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The *in Situ* structure of a Parkinson's Disease Mutant LRRK2 Bound to Cellular Microtubules Revealed by Cryo-electron Tomography

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Mutations in the leucine-rich repeat kinase 2 (LRRK2) are the major cause of familial Parkinson's disease (PD) (Roosen and Cookson, 2016). LRRK2 is a very intriguing protein composed of one kinase and one GTPase domains surrounded by multiple domains involved in protein interactions. Most of the pathogenic mutations are found in two catalytic domains and result in hyperactivity of the LRRK2 kinase(Steger et al., 2016). Hyper-activation of LRRK2 kinase is also reported in idiopathic PD cases, suggesting that suppression of kinase activity might be beneficial for a wide range of PD patients(Di Maio et al., 2018). Despite being a promising drug target, structural information of LRRK2 has been limited so far. Several pathogenic mutations cause LRRK2 to form filamentous structures co-localizing with microtubules (MTs), unlike wild-type protein which is diffuse throughout the cytoplasm(Blanca Ramirez et al., 2017; Kett et al., 2012; Schmidt et al., 2019). Pharmacological LRRK2 kinase inhibition also causes recruitment of both wild-type and mutant LRRK2 to MTs(Blanca Ramirez et al., 2017; Deng et al., 2011) emphasizing the need for detailed structural information of LRRK2 bound to MTs for designing effective PD therapeutics.

We have revealed that the *in situ* structure of the pathological mutant of LRRK2 (I2020T) forms regular double helices surrounding microtubules using cryo-electron tomography (cryo-ET) and subtomogram averaging (Fig.1). Interestingly, mutant LRRK2 preferentially decorates atypical MTs composed of 11- and 12- protofilaments (PFs) as opposed to the conventional 13-PF MTs typically found in eukaryotic cells. Based on the cryo-ET structure of LRRK2 combined with integrative modeling, we have determined the architecture of the C-terminal half of LRRK2 bound to microtubules (Fig.2). Our model shows two oligomerization interfaces of LRRK2, in the COR and WD40 domains dimerizing to form filaments and in the ROC, a GTPase domain points to the microtubule. We have also found that in a known risk factor for Parkinson's disease, a mutant LRRK2 (G2385R), the mutation is located in the WD40 dimerization interface, and no longer forms filamentous structures even in the presence of LRRK2 kinase inhibitor unlike wild-type LRRK2. This suggests a critical role of WD40 dimerization for MT recruitment and/or helix formation. Our work provides the first example of a protein structure being determined inside the cell before it was done in vitro and yields unprecedented insight into the interaction between LRRK2 and MTs as well as the dimerization interfaces that lead to the putative pathogenic state. Our structure will help in the design of inhibitors, and to understand the mechanistic details of LRRK2 function.

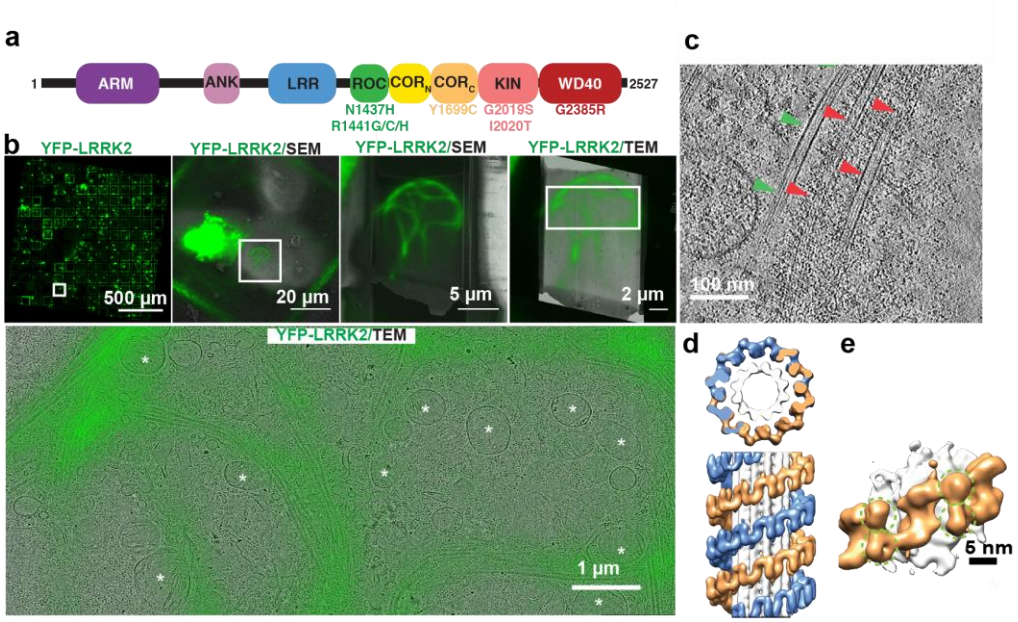

Figure 1. a) Domain structure of human LRRK2 protein. b) Cryo-correlative microscopy targeting LRRK2 in cells. Asterisks show mitochondria. c) The slice of tomogram showing LRRK2-decorated MT (red arrowhead) and non-decorated MTs (green arrowhead). d) Top and side view of LRRK2 double-helical structure along MTs. Note that MT shown here is atypical MT with12 protofilaments. e) Higher-resolution average containing an asymmetric unit of LRRK2. Green circles show typical donut-shaped of the WD40 domain.

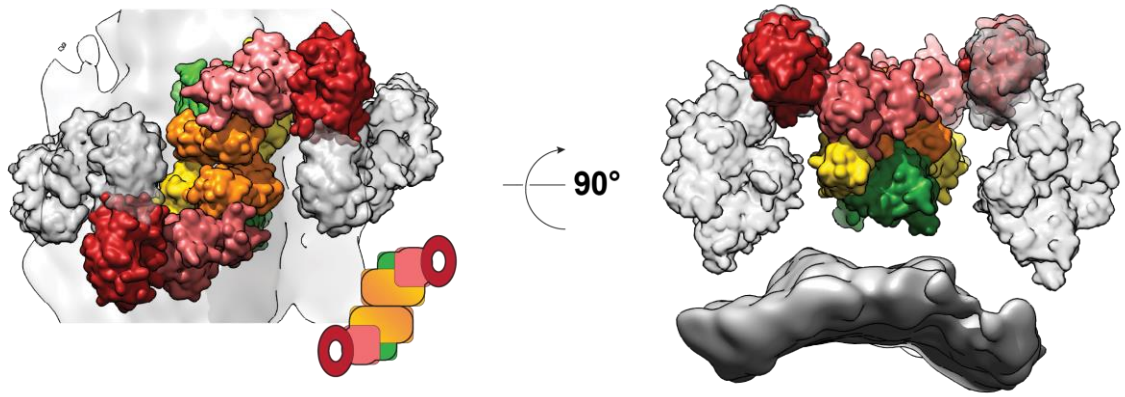

Figure 2. A potential LRRK2 structural model based on integrative modeling.

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