from Bismuth-treated and untreated pre- and post SCV-infected cultures) were washed twice with PBS, and total RNA was extracted using the RNeasy Mini kit (Qiagen, Germany) in accordance with the manufacturer’s instructions. Reverse-transcription was performed using random hexamers with the ThermoScript RT system (Invitrogen, CA). Intracellular viral RNA was quantified using Q-RT-PCR, using the forward primer 5'-GCT TAG GCC CTT TGA GAG AGA CA-3' and the reverse primer 5'-GCC AAT GCC AGT AGT GGT GTA AA-3' (final concentration 200 nM), the fluorescent probe 5'-CCT GAT GGC AAA CCT TGC AC-3' and phosphate probe 5'-(LC640)CAC CTG CTC TTA ATT GTT ATT GGC C-3' (final concentration 800 nM) which anneal to the spike protein region of the SCV genome. Real-time quantification was carried out using LC Faststart DNA Master Hyb Probes and a LightCycler (Roche Diagnostics, USA). PCR conditions employed were: 95 °C for 10 min; and then 50 cycles at 95 °C for 10 sec, 60°C for 5 sec, 72°C for 5 sec and 40°C for 30 sec. The increase in PCR products was monitored for each amplification cycle by measuring the increase in fluorescence caused by the binding of SYBR Green I to double strand DNA. The crossing point values were determined for each sample and specificity of the amplicons was measured by melting curve analysis and visualized by agarose gel electrophoresis. A ten-fold serial dilution of plasmid ranging from 1.5 pg/ml to 1.5 × 10⁶ pg/ml were used as standards, and the housekeeping gene β-actin was used as an endogenous control to normalize for inter-sample variations in the amount of total RNA.
Figure S1. a) The gel filtration profile of Zn\(^{2+}\)-MBD, b) Bi\(^{3+}\)-MBD and c) apo-MBD (MBD incubated with excess EDTA).

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