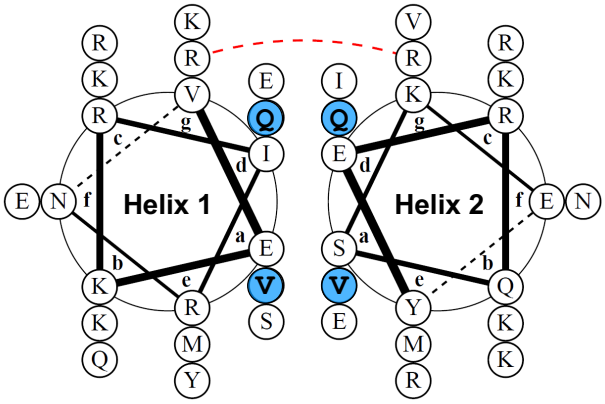
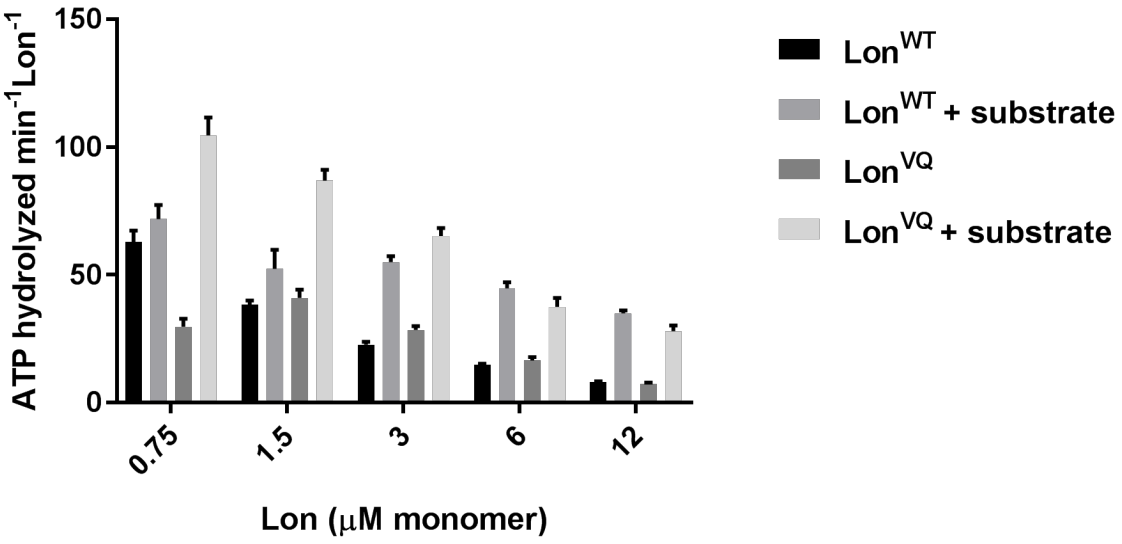


Supplemental Figure 1. Bioinformatics analysis of hypothesized coiled-coil register. Related to Figure 1B-E

E. coli Lon N-domain residues 209-228 were entered in DrawCoil 1.1 (http://www.grigoryanlab.org/drawcoil/) to generate the helical wheel diagram for the antiparallel coiled coil. Residues V217 and Q220 (highlighted in blue) can form disulfide bonds (Fig 1C-D), supporting their role in mediating antiparallel coiled-coil interactions. This register positions V217 and Q220, heptad positions a and d, respectively, in the closest possible proximity to one another. Although V217C and Q220C would not be positioned directly across from one another, the a and a' and d and d' positions of the respective helices can interact in a non-canonical antiparallel coiled-coil configuration. The red dashed line indicates a potential interfacial electrostatic bridge.



Supplemental Figure 2. Lon ATP hydrolysis activity in the presence of substrate. Related to Figure 4A

ATPase activity of Lon^{WT} and Lon^{VQ} was measured with and without 40 μM carboxymethylated $titin^{I27}$-sul20 substrate. Activity rates were determined as described in text and were determined from three independent experiments, each performed in triplicate. Error bars represent SEM.