Supplementary Materials

Evolution of Plant Na^+-P-Type ATPases: From Saline Environments to Land Colonization

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Figure captions

Supplementary Table S1. 1. Domain-based classification of the P-ATPases from species used in this study. Known Na^+/K^+-transporting P-ATPases are marked with green. Unusual metal-binding domain (PF12156) is marked with yellow. 2. List of species used in this study. Taxonomy was simplified. 3. List of Na^+/K^+-transporting ATPases from green and red Algae and Animalia used to extract β-like subunit insertions (algae) and compare to the β subunit from Animalia. 4. List of Bacteria species with β subunit and algae with β-like subunit insertions.

Supplementary Figure S1. **(a)** Comparison of Ca^{2+} (depicted in yellow circles) binding sites from known sodium/potassium-transporting P-ATPases (identified by [51,56]). AtACA4 and AtACA2 proteins, known to be involved in NaCl tolerance [71,72], highlighted in bold. **(b)** Comparison of Na+ binding sites from known sodium/potassium-transporting P-ATPases identified by [51] (depicted in magenta circles) and [57] (depicted in green squares). AtACA4 and AtACA2 proteins, known to be involved in NaCl tolerance [72,73], highlighted in bold. **(c)** The first C-terminal insertion into the green (*Ostreococcus lucimarinus*, *Micractinium conductrix*, and *Chlamydomonas reinhardtii*) and red (*Porphyridium purpureum* and *Gracilariopsis chorda*) algae's α-subunit with a high similarity to the animal's β-subunit. **(d)** Conserved catalytic D site, identified in α-subunit, depicted in magenta. AtACA4 and AtACA2 proteins known to be involved in NaCl tolerance [72,73], highlighted in bold. **(e)** Amino acids of the α-subunit important for the interaction with γ-subunit (identified in *Sus scrofa* (4HQJ) [57], depicted with yellow dots). AtACA4 and AtACA2 proteins, known to be involved in NaCl tolerance [72,73], are highlighted in bold.

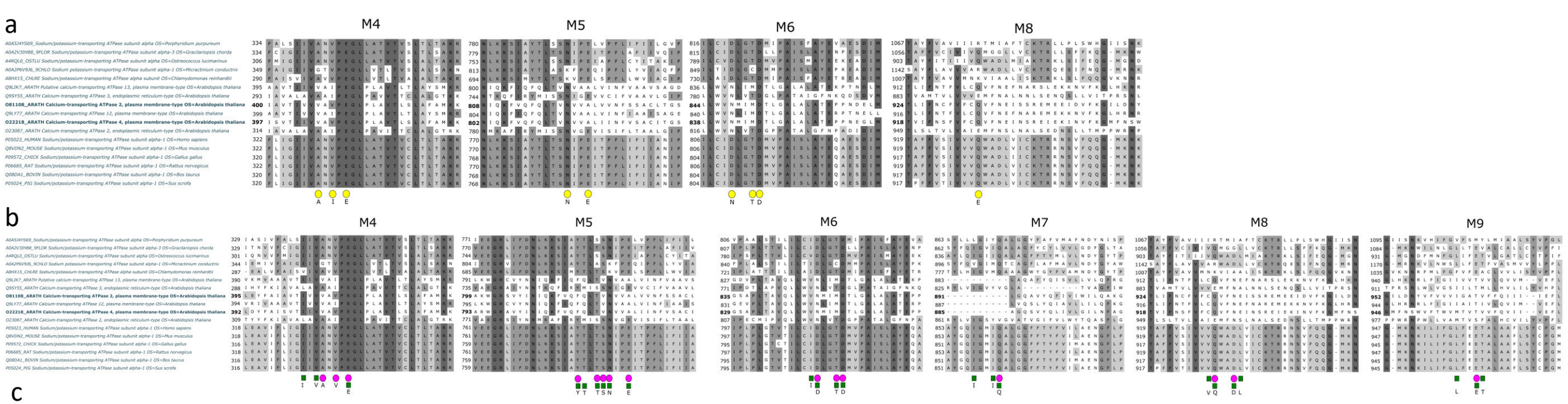
Supplementary Figure S2. Structural alignment of the β-subunits from human (P05026) (magenta), *Anthurium amnicola* (A0A1D1Y1U2) (green) and *Gammaproteobacteria bacterium 2W06* (PYZ99283.1) (cyan). Alignment scores: (A) human vs. bacteria 164.32, RMSD 0.70; (B) human vs. plant 182.51, RMSD 0.67; (C) bacteria vs. plant 305.99, RMSD 0.98. iPBA webserver was used for the pdb structures alignment (https://www.dsimb.inserm.fr/dsimb_tools/ipba/index.php).

Supplementary Figure S3. The second C-terminal insertion into the green (*Ostreococcus lucimarinus*, *Micractinium conductrix* and *Chlamydomonas reinhardtii*) and red (*Porphyridium purpureum* and *Gracilariopsis chorda*) algae α-subunit with high similarity to the animal's β-subunit.

Supplementary Figure S4. Amino acids of the α-subunit important for the interaction with β-subunit (identified in *Sus scrofa* (4HQJ) [57]), depicted with blue dots. AtACA4 and AtACA2 proteins, known to be involved in NaCl tolerance [72,73], highlighted in bold.

Supplementary Figure S5. Alignment of the β-subunit-like insertions of the algae (highlighted in bold) with β-subunits from animals, *Anthurium amnicola* (A0A1D1Y1U2), and *Gammaproteobacteria bacterium 2W06* (PYZ99283.1). Amino acids, important for the interaction with β-subunit (depicted with blue dots) and γ-subunit (depicted with orange dots) identified in *Sus scrofa* (4HQJ) [57].

Supplementary Figure S6. Sites evolution. Evolution of the Ca^{2+} and Na^+ binding sites throughout the simplified tree of life. LUCA (last unicellular common ancestor) represents an ancient cell-structure before the split on the "plant" and "animal" lines. The fungi line is not presented. The evolution of the plant started from the incorporation of the cyanobacteria to the ancestor (proto algae), giving rise to the red and green algae lineages. Further evolution line includes "low" plants (mosses) and flowering plants.


a
M4
M5
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M8
b
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M7
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A4RQL0_Sodium/potassium-transporting ATPase subunit alpha OS=Ostreococcus lucimarinus
A0A2P6V9J6_Sodium potassium-transporting ATPase subunit alpha-1 OS=Micractinium conductrix
A8HX15_Sodium/potassium-transporting ATPase alpha subunit OS=Chlamydomonas reinhardtii
P05023_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens
Q8VDN2_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Mus musculus
P09572_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Gallus gallus
P06685_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Rattus norvegicus
Q08DA1_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Bos taurus
P05024_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Sus scrofa
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Q9SY55_Calcium-transporting ATPase 3, endoplasmic reticulum-type OS=Arabidopsis thaliana 343 ICSDKTGTLTTN
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Q9LY77_Calcium-transporting ATPase 12, plasma membrane-type OS=Arabidopsis thaliana 449 ICTDKTGTLTLN
O22218_Calcium-transporting ATPase 4, plasma membrane-type OS=Arabidopsis thaliana 447 ICTDKTGTLTTN
O23087_Calcium-transporting ATPase 2, endoplasmic reticulum-type OS=Arabidopsis thaliana 364 ICSDKTGTLTTN
P05023_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens 372 ICSDKTGTLTQN
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P09572_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Gallus gallus 370 ICSDKTGTLTQN
P06685_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Rattus norvegicus 372 ICSDKTGTLTQN
Q08DA1_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Bos taurus 370 ICSDKTGTLTQN
P05024_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Sus scrofa 370 ICSDKTGTLTQN
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P05023_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens 947 QG-MKNKILIFGLFEETALAAFLSYCPGMGVALRMYPLKPTWFCA
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Figure S1

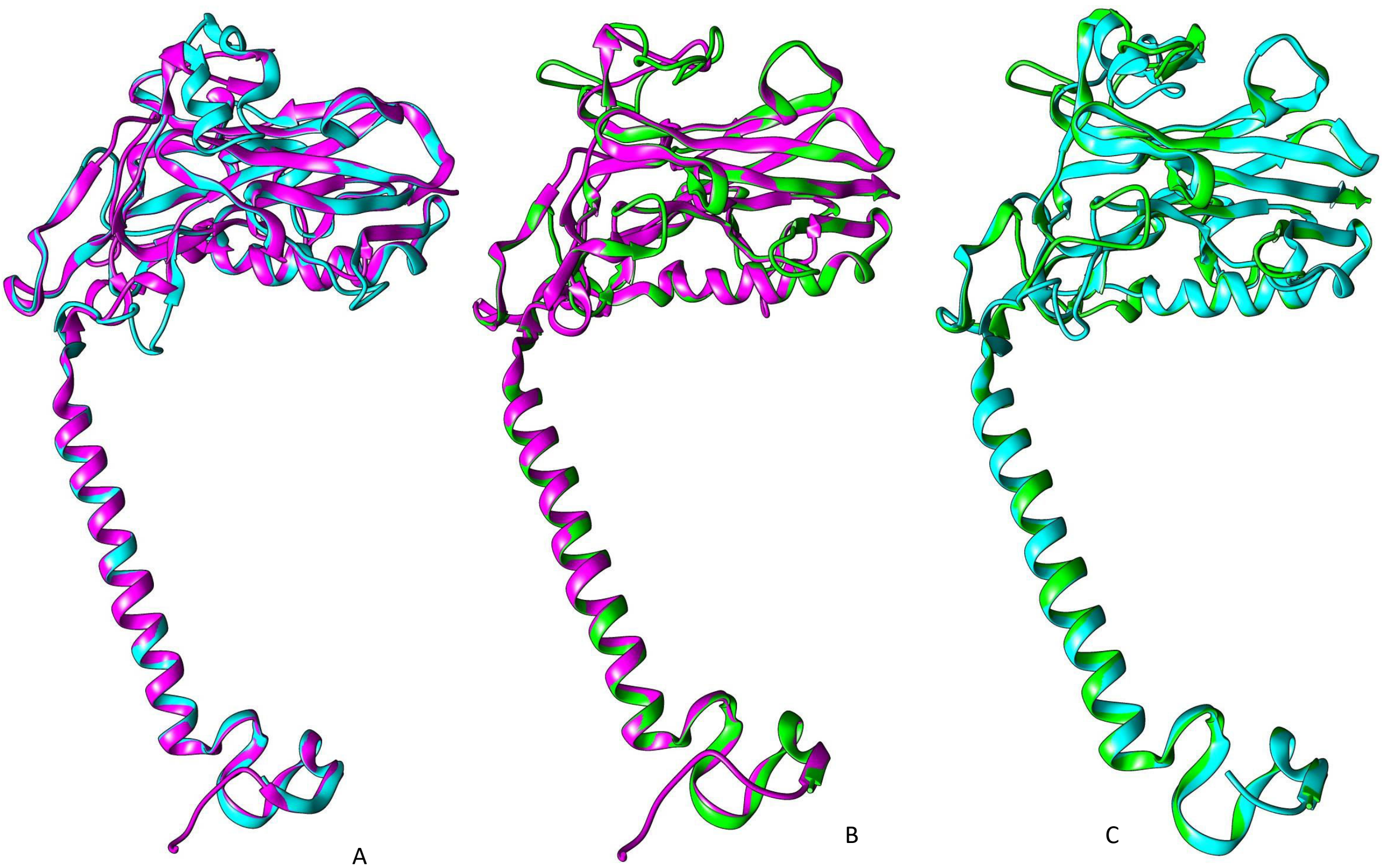

Figure S2


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P09572_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Gallus gallus 897 924
P06685_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Rattus norvegicus 899 926
Q08DA1_Sodium/potassium-transporting ATPase subunit alpha-1 OS=Bos taurus 897 924
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Figure S3

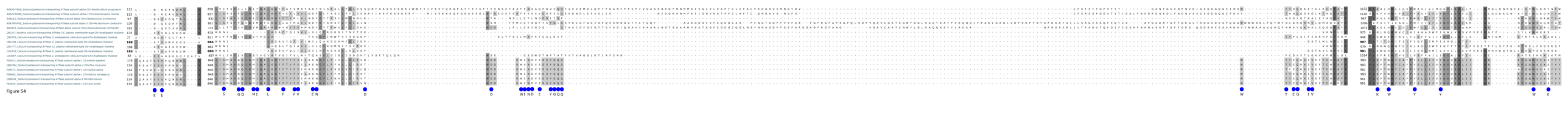

Figure S4

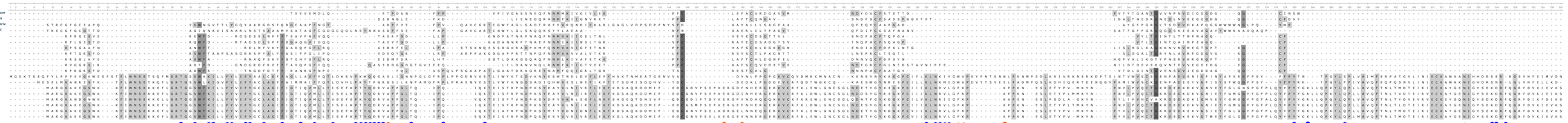
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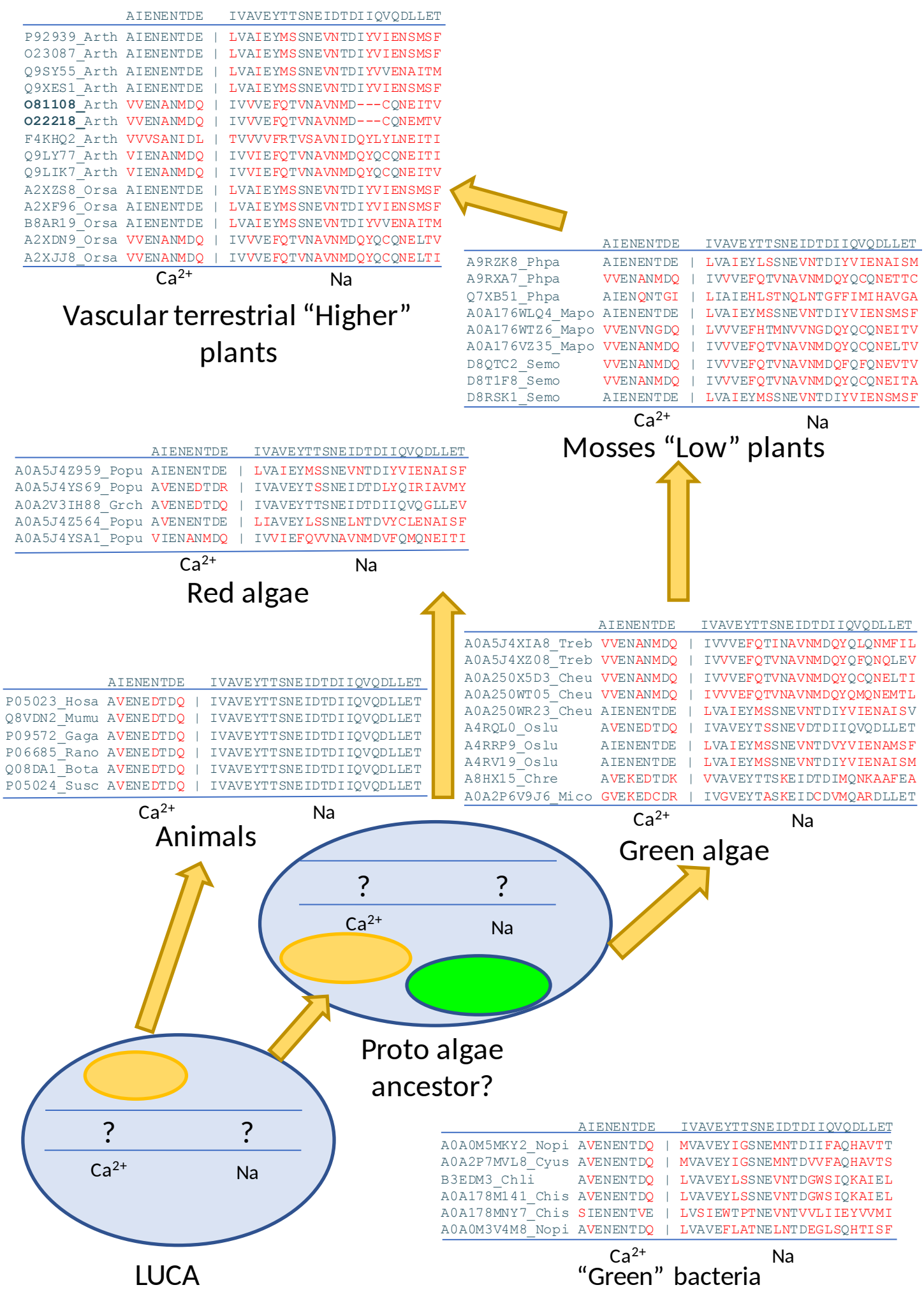
Figure S5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Ca2+
Na
Vascular terrestrial “Higher” plants
AIENENTDE IVAVEYTTSNEIDTDIIQVQDLLET
A9RZK8_Phpa AIENENTDE | LVAIEYLSSNEVNTDIYVIENAISM
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D8RSK1_Semo AIENENTDE | LVAIEYMSSNEVNTDIYVIENSMSF
Ca2+
Na
Mosses “Low” plants
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Ca2+
Na
Red algae
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Ca2+
Na
Green algae
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Q8VDN2_Mumu AVENEDTDQ | IVAVEYTTSNEIDTDIIQVQDLLET
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P06685_Rano AVENEDTDQ | IVAVEYTTSNEIDTDIIQVQDLLET
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Ca2+
Na
Animals
? ?
Ca2+
Na
Proto algae ancestor?
? ?
Ca2+
Na
LUCA
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Ca2+
Na
“Green” bacteria

Figure S6