Supplemental Material:

Transient sampling of aggregation-prone conformations causes pathogenic instability of a parkinsonian mutant of DJ-1 at physiological temperature

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Figure S1

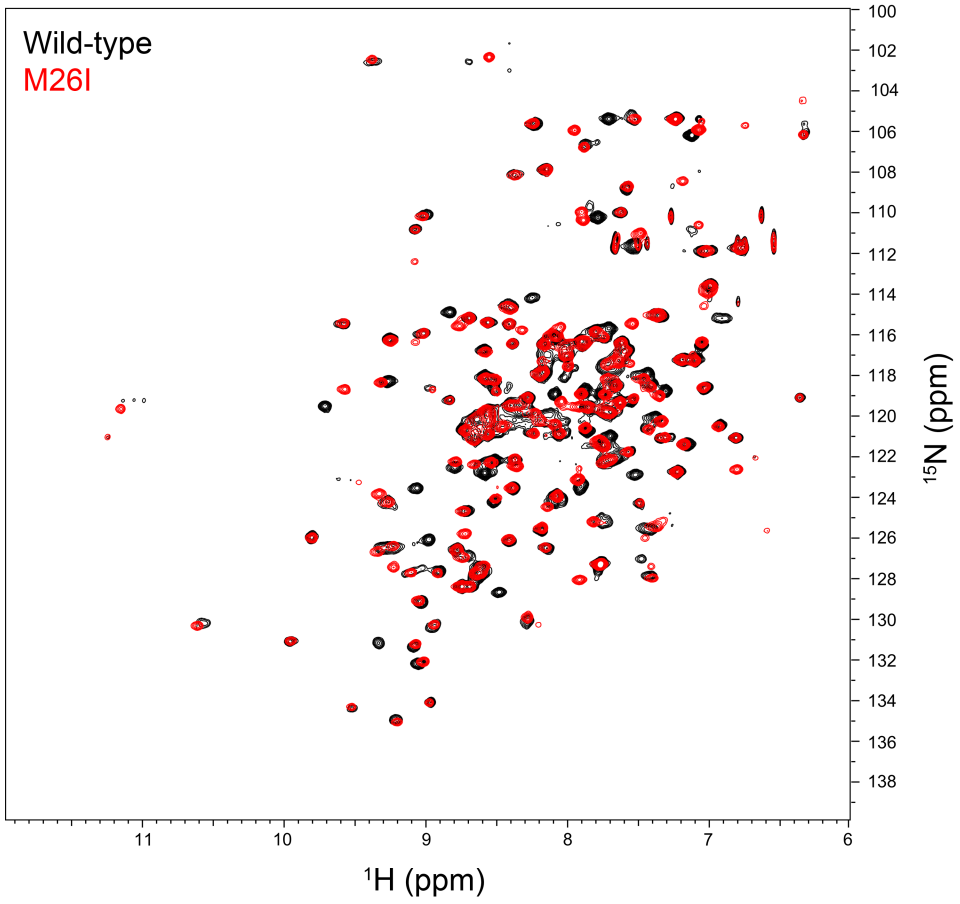

Fig. S1: Wild-type and M26I DJ-1 have similar 2D ^{1}H-^{15}N HSQCs at 35°C. The 2D ^{1}H-^{15}N HSQC spectrum for wild-type (WT, black) is overlaid with the spectrum for M26I DJ-1 (red). M26I DJ-1 resonances mostly overlap with those observed for WT DJ-1, as was previously shown by Malgieri and Eliezer [17].

Figure S2

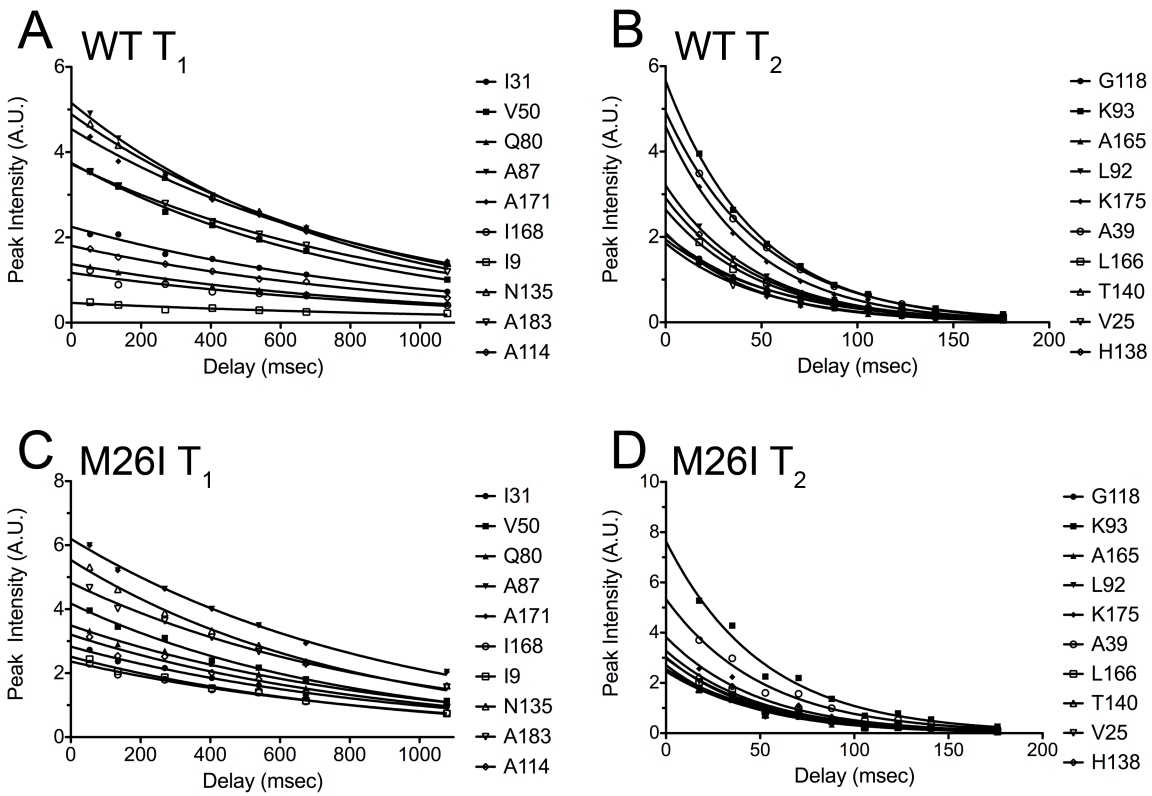

Fig. S2: T_1, T_2 relaxation curves of select residues in wild-type and M26I DJ-1 at 35°C.

A, C: Wild-type (WT) and M26I T_1 relaxation fits for ten randomly chosen amino acids. **B, D**: WT and M26I T_2 relaxation fits for the same amino acids as in **A**. All data were fitted to EQ. 1 in 'Materials and Methods'.

Table S1

ModelFree Parameters for Wild-type and M26I DJ-1

	Wild-type DJ-1	**M26I DJ-1**
Model Free Input		
NOE[1]	0.779 ± 0.0997	0.770 ± 0.0871
R_1	1.158 ± 0.1398	1.219 ± 0.288
R_2	19.303 ± 2.309	18.821 ± 1.727
R_2/R_1	16.877 ± 2.643	15.911 ± 2.271
Model Free Output		
S^2	0.912 ± 0.0788	0.934 ± 0.0544
S^2_f	0.876 ± 0.0492	---
S^2_s	0.916 ± 0.0724	0.934 ± 0.0544
t_e	1313.9 ± 714.923	622.75 ± 656.975
R_{ex}[2]	3.188 ± 1.688	1.356 ± 1.917
SSE	1.977 ± 2.742	1.545 ± 2.238

Values in the table are averages ± SD

[1]The NOE values are reported after the removal of NOE values > 1, which include residues:

WT: 27 (1.00±0.196), 80 (1.20±0.34), 112 (1.04±0.78), 156 (1.06±0.60), 157 (1.04±0.49), and 168 (1.10±0.36)

M26I: 15 (1.06±0.21), 133 (1.64±0.12), 161 (1.62±0.11), 169 (1.62±0.11), 186 (1.64±0.16)

[2]The R_{ex} values reported may include values of zero in the average and standard deviations

Figure S3

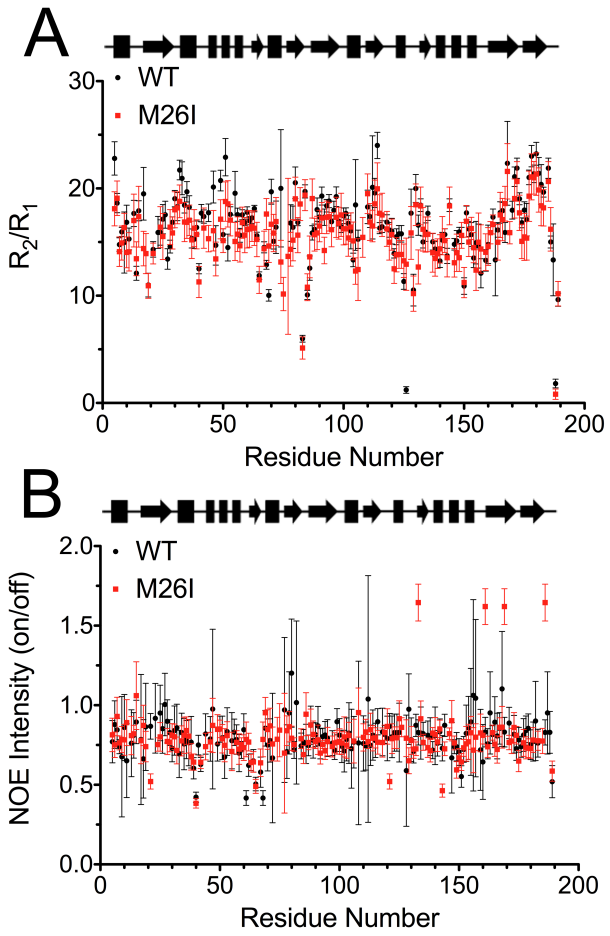

Fig. S3: The R_2/R_1 and heteronuclear NOE ratios are similar for WT and M26I DJ-1 at 35°C. **A**: The ratio of relaxation rates (R_2/R_1) is plotted for every observed backbone amide resonance in both wild-type (WT, black) and M26I (red) DJ-1. The R_2/R_1 ratio is similar for both proteins at 35°C. **B**: WT (black) and M26I (red) heteronuclear NOE intensity ratios (see EQ. 2 in 'Materials and Methods) are plotted. Four heteronuclear NOE ratios for M26I DJ-1 are not bound by 0 and 1; Met133, Ser161, Val169, and Val186. This is likely due to inadequate equilibration of NOE saturation for these few residues and they were omitted from the fitting.

Table S2

S^2 Values for Assigned Residues in Wild-type and M26I DJ-1

See attached Excel File: “TableS2_S2_WT_M26I.xlsx”

Figure S4

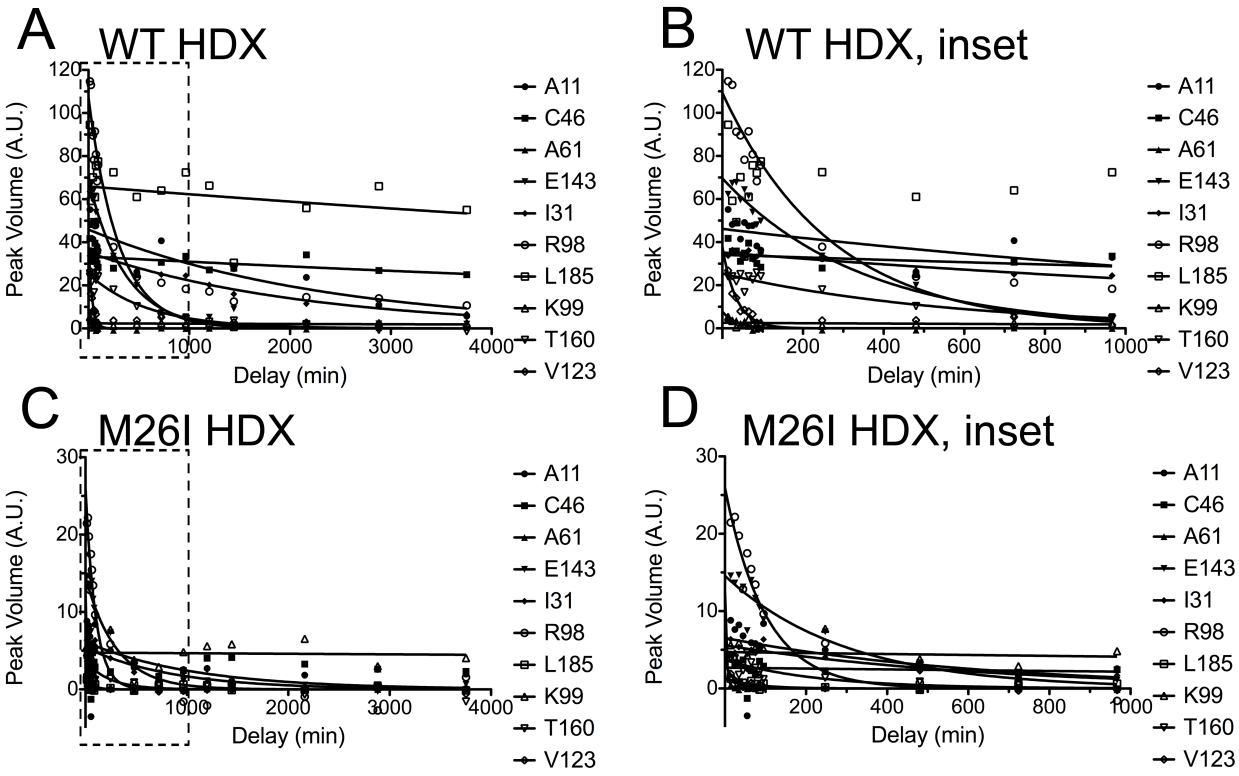

Fig. S4: HDX curves of select residues in WT and M26I DJ-1. **A, C**: Fitted decay curves for ten amino acids in wild-type (WT) and M26I DJ-1 HDX, spanning a range of different $\log_{10}$PF values. **B, D**: Expanded view of the boxed region in **A** and **C**, respectively. For WT HDX, Leu185 (open squares) data could not be adequately fitted to an exponential decay function due to lack of exchange with D_2O on this time scale (compare L185 in **A, B**). This was uncommon in the dataset and is shown to represent the full range of data quality obtained. All data were fitted to EQ. 1 in 'Materials and Methods'.

Table S3

Log_{10}PF Values for Assigned Residues in Wild-type and M26I DJ-1

See attached Excel File: “TableS3_WT_M26I_ProtFactCalc.xlsx”

Figure S5

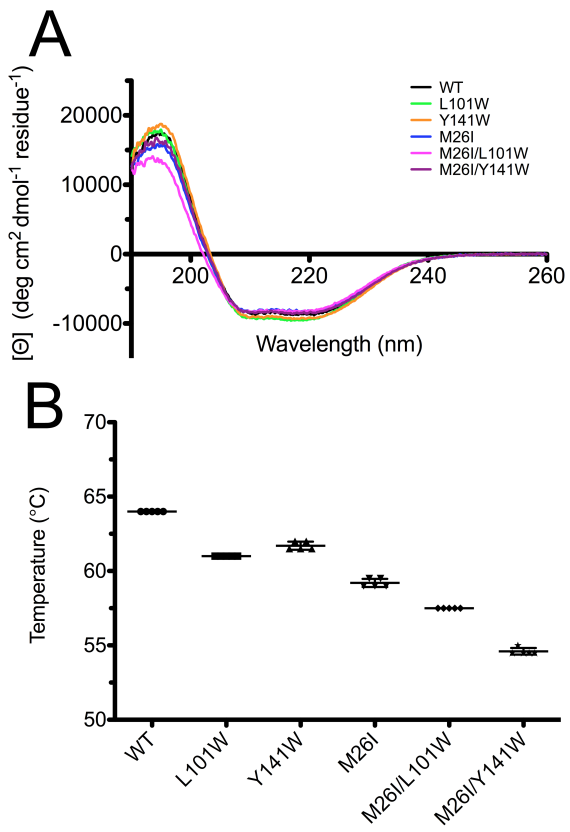

Fig. S5: Tryptophan mutations have modest effects on thermal stability and secondary structure of DJ-1 at 37°C. **A:** Circular dichroism (CD) spectra were collected at 37°C for wild-type (WT) (black), M26I (blue), L101W (green), M26I/L101W (magenta), Y141W (orange), and M26I/Y141W DJ-1 (purple). These proteins show no significant differences in the mean residue molar ellipicity ([Θ]) as a function of wavelength, indicating that the Trp mutations are not highly disruptive to DJ-1 structure. **B**: Thermal stability for all proteins in **A** was measured using the thermofluor assay in five separate experiments per sample. Consistent with the CD spectroscopy data (**A**), only small changes are observed. Mean melting temperatures (T_m, °C) are as follows: WT (64.0), L101W (61.0), Y141W (61.7±0.3), M26I (59.2±0.3), M26I/L101W (57.5), and M26I/Y141W (54.6±0.2).

Figure S6

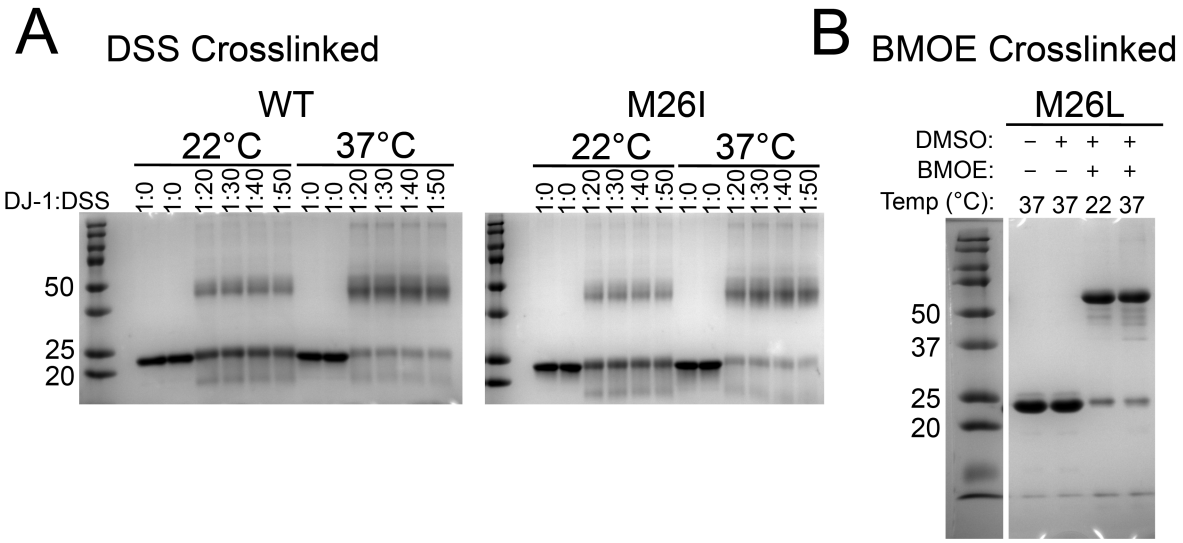

Fig. S6: Cross-linking behavior of DJ-1 proteins. A: Primary amine cross-linking with DSS for wild-type (WT, left) and M26I (right) DJ-1 resolved by SDS-PAGE. Cross-linking was performed at 1:20, 1:30, 1:40, and 1:50 (DJ-1:DSS) at both 22 and 37°C. Samples in lanes 1 and 2 for both gels are the input and protein + DMSO controls. There is no difference in DSS cross-linking between WT and M26I DJ-1 at either temperature. **B**: BMOE cross-linking for M26L DJ-1 is similar to that of wild-type DJ-1 (see Fig. 4A), consistent with this mutation being non-disruptive.