Supplement S1

Calculation of phototrophic energy fluxes per unit mass at different light levels

The 'return on investment' of a phototrophic system is taken in this work to be the energy flux per unit mass of the dedicated phototrophic machinery at a given light level. Recycling rate of individual proteins within the phototrophic machinery was beyond the scope of this review to incorporate due to the widely varying half-life or dilution rates of different components, and as such energy fluxes are measured in energy flux per unit mass rather than energy yield per unit of protein synthesis.

To calculate the maximum mass-specific energetic rate of return (V_{max}) on investment of retinalophototrophic versus chlorophototrophic systems, the total mass per functional phototrophic unit (M_{total}), the cycling rate (R_{max}), and the protons pumped per cycle (N_p) must be known. V_{max} is equal to the product of N_p and R_{max} divided by M_{total}, as described in the following equation:

$$\text{Equation 1: } V_{max} = N_p \cdot R_{max} / M_{total}$$

Two retinalophototrophic systems, bacteriorhodopsin and proteorhodopsin, and two chlorophototrophic systems, oxygenic photosynthesizers and purple bacteria, were considered for breadth. Bacteriorhodopsin is described as a functionally monomeric 26 kDa protein [1]and proteorhodopsin is described as a functionally monomeric 27 kDa protein [2].

In the case of complex chlorophototrophic systems, the total mass per functional phototrophic unit must be calculated to include the mass of any antenna complexes associated with a

chlorophototrophic reaction center. This was done by multiplying the mass of each antenna complex by the number of antenna complexes per photosynthetic unit at their in vivo stoichiometry, and adding this to the total mass of the active center. There are few cases in which every reaction center and antenna complex in a single organism are structurally understood and the stoichiometry of each component is known. Thus, in order to estimate the total mass of a functional unit of chlorophototrophic machinery it is necessary to use data from multiple functionally similar organisms to infer an approximate value.

An oxygenic chlorophototrophic reaction center was taken to be a single instance of either photosystem I (PSI) or photosystem II (PSII). As described in Cunningham et al., 1989 [3], the ratio of PSII : PSI : Phycobilisome in the oxygenic red algae *Porphyridium cruentum* (ATCC 50161) in low light (6 μmol photons m^{-2} s^{-1}) is 2.85 : 5.35 : 1 and in high light (280 μmol photons m^{-2} s^{-1}) the ratio is 3.96 : 7.59 : 1. The average across all conditions tested was 3.13 PSII : 6.50 PSI : 1 phycobilisome in this red algae. The mass of PSII is taken to be 350 kDa as described for *Thermosynechococcus vulcanis* in Umena et al., 2011 [4], the mass of PSI is taken to be 356 kDa as described for *Synechococcus elongates* in Fromme et al., 2001 [5], and the mass of a phycobilisome antenna complex is taken to be 16.2 mDa as described for the red algae *Griffithsia pacifica* in Zhang et al., 2017 [6]. This brings the total mass of an approximated stoichiometric unit with 9.63 reaction centers (RCs) and one phycobilisome to approximately 20.2 mDa and the total mass per reaction center to 2098 kDa.

The mass of antenna complexes other than phycobilisomes in oxygenic chlorophototrophs was not considered due to diversity in antenna complexes present in different organisms. The mass of other electron transport chain components or membrane ATPases was not considered due to

likely low but poorly constrained stoichiometries [7], low masses compared to phycobilisomes [8], and their use in multiple cellular processes compared to the comparatively dedicated chlorophototrophic machinery.

Table S1: Mass estimation per oxygenic chlorophototrophic reaction center

Component	Number per stoichiometric unit	Mass
PSII	3.13	350 kDa
PSI	6.50	356 kDa
Phycobilisome	1	16.2 mDa
Total (9.63 RC plus phycobilisome)		20.2 mDa
Mass per RC (M_{total})		2098 kDa

A representative anoxygenic chlorophototrophic system from the purple bacteria species *Rhodospirillum photometricum* was examined for comparison, as described by Scheuring & Sturgis, 2009 [9]. In this species, each type-II reaction center is associated with one complex of 16 LH1 antenna proteins, forming a 'core complex', and multiple rings of 9 LH2 antenna proteins. The core complex:LH2 complex ratio was taken to be 3.5, as observed at high light adaptation in this work. The mass of the RCII/LH1 core complex described in Niwa et al., 2014 [10] in the purple bacterium *Thermochromatium tepidum* is 380 kDa. The measured mass of the LH2 complex described by Cherezov et al., 2006 [11] in the purple bacterium *Rps acidophila* is 130 kDa. This brings the total mass of a stoichiometric unit containing a single RC to approximately 835 kDa. Again, the mass of additional electron transport chain components was

not considered due to the multiple roles of these components in other cellular processes and low apparent stoichiometry relative to other chlorophototrophic machinery [7].

Table S2: Mass estimation per anoxygenic purple bacteria chlorophototrophic reaction center

Component	Number per stoichiometric unit	Mass
RC + LH1	1	380 kDa
LH2	3.5	130 kDa
Mass per RC (M_{total})		835 kDa

Field measurements of the maximum cycling rate of chlorophototrophic reaction centers from Kolber et al., 2000 [12] were used along with these figures to estimate the maximum energy flux per unit protein available to chlorophototrophs. In saturating light levels, oxygenic phototrophic phytoplankton were measured to have a maximum sustainable rate of reaction center photocycling of approximately 350 per second, and aerobic anoxygenic phototrophs were measured to reach up to approximately 150 per second. These are broadly consistent with in vitro measurements of photosystem II photocycle rate of more than 200 cycles per second observed in isolated photosystems by Lubner et al., 2011 [13]. Each photocycle of a reaction center was taken to represent two protons pumped across the photosynthetic membrane by the cytochrome b_6f complex in oxygenic chlorophototrophs or other electron transport chain components in anoxygenic chlorophototrophs. Electron transport chains containing the Complex-I like NDH complex (likely capable of pumping additional protons) rather than cytochrome b_6f alone was not considered due to a low apparent rate of cycling and low

stoichiometry, suggesting that while necessary for regulatory purposes it is not a primary player in energy metabolism [14].

Microbial rhodopsins were represented by proteorhodopsin which has been measured at being capable of 25 pumping protons per second in Friedrich et al., 2002 [15] and bacteriorhodopsin measured at approximately 50-100 protons per second in Béja et al., 2000 [2] and Lanyi, 2006 [16]. We conservatively took the bacteriorhodopsin maximum cycling rate to be 50 protons per second. In all cases, energy flux was calculated as described in equation 1.

Table S3: Maximum energy flux per unit mass for chlorophototrophy and retinalophototrophy

	Proteorhodopsin	Bacteriorhodopsin	Oxygenic RC	Anoxygenic RC
M_{total} (kDa)	27	26	2098	835
R_{max} (cycles s^{-1})	25	50	350	150
N_p (protons $cycle^{-1}$)	1	1	2	2
$R_{max}*N_p$ (Protons s^{-1})	25	50	700	300
V_{max} (Protons kDa^{-1} s^{-1})	0.93	1.92	0.33	0.36

The response of the energy flux per kilodalton of protein mass in chlorophototrophs and retinalophototrophs to varying light levels was modeled via simple Michaelis–Menten kinetics, using the following equation:

Equation 2: $$\boldsymbol{V} = \frac{\boldsymbol{V_{max}}\cdot[\boldsymbol{lightintensity}]}{\boldsymbol{K_m}+[\boldsymbol{lightintensity}]}$$

V_{max} was taken to be the previously calculated maximum energy flux rate of a phototrophic system in protons kDa^{-1} s^{-1}. K_m represents the light level at which the energy flux per functional unit reaches its half-maximum. The K_m for both proteorhodopsin and bacteriorhodopsin was taken to be 2700 μmol m^{-2} s^{-1}, as described for proteorhodopsin in Walter et al., 2007 [17]. The Km for oxygenic RCs was taken to be 40 μmol m^{-2} s^{-1} and the K_m for an anoxygenic RC was taken to be 191 μmol m^{-2} s^{-1}, as described in Kirchman and Hanson, 2013 [18].

Table S4: K_m, V_{max} for energy flux per unit mass for chlorophototrophy and retinalophototrophy

	Proteorhodopsin	Bacteriorhodopsin	Oxygenic RC	Anoxygenic RC
K_m (μmol m^{-2} s^{-1})	2700	2700	40	191
V_{max} (Protons kDa^{-1} s^{-1})	0.93	1.92	0.33	0.36

The relationship between light intensity and the energy flux per unit incident light for each phototrophic system was calculated using equation 3, derived by dividing Equation 2 by [light intensity], yielding the following equation.

$$\text{Equation 3: } Y = \frac{V_{max}}{K_m + [light intensity]}$$

The yield Y, in units of protons kDa^{-1} s^{-1} / (μmol m^{-2} s^{-1}), represents the specific energy flux of a unit of protein machinery per unit incident light, and is reduced upon saturation of the machinery with light. It is thus maximized at low light. The form that this efficiency curve takes is similar to that determined from first-principles modeling of anoxygenic chlorophototrophic machinery in Sener et al., 2019 [19].

The maximum yield per unit incident light was calculated by setting [light intensity] equal to zero, at which point the value of Equation 3 is maximized and the largest marginal return per unit incident light is achieved. This value is represented by the following equation:

$$\text{Equation 4: } Y_{max} = V_{max}/K_m$$

This maximum yield represents the highest efficiency available per available light resource, reached at infinitesimal light levels.

Table S5: Maximum yield Y_{max} for chlorophototrophy and retinalophototrophy

	Proteorhodopsin	Bacteriorhodopsin	Oxygenic RC	Anoxygenic RC
Y_{max} (protons kDa^{-1} s^{-1} / (μmol m^{-2} s^{-1}))	$3.43*10^{-4}$	$7.12*10^{-4}$	$8.34*10^{-3}$	$1.88*10^{-3}$

It is important to note that a large fraction of the difference in efficiency per unit incident light between chlorophototrophic and retinalophototrophic machinery is due not to the greater quantum yield of chlorophototrophic machinery per unit absorbed photon, but instead is simply due to a much larger absorption cross section per functional unit due to a much larger amount of dedicated light-gathering protein machinery per unit. However, the total absorption cross section per unit infrastructure for chlorophototrophs and retinalophototrophs is quite similar.

The protein infrastructure mass per unit absorption (M_{cs}) of a phototrophic system measures how efficiently light is captured. It is calculated from the mass per functional unit (M_{total}) and the absorption cross section per functional unit (Cs). The cross-section per functional unit is taken

from Kirchman and Hanson, 2013 [18], in which it is approximated for all rhodopsins (2 $Å^2$), an oxygenic phototroph (100 $Å^2$), and an anoxygenic phototroph (50 $Å^2$) based on a compilation of experiments and the light spectrum available in a marine environment. This is represented by the following equation:

Equation 5: $M_{cs} = M_{total}/Cs$

The mass per unit light-absorbing cross section is measured in in units of kDa $Å^{-2}$.

While a large amount of the difference in efficiency per unit incident light is explicable in terms of the difference in total mass per functional unit, the enhanced quantum yield of chlorophototrophic machinery in terms of protons pumped per photon absorbed is an important difference between the two forms of phototrophy. Thus, to account for the greater yield per absorbed photon, the mass per unit cross section is normalized using the following equation:

Equation 6: $NormalizedM_{cs} = M_{cs}/Yield$

This normalized mass per unit cross section accounts for the fact that photons absorbed by chlorophototrophic machinery are used to energize an electron transport chain and are capable of pumping ~2 protons per photon rather than the 1 proton per photon of retinalophototrophic machinery, and are thus twice as efficient in terms of yield per unit incident light.

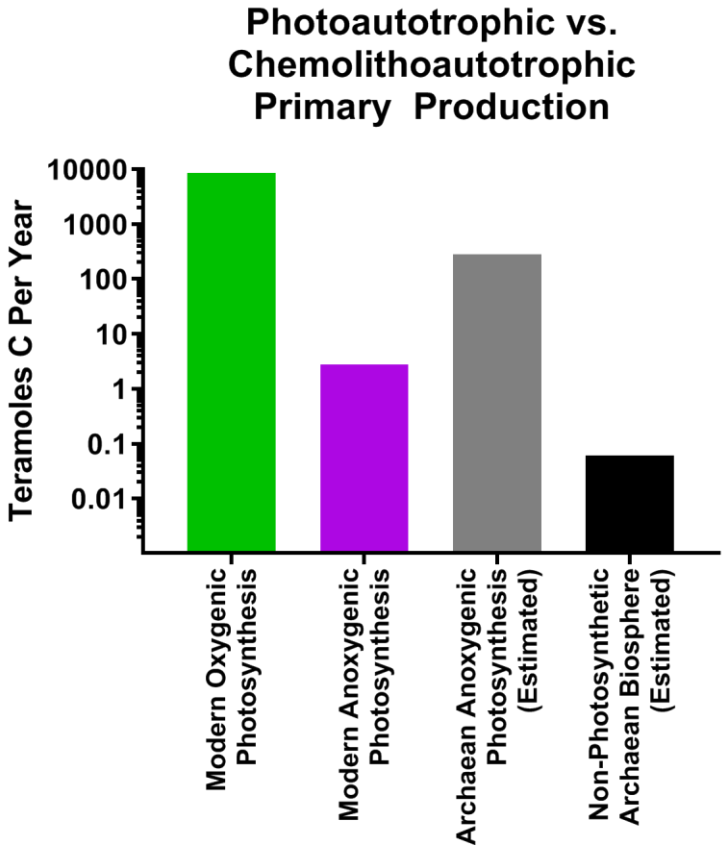
Table S6: mass per unit cross section calculation for chlorophototrophy and retinalophototrophy

	Proteorhodopsin	Bacteriorhodopsin	Oxygenic RC	Anoxygenic RC
M_{total} (kDa)	27	26	2098	835

Cs ($Å^2$)	2	2	100	50
M_{cs} (kDa $Å^{-2}$)	13.5	13	20.98	16.7
Yield (protons/photon)	1	1	2	2
Yield-Normalized M_{cs} (kDa $Å^{-2}$)	13.5	13	10.49	8.35

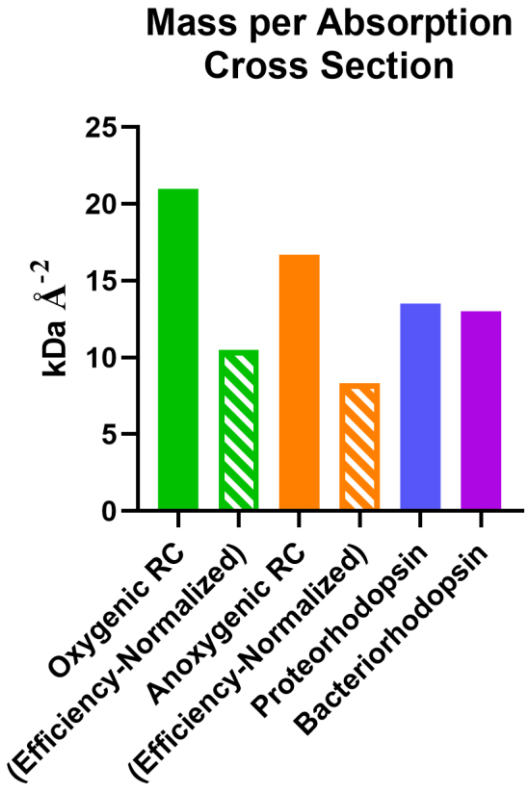
Strikingly, the mass per unit cross section for all phototrophic machineries, chlorophototrophic and retinalophototrophic, are calculated to be within a factor of ~1.6 regardless of yield normalization. This suggests that this approximate level of absorption cross section per unit mass represents a biophysical constraint of proteins bound to optically active cofactors, rather than being a detail specific to each form of phototrophic machinery (see Supplemental Figure 2). This may, however, be violated in the case of anoxygenic phototrophs bearing chlorosomes – the protein mass per chlorophyll molecule in these specialized structures present in bacteria adapted for extremely low-light conditions is estimated to be less than one fifth that of other chlorophototrophs [20]. These extreme low-light adapted phototrophs likely extend the trade-off between efficiency per unit resource and efficiency per unit light beyond that observed in other chlorophototrophs, as the mass per functional unit rises drastically while capturing dimmer light.

Supplemental Figure 1:



Legend: Comparison between carbon flux through biospheres driven by photosynthesis and chemolithoautotrophy. Modern oxygenic photosynthesis drives carbon fixation of nearly 9,000 teramoles per year [21]. While modern anoxygenic photosynthesis represents at most ~2.7 teramoles per year [22], estimates of anoxygenic photosynthesis in the Archaean are ~280 teramoles per year [23]. Estimates of possible carbon flux through a pre-photosynthetic, entirely chemolithotrophic biosphere driven by Archaean geochemical fluxes alone are circa 0.06 teramoles per year [24].

Supplemental Figure 2:



Legend: In solid colors, the mass per unit absorption cross section of chlorophototrophic and retinalophototrophic machinery as calculated from table S6. In striped colors, the yield-normalized mass per unit absorption cross section. Chlorophototrophic machinery is slightly more massive per unit light absorbed before normalization for quantum yield, and slightly less massive per unit light absorbed after normalization. No systems vary by more than approximately a factor of 1.6.

Works Cited:

1. Oesterhelt, D. and W. Stoeckenius, *Rhodopsin-like protein from the purple membrane of Halobacterium halobium.* Nature new biology, 1971. **233**(39): p. 149-152.
2. Béja, O., et al., *Bacterial rhodopsin: evidence for a new type of phototrophy in the sea.* Science, 2000. **289**(5486): p. 1902-1906.
3. Cunningham, F.X., et al., *Stoichiometry of photosystem I, photosystem II, and phycobilisomes in the red alga Porphyridium cruentum as a function of growth irradiance.* Plant physiology, 1989. **91**(3): p. 1179-1187.
4. Umena, Y., et al., *Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å.* Nature, 2011. **473**(7345): p. 55-60.
5. Fromme, P., P. Jordan, and N. Krauß, *Structure of photosystem I.* Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2001. **1507**(1-3): p. 5-31.
6. Zhang, J., et al., *Structure of phycobilisome from the red alga Griffithsia pacifica.* Nature, 2017. **551**(7678): p. 57-63.
7. Singharoy, A., et al., *Atoms to Phenotypes: Molecular Design Principles of Cellular Energy Metabolism.* Cell, 2019. **179**(5): p. 1098-1111. e23.
8. Muench, S.P., J. Trinick, and M.A. Harrison, *Structural divergence of the rotary ATPases.* Quarterly reviews of biophysics, 2011. **44**(3): p. 311-356.
9. Scheuring, S. and J.N. Sturgis, *Atomic force microscopy of the bacterial photosynthetic apparatus: plain pictures of an elaborate machinery.* Photosynthesis research, 2009. **102**(2-3): p. 197-211.
10. Niwa, S., et al., *Structure of the LH1–RC complex from Thermochromatium tepidum at 3.0 Å.* Nature, 2014. **508**(7495): p. 228-232.

11. Cherezov, V., et al., *Room to move: crystallizing membrane proteins in swollen lipidic mesophases.* Journal of molecular biology, 2006. **357**(5): p. 1605-1618.
12. Kolber, Z.S., et al., *Bacterial photosynthesis in surface waters of the open ocean.* Nature, 2000. **407**(6801): p. 177-179.
13. Lubner, C.E., et al., *Solar hydrogen-producing bionanodevice outperforms natural photosynthesis.* Proceedings of the National Academy of Sciences, 2011. **108**(52): p. 20988-20991.
14. Nawrocki, W., et al., *The mechanism of cyclic electron flow.* Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2019.
15. Friedrich, T., et al., *Proteorhodopsin is a light-driven proton pump with variable vectoriality.* Journal of molecular biology, 2002. **321**(5): p. 821-838.
16. Lanyi, J.K., *Proton transfers in the bacteriorhodopsin photocycle.* Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2006. **1757**(8): p. 1012-1018.
17. Walter, J.M., et al., *Light-powering Escherichia coli with proteorhodopsin.* Proceedings of the National Academy of Sciences, 2007. **104**(7): p. 2408-2412.
18. Kirchman, D.L. and T.E. Hanson, *Bioenergetics of photoheterotrophic bacteria in the oceans.* Environmental microbiology reports, 2013. **5**(2): p. 188-199.
19. Sener, M., et al., *Overall energy conversion efficiency of a photosynthetic vesicle.* elife, 2016. **5**: p. e09541.
20. Bryant, D.A. and D.P. Canniffe, *How nature designs light-harvesting antenna systems: design principles and functional realization in chlorophototrophic prokaryotes.* Journal of Physics B: Atomic, Molecular and Optical Physics, 2018. **51**(3): p. 033001.
21. Field, C.B., et al., *Primary production of the biosphere: integrating terrestrial and oceanic components.* science, 1998. **281**(5374): p. 237-240.

22. Raven, J.A., *Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments.* Aquatic Microbial Ecology, 2009. **56**(2-3): p. 177-192.

23. Canfield, D.E., M.T. Rosing, and C. Bjerrum, *Early anaerobic metabolisms.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2006. **361**(1474): p. 1819-1836.

24. Sleep, N.H. and D.K. Bird, *Niches of the pre-photosynthetic biosphere and geologic preservation of Earth's earliest ecology.* Geobiology, 2007. **5**(2): p. 101-117.