Supplementary Materials for

The SARS-CoV-2 spike reversibly samples an open-trimer conformation exposing novel epitopes

Shawn M. Costello†, Sophie R. Shoemaker†, Helen T. Hobbs, Annalee W. Nguyen, Ching-Lin Hsieh, Jennifer A. Maynard, Jason S. McLellan, John E. Pak, Susan Marqusee*

† These authors contributed equally to this work
* Correspondence to: Susan Marqusee, marqusee@berkeley.edu

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Supplementary Note
Figs. S1 to S5

Other Supplementary Materials for this manuscript include the following:

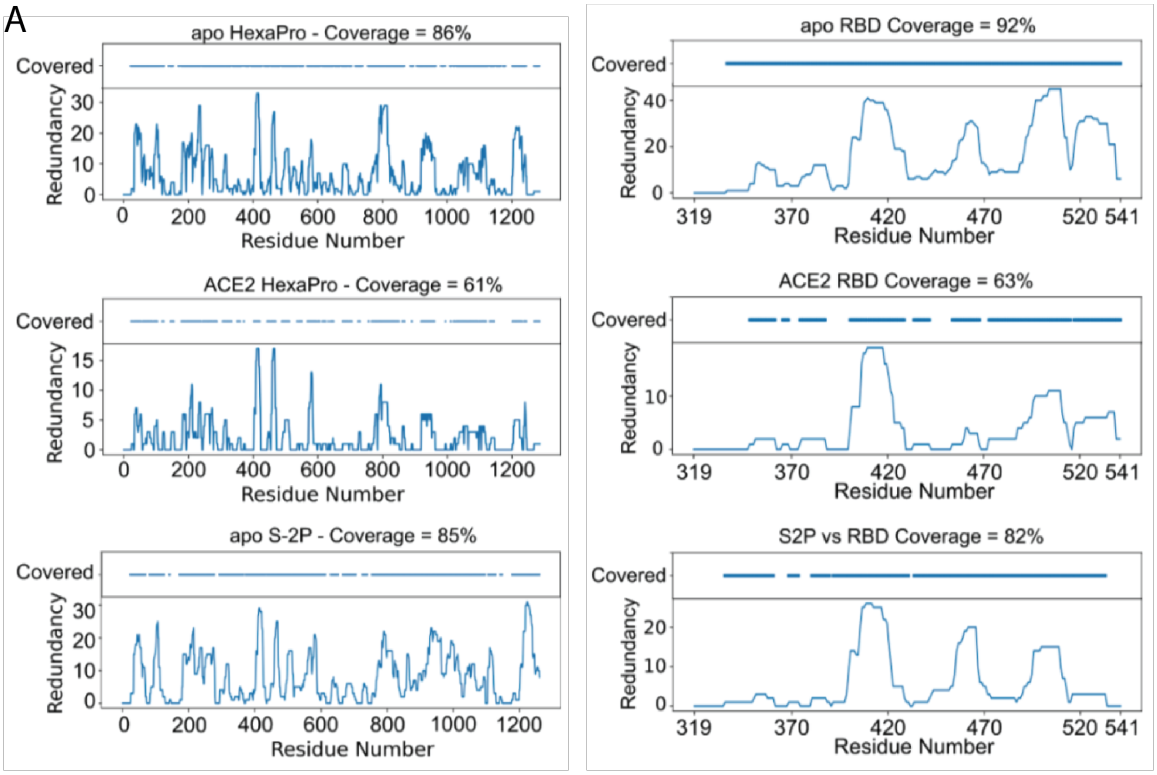
ApoS2P_UptakeResultsSummary.csv
ApoHP_UptakeResultsSummary.csv
ACE2_HP_UptakeResultsSummary.csv
ApoRBD_vs_ACE2RBD.csv
S2P_BackExchangeControl.csv
Alpha_S1_ExampleBimodals.zip
Alpha_S1_RawData.zip
HP_ExampleBimodals.zip
HP_RawData.zip
S2P_ExampleBimodals.zip
S2P_RawData.zip

Supplementary Note

Lack of observed bimodal peptides in previous SARS-CoV-2 spike HDX-MS studies

Other groups have studied the spike protein by HDX-MS yet did not initially report the presence of bimodal mass distributions for any peptides (27, 28). After discussion with the authors of these other studies, we have determined there are no inconsistencies between our data and theirs. In the case of Huang et al. (28), bimodal behavior may be less obvious for several reasons, one is the length of the peptide. Shorter peptides with bimodal behavior will have less difference between the two distributions, making them appear more asymmetrical rather than two clear different distributions. Maximum deuteration and back exchange are two other factors that will influence how obvious the bimodal behaviors are. Indeed Huang et al. report that re-examination of the data reveals a notable increase in the width of the mass envelope for the peptides we report to be bimodal, consistence with the presence of bimodal behavior. In the case of Raghuvamsi et al. (27), in addition to smaller peptides, the HDX experiments were carried out at a higher temperature. (Raghuvamsi et al. labeled at 37°C, instead of 25°C as in our study). This higher temperature results in faster intrinsic exchange rates and thus the lighter envelope (of the bimodal peaks) will exchange more than in our study, but the higher molecular weight envelope would not change much (because they are nearly fully deuterated in our experiments), and thus the two peaks would have substantial to total overlap. Indeed, Raghuvamsi et al. report that they repeated their experiments with labeling at 25°C and see the same bimodal distributions that we report (personal communication). Furthermore, differences in back exchange and theoretical maximum deuteration could further obscure the two distributions. Our back exchange is estimated to be an average of 25% among peptides, Raghuvamsi et al. estimate their back exchange to be an average of 34%. Finally, the construct in Raghuvamsi et al. is different from the ones we used in this study, notably it does not have an appended trimerization domain and appears to be more heterogeneously glycosylated, as evidenced by the detection of non-glycosylated peptides spanning 12 of the known N-linked glycosylation sites.

A



B

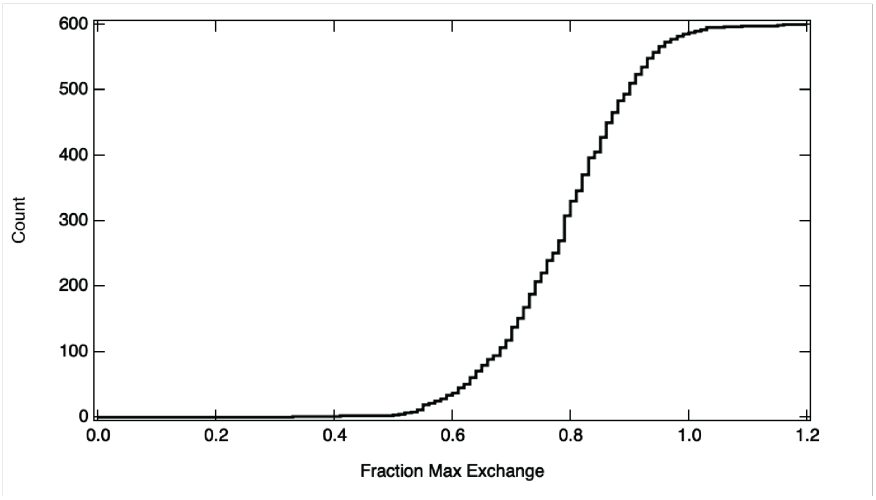
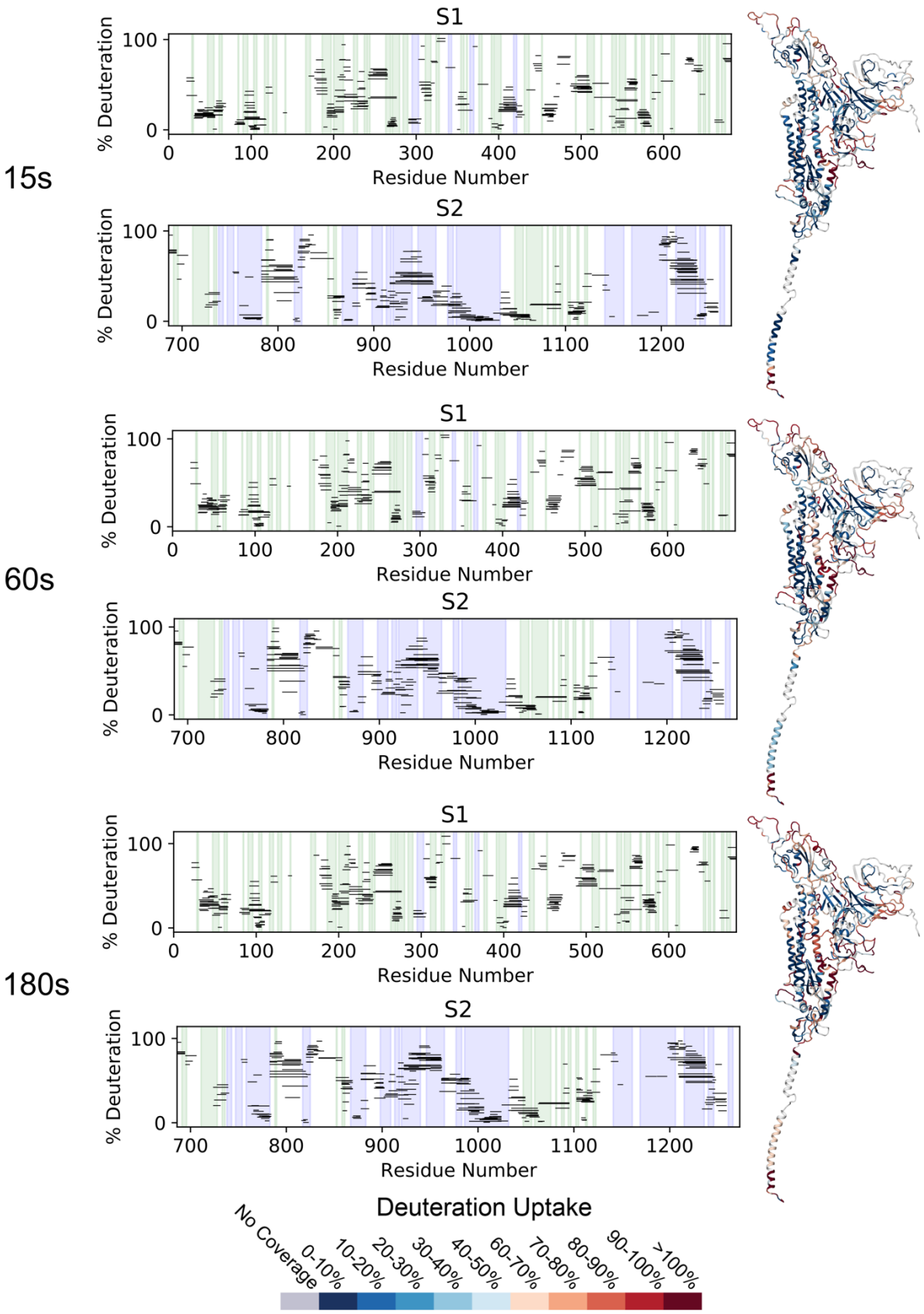


Fig. S1. Coverage, redundancy, and back exchange results from HDX-MS experiments

(A) Peptide coverage and redundancy at each residue for all HDX-MS experiments. **(B)** Back-Exchange Control: Cumulative histogram of the fractional deuterium maintained during workup of a fully deuterated sample. Fraction max exchange is corrected for the 90% D_2O experimental conditions.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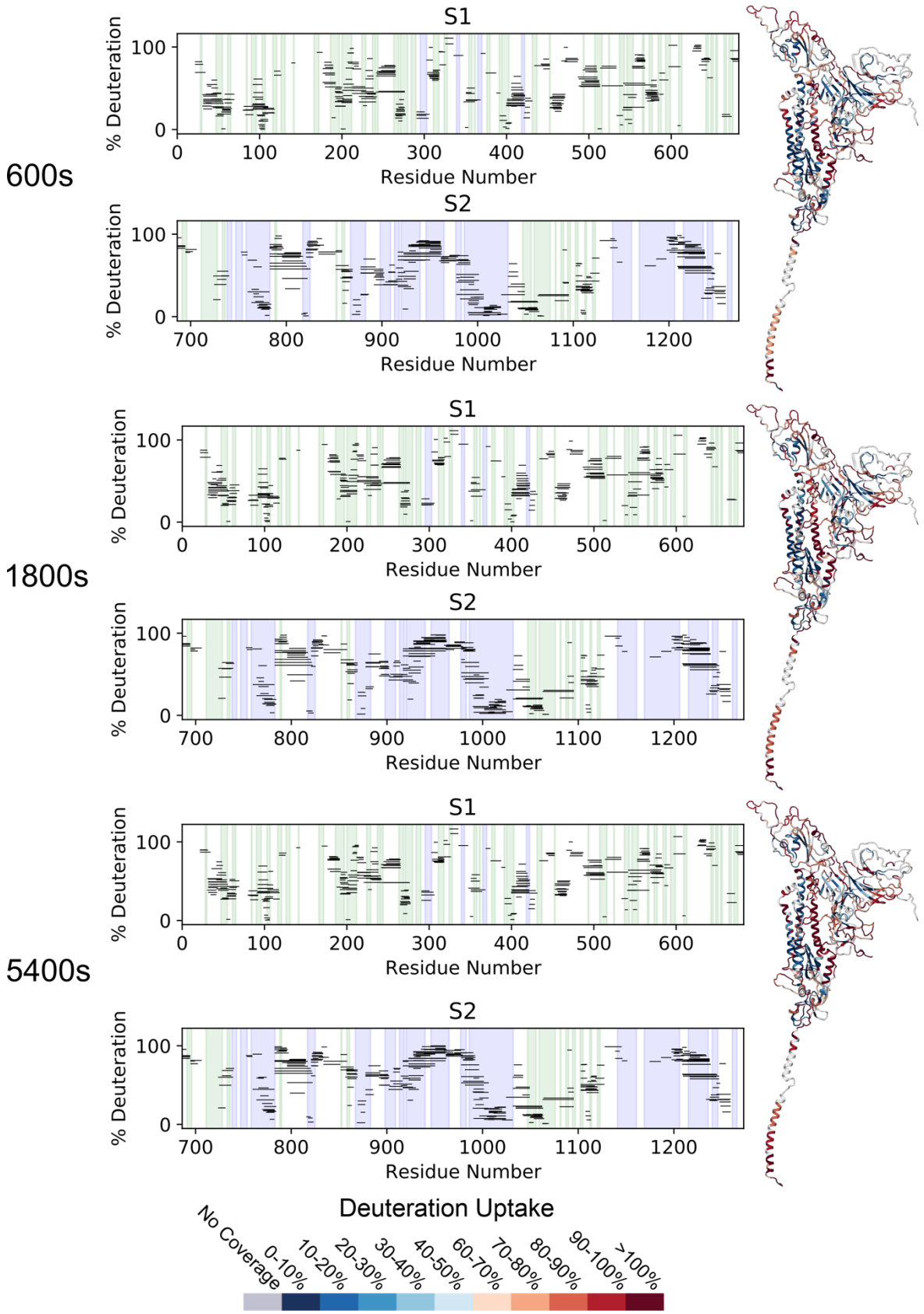
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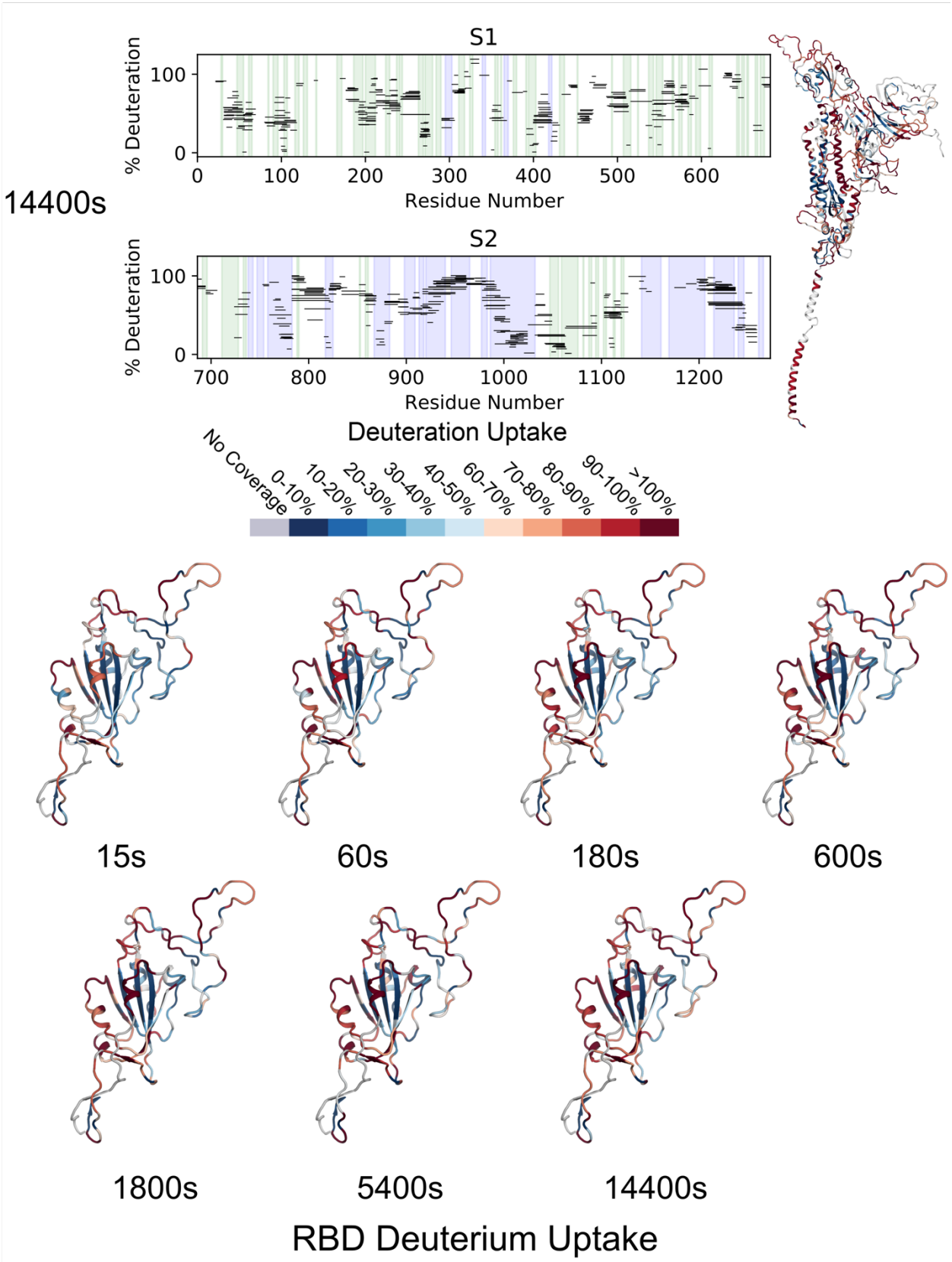
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RBD Deuterium Uptake

Fig. S2. Spike HDX-MS results as a function of time
(Top) Deuterium uptake for each S-2P experimental time point (left) shown as a function of sequence position for every peptide analyzed (center) and with per-residue deuteration uptake (scale shown at bottom) mapped to the structure a single protomer of a full length prefusion spike trimer model from (24) (right). Secondary structures in the prefusion structure are shaded in blue (alpha helices) and green (beta strands). (Bottom) Per-residue deuterium uptake for each Apo-RBD experimental time point mapped to the structure of the RBD (single RBD from a full-length spike trimer model from (24)). Per-residue deuteration calculated from all peptide data by HDExaminer 3.

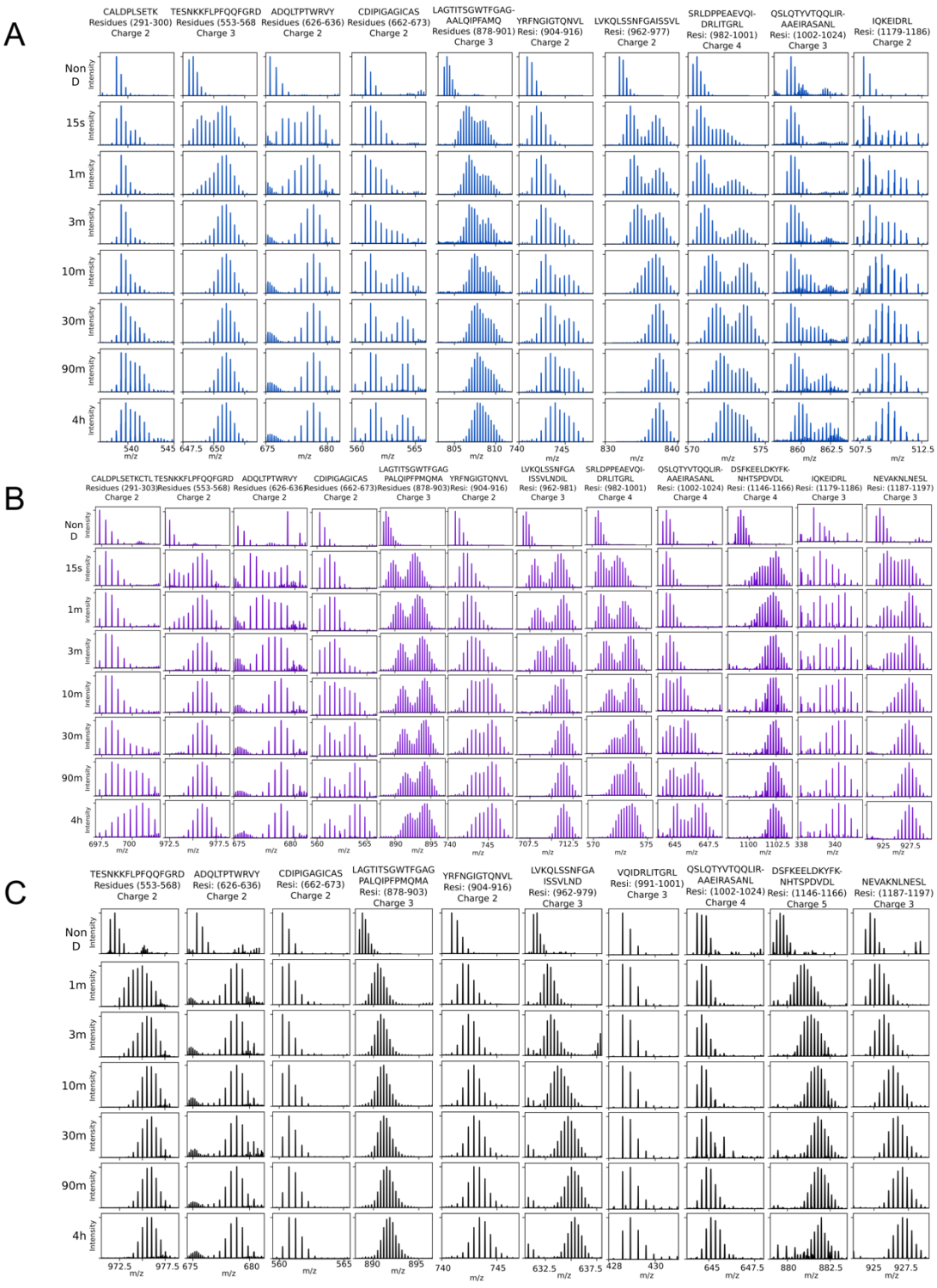

Fig. S3. Bimodal peptide spectra observed in continuous labeling HDX-MS experiments

Observed spectra of a representative set of peptides with observed bimodal behavior for **(A)** S-2P and **(B)** HexaPro **(C)** Disulfide-locked HexaPro

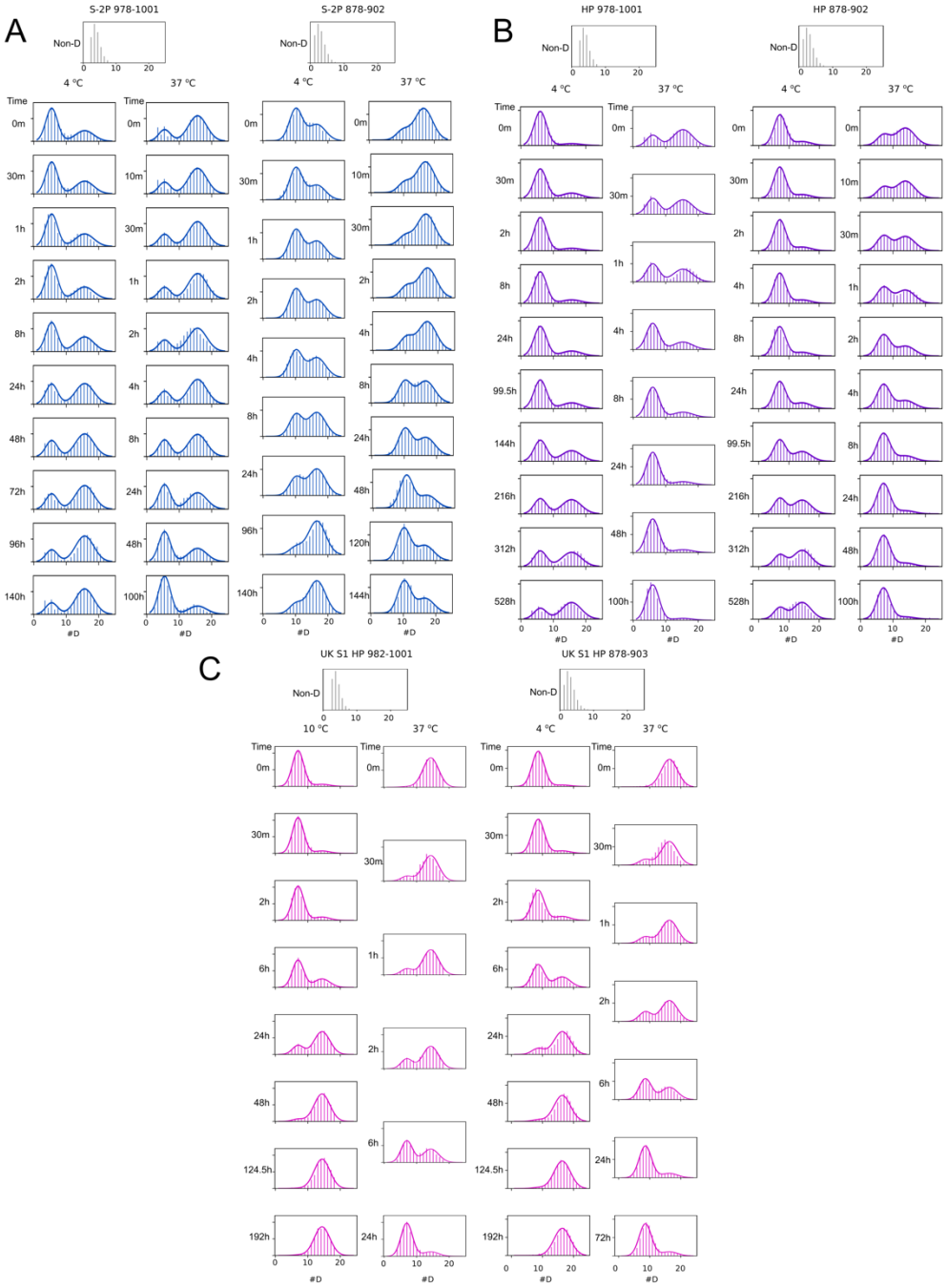

Fig S4. Example bimodal peptide spectra observed in pulsed-labeling HDX-MS experiments One example time course for a peptide from each of the two regions used to quantify states A and B in (A) S-2P, (B) HexaPro, and (C) alpha S1 HexaPro. The bimodals in the time course were globally fit to a sum of two gaussians to determine the distributions for state A and state B, the fits were then used to quantify the relative populations of state A and state B (see methods), the resulting gaussian fits are overlaid. Undeuterated spectra are shown at the top in gray.

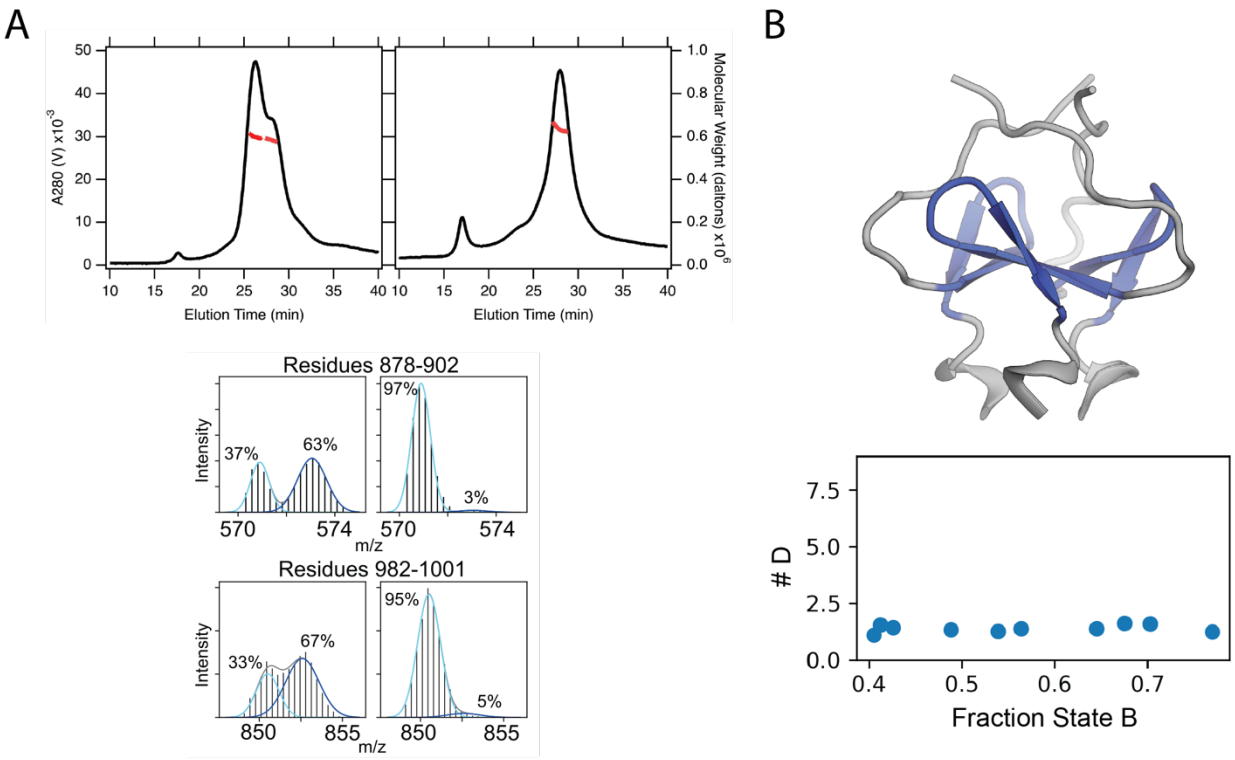

Fig. S5. State A and state B are both Trimeric.
(A) Top: SEC-MALS (Superose 6 increase 10/300) UV (black line, left axis) and estimated molecular weight (red line, right axis) traces from S-2P performed after four days of incubation at 4 °C (left) and 37 °C (right). The molecular weights of all primary species are most consistent with the spike ectodomain trimer (~600 kilodaltons). Bottom: MS spectra of a bimodal peptides from each sample taken immediately before SEC-MALS experiment with gaussian fits and estimated populations shown. Populations of states A and B are consistent with the observed peaks shapes in the SEC-MALS experiments (one predominant peak at 37 °C, and two predominant peaks with a ratio of ~3:2 at 4 °C) the earlier elution volume of state B supports a model of a more expanded conformation (Fig. 7) (B) Top: Structure of the T4 fibritin trimerization domain (PDB 1RFO) with peptide shown in blue. Bottom: peptide deuterium uptake at one minute as a function of fraction state B. The observed lack of dependence is consistent with the maintenance of the trimerization domain structure in both states A and B.