Molecular mechanisms of the unfolded protein response

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The unfolded stress response (UPR) is one of the most ubiquitous signalling pathways in the eukaryotic cells, enabling real-time adaptive adjustments of the folding capacity in the endoplasmic reticulum (ER) and cell survival. Similar to other transmembrane signalling systems, UPR activation is a fast and reversible process that relies on multistep activation of three stress sensors: IRE1, PERK and ATF6. The initial step of UPR signalling has been suggested to involve dissociation of the most abandon ER molecular chaperone BIP from luminal domains of IRE1, PERK and ATF6, dimerization (or/and homooligomerization) of these stress sensors in the plane of the ER membrane resulting in activation of their cytoplasmic domains. The aim of this project is to elucidate how interactions between Hsp70 molecular chaperone BIP and the ER stress sensor IRE1 trigger the UPR cascade and to understand how the IRE1 multicomponent signalling pathway is regulated at the molecular level by physiological and pathological factors as well as through evolution.

Results

Conformational flexibility of the molecular chaperone BIP: To investigate the molecular basis of BIP function and regulation and well as better understand allosteric signal transduction in large multidomain and multicomponent biological machines, we have employed solution NMR spectroscopy. We have designed several BIP constructs, including truncations and mutations in functionally important regions, which allows us to use the "divide-and-conquer' strategy to dissect and characterize individual functional steps of the BIP chaperone cycle.

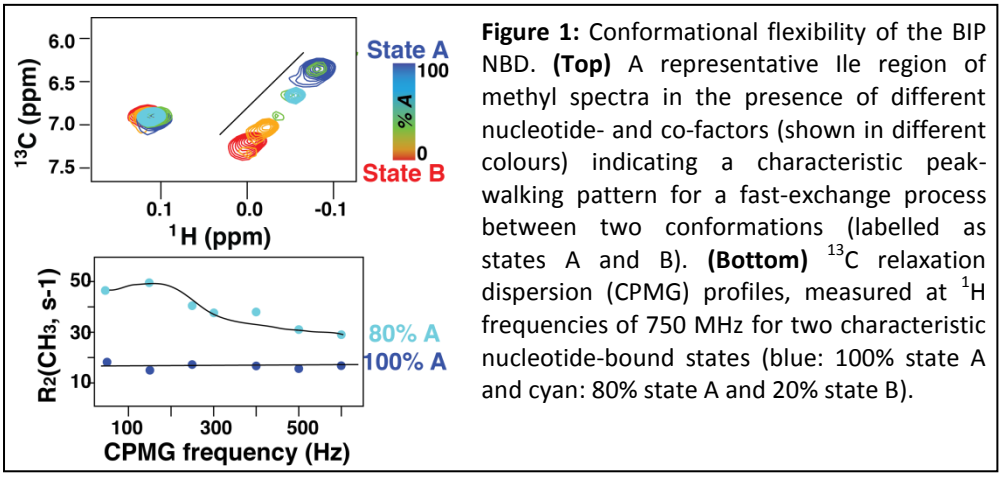

Figure 1: Conformational flexibility of the BIP NBD. **(Top)** A representative Ile region of methyl spectra in the presence of different nucleotide- and co-factors (shown in different colours) indicating a characteristic peak-walking pattern for a fast-exchange process between two conformations (labelled as states A and B). **(Bottom)** ^{13}C relaxation dispersion (CPMG) profiles, measured at ^{1}H frequencies of 750 MHz for two characteristic nucleotide-bound states (blue: 100% state A and cyan: 80% state A and 20% state B).

The structural and dynamics features of BIP were investigated in the presence and the absence of different ligands, including phisiologically relevant nucleotides, substrates, and ions. Our results demonstrated that BIP is an extremely flexible protein that co-exists as an ensamble of functianaly distinct conformations. The conformatinal equilibrium is under the tide control of several factors, such as fluxes of substrates, nucleotides and Ca^{2+} as well as redox conditions. These findings reveal several unique structural and dynamic features of the full length BIP and its nucleotide binding domain NBD (Figure 1), suggesting that conformational flexibility are extremely important for NBD function and can be fine-tuned by several ER enviromental factors.

Communication between the UPR stress sensor IRE1 and molecular chaperone BIP: The aim of this project is to build a structural model for the initial steps of IRE1 activation,

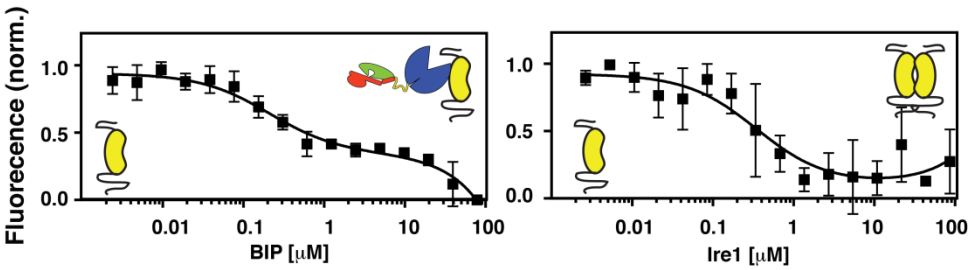

Figure 2: IRE1-BIP (left) and IRE1-IRE1 (right) interactions studied by MST. Cartoons indicate expected transitions upon interactions. Normalised MST data are shown; error bars indicate ±SE.

including IRE1 interactions with ER molecular chaperone BIP and unfolded protein substrates, dimerization and oligomerization of the IRE1 luminal domain. To understand this challenging, multistep process, we are using several BIP and IRE1 constructs to 'trap' individual steps of the IRE1-BIP activation cascade and characterize conformational features of 'trapped' steps using NMR, MicroScale Thermophoresis (MST), Mass Spectrometry (MS), Size-Exclusion Chromatography (SEC), and other biophysical methods. Interactions between IRE1 and BIP were characterized by MST are shown in Figure 2. The results suggest that at low (μM) IRE1 concentrations IRE1 dimerization and IRE1 binding to BIP are competitive processes with the dissociation constants around 0.5 μM.

The current work is focused on examination of the structural details of the transient IRE1-BIP complex as well as identification of binding interfaces for both proteins. We are employing an array of approaches: including methyl NMR for detailed characterization of IRE1-BIP interactions at near native conditions and concentrations (Figure 3); mass spectrometry to characterize high order BIP and IRE1 oligomerization at higher (tens μM) protein concentrations, mutagenesis to control protein-protein interactions and conformational changes, and computational approaches to analyse and interpret sparse experimental data. We hope to answer several fundamental questions regarding the molecular basis of IRE1 activation and regulation and well as better understand signal transduction in large, multicomponent protein-protein complexes.

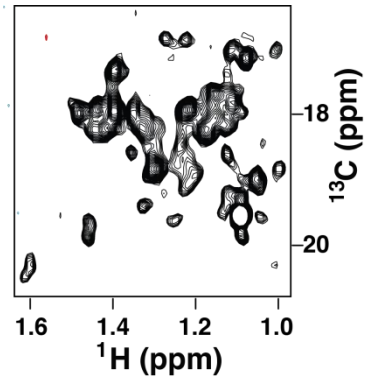

Figure 3: Two dimensional Alaβ HMQC spectrum of 1 μM BIP NBD, measured at ^{1}H frequencies of 950 MHz.

Publications

Zhuravleva A. & Gierasch L.M. (2015) Substrate-binding domain conformational dynamics mediate Hsp70 allostery. *Proc. Natl. Acad. Sci. USA* **112**:E2865-E2873.

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