A.

Domain I	**1**	MKFTVEREHLLKPLQQVSGPLGGRPTLPILGNLLLQVADGTLSLTGTDLE
Domain II	**126**	VEFTLPQATMKRLIEATQFSMAHQDVRYYLNGMLFETEGEELRTVATDGH
Domain III	**254**	----KHLEAGCDLLKQAFARAAILSNEKFRGVRLYVSENQLKITANNPEQ

```
                     ::  .      .        .   *    .     .  .  .
```

Domain I	MEMVARVALVQPHEPGATTVPARKFFDICRGLPEGAEIAVQLEGERMLVR
Domain II	RLAVCSMPIGQSLPSHSVIVPRKGVIELMRMLDGGDNPLRVQIGSNNIRA
Domain III	EEAEEILDVTYSGAEMEIGFNVSYVLDVLNALK----------CENVRM

```
            : :  .          .       .::: . *            . :
```

Domain I	SGRSRFSLSTLPAADFPNLDDWQSE---	**125**
Domain II	HVGDFIFTSKLVDGRFPDYRRVLPKNPD	**253**
Domain III	MLTDSVSSVQIEDAASQSAAYVVMPMRL	**366**

```
         .  .       :    .    .
```
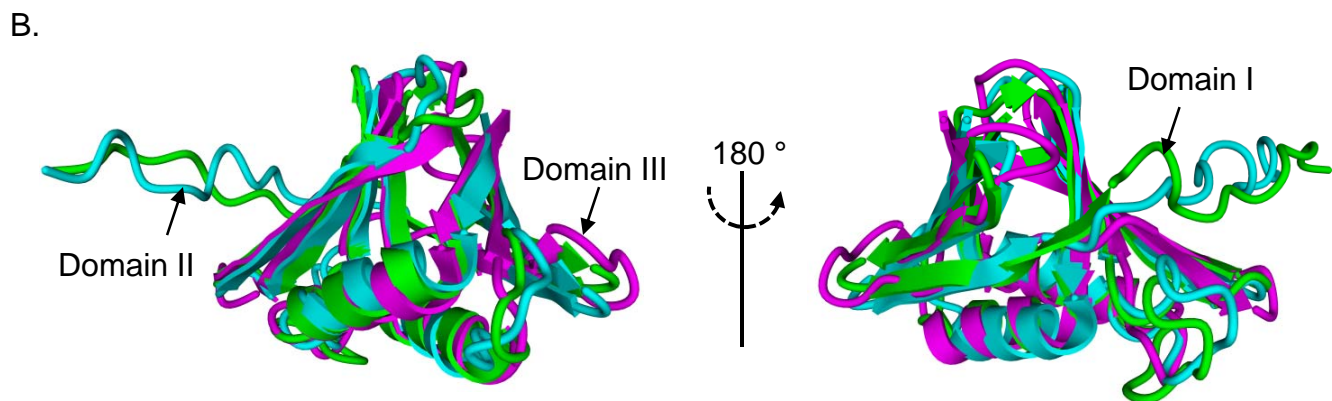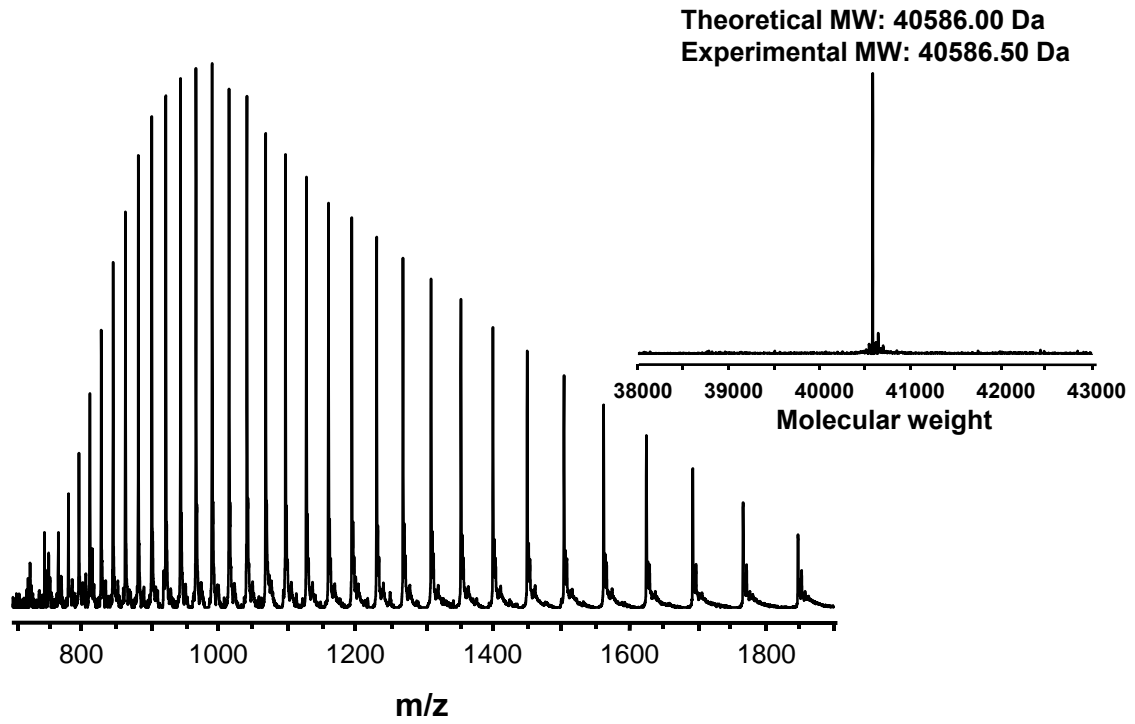
B.

Figure S1. Domain alignments. (A) The sequence of the three domains are aligned using CLUSTAL W (1.81) multiple sequence alignment software [1]. The numbers to the left and right are the first and last residue numbers in each domain '*' indicates positions which have a single, fully conserved residue. ':' indicates that one of the following 'strong' groups is fully conserved: STA; NEQK; NHQK; NDEQ; QHRK; MILV; MILF; HY; FYW. '.' indicates that one of the following 'weaker' groups is fully conserved: CSA; ATV; SAG; STNK; STPA; SGND; SNDEQK; NDEQHK; NEQHRK; FVLIM; HFY. (B) Alignment of tertiary structure of the three domains (Domain I shown in green, Domain II shown in cyan and Domain III shown in magenta). The alignment is shown in two orientations: the left view turned 180° to obtain on the right view.

[1] Larkin, M. A., Blackshields, G., Brown, N. P., Chenna R., McGettigan P. A., *et al*, (2007) Clustal W and Clustal X version 2.0., *Bioinformatics*, 23, 2947-2948.
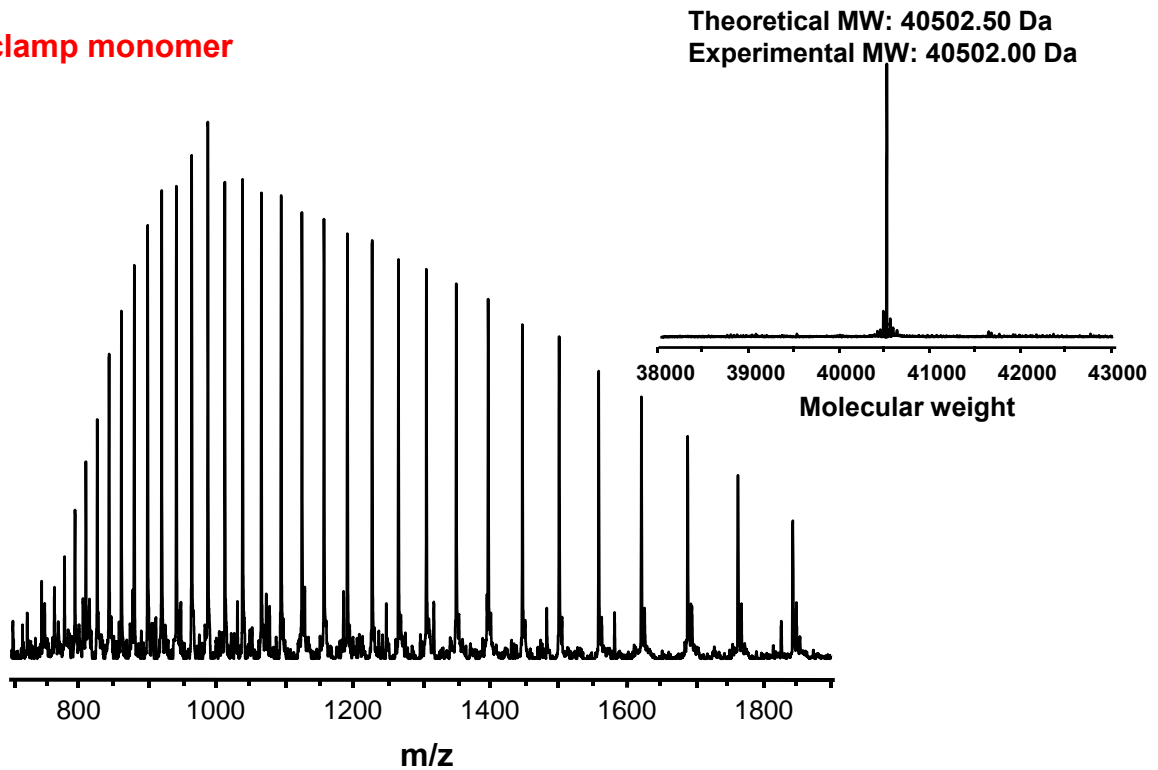
(A) WT β clamp

Theoretical MW: 40586.00 Da
Experimental MW: 40586.50 Da

38000 39000 40000 41000 42000 43000
Molecular weight

800   1000   1200   1400   1600   1800

m/z

(B) β clamp monomer

Theoretical MW: 40502.50 Da
Experimental MW: 40502.00 Da

38000 39000 40000 41000 42000 43000
Molecular weight

800   1000   1200   1400   1600   1800

m/z

Figure S2. Mass verification of (A) WT and (B) monomer variant. The raw m/z data are shown on the left and the transformed spectra are shown on the right. The measured and theoretical molecular masses are indicated.
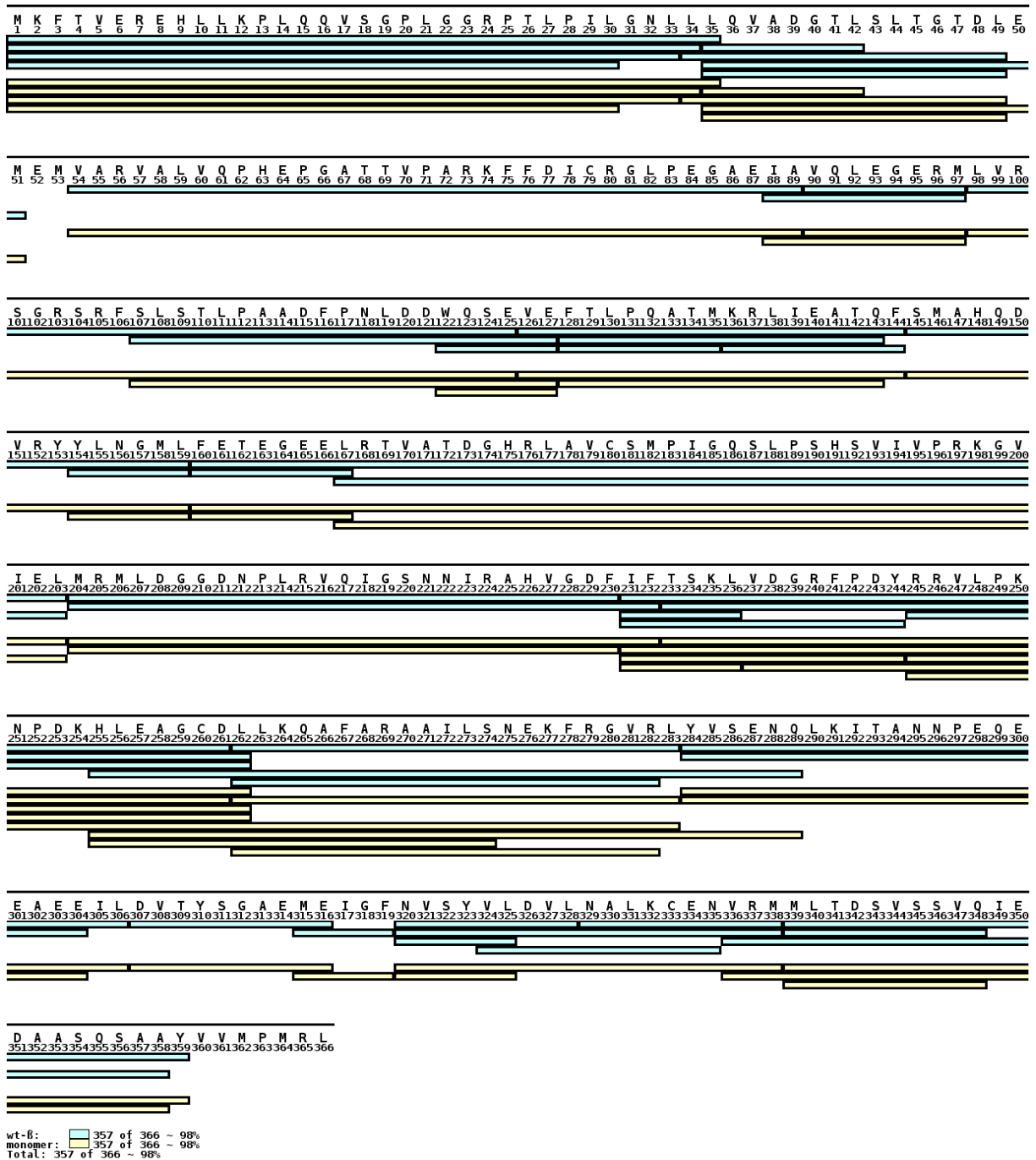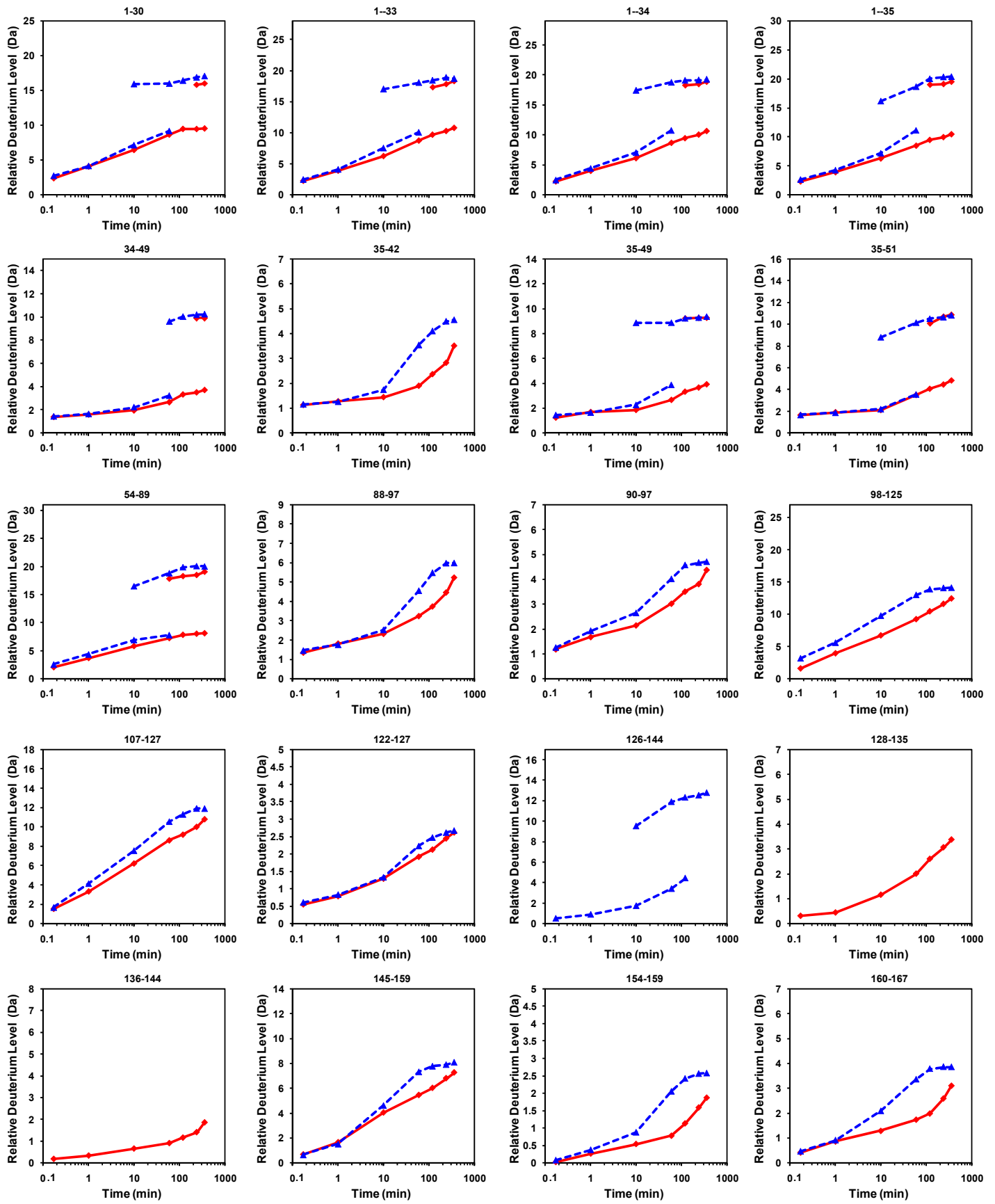
Figure S3. Peptide map of WT beta and monomer variant. The rectangular boxes under the sequence indicate the peptic peptides that were identified and monitored by HXMS, cyan represents the WT β clamp and yellow represents the β clamp monomer. Identification of the peptic fragments was accomplished through a combination of exact mass analysis and MS^E [2], using IdentityE Software from Waters. The map was made by online software MS tools [3].

[2] Geromanos, S. J., Vissers, J. P. C., Silva, J. C., *et al.,* (2009) The detection, correlation, and comparison of peptide precursor and product ions from data independent LC-MS with data dependent LC-MS/MS., *Proteomics,* 9, 1683-1695.

[3] Kavan, D., Man, P., (2011) MSTools–Web based application for visualization and presentation of HXMS data., *Int. J. Mass Spectrom.* 302, 53-58.
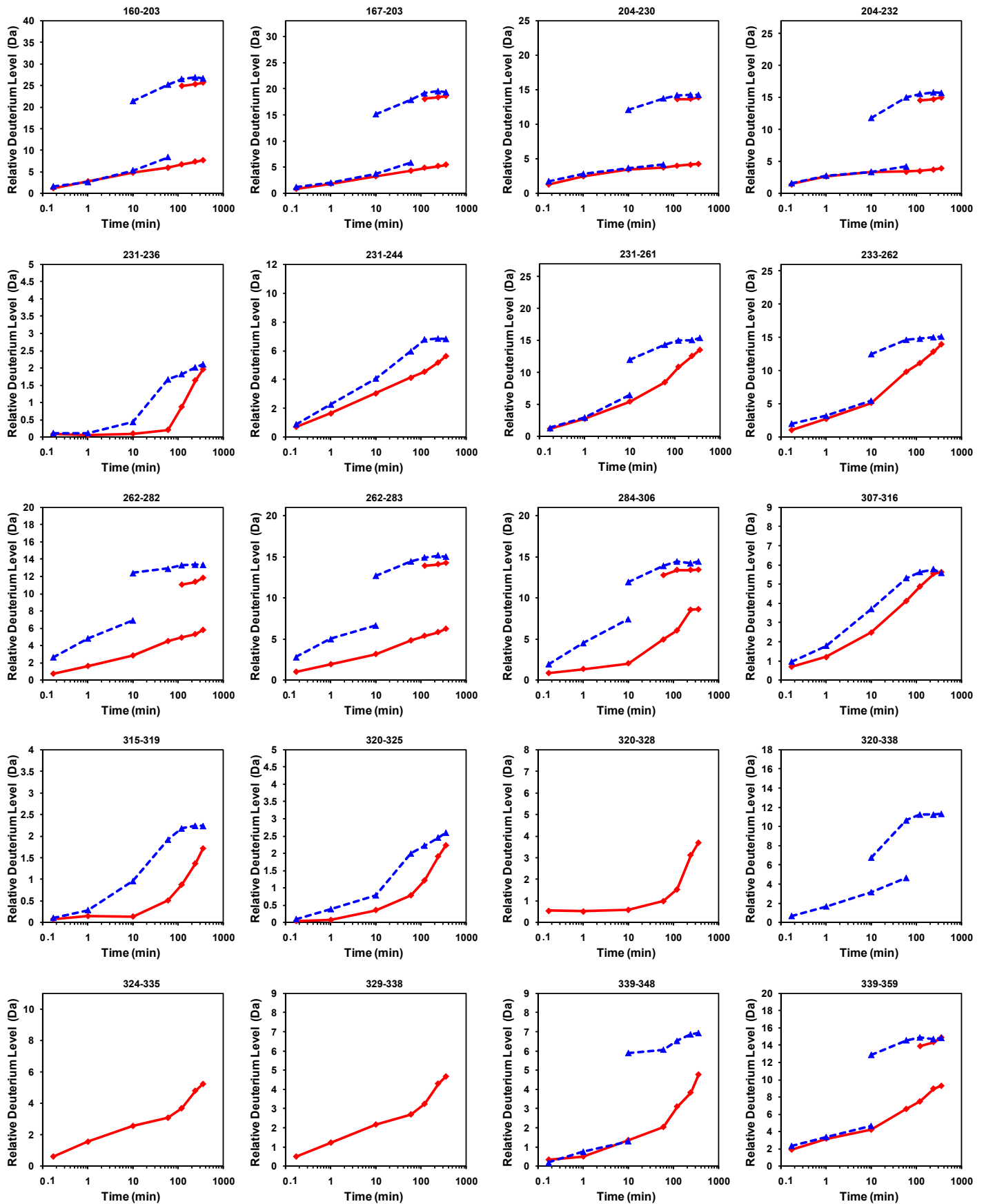
Figure S4. Deuterium incorporation

Figure S4. Deuterium incorporation (cont.)

Legend: WT beta-clamp; Beta monomer

Figure S4. Comparison of relative deuterium incorporation in WT β and monomer variant at the peptide level. Red represents WT β clamp and blue represents β monomer. The maximum of the y-axis in each graph is the maximum number of exchangeable NHs in that peptide. The data shown here are based on a set of data from more than three experiments. The error of determining the deuterium levels was ± 0.2 Da in the experimental system.
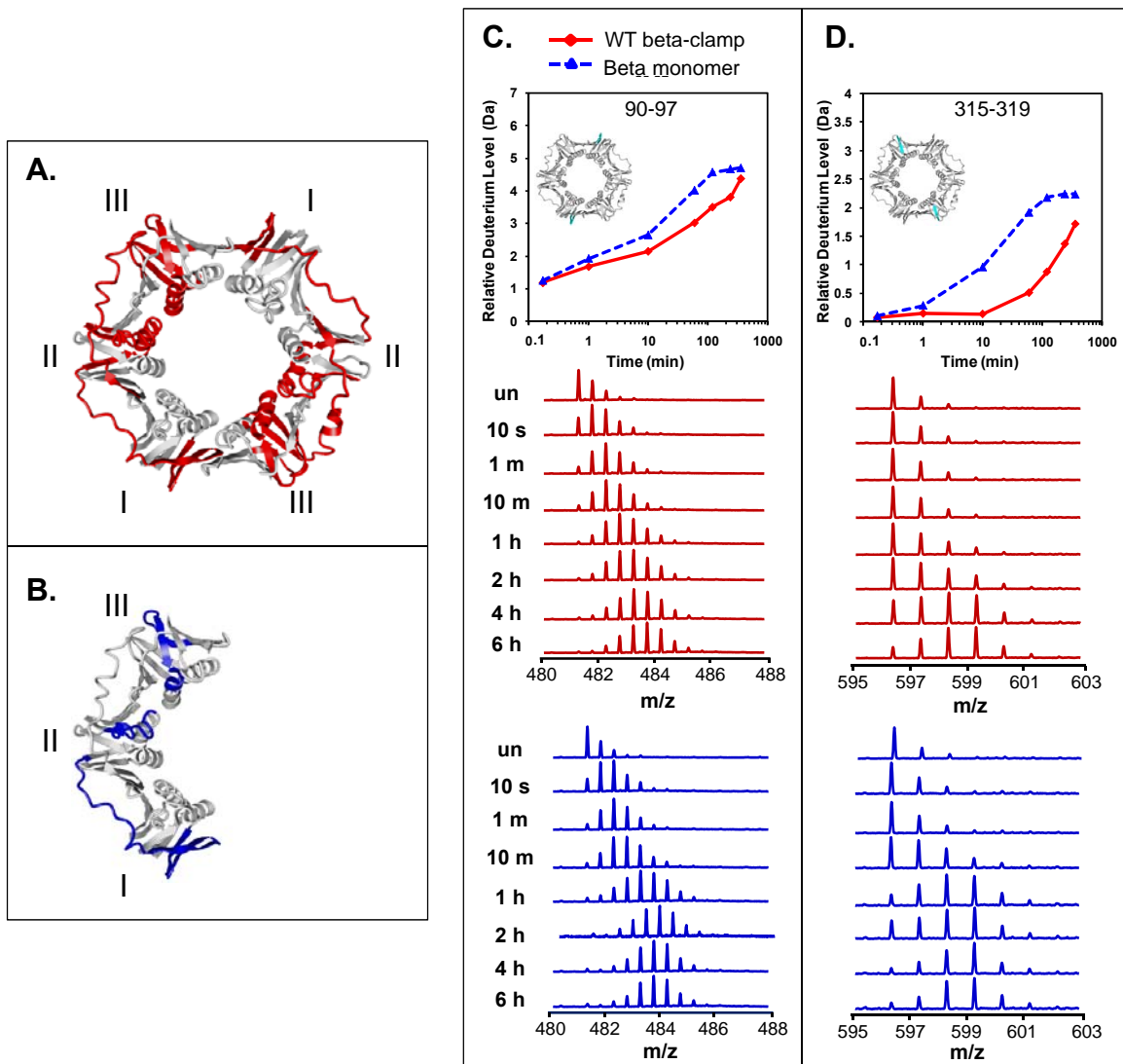
Figure S5. Residues showing EX2 kinetics in WT β clamp and monomer variant. All residues that showed EX2 kinetics are highlighted in the crystal structure of β clamp: (A) for WT β and (B) for β clamp monomer. Deuterium uptake and raw spectra are shown for two representative peptides, residues 90-97, m/z=481.3, +2 charge state, (C) and residues 315-319, m/z=596.3, +1 charge state, (D). Data for the WT are shown in red and data for the monomer are shown in blue. In the deuterium incorporation curves for the two peptides, the inset shows the locations of the peptides, highlighted in cyan and the two sets of lines indicate the components of the EX2 distributions.