# SUPPORTING INFORMATION

## Synergistic Structural Information from Covalent Labeling and Hydrogen-Deuterium Exchange Mass Spectrometry for Protein-Ligand Interactions

Tianying Liu, Patanachai Limpikirati, Richard W. Vachet*

*Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, United States*

* Prof. Richard W. Vachet, Department of Chemistry, University of Massachusetts, Amherst, MA 01003. E-mail: rwvachet@chem.umass.edu. Phone: (413) 545-2733.

# Table of contents:

## Materials and Sample Preparation

Myoglobin from equine skeletal muscle, apomyoglobin from equine skeletal muscle and carbonic anhydrase isozyme II from bovine erythrocytes (BCA) were purchased from MilliporeSigma (St.Louis, MO). Apomyoglobin is prepared using the method described by Rothgeb and Gurd (Rothgeb T. M.; Gurd F. R. N. Physical methods for the study of myoglobin. *Methods Enzymol.* **1978**, *52*, 473-486.) that involves 2-butanone extraction. Maltose binding protein (MBP) was obtained from MyBioSource.com (San Diego, CA). Brinzolamide, deuterium oxide (99.9 atom %D), diethylpyrocarbonate (DEPC), dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium bromide (HNSB), guanidine hydrochloride (GuHCL), imidazole, maltose monohydrate, 3-(N-morpholino)propanesulfonic acid (MOPS), MOPS sodium salt, and L-tryptophan were also purchased from MilliporeSigma. Acetonitrile, formic acid, sodium phosphate, sodium phosphate monobasic monohydrate, HPLC grade water, and a 1 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8) stock solution were all purchased from Fisher Scientific (Fair Lawn, NJ). Centricon molecular weight cutoff (MWCO) filters were obtained from Millipore (Burlington, MA). Sequencing grade modified trypsin and sequencing grade chymotrypsin were obtained from Promega (Madison, WI).

*Buffer exchange procedure for the maltose-binding protein (MBP):* MBP comes from the vendor in a 20 mM Tris (pH 8.0) buffer. Before analysis, the buffer was exchanged with a 20 mM phosphate buffer at pH 7.5 using the 10K MWCO filters by three cycles of concentration and reconstitution.

*Protein digestion procedure after the covalent labeling reactions:* diluted with water to a final volume of 400 µL and concentrated using a 10,000 MWCO filter to a final volume of 40 µL. Then, the samples were reconstituted in 0.25 M Tris (pH 8.0) and 6 M GuHCl at 55 °C for 1 h to denature the protein. The samples were then diluted in 300 µL water and concentrated again by a 10,000 MWCO filter to volume of 40 µL. This dilution-concentration step was repeated twice to decrease the GuHCl concentration. The resulting sample was diluted to 100 µL with 0.1 M Tris (pH 8.0) buffer and digested with trypsin for 2 h at 37 °C if the protein was HNSB labeled, or with chymotrypsin for 2 h at 25 °C if the protein was DEPC labeled.

## HDX procedures

The HDX procedure consisted of the following steps: To initiate the HDX, 3.8 µL of the prepared protein or protein-ligand complex sample was diluted into 52.2 µL $D_2O$ buffer and allowed to exchange for different amounts of time at 10 °C. At the end of each exchange period, the reaction was quenched by mixing the sample with a quenching buffer (1:1, v/v) that contained 3.6 M GuHCl and ~ 0.8% formic acid in water (pH = 2.25) at 1 °C for 1 min. After the quench step, the sample was transferred and injected into the Waters ACQUITY UPLC System. Online digestion was performed using a Waters ENZYMATE immobilized pepsin column (ID: 2.1 length: 30 mm). The proteolytic products were collected by a trap column (HSS T3 pre-column, 100 Å, 1.8 µm, 2.1 mm X 5 mm, Waters) for 4 min. Then, trapped peptides were eluted by a Waters ACQUITY C18 column (2.1 x 50 mm, 1.8 µm) at 0 °C with a linear gradient of acetonitrile (with 0.1% formic acid) that was increased from 5% to 35% over 7 min and then increased from 35% to 85% in 1 min at a flow rate of 40 µL/min. The eluent was then directed into a Waters SYNAPT G2Si mass spectrometer for analysis in MSE mode over the m/z range of 50 - 2000. The deuterium uptake level of each measured peptide at different exchange time points

was automatically calculated using the DynamX 3.0 software (Waters). Averaged values from triplicate experiments with propagated error are reported. The reported deuterium uptake values are not corrected for back-exchange. Back exchange values ranged from 30 to 50% for the measured peptides.

Calculations of the Relative Fractional Uptake of deuterium, as shown in Figures 1, 3, and 5, were performed using Waters DynamX 3.0 software. Differences in exchange at the peptide level upon comparing ligand-free and ligand-bound states were calculated by DynamX for peptides that were measured in all three samples. In the case of overlapping peptides for any given residue, the exposure data rendered is determined by the shortest peptide. Where multiple peptides are of the shortest length, the DynamX software uses the peptide with the residue closest to the peptide C-terminus, to render the exposure data. DynamX software does not use the N-terminus of any peptide to render the data. The resulting numbers provided by DynamX were then imported into PyMol (The PyMOL Molecular Graphics System, Version 2.3 Schrödinger, LLC) to map regions of increased (red) or decreased (blue) exchange.

## LC-MS parameters for CL-MS

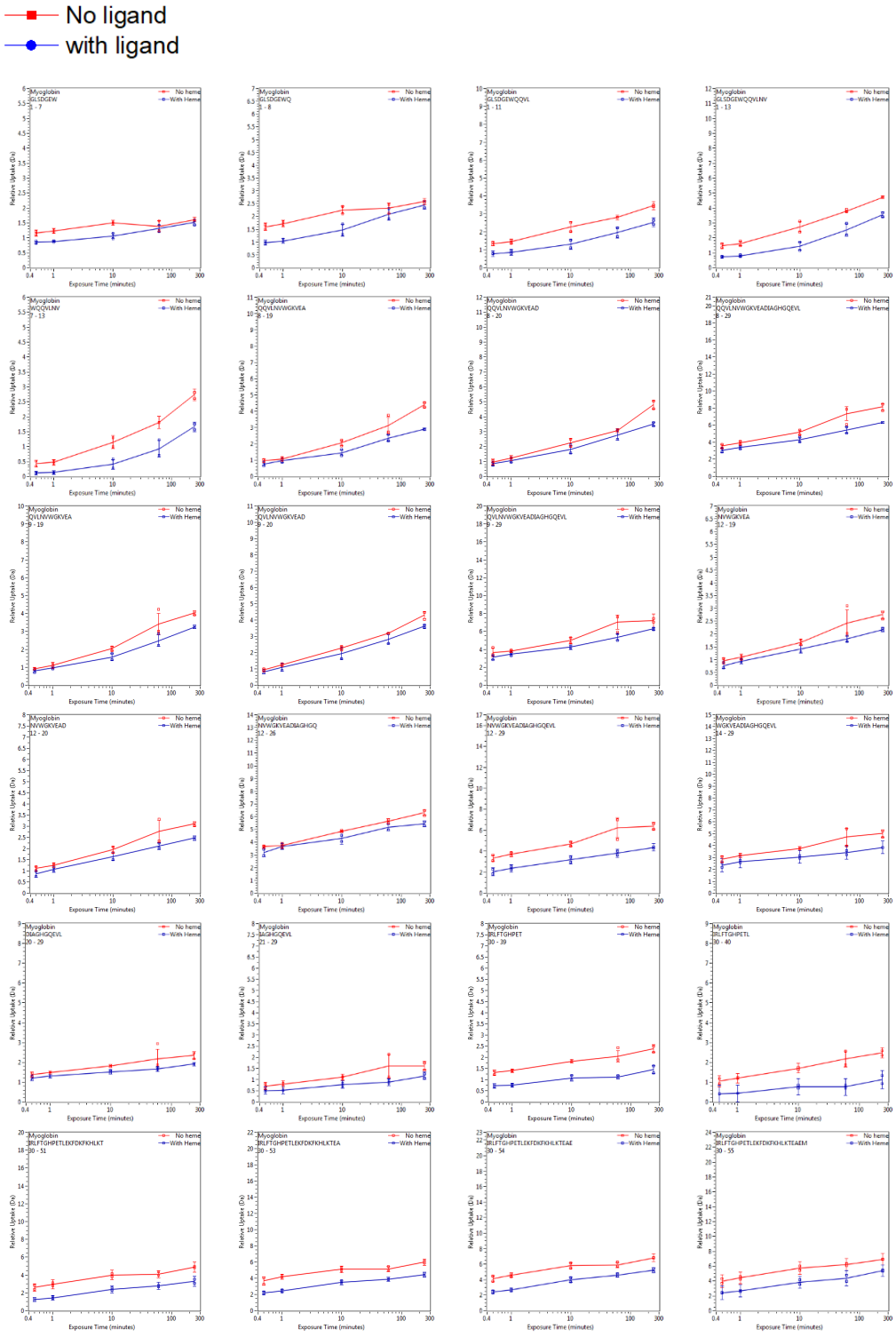
The electrospray voltage was settled at 2.1 KV and the ion transfer tube temperature was set at 325 °C. The resolution of Orbitrap was set at 60,000, the $MS^1$ AGC target was set at $4 \times 10^5$ ions with a maximum injection time of 50 msec. Collision-induced dissociation (CID) with a normalized collision energy of 35% was used for tandem mass spectrometry (MS/MS) experiments. Data-dependent selection of the precursor ions with ion abundances above 5,000 was applied. On-line HPLC separation of the digested protein samples was conducted using a Thermo Scientific Easy-NanoLC 1000 system with a Thermo Scientific Acclaim PepMap C18 nanocolumn (15 cm x 75 µm ID, 2 µm, 100 Å). Peptides were eluted using a gradient of acetonitrile containing 0.1% formic acid that increased from 0 to 50% for 60 min at the flow rate of 0.3 µL/min.

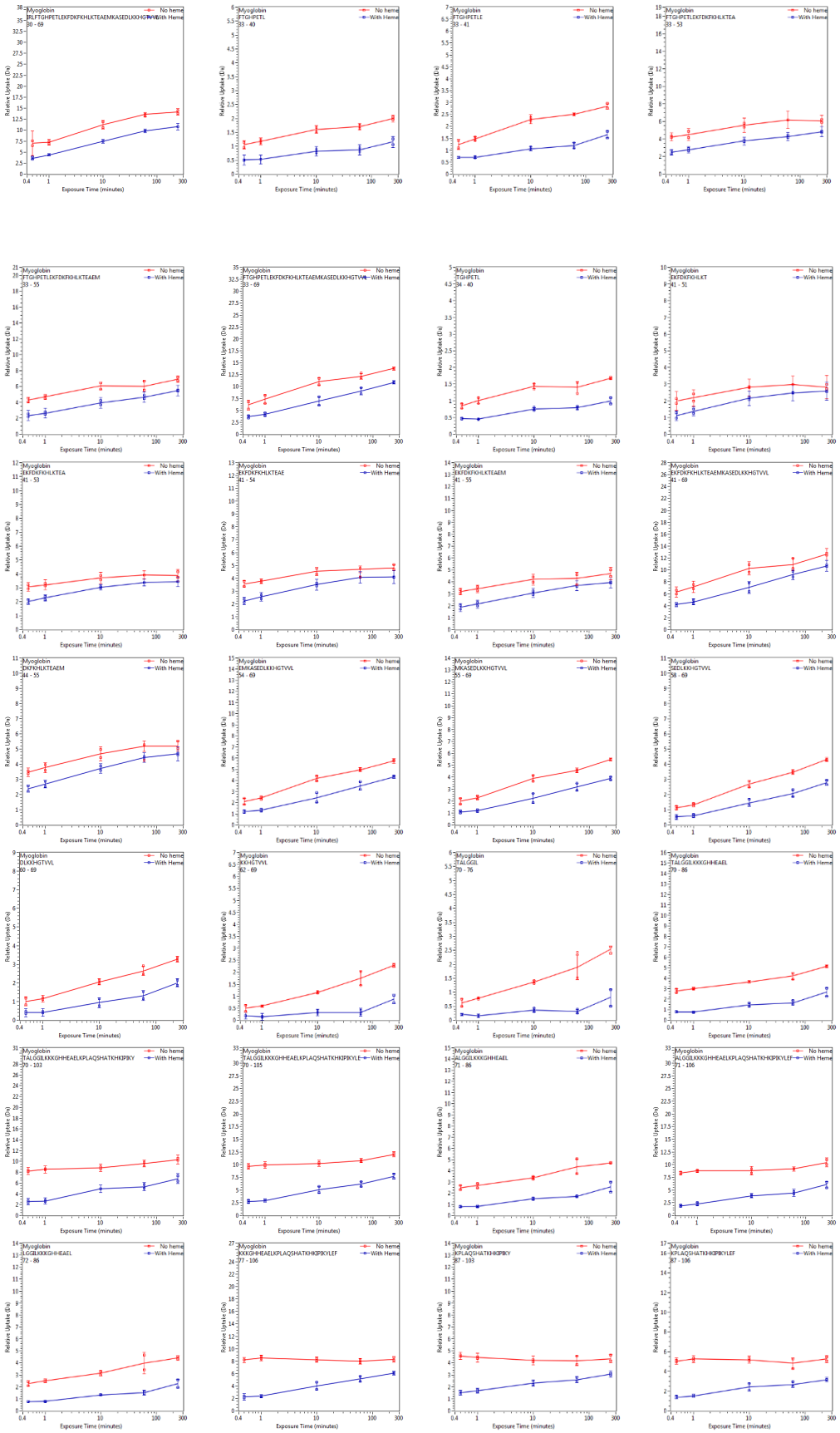
**Table S1.** Peptides from myoglobin with statistically significant differences in the relative deuterium uptake, as determined using the criteria described in experimental section.*

| Region | Sequence position | Sequence |
|---|---|---|
| 12 – 29 | 12 – 29 | NVWGKVEADIAGHGQEVL |
| 30 – 69 | 30 – 53 | IRLFTGHPETLEKFDKFKHLKTEA |
| | 30 – 54 | IRLFTGHPETLEKFDKFKHLKTEAE |
| | 33 – 69 | FTGHPETLEKFDKFKHLKTEAEMKASEDLKKHGTVVL |
| | 54 – 69 | EMKASEDLKKHGTVVL |
| | 55 – 69 | MKASEDLKKHGTVVL |
| 70 – 106 | 70 – 86 | TALGGILKKKGHHEAEL |
| | 70 – 103 | TALGGILKKKGHHEAELKPLAQSHATKHKIPIKY |
| | 70 – 105 | TALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLE |
| | 71 – 86 | ALGGILKKKGHHEAEL |
| | 71 – 106 | ALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEF |
| | 72 – 86 | LGGILKKKGHHEAEL |
| | 77 – 106 | KKKGHHEAELKPLAQSHATKHKIPIKYLEF |

| | | |
|---|---|---|
| | 87 – 103 | KPLAQSHATKHKIPIKY |
| | 87 – 106 | KPLAQSHATKHKIPIKYLEF |
| 137 – 153 | 137 – 151 | LFRNDIAAKYKELGF |
| | 137 – 153 | LFRNDIAAKYKELGFQG |
| | 138 – 146 | FRNDIAAKY |
| | 138 – 151 | FRNDIAAKYKELGF |
| | 138 – 153 | FRNDIAAKYKELGFQG |
| | 143 – 151 | AAKYKELGF |

***** The global deuterium uptake difference significance threshold for myoglobin is 1.43 Da.


No ligand
with ligand
Myoglobin
GLSDGEW
2 - 7
Myoglobin
GLSDGEWQ
2 - 8
Myoglobin
GLSDGEWQQVL
2 - 11
Myoglobin
GLSDGEWQQVLNV
2 - 13
Myoglobin
WQQVLNV
7 - 13
Myoglobin
QQVLNVWGKVEA
9 - 19
Myoglobin
QQVLNVWGKVEAD
9 - 20
Myoglobin
QQVLNVWGKVEADIAGHGQEVL
9 - 29
Myoglobin
QVLNVWGKVEA
9 - 19
Myoglobin
QVLNVWGKVEAD
9 - 20
Myoglobin
QVLNVWGKVEADIAGHGQEVL
9 - 29
Myoglobin
NVWGKVEA
12 - 19
Myoglobin
NVWGKVEAD
12 - 20
Myoglobin
NVWGKVEADIAGHGQ
12 - 26
Myoglobin
NVWGKVEADIAGHGQEVL
12 - 29
Myoglobin
WGKVEADIAGHGQEVL
14 - 29
Myoglobin
DIAGHGQEVL
20 - 29
Myoglobin
IAGHGQEVL
21 - 29
Myoglobin
RLFTGHPET
30 - 39
Myoglobin
RLFTGHPETL
30 - 40
Myoglobin
RLFTGHPETLEKFDKFKHLKT
30 - 51
Myoglobin
RLFTGHPETLEKFDKFKHLKTEA
30 - 53
Myoglobin
RLFTGHPETLEKFDKFKHLKTEAE
30 - 54
Myoglobin
RLFTGHPETLEKFDKFKHLKTEAEM
30 - 55
Relative Uptake (Da)
Exposure Time (minutes)


Myoglobin
No heme
With Heme
Relative Uptake (Da)
Exposure Time (minutes)

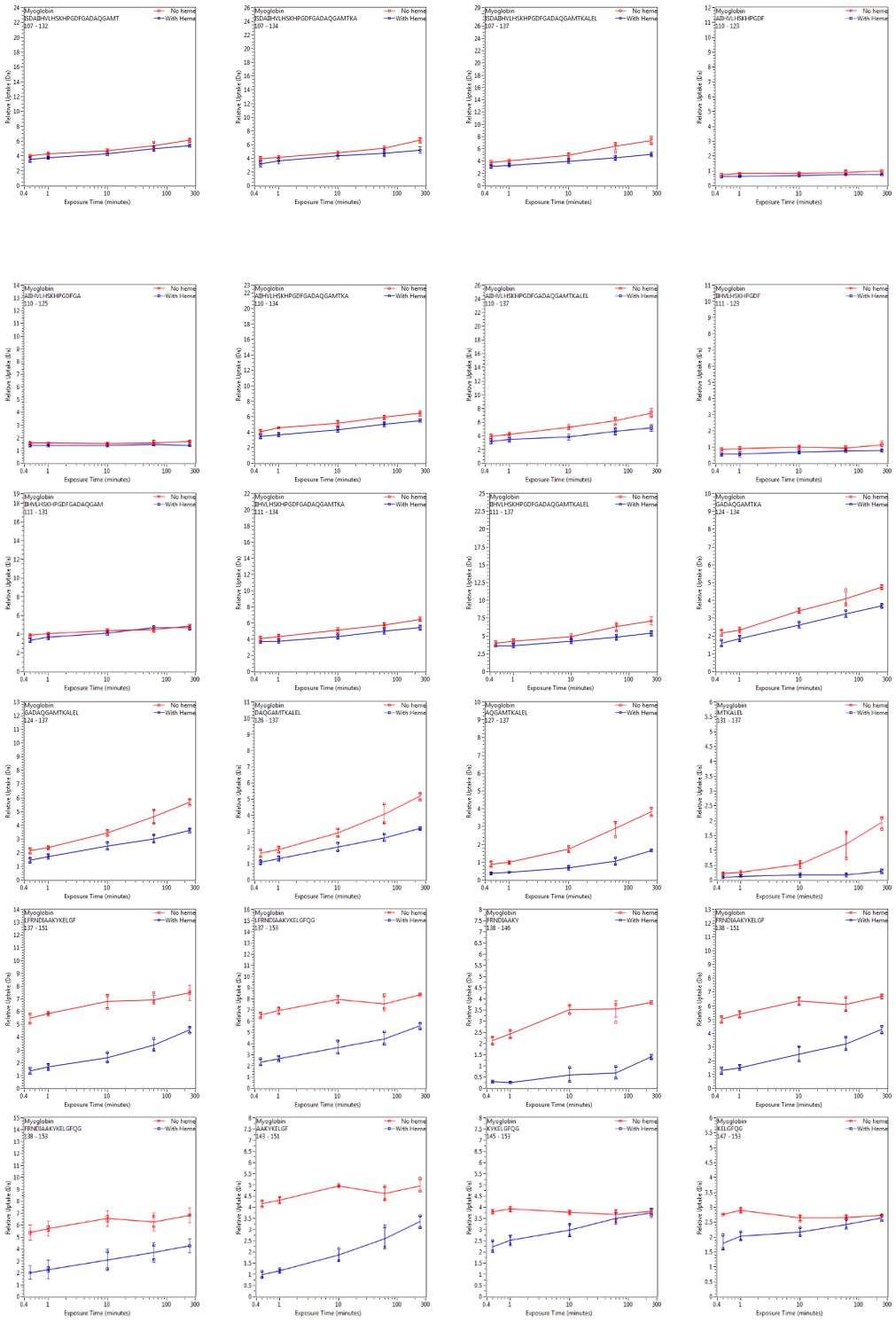


**Figure S1.** Deuterium uptake plots for myoglobin with (blue) and without (red) the heme bound.

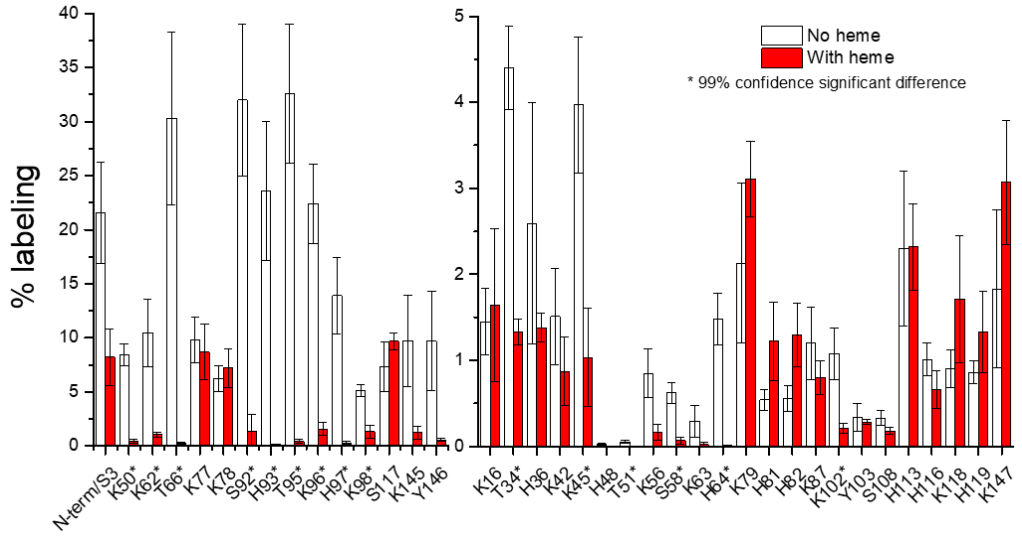



**Figure S2.** Summary of DEPC labeling percentages for myoglobin with and without heme bound. Residues that undergo significant decreases in DEPC labeling at a 99% confidence interval are marked with an asterisk "*" on the x-axis next to their single-letter code. The labeling ratio of K145 is 10 ± 4% and 1 ± 1% without heme and with heme, respectively, and the labeling ratio of Y146 is 10 ± 5% and 0.6 ± 0.2% without heme and with heme, respectively. While these two residues show a labeling decrease, they are not significant at the 99% confidence interval that was chosen for the rest of the data.

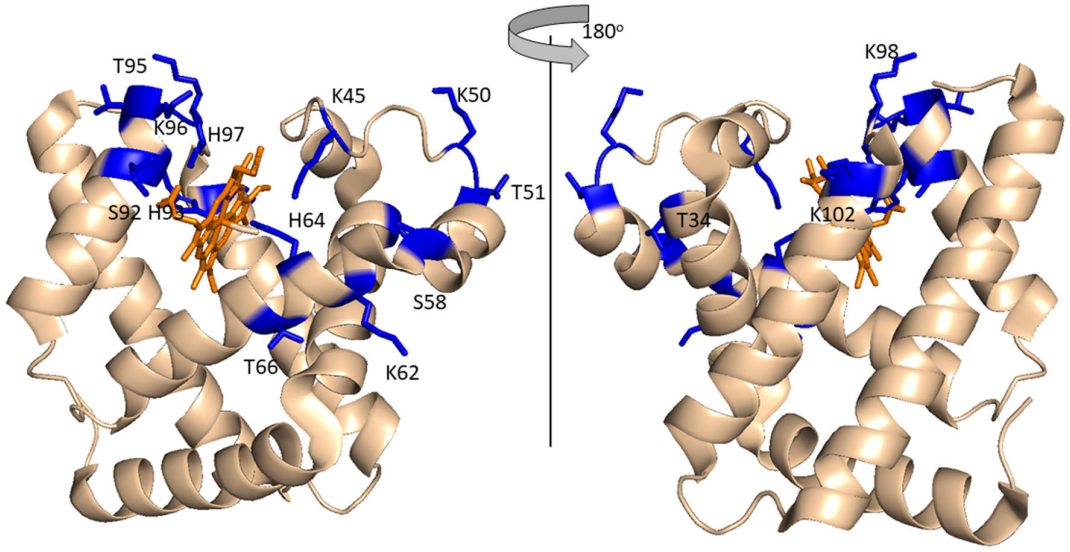



**Figure S3.** Mapping on the holo-myoglobin structure (adapted from PDB 1DWR) the residues identified to undergo significant decreases in DEPC labeling upon heme binding (blue).

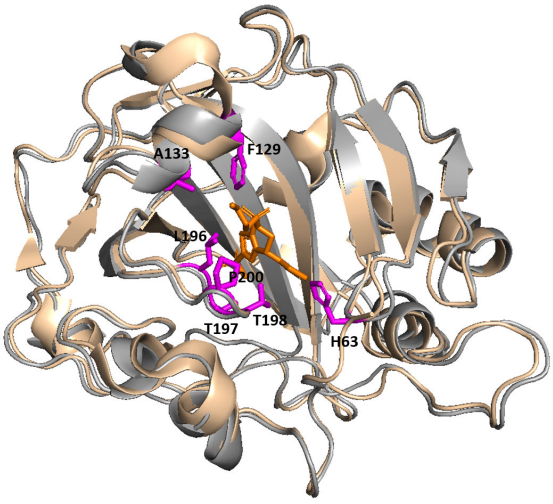
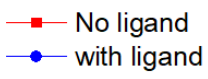
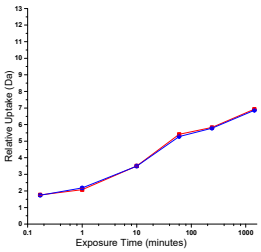



**Figure S4.** Overlay of BCA (PDB 1V9E, light brown) and HCA-brinzolamide (PDB 1A42, grey) structures with residues interacting with brinzolamide (orange) shown in magenta.



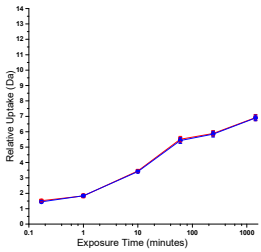

32-47

IDTKAVVQDPALKPLA



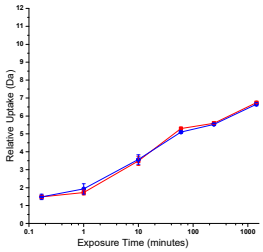

32-48

IDTKAVVQDPALKPLAL



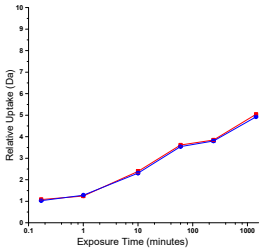

34-48

TKAVVQDPALKPLAL



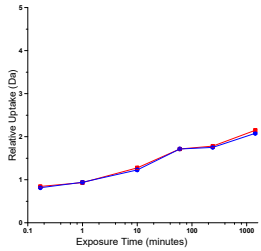

35-47

KAVVQDPALKPLA



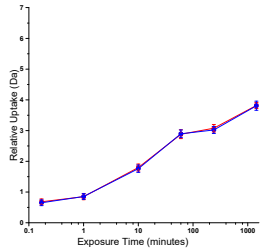

37-43

VVQDPAL



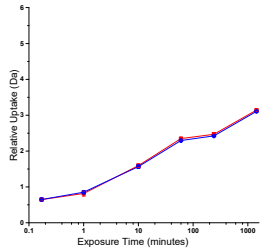

39-48

QDPALKPLAL



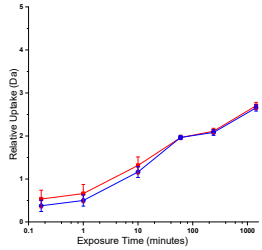

40-48

DPALKPLAL



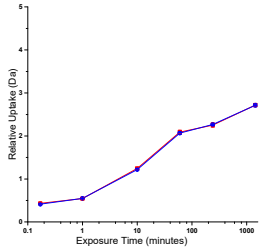

41-48

PALKPLAL



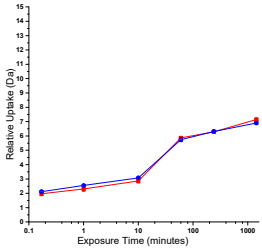

42-48

ALKPLAL



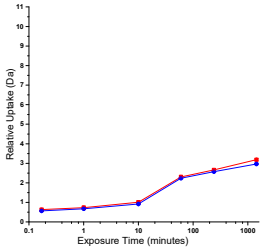

50-65

YGEATSRRMVNNGHSF



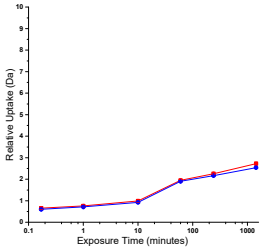

54-65

TSRRMVNNGHSF



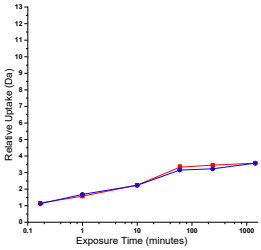

55-65

SRRMVNNGHSF



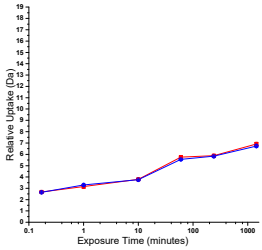

65-78

FNVEYDDSQDKAVL



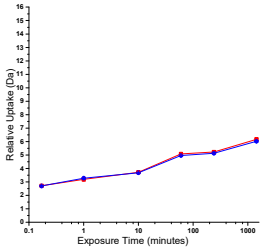

66-86

NVEYDDSQDKAVLKDGPLTGT



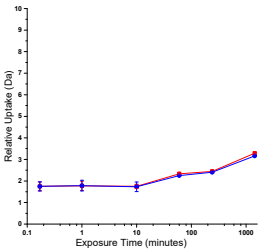

69-86

YDDSQDKAVLKDGPLTGT



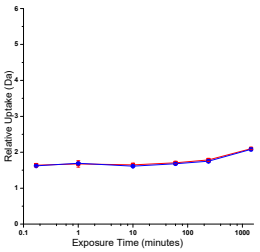
75-86

KAVLKDGPLTGT



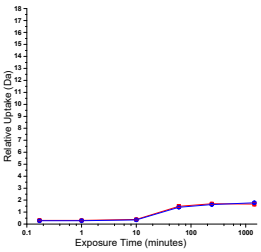

79-86

KDGPLTGT



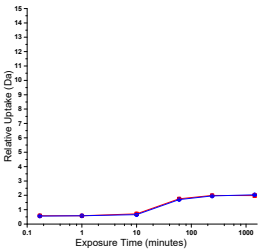

87-105

YRLVQFHFHWGSSDDQGSE



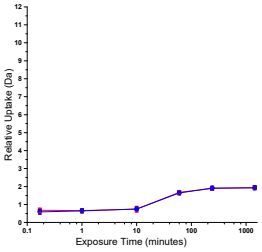

90-105

VQFHFHWGSSDDQGSE




93-105

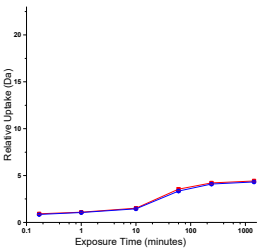
HFHWGSSDDQGSE




93-116

HFHWGSSDDQGSEHTVDRKKY

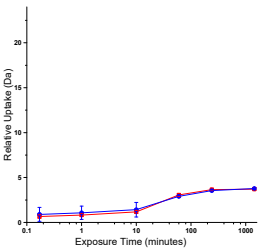
AAE




93-117

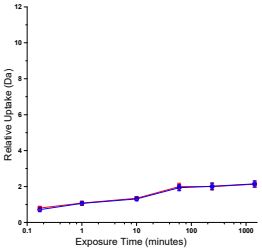
HFHWGSSDDQGSEHTVDRKKY

AAEL




117-129

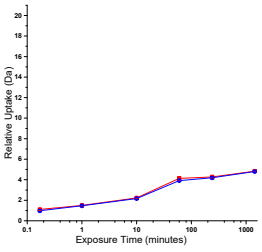
LHLVHWNTKYGDF




117-139

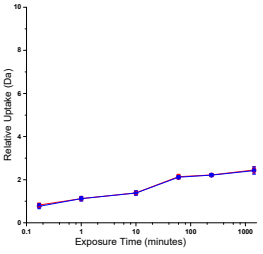
LHLVHWNTKYGDFGTAAQQPD

GL



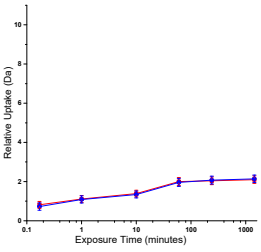

118-128

HLVHWNTKYGD



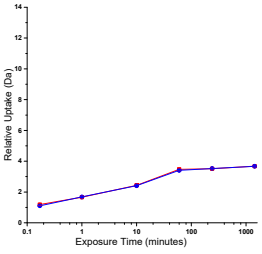

118-129

HLVHWNTKYGDF



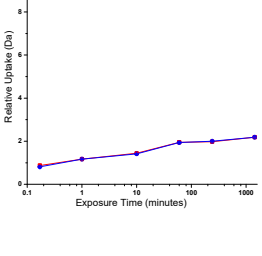

118-132

HLVHWNTKYGDFGTA



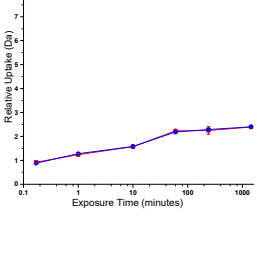

119-129

LVHWNTKYGDF



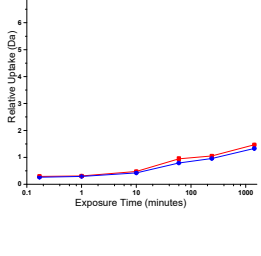

120-129

VHWNTKYGDF



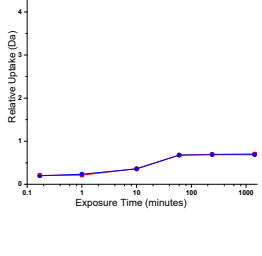

130-139

GTAAQQPDGL



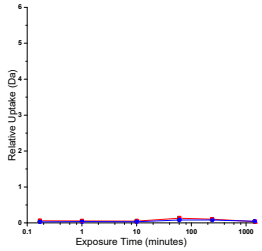

133-139

AQQPDGL



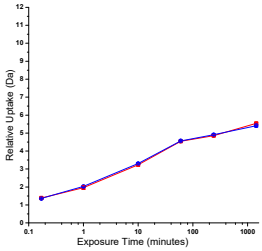
140-146

AVVGVFL



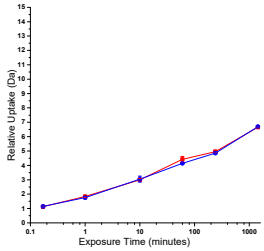

146-159

LKVGDANPALQKVL



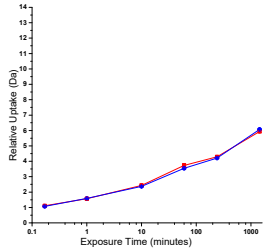

146-162

LKVGDANPALQKVLDAL



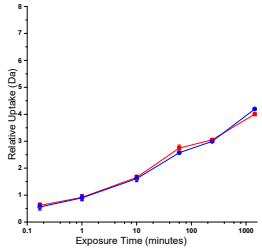

147-162

KVGDANPALQKVLDAL



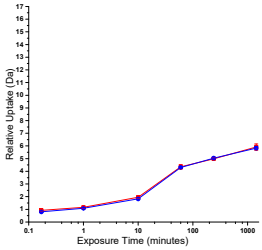

153-162

PALQKVLDAL



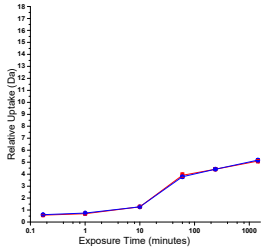

163-182

DSIKTKGKSTDFPNFDPGSL



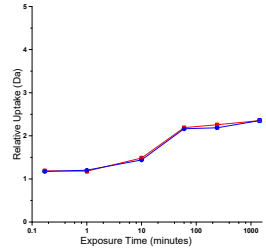

163-183

DSIKTKGKSTDFPNFDPGSLL



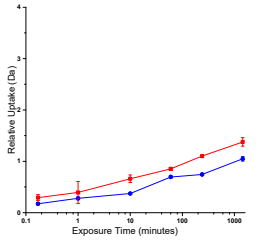

183-189

LPNVLDY



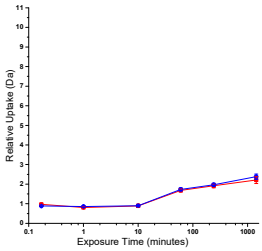

197-203

TTPPLLE



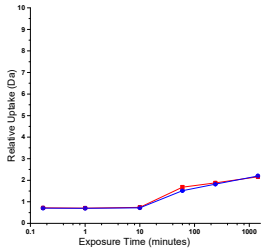

209-221

VLKEPISVSSQQM



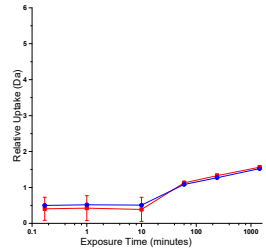

210-221

LKEPISVSSQQM



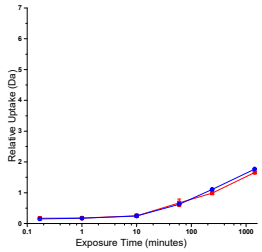

215-221

SVSSQQM



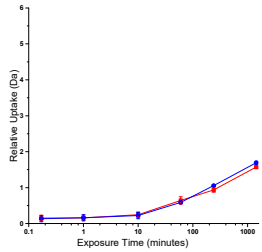

222-229

LKFRTLNF



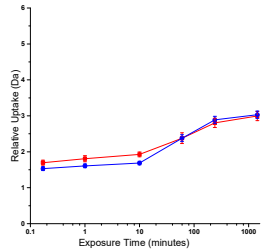

223-229

KFRTLNF



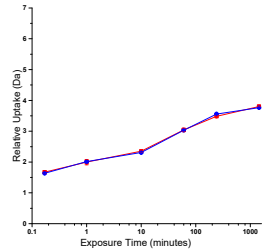

230-237

NAEGEPEL



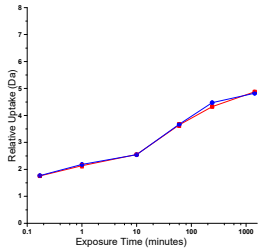

230-238

NAEGEPEL



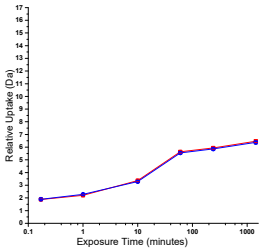
230-239

NAEGEPELLM



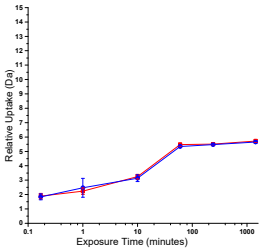

239-259

MLANWRPAQPLKNRQVRGFPK



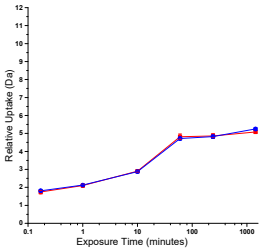

241-259

ANWRPAQPLKNRQVRGFPK




244-259

RPAQPLKNRQVRGFPK




245-259

PAQPLKNRQVRGFPK

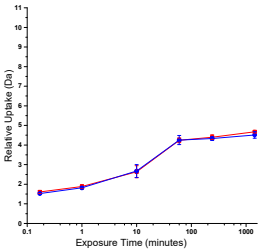



**Figure S5.** Deuterium uptake plots for BCA for with (blue) and without (red) brinzolamide bound.

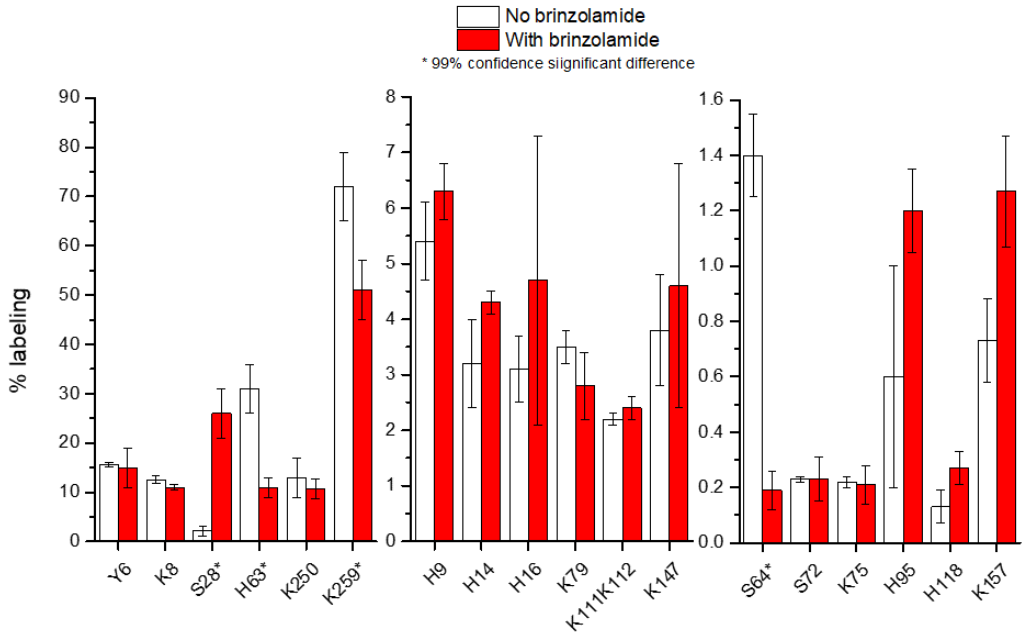



**Figure S6.** DEPC labeling results for BCA with (red) and without (white) brinzolamide bound. Residues that undergo significant decreases in DEPC labeling at a 99% confidence interval are marked with an asterisk "*" on the x-axis next to their single-letter code.

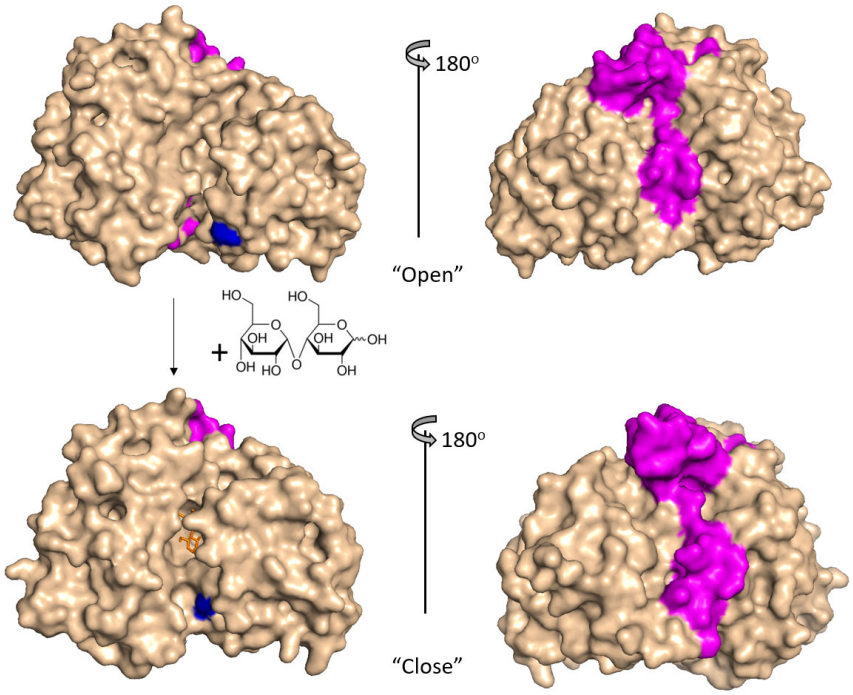
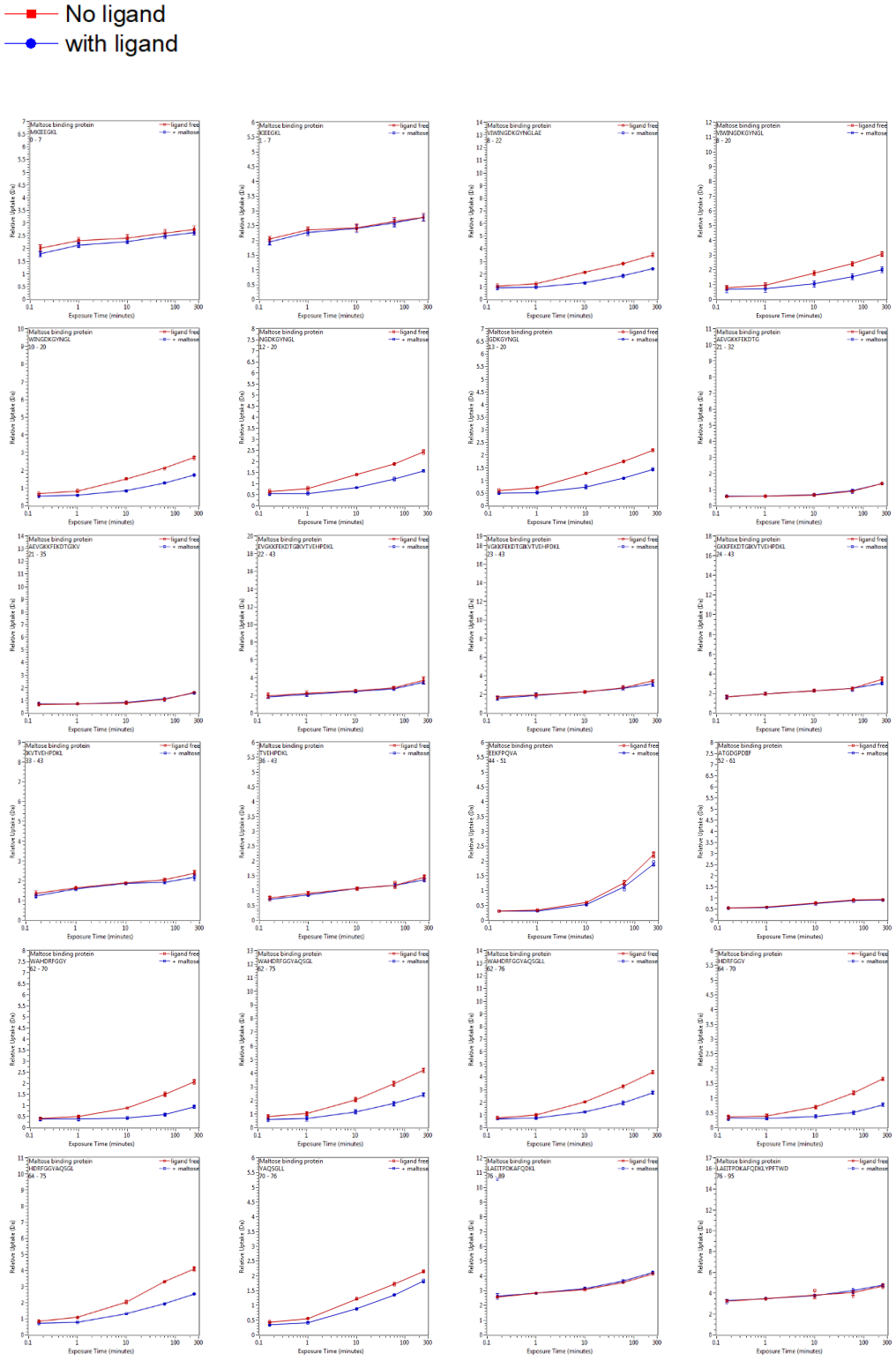


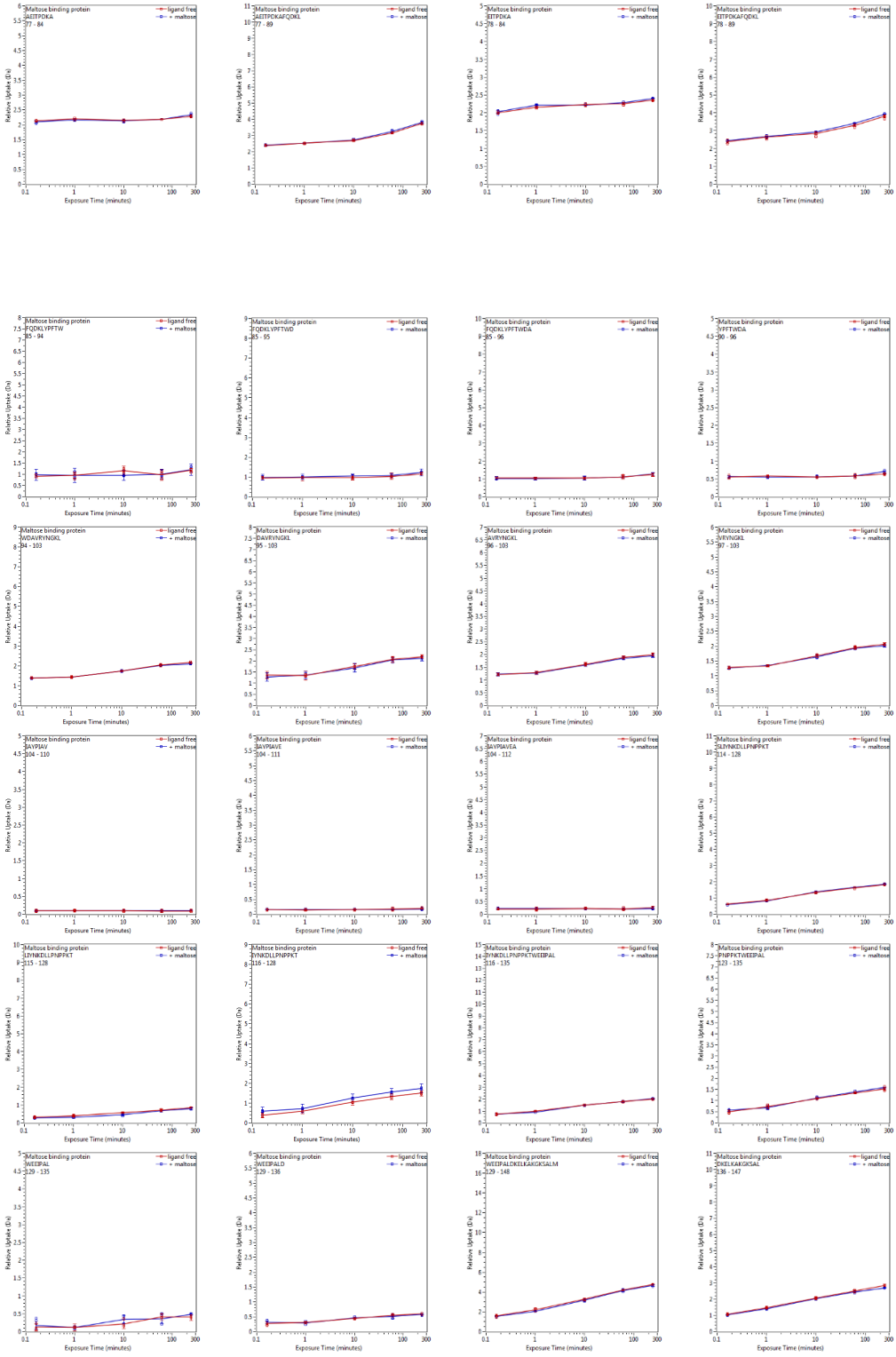

**Figure S7.** Ligand free "open" state (PDB 1OMP) and maltose bound "closed" state (PDB 1ANF) for the maltose binding protein. The residues that are part of the hinge region are indicated in magenta. K297 is indicated in blue.

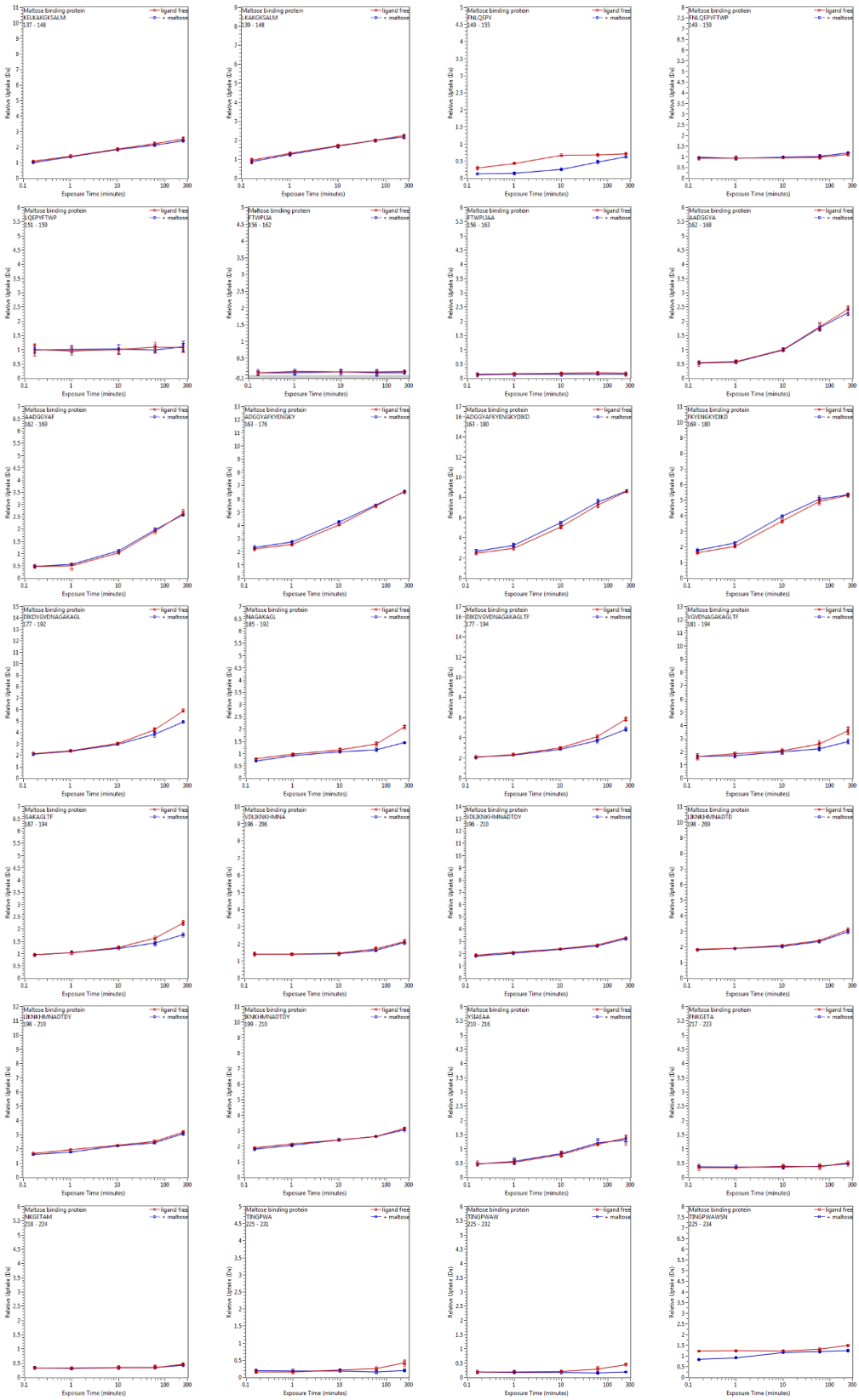
**Table S2.** Peptides from the maltose-binding protein with statistically significant differences in the relative deuterium uptake, as determined using the criteria described in experimental section.*

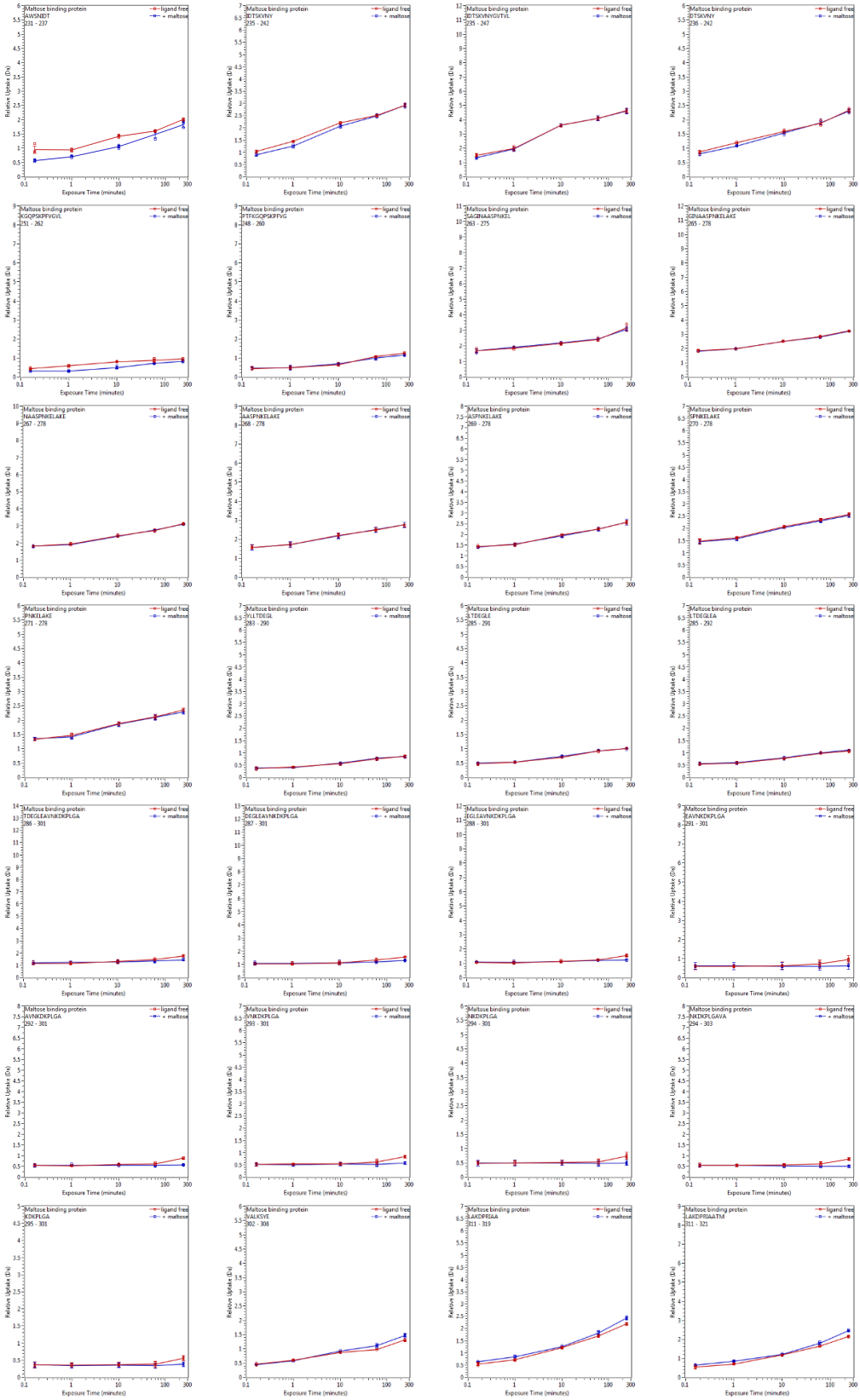
| Region | Sequence position | Sequence |
|---|---|---|
| 8 – 22 | 8 – 22 | VIWINGDKGYNGLAE |
| | 10– 20 | WINGDKGYNGL |
| | 12 – 20 | NGDKGYNGL |
| | 13 – 20 | GDKGYNGL |
| 62 – 76 | 62 – 70 | WAHDRFGGY |
| | 62 – 75 | WAHDRFGGYAQSGL |
| | 62 – 76 | WAHDRFGGYAQSGLL |
| | 64 – 75 | HDRFGGYAQSGL |
| 322 – 336 | 322 – 336 | ENAQKGEIMPNIPQM |
| 346 – 361 | 346 – 361 | AVINAASGRQTVDEAL |

***** The global deuterium uptake difference significance threshold for MBP is 0.34 Da.


No ligand
with ligand


Maltose binding protein
ligand free
+ maltose
Relative Uptake (Da)
Exposure Time (minutes)


Maltose binding protein
ligand free
+ maltose
Relative Uptake (Da)
Exposure Time (minutes)


Maltose binding protein
ligand free
+ maltose
Relative Uptake (Da)
Exposure Time (minutes)

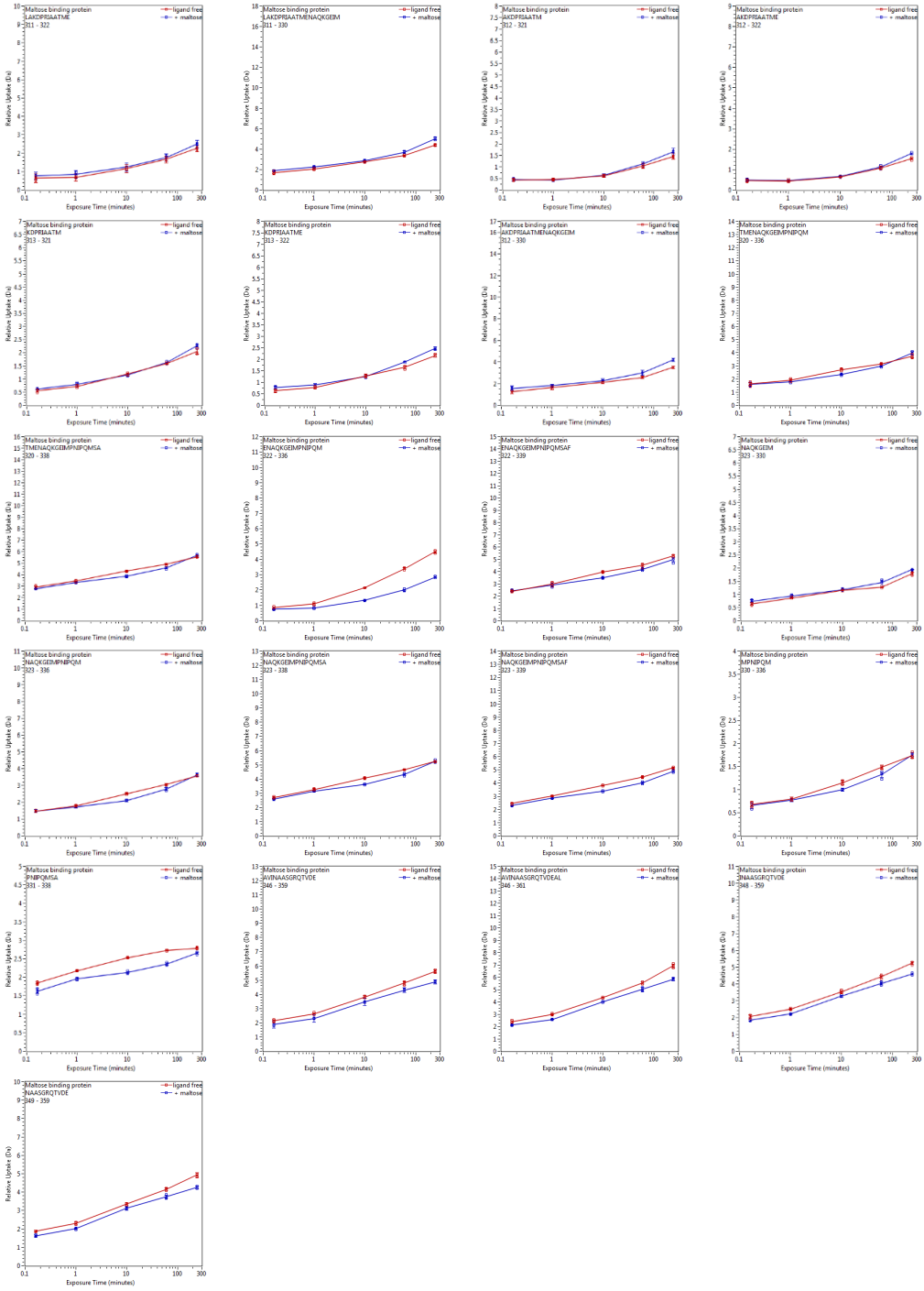


**Figure S8.** Deuterium uptake plots for MBP with (blue) and without (red) maltose bound.

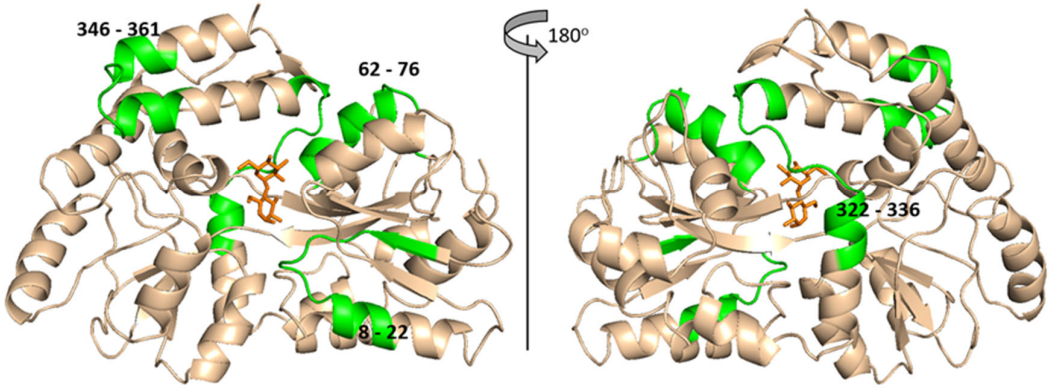



**Figure S9.** Regions identified to undergo significantly decreased HD exchange (green) upon maltose binding of MBP. The structure is from PDB 1ANF.

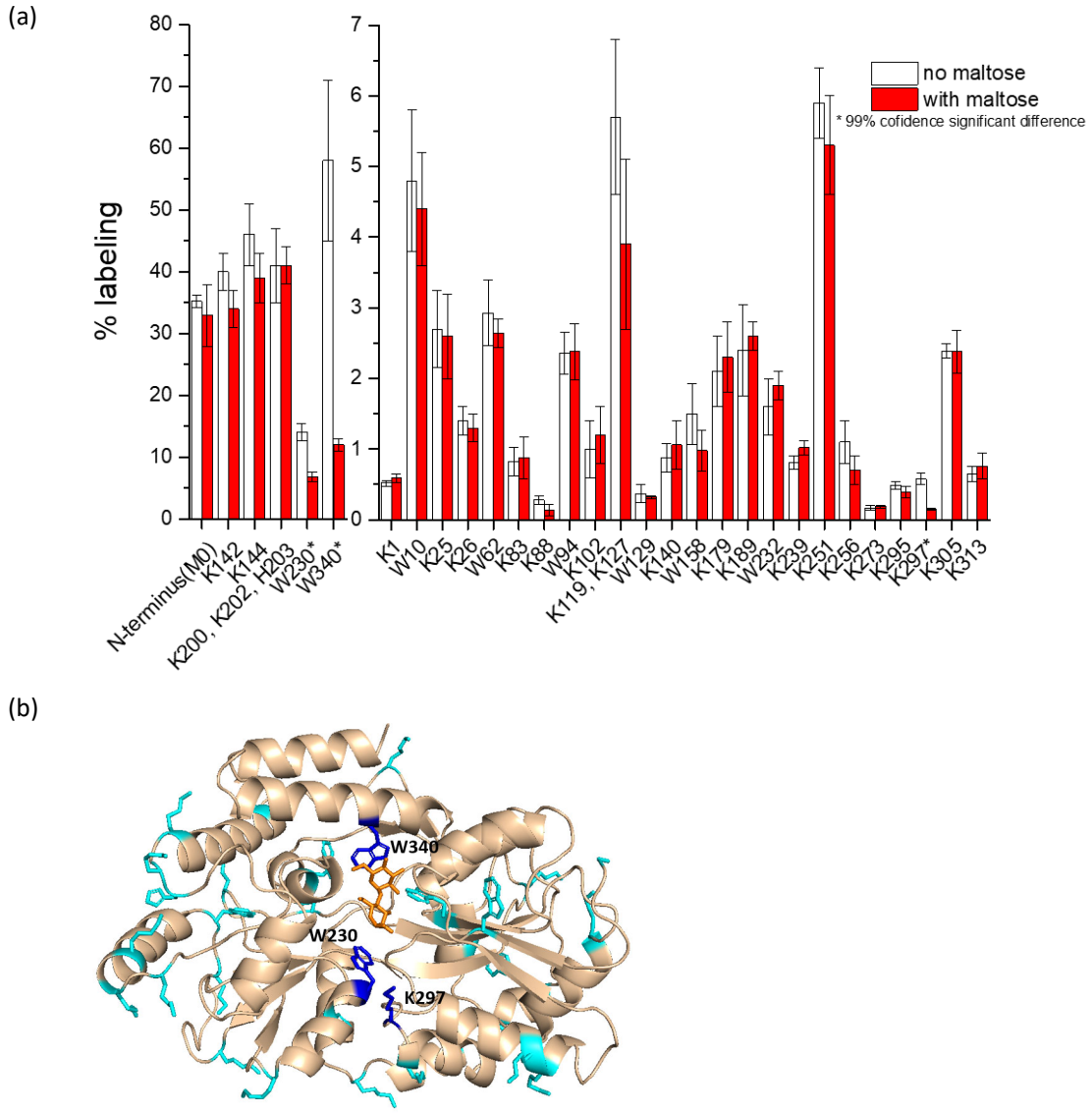



**Figure S10.** Summary of DEPC and HNSB labeling results for MBP upon maltose binding, and the location of modified residues mapped on the MBP-maltose complex structure (PDB ID 1ANF). (a) Relative covalent labeling percentages with (red) and without (white) ligand bound. Residues that undergo significant decreases in labeling at a 99% confidence interval are marked with an asterisk "*" on the x-axis next to their single-letter code. (b) Residues undergoing significant decreases in labeling are indicated in blue. Residues that are covalently labeled but undergo no significant change in labeling percentage are indicated in cyan.

**Table S3**. SASA values of residues on MBP that are modified by DEPC or HNSB. The residues indicated in bold are the residues that undergo statistically significant decreases in labeling.

| Residue | No ligand<br>PDB 1OMP | With ligand<br>PDB 1ANF |
|---|---|---|
| K1 | Not available in the structure | Not available |
| W10 | 11.9 | 10.6 |
| K25 | 54.2 | 83.4 |
| K26 | 67 | 64.7 |
| W62 | 22.5 | 22.7 |
| K83 | 96 | 94.1 |
| K88 | 63.1 | 57.8 |
| W94 | 3.8 | 3.6 |
| K102 | 67.1 | 66.3 |
| K119 | 48.3 | 47.1 |
| K127 | 64.6 | 83.8 |
| W129 | 0.3 | 1.1 |
| K140 | 46.2 | 43.2 |
| K142 | 62.5 | 48.5 |
| K144 | 35.2 | 30.6 |
| W158 | 0.1 | 1.4 |
| K179 | 62.3 | 68.9 |
| K189 | 30.5 | 39.9 |
| K200 | 67.2 | 62.2 |
| K202 | 68.3 | 79.9 |
| H203 | 15.7 | 16.4 |
| **W230** | **34.3** | **5.2** |
| W232 | 10.3 | 1.2 |
| K239 | 87.3 | 82.1 |
| K251 | 48.6 | 56.6 |
| K256 | 26.5 | 30.8 |
| K273 | 43.7 | 44.7 |
| K295 | 69.6 | 67.1 |
| **K297** | **38** | **23.9** |
| K305 | 61.5 | 47 |
| K313 | 87.8 | 71.7 |
| **W340** | **25** | **7.1** |