SUPPORTING INFORMATION

Materials and Methods

Fluorescence microscopy and amyloid staining

1-day or 4-days transgenic worms were placed in PBS buffer drop with 5% glycerol and anesthetized with 1% sodium azide. GFP fluorescence was observed using a Leica Mod. TCS-SP2 (Leica Microsystem) and image processing was performed with Leica Confocal Software (LCS). For the X-34 staining, worms were incubated with 1 mM X-34 in 10 mM Tris/HCl pH 8.0 for 2 h, and then analyzed as above.

Fig. S1 SEC profiles of AT3Q55 on a Superose 12 10/300 GL in PBS buffer. (A) 10 mg His-tagged AT3Q55 was loaded onto a gel filtration column. The arrow indicates the peak corresponding to the AT3Q55 monomeric form. (B) 2 mg of monomeric AT3Q55 was reloaded onto the same column.

Fig. S2 SDS-PAGE (12%) of freshly purified AT3Q55. (A) SDS-gel (5 µg protein) stained with Imperial Protein Stain (Thermo Scientific Rockford, IL USA), along with molecular weight markers; (B) western blot (1 µg protein) performed using anti-AT3 Z46 polyclonal antibody, as previously reported (31).

Fig. S3 SDS-PAGE (12%) of the soluble protein fraction of AT3Q55-EGCG 1:1 and AT3Q55-tetracycline 1:1. The gels were stained with Imperial Protein Stain (Thermo Scientific Rockford, IL USA).

Fig. S4 SEC profiles of AT3Q55 on a Superose 12 10/300 GL in PBS buffer after incubation with either EGCG or tetracycline. 500 µg AT3Q55 was loaded onto a gel filtration column after the indicated incubation times with either drugs. Other details are reported in the Section Materials and Methods.

Fig. S5 FTIR spectra of AT3Q55 fibrils. (A) Absorption spectrum (bold line) and its second derivative in the amide I region of AT3Q55 fibrils obtained by centrifugation of the protein solution upon incubation at 37°C for two weeks. (B-C-D) Second derivative spectra of AT3Q55 fibrils resuspended in PBS (B), EGCG (C) and tetracycline (D) collected at different times of incubation at 37°C. Band assignment of the main components is reported in (B).
**Fig. S6** Confocal microscopy analyses of AT3 expression. AT3Q17-GFP and AT3Q130-GFP expression in the 1-day animals (A) and in the 4-days animals (B) grown at 25°C was detected by GFP fluorescence. 4-days animals expressing the AT3 variants were stained for 2 h with 1 mM X-34 dye (C).

**Fig. S7** Effect of EGCG and tetracycline on the time course of AT3Q55 aggregation. No drug added (A), EGCG (B), tetracycline (C). Protein-drug molar ratio was 1:5. Total soluble protein fraction was determined by the Bradford assay on supernatants after centrifuging the incubation mixtures at 14,000 x g. SDS-soluble fraction was quantified densitometrically in SDS-PAGE of the supernatants. Both were expressed as percentage of zero time. Soluble, SDS-resistant fraction was quantified by subtracting SDS-soluble from total soluble fraction. Insoluble fraction (expressed as percentage of total protein) was calculated as: 100 – total soluble fraction.

**Fig. S8** Subtraction procedure in the ATR/FTIR analyses. A representative spectrum subtraction procedure is shown, as accomplished in the case of AT3Q55 in the presence of tetracycline. The following absorption spectra (bottom panel) are shown: 25 µM freshly purified AT3Q55 in PBS and in the presence of 125 µM tetracycline (A); 125 µM tetracycline in PBS (B); AT3Q55 after reference spectrum subtraction (C=A-B). In the second derivative (top panel) of AT3Q55 subtracted spectrum (D) the ≈ 1595 cm⁻¹ tetracycline peak (see spectra D and E) is not present, highlighting a successful subtraction procedure.
Fig S1
Fig S4
Fig S5

A. AT3Q55

Time
- 1 h
- 24 h
- 48 h
- 96 h
- 1W

Absorbance

Second derivative

Wavenumber (cm⁻¹)

1700 1680 1660 1640 1620 1600

B. AT3Q55

Second derivatives

- 1609
Intermolecular β-sheets

- 1657
Glu C=O

- 1634
Intermolecular β-sheets

Wavenumber (cm⁻¹)

1700 1680 1660 1640 1620 1600

C. AT3Q55-EGCG

Second derivatives

Wavenumber (cm⁻¹)

1700 1680 1660 1640 1620 1600

D. AT3Q55-tetracycline

Second derivatives

Wavenumber (cm⁻¹)

1700 1680 1660 1640 1620 1600
Fig S6
Fig S8

Spectrum A) AT3Q55 25μM and Tetra 125μM in PBS
Spectrum B) Tetra 125μM in PBS
Spectrum C) Subtracted spectrum: A-B

Second derivative of spectrum C
Second derivative of spectrum B

Absorbance vs. Wavenumber (cm⁻¹)