

Regulation of carbon assimilation by Rubisco in wheat



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Photosynthesis team at LEC



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Carmo-Silva**



**Marjorie
Lundgren**



**Doug
Orr**



**Sam
Taylor**



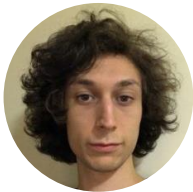
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Long**



**Martin
Parry**



**Joana
Amaral**



**Duncan
Bloemers**



**Nathália
Bonetti**



**Louis
Caruana**



**M. Waqas
Khokhar**



**Ana
Lobo**



**Nandhara
Mendes**



**Mike
Page**



**Rhiannon
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**Ingrid
Robertson**



**Lisa
Stout**



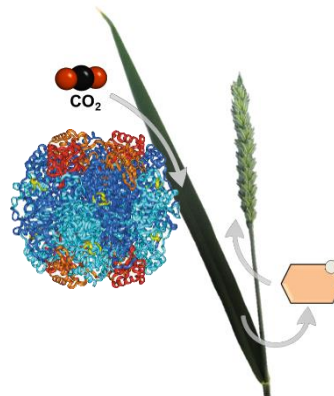
**Supreeta
Vijaykumar**



**Dawn
Worrall**



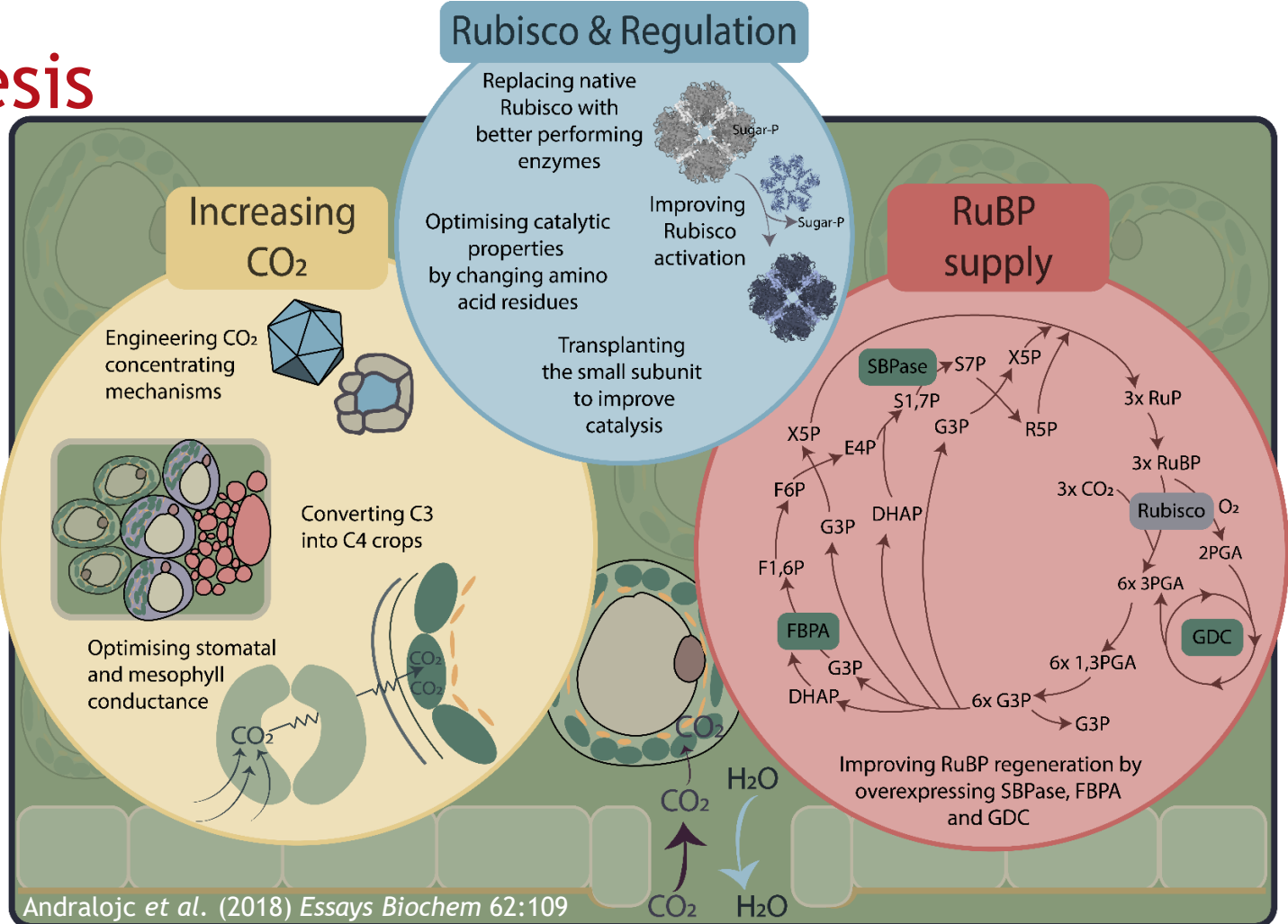
Improving photosynthetic efficiency



Sustainable agricultural crop production

Improving photosynthesis

- Target crop
- Target environment





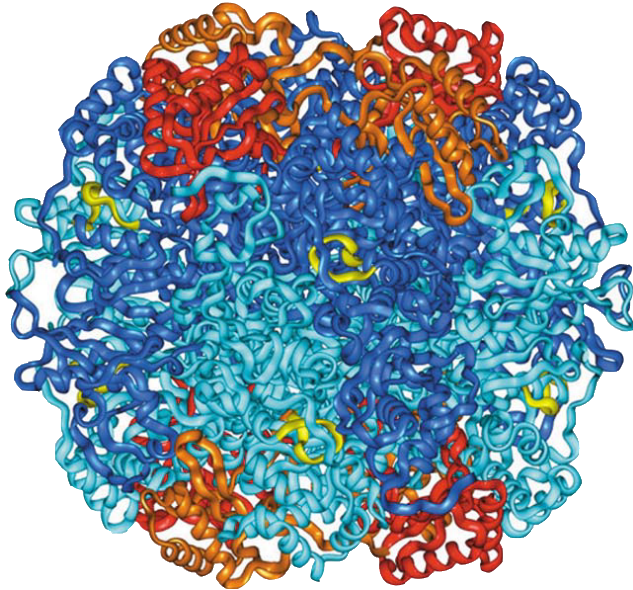
Rothamsted, UK, 20/06/2014

Flag leaf photosynthesis in field-grown UK wheat cultivars

- ✓ Genetic variation & heritability of photosynthetic traits
- ✓ Correlation to harvest index & grain yield

Trait	Potential
Pre-anthesis A	Increase photosynthetic efficiency when flag leaves are most active
Post-anthesis A	Increase photosynthetic efficiency at a critical stage for grain filling
Light response of A	Improve photosynthetic radiation use efficiency
Rubisco amount	Optimise allocation of resources and N use efficiency (NUE)
Cultivar	Traits/Potential
Mercato, Zebedee	High-yielding cultivar; high pre-anthesis A_{400} + high HI
Gladiator	High-yielding cultivar; high post-anthesis A_{400} + high HI
Gatsby	High photosynthetic rates + low Rubisco amount (improve NUE?)

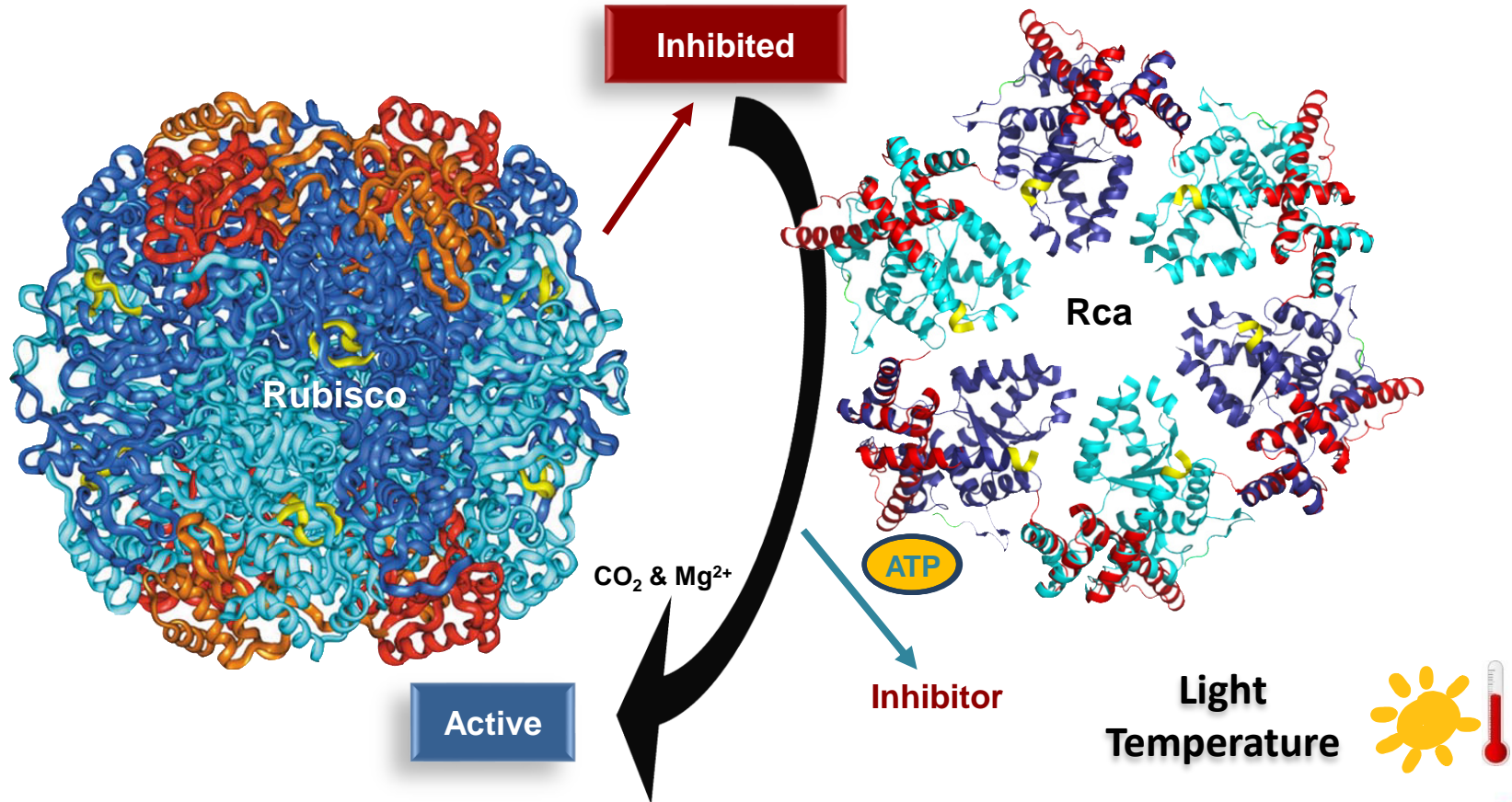
Rubisco - the holy grail



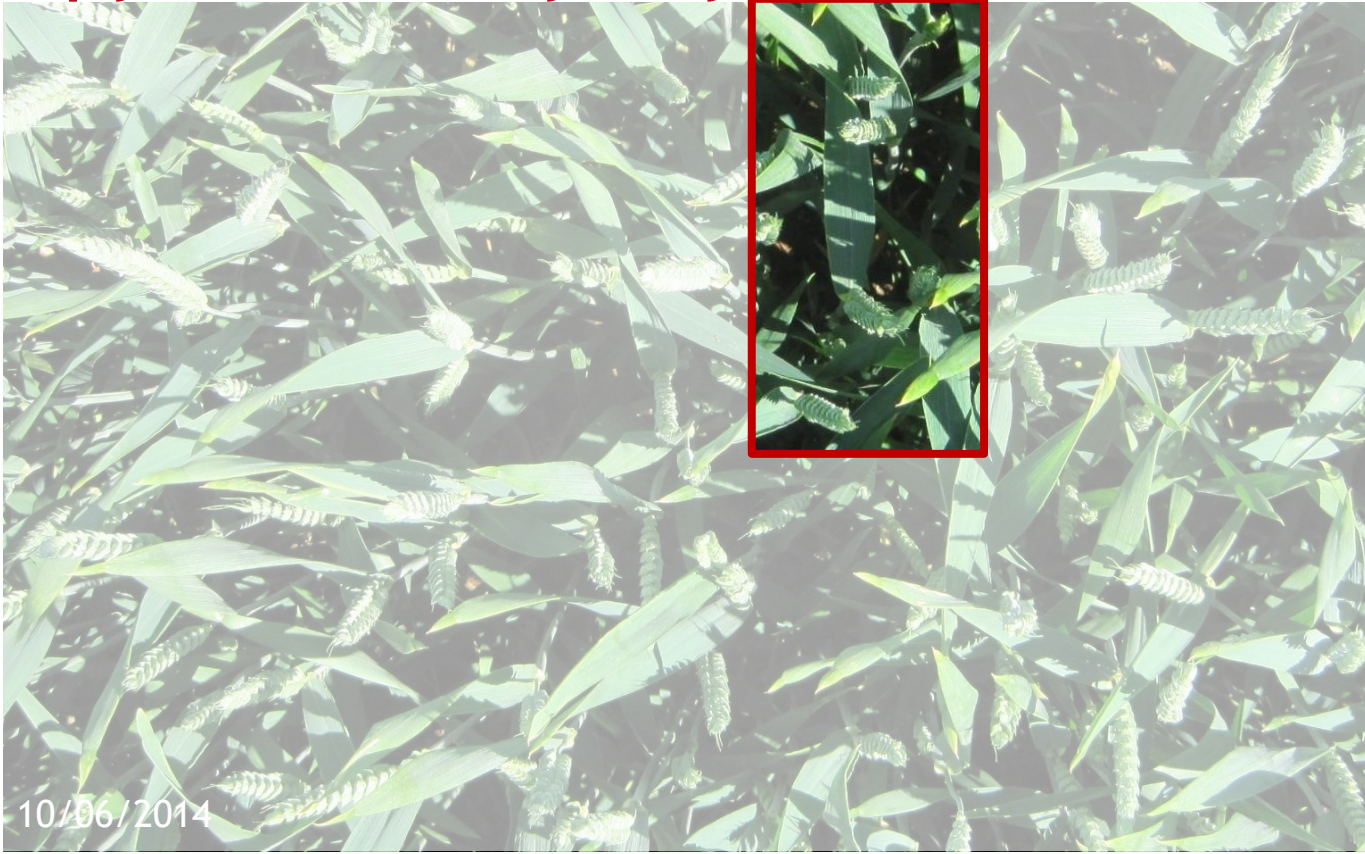
20-70% of leaf TSP
10-35% of leaf N

- **Point of CO₂ entry into biosphere**
- **Unique & essential, yet imperfect**
- **Biogenesis and abundance (N investment)**
- **Complex reaction mechanism**
- **Catalytic properties**
Catalytic rate, CO₂ vs. O₂
- **Regulation**
Carbamylation (CO₂-Mg²⁺), inhibitors, Rca, phosphatases, ...

Regulation of Rubisco activity



The light level that reaches the top of a canopy on a sunny day fluctuates

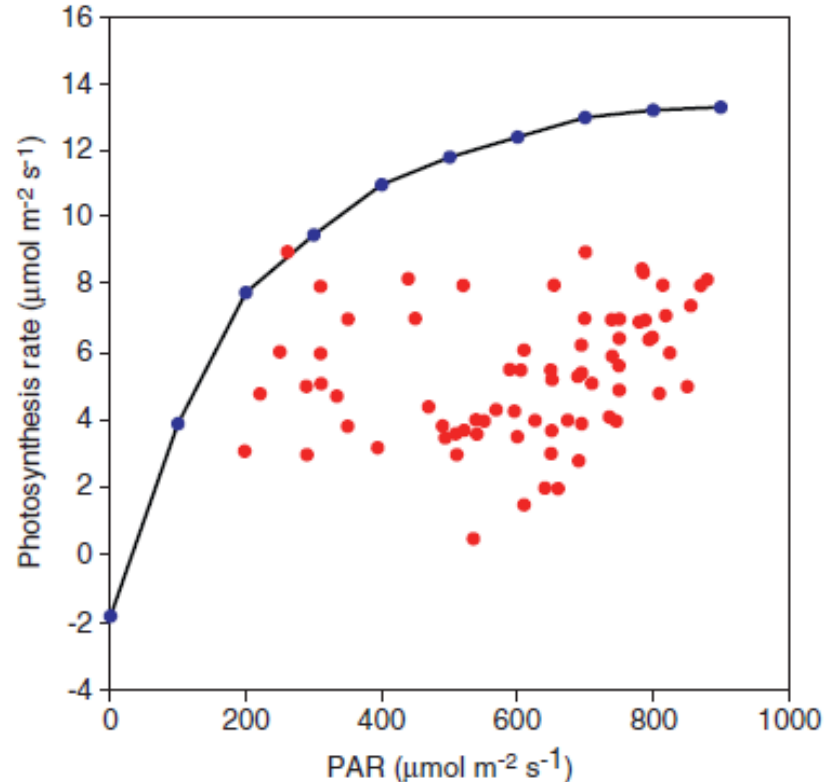


10/06/2014

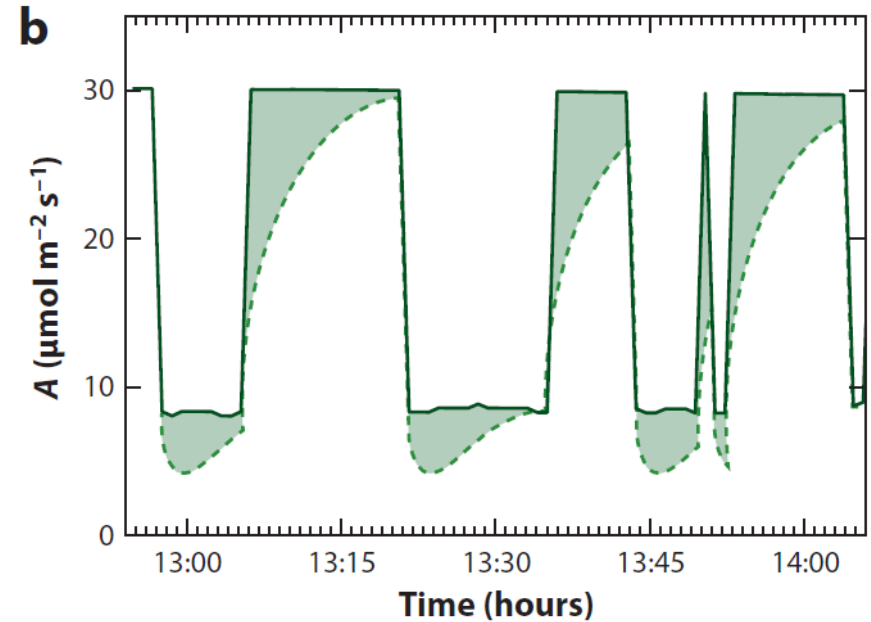
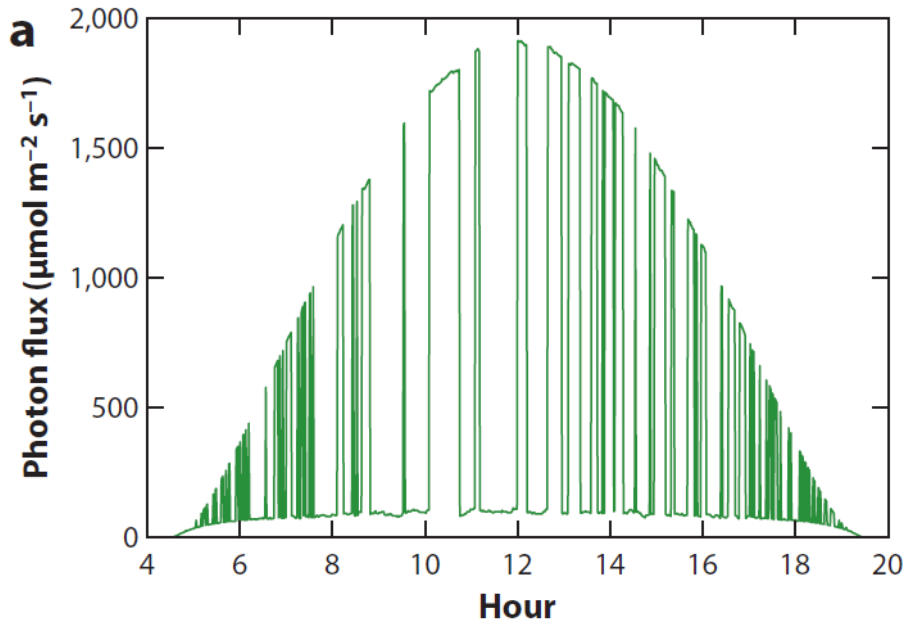
Potential photosynthesis is rarely observed under fluctuating conditions

- Steady-state photosynthesis
- Instantaneous, non-steady-state photosynthesis

Plants in natural, fluctuating light environments rarely attain steady-state photosynthesis



Photosynthesis in fluctuating light

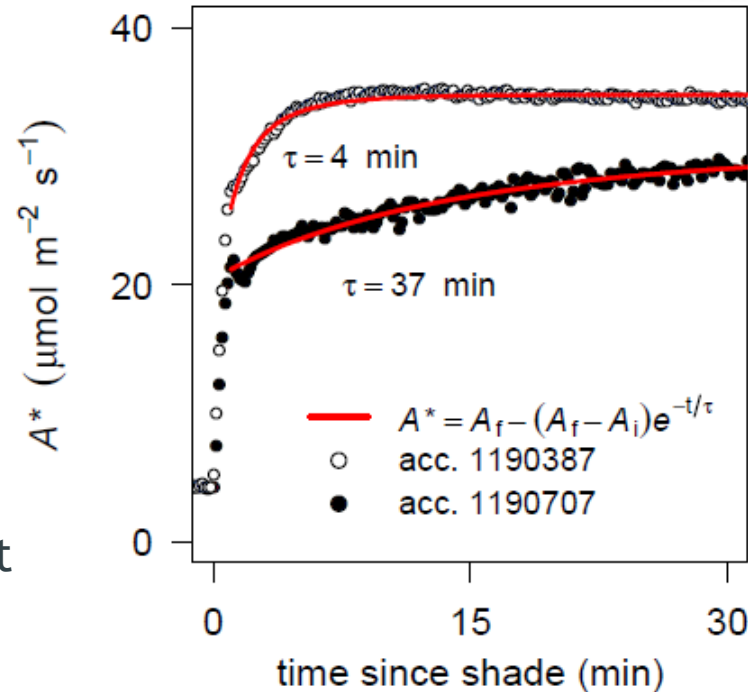


- Predicted CO_2 uptake rate
- - - Typical lags in response
- Loss in photosynthetic efficiency during light fluctuations

Induction: RuBP + Rubisco + gs + gm
(shade duration & intensity)

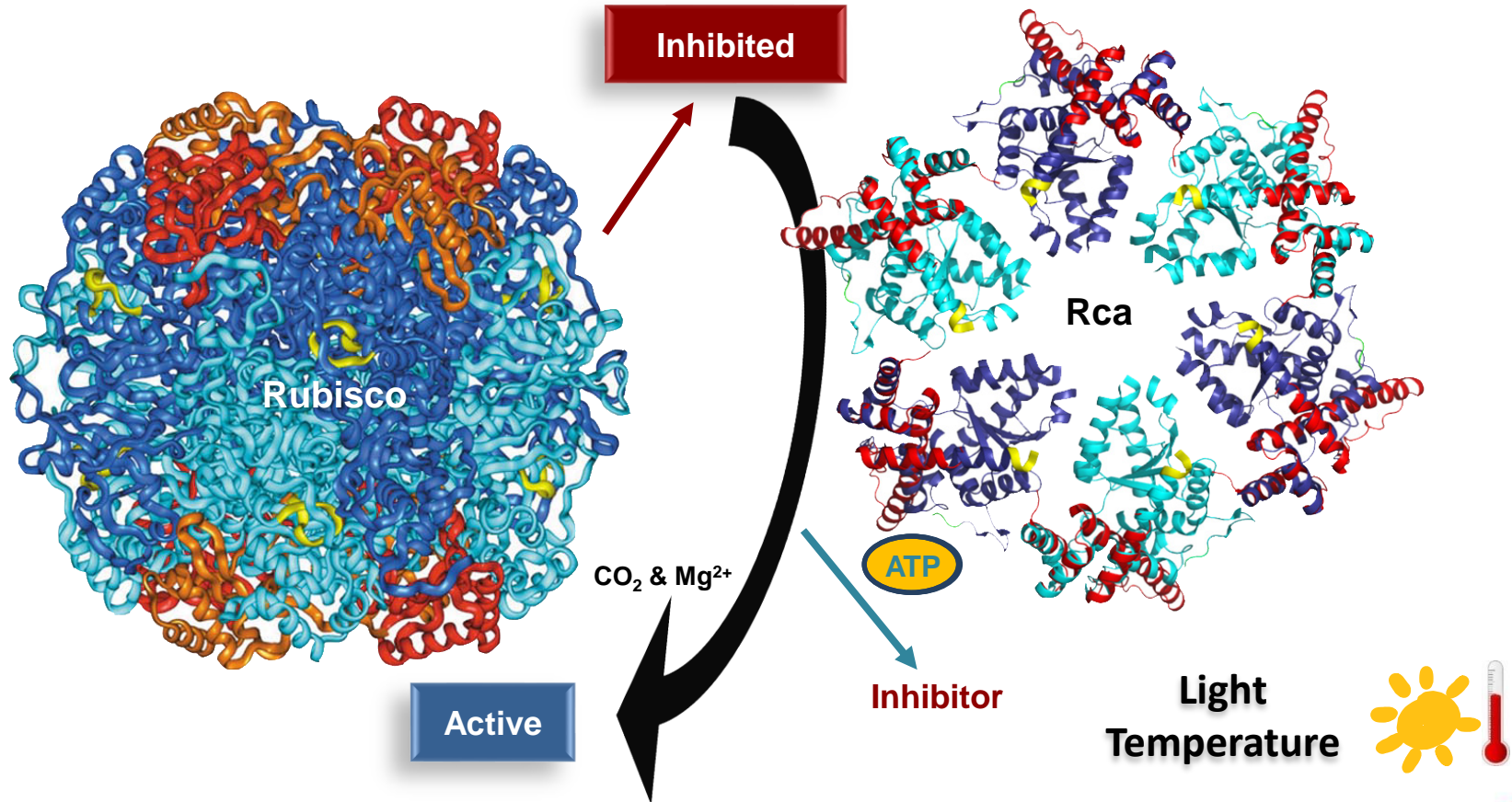
Faster Rubisco activation may improve wheat photosynthetic productivity

- Wheat physiology: photosynthetic induction limited by **Rubisco regulation**
A corrected for stomatal limitation*
- Modelling: scope for improving wheat carbon assimilation by **20%**
- Screening: variation exists in wheat germplasm



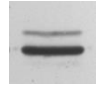
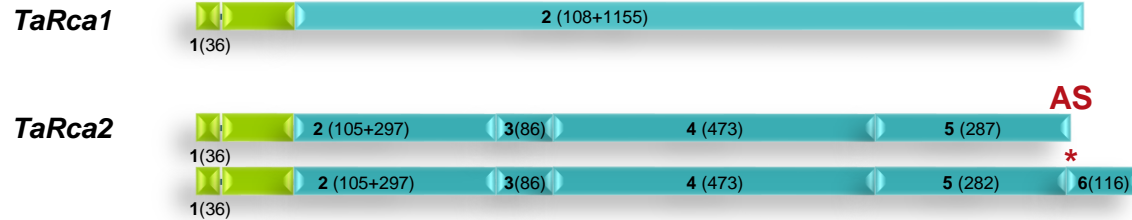
Sam Taylor

Regulation of Rubisco activity



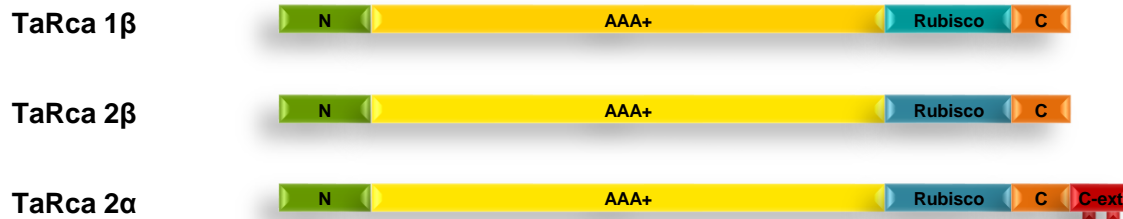
Wheat Rubisco activase

2 genes, one alternatively spliced



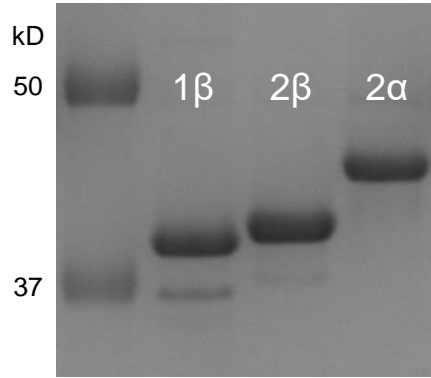
Rca- α
8-20%

3 protein isoforms, two short & one long

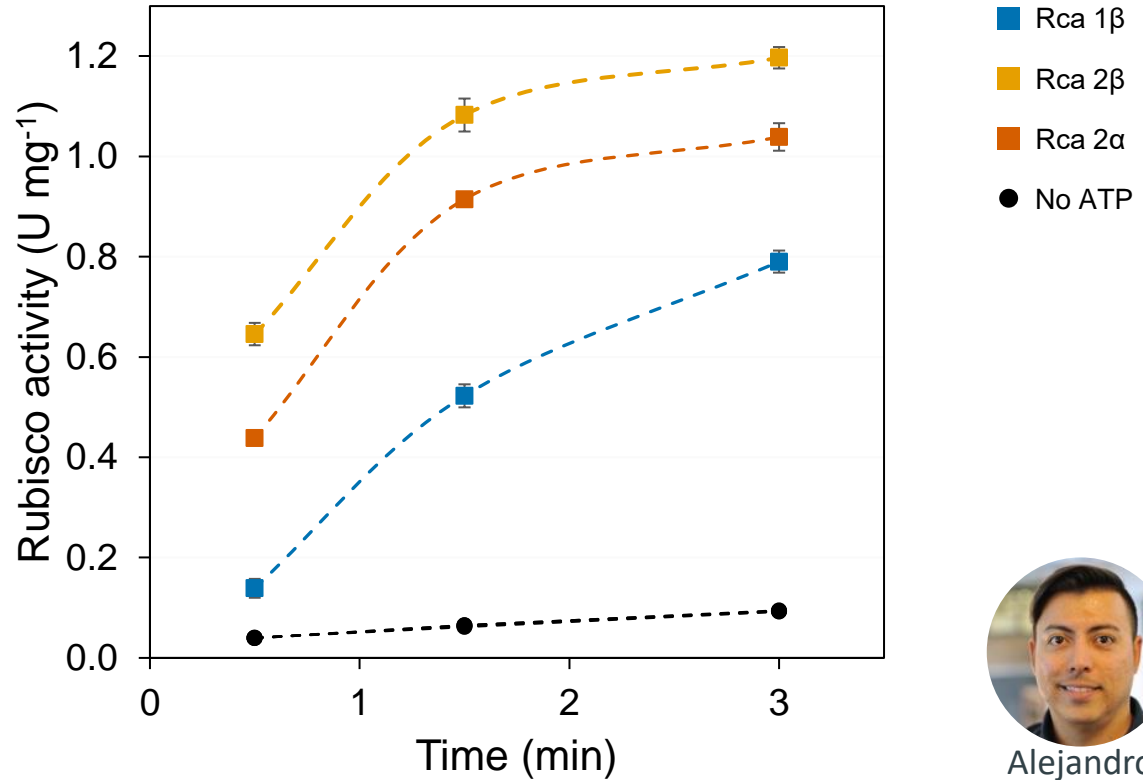


Joanna Scales

Rubisco reactivation by wheat Rca

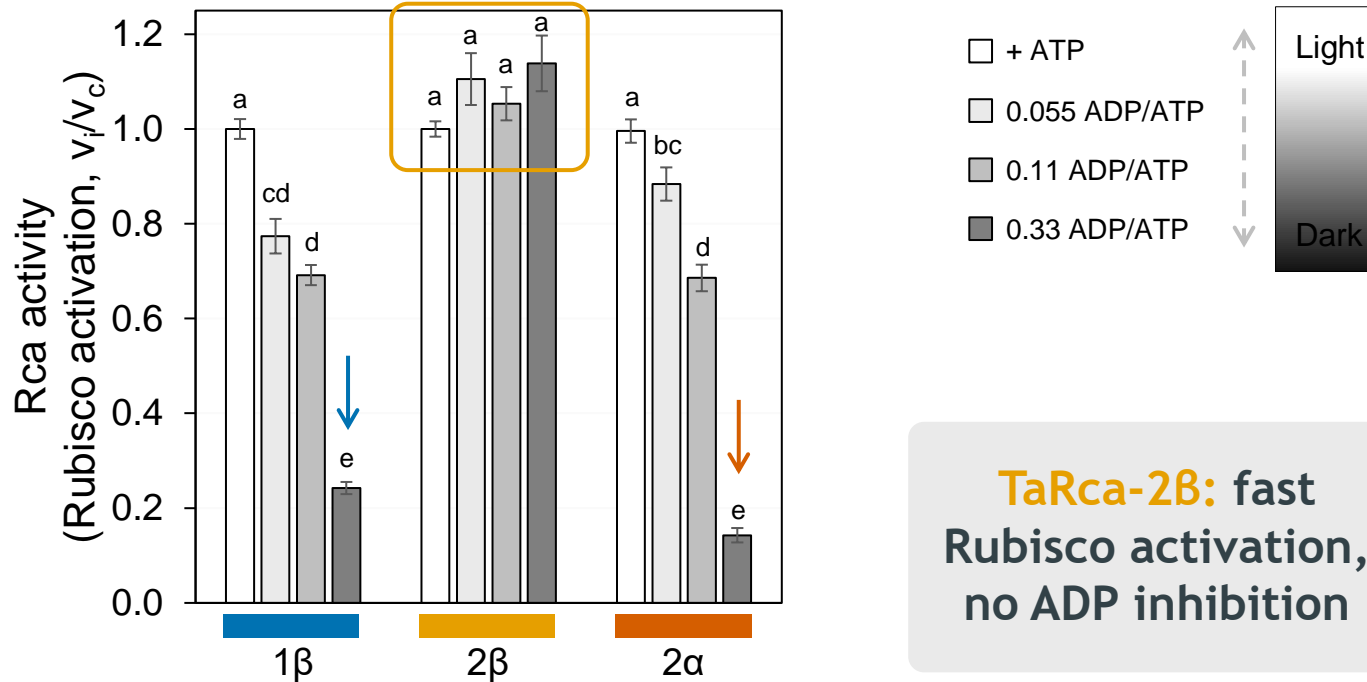


TaRca-1 β : slower Rubisco activation



Alejandro Perdomo

ADP sensitivity of wheat Rca



TaRca-2 β : fast Rubisco activation, no ADP inhibition



Alejandro Perdomo

Site-directed mutants

Wheat 1 β \rightarrow N domain (A1 – Y65) \rightarrow α/β sub-
 AA~~K~~ELDEGKQ~~T~~NADRWKGLAYDISDDQ~~Q~~DITR~~G~~GKGI~~V~~DSL~~F~~QAPMGDGT~~H~~EAILSS~~S~~YEYISQGLRKYDFDNTMD
 Wheat 2 β AAEN – LDEK~~R~~NT – – DKWKGLAYDISDDQ~~Q~~DITR~~G~~GKGI~~V~~DSL~~F~~QAPTGDGT~~H~~EAVLSS~~S~~YEYVSQGLRKYDFDNTMG
 Tobacco – EEKDAD~~P~~KKQ~~T~~SDRWKGLVQ~~D~~FSDDQ~~Q~~DITR~~G~~GKGM~~V~~DSL~~F~~QAPTGT~~G~~THHAVL~~S~~QSYEYVSQGLRQYNLDNKLD
 Arabidopsis – – – AVKEDKQ~~T~~DGDRWRGLAYD~~T~~SDDQ~~Q~~DITR~~G~~GKGM~~V~~SV~~F~~QAPMG~~T~~GT~~H~~HAVL~~S~~SYEYVSQGLRQYNLDN~~M~~MD

domain (D66 – P250)
 Wheat 1 β GLYIAPAFMDKLI~~V~~H~~L~~AKNFMTLPNIK~~V~~PLILGIWGGK~~G~~Q~~G~~KS~~F~~QCELVFAKMGINPIMMSAGELES~~G~~NA~~G~~EPAK
 Wheat 2 β GFYIAPAFMDKLI~~V~~H~~L~~SKNFMTLPNIK~~I~~PLILGIWGGK~~G~~Q~~G~~KS~~F~~QCELVFAKMGINPIMMSAGELES~~G~~NA~~G~~EPAK
 Tobacco GFYIAPAFMDKLI~~V~~H~~L~~IKNFMTLPNIK~~I~~KVPLILGIWGGK~~G~~Q~~G~~KS~~F~~QCELVFAKMGINPIMMSAGELES~~G~~NA~~G~~EPAK
 Arabidopsis GFYIAPAFMDKLI~~V~~H~~L~~ITKNFL~~L~~PNIKVPLILGIWGGK~~G~~Q~~G~~KS~~F~~QCELVMAKMGINPIMMSAGELES~~G~~NA~~G~~EPAK

Wheat 1 β LIRQRYEAADI~~I~~INKGK~~M~~C~~L~~FLINDLDAGAGRMGGT~~T~~QYTVN~~N~~QMVNATLMNIADAP~~T~~NVQLPGMYNKEENPRVP
 Wheat 2 β LIRQRYEAAD~~M~~IKK~~G~~K~~M~~C~~L~~FLINDLDAGAGRMGGT~~T~~QYTVN~~N~~QMVNATLMNIADAP~~T~~NVQLPGMYNKEENPRVP
 Tobacco LIRQRYEA~~A~~E~~I~~IRKGN~~M~~C~~L~~FLINDLDAGAGRMGGT~~T~~QYTVN~~N~~QMVNATLMNIADN~~P~~TNVQLPGMYN~~K~~QENARVP
 Arabidopsis LIRQRYEAAD~~L~~I~~K~~K~~G~~K~~M~~C~~L~~FLINDLDAGAGRMGGT~~T~~QYTVN~~N~~QMVNATLMNIADN~~P~~TNVQLPGMYN~~K~~EENARVP

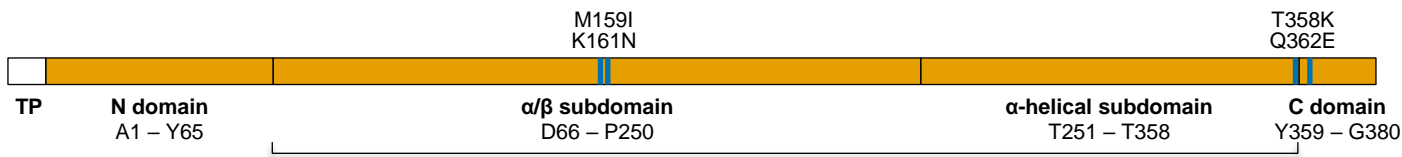
Wheat 1 β IIVTGNDFSTLYAPLIRDRGRMEK~~F~~YWAP~~T~~REDRIGVCKGIF~~R~~TDN~~V~~DEAVVRLVDTFFGQSIDFFGALRARVYD
 Wheat 2 β IVV~~T~~GNDFSTLYAPLIRDRGRMEK~~F~~YWAP~~T~~RDRIGVCKGIF~~Q~~TDN~~V~~SDES~~V~~VKIVDTFFGQSIDFFGALRARVYD
 Tobacco IIVTGNDFSTLYAPLIRDRGRMEK~~F~~YWAP~~T~~REDRIGVCTGIF~~R~~TDN~~V~~PAEDVVKIVDN~~F~~FPGQSIDFFGALRARVYD
 Arabidopsis IICTGNDFSTLYAPLIRDRGRMEK~~F~~YWAP~~T~~REDRIGVCKGIF~~R~~TDKIKDEDIVTLVD~~P~~FPGQSIDFFGALRARVYD

Wheat 1 β DEVRK~~V~~WGEIGVENISKRLVNSREG~~P~~PT~~F~~DQPKMTIEK~~L~~MEYGHMLVQE~~Q~~ENV~~K~~RVLADKYLSEAAALGDAN~~D~~DA
 Wheat 2 β DEVRK~~V~~W~~T~~STGIENIGKRLVNSRDGP~~V~~TFE~~Q~~PKMTIEK~~L~~MEYGHMLVQE~~Q~~DNV~~K~~RVLAD~~T~~YMSQAALGDAN~~O~~DA
 Tobacco DEVRK~~V~~W~~S~~GTGIEKIGDKLLNSFDG~~P~~PT~~F~~E~~Q~~PKMTIEK~~L~~MEYGHMLVQE~~Q~~ENV~~K~~RVLADKYLKEAALGDAN~~A~~DA
 Arabidopsis DEVRK~~V~~ESL~~G~~VEKIGKRLVNSREG~~P~~PP~~V~~FE~~Q~~PEMTY~~E~~KLMEYGNMLVME~~Q~~ENV~~K~~RVLAE~~T~~YLSQAALGDAN~~A~~DA

Wheat 1 β MKTGA~~F~~Y~~G~~K
 Wheat 2 β MKTGS~~F~~Y~~C~~ –
 Wheat 2 α MKTGS~~F~~Y~~G~~KGAQ~~Q~~GTLPVPAGCTDQTAKNFDPTARSDDG~~S~~CLYTF
 Tobacco INNGS~~F~~FFAS
 Arabidopsis β IGRGTFY~~G~~K
 Arabidopsis α IGRGTFY~~G~~KGAQ~~Q~~VNLPVPEGCTDPVAENFDPTARSDDG~~T~~CVYNF



4 residues: ADP sensitivity?

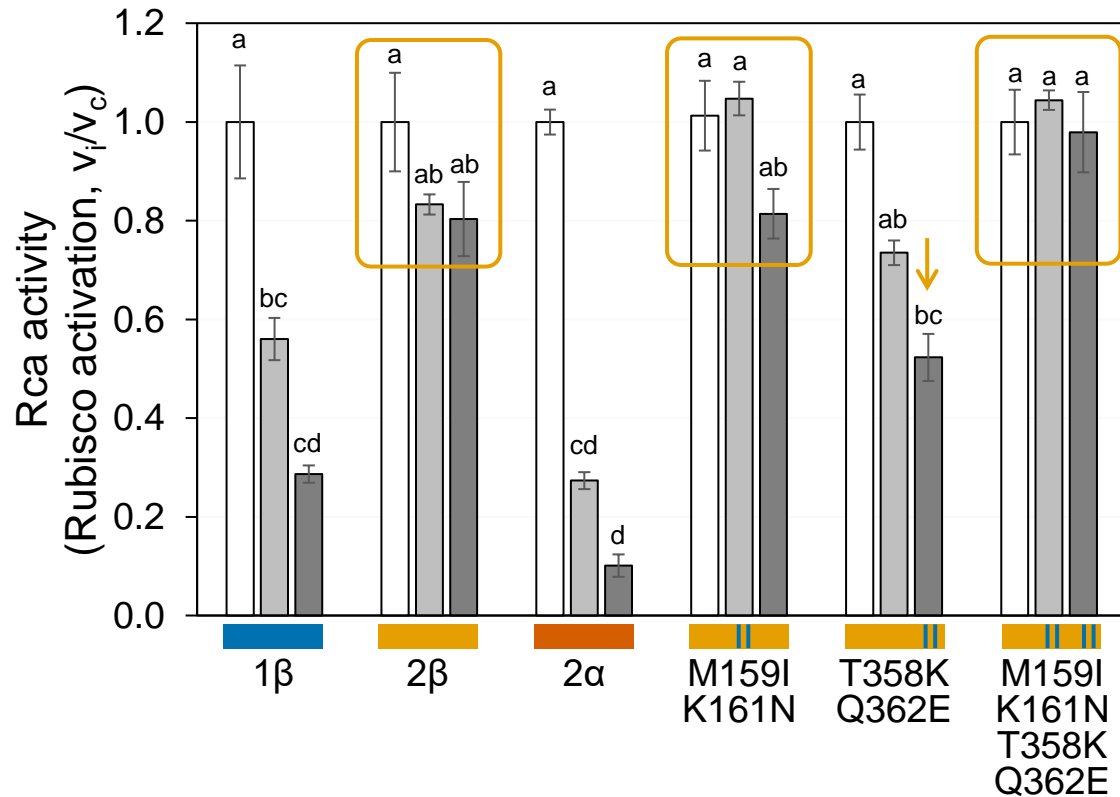


Dawn Worrall





Gustaf Degen

Rca residues associated with inhibition



T358K/Q362N more sensitive to ADP inhibition than 2 β

-  + ATP
-  0.11 ADP/ATP
-  0.33 ADP/ATP



Dawn Worrall



Gustaf Degen

Wheat Rubisco activase isoforms

- ✓ Rca-2B is faster at activating Rubisco and is insensitive to ADP inhibition
- ✓ Site-directed mutants partly explain ADP sensitivity of Rca (T358K/Q362E)



Alejandro Perdomo



Dawn Worrall



Gustaf Degen

Conclusions

- Large N investment to make Rubisco
- Rubisco activity: abundance + activation
- Diversity in Rubisco activase isoforms
- Interactions between processes matter



Photosynthesis team at LEC



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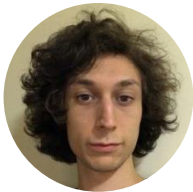
**Martin
Parry**



**Biotechnology and
Biological Sciences
Research Council**



**Joana
Amaral**



**Duncan
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**Foreign, Commonwealth
& Development Office**

**BILL & MELINDA
GATES foundation**



**Mike
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**Ingrid
Robertson**



**Lisa
Stout**



**Supreeta
Vijaykumar**



**Dawn
Worrall**



Thank you!

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<http://wp.lancs.ac.uk/lancsphotosynthesis/>