Supporting Information for Publication

A Nanobody Binding to Non-amyloidogenic Regions of the Protein Human Lysozyme Enhances Partial Unfolding but Inhibits Amyloid Fibril Formation

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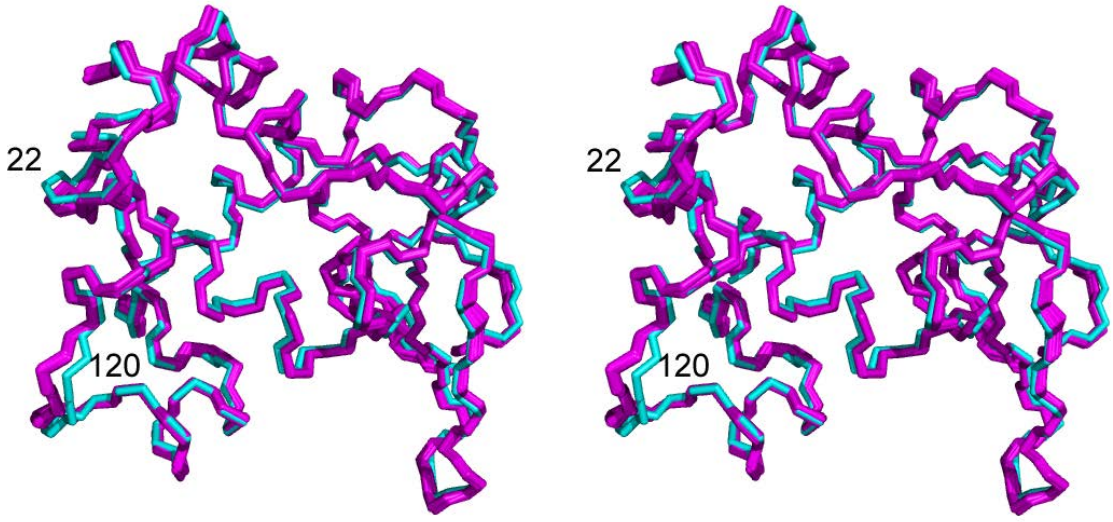

Figure S1. Stereoscopic representation of the three dimensional alignment of human lysozyme bound to cAb-HuL5 with known non-complexed human lysozyme structures taken from the Protein Database (Pdb n°:, 1iwy, 1iwx, 3fe0, 2zwb, 1iww, 1iwv, 1iwu, 1iwt, 1jwr, 2zij, 2zik and 1lzr). The backbone atoms Cα, C and N are shown; the carbonyl oxygen is omitted for clarity. The lysozyme molecule bound to cAb-HuL5 is shown in light blue and the other lysozyme molecules are shown in magenta.

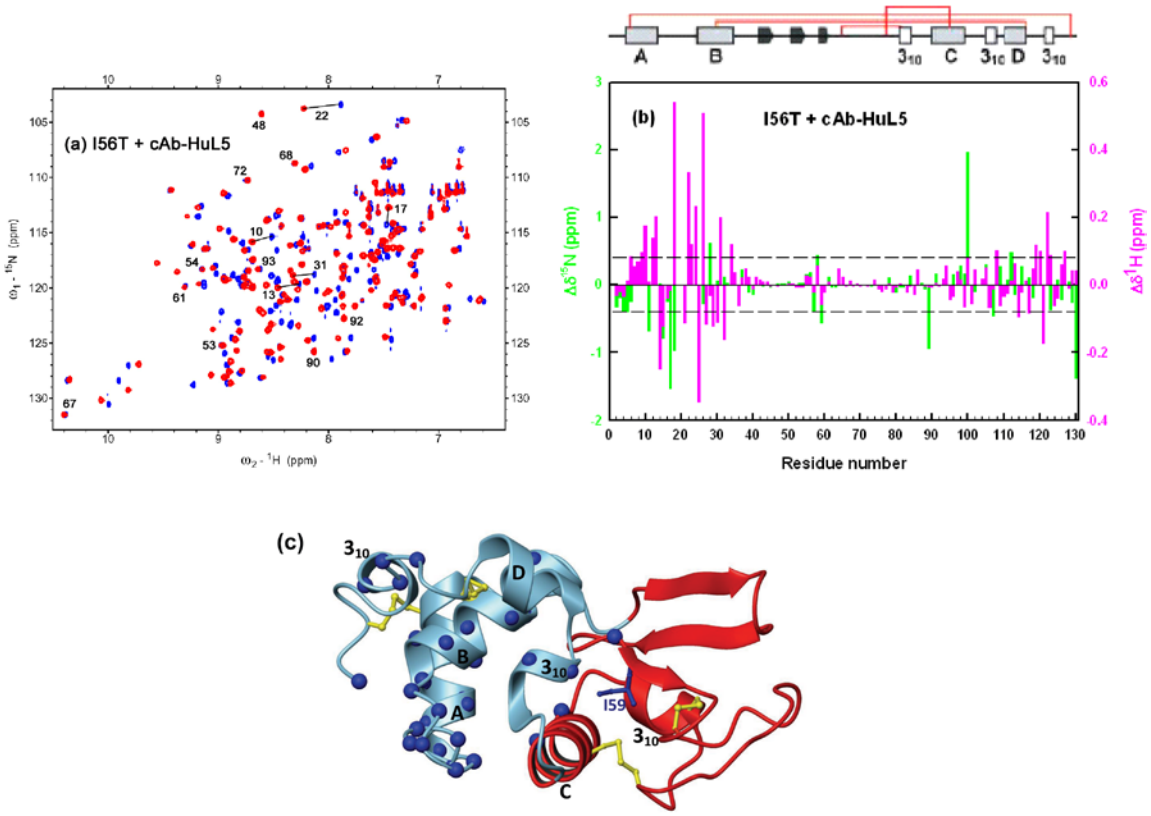

Figure S2. Overlaid [^{15}N-^{1}H]-HSQC NMR spectra of (a) the I56T lysozyme variant (pH 5.0 and 37 °C) in the absence (blue) or presence (red) of the cAb-HuL5 fragment. The chemical shift perturbations of the I56T variant caused by the binding of the cAb-HuL5 fragment are shown in (b). The magenta and green bars represent the chemical shift perturbations of the ^{1}H and ^{15}N resonances, respectively. Residues experiencing a chemical shift change ≥ |0.4| ppm for ^{15}N or ≥ |0.1| ppm for ^{1}H resonances are considered to be affected significantly by the interactions with the antibody fragment. (c) Ribbon diagram of the I56T variant showing the C_α atoms of the residues affected significantly by the binding of the cAb-HuL5 fragment (blue spheres). The four disulfide bonds are shown in yellow. The β-domain and the adjacent C-helix that cooperatively unfold in the amyloidogenic intermediate is colored red. The peaks corresponding to G19 and Y20 (located in the loop between the A- and B-helices) in the I56T/cAb-HuL5 complex have not been assigned; their chemical shift differences are therefore not shown in the histograms. No unassigned peaks are visible in the HSQC spectra of the complexes within 0.1 ppm (^{1}H) and 0.4 ppm (^{15}N) of the peak positions of G19 and Y20 in the HSQC spectrum of the unbound lysozyme variant, suggesting that these residues experience significant chemical shift changes upon binding to the antibody fragment. Note that I59 whose side chain points towards the interface between the α- and β-domains are significantly affected by the binding of the cAb-HuL5 fragment. The structure of the I56T variant was generated from its X-ray coordinates (Pdb n^{o}: 1loz) and the diagram produced using MOLMOL.

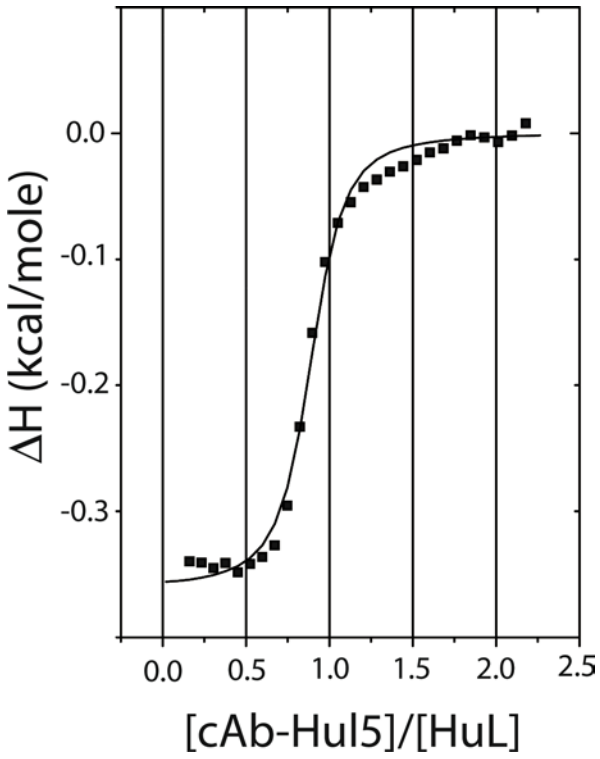

Figure S3. ITC binding isotherm for the cAb-HuL5G/WT-HuL interaction at 50 °C and in 0.1 M sodium citrate buffer pH 5.5 containing 3M urea. The integrated enthalpy changes of binding are plotted versus the molar ratio of cAb-HuL5/WT-HuL. The curve predicted using the fitted parameters by non-linear regression to a 1:1 bimolecular binding model is shown as a solid line.

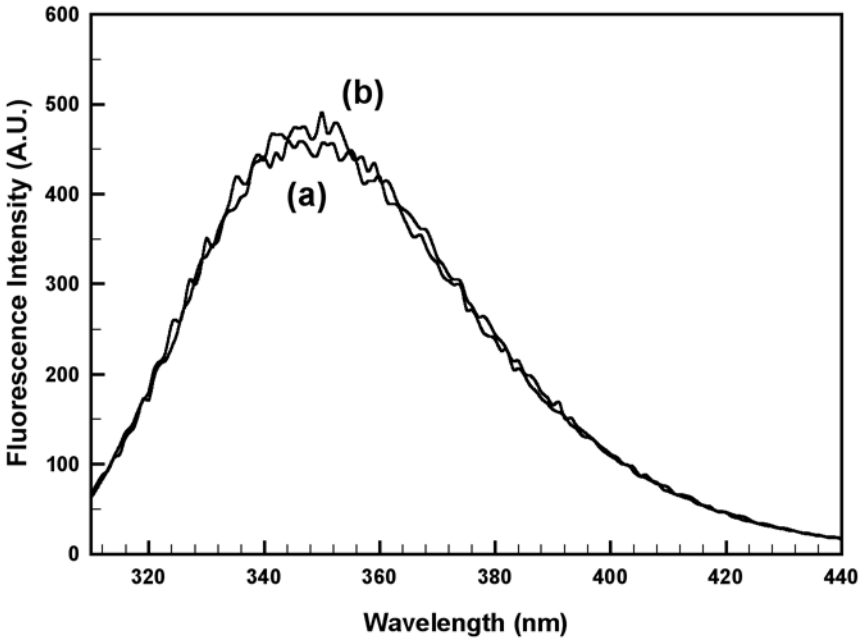

Figure S4. Fluorescence spectra of (a) the cAb-HuL5 fragment (0.4 mg/ml) incubated for 1h with the fibrils of the D67H variant (~0.4 mg/ml) and (b) the cAb-HuL5 alone (0.4 mg/ml) in 0.1 M sodium acetate buffer pH 5.5 at 25 °C. The samples were then centrifuged (353 200 g, 1 h) and the fluorescence spectrum of each supernatant was recorded. The superposition of the two curves indicates that cAb-HuL5 does not bind to the fibrils.

Table S1. RMSD values for the Cα atoms of lysozyme structures compared to the average coordinates.	
Structure	RMSD (Å)
WT-HuL in complex with cAb-HuL5	0.43
[a]1iwy	0.11
[a]1iwx	0.11
[b]3fe0	0.16
[b]2zwb	0.16
[a]1iww	0.10
[a]1iwv	0.11
[a]iwu	0.11
[a]1iwt	0.12
[c]1jwr	0.12
[d] 2zij	0.15
[d] 2zik	0.19
[e] 1lzr	0.18

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[b] Chiba-Kamoshida, K., Matsui, T., Chatake, T., Ohhara, T., Ostermann, A., Tanaka, I., Yutani, K., Niimura, N. Site-specific softening of peptide bonds by localized deuterium observed by neutron crystallography of human lysozyme (http://www.rcsb.org/pdb/)

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[d] Shoyama, Y., Tamada, T., Nitta, K., Kumagai, I., Kuroki, R., Koshiba, T. Preparation and characterization of methyonyl-lysine attached human lysozyme expressed in Escherichia coli and its effective conversion to the authentic-like protein (http://www.rcsb.org/pdb/)

[e] Song, H.; Inaka, K.; Maenaka, K.; Matsushima, M. *J. Mol. Biol.* **1994**, *244*, 522.