PÁZMÁNY PÉTER CATHOLIC UNIVERSITY
ROSKA TAMÁS DOCTORAL SCHOOL OF SCIENCES AND TECHNOLOGY

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Structural and functional investigation of the postsynaptic scaffold protein GKAP

Theses of PhD Dissertation

Thesis supervisor:

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2025

Introduction

Intrinsically disordered proteins/regions (IDPs/IDRs) do not adopt a stable 3D structure, rather exist as a dynamic ensemble of diverse, interconverting conformers. Because of this high plasticity, IDPs/IDRs can perform immensely diverse tasks in living cells. They typically bind to their partners via Short Linear Motifs (SLiMs) with high specificity and tunable affinity; hence they might endure sophisticated regulatory and modulatory mechanisms.

Complicated and diverse molecular mechanisms underlie the phenomenon of memory, learning and synaptic plasticity. The dense, elaborate and complex protein network located on the cytoplasmic site of the membrane in the dendritic spines of neurons is called the postsynaptic density (PSD) and is most prevalent in excitatory synapses. The PSD is a fundamental processing unit of synaptic signal transmission. It is populated by neurotransmitter receptors, scaffold proteins, cytoskeletal proteins, and signalling molecules. All components are relevant participating in establishing the network: proteins connecting to each other, forming complexes and supercomplexes. The exact composition and distribution of this network is variable between different neurons and brain regions [1], [2], [3], [4], [5], [6], [7], [8], [9], [10], [11]. Any disturbance in this complex network might result in substantial functional and structural impact in the neurons affected. Many alterations or mutations of PSD proteins have been associated with neurodegenerative diseases [12], [13], [14], [15], [16]. Mapping atomic details and residue-level nuances of structural rearrangements and interaction mechanisms can provide insights in such a depth that might lead to novel therapeutic or diagnostic approaches.

Scaffold proteins creating and maintaining the core of the PSD can be classified into four protein families: MAGUK, SHANK, GKAP and HOMER families. Enzymatically inactive guanylate kinase (GK) domain of the MAGUK protein family can bind to the GK-binding region of SAPAP proteins (also called GKAP, GK-associated protein or DLGAP1, Discs large-associated protein). SAPAP proteins then

bind to the PDZ domain of SHANK family proteins through their C-terminal motifs. The supercomplexes formed are then arranged to sheet-like tertiary structure, forming as a base for connecting receptors and anchoring downstream signalling molecules [17], [18], [19].

GKAP is a member of the five-membered SAPAP protein family. It is predicted to be mostly intrinsically disordered, showing high degree of flexibility (Figure 1). Several SLiMs have been described along its sequence. It associates with the GK domain of the PSD95 MAGUK protein (hence the name, GK-associated protein), the PDZ domain of the Shank1 protein and also the dynein light chain protein (DLC, or LC8). Several of the mentioned regions are bivalent/multivalent, meaning the ability to bind several partner molecules simultaneously. GK-binding segment consists of five 14-residue repeats and DLC-binding domain contains two binding motifs. The Shank1 PDZ binding domain is a C-terminal SLiM [20], [21]. For the functional features of GKAP, see Figure 1.

The LC8 protein has been identified as a subunit of the dynein motor complex, but emerging evidence revealed this molecule to be a so-called hub with more than a hundred different interacting partners, localizing to different cellular compartments. It can act as a dimerization engine enhancing the self-association (dimerization or multimerization) of its originally monomeric partners, this way forming bivalent or polyvalent networks. It was suggested in this role as an associating partner of GKAP, while making connection between the PSD scaffold protein complexes and motor complexes, thus taking part in microtubule dynamics, centrosome positioning and cell polarity of neurons [22], [23].

The C-terminal region of GKAP binds to the PDZ domain of Shank, which is a member of another postsynaptic scaffold protein family, SHANK. This group is also characterized with substantial intrinsic disorder [24].

One of the currently available atomic-resolution structural investigation methods is NMR (nuclear magnetic resonance) spectroscopy. Atomic level information can be

obtained from NMR experiments regarding the 3D structure of a molecule, furthermore, investigation of flexible molecules with continuously changing conformations is also possible. NMR is the only method capable of detailed characterization of protein molecules with high conformational dynamics. We can not only obtain information on the structural preferences of the given segments with such proteins, but also map regions affected by protein:protein interactions and analyse changes upon partner binding.

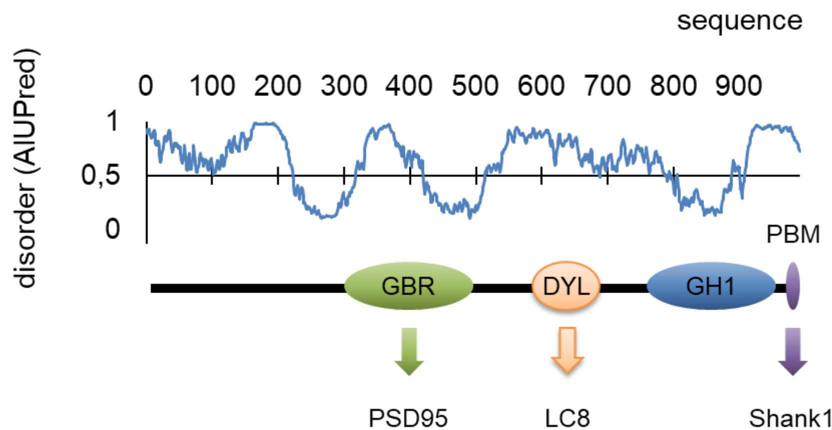


Figure 1: AIUPred disorder prediction of the full-length Rattus norvegicus GKAP protein (isoform 1) [25], and regions and interaction partners described in the literature.

Research objectives

The aim of my work was the structural and functional characterization of longer regions of the GKAP postsynaptic protein. I chose NMR spectroscopy as the primary method of structure investigation. Examining the structural properties of the emerging GKAP:LC8 complex with respect to both partners was considered relevant, therefore the NMR interaction study was performed with two setups, with alternating isotope labelling of the two partner proteins. I complemented the NMR studies with computational methods along with other biochemical analytical techniques to comprehensively characterize the GKAP segments and their complexes.

My aims were the following:

- To produce and purify selected disordered regions of GKAP for further analysis, specifically the DLC binding region and the C-terminal PDZ binding domain.
- To characterize the purified disordered segments and complexes with different biochemical analytical techniques, with special attention to their size, molecular weight and hydrodynamic properties.
- To study the appropriately produced and purified constructs with NMR spectroscopy, focusing on their global and local, and also their residue-level structural properties.
- To analyse these regions in complex with their partners, describe their binding kinetics, and give a detailed structural description based on NMR measurements complemented with molecular dynamics simulations.

Theses of the Dissertation

Thesis 1

I produced and purified two regions of the postsynaptic density protein GKAP and the dynein light chain protein (LC8), all of them suitable for NMR spectroscopy measurements (in appropriate purity and concentration). I refined and optimized the production and purification protocol for the dynein light chain binding region of GKAP to achieve the best possible signal/noise ratio in higher dimension NMR measurements.

Publications related to this thesis point: [J1]
Oral communications and poster presentations related to this thesis point: [O2]-[O6], [P3]-[P11]

Thesis 2

I performed chemical shift assignments for GKAP segments not described earlier with NMR spectroscopy, and LC8 that has previously been analysed under different conditions.

2.a I performed the complete backbone and partial sidechain chemical shift assignment of the LC8 binding segment of GKAP (100% of N, HN, C', Cα, Cβ, Hα and Hβ atoms, and 52% and 61% of Cγ and Hγ atoms, respectively – see Figure 2).

2.b I performed the partial backbone assignment of the dynein light chain molecule, because neither the literature nor the BMRB database contains any dataset that was measured with the reducing agent TCEP present in the sample solution.

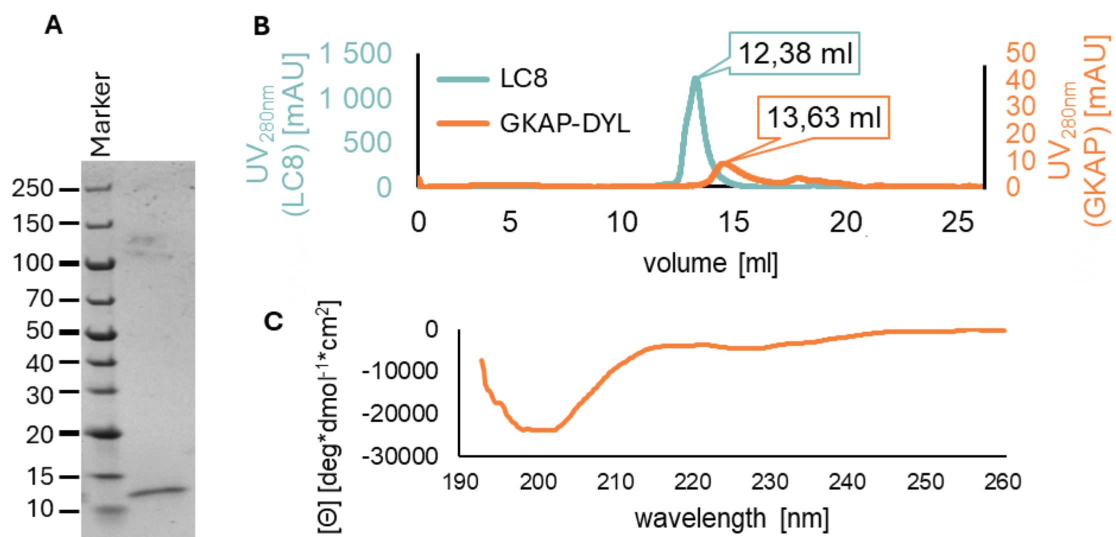
2.c I performed the complete backbone and partial sidechain chemical shift assignment of the C-terminal 43 residues of GKAP (see Figure 4).

Publications related to this thesis point: [J1]-[J2]
Oral communications and poster presentations related to this thesis point: [O1]-[O6], [P1]-[P11]

Thesis 3

I confirmed with NMR measurements, SDS-PAGE runs, SEC analytical chromatography and CD spectroscopy that the dynein binding region of GKAP is functionally disordered in its full length. Cα-Cβ secondary chemical shift evaluation showed, that the flanking region of the first binding site can be characterized with slight extended preference, while segments outside the binding motifs harbour varying extent of helical propensity (see Figure 2).

Publication related to this thesis point: [J1]
Oral communications and poster presentations related to this thesis point: [O2]-[O6], [P3]-[P11]


A
Marker
250
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B
UV280nm (LC8) [mAU]
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LC8
GKAP-DYL
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13,63 ml
50
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UV280nm (GKAP) [mAU]
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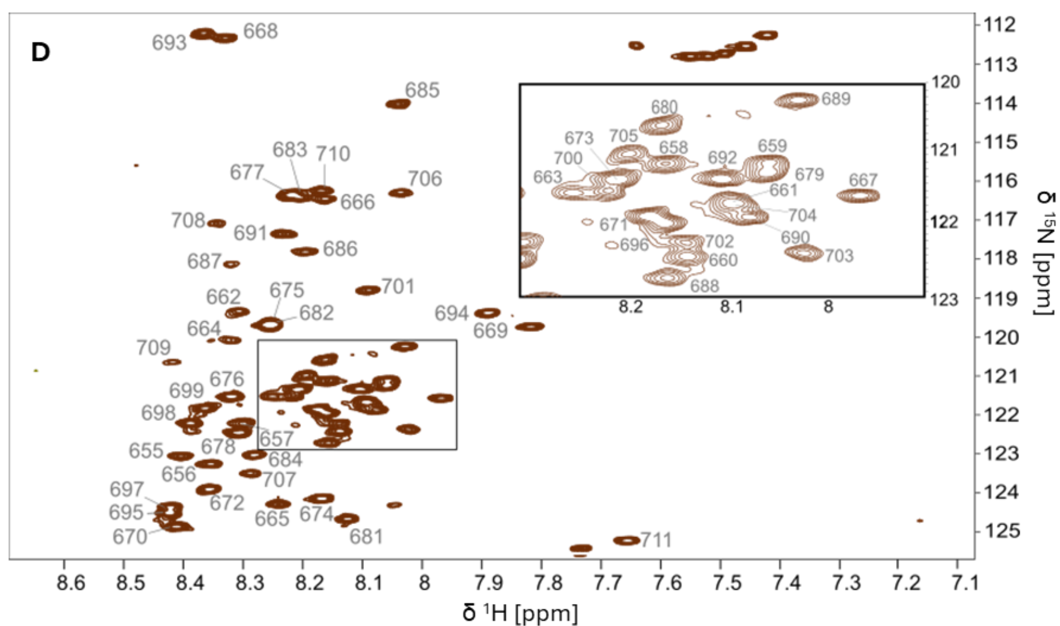
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δ 1H [ppm]
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124
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E
ΔδCα-ΔδCβ [ppm]
1,5
0,5
-0,5
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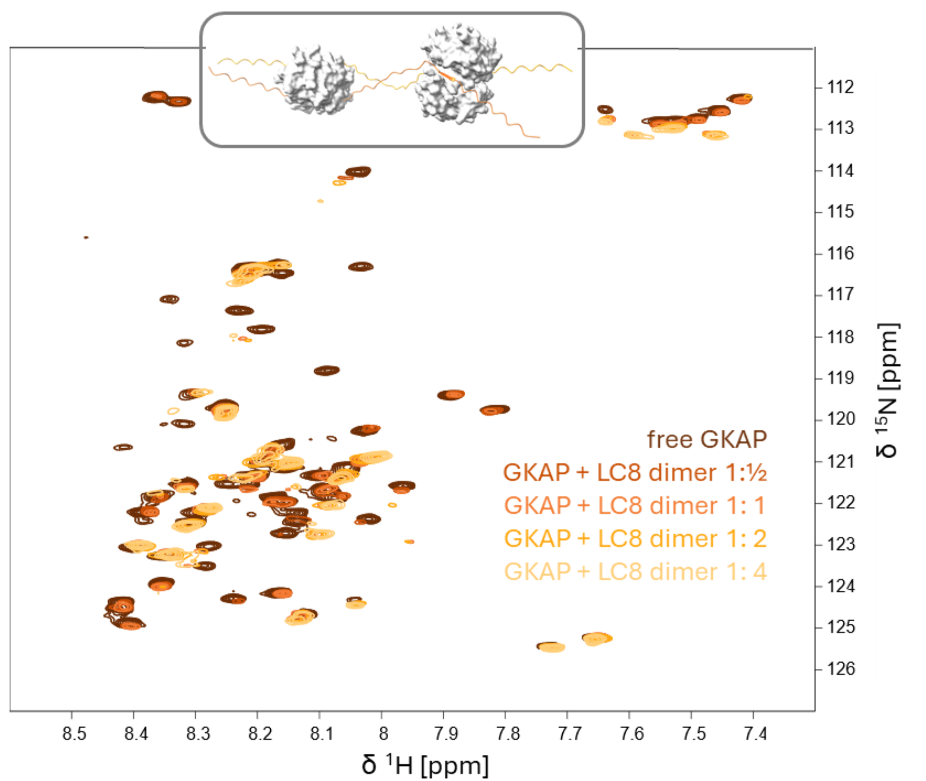
F
ΔδHα [ppm]
0,05
-0,05
RKKDFKKNRCLSIGIQVDDAEESEKMAESKTSSKFQSVGVQVEEEKCFRRFTRSNSV

Figure 2: Experimental analysis of the LC8-binding segment of GKAP in free form. (a) SDS-PAGE run, where the GKAP-DYL migrates slower than expected, seems to be 13 kDa instead of 7 kDa. (b) Analytical size exclusion chromatography chromatogram: GKAP-DYL seems to have higher hydrodynamic dimensions than expected from a similar molecular weight globular protein (like LC8). (c) Far-UV CD spectrum of GKAP-DYL confirms it to be disordered. (d) Low signal dispersion is visible on the ^{1}H-^{15}N HSQC spectrum of GKAP-DYL. (e, f) Cα-Cβ, and Hα secondary chemical shifts also confirm disorderedness, with very low secondary structure preferences. The binding motifs are highlighted with light yellow.

Thesis 4

I characterized the GKAP-DYL + LC8 complex structure with NMR. I concluded that specific segments of GKAP retain their flexibility even in the bound form. I also established that a well-defined stoichiometry complex is formed, as there is no sign of a newly emerging group of peaks on the HSQC spectrum during titration. I corroborated this finding with the LC8 NMR measurements: the disappearance of signals along with the absence of different, newly emerging peaks prove the formation of a large complex. I showed that the two LC8 binding motifs of GKAP behave differently upon binding, and that the flanking regions (up to 10-14 amino acids) around the SLiM residues are also involved in the interaction (see Figure 3).

I described the GKAP + LC8 complex with additional experimental methods. I confirmed with CD spectroscopy that no major change occurs in the secondary structure of the partners upon binding. I showed with BLI measurements that LC8 binds simultaneously to both binding sites on GKAP, and the dissociation constant is: Kd = 0.29 μM. I estimated the hydrodynamic properties of the complex with DLS measurement, and it was comparable to the previously calculated value (56 kDa).



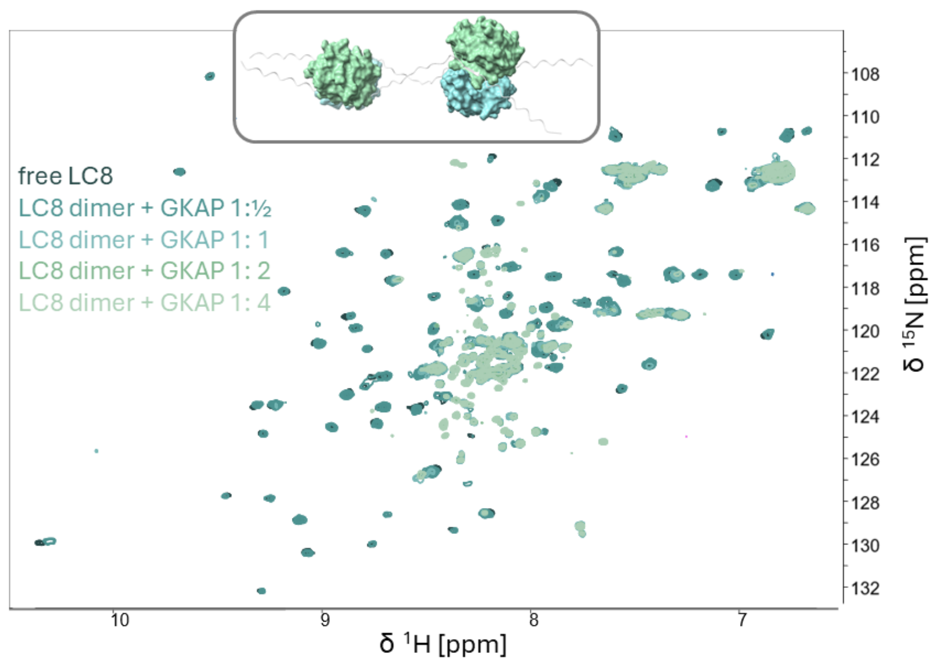


Figure 3: Isotopically labelled GKAP-DYL + unlabelled LC8 „forward" titration, ^{1}H-^{15}N HSQC spectra; and isotopically labelled LC8 + unlabelled GKAP-DYL „reverse" titration, ^{1}H-^{15}N HSQC spectra.

Publication related to this thesis point: [J1]

Oral communications and poster presentations related to this thesis point: [O2]-[O6], [P3]-[P11]

Thesis 5

I provided evidence that the C-terminal region of GKAP is intrinsically disordered almost along its entire length (see Figure 4). Based on NMR measurements, I concluded that a slight helical preference is visible between residues 950-970. There is a turn structure immediately before the binding motif, that is comparable to features of other extended PDZ-binding constructs described in the literature. The C-terminal region of GKAP largely retains its flexibility during binding, and positions far from the SLiM sequence are also involved in complex formation. These results highlight that complexes of full-length proteins cannot be modelled realistically based on experiments with only short peptides (SLiM sequences), but longer protein segments are necessary for more precise descriptions.

Publication related to this thesis point: [J2]

Oral communications and poster presentations related to this thesis point: [O1], [P1]-[P2]

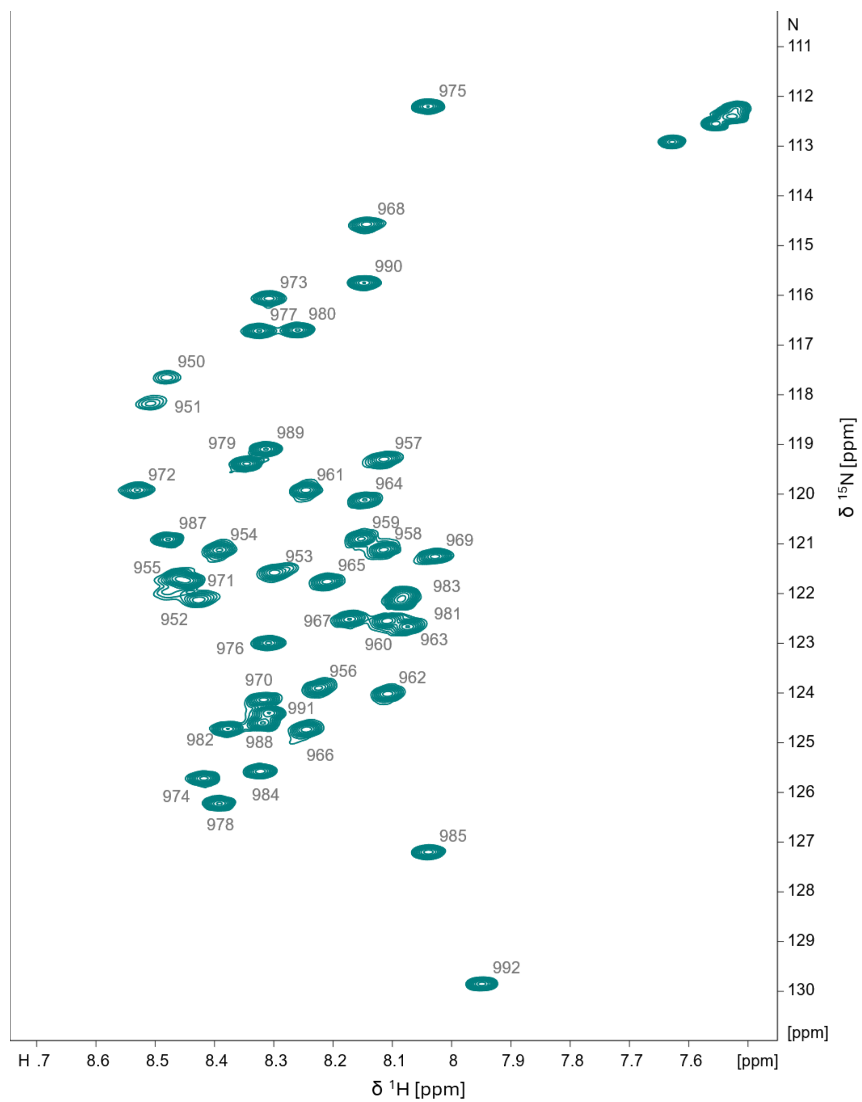


Figure 4: ^{1}H-^{15}N HSQC spectrum of GKAP-Ct43.

Discussion

During my PhD work I prepared and examined two separate disordered regions of the postsynaptic density scaffold protein GKAP. I used NMR spectroscopy and other experimental techniques. I provided experimental evidence that these segments are intrinsically disordered, and I characterized their structures. I showed that NMR and molecular dynamics calculations provide relevant insights when used to describe a complicated and flexible system. Based on my results I can conclude that flanking regions surrounding SLiMs (short linear motifs) might have high relevance in protein-protein interactions, therefore their inclusion in the structural investigation of disordered proteins and their complexes is fundamental.

I evaluated NMR spectroscopy measurements and molecular dynamics calculations regarding the GKAP dynein light chain binding region and LC8. I provided a detailed description of both global structural properties and residue-level details of their complex, pointing out the flexibility of the linker region and the flanking residues around the SLiMs participating in the interaction. Besides showing evidence on the disordered nature of the C-terminal region of GKAP, I also showed that the segment between residues 950-970 has slight helical preferences. My results confirm the conformational preferences of this segment in solution.

Structural and functional investigation of the postsynaptic density proteins is fundamental to understand cellular processes like neuronal signal transduction better. To design therapeutic procedures and potential medications, not only the structural properties of the postsynaptic proteins should be described in detail, but the connections between these elements and their dynamics. Understanding better the smaller steps will lead us to understand better the molecular mechanisms behind learning, memory and brain plasticity [13]. With describing the residue-level and functional unit-level structural properties of GKAP, I also contributed to the better understanding of molecules constructing the postsynaptic density.

Author’s list of publications

Author’s publications related to the Theses

[J1] **Eszter Nagy-Kanta**, Zsófia E. Kálmán, Helena Tossavainen, Tünde Juhász, Fanni Farkas, József Hegedüs, Melinda Keresztes, Tamás Beke-Somfai, Zoltán Gáspári, Perttu Permi, & Bálint Péterfia (2025). Residual flexibility in the topologically constrained multivalent complex between the GKAP scaffold and LC8 hub proteins. The FEBS Journal. 2026 Jan;293(1):76-95. doi: 10.1111/febs.70219.

[J2] **Eszter Nagy-Kanta**, Anna Sánta, Zsófia E. Kálmán, Jessica Amy Li, Perttu Permi, Zoltán Gáspári, Bálint Péterfia (2025). 1H, 13C, and 15N resonance assignment of the C terminal region of the disordered postsynaptic scaffold protein GKAP. Journal of Biomolecular NMR Assignments. 2025 Nov 5;20(1):2. doi: 10.1007/s12104-025-10253-2.

Author’s oral talks and poster presentations related to the Theses

[O1] **Eszter Nagy-Kanta**, Anna Sánta, Zsófia E. Kálmán, Perttu Permi, Zoltán Gáspári, Bálint Péterfia. Insights on the interaction between the postsynaptic GKAP and Shank scaffold proteins. September, 2025 – FEBS3+ Meeting, Belgrade, Serbia. Short talk.

[O2] **Eszter Nagy-Kanta**, Zsófia Dobson-Kálmán, Helena Tossavainen, Zoltán Gáspári, Perttu Permi, Bálint Péterfia. Structural description of the multivalent interaction of the post-synaptic scaffold protein GKAP and dynein motor molecule. June, 2024 – FEBS Congress, Milano, Italy. Speed talk.

[O3] **Nagy-Kanta Eszter**, Kálmán Zsófia, Gáspári Zoltán. A GKAP és a DLC2 multivalens komplexének jellemzése NMR és molekuladinamika segítségével. May, 2024 – Hungarian NMR Discussion Group Meeting, Balatonszemes, Hungary. Scientific talk.

[O4] **Eszter Nagy-Kanta**, Helena Tossavainen, Zoltán Gáspári, Perttu Permi, Bálint Péterfia. A posztszinaptikus GKAP fehérje és a dinein könnyűlánc (DYNLL2) multivalens interakciójának vizsgálata. October, 2022 - Hungarian NMR Discussion Group Meeting, Balatonszemes, Hungary. Scientific talk.

[O5] **Eszter Nagy-Kanta**, Bálint Péterfia, József Hegedüs, Melinda Keresztes, Viktor Farkas, Perttu Permi, Zoltán Gáspári. Functional and structural investigation of an

intrinsically disordered segment of the post-synaptic scaffold protein GKAP. October, 2019 – From Protein Complexes to Cell-Cell Communication, Esztergom, Hungary. Scientific talk.

[O6] **Eszter Nagy-Kanta**. Functional and structural investigation of the post-synaptic scaffold protein GKAP. June, 2019 – PhD Proceedings, Budapest, Hungary. Scientific talk.

[P1] **Eszter Nagy-Kanta**, Anna Sánta, Perttu Permi, Zoltán Gáspári, Bálint Péterfia. Connecting the dots in the postsynapse: interaction between the postsynaptic GKAP and Shank scaffold proteins. July, 2025 – 49th FEBS Congress, Istanbul, Turkey.

[P2] **Eszter Nagy-Kanta**, Anna Sánta, Perttu Permi, Zoltán Gáspári, Bálint Péterfia. Connecting the dots: the interaction between the postsynaptic GKAP and Shank scaffold proteins. March, 2025 – Hungarian Molecular Life Sciences Conference, Eger, Hungary.

[P3] **Eszter Nagy-Kanta**, Zsófia E. Kálmán, Zoltán Gáspári. Diversity in the bivalent and multivalent interactions of the LC8 hub protein. September, 2024 – FEBS3+ Meeting: Exploring Molecular Frontiers, Pula, Croatia.

[P4] **Eszter Nagy-Kanta**, Zsófia E. Kálmán, Bálint Péterfia, Zoltán Gáspári. Structural investigation of the multivalent complex of the GKAP scaffold and LC8 hub protein. August, 2024 – Annual Meeting of the Hungarian Biochemical Society, Budapest, Hungary. Poster prize, 2nd place.

[P5] **Eszter Nagy-Kanta**, Zsófia E. Kálmán, Helena Tossavainen, Zoltán Gáspári, Perttu Permi, Bálint Péterfia. Structural description of the multivalent interaction of the post-synaptic scaffold protein GKAP and dynein motor molecule. June, 2024 – 48th FEBS Congress, Milano, Italy; and Young Scientists' Forum, Pavia, Italy.

[P6] **Eszter Nagy-Kanta**, Zsófia E. Kálmán, Bálint Péterfia, Zoltán Gáspári. Egy multivalens fehérje-fehérje interakció elemzése NMR mérésekkel és bioinformatikai módszerekkel. April, 2024 – Biotechnology Days of the Hungarian Biotechnology Students' Association, Budapest, Hungary.

[P7] **Eszter Nagy-Kanta**, Bálint Péterfia, Fanni Farkas, Perttu Permi, Maarit Hellman, Helena Tossavainen, Zoltán Gáspári. NMR-based structural characterization of the post-synaptic density scaffold protein GKAP. November, 2021 – Hungarian Molecular Life Sciences, Eger, Hungary.

[P8] **Eszter Nagy-Kanta**, Anna Sánta, Fanni Farkas, Perttu Permi, Maarit Hellman, Helena Tossavainen, Zoltán Gáspári, Bálint Péterfia. NMR-based structural

characterization of the post-synaptic density scaffold protein GKAP. July, 2021 – The 45th FEBS Congress, Ljubljana, Slovenia (online conference).

[P9] **Eszter Nagy-Kanta**. Structural characterization of the postsynaptic density scaffold protein GKAP with NMR spectroscopy. June, 2020 – PhD Proceedings, Budapest, Hungary.

[P10] **Eszter Nagy-Kanta**, Bálint Péterfia, József Hegedüs, Fanni Farkas, Zita Harmat, Viktor Farkas, Gyula Batta, Zoltán Gáspári. Structural and functional characterization of the GKAP post-synaptic density scaffold protein. July, 2019 – The 44th FEBS Congress, Krakow, Poland.

[P11] **Eszter Nagy-Kanta**, Zita Harmat, Bálint Péterfia, József Hegedüs, Gyula Batta, Zoltán Gáspári. Structural and functional characterization of the GKAP post-synaptic density scaffold protein. March, 2019 – Hungarian Molecular Life Science 2019, Eger, Hungary.

Other publications not related to the Theses

[J3] Szabó, A.L., **Nagy-Kanta, E.**, Varga, S., Jáger, E.A., Pongor, C.I., Laki, M., Laki, A.J. and Gáspári, Z. Diffusion-based size determination of solute particles: a method adapted for postsynaptic proteins. FEBS Open Bio. 2026 Jan;16(1):25-40. doi: 10.1002/2211-5463.70111.

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