EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title: The molecular mechanism of steroid receptor kinase activation at plant membranes**	**Experiment number**: MX-1517
Beamline: ID29	**Date of experiment**: from: 06/07/13 to: 07/07/13	**Date of report**: 20/02/14
Shifts: 3	**Local contact(s)**: Christoph Mueller-Dieckmann	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Michael Hothorn**

***Julia Santiago**

***Daniel Bojar**

Report:

Plants have evolved a unique set of membrane receptor kinases that sense growth promoting steroid hormones. We have previously shown that the receptor kinase BRI1 binds the steroid hormone using its extracellular spiral shaped leucine-rich repeat (LRR) domain, and that binding of the hormone creates a docking platform for a shape-complementary co-receptor protein (Hothorn et al., **Nature**, 2011).

We have used the 3 shifts assigned to us at ID29 to characterise crystals of a ternary complex, consisting of the LRR domain of the receptor BRI1 (a 120 kDa glycoprotein) and shape-complementary co-receptor protein SERK1 (a 35 kDa glycoprotein) held together by the steroid hormone, which acts as a molecular glue (Figure 1a,b). The data collected at the ESRF confirmed a long c-axis (874 Å, Figure 1c) and the presence of perfect merohedral twinning (apparent space-group $P6_522$, model refined in $P6_5$ with twin law *k*,*h*,-*l*) (Santiago, Henzler & Hothorn, **Science**, 2013), but did not offer improved diffraction over crystals collected some weeks earlier at SLS beamline PIIX (where they diffracted up to 3.3 Å).

Because the steroid hormone acts as a molecular glue, we next tested if chemically modified steroid analogues could disrupt the complex and thus act as receptor antagonists. We collected two datasets of the receptor BRI1 in complex with 2' and 2'-hydroxy-modified brassinosteroids, which indeed target the co-receptor binding site (Figure 1d). We now use these structures to design and synthesise more potent BRI1 receptor antagonists.

The cytoplasmic part of the receptor folds into a kinase domain. We could determine the crystal structure of the active, phosphorylated BRI1 kinase domain in complex with ADP, diffracting to 2 Å (Figure 1e). This structure helped us to understand: a) why plant membrane receptor kinases are dual-specificity kinases that are able to phosphorylate on Ser/Thr and Tyr residues, b) how the different auto- and transphosphorylation sites contribute to receptor activation, c) how the receptor kinase domain interacts with substrate kinases and with

a peptide inhibitor protein (Bojar et al., **Plant J**, 2014 10.1111/tpj.12445; this publication acknowledges use of ESRF ID29 and has been registered as an ESRF publication).

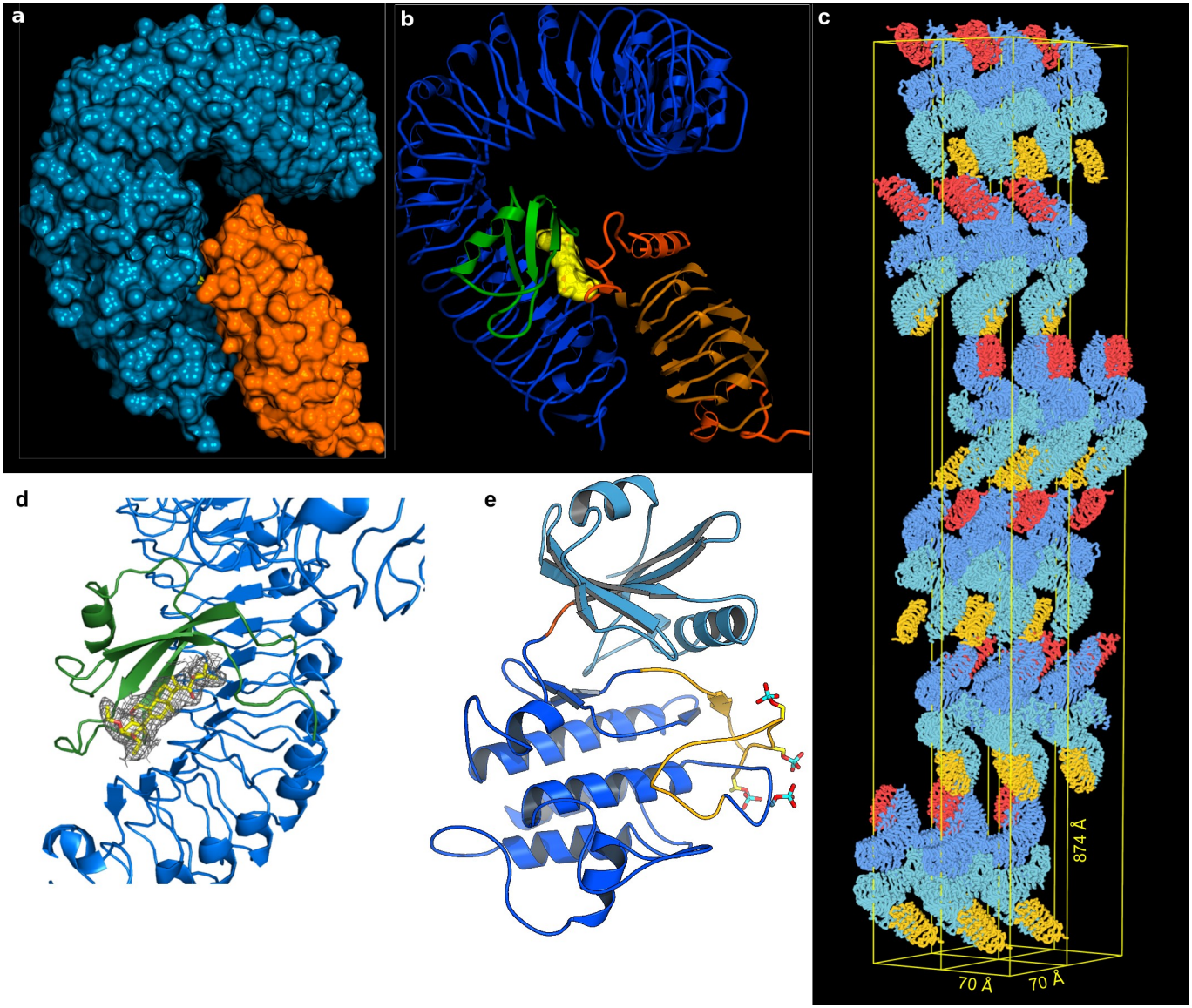

Figure 1 Crystal structures of the extracellular and cytoplasmic portions of a plant steroid receptor signalling complex. a) Surface view of the BRI1-SERK1 ectodomain complex structure. The BRI1 LRR domain is shown in blue, the SERK1 LRR domain in orange. b) Same complex as ribbon diagram. The steroid hormone (in yellow, surface representation) acts as 'molecular glue' promoting association of receptor and co-receptor LRR domain. c) Unit cell of BRI1-SERK1 complex crystals, depicting the long c-axis. d) BRI1 LRR domain in complex with a first receptor antagonist, which binds to BRI1 with nanomolar affinity but does not allow for the binding of the co-receptor. e) Crystal structure of the BRI1 cytoplasmic kinase domain. The fully phosphorylated activation loop is shown in yellow, the N- and C-lobes are shown in light- and dark-blue, respectively.