Structural basis of steroid hormone perception by the receptor kinase BRI1

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Abstract

Polyhydroxylated steroids are regulators of body shape and size in higher organisms. In metazoans intracellular receptors recognise these molecules. Plants however perceive steroids at membranes, using the membrane-integral receptor kinase BRASSINOSTEROID INSENSITIVE 1 (BRI1). The BRI1 ectodomain structure at 2.5 angstrom resolution reveals a superhelix of 25 twisted leucine-rich repeats (LRRs), an architecture that is strikingly different from the assembly of LRRs in animal Toll-like receptors. A 70 amino-acid island domain between LRRs 21 and 22 folds back into the interior of the superhelix to create a surface pocket for binding the plant hormone brassinolide. Known loss- and gain-of-function mutations closely map to the hormone-binding site. We propose that steroid binding to BRI1 generates a docking platform for a co-receptor that is required for receptor activation. Our findings have mechanistic implications for hormone, developmental and innate immunity signaling pathways in plants that use similar receptors.

Signal perception at the cell surface and transduction of this signal to the cell's interior is essential to all life forms. Plants have met this challenge in part by evolving membrane-integral receptor kinases (RKs). Many RKs are comprised of an extracellular Leucine-Rich Repeat (LRR) module and a cytoplasmic kinase domain, connected by a single membrane-spanning helix[1]. Receptors with this architecture (LRR-RKs) for example regulate plant growth[2], development[3,4] and interactions with the environment[5,6]. Their corresponding ligands range from small molecules[7] and peptides[8] to entire proteins[5].

The LRR-RK BRI1[2,9] controls a steroid signalling pathway essential for plant growth[10]. While animal steroid receptors are found predominantly in the nucleus[11], BRI1 is localised at the plasma-membrane and in endosomes[12]. The following model for BRI1 activation has been proposed: In the absence of brassinosteroid, BRI1's kinase domain is kept in a basal state by its auto-inhibitory C-terminal tail[13], as well as by interaction with the inhibitor protein BKI1[14]. Hormone binding to the extracellular domain of BRI1[7,15] in a region that includes a ~70 amino acid 'island' domain[16], causes a change in the receptor (a conformational change in a preformed homodimer[13] or receptor dimerisation), leading to autophosphorylation of the BRI1 kinase domain[17], release of its C-terminal tail[13], and trans-phosphorylation of the inhibitor BKI1[14,18]. BKI1 then dissociates from the membrane, allowing BRI1 to interact with a family of smaller LRR-RKs[19], including the BRI1 ASSOCIATED KINASE 1 (BAK1)[20,21]. The kinase domains of BRI1 and BAK1 trans-phosphorylate each other on multiple sites[22], and the fully activated receptor triggers downstream signalling events[23], resulting in major changes in nuclear gene expression[10].

BRI1's architecture is reminiscent of animal Toll-like innate immunity receptors (TLRs), and notably several plant LRR-RKs are immunity receptors[5]. It was, thus, reasonable to assume that the BRI1 ectodomain would form a TLR-like horseshoe structure[24] that binds its ligand along a dimer interface, as observed in several TLRs[25,26]. Here, we report the structure of the ligand binding domain of *Arabidopsis* BRI1 in its free form and bound to the steroid brassinolide, and show that BRI1 folds into a superhelical assembly, whose interior provides the hormone-binding site. Comparison of the free and hormone-bound structures, combined with genetic data, suggests a novel activation mechanism for BRI1 that is distinct from TLRs.

Overall structure of the BRI1 ectodomain

BRI1 was expressed in baculovirus-infected insect cells and the secreted ectodomain was purified by tandem-affinity and size-exclusion chromatography. The crystal structure was solved to 2.5 Å resolution by single isomorphous replacement (see Methods, Supplementary Table 1 and Fig. 1). BRI1 does not adopt the anticipated TLR-horseshoe structure but forms a right-handed superhelix composed of 25 LRRs (Fig. 1a). The helix completes one full turn, with a rise of ~70 Å. The concave surface, that determines the curvature of the solenoid[27], is formed by α- and 3_{10} helices (green in Fig. 1a) that cause inner and outer diameters of ~30 and ~60 Å, respectively. The overall curvature of BRI is similar to TLR3[24] (Fig. 1b), but, while the TLR3 ectodomain is essentially flat, BRI1 is highly twisted (Fig. 1b). Such twisted assemblies of LRRs have been observed previously with bacterial effector[28] and adhesion proteins[29], and with the plant defence protein PGIP[30] (Supplementary Fig. 2). The twist of PGIP's LRR domain is caused by a non-canonical, second β-sheet that is oriented perpendicular to the central β-sheet forming the inner surface of the solenoid[30]. Additional β-sheets are also present in our structure (blue in Fig. 1a, Supplementary Fig. 3), but in the case of the much larger BRI1 ectodomain result in a superhelical assembly (Fig. 1a). The second β–strand in PGIP and in BRI1 is followed by an Ile-Pro spine that runs along the outer surface of the helix and provides packing interactions between consecutive LRRs (Fig. 2a and ref.[30]). Both structural features are directly linked to the Lt/sGxIP consensus sequence of the plant-specific LRR subfamily[31] (Fig. 2c, Supplementary Fig. 4 and Table 2). Because this consensus sequence is found in other plant RKs, these receptors may also harbour twisted LRR domains (Fig. 2c), making BRI1 the primary template for the study of diverse signalling pathways in plants[3–5].

N- and C-terminal flanking regions that cap the hydrophobic core of the BRI1 solenoid are similar to caps previously described for PGIP[30] (Supplementary Figure 5). Notably, not are only these caps stabilised by disulfide bridges, but 5 additional disulfide bonds link

consecutive LRR segments in the N-terminal half of the BRI1 ectodomain (Fig. 2b, Supplementary Fig. 4 and Table 2).

The island domain

The island domain in BRI1 corresponds to a large insertion in the regular repeat-structure between LRRs 21 and 22 (residues 584–654) (Fig. 1a). The resulting ~70 residue segment forms a small domain that folds back into the interior of the superhelix, where it makes extensive polar and hydrophobic interactions with LRRs 13–25 (Fig 1a, Supplementary Fig. 6 and Table 2). The domain fold is characterised by an anti-parallel β-sheet, which is sandwiched between the LRR core and a 3_{10} helix and stabilised by a disulfide bridge (Fig. 3a, Supplementary Fig. 4). The loss-of-function alleles *bri1-9* (Ser662Phe, weak)[32] and *bri1-113* (Gly611Glu, strong)[2] map to this island domain – LRR interface (Supplementary Fig. 6), and likely interfere with folding of the island domain[33]. Two long loops that connect the island domain to the LRR core appear partially disordered in the unliganded receptor (Supplementary Fig. 7). The insertion of a folded domain into the LRR repeat has not been observed in other LRR receptor structures and is likely an adaptation to the challenge of sensing a small steroid ligand, compared, for example, to recognising larger ligands, such as proteins, nucleic acids, or lipids[25,26].

We next solved a 2.5 Å co-crystal structure with brassinolide, a potent *Arabidopsis* steroid that binds BRI1 with nanomolar affinity[7,34]. One molecule of brassinolide per BRI1 monomer binds in close proximity to the island domain (Fig. 3 a–c), which was previously implicated in steroid binding[7,16]. Our structure reveals that the LRR superhelix and the island domain both extensively contribute to formation of the hormone binding site. The A–D rings of the steroid bind to a hydrophobic surface which is provided by LRRs 23–25 and that maps to the inner side of the BRI1 superhelix (Fig. 3b,d, Supplementary Fig. 8). The alkyl chain of the hormone fits into a small pocket formed by residues originating from LRRs 21 and 22 (Ile563, Trp564, Met657, Phe658) and from two loops connecting the island domain with the LRR core (Fig. 3d). The hydrophobic nature and restricted size of this pocket now explain why steroid ligands with bulkier or charged alkyl side chains, such as the arthropod steroid ecdysone (Supplementary Fig. 8), cannot be recognised by BRI1[7]. A few polar interactions with brassinolide's second diol moiety (Fig. 3d) are established with Tyr597 and main chain atoms from His645 and Ser647 in the island domain, and are mediated by water molecules (Fig. 3d). Mutation of the neighbouring Gly644 to Asp may interfere with this hydrogen bonding network, and explain why this mutation greatly reduces the binding activity of the receptor[7] and causes the loss-of-function phenotype *bri1-6*[32] (Fig. 3d). No polar contacts are observed with the seven-membered B-ring lactone (Fig. 3d), consistent with B-ring modifications as found in e.g. castasterone (Supplementary Fig. 8) being tolerated by BRI1[7,35].

The steroid-complex reveals a hormone-binding site that involves a much larger portion of the LRR domain than previously anticipated[16]. Major interactions between the steroid and the BRI1 ectodomain originate from the very C-terminal LRRs 23–25, which brings the hormone in close proximity to the membrane (Fig. 3a,d). Importantly, while there is a significant hormone-receptor interface (550 Å^2) for such a small molecule ligand, large parts of the steroid are exposed to the solvent, including the 2α,3α-diol moiety in brassinolide that is important for biological activity[35]. Thus, protein-protein interactions may be involved in the recognition of the steroid ligand, with the hormone itself providing a docking platform. Importantly, steroid binding induces a conformational rearrangement and fixing of the island domain, which becomes fully ordered and competent to participate in protein-protein interactions that could be critical for receptor activation (Supplementary Fig. 7).

A protein interaction platform

Four known BRI1 missense alleles map to the inner surface of last 5 LRRs (Fig. 5a). This surface is not masked by carbohydrate (Supplementary Fig. 9) and contains both the hormone-binding site and the island domain (Fig. 3a,d and 4a,c). Three mutations cluster in a loop connecting the island domain with LRR 22 (Fig. 5a). This loop is partially disordered in the unliganded structure but is well-defined in the brassinolide complex (Supplementary Fig. 7). We speculate that this loop, when ordered, is engaged in protein-protein interactions that are critical for receptor activation, and that missense alleles in BRI1 modulate these interactions: The gain-of-function allele *sud1*[36] (Gly643-Glu) may establish contact with Ser623 in the island domain, and lead to an ordered loop even in the absence of steroid ligand (Supplementary Fig. 10). Mutation of the neighbouring Gly644 to Asp causes the loss-of-function phenotype *bri1-6*[32] (see above, Fig. 3d, 4a), and mutation of conserved Thr649 to Lys inactivates barley BRI1[37]. These mutations, when modelled *in silico*, induce steric clashes with residues in the island domain and in the underlying LRR domain (Supplementary Fig. 10), and thus may distort the position of the loop. Interestingly, *bri1-102*, a strong loss-of-function mutation (Thr750-Ile)[38] that does not affect steroid binding[7], maps to a distinct surface area in LRR 25 (Fig. 4a). Thus protein-protein interactions critical for receptor activation may not be restricted to the island domain, but also involve residues from the LRR core.

Receptor activation

BRI1 has been shown to exist at least partially as a omooligomer *in planta*[13,39,40]. Thus steroid binding to the island domain and the concomitant rearrangements of the island domain loop could induce a conformational change in a preformed BRI1 homodimer[13], or allow for ligand-dependent dimerisation of the BRI1 ectodomain. However, models of BRI1 dimers that bring the C-termini of their ectodomains into close proximity (n.b. it is known that the cytoplasmic kinase domains of BRI1 can interact[13,18]) and that make use of the interaction surface outlined above, encounter steric clashes with the N-terminal LRRs (Supplementary Fig. 11). Furthermore, in contrast to TLR ectodomains, which in crystals tend to form homodimers even in the absence of ligand[24,25], dimers cannot be seen in BRI1 crystals, which grew under the same acidic pH conditions that are typically associated with the plant cell wall. The largest interface area between two neighbouring BRI1 molecules amounts to only ~1.5% of the total accessible surface area, consistent with the high solvent content of our crystals (see Supplementary Methods). The main crystal contact involves a head-to-head arrangement of two BRI1 monomers, a configuration that would place the cytoplasmic kinase domains far apart (Supplementary Fig. 12). The recombinant BRI1 ectodomain eluates as a monomer in the absence of steroid ligand, and shows no tendency to dimer- or oligomerise in the presence of a ~4x molar excess of brassinolide in size-exclusion chromatography experiments (Fig. 4b).

Our analyses suggest that the superhelical BRI1 LRR domain alone has no tendency to oligomerise, indicating that BRI1 receptor activation may not be mediated by ligand-induced homodimerisation of the ectodomain (as described for TLRs[25,26]) or by conformational changes in preformed homodimers[13]. We do not want to dismiss the fact that the cytoplasmic kinase domain of BRI1 can dimerise[18], or that BRI1 homooligomers are present *in vivo*[13,39,40]. However, our structures reinforce the notion that homooligomerisation of BRI1 may be constitutive and independent of ligand stimulus[39]. The presence of an interaction platform that undergoes conformational changes upon steroid binding and that harbours several loss- and gain-of-function alleles suggests that interaction with another protein factor may control BRI1 activation.

Discussion

The structure of the BRI1 ectodomain offers several new insights, and its twisted shape will likely characterise the architecture of many plant LRR-RKs[1]. The presence of a folded domain-insert appears to be an adaptation to recognition of a small molecule ligand, a challenge that smaller LRR proteins have met by generating loop insertions into their capping motifs[41]. This fascinating mode of ligand recognition reveals how steroids can be sensed at membranes and rationalises a large set of genetic and biochemical findings[2,7,32,38].

Different BRI1 receptor activation mechanisms have been proposed including ligand-dependent dimerisation as seen for TLRs[25,26] and ligand-induced conformational changes in preformed homodimers[13]. Our analyses suggest that the superhelical shape of the BRI1 ectodomain is incompatible with homodimerisation, and that the isolated ectodomain behaves as a monomer even in the presence of steroid. These findings leave us with the alternative hypothesis that another protein factor could bind to the interaction platform in BRI1 that would minimally encompass the steroid ligand, LRRs 21–25 and parts of the island domain (Fig. 4a). While it is possible that an unknown protein fulfils this role and provides a dimerisation interface for two BRI1 molecules (as seen for example for TLR4[26]), genetic and biochemical screens have not uncovered this protein. It is thus possible that the small receptor kinase BAK1 acts as a direct brassinosteroid co-receptor, as suggested previously[10,20]. It has been demonstrated that BAK1 is a genetic component of the brassinosteroid pathway[20,21], that BRI1 and BAK1 interact in a steroid-dependent manner[22] and that both receptors trans-phosphorylate each other upon ligand stimulus[22]. Notably, a homology model of the BAK1 ectodomain (Supplementary Fig. 13) is compatible in size and shape with the interaction platform in BRI1, and the BAK1 *elg* allele, which maps to the BAK1 ectodomain (Supplementary Fig. 14), renders plants hypersensitive to brassinosteroid treatment[42]. We speculate that the *sud1*, *bri1-6*, *bri1-102* and *elg* mutations modulate the interaction between the BRI1 and BAK1 ectodomains in a brassinosteroid-dependent manner (Supplementary Fig. 14). The demonstration that BAK1 is essential for brassinosteroid sensing may have been obscured owing to genetic redundancy[20], with at least two BAK1-like proteins interacting with BRI1 *in vivo*[43,44]. We recently overcame this limitation by showing that the BRI1 inhibitor protein BKI1 blocks the interaction between the BAK1 and BRI1 kinase domains[18]. Importantly, transgenic lines that constitutively deliver BKI1 to the site of BRI1 signalling, resemble strong BRI1 loss-of-function mutants, suggesting an important role for receptor - co-receptor association in brassinosteroid signal initiation[18].

Future studies will undoubtedly test this heteromerisation model and dissect the relative contributions of the BRI1 and BAK1 ectodomains, their transmembrane segments and their cytoplasmic kinase domains to receptor activation. It will become important then to understand how BAK1 could serve as co-receptor for other LRR-RK signalling pathways[5,19].

Methods Summary

The StrepII-9xHis fused BRI1 ectodomain (residues 29–788) was produced by secreted expression in baculovirus-infected insect cells, harvested 4 d post-infection by ultrafiltration and purified by tandem affinity chromatography, and by gel filtration. BRI1 was concentrated to 15 mg/ml and crystallised by vapour diffusion using a reservoir solution containing 14% PEG 4,000, 0.2 M $(NH_4)_2SO_4$, 0.1 M citric acid (pH 4.0). The brassinolide complex was obtained by co-crystallisation. Diffraction data to 2.5 Å resolution were collected on a rotating anode X-ray generator and at beam-line 8.2.1 of the Advanced Light

Source (ALS), Berkeley. The structure was solved using the SIRAS method. Data and refinement statistics are summarised in Supplementary Table 1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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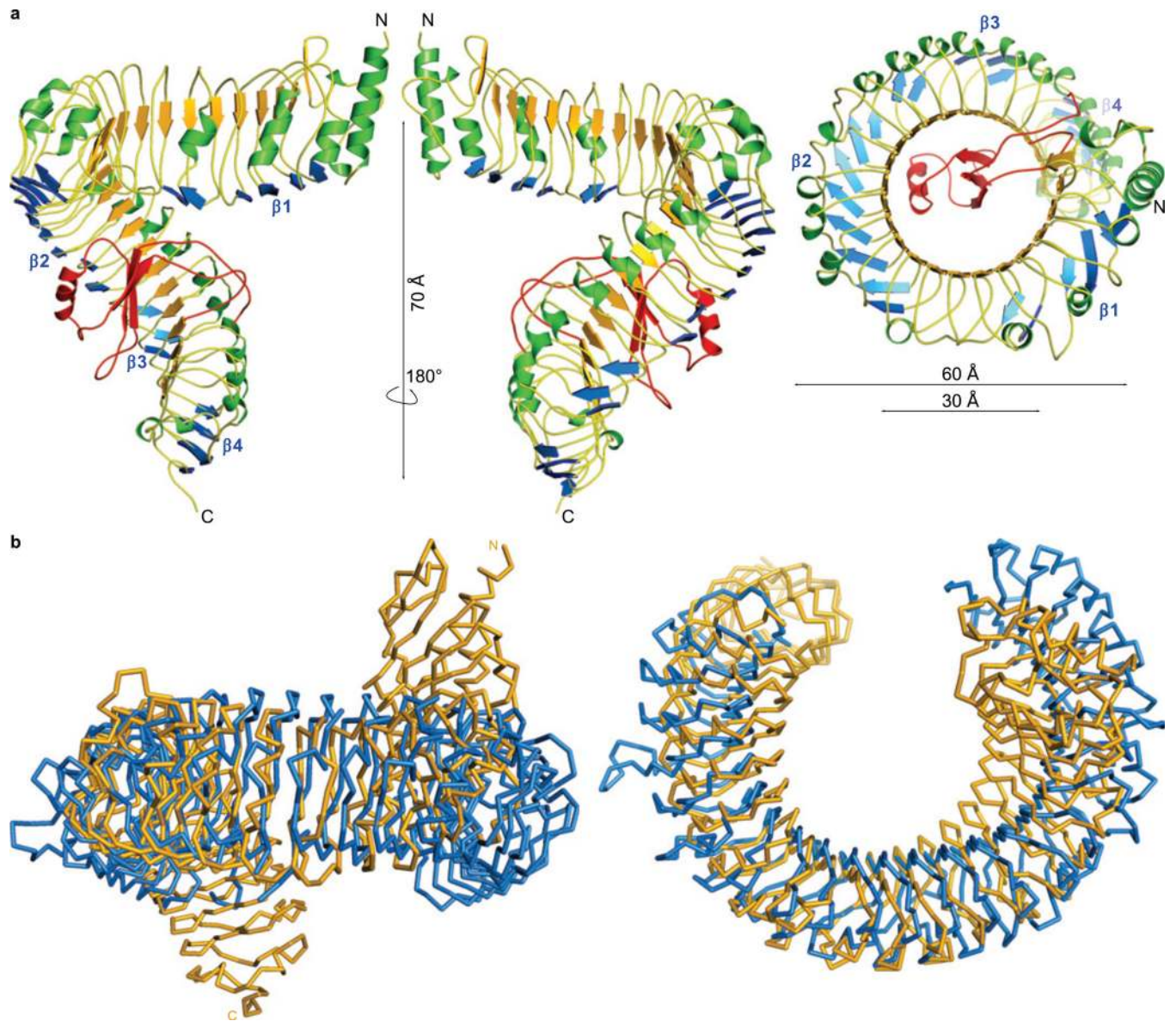
Figure 1. The BRI1 ectodomain forms a superhelical assembly
a, Ribbon diagram of the BRI1 LRR domain (front, back and top). The canonical LRR β-sheet is shown in orange, and the additional plant-specific β-sheets in blue. Helices are shown in green, and the island domain is depicted in red. **b,** Structural comparison of the BRI1 (shown as yellow C_α trace) and TLR3 (in blue, pdb-id: lziw)27 ectodomains. The structures superimpose with an r.m.s.d. of 4.2 Å between 341 corresponding C_α atoms. Side and top views are shown. The island domain has been omitted for clarity.
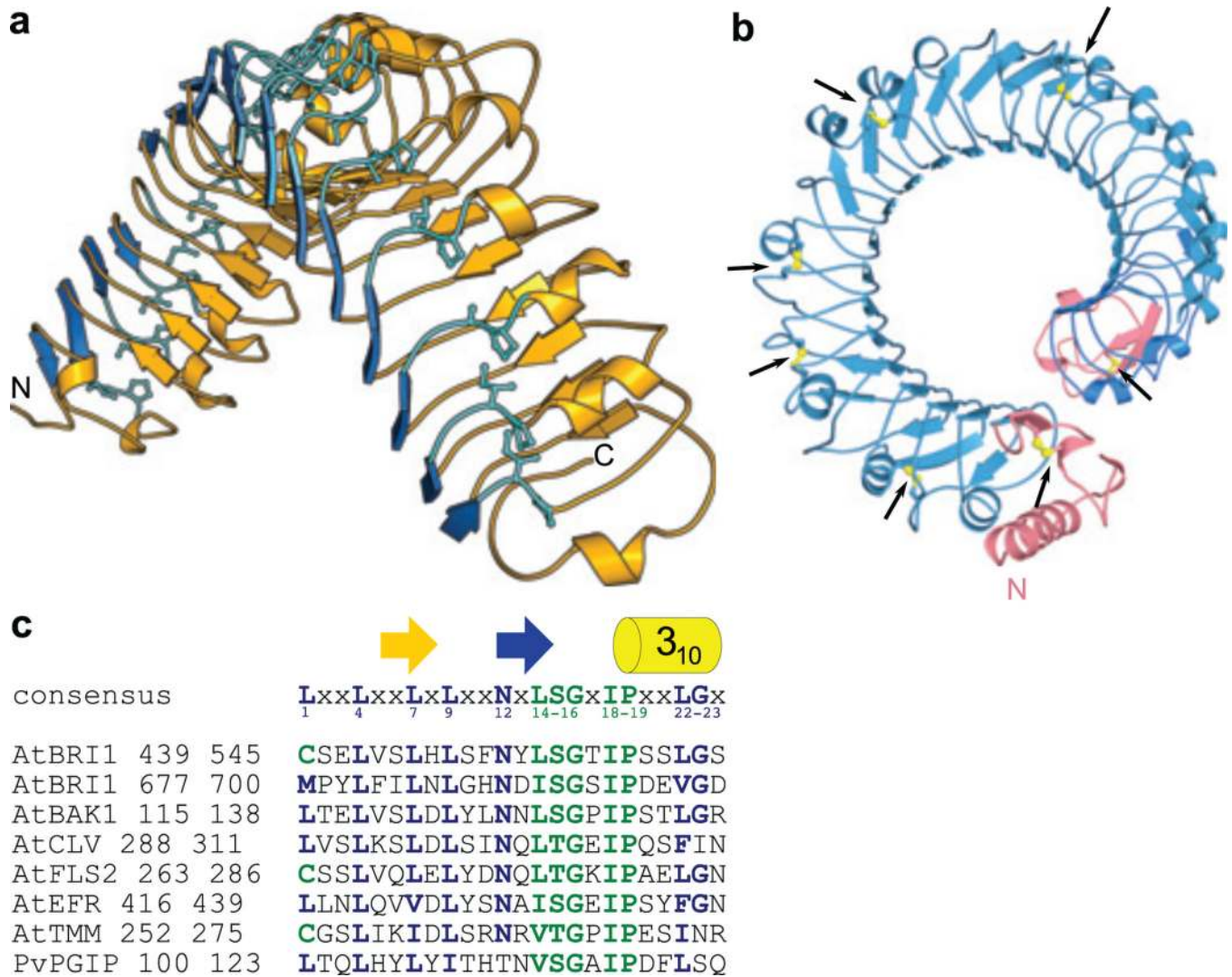
Figure 2. Plant-specific sequence fingerprints result in a superhelical BRI1 ectodomain
a, Ribbon diagram of the convex side of BRI1 LRRs 9–25 (in yellow). The non-canonical β-strands and the Ile-Pro spine are shown in dark and light blue, respectively, **b,** Top view of the BRI1 ectodomain (in blue) with disulfide bridges shown in yellow. The N- and C-terminal caps are highlighted in pink, **c,** Sequence alignment of LRRs in BRI1, other plant receptor kinases[4–7,23,24] and PGEP[33]. The canonical LRR consensus sequences are highlighted in blue, and plant-specific motifs are in green.
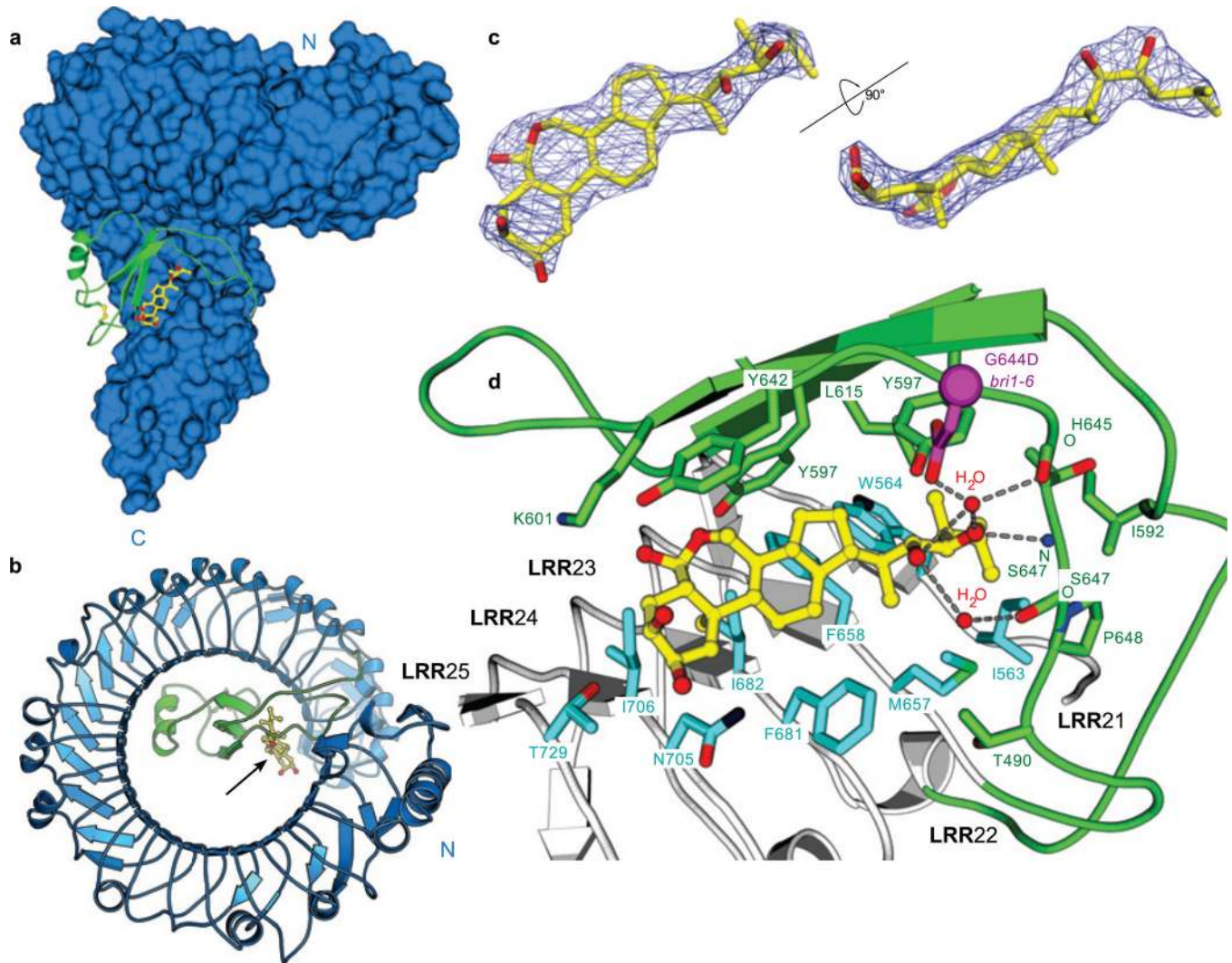
Figure 3. The steroid hormone binding site maps to C-terminal inner surface of the superhelix
a, Brassinolide (in yellow sticks) binds to a surface provided by the LRR domain (in blue) and by parts of the island domain (green ribbon), **b,** Location of the steroid in centre of the BRI1 superhelix. **c,** Close-up view of the brassinolide in two orientations, including an omit $2F_O-F_c$ electron density map contoured at 1.5 σ. **d,** Protein-hormone interactions in the BRI1 steroid binding site. Ribbon diagram of LRRs 21–25 (in grey) are shown together with parts of the island domain (in green). Contacting residues are shown in full side-chain representation, polar interactions as dotted lines, and water molecules as red balls, *bril-6* (Gly644Asp) is depicted in magenta.
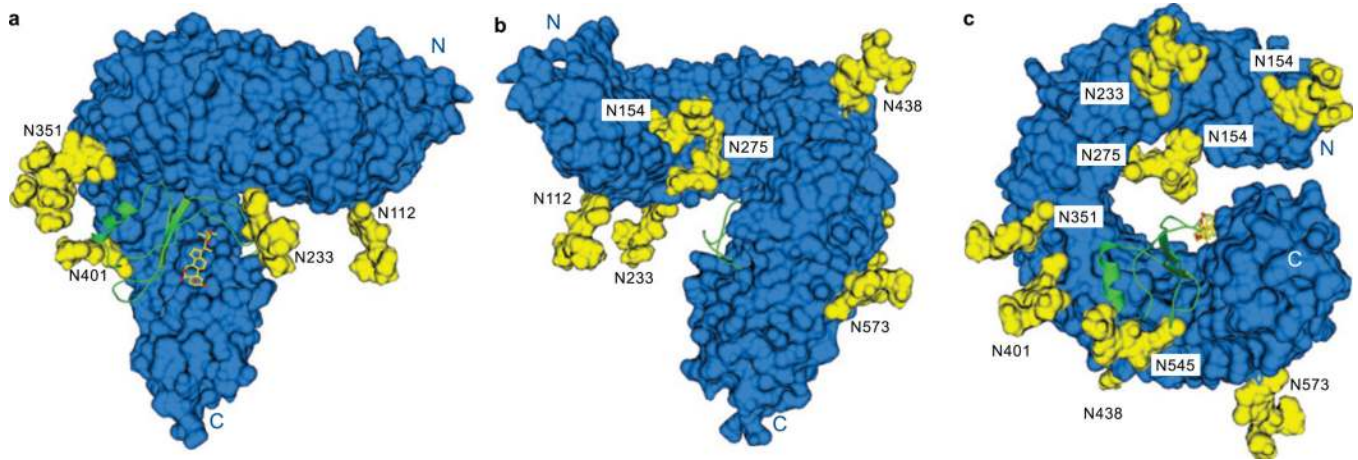
Figure 4. N-linked glycans mask large surface areas of the BRI1 superhelix
Oligomannose core stmctures (containing two N-acetylglucosamines and three mannose units) as found in insect cells and plants were modelled onto the nine glycosylation sites in the structure to visualise the BRI1 surface that is potentially masked by carbohydrate. The LRR domain in surface representation is shown in blue, and the glycan structures are highlighted in yellow. A ribbon diagram of the island domain (in green) and the steroid ligand (in yellow) is included. The views are **a,** front **b,** back and **c,** perspective along the superhelix from the C-terminus.
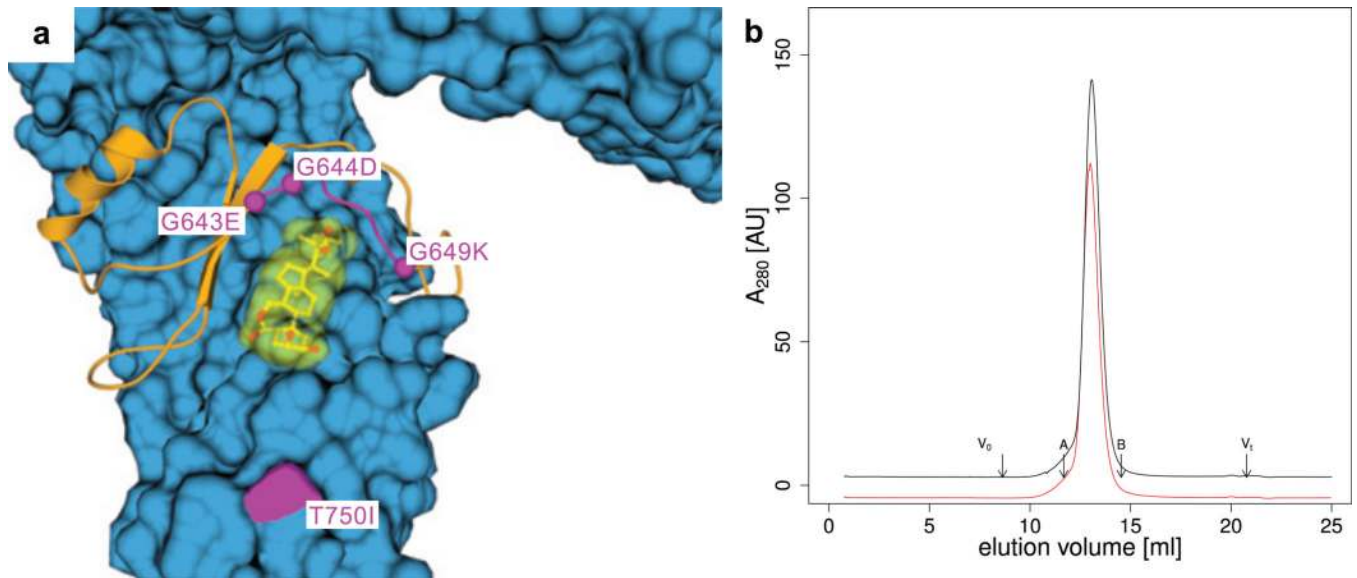
Figure 5. An accessible membrane-proximal region of BRI1 may provide a protein-protein interaction platform

a, Overview of the C-terminal surface area (in blue) that is not masked by carbohydrate. Brassinolide is shown in yellow, the island domain in orange, and genetic alleles connected in magenta, **b,** Analytical gel-filtration 280 nm absorbance trace. The free ectodomain eluates as a monomer (black line), as does a putative complex with brassinolide (red line). Void (V_0) and total volume (V_t) are shown together with elution volumes for molecular weight standards (A, aldolase, MW 158,000 Da; B, conalbumin, MW 75,000 Da). The estimated molecular weight for the monomer peak is ~125 kDa. The approximate molecular weight of the purified BRI1 is 110 kDa.