The influence of adnectin binding on the extracellular domain of epidermal growth factor receptor

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Supplemental Figures

Figure S1

Amino acid sequence and peptic peptide coverage for exEGFR when digested alone. Each blue bar under the sequence corresponds to a peptic peptide that was identified. A total of 84.1% linear coverage was obtained for exEGFR. This protein coverage map was generated using the DynamX 2.0 software package (Waters Corp).

Figure S2

Amino acid sequence and peptic peptide coverage of exEGFR in the presence of Adnectin 1. Each red bar under the sequence corresponds to a peptic peptide that was identified and for which deuterium uptake was followed (as in Supplemental Figure S3). The followed peptides correspond to 80.3% linear sequence coverage. This protein coverage map was generated using the DynamX 2.0 software package (Waters Corp).

Figure S3

Deuterium incorporation graphs for the peptic peptides that were followed by HDX MS for exEGFR free (blue) and bound to Adnectin 1 (red). Each datapoint represents the average of two independent measurements. The green boxes indicate the regions where differences in deuterium uptake were observed between free exEGFR and Adnectin 1-bound exEGFR. The graphs were produced by DynamX 2.0 software (Waters Corp.).

Figure S4

A. Amino acid sequence and peptic peptide coverage of Adnectin 1 when digested alone. Each red bar under the sequence corresponds to a peptic peptide that was identified and for which deuterium uptake was followed. **B.** Deuterium incorporation graphs for Adnectin 1 peptic peptides that were followed by HDX MS. Each datapoint represents the average of two independent measurements. The graphs were produced by DynamX 2.0 software (Waters Corp.).

Figure S5

Verification that deuterium scrambling was negligible during ETD. The relative deuterium content of c ions for triply charged peptide P1 [as described in [1], sequence shown in top panel] was measured using a Synapt G2-S Q-Tof MS. These data are in agreement with previously published results for low scrambling conditions using the Synapt G2 MS in ETD fragmentation mode [2].

Figure S6

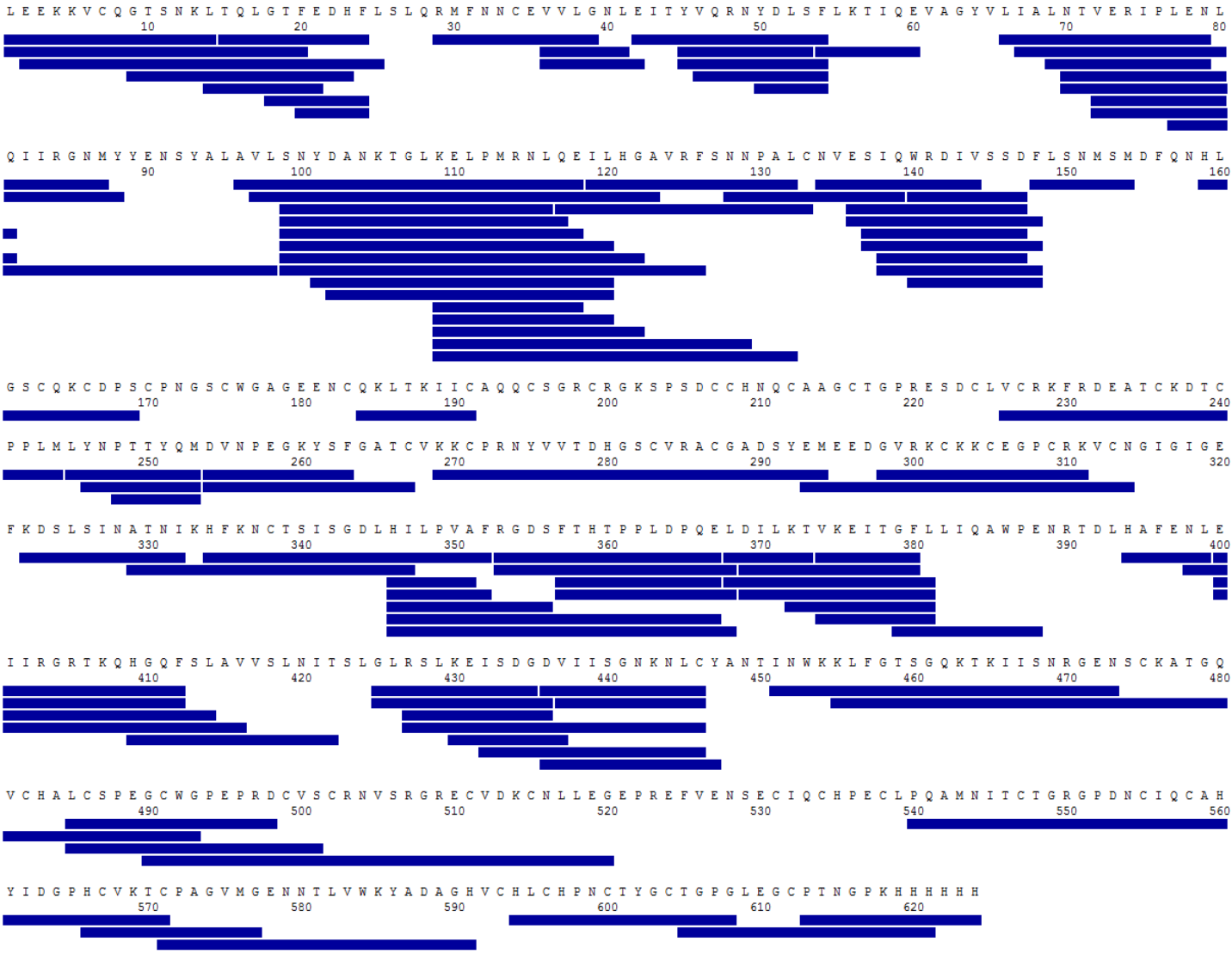
Mass spectra of z_{17}, z_{16}, z_9, z_7, $c_{10,}$ and c_2 ions from the ETD fragmentation of exEGFR peptide 1-19. **A.** z ions z_{17}, z_{16}, z_9, z_7 showed uptake differences between unbound (blue) and Adnectin 1-bound (red) versions of exEGFR peptide 1-19 whereas **B.** c ions c_{10} and c_2 did not. These data were acquired after labeling exEGFR or exEGFR:Adnectin 1 complexes for 30 minutes with deuterium (see main text Methods section).

Supplemental References

(1) Rand, K. D.; Jorgensen, T. J. Development of a peptide probe for the occurrence of hydrogen (1H/2H) scrambling upon gas-phase fragmentation. *Anal Chem* **2007***,* 79, 8686-8693.

(2) Rand, K. D.; Pringle, S. D.; Morris, M.; Engen, J. R.; Brown, J. M. ETD in a traveling wave ion guide at tuned Z-spray ion source conditions allows for site-specific hydrogen/deuterium exchange measurements. *J Am Soc Mass Spectrom* **2011***,* 22, 1784-1793.

Supplemental Figure S1



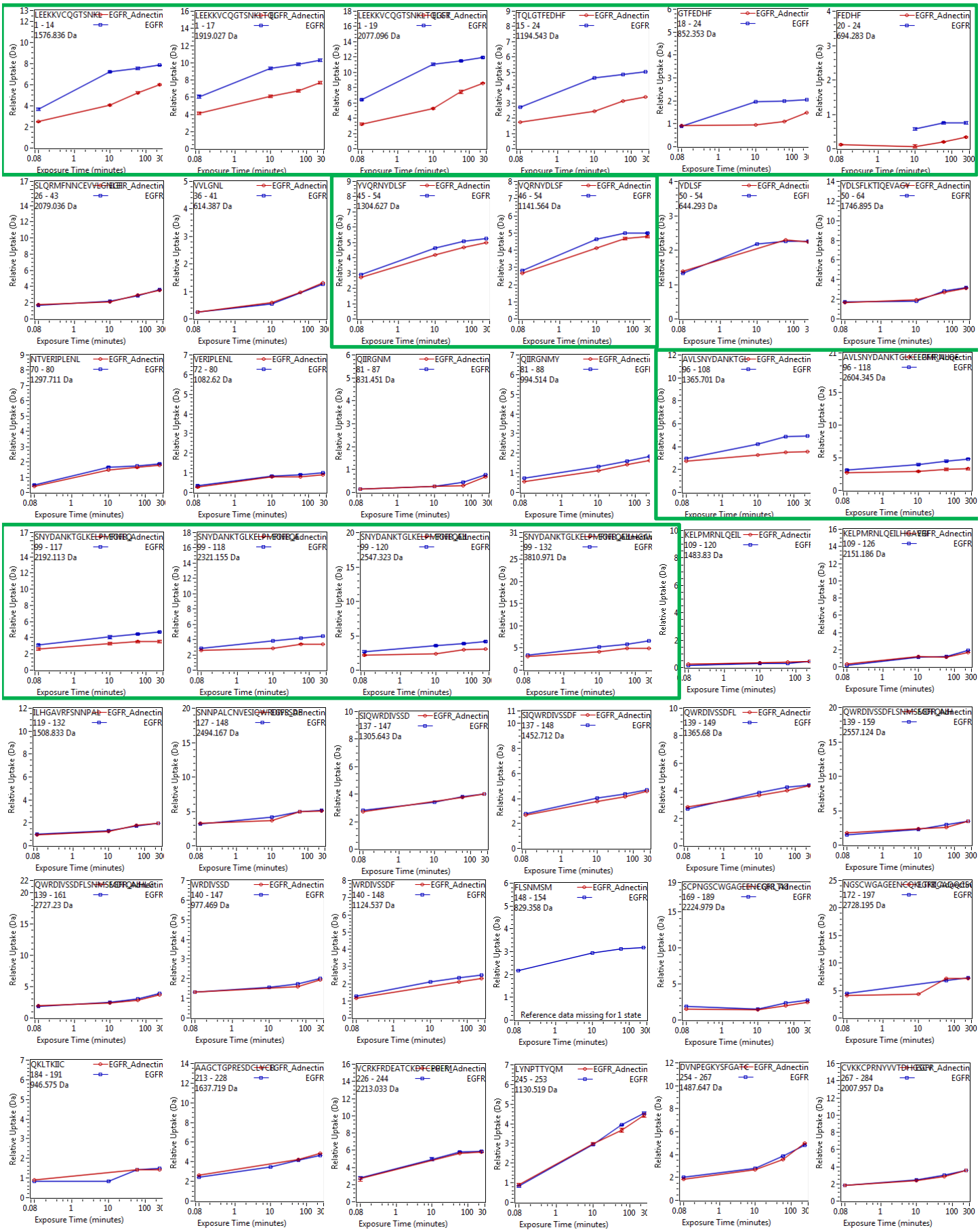
Total: 112 Peptides, 84.1% Coverage, 2.98 Redundancy

Supplemental Figure S2

Total: 76 Peptides, 80.3% Coverage, 2.10 Redundancy

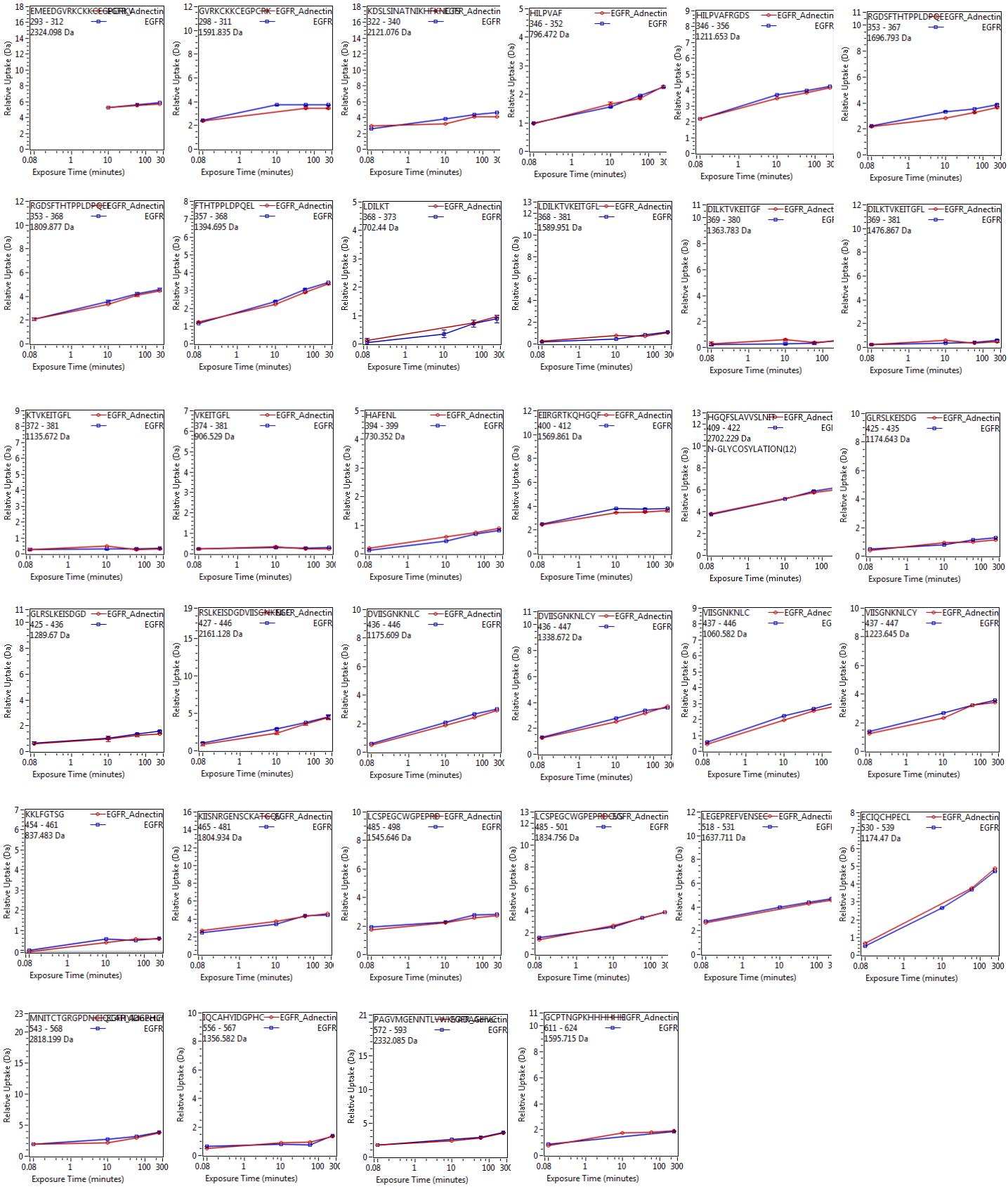
Supplemental Figure S3

exEGFR + Adnectin
exEGFR



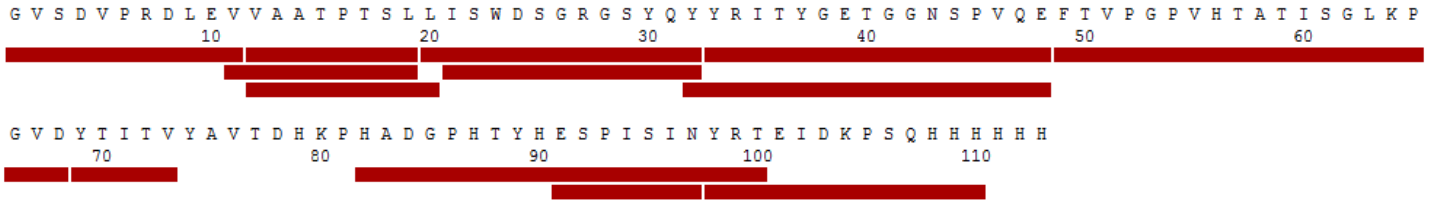
Supplemental Figure S3, cont.

exEGFR + Adnectin
exEGFR



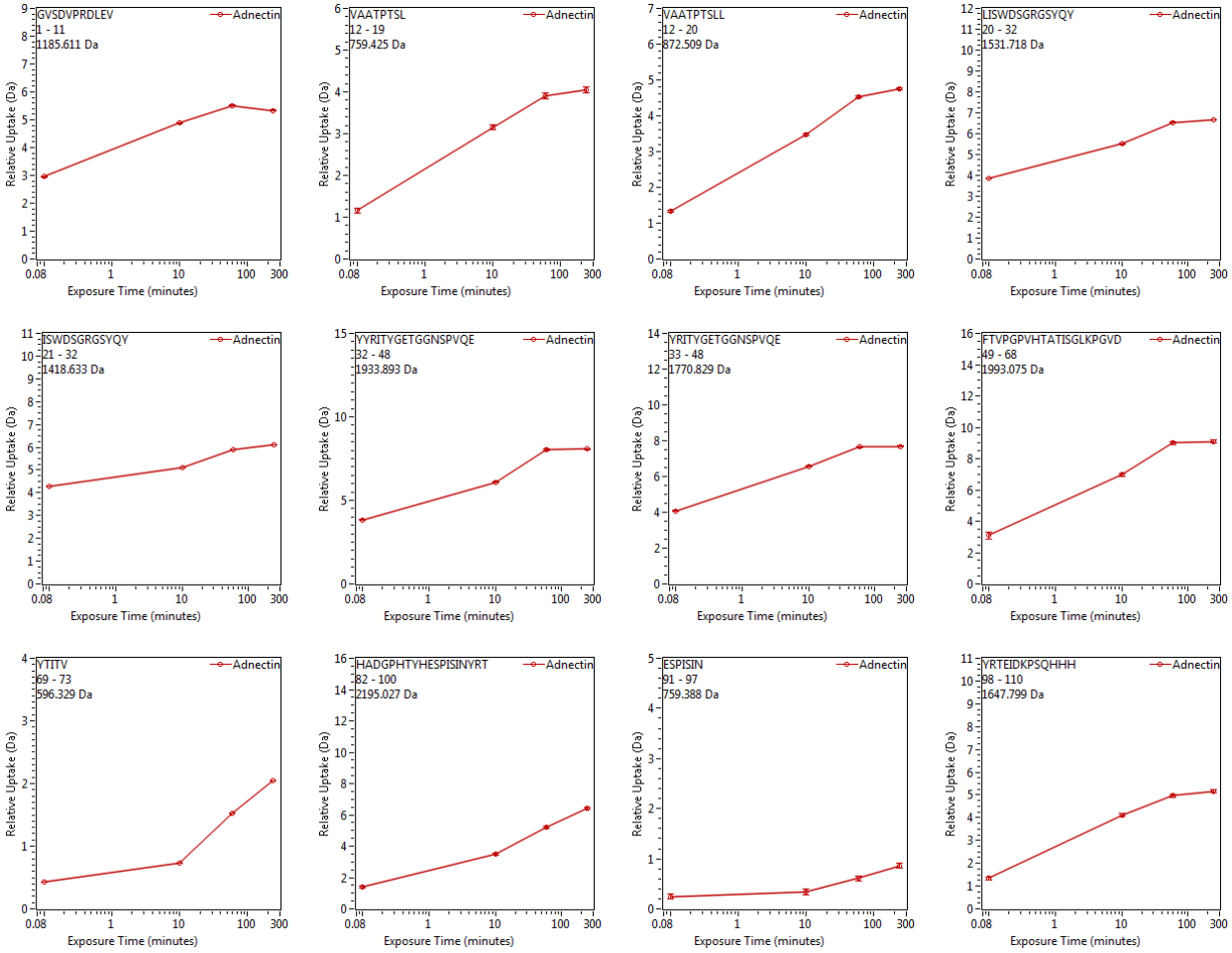
Supplemental Figure S4

A.

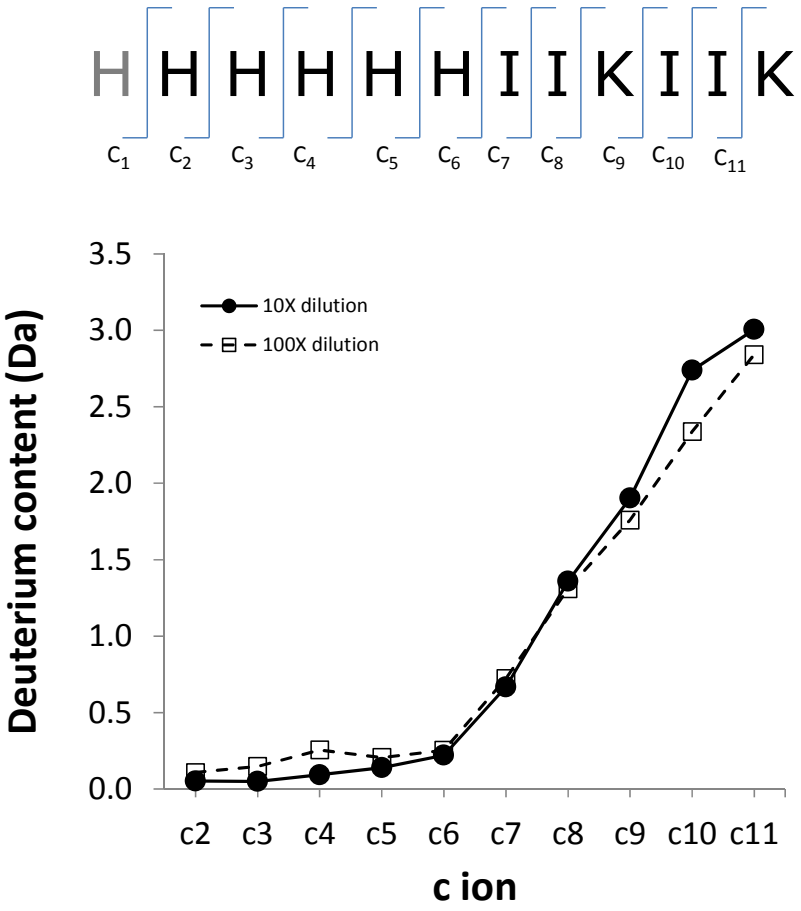


Total: 13 Peptides, 90.3% Coverage, 1.56 Redundancy

B.



Supplemental Figure S5



Supplemental Figure S6