Characterization of the intramolecular interactions of the protein Tks4

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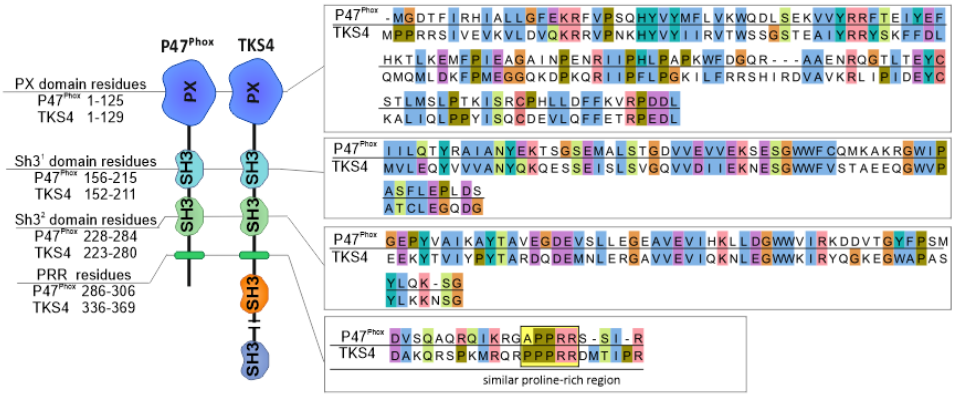
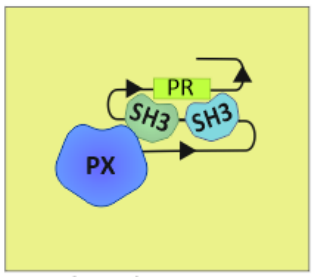
Introduction

- Tks4 (Tyrosine kinase substrate with four Src homology 3 domains)
- It belongs to the p47Phox-related protein superfamily
- Scaffold protein, which builds up a lipid-binding phox (PX) domain, four SH3 domains and several proline-rich (PR) regions
- It has a role in the EGFR signalling and require for the formation of actin-rich membrane protrusions
- Mutations in the Tks4 gene result in a rare genetic disease (Frank-Ter Haar syndrome)

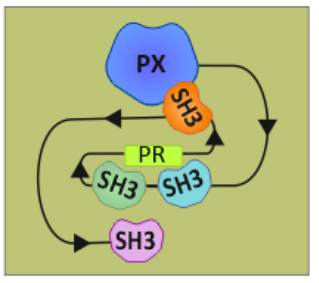
Sequence and conservation analysis of human Tks4 reveals Tks4 and p47Phox share many similarities in stucture



- p47Phox protein is autoinhibited via intermolecular interactions
- The second SH3 domain of p47Phox binds to the proline rich region of the PX domain
- The tandem SH3 domains of p47Phox binds to the PR region



closed stucture of P47Phox



supposed closed stucture of Tks4

- We suppose that the Tks4 has a closed conformation in solution
- Sequence and conservation analysis of human Tks4 revealed that the N-terminal region of Tks4 and p47Phox share many similarities (similar proline-rich region that potentially binds to the tandem SH3 domain)
- Third SH3 domain of Tks4 binds the PX domain (A novel gene, fad49, plays a crucial role in the immediate early stage of adipocyte differentiation via involvement in mitotic clonal expansion. (Hishida T, Eguchi T, Osada S, Nishizuka M, Imagawa M. FEBS J. 2008 Nov;275(22):5576-88.)

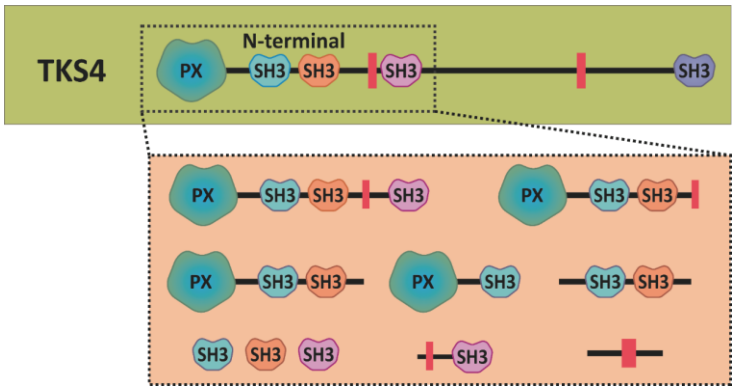
Goals

Our goal is to investigate whether Tks4 is capable of the predicted autoinhibitory comformation.

By using recombinant protein fragments of the N-terminal part of Tks4:

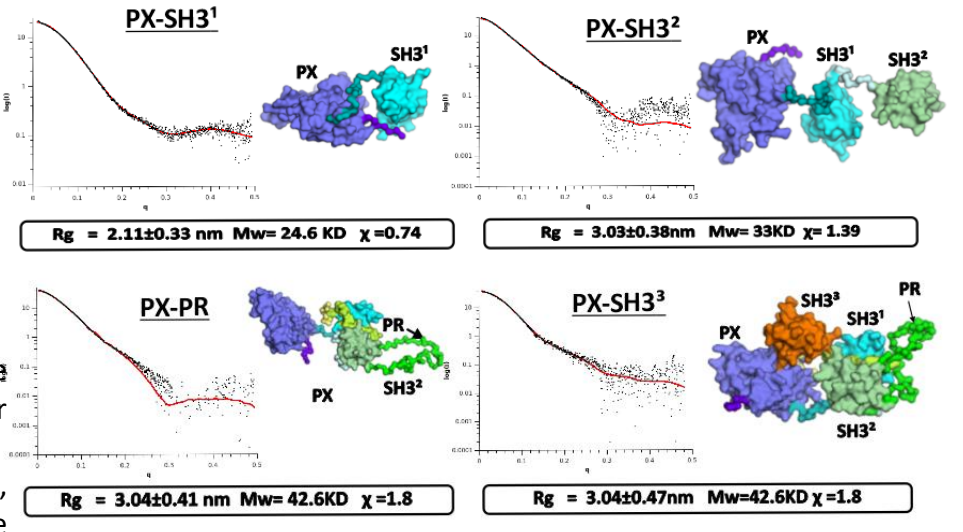
- Intramolecular interactions will be investigated by Surface plasmon resonance, Fluorescence spectroscopy and/or Isothermal titration calorimetry.
- Stuctural details of autoinhibitory interactions will be elucidated by X-ray crystallography and SAXS
- Inhibition of the PX domain will be investigated in lipid binding assays (e.g. SPR)

Results 1. We successfully expressed recombinant protein fragments of the N-terminal part of Tks4 protein

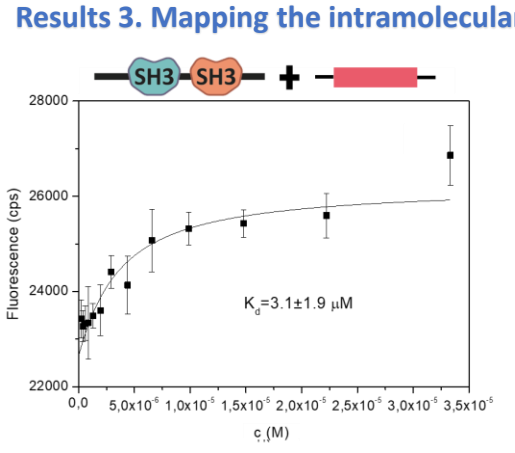


Results 2. SAXS analysis confirms the closed conformation of Tks4

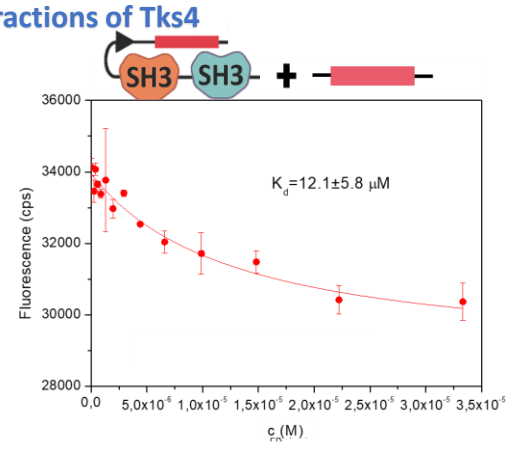
- Radius of gyration (Rg) of the longer N-terminal fragment of Tks4 does not increase, which unequivocally indicates intramolecular interactions
- Based on this observation and SAXS molecular modelling, in solution the N-terminal region of Tks4 has a closed conformation



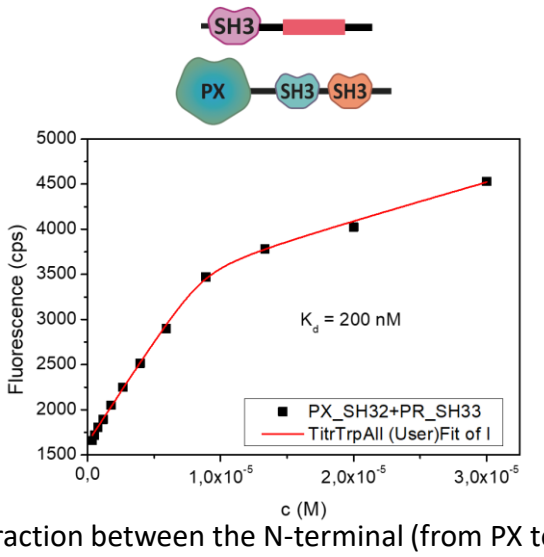
Results 3. Mapping the intramolecular interactions of Tks4



Proline rich region (red bar) binds to the double SH3 domains with a **$K_d \approx 3$ µM**



If the intramolecular proline rich region is present the binding affinity drops to **$K_d \approx 12$ µM**



Interaction between the N-terminal (from PX to SH3²) and the thord SH3 domain with the proline rich region displays a two pronged binding, hence the crooked curve. The double binding also answers the increased **$K_d \approx 200$ nM**

The intramelucar ligand of the double SH3 domains is the proline rich region