The Parkinsonism-associated protein DJ-1 is an antagonist, not an eraser, for protein glycation

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Supporting Information

Supporting Figures

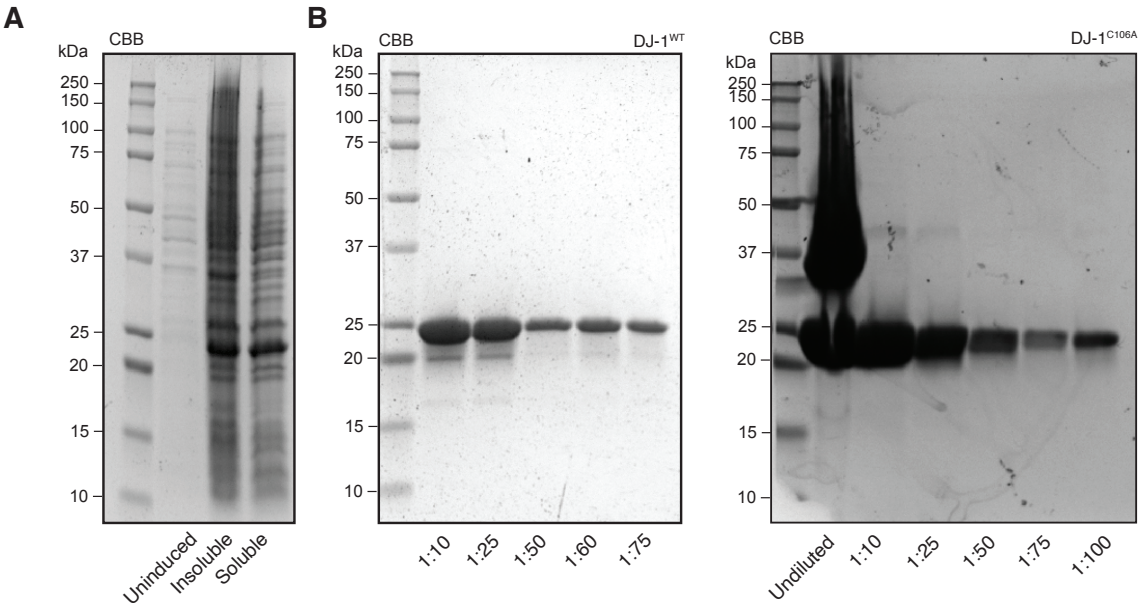

Figure S1. DJ-1 Expression and Purification. A) His-tagged DJ-1^{WT} was expressed in BL21(DE3) *E. coli*. After sonication, protein was centrifuged and purified using a Ni-NTA column and then buffer exchanged into 20 mM PBS. **B)** Using serial dilution, purity was estimated to be >95% by densitometry for both DJ-1^{WT} and DJ-1^{C106A} using SDS-PAGE and Coomassie Brilliant Blue staining.

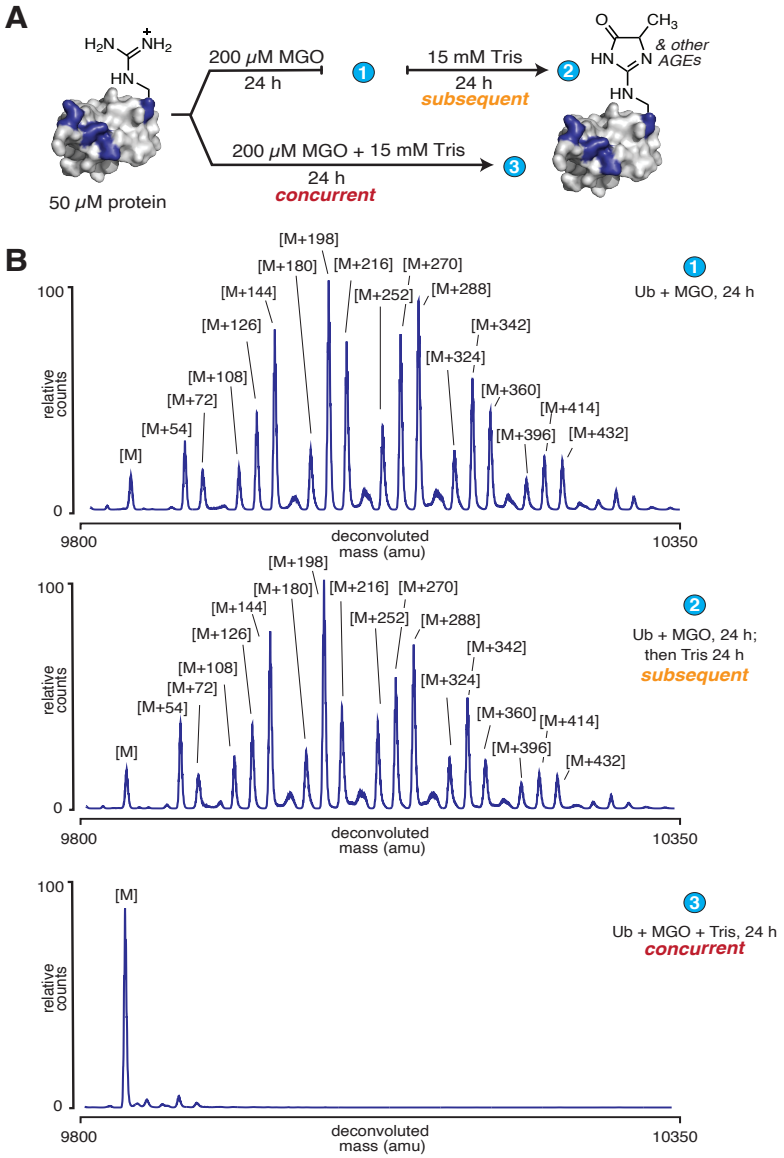

Figure S2. Tris is a robust quench for glycation. A) To evaluate the use of Tris as a robust quench for MGO, 50 μM Ub was treated with 200 μM MGO for 24 h at 37 ºC in the presence or absence of 15 mM Tris buffer. For the sample treated only with MGO, after 24 h of incubation, 15 mM Tris was added and the reaction was incubated at 37 ºC for another 24 h. **B)** Representative intact protein mass spectrometry data for each of the indicated samples (1, 2, and 3, as indicated). These data show that there are no substantive changes in overall glycation of Ub during the additional 24 hours of incubation with 15 mM Tris (comparing 1 to 2). These data also show that glycation cannot occur in the presence of Tris (comparing 1 to 3). These data demonstrate that Tris is an effective quench for glycation.

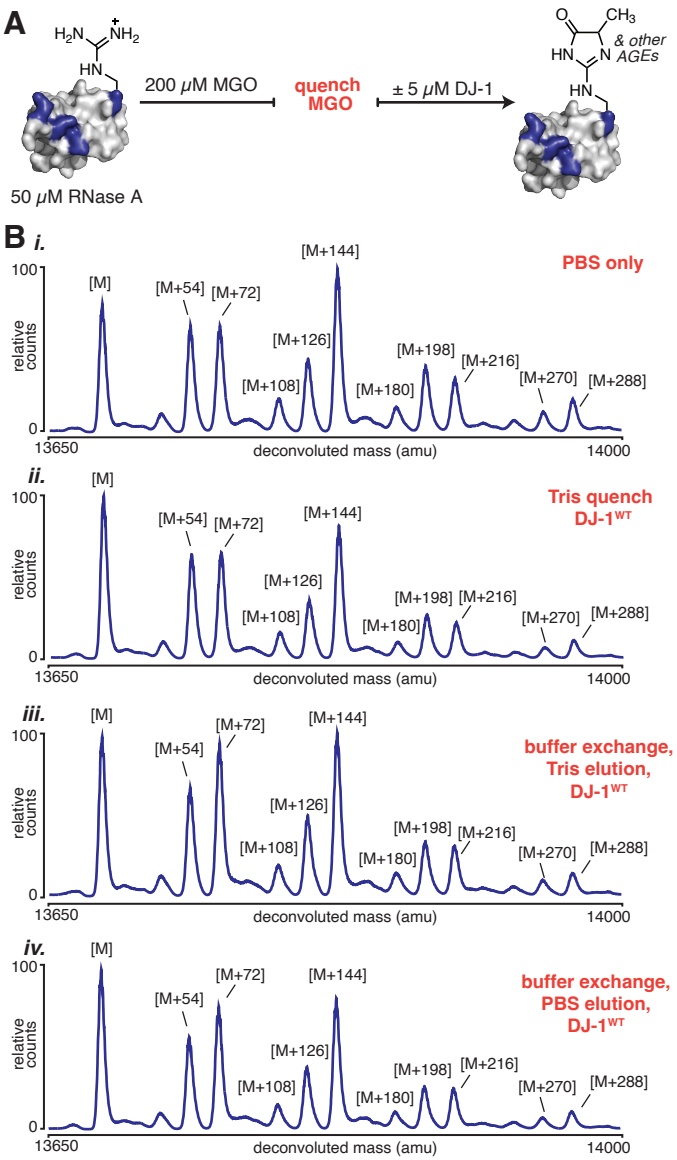

Figure S3. Removal of MGO by different methods does not impact DJ-1 activity. A) To determine the effects of Tris buffer on DJ-1 activity, 50 μM RNAse was treated with 200 μM MGO for 24 h at 37 °C before undergoing different methods of MGO quenching. After quenching or removal of MGO, the glycation reactions were subjected to subsequent treatment with DJ-1^{WT} for 24 h at 37 °C and then analyzed by LC-MS. **B)** Representative intact protein mass spectrometry data for each protocol used to remove MGO: ***i.*** PBS buffer only (no DJ-1^{WT}); ***ii.*** Tris buffer quench (15 mM final concentration) with subsequent DJ-1^{WT} treatment; ***iii.*** buffer exchange (Nap5 columns, Cytiva) into 20 mM Tris buffer with subsequent DJ-1^{WT} treatment; and ***iv.*** buffer exchange (Nap5 columns, Cytiva) into 20 mM PBS with subsequent DJ-1^{WT} treatment. No differences in DJ-1^{WT} activity were detected between the different quench methods.

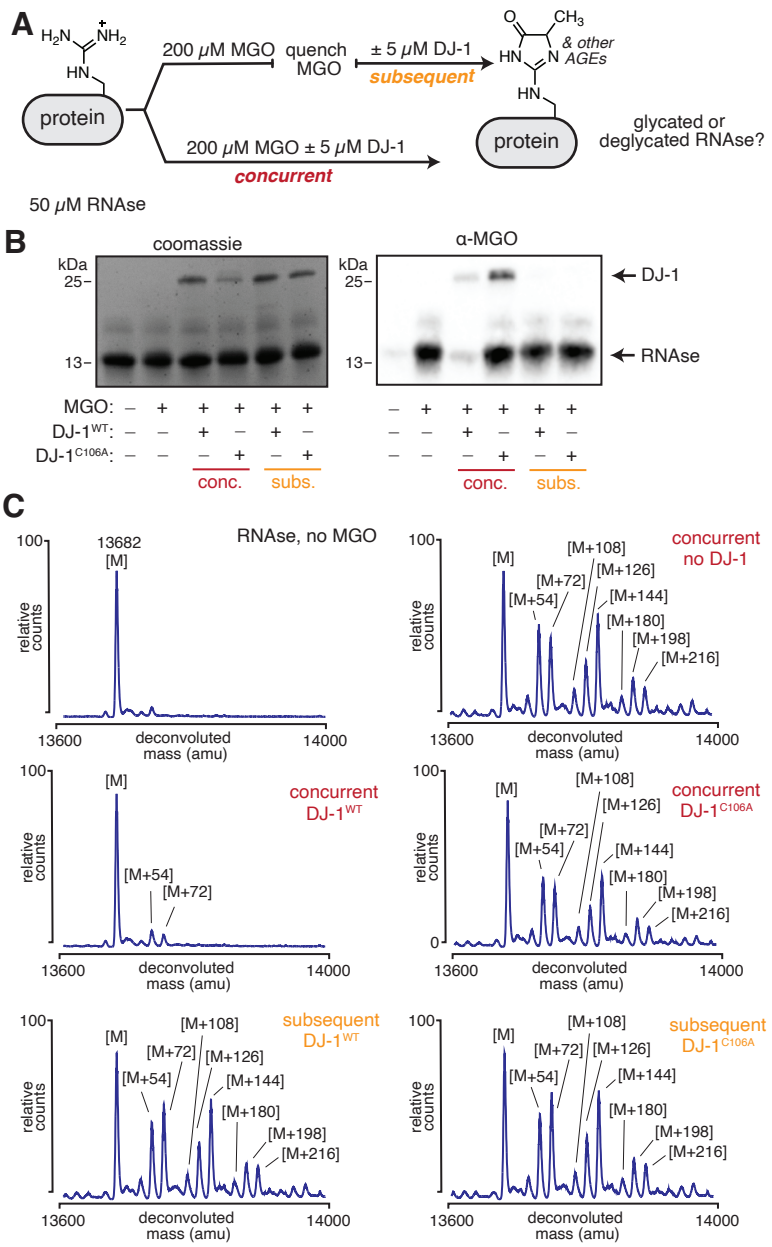

Figure S4. DJ-1^{WT} prevents glycation of ribonuclease A during concurrent incubations. A) To assess the potential deglycase activity of DJ-1, two sets of conditions were evaluated. In the "concurrent" treatment, DJ-1 was co-incubated with both RNAse A and MGO for 24 h at 37 ºC. In the "subsequent" treatment, RNAse A was first glycated by MGO for 24 h at 37 ºC and the excess MGO was quenched with Tris buffer. Afterwards, DJ-1 was incubated with the glycated protein for an additional 24 h at 37 ºC. **B)** Using SDS-PAGE and western blotting against MGO, it was possible to observe a decrease in glycation from concurrent treatments with wild-type DJ-1 (DJ-1^{WT}), but not a catalytically-inactive DJ-1 variant (DJ-1^{C106A}). **C)** These same findings were obtained using intact protein mass spectrometry following subsequent or concurrent DJ-1 treatments.

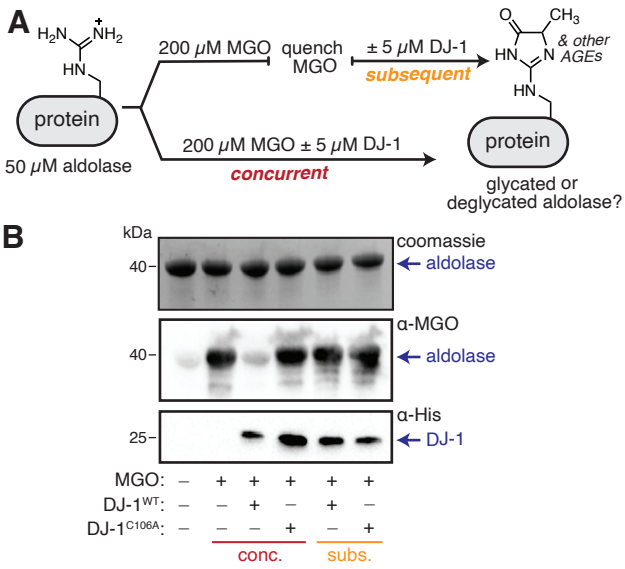

Figure S5. DJ-1^{WT} prevents glycation of aldolase during concurrent incubations. A) To assess the potential deglycase activity of DJ-1, two sets of conditions were evaluated. In the "concurrent" treatment, DJ-1 was co-incubated with both aldolase and MGO for 24 h at 37 ºC. In the "subsequent" treatment, aldolase was first glycated by MGO for 24 h at 37 ºC and the excess MGO was quenched with Tris buffer. Afterwards, DJ-1 was incubated with the glycated protein for an additional 24 h at 37 ºC. **B)** Using SDS-PAGE and western blotting against MGO and the DJ-1 His-tag (loading control), it was possible to observe a decrease in glycation only from concurrent treatments with wild-type DJ-1 (DJ-1^{WT}), but not a catalytically inactive DJ-1 variant (DJ-1^{C106A}).

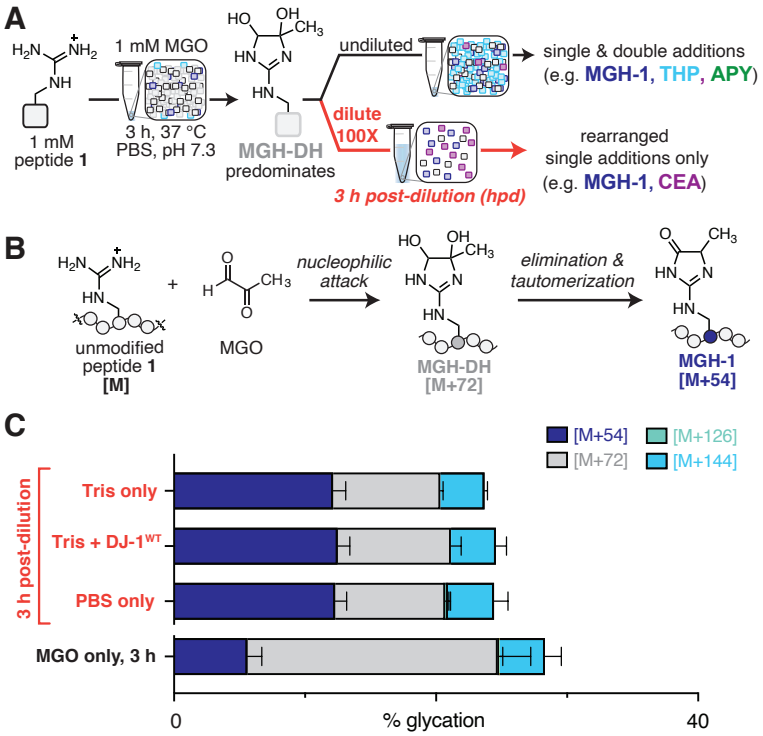

Figure S6. DJ-1^{WT} does not affect the conversion of MGH-DH to MGH-1. A) Treatment of peptide **1** with equimolar concentrations of MGO (1 mM) leads to the formation of many AGEs including those generated from the double addition of MGO (see also main text **Figure 1**). To evaluate the potential for DJ-1 to impact the proportional distribution of AGEs, we used a dilution protocol we previously developed.[1] Briefly, peptide **1** was treated using our standard reaction conditions for 3 h and was then diluted 100X. This slows the formation of AGEs that require a second MGO equivalent, but allows intramolecular reactions and/or rearrangements to proceed without competition. **B)** In our past work, use of this protocol allowed us to confirm that MGH-1 spontaneously forms from MGH-DH, through a likely elimination and tautomerization mechanism.[1] **C)** To assess the potential for DJ-1 to influence the conversion of MGH-DH to MGH-1, we incubated peptide **1** with equimolar MGO (1 mM) for 3 h at 37 ºC. At this point, the reaction was diluted 100X in either phosphate-buffered saline (PBS), Tris buffer alone, or Tris buffer with DJ-1^{WT}, and the reaction was allowed to incubate at 37 ºC for another 3 **h**ours **p**ost-**d**ilution (hpd) before analysis by LC-MS. Stacked bar graphs show the distribution of AGE adducts. This experiment reveals that the formation of MGH-1 from MGH-DH is unaffected by the presence of DJ-1^{WT}. It also further confirms our previous findings that MGH-DH is meta-stable and can either proceed to form MGH-1, or reverse to release free MGO and increase levels of unmodified peptide.[1]

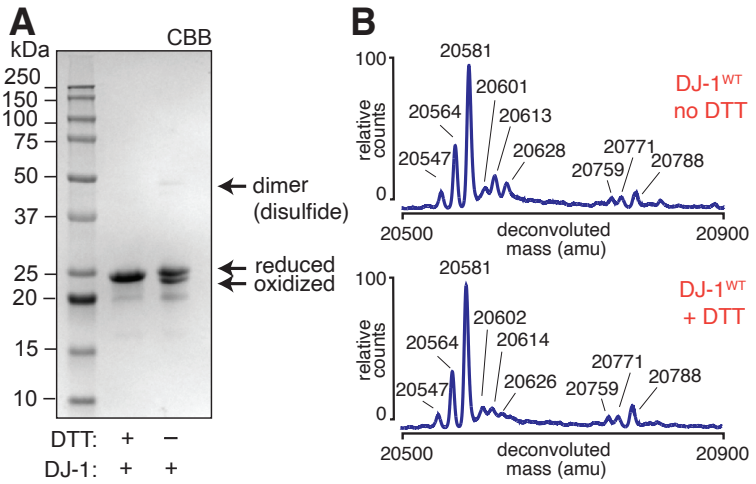

Figure S7. Purified DJ-1 is minimally oxidized A) We examined DJ-1^{WT} for disulfide linkage or other oxidative modifications by comparing DJ-1^{WT} treated with and without the reducing agent dithiothreitol (DTT) using SDS-PAGE. **B)** Intact protein mass spectrometry was also used to evaluate DJ-1 oxidation by treating DJ-1^{WT} with and without DTT prior to analysis by LCMS. We found that even when stored solely in PBS, purified DJ-1^{WT} exhibits minimal oxidative modifications. An intramolecular disulfide bond is known to occur between residues Cys46 and Cys53 and is necessary for tertiary structure.[2]

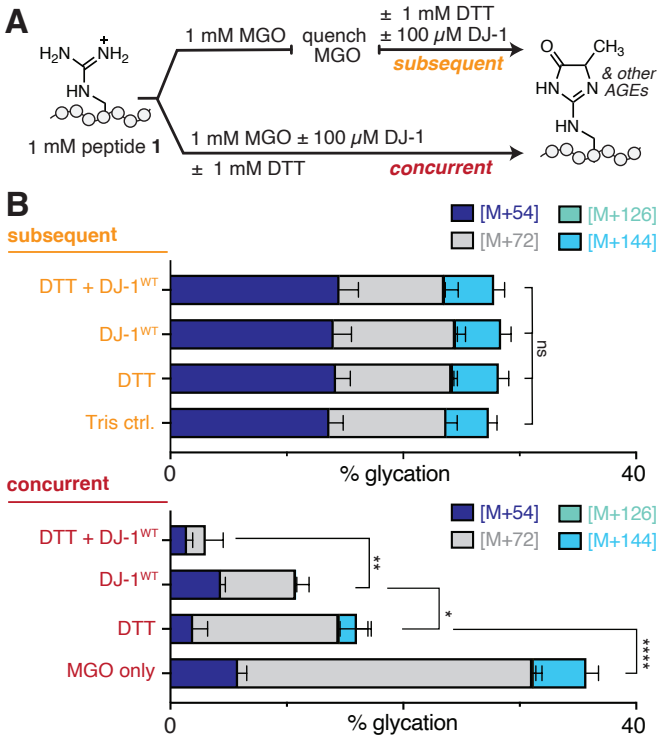

Figure S8. Free thiols inhibit glycation independent of DJ-1 activity A) Ac-LESRHYA, (peptide **1**) was allowed to react with equimolar MGO (1 mM) in 20 mM PBS at pH 7.3 for 3 h at 37 °C, and was then analyzed by LC-MS after "subsequent" or "concurrent" treatment with DJ-1 and/or DTT. **B)** Distribution of glycation products observed by LC-MS after DJ-1 and/or DTT treatment. Decreases in glycation were only observed in "concurrent", not "subsequent", treatments. While the addition of either DTT or DJ-1^{WT} was able to reduce glycation during concurrent incubations, there was also an additive effect where even greater reductions in glycation were observed in the presence of both DTT and DJ-1^{WT}. Stacked bar graphs are plotted as mean ± standard deviation for each mass adduct. A non-directional (two-tailed) one-way ANOVA using Dunnett's multiple comparison test was used to determine statistically significant differences in total glycation compared to peptide **1** treated with MGO, $p<0.0001$(****), $p<0.01$(**), $p<0.05$(*).

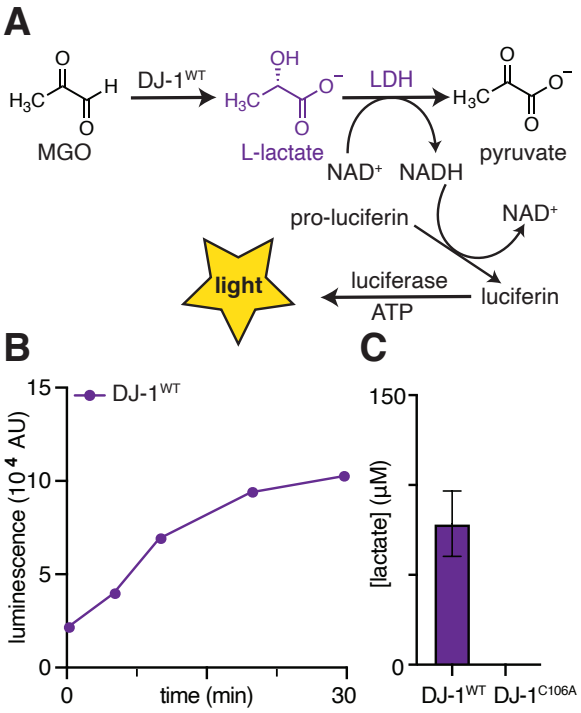

Figure S9. DJ-1 generates lactate from MGO. A) Using a commercially available kit (Lactate-Glo™, Promega), the formation of L-lactate can be monitored by an enzyme-coupled assay that generates detectable luminescence in the presence of L-lactate. **B)** When 1 μM DJ-1^{WT} was incubated at 37 ºC in 20 mM PBS at pH 7.3 with 200 μM MGO, it was possible to observe the formation of lactate within 30 min. **C)** It was possible to quantify the amount of lactate produced after 200 μM MGO was incubated for 30 min at 37 ºC with either 10 μM DJ-1^{WT} or DJ-1^{C106A} in 20 mM PBS at pH 7.3.

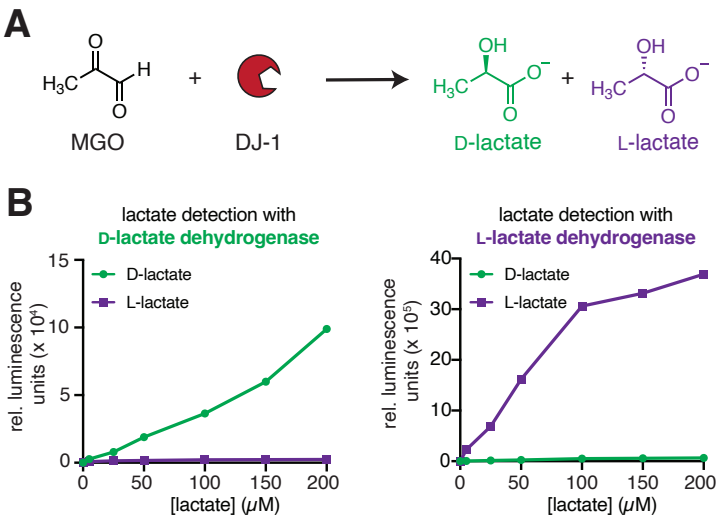

Figure S10. L- and D-lactate dehydrogenases are stereospecific. A) Previous work has suggested that DJ-1 glyoxalase activity stereospecifically generates L-lactate, while DJ-1 "deglycase" activity leads to a mixture of L- and D-lactate stereoisomers.[3,4] **B)** To evaluate the stereospecificity of DJ-1 glyoxalase activity, we adapted the commercially available lactate detection kit for use with L- and D-lactate dehydrogenases (LDH). We confirmed that when L- or D-LDH are incubated with their proper substrate, significant increases in luminescence are observed as lactate concentration increases. However, when lactate and LDH are mismatched, minimal luminescence is detected even as the mismatched substrate concentration increases.

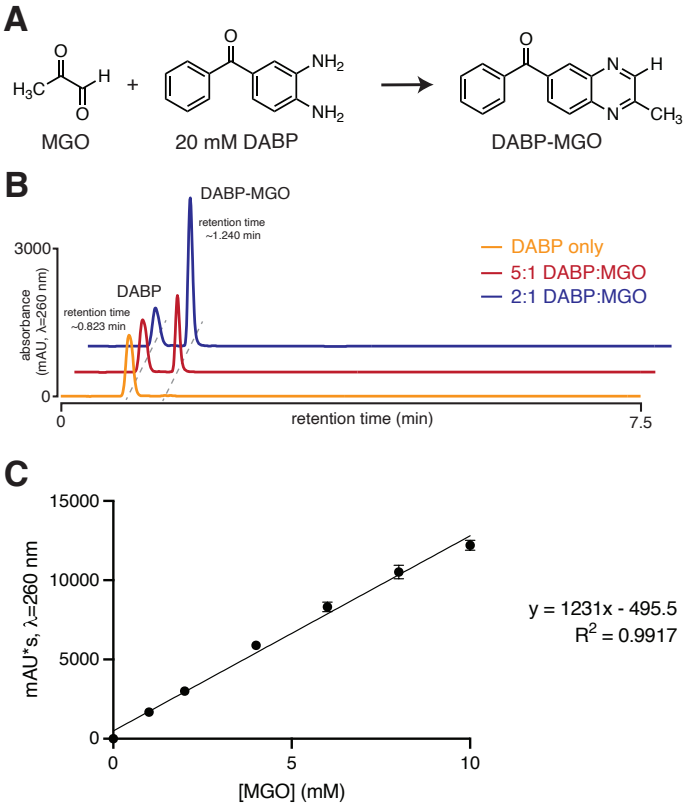

Figure S11. A quantitative HPLC assay to quantify MGO. A) DABP binds free MGO in solution to form a product that can be tracked by HPLC using an absorbance wavelength of 260 nm. **B)** Representative chromatograms shows that DABP elutes at approximately 0.823 minutes while the MGO-DABP peak elutes at approximately 1.240 minutes. Peaks are inversely proportional: as the concentration of MGO increases, the unmodified DABP peak decreases in absorbance, while the MGO-DABP peak increases in absorbance. **C)** A representative standard curve was generated using a 20 mM DABP stock solution and MGO concentrations varying from 0 mM to 10 mM. Each MGO concentration was replicated in triplicate and a high degree of linearity was displayed between MGO concentrations and the corresponding integrated MGO-DABP peak.

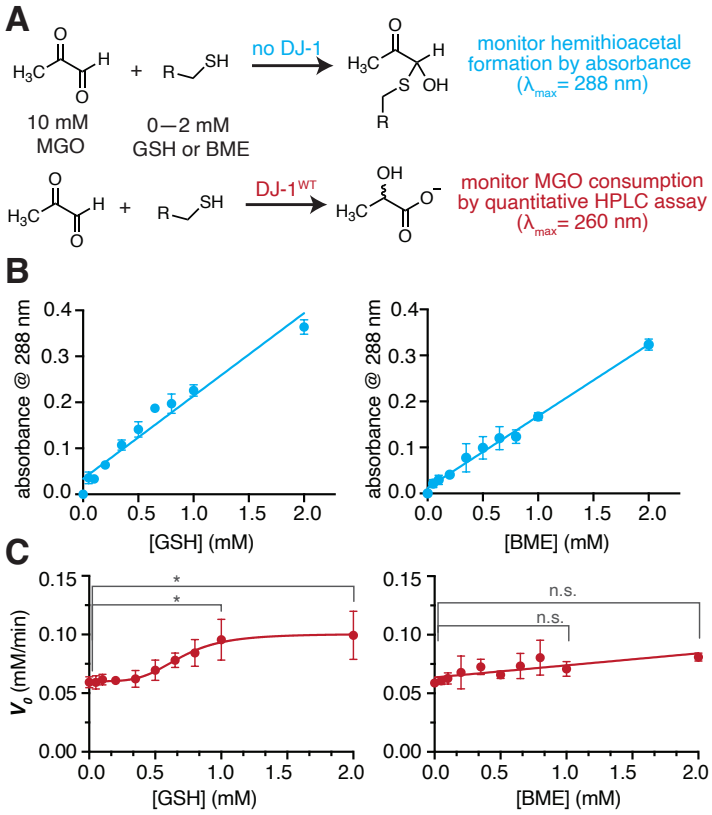

Figure S12. Glutathione is an allosteric activator for DJ-1. A) To further assess the hypothesis that GSH is an allosteric activator for DJ-1, we evaluated hemithioacetal formation and the DJ-1 reaction velocity (V_0) for 10 mM MGO co-incubated with a range of thiol concentrations (0—2 mM). The same protocols were performed for GSH or another molecule containing a single thiol, β-mercaptoethanol (BME). **B)** The extent of hemithioacetal formation was similar for both GSH (left) and BME (right), based on absorbance measurements taken at 288 nm. C) The quantitative HPLC assay using DABP (see **Figure S11**) was used to monitor the reaction velocity (V_0=-d[MGO]/dt) over 30 min of treatment with 100 mM DJ-1^{WT} and MGO (10 mM initial concentration), while titrating the GSH or BME concentrations from 0—2 mM. There was a sigmoidal relationship between GSH concentration and V_0, while the addition of BME did not appear to influence the reaction velocity. An unpaired student's T-test was used to determine statistically significant differences between V_0 at 0 mM and either 1 mM or 2 mM GSH ($p<0.05$(*)) or BME, (n.s.). We note that a newly prepared stock of DJ-1^{WT} exhibited higher specific activity than previous preparations of enzyme that were used in main text Figure 5. Nonetheless, the trends we observe are consistent across experiments with different enzyme stocks. Taken together, we can conclude that the observed increase in DJ-1 glyoxalase activity in the presence of GSH is more likely due to cooperativity between DJ-1 and GSH, rather than preferential deglycation of hemithioacetals.

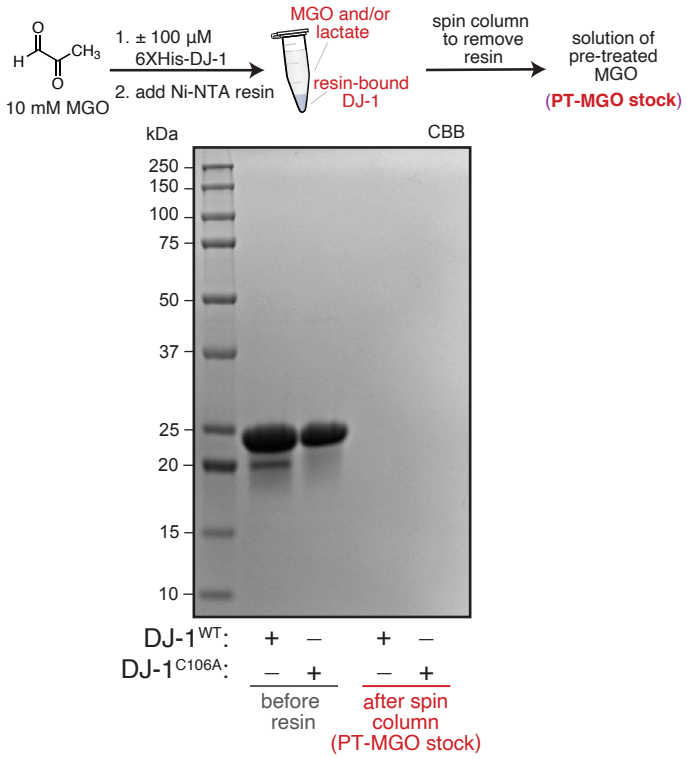

Figure S13. Controls for pre-treatment assay. SDS-PAGE and Coomassie Brilliant Blue staining of flow-through from spin column following the addition of Ni-NTA resin indicate full removal of DJ-1 from pre-treatment sample. Samples with either DJ-1^{WT} or DJ-1^{C106A} are both shown to have an absence of protein after incubation with resin and collection of the supernatant using a spin column.

Supporting References

(1) McEwen, J. M.; Fraser, S.; Guir, A. L. S.; Dave, J.; Scheck, R. A. Synergistic Sequence Contributions Bias Glycation Outcomes. *Nat Commun* **2021**, *12* (1), 3316.

(2) Song, I. K.; Kim, M. S.; Ferrell, J. E.; Shin, D. H.; Lee, K. J. Stepwise Oxidations Play Key Roles in the Structural and Functional Regulations of DJ-1. *Biochem J* **2021**, *478* (19), 3505–3525.

(3) Choi, D.; Kim, J.; Ha, S.; Kwon, K.; Kim, E.-H.; Lee, H.-Y.; Ryu, K.-S.; Park, C. Stereospecific Mechanism of DJ-1 Glyoxalases Inferred from Their Hemithioacetal-Containing Crystal Structures. *FEBS J.* **2014**, *281* (24), 5447–5462. https://doi.org/10.1111/febs.13085.

(4) Richarme, G.; Mihoub, M.; Dairou, J.; Bui, L. C.; Leger, T.; Lamouri, A. Parkinsonism-Associated Protein DJ-1/Park7 Is a Major Protein Deglycase That Repairs Methylglyoxal- and Glyoxal-Glycated Cysteine, Arginine, and Lysine Residues. *J Biol Chem* **2015**, *290* (3), 1885–1897.