Accelerating bioactive discovery for human and planetary health using artificial intelligence and animal-free testing

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Competing interest statement

Siva Vanapalli and Martin Kussmann are equity holders in NemaLife Inc.

Bio

Professor Dr Siva Vanapalli is the CEO of NemaLife and a Professor in Chemical Engineering at Texas Tech University. At NemaLife, he drives vision, team building and growth strategy, transforming preclinical R&D for global brands in the functional ingredient and pharmaceutical markets. He has published more than 100 journal articles in diverse areas including artificial intelligence, microfluidics, complex fluids, food science, cancer, healthy ageing, and space biology.

Dr Martin Kussmann is CEO and founder of Kussmann Biotech GmbH, a biotechnology consulting firm in health, nutrition, and sustainability. Dr Kussmann is also Head of Science at the Competence Center for Nutrition (KErn), an institute within the Bavarian State Ministry. Trained as a biochemist, Dr Kussmann has a 30-year corporate/academic career in nutrition, pharma, and biotechnology including professorships at EPF Lausanne, Switzerland; Aarhus University, Denmark; and Auckland University, New Zealand. He is credited with more than 200 publications.

Collaborators

Dr Mizanur Rahman, CTO & Founder, NemaLife Inc., USA

Dr Jim Kaput, CSO & Founder, Vydiant Inc., USA

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Accelerating bioactive discovery for human and planetary health using artificial intelligence and animal-free testing

- This article summarises the work of Professor Dr Siva Vanapalli and Dr Martin Kussmann, who are the CEOs and Founders of NemaLife Inc and Kussmann Biotech GmbH, respectively.
- NemaLife Inc is a TechBio company in Texas, USA, that harnesses the power of artificial intelligence and microfluidics on human-relevant *C. elegans* biology to transform the discovery and development of bioactives.
- Kussmann Biotech GmbH, Germany, is a biotechnology consulting firm in health, nutrition, and sustainability.

Our planet is witnessing an unprecedented global health crisis, with more than one billion people suffering from metabolic disorders and about half a billion suffering from age-related diseases. The socio-economic burden associated with such a health crisis is staggering and warrants new approaches to provide affordable and accessible human health solutions. While the advances in medical sciences helped treat or mitigate many of the above-mentioned conditions, this reactive approach comes at a high cost, including (1) expensive clinical trials for drugs across the pharmaceutical industry, and (2) poor adherence to both dietary and medical regimens by many patients [Berciano, S, et al, 2022].

Food as medicine: nature's bioactives for proactive human health management

We therefore need a more proactive and preventative approach to health and well-being, which better leverages the power of the food we ingest in our daily lives. With the advent of personalised health-tracking devices and applications, the consumer is increasingly aware of their daily goals regarding nutrition, physical activity, and sleep. It is through this awareness that food and nutrition science and industry have identified their next big goal: discovering natural food ingredients, ie, bioactive compounds from the food chain, and understanding their relationships with human health [Kussmann, M, et al, 2023].

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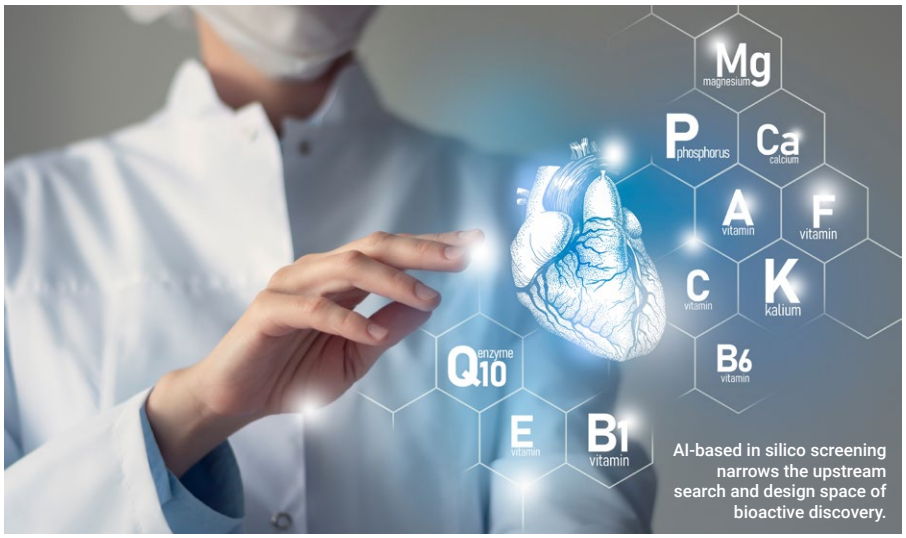
Bioactive compounds are abundant in nature.

Natural, edible bioactive compounds are classified into macronutrients (proteins, peptides, carbohydrates, and lipids), micronutrients (vitamins and minerals), phytonutrients (secondary plant metabolites, phenolic compounds, alkaloids, and terpenes), and microbiome regulators (prebiotics, probiotics, and synbiotics). These bioactives are found in natural food sources and plants, which can be incorporated as active ingredients in nutritional products and supplements for an overall improved quality



of life and as an eco-friendly nutritional approach to help meet the UN's sustainability development goals [Kussmann, M, et al, 2023].

Accelerating bioactive discovery

While bioactive compounds are abundant in nature, they are difficult to rapidly discover and efficiently translate into functional ingredients with unique health benefits. Previously, bioactive discovery depended on serendipity and observations of relationships with particular health conditions, eg, deficit symptoms in the absence of certain micronutrients. With the advent of high-throughput screening (HTS), the world of medicine saw a significant upgrade in this discovery process. The traditional approach for HTS involves in vitro studies, followed by testing in animal models, and, finally, human clinical trials. Therefore, HTS is still resource-intensive, and translating its discoveries into clinical practice has proven challenging. By integrating artificial intelligence (AI), HTS workflows are becoming cost-effective and faster, providing rich human-relevant data sets to de-risk clinical trials [Kussmann, M, 2022].

Given the vast search space for discovering bioactives from nature, AI can be used upstream of wet lab experiments and preclinical studies to significantly reduce time and cost. This in silico approach involves mapping the interactions between bioactive compounds and their effects on the human body. Data on proteins and their interactions (proteomics), metabolites and their effects (metabolomics), as well as gut microbes and their symbiotic relationship with food molecules (microbiomics) are essential to building foundational AI models. Starting with a desired and defined benefit, be it for human/animal health or a healthier and more sustainable food solution, AI models can be used to predict bioactives that can exert such function. Analytical techniques like mass spectrometry, liquid chromatography, and isotope-labelling assays are helpful to structurally characterise, purify, and quantify the bioactive compounds predicted by AI, which can be subsequently validated by in vitro and in vivo testing [Doherty, A, et al, 2021].

Validating the efficacy and safety of bioactives

While AI-based in silico screening narrows the upstream search and design space of bioactive discovery, a major choke point is still the validation of the efficacy and safety in an in vivo model. Testing bioactives in traditional animal models (eg, rodents) for human health conditions such as metabolic disorders, ageing, and cognitive health is expensive, time-consuming, and increasingly challenged due to animal welfare reasons. Moreover, animal husbandry causes greenhouse emissions and requires significant energy and water resources – all of which negatively impact planetary health.

The microscopic roundworm *Caenorhabditis elegans* (*C. elegans*) has been a cornerstone for many human-relevant biological discoveries, including three Nobel-prize-winning investigations within a period of 10 years – an astonishing achievement in the history of science, highlighting the relevance of this non-mammalian model for human biology.

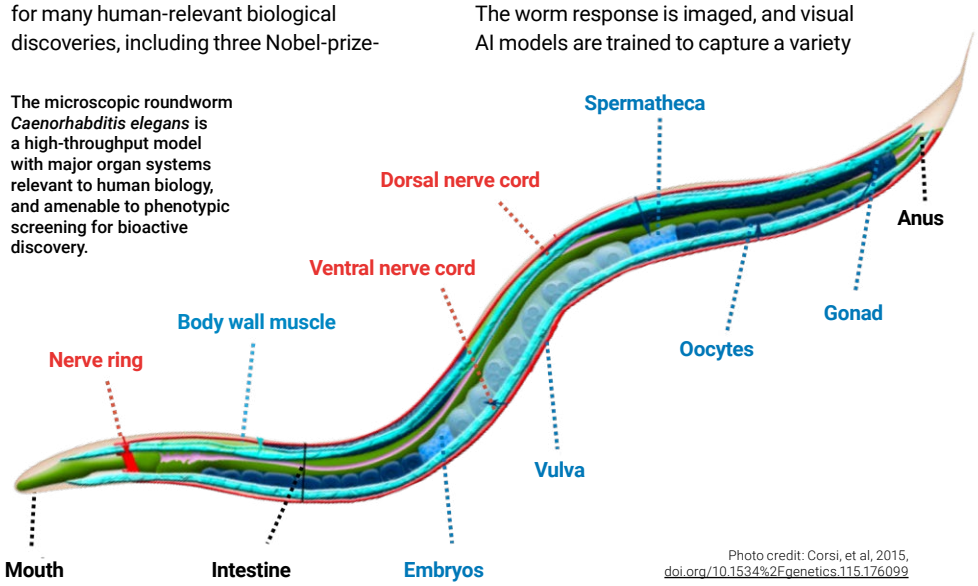
C. elegans has a short lifespan of ~ 3 weeks. It is addressing the need for an in vivo model that is both cost- and time-efficient for preclinical studies (eg, 100,000+ worm subjects can be recruited in about one week). 60–80% of the human-encoding genes have *C. elegans* homologs. 42% of the human disease-causing genes have a homolog in *C. elegans*. Many cellular and molecular signalling pathways related to metabolism, neuronal function, stress regulation, muscle force generation, and longevity are conserved between *C. elegans* and mammals.

From a regulatory perspective, *C. elegans* is excluded from the definition of an animal due to its invertebrate status. The '3R' principles of replace, reduce, and refine are certainly relevant when using the *C. elegans* model, and have become a part of corporate governance. With the signing of the FDA Modernization Act 2.0 in 2022, new medicines need not be tested in traditional animals to receive US FDA approval. *C. elegans* can address this gap of evaluating safety and efficacy in an intact living system, providing data that is actionable and qualifies safe bioactives for human studies.

> **'If we don't understand the worm, we don't understand life' – John Sulston, Nobel Prize, 2002.**

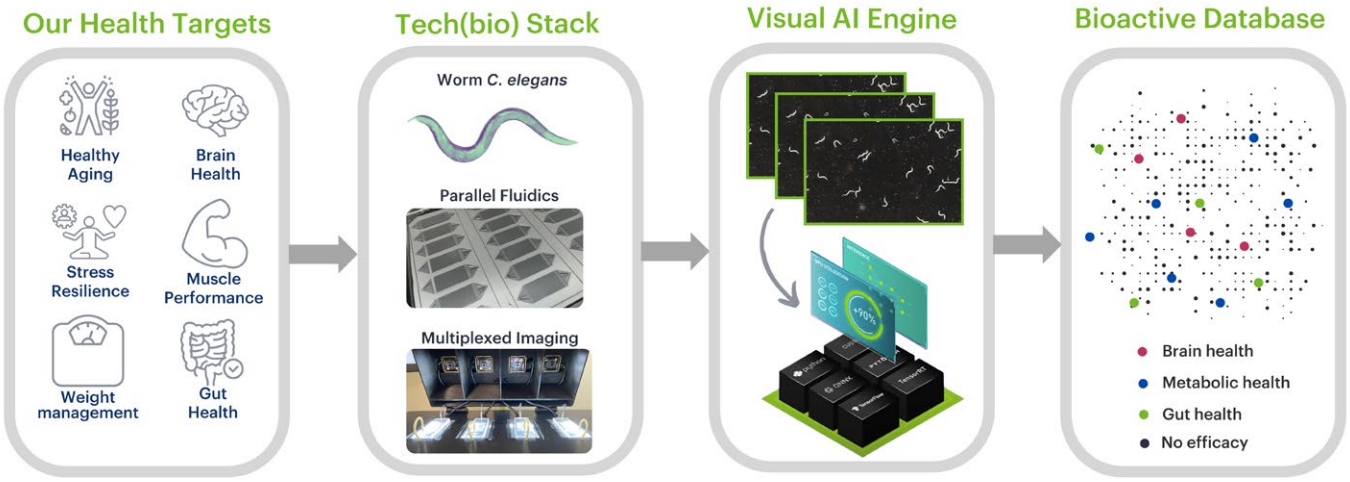
AI-powered organism-on-chip platform for bioactive discovery

To perform high-throughput screening and validation of bioactives in *C. elegans*, NemaLife has developed a microfluidic chip along with visual AI. The microfluidic chip enables effortless culture of *C. elegans* across its life span, along with flexible scheduling and dosing of bioactives [Rahman, M, et al, 2020]. The worm response is imaged, and visual AI models are trained to capture a variety



The microscopic roundworm *Caenorhabditis elegans* is a high-throughput model with major organ systems relevant to human biology, and amenable to phenotypic screening for bioactive discovery.

Photo credit: Corsi, et al, 2015, doi.org/10.1534%2Fgenetics.115.176099



The organism-on-chip platform is powered by *C. elegans*, parallel microfluidics, multiplexed imaging and visual AI to screen at high throughput multiple phenotypes that provide a living proof of human health benefits.

The NemaLife Discovery Flywheel and the Efficacy Matrix create enormous ingredient innovation opportunities including adding new benefits to existing products, mining collections and side/waste streams, and designing combinations with synergistic outcomes.

of phenotypes based on the health benefit of interest and safety end points. Thus, microfluidics allows scalable worm husbandry, while visual AI facilitates high-volume image processing, providing a high-throughput in vivo alternative to traditional animal testing.

NemaLife's first generation organism-on-chip platform has shown that bioactive compounds, present in tart cherry extract, confer longevity benefits in *C. elegans* by improving mitochondrial function and reducing oxidative stress [Jayarathne, S, et al, 2020]. Bioactive hemp compounds, such as cannabidiol [Land, MH, et al, 2021] and cannabigerol [Kulpa, J, et al, 2023], as well as oleuprin [De Marchi, U, et al, 2022] (from olive leaves) and trigonelline [Feige, J, et al, 2020] (present in fenugreek seeds) were found to improve healthy ageing outcomes.

The organism-on-chip platform has been expanded to develop the NemaLife Discovery Flywheel, which screens concurrently several health benefits, such as stress resilience, cognitive health, gut health, metabolic health, muscle performance, and longevity. The Discovery Flywheel profiles each bioactive comprehensively across a range of health benefits, providing an Efficacy Matrix. The NemaLife Discovery Flywheel and the Efficacy Matrix create enormous ingredient innovation opportunities including adding new benefits to existing products, mining collections and side/waste streams, and designing combinations with synergistic outcomes.

The future of bioactive discovery

AI enables both efficient discovery of bioactives and rapid processing of real-time in vivo data on those very bioactives. Harnessing the power of AI in discovering bioactives from plant and food sources in a high-throughput, in-vivo setting not only makes the process more efficient and sustainable but also provides the scope to proactively address important human health conditions. Moreover, using animal-free methods to validate these bioactives contributes to the global sustainability goals – a win-win situation for science and the planet.

Personal response

What inspired you to conduct these studies?

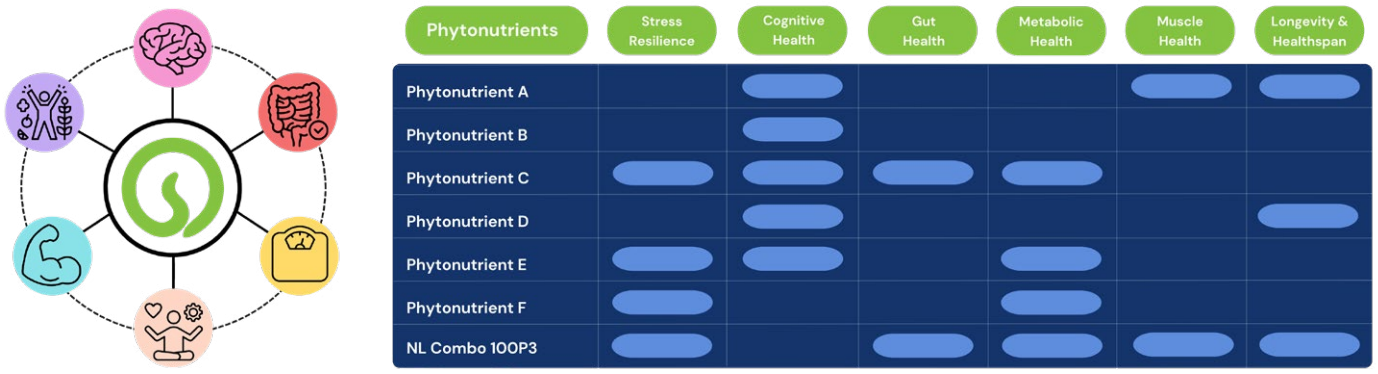
There is an urgent need to develop science and data-backed bioactives for human health benefits since traditional animal testing is antiquated, animal welfare activism is rising, and regulatory bodies have mandates to ban animal testing. The development of the organism-on-chip platform and its validation for bioactive discovery has been motivated by the need to solve this crisis and unlock transformative natural health solutions.

Apart from longevity and health span, can the C. elegans platform test for bioactives supporting cognitive or mental health?

Yes. *C. elegans* neurobiology is highly conserved with similar ion channels, receptors, vesicular transporters, and synaptic components. Similar to mammals, it uses neurotransmitters such as glutamate, γ-aminobutyric acid, dopamine, serotonin and acetylcholine. This conserved biology lends to modelling learning, memory, and depression to screen for novel bioactives.

What is next for your research?

We aim to drive this AI-powered bioactive discovery and validation technology to develop the best-in-class bioactive combinations by leveraging nature's treasures for human and planetary health at an unprecedented scale, speed, and cost efficiency.



NemaLife Discovery Flywheel and Efficacy Matrix; Comprehensive parallel profiling provides a new strategy to re-purpose bioactives and design combinations that confer resilience against several health conditions.

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