HelmholtzZentrum münchen
German Research Center for Environmental Health

Scientific Highlight April 2010

[X] co-ordinated with the Director of the Institute / Head of Department

Institute / Independent Department / Clinical Co-operation Group / Junior Research Group:
Department of Protein Science

PSP-Element:
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Title of the Highlight:
Analysis of LRRK2 autophosphorylation by systematic mapping of LRRK2 phosphorylation sites

Keywords:
LRRK2, kinase, autophosphorylation, PTM analysis, mass spectrometry

Central statement of the Highlight in one sentence:
A clustering of autophosphorylation sites within the Parkinson-disease associated LRRK2 kinase with its GTPase domain Roc as major target directs towards an intramolecular cross regulation mechanism between Roc and kinase domain.

Text of the Highlight:

The Parkinson disease (PD) is the most common movement disorder, severely afflecting dopaminergic neurons within the *substantia nigra*. Mutations within the *Leucine-rich Repeat Kinase 2* (*LRRK2*) gene have been recently identified in autosomal dominant inherited forms of PD. Among the PD-genes identified so far, *LRRK2* mutations are the most common ones making LRRK2 a key towards understanding the ethiology of PD. *LRRK2* encodes a 280 kDa protein which consists of multiple domains, among them the GTPase domain Roc (Ras of complex proteins) and a kinase domain with high similarity to those of mixed-lineage kinases (MLKs). The dominant nature of the *LRRK2* mutations is most likely caused by an increased kinase activity as demonstrated recently. Phosphorylation by upstream kinases as well as autophosphorylation is an important regulatory mechanism for kinases and PD-associated LRRK2 variants show an increased autophosphorylation. Therefore, LRRK2 phosphorylation sites

have been systematically mapped by the means of mass spectrometry. The mapping approach revealed several distinct clusters of phosphorylation sites. Besides a cluster of constitutively phosphorylated serine residues within the LRRK2 N-terminus, several distinct regions of LRRK2 autophosphorylation have been identified by combining an auto-kinase assay with phosphopeptide enrichment and subsequent mass spectrometry. Interestingly, the GTPase domain Roc is a major target for LRRK2 autophosphorylation indicating a cross regulation between the GTPase domain Roc and the kinase domain of LRRK2.

Publication:

Gloeckner, C.J., Boldt K., von Zweydorf F., Helm S., Wiesent L., Sarioglu H., Ueffing M. (2010). Phosphopeptide analysis reveals two discrete clusters of phosphorylation in the N-terminus and the Roc domain of the Parkinson-disease associated protein kinase LRRK2. *J. Proteome Res.*, **9**:1738-1745.

Taking account of the HMGU mission:

Our research aims at gaining better mechanistic insights into the regulation of the PD-associated Leucine-rich Repeat Kinase 2 (LRRK2). Mutations within *LRRK2* explain up to 7% of familial PD cases making *LRRK2* to the most important PD-gene so far.

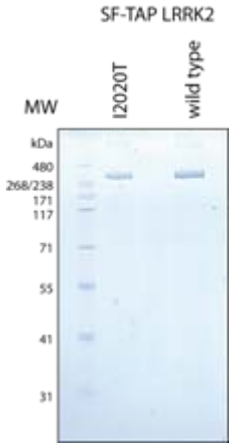
The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:

Mapping of phosphorylation sites of the PD-associated Leucine-rich Repeat Kinase 2 (LRRK2)

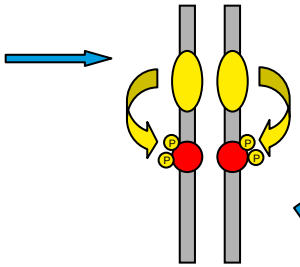
Department of Protein Science

Dept. Protein Science

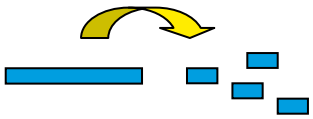
Purification of active LRRK2



Autokinase assay



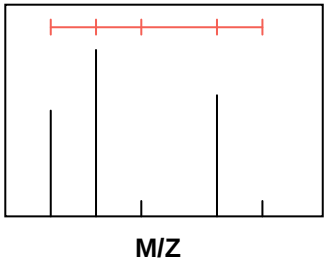
Proteolysis



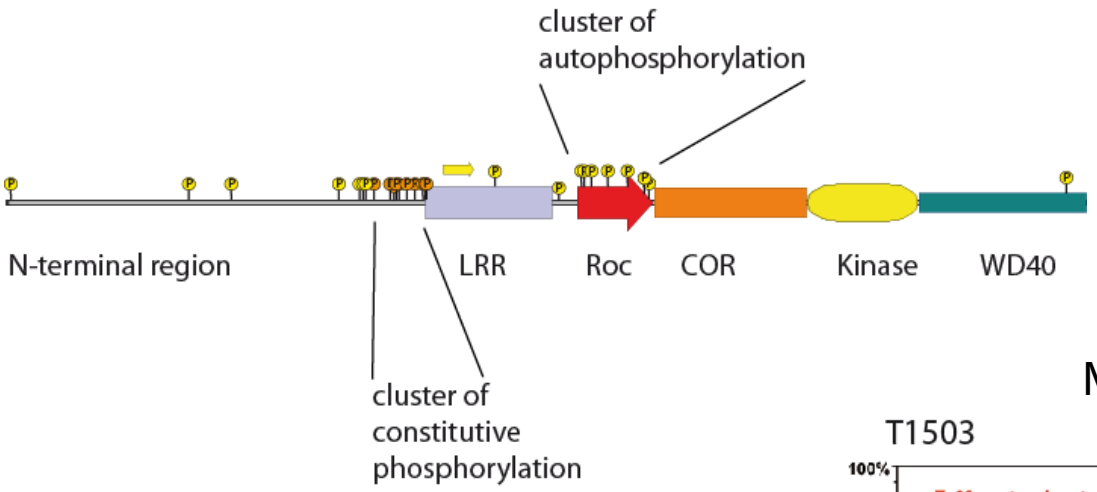
Phosphopeptide enrichment via binding to Titaniumdioxide

TiO_2

Mass spectrometry



Distinct clusters of constitutive LRRK2 phosphorylation and autophosphorylation sites



Domains:

LRR: Leucine-rich repeats

Roc: Ras of complex proteins (GTPase)

COR: “C-terminal of Roc“ domain

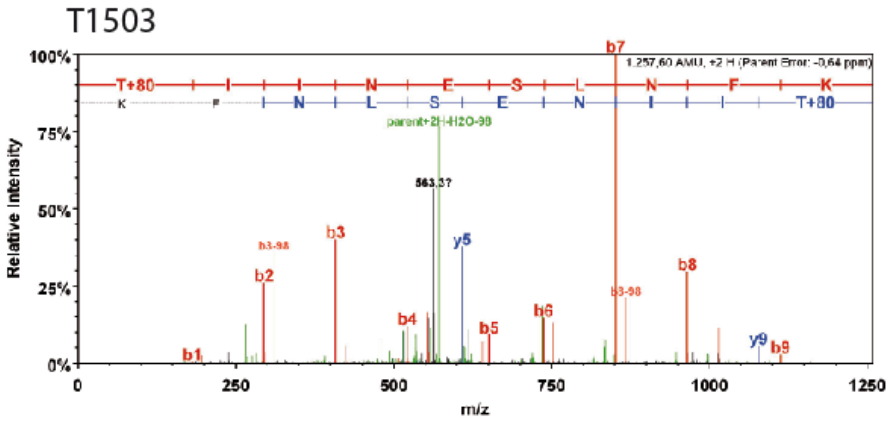
Kinase: Kinase domain

WD40: WD40 repeat domain

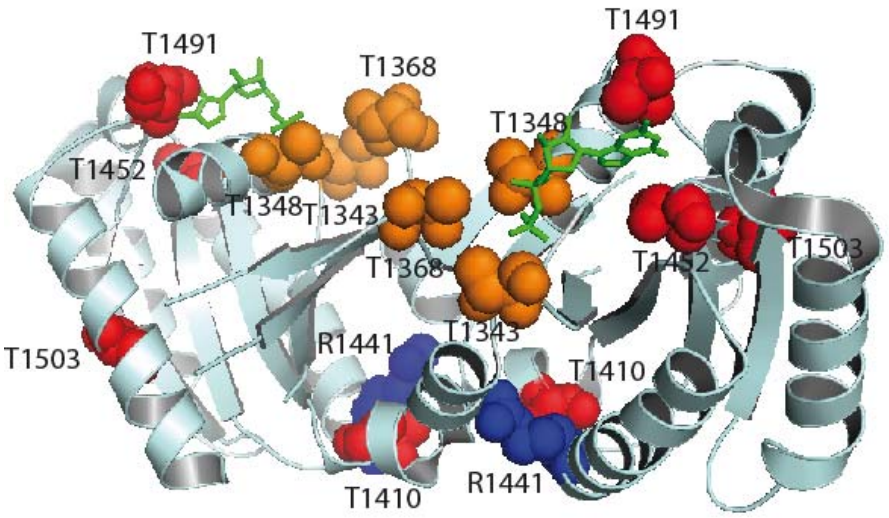
orange: constitutive phosphorylation

yellow : autophosphorylation

MSA (peudo MS^3) spectrum

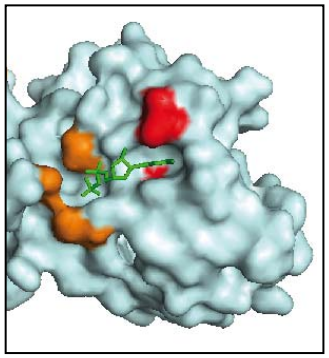


The GTPase domain Roc is a major target for LRRK2 autophosphorylation – a possible GTPase / kinase cross-regulation mechanism?



- unique phosphorylation sites
- P-sites with alternative choices (multiphosphorylation possible)
- PD-associated mutations (codon R1441)

GTP binding pocket



Hypothesis: domain cross-regulation