

## SUPPORTING INFORMATION

**Figure S1:** Enhanced mass envelope of 0.5, 5, 30 and 120 min deuterated glucagon monomer ( $m/z = 871.1729$ ,  $z = +4$ ). Spectra show single Gaussian peak distribution with no significant peak broadening. #D = number of deuterons incorporated.

**Figure S2:** Amino acid sequence of human glucagon showing the secondary structural elements with cylinders representing  $\alpha$ -helix. Dotted lines indicate the 9 pepsin digest fragments analyzed in this study, with a total sequence coverage of 100%.

**Figure S3:** Percent deuterium uptake for Peaks I (closed circle) and II (closed square) populations as a function of fibrillation time ( $n = 3$ , mean  $\pm$  SD).

**Figure S4:** Enhanced mass spectra of the four selected pepsin digest fragments, 1-6 ( $m/z = 676.2988$ ,  $z = +1$ ), 1-9 ( $m/z = 979.4110$ ,  $z = +1$ ), 10-21 ( $m/z = 376.1892$ ,  $z = +4$ ) and 22-29 ( $m/z = 519.7599$ ,  $z = +2$ ). D = average percent deuterium uptake ( $n = 3$ ). Gray and black broken lines represent the centroids of deuterated peptide mass envelopes after 0 and 1440 min of fibrillation, respectively.

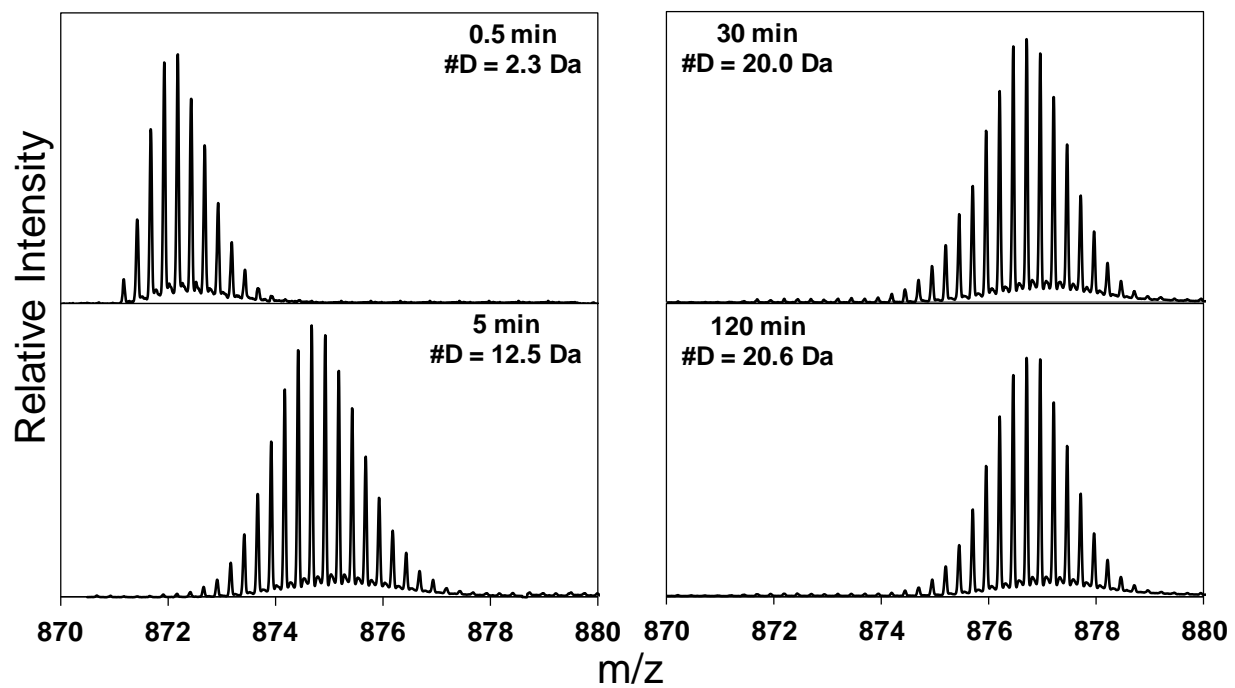
**Figure S5:** Percent deuterium uptake for pepsin digest peptides as a function of time of fibrillation. The data were fitted to an equation for one-phase exponential decay using GraphPad Prism 5 software (San Diego, CA) ( $n = 3$ , mean  $\pm$  SD).

**Figure S6:** Prediction of aggregation prone regions in glucagon using AGGRESCAN (A) and TANGO (B) software.

**Figure S7:** Enhanced mass spectra of the three selected pepsin digest fragments, 1-9 ( $m/z = 979.4110$ ,  $z = +1$ ), 10-21 ( $m/z = 376.1892$ ,  $z = +4$ ) and 22-29 ( $m/z = 519.7599$ ,  $z = +2$ ). Gray and black broken lines represent the centroids of deuterated peptide mass envelopes for Peaks I' and II', respectively.

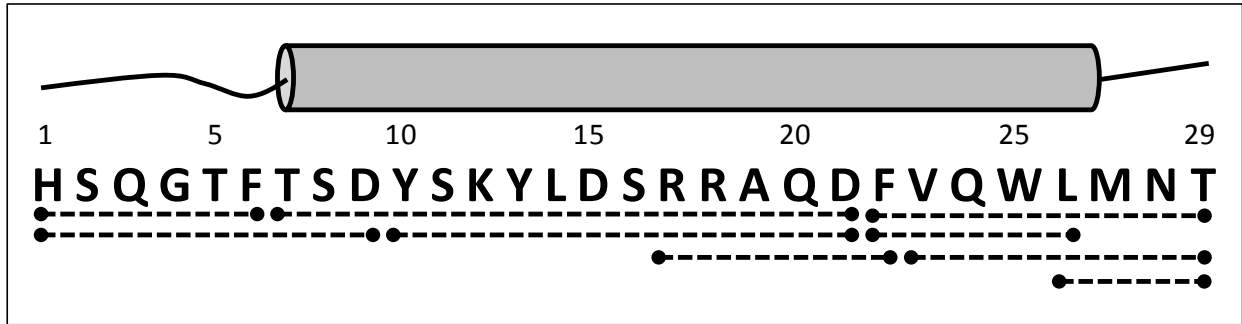
**Figure S8:** Simulations of  $\alpha$ -helix content of glucagon-derived peptides: **(A)** a single molecule of fragment 1-8, **(B)** two molecules of fragment 1-8, **(C)** a single molecule of fragment 22-29 and **(D)** two molecules of fragment 22-29. The  $\alpha$ -helix content for each amino acid in the fragment is shown.

**Figure S1**



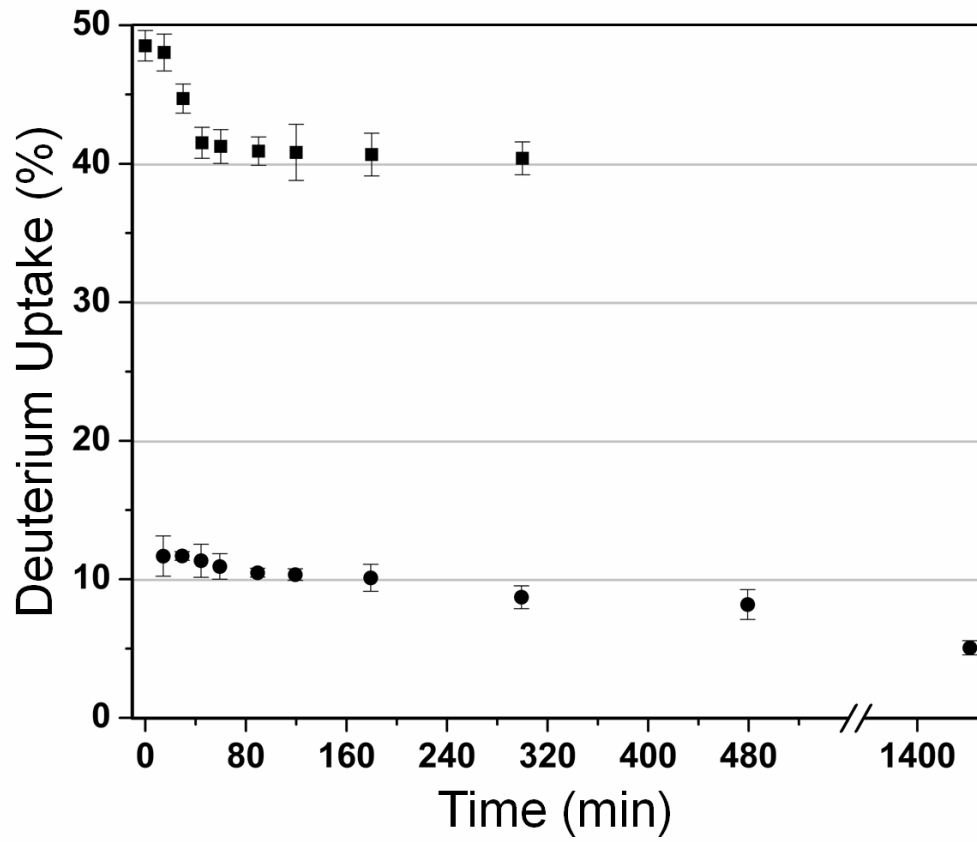
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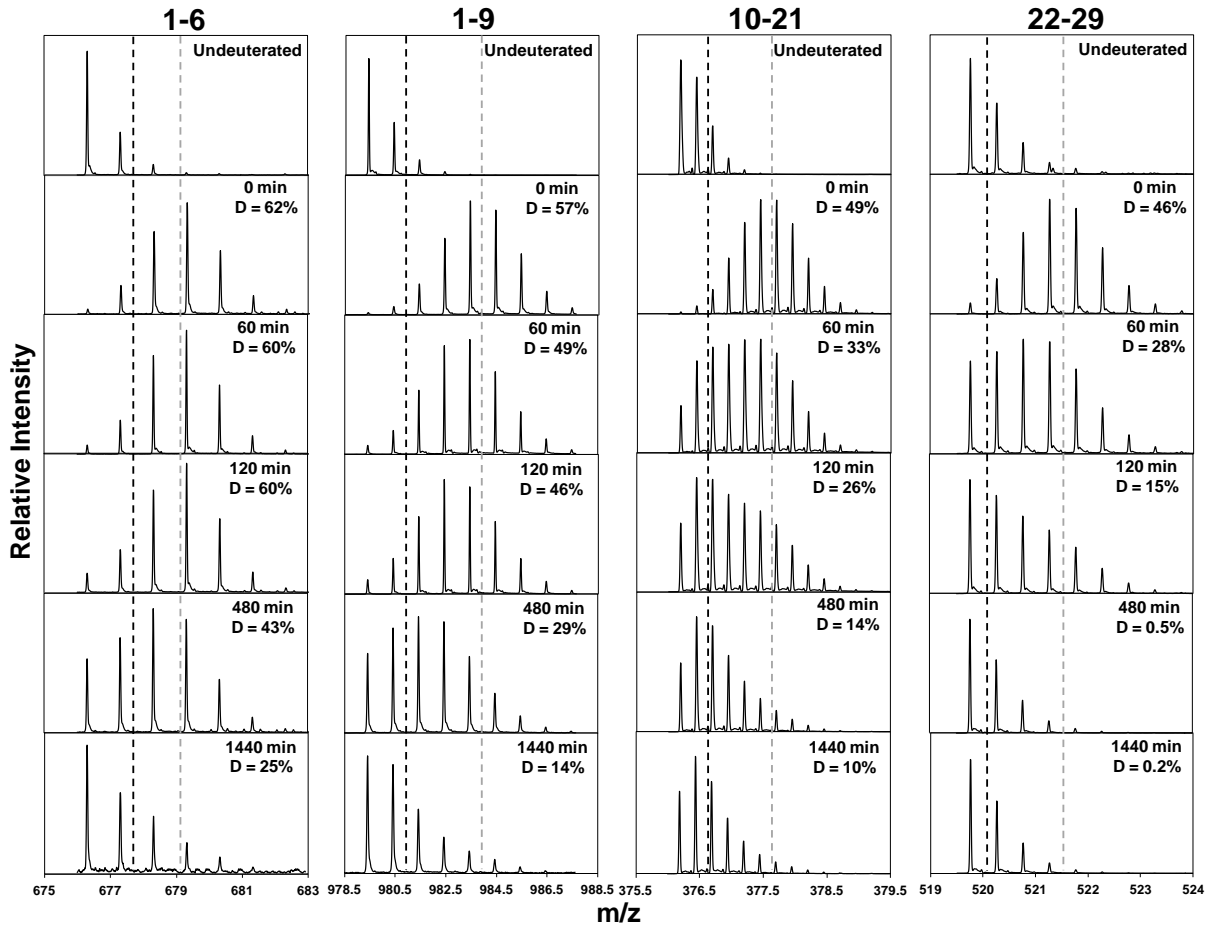
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Figure S3



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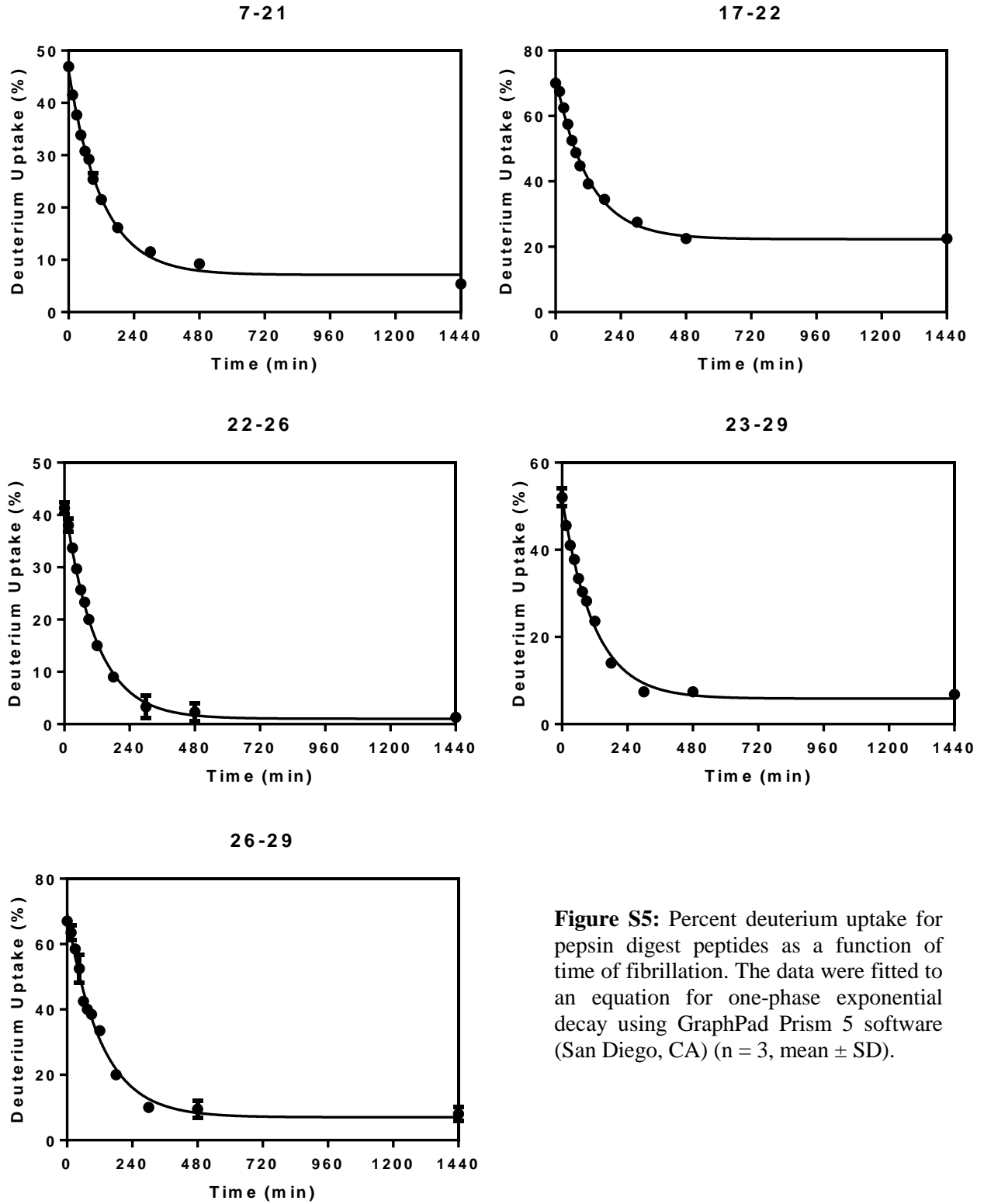
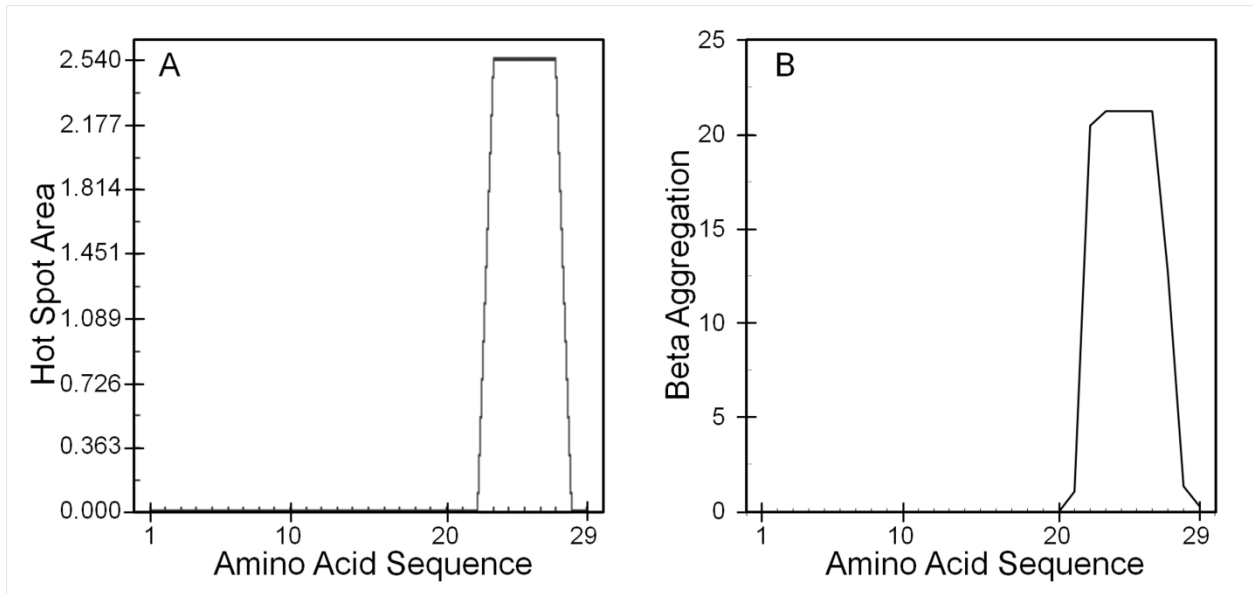


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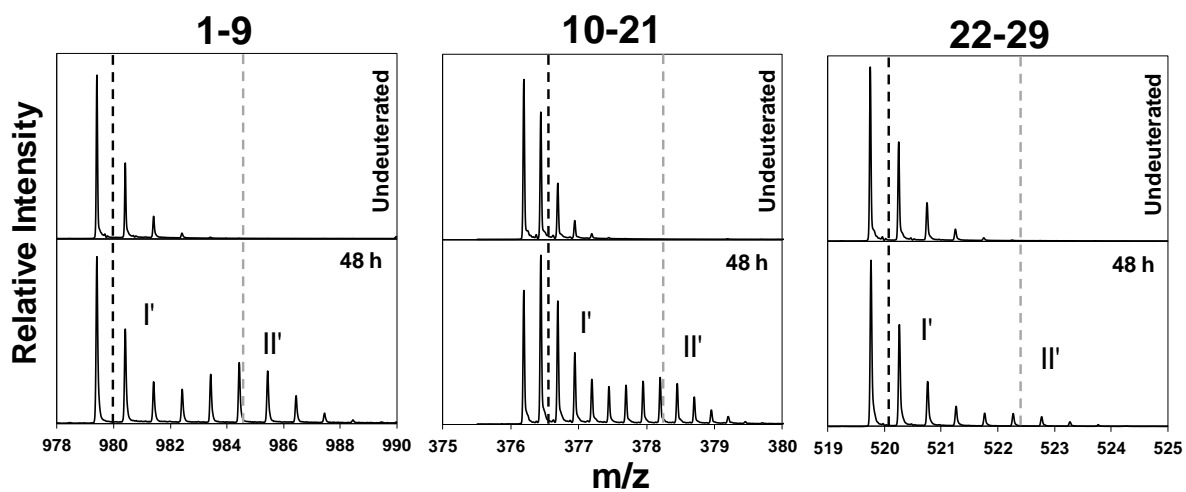
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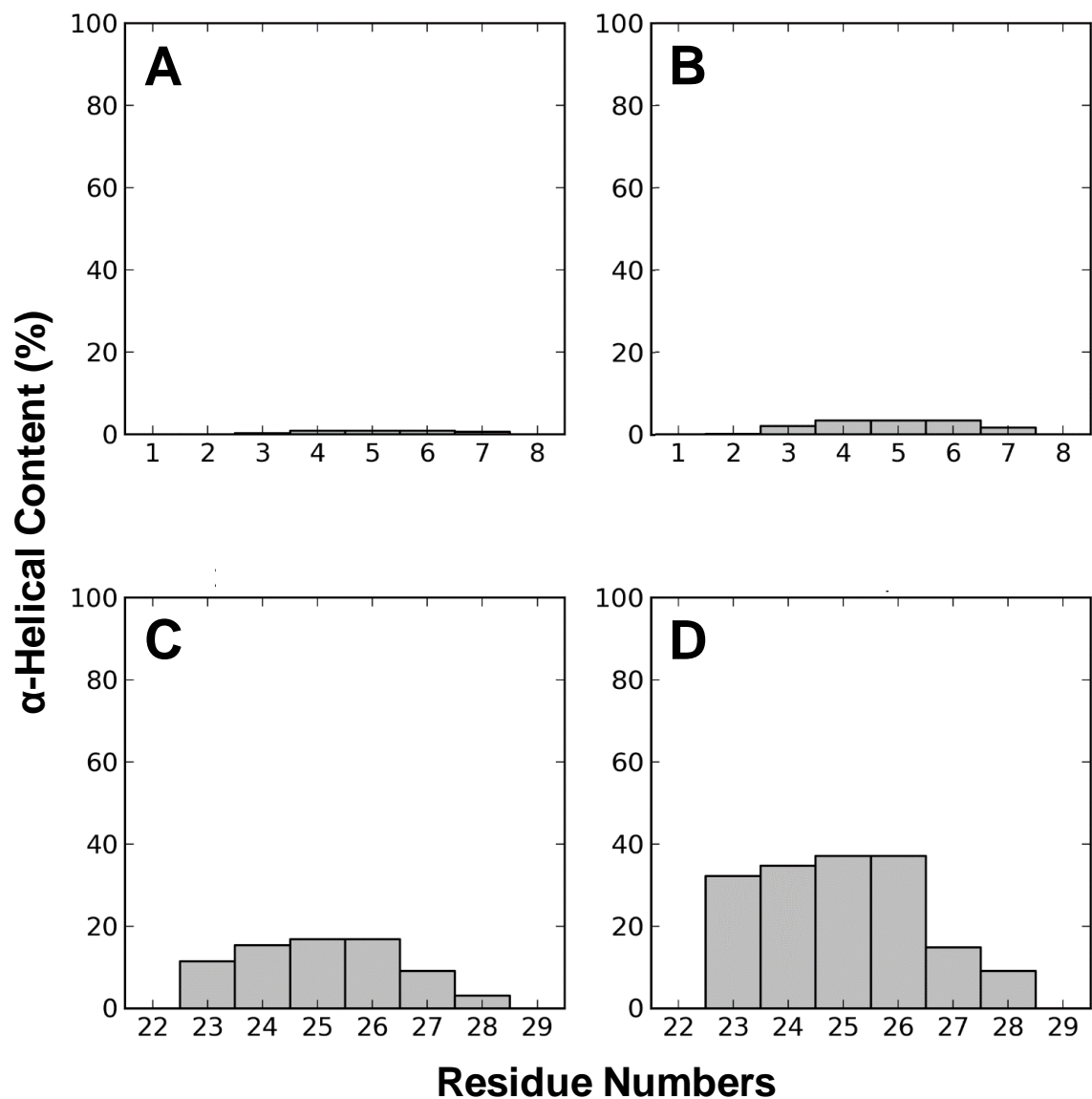


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