Caspase-6 Undergoes a Distinct Helix–Strand Interconversion Upon Substrate Binding

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Running title: Caspase-6 helix–strand interconversion

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Keywords: cysteine protease, apoptosis, neurodegeneration, hydrogen/deuterium exchange mass spectrometry, molecular dynamics

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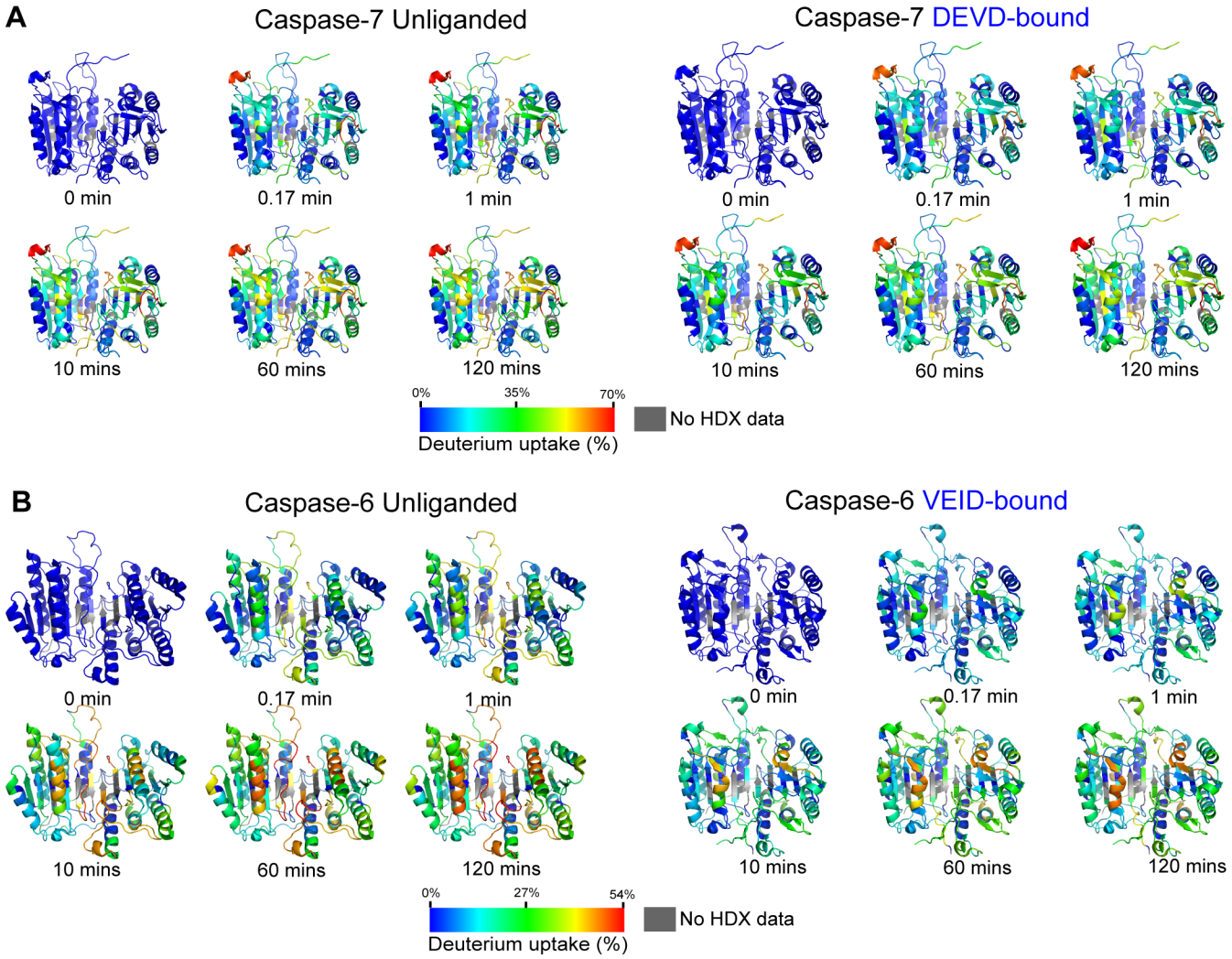
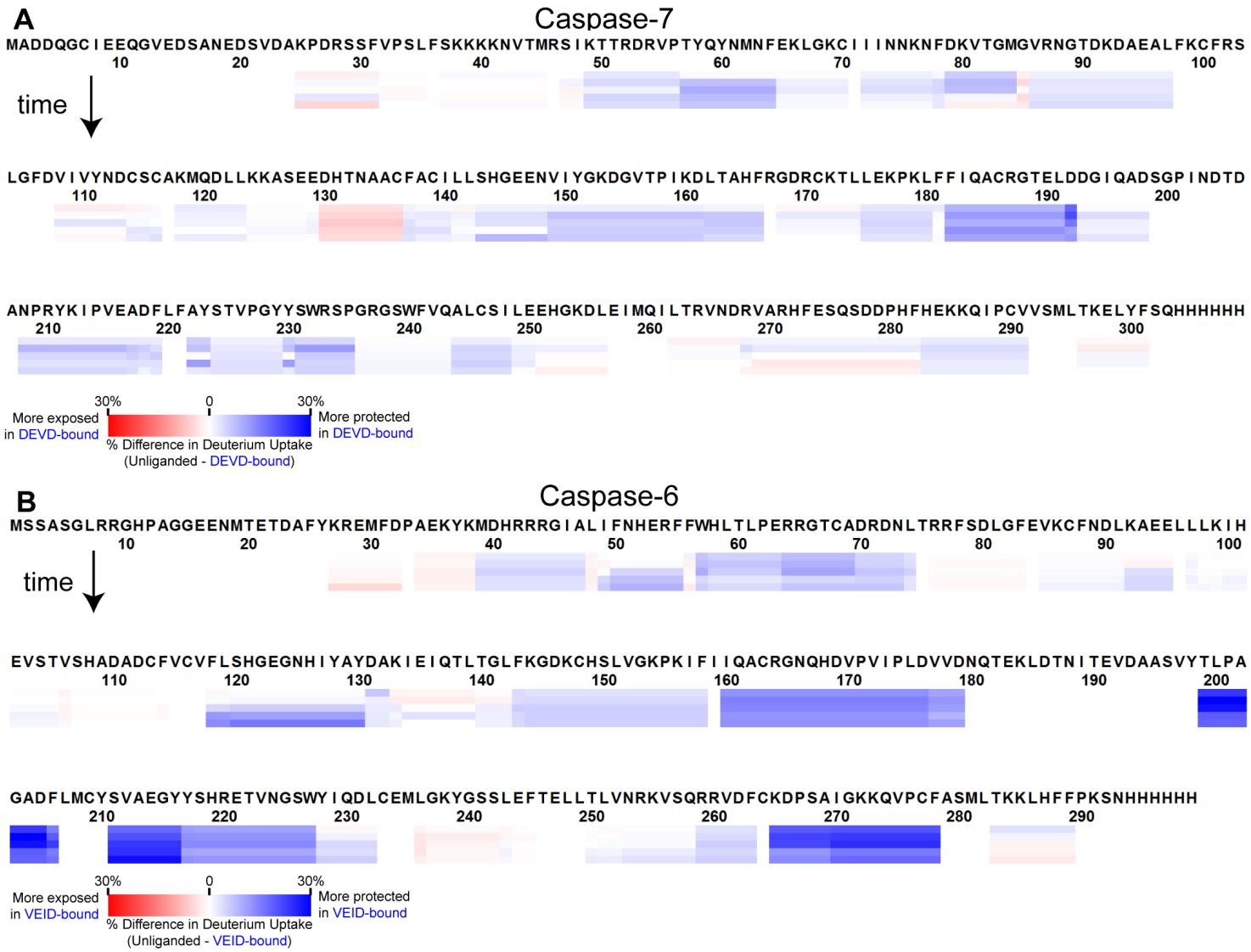
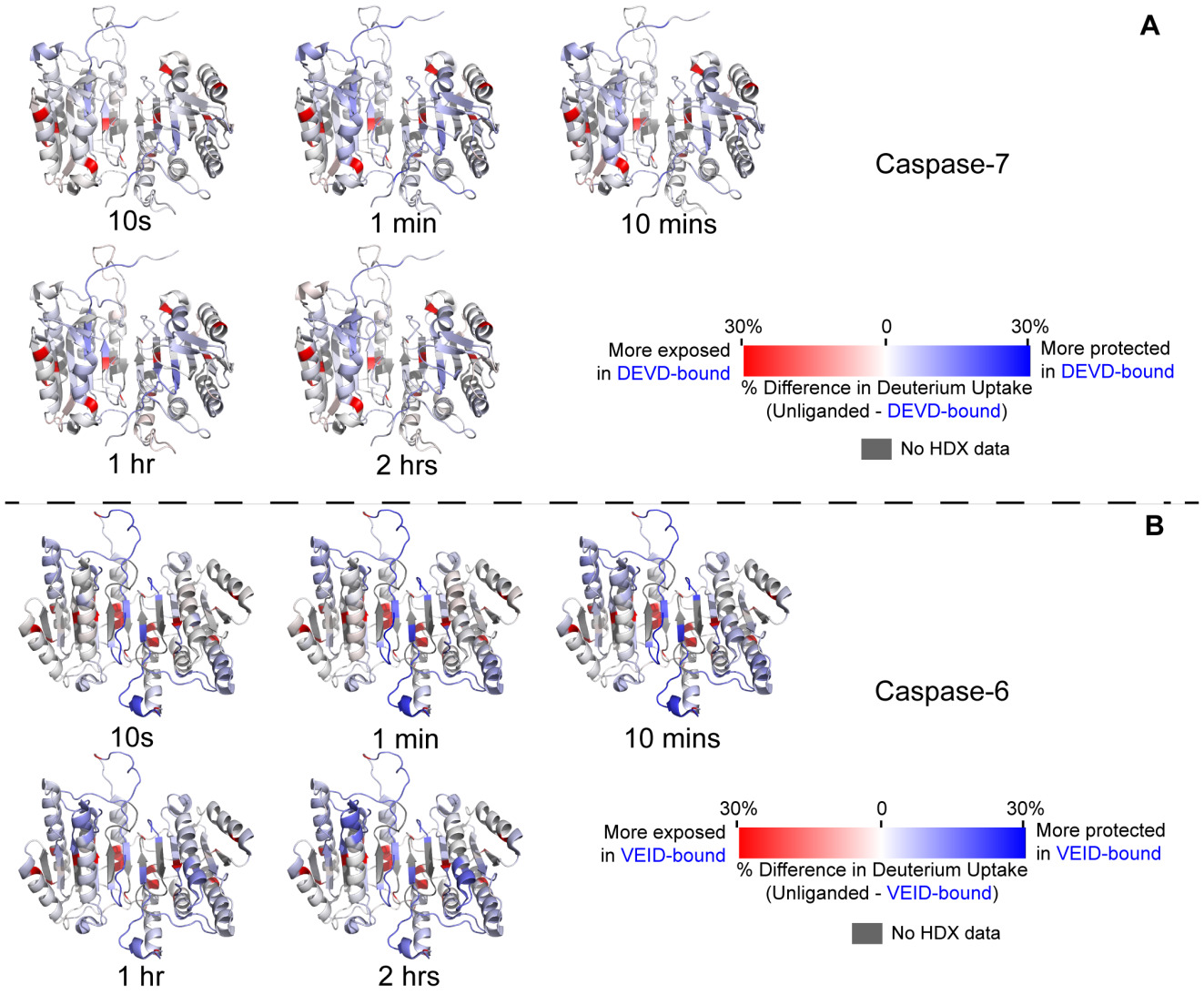
SUPPLEMENTAL FIGURE LEGENDS



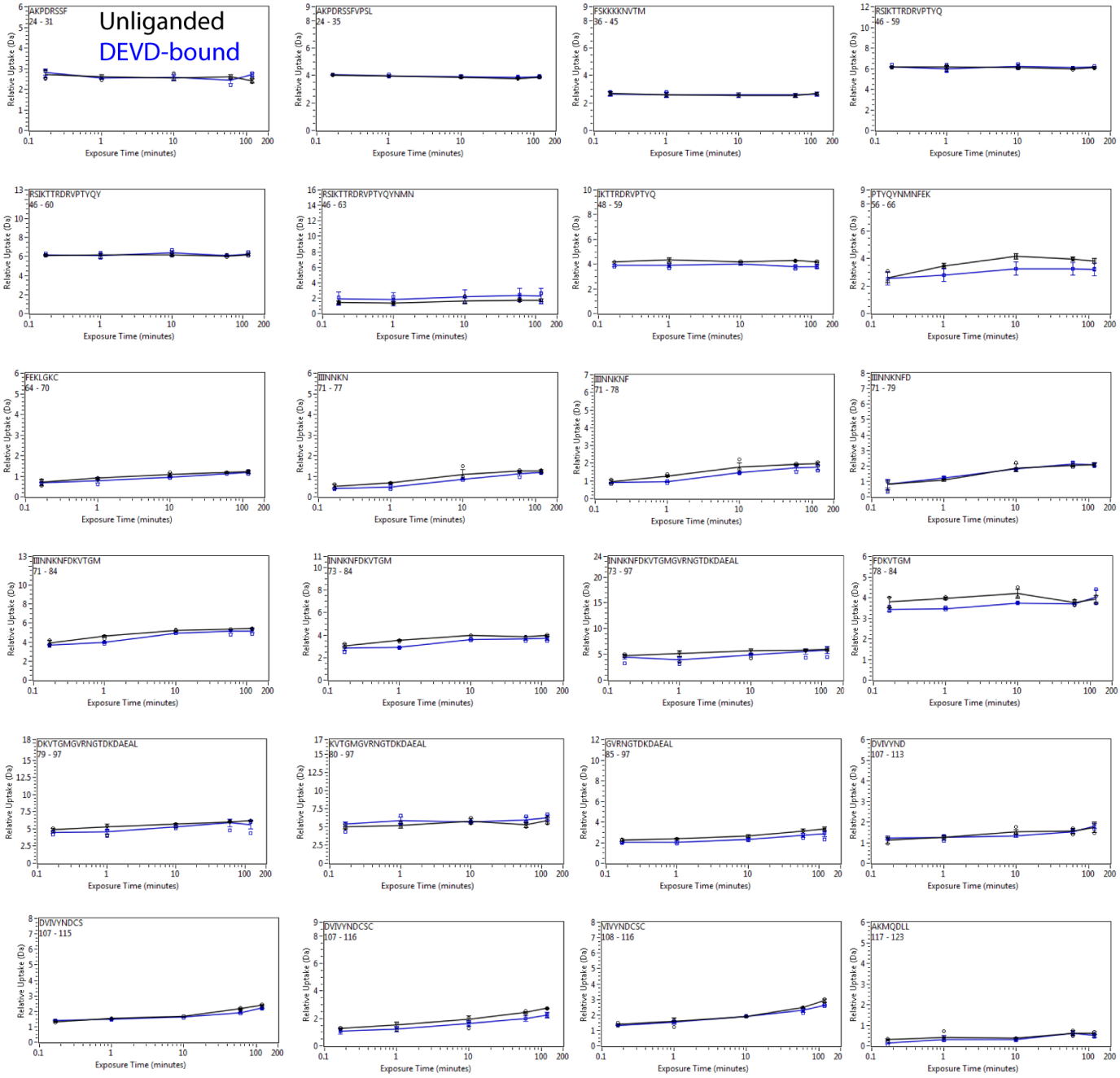
Supplemental Figure S1. The percent relative deuterium incorporation of caspase-7 (A) and caspase-6 (B) before and after binding to peptide-based substrate mimic as a function of H/D exchange incubation period are shown mapped onto its corresponding crystal structures (PBD code IK86 for caspase-7; PBD code 2WDP for caspase-6). The percent relative deuterium incorporation is calculated by dividing the observed deuterium uptake by the theoretical maximum deuterium uptake for each peptide.Regions of the protein without H/D exchange data are shown in *gray*.



Supplemental Figure S2. Heat maps of the percent difference in the deuterium uptake level between the unliganded and the peptide-based substrate mimic-bound states of caspase-7 (A) and caspase-6 (B) are shown mapped onto its corresponding linear sequence. The H/D exchange incubation period (*minutes*: 0.17, 1, 10, 60, and 120) are shown from *top* to *bottom*. The percent relative deuterium incorporation is calculated by dividing the observed deuterium uptake by the theoretical maximum deuterium uptake for each peptide. *Red* regions undergo more H/D exchange (less protected, more flexible) in the unliganded state compared to the peptide-based substrate-bound state. *Blue* regions undergo less H/D exchange (more protected, less flexible) in the unliganded state compared to the peptide-based substrate-bound state. Peptic peptides with no H/D exchange data are colored *white*.

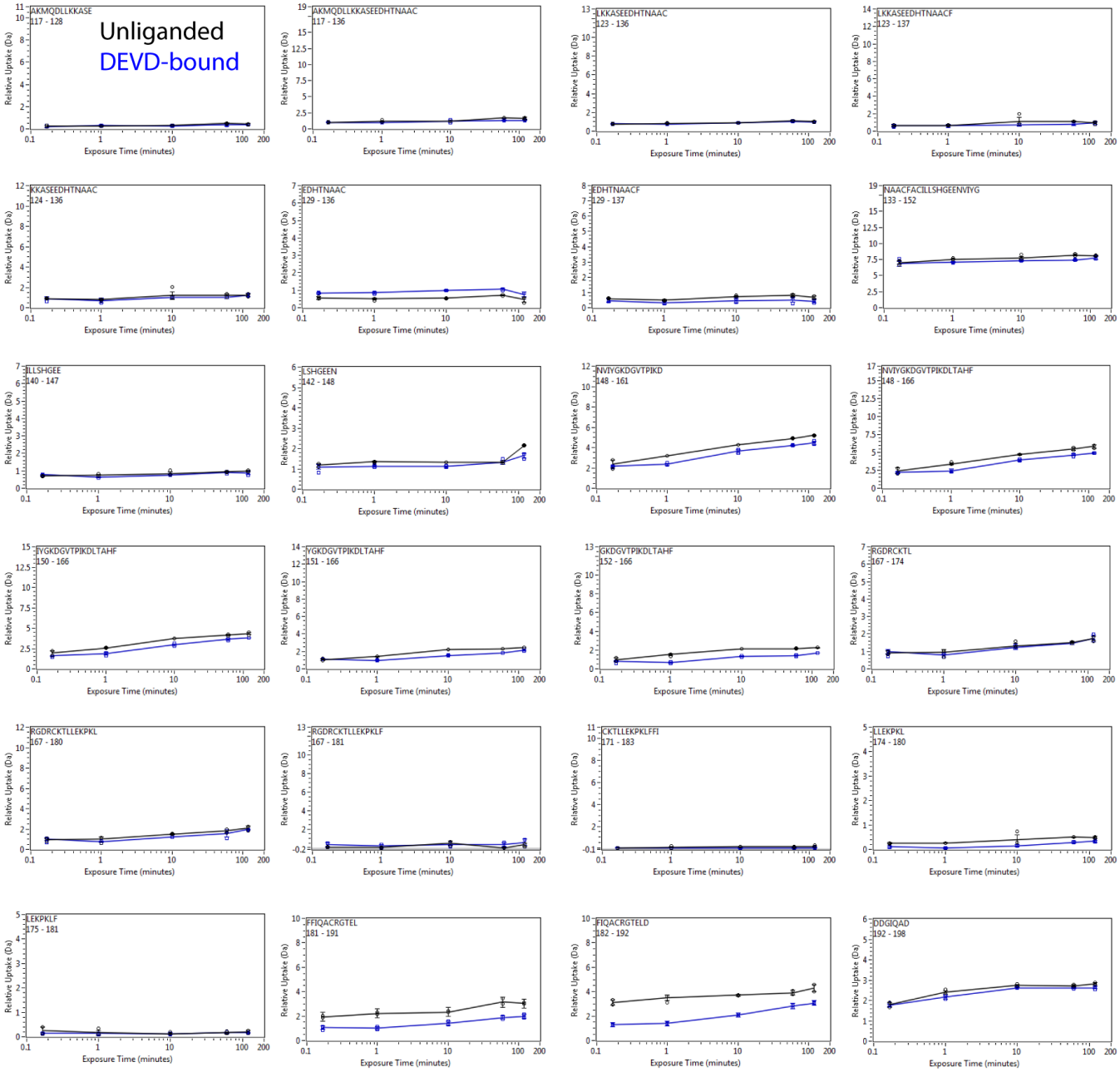


Supplemental Figure S3. Heat maps of the percent difference in the deuterium uptake level between the unliganded and the peptide-based substrate mimic-bound states of caspase-7 (A) and caspase-6 (B) are shown mapped onto its corresponding crystal structures (PBD code 1K86 for caspase-7; PBD code 2WDP for caspase-7). Regions of the protein without H/D exchange data are shown in *gray*.

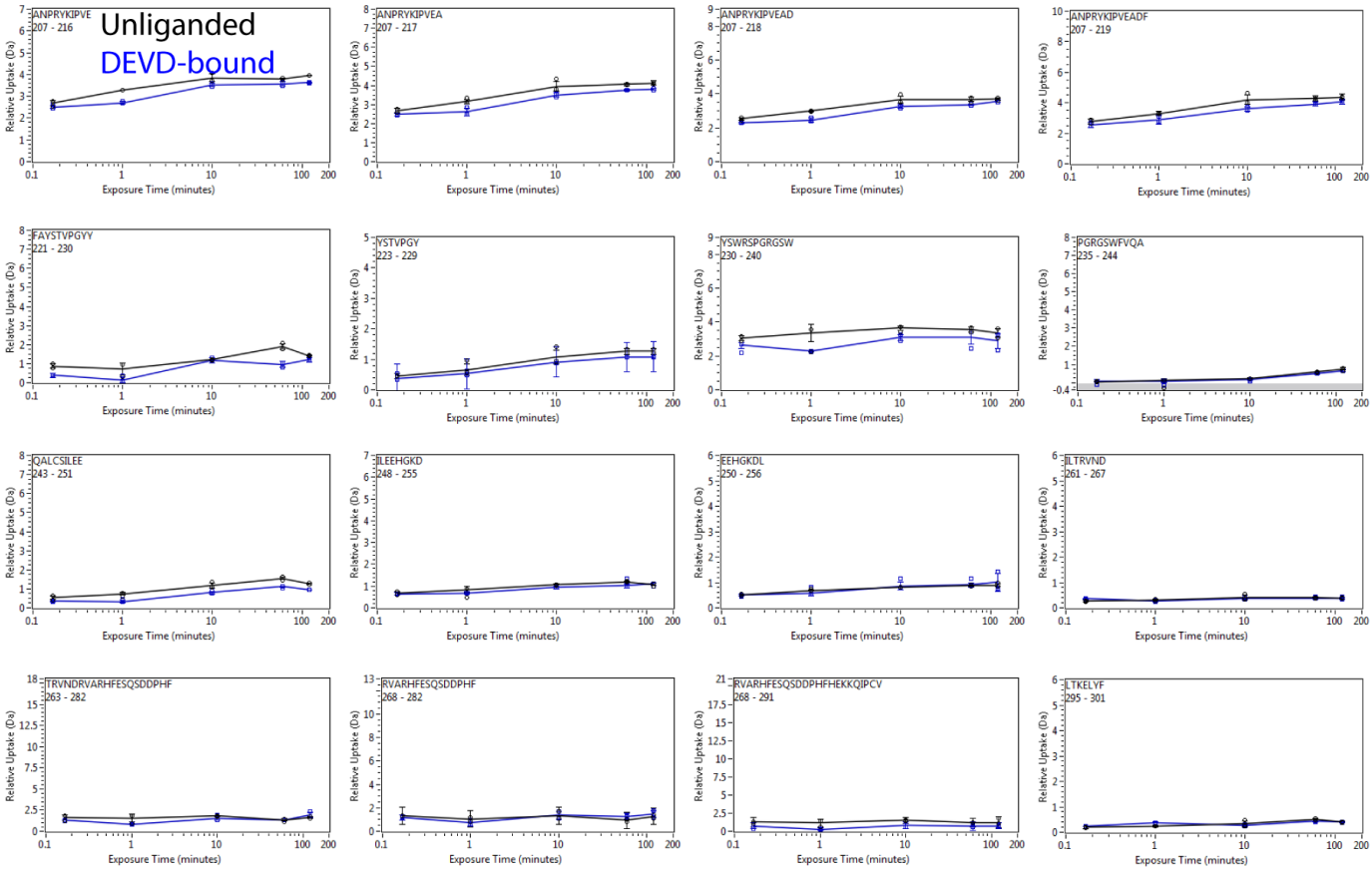


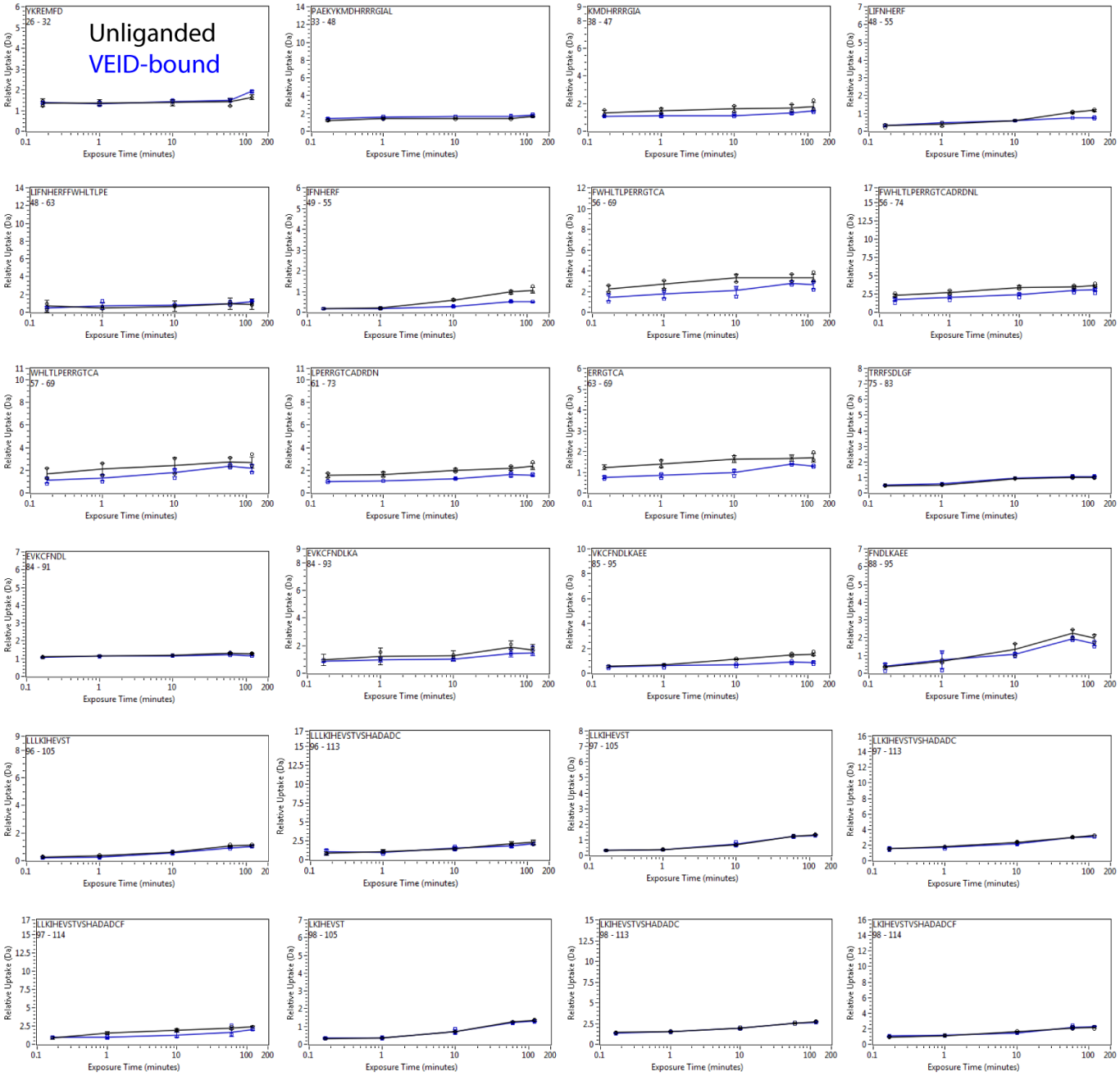
Supplemental Figure S4. Relative deuterium uptake plots comparing the unliganded (*black line*) and the DEVD-bound (*blue line*) states of caspase-7 as a function of H/D exchange incubation period. The amino acid sequence and number are shown for each peptic peptide of caspase-7. The standard deviations (SD) of two independent H/DX-MS experiments performed in two separate days are indicated.

Supplemental Figure S4
(continued)



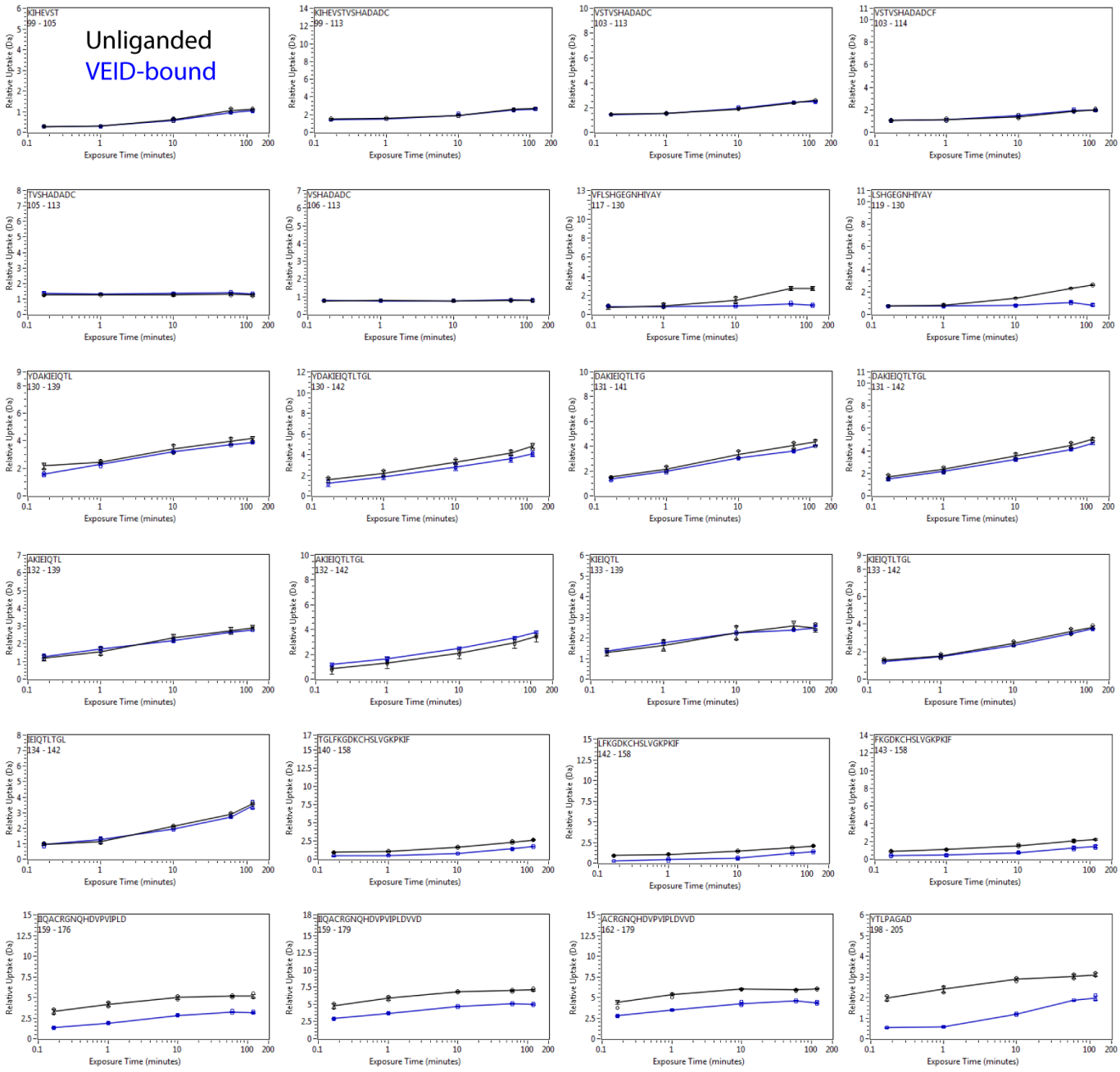
Supplemental Figure S4
(continued)



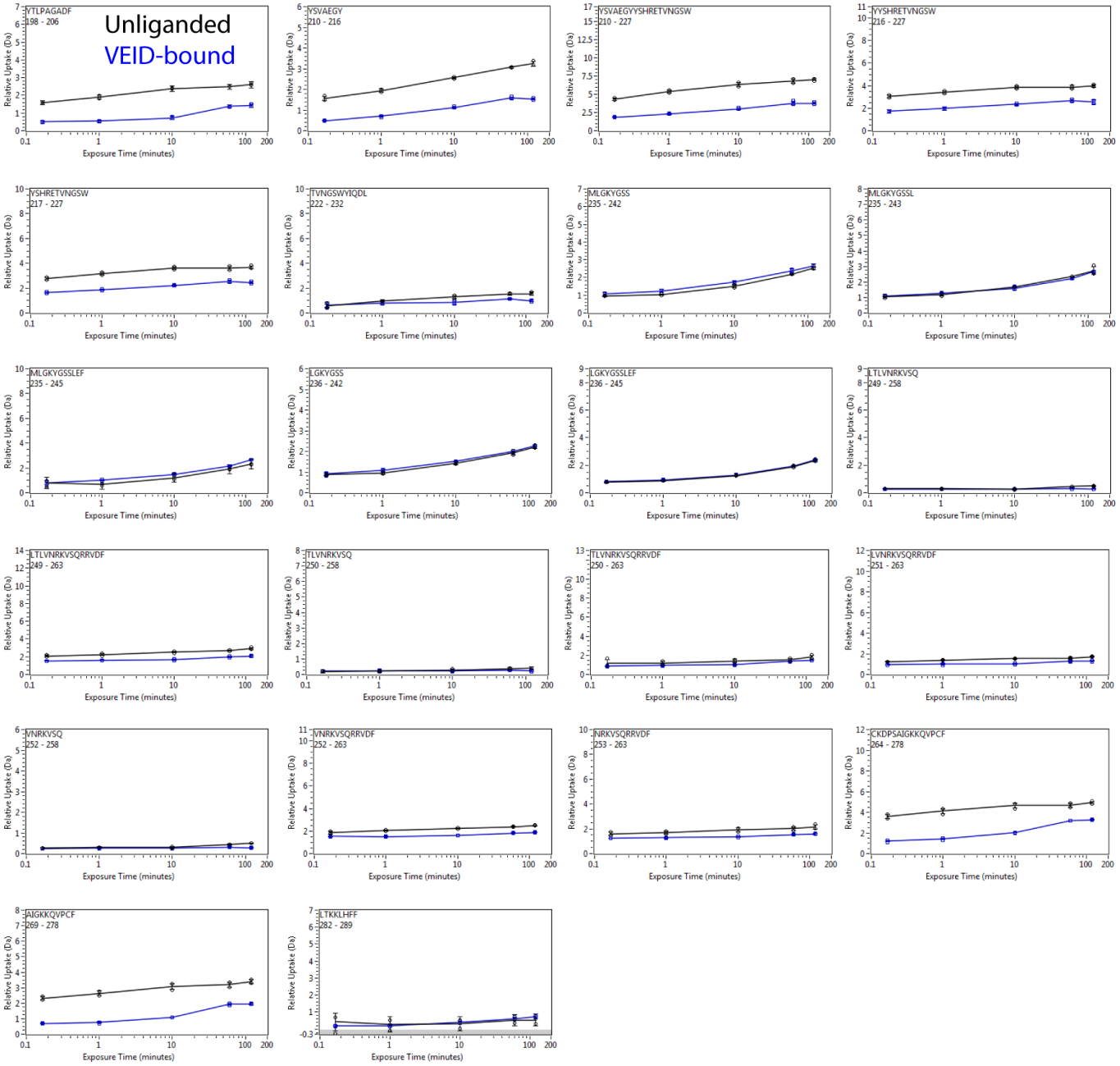


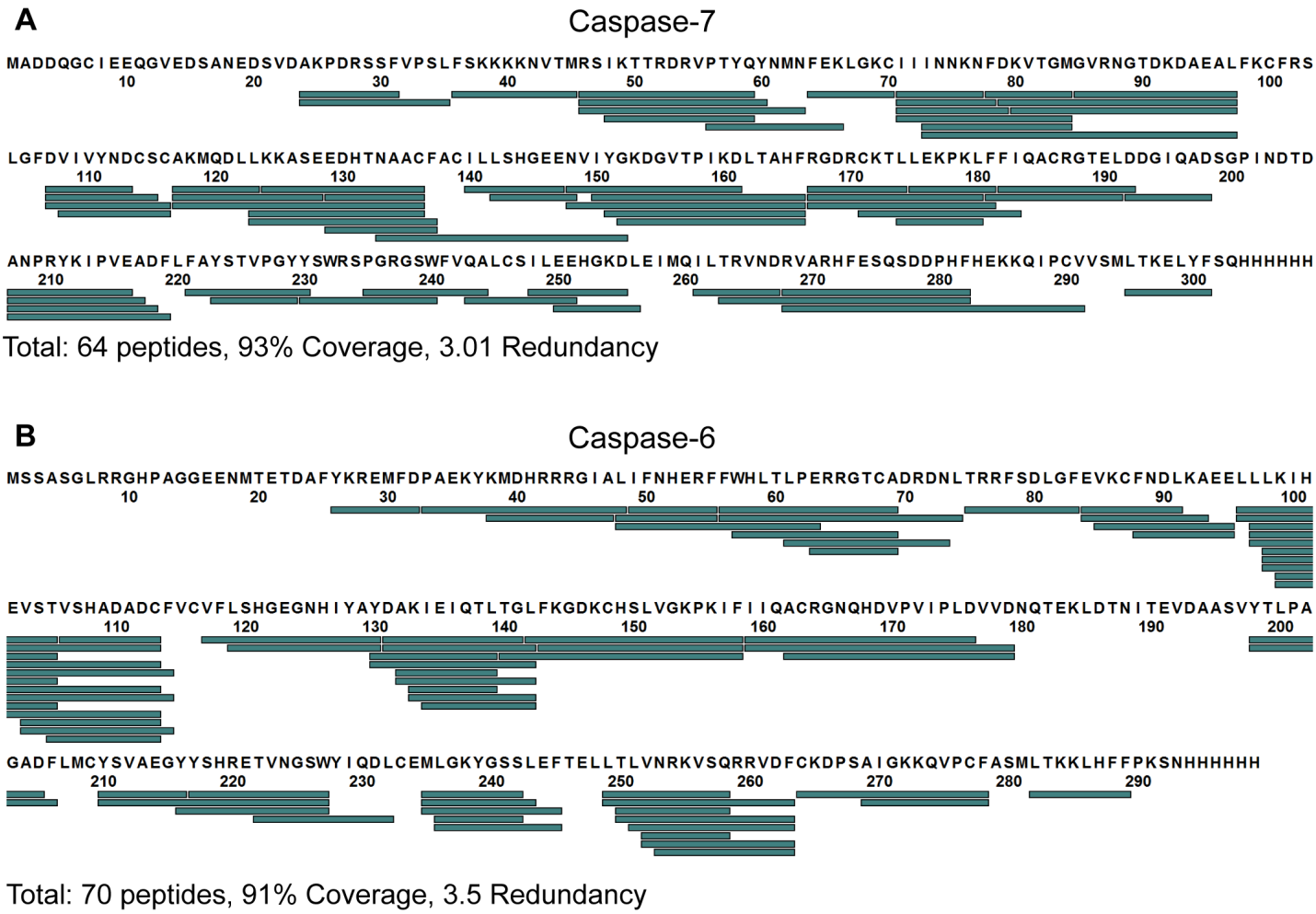
Supplemental Figure S5. Relative deuterium uptake plots comparing the unliganded (*black line*) and the VEID-bound (*blue line*) states of caspase-6 as a function of H/D exchange incubation period. The amino acid sequence and number are shown for each peptic peptide of caspase-6. The standard deviations (SD) of two independent H/DX-MS experiments performed in two separate days are indicated.

Supplemental Figure S5
(continued)



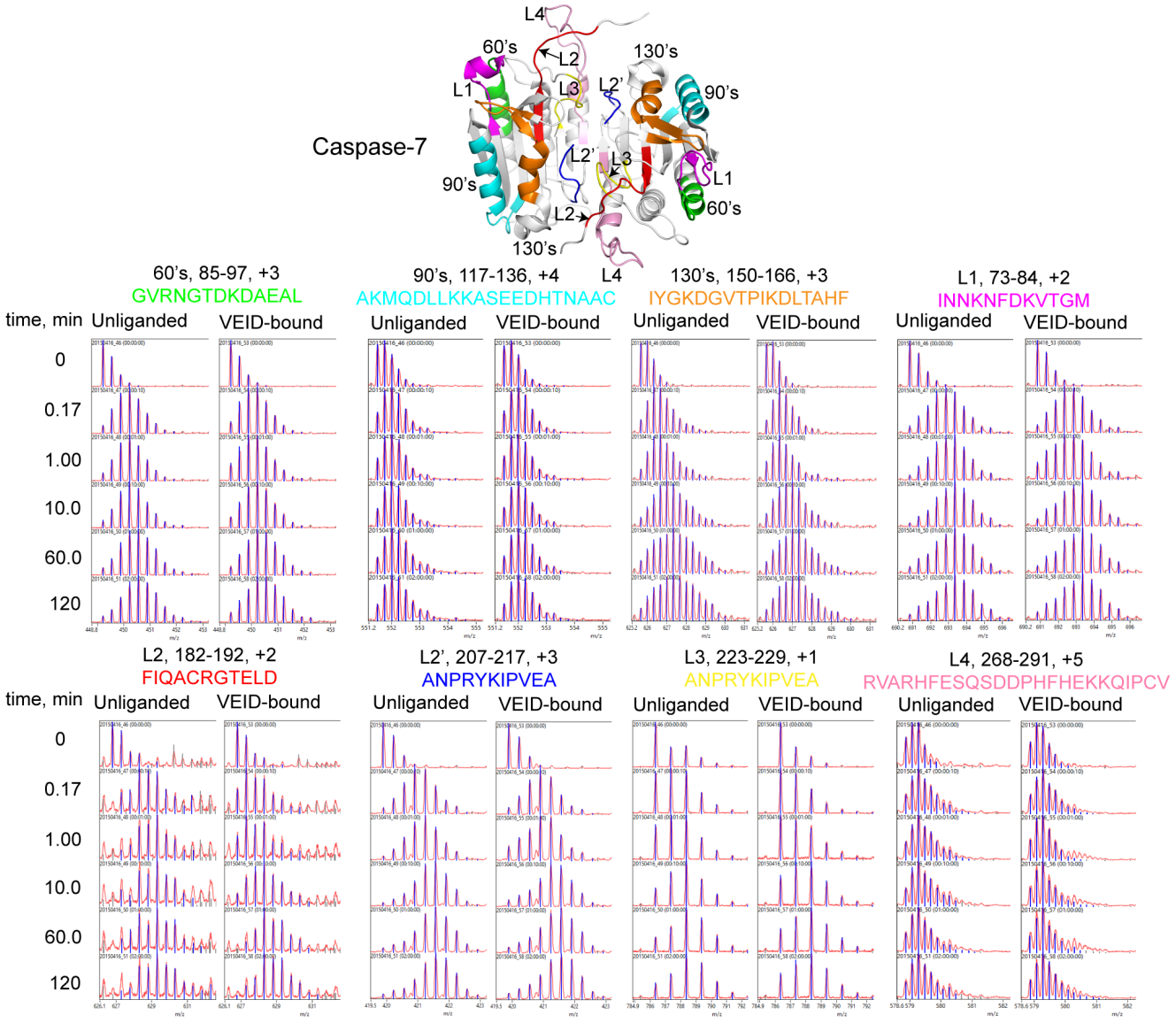
Supplemental Figure S5
(continued)





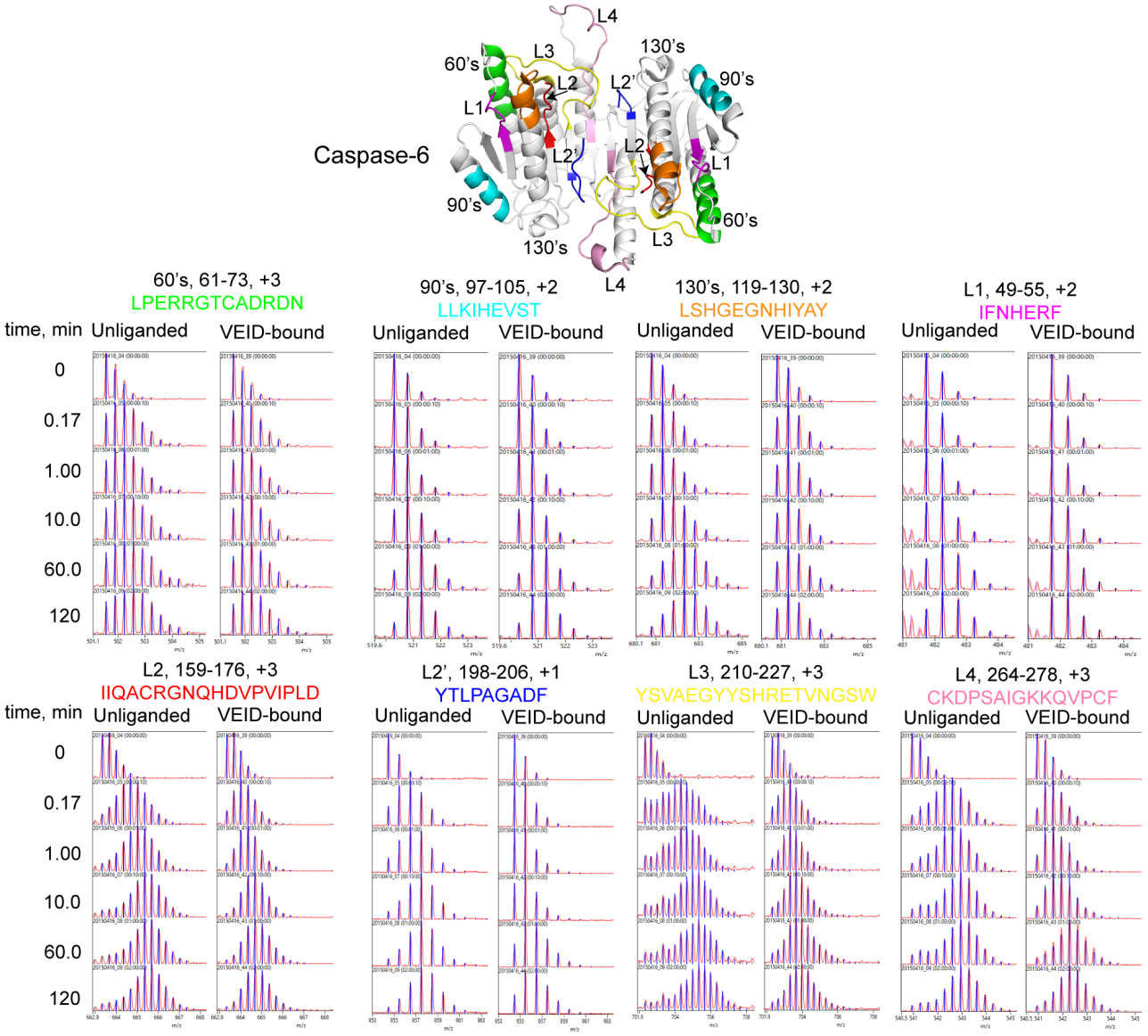
Supplemental Figure S6. Coverage Map of the peptic peptides identified from H/DX-MS experiments for caspase-7 (A) and caspase-6 (B) in both the unliganded and the peptide-based substrate mimic-bound states. In both caspase-6 and caspase-7, the protein constructs used were designed to exclude the prodomain and linker, which represent the fully cleaved and active form of the enzymes.

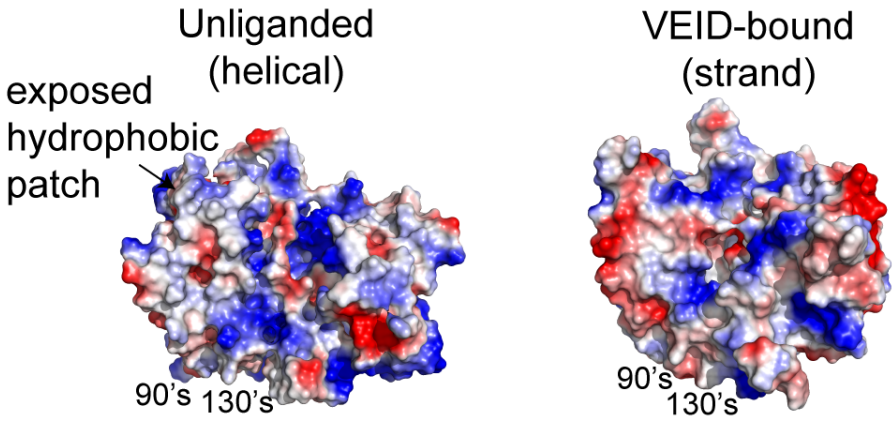
Supplemental Figure S7A



Supplemental Figure S7. Representative MS spectra of the peptic peptides following H/D exchange experiments of the unliganded and peptide-based substrate mimic-bound states of caspase-7 (A) and caspase-6 (B). The relative location of the highlighted regions (60's, *green*; 90's, *cyan*; 130's, *orange*; L1, *magenta*; L2, *red*; L3, *yellow*; L4, *pink*) and its representative peptic peptides are shown mapped onto the corresponding crystal structures of casapse-7 (PBD code 1K86) and capase-6 (PDB code 2WDP). The amino acid sequence, the charge state and the residue number covered by the representative peptic peptide are also indicated.

Supplemental Figure S7B





Supplemental Figure S8. Map of the electrostatic potential of caspase-6 in the unliganded (*left*, PDB code 2WDP) and the VEID-bound (*right*, PDB code 3OD5) states are shown (*red*, acidic regions; *blue*, basic regions; *white*, hydrophobic/neutral regions). The vacuum electrostatic potential maps were generated using Pymol (Schrödinger) and the arrow indicates electrostatic potential map around the hydrophobic patch.