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## Supplementary Materials

### **Functional characterization of amphipathic $\alpha$ -helix in the osmoregulatory ABC transporter OpuA**

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| Mutant   | Modification   | Primers (5'- 3')   |
|----------|--|--|
| T23C     | T23C   | <u>CCATAGCAA</u> ACTGGGTGTCATCGGCAACAGATTGGATT <b>TG</b> TAGACTTTTAGTT<br>CAGGATTT <b>GACGT</b><br><u>CAAATCCTGAACTAAA</u> AGTACTAC <b>CA</b> AATCCAATCTGTTGCCGATGACACCCAGT<br>TTGCTAT <b>GGTAC</b>          |
| S24C     | S24C   | <u>CCATAGCAA</u> ACTGGGTGTCATCGGCAACAGATTGGACT <b>TT</b> GTACTTTTAGTTC<br>AGGATTT <b>GACGT</b><br><u>CAAATCCTGAACTAAA</u> AGTAC <b>AA</b> GTAAATCCAATCTGTTGCCGATGACACCCAGTT<br>TGCTAT <b>GGTAC</b>           |
| T25C     | T425C  | <u>CCATAGCAA</u> ACTGGGTGTCATCGGCAACAGATTGGACTAG <b>TTG</b> TTTTAGTTC<br>AGGATTT <b>GACGT</b><br><u>CAAATCCTGAACTAAA</u> CAACTAGTAATCCAATCTGTTGCCGATGACACCCAGTT<br>TGCTAT <b>GGTAC</b>                       |
| Scramble | N13W, W14N,<br>S17A, A18S,<br>I22T, T23I ,<br>F26S, S27F | <u>CCATAGCA</u> <b>TGGAATGTGTCAGCTTCAACAGATTGGACAATTAGTACTTCATTTCA</b><br>GGATTT <b>GACGT</b><br><u>CAAATCCTGAA</u> <b>AATGAAGTACTAATTGTCCAATCTGTTGAAGCTGACACATTCCA</b><br>TGCTAT <b>GGTAC</b>               |
| Pltp1    | FKKVYDFLSTFI<br>TSGMRF                                   | <u>CCTTTAAAAA</u> AGTTTATGATTTTTTATCA <b>ACA</b> TTTATTACTAGTGGTATGCGTTTTG<br>GATTT <b>GACGT</b><br><u>CAAATCCAAAACGC</u> ATACCACTAGTAATA <b>AA</b> TGTTGATA <b>AAAAATCATAAACTTTT</b><br><b>TTAAAGGGTAC</b>  |
| Pltp2    | FANVYDFLSTFI<br>TSGMSF                                   | <u>CCTTTGCTAA</u> TGTTTATGATTTTTTATCAACATTTATTACTAGTGGTATG <b>TC</b> ATTTG<br>GATTT <b>GACGT</b><br><u>CAAATCCAAA</u> TGACATACCACTAGTAATAAAATGTTGATA <b>AAAAATCATAAACTTA</b><br><b>GCAAAGGGTAC</b>           |
| Pltp3    | FANVYSFLSDFI<br>TSGMSF                                   | <u>CCTTTGCTAA</u> TGTTTAT <b>TC</b> ATTTTTATCAGATTTTATTACTAGTGGTATG <b>TC</b> ATTTG<br>GATTT <b>GACGT</b><br><u>CAAATCCAAA</u> TGACATACGACTAGTAATA <b>AAATCTGATAAAAAATGAATAAACATT</b><br><b>AGCAAAGGGTAC</b> |
| 3K       | S17K, S24K,<br>S28K                                      | <u>CCATAGCAA</u> ACTGGGTGTC <b>AAA</b> GCAACAGATTGGACTACT <b>AAA</b> ACTTTTAGT <b>A</b><br><b>AAGGATTTGACGT</b><br><u>CAAATCCTTTACTAAA</u> AGTTTAGTAATCCAATCTGTTGCTTTTGACACCCAGT<br>TTGCTAT <b>GGTAC</b>     |
| A        | SGTVLMN <b>AGI</b><br>GAL                                | GCACTG <b>GGCGCGCTA</b> ACTGCTGTTCCATTCTGGTTGATG (fwd A)<br>CAGTCG <b>TAGCGCGCC</b> TGTAATACC <b>AGC</b> GTTTCATCAATACCGTTCC   |
| AA       | SGTVLMN <b>AAGI</b><br>TGAL                              | GCACTG <b>GGCGCGCTA</b> ACTGCTGTTCCATTCTGGTTGATG (fwd A)<br>CAGTCG <b>TAGCGCGCC</b> TGTAATACC <b>AGCAGC</b> GTTTCATCAATACCGTTCC  |
| AAA      | SGTVLMN <b>AAA</b><br>GITGAL                             | GCACTG <b>GGCGCGCTA</b> ACTGCTGTTCCATTCTGGTTGATG (fwd A)<br>CAGTCG <b>TAGCGCGCC</b> TGTAATACC <b>AGCAGCAGC</b> GTTTCATCAATACCGTTCC   |
| AAAA     | GTVLMN <b>AAAA</b><br>GITGAL                             | GCACTG <b>GGCGCGCTA</b> ACTGCTGTTCCATTCTGGTTGATG (fwd A)<br>CAGTCG <b>TAGCGCGCC</b> TGTAATACC <b>AGCAGCAGCAGC</b> GTTTCATCAATACCGTTCC  |

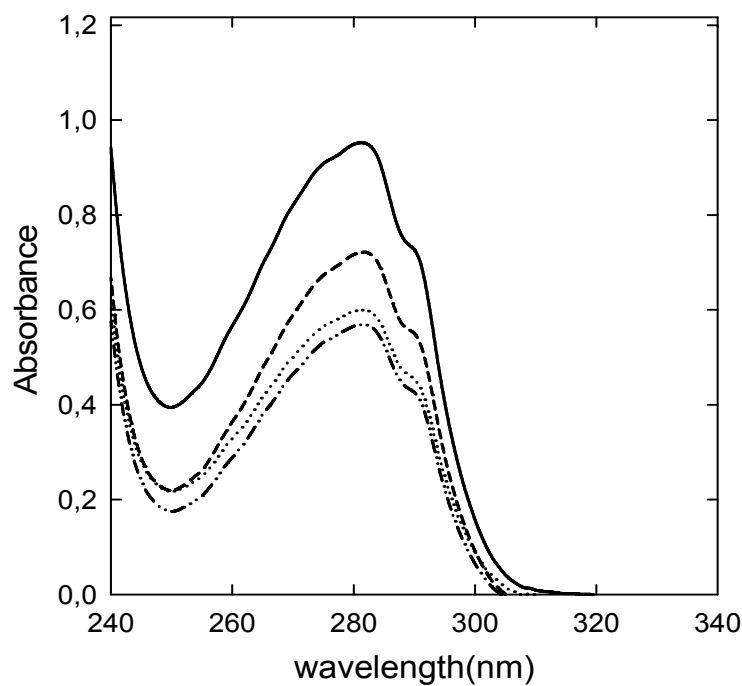
**STable1:** Primers used for construction of the mutants. Restriction sites are indicated in bold and underlined. Nucleotides different from wild type OpuA are indicated by red color.

| Protein          | Labeling efficiency (%) | ATPase activity (mmol/min*mg of OpuA) |
|------------------|-------------------------|---------------------------------------|
| WT               | -                       | 3.5 ±0.1                              |
| S24C-5FM         | 80                      | 3.74 ±0.04                            |
| V295C-5FM        | 89                      | 3.1 ±0.1                              |
| nanodisc wo OpuA | -                       | 0.26 ±0.01                            |

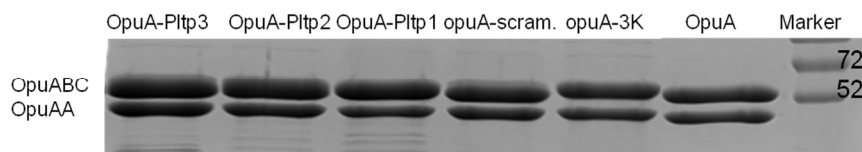
**STable 2.** Labeling efficiency and ATPase activity of Cys mutants.

The labeling efficiency was determined from the molar ratio of OpuA and F5M calculated from the absorbance at 280 nm and 495 nm and the molar extinction coefficient as described in Materials and methods. The ATPase assay was performed in 50 mM KPi, pH 7.0, plus 300 mM KCl as described in Materials and methods.

A



B



**Figure 1: Purification and stability of wildtype OpuA and mutant derivatives.**

(A): UV spectra of Ni-Sepharose purified proteins: wildtype OpuA (solid black line), OpuA3K (dashed and dotted line), OpuA-Pltp1 (dotted line) and OpuA-scrambled (dashed lines).

(B): SDS-PAGE of purified proteins. The proteins were separated on a 12.5% polyacrylamide gel and stained with Coomassie brilliant blue. 5  $\mu$ g of protein was loaded per lane.