ESF Studies on Large Research Facilities in Europe

Advanced Protein Crystallisation Facility (APCF)

ESF Review of the ESA Microgravity Programme APCF

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1. Background

The Advanced Protein Crystallisation Facility (APCF) is a satellite-borne facility developed by the European Space Agency (ESA). The APCF first flew in space in 1993, and, since then, has been the primary facility for microgravity protein crystallisation activities in Europe. The Protein Crystallisation Diagnostics Facility (PCDF) is the follow-on instrument to APCF and, from 2004, should enable scientists to quantitatively investigate gravity-dependent effects on crystal growth. In 1999, ESA secured the operation of an APCF on a 10-week mission to the International Space Station which began in August 2001, and one on a Spacelab mission at present scheduled for spring 2002.

In response to opinions expressed by external peer reviewers and to a recommendation by the ESA Microgravity Programme Board, ESA asked the ESF and its Standing Committee for Physical and Engineering Sciences (PESC) to undertake a review of the scientific results achieved by, and anticipated for, the missions of APCF in the overall context of ESA's activities in the field of protein crystal growth. The review was to form a conclusion on the value of the APCF facility for protein crystallisation studies, and to recommend future activities. ESA requested that the scope of the review be trans-disciplinary, focusing on physical protein crystallisation research in microgravity fields.

The Executive Board of the ESF and PESC discussed ESA's request during 2000, culminating in November 2000 with the approval of terms of reference, a chair, review criteria and procedural arrangements. A Review Panel (consisting of a Review Group and an Expert Reference Group) was established with external members and experts nominated by the ESF Committees (PESC, LESC, EMRC, ESSC) in the areas of natural and technical sciences, life sciences and medical sciences, and space sciences. ESA was kept fully informed during this process.

2. Structure of the review

The Review Panel

The Panel was chaired by Professor Sir Peter Swinnerton-Dyer, Emeritus Professor of Mathematics at the University of Cambridge, and member of PESC 1995-2000. The Panel consisted of two groups:

- The **Review Group** consisted of the chairman and four members, selected for their broad experience and expertise in areas other than space research.
- The **Expert Reference Group** consisted of five experts in ground-based and space-based crystal growth and diagnostics, and in protein research and crystallography.

Full membership details are given in the appendix.

Scope of the review

The review covered the scientific case, the experimental-technical case, and the related organisational-managerial aspects of the ESA Microgravity Programme, based on the space facility APCF and its past and planned flight-mission programme.

The Review Panel considered substantial background documentation from ESA, including:

- a description of both the APCF and PCDF instruments,

- scientific papers reporting the results of APCF experiments,
- summary reports on APCF missions as submitted to ESA's microgravity database by the scientists participating in these missions, and
- a range of reports by ESA and by the US National Research Council.

The Panel also received a detailed briefing from ESA's microgravity programme lead scientist for physical sciences, Dr Olivier Minster.

Panel meetings

The full Panel, i.e. the Review and Reference Groups combined, met on 29-30 May 2001. Both groups had received the same documentation in advance and met jointly to be briefed by ESA and for a general discussion session. The groups separated to discuss and produce their specific recommendations. Due to scheduling conflicts the Review Group was unable to meet again in the time available and members commented electronically on the draft report.

3. Summary of the views of the Expert Reference Group

1. The results from the experiments performed to evaluate the effect of microgravity on protein crystal growth are at present inconclusive. This is both due to the low number of experiments carried out and the insufficient, or non-systematic, analysis of the crystals obtained. No major breakthrough has been made so far and the documented improvements all appear incremental.

2. Improving the quality of crystals remains an important goal for structural biology. The structural and functional relevance of any such improvement depends not only on the resolution range and extent of the improvement but also on the specific structural questions that are being addressed in each project. Crystal growth under microgravity conditions is one important tool that could contribute to crystals of higher quality. It should be noted that the programme has provided technological innovations that benefit the ground-based crystal growing community.

3. The programme should continue with the main thrust divided between:

- The physics of crystal growth.
- Improving crystal quality of important molecules/complexes selected on the basis of the expected impact or relevance of an anticipated improvement.

There should be a better integration between the two sub-programmes. Furthermore, to ensure biologically significant results a better selection system is required.

4. ESA needs to make itself more attractive to scientists. This could be achieved by a number of different strategies.

- The greatest impact on the programme would be achieved by ESA providing peer reviewed research awards (as NASA does). This would have the added advantage that only the best research would be funded.
- If direct funding is not possible, ESA should form links with funding agencies.
- ESA needs to be more visible to attract the best scientists into the programme.

5. There is clear need for a more focused and coordinated programme. Focusing the programme would make better use of the limited facilities, i.e. relatively few experiments (when compared to the thousands of crystallisation experiments carried out on the ground with any single macromolecule) can be carried out per mission. Coordination via a project leader, or the setting up of a dedicated group, would ensure optimal use of the facilities (reactors) and even more importantly, the application of technical standards for setting up and evaluating experiments. It is essential, if the evaluation of results is to have any scientific meaning, that crystals when returned to Earth are analysed in a systematic and standardised manner i.e. on the same synchrotron source (and the same experimental station where possible), data processed with the same computer programmes etc. One way of ensuring systematic and uniform analysis of crystals would be for ESA to enter into a collaboration with one of the European synchrotron facilities.

6. The group arrived at many of the same conclusions reported in the NASA report. Our final evaluation concurs with NASA's recommendation that despite inconclusive results, the microgravity programme should continue.

4. Summary of the views of the Review Group

The Review Group fully accept the assessments made by the Expert Reference Group in 3.1 and 3.2. They do not agree with the conclusions that the Expert Reference Group draw in 3.3 and 3.6 from these assessments. However, they accept that if the protein crystallisation programme is to continue, it should only do so subject to what is said in 3.4 and 3.5.

Evaluation of the APCF programme to date

1. The experiments reported on microgravity effect in protein (or macromolecular) crystallisation arising from the APCF programme are disappointing and have not yielded any interesting progress or breakthroughs, either in the understanding of crystal growth or in the production of better crystals. In consequence, they have not made any substantial contribution to the determination of protein structures. A test of the scientific performance of the APCF programme, by results published in internationally recognised journals, shows a poor result. The visibility of the APCF programme in the scientific community is low and in general it has not engaged the interest of the ground-based biological community.

2. The poor presentation of the results by participating scientists and the lack of open scientific debate on the experiments (e.g. at conferences) makes it difficult for the Review Group to evaluate the programme. Some part of our adverse judgments may therefore be due to faults of exposition or presentation, rather than to fundamental weaknesses in the programme itself; but we

have not found evidence on which a favourable judgment could be based, in view of the high cost of space flight experiments. The evaluation processes of the programme and the standardisation of experimental technique and analysis is poor. There appears to have been little or no systematic control over the selection of experiments, their coordination with ground-based experiments, or their subsequent reporting and publication. It should be noted that none of these defects relate to the limitations of the APCF as an instrument, and therefore none of them would necessarily be cured by its replacement by the PCDF.

3. The Panel recognised that if judged by the standards of national research councils and foundations, the APCF programme would be ended immediately. However, this option appears not to be realistic since two more space flights are scheduled, the first August 2001 flight will have taken place by the time this report is submitted and planning for the April 2002 flight must already be far advanced. But there should be a strong presumption that there should be no more APCF flights after these two, and the onus should be on the participating scientists to provide convincing reasons why this microgravity programme should continue at all. Those reasons should include an in-depth analysis of the outcomes of the two 2001-02 flights of APCF, as well as of previous flights. We suggest that ESA may wish to use the existing ESF Review Group to assess the case made by the participating scientists.

Specific recommendations

We now turn to the issues that the participating scientists will have to address, and to the conditions which in our view any microgravity programme will have to satisfy if it is to continue.

4. The experiments in any subsequent space flights, whether on APCF or not, should be designed in a focused and more structured way. There should be a major rearrangement of the procedures to select, perform and analyse the experiments of all flights.

5. Any future protein microgravity programme should be much more tightly focused, and the experiments flown should be restricted to ones that fit into this programme. This should involve the co-operation of national research councils, both because the space programme should be integrated with ground-based experiments and because it would need assured beam time for analysis – but it is not suggested that ESA should pay for either of these. For the purposes of reproducibility and comparability, ESA should establish a protocol on the collecting and handling of data.

6. All researchers should be required to scientifically justify at the time of the proposal the need for their research to be conducted in microgravity. If possible, the reports of the two APCF flights in 2001-02 should also include such justification. All selected experiments should clearly demonstrate both scientific value and the potential for new knowledge, and include a well-defined follow-up scheme including publication and other dissemination plans.

7. Experimental results, whether positive or negative, must be presented at national or international meetings in their field within one year of flight. A complete report must be produced within one year to allow full peer review evaluation by an independent panel. Results must also be reported to ESA within six months of flight, detailing how the results agreed or disagreed with their microgravity hypothesis and detailing analysis of resulting crystals or lack of crystals. As the evaluation of the results of the two APCF flights in 2001-02 will be critical to the future of that programme, this fact must be clear to the participants.

8. Future activity in microgravity protein crystal growth should concentrate on three areas:

- **The physics of crystal growth of proteins.** Emphasis should be placed on the basic physics of crystal growth using "model or representative proteins" to learn more about crystal growth on Earth and simultaneously in space. The potential of crystal growth in gels should be explored. This approach should be supported by one or two strong biophysical-biochemical groups in order to provide the physical studies with a broad platform with respect to the variety of the proteins, and their properties, involved. This coordinated approach should maximise the chances of success from the relatively small number of experiments that can be flown.
- **Statistical crystallisation tests using a large number of samples in high density experiments.** For example, a team, funded by ESA, could prepare the experiments and their analysis using samples provided by the biological community. The work of Garcia-Ruiz and associated groups using the counter diffusion technique has provided good examples.
- **Coordinated 3D structural analysis of proteins by neutron and X-ray diffraction.** Crystals resulting from experiments should be analysed at the earliest opportunity (to prevent degradation) using state-of-the-art facilities and experimental stations. Wherever feasible, crystals should be analysed using the same facility, experimental stations and analytical equipment to maximise comparability of samples. Whilst recognising the principles involved in application to and selection by facilities, a primary consideration for ESA and its researchers must be to ensure that crystals produced by microgravity experiments are analysed quickly and in an optimal manner.

9. The scientific community involved so far in the microgravity experiments has been largely insular. There is a urgent need to advertise the potential of microgravity crystal growth experiments in order to attract new and highly motivated groups (physicists, biologists, chemists) willing to do a long term scientific effort in multi-disciplinary teams. Amongst other mechanisms, this could be done via plenary lectures at major conferences and/or by review articles in leading journals.

10. Future flight hardware, for example PCDF, should be evaluated to make sure it meets the needs of all scientific investigations.

5. Recommendations of the review

1. If the participating scientists wish to make a case for continuing the APCF flights, a new evaluation of the APCF programme should be made as soon as possible after the 2001-02 flights. (The Review Group recognise that this may take some time, not least because the participants in the experiments must be allowed enough time to write up their results and, if they wish, to provide a synoptic view of the results of the programme so far. The evaluation could be made by the existing ESF Review Group) Until this evaluation is complete, ESA should make no commitment to further APCF flights. Results at the level achieved so far are not satisfactory and unless the APCF flights in 2001-02 either produce a major breakthrough in the theory of crystallisation, or produce results that are of real interest to the biological community, the APCF programme should be cancelled. Moreover it should be brought to life again only if ESA are satisfied that the new APCF programme is substantially more likely to succeed than the old one was.

2. If the APCF Programme is continued beyond the two flights in 2001-02, or if it is discontinued but subsequently revived, then the following changes, which will apply equally well to future microgravity protein crystallography programmes, should be made:

(i) There is a need to establish a stronger regime of evaluation, both so that new experiments fit with the goals of the programme, and that completed experiments are fully and properly analysed and fully reported upon.

(ii) Steps should be taken to broaden the scientific community informed about and attracted to the opportunities presented by the microgravity protein crystallography programme. Particular effort should be made to involve and bring together leading groups in the two key areas of physics of crystal growth and the analysis of biologically important proteins.

(iii) There must be an increased focus to the programme, using clear and improved coordination with ground-based activity to identify areas of opportunity for the space-based programme. Increased cooperation with both national funding agencies and major structural analysis facilities should be established.

(iv) Significant scientific progress should be demonstrated in either, preferably both, of the scientific areas of physics of crystal growth and analysis of biologically important proteins.

Review Panel

Review Group

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