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Self-assembling protein nanostructures - incorporating active functionality

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5e. TASK NUMBER

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14. ABSTRACT
This three year programme explored ways to engineer useful protein scaffolds that had functional components. The two objectives explored different approaches to this problem. Objective one successfully built high surface area nanoscaffolds from amyloid fibrils, and demonstrated that enzyme activity could be attached to this scaffold. These active nanoscaffolds were also embedded in permeable films and grown from surfaces. In the case of organophosphorous hydrolase, a significant increase in thermal stability was observed. Two papers were published

15. SUBJECT TERMS
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					643-364-2987

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Report Title

Self-assembling protein nanostructures - incorporating active functionality

ABSTRACT

This three year programme explored ways to engineer useful protein scaffolds that had functional components. The two objectives explored different approaches to this problem. Objective one successfully built high surface area nanoscaffolds from amyloid fibrils, and demonstrated that enzyme activity could be attached to this scaffold. These active nanoscaffolds were also embedded in permeable films and grown from surfaces. In the case of organophosphorous hydrolase, a significant increase in thermal stability was observed. Two papers were published in Biotechnology Progress and a new collaboration formed with Dr Charlene Mello (funded by ITC-PAC and SERDP).

Objective two explored domain swapping to build higher order structures from model protein ribonuclease and the biophysical and biochemical properties of this thermally stable enzyme were retained, irrespective of the quaternary structure. This work was published in Biochemistry and Biophysics Research Communications. Attention then switched to a more versatile fold for catalysis, the (alpha-beta)8 barrel. A model thermophilic enzyme, natively a tetramer, was engineered as a monomer, a dimer (submitted to Biochimica et Biophysica Acta) and preliminary characteristaion of higher order structures was obtained. Quaternary structure altered activity a controlled way, opening up the potential of triggered assembly of protein structures.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Number of Papers published in peer-reviewed journals: 1.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Number of Presentations: 2.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

Number of Manuscripts: 1.00

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Jagan Bilakanti	0.75
FTE Equivalent:	**0.75**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Juliet Gerrard	0.20	No
FTE Equivalent:	**0.20**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale): 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering: 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

see attachment

Technology Transfer

Self-assembling protein nanostructures – towards active functionality

Overview

Project AA06SPO0003_AB06CBT024 commenced its third and final year of funding in March 2009. The objectives for the third year were revised to include the outcomes from years one and two and to accommodate an alteration to the contract as a result of Crop & Food, our sub-contractors, being restructured to become Plant and Food. This programme explored ways to engineer useful protein scaffolds that had functional components. The two objectives explored different approaches to this problem.

Objective one, which was leveraged by funding from the NZ government, successfully built high surface area nanoscaffolds from amyloid fibrils, and demonstrated that enzyme activity (glucose oxidase and organophosphorous hydrolase) could be attached to this scaffold. These active nanoscaffolds were also embedded in permeable films and grown from surfaces. In the case of organophosphorous hydrolase, a significant increase in thermal stability was observed. Two papers were published in Biotechnology Progress describing this work, and a new collaboration formed with Dr Charlene Mello to explore the scale up and production of amyloid fibril nanoscaffolds from wastestream materials (funded by ITC-PAC and SERDP).

Objective two explored an alternative approach to building supramolecular structures from proteins, in which the original activity of the building block protein was retained. In a proof of principle study, domain swapping was used to build higher order structures from ribonuclease and the biophysical and biochemical properties of this thermally stable enzyme were retained, irrespective of the quaternary structure. This work was published in Biochemistry and Biophysics Research Communications. Attention then swithched to a more versatile fold for catalysis, the (alpha-beta)8 barrel. A model enzyme, natively a tetramer, was engineered as a monomer, a dimer and preliminary characteristaion of higher order structures was obtained. The work translated well to a thermophilic protein. This work has been submitted to Biochimica et Biophysica Acta. In this system, quaternary structure did impact on catalytic activity, but in a controlled way. Future work will explore the potential of triggered assembly of protein structures in response to an environmental trigger.

Technical update

Objective 1: Functional fibers & films

We successfully generated large (gram) quantities of amyloid fibrils from aqueous solution, and demonstrated that these could be functionalized with different enzymes. The chemistry of attachment needs to be optimized for each system, and not all enzymes trialed were successful (eg cytochrome P450 proved incompatible with the scaffold). However, for organophosphorous hydrolase, a significant increase in thermal stability was achieved.

A selection of other enzymes are currently being explored to establish the generality of this technology in our related NZ-government funded programme, which has been extended. The functionalized nanoscaffold can be incorporated into permeable films, and activity retained.

We are continuing development of templated growth of fibrils on a solid phase in our NZ-government funded program and have encouraging results in a relatively high throughput system in which the fibrils are grown from the surface of glass beads. Encouraging data continues to be obtained as we optimize the system. This technology can also be adapted as a means to functionalize surfaces, e.g. cotton, with our high surface area functionalizable scaffold.

Collaborative work in Dr Charlene Mello's laboratory continues to optimise the use of concentrated crude fish crystallins in glutaraldehyde crosslinked fibres.

Objective 2: Active self-assembled protein nanostructures

Our model study on domain swapping in ribonuclease was successfully completed and published. Final experiments and publication from the rest of this objective were somewhat disrupted by the Christchurch earthquakes, but resources are available to complete these.

Controlled assembly and characterisation of high order structures is ongoing for our model protein (DHDPS). In an effort to extend our studies of the DHDPS quaternary structure, we have successfully cloned and expressed *Arabidopsis* DHDPS enzyme. Characterisation was disrupted by the earthquakes, but will be completed shortly and we expect a further publication to arise from this work.

One of the challenges encountered in the re-engineering of the *E. coli* DHDPS enzyme to resemble that of *Nicotiana* (Figure 1), is that the mutants tend to form large aggregates. It is possible that these aggregates are the result of large assemblies of subunits using both interfaces, and characterisation is ongoing. While this aggregation is encouraging, we need to design methods to control it. Currently we are looking at two techniques to achieve this: engineering of disulfide crosslinks at key interface positions and control of assembly using pyruvate as a ligand.

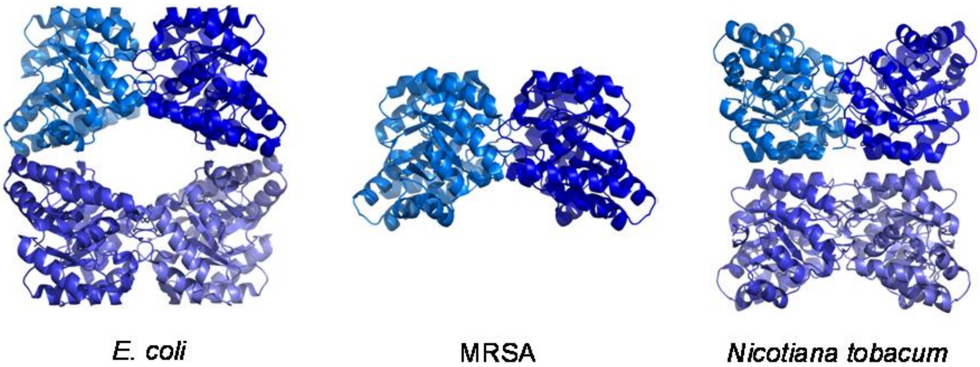

Figure 1. E. coli and Nicotiana tobacum DHDPS enzymes are naturally tetrameric, which MRSA is naturally a dimer. The E. coli and Nicotiana tetramers have different arrangements of the subunits, each made up of the basic dimeric unit.

After ruling out a variety of common methods employed to form disulfide crosslinks, we pursued the use of diamide for covalent scaffold assembly. We aim to engineer disulfide bonds at the dimer interface of *E. coli* and *Thermotoga* DHDPS. *Thermotoga* DHDPS naturally has a pair of cysteine residues at the dimer interface, but there is no evidence of disulfide bond formation. A variant of *E. coli* DHDPS was engineered to include a cysteine residue at this interface (L167C), but non-specific disulfide bonding was problematic during the oxidation stage. A naturally occurring cysteine residue at position 20 was removed, creating L167C/C20S, which partially resolved the problem. A triple mutant, L167C/C20S/C218S was also engineered, but expression and purification proved problematic.

Thus although higher order assembly was successful, and activity was retained in part, non specific chemistry confounded a detailed analysis of the product supramolecular assemblies.

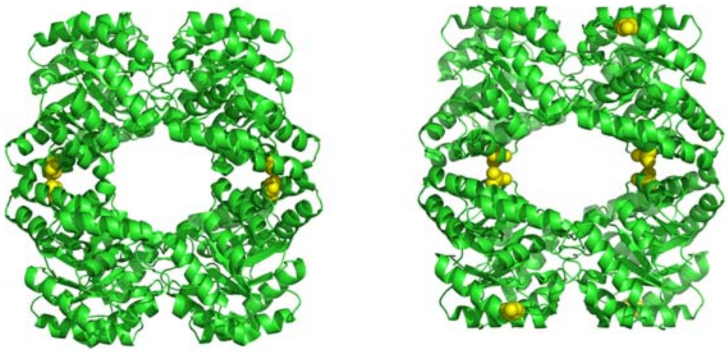

Figure 2: the location of cysteine residues at the interface of Thermotoga *DHDPS (left),* and E. coli *DHDPS (right).* E. coli *DHDPS has an additional cysteine residue at position 20 (also shown in the figure).*

More success was met in our exploration of thermostable building blocks. A clear relationship between thermostability and oligomeric state has been demonstrated for a thermostable variant of our model protein system. Structural analysis of variant proteins is being completed and a manuscript has been submitted to Biochimica et Biophysica Acta on the generation of thermostable building blocks. Another advantage of the *T. maritima* system is that it is possible to control the formation of higher order structures through the addition of substrate pyruvate, which opens up the exciting possibility of triggered protein assembly in future programmes.

Following successful applications for beamtime at the Australian Synchrotron, work continues in developing techniques to determine the size and shape of our protein assemblies in solution. This will be critical for the characterisation of our supramolecular structures in future programmes.