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Structural and functional analysis of p47 cofactor binding on the p97 disease mutant

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Human p97/VCP (valosin-containing protein) is a hexameric AAA+ (ATPase associated with diverse cellular activities) ATPase plays a pivotal role in the regulation of multiple cellular activities by interacting with various cofactor proteins. Critical roles of p97 involve ubiquitin-dependent protein quality control and regulation of membrane fusion in the Golgi apparatus in the presence of cofactor p47. Heterozygous missense mutations of p97 have been implicated in numerous neurodegenerative diseases, such as IBMPFD (Inclusion body myopathy with early-onset Paget's disease and frontotemporal dementia) and ALS (amyotrophic lateral sclerosis). The disease mutations of the p97 are mostly clustered on the N-domain or the connection between N and D1-domain. The single amino acid mutation of R155H on the N-domain is the highest mutated sites, leading to a rare degenerative disease multisystem proteinopathy 1 (MSP1) and resulting in abnormal ATPase activity and cofactor dysregulation. The structural details of the interaction between $p97^{R155H}$ and p47 cofactor. Our study aims to characterize the protein interactions and identify the key complex structure to answer disease relevance of the $p97^{R155H}$-p47 complex.

We pursued biochemical characterization in combination with single-particle cryo-electron microscopy (cryo-EM) to study $p97^{R155H}$ mutant interaction with p47 in the presence or absence of nucleotides. Additionally, to understand the functional behaviors of the complexes, we performed ATPase activity assays and fluorescent labeling to detect the temperature-related intensity change signals to guide quantitative determination of binding affinities of p47 for $p97^{R155H}$.

Our results report the cryo-electron microscopy (cryo-EM) structures of the full-length $p97^{R155H}$-p47 complex for the first time in different nucleotide-binding states. In the absence of nucleotides in the D1 nucleotide binding pocket, $p97^{R155H}$ is still stable either as a hexamer or stacked as $p97^{R155H}$ dodecamer. The $p97^{R155H}$ dodecameric structure revealed a highly ordered C-terminal tail density, not reported previously. This $p97^{R155H}$ dodecamer does not bind to p47 or nucleotides and bears close resemblance to the inhibitor bound CB-5083:p97 structure, implying that the dodecameric form is inactive. The highly symmetric dodecamer thus limits the mutant p97 ATPase activity and prohibits it from participating in any downstream substrate processing.

In the full-length $p97^{R155H}$-p47 complex structure, the p47 interacts through its UBX domain in an asymmetric manner to bind the $p97^{R155H}$ N-domain. In the absence of nucleotides, four N-domain densities and one $p47^{UBX}$ domain were identified by docking the atomic models into the density map. Consistent with previous findings, we saw that p47 does not bind to $p97^{R155H}$ as a trimer, instead there is a dynamic equilibrium for this $p97^{R155H}$-p47 interaction when the D1 and D2 nucleotide binding pockets are empty. We further employed deep coordinate neural network analysis to analyze data heterogeneity of the nucleotide bound states of $p97^{R155H}$-p47.The flexibility of the $p47^{UBX}$ bound N-domains were less for the $p97^{R155H}|_{ATP\gamma S}$-p47 dataset, whereas it is fragmented in $p97^{R155H}|_{ADP}$-p47 dataset. Superimposition of the D1 and D2 domains of the three different nucleotide states revealed that the D1 domains exhibit negligible structural change, whereas the D2 HTH motifs of the complexes with nucleotides are tilted downward. The C-terminal tails of the $p97^{R155H}|_{ATP\gamma S}$-p47 structure points in an opposite direction than that of $p97^{R155H}$-p47 and $p97^{R155H}|_{ADP}$-p47, hence implying the C-terminal tail conformation may influence the

p97 D2 ATPase activity. The structures also established that the D1 and D2 arginine fingers play a critical role for the elevated $p97^{R155H}$ ATPase activity. These recent findings give us an insight into the disease mechanism of the R155H mutation regarding its role in its pathological functions.

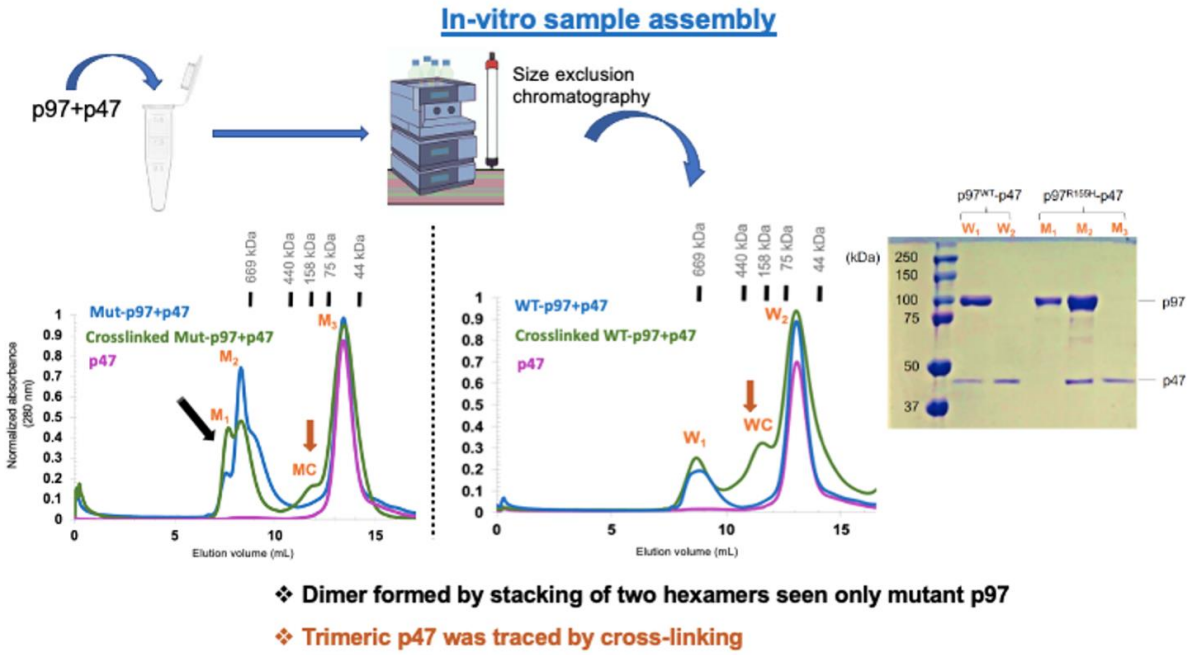

Figure 1. Size-exclusion chromatographic profile of the WT p97-p47 (red) and R155H p97-p47 assemblies (blue) on a Superose 6 Increase 10/300 GL column, respectively. The assemblies were identified on the first eluted peak fraction, but for the R155H mutant, an additional leading shoulder was appeared. b, SDSPAGE electrophoresis gel of the assemblies. The bands of the first elution peaks (#1 and #4) for both WT and mutant assemblies showed the presence of the p97 and p47

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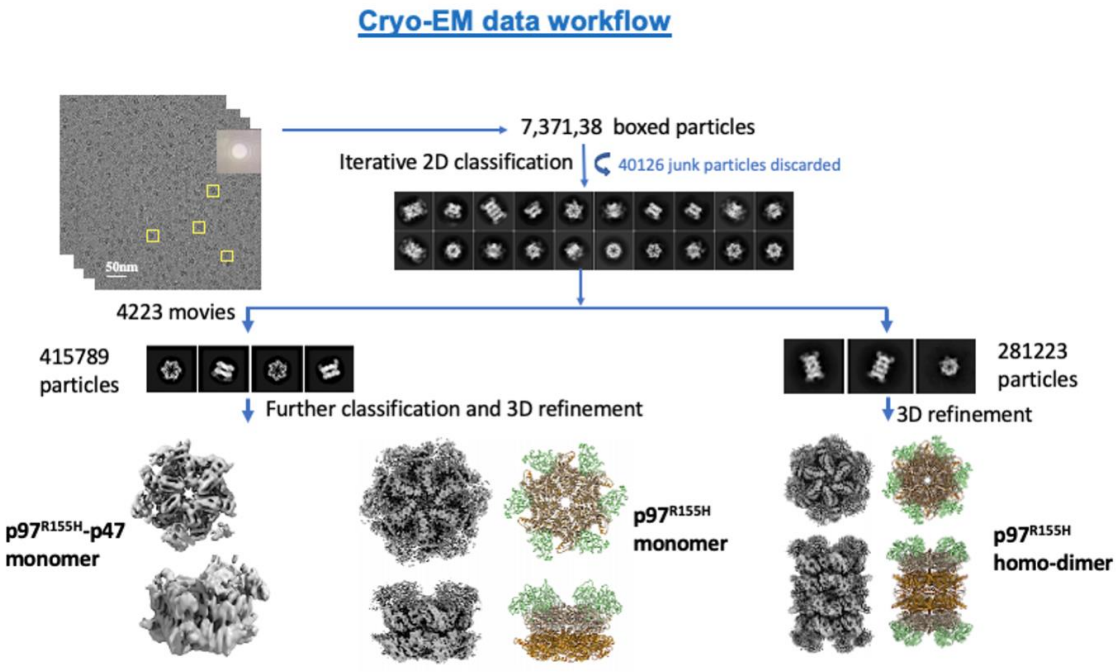

Figure 2. Representative electron micrograph of the cryogenic mutant p97-p47 assembly. The complex was shown in black and background as white. Scale bar indicates 50 nm. The insets are the representative 2D class averages of two distinct molecular species. The box side length of the upper inset is 269 Å and that of the lower is 348 Å. d, Cryo-EM reconstructions of different molecular species. Three models were reconstructed: a dimeric assembly of the p97 hexamers (left), a p97 hexamer (middle), and a p97 hexamer bound with a p47 trimer (right). The atomic coordinates were built along the cryo-EM densities. Light green is for N-domain, orange white for D1 ring, orange for D2 ring, and dark pink for p47 UBX domain.

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