



Expression of Proteins from Mammalian Cells for Structural Studies

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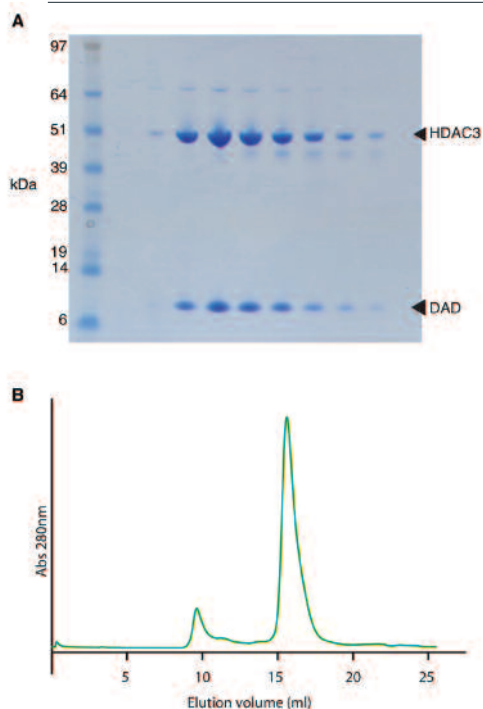
Our research focuses on using structural and functional studies to understand the assembly and mechanism of action of transcriptional regulatory complexes. To overcome difficulties in expressing these complexes in bacteria we have recently moved toward using large-scale suspension grown HEK293 cell cultures for protein complex expression. This approach has been greatly aided by the use of the Anicell shaking CO₂ incubators which enable us to maintain our cells under optimum conditions thus ensuring consistent, high-levels of protein expression as well as offering time-saving and convenience of handling.

Methods

Recombinant protein complex (full length Histone Deacetylase 3 with the deacetylase activation domain (DAD) from the SMRT co-repressor) was expressed by transient transfection in suspension grown 293F cells (Invitrogen), and purified using FLAG resin (Sigma) followed by gel filtration (Watson et al. 2012). Cells were grown and maintained at 37°C, 5% CO₂ in an Anicell shaking CO₂ incubator.

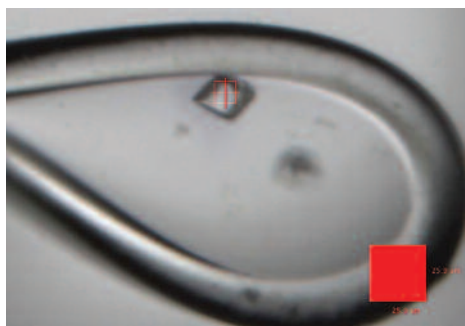
Watson, P.J. et al., 2012. Structure of HDAC3 bound to co-repressor and inositol tetraphosphate. *Nature*.

Results



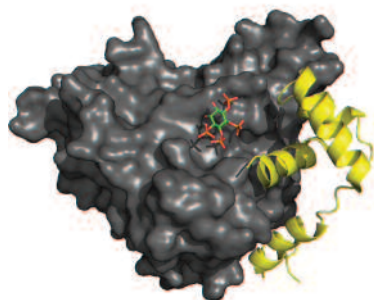
1. Purification of protein complex from HEK293F cells

A/B SDS PAGE gel and gel filtration trace showing purification of the HDAC3 / DAD complex.



2. Crystallisation of the HDAC3/ DAD complex

Crystal of the HDAC3/DAD complex, mounted in a cryo loop at the I24 beam line Diamond Light Source.



3. Structure of the HDAC3 / DAD complex

Structure of the HDAC3/DAD complex, the HDAC3 is shown as a dark grey surface, the DAD as a yellow cartoon and the D-myoinositol-(1,4,5,6)-tetrakisphosphate as a stick model.

Summary

The use of large scale mammalian cell culture to produce recombinant proteins for structural studies has allowed us to study proteins that could not be produced reliably in other systems (E. coli / insect cell), and has revealed the presence of novel co-factors. The Anicell shaking CO2 incubator is a cost effective and reliable solution for the maintenance and expansion of suspension grown mammalian cells for production of proteins for structural studies.

The Anicell is supplied by
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