Supporting Information

for

An Asymmetric Runaway Domain Swap Antithrombin Dimer as a Key Intermediate For Polymerization Revealed by Hydrogen/Deuterium-Exchange Mass Spectrometry

Morten Beck Trelle[a], Shona Pedersen[bc], Eva Christina Østerlund[a], Jeppe Buur Madsen[a], Søren Risom Kristensen[bc], Thomas, J.D. Jørgensen[a]*

[a] Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark
[b] Department of Clinical Biochemistry, Aalborg University Hospital, Hobrovej 18 , 9000 Aalborg, Denmark
[c] Department of Clinical Medicine, Aalborg University, Sdr. Skovvej 15, 9000 Aalborg, Denmark

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Fig. S1

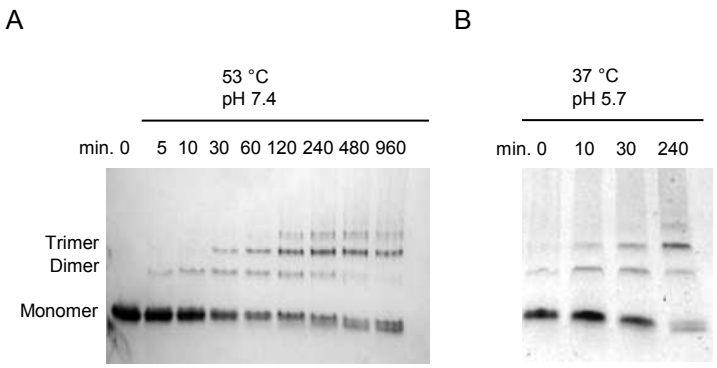
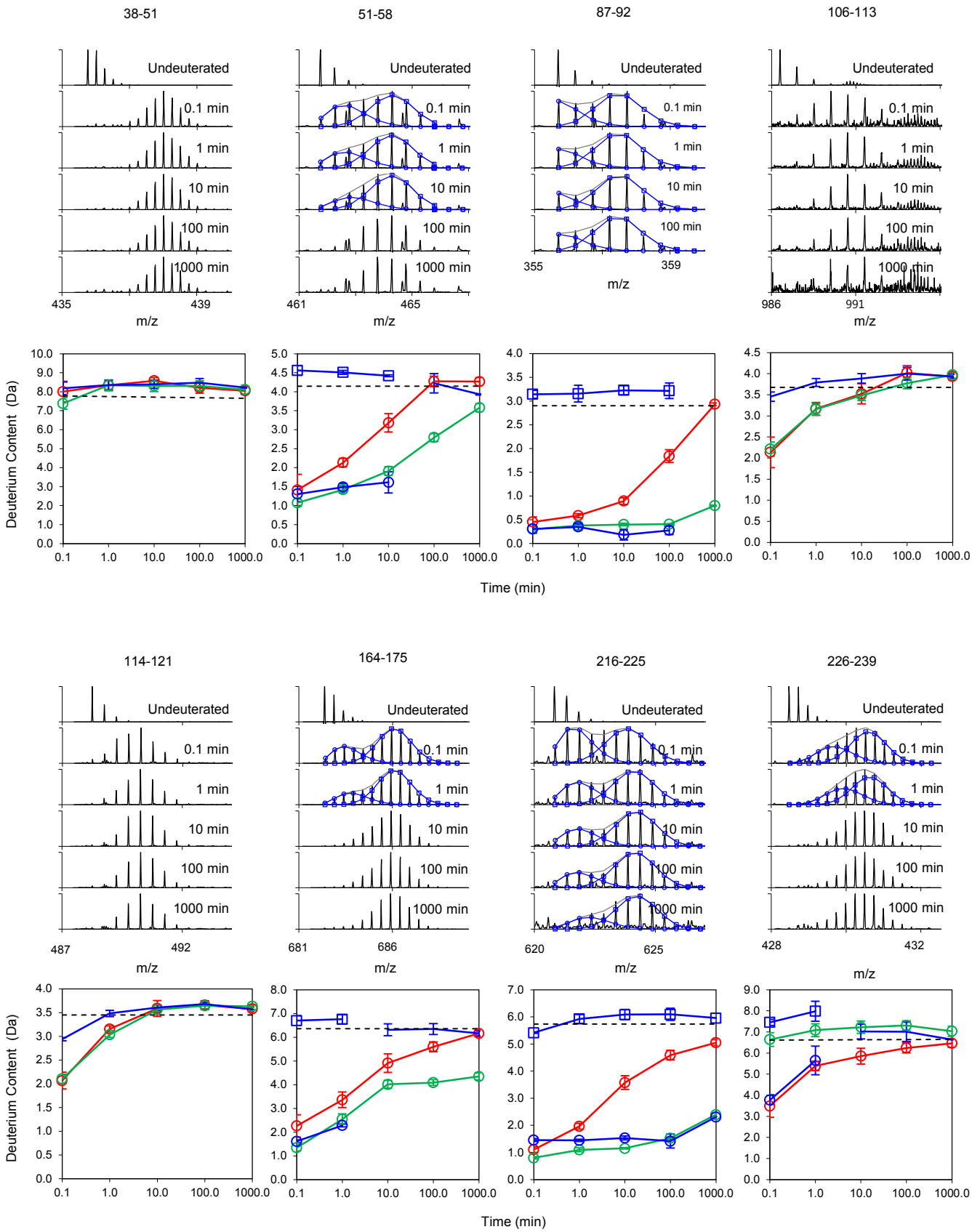
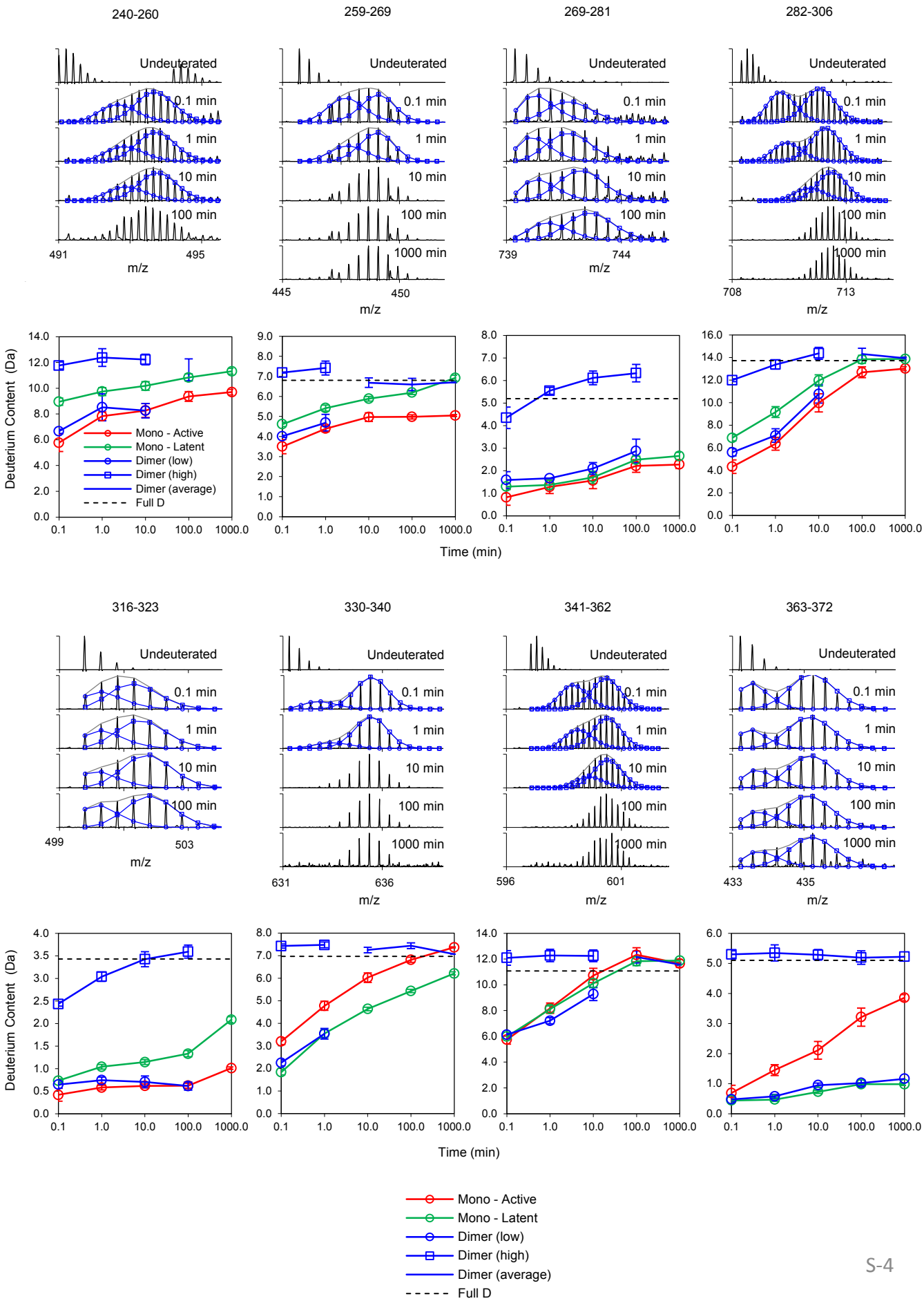


Fig. S1. Polymerization of antithrombin. Native gel electrophoresis (4-15% Tris-HCl) of antithrombin is shown following incubation at ***A)*** 53 °C at pH 7.4 and ***B)*** at 37 °C at pH 5.7 for the indicated times.

Fig. S2


38-51
51-58
87-92
106-113
Undeuterated
0.1 min
1 min
10 min
100 min
1000 min
m/z
Deuterium Content (Da)
Time (min)
114-121
164-175
216-225
226-239
Mono - Active
Mono - Latent
Dimer (low)
Dimer (high)
Dimer (average)
Full D


240-260
259-269
269-281
282-306
Undeuterated
0.1 min
1 min
10 min
100 min
1000 min
491
495
445
450
739
744
708
713
m/z
Deuterium Content (Da)
Mono - Active
Mono - Latent
Dimer (low)
Dimer (high)
Dimer (average)
Full D
Time (min)
316-323
330-340
341-362
363-372
499
503
631
636
596
601
433
435

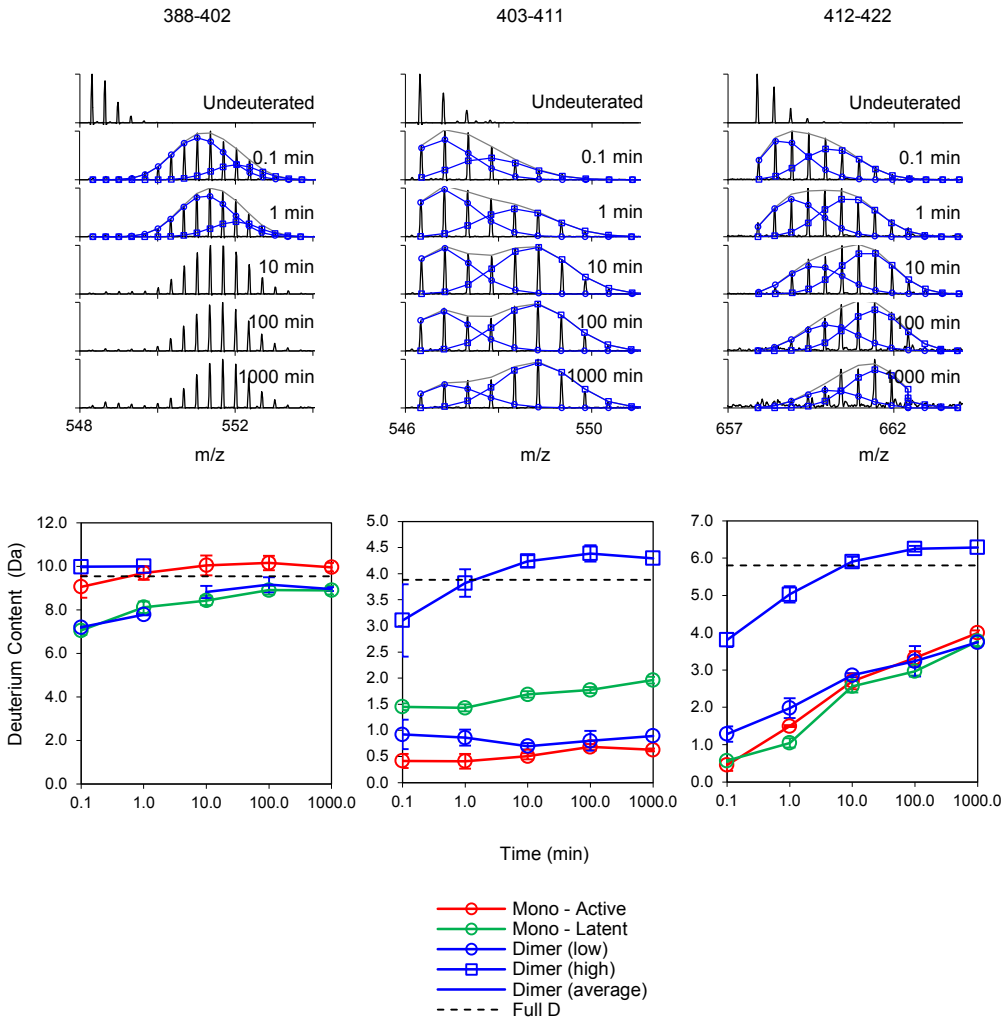


Fig. S2. Hydrogen/deuterium-exchange data for a representative set of antithrombin peptides. Stacked peptide mass spectra are shown from 1 replicate of the antithrombin dimer data after incubation in 90% D_2O for 0 (undeuterated), 0.1, 1 , 10, 100 and 1000 minutes (top to bottom). Below each set of stacked spectra is shown the peptide deuterium uptake plot from all analyzed states of antithrombin. Error bars indicate the standard deviation between replicates (n=3). All spectra where bimodal distributions are observed were fitted to two binomial functions and the deuterium content for each mass population was calculated and plotted individually. In certain dimer HDX mass spectra the bimodal isotope distributions were only observed at some of the earlier time points. The deuterium uptake at later time points, where bimodality was not observed, was calculated based on the average mass of the isotopic distribution and indicated by the blue dimer (average) line.

Fig. S3

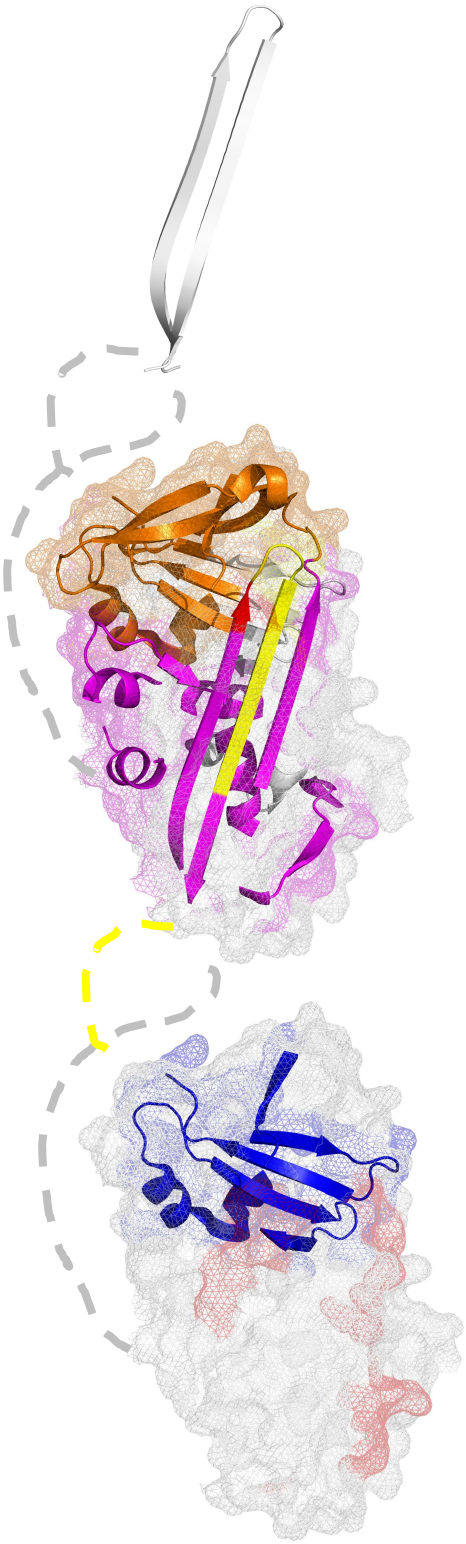


Fig. S3. Phenotypic characterization and mapping of dimer peptide deuterium uptake profiles on the hypothetical propagated runaway version of the β5A-β4A domain swap dimer. The mapping from **Fig. 4** is concatenated on the hypothetical propagated runaway version of the β5A-β4A domain swap dimer (the model is build using the 2ZNH structure[7]). All peptides for which the high-mass population show protection against isotopic exchange are colored blue on the structure of the ”unstable” subunit of the dimer. All peptides for which the low-mass population show active-like deuterium uptake profile are colored orange on the structure of the ”stable” subunit of the dimer. All peptides for which the low-mass population show latent-like deuterium uptake profiles are colored purple on the structure of the ”stable” subunit of the dimer. Peptides for which protection against isotopic exchange is observed is shown in cartoon representation and the remaining structure is shown in a 60% transparent surface wireframe representation.

Fig. S4

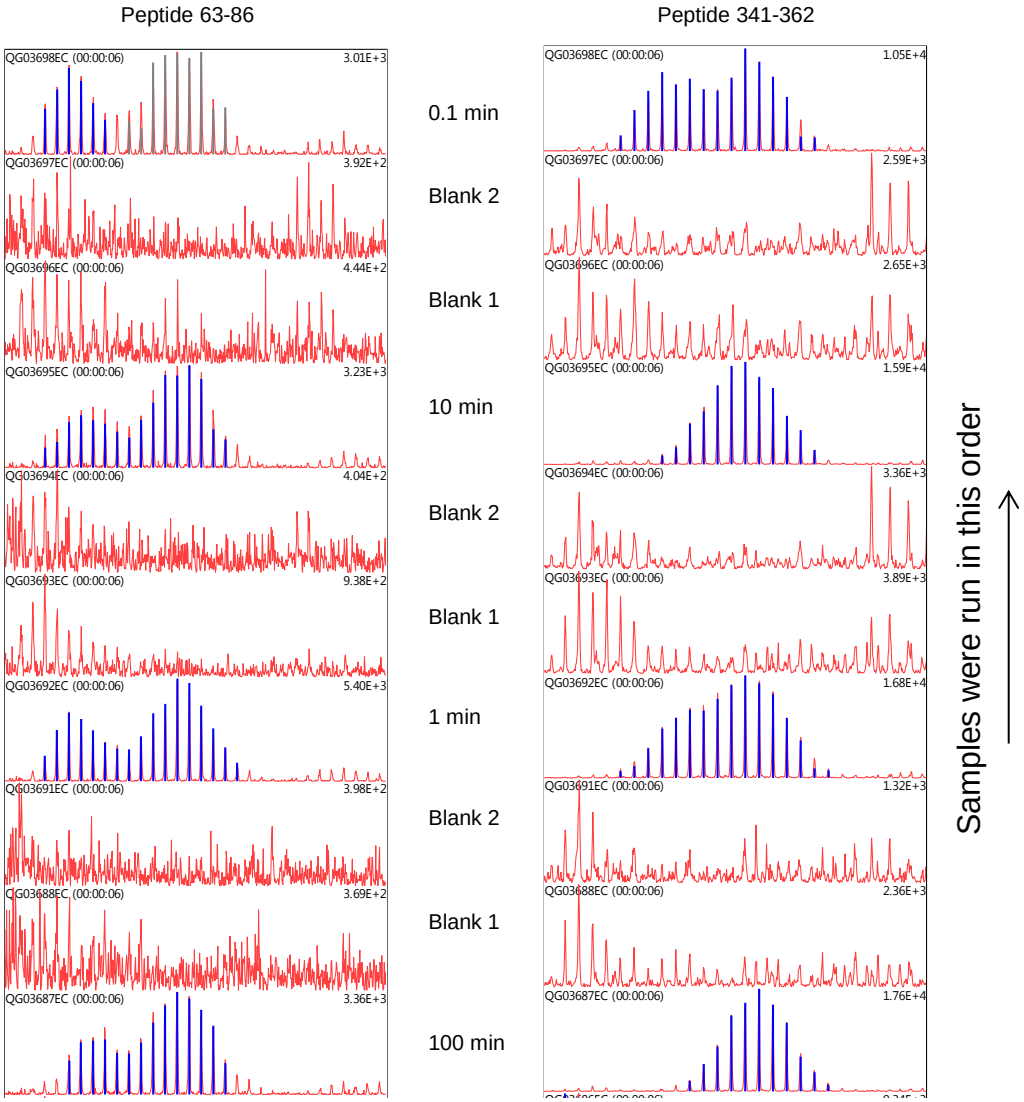


Fig S4. Hydrogen/deuterium-exchange data from blank injections following real sample injection. Two blank injections were conducted in between every real sample to evaluate the level of carry-over in the LC system and to wash away carry-over proteins and peptides. Mass spectra of two peptides (63-83 and 341-362) are shown of real labeled dimer sample injections (indicated by labeling time points) followed by 2 blank injections (blank 1 and blank 2) from bottom to top, as example. The spectra are exported from the software DynamX (Waters) and the absolute intensity of the base peak in each spectrum is indicated in the upper right corner. Generally, the carry-over level is reduced to background noise levels following 2 blank injections. Furthermore, the deuterium content of peptides in the first blank (blank 1) is typically lower than the deuterium content of the low mass population in the real dimer sample injections. Taken together, it is clearly evident that the bimodality observed in the spectra of the dimer samples is not caused by reminiscent carry-over peptides from previous injections.