CONFORMATIONAL DYNAMICS IN THE DISORDERED REGION OF HUMAN CPEB3 LINKED TO MEMORY CONSOLIDATION

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Abstract

Background: Current understanding of the molecular basis of memory consolidation points to an important function of amyloid formation by neuronal-specific isoforms of the cytoplasmic polyadenylation element binding (CPEB) protein family. In particular, CPEB is thought to promote memory persistence through formation of self-sustaining prion-like amyloid assemblies at synapses, mediated by its intrinsically disordered region (IDR) and leading to permanent physical alterations at the basis of memory persistence. Although the molecular mechanisms by which amyloid formation takes place in CPEB have been described in invertebrates, the way amyloid formation occurs in the human homolog CPEB3 (hCPEB3) remains unclear. Here, we characterize by NMR spectroscopy the atomic level conformation and ps-ms dynamics of the 426-residue IDR of hCPEB3, which has been associated with episodic memory in humans.

Results: We show that the 426-residue N-terminal region of hCPEB3 is a dynamic, intrinsically disordered region (IDR) which lacks stable folded structures. The first 29 residues, M1QDDLLMDKSKTQPQPQQQQRQQQQPQP29, adopt a helical + disordered motif, and residues 86–93: P83QQPPPP93, and 166–175: P166PPPAPAPQP175 form polyproline II (PPII) helices. The (VG)₅ repeat motif is completely disordered, and residues 200–250 adopt three partially populated α-helices. Residues 345–355, which comprise the nuclear localization signal (NLS), form a modestly populated α-helix which may mediate STAT5B binding. These findings allow us to suggest a model for nascent hCPEB3 structural transitions at single residue resolution, advancing that amyloid breaker residues, like proline, are a key difference between functional versus pathological amyloids.

Conclusion: Our NMR spectroscopic analysis of hCPEB3 provides insights into the first structural transitions involved in protein–protein and protein–mRNA interactions. The atomic level understanding of these structural transitions involved in hCPEB3 aggregation is a key first step toward understanding memory persistence in humans, as well as sequence features that differentiate beneficial amyloids from pathological ones.

Areas: Biophysics, Structural Biology, Biochemistry & Neurosciences.

Keywords: Memory consolidation, Intrinsically disordered proteins, NMR spectroscopy

Background

The molecular basis of long-term memory, which endures decades despite being built by ephemeral biomolecules, has long fascinated biochemists [1]. Seminal findings by Si, Lindquist, and Kandel suggested that long-term changes in synaptic efficacy require a self-perpetuating amyloid state in the Aplysia CPEB (ApCPEB)
This change in CPEB’s conformation would lead to permanent alterations at the synapse constituting a physical substrate of memory persistence. To attain the aggregated state necessary to stabilize memory, ApCPEB contains an N-terminal IDR that is very rich in glutamine (Q) residues which losses α-helix and gains coiled-coil and β-sheet structure during amyloid formation in vitro [4, 5]. The Drosophila homolog of CPEB, called Orb2, behaves in a similar fashion even though its N-terminal IDR has a lower glutamine residue content and a more tightly regulated amyloid formation [6–9]. Indeed, inhibition of Orb2 amyloid formation targeting the N-terminal IDR specifically impairs memory consolidation, but not short-term memory in Drosophila [6, 10]. Due to numerous histidine (H) residues in the Orb2 Q/H-rich amyloid core comprised in the N-terminal IDR, pH may regulate this structure’s stability as suggested by CryoEM analysis [5] and characterization by NMR spectroscopy [11]. In mammals, the N-terminal IDR of the neuronal-specific isoform of CPEB3 is crucial for amyloid formation and memory consolidation [12, 13]. The regulation of functional amyloid formation in mammalian CPEB3 appears to be even more sophisticated due to multiple mechanisms involving post-translational modifications [14] and feedback loops to maintain hCPEB3 expression levels [13]. Compared to the Aplysia and Drosophila homologs, hCPEB3’s content of glutamine residues in its 426-residues long IDR is lower, and it contains diverse segments which are enriched for certain residues such as Ser, Ala, Pro, Gly + Val, and hydrophobic residues (Table 1).

Mammalian CPEB3 travels to distinct neuronal regions to carry out multiple functions, where the 426-residue long IDR plays a key role (Fig. 1A). Following its synthesis, CPEB3 is SUMOylated, which has been reported to block CPEB3 aggregation [14]. Upon neuronal stimulation, CPEB3, which is mostly cytoplasmic, travels to the nucleus. This process is mediated by the karyopherin IPO5 through interactions with the NLS in the first RNA recognition motif (RRM) of hCPEB3 [15]. Inside the nucleus, CPEB3 interacts with STAT5B, which normally activates the transcription of genes such as EGFR, triggering signaling cascades thought to promote memory consolidation [16, 17]. CPEB3-STAT5B binding, driven by interactions between the IDR of hCPEB3 and residues 639–700 of STAT5B, downregulates STAT5B-dependent...
transcription [17]. However, the details of this interaction have not yet been addressed. By contrast, the 3D structure of the first CPEB3 RRM domain has been elucidated and revealed a β-hairpin (W471-G485) proposed to play a key role in RNA recognition [18]. This domain, as well as the second RRM domain and zinc finger (ZnF) motif, were reported to bind specifically to the 3′UTR mRNA of the AMPA receptor subunit GluR2 [19]. Together, CPEB3 and its target mRNA eventually exit the nucleus and can join distinct biomolecular condensates such as stress granules and neuronal granules, which provide physiological transport to dendritic spines, or to dendritic P-body-like granules [20], where CPEB3 stores GluR2 mRNA and downregulates its translation [19].

After synaptic activity in the hippocampus, SUMOylation of CPEB3 decreases [14] and CPEB3 converts, mediated by the IDR, from a translation repressor into a self-sustaining activator, promoting the translation
of AMPA receptors [12]. This leads to structural modifications, including a more robust actin network, which fortify the spine and permanently enhance neurotransmission at a given particular synapse [13]. The hypothesis that CPEB3 functional amyloid formation is key for memory persistence in mammals is supported by the impairment in long-term memory and long-term potentiation in the CPEB3 conditional knockout mice [12]. In our species, the causal role of human CPEB3 (hCPEB3) in memory is corroborated by observations that persons carrying a rare CPEB3 allele, which leads to a decreased production of hCPEB3 protein, have episodic memory impairments [23].

The 426-residue long IDR of hCPEB3 plays a key role mediating memory persistence through this prion-like mechanism. It contains an amyloid-forming region spanning residues 1–200 and a condensate-promoting region formed by residues 250–426 which are linked by an alanine rich segment [24]. The full IDR is followed by two folded RRM which bind RNA and finally a ZZ-type ZnF domain (Fig. 1B). Recent sequence and deletion mutational analyses of the IDR have begun to identify subregions key for aggregation, such as the first 30 residues [13]. However, for the hCPEB3 IDR, programs to predict secondary structure tendencies give different outputs, Alpha Fold 2 structural predictions [25] are marked as low to very low confidence (see https://alphafold.ebi.ac.uk/entry/Q8NE35), and, to date, no high-resolution experimental data on the partial structures or motions have been reported. Here, motivated by the key roles of the IDR in CPEB prion-like aggregation required for memory persistence, we characterize the atomic level conformations and dynamics of the complete IDR of hCPEB3 by NMR spectroscopy.

**Results**

**hCPEB3’s IDR is chiefly disordered**

As a first step to experimentally characterize hCPEB3’s IDR, we probed the complete 426-residue IDR of hCPEB3 by biophysical techniques and homonuclear NMR. Its fluorescence emission spectra, recorded at temperatures ranging from 2 to 70 °C, show emission maximum > 350 nm. This is consistent with its six Trp residues being solvent exposed and not buried in the hydrophobic core of a folded domain (Additional File 1. Fig. S1A) [26]. The far UV CD spectra of the hCPEB3 IDR also shows the hallmarks of a disordered protein, namely a minimum near 200 nm [27]. No spectral features indicative of α-helix and β-sheet; namely, minima at 208, 218, or 222 nm and no maximum at 195 nm, are evident (Additional File 1. Fig. S1B). The 1D 1H and 2D 1H-1H NOESY spectra show 1H signals clustered into narrow bands near the values observed for short, unstructured peptides (Additional File 1. Fig. S1C) [28, 29]. The sequence alignment of several representative vertebrate CPEB3 proteins using the T-Coffee program is shown in Additional File 1. Fig. S2. Very similar results were obtained from the Omega Clustal program (not shown). Whereas most IDPs show poor levels of sequence conservation, some stretches rich in hydrophobic residues, such residues M1-T12, W111-F139, and Y341-I357, are highly conserved. By contrast, glutamine rich, alanine rich, and some proline rich segments are present only in mammals. Taking all these data together, the presence of large, stably folded domains in the IDR can be ruled out, but short segments with partly populated secondary structures could still be present.

**Atomic level characterization reveals partially structured elements in hCPEB’s N-terminal “disordered” region**

To discover and characterize possible segments with partial secondary structure, we applied multidimensional heteronuclear NMR. As the full length IDR is too long to characterize by this methodology, we have followed the “divide and conquer” approach implemented by Zweckstetter et al. to characterize tau, a similarly sized IDP implicated in Alzheimer’s disease and other tauopathies [30]. As described in the “Methods” section, and shown in Table 1, eight overlapping segments of 100 residues were characterized.

Using our powerful 13CO, 15 N, 1HN-based assignment strategy, over 99% of the main chain 13CO, 13Ca, 15 N, 1HN, and the 13CB resonances were assigned for residues 1–450 of hCPEB3. The chemical shifts of the complete IDR of hCPEB3 are reported in the BMRB (entry number 50256), and the original 2D and 3D spectral data have been deposited in the Mendeley data repository. The assigned 2D 1H-15 N HSQC and 2D 13CO15N spectra of segment 4 are shown in Additional File 1. Fig. S3 and Additional File 1. Fig. S4, respectively. The 2D 1H-15 N HSQC spectra of segments 1, 3, 4, 5, 6, 7, and 8 are shown in Additional File 1. Fig. S5. The similar positions of most 1H-15 N signals in neighboring segments additionally suggest a sparsity of long range interactions under these conditions. Likewise, the majority of the crosspeaks of the same residues in adjacent segments also overlap or are close together in the 2D 13CO15N spectra of segments 1, 3, 4, 5, 6, and 8 (Additional File 1. Fig. S6).

Multiple attempts to express and purify hCPEB3 segment 2, which spans residues 51–150, by recombinant methods were unsuccessful. Nevertheless, all the residues within segment 2 are present and have been characterized structurally in the context of segments 1 and 3. To test if there might be some structure in the neighborhood
of residues 91–110 located in the middle of segment 2 and the C- and N-termini of segments 1 and 3, respectively, we studied the conformation of a twenty-residue peptide corresponding to this region by NMR spectroscopy. No significant trends towards structure formation were detected (Additional File 1. Fig. S7).
The $^{13}\text{C\alpha}$ and $^{13}\text{CO}$ conformational chemical shifts ($\Delta\delta$) of the hCPEB3 segments 1 and 3–8 are plotted in Additional File 1, Fig. S8. These data show five segments, comprising residues 1–10, 202–210, 222–234, 238–246, and 346–356, with significantly high $\Delta\delta^{13}\text{C\alpha}$ and $\Delta\delta^{13}\text{CO}$ values. Such values are characteristic of partially populated $\alpha$-helices and are examined in detail in the following paragraphs.

The first residues of hCPEB3 adopt a partly populated $\alpha$-helix which precedes the Q-rich stretch
The conformational chemical shifts point to the formation of partly populated (20%) $\alpha$-helix in the first ten residues of the protein (Fig. 2A, B). Increased conformational chemical shifts are observed at 5 °C, reflecting a higher amount of helical structure upon cooling. Standard $^1\text{H}$-detected $^{15}\text{N}$ relaxation measurements detect that these residues are the most rigid part of segment 1 (Fig. 2C, D). These results are corroborated by $^{13}\text{C}$-detected $^{15}\text{N}$ relaxation experiments (Additional File 1 Fig. S9).

The $\alpha$-helix detected for these first residues extends N-terminally into the His/Tev tag. To rule out a possible structure-promoting effect on segment 1, we tried to remove it by proteolytic cleavage with the TEV protease. Multiple attempts failed, which suggests that the helix spanning the last residues of His/Tev tag and the first residues of the hCPEB3 IDR is present and impedes the proteolytic cleavage. Therefore, we characterized a dodemeron peptide whose sequence corresponds to the first twelve residues, $\text{M}_{1}\text{QDDLLMDKSKT}_{12}$, of the hCPEB3 IDR. The observation of a series of weak $^1\text{HN}_{i}-^1\text{HN}_{i+1}$ nuclear Overhauser enhancement (NOE) crosspeaks reveals that this peptide has a slight tendency to form $\alpha$-helix in aqueous buffer (Additional File 1 Fig. S10A).

Fluorinated alcohols like trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP) are known to increase the population of helical conformations in peptides which contain numerous aliphatic and aromatic residues; namely $+0.6$ ppm, $-1.0$, and $-0.3$ for $^{13}\text{C\alpha}$, $^{13}\text{C\beta}$, and $^{13}\text{CO}$, respectively (Table 2, Additional File 1 Fig. S11). Not observed in isolated proline residues, we advance that they are hallmarks of a PPII helical conformation.

Residues 101–200 of hCPEB3 contains a rigid nonpolar segment and a PPII helix
Regarding residues 101–200, no strong trends to adopt $\alpha$-helical or $\beta$-structures are detected. Nevertheless, the stretch composed of residues, $\text{W}_{111}\text{STGTTTNAVEDSFFQG1TPVNGTHMLFQNF}_{139}$ which contain numerous aliphatic and aromatic residues, shows relatively high rigidity, both on fast ns/ps as well as slower µs/ms timescales (Additional File 1 Fig. S12). This finding is interesting considering that this relatively hydrophobic segment also appears to be essential for hCPEB3 amyloid formation in vitro [24], and very recently, it has been reported to form amyloid in mouse CPEB3 [37]. In addition, the stretch of residues 161–190 $\text{Q}_{166}\text{HHQPPPPPA}_{170}\text{PAPQPAQPAQ}_{180}\text{PQAQPQQPQ}_{190}$ has a very high $Q/P$ content, and Pro and Gln are the residues with the highest intrinsic tendencies to adopt PPII helices [38]. The consecutive proline residues, $\text{P}_{166}\text{PPPAPAPQ}_{179}$ also display the characteristic PPII pattern of conformational chemical shifts (Additional File 1 Fig. S11 ABC) seen for residues 86–93. Although more weakly than long stretches of pure polyproline [39, 40], a synthetic peptide corresponding to residues P166-P175 of hCPEB3 binds to human Profilin 1, a known mediator of interactions with actin (Additional File 1 Fig. S11 D).

Residues 201–300 contain three $\alpha$-helical segments and a disordered (VG) segment
Significant $^{13}\text{C\alpha}$ and $^{13}\text{CO}$ chemical shift deviations with respect to values predicted for a statistical coil,
for three residue segments spanning A202QRSAAAY210 GHQPMTSKP220-S221SSAVAAAA230AAAAA) SSASS240SWNTHQSVHAA250 (Fig. 3A). These results indicate that the three segments of underlined residues adopt partially populated α-helices. Based on the magnitude of the conformational chemical shifts, the helical populations are different, being about 30% for the A202-Y210 α-helix, 80% for the S223-A234 α-helix and 20% for the A238-Q246 α-helix at 5 °C; whereas these populations decrease at 25 °C to approximately 10%, 40%, and 15%, respectively, they are still significant (Fig. 3B). The presence of the first and second helices are confirmed by 1HN-1Hα coupling constants (Additional File 1 Fig. S13). Moreover, analysis with TALOS+, which predicts secondary structure taking into account 13Cβ, 15N and 1Hα chemical shifts in addition to 13Cα and 13CO, confirms the presence of these three helical segments and structural calculations with CYANA suggest that the three helices do not tend to adopt a preferred alignment relative to each other (data not shown). The helices are not especially rigid on fast ps-ns time scales (Fig. 3C) or the slower µs-ms time domain (Fig. 3D) at 25 °C but do show a heightened stiffness at 5 °C (Additional File 1. Fig. S12). Helical wheel projections (Additional File 1. Fig. S14) suggest that different interactions contribute stability to these α-helices. Gly 211 and His 212 are positioned to stabilize the A202-Y210 α-helix by a C-capping motif [41]. Whereas Ala has a very high intrinsic helix forming propensity, the propensity of Ser is low [42]. In this segment, however, the Ser residues are positioned at the N-terminus of the α-helices, where adding negative charge via phosphorylation would increase the helical population, considering the well-known stabilizing effects of charge/macro dipole interactions and N-capping H-bonds [43]. This proposal is supported by NMR spectroscopic characterization of a peptide EAVAAAAAAAKK, with a phosphomimetic N-terminal Glu residue, which reveals a modest increase in helicity as the pH is raised from three, where the Glu is mostly neutral to five, where the Glu is chiefly anionic (Additional File 1. Fig. S15). Although this sequence’s insolubility thwarts attempts to more directly test the impact of phosphorylation, we note that these Ser residues are placed at the positions where phosphorylation is expected to increase α-helix stability the most [44]. Moreover, at neutral pH, where phosphoserine carries two negative charges, the stabilization is substantially greater than at pH 4, where it carries one [44]. The last α-helix, A238-Q246, is less populated, but its stability might increase if W242 were to engage in long-range interactions, such as with the hydrophobic or cationic residues of the first α-helix, i.e. MQDDELMDKSKT. To test this possibility, we studied two polypeptides containing the M1-T12 and A238-Q236 helical segments with and without an N-terminal Dansyl group, connected by a flexible (Gly), linker. The results of FRET and 2D NMR spectroscopy evince that this polypeptide adopts a conformational ensemble significantly more compact than a

| α-Helix | β-Strand | Gly-rich PPII helical bundle | Isolated Pro-rich PPII helix |
|---------|---------|-----------------------------|-----------------------------|
| δΔ 13Ca | 3.1b/2.9b | −1.5b/−1.8a | −0.6d | +0.6d |
| δΔ 13Cβ | −0.4b/−0.8b | 2.2b/2.7b | +0.3d | −1.0d |
| δΔ 13CO | 2.2b/2.2b | −2.1b/−2.1b | −0.2d | −0.3d |

* From Spera and Bax (1991) [34]
* From Wang and Jardetzky (2002) [35] for alanine
* Calculated from Wishart and Skyes (1994) [36]
* From Treviño et al. (2018) [33]
* This study
Fig. 3 (See legend on previous page.)
statistical coil but that the α-helix contents are not significantly altered (Additional File 1, Fig. S16).

One of the most striking features in hCPEB3’s sequence is a short dipeptide protein motif (Val-Gly)$_5$ spanning residues 271–281, which is reminiscent of longer (Ala-Gly)$_n$ and (Pro-Gly)$_n$ and (Arg-Gly)$_n$ dipeptide repeat proteins encoded by mutant C9orf72 which have been implicated in ALS [45, 46]. Our in silico analysis identified this segment as having a high potential to form amyloid [24]. In the context of hCPEB3, however, this segment is among the most disordered and flexible of all the zones of the IDR (Additional File 1, Fig. S8, S12). Just beyond the (VG)$_3$ segment, there is a stretch of 15 residues, S$_{284}$PLNPISSLKPFS$_{298}$, whose NMR parameters indicate disorder and flexibility (Additional File 1, Fig. S8, S12). Nevertheless, this stretch contains four Ser residues reported to be phosphorylated by protein kinase A (PKA) or calcium/calmodulin-dependent protein kinase II [47] (Table 1) and therefore might be important for the transition between short- and long-term memory. Residues P$_{303}$PKFRAAP$_{311}$ are proline rich. Predictions suggest that Arg 308 can be methylated (Table 1). This modification, whose impact has not been probed here, was reported to fortify cation–π interactions, reduce interactions with RNA, and destabilize condensates in other proteins [48].

The residues forming the Nuclear Export Signal (NES) show a marked tendency to adopt α-helical structures

Significant conformational chemical shifts were also observed for residues L349-L353 which form the NES (Fig. 4) indicating the presence of helical structure. Using the $^{13}$C$_O$, $^{15}$N, $^1$HN, $^{13}$Ca, and $^{13}$CB chemical shift data as input, a family of conformers was calculated using the programs TALOS+ and CYANA for residues P333-P363. This 31-residue segment is rich in aromatic (five) and aliphatic (six) residues, which is unusual for a disordered polypeptide. The resulting structures reveal that residues L346-L349 adopt one turn of α-helix and residues S352-M356 form a short α-helix (Fig. 4A). It is notable that this conformer positions five nonpolar residues: L346, L349, L353, M354, and I357 on the same face of the α-helices. Y341, the putative phosphorylation site, is in an extended portion of the backbone and would be accessible for this post-translational modification (PTM). Whereas the conformational ensemble will contain many other structures, based on conformational chemical shifts as illustrated by the $\Delta \delta^{13}$Ca and $\Delta \delta^{13}$CO values shown in Fig. 4B, the α-helical population is about one third. The presence of rigid conformers is corroborated by relatively high $^1$H–$^{15}$N NOE ratios (Fig. 4C) and elevated transverse relaxation ratios (Fig. 4D).

Beyond the NES α-helix, no segments with preferred secondary structure are detected. The last residues of the segment 8 construct S$_{426}$-RKVFVGLLPPDIDEDEITASFRRF$_{450}$ belong to the RRM1 domain. According to the 3D structure [18], residues K428–G432 adopt a β-strand and residues E440–R449 form an α-helix in the context of the complete RRM1 domain. Here, these segments appear to be largely disordered. After a proline-rich zone ending around residue 380, the next fifty residues have a higher content of nonpolar residues and tend to be more rigid (Additional File 1, Fig S12). Residues 400–412 SHGDQALSSGLSS contain five Ser residues reported to be phosphorylated [47] (Table 1).

Discussion

Like its homologs in Aplysia and Drosophila, hCPEB3 resembles the diverse superfAMILY of RNA-binding proteins that contain RRM and/or ZnF domains as well as intrinsically disordered prion-like regions, such as fused in sarcoma (FUS) or transactive response DNA-binding protein of 43 kDa (TDP-43). FUS and TDP-43 are essential proteins, but their anomalous aggregation has been implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Thus, comparing the CPEB3 and TDP-43 IDR conformational tendencies and dynamics may reveal why the latter can become pathological. The biophysical analysis of the full length hCPEB3 IDR shows that it lacks stable secondary structure. Disordered regions tend to change their sequence much more rapidly over the course of evolution due to a lack of structural constraints [49]. The strong conservation in vertebrate CPEB3 of the N-terminal α-helix and the 350’s (NES) α-helix and the nearby putative phosphoTyr site suggests physiological importance (Additional File 1, Fig. S2). The latter’s hypothetical binding to the SH2 domain of STAT5B might occlude the NES, leading to nuclear retention. Additional segments, like the hydrophobic stretch key for amyloid formation [24] and a cluster of three Trp residues (W242, W252 and W259), are also conserved from mammals to fish. In contrast, the N-terminal Gln-rich segment, the Pro-rich “breaker” regions and the Ala-rich helices are well conserved in mammals but not across all vertebrates. In some lower vertebrates, there is an alternative Q-rich region positioned after the 100’s hydrophobic segment. These elements’ rapid evolution could be related to the development of the mammalian brain. By contrast, the ability to move the poly-Q segment or substitute it for a hydrophobic amyloidogenic segment highlights the cassette or modular nature of PLDs, which was previously established for the Drosophila CPEB homolog [5].
PPII helices may regulate amyloid formation

Whereas hCPEB3 and its homologs Orb2 and ApCPEB have α-helix destabilizing residues between the N-terminal α-helix and the amyloidogenic Q-rich segment, such as proline in hCPEB3 and Orb2 or serine and valine in the case of ApCPEB, no such α-helix busters are present in the analogous segments of huntingtin, the androgen receptor, or TDP-43 (Table 3). This allows the poly-Q segment to interact with the α-helix through the formation of sidechain to backbone hydrogen bonds in the androgen receptor [50] and to form part of the amyloid structure as proposed for TDP-43 [51] and found in ALS/FTLD patient brains [52]. The presence of α-helix breakers between the α-helix and the poly-Q(/N) segments in functional amyloids and their absence in pathological amyloids may be a fundamental difference behind their radically different toxicities.

PPII helices, well known in collagen and certain glycine-rich proteins [53], also play key roles in mediating protein–protein interactions in biomolecular condensates [54]. Whereas conformational chemical shifts have proven to be extremely useful tools to identify α-helices and β-strands in folded and disordered proteins, conformational chemical shifts for PPII helices were less known. The first 450 residues of hCPEB3 include no less than 66 proline residues (15% of the total). We hypothesize that

![Fig. 4](image-url)

Fig. 4 Conformation of the NES and nearby putative phosphoTyr site. A (Top) Residues L346-L349 and S352-M356 adopt two short, partially populated α-helices. (Bottom) Representative conformer is shown with cationic residues (R and K) colored blue, aromatics (F, Y, and H) = purple, anionic (E and D) = red, aliphatic (I, L, M) = dark gray, amyloidogenic (N and Q) = magenta, hydroxyl bearing (S and T) = cyan, and proline = green. Spiral ribbons mark the helical segments spanning residues 346–349 and 352–356. B Conformational chemical shifts of $^{13}C\alpha$ (blue bars) and $^{13}CO$ (black narrow bars) afford detection of helical conformations. Uncertainties in the conformational chemical shifts (Δδ) are 0.02 and 0.10 ppm for $^{13}CO$ and $^{13}C\alpha$, respectively. C $[^1H]^{15}N$ NOE ratios of 0.85 and −0.20 are indicative of high rigidity and flexibility, respectively, on ps-ns time scales. D Higher $R_1\rho$ rates are diagnostic of rigidity on μs-ms time scales.
segments such as G_{162}PPPAPAPQ_{175} may bind profilin, which has specific domains to interact with proline-rich polypeptides [39] and to mediate interactions with actin, whose levels rise at the synapse following CPEB3 aggregation [13]. On the basis of the thorough set of chemical shifts obtained here, including Pro $^{15}$N assignments which are rare in the literature, we propose a pattern of conformational chemical shifts that define PPII helices (Table 2). With standards for glycine-rich PPII helical bundles [33], these values should aid the detection of PPII helices in biomolecular condensates and the elucidation of their roles in physiology and pathology.

Towards an atomic-level description of the first steps in hCPEB3 IDR self-association

Based on the NMR results, we suggest a speculative working hypothesis for hCPEB3 conformational changes (Fig. 5). Initially, the first 200 residues of hCPEB3, which are necessary and sufficient for aggregation and amyloid formation in vitro [12, 24], are mostly disordered except for the relatively stable α-helices formed by residues 222–234 and modestly populated α-helices at the N-terminus and spanning residues 202–212 and 237–245 (Fig. 5A). The Q$_{RQ_4}$ motif and hydrophobic segment F$_{123}$FQGIT-PVNGT-MLFQNF$_{139}$ are initially disordered and premature amyloidogenesis is discouraged by SUMOylation [14] and proline breaker motifs. Association among the α-helices into more compact ensembles, as suggested by FRET results (Additional File 1. Fig. S16), would become possible and might be strengthened by hydrophobic interactions between Met 7 and Trp 242 or cation-π interactions between Lys 11 and Trp 242 (Fig. 5B), analogous to the long range contacts seen in a TDP-43 amyloid [55]. The increased production of hCPEB3 upon neuronal stimulation [12] as well as the proximity of the serine and alanine rich α-helix (residues 222–234) may promote α-helix formation by the QQQQRQQQQ motif. These could then associate to form a coiled-coil, as has been demonstrated in model polypeptides [56–58]. Moreover, completely helical polyalanine peptides, some of which are linked to polyalanine expansion diseases, were reported to promote coiled-coil mediated aggregation, and no conversion into β-sheet structures was observed [57, 59]. Although future studies are necessary to provide additional evidence and test plausible hybrid aggregation mechanisms with several elements of structure acting in parallel, this model is supported by analogous results on ApCPEB [4, 60] and polyalanine expansions [59], which led to the proposal of similar mechanisms.

These events would reinforce intermolecular contacts within the dendritic P-body like granule [20] leading to gelification and, eventually, amyloid formation (Fig. 5C). The final amyloid structure could be comprised of the Q$_{RQ_4}$ motif and the hydrophobic segment as evidenced by hCPEB3 fibril formation kinetics [24]. A construct spanning residues 1–200 of hCPEB3, which contains the Q$_{RQ_4}$ motif, plays a role in hCPEB3 amyloid formation as indirectly inferred by the anti-amyloid action of QBP1 [24]. For the hydrophobic segment, its role in mouse CPEB3 amyloidogenesis has recently been corroborated [37]. In addition, the poly-A segment of helices 202–212 and 222–234 may also transform into amyloid (Fig. 5C). Such α-helix to amyloid conformational transformations have been previously described in alanine-rich polypeptides as diverse as a fish antifreeze protein [61] and a synthetic (Ala)$_{10}$-(His)$_{6}$ hexadecapeptide [62] and could form pathological amyloids in several diseases involving polyA expansions [63].

Residues 217–284 of CPEB3 have been reported to be key for interactions with actin [13]. Considering this, the hypothetical associations among the α-helices formed by residues M$_{1}$–T$_{12}$ and residues A$_{202}$–Q$_{246}$ could dispose the PPII helices formed by residues P$_{166}$–Q$_{175}$ and P$_{166}$–P$_{175}$ (Additional File 1. Fig. S10) to bind profilin. This protein contains a second binding site specific for actin and

| PROTEIN      | SEQUENCE* |
|--------------|-----------|
| Huntington   | M$_{305}$ATLEKLMKA FESLKSFOQQ 0000000000 00000000PP PPPPPPPPQP LQDPPQ0ADP |
| ANDROGEN RCPT| H$_{404}$EAASAAAPGASLLLL000 000000000Q 000000000Q ETSF000000GQEDGSAQAH |
| TDP-43       | P$_{359}$AMMAAAQAALQSWQ50MGN LASQSQNSGQ SGANNQOCMNQIVNAQFGS FNNSYSSSNI |
| Ataxin-7     | A$_{365}$AAAACCCAAA AAR00QQQ0QQ0QQQ0QQQ HPPRRTRRTEDGEN GAASSTAAAAM |
| hCPEB3       | M$_{305}$QLDDLMKDS KITQ0QQQ0QQ OR0QQQ0QFE SSVEASEST EQSETKTEE NSALBLSL |
| Drosophila 0rb2A | M$_{305}$YNKENFVNIC GGMLNLNNK RP14LH0000Q 00Q0QD0QD 0QQQ0QHHGGQ LSNLNALH |
| Apysia CPEB  | F$_{358}$IEERRTAL LDLSLEAMNV SCSST5000 0000000000 00000000MLQ DVOOOQALLQ |

* In these sequences, nonpolar residues are shaded gray, Gln and Asn residues are shaded magenta, and prolines are shaded green.
promotes actin filament network formation [64]. Such actin networks are known to become more robust as a dendritic bud strengthens during memory consolidation [65].

Conclusions
In summary, by detecting several partly populated α-helices, PPII-helices, and hydrophobic segments in the hCPEB3 IDR, these NMR spectroscopic results provide clues for comprehending the first structural transitions involved in protein/protein and protein/RNA interactions which may be key for memory consolidation as well as motifs that discriminate functional versus pathological amyloids.

Methods
Materials
$^{15}$NH$_4$Cl and $^{13}$C-glucose were purchased Tracetec (Madrid, Spain); D$_2$O was a product of Euroisotop. Deuterated acetic acid was from Sigma/Aldrich, and 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as the internal chemical shift reference, was from Stolher Isotopes Chemical Company.

A twelve residue peptide, called hCPEBpep1, whose sequence corresponds to the protein's first 12 residues (M7QDDLLMDKSKT12) and a twenty residue peptide, called hCPEBpep2 with the sequence, P$_{91}$PPQEPAPGASLSPFSGT$_{110}$ in hCPEB3 were purchased from Genscript. hCPEBpep2’s sequence overlaps with the C-terminus of Segment 1 and the N-terminus of Segment 3. A proline-rich peptide acGPPPPAPAPQ-Pam, with acetylated and amidated termini and whose sequence covers residues 166–175 in hCPEB3, two poly-peptides with the sequence MQDDLLMDKSKTGGAGSSWNTHQ (with and without an N-terminal Dansyl group) were also obtained from Genscript. Finally, another peptide, acEAVAAAAAKKam, which corresponds to residues 224–234 but with S224 substituted by E as a phosphomimetic residue and A234 and A235 substituted by K for solubility, was obtained from the Proteome Service of the Centro Nacional de Biotecnologia, CSIC. All the peptides were over 95% pure, as assessed by HPLC, and their identities were confirmed by mass spectrometry and NMR spectroscopy. Recombinant Human Profilin 1, produced recombinantly in E. coli, was obtained from Abcam (reference number ab87760). It is over 95% pure as judged by SDS PAGE.

Sample production
Coding mRNAs of hCPEB3 vary in length due to an embedded human delta virus-like ribozyme which slowly splices out introns, leading to the generation of multiple isoforms [66]. It is also noteworthy that Orb2A’s pre-mRNA contains an intron with multiple stop codons which is only spliced out when certain “memorable” stimuli are experienced [9]. Here, the hCPEB3 isoform 2, Uniprot Q8NE35-2/Genbank CAI14105.1, is studied.

Plasmid construction, protein expression, and purification
The hCPEB3 IDR, which corresponds to the first 426 residues of the protein whose sequence is shown in Table 1,
was expressed at eight highly overlapping one-hundred residue segments. To control for end effects and to check the reproducibility, each segment overlaps by 50 residues with the preceding and successive segments.

Each segment was cloned into the pET-28a(+) plasmid by PCR using the full length human CPEB3-2 in plL3.7 plasmid as the template kindly provided by Dr. Yi-Shuian Huang [67]. The DNA amplified fragments were digested with Xhol and Nhel. Expression of the resulting clones led to fusion proteins containing a His6 tag and a TEV Nla protease cleavage site. Thus, each segment studied had the sequence MGSSHHHHHHHHSSGLVPRGSHMASENLYFQ, at its N-terminus.

All overlapping segments were expressed in the E. coli BL21 Star (DE3) strain using the T7 expression system (Novagene). 15C/15N isotopic labeling of each segment was done by using a previously published protocol [68]. Briefly, cells were grown in 1 L of LB at 37 °C by shaking at 280 rpm upon reaching optical cell densities at 595 nm (OD595) ~ 0.6–0.7. Cells were pelleted by a 30 min centrifugation at 5000 × g and washed using a M9 salt solution (15.0 g/L KH2PO4, 34.0 g/L Na2HPO4 and 2.5 g/L NaCl for 1 L of 5 × M9 salts) excluding nitrogen and carbon sources. Cell pellets were resuspended in 250 mL of isotonically labeled minimal media M9 salt solution supplemented with 15C D-glucose 4.0 g/L and 15NH4Cl 1.0 g/L (Cambridge Isotope Laboratories, Inc.) and then incubated to allow the recovery of growth and clearance of unlabeled metabolites. Protein expression was induced after 1 h by addition of IPTG to a concentration of 1 mM. After a 4.5 h incubation period, the cells were harvested.

Cell pellets were lysed with buffer with the following composition: 50 mM NaH2PO4/Na2HPO4, 500 mM NaCl, 50 mM imidazole, 6 M guanidinium chloride (GdmCl), pH 7.4, and then sonicated. Each recombinant segment was purified by Ni2+-affinity chromatography using HisTrap HP purification columns with a FPLC system (ÄKTA Purifier, GE Healthcare) with elution buffer consisting of 50 mM NaH2PO4/Na2HPO4, 500 mM NaCl, 500 mM imidazole, 6 M GdmCl, and pH 7.4.

If necessary, the pure segments were then incubated with TEV Nla protease O/N at 4 °C [69]. The cleaved protein was then subjected to dialysis and recovered. Protein samples were stored at −80 °C until use. Eventually, they were desalted to 1 mM DAc, pH4.0 by gel filtration chromatography using a PD-10 column (GE Healthcare), and concentrated to a final protein concentration of 1.0–1.5 mM in 250 μL using a Vivaspin microfiltration device and placed in a 5-mm Shigemi reduced volume NMR tube for measurements. These low pH, low ionic strength conditions have been reported [70] and corroborated [71] to maximize solubility by increasing charge-charge repulsion between protein molecules. Intrinsically disordered proteins with large net positive [72] and negative charges [73] have been reported to show highly mobile main chains, either alone or in complex. This strongly suggests that the different net charge on hCPEB3 at pH 4 versus pH 7 is unlikely to significantly impact the local chain dynamics. α-helix stability depends on pH due to charge–charge interactions, charge–helix macrodipole interactions, and intrinsic helix propensities [43]. A consideration of these factors suggests that the effect of increasing the pH from 4 to 7 would be mildly stabilizing for the first α-helix and insignificant for the four remaining α-helices detected in the hCPEB3 PLD.

Non-labeled full length hCPEB3-IDD expression and purification was carried out essentially as described in [24]. Briefly, cells were grown in 1 L of LB medium at 37 °C until reaching an OD595 = 0.6–0.7, and protein expression was induced for 4 h by adding IPTG at 1 mM final concentration. Cells were harvested and following sonication, then lysed with buffer 50 mM Na2HPO4, 500 mM NaCl, 50 mM imidazole, 6 M GdmCl, and pH 7.4. After centrifugation at 18,000 rpm for 45 min, supernatants were purified with Ni2+-affinity chromatography, and elution was performed in buffer containing 50 mM NaPO4, 500 mM NaCl, 500 mM imidazole, 3 M GdmCl, and pH 7.4. Purified CPEB3-IDD was diluted to PBS, 1 M GdmCl pH 7.4, dialyzed against PBS pH 7.4 at 4 °C, and finally concentrated by using Amicon Ultra-15 centrifugal filters with a 10-kDa cutoff.

Sequence alignment
The conservation of vertebrate CPEB3 protein sequences was assessed using the programs T-coffee [74] and Clustal Omega [75] using the default settings. The sequences chosen as representative are human isoform 1 NP_001171608.1, human isoform 2 CAI14105.1, mouse NP_001277755.1, chicken XP_105144323.1, turtle (Chrysemys picta bellii) XP_005301348.1, frog NP_0001015925.1 (Xenopus tropicalis), and fish (Danio rerio) XP_009305819.1.

Fluorescence and circular dichroism spectroscopies
Fluorescence spectra on the full length, unlabeled hCPEB3-IDD at ca. 5 μM in 1.0 mM deuterated acetatic acid at pH 4.0 were recorded on a Horiba FluorMax 4 instrument equipped with a Peltier temperature control device using a 0.2 s-nm−1 scan speed and three nm excitation and emission slit widths. The excitation wavelength was 280 nm, and the emission was scanned over 300–400 nm. A series of spectra were recorded at 2, 10, 20, 30, 40, 50, 60, and 70 °C.
To test for binding of a segment of hCPEB3 rich in proline residues to Profilin, fluorescence spectra were recorded at 20 °C on 10 μM human Profilin 1 using 2-nm excitation and emission slits, a 2-nm s⁻¹ scan speed, and an excitation wavelength of 295 nm, and emission was recorded from 300 to 400 nm in the absence and presence of 4.5 mM of the peptide acGPPPPAPAPQ[pnm, which corresponds to residues P166-Q175 of hCPEB3, with an N-terminal acetyl and glycine residue and a C-terminal amide group added to avoid end charges. This assay is based on blue shift and enhanced emission of Trp3 and Trp31 of human Profilin 1 as the environment surrounding their indole moieties becomes less solven exposed upon polyproline ligand binding [39].

Fluorescence spectra on Dansyl-MQDDLLMDKSK-TGGGGASSWNTHQ and the control polypeptide without Dansy, MQDDLLMDKSKTGGGGASSWNTHQ, were recorded using an excitation wavelength of 295 nm, which is selective for the donor Trp, and scanning the emission over 300–580 nm, using a 2 nm s⁻¹ scan speed and slit widths of 2 nm. The final concentration of both polypeptides was matched at 10.0 μM. Spectra were recorded in 100 mM KCl, 20 mM KH₂PO₄/KH₂PO₄ (pH7) with or without the denaturant GdmCl present at a final concentration of 7.4 M. Assuming randomized orientations of the donor and acceptor groups and a Förster distance (\(R_0\)) of 23.6 angstroms for the Dansyl/Trp pair, their average distance, \(<r>\), can be estimated using the equation \(<r> = \frac{\sqrt{6}}{\sqrt{E}} (R_0^6 - E R_0^6)/E\), where \(E\) is the fraction of energy transferred from donor to acceptor [76].

A Jasco 810 spectropolarimeter fitted with a Peltier temperature control unit was used to record far UV-CD spectra at 5 and 35 °C on the complete hCPEP3 IDD at ca. 5 μM in 1 mM deuterated acetic acid (pH4.0) using a 1.2-nm bandwidth scanning from 260 to 190 nm in a 0.1-cm quartz cuvette at 50 nm min⁻¹. Eight scans were recorded and averaged for each spectrum.

**NMR spectroscopy: instrumentation**

All spectra for the hCPEB3 segments were recorded on a Bruker 800 MHz (¹H) Avance spectrometer fitted with a triple resonance TCI cryoprobe and Z-gradients. The ¹H chemical shift was referenced to 50 µM DSS measured in the same buffer and at the same temperatures as those used for the hCPEB3 segments. Since DSS can sometimes bind to intrinsically disordered proteins [77], the DSS signal was recorded in an independent reference tube. The ¹³C and ¹⁵N chemical shift references values were calculated by multiplying by their respective gyromagnetic ratios with ¹H; that is \(\Xi = \frac{13\text{C}}{1\text{H}} = 0.251449530\) and \(\Xi = \frac{15\text{N}}{1\text{H}} = 0.101329118\) [78]. NMR spectra were recorded and transformed using TOPSPIN (versions 2.1) (Bruker Biospin).

**¹³C-detection assignment strategy**

Among the NMR approaches for studying disordered proteins (reviewed by [79]), ¹³C-detection has been gaining in popularity as it affords the characterization of proline residues [80, 81] and offers superior signal dispersion [82]. Here, to speed and improve the assignment of the backbone, we used a “proton-less” NMR approach for segments 1, 4, 5, 6, and 8 based on 2D CON spectra in which successive ¹⁵N-¹³CO nuclei correlations are obtained in two 3D spectra called hacacoNcaNCO and hacaCOncaNCO [83]. For segments 7 and 8, which tend to form condensates [24] and seem to be more rigid, this strategy afforded less intense spectra and in particular about 35 CON crosspeaks were missing. Therefore, an additional strategy based on ¹⁵CO connectivities from 3D HNCO and HNcaCO spectra as well as ¹³HN and ¹⁵N connectivities of consecutive residues from 3D HncoCaHN and hNccaNH spectra [84, 85] was utilized to check and complete the backbone assignments. The success of this approach seems to be due to a very slow equilibrium between aggregated protein molecules and those remaining in solution, which are detectable by NMR. The latter strategy was also employed for segment 3, which was less soluble. For all segments, further corroboration was obtained by conventional 2D ¹H-¹⁵N HSQC and 3D HNCO spectra as well as 3D CCON to confirm the residue identity and obtain the chemical shift values of ¹³C nuclei of the side chains.

Of the eight segments, only segment 2 failed to yield a soluble sample. Whereas the sequence assignments are complete thanks to the analysis of segments 1 and 3, to test for possible end effects, a 20 residue peptide, hCPEB-ep2, corresponding to residues 91–110 of the hCPEB3 sequence was assigned and characterized structurally by 2D ¹H-¹H COSY, ¹H-¹H TOSCY, ¹H-¹H NOESY, and 2D ¹H-¹³C HSQC NMR spectra at 5.0 °C on a Bruker 600 MHz spectrometer fitted with a cryoprobe and Z-gradients. A 2D ¹H-¹⁵N HSQC was also recorded on the 800-MHz Bruker spectrometer. Both the ¹H-¹⁵N HSQC and the ¹H-¹³C HSQC spectra were recorded at the natural abundance of ¹⁵N and ¹³C. The program NMRFAM-Sparky [86] was used to facilitate manual spectral assignment. The NMR spectral parameters are summarized in Additional File 1. Table S1.

Theoretical chemical shift values (\(\delta_{	ext{calc}}\)) for statistical coil ensembles were calculated using the parameters tabulated by [87] and [88], as implemented on the server at the Bax laboratory. These values were used to calculate conformational chemical shifts (\(\Delta\delta\)), as the experimentally measured chemical shift (\(\delta_{	ext{exp}}\)) minus
the calculated chemical shift ($\delta_{\text{calc}}$). Segments of five or more residues with $\Delta\delta^{13}\text{C}_\alpha > 0.3$ ppm and $\Delta\delta^{13}\text{CO} > 0.3$ were considered to have a significant preference to form an $\alpha$-helical segment. When appropriate, families of representative preferred conformers were obtained using the program CYANA 3.98 [89] using the chemical shift data to delimit helical segments. The conformers with the lowest energy functions were chosen to be represented in the figures.

**Coupling constants**

For segment 5, as an additional, independent test, a 3D HNHA spectrum were recorded and the ratio of the $^1\text{H}_\alpha$-$^1\text{HN}$ crosspeak to the $^1\text{HN}$-$^1\text{HN}$ diagonal peak intensities in the 3D HNHA spectrum was utilized to calculate the $^{3}J_{\text{HNC}}^{\alpha}$ coupling constants following the procedure of [90]. $^{3}J_{\text{HNC}}^{\alpha}$ coupling constants were also measured for hCPEBpep2 using the 2D $^1\text{H}$-$^1\text{H}$ COSY spectrum. Utilizing the Karplus equation [91], these $^{3}J_{\text{HNC}}^{\alpha}$ coupling constants can be related at the backbone $\Phi$ angle, which is different for $\alpha$-helical, statistical coil, and $\beta$-strands.

**Relaxation**

To assess the dynamics on the ps–ns time scales, the heteronuclear $^{15}\text{N}[^1\text{H}]$ NOE (hNOE) of backbone amide groups was registered as the ratio of spectra recorded with and without saturation in an interleaved mode. Long recycling delays of 13 s were used. Two sets of experiments, one at 25 °C and one at 5 °C, were recorded at 800 MHz. Uncertainties in peak integrals were determined from the standard deviation of intensities from spectral regions devoid of signal which contain only noise.

In addition, $R_{1\rho}$ relaxation rates which are sensitive to the presence of preferred, rigid conformers on slower $\mu$-ms timescales were measured by recording two sets of ten $^1\text{H}$-$^{15}\text{N}$ correlation spectra with relaxation delays at 8, 300, 36, 76, 900, 100, 500, 156, 200, and 700 ms. One set of experiments was recorded at 25 °C, and the second was recorded at 5 °C. The relaxation rates were calculated by least-squares fitting of an exponential decay function to the data using NMRPipe [92]. As an additional check, the data were also analyzed independently by using the program DynamicsCenter 2.5.2 (Bruker Biospin).

$^{13}\text{C}$-detected relaxation experiments were measured for segment 1 to confirm the presence of a rigid N-terminus and to determine proline residue imino $^{15}\text{N}$ relaxation rates. Transverse relaxation rates ($R_T$) of hCPEB3 segment 1 imino $^{15}\text{N}$ nuclei were measured by a $^{13}\text{C}$-detected c_hacon_nt2_ia3d pulse sequence [80] as a pseudo 3D experiment time composed of nine 2D experiments with relaxation delays of 15.9, 79.2, 158.4, 269.3, 396.0, 554.4, 712.8, 871.2, and 1030 ms over a $^{15}\text{N}$ chemical shift range that is selective for the Pro $^{15}\text{N}$ chemical shifts (132–140 ppm). This experiment, and a similar one with a wider sweep width to also measure the $^{15}\text{N}$ $T_2$ relaxation of all 20 imino/amino-acid residues, was recorded at 25 °C, without non-uniform sampling or linear prediction. Following Fourier transformation, IPAP virtual decoupling, and baseline correction, the peaks were integrated with TOPSPIN 4.0.8 or alternatively NMRPipe by a different operator for comparison. A single exponential decay curve was then fitted to the peak integral versus time data to calculate $R_2$ rates for each position.

**Abbreviations**

ApCPEB: Aplysia CPEB; AMPA receptors: $\alpha$-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CPEB: Cytoplasmic Polyadenylation Element Binding protein; DSS: 4,4-Dimethyl-4-silapentane-1-sulfonic acid; FRET: Föster resonance energy transfer; GdmCl: Guanidinium chloride; HFIP: Hexafluoroisopropanol; hCPEB3: Human homolog CPEB3; IDR: Intrinsically disordered region; IPTG: Isopropyl $\beta$-D-1-thiogalactopyranosidase; NLS: Nuclear localization signal; PPII: Polyproline II; PLD: Prior-like domain; RRM: RNA recognition motif; STATS: Signal transducer and activator of transcription; TFE: Trifluoroethanol; ZnF: Zinc Finger.

**Supplementary Information**

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Availability of data and materials
All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. All data generated or analyzed during this study are included in this published article, its supplementary information files, and publicly available repositories. In particular, NMR chemical shift data are deposited in the BMRB database under file number 50256 [93], and NMR raw spectral data are deposited in the Mendeley data repository at Lauretts, Douglas; Pantajo, David (2021) “hCPEB3_NMR_Spectra” Mendeley Data v1, doi:10.17636/hpypjd35sx4/2, at this link: https://data.mendeley.com/datasets/hpypjd35sx4/2.

Declarations

Ethics approval and consent to participate
No animal or human participation was involved in this research.

Consent for publication
All authors given their consent for publication.

Competing interests
The authors declare that they have no competing interests.

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