Designing an iGEM Project Biosecurity Framework

1. Foreword

The UK Biological Security Strategy underlines four pillars adopted by the UK to appropriately and efficiently respond to biological risks: 1) Understanding the biological risks faced today and those that could be faced in the future, 2) Preventing biological risks from emerging, where possible, or from threatening the UK and its interests, 3) Detecting, characterising and reporting biological risks when they do emerge as early and reliable as possible, and 4) Responding to biological risks that have reached the UK or its interests in order to lessen their impact and allow a rapid return to normal business practices. In part 1 of this document, we have designed a general framework which that can be utilised by any iGEM team for their project, especially those working on a platform technology. In part 2, we then explore the application of biosecurity pillars to the development, distribution and downstream use of our HET-s protein hydrogel platform. At the end of this section, we provide a Biosecurity Framework Deliverables Checklist inspired by our own project that can be used by other teams to utilise specific components in their biosecurity risk assessments.

This framework is built from a bottom-up rather than top-down approach, following our consultation with Mr. Edward You, former Supervisory Special Agent in the FBI's Weapons of Mass Destruction Directorate. We also followed the recommendation of Mr. Henrik Hagemann, former iGEMer turned entrepreneur, to anticipate and overcome regulatory hurdles and difficulties related to stakeholder and public acceptance. The governance of biological materials, which are inherently open-source and dual-use and increasingly utilised at bench scale in ordinary laboratories, can be rendered effective not by regular surveillance of infrastructure which may function for other weapon categories, but rather by embedding our design, documentation and distribution strategies within a defined framework.

PART 1

2. Communicating a dual-use material

The feature that makes a platform technology commercially attractive - that is works with an arbitrary input - is the same feature that renders it a biosecurity concern. This double-edged feature must be honestly communicated to investors and regulators in a biosecurity framework that explicitly states the points of potential beneficial use and misuse. This framing is not solely an act of transparency, but also a way to protect a team commercially, as an investor who later learns of dual-use concerns from a third party could treat this finding as a concealed liability. An investor who is shown that due diligence has already been performed by scoping risk, red-teaming and turning this into written conditions of use provides credibility for a team and

incentive to invest. Two points can be outlined for any audience when introducing them to your technology:

1) **Where the risk actually sits**, which is rarely identified by a non-specialist. Unfamiliar words can attach an element of alarm rather than understanding mechanisms that could cause harm. Naming a risk that can be misidentified avoids any uncertainty.
2) **What the technology adds** to a harm that is already possible.

3. Developing controls

A platform technology ships without its payload, so there is nothing in the material itself to screen at the point of sale. Control is exercised at several points across the material lifecycle. But any biosecurity risk assessment should state which control is attached where:

Control	Function
Scope by application	Establishes a separate risk profile per downstream use case.
Governance of the interface	Including a payload policy, performing red-teaming and declaring objects of concern when a user's material joins the team's material.
Governance of the design	Decides between direct release, release with a stewardship note or withholding.
Payload sign-off	Named reviewers must approve any new payloads.
Distribution controls	Involves customer verification, terms of use and a provenance record per batch.
Decommissioning	Describes a validated off-switch and minimum effluent testing panel for any party who intends to deploy the material at scale.

4. Avoiding regrettable substitution

A regrettable substitution occurs when a material is replaced by an alternative that avoids a known hazard while introducing an unassessed hazard of its own, the latter of which only being identified once the substitute has been widely adopted. This problem was pointed out to us by Mr. Harry MacPherson and arises when a substitute is adopted on the strength of a single property being avoided rather than

conducting a full hazard profile comparing both materials. Synthetic biology is structurally exposed to this contingency, as projects are frequently pitched as more efficient or sustainable replacements for current technologies, and because biological material is often perceived as safer than chemical material by default. Yet a protein-based material remains a substance that can shed, leach whatever payload it carries, accumulate chemicals that it absorbs and generate a waste stream that must be managed. A team proposing a substitute should therefore assess the replacement against a panel of identical criteria as the original/traditional technology (the incumbent). Teams should:

- Identify points where the technologies differ, and why.
- List the hazard classes on which the incumbent is being assessed, and assess the substitute on all of them as well.
- Identify hazards that are novel to the substitute yet absent in the incumbent.
- Publish the comparison, including the axes on which the substitute performs worse.

This maps directly onto the requirements of an Environmental Impact Assessment, where the Environmental Statement must describe alternatives considered that may be less hazardous than the substitute proposed, and perform baseline studies to measure the impact of the novel technology once it has been implemented. A substitution argument made without a comparative panel will not be seen as credible.

5. Communication with beneficiary communities

Biosecurity expert Mr. Edward You's closing advice was to engage the communities who would use technology before approaching those who would govern it. A regulator that is asked to assess a technology that nobody is currently using is likely to classify it in whatever category appears the most appropriate, which may not fully assess the scope of its risk. However, a regulator that is approached by an established user community and the experts building it can respond to evidence of how the technology is actually handled, at what scale, and with what controls are already in place.

This approach is tractable for platform technologies in particular as their users are not the general public: teams should identify the set of research groups, developers and industrial buyers that would adopt the material, approach them individually and treat each conversion as a test of whether the technology truly offers an advantage over the incumbent that the community already uses, and whether the controls proposed by the framework are within the capabilities of the community's current workflow - a control that a user community cannot implement cannot serve as a control. Questions of waste, effluent and end-of-life for the technology should be

raised in these conversions as they will be used to guide the Environmental Statement.

PART 2

Each section described below addresses the corresponding section of Part 1 to show how the framework can be applied for our HET-s hydrogel. We discuss the dual-use framing we used with investors and regulators, our substitution claims against the incumbents we displace and the communities we engaged before attempting to scale-up.

6. Communicating our dual-use material

The commercial case to be made for our hydrogel rests on the LPETG/triple-glycine sortase junction. The scaffold is payload-agnostic, allowing any protein bearing a GGG terminus to be ligated onto it. The scaffold itself represents the product sold: an immobilisation platform designed to accept an arbitrary enzyme proposed by a customer. Immobilisation can then confer increased proteolytic activity, stability on the amyloid core, retained catalytic activity, all delivered in a form that is relatively simple to handle, store and transport. The breadth of the market (prion and functional amyloid research, multi-enzyme cascade design, biocatalysis and industrial effluent treatment), is inseparable from the breadth of payloads that can be attached.

6.1 Governance of the Interface

This technology without conditions of use could not be defended by a biosafety committee, an export control authority or an insurer, and would therefore not be marketable. Thus, justifying the need for the following controls before agreeing to sell the platform:

- A **payload policy** should be published alongside the part, containing an explicit list of enzyme classes that the scaffold must not be used to immobilise. This includes toxins, select-agent-derived enzymes, or agents on the UK Strategic Export Control list which compile lists of dual-use items from 7 pieces of international legislation to set out which goods require export authorisation from the United Kingdom. This policy should be treated as a condition of use rather than expectation, with customer intended payloads screened before sharing of the functionalisation protocol.
- **Red-teaming** on behalf of our team should be performed ahead of time to adopt a white-hat approach and find vulnerabilities in the system following a payload request. The following questions should be asked: what are the most harmful GGG-tagged payloads, what does immobilisation add, would any design adjustment alter potential risks?

- An **object of concern** should be declared for the functionalisation protocol, which allows individual to take an arbitrary protein and immobilise it in a stable and active form (not the HET-s sequence, which is already published, non-pathogenic and possesses no mammalian transmission route).

A distribution model would be governed by our team, as we would be the initiating point in the supply chain.

6.2 Responsible Distribution

Once our material exits the laboratory, none of our internal controls would remain attached, except for the conditions established at the time of sale. We would need to verify the customer and set of conditions of use rather than screening the material pre-sale, and we recognise onward transfer as the point where these controls would be the most easily lost.

- **Terms of use** would carry the payload policy, a no-transfer clause once purchased and acknowledgement of the decommissioning protocol.
- **Customer verification** would be applied for the scaffold, with institutional affiliation checked and an internal escalation route already prepared in the case of suspicious applications.
- **Stated intended use** would ask customers what they intend to immobilise, creating a record for future reference if misused .
- **A provenance record and certificate of analysis** should be documented per batch to track units in the case of misuse downstream. This should include the lot number, concentration and how it was determined, purity by SDS-PAGE with a densitometry figure, buffer composition, storage conditions and expiry. Fibril-specific data such as ThT anisotropy endpoint, TEM length distribution and the density of biotin labelling should also be provided.

6.3 Guardianship

Mr. You emphasised the notion of all individuals acting to safeguard science, with biologists in particular assuming personal responsibility to identify misuse rather than presuming that someone else has verified. This will require interdisciplinary guardianship at multiple stages of the production and sales process, in the forms of:

- **A payload sign-off**, whereby no new effector protein enters the sortase pipeline without a dedicated individual signing off on the payload in question.
- **Interdisciplinary composition** of the team spanning wet-lab, dry-lab, business and policy. This will ensure that suspicious instances are flagged before the product can be distributed further down the supply chain. As highlighted by Mr. You, the UK Home Office and Metropolitan suggested that the UK does not currently possess coordinators who are in contact with local

universities, laboratories and community DIY-bio spaces. In the case of a suspicious instance, a contact should have been established prior to trigger a parallel law-enforcement and public-health response communicated through human contact, as opposed to a more general emergency call response or AI processing of a raised alarm.

6.4 Governance of the Design

Constructs, dry-lab parameters and model outputs should be appropriately documented via direct release into the wiki and/or parts registry, or released alongside warrant stewardship notes if the output constitutes a transferable capability rather than a result.

- The HET-s protein forming domain sequence, all constructs maps, streptavidin, streptavidin variants are already published and carry no restriction. The node architecture, ratio-tuning approach, expression & purification protocols, fibrillation conditions and ThT kinetics, rheology, pore size and swelling should also also be directly released into the wiki and registry. The biotin disassembly protocol in particular should be pushed the hardest to release, given its value as a reversible assembly mechanism scales with adoption. All negative results, failed constructs and structural prediction outputs will equally be released.
- The HET-s strut parts, LPETG and GGG-tagged variants and sortase A coding sequence are reusable units suited for the parts registry for others to build on.
- Outputs that generalise beyond our own system warrant a stewardship note, rather than withholding. A validated relationship between linker length, node ratio and retained activity across enzyme classes, or a model predicting retained activity for an untested payload, would describe a method for immobilising an arbitrary protein in a stable and active form rather than a result about our hydrogel. These should be released alongside the payload policy in the same document, so that the gel capabilities and conditions of use travel together.
- The only element to withhold should be procurement-identifying details for payloads named on our own exclusion list. The red-team reasoning should be published without a route that traces back to the source of these results.
- A disclosure decision record should report which components were released and why so that the reasoning behind each decision is auditable.

6.5 Application risk scoping

Following Mr. You's initial recommendation to define the specific downstream applications of our hydrogel, in order to determine both our target audience and risk profile, three primary applications are underlined. Identifying the varying severity

profiles of each application opposes the use of a one-size-fits all restriction tiered by a party that may not possess the knowledge to tier these accurately, thereby reducing the risk of their misuse on a smaller scale.

Application	Product	Primary audience	Dominant Risk
Core hydrogel scaffold	HET-s struts, streptavidin nodes, eSortase A kit, customer-appended GGG-tagged protein	Synthetic biologists, prion and functional-amyloid researchers, biocatalysis groups	Payload does not exist when product is shipped - screening must be performed as customer verification and provision of conditions of use, given its dual-use capacity.
Cellulosome mimic	Co-localised CBH and glucose-tolerant BG, proximity-driven synergy	Academic kinetic groups, biomass processing R&D	Lowest risk application as laboratory use will be contained. Fibril persistence in waste presents a risk depending on disassembly efficiency.
CotA laccase gel	Immobilised CotA for dye decolourisation and industrial effluent	Textile, dye and wastewater treatment plants	Deliberate environmental release (see further details below)

The CotA application serves as an example of how a purchase enquiry would be assessed. Suppose that a customer proposing to immobilise CotA for dye decolourisation asks to deploy our material into an environmental discharge stream, which raises concerns beyond contained laboratory uses. Laccase oxidation degrades the chromophore without causing mineralisation of the dye, therefore, a colourless effluent would exit while carrying transformation products that the absorbance readout cannot detect, and these may retain or exceed the toxicity of the parent compound. Furthermore, the gel itself may shed HET-s fragments, streptavidin nodes, biotin linker and copper leached from CotA's four-copper centre which would exit alongside the treated water. Copper is an ecotoxic heavy metal under consented parameters in UK trade effluent permits. Furthermore, the π–π stacking and hydrophobic interactions we propose to concentrate aromatic pollutants near CotA would render the gel a loaded adsorbent requiring a defined disposal

route, unlike an unexposed scaffold which does not carry a payload. We would therefore ask this customer to confirm their discharge consent and their plan for ecotoxicity screening alongside the absorbance readout, since what actually exits a site depends on flow rate, dilution, effluent matrix and treatment train, which cannot be determined from our side. In return, we would supply the shed-component panel, including the quantity of copper leached out of material per reuse cycle. By characterising the biomaterial ourselves and asking customers to characterise its deployment before a sale can be made, we would establish where responsibility is transferred at the point of future delivery.

7. So where does our risk sit?

The term that generates alarm when presenting our project is ‘prion’ - but this serves as an example of risk misidentification. HET-s is a functional prion mediating heterokaryon incompatibility in *Podospora anserina.* It has no PrP homology, no mammalian transmission route and no infectivity in the sense that this word implies. Its sequence is also already published and carries no restriction. We state this early, clearly (in layman terms if necessary) and with citations in every document that reaches a non-specialist.

7.1 Decommisionning

The containment issue with our project is much narrower and unrelated to transmission: HET-s fibrils are protease and heat resistant, so standard autoclaving is not a reliable decontamination route. In order to address issues of containment and decommissioning, the following strategies should be adopted:

- **Validation of the ‘off-switch’** should be performed to confirm biotin- or low-pH-triggered disassembly, which currently remains a proof-of-concept endpoint. Safety data should report metrics such as disassembly efficiency, residual fibril content and time-until-inactivation.
- **Waste stream definition** should be provided within the distributed protocol and internal SOP. The cell-free design of the core gel removes the biological waste requirement entirely across all use cases, with the only disposal constraint remaining payload-specific and arising from what the gel has been exposed to rather than the gel itself. In the case of CotA immobilisation, for example, adsorbed aromatics would require a dye-waste route that follows protocols of the site’s existing spend-adsorbent route and copper leached from CotA would require a consented parameter in UK trade effluent permits based on its customer-measured concentration in the discharge. Because what the gel accumulates is determined entirely by what passes through it, the disposal route cannot be specified from our side. We will therefore ask that customers declare disassembly (once the efficiency is proven) and then disposal as a condition of sale a compliant disposal route for the spent

cartridge themselves at end-of-life, and that they will classify the waste against their own dye inventory. We will also supply the shed-component panel that includes copper leached per reuse cycle so that this classification can be made against our material.

- **Clear statement of the non-infectivity of HET-s**, which is a functional prion mediating heterokaryon incompatibility in *Podospora anserina*. It does not possess PrP homology, nor a transmission route. In official documents, this should be stated clearly, early on and supported by citations, as the word 'prion' is a source of alarm. The approach of familiar framing when it comes to alarming terms described in the Ethics handbook can be reserved for education around prions.
- **Effluent characterisation** is required as the output stream generated from the immobilisation of an arbitrary input cannot be predicted and must be measured first. For example, scaffold may shed in the effluent, copper will be present from payload metalloenzymes and the gel may have altered kinetics from high local enzyme density. A platform-level obligation should be stated so that any individual or group deploying the gel at scale first characterises the discharge stream and specifies the minimum testing panel (chemical oxygen demand, ecotoxicity, leached metals, shed protein, etc). Each use case should then publicly document its payload-specific parameters.

Hence, the main biosecurity risk remains our functionalisation protocol - not the material itself. The HET-s sequence, construct maps and traptavadin and streptavidin variants are published and confer nothing. What constitutes a transferable capability is the documented method for attaching an arbitrary GGG-tagged protein onto the scaffold, and the relationship behind this mechanism. A validated relationship between linker length, node ratio and retained activity across enzyme classes or a model predicting retained activity for an untested payload is not a result about our hydrogel - it is a method for immobilising an arbitrary protein. This is why such outputs are released with a stewardship note and the payload policy in the same document: so that the standardised parts and their conditions of use can travel together. The only element we withhold outright is procurement-identifying detail for payloads on our exclusion list.

Two features of our platform restrain risk considerably:

1) The platform is **cell-free**: the product is designed to be purified HET-s struts, streptavidin nodes and enhanced sortase A. Although engineered organisms are required during the production process, none will exit the laboratory, thereby removing the risks of environmental release and horizontal-transfer which are highly prominent in synthetic biology risk assessments.
2) The platform has a designed **disassembly switch**: free biotin is able to outcompete the biotin linker, saturating the streptavidin nodes and leading to network dissociation, with sonication/vortexing completing the breakdown of

struts. HET-s disassembly is also possible at low pH, as outlined by Prof. Salvador Ventura.

The scaffold will also ship without a payload, so no screening of the material itself is possible or meaningful. Controls will instead be conducted through customer verification and conditions of use at the point of sale, hence why our framework is focused on distribution controls rather than material controls.

8. Our substitution claims

Communities must be shown that our hydrogel offers advantages of current market options. We are more expensive per gram than every incumbent we are positioned against (see our Entrepreneurship Guide), which means that the case for paying more is dependent on the different function of the gel. A substitution argument on those terms is only credible if we also report where we perform worse compared to benchmarks. For us, this includes assessing stability advantages over repeated cycles once the gel is formed, whether orientation control is worth the cost to customers, residual PEG in our own linker (when comparing to current PEG hydrogels, for instance), and transformation product toxicity compared to current colour removal methods in dye effluents. Overall, the cartridge format as per Dr. Ben Reeve's suggestion allows current treatment trains to be altered at one specific step without causing major downstream changes. This format keeps the gel contained and allows us to take the material back at the end of its life rather than leaving it in the customer's waste stream.

9. Communication with Beneficiary Communities of our Hydrogel

Mr. You's closing advice was to engage with the communities who would employ our technology before approaching those who would govern it. This reasoning is practical in the sense that a regulator asked to assess a technology that nobody is using yet will tier it in whatever category seems the most appropriate, whereas one approached by an established user community would respond to evidence of how it is actually handled. This approach would be manageable, given that our users are not the general public: what will reach customers are fermentation feedstocks, bleached fibre, etc, which reside several downstream of our hydrogel application. Therefore the communities to engage with are a smaller and identifiable set of research groups and industrial buyers that can be reached individually. As previously mentioned, our target audiences are primarily:

1) Synthetic biologists building multi-enzyme cascades and seeking spatial organisation without a cell-dependent chassis
2) 2) Prion and functional amyloid researchers, which represent a community that possesses a direct stake in reframing prions as biomaterials rather than pathogens

3) 3) Biocatalysis, hydrogel and enzyme immobilisation groups working on optimising reusability and operational stability.

Engaging in discussions with industrial partners such as biorefineries, textile finishing and firms attempting to convert lignin-rich waste into useful commercial products for the maintaining of a circular bioeconomy would establish the level of demand and concerns regarding for our product before implementing specific protective policies. We engaged the following stakeholders to have such conversations:

Stakeholder	**Role and Expertise**	**What the conversation tested**
Hilary Sherman	Senior Scientist at Corning Life Sciences Led application development for Matrigel	Verified our substitution claim against the closest commercial equivalents to our material. Hilary identified ordered, controlled synthetic hydrogels as a genuine market gap.
Harry MacPherson	Materials scientist at Deep Science Ventures specialised in carbon capture Author of a toxicity report with the Grantham Foundation on chemical pollution as a global health emergency	Proposed that we adopt the regrettable substitution guideline and redirected our success metric for enzyme immobilisation from catalytic efficiency to enzymatic lifetime and stability.
Salvador Ventura	PI of functional prions, amyloid and protein aggregation group Expertise in HET-s hydrogels	Challenged the novelty of the matrix itself, citing prior functional prion work on glucose biosensors, antibody-binding fibrils, and more. Advised selling the application rather than the platform which reshaped our IP strategy around sortase A, the one element he saw as unexplored. He also identified our production cost (rather than stigma or regulation) as the barrier for scale-up and noted HET-s disassembly at low pH, offering us a second disassembly route.
Dr. A	Expertise in synthetic biology and hydrogels	Assessed that our monolayer gel is unlikely to have the structural integrity or thickness to be characterised or marketed as robust, pushing us towards a multilayer design and cross-linking molecules for stability which led to the

		strut-and-node architecture. Also proposed benchmarking against a collagen or alginate hydrogel to strengthen our applications assessment.

10. Conclusion

Overall, our project sits in the under-indexed space mentioned by Mr. You, whereby our hydrogel is a biotechnology which can act as a platform in infrastructure for agriculture, manufacturing and other industries. The governing questions therefore become, “who depends on this technology, what if it breaks or fails, who becomes accountable once it is embedded in another party’s process?”.

Biosecurity Framework Deliverables Checklist

These are the following deliverables of a platform technology framework that can be used by other teams.

Pillar	Deliverable	Content
1	Risk assessment per application	● Intended use ● Foreseeable misuse ● Exposure route ● Scale ● Downstream parties involved
2	Payload policy	● Excluded enzyme classes ● Condition of use attached to the part
3	Red-team memo	● 3 worse-case payloads ● What immobilisation would add ● Mitigations to consider
4	Decommissioning protocol	● Biotin disassembly efficiency and residual fibril data ● Waste disposal route
5	Terms of use	● Payload policy ● No onward transfer of technology ● Decommissioning acknowledgement
6	Customer verification checklist	● Institutional affiliation ● Reason for application ● Detailed escalation route
7	Payload sign-off log	● Named reviewer per effector protein entering the pipeline
8	Disclosure decision	● What is published or unpublished

	record	● What carries stewardship note and why
9	Beneficiary and workflow map	● Current hydrogel-dependent workflows and their components and incumbents ● Substitution cases

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