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The original SPR-based Hsp70 biosensor for rapid quantification and revelation of Hsp70 substrates in complex solution

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The Hsp70s (heat shock 70kDa) proteins are ubiquitous molecular chaperones forming the center of protein folding, refolding and trafficking in all organisms. Hsp70 interacts with hydrophobic peptide segments of non-folded chains, as well as near-native, misfolded and aggregated proteins (clients), both at normal and at stressed conditions. To date, mostly the ADP-affinity chromatography or affinity purification chemical tool is generally used to detect the Hsp70 substrates. These techniques are time consuming and samples may contain non-specific ADP-like proteins or should be modificated before subsequent identification through biochemical techniques. We designed a new assay which allows real-time monitoring of the (i) trapping and retention of Hsp70 client proteins from blood plasma samples and (ii) highly efficient elution (>90%) of retained proteins. The Hsp70 trap assay has been implemented using a multichannel surface plasmon resonance (SPR) biosensor functionalized by Hsp70. Trapping and elution of client proteins is controlled by ATP/ADP exchange. The nucleotide and cations requirements were investigated and set up. In the three-step assay, the reference channel represents non-specific proteins was designed to control of elution. The SPR biosensor was used for the screening of healthy controls and clinical samples – myelodysplastic syndromes patients (MDS, subgroups – RARS, RAEB and AML). We observed significant differences between the tested groups and that the amount of misfolded proteins correlated with the progression of the disease. Our results are in a good agreement with the fact that oxidative stress as one of the main factor in MDS disease progression leads up to covalent modifications that destabilize and inactivate proteins. To the best of our knowledge, this is the first biosensor using Hsp70 to reveal a misfolded protein complexes. The Hsp70 trap assay seems to be a promising tool for general profiling of disease pathogenesis and progression. Using three-step assay, Hsp70 client proteins were detected in blood plasma in ~35 min at 25 ng/cm^2 to 150 ng/cm^2 concentration for healthy controls and AML patient, respectively.

Biography

Ondřej Pastva is a PhD student in the Biochemistry group at the Institute of Hematology and Blood Transfusion, Prague, Czech Republic. His main research interest is in utilizing of optical biosensors and bioassays for improving of diagnostics in Leukemias.

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