Supplemental Materials: DeltaPsi.py Version 1.0

DeltaPsi.py is a program for first-order, exploratory simulations of the effects of capacitance, proton buffering capacity and counter-ion movements on the thylakoid proton motive force (*pmf*), transthylakoid electric field ($\Delta\psi$), stroma-lumen pH difference (**ΔpH)**, linear electron flow (LEF), the ratio of ATP/NADPH produced by LEF, the activation and recovery of the lumen pH-dependent form of nontphotochemical quenching (NPQ), termed q_E, photosystem II (PSII) activity and recombination rates and $^1O_2$ production.

The code is based on the simulations in (Cruz et al. 2001) because simulations using this code were validated by the review process in both the original publication and in a separate, follow up publications (Zaks et al. 2012). The following describes changes in this version.

1) The code was updated to run on modern, open-source, cross-platform and freely-available Python platform. Examples below were performed using the open source Jupyter notebook platform (www.jupyter.org), but code can also be run on any Python platform.
2) To provide the maximal transparency and allow others to repeat and modify our code and simulations, the full code is made available on GitHub (www.github.com) with extensive annotation. Readers are encouraged to fork the code and add their modifications, improvements and tests. It is hoped that these open source tools will provide the capacity to extend or modify and extend and validate the simulations, especially to test new hypotheses.
3) A more efficient ODE solver, odeint (scipy.integrate.odeint https://docs.scipy.org/doc/scipy-0.18.1/reference/generated/scipy.integrate.odeint.html), was used to increase the speed and accuracy of the simulations.
3) Several improvements in the formulae were made to include several recent advances, as outlined in the following.
    a) Explicit simulation of Q_A and plastoquinone (PQ) redox states were included to simulate PSII quantum yields and to account for the effects of $\Delta\psi$ on PSI recombination reactions.
    b) The lumen pH-dependence of the xanthophyll cycle and the protonation of PsbS were updated to simulate the pH dependencies reported in Takizawa et al. (Takizawa et al. 2007) and Zaks et al. (Zaks et al. 2012).
    c) The effects of lumen pH on plastoquinol (PQH_2) oxidation at the cytochrome b_6f complex were included to account for the observed "photosynthetic control" of electron transfer by the lumen pH, using the model and pK_a values reported in (Takizawa et al. 2007).
    d) The driving force for ATP synthase reaction (and thus the efflux of protons) is now taken as being equal to the difference in the proton motive force (*pmf*) and the free energy storage in ATP (ΔG_{ATP}) divided by *n*, the stoichiometry of H^+ translocated through the ATP synthase over the number of molecules of ATP formed, as described in more detail below.
    e) A more realistic model for the kinetics of the cytochrome b6f complex were developed, to account for the thermodynamic and kinetic effects of pmf components on these reactions. In particular, the code now considers the b_6f complex to be fully reversible, so that both Dy and DpH alter the equilibrium constant.
    f) An equation was derived and implemented to calculate the quantum efficiency of PSII based on NPQ and Q_A redox state.

Table S1. Static and slowly-changing parameters. Table 1 describes the static, or slowly changing, parameters used in model; these constants are not updated by the odeint package, but can be updated between sub-sets . Baseline (standard) values and descriptions of the major parameters used in the simulations together with citations are given in Tables 1 and 2, but can be modified at run time, or during the simulations.

Parameter, variable name	**Initial Value**	**Units**	**Description**
standard PSII content, max_PSII	1	X $2 \cdot 10^{-13}$ moles cm^{-2}, X $6 \cdot 10^{10}$ complexes cm^{-2}	To simplify the calculations, the contents of photosynthetic complexes are normalized to a certain area of the thylakoid membrane, the standard PSII content, or max_PSII. The initial value is taken from the literature to be $6 \cdot 10^{-10}$ complexes cm^{-2}, see (1, 2)
cytochrome b_6f content, b6f_content	1	complex standard PSII content $^{-1}$	b6f_content describes the content of cytochrome b_6f complex relative to the standard PSII content. The content and properties of the cytochrome b6f complex are critical for maintaining photosynthetic control (3-9). Literature values range from substoichiometric to equal to that of PSII, and may change in response to environmental stresses or developmental regulation (10). Changes in this parameter can be compensated by changes in the rate constants.
PSI content, PSI_content	1	complex standard PSII content $^{-1}$	Describes the content of PSI centers relative to the standard PSII content. Changes in this parameter can be compensated by changes in the rate constants. A recent review of the literature (10) concluded that the ratio of PSI to PSII is relatively close to 1:1, but can vary from species to species, and in response to environmental stresses or developmental regulation (10).
ATP synthase content, ATP_synthase_content	1	complex standard PSII content $^{-1}$	Describes the content of ATP synthase centers relative to the standard PSII content. Changes in this parameter can be compensated by changes in the rate constants.
lumen volume per standard PSII content lumen_protons_per_turnover	$6.7 \cdot 10^{-21}$	L complex^{-1}	The lumen volume for each standard PSII content, derived from the estimated lumen volume per area of thylakoid (11). In this version, the volume is held constant, but in reality is expected to change with altered stromal and lumenal osmotic potentials, as reviewed in (12).
n, n	4.67	scalar	The stoichiometry of moles of protons translocated through the ATP synthase per mole of ATP produced. The value of *n* can be estimated based on the mechanistic model for ATP synthase and the observed stoichiometry of the c subunit ring in thylakoids (c_subunits_per_ATP_synthase = 14 (13), indicating 14 protons translocated for a complete rotation of the and the number of ATP molecules made for a full rotation of the F_1 subcomplex = 3 (14), suggesting that n = 4.67
ΔG_{ATP} , DeltaGatp_KJ_per_mol	40-45 kJ/mol	kJ/mol	ΔG_{ATP} is the free energy stored in stromal ATP/ADP + Pi couple. ΔG_{ATP} is thought to remain relatively constant under steady-state conditions, between 40-45 kJ/mol, but can change under fluctuating light (15).
ΔG_{ATP} (eV) DeltaGatp	0.42-0.47	eV	ΔG_{ATP} , expressed in electron volts.
pH_{stroma}, pHstroma_initial	7.8	pH units	The pH of the stromal compartment. Literature values for pH_{stroma} range from 7.5 to 8, and is thought to be higher during photosynthesis (16, 17). The pH of the stroma (considered to be constant in this version of the simulation).

V_{max}(VDE) , VDE_max_turnover_number	1	s^{-1} $PSII^{-1}$	Because we do not have reliable information on the ratio of VDE to PSII, this value is modulated to roughly match the observed rates of zeaxanthin accumulation.
pKa(VDE), pKvde	5.8	pH units	pK_a(VDE) is the effective pK_a for protonation of the violazanthin de-epoxidase (VDE), that results in the activation of the enzyme (18).
Hill coefficient for VDE activation, VDE_Hill	4	scalar	Empirical fitting of Z accumulation to in vivo estimates of lumen pH are consistent with a range of values reported for isolated thylakoids (18-20). Modulating this parameter from 1-5 affected results quantitatively, but did not alter the major trends.
rate constant for ZE, kZE	0.01	s^{-1}	The value of kZE was taken from Takizawa et al. (2007), to account for the apparent difference between the pKa for activation of VDE and the apparent pKa for accumulation of Z. Note that the zKE reaction is considered to be pseudo-first order with respect to the content of Z, and that any dependence on other substrates (NADPH and O_2) are ignored.
pK_a(PsbS), pKPsbS	6.0	pH units	pK_a(PsbS) describes the pKa for protonation of PsbS, which results in activation of the rapid qE response. The value of kZE was taken from Takizawa et al. (7), based on empirical fits to in vivo estimates.
$NPQ_{max,}$ max_NPQ	5.8	pH units	NPQ_{max} is an empirically-derived term describing the relationship between maximum level of NPQ at saturation, when all xanthophyll components are in the Z form, and all PsbS is protonated, so that: NPQ=max_NPQ [PsbS protonation][Z]. The maximum NPQ is known to be dependent on genotype and growth conditions.
Vmax(ATP synthase), ATP_synthase_max_turnover	1000	ATP s^{-1} $complex^{-1}$	The maximum turnover rate of ATP synthase.
$C_{thylakoid}$, Thylakoid_membrane_capacitance	0.6	µF cm^{-2}	The thylakoid membrane electrical capacitance (1, 2, 11).
Inverse thylakoid membrane capacitance, Volts_per_charge	0.033	V $charge^{-1}$ standard PSII content $^{-1}$	Volts_per_charge is used to rapidly calculate the membrane voltage generated by chare movements.
P_{K+}, perm_K	60-6000	ions s^{-1} V^{-1} standard PSII content $^{-1}$	P_{K+} describes the permeability of the thylakoid to K^+ or similar counter-ions. This term is adjusted empirically to modulate the simulated rates of appearance and dissipation of the ΔpH component of *pmf*. The units are in V^{-1} because as in Cruz et al. 2001, the simulation considers the rate of K+ movement through the channels to be proportional to pK+ and the potassium motive force, Kmf, i.e. the sum of the electrical field and concentration differences, calculated as for the *pmf*, see also below.
$V_{max}(b_6f)$, max_b6f	300	electrons from PQH_2 to PC $complex^{-1}$ s^{-1}	Maximum rate of the cytochrome b_6f complex, when the plastoquinone pool is in the reduced form (PQH_2) and lumen pH is well above the regulatory pK_a. The rate is an average of the individual partial reactions, and a value of about 300 is consistent with the observed rates of photosynthesis and complex stoichiometries.
$pK_{reg,}$ pKreg	6.0	pH units	The pKreg determines pH-dependence of PQH_2 oxidation at the cytochrome b6f complex. This is a kinetic constraint, likely related to the deprotonation of a His residue on the Rieske FeS protein (21-23). The empirically measured value is between 6.0 and 6.5 (6-9).
$E_{m,7}$ (PC), Em7_PC	0.37	V (versus SHE)	The midpoint potential of plastocyanin at pH=7, expressed as V against standard hydrogen electrode. The midpoint potential of PC is considered to be pH-independent over the physiological range (24).
$E_{m,7}$ (PQ/PQH_2), Em7_PQH2	0.11	V (versus SHE)	The midpoint potential of the PQ/PQH2 couple at pH=7. This value is pH dependent (22) and is adjusted in the program by -0.06 V per pH unit changes in lumen pH from 7.0.

β_{lumen}, buffering_capacity	0.03	M/pH unit	β_{lumen} describes the dependence of lumen pH on the number of protons introduced. the buffering capacity of the lumen
$\sigma_{PSI,}$ PSI_antenna_size	1	ratio	σ_{PSI} describes the relative PSI cross section (compared to that of PSII). Setting σ_{PSI} and σ_{PSII} equal will ensure that photosystems receive the same light flux.
$k_{PC\rightarrow P700}$+, k_PC_to_P700	500	PC^{-1} s^{-1}	The rate constant for oxidation of PC by P_{700}^{+}, with unity concentrations of reduced PC and P_{700}^{+}. The reaction is bimolecular, and the rate is determined by the product of $k_{PC\rightarrow P700}$+, the concentration of reduced PC and P_{700}^{+}. In version 1.0, the potential complexities of PC interactions with PSI and cytochrome *f* (25, 26) are simplified in this version of the simulation.
$\sigma_{PSII,}$ PSII_antenna_size	1	ratio	Describes the relative PSII cross section. See comments on $\sigma_{PSI,}$
k_{QA}, kQA	1000	s^{-1}	The rate constant for oxidation of Q_A^- by PQ. The reaction is simplified as a second-order process between reduced Q_A- and oxidized PQ, and thus a rough average of the individual partial reactions (27) that involve a series of electron transfer to Q_B, exchange of PQ and PQH_2 at the Q_B site etc.
k_{recomb}, k_recomb	0.33	s^{-1}	The rate of charge recombination in PSII. The value of k_{recomb} is that for recombination from the S_2Q_A- state in the absence of $\Delta\psi$ and ΔpH. The actual value will depend on a number of factors, including the redox state of the oxygen evolving complex, i.e. the S-state as well as $\Delta\psi$ and ΔpH. Some S-states are more or less stable than the S2 state, so the current code assumes the average is close to that from S_2Q_A-, which measured in the presence of DCMU but in the absence of an electric field is about 0.3 s-1 The effects of $\Delta\psi$ and ΔpH are discussed below.
$\phi_{triplet}$, triplet_yield	0.45	fraction	The yield of $^3P_{680}$ triplets arising from PSII recombination events. Recombination produces a high yield of $^3P_{680}$ ()
$\phi^1{}_{O2}$, triplet_to_singletO2_yield	1.0	fraction	The yield of $^1O_2$ from chlorophyll triplets, which is likely to be near unity ().

Table S2. Variable states. Table S2 describes the rapidly changing states of the model, which are updated by the odeint routines. Each of these parameters can change rapidly, and are modified by the odeint solver**.**

Parameter, variable name	**Initial Value**	**Units**	**Description**
Violaxanthin content, V	1	Relative	The content of violaxanthin in the thylakoid, expressed in relative units.
Zeaxanthin content, Z	1	Relative	The content of zeaxanthin in the thylakoid, expressed in relative units.
PQ, PQ	6	Relative	The number of molecules of plastoquinone (PQ) in its oxidized form, relative tostandard PSI content.

PQH_2, PQH2	1	Relative	The number of molecules of plastoquinol (PQH_2) in its oxidized form, relative tostandard PSI content.
PC(ox), PC_ox	0	Relative	The number of molecules of plastocyanin (PC) in its oxidized form, relative tostandard PSI content.
PC(red), PC_ox	2	Relative	The number of molecules of plastocyanin (PC) in its reduced form, relative tostandard PSI content.
pmf, pmf	0.06(ΔpH) + Δψ	V	The total proton motive force, *pmf* The initial value is calculated from the sum of energetic contributuions from ΔpH and Δψ.
ΔG_{ATP} , DeltaGatp_KJ_per_mol	40-45 kJ/mol	kJ/mol	ΔG_{ATP} is the free energy stored in stromal ATP/ADP + Pi couple. ΔG_{ATP} is thought to remain relatively constant under steady-state conditions, between 40-45 kJ/mol, but can change under fluctuating light (15).
ΔG_{ATP} (eV) DeltaGatp	0.42-0.47	eV	ΔG_{ATP} , expressed in electron volts.
pH_{stroma}, pHstroma	7.8	pH units	The pH of the stromal compartment. Literature values for pH_{stroma} range from 7.5 to 8, and is thought to be higher during photosynthesis (16, 17). The pH of the stroma (considered to be constant in this version of the simulation).
pH_{lumen}, pHlumen	*calculated at run time	pH units	The pH of the lumen.
K^+_{lumen}, Klumen	0.01-0.1	M	The concentration of K+ in the lumen. The current model assumes that all K+ is free, i.e. not bound.
K^+_{stroma}, Kstroma	0.01-0.1	M	The concentration of K+ in the stroma. The current model assumes that all K+ is free, i.e. not bound. In the current model, K^+_{stroma} is assumed to be constant.
Q_A, QA_content	1	relative	The content of oxidized QA relative to standard PSII.
Q_A^-, QAm_content	0	relative	The content of semiquinone Q_A- relative to standard PSII.
P_{700}^+, P700_ox	0	relative	The content of oxidized P_{700}^+ relative to PSI.
P_{700}, P700_red	1	relative	The content of reduced P_{700} relative to PSI.
Fd(ox), Fd_ox	1	relative	The content of oxidized ferredoxin (Fd) relative to standard PSII.
Fd(red), Fd_red	0	relative	The content of reduced ferredoxin (Fd) relative to standard PSII.
NPQ	0	relative	The ratio of rate constant (kNPQ) for regulated nonphotochemical quenching (NPQ) NPQ (kNPQ) relative to the sum of rate constants for non-radiative decay and fluorescence, see text for details.
$^1O_2$, singletO2	0	relative	The number of $^1O_2$ molecules produced per standard PSII.

[ATP], ATP_pool	Set by DGATP,	relative	The content of ATP in the stroma relative to standard PSII. The value is set from the initial DGATP value, with ATP+ADP=60
[ADP], ADP_pool	30		The content of ADP in the stroma relative to standard PSII.

Description of updated code.

The following describes the equations used in the simulations, focusing especially on those that extend the previous versions. Details of the calculations are embedded in the code and described in the code and the text.

The *pmf* and its partitioning into Δψ and ΔpH.

The theory and equations used to describe *pmf* components are essentially as described in earlier work by Cruz et al., (2001) and Zaks et al. (2012), with two updates. First, the new code allows for addition of antiporters or symporters, as described in the code for KEA3, which is proposed to act as a K+/H+ antiporter in the thylakoid membrane (28, 29):

#the KEA reaction looks like this:

$$H^+_{lumen} + K^+_{stroma} \rightleftharpoons H^+_{stroma} + K^+_{lumen} \quad \text{(Eq. 1)}$$

Where H^+_{lumen}. H^+_{stroma}, K^+_{lumen}, and K^+_{stroma} represent protons in the lumen and stroma and K^+ ions in the lumen and stroma, respectively. The reaction is electroneutral, so the forward reaction will depend on Δ[H^+]and Δ[K^+] as:

$$v_{KEA} = v_{KEA}\,(\,[H^+_{lumen}]\cdot[K^+_{stroma}] - [H^+_{stroma}][\,[K^+_{lumen}]) \quad \text{(Eq. 2)}$$

v_KEA = k_KEA*(Hlumen*Kstroma - Hstroma*Klumen) (Python)

where H^+_{lumen} (Hlumen), H^+_{stroma} (Hstroma), K^+_{lumen} (Klumen), K^+_{stroma} (Kstroma) are the concentrations of free H+ and K+ ions in the lumen and stroma, respectively.

#Next, the following is used to calculate K+ flux, which depends on the permeability of electrogenic K+ channels and the KEA reaction. The permeability of K^+ through the K^+ channel is perm_K. The K^+ flux through the electrogenic channel depends on both the K^+ concentration gradient and the electric field, i.e. $\Delta\tilde{\mu}_{K^+}$,

$$\Delta\tilde{\mu}_{K^+} = .06(\log_{10}\left(\frac{[K^+]_{stroma}}{[K^+]_{lumen}}\right) - \Delta\psi \text{ (Eq. 3)}$$

K_deltaG=(.06*np.log10(Kstroma/Klumen) - Dy) (Python)

where K_deltaG $= \Delta\tilde{\mu}_{K^+}$ and Dy=$\Delta\psi$, the transthylakoid electric field (stroma-lumen) in V.

The instantaneous net change in lumen [K^+] per unit time is given by:

$$\frac{d[K^+]}{dt} = v_{KEA} + P_{K^+}\Delta\tilde{\mu}_{K^+}([K^+]_{lumen} + [K^+]_{stroma})/2 \text{ (Eq. 3)}$$

net_Klumen = perm_K * K_deltaG*(Klumen+Kstroma)/2 + v_KEA (Python)

where net_Klumen = $\frac{d[K^+]}{dt}$. In this simplified form, we assume that the flux through the K^+ channel is proportional to the driving force and the average concentration of K^+ in the stroma and lumen.

PSII recombination reactions and singlet O_2 production

Earlier work (e.g. (30) (31, 32)) shows that both $\Delta\psi$ and ΔpH should increase PSII recombination rates, and thus should increase $^1O_2$ production. We derived an equation for estimating recombination rate in PSII is described in Davis et al (2016) considering the effects of ΔpH and $\Delta\psi$ on energetics of electron sharing between redox intermediates:

:

$$v_{recomb} = [S_2Q_A^-] * k_{recomb}10^{(f_{\Delta\psi}\frac{-\Delta\psi}{0.06} + f_{pH}\cdot\Delta pH)} \quad \text{(eq. 4)}$$

v_recomb = k_recomb*QAm*(10**(fraction_Dy_effect *Dy/.06 + fraction_pH_effect*(7.0-pHlumen)) (Python)

where v_{recomb} is the rate of recombination, $[S_2Q_A^-]$ is the concentration of PSII centers with reduced QA and the oxygen evolving complex in the S_2 state, k_{recomb} the intrinsic rate of recombination from $S_2Q_A^-$ in the absence of $\Delta\psi$ and ΔpH, ΔE_{stab} the stabilization free energy of the charge separated state, expressed in eV (Davis et al. 2016), $f_{\Delta\psi}$ (fraction_Dy_effect) and $f_{\Delta pH}$ (fraction_pH_effect) represents the fraction of quasi-stable S-states (that are able to recombine) and are sensitive to $\Delta\psi$ and ΔpH respectively (see text for more detail).

Normalizations of parameters

One (trivial) difference with our earlier model Cruz et al., (2001) is that content and concentration parameters are normalized to PSII content rather than thylakoid surface area. This will make it easier to account for lumen volume changes. We use the general parameters from the literature, as reviewed in Cruz et al., (2001), including the following. From Cruz et al., 2001 "...we estimated that there were approximately 2 × 10^{-13} mol of PSII cm^{-2} thylakoid mmbrane, or 6 X 10^{10} PSII complexes cm^{-2}. If all PSII centers were hit, on average there would be 6 x 10^{10} cm^{-2} protons delivered into the lumen.

2 × 10^{-13} mol of PSII cm^{-2} thylakoid membrane
6.02 x 10^{23} molecules per mole × 10^{-13} moles of PSII cm^{-2} thylakoid membrane
1.2 x 10^{11} PSII centers per cm^{-2} thylakoid membrane
1.2 x 10^{11} protons cm^{-2}, into 0.8 10^{-9} L cm^{-2} i.e. 23 10-6 moles/L are moved, equivalent to a change in the concentration of protons (both bound and unbound) of 2.3 10^{-5} moles or about 12 μM.

With 0.8 x 10^{-9} L lumen volume cm^{-2} thylakoid membrane, yield 1.2x10^{11} PSII centers per cm^{-2} thylakoid membrane, or 6.7x 10^{-21} L per PSII center. Therefore, one turnover of PSII should introduce about 1.7 10^{-24} moles H+ /6.7 10^{-21} L equivalent to a change in concentration of about 2.5 10^{-4} M.

Given the large lumen buffering capacity, β=0.03 M/pH unit (), the vast majority of these protons become buffered, and a single turnover flash should yield a **ΔpH** change of only about 0.008 units. On the other hand, the capacitance of the thylakoid membrane is expected to be small, about 0.6 μF/cm^2 (see text) so that moving one charge per PSII center should produce a substantial $\Delta\psi$,

Hitting all PSII centers with a single turnover flash should move 1.2 x 10^{11} charges cm^{-2} or 1.2 x 10^{11} charges/6.242×10^{18} charges $coulomb^{-1}$ =1.9E-8 C, and with 1.9E-8C/0.6 E-6C/V, and thus:

$\Delta\psi$(flash)=0.033 V

We use this value to indicate the effective $\Delta\psi$ for a transthylakoid movement of one charge per PSII equivalent.

The reactions of the cytochrome b_6f complex

The overall reaction for the cytochrome b_6f complex is takes to be:

PQH_2 + PC(ox) <-- b_6f--> PQ + *pmf* + PC(red) (Eq. 6)

where PQ and PQH_2 are the oxidized and reduced forms of plastoquinone, PC(ox) and PC(red) are the oxidized and reduced forms of plastocyanin, and pmf is the proton motive force. There are several factors to consider. First, there is a kinetic effect of lumen pH on the binding of PQH_2 to the Rieske Fe_2S_2 protein (21), with a pK_a near 6-6.5 (see (7) and references within), so that:

$b_6f(H^+)$ <-- --> b_6f(active), pKreg~6.5 (Eq. 7)

Where b_6f(active) is the deprotonated, active and $b_6f(H^+)$ is inactive forms of the complex. This is a kinetic constraint, likely related to the deprotonation of a His residue on the Rieske FeS protein, see Wang et al. (21), for a detailed description of the experimental bases of this interpretation in the cytochrome bc1 complexes. The term, pKreg, describes the pH-dependence of activation of the cytochrome b6f complex. The empirically measured value is between 6.0 and 6.5 (7).

Next, we need to consider the redox states of PQH_2, PC as well as the $\Delta\psi$ and ΔpH components of pmf. Because the Q-cycle releases 2 H^+ into the lumen for each electron passed from PQH_2 to PC, there should be a thermodynamic constraint to the forward reaction:

0.5PQH_2 + b_6f*(active)* + *PC(ox)* --*k_b6f*--> *PQ* + $b_6f(H^+)$ + *PC(red)* + 2H^+(lumen) (Eq. 8)

This is somewhat simplified in that the intermediate reactions involving electron transfer through the low potential cytochrome b chain are ignored and it is assumed that the electron from the semiquinone generated in the Qo site of the complex is immediately transferred to PQ, so that while one PQ is produced during the Qo site reaction, it is partially reduced with the net effect of oxidation of a 0.5 PQH_2.

The forward rate constant is k_b6f, but the reaction is reversible, so that

0.5PQH_2 + b_6f*(active)* + *PC(ox)* <--*k_b6f_reverse*-- *PQ* + $b_6f(H^+)$ + *PC(red)* + 2Hin (Eq. 9)

k_b6f_reverse is a function of *pmf* because the Q-cycle in the forward direction works against both ΔpH and $\Delta\psi$. Note that this thermodynamic effect is in addition to the kinetic effect on the deprotonation of the Rieske protein. We simulate this as follows:

$$K_{eq,b_6f} = E_m\left(\frac{PC_{ox}}{PC_{red}}\right) - E_m\left(\frac{PQ}{PQH_2}\right) - n \cdot pmf \quad \text{(Eq. 10)}$$

Keq_b6f = E_m(Pc(ox)/PC(red)) - E_m(PQ/PQH_2) – n**pmf* (Python)

where K_{eq,b_6f} is the equilibrium constant for the forward reaction, and E_m(Pc(ox)/PC(red))=) = 0.370 V and E_m(PQ/PQH_2)=0.11 V at pH=7, are the effective redox midpoint potentials for the PC and PQ couples at pH=7, but are pH-dependent so that:

$E_m(PQ/PQH_2)$ = 0.11 V - (7-pH_{lumen}) * 0.06 (Eq. 12)

. In other words, the overall equilibrium constant is determined by the redox potentials of the donor and acceptor together and the pmf. We use unity as the scaling factor for the *pmf* contributions because one proton translocated to the lumen per e- transferred (together with one e^- charge moved from the p- to the n-side) equilibrium.

$k_{b_6f,reverse} = \frac{k_{b6f,forward}}{K_{eq,b_6f}}$ (Eq.13)

k_b6f_reverse = k_b6f / Keq_b6f (Python)

In principle, we could simulate the effects of changing PQH_2 and PC redox states in two ways, either using the simulated concentrations of PQH_2 and PC together with the standard E'^0 (E_m) values, or accounting for the concentrations in the E_m values. We chose the former because it better fits the form of the ODE equations and is a bit simpler to calculate. Thus,

$v_{b6f} = [PQH_2][PC_{ox}]k_{b_6f,forward} - [PQ][PC_{red}]\, k_{b_6f,reverse}$ (eq. 14)

v_b6f=[PQH2]*[PC_ox]*k_b6f - [PQ]*[PC_red]*k_b6f_reverse (Python)

The midpoint potential of PC is pH-independent under our conditions, but E'0(PQ/PQH2) = 0.11 V at pH=7, but pH-dependent (see Eq. 12) so that:

$K_{eq,b_6f} = E_m\left(\frac{PC_{ox}}{PC_{red}}\right) - E_m\left(\frac{PQ}{PQH_2}\right) - n \cdot pmf = 0.370 - 0.11 + .06 * (\text{pHlumen} - 7.0) - n \cdot \text{pmf}$ (Eq. 15)

Keq_b6f = E'0(Pc_ox/PC_red) - E'0(PQ/PQH2) - pmf = 0.370 - 0.11 + .06 * (pHlumen - 7.0) – pmf (Python)

So, the full set of equations in Python is:

Em7_PC=0.37 Em_PC=Em7_PC Em7_PQH2 = 0.11 Em_PQH2= Em7_PQH2 + 0.06*(pHlumen-7.0) #(Eq. 16)

Keq_b6f = 10**((Em_PC - Em_PQH2 - pmf)/.06) #(Eq. 17)

k_b6f_reverse = k_b6f / Keq # (Eq. 18)

v_b6f=PQH2*PC_ox* k_b6f - PQ*PC_red*k_b6f_reverse # (Eq. 19)

Calculating Phi2

The following is a derivation for determining Phi2 from NPQ and QA redox state. The equations and derivations are based on those presented in Kramer et al. (33).

Recall that NPQ is the ratio of:

$$NPQ = \frac{k_{NPQ}}{k_f + k_d} \text{ (Eq. 20)}$$

where k_{NPQ}, k_f and k_d are the intrinsic rate constants for NPQ, fluorescence and non-radiative decay of excitons in the photosynthetic antenna. Also, maximal PSII quantum yield is:

$$\phi_{II,max} = \frac{k_{pc}}{k_d + k_f + k_{pc}} \sim 0.8 \text{ (Eq. 21)}$$

$$\frac{1}{\phi_{II,max}} = \frac{k_d + k_f + k_{pc}}{k_{pc}} = 1 + \frac{k_d + k_f}{k_{pc}} \text{ (Eq. 22)}$$

$$\frac{k_{pc}}{k_d + k_f} = \frac{1}{\frac{1}{\phi_{II,max}} - 1} \sim 4.88 \text{ (Eq. 23)}$$

where k_{pc} is the maximal rate constant for PSII photochemistry.

The realized ϕ_{II} at any time is given by:

$$\phi_{II} = \frac{[Q_A] k_{pc}}{k_d + k_f + k_{NPQ} + [Q_A] k_{pc}} \text{ (Eq. 24)}$$

where [QA] is the fraction of open PSII centers with oxidized Q_A.

$$\frac{1}{\phi_{II}} = \frac{k_d + k_f + k_{NPQ} + [Q_A] k_{pc}}{[Q_A] k_{pc}} = 1 + \frac{k_d + k_f + k_{NPQ}}{[Q_A] k_{pc}} = 1 + \frac{k_d + k_f}{[Q_A] k_{pc}} + \frac{k_{NPQ}}{[Q_A] k_{pc}} = 1 + \frac{k_d + k_f}{[Q_A] k_{pc}} + \frac{NPQ(k_f + k_d)}{[Q_A] k_{pc}} \text{ (Eq. 25)}$$

$$[Q_A]\left(\frac{1}{\phi_{II}}-1\right)=\frac{k_d+k_f}{k_{pc}}+\frac{NPQ(k_f+k_d)}{k_{pc}}=\frac{k_d+k_f}{k_{pc}}(1+NPQ)=\frac{(1+NPQ)}{4.88}$$ (Eq. 26)

$$\frac{1}{\phi_{II}}-1=\frac{(1+NPQ)}{4.88[Q_A]}$$ (Eq. 27)

$$\frac{1}{\phi_{II}}=\frac{(1+NPQ)}{4.88[Q_A]}+1$$ (Eq. 28)

$$\phi_{II}=\frac{1}{\frac{(1+NPQ)}{4.88[Q_A]}+1}$$ (Eq. 29)

In Python, this translates to:

```
def Calc_Phi2(QA, NPQ):
    Phi2=1/(1+(1+NPQ)/(4.88*QA))
    return Phi2
```

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