VitaLabs Project Submission Form

Please complete all sections clearly. Each question has specified character or word limits.

Part 1: Project Overview

1. **Project Title**
   Enzyme engineering for crosslinked collagen turnover in the extracellular matrix

2. **Short Description**
   The project postulates extracellular matrix (ECM) stiffening as the upstream cause of the hallmarks of aging and proposes leveraging generative AI to engineer matrix metalloproteinases (MMPs) capable of degrading sugar-modified, or glycated, collagen that resists normal remodeling. The initial experimental framework encompasses in vitro studies using hydrogels with tunable stiffnesses to probe cellular responses to different mechanoenvironments, and further in vivo validation in a murine liver regeneration model to build evidence for the proximal role of ECM stiffening in aging. The work aims to overcome hitherto irreparable forms of damage through engineered ECM rejuvenation, offering its first therapeutic application in diabetic nephropathy using enhanced glyoxalase I and MMPs-3, -9, targeting both intracellular and extracellular glycation damage in kidneys.

3. **Project Keywords**
   List 4-5 keywords that summarize the project focus.
   - Keyword 1: enzyme engineering
   - Keyword 2: extracellular matrix
   - Keyword 3: matrix metalloproteinase
   - Keyword 4: mechanobiology
   - Keyword 5: protein turnover

4. **At-a-Glance Questions**
   Answer these questions to provide an immediate snapshot of the project:
   - **What problem does the project address?**
     ECM stiffening due to irreversible accumulation of

glucose-derived crosslinks resists natural remodeling, leading to tissue aging.

- **Why is the project relevant for longevity science?**
  It proposes ECM stiffening as the upstream cause of the hallmarks of aging, offering a new paradigm for treating age-related diseases.

- **What makes this project innovative or distinctive?**
  The project uses AI to engineer novel MMPs capable of degrading crosslinked ECM, addressing previously irreparable damage.

- **What will be the main outcome of this project?**
  Enhanced MMPs for engineered ECM remodeling, with first application in diabetic nephropathy targeting glycation damage.

- **How much funding are you requesting?**
  50,000 USD.

Part 2: Detailed Project Description

5. **The Problem**

   There's no definite consensus on what aging is (1). To contextualize geroscience research, mechanisms of aging have been organized into the framework of hallmarks (2). The hallmarks concept, however, does not conform to a full-fledged scientific paradigm, leaving out a few prominent characteristics of the aging process and not comprehensively explaining why the hallmarks emerge in the first place (3, 4). Recently, I proposed that the hallmarks of aging are emergent and subordinate entropic phenomena in relation to aging-associated extracellular matrix (ECM) stiffening (5).

   Mechanical properties of cells and their environment, represented by the ECM, have long been recognized as a factor in a plethora of age-related conditions (6). An important source of ECM stiffness is non-enzymatic glycosylation (glycation) and the resultant formation of glucose-derived intermolecular crosslinks (7). Given the high concentration of glucose in the blood (~5 mM) and a low turnover of

ECM proteins, e.g. collagens, the crosslinks inevitably accumulate with age, resulting in tissue stiffness (8, 9).

ECM turnover is mediated by **matrix metalloproteinases** (MMPs) (10). Mice expressing collagen resistant to MMP degradation show impaired ECM remodeling (11) and signs of premature aging (12). Further, the crosslinked collagen is resistant to proteolysis by MMPs (13, 14).

6. **The Solution**

There are no means to target the crosslinks, i.e. once formed, they are irreversible (9). On top of different sugar- and sugar metabolite-derived crosslinks and adducts, the ECM sustains a range of additional stochastic chemical damage. To address all damage at once, it's sensible to promote ECM turnover, leveraging the cell's natural ability to remodel its environment toward the preferred biomechanical composition.

Latest developments in generative AI allowed substrate-conditioned de novo enzyme engineering (15). To overcome the resistance of crosslinked ECM to normal remodeling, I propose to generate MMPs that would degrade glycated collagen, facilitating its turnover.

To establish a preliminary experimental foundation for AI-inspired engineered ECM remodeling, I first will perform several in vitro experiments to ascertain the causal link between substrate stiffness and the hallmarks of aging. Building on my previous work (16), I will use hydrogels with tunable mechanical properties to probe into cells', e.g. primary fibroblasts', responses to different mechanoenvironments. Using translation fidelity and DNA double-strand break accumulation as readouts, I will test the positions of ECM stiffening as the upstream cause and of the hallmarks as downstream effects.

Secondly, I will use a murine liver regeneration model to test my assumptions in vivo (17). The regenerated portion of the liver, residing in the same organ, in the same organism, and subjected to the same systemic circulation, is only different in that it has a newly synthesized ECM. Recent research suggests embryonic-like epigenetic reprogramming in hepatocytes during regeneration (18, 19). By applying methylation and transcriptomic aging clocks to intact and regenerated portions of the liver, I will reveal if the latter part is indeed biologically younger, integrating both lines of evidence into a rationale for engineered ECM rejuvenation.

7. **What Makes This Project Distinctive?**
   Taking into account both theoretical and applied nature of the proposal, it offers a comprehensive framework for targeting hitherto irreparable forms of damage. With its scope outside the cell-centric view on aging, the project addresses a critical conceptual and technological gap, aiming to solve most ailments of old age by leveraging the body's intrinsic ability to restore itself, provided that it's given a slight "nudge" in the shape of MMPs that would degrade ECM components not normally amenable to remodeling. De novo engineering of enzymes with enhanced functionalities and/or custom target specificities opens broad possibilities for achieving cellular resilience, damage repair, and carrying out novel reactions, working towards optimal human health and longevity.

8. **Expected Impact and Innovation**
   Methylglyoxal (MGO), a byproduct of glycolysis, is a potent electrophile that accounts for the bulk of the intracellular glycation damage (20). Cells have mechanisms that detoxify MGO – **glyoxalases I** and **II** (Glo1 and Glo2, respectively) that work in combination to convert MGO to lactic acid (21). Previously, I suggested an enhanced version of Glo1 to alleviate nephropathy in diabetic patients (22). In addition to the intracellular damage, glycated collagen IV in the glomerular basement membrane, resistant to MMP cleavage, is implicated in some hallmarks of diabetic nephropathy, i.e. glomerular membrane thickening (23). Consequently, I envisage engineered **Glo1**, **MMP-3**, and **MMP-9** to address the issues of both intracellular and extracellular glycation damage to kidneys in diabetes, forming the basis of my IP pursuits in the near term.

Part 3: Funding Request and Budget

9. **Requested Funding Amount**
   Total funding requested for the project (USD): **50,000**

10. **Milestone-Based Budget and Timeline**
    Break down the funding request for the duration of VitaLabs (4 months total) into key project milestones. Include descriptions, expected outcomes, duration, and costs.

Milestone	Description	Duration	Cost (USD)
In vitro mechanoenvironment studies	- Establish a hydrogel system with varying stiffnesses (different kPa range). - Culture primary fibroblasts on hydrogels. - Measure translation fidelity readouts. - Quantify DNA double-strand breaks. -Data analysis and correlation with substrate stiffness.	1.5 mo.	20,000
Liver regeneration model	- Establish a partial hepatectomy model in mice. - Collect tissue samples from regenerated and intact portions. - Apply methylation aging clocks. - Perform transcriptomic analysis. - Compare biological age markers between regions.	1.5 mo.	25,000
Data integration and analysis	- Statistical analysis of both experimental phases. - Integration of in vitro and in vivo findings. - Reporting. - Development of specifications for AI-engineered MMPs.	1 mo.	5,000
Total		4 mo.	**$50,000.00**

11. Long term vision

Cell-mediated ECM degradation based on MMP production has been

proposed as a therapeutic strategy (24). Therapies centered around engineered enzyme applications will require additional considerations in terms of delivery, in situ activity monitoring, controlled release, etc., driving innovation and opportunities for novel patentable interventions.

It's possible that the expression of engineered MMPs alone would be sufficient for therapeutic benefits. For example, replicatively aged fibroblasts reverted some of the signs of senescence after having been cultivated on youthful ECM (25).

Should cells require extra help in getting rid of cleaved collagen and building a new matrix, a more comprehensive approach can be employed:

- Engineered MMPs for breaking down glycated collagen;
- Targeted removal of the resulting collagen fragments from the extracellular space;
- Enhanced intralysosomal degradation of the fragments by recombinant **cathepsin D**.

Read notes for details.

Part 4: Team and Contact Details

12. **Lead Researcher Information**

- **Full Name:** Rakhan Aimbetov
- **Email:** r@overlake.bio
- **Institution/Organization:** Overlake
- **Position/Role:** R&D lead
- **LinkedIn Profile:** https://www.linkedin.com/in/raimbetov/
- **Twitter (if applicable):**

12. **Supporting Team Members**

List key contributors to the project and their roles.

- **Team Member 1:** -
- **Team Member 2:** -
- **Team Member 3:** -

13. **Project Website or Additional Resources**

If available, provide links to supporting websites or documents relevant to the project.

Project website – https://overlake.bio/

Part 5: Additional Notes and References

14. **POI link**

Root: 0xce29ab2a9e081048ff74312462a9680b8344d2e2248ee1d8a1ad69d0dbac3af1

Txn hash: 0xc654a43ac76a3e36ead3d888efb181a62d41af031558a6dec45b9f01f33edd81

15. **Discord thread link**

https://discord.com/channels/810893413880561704/1316093489586372768

16. **Additional Notes**

My overall overarching vision for the engineered ECM turnover is three-fold:

1. Engineered MMP-mediated proteolysis of glycated collagen, as explained above;

2. One of the existing techniques for the targeted degradation of extracellular proteins to selectively take up proteolytic collagen fragments (26);

3. Next, overexpressed cathepsin D, responsible for the proteolysis of most glycated proteins inside cells (27), can facilitate collagen breakdown in the lysosome. Enzyme replacement therapy with recombinant cathepsin D has been used to ameliorate defective autophagy in **neuronal ceroid lipofuscinosis** (28), with ceroids in part consisting of glycation endproducts.

Finally, an optional combinatorial approach to stimulate ECM synthesis can further strengthen the desired effects (29), achieving a newly reconstituted matrix relieved of chemical damage.

17. **Attachments (if applicable)**

Provide links or upload relevant files (e.g., research papers, diagrams).

Delocalized entropy aging theorem – https://doi.org/10.31219/osf.io/anc8u

Project slides – https://doi.org/10.6084/m9.figshare.28099607.v1

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