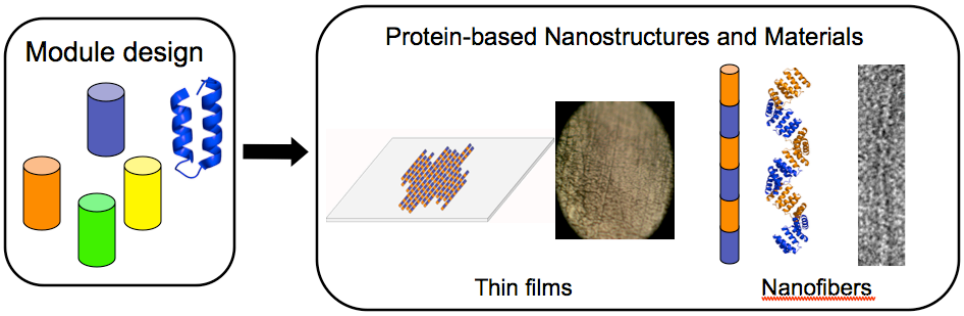
Publishable summary

The overall objective of the "BioNanotools" project was the understanding of the principles that underlie protein structure, stability, and function by protein design. This research not only contributes to our understanding of the fundamental physics of protein folding, but also provides guidelines for the design of functional proteins with new and useful activities and protein-based nanostructures.

In particular, we focus on a type of proteins called tetratricopeptide repeats (TPR). They present a simple modular structure, where a small structural unit is repeated in tandem. Overall TPR domains represent a very robust system to study protein structure, folding, and function, and to use them as building blocks for protein engineering to generate new functional nano-molecules.

The main objectives of the "Bionanotools" project are:
(1) Characterization of thermodynamic stability of scaffold proteins
(2) Design of novel stable scaffolds
(3) Design of protein libraries for selection of novel functional domains
(4) Application of designed modules in nanotechnology: functional materials and nanostructures



Towards these objectives the "Bionanotools" project has made the following progresses:

We performed studies on protein stability and folding of designed proteins to gain a better understanding on how the protein sequence determines the structure and thermodynamic stability of the proteins. First, we studied the thermodynamic stability of newly designed repeat proteins. In this work, we showed that the modular structure of the proteins translates into a modular stability.[1] Additionally, we showed how by using simple design principles, the protein stability can be increased in a predictable fashion.[2] Therefore, we can manipulate the properties of individual units to generate a collective and predictable effect on the ensemble.

We have also successfully designed repeat protein modules that bind novel target peptides and demonstrated that these novel functional modules exhibit the desired binding activity *in vitro* and are also active in cells.[3]

Once we design TPR modules with desired stability and binding properties, we focused on the use of our designed recognition modules for their immobilization onto ordered surfaces synthesized of block-copolymers to create novel biorecognition platforms for specific immobilization and patterning of proteins and bacteria.[4,5]

Finally, we use the intrinsic self-assembly properties of redesigned stable repeat proteins to assemble different protein based ordered nanostructures: nanostructured thin films made purely of proteins[6] and nanometric fibers.[7]

The main results achieved in this project are:

(1) The generation by rational design of extremely stable protein frameworks. These proteins with improved physical and biological properties are useful in biotechnological applications, such as the generation of novel biomaterials and will present enhanced efficiency as therapeutics.

(2) The design and selection of novel binding modules that have many potential applications, such as functional substitutes for antibodies, and represent new tools for cellular and molecular biology.

(3) The combination of those designed modules with block-copolymer technologies to generate biofunctional patterned surfaces.

(4) The generation of ordered functional nanostructures and materials by self-assembly of designed protein modules.

At the end of Bionanotools project we have achieved a profound understanding of the thermodynamics and functionality of designed TPR protein modules. Therefore, we can successfully generate arrays of proteins with defined structure, stability and function. Those proteins represent extremely useful nanotools that are being applied to monitor biological processes *in vivo,* as biorecognition molecules for patterning and sensing.

The results of Bionanotools project provide us a better understanding of the protein modules for their use as building blocks in nanofabrication and design of biomaterials, offering a new perspective of proteins in these novel fields of research. In this project we successfully used the CTPR building block to assemble in a control manner nanofibers and ordered thin films. These nanostructures and biomaterials can be endowed with reactive groups to generate nanostructures and materials with tailored morphology and function. Overall, these results supply generally applicable concepts for the rational and efficient fabrication of novel materials and nano-devices for a wide range of applications: from nanotechnology to biomedicine, and therefore have a ground-breaking impact.

References:

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