A Structural Approach to Discover an Achilles'heel of fungi : the case of Knr4/Smi1

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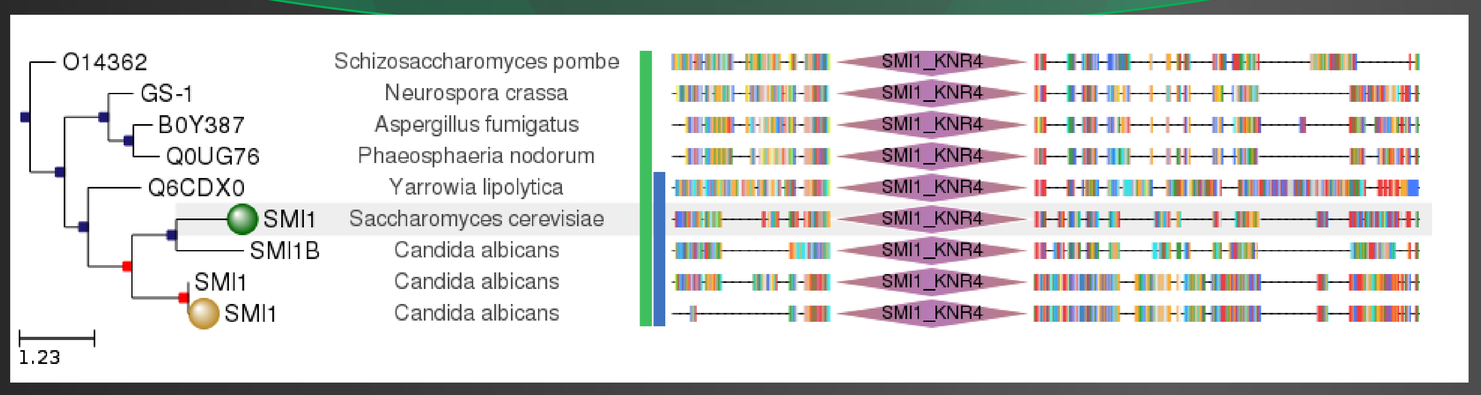
Background

Intrinsically disordered proteins (IDPs) are involved in numerous essential biological processes. Their conformational flexibility gives them the ability to be involved in one-to-many binding (a single disordered domain is able to bind several structurally diverse partners). Highly connected proteins located at **nodes in interactions networks or "Hubs"** are significantly enriched in disordered domains which allow them to fulfil their multiple interactions.

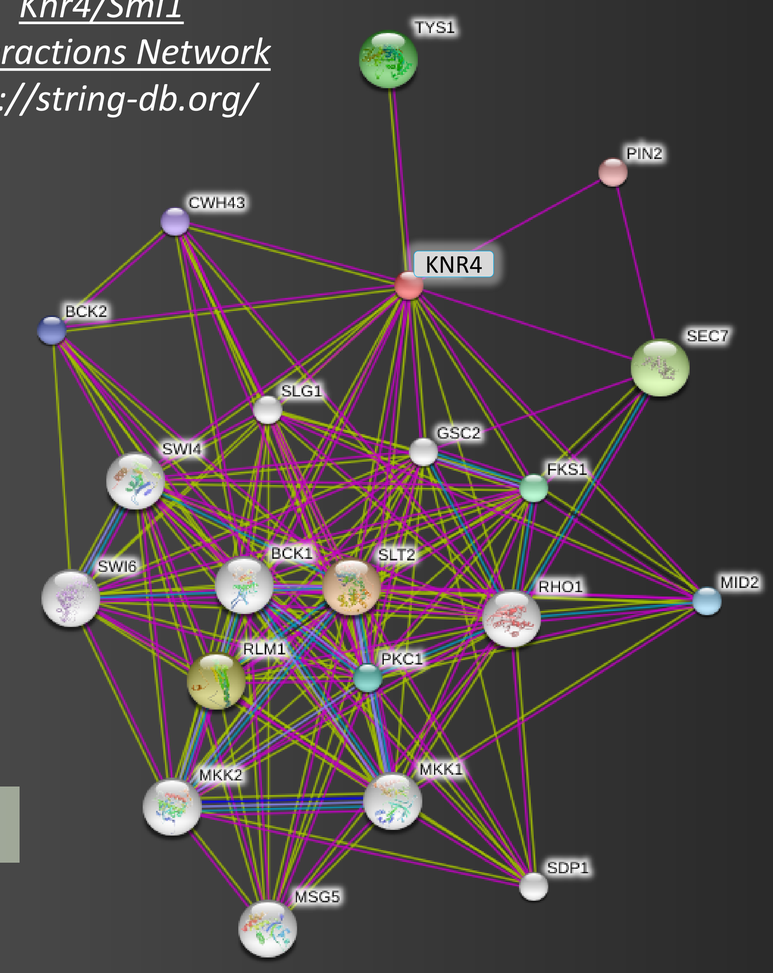
Knr4/Smi1 : a *S. cerevisiae* hub protein

- functions in signaling, gene transcription, cell cycle progression and morphogenesis[1];
- *knr4Δ* : hypersensitive to stress conditions (high θ°, SDS, caffeine, CFW, …);
- multiple physical interacting partners;
- over 280 synthetic lethal or sick interactions.

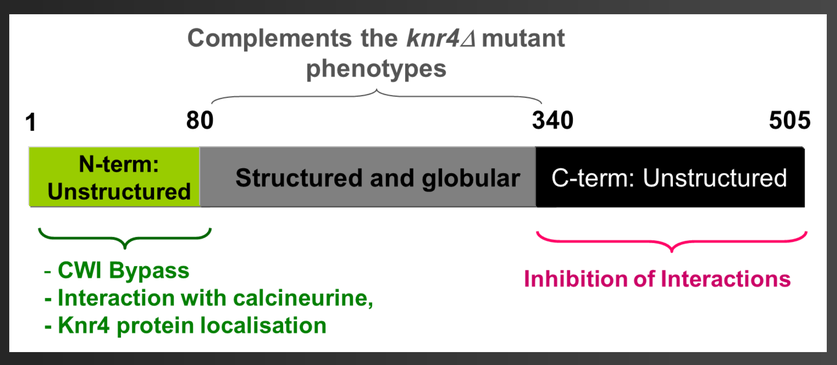


Conservation of Knr4 gene among the fungal kingdom.
Phylogenetic tree made from T. Gabladeon PhylomeDB web site http://phylomedb.org/.

Knr4/Smi1 Interactions Network
http://string-db.org/



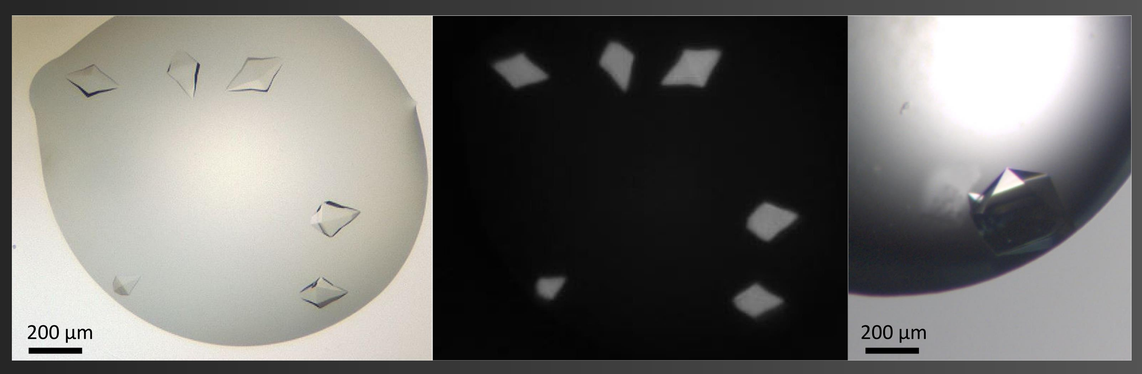
Disordered domains of Knr4



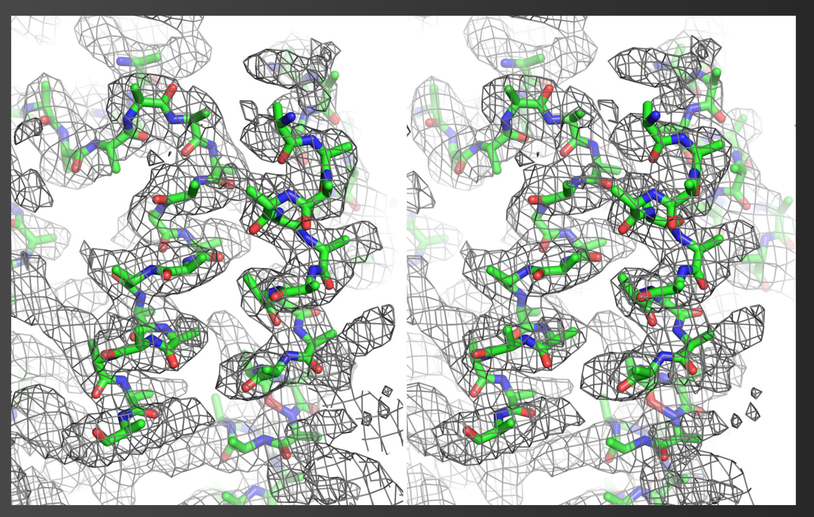
3D structure of fungal Knr4 is unknown to date.
In silico, biophysical and biochemical methods have shown that it contains large disordered N-terminal (1-80) and C-terminal (341-505) parts and a structured and globular central core, which holds the essential of the biological functions of the protein[2]. The disordered N and C parts control the interactions of the protein with its partners.

Crystals

We have expressed in *E. coli* , purified and crystallized this core domain, as well as a modified Selenomethionine containing corresponding protein [3].



First structural elements solved



Stereoview of the electron-density map
obtained after SAD phasing with SHELXC/D/E and further density modification with DM.
The electron-density map is displayed as a grey mesh contoured at 1σ.

Conclusions and outlook

We have found crystallization conditions allowing the identification of secondary structure elements of *S. cerevisiae* Knr4 central core domain[3]. Recently, we obtained new Knr4 crystals diffracting at 2.5 Å, a resolution which should allow us to rapidly decipher the 3D structure of the protein core of this unique fungal IDP. Our goal is to finally decipher the global 3D structure of the complete protein, in complex with relevant essential proteins partners, in order to later define targetable regions for complex formation inhibition.

1. Dagkessamanskaia A., …. and Martin-Yken H. 2010, Knr4 N-terminal domain controls its localization and function during sexual differentiation and vegetative growth, Yeast, 27(8), 563-74.
2. Dagkessamanskaia A., …, and Martin-Yken H. 2010, Functional dissection of an intrinsically disordered protein: understanding the roles of different domains of Knr4 protein in protein-protein interactions. Protein Sci, 19(7):1376-85.
3. Julien S. ,… and Maveyraud L., (2015), Crystallographic studies of the structured core domain of Knr4 from *Saccharomyces cerevisiae*. Acta Cryst. F.