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Characterization of a new Post Translational Modification of Neurofibromin: atypical structural requirements for its SUMOylation

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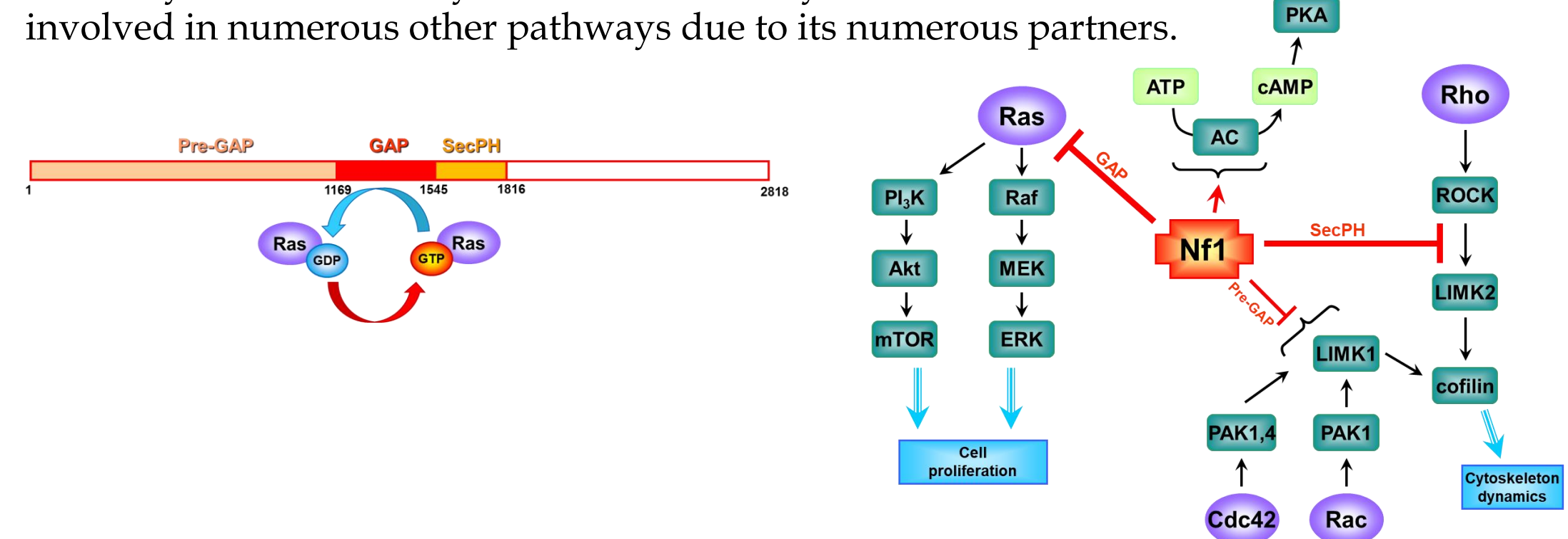
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Nf1 and SUMO

Neurofibromin, Nf1, the protein encoded by NF1 gene

- NF1 is a tumor-suppressor gene which encodes Neurofibromin, Nf1.
- Nf1 is a giant multidomain protein (isoform 1/2 2818/2839 aa).
- Initially characterized by its Ras-GAP activity, Nf1 is also involved in numerous other pathways due to its numerous partners.

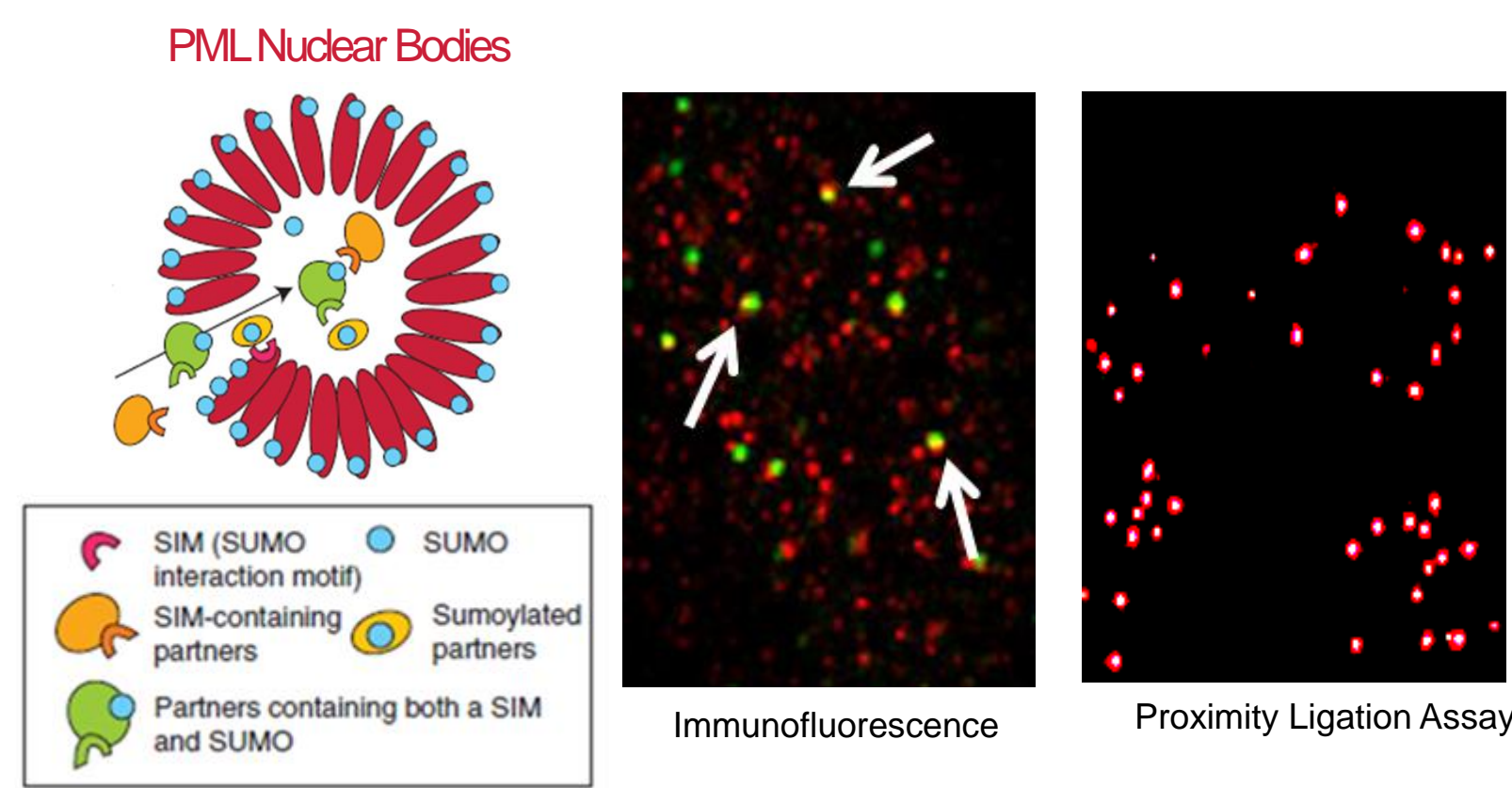


Nf1 post-translational modifications

- Phosphorylation:** C-Terminal Domain has been shown to be phosphorylated by PKA (interaction with 14-3-3) and PKC ϵ (nuclear entry).
- Ubiquitination:** Nf1 is negatively regulated by different ubiquitin ligases which induce its proteasomal degradation.

Nf1 colocalizes with PML nuclear bodies

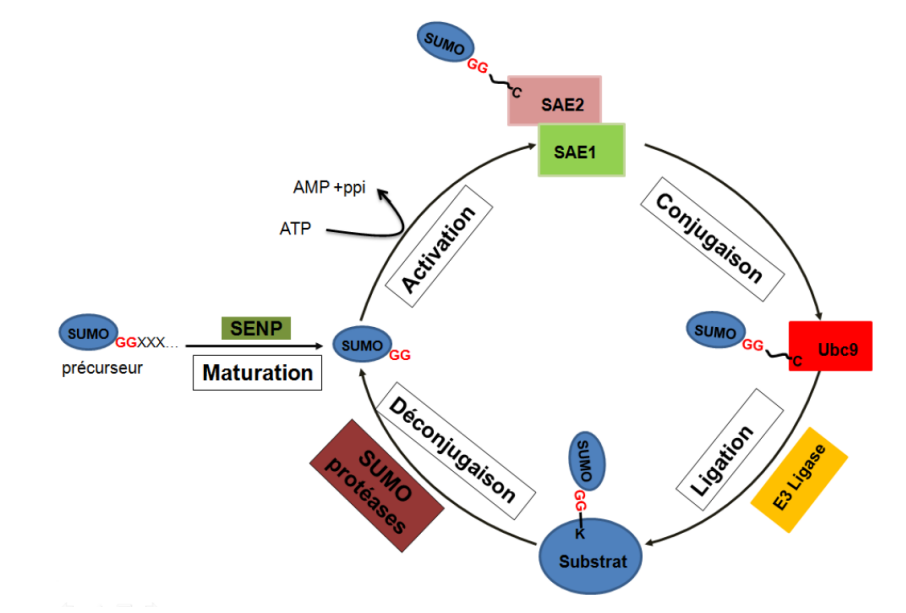
PML: ProMyelocytic Leukemia (Godin et al., 2012)



- Nf1 (green) partially localizes with PML Bodies (red, PML-NBs) which are hot spots of SUMOylation.
- Nf1 and PML proteins belong to the same multiprotein complex

SUMO, a post-translational modification

- Reversible Post Translational Modification (PTM)
- Rare event: steady-state, SUMOylation is usually less than 5%
- Multiple functional roles: protein/protein interaction, protein stability, protein subcellular localization
- Several neurodegenerative diseases rely on SUMO modification
- SUMO-based therapies are currently developed

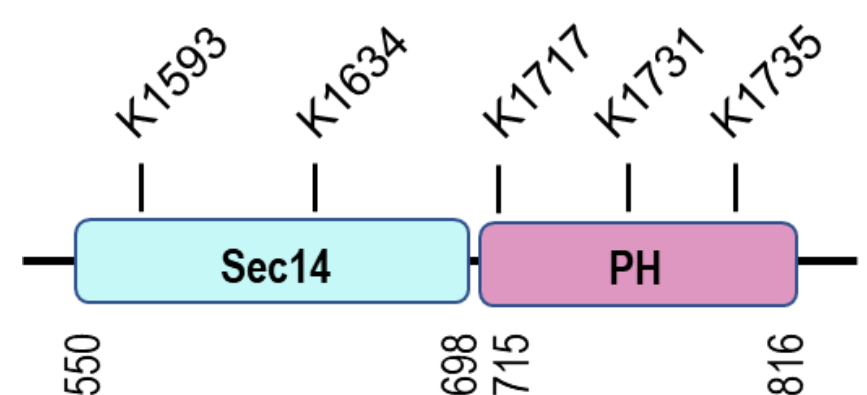


- SUMOylation occurs on Lys residues belonging to a consensus sequence (Ψ KxE/D, Ψ being an hydrophobic residue, x any residue) or via the help of the SIM domain of the substrate which allows the recruitment of the E2, Ubc9.

The SecPH domain of Nf1 is SUMOylated on its Lys1731 and 1634

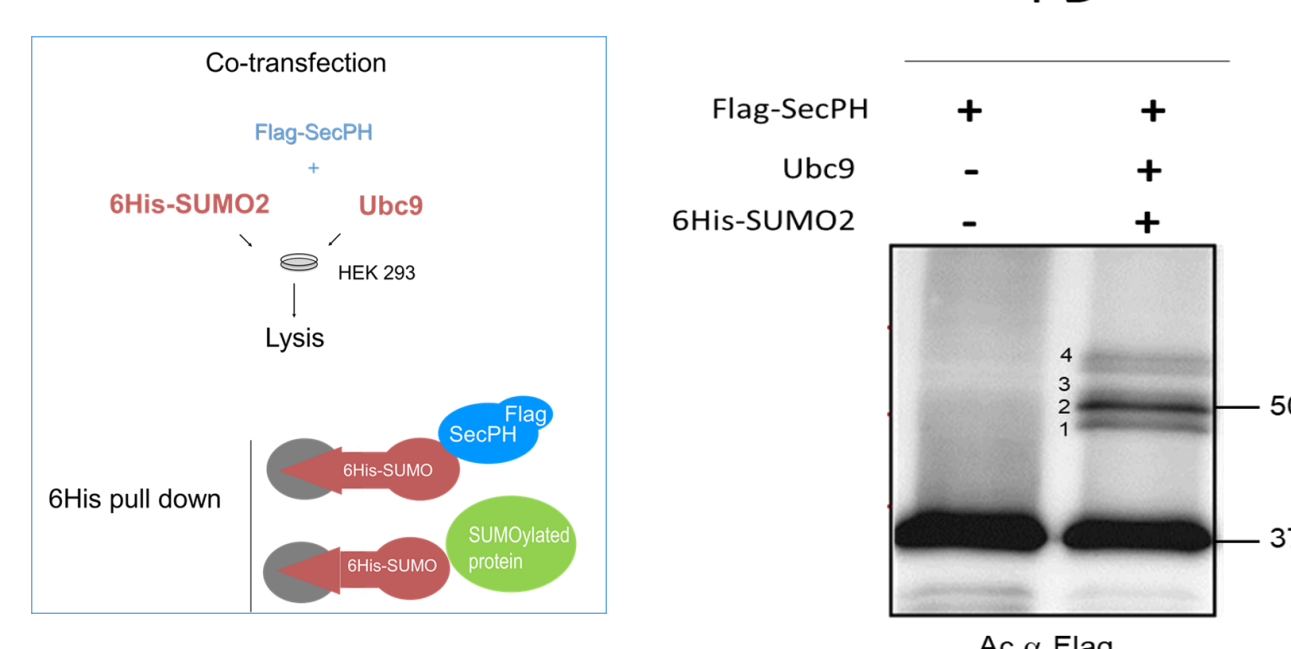
SecPH domain of NF1

- The SecPH domain contains the highest number of SUMOylation consensus sites, 5 sites are predicted to be SUMOylated



- As evidenced by the recently resolved CryoEM structures of Nf1 dimer, it is exposed at the surface of the dimer and it is prone with its adjacent GRD domain, to a structural rearrangement.
- Pleckstrin Homology (PH) domains are enriched in SUMOylation substrates.
- SecPH might play an important role in Nf1 function as it could allosterically affect GRD Ras-GAP function and as it interacts with many partners (lipids, VCP, LIMK2, 5HT6, caveolin).

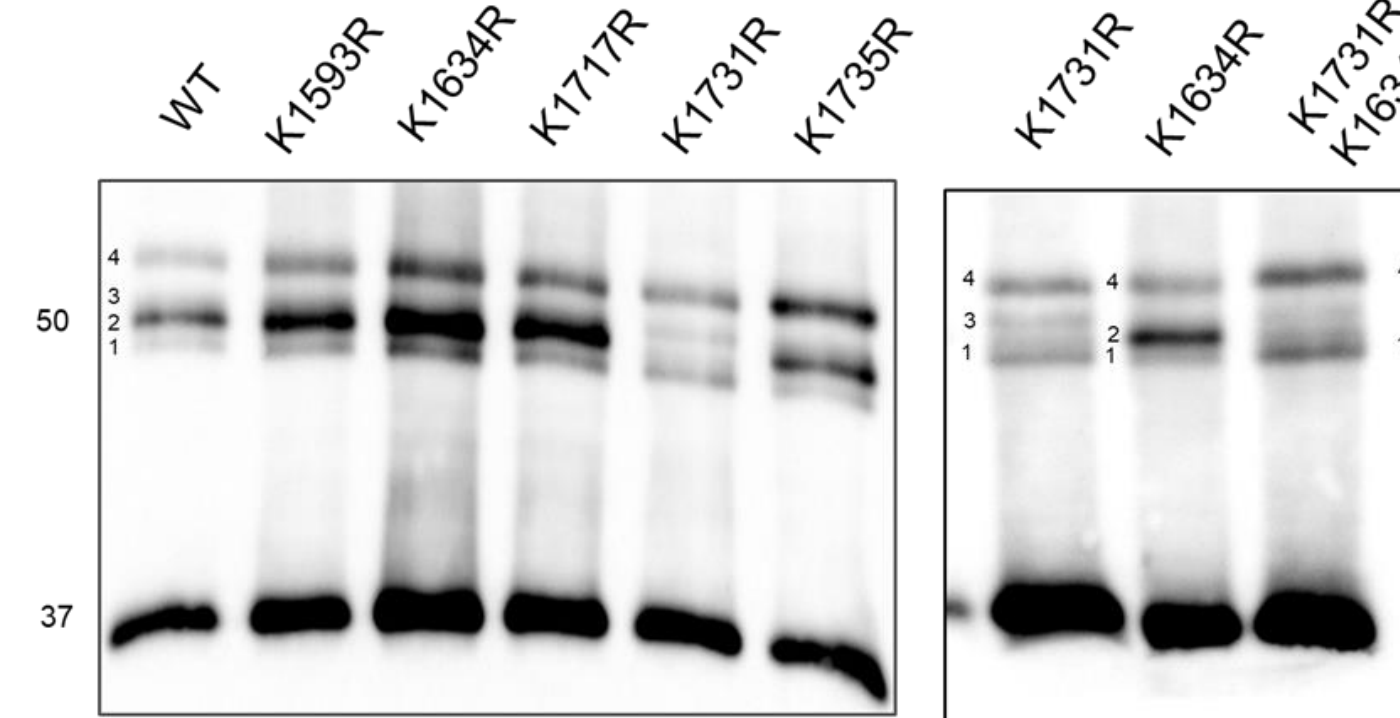
SecPH SUMOylation profile



- SecPH is characterized by 4 bands of monoSUMOylation

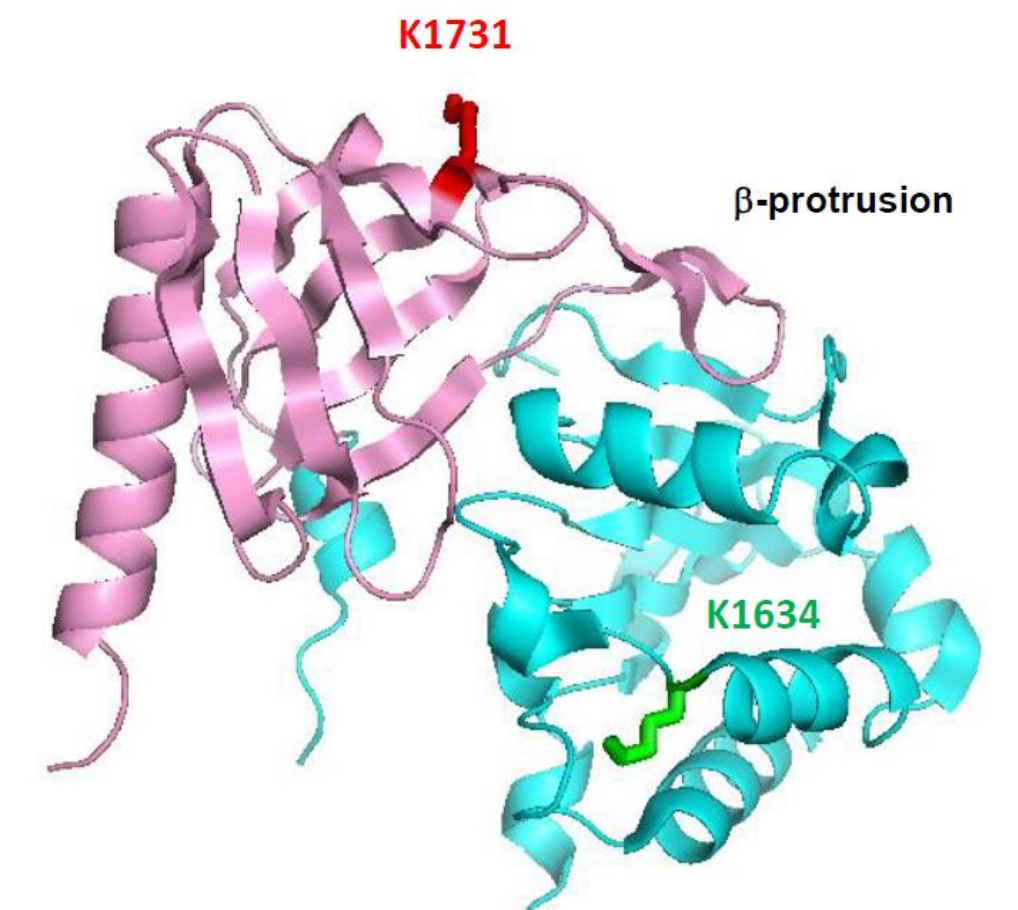
SecPH SUMOylated Lys determination

Each Lysine has been substituted by Arginine either individually (left), in combination (right) and analysed by PD (pull-down) affinity on cobalt beads



- Band 2 disappears in K1731R mutant
- Band 3 disappears in K1634R mutant

K1731 and 1634 localization

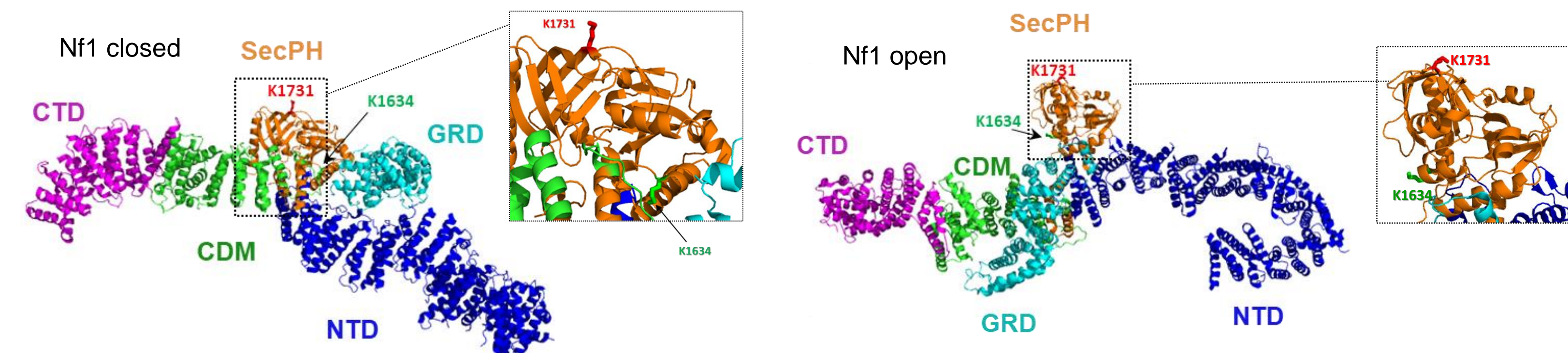


- K1731 and 1634 are surface exposed

K1731 in Nf1

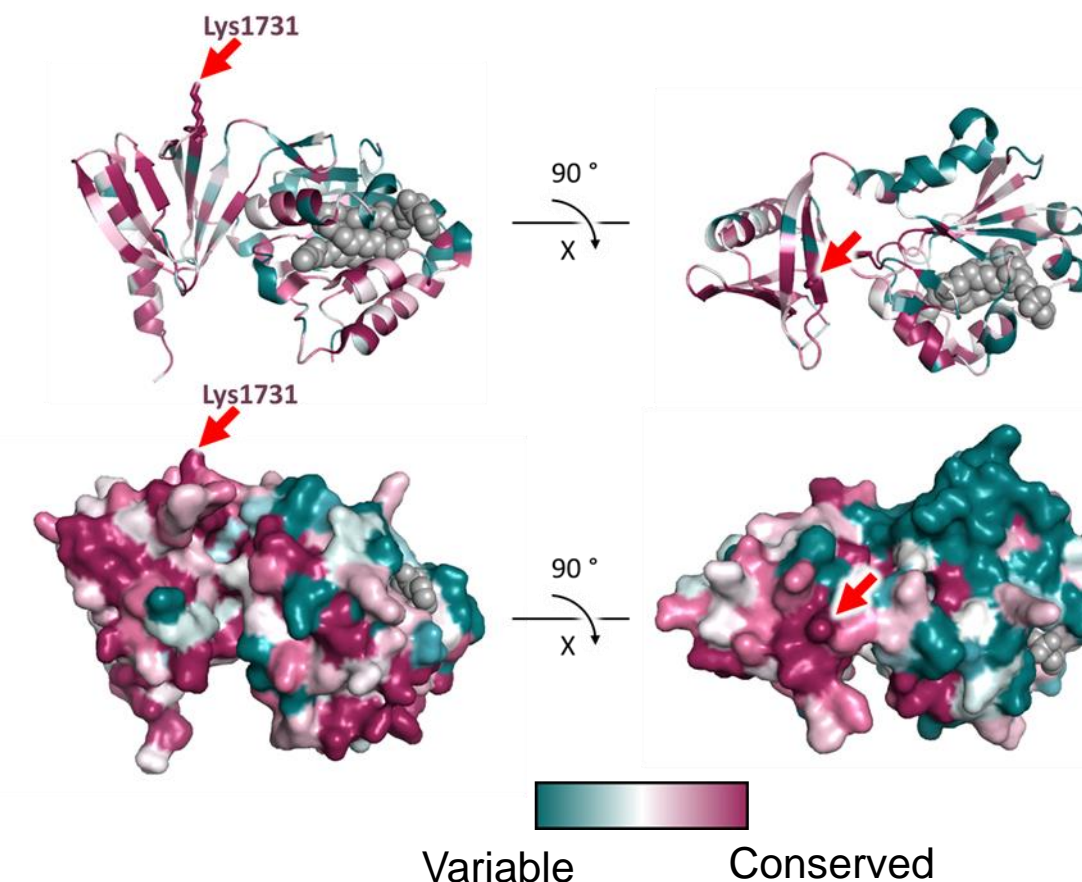
3D structure of Nf1

Nf1 isoform 1 forms a head-to-tail dimer which oscillates between an open active conformation able to negatively regulate RAS and an occluded conformation unable to inactivate RAS.



- Whatever Nf1 conformation, K1731 is surface exposed and may interact with SUMO machinery

K1731 conservation



- K1731 is highly conserved in most metazoan Nf1 orthologues

K1731 pathogenicity

In the gnomAD database a c.5192A>G (p.Lys1731Arg) missense variant of NF1 was found associated with two NF1-patients for which the *in silico* predictions of deleterious effects by SIFT and PolyPhen-2 software were inconclusive.

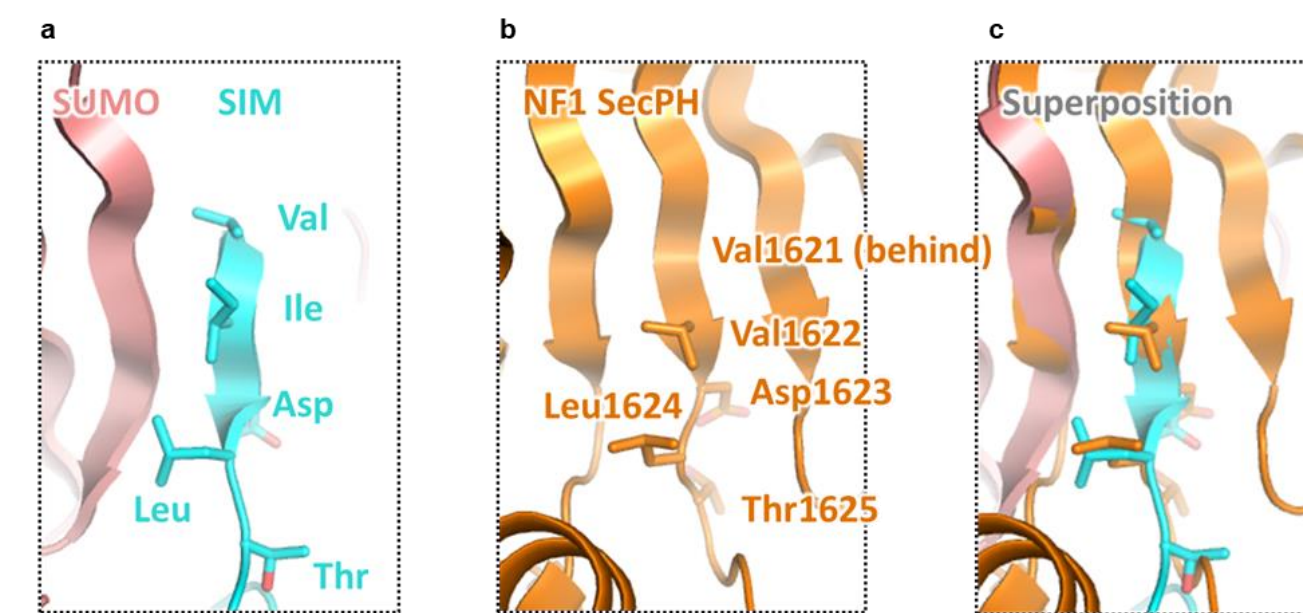
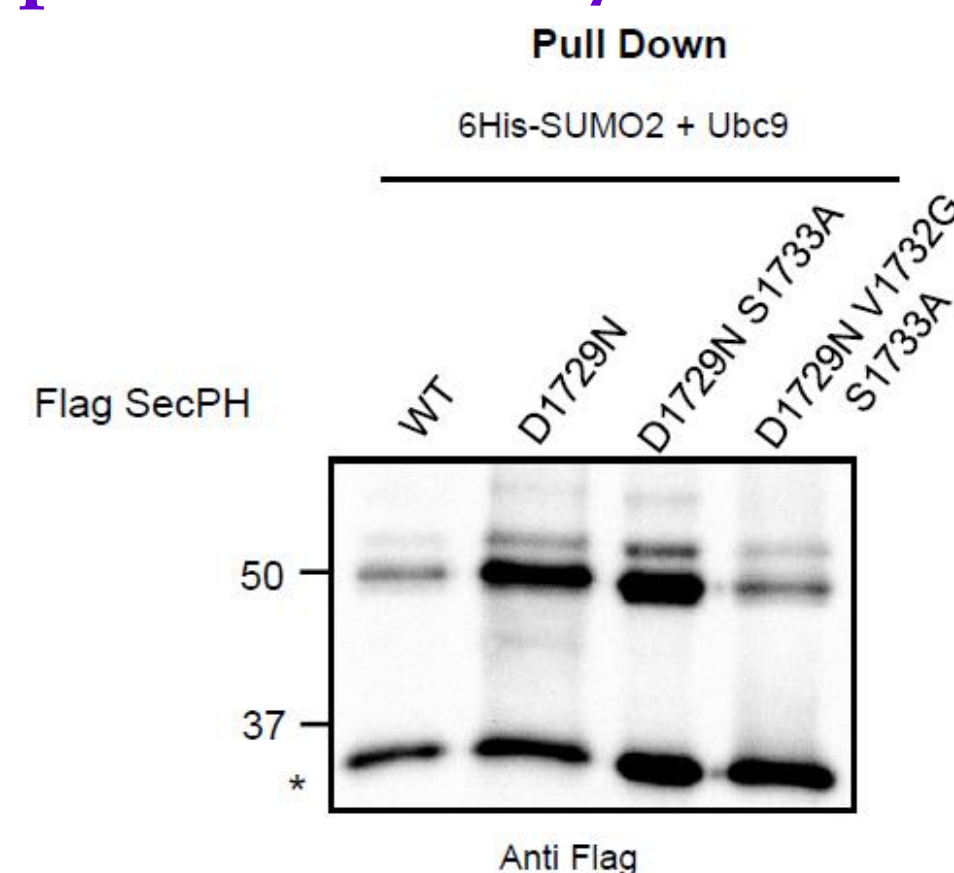
Molecular mechanisms of SUMOylation

K1731 SUMOylation does not depend on Ψ KxE/D consensus site

K1731 SUMOylation does not depend on its SIM domain

AHKDTKVSIVK (WT)
AHKNTKVSIVK (D1729N)
AHKNTKVAIVK (D1729N S1733A)
AHKNTKGAIVK (D1729N V1732G S1733A)

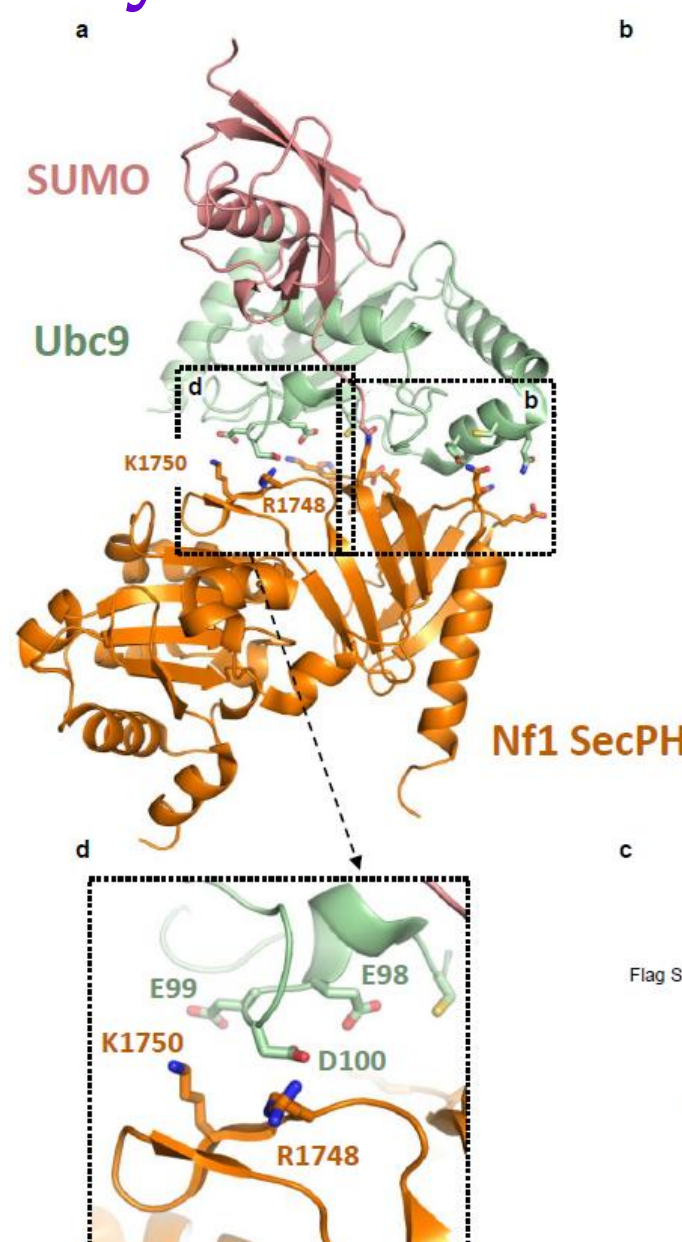
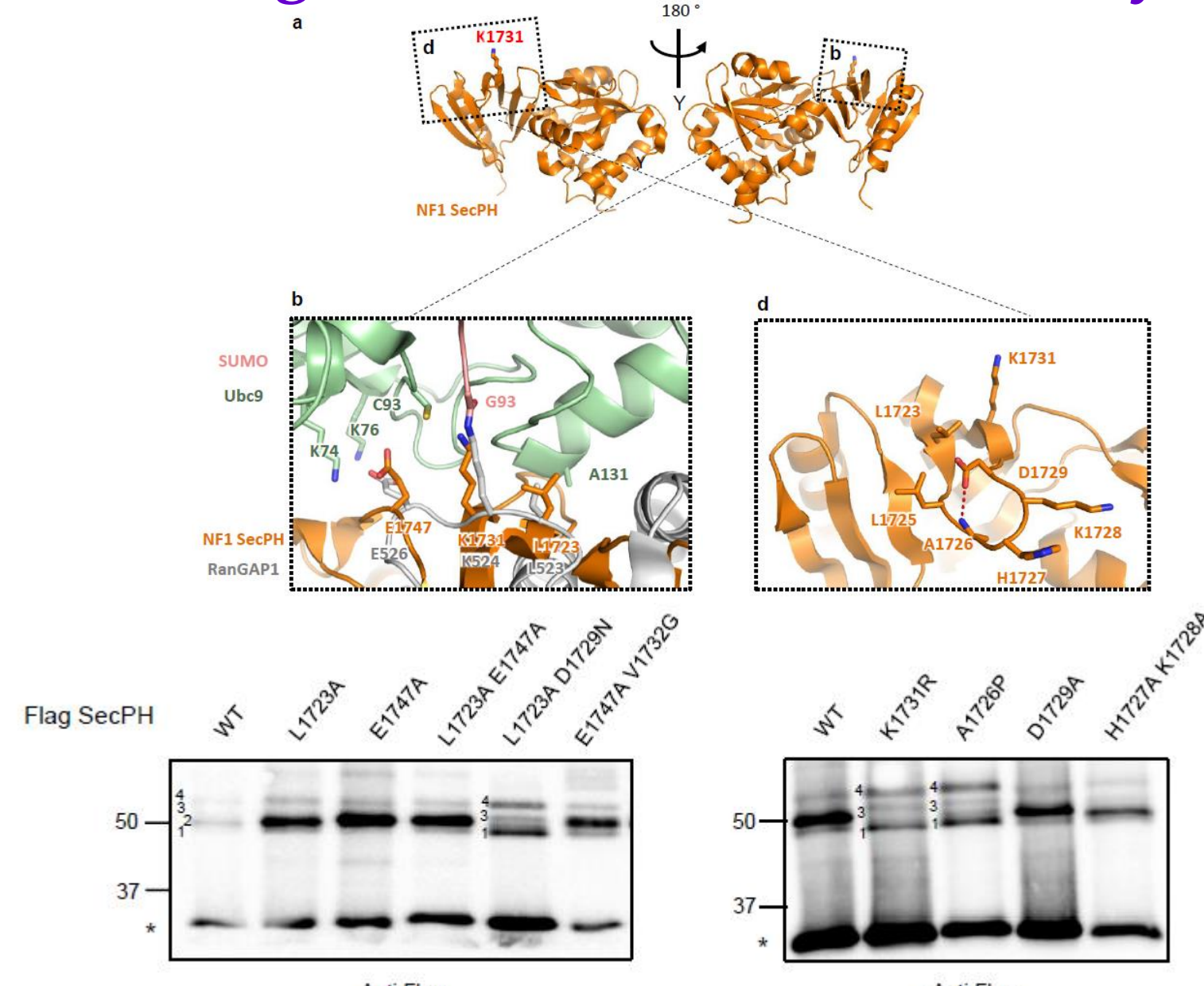
- K1731 remains modified independently of the inverted SUMO consensus sequence Ψ KxE/D surrounding it



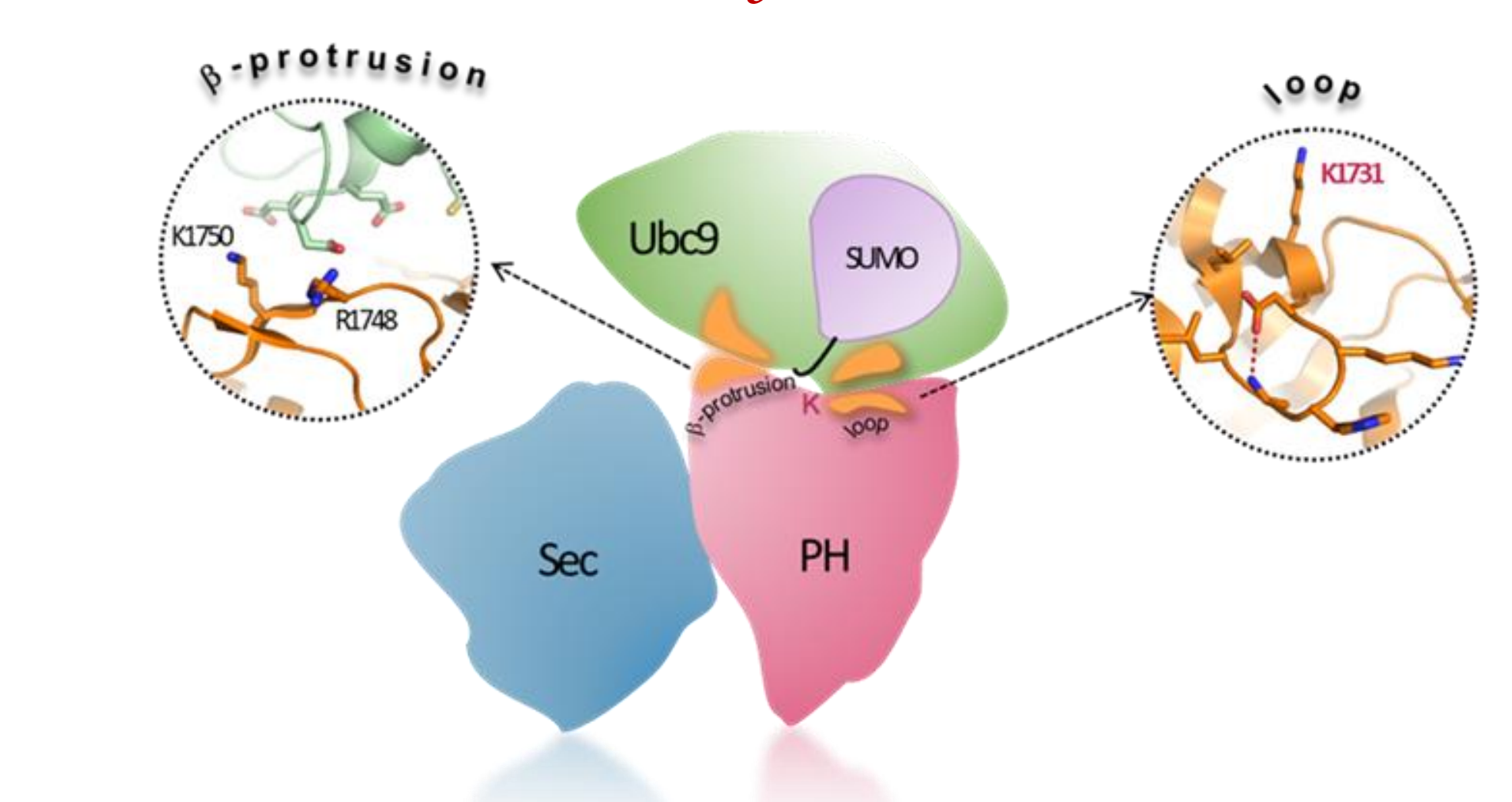
- SecPH SIM domain is not accessible to SUMO binding as it is surrounded by β -strands

The loop 1723-1729 of SecPH contributes to the recognition of K1731 for SUMOylation

Residues R1748 and K1750 of the β -protusion are essential for SecPH SUMOylation on K1731



Proposed model for SecPH SUMOylation on K1731



- K1731 SUMOylation of SecPH is mediated by two surface-exposed structural elements the PH domain, the loop 1723-1729 and the β -protusion, which form a complex composite "structural motif" for recruiting Ubc9 and positioning it on K1731.
- This mechanism is totally atypical.

- SecPH SUMOylation on its K1731 relies on a tertiary structural environment around this amino acid, which has never been described in the literature so far.

Link to NF1 disease: some pathogenic patients harbour single mutation in this loop or in the β -protusion.