Original Work Part 2

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Independent Study and Mentorship

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Bouin, Alexis, et al. “New Rabies Viral Resources for Multi-scale Neural Circuit Mapping.” *Molecular Psychiatry*, vol. 29, no. 7, Feb. 2024, pp. 1951–67. https://doi.org/10.1038/s41380-024-02451-6.

Research Assessment - 2

This article introduces a suite of 20 newly engineered rabies virus (RV) ΔG vectors designed for high-resolution, multi-scale mapping of neural circuits. The authors describe enhancements across multiple domains, including brighter cytoplasmic fluorescent reporters (e.g., mNeonGreen, tdTomato), expanded spectral options (blue to far-red), organelle-specific localization tags (mitochondria, nucleus, plasma membrane, and somatodendritic compartments), and dual reporters for both fluorescence and electron microscopy through ferritin expression. They also introduce functional reporters, such as nanoluciferase and fluorescent timers, to study infection kinetics. The research demonstrates how these viral tools enable the precise mapping of synaptic connections, the detection of microstructural changes in aging and Alzheimer’s disease models, and the real-time analysis of organelle dynamics, such as mitochondrial transport. Overall, the paper expands the rabies virus toolkit beyond mesoscale imaging toward multi-modal, cross-scale connectomics.

This information is highly relevant to my ISM project, which focuses on AI-driven design of rabies virus glycoprotein (RVG) variants to optimize blood–brain barrier crossing and

neuronal targeting in Alzheimer's disease. The paper clarifies how structural modifications to rabies vectors influence tropism, expression patterns, and subcellular localization, knowledge fundamental to rational glycoprotein engineering. Understanding how pseudotyping, ΔG deletion systems, and helper-virus frameworks control viral spread allows me to conceptualize better how an AI-designed RVG variant must behave in vivo to enable safe, single-step delivery across the BBB. The Alzheimer's disease applications in the study directly reinforce the therapeutic relevance of my own work. Their ability to detect mitochondrial size reduction and dendritic spine loss using RV-based labeling demonstrates how optimized viral entry proteins can serve as powerful research and diagnostic tools in AD. In short, the article provides biological grounding and experimental context for why improving RVG through computational design could meaningfully assist disease-targeted neural delivery.

The information can be broken down into several key components: recombinant design, fluorescent reporter engineering, organelle-specific targeting, viral packaging/production workflows, and in vivo applications. The article categorizes RV tools into four types: cytoplasmic reporters, subcellular reporters, dual EM/fluorescence reporters, and functional/bioluminescent reporters. When compared to my prior knowledge, I have a better understanding of how RV entry efficiency is influenced not only by the glycoprotein but also by downstream expression, toxicity, and MOI-dependent kinetics. This article expands my knowledge of the constraints of RV-based systems. For example, replication competence, despite spread deficiency, introduces dose-dependent toxicity, which impacts the therapeutic viability of RVG-based vectors. It also reframes the RV system as not just a transsynaptic tracer but a modular genetic delivery platform whose specificity depends heavily on envelope protein. This reinforces how my AI-based RVG optimization could fit within existing viral workflows without altering the backbone architecture.

This article inspires several new directions for my ISM project. First, the subcellular targeting approach suggests that future RVG variants could be engineered via AI to preferentially engage receptors enriched in disease-affected compartments, such as postsynaptic densities in AD. Second, the mitochondrial reporter applications encourage me to explore how RVG-Xplorer 2.0 could be integrated with fluorescent or enzymatic reporters to analyze therapeutic impact after BBB delivery. Third, the authors' modular cloning system gives me a conceptual blueprint for how an AI-designed glycoprotein might be inserted and tested within a ΔG system. I now plan to model multi-receptor targeting (e.g., nAChR, NCAM1, and LDLR family) using transformer-based architectures, guided by the biological insight that viral tropism is combinatorial rather than singular. A new question emerged: Could AI-assisted glycoprotein designs also modulate intracellular trafficking to favor organelles relevant to AD pathology?

This article was extremely effective in advancing my project goals. It was both motivating and clarifying, especially in showing how rabies-based vectors are already being applied to Alzheimer's research. The findings were encouraging because they provided objective evidence that rabies-derived tools can detect AD-related structural degeneration with high precision, supporting the premise that enhanced RVG variants could enhance disease-targeted delivery. I did not expect how much subcellular targeting mattered for accurate neural mapping, and this shifted my perspective on what an "optimized" RVG might need to accomplish. The only discouraging aspect is the toxicity risk at high MOI, which reinforces that therapeutic applications must remain highly controlled. Still, the knowledge broadened my understanding of the technology's limitations and convinced me that computational glycoprotein design has real potential to address unmet needs in brain-targeted delivery.