Development of targeted mass spectrometry-based proteomic assays for the discovery of a novel endothelium-specific pharmaceutical for the treatment of human vascular diseases

A. Project Significance and Specific Aims

The goal of the following proposal is to bring novel mass spectrometry know-how in the field of protein biochemistry to Poland from Canada and apply this knowledge through a collaboration in Warsaw involved in drug development for human vascular disease. The candidate, Dr. Dominik Domanski, is a Canadian scientist with expert knowledge in the field of *proteomics* which is the study of proteins through the use of mass spectrometry (MS), gained at the University of Victoria-GBC Proteomics Centre. Specifically, the candidate brings three years of experience in a novel and flourishing technique in quantitative proteomics called *multiple reaction monitoring* (MRM) which allows for the absolute quantitation of proteins in cells and blood samples. In the proposed project the candidate will work at the Mass Spectrometry Laboratory led by Dr. Michal Dadlez at the Institute of Biochemistry and Biophysics (IBB) at the Polish Academy of Sciences in Warsaw. The MRM knowledge, developed platform and applications will be applied in a collaboration with Dr. Katarzyna Koziak at the Warsaw Medical University (WMU) to the study of new escin derivatives which are potential drug compounds for the treatment of human circulatory diseases such as chronic venous insufficiency.

The ultimate goal is to bring the knowledge and expertise on the use of MRM methods in mass spectrometry-based proteomics to Poland by developing an MRM platform at the MS Laboratory at the IBB. In the last two years MRM has been a method in proteomics that has seen a tremendous increase in application by academic and industrial research centres for the measurement of protein targets from a variety of sources ranging from plants in agricultural research to measurement and validation of biomarkers in human plasma samples in clinical studies. Such research centres rely on the expertise and services provided by MS laboratories and have greatly shifted their demand in the last two years from *global* proteomic analyses to the *targeted* quantitative proteomic analysis of specific proteins provided by MRM assays. MRM has become a key technology with increased demand in proteomic service laboratories. The importance of this quantitative proteomics tool has been noticed by the scientific community as publicized in Nature Methods which has selected targeted proteomics by MRM as the potential method of the year in 2011 (Doerr, 2011). The introduction of such a powerful method to the research environment in Poland would result in significant scientific gains matching those of the international scientific community. The proposal aims to develop an MRM platform at the MS laboratory at the IBB which would include the development of a number of MRM assays and techniques capable of detecting and measuring specific protein targets of interest in cells and biological fluids such as blood plasma. The MRM assays through the use of synthetic peptide standards, labelled with stable-isotopes, would provide the ability to measure absolute protein concentrations at a specificity, accuracy, reproducibility and sensitivity not achievable using other analytical techniques. Additionally, the method's multiplexed ability allows dozens to hundreds of proteins to be measured in a single hour-long analysis making MRM a highly economical and fast analytical technique.

The developed MRM platform and assays would be valuable tools for a variety of proteomic studies in academic and industrial-biotech research groups, and we propose the platform's first application to be to the study of new escin derivatives as innovative endothelial drugs carried at the WMU. Escin, the extract derived from the horse chestnut (*Aesculus hippocastanum*) has shown potential in treatment of a number of human vasculature related ailments, and could be a source of novel drug compounds (Sirtori, 2001). Cardiovascular disease is the leading cause of death in Poland amongst men and women, and there is a search for new pharmacological compounds which are safe, effective and economical against this group of ailments. New and specific compounds derived from the natural plant product escin have the potential to fit these criteria and reduce the morbidity due to vascular diseases. The ongoing project by Dr. Koziak aims at synthesizing new escin derivatives and testing their biological effects with the final aim of producing a pharmacological compound. Initial studies with pure specific compounds on vascular endothelial cells *in vitro* will be followed by *in vivo* animal model studies analyzing blood plasma. Compounds will be selected based on their protective effects of the endothelium and vascular change, with the goal of discovering a single therapeutic pharmaceutical. In each case, a panel of specific biomarker proteins needs to be detected and accurately measured in abundance. MRM-based assays are an ideal technique to study such specific protein abundance changes in cultured cells and for the measurement of concentration changes in plasma proteins. The measurement of these proteins by MRM will bring a specificity, accuracy and protein target throughput that is not

achievable by any other analytical technique in biochemistry. The candidate has experience in developing a number of MRM assays for the absolute quantitation of proteins from plants, cells in cell culture or tissues, and from blood plasma. The experience covers the development of the assay from conception to validation and finally to implementation in pre-clinical research. This includes development of a variety of assays ranging from targeting tree enzymes not detectable by other analytical techniques, to discovery and validation of biomarkers in human plasma, to developing sensitive MRM assays in conjunction with antibodies for the detection of a low-level cancer biomarker. Therefore, the knowledge and experience the candidate brings and the strengths of the MRM assays will bring great novel benefits to the escin project increasing the quality of the results and strengthening its findings, ultimately increasing the chances of discovering a novel pharmacological compound.

In summary, the proposal for this project consists of two specific aims as follows:

Specific Aim 1: To build an MRM platform, at the IBB MS Laboratory, which is a novel and powerful analytical technique for the absolute and accurate measurement of proteins in biological samples, and to provide this technique in the future to the Polish research and industrial-biotech community thereby increasing the country's scientific competitiveness and progress.

Specific Aim 2: To apply this MRM platform and specific assays to the escin project carried out by Dr. Koziak at the WMU providing a means of measuring specific proteins in cell culture and animal model plasma at a specificity, accuracy and protein target throughput not achievable by other analytical techniques, therefore strengthening the quality of the escin project results and increasing the success of producing a therapeutic pharmacological compound for the treatment of vascular diseases.

B. Background

Mass spectrometry-based proteomics and Multiple Reaction Monitoring

In comparison to genomics, which strictly looks at the genetic information and expression of genes, proteomics has the advantages of analyzing the functional components of the cell, the proteins. Proteins are the machinery of the cell and their abundance and function ultimately define the organism's phenotype. In the last decade, the analysis and measurement of proteins in life science has been revolutionized by new technological developments in mass spectrometry (MS) for protein analysis, causing an exponential growth in the field of mass spectrometry-based proteomics. Initial work focused on the use of MS for specific protein characterization and global analysis of the protein complement of an organism in an attempt to identify all the proteins present. This was subsequently followed by the analysis of global protein expression, where changes in relative protein abundance were detected between different samples, amongst thousands of indentified proteins. Techniques such as iTRAQ (**i**sobaric-**t**ags for **r**elative and **a**bsolute **q**uantitation), which use stable-isotopic labeling tags to differentiate between 4-8 different samples, allow the identification of thousands of proteins with simultaneous information on *relative* protein abundance amongst these 4-8 samples (Ross *et al.*, 2004). The method is a non-targeted approach used in *discovery* type of experiments where any detectable and identifiable proteins that are altered in abundance relative to other samples are identified. iTRAQ has been widely used in research in the biomarker discovery phases. Such global methods, however, lack the specificity, accuracy, reproducibility, and most importantly the ability to report *absolute* protein amount quantitation that is obtainable by the MRM method. The trend within proteomics in the last two years has been towards hypothesis-driven type of proteomic experiments using the *targeted* approach of MRM. The protein targets were often identified in global-type *discovery* experiments and now the need exists to accurately quantify them with higher sensitivity by MRM. MRM is now often used for biomarker verification and validation, and when a highly sensitive and accurate protein measurement is required (Doerr, 2011, Kuzyk *et al.*, 2009).

MRM, also referred to as Selected Reaction Monitoring (SRM), can be best performed on triple quadrupole mass spectrometers (QqQ). Visualizing the components in linear sequence, these machines consist of an ionization source where biological molecules are first ionized and begin to fly, the first quadrupole mass filter (Q1), a collision cell (q) where ions are fragmented, and a second quadrupole mass filter (Q3) where passing fragment ions are selected before hitting a detector. Prior to MS analysis a protein sample such as blood plasma or a cell lysate is broken down into peptides by specific enzymatic trypsin digestion and these peptides are then separated by on-line reversed-phase liquid chromatography (LC) before entering the mass spectrometer. The LC separation allows for the sensitive detection of

individual peptides within a complex sample as they elute from the column. Peptides, ranging in size from 5 to 30 amino acids in length, are unique enough in sequence to serve as specific identifiers of specific proteins in a sample. The prior knowledge of peptide sequences is used to direct the MS in MRM mode. In MRM mode, the mass spectrometer filters a specific ion mass (with Q1) specific to a peptide of interest, subsequently fragments this parent peptide to produce fragment ions (in q), and further filters a specific ion mass (with Q3) which is now specific to a fragment of the peptide of interest and allows it to pass to a detector for quantitation of the signal. This mass filtering process is called an *MRM transition*, and is specific to a unique peptide which represents a specific protein being targeted. Therefore, the MRM signal for this unique peptide represents the level of the protein in the original sample. The QqQ machine can cycle through hundreds of different MRM transitions within a second and therefore the mass spectrometer is constantly targeting, detecting and measuring hundreds of specific peptides. The list of MRM transitions is specified by the user and targets specific proteins of interest in the biological system being studied. Within an hour-long LC separation and MRM analysis, hundreds of peptides (200-500), representing hundreds of proteins can be quantified. This is analogous to performing hundreds of highly specific and sensitive immunological assays like the Western blot analyses or ELISA (enzyme-linked immunosorbent assay) within an hour. Due to the high sensitivity of QqQ mass spectrometers, peptides in the attomole (10^{-18} mole) range can be detected making analysis of low abundance proteins possible in complex mixtures such as cell lysates and plasma (Kuzyk *et al.,* 2009). The specificity and accuracy of MRM is often increased through the inclusion of stable-isotope-labeled standard (SIS) peptides for each target of interest within the sample. SIS peptides are chemically identical, carrying the same amino acid sequence as the target peptide and therefore co-elute from the LC, but are distinguished within the MS due to isotopic differences (^{13}C,^{15}N). The inclusion of multiple (3-5) MRM transitions per peptide, with three selection criteria of *two specific masses* (Q1: parent peptide and Q3: fragment ion) accurate within 0.5 atomic mass unit resolution, and the specific *LC retention time* (accurate within seconds based on the SIS) makes MRM more specific than either Western or ELISA antibody-based type of assays. The high accuracy and reproducibility of the method is achieved by the inclusion of SIS peptides which compensate for variability introduced in sample preparation and variability within the MS signal over time. Additionally, the method can be made even more accurate by including multiple SIS peptides per protein. The inclusion of a known amount of SIS peptides allows for *absolute* quantitation of the peptide targets and therefore the proteins within the samples being analyzed, making it an ideal tool for accurate quantitation of proteins in research and pre-clinical analysis. The candidate has specialized in the use of MRM with SIS peptides, and in one study (with Dr. El-Sohemy, University of Toronto) has demonstrated a reproducibility in abundance measurement below 10% CV for 50 of 57 plasma protein targets in a study of 1088 human plasma samples. For these 57 plasma proteins the lowest level of detection achieved for a target was demonstrated to be 15 amol, with a linear concentration range of over 30,000-fold, and accurate (<20% error) measurements ranging from a concentration of 76 nM to 1100 μM of different proteins in plasma (Kuzyk *et al.*, 2009). Currently, the UVic-GBC Proteomics Centre, where the candidate is research leader, is capable of analyzing >200 protein targets in a single analysis. The economics of MRM analysis far outweigh those methods requiring antibodies for detection (Western or ELISA), especially considering the fact that an MRM assay can target proteins for which no antibodies are available and the development of an MRM assay takes days versus months required for antibody development.

New derivatives of escin as novel endothelium specific compounds for the treatment of vascular diseases

Herbal remedies, such as horse chestnut (*Aesculus hippocastanum*) have been used as medical treatments since the beginning of civilization. Its active ingredient, escin, despite its broad use as the remedy for chronic venous insufficiency, has not undergone careful scientific assessment. This is probably due to the fact that escin is a mixture of tens of structurally closely related triterpene saponin compounds and the analysis of cellular responses following its administration may vary depending on the preparation. The properties of escin, however, are well known as acting on the vascular endothelium in an anti-inflammatory, contractile and blood vessel sealing fashion (Sirtori, 2001). The project led by Dr. Koziak is based on the search for a novel endothelial drug amongst created, structurally novel escin derivatives. Dysfunction of endothelial cells contribute not only to the development of dysfunction of the circulatory system, leading to chronic venous insufficiency disease, with which the origin of the escin project is connected, but dysfunction of the endothelium also plays a major role in the rise of many other developed-world diseases such as atherosclerosis, neurodegenerative diseases, cancer, and diabetic complications. Venous insufficiency, characterized by chronic swelling of the legs and ankles often requiring medical intervention, is only one of the diseases caused by endothelium dysfunction but it occurs in 50-60% of woman and 10-55% of man, mostly after the age of 50 (Frick,

2000). Such vascular dysfunctions are often chronic causing large clinical and societal strains, and the financial burden on society from these will only grow with an increase in an aging population. Considering that these vascular diseases are mainly related to endothelial dysfunction, a new endothelial drug, based on the properties of escin, could gain very strong position in the fight against such diseases.

Dr. Koziak's escin project is based on the synthesis of pure new escin derivatives, initial *in vitro* analysis of their biological activity, and selection of compounds acting on the endothelium. These compounds will then be candidates for the new drug, which *in vivo* will inhibit the endothelium's activation and inflammatory response, and protect blood vessels from pathological change. The escin project aims at obtaining the novel active compound in as few steps, taking into consideration the economics of future synthetic technology of the therapeutic substance. Therefore, natural escin will be used from renewable resources, compared to chemical synthesis of a substance of same degree of structural complexity, creating a great benefit for the environment and Poland's energy resources.

C. Research Design and Methods

Phase 1 (Timeline: first 3 periods): Development of MRM platform and phase 1 of escin project - *in vitro* tests

Endothelial cell activation caused by exposure to hypoxic conditions, such as those that occur during blood stasis in chronic venous insufficiency patients, triggers an inflammatory response in the vein. Neutrophils are recruited and activated and are responsible for alterations of the venous wall, typically observed as varicose veins. The group of Dr. Koziak will synthesize a number of novel compounds based on the main active component of natural escin, which is β-escin, in the search for a well-defined and effective venotropic drug with minimum side effects. To this aim, in phase 1, the escin project will employ several *in vitro* techniques assessing endothelial cell function. These will include analysis of endothelial cell activation, via assessment of various signaling pathways, gene activation and protein expression. The proposed collaboration with Dr. Domanski will provide mass spectrometry-based proteomic analyses that would normally not be available to the escin project. Dr. Koziak's group will be in charge of performing the biological experiments and providing samples to Dr. Domanski who will design and perform the different type of proteomic analyses:

Pilot global proteomic analyses: In phase 1 a number of novel compounds will be tested on human endothelial cells in culture, with or without cytokine activation. Although many proteins that indicate endothelial cell activation are known and can already be selected as targets for MRM analysis, first a pilot global proteomic analysis, using the unbiased iTRAQ labelling technique, will be performed to identify additional potential targets. Using this method, proteins that are altered in abundance due to the effects of the tested compounds and endothelial cell activation will be identified. The candidate has experience in global proteomic analysis using iTRAQ labelling (Domanski *et al.,* 2007). Additional protein targets indicative of endothelial cell activation will also be discovered by looking at alterations in the phosphoproteome which is composed of phosphorylated proteins involved in various cellular signaling pathways. This will be performed by titanium dioxide (TiO_2) purification of phosphopeptides, due to the low abundance of phosphoproteins, prior to global expression analysis by iTRAQ, a method in which the candidate has experience (*Hem et al., 2010*). These initial global-type of analyses will provide a list of protein markers that are indicative of endothelial cell activation, and also guide the first selection of biologically active escin derivatives. Dr. Koziak's group will decide on the biological relevance of the altered proteins and phosphoproteins and decide for which MRM assays will be generated. **Milestone 1: Identify new targets for MRM and select biologically active compounds.**

Targeted MRM analyses: The *non-targeted* iTRAQ method, although unbiased and capable of identifying unknown proteins responding to escin derivatives, lacks precision in quantitation (CV>20%) and sensitivity in detection. iTRAQ can miss low abundance proteins who's quantitation may be vital in defining a beneficial response to the test compounds. Therefore in the next phase, MRM assays, with absolute quantitation capability and higher sensitivity, will be developed to measure, in a *targeted* fashion, specific proteins known to be involved in specific cellular responses, and those protein targets selected from the iTRAQ analyses. Of interest will be the response of proteins involved in the inflammatory response, cell migration, cell metabolism, cell proliferation and apoptosis. An indication of a potentially beneficial compound will be the reduction in the endothelial cells' activation and inflammatory response. These specific MRM targets will include 50-75 proteins known to be related to escin function and proteins involved in endothelium activation such as: adhesion molecules used for leukocyte migration (PECAM-1, CD99, ICAM-1, VCAM-1, α4β1-integrin), inflammatory chemokines and cytokines, cytoskeletal proteins (β-,γ-catenins, α-actinin, β-tubulin), endothelial junction proteins (JAMs, cadherins), endothelial matrix-metallo proteases (MMPs), cell

metabolism proteins (NADPH oxidase, eNOS, phospholipases), and a variety of signaling proteins (ERK1,2, JNK, p38 MAPK, PKC, PI-3K, αvβ3-integrin, Ras) (Huang *et al.*, 2010, Cook-Mills *et al.*, 2005, Carrasco *et al.*, 2007, Sirtori 2001). In addition to measuring the levels of these proteins, assessing the activation of signaling pathways, as indicated by changes in phosphorylation levels of specific pathway proteins, will be equally important for the determination of response in terms of the endothelial cells' activation and inflammatory response. MRM assays will also be developed to specifically target signaling pathway phosphoproteins for some targets mentioned above. This will be done as described by the candidate in his publication using the PPQ-MRM/direct-MRM methods that use phosphatase treatment or phosphorylated SIS peptides (Domanski *et al.* 2010). These methods will allow for absolute quantitation of phosphorylation changes indicating which signaling pathways are activated adding a vital additional layer of information to the protein abundance, which is protein activity.

The MRM platform: The development of MRM assays will include bioinformatics analysis to select unique peptide sequences that target each protein specifically, and then use these sequences to synthesize stable-isotope-labeled standard (SIS) peptides. These SIS peptides, added to the sample at a known concentration, not only allow for absolute quantitation of protein abundance, but also make the method more accurate and reproducible as they compensate for variability in sample handling and LC-MS machine performance (Kuzyk *et al.*, 2009). Additionally, specificity of identification is increased as the SIS co-elutes exactly with the natural peptide target. A library of SIS peptides will be generated for the protein targets of interest based on 3 to 5 highly detectable unique peptides. Using the pure SIS peptides, the sensitivity of the MRM assays will further be increased by optimizing the mass spectrometer parameters specific for each MRM transition to provide the highest possible signal. The linearity and accuracy of detection will be experimentally determined for each SIS peptide. The candidate has experience in the development and implementation of such MRM assays and would further expand his knowledge and experience by generating "winged-SIS peptides" which will take into account protein digestion efficiency making the MRM assay even more accurate than what has been performed up to date at the UVic-GBC Proteomics Centre in Canada. The candidate will therefore bring a wealth of know-how and experience, but also further his development by novel experimentation and a more independent research experience. The development of the above SIS library, the associated MRM assays and developed methods will constitute the "MRM platform" which can be applied to other experiments for other academic or private biotech groups in the future requiring a service from the Mass Spectrometry Laboratory at the IBB. Once the methods are in place, new additional SIS targets can be easily generated and samples can be easily and quickly assessed for specific and accurate protein abundance in a variety of samples.

Experimental problems and solutions: It is possible that certain protein targets of interest might not be detectable by direct MRM analysis of the sample due to very low cellular abundance. In these cases the candidate has experience in a number of techniques to solve such a problem. One method, termed GeLC-MS, was used by the candidate to quantify previously undetectable tree enzymes by gel-electrophoresis (SDS-PAGE) fractionation of the samples prior to MRM analysis (Zulak *et al.*, 2009). Another method, which the candidate is currently optimizing is fractionation of proteins or peptides based on isoelectric focusing (IEF) using the Agilent OFFGEL fractionator. This fractionation of a complex peptide sample prior to MRM analysis allows very low abundance protein targets to be detected.

The final multiplexed MRM assay (goal: ~50-75 protein targets) will allow all protein and phosphoprotein targets to be assessed in a single analysis, testing endothelial cells exposed to a number of novel compounds with or without cytokine activation. The MRM assay, due to its high accuracy, sensitivity, high reproducibility, high target throughput and ability to provide absolute quantitation of proteins in the sample, will be capable of verifying and validating the endothelial cells' response to the different test compounds and allow the selection of escin derivatives with potential therapeutic effects for subsequent animal model studies. Additionally, these global and targeted proteomic analyses will provide information on the molecular mechanisms of escin action which is currently only vaguely understood. After completion of phase 1, at least one publication on the results of the *in vitro* studies would result, presenting the scientific data as per Dr. Koziak's guidance. **Milestone 2: Development of MRM platform including a 50-75 MRM (phospho)protein assay, and selection of compounds for *in vivo* studies.**

Phase 2 (Timeline: last two periods): MRM analysis of effects of new escin derivatives *in vivo* for selection of effective pharmacological compound

The above *in vitro* tests will provide data to choose the best drug candidates to perform *in vivo* assays using a murine model of chronic venous insufficiency. The group of Dr. Koziak will use histological techniques to evaluate the efficacy of newly synthesized escin derivatives as endothelial cell protective agents. In addition, if the proposed

project is successful, blood plasma samples from these animal model experiments will be provided for proteomic analyses by Dr. Domanski. A number of selected escin derivative compounds from phase 1 will be tested for their effect on specific plasma protein concentrations using MRM, indicating a protective effect on the endothelium. The protein targets (~50) will include plasma proteins known to be related to cardiovascular disease, procoagulant, endothelial and oxidative stress markers, as well as protein markers of inflammation which may include some for which SIS peptides were generated in phase 1. Examples of plasma proteins involved in chronic venous insufficiency, escin action, and that may also be related to atherosclerosis include: CRP, IL-1β, TNF-α, soluble TNFα-receptor II, soluble intercellular adhesion molecule 1, leptin, endothelial lipase, protein C, protein S, fibrinogen, von Willebrand factor, tPA, VEGF, IL-6, and IL-12 (Huang *et al.*, Sirtori 2001). There are currently over 100 known cardiovascular and inflammation related plasma proteins that can be detected and quantified by MRM (Anderson 2005, Kuzyk *et al.*, 2009). The candidate is involved in projects at the UVic-GBC Proteomics Centre where 170 plasma proteins, including many of the above targets, can be detected and quantified by MRM within a 2 hour analysis. The development of SIS peptides and MRM assays for the new targets will be performed as in phase 1. Standard curves generated using SIS peptides will provide absolute plasma concentration values that can be compared over many analyses and experiments and not just a side-by-side few sample comparison that is obtainable by global proteomic analyses. High-flow liquid chromatography (0.3 mL/min) will be used during LC-MS analysis versus the commonly used in proteomics nano-liquid chromatography (0.3 nL/min). Recently, high-flow LC such as ultra performance liquid chromatography (UPLC) has been determined to be more robust, allowing more reproducible and higher throughput sample analysis in shorter times, with sensitivities surpassing those of nano-LC due to increased sampling. Since plasma is available in large amounts all analyses will be performed using the UPLC systems available at the IBB Mass Spec Laboratory. The candidate has experience in using such UPLC-MRM systems from the UVic-GBC Proteomics Centre. Plasma samples will initially be analyzed by MRM without complex sample pre-treatment. However, if targets of interest are not detected due to their lower abundance, plasma depletion of the 14 most abundant plasma proteins that makeup ~95% of the plasma proteome, will be performed using commercial columns. The candidate is currently working on optimizing methods to remove the top 2 or top 14 plasma proteins to reveal lower abundance targets of interest. An additional method that will be used to detect lower abundance targets is IEF fractionation using the Agilent OFFGEL apparatus. The candidate is currently using such methods and has observed an enhancement in detection of over 50% of certain cardiovascular related plasma protein markers that could not be observed by analyzing unfractionated plasma. An additional method that can be employed for the detection of extremely low level plasma proteins relies on the use of peptide antibody capture in combination with MRM detection, termed *stable isotope standards and capture by anti-peptide antibodies* (SISCAPA) (Anderson et al., 2004). This method has been developed by the candidate for the detection of a very low abundance cancer biomarker present in human plasma for CARE Biotechnologies. This allowed the detection and absolute quantitation of attomole amounts of the target per μL of plasma. This approach will only be employed for important targets that cannot be assessed by other means and for which a peptide antibody is easily commercially available. **Milestone 3: Develop MRM assay for ~50 plasma proteins to allow selection of therapeutic escin derived compound.**

The candidate will bring a large amount of experience in cell and plasma analysis by MRM and will further develop ongoing improvements of the methods from experience on the proposed project. The professional expertise of the candidate and the proposed project will significantly boost the chances of accomplishing the project goals of Dr. Koziak of discovering a novel endothelium-specific pharmaceutical for the treatment of human vascular diseases. Additionally, the success of the project will allow to establish a long lasting professional cooperation and leave long-term gains for the Polish scientific community which will be able to take advantage of the MRM platform developed at the IBB Mass Spectrometry Laboratory.

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