European Cooperation in the field of Scientific and Technical Research
- COST -

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COST 047/13

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM1307: European network to integrate research on intracellular proteolysis pathways in health and disease (PROTEOSTASIS)

Delegations will find attached the Memorandum of Understanding for COST Action BM1307 as approved by the COST Committee of Senior Officials (CSO) at its 188th meeting on 14 November 2013.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action BM1307
EUROPEAN NETWORK TO INTEGRATE RESEARCH ON INTRACELLULAR PROTEOLYSIS PATHWAYS IN HEALTH AND DISEASE (PROTEOSTASIS)

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4114/13 "COST Action Management" and document COST 4112/13 "Rules for Participation in and Implementation of COST Activities" , or in any new document amending or replacing them, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to promote collaboration among experienced and early-stage academic, clinical and industry-based European researchers involved in intracellular proteolysis research and to translate scientific knowledge into cutting edge innovations that will improve human health.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 68 million in 2013 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of section *2. Changes to a COST Action* in the document COST 4114/13.

A. ABSTRACT AND KEYWORDS

Intracellular proteolysis is critical for cell homeostasis and to prevent pathologies such as cancers, immune diseases and neurological disorders. Its involvement in the control of almost every biological process has generated a huge interest amongst scientists from very diverse backgrounds, which in turn has resulted in both a tremendous advance of the knowledge and an important fragmentation of the field. The COST Action PROTEOSTASIS will coordinate and integrate the efforts made by European research teams to better understand intracellular proteolysis and to translate novel discoveries into products of clinical and/or economical values. It will gather all European academic, clinical and industrial partners willing to foster collaboration and training in the field through the organization of meetings, workshops and exchange programs. The implementation of different translational projects within the network will generate a "mind-agitating" atmosphere that will promote both creativity and reactivity. To help overcome the energy-barrier that too often limits development of novel and original ideas and concepts, a core dedicated think-tank created within PROTEOSTASIS will detect outstanding and clinically relevant projects that cannot be productively tackled by individual teams and help to assemble both the appropriate funding and workforce required to translate them into medically-valuable applications.
Keywords: Ubiquitin, Ubiquitin Proteasome System, Ubiquitin-like proteins, Proteases, Autophagy

B. BACKGROUND

B.1 General background

The Ub-Proteasome (UPS) and the Autophagy-Lysosomal (ALS) systems are two major intracellular pathways for selective degradation of most cellular proteins. Since proteolysis is the ultimate regulator of protein function as it leads to definitive and irreversible inactivation of target proteins, the role of these two pathways is particularly important for the control of regulatory proteins whose functions and thus expression window must be tightly regulated. Moreover, an important feature of both UPS and ALS is that their effective operation is intrinsically coupled to an intricate system for post-translational "tagging" of targets by ubiquitin (Ub) and Ub-like molecules. Together, both systems involve hundreds of components and control most if not all cellular processes, including cell cycle progression, intracellular signalling, transcription or apoptosis. They are therefore very attractive targets for therapeutic intervention, as their complexity and selectivity open in principle the possibility to modulate the expression of any protein of interest, and thus to

correct any pathological alteration.

Pharmaceutical companies are now investing a considerable amount of effort developing small molecule chemotherapeutics in the Ub pathway. The great success of the proteasome inhibitor bortezomib/Velcade™ in the treatment of multiple myeloma and mantle cell lymphoma illustrates this potential and has indeed encouraged the development of strategies targeting components of the UPS and ALS systems. Indeed, many experts now believe that drug discovery in the Ub/Ub-like molecules pathways will rival the kinase drug discovery in the next decade. The growing realisation of the biological importance of Ub and Ub-like molecule signaling has prompted the development of specific inhibitors for each conjugation machinery. The specific inhibitor for protein NEDDylation (MLN4924) is in clinical trials for the treatment of cancer whereas specific inhibitors for the Ub, SUMO and autophagy pathways have been developed and anticipated to enter clinical trials in the near future.

However, many unresolved scientific questions must be addressed to accelerate the successful translation of basic research into medical application. Typically, in most cases the identity of the enzymes that control the degradation of a protein of interest is just not known. Another example is that, although it is now clear that in many debilitating neurological disorders a deleterious aggregation of abnormal proteins overrides proteolytic pathways, the Action still have a very limited understanding of (i) the specific roles of each proteolytic pathways in the degradation of abnormal proteins and (ii) the means to globally enhance the activities of these pathways.

Moreover, some outstanding questions in the field also raise organization issues, as their resolution requires convergence of scientific domains that were traditionally separated. This is the case for example for the question of how UPS and ALS deal with the emerging complexity of the formation and decoding of the Ub signal. To realize significant progress on how to target protein turnover mechanisms to treat specific diseases, it is therefore crucial to advance our understanding of both the physiological and pathological functions of intracellular proteolysis, and to promote synergy and integration in this multidisciplinary and promising field of research.

COST countries are well represented and contribute heavily in the worldwide research effort being made on UPS and ALS, and on proteostasis in general. However, up to now and particularly in Europe, this effort is highly fragmented whereas the complexity of these systems and the number of their components requires integrated approaches that favour sharing the intellectual and technical expertise, as well as the generation of knowledge, and that limit research redundancy. Even though funding of science remains an issue, it is clear that one of the priorities in the field is to develop cooperation between the many groups involved in this research.

COST is the only programme that is appropriate for this type of effort, as it is the only funding tool

that works at the broader level of the research community. In particular, it will support the integration of a large number of already existing research endeavours, using already implemented national research investments addressing issues of global relevance, as is the case in this highly relevant area of UPS and ALS research. Other funding programmes are not adequate for this Action, as they either work at the level of the individual researcher/research entity, or they are limited to specific research topics. The stated mission of this COST action is to forge a cohesive scientific community linking multiple teams throughout Europe. This community will support research activity, training, and idea-sharing on broad aspects of protein network complexity. Taken together, the integration of multidisciplinary research approaches in a bona fide pan-European collaborative community of researchers is expected to lead a paradigm shift for investigation of protein networks with the potential to impact bio-medical research.

B.2 Current state of knowledge

The UPS is one of the two major intracellular proteolytic systems with ALS (see below). Schematically, it functions in two main steps: (i) first, protein substrates are tagged by covalent addition of a poly-Ub chain (poly-ubiquitylation) and (ii) the poly-ubiquitylated protein is then recognized and degraded into peptides by a giant protease called the proteasome. Poly-ubiquitylation is performed by an enzymatic cascade that involves three types of components called E1, E2, E3, among which E3s (or Ub-ligases) are the substrate-recruiting factors and are thus responsible for most of the specificity of the reaction. Importantly, ubiquitylation is a reversible process, thanks to many de-ubiquitylases (DUB) that can remove Ub from the substrate. As highlighted by the Nobel prize attributed to its discoverers in 2004, protein ubiquitylation is now recognized as an essential biological process. Of note, although it has been discovered and is best known as a modification targeting proteins for degradation by the proteasome, protein ubiquitylation is now considered as a major and highly versatile post-translational modification that plays many other functions, including enzyme activation, membrane protein endocytosis and control of gene transcription. The exact effect of protein ubiquitylation is determined by the type of Ub (Ub) chain built on the substrate, and by a plethora of Ub-binding proteins that target the ubiquitylated protein to various fates.

Overall, UPS is an extremely complex multi-enzymatic system comprising hundreds of components and is involved in the regulation of most cellular pathways and processes. Moreover, this system is functionally connected to other systems of protein conjugation that involve Ub-like proteins (such as SUMO or NEDD8). These Ub-like systems use their own conjugation cascades, which

mechanistically are similar to Ub conjugation and are also highly dynamic thanks to multiple deconjugating enzymes. SUMOylation controls the activity of various proteins involved in most cellular pathways. Its best-characterized role is in transcriptional regulation via modification of many transcription factors and co-regulators. In addition, SUMOylation plays important roles in DNA damage repair and maintenance of genome integrity. NEDDylation (conjugation of NEDD8) plays an important role in the regulation of cell viability, development and growth. Importantly, inhibitors of NEDDylation are in clinical trials for the treatment of cancer.

Very interestingly, the other major intracellular proteolytic system, APS, also relies on the use of Ub-like proteins for its functioning. During autophagy, proteins or portions of cytoplasm are digested and recycled by lysosomes. It is a constitutive mechanism that can be activated by multiple forms of cellular stress and initiates with the formation of an isolation membrane, or phagophore. Elongation and closure of the phagophore form a double membrane vacuole, the autophagosome, which engulfs cytoplasmic material, including long-lived proteins, protein aggregates and damaged organelles. Following maturation, the autophagosome fuses with a lysosome where the sequestered material is digested. Two Ub-like conjugation systems mediate vesicle elongation and sealing of the autophagosome. Atg12 is conjugated to Atg5, in a reaction mediated by the E1-like enzyme Atg7 and the E2-like enzyme Atg10. No E3-like enzyme has been discovered. The second conjugation system is unusual, as LC3 (Atg8 in yeast) is conjugated to phosphatidylethanolamine (PE), a lipid, through a mechanism controlled by Atg7 and Atg3, another E2-like enzyme. In this case, the Atg12-Atg5 conjugate acts as an E3 for the conjugation of LC3 to PE.

Importantly, there are many functional cross-talks between the different Ub-like systems as well as between UPS and APS. For example: (i) NEDD8, through modification of cullins, controls the activity of numerous cullin-based Ub-ligases and thus the ubiquitylation of multiple substrates; (ii) a family of Ub-ligases can recognize poly-SUMOylated proteins and target them for Ub-mediated degradation; (iii) proteins that possess Ub-binding domains, such as p62 (also called SQSTM1/A170), NBR1 (neighbour of the *BRCA1* gene 1), and HDAC6 (Histone deacetylase 6) can interact directly or indirectly with both Ub and components of the autophagic machinery; (iv) finally, many proteins are known to be eliminated both by the UPS and autophagy, and, in certain conditions, ubiquitylated proteasomal substrates, which are normally degraded by the UPS, can also be digested by autophagy and vice-versa.

This brief summary illustrates both the complexity of the mechanisms dedicated to intracellular proteolysis and the importance of these processes in the control of all cellular pathways. Not surprisingly, research in this area is extremely diverse and, consequently, highly fragmented. The participants of this Action believe that it is now time to move towards a more integrated approach,

and to develop a common strategy that will allow the community to (i) identify actions or research areas that could constitute major routes of progress and (ii), when possible, share expertise and resources to address key issues that cannot be tackled by individual groups. The participants believe that the European level is the appropriate level for this challenge, as it is large enough to cover all scientific issues in the field but coherent geographically and in terms of possible future shared funding. To the best of our knowledge, there is no similar effort funded at the European level to unite this large and essential community. The COST action PROTEOSTASIS will be innovative *per se* as it will help to bring together experienced and young researchers from academia, industry and clinics to raise cooperation and synergy in a field of research extremely promising in terms of possible medical applications. It will create a fertile environment for the development of a true community spirit that will favour cooperation and creativity, and will thus foster future collaborations between different groups of the network.

B.3 Reasons for the Action

The main reason for the Action is to improve high-quality collaborations and valorisation at all levels in the COST countries, in an important field of research: important in view of the number of research groups working in this field in Europe and worldwide, important in view of the biological roles of UPS and ALS and important also being at the cutting edge of identifying and validating novel targets for therapeutic intervention.

The participants of this COST Action confronted by the complexity of the intracellular proteolytic systems and of the biological processes controlled by these systems, recognize that the current fragmentation of their field of research is becoming a limiting factor to develop ambitious strategies aiming at exploiting these systems or at correcting their dysfunctions to cure human diseases in which proteolysis is involved. This includes certain cancers or certain neurodegenerative diseases. To resolve this crucial point, the participants of the Action have decided to join their research efforts to construct a network that will directly address the question of fostering cooperation and collaboration in the field. For this, the objectives of the Action will be aimed towards a scientific/technological advance at a European level, and include:

1. To defragment European research in UPS/ALS.

Result: Improved coordination among the different labs and generation of new collaborative projects in the European Research Area

2. To increase the transfer of knowledge from basic to applied science.

Result: development of new diagnostic and therapeutic techniques and tools.

3. To foster technological developments and knowledge in proteolysis.
Result: faster project implementation and completion resulting in improved scientific productivity and optimisation of resources.
4. To create a strong European scientific core in UPS/ALS and consolidate training efforts.
Result: to maintain and improve competitive know-how in a European context.
These objectives will be met primarily by the implementation of several Horizontal Actions (HA) (see section C3) that will serve to promote scientific interactions. The common theme of all HA will be to favour exchange and to identify conceptual and/or technical nodal points that must be overcome to allow a significant leap forward in the field and in its sub-domains.

B.4 Complementarity with other research programmes

PROTEOSTASIS will integrate numerous COST country scientists, many of them developing projects competitively funded from national and/or European sources or by independent grants. All Action members have demonstrated enthusiasm for joining forces, sharing information and expertise, and arranging meetings and workshops as exemplified by their common cooperative networks, joint grants, and shared publications.
Many of them have already worked together within previously/currently existing academic networks, such as the informal INPROTEOLYS (www.inproteolysis.eu) and ZOMES groups (http://ibis.tau.ac.il/twiki/bin/view/Plant_S/ZOMEBase/ZomesMeetings), or the EU-funded Marie Curie ITN UPStream and RUBICON (NoE) European networks. In FP7, the majority of currently executing grants related to UPS research are Marie Curie or ERC grants made to individual researchers. However, to our knowledge, there is presently no running project dedicated to fostering European-wide cooperation in the field of intracellular proteolysis of the magnitude of the COST Action PROTEOSTASIS. For this very reason, seeing the substantial, but independent efforts being made at a European level, the importance of a unifying COST Action takes on an extra imperative.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The aim of the Action is to promote high-quality collaboration and valorisation among experienced and early-stage academic, clinical and industry-based European researchers involved in intracellular proteolysis research. The expected scientific impact of this initiative is twofold: to increase Europe’s leadership in this highly competitive field; and to translate scientific knowledge into

cutting edge innovations that will improve human health.

C.2 Objectives

Researchers in this field will work together to achieve the following objectives:

(i) **To defragment European research in UPS/ALS**

This objective will improve coordination among the different labs and generate new collaborative projects in the European Research Area. It will provide an opportunity to previously disparate groups to develop new and beneficial collaborations through open discussion and the exchange of new know-how. Part of this defragmentation process is illustrated by the creation of collaborative translational projects (up to 10 are envisioned).

(ii) **To increase the transfer of knowledge from basic to applied science**

An important aspect of this objective is to develop appropriate approaches to translate this knowledge and expertise into innovative medical applications, through the creation of a translational think-tank. It will promote the development of new projects aimed to progress on the development of novel diagnostic and therapeutic techniques to control proteolytic pathways and corresponding diseases. Various biomarkers in immunological disorders, neurological diseases and cancer are expected to be discovered as a result of this collaborative effort.

(iii) **Foster technological developments in proteolysis**

This Action will develop a forum for communication and free exchange of protocols, ideas and data, helping to both critically evaluate “state-of-the-art” questions and to implement the appropriate approaches to tackle them. This will facilitate technological collaboration and future developments in both basic and clinical research. The integration of available data on pathophysiological roles of intracellular proteolysis will help to elucidate its relation with diseases such as cancer, neurodegenerative disorders, allergic and immune diseases.

(iv) **To create a strong European scientific core in UPS/ALS and consolidate training efforts**

This Action will bring together a solid core of researchers working in this field, uniting their expertise in the varied research disciplines. A primary aspect of this objective is to transmit this knowledge to the younger generations of researcher through the organisation of hands-on and theoretical courses, Short Term Scientific Missions, Training Schools and conferences.

C.3 How networking within the Action will yield the objectives?

To achieve the objectives mentioned in C2 above, the network will implement several HAs to

promote the necessary scientific interactions:

(i) **Translational projects**: these will involve a variety of different working groups with common but complimentary interests, working towards more medically and commercially relevant research. (Objectives i, ii)

(ii) Creation of a **"translational" think-tank** (embodied in the Strategic Committee) specifically dedicated to the selection and strategic support of outstanding and clinically relevant projects to translate the new research findings into medically-valuable applications. (Objectives i, ii)

(iii) **Working Groups meetings**: PROTEOSTASIS will organise annual meetings between working groups to promote synergies and support specific translational research actions. (Objectives ii, iii)

(iv) **Annual workshops:** the Action will organise a series of concrete workshops on the most relevant and innovative research topics in the field. (Objectives i, iii, iv)

(v) **Creation of an interactive website**: will include lists of cellular/molecular tools, available techniques and expertise per laboratory, technical platforms, technologies, and useful information for the members. (Objectives i, ii, iii, iv)

(vi) **Knowledge transfer activities:** Academic and technical training like Short Term Scientific Missions in specialized laboratories, or Training Schools (organised within the Action and open to the community) for the formation of technicians, PhD students, and post-doctoral members of the network. (Objectives iii, iv)

(vii) **Networking**: the Action will strongly encourage the collaboration with other projects, other scientists and scientific networks in other countries, and other non-scientific interested stakeholders. (Objectives ii, iii, iv)

C.4 Potential impact of the Action

This inclusive interdisciplinary COST action will be a perfect means to **synergise the participants' existing research activities in this field, and to develop novel approaches**. The PROTEOSTASIS network will immediately improve exchange of knowledge and people within the scientific and business community, accelerating solutions to many important scientific problems. It will advance basic scientific and early clinical progress and build strong foundations for future scientific and clinical applications. In particular, considerable benefits are expected:

- **At the scientific and technological level**: the fostering of European collaborations will boost creativity, technological improvement and mentoring thanks to the sharing of experience between more experienced and ESR scientists, and between different groups specialising in basic and

applied research. This open discussion and the supportive spirit at the core of the network will help to focus ideas and to break the knowledge barrier that often limits the translation of original concepts into pertinent applications.

- **At the level of economy and society:** the training and valorisation in the field of intracellular proteolysis will be translated through a deeper diffusion within the European population of scientific knowledge and also into a number of applications with high clinical impact. These will also support related economic activities, such as the establishment of related research projects in small research accelerators, incubators or start-ups. The advantages of new and innovative medical applications resulting from the collaborative research in a variety of different diseases will be finally translated into benefits for society in general.

C.5 Target groups/end users

The Target Groups of this Action will be academic, clinical and industrial researchers. Particular benefit will be obtained from early-stage researchers implicated in the distinct scientific programs of this Action. Students of the institutions participating in this Action will learn the latest techniques and concepts emerging in the field. Spin-off companies and Small and Medium Enterprises (SMEs) and Industrial groups exploiting technology for academic research, diagnosis and/or therapeutic purposes could also benefit from the Action. In fact, some of the members of the Action are SMEs that have an interest in exploiting the results. In general, scientists, students, health care providers and patients will be the ultimate “end-users”.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

This Action is a commitment to develop and integrate translational research projects linked to distinct proteolytic pathways that were traditionally studied separately. Recent evidence suggests that these proteolytic pathways are interconnected and the central goal of the Action is to better integrate the efforts to deepen our understanding of their underlying mechanisms, and to exploit them for therapeutic intervention. While competition is often an important driving force for scientific progress, PROTEOSTASIS will concentrate on coordination, cooperation and exchange among European participants in order to improve their contribution to the worldwide effort made in this field. Indeed participants of this COST Action agree that reciprocal basic/fundamental knowledge transfer within European academic, clinical and industrial research represents the best

means to address current challenges. The scientific innovation of this Action lies in its comprehensive and collaborative approach, which will allow access to multiple state-of-the-art technologies and integration of the data generated by each Working Group (WG). The ultimate goal is to fully exploit the potential of the scientific domain to develop translational research projects that will improve the quality of life of European citizens. To this purpose, the translational efforts of the Action are organized around two main axes:

-*Translation of findings into clinical practice:* The Action will aim at exploiting when interesting and feasible our knowledge and expertise to develop novel strategies to fight human diseases. This includes the identification and validation of novel biomarkers for early diagnosis, prognosis, and prediction of response to treatment for diseases such as cancer, neurodegeneration and immune disorders.

-*Development of therapeutic approaches towards individualised treatment*: Clinical trial design and development of personalized therapies require collaboration of multiple institutions and scientists having different background and expertise to harmonize strategies, combine results, integrate knowledge and thus facilitate progress. Several groups involved in this Action plan to develop molecular and gene target therapies. The coordination of their work will accelerate discovery, validation, and clinical implementation of novel therapies.

In line with these objectives, this Action will develop a number of translational projects representing collective efforts to improve efficiency and success in the field of PROTEOSTASIS research in Europe, as described in section D2. To illustrate these collaborative efforts, five examples of such projects are presented below. Each translational project will be selected according to the degree of maturity in the area of application and will require participation of different WGs. This transversal organization involving participation of different WGs in each translational project appears to be the most efficient way to integrate the European know-how on the distinct proteolytic pathways into dedicated workforces that will tackle specific objectives. WGs and translational projects will remain open to the participation of members and non-members of this Action, and participants will facilitate integration of new groups from academia, clinics or industry when useful. Additional tasks could be undertaken according to the progression achieved within each research challenge.

All translational projects are state-of-the-art research initiatives that will be implemented with original technologies developed by some of the partners. For example, one of the most versatile and original approaches will be the use of organic synthesis for the development of tools allowing investigation of biochemical processes in relation to cancer or neurodegeneration. The method is pragmatic, focusing on the search for inhibitors of enzymatic activities by high and ultra-throughput

screening of small molecule libraries followed by lead-optimization and on the development of biochemical tools by rational design. Also, several techniques to identify and characterize proteolytic substrates, particularly molecular traps (developed by partners of this Action) to capture ubiquitylated, SUMOylated or NEDDylated proteins from cell cultures, tissues, organs or organisms will be implemented when required. The combination of these techniques with mass spectrometry will help understanding proteolytic processes and also provide biomarkers for prognosis and diagnosis as well as potential drug targets. WGs will exploit these types of approaches and tools to profile cellular enzymatic activities associated primarily with Ub and Ub-like systems, as well as the activity of the proteasome and other proteases. They will provide all the molecular and cell biology systems necessary for these studies, as well as the animal models for disease when available. Using in-house innovative tools and state-of-the-art facilities, or in collaboration with external basic research groups and industrial partner, WG will also develop HTS assays for screening modulators of Ub and Ub-like enzymes and perform profiling of their activities. Particularly interesting in this context will be the study of deconjugating enzymes.

D.2 Scientific work plan methods and means

This Action will contribute to the generation of an integrated view and a better understanding of the regulatory and action mechanisms of different interconnected proteolytic pathways, increase our knowledge of the molecular mechanisms involved during physiological and pathological processes, identify novel targets for drug development and validate susceptibility or biological markers that could be instrumental for better diagnosis and follow-up of patients treated with drugs targeting proteolytic pathways. These goals will be accomplished by 6 interacting WGs, as detailed below. The participants of the COST Action will use their current manpower, laboratory equipment and facilities to pursue their scientific objectives. The network comprises at this stage 80 laboratories, but the number of participants is expected to increase during the implementation of the Action. Research teams already own or will gain access, through new collaborations, to state-of-the-art equipment and expertise on the latest technologies including cell and animal imaging, NMR, X-ray crystallography, chemical peptide modification, quantitative proteomics, biochemistry, molecular and cellular biology, molecular traps, high throughput screening methods, animal and plant model systems, etc. This will be used to develop outstanding translational projects and progress in the tasks. WGs will design their own objectives and decision-making processes, define with the other WGs their contribution to the different translational projects, and specify the responsibilities among the WG members. WG members will present their results at workshops and meetings, as well as in

international peer-reviewed journals. All the six WGs will be responsible for making public new results, initiatives, guidelines, etc, via the Action website. WGs will be organized around specific scientific issues, as follows:

- WG1. Protein modification in intracellular proteolysis: mechanisms, roles and regulation. The aim of this WG is to investigate (i) the mechanisms of ubiquitylation, SUMOylation and modifications by other Ub-like proteins, (ii) the crosstalks of factors involved in protein modification under basal and stress conditions, (iii) the post-modification mechanisms responsible for the physiopathological properties of the targeted proteins, (iv) the structural bases of protein recognition and (v) the characterization of Ub- or Ub-like receptors involved in the processes under investigation.

- WG2. Intracellular proteolytic systems: mechanisms, structures and regulation. This WG will aim to understand (i) proteasome biogenesis and regulation, (ii) the molecular mechanisms controlling autophagy and (iii) the Ub-dependent and -independent processes regulating programmed cell-death/apoptosis.

- WG3. Regulation of cell signalling. This WG will address (i) the regulation of signalling cascades under basal and stress conditions, (ii) the intracellular traffic of modified proteins and the identification of factors regulating these mechanisms in physiological and pathological situations, and (iv) the molecular mechanisms regulating transcription.

- WG4. Protein quality control, misfolding and aggregation. This WG will investigate (i) the regulation of protein folding in physiology and pathology, (ii) the molecular mechanisms controlling the ERAD pathway and (iii) the functional characterization of chaperones involved in protein quality control.

- WG5. Regulation of cell proliferation and differentiation. This WG will study (i) the regulation of proteolysis during the cell cycle and (ii) the gene products regulating essential cellular processes during cell growth and differentiation.

- WG6. Molecular bases of diseases, biomarkers, drug targets and biotechnology. This WG will (i) address issues connected to disease understanding, diagnosis and treatment, with an emphasis on cancer and immune and neurological disorders, and (ii) deal with biotechnology challenges involving protein engineering, and plant and animal breeding.

Each WG will identify projects of high potential clinical value that could be integrated into translational projects and define when applicable its participation in already initiated translational projects. During the elaboration of this application, the Action already identified five translational projects that are detailed below as an illustration of the potential of the Action:

(I) Elucidation of the role of TRIM Ub ligases in diseases and development of small molecule modulators towards therapeutic aims. Participants: WG1, WG3, WG6.

The TRIM family of E3 - Ub ligases is implicated in many human diseases, including cancer, autoimmune and neurological disorders. This makes certain TRIMs particularly relevant biomarkers and therapeutic targets in systemic lupus erythematosus (SLE) and Parkinson's Disease (PD). This project is therefore at the forefront of translational efforts within PROTEOSTASIS as it will aim at: (i) better understanding the pathological roles of TRIM in SLE, (ii) studying the TRIM18 and TRIM1 family members implicated in genetic syndromic forms of neurological disorders as well as in innate immunity, (iii) elucidating the crucial roles that TRIM17, another member of the family, plays in the triggering of apoptosis and most likely in PD pathogenesis. Using systematic bioinformatic, molecular and cell biology and biochemical approaches, this project will explore the possible use and development of small molecule agonists or antagonists targeting either the ligase activity or the ligase substrate recognition motif.

Work plan and methods: (1) Undertake a rational drug design and *in silico* screening for the development of small molecule modulators (SMMs) of TRIMs involved in diseases; (2) Develop miniaturized *in vitro* assays to screen chemical compounds libraries to select lead molecules with agonist or antagonist activity; (3) Coordinate access to relevant *in vivo* models and biobank material to test lead SMMs in the context of disease; (4) Advance our understanding of the physiological mechanisms of TRIM family members function. Both chemical libraries and biobanks are available for this project.

(II) Tackling proteases: from fundamental mechanisms to clinical implications. Participants: WG1, WG2, WG3, WG6.

A big success in the field has been the validation of the proteasome inhibitor bortezomib/Velcade™ as a drug against multiple myeloma. More specific/limited targets are expected to be affected by tackling other proteases. Deubiquitylases (DUB) have three generic functions that render them particularly interesting therapeutic targets: (i) they maintain free Ub levels, (ii) they rescue proteins from Ub-mediated degradation, and (iii) they control the dynamics of Ub-mediated signalling events. Different groups participating in this project have developed a suite of tools around this family, which provides a platform for cell biology, biochemical and structural studies. Cell biological studies seek to link DUBs with pathways germane to cancer or neurodegenerative diseases (e.g. PD). Collaborations between academia and private companies involve drug discovery programmes targeting the most promising DUBs.

Work plan and methods: (1) Identify DUBs that can be associated to physiological processes or

pathologies such as cancer and neurodegeneration; (2) Development of technology to characterize DUBs activity and specificity; (3) Identification of inhibitors for DUBs involved in diseases; (4) Clinical and/or preclinical studies using DUBs inhibitors.

(III) Autophagy in physiology and pathology. Participants: WG2, WG3, WG4, WG6.

This project will use yeast and *Dictyostelium*, two model organisms frequently used to study the function of human proteins. Its goal is to better understand two inherited human diseases, choreoacantocytosis and Cohen syndrome, related to hVPS13 genes. Indeed the four human hVPS13A-D proteins, besides homology to yeast Vps13, show homology to the autophagy-related yeast protein Atg2 and its human homologues hATG2A and hATG2B, suggesting that they have some functions in common. The functions and interactions of hVPS13A and hVPS13B and their mutant variants found in patients will be studied using yeast. These studies will contribute to broaden our knowledge of the autophagic process and, more importantly, to understand the molecular bases of the two above-mentioned rare diseases which have been associated with loss-of-function mutations in vps13 genes. Further studies in mammalian cells should confirm our observations and help us to set up the fundaments for future preclinical studies.

Work plan and methods: (1) Study the roles of the VPS13 family of proteins in autophagy and other membrane-traffic processes using Yeast and Dictyostelium as model systems; (2) Identification and study of possible interactors of the VPS13 family of proteins; (3) Study a possible connection between autophagy and the disease choreocanthocytosis and Cohen syndrome using human cell lines; (4) Develop yeast and mammalian cell models of the diseases for drug screening.

(IV) Cancer: Molecular pathways and clinical challenges. Participants: WG1, WG3, WG5, WG6.

In breast cancer, the promyelocytic leukemia protein PML is over-expressed in a subset of aggressive tumors with poor prognosis and therapy resistance and provides a selective advantage to tumor cells. Arsenic trioxide (ATO) promotes UPS-mediated degradation of PML. A combinatorial approach to exert a lethal activity on breast cancer cells will be defined for preclinical and clinical evaluation. Hospitals participating in this Action will integrate all tasks in their Translational Cancer Medicine Programme focused on biomarker-led therapeutic combination of pharmacological agents. The staining of PML can be automated, quantified, and is currently a diagnostic tool in APL. The application of ATO to breast cancer therapy is feasible since it is a safe drug already used in the clinic. The Action hypothesize that smart FDA-approved drug combinations will enhance potency and quickly generate positive results. Implicated WG will also contribute to a biobank of tumor and blood derivatives.

Work plan and methods: (1) To automate PML staining and quantification in breast tumor samples*;*

(2) To corroborate the antitumor effect of ATO in PML-high expressing breast cancers using primary samples *in vitro* and in immunocompromised mice; (3) To screen for pharmacological combinations of ATO and FDA approved drugs (preferentially those used in breast cancer) in order to define effective therapies; (4) To evaluate the status, relevance and druggability of the main PML E3 ligase, RNF4, in breast cancer.

(V) Aging and Neurodegeneration: Diagnosis and therapeutics. Participants: WG2, WG4, WG5, WG6.

This project will study the cause and effect of disturbed proteostasis in Alzheimer's disease (AD) focusing on early factors that drive the pathogenesis in sporadic AD. Translational work combines cell and animal models, post-mortem brain material, and bio- and physicochemical protein analysis. The unfolded protein response (UPR) is functionally connected to the early stages of AD, indicating both a reversible stage in the pathogenesis and that the UPR may be an early therapeutic target for intervention. Groups will investigate the potential of targeting UPR and the different proteolytic systems, particularly the autophagy/lysosomal pathway which is the major proteolytic system during UPR activation and that is severely impaired in AD brain. The aim is to identify UPR related early diagnostic biomarkers. The function and regulation of Tau in AD will be explored, using a mouse line that expresses all human isoforms and develops cognitive symptoms and neurodegenerative phenotypes akin to human Alzheimer's or Pick's diseases. A number of human post-mortem samples form different brain regions will be analyzed. Finally, the Tauopathy Proteome for Identification of Disease Markers will be studied and its utility as potential drug target will be explored. Screening of compounds libraries based on their chemical properties will be performed.

Work plan and methods: (1) Study of the proteostatic disturbance as a disease mechanism in neurodegeneration; (2) Exploring proteostatic disturbance as biomarker in neurodegenerative diseases*;* (3) Identification of new drugs to treat neurodegenerative diseases*;* (4) Restoring proteostasis as therapeutic strategy for neurodegenerative diseases.

E. ORGANISATION

E.1 Coordination and organisation

Three levels of organization are envisaged:

1. Management Committee.

ESRs will be expected to play an active role in the MC. In addition, the MC will be responsible for the following activities:

(i) Coordinating the participation of the different members of the Action, with respect to the WGs and other ad-hoc groups that may be formed.
(ii) Establishing and maintaining a COST website for both internal communication between the parties and external dissemination activities (conferences, workshops and Training Schools, working opportunities, overall scientific production and innovation of the Action). The website will be the primary dissemination and communication tool of the Action.
(iii) Planning and management of Meetings, Conferences, Workshops, Short Term Scientific Missions (STSM) and Training Schools, etc., related to the Action.
(iv) Reviewing and approving applications to STSMs and Training Schools.
(v) Managing of reporting obligations of the Action, including revision of yearly and final Action reports, and intermediate reports produced by the different WGs.
(vi) Managing of the introduction of new participants to the Action.
(vii) Coordinating the management of intellectual property produced as a result of the work in translational projects within the different WGs. Providing recommendations for the future protection/use/publication of any foreground produced.
(viii) Acting on the recommendations provided by the SC on the strategic direction of the Action.
(ix) Implementing links to other relevant programmes and bodies at a European and international levels.
(x) Defining a realistic scheme for an added-value post-COST collaboration of the parties to ensure the continuity of the current network.
The MC is expected to meet twice a year. People responsible for the management of a particular task will be selected among the MC, and specialised supporting sub-committees will be formed where necessary (for example, for website establishment and maintenance or workshops' organization).
2. Strategic Committee. The next level in the management scheme is occupied by the Strategic Committee (SC), which is formed of the Chair, vice-Chair and WGLs. This Core Group is the technical nucleus of the Action and will be responsible for the strategic direction of the Action. Its main task will be to identify and manage the implementation of the translational projects, which will be defined to encompass two or more different WGs as required. The SC will report to the MC on regular basis (minimum every 6 months), and will meet in person during the MC meetings, but is expected to be in contact by teleconference on a regular basis.
Due to the complexity and the essential roles of intracellular proteolysis in homeostasis, many translational projects could be developed within the PROTEOSTASIS COST Action. Some translational projects have already been defined and can be seen in section D1 and D2; it is foreseen

that new translational projects may be defined and implemented as the Action progresses, depending on the development and results of the Action. However, which translational project to tackle within the Action requires careful evaluation of many parameters, including the real clinical potential of the project, the strengths (both theoretical and technical) present in the network, the added-value of tackling the project within the Action compared to the commitment of individual teams, etc. Therefore, particularly during the starting period of the Action, but also during its whole duration, a considerable effort will be made within the network to assess the different possibilities, and to help for the maturation, the funding and the valorisation of those that will appear to be the most promising. This evaluation/support role will be the main task assigned to a dedicated think-tank that will be created within the SC. This think-tank will be composed of a small group of participants chosen within those who have the largest experience with applied project (clinical or industrial) implementation, and whose scientific reputation could help to establish appropriate contacts with clinical teams and possible sponsors. The functions of this structure will be to: (1) Collect from the WGs the information about the projects that could have a translational dimension; (2) Assess the interest/feasibility of these projects (according to the parameters mentioned above); (3) Select the most promising ones and organize their implementation as a translational project of the Action. (4) Act as a consulting board for the different translational projects of the Action, providing advice on the progression of their objectives. (5) Finally, as the goals of this Action go beyond its lifespan, an important role of the think-tank will be to foresee and help organize the continuation and the development of the translational projects after the end of this Action. Here the experience of the members of the think-tank will be crucial to help aggregating a sufficient workforce (including when necessary scientists outside of the Action) and finding the financial support required for the full-maturation into independent clinical or industrial projects of the most advanced translational projects of the Action. Obviously at this stage, and in view of the structure and the ambition of this Action, it is difficult to be very specific about which translational projects will be able to develop into a full spin-off of the Action. There are too many variables that cannot be easily anticipated and a large part of the process will have to be dynamically managed during the Action. This is why the think-tank dedicated to the evaluation and the support of the translational projects is an important and original structure implemented by our Action. The Action strongly believe that the experience of the members of this structure, associated to their constant interaction with the WGs, will be an important asset for the success of the Action in terms of translational outputs.

3. Working Groups. Finally, the technical level of the project is provided through the WGs, the responsibilities of which are defined in section E2.

The principle milestones are mentioned in section F. Other more diffuse milestones include the successful implementation and completion of the objectives of the translational projects, although some of these may have a lifespan that is longer than the current Action.

E.2 Working Groups

The Action has been designed around 6 WGs defined in section D2. Each WG will appoint a leading scientist during the kick-off meeting. All participants in the network will be invited to join at least one WG. The active involvement of Early Stage Researchers in their functioning will be strongly encouraged. The integration of the WGs will be ensured through the SC. In addition, the sharing of knowledge and costly technological resources amongst the different WGs will be encouraged, in order to favour access to the latest technologies to all researchers. The WGs will meet once a year, coinciding with the MC meeting in order to save costs. They will report once a year to the MC. Other meetings may be organised depending on the necessities of the group members; these may be in-person, if the budget allows, or by teleconference. The main responsibilities of each WG are:
(i) To coordinate and participate in the research activity of the WG, ensuring that it meets the objectives of the Action.
(ii) To monitor the state-of-the-art of their topic, identify innovative developments, and communicate any advances to the rest of the network through the MC.
(iii) To implement translational projects identified by the SC as being critical to the Action.
(iv) To promote translational aspects of the Action, represented by translational projects.
(v) To coordinate integration of new members recommended by the MC to the WGs as the Action progresses.
(vi) To manage the adequate protection of innovative foreground produced as a result of the Action, respecting the rulings of the MC.

E.3 Liaison and interaction with other research programmes

To meet the dynamism of the American and Asian academic systems, it is necessary to integrate all technical expertise and creativity to exploit the full potential of European science in order to generate basic, clinical and translational research. PROTEOSTASIS takes a leap forward in this direction, and integrates many of the leading European scientific groups working in intracellular proteolysi, most of them developing projects competitively funded by national and / or regional

funding or independent grants. Many of the participant laboratories are or were also involved in previously or currently existing academic networks (INPROTEOLYS and ZOMES groups), or the FP7 Marie Curie ITN UPStream (http://upstreamproject.eu) and the FP6 Network of Excellence RUBICON (http://www.rubicon-net.org/index.php). There is also a number of individual Marie Curie grants and European Research Council Starting and Advanced grants touching on this theme, and an important effort will be made to invite those who are not yet integrated to take an active part in this Action, thereby helping to further consolidate the fragmented research pattern of this particular scientific area within the ERA.

In addition, active contact will be made with a variety of non-European networks, such as the International Proteolysis Society (http://www.protease.org/index.html) and the International Protease Network (http://www.protease.net). Through these networks, contact will be made in particular with the North American Degradomics Group (USA and Canada). In the short term, members of these groups will be invited to take part as researchers/speakers in the workshops/conferences and, in the long term, research partnerships with members of the Action will be developed and contact opportunities for ESRs within the Action will be favoured.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers. This item will also be placed as a standard item on all MC agendas.

Gender balance:

Within the current structure of the Action (80 scientists from 17 countries), 36.7% of the laboratory leaders are women. As the group grows, and more researchers are added, the Action expects that this percentage will grow due to the high presence of female researchers in this scientific domain. It should also be noted that the coordinator of the network is a female scientist, who is also coordinator of the UPStream ITN network. It is important to emphasize that female PIs participated intensively in the initiation, coordination and preparation of this proposal. Females presence and visibility in the network will be achieved through (i) female participation in the MC and as coordinators for the WGs, (ii) female participation in the organization of meetings and (iii) female selection as Chairs of the meetings. Accordingly the presence of female ESRs in the groups will be encouraged.

Early Stage Researchers:

One of the main objectives of the Action is to foster the involvement and training of a new generation of European scientists in a critical and complex field of modern biology. The Action will include researchers at both PhD level and particularly at post-doctorate level. STSMs, summer training schools and workshops are all designed to increase and advance the ESR research capacity in this exciting area. The ESRs will be encouraged to (i) participate and make oral presentations at the meetings, (ii) organize ESR reunions during the yearly meetings of the Action and courses oriented to ESR training, (iii) to organize secondments in other groups of the Action for collaborations and training.

F. TIMETABLE

	YEAR 1		YEAR 2		YEAR 3		YEAR 4		
ACTIVITY	SEM1	SEM2	SEM3	SEM4	SEM5	SEM6	SEM7	SEM8	**MILESTONE**
Kick-off meeting	**X**								Meeting completed
Website building and maintenance	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	Website running
MC meetings		**X**		**X**		**X**		**X**	Meeting completed
WG meetings		**X**		**X**		**X**		**X**	Meeting completed
Translational projects	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	Objectives completed
SC meetings		**X**		**X**		**X**		**X**	Strategic changes implemented
Continuous Call for STSM	**X**	**X**	**X**	**X**	**X**	**X**	**X**		Missions completed/ reports produced
Training schools			**X**		**X**		**X**		Schools completed
Workshop		**X**		**X**		**X**			Workshops completed
Annual reports		**X**		**X**		**X**		**X**	Reports completed
Final conference								**X**	Conference completed

The network is designed to last for 4 years, but, hopefully, continuation of research excellence on this topic in Europe will be assured through the development of the translational projects.
Apart from the standard meetings for management bodies, an intensive effort will be made to include ESRs in the network and improve the possibilities for trans-national collaboration, through STSMs and workshops. Depending on the techniques to be learned/taught, STSMs will be organised for differing lengths, but will tend to last days or a couple of weeks at most. A few longer, more personalised STSMs may be permitted as required if more specific, long-term

collaborations are to be attempted. A major aim is to foster technological developments in proteolysis through a “translational think-tank” aimed at translating new research findings to medically relevant applications – this think-tank will have a fluid meeting arrangement, and may be called at any time during the 4 years, as deemed necessary by its composite members. An effort will be made to include ESRs within its structure.

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, DE, DK, EL, ES, FI, FR, IE, IL, IT, NL, PL, PT, SE, SI, TR, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 68 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

Dissemination of the Action will be developed at different levels. The Action identified the following target audiences:

- All PROTEOSTASIS participants, including academic, clinical and industrial partners. Special attention will be paid to ESRs.
- Scientific community in the field of the UPS and other societies and consortiums with similar interests (i.e. The International Proteolysis Society, UPStream, etc.)
- General scientific community in other fields of research; Scientific societies, Research Institutes, Academia (i.e. Biochemistry and Molecular Biology National and International Societies, etc.)
- ESRs in both the general scientific and medical communities.
- Industry.
- Policy makers at local, national and European levels.
- The general public.

H.2 What?

Different methods of dissemination will be selected for each targeted audience, from the members

of the Action to the general public.

Website generation:

The PROTEOSTASIS website will be a central instrument for the communication of the members of the Action among themselves and to other various audiences. The website will include the following information:

- Aims, objectives and purpose of the Action.
- Information about the members, contact information and organization of the Action.
- Information on cellular, molecular, genetic or biochemistry tools and techniques.
- Calendars and information about the courses and hosting stages organized by the members of the action, principally targeted to ESRs.
- Calendars and information about the meetings organized by the Action.
- Information and calendars about courses and meeting organized by other agents.
- Links to other Actions, societies, consortiums, of interest.
- Information written for non-specialists in various European languages, directed to the general public.
- Forums for internal discussion and interchange of material.

The Action will promote the organization and participation in meetings, conferences and workshops:

- General annual meeting of all the participants of the Action, where participation of ESRs will be encouraged.
- Meetings of the Working Groups with special dedicated sessions.
- Annual workshops on specific topics open to groups not participant in the Action.
- Meetings of the Strategic Committee.
- Oral or poster presentations in national and international conferences and meetings.

Training and education:

Academic and technical training will be one of the activities promoted by the Action, which will specially favour ESRs. Training will cover the following aspects:

- Specialized hands-on courses organized by members of PROTEOSTASIS.
- Specialized theoretical courses organized by members of PROTEOSTASIS.
- Facilitate the assistance to specialized courses organized by other actors.
- Promote the interchange of ESRs among the participants' laboratories, which will stimulate collaborations and interchange of know-how.
- Facilitate protocols, tools and reagents among members of the Action.

- Members of PROTEOSTASIS will be encouraged to participate in courses, seminars, master courses, conferences, etc., on the topic.
- Members would be encouraged to introduce the topic to undergraduate, graduate or postgraduate students in their laboratories or institutions.

Publications:

The Action will favour scientific and educational publications in the following way:
- Original scientific publications in international peer-reviewed journals by members of the Action.
- Review articles on the topic in international peer-reviewed journals.
- Publication in the web page of summaries of the meetings and conferences, as well as periodic reports.
- Generation of a brochure with information about the Action.
- Press releases directed to the local media.
- Generation of a Summary report of the Action at the late stages, which will address future scientific goals in the field.

Networking:

Networking will be promoted at different levels:
- Promote and encourage interactions among the members of the network with common interests.
- Promote interactions with other projects, actors, scientific networks or societies, either at the European or international levels.
- Promote the contact with Industry in the biotechnological and pharmacological areas.

H.3 How?

Website:

The Website will have sections devoted to the internal use by the participants of the Action, but also to the rest of the scientific medical and biotechnological communities, enterprises, policy makers and general public. A member of the Management Committee will be designated as Website Coordinator during the kick-off meeting and will be responsible for the creation and maintenance of the website. Regular information updates will be done.

Meetings and conferences:

Either organized by the Action or by other scientific agents, meetings and conferences are an important tool of dissemination through oral or poster presentations, as well as organization of specific sessions on the topic. This will be directed to the scientific community, enterprises and policy makers. Organization of informative talks within the scientific program will make meetings

accessible to the general public. Again, a specific member of the Management Committee will be designated as the main person responsible for the coordination of these tasks.

Training and education:

To consolidate the field within Europe and promote the integration of your researchers, training is the tool that will be directed to ESRs and general scientific community through the organization of courses and training stages by participants of the Action. Spreading the word in universities, schools and other forums, will make the Action accessible to the general public. The Management Committee will be responsible for the overall coordination of training activities.

Publications:

The different levels of publications will reach different societal agents, from the scientific community through publications in scientific journals, to the general public through publication of general information in our web page. A member of the Management Committee will be designated as Publications Editor and will be responsible for these tasks. This person will liaise closely with the Strategic Committee responsible for the monitoring of IPR within the Action, in order to ensure that aspects of publication and Open Access do not infringe on the intellectual property rights of the members of the Action.

Networking:

Interaction with other scientific agents will be promoted through the organization of general meetings, invitations to conferences and participation in other societies and networks. This will be directed to the scientific community, the industry partners and other interested stakeholders, such as policy makers and the scientific media.