The principal objective of this project is to develop an understanding of the molecular self-organisation process in elastic protein resilin in aqueous dispersion. This research will promote fundamental knowledge relating to resilin protein’s structure & special organization, which is responsible for the formation of unique and highly responsive functional elastomeric gels from such protein. The sensitivity of the organisation process on variables such as temperature, concentration and pH will be quantified.

Research outcomes
The biomimetic synthetic resilin developed was confirmed to have elasticity and resilience (~94%) comparable to the natural resilin and also observed to be less sensitive to temperature [1-3]. However, resilin is highly resilient only in hydrated state and structural organization of synthetic resilin in presence of water and other polar solvent is extremely important. Small angle neutron scattering (SANS) and TEM was used to investigate the effect of water in the molecular organization of resilin. This will also provide information about the hydrophobic aggregate size and shape. To support the size and shape of the resilin, TEM and Micro-TA instruments was used.

Experimental
Details about the cloning, expression and invitro crosslinking of insect proresilin using dityrosine cross-links via Ru (II)-mediated photochemistry to prepare crosslinked resilin like polypeptide has been discussed elsewhere [1]. Small Angle Neutron Scattering (SANS): Small-angle neutron scattering (SANS) experiments were performed with the small-angle neutron diffractometer at Bragg Institute, ANSTO, Lucas Height, NSW using the AUSANS instrument. The sample to detector distance was fixed at 5 meters. The sample temperature was controlled to within ± 0.5 °C using a circulating bath. The two-dimensional data were circularly averaged to produce one-dimensional scattering intensity I(Q) versus Q. In the experiments, heavy water was used.

Results and Discussion
SANS investigation
SANS has been proven to be effective method in providing mesoscale structural information for gel systems. Many different proteins such as ribosomal proteins [4], bovine serum albumin and ovalbumin [5,6], bovine pancreatic trypsin inhibitor [7], human serum albumin [8], horse heart cytochrome C [9,10], wheat gluten proteins [11], spider dragline silk [12] etc. have been investigated using scattering techniques and information on their shape, size has also been reported. Many attempts have been made to describe soft-gel scattering data by using two or multiple correlation length, however no theory exists that satisfactorily describe the gel structure.
Table 1: Change in power law exponent ‘D’ with hydration

<table>
<thead>
<tr>
<th>Water uptake (wt%)</th>
<th>D</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>2.7</td>
</tr>
<tr>
<td>13</td>
<td>1.7</td>
</tr>
<tr>
<td>20</td>
<td>1.9</td>
</tr>
<tr>
<td>24</td>
<td>1.7</td>
</tr>
<tr>
<td>85</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Figure 1: SANS profiles of resilin at various water uptakes, equilibrate state (A), 20% water uptake (B) and dry condition (C); solid line is Power Law curve.

Figure 2: Water uptake by resilin wt% with time

The evolution of intensity and shape of the scattering cross-section with hydration is clearly visible in the low and intermediate q regime. For the q range between 0.01 and 0.03 Å⁻¹, the intensity values increase with increase in water content. This behaviour at low q reflects the role of the structure factor and thus depends on protein-protein interaction. The neutron scattering spectra of different samples overlap in the range 0.03 to 0.1 Å⁻¹ except the fully hydrated sample. A broad peak at q* ~ 0.015 Å⁻¹ was identified for the dry sample indicating presence of aggregate structure with a predominant characteristics length (q=2π/q*) of 418 Å. For globular particle systems in solutions, the repulsive interaction between the particles usually produces only a broad single peak at the small ‘q’ region. The distinct characteristics q* is not observed for the hydrated sample within the experimental scale, which may be due to the shift of the domain peak of the wet sample towards lower q regime. It should be noted that lower q range SANS instrument needs to be used in order to have a whole concept of the water structural organization in resilin. The anisotropic pattern of the scattering cross-section also observed to be changed with absorption of water, confirming the change in the anisotropic structure of resilin. The other interesting profile is the change in the slope at low q region with water sorption. The SANS intensities in the regions follow a power-law (I(Q) ~ Q⁻D) variation with respect to q for q<0.04Å⁻¹; for higher values of q they are determined by the micellar form factor. The exponent D extracted using the power law model shows a systematic variation with hydration level. With the use of simple models of structure factor of fractal aggregates; it shows a large change in fractal dimensionality from ~2.5 in dry sample to 1.1 (linear structure) in fully hydrated conditions.

The values in Table 1 show the transformation of the protein aggregate shape that leads to change the physical characteristics of resilin from glassy to elastic state. The dry sample (glassy state) has a disc shape, which is corresponded to an exponent of ~3. The protein aggregate shape transforms to thin disc with increasing of water content that is corresponded to an exponent of ~2 for the resilin contained from 13 to 24 wt% water uptake. At the equilibrium hydration level (fully elastic state), the protein aggregate shape transforms to thin rod that is corresponded to an exponent of ~1.
Figure 3 shows a TEM image of dry stained resilin sample, which clearly indicate the aggregate nature of the dry resilin sample. Temperature between 10 to 60 °C and pH from 5-10 does not show significant influence on the self-organization in resilin gels.

Figure 3: TEM image of Dry resilin hydrogel from 2 mg/ml proresilin (bottom). The scale bar represents 200 nm

Conclusions and future works
Self-organisation of the protein in hydrated state is responsible for the unusual elastic behaviour. Hydrophobic aggregate shape of resilin is disc-like in dry state with strong intermolecular interaction and exhibit brittle plastic-like behavior. With increase in hydration the aggregate shape change from thick disc through to thin disc to thin rod shape and physical properties change progressively change from plastic to viscoelastic to highly elastic material.

Further Investigation
The details of the local structure of the synthetic resilin cluster are not fully understood yet from this investigation as further investigation using lower and higher Q ranges are essential.

References

Signature of Investigator preparing the report
After signing this report please fax this page with your signature for our files

[Signature]

Date:
