For *Metallomics*

**Supporting Information**

The S2 Cu(I) site in CupA from *Streptococcus pneumoniae* is required for cellular copper resistance

Yue Fu, Kevin E. Bruce, Hongwei Wu and David P. Giedroc

This file contains **Supporting Tables S1-S2** and **Supporting Figures S1-S6**.
Supporting Table S1. Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Genotype (description)</th>
<th>Antibiotic resistance</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>K643</td>
<td>D39 Δcps ΔcopA:: P_c[kanR-rpsL'] (IU1945 transformed with ΔcopA:: P_c[kanR-rpsL'] amplicon)</td>
<td>Str^R Kan^R</td>
<td>1</td>
</tr>
<tr>
<td>K645</td>
<td>D39 Δcps ΔcupA:: P_c[kanR-rpsL'] (IU1945 transformed with ΔcupA:: P_c[kanR-rpsL'] amplicon)</td>
<td>Str^R Kan^R</td>
<td>1</td>
</tr>
<tr>
<td>IU1690</td>
<td>D39 (Single colony isolate of serotype 2 strain encapsulated D39 NCTC 7466)</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>IU1781</td>
<td>D39 rpsL1 (IU1690 transformed with pulA-rpsL1-rpsG-fusA amplicon)</td>
<td>Str^R</td>
<td>3</td>
</tr>
<tr>
<td>IU1945</td>
<td>D39 Δcps</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>IU5921</td>
<td>D39 rpsL1 ΔcupA:: P_c[kanR-rpsL'] (IU1781 transformed with ΔcupA:: P_c[kanR-rpsL'] amplicon from K645)</td>
<td>Str^R Kan^R</td>
<td>1</td>
</tr>
<tr>
<td>IU5923</td>
<td>D39 rpsL1 ΔcopA:: P_c[kanR-rpsL'] (IU1781 transformed with ΔcopA:: P_c[kanR-rpsL'] amplicon from K643)</td>
<td>Str^R Kan^R</td>
<td>1</td>
</tr>
<tr>
<td>IU5971</td>
<td>D39 rpsL1 ΔcupA (IU5921 transformed with ΔcupA amplicon)</td>
<td>Str^R</td>
<td>1</td>
</tr>
<tr>
<td>IU5975</td>
<td>D39 rpsL1 ΔcopA (IU5923 transformed with ΔcopA amplicon)</td>
<td>Str^R</td>
<td>1</td>
</tr>
<tr>
<td>IU6041</td>
<td>D39 rpsL1 cupA-(C)-FLAG (IU5921 transformed with cupA-(C)-FLAG amplicon)</td>
<td>Str^R</td>
<td>1</td>
</tr>
<tr>
<td>IU6084</td>
<td>D39 rpsL1 cupA (Δ(2-28)) (IU5921 transformed with cupA Δ(2-28) amplicon)</td>
<td>Str^R</td>
<td>1</td>
</tr>
<tr>
<td>IU6242</td>
<td>D39 rpsL1 copA (C49S) (IU5923 transformed with copA (C49S) amplicon)</td>
<td>Str^R</td>
<td>This study</td>
</tr>
<tr>
<td>IU6458</td>
<td>D39 rpsL1 copA (C49S M172A E216A D347A) = copA MBS (IU5924 transformed with copA MBS fusion amplicon)</td>
<td>Str^R</td>
<td>This study</td>
</tr>
<tr>
<td>IU6585</td>
<td>D39 rpsL1 cupA (M113A; M115A) = cupA (2A) (IU5921 transformed with cupA (2A) amplicon)</td>
<td>Str^R</td>
<td>1</td>
</tr>
<tr>
<td>IU6618</td>
<td>D39 rpsL1 copA (K155E K166E K167E) (IU5924 transformed with copA P Helix change revertant fusion amplicon)</td>
<td>Str^R</td>
<td>This study</td>
</tr>
<tr>
<td>IU7391</td>
<td>D39 rpsL1 copA (C49S K155E K166E K167E) (IU5923 transformed with copA C49S K155E K166E K167E fusion amplicon)</td>
<td>Str^R</td>
<td>This study</td>
</tr>
<tr>
<td>IU7487</td>
<td>D39 rpsL1 cupA_{aa28-123}::copZ_Bst (aa2-69)* (C)-FLAG (IU5921 cupA::kanrpsL1 transformed with cupA_{aa1-27}* copZ_Bst(NC: 000964 aa2-69)*FLAG fusion amplicon)</td>
<td>Str^R</td>
<td>This study</td>
</tr>
<tr>
<td>IU7620</td>
<td>D39 rpsL1 cupA C111S (IU5921 D39 rpsL1 1781 ΔcupA::kanrpsL1 transformed with cupA C111S fusion amplicon)</td>
<td>Str^R</td>
<td>This study</td>
</tr>
</tbody>
</table>
Primers used to synthesize fusion amplicons for this study are listed in Supplemental Table S2. All FLAG-tagged fusions ((C)-FLAG) were made to the carboxyl end of reading frames. The amino acid sequence for the FLAG epitope is DYKDDDDK. aa = amino acids.

Antibiotic resistance markers: KanR, kanamycin; StrR, streptomycin. Concentrations of antibiotics used for S. pneumoniae strains: 250 µg Str per mL (Sigma S6501) and 250 µg Kan per mL (Sigma K0254).

Supporting Table S2. Oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Template</th>
<th>Amplicon Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For construction of IU6242 (copA (C49S))</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1360</td>
<td>TTCGACTGTCCAAGTGTCAGACA</td>
<td>D39</td>
<td>5’ flanking fragment</td>
</tr>
<tr>
<td>TT331</td>
<td>CTTCTTCTAAACAGAATTTCTATTATAAGAGTTGAAGGATGACAGGTAAAG</td>
<td>D39</td>
<td>3’ flanking fragment with copA-FLAG</td>
</tr>
<tr>
<td>TT332</td>
<td>CGTGCTACTCTCCAAACTCTTTATAAGGAATTTCTGTGGAAAGAAGGTAT</td>
<td>D39</td>
<td></td>
</tr>
<tr>
<td>P1361</td>
<td>TCGTTCAAGACGAGGCGATGAATGACGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>For construction of IU6458 (copA (C49S M172A E216A D347A)) = copA MBS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1360</td>
<td>TTCGACTGGTCCAAGTGTCAGACA</td>
<td>IU 6242 (CopA: C49S)</td>
<td>5’ flanking fragment</td>
</tr>
<tr>
<td>KB29</td>
<td>CCAGGGAACCAAGGTATCGCAGCGTTCAGTGC</td>
<td>IU 6242 (CopA: C49S)</td>
<td>MBS mid 1</td>
</tr>
<tr>
<td>KB30</td>
<td>TTGGTAAAGGCAATGCGAAGCTGATACCTTGGTTGCCCTGGGAAC</td>
<td>IU 6242 (CopA: C49S)</td>
<td></td>
</tr>
<tr>
<td>KB31</td>
<td>TGAGACGTATTTTTCATTTTTTCAGCAGAAAAACTGCTCCAAAGAAACG</td>
<td>IU 6242 (CopA: C49S)</td>
<td></td>
</tr>
<tr>
<td>KB32</td>
<td>GTCTTTTTGGGACGAGGTTTTTGTGAAATGAGAATATACGTCTCAAGC</td>
<td>IU 6242 (CopA: C49S)</td>
<td>MBS mid 2</td>
</tr>
<tr>
<td>KB33</td>
<td>GACACACCAATCCCTGAAATTTAGCTGACTGCATGCCAAGCGTCAATCGGCC</td>
<td>IU 6242 (CopA: C49S)</td>
<td></td>
</tr>
</tbody>
</table>

IU9405: D39 rpsL1 cupA C111S-(C)-FLAG (IU5921 D39 rpsL1 1781 ΔcupA::kanrpsL transformed with cupA C111S-(C)-FLAG fusion amplicon) StrR This study

IU9584: D39 rpsL1 cupA H117A-FLAG (IU5921 D39 rpsL1 cupA::kanrpsL transformed with cupA H117A-FLAG fusion amplicon) StrR This study

IU10134: D39 rpsL1 copA ΔMBD-FLAG (Δa2-98) (IU5923 D39 rpsL1 copA::kanrpsL transformed with copA ΔMBD-FLAG fusion amplicon) StrR This study

IU10148: D39 rpsL1 copA WF-to-AA-FLAG (W162A/F165A) (IU5923 D39 rpsL1 copA::kanrpsL transformed with copA WF-AA-FLAG fusion amplicon) StrR This study

IU10316: D39 rpsL1 copA WF-to-LL-FLAG (W162L/F165L) (IU5923 D39 rpsL1 copA::kanrpsL transformed with copA WF-to-LL-FLAG fusion amplicon) StrR This study

IU9405: D39 rpsL1 cupA C111S-(C)-FLAG (IU5921 D39 rpsL1 1781 ΔcupA::kanrpsL transformed with cupA C111S-(C)-FLAG fusion amplicon) StrR This study

IU9584: D39 rpsL1 cupA H117A-FLAG (IU5921 D39 rpsL1 cupA::kanrpsL transformed with cupA H117A-FLAG fusion amplicon) StrR This study

IU10134: D39 rpsL1 copA ΔMBD-FLAG (Δa2-98) (IU5923 D39 rpsL1 copA::kanrpsL transformed with copA ΔMBD-FLAG fusion amplicon) StrR This study

IU10148: D39 rpsL1 copA WF-to-AA-FLAG (W162A/F165A) (IU5923 D39 rpsL1 copA::kanrpsL transformed with copA WF-AA-FLAG fusion amplicon) StrR This study

IU10316: D39 rpsL1 copA WF-to-LL-FLAG (W162L/F165L) (IU5923 D39 rpsL1 copA::kanrpsL transformed with copA WF-to-LL-FLAG fusion amplicon) StrR This study
<table>
<thead>
<tr>
<th>KB34</th>
<th>GCCGATTCAGGACTTGACAGCTAAGATTTCAGGA</th>
<th>IU 6242 (CopA: C49S)</th>
<th>3’ flanking fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1361</td>
<td>TCGTTCAAGCAGGAGCGATGAATGA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For construction of IU6618 (copA C49S K155E K166E K167E) = copA P-helix

<table>
<thead>
<tr>
<th>P1360</th>
<th>TTCGACTCTGGTCCAAGTCAACGGGTTCA</th>
<th>D39 genomic</th>
<th>5’ flanking fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB69</td>
<td>CCAAAGCAGCTCTGGATAGGAAGCTCAGTCAGAACTTCAGGTAATCGGTTG</td>
<td>IU6618 copA K155E K166E K167E</td>
<td></td>
</tr>
<tr>
<td>KB70</td>
<td>CGCCTATTAGTTGGATAGGAAGCTCAGTCAGAACTTCAGGTAATCGGTTG</td>
<td>IU6618 copA K155E K166E K167E</td>
<td></td>
</tr>
<tr>
<td>KB71</td>
<td>AGGTATCCATGGGCTCLCTCCATCATCATCATGAGCTCAGTCAGAACTTCAGGTAATCGGTTG</td>
<td>IU6618 copA K155E K166E K167E</td>
<td></td>
</tr>
<tr>
<td>KB72</td>
<td>GAGTGCTTTGGGCAGTTTTTGAGGAGCGACAAATGCACCAGATCATCATCATGAGCTCAGTCAGAACTTCAGGTAATCGGTTG</td>
<td>IU6618 copA K155E K166E K167E</td>
<td></td>
</tr>
<tr>
<td>P1361</td>
<td>TCGTTCAAGCAGGAGCGATGAATGA</td>
<td>D39 genomic</td>
<td>3’ flanking fragment</td>
</tr>
</tbody>
</table>

For construction of IU7391 (copA C49S K155E K166E K167E)

<table>
<thead>
<tr>
<th>P1356</th>
<th>AGTCCCTGCAATGCTCAACCG</th>
<th>IU6041 cupA-FLAG</th>
<th>5’ flanking fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT331</td>
<td>CTTCCTCAAAACAGAATTTACTTATAGGAAGTTGGAAG</td>
<td>IU6041 cupA-FLAG</td>
<td>5’ flanking fragment</td>
</tr>
<tr>
<td>TT332</td>
<td>CGTGCCTACTTCAACTTCTAAGGGAATTCCTGTCTTCGAAAGATCAATG</td>
<td>IU6041 cupA-FLAG</td>
<td>3’ flanking fragment</td>
</tr>
<tr>
<td>P1361</td>
<td>TCGTTCAAGCAGGAGCGATGAATGA</td>
<td>D39 genomic</td>
<td>3’ flanking fragment</td>
</tr>
</tbody>
</table>

For construction of IU7487 (cupA(aa28-123)::copZ_Bsu (aa2-69)-(C)-FLAG)

<table>
<thead>
<tr>
<th>P1356</th>
<th>AGTCCCTGCAATGCAACGGG</th>
<th>IU6041 cupA-FLAG</th>
<th>5’ flanking fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB208</td>
<td>TCAACTTGCAATGCTCAACCG</td>
<td>IU6041 cupA-FLAG</td>
<td>5’ flanking fragment</td>
</tr>
<tr>
<td>KB207</td>
<td>TTCAAAAAGCCTGAAAAAGAAGATACATGCGAAGTAAGTGGAGGAATTCCTGTCTTCGAAAGATCAATG</td>
<td>IU6041 cupA-FLAG</td>
<td>5’ flanking fragment</td>
</tr>
<tr>
<td>KB209</td>
<td>TATTTATCATCATCATATTTATATATTCCTGTGCTACGTCTAGCACCTGTATCT</td>
<td>IU6041 cupA-FLAG</td>
<td>3’ flanking fragment</td>
</tr>
<tr>
<td>KB210</td>
<td>AGGGCTATAGCTAGCCAAGGATATTATAAGATGATGATGATAATAGTGGAGAGT</td>
<td>IU6041 cupA-FLAG</td>
<td>3’ flanking fragment</td>
</tr>
<tr>
<td>P1357</td>
<td>AGGTCGCTACTTCAACTTGACTTTTCAACGGG</td>
<td>D39 genomic</td>
<td>3’ flanking fragment</td>
</tr>
</tbody>
</table>

For construction of IU7620 (cupA C111S)

<table>
<thead>
<tr>
<th>P1356</th>
<th>AGTCCCTGCAATGCTCAACCG</th>
<th>IU6041 cupA-FLAG</th>
<th>5’ flanking fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB271</td>
<td>CATACCCGAAAGCAAGAGCTAACTCTCCAG</td>
<td>IU6041 cupA-FLAG</td>
<td>5’ flanking fragment</td>
</tr>
<tr>
<td>KB270</td>
<td>CTTTGCCTCTGGTATAGAATGATGAGC</td>
<td>IU6041 cupA-FLAG</td>
<td>3’ flanking fragment</td>
</tr>
<tr>
<td>P1357</td>
<td>AGGTCGCTACTTCAACTTGACTTTTCAACGGG</td>
<td>D39 genomic</td>
<td>3’ flanking fragment</td>
</tr>
</tbody>
</table>

For construction of IU9405 (cupA C111S)-(C)-FLAG

<table>
<thead>
<tr>
<th>P1356</th>
<th>AGTCCCTGCAATGCTCAACCG</th>
<th>IU6041 cupA-FLAG</th>
<th>5’ flanking fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB271</td>
<td>CATACCCGAAAGCAAGAGCTAACTCTCCAG</td>
<td>IU6041 cupA-FLAG</td>
<td>5’ flanking fragment</td>
</tr>
<tr>
<td>KB270</td>
<td>CTTTGCCTCTGGTATAGAATGATGAGC</td>
<td>IU6041 cupA-FLAG</td>
<td>3’ flanking fragment</td>
</tr>
<tr>
<td>P1357</td>
<td>AGGTCGCCTACCTTGACTTGTTCCAA</td>
<td>fragment</td>
<td></td>
</tr>
<tr>
<td>P1356</td>
<td>AGTCCCTGCAATGGTCAAAGCACCGG</td>
<td>IU6041 copA-FLAG</td>
<td></td>
</tr>
<tr>
<td>KB358</td>
<td>CTCTCAAATCATCTTGCACACCATCATGTCATACCAACAAACAAAGC</td>
<td>5’ flanking fragment</td>
<td></td>
</tr>
<tr>
<td>KB359</td>
<td>CTTGTGTTATGACATGATGCTGCAAGATGATTGTAGGAGATTATAGATG</td>
<td>3’ flanking fragment</td>
<td></td>
</tr>
<tr>
<td>P1357</td>
<td>AGGTCGCCTACCTTGACTTGTTCCAA</td>
<td>fragment</td>
<td></td>
</tr>
</tbody>
</table>

**For construction of IU9584 (copA H117A-(C)-FLAG)**

| P1360 | TTGGACTGGTCCAAGTGGAAGGTTCAGCA | IU6044 copA-FLAG |
| KB387 | GATTTTCGAGTCTTCATAGTCTCCACCTATCTCTACAATCATCCTGC | 5’ flanking fragment |
| KB388 | GATTTTCGAGTCTTCATAGTCTCCACCTATTTATCATCATCTTTATATACCTCTAC | 3’ flanking fragment |
| P1361 | TCGTTCAAGAGTGAGGAGTGATGAATGA | |

**For construction of IU10134 (copA AMBD-(C)-FLAG)**

| P1360 | TTGGACTGGTCCAAGTGGAAGGTTCAGCA | IU6044 copA-FLAG |
| KB389 | CATGTTGGCATTTGCTTTTTAGCACTGGCAGCAGCAGCAGCACTCTGGATATATGGCTTACCTGC | 5’ flanking fragment |
| KB390 | AAGCCTATATCCAGAGTGCTGCTGCTCGAGGTGCTAA | 3’ flanking fragment |
| P1361 | TCGTTCAAGAGTGAGGAGTGATGAATGA | |

**For construction of IU10148 (copA WF-to-AA-(C)-FLAG)**

| P1360 | TTGGACTGGTCCAAGTGGAAGGTTCAGCA | IU6044 copA-FLAG |
| KB391 | CATGTTGGCATTTGCTTTTTTAGAAGACTGGCAGCAGCAGCAGCACTCTGGATATATGGCTTACCTGC | 5’ flanking fragment |
| KB392 | AAGCCTATATCCAGAGTGCTGCTGCTCGAGGTGCTAA | 3’ flanking fragment |
| P1361 | TCGTTCAAGAGTGAGGAGTGATGAATGA | |

**For construction of IU10316 (copA WF-to-LL-(C)-FLAG)**

| P1360 | TTGGACTGGTCCAAGTGGAAGGTTCAGCA | IU6044 copA-FLAG |
| KB391 | CATGTTGGCATTTGCTTTTTTAGAAGACTGGCAAGGCAGCAGCAGCAGCAGCACTCTGGATATATGGCTTACCTGC | 5’ flanking fragment |
| KB392 | AAGCCTATATCCAGAGTGCTGCTGCTCGAGGTGCTAA | 3’ flanking fragment |
| P1361 | TCGTTCAAGAGTGAGGAGTGATGAATGA | |

*Sequences in italic and bold letters represent FLAG tag. Underlined sequences indicate sequence changes to produce the desired mutation. Genomic DNA of the indicated S. pneumoniae strains was used as templates for PCR reactions. aa = amino acids.*
Supporting Figure S1. Build-up curves as a function of intensity ratio ($I_a/I_b$) (see Methods, main text) used to determine the intramethyl $^1$H-$^1$H dipole-dipole cross-correlated relaxation rate ($\eta$) for each of the seven Met $\epsilon$CH$_3$ groups in sCupA in each of three ligation states, apo (red), 1 Cu bound to the S1 sites (blue), and both S1 and S2 sites filled (green). $\eta$ is related to the axial order parameter, $S^2_{\text{axis}}$ and the molecular rotational correlation time, $\tau_c$ (see Figure 4, main text). Data for Met46 in the Cu$_1$ state were obscured by resonance overlap and are therefore not shown.
Supporting Figure S2. Cu(I) binding affinity titrations obtained for (A) C111S sCupA and (B) sCupA^{2MA}. (A) For C111S sCupA, 21 or 30 µM CuCl was mixed with 50 µM (red) or 70 µM (blue) BCA and protein was titrated into this mixture. The absorbance of BCA:Cu(I) complex was monitored at 562 nm. (B) For CupA^{2MA}, 18, 28, 37 or 48 µM CuCl was mixed with 44 µM (red), 60 µM (blue), 71 µM (cyan) or 93 µM (green) BCS and protein was titrated into this mixture. The absorbance of BCS:Cu(I) complex was monitored at 483 nm. Lines in each panel represent the results of a global fit of a 1:1 binding model as described previously.\textsuperscript{1}
Supporting Figure S3. $^1$H-$^{13}$C HSQC spectra showing 1H-13C crosspeaks of the $\varepsilon$CH$_3$ Met methyl groups recorded for sCupA$^{2MA}$ (A), C111S sCupA (B) and the triple substitution mutant C111S/M116A/H117A (C) in the absence (apo, red crosspeaks) and presence (blue crosspeaks) of 0.9 mol equivalents Cu(I). These data reveal that all mutant proteins are folded and that the C111S substitution introduces considerable chemical exchange broadening into the S2 Cu-binding site region that is not quenched by Ala substitution of immediately adjacent, potential non-native Cu(I)-ligating residues M116 and H117. The methyl groups of S2 Cu(I)-ligating M113 and M115 are significantly broadened in panels B and C when Cu(I) is added. No $\varepsilon$CH$_3$ Met resonances outside of this spectral window were observed, in contrast to the wild-type protein.\(^1\)
Supporting Figure S4. Microaerophilic growth rate analysis of $cupA^{C111S}$ and $cupA^{2MA}$ strains compared to parent isogenic $\Delta cupA$ and wild-type (WT) strains in the presence of 0.5 mM Cu. A $cupA^{2MA}$ mutant strain (red symbols) exhibits a growth phenotype indistinguishable from a $\Delta cupA$ strain (X), while a $cupA^{C111S}$ mutant (blue symbols) grows like WT (black filled circles) when 0.5 mM Cu is added to the BHI growth medium at $t=0$. 
Supporting Figure S5. Total [Cu] (ng Cu/mg protein) in the indicated strains as measured by ICP-MS in the absence (open bars) or presence (filled bars) of 0.5 mM Cu added to cultures at OD620≈0.03. These data represent mean ± S.E. from three biological replicates. Statistical significance was determined using one-way ANOVA with Tukey post-test where ***p<0.0001; *, p<0.05. WT, wild-type strain; MBS, M172A/E216A/D347A copA strain; ΔMBD, copA lacking the N-terminal metal binding domain (MBD; residues 1-99), WF-to-AA, W162A/F165A copA strain. The MBS strain also carries a copAC49S substitution which has no detectable growth phenotype.¹
Supporting Figure S6. (A) Representative growth curves (BHI + 0.5 mM Cu) obtained for wild-type (WT) (black filled circles), a copA strain expressing a triple mutant (K155E/K166E/K167E) CopA (P-helix) (blue filled circles), a copA strain expressing a quadruple mutant (C49S/K155E/K166E/K167E) CopA (P-helix C49S) (red filled circles), a copA strain expressing the triple MBS mutant in a C49S background (MBS C49S) (green filled circles), compared to a ΔcopA strain (x) which fails to grow under these conditions. The copA<sup>C49S</sup> strain (not shown) grows like WT under these conditions.<sup>1</sup> (B) Space filling model of the MBS region in a homology model of <i>S. pneumoniae</i> CopA threaded through <i>Lp</i> apo-CopA<sup>4</sup> with basic residues surrounding the MBS and the platform helix (P-helix) highlighted. Surface electrostatic charge is represented by blue (positive potential) and red (negative potential). The approximate placement of the CopA<sup>MBD</sup> of known structure<sup>1</sup> within the CopA model is indicated; the work presented here is consistent with a model in which the S2 Cu(I) of CupA delivers Cu(I) directly to the MBS as indicated by the grey arrow (right). The direction of the Cu transport is indicated by the light blue arrow.
REFERENCES


