Title: Confronting the unprecedented risks of mirror life

Authors: Katarzyna P. Adamala[1], Deepa Agashe[2], Yasmine Belkaid[3], Daniela Matias de C. Bittencourt[4, 5], Yizhi Cai[6], Matthew W. Chang[7, 8, 9, 10], Irene A. Chen[11, 12], George M. Church[13, 14], Vaughn S. Cooper[15], Mark M. Davis[16, 17, 18], Neal K. Devaraj[19], Drew Endy[, 20, 21, 22, 23], Kevin M. Esvelt[24], John I. Glass[4]*, Timothy W. Hand[25], Thomas V. Inglesby[26, 27, 28], Farren J. Isaacs[29, 30, 31], Wilmot G. James[32,33], Jonathan D. G. Jones[34], Michael S. Kay[35], Richard E. Lenski[36, 37], Chenli Liu[38], Ruslan Medzhitov[39, 40, 41, 42], Matthew L. Nicotra[26], Sebastian B. Oehm[4, 43, 44], Jaspreet Pannu[26, 45, 46], David A. Relman[15, 46, 47], Petra Schwille[48, 49, 50], James A. Smith[4], Hiroaki Suga[51], Jack W. Szostak[52]*, Nicholas J. Talbot[34], James M. Tiedje[53, 54], J. Craig Venter[4, 55], Gregory Winter[43, 44], Weiwen Zhang[56, 57], Xinguang Zhu[58], Maria T. Zuber[59]

Affiliations:

[1]Department of Genetics, Cell Biology and Development, University of Minnesota; Minneapolis, MN, USA.

[2]National Centre for Biological Sciences, Tata Institute of Fundamental Research; Bengaluru, India.

[3]Institut Pasteur; Paris, France.

[4]J. Craig Venter Institute; La Jolla, CA, USA.

[5]Embrapa Genetic Resources and Biotechnology/National Institute of Science and Technology in Synthetic Biology (INCT BioSyn); Brasília, DF, Brazil.

[6]Manchester Institute of Biotechnology, University of Manchester; Manchester, United Kingdom.

[7]NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI), National University of Singapore; Singapore, Singapore.

[8]Synthetic Biology Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore; Singapore, Singapore.

[9]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore; Singapore, Singapore.

[10]National Centre for Engineering Biology (NCEB); Singapore, Singapore.

[11]Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, CA, USA

[12]Department of Chemistry and Biochemistry, University of California, Los Angeles, CA, USA

[13]Department of Genetics, Harvard Medical School; Boston, MA, USA.

[14]Wyss Institute for Biologically Inspired Engineering; Boston, MA, USA.

[15]Department of Microbiology and Molecular Genetics, University of Pittsburgh; Pittsburgh, PA, USA.

[16]Institute for Immunity, Transplantation and Infection, Stanford University School of Medicine, Stanford University; Stanford, CA, USA.

[17]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford University; Stanford, CA, USA.

[18]Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford University; Stanford, CA, USA.

[19]Department of Chemistry and Biochemistry, University of California, San Diego; La Jolla, CA, USA.

[20]Department of Bioengineering, Stanford University; Stanford, CA, USA.

[21]Stanford d.school, Stanford University; Stanford, CA, USA.

[22]Freeman Spogli Institute for International Studies, Stanford University; Stanford, CA, USA.

[23]Hoover Institution, Stanford University; Stanford, CA, USA.

[24]Media Laboratory, Massachusetts Institute of Technology; Cambridge, MA, USA.

[25]Pediatrics Department, Infectious Disease Section, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine; Pittsburgh, PA, USA.

[26]Center for Health Security, Bloomberg School of Public Health, Johns Hopkins University; Baltimore, MD, USA.

[27]Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University; Baltimore, MD, USA.

[28]Johns Hopkins Bloomberg School of Medicine, Johns Hopkins University; Baltimore, MD, USA.

[29]Department of Molecular, Cellular, and Developmental Biology, Yale University; New Haven, CT, USA.

[30]Systems Biology Institute, Yale University; West Haven, CT, USA.

[31]Department of Biomedical Engineering, Yale University; New Haven, CT, USA.

[32]Department of Health Services, Policy and Practice, School of Public Health, Brown University; Providence, RI, USA.

[33]The Pandemic Center, School of Public Health, Brown University; Providence, RI, USA.

[34]The Sainsbury Laboratory, University of East Anglia; Norwich, United Kingdom.

[35]Department of Biochemistry, University of Utah School of Medicine; Salt Lake City, UT, USA.

[36]Department of Microbiology, Genetics, and Immunology, Michigan State University; East Lansing, MI, USA.

[37]Program in Ecology, Evolution, and Behavior, Michigan State University; East Lansing, MI, USA.

[38]Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences; Shenzhen, China.

[39]Department of Immunobiology, Yale University School of Medicine; New Haven, CT, USA.

[40]Howard Hughes Medical Institute; Chevy Chase, MD, USA.

[41]Center for Infection and Immunity, Yale University School of Medicine; New Haven, CT, USA.

[42]Tananbaum Center for Theoretical and Analytical Human Biology, Yale University School of Medicine; New Haven, CT, USA.

[43]Medical Research Council Laboratory of Molecular Biology, University of Cambridge; Cambridge, United Kingdom.

[44]Trinity College Cambridge, University of Cambridge; Cambridge, United Kingdom.

[45]Department of Health Policy, Stanford School of Medicine, Stanford University; Stanford, CA, USA.

[46]Department of Medicine, Stanford University School of Medicine, Stanford University; Stanford, CA, USA.

[47]Infectious Diseases Section, Veterans Affairs Palo Alto Health Care System; Palo Alto, CA, USA.

[48]Department of Cellular and Molecular Biophysics, Max Planck Institute for Biochemistry; Martinsried, Germany.

[49]Excellence Cluster ORIGINS; Garching, Germany.

[50]Center for Nanoscience; Munich, Germany.

[51]Department of Chemistry, Graduate School of Science, The University of Tokyo; Bunkyo-ku, Tokyo, Japan.

[52]Howard Hughes Medical Institute, Department of Chemistry, The University of Chicago; Chicago, IL, USA.

[53]Center for Microbial Ecology, Michigan State University; East Lansing, MI, USA.

[54]Interdisciplinary Research Center for Soil Microbial Ecology and Land Sustainable Productivity in Dry Areas, Northwest A&F University; Shaanxi, China.

[55]J. Craig Venter Institute; Rockville, MD, USA.

[56]School of Chemical Engineering and Technology, Tianjin University; Tianjin, China.

[57]Center for Biosafety Research and Strategy, Tianjin University; Tianjin, China.

[58]Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences; Shanghai, China.

[59]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology; Cambridge, MA, USA.

*Corresponding authors. Email: jglass@jcvi.org; jwszostak@uchicago.edu

Abstract: Mirror bacteria could pose unprecedented risks; broad discussion is needed to chart a path forward.

Main text:

Chirality—the intrinsic "handedness" of molecules—has intrigued scientists since its discovery by Louis Pasteur. All known life consistently builds its DNA and RNA from 'right-handed' nucleotides, and its proteins from 'left-handed' amino acids. Driven by curiosity and plausible applications, some researchers have begun early work towards creating mirror organisms—lifeforms composed entirely of mirror-image biological molecules. A mirror organism would constitute a radical departure from life as we know it, and its creation warrants careful consideration. We now offer an in-depth analysis suggesting that mirror bacteria could pose unprecedented and largely overlooked risks to much of existing life (*1*). The capability to create mirror life is likely at least a decade away and would require large investments and major technical advances; we thus have an opportunity to consider and preempt risks before they are realized. Here, we call for broader discussion amongst the global scientific community, policymakers, research funders, industry, civil society, the public, and others, to chart an appropriate path forward.

Others have considered the dangers from mirror life (*2*, *3*), but a thorough analysis of the risks has not previously been completed. The need for such an analysis has grown with advances in key technologies. To address this gap, we have examined the feasibility of creating mirror bacteria and the associated risks in detail. Our group includes expertise in synthetic biology, human, animal and plant physiology and immunology, microbial ecology, evolutionary biology, planetary life detection, and biosecurity, and includes researchers who have held the creation of mirror bacteria as a long-term aspirational goal. The findings are summarized below and detailed in a separately released, in-depth technical report (*1;* see supplementary material for a version of this article thoroughly referenced to specific sections of that report). We focus on mirror bacteria, but note that many of the considerations might also apply to other forms of mirror life.

Our analysis suggests that mirror bacteria would likely evade many chirally mediated immune mechanisms, potentially causing lethal infection in humans, animals, and plants. They are likely to evade predation from natural-chirality phage and many other predators, facilitating spread in the environment. We cannot rule out a scenario in which a mirror bacterium acts as an invasive species across many ecosystems, causing pervasive lethal infections in a substantial fraction of plant and animal species, including humans. Even a mirror bacterium with a narrower host range and the ability to invade only a limited set of ecosystems could still cause unprecedented and irreversible harm.

Most of us were initially skeptical that mirror bacteria could pose major risks. However, although our analysis is necessarily uncertain, we have become deeply concerned about the plausibility of widespread harm. We call for additional scrutiny and engagement with this topic. In the absence of compelling evidence for reassurance, our view is that mirror bacteria and other mirror organisms should not be created, and that action should be taken to ensure this.

Towards mirror life

Mirror life could be just as functional as natural-chirality life, but it cannot arise from existing life: evolution proceeds in incremental steps and would be unable to invert the chirality of complex biomolecules such as DNA or proteins, let alone all biomolecules simultaneously. However, with scientific advances, a mirror organism might in the future be created in a laboratory.

Creating a mirror organism, even one as simple as a bacterium, would be a far more complex feat of biological engineering than has ever been accomplished. Yet, progress on key enabling technologies is underway. Scientists are increasingly able to synthesize complex mirror-image biomolecules (*4*, *5*); recent advances have enabled the chemical synthesis of mirror-image kilobase-length nucleic acids and large functional proteins (*6*). Their reversed chirality makes these biomolecules resistant to normal forms of biological degradation, leading to emerging applications such as long-lasting and non-immunogenic therapies (*1, 4, 5*).

In parallel, researchers are making rapid progress towards constructing synthetic cells (of natural chirality) from non-living parts (*7*, *8*). Once a method is developed that enables the construction of a natural-chirality bacterium entirely from synthetic DNA, synthetic proteins, and synthetic lipids, and once mirror versions of these components can also be synthesized, a living mirror bacterium could be constructed in the same way (*1, 9*). Other pathways to constructing a mirror bacterium are also plausible; for example, with further advances in synthetic biology, a natural-chirality bacterium might be engineered to produce mirror proteins and nucleic acids *in vivo*, which could provide a starting point for stepwise conversion into a mirror bacterium (*1*).

In isolation, mirror bacteria would function identically to their natural-chirality counterparts if provided with achiral or mirror-image nutrients—and be as feeble or robust as the strain that served as their template. Genetic engineering could transform a slow-growing, specialized mirror bacterium into a mirror version of a fast-growing, generalist bacterial strain (*1*). Many bacteria, including *Escherichia coli*, can grow robustly in growth media without chiral nutrients (*10*); hence, mirror versions of those bacteria would do the same. Achiral nutrients are available in quantities sufficient for growth of common bacteria in a wide range of natural environments, including within potential hosts (*1*). Further genetic engineering could provide mirror bacteria with pathways needed to consume abundant chiral nutrients such as D-glucose.

Growth of mirror bacteria outside of the laboratory is therefore plausible. However, their interactions with other lifeforms would differ profoundly as a result of their reversed chirality.

Potential for broad immune evasion and ecosystem invasion

Our analysis suggests that mirror bacteria could broadly evade many immune defenses of humans, animals, and plants. Chiral interactions are central to immune recognition and activation in multicellular organisms, and these chiral interactions would be impaired with mirror bacteria. This could result in weakened immune recognition, a weakened response by innate immune systems, and (in vertebrates) limited downstream activation of adaptive immune functions (*1*). For example, experiments show that mirror proteins resist cleavage into peptides for antigen presentation and do not reliably trigger important adaptive immune responses such as the production of antibodies (*11*, *12*). We are thus concerned that the function of many vertebrate

immune systems against mirror bacteria would be severely impaired. Invertebrate and plant immune systems are less well studied but appear to suffer analogous limitations (*1*).

Given the potential for severe immune evasion, mirror bacteria might not require host-specific factors to invade hosts and cause infection. In animals (including humans), bacteria regularly cross barriers in the skin, mouth, gut, lungs, and other mucosal surfaces due to routine damage and intrinsic leakiness (*13*, *14*); mirror bacteria would be expected to do the same. In healthy animals, translocated natural-chirality bacteria are typically cleared by immune defenses. However, if the immune response against mirror bacteria is severely impaired, translocated mirror bacteria might replicate within the host and establish an infection. Unchecked replication of mirror bacteria within internal tissues is likely to be deleterious to the host organism, and may be lethal (*1*). We are therefore concerned that mirror bacteria might act as a serious pathogen with an unusually broad host range.

Mirror bacteria could also pose ecological risks more broadly. By virtue of their reversed chirality, mirror bacteria may evade many forms of predation and microbial interference. They would be intrinsically resistant to infection by natural-chirality bacteriophages, may be resistant to consumption by many predators, and may be resistant to most antibiotics produced by microbial competitors. This resistance could allow mirror bacteria to be unusually persistent outside of multicellular hosts, facilitating transmission. Reduced mortality due to predation could provide a fitness advantage that might allow colonization of some external environments, despite potential disadvantages such as reduced ability to acquire chiral nutrients (*1*). Transport by multicellular hosts could disperse mirror bacteria across many environments. Much like an invasive species with few natural predators, we are concerned that mirror bacteria could rapidly proliferate, evolving and diversifying as they spread. Persistent and potentially global presence of mirror bacteria in the environment could repeatedly expose human, animal and plant populations to the risk of lethal infection.

Biosafety and biosecurity considerations

Biocontainment and biosafety approaches might be proposed to reduce these risks. Scientists could intentionally hobble mirror bacteria by engineering dependence upon molecules not present in nature (i.e., synthetic auxotrophy), safeguards intended to prevent growth outside controlled laboratory environments. However, escape from these safeguards due to evolution or human error could occur. Multiple auxotrophies would reduce the chance of escape but would not eliminate it. Physical containment approaches could be used, but laboratory accidents happen with some regularity, even in high containment laboratories, due to human error and equipment failure (*15*).

Even if a mirror bacterium unable to grow outside controlled lab environments could be created, it would not be secure—that is, permanently controlled in such a way that would prevent large-scale harm through negligence or intentional misuse. Once a biocontained mirror bacterium has been created, it would be comparatively straightforward to engineer it to be free of safeguards (*1*). Methods for the construction of mirror bacteria could also be replicated by others in pursuit of various (perhaps safeguard-free) mirror bacteria.

Countermeasures, such as mirror antibiotics, crops engineered to be resistant to mirror bacteria, and mirror phages, appear very unlikely to be sufficient to stop or reverse the spread of mirror bacteria throughout global ecosystems, nor to prevent unacceptable loss of life and irreversible ecological changes that could result. The primary challenge with these countermeasures is our inability to deploy them throughout the ecosphere at sufficient scale to prevent or counter dissemination and evolutionary diversification of mirror bacteria in the wild. They could therefore only protect against a fraction of the potentially immense harm.

Foreseeable benefits of the creation of mirror bacteria are limited. Mirror biomolecules have scientific and potential therapeutic applications that are worth pursuing; however, while mirror bacteria could plausibly help to manufacture them, such molecules can be made through other means. More speculatively, mirror bacteria might be pursued as a chassis for live cell therapeutics, but again, alternative pathways are available. The potential risks of creating mirror bacteria cannot be justified by the relatively limited potential benefits.

The path forward

We encourage relevant expert communities to critically engage with the analysis summarized here and detailed in the accompanying technical report (*1*), and welcome arguments and evidence about mirror life we have not yet considered. In light of our initial findings, we believe it is important to begin a conversation on how the risks can be mitigated, and call for collaboration among scientists, governments, funders, and other stakeholders to consider an appropriate path forward. Below is our recommendation, which we offer as a starting point for further discussion.

Unless compelling evidence emerges that mirror life would not pose extraordinary dangers, we believe that mirror bacteria and other mirror organisms, even those with engineered biocontainment measures, should not be created. We therefore recommend that research with the goal of creating mirror bacteria not be permitted, and that funders make clear that they will not support such work. Governance of a subset of enabling technologies should also be considered: we recommend that steps be taken to prevent the production of certain upstream components of a mirror bacterium, including 1) a mirror genome or equivalent set of mirror genes and 2) a full mirror proteome or an equivalent set of mirror proteins sufficient to enable the construction of a mirror cell. Further, we recommend consideration of systems for monitoring the purchase of mirror nucleosides and mirror oligonucleotides, and additional regulations and laws to prevent the creation of mirror life. Governance measures should ensure that anyone attempting to create mirror bacteria would be hindered by multiple scientifically challenging, expensive, and time-consuming steps. As science progresses and opens up additional pathways to the creation of mirror life, these measures should be regularly reviewed.

Many related technologies, such as the chemical synthesis of mirror-image nucleic acids and proteins—not aimed at the creation of a mirror bacterium—have scientific and potential therapeutic applications. Diverse mirror proteins and RNAs could be made for research applications such as aptamers, biocatalysis, and phage display, and D-amino acids could be incorporated into synthetic peptide or protein drugs. We do not recommend any new restrictions on such research. Similarly, much synthetic cell research does not directly enable the creation of a mirror bacterium, is of great value to basic science, and should continue.

Transparent and international research programs might be considered to understand and prepare for risks from mirror bacteria, in case efforts to prevent their creation fail. Such research might include studying the interaction of mirror biomolecules with the immune system, as well as developing detection methods and biosurveillance systems. While countermeasures could not prevent widespread harm, they might offer some limited or localized protection. None of these research directions would require building mirror bacteria, and neither mirror bacteria nor any key enabling precursors should be produced in the course of any such research. Any research on countermeasures should take place in change line number an open, international setting to engender trust.

We believe there is a productive path ahead in which a range of stakeholders collaboratively consider the risks from mirror life and develop appropriate governance without unnecessarily impeding scientific research. Drawing inspiration from the Tianjin Biosecurity Guidelines (*16*) and other relevant frameworks, we invite the global scientific community, policymakers, research funders, industry, civil society, and the public to join this discussion. To facilitate greater understanding of the risks associated with mirror life and further progress on governance, we plan to convene discussions on these topics in 2025. We are hopeful that scientists and society at large will take a responsible approach to managing a technology that might pose unprecedented risks.

References and Notes

1. Adamala K. P. et al. "Technical Report: Mirror Bacteria, Feasibility and Risks". November 2024, https://doi.org/10.25740/cv716pj4036

2. J. H. Brewster, M. Laskowski Jr, Left-handed comments. *Science* **258**, 1289 (1992).

3. J. Bohannon, Mirror-Image Cells Could Transform Science — or Kill Us All, *WIRED* (2010). https://www.wired.com/2010/11/ff-mirrorlife/.

4. B. E. Young, N. Kundu, J. T. Sczepanski, Mirror-image oligonucleotides: History and emerging applications. *Chemistry* **25**, 7981–7990 (2019).

5. K. Harrison, A. S. Mackay, L. Kambanis, J. W. C. Maxwell, R. J. Payne, Synthesis and applications of mirror-image proteins. *Nat. Rev. Chem.* **7**, 383–404 (2023).

6. Y. Xu, T. F. Zhu, Mirror-image T7 transcription of chirally inverted ribosomal and functional RNAs. *Science* **378**, 405–412 (2022).

7. N. J. Gaut, K. P. Adamala, Reconstituting natural cell elements in synthetic cells. *Adv. Biol. (Weinh.)* **5**, e2000188 (2021).

8. D. G. Gibson, J. I. Glass, C. Lartigue, V. N. Noskov, R.-Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krishnakumar, N. Assad-Garcia, C. Andrews-Pfannkoch, E. A. Denisova, L. Young, Z.-Q. Qi, T. H. Segall-Shapiro, C. H. Calvey, P. P. Parmar, C. A. Hutchison 3rd, H. O.

Smith, J. C. Venter, Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* **329**, 52–56 (2010).

9. F. Rohden., J. D. Hoheisel, H. J. Wieden, Through the looking glass: milestones on the road towards mirroring life. *Trends Biochem Sci.* **46**(11):931-943. (2021).

10. M. Tong, S. French, S. S. El Zahed, W. K. Ong, P. D. Karp, E. D. Brown, Gene dispensability in *Escherichia coli* grown in thirty different carbon environments. *mBio* **11** (2020).

11. H. M. Dintzis, D. E. Symer, R. Z. Dintzis, L. E. Zawadzke, J. M. Berg, A comparison of the immunogenicity of a pair of enantiomeric proteins. *Proteins* **16**, 306–308 (1993).

12. M. Uppalapati, D. J. Lee, K. Mandal, H. Li, L. P. Miranda, J. Lowitz, J. Kenney, J. J. Adams, D. Ault-Riché, S. B. H. Kent, S. S. Sidhu, A potent d-protein antagonist of VEGF-A is nonimmunogenic, metabolically stable, and longer-circulating in vivo. *ACS Chem. Biol.* **11**, 1058–1065 (2016).

13. P. C. Sedman, J. Macfie, P. Sagar, C. J. Mitchell, J. May, B. Mancey-Jones, D. Johnstone, The prevalence of gut translocation in humans. *Gastroenterology* **107**, 643–649 (1994).

14. S. Jin, D. Wetzel, M. Schirmer, Deciphering mechanisms and implications of bacterial translocation in human health and disease. *Curr. Opin. Microbiol.* 67, 102147 (2022).

15. S. D. Blacksell, S. Dhawan, M. Kusumoto, K. K. Le, K. Summermatter, J. O'Keefe, J. P. Kozlovac, S. S. Almuhairi, I. Sendow, C. M. Scheel, A. Ahumibe, Z. M. Masuku, A. M. Bennett, K. Kojima, D. R. Harper, K. Hamilton, Laboratory-acquired infections and pathogen escapes worldwide between 2000 and 2021: a scoping review. *Lancet Microbe* **5**, e194–e202 (2024).

16. "The Tianjin Biosecurity Guidelines for Codes of Conduct for Scientists" (Tianjin University, 2021); https://tjusa.tju.edu.cn/info/1093/1443.htm.

Acknowledgements: This article emerged from the activities of a working group chaired by JIG and JWS. The Mirror Biology Dialogues Fund, established to support discussions and research on this topic, and enabled by contributions from Open Philanthropy, Patrick Collison, the David and Lucile Packard Foundation, and the Gordon and Betty Moore Foundation, supported the working group via support from staff and via funding to MLN, SBO and JAS. Open Philanthropy also supported the working group via support from staff and via funding to KE, MLN, SBO and JAS for work contributing to this article and/or the accompanying technical report; DAR also acknowledges past support for work on the same topic. Y.C. acknowledges support from UKRI Engineering and Physical Sciences Research Council EP/V05967X/1 (Open Plus Fellowship: Engineering and safeguarding synthetic genomes). Other authors acknowledge general support from a wide range of sources. A list of competing interests for all authors is

provided in the Supplementary Materials. The views expressed here are those of the individuals and not those of any organizations with which they are affiliated.

Author contributions: Authors are listed alphabetically by surname. JP led the writing, including writing the first draft outlining the working group's position. This was reviewed and revised by a core drafting group comprising JP, JAS, SBO, DAR, JIG, JWS. Further input from all authors was incorporated in subsequent drafts. The following authors contributed to writing the separately released technical report: KPA, DA, YC, VSC, KME, JIG, TWH, TVI, FJI, JDGJ, REL, RM, MLN, SBO, JP, DAR, HS, JWS, NJT.