SUPPORTING INFORMATION

Characterizing the Structure and Oligomerization of Major Royal Jelly Protein 1 (MRJP1) by Mass Spectrometry and Complementary Biophysical Tools

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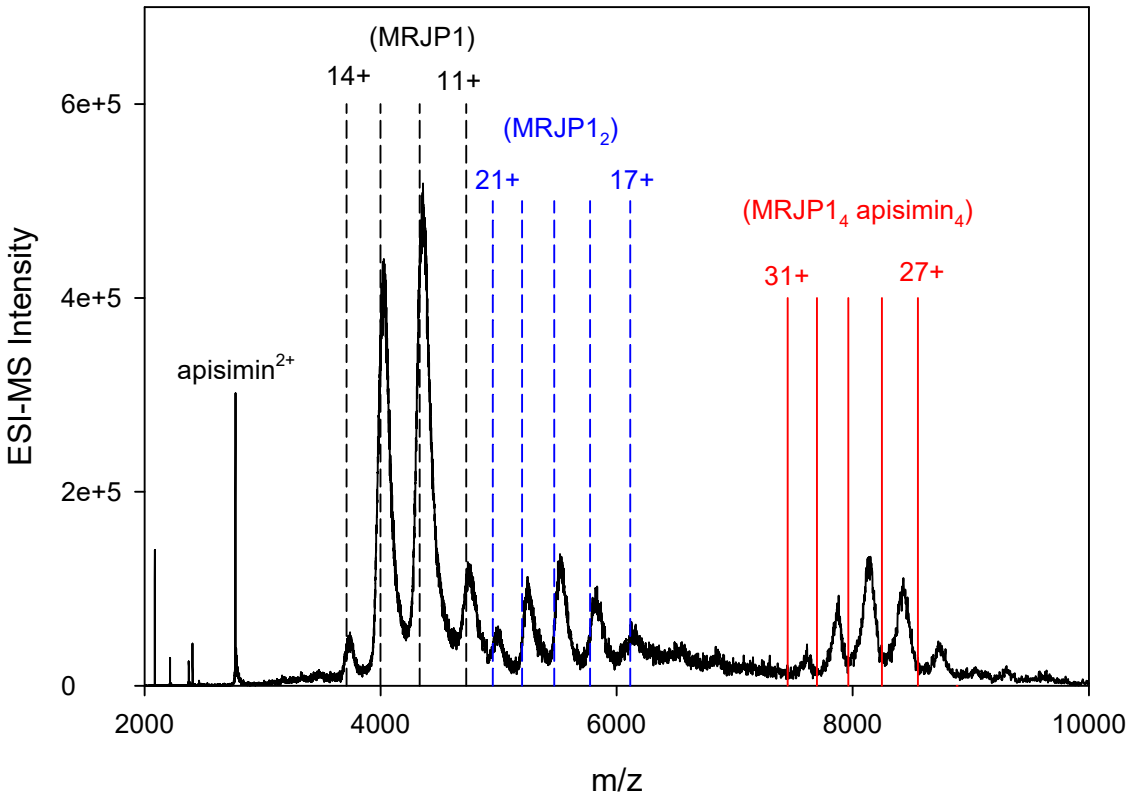
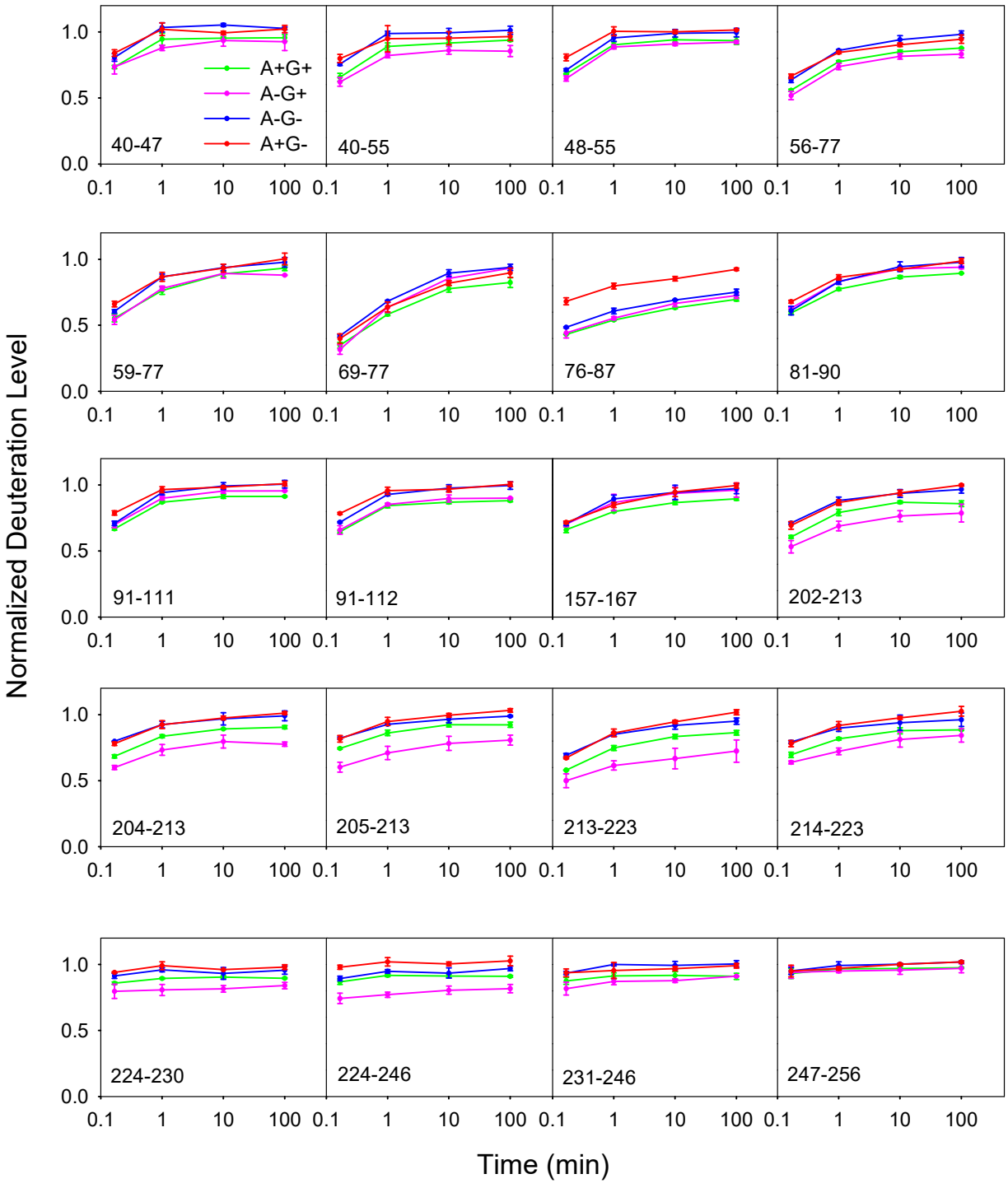
Supporting Figure S1. MRJP1 peptic peptides generated during the HDX-MS workflow. The sequence numbering represents that of the unprocessed full-length protein, as deduced from cDNA.[(1)] The sequence coverage is 55%.

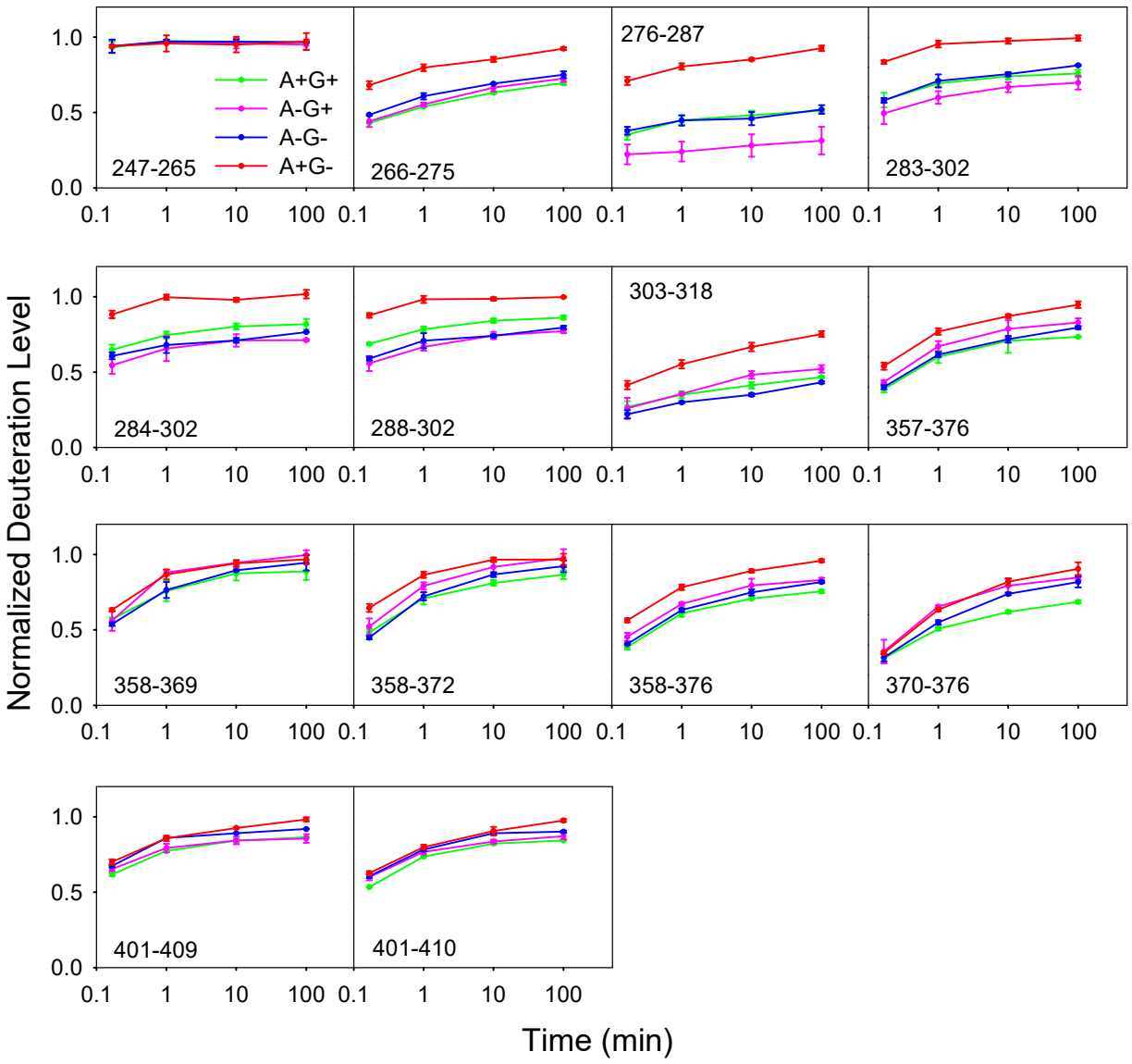
Supporting Figure S2. MRJP1 peptides identified after tryptic digestion, using the cDNA derived published protein sequence.[(1)] The sequence coverage is 64%.



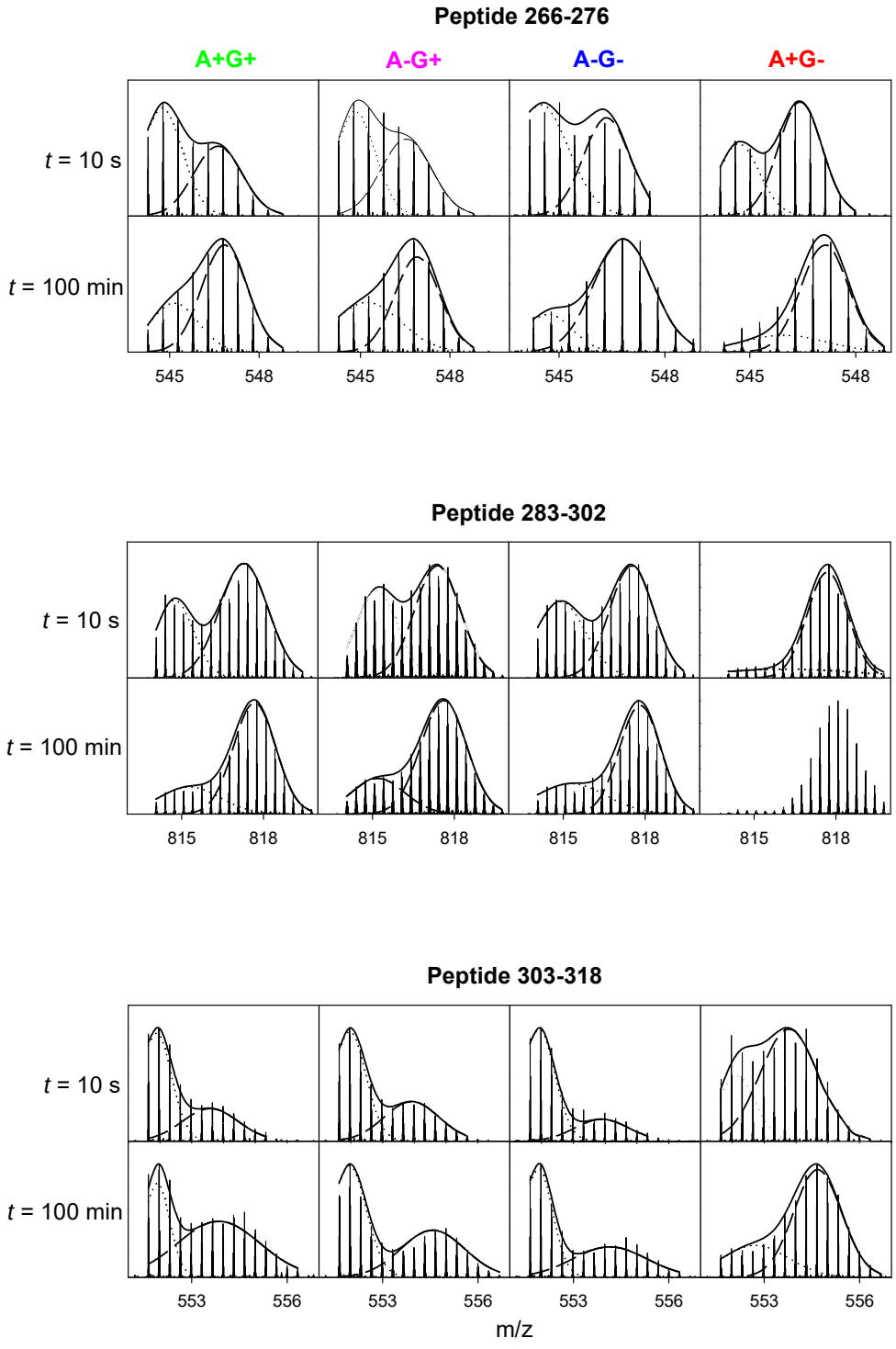
Supporting Figure S3: Native ESI mass spectrum of MRJP1/apisimin (A+G+) acquired under gentle source conditions. Peaks are assigned based on the mass values deduced from Figure 1 (main text). The full spectrum shown here indicates that the protein in solution exists as a mixture of tetrameric complexes (MRJP1$_4$ apisimin$_4$) and free monomeric MRJP1. Some dimeric MRJP1 is seen as well. The peak intensity ratios seen here cannot be directly related to solution phase concentrations due to differences in ionization efficiency and m/z bias of the mass analyzer used.[(2)] Both of these factors are expected to diminish the magnitude of (MRJP1$_4$ apisimin$_4$) signals. All peaks are shifted to higher m/z due to the retention of nonspecific adducts.[(3)] For the gentle source conditions used here, this effect is particularly pronounced for (MRJP1$_4$ apisimin$_4$). Much better agreement between expected and measured (MRJP1$_4$ apisimin$_4$) is obtained when applying slightly more extensive source activation of the complex (see main text and Figure 1a).



Supporting Figure S4 – Part I. (see caption on next page)



Supporting Figure S4 – Part II. Complete summary of the HDX kinetics measured (normalized deuteration level *vs*. labeling time) for all the peptic peptides monitored for A+G+, A-G+, A-G-, and A+G- samples. The sequence range covered by each peptide is indicated in the individual panels. Error bars (representing standard deviations) are shown for each point.



Supporting Figure S5. Examples of bimodal (EX1) MRJP1 isotope distributions after 10 s (top panels) and 100 minutes (bottom panels) of deuteration. Smooth lines indicate Gaussian deconvolutions into high mass and low mass components.

SI References

1. Schmitzova, J., Klaudiny, J., Albert, S., Schroder, W., Schreckengost, W., Hanes, J., Judova, J., and Simuth, J. (1998) A family of major royal jelly proteins of the honeybee Apis mellifera L, *Cell. Mol. Life Sci. 54*, 1020-1030.
2. Kuprowski, M. C., and Konermann, L. (2007) Signal Response of Co-Existing Protein Conformers in Electrospray Mass Spectrometry, *Anal. Chem. 79*, 2499-2506.
3. Benesch, J. L. P., Ruotolo, B. T., Simmons, D. A., and Robinson, C. V. (2007) Protein Complexes in the Gas Phase: Technology for Structural Genomics and Proteomics, *Chem. Rev. 107*, 3544-3567.