Taking steps towards engineering a modular HET-s prion-based hydrogel for biomaterial functionalisation.

Hector Cross[1], Mateo Martín-Nieto[1], Katie Wei[1], Vaiva Knabikaitė[1], Vasily Vanifatov[1], Daphne Armengol[1], Leah Gibson-Duff[1], Harrish Shivakumar[1], Debabrata Dutta[2], Lenka Mrazova[2], Ben Shin[2], Stephen Matthews[3], Szymon W. Manka[2]

[1] Imperial College London iGEM 2026, Imperial College London, London, UK
[2] Department of Infectious Disease, Imperial College London, London, UK
[3] Department of Life Sciences, Imperial College London, London, UK

The intersection of prion biology and synthetic biology has long remained somewhat of an uncharted territory. The ability to manipulate the structure and role of functional prions with synthetic biology would open new gateways for prionic research, in fields, such as biomaterial design, where the high stability and aggregational properties of these molecules are highly lucrative. The mechanical strength, high thermodynamic stability, resistance to proteolysis, and templated self-assembly place functional prions at the frontier of attractive scaffolds. Contrary to prion-based hydrogels, protein-based materials, such as collagen, gelatin, and fibrin hydrogels, are proteolytically unstable and mechanically weak without cytotoxic crosslinking, limiting their functional applications.

We selected the HET-s prion-forming domain that plays a role in fungal heterokaryon incompatibility in the filamentous fungus *Podospora anserina* as a chief component of our biomaterial, due to its conformational fidelity. Unlike Sup35p, the functional prion predominantly used by previous iGEM teams, whose polymorphism yields variable assembly geometry, HET-s adopts a well-defined two-rung β-solenoid with a triangular hydrophobic core stabilised by asparagine ladders and salt bridges.

In our project, we utilise the very same structural consistency of HET-s to build a modular and self-assembling prion-based hydrogel. We engineer multiple HET-s constructs, varying features, such as tandem repeat number and cysteine content, to form the structural strut of the hydrogel. We further crosslink the prion fibril termini to streptavidin tetrahedral nodes via site-specific cysteine handle conjugation to desthiobiotin. Reversible crosslinking is achievable through competitive biotin displacement. All necessary protein components have been successfully expressed and purified. Electron microscopy confirmed wild-type HET-s fibrillation at pH 7. Preliminary one-dimensional NMR data are consistent with partial aggregation of two cysteine-mutated HET-s constructs into higher-molecular-weight species, with their fibrillar identity awaiting confirmation.

To enhance the versatility of our hydrogel, we have engineered a subset of our constructs to carry enhanced Sortase A LPETG recognition motifs at the N-termini, enabling covalent and site-specific post-assembly ligation with arbitrary cargo proteins. As a proof-of-concept of such functionalisation, we aim to co-localise two cellulose-degrading enzymes, a cellobiohydrolase and the Cel1A β-glucosidase, on the hydrogel to test whether this fibril-based architecture can reproduce the proximity-driven catalytic synergy of the natural cellulosome, a combination not previously attempted with an amyloid scaffold. Evaluation of enhanced Sortase A variants for cargo ligation is underway, using eGFP as an initial model substrate.

All genetically-encoded components of our hydrogel have been deposited as BioBricks in the iGEM Registry of Standard Biological Parts, making the material accessible to any group without proprietary reagents. This work was carried out by an undergraduate team within the iGEM competition. To our knowledge it is the first iGEM project to employ HET-s and explore its potential in biomaterials; it is also the first prion-focused project in the last 9 years of iGEM. Beyond the material itself, this work bridges prion biology and synthetic biology, two fields whose translation has been slowed by public scrutiny. By combining our research with public engagement, we demonstrate the profound potential of functional prions in hopes of paving an exciting future for their applications.