ERC Synergy Grant 2018

Research proposal [Part B2][[1]](#footnote-1)

*(not evaluated in Step 1)*

* Name of the corresponding Principal Investigator (cPI) and corresponding Host Institution (cHI)
* List the other PIs, indicating the Host Institution of each PI

**Part B2: *The scientific proposal* (max. 15 pages, excluding the Resources section and References)**

**Section a. State-of-the-art and objectives**

In its largest acceptation, the prion phenomenon involves the self-propagation of a biological information through structural information transfer from a protein in a prion-state to the same protein in a non-prion state. Such concept is key to the regulation of diverse physiological systems and to the pathogenesis of prion diseases (*1-3*). Recently, prion-like mechanisms have been involved in the propagation and gain of toxic functions of proteins or peptides associated with commoner neurodegenerative disorders such as Alzheimer, Parkinson and Huntington diseases (*4*).

Mammalian prions cause inexorably fatal neurodegenerative diseases (also known as transmissible spongiform encephalopathies, i.e. TSE) in human and animals, including human Creutzfeldt-Jakob disease (CJD), sheep scrapie, bovine spongiform encephalopathy (BSE) and chronic wasting disease (CWD) of cervids (*1*). Both prion replicative information (i.e. infectivity) and pathogenicity are encoded in the structure of PrPSc assemblies, a misfolded, β-sheet rich conformer of the ubiquitously expressed, host-encoded prion protein PrPC (*5*). In these susceptible host-species and in laboratory rodent models, PrPSc assemblies show a remarkable ability to form stable, structurally distinct PrPSc conformers (*6-9*), known as prion strains, and encoding unique stereotypical biological phenotypes (*10-13*). How PrPSc structural polymorphism causes these distinct phenotypes remains poorly defined.

Kinetic aspects of prion replication have been well described by measuring infectivity or PrPSc levels in the brain (*14, 15*). Plotting prion accumulation as function of time provides a sigmoidal shape, which has served to roughly elaborate theoretical and mathematical models for prion replication. The autocatalytic conversion model (ACM) proposed by Griffith in 1967 (*16*) and the nucleated-polymerization model (NPM) by Lansbury and Caughey in 1995 (*17*) remain so far the most accepted theoretical mechanisms describing the conversion process. However, both models fail to describe neither the molecular mechanism of the templating process nor the multiplication of the templating interface which is at the base of the amplification and spreading phenomenon in the prion paradigm. Based on yeast prions, Shorter and Lindquist (*18*) introduced the concept of active fragmentation mediated by housekeeping proteins such as HSPs as critical underpinning for fungus-prion spreading. Since, fragmentation has been commonly accepted as the basis of spreading and amplification for mammalian prions, despite the lack of relevant biochemical and biological evidence. Recently, by correlating the PrPSc quaternary structural transition with biological activity during unfolding and refolding, we revealed the existence of a dynamic equilibrium between PrPSc and a highly stable oligomeric elementary PrP subunit termed suPrP. suPrP contains the strain structural determinant (SSD) information. The existence of suPrP as a highly-stable elementary building block in equilibrium with PrPSc assemblies truly challenges the actual model of prion replication as well as the contribution of fragmentation process to the amplification and spreading phenomenon (*19*).

Evidence for a broad degree of heterogeneity within PrPSc assemblies within prion strains or isolates was inferred from several reports, including: i) transmission of TSE field isolates (*20-22*) or experimental prion strains (*23*) with species or transmission barrier, - certain substrains or subpopulations present as minor component emerging preferentially due to the selection pressure, ii) size- or density-fractionation studies supporting the existence of a heterogeneous spectrum of PrPSc assemblies with respect to their tertiary and quaternary structure and biochemical properties (*24-31*). Characterization of the specific biological activity (templating activity and infectivity) of certain of these species led to the conclusions that it varied markedly with the size of the assembly (*24-26*) and importantly, in a strain-specific manner (*24, 26*), iii) kinetic studies of prion pathogenesis revealed that the formation of neurotoxic PrPSc species (*32*), which would cause neuronal damage/death in specific brain regions would be produced during a late phase, whereas PrPSc assemblies harbouring the replicative information would be formed during the early stage of prion infection (*33, 34*).

Even if the implication of PrPSc structural heterogeneity is at the backdrop of each article about prion strains and cross-species barrier (*8, 20, 23, 35, 36*), little is known about the mechanisms that govern their dynamic and how this heterogeneity contributes to the evolution and adaptation of the prion as a pathogen. This void of knowledge is inherent to prion paradigm which implicitly considers that within the substrain diversity only one subtype of PrPSc primarily drives the pathological phenotype. Furthermore, both Griffith, Lansbury-Caughey and derived models (*37, 38*) merely consider prions as a single/homogeneous entity all along the pathology progression.

As the physico-chemical properties of an assembly are dictated by its structure, two structurally distinct PrPSc subsets will exhibit two distinct dynamic and stability and more specifically they will compete for PrPC (i.e. the more replicative assembly subset should be selected during serial passaging). However, this is not the case and the structural diversity of PrPSc assemblies is maintained on serial transmission in the same host (*20, 23, 36*). One of the explanation for PrPSc substrain/subassemblies maintaining within the same biological environment is the existence of a **network of exchange** between different PrPSc subassemblies. Our preliminary investigation based on the kinetic of the quaternary structure evolution during the progression of the pathology revealed a quasi-oscillatory and dumped behavior. Furthermore, size-relaxation kinetic after a temperature perturbation of extractive PrPSc assemblies revealed an unexpected oscillatory behavior (with a period of 2 min). These unprecedented observations could be explained in term of biochemical process if different PrPSc subassemblies **exchange material** in a network of exchange.

The great challenge of the PeRCID project is to move forward the framework of prion paradigm by introducing the concept of exchange network between PrPSc subassemblies or ‘quasi-species’. The network of exchange concept is extensively used in **constitutional dynamic chemistry** and in the field of **self-organization biological network**. The introduction of exchange network concept to the prion paradigm requires mathematical mechanistic model based on an inventory of different PrPSc subassemblies as well as their dynamic. Therefore, the present project is based on a strong synergy between biology, biochemistry, biophysics and mathematic applied to biology and complex system in order to drive forward the current prion replication dogma.

The PeRCID project is structured in three major work-packages (WP). The first WP has for objective to explore the dynamic of PrPSc subassemblies within diverse prion strains and/or TSE cases in order to extract the phenomenological law driving the exchange process. Based on the WP1, the WP2 has for objective to explore the adaptive response of the exchange network during the templating process. WP2 will also explore the allosteric structural rearrangement of the substrate during the templating process. Even if WP1 and WP2 involve mathematical approach at each stage, the WP3 will address specific mathematical problems which raise the dynamic of exchange between different PrPSc subassemblies in order to obtain a holistic description of prion conversion, evolution and adaptation.

**Section b. Methodology**

**Work package 1: the repertoire of prion assemblies generated during prion replication, their dynamic and mechanistic relationships**

**Start**: year 1, **Duration**: 4 years, **Risk**: moderate/high

**Staff**: H. Rezaei, V. Beringue, M. Moudjou, S. Truchet, Posdoctoral fellows to be appointed, M. Doumic **Collaborators**: A. Deniset (Université Paris-Saclay; structural analyses), P. Minard (CNRS, PrP ligands), J.L Vilotte (INRA Jouy-en-Josas; transgenic mouse models), O. Andréoletti (INRA ENV Toulouse; neuropathological studies, human and animal prion isolates), JM Peyrin (Paris Neuroscience, primary cultured neurons in microfluidic devices).

**General objective**

The broad degree of heterogeneity within prion assemblies seen on transmission of prion strains/isolates with transmission barriers (*20, 21, 23*) and size-fractionation experiments (*24, 26*) raises the important question of their dynamic during the pathology evolution. Pursuing on delineating the reason for such PrPSc heterogeneity, the INRA group demonstrated recently that prion assemblies result from a **reversible condensation** of an elementary oligomeric PrP subunit (namely suPrP), which encodes the Strain Structural Determinant (SSD) (*19*). As the physico-chemical properties of an assembly are dictated by its structure, two structurally distinct PrPSc subsets will have two distinct dynamic and stability. Therefore, the coexistence of structurally different PrPSc subassemblies within the same biological environment raises the possibility of the existence of a **network of exchange** between two subtypes of PrPScassembliesthat could be mediated by their suPrP. The dynamic of the network should be at the basis of unexpected kinetics behavior during prion replication, including oscillations in the size evolution and exchange process between different subtypes of prion assemblies. It is evident that such complex kinetic pathways require new mathematical tools for its formalization.

The WP1 has for objective to **explore the dynamic of quaternary structural diversity** of PrPSc during the disease progression and to detail the network of exchange between PrPSc assemblies. The first task aims at establishing a time-course inventory of PrPSc structural diversity within different field isolates or strains and at delineating the contribution of the host and microenvironment to the dynamic observed (task1.1). By using notably an original single molecule approach currently developed in our consortium, the second task will quantify the structural heterogeneity of PrPSc assemblies. The structural determinant of this heterogeneity will also be explored through the biochemical, biophysical and structural characterization of PrPSc subassemblies as well as their elementary building block (task 1.2). In the third task, we will more specifically study the intrinsic dynamic and the exchange process within PrPSc assemblies outside a replicative context in order to explore the network of exchange between different PrPSc subassemblies (task 1.3).

WP1 outcomes will consciously feed the WP3 in order to build a model describing the network of exchange dynamic.

Task.1.1 Dynamic and structural diversification of prion assemblies during prion replication

The present task will define the PrPSc structural-to-activity landscape during the evolution of the pathology and the potential prion strain- and host-specific modifiers. Considering the total absence of knowledge on the structural diversification of PrPSc during the pathogenesis of a given prion strain/isolate, the INRA group has initiated studies on the time-course evolution of PrPSc quaternary structure in prion-infected transgenic mice or hamsters by fractionation based methods (*19, 24, 26*). Unexpectedly, these preliminary observations reveal that PrPSc distribution is evolving in a non-asymptotic manner: i) small PrPSc assemblies are predominantly formed at the early stages of priogenesis, 2) during the time evolution of the pathology, larger-size oligomers are detected, with a mean size fluctuating until the terminal stage of the disease. **This ‘quasi-oscillatory’ behavior is a hallmark of a complex kinetic process of prion conversion in which exchanges, feedback pathway and delay process should contribute to generate oscillations** (Figure 1). Thus, the following subtasks aim at consolidating these initial observations and precisely define the interactions between the observed population.

Subtask 1.1.1: Strain-dependent dynamic of PrPSc assemblies along the evolution of prion pathology

**Objectives.** The preliminary observations shown in Figure 1 have been obtained with 139A mouse prion strain (*39*) replicating in tga20 transgenic mice. Even if the 139A prion strain, as a cloned strain, is considered highly homogenous in term of PrPSc structuration (*40*), the quasi-oscillations strongly suggest structurally different assemblies evolving in a multiscale and complex conversion kinetic. The objective of this subtask is to determine if the initial formation of small PrPSc assemblies and the quasi-oscillatory behavior of size variation during the time-evolution of the pathology is a generic hallmark of the replication of mammalian prion strains.

**Methodology.** A large library of mammalian (mostly cloned) and synthetic prion strains is available at INRA, all passaged in *ad hoc* transgenic mouse lines and deeply characterized phenotypically and biochemically ((*6, 20, 21, 35, 36, 41-45*) and unpublished, Figure xxx). For most prion strains, the replication kinetics have been studied at the level of total PrP. The infectivity and PrPSc sedimentograms of a number of strains have already been defined at disease end stage ((*26*) and unpublished). These data will be completed by SV-fractionation sizing of prion assemblies at defined time-points of prion pathogenesis. Of particular interest are the origin of early/primary assemblies of small size. Comparison of *fast* and *slow* prion strains will allow determining whether those assemblies originate from the replication of the fittest, small-sized assemblies at disease terminal stage (*24, 26*) or from a more generic mechanism.

To complete the results obtained with ‘cloned’ or terminally adapted prions, the transmission experiments will been extended to i) so-called ‘field’ prion isolates, in particular those known to contain or to favor emergence of at least 2 substrain components, even in homotypic transmissions (*20, 36*), ii) to prions, which despite a fixed biological phenotype in the host species (incubation times, distribution of PrPSc and vacuolation in the brain) are still evolving/adapting (over > 4-5 passages) on iterative passage, as shown by retrotransmission to the parental host PrP or to a third species (unpublished data). These features are suggestive of subtle PrPSc structural evolution that are worth investigating because they are highly relevant to the ‘natural’ transmission events.

**Outcome.** As stated in the methodology, the library contains mostly cloned mammalian prion strains. It is expected from “cloned” terminology a structural homogeneity in term of PrPSc structuration. The existence of quasi-periodical or at least non-asymptotical evolution of size distribution will strongly suggest complex competition and exchange between structurally distinct assemblies and will argue in favor of prion strain defined by a convolution of structurally different subsets of PrPSc assemblies. Thus, the main outcome of this task is the identification of prion strains for which it exists a non-asymptotical size variation. This last being a **direct proof** of an *in vivo* structural diversification and exchange process during prion replication.

**Synergetic outcome:** Sinal traitement, Mathematical modeling based on size distribution kinetic,

Subtask 1.1.2: Effect of host determinants and cell-microenvironments on the dynamic of prion assemblies

**Objectives.** In the continuity of the previous task, we will address whether host factors participate to the dynamic of PrPSc assemblies, including fluctuations in the tissue/microenvironment (i.e. specific brain areas), the cells-diversity constituting each brain area (i.e. astrocytes and neurons) ((*35, 46, 47*) and unpublished) and PrPC levels, all these factors being intertwined.

**Methodology.** Two kinds of potential influence of fluctuation in the amount of brain PrPC substrate will be tested: i) exhaustion of PrPC substrate due to proteostatic response during disease evolution as published earlier (*48*). We will determine variations in PrPC expression levels (and the transcripts) throughout the pathogenesis in the most relevant prion strains/rodent models and if any examine correlation with oscillations in PrPSc assemblies; ii) excess or controlled levels of PrPC, by sizing prion assemblies during prion pathogenesis in proprietary transgenic mice expressing ovine PrPC at different levels, from physiological to 8-10 fold overexpression (*36*). Depending on the results, we will create new mouse models with <0.5x expression level to overtly delay the clinical phase of disease and examine prion dynamical behavior during the replicative phase.

Rather than being inherent to the dynamics of misfolding and aggregation process, the multiplicity of assemblies with differing structuration/arrangements may be due to production of different assemblies by different brain areas and/or cell types in the brain (or loss). To examine this possibility, we will make punches at different stage of the pathogenesis in brain areas with prominent PrPSc deposition at terminal stage of disease (e.g. (*14*)) from the most pertinent mouse models/prion strains combination and will establish as in tasks 1.1.1 the inventory of the PrPSc assemblies and dynamic of evolution.

To complete these data, we will perform similar studies in a simplified *in vitro* context as some prions can be replicated in immortalized in primary cultured (neurons, astrocytes) cells (*20, 47, 49-52*). Primary neurons and astrocytes (coming from the same brain area or not) will be eventually cultivated in compartmentalized microfluidic assays (*53, 54*). A further refinement with such tool is the potential use of membranes with specific cut-off to control the size of PrPSc assemblies spreading from one compartment to another (and see WP2, task xxx). This will allow studying (as in the brain) the effect of replication starting at different time points in different prion-permissive compartments.

We will then combine the curves obtained for each specific brain area with that of the total brain to examine the contribution of local replication to the overall distribution of PrPSc assemblies and oscillatory behavior.

In a last experimental paradigm, we will transpose our studies to the spleen tissue for prions able to replicate in the lymphoid tissue (e.g. (*14, 35, 43*)). It combines a simplified tissue where one type of cells, - the follicular dendritic cells, mostly replicate prions and different prion replication curve as replication tends to plateau early after the infection until the disease terminal stage (*35, 43, 55*).

**Outcome.** Host-determinants (PrPC/tissue/cells) to the dynamic of misfolding and aggregation process, vs. genericness of the dynamic behavior. Contribution of the dynamic of prion replication locally to the global dynamic in the brain, (a)synchronicity, local evolution.

Task.1.2 Structural and biochemical characterization of the PrPSc subassemblies

Task 1.1 aims at exploring the dynamic of PrPSc quaternary structure within a given strain or field-isolate *in vivo* and *ex vivo*. Through biochemical, biophysical and low-resolution structural biology at single molecule level, task 1.2 aims at exploring the structural underpinnings of this diversity and its strain dependency at the level of both the PrPSc polymers and the constitutive suPrP subunits. A particular emphasis will be put on the **quantification of the structural heterogeneity**.

Subtask 1.2.1: Inventory of PrPSc substructures within given prion strain

**Objective.** The objective is to setup an inventory method based on structural and biochemical properties of PrPSc subassemblies. A specific pioneering objective here is to develop new tools to characterize the underlying heterogeneity at the structural level.

**Methodology:** The approaches for setting up the inventory of PrPSc substructure within prion populations (according to task 1.1) will consist in three different and complementary strategies. The first strategy is based on a quantitative-epitope mapping after SV-fractionation methods. The epitope mapping will use either a library of available anti-PrP antibodies (e.g. (*56, 57*)) covering the entire amino acid sequence or an alpha-P protein designed ligands for different conformation or domain of PrP protein (Valerio-Lepiniec M, 2015 Biochem Soc Trans PMID: 26517888; Urvoas A, Trends Biotech 2012 PMID: 22795485 ). Limited proteolysis of fractionated assemblies incubated with different concentrations of chaotropic agent will also lead to further structural discrimination. The latter strategy will be particularly informative to pinpoint any correlation between small-sized assemblies and suPrP which is specifically evidenced at high concentration of urea (*19*). The second strategy is based on the matrix of differential seeding-activity of structurally different PrPSc assemblies explored by RT-QuIC (*58, 59*), by using as PrP substrate a unique collection of recombinant PrP from different mammalian species and with specific mutations found to be key in the templating process (unpublished, Figure 2). This technique is routinely used at INRA. These two first methods could be performed on SV-fractionated PrPSc assemblies. The third method takes advantage of our ability to purify SV-fractionated (or not) PrPSc assemblies. The morphologies of the purified and concentrated (e.g. (*60*)) assemblies will be analyzed by Atomic Force Microscopy techniques by an automatic image analysis algorithm already developed by Marie Doumic group at INRIA (unpublished). By using a unique in-house instrument combining atomic force microscopy with infrared spectroscopy (NanoIR), we will have access for the first time to the secondary structure of each individual assemblies. The NanoIR spectroscopy technique has been already successfully implanted in close collaboration with Ariane Deniset (who will participate to this project at 30% of her research time). This technique has been implemented to be used with prion strains requiring a safety II containment level.

**Outcome.** This task will lead for the first time (to our knowledge) to estimate the degree of structural polydispersity within a given prion strain or field isolate. The measurement of IR spectra of each individual assembly obtained by NanoIR spectroscopy associated to a statistical analysis will lead the calculation of the IR spectrum-variance which will constitute a direct parameter of the heterogeneity in PrPSc subassemblies.

**Synergetic outcome:** Identification of number of species that should be include in the model building and kinetic modeling and if possible their phylogeny

Subtask 1.2.2: Structural heterogeneity of the oligomeric elementary subunit suPrP

**Objective.** At mesoscale, prion SSD is encoded in the structural landscape of PrPSc assemblies (reviews (*6, 8*)). At the molecular level, the INRA group demonstrated that the SSD is encoded in the conformation of an oligomeric elementary PrPSc subunit termed suPrP (*19*). The existence of strains and PrP subassemblies implicates *de facto* a certain degree of variability in suPrP structuration. It raises then the perplexing question how the strain information as well as the heterogeneity of PrPSc subassemblies could be encoded in the structure of an oligomeric subunit as suPrP. A pertaining possibility is the existence of suPrPs specific of each subassembly. While subtask 1.2.1 has for objective an inventory of PrPSc substructures, we will dissect in this task the molecular architecture of suPrP obtained from these different PrPSc subassemblies in order to determine the origin of their variability.

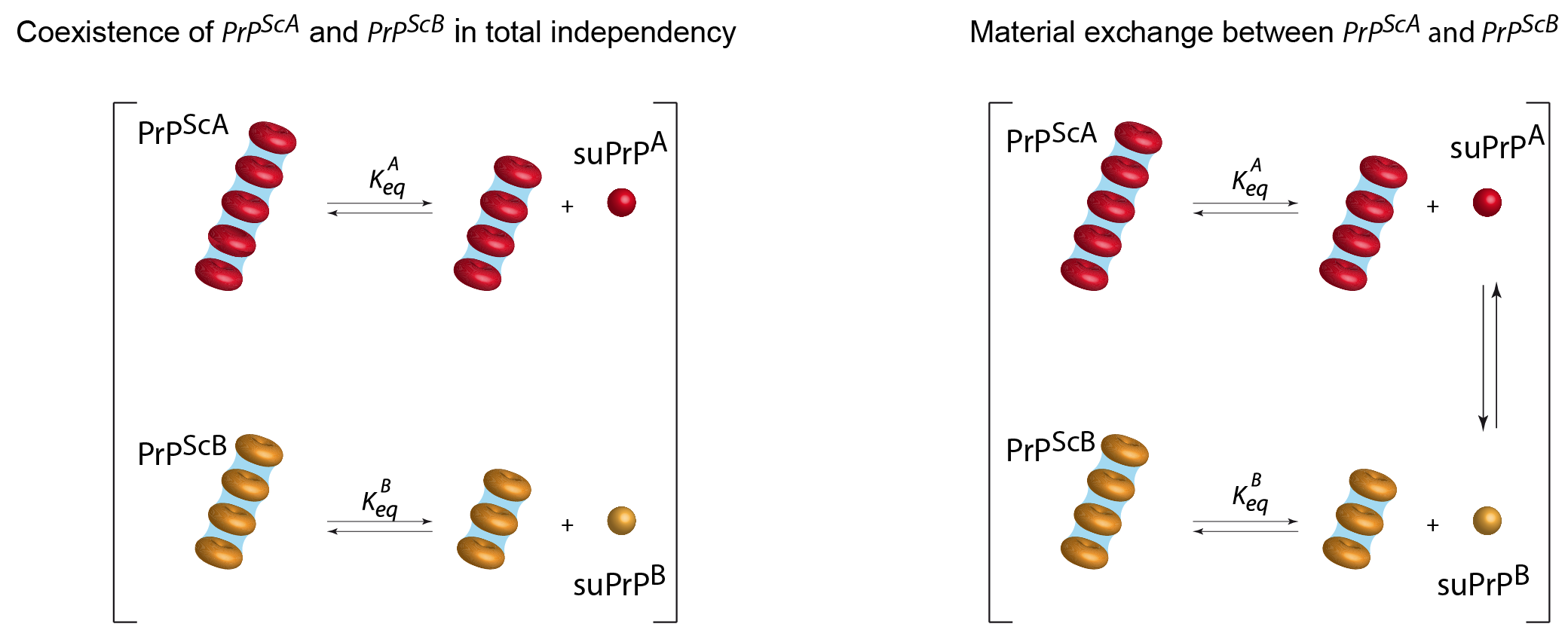
**Methodology.** suPrP from the fractionated subassemblies will be generated according to a routine protocol based on urea-induced unfolding (*19*) and analyzed by FFFF coupled to MALS (instrument will be financed by the ERC grant) in order to have access to its molecular weight, hydrodynamic and gyration radius values. As suPrP is protease sensitive (*19*), hydrogen-deuterium (HDX) approaches followed by pepsin digestion will be performed. The resulting peptides will be analyzed by mass-spectroscopy and the assignments will inform us about the domain of PrP involved in suPrP formation. The multi-strain approach constitutes an important key-point in this task as it will allow us to compare outcomes data and establish a differential approach to characterize the difference of SSD at the level of suPrPs. A second degree of comparison will be performed with prion isolates formed from a mixture of substrains. For example, prion strains called T1Ov (21K PrPSc electrophoretic pattern, (*20*)) and T2Ov (19K PrPSc electrophoretic pattern, (*20*)), upon isolation have their respective suPrP containing the information relative to their differential proteolysis pattern (*19*). These two strains can be stably co-propagated in the brains of experimentally infected transgenic mouse (*20*), thus questioning the potential relationships between their SuPrP. It will thus be readily possible to study the suPrP from the T1Ov and T2Ov subassemblies upon isolation or as mixture.

**Outcome.** This subtask will lead us to determine if the PrPSc subassemblies differ by the architecture of their respective suPrP. In other words, if it exists an intrinsic heterogenic structuration of suPrP leading to the formation PrPSc substructure. Or structural diversification of PrPSc assemblies occurs during structural fluctuations during the condensation of a commune suPrP. The fluctuation could be environmental and joints the subtask 1.1.4.

**Synergetic outcome:** The synergetic outcome of this subtask will have important impact on the modeling of network of exchange. Indeed if it exists two type suPrP differing in size their interconversion will involve either a relies or recapture of monomer.

Task 1.3 Dynamic of PrPSc assemblies and exchange process.

Out of the replicative context, the coexistence of substructures or substrains within the same media/environment is questionable if we consider that each subassembly is in an equilibrium with its elementary subunit (*19*). Two situations could be envisaged in the case of the coexistence of PrPSc subassemblies (Figure 3). The first situation could correspond to a total independency between each subtype of PrPSc and their respective elementary building block. The second possibility could correspond to an interconnection between subassemblies through the existence of an equilibrium between their respective elementary building block. This last situation generates an **interconnection between PrPSc subgroups** through a **network of exchange** in which an implication of PrPC could be also envisaged.



**Figure 3.** The Dynamic of two PrPSc subassemblies PrPScA and PrPScB within the same environment. PrPScA and PrPScB could evolve in total independence if no material is shared. Their dynamic becomes entangled if they exchange material. Here the exchange occurs through the existence of an equilibrium: . Even if not mentioned PrPC could participate to the exchange process.

Preliminary relaxation kinetic experiments after thermal perturbation using 263K, mouse ΔGPI prion strains or infectious synthetic human-Prion assemblies revealed the existence of highly periodical variation in the size mean average (measured by few molecule static light scattering, figure?), highlighting a complex exchange process through a network. This network of exchange could be at the origin of the dumped-oscillation observed during 139A prion replication *in vivo* (Figure 1). Therefore, the objective of task 1.3 is to highlight and explore the exchange process between different subsets of PrPSc assemblies within a strain or field-isolate and to determine for the first time the contribution of PrPSc subassemblies to prion evolution and adaptation to a new host. One of the major objective of this task is the introduction of the key-principles of Constitutional Dynamic Chemistry (CDC) concept (Lehn, 2012, PMID: 22169958 ) into prion paradigm, to consider prion replication as a replication of a network through material exchange rather than a simple polymerization process of a given PrPSc assembly or subassembly.

Subtask 1.3.1: Mechanisms of the oscillatory behavior of PrPSc assemblies during depolymerization

**Objective.** For certain concentration, the depolymerization kinetic of PrPSc assemblies after a thermal perturbation revealed an oscillatory behavior in size variation (Figure?). This oscillatory behavior highlights the existence of complex exchange network (ref CDC) within different subassemblies. This is the first time that an oscillatory behavior is observed for biological assemblies without energy input as is the case for microtubules filament (PMID: 9442869 ). The objective here is to precisely define the origin of the oscillations during the dilution-induced depolymerization kinetic of PrPSc assemblies and to characterize the structural rearrangement associated with these oscillations

**Methodology.** To identify the biochemical process responsible for the observed oscillations, we will combine biochemical and biophysical approaches such as thermal perturbation using few molecules static light scattering measurement approaches with oscillation analysis in term of signal. Single molecule approach such as TIRF will be used to explore at single-assembly level the oscillatory phenomenon responsible for size variations of the assemblies. Kinetic modelling, phase-space analysis, bifurcation point identification using either discrete of continuous approaches will be performed in order to establish a kinetic model describing the occurrence of oscillations.

**Outcome.** The outcome oftheseexperimental approaches will be used to mathematically model the kinetic process and validate different pathways.

**Synergetic outcome:** The oscillatory kinetic will serve as an output of kinetic modeling and model building

Subtask 1.3.2: Evidence for material exchange between PrPSc subassemblies

**Objective.** The coexistence of substrains or structurally distinct PrPSc assemblies within the same environment implicates two phenomena during the evolution of the pathology. The first phenomenon is a competition for PrPC during the conversion process. Two structurally distinct assemblies should have two different templating rates. The second phenomenon will consist to an exchange process if they have in common a reactant such as their respective elementary building bloc suPrP. The objective of this task is to explore the exchange process between different PrPSc subassemblies within a given prion population (strain or field-isolate).

**Methodologies.** It is expected from our mathematical modeling that two structurally distinct assemblies exchanging material should have an intertwined relaxation kinetic (*61*). We will thus i) explore the exchange process by a kinetic analysis of the dilution-induced displacement of the suPrP-PrPSc equilibrium (*19*) and ii) characterize the PrP conformer involved in this exchange. The experimental procedure is based on the separation, by a low-pass molecular weight membrane filter, of two distinct compartments containing two different concentrations of the same PrPSc assembly preparation (Figure 4). In the case of exchange between two subsets of PrPSc assemblies, e.g. attractor and donor, the flux of material will be directed toward the compartment containing the highest concentration of attractor assemblies before reversing in order to reach an equilibrium. We already build a homemade device using millifluidic system coupled to two different measurement systems as fluorescence, few-molecules light scattering spectroscopy and PMCA anlysis (Figure 4). By using different Mw cutoff for the membrane, we will then be able to estimate the size of the conformer involved in the exchange process and examine whether it is consistent with suPrP.

**Outcome.** Determination of a kinetic model of the exchange process in order to establish the base of the dynamic of the network of exchange.

**Synergetic outcome:** mathematical model improvement based on accurate kinetic data

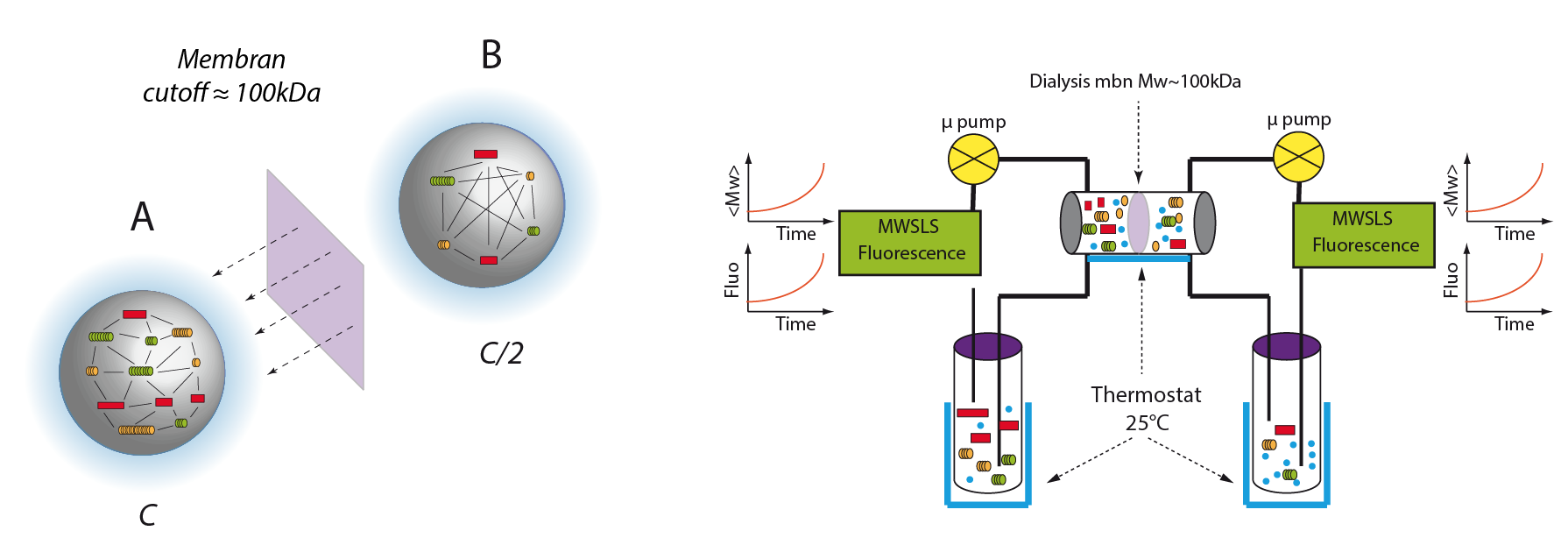


Figure 4. Experimental design to determine the maximal size of the PrP conformer serving as vector of exchange between structurally distinct subset of assemblies.

Subtask 1.3.3: Formation of hetero-strain from two distinct elementary building blocks

**Objective.** The co-propagation of two distinct strains within the same animal and their maintaining on iterative passage (*20, 36*) constitutes a paradox, as it is expected that structurally distinct assemblies even with tiny difference in replication rate would be differentially selected on serial passage. One explanation could be the existence of hetero-strain assemblies. In this task, we will explore the possibility that two pairs of strains (e.g. LA21K and LA19K sheep scrapie prions (*36*) or CJD-derived T1Ov and T2Ov prions (*20*)) form hetero-strain assemblies.

**Methodology.** The strains mentioned above can be isolated from each other by limiting dilution or passage by PMCA or transgenic mouse models expressing PrPC at variable levels ((*20, 36*) and unpublished) or be stably co-propagated. The approach adopted in this task will consist to disassemble these prions alone or in combination in their respective suPrP (*19*), then mix their elementary building block and proceed to the refolding. As we are able to work with purified PrPSc material, chemical labelling such as addition of biotin, tags or fluorescent dye could be performed to track the formation of hetero-assemblies through pulldown or FRET technique. Bioassay experiments with hetero-strains assemblies will be envisaged to determine their replicative propensity to coevolve.

**Outcome**. The major outcome of this task is to establish if different strains can share elementary building block even if they are structurally different. The existence of such hetero-strain assemblies constitutes an unexpected extension in the prion paradigm and could explain cross species barrier (evocated in task 1.4.5), strain mutation and adaptation phenomenon.

**Synergetic outcome:** kinetic model improvement. This task will lead to determine if the kinetic model should envisage the emergence of “new” subassemblies initially inexistent (within the strain and field isolate) through the condensation of suPrP structurally different.

Subtask 1.3.4: The contribution of structural heterogeneity to prion adaptation during cross-species transmission events

**Objective.** Conceptually, the species barrier that limits prion propagation in new hosts or new host PrP species is believed to be the result of a misfit between PrPC and PrPSc contained in the infecting prion (*6, 8*). Thus, both host PrPC sequence and the infecting strain type are key determinant. The objective is to define the contribution of the dynamic of material exchange/prion structural dispersity to across-species prion adaptation.

**Methodology**. We will compare the relative capacity of SV-isolated PrPSc assemblies from multiple prion strains to cross mounting species barriers, as modelled by transgenic mice expressing heterotypic PrPC (e.g. (*41*)). Preliminary data from the INRA group suggest that overcoming prion species barrier somehow requires cooperation between structurally distinct PrPSc assemblies (prelim figure or Hlink). As second step, we will reconstitute different sets of assemblies and examine their cross-species capacities in relation with their structural variability. Having identified the number of minimal PrPSc interactants for efficient crossing, we will then particularly study which type of material is exchanged and at which rate, as in subtasks 1.3.1 and 1.3.2.

**Outcome.** The main outcome of this subtask will be a better understanding of the CDC in the overall prion fitness and new important insights on prion species barrier concept. From a public health perspective, this is a key issue given the zoonotic potential of animal prions (*35, 42, 62, 63*).

Subtask 1.3.5: Contribution of structural heterogeneity to life-long prion persistence

**Objective.** By virtue of absence of substrate to convert, prions cannot replicate in PrP0/0 transgenic mice (*64-66*). Preliminary data from the INRA group reveal that prions responsible for human CJD (MM1 subtype) can persist in the brain of PrP0/0 mice from one to two years after experimental challenge, as detected by bioassay of PrP0/0 brains in reporter human PrP mice (*43*). These dormant prions appeared able to initiate disease back in human PrP mice at near full attack rate, despite very long incubation periods and low seeding activity suggesting an apparent very low infectious titre in contradiction with the full-attack rate. The disease phenotype was unchanged indicating that the CJD SSD was conserved in the dormant prions. The objectives here are to identify how prions can persist. Two exclusive hypotheses will be examined, i) displacement of equilibrium until suPrP and initiation of disease (possibly stochastically) back to the replicative context, ii) persistence of certain PrPSc subassemblies that are more prone than other to persist and re-initiate replication.

**Methodology**. We will establish the size of the remnant PrPSc assemblies in PrP0/0 mice by coupling SV with RT-QuIC detection which allows exquisite detection of highly diluted CJD prions (ref and unpublished data) and will pinpoint any correlation with CJD suPrP. As second step, we will explore by RT-QuIC the seeding-activity of the remnant assemblies towards the matrix of recombinant PrP from different mammalian species and with specific mutations found to be key in the templating process (Cf. task 1.2.1). Finally, we will perform again this type of experiment but by controlling the PrPSc assemblies left to perdure in PrP0/0 brains (e.g. T1Ov and T2Ov in combination at certain concentration vs. in isolation) to better identify the number of PrPSc assemblies and the interactions necessary to remanence.

**Outcome.** The main outcome of this subtask will be a better understanding of the CDC in the overall prion persistence in non-replicative environments.

**Synergetic outcome:** The evolution dynamic of PrPSc subassemblies within prion strain or field isolate in vivo out of the replicative context.

Subtask 1.3.6: Effect of quaternary structure homogenization on prion structural diversification

**Objective.** A pertaining question to the existence of the network of exchange (and by homology to self-organization properties of complex systeme (PMID: 21419158)) is if, - within by homology/contrast? with the quasi-species concept (*67, 68*), one PrPSc isolated subassembly is able to generate the whole the whole structural heterogeneity of the strain on transmission. The aim of this task is to explore the effect of quaternary structure homogenization on generation of PrPSc diversity.

**Methodology.** We will use the fact that partial unfolding and refolding of PrPSc assemblies by urea leads to relative quaternary structure homogenization (*19*). Prion strains for which the specific templating activity correlate with size distribution, or oppositely, with templating activity associated with oligomeric subsets (*24, 26*) will be used. Their quaternary structure homogenization by unfolding and refolding followed by templating activity measurement by PMCA and bioassay after SV-fractionation will address the question of quaternary structure homogenization on prion diversification into diverse assemblies.

**Outcome.** This subtask will provide key insight into the “quasi-species” behaviour of the structurally distinct PrPSc assemblies constituting prion strains.

**Synergetic outcome:**

**WP2: Mechanisms of structural information transfer and spreading during templating process**

**Start**: year 1, **Duration**: 5 years, **Risk**: moderate/high

**Staff**: H. Rezaei, V. Beringue, M. Moudjou, S. Truchet, Posdoctoral fellows to be appointed, M. Doumic **Collaborators**: A. Deniset (Université Paris-Saclay; structural analyses), P. Minard (CNRS, PrP ligands), J.L Vilotte (INRA Jouy-en-Josas; transgenic mouse models), O. Andréoletti (INRA ENV Toulouse; neuropathological studies, human and animal prion isolates), JM Peyrin (Paris Neuroscience, primary cultured neurons in microfluidic devices). C. Sizun, CNRS, ISN, gif-Sur Yvette, France (RMN), F. Fraternali, King’s College, London, UK (Molecular dynamic)

The base of the prion paradigm considers prion replication as a process of **Structural Information Transfer** (SIT) from a template (i.e. PrPSc) to a substrate (i.e. PrPC) (Fig?). The transfer leads to a deep structural remodeling of the substrate, which will adopt the structure of the template containing also the strain structural determinant. Conventional methodologies have so far failed to provide a precise view of the molecular process of the SIT. In the literature, only eight publications based on theoretical approaches tried to address this topic (ref). This failure is mainly due to the fact that **there is not a unique templating interface** but potentially as much as the number of PrPSc subassemblies/substrains. Considering the achievements expected from WP1, the WP2 has for objective to address the molecular mechanisms of the SIT by considering two major mechanistic issues: i) The first one will take into account the structural heterogeneity of the template and the existence of a network of exchange between PrPSc subassemblies within a given strain or field isolate. The structural heterogeneity and the network of exchange impose us to explore the response of the network (i.e. a global response) to a given type of substrate rather than the efficiency of a unique PrPSc subassemblies to template a given substrate. ii) In the frame work of SIT how suPrP is formed (*19*). Beyond, we will address the contribution of equilibrium to prion spreading and amplification based on the spatial diffusion of suPrP, which may bypass the contribution of active fragmentation (i.e. that required contribution of housekeeping protein and energy).

Task 2.1. Adaptive templating response of a strain or brain isolate to a given PrPC substrate

As mentioned above, the SIT mechanisms gain in complexity with the existence of different PrPSc subassemblies, each one with a given templating efficiency. In addition, a given strain has a preference for a given PrPC substrate (PrP polymorphism, mutations, PrPC glycoforms, etc.) (refs). Thus, by taking into account the existence of PrPSc substrain forming a network of exchange, it is expected that depending on the type of PrPC substrate, the subset of PrPSc subassemblies with the higher templating rate will be promoted and other subassemblies sub-served. This preferential templating should shift the state of the network by modifying the partition between different PrPSc subassemblies. We call this phenomenon an **Adaptive Templating Response (ATR)** of the exchange network. Preliminary exploration of an exchange network composed from 3 different subassemblies suggested that for certain parameters of the exchange process, the preferential templating of one subtype will not eliminate other subtype but in opposite contribute to their maintaining. The aim of this task is to explore the **ATR** of a given strain with regard to a defined substrate and to address the orthosteric mechanism of PrPC structural rearrangement during the templating process.

Subtask 2.1.1: Characterization of the adaptive templating response to different PrPC substrate.

**Objective.** It is well documented in the prion field that during homologous transmission (i.e when the primary structure of PrPSc and PrPC are identical), the prion strain characteristics (quaternary structure diversity and all physio-pathological phenotypes) are faithfully conserved on iterative passage (ref). This affirmation can become false in an absolutely unpredictable manner when the primary structure of PrPC serving as substrate changes (ref). Thus, during prion replication in the presence of homologous PrPC, the structural diversity of PrPSc subassemblies will remain invariant, whereas in the presence of heterologous PrPC, the ATR of the network will be at the origin of a change in the partition of different PrPSc subassemblies. The objective of this task is to characterize the response of different PrPSc subassemblies from specific strains to different PrPC in order to identify on the substrate scaffold a selective structural determinant that will affect specifically the dynamic of a PrPSc subpopulation during the templating process.

**Methodology.** The INRA prion strain library will serve for the purification of extractive infectious prion subassemblies (refs). The purified assemblies will be used to template a library of structurally well characterized PrP substrate (extractive PrPC and recombinant PrP). The substrate library comprises PrP mutants for which single specific mutation, substitution or deletion has been introduced in a precise region in order to tune either the overall conformational dynamic of PrP or to constraint a specific region (as example, introduction of S-S bond, ref). Preliminary experiments using as template vCJD, 263K or synthetic human infectious prions and as substrate human or bovine PrP, revealed that the best technique to evaluate the ATR is the evolution of the variation kinetic of weighted-average molecular weight during the templating, as determined by few-molecule light scattering approach. More conventional approaches as Thioflavin T fluorescence measurement as function of time and the increase of size of assemblies estimated by atomic force microscopy images analysis by an automatic image analysis algorithm already developed by Marie Doumic group at INRIA will also be used. The estimation of the heterodispersity parameter (cf. substask xxx) using single molecule NanoIR technique will be used to determine the effect of different PrP substrate on the inflation of PrPSc subpopulation (Contribution of Ariane Deniset from Soleil synchrotron who will dedicate 30% of her research time). By combining this last technique to the use of 13C labelled PrP substrate, we will be able to distinguish the infrared absorbance of the PrPSc subassemblies that will serve as template from those which will not participate. By using isothermal titration calorimetric (ITC) measurement during iterative addition of substrate on the template, we will explore the amplitude of the structural rearrangements of the substrate through the excess of heat-release during the templating process. The ITC device will be funded by the ERC grant. The combination of all these readouts will lead us to have a quantitative estimation of adaptive response of the network of exchange during the templating process.

**Outcome.** The use of different PrP mutants with deletion or substitution in a specific region will inform us about the importance of these regions to induce an adaptive templating response. The use of structurally different prion strains will lead also to explore whether different prion strains could share a common domain for an ATR.

**Synergetic outcome:** The information that will obtained through this subtask will lead to build and improve the mathematic model of exchange between different subassemblies in the context of replication. Beyond the fact that we will build a kinetic templating model, we will also start to analyze mathematically the response of the model of network of exchange to a templating perturbation (here the templating of a specific subassemblies by a PrPC) and analyze the adaptive response of the network.

Subtask 2.1.2: Adaptive response to therapeutic molecules and specific ligands

**Objective.** The adaptive partition of different PrPSc subassemblies within the network of exchange is not restricted to the templating process and a ligand that stabilizes or destabilizes a subpopulation should also induce **an adaptive response** of the network of exchange. A panel of molecules and therapeutics ligands exists modulating modestly the disease evolution (refs). In this task, we will determine the effect of certain (sometimes proprietary) ligands to induce an adaptive response from the network of exchange.

**Methodology.** The approaches adopted here are similar to those used in subtask 2.1.1 for ATR exploration. Indeed, the ligands used in this task could be assimilated to a PrP substrate with the difference that they will not be converted. We envisage to use three types of ligands. This first type will correspond to classical therapeutic ligands already well described for their partial effect on prion replication (e.g. Congo-Red, Pentosan Polysulphate, Polyene antibiotics, etc…(*46, 55*)). The second type of ligand will be monoclonal antibody directed against specific regions of PrP. A large panel of antibodies covering different regions of PrP is available in the INRA group (e.g. (*57*)). The third panel of ligand will correspond to alpha-P protein designed ligands for different conformations or domains from PrP. This last type of ligand will require the contribution of P. Minard’s group, an expert on alpha-P protein design and evolution.

**Outcome.** The major outcome of this task is the adaptive response of the PrPSc subassemblies to a ligand-induced perturbation. This is the first time to our knowledge in the prion field that the effect of a ligand or a therapeutic compound is investigated by taking into account the polydispersity of the target.

Subtask 2.1.3: Allosteric mechanism of PrPC structural rearrangement during the templating

**Objective.** Among prionoids, PrP is the unique example for which the native state is folded. For other prionoids, the substrate of the conversion is usually either an internal disordered protein or at least the domain involved in the conversion is a disordered domain (ref). Thus, during the templating process of the monomeric PrP, at least a partial unfolding followed by an assisted refolding process occurs. These two processes are induced by the interaction between the template interface and the templating target interface of the substrate (Fig.xx). The aim of this task is to explore the allosteric linkage between the templating target interface and other domain of the substrate during the templating process.

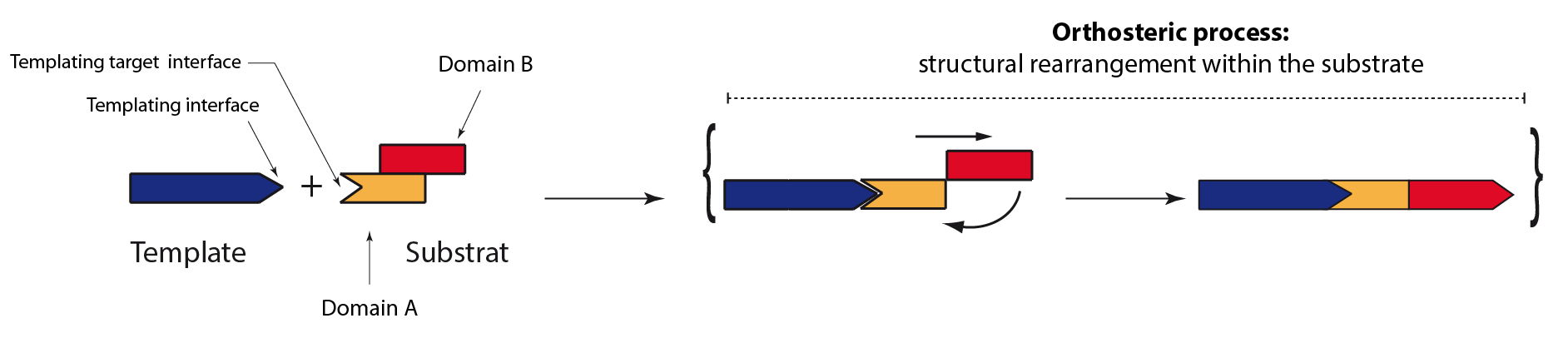


Fig.xxx

**Methodology.** The methodology adopted here will combine two approaches. The first approach that will be undertaken is to identify the allosteric linkage between different PrPC subdomain through the use of different PrP mutants out of a templating context (we will use mainly recombinant PrP). These PrP mutants could be divided into two groups. The first group corresponds to a library of PrPC with a unique replacement of a stretch of amino-acid with (GSGG)2 in a specific PrPC subdomain in order to induce a local unfolding (flexible mutant). The second group of PrP mutants will correspond to the introduction of covalently structural constraint by an additional S-S bond (constrained mutants, (*69*)). A structural approach using liquid NMR (participation of Christina Sizun) will lead to accurately assign the allosteric linkage between perturbed domain and other regions of PrP (*70*). Furthermore, NMR structural approach will generate experimental constrains in order to perform molecular dynamic simulation through the participation of Franca Fraternali. In the paste, these two approaches have been successfully used (independently of each other) to identify the allosteric sequence of events for PrP oligomerization (*71*) and to generate a bona fide mammalian prion with internal deletions in PrP (*70*).

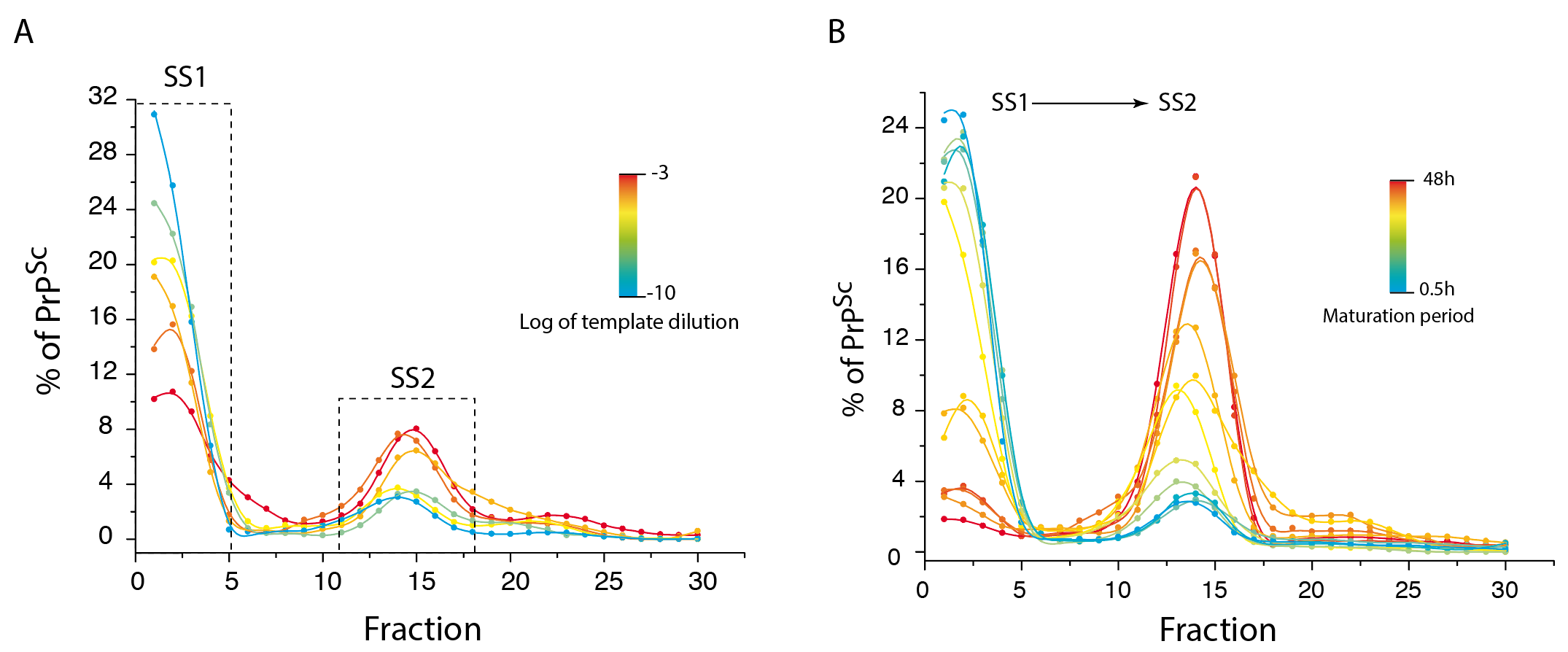
The second approach will require templating experiments and explore the response of the substrate to the adaptive templating (in the subtask 2.1.2 we investigate the ATR to a given substrate). To address this issue, we will use experimental approaches leading to explore specifically the structural variation of the substrate during the templating. By using flexible or constrained mutants of PrP isotopically 13C or 13C, 15N labeled as substrate of templating we can therefore monitor fine structural modifications after templating using solid state NMR (contribution of Christina Sizun). Due to the safety legislations, the template library that will be use should comprise only safety level 2 prion strains.

**Outcome.** The first part of this approach will lead to identify on PrPC scaffold PrP subdomain that are allosterically linked out of a templating context. For example, preliminary study leads us to find a strong allosteric linkage between PrP L3 loop and the N-terminal region of H3 even if they are at 20Å distance. The second approach which is complementary of the approach developed in subtask 2.1.2 will lead to specifically investigate the propagation of structural information toward constrained or flexible mutants.

Subtask 2.1.4. ~~Prion assemblies dynamic behavior and intervention of fragmentation during prion replication in cell-free assays.~~ Early stage of structural PrPSc diversification during the conversion process

The mechanisms of structural diversification within a given strain constitutes a poorly explored field, even if the diversity is often evocated to explain the cross-species barrier and prion adaptation (ref Collinge &clarck).

The PMCA technique that leads to a bona-fide prion replication amplification (refs) in vitro leads us to demonstrate that during the early stage of the prion conversion two distinct subsets of PrPSc assemblies are generated (SS1 and SS2, FigXXA) both sharing the same strain structural determinant (?). Depending on the initial template concentration one species is favored compared to the other one suggesting two distinct kinetic order governing their respective formation (ref Eghaian as example ?). The maturation of SS1 and out of PMCA context but in the presence of PrPC (on va faire comme si…) leads to an interconversion of SS1 to SS2. This very simplistic system of prion replication reflects both the step of bona fide templating and the evolution of this PrPSc subassemblies by an exchange process



Figxx: 127S (?) prion strain amplification by PMCA technique. A) template concentration-dependency of size distribution revealed two subset of PrPSc assemblies (SS1 and SS2). B) Post PMCA evolution of SS1 and SS2 as function of time reveals a delay-transformation of SS1 to SS2

**Objective:** the objective of this subtask is to explore the diversification and maturation of PrPsc subassemblies at the early and late stage of conversion process.

**Methodology.** The methodology here is

First, the size of the SS1 and SS2 PMCA products (from different strains with diverse size for the fittest assemblies) will be precisely determined by a combination of techniques including SV, size exclusion chromatography, and Asymmetric Flow Field-Flow Fractionation (AF4) coupled with multi-angle light scattering. Second, the morphology and the conformational heterogeneity of the replication products will be examined as in task 1.2.1. A major advantage of the PMCA is that this technique allows endless production of infectious material amenable to a high degree of purity. Third, we will address the effects of unfolding and refolding approaches coupled to size distribution analyses to make the link with SuPrP for each subassemblies. Fourth, based on our expertise in analyzing by static light scattering the evolution of prion polymerization with purified material (ref and subtask 1.3.2), we will put hard work on developing a real-time PMCA technology coupled with fractionation and/or detection of the formed assemblies, so as to trap the intermediate assemblies at work in PMCA. Developing this aspect is of key importance to quantitate the respective effects of sonication vs. incubation on PrPSc assembling. ~~Five, kinetic~~~~refinement with adjustments of duration of incubation vs. sonication and use of RT-PMCA (as above) will be performed to better follow the evolution of PrP~~~~Sc~~ ~~quaternary structure and the amount of PrP~~~~Sc~~ ~~as a function of time within the 48h and after the PMCA reaction. This will define the respective contribution of sonication vs. incubation to the size of the assemblies generated.~~ In parallel, the time-evolution of the specific biological/templating activity of the assemblies, in combination with their biochemical evolution (resistance to sonication, PK-resistance, etc.) will be addressed to decipher which types of arrangements and what type of evolution are at work. ~~Finally, we will leave the pA2 assemblies maturate/incubate even longer to examine whether this modifies the types and structures of PrP~~~~Sc~~ ~~assemblies in a network that would tend to that observed in vivo.~~

**Outcome.** The major outcome of this subtask will consist to explore the kinetic aspect of the hole process : templating, structural diversification and evolution of the network of exchange as reported in figXX. The development of specific biophysical instrument combining millifluidic system as reported in subtask 1.3.2 will lead to trap and identify the vector of exchange leading the evolution of the PrPSc subassemblies within the network of exchange.

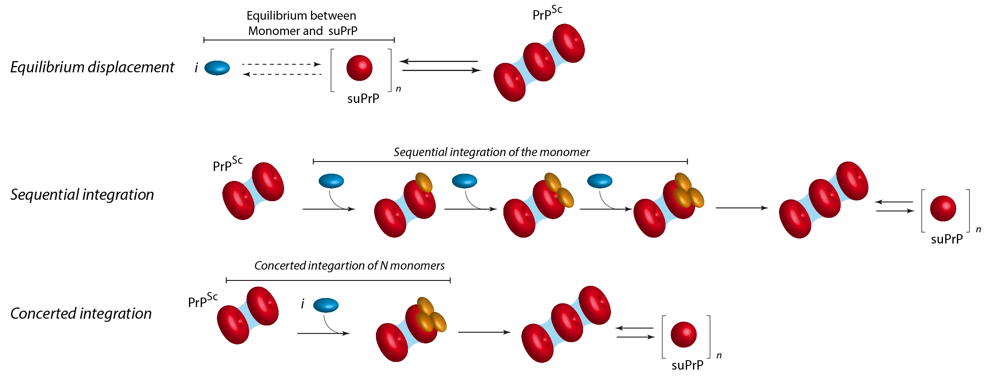
**Synergetic outcome:** The time dependency of size distribution variation as function of seeds will strongly help to improve the mathematical models. Furthermore, as shown in FigXX the kinetic readout could be used in a data assimilation pipeline in order to have a quantitative physico-chemical analysis of the process

Task 2.2: Mechanism of suPrP formation and its contribution to prion amplification and spreading

The prion paradigm leads to describe the perpetuation of some pathological information in an unprecedented perspective, however it fails to bring molecular explanation to how this biological information could be amplified and spread. since the seminal work from Lindquist and Shorter (*18*), fragmentation is now commonly proposed to be a central event in prion paradigm for the spreading and amplification of structural information. However, the fragmentation hypothesis presents major flaws in the case of mammalian prion, as the propagation takes place outside the cell where no cell components with desaggregase activity have been described. Therefore, the fragmentation process as a mechanism for PrPSc amplification and spreading of the replicative centre is likely not a generic mechanism. In this task, two intertwined objectives will be addressed, i) whether fragmentation is involved in prion replication by studying the mechanisms at work in prion replication in cell-free assays, and ii) to examine the potential contribution of suPrP to the spreading phenomenon through the equilibrium .

Subtask 2.2.1: Mechanism of suPrP formation during the templating process.

**Objective.** The recent demonstration of an oligomeric elementary building block (suPrP) in PrPSc assemblies (*19*) raised the question of its mechanism of formation and its role in the polymerization/templating process. Two formation pathways could be envisioned. The first one would consist in the building block formation induced by the template at the templating interface. This mechanism could either be concerted with the addition at once of all the protomers of the elementary brick or sequential with the addition of the protomers one after the other on the templating interface. The second possibility could imply that in a cellular context, PrPC and suPrP are in equilibrium, with a disadvantage for suPrP species. The presence of prion assemblies in the brain for example would displace this equilibrium towards suPrP by integrating them. The objective of this task is to discriminate between these hypotheses without neglecting other possibilities using kinetic models and parameter estimation.



**Methodology.** The approach we will adopt will combine single molecule fluorescence spectroscopy with FRET technic resolved in time with discrete kinetic modeling. A complementary approach will be to study the kinetics of templating reaction in different conditions. The kinetics will be followed by static light scattering and Thioflavin T fluorescence. An analysis of the initial rate as a function of the concentration of the reactants for example will give pieces of information about the order of the templating reaction. Analyses of these data with different kinetic models will be carried out to distinguish between them.

**Outcome.** The estimation of the kinetic order of the templating reaction will lead to differentiate between the proposed processes of suPrP involvement in the polymerization process. This will also allow us to understand the role of suPrP in the polymerization process.

**Synergetic outcome:** In term of synergy, the outcome of this subtask will directly be injected in the model building section of WP3. Indeed, kinetics data obtained here will be challenged by the three models of figure xx (and some variant of these three models) in order to determine the kinetic process of suPrP formation during the templating process.

Subtask 2.2.2: Relation between depolymerization of PrPSc assemblies into suPrP and the spreading phenomenon.

**Objective.** The widespread idea concerning the mechanisms of the replication center spreading refers to an active (i.e. energy dependent) fragmentation process (*18*)+refs) leading to generate *de novo* interface of templating. According to the fact that PrPSc assemblies are highly dynamic and are in equilibrium with a small oligomeric conformer which is called suPrP the simple spatial diffusion of suPrP should be sufficient to delocalized and spread the templating interface (Figure?). The aim of this task consists to explore the propensity of depolymerization of PrPSc assemblies into suPrP as a mechanism to spread the replication center.

**Methodology.** The methodology used here consists to spatially separate in two different compartments the suPrP compound of the equilibrium PrPSc/suPrP through the use of low-pass molecular weight membrane filter and to monitor the propensity of the isolated suPrP to initiate de novo conversion. Preliminary experiments performed using a milli-fluidic approach were very promising using 263K prion strain. The same strategy will be translated to cell assays with primary neurons cultivated in compartmentalized microfluidic assays, the use of membranes with specific cut-off allowing to control the size of PrPSc assemblies spreading from one compartment to another (ref antoine Orsay?). The possibility to use neuron permissive or not (from PrP0/0 neurons) to prion replication in donor or receptor compartment will allow deciphering which species diffuse and to correlate their genesis with the replication steps.

**Outcome.** The outcome of this task will lead to proposed a molecular mechanism of the spreading and amplification phenomenon of mammalian prion based on the intrinsic dynamic of PrPSc assemblies and the existence of equilibrium between PrPSc and suPrP.

**Work Package 3: Mechanistic modelling of the network of exchange.**

**Start:** year 1, **Duration:** 6 years, **Risk:** moderate/high

**Staff:** M. Doumic, in constant interaction with H. Rezaei and V. Béringue and their team;

Post-doctoral fellows and Ph.D students to be appointed and co-supervised with our main collaborators:

**Klemens Fellner** (Prof. in University of Graz, Austria), **Marc Hoffmann** (Prof. in University Paris-Dauphine, France), **Philippe Robert** (Prof. in Inria, Paris)

**General objectives.**

This work package gathers all the mathematical and numerical methods, which are in turn used in WP1 and WP2, in an iterative way. Its general objectives are twofold. **First**, it aims at providing mechanistic models able to be qualitatively and quantitatively confronted to the experimental data of WP1 and WP2, ultimately able to predict the dynamics of new experiments, especially concerning *in vivo* and *ex vivo* data. Second, it shall pave the way for original mathematical problems, interesting for the mathematical community in their own right. Of note, the questions raised by the modelling of different types of interacting assemblies are renewing the field of aggregation-fragmentation equations and processes, which may exhibit new kinds of behaviours such as **sustained or damped oscillations, periodicity, slow-fast dynamics etc**. The study of these new phenomena, together with the links between stochastic and deterministic modelling and the theoretical analysis of data assimilation for structured equations problems, are the main mathematical objectives.

The previous ERC Starting Grant project SKIPPERAD, which involved both M. Doumic (P.I.) and H. Rezaei (30% of time), will be very useful to us, because it provides us with a solid working arrangement. The methodological approach we have developed during the SKIPPERAD project has proved successful on specific questions (*61,****Armiento JTB***), which were preliminary results for this proposal. However, the major aim of the PeRCID project, which is to unravel the network of exchange between different PrPSc subassemblies, represents a break-through compared to the SKIPPERAD project, which did not address this issue and concentrated on models where polymers are only described by their size, *i.e.,* the number of protomers they contain.

**Subtask 3.1 Model building and model selection : from data to model**

In this first subtask, we shall analyse the experimental data (incoming from subtasks: 1.1.1, 1.2.1 1.3.1, 1.3.2, 2.2.1and 2.14) and discuss them in deep to design, simulate, adapt mathematical models: deterministic ones, based on systems of biochemical reactions – the major difficulty being to take into account several species of assemblies, and the fact that the size of polymers can be arbitrarily large - or stochastic ones, when the number of objects are so small that stochastic effects can no more be negligible. We detail this into three successive steps, as follows.

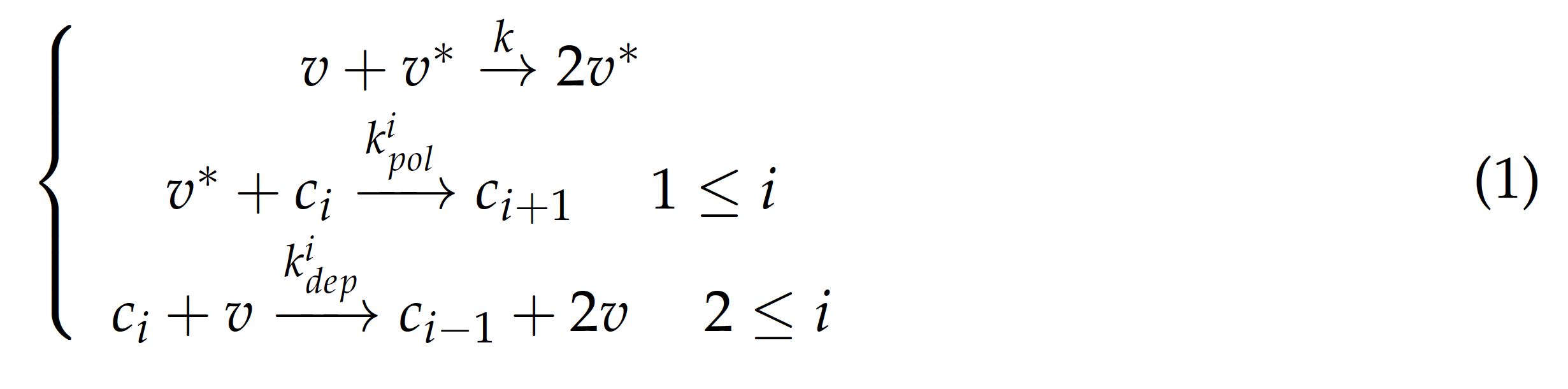
**First step: Noise analysis and image analysis**

In collaboration with M. Hoffmann and a post-doctoral fellow to be recruited, and based on the image analysis software built by Y. Yin (in progress) to analyse electron microscopy images, we shall

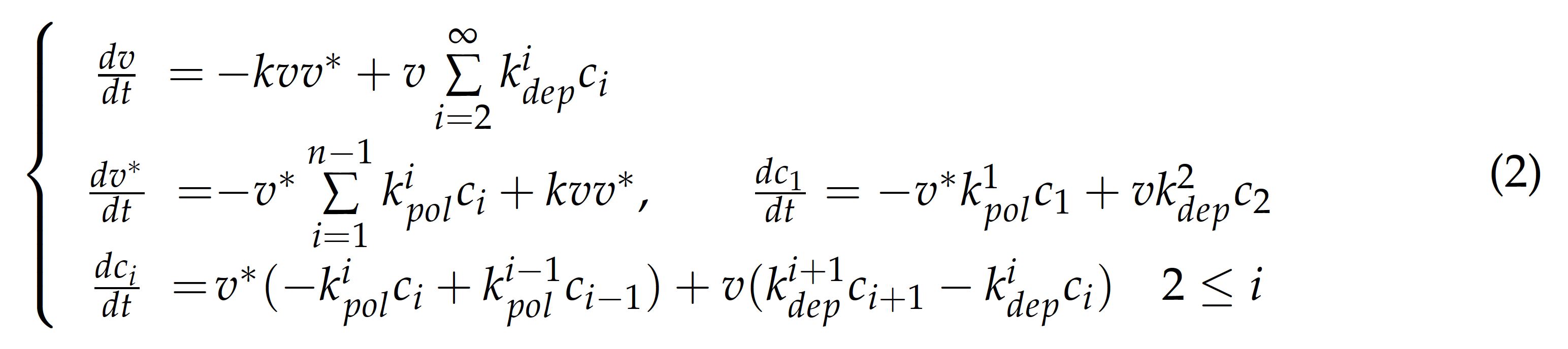
* extend the software to be able to analyse 3D images coming from AFM and NanoIR, and develop a biologist user-friendly software (with Y. Yin and an engineer to be recruited) (***Yin et al, 2012****,* )
* propose statistical tests to assess the presence or not of periodic oscillations in the signal based on wavelet transformation of the signal, and prove its statistical power.

**Second step: Kinetic pathway modelling (based on data from subtasks** 1.2.1, 1.3.1, 1.3.2, 2.2.1, 2.14**)**

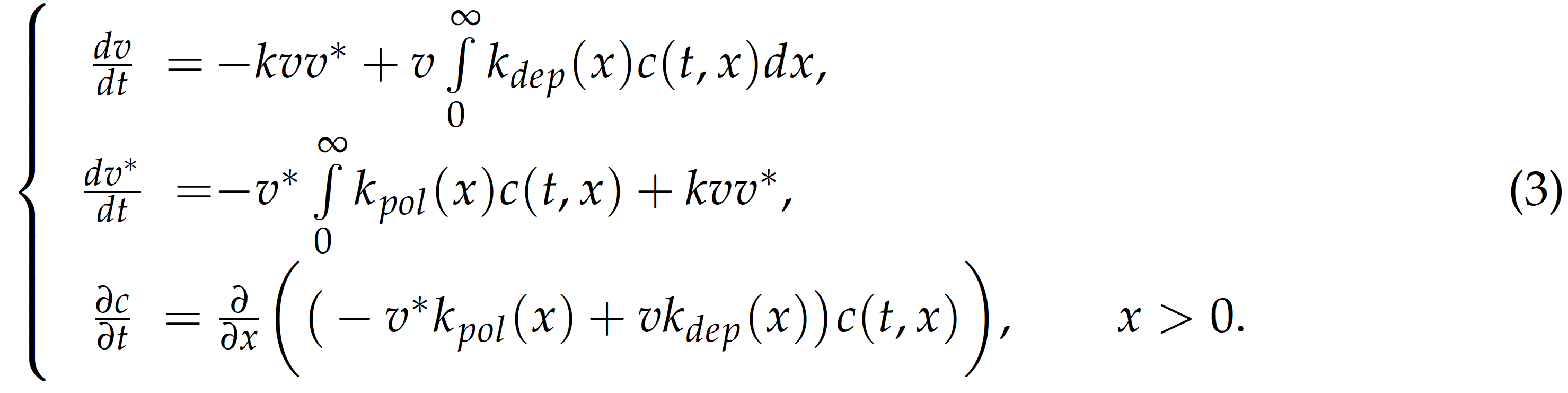
This step consists not only in designing one model, but rather a family of models, based i) on the models developed in the previous project SKIPPERAD (*61,* ***Armiento JTB***), and ii) on models taking into account several interacting species, and possibly also spatial diffusion **(Desvillettes, Fellner, Tang, SIAM, 2017)**. As a preliminary result, the following system, inspired from Becker-Döring system but with a nonlinear depolymerisation reaction and two monomeric species, is able to exhibit persistant or damped oscillations (work in progress) and will serve as a first building block



where *ci* denotes a PrPSc substructure formed of *i* elementary block, and *v* and *v\** denote two types of elementary building blocks (suPrP). The differential system is then



In a first approximation for a continuous version – valid for large sizes, and to be studied, together with more complex ones, in Task 3.2. - it gives



More complex models include other species. To take into account a whole- brain system, non-homogeneous space diffusion could also be added (see subtask 1.3.2)

**Third step: introduction of stochasticity in the exchange network** (with P. Robert and Ph.D students)

To model the adaptive templating response, which could be, at the level of subassemblies, assimilated to a nucleation elongation step, or when there is a failure in the environment (species barrier or very large dilutions), it may be necessary to use the stochastic system underlying the chemical balance equation, and to link it with its deterministic limit, in the spirit of (*W. Sun, 2017,* ).

**3.2 : Model analysis: understanding the kinetic models, elucidating the key mechanisms**

This part gathers the most theoretical part of WP3: it consists in the mathematical and numerical analysis of the models developed in 3.1. Among the many challenging mathematical questions we will address, in collaboration with K. Fellner, P. Robert and M. Tournus, we can cite the following.

* Under which conditions can periodic or pseudo-periodic oscillations appear ? Analyse and simulate the models to evidence the presence of slow/fast dynamics. Determine which systems may exhibit damped or sustained oscillations (with M. Tournus)
* Sensitivity analysis: which are the key mechanisms, along which bifurcation of the system is possible ?
* Long-time asymptotics for nonlinear reaction-diffusion systems. In collaboration with K. Fellner, we shall use the results (*1-3*), where entropy methods for complex-balanced systems have been developed, and try to extend them to our models which are neither detailed nor complex-balanced but may exhibit other interesting features like Hamiltonian or could be simplified using a quasi-steady state approximation.
* From discrete to continuous models: in which conditions and in which sense can systems of the type (2) be the limit of systems of the type (1)?
* Central limit theorems for stochastic Bekker-Döring-like systems, in the spirit of (*1-3*) (with P. Robert and his students), and combination of stochastic and deterministic models in a given experimental timecourse.

**3.3: Data assimilation and model selection: from model to data**

For this part, we use the approach already developed with the consortium by M. Doumic and P. Moireau (ref Armiento et al). In (*61,* ***Armiento JTB***), we have applied our approach to two specific kind of experimental data, and we now aim at a very systematic approach, concerning theoretical as well as experimental results. P. Moireau will be involved in this task, together with a 3-year post-doctoral fellowship and then a 3-year engineer, who will use the results previously obtained to develop a software in link with the existing data assimilation library called Verdandi (http://verdandi.gforge.inria.fr/)

**First step** : design of the data assimilation problem for a framework model, in the spirit of (*Armiento JTB*) but for general models, either discrete or continuous : variational approach and filtering methods. Theoretical analysis (can we prove an observability condition?).

**Second step :** numerical implementation on synthetic data – discussion with H. Rezaei and V. Béringue, in the spirit of (*61*) but in a systematic way, to design new experiments in the cases where the data assimilation problem is proved to be not observable.

**Third step:** back to the data: application to data from WP1 and WP2, in the spirit of (*61, Armiento JTB*) and beyond : use the results to predict in vivo data (which are most probably not rich enough to be observable, but can be used to validate or invalidate predictions done on in vitro data).

References (peut-etre que Wen Sun ça ne passe pas car preprint?):

A. Armiento *et al*., Estimation from Moments Measurements for Amyloid Depolymerisation. *J. Theor. Biol.* 387, 68-88 (2016).

Y. Yin *et al.,* Retinal vessel segmentation using a probabilistic tracking method, *Pattern Recognition* **45**, 1235-1244 (2012).

S. Eugène *et al.*, Insights into the variability of nucleated amyloid polymerization by a minimalistic model of stochastic protein assembly. *J. of Chem. Phys.*, **144** (17):12 (2016).

W. Sun, 2017, *A functional central limit theorem for the Becker-Döring model,* [*https://arxiv.org/abs/1710.04059*](https://arxiv.org/abs/1710.04059)

L. Desvillettes, K. Fellner, B.Q. Tang, Trend to equilibrium for reaction-diffusion systems arising from complex balanced chemical reaction networks, SIAM Journal on Math. Analysis, 49 no.4 (2017) 2666—2709.

S. Eugène et al., Insights into the variability of nucleated amyloid polymerization by a minimalistic model of stochastic protein assembly, Journal of Chemical Physics, American Institute of Physics, 2016, 144 (17), pp.12.

**Section c. Resources (including project costs)**

(Note: Describe the resources needed according to the indications in the *Information for Applicants to the Synergy Grant 2018 call*, section 1.1.2.2 *Instructions for completing 'Part B' of the proposal*.

**Each PI is required to fill in their budget breakdown using the following budget table and the declaration of their level of commitment to the project.** Depending on the number of PIs you may delete unneeded columns**.** All eligible costs requested should be included in the budget. **Please use whole euro values only**. In case you are requesting additional funding (up to EUR 4 million) above the normal EUR 10 million, include these top-up costs in the budget table as well and justify your request in the second table at the end. In addition to the budget table, please describe and **fully justify** the amount of funding considered necessary to fulfil the objectives throughout the duration of the project.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Cost category** | | | **Corresponding PI** | **2nd PI** | **3rd PI** | **4th PI** | **Total in euro** |
| **PI name** | | |  |  |  |  |  |
| **Host Institution** | | |  |  |  |  |  |
| **Direct Costs[[2]](#footnote-2)** | **Personnel** | PI[[3]](#footnote-3) |  |  |  |  |  |
| Senior Staff |  |  |  |  |  |
| Postdocs |  |  |  |  |  |
| Students |  |  |  |  |  |
| Other |  |  |  |  |  |
| *i. Total Direct costs for Personnel (in euro)* | |  |  |  |  |  |
| **Travel** | |  |  |  |  |  |
| **Equipment** | |  |  |  |  |  |
| **Other goods and services** | Consumables |  |  |  |  |  |
| Publications[[4]](#footnote-4) |  |  |  |  |  |
| Other (please specify) |  |  |  |  |  |
| *ii. Total Other Direct Costs (in euro)* | |  |  |  |  |  |
| **A – Total Direct Costs (i + ii)** (in euro) | | |  |  |  |  |  |
| **B – Indirect Costs (overheads)** 25% of Direct Costs[[5]](#footnote-5) (in euro) | | |  |  |  |  |  |
| **C1 – Subcontracting Costs** (no overheads) (in euro) | | |  |  |  |  |  |
| **C2 – Other Direct Costs with no overheads[[6]](#footnote-6)** (in euro) | | |  |  |  |  |  |
| **Total Estimated Eligible Costs (A + B + C)** (in euro) | | |  |  |  |  |  |
| **Total Requested Grant** (in euro) | | |  |  |  |  |  |

The project cost estimation should be as accurate as possible. Significant mathematical mistakes may reflect poorly on the credibility of the budget table and the proposal overall. The evaluation panels assess the estimated costs carefully; unjustified budgets will be consequently reduced. The Total Estimated Eligible Costs and the Total Requested Grant amounts in the table MUST match those presented in the online proposal submission form, section 3 – Budget.

In case you are requesting additional funding (up to EUR 4 million) above the normal EUR 10 million, fully justify your request by filling in the table below (please delete the table if not applicable). Include these costs in the above budget table.

**Project costs.**

The costs of the project are summarised in the table. The total amount of the proposal reaches € 10 000 0000.

Personnel costs

The personnel costs cover the PI’s and their team members involved in the project, at a percentage of their working time. The Responsible PI (H. Rezaei) will devote 80 % of his working time to the project. Besides the overall supervision, he will pay particular attention to the synergic aspects of the project His experience in mathematics due to past-collaboration with the INRIA PI (M. Doumic) will help establishing strong links with the mathematicians involved. The second INRA PI (V. Beringue) will devote 70% of his working time to the project. He will more specifically cover all the aspects regarding in vivo experiments. For INRA, the project is in the direct continuity of the work done at Jouy-en-Josas over the last years. This means that key persons from the INRA team will be involved due to their respective expertise that is requested in PeRCID: as scientists, Dr. D. Martin (biophysicist, chemist) will be in charge of the templating experiments and RT-QuIC analyses (60% of working time), Dr. M. Moudjou of all the mechanistic and bioassay aspects necessitating cell-free prion amplifications (*72, 73*) (50% of working time), Dr. S. Truchet having recently joined the INRA team for the ex vivo culture systems (40% or her working time). Research assistants will assist in animal experimentation and in the administrative part of the project.

INRIA

Due to their complementary scientific or technical expertise, long-term collaborators from the host institutions will participate to the project due to their unique expertise:

* Dr Jean-Luc Vilotte (UMR GABI, INRA Jouy-en-Josas, France) and two research assistants will be involved in all aspects necessitating the creation of transgenic mouse models expressing PrP from different species or at different levels (*21, 36, 74*).
* Dr Olivier Andréoletti (UMR IHAP, ENV Toulouse, France) will provide relevant sheep and human TSE cases and provide expertise in all the neuropathological aspects of prion strain characterization (*14, 21, 62*)
* Bernard Cayron (Mouse Experimental Unit, INRA Jouy-en-Josas) and his team will breed certain mouse models and lodge the prion experimentation in his BSL III facilities.

INRIA

External (most often long-term) collaborators have agreed to participate to the project. Each of them is an internationally-renowned specialist in a field of research that is not covered by the PI host institutions and will provide strong input to specific part of the project:

* Dr Philippe Minard (CNRS, i2BC, Orsay, France) will develop artificial PrP protein ligands with high affinity using proprietary strategies (ref REP).
* Dr Jean-Michel Peyrin (CNRS, IBPS, Paris, France) will help implanting compartmentalized microfluidic cell culture chambers for handling primary neurons. He has pioneered the reconstruction of rodent oriented neuronal networks in microfluidic devices (*46, 47, 50, 53, 54*)
* Dr Christina Sizun (CNRS, ICSN, Gif-sur-Yvette, France), liquid and solid-state RMN (*70*)
* Pr. Franca Fraternali (King’s college, London, UK), molecular dynamic (*71*)
* Dr Michel Dron, actually in the INRA Unit but scientist from INSERM for aspects necessitating PrP domain involved in prion conversion, as modelled in cell assays (*70, 75-77*)
* INRIA

Of course, the list is not exhaustive and new collaborations are likely to emerge, either with mathematicians, biologists and physicists: one of the goals of this project is precisely to encourage and develop new collaborations on prion assemblies networking and exchange.

* Ref to Strasbourg gourp ? Bring people from this field to the prion field ?

Postdoc and PhD students

Emphasis, training of youngesters on a hih-profile, synergic aspects, etc.

the first in year 2, the second in year 3, each of them being recruited for 2 years. Due to the interdisciplinary character of the project, it will be essential that at least one of them has experience in the mathematical modelling of biological processes. The best set-up would be one student having gained his/her PhD in biomathematics, and one student either in biomathematics or in experimental biology on proteopathies but with an MSc in mathematics or informatics.

At the interface….

Equipment

A number of technical equipment which are not available in the framework of the project in BSL II and BSL III laboratories are requested.

Other

Workshops, free….

Consumables, in the standards….

|  |  |
| --- | --- |
| **Request for additional funding above**  **EUR 10 000 000 for** | **Justification** |
| Keep only the category(ies) that apply to the project.  (a) covering eligible 'start-up' costs for a PI moving from another country to the EU or an Associated Country as a consequence of receiving an ERC grant and/or,  (b) the purchase of major equipment and/or,  (c) access to large facilities. |  |

|  |  |
| --- | --- |
| **Please indicate the duration of the project in months[[7]](#footnote-7):** |  |
| **Please indicate the % of working time the PI dedicates to the project over the period of the grant:** | **%** |
| **Corresponding PI name:** |  |
| **2nd PI name:** |  |
| **3rd PI name:** |  |
| **4th PI name:** |  |

Each PI must specify briefly their commitment to the project and how much time each one of them is willing to devote to the proposed project. Please note that each PI is expected to devote at least 30% of their total working time to the ERC project.

1. J. Collinge, Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* **24**, 519-550 (2001).

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1. Instructions for completing Part B2 can be found in the ‘*Information for Applicants to the Synergy Grant 2018 Call’*. [↑](#footnote-ref-1)
2. An additional cost category 'Direct costing for Large Research Infrastructures' applicable to H2020 can be added to this table (below ‘Other Goods and services’) for PIs who are hosted by institutions with Large Research Infrastructures of a value of at least EUR 20 million and **only** after having received a positive ex-ante assessment from the Commission's services. [↑](#footnote-ref-2)
3. 3 When calculating the salary, please take into account the percentage of each PI's dedicated working time to run the ERC funded project (i.e. minimum 30% of the total working time). [↑](#footnote-ref-3)
4. **Include** in the Publications costs **Open Access fees**, dissemination activities, etc. [↑](#footnote-ref-4)
5. Please note that the overheads are fixed to a flat rate of exactly 25%. [↑](#footnote-ref-5)
6. Such as the costs of resources made available by third parties which are not used on the premises of the beneficiary [↑](#footnote-ref-6)
7. The maximum award is reduced pro rata temporis for projects of a shorter duration than 72 months (e.g. for a project of 60 months duration the maximum requested EU contribution allowed is EUR 8 333 333). Additional funding to cover major one-off costs is not subject to pro-rata temporis reduction for projects of shorter duration (e.g. with additional funding it is possible to request a maximum EU contribution of EUR 12 333 333 million for a project of 60 months duration). [↑](#footnote-ref-7)