Comisión Nacional de Investigación Científica y Tecnológica – CONICYT

RESEARCH TEAM GRANTS IN SCIENCE AND TECHNOLOGY

FINAL REPORT

I. PROJECT PRESENTATION

PROJECT TITLE	CODE
Integration of Structural Biology to the development of Bionanotechnology.	**ACT 1107**
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Universidad de Talca **Universidad de Chile**	
PERIOD INFORMED	
Year 3; From January to December 2015	

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RESUMEN EJECUTIVO

Este proyecto constituye un esfuerzo multidisciplinario para estudiar aspectos fundamentales y aplicados de sistemas bionanotecnológicos mediante el uso tanto de herramientas, así como de conceptos importados desde la Biología Estructural y otras disciplinas científicas. El logro de esta integración es una tarea compleja que requiere la fusión de un un amplio número de áreas de especialización, representadas por las líneas de investigación de cada uno de investigadores los investigadores principales. Al finalizar este proyecto, estamos orgullosos de ofrecer nuevas herramientas así como nuevos conocimientos, útiles para el desarrollo de la bionanotecnología, la biología estructural, así como decenas de nuevos científicos y estudiantes entrenados en estas materias.

Nuestros esfuerzos de investigación incluyeron aspectos teóricos y experimentales. Por un lado, se trabajó en el desarrollo de métodos de vanguardia para la síntesis de nanopartículas, en el uso de proteínas y bacterias como biorreactores, así como en el uso métodos sintéticos de química orgánica. Por otro lado, hemos ampliado las aplicaciones tecnológicas de la interacción de dendrímeros con diferentes ligandos (incluyendo ADN y péptidos). Asimismo, hemos probado el uso de "proteínas sintéticas" como herramientas de transfección de ADN. Además, hemos desarrollado nuevos métodos para la generación de nanointerfaces de bio-silicio. Estas interfaces, nos han permitido la integración de un canal de iones, creando las bases para la generación de nanosensores moleculares.

Dada la importancia del desarrollo de la Biología Estructural para este proyecto, dimos pasos importantes para estudiar la estructura y función de proteínas, implementando métodos para su expresión, cristalización, y caracterización termodinámica. Entre las diferentes proteínas estudiadas destacan enzimas redox, canales iónicos, canales de connexina y factores de transcripción. Desde el punto de vista teórico, integramos diversos métodos de simulación molecular para caracterizar nanomateriales y su interacción con biomoléculas tales como proteínas, ADN y fosfolípidos. De este modo, utilizamos diversos nanomateriales como sistemas modelo para estudios experimentales y teóricos, cubriendo nanopartículas metálicas, dendrímeros, nanocompuestos y nanotubos de carbono, entre otros. Resulta importante destacar que varios de estos estudios derivaron tanto en la generación de conocimiento fundamental, así como en aplicaciones en biotecnología, cumpliendo de esta forma el objetivo central de nuestro proyecto. De esta forma, hemos abierto un nicho fructífero para la aplicación de una amplia gama de técnicas de biología estructural para el diseño de nuevos sistemas bionanotecnológicos.

Todos estos esfuerzos se tradujeron en la publicación de 49 artículos en revistas ISI, así como 9 solicitudes de patentes. En nuestra opinión, estos logros revelan tanto el alto nivel, así como el alcance internacional del fruto de nuestra investigación científica y tecnológica.

En lo que respecta a la generación de capital humano avanzado, 32 tesis se desarrollaron bajo la dirección del equipo de investigación, principalmente de estudiantes de postgrado. Es importante destacar que el entrenamiento de estos estudiantes nos permitió generar fuentes de colaboración e integración entre nuestros equipos de investgación científica, generando 3 publicaciones ISI colaborativas.

Las redes nacionales e internacionales de cooperación aportadas por cada uno de los investigadores de este proyecto fueron fortalecidas a través de la organización de eventos, incluyendo 4 Cursos y 4 Talleres, 10 visitas al extranjero, y mediante la participación de nuestros investigadores en 4 proyectos internacionales de I+D. En el ámbito local, nuestro proyecto sirvió como plataforma para la adquisición de nuevo equipamiento a través del programa FONDEQUIP, permitiéndonos la apertura de nuevas líneas de investigación y aumentando nuestra competitividad. Dado que estos equipos están destinados al apoyo de la investigación colaborativa, su adquisición y puesta en marcha revela nuestro compromiso de fomentar la investigación científica nacional sobre la base de los nuevos conceptos y herramientas aportatas por nuestro proyecto.

Con el fin de llegar a diferentes públicos de la sociedad nacional e internacional, nuestros investigadores han participado activamente en diversas actividades de difusión tales como la "XI Bienal de Artes mediales", "1000 Científicos 1000 Aulas", así como eventos internacionales tales como el 7º Foro Mundial de la Ciencia de la UNESCO.

II. EXECUTIVE SUMMARY

This project constitutes a multidisciplinary effort to study fundamental and applied aspects of bionanotechnolgical systems through the use of tools and concepts brought from Structural Biology and other disciplines. Achieving such an integration was a complex task that required to coalesce a broad number of expertises, represented by the research lines lead by the main investigators. At the end of this project we are proud to deliver novel and useful knowledge within the fields of bionanotechnology and structural biology, as well as tenths of new trained scientists.

Our initiatives involved both experimental and theoretical studies. On one hand, we worked on forefront methods for the synthesis of nanoparticles, by the use of proteins and bacteria as bioreactors, and also the use of organic synthetic chemistry. Also, we expanded the applications of the interaction of dendrimers with different ligands (including DNA and peptides). Noteworthy, we developed the use of "synthetic proteins" as DNA transfection tools. Additionally, novel silicon-lipid nanointerfaces were developed that, together with an ion channel, could perform as a nanosensor when connected to a circuit. Also, since we gave importance to the research on structural biology, studies on proteins structure, function and production deserved high dedication. On the other hand, from the theoretical point of view, we used different molecular simulation methods to analyze nanomaterials and their interaction with biomolecules such as proteins and DNA.

The nanomaterials used as model systems for experimental and theoretical studies covered metal nanoparticles, dendrimers, nanocomposites and carbon nanotubes. Among the different proteins studied are redox enzymes, ion channels, connexins and transcription factors, deserved a long standing attention during the project, and even the generation of new research objectives (most of them clustered under obj. 10). Several of them known applications in biotechnology. The accomplishment of our objectives allowed us to find a fruitful niche for the application of a diverse array of structural biology techniques for the design of novel nano-biosystems.

All these efforts translated into almost 50 papers in ISI journals with high impact factors including ACS Nano (IF 12.881) and ACS Catalysis (IF 9.312) and 9 patent applications. In our opinion, these achievements reveal both the high-level and international scope of our scientific and technological research. In regard to human capital generation, 32 theses have been developed under the direction of the research team, mainly from posgraduated students. These work also served in several instances for the integration among the research lines by means of co-tutoring students.

The existing international cooperation networks were strengthen through different means, such as the organization of international events (4 Courses and 4 Workshops) 10 visit abroad, participation in 4 International R&D projects. At the national level, our project also served as platform for acquisition of major equipment from the national competition FONDEQUIP. Since this equipment are intended for collaborative research, including these initiatives under the umbrella of our project, reveals our commitment to foster national scientific research on structural biology and bionanotechnology. In order to reach different audiences of the national and international society, our researchers actively participated in communication activities such as the."XI Bienal de Artes Mediales" and "1000 Cientificos 1000 Aulas", as well as international events such 7th UNESCO World Science Forum.

III. ACTIVITIES

Objective 1: Structural Biology of Biomineralization Nanoreactors.

From the beginning of the project, the concept of a reactor for metallic nanoparticles and quantum dots production was advantageously expanded from the protein shell mechanism provided by ferritin to the bacterial synthesis processes.

In the third year of the project Dr. Yévenes conducted a research stay in the laboratory of Dr. Ehmke Pohl, Durham University (supported by Pontificia Universidad Católica de Chile) to prepare crystals of a mutant form of ferritin from *Chlorobium tepidum* **[Act 1]**. Although it was not possible to get useful diffraction patterns to determine the structure, this mutant presented interesting properties in nanoparticle synthesis (ongoing research). It is worth to acknowledge the production of an article in collaboration with Dr. González-Nilo about the interaction of PAMAM G4 and human L-chain ferritin (Camarada et al. 2015, Phys. Chem. Chem. Phys. doi: 10.1039/c5cp02594j), and a theoretical study of the properties of *Pyrococcus furiosus* ferritin, that was presented in the Annual Meeting of the Biochemistry and Molecular Biology Society 2015.

Regarding the use of bacteria as reactor for Quantum dots synthesis, we previously reported the effect of different sulfur sources on the biosynthesis of fluorescent nanoparticles, the metabolic analysis of *E. coli* cells synthesizing CdS quantum dots, and the purification and characterization of the produced NPs. During the last year we completed **[Act 2]** by studying the production of fluorescent nanoparticles by *T. thioxidans* in minimal media amended with sulfur. Different factors were evaluated such as growth media, pH, time, presence of phosphate, etc) in order to improve the conditions for the production of NPs. The chemical and structural characterization of the NPs produced by acidophilic bacteria was performed by DLS, spectroscopy (absorbance and emission spectra) and TEM.

Objective 2: Bioeletrochemical Devices.

Although the original proposal involved the immobilization of redox enzymes on nano-structured systems, we paid much attention to the production and structural characterization of two different Cu-oxidases (laccase and hemocyanin) that have been proposed as electron transfer catalysts in electrochemical devices.

To accomplish **[Act 3]** the ABTS complexes of laccase-α and mutants were generated by comparative modelling followed by molecular dynamics and ligand docking. The wild-type enzyme was expressed in the supernatant of *S. cerevisiae* cultures induced with galactose. Initial low expression levels were optimized 25 fold by changes in the culture media. The enzyme was partially purified using hydrophobic interaction chromatography, obtaining an increase of 480-fold in specific activity. This work was produced by a Master thesis.

The phenoloxydase activity of Hemocyanins has been used recently in an electrochemical sensing platform along with gold nanoparticles (Yang et al, 10.1039/C3AY40654G). According to **[Act 4]**, we purified and studied the properties of hemocyanins from chilean tarantulas *Euathlus condorito* and *Grammostola rosea*, in order to establish their use in such applications. Both hemocyanins presented a phenol-oxidase activity induced by low concentrations of SDS *in vitro*. Using dopamine as the substrate, the reaction was found to be dependent on a divalent cation such as Ca^{+2} o Mg^{+2}.

Objective 3: Biospecific targeting.

This objective has been not complete. That goal requires an infrastructure and expertise that is still not installed in Chile. The use of antibody has been explored by other groups in the word, for this reason we beginning to explore the use of aptamers, with the objective to find a new approach and potential IP. Unfortunately, this field has too much IP restriction and we do not have

enough experts to reach that goal successfully, at the same time this project do not have enough budget to support that initiative.

Objective 4: Synthetic protein development:
Generation of synthetic proteins based on "smart polymers" for controlled drug (DNA/RNA-like and small molecules) delivery.

Synthetic protein is an approach that mimics the structural properties of proteins to interact with DNA fragments, using as a support dendrimers functionalized with a mixture of amino acids. To design the best combination of this amino acids, we used an approach described in Marquez-Miranda et al, PLOS One, 2015 [1]. As a result of this approach, we have molecularly designed a new dendrimer with a combination of amino acid residues as terminal end-groups. This new dendrimer was synthesized by FCR-UNAB and then, tested in *in-vitro* cell cultures as a transfection reagent. The efficiency and cytotoxicity of this dendrimer have shown to be better than commercial reagents, like Lipofectamine 2000 and 3000 and dendrimer-based Superfect. Our experimental results have been recently successfully validated in Fraunhofer Institute IME, Germany. The intellectual property of this system is currently under revision. This system is the first transfection reagent designed, synthesized and tested in Chile. We expect to refine this new product and commercialize it with the support of Fraunhofer Institute IME Germany, Fraunhofer Chile Research and UNAB.

The synthesis of Megamers **[Act 7]** based on PAMAM showed to be a great alternative to classic PAMAM dendrimers. In-situ studies of dendrimers with water contaminants were published. This study reported the photophysical behavior of PAMAM-G0 in the presence of Cu(II) and Zn(II) ions in aqueous solutions. Theoretical and experimental results confirmed the presence of a strong covalent metal–ligand interaction between PAMAM-G0 and copper ion that favored the formation of a ligand–metal charge transfer band coordination complex. This work was published in J. of Luminescence 2015. Furthermore, we synthesized different PAMAM-G4 and G5 derivatives in order to prove their interaction with uranium and their effect on the viability of red blood cells in vitro **[Act 8, 10]**. In this work, we proved the effectiveness of the selected dendrimers in an animal model of acute uranium intoxication. The dendrimer PAMAM G4-Lys-Fmoc-Cbz demonstrated the ability to chelate the uranyl ion in vivo, improving the biochemical and histopathologic features caused by acute intoxication with uranium. This work was published in Molecules 2015. We also reported an study based on small molecules on the molecular structure and electrochemical behavior of a series of methoxylated 20-hydroxychalcones, whose antitumor activity has been previously described **[Act 8, 10]**. A direct relation among the substitution pattern on rings A and B, the strength of the IHBs and the reduction potentials was found. The results showed the importance of the methoxy-substitution pattern on the IHB and redox properties of these compounds. Our findings have potential implications in the design of antitumor chalcones. This work was published in RCS Advances 2015.

Regarding the formation of new high-density dendrimers, we explored a cationic pre-catalyst, methallyl 2-pyridine-4,7-dimethoxybenzimidazole nickel, to produce new functionalized dendrimers by dimerization to 1-butene. This work was published in a high impact factor ISI magazine (ACS Catalysis) and according to it, a new postdoc associate joined our group (UTALCA) to develop new catalysts in the synthesis PAMAM-Polymer nanocompounds.

Thermodynamic characterization of PANAM complex with biologically active molecules using isothermal microcalorimetry.
Since the microcalorimeter (FONDEQUIP EQM140174) was just installed a month ago, the experiments regarding to the study of the complex formation between dendrimers and nucleic acids have been not implemented yet **[Act 12]**. Instead, we have implemented an alternative method to determine indirectly dendrimer-DNA affinity. To this end, we considered the fact that the complexation can be studied by following the fluorescence quenching of DNA. Our results show that all the dendrimers are able to generate complexes with DNA, at a ratio of 10:1

weight/weight. These kind of assays resulted very useful to determine in a straightforward way the ability of dendrimers to form complexes with DNA.

Characterization of PAMAM-DNA, PAMAM-Peptides and PAMAM-Antibiotic complexes:
During the last year, we identified the most frequent residues in the Protein-DNA interaction complexes **[Act 11]**. This information was used to suggest new functionalizations for the chemical synthesis of dendrimers. These results were published in an ISI article (Biomimetics: From Bioinformatics to Rational Design of Dendrimers as Gene Carriers). From this study, and in collaboration with Fraunhofer Chile, the chemical synthesis of new dendrimers with aminoacids was carried out. Our results demonstrated that these new prototypes are very promissory, due to their higher efficiency compared to commercial products, such as Superfect and Lipofectamine.

Objective 5: Molecular nanosensornig systems:
Studies on the biophysical properties governing the interactions between inorganic surfaces and polysaccharides, biological membranes and G-protein coupled receptors

Relying on our technological achievement to create novel nano-interfaces (see objective 6), we moved towards the creation of nano-sensors **[Act 15]**. To do so, we decided to embed an ion channel in the DPPC membrane that belongs to our nano-interface: we selected the gramicidin protein. This protein, when folded as an alpha-helix bundle within a biological membrane, produces a non-selective cation channel. Once inserted in the membrane, we created a circuit capable to produce a potential difference controlled by an oscilloscope between the membrane and the SiO_2 surface. Importantly, our design (which is in the process to be submitted to a PCT patent application) considered that the nano-interface will perform as a condenser. Therefore, any ionic movement through the ion channel will be affected by the discharge rate of the condenser (the tau characteristic time of discharge), producing a current difference between the input and the output current. This current difference could be used to calibrate our sensor. Despite further studies are necessary to calibrate this device and/or to prove the ability of using other proteins such as GPCRs, due to the finalization of this project, they will remain out of the scope of this project.

Objective 6: Bio-silicon interfaces:
Studies on signal transduction from protein-ligand binding events towards electronic devices through SiO2 surfaces.

During the execution of this project, we intended to get insights on the experimental and theoretical foundations that will allow us to eventually, design novel nano-sensors **[Act 15]**. In this context, we successfully developed nano-interfaces composed by inorganic SiO_2 surfaces used to support biological membranes formed by DPPC. In doing so, we created a novel method to develop synthetic membranes supported on SiO_2 substrates and using chitosan as hydrating mattress (Retamal, Cisternas et al. 2014). Due to the novelty of our method and its implications for future developments related to nano-sensors technology, we submitted a provisional patent (Volkmann, Perez-Acle et al. 2014). Denoting its importance to the field, this contribution has been highlighted by both specialized and general audience media (Bardi 2014, USANews 2014).

Objective 7: Structural and electronic characterization of the interaction of biomolecules with surfaces and nanocomposites by theoretical methods.
Use of multiscale QM-MM theoretical methods for the study of binding and electron transfer properties of nanostructured materials such as SIC and TIO2 and carbon nanotubes.

In the Original Proposal (Objective 7) we planned to study the interaction of biomolecules with surfaces and nanostructures by first-principles theoretical methods. However, in the last Annual Activity Plan, we committed to finish three lines of research: (1) The functionalization of carbon nanotubes with porphyrins chromophores, (2) the characterization of the hydrogenated SiC(001) surface, and (3) The characterization of nanoporous graphene as membranes for water

desalination. Additionally (4), we committed to finish a work in collaboration with the postdoctoral researcher Germán Miño on the characterization of Hv1 proton channels using ab-initio molecular dynamic simulations. All four commitments were accomplished during the last period. For the line (1) we published two articles, one in the Journal of Material Science and the other in *Chemical Physics Letters*. For the line (2) we published one article in *Applied Surface Science*. The line (3), which was not in the original proposal, was the thesis project of the undergraduate student Raúl Guerrero (Engineering Physics) under the supervision of Walter Orellana. This work was presented on the international conference *22nd Latin American Symposium on Solid State Physics,* on last November. The collaboration work with Dr. Germán Miño was also finished and the corresponding article, is currently under consideration in *Nature Communications*.

Objective 8: Ferric-Siderophores as "Trojan Horses" for Toxic Metals via Atomic Layer Deposition processes.
Structural characterization and engineering of bacterial siderophores for the control of metal specificity and its application as novel bactericides

In the third year of the project, we conducted a theoretical and experimental study of the reactivity of Zinc-modified Enterobactin and Fe-Enterobactin by means of the ALD process, finding an innovative methodology to functionalize drugs. Siderophores are efficient iron carriers synthesized and secreted by microbial species. Herein, the atomic layer deposition (ALD) process has been used to functionalize Ferric Enterobactin (FeH3EB) and Enterobactin (H6EB) by linking ZnOH fragments. IR spectrums and NMR were successfully correlated by DFT calculation and molecular dynamics studies, explaining the effect of ZnOH fragments over FeH3EB and H6EB structures. As a whole, our studies probed the successful Zn-modification of FeH3EB and H6EB as well as the viability of using the ALD process to attach metals to soft materials. Docking results between Siderophore ZnOH and FepA showed an affinity similar to (or in some cases, even higher than) that of their bare counterparts (FeH3EB and H6EB). The ALD process could be a potential route to functionalize soft organic molecules with potential application in the pharmaceutical field.

Objetivo 9: Bionano- and nanobiomaterials comparative structure analysis for the development of novel analytical methods for structure annotation and shape decomposition for their use in structure activity correlation studies.
Through the execution of this project, we implemented a number of computational tools to characterize the structure of dendrimers and to get insights on how different functional groups drive the interaction with targets, specifically, small molecules and nucleotides. All these approaches have been described in detail in Gonzalez-Nilo's articles. In summary, we found that the dendron flexibility is a key property that is governed by the end-groups, therefore, a balance between the affinity of the end-group/substrate and the flexibility of the branches is key to modulate the affinity of a dendrimer again to a specific target..

Objective 10: Application of structural biology tools to the study of bionanosystems (new)

Establishment of a protein crystallization facility.
During this project, two workshops of Protein Crystallization and Crystallography were organized giving scenario for lectures of renowned crystallographers from South America **[Act 5]**. More than 80 participants from different universities from Chile attended these events. In the last year, we conditioned the crystallization room that contains the protein crystallization equipment. Furthermore diffractions patterns were obtained for two proteins grown in our facility, but not yet with enough quality to their atomic structure determination. New crystallization experiments are being conducted. Since the equipment was set up, 28 crystallization experiments were made in which more tan 6500 crystallization conditions were tested.

The effect of the protein topology in the mechanical and thermodynamic stability of knotted proteins

We used comparative modelling to predict the presence of a knot in the structure of the transcription factor TraY, performed the optimization of purification and solubility of this protein and different mutants, improving conditions for monodisperse solutions. This final year we put forward **[Act 5]** the determination of the structure and folding mechanism of TraY. Furthermore, single molecule studies of protein stability by means of optical tweezers were performed. Comparison of non-equilibrium thermodynamic analysis of single molecule force induced transitions from knotted or unknotted configurations of our ARC-L1-ARC construction indicated that the energetic cost paid to knot a polypeptide chain (6-7 kcal/mol) is marginal with respect to the huge free energy associated with the configurational entropy of folding. These results have been presented in national and international congresses.

Development of molecular models to describe the structural properties that modulate the dendrimer/peptide interaction.
The peptide being studied has beneficial effects on the treatment of circulatory system, skeletal muscle and nervous system diseases. However, due to their instability, peptides cannot be efficiently administered. Therefore, dendrimers have emerged as promissory vehicles to protect and transport a wide range of bioactive molecules **[Act 13]**. In this work, we explored the use of PAMAM-NH_3^+, PAMAM-OH and PAMAM-COO^- dendrimers as carrier of peptides by molecular dynamics simulation (MD). Results showed that, over 60 ns of MD simulation, peptide-binding capacity of the dendrimer was 2:1 molar ratio. MD analysis also revealed the capacity of PAMAM-OH to protect Ang1-7 and form stable complexes. Currently, that article has been submitted to review.

Establishment of an expression system for membrane channels.
Regarding to the purification of membrane channels, hSlo and *Pichia pastoris* were chosen as target protein and expression system, respectively. We are currently testing different protocols to improve protein production and purification **[Act 12]**. In that context, also was exploring the use of dendrimers as "syntactic toxins" again to ion channels. That study was validated experimentally (electrophysiology assays) that low generation of PAMAM can be used as blockers of ion channels. Interestingly, we observe that the effect of PAMAM G2 is produced by the intracellular region. More studies are in progress.

Structure and dynamics of nano-confined water
Hydrophobicity and Polarity effects on carbon-nanotube assisted solvation.
By conducting out of equilibrium MD simulations we have demonstrated that water filling of SWCNT (single walled carbon nanotubes) is a spontaneous phenomenon, primarily commanded by an entropy gradient (Garate, Perez-Acle et al. 2014) **[Act 16]**. Considering this evidence, we hypothesize that thermodynamic properties of water should be considered when characterizing protein- protein interactions and when dealing with protein-ligand interactions. Our preliminary data suggest that increased water entropy should be considered as an important indicator of energetically favored interactions.

Is the Arg143 the voltage sensor in human Cx26 hemichannels?
Using MD simulations to study electrical synapsis mediated by Gap Junction channels (GJC) formed by the human connexin (hCx) 26 hemichannel (HC), we have recently discovered a water pocket within the intracellular portion of the hCx26 HC (Araya-Secchi, Perez-Acle et al. 2014). Functional assessments demonstrated that Arg143 is a key residue to the hCx26 HC activity, suggesting that this residue is part of the fast-voltage sensor. To confirm this hypothesis, we are conducting out of equilibrium MD simulations that will be complemented with molecular biology and electrophysiological experiments. If our hypothesis is correct, then we will propose the existence of a voltage-controlled hydraulic gating (VCHG) mechanism in hCx26. Notably, this mechanism will be the very first of its type ever described for ion channels **[Act 16]**.

IV. OUTPUTS

During the execution of this project we may report the following indicators:

1. 49 ISI publications ISI: 3 of them have been written between the main researchers of this project. Several cross-collaborations were generating during these 3 year, many of them are in progress and evaluation new concepts to apply to new grants.

2. 32 theses students: 12 PhD, 11 MsCs and 9 undergrad students. In that context the PI were actively involved, that motivate a cross discussion with exiting and innovative concepts.

3. 9 patent applications. Dr. Leonardo Santos has had high productivity in that field, with smart and novel application of dendrimer technology in the agroindustry. At the same time, Dr. Perez-Acle contribute with a novel strategy to create synthetic membranes. Finally, Dr. Gonzalez-Nilo develops a novel system for transfection using concept obtained fro bioinformatics analysis. The transfection prototype has been recently validated in Fraunhofer Institute IME in Germany, showing a really competitive transfection efficiency and low cytotoxicity.

V. HIGHLIGHTS

1. This project allowed assembled a multidisplinary team of young and prolific researchers coming from three academic institutions. The commitment of our researchers allows successfully fulfill the main objectives of that project through a multidisplinary discussion that combine computational biology, chemistry, molecular biology and biophysics. The intensive multidisciplinary discussion promoted by that Grant, probably is one of the first in Chile in an academic environment.

2. We explored the outcome of diverse structural biology techniques to be applicable for the design of novel nanobiosystems ranging from molecular simulations, chemical synthesis to electron microscopy.

3. We used computational tools to design and develop of synthetic nano-systems such as synthetic proteins, smart polymers and molecular nano-interfaces. All these systems have been protected by patents, and some of them have been already accepted. Therefore was possible to integrate from computational molecular design until the production of suitable prototypes.

4. We explored the application of organic synthetic chemistry with structural biology tools to support the design of novel nano-particles suitable for applications in drug delivery, water remediation and agriculture.

VI. LESSONS LEARNED

1. **Research Team Grants are very complex projects in two ways:**
   a. Proposal assembly: In order to assemble a successful application, these projects require an interdisciplinary team composed by both young and prolific researchers. Despite we succeed in the application, to be competitive we needed to involve 5 main researchers together with their lab members.
   b. Project execution: As mentioned before, this project was executed by a team of 5 research groups. Considering the number of people involved in the execution of this project, the budget is certainly scarce. Therefore, financial and administrative issues arose during the execution of this project that, to be solved, required budgetary changes. From our point of view, requiring CONICYT acknowledge for every budgetary change is an administrative overhead that could be easily simplified by using a web platform as in the case of the FONDECYT projects.

2. **Budget is scare.**
   As mentioned before, the assembly and execution of a Research Team Grant requires the involvement of several main researchers and their labs. Therefore, the available budget results scarce compared to the obtained output. PIA-CONICYT should evaluate the success of this program and, consequently, increase the available budget per grant.

3. **The execution time of a Research Team Grant is too short**
   a. Team complexity: As part of this program rules, teams applying to Research Team Grants should be young, prolific and multidisciplinary. Assembling and coordinating such a group of people is a complex task that requires a lot of administrative work. Moreover, the establishment of collaboration efforts between diverse groups requires time, money and ideas to be pursued. Therefore, a 3-year period of execution is certainly a short period of time that should be extended to 5 years: even FONDECYT acknowledges this issue by allowing projects spanning by 4 years.
   b. Team dissolution: On the other hand, once the team has finally been assembled, establishing joint research efforts and other collaborative activities, the grant comes to and end. Thus, hired people and their accumulated knowledge, tend to move out from the groups belonging to the Research Team Grant. Therefore, a 3-year period of execution is certainly a short period of time that should be extended to 5 years.

VII. COMMENTS TO PREVIOUS EVALUATIONS