# OVER-EXPRESSION OF RECOMBINANT PROTEINS BY A SELF-INDUCTION METHOD FOR NANOTECHNOLOGY APPLICATIONS

*Edurne Teiletxea Malda[1], Aaron Cabrera[2], Jose F. Moran[2]*
*[1]FideNa PrincipiaTech Navarra, Edificio CIDEC, C/ Tajonar s/n, 31006 Pamplona, Navarra, Spain*
*[2]Instituto de Agrobiotecnología, Universidad Publica de Navarra-CSIC-Gobierno de Navarra, Campus de Arrosadia s/n, 31006, Pamplona, Navarra, Spain*
*jose.moran@unavarra.es*

The production of high quantities of a pure biological macromolecule is a very important subject in many nanobiotechnological applications, during the investigation phase, as well as, in the posterior industrialization phase. Protein production may be carried out by over-expression of foreign gene in bacteria followed by protein purification and characterization of the recombinant protein. Former bacterial expression systems involved the use of plasmids expressing proteins under the control of constitutive promoter, which we found to render low protein concentration [1], and some authors reported protein expression unable to characterized [2]. The use of newer inducible plasmids may allow much higher concentrations of expressed proteins. However, these expression systems often results in high formation of insoluble protein into “inclusion bodies” or even, it could lead to unpaired synthesis of protein and prosthetic group as the heme group in hemoglobins. Therefore, when using systems based on the de-repression of an inducible promoter with Iso-propyl thiogalactoside (IPTG) some authors had employed denaturing buffers to isolate the apo-proteins, and at a later stage, prosthetic group was added [3].

In this work we describe the production of recombinant proteins under a self-expression system [4], and report the purification of iron-superoxide dismutase (FeSOD) from cowpea (*Vigna unguiculata*). FeSOD is a metallo-enzymes that catalyzes the dismutation of superoxide radicals into hydrogen peroxide, thus preventing oxidative damage [5]. The over-expression of FeSOD in Escherichia coli using self-induction of the bacterium may take between 16-24 hours with optimal results, after which period cells are harvest by centrifugation and either used or stored at – 80º C. FeSOD is over-expressed using plasmid pET28a(+), and the gene has been cloned at the NdeI site of this vector, which implies the synthesis of the protein with a 6(Hys)-tag [5]. Up to 50 mg of protein can be affinity purified from 1L of bacteria culture in a single chromatography step with a 5 mL NTA-Ni column (Amersham-Pharmacia). The process can be completed with the treatment of the FeSOD with

thrombin to remove the 6(Hys)-tag, and appropriate capture of the thrombin and dialysis with desired buffer. Full process of purification can be easily accomplished in only 3 days.

This expression and purification system produce higher quantities of recombinant protein, reducing the formation of inclusion bodies and contributing to the better purification and characterization of the protein, as well as, minimizing the production cost. In our lab, we have produced several recombinant bacteria with a high effectiveness using this system obtaining improved yields which ranges between 4-10 times higher. We have reported that this system is optimal to over-express and purify flavoproteins which contain FAD as prosthetic groups [6]. For the FeSOD the production has been shown to be 3.5 times higher. The chromatography step renders protein extremely pure and we have produced high quantities that have made possible the crystallization of the protein and the research by X-ray of the protein crystals [7].

Currently, we are using these and other macromolecules in nanodevices for different applications.

**References:**

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