MD and docking studies reveal that the functional switch of CYFIP1 is mediated by a butterfly-like motion

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Keywords: CYFIP1, FMRP, eIF4E, WRC, Molecular Dynamics simulation, Protein domain communication, docking, conformational change.

The Fragile X syndrome is the most common form of inherited intellectual disability, affecting 1 in 4000 males and 1 in 8000 females [1]. Most cases are due to the transcriptional silencing of the FMR1 gene as a consequence of a CGG expansion within its 5'UTR1, [2]. The FMR1 gene encodes the Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein that represses the translation of associated mRNAs [3, 4]. A mechanism for the FMRP-mediated translation involves the Cytoplasmic FMRP-Interacting Protein 1 (CYFIP1) and the translation initiation factor eIF4E5. Similarly to general eIF4E-binding proteins (4E-BPs), CYFIP1 blocks access of eIF4G to eIF4E, thus inhibiting the assembly of the translation initiation machinery. Notably, CYFIP1 binds eIF4E through a non-canonical 4E-binding site (residue 724-732), which primarily implicates a lysine (Lys725) establishing an electrostatic interaction with a glutamate (Glu132) of eIF4E [5]. Moreover, the CYFIP1-eIF4E inhibitory complex is tethered onto specific mRNAs through FMRP5. In response to synaptic stimulation, the FMRP-CYFIP1 complex dissociates from eIF4E, and translation of FMRP-repressed mRNAs ensues5. This event requires activation of the small GTPase RAC1, which triggers the dissociation of CYFIP1-eIF4E [6].

CYFIP1 is also part of the WAVE Regulatory Complex (WRC), a heteropentamer that controls actin rearrangements through the ARP2/3 complex [7-9]. The structure of the WRC has been solved9: CYFIP1 has a planar conformation and interacts with NCKAP1 via a large surface that accommodates the heterotrimer WAVE1:ABI2:HSPC300. CYFIP1 participates in the auto-inhibition of the WRC by contributing to the surface that buries the VCA (verprolin-homology, central and acidic region) motif of WAVE19, precluding activation of the ARP2/3 complex. Binding of active RAC1-GTP to CYFIP1 to a region adjacent to the inhibitory surface frees the VCA and thus activates ARP2/3 [9]. This event is supported, in a cooperative manner, by binding to phospholipids, binding to a WIRS motif found in several membrane proteins, or by WAVE phosphorylation [10-14]. In summary, CYFIP1 interacts with numerous proteins, serving as a platform for the assembly of two independent complexes (see Scheme 1 for a summary of the interactions relevant for this study).

Importantly, the planar conformation of CYFIP1, based on the published structure of the WRC, does not allow an easy access to the eIF4E-binding region [6, 9] : CYFIP1 can only associate with one complex at a time, either the WRC or the translation-inhibiting complex, thus creating a homeostasis between the two complexes6. We hypothesized that a conformational switch in CYFIP1 allows access of either eIF4E or the WRC components and therefore dictates its partitioning between the two complexes [6].

Here, we used molecular dynamics (MD) simulations of 135 ns each to study possible conformational changes in CYFIP1, following the free protein and the NCKAP1 complex. We show that free CYFIP1 undergoes a butterfly-like motion that bends the N- and C-terminal domains toward the central one, resulting in a more globular conformation. Conversely, when bound to NCKAP1, CYFIP1 maintains a planar conformation. The conformational change reorients helix H8b, part of the eIF4E binding site, rendering it more accessible for eIF4E. No similar motion is observed in the simulation of the CYFIP1-NCKAP1 dimer. Accordingly, docking experiments indicate an increased affinity of CYFIP1 for eIF4E in the globular formation with respect to the formation in the dimer. Furthermore, the globular formation has a steeply decreased affinity for RAC1-GTP suggesting how RAC1 could shift CYFIP1 from one complex to the other. These findings provide a detailed structural explanation for the dual role of CYFIP1.

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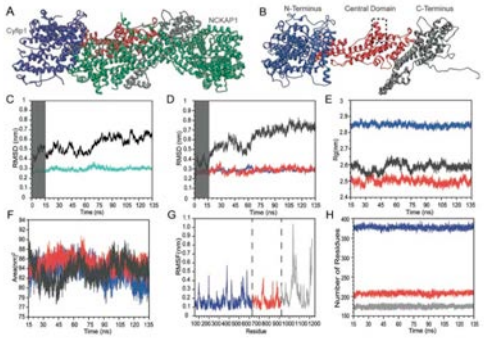

Figure 1. Domain structure and conformational evolution of CYFIP1

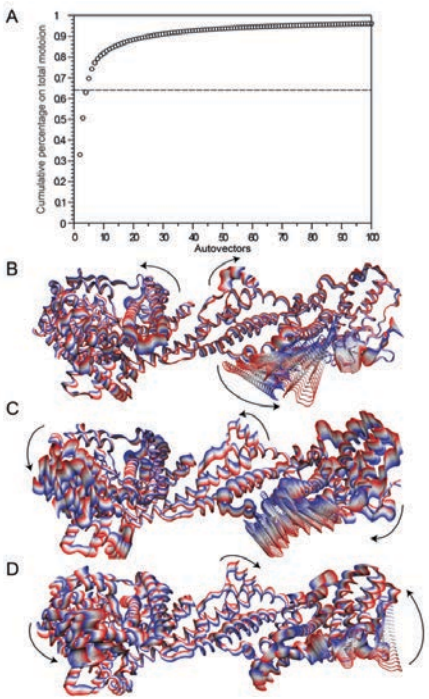

Figure 2. Analysis of the principal components of the motion in CYFIP1

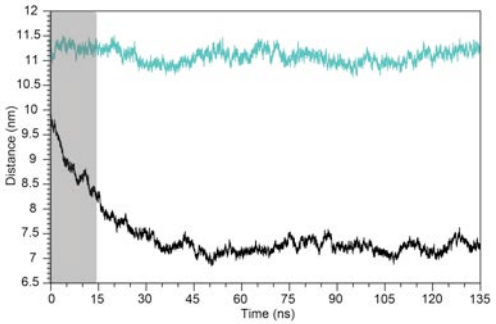

Figure 3. The terminal domains of CYFIP1 move closer to each other during simulation

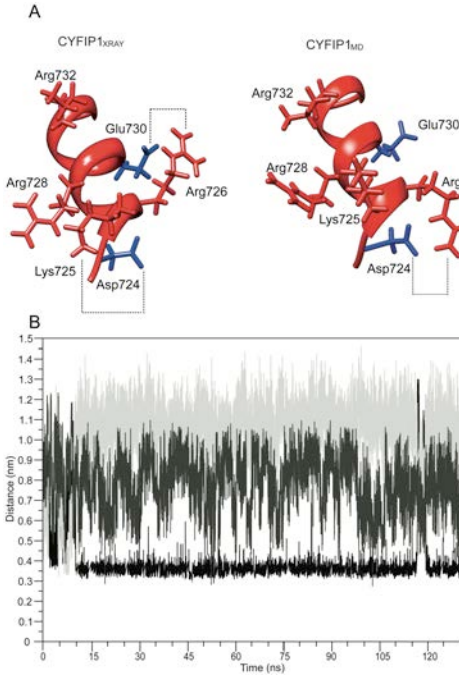

Figure 4. Electrostatic features of CYFIP1 helix H8b, involved in the interaction with eIF4E.