Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XL

In silico characterization of the CYFIP1-eIF4E complex via coarse-grained funnel metadynamics

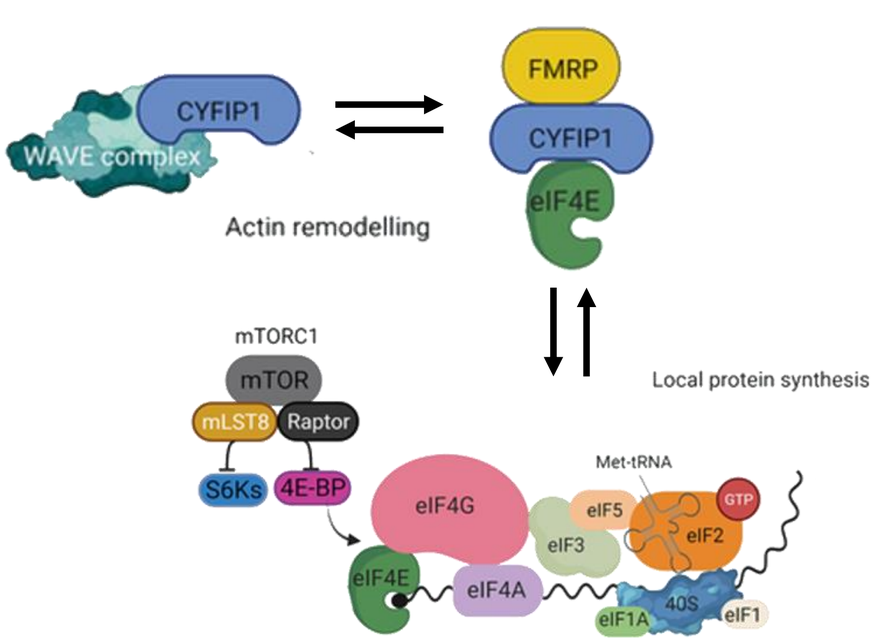
Francesco Pignotti, Giovanni Di Muccio[1], Alice Romagnoli[1], Anna la Teana[1]

[1] New York-Marche Structural Biology Centre (NY-MaSBiC), DISVA, Polytechnic University of Marche, Ancona, Italy

Abstract

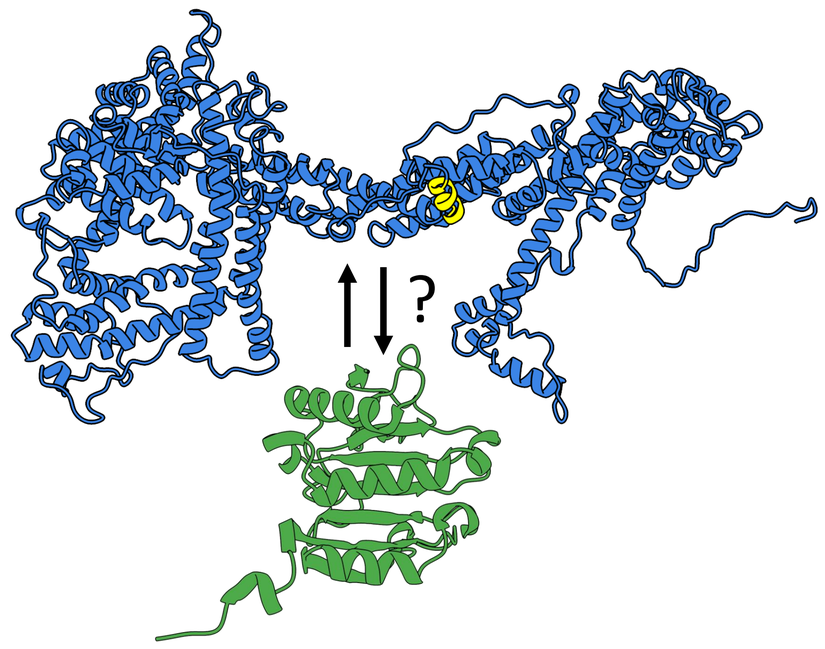
CYFIP1 (Cytoplasmic FMR1-Interacting Protein 1) plays a dual role in cellular functions. It interacts with the WAVE Regulatory Complex (WRC), which is essential for actin polymerization [1], and additionally functions as a "bridge protein," mediating the interaction between FMR1 (Fragile X Messenger Ribonucleoprotein 1) and eIF4E (Eukaryotic Translation Initiation Factor 4E), thereby regulating translation initiation [2]. CYFIP1 is critical for neural development and synaptic maturation and has been implicated in several neurological disorders, including schizophrenia, autism spectrum disorders, and various types of cancer [3,4]. To date, all available structural data on CYFIP1 pertain to its role within the WRC complex. The only known structural detail about the FMR1–CYFIP1–eIF4E complex is a helical motif of CYFIP1 that binds the canonical 4E-binding protein (4E-BP) interaction site on eIF4E [5,6]. Given that the full molecular picture of the CYFIP1–eIF4E interaction remains unclear, the primary objective of this research project is to model the complex entirely in silico, employing coarse-grained molecular dynamics simulations and enhanced sampling techniques. The goal is to elucidate the structural basis of this interaction and determine whether CYFIP1 contains an additional eIF4E-binding motif, as described for other 4E-BPs [6].

A picture of CYFIP1 interactions



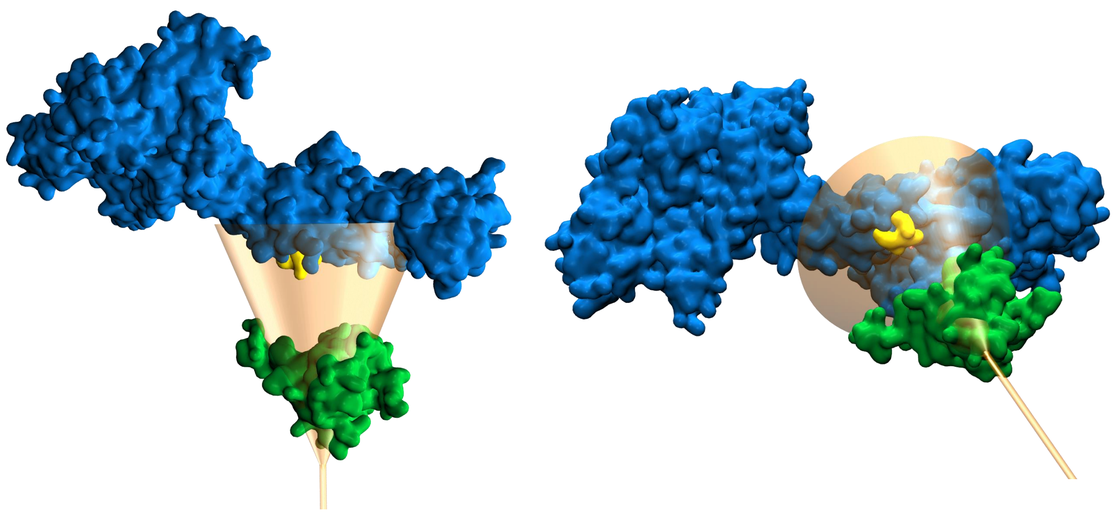
The molecular mechanism of CYFIP1 (shown in blue). CYFIP1 can switch between the eIF4E–FMRP complex (green-yellow) and the WAVE Regulatory Complex (WRC), thereby regulating translation and actin remodelling. *Picture adapted from Romagnoli & Di Marino (2021).*

Aim of the project



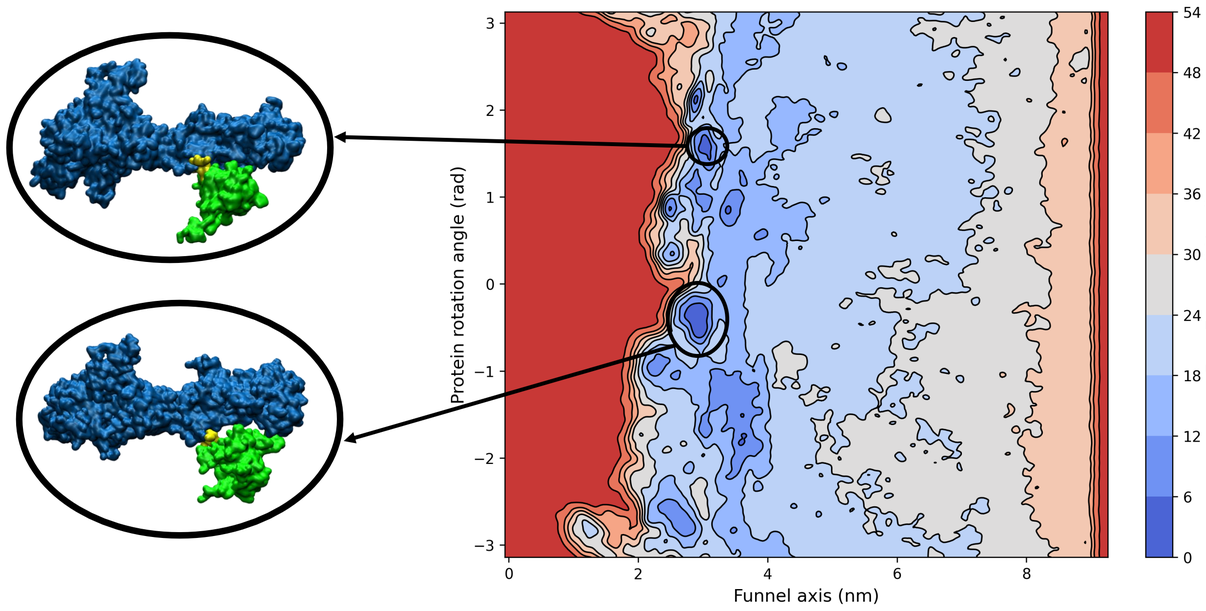
Structure of CYFIP1 (blue) and eIF4E (green). The aim of this project is to gain a detailed understanding of how these two proteins interact by generating a structural model of their complex. Particular attention will be given to the known eIF4E-binding motif of CYFIP1, highlighted in yellow.

MD simulation setup



Representation of the simulation setup. The system was coarse-grained using the Martini 3 force field due to its large size. Additionally, funnel metadynamics was employed, using a funnel-shaped restraint based on previously identified binding region of CYFIP1 to reduce the sampled space and capture multiple binding/unbinding events. This motif (shown in yellow and blue) is responsible for interacting with eIF4E (green).

Preliminary results



The energy landscape derived from two collective variables (CVs), obtained after 10 microseconds of simulation. From each minimum, a set of representative structures can be extracted and clustered, enabling the characterization of potential binding modes. Additionally, the free energy difference between the minima and the isosurface around 8 nm can be used to estimate the dissociation constant (Kd) of the complex.

References

[1] Han, K., & Ko, J. (2023). Orchestration of synaptic functions by WAVE regulatory complex-mediated actin reorganization. Experimental & Molecular Medicine, 55(6), 1065–1075.

[2] Napoli, I. et al (2008). The Fragile X Syndrome Protein Represses Activity-Dependent Translation through CYFIP1, a New 4E-BP. Cell, 134(6), 1042–1054.

[3] Domínguez-Iturza, N. et al (2019). The autism- and schizophrenia-associated protein CYFIP1 regulates bilateral brain connectivity and behaviour. Nature Communications, 10(1).

[4] Romagnoli, A. et al (2024). Translational takedown: CYFIP1-based peptidomimetic against cancer and fragile X Syndrome. Biophysical Journal, 123(3), 184a.

[5] Di Marino, D. et al (2015). A unique binding mode of the eukaryotic translation initiation factor 4E for guiding the design of novel peptide inhibitors. Protein Science, 24(9), 1370–1382.

[6] Romagnoli, A. et al (2021). Control of the eIF4E activity: structural insights and pharmacological implications. Cellular and Molecular Life Sciences, 78(21–22), 6869–6885.

MOLecular LAB
Molecular Biology & Computational Biophysics