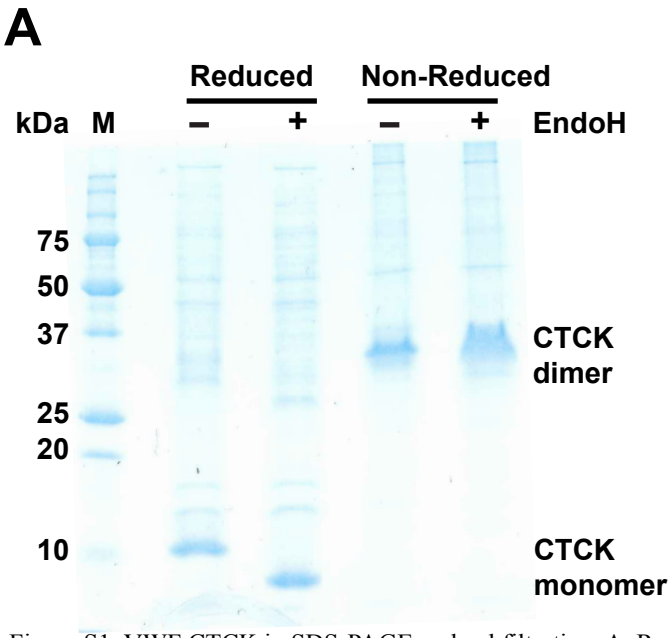


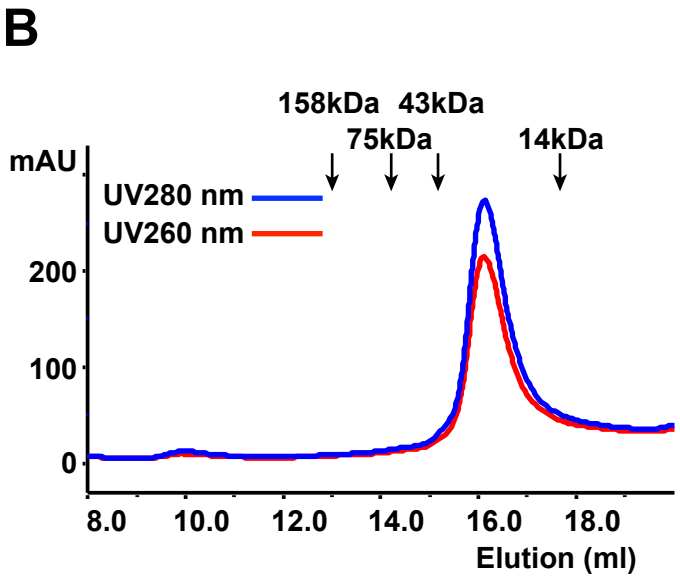
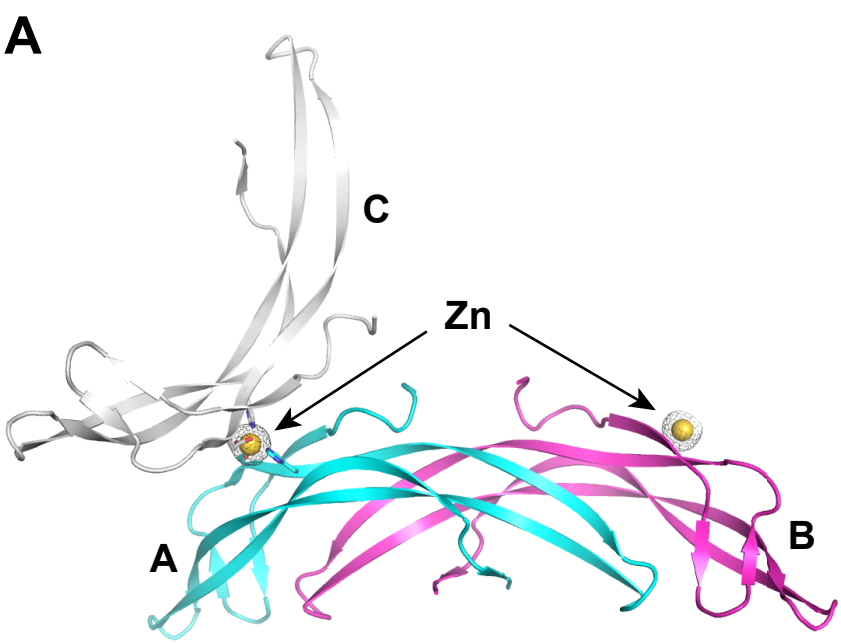


Figure S1. VWF CTCK in SDS-PAGE and gel filtration. A. Reduced and non-reduced SDS-PAGE of CTCK after ion exchange purification. Incubation with EndoH decreased the molecular weight in reducing conditions by 1.9 kDa according to the protein standards. The same sample run under non-reducing conditions reproducibly showed no change in migration. This might relate to the adjacency of the N-linked site to the C2-C8 disulfide, or its presence at the tips of dimers (Fig. 1A). SDS-PAGE works because unfolded polypeptides assume similar rod-like shapes related to their polypeptide chain lengths, it is fortuitous that N-linked sites show up relatively accurately as additional weights, and in non-reducing SDS-PAGE protein shapes differ from the rod-like shape in reducing page, and thus the disulfides perturb migration. B. Superdex 200 10/300 GL chromatography of EndoH-digested CTCK. Arrows show positions of protein standards.



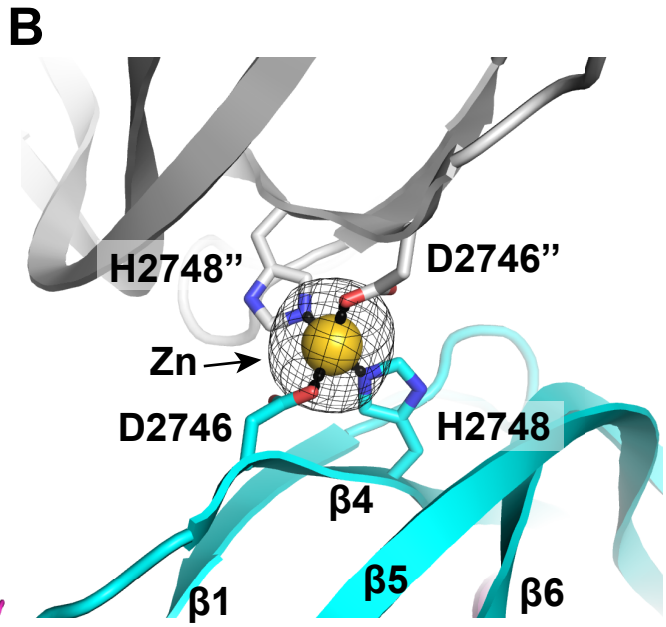


Figure S2. The zinc atom in a crystal lattice contact. A. Three symmetry-related monomers. Monomers A and B form the physiologically relevant dimer; whereas monomers A and C jointly coordinate one zinc atom. B. Details of zinc coordination, showing sidechains of coordinating residues in stick. The anomalous difference Fourier map contoured at 6σ is shown in mesh. Zinc atoms are shown as spheres.

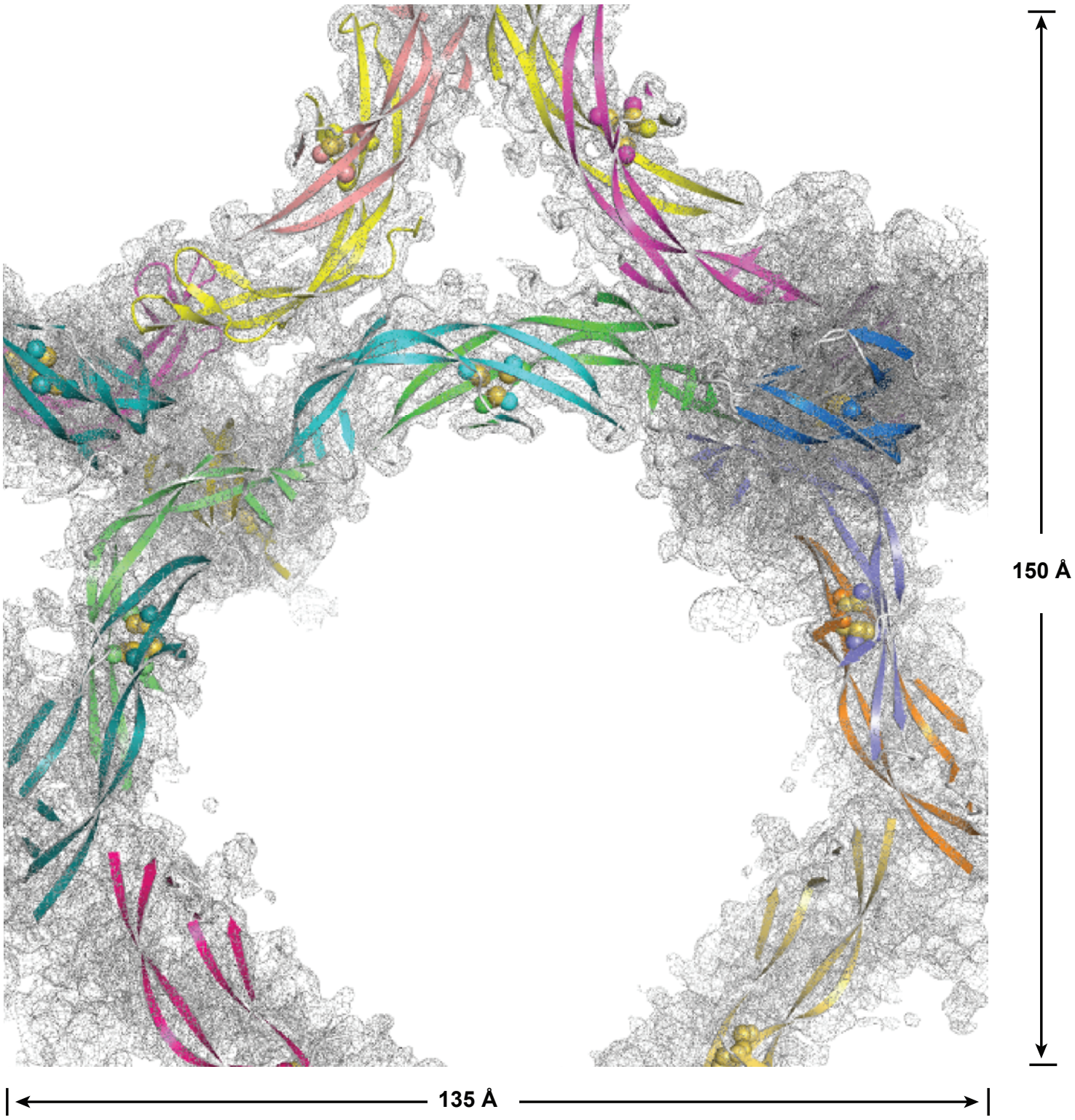


Figure S3. VWF CTCK packing in crystals. All 2Fo-Fc electron density contoured at 1σ within a slab approximately 150 Å x 135 Å x 110 Å is shown as grey mesh. CTCK monomers in the same region are individually colored, and shown in cartoon with their cysteines that form inter-chain disulfides shown as Cβ spheres.

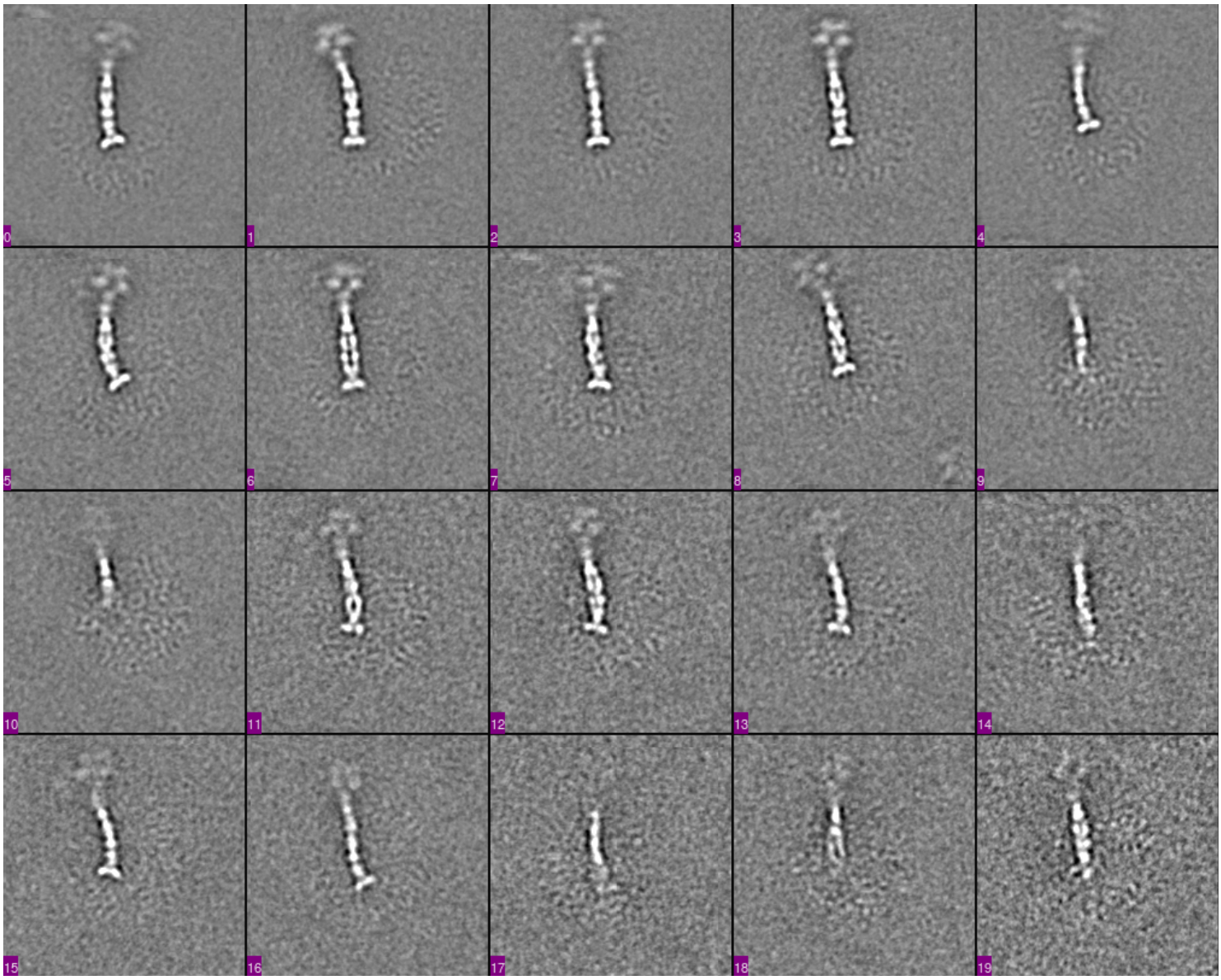


Figure S4. All twenty class averages of fragment A3-CK centering on the CTCK region. 5454 particles were grouped to 20 classes, with a radius for alignment and averaging of 22 nm. Classes are arranged by the number of particles in each class, from left to right, and from top row to bottom. Class number, from 0 (most populous) to 19 (least populous), is shown in lower left of each class.

A **Structural relationships to other CK domains.** [a]

Structure PDB code	PDGF 1pdg	Artemin 2gh0	Gonadotropin 1qfw	Sclerostin 2kd3	Interleukin-17 3jvf	Noggin 1m4u	Neurotrophin 1hcf	Coagulogen 1aoc
Z-score [b]	7.8	7.1	7.1	5.3	4.6	4.0	4.0	2.9
RMSD (Å) / residues [c]	2.8/76	3.8/76	2.8/83	3.2/84	3.8/68	3.3/70	4.5/76	3.6/67

[a] DALI search (Hasegawa, et al.) of VWF CTCK revealed structural similarity between CTCK and CK cytokines. Structures of CK cytokines were ranked by Z-scores in DALI, and the structure giving the highest Z-score in each of seven different SCOP families (g.17.1.1-7) is listed in the table. Sclerostin was not included in SCOP families of CK cytokines.

[b] DALI Z-score is a measure of similarity by comparing intramolecular distances (Cα-Cα) (Hasegawa, et al.). A Z-score over 2 indicates significant structural similarity and most likely same fold.

[c] The number of structurally equivalent residues are listed.

B **superimposed monomers**

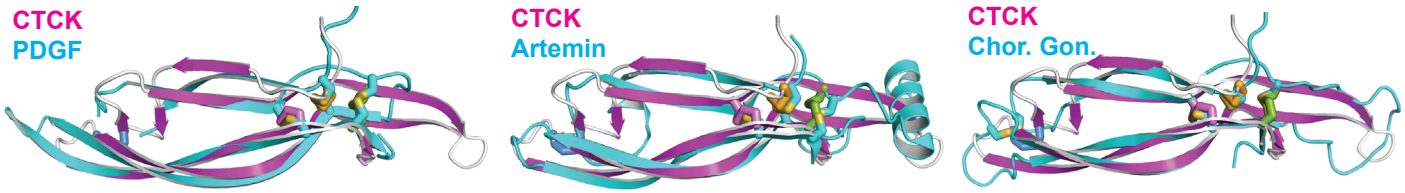


Figure S5. Superposition of the VWF CTCK monomer on cytokine CK domain monomers. A. Summary of DALI results. The highest scoring member of each subfamily of CK cytokines listed in SCOP is shown. B. Representative monomer superpositions.

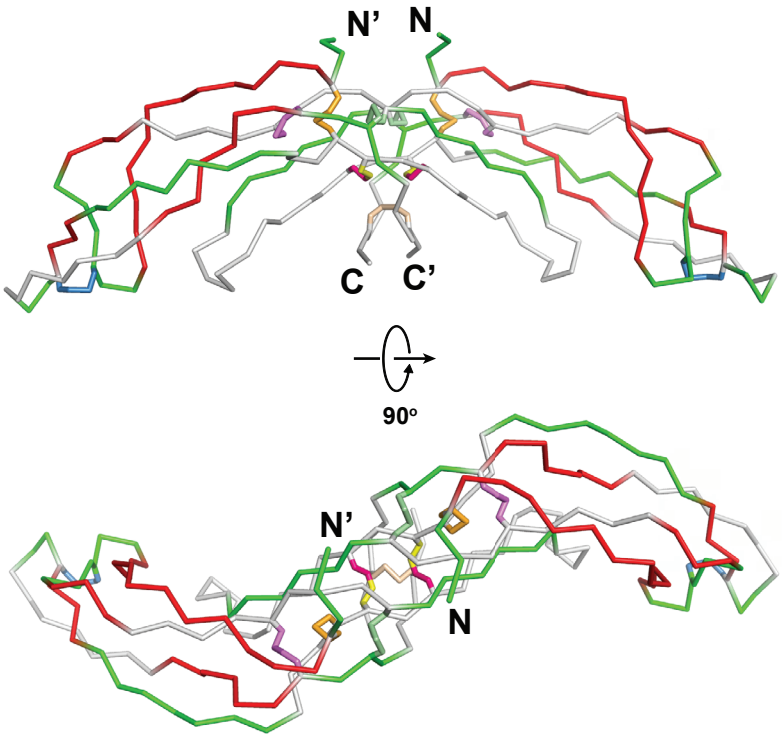


Figure S6. The frizzled4 binding site in norrin. Using the alignment to VWF CTCK sequence shown in Fig. 5, norrin mutational effects are displayed on the VWF CTCK structure. Alanine mutations of residues with less than or more than 25% of residual frizzled4 binding are shown in red and green on the Cα trace, respectively (Smallwood et al. 2007). In other words, the frizzled4 binding site on each monomer is in red. The alignment to VWF CTCK generates a single contiguous binding site in each monomer, whereas several non-contiguous regions are apparent in Fig. 6D of Smallwood et al. 2007 in which the alignment is to BMP2.