Expanded View Figures

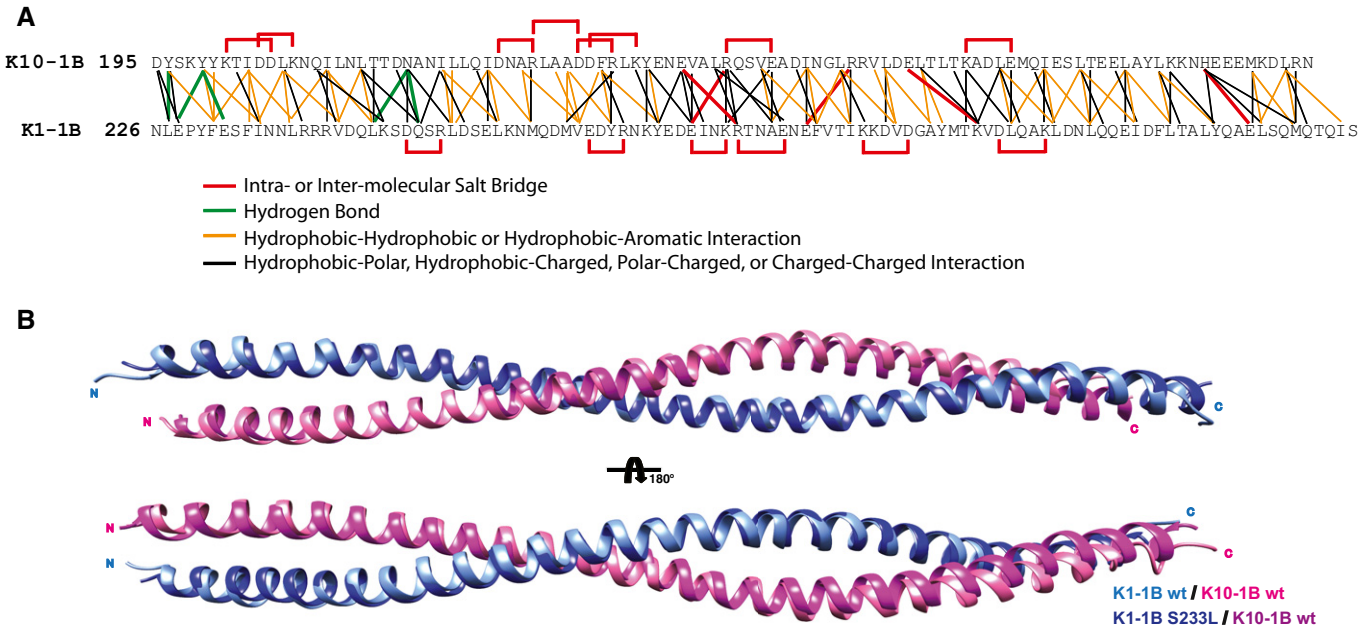

Figure EV1. Structure analysis of wild-type K1/K10-1B and mutant K1^{S233L}/K10-1B heterodimer structures.

A The amino acid contacts at the heterodimer interface for both wild-type K1/K10-1B and mutant K1^{S233L}/K10-1B X-ray crystal structures were analyzed and plotted onto a single residue contact map. Intramolecular (within K1 or K10 only) and inter-molecular (between K1 and K10) salt bridges are plotted red. Hydrogen bonds are plotted green. Interactions between hydrophobic residues are plotted orange (hydrophobic residues are defined as A, I, L, F, V, P, M, W), including hydrophobic interaction with the aromatic residue tyrosine. Other types of molecular contacts are plotted black. Analyses were performed using WHAT IF (defines atoms as "in contact" when the distance between their van der Waals surfaces is < 1.0 Å), ESBRI, and PDBePISA. Since S233^{K1} is a surface-exposed residue, its mutation to L233 does not impact the heterodimer interface. Hence, the analysis of both heterodimer interfaces was used to obtain the contact map.

B The wild-type K1/K10-1B and mutant K1^{S233L}/K10-1B heterodimer structures were superimposed and have a root-mean-square deviation (RMSD) of 0.736 Å. The superposition shows slight variation in the positioning of the K10 C-terminus.

A

Crosslinked Mouse K1/K10-1B Trypic Peptides	Expected Corresponding Human K1/K10-1B Trypic Peptides
mK10-1B YYKTIEDLK	hK10-1B 199 YYKTIDDLK 207
mK10-1B EMQIESLNEELAYLKK	hK10-1B 271 EMQIESLTEELAYLKK 285
mK1-1B VDSLKSDQSR	hK1-1B 242 VDQLKSDQSR 251
mK1-1B VELQAKR	hK1-1B 299 VDLQAKL 305
mK1-1B YEDEINKR	hK1-1B 270 YEDEINKR 277
mK1-1B YEDEINKR	hK1-1B 270 YEDEINKR 277
mK1-1B TNAENEFVTIKK	hK1-1B 278 TNAENEFVTIKK 289
mK10-1B LKYENEVTLR	hK10-1B 236 LKYENEVALR 245
mK1-1B TKYEDEMNKR	hK1-1B 268 NKYEDEINKR 277
mK10-1B LKYENEVTLR	hK10-1B 236 LKYENEVALR 245

B

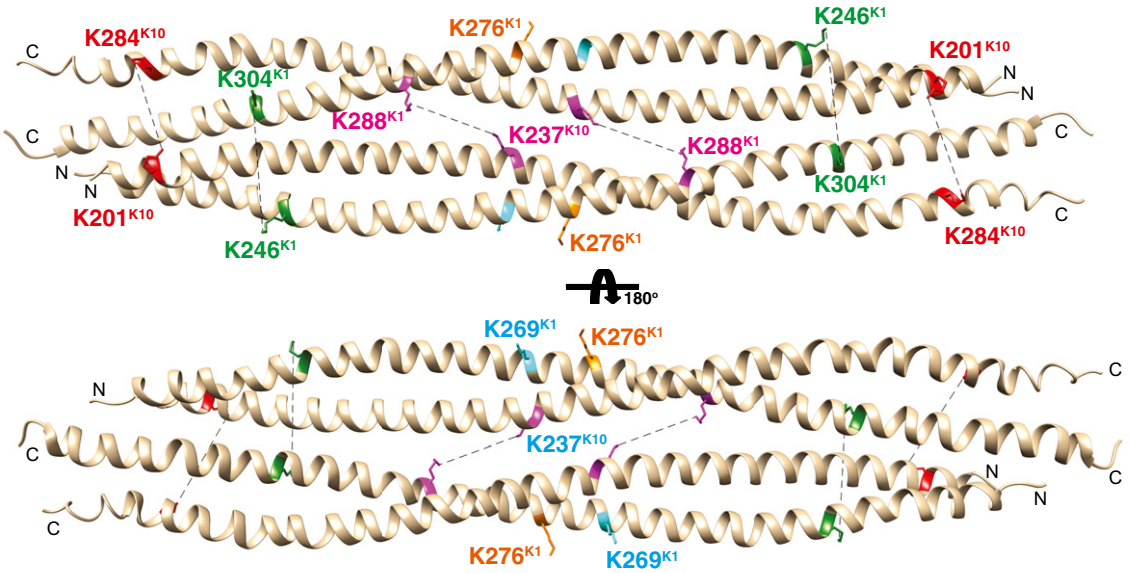

Figure EV2. Mapping of cross-linked mouse K1/K10-1B tryptic peptide data onto the human K1/K10-1B tetramer structure.

A (Left) Five cross-linked mouse K1/K10-1B tryptic peptides previously described (Steinert *et al*, 1993a). (Right) The expected corresponding cross-links in human K1/K10-1B. Cross-linked lysines are color-coded for easier identification in panel (B). Lysine 237 from K10 appears in two distinct cross-linked species (magenta and blue).

B Crystal structure of human K1/K10-1B with backbone presented in ribbon diagram and pertinent lysine side chains as sticks. Steinert and colleagues used DST (disulfosuccinimidyl tartrate) as cross-linking reagent (cross-link arm of 0.6 nm); this means lysines within ~ 15 Å can cross-link. In the structure, $K288^{K1}$ and $K237^{K10}$ are 13.4 Å apart. $K201^{K10}$ and $K284^{K10}$ are 16.8 Å apart, and $K246^{K1}$ and $K304^{K1}$ are 21.6 Å apart: Both of these lysine pairings are structurally adjacent and can be expected to form the observed lysine cross-links when factoring in protein dynamics in solution (i.e., the crystal structure is a static picture of a protein moment in time, but in solution, the lysine side chains, based on proximity in the tetramer structure, should be able to move into a conformation amenable to cross-link formation with DST). It is also important to note Steinert and colleagues had peptide cross-links of various oligomer sizes; thus, the presence of an octamer species (two tetramers) will influence the cross-links observed. Both $K269^{K1}$ and $K276^{K1}$ are surface-exposed residues that structurally appear to require the higher order oligomer to explain the observed cross-links. The key point is the positions of the lysines in the wild-type K1/K10-1B tetramer structure are consistent with the biochemical cross-linking data.

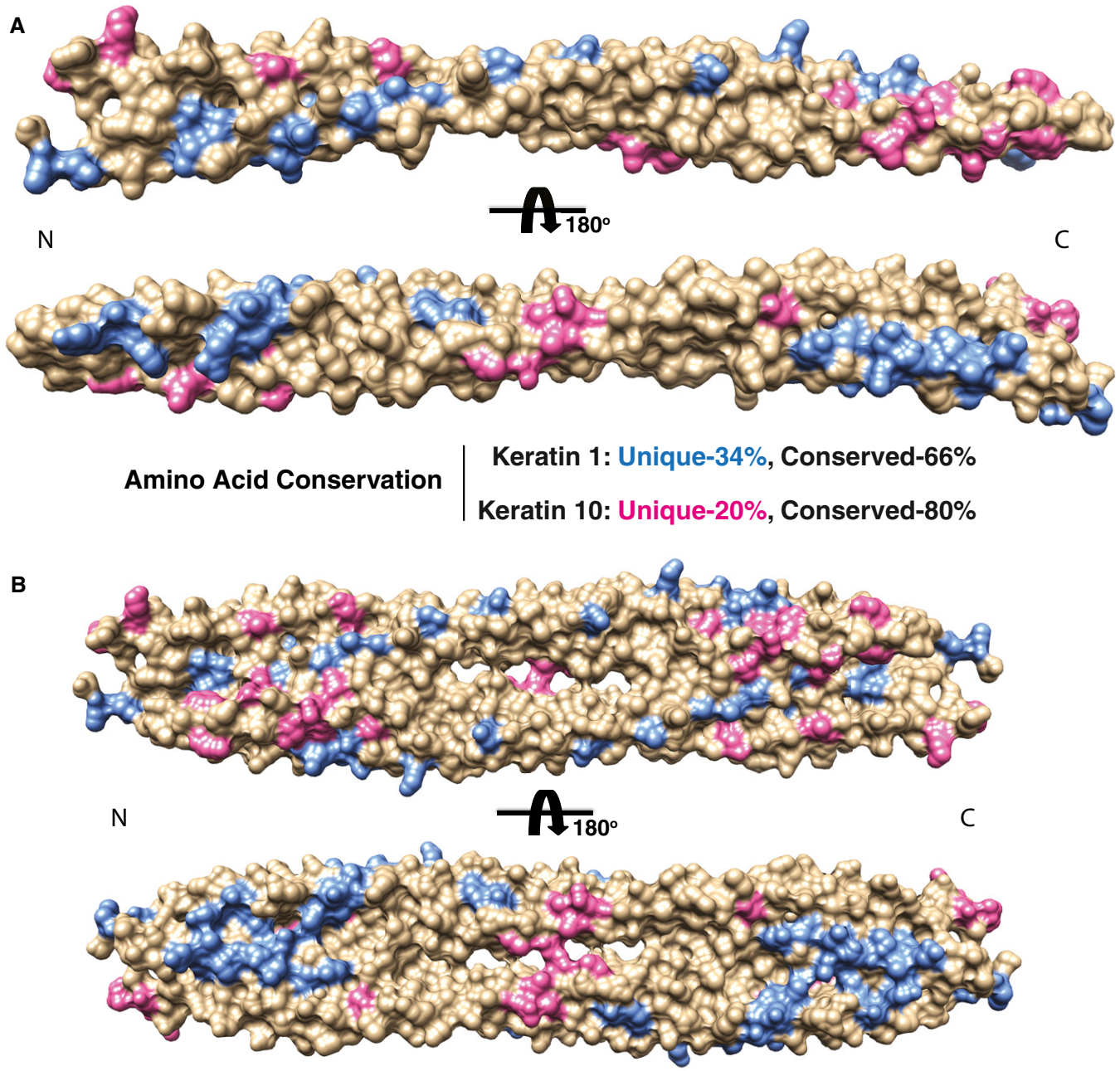

Figure EV3. Mapping of amino acid sequence differences between K1/K5 and K10/K14 onto the molecular surface of the wild-type K1/K10-1B structure.

A Residues in K1 (blue) and K10 (pink) that are unique (non-identical) compared with basal keratins K5 and K14, respectively, are mapped onto the K1/K10-1B heterodimer structure (represented as a molecular surface). Residues that are conserved/identical are tan. K1 residues that are non-identical to K5 are colored blue, while K10 residues that are non-identical to K14 are colored pink. The majority of unique residues for K1 and K10 align along the outer aspect of the helices. The majority of the conserved residues lie along the dimer interface. This distribution of unique residues enables keratins to maximize the diversity of surface properties, such as surface charge and shape, while maintaining a common structural coiled-coil fold.

B The same mapping and coloring of unique residues as in panel (A) is applied to the K1/K10-1B tetramer structure.

Figure EV4. Comparison of K1/K10-1B and vimentin-1B tetramer structures.

A Sequence alignments of K1-1B and K10-1B each with vimentin 1B. There is 36% sequence identity between K1-1B and vimentin 1B (yellow), and 33% sequence identity between K10-1B and vimentin 1B (green).

B The wild-type K1/K10-1B and vimentin-1B (PDB Code 3UF1) tetramer structures were superimposed and have a RMSD of 1.3 Å.

C The keratin residues that are identical to vimentin 1B were mapped onto a molecular surface representation of the K1/K10-1B tetramer structure and colored yellow (K1-1B) or green (K10-1B). All K1/K10-1B residues not identical to vimentin 1B were mapped onto the molecular surface of the K1/K10-1B tetramer structure and colored red, demonstrating a significant proportion of the K1/K10-1B molecular surface will have differences in chemistry compared to vimentin 1B.

D To further illustrate how the molecular surface of the K1/K10-1B tetramer differs from vimentin 1B, both identical residues (lighter colors) and residues unique to K1 or K10 (darker colors) were divided into hydrophobic, positively charged, and negatively charged groups and mapped onto the K1/K10-1B tetramer molecular surface. The majority of the unique hydrophobic residues are located in the central region of the 1B domain, whereas the unique charged residues appear evenly distributed. Definitions of residues were acidic (D, E), basic (K, R), and hydrophobic (A, F, I, L, M, P, V, W).

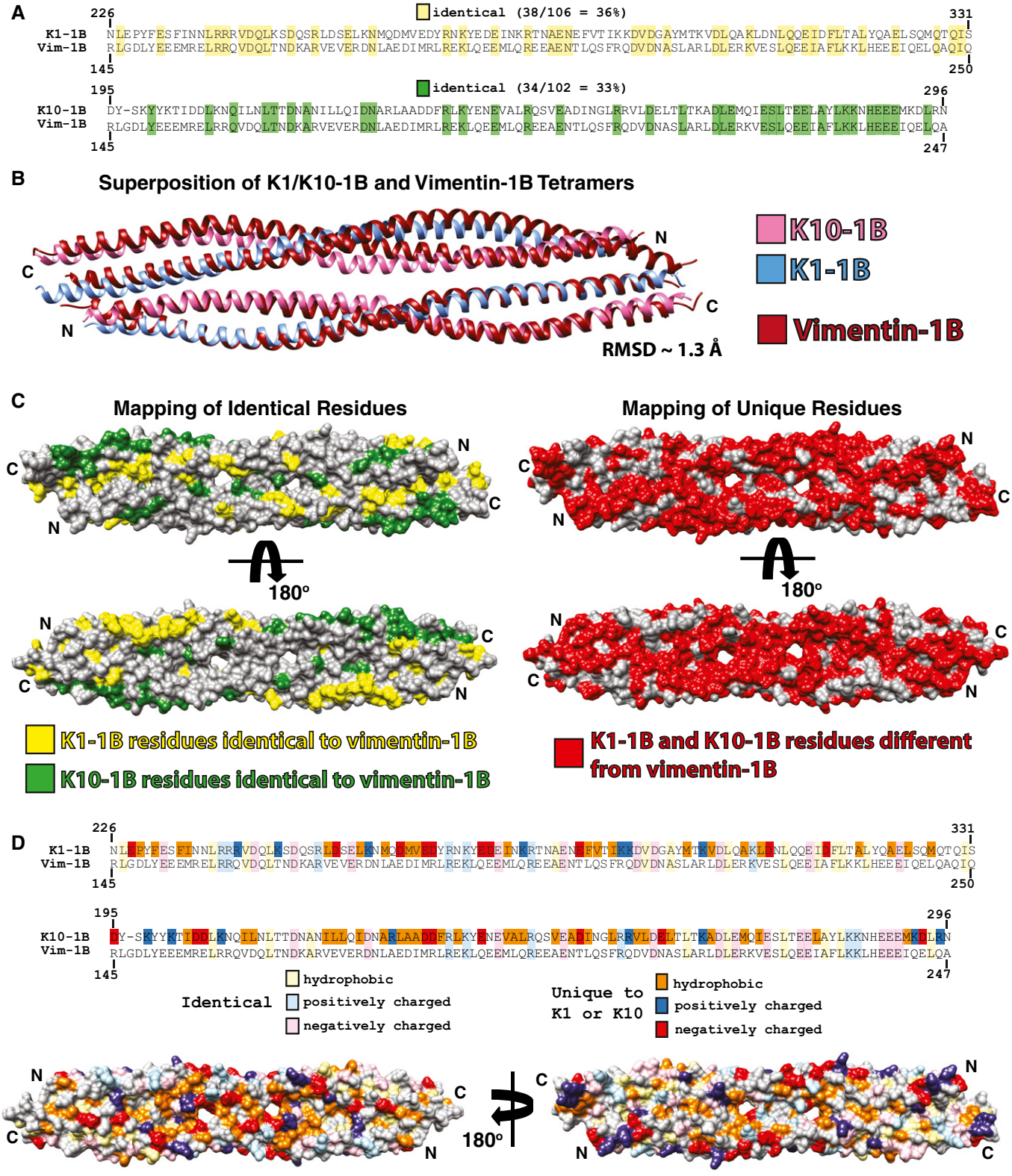

Figure EV4.

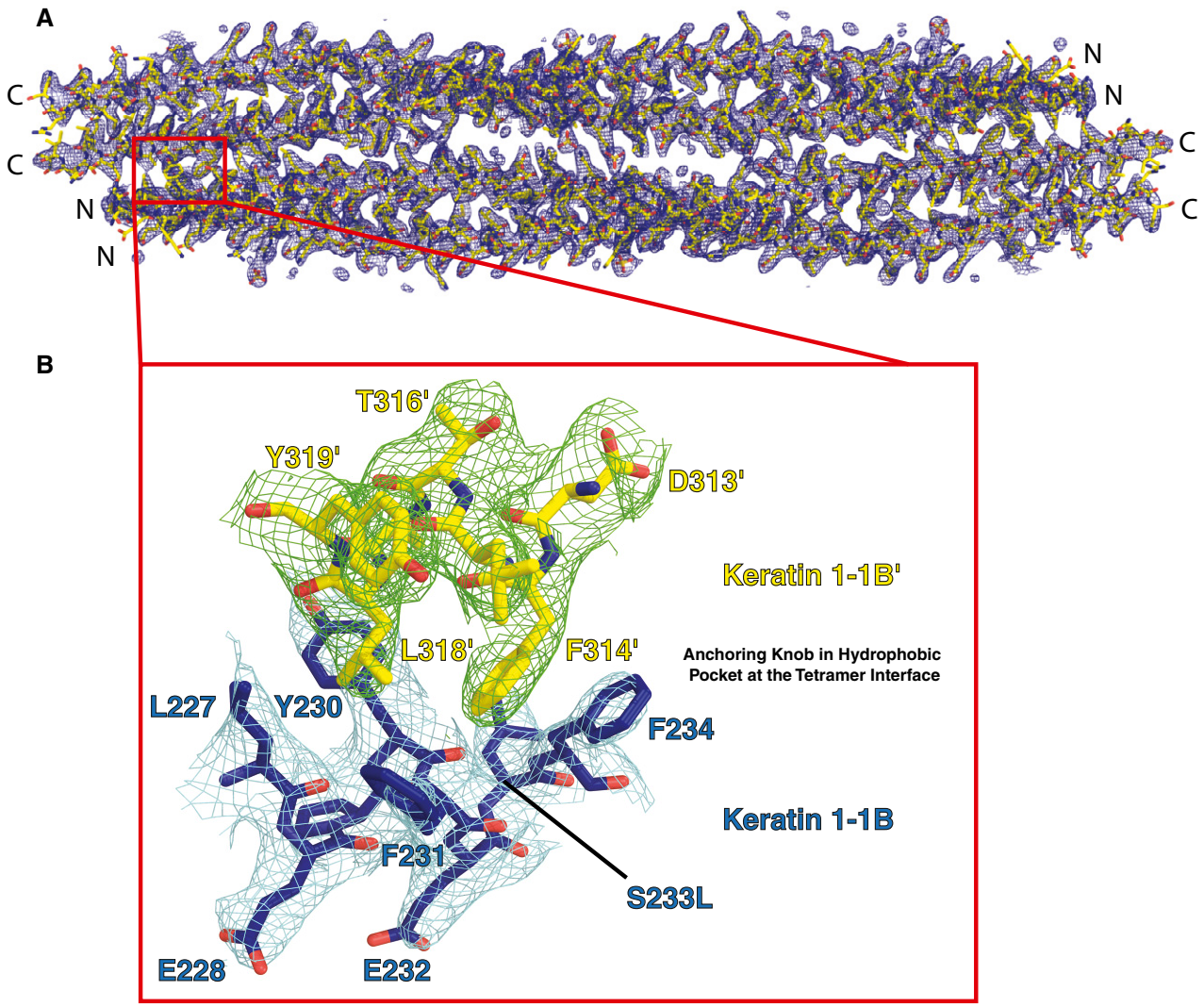

Figure EV5. Observed electron density throughout the K1^{S233L}/K10-1B crystal structure.

A The two K1^{S233L}-1B and two K10-1B molecules composing the K1^{S233L}/K10-1B tetrameric crystal structure are depicted as yellow sticks in electron density (blue, contoured at 0.8σ). A red box highlights one anchoring knob/hydrophobic pocket site in the symmetric, anti-parallel tetramer.

B A close-up view of one anchoring knob/hydrophobic pocket site in the K1^{S233L}/K10-1B crystal structure (K10 residues have been omitted for clarity). The K1-1B containing the hydrophobic pocket is depicted as blue sticks in electron density (cyan, contoured at 0.8σ). The K1-1B′ from the partner heterodimer contains the anchoring knob and is depicted as yellow sticks in electron density (green, contoured at 0.8σ). N, N-terminus; C, C-terminus.