Executive Summary:

The Center for Proteomics was founded at the University of Rijeka in 2006, with the focus on monoclonal antibodies (mAb) development for cutting-edge applications, including proteome analysis. In frame of its core activity, the Center has generated several hundred hybridoma cell lines secreting mAbs to a variety of target proteins and has invested substantial efforts to make this collection available to the scientific community.

The major objective of the CAPRI2010 project has been to extend the mission of the Center in order to attract research entities with interests in mAb technology and proteomics. The key activities have consisted in upgrading of the infrastructural resources and mobilization of human resources through training and exchange of know-how actions.

The first major objective, the upgrading of the technological capacities, has been reached by procuring and putting into use the instrumentation (including high-speed centrifuge, ELISA multimode reader, mAb purification devices, high-capacity liquid nitrogen tank, nucleofector, ultracentrifuge, flow cytometer) for hybridoma processing and mAb development and characterization optimization. These procurements have enabled the Center to upgrade the laboratories for prokaryotic and eukaryotic protein expression and production of hybridoma and recombinant proteins based on secreted monoclonal antibodies, to upgrade its IT, dissemination and lab management capacities and to set up the laboratory for work with human cytomegalovirus.

Second, the Center has recruited research personnel to be responsible for the optimization of its workflow. In total 13 newly recruited persons and 4 permanent personnel were appointed to CAPRI2010 during the course of the project, with partial or full effort. Among these, two researchers with experience in both the academia and industry were affiliated to this project to work as leaders of two teams, one oriented at protein expression and mAb purification, characterization and quality control, and the other in charge of the hybridoma production optimization. Moreover, three of the newly recruited scientists were repatriated experienced researchers, which is particularly encouraged within the REGPOT scheme. The project team also involved six experienced laboratory engineers and one early-stage researcher. The coordinating person and all team members were supported by an experienced project manager.

The third project objective, the facilitation of knowledge transfer between the Center and its partner institutions, was accomplished by organizing two seminar-conferences at which international researchers of the highest rank in the field of immunology (from Washington University in St. Louis, University of Western Australia and Humanitas Clinical Institute Milan) gave talks at the Center. Furthermore, three advanced laboratory workshops were organised, with speakers from reputable institutions, including Hebrew University Jerusalem, Hannover Medical School, Oxford University, EMBL Grenoble, Cambridge University and Technical University of Braunschweig.

Fourth, to promote the exchange of know-how, a number of short-term visits of researchers from reputable institutions, including Medical University of Vienna, Helmholtz Centre for Infection Research and ETH Zurich, were organized at the Center. Also, four staff members were seconded to partner laboratories at Hebrew University Jerusalem, Max von Pettekonfer Institute Munich, Hannover Medical School, Pasteur Institute, EMBL and University of Zurich. Two training sessions, on usage of ELISA robot for the measurement of luminescent samples and use of siRNAs as a technique enabling mAb specificity determination as well as on analysis of the Varicella Zoster virus

proteome, were organised at the Center, in collaboration with Heinrich Heine University Duesseldorf and Max von Pettenkofer Institute Munich.

Finally, numerous actions undertaken to increase the visibility of the Center include the design of the project website (http://www.capri.com.hr/capri2010) and enhanced networking, which resulted in the publication of 14 articles in highly ranked journals and in joint applications of 25 project proposals to FP7 and other calls.

The immediate benefit of CAPRI2010 consists in the upgrade of the Center's technological setup, which has enabled the full implementation of protein expression and mAb production and characterization techniques. The result is an enhanced ability of the Center to produce the mAbs of the highest quality, which have been demanded worldwide due to their great significance in the studies of pathogenesis of viral disease. Moreover, this project ensured highly-qualified positions for researchers from Croatia and abroad. Through networking with excellent research institutions, either via two-way visits or collaborative research efforts, CAPRI2010 has also contributed to overcoming the problem of research fragmentation in the European Research Area.

Project Context and Objectives:

The Center for Proteomics was established at the University of Rijeka in 2006, with the focus on monoclonal antibodies (mAb) development for cutting-edge applications, including proteome analysis. Since its inception, it has launched several high-level scientific collaboration projects in EU and wider. In frame of its core activity, the Center has generated several hundred hybridoma cell lines secreting mAbs to a variety of target proteins and has invested substantial efforts to make this collection available to the scientific community.

Although the competence of the Center in protein expression and mAb production was proven in a short time upon its establishment, further development of the Center required specific qualitative and quantitative improvements of its capacities in terms of optimization of hybridoma processing and final mAb products as well as broadening of mAb-based tools for protein research. To reach this goal, five key objectives were identified within the CAPRI2010 project.

The first objective was to upgrade the existing technological capacities of the Center. The major obstacle in the establishment of a comprehensive antibody collection against proteins of interest was the shortage of equipment used on a daily basis and lack of instrumentation needed to produce and characterize mAbs of the highest quality. The aim of CAPRI2010 was to support the equipping of the Center with new research equipment (e.g. high speed-centrifuge, Western blot (WB) and ELISA robot as well as antibody purification devices). The growing number of new proteins for immunization, hybridoma cell lines and respective mAbs required the upgrade of storage capacities and the improvement of the information retrieval at the Center for Proteomics. Therefore, a new high-capacity liquid nitrogen tank was essential. Moreover, the existing lab management software needed to be upgraded to a fully operational level. Next, the production of mAbs in ascites is no longer a standard procedure in EU countries or in Croatia. To that aim, the Center needed to adopt alternative techniques for the production of larger amounts of mAbs. Finally, there is a strong need for delivering concentrated and fully characterized mAbs of the highest purity. For that reason, a liquid chromatography system for the optimization of the mAb purification process from supernatants was required.

The second project objective referred to recruitment of research personnel who would optimize the Center's workflow and disseminate its potential. Two research investigators, one with the background in biotechnology, and the other with the background in chemistry and expertise in immunology, were essential as the backbone of two teams, one focused on various methods of protein expression and the other oriented at hybridoma production optimization and protein purification in high yield, including their final characterization and quality control. The teams needed to comprise additionally experienced laboratory engineers (three in total). Since the increase in the visibility of the Center was an important objective of the CAPRI2010 project, an additional, third research investigator was required, with the focus on dissemination activities, like systematically approaching all relevant institutions in the area. Regarding the non-research personnel, the Center's expansion required a highly educated person, preferably with a degree in international economics, who would be able to cope with administrative and financial issues, organizing workshops and conferences but also with the dissemination activities to ensure visibility of the research entity.

The third project objective was to facilitate knowledge transfer between the Center and its partner institutions. The Center for Proteomics needed to acquire sustainable human and technological resources to organize annual two-week experimental, i.e. hands-on workshops for approximately 8 participants and conferences aimed at network building between regional and international experts,

researchers and former workshop participants. Together with their international colleagues, the research staff of the Center for Proteomics needed to prepare and schedule workshops, which would enable all participants to perform theoretically and practically all steps of the technology to generate mAbs and acquire various techniques in protein analysis. The researchers from reputable international institutes with expertise in this field of science needed to be invited to the Center to be the key figures in the promotion of the topics described.

In order to reach the fourth project objective, the promotion of exchange of know-how and development of strategic partnerships with institutions of similar interests, a number of short-term visits needed to be organized in both directions. Also, long-term research-oriented secondments between the Center for Proteomics and the partner institutions were essential. To enhance the research capacity of the Center for Proteomics, the intention was to build up essential know-how in mAb technology and associated techniques in protein analysis. This required opportunities for long-term professional development of qualified research staff/laboratory staff from the Center. The organisation of short-term visits to the partner organizations related to ongoing research projects was required. At the same time, scientists from the collaborating institutions should, by visiting the Center and directly taking part in its activities, significantly contribute to strengthening the research capacity of the Center and the Faculty of Medicine as such.

Finally, the overall objective of the CAPRI2010 project was to implement an action plan that would consist of various activities necessary to increase the visibility of the Center for Proteomics in the EU and associated countries: improvement of the existing web-site, reviews published in international research magazines, cooperation with National European Proteomic Societies and European Proteomic Association (EuPA), and the improved advertising policy with regular press conferences. The dissemination activities should target two audience categories: scientific community (both from academia and industry) and non-scientific community (public bodies, private investors and the general public).

Project Results:

The first major objective, the upgrading of the technological capacities of the Center for Proteomics, was reached by procuring and putting into use the instrumentation for building up a complete work flow line. This included the equipment for a) hybridoma development, screening and storage, b) mAb and protein production and purification in high yield, including final characterization, quality assurance and function analysis, c) protein expression, for work with baculovirus vectors and insect cells as well as for eukaryotic expression in mammalian cells and d) improvement of IT and dissemination capacities.

Concerning the equipment for hybridoma development, screening and storage, the growing number of new proteins for immunization, hybridoma cell lines and respective mAbs required the upgrade of storage capacities and the improvement of the information retrieval at the Center. Cryoconservation and storage facilities were upgraded by procuring a new high-capacity liquid nitrogen tank, freezers, large and combined refrigerators, cryo-cooler coldbox (for cuvettes) and control board (upgrade of the freezer). The existing data organization system for accurate management of the large numbers of hybridoma and mAb (lab management program) was adapted to the Center's specific needs and upgraded to a fully operational level. Its regular maintenance was ensured. The instalment of the microbiological safety cabinet upgraded the capacities of the Center for work with human cytomegalovirus, supporting hybridoma screening on antigens from relevant human pathogens. The procurement of fume hood significantly reduced the harm for health caused by methanol, acryl-amide or b-mercaptoethanol.

The original project proposal foresaw the procurement of a gel/blot imager, which would improve the Center's hybridoma development and screening potential. Since in the meantime this device was procured at the Faculty of Medicine from another source, the request for amendment of the Grant Agreement (GA) was sent to the European Commission in terms of deletion of this item from the proposed plan and its replacement with another piece of equipment (Individually ventilated cages (IVC) system). Following the approval of amended GA, the IVC system was procured, which satisfied the Center's increasing need for mice used for immunization, taking into account its core activity of high-throughput mAb production and different modes of immunization, for example, with inactivated mouse cytomegalovirus, leading to production of mAb against viral structural proteins.

Next, Annex I to GA originally foresaw the procurement of the hollow fibre system, which would enable mAb production in high yield. Since bioreactors already obtained for the same purpose fulfilled all the requirements of the Center for Proteomics regarding the high yield mAb production; the procurement of the hollow fibre system was evaluated by the project team as redundant for the time being. Following the approval of the European Commission, the following replacement devices were procured: sonicator system with accompanying components, which is crucial in several working lines of optimization of hybridoma production and screening, cooling microcentrifuge, which enabled the strict separation of the protein production and hybridoma production facilities, necessary for the prevention of cross-contamination, and compact flow cytometer, which enabled high-throughput (96-well) screening of hybridomas and thus met the research needs of the Center, which were previously unmet due to the inability of the old system to perform screening in a multi-well format.

Concerning the equipment for mAb and protein production and purification in high yield, including final characterization, quality assurance and function analysis, high speed-centrifuge and ELISA multimode reader were procured. New ELISA system significantly reduced time needed to characterise hybridoma cell lines and mAb quality and thus improved two major steps in a workflow.

It also has the ability to read luciferase-based assays, which in parallel with luciferase-engineered viruses, speeds up the process of mAb characterisation in terms of their in vitro blocking capacity, being a relevant functional characteristic. The same system enabled the Center to routinely test the hybridoma cell lines on the existence of mycoplasma, which is a crucial step in their quality control. In addition, bioflasks were acquired, which help move the mAb production in high yield away from ascites in the direction of cell culture-based systems. For this purpose, the workflow needed to be optimized by adapting all hybridoma cell lines (one hybridoma cell line per antigen) for which mAb was fully characterized (isotype determined, production in serum-free media confirmed, negative mycoplasma test, specificity confirmed in ELISA and at least one additional technique: Western Blot, immunohistochemistry, immunofluorescence or flow cytometry).

Next, the existing technological setup for mAb purification and characterization was upgraded: the liquid chromatography system (HPLC) was installed in order to reach high purity and concentration of produced mAbs or of expressed target proteins. This system is used on a daily basis for the extraction of mAbs from the hybridoma supernatant medium, serum-free medium collected in bioflasks as well as for the purification of His-tagged proteins produced in E. Coli and fc-tagged proteins produced in the eukaryotic 293T cells. Also, the supporting instruments-components (platforms, columns, column adaptors, concentrators, membranes, superloop for liquid chromatography system, gel electrophoresis system) and small laboratory devices (advanced MidJet System with accompanying start kits, Maxi gel system with microtiter combs, peristaltic pump, inverse microscope, pipettes, pipettors, multiple pipettes, thermoshaker, personal vortex, magnetic stirrer, laboratory dishwasher, precision balance, orbital shakers with platforms; horizontal DNA electrophoresis systems, MiniVE protein integrated vertical electrophoresis system, Multi Bio 3D shaker) were procured in accordance with Annex I of the GA in order to upgrade the existing technological capacities. Finally, the procurement of ultracentrifuge with supporting components was needed to upgrade the Center's capacity in terms of mAb characterization. It was approved by the Commission to be a replacement for an item originally planned but in the meantime bought from another source (lyophilisator).

Concerning the equipment for protein expression, the Center procured the 4D-nucleofector, equipment for transfection of eukaryotic cells, which combines electroporation with the chemical transfection and is very efficient in the DNA intake directly to the nucleus. It enabled the Center to improve its production line of proteins in eukaryotic cells, which is a better option when compared to E.coli production. The newly acquired device has been used for generation of many transient and stable transfectant cell lines, while the most straightforward way to improve the protein production capacity is the ability of nucleofector to introduce DNA in the nucleus of SF9 cells. This insect cell line (SF9) is most popular for the generation of eukaryotic proteins since it ensures sufficient quantity of the protein with significantly improved status of glycosylation in comparison to E.coli.

In addition, Annex I to GA originally foresaw the procurement of the automated cell-free protein expression system. However, during the course of the project, the Center's research team concluded that it was more reasonable to invest the efforts in the other direction and not to the immediate implementation of this system. Namely, the Center for Proteomics' main interest in protein expression is their usage as antigens to generate monoclonal antibodies as well as the production of recombinant proteins that my serve as models for potential therapeutic agents. The Center has adapted the production in prokaryotic (E.coli) system and eukaryotic cells. Eukaryotic expression is based mainly, but not only, on the baculoviral expression in the insect SF9 cells and fc-fusion protein expression in 293T cells. Immunizations performed when ordered peptides of the targeted antigens were used had successful outcomes. Thus, for each antigen, diverse and mostly sequential (parallel) modes of

expression have been in place. Therefore, it was found unreasonable to implement RTS cell-free expression system, mostly because of its main intrinsic constraint: it cannot introduce post-translational glycosylation, not even the disulfide bond formation or signal sequence cleavage. Following the approval from EC, the following apparatuses were procured instead of the cell-free expression system: NanoPhotometer, which allows the quantification of nucleic acids and proteins in a very low volume, reducing thereby substantially the consumption of precious protein-, DNA- as wells as mRNA- preparations, enlarging the linear detection range, omitting the need for sample dilution and improving the accuracy and speed of analysis, and orbital shaker for bacterial growth, which enabled the expression of larger quantities of proteins and facilitated bacterial and baculoviral expression as well as simultaneous expression of multiple constructs, thus speeding up the established protein expression processes.

Using the above described equipment, the following new protein expression techniques have been introduced or refined by the Center: periplasmic space bacterial expression system, MultiBac - a baculoviral expression system for co-expression of proteins (including mouse and human antigens - type I and type II membrane proteins) using multigene baculoviral vectors, and eukaryotic expression in mammalian 293T (human, kidney, embryonic) cells.

Finally, IT and dissemination capacities were upgraded through the procurement of one server, two personal computers with components, two laptop computers and FlowJo 7/9 Dongle for analyzing flow cytometry data on multiple computers. IT software capacities were upgraded with the software for basic biostatistics, curve fitting and scientific graphing, software for editing digital images and creating vector graphics, and software for data analysis. In addition, the existing project financial management software was upgraded in order to completely meet the project reporting requirements. The dissemination capacities of the Center were upgraded through the acquisition of an LCD projector and conference equipment in the seminar and conference room.

All in all, these procurements enabled the Center to upgrade the laboratories for prokaryotic and eukaryotic protein expression and production of hybridoma and recombinant proteins based on secreted monoclonal antibodies, to upgrade its IT, dissemination and lab management capacities and to set up the laboratory for work with human cytomegalovirus (HCMV). The latter is of particular importance for the Center, having in mind the aim to develop a prototype of a vaccine platform based on a live attenuated herpes virus. The relevance draws from the huge demand for new and efficient approaches in designing vaccines to various pathogens and tumours. There is an excellent research potential focused on vaccine development at the Center, which has already been evaluated as the highest level research endeavour with attractive potential for clinical studies, not only through publications in the top scientific journals (Slavuljica et al., Journal of Clinical Investigation, 2010), but also by the leaders in the field of virology and immunology (Schleiss, Journal of Clinical Investigation, 2010). In the short run, the set-up of this laboratory will significantly speed up the development of antibodies to HCMV at the Center, enabling the test of mAb specificity in conditions of virally infected cells.

The second project objective was to recruit research personnel to be responsible for the optimization of the workflow. In total 13 newly recruited persons and 4 permanent personnel were appointed to CAPRI2010 during the course of the project, with partial or full effort. Among these, two researchers with experience in both the academia and industry were affiliated to this project to work as leaders of two teams, one (Dr. Tihana Lenac Rovis) oriented at protein expression (30% effort) and the other

(Antonija Zurunic) in charge of the hybridoma production optimization and mAb purification, characterization and quality control (50% effort).

Moreover, three of the newly recruited scientists were repatriated experienced researchers, which is particularly encouraged within the REGPOT scheme: Dr. Anto Vrdoljak, with the background in biochemistry, responsible for coordination of mAb production optimization activities, quality control and dissemination (100% effort), Dr. Alexej Schmidt, with the background in biology, responsible for coordination of hybridoma cloning; recombinant mAb expression and recombinant fusion protein expression optimization activities as well as dissemination activities (50% effort on the project), and Mijo Golemac, MD, with the background in experimental pathology, responsible for support activity of hybridoma development, screening and generation of mAb database suitable for application in immunohistochemistry (50% effort on the project). These three researchers were appointed to the project in the 2nd project period, since there were some difficulties related to the selection of candidates and recruitment process (administrative procedures of hosting international researchers). All three researchers were offered positions which could be extended even after the expiry of the CAPRI2010 project (which is one of the missions of the REGPOT scheme), which were accepted by two appointed researchers.

The project team also involved two permanent experienced researchers, Dr. Marina Babic Cac and Vanda Juranic Lisnic (no contribution requested from EC), six newly recruited experienced laboratory engineers (50% effort each) and one newly recruited early-stage researcher, Maja Gulin (100% effort), who was responsible for the support activity of protein production optimization, i.e. establishment of the insect and mammalian cell expression systems for the generation of various recombinant constructs. Not all of the six engineers were affiliated to the project over the whole 36 months: there were in fact three positions for lab engineers foreseen in the project, in accordance with Annex I to GA. The first lab engineer (Karmela Miklic), responsible for the support of the project team working on hybridoma development, screening and storage optimisation, was recruited to the project from M5 to M36. The second lab engineer (Marko Lapat), also responsible for the support of the project team working on hybridoma development, screening and storage optimisation, was recruited to the project from M1 to M7 and was replaced in M21 with another engineer (Ante Mise). The third lab engineer (Suzana Malic), responsible for support of the project team working on protein production and purification in high yield as well as introduction of new and upgrade of existing protein expression systems, worked on the project from M1 to M20 and was (during the leave of absence) replaced with two lab engineers (Doris Malnar and Danica Rebic), one from M22 to M25 and the other from M26 to M36. The coordinating person, Stipan Jonjic (10% effort), and all team members were supported by an experienced project manager, Ani Gerbin (100% effort).

To summarize the work of the two project teams, the specific tasks of the team responsible for protein expression optimization included: establishment and upgrading of target protein expression systems with bacterial or eukaryotic techniques - growing of newly introduced cell lines 293T and especially Sf9 insect cells; transfection of both cell lines as well as additional cell lines as COS-7 eukaryotic cell line; protein purification adapting various programmes of the purchased HPLC system; protein characterisation using western blot technique; implementation of immunoprecipitation using antibodies of unknown specificity, silver stain detection and mass spec determination of antigens; testing of varicella zoster virus (VZV) mAb database on VZV lysates, including kinetic experiments; set-up of the laboratory for work with human cytomegalovirus at the Center for Proteomics.

Specific tasks of the team responsible for hybridoma production included: mice immunisation (2-3 immunisations per antigen; difference modes of immunisation with purified proteins, cell lysates,

virion particles and attenuated virus); testing of mice sera, fusion of mice splenocytes with SP2O cell lines in the process of hybridoma production; hybridoma selection using ELISA; cloning of perspective hybridoma cell lines and mAb production in high yield in flasks or in bioflasks after their adaptation to serum-free medium. They also supported the process of mAb purification optimisation using HPLC and performed basic characterization (e.g. Western Blot, UV absorption chromatography, FACS and, optionally, project specific testing according to collaboration partner's requirements). By the end of the 2nd project period, in total 146.2 person-months were consumed, which is close to 90% of the total number of person-months planned (165.6).

Related to this project objective, another task was to enter into at least three additional collaborative projects with innovative applications of mAbs by the end of the project. During the course of the project, the Center applied as a consortium partner or single beneficiary to in total 25 FP7, National Institutes of Health (NIH) and other calls for project proposals. Five were selected for financing (by NIH, Helmholtz Association and Public Agency for Research of the Republic of Slovenia), twelve were not on the priority list for financing and eight are still in evaluation.

The third project objective, the facilitation of knowledge transfer between the Center and its partner institutions, was accomplished by organizing two seminar-conferences at which international researchers of the highest rank in the field of immunology gave talks at the Center. The 1st CAPRI2010 seminar-conference was organised on 20th April 2010, with Prof. Wayne Yokoyama (Rheumatology Division, Department of Medicine, HHMI, Washington University School of Medicine, United States of America) and Prof. Mariapia Degli-Esposti (Centre for Experimental Immunology, Lions Eye Institute, University of Western Australia, Australia) as speakers. Prof. Wayne Yokoyama, also the President of the Society for Natural Immunity, gave talk on genomic analysis of NK cells, whereas Prof. Mariapia Degli-Esposti gave talk on NK cells regulation of adaptive immunity. This seminar-conference has significantly broadened the Center's research staff's perspectives to start the process of making the Center's mAbs visible to the scientific community, since it was decided that the first battery of mAbs that should be offered as a full product is the one covering mAbs to cell surface markers on mouse and human NK cells and their ligands. In addition, Prof. Yokoyama's presence contributed to one of the alternative approaches the Center intends to use in order to provide the most relevant antigens for immunisation: it was planned that, instead of using computationally predicted genes of e.g. VZV or mouse cytomegalovirus, a more direct approach based on the transcriptomic analysis should be used.

The 2nd CAPRI2010 seminar-conference was held on 20th September 2011 at the University of Rijeka, Faculty of Medicine. It was oriented specifically at establishing collaboration between two research groups: Antonella Viola group from the Humanitas Institute, Italy, and Stipan Jonjic group. The members of the two research groups and conference participants from other departments of the University of Rijeka Faculty of Medicine received an opportunity to exchange knowledge and obtain insights into the most recent research efforts on the topic of cellular immune mechanisms to tumours and viruses. This seminar-conference also aimed to broaden the Center's research staff's perspectives to start the process of making the mAbs towards the relevant human antigens, in particular the tumour markers. The majority of the Center's mAbs are directed toward herpesviral or cell surface markers on mouse and human lymphocytes and their ligands. However, the Center already made some efforts to develop markers that would be able to discriminate between undifferentiated and malignant differentiated cells in pancreatic tumours as well as mAb towards the pregnancy specific factor 1 (PSG), which is also implied to be upregulated in several malignant disorders, including colon.

Furthermore, three advanced laboratory workshops were organised in the 2nd project period, with speakers from reputable institutions. The 1st CAPRI2010 Advanced Laboratory Workshop 'Fc protein expression of type I and type II proteins' was organised from May 2 8, 2011 at the Center for Proteomics, with Prof. Dr. Ofer Mandelboim, Dr. Noah Stanietsky, Rachel Yamin, Yotam Bar-On, Yoav Bauman (Hebrew University of Jerusalem, Israel); Martin Messerle, Hannover Medical School, Germany and Antonella Viola, Humanitas Clinical Institute, Rozzano, Italy as international speakers and practical sessions organisers. The aim of the workshop was to provide intensive theoretical and practical overview of Fc-fused protein expression technique. The topics included: cloning, eukaryotic cell transfection, protein expression, ELISA and flow cytometry analysis and seminars on Natural Killer cell biology.

The 2nd CAPRI2010 Advanced Laboratory Workshop 'Advanced production techniques for complex protein biologics' was held from October 24-27, 2011 at the Center, with Dr. Imre Berger, Dr. Christoph Bieniossek, Frederic Garzoni, European Molecular Biology Laboratory (EMBL), Grenoble, France, Dr. Philipp Berger, Paul Scherrer Institut, Villigen, Switzerland, Dr. Raymond Owens, University of Oxford, Oxford, UK, and Dr. Christophe Romier, Institute of Genetics and Molecular and Cellular Biology, Illkirch, France as speakers and practical part organisers. The aim was to provide intensive theoretical and practical overview of protein expression and analysis with the focus on multi-expression systems and protein complexes. The topics included: multi-expression in E.coli, baculoviral system, mammalian cells and multi labelling technology.

The 3rd CAPRI2010 Advanced Laboratory Workshop 'Antibody Engineering from murine hybridoma to therapeutics' was organised from February 13-15, 2012 at the Center, with Prof. Dr. John McCafferty, University of Cambridge, UK, Prof. Dr. Stefan Dübel, Dr. Thomas Schirrmann, Technical University of Braunschweig, Germany, Dr. Dafne Müller, University of Stuttgart, Germany, and Prof. Dr. Marko Dolinar, University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia as international speakers and practical part organizers. The aim was to provide intensive theoretical and some practical overview of antibody engineering techniques and their application to develop antibody-based therapeutics. The workshop topics included: phage display method, cloning of hybridoma, transient expression in eukaryotic cells, humanization of a murine monoclonal antibody and bi-specific antibody constructs.

All workshops targeted PhD level students from University of Rijeka and other universities in the region (from Croatia, Slovenia and Bosnia and Herzegovina), and the number of participants was restricted to 50 in theoretical sessions and 12 in practical sessions. The main impact of these workshops on the Center for Proteomics is reflected in enhanced capacities of the Center's personnel for competitive research and built new / strengthened existing networks with regional and international researchers of the highest rank in immunology and virology-related research.

The fourth project objective was to promote the exchange of know-how. To this aim, ten short-term visits of researchers from reputable institutions were organized at the Center. The visit of Dr. Marko Poglitsch (Medical University of Vienna) was related to the collaboration of the Center with Dr. Marcus Saemann and Dr. Thomas Weichhart from the Medical University of Vienna on the immunomodulatory potential of the Rapamycin, the inhibitor of mTOR (mammalian target of rapamycin). In addition to in vivo studies aimed to determine the importance of mTOR signalling during cytomegalovirus infection, the participants discussed the development of monoclonal antibodies to mTOR and related signalling molecules. Because these proteins are much conserved, the

development of monoclonal antibodies is not an easy task. To that aim, the possibility of developing rabbit monoclonal antibodies or at least rabbit polyclonal sera suitable to study the molecular events involved in mTOR signalling was discussed. Concerning the visit of Dr. Stefan Jordan (Max von-Pettenkofer Institut, LMU, Munich, Germany), he recently discovered a new subset of dendritic cells (DCs) in spleen. He also showed that this DCs subset was depleted if mice were infected with the virus. The idea of his visit was to test a variety of available monoclonal antibodies to DCs in order to characterize the new subset. In addition, the task of the Center for Proteomics was to try to develop monoclonal antibodies to this DC subset. Stefan Jordan also conducted several practical courses at the Center for Proteomics to train PhD students and technical staff in various methods of generating and growing this DC subset. The aim of Dr. Torsten Sacher's visit (Max von-Pettenkofer Institut, LMU, Munchen, Germany) was the set up of experimental protocols and tools to study virus spread. His task was to introduce the team at the Center for Proteomics to different uses of recombinant viruses expressing inducible gfp (green fluorescent protein) to study the virus spread and tissue tropism. Dr. Daniel Giner-Sanchez (from: University General Hospital Alicante, Alicante, Spain) visited the Center to discuss the possible post doc position dedicated to further development of the hybridoma production, monoclonal antibody quality and processing. Annette Fink (from: Johannes Gutenberg-University, Mainz, Germany) visited the Center to discuss glycosylation in protein interactions. The purpose of visit of Dr. Jurica Arapovic (from: University of Mostar Faculty of Medicine, Bosnia and Herzegovina) was the establishment of the human cytomegalovirus (HCMV) analysis platform using human material. Dr. Luka Cicin-Sain (from: Helmholtz Centre for Infection Research, Braunschweig, Germany) discussed the development of mAb database directed at mouse cytomegalovirus (MCMV) at the Center. The discussion with Dr. Cicin-Sain at the Center for Proteomics resulted in the identification of most attractive targets within MCMV genome as well as in joint efforts on antigen production for the generation of respective mAb. The discussion with Dr. Annette Oxenius (from: ETH Zurich, Switzerland) was focused on immunodominant CMV proteins as potential targets for antibody production. The visit of Annette Fink and Vanessa Wilhelmi (from: Johannes Gutenberg-University, Mainz, Germany) was related to reporter cell assay tools and generation of mAbs to MCMV immunoevasins. Finally, Dr. Alexej Schmidt (from: HeliCure AB, Sweden) visited the Center to provide expertise in recombinant fusion protein expression for the design of the Thy1-Fc fusion protein used for mouse immunization and an immunoconjugate that aims to target NK cells to MCMV infected cells. The added value of these visits for the Center's research personnel lies in the useful insights into usage of mAb as tools in the analysis of certain protein groups. Since there is a constant lack of high quality mAbs able to support investigations under different conditions and in various techniques, the possible cooperation with the Center was discussed.

Also, four staff members were seconded to partner laboratories. One outgoing researcher was Maja Smurinic and her trainer in Israel (Hebrew University Jerusalem, Hadassah Medical School) Prof. Ofer Mandelboim, a long-standing collaborator of S. Jonjic's group. The purpose of Ms. Smurinic's stay in Jerusalem was the isolation of genetic material encoding the variable region of several Center's mAbs directed against the proteins of murine cytomegalovirus. Adriana Tomic visited Hannover Medical School, Germany to be trained in development of new constructs based on cytomegalovirus vector used to develop potential viral vaccines. The generated vector will be used at the Center for immunization and mAb generation to antigens inserted into it (for example, hemagglutinin, RAE-1 family of molecules, etc.). In addition, the results that will be published in prestigious journals will include anti-MCMV antibodies generated at the Center. The purpose of Dr. Marina Babic Cac's visit to University of Zurich, Switzerland was the training in techniques of work with mice with reconstituted human immune system and exchange of knowledge on the role of NK and CD8 T cells in Epstein-Barr Virus (EBV) infections using the humanized mouse model. This will specifically

boost the development of the Center's monoclonal antibody pool directed towards the receptors on the human and mouse immune cells and their ligands. Dr. Marina Babic Cac also visited Pasteur Institute, Paris, France, to exchange knowledge in research on the role of Ly49 receptors during cytomegalovirus infection and obtain insight into research performed at the guest institution, in particular on the role and lineage relationship of lymphoid tissue inducer cells in lymphoid organogenesis. This will equally support the development of antibodies towards important immune receptors and CMV proteins. Next, Adriana Tomic was seconded to European Molecular Biology Laboratory - EMBL, Grenoble, France, to be trained in MultiBac systems for expression of protein complexes. The MultiBac system enables the Center to co-express eukaryotic proteins in high amounts, thus facilitating the subsequent interactinal studies. It is an invaluable tool for simultaneous expression of different proteins. Therefore, this approach will be needed for the proteomes of larger viruses (larger than VZV), for example MCMV and HCMV, that are now in the focus of the Center's mAb development strategy. Specifically, some proteins form complexes and cannot be produced outside the complex stabilisation conditions. For such proteins, MultiBac is the only choice of antigen expression. Finally, Ilija Brizic was trained at Max von-Pettenkofer Institute, LMU, Munich, Germany) to acquire expertise on human cytomegalovirus (HCMV) growth and analysis in order to support the existing and develop new anti-HCMV mAbs and recombinant tools based on them. This is achieved through participation in HCMV projects running at the guest institution, primarily the characterization of envelope gH/gL complexes and their receptors. These proteins are involved in virus entry into cells and spread and hence, they are a potential target of antiviral treatment. Moreover, one part of the stay of I. Brizic at the guest institution was focused on MCMV gH/gL complexes. HCMV and MCMV protein complexes are of special interest for the Center due to the possibility that the generated antibodies have a blocking and neutralising capacity.

In addition, two visits of experienced scientists from partner institutions were supported, for essential training of the Center's qualified staff in order to enhance their competence to take part in the cutting-edge research projects. In the first project period, one training for qualified research staff was organised at the premises of the Center, with Dr. Mirko Trilling (Heinrich-Heine-University, Düsseldorf; Germany) as a trainer. The topics of the training covered: usage of ELISA robot for the measurement of luminescent samples; defining the blocking capacity of mAb, as its important functional property; using siRNAs as a technique enabling mAb specificity determination through the specific protein expression shut-down. The added value of this training for potential increase of the research capacity/quality of the Center reflects in the development and implementation of further techniques of mAb characterisation. Moreover, different possibilities related to the Center's growing collection of mAbs directed to cytomegaloviral proteins were discussed. In the second project period, another training session was organised at the Center, with Dr. Susanne Bailer from the project partner institution Max von-Pettenkofer Institute, LMU, Munich, as a trainer. The following training topics were discussed with Dr. Bailer: analysis of the varicella zoster virus (VZV) proteome; production of recombinant VZV proteins in prokaryotic and eukaryotic systems; functional analysis of the Center's antibody bank directed toward the VZV proteins.

The fifth objective of the CAPRI2010 project was to implement an action plan that would consist of various activities necessary to increase the visibility of the Center for Proteomics in the EU and associated countries. The numerous actions which were undertaken targeted both the scientific community (from the academia and the biotechnology industry) and the wider audience, including the general public. As to the dissemination activities targeting the scientific community, first, the existing Center for Proteomics' web-site (http://www.capri.com.hr) was improved. The website is designed in

a way that informs the scientific community about the ongoing activities and current programmes at the Center. The main concept of the Center's website includes: its main research activities (projects), workshop and research training opportunities, collaborators, lab members and job opportunities; products and services (custom hybridoma development, catalogue of specific hybridoma cell lines and mAbs at the Center: to cytomegalovirus proteins, to leukocyte receptors and ligands and to Varicella Zoster virus proteins) as well as contact forms. Next, the CAPRI2010 project web site (http://www.capri.com.hr/capri2010) was designed, with the following sections: general information, summary, collaborators, objectives, activities, results, dissemination, contact and links. The Results and Dissemination sections are regularly updated.

Furthermore, the Center's research personnel participated with invited talks, oral or poster presentations in more than 30 scientific conferences and seminars. These include: the European Proteomic Forum 2009, Berlin, Germany; European Congress of Immunology (ECI2009), Berlin, Germany; Macroregional Network for Cell Therapy in Hematopoietic Stem Cell Transplant Recipients, 2009, Brixen (Bolzano), Italy; 12th Cytomegalovirus Workshop, 2009, Boston, Massachusetts, USA; Annual Meeting of the Croatian Immunological Society, 2009-2011, Croatia; 35th International Herpesvirus Workshop, 2010, Salt Lake City, Utah, USA; 14th International Congress of Immunology, 2010, Kobe, Japan; Annual Meeting of the Austrian Society for Allergology and Immunology, 2010, Vienna, Austria; 20th Annual Conference of the International Association for Management of Technology (IAMOT2011), Miami Beach, Florida, USA, NK2011, Mainz, Germany; 21st Meeting of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and 27th Meeting of the International Society of Chemotherapy (ISC), 2011, Milan, Italy, 13th International Cytomegalovirus/BetaHerpesvirus Workshop, 2011, Nurnberg, Germany; 56th Annual Conference of the International Council for Small Business (ICSB 2011), 2011, Stockholm, Sweden; 7th ISABS Conference in Forensic, Anthropological and Medical Genetics and Mayo Clinic Lectures in Translational Medicine, 2011, Bol, Brac, Croatia; 36th Annual International Herpesvirus Workshop, 2011, Gdansk, Poland, 3rd EFIS-EJI South East European Immunology School (SEEIS2011), Arandelovac, Serbia, The British Society for Immunology Congress (BSI 2011), Liverpool, UK, 13th Meeting of the Society for Natural Immunity and NK2012, Asilomar, USA.

The enhanced networking activities and increased capacities for competitive research resulted in 14 scientific papers published by the Center's staff in highly-ranked journals (e.g., The Proceedings of the National Academy of Sciences of the USA, Current Opinion in Microbiology, Journal of Immunology, PLoS Pathogens, Journal of Clinical Investigation, Journal of Experimental Medicine, European Journal of Immunology, Journal of Virology, Trends in Molecular Medicine), in collaboration with the partner research groups.

In addition to the dissemination in scientific journals and at international conferences, numerous measures were undertaken in order to enhance the dissemination and exploitation of the research results generated at the Center for Proteomics. These measures were implemented A) either solely by the Center, B) or in collaboration with the project partners from reputable institutions in EU and associated countries or C) with the help of external consultants specialised in knowledge transfer and commercialisation in the life science field.

Concerning the dissemination activities targeting the general public and broader scientific community, in 2010 a press conference was organised to promote CAPRI2010 and TransMedRi, another FP7-REGPOT project of the University of Rijeka, Faculty of Medicine. The invited guests were all department chairs of the Faculty of Medicine, Chancellor of the University of Rijeka, representatives

from the Ministry of Science, Croatian FP7 National Contact Points, PhD students in the biomedical field, political representatives from the local government and Rijeka county as well as scientists from international partner institutions. In addition, the CAPRI2010 project coordinating person, S. Jonjic, gave several talks about CAPRI2010 at national-level workshops promoting the participation of Croatian research organisations in the 7th Framework Programme, including the FP7 Info Day 2008, University of Rijeka Faculty of Medicine, Rijeka, FP7 Info Workshop on newly published REGPOT calls for proposals, Faculty of Electrical Engineering and Computing, Zagreb, 2009, REGPOT Info Day 2010, University of Rijeka Faculty of Medicine, Rijeka and FP7 Health Info Day 2011 at the University of Rijeka Faculty of Medicine.

Also, the Center published a review on CAPRI2010 in the Projects magazine, which targets the EU and international research, business and policy making community. Next, the Center's research staff participated in the 2011 edition of the Festival of Science, a chain of events aimed at the popularization of science and the promotion of the University of Rijeka as a whole. Finally, the other conducted dissemination activities, referring to the presentation of CAPRI2010 and/or the Center's activities in the media, include 13 articles in the daily newspapers (Novi List, Vecernji list, Slobodna Dalmacija), 7 interviews on the radio and national television and 4 articles published on Internet portals.

In conclusion, it can be claimed that the CAPRI2010 project has achieved the great majority of objectives defined in the proposal. The immediate benefit of CAPRI2010 is the upgrade of the Center's technological setup, which has enabled the full implementation of protein expression and mAb production and characterization techniques. The result is an enhanced ability of the Center to produce the mAbs of the highest quality, which have been demanded worldwide due to their great significance in the studies of pathogenesis of viral disease. Moreover, this project ensured highly-qualified positions for researchers from Croatia and abroad. Through networking with excellent research institutions, either via two-way visits or collaborative research efforts, CAPRI2010 has also contributed to overcoming the problem of research fragmentation in the European Research Area.

Potential Impact:

First, the impact of CAPRI2010 on upgrade of the RTD capacity and capability of the Center for Proteomics in terms of the human potential (number of new researchers and training of research staff, improvement of research management), the educational significance (annual workshops and conferences, long-term training programs) as well as the quality of research (scientific equipment) carried out by the Center is assessed.

Scientists at the Center for Proteomics have been strongly embedded into the international scientific community, with more than 120 research papers published in prestigious research journals jointly with colleagues from partner institutions. In addition to collaboration through formal research projects (EU, NIH and others), a large part of this collaborative work has been based on the production and characterization of mAbs to cellular and microbial antigens. The antibodies produced at the Center have been of tremendous significance in studies of pathogenesis of viral diseases and some of these have been used worldwide. Although huge progress has been made at the Center for Proteomics during the first five years of its existence, before the commencement of the CAPRI2010 project its technological setup was still insufficient to fulfil the needs and expectations from the scientific community. In order to respond to all the challenges in the field and requirements by the collaborative groups and laboratories from both the academic and the biotech settings, significant improvements in protein expression technology and in production and characterization of monoclonal antibodies were required. In frame of CAPRI2010, several key measures were proposed in order to improve the capacities and competitiveness of the Center and as such, enable it to play an important role in the development of biomedical science, not only in Croatia, but also in South Eastern Europe.

As a result, the project directly enabled 13 new highly-qualified positions for perspective researchers and support personnel, with full or partial effort on the project. Next, two seminar-conferences, three advanced laboratory workshops, two training sessions, ten short visits from researchers from reputable institutions, six outgoing visits for training in partner institutions as well as numerous other networking and exchange of know-how activities were organised, which have had tremendous educational significance and substantial impact on the human potential of the Center.

Regarding the impact of CAPRI2010 on the quality of research, the realization of the project enabled the procurement of more than 50 larger and smaller apparatuses, essential for the upgrade of the laboratories for prokaryotic and eukaryotic protein expression and production of hybridoma and recombinant proteins based on secreted monoclonal antibodies, upgrade of the IT, dissemination and lab management capacities and set-up of the laboratory for work with human cytomegalovirus (HCMV). The latter is of particular importance for the Center, having in mind the aim to develop a prototype of a vaccine platform based on a live attenuated herpes virus. The relevance draws from the huge demand for new and efficient approaches in designing vaccines to various pathogens and tumours. There is an excellent research potential focused on vaccine development at the Center, which has already been evaluated as the highest level research endeavour with attractive potential for clinical studies, not only through publications in the top scientific journals (Slavuljica et al., Journal of Clinical Investigation, 2010), but also by the leaders in the field of virology and immunology (Schleiss, Journal of Clinical Investigation, 2010). In the short run, the set-up of this laboratory will significantly speed up the development of antibodies to HCMV at the Center, enabling the test of mAb specificity in conditions of virally infected cells.

In a broader sense, the procured scientific equipment will create a firm platform for other ongoing activities and future collaborative research and technological projects. Many of these items would

probably be much harder to acquire without the Research Potential programme support. The same applies to the recruitment of highly-skilled personnel, especially of nationals having left the country: the mechanisms to attract and retain these exist on a national (Unity through Knowledge Fund, National Science Foundation) and international level (FP7 People Programme), but those on a national level are still very limited in scope and highly depend on the availability of funds in the state budget. The procured equipment as well as exchange of know-how and personnel between the Center for Proteomics and its partner institutions thanks to the CAPRI2010 support have significantly contributed to upgrading of its capacities for cutting-edge research, which is being reflected in the increased number of papers published in reputable scientific journals and increased number of research results with potential commercial application. On the long run, these results contribute to strengthening the national, regional and European innovative capacity and competitiveness.

The measures foreseen to sustain the RTD capacity of the Center after the end of the CAPRI2010 project include: the application of at least five research proposals every year to calls for proposals published by the European Commission, National Institutes of Health as well as by national science and technology funding agencies (Croatian Ministry of Science, Education and Sports, Unity through Knowledge Fund, Business-Innovation Centre of the Republic of Croatia, National Science Foundation) and transfer of knowledge and technology activities from the academia to the business sector by commercialising the Center for Proteomics' research results (via licensing and spinning-off) or increased collaboration with the industry through sponsorship and consulting agreements.

Second, the impact of CAPRI2010 on integration of the Center for Proteomics in the European Research Area as a whole (emphasizing the cooperation with the more experienced organizations in other EU countries (partnerships, including twinning, exchange of know-how) and regionally based cooperation) is assessed. Strengthening research institutions is a key to stimulating business R&D investment in Europe. Related to the general aims of the European Research Area, one of the most prominent impacts of the CAPRI2010 project is in its small, but significant contribution to overcoming the fragmentation of research activities across Europe. The fragmentation as the prevailing characteristic of the European research base prevents Europe from fulfilling its research and innovation potential. Through networking with excellent research institutions from EU member states and from the region of SE Europe via the organised laboratory workshops, seminar-conferences, training sessions and individual secondments, CAPRI2010 has given its contribution to surpassing this problem. To the same aim, during the course of the project the Center applied, either as a single beneficiary or consortium partner, to 25 calls for proposals published by EC (FP7), NIH and other funding agencies. In total five proposals were selected for financing and eight are still in evaluation. Regardless of the evaluation outcomes, the Center has entered into numerous new informal collaboration projects with excellent research institutes and universities (e.g., European Molecular Biology Laboratory (EMBL Grenoble), Pasteur Institute, Helmholtz Centre for Infection Research, Utrecht University, Jozef Stefan Institute Ljubljana) and SMEs (Bia Separations, Slovenia, Genos, Croatia). Moreover, the already existing collaborative efforts with strategic partners from reputable institutions (Max von Pettenkofer Institute Munich, Hannover Medical School, Heinrich-Heine-University, Dusseldorf, Johannes Gutenberg University Mainz, Medical University of Vienna, Hebrew University Jerusalem, etc.) were additionally strengthened owing to a spectrum of exchange of know-how activities organised in frame of CAPRI2010.

Third, the contribution of CAPRI2010 to regional capacity building is assessed. Raising the country's competitiveness and international cooperation in biotechnology is a priority scientific field of intervention cited in the EU-Balkan countries Action Plan in Science and Technology. In view of the general needs in the area of biomedical research, a qualitatively and quantitatively satisfying protein development and production of mAbs present in the Center for Proteomics is one of the most promising approaches for developing and upgrading of the research capacities in biotechnology. Since biotechnology is not adequately developed in the region of South Eastern Europe, the Center's ability to serve as an example and a motivator to other institutions of the region should have an impact on the development of their biomedical research potential to organize similar start-up activities in that field.

Besides being a positive example, the Center for Proteomics identifies and supports centres that have the potential of developing into the institutions with capabilities comparable to those in the EU. CAPRI2010 enabled the Center to strengthen the collaboration with the University of Sarajevo and University of Mostar, Bosnia and Herzegovina, by organising incoming short visits and hosting PhD students during the advanced laboratory CAPRI2010 workshops. The hosted researchers have in that way increased their skills and competence for high-quality research at their institutions. This practice will certainly be continued in the future. Another important aspect of regional capacity building is the contribution to reduction in the regional disparities, not only regarding the whole region of South-Eastern Europe, but also taking into consideration the Croatian regions. Highly-qualified laboratory and research activities are concentrated almost exclusively in the Croatian capital city of Zagreb. Therefore, the formation of the collaborative environment between the Center and different scientific groups and institutions within the region and internationally has contributed to a more geographically balanced scientific and economical development. Finally, a very important factor is the economic situation in the region of South-Eastern Europe. Croatia lacks initiatives and funds which would improve its scientific status. CAPRI2010 has made its contribution to the improvement of the economic situation in the region, before all by opening new and sustainable positions for highly-educated personnel. Three organised international workshops and two seminar-conferences gathered experienced researchers from the EU as well as young researchers from the region, thus facilitating communication between research entities having similar scientific interests. This improved the responses to socio-economic needs of the region. What is more, three of the experienced researchers recruited to work on CAPRI2010 were nationals returning to Croatia after a period of appointment at institutions in Ireland, Sweden and Bosnia and Herzegovina. This has had a positive impact on the decision of experienced national researchers working abroad to return to the country. Consequently, the project has also contributed to preventing the brain-drain process, which currently represents a significant problem in Croatia and the bordering countries.

Fourth, the impact of CAPRI2010 on improvement of the potential of the Center to participate in FP7 projects is assessed. Even before the commencement of CAPRI2010, the Center successfully implemented three FP6 projects, either as a coordinator (CAPRI - INCO) or as a consortium partner (NK Defense and Therapy Marie Curie RTN; ProteomeBinders - Infrastructures). As described above, during the course of the CAPRI2010 project the Center applied, either as a single beneficiary or consortium partner, to 25 calls for proposals published by EC (FP7), NIH and other funding agencies, with in total five proposals selected for financing and eight still in evaluation. The numerous transfer of knowledge and networking activities organised in frame of CAPRI2010 have had a substantial impact on forming new consortia and enhancing those already existing. The collaboration with the academia and the industry has had the form of both formal (joint applications to FP7 and other research grants, implementation of joint research projects funded by national and international

funding agencies) and informal (exchange of research information and materials) collaboration in the field of immunology (primarily NK cell biology research) and virology (primarily cytomegalovirus research). Thus, the implementation of CAPRI2010, as a capacity building project, also complemented and facilitated the implementation of ongoing scientific projects of the Center, funded by the National Institutes of Health (NIH), Croatian National Science Foundation, Helmholtz Association and other funding agencies, which resulted in 14 publications in top journals (e.g., The Proceedings of the National Academy of Sciences of the USA, Current Opinion in Microbiology, Journal of Immunology, PLoS Pathogens, Journal of Clinical Investigation, Journal of Experimental Medicine, European Journal of Immunology, Journal of Virology, Trends in Molecular Medicine) during the course of the project. Thus, although the Center showed ability to attract funding from EU and other sources even before the start of CAPRI2010, this project has certainly had a positive impact on improvement of the potential of the Center to participate in FP7 and other projects, as it has served as a proof of continuous scientific excellence.

Finally, the main dissemination and exploitation activities are described. A crucial factor for success is to make the Center for Proteomics visible as a regional and international centre of excellence in the emerging field of antibody development for proteomics. Without this skill, the Center would have only a minor chance to compete successfully at the European level with other institutions for antibody development as well as for the know-how expertise in the same field. This is why a tremendous effort within the CAPRI2010 project was focused on increasing the visibility in EU and associated countries, by using the widest possible range of available means, from the web-page and personal contacts, to conferences, workshops and seminars, aimed to establish new collaborations with partners from the scientific community and from industry. The numerous actions which were undertaken targeted both the scientific community (from the academia and the biotechnology industry) and the wider audience, including the general public.

As to the dissemination activities targeting the scientific community, first, the existing Center for Proteomics' web-site (http://www.capri.com.hr) was improved. The website is designed in a way that informs the scientific community about the ongoing activities and current programmes at the Center. The main concept of the Center's website includes: its main research activities (projects), workshop and research training opportunities, collaborators, lab members and job opportunities; products and services (custom hybridoma development, catalogue of specific hybridoma cell lines and mAbs at the Center: to cytomegalovirus proteins, to leukocyte receptors and ligands and to Varicella Zoster virus proteins) as well as contact forms. Next, the CAPRI2010 project web site (http://www.capri.com.hr/capri2010) was designed, with the following sections: general information, summary, collaborators, objectives, activities, results, dissemination, contact and links. The Results and Dissemination sections are regularly updated.

One of the specific tasks of CAPRI2010 was the establishment of a large database and bioinformatics unit, which follows the flow of data regarding mAb production and hybridoma and antibody bank formation. This database provides scientists with various and accurate information about the mAbs and their application, and creates a collaborative environment between the Center and different scientific groups and institutions within the region and internationally. An enhanced cooperation in the area of protein research could significantly contribute to overcoming the technological gap and to enhance the economical growth of Croatia and the whole region. The two main categories of end-

users of the Center's mAb are the basic research community (academia) requiring monoclonal antibodies as inputs for research on biology of different animal or human viruses and cell receptors, and applied research community (dominated by biotechnology SMEs), which use the Center's antibodies as tools for development of different diagnostic or therapeutic products.

Furthermore, owing to the CAPRI2010 project funds, the Center's research personnel participated with invited talks, oral or poster presentations in more than 30 scientific conferences and seminars worldwide. In addition to the dissemination in scientific journals and at international conferences, numerous measures were undertaken in order to enhance the exploitation of the research results generated at the Center for Proteomics.

These measures were implemented A) either solely by the Center, B) or in collaboration with the project partners from reputable institutions in EU and associated countries or C) with the help of external consultants specialised in knowledge transfer and commercialisation in the life science field. The major step consisted in the identification and analysis of the Center's research results with commercial potential. Also, contacts with consultants-experts for intellectual property and the University Technology Transfer Office were intensified, which resulted in the adoption and implementation of the intellectual property protection and commercialisation strategy of the Center for Proteomics.

Measures taken by the research and management personnel of the Center for Proteomics in frame of CAPRI2010 include the participation in six workshops on knowledge transfer and academic entrepreneurship, aimed at enhancing the capacities of the Center for business-oriented thinking and industry-oriented research. Next, Expression of interest for the Technology Transfer Office of the University of Rijeka was prepared, which used it to assess the possibilities for intellectual property protection of the research results generated at the Center; help the Center find strategic partners from the business sector and help the Center in establishing a spin-off company, the first biotech spin-off of the University of Rijeka. Moreover, the Center created the Searching for Partners Sheet, aimed at finding partners interested in the submission of proposals to collaborative FP7 Health funding opportunities. The Center also contributed to the Competence Database of the University of Rijeka, aimed to identify the knowledge base and intellectual property existing at the University and form a public database which will comprise these data at the level of University. The collected data should facilitate the establishment of collaboration of researchers from the University of Rijeka with different stakeholders on the market, locally and internationally. In the questionnaire, the Center described its skills, competences, expertise, key research interests, major facilities and equipment, previous experience with knowledge transfer activities and products and services of potential commercial value and previous collaboration with academic and profit institutions. Finally, three project proposals were submitted to calls published by BICRO, an agency which funds the initial stage of innovative scientific-entrepreneurial projects. Two proposals ('Croatian Monoclonal Antibodies' and 'MAT promoter a novel DNA region for enhanced protein expression') were not on the list for financing and one ('A novel, strong CMV-promoter for expression of proteins and RNA molecules') is still in evaluation.

Measures taken by the research and management personnel of the Center for Proteomics in collaboration with the project partners from EU and associated countries include the creation of the Business plan of the Center for Proteomics, in collaboration with Vienna University of Economics and Business, Institute for Entrepreneurship and Innovation, headed by Prof. Dr. Nikolaus Franke,

and submission of a project proposal 'Becoming entrepreneurial: Knowledge transfer from the University of Rijeka Faculty of Medicine to the biotechnology business sector' to the call published by the Science and Innovation Investment Fund (IPA), in collaboration with Prof. Martin Messerle, Medical School Hannover (the evaluation of the proposal is still in progress).

Measures taken by the research and management personnel of the Center for Proteomics with the help of external consultants specialised in knowledge transfer in life sciences include: 1) the evaluation of the business strategy of the Center by Chedo Bagi, MD, PhD, a Senior Research Fellow in Pfizer, Inc, and a freelance consultant specialized in organizational and research setup in biomedical research organizations, including optimization of science and technology groups, protection and utilization of intellectual property, and due diligence services and 2) evaluation of the Business Plan of the Center by Isis Innovation Ltd consultants, a technology transfer company of the University of Oxford, which has assisted in the formation of more than 70 spin-out companies and has negotiated in more than 800 commercial agreements with more than 500 technology licenses / options. They reviewed the initial Business Plan of the Center and proposed some solutions in regards to the Center's commercialisation strategy.

Concerning the dissemination activities targeting the general public and broader scientific community, in 2010 a press conference was organised to promote CAPRI2010 and TransMedRi, another FP7-REGPOT project of the University of Rijeka, Faculty of Medicine. The invited guests were all department chairs of the Faculty of Medicine, Chancellor of the University of Rijeka, representatives from the Ministry of Science, Croatian FP7 National Contact Points, PhD students in the biomedical field, political representatives from the local government and Rijeka county as well as scientists from international partner institutions. In addition, the CAPRI2010 project coordinating person, S. Jonjic, gave several talks about CAPRI2010 at national-level workshops promoting the participation of Croatian research organisations in the 7th Framework Programme (FP7 Info Days). These activities were organised either by the University of Rijeka Faculty of Medicine or by the Croatian National Contact Points for FP7, and they represented an opportunity to meet with the national policy-makers and discuss the directions of the country's scientific and technological policy. Here, it is very difficult to estimate the actual impact of CAPRI2010 on the national S&T policy because many other factors have a more prominent influence on it (e.g. economic situation which determines the state budget for science). Next, the Center published a review on CAPRI2010 in the Projects magazine, which targets the EU and international research, business and policy making community. Also, the Center's research staff participated in the 2011 edition of the Festival of Science, a chain of events aimed at the popularization of science and promotion of the University of Rijeka as a whole. Finally, the other conducted dissemination activities, referring to the presentation of CAPRI2010 and/or the Center's activities in the media, include 13 articles in the daily newspapers (Novi List, Vecernji list, Slobodna Dalmacija), 7 interviews on the radio and national television and 4 articles published on Internet portals. Success of the University of Rijeka Faculty of Medicine, Rijeka, Croatia with two applications to Research Potential calls for proposals (CAPRI2010 and TransMedRi) has been widely disseminated in Croatia and has boosted the applications of other laboratories, departments and institutions to FP7 Capacities calls for proposals, which in the case of positive evaluations directly results in new highly-qualified positions as well as extended capacities for competitive research in Croatia.

List of Websites:

The project website address is: http://www.capri.com.hr/capri2010.html.

Contact details are as follows: Prof. Dr. Stipan Jonjic, Center for Proteomics, University of Rijeka Faculty of Medicine, Brace Branchetta 20, 51000 Rijeka, Croatia; E-mail: jstipan@medri.hr; phone: +385-51-651-206; fax: +385-51-651-261