

## Peer-Review

Filippone-Thaulero, Federico. 2026. "From Convergence to an Iterative Optimization-Circumvention-Collapse Framework in CRISPR Bioengineering." *Journal of High School Science* 10 (3): 177–96. <https://doi.org/10.64336/001c.165176>

Interesting review but is not forward looking. Please see the separate email that contains the chatgpt file for my interaction with AI regarding your manuscript. The conversation is self-explanatory. I would like you to make your manuscript more forward looking - see what you can do with the optimization-circumvention-collapse paradigm - and revise accordingly.  
If you have trouble accessing the chatgpt file, please let me know.

| Reviewer comment                                                                                                                                                                                                                                                                                                                                       | Extracts / response from revised manuscript                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| 1. The claim that OTE reduction and HDR maximisation have conventionally been pursued independently is overstated. Modern CRISPR engineering already evaluates activity, specificity, HDR, viability, delivery, and genomic stability together. The manuscript should instead argue that it formalises this interdependence within a Pareto framework. | <b>Addressed / partially reconsidered.</b> The manuscript now softens the claim: "Although modern CRISPR engineering increasingly co-evaluates activity, specificity, repair outcome, delivery, and genomic stability, OTEs and HDR are often analysed through distinct intervention frameworks." Also: "This review formalises this interdependence as an optimisation frontier generated by three different levels of convergence." The abstract, introduction, Section 2.1, and other sections were revised to avoid implying that interdependence has been ignored, and instead present the manuscript as formalising this interdependence. |
| 2. The manuscript should clarify what the Pareto model adds beyond reorganising known trade-offs. Its value is strongest if presented as a benchmarking and design framework, not as a biological solution to OTEs, HDR inefficiency, or cancer risk.                                                                                                  | <b>Addressed.</b> The manuscript now states: "It must be noted that the model is purely theoretical and not empirically derived: it does not solve OTEs, HDR inefficiency, or genomic instability, but provides a benchmarking framework for comparing interventions against application-specific clinical requirements."                                                                                                                                                                                                                                                                                                                       |
| 3. Empirical Pareto reconstruction is a useful future direction, but the claim should be tempered. There will not be one universal frontier; frontiers will depend on cell type, locus, delivery system, guide RNA, donor design, and clinical context.                                                                                                | <b>Addressed.</b> The conclusion now states: "Future research should therefore reconstruct context-specific frontiers rather than a singular, universal one. Such frontiers will vary with cell type, locus, guide RNA, donor design, delivery system, and clinical context."                                                                                                                                                                                                                                                                                                                                                                   |
| 4. Mapping a frontier is necessary for rational engineering, but not sufficient for innovation. The manuscript should explain how frontier analysis would guide experimental decisions or identify which mechanisms should be targeted next.                                                                                                           | <b>Addressed.</b> The conclusion now states: "Frontier mapping would guide clinical and engineering decisions by exposing which variables should be tuned to maximise intersection with the clinical acceptance region, and therefore whether the next intervention should tune delivery or dosage, target a coupling mechanism, or test an alternative architecture."                                                                                                                                                                                                                                                                          |
| 5. The discussion should distinguish three modes of progress: optimization, circumvention, and collapse. Optimization finds a better compromise within the current system; circumvention avoids the limiting mechanism through a different architecture; collapse weakens the mechanistic coupling that creates the trade-off.                         | <b>Addressed.</b> Section 3.1 states: "Optimisation refers to the navigation of the existing Pareto frontier within a given architecture. Circumvention refers to the invention or adoption of a new editing paradigm whose required mechanistic dependencies differ from those of the original architecture... Collapse refers to the dissociation of conventionally coupled variables, such that improvement in one variable no longer necessarily worsens another."                                                                                                                                                                          |
| 6. Base and prime editors are good examples of circumvention because they reduce dependence on DSB/HDR-based editing. However, they introduce new trade-offs such as edit scope, product purity, efficiency, and delivery. The key question is whether their new frontier is more clinically favourable than that of DSB-based systems.                | <b>Addressed.</b> Section 3.2 states: "Base and prime editors both represent empirical examples of circumvention." It then explains that base editors use "Cas-guided chemical base conversion," while prime editors use "pegRNA-guided reverse transcription and incorporation of the intended edit." It also notes: "broader optimisation variables such as product purity, efficiency, delivery, and bystander editing still emerge."                                                                                                                                                                                                        |
| 7. The manuscript should discuss collapse more explicitly. In biotechnology, transformative progress often occurs when a trade-off is weakened rather than merely optimized. Nucleoside-modified mRNA vaccines are a useful analogy: innate immune activation was reduced without sacrificing protein translation.                                     | <b>Addressed.</b> Section 3.3 states: "Collapse is sought because of the extreme return on investment: a successful collapse weakens or eliminates one mechanistic coupling." It adds the mRNA analogy: "The incorporation of modified nucleosides, namely pseudouridine and later N1-methylpseudouridine, weakened the coupling by maintaining high translation with low innate immune activation."                                                                                                                                                                                                                                            |
| 8. Optimization should be framed not only as a way to choose the best point, but also as a process that can expose hidden constraints. Pushing one variable often reveals new limiting variables, which can then motivate circumvention or collapse.                                                                                                   | <b>Clarified rather than redefined.</b> The manuscript preserves its hierarchy, where convergence analysis diagnoses the frontier and optimisation acts on it. Section 3.4 now states: "Convergence analysis therefore diagnoses the Pareto frontier that optimisation subsequently navigates, although optimisation-oriented experiments may later reveal additional limiting variables."                                                                                                                                                                                                                                                      |

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| 9. The clinical implications need to be sharper. Even an optimal point on a Pareto frontier may still carry unacceptable risks, including oncogenic mutations, chromosomal rearrangements, large deletions, translocations, p53 selection, or other genomic instability. | <b>Addressed.</b> Section 3.2 now states that DSB-based precise editing must avoid “NHEJ, MMEJ, large deletions, indels, rearrangements, p53 activation, and translocations.” It also clarifies that “even a Pareto-optimal point may be disqualifying if it possesses unacceptable risks of oncogenic mutations, translocations, deletions, or chromosomal rearrangements.”                                                                                                                                                                                                                                                                       |
| 10. Figure 3 should be revised because it is the central conceptual figure but currently looks too abstract. A ternary diagram or 3D trade-off surface showing activity, specificity, and HDR would better communicate the author’s argument.                            | <b>Reconsidered.</b> A 3D surface was not adopted because the manuscript treats HDR as partly downstream of activity in DSB-based precise editing, rather than as an independent third axis. Instead, Figure 3 was revised into a two-dimensional conceptual Pareto-like frontier comparing specificity/off-target rejection with effective precise-edit output. The figure now distinguishes the conventional DSB-HDR frontier, a possible reshaped frontier, intervention positions, and application-specific clinical acceptance regions. The caption also clarifies that positions are schematic, relative, and not empirically reconstructed. |
| 11. Figure 2 should more clearly show why S1mplex represents convergence. The figure currently shows the architecture, but not how donor tethering and transient RNP delivery jointly affect HDR and off-target exposure.                                                | <b>Reconsidered / addressed in caption rather than redrawing.</b> The figure remains molecular because its purpose is to visualise the S1mplex architecture. However, the caption now explicitly explains convergence: “The convergence lies in the combination of transient RNP delivery, which limits Cas9 exposure and OTE accumulation, with donor tethering, which increases local donor availability for HDR.”                                                                                                                                                                                                                               |
| 12. The conclusion should avoid implying that progress simply depends on mapping each technology’s frontier. A stronger claim is that frontier mapping helps identify whether the next step should be optimization, circumvention, or collapse.                          | <b>Addressed.</b> The conclusion now states: “Frontier mapping would guide clinical and engineering decisions... whether the next intervention should tune delivery or dosage, target a coupling mechanism, or test an alternative architecture.” It also ends: “progress in gene editing depends on choosing, reshaping, and optimising the frontier most compatible with a given clinical objective.”                                                                                                                                                                                                                                            |

Thank you for addressing my concerns. Accepted.