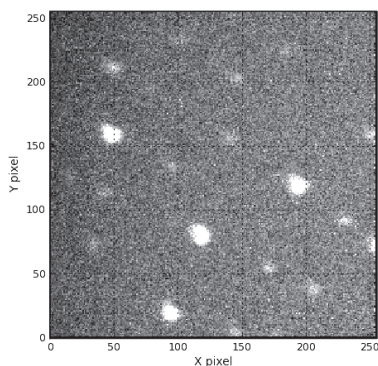


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Transthyretin (TTR) is one of more than 30 nonhomologous proteins linked to human amyloid disease. TTR is found in the blood and cerebrospinal fluid, and is composed of four identical 127-aminoacid β -sheet-rich subunits. Dissociation of TTR appears to be the rate-determining step that allows subsequent partial misfolding and misassembly, leading to the formation of cross β -sheet amyloid fibrils, as well as several other aggregate morphologies. Deposition of TTR amyloid fibrils is linked to four different amyloid diseases: senile systemic amyloidogenesis, familial amyloid cardiomyopathy, familial amyloid polyneuropathy, and central nervous system selective amyloidosis. In spite of the many high-resolution crystal structures of wild-type and amyloidogenic mutant TTR, comparisons of mutant amyloidogenic TTR structures to native TTR structures do not reveal any significant structural diversity that could account for amyloidogenesis. In order to investigate molecular property of TTR, the neutron diffraction experiments of N-terminal truncated mutant TTR crystals were performed at J-PARC BL-03 iBIX, Japan. The mutant TTR crystals were grown using sitting drop vapor diffusion method up to 2.5mm³ in volume after 3 months. Measurement conditions are as follow: the accelerator beam power: 120-220kW, the pulse repetition: 25Hz, the range of wavelengths: 2.5~6.5 Å (the 1st frame), 5.0~9.0 Å (the 2nd frame), the number of measurement settings: 82 settings (1st frame: 41 settings, 2nd frame: 41 settings). The neutron diffractions were observed up to 2.5-3.0 Å resolution (Figure). We present the detailed data statistics of the preliminary neutron crystallographic experiments including the data processing and the structural refinement.



(Figure) TOF diffraction image of mutant TTR ($2\theta=51.13^\circ$).

Keywords: transthyretin, neutron protein crystallography, amyloidosis

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Structural study of protein-bound CNG-repeats

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CNG repeat expansions in DNA cause many human diseases. The CNG-repeating RNAs produced during transcription are hypothesized to trigger these diseases via the ability to form double-helical hairpins and subsequently intervene with the cell silencing machinery [1], [2].

We performed the crystallographic study of double-helical RNAs that contained 5 to 6 consecutive CNG repeats (N = U, A, G or C) bound to silencing suppressor p19.

All CNG-repeats were found to form A-RNA double helix with

N•N mismatches flanked by Watson-Crick G•C base pairs at both ends. The dominant stabilizing feature of these RNA structures is an intensive intrastrand stacking-interaction between two consecutive base pairs, observed at the GC/GC steps, which are periodically located at each third position of all CNG-repeating sequences. Stabilized by these interactions, the double helix easily accommodates N•N mismatches, with Pyr-Pyr 'pairs' showing no regular H-bonding between bases, but being supported by short repulsive contacts. In contrast, Pur-Pur arrangements are stabilized by hydrogen bonds, with G•G and A•A displaying *anti-syn* and *anti-anti* alignments, respectively. Interestingly, our CAG- and CUG-repeating RNA structures differ from protein-unbound short counterparts described in [3], [4], although overall RNA structures are similar. The observed structural differences between protein-bound and protein-free N•N 'pairing' in CNG-repeating RNAs point on adaptive role of these mismatches in facilitating the process of protein-RNA interaction in case of these sequences. In particular, the helical bending observed in [5], [6] for siRNA bound to p19 is most likely to be provided by the structural 'mobility' of these mismatches in case of CNG-repeating RNAs.

This structural study of CNG-repeating RNAs bound to p19 further supports the hypothesis of possible involvement of RNA silencing machine in development of human diseases caused by the trinucleotide expansions in genes. Our high-resolution structures of CNG-repeating RNAs complexed with p19 shed the light on what is happening with these repeats when they are getting bound to a protein.

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Structural characterization of atypical PKC iota complexes with substrates

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PKC iota is a human oncogene, a useful prognostic marker and validated target for chemical intervention in non-small cell lung cancer (NSCLC) and ovarian cancer [1]. This atypical PKC isoform has a C-terminal serine/ threonine protein kinase domain (AGC class) that is auto-inhibited by a pseudosubstrate (PS) region until a poorly characterized allosteric input releases it from the kinase active site. PKC iota associates with the polarity proteins PAR-3 and PAR-6 to form a conserved apical polarity complex found in both vertebrates and invertebrates [2].

The PKC iota kinase domain interaction with substrate and pseudosubstrate (PS) peptides has been characterized using fluorescence polarisation assay and kinetic assays. Optimized peptide/kinase domain complexes were then screened in crystallization trials and crystals were obtained diffracting to 4.0 Å. The peptide optimization was essential to obtaining crystals of the peptide/kinase complex.

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