Structure of the human Parkin ligase domain in an autoinhibited state

Supplementary Material

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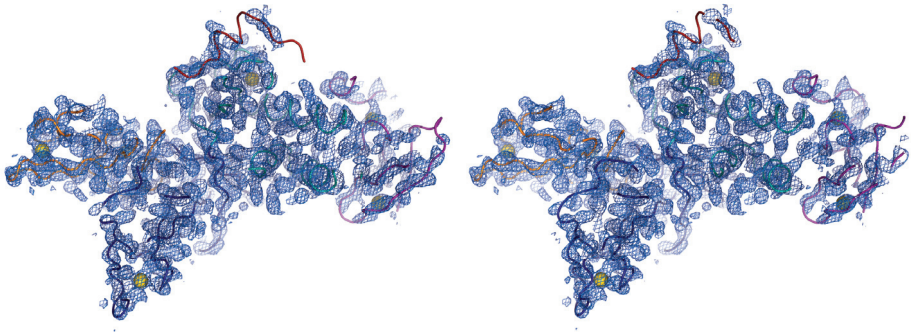
Running title: Structure of the Parkin UPD-RBR

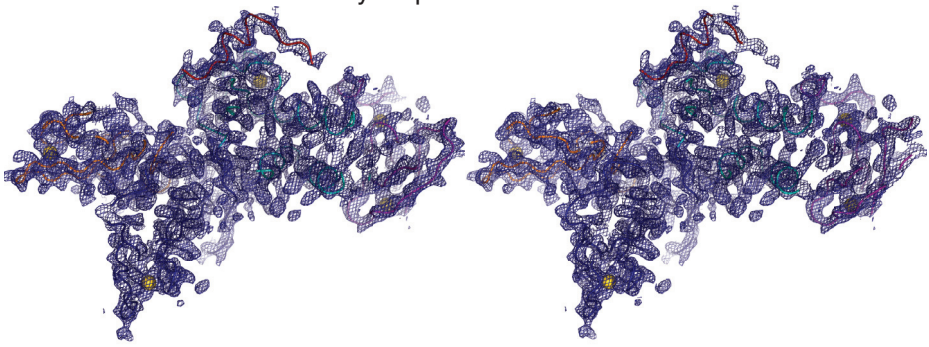
Contents:

Supplementary Figure 1

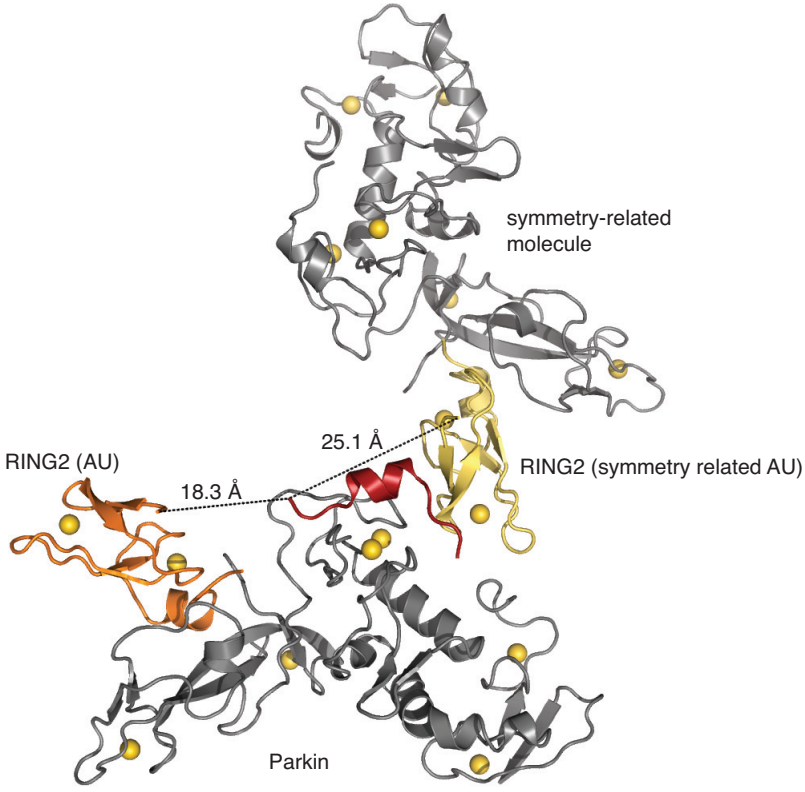
A Experimental electron density map, phased by Zn-SAD



B Final refined electron density map



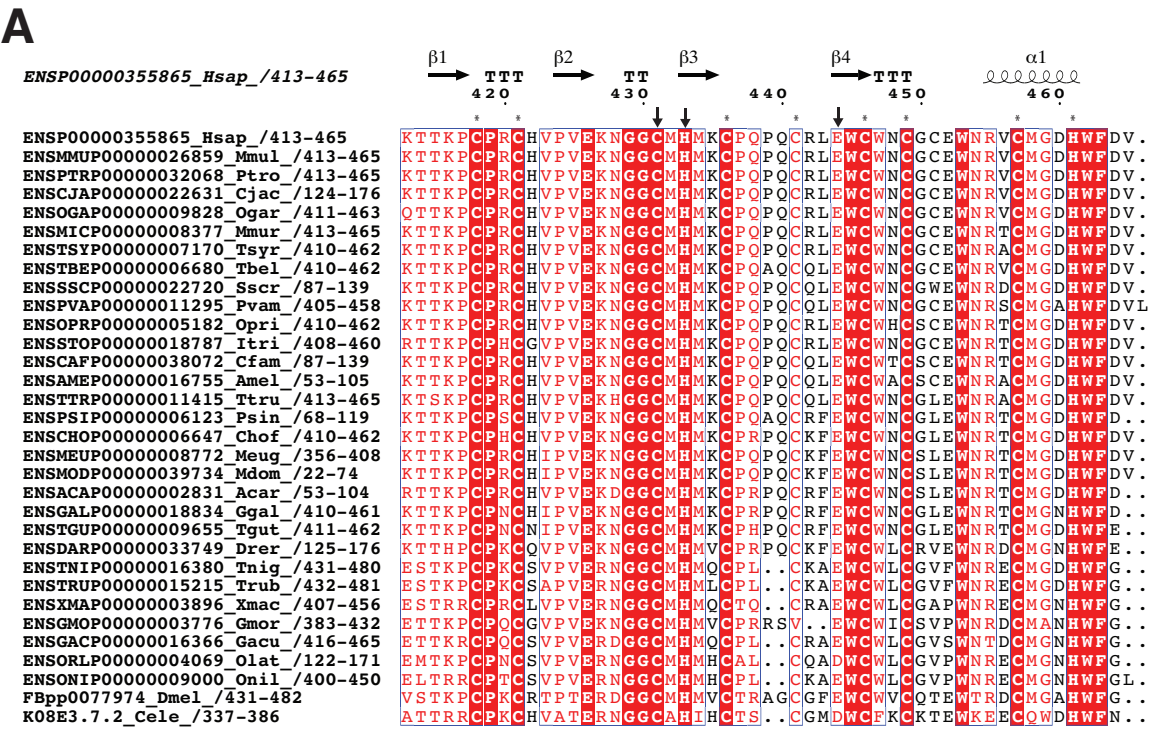
C RING2 positioning in crystal structures

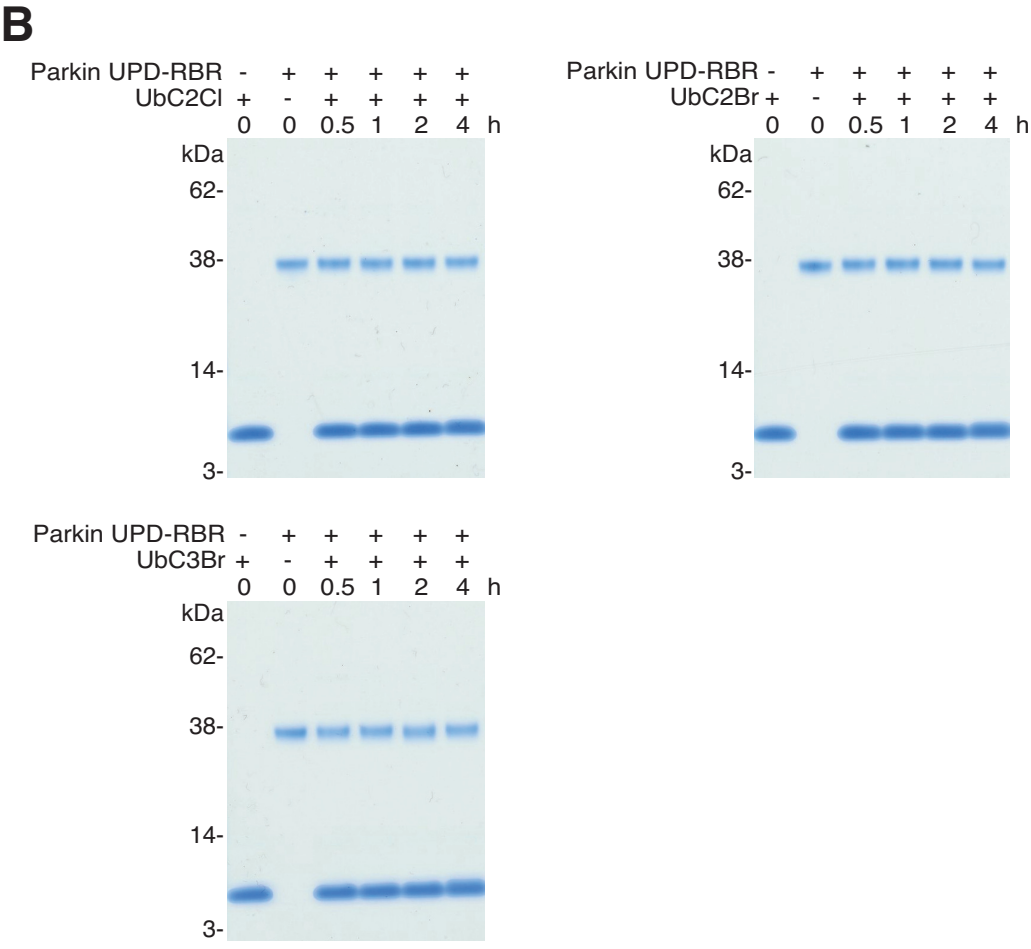


Supplementary Figure 1. Electron density maps

Stereo picture of electron density covering Parkin in the asymmetric unit, shown as a ribbon diagram coloured as in **Figure 1B**. Zn atoms are shown as yellow spheres. **(A)** Experimental electron density map, contoured at 1 σ, after density modification by SHELXE. **(B)** Refined 2|Fo|-|Fc| electron density map contoured at 1 σ. **(C)** Subdomain connectivity was initially ambiguous, as the disordered region between the linker helix and RING2 (aa 405-412) could reach RING2 of two neighbouring asymmetric units (AUs). The shorter distance (18.3 Å) connects the linker to the RING2 in the AU, as this domain forms strong hydrophobic contacts with the UPD, and RING2 would be autoinhibited. The longer distance (25.1 Å) would connect to a RING2 domain that would expose its catalytic Cys, and contact RING1, IBR and the linker helix, albeit only with few polar contacts. Our mutational and biochemical analysis makes the latter interface more unlikely. It was a possibility that the alternative RING2 conformation resembled that of an active 'open' state of Parkin (compare **Figure 5B**), but modelling of the E2~Ub (as in **Figure 5A**) led to significant clashes of the E2 with RING2 in the alternative position.

Supplementary Figure 2

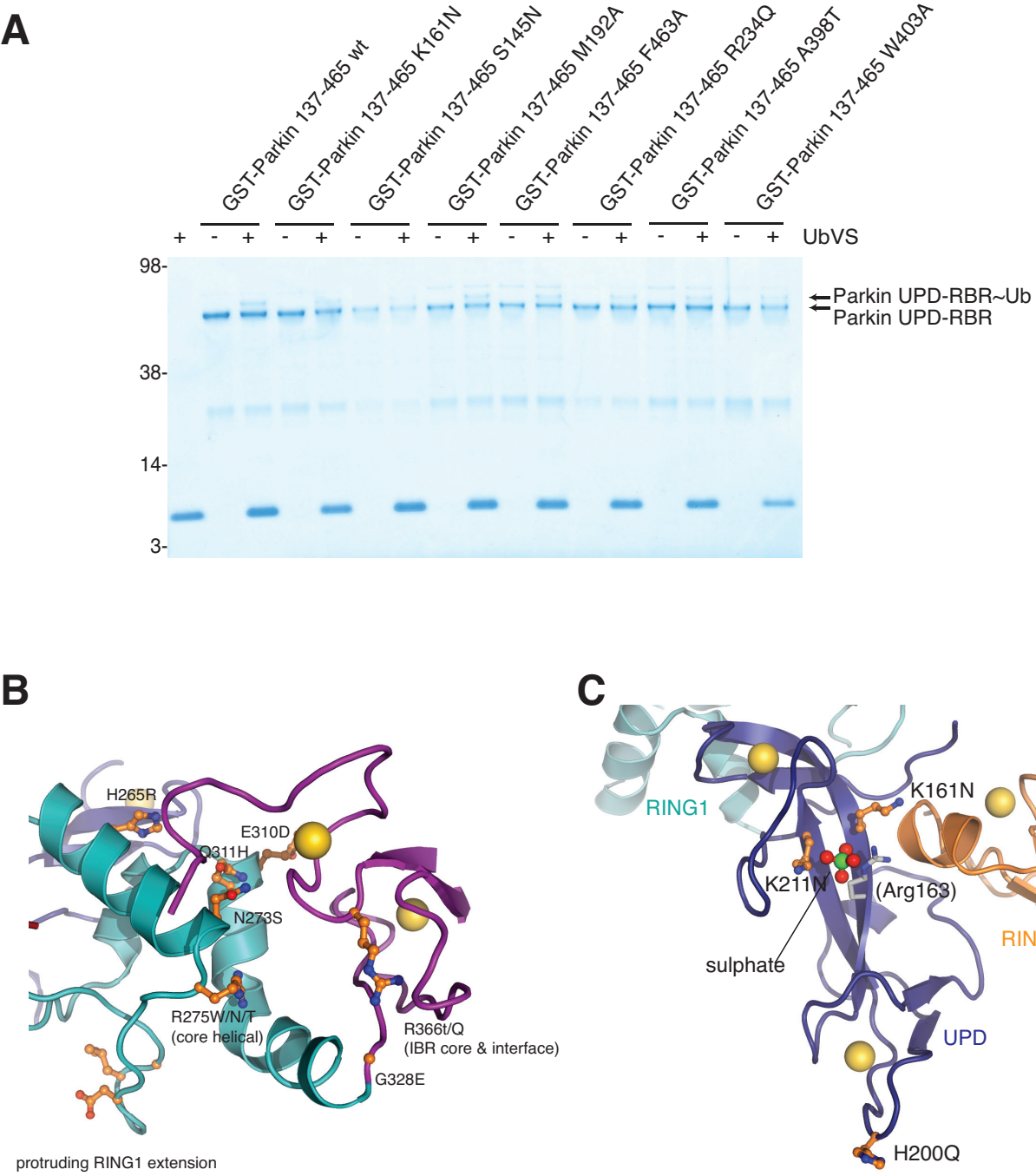




Supplementary Figure 2. RING2 conservation in Parkin

Species alignment based on Parkin sequences annotated in ensemble (http://www.ensembl.org/Homo_sapiens/Gene/Sequence?g=ENSG00000185345;r=6:161768452-163148803) that contain a fully annotated RING2 sequence. Secondary structure and numbering based on the human sequence and according to the crystal structure is indicated above the alignment. **(B)** Ub-based suicide inhibitors are unable to modify the crystallised Parkin UPD-RBR fragment. Coomassie-stained SDS-PAGE gels are shown for incubation of Parking with Ub-based suicide probes for indicated amount of time. The used Ub-haloalkyl probes are described in (Borodovsky *et al*, 2002).

Supplementary Figure 3



Supplementary Figure 3. Structural detail of Parkin mutations

(A) modification of indicated GST-tagged Parkin UBP-RBR variants with Ub-vinylsulfone (UbVS). The GST-tag on wt UPD-RBR fragment activated the protein, which is modified with Ub-VS, and an increase in reactivity cannot be observed in this assay. **(B)** Parkin mutations in the RING1:IBR domain interface, including two core residues mutation of which may change the structure. Also, the three surface exposed mutations in the unique protruding β-hairpin of the RING1 domain are shown. This mutations hotspot indicates functional relevance, most likely in interactions with other proteins. **(C)** View of the UPD in a ~180º rotated orientation as in **Figure 1B** (note RING2 on right hand side) showing three patient mutations. His200 is fully exposed. The sulphate ion is shown in ball-and-stick representation with a green sulphur atom and red oxygen atoms.

Supplementary Table I. Known Parkin mutations in the crystallized construct

Mutation	Domain	Predicted structural rationale	Biochemical effect
Zn-coordinating residues			
C166Y	UPD	Coordinates UPD Zn2	
C212G **C212Y**	UPD	Coordinates UPD Zn1	Reduced solubility (Wang *et al*, 2005)
H215Q	UPD	Coordinates UPD Zn1	
C238Q	RING1	Coordinates RING1 Zn1	
C253Y **C253W**	RING1	Coordinates RING1 Zn2	
R256C	RING1	Creates a competing Cys for RING1 Zn2 binding	- Reduced complex formation (Van Humbeeck *et al*, 2008) - Reduced solubility (Wang *et al*, 2005) - Autoubiquitination, but reduced substrate ubiquitination (Sriram *et al*, 2005)
H257R	RING1	Coordinates RING1 Zn2	
C289G	RING1	Coordinates RING1 Zn2	- Reduced solubility (Wang *et al*, 2005; Gu *et al*, 2003)
R334C	IBR	Creates a competing Cys for IBR Zn1 binding	- Reduced solubility (Wang *et al*, 2005)
R402C **R402H**	Linker helix	May interfere with Zn1 binding of RING1	
C418R	RING2	Coordinates RING2 Zn1	- Reduced solubility (Gu *et al*, 2003)
C441R	RING2	Coordinates RING2 Zn1	- Reduced solubility (Wang *et al*, 2005; Hampe, 2006)
Domain core residues			
P153R	UPD	In UPD Zn1 binding loop	
T173M	UPD	In UPD Zn2 binding loop	
T240R **T240M**	RING1	In RING1 Zn1 binding loop May affect E2 binding	- Ligase dead (Sriram *et al*, 2005) - Reduced mitochondrial localization (Matsuda *et al*, 2010)
V258M	RING1	RING1 core residue, may affect linker helix binding	
Y267H	RING1 helical	Core residue of RING1 helical domain	
C268R	RING1 (helical)	Core residue of RING1 helical domain	
R275N **R275W** **R275T**	RING1 (helical)	RING1 helical domain core residue	- Reduced solubility (Wang *et al*, 2005) - Increased ubiquitination activity (Sriram *et al*, 2005) - No mitophagy (Narendra *et al*, 2010; Geisler *et al*, 2010)
I298S **I298L**	RING1	RING1 core residue	

T351P	IBR	Disrupt IBR β-sheet, next to Zn-coordinating Cys	IBR domain destabilisation (Beasley *et al*, 2007)
R366T **R366Q**	IBR	IBR core residue, also on interface with RING1	
P437L **P437I**	RING2	Near Zn1 binding site	Ligase dead (Sriram *et al*, 2005)
Residues affecting ubiquitination mechanism			
D243N	RING1	Forms contact with conserved Lys on E2 helix 1	
C431F	RING2	Active site Cys	- Reduced solubility (Wang *et al*, 2005; Sriram *et al*, 2005) - Increases autoubiquitination (Sriram *et al*, 2005) - No substrate ubiquitination (Sriram *et al*, 2005) - Reduced translocation to mitochondria (Lazarou *et al*, 2013)
E444Q	RING2	Potential catalytic triad residue (unconfirmed)	
Interface residues between RING2 & UPD			
S145N	UPD	Destabilises interface?	
M192L **M192V**	UPD	Destabilises interface?	
S193I	UPD	Destabilises interface?	
G429D	RING2	Destabilises interface?	
G430D	RING2	Destabilises interface?	- Ligase dead (Chew *et al*, 2011) - Increased auto-ubiquitination (Sriram *et al*, 2005) - Reduced substrate ubiquitination (Sriram *et al*, 2005)
M458L	RING2	Near RING2 UPD interface but exposed	
A230T	RING1	May create RING1/RING2 interface. May affect Linker helix binding May affect RING1 structure	
Interface residues between RING1 and linker helix			
R234Q	RING1	May affect linker helix binding	
A398T	Linker helix	Disrupts linker helix binding	
A401D	Linker helix	Disrupts linker helix binding	
Residues in RING1:IBR interface			
N273S	RING1	Destabilises interface?	
E310D	RING1	Destabilises interface?	

Q311H	RING1	Destabilises interface?	
G328E	RING1	Loop between RING1 and IBR Destabilises interface?	- Increased auto-ubiquitination (Sriram *et al*, 2005) - Reduced substrate ubiquitination (Sriram *et al*, 2005) - Reduced solubility (Wang *et al*, 2005)
Putative phospho-peptide binding site			
K161N	UPD	- Phospho-peptide binding site? - Salt bridge to Ring2	- Reduced complex formation (Van Humbeeck *et al*, 2008) - Ligase dead (Sriram *et al*, 2005) - Ubiquitinates substrate (Hampe *et al*, 2006) - Reduced mitochondria clearance (Geisler *et al*, 2010)
K211N	UPD	Phospho-peptide binding site?	- Reduced complex formation (Van Humbeeck *et al*, 2008) - Reduced mitochondrial localization (Matsuda *et al*, 2010)
Residues with unclear effects on Parkin function			
H200Q	UPD	Exposed in UPD	
V244I	RING1	On RING1, exposed	
H265R	RING1 helical	On RING1, exposed	
D280N	RING1	β-sheet insertion in RING1	Reduced solubility (Wang *et al*, 2005)
L283P	RING1	β-sheet insertion in RING1	
G284R	RING1	β-sheet insertion in RING1	
T415N	RING2	Exposed in RING2.	Ligase dead (Sriram *et al*, 2005; Matsuda *et al*, 2010)

Supplementary References

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