Supporting Information

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SI Methods

Protein Production and Purification. C_L^{wt} and C_L^{P35A}, the mutant generated by site-directed mutagenesis (Stratagene), were expressed, refolded, and purified as described in ref. 1. The refolding buffer was modified [250 mM Tris/HCl (pH 8.0), 100 mM L-Arg, 10 mM EDTA, 1 mM GSSG, 0.5 mM GSH]. β_2m was cloned from human cDNA (RZPD), the gene for β_2m^{toCL} was synthesized (GATC Biotech). Both proteins were cloned into the pET28a vector (Novagen) without a His-tag and expressed overnight (o.n.) at 37°C in *Escherichia coli* BL21 DE3 cells, and the inclusion bodies (IBs) were prepared as described in ref. 1. IBs were solubilized in 50 mM Tris/HCl (pH 7.5), 8 M urea, 50 mM 2-mercaptoethanol, 10 mM EDTA and applied to a Q-Sepharose column equilibrated in 50 mM Tris/HCl (pH 7.5), 5 M urea, 10 mM EDTA. The protein of interest in each case did not bind to the column, and the flow-through was refolded as described above. A Superdex 75 26/60 column (GE Healthcare) equilibrated in PBS was used as a final purification step for all proteins. Isotope-labeled proteins were expressed in M9 minimal medium containing either ^{15}N ammonium chloride as the only nitrogen source or additionally ^{13}C glucose as the only carbon source. All plasmids were sequenced, and the mass of each protein was confirmed by MALDI-TOF MS.

CD Spectroscopy. CD measurements were performed in a JASCO J-715 spectropolarimeter. CD spectra were accumulated 16 times and buffer-corrected. For far-UV CD measurements, 10 μM protein in a 1-mm quartz cuvette was used; near-UV CD measurements were carried out at 50 μM protein concentration in a 5-mm quartz cuvette. To obtain far-UV as well as near-UV CD spectra of the intermediate, three spectra were measured and averaged beginning after 2 min of refolding. At the chosen instrumental parameters this corresponds to a maximum measuring time of 10.5 min in which maximally 5% of the molecules fold to the native state at 2°C. The same experiment was repeated seven times independently for far-UV and three times for near-UV CD spectra and subsequently averaged. The individually averaged spectra were identical within ±5%. The spectra of the intermediate were corrected for the 10% of native molecules that possess the correct Pro-isomerization state and hence refold to the native state within the dead time of the experiment (1). For pH transitions, 10 μM protein was incubated o.n. at the different pH values in Theorell–Stenhagen buffer (2) at 20°C before the far-UV CD signal at 218 nm was recorded. Temperature-induced unfolding transitions were monitored by the change in the far-UV CD signal at 205 nm with a heating rate of 20°C/h. Because all temperature-induced unfolding transitions were not completely reversible, an apparent melting temperature was derived by a Boltzmann fit.

NMR Spectroscopy. For C_L^{wt} as well as C_L^{P35A}, backbone sequential assignments were obtained by using standard triple resonance experiments implemented with selective proton flip-back techniques for fast pulsing (3). Aliphatic side chain assignments were completed by using a combination of CCH-TOCSY and CCH-COSY experiments. Distance information was derived from a set of 3D-NOESY spectra, including NNH- and CNH-NOESY spectra (4), in addition to a ^{15}N-HSQC-NOESY spectrum. Dihedral angle restraints were determined for backbone Φ and Ψ angles based on C^α, C^β, C′, and H^α chemical shifts using the program TALOS (5).

MD Simulations. The restraints for simulations of C_L^{P35A} were derived from a comparison of the NMR chemical shifts of C_L^{wt} and C_L^{P35A}. (*1*) C^α, NH, C′, and C^β atoms of residues with chemical shifts similar to C_L^{wt} were restrained by minimizing the RMSD of the interatomic distances of the four atoms with respect to the crystal structure. (*2*) C^α, C′, and C^β atoms were restrained as described for restraint *1* for residues where the NH chemical shift could not be determined but otherwise had similar chemical shifts to the wild type. (*3*) Residues that were unassigned in the C_L^{P35A} mutant alone were restrained toward zero native contacts. In this regard, native contacts were computed by using only C^α, NH, C′, and C^β atoms, a cut-off of 0.65 nm, and for pairs of residues separated by at least five other residues in the primary sequence. (*4*) Φ and Ψ dihedral angles were restrained toward values determined by TALOS if available.

To minimize the number of restraints, restraints *1–3* were only applied to residues located in secondary structure elements. The ensembles of structures obtained with and without the dihedral restraints were largely similar. The restraint potential, V, used was $V(\rho, t) = (\alpha_i/2)(\rho - \rho_i)^2$, where $i = 1, 2, 3, 4$ was the type of restraint used and corresponded to *1–4* described above, ρ was defined as the mean squared difference between the calculated and target quantities to be restrained, ρ_i was the target value of the restrained quantity, and α_i was the value of the bias (kcal/mol) used to drive the restrained quantities toward the target values. A simulated annealing protocol was used to enhance the sampling of the conformational space. It was composed of six stages lasting a total of 452 ps: (*i*) 520 K for 62 ps, (*ii*) 650 K for 62 ps, (*iii*) 500 K for 62 ps, (*iv*) 420 K for 82 ps, (*v*) 350 K for 82 ps, and (*vi*) 300 K for 102 ps. A total of 100 cycles were performed.

AFM Measurements. For fibrillization experiments, a 100 μM protein solution in PBS was mixed 1:1 with buffer A (25 mM sodium acetate, 25 mM sodium phosphate) at pH 1.5 or 2.5 (final pH: 1.5 or 3.0 respectively, pH-meter reading) or PBS. The solution was incubated for 7 days, under slight shaking at 37°C. Seeds were prepared from β_2m protein fibrils (grown at pH 1.5 for 7 days) by sonication for 15 min in a sonication bath. Two microliters of seeds were used to seed 100 μl of solution, which corresponds to 1 μM seeds for 50 μM protein. For β_2m^{toCL}, seeds were prepared analogously. To prepare AFM samples, 20 μl of protein solution was spotted on freshly cleaved mica, incubated for 2 min at room temperature, washed three times with 100 μl of MilliQ water (Millipore), and dried overnight at room temperature. AFM measurements were done in contact mode with a Digital Instruments multimode scanning probe microscope (Veeco) at a scanning speed of 1.5 μm/min. DNP-S20 tips were used for all measurements (Veeco). Each experiment was repeated seven times.

1. Feige MJ, Hagn F, Esser J, Kessler H, Buchner J (2007) Influence of the internal disulfide bridge on the folding pathway of the CL antibody domain. *J Mol Biol* 365:1232–1244.
2. Theorell T, Stenhagen E (1938) Ein Universalpuffer fiir den pH-Bereich 2.0 bis 12.0. *Biochem Z* 299:416.
3. Diercks T, Daniels M, Kaptein R (2005) Extended flip-back schemes for sensitivity enhancement in multidimensional HSQC-type out-and-back experiments. *J Biomol NMR* 33:243–259.
4. Diercks T, Coles M, Kessler H (1999) An efficient strategy for assignment of cross-peaks in 3D heteronuclear NOESY experiments. *J Biomol NMR* 15:177–180.
5. Cornilescu G, Delaglio F, Bax A (1999) Protein backbone angle restraints from searching a database for chemical shift and sequence homology. *J Biomol NMR* 13:289–302.

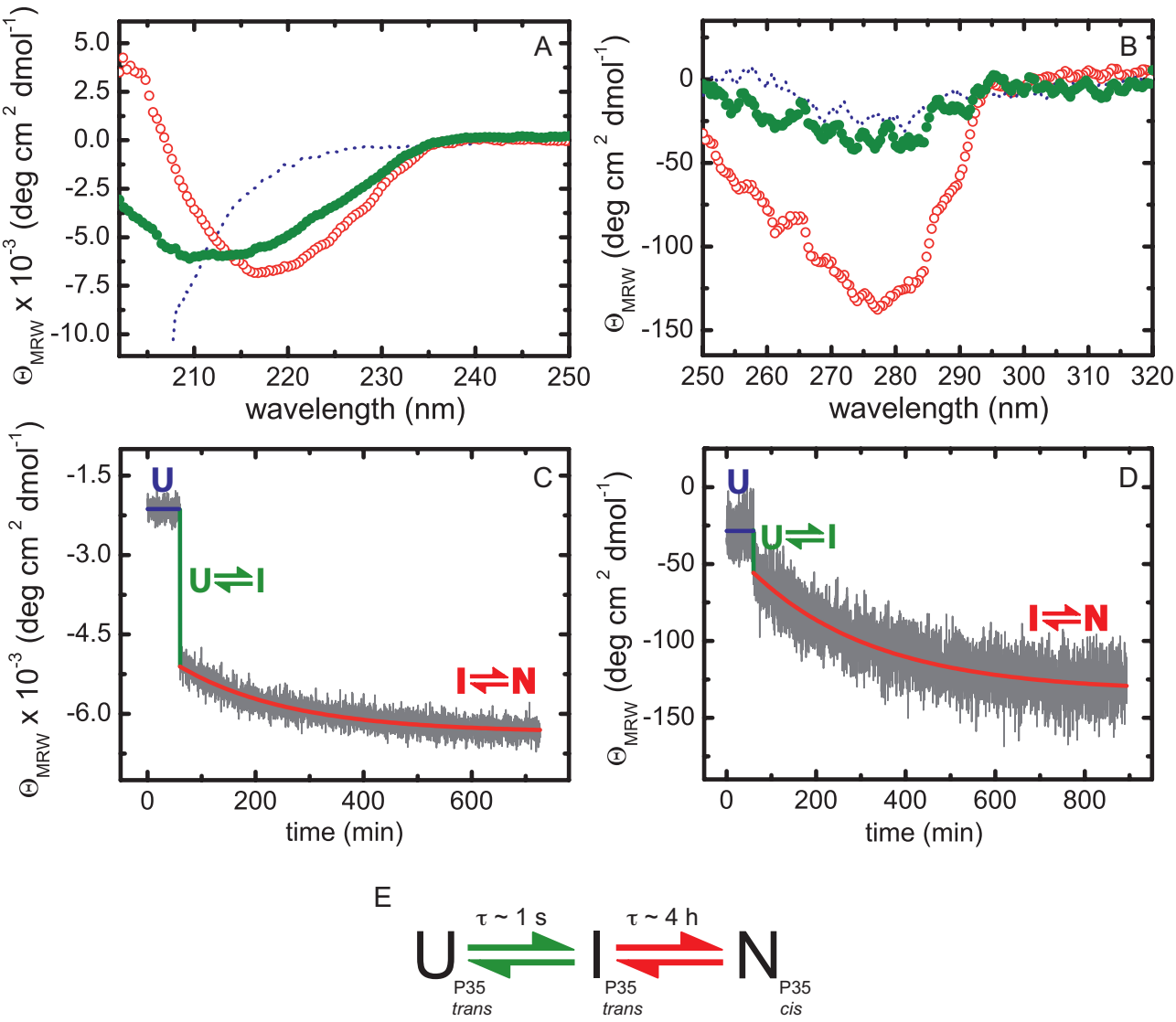


Fig. S1. Structural and kinetic characterization of the C_L domain and its major folding intermediate by CD spectroscopy. The secondary structure of the native (red open circles) and the unfolded C_L domain (blue dots) was investigated by far-UV CD spectroscopy (*A*) and its tertiary structure by near-UV CD-spectroscopy (*B*). During refolding, spectra of the major folding intermediate were measured (green, filled circles) (*A* and *B*). The spectra of the intermediate were corrected for 10% of the molecules that already possess the correct Y34–P35 isomerization state and therefore refold to the native state during the dead time of the experiment. Refolding kinetics were followed by far-UV CD spectroscopy at 218 nm (*C*) as well as by near-UV CD spectroscopy at 280 nm (*D*). The signal of the unfolded domain is shown in blue, refolding to the intermediate in green, and folding from the intermediate to the native state as a single exponential fit in red (*C* and *D*). (*E*) The overall folding mechanism on the slow C_L folding pathway can be described as a three-state process. Time constants for each reaction are indicated. For simplicity, the folding mechanism does not show the parallel folding pathway of the C_L domain with the Y34–P35 bond in the correct isomerization state. For far-UV CD measurements, 10 μM protein was used, for near-UV CD measurements 50 μM protein was used. All measurements were carried out at 2°C in PBS in the presence of a GdmCl concentration of 2 M for the unfolded protein and 0.2 M for the folded protein.

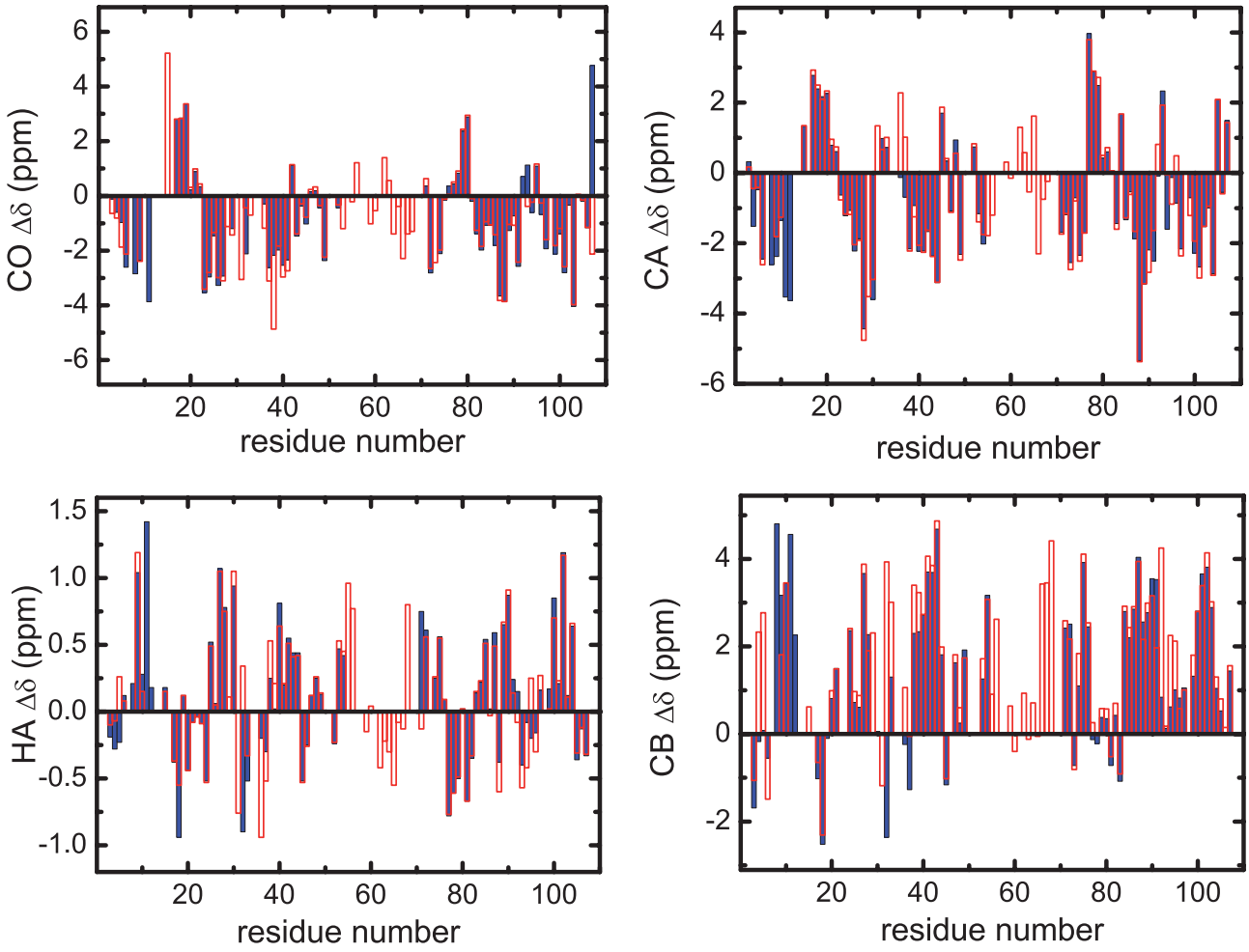
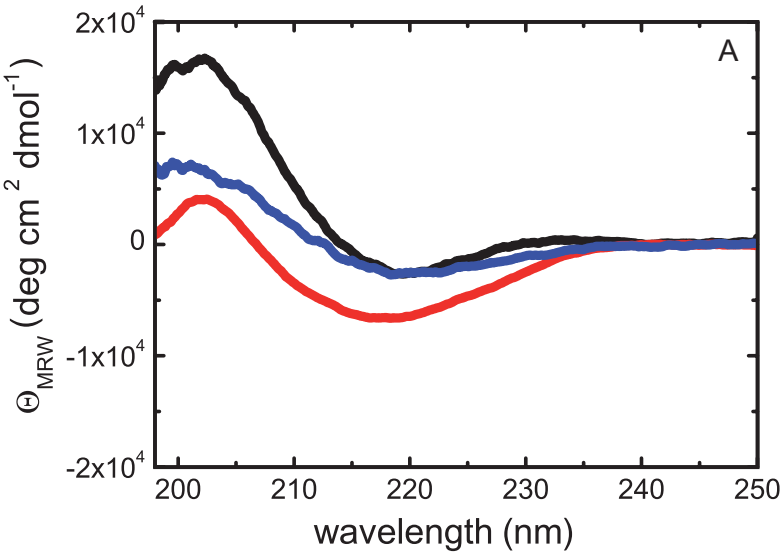
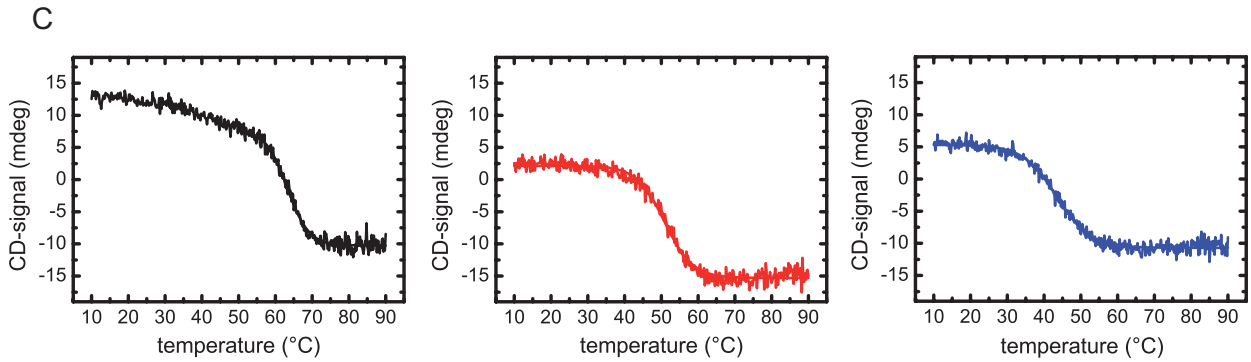


Fig. S2. Chemical-shift deviations of the nuclei C′, C^{α}, C^{β}, and H^{α} from the random coil values published by Wishart and colleagues [Wishart DS, Sykes BD, Richards FM (1992) *Biochemistry* 31:1647–1651; Wishart DS, Sykes BD (1994) *J Biomol NMR* 4:171–180] for C_L^{wt} shown in blue and for the mutant C_L^{P35A} shown in red.



B

b_2m	...IQVYSR **HPAENGKS** NFLNCY...YYTEFT **PTEK** DEYACR...
C_L	...TVSIF **PPSSEQLTSGG** ASVVCF...STLTLT**KDEYERH**NSYTCE...
b_2m^{toCL}	...IQVYSR**PPSSEQLTSGG**NFLNCY...YYTEFT**KDEYERH**DEYACR...



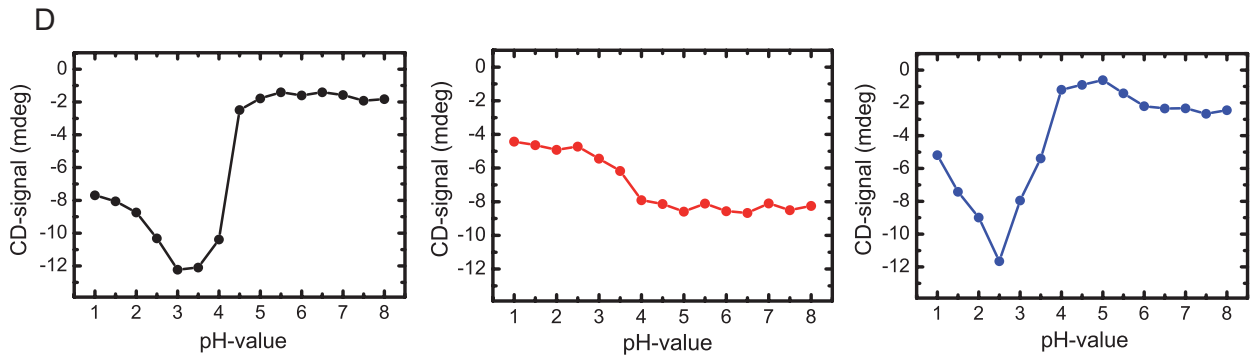


Fig. S3. Secondary structure, sequence and stability of β_2m, C_L and the β_2m^{toCL} exchange mutant. (*A*) Far-UV CD spectra were measured at 20°C in PBS at 10 μM protein concentration. β_2m is shown in black, C_L in red, and β_2m^{toCL} in blue. (*B*) Sequence elements from β_2m (black), C_L (red), and β_2m^{toCL} (blue). Exchanged elements and the corresponding wild-type sequences are depicted in bold. The temperature-induced unfolding of all three proteins is shown in *C*. It was followed by far-UV CD spectroscopy at 205 nm. β_2m (black) shows a transition midpoint of 63°C, for C_L (red) the midpoint is at 51°C, and for β_2m^{toCL} (blue) at 43°C. (*D*) The pH stability of β_2m (black), C_L (red), and β_2m^{toCL} (blue) at 20°C was investigated at 218 nm by far-UV CD spectroscopy.

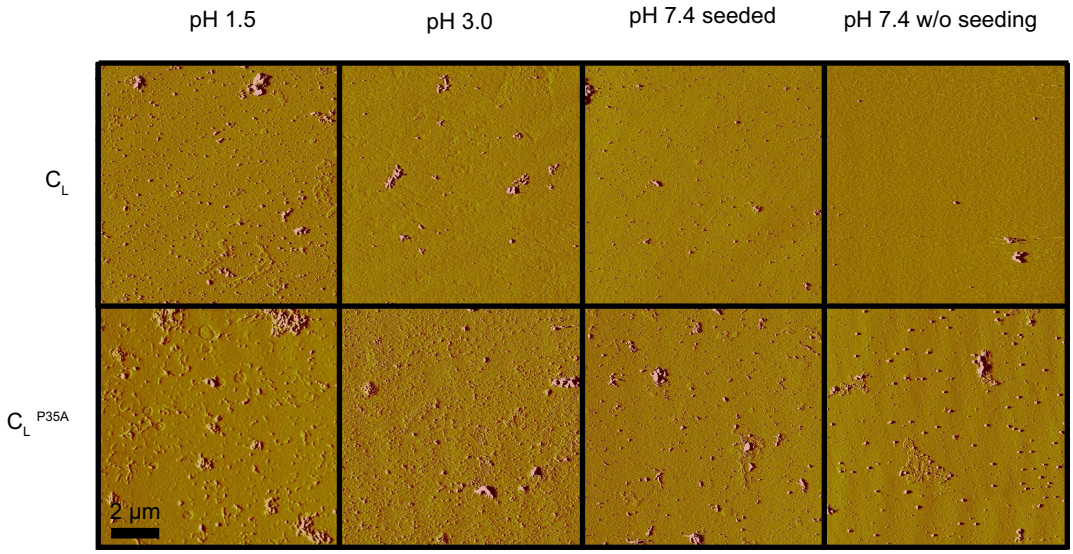


Fig. S4. Amyloidogenic properties of C_L and C_L^{P35A}. The proteins were incubated at pH 1.5 or 3.0 at 37°C for 7 days at a concentration of 50 μM. Additionally, both proteins were incubated under physiological conditions (PBS, 37°C) either seeded with β_2m fibrils or not. Formation of amyloid fibrils was assessed by AFM measurements, and representative pictures of each sample are shown.