

Final report of grant (67p12) within the Aktion framework – „Structural analysis of the binding of tau protein to Plectin’s SH3 domain using NMR.

Bedingungen der Förderung/Podmínky podpory

2.1. Zur Förderung Arden folgende Profgrammteile bzw. Aktivität des Projektantrages genehmigt/Byly schváleny tyto části resp. aktivity uvedené v návrhu projektu:

EUR	2.148	– laut Projektantrag/dle návrhu projektu
CZK	2013	34.000 – laut Projektantrag/dle návrhu projektu
	2014	11.000 – laut Projektantrag/dle návrhu projektu

II) Přehled nákladů na řešení projektu vznikajících na území ČR v Kč Übersicht der auf tschechischem Gebiet anfallenden Kosten in CZK

typ nákladů / Kostenart	2013 Kč	2014 Kč
Úhrada ubytování účastníků projektu v ČR (viz Pokyny) / Unterkunftskosten für Projektteilnehmer in d. ČR (s. Merkblatt)	9 000 CZK	4 500 CZK
Stravné a kapesné účastníků projektu v ČR (s. Merkblatt) Verpflegung für Projektteilnehmer in der ČR (viz Pokyny)	4 000 CZK	1 500 CZK
Jízdné česk. účastníků do Rak. a účastníků projektu po ČR (viz Pokyny) / Reisespesen: Reisen tsch. Projektteilnehmer nach Ö., Reisen von Projektteilnehmern auf tschech. Gebiet (s. Merkblatt)	7 398 CZK	868 CZK
Odměna za výuku českým účastníkům projektu (viz Pokyny) / Honorar für Lehrtätigkeit tsch. Projektteilnehmer (s. Merkblatt)	0	0
Publikační a ediční náklady (lektorský posudek, tisk)/ Publikations- und Editions-kosten (Rezensenten-honorar, Druckkosten)	0	0
Ostatní náklady / sonstige Kosten	13 000 CZK	3 500 CZK
Verwaltungs- und Regiekosten (nur bei den Sommerkollegs)/ správní a režijní náklady (pouze pro letní kolegia)		
Celkové náklady v Kč / Gesamtkosten in CZK	33 398 CZK	10 368 CZK

The Implementation Plan for grant 67p12:

Month	Person	Function	Duration	Purpose	Place
September 2013	L. Janda	Applicant	1 week	data acquisition	Vienna
October 2013	G. Wiche	Professor, co-applicant	2 days	lecture	Brno
October 2013	RG. Valencia	PostDoc	3 days	lecture, data acquisition	Brno
November 2013	E. Mihailovska	PostDoc	3 days	data acquisition	Brno
November 2013	J. Nováček	Post Doc	1 week	data acquisition	Vienna
February 2014	J. Nováček	Post Doc	1 week	data acquisition	Vienna
April 2014	L. Janda	Applicant	1 week	data acquisition	Vienna
May 2014	RG. Valencia	Post Doc	3 days	data acquisition	Brno

The Implementation Plan:

September 2013/Vienna: The task was to transfer the know-how in the preparation of proteins (tau protein and tubulin) to the Vienna laboratory (Lubomir Janda).

October 2013/Brno: The aim was to familiarize the partner laboratory about the details of the project in form of a lecture and to formulate specific objectives based on the measured data from Vienna (Prof. Wiche).

October 2013/Brno: R. Garcia Valencia prepared the proteins for NMR measurement.

November 2013/Vienna: Jiri Novacek was testing the biological activity of above mentioned proteins in Vienna (co-sedimentation assays, turbidity measurements).

November 2013/Brno: Eva Mihailovska prepared proteins for NMR measurements.

February 2014/Vienna: Jiri Novacek continued with tests of the biological activity of above mentioned proteins and completed measurements in order to publish the results.

April 2014/Vienna: The goal was to process the results from both institutions, and/or complete the measurements in order to publish the results (Lubomir Janda).

May 2014/Brno: Processing of results and writing the publication (R. Garcia Valencia).

The actual implementation of the plan for grant 67p12

Month	Person	Function	Duration	Purpose	Place
December 2013	L. Janda	Applicant	1 week	data acquisition	Vienna
December 2013	J. Nováček	Post Doc	1 week	data acquisition	Vienna
December 2014	M. Huber	PhD student	1 week	data acquisition, lecture	Brno
December 2014	L. Janda	Applicant	1 week	Data acquisition	Vienna
December 2014	Z. Máčalová	Diploma student	2 week	Data acquisition	Vienna

The implementation Plan for grant 67p12 was as follows:

December 2013/Vienna: J Nováček and L. Janda

Due to the reorganization of the laboratory of prof. Wiche (prof. Wiche, dr. Valencia and Mgr. Mihailovska), it was not possible to perform all scheduled visits during the fall 2013. Both scheduled visits (dr. Janda, dr. Nováček) were postponed to December 2013 and unclaimed finances were refunded.

Main topic of the visit was the discussion of publication B., which was a result of the collaboration with the laboratoř in Vienna, and scheduling experiments leading to the next publication, studying the interactions of microtubule associated protein 2c in neuronal cells. The evidence of interaction of Plectin SH3 domain with microtubule-associated proteins, such as MAP2c or tau protein, will be of great value.

We have acquired the 3D HNC0 spectra at 600MHz of the ¹³C,¹⁵N labeled MAP2c in its free form and after addition of Plectin SH3 domain (molar ratio MAP2c:SH3 ~ 1:2). The data show that the interaction interface of Plectin SH3 domain with MAP2c, is in the region of aminoacids 208-218. This region is relatively close to two residues S184, T220, predicted to be phosphorylated by the PKA kinase. Apart from that, we can also observe changes in the ratio of signal intensities for the region of aminoacids 330-370, which correspond to the central microtubule binding domain and both inter-repeat regions.

Due to the moving and reorganization of laboratory in Brno in spring 2014, we applied for grant extension, which was accepted and the grant was extended until the end of 2014.

December 2014/Vienna: Markus Huber:

During 2014, R. Valencia went to the a new postdoctoral position and E. Mihailovska took maternity leave. Their role in prof. Wiche lab was substituted by Markus Huber. He spent one week in Brno, where he helped to prepare actin binding domain of plectin for NMR analysis, which was successfully measured and data of well folded protein were obtained. On HSQC we identifyied 192 peaks, which is 75% of expected peaks. Additionally, Markus Huber gave a talk entitled “Determiningthe structure ofsmallersubunitsMAP1b(Microtubule-associated protein1b) by NMR analysis”.

December 2014/Vienna: Z. Máčalová and L. Janda

In 2014, Dr. Nováček went to a new position and was replaced by Zuzana Máčalová. She started work in Brno laboratory since fall 2014 and she will be working on her thesis “Study of post-translational modifications ABD domain of plectin”. The visit in Vienna resulted in a decision, that both laboratories will apply for a new bilateral grant FWF-GACR, and preparations for the grant started during this visit.

Publications arising from the collaboration between both laboratories.

A.

Intermediate filament-associated cytolinker plectin 1c destabilizes microtubules in keratinocytes

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B.

Efficient protocol for backbone and side-chain assignments of large, intrinsically disordered proteins: transient secondary structure analysis of 49.2 kDa microtubule associated protein 2c

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C.

Detailní popis struktury plektinové aktin vazebné domény (Detailed description the structure of the Plectin actin-binding domain). (Diana Lamborová, Bachelor thesis, Masaryk university 2014).

D.

Studium postranlačních modifikací u Plektinové ABD domény (Study of posttranslational modifications ABD domain of plectin). (Zuzana Máčalová, Bachelor thesis, Masaryk university 2015 in preparation)

26. 1. 2015 Brno , Mikuláš Bek , rektor

RNDr. Lubomír Janda, Ph.D.

26. 1. 2015

Ort, Datum Unterschrift des Antragstellers
Místo, datum podpis předkladatele

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