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Supporting Material

A yeast toxic mutant of HET-s(218-289) prion displays alternative intermediates of amyloidogenesis

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIALS AND METHODS

ATR-FTIR spectra analysis

For clarity, the maximum absorbance for the amide I band of all spectra shown have been normalized to the same value. The resulting spectra were analyzed with an algorithm based on a second derivative function (OMNIC software, Thermo Fisher Scientific) and self-deconvolution procedure (GRAMS software, Thermo Fisher Scientific) to determine the number and wavenumber of individual bands within the spectral range 1485-1750 cm^{-1}. The amide I band of both spectra could be fitted by five components assigned to the vibration of amide I for different secondary structures. The relative contribution of the various bands was obtained by a band-fitting procedure keeping wavenumber, width at half-height and band profile constant. Only intensity was allowed to vary. The fit for both spectra was obtained with a mixed Lorentzian (70%)-Gaussian (30%) band profile and width at half-height included between 18 and 40 cm^{-1}. Fit parameters have been kept the same for all spectra. The amide II band was fitted by five components (data not shown). Less sensitive to the secondary structure of proteins as amide I in infrared, fit parameters were allowed to freely fluctuate in order to obtain the best fit for this vibration band. Consequently, the relative contributions of the different bands were determined from the fit results obtained for the amide I band. The amount of each secondary structure element is given as a percentage, by dividing the integral intensity of one amide I band component by the total intensity of all amide I band components. For both spectra, fit results correspond to a local minimum at the end of the iteration procedure. The standard error does not exceed 1.5%. Assignment of amide I and I' (in D_2O) were made according to Goormaghtigh *et al.*, 1994a/b.

SUPPLEMENTARY RESULTS

SUPPLEMENTARY TABLE 1 Vibrational assignments of the ATR-FTIR band components in the amide I region for WT and M8 air-dried amyloids

Band	Secondary structure elements*	Wavenumber (cm^{-1})		Width (cm^{-1})	Profile
		Wild-type	Mutant 8		
Band 1	Antiparallel ß-sheet	1692.3	1692.3	18.3	70% Lorentzian
Band 2	ß -turn	1669.4	1672.0	36.7	70% Lorentzian
Band 3	Random coil	1655.0	1651.0	39.9	70% Lorentzian
Band 4	Parallel ß-sheet	1630.0	1630.0	29.6	70% Lorentzian
Band 5	Antiparallel ß-sheet	1619.5	1619.8	26.1	70% Lorentzian

* Assignments were made according to Goormaghtigh *et al.*, 1994a.

SUPPLEMENTARY TABLE 2 Physico-chemical parameters of His-tagged WT and M8 proteins

Proteins	MW	pI	Net charge	Hydropathicity
WT	9548.7	8.15	+1	-0.442
M8	9347.4	8.22	+1	-0.474

Molecular weight, isoelectric point, net charge at pH 7.4, and grand average of hydropathicity (GRAVY) were estimated from the amino-acid sequence of each protein fused to the His tag (data from ExPASy Proteomics Server, http://expasy.org/tools/protparam.html)

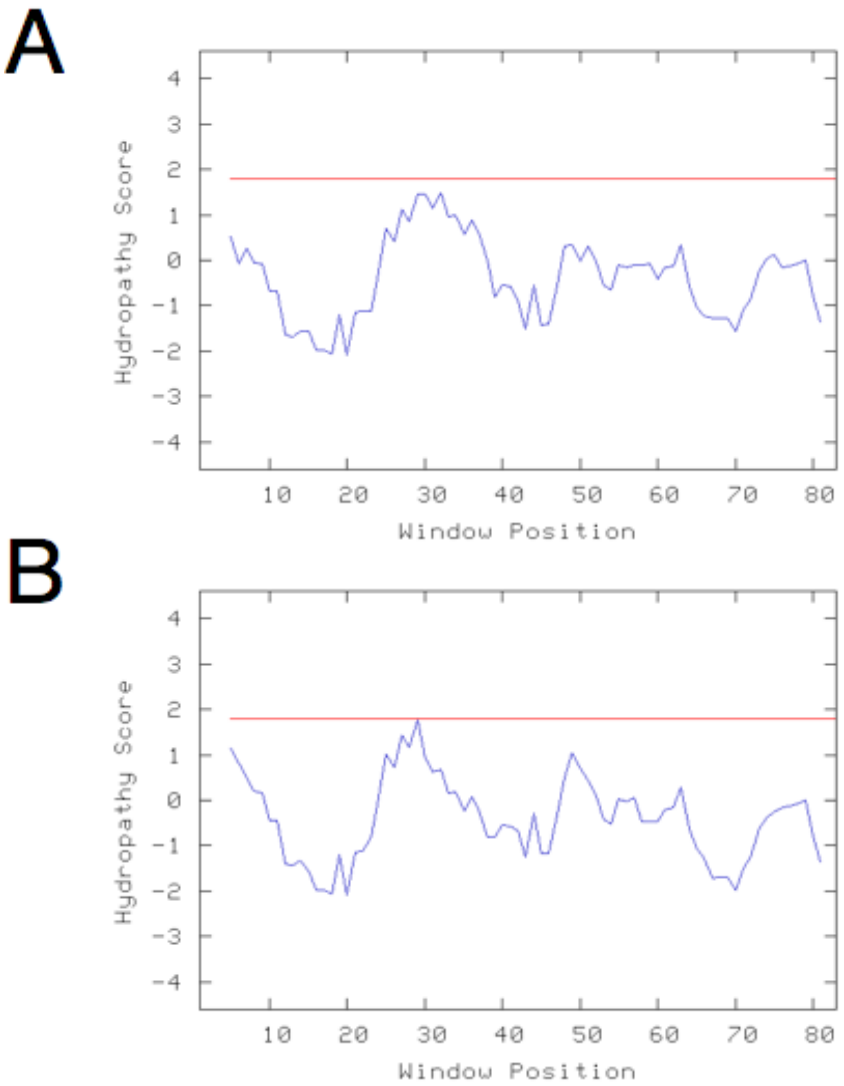

FIGURE S1 Kyte-Doolittle hydropathy plots of WT (*A*) and M8 (*B*) obtained at http://gcat.davidson.edu/rakarnik/kyte-doolittle.htm (Kyte & Doolittle, 1982). Window size 9 was applied.

SUPPLEMENTARY REFERENCES

Goormaghtigh, E., Cabiaux, V., and J. M. Ruysschaert. 1994. Determination of soluble and membrane protein structure by Fourier transform infrared spectroscopy. I. Assignments and model compounds. *Subcell. Biochem.* 23:329-362.

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Kyte, J., and Doolittle, R.F. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157:105-132.