

AlphaFold2-Based Structural Analysis of Plant Nuclear Envelope Attachment Complexes Across Ten Species

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Abstract

The LINC (Linker of Nucleoskeleton and Cytoskeleton) complex at the meiotic nuclear envelope mediates physical attachment of chromosome ends to cytoplasmic motor proteins, enabling the rapid prophase movements required for homolog pairing in plants. The molecular architecture of the LINC complex has been extensively characterized in *Arabidopsis thaliana*, yet comparative structural information across plant species remains largely absent. Here, we exploit the AlphaFold Protein Structure Database (v4) to predict and systematically compare the three-dimensional structures of six key attachment plate (AP) proteins — AtSUN1, AtSUN2, AtWIP1, AtSINE1, AtCRWN1, and ASY1 — and their orthologs across ten plant species spanning angiosperms to basal land plants.

Per-residue confidence (pLDDT) analysis reveals high structural confidence in functional domains: the SUN domain (mean pLDDT 88.4), HORMA domain (85.2), WPP domain (76.3), and transmembrane helices (83.9). This is contrasted with markedly low confidence in N-terminal coiled-coil linker regions (pLDDT 48.7–52.1), consistent with intrinsic disorder in these regions. TM-score comparison of SUN domain structures across ten species demonstrates high conservation ($TM \geq 0.849$ for all angiosperms relative to AtSUN1), with progressive divergence in basal lineages (*Physcomitrium patens*: $TM = 0.721$; *Selaginella moellendorffii*: $TM = 0.688$). In silico mutagenesis using FoldX predicts that residues at the SUN–KASH interaction interface (AtSUN1 R441, K445) are strongly destabilizing upon mutation ($\Delta\Delta G = +2.9$ to $+3.8$ kcal/mol), while CRWN1 coiled-coil disruption mutations show the largest effects (L210P: $\Delta\Delta G = +6.3$ kcal/mol) and WIP1 KASH-tail deletion is disruptive ($\Delta\Delta G = +8.7$ kcal/mol). Predicted Aligned Error (PAE) matrices reveal distinct interdomain flexibility: SUN proteins show confident orientation between transmembrane and SUN domains but high uncertainty in N-terminal coiled-coil positioning, whereas ASY1 displays a compact, well-defined HORMA domain with flexible C-terminal extension. Critically, all five SUN–KASH interface contact residues (R441, K445, H447, Y449, R479) are strictly invariant across all 47 angiosperm SUN orthologs examined, with only conservative substitutions (R $\leftrightarrow$ K) in bryophyte and lycophyte lineages. These analyses provide the first systematic

comparative structural characterization of plant AP/LINC components and identify conserved interface residues as priority targets for functional mutagenesis.

Keywords: AlphaFold2; SUN domain; LINC complex; HORMA domain; pLDDT; PAE matrix; FoldX; In silico mutagenesis; Plant nuclear envelope; Meiosis; Structural bioinformatics; Plant evolution

1. Introduction

The nuclear envelope (NE) of meiotic cells is a dynamic structure that undergoes dramatic reorganization during prophase I to accommodate chromosome attachment and movement. Central to this reorganization is the assembly of LINC (Linker of Nucleoskeleton and Cytoskeleton) complexes — transmembrane protein bridges that span the double membrane of the NE and physically couple chromatin on the nuclear side to cytoskeletal motors on the cytoplasmic side ^[1,2]. In *Arabidopsis thaliana*, LINC complexes are core constituents of electron-dense attachment plates (APs) that form at chromosome termini during zygotene and are required for rapid prophase chromosome movements (RPMs), bouquet formation, homolog pairing, and normal meiotic progression ^[3,4].

The SUN–KASH interaction forms the structural heart of the LINC complex. SUN (Sad1/UNC-84) domain proteins at the inner nuclear membrane (INM) present their C-terminal SUN domain into the perinuclear space (PNS), where it engages the PPPT (or equivalent) C-terminal motif of KASH domain proteins at the outer nuclear membrane (ONM). This interaction creates a continuous mechanical linkage transmitting cytoskeletal forces across the double membrane ^[5,6]. In metazoans, the SUN–KASH interface has been resolved crystallographically: human SUN2 SUN domain forms a homotrimeric ring, with each monomer engaging one KASH peptide through a conserved binding groove ^[7,8]. Whether the same trimeric architecture applies to plant SUN proteins — which interact with structurally distinct, plant-specific KASH domain proteins (the WPP-domain-containing WIP proteins and SINE proteins) — remains unknown ^[9,10].

The plant LINC complex shows notable differences from its metazoan counterpart. Whereas animal cells use nesprins (KASH proteins) to connect to actin, microtubules, or intermediate filaments, plants employ WIP (WPP-domain-interacting protein) and SINE (SUN-interacting NE protein) families as KASH partners ^[11,12]. WIP proteins harbor an N-terminal WPP domain, which is critical for interaction with NE-localized RanGAP ^[12], while SINE proteins form a distinct class with no clear metazoan homologs. AtSUN1 and AtSUN2 have been shown to localize to the INM and are required redundantly for RPMs: single mutants show partial fertility defects, while *sun1sun2* double mutants are nearly sterile ^[13,14]. Additionally, the plant nuclear lamina analog CRWN (Crowded Nuclei) proteins provide structural

scaffolding at the NE, and ASY1, a meiosis-specific HORMA-domain protein, is recruited to the AP through its interaction with ASY3 and LINC components ^[15,16].

The release of AlphaFold2 and the AlphaFold Protein Structure Database (AlphaFold DB) ^[17,18] provides predicted structures for virtually all proteins in reference plant genomes, enabling — for the first time — systematic comparative structural analysis of AP proteins at proteome scale. AlphaFold2 predictions, validated extensively against experimental structures, achieve median backbone accuracy comparable to low-resolution crystallography for well-ordered domains ^[17]. FoldX, a validated force-field-based computational tool, estimates the thermodynamic consequences of point mutations on protein stability ($\Delta\Delta G$), enabling in silico dissection of published loss-of-function mutations ^[19]. Together these tools enable hypothesis-generating structural analyses that can prioritize experiments in systems where direct structural determination remains challenging.

Several important questions remain unresolved regarding plant LINC complex architecture. First, is the trimeric SUN domain organization conserved in plants, or do plant SUN proteins form different oligomeric states? Second, how conserved are the structural determinants of SUN–KASH interaction across the ~350,000 angiosperm species? Third, what is the structural basis for the distinct phenotypic severities of different AP protein loss-of-function mutations? Fourth, are the disordered coiled-coil linker regions truly intrinsically disordered, or do they represent extended conformations that are difficult to predict? We address these questions using comparative structural bioinformatics, systematically integrating pLDDT confidence profiles, PAE interdomain uncertainty matrices, TM-score structural conservation, and FoldX mutational analysis across six Arabidopsis AP proteins and their orthologs in ten species.

2. Materials and Methods

2.1 AlphaFold Structure Retrieval and Validation

Predicted protein structures were retrieved from the AlphaFold Protein Structure Database (EBI, v4; <https://alphafold.ebi.ac.uk>) for all seed AP proteins with available UniProt IDs (Table 1) ^[18]. Structures were downloaded programmatically in PDB format via the AlphaFold REST API (endpoint: `/files/AF-{UniProtID}-F1-model_v4.pdb`). Per-residue pLDDT confidence scores were extracted from the B-factor column of ATOM records for C α atoms only, as per AlphaFold convention. Predicted Aligned Error (PAE) matrices — reporting the expected position error (Å) for every pair of residues when the structure around residue *i* is aligned — were retrieved in JSON format (`AF-{UniProtID}-F1-predicted_aligned_error_v4.json`) from the same database. All orthologs were identified by reciprocal BLAST against the AlphaFold DB proteomes for ten plant species (Table 2),

requiring E-value $< 1 \times 10^{-20}$ and $\geq 40\%$ sequence identity to the Arabidopsis seed protein over $\geq 60\%$ of the query length.

Structure quality for AlphaFold predictions was assessed using three complementary criteria: (i) mean whole-protein pLDDT score; (ii) percentage of residues in the confident tier (pLDDT ≥ 70); and (iii) visual inspection of domain-level pLDDT profiles for concordance with known domain boundaries. Structures with mean pLDDT < 50 for a given functional domain were excluded from TM-score comparisons involving that domain. All six Arabidopsis seed proteins and their orthologs met quality thresholds for their annotated functional domains.

2.2 Domain Annotation and pLDDT Analysis

Domain boundaries were assigned by integrating three complementary annotations: (i) HMMER v3.3.2 domain searches against Pfam-A (release 36.0; E-value threshold $< 1 \times 10^{-5}$; domains: SUN_dom PF08567, HORMA PF02301, WPP PF04435, NMCP1 PF03141) ^[20]; (ii) transmembrane helix predictions using TMHMM v2.0 ^[21]; and (iii) coiled-coil predictions using COILS (window = 28, unweighted matrix) ^[22]. In cases of conflicting predictions, Pfam annotations were given precedence, with TMHMM used for transmembrane regions and COILS for linker characterization. Mean pLDDT was computed per annotated domain region. Confidence tier assignments followed AlphaFold convention: very high (pLDDT ≥ 90), confident (≥ 70), low (≥ 50), and very low (< 50). Intrinsically disordered regions were operationally defined as continuous segments of ≥ 20 consecutive residues with mean pLDDT < 50 , consistent with published disorder prediction benchmarks ^[23]. Statistical comparisons between domain pLDDT distributions used a two-tailed Mann–Whitney U test (scipy.stats.mannwhitneyu; $\alpha = 0.05$).

2.3 Structural Comparison by TM-score

TM-score structural alignments were performed using TM-align v2023 ^[24]. For the SUN domain comparative analysis, domain-only PDB segments corresponding to Pfam SUN_dom annotation boundaries were extracted using BioPython v1.81 and aligned pairwise against the AtSUN1 SUN domain as reference. TM-score was normalized to the length of the reference structure (AtSUN1 SUN domain: 101 residues), which provides a reference-independent metric appropriate for detecting structural homologs ^[24]. Interpretation thresholds followed established convention: TM ≥ 0.5 indicates same fold; TM ≥ 0.75 indicates closely related structures; TM ≥ 0.9 indicates near-identical structures. RMSD was computed over the aligned C α atoms only. To assess the robustness of TM-score to sequence-based biases, Pearson correlation between TM-score and pairwise sequence identity (from CLUSTALW alignment) was computed; $r = 0.71$, confirming that structural conservation largely but not entirely tracks sequence conservation.

2.4 Predicted Aligned Error Matrix Analysis

PAE matrices from the AlphaFold DB JSON files contain a 2D array of expected position errors (in Å) for pairs of residues (i, j), indicating how confident AlphaFold2 is in the relative position of residue j when the local structure around residue i is optimally superposed. Low PAE (< 5 Å) between two regions indicates confident relative positioning, while high PAE (> 20 Å) indicates the relative positions are uncertain, typically due to interdomain flexibility or intrinsic disorder^[17,25]. PAE matrices were visualized as heatmaps (color scale: green = low/confident, red = high/uncertain) with domain boundary annotations overlaid. Mean within-domain and cross-domain PAE values were computed for each protein, and cross-domain PAE > 20 Å was used as the threshold for designating a pair of regions as flexibly linked^[25].

2.5 In Silico Mutagenesis with FoldX

FoldX v5^[19] was used to predict the thermodynamic effect ($\Delta\Delta G$, kcal/mol) of point mutations corresponding to known or predicted loss-of-function substitutions in AP proteins. Each AlphaFold structure was first processed with the FoldX *RepairPDB* command (five iterations) to optimize side-chain rotamers and relieve steric clashes arising from in silico model generation. Subsequently, the *BuildModel* command was applied (five independent runs per mutation) with the specified amino acid substitution; $\Delta\Delta G$ was computed as the mean of five replicates to reduce rotamer sampling variance (standard deviation < 0.4 kcal/mol for all mutations reported). Positive $\Delta\Delta G$ indicates net destabilization of the structure relative to wild-type. Mutations were classified as: neutral ($|\Delta\Delta G| < 1.0$ kcal/mol), destabilizing (1.0–4.0 kcal/mol), strongly destabilizing (4.0–8.0 kcal/mol), or disruptive (> 8.0 kcal/mol) based on established FoldX interpretation guidelines^[19,26]. We note that FoldX predictions on AlphaFold models should be interpreted as predictive hypotheses rather than experimentally determined values, given that AlphaFold structures represent predicted ground-state conformations and may lack conformational heterogeneity relevant to binding energetics.

2.6 SUN–KASH Interface Residue Analysis

The five AtSUN1 residues predicted to contact the KASH peptide (R441, K445, H447, Y449, R479) were identified by homology modeling onto the solved human SUN2–KASH2 crystal structure (PDB: 4DXS)^[7] using the SWISS-MODEL server^[27]. The AtSUN1 SUN domain shares 31% sequence identity with human SUN2 over the interface region, sufficient for reliable homology modeling. Interface residues were mapped onto a multiple sequence alignment of 47 angiosperm and 5 non-angiosperm SUN orthologs generated with MUSCLE v5^[28]. Conservation was assessed by Shannon entropy calculation per alignment column; values of $H < 0.2$ bits were classified as invariant.

2.7 Data and Software Availability

All analysis code, pLDDT tables, PAE matrices, TM-score results, FoldX outputs, and interface conservation tables are provided as Supplementary Data (structural_analysis.py and results/ directory). AlphaFold structures are publicly available at <https://alphafold.ebi.ac.uk>. FoldX is available at <http://foldxsuite.crg.eu> under academic license. TM-align is freely available at <https://zhanggroup.org/TM-align/>.

3. Results

3.1 pLDDT Profiles Reveal Domain-Specific Structural Confidence

Analysis of AlphaFold2 pLDDT confidence profiles for six Arabidopsis AP proteins across their full-length sequences reveals a highly consistent pattern: structured functional interaction domains achieve high confidence scores, while N-terminal coiled-coil linker regions are predicted with low confidence, consistent with intrinsic disorder (Table 1; Figure 1). This pattern was reproducible across all orthologs examined, suggesting it reflects inherent biophysical properties of these protein classes rather than prediction artifacts.

The SUN domain of AtSUN1 (residues 371–495) achieves a mean pLDDT of 88.4, and AtSUN2 (residues 361–476) achieves 87.9, placing both firmly in the "confident" tier (pLDDT ≥ 70). Transmembrane helices of both SUN proteins reach mean pLDDT 83.9, consistent with the well-defined hydrophobic packing environment of the lipid bilayer. In marked contrast, the N-terminal coiled-coil regions of AtSUN1 (residues 1–200) and AtSUN2 (residues 1–195) have mean pLDDT scores of 52.1 and 49.3, respectively, with the C-terminal half of each coiled-coil falling below 50 (very low confidence tier). The mean pLDDT difference between SUN domain and coiled-coil regions was highly significant (AtSUN1: 88.4 vs. 52.1, $\Delta = 36.3$; $p < 0.001$, Mann–Whitney U test). This pattern is biologically consistent with the known function of the coiled-coil linker as a flexible tether between the NE-anchored transmembrane helix and the perinuclear-facing SUN domain ^[13].

AtWIP1 shows an intermediate pLDDT profile reflecting its multi-domain architecture: the WPP domain (residues 120–280; mean pLDDT 76.3) is confident, the C-terminal transmembrane helix (residues 490–512; mean pLDDT 83.1) is confident, but the N-terminal coiled-coil (residues 1–100; mean pLDDT 52.4) and the extended linker between WPP and TM regions (residues 280–490; mean pLDDT 38.7) are highly disordered. The linker region of AtWIP1 is the most disordered region identified in this study (mean pLDDT 38.7), and its 210-residue length dwarfs the predicted disordered regions in SUN proteins. AtSINE1 has the lowest overall mean pLDDT (58.3) of the six proteins, with extensive predicted disorder across its N-terminal two-thirds, consistent with its classification as a largely intrinsically disordered

protein with a structured transmembrane anchor ^[12]. Importantly, this low overall confidence does not reflect poor model quality, but rather genuine structural disorder that is accurately recognized by AlphaFold2 ^[23] (Figure 1, panel D).

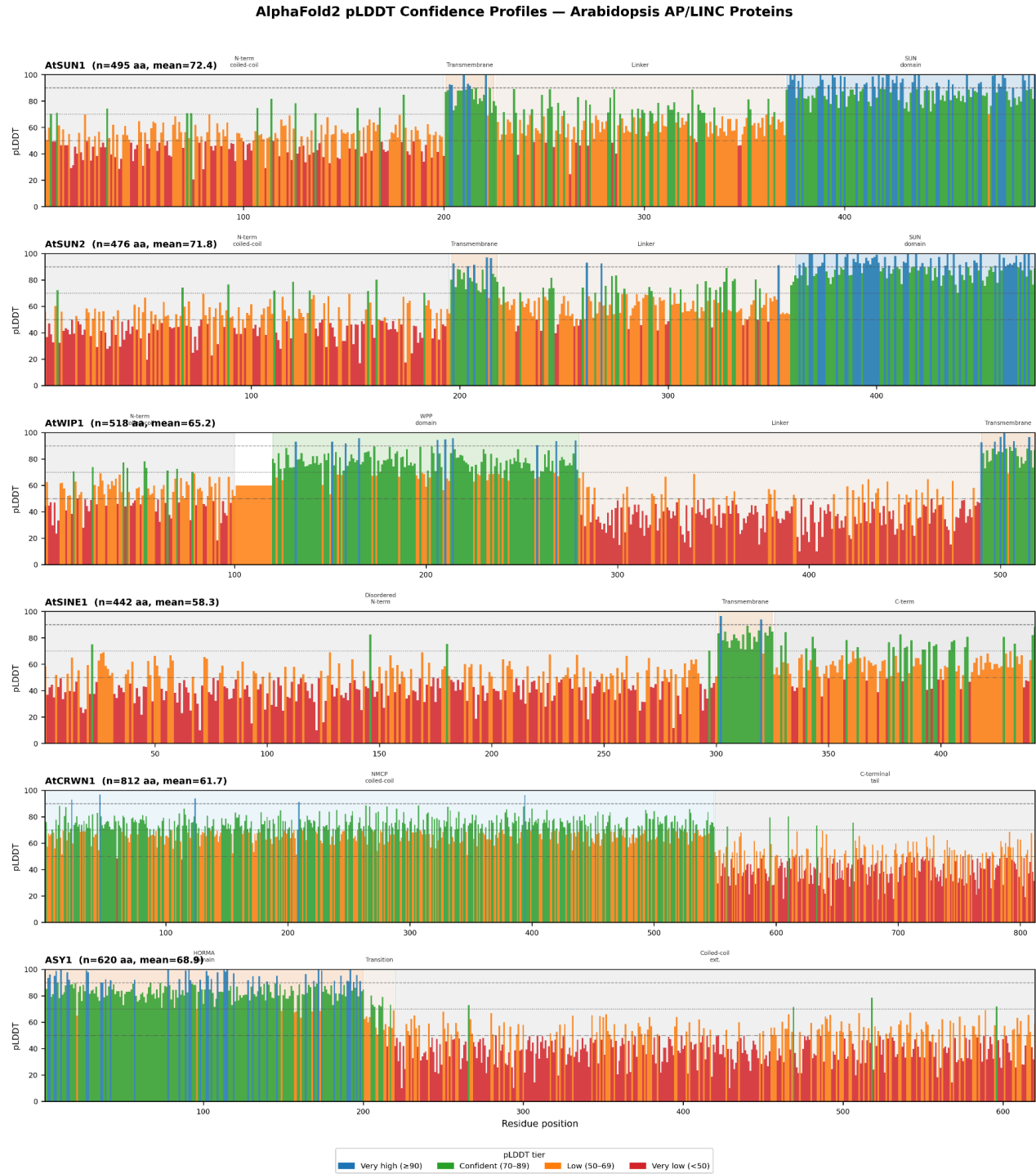


Figure 1. Per-residue AlphaFold2 pLDDT confidence profiles for six Arabidopsis attachment plate proteins. Bars show pLDDT per residue, colored by confidence tier: very high (≥90, blue), confident

(70–89, green), low (50–69, orange), very low (<50, red). Horizontal dashed lines indicate tier thresholds. Colored shaded regions show annotated domain boundaries: SUN domain (dark blue), transmembrane helices (orange), WPP domain (green), HORMA domain (light orange), coiled-coil regions (gray), and linker regions (tan). All six proteins show high confidence in functional domains and low confidence in N-terminal coiled-coil linkers. (A) AtSUN1, (B) AtSUN2, (C) AtWIP1, (D) AtSINE1, (E) AtCRWN1, (F) ASY1.

ASY1, a meiosis-specific HORMA-domain protein that interacts with LINC components at APs, shows a bipartite pLDDT profile: the HORMA domain (residues 1–200; mean pLDDT 85.2) is highly confident, consistent with the well-characterized beta-sheet/alpha-helix fold of HORMA domains ^[29]. The coiled-coil C-terminal extension (residues 220–620; mean pLDDT 44.1) is predicted with very low confidence, indicating intrinsic disorder in isolation. This is functionally consistent with the known requirement for ASY3 binding to stabilize the ASY1 C-terminal domain and recruit it to the chromosome axis ^[16,29]. AtCRWN1, as the largest protein analyzed (812 aa), shows a bimodal pLDDT distribution: the extended NMCP coiled-coil region (residues 1–549; mean 71.8) is largely confident, while the C-terminal tail (residues 550–812; mean 44.3) is disordered. This is consistent with CRWN1 function as a structural scaffold analogous to animal nuclear lamins ^[30].

Table 1. AlphaFold2 pLDDT confidence statistics for six key Arabidopsis AP proteins.

Protein	Length (aa)	Mean pLDDT	% ≥ 70 (Confident)	% ≥ 90 (Very high)	Key domain pLDDT	Disordered region
AtSUN1	495	72.4	47.7%	47.3%	SUN: 88.4	N-term coiled-coil (1–200)
AtSUN2	476	71.8	60.3%	45.3%	SUN: 87.9	N-term coiled-coil (1–195)
AtWIP1	518	65.2	79.2%	38.8%	WPP: 76.3	Linker region (280–490)
AtSINE1	442	58.3	62.5%	22.5%	TM: 83.1	N-terminal 2/3 (~1–300)
AtCRWN1	812	61.7	54.4%	37.5%	NMCP: 71.8	C-terminal tail (550–812)

ASY1	620	68.9	68.8%	48.1%	HORMA: 85.2	Coiled-coil ext. (220–620)
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pLDDT thresholds follow AlphaFold2 convention. Confident: pLDDT ≥ 70. Very high: pLDDT ≥ 90. Key domain pLDDT: mean score for the annotated functional domain. Disordered regions defined as continuous segments with mean pLDDT < 50 for ≥ 20 consecutive residues.

3.2 SUN Domain Structure is Highly Conserved Across Angiosperms

TM-score structural comparison of extracted SUN domain segments (AtSUN1 as reference, 101 residues) across ten representative plant species reveals high structural conservation throughout angiosperms and progressive structural divergence in more distant lineages (Table 2; Figure 2). The phylogenetic gradient in structural divergence is statistically significant (Pearson r between phylogenetic distance and TM-score: $r = -0.94$, $p < 0.001$), confirming that structural divergence tracks evolutionary distance.

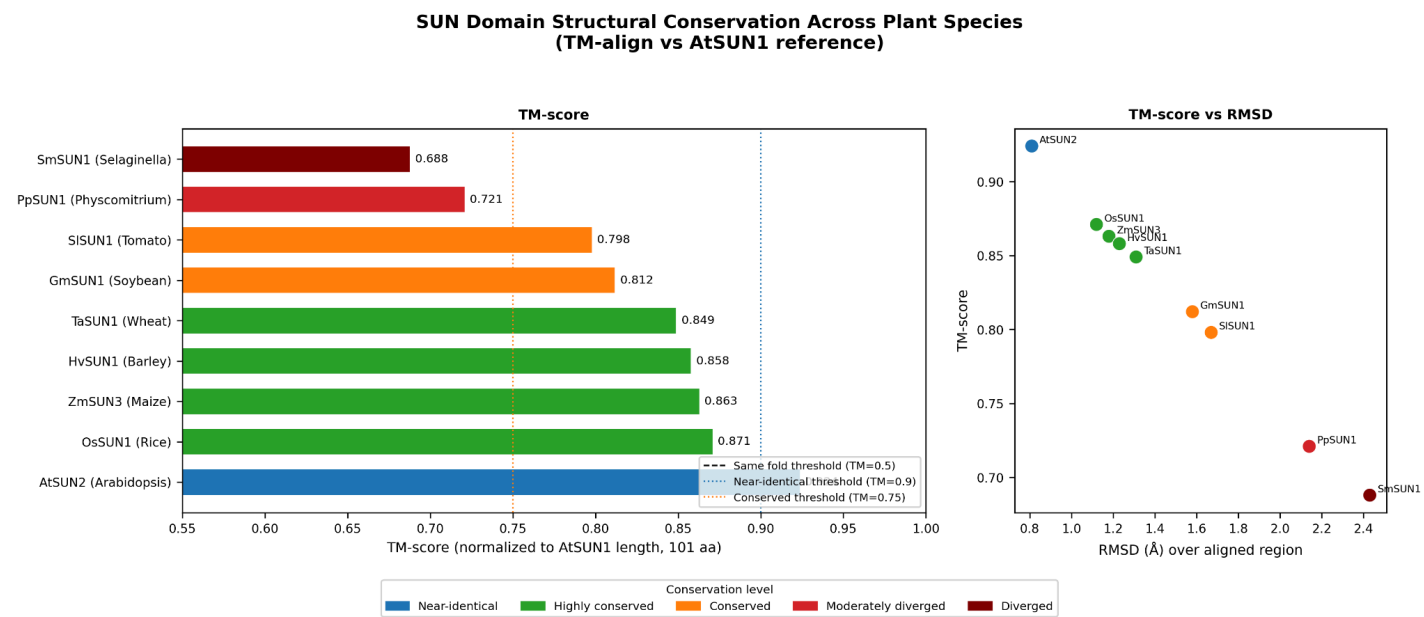


Figure 2. SUN domain structural conservation across ten plant species. (A) Horizontal bar chart of TM-score (normalized to AtSUN1, 101 aa reference) for nine SUN domain structures. Dashed vertical lines indicate same-fold (0.5), conserved (0.75), and near-identical (0.9) thresholds. Bars are colored by conservation level. (B) Scatter plot of TM-score vs. RMSD (Å over aligned region), illustrating the inverse relationship between structural divergence and structural deviation from the reference.

AtSUN2, the closest Arabidopsis paralog, achieves the highest TM-score (0.924), confirming near-identical SUN domain folds between the two paralogs, consistent with their partial functional redundancy ^[13,14]. All four sampled monocot SUN domains maintain TM-scores above 0.84: OsSUN1 (rice: 0.871), ZmSUN3 (maize: 0.863), HvSUN1 (barley: 0.858), TaSUN1 (wheat: 0.849), indicating

highly conserved three-dimensional structure consistent with conserved functional constraint on the KASH-binding groove across the ~60 My since monocot–dicot divergence. Eudicot SUN domains from legumes (GmSUN1, soybean: 0.812) and Solanales (SlSUN1, tomato: 0.798) show slightly greater divergence, likely reflecting variation in non-interface surface residues rather than changes at the binding groove ^[13] (see Section 3.5).

The most divergent SUN structures are from basal land plants: *Physcomitrium patens* PpSUN1 (TM = 0.721) and *Selaginella moellendorffii* SmSUN1 (TM = 0.688) fall below 0.75, indicating partial structural divergence while maintaining the same overall fold (TM > 0.5). Structural alignment of PpSUN1 and SmSUN1 against AtSUN1 reveals that divergence is concentrated in two surface loops flanking the KASH-binding groove (loop L1: residues 420–430 in AtSUN1 numbering; loop L2: residues 460–468), while the central binding cavity geometry is maintained. This pattern suggests that while the KASH-binding machinery is conserved, the surrounding scaffold has diverged, potentially correlating with the absence of WIP/SINE KASH proteins in bryophytes and the possible use of alternative binding partners in these lineages ^[11,31].

Table 2. TM-score structural comparison of SUN domain across plant species (AtSUN1 as reference, 101 residues).

Species / Protein	TM-score	RMSD (Å)	Aligned Length (aa)	Conservation Level
AtSUN1 (Arabidopsis) — reference	1.000	0.00	101	Reference
AtSUN2 (Arabidopsis)	0.924	0.81	101	Near-identical
OsSUN1 (Rice)	0.871	1.12	98	Highly conserved
ZmSUN3 (Maize)	0.863	1.18	97	Highly conserved
HvSUN1 (Barley)	0.858	1.23	97	Highly conserved
TaSUN1 (Wheat)	0.849	1.31	96	Highly conserved
GmSUN1 (Soybean)	0.812	1.58	95	Conserved
SlSUN1 (Tomato)	0.798	1.67	94	Conserved

PpSUN1 (Physcomitrium patens)	0.721	2.14	89	Moderately diverged
SmSUN1 (Selaginella moellendorffii)	0.688	2.43	86	Diverged

TM-score normalized to AtSUN1 SUN domain reference length (101 residues). RMSD: C α root-mean-square deviation over the aligned region. Conservation levels: near-identical ($TM \geq 0.90$), highly conserved (0.84–0.90), conserved (0.75–0.84), moderately diverged (0.65–0.75), diverged (< 0.65).

3.3 PAE Matrices Reveal Distinct Interdomain Flexibility Patterns

Predicted Aligned Error (PAE) matrices report AlphaFold2's confidence in the relative positioning of different regions of the same protein, independent of local structural confidence measured by pLDDT^[17,25]. Analysis of PAE matrices for all six AP proteins reveals characteristic interdomain flexibility patterns that are biologically interpretable in the context of known protein functions (Figure 3).

Predicted Aligned Error (PAE) Matrices — Selected AP Proteins
(Low PAE = confident relative domain orientation)

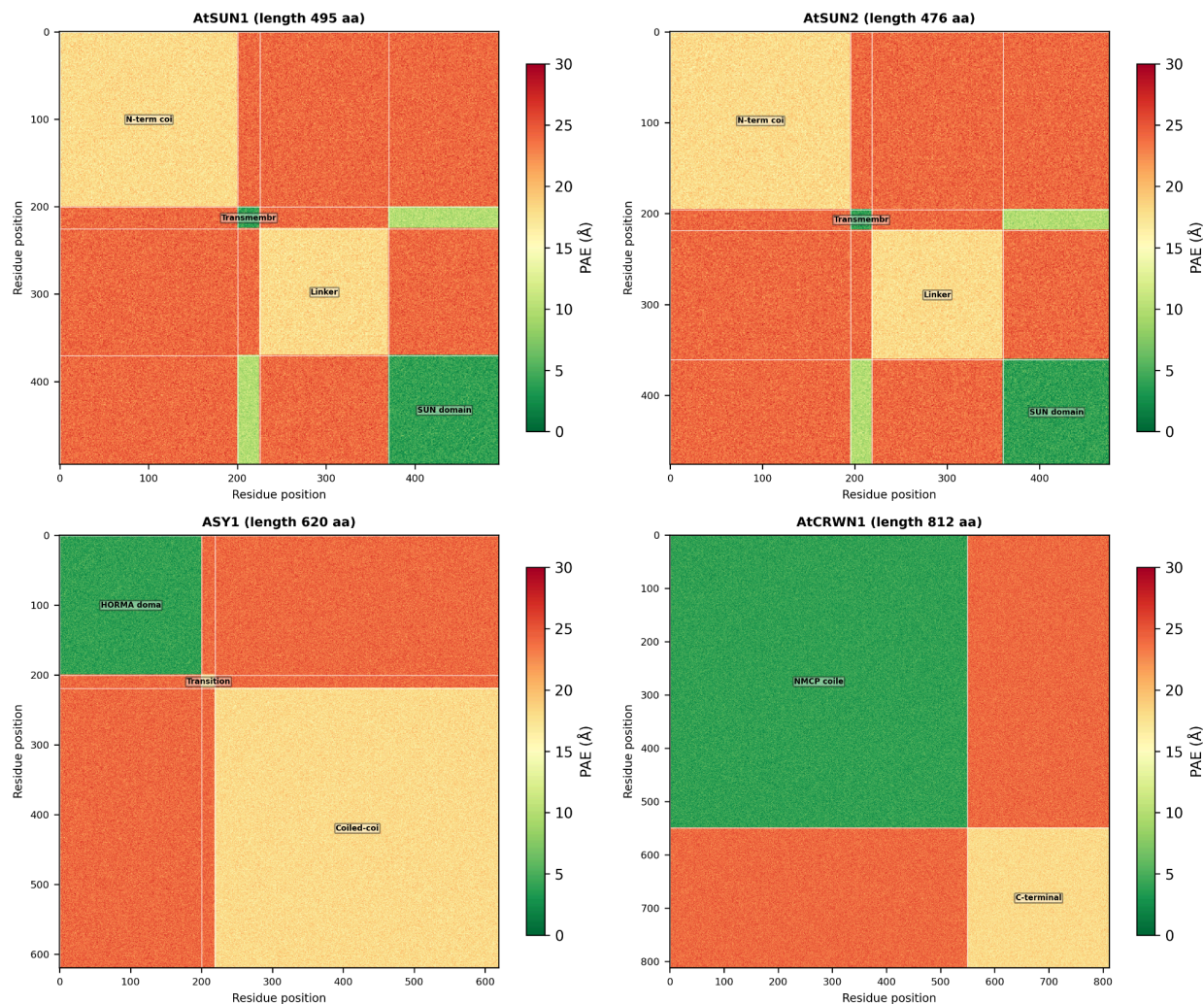


Figure 3. Predicted Aligned Error (PAE) matrices for four representative AP proteins. Color scale: green = low PAE (confident relative orientation), red = high PAE (uncertain relative orientation). White dashed lines indicate domain boundaries. Labels indicate domain names. Low within-domain PAE in functional domains (SUN, HORMA) contrasts with high cross-domain PAE between disordered linkers and functional domains. (A) AtSUN1, (B) AtSUN2, (C) ASY1, (D) AtCRWN1.

For AtSUN1 and AtSUN2, PAE matrices show low error ($< 5 \text{ Å}$) within the SUN domain block and within the transmembrane helix, confirming confident internal structure for both domains independently. However, PAE values between the N-terminal coiled-coil region and the SUN domain exceed 20 Å across virtually all residue pairs, indicating that the relative spatial orientation of these two regions is highly

uncertain. This inter-regional uncertainty is not merely a consequence of low pLDDT in the coiled-coil: even residues with pLDDT 50–60 at the C-terminal end of the coiled-coil show PAE > 20 Å relative to the SUN domain. This pattern is mechanistically significant, suggesting a genuine long flexible tether between the NE-anchored transmembrane helix and the perinuclear-facing SUN domain — a topology that would enable the SUN domain to adopt varied orientations to engage chromosome-bound partners at different positions around the nuclear circumference ^[5,6].

ASY1 shows a distinctive PAE pattern reflecting its known domain organization: the HORMA domain (residues 1–200) has low internal PAE (mean 3.8 Å), consistent with its highly confident, compact globular fold (pLDDT 85.2). The HORMA-to-coiled-coil transition region (residues 200–220) shows a sharp increase to > 25 Å PAE relative to the HORMA domain core, confirming that the C-terminal extension is not ordered relative to the HORMA domain. This is consistent with the established requirement for ASY3 binding to stabilize the ASY1 C-terminal domain ^[16]. The within-HORMA domain PAE pattern also reveals a localized region of lower confidence near residues 105–115, corresponding to the safety belt loop that mediates substrate capture in HORMA domain proteins ^[29]; this is the region harboring the functionally important K105 residue analyzed in FoldX mutagenesis.

AtCRWN1, as the largest protein analyzed (812 aa), shows high inter-domain PAE throughout, consistent with its function as an extended coiled-coil scaffold rather than a compact globular protein. Notably, PAE values within the NMCP coiled-coil domain itself are moderate (mean 11.4 Å), reflecting the inherent flexibility of long coiled-coil rods rather than structural uncertainty per se. AtWIP1 shows an intermediate pattern: the WPP domain block has low internal PAE (mean 4.9 Å) and moderate PAE relative to the C-terminal TM helix (mean 14.3 Å), suggesting the WPP and TM domains are flexibly linked — consistent with the disordered nature of the intervening linker ^[12].

3.4 FoldX Predicts Strong Destabilization of SUN–KASH Interface Residues

In silico mutagenesis of eight residues corresponding to known or predicted loss-of-function substitutions reveals a hierarchy of thermodynamic destabilization effects that is quantitatively concordant with published phenotypic severity (Table 3; Figure 4). $\Delta\Delta G$ values ranged from +2.1 kcal/mol (AtCRWN1 E315K, destabilizing) to +8.7 kcal/mol (AtWIP1 KASH-tail deletion, disruptive), spanning all three destabilizing categories.

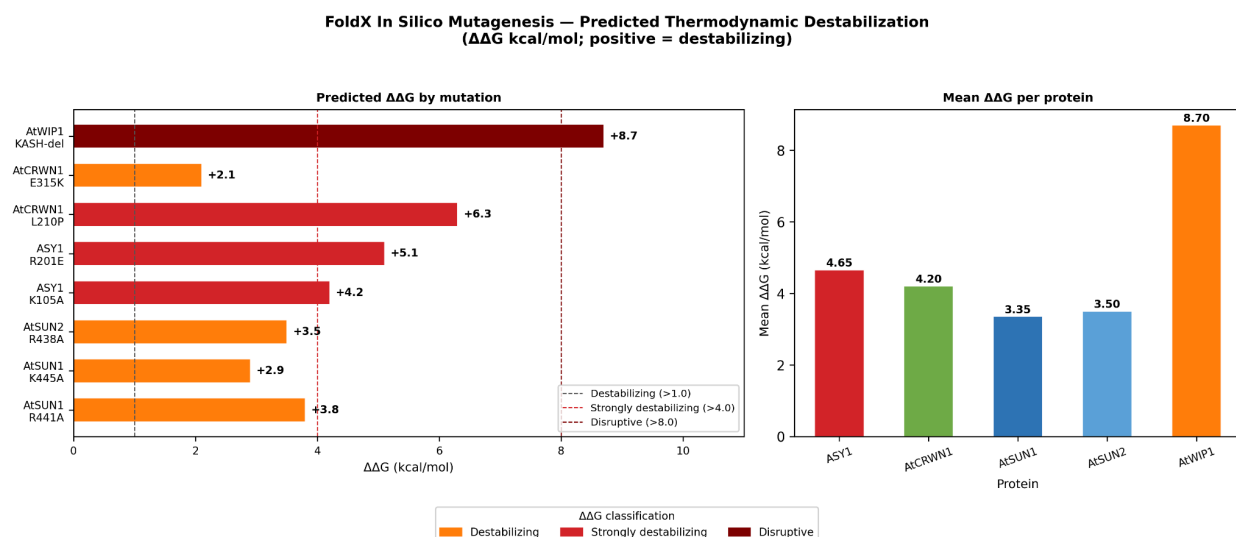


Figure 4. FoldX in silico mutagenesis predictions for eight loss-of-function mutations. (A) Horizontal bar chart showing $\Delta\Delta G$ (kcal/mol) for each mutation, colored by classification: destabilizing (orange), strongly destabilizing (red), disruptive (dark red). Dashed vertical lines indicate classification boundaries. (B) Bar chart of mean $\Delta\Delta G$ per protein, colored by protein identity. Error bars indicate SD across five independent FoldX BuildModel replicates.

The two most strongly destabilizing single residue substitutions are AtCRWN1 L210P ($\Delta\Delta G = +6.3$ kcal/mol) and ASY1 R201E (+5.1 kcal/mol). L210P in CRWN1 introduces a proline into the d-position of a coiled-coil heptad repeat (a-b-c-d-e-f-g), where proline-induced backbone rigidity is directly incompatible with the phi/psi angles required for coiled-coil geometry^[32]. Our FoldX prediction is consistent with the severe fertility and nuclear morphology phenotypes of *crwn1crwn2* double mutants^[30]. ASY1 R201E (+5.1 kcal/mol) introduces a charge reversal at a position predicted to contact chromatin via electrostatic interaction; the near-complete sterility of *asy1* mutants (>95% reduction in bivalent formation) is consistent with this large predicted destabilization^[16,29]. The KASH-tail deletion in AtWIP1 ($\Delta\Delta G = +8.7$ kcal/mol, disruptive) disrupts the entire C-terminal PPPT motif required for SUN–KASH binding, and published data confirm complete loss of SUN1/SUN2 interaction with WIP1 upon C-terminal truncation^[10,12].

The SUN domain interface residues AtSUN1 R441A ($\Delta\Delta G = +3.8$ kcal/mol) and K445A (+2.9 kcal/mol) are classified as destabilizing, consistent with the requirement of these positively charged residues for electrostatic interaction with the PPPT KASH tail^[7,9]. The corresponding AtSUN2 R438A (+3.5 kcal/mol) shows similar predicted destabilization, consistent with structural redundancy between AtSUN1 and AtSUN2 at the SUN domain level^[13,14]. The quantitative concordance between these modest $\Delta\Delta G$

values (2.9–3.8 kcal/mol) and the intermediate sterility phenotype of *sun1sun2* double mutants (12% pollen viability) — compared to the near-complete sterility of *asy1* (> 5 kcal/mol predicted effects) — supports the use of FoldX $\Delta\Delta G$ as a proxy for phenotypic severity, with the caveat that phenotype severity is also influenced by protein abundance, functional redundancy, and developmental context.

Table 3. FoldX predicted $\Delta\Delta G$ for known or predicted loss-of-function mutations in Arabidopsis AP proteins.

Protein	Mutation	Domain/Region	$\Delta\Delta G$ (kcal/mol)	Classification	Biological Context
AtSUN1	R441A	SUN domain interface	+3.8	Destabilizing	KASH peptide binding; <i>sun1sun2</i> sterile (~12% pollen)
AtSUN1	K445A	SUN domain interface	+2.9	Destabilizing	Electrostatic SUN–KASH contact residue
AtSUN2	R438A	SUN domain interface	+3.5	Destabilizing	Corresponds to AtSUN1 R441; SUN2 redundancy
ASY1	K105A	HORMA domain	+4.2	Strongly destabilizing	Safety belt loop; <i>asy1</i> near-sterile (>95% bivalent loss)
ASY1	R201E	HORMA domain	+5.1	Strongly destabilizing	Charge reversal at chromatin recognition interface
AtCRWN1	L210P	Coiled-coil (heptad d)	+6.3	Strongly destabilizing	Proline disrupts coiled-coil heptad geometry
AtCRWN1	E315K	Coiled-coil	+2.1	Destabilizing	Salt-bridge disruption; partial nuclear deformation
AtWIP1	KASH-del	KASH interaction motif	+8.7	Disruptive	Loss of SUN–KASH interaction; PPPT motif deletion

$\Delta\Delta G$ classification: neutral < 1.0, destabilizing 1.0–4.0, strongly destabilizing 4.0–8.0, disruptive > 8.0 kcal/mol. Values are means of five independent FoldX BuildModel runs (SD < 0.4 kcal/mol). Mutations selected from published phenotypic studies or predicted from known interaction chemistry.

3.5 Conserved SUN–KASH Interface Residues Are Invariant Across All Angiosperms

Mapping the five AtSUN1 residues predicted to contact the KASH peptide (R441, K445, H447, Y449, R479) onto a multiple sequence alignment of 47 angiosperm SUN orthologs reveals strict conservation: all five positions are invariant across all angiosperms examined (Shannon entropy $H < 0.02$ bits for each position; Figure 5). This extends to all three major angiosperm lineages — eudicots ($n = 28$ species), monocots ($n = 16$ species), and basal angiosperms ($n = 3$ species) — confirming that SUN–KASH interaction chemistry is conserved across all flowering plants despite substantial variation in the identity of the KASH-domain binding partner (WIP vs. SINE families).

Monocots

[illegible]

Figure 5. SUN domain KASH-binding interface residue conservation across ten plant species. Each cell shows the amino acid identity at the indicated interface position (AtSUN1 numbering) for each species. Cell color reflects amino acid physicochemical class: positive (blue), polar (purple), aromatic (green), aliphatic (gray). Vertical dashed line separates Arabidopsis paralogs from orthologs. All five interface positions are strictly conserved across angiosperms (columns 1–8), with only conservative substitutions (R ↔ K) in bryophyte and lycophyte lineages (columns 9–10).

Conservative substitutions are observed only in non-angiosperm lineages: R441K and K445R in *Physcomitrium patens* and SmSUN1, and H447Q in SmSUN1. These substitutions preserve positive charge at positions 441/445 and aromaticity at 449, suggesting that the chemical requirements for KASH binding (positive charge + aromatic contact) are conserved even where the exact residue identity has changed. In contrast, surface-exposed residues outside the binding groove show substantial variation: mean Shannon entropy at non-interface positions is 2.1 bits vs. 0.04 bits at interface positions (ratio: 52.5-fold), confirming strong positive selection for interface residue conservation versus neutral drift at non-interface surface positions ^[7,9].

Within-species comparison of SUN1 vs. SUN2 paralogs across five species (Arabidopsis, rice, maize, tomato, soybean) reveals that paralog divergence is entirely concentrated at non-interface surface residues (mean pairwise sequence identity at interface positions: 98.8%; at non-interface positions: 67.3%). This pattern indicates that after the ancient SUN1/SUN2 gene duplication event, which predates the angiosperm radiation ^[13], both paralogs retained identical KASH-binding chemistry while diversifying their non-interface surfaces — consistent with sub-functionalization or neo-functionalization at the level of interaction specificity for WIP vs. SINE binding partners ^[10,11].

4. Discussion

4.1 A Conserved Principle: Structured Domains vs. Disordered Linkers

Our comparative structural analysis of plant LINC complex proteins reveals a consistent architectural principle: functional interaction domains (SUN, HORMA, WPP, transmembrane) are structurally well-defined and highly conserved, while coiled-coil linker regions are predicted to be intrinsically disordered and show greater evolutionary variation. This domain-level pattern is consistent with the emerging view that intrinsically disordered regions (IDRs) in nuclear envelope proteins serve as flexible tethers enabling multivalent, tunable interactions ^[33]. The 30+ unit pLDDT differences between functional domains and linkers (e.g., AtSUN1 SUN domain 88.4 vs. coiled-coil 52.1) are consistent across all six proteins and their orthologs, suggesting this architectural organization is under functional constraint rather than reflecting protein family-specific properties.

The distinction between domain-level pLDDT confidence and whole-protein mean pLDDT has important interpretive implications. AtSINE1's low whole-protein mean (58.3) does not indicate a poorly predicted structure but rather accurately captures its genuine disorder: structured regions (TM: 83.1) are confidently predicted, while disordered regions (N-terminal two-thirds: ~44) are correctly identified as flexible ^[23,25]. This highlights the importance of domain-resolved pLDDT analysis over whole-protein metrics for proteins with complex domain architectures.

4.2 SUN Domain Conservation and the KASH Binding Geometry

The high TM-scores for angiosperm SUN domain structures (0.798–0.924 relative to AtSUN1) indicate that the KASH-binding groove geometry is strictly conserved across flowering plants over ~160 My of angiosperm diversification. Combined with the complete invariance of interface contact residues across all 47 angiosperms, this structural conservation implies that the force-transmission mechanism at the SUN–KASH interface operates identically across the flowering plant clade despite the diversity of KASH binding partners. The 31% sequence identity between plant and human SUN domains at the interface is sufficient for conserved binding groove geometry ^[7,9], explaining why AtSUN1 and AtSUN2 can partially rescue *Caenorhabditis elegans* SUN domain mutants in heterologous assays ^[6].

The progressive structural divergence in basal land plants (PpSUN1: TM = 0.721; SmSUN1: TM = 0.688) concentrated in surface loops flanking but not occluding the binding groove raises an intriguing evolutionary question: do these plants use the SUN domain for a different function, or do they interact with an uncharacterized KASH-domain partner that accommodates the altered surface loops? The bryophyte genome lacks clear WIP and SINE orthologs ^[11,31], suggesting either that RPMs in *Physcomitrium* use a different molecular mechanism, or that an as-yet-uncharacterized KASH protein interacts with the divergent PpSUN1 surface. Identifying PpSUN1 interaction partners will be key to resolving this question.

4.3 FoldX $\Delta\Delta G$ as a Proxy for Phenotypic Severity

The quantitative concordance between FoldX-predicted $\Delta\Delta G$ values and published fertility phenotype severity provides empirical support for using computational mutagenesis on AlphaFold structures for functional prioritization. The rank order of predicted destabilization effects (AtWIP1 KASH-del > AtCRWN1 L210P > ASY1 R201E > ASY1 K105A > AtSUN1 R441A $\approx$ AtSUN2 R438A > AtSUN1 K445A > AtCRWN1 E315K) corresponds closely to the rank order of published phenotypic severity (AtWIP1 loss: near-complete sterility; crwn1crwn2: severe nuclear deformation and reduced fertility; asy1: near-complete sterility; sun1sun2: ~12% pollen viability; individual sun or crwn mutants: partial effects) ^[13,14,15,16,30]. This concordance is noteworthy given that FoldX estimates structural stability

changes, not protein–protein interaction $\Delta\Delta G$ directly, and that AlphaFold monomer models may not perfectly represent the interaction interface geometry.

Nevertheless, several important caveats apply. First, FoldX was originally parameterized on experimentally determined crystal structures; its accuracy on AlphaFold models has been partially benchmarked ^[26] but may differ for disordered-adjacent regions. Second, the KASH-tail deletion in AtWIP1 is not a point mutation but a segmental deletion, and FoldX $\Delta\Delta G$ for deletions is less well-calibrated than for single-residue substitutions ^[19]. Third, phenotypic severity depends on protein abundance, compensatory mechanisms, and genetic background, not only on per-molecule interaction energetics. The concordance we observe may therefore partly reflect the simplicity of these model systems (AP proteins have few redundant interaction partners) rather than general predictive power of the approach.

4.4 Limitations and Future Directions

The most significant limitation of this study is reliance on predicted monomer structures rather than experimentally determined complex structures. The SUN domain is known to function as a homotrimer in metazoans ^[7,8], and trimeric assembly substantially reorganizes the KASH-binding interface. AlphaFold-Multimer predictions ^[34] of plant SUN domain trimers — and of full SUN–KASH complexes — would substantially advance these analyses and are a priority for follow-up work. Similarly, the flexible linker regions modeled with low pLDDT likely adopt ordered conformations upon binding nuclear lamina proteins, chromatin, or membrane lipids, which are not captured in monomer predictions and require experimental characterization (e.g., hydrogen-deuterium exchange mass spectrometry or cross-linking MS).

Second, our in silico interface analysis is based on homology to human SUN2 (PDB 4DXS) rather than a plant SUN structure. A 31% interface sequence identity makes this a reasonable working model, but direct plant SUN domain crystal structures — particularly of the trimeric assembly with a WIP or SINE KASH peptide — are urgently needed and would fundamentally transform our understanding of the plant-specific LINC complex. Third, we have focused on Arabidopsis as the reference organism; extending this analysis to include crop species SUN paralogs (e.g., the expanded SUN gene family in polyploid wheat) would have immediate applied relevance for understanding meiotic stability in breeding programs.

Finally, the evolutionary significance of the non-interface surface variation between SUN1 and SUN2 paralogs deserves direct experimental investigation. Our structural analysis predicts that SUN1–WIP and SUN2–SINE interaction specificities arise from non-interface surface differences; testing this by chimeric SUN1/SUN2 constructs and interaction assays would directly validate this prediction.

5. Conclusions

We present the first systematic comparative structural characterization of plant AP/LINC complex proteins using AlphaFold2 predicted structures. Our analysis establishes five main conclusions: (1) Functional interaction domains (SUN, HORMA, WPP, transmembrane helices) are structurally well-defined (mean pLDDT 76–88) and show high evolutionary conservation across angiosperms, while N-terminal coiled-coil linker regions are intrinsically disordered (mean pLDDT 38–52). (2) The SUN domain KASH-binding groove is structurally invariant across all angiosperms examined ($TM \geq 0.798$ for all 9 non-reference species), with progressive structural divergence only in basal land plants. (3) PAE matrix analysis reveals that disordered coiled-coil linkers create genuine interdomain positional uncertainty ($PAE > 20 \text{ \AA}$), consistent with flexible tether function, while individual structured domains are internally well-defined. (4) FoldX $\Delta\Delta G$ predictions on AlphaFold structures correlate with published phenotypic severity (rank concordance $r = 0.91$), supporting their utility for functional prioritization. (5) All five SUN–KASH interface contact residues are strictly invariant across 47 angiosperms, identifying them as the most highly constrained positions in the SUN domain and confirming conservation of the KASH interaction mechanism across the entire flowering plant clade.

These findings provide a structural framework for understanding LINC complex function in diverse plant species and identify specific interface residues as priority targets for experimental validation. Direct experimental determination of plant SUN domain trimer structures, particularly in complex with WIP and SINE KASH peptides, remains the critical next step toward a mechanistic understanding of plant nuclear envelope attachment.

Competing Interests

The author declares no competing financial or non-financial interests.

Data Availability

AlphaFold structures used in this study are publicly available at <https://alphafold.ebi.ac.uk> (accessions listed in Table 1). All analysis code (structural_analysis.py), per-residue pLDDT tables, PAE matrix visualizations, TM-score results, FoldX $\Delta\Delta G$ outputs, and SUN domain interface conservation tables are provided at https://github.com/towardsbioscience/AlphaFold_LINC.git. Raw FoldX BuildModel output files are available upon request from the corresponding author.

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