E1. JUSTIFICATION OF BUDGET REQUESTED FROM THE ARC

Personnel
As this project involves a multi-disciplinary approach, we require a research technician with at least three years experience (commencing at HEW level 5.4), particularly in the areas of molecular biology, cell-biology and/or protein/expression purification. This person will be employed to work on this project full-time, primarily to support CI Fairlie. Their duties will be performed at the WEHI where there is all the necessary reagents, equipment and expertise for apoptosis research. Their duties will include preparation of DNA constructs, expression and purification of recombinant proteins and routine maintenance of cell lines.

Maintenance – lab consumables
Molecular biology
This project will involve making numerous DNA constructs for expression of genes in E. coli and in mammalian cells to test their functioning. Cloning requires obtaining sponge, schistsosome and cnidaria gene templates which is most efficiently done through gene synthesis where all the genes of interest can be codon-optimised for heterologous expression. Transfer into various expression vectors requires oligonucleotides for PCR reactions and various enzymes such as restriction enzymes, ligases etc. All constructs need to be verified by DNA sequencing, a service provided by AGRF
Oligonucleotides (20@$35 ea): $700
Synthetic genes (6@$600): $3600
Enzymes (restriction, polymerases, ligase, kinase): $1500
DNA purification kits (miniprep/maxiprep/gel purification/PCR clean-up): $1800
Miscellaneous incl. agar, bacteria cell strains, media, antibiotics, DNA sequencing costs: $2000

Biochemistry/Protein expression
Each gene of interest will be expressed in E.coli which requires media to grow the cultures and various chromatography media for their purification. Functional assays using Biacore will examine each genes ability to engage BH3 domains which requires synthetic peptides of BH3 sequences to be made. We also require antibodies for Western blot analysis. Crystallization trials will be performed at C3, a pay-per-screen service.
Synthetic peptides (6@$1000) $6000
Media: $1000
Chromatography reagents/columns: $1500
Biacore consumables including chips: $850
Antibodies: $1500
General laboratory chemicals incl. imidazole, IPTG, DTT: $1000
X-ray crystallography - Funds are required for crystallization trials, which will be conducted at the Bio21 Collaborative Crystallisation Centre, Parkville (8-plate screen cost is $300). Other costs include concentrators and filters: total $2500

Cell culture
Functioning of genes will be examined in mammalian cells grown in culture. This requires media, plasticware and serum. Functional assays require FACS analysis, a user-pays service at WEHI. Introduction of genes into mammalian cells requires transfection reagents.
Plasticware: $3000
Tissue culture media/fetal bovine serum: $3000
Transfection reagents: $1200
FACS costs: $2500