Thioflavin T forms a non-fluorescent complex with α-helical poly-L-glutamic acid

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ELECTRONIC SUPPLEMENTARY INFORMATION

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1. Materials and Methods

Samples

PLGA and PDGA (sodium salts) with molecular weight (M.W.) 15 - 50 kDa were purchased from Sigma. As predisposition of homopolypeptides to acquire particular conformations, and their affinity towards small ligands (such as ThT) may depend on molecular weight, additional characterization of M.W. was carried out using size exclusion chromatography (SEC), which confirmed M.W. and showed no evidence of main-chain-branching.

CD, ICD and ThT fluorescence measurements

For far-UV CD measurements, approx. $2{,}25 \cdot 10^{-2}$ wt. % aqueous samples of PLGA (PDGA) were first pH-adjusted to the desired values, and subsequently transferred to quartz cuvettes with 1-mm-long optical pathlengths. For ICD measurements, 0,3 wt. % fresh aqueous solution of ThT was added to $2{,}25 \cdot 10^{-2}$ wt.

% samples of PLGA (PDGA) to the final dye concentration of 70 μM. A 10-mm quartz cuvette was used for ICD measurements. All CD and ICD spectra were collected on a Jasco J-815 S spectropolarimeter equipped with a temperature-control unit. Control experiments have shown that the presence of ThT at this low concentration has no measurable impact on the course of RC-to-α-helix transition in polyglutamic acid. Other experimental details were the same, as described earlier.[SI1-SI3]

For fluorescence measurements, aqueous samples containing 0,1 wt. % of PLGA (PDGA) and 30 μM ThT were adjusted to desired pH values. Samples of β_2-fibrils of polyglutamic acid were obtained upon incubation of α-helical PLGA (PDGA) at 65 ^{o}C, as described earlier.[SI4] Aqueous suspensions of β_2-fibrils used for fluorescence measurements were also 0,1 wt. %, with pH set at 4.3. Emission spectra were excited at 450 nm, while excitation spectra were collected at 482 nm. Measurements were carried out using AMINCO Bowman Series 2 luminescence spectrometer.

2. Additional UV absorption data

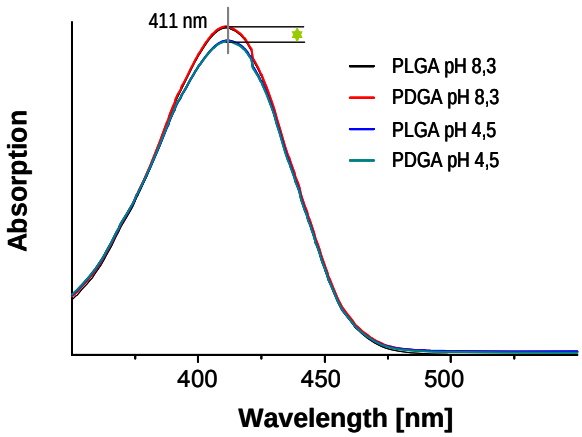

Figure SI 1. UV-absorption spectra of ThT in the presence of PDGA (PLGA) in RC (pH 8.3) and α-helical (pH 4.5) conformations.

The spectra show a small, yet detectable decrease in ThT electronic absorption intensity coinciding with the conversion of random coil conformation to α-helical structure. This spectral effect has been interpreted as a consequence of pronounced twisting of PGA-bound ThT molecules. Recent theoretical works have indicated that extreme twisting of a ThT molecule will lead to distortion of the conjugated π-electron system of ThT's benzothiazole and dimethylaminobenzene rings, ultimately resulting in the two rings acting as separate chromophores – shifting to shorter wavelengths.[SI5] Strong light-scattering on large PGA chains rendered precise estimation of absorption in the UV range problematic.

ESI References

(ESI1) W. Dzwolak and M. Pecul, *FEBS Lett.*, 2005, **579**, 6601.

(ESI2) W. Dzwolak, A. Loksztejn, A. Galinska-Rakoczy, R. Adachi, Y. Goto and L. Rupnicki, *J. Am. Chem. Soc.*, 2007, **129**, 7517.

(ESI3) A. Loksztejn and W. Dzwolak, *J. Mol. Biol.*, 2008, **379**, 9.

(ESI4) A. Fulara and W. Dzwolak, *J. Phys. Chem. B*, 2010, **114**, 8278.

(ESI5) A. A. Maskevich, V. I. Stsiapura, V. A. Kuzmitsky, I. M. Kuznetsova, O. I. Povarova, V. N. Uversky and K. K. Turoverov, *J. Proteome Res.*, 2007, **6**, 1392.