Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Supporting information for "Conservation and Diversity in the Secondary Forward Photodynamics of Red/Green Cyanobacteriochromes"

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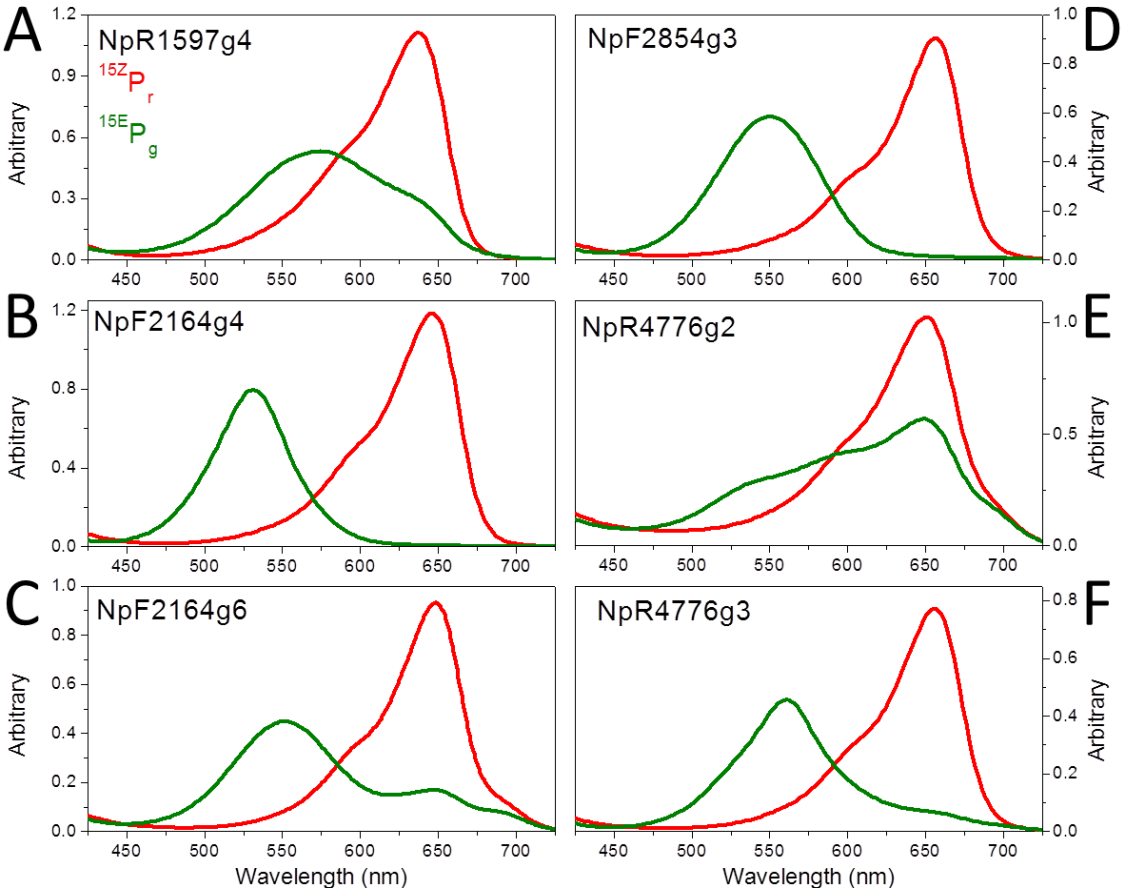

Figure S1. Ground state $^{15Z}P_r$ (red curves) and $^{15E}P_g$ (green curves) absorption spectra for the remaining CBCRs in this study. Several CBCRs do not fully convert from the $^{15Z}P_r$ to the $^{15E}P_g$ state, as shown by the distorted $^{15E}P_g$ spectra for NpR1597g4, NpR4776g2, and NpF2164g6.

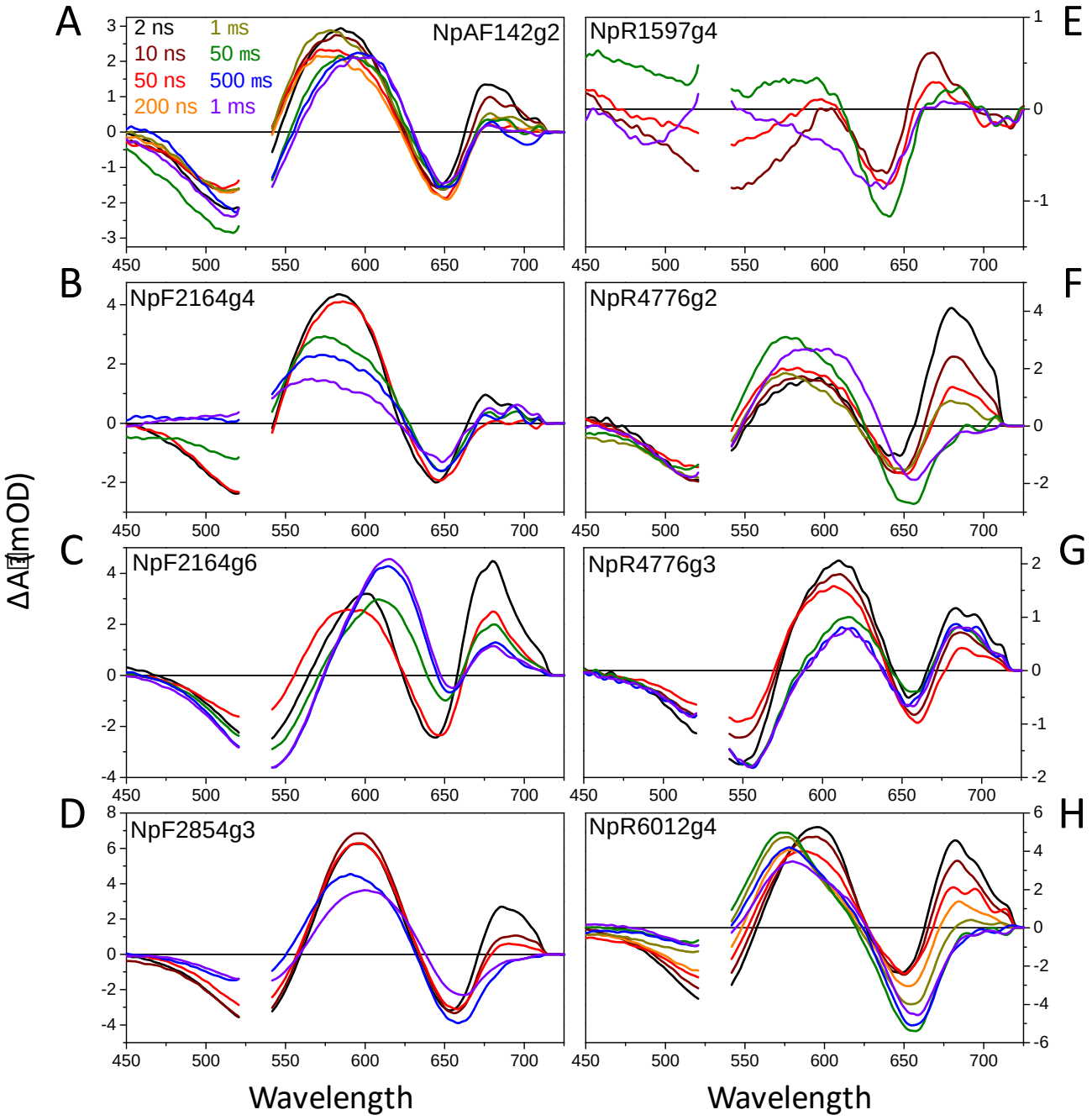

Figure S2. Raw (uncorrected) transient absorption spectra after 532-nm excitation for (A) NpAF142g2 (B) NpF2164g4, (C) NpF2164g6, (D) NpF2854g3, (E) NpR1597g4 (F) NpR4776g2, and (G) NpR4776g3 and (H) NpR6012g4. Signals exhibit signatures of forward and reverse reaction dynamics overlapping. Spectra are color-coded to the probe times specified in the legend in panel A. Note that not all probe times are represented for each CBCR.

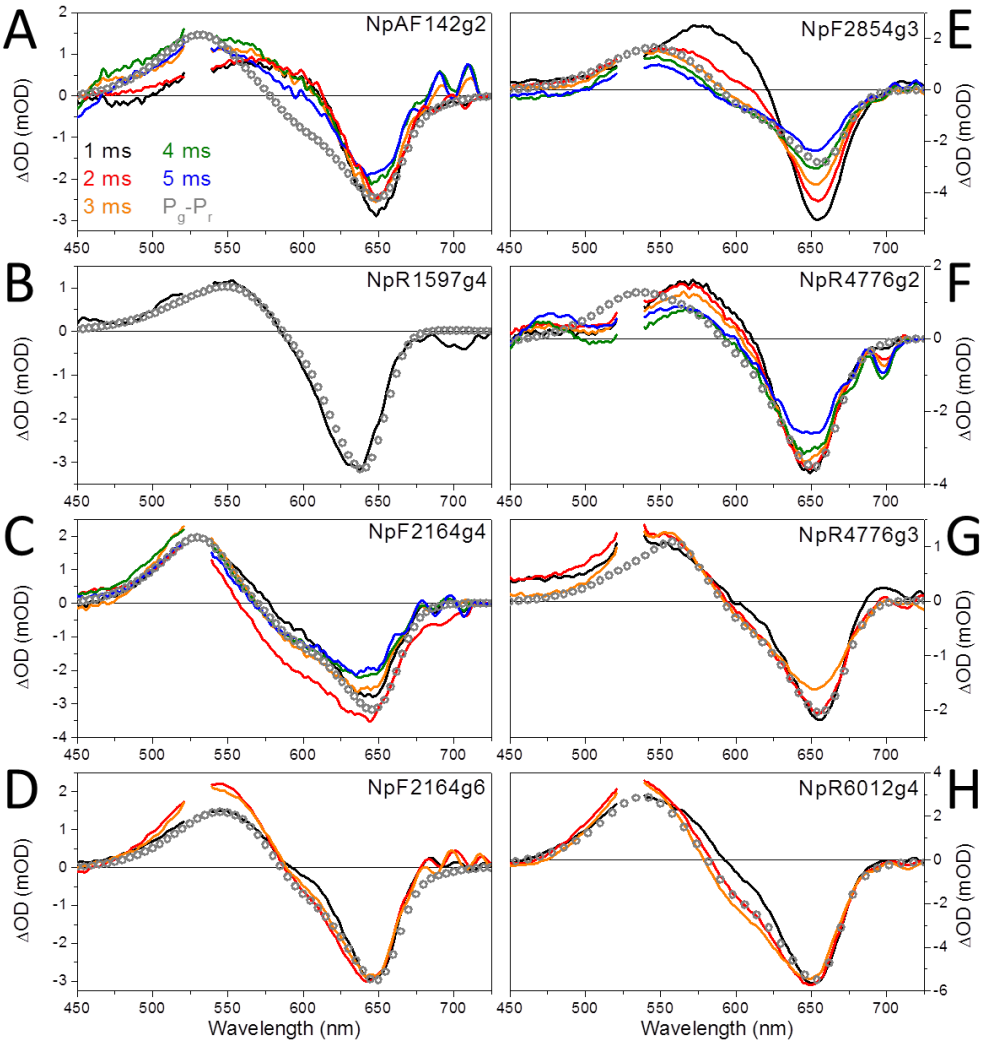

Figure S3. Transient absorption difference spectra for the CBCRs past 1 ms. Signals for (B) NpR1597g4 were not collected past 1 ms. Signals for (D) NpF2164g6, (G) NpR4776g3 and (H) NpR6012g4 were not collected past 3 ms. The signals for the remaining CBCRs suffer from low S/N after 5 ms. This is especially true for (E) NpF2854g3. Spectra colors correspond to the probe times specified in the legend in panel A. Ground state difference spectra (gray circles) are shown to determine completeness of the photoreaction for each CBCR.

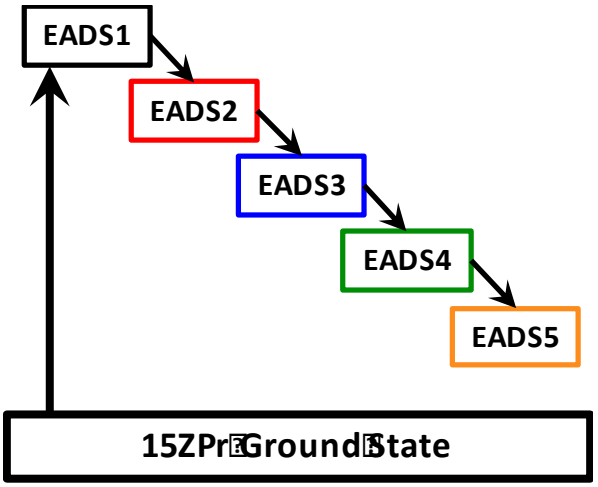

Figure S4. Sequential model used to extract Evolution Associated Difference Spectra (EADS) for each CBCR.

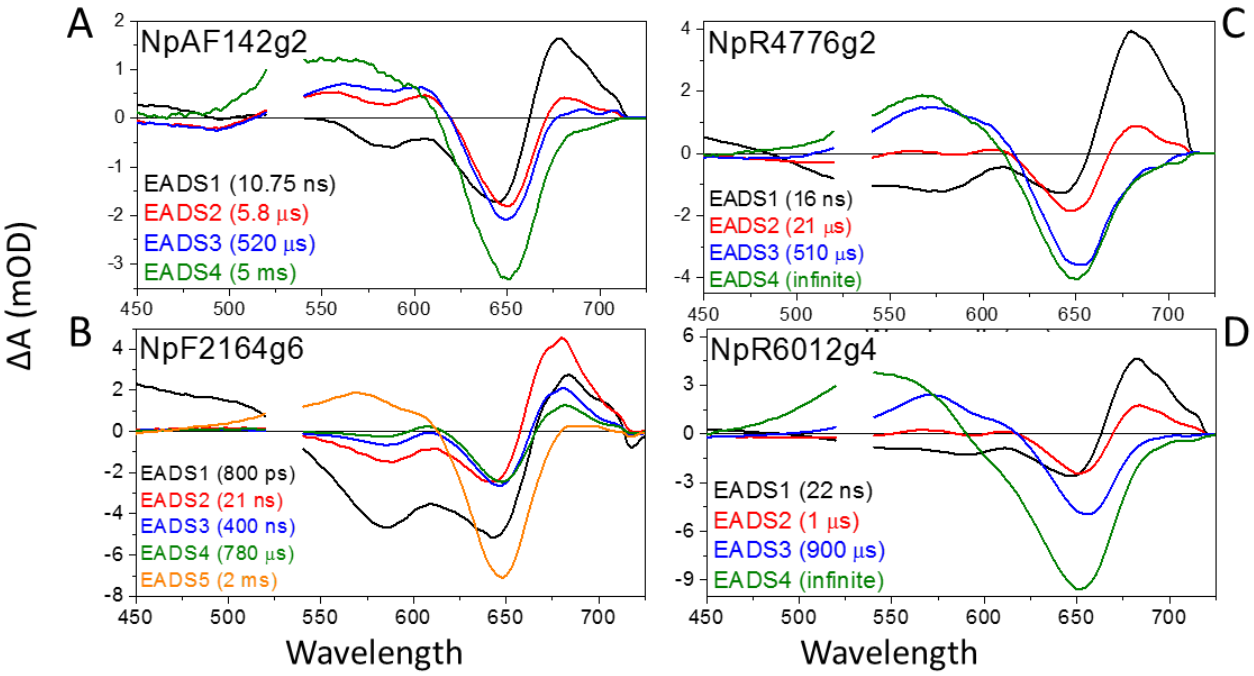

Figure S5. EADS (color coded to model in Figure S4) for the CBCRs that exhibit the unproductive Lumi-O_f pathway using the sequential model presented in Figure S4: (A) NpAF142g2 (B) NpF2164g6 (C) NpR4776g2 and (D) NpR6012g4. The static $^{15E}P_g$ - $^{15Z}P_r$ difference spectrum (dashed grey curves) are shown for comparison. The EADS of the CBCRs that did not exhibit the unproductive Lumi-O_f pathway can be found in Figure 6.

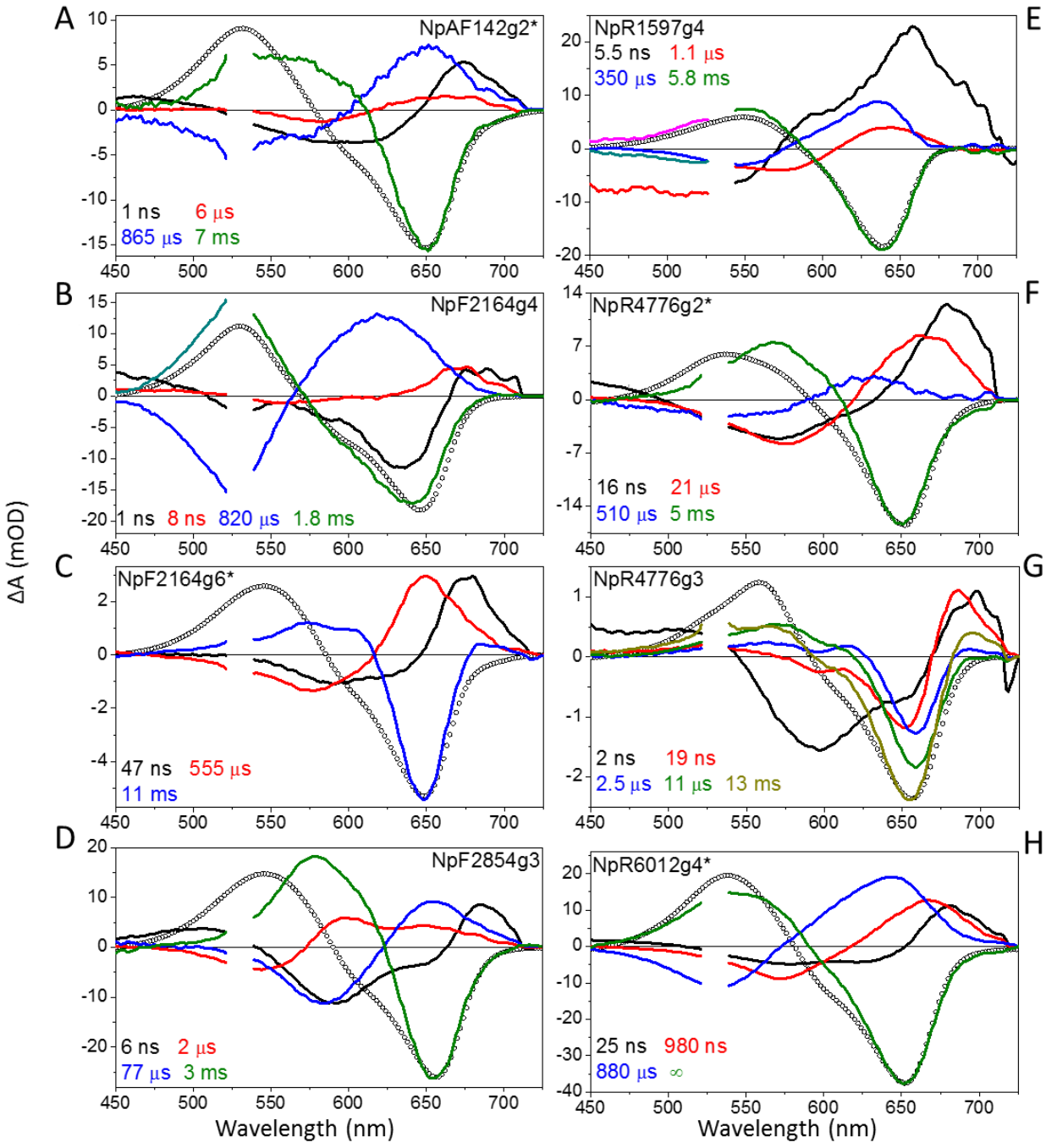

Figure S6: DADS of the various CBCR domains using a parallel model incorporating the same time constants as the EADS analysis (sequential model) given in Table 1.

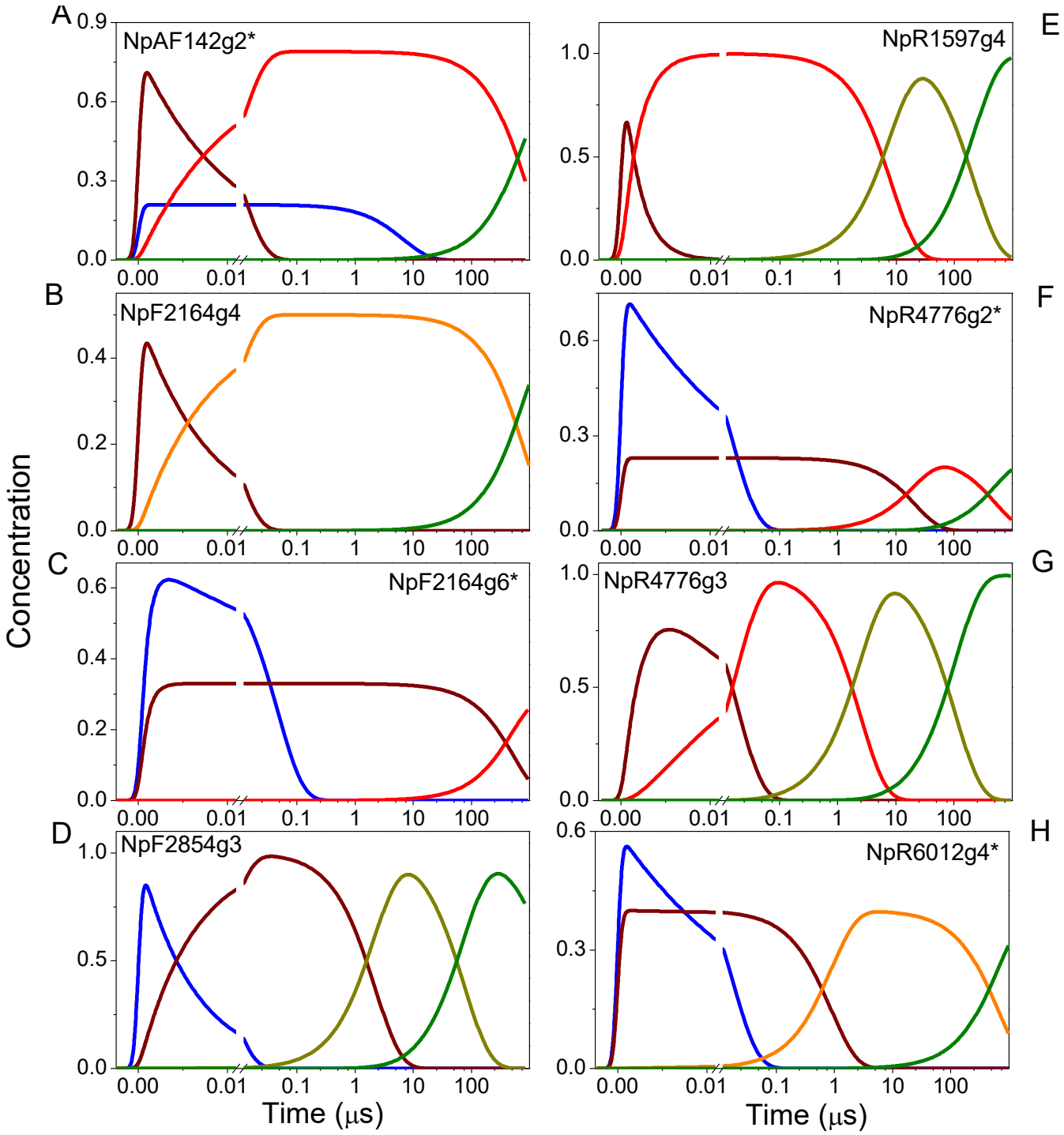

Figure S7: Concentration profiles from the fitting of the various CBCR domains using the model presented in Figure 5. The curves are color coded to match the EADS in Figure 6.

NpAF142g2: ----------VGMVWDDTYLQETQGGRYRNNETF

NpF2164g6: ----------IRTIWEDEHLQQTQGGRYRNNETF

NpR4776g2: NESEIGINTVWNDTYL

NpR6012g4: ----------IKTVWEDTHLQETQGGRYAQGENF

NpF2164g4: ----------IKTVWEDEHLQQTQGGRYRNNETL

NpF2854g3: ----------KNLVWEDTHLQETKGGRYRNNENF

NpR1597g4: ----------IVPVVADDYLQETQGRNFANGKSI

NpR4776g3: ----------TKTVWQDSYLQETQGGRYRHNETF

NpF2164g5*:---------- IKTIWMDEHLQETQGGRYRNHETF

Figure S8. Sequence alignment of the 7 out of 8 of these proteins, excluding NpR4776g2, were previously reported by Rockwell et al.[1] The proteins with names written in blue exhibited the unproductive Lumi-O_f and those written in red did not. Given the protein sequence obtained for NpR6012g4 and the protein sequence alignment from Rockwell et. al,[1] the tryptophan (W) written in red was identified as W655 and the aspartic acid (D) written in blue was identified as D657. The orientation of these two specific amino acid residues were shown to be responsible for the spectral inhomogeneity in NpR6012g4 and the unproductive $^{15Z}P_o$ subpopulation.[2] The corresponding residue in the 7 other proteins previously reported by Rockwell et. al. and NpR4776g2 were also highlighted.[1] Every protein studied had a W at this corresponding location (written in red), except for NpR1597g4 which incorporated a valine (V) at this location which is written in green. All the domains studied also incorporated Asp (blue) at the same location in the amino acid sequence as NpR6012g4. The sequence alignment for 4776g2 was obtained by preforming a BLASTp search comparing the amino acid sequence for NpR6012g4 and NpR4776g2 obtained from a patent belonging to Dr. Lagarias.[3] *NpF2164g5 secondary dynamics were not probed, hence not included in this study, because the primary dynamics revealed no measurable Lumi-R quantum yield,[4] but was added as a comparison for NpR159g4 that also exhibited a low Lumi-R quantum yield but replaced the conserved Trp with Val.

Table S1. Amino acid present at key locations shown to effect CBCRs.[5] The domain names highlighted in orange displayed features consistent with the unproductive $^{15Z}P_o$ subpopulations and the amino acid residues highlighted in red differ from the more consistently presented amino acid residues at the given positions.

Domain	B1 notch	B2 Phe	Lid Trp	Asp	Cys	His	Helix Phe
NpAF142g2	Ser	Phe	Trp	Asp	Cys	His	Phe
NpF2164g6	Ala	Phe	Trp	Asp	Cys	His	Phe
NpR4776g2	Ser	Phe	Trp	Asp	Cys	His	Phe
NpR6012g4	Ala	Phe	Trp	Asp	Cys	His	Phe
NpF2164g4	Ala	Phe	Trp	Asp	Cys	His	Phe
NpF2854g3	Gly	Phe	Trp	Asp	Cys	His	Phe
NpR1597g4	Thr	Phe	Val	Asp	Cys	His	Met
NpR4776g3	Ala	Tyr	Trp	Asp	Cys	His	Ile

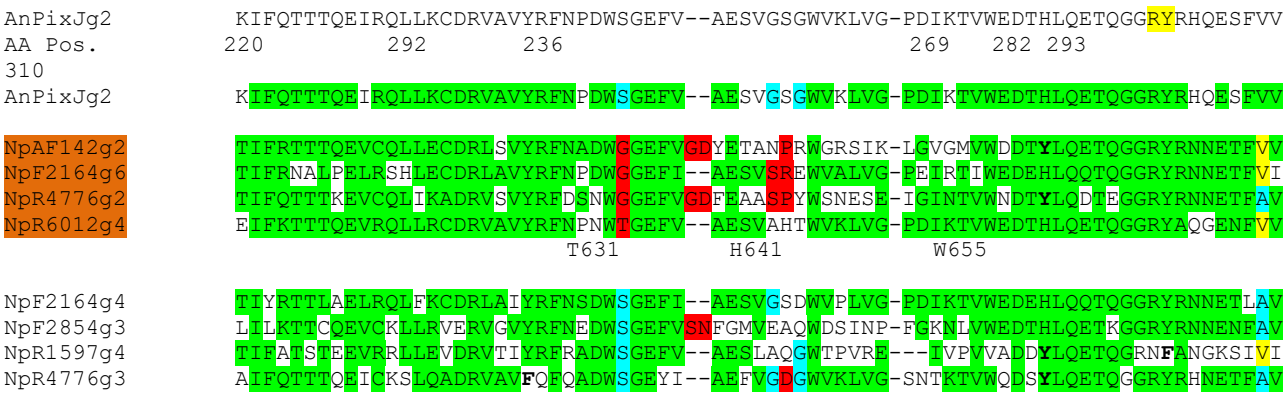

Figure S9. Partial amino acid sequence of the Red/Green CBCRs presented here reported by Rockwell et al.[1, 5] The domain names highlighted in orange displayed features consistent with the unproductive $^{15Z}P_o$ subpopulations The amino acid residues highlighted in green were consistent in both subclasses. The amino acids highlighted in red were consistent with the domains that possessed the $^{15Z}P_o$ subpopulations and the amino acids highlighted in blue are consistent with the domains that did not have the $^{15Z}P_o$ subpopulations. Interestingly, at the T631 position of NpR6012g4, all of the $^{15Z}P_o$ subpopulations containing domains had a glycine residue (other than NpR6012g4) and all of the non $^{15Z}P_o$ subpopulations containing domains had a serine residue.

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NpAF142g2   WGGEFVGDYETANPRWGRSIKLG
NpF2164g6   WGGEFIAES
NpR4776g2   WGGEFVGDFEAASPYWSNESEIG
NpR6012g4   WTGEFVAES

All2699g2   WGGEFVGDFEATSPHWSNESKIS
AnpixJg3    WGGEFVAES
AnpixJg4    WGGEFVAES

NpF2164g4   WSGEFIAES
NpF2854g3   WSGEFVSNFGMVEAQWDSINPFG
NpR1597g4   WSGEFVAES
NpR4776g3   WSGEYIAEF


AnpixJg2    WSGEFVAES
NpF2164g5   WSVEFVAES
NpF2854g1   WSGSFINRFGFAEHPWDALTAFG
NpF2854g2   WSGEFVSQFGMLEPQWHRIHPFG
NpR5113g2   WSGNFVAES
NpR6012g3   WGGEFVAES
NpR6012g2   WSGEFVAES
slr1393g3   WSGEFIHES
NpF2164g7*  WSGEFVVES *Orange/Green CBCR
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Figure S10. Partial amino acid sequence around T631 (highlighted in purple) position of NpR6012g4 believed to be of importance of several Red/Green CBCRs reported by Rockwell et al.[1, 5] Multiple residues incorporated a serine residue (highlighted in blue) at this location, consistent with not having the unproductive $^{15Z}P_o$ subpopulation and a few incorporated the glycine (highlighted in red) that was consistent with having the unproductive $^{15Z}P_o$ subpopulation. The names written in red are the domain that were characterized and did not have the unproductive $^{15Z}P_o$ subpopulations and the domains that did exhibit the unproductive $^{15Z}P_o$ subpopulation are written in orange. If the serine/glycine residue is indicative of the presence of the unproductive $^{15Z}P_o$ subpopulation then this suggest that a larger percentage of the Red/Green CBCRs presented in this list do not incorporate the unproductive $^{15Z}P_o$ subpopulations than the 50% of samples whose secondary dynamics were reported here.

References

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3. Lagarias, J. C., N. C. Rockwell and S. S. Martin (May 5, 2016) Cyanobacteriochriomes as color-fast or color -switching food additives. (Edited by U. P. 201662339034).
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5. Rockwell, N. C., S. S. Martin, A. G. Gulevich and J. C. Lagarias Conserved phenylalanine residues are required for blue-shifting of cyanobacteriochrome photoproducts. *Biochemistry*, 2014, **53,** 3118-3130.