PCUBE: Infrastructure for Protein Production Platforms

Purified macromolecular samples are a prerequisite for any type of biology research including X-ray crystallography – a method of determining the arrangement of atoms within a crystal – and electron microscopy. Methods used to produce these macromolecules and grow well-diffracting crystals are significantly less sophisticated than those used for X-ray crystallography structure determination. Various projects have tried to tackle this problem and established high-throughput cloning, expression, purification and crystallisation methods in a select few specialist laboratories. The EU-funded PCUBE project offers access to all currently available protein production and crystallisation facilities. This will allow biologists and biomedical researchers conduct better investigations with better structural information.

PRODUCING PROTEINS

In contemporary structural biology project, the gene encoding of a protein of interest is transferred using conventional molecular biology cloning methods into a series of expression plasmids, or extra-chromosomal DNA, with a variety of expression properties, including purification tags. To improve PCUBE's success, different truncation and mutation variants of the gene of interest are included.

The next step is the expression of this assortment of protein-encoding DNA molecules. Up to this point, the expression is carried out mainly in bacterial cells (predominantly *E.coli* strains), but increasingly in eukaryotic host cells (mammalian and insect cell lines). Both approaches have been significantly enhanced in recent years through the installation of high-throughput technologies and miniaturised cell cultivation. When large macromolecular specimens are studied - which is increasingly the case - single-particle electron microscopy can then be used to determine structural integrity and confirm homogeneity of the sample at low and medium resolution.

Crystallisation screening of highly purified protein samples is carried out in a fully automated setup. The resulting protein crystals are subjected to X-ray diffraction analysis with high brilliance synchrotron radiation, followed by structure determination, generally employing single or multiple isomorphous replacement routines and computer-assisted model building.

All aspects of modern structural biology have greatly benefited from the development and installation of robotics, high throughput and miniaturisation. The PCUBE project integrates existing platforms with emerging, powerful new technologies for transnational access.

AUTOMATING RESEARCH

There is still a need for marked methodological and automation improvements in protein production and crystallisation. This is needed for higher throughput in the study of large complexes, the units that control many cellular functions. For this, the automated protein production technologies developed for bacterial expression system will be extended in PCUBE to membrane-bound expression systems. PCUBE's research is aimed at creating automated protein production through the development of robot prototypes that will be integrated into access platforms.

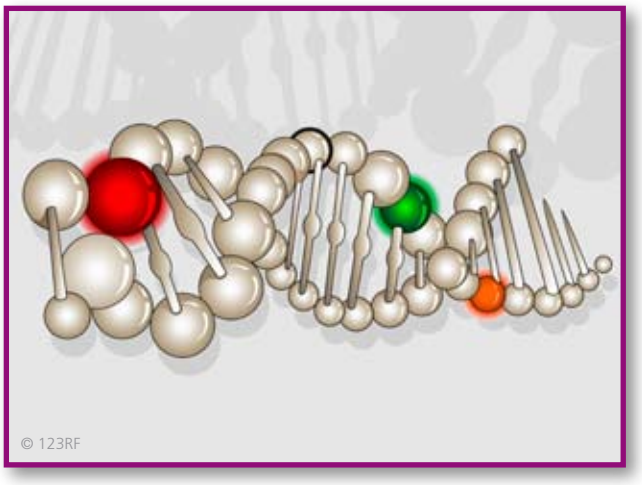
PCUBE is working to provide better access to the most advanced techniques in cloning, expression, protein characterisation and crystallisation available in specialised European laboratories.

SEVENTH FRAMEWORK PROGRAMME

EUROPEAN COMMISSION

Specific elements of the project are focused on opening laboratory doors and informing European researchers of the facilities that are available for their use. PCUBE is also developing channels so that partner laboratories can share information and expertise in order to improve technologies and standardise procedures. The ultimate aim is to share this expertise throughout Europe so the maximum number of researchers can take advantage of it. This will come through common training exercises, annual user meetings and websites.

Moreover, project partners are working to improve current technologies and optimise research platforms. Efforts are also underway to automate partner facilities and identify and overcome bottle-necks in functional protein complex assembly. This will maximise chances of obtaining well-ordered crystals of the specimens which will then be suitable for structure determination.



Protein Production Platform P-CUBE

Project acronym: PCUBE

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EU project officer: Brigitte Sambain

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Partners:
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University of Oxford (UK)
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