Unraveling Cooperativity in Molecular Machines Using Methyl-TROSY

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Developments of TROSY-based NMR experiments and selective labeling schemes have enabled high-molecular-weight complexes and molecular machines to be investigated by solution NMR. Molecular machines are usually comprised of multiple subunits which often work in a concerted manner to achieve their biological functions. In order to understand cooperative structural changes and dynamics in homo-oligomeric molecular machines, we develop an approach where distinctively labeled subunits are constructed into the complex and investigated by methyl-TROSY-based experiments. We present here two examples of such an approach on (i) heptameric α_7-ring of the proteasome core particle from *Thermoplasma acidophilum* (180 kDa) and (ii) hexameric ND1 domain of human AAA+ ATPase p97 (320 kDa).

Proteasome plays an indispensable role in cellular homeostasis by degrading proteins no longer required for function. The process is in part controlled via gating residues localized to the ends of the barrel-like proteasome core particle (CP), which occlude substrate entry pores and prevent unregulated degradation (Figure 1A). In order to elucidate the mechanism of gating, we constructed a series of mixed proteasome rings such that the percentage of gate-containing subunits is varied, through which we addressed the energetics of gating and established whether gating is a cooperative process involving concerted action of residues from more than a single protomer. Our results showed that the intrinsic probability of a gate entering the lumen highly favors the *in* state (by close to 20-fold compared to the *out* state), that entry of each gate is noncooperative, with the number of gates accommodated inside the lumen a function of the entry pore size and the bulkiness of the gating residues. We also obtained insight into the origin of the high affinity for the *in* state from spin-relaxation experiments.

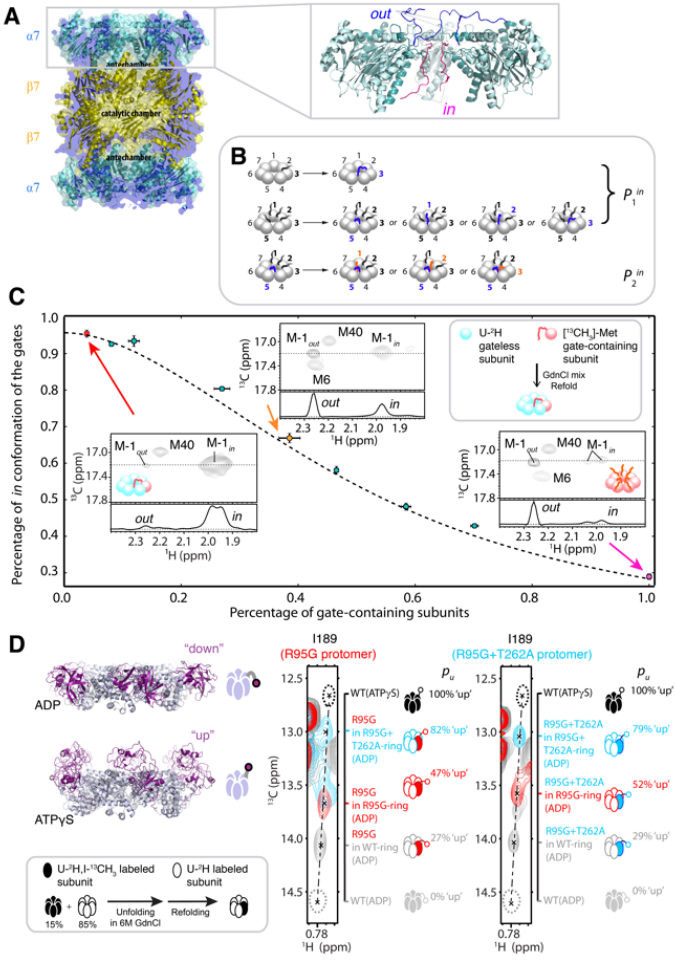

Figure 1. Probing the cooperativity of proteasome gating (A-C) and subunit dynamics in p97 (D) using methyl-TROSY. (A) Side view of the proteasome CP with α_7-ring enlarged highlighting the N-terminal gates. (B) Probabilities of the first and second gate adopting the *in* conformation, described by a probabilistic model. (C) Titration with varied percentages of gate-containing subunits in the complex. (D) Cooperative NTD dynamics in p97.

p97 is an essential hexameric AAA+ ATPase involved in a wide range of cellular processes. Mutations in the enzyme are implicated in the etiology of an autosomal dominant neurological disease affecting musculature, bone, and brain function. The N-terminal domain (NTD) of p97 undergoes conformational changes between a *down* and an *up* position during nucleotide hydrolysis; disease mutations shift the equilibrium from the *down* conformation in the ADP-bound state progressively toward the *up* conformation as a function of disease severity. Given that patients are heterozygous containing one copy each of WT and disease-causing mutant genes, *in vivo*, p97 molecules can be heterogeneous in subunit composition. To understand NTD functional dynamics in biologically relevant p97 heterohexamers comprising both WT and disease-causing mutant subunits, we performed a study on a series of constructs in which only one of the protomer types is NMR-labeled. Our results showed positive cooperativity of NTD *up/down* equilibria between neighboring protomers, allowing us to define interprotomer pathways that mediate the allosteric communication between subunits. Notably, the perturbed *up/down* NTD equilibrium in mutant subunits is partially restored by neighboring WT protomers, as is the two-pronged binding of the UBXD1 adaptor that is affected in disease.

References

1. Huang R, Pérez F, Kay LE (2017) *Proc Natl Acad Sci USA* 114:E9846-E9854.
2. Huang R, Ripstein ZA, Rubinstein JL, Kay LE (2019) *Proc Natl Acad Sci USA* 116:158-167.