A hereditary spastic paraplegia–associated atlastin variant exhibits defective allosteric coupling in the catalytic core

The dynamin-related GTPase atlastin (ATL) catalyzes membrane fusion of the endoplasmic reticulum and thus establishes a network of branched membrane tubules. When ATL function is compromised, the morphology of the endoplasmic reticulum deteriorates, and these defects can result in neurological disorders such as hereditary spastic paraplegia and hereditary sensory neuropathy. ATLs harness the energy of GTP hydrolysis to initiate a series of conformational changes that enable homodimerization and subsequent membrane fusion. Disease-associated amino acid substitutions cluster in regions adjacent to ATL’s catalytic site, but the consequences for the GTPase’s molecular mechanism are often poorly understood. Here, we elucidate structural and functional defects of an atypical hereditary spastic paraplegia mutant, ATL1-F151S, that is impaired in its nucleotide-hydrolysis cycle but can still adopt a high-affinity homodimer when bound to a transition-state analog. Crystal structures of mutant proteins yielded models of the monomeric homodimer when bound to a transition-state analog. Together, these findings define a mechanism for allosteric coupling in which Phe151 is the central residue in a hydrophobic interaction network connecting the active site to an interdomain interface responsible for nucleotide loading.

Hereditary spastic paraplegia (HSP) comprises a group of genetic neurological disorders that manifest as spasticity and weakness in the distal extremities. One of the predominantly mutated loci contributing to HSP (subtype SPG3A) is the atlastin-1 (atl1) gene, which accounts for ~10% of autosomal dominant and the majority of early onset cases (1). Similarly, mutations in atl1 and atl3 were identified recently as a cause of hereditary sensory neuropathy (HSN) type I (2, 3), emphasizing the crucial role of atlastins (ATLs) in axonopathies and for normal cellular physiology (4, 5).

The atl genes encode for a subclass of the dynamin-related protein (DRP) superfamily (6). DRPs use the energy from GTP hydrolysis to control a multitude of cellular events, including vesicle scission, fusion and fission of organelle membranes, cytokinesis, and antiviral activity (7). DRPs often contain a GTPase (G) domain, helical middle domain, motifs for membrane association, and a GTPase effector domain (GED) (7, 8). The GED forms part of the protein’s stalk that is important for oligomerization and assembly-dependent enzyme stimulation. Similarly, ATLs consist of G and middle domains and a transmembrane anchor but lack the canonical GED and consequently appear not to form regular higher-order complexes akin to those observed with dynamin and Mxa (9–12). Instead, ATLs contain a C-terminal, cytoplasm-facing amphipathic helix that is crucial for lipid bilayer thinning and membrane fusion, the main function described for this class of DRPs (13–15).

ATLs localize to the endoplasmic reticulum (ER), where they catalyze the fusion of membrane tubules to form three-way junctions, establishing the highly reticular morphology of the smooth ER found at the periphery of the cell body (16–18). The branched structure of the peripheral ER is suppressed upon the expression of dominant-negative ATL alleles, including disease mutations, gene deletions, and transient siRNA knockdowns (16, 18–20). In Xenopus egg extracts or reconstituted systems using an invertebrate Drosophila melanogaster ortholog, catalytically active ATL is required to generate and maintain a branched, tubular network; Drosophila ATL is also sufficient to create a stable tubular membrane network from synthetic proteoliposomes (19, 21, 22). Effective fusion of ER membranes both in vitro and in the cell relies on ATLs traversing multiple cycles of conformational changes and oligomerization events that are driven by both GTP binding and hydrolysis (23–26).

Genomes of higher eukaryotes typically encode three main ATL isoforms (ATL1–3), with ATL1 being enriched in neural tissues and ATL2 and ATL3 showing more ubiquitous expression patterns (16). On a molecular level, ATL isoforms vary in GTPase activity and their apparent distribution across the ER (27, 28). ATL1 is the most active isoform in vitro and decorates...
the tubular ER network evenly in cells, whereas ATL3 is a less efficient enzyme and localizes preferentially to junction points in the ER network that appear to be associated with membrane fusion events (27–29). A closer structural comparison of two ATL isoforms (ATL1 and ATL3) indicates that despite differences in GTP-hydrolysis kinetics, the overall sequence of molecular events leading to ATL dimerization (and presumably membrane fusion) is conserved (26). In both cases, monomeric ATL binds GTP, undergoes an intramolecular conformational change, and hydrolyzes GTP, which enables the sequential dimerization of the G and middle domains. Dimerization precedes phosphate release. As indicated by a structural study, an intramolecular arginine residue displaces the catalytic magnesium, allowing the cycle to be reset (26). Compromising the overall fidelity of this system is a hallmark in mutant variants that are associated with neurological disorders (e.g. HSP and HSN) (2, 16, 23, 30, 31).

There is an emerging theme that neurological disorders are caused or affected by defects in organelle structure and membrane trafficking, exemplified by HSP, HSN, and related neurodegenerative diseases (32). However, the underlying structural and functional principles often remain poorly understood. In the case of ATLs, part of the knowledge gap can be attributed to the lack of reoccurring mutations that would define residue-specific hot spots within the proteins. Overall, mutations appear family-specific and distribute across ATL’s primary sequence (1, 30). When mapped onto the three-dimensional structures of ATL, mutants predominantly cluster in or near the protein’s active site or a region connecting the active site to an allosteric interface between the G and middle domains responsible for nucleotide loading (23, 30, 33). The majority of reported HSP/HSN mutations elicit dominant-negative phenotypes, yet the mutations are buried within the folded protein (16, 31, 34–36), supporting the notion that the mutations’ primary defects are intrinsic to ATLs. However, there is no unifying mechanistic model for how mutations affect ATL function, as the disease variants impair nucleotide hydrolysis, protein oligomerization, or both (30, 31, 33). Unfortunately, characteristic defects often cannot be gleaned from their location in the protein’s structure, and this lack of predictive power necessitates studies on individual mutants to elucidate pathogenic mechanisms. Here, we biochemically characterize ATL1 harboring the HSP mutation F151S (34, 35) and show that despite being able to bind nucleotide, the mutant enzyme is deficient in forming higher-order oligomers in the presence of GTP or non-hydrolyzable GTP analogs. However, this HSP mutant can dimerize through G and middle domains when bound to a transition-state analog, GDP-AlF<sub>4</sub>. Phe<sup>151</sup> clusters with other HSP/F151S mutations elicit dominant-negative phenotypes (32); however, it cannot dimerize when bound to Gpp(NH)p, and R217Q, which cannot hydrolyze GTP or dimerize in the presence of either GDP-AlF<sub>4</sub> or Gpp(NH)p (30) (Fig. 1A). Effects of Q191R on ATL1’s molecular mechanism have not been examined in greater detail, and hence it is not clear how the apparent defect in oligomerization propensities manifests. In contrast, the cause of R217Q’s negative effect on ATL function is explicable. The guanidinium group of Arg<sup>217</sup> directly contributes to the binding of the nucleotide by coordinating the guanine base; when mutated to glutamine, the affinity for nucleotide is lost (30). Similar to the R217Q mutant, engineered ATL variants such as R77E, a charge reversal mutation of the catalytic arginine, and a truncated construct expressing the isolated G domain both have impaired GTPase activity and cannot dimerize in GDP-AlF<sub>4</sub> (30, 33) (Fig. 1A, pink x and circles). The loss of function for both R77E and the isolated G domain can be attributed to a decreased affinity for nucleotide (23, 33). A more subtle mutation at the catalytic arginine finger, R77A, renders the protein’s GTPase inactive despite preserving GDP and GTP binding with affinities comparable with wild-type enzyme; nevertheless, if left to equilibrate for extended periods of time, the enzyme begins to exhibit the propensity to dimerize in Gpp(NH)p and GDP-AlF<sub>4</sub> (23) (Fig. 1A, pink plus symbol). Overall, these mutational analyses show that even in cases where GTPase activity and nucleotide binding are compromised, ATLs are still able to oligomerize at equilibrium. At the same time, equilibrium oligomerization does not equate to proper function, as cellular ATL function depends on coordi-
Allosteric defect in disease-associated atlastin mutant

Figure 1. ATL1 with the atypical HSP mutation F151S is catalytically deficient but retains ability to bind nucleotide. A, re-evaluation of the correlation between GTPase activity and dimerization propensities of HSP mutations found in ATLs. B, the atypical F151S mutation is located between the active site and intramolecular domain interface responsible for nucleotide loading. Motifs characteristic of G proteins including the switch regions are shown in color. G1/P-loop (pink), G2/switch 1 (teal), G3/switch 2 (dark purple), and G4 (blue/purple) are indicated along with HSP mutations proximal to Phe151. A schematic depicting the engaged, tight cross-over, and relaxed cross-over states is shown above the panels to indicate the crystallographically determined conformations. C, turnover number (kcat) of phosphate release for wild-type ATL1, catalytically deficient ATL1-R77A mutant, and ATL1-F151S. Kinetic experiments were conducted with the soluble catalytic core fragments, comprising G and middle domains. D, affinity of wild-type and F151S-containing ATLs for GTP and GDP were calculated using mant-nucleotides. Graphs showing means and S.D. (error bars) are plotted from two biological replicates with three technical repeats each.

ATL1-F151S dimerizes only when bound to the transition-state analog at equilibrium

Considering that ATL1-F151S is catalytically inactive but retains the ability to bind nucleotide (Fig. 1, C and D), we would predict that its dimerization profile would be similar to that of the R77A mutant (23). The oligomerization capability of ATL1-F151S was assessed upon incubating the protein with various nucleotides and nucleotide analogs and measuring the molecular weights of the protein/nucleotide mixtures using size exclusion chromatography–coupled multiangle light scattering (SEC-MALS) (39). The wild-type ATL1 catalytic core exhibited nucleotide-dependent dimerization consistent with previous studies where nucleotide-free (apo) and GDP-bound proteins were monomeric with a molecular mass of ~50 kDa, and Gpp(NH)p and GDP-AlF4- were dimeric with a molecular mass of ~100 kDa (26, 30) (Fig. 2A). GTPγS-bound, wild-type ATL1 comprises a mixture of dimeric and monomeric species (60% dimer, 40% monomer; cumulative weight analysis), which can be rationalized by either hydrolysis of GTPγS at high protein concentrations (40 μM) or weaker overall dimerization that is more affected by buffer exchange (GTPγS was not present in the mobile phase) (Fig. 2A). ATL1 equilibrated with GTP eluted as monomers due to both substrate consumption and buffer exchange into the mobile phase void of GTP (as indicated above) (Fig. 2A).

Analogous to wild-type protein, apo- and GDP-bound species of ALT1-F151S eluted as monomers (Fig. 2B). The light-
scattering chromatogram from GTP-bound ATL1-F151S displays two interesting signatures; the GTP-bound species eluted ~0.4 ml earlier and was band-broadened by ~0.3 ml, as determined from the difference between initial and final elution volumes at half-maximal intensities when compared with other monomer SEC profiles from both wild-type ATL1 and ATL1-F151S (Fig. 2, A and B). Because the molecular weight is consistently monomeric across the peak, this observation suggests that ATL1-F151S is able to undergo a specific conformational change when bound to GTP that has not been observed in other protein-nucleotide complexes. We expected ATL1-F151S to dimerize in the presence of GTPγS and Gpp(NH)p due to the similar nucleotide-binding abilities shared with R77A (23); however, ATL1-F151S remained monomeric when bound to GTPγS, and cumulative weight fraction analysis indicated that only ~5% of the population was dimeric when bound to Gpp(NH)p (Fig. 2B). In contrast to the GTPγS and Gpp(NH)p results, ATL1-F151S can fully dimerize when bound to GDP-AlFx. This nucleotide-dependent dimerization profile makes ATL1-F151S unique and the only HSP mutant protein characterized to date that is deficient in catalytic cycling, unable to dimerize when bound to non-hydrolyzable GTP analogs, but capable of dimerizing with a transition-state analog. 

ATL1-F151S retains its ability to dimerize through both G and middle domains when bound to GDP-AlFx.

SEC-MALS analysis reports on the absolute molecular weight of the species in solution but cannot discriminate between the relative orientation of the G and middle domains in dimeric assemblies. Using FRET-based approaches, we have
previously shown that ATLSs proceed through a series of conformational changes where dimerization of the G and middle domains occurs in close succession (23, 26). To determine the conformations that ATL1-F151S is able to adopt, we used the same FRET sensors to differentiate between G and middle domain dimerization (Fig. 3). In short, engineered surface-exposed cysteine residues were incorporated into the G or middle domains and labeled with small organic donor or acceptor fluorophores (23, 26) (Fig. 3, A and B, top). Equilibrium FRET efficiencies in the presence of various nucleotides were calculated for ATL1 wild type and the corresponding F151S variant; the duration required to reach equilibrium was determined via kinetic time courses (Fig. S1). As in previous studies (23, 26), the G and middle domains of ATL1 wild type formed homodimers in the presence of Gpp(NH)p, GTPγS, and GDP-AlFx, whereas the enzyme remained monomeric when bound to GDP or in the absence of nucleotide (Fig. 3, A and B). In agreement with the SEC-MALS molecular weight determination, the ATL1-F151S catalytic core fragment was unable to dimerize with non-hydrolyzable nucleotides but formed G and middle domain homodimers when bound to GDP-AlFx. Because the middle-domain FRET efficiency for ATL1-F151S bound to GDP-AlFx was greater than wild type bound to non-hydrolyzable analogs (***, p ≤ 0.0001), we can conclude that the mutant is competent in forming the high-affinity transition-state dimer (25, 26) (Fig. 3B). A non-equilibrium measurement was conducted at steady state in the presence of GTP. The wild-type G and middle domain FRET exhibit intermediate efficiencies, which is in agreement with a cycling system that samples both monomeric and dimeric states. In contrast, F151S lacks pronounced G and middle domain FRET under the same conditions (Fig. 3, A and B).

Structural characterization of ATL1 variants with diverse dimerization capacities

GTase-driven dimerization is crucial for ATL function (19, 21, 22, 24). Previous studies shed light on the dimer conformations that ATLSs sample (23, 26, 30, 33, 40, 41), but the conformations of the monomeric states are less well-defined. Here, we determined crystal structures of ATL1 mutants R77A, R77A/F151S, and F151S, all of which display distinct oligomerization propensities. We also crystallized the isolated G domain in the presence of GDP-Mg2+ as another reference model for a catalytic and dimerization-incompetent state. The G domain fails to dimerize in any nucleotide because it cannot adopt the GTP loading—competent state without the middle domain (23).

A structure for the isolated G domain of ATL1 (residues 1–339) was solved in space group P21, containing four molecules per asymmetric unit, at a maximum resolution of 3.0 Å (Table 1). The four GDP-Mg2+-bound protomers assemble into two identical crystallographic dimers via a G domain dimer interface seen in previously published, GDP-Mg2+-bound ATL structures despite the protein’s inability to oligomerize in solution (23, 26, 30, 33, 42) (Fig. 4A). One key feature of this structure is the configuration of the fourth α-helix (α4) of the G domain fold, which is straight. Based on previous ATL1 structures, this helix can adopt two principal configurations, straight and bent (26, 30, 33, 42) (Fig. 4B). When bent, G and middle domains are engaged, and this interaction enables GTP loading (23). GTP binding is followed by an intramolecular conformational change that is thought to represent the release of the middle domain from the G domain (26). The straightening of helix α4 is one potential mechanism for the kick-off of the middle domain. In conclusion, the structure of the isolated G domain represents a GTase cycling—competent state and...
provides further structural evidence that helix $\alpha 4$ bending requires middle domain docking, which is associated with GTP binding. Of note, Phe$^{151}$ is resolved in the isolated G domain structure. The residue shares the same conformation and environment as seen in a crystallographic state of ATLs bound to GDP-Mg$^{2+}$ (see Fig. 1B (right) for reference), where the middle domain is dislodged from the G domain, and G and middle domains form weak homodimers with an adjacent protomer in the crystal lattice, which is thought to represent a post-hydrolysis dimer (30, 33).

One caveat with previous structural studies of ATL, including the isolated G domain above, stems from a preferred crystal packing interaction where the G domains of two molecules form a loose dimer even when the protein is bound to GDP-Mg$^{2+}$ (23, 26, 33, 42). This conformation is reminiscent of the tight dimer observed in the transition state and involves the switch regions (G1–G4) of the G domains but has a smaller interfacial area than the transition state (23).

Table 1

| X-ray data collection and refinement statistics |
|-----------------------------------------------|
| ATL-R77A; Mg$^{2+}$, GDP 1–446               |
| X-ray source                                  |
| CHESS                                        |
| Wavelength                                   |
| 0.9759                                       |
| Unit cell                                    |
| $a$, $b$, $c$ (Å)                            |
| 51.1, 68.4, 75.9                             |
| Resolution (Å)                               |
| 42.36–1.95 (2.06–1.95)$^*$                  |
| No. of reflections                           |
| 153,067 (22,418)                             |
| Total                                        |
| 63,315 (9210)                                |
| Completeness (%)                             |
| 96.4 (96.2)                                  |
| Multiplicity                                 |
| 2.4 (2.4)                                    |
| $I/\sigma(I)$                                 |
| 7.1 (2.1)                                    |
| $R_{merge}$ (%)                              |
| 10.3 (56.0)                                  |
| $R_{res}$ (%)                                |
| 7.9 (43.0)                                   |
| $CC_{1/2}$                                   |
| 0.991 (0.784)                                |
| Protein (%)                                  |
| 5872                                         |
| Ligands (%)                                  |
| 94                                           |
| Water (%)                                    |
| 281                                          |
| Average $B$-factors (Å$^2$)                  |
| 43.3                                         |
| Protein (%)                                  |
| 6088                                         |
| Ligands (%)                                  |
| 63                                           |
| Water (%)                                    |
| 198                                          |
| Ramachandran (%)                             |
| 97.7                                         |
| Favored (%)                                  |
| 98.0                                         |
| Outliers (%)                                 |
| 0                                            |
| Quality (%)                                  |
| 17.8 (22.2)                                  |
| RMSD                                         |
| 0.007                                        |
| No. of atoms                                 |
| 6879                                         |
| Protein (%)                                  |
| 6088                                         |
| Ligands (%)                                  |
| 63                                           |
| Water (%)                                    |
| 198                                          |
| Ramachandran (%)                             |
| 97.7                                         |
| Favored (%)                                  |
| 98.0                                         |
| Outliers (%)                                 |
| 0                                            |
| Quality (%)                                  |
| 17.0 (20.5)                                  |

$^*$ Values in parentheses are for the highest-resolution bin.

Figure 4. Crystal structure of the isolated G domain of ATL1 bound to GDP-Mg$^{2+}$. A, the G domain structure displays weak crystal packing interactions that involve switch regions. The G domain (colored salmon), helix $\alpha 4$ (red), and a second protomer (white) are illustrated. B, comparison of the isolated G domain with either the engaged (PDB code 3Q5E; left) or relaxed cross-over (PDB code 3Q5D; right) structures (both black).
Allosteric defect in disease-associated atlastin mutant

In spite of the high degree of similarity between the two structures of the F151S mutation into both wild-type and R77A con-
major, second transition occurring at 56 °C. Upon the addition of GDP, wild-type and R77A proteins exhibit two phases of unfolding, with a minor unfolding transition occurring at 40 °C (accounting for 20% of the total fluorescence signal) and a major, second transition occurring at 56 °C. Upon the addition of the F151S mutation into both wild-type and R77A con-
struc-
structs, the initial unfolding phase at 40 °C was not observed (Fig. 5B, pink halo). Despite the high degree of similarity between the two structures (RMSD = 0.25 Å), the ATL1-R77A/F151S structure has fewer disordered loops than ATL1-R77A, which is most likely due to the addition of the F151S mutation (Fig. 5B).

We used an established fluorescence-based thermal shift assay to determine whether the F151S mutation confers temperature-dependent stability in solution. When bound to GDP, wild-type and R77A proteins exhibit two phases of unfolding, with a minor unfolding transition occurring at 40 °C (accounting for 20% of the total fluorescence signal) and a major, second transition occurring at 56 °C. Upon the addition of the F151S mutation into both wild-type and R77A con-
struc-
structs, the initial unfolding phase at 40 °C was not observed (Fig. 5C, left). Tm values for the GDP-bound proteins were cal-
culated for the predominant unfolding phase, and they were significantly different between ATL1-R77A and -R77A/F151S with a difference of 1.0 °C (**, p = 0.01) (Fig. 5C, right). We also tested the thermal stability of ATL1-R77A, -F151S, and -R77A/ F151S bound to GTP and determined that the F151S mutations alone or in combination with R77A confers a 5.2 °C and 2.8 °C stabilization, respectively (Fig. 5D). The stabilization exhibited by F151S-GTP is additional evidence for the conformational change that resulted in the shifted elution and peak broadening determined via SEC-MALS (Fig. 2B).

To further delineate the effect that the addition of R77A contributes to the double mutant, we conducted SEC-MALS–based molecular weight determination for both ATL1-R77A and -R77A/ F151S in the presence of various nucleotides (Fig. S4). The data indicate that ATL1-R77A/F151S does not dimerize with GTP, non-hydrolyzable analogs, or the transition state, unlike the variant with the R77A mutation alone. Arg77 is also required for the apparent band broadening and peak shift observed with ATL1- F151S (Fig. 2B), as elution peak volume and peak width changes are affected in the ATL1-R77A/F151S double mutant compared with ATL1-F151S (Fig. S4B and Fig. 2B). This result suggests a conformation where Arg77 assumes its nucleotide-coordinating conformation in ATL1-F151S-GTP, and, based on the SEC-MALS data above, this occurs in a monomer.

Additional structural differences pertain to the guanine cap, a motif that is important for guanine-base binding and specificity (37). In the structure of GDP-bound ATL1-R77A/F151S, this motif adopts an open conformation (Fig. 5E [dark red trace] and Fig. S5). Based on an overlay with all other crystal forms (i.e. G/M-engaged, transition state, and relaxed crossover (23, 30)), this guanine cap conformation and the resolved portion of G2 mirrors the transition-state form of ATL1 (Fig. 5E, green traces). In contrast, ATL1-R77A adopts a conformation more similar to all other GDP-bound structures, with the guanine cap adopting a closed configuration (Fig. 5E). We were also able to attain the transition-state structure of ATL1-F151S with crystals diffracting X-rays to 1.9 Å. Phasing by molecular replacement and subsequent refinement in space group P2_12_1 yielded the final model with two molecules per asymmetric unit (Table 1 and Fig. 5F). Despite including a non-
conser-
vantive mutation in the buried core of the G domain, the structure is well-resolved and displays the anticipated change in electron density at position 151 (Fig. 5F, inset). ATL1-F151S bound to GDP-AIF_3 is virtually identical to the synonymous wild-type transition-state structure (PDB code 4IDO) with an RMSD of 0.22 Å (23). This structure indicates that the protein with the F151S mutation is fully capable of equilibrating into the high-affinity transition state but is unable to reach this ener-
get-
etic state with the endogenous substrate GTP.

Discussion

Here we have characterized the atypical HSP mutation ATL1-F151S that is deficient in GTP-hydrolysis and cannot dimerize when bound to GTP or non-hydrolyzable analogs but retains the ability to adopt the dimeric transition state when bound to GDP-AIF_3. Mechanistically, this implicates Phe151 in reducing the activation energy required for GTP hydrolysis and/or interdomain allostery linking nucleotide hydrolysis and middle domain engagement. Structural comparisons suggest a model where Phe151 may represent the central residue that couples the enzyme’s active site with the functionally important
Allosteric defect in disease-associated atlastin mutant

Figure 5. Structures of ATL1 mutants have diverse dimerization capacities and guanine cap configuration. A, ATL1-R77A bound to GDP-Mg\(^{2+}\) (light gray) exists in a monomeric state with G and middle domains engaged. Switch regions G1 (pink), G2 (teal), G3 (dark purple), and G4 (blue/purple)) are largely disordered, and their hypothetical locations are indicated (red dots highlighted yellow). Inset, α-carbon backbone of loop containing R77A and the β-carbon of residue 77 are not perturbed. B, ATL1-R77A/F151S bound to GDP-Mg\(^{2+}\) (dark gray) also exists in a monomeric state with G and middle domains engaged. Switch regions, colored as in A, are more ordered than in the isomorphic ATL1-R77A structure, and their hypothetical locations are indicated (red dots highlighted yellow). The F151S mutation is part of the G3 switch region and is disordered in this structure; hypothetical location of the mutation is outlined by a pink halo. C, thermal melting data for wild-type ATL1, ATL1-R77A, ATL1-F151S, and ATL1-R77A/F151S bound to GDP. The T_m associated with the major unfolding phase is significantly different for ATL1-R77A and ATL1-R77A/F151S (**, p < 0.01). D, thermal melting data for ATL1-R77A, ATL1-F151S, and ATL1-R77A/F151S bound to GTP. A single unfolding phase exhibits very different T_m values for the three mutant proteins (****, p < 0.0001 for all possible comparisons). E, the indicated crystal structures were aligned using the G domain as a reference to observe the conformation of the guanine cap. The ATL1-R77A structure (yellow) had this loop closed onto the guanine base like other GDP-Mg\(^{2+}\)-bound structures (orange, blue, and purple). However, both the ATL1-R77A/F151S GDP-bound (red) and the ATL1-F151S GDP-AIF\(_4\) bound (light green) structures mirror the wild-type transition-state structure (dark green) and have a retracted guanine cap. F, ATL1-F151S bound to GDP-AIF\(_4\) (protomer 1 (green) and protomer 2 (white)) is fully capable of equilibrating into the high-affinity transition state despite the F151S mutation being buried within the core of the enzyme. Stepwise map for the F151S mutation contoured to 1σ is depicted in black mesh. In C and D, melting curves showing means and T_m values showing means ± S.D. (error bars) are plotted from two biological replicates with a minimum of three technical repeats each.
toward the bound GDP (Fig. 6A). These monomeric ATL structures may represent the hitherto elusive nucleotide-loading state, as they are the first structures where the switch regions and guanine cap are not stabilized through crystallographic contacts (Fig. 6A). Weak crystal packing interactions that involve the G domain and its switch regions (PDB code 3Q5E) allow Phe76 to rotate $\sim 180^\circ$ toward switch 1 (Fig. 6B). In the presence of a nucleotide with $\gamma$-phosphate or a transition-state analog, Phe76 moves outward in concert with major reorientations of the switch regions that stabilize the phosphate moieties and align Arg77 for nucleophilic attack (Fig. 6C) (23). In the post-hydrolysis states, Phe76 may return to the configuration seen in the engaged monomeric structures, which may aid phosphate release and resetting of the enzymatic cycle (Fig. 6D).

Phe76 is proximal to Phe151, the site of the HSP disease mutation investigated above. Phe151 adopts a number of conformations when comparing the various crystallographic states and is not resolved in the monomeric, engaged structures where the switch regions are largely disordered (Fig. 6A). During the large reorganization of the switch regions leading to the conformation depicted in the transition-state crystal structures, Phe151 transitions from interacting with a single hydrophobic residue (Phe164) to an environment that is highly hydrophobic. As Phe151 rotates away from the active site, it pushes against His189 and Leu157 located on helix 4 (Fig. 6, B and C). Pushing against these residues coincides with a rotation of helix 4, which results in its straightening. The straightening of helix 4 also involves Phe193, which is located at the helix’s bend and may contribute energetically to helix straightening, rotates to form hydrophobic interactions with other residues labeled in this panel. D, structures resembling the post-hydrolysis state (PDB code 3Q5D, pink/dark gray) and isolated G domain (purple/light gray) exhibit a configuration that is en route to resetting the cycle. The helix a4 remains straight, and upstream residues (Phe76 and Phe193) begin to take on conformations seen in A.

Figure 6. Structure-based model for a hydrophobic interaction network that establishes interdomain allostery. Hydrophobic residues connect the active site (GDP (white/black) and guanine cap (green/salmon)) to helix a4 (schematic helix). A, structures depicting crystallographic monomers (ATL1-R77A (pink/dark gray) and ATL1-R77A/F151S (purple/light gray)) are flexible with Phe76 (in ATL1-R77A structure), F151S, and Leu157 being disordered and helix a4 bent. B, when switch regions are stabilized via homotypic crystallographic dimer contacts, the G/middle domain-engaged structure (PDB code 3Q5E, pink/dark gray) shows Phe76 rotating away from the nucleotide and Phe151 being resolved. C, both wild-type (PDB code 4IDO, pink/dark gray) and ATL1-F151S (purple/light gray) transition-state structures exhibit a switch reorganization that is accompanied with a downward rotation of Phe151 that pushes against His189 and Leu157. The helix a4 becomes straight, and Phe193, which is located at the helix’s bend and may contribute energetically to helix straightening, rotates to form hydrophobic interactions with other residues labeled in this panel. D, structures resembling the post-hydrolysis state (PDB code 3Q5D, pink/dark gray) and isolated G domain (purple/light gray) exhibit a configuration that is en route to resetting the cycle. The helix a4 remains straight, and upstream residues (Phe76 and Phe193) begin to take on conformations seen in A.
Allosteric defect in disease-associated atlastin mutant

family, considering the high conservation of these residues in ATL orthologs, including isoforms from Drosophila, silkworm, zebrafish, and higher eukaryotes.

The two F151S-containing crystal structures establish that the mutant proteins in principle can sample the mechanistically relevant nucleotide-loading and transition states, but cannot adopt the latter under physiological conditions due to lack of GTP hydrolysis (Fig. 6, A and C). In the transition-state structure, Arg\(^{77}\) adopts its catalytically competent conformation by coordinating the phosphate moieties, identical to the conformation of the wild-type protein in this state (Fig. 6C) (23). Arg\(^{77}\) is also involved in the GTP-induced changes in hydrodynamic properties of monomeric ATL1-F151S (Fig. 2E and Fig. S4). Together, these results suggest that Arg\(^{77}\) not only serves as the catalytic arginine finger but may also contribute to the allosteric network by communicating with Phe\(^{76}\) and Phe\(^{151}\), and this communication is broken in the F151S mutant. Curiously, the protein with the F151S mutation has an even higher affinity for GTP than the corresponding wild-type ATL1, which can be rationalized based on the crystal structure of ATL1-R77A/F151S (G/middle-engaged) (Fig. 5E and Fig. S5). With F151S, the monomeric protein exhibits an open conformation of the guanine cap that was previously reserved for structures adopting the tight cross-over dimer (e.g. the transition-state structure) (23). The guanine cap is not stabilized through crystal packing in either ATL1-R77A or ATL1-R77A/F151S structures, indicating the side chain identity at position 151 as the intrinsic reason for the apparent differences in ATL conformation and/or dynamics observed in the crystal structures.

Notably, two residues that Phe\(^{151}\) interacts with (Leu\(^{157}\) and His\(^{189}\)) are both HSP disease mutants (46). Biochemical characterization of the corresponding disease variants, ATL1-L157W and ATL1-H189D, established that they are both catalytically active and able to fully dimerize when bound to either Gpp(NH)p or GDP. This is consistent with what is known from structures of other DRPs (24–27). The two F151S-containing crystal structures establish that they are both catalytically active and able to fully dimerize when bound to either Gpp(NH)p or GDP.

**Experimental procedures**

**Phosphate release kinetics**

The release of free phosphate was detected using the Enzchek phosphate assay kit and the manufacturer’s protocol (Molecular Probes, Inc., Eugene, OR) with the exception of the addition of 100 mM NaCl to the reaction buffer. Reactions consisted of a 1 μM nucleotide and increasing amounts of ATL (5–20 μM) at 25 °C. Samples were excited at 366 nm, and signal from emission filter HQ460/40 M (Chroma Technology Corp., Rockingham, VT) was collected. Data were fit to exponential association equations, and the rate constants were plotted versus ATL concentration; the

Determination of dissociation constants with N-methylanthraniloyl (mant)-nucleotides

On and off rates were determined from mant-nucleotide binding experiments (23, 55) and used to calculate dissociation constants for mant-GDP and mant-GTP (Thermo Fisher Scientific, Waltham, MA), incubated in half-area, 96-well plates (Corning, Inc., Corning, NY) at 25 °C. Data were recorded at an absorbance of 366 nm using a BioTek Synergy II plate reader, and the initial rate before 10% substrate conversion was fit to a linear regression and converted to units of μM/min using a standard curve. Turnover number is defined as \(k_{cat} = V/[enzyme]\).
resulting slope of the linear fit corresponds to $k_{on}/k_{off}$ was directly measured by preloading ATL with mant-nucleotide and chasing with a high concentration of nucleotide (final concentrations of 2.5 mM ATL-mant-nucleotide and 2.5 mM unlabeled nucleotide). The resulting data were fit to a single-exponential decay curve where the observed rate constant corresponds to $k_{off}$. Dissociation constants ($K_d$) were calculated as $k_{off}/k_{on}$.

**Size-exclusion chromatography coupled to multiangle light scattering**

Protein/nucleotide mixtures (40 μM ATL and 1 mM nucleotide) were injected onto a Superdex 200 Increase 10/300 column (GE Healthcare), equilibrated with 25 mM HEPES, pH 7.5, 100 mM NaCl, 4 mM MgCl$_2$, and 2 mM EGTA. The GDP-AIF$_x$ sample was supplemented with 2 mM aluminum chloride and 20 mM sodium fluoride. Before injection, protein/nucleotide mixtures were incubated for 1 h. The gel filtration column was coupled to a static 18-angle light scattering detector (DAWN HELEOS-II) and a refractive index detector (Optilab T-rEX) (Wyatt Technology, Goleta, CA). Data were collected every second at a flow rate of 0.7 ml/min. Data analysis was carried out using ASTRA VI, yielding the molar mass and mass distribution (polydispersity) of the sample. The monomeric fraction of BSA (Sigma; 5 mg/ml) was used to normalize the light scattering detectors and used as a positive quality control.

**Dye labeling and FRET measurements**

Site-specific dye labeling was achieved by replacing non-conserved surface amino acids with cysteine residues within the G or middle domains (23, 26). To obtain singly labeled protein, a surface-exposed cysteine residue was replaced with an alanine residue (C375A). A cysteine residue for site-specific labeling was introduced at a surface-exposed position on either the G (K295C) or middle (K400C) domain. Labeling was conducted at 100 μM protein, 150 μM Alexa Fluor 488 or Alexa Fluor 647 (Molecular Probes) in a buffer containing 25 mM HEPES, pH 7, 100 mM NaCl. This reaction was incubated on ice for 30 min, and excess dye was removed with a NAP-5 column (GE Healthcare) pre-equilibrated with buffer containing 25 mM HEPES, pH 7.5, 100 mM NaCl, 2 mM MgCl$_2$. Data for FRET equilibrium and time-course measurements were collected with a Gemini EM microplate reader (Molecular Devices, Sunnyvale, CA) with sensor excitation at 473 nm and fluorescence recorded at 515 and 665 nm for donor and acceptor channels, respectively. All experimental reactions consist of 1 μM protein and 500 μM nucleotide in a buffer containing 25 mM HEPES, pH 7.5, 100 mM NaCl, and 2 mM MgCl$_2$. Raw FRET donor and acceptor signals were used to calculate a FRET efficiency, where FRET efficiency = $I_{acceptor}/(I_{donor} + I_{acceptor})$.

**ATL mutant and isolated G domain crystallization and structure determination**

Proteins were expressed and purified as reported previously (23, 26, 30). ATL crystals were obtained with the catalytic core fragment for mutants (residues 1–446) and the isolated G domain (residues 1–339) through sitting drop vapor diffusion. Protein (10–30 mg/ml) was incubated with 2 mM nucleotide and 4 mM MgCl$_2$ for 1 h at 25 °C, subsequently mixed with an equal volume of reservoir solution, and stored at 20 °C. Initial crystal hits were optimized via hanging drop vapor diffusion at a protein concentration of 30 mg/ml. Final optimized conditions are as follows: ATL1-R77A, 0.2 M sodium malonate, pH 6, 20%...
Allosteric defect in disease-associated atlastin mutant

PEG 3350, 1 mM GDP, 2 mM MgCl₂; ATL1-R77A/F151S, 0.1 M BisTris, pH 5.5, 0.2 M ammonium sulphate, 22% PEG 3350, 1 mM GDP, 2 mM MgCl₂, 10% xylitol (cryoprotectant); ATL1-F151S, 0.1 M ammonium citrate tribasic, pH 7, 0.1 M imidazole, pH 7, 20% PEG monomethyl ether 2000, 1 mM GDP, 2 mM MgCl₂; ATL1 G domain, 0.2 M D- malic acid, pH 7, 20% PEG 3350, 1 mM GDP, 2 mM MgCl₂. Single crystals appeared between 24 and 48 h at 20 °C, and if crystals were not grown in a cryoprotectant (see above), they were supplemented with 25% glycerol for 5 min and frozen in liquid nitrogen.

X-ray diffraction data were collected at the Cornell High Energy Synchrotron Source (CHESS), and data processing and scaling were carried out with X-ray Detector Software (XDS) and CCP4 software suite (56, 57). Phases were attained through molecular replacement methods using the PHENIX software package (58) with the coordinates of ATL1 (3Q5E, GDP-bound structures; 4D0, GDP-AlF₃-bound structure) as search models. Because space groups varied between crystallographic states, a new test set was chosen for each structure before refinement. To reduce model bias, we included simulated-annealing steps early in the refinement. In addition, composite OMIT maps were used for each structure refinement. Refinements were carried out in PHENIX (58) and COOT (59) to produce the final models. Data collection and model statistics are summarized in Table 1. Resolution cutoffs were selected based on a CCₕₘ > 0.4 (60). Figures were made in PyMOL (version 1.8.4; Schrodinger, LLC). The aforementioned software packages were accessed through SBGrid (www.sbggrid.org (61)).

Thermal shift assay

A fluorescence-based thermal shift assay using SPYRO Orange (Invitrogen) was used to generate protein melting curves and calculate Tₘ values (62). The reaction contains 2 μM ATL, 1 mM nucleotide, 2 mM MgCl₂, and 5X SYPRO Orange in 25 mM HEPEs, pH 7.5, 100 mM NaCl. A 20-μl reaction was melted in a MicroAmp 384-well plate (Applied Biosystems, Foster City, CA) using a Viia 7 real-time PCR system (Applied Biosystems, Foster City, CA). The protocol was initiated with a 5-min incubation at 10 °C followed by increasing the temperature to 95 °C at the rate of 0.03 °C/s; excitation and emission wavelengths were 470 ± 15 and 586 ± 10 nm, respectively. Tₘ values are defined by calculating the temperature where the second derivative of the thermal unfolding curve is equal to zero using GraphPad Prism (GraphPad, La Jolla, CA).

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References

1. Fink, J. K. (2013) Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol. 126, 307–328 CrossRef Medline

2. Guelli, C., Zhu, P.-P., Leonards, L., Papić, L., Zidar, J., Schabbißt, M., Strohmaier, H., Weis, J., Strom, T. M., Baets, J., Willems, J., De Jonghe, P., Reilly, M. M., Frohlich, E., Hatz, M., et al. (2011) Targeted high-throughput sequencing identifies mutations in atlastin-1 as a cause of hereditary sensory neuropathy type I. Am. J. Hum. Genet. 88, 99–105 CrossRef Medline

3. Fischer, D., Schabbißt, M., Wieland, T., Windhager, R., Strom, T. M., and Auer-Grumbach, M. (2014) A novel missense mutation confirms ATL3 as a gene for hereditary sensory neuropathy type I. Brain 137, e286–e286 CrossRef Medline

4. Hübner, C. A., and Kurth, I. (2014) Membrane-shaping disorders: a common pathway in axon degeneration. Brain 137, 3109–3121 CrossRef Medline

5. Li, J., Yan, B., Si, H., Peng, X., Zhang, S. L., and Hu, J. (2017) Atlastin regulates store-operated calcium entry for nerve growth factor-induced neurite outgrowth. Sci. Rep. 7, 43940 CrossRef Medline

6. Ferguson, S. M., and De Camilli, P. (2012) Dynamin, a membrane-remodeling GTPase. Nat. Rev. Mol. Cell Biol. 13, 75–88 Medline

7. Chappie, J. S., Acharya, S., Leonard, M., Schmid, S. L., and Dyda, F. (2010) G domain dimerization controls dynamin’s assembly-stimulated GTPase activity. Nature 465, 435–440 CrossRef Medline

8. Daumke, O., and Praefcke, G. J. K. (2016) Invited review: mechanisms of GTP hydrolysis and conformational transitions in the dynamin superfamily. Biopolymers 105, 580–593 Medline

9. Ford, M. G. J., Jenni, S., and Nunnari, J. (2011) The crystal structure of Atlastin-1. EMBO Rep. 12, 978–983 CrossRef Medline

10. Moss, T. J., Andreazza, C., Verma, A., Daga, A., and McNew, J. A. (2011) Dynamin-like MxA GTPase: structural insights into oligomerization and implications for antiviral activity. J. Biol. Chem. 285, 28419–28424 CrossRef Medline

11. Liu, T. Y., Bian, X., Sun, S., Ku, H. W., Klemm, R. W., Prinz, W. A., Rapoport, T. A., and Hu, J. (2012) Lipid interaction of the C terminus and association of the transmembrane segments facilitate atlastin-mediated homotypic endoplasmic reticulum fusion. Proc. Natl. Acad. Sci. U.S.A. 109, 11133–11138 CrossRef Medline

12. Haller, O., Gao, S., Malsburg von der, A., and Daumke, O. (2010) Dynamin-like MxA GTPase: structural insights into oligomerization and implications for antiviral activity. J. Biol. Chem. 285, 28419–28424 CrossRef Medline

13. Faust, I. E., Desai, T., Verma, A., Ullingen, L., Sun, T.-L., Moss, T. J., Betancourt-Solis, M. A., Huang, H. W., Lee, T., and McNew, J. A. (2015) The atlastin C-terminal tail is an amphipathic helix that perturbs the bilayer structure during endoplasmic reticulum homotypic fusion. J. Biol. Chem. 290, 4772–4783 CrossRef Medline

14. Masson, N., Soderblom, C., Stadler, J., Zhu, P. P., and Blackstone, C. (2008) Atlastin GTPases are required for Golgi apparatus and ER morphogenesis. Hum. Mol. Genet. 17, 1591–1604 CrossRef Medline

15. COURT-SOLS, M. A., Huang, H. W., Lee, T., and McNew, J. A. (2015) The atlastin C-terminal tail is an amphipathic helix that perturbs the bilayer structure during endoplasmic reticulum homotypic fusion. J. Biol. Chem. 290, 4772–4783 CrossRef Medline

16. Rismarchni, N., Soderblom, C., Stadler, J., Zhu, P. P., and Blackstone, C. (2008) Atlastin GTPases are required for Golgi apparatus and ER morphogenesis. Hum. Mol. Genet. 17, 1591–1604 CrossRef Medline

17. Orso, G., Pendin, D., Liu, S., Tosetto, J., Moss, T. J., Faust, I. E., Micaroni, M., Egorova, A., Martinuzzi, A. and McNew, J. A. and Daga, A. (2009) Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. Nature 460, 978–983 CrossRef Medline

18. Liu, J., Shibata, Y., Zhu, P.-P., Voss, C., Rismarchni, N., Prinz, W. A., Rapoport, T. A., and Blackstone, C. (2009) A class of dynamin-like GTPases involved in the generation of the tubular ER network. Cell 138, 549–561 CrossRef Medline

19. Wang, S., Tuchynskiy, H., Romano, F. B., and Rapoport, T. A. (2016) Cooperation of the ER-shaping proteins atlastin, lunapark, and reticu-
Allosteric defect in disease-associated atlastin mutant

lons to generate a tubular membrane network. eLife 5, e18605 CrossRef Medline
30. Zhao, G., Zhu, P.-P., Renvoisé, B., Maldonado-Báez, L., Park, S. H., and Blackstone, C. (2016) Mammalian knock out cells reveal prominent roles for atlastin GTPases in ER network morphology. Exp. Cell Res. 349, 32–44 CrossRef Medline
31. Wang, S., Romano, F. B., Field, C. M., Mitchison, T. J., and Rapoport, T. A. (2013) Multiple mechanisms determine ER network morphology during the cell cycle in Xenopus egg extracts. J. Cell Biol. 203, 801–814 CrossRef Medline
32. Powers, R. E., Wang, S., Liu, T. Y., and Rapoport, T. A. (2017) Reconstitution of the tubular endoplasmic reticulum network with purified components. Nature 543, 257–260 CrossRef Medline
33. Byrnes, L. J., Singh, A., Szeto, K., Benvin, N. M., O’Donnell, J. P., Zipfel, T. A., and Reid, E. (2011) Hereditary spastic paraplegias: membrane traffic and the motor pathway. Nat. Rev. Neurosci. 12, 31–42 Medline
34. Ivanova, N., Claeys, K. G., Deconinck, T., Litvinenko, I., Jordanova, A., Mercelis, R., Plecko, B., Priller, J., Zámecník, J., Ceulemans, B., et al. (2007) Hereditary spastic paraplegia 3A associated with axonal neuropathy. Arch. Neurol. 64, 706–713 CrossRef Medline
35. Namekawa, M., Muriel, M.-P., Janer, A., Latouche, M., Dauphin, A., Debeir, T., Martin, E., Duyckaerts, C., Prigent, A., Delpierre, C., Sittler, A., Brice, A., and Ruberg, M. (2007) Mutations in the SPG3A gene encoding the GTPase atlastin interfere with vesicle trafficking in the ER/Golgi interface and Golgi morphogenesis. Mol. Cell. Neurosci. 35, 1–13 CrossRef Medline
36. Khan, T. N., Klar, J., Tariq, M., Anjum Baig, S., Malik, N. A., Youssaf, R., Baig, S. M., and Dahl, N. (2014) Evidence for autosomal recessive inherit-

ance in SPG3A caused by homozygosity for a novel ATL1 missense mutation. Eur. J. Hum. Genet. 22, 1180–1184 CrossRef Medline
37. Wittinghofer, A., and Vetter, I. R. (2011) Structure-function relationships of the G domain, a canonical switch motif. Annu. Rev. Biochem. 80, 943–971 CrossRef Medline
38. Zhao, X., Alvarado, D., Rainier, S., Lemons, R., Hedera, P., Weber, C. H., Tukel, T., Apak, M., Heiman-Patterson, T., Ming, L., Bui, M., and Fink, J. K. (2001) Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. Nat. Genet. 29, 326–331 CrossRef Medline
39. Säini, S. G., Liu, C., and Lee, T. H. (2014) Membrane tethering by the atlastin GTPase depends on GTP hydrolysis but not on forming the cross-over configuration. Mol. Biol. Cell. 25, 3942–3953 CrossRef Medline
40. Winsor, J., Hackney, D. D., and Lee, T. H. (2017) The crossover conformational shift of the GTPase atlastin provides the energy driving ER fusion. J. Cell Biol. 216, 1321–1335 CrossRef Medline
41. Liu, T. Y., Bian, X., Liu, X., Xiu, X., and Hu, J. (2015) Cis and trans interactions between atlastin molecules during membrane fusion. Proc. Natl. Acad. Sci. U.S.A. 112, E1851–E1860 CrossRef Medline
42. O’Donnell, J. P., Cooley, R. B., Kelly, C. M., Andersen, O. S., and Daga, A. (2011) GTP-dependent packing of a three-helix bundle is required for atlastin-mediated fusion. Proc. Natl. Acad. Sci. U.S.A. 108, 16283–16288 CrossRef Medline
43. Liu, T. Y., Bian, X., Romano, F. B., Shemesh, T., Rapoport, T. A., and Hu, J. (2015) Cis and trans interactions between atlastin molecules during membrane fusion. Proc. Natl. Acad. Sci. U.S.A. 112, E1851–E1860 CrossRef Medline
44. Byrnes, L. J., Singh, A., Szeto, K., Benvin, N. M., O’Donnell, J. P., Zipfel, T. A., and Reid, E. (2011) Hereditary spastic paraplegias: membrane traffic and the motor pathway. Nat. Rev. Neurosci. 12, 31–42 Medline
Allosteric defect in disease-associated atlastin mutant

ing protein 1 (hGBP1). Methods Enzymol. 404, 512–527 CrossRef Medline

56. Kabsch, W. (2010) XDS. Acta Crystallogr. D Biol. Crystallogr. 66, 125–132 CrossRef Medline

57. Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G. W., McCoy, A., McNicholas, S. J., Murshudov, G. N., Pannu, N. S., Potterton, E. A., Powell, H. R., et al. (2011) Overview of the CCP4 suite and current developments. Acta Crystallogr. D Biol. Crystallogr. 67, 235–242 CrossRef Medline

58. Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. J., Richardson, D. C., et al. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221 CrossRef Medline

59. Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132 CrossRef Medline

60. Karplus, P. A., and Diederichs, K. (2012) Linking crystallographic model and data quality. Science 336, 1030–1033 CrossRef Medline

61. Morin, A., Eisenbraun, B., Key, J., Sanschagrin, P. C., Timony, M. A., Ottaviano, M., and Sliz, P. (2013) Collaboration gets the most out of software. eLife 2, e01456 Medline

62. Lo, M.-C., Aulabaugh, A., Jin, G., Cowling, R., Bard, J., Malamas, M., and Ellestad, G. (2004) Evaluation of fluorescence-based thermal shift assays for hit identification in drug discovery. Anal. Biochem. 332, 153–159 CrossRef Medline