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# Levels of supramolecular chirality of polyglutamine aggregates revealed by vibrational circular dichroism

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ABSTRACT

**Polyglutamine (PolyQ) aggregates are a hallmark of several severe neurodegenerative diseases, expanded CAG-repeat diseases in which inheritance of an expanded polyQ sequence above a pathological threshold is associated with a high risk of disease. Application of vibrational circular dichroism (VCD) reveals that these PolyQ fibril aggregates exhibit a chiral supramolecular organization that is distinct from the supramolecular organization of previously observed amyloid fibrils. PolyQ fibrils grown from monomers with Q repeats 35 and above ($Q \geqslant 35$) exhibit approximately 10-fold enhancement of the same VCD spectrum compared to the already enhanced VCD of fibrils formed from Q repeats 30 and below ($Q \leqslant 30$).**


## 1. Introduction

Supramolecular chirality is a distinct property of all macromolecules found in living organisms. Beyond individual small molecules, macromolecular structures can adopt a higher level of chirality, such as the α-helix of proteins and the B-helix of DNA, in which the chiral sense of helical structure has its origins in the chirality of its individual amino acid or sugar constituents. The hierarchy of chiral structure and organization can be traced to even higher levels of supramolecular chirality leading, in most cases, to a distinct handedness in the resulting biological structure, such as protein–RNA complexes, DNA–histone assemblies, and eventually to even higher levels of biological organization associated with physical morphology. Here we demonstrate that vibrational circular dichroism (VCD) reveals two levels of supramolecular chiral organization in polyglutamine (PolyQ) fibrils having their origins beyond the local secondary structure of the constituent polypeptide molecules. This structural distinction has not been detected by any previous imaging or spectroscopic analyses.

Amyloid fibrils are β-sheet rich protein aggregates that are often detected in organs and tissues of patients diagnosed with different neurodegenerative diseases and other maladies associated with protein misfolding [1,2]. Application of electron microscopy (EM) and atomic force microscopy (AFM) has revealed a high morphological heterogeneity of amyloids, a phenomenon known as fibril polymorphism [3–6]. These and other microscopic tools, however,

*Abbreviations:* PolyQ, polyglutamine; VCD, vibrational circular dichroism; DUVRR, deep UV resonance Raman; IR, infrared; AFM, atomic force microscopy; EM, electron microscopy; H/D, hydrogen-deuterium

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may provide only limited information about fibril chirality [7]. This limit lies at the level of imaged fibril handedness and none of these techniques appears to probe down to the molecular level of fibril chiral organization [8].

Recently it was reported that VCD shows an enhanced sensitivity to amyloid fibril formation and development as demonstrated for lysozyme and insulin [9]. The observed VCD intensities from fully developed fibrils are one to two orders of magnitude larger than VCD intensities observed from solutions of isolated proteins. It has now been unambiguously demonstrated that this enhanced VCD sensitivity arises from the long-range supramolecular chirality of fibril structure and dynamics at all hierarchical levels [8]. This sensitivity makes VCD a unique solution-phase, stereo-specific probe of protein fibril chiral structure and correlated fibril morphology [10]. Basic theoretical concepts lead to the conclusion that enhanced VCD spectra as large as those observed from protein fibrils can originate only from supramolecular aggregates that have a long-range chiral organization [10]. For example, insulin and lysozyme fibrils that have an imaged left-handed twist morphology and possess *l*eft-handed helical proto-filaments, exhibit a distinct VCD spectrum with a sign pattern ( + + – + + ), named 'normal VCD' [11]. Microscopic examination of protein aggregates formed from other amyloid-associated proteins reveals in some cases a presence of flat, tape-like fibril polymorphs [12–15]. None of the conventional microscopic techniques, such as cryo-S.E.M. and AFM, have been able to visualize any twist on their surface. Nevertheless, VCD reveals a presence of supramolecular chirality in their supramolecular structure indicating that these tape-like fibrils are composed of right-handed helical proto-filaments [8]. It was found that these tape-like fibrils exhibit a unique VCD spectrum that is close to mirror image with respect to the signs, relative intensities and peak locations, compared to a 'normal' (left-handed) fibril VCD spectrum.

Herein we investigate the supramolecular organization of polyQ fibrils formed from monomers having differing lengths of Q residues. Such fibrils are thought to be important players in the pathology of the expanded CAG-repeat diseases, a family of at least 9 neurodegenerative diseases [16] linked to expanded polyQ sequences in 9 different disease proteins. For example, polyQ aggregates are found in brain autopsies [16], and the repeat-length dependence of spontaneous amyloid formation [17–19] parallels the repeat length dependences of most of these diseases [16]. Thus, disease risk is minimal for most of these diseases if polyQ repeat length is below about 35, but is high for longer repeat lengths. In contrast to the correlation of aggregation tendencies and rates of disease risk, there has been little evidence for any systematic variation of aggregate morphology with repeat length.

## 2. Materials and methods

### 2.1. Preparation of polyglutamine aggregates

All polyglutamine peptides were synthesized at the Small Scale Synthesis facility at the Keck Biotechnology Resource Laboratory of Yale University (http://keck.med.yale.edu/) and supplied crude. All peptides were purified and disaggregated as described [20]. Aggregation reactions at 37 °C in PBS (pH 7.4) were initiated, and monitored by an HPLC-based sedimentation assay. Fibrils of polyglutamine peptides were isolated by centrifuging the final aggregate suspension at 14000 rpm and re-suspending the pellet with appropriate volume of water to achieve the desired concentration.

### 2.2. Vibrational circular dichroism spectroscopy

VCD and IR spectra were measured at BioTools, Inc, Jupiter, FL using Chiral*IR-2X* Fourier transform VCD (FT-VCD) spectrometer equipped with an MCT detector and the Dual*PEM* option for enhanced VCD baseline stability. For each measurement, ~10 μl of polyQ sample was placed in a *Bio*Cell (BioTools, Inc.) with $CaF_2$ windows and a 6 μm pathlength. During measurements the *Bio*Cell was rotated at a constant velocity about the beam IR axis using SyncRoCell (BioTools, Inc.) to eliminate cell and possible sample birefringence. VCD and IR spectra were acquired for 2–4 h at 8 $cm^{-1}$ spectral resolution. Spectral baselines for VCD and IR were determined from measurements of water in the same *Bio*Cell for the same length of time as sample measurements. All subsequent data processing leading to final spectra was carried out in GRAMS/AI 7.0 (Thermo Galactic, Salem, NH).

### 2.3. Deep ultraviolet resonance Raman (DUVRR) spectroscopy

DUVRR spectra were obtained at the University at Albany using a home-built Raman spectrometer with 199 nm excitation wavelength [21]. A spinning NMR tube with a magnetic stirrer inside was used for sampling. All reported Raman spectra are an average of at least three independent measurements. In hydrogen–deuterium (H/D) exchange experiments, aggregated polyQ samples were centrifuged at 14000 rpm for 30 min. The precipitate was separated from the supernatant and washed with $D_2O$. This procedure was repeated several times. The resulting dispersion of polyQ aggregates in $D_2O$ was incubated overnight at 25 °C prior to recording DUVRR spectra. GRAMS/AI 7.0 (Thermo Galactic, Salem, NH) was used for spectral data processing.

### 2.4. Electron microscopy

An aliquot of 5 μl from the fibril suspension was placed on freshly glow-discharged carbon-coated 400-mesh-size copper grid and kept for 2 min before the grid was washed with a drop of deionized water. After that, the sample was stained with freshly filtered 5 μl of 1% (w/v) uranyl acetate for 2 s. The excess of sample, washes and stains was gently blotted off with filter paper. Images were obtained using a Tecnai T12 microscope (FEI) operating at 120 kV and 30000xg magnification and equipped with an UltraScan 1000 CCD camera (Gatan) with post-column magnification of ×1.4 (EM facility at Structural Biology Department).

## 3. Results and discussion

We utilized VCD to investigate the supramolecular chiral organization of polyQ fibrils formed from monomers with $Q \leqslant 30$, and $Q \geqslant 35$. We also employed deep UV resonance Raman (DUVRR) spectroscopy coupled with hydrogen–deuterium exchange (H/D) to probe their secondary structure organization and evaluate the solvent accessibility of their β-sheet cores. We found that polyQ monomers of different repeat length not only aggregate at different rates [18] but also form amyloid-like structures with different morphologies as revealed by vibrational spectroscopy. VCD and IR of mature polyQ fibrils in Fig. 1 were measured using a Chiral*IR-2X* VCD spectrometer equipped with a Dual*PEM* accessory (BioTools, Inc.).

It was previously shown that IR spectra of polyQ aggregates exhibit typical vibrational modes of Q amino-acid residues (C=O at 1657 $cm^{-1}$ and $NH_2$ at 1608 $cm^{-1}$), as well as vibrational modes that originate from the amide chromophores (Amide I at 1626 $cm^{-1}$ and Amide II at 1533 $cm^{-1}$) [22–25]. While it cannot be ruled out that 1657 $cm^{-1}$ band also contains amide I contributions from other minor amounts of secondary structure, such as α-helix or polyproline II, the predominant contribution has been previously established to be side-chain Q C=O stretching. Further, all models of the structure of polyQ fibril aggregates consist of