DEPARTAMENT OF CHEMISTRY

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

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BSc in Biochemistry

SUSTAINABLE FIBERS FOR BIOSEPARATION: INNOVATION, MARKET TRANSFER, AND A COLLABORATIVE MODEL

MASTER IN BIOTECHNOLOGY

NOVA University Lisbon

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MASTER IN BIOTECHNOLOGY

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Sustainable Fibers for Bioseparation: Innovation, Market Transfer, and a Collaborative Model

To my parents,

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I would like to express my thanks to my supervisor, Professor Fernanda Llussá for the advice and encouragement to embrace a completely new and challenging theme. To Professor Paula Urze, my co-advisor, thank you for your insight and for introducing me to sociology.

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Finally, I would like to thank my parents because none of this would ever be possible without you!

ABSTRACT

The present thesis was performed within the framework of a collaborative European research project, the PURE project integrated in the EU FET-OPEN program. The research approach of this thesis involves the combination of three areas (sociology, economics, and biotechnology) to develop a go-to-market strategy and an attempt to design a preliminary collaborative model for PURE.

The PURE consortium is a network formed by three academic institutions and one SME. The nature of the network was studied to develop a collaborative model. The theoretical approach in this work followed Gibbons' Mode 2 Production of Knowledge Theory and it was found that transdisciplinarity, mechanisms for sharing knowledge, communication and trust were key factors for collaboration in this organization.

The technology explored is the production of adsorbents modified with affinity ligands for the purification of biopharmaceuticals, like monoclonal antibodies and virus-like particles (VLPs). In this thesis, we showed the successful modification of adsorbents with ligands for the capture of VLPs in purification processes.

For the research of applications, only two applications were considered viable since they were the most innovative and with a higher market need: adsorbents for the purification of monoclonal antibodies and for the purification of virus-like particles.

For the selected applications, competitive and market growth analysis were performed. The preferred application is the purification of virus-like particles given that it has less competition and a greater market growth. Finally, regarding the business model, the possibility of licencing of the technology is considered an interesting option for the chosen application but other business models can be considered according to the application or product that is developed from the PURE technology.

Keywords: go-to-market; knowledge production; purification; biopharmaceuticals; innovation; collaboration

RESUMO

Esta tese foi realizada no âmbito de um projeto de investigação de colaboração europeu, o projeto PURE integrado no programa FET-OPEN da UE. A abordagem de investigação desta tese envolve a combinação de três áreas (sociologia, economia e biotecnologia) para desenvolver uma estratégia de transferência de mercado e uma tentativa de conceber um modelo colaborativo preliminar para o PURE.

O consórcio PURE é uma rede formada por três instituições académicas e uma PME. A natureza da rede foi estudada para desenvolver um modelo colaborativo. O enquadramento teórico deste trabalho seguiu a Teoria da Produção de Conhecimento de Modo 2 de Gibbons e verificou-se que a transdisciplinaridade, mecanismos de transferência de conhecimento, comunicação e confiança são fatores chave para a colaboração nesta organização.

A tecnologia explorada é a produção de adsorventes modificados com ligandos de afinidade para a purificação de biofarmacêuticos, como anticorpos monoclonais e partículas semelhantes a vírus (VLPs). Nesta tese, mostrámos a modificação bem-sucedida de adsorventes com ligandos para a captura de VLPs em processos de purificação.

Para a pesquisa de aplicações, apenas duas aplicações foram consideradas viáveis por serem as mais inovadoras e com maior necessidade de mercado: adsorventes para a purificação de anticorpos monoclonais e para a purificação de partículas semelhantes a vírus.

Para as aplicações selecionadas, foram realizadas uma análise da concorrência e do crescimento do mercado. A aplicação selecionada é a purificação de partículas semelhantes a vírus, dado que tem menos concorrência e um maior crescimento do mercado. Finalmente, em relação ao modelo de negócio, a possibilidade de licenciamento da tecnologia é considerada uma opção interessante para a aplicação escolhida, mas outros modelos de negócio podem ser considerados de acordo com a aplicação ou produto que é desenvolvido a partir da tecnologia PURE.

Palavras-chave: estratégia de transferência de mercado; produção de conhecimento; purificação; biofarmacêuticos; inovação; colaboração

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ABBREVIATIONS

BOKU	University of Natural Resources and Life Sciences.
EU	European Union.
FT	Flow-through.
iBET	Institute of Experimental and Technological Biology.
L	Loading.
mAbs	Monoclonal Antibodies
NOVA	NOVA School of Science and Technology.
SME	Small-Medium Enterprise.
VLP	Virus-like Particles.
W	Wash.

1. INTRODUCTION

The production of knowledge in contemporary society is related to a diverse number of areas, ranging not only from social sciences and humanities, but also engineering, science, and technology.

Nowadays, the collaboration between two or more partners from academia and industry is essential to the achievement of high-level performance by companies to respond to an environment of emerging competitiveness and to create the opportunity to generate the production of innovative products [1]. In most cases, companies do not have in their organization all the required skills to succeed. Therefore they rather benefit from a collaboration with parties that add value, such as: expertise in a specific field, the utilization of a multidisciplinary approach, the reputation of such entity, and the reduction of costs [1].

From the academic side, a study done by Draghici et al. [2], concluded that in the knowledge-based society, universities play an enhanced role in innovation as entrepreneurs adding that the entrepreneurial outcome can be considered because of the university-industry-government relationship. Universities are considered knowledge transfer organizations, and the knowledge that is traded is related to research collaborations, property rights (patents), start-ups, and spin-off companies. However, in recent years, the management of knowledge transfer activities is mainly focused on the transfer of technology. It is specifically centred on evaluating and protecting intellectual property to make it available to the industry field [3].

The EU FET-Open Project, a European Commission funding program derived from the Horizon 2020 Program, has as its main objective to support the development of innovative technologies and scientific research to generate new knowledge for the future generation of European industries, investing primarily in interdisciplinary and transdisciplinary collaborative research [4]. Furthermore, FET-OPEN is a program that envisions the link between science and society by encouraging research driven by societal or industrial challenges, which is increased by a strong collaboration between several scientific and technological disciplines allowing a synergy between natural sciences and exact sciences (biology, material sciences, mathematics, chemistry), medicine, engineering, social sciences (sociology and economy) and also arts and humanities [4].

Therefore, to achieve this collaboration, key players in academic and industry fields must be involved, like research-intensive SMEs, civil society, along with senior and young researchers [4]. These projects are comprised of a network of two or more industry- and academia-based institutions that collaborate to develop a product and process that will impact the general society.

It has been shown that no universal model of scientific production is viable for all areas, declaring that different fields of science generate knowledge in their own way and are dependent on dissimilar dynamics [5].

A network form of organization contrasts with other forms of the organization due to the more permeable boundaries and the reliance on close and informal connections for all organizational aspects of the collaboration [6]. For example, a biotechnology network collaborates with other firms, universities, and even non-profit research institutes on their research and development projects [6]. Hence, a core competency for network organizations is establishing and maintaining inter-organizational relationships, which can give an individual member of this network access to diverse information, accelerating innovation [6].

The present work has been developed under the scope of the European Union's Horizon 2020 research and innovation program under the grant agreement No. 899732 PURE (Precisely Patterned Nanofibers for High-Performance Bioseparations), a multidisciplinary project that envisions the development of purification adsorbents, using principles of scalable white biotechnology and environmentally friendly processes.

1.1 PURE: A vision to revolutionize the biopharmaceutical industry

The biotechnology industry appeared in the 1970s with the development of genetic engineering, recombinant DNA technology, and monoclonal antibody technologies.

Biopharmaceuticals are biological drugs with therapeutic effects. The medicinal uses of biopharmaceuticals mainly include recombinant protein therapy, antibody therapy, cell therapy, and gene therapy. Biopharmaceuticals can be grouped into multiple categories according to the type of biological entity. They encompass a large group of monoclonal antibodies, enzymes, growth factors, hormones, vaccines, and cell and gene therapeutical drugs (Figure 1.1).

The development of biopharmaceuticals is a very complex process that involves numerous steps [8]. According to a report on the global biopharmaceutical market field [9], this market was valued at US$ 401.32 billion in 2021, expected to be US$ 534.19 billion by 2027 [10]. New anticancer biopharmaceuticals (immunological and other) and gene therapy are the categories in more significant development (Figure 1.1). Monoclonal antibodies and antiviral drugs amount to 8% and 6.4%, respectively. With respect to sales, the leading top biopharmaceutical products worldwide in 2021 (Figure 1.2) are mRNA vaccines (Comirnaty [11] and Spikevax [12]), monoclonal antibodies for cancer (Humira [13], Stelara [14], Keytruda [15] and Opdivo [17]), anticancer small molecule drugs (Revlimid [16]), antiviral small molecule drugs (Biktarvy [18]) and antidiabetic monoclonal antibody (Trulicity [19]; small peptide fused to an antibody, so it is an antibody-like structure).

With the global pandemic, vaccines were the leading biopharmaceutical product sold in 2022. The RNA vaccines are the latest development. However, VLPs are a very robust technology and are still mainly used. Therefore, virus-like particles have grown in importance due to the greater need for vaccine development and the growth of gene therapy processes (Figure 1.2).

The development of a biopharmaceutical product should be considered clinically effectiveness, regulatory approval, and commercial viability [7].

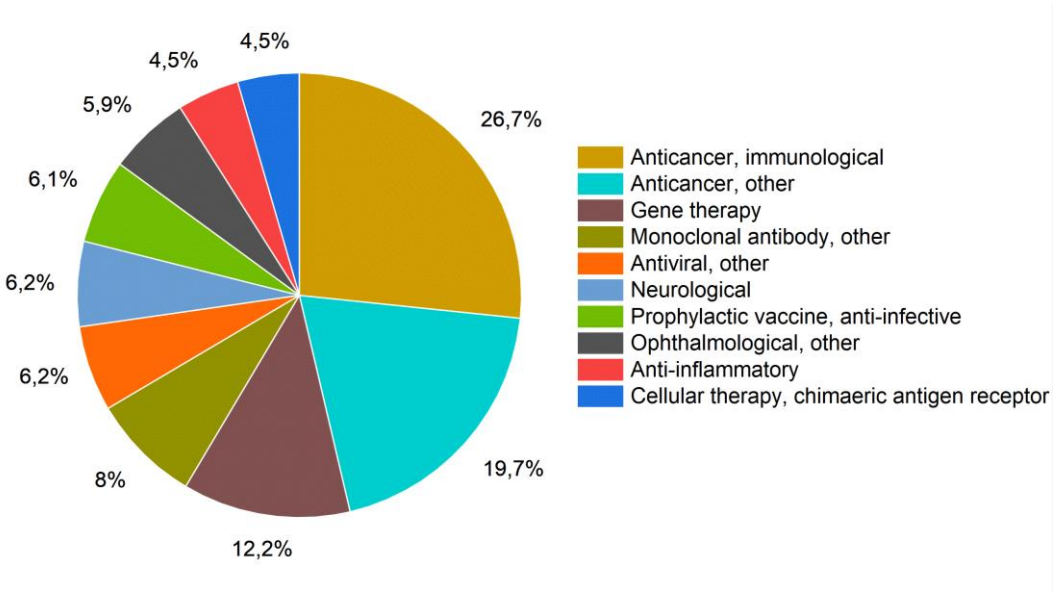

Figure 1.1 - Top therapeutic categories worldwide 2022, by a number of R&D products [8].

According to a report on the global biopharmaceutical market [8], this market was valued at US$ 401.32 billion in 2021, expected to witness a revenue of US$ 534.19 billion by 2027 [9] which represents a growth of 4.88% being a very attractive market.

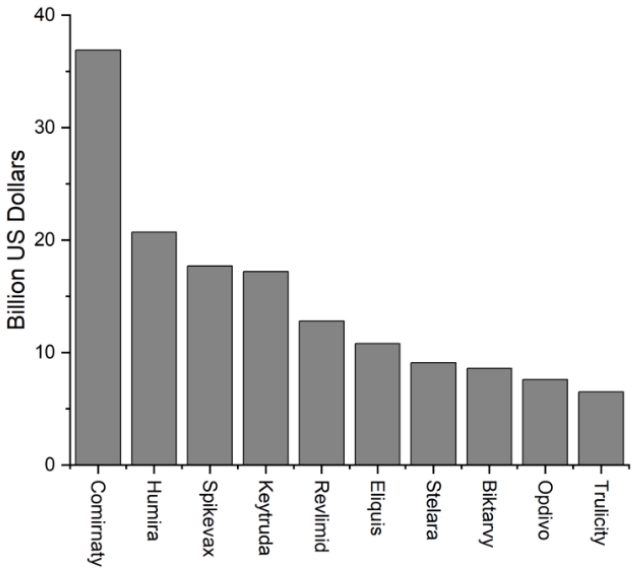

Figure 1.2 - Number of sales in US$ of biopharmaceuticals worldwide in 2021 [10].

1.2 Biopharmaceutical manufacturing: downstream processing

Biopharmaceutical companies face intense pressure to improve the way biopharmaceuticals are manufactured, namely product quality, time-to-market, and manufacturing costs [11].

In particular, the biopharmaceutical manufacturing costs are one of the industry's main priorities. The biopharmaceutical process consists of a sequence of unit operations divided into two main parts: upstream and downstream [12].

Upstream processing has the goal of biopharmaceutical production typically performed in host cells [13]. In the case of VLPs, depending on their specific target's biological function, protein expression occurs in varied platforms. Several expression systems exist for the expression of the required subunits: bacteria, yeast, mammalian cells, plant cells, and insect cells can be used [14].

However, the virus-like particle production generates a vast number of impurities, which can have process-related or product-related origins. Therefore several purification steps are required to meet purity criteria for recombinant VLPs [15]. The downstream processing stage will address the removal of impurities and enrichment of the main biological product.

The downstream includes several separations and purification steps, including unit operations like clarification, capturing, intermediate purification, and polishing [12,16]. The techniques in these steps are high-pressure homogenization, centrifugation, filtration, and chromatography (Table 1.1). Chromatography is the central technique of downstream processes in the biopharmaceutical industry. Affinity, ion exchange, hydrophobic and mixed mode chromatography are distinct types of this technique that differ in the interaction mode for capturing VLPs in bind-and-elute mode [15]. Affinity chromatography is the most used purification method once it allows for the separation of a wide number of contaminants in a single process step.

Hence, affinity chromatography separates proteins based on the reversible interaction between a protein and a specific ligand (e.g. small synthetic ligands [17], peptides [18], small proteins [19], fragments [20] or full size antibodies [21]) coupled to a chromatography matrix, where the protein of interest is captured and collected [22]. The biological interactions between ligand and target molecule can be a result of electrostatic or hydrophobic interactions, van der Waals' forces and/or hydrogen bonding.

Table 1.1 - Downstream processing steps, purification methods and techniques for purification of virus-like particles [15].

Step	*Purification methods*
Cell Lysis	High-pressure homogenization
Clarification	Centrifugation Depth filtration Tangential flow filtration
Capturing	Ultrafiltration/Diafiltration Affinity Chromatography Ion Exchange Chromatography Hydrophobic Interaction Chromatography Mixed Mode Chromatography
Intermediate Purification	Ion Exchange Chromatography Hydrophobic Interaction Chromatography Mixed Mode Chromatography Size-exclusion Chromatography
Polishing	Size-exclusion Chromatography Ultrafiltration/Diafiltration

Packed columns are the most common matrices for affinity chromatography as they are established as primary tools for bioseparation. However, it suffers from a number of problems such as slow flow speed through the column and low capacity, which leads to low process productivity [23].

In contrast, membrane chromatography is able to overcome the mentioned problem of packing column and minimize it once it is able to generate a higher flow rate, low pressure drops, and has higher productivity per unit time [23].

These reported advantages are due to the flow-through pores of the membrane allowing the fast transport of the target protein to the correct binding site [23]. In addition, it has been reported that, monoliths, nanofibers, and membrane adsorbers are viable options to conventional chromatography on bead supports, especially for large biomolecules such as DNA, virus, and virus-like particles [24].

1.3 Alternative bioseparation adsorbents

Polymeric fibers are materials with elongated structures, composed by identical and repeating basic chemical structured units, called monomers, linked together to form wide variety of macromolecular structures [25]. These materials can be divided by origin in two distinct classes: synthetic and natural based fibers. Nowadays, the most widely used materials come from chemically synthetic production processes e.g., polyvinyl alcohol (PVA) [26], polysulfone (PSF) [27], poly(methyl methacrylate) (PMMA) [28] and polyethylene vinyl alcohol (PEV) [29]. These fibers are produced from materials that are chemically synthesized. New regulations and an increase in concern about carbon footprint, as well as the environmental impact of technologies has led to an extensive research and need of new sustainable materials that can replace petroleum-based polymeric fibers in several industries [30], such as the biopharmaceutical industry [21,27,31,32], cosmetics [33,34], water filtration [35], food [36] and agriculture [37].

There has been an increased effort in the production of natural fibers as bioseparation adsorbents. These fibers are produced in geological processes, plants, or animals, and are biodegradable overtime depending on the environmental conditions in their environment. The most notable natural materials being produced for bioseparation of biopharmaceuticals are regenerative cellulose based materials, functionalized with Protein A for purification of monoclonal antibodies (mAbs) [22,38–40].

The production of electrospun nanofibers has contributed to a new generation of nonwoven fabric-based materials for useful applications in multi-disciplinary research areas. Electrospun nanofibers are produced by a technique named electrospinning, which produces materials with valuable characteristics such as a high surface-to-volume ratio, interconnected ultrafine fibrous structure, high tortuosity, high permeability and light weight [41].

Given this contextualization, the PURE project envisions the development of a new purification of biopharmaceuticals using functionalised nanofibers and ruled by the basic principles of scalable biotechnological processes.

Thus, PURE intends to solve the problems the biopharmaceutical industry has in the bioseparation step in the manufacturing of these compounds, more specifically due to its low productivity, and the impact it can have on the environmental footprint and high cost of the processes.

1.4 PURE Consortium

The consortium built around the PURE Project fits the same narrative as previously described for the FET-OPEN Projects, as it is a multidisciplinary project that combines different fields of science and research like biology, chemistry, computer science, material science, engineering, and social sciences (Figure 1.3).

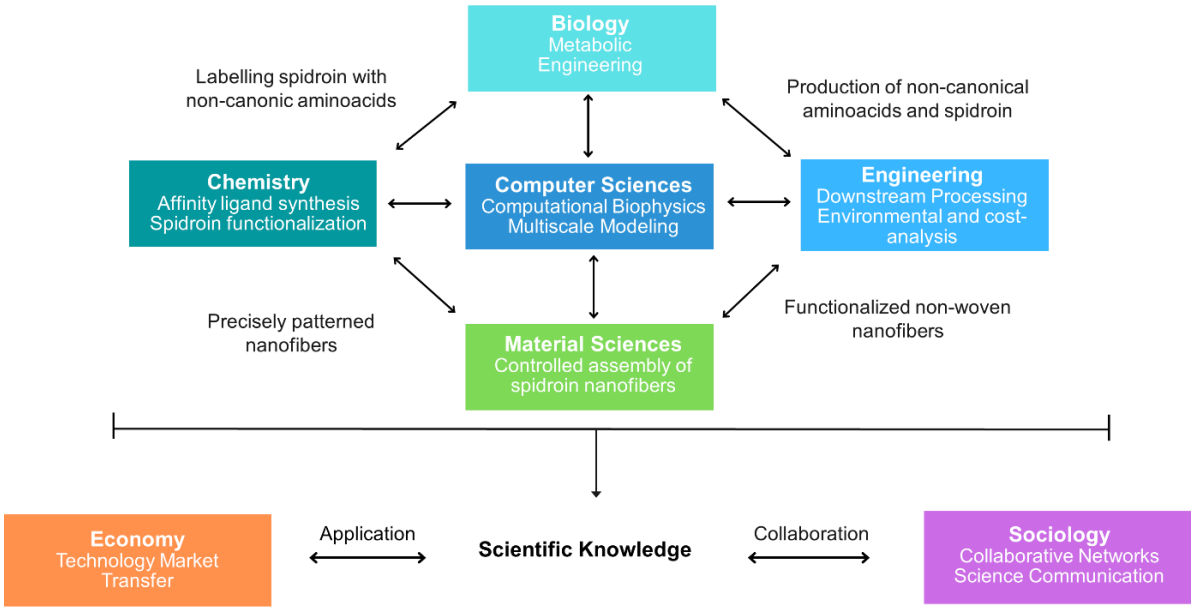

Figure 1.3 - Different scientific fields in PURE and the interplay between them.

Beyond the technical component of the project, there is a part of the project that also is particular for the FET-OPEN Projects, which is the study of the knowledge transfer in the consortium to design a go-to-market strategy and a collaborative network framework. Both these research avenues are unique in a technical project like PURE because they introduce a new bridge to the social scientific research, which will increase innovation and allow for the tuning of the scientific methodology and implementation strategies in the project.

As PURE is a multidisciplinary consortium with several tasks and goals, the tasks of the project were organized different work packages that outline the research methodology and milestones to be achieved during the project runtime, as well as the deliverables. Thus, the division of work packages in the PURE Project is depicted in Table 1.2.

Table 1.2 – Work Package division in the PURE consortium by lead partners and contributors.

Work Package	*Lead Partner*	*Other Partners*
WP1: Project management and coordination	NOVA	NOVA
WP2: Bioproduction of ncAA of modified spidroin	BOKU	NOVA, Bayreuth
WP3: Spidroin non-woven nanofibers	Bayreuth	NOVA
WP4: Spidroin nanofibers in bioseparation	NOVA	iBET, NOVA
WP5: Dissemination, exploitation, and communication	iBET	NOVA

For each of these work packages, experts in different fields are involved in different tasks within the project once, as described before, each one of the tasks within the work package requires expertise and experience in that specific scientific field.

The team in the PURE Project is a multidisciplinary team that is constituted by three academic institutions from the European Union and one SME. The institutions involved are as follows:

a) **NOVA School of Science and Technology**, in Portugal, whose expertise on the technical side rely on the fields of chemistry, engineering, and computational biophysics. In addition, NOVA is also responsible for creating the bridge with social sciences by having a team from Sociology and Economy.
b) **University of Natural Resources and Life Sciences (BOKU)**, in Austria, that has the main goal of the biosynthesis of non-canonical amino acids for the purpose of labelling the spider silk proteins and of scaling-up the bioproduction of non-canonical amino acids. In addition, BOKU also is working in the production of model biopharmaceuticals, as well as process optimization and environmental footprint assessment.
c) **University of Bayreuth, in Germany**, is responsible for the recombinant production of spider-silk proteins, the processing of these proteins into membranes and, along with BOKU, contributing for the environmental footprint assessment.
d) **Instituto de Biologia Experimental e Tecnológica (iBET)**, is a SME whose responsibility in the project is mainly performing a benchmarking of the process by comparing the results obtained from the testing of the adsorbents for different targets to the results obtained using the modified spidroins.

Overall, all the institutions are collaborating and play a role in all the work packages, due to the multidisciplinary nature of the project. However, each one of the partners previously described leads a work package according to their expertise and its research is based on their facilities. This means that there is a continuous collaboration between all actors within the project given that there are tasks that require materials or results from the other partners.

Apart from the institutions previously described there is an Advisory Board in the project composed of both key players in the industry and academia. The principal purpose of this body is to essentially give external feedback on both the scientific and impact developments in PURE. Beyond this, the Advisory Board is also responsible for the promotion of the PURE technology with industry stakeholders during the project and, in the end, of the results it may produce.

There are several members and actors in integrated in the PURE Project with several different roles. The roles in the project are as follows: Project Coordinator; Team Coordinator; Senior Researcher; Researcher; Research Assistant; PhD Students; MSc Students; Advisory Board Member.

1.5 Research Aim

The aim of this thesis is to study two main aspects of the project: the consortium network existent in the project and the potential exploitation of the research being developed. Moreover, what this work proposes to achieve is to, first, lay the basis for a design of a collaborative framework network model by conducting a deep analysis into the structure of the network that composes the PURE consortium, the key players and what factors play a role in the production of knowledge in a network. Second, after understanding the foundations of the research being developed and how the relationships are built in PURE, a deep dive into the commercialization and implementation is done to assess the impact the Project can have into society and industry. In addition, even though PURE is a project to prove a concept, there will be experimental tests are performed to assess the feasibility and validity of the technology in the proposed field.

Moreover, a Market Analysis and Business Model are performed to understand the potential applications the technology can have, the markets where this technology being developed can be introduced in, as well as the value proposition of the technology considering the technical description and proposition for the PURE technology.

In the end, this work intends to give more insight into the possibilities of exploitation of this technology to generate more applications and impact in society.

This thesis addresses knowledge production under PURE consortium as well as a market strategy for commercialization of the PURE technology. It is divided in the following chapters: Chapter 2 deals with knowledge production and a preliminary collaborative model; Chapter 3 explores the PURE technology directly applied for the purification of HIV-Gag virus-like particles; Chapter 4 deals with the exploitation and go-to-market plan of the PURE technology, outlining several potential applications for this technology and the study of a particular application selected based on market growth and competitive analysis; Chapter 5 studies two different proposals for a business model of the PURE technology in the future; finally, Chapter 6 discusses the general conclusions and future steps in this study.

2. PRODUCTION OF KNOWLEDGE AND COLLABORATIVE MODEL

2.1 Background: Mode 2 of Production of Knowledge

Most studies state that the gap between the production of knowledge in academia and the industry sector has been significantly diminished [42]. Moreover, it has been thoroughly described in literature that the academic culture, throughout the years, has been increasingly adopting corporate values and practices, which lead to the inevitable commercialization of science and innovation. Thus, the production of knowledge in academia is no longer exclusively destined to discover new scientific breakthroughs, it also serves as a new way to create and develop new products that can have market value.

Gibbons et al. [43] has defined in his work a thesis for how knowledge is produced in contemporary society. Hence, he divides knowledge production into two different modes: Mode 1, that incorporates traditional knowledge, created by disciplinary and cognitive context; and Mode 2, that comprises necessity to adapt to new changes and converge with other areas [43]. Therefore, Gibbons states that the insurgency of Mode 2 calls into question the methodologies of fundamental knowledge production institutions like universities, government research facilities, or corporate laboratories [43].

The term Mode 1 refers to a form of knowledge (or a complex of ideas, methods, values, norms) that is meant to summarize in a single phrase the cognitive and social norms which must be strictly followed in the production, legitimation, and diffusion of knowledge [44]. In science, its norms determine what counts as significant problems, who is legitimate enough to practice science and what constitutes good science. Thus, everything that is outside these guidelines is not considered as being scientific or trustworthy in the science world. In Mode 2, however, even though the production of knowledge is not conventional, this does not necessarily mean that it doesn't follow the norms of scientific methods. Consequently, comparing the difference of attributes between Mode 1 and Mode 2 allows the understanding of the characteristics of each mode.

First, in Mode 1, problems are set and solved in a context ruled by academia and the interests of a specific community. Thus, it is a form of production of knowledge that is disciplinary, since it is concentrated by actors of a certain field of knowledge, and it is also homogeneous, because it only comprises a specific way of creating knowledge. Organizationally, Mode 1 is hierarchical and not flexible. However, Mode 2 is different from Mode 1. In this case, Mode 2 is carried out in a context of application where it includes a wider, more temporary, and heterogeneous set of practitioners,

collaborating on a problem defined in a specific and localized context. Moreover, it is also characterized by its transdisciplinary and heterogeneity, and it is considered as being heterarchical and transient. All in all, Mode 2 is much more socially accountable and reflexive.

In Mode 1, problem solving occurs according to the rules the codes of practice of a specific scientific field. Usually, knowledge production in academic science is generated in the absence of a practical goal. By contrast, knowledge, in Mode 2, can be used by actors in industry or government, or society in general. This notion is implemented right at the start of the research. Furthermore, there is constant and continuous negotiation where all actors are involved, and their interests must be considered to proceed with knowledge production. In academia, basic or fundamental research is predominant and is defined as a type of research undertaken with a primary purpose of the advancement of knowledge for its own sake [45]. The Mode 2 thesis, first outlined in The New Production of Knowledge by Gibbons et al. [43], argued that academic-oriented research conducted exclusively within universities is no longer the core mode of knowledge production. Mode 2 knowledge, produced within the context of application, has become the dominant form.

Applied disciplines within the technological sciences are purposive and pragmatic in their knowledge resulting in new products and techniques with commercial purposes. Therefore, the generation of practical applications which implies the collaboration and synergy between several areas to create more innovative outcomes. The ability to produce knowledge that combines different fields is called transdisciplinarity [46].

Furthermore, transdisciplinarity involves the integration of different skills in a framework of action. First, it develops a distinct but involving framework to guide problem solving efforts. It is generated in a context of application. Second, the solution developed combines both empirical and theoretical elements. Third, the results obtained are communicated to those who have participated, and the dissemination of the results is accomplished in the process of the production, through journals or at conferences. Fourth, transdisciplinarity is dynamic, where solutions developed are a starting point for other possible discoveries. Thus, Mode 2 is marked by the close interactions of knowledge production with a succession of problem contexts.

In addition, Mode 2 Knowledge production is heterogeneous in terms of skills and experience people bring to it. The composition of a problem-solving team changes over time as requirements evolve.

Growing awareness about the variety of ways in which advances in science and technology can affect the public interest has increased the number of groups that wish to influence the outcome of the research process. Social scientists work alongside natural scientists, engineers, lawyers and businesspeople because the nature of the problems requires it [43].

Therefore, considering the characteristics of Mode 2 Production of Knowledge explained above, it is relevant to study how these characteristics take place in a collaborative network like PURE, composed by actors with distinct experiences and backgrounds, but with the common goal of translating the knowledge they are producing in the PURE Project into a practical application. To do so, the next section lays out the specific characteristics of a collaborative network in a theoretical perspective.

2.2 Collaborative Network: A Theoretical Perspective

In science, research is conducted by groups, assessed by peer reviews and built upon collaborations [47]. Collaborative efforts generally lead to most productive, creative, and innovative results. Hence, experts and actors from diverse backgrounds organize themselves into collaborative networks, composed of autonomous, geographically distributed and transdisciplinary components, which are drivers for value creation [48]. Collaborative networks are dynamic structures governed by the emergence of the process and interpersonal relations by exchanging ideas, resources, trust and lead to innovation [49].

Typically, a collaborative network is formed for the research and development of an innovative idea, new methodology or product. Therefore, there are four distinct approaches to assemble a collaborative network:

First, formal collaborative networks use past projects successful experiences, taking advantage of members' previous experiences where they collaborate exclusively within their own network. However, the second approach uses informal collaborative networks, which involves the use of outside channels to construct its network, such as online tools, social media channels or mailing lists. It is reported that the latter approach can augment the possibility of integrating members with greater expertise and that they require coordinators who know well the networks of the network members. The third approach involves networking events, typically organized adjacent to a scientific conference or as standalone events. These events are organized in a way when specific funding opportunities are open, this allows participants to seek collaborations that add specific expertise currently outside existing collaborative networks. Finally, the fourth approach for constructing collaborative networks allows a broader display of expertise, using online tools [46].

Considering these theoretical aspects of a collaborative network, it is intended to design a preliminary collaborative model of PURE where several components regarding the nature of its network are going to be addressed, such as: background and expertise of the PURE Members, to characterize the actors in this project regarding their industry or academic backgrounds, as well as their main field of study; the applied and fundamental research components of the project and how it reflects in terms of the research process in the project; the external relationships (particularly with industry), and the dissemination drivers most relevant to the diffusion of the knowledge produced within the project; transdisciplinarity and how the diversity of disciplines and backgrounds in the project interact to produce a knowledge in context of application with social implications; and finally, how trust influences the relationships and knowledge production within the project. Therefore, a qualitative approach was taken in the form of semi-structured interviews, whose methodology will be explained in the next section.

2.3 Methodology

This research work has the main objective of studying the research and development within the context of PURE Technology and its future exploitation. In addition, this work also lays out criteria

for the development of a collaborative framework model for European FET-OPEN Projects using this consortium as baseline. On the other hand, when addressing the technology market transfer, the focus was on four points: the differentiation of the proposed technology compared to existing ones, the industry scale-up and replicability, attending to the needs and wants of the customers and applications in other markets outside of what is proposed in the project.

This empirical work was based mainly on semi-structured interviews (that obey to a script). The interviews were divided in two sets. First, six members of the PURE Consortium were interviewed based on their role in the project, background, and their institution. Seniority and institution diversity were both prioritized. The interviews took place during conducted during January and February 2022. The interviews took approximately 360 minutes in total (1 hour per interview), they were recorded upon consent and transcribed with Otter.ai software. A pilot interview was conducted to review the script and to select the topics and the questions that were the most relevant. All the information related to the interview is described in Table 2.1.

Table 2.1 – Interviewee identification by institution. Interview information by date and duration in minutes.

Interviewee	*Institution*	*Interview Date*	*Duration (min)*
Interviewee A	BOKU	27/01/2022	55
Interviewee B	FCT NOVA	25/01/2022	70
Interviewee C	BOKU	31/01/2022	77
Interviewee D	iBET	31/01/2022	47
Interviewee E	Bayreuth	14/02/2022	48
Interviewee F	FCT NOVA	17/01/2022	92
Interviewee AB 1	Oxford University	04/05/2022	58
Interviewee AB 2	3M	06/05/2022	48

The interviews were divided in six sections. First, the Prior Knowledge section focuses on interviewees' professional and academic background, European project management/participation experience and their role within the project. The second section was knowledge production and transference with the focus on applied and fundamental, as well as how interviewees view the share of knowledge with industry. Third, this section explores the transdisciplinarity strand in the project, specifying the how it can influence the research path and innovation opportunities, as well as be a barrier to the advancement of the project's goals. Next, the fourth section focuses on trust and communication between the project's members and the industry actors in the Advisory Board. These are all fundamental components of the collaborative framework model being designed. The fifth and sixth sections are part of the technology market transfer strategy and intended to assess the inventors' take on the PURE Technology's value proposition and attributes, as well as potential applications for further exploitation purposes.

The second set of interviews were market research oriented and served as the primary data of the market analysis section of this work. The interviewees were four members from the Advisory Board, with academic and industry experience. The length of the interviews was approximately thirty minutes and were conducted during May 2022. The topics of the interviews consisted in sixth questions on the stakeholder's know-how and expertise, the project's value proposition, attributes, possible the barriers of implementation of this technology as well as sustainability parameters. These interviews will be explored in Chapter 4 and 5 of this work as basis for the Technology Market Transfer.

The data analysis of the interviews conducted was based on content analysis methods. The topics of the interviews were divided into codes and subcodes, each one corresponding to the segments of the transcribed interviews (Table 2.2). All data was processed and analysed using MaxQDA Pro 2022.

Table 2.2 - Interview categories, subcategories, and indicators.

Categories	Subcategories	Indicators
Background	Career path	Expertise
		Past Projects
	Current role	Role in the consortium
Applied and fundamental research	In the project	Both
	Throughout career	Fundamental research
		Applied research
Knowledge transfer	Relationships	External relationships
		Internal relationships
	Drivers for knowledge transfer	PhD students in industry
		Papers
		Conferences
		Meetings
Transdisciplinarity	Transdisciplinary environment	
	Communication	
Trust	Trust inside the consortium	
	Trust outside the consortium	

In Table 2.2, the codes resulting from the interview analysis obtained by MaxQDA Pro 2022 are represented. The method of data analysis followed in this study was subjected to the organization and division of the data into categories, subcategories, and indicators. The categories in this study were respective to the main topics of the interview, which amounted to five different categories (Background, Applied and fundamental research, Knowledge Transfer, Transdisciplinarity and Trust). During the content analysis of the interviews, the segmentation of the knowledge took place due to the variety of themes within each category covered. Therefore, several subcategories were created to classify the content in a more effective way. Each subcategory, however, may also demand a deeper specificity in that topic, which required the creation of indicators which adds another layer to this analysis. In the next

section, the results of the interviews will be discussed within the framework of content analysis previously explained.

2.4 Results and Discussion

In this section, the results of the qualitative semi-structured interviews will be discussed related to PURE consortium and the functioning of the collaborative network. However, only the first four sections of the interviews will be presented in this chapter because they are related to PURE consortium functioning (the others are related to technology market transfer). The script of the interviews in Appendix 1**.**

2.4.1 Background and expertise

In this first section, the background and expertise of PURE Members was assessed to give a deep understanding and context of the actors who take part in this project. In these interviews, all members' names were pseudonymised and will be referred to with their code names according to EU Data Protection and a consent form was signed. Therefore, the following paragraphs describe in detail the information about each interviewee.

First, Interviewee A is a senior researcher from BOKU with a high level of experience, having worked his entire life in academia, however given his vast career he stated that he has had interactions with industry as evidenced by the following quote "(…) my entire professional life I worked in academia, but all my career I had interactions with industry." In addition, regarding his role nowadays in his institution, this interviewee has noted that his main role beyond being a researcher is "(…) acquiring research brands and making strategic decisions for research programs." In the consortium, this interviewee has stated that he performs supervision roles, specifically of young researchers, and "(…) guide the entire project into the right direction and to support the project coordinator." This interviewee, in his own words, has a very strong view on the needs of the biotech industry. Moreover, he applies his expertise on tech transfer to the PURE Project, specifically in the translation of materials for bioseparation and in downstream bioprocessing, as well as the field of ligand design and generation. In the opinion of Interviewee A, there is a distinction of positive experiences in respect to the administrative and the scientific aspect of the projects. In terms of the administration of the projects, according to this interviewee, the projects usually run in the right direction because the deadlines are met and every partner delivers their work on time ("(...) the projects ran very well, all participants delivered on time so that was a positive moment."). On the other hand, related with the scientific point of view, because there are a lot of scientific areas in the project and the findings are always reported through meetings, reports or discussion with project management, the Interviewee recognizes that he gains more insight in other areas other than their own ("Now, I have quite a good insight in genetic engineering, cell biology and so on while I am not a cell biologist (...) through reports, project management, meetings and so you get really prime and up to date information.").

Interviewee B is a Junior Researcher at FCT-NOVA and has key roles in the project as a “(…) researcher and I perform management roles (…)”. In addition, Interviewee B has started their career in academia by being enrolled in a PhD. Then, this interviewee worked in industry where they have experience with project management and participation in various projects, as evidenced by the quotes "(...) ten projects in industry (...)" and "In industry, I also have project management experience and have participated in several projects.". In industry, it was stated that this interviewee has project management experience in pharmaceutical companies, as it is evidenced by the quote "I was in projects that were part of a team that provided services to pharma companies." Relating to their previous experience in industry, this interviewee refers to industry has had much tighter deadlines where, according to this interviewee, "(…) you have very high expectations from the people who hire you." However, in academia there is more flexibility, given that "(…) there are less resources (…)" and that the research is more basic or fundamental, as opposed to industry where you have "(…) streamlined processes (…)" and "(…) quality-controlled checkpoints (…)". Overall, the most challenging issue in both industry and academia would be the team. In the opinion of this interviewee, the main considerations taken from past project participation is to have good objectives to fulfil and that working in a team can be challenging at times.

Interviewee C is from BOKU and is a research assistant who runs her own group. Regarding past project participation, in the opinion of Interviewee C, if you work with companies, you always have stricter deadlines, as is evidenced by the quote "(...) if you work with companies you have to deliver on time." Moreover, there is difficulties on the part of students in academia to keep up with the pressures of meeting industry deadlines. With respect to European Projects, there are defined deadlines and deliverables but, because interviewee C has experience in working with companies, she got used to meeting those deadlines. The main positive lessons taken from past project participations for interviewee C were the team and the co-workers of past projects. Overall, there were not negatives but it was stated that sometimes there are not completely satisfied with the results or don't comply with the tasks and the worked they were previously assigned to do. The main expertise of this member is the biosynthesis of non-canonical amino acids, and in the project, she is not only responsible for the team who produces these molecules, but also in charge of their supply. Beyond this, interviewee C and her team are also the ones who have the most expertise in the modification of the proteins with the non-canonical amino acids.

Furthermore, interviewee D works at iBET and has had a diverse career path, what is shown by the fact that she has worked in different types of institutions, giving as an example having worked in a “(…) public school (…)”. In addition, Interviewee D is coordinating a laboratory and a unit in her institution. She has experience in making the bridge between industry and academia, giving their experience in working with industry partners. Interviewee D considers that having more than four or five institutions in the project, increases the difficulty in coordinating all institutions given that there are different fields involved and the communication needs to be "(...) very clear and transparent (...)". This interviewee also considers that in industry, there is pressure to enforce deadlines and to meet them. In opposition to this, in academia there is less pressure to develop your research and to resolve different

issues, because you don't have time restrictions. The main lessons for Interviewee D were working and interacting with a team where complementary knowledge is shared. The main negative, according to Interviewee D, is that the communication is "(...) not clear enough (...)" and the number of partners can affect the direction of the project, given the difficulty to integrate everyone's perspective ("(...) if the project has a lot of partners, it is difficult to be integrated for things to go in the right direction.").

Interviewee E is a member of Bayreuth University and is one of the PIs of the project, running the project in his institution. According to the quotes from the interview, he is responsible for not only the technical aspect of the task but also the supervision of his team. Interviewee E has been part of several projects, ranging from forty to fifty. This interviewee has done their PhD and has worked as a research assistant in various universities and has been a group leader of several research units. He also founded two different companies. It can be inferred that he has vast experience in both academia and industry. This interviewee considers that, from their experience, projects are "(…) highly dynamic processes (…)" which requires you to always adjust, because there are a lot of issues constantly surfacing. Therefore, Interviewee E always tries to "(...) dynamically adjust things from the fly (...)", which, from his experience, didn't happen with other consortia where "(...) the biggest problem that many people have is that they set up a project and they think everything is taken for granted (...)".

Finally, Interviewee F works at FCT-NOVA and is the project coordinator. In her institution, she is an associate professor and a researcher within her institution. This interviewee has never worked in industry, however, she has vast experience in projects that involve industry, as evidenced by the quote "Although I've never worked in industry, we did have some projects in collaboration with industry."

Overall, we can affirm that of the six interviewees most of them has large experience participating in consortium networks and have already been coordinators in some projects. Most of them has extensive industry networks or constant contact with industry. However, only one has worked in industry. Moreover, most of the participants stated that the main difference from academia and industry is the deadlines imposed, where in academia there are much looser deadlines than in industry. In addition, in a European Project like PURE it is of utmost importance the compliance with deadlines since these are the rules adopted by the FET-OPEN project framework.

Therefore, in the next section the fusion of applied and fundamental research methodologies will be addressed since to assess how industry-like methodologies and rules coexist within a mainly academic research project.

2.4.2 Applied or Fundamental Research

In this section, the research aims to verify if this consortium follows the Mode 2 Production of Knowledge criteria of knowledge produced in context of application, given that this project, as stated before, has the main goal of application and, therefore, implies the fusion and collaboration with industry practices and methodologies. Therefore, three subcategories were studied: first, the methodology

practiced by each interviewee in their careers and in the PURE consortium; then, it was asked what are the most relevant drivers for transferring the knowledge produced in the project to industry stakeholders; and finally, the last subcategory intended to study the relationships in the project between the core members and the Advisory Board, the peer-review body in the project that provides the more direct connection with industry.

2.4.2.1 Type of research methodology in PURE

PURE Members' background is mainly academic; therefore, it was expected that their experience would be mostly with fundamental research. However, since they have some sort of contact with industry it was poignant to investigate what the patterns of research methodology were throughout PURE Members' careers, and how they translate to the methodology of the project.

Interviewee A has stated that he has expertise in translating materials for bioseparation into practice, which can be classified as applied research experience throughout his career. Therefore, he perceives fundamental research as the cornerstone of PURE, stating that "PURE has a very strong element of fundamental research. It is rooted in the project." In addition, he also affirms that applied and fundamental research are equally important in the project, and that they are not so easily distinguished. Although, he believes that applied research only should be thought about if the fundamental research problems can be solved.

In the case of Interviewee B, she has been more applied oriented since the beginning of her career, as evidenced by the quote "Even in my PhD I did more applied research." This interviewee has had experience working in industry which reveals the applied focus, but also within her career in academia today, Interviewee B is preoccupied in merging the two types of research together. Regarding the PURE Project, similarly as Interviewee A, Interviewee B states that the focus now should be first in the proof-of-concept that the project proposed, which is achieving the "(…) precise modification of the fibers (…)", and then as soon as the fundamental research is completed, then the project should focus on the performance for the application of the fibers.

On the other hand, Interviewee C has worked most of her career in a company that combines fundamental research with applied research, highlighting that she has experience on both ends "(…) I have done fundamental research for application." Furthermore, she also asserts that her own research has been transferred to patents that, nowadays, are being used by a company. Additionally, Interviewee C is also of the opinion that the project, at the time in which interviews were conducted, the fundamental research should be the focus because of the novelty of the application. However, once again, a key element of Mode 2 Production of Knowledge is evidenced when Interviewee C mentions that even though the project is developing fundamental research, the focus should be application ("I think you can only do the best out of everything to pursue an application. Anything that is done in our group is fundamental research, but we always have a focus on application.").

Next, Interviewee D, a member from iBET, made the distinction between her institution and the other institutions in the project, enforcing that her institution is "(…) more applied oriented (…)" and

all the other partners are “(…) really strong in fundamental research (…)”. This statement adds on to the general notion that the project consists of a fusion between fundamental and research elements, in which one complements the other by having an institution responsible for the application and the scalability of the technology being developed at the fundamental stage. Moreover, this thesis is sustained because Interviewee D also believes that fundamental science is essential for the development of any application and that fundamental and applied research are difficult to distinguish.

Interviewee E, in similarity to the other interviewees, states that both fundamental and applied research are equally important to the project. On one hand, Interviewee E says that “(…) you always need to keep in mind that there is the other end therefore, it helps if you know both ends (…)”, which means that there is a combination of both methodologies for the project at-large to succeed. In addition, Interviewee E notes that the senior members in the consortium have great experience with both methodologies, attributing this as an advantage.

Finally, Interviewee F is of the same opinion as their colleagues that there should be a combination of both methodologies. However, she goes one step forward in asserting that technology design is greatly affected by the fundamental research performed, because if a researcher doesn’t comprehend the fundamentals of their research, they will never be able to control their work or predict their system, which will compromise technology development (“(…) Then you start finding out that something is happening as a result, so most of the times you need to do things that you don’t know exactly what is going to happen but then if you don’t try to understand why this has happened, you will never be able to predict or design your system so you cannot have a technology.”).

2.4.2.2 Relationships with industry

In this subsection, relationships in the project were studied to give a broader notion on how the project members collaborate with internal and external industry actors. Therefore, we can classify the relationships between members of the project with the Advisory Board as inside the project, whereas with industry are called relationships outside the Project.

First, Interviewee B considers that the proximity of this project to industry is shown in two different fronts. On one hand, she considers that inside the project there is a SME, iBET, that works directly with industrial scale-up of these “(…) supports for bioseparation (…)” and has deep knowledge of the needs of the market. The presence of such company is essential, as evidenced by the quote “(…) we need to have those kinds of people on board.” On the other hand, Interviewee B infers that the Advisory Board is also an asset because they have industry experience with the materials that the Project is working with, which is as an advantage. Interviewee D, a member from iBET, also is of the opinion that the Advisory Board and the network of each partner are extremely helpful for expanding and deepening the project’s connection with industry.

In the case of Interviewee C, she recognizes that the Advisory Board is a peer advisory body that is constituted by a “(…) nice mixture of people and even the academic partners have close ties to industry.” Furthermore, Interviewee C also considers that the role of this body is more than consulting

on technical and market issues. She also perceives them as being key knowledge transfer drivers and vehicles of dissemination of the project's results and vision ("It happens very often that they talk to each other and get to know things from each other, so it is good to have company partners in this project not only in the advisory board"). This way, the project gains notoriety and credibility because valued members in industry and academia spread the word on their work. However, the relationship with these industry stakeholders can hit roadblocks, because the companies that are involved can be considered competitors, so this issue has to be resolved in the form of technology licensing and IP Protection, as it was illustrated by Interviewee C's own personal experience ("I sold methods and licensing which is not all patents because we cannot afford it, so we sell the patents to company partners meaning they keep the materials or the knowledge secrets that we produce."). Finally, it was also noted that the relationship with industry in PURE is satisfactory, however this interviewee believes the project should invest more in expanding its industry network "I think we have a very good connection to industry, but I think there should be more." The approximation to industry should only be done as soon as the project's proof-of-concept is proved and that there are opportunities for commercialization.

Ultimately, Interviewee F affirms that the Advisory Board is the Project's closest relationship with industry ("Our closest relationship in the industry is really in the advisory board (…)"), alongside with the connections from the project's members own professional networks network. However, these relationships are a way to complement a lack of presence of an industrial partner in the project, which was similarly referred by Interviewee C. The SME present in this project is viewed as "(…) providers of services to industries and customers so it means that in reality in this project we don't really have a company that is an industrial producer."

The connections between the consortium and industry are mainly built around the members' connections and network with the industry. In addition, the Advisory Board is the main reflection of the members' connections given that this body was assembled via previous relationships established.

However, there is a need for the establishment of new connections to expand the PURE's industry network, even though the project is not at the industry scale-up phase. Therefore, in the next subsection some dissemination and knowledge transfer strategies are going to be discussed.

2.4.2.3 Dissemination drivers and strategies

The dissemination and knowledge exchange must consider the evolution path of technology development. As such, this path is as follows: first, there is early stage invention, where the brainstorming and discussion of possible applications takes place; secondly, the proof-of-concept where fundamental research dominates the overall research in project development; then, in third place, the reduction to practice, which is the optimization of the methodology and scaling-up; finally, there is the prototyping, formulation, and compound for the commercialization and transfer to the market of this technology [50].

The PURE Project is nowadays in the proof-of-concept stage, where researchers are currently attempting to proving the concept proposed by the Project. However, it is important to discuss the

dissemination of this technology to the industry to spread the word and improve the industry network to aid in the other stages of technology development. Therefore, in literature there are several knowledge dissemination strategies with industry [51]. In this study, there were four mechanisms that stood out from the interviews, and they were: conferences, meetings, PhD students working in industry and publications.

First, Interviewee A considers that the knowledge transfer drivers that are the most important to contact the industry in the consortium are international conferences, meetings, and publications. However, he states that "(…) the maintenance of the website (…)" is also extremely poignant given that this is also a mean of contact with industry as they are a way to make project advancements relevant to the public. On the other hand, Interviewee B considers that having experienced people in the Advisory Board working in companies is sufficient for knowledge transfer ("(…) even in our Advisory Board we have someone who has experience with that by working in a company.").

Furthermore, Interviewee C, as it was reinforced before, when speaking about consortium-industry relationships, mentions again that having industry partners is beneficial for spreading the word, and to disseminate the knowledge produced in the PURE Consortium. In addition, when speaking about publications, she states that "(…) if you only publish maybe, you don't address industry so well." This suggests that publications themselves are important, but they need to be accompanied by other vehicles of dissemination.

On the contrary, Interviewee D gives an opposing opinion to Interviewee C, stating that "(…) my experience is that the scientific manuscripts are the stronger ones.", also adding that in most cases, stakeholders read a publication about a specific topic and then they contact iBET to receive consultation on that same topic. As such, there is two contrary views on publications as mechanisms of knowledge transfer. Despite these contrary views, Interviewee D also agrees with Interviewee C that the connections/network of all partners are important and will help with knowledge transfer in this consortium. Interviewee D also adds that conferences are means of dissemination of knowledge of fast impact, a view that is complemented by the opinions of Interviewee F that says that interactions in conferences are good platforms to getting to know your own field and to discuss innovation, ideas and problem solving.

Lastly, Interviewee E is the interviewee with the most different view out of all the other interviewees. First, he doesn't believe that PhD students working in industry is a driving force for knowledge transfer. All the other interviewees didn't mention this mechanism, which signals the lack of importance given to this mechanism in this consortium. However, Interviewee E believes that the greatest driver of knowledge transfer is himself, justifying this statement by stating "(…) I have the networks and the company contacts and have also the network in terms of media, press and TV (…)", meaning that he brings to the consortium multiple assets that can help disseminating knowledge and bring more industry contacts to accelerate the process of knowledge transfer.

In the next section, transdisciplinarity will be addressed to assess how the knowledge production, the relationships within the consortium and the mechanisms for dissemination above described are affected by the diversity of scientific fields and backgrounds in the PURE Project.

2.4.3 Transdisciplinarity

Transdisciplinarity, as stated before, is often characterized by the inclusion of non-academic stakeholders in the process of knowledge production and into problem-solving approaches that are applied to tangible, real-world problems [46,52,53]. Therefore, especially in sciences that directly address societal challenges, transdisciplinary teams should be involved in increasing the diversity of fields of expertise, which stimulates innovation and productivity and can outperform competition [46]. In the case of PURE, since it is a consortium formed in a framework of innovation to develop an application that could impact society, it was necessary to investigate how transdisciplinarity affects the research process in PURE.

In PURE, the members of this consortium are from vastly different areas as previously described, and they are: downstream processing, bioengineering, chemistry, bioinformatics, material sciences, economy, and sociology.

In such a diverse team, the dynamics of collaboration were investigated, which involves the understanding of how actors communicate through multiple areas of knowledge, how innovation is created and the barriers that may exist.

The diversity of PURE's expertise is confirmed by Interviewee C, as is evidenced by the quote "(…) the NOVA group in the modification of proteins and small molecules, chemistry and bioinformatics which is nice, then we contribute with downstream processing the bioseparation topic which is really in BOKU's heart of expertise (…) particularly the Bayreuth group are experts in the spider silk proteins (…)". Moreover, Interviewee C believes that the diverse expertise in the project gives a synergetic effect since the problem that the projected proposed to resolve is being addressed from different angles. This effect is also caused by the presence of social scientists in the project, which in her view, is a new opportunity have a new perspective on the project applicability since the members of the consortium are all natural scientists, therefore this approach would never been achieved without having "(…) economists and socio-economists there (…)". Furthermore, there is also a general belief that the integration of different natural sciences enables the combination of two completely different techniques that have never been put together before.

First, Interviewee B mentions that the spidroin membranes without the precise ligand modification would never have been used for bioseparation alone, which means that one technique complements the other. In addition, this interviewee also reaffirms that the diversity of members' backgrounds leads to an improvement in data quantity and therefore the technology impact is much greater than if you work exclusively in one fundamental aspect of the technology. Also, this allows the researchers to further explore and analyse in depth the properties of the material, process, and impact.

Interviewee D reinforces that complementary backgrounds of the members of the consortium allows the team to tackle the challenges in a faster and easier way. Interviewee E also believes that transdisciplinarity and the diversity of disciplines in this consortium is an advantage towards the commercialization of PURE's technology, reinforcing that "(…) if you have absolutely super experts in

one field you will never be successful in creating new and novel products for the market." Furthermore, Interviewee F resumes transdiciplinarity as a competitive advantage of the project.

However, there is one element of collaboration within a transdisciplinary consortium that the members interviewed mentioned as relevant and that is communication. On one hand, Interviewee D believes that due to different visons it is extremely important to communicate among consortium members to coordinate strategies and to follow a common research path. In the case of Interviewee E, he believes that communication between people from different areas must be done at a lower level, meaning that the consortium members must have clear mechanisms to communicate their data for members from other areas to understand. Otherwise, according to Interviewee F, it is difficult to share knowledge and communicate your data and work in a clear way, adding that time is needed for learning how to communicate with a fellow partner.

According to the PURE Members, there are several key aspects that define good communication in the consortium. First, the coordinator is key for the mediation and is responsible for bringing the consortium together as a whole, according to Interviewee A, Interviewee C, and Interviewee D. Moreover, in this consortium, the coordination is doing a good job, since the communication is clear, effective, the deadlines are imposed and there is organization. This leadership foments a good environment where everyone respects each other's expertise.

Besides the role of the coordinator, there are other communication aspects to consider. One of them, according to Interviewee A is formal communication in a legal framework that respects authorships to validate future patents and technology licencing. Additionally, Interviewee B mentions that having regular meetings increases communication ease and leads everyone in the consortium to be aware of the work being done, while also clarifying more specific terms to respective areas ("(…) It is important to have regular meetings and also agree on the meaning of the words that you use, because sometimes the words you use don't mean the same in all scientific fields."). Moreover, having meetings between the smaller teams is also important because it helps resolve issues within specific tasks and is better to resolve minor issues ("(…) we also have to have meetings between the smaller teams that are solving specific problems within the different tasks instead of having everyone involved.").

However, good communication depends greatly on how the consortium members and partners trust each other on a personal level and, more importantly, in a professional level. Since trust is the foundation of any project, it is going to be explored in the next section.

2.4.4 Trust

Trust is one of the most important factors to take into account in collaborative networks [54]. Moreover, it has been stated to improve cooperation, partner satisfaction and reduces conflicts [55,56]. Therefore, in this project the intention was to investigate how trust impacts the collaboration between partners and external relationships that exist with the industry.

First, to investigate trust inside the project, it was key to understand what the key factors when it comes to trust in the consortium. According to Interviewee A, the two main factors that affect trust are

previous professional experiences partners and their reputation. This opinion is shared by Interviewee C, stating that trust is helped by the fact that the majority of the project members' have worked together, which is also reinforced by respect of the expertise and knowledge of each partners' fields of science. According to Interviewee B, publication track record is extremely important because not only assures the proficiency that they have in their area, but also their ability to "(…) introduce novelty on the top of their research field (…)". Moreover, there are other particularities associated with trust observed in these interviews. Interviewee B and Interviewee D affirm that trust is also derived from rapid email response and frequent interactions, within a reasonable time frame, as well as following the project's rules and deadlines previously established by the project coordination team.

On the other hand, trust outside the Project is also investigated and it can be defined by trust with actors external to the project, more specifically the Advisory Board. Therefore, there is a clear concern about the presence of competitors in this body, stating the NDA signed in the beginning of the project is of high importance given it protects intellectual property, according to Interviewees B and D. Moreover, on the issue of Advisory Board member selection, Interviewee A adds that a key factor should be that the members are not competitors otherwise, it could compromise the project's advancements and, as Interviewee C clarifies, prevents leaks from happening. However, since the Advisory Board has a high level of importance because of their expertise and experience with market transfer, the PURE Members suggest that more frequent communication would help the Project, as suggested by Interviewee B in the following quote "So, it would be interesting to communicate every 6 months or even every 3 months."

2.5 Collaborative Model for PURE: A Preliminary Attempt

In the latter section, several parameters were laid out that influence the collaboration between partners in the PURE Project. Of all those parameters, four key elements of collaboration stood out as being extremely important for successful collaboration in this consortium: members, research methodology, dissemination, and trust. In detail, in Figure 2.1 every parameter is represented.

The first element of the collaborative model is the members that compose this project. Moreover, the research presented in the section above outlined that the actors of this consortium are from diverse backgrounds within the natural sciences (Biomaterial Science, Chemistry, Molecular Biology, Bioengineering) and the exact sciences (Computational Sciences). However, one characteristic that distinguishes this consortium is the presence of Social Sciences (Economics and Sociology) as integrative part of the research conducted, where multi and interdisciplinary research is conducted, since there is collective knowledge being produced within the consortium in all these areas.

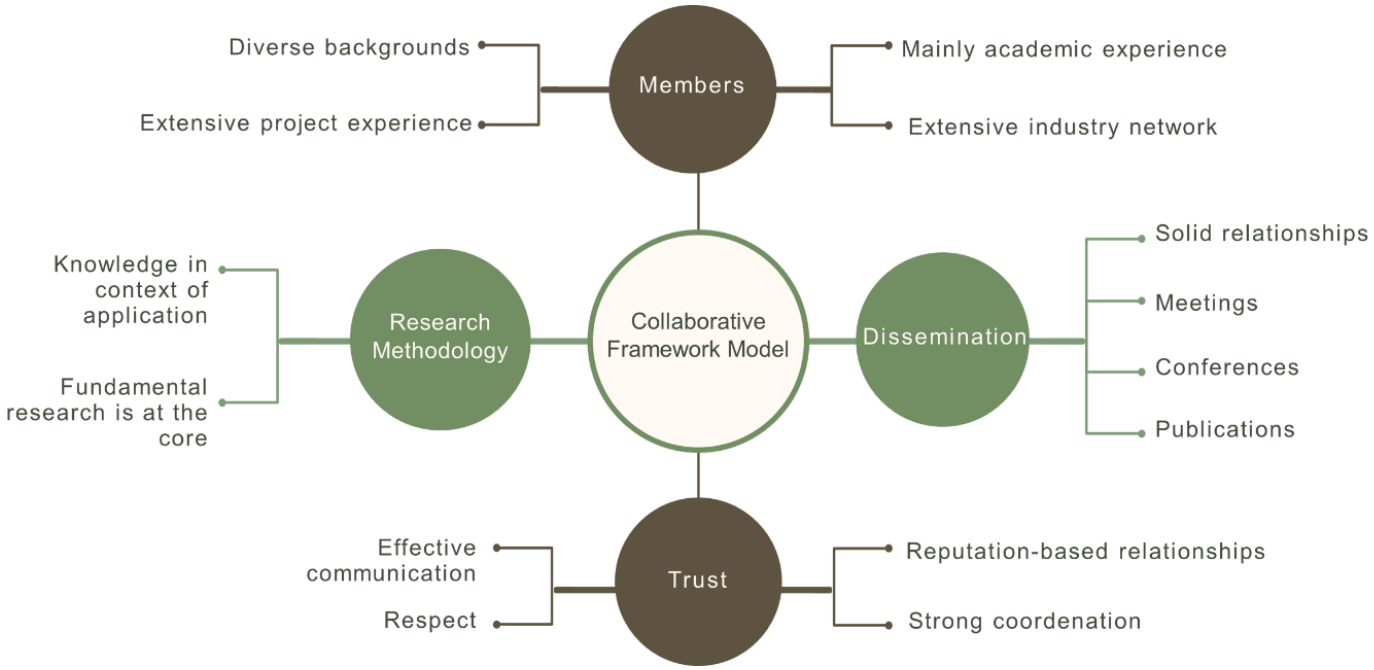

Figure 2.1 – Preliminary collaboration network model for PURE.

In addition, the EU FET-OPEN Project framework demands an innovative and socially conscious research approach, to create policy to affect societal change. Thus, in the PURE Project the technology being developed is in Bioprocessing, which demands an effective laboratory-to-market approach since its goal is to reach industry-scale production. Therefore, an industry mindset and methodology are required. Nonetheless, from Figure 2.1, it is possible to observe that this Project is composed of mainly academic actors who have had limited experience with industry projects. Yet, extensive industry network and European project experience contribute to an application focused consortium, given that they are familiar with the industry process and a SME in the Project who on a day-to day basis contacts with the bioprocess and biopharmaceutical industries give enough industry support to the project, according to most of the interviewees.

As described above, the research process in this project is applied oriented, though these findings suggests that fundamental research plays a significant role in this consortium. Given that natural sciences dominate the knowledge production in this consortium, academic practices must be considered by the actors during the proof-of-concept stage of the project, meaning that the goal of application is always present, but the actual research process maintains according to fundamental research practices. It is concluded then that a fusion of both methodologies is required, since in the first stages of project development fundamental research is dominant and in the last stages applied research is the reigning methodology. In addition, Gibbons' Mode 2 Production of Knowledge Theory applies in this case because the knowledge produce is always in the context of application and involves industry methodologies, notwithstanding the core fundamental aspect of the project.

A third element of the collaborative model represented in Figure 2.1, is dissemination because it evaluates the way the project communicates and translates the knowledge produced to the industry and to society, since it is one of its main objectives. Dissemination in this project, according to these findings relies on four factors: solid relationships, meetings, conferences, and publications. On one hand, relationships with industry are of extreme importance because they enable the spreading of the word and diffusion of the project's ideas. These relationships allow for the opportunity of meetings and the attendance of conferences to expose the project's findings, as well as the creation of partnerships with key industry stakeholders. On the other hand, publications are going to be crucial to give a scientific credibility to the project because it is what builds confidence in the scientific community about the validity of the results and the outcome of the PURE Project.

Finally, the fourth key element of the preliminary collaborative model designed in this work is trust. Trust is the foundation of collaboration because it enables for the actors to have confidence in each other professionally. Respect and reputation-based relationships are essential to build trust and are the backbone in this project, given that this project was built with people that had previous collaborative experience and respect each other's work. In addition, trust is reflected in PURE by an effective communication lead by strong leadership of the project coordinator.

All in all, it was possible to design a preliminary model of collaboration for PURE that possibly can be applied to other projects within the EU FET-OPEN Project Framework.

2.6 Final Remarks

After the empirical evidence and the results demonstrated, it is possible to also affirm that PURE fits the narrative of Mode 2 Production of Knowledge Theory, given that, as evidenced there is heterogeneity in background and expertise. Moreover, a collaborative network preliminary model is designed based on the results in this study. However, it is shown that the project lacks industry presence in the current scientific development and advancements, relying mostly on each partners connections and expertise. The presence of industry in this consortium is reflected in the Advisory Board.

Therefore, there is a clear need for an approximation to industry that should be done through, according to the findings, international conferences as way to expand the industry network and connections, and publication of findings as soon as possible as a mechanism of giving credibility to the work while also spreading the word about the project's achievement and goals.

In addition, there is a common and established notion of the project application roots despite the current research status being uniquely fundamental. Despite this, the members are clearly aware of this context and the methodology of research in the project combines both applied and fundamental research as they complement each other, which matches Gibbons' Theory that there is synergy between industry and academic practices in Mode 2.

3. PROOF-OF-CONCEPT FOR VIRUS-LIKE PARTICLE PURIFICATION

3.1 Background

In this Chapter, a preliminary test to assess the possibility to capture HIV-Gag VLPs using spidroin membranes functionalized with heparin was done.

Virus-Like Particles range between 100-200 nm in diameter [57] and are self-assembled, non-infectious, and structurally authentic to viruses that is able to conformationally display antigens on their surface and contribute to more robust humoral and cell-mediated immunity against viral infection [58]. In addition, VLPs are highly ordered repetitive structures that can stimulate both innate and adaptive immune responses [59]. There are several types of Virus-like particles [60], such as Human immunodeficiency virus (HIV) [61–63], influenza virus A [64], chikungunya virus (CHIKV) [65,66], severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [67,68], among others [60].

The main product of human immunodeficiency virus type 1 (HIV-1) and related retroviruses is a cytoplasmic polyprotein necessary and sufficient for the assembly and release of virion material, the Gag polyprotein.[69] HIV-1 Gag virus-like particle production requires the expression of this protein producer cell line [70] and, upon expression, it is able to self-assemble, giving rise to non-infectious VLPs in the absence of any other viral protein or virus RNA [71].

Currently, there are significant challenges associated with VLPs, such as lower stability, high production costs, sensitivity to environmental conditions, and difficult downstream processing [16]. This latter challenge is going to be further explored in his work.

Spidroin, or the eADF4(C16) protein, is the recombinant spider silk protein based on the consensus sequence of one of three spidroins of the dragline silk of the European garden spider (*A. diadematus)* [75,] and it is expressed in *E.coli* [72]. This protein due to the glutamic acid residue as a negative charge [73]. The consensus motif (C-module) of ADF4(C16) is repeated 16 times in the recombinant protein, and each repeating motif contains one glutamic acid residue. However, it was reported previously that polyanionic eADF4(C16) films were not suitable substrates for cell adhesion, specifically due to extracellular negative charge [72]. Therefore, a new eADF4(κ16) protein was engineered where the C-module was replaced by the κ-module, which replaces the naturally occurring glutamic acid residue in the repetitive units of the eADF4 core domain with lysine. Figure 3.1 illustrates the structure and composition of eADF4(C16) and eADF4(κ16) proteins.

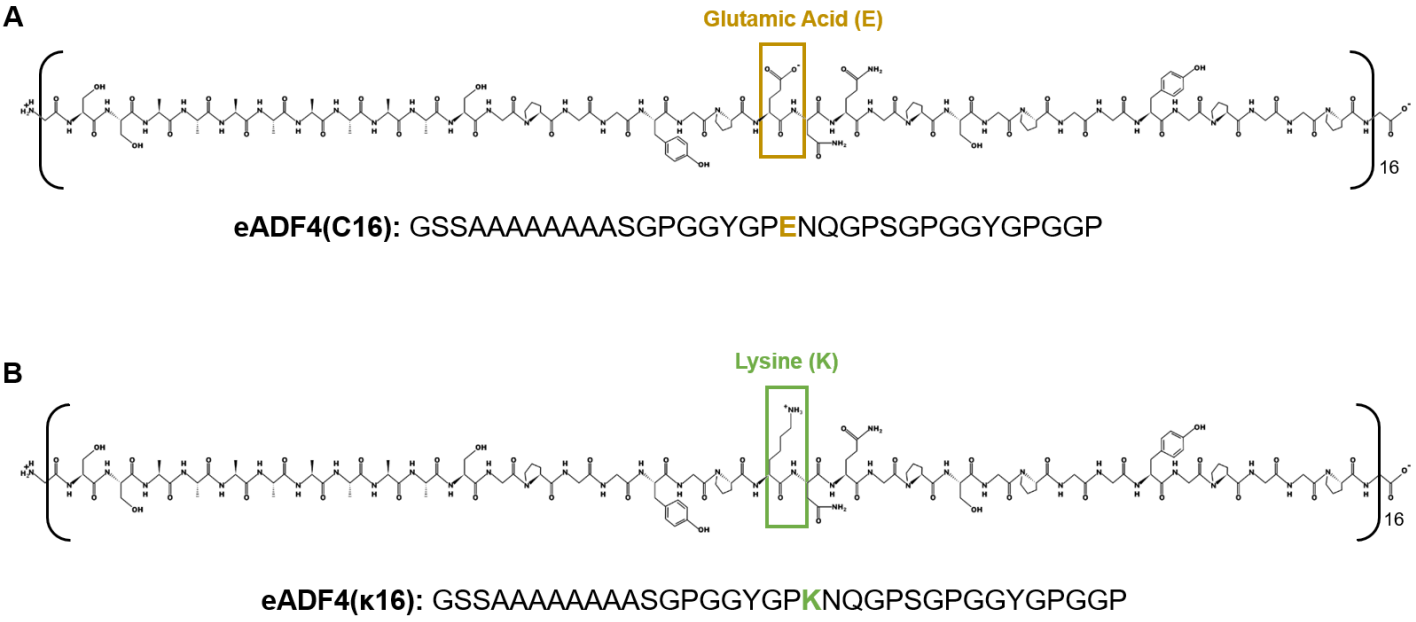

Figure 3.1 - Structure of recombinant spider silk peptides. (A) Repeating unit of eADF4(C16) peptide. A glutamic acid residue is repeated 16 times, conferring the protein a negative charge. (B) Repeating unit of eADF4(κ16) peptide. The glutamic acid residue is replaced by a lysine residue, conferring the protein a positive charge. Structures were designed with PepDraw (http://pepdraw.com/).

Heparin is a large polysaccharide with an average molecular weight of 12 kDa, composed of repeating units of disaccharides. All these repeating units contain a carboxyl group, 1/2 hydroxyl groups, and 2.5/3 O-sulfo and N-sulfo groups. Each chain contains a reducing end hemiacetal, which is a mask aldehyde group [74]. In this study, an alternative method was used to determine the amount of immobilized heparin.

Heparin is a natural receptor for many viruses since it is a relatively inexpensive and stable affinity chromatography ligand used to purify protein mixtures due to interactions with a variety of proteins [75]. In addition, it was described that heparin interacts with these heparin-binding proteins via ionic or hydrogen bonds between heparin's sulfo- and the amino- groups of the protein [75]. The heparin-specific interactions with various proteins enable protein purification processes, where heparin is covalently immobilized on a porous bead and acts as a specific affinity ligand, and the mixtures of proteins can be separated using heparin affinity chromatography columns[75].

Sasaki et al. introduced a new mechanism for coupling heparin at the reducing end to free lysins at the surface of Amino and Hydrazino Sepharose [76]. This approach is an improvement to previous studies that required reductive amination of the formyl group and amino-agarose while using cyanoborohydride as a reducing agent[77]. Figure 3.2 represents the immobilization reaction in this work.

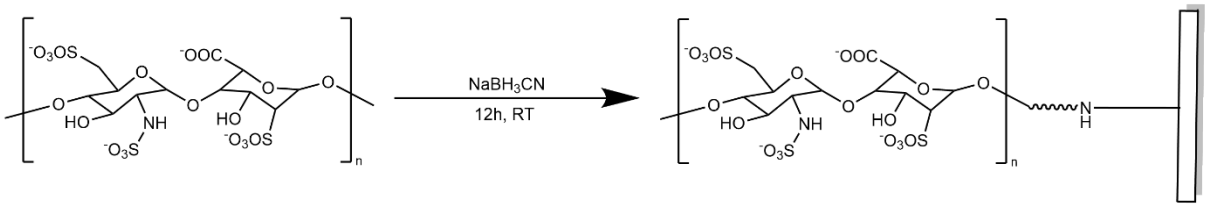

Figure 3.2 - Immobilization reaction of heparin in eADF4(K16) membranes. Reaction occurs at room temperature and 12 hours. Cyanoborohydride acts as a reducing agent of the terminal aldehyde group, forming a transient imine when reacting with the free lysins in the eADF4(κ16).

Heparin-binding technologies are achievable through alternative methods for coupling heparin, not just the single end-point covalent linkage of heparin, where heparin attaches to a surface via its reducing end. Hence, it can also be done at multi-point covalent linkage in which heparin can generally be classified as differing in the number (single- or multi-) and type (chemical groups used for bonding) of linkage points between the heparin chain and the biomaterial [78].

Sasaki *et al.*. [76] developed an immobilization strategy where the covalent linkage of heparin is done via reductive amination in the end-point aldehyde group in heparin, which enables a shorter conjugation time. However, other immobilization strategies can also be employed. Kolar et al. [79] developed an alternative strategy where a crosslinking agent, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), is used in a condensation reaction between an activated carboxyl group of heparin and a primary amine on the material surface.

The objective of this study was to test one of the applications currently being developed by PURE: functionalized spidroin non-woven nanofibers for purification of Virus-Like Particles. As described before, heparin-based affinity chromatography is an established method used in downstream process applications for VLPs. Hence, the aim is to functionalize the material support developed in the PURE Project with this widely used ligand.

In this work, as a model VLP, it was selected HIV 1-Gag particles to test the binding capacity of spidroin eADF4(κ16) modified with heparin.

3.2 Materials and Methods

3.2.1 Materials

3.2.1.1 Reagents

The reagents used were of the highest grade available. Heparin Sodium Salt from Porcine Intestinal Mucosa (CAS Number: 9041-08-1), Sodium Cyanoborohydride ($NaBH_3CN$; 71435; CAS Number: 25895-60-7), Quantipro™ BCA Assay Kit for 0.5-30 µg/mL and Potassium Chloride (KCl; CAS Number: 7447-40-7) were all purchased from Sigma-Aldrich (Sintra, Portugal). The 2 mg/mL Bovine Serum Albumin (CAS Number: 9048-46-8), 0.02% (w/v) Coomassie Brilliant Blue R-250 Staining

Solution (CAS Number: 6104-59-2), 24% (w/v) of glycerol (CAS Number: 56-81-5), and 4% (v/v) β-mercaptoethanol (CAS Number: 60-24-2) were all obtained from Merck KGaA (Darmstadt, Germany). The 1% (w/v) Sodium Dodecyl Sulfate (Number: 151-21-3) and Silver Stain Plus Kit (Catalog Number: 1610449) were obtained from Bio-Rad (Germany). The HEPES ($C_8H_{18}N_2O_4S$; Catalog Number: H3375; CAS Number 7365-45-9), Sodium Chloride (NaCl, CAS Number: 7440-23-5) were obtained from PanReac Applichem GmbH (Darmstadt, Germany).

Sodium Phosphate Dibasic (CAS Number: 7558-79-4) and Potassium Phosphate Monobasic (CAS Number: 7778-77-0) were purchased from Biogen Cientifica S.L. (Portugal).

The spidroin eADF4(κ16) and eADF4(C16) membranes were produced and assembled at the Institute of Biomaterials in Bayreuth, Germany.

3.2.1.2 Equipment

An Optic Ivymen® Orbital Shaking was used for agitation purposes. The Microplate Reader – Tecan Infinite F200 from Tecan was used for all spectrophotometric assays. The gels were photographed in a Gel Doc™ XR+ Molecular Imager® (BioRad). The densitometry of the gel bands was quantified using Image Lab™ Software (BioRad).

3.2.1.3 Software

For chemical reaction design, ChemDraw® 21.0.0 from PerkinElmer Informatics. The densitometry of the gel bands was quantified using Image Lab™ Software.

Data was processed into plots using Origin (Pro) (Version 2022) OriginLab Corporation, Northampton, MA, USA.

3.2.2 Methods

3.2.2.1 Conjugation of heparin in eADF4(K16) and eADF4(C16) membranes

The aminated surface used in this assay were the eADF4(κ16) membranes (free amine in the Lys side chain and N-terminal) and eADF4(C16) membranes (free amine in the N-terminal). These membranes were produced by electrospinning of spidroin protein solutions into aluminium foil at Bayreuth University (Germany). To initiate the conjugation reaction of heparin to eADF4(κ16) and eADF4(C16) membranes, 55.2 mg of heparin and 5.52 mg of cyanoborohydride ($NaBH_3CN$) were dissolved in 1 mL of 1xPBS pH 7.4 (10 mM Sodium Phosphate dibasic, 10 mM of Potassium Phosphate Monobasic, 2.7 mM Potassium chloride and 137 mM of Sodium Chloride), and 200 µL of this solution

were added immediately to the membranes and incubated overnight (12h) at room temperature with agitation. After conjugation, the membranes were washed 15 times with 200 µL of 1xPBS, pH 7.4, for 5 minutes. The recovered solutions were stored in the fridge until quantification.

In the heparin conjugation assay, **Equations (1)** and **(2)** represent the total heparin bound (mol) and percentage of heparin bound to the membranes calculated.

$$Heparin\ Bound\ (mol/cm^2) = heparin\ loaded\ (mol/cm^2) - (heparin\ flowthrough\ (mol/cm^2) + heparin\ washed\ (mol/cm^2)) \quad \textbf{(1)}$$

$$Heparin\ Bound\ (\%) = \frac{Amount\ heparin\ flowthrough\ (mol/cm^2) + Amount\ heparin\ washed\ (mol/cm^2)}{Amount\ heparin\ loaded\ (mol/cm^2)} \times 100 \quad \textbf{(2)}$$

3.2.2.2 Quantification of Heparin functionalization in spidroin membranes through ICP-AES

For the quantification of functionalized heparin in eADF4(κ16) and eADF4(C16) membranes, each sample corresponding to the loading, flow-through, and washes (combined total amount of washes) was analysed via Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), at Laboratório de Análises REQUIMTE – LAQV. For the analysis, the samples collected were diluted 1:10 in 1xPBS, pH 7.4 buffer.

3.2.2.3 Binding assays between HIV-Gag Virus-Like Particles and heparin modified and unmodified eADF4(C16) membranes

3.2.2.3.1 Screening

For the screening of HIV-Gag Virus-like Particles in unmodified and heparin-modified eADF4(C16) membranes, 60 µL of an HIV-Gag VLP (stock solution at protein concentration 750 ± 60 µg/µL) was added to 1440 µL of 10mM HEPES, 150 mM NaCl, pH 7.4. From the previous solution, 200 µL was added to two Heparin-modified and two unmodified eADF4(C16) membranes and incubated for 1h at room temperature with agitation at 50 rpm. The flow-through solution was collected and stored at 4 °C. To wash the membranes, 200 µL of HEPES, 150 mM NaCl, and pH 7.4 was added to each membrane, and it was collected and stored at 4 °C. This procedure was repeated five additional times. Duplicates were prepared for this experiment.

3.2.2.3.2 Micro BCA Assay for the Quantification of Total Protein

For the quantification of total protein in each sample, the Micro BCA Assay was used. For the BSA protein standard solution, 300 µL at 50 µg/µL was prepared from 1 mg/mL stock BSA solution. For the calibration curve in the range of 0.5-30 µg/mL (0; 0.5; 1.5; 10; 20; 30) and 10 mL of working reagent (25A:25B:1C) was prepared in accordance with manufacturer instructions. The assay was prepared by using 75 µL of the protein standard solution in the range of 0.5-30 µg/mL and 75 µL of working reagent in a Greiner 96-well Flat Bottom Transparent Polystyrene Microplate. The same procedure was adopted for the quantification of total protein in the flow-through, loading, and washes. The microplates were incubated for 1h at 60 °C, covered with aluminium foil, and the absorbances were read at 560 nm in an Infinite 200 Tecan Microplate Reader.

3.2.2.4 Quantification of Total Protein Leached from Unmodified eADF4(C16) membranes

3.2.2.4.1 Binding Buffer Assay

For the determination of total protein in unmodified eADF4(C16) membranes, 200 µL of 10mM HEPES, 150 mM NaCl, pH 7.4, and incubated for 1h at room temperature with agitation at 50 rpm. The flow-through solution was collected and stored at 4 °C. To wash the membranes, 200 µL of HEPES, 150 mM NaCl, and pH 7.4 was added to each membrane, and after 5 minutes of incubation, each sample was collected and stored at 4 °C. This procedure was repeated 5 additional times. Samples were quantified for total protein using Micro-BCA assays as previously described.

In the VLP binding assay, the total protein bound (µg/mL) was calculated according to **Equation (3).**

$$Total\ protein\ bound\ \ (\mu g/mL)\ = total\ protein\ loaded\ (\mu g/mL) - (total\ protein\ flowthrough\ (\mu g/mL) + total\ protein\ washed\ (\mu g/mL)) \quad \textbf{(3)}$$

3.2.2.4.2 Analysis of protein collected through a Tris-Tricine gel

The samples from loading and washes were analysed with Tris-Tricine gel. Before they were loaded in the gel, 15 µL of the samples were mixed with 20 µL of loading buffer (100 mM Tris-HCl pH 6.8, 1% (w/v) SDS, 4% (v/v) β-mercaptoethanol, 0.02% (w/v) Coomassie Brilliant Blue (CBB) and 24% (w/v) of glycerol). The samples were incubated at 100 °C for 10 mins. After the incubation, 15 µL of the samples were loaded in the gel and were run for 90 min at 60 mA and 150 V.

For gel staining, the Bio-Rad Silver Stain kit was used following the manufacturer's instructions. The gels were visualized in GelDoc and analysed by ImageLab software.

3.3 Results and Discussion

3.3.1 Heparin Conjugation in eADF4(κ16) and eADF4(C16) membranes

There are several immobilization strategies and techniques. In this work, the immobilization technique was single end-point covalent linkage via reductive amination by using a reductive agent (cyanoborohydride), where the end point aldehyde group in heparin reacts to the free amine groups in the membrane surface. This method is based on a study done by Sasaki et al. [76] where heparin was immobilized via reductive amination in Hydroazino Sepharose carriers for 48h. However, there are other methods of immobilization, such as multi-point covalent linkage where heparin is attached via the carboxyl groups via N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) condensation (Table 3.1).

Table 3.1 - Immobilization techniques of heparin to different surfaces.

	This work	*Sasaki et al..* [76]	*Kolar et al..* [79]
Type of Immobilization	Single, end point covalent linkage in eADF4(K16) membranes	Single, end point covalent linkage in Amino Sepharose	Multi-point covalent linkage in PET materials
Buffer	PBS, pH 7.4	Phosphate Buffer	50 mM MES buffer
Reducing/Crosslinking Agent	Sodium cyanoborohydride	Sodium cyanoborohydride	N-(3-dimethylaminopropyl)- N′-ethylcarbodiimide hydrochloride (EDC)
Type of Reaction	Reductive Amination	Reductive Amination	EDC Condensation
Incubation Time	12h	48h	4h

For the heparin immobilization studies, the ICP-AES Spectroscopy was used to determine the amount of heparin functionalized in the membranes. This technique allows for the determination of over 70 elements in liquid, solid, or gas samples [80]. In these systems, the species are atomized and ionized in high-temperature plasma, given the ions are excited, which leads the electrons to be elevated to higher energy levels [81]. The optical spectrometer in the ICP-AES system collects the light emitted with a specific wavelength that corresponds to the energy difference between the excited levels and the ground state level. When compared to other techniques, the ICP-AES has the advantage for the determination of non-metallic elements like C, S, P, and Cl. Furthermore, as heparin has 2.5/3 sulfo groups in its repeating unit, the immobilized heparin was calculated through the amount of sulfur present in the samples. The amount of heparin can be estimated since it is 1/3 of the amount of sulfur determined in the solutions.

Therefore, in the immobilization studies, the samples analysed were the loading solution, the amount of heparin loaded in the membranes; the flow-through, the amount of heparin that was collected upon 12h of incubation; and the washes, the total amount of solution washed in this assay. The amount of heparin bound was calculated in mol/cm^2, considering that each membrane has a total area of 0.38 cm^2. These results are represented in Table 3.2 and Figure 3.3.

Table 3.2 - Amount of heparin immobilized in mol/cm^2 and percentage of heparin immobilization in eADF4(C16) and eADF4(κ16) membranes.

Membrane Type	Bound Heparin (mol/cm^2)	Bound Heparin (%)
eADF4(C16)	1.9×10^{-6}	7.8
eADF4(K16)	2.0×10^{-7}	0.8

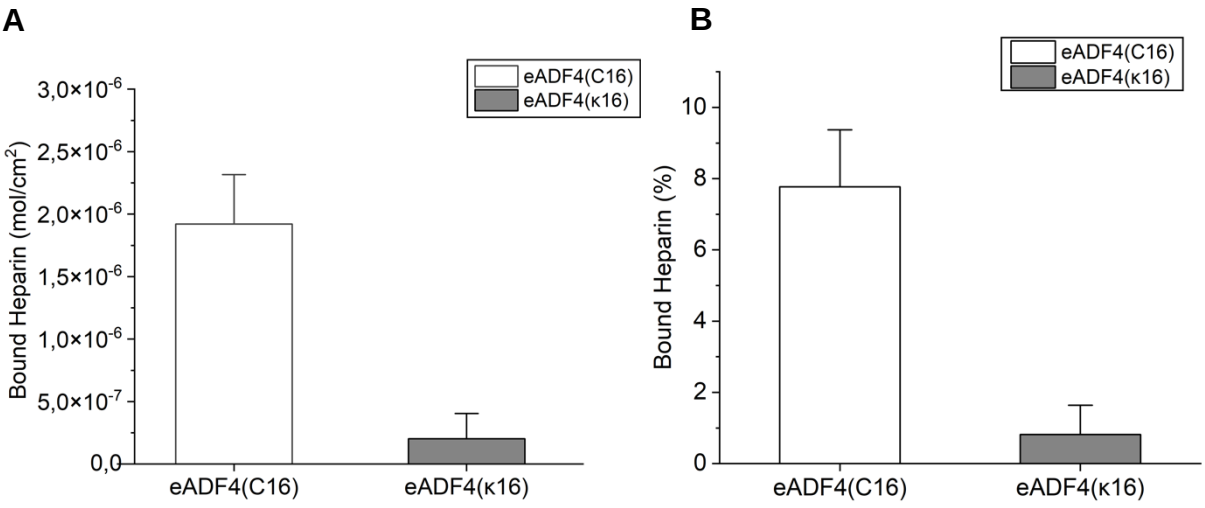

Figure 3.3 - Comparison of amount of heparin bound to eADF4(C16) and eADF4(K16) membranes. (A) Amount of heparin bound in mol per area of membrane. (B) Percentage of heparin bound to each membrane type.

In Figure 3.3, the results of the heparin immobilization show that the eADF4(C16) membranes exhibit more immobilization of heparin than in the eADF4(κ16) in the amount of heparin bound, which indicates more heparin immobilized in the eADF4(C16) membranes. Furthermore, it can be inferred that the eADF4(C16) membranes were modified in a higher percentage than the eADF4(κ16) membranes. This was not expected based on the free amine content, which is higher in eADF4(κ16) membranes. However, it indicates that the self-assembly of the proteins is not the same, and the free amines from the lysine groups are not easily accessible for conjugation.

It has been suggested that positively charged surfaces can be subjected to non-specific interactions, where heparin is binding to other sites in the protein rather than the free amines in the lysine residues. Thus, for the VLP binding assay, only the eADF4(C16) membranes were used.

3.3.2 Determination of VLP binding to eADF4(C16) membranes

In this assay, the aim was to determine the amount of VLP binding to eADF4(C16) membranes. Therefore, the heparin-modified eADF4(C16) (eADF4(C16)_Hep) membranes were used and, as a control, unmodified eADF4(C16) membranes.

First, the screening of the HIV-Gag VLPs was done with a binding buffer (10mM HEPES 150 mM NaCl pH 7.4); this buffer was selected because it is commonly used for VLP purification with heparin-based supports. The VLPs solution was incubated for 1h of incubation; the flow-through was collected and stored at 4°C. Moreover, the membranes were washed 6 times with the binding buffer. The fractions collected from the binding assay were quantified for total protein through a colorimetric assay. Table 3.3 summarizes the results obtained from these assays.

Table 3.3 - Amount of total protein washed and bound to the modified and unmodified eADF4(C16) membranes. eADF4(C16) _Hep corresponds to the modified eADF4(C16) membranes with heparin, and eADF4(C16) corresponds to the unmodified eADF4(C16) membranes. The HIV-Gag VLP loading solution corresponded to 23.44 ± 8.04 μg/mL.

Membrane Type	[Total Protein] washed (μg/mL)	[Total Protein] bound (μg/mL)
eADF4(C16)_Hep	46.97 ± 2.81	-23.53 ± 2.81
eADF4(C16)	42.44 ± 0.63	-19.01 ± 0.63

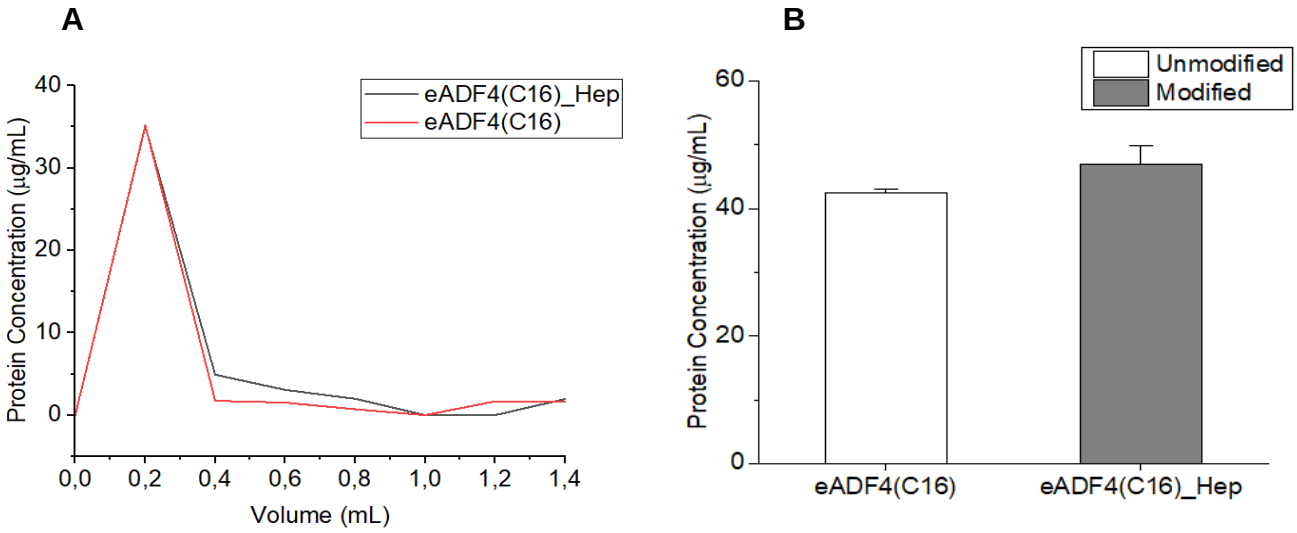

Figure 3.4 - Binding assay of HIV-Gag VLPs in heparin-modified eADF4(C16) membranes. (A) Chromatogram for the binding assay of VLPs for heparin-modified eADF4(C16) membranes (eADF4(C16) _Hep, black line). Unmodified eADF4(C16) membranes were used as a control (eADF4(C16), red line). (B) Comparison of total washed protein (µg/mL) in the binding assay.

The results show that for the modified and unmodified eADF4(C16) membranes, the total amount of protein that is collected from the membrane is higher than the loading ([VLPs] 23.44 ± 8.04 µg/mL, Table 3.3, Figure 3.4). Moreover, this value indicates that not only the VLP loading solution is washed out in its entirety, but also additional protein is leaching out from the spidroin membranes, which indicates that the conditions of the binding assay are unfavourable to the spidroin membrane surface.

3.3.3 Quantification of Total Protein Leached in Unmodified eADF4(C16) membranes

The results of the binding assay, as it was described previously, might indicate that in the flow-through and washes, there are other proteins besides VLPs coming out in the washes. Thus, it is suggested that spidroin membranes are susceptible to leaching. To respond to this question, we prepared an assay with unmodified spidroin membranes with binding buffer and proceeded to determine the total protein that comes out in the washes.

Table 3.4 - Amount of protein washed out by fraction in the binding assay. L – Loading sample FT – Flow-through sample, W1 – First wash sample, W2 – Second wash sample, W3 - Third wash sample, W4 – Fourth wash sample, W5 – Fifth wash sample, W6 – Sixth wash sample

Fraction	Volume (mL)	Protein (µg/mL)
L	0.00	0.00
FT	0.20	5.61
W1	0.40	3.10
W2	0.60	1.57
W3	0.80	1.77
W4	1.00	1.49
W5	1.20	2.61
W6	1.40	2.88

Table 3.5 - Total protein washed out in eADF4(C16) membranes

Membrane Type	Total Washed Protein (µg/mL)
eADF4(C16)	19.02 ± 4.52

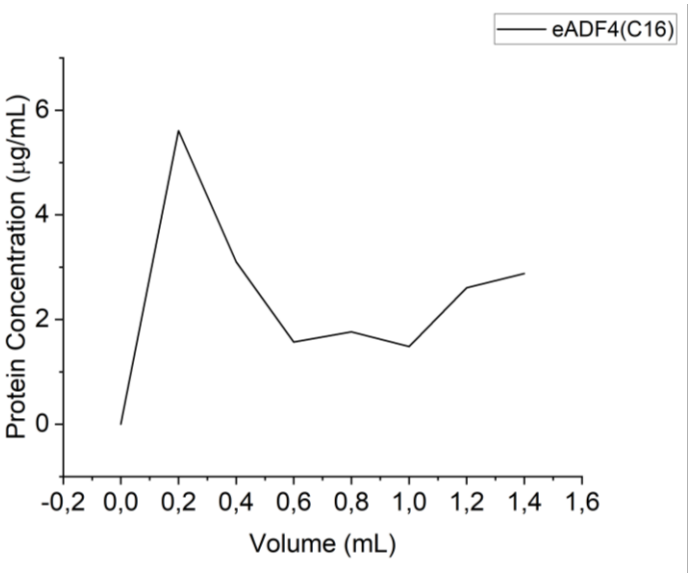

Figure 3.5 - Leaching protein assay for the eADF4(C16) membranes. Chromatogram of binding assay for the flow-through and washes steps representing total protein (µg/mL) in each fraction's volume.

According to the results in Figure 3.5, Table 3.4, and Table 3.5, it is observed that there is a protein present in the flow-through and washes fractions when we use a mild binding buffer. Moreover, at a volume 200 µL, the flow-through fraction, the concentration of total protein is the highest protein out at 5.61 ± 1.83 µg/mL. In addition, in the wash fractions, there is a significant concentration of total protein

present. To complete the quantitative analysis, a Tris-Tricine gel was performed to verify the type of proteins or protein fragments that were leaching from the spidroin membranes (Figure 3.6).

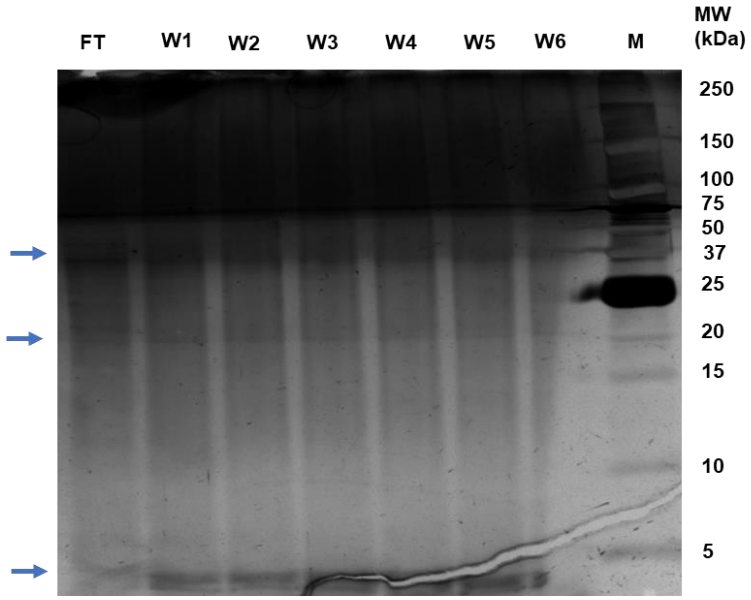

Figure 3.6 - Tricine-SDS-PAGE analysis for the incubation of eADF4(C16) membranes with HEPES 150 mM NaCl pH 7.4 binding buffer. FT – Flow-through sample, W1 – First wash sample, W2 – Second wash sample, W3 - Third wash sample, W4 – Fourth wash sample, W5 – Fifth wash sample, W6 – Sixth wash sample, M – Molecular marker.

In Figure 3.6, it is possible to observe that there are three main bands visible: 40 kDa, 20 kDa, and 5 kDa. By gel densitometry, it was possible to verify that the bands with the highest intensity are the band at 5 kDa, followed by the band at 40 kDa, and the band at 20 kDa. As was previously discussed, the eADF4(C16) protein has a molecular weight of 47.6 kDa. In a previous SDS-PAGE analysis of purified eADF4(C16) done in the PURE Project, it was verified that the fluorescence detected gel exhibited a clearer protein band and weaker degradation bands [82].

This work confirms that spidroin and spidroin fragments are leaching from the electrospun membranes. Thus, new methods to increase the mechanical robustness of the spidroin membranes are required before proceeding with conjugation and binding assays.

3.4 Final Remarks

In this preliminary proof-of-concept test, it was shown that spidroin membranes could be functionalized with heparin, here used as an affinity ligand for VLPs. We attempted a covalent functionalization, using free amines in spidroin as the anchoring points. The membranes made from eADF4(C16) spidroin, displaying only free amine terminals from the polypeptide chain, yielded the best heparin coupling results when compared with the immobilization values obtained for eADF4(κ16)-based

membranes. This may indicate that the self-assembly of the proteins is not the same, and the free amines from the lysine groups are not easily accessible for conjugation.

After successful heparin conjugation, we performed tests to assess VLP binding. It was observed that there is more protein washed out than in the initial HIV-1 Gag VLP loading solution (23.44 ± 8.04 µg/mL). This indicates that probably spidroin protein is leaching out. After doing a test with unmodified membranes in the binding buffer, we verified that there was protein coming out (19.02 ± 4.52 µg/mL of total protein). By comparing these results, it is suggested that in the VLPs binding assay, we have contamination with the spidroin from the membranes. Therefore, in the conditions demonstrated in this work, the membranes are not suitable for bioprocessing. The next steps should include a strong effort to optimize the mechanical robustness of the spidroin membranes to common chromatographic buffers.

Despite the results not being optimal for proof-of-concept, given that the PURE Project is an ongoing project and is a proof-of-concept itself, new approaches are currently being researched for optimizing both heparin functionalization and VLP binding to increase maximum ligand capacity and binding.

4. TECHNOLOGY MARKET TRANSFER PLAN

4.1 Background

The first challenge of a technology market transfer plan for a platform technology like PURE is to understand very well the technology, its attributes, and uniqueness and, based on that, to identify possible applications where the technology could fit market needs.

Throughout the years, multiple frameworks have been developed with the intent to identify, analyse, and solve problems to create value for society [83]. These frameworks are based on trade-offs, autonomous steps, and decisions that consider the business environments and ecosystems because it is in constant change and constantly mutate due to the dynamics of society [83]. There are several tools developed with this intent, e.g., Balanced Score Card [84], Business Model Canvas [85,86], Business Plans [87], Cooper Stage-Gate Model [88,89], Design Thinking [90], Lean Manufacturing [91], TQM [92], SERVQUAL [93], and Porter's 5 Forces Model [94]. However, a new tool has been developed that can be applied to numerous organizations to find new market solutions to answer a wide variety of problems. This framework is called Value Creation Wheel (VCW) [83,95].

The VCW is an extremely dynamic tool once the challenges, markets, and filters, are defined at the right time by the key stakeholders together (manager team, inventors of the technology, potential investors, etc.) and not imposed by the framework itself [95]. The VCW is composed of a theoretical framework, DIANA (Define, Increase, Assess, Narrow, Act), and a customizable tool that adjusts to the problem and context of implementation called TIAGO (Tap, Induce, Analyse, Ground, Operate) [83]. The VCW framework can be applied in various areas like astronomy, biotechnology, business, chemistry, design, energy, engineering, healthcare, and public policy making [83].

Overall, in a selection process of a technology application, the main purpose of this tool can be to narrow down several applications generated by applying the appropriate filters selected.

In this work, the Value Creation Wheel is used to explore application opportunities for the PURE Technology by addressing the potential applications that can arise from this technology in several industries considering both the inventors' and stakeholders' perspectives. The filters used for the selection of potential applications were market and technology-oriented filters. Then, with the inventors and stakeholders, the selected applications were classified and ranked according to their interests.

In this work, this exercise was achieved by conducting semi-structured interviews as the source for primary data collection. These interviews were done simultaneously with the ones previously described in Chapter 2 and addressed the inventors' and stakeholders' views on the technology's attributes, as well as their ideas and inputs regarding potential future exploitation avenues for this in-development technology. The latter is the focus of study in this Chapter. Secondary data collection was also pursued to complement the market strategy of this technology in the form of relevant and updated quantitative, obtained data in two different databases (Statista, Frost&Sullivan), and literature research to obtain information on the latest developments and the state-of-the-art of specific applications (Figure 4.1).

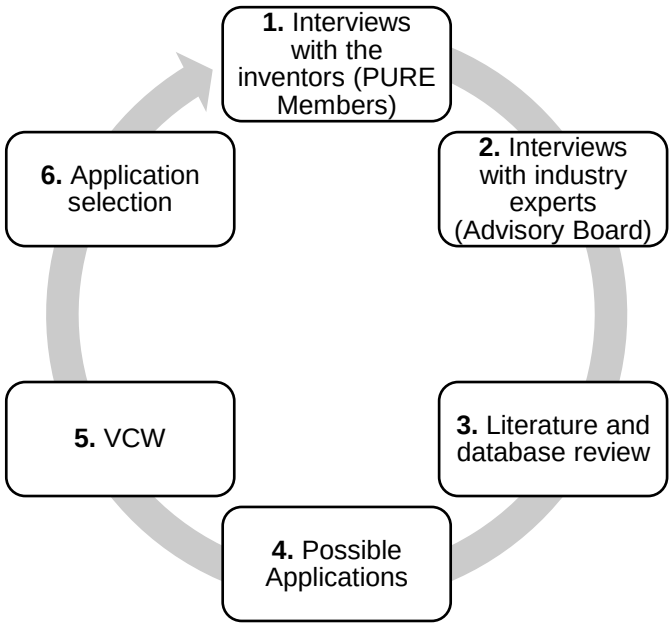

Figure 4.1 - Schematic representation of the workflow in the technology assessment

From the interviews with Pure Members, it was asked which industries would be interesting to explore this technology in and what suggestions of applications would be suitable for the technology being developed. Therefore, five industries were selected as the most relevant for exploitation, according to the opinions of the interviews: the biopharmaceutical industry, the biomedical industry, the cosmetic industry; the food industry; and the water filtration industry (Figure 4.2). Moreover, the Interviews with the Advisory Board Members also gave some insight into the possible applications of the PURE Technology and what would be the adequate market entry for this technology.

First, Advisory Board Member 1 adds that since precisely functionalized recombinant spider silk non-woven nanofibers are a novelty, PURE probably doesn't have much competition; therefore, an interesting starting point could be the engineering of tissue scaffolds. On the other hand, Advisory Board Member 2 suggests that purification of recombinant proteins and virus-like particles would be a great start due to high market need ("I would start certainly with recombinant proteins, and maybe move on to viral particles or other modalities primarily, because this is needed (e.g., COVID).”). Additionally,

according to this interviewee, monoclonal antibodies are the biggest and fastest-moving market, noting as well that gene therapy and cell therapy will require more time to grow.

Given all the data collected in the interviews, further data is also required to be collected. Therefore, we proceeded with research on potential applications from the five industries selected.

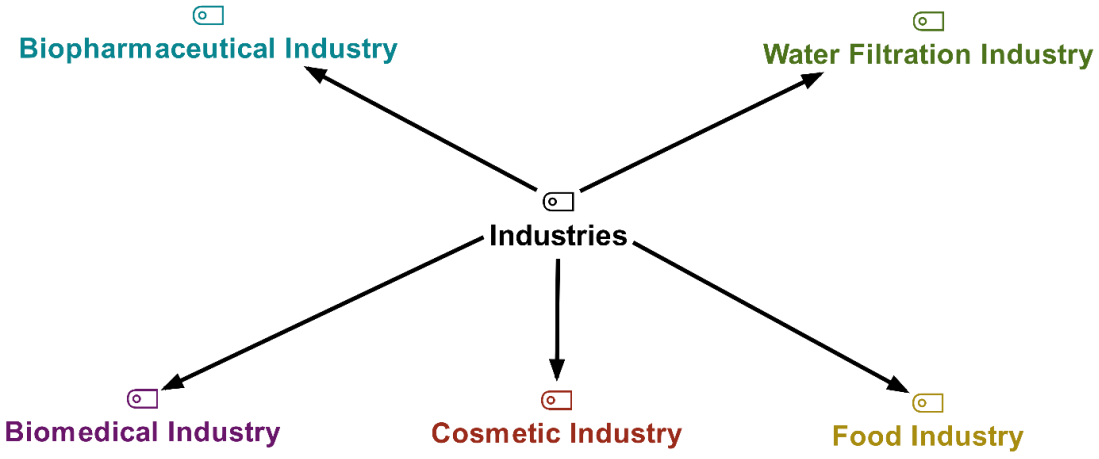

Figure 4.2 - Schematic representation of industries potential for development of applications for the PURE technology.

4.2 Brainstorming of Applications

As previously described before, the VCW methodology was used to select the most interesting application. The first step was the brainstorming of possible applications which included the applications mentioned by the Advisory Board members. In the next sections we discuss interesting areas of applications for PURE technology. The possible applications were divided into five industries that are briefly presented and discussed below: the biopharmaceutical industry, the biomedical industry, the cosmetic industry, the water filtration industry, and the food industry.

4.2.1 Biopharmaceutical Industry

In the biopharmaceutical industry, the possible applications are illustrated in Table 4.1.

Table 4.1 – Researched applications for the biopharmaceutical industry.

Researched applications
a) Precisely functionalized spidroin nanofibers as in vivo drug delivery systems.
b) Precisely functionalized spidroin non-woven nanofibers for bioseparation of Virus-like Particles.
c) Precisely functionalized spidroin non-woven nanofibers for bioseparation of Monoclonal Antibodies.
d) Precisely functionalized spidroin non-woven nanofibers for bioseparation of SPIKE Protein.

First, electrospun nanofibers have been used as drug delivery vehicles, where the drug delivery mechanism is based on the polymer degradation and diffusion of the biomolecules it carries within its nanostructures [96]. These nanofibers can be coupled with a vast number of therapeutic agents and have several surface coating formulas. With precisely functionalized non-woven nanofibers with ligands suitable for targeting these molecules, the efficiency of the coupling of these targets can be improved due to controlled ligand density, and therefore the drug loading is higher. Moreover, this material has biodegradable properties that are advantageous for drug delivery systems.

On the other hand, Virus-Like Particles are nanoscale structures composed of assembled viral proteins with non-infectious properties with the possibility of being produced in several organisms, ranging from mammals and insects to plants and bacteria [97]. They are used for a number of applications [98] such as medical, analytical, and scientific applications. Their market is growing due to an increase in interest in vaccines [98] and gene therapy [98].

However, most downstream processes for these types of biomolecules lack efficiency due to friction occurring in porous beads, given that the pore dimensions exclude viruses. Membrane chromatography circumvents this problem because it allows for higher volumetric throughput, leading to increased speed and productivity [99]. PURE's non-woven silk-inspired membrane adsorbents will allow for an ultrafast bioseparation process due to high surface area and high porosity, with pore size at a nanometre scale. Moreover, modification of the membrane surface is done before assembly, allowing for a controlled ligand density and, therefore, precise target-ligand coupling.

Another high-interest class of biopharmaceuticals is Monoclonal antibodies (mAbs), having their market valued at $146,642 million in 2020, and is projected to reach $390,582 million by 2030 [100]. These molecules derived from identical immune cells that can bind to a specific antigen when administered are used as a form of immunotherapy [101]. The main bioseparation process for these molecules is Protein A affinity chromatography. However, current methods are too costly, time-consuming, and require high consumable use. Alternative methods to resin-based columns have been widely researched to combine this well-established method that can improve efficiency, productivity, and recovery yields. PURE's combination of non-woven nanofibers with precise ligand functionalization can ensure, on one end, target-specific binding of the mAbs at predicted sites as well as developing membrane adsorbents with Protein A and new synthetic ligands for high-performance bioseparations.

4.2.2 Biomedical Industry

In the biomedical industry, the possible applications are illustrated in Table 4.2.

First, it has been reported that nanofiber membranes can capture a wide variety of contaminants and can qualify as high-efficiency air filters [102,103]. A surface modification confers electrospun nanofibers a specific function [103]. With the precise modification of non-woven nanofibers, it is possible to couple ligands for the targeting of a wide variety of pathogenic organisms to prevent infectious diseases. Moreover, this technique could enable the production of masks already

functionalized with biomolecules and retain the pathogenic particles and therefore protect the user from infection in high-risk situations.

Table 4.2 - Researched applications for the biomedical industry.

Researched Applications
e) Precisely functionalized spidroin for coating of surgical masks.
f) Precisely functionalized spidroin non-woven nanofibers for skin regeneration.
g) Precisely functionalized non-woven nanofibers for removal of uremic toxins in haemodialysis.
h) Precisely functionalized non-woven nanofibers for wound-healing in burn repair.

Another application is to address separation in haemodialysis. Haemodialysis eliminates the toxic metabolites, like uremic toxins, from the blood via diffusive or convective transport across the membrane. This process requires membranes to have high permeability and uniform pore distribution for greatest selectivity, and high blood compatibility [104]. Moreover, heparin has become a standard anticoagulant to prevent clotting haemodialysis therapy[105] due to its effectiveness, low toxicity and reduced costs [106]. However, heparin has been said to cause thrombocytopenia and high bleeding risk [106]. New approaches have been made to reduce the dose of heparin, like membranes capable of absorbing heparin. In this case, a new coating with spider-silk non-woven nanofibers would be developed to capture heparin precisely in haemodialysis membranes.

Another biomedical application for precisely functionalized non-woven nanofibers is wound dressing in burn repair. Wounds are defined as deformities in the skin upon injury, whether they are physical, thermal or from other origins [107]. Wound dressings are responsible for healing the wound by protecting it from external risk factors and enabling the control of wound infections [108]. Due to their structure and large surface area, natural nanofibers enable the successful loading and transporting of bioactive ingredients such as drugs and growth factors. The PURE technology can improve the loading of biomolecules in wound healing since it allows for the precise conjugation of biomolecules to specific ligands.

4.2.3 Water Filtration Industry

In the water filtration industry, the possible applications are illustrated in Table 4.3.

As described before, a non-woven functional biopolymer can improve the adsorption capacity of a process due to its high porosity and large area [104]. These membranes can be used as membrane chromatography adsorbents for the removal of several type of toxins or pollutants from water, like endotoxin, metal content, dyes or reduce the concentration of sodium chloride in saline water. This non-woven nanofiber when combined with a suitable affinity ligand may function as a water purification device.

Table 4.3 - Researched applications for the water filtration industry.

Researched Applications
i) Functionalized spidroin non-woven nanofibers to reduce endotoxin levels in water.
j) Functionalized spidroin non-woven nanofibers for removal of metal content in water.
k) Functionalized spidroin non-woven nanofibers for dye removal.
l) Functionalized spidroin non-woven nanofibers to produce potable water.

4.2.4 Cosmetic Industry

In the cosmetic industry, the possible application is illustrated in Table 4.4.

Table 4.4 - Researched applications for the cosmetic industry.

Researched Applications
m) Precisely functionalized spidroin non-woven sheet masks

In the cosmetic industry, there are several functional polymer-based materials used as the origin for product development, such as synthetic functional materials (e.g., metals, metal oxides, carbon based, ceramics) and natural based functional materials (e.g., fibrin-based, elastin-based, collagen based, silk-based). These functional materials can be coupled with affinity ligands for the development of sheet masks with antimicrobial properties.

4.2.5 Food Industry

In the food industry, the possible application is illustrated in Table 4.5.

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Table 4.5 - Researched applications for the food industry.

Researched Applications
n) Functionalized spidroin non-woven nanofibers for antimicrobial protection in packaging
o) Precisely functionalized nanofibers as food quality sensors to detect pH range in meat-based products

Nanofibers have been reportedly used as food packaging applications to encapsulate bioactives to the polymers surface, for antioxidant properties [109]. Moreover, nanofibers could function as pH detectors when coupled with specific ligands [109].

4.3 Application selection

Table 4.6 summarizes the applications discussed in section 4.1. above. The biomedical, biopharmaceutical and water filtration industries generated a greater number of applications as this technology has a greater potential innovation in liquid-liquid separations.

Table 4.6 - Applications for the PURE technology divided by industry.

Application	Industry
a) Precisely functionalized spidroin nanofibers as *in vivo* drug delivery systems	**Biopharmaceutical**
b) Precisely functionalized spidroin non-woven nanofibers for bioseparation of Virus-like Particles	
c) Precisely functionalized spidroin non-woven nanofibers for bioseparation of Monoclonal Antibodies	
d) Precisely functionalized spidroin non-woven nanofibers for bioseparation of SPIKE Protein	
e) Precisely functionalized spidroin for coating of surgical masks	**Biomedical**
f) Precisely functionalized spidroin non-woven nanofibers for skin regeneration	
g) Precisely functionalized non-woven nanofibers for removal of uremic toxins in haemodialysis	
h) Precisely functionalized non-woven nanofibers for wound-healing in burn repair	
i) Functionalized spidroin non-woven nanofibers to reduce endotoxin levels in water	**Water Filtration**
j) Functionalized spidroin non-woven nanofibers for removal of metal content in water	
k) Functionalized spidroin non-woven nanofibers for dye removal	
l) Precisely functionalized non-woven nanofibers with cell-binding peptides for medical implants	
m) Precisely functionalized spidroin non-woven sheet masks	**Cosmetic**
n) Functionalized spidroin non-woven nanofibers for antimicrobial protection in packaging	**Food**
o) Precisely functionalized nanofibers as food quality sensors to detect pH range in meat-based products	

According to the VCW framework, after the brainstorming of possible applications, it is necessary to select technology and market criteria that are going to be used to choose the most interesting application. Examples of technology related criteria are proof-of-concept, intellectual property protection, innovation, regulation, among others. Examples of market related criteria are market dimension and growth potential, market need, among others. This process was done with PURE's coordination team whose choice were innovation and market need as the most important criteria to select a preferred application for the technology.

Innovation refers to the use of a new idea or method that was not thought of yet, but it does not imply that it is useful for a specific target customer. On the other hand, market need refers to the need or utility of a product or service to a specific customer.

All applications in section 4.1, were subject to classification in a scale from 0-3, according to their level of innovation (0 – no innovation; 1- low innovation; 2 – medium degree of innovation; 3 – degree of innovation) and market need (0 – no market need; 1 – low market need; 2 – medium market need; high market need).

According to Table 4.7, it is possible to categorize each group of application given the total classification attributed.

Table 4.7 - Classification of each application according to its innovation and market need.

	Application	Innovation	Market Need	Total
a)	Precisely functionalized spidroin nanofibers as *in vivo* drug delivery systems	**3**	**2**	**5**
b)	Precisely functionalized spidroin non-woven nanofibers for bioseparation of Virus-like Particles	**3**	**3**	**6**
c)	Precisely functionalized spidroin non-woven nanofibers for bioseparation of Monoclonal Antibodies	**3**	**3**	**6**
d)	Precisely functionalized spidroin non-woven nanofibers for bioseparation of SPIKE Protein	**3**	**2**	**5**
e)	Precisely functionalized spidroin for coating of surgical masks	**2**	**2**	**4**
f)	Precisely functionalized spidroin non-woven nanofibers for skin regeneration	**2**	**2**	**4**
g)	Precisely functionalized non-woven nanofibers for removal of uremic toxins in haemodialysis	**2**	**1**	**3**
h)	Precisely functionalized non-woven nanofibers for wound-healing in burn repair	**2**	**1**	**3**
i)	Functionalized spidroin non-woven nanofibers to reduce endotoxin levels in water	**2**	**1**	**3**
j)	Functionalized spidroin non-woven nanofibers for removal of metal content in water	**2**	**1**	**3**
k)	Functionalized spidroin non-woven nanofibers for dye removal	**2**	**1**	**3**
l)	Precisely functionalized non-woven nanofibers with cell-binding peptides for medical implants.	**1**	**1**	**2**
m)	Precisely functionalized spidroin non-woven sheet masks	**1**	**1**	**2**
n)	Functionalized spidroin non-woven nanofibers for antimicrobial protection in packaging	**1**	**1**	**2**
o)	Precisely functionalized nanofibers as food quality sensors to detect pH range in meat-based products	**1**	**1**	**2**

In addition, Table 4.8 below organizes the applications according to this classification. Moreover, we can identify five different categories according to the total score given: applications with low innovation and low need in the market (total score of 2); applications with some level of innovation but with a low need in the market (total score of 3); applications with some level of innovation and a moderate market need (total score of 4); applications with a high level of innovation and a moderate market need (total score of 5); and, applications with both a high level of innovation and a high need in the market (total score of 6).

Table 4.8 – Applications according to classification and score.

Classification	Score	Application (s)
Applications with low innovation and reduced need in the market	**2**	**l)** Precisely functionalized non-woven nanofibers with cell-binding peptides for medical implants **m)** Precisely functionalized spidroin non-woven sheet masks **n)** Functionalized spidroin non-woven nanofibers for antimicrobial protection in packaging **o)** Precisely functionalized nanofibers as food quality sensors to detect pH range in meat-based products
Applications with moderate innovation and reduced need in the market	**3**	**g)** Precisely functionalized non-woven nanofibers for removal of uremic toxins in haemodialysis **h)** Precisely functionalized non-woven nanofibers for wound-healing in burn repair **i)** Functionalized spidroin non-woven nanofibers to reduce endotoxin levels in water **j)** Functionalized spidroin non-woven nanofibers for removal of metal content in water **k)** Functionalized spidroin non-woven nanofibers for dye removal
Applications with moderate innovation and moderate need in the market	**4**	**e)** Precisely functionalized spidroin for coating of surgical masks **f)** Precisely functionalized spidroin non-woven nanofibers for skin regeneration
Applications with high innovation and moderate need in the market	**5**	**a)** Precisely functionalized spidroin nanofibers as *in vivo* drug delivery systems **d)** Precisely functionalized spidroin non-woven nanofibers for bioseparation of SPIKE Protein
Applications with high innovation and high need in the market	**6**	**b)** Precisely functionalized spidroin non-woven nanofibers for bioseparation of virus-like particles **c)** Precisely functionalized spidroin non-woven nanofibers for bioseparation of monoclonal antibodies

The first group of applications (Applications with low innovation and reduced need in the market) comprises four applications in areas where there are several applications like them, and therefore the need in the market is low. For example, in the case of sheet masks, there are a high number of companies that already develop spider-silk inspired products which significantly reduces the market need and level of innovation.

Next, the second group of applications comprehend applications with moderate innovation and reduced need in the market. These applications represent a moderate innovation level because, on one hand, four of them - **g)**, **i)**, **j)**, **k)** - introduce this material for the first time into liquid-liquid separation. On the other hand, application **h)** is also innovative because combines this technology with burn repair, which is a novelty as well. In terms of market need, all these applications are in extremely competitive markets with no demand for new applications.

The third group of applications is categorized as applications with moderate innovation and moderate need in the market because they introduce novelty in the biomedical industry to respond to skin regeneration and to microbial air transmissions in high-risk situations. In the latter situation, there is need in the market due to the COVID-19 pandemic increasing the need for development of new materials for protection to viral infections. However, these applications are not considered high innovative opportunities because the markets they act on are already well developed and with other technologies currently with higher demand.

The fourth category refers to applications that are of high innovation and with moderate market need. The two applications in these group belong to the biomedical and biopharmaceutical industries, and present innovative opportunities for these markets. However, previous studies have already demonstrated the potential for spider silk particles as drug delivery vehicles [110], which can be a bottleneck to introduce this technology into the market. Moreover, regarding using this technology for bioseparation of SPIKE protein of SARS-Cov2 is an extremely innovative idea, however the need in the market is affected by great research and development of other alternatives for these particles increasing the competition potential. In addition, the membrane produced would be too specific and wouldn't be compatible with other biopharmaceutical classes.

Finally, the last group comprises the selected applications because they scored the highest, being the most innovative and with the highest market need. This is due to the strong necessity of creating new alternatives to downstream processing for monoclonal antibodies and virus-like particles. On one hand, monoclonal antibodies are a continuous market of interest, due to being in the top biopharmaceutical drugs sold worldwide. On the other hand, virus-like particles are a growing market and its bioseparation process is met with extreme difficulties and with very few cost-effective alternatives. Therefore, in the next chapter a market analysis will be performed of these two applications selected to justify this conclusion.

4.4 Market Analysis

As described in Chapter 1, PURE focus on increasing the productivity of bioseparation processes in the biopharmaceutical industry. The COVID-19 pandemic has had a negative impact on many businesses, and the chromatography sector is no exception. Bioprocessing is the most expensive and time-consuming step in biopharmaceutical manufacturing. These types of systems are employed in the separation of biological products such as biochemicals, biopharmaceuticals, among others. Some examples of these systems are membrane/filters, chromatography, and centrifuges. The most used

system employed in the separation of biological products is chromatography. The chromatography market is projected to reach US$ 15,3 billion by the end of 2030 [111].

Supply chain and manufacturing are greatly affected by an increased demand in biomanufacturing techniques, where companies have difficulty in developing and delivering their product on time for end-users. Therefore, this market is facing a period of short-term negative growth, which can be attributed to factors such as a decline in the product demand from major end-users, limited operation capacity, lack of funding to research and academic institutes, and disrupted supply chain [112].

Many pharmaceutical and biopharmaceutical companies are focusing on new drug development to combat the pandemic, this has significantly increased the use of chromatographic materials [112].

In addition to the biopharmaceuticals targeted in PURE, non-woven nanofibers can be applied in the purification of other relevant biological products by tuning the affinity ligand coupled. The expected high productivity of the spidroin nanofiber bioseparation will contribute to reduce the environmental footprint of the manufacturing process. Considering the increasing trend on the use of single use and disposable components in bioprocessing, the biobased nature and biodegradability of the nanofibers is also considered favourable from an environmental perspective.

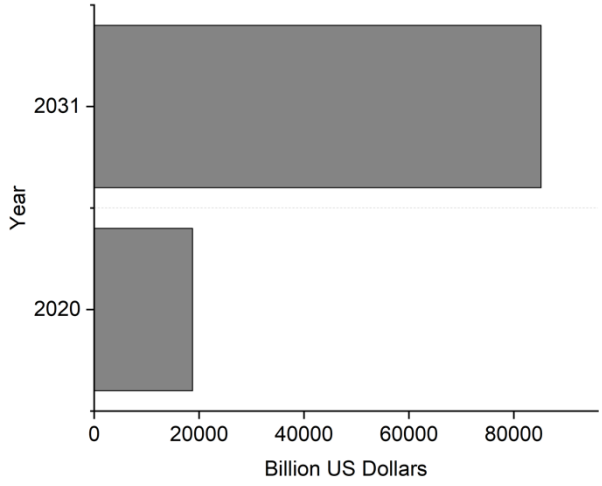

Figure 4.3 - Global next-generation biomanufacturing market size in 2020 and 2031 (in million U.S. dollars) [113].

The global market for Biopharmaceutical Bioseparation Systems estimated at US$ 18,8 billion in the year 2020, is projected to reach a size of US$85,2 Billion by 2030 which is surprisingly more than 4 times bigger than in 2020 (Figure 4.3). The next section is going to focus on the global chromatography market given that it is the most used technique in bioprocessing.

4.4.1 Global Chromatography Market

Chromatographic devices are used in several industries such as: oil and gas; food and beverage; biotech and pharmaceutical industries; and as research and academic institutions. The global

chromatography instruments market has been categorized based on four major regional segments (North America, Europe, Asia Pacific, and the Rest of the World) (Table 4.9). Asia Pacific is the region expected to grow at the highest, influenced mainly by environmental protection activities and strategic expansions by key players in China, growth in biomedical and medical research and an increase in awareness about chromatography in Japan, and government initiatives supporting the growth of the pharmaceutical industry in India [112]. Moreover, the current outbreak of COVID-19 in the region has resulted in an increased patient pool, leading to subsequent surge in research and developing activities.

Table 4.9 - Segmentation of the chromatographic market by geography [111].

Region	Country/Group of Countries
North America	United States
	Canada
Europe	Germany
	United Kingdom
	France
	Italy
	Spain
	Eastern Europe
Asia-Pacific	CIS
	China
	Japan
	India
	Australia
	Others
Middle-East and Africa	GCC
	South Africa
	Rest of Middle East and Africa

4.4.2 Membrane Chromatography Market

This high demand for biopharmaceutical drugs has presented huge opportunity for manufacturers. The increase in demand for vaccine and treatment drugs for COVID-19, for example, the pharmaceutical and biotechnology industry is expected to witness a lucrative growth in the future [112]. Membrane chromatography is a technique needed in research and development of new drugs as well as in the production of them. The membrane chromatography market can be divided into various segments, such as by technique, operation mode, end-user, and key market players (Table 4.10).

Table 4.10 - Segmentation of the membrane chromatography market by technique, operation mode, end user, region. Key market players are also identified [114].

Segments	Sub-segments
By Technique	Ion Exchange Membrane Chromatography
	Anion Exchange Membrane Chromatography
	Cation Exchange Membrane Chromatography
	Affinity Membrane Chromatography
	Hydrophobic Interaction Membrane Chromatography
By Operation Mode	Flow-through Membrane Chromatography
	Bind-elute Membrane Chromatography
By End User	CROs
	Pharmaceutical and Biopharmaceutical Companies
	Academic and Research Institutes
By Region	North America (U.S., Canada, Mexico)
	Europe (Germany, France, UK, Italy, Spain, Rest of Europe)
	Asia-Pacific (Japan, China, India, Australia, South Korea, Rest of Asia-Pacific)
	LAMEA (Brazil, South Africa, Saudi Arabia, Rest of LAMEA)
Key Market Players	Danaher Corporation, Sartorius AG, Merck Millipore, Purilogics, Thermo Fisher Scientific, 3M Company, Asahi Kasei Corporation, Membrane Solutions LLC, Cole-Parmer Instrument Company, GVS Group

4.4.3 Competitors in Membrane Chromatography

The main players are Danaher Corporation, Sartorius AG, Merck Millipore, Purilogics, Thermo Fisher Scientific, 3M Company, Asahi Kasei Corporation, Membrane Solutions LLC, Cole-Parmer Instrument Company, GVS Group. However, the key market players identified as direct competitors of the PURE Technology are three membrane adsorbent devices derived from biological sources for the bioseparation of monoclonal antibodies (Table 4.10). These devices are from two different players: Cytiva (*HiTrap™ Fibro PrismA* and *HiScreen™ Fibro Prism A*) and Sartorious (*Sartobind® Rapid A*).

A few examples of the competitors of this technology are in Table 4.11. All the materials described in are derivatized from cellulose, where *HiTrap™ Fibro PrismA and HiScreen™ Fibro PrismA* are made from stabilized reinforced cellulose. However, despite cellulose fibers having a great potential in pharmaceutical applications [115], controlled ligand functionalization of these fibres has yet to be proven successful. Therefore, PURE presents a better opportunity since functionalization is done upon the fibre assembly.

Moreover, the examples of competitors show, even though there are few technologies, that there are several more opportunities and options for the bioseparation of monoclonal antibodies. Thus, PURE allows for the creation of more opportunities for the bioseparation of other targets, especially virus-like particles.

Table 4.11 - Main competitors in membrane adsorbents for capture of mAbs and VLPs.

	PURE	**HiTrap™ Fibro PrismA**	**HiScreen™ Fibro Prism A**	**Sartobind® Rapid A** [116–119]
Material	Recombinant spidroin non-woven nanofibers	Derivatized eletrospun cellulose fibers	Derivatized electrospun cellulose fibers	Stabilized reinforced cellulose
Targets	Monoclonal Antibodies Virus-like particles SPIKE Protein	Monoclonal antibodies	Monoclonal antibodies	Monoclonal antibodies
Ligands	Protein A Heparin Synthetic ligands	Protein A from E. coli	Protein A from E. coli	Protein A from E. coli
Recovery yield (%)	>90	≈90	≈90	≈99
Binding Capacity (mg/mL)	>10	30	30	30-58
Adsorbent Re-use (cycles)	>50	200	200	>40
Ligand Coupling	Single point attachment at predicted site	Single Point Attachment	Single Point Attachment	Covalent bond

4.5 Final Remarks

After the literature search and the conduction of the semi-structured interviews with both stakeholders and the inventors, several applications in five distinct industries were identified (biopharmaceutical, biomedical, cosmetic, water filtration and food industries). Through the framework Value Creation-Wheel, it was possible to narrow down the selection of an application that responded to needs and wants of both the stakeholders and the inventors. Therefore, the most important factors were innovation and market need. Of the applications, only two of them had significant market need and

innovation: precisely functionalized non-woven nanofibers for the bioseparation of monoclonal antibodies and precisely functionalized non-woven nanofibers for the bioseparation of virus-like particles.

PURE intends to develop a new sustainable technology for the bioseparation of biopharmaceuticals with a high innovation to be introduced in the Market for Biopharmaceutical Bioseparation Systems estimated to reach a size of US$ 85,2 Billion by 2030 which is surprisingly more than 4 times bigger than in 2020, and more specifically, competing with chromatography companies whose market is projected to reach US$ 15,3 billion by the end of 2030 [111]. However, there are more competitors in the market in the bioseparation of monoclonal antibodies than for the bioseparation of virus-like particles. Therefore, this last application is the best entry in the market for the PURE Technology.

In the next chapter, a business model proposal is going to be discussed.

5. BUSINESS MODEL

5.1 Background

Nowadays, universities and scientific institutes are considered significant economic drivers in the development and transfer of knowledge to the commercial market place, as well as great contributors to policy making and change in the Europe [120]. In particular, the European Union has various research and innovation programs (e.g., Horizon 2020, FET OPEN) that incentivize the cooperation between science and technology to solve numerous social problems, which has greatly diminished European innovation in comparison to the United States and Asia. Therefore, an increased effort has been made to develop innovative technologies and commercialize them as products, services, and/or products. Hence, this process of transferring academic knowledge to a commercialization stage is called technology transfer.

Technology transfer in academia-to-market usually happens in two different ways: through licensing patented intellectual property to corporations (e.g., small medium enterprises (SMEs), large companies) or the creation of a start-up company, which also licenses the technology developed. The most suited alternative depends on several factors that are discussed in the next session on business models.

This section aims to lay out potential business models to further adopt when the PURE Project reaches the commercialization stage. In the case of the PURE Technology, since it is a proof-of-concept and there isn't a finished product the designing of a business model is a much more complicated effort.

Several possible applications of PURE technology were previously discussed, and the most suited business model will depend on the type of application that is chosen. In the next sections, we will address business models from a theoretical perspective, as well as applied to PURE project possible applications.

5.2 Business Model Definition

A business model is defined as an outline for how a company or enterprise can profit from their products or services. Moreover, it also helps to define what types of profits or services a company will sell and how it intends to market that product or service. In addition, it also helps to preview what kind of expenses the company will face.

In the case of commercialization of a technology, intellectual property is a key element and an instrument to help assure the ownership over findings develop by a certain individual or organization as said by World Intellectual Property Organization (WIPO). Moreover, the main legal mechanisms for protecting intellectual property are patents, trademarks and copyright [121]. The legal protection of the research is essential to commercialization of a technology and to design a business model.

To design a Business Model, we need to consider three fundamental steps: (i) value proposition, where key attributes of the technology are defined; (ii) value capturing, where a strategy for attracting investment; (iii) the value delivery, where return of investment and profitability are estimated. (Figure 5.1).

In this chapter, two different visions (start-up creation and technology licensing) are going to be discussed and, according to the technology developed, one of these strategies is going to be adopted for future commercialization.

Figure 5.1 - Schematic representation of business model strategy.

5.3 Value Proposition of PURE technology

Non-woven and fibrous materials have a big advantage because of convective properties which enable them to be very fast. Therefore, this enables the creation of a high-performance system, which will allow for a faster flow. Figure 5.2 outlines the problem, attributes, and solution that PURE Technology presents.

In the case of PURE, the key unique attributes of the technology are high performance, increased selectivity, reduced environmental footprint and versatility of the technology which will result in a better flow; therefore, a system is created where biomolecules are processed more efficiently and in a shorter time.

Moreover, PURE technology will allow for process intensification where there is a smaller system which is capable of processing more material. Since this is a smaller system, less volumes are required for the purification processes, thus there is a higher environmental footprint due to water reduction.

As it was demonstrated before this technology can have several applications in multiple industries, which can open the door for the development of the exploitation of this technology through several markets.

Finally, this technology has never been introduced in the biopharmaceutical industry, representing high innovation for combining several areas into one Project to develop a new technology.

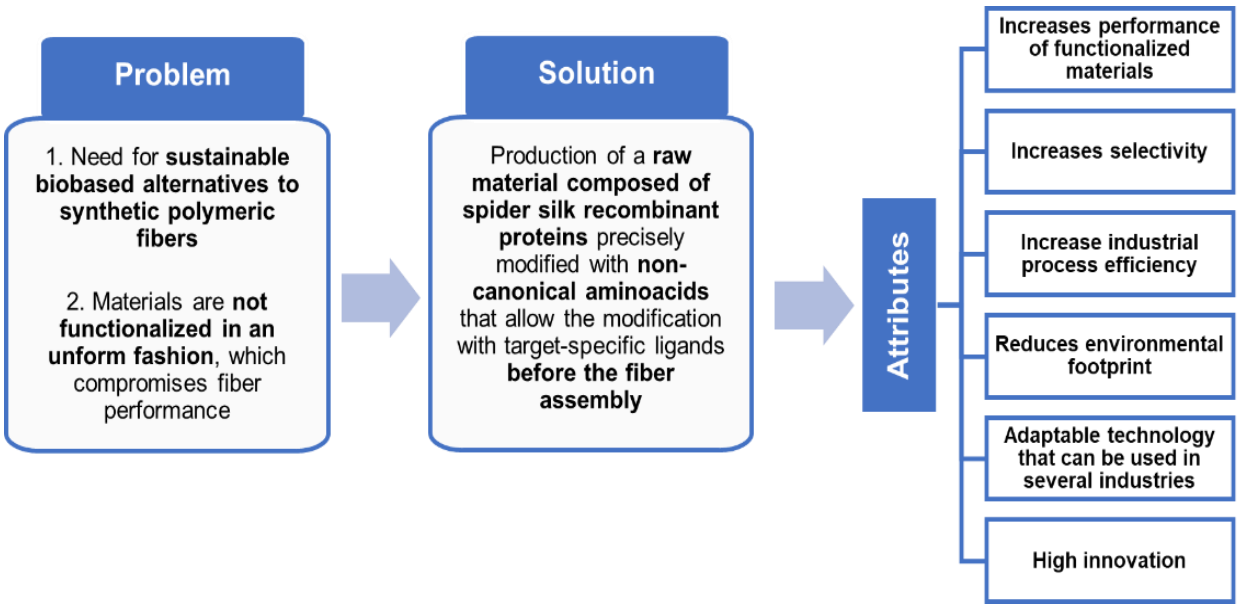

Figure 5.2 - Problem, solution, and attributes of the PURE Technology.

5.4 Value Capturing of PURE technology

The next step in the business model strategy of PURE is to define how value is going to be captured to attract investment and to introduce this innovative technology into the market. The intellectual property rights (IPR) strategy is essential to protect an invention and capture the value created by the technology making it more difficult to copy the innovation involved.

The IPR strategy it is a key choice for development of a business model, since attracts external investment as well as may be responsible for a successful commercialization and future profitability.

Note that external funding is fundamental as life science products generally involve significant costs of development, since these industries are highly regulated, and time-to-market is high. In addition, marketing and distribution costs are also large for these types of products [122].

Usually, universities are not in position of commercializing technologies themselves. Therefore technology licencing can be an alternative to put a technology to use [123].

5.5 Value Delivering of PURE technology

The value created by PURE technology and captured through patenting the innovations with the development of the technology as it is being done by PURE can be delivered through technology licensing, selling the patent or through the creation of a start-up which will sell the product or do a purification service.

5.6 Licensing or Creating a Start-up from PURE Technology: A discussion

5.6.1 Technology Licensing

According to the WIPO, successful technology licencing is based on involves six main principles [124]. First, it occurs solely when one of the parties owns Intellectual Property (IP), and because of that it has legal right to prevent others from reproducing it. Secondly, there are three types of technology licencing: licenses for a specific patent or work of authorship of a technology; licenses for all aspects and components of that technology; or licenses to create and market a product to comply to a technical standard. Third, technology licensing is dependent of the business relationship previously established, where manufacturing and marketing strategies must be previously agreed. The fourth principle implies that even though all parties have different interests, all must coincide to reach an agreement. Fifth, businesses objectives must be clearly defined before an agreement is reached. Finally, the sixth principle relates to the fact that technology licencing on its own is not technology transfer, meaning that for successful technology transfer the inventors deliver the technology and knowledge to the licensing partner for them to learn how to adapt, effectively use and build on the knowledge transferred.

5.6.2 Start-up Creation

An alternative business model is to sell a product or service based on PURE Technology through a new start-up.

Licencing a technology may also be used as way to create an exit for a business, if it becomes clear that the business cannot fund the marketing, sales, and distribution of the product from existing resources and additional financing is not available [125].

If this new start-up manages to raise large amounts of funding or loan and to sign up a contract with potential customers that could impact in a positive cash flow that warranties its survival and growth the profitability will be even higher than in the licencing case.

5.6.3 Discussion: Licencing versus Start-up creation

Interviews were conducted with Advisory Board members of PURE Project to understand their perspective on the best business model strategy for PURE (see Appendix). Interestingly, two different approaches were discussed.

On one hand, Advisory Board Member 1 who has mainly academic and spin-off experience states that Small Medium Enterprises (SMEs) or a start-up is a good option for the exploitation of the PURE Technology, once there is a different risk assessment between an SME and big companies, given that SMEs as they are smaller companies, they can afford to take more risks.

Therefore, SMEs adapt technology transfer to support their needs, address obstacles and challenges, acquire and develop technologies and access new research that they can take forward, which allows for to form alliances with fellow companies and research institutes to produce innovations, reduce financial risks or share technologies [126].

On the other hand, Advisory Board Member 2, who is a member of a large tool provider and has vast experience with bioprocessing in the biopharmaceutical industry, outlined the typical value chain for a bioseparation system (Figure 5.3).

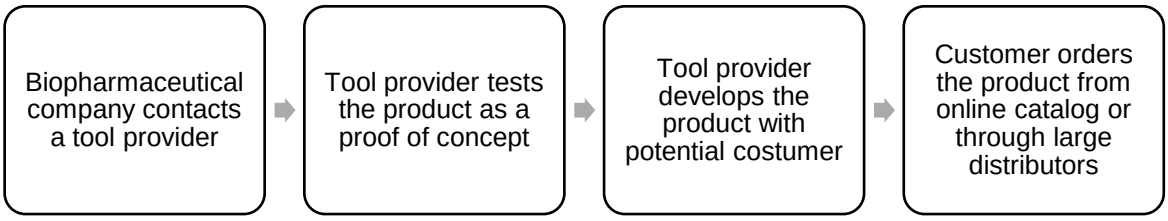

Figure 5.3 - Value chain in the biopharmaceutical industry.

In this case, the biopharmaceutical companies (e.g., Amgen Inc., Abbvie Inc., Bristol-Myers Squibb Company, Eli Lilly and Company, Norvo Nordisk) may develop new products that require different downstream process methods; therefore, they must partner with a tool provider (e.g., 3M, Sartorius, Pall Corporation, GE Healthcare Life Sciences, Thermo Fisher Scientific), which is then responsible for testing the product as proof-of-concept. Therefore, if the costumer agrees with the plan developed by the tool provider, then the two companies develop the product together. Finally, the customer orders the product from an online catalogue or through large distributors.

In addition, both interviewees state that large companies are very resistant to radical change ("Big companies are very resistant to radical changes because if it doesn't work, they have a real problem as the cost is too high."). Moreover, other concerns to industry are the scaling-up, meaning that the process being developed needs to be adaptable into a large production scale, and in compliance with Good Manufacturing Practices (GMP).

5.7 Final Remarks

In the case of PURE, since the biopharmaceutical application is the primary chosen one, and as this industry have established players, the processes take a long time to be implemented and used by the biopharmaceutical companies ("A drug manufacturer has to be sure that this product will be made exactly the same way for the next 25 years", Advisory Board Member 2), a start-up might be a greater risk.

For the drug manufacturers, a SME or start-up will likely not have the necessary means for the necessary development of the product to comply with the clients' high demands. However, if the

technology is licensed and the IP sold to a tool provider, it may reduce the risk because a tool provider with higher capital and scalability capacity is able to develop the product with the client. Finally, since this is a versatile technology that can have multiple applications in other industries, a start-up might be an option that should not be discharged and could even be a preferred alternative according to the characteristics of other entry markets for a product or service that comes from PURE Technology.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

The aim of this thesis is threefold: to understand the collaborative network involved in PURE FET-OPEN Project, to study the development of the technology and do laboratory proof-of-concept work for a preferred application and to analyse the technology market transfer alternatives for the exploitation of the PURE Technology.

First, the study begun with the composition the PURE Project, more specifically its background and its organizational structure. These two elements are fundamental to execute a comprehensive study on the production of knowledge in the PURE Project. Therefore, the parameters involved upon the collaboration between partners with the goal of constructing a framework for collaboration in transdisciplinary projects were defined. This approach was done through a qualitative empirical study of several topics (conduction of semi-structured interviews), such as background of the actors involved, the methodology of research, transdisciplinarity, and trust.

Second, a particular application of the PURE technology was studied: heparin-functionalized spidroin non-woven nanofibers for purification of recombinant VLPs. These experimental results had the purpose of testing the technology at a fundamental stage for verification of feasibility and to verify what aspects work and don't work.

Finally, the third approach was a technology market transfer plan and business model strategy based on qualitative data obtained from the semi-structured interviews conducted with project members, and industry and academic stakeholders' part of the Advisory Board.

The production of knowledge in this Project is founded on the principal aim of the Project: enhancing the productivity, reduce environmental impact and reduce time-to-market of biopharmaceutical manufacturing. As evidenced by the results of the semi-structured interviews, most actors in the project consider that PURE is application oriented. However, they view fundamental research as key at this stage of the project since the technological innovation level is extremely high and has never been achieved before. Moreover, this fact coincides with Gibbons' Mode 2 of Knowledge Production Theory, which states that for application-oriented collaborations, there is a fusion of methodologies and an integration of different backgrounds, which is mirrored in this project by the integration of industry practices in academic research.

A collaborative framework model was also explored in this project. From the empirical evidence described before it is possible to conclude that there are three pillars for collaboration. First, transdisciplinarity is the characteristic that defines the Project and its core foundation, since the makeup of this network is multidisciplinary and incorporates three academic institutes, a SME and a peer-review

body with industry and academic experts. Second, communication and trust are key in this Project and constitute a significant part of collaboration, which is achieved via regular meetings and through respect between partners' reputation and expertise. Finally, industry collaboration and knowledge exchange are established in the form of international conferences, publications, and industry meetings.

Experimental verification of a technology is key for a technology market transfer strategy. However, the experimental tests conducted in this work did show that this technology is still at a very preliminary stage and several key parameters need to be adjusted. First, in the heparin immobilization studies, the percentage of functionalization for the eADF4(κ16) was significantly lower than the control eADF4(C16), which shows a deficiency in the covalent immobilization of heparin in the membrane surface. This can be due to the amine surfaces not having enough free amines in its composition, and/or heparin is being bound to the N-terminal in the eADF4(C16) membranes.

Therefore, a new strategy must be investigated to estimate the amount of free amine groups in membrane the membrane surface, as well new immobilization techniques might also be explored. Since the immobilization in the eADF4(C16) membranes was the most effective, the VLP binding tests were performed using solely this type of membranes. The results show that there was no VLP binding to the membrane surface and that more protein was being washed out during the assays. Therefore, we tested if the membranes were leaching protein. From the results obtained we can conclude that spidroin protein fragments were washed because in the binding buffer assay, both quantitative and gel analysis confirm that spidroin is being washed out under the conditions employed in these assays. Overall, new procedures need to be studied, such as new buffer conditions, to test the binding of VLPs in the surface of the membranes.

The PURE technology has the purpose of enhancing the capture of biomolecules through the precise ligand functionalization of spidroin non-woven nanofibers with the objective of increasing process productivity and reducing time-to-market. From the results, several applications were investigated; however, due to an increased research and development of new biopharmaceuticals, such as vaccines, gene therapy vectors, the growing reliance of monoclonal antibodies, and the novelty of this application, only the applications towards the purification of VLPs and mAbs were considered at this stage. Furthermore, the bioseparation market for antibodies is a very established market, with numerous applications already in the market with very big players dominating the market. In the case of VLPs, there are few affinity membrane chromatography technologies, with most biopharmaceutical companies still relying on resin-based chromatography for the downstream process. Thus, VLPs are a viable entry in the market.

Regarding the application chosen a business model was studied. Considering the value proposition, this technology will be an asset to reduce the costs related to biopharmaceutical manufacturing, once it decreases consumable use, water use, and increases productivity once more product is captured by the ligands attached to the membranes. However, to create revenue, it is necessary to patent this technology. When it comes to value delivering strategy, there are two ways this technology can reach the market: either from licensing the technology and selling the intellectual property to a larger company or creating a start-up to sell the product directly to biopharmaceutical

companies. Since the biopharmaceutical industry is dominated by larger companies, technology licensing is the ideal strategy for this application. However, for other applications the creation of a start-up might be a more interesting strategy depending on the industry.

To conclude, PURE is an extremely innovative application that has the potential to have an impact on the market and industry in general. However, it is still a technology in development and a lot of other factors need to be further studied to successfully transfer this technology to the market.

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A. INTERVIEW INFORMATION

A.1 PURE Member Interviews Script

I. Prior Knowledge

1. What is your role within your organization? In what institutions have you worked previously?

2. How many projects have you coordinated/been part of? Which positive and negative lessons have you taken from those experiences?

3. Can you describe your role and tasks within the consortium? Do you think you are playing other roles inside the project?

II. Knowledge Production and Transference

1. In your experience, have you focused more on applied or fundamental research?

2. In your point of view, for a project like PURE that has the main goal of application, do you think the way of producing knowledge should follow both the applied research path and fundamental research path? Are there important methods in fundamental research that also need to integrate the research development in PURE?

3. What specifically do you believe should be explored in PURE beyond the commercialization of the research being developed? Can you list other exploitation avenues that you would like to be pursued and possibly rank them from 1 (irrelevant) to 5 (most relevant)?

4. What are the main drivers in the process of knowledge transference between industry and academia in PURE? Can you give concrete examples?

III. Transdisciplinarity

1. In PURE, how does the integration of members from different scientific areas contributes to:

   a) better science to cope with complex challenges
   b) more opportunity to innovate
   c) creating barriers to define a research path
   d) deepening the research path

2. Which factors play an important role in the process of communication between people from distinct scientific areas?

IV. Trust

1. What makes you trust in your colleagues from industry/academia when sharing knowledge?

2. What suggestions do you have to enhance trust among PURE members and advisory board?

V. Value Proposition

1. What attribute of the technology has a more competitive advantage?

2. In PURE, a Comparative Life Cycle Assessment will be performed to evaluate the environmental impact of the process developed by comparing it with current chromatographic methods. What are your expectations regarding the environmental impact of the process being developed and has the project produced any data so far that can sustain your view?

3. What has the project done to prove the scalability of this technology?

4. What other concerns/bottlenecks should be taken into consideration to ensure the success of this project?

VI. Applications

1. Among the biopharmaceutical drug(s) PURE Project has proposed to purify, which would be more interesting to explore considering the technology attributes and market need?

2. What other industries present an opportunity for implementation of this technology? (e.g., food industry, cosmetics, agro-food industry)

A.2 Advisory Board Script

Introduction: PURE has a vision of producing biobased fibers precisely functionalised with ligands to give a function to these materials, enabling them to be used in various applications. Currently, most used materials are not sustainable and ligand coupling is done in a random fashion. We aim to produce spider-silk inspired non-woven nanofibers modified with reactive non-canonical amino acids that will allow the site-specific ligand functionalization. The end goal is to introduce this technology in the field of bioseparation to increase productivity and process performance. We envision the introduction of the non-woven nanofibers in filters already used in chromatographic methods.

1. Regarding your role as an Advisory Board Member, what is the know-how you bring to the consortium?

2. Assume we can produce filters composed by non-woven nanofibers precisely functionalized with any type of ligand and introduce them in filters. Where do you think it would be more useful?

3. What are the key attributes that will make you use this new bioseparation material?

4. From your experience, what is the burden of change for the PURE technology in the industry? What are the barriers for implementation of this new technology?

5. Regarding sustainability, which parameters do you think are most critical for adopting PURE technology?

A.3 Consent Form

I, _____

confirm that I have been previously informed about the main objectives of this study, in which I agree to collaborate in the form of an interview, which aims to evaluate the socio-economic impact of PURE Project and focuses on the processing of personal opinions, marketing preferences and prior knowledge. The interviews will be divided in the following sections:

i) Study of the consortium and its organization where questions will focus on members' curriculum/experience and how knowledge is produced and transferred within the

different actors in the PURE Project, while also address how knowledge production is influenced by transdisciplinarity and how much social accountability drives research in the PURE Project.

ii) Evaluation of the economic exploitation and technology market transfer strategy of PURE Project by assessing their curriculum, prior knowledge, the value proposition and applicability of the technology currently being developed.

In this context, personal data will be processed by the Biomolecular Engineering Lab of UCIBIO of FCT-NOVA (belonging to Universidade Nova de Lisboa – UNL), which shall be considered the data controller, according to the definitions stated in the General Data Protection Regulation (GDPR).

The interview will be audio-recorded in its entirety for later transcription, which can be reviewed and corrected upon request.

I understand that only members of the PURE Project research team will have access to the original recording and that the data resulting from the interview are relevant and limited to the purposes of the PURE Project.

The processing of personal data in the context of the PURE Project is based on the explicit informed consent of the data subjects.

To ensure that personal data is stored only for the necessary period, UNL will store personal data only during the PURE Project, which is until September 2024. In some cases, the storage of personal data might occur for longer periods of time, namely when the law so requires.

In order to ensure the security of personal data, a set of technical and organisational measures has been implemented, namely:

- Members of the PURE Project research team will have access to personal data on a need-to-know basis.
- All information obtained in connection with this study will be strictly confidential.
- Basic information and contact details will be pseudonymized, therefore not allowing the direct identification of data subjects.
- Personal data will only be stored on a need-to-know basis.
- In the context of these interviews, personal data will not be transferred outside of the European Economic Area.
- I understand that my participation in this study is voluntary, without any direct or indirect benefit having been agreed upon, and that I may withdraw my consent at any time, without this decision being of any consequence.

At any moment, I may exercise my rights, namely, the right to request more information regarding the processing of my personal data, the right to rectification, to erasure, to oppose to the processing activities, among others stated in the GDPR.

I can exercise my rights by contacting the PURE Project team at [cecilia.roque@fct.unl.pt] or contacting UNL's Data Protection Officer at dpo@unl.pt.

In certain cases (for example, due to legal requirements), requests might not be complied with. In any case, data subjects will be informed of the measures taken within one month of the placement of the request.

Additionally, I have the right to lodge a complaint with the competent supervisory authority. In Portugal it is Comissão Nacional de Proteção de Dados (CNPD), which can be contacted at https://www.cnpd.pt/.

I hereby consent with the processing of my personal data, collected in the form of an interview, for the purposes of the PURE Project.

Research Participant's Name: _____

Research Participant's Signature: _____

Date: ____ / ____ / ___