PROGRESS REPORT FOR AINGRA09137

PROJECT TITLE
Integrating structural biology techniques to understand endogenous Src-family kinase inhibition toward the development of novel anti-cancer drugs

INVESTIGATOR(S)

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Students
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ANSTO Investigators
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Specialist Committee
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SCIENTIFIC OBJECTIVES

Use small-angle x-ray scattering (SAXS) to identify the mode of inhibitory interaction between Src-family kinases (SFKs) and Csk-family kinases (CFKs).

For each SFK/CFK subunit and the SFK-CFK complex:
(a) Measure intensity at zero angle (I(0)) to characterise the subunit stoichiometry for self-association and complex formation
(b) Determine the radius of gyration (Rg)
(c) Estimate the maximum dimension (Dmax)
(d) Calculate dummy atom models
(e) Compare SAXS profiles with atomic resolution models derived from x-ray crystallography
(f) Establish whether the known modes of interaction fit with the complex formed in solution
(g) Indicate initial feasibility of contrast variation SANS studies of the SFK-CFK complex

During the course of the Award it was established that the initial hypothesis was flawed and the project aims were realigned to address the following hypothesis:

The unique regions (URs) of SFKs have not been crystallised, presumably due to their intrinsically unfolded nature. For several Src-family kinases there is evidence that phosphorylation of a tyrosine residue in the unique region is involved in an additional regulatory mechanism. We propose that the flexibility of the unfolded unique region allows it to bind to the SH2 domain, thereby displacing the C-terminal tail and stabilising an active conformation of the kinase domain.

PROGRESS REPORT and RESEARCH OUTCOMES

Summary of results:
The SH2 domain of haematopoitic cell kinase (Hck) UR/SH3/SH2 is capable of binding a phosphopeptide containing the Hck UR autophosphorylation motif pYVPDPT (pUR). There is no secondary structure change in the presence of the pUR ligand. The affinity of pUR for Hck SH2 domain alone was similar to that of the Hck-UR/SH3/SH2 construct, suggesting that the unphosphorylated unique region has little influence on SH2 ligand binding. Initially, aggregation interfered with shape determination; however, refinement of the purification protocol enabled structural parameters of the nonphosphorylated Hck-UR/SH3/SH2 construct to be elucidated by analytical ultracentrifugation and Small-angle x-ray scattering (SAXS).

Outcomes:
Results from this AINSE Award were presented at the following conferences:
DATA

To determine the shape of the Hck-UR/SH3/SH2 construct small-angle x-ray scattering was used. A small amount of aggregation was observed. Interestingly, the aggregation was eliminated upon addition of a peptide containing the sequence pYEEI. The radius of gyration and $D_{\text{max}}$ were measured in the presence and absence of the pYEEI ligand.

Small-angle x-ray scattering profiles of Hck-UR/SH3/SH2 with and without the pYEEI peptide. Aggregation, indicated by the upturn at low $q$, disappeared upon ligand binding.

$D_{\text{max}} = 93 \text{ Å}$
Pair distribution function of Hck-UR/SH3/SH2 in the presence of the pYEEI peptide. The presence of multiple peaks is consistent with the expected structure of the multidomain Hck-UR/SH3/SH2 construct.

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<tr>
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<th>$R_g$</th>
<th>$D_{max}$</th>
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<tbody>
<tr>
<td>Hck-UR/SH3/SH2</td>
<td>32.3 ± 0.4 Å</td>
<td>125 Å</td>
</tr>
<tr>
<td>Hck-UR/SH3/SH2 +pYEEI</td>
<td>28.4 ± 0.6 Å</td>
<td>93 Å</td>
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Signature of Investigator preparing the report for
After signing this report please fax this page with your signature for our files

| Proj: AINGRA09137 
| Date: |

PUBLICATIONS / REPORTS arising as a result of your work.

None as yet, planned for submission during 2011

**PhD STUDENTS**

Natalie J. Gunn, due to Complete September 2011 (3.5 years) “Structural Characterization of SH2-mediated regulatory mechanisms for Src-family kinases and their upstream regulators”