Supplementary material

Biochemical signatures of acclimation by *Chlamydomonas reinhardtii* to different ionic stresses. Elia D. Charles, Howbeer Muhamadali, Royston Goodacre, and Jon K. Pittman

Supplementary Figure 1

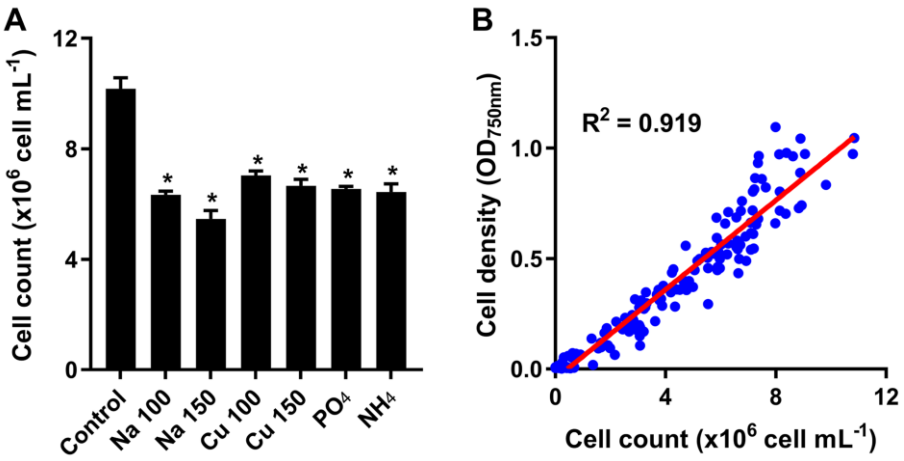

Fig. S1. Cell numbers of *Chlamydomonas reinhardtii* in response to excess Na^{+}, Cu^{2+}, PO_4^{3-} and NH_4^{+}. (A) Cell density determined by cell counts after 7 d batch culture growth of non-acclimated cells in TAP medium amended with Na^{+}, Cu^{2+}, PO_4^{3-} or NH_4^{+} conditions as indicated. Control cells show growth in non-stressed conditions (standard TAP medium). All data are mean values ± SEM from 3 - 4 measurements. An asterisk (*) indicates significant difference ($p < 0.05$) of acclimated cells compared to non-acclimated cells. (B) Correlation plot of cell density values of *C. reinhardtii* determined by optical density at 750 nm (OD_{750nm}) against cell number. Data points are values of individual culture samples taken throughout the 7 d batch culture growth period of non-acclimated and acclimated cells in control conditions or TAP medium amended with Na^{+}, Cu^{2+}, PO_4^{3-} or NH_4^{+} conditions.

Supplementary Figure 2

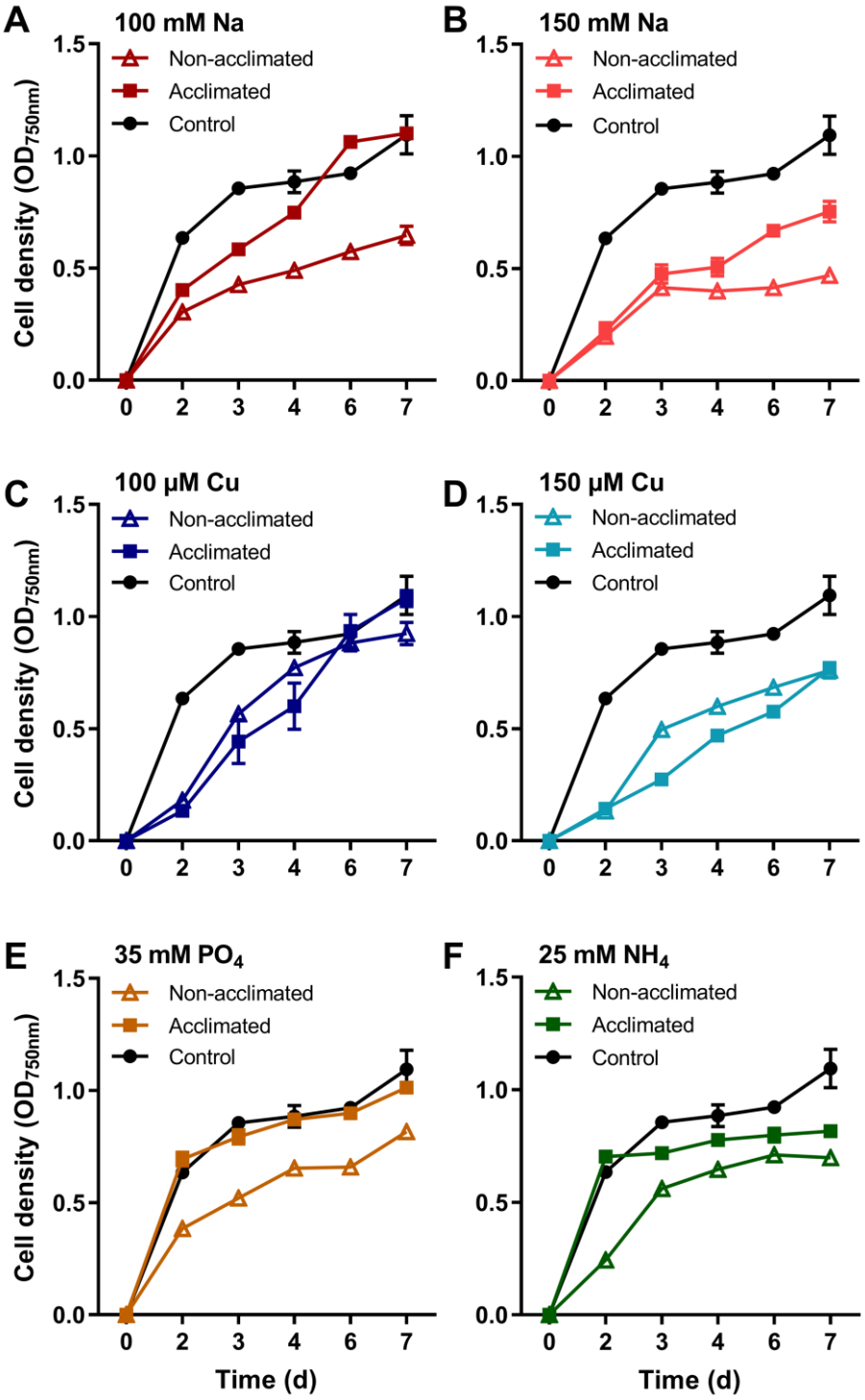

Fig. S2. Batch culture growth profiles in acclimated *Chlamydomonas reinhardtii* cells. Acclimated cell growth compared with non-acclimated cells grown in Na^{+}, Cu^{2+}, PO_4^{3-} or NH_4^{+} conditions as indicated. Control cells show growth in non-stressed conditions (standard TAP medium). Cell density was determined by OD 750nm measurement. All data are mean values ± SEM from measurements from 3 different culture bottles, and are representative of a batch culture growth profile of one of the independent sets of acclimated and non-acclimated cultures.

Supplementary Figure 3

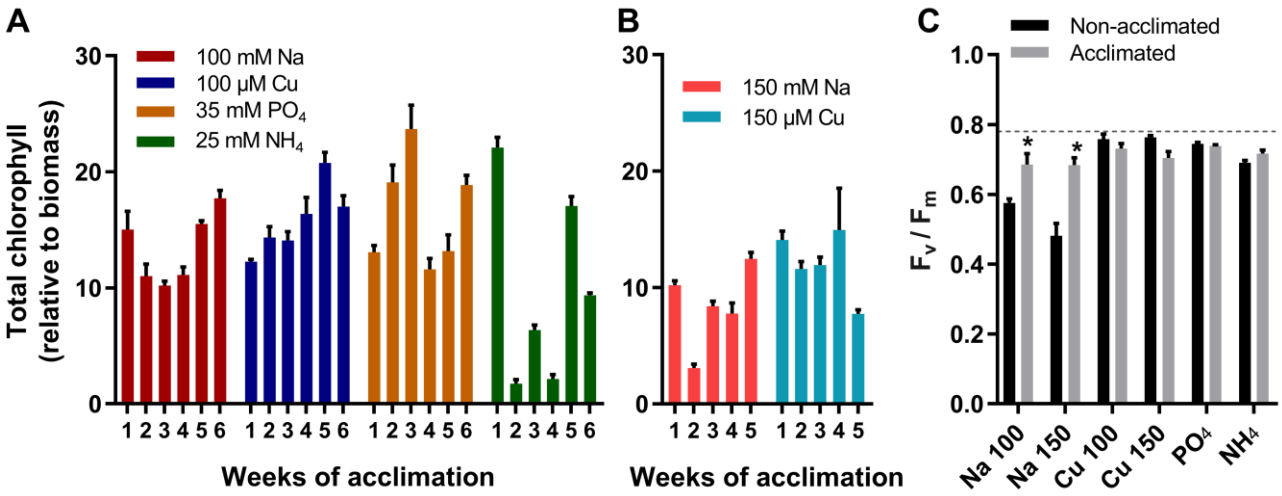

Fig. S3. Acclimating *Chlamydomonas reinhardtii* to excess Na^{+}, Cu^{2+}, PO_4^{3-} and NH_4^{+}. (A) Total chlorophyll concentration measured after 4 d of batch culture growth at each week of serial inoculation in TAP medium amended with 100 mM Na^{+}, 100 µM Cu^{2+}, 25 mM NH_4^{+} and 35 mM PO_4^{3-}. (B) Total chlorophyll concentration measured after 4 d of batch culture growth at each week of serial inoculation of cells originally acclimated to 100 mM Na^{+} and 100 µM Cu^{2+} in TAP medium amended with 150 mM Na^{+} and 150 µM Cu^{2+}, respectively. Data shown (A and B) is total chlorophyll concentration relative to amount of biomass. (C) F_v / F_m ratio for acclimated and non-acclimated cells grown in Na^{+}, Cu^{2+}, PO_4^{3-} or NH_4^{+} conditions as indicated. An asterisk (*) indicates significant difference ($p < 0.05$) of acclimated cells compared to non-acclimated cells. The dashed line indicates the mean non-stressed control value of F_v / F_m ratio. All data are mean values ± SEM from 3 - 4 measurements.

Supplementary Figure 4

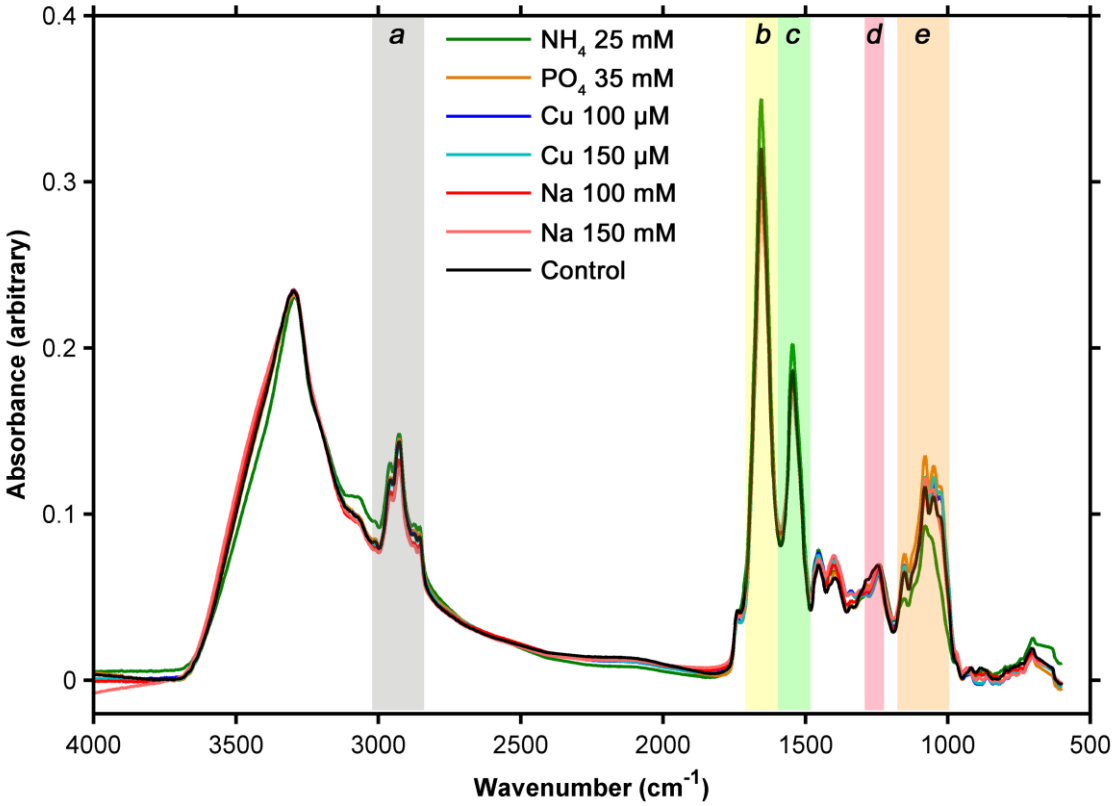

Fig. S4. FT-IR spectra in response to different ionic stresses in non-acclimated *Chlamydomonas reinhardtii* cells. Mean spectra derived from replicate EMSC2 normalized spectra of non-acclimated cells grown in Na^{+}, Cu^{2+}, PO_4^{3-} or NH_4^{+} conditions as indicated. Control cells were grown under non-stressed conditions. Key features of the spectra are highlighted: *a*, $\nu_s CH_2$ and $\nu_{as} CH_2$, $\nu_s CH_3$ and $\nu_{as} CH_3$ of fatty acids; *b*, νC=O of amides associated with protein (Amide I); *c*, δN-H of amides associated with protein (Amide II); *d*, ν_{as}P=O of nucleic acids, phosphoryl groups, phosphorylated proteins; *e*, νC-O of carbohydrates.

Supplementary Figure 5

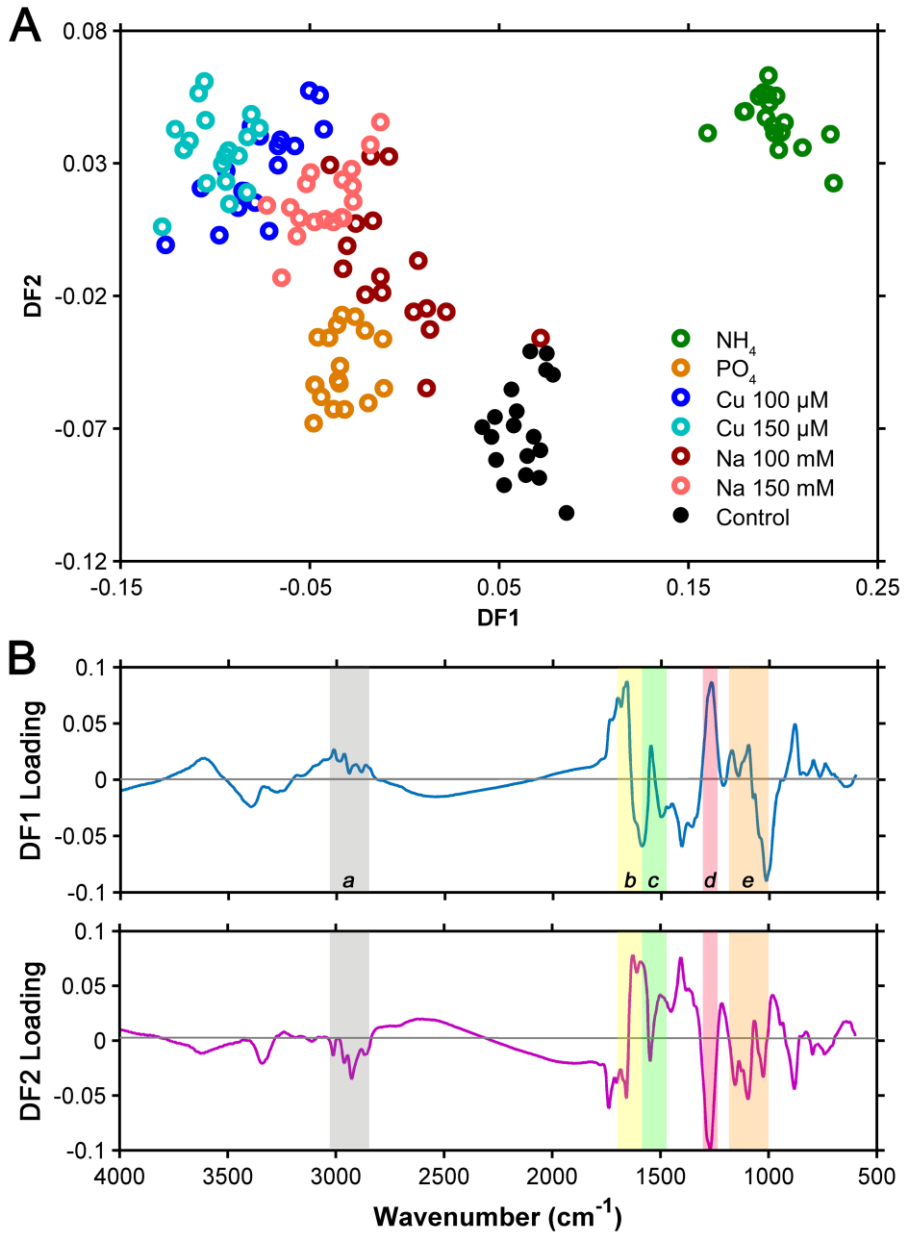

Fig. S5. FT-IR spectroscopy signatures in response to different ionic stresses in non-acclimated *Chlamydomonas reinhardtii* cells. (A) DF-PCA scores plot derived from replicate EMSC2 normalized spectra of non-acclimated cells grown in Na^{+}, Cu^{2+}, PO_4^{3-} or NH_4^{+} conditions as indicated. Control cells were grown under non-stressed conditions. (B) DF1 and DF2 loading plots derived from the scores plot shown in panel (A). Key features of the spectra are highlighted: *a*, $\nu_s CH_2$ and $\nu_{as} CH_2$, $\nu_s CH_3$ and $\nu_{as} CH_3$ of fatty acids; *b*, νC=O of amides associated with protein (Amide I); *c*, δN-H of amides associated with protein (Amide II); *d*, ν_{as}P=O of nucleic acids, phosphoryl groups, phosphorylated proteins; *e*, νC-O of carbohydrates.