P-451

Structure and binding to Phosphorylated Phosphoinositides of the RGD1-RhoGAP domain of S.cerevisiae

D. V. Martinez[1], V. Prouzet-Mauléon[2], A. Clavères[2], M. Hugues[2], É. J. Dufourc[1], F. Doignon[2], B. Odaert[1]
[1]CBMN, UMR 5248, 14 bis allée Saint-Hilaire, 33607, Pessac, France, [2]LBM, UMR 5200, 71 avenue Edouard Bourlaux, 33883 Villenave d´Ornon, France

Lipid binding to proteins is driven by hydrophobic and electrostatic interactions, mediated through their acyl chains and headgroups. Among lipids, Phosphorylated Phosphoinositides (PIP) regulate essential biological processes at the membrane-cytosol interface, such as membrane dynamics in cell division. We focus our attention on the Rgd1 protein involved in polarized growth and mitosis in yeast. PIPs not only bind to Rgd1p, but also regulate its activity and cellular distribution. Rgd1p is composed of a F-BAR domain and a Rho GTPase activating protein (RhoGAP). To understand the effect of PIPs on Rgd1p, a solution NMR study has been performed on its RhoGAP domain. A complete resonances and secondary structure assignment has been achieved on the $^{15}N^{13}C$ labeled protein. The 3D structure has been elucidated with residual dipolar couplings and NOEs. Titration studies have been performed.with $PI(4,5)P_2$. The binding site involves a non conserved region of the RhoGAP family. A solid state ^{2}H and ^{31}P NMR study has been performed for deciphering the protein effect on membrane dynamics. This NMR study of the RhoGAP domain should allow us to understand at the residue level the regulation of the Rgd1p activity by PIP.

P-452

Tracking membrane-driven protein oligomerization using fluorescence lifetime and homo-FRET studies

A. M. Melo[1], A. Fedorov[1], M. Prieto[1], A. Coutinho[2]
[1]CQFM/ IN, IST, UTL, Lisbon, Portugal, [2]CQFM/ IN, IST, UTL and DQB/FCUL, Lisbon, Portugal

It has been proposed that anionic lipid membranes can promote "amyloid-like" fiber formation of several non-amyloidogenic proteins, *e.g.* lysozyme (Lz) [1]. To obtain information about the factors that govern the formation of these structures, Lz interaction with anionic lipid vesicles was studied using both steady-state and time-resolved fluorescence techniques. The biphasic variation of the mean fluorescence lifetime of Lz fluorescently-labeled with Alexa 488 (Lz-A488) as a function of the surface coverage of the liposomes was quantitatively described by a three-state model. This cooperative model assumes that monomeric Lz molecules partition into the bilayer surface and reversibly assemble into oligomers with k subunits ($k \geq 6$) [2]. The global fit to the data was done using the partition coefficients previously determined by FCS [3] and by taking into account electrostatic effects by means of the Gouy-Chapman theory. Finally, the oligomer stoichiometry was further narrowed down to $k = 6 \pm 1$ by homo-FRET measurements, which takes into account the binomial distribution of fluorescently-labeled monomers among the oligomers. Supported by project PTDC/QUI-BIQ/099947/2008 FCT/Portugal.

[1] Zhao et al. **2004** *Biochemistry* 43: 10302
[2] Melo et al. **2013** *J. Phys. Chem. B* 117: 2906
[3] Melo et al. **2011** *Biochim. Biophys. Acta* 1808: 2559

P-453

Biophysical features of electrostatically-driven lipid-protein fibers

A. M. Melo[1], L. M. S. Loura[2], J. Villalaín[3], F. Fernandes[1], M. Prieto[1], A. Coutinho[1]
[1]CQFM and IN, IST, UTL, Lisbon, Portugal, [2]Faculty of Pharmacy and Centre for Chemistry-Coimbra, UC, Coimbra, Portugal, [3]IBMC, UMH, Elche-Alicante, Spain

Anionic lipid membranes have been proposed to trigger "amyloid-like" fiber formation of several non-amyloidogenic proteins, *e.g.* lysozyme.[1] Here, the structural properties of the mixed lipid-protein fibers formed upon lysozyme interaction with POPC:POPS 80:20 LUVs at a low L/P molar ratio were studied in detail. Using complementary time-resolved FRET measurements, at the single-fiber (FLIM-FRET) and macroscopic (bulk) level, it is shown that these fibers display a multilayer structure, in which predominantly oligomeric lysozyme[2] is sandwiched between adjacent lipid bilayers. Additionally, FRAP measurements showed that both lipids and lysozyme display a slow lateral diffusion in these mesoscopic structures, due to extensive membrane surface crowding[2] and/or protein confinement between cross-bridged bilayers. Furthermore, 2PE Laurdan generalized polarization revealed that the formation of these fibers is accompanied by extensive membrane surface dehydration. Finally, IR measurements support that anionic lipid membranes cannot generically trigger "amyloid-like" fiber formation of lysozyme, since these supramolecular assemblies do not exhibit a rich β-sheet structure. Supported by FCT.

1. Zhao *et al.*, *Biochemistry*, **2004**, *43*, 10302.
2. Melo *et al. J. Phys. Chem. B*, **2013**, *117*, 2906.

P-454

Cholesterol effects on stability and intracellular processing of melanosomal membrane proteins

A. L. Milac, P. R. Alexandru, M. Chiritoiu, C. Tanase, G. Negroiu
Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, 060031, Bucharest 17, Romania

Malignant melanoma is one of the most insidious types of cancer. Melanosomal proteins members of the TRP-family are enzymes modulating the amount / quality of melanin. TRPs are also melanoma markers and immunological targets, so understanding their structure and function is crucial for their use in diagnosis and therapy. TRPs are type I membrane proteins, with an N-terminal catalytic domain in the lumenal side of the membrane, followed by a transmembrane (TM) segment and a C-terminal cytosolic short domain. Despite high sequence similarity, TRPs display different processing and trafficking along the secretory pathway and different responses to cholesterol-blocking agents, as shown by *in vitro* assays. To understand the structural basis of differences in interaction with membranes, we use molecular dynamics simulations of TM segments embedded in lipid bilayers, in the presence and absence of cholesterol. Our study is one of the few simulation studies on the importance of cholesterol for TM type I protein stability and trafficking. We discuss simulation data in correlation with experimental results and the possible impact on melanoma progression and therapy.

Financial support: Exploratory Research Projects, grant 156/ PN-II-ID-PCE-2011-3, Romanian Ministry of Education and Research.