Structural Impact of Three Parkinsonism-Associated Missense Mutations on Human DJ-1

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SUPPORTING INFORMATION

Protein	Dimeric molecular weight (calculated; Da)	Dimeric molecular weight (fit; Da)
Wild-type	40345	37793
A104T	40405	35264
E163K	40343	40876
M26I	40309	36407

Table S1. Comparison of theoretical and fit molecular weights for dimeric DJ-1 determined from the representative sedimentation equilibrium ultracentrifugation experiment illustrated in Figure 3. The theoretical molecular weights are those calculated for the tag-cleaved protein (see Experimental Procedures), which includes a GSH- "scar" sequence at the N-terminus of each protein resulting from thrombin cleavage of the N-terminal hexa-histidine tag.

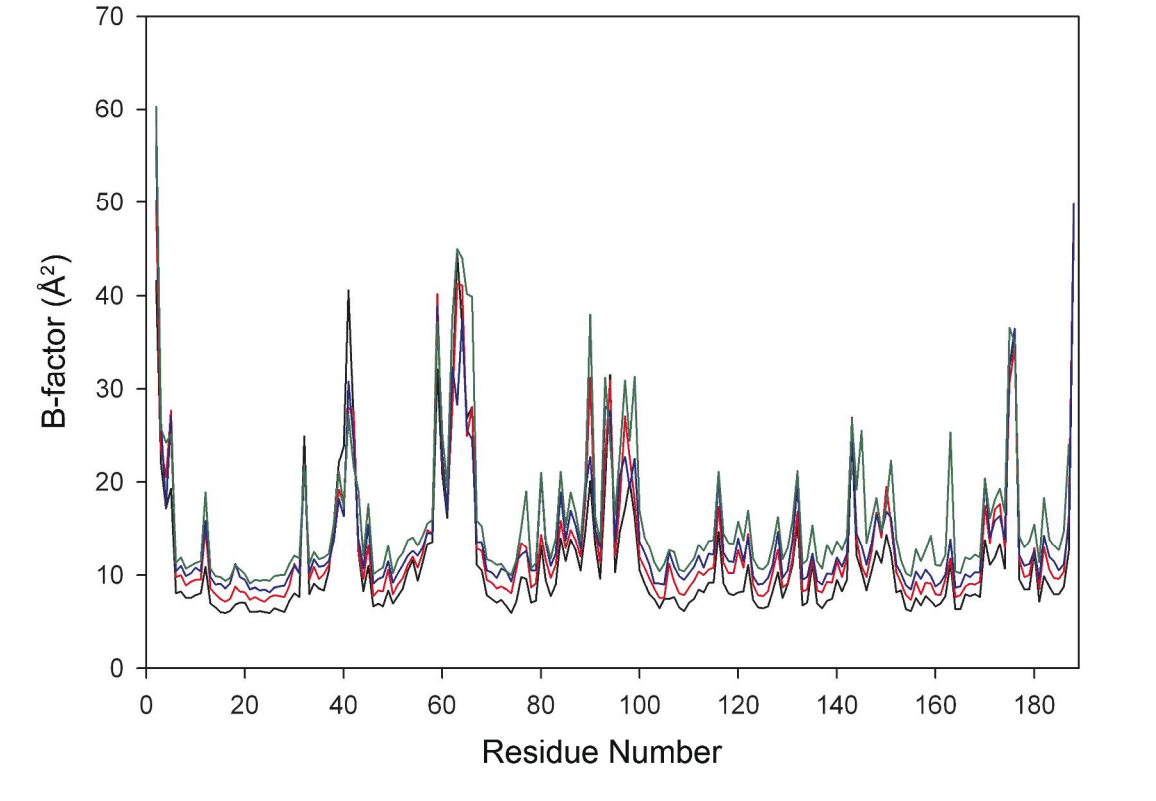
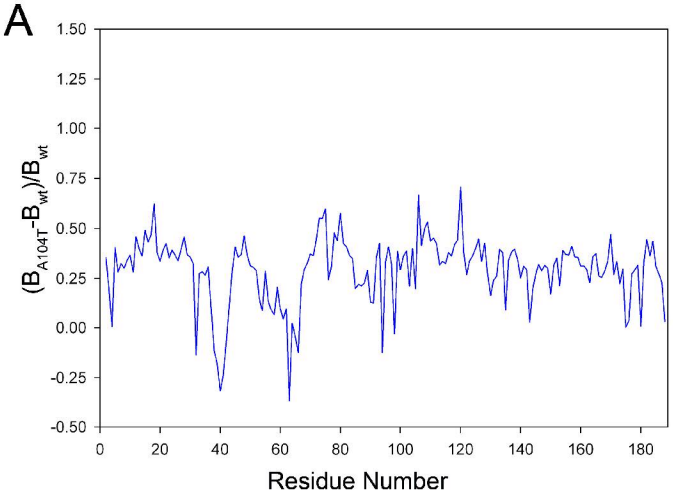


Figure S2. Comparison of refined B_{eq} factors for DJ-1. The residue averaged B-factors for wild-type (PDB 1SOA; black), M26I (red), A104T (blue) and E163K (green) DJ-1 are shown as a function of residue number. Each structure was refined in SHELX-97 with anisotropic displacement parameters; therefore the B_{eq} is the isotropic equivalent B-factor as reported in the PDB. Overall, the four proteins display very similar distributions of B-factors, with some noticeable differences for E163K from residues 140-180. Interestingly, the average B-factor for each protein is inversely correlated with its T_m (see Table 1 and Figure 2), as demonstrated above by the B-factor baseline offsets that increase as measured stability of the protein decreases (rank order of stability: wt>M26I>A104T>E163K). We note that many experimental factors can influence the overall B-factor, however, and this correlation may be coincidental.

A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40
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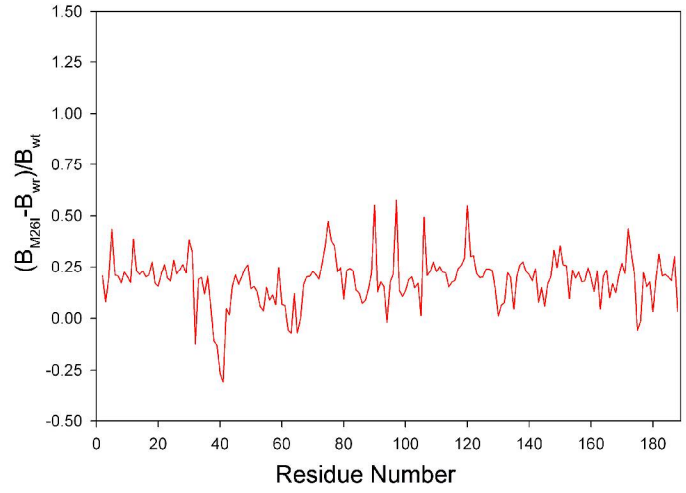
80
100
120
140
160
180
Residue Number
(B_A104T-B_wt)/B_wt

B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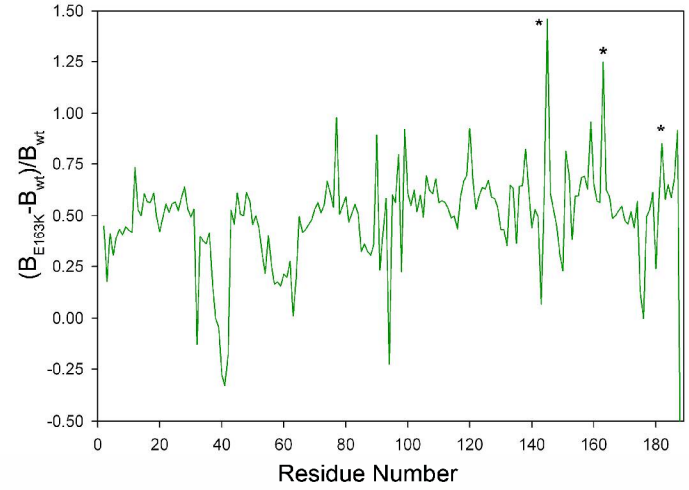
Residue Number
(B_M26I-B_wt)/B_wt

C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40
60
80
100
120
140
160
180
Residue Number
(B_E193K-B_wt)/B_wt
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Figure S3. Normalized difference B-factors for each of the three PD-associated DJ-1 mutations. In each panel, the difference between the residue averaged B-factor of the mutant protein and wtDJ-1 (PDB 1SOA) is divided by the residue averaged B-factor of the wild-type protein ($\frac{\{B_{mut} - B_{wt}\}}{B_{wt}}$) to provide a residue averaged difference B-factor that is weighted by the B-factors of the wild-type protein. This is an approximate form of error scaling, since larger B-factors in the wild-type protein will be associated with larger errors, and the corresponding normalized difference B-factor will be smaller. Panel A shows A104T DJ-1 (blue), Panel B shows M26I DJ-1 (red) and Panel C shows E163K DJ-1 (green). All three mutants show lower mobility than wtDJ-1 in two regions centered around residues 40 and 60. The most significant elevations in normalized difference B-factors are seen in E163K (Panel C), where residues 145, 163 and 186 (indicated with asterisks) are significantly more mobile in the mutant protein. These residues are involved in a dimer-spanning network of hydrogen bonds that is disrupted by the E163K substitution (Figure 7).