Engaging inexpensive hands-on activities using *Chlamydomonas reinhardtii* (a green micro-alga) beads to teach the interplay of photosynthesis and cellular respiration to K4 - K16 Biology students.

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Background. Photosynthesis and cellular respiration play major roles in energy metabolism and are important Life Science topics for K16 Biology students. Currently there are a few companies that sell biology educational kits for making algae beads using non-motile green micro-algae to introduce students to photosynthesis. These kits are expensive, can be applied to only non-motile algae, protocols lack detailed specifics for trouble shooting and does not provide customization guidelines for different grade levels. *Chlamydmonas reinhardtii* is a motile green micro-alga and is an excellent model system for photosynthesis studies. We have generated a protocol which can be used to make *Chlamydomonas* beads. We have used these beads to design two simple and inexpensive plant biology hands-on activities. These laboratory activities have been customized to teach the interplay of photosynthesis and cellular respiration to K4 – K16 Biology students. This work is a supervised collaborative effort of three undergraduates and one high school student.

Methods. *Chlamydomonas* beads were used for two different laboratory activities that involved monitoring pH changes over time using a pH indicator. First activity centers on making light-powered algae bead bracelets and using these bracelets to monitor dramatic color/pH change over time when exposed to darkness or light. The second activity focuses on comparative studies of photosynthesis and cellular respiration in a *Chlamydomonas* wild type (4A+) and a pigment-deficient high light-sensitive photosynthetic mutant strain (*10E35/lsr1a*), using strain-specific algae beads having approximately equal cell numbers.

Results. We optimized our experimental protocol using algae beads in a 5.5 mL screw capped glass vials before performing the same experiment in algae bead bracelets. We found that the algal cell number in the individual bead, type of water used to immerse the algal beads and the duration of dark exposure of algal beads used in the experiment are important factors that affect successful implementation of the lab activities. Light-powered algae bead bracelets showed dramatic color/pH change within 3 hours upon exposure to light or darkness. These bracelets can be switched back and forth between darkness and light multiple times for within 48-72 hours to display color/pH changes, provided prior dark exposure time does not exceed 9 hours. Our comparative studies of photosynthesis and cellular respiration in the *10E35* and in 4A+ showed that *10E35* relatively has a higher respiration rate and a lower photosynthetic rate than 4A+. Additionally, *10E35* fails to display the expected photosynthesis-induced pH/color changes in the light after prolonged exposure to darkness indicating prolonged dark exposure of *10E35* is

detrimental for photosynthesis.

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19 ## Abstract

20 **Background.** Photosynthesis and cellular respiration play major roles in energy metabolism and
21 are important Life Science topics for K16 Biology students. Currently there are a few companies
22 that sell biology educational kits for making algae beads using non-motile green micro-algae to
23 introduce students to photosynthesis. These kits are expensive, can be applied to only non-motile
24 algae, protocols lack detailed specifics for trouble shooting and does not provide customization
25 guidelines for different grade levels. *Chlamydmonas reinhardtii* is a motile green micro-alga and
26 is an excellent model system for photosynthesis studies. We have generated a protocol which can
27 be used to make *Chlamydomonas* beads. We have used these beads to design two simple and
28 inexpensive plant biology hands-on activities. These laboratory activities have been customized
29 to teach the interplay of photosynthesis and cellular respiration to K4 – K16 Biology students.
30 This work is a supervised collaborative effort of three undergraduates and one high school
31 student.
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33 **Methods.** *Chlamydomonas* beads were used for two different laboratory activities that involved
34 monitoring pH changes over time using a pH indicator. First activity centers on making light-
35 powered algae bead bracelets and using these bracelets to monitor dramatic color/pH change
36 over time when exposed to darkness or light. The second activity focuses on comparative studies
37 of photosynthesis and cellular respiration in a *Chlamydomonas* wild type (4A+) and a pigment-

38 deficient high light-sensitive photosynthetic mutant strain (*10E35/lsr1a*), using strain-specific
39 algae beads having approximately equal cell numbers.
40 **Results.** We optimized our experimental protocol using algae beads in a 5.5 mL screw capped
41 glass vials before performing the same experiment in algae bead bracelets. We found that the
42 algal cell number in the individual bead, type of water used to immerse the algal beads and the
43 duration of dark exposure of algal beads used in the experiment are important factors that affect
44 successful implementation of the lab activities. Light-powered algae bead bracelets showed
45 dramatic color/pH change within 3 hours upon exposure to light or darkness. These bracelets can
46 be switched back and forth between darkness and light multiple times for within 48-72 hours to
47 display color/pH changes, provided prior dark exposure time does not exceed 9 hours. Our
48 comparative studies of photosynthesis and cellular respiration in the *10E35* and in 4A+ showed
49 that *10E35* relatively has a higher respiration rate and a lower photosynthetic rate than 4A+.
50 Additionally, *10E35* fails to display the expected photosynthesis-induced pH/color changes in
51 the light after prolonged exposure to darkness indicating prolonged dark exposure of *10E35* is
52 detrimental for photosynthesis.
53

Introduction

55 Photosynthesis and cellular respiration are complementary life supporting fundamental
56 biochemical reactions on the planet. These biochemical reactions are important Biology topics
57 listed in the Next Generation Science Standards (NGSS) Life Science core idea LS1C: From
58 Molecules to Organisms: Structures & Processes which align with principles 1, 2, 3, 5, 10 and 11
59 of the 12 Principles of Plant Biology listed by the American Society of Plant Biologists ("The 12
60 Principles of Plant Biology", ASPB; Article S1). Guidelines for photosynthesis laboratories
61 using non-motile green micro-algae bead are available on the websites of Carolina Biological
62 (Burlington, NC, USA) and Gene Technology Access Center (GTAC; Victoria, Australia) for
63 classroom use ("Algae Immobilised in Alginate balls", GTAC, 2016; "Carolina Quicktips
64 Making Algae Beads", Carolina Biological). Traditionally, non-motile algae have been used to
65 generate algae beads as non-motile algae can be trapped and immobilized easily ("Carolina
66 Quicktips Making Algae Beads", Carolina Biological; "Algae Immobilised in Alginate balls",
67 GTAC, 2016). To the best of our knowledge there are currently no protocols available for
68 making algae beads using a motile alga. *Chlamydomonas reinhardtii* is motile green micro-alga
69 and is an excellent model system for photosynthesis and bioenergy researchers (Merchant et al.,
70 2007; Scranton et al., 2015; Radakovits et al., 2010). Available hands-on activity resources for
71 classroom photosynthesis labs associated with educational kits in the market do not work well
72 most of the time and lack specific guidelines for optimizing the experiment and troubleshooting.
73 Additionally, the educational kits for the classroom photosynthesis labs are expensive. Hence a
74 well–defined protocol with proper detailed guidelines for conducting lab activities & managing
75 class times and information for acquiring lab materials inexpensively, will be useful Biology
76 educators at schools and institutions that have limited resources.

77   Calcium alginate is used to trap and immobilize living cells in industrial procedures ("Carolina
78   Quicktips Making Algae Beads, Carolina Biological; "Algae Immobilised in Alginate balls",
79   GTAC, 2016). For example, immobilized non-motile colonial algae are being tested for biofuel
80   production, immobilized yeast cells are being used for alcoholic wine fermentation, and
81   immobilized bacterial cells are being used for water disinfection (Kröger & Müller-Langer,
82   2012; Gotovtsev et al., 2015). To entrap algae in beads, cell suspension and 2% sodium alginate
83   are mixed at a specific ratio and added drop-wise to chilled calcium chloride solution. Calcium
84   ions link the alginate monomers together to make a gel-like polymer of calcium alginate which
85   trap cells and immobilize them in beads. These algal beads can be used for biological
86   experiments or other biotechnological applications.
87   We have generated a detailed protocol of making *Chlamydomonas* beads and two simple plant
88   biology hands-on activities. These laboratory activities were used to teach the interplay of
89   photosynthesis and cellular respiration to Biology students in nine schools and two universities
90   in Georgia in a fun way. The presented work is a product of supervised collaborative efforts of
91   three undergraduate students and one high school student in Georgia, USA.
92   In the two designed laboratory activities students make *Chlamydomonas* beads and use these
93   beads to conduct their experiments. In the first lab activity students make light-powered green
94   algae bead bracelets and use these algae bracelets to perform time course experiments in light
95   and in dark to study the interplay of photosynthesis and cellular respiration. In the second
96   activity, students compare relative ratios of photosynthesis and cellular respiration in a
97   *Chlamydomonas* wild type (4A+) and a chlorophyll-deficient high-light sensitive mutant strain,
98   *10E35/lsr1a* using strain-specific algae beads. *10E35* is a random insertional mutant generated
99   by our research lab with a mutation in a novel functionally uncharacterized gene of unknown
100   function and is the center of an on-going research project at our laboratory [Nguyen et al., 2017;
101   Article S2].
102   Cellular respiration oxidizes organic chemicals and releases CO_2 into the environment and
103   photosynthesis converts CO_2 into fixed carbon in the presence of light. Irrespective of the light
104   conditions, cellular respiration in live cells in the beads will release CO_2 that will dissolve in
105   water in which the beads are immersed to generate carbonic acid. Conversely in the light,
106   photosynthesis in the algal cells in the beads will remove CO_2 from the water surrounding the
107   beads. Hence pH of the water will be acidic in the dark and alkaline in the light. In the two
108   activities designed by us, students will monitor photosynthesis and cellular respiration-induced
109   pH changes in the water by color changes of a pH indicator as well as by measuring the pH with
110   pH testing strips and/or a pH electrode.
111   We present in this article the protocol for making *Chlamydomonas* beads (including some
112   preliminary testing data that helped us to refine the protocol), two new plant biology teaching
113   tools and teaching resources for educators. We hope that the teaching resources will help plant
114   biology educators to customize the labs according to the K4 - college level grade level
115   curriculum and availability of resources at their institutions and allow them to better manage
116   times in classrooms. The designed lab activities support active learning and contributes toward

117   the following: **1)** NGSS Science and Engineering Practice: Developing and using models;
118   Planning and carrying out investigations and **2)** NGSS Core Idea: Life Science LS1C: From
119   molecules to organisms: Structures and processes.
120
Materials & Methods
122   **Material information for educators**
123   Materials and general equipment required for the lab activities are given in Text S1. Information
124   (vendors and catalog numbers) for ordering specific items related to the project like algal strains,
125   algal growth media, inoculating loops, flasks, plastic transfer pipettes, Eppendorf tubes, pH
126   indicators, pH test strips, bracelet tubing, glass vials, yarns for bracelet braids, sodium alginate,
127   calcium chloride, counting chambers etc. are given in the Text S2.

128   **Algal media and cultures**
129   *Chlamydomonas* wild type strain 4A+ (CC- 4051 4A+ mt+) strain was a gift from Dr. Krishna K.
130   Niyogi (UC Berkeley, CA). *10E35/lsr1a* (light-sensitive related 1a) is a random insertional
131   nuclear mutant generated by our lab which has a mutation in a novel uncharacterized gene
132   encoding a protein of unknown function [Nguyen et al., 2017; Article S2]. 4A+ and *10E35*
133   strains were maintained in the lab on Tris-Acetate Phosphate (TAP) agar media plates (Text S3)
134   in dim light intensities (15-20 µmol m^{-2}s^{-1}) at 25°C. A starter culture of 4A+ was started
135   approximately 11-12 days ahead of the lab activity by inoculating 10 mL of liquid TAP media in
136   a 50 mL flask with 4A+ cells from a 5-day old TAP agar media plate (Text S3). After 5 days of
137   growth, 1 mL of the starter culture was used to inoculate 300 mL of fresh TAP media in a 1L
138   flask. The TAP liquid 4A+ culture was grown for 6-7 days for dense dark green growth. *10E35*
139   grows slower than 4A+. Hence *10E35* liquid TAP cultures should be started at least 3-4 days
140   before starting the 4A+ liquid TAP cultures. Algal liquid cultures were grown under 25°C under
141   continuous illumination of 80-100 µmol photons m^{-2} s^{-1} provided by the combined light
142   intensities of four to six cool white fluorescent lights. Cultures were shaken continuously on an
143   open-air orbital shaker at a speed of 150-180 rpm to ensure a uniform illumination of the cells
144   and to prevent cells from settling down. Light intensities were measured using a LI-250A Light
145   Meter (LI-COR, Inc., Lincoln, NE).

146   **Preparation of 2% sodium alginate and 3% calcium chloride solutions**
147   2 grams of sodium alginate (Fisher Scientific, Waltham, MA) was dissolved in 100 mL of E-pure
148   water overnight at room temperature by stirring at a speed of 400 rpm using a magnetic stirrer.
149   [Note: sodium alginate forms a very viscous solution when dissolved at 1.5% - 4%]. 2% sodium
150   alginate solution was stored at room temperature. 30 grams of calcium chloride was dissolved in
151   1000 mL of E-pure water and stored at 4ºC in a fridge.

152   **Cell counts**
153   Cell density (number of cells per mL of the culture) was determined before harvesting
154   *Chlamydomonas* cells from the TAP liquid culture to estimate the volume of culture needed to
155   harvest specific number of cells per 50 mL falcon tube. Cell density was calculated by counting

156   the cells using a Hausser Scientific Bright-Line™ Counting Chamber (Hausser Scientific,
157   Philadelphia, PA).

158   **Preparation of *Chlamydomonas* 4A+ and *10E35* beads**
159   A detailed version of the *Chlamydomonas* bead-making protocol is available at
160   dx.doi.org/10.17504/protocols.io.bgpyjvpw/. *Chlamydomonas* strain 4A+ or *10E35* cells were
161   harvested by spinning down dense TAP liquid strain-specific cultures at 1,000-1,500 g for 3
162   minutes in a benchtop centrifuge. The supernatant was discarded and the cell pellet was
163   collected. Harvesting 100 mL of dense *Chlamydomonas* culture generated 200-300 beads of 4-5
164   mm in diameter. 2% well mixed-sodium alginate was added to the cell pellet in a 4:1 or 5:1 ratio
165   (depending on the total number of cells harvested; see results and detailed protocol on
166   dx.doi.org/10.17504/protocols.io.bgpyjvpw/). The algae and 2% sodium alginate were gently
167   mixed till the entire cell pellet was completely resuspended without any visible cell clumps.
168   Maximum number of total cells used for resuspension in sodium alginate was either 395×10^6 or
169   790×10^6 cells depending on the experiment (see result section). We resuspended the cell pellets
170   containing 395×10^6 cells and 790×10^6 cells in 5 mL of sodium alginate to get an approximate
171   final cell density of 66×10^6 cells/mL and 132×10^6 cells/mL in the cell suspension, respectively.
172   1 mL of sodium alginate-algal cell suspension gave us approximately 32-35 beads depending on
173   pipetting techniques. Hence the cell suspension with cell density of 66×10^6 cells/mL will form
174   beads that have approximately 1.89×10^6 -2×10^6 cells/bead while the cell suspension with cell
175   density of 132×10^6 cells/mL will have 3.77×10^6 - 4.1×10^6 cells/bead. We used 8 beads of
176   similar sizes (4-5 mm in diameter) for glass vial experiments.
177   The algae-sodium alginate mix was added drop wise steadily and quickly with uniform pipetting
178   by using a micropipette or a plastic transfer pipette into a beaker of pre-chilled 3% calcium
179   chloride kept on ice. If pipetting is not smooth and regular and, the algae-sodium alginate
180   mixture is not mixed by swirling in between pipetting, irregular shaped and beads with different
181   cell numbers/bead (light and dark green beads) will form (Fig. S1). As soon as the algae-sodium
182   alginate mixture touches the chilled calcium chloride liquid surface, the mixture solidified into
183   tiny beads. The calcium chloride beaker containing the beads were kept on ice for 10-15 minutes
184   to allow complete solidification of the algal beads.
185   The beads are separated from the calcium chloride solution by filtering through an oil strainer.
186   Algal beads on the strainer were washed with tap water. The beads were kept temporarily in a
187   petri dish containing small amount of tap water till the bracelets were made. Surplus beads were
188   stored in tap water in a beaker for future use within 1-2 days. Algae bead making demonstration
189   video clips are available at: https://youtu.be/u4BbZ29qlWQ and at
190   https://youtu.be/eIxbzeHW8IM.
191   **Preparation of *Chlamydomonas* 4A+ bead bracelet**
192   Flexible tubing was cut into 10 pieces, each 5 inches long. Caps of 1.5 mL Eppendorf tubes were
193   cut off with a scissor. De-capped Eppendorf tube was used to plug the ends of the bracelet tubing
194   (one de-capped tube at each end of the cut tubing). Colorful cotton yarn was cut according to the
195   wrist width, intertwined and yarn braids were made. One braid was looped tightly onto the
196   mouth of each de-capped Eppendorf tube at each end of the bracelet. Next, one end of the

197 bracelet tubing was unplugged by removing the de-capped Eppendorf tube that was sealing the
198 end. About 3.5 mL of tap water [pH 7.2 -7.3] was introduced into the bracelet flexible tubing.
199 15- 38 algae beads (depending on the experiment) were gently introduced into the water inside
200 the tubing. 8-10 drops of the bicarbonate indicator (Carolina Biological, Burlington, NC) were
201 added into the water in the tubing and the end of the tubing was plugged back with the de-capped
202 Eppendorf tube. Precautions were taken to avoid acidic or alkaline contamination of the flexible
203 tubing, plastic spoon, transfer pipettes, petri dishes etc. used in our experiments, since the
204 bicarbonate indicator is not directly specific to gases like carbon dioxide. About 0.5 cm-1 cm air
205 gap was left at each end inside the tubing to provide enough air for cells. The bracelet was
206 imaged and the pH of the water inside the bracelet was measured using pH testing strips (Fisher
207 Scientific, Waltham, MA) before shifting it to light or to darkness for the lab activity. All
208 experiments described below were performed with the same batch of beads. A detailed version
209 of the protocol is available at dx.doi.org/10.17504/protocols.io.bgpyjvpw. Demonstration of
210 algae bead bracelet making video clips available at: https://youtu.be/A7VIjLDGSCc and
211 https://youtu.be/vh_1ASpQgS8   and https://youtu.be/enctr0yhWQ8.

Light and dark exposure experiments with *Chlamydomonas* bead bracelets

213 For the constant light/dark exposure experiment, one bracelet was kept under 150-200 µmole m^{-}
214 ^{2}s^{-1} light intensity [equivalent to the combined light intensities of 12 to 14 cool white fluorescent
215 lights] and another one was kept in the dark inside a lab cabinet drawer. After 3 hours of
216 light/dark exposure, the bracelets were imaged. pH of the water inside the bracelets were
217 measured using pH testing strips (Fisher Scientific, Waltham, MA).
218 For dark shift experiment, the bracelet was first light-adapted for 4 hours and then shifted to
219 darkness. For light shift experiment, the bracelet was dark-adapted for 4 hours and then shifted to
220 light. After every 1 hour over a period of 4 hours during light exposure or over a period of 3
221 hours during dark exposure, the bracelet was imaged to monitor the carbon dioxide percentage
222 change inside the bracelet tubing. The carbon dioxide percentage change is monitored indirectly
223 by the color changes of the bicarbonate indicator. pH was not measured for the light/dark shift
224 experiments with algae bead bracelets. For testing the effect of different dark exposure times on
225 photosynthesis, one algae bead bracelet was exposed to 9 hours of darkness and the other was
226 exposed to 15 hours of darkness. After the dark exposure, the 9 hours- and 15 hours- dark-
227 adapted bracelets were exposed to light for 4 hours and 12 hours, respectively and were imaged
228 after the light exposure. pH of the water inside these light and dark-exposed bracelets were
229 measured using pH testing strips (Fisher Scientific, Waltham, MA).

**Light and dark exposure experiments with *Chlamydomonas* strain-specific beads in glass
vials**

232 For testing the effect of water quality on photosynthesis, eight 4A+ beads were either introduced
233 into 2.5 mL of tap water (pH 6.9 -7.3) or into de-ionized [DI] water (pH 7.1-7.2) in 5.5 mL screw
234 capped glass vials (Fisher Scientific, Waltham, MA). For testing the effect of cell density on
235 photosynthesis, eight 4A+ beads were either introduced into 2.5 mL of tap water (pH 7.2 -7.3).
236 For both stated experiments, 125 µL of the 0.02% phenol red solution (Fisher Scientific,
237 Waltham, MA) was added to the algae bead vials and the vials were capped tightly. One set of

238   4A+ bead and the control vials were exposed to 150-200 µmole m^{-2}s^{-1} light intensity and the

239   other set to darkness for 2 hours. After 2 hours of light or dark exposure, vials were imaged and

240   pH of the water in the vials were measured using a Thermo Fisher Scientific Orion-3 Star

241   benchtop pH meter (Fisher Scientific, Waltham, MA).

242   For comparative analyses of photosynthesis and cellular respiration in 4A+ and *10E35* strains

243   under constant light/darkness, beads having approximately 1.89 -2 X 10^6 cells/bead for each

244   strain were used (Fig. S2). Eight 4A+ and *10E35* beads were introduced into 2.5 mL of tap water

245   (pH 6.9 -7.3) in 5.5 mL screw capped glass vials (Fisher Scientific, Waltham, MA). 125 µL of

246   the phenol red solution (Fisher Scientific, Waltham, MA) was added to the 4A+ and *10E35* bead

247   vials and the vials were capped tightly. One set of 4A+, *10E35* and control vials was exposed to

248   light intensity of 150-200 µmole m^{-2}s^{-1} and the other set was exposed to darkness for 1 hour. The

249   algae bead and control vials were imaged after every 30 minutes over a period of 1 hour and pH

250   of the water in the vials were measured. The 1-hour light adapted 4A+, *10E35* and the control

251   vials were exposed to light for an additional 3 hours and then shifted to dark. The vials were

252   imaged after every 15 minutes for a period of 1 hour during dark exposure. After 1 hour, these

253   dark -exposed vials were kept under dark for additional 5 hours. After 6 hours-of dark exposure,

254   vials were shifted to light [150-200 µmole m^{-2}s^{-1}] and imaged after 30 minutes, 1 hour, 2 hours, 3

255   hours and 48 hours. pH of the water in the glass vials in the above stated experiments were

256   measured using a Thermo Fisher Scientific Orion-3 Star benchtop pH meter (Fisher Scientific,

257   Waltham, MA).

258   **Data analyses**

259   Statistical analyses of the recorded pH data were performed using Microsoft Excels' t-Test:

260   Paired Two Sample for Means tool in the analysis ToolPak. Both One-Tailed and Two-Tailed

261   Hypothesis Tests were performed. Standard deviations shown in Tables under result section was

262   calculated using Excel. The raw statistical analyses data from three biological replicates per

263   experiment have been deposited in Figshare (10.6084/m9.10.6084/m9.figshare.12344024) and

264   are presented in the supplementary Data S1 file. Data S2 file contains the raw pH data, mean and

265   standard deviation information.

266

267   # Results

268   **Photosynthesis and cellular respiration-induced pH changes in de-ionized (DI) water and**

269   **tap water vials containing *Chlamydomonas* 4A+ strain beads.**

270   DI water is known to contain less dissolved gases and minerals than tap water ["The Lab Depot,

271   What is Pure Water?"; Whitehead, 2020]. Amounts of dissolved oxygen and carbon-dioxide in

272   the water used for photosynthesis monitoring experiment will affect the results in a

273   photosynthesis lab. Hence, we monitored photosynthesis and cellular respiration of the wild type

274   *Chlamydomonas* strain, 4A+ beads in DI water and in tap water to see which one would be

275   suitable for designing the photosynthesis lab (Fig. 1; Table 1). The pH indicator phenol red

276   exhibits a gradual transition from light orange to red over the pH range 6.8 to 8.2. Phenol red

277   turns yellow below pH 6.7 and turns to a bright pink (fuchsia) color above pH 8.2. Our results

278   show that there was no significant difference in the water color or pH between the control and
279   experimental vials in de-ionized water in the light and in the dark. p values from the 1-tailed and
280   2-tailed hypothesis tests for the DI water control and 4A+ DI water light set was 21% and 42%,
281   respectively (Data S1). p values from the 1-tailed and 2-tailed hypothesis test for the DI water
282   control and 4A+ DI water dark set was 9% and 18%, respectively. (Fig. 1A; Table 1;
283   10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). p-values for the control DI water
284   and control tap water under light from the 1-tailed and 2-tailed hypothesis tests were 11% and
285   22%, respectively. p-values for the control DI water and control tap water in dark from the 1-
286   tailed and 2-tailed hypothesis tests were 9% and 18%, respectively. Hence there were no
287   significant differences in pH in the control vials with DI and tap water under light and dark.
288   Dark-exposed tap water experimental vial displayed acidic pH (yellow color) while the light-
289   exposed tap water experimental vial displayed alkaline pH (fuchsia color) relative to the
290   respective control vials (Fig.1B; Table 1; 10.6084/m9.10.6084/m9.figshare.12344024; Data S1;
291   Data S2). There was a difference of approximately 2 pH units between the dark and light-adapted
292   tap water experimental vials in contrast to the 0.07 pH unit difference between the DI water in
293   the dark-and in the light-adapted experimental vials (Table 1;
294   10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). p-values from the 1-tailed and 2-
295   tailed hypothesis tests for the tap water 4A+ vial under light and that in the dark were 0.01% and
296   0.02%, respectively (Data S1). p-values were undefined from the 1-tailed and 2-tailed hypothesis
297   tests for the DI water 4A+ vial under light and that in the dark as the means and standard
298   deviations were identical for the two samples (Table 1; Data S1 and Data S2). p-values from the
299   1-tailed and 2-tailed hypothesis tests for the DI water 4A+ vial and the tap water 4A+ vial in the
300   light were 0.1% and 0.2%, respectively (Data S1). p-values from the 1-tailed and 2-tailed
301   hypothesis tests for the DI water 4A+ vial and the tap water 4A+ vial in the dark were 0.3% and
302   0.6%, respectively (Data S1). In the light-exposed tap water algal bead vial, buoyancy of a bead
303   can be seen which is indicative of O_2 production in photosynthesis (Fig. 1B). Taken together our
304   results show that tap water is preferable over DI water for performing photosynthesis lab
305   activities.

306   **Effect of total cell numbers in *Chlamydomonas* 4A+ strain beads on photosynthesis and**
307   **cellular respiration-induced color/pH changes in tap water.**
308   We used two types of beads that have two-fold difference in total cell numbers/bead: 1) beads
309   that have approximately 2 x10^6 cells/bead and 2) beads that have approximately 4 x 10^6
310   cells/bead. It is expected that a high cell number in a bead will increase cellular respiration as a
311   high cell density in the bead will create oxygen stress. The pH in the light-exposed vial
312   containing 4 x 10^6 cells/bead was 6.1 and the pH in the light exposed vial containing 2 x10^6
313   cells/bead was pH 8.4 for the same duration of light exposure (Fig. 2; Table 2;
314   10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). The pH in the light- and dark-
315   exposed vials containing 4 x 10^6 cells/bead differed by only 0.1 pH unit while the pH in the
316   light- and dark-exposed vials containing 2 x 10^6 cells/bead differed approximately by 2 pH units
317   for the same duration of light exposure (Fig. 2; Table 2;

318   10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). p-values from the 1-tailed and 2-
319   tailed hypothesis tests for the light-exposed vials with 4 x 10^6 cells/bead and 2 x 10^6 cells/bead
320   were 0.01% and 0.02%, respectively (Data S1). p-values from the 1-tailed and 2-tailed
321   hypothesis tests for the dark-exposed vials with 4 x 10^6 cells/bead and 2 x 10^6 cells/bead were
322   1% and 2%, respectively (Data S1). pH differences between the light and dark control vials were
323   insignificant as the p-values were higher than 5% in both 1-tailed and 2-tailed hypothesis tests
324   (Data S1). Our results show a high cell numbers/bead will hinder observation of pH changes in a
325   photosynthesis lab. In the light-exposed experimental vial, partial buoyancy of one bead can be
326   seen, indicative of O_2 production in photosynthesis (Fig. 2B).

327   **Indirect detection of carbon dioxide concentration in the 4A+ bead bracelet under light and**
328   **darkness using the bicarbonate indicator.**
329   Bicarbonate indicator is commonly used in photosynthesis and respiration experiments to detect
330   indirectly the percentage of carbon dioxide in a sample. When the carbon dioxide content in
331   water is higher than 0.04%, pH becomes acidic. Acidic pH changes the red color of the indicator
332   to yellow. If the carbon dioxide content is lower than 0.04%, pH gets alkaline and the indicator
333   changes color from red to magenta and, under very low carbon dioxide concentrations the color
334   of the indicator changes to purple. We used three bracelets (with algal beads ranging from 30-38)
335   to monitor color changes of the bracelet water containing the bicarbonate indicator. These are
336   designated as control, dark-exposed and light-exposed bracelets in Fig. 3. The color of the water
337   in the control bracelet (not exposed to dark or light), dark- and light-exposed bracelets were,
338   light red, bright yellow and dark blue, respectively (Fig. 3). The objective of the experiment was
339   to simply determine the color/pH changes of the water in the experimental bracelets in the light
340   or in the dark relative to the control. The average pH of the water in the control algal bracelet
341   was around 7 (Fig. 3A; Table 3; 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2).
342   pH of the water in the dark-exposed algal bracelets ranged between 6 and 6.5 with STDEV ±
343   0.24 (Fig.3B; 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2) indicating a high
344   percentage of carbon dioxide because of cellular respiration. pH of the water in the light-exposed
345   algal bracelets ranged between 8.5 and 9 with STDEV ± 0.24 indicating a low percentage of
346   carbon dioxide because of photosynthesis (Fig. 3C; Table 3;
347   10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). p-values from the 1-tailed and 2-
348   tailed hypothesis tests for the dark-adapted and light-adapted algal bracelets were 0.1% and
349   0.3%, respectively (10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). Our results
350   clearly show that carbon-dioxide percentage can be monitored indirectly under light/darkness via
351   sharp pH/color changes in the water in the bracelet in the presence of the bicarbonate indicator.

352   **Time course monitoring of photosynthesis-induced color changes in the dark adapted- 4A+**
353   **bead bracelet when shifted to light.**
354   An algal bracelet was dark-adapted for 4 hours. After dark-adaptation the bracelet was exposed
355   to light for 4 hours. This light-exposed bracelet was imaged after every 1 hour during light
356   exposure to monitor the gradient color changes over time without disturbing the bracelet (Fig. 4).
357   The results show that if the algal bracelet is left undisturbed, one can pinpoint specifically which

358 beads were actively photosynthesizing from the red-magenta-purple color streaks in the water on
359 top of these beads that were removing carbon-dioxide from the tubing water (Fig. 4 B-D). pH
360 was not measured in these bracelets as the objective of this experiment was to determine if
361 differences exist in photosynthetic rates among different beads by visually observing the gradual
362 color change of the water in the bracelet in light.

363 **Time course monitoring of cellular respiration-induced color changes in the light adapted-**
364 **4A+ bead bracelet when shifted to darkness.**

365 An algal bracelet was light adapted for 4 hours. After light-adaptation the bracelet was exposed
366 to dark for 3 hours. The dark-exposed bracelet was imaged after every 1 hour during the light
367 exposure to monitor the gradient color changes over time (Fig. 5). The results show that distinct
368 pH gradient can be observed in a colorful way in an undisturbed algal bracelet (Fig. 5B). pH was
369 not measured in these bracelets as the objective of this experiment (in conjunction with the Fig. 4
370 experiment) was to teach students in a fun way, the "tug of war" between photosynthesis and
371 cellular respiration by visually observing the dramatic color changes of the water in the bracelet
372 upon exposure to darkness or light.

373 **Effect of prior dark exposure duration on photosynthesis-induced pH changes in the 4A+**
374 **bead bracelet in light.**

375 One algal bracelet was kept in the dark for 9 hours and the other one was kept in the dark for 15
376 hours. After dark incubation both bracelets were imaged and the pH was measured using pH
377 testing strips and then shifted to light. (Fig. 6). There was no significant difference in pH
378 between the 9-hours dark-adapted and 15-hours-dark adapted bracelets (p values from 1-tailed
379 and 2-tailed tests were11% and 22%, respectively) (Data S1). There was a significant difference
380 in pH between 9-hours-dark adapted bracelet and 15-hours-dark adapted bracelet when these
381 were exposed to light for 4 hours and 12 hours, respectively (p values from 1-tailed and 2-tailed
382 tests were 0.2% and 0.5%, respectively) (Data S1). The bracelet that was kept in dark for 9 hours
383 showed increase in pH from pH 6 [STDEV ±0] to pH 8.67 [STDEV ±0.24] within 4 hours under
384 light because of photosynthesis (Fig. 6A & 6B; Table 4;
385 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). The bracelet that was kept in
386 dark for 15 hours showed a small increase in pH from 5.5 [STDEV ±0.41] to 6.3 [STDEV
387 ±0.24], despite being exposed to light for 12 hours. This indicates prior prolonged exposure to
388 darkness hinders photosynthesis in algal beads in light (Fig. 6C & 6D; Table 4;
389 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2).

390 **Comparative studies of photosynthesis and cellular respiration-induced color/pH changes**
391 **in vials containing wild type 4A+ and *10E35* mutant beads.**
392 4A+ and *10E35* beads have approximately 2×10^6 cells/bead (Fig.S2). Each light and dark set
393 comprised of a control and experimental vials of *10E35* and 4A+ (Fig.7). Images of the vials in
394 each light and dark set were taken before light or dark exposure (Fig.7A and 7D). Each light and
395 dark vial sets were imaged after 30 minutes of light and dark exposures, respectively for a period
396 of 1 hour. Results show a slow increase in pH in *10E35* vial under light compared to that in the

397 4A+ vial (Fig.7B -7C; Table 5; 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2).
398 This could be due to a slow rate of photosynthesis or a high rate of cellular respiration or a
399 combination of both phenomena in *10E35* relative to that in 4A+. p-values from the 1-tailed tests
400 for pH differences between 4A+ and *10E35* for the time course experiment in the light ranged
401 from 0% to 1.5%. p-values from the 2-tailed tests for pH differences between 4A+ and *10E35* for
402 the time course experiment in the light ranged from 0% to 3.1% (Data S1). p-values from the 1-
403 tailed tests for pH differences between 4A+ and *10E35* for the time course experiment in the
404 dark ranged from 0.2% to 1.1%. p-values from the 2-tailed tests for pH differences between 4A+
405 and *10E35* for the time course experiment in the dark ranged from 0.5% to 2.2% (Data S1).
406 *10E35* displays relatively a higher rate of cellular respiration in dark compared to that in 4A+ as
407 indicated by the fast pH drop in dark in *10E35* vial over time compared to that in the 4A+ vial
408 (Fig.7E- 7F; Table 5, 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2).

409 **Time course monitoring of cellular respiration-induced color/pH changes in the dark in**
410 **vials containing 4A+ and *10E35* beads that were exposed to light for 4 hours.**
411 Light-exposed *10E35*, 4A+ and control vials from Fig. 7 experiment were exposed to light for
412 additional three hours. Hence this set was light-exposed for a total of 4 hours. After fours of light
413 exposure, images were taken and the vials were exposed to dark. Images of the dark-exposed
414 vials were taken every 15 minutes over a period of 1 hour during dark exposure (Fig. 8). *10E35*
415 shows relatively a higher cellular respiration rate compared to that in 4A+ as indicated by the
416 rapid drop in pH in the *10E35* vial compared to that in the 4A+ vial (Fig. 8B - 8E; Table 6;
417 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). p-values from the 1-tailed test
418 for pH differences between 4A+ and *10E35* for the time course experiment in the dark ranged
419 from 0.05% to 0.1% (Data S1). p-values from the 2-tailed test for pH differences between 4A+
420 and *10E35* for the time course experiment in the dark ranged from 0.1% to 0.3% (Data S1). p-
421 values from the 1-tailed and 2-tailed hypothesis tests were less than 5 % for all control vials
422 (Data S1). The results re-confirm the results shown in Fig. 7E-7F (Table 5,
423 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2).
424 **Time course monitoring of photosynthesis-induced pH changes in the light in 4A+ and**
425 ***10E35* bead vials that were exposed to dark for 6 hours.**
426 Dark-exposed *10E35*, 4A+ and control vials from Fig. 8 experiment were exposed to dark for
427 additional five hours. Hence this set was dark-exposed for a total of six hours. After six hours of
428 dark exposure, images were taken of the dark-exposed vials and the vials were exposed to light.
429 Images of these light-exposed vials were taken after 30 minutes, 1 hour, 2 hours, 3 hours and 48
430 hours of light exposure (Fig. 9). The results show that 4A+ could photosynthesize at a faster rate
431 compared to *10E35* after 6 hours of dark exposure to cause a distinct water color/pH change
432 (Table 7; 10.6084/m9.10.6084/m9.figshare.12344024; Data S1; Data S2). p-values from the 1-
433 tailed test for pH differences between 4A+ and *10E35* for the time course experiment in the light
434 ranged from 0.01% to 1.75% (Data S1). p-values from the 2-tailed test for pH differences
435 between 4A+ and *10E35* for the time course experiment in the light ranged from 0.02% to 3.5%
436 (Data S1). The p values for the 1-tailed and 2-tailed tests for pH difference between the *10E35*

437 vial before light shift and the *10E35* vial exposed to light for 48 hours were 0.2% and 0.5%,
438 respectively. Despite the significant pH difference in these two vials of *10E35*, pH in the 48
439 hours-light exposed *10E35* vial was acidic (pH= 6.43±0.06) compared to the alkaline pH
440 (8.47±0.06) in the 48 hours-exposed 4A+ vial. It is known that *10E35* progressively photo-
441 bleaches with increase in light intensity [Nguyen et al., 2017; Article S2]. Figure 9F shows that
442 *10E35* beads were photo-bleached in the light-exposed vial after 48 hours of light exposure.
443 Photo-bleaching indicates that there is a chlorophyll breakdown in the beads.
444

Discussion

446 *Chlamydomonas reinhardtii* is a unicellular micro-green alga (a Chlorophyte) that retains many
447 of the features of green plants and of the common ancestor of plants and animals, although its
448 lineage diverged from Streptophytes over one billion years ago. *Chlamydomonas* is used to study
449 eukaryotic photosynthesis because, unlike angiosperms, it can use acetate to grow in the dark
450 while maintaining a functional photosynthetic apparatus (Merchant et al., 2007). It is also a
451 model organism for elucidating eukaryotic flagella and basal body structure and functions which
452 can be linked to various ciliopathies (Silflow & Lefebvre, 2001). More recently,
453 *Chlamydomonas* research has been developed for bioremediation purposes, generation of
454 biofuels and has led to breakthroughs in optogenetics (Merchant et al., 2007; "Critical tool for
455 brain research derived from 'pond scum" NSF, 2013; Zhang, 2015; Scranton et al., 2015).
456 Currently, the *Chlamydomonas* Resource Center [https://www.chlamycollection.org/]
457 offers number of educational kits ("Resources For Teaching" - Chlamydomonas Resource
458 Center") including instructions and strains on its website; however, these tools barely scratch the
459 surface of what could be taught using *Chlamydomonas* to students enrolled in K12 Biology and
460 in college Biology undergraduate courses. Hence there is a huge potential to develop
461 *Chlamydomonas* an under-utilized teaching tool, into a powerful popular teaching tool which
462 will complement existing plant science teaching strategies. Our objective for the American
463 Society of Plant Biologists' (ASPB) Plant-BLOOME project was to design fifteen simple hands-
464 on activities that can not only educate and excite high school students about *Chlamydomonas* but
465 can be also included as a component in college Biology laboratory courses. The activities
466 described in this manuscript is centered on one such designed activities.
467 We have found that the *Chlamydomonas* culture should be grown under low light (80-100 micro
468 mol photons m^{-2} s^{-1}) to obtain a healthy culture that is not photo-oxidatively stressed to be used
469 for our lab activities. The culture should be a dense culture and have a cell density ranging from
470 18 x10^6 cells/mL to 22 x 10^6 cells/mL to get enough cells for a class of 24 students, working in
471 groups of two to three. Algal beads once made, should be rinsed thoroughly with tap water for at
472 least five minutes to remove residual sodium chloride that is formed as a product in the reaction
473 between sodium alginate and calcium chloride during bead-making step. This step is a very
474 important step and must not be skipped as any residual sodium chloride will hinder
475 photosynthesis in the experiment. As shown in the Fig. 2, high cell numbers in a bead has a
476 negative effect on photosynthesis. We have found best results can be achieved when the

477   harvested cell numbers are between 250 x 10^6 - 395 x10^6 cells/ 50 mL falcon tube. Cells inside
478   the beads are oxygen-stressed. Hence it is important to leave air gaps inside the bracelet at each
479   end of the flexible tubing (see Materials and Methods). The same rule applies when performing
480   the experiment in a 5.5 mL glass vial. It is important to leave air gap of half the volume of the
481   vial.
482   Results in Fig. 6 showed that prolonged dark exposure of 15 hours has a negative effect on
483   photosynthesis. Algae bead bracelets exposed to 9 hours of darkness (Fig. 6) can be shifted back
484   and forth between dark and light to display color changes over a period of 24-48 hours (the color
485   changes slowly after 24 hours; data not shown). We have also found that once the bracelet is
486   assembled, if it is exposed to light for about 3-4 hours (which we call in our lab as the "light
487   charging of the bracelet") and, then switched to dark for 2-3 hours ["discharging of the
488   bracelet"], the bracelet displays fast color changes as long as the dark exposure time was not
489   exceeded beyond 9 hours (data not shown). But if the assembled bracelet is shifted to dark
490   immediately after assembly, the bracelet fails to display fast color changes. Immobilized oxygen
491   stressed-*Chlamydomonas* cells in the beads are dependent on photosynthesis for glucose
492   biosynthesis as they are immersed in tap water in the bracelet. Tap water lacks acetate and other
493   nutrients. We hypothesize that the initial light exposure allows the cells to synthesize
494   glucose/starch by photosynthesis which is later used to support the high rate of cellular
495   respiration in beads for energy production. If the bracelet is shifted to dark without prior light
496   exposure, the high cellular respiration rate consumes the existing starch in the cells. Hence when
497   this dark-exposed bracelet is exposed to light, the cells will have to synthesize enough
498   glucose/starch via photosynthesis to support the high rate of cellular respiration in the beads and
499   this will take some time. This is reflected in the slow color/pH changes of a bracelet that is
500   exposed to dark immediately after assembly compared to the one that is exposed to light
501   immediately after assembly.
502   *Chlamydomonas* can take up exogenous acetate from the TAP medium to make net synthesis of
503   glucose via the Glyoxylate/C2 cycle, which is present in many bacteria, micro-algae and plants
504   (Kunze et al., 2006). Substituting tap water with acetate containing-TAP growth media
505   (heterotrophic and photo-heterotrophic media; Text S3) inside the bracelet will hinder color
506   change in bracelets/vials as TAP medium has Tris buffer, which has a pKa value of 8.06 at 25°C
507   and a buffering range of pH 7-9. *Chlamydomonas* grows slower in High Salt (HS) photosynthetic
508   media than in TAP medium as HS medium lacks acetate (Sueoka, 1960). HS medium cannot be
509   used as a substitute for tap water inside the bracelet as we have tried it and have found that the
510   bracelets do not show color changes even if the beads are exposed to light for 48 hours (data not
511   shown).
512   During spring 2018- fall 2019, the described laboratory activities were incorporated in Biology
513   classes in nine schools and in Biology labs at the University of West Georgia and at the
514   Perimeter College [Georgia State University]. To date, we have targeted of about 947 school
515   students in Georgia and hope to target more college students in future. We are proposing a class
516   workflow in Table 7, which is based on the feedback of 12 school teachers and 2 college

517 instructors who participated in the Plant-BLOOME project. Regardless of the suggested time
518 line, instructors can adjust lab times according to their teaching agenda by either spreading the
519 lab activities across multiple classes or by removing one or more activities (Table 8). This will
520 allow the instructor to involve the class in discussion after each activity. Alternatively, students
521 can perform an outdoor experiment by wearing these bracelets/necklaces (as you can also make
522 algae bead necklaces) during day time and exposing these bracelets to strong sunlight or wear
523 them in the night and to see the water color changes. Conducting the experiment in a 5.5 mL
524 capped glass vials will expedite the experiment completion within 1.5-2 hours in classrooms.
525 The advantage of performing the experiment in glass vials is that students can clearly monitor
526 oxygen production in photosynthesis by monitoring the buoyancy of the algal beads over time.
527 Bead buoyancy is difficult to clearly visualize in a bracelet because of the narrow diameter of the
528 bracelet tubing.
529 Photosynthetic efficiencies of *Chlamydomonas* strains are measured in a laboratory by an oxygen
530 electrode. But many financially disadvantaged schools and institutions of higher learning do not
531 have access to an oxygen electrode. Our hands-on activity can be used to compare the
532 photosynthetic efficiencies of *Chlamydomonas* wild type and photosynthetic mutant strains in a
533 classroom setting. This will allow educators at institutions with limited resources to engage
534 students in critical thinking based on observations of a scientific experiment.
535 Our lab activities can be customized for different grade levels by adding or removing layers of
536 lab components. For example, for middle school students the algae bead bracelet or the vial
537 version of the experiment can be used. Students can observe under light microscopes, swimming
538 *Chlamydomonas* and its bright orange eyespot which is used by the cell for light sensing and aids
539 photo-taxis [Ueki et al., 2016). Photosynthesis is modulated by light color and light intensities.
540 Red and blue light stimulates photosynthesis and other colored light are not utilized for
541 photosynthesis. Hence algae bead bracelets can be used by high school students to test the
542 effects of different light intensities and colored light using different colored light filters.
543 High school students can also conduct a vial experiment a wild type strain and any well
544 characterized photosynthetic mutant strain. The cytochrome *f* deficient mutant (*ΔpetA*) [CC-3737
545 petA (N153Q)]; the D1-less mutant (*Fud7)* [CC-4147 FUD7 (psbA deletion) mt+] and the D2-
546 less mutant (*ΔPsbD*) [CC-4385 PsbD (deletion) mt+ are available via *Chlamydomonas* Resource
547 Center. Additionally, a basic bioinformatic laboratory can be added to the high school Biology
548 lab. The DNA sequence of the mutated gene in the photosynthetic *Chlamydomonas* mutant can
549 be given to students and they can use the DNA sequence to BLAST the NCBI database to
550 identify the gene and the protein. Students can also check for paralogs/orthologs of the identified
551 gene/protein. For college undergraduate level Biology labs, additional molecular and
552 biochemical layers can be added on top of the high school lab components as shown in Table 9.
553 We have provided sample pre- and post-lab questions (Text S4) and a rubric for grading pre- and
554 post-lab assignments that can be used by educators (Table S1). The assignments and the rubric
555 can be customized according to the knowledge base of students in the class. In summary, science
556 literacy in young students can be improved by studying a "pond-scum" which is used by plant

557   biologists, neuroscientists, biomedical and renewable energy researchers and can show them the
558   inter-disciplinary nature of 21st century Biology.
559

Conclusions

561   Our designed protocol can be used to make beads using motile micro-alga like *Chlamydomonas*
562   *reinhardtii.* These algal beads can be used for basic photosynthesis labs or for comparative
563   studies of relative rates of photosynthesis and cellular respiration in *Chlamydomonas* wild type
564   and mutant strains. Although our work was performed with the objective of designing engaging
565   hands-on plant biology labs for K16 Biology students, it might be useful to bioenergy
566   researchers who are interested in exploring the use of immobilized *Chlamydomonas* or other
567   motile green algae for biofuel production (Scranton et al., 2015; Radakovits et al., 2010). Our lab
568   activities using the wild type *Chlamydomonas* strain can be performed both in glass vials and in
569   bracelets. Based on our class room experiences at nine schools and two colleges in Georgia and
570   the enthusiasm of the plant community members at the educational booths at the Plant Biology
571   meetings organized by ASPB, we envision that young students will find the 'bracelet" approach
572   more enjoyable than conducting the same experiment in glass vials. Our lab activities are
573   inexpensive and can been customized according to grade levels.
574

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592

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660

Figure 1

Photosynthesis and cellular respiration-induced pH/color changes in vials containing *Chlamydomonas* 4A+ strain beads in de-ionized water and tap water .

C stands for control vials which do not contain algae beads and E stands for experimental vials containing algae beads. (A) Color changes in de-ionized water. (B) Color changes in tap water. Vials were exposed to light and darkness for 2 hours. Algal beads had approximately 2 x10^6 cells/beads and 8 algal beads were used per experimental vial.

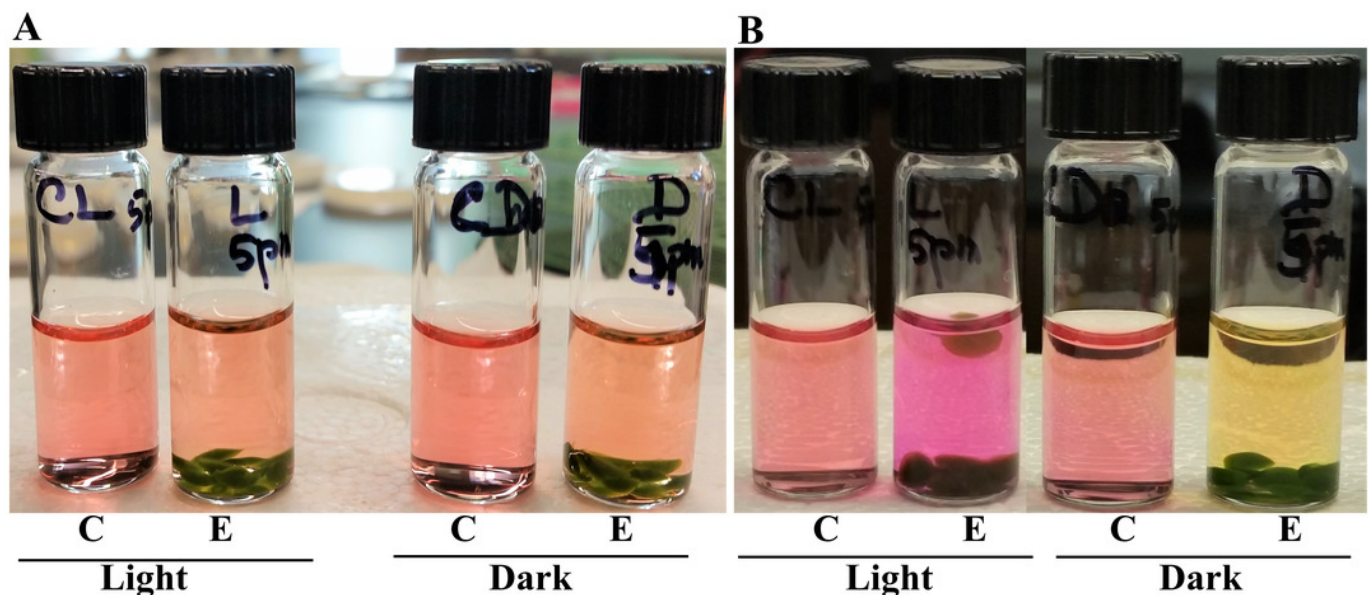



Figure 2

Effect of cell numbers per *Chlamydomonas* 4A+ strain bead on photosynthesis and cellular respiration-induced pH/color changes in tap water.

"C" stands for control vials that do not contain algae beads. "E" stands for experimental vials containing algae beads. (A) Color changes in experimental vials that contained beads that had approximately 4 x 10^6 cells/bead. (B) Color changes in experimental vials that contained beads that had approximately 2 x 10^6 cells/bead. Light and dark exposure of vials was for 2 hours. 8 algal beads were used per experimental vial.

A

C	E	C	E
Light		Dark	

B

C	E	C	E
Light		Dark	

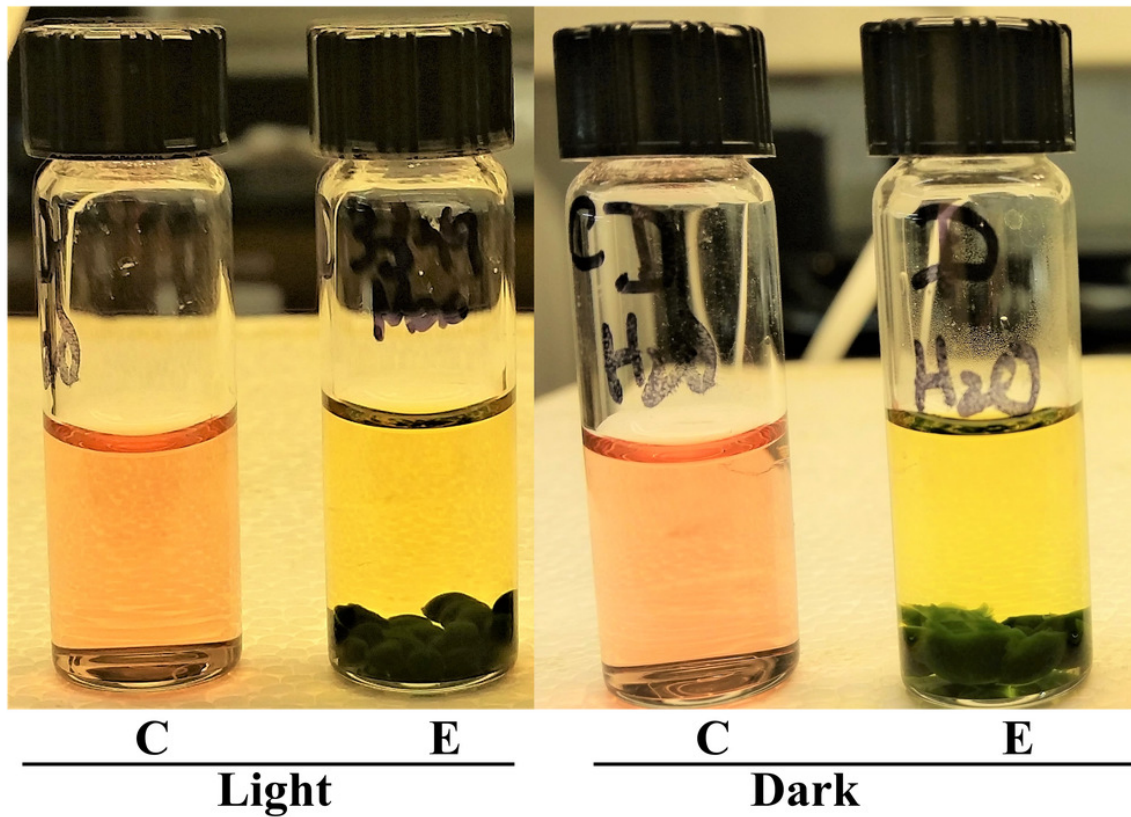
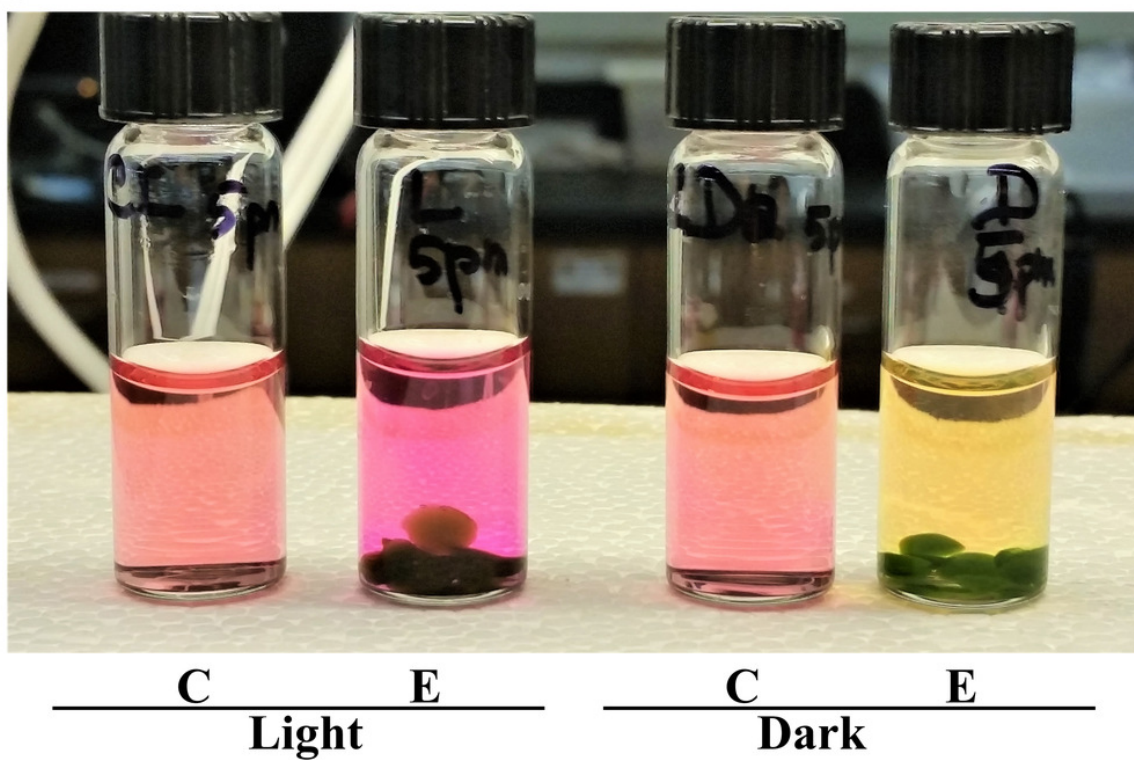
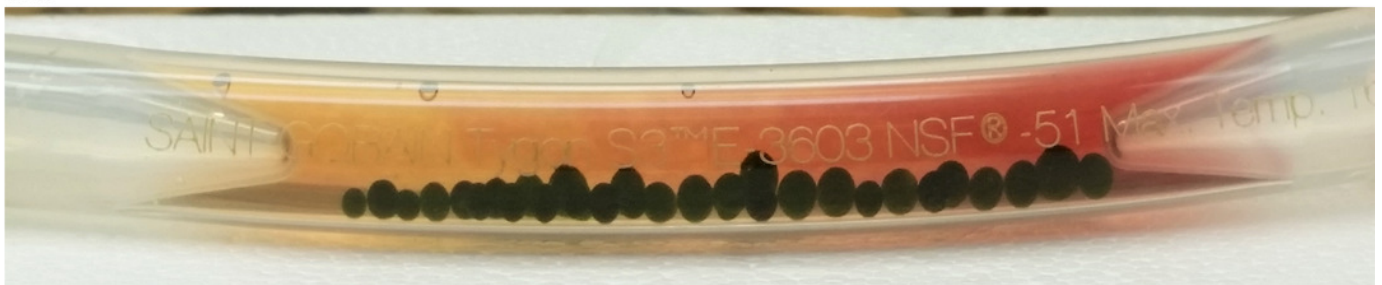
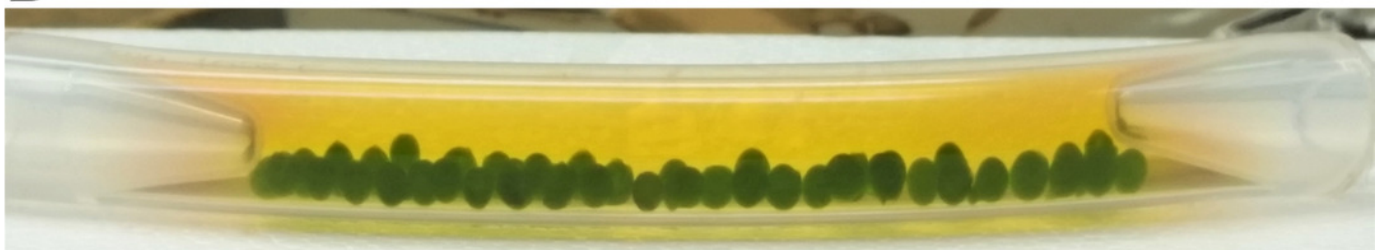



Figure 3

Indirect monitoring of carbon dioxide percentage inside the 4A+ bead bracelet under constant light and darkness using bicarbonate indicator.

(A) Control algal bracelet that has not been exposed to light or to darkness (zero time point).

(B) An algal bracelet that was exposed to darkness for 3 hours. (C) An algal bracelet that was exposed to light for 3 hours. Water color change was monitored in dark and light. Algal beads had approximately 2 x10^6 cells/bead. Number of algal beads in the control, dark- and light-exposed bracelets were 32, 38 and 38, respectively.

A

B

C

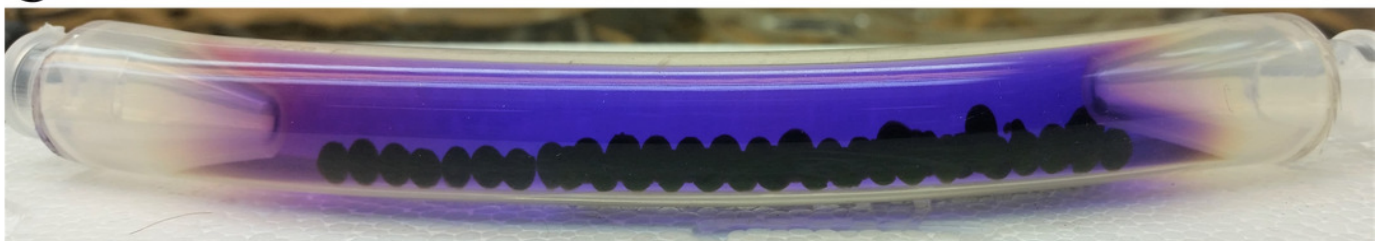



Figure 4

Time course monitoring of photosynthesis-induced color/pH changes in the dark-adapted 4A+ bead bracelet when shifted to light.

(A) An algal bracelet that was dark adapted for 4 hours. (B) Dark-adapted bracelet exposed to light for 1 hour. (C) Dark-adapted bracelet exposed to light for 2 hours. (D) Dark-adapted bracelet exposed to light for 3 hours. (E) Dark-adapted bracelet exposed to light for 4 hours.

Algal beads have approximately 2×10^6 cells/bead. Number of algal beads in the bracelet was 15. Bicarbonate indicator was used as a pH indicator.

Figure 5

Time course monitoring of cellular respiration-induced color/pH changes in the light-adapted 4A+ bead bracelet when shifted to darkness.

(A) An algal bracelet that was light adapted for 4 hours. (B) Light-adapted bracelet exposed to dark for 1 hour. (C) Light-adapted bracelet exposed to dark for 2 hours. (D) Light-adapted bracelet exposed to dark for 3 hours. Algal beads have approximately 2 x10^6 cells/bead. The bracelet contained thirty-six 4A+ strain beads. Bicarbonate indicator was used as a pH indicator.

A

B

C

D

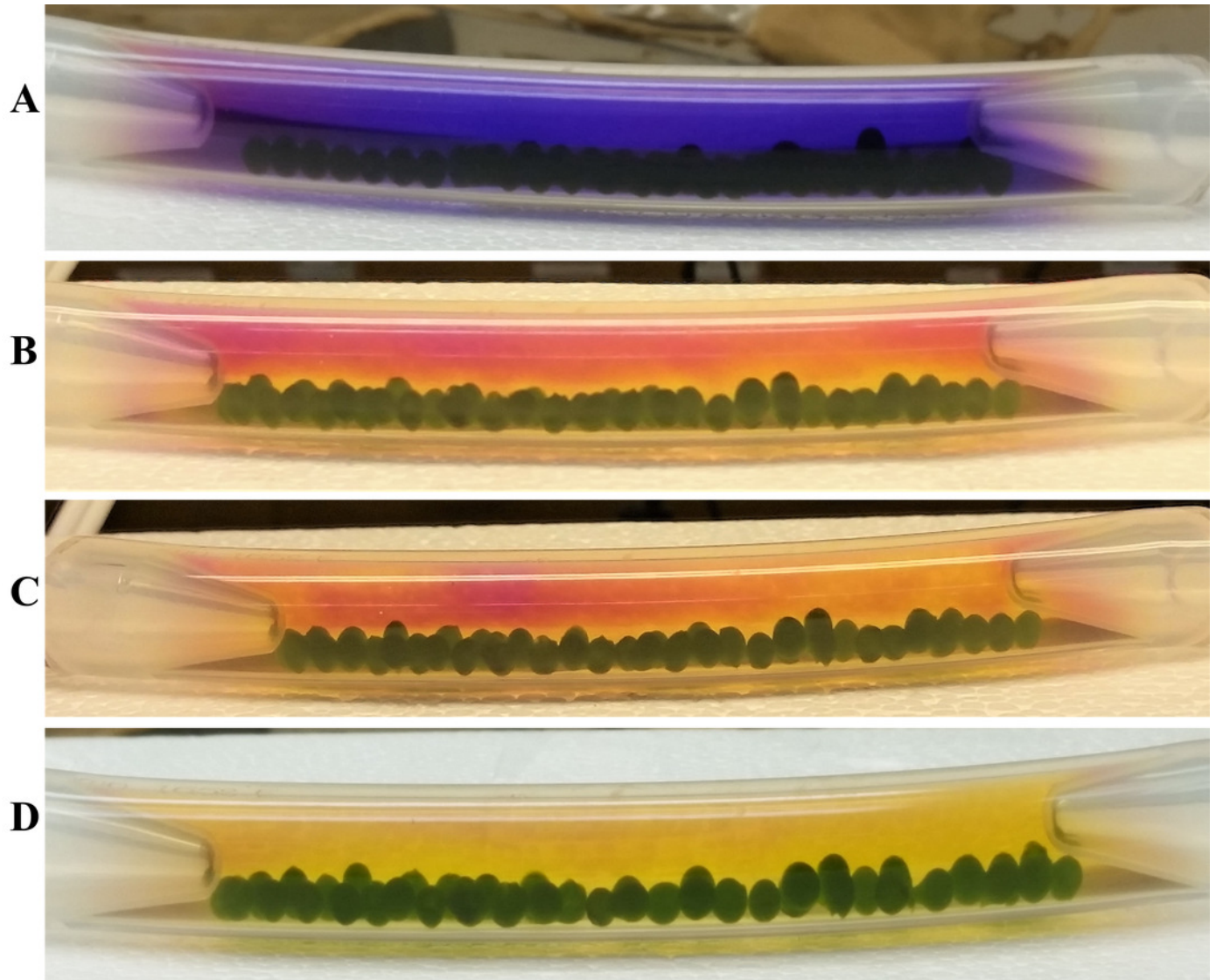



Figure 6

Effect of prior dark exposure duration on photosynthesis-induced color/pH changes in 4A+ bead bracelet in light.

(A) An algal bracelet that was dark adapted for 9 hours. (B) 9 hours-dark adapted-bracelet shifted to light for 4 hours. (C) An algal bracelet that was dark adapted for 15 hours. (D)15 hours-dark adapted-bracelet shifted to light for 12 hours. Algal beads had approximately 2 $x10^6$ cells/bead. Both bracelets contained 38 beads.

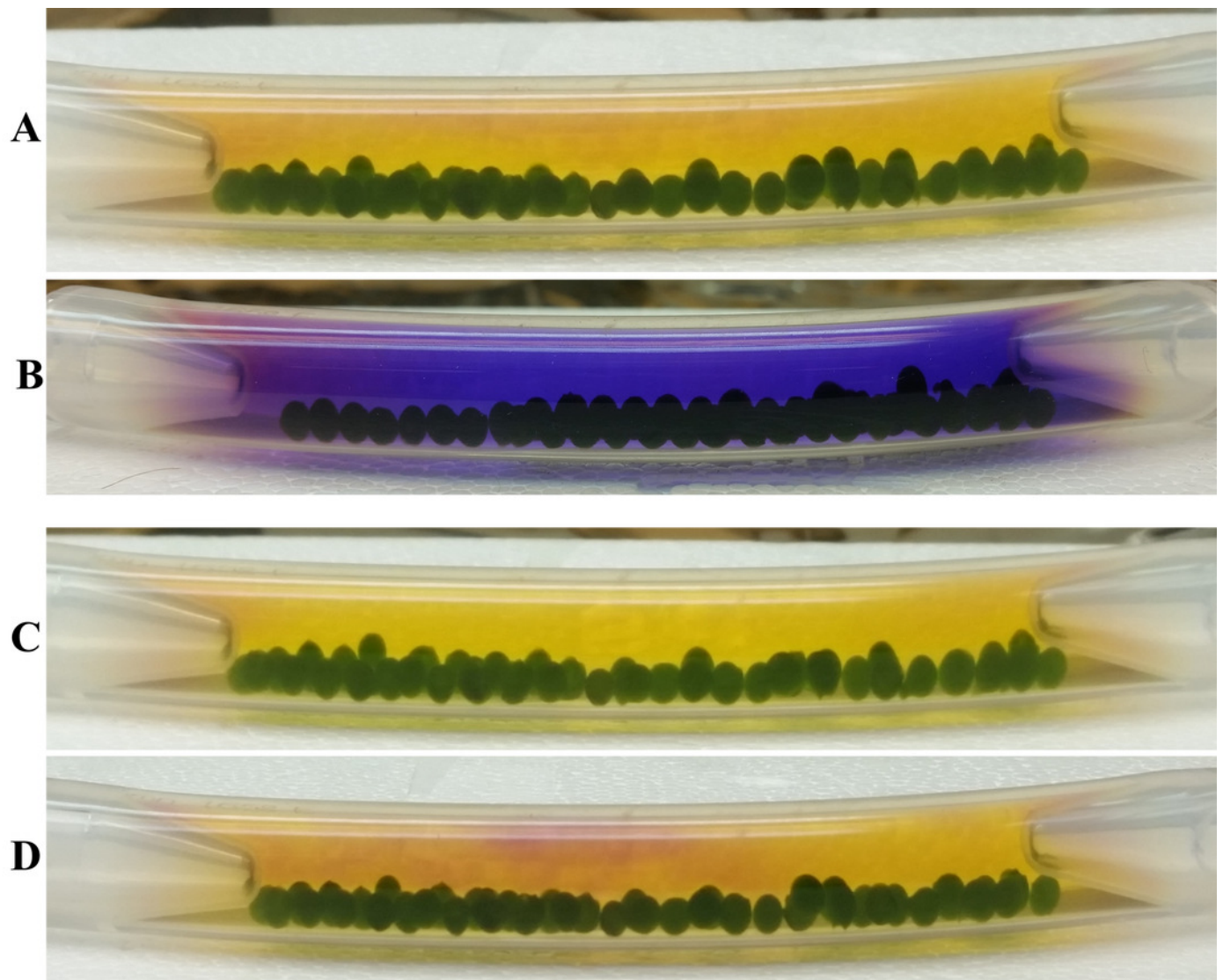



Figure 7

Comparative studies of photosynthesis and cellular respiration-induced pH/color changes in wild type 4A+ and *10E35* bead vials under light and darkness.

(A) Control, *10E35* and 4A+ bead vials before light exposure. (B) Control, *10E35* and 4A+ bead vials after 30 minutes of light exposure. (C) Control, *10E35* and 4A+ bead vials after 1 hour of light exposure. (D) Control, *10E35* and 4A+ bead vials before dark exposure. (E) Control, *10E35* and 4A+ bead vials after 30 minutes of dark exposure. (F) Control, *10E35* and 4A+ bead vials after 1 hour of dark exposure. Algal beads of each strain had approximately 2 $\times10^6$ cells/bead. Eight beads of each strain were used per experimental vial for the experiment. The order of the vials from left to right: control, *10E35* and 4A+ vials.

Light set

Dark set

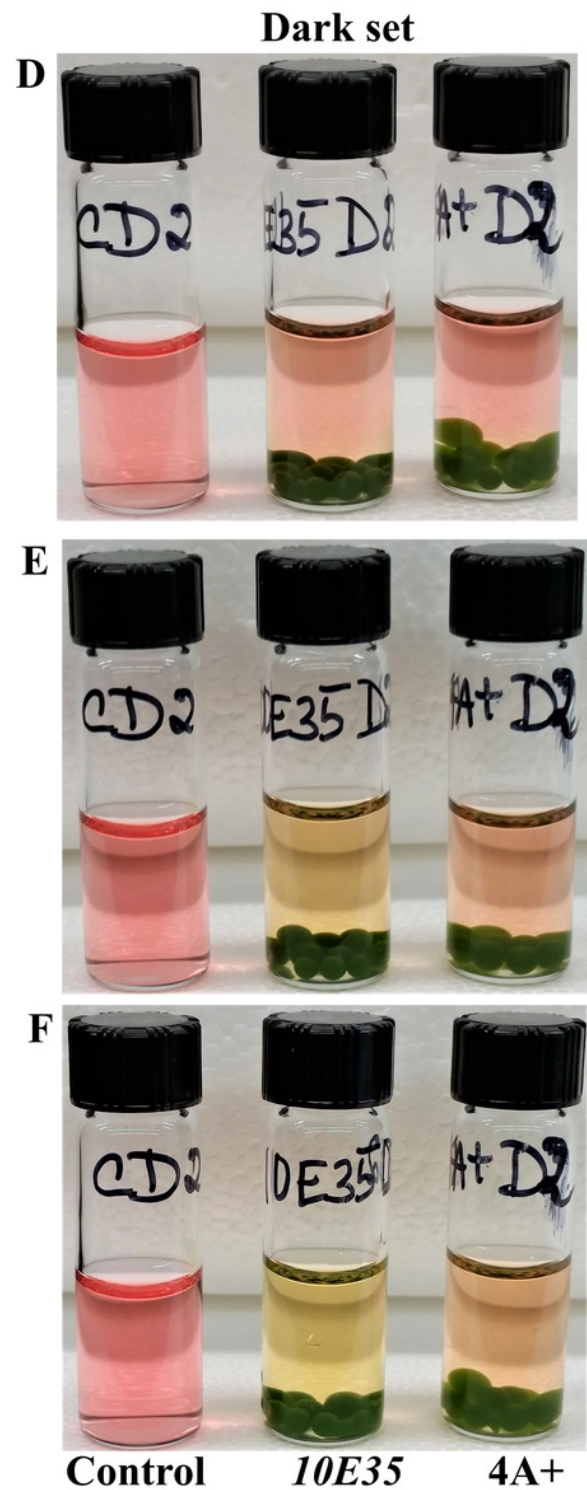
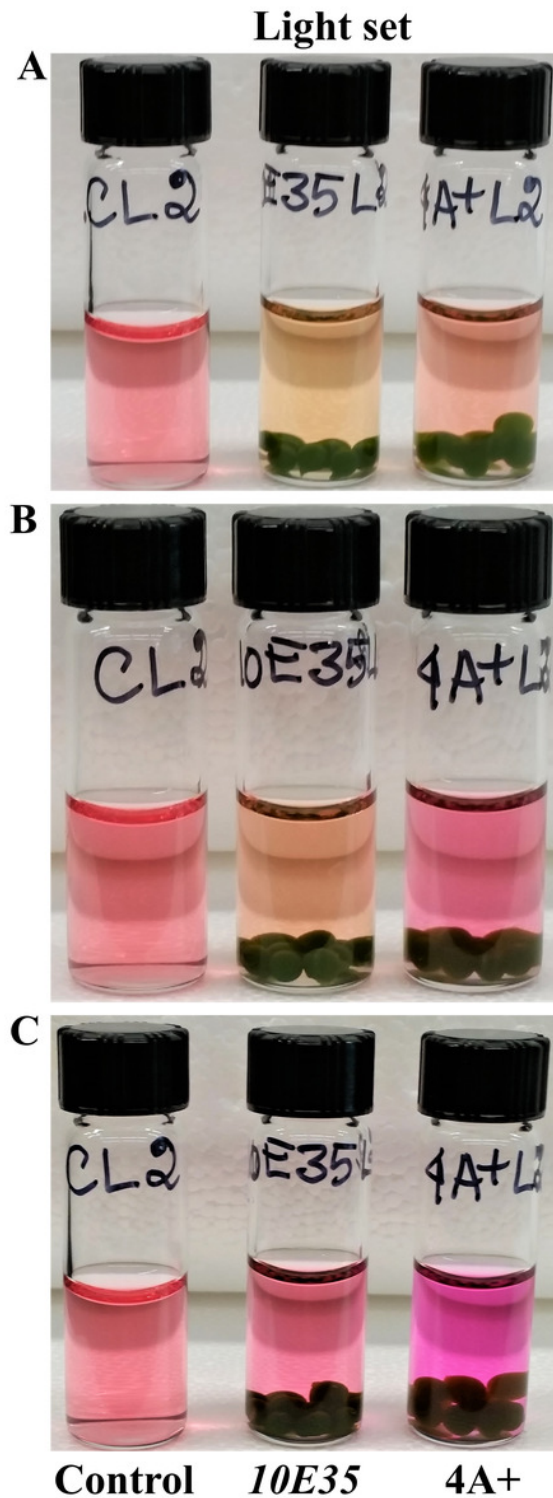



Figure 8

Time course monitoring of cellular respiration-induced color/pH changes in the dark in 4A+ and *10E35* bead vials that were adapted to light for 4 hours.

These vials are the light-adapted vials from the Fig. 7 experiments. (A) Light-adapted control, *10E35* and 4A+ bead vials before dark exposure. (B) Control, *10E35* and 4A+ bead vials after 15 minutes of dark exposure. (C) Control, *10E35* and 4A+ bead vials after 30 minutes of dark exposure. (D) Control, *10E35* and 4A+ bead vials after 45 minutes of dark exposure. (E) Control, *10E35* and 4A+ bead vials after 1 hour of dark exposure. The order of the vials from left to right: control, *10E35* and 4A+ vials.

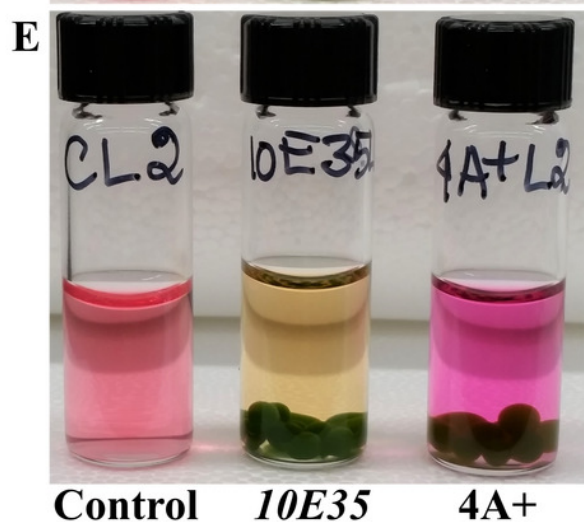
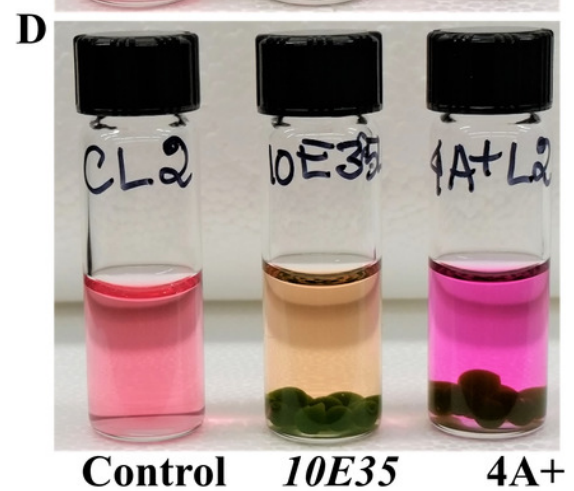
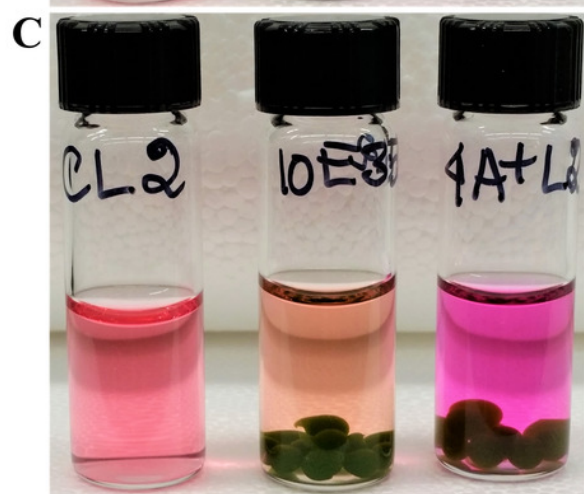
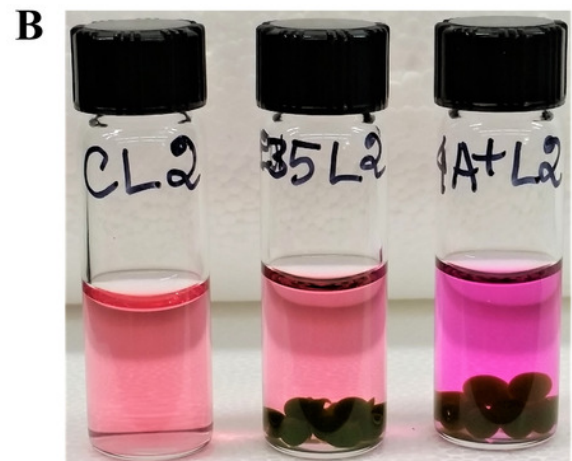
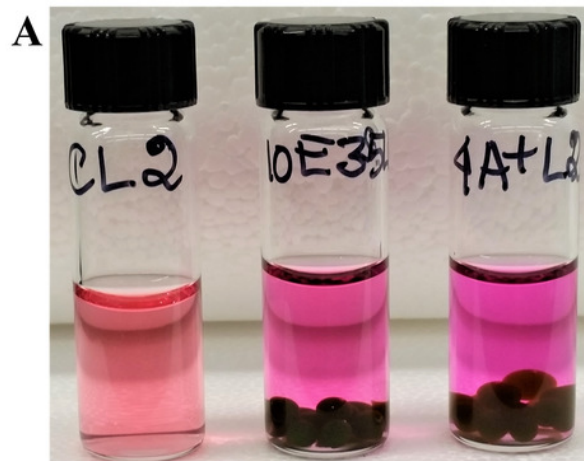
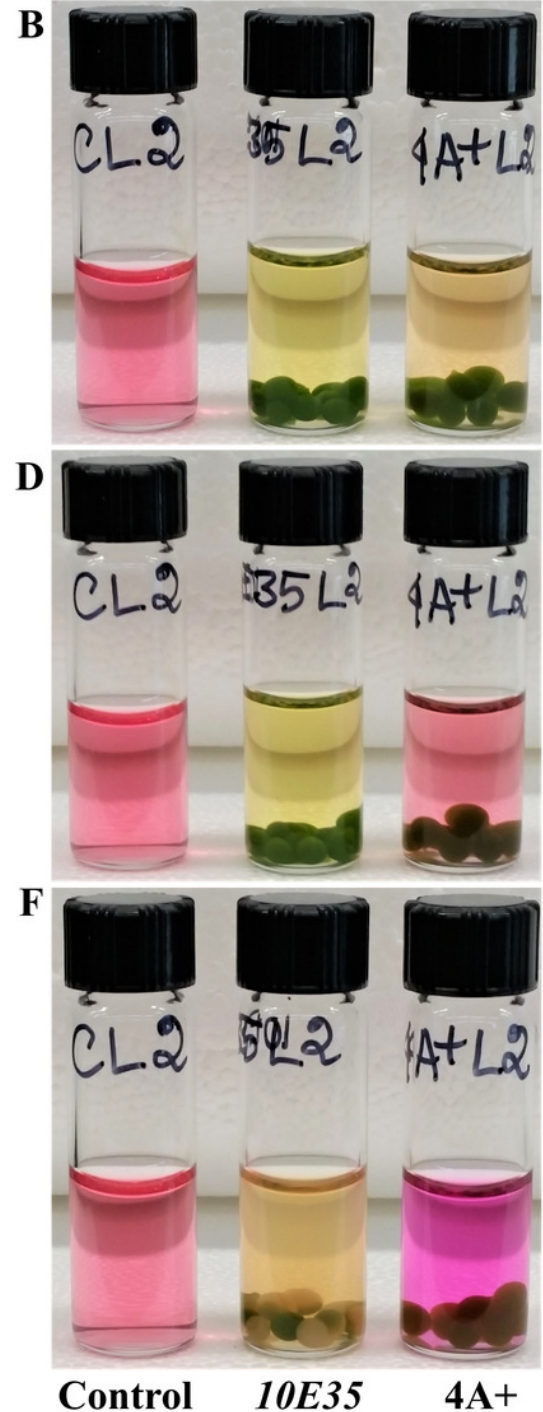
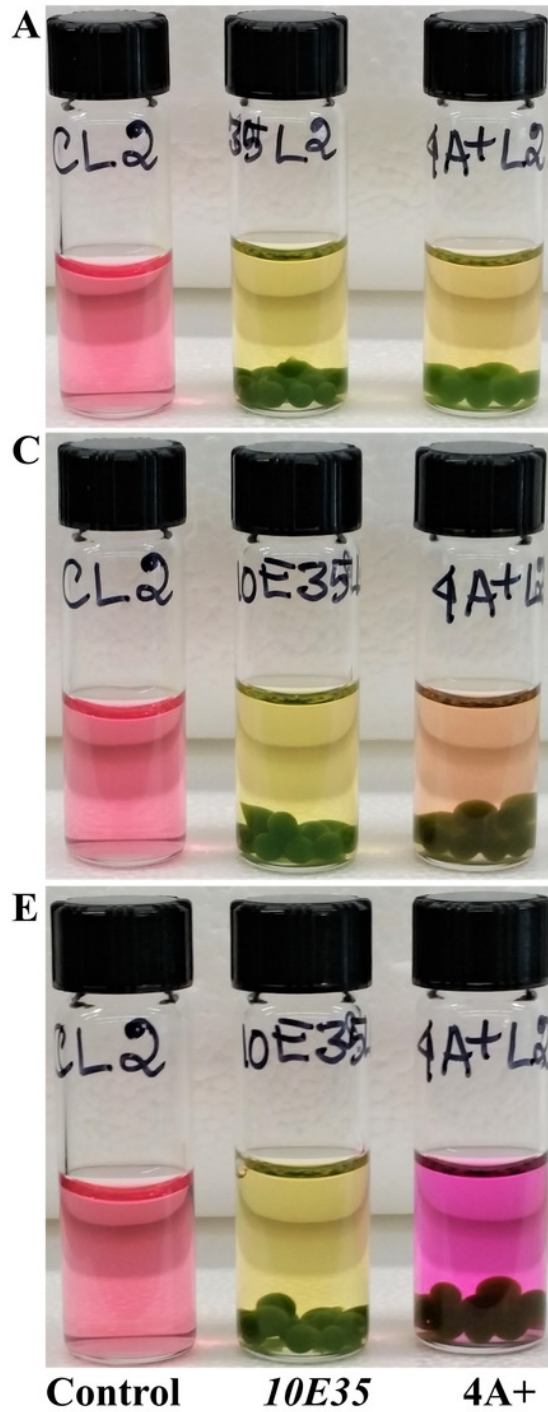



Figure 9

Time course monitoring of photosynthesis-induced color/pH changes in the light in 4A+ and *10E35* bead vials that were adapted to darkness for 6 hours.

These vials are the dark-adapted vials from the Fig. 8 experiments. (A) Dark-adapted control, *10E35* and 4A+ bead vials before light exposure. (B) Control, *10E35* and 4A+ bead vials after 30 minutes of light exposure. (C) Control, *10E35* and 4A+ bead vials after 1 hour of light exposure. (D) Control, *10E35* and 4A+ bead vials after 2 hours of light exposure. (E) Control, *10E35* and 4A+ bead vials after 3 hours of light exposure. (F) Control, *10E35* and 4A+ bead vials after 48 hours of dark exposure. The order of the vials from left to right: control, *10E35* and 4A+ vials.

pH changes in dark-and light-exposed vials containing *Chlamydomonas* 4A+ strain beads in de-ionized (DI) water and tap water.

The table shows the mean pH with standard deviations based on data from three biological replicates. Phenol red was used as the pH indicator in the control and experimental vials. pH was measured using a pH meter. Raw pH data of three biological replicates with statistical analyses can be found in 10.6084/m9.figshare.12344024 and in the Data S1 file . Data S2 file contains the raw pH data with mean and standard deviation information.

Samples	Mean
Control, light, DI water	7.17±0.06
4A+ beads, light, DI water	7.13±0.06
Control, dark, DI water	7.20±0.00
4A+ beads, dark, DI water	7.13±0.06
Control, light, tap water	7.27±0.06
4A+ beads, light, tap water	8.40±0.10
Control, dark, tap water	7.27±0.06
4A+ beads, dark, tap water	6.43±0.06

pH changes in dark-and light-exposed vials containing *Chlamydomonas* 4A+ strain beads differing in cell numbers per bead in tap water.

The table shows the mean pH with standard deviations based on data from three biological replicates. Phenol red was used as the pH indicator in control and experimental vials. Cell numbers shown below are approximate estimates. pH was measured using a pH meter. Raw pH data of three biological replicates with statistical analyses can be found in 10.6084/m9.figshare.12344024 and in the Data S1 file. Data S2 file contains the raw pH data with mean and standard deviation information.

Samples	Mean
Control for 4A+ 4 x 10^6 cells/bead set, light	7.00±0.00
4A+ 4 x 10^6 cells/bead, light	6.17±0.06
Control for 4A+ 4 x 10^6 cells/bead set, dark	7.10±0.10
4A+ 4 x 10^6 cells/bead; dark	6.07±0.06
Control for 4A+ 2 x 10^6 cells/bead set, light	7.17±0.15
4A+ 2 x 10^6 cells/bead, light	8.43±0.06
Control for 4A+ 2 x 10^6 cells/bead, dark	7.13±0.06
4A+ 2 x 10^6 cells/bead, dark	6.47±0.06

pH changes in the 4A+ bead bracelet that was exposed to light/darkness for three hours.

The table shows the mean pH with standard deviations based on data from three biological replicates. Control algal bracelet was not exposed to light or to darkness (zero time point). Bicarbonate indicator was used as the pH indicator in the algal bead bracelets. pH was measured using pH testing strips. Raw pH data of three biological replicates with statistical analyses can be found in 10.6084/m9.figshare.12344024 and in the Data S1 file. Data S2 file contains the raw pH data with mean and standard deviation information.

Samples	Mean
Control bracelet	7.00±0.00
Dark-exposed 4A+ algal bracelet	6.17±0.24
Light-exposed 4A+ algal bracelet	8.83±0.24

pH changes in 9 hours- and 15-hours dark adapted bracelets that were shifted to light for 4 hours and 12 hours, respectively.

The table shows the mean pH with standard deviations based on data from three biological replicates. Bicarbonate indicator was used as the pH indicator in the algal bead bracelets. pH was measured using pH testing strips. Raw pH data of three biological replicates with statistical analyses can be found in 10.6084/m9.figshare.12344024 and in the Data S1 file. Data S2 file contains the raw pH data with mean and standard deviation information.

Samples	Mean
9 hour-dark-exposed 4A+ algal bracelet	6.00±0.00
9 hour-dark-exposed 4A+ algal bracelet exposed to light for four hours	8.67±0.24
15 hour-dark-exposed 4A+ algal bracelet	5.50±0.41
9 hour-dark-exposed 4A+ algal bracelet exposed to light for 12 hours	6.33±0.24

pH changes in dark-and light exposed-vials containing wild type 4A+ and *10E35* mutant beads in tap water.

The table shows the mean pH with standard deviations based on data from three biological replicates. Phenol red was used as the pH indicator in control and experimental vials. pH was measured using a pH meter. Raw pH data of three biological replicates with statistical analyses can be found in 10.6084/m9.figshare.12344024 and in the Data S1 file. Data S2 file contains the raw pH data with mean and standard deviation information.

Samples	Average
Control before light shift	7.27±0.06
10E35 before light shift	6.73±0.06
4A+ before light shift	7.10±0.10
Control after 30 minutes of light exposure	7.23±0.06
10E35 after 30 minutes of light exposure	6.87±0.06
4A+ after 30 minutes of light exposure	7.63±0.06
Control after 1 hour of light exposure	7.33±0.06
10E35 after 1 hour of light exposure	7.57±0.06
4A+ after 1 hour of light exposure	8.77±0.06
Control before dark shift	7.20±0.10
10E35 before dark shift	6.80±0.10
4A+ before dark shift	7.07±0.06
Control after 30 minutes of dark exposure	7.10±0.10
10E35 after 30 minutes of dark exposure	6.53±0.06
4A+ after 30 minutes of dark exposure	6.97±0.06
Control after 1 hour of dark exposure	7.13±0.06
10E35 after 1 hour of dark exposure	6.33±0.06
4A+ after 1 hour of dark exposure	6.77±0.06

Cellular respiration-induced pH changes in the dark in 4A+ and *10E35* bead vials that were exposed to light for 4 hours.

The table shows the mean pH with standard deviations based on data from three biological replicates. Phenol red was used as the pH indicator in control and experimental vials. pH was measured using a pH meter. Raw pH data of three biological replicates with statistical analyses can be found in 10.6084/m9.figshare.12344024 and in the Data S1 file. Data S2 file contains the raw pH data with mean and standard deviation information.

Samples	Average
Control before dark shift	7.37±0.06
10E35 before dark shift	8.33±0.06
4A+ before dark shift	8.87±0.06
Control after 15 minutes of dark exposure	7.33±0.00
10E35 after 15 minutes of dark exposure	7.37±0.06
4A+ after 15 minutes of dark exposure	8.73±0.06
Control after 30 minutes of dark exposure	7.37±0.06
10E35 after 30 minutes of dark exposure	6.93±0.06
4A+ after 30 minutes of dark exposure	8.53±0.06
Control after 45 minutes of dark exposure	7.33±0.06
10E35 after 45 minutes of dark exposure	6.73±0.06
4A+ after 45 minutes of dark exposure	8.53±0.06
Control after 1 hour of dark exposure	7.33±0.06
10E35 after 1 hour of dark exposure	6.47±0.06
4A+ after 1 hour of dark exposure	8.40±0.10

Photosynthesis-induced pH changes in the light in 4A+ and *10E35* bead vials that were exposed to dark for 6 hours.

The table shows the mean pH with standard deviations based on data from three biological replicates. Phenol red was used as the pH indicator in control and experimental vials. pH was measured using a pH meter. Raw pH data of three biological replicates with statistical analyses can be found in 10.6084/m9.figshare.12344024 and in the Data S1 file. Data S2 file contains the raw pH data with mean and standard deviation information.

Samples	Average
Control before light shift	7.33±0.06
10E35 before light shift	5.97±0.06
4A+ before light shift	6.27±0.06
Control after 30 minutes of light exposure	7.30±0.00
10E35 after 30 minutes of light exposure	6.00±0.00
4A+ after 30 minutes of light exposure	6.47±0.06
Control after 1 hour of light exposure	7.37±0.06
10E35 after 1 hour of light exposure	6.17±0.06
4A+ after 1 hour of light exposure	6.83±0.06
Control after 2 hours of light exposure	7.27±0.06
10E35 after 2 hours of light exposure	6.20±0.00
4A+ after 2 hours of light exposure	7.37±0.06
Control after 3 hours of light exposure	7.20±0.10
10E35 after 3 hours of light exposure	6.23±0.06
4A+ after 3 hours of light exposure	8.37±0.06
Control after 48 hours of light exposure	7.30±0.00
10E35 after 48 hours of light exposure	6.43±0.06
4A+ after 48 hours of light exposure	8.47±0.06

Class workflow for photosynthesis/cellular respiration lab.

Activity #	Time Required	Can Activity be Split Across Different Classes?		Can this activity be grouped with other activities?
1. Inoculating algae growth media to culture algae and preparing bracelet tubing and yarn braids	Inoculation takes 10 minutes. It takes 30 minutes to prepare bracelet tubing and yarn braids	Not applicable as cultures need to be started 1-2 weeks ahead of bead making.		No
2. Algae bead making	35-40 minutes	Yes; beads can be made one-1 day before activities 3, 4 and 5 are performed		Yes; Activity 2 can be performed alone or combined with Activities 3 and 4
3. Bracelet Making	10-15 minutes	Yes, can be separated from activity 2 (see above)		Yes, Activities 3 & 4 can be combined
4. Light adaptation and light to dark shift of algae bracelets	3-4 hours for light adaptation on day 1 and 1.5 - 4 hours for dark shift on day 2	Yes, can be separated from activity 2 and 5 (see above)		Yes, Activities 2, 3 & 4 can be combined or Activities 3 & 4 can be combined.

Customization of the photosynthesis lab for college undergraduates.

Customization of the photosynthesis lab for college undergraduates.

Activities	Comments
1. Comparative photosynthesis studies of a wild type and a photosynthetic mutant strain using strain-specific algae beads in vials.	Same type of experiment as that described for 4A+ and 10E35 in the article.
1. PCR using mutated gene-specific primer using genomic DNA of the mutant and the wild type strain	Students can learn how to isolate genomic DNA
2. DNA gel electrophoresis and agarose gel extraction of the PCR product	Students learn molecular techniques
3. Cloning of the gel extracted PCR product, DNA sequencing of the clone and analyses of DNA sequencing data	Students learn molecular techniques
4. Western blotting to detect presence or absence of the protein in the wild type and mutant strains	If the protein-specific antibody is available.
5. Bioinformatic labs	Using various web-based free programs to perform multi-sequence alignments of DNA/protein sequences and generating phylogenetic trees, identifying conserved domains, studying gene expression and gene co-expression and generating gene network; prediction of protein location in cell, learning to use different gene/protein databases etc.
6. Photosynthetic pigment analyses by pigment extraction and spectrophotometry; Paper chromatography or thin layer chromatography- based labs.	Many photosynthetic mutants are deficient in chlorophyll and carotenoids. Chlorophyll and carotenoids can be extracted by 100% acetone.
7. Photosynthesis and Non-photochemical quenching studies using sophisticated equipment.	Only institutions that have an oxygen electrode and a PAM fluorometer can perform these activities.