Supporting Information: Arabinose Alters Both Local and Distal H-D Exchange Rates in the *Escherichia coli* AraC Transcriptional Regulator

Alexander Tischer[1], Matthew J. Brown[2], Robert F. Schleif[2*], and Matthew Auton[1*]

[1] Division of Hematology, Departments of Internal Medicine and Biochemistry and Molecular Biology, Mayo Clinic, Rochester, Minnesota, 55905, USA.

[2] Department of Biology, Johns Hopkins University, Baltimore, Maryland , 21218, USA.

[*] To whom correspondence is addressed, auton.matthew@mayo.edu and rfschleif@jhu.edu.

Author Contributions: AT performed and analyzed the Hydrogen-Deuterium Exchange Mass Spectrometry (HXMS) experiments. MJB expressed and purified the AraC dimerization domain and the full-length AraC protein. RFS and MA designed the research. MA wrote the paper.

Running Title: H-D Exchange in AraC.

Keywords: HXMS, Allostery, Arabinose, Operon, AraC, Dimerization Domain, DNA Binding Domain, Linker, Helix Capping.

Abbreviations Used: HXMS, Hydrogen Deuterium Exchange Mass Spectrometry.

1 Contents:

This Supporting Information document contains supplemental figures for the hydrogen deuterium exchange mass spectrometry figures in the main manuscript. **Fig.1** contains peptide maps obtained from on-column pepsin cleavage of the AraC dimerization domain and full-length AraC containing both the dimerization and DNA binding domains. These maps were used by EXMS2 and HDSite to identify deuterated peptides [1] and resolve residue specific hydrogen exchange [2] . Peptides covering the structural regions of importance including the arabinose binding and allosteric regions are indicated. **Fig.2** shows exchange fraction area plots as a function of residue number at the indicated incubation times from 10s to 24hrs. **Figs.3-4** show exchange fraction kinetics for various residues in the AraC dimerization domain from 10s to 24hrs. Likewise, **Fig.5** shows exchange fraction area plots as a function of residue number at the indicated incubation times from 10s to 1h. Note that at the higher incubation times we see less coverage of the protein exchanging due to aggregation of the full-length AraC protein in D_2O. This is likely a result of a slight kosmotropic effect of D_2O as the hydrogen bonding of D_2O is weaker than H_2O [3]. Arabinose appears to stabilize full-length AraC against aggregation, but ultimately the arabinose bound AraC also aggregates at incubation times of hours or longer. **Figs.6-7** show exchange fraction kinetics for various residues in the full-length AraC protein between 10s and 30min.

2 Accession ID

The uniprot accession ID for AraC is P0A9E0 (ARAC_ECOLI).

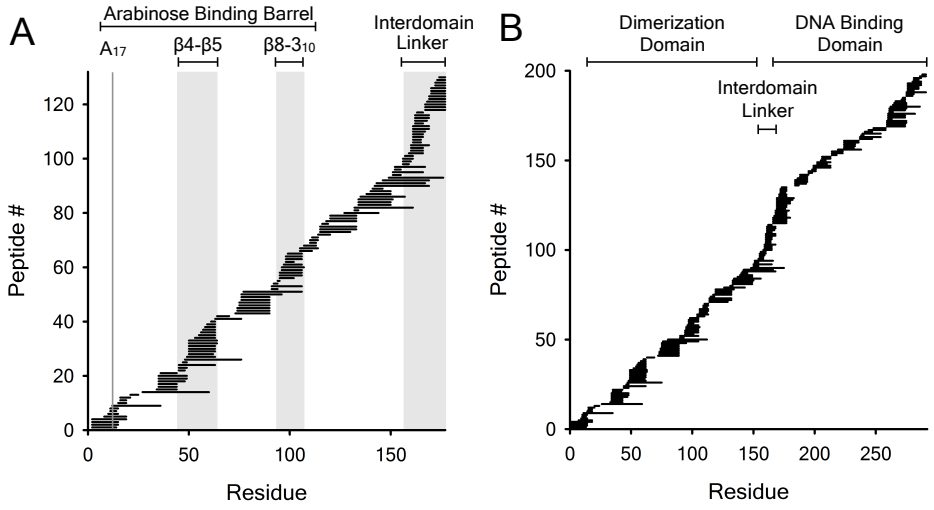

Figure S1: A) Pepsin peptide coverage map of the AraC dimerization domain. B) Pepsin peptide coverage map of the full length AraC protein containing both the dimerization and DNA binding domains. The coverage shows that the domains are mapped by many overlapping peptides that provide high resolution of the dynamic flexibility of the AraC transcription repressor.

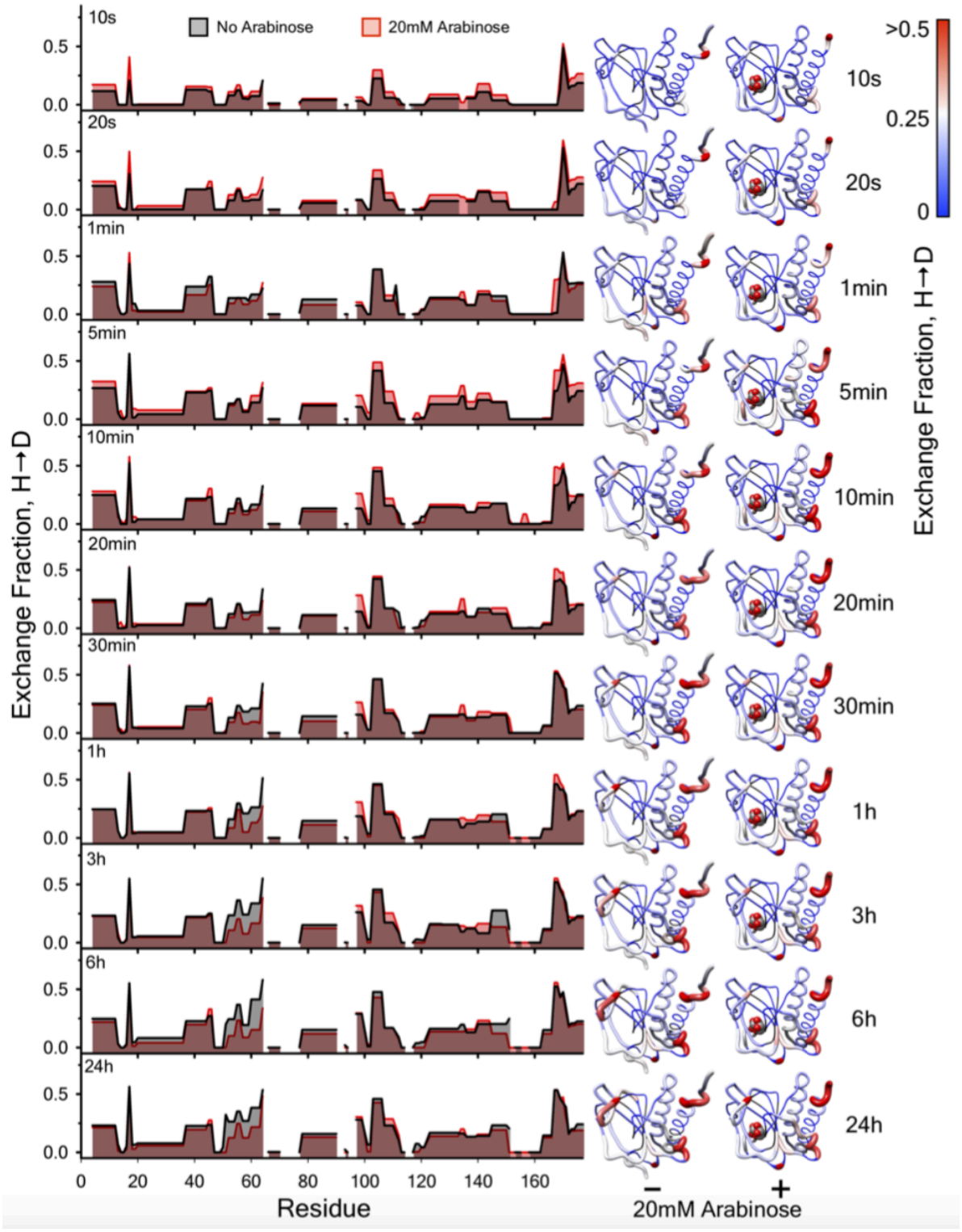

Figure S2: Left: Site resolution of the HX exchange fraction throughout the AraC dimerization domain in the presence (red) and absence of 20mM arabinose (black) plotted as a function of the residue number. Right: HX exchange fraction is mapped onto the structure of the AraC dimerization domain (pdb ID = 2ARC [4]). Black = not resolved, blue = 0, white = 0.25, red $\geq$ 0.5. Rendered using UCSF Chimera [5].

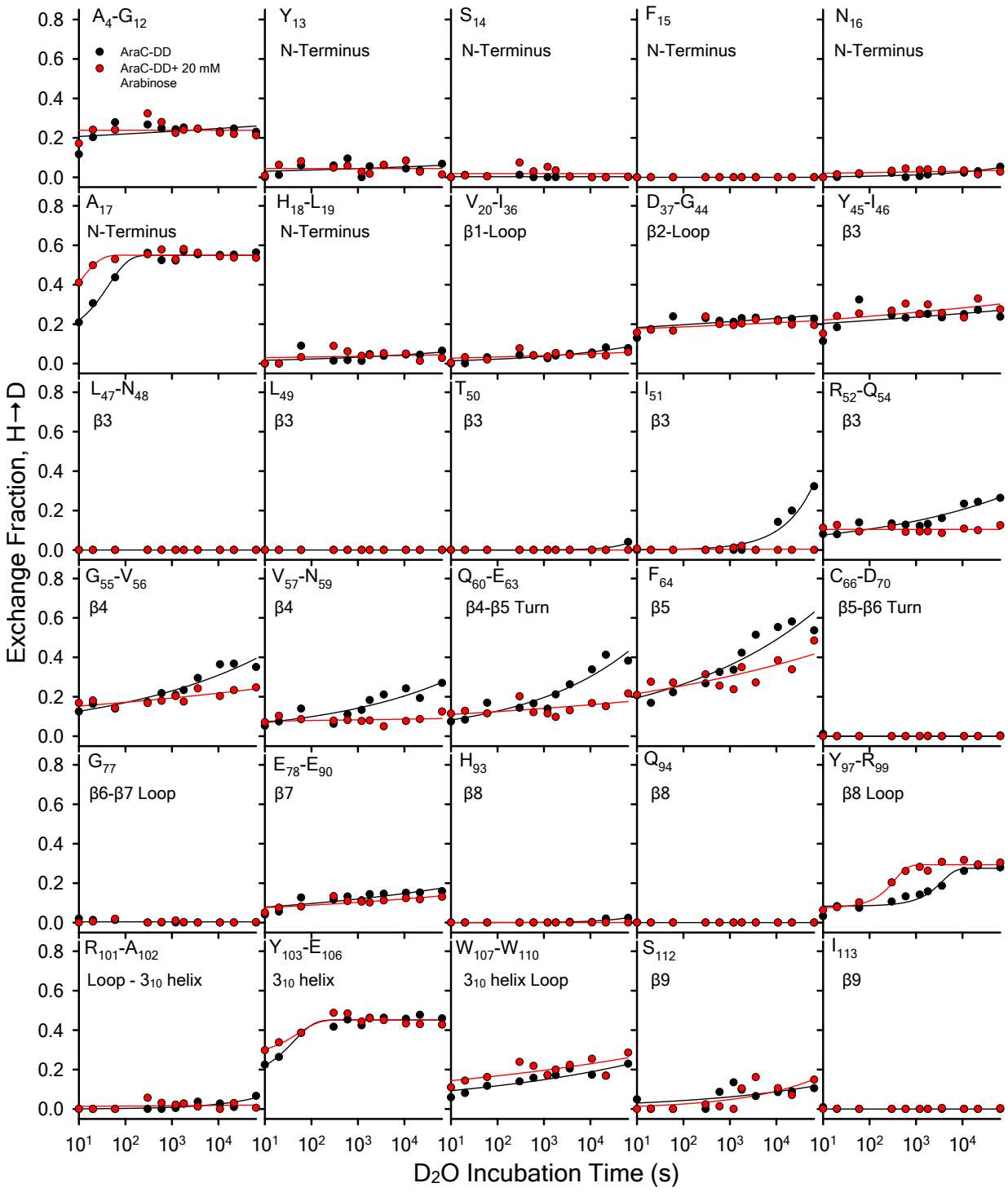

Figure S3: HX Kinetics of site-resolved residues throughout the AraC dimerization domain in the presence (red circles) and absence of 20mM arabinose (black circles). Part 1 of 2.

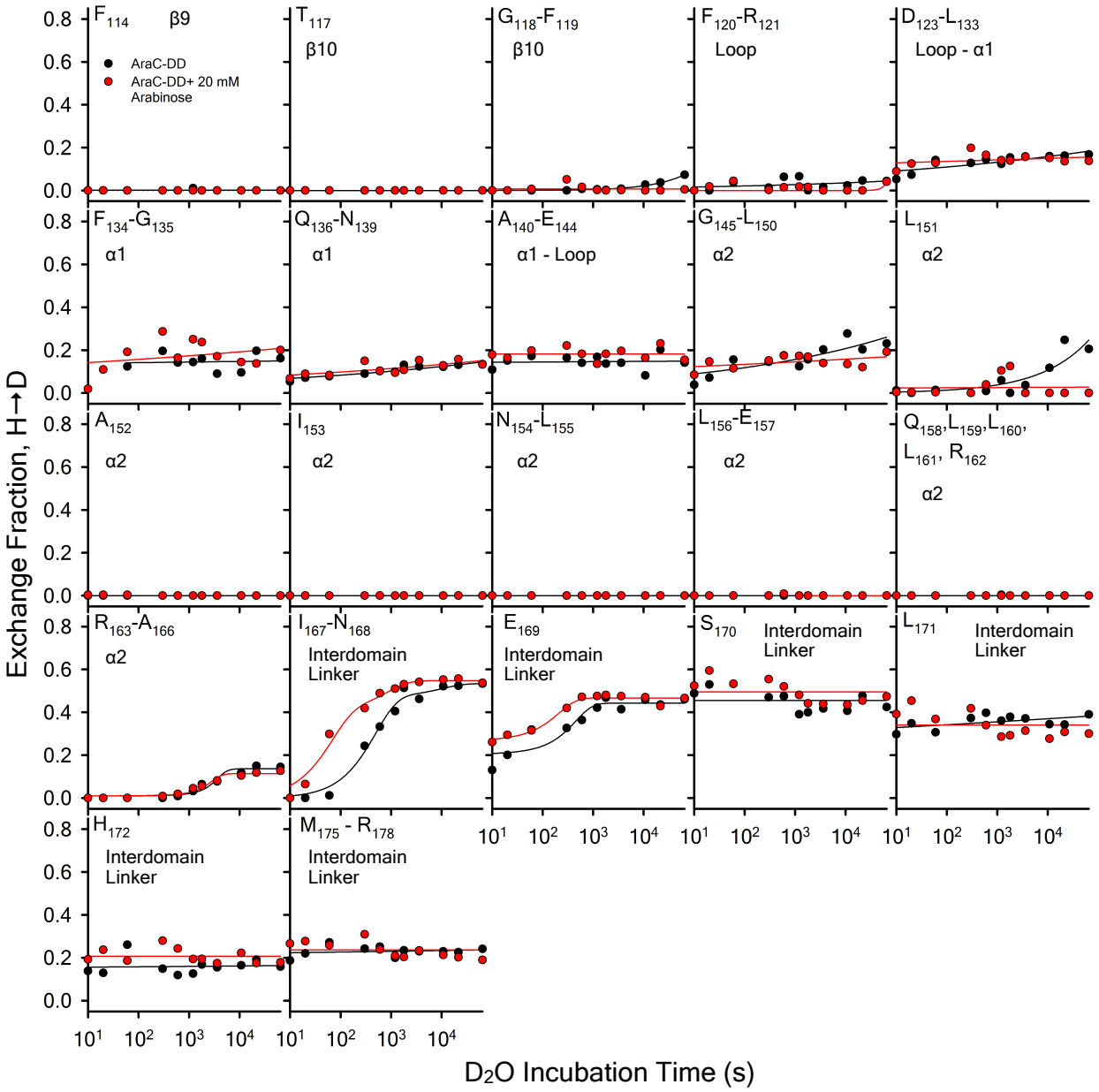

Figure S4: HX Kinetics of site-resolved residues throughout the AraC dimerization domain in the presence (red circles) and absence of 20mM arabinose (black circles). Part 2 of 2.

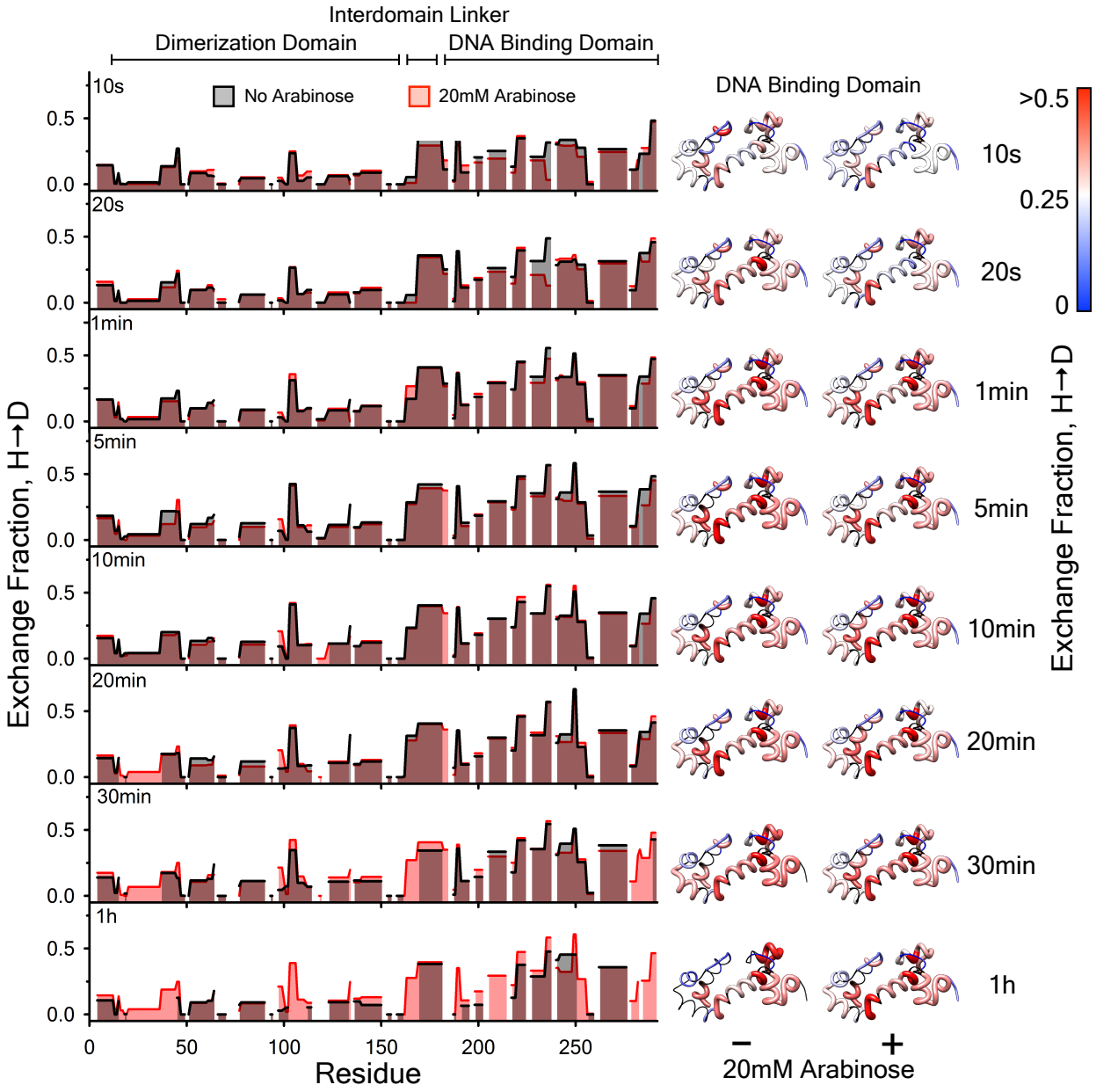

Figure S5: Left: Site resolution of the HX exchange fraction throughout the full length AraC protein containing both the dimerization and DNA binding domains in the presence (red) and absence of 20mM arabinose (black) plotted as a function of the residue number. Right: HX exchange fraction is mapped onto the structure of the AraC dimerization domain (pdb ID = 2K9S [6]). Black = not resolved, blue = 0, white = 0.25, red $\geq$ 0.5. Rendered using UCSF Chimera [5].

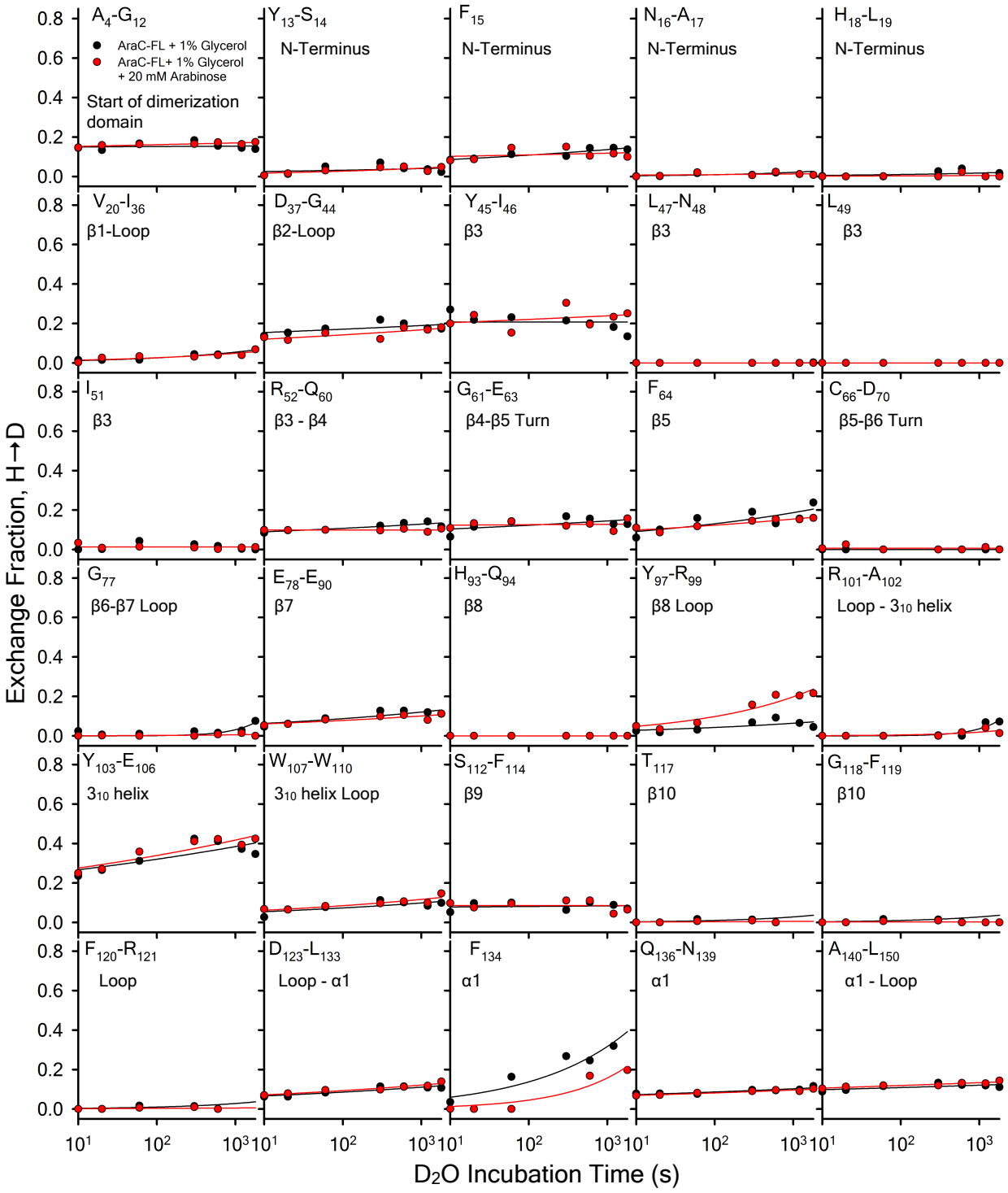

Figure S6: HX Kinetics of site-resolved residues throughout the full length AraC protein containing both the dimerization and DNA binding domains in the presence (red circles) and absence of 20mM arabinose (black circles). Part 1 of 2.

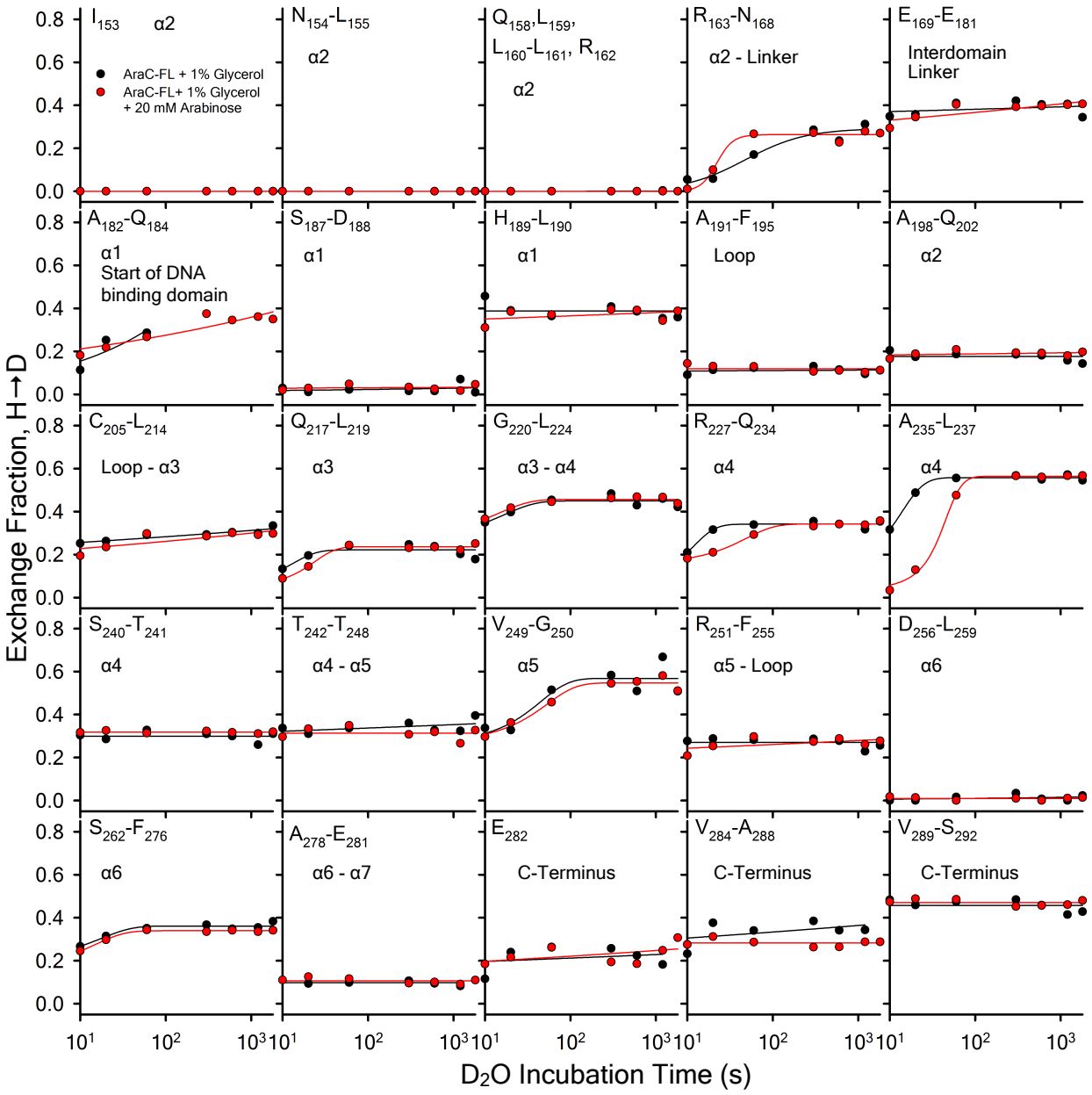

Figure S7: HX Kinetics of site-resolved residues throughout the full length AraC protein containing both the dimerization and DNA binding domains in the presence (red circles) and absence of 20mM arabinose (black circles). Part 2 of 2.

References

[1] Kan, Z. Y., Mayne, L., Chetty, P. S., and Englander, S. W. (2011) ExMS: data analysis for HX-MS experiments., *J Am Soc Mass Spectrom 22*, 1906-1915.

[2] Kan, Z. Y., Walters, B. T., Mayne, L., and Englander, S. W. (2013) Protein hydrogen exchange at residue resolution by proteolytic fragmentation mass spectrometry analysis., *Proc Natl Acad Sci U S A 110*, 16438-16443.

[3] Schott, H. (1988) Direct comparison of the strength of hydrogen bonds formed by H2O and D2O, *Journal of Macromolecular Science, Part B: Physics 27*, 119-123.

[4] Soisson, S. M., MacDougall-Shackleton, B., Schleif, R., and Wolberger, C. (1997) Structural basis for ligand-regulated oligomerization of AraC., *Science 276*, 421-425.

[5] Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera–a visualization system for exploratory research and analysis., *J Comput Chem 25*, 1605-1612.

[6] Rodgers, M. E., and Schleif, R. (2009) Solution structure of the DNA binding domain of AraC protein., *Proteins 77*, 202-208.