**Specific immobilization and purification of recombinant proteins using diethylaminoethyl-functionalized magnetic nanoparticles**

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The affinity of proteins for their ligands is usually employed in several biotechnological processes that involve their immobilization on solid surfaces, among which affinity chromatography is perhaps the best known application. In this sense, the choline-binding modules (CBMs) such as the C-LytA protein, have been used in these tasks with appreciable success [1-4]. CBMs constitute a family of polypeptides that are part of several enzymes such as the murein hydrolases from *Streptococcus pneumoniae* (pneumococcus) [5-6] and that present a high affinity for choline and other tertiary and quaternary amines [7], as well as for supports that contain these groups, such as diethylaminoethyl (DEAE)-cellulose. Fusion proteins containing one of these choline-binding modules as affinity tags can be immobilized and/or purified in a single chromatographic step by means of a simple, gentle, non-covalent procedure [1-4,8].

With the aim of checking the performance of the CBM system on the immobilization of proteins in nanostructures, we have evaluate the binding of the C-LytA and C-LytA fusion proteins on several classes of magnetic nanoparticles. Firstly, we carried out the synthesis of $Fe_2O_3$ magnetic nanoparticles by coprecipitation methods which were subsequently coated with DEAE-dextran, with a resulting diameter of ~ 10 nm. On the other hand, we also assayed commercial magnetite nanoparticles coated with DEAE-starch (Fig. 1) (200 nm diameter) or with DEAE-silica (750 nm diameter). In all cases we observed a very efficient binding of the C-LytA module, either isolated or fused to the green fluorescent protein (C-LytA-GFP) or to the β-galactosidase (C-LytA-β-gal). Bound proteins could withstand strong washes with up to 1 M NaCl, conserved their structure and activity, and could be specifically eluted upon addition of 150 mM choline as a competitor ligand (Fig. 2). With this in mind, we set up a simple purification procedure of recombinant proteins from a bacterial extract using the 200-nm particles and the protocol depicted in Fig. 3. Proteins could be purified this way to electrophoretic homogeneity (Fig. 4), with a yield of 50 mg per gram of nanoparticles, and the whole procedure could be accomplished in as low as 20 minutes.

The procedure described here displays a series of advantages with respect to other current methods: DEAE nanoparticles are easy to synthetize and the protein immobilization is fast, efficient and strong although, due to its non-covalent nature, the regeneration of the particles can be easily carried out. Moreover, buffers are simple and do not contain components that may potentially inactivate enzymes or be harmful to the human body. Besides the rapid purification of proteins, DEAE-containing magnetic nanoparticles may act as protein carriers to be used in a wide panoply of applications, such as the construction of enzymatic electrodes, recyclable enzymatic bioreactors, or *in vivo* delivery of proteins to specific tissue targets by means of the application of an external magnet.

## References

[1] Sánchez-Puelles, J.M. et al. Eur. J. Biochem. **203**, (1992),153-159.
[2] Ortega, S. et al Bio/Technology **10** (1992), 795-798.
[3] Akerstrom, B. et al. Biochim. biophys. Acta **1482** (2000),172-184.
[4] Caubin, J.et al. Biotechnol. Bioeng. **74** (2001), 164-171.
[5] García,P.et al. J. Appl. Biomed. **8**, (2010), 131–140.
[6] CW_binding_1 family: Pfam code PF01473; http://pfam.sanger.ac.uk/family?PF01473
[7] Sanz,J.M. et al. FEBS Lett. **232**, (1988), 308-312.
[8] *LYTAG* and *LYTAG-Two-Phase* user manuals. Biomedal, S.L. http://www.biomedal.es

## Figures

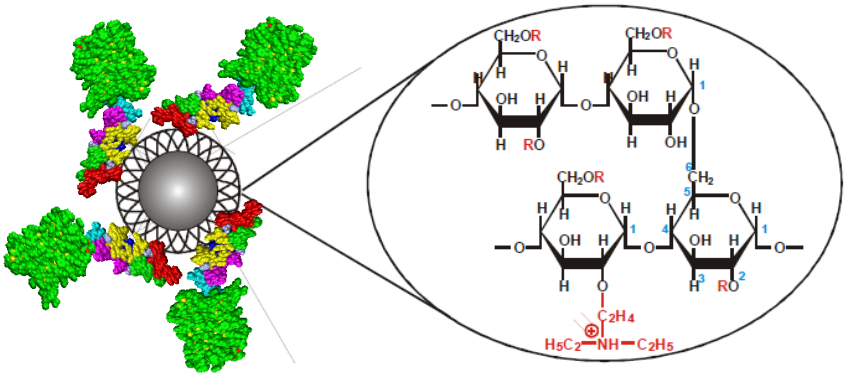


***Figure 1**. Scheme of magnetic nanoparticles derivatized with DEAE-starch (taken from Chemicell GmbH) coated with C-LytA-GFP.*

***Figure 2.** Left to right: C-LytA-GFP solution prior to binding to magnetic nanoparticles; two washes with 1M NaCl; elution of protein from the particles with choline.*

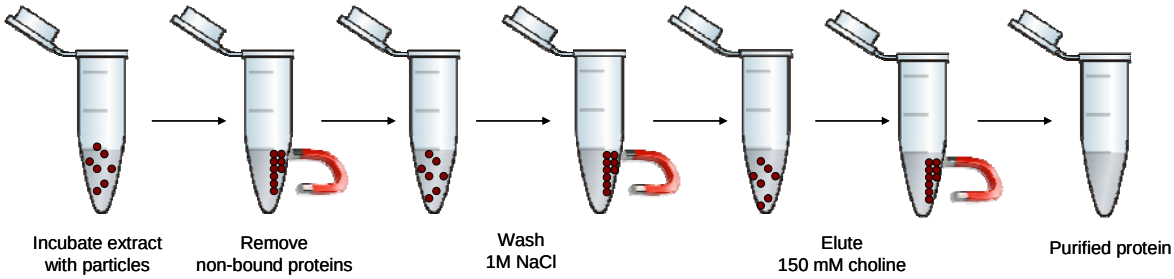


***Figure 3**. Scheme of the purification method of CBM-tagged proteins*

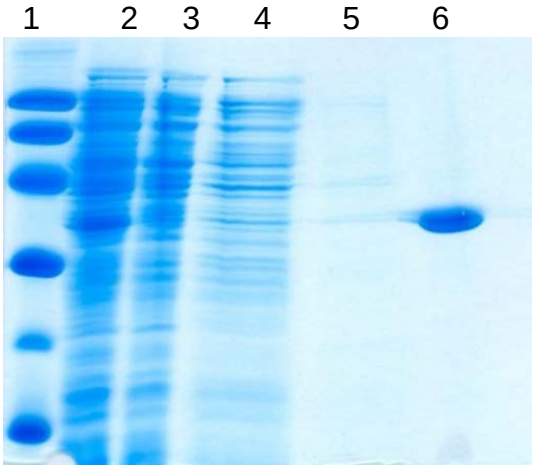


***Figure 4**. Polyacrylamide gel electrophoresis in the presence of SDS showing the purification of C-LytA-GFP. Lane 1, protein molecular weight markers; Lane 2, total extract of Escherichia coli RB791 overproducing C-LytA-GFP; Lane 3, non-bound proteins upon incubation with magnetic nanoparticles; Lane 4, first wash with NaCl; Lane 5, last wash with NaCl; Lane 6, protein eluted with choline.*