Crystallisation of plant phytochrome A signalling states

Kaoling Guan, Soshichiro Nagano, Jon Hughes
Institute for Plant Physiology, Justus Liebig University, Giessen, Germany

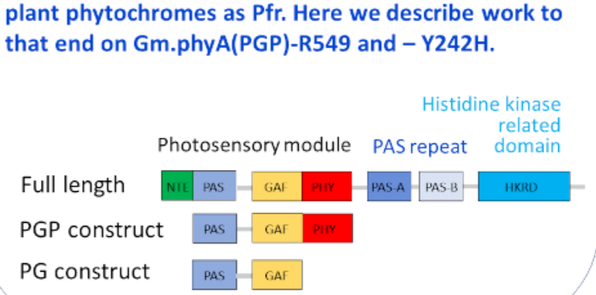
Introduction

Plant phytochrome crystal structures

- Pr state
  - phyB(PG) from *Sorghum bicolor*
  - phyB(PGP) from *Glycine max*
  - phyA(PG)

  } Nagano *et al.* (2020) Nature Plants
  - phyB(PGP) from *Arabidopsis thaliana* (Burgie *et al.* 2014.)
- Structures in Pfr signalling state remain elusive

Aim: to determine high resolution crystal structures of plant phytochromes as Pfr. Here we describe work to that end on Gm.phyA(PGP)-R549 and – Y242H.



Future work

- Optimization of conditions for Gm.phyA(PGP)-R549A and -Y242H crystals
- Crystallisation of Gm.phyA(PG)-Y242H
- Crystallisation of the equivalent mutations in Gm.phyB(NPGP) or (PGP)

Gm.phyA(PGP)-R549A

- PRxSF→PAxSF: a Pfr-stabilizing mutation

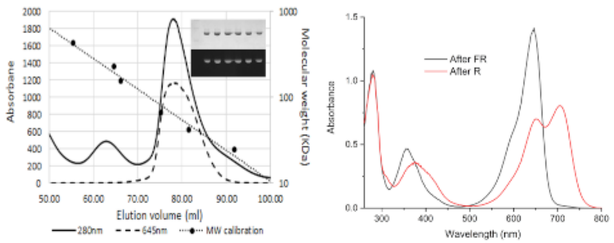


Fig.1 Sample characterization.
Left: SEC profile and SDS-PAGE of peak fractions (inset: top, Coomassie stained, bottom: Bilin fluorescence with Zn^{2+}). Right: UV-Vis absorbance spectra.

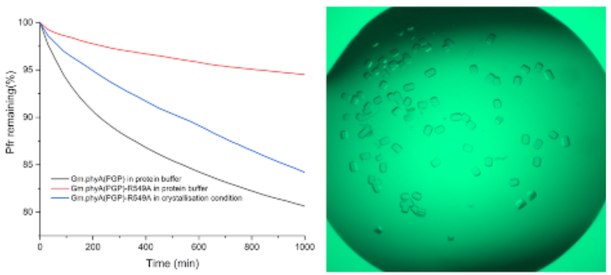
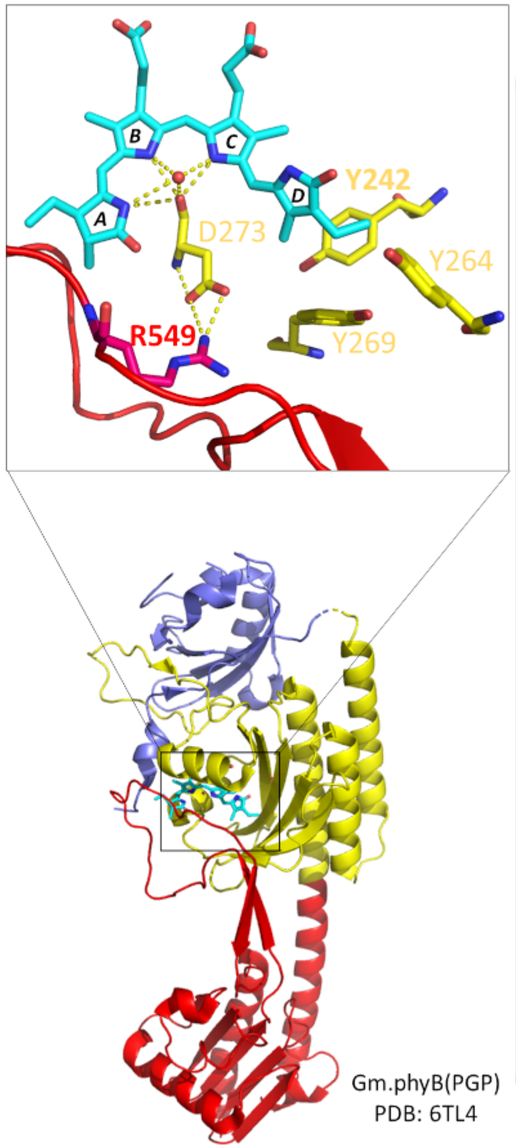


Fig.2 Left: Pfr→Pr thermal reversion at 25 °C of Gm.phyA(PGP) wild type and R549A in sample buffer and crystallisation conditions as measured by absorbance at 720 nm. Right: Gm.phyA(PGP)-R549A crystals grown in 0.1 M Bicine pH 9.0, 10 % (v/v) 2-Methyl-2,4-pentanediol.



Gm.phyB(PGP)
PDB: 6TL4

Gm.phyA(PGP)-Y242H

- Equivalent mutation to Cph1 Y176H
- Constitutively photomorphogenic in plants → mimics Pfr signal

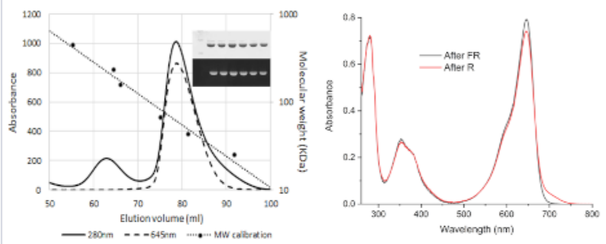


Fig.3 Sample characterization.
Left: SEC profile and SDS-PAGE of peak fractions (inset: top, Coomassie stained, bottom: Bilin fluorescence with Zn^{2+}). Right: UV-Vis absorbance spectra.

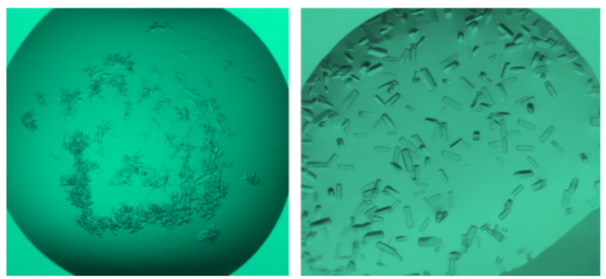


Fig.4 Gm.phyA(PGP)-Y242H crystals grown under different conditions. (Left: 0.1 M sodium acetate pH 4.5, 0.2 M zinc acetate and 10 % (w/v) polyethylene glycol 3000. Right: 0.1 M magnesium formate, 50% glycerol)