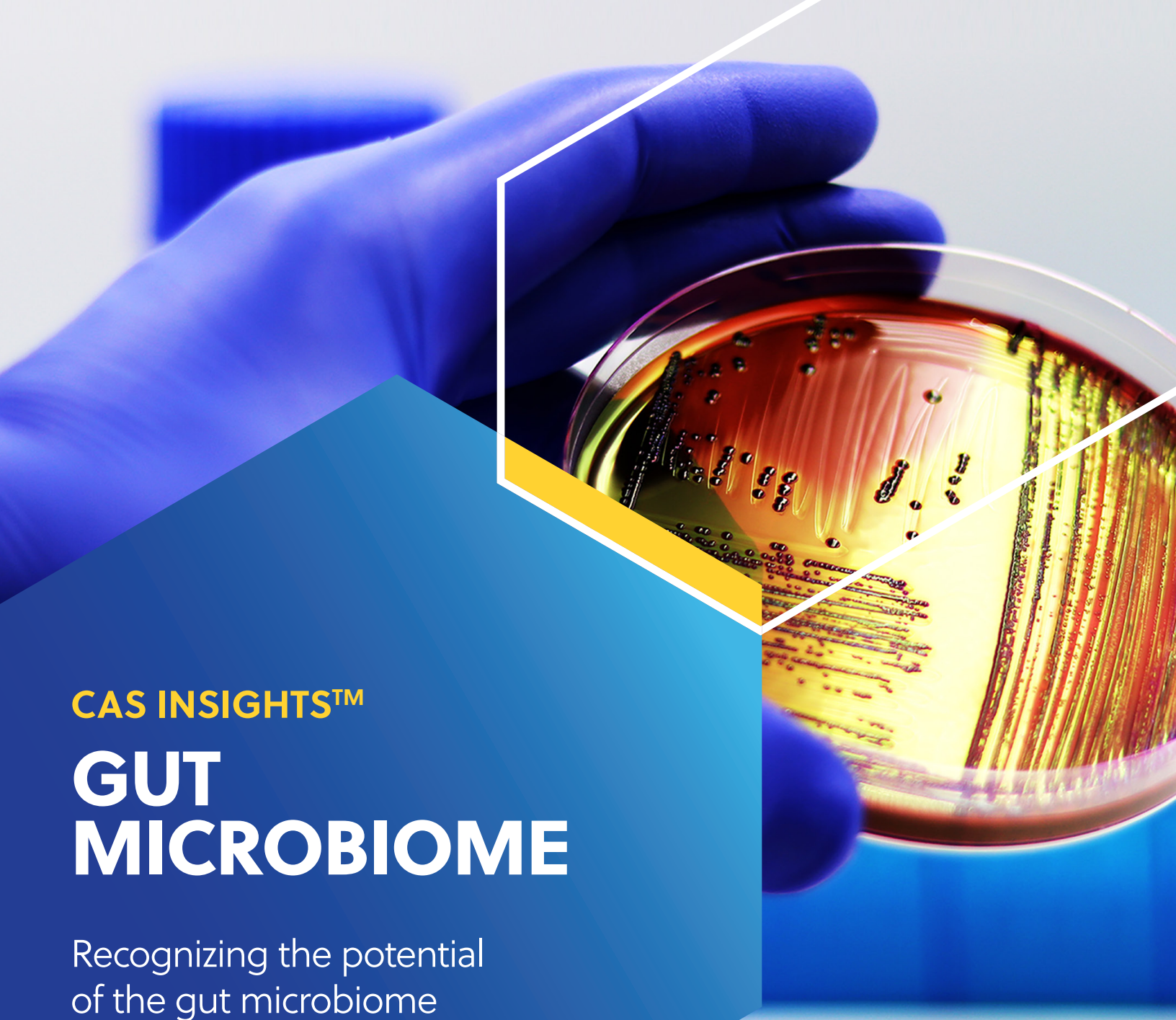

CAS INSIGHTS™

GUT MICROBIOME

Recognizing the potential of the gut microbiome

BAYER

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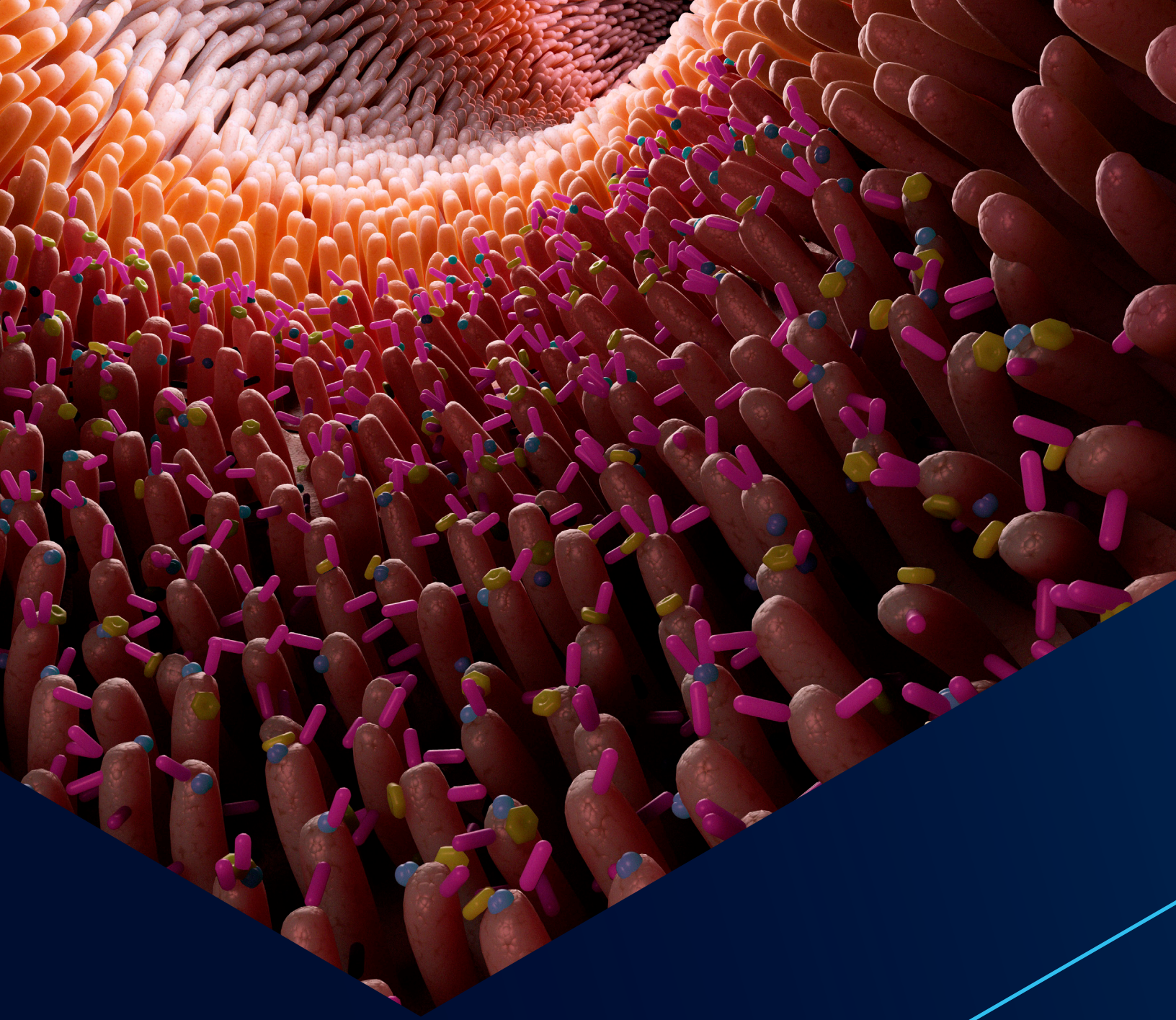


A forgotten organ

The human body comprises an unthinkable 30 trillion human cells, which is overshadowed by the amount of resident bacterial cells, estimated at approximately 40 trillion.[1] The impact of the microbiome on human physiology and pathology is extensive; it has been suggested to function as an essential, yet 'forgotten,' organ.[2–4] The gut microbiome alone is estimated to be more than 100 times larger than the human genome, containing >22 million microbial genes vs. 22,000 human genes.[5,6] Substantial data suggest the gut microbiome plays a role in virtually all physiological processes in the human body, including metabolism and immune homeostasis.[7,8] Over a century ago, the Russian biologist Élie Metchnikoff advocated for exploiting the intestinal microbiome using host bacteria in yogurt, owing to enhanced health benefits.[9] While Metchnikoff's hypothesis didn't gain traction, the popularity of the gut microbiome had grown substantially since the 2010s, when we witnessed a stream of significant developments in the field, leading to Forbes naming this period The Decade of the Microbiome (**Figure 1**):[10]

- **2010:** The first extensive catalog of human intestinal microbial genes based on studies on 124 individuals was published.[11]
- **2011:** The National Institutes of Health initiated "The Human Microbiome Project" and published the sequences of 178 bacterial species.[12,13]
- **2012:** The METAgenomics of the Human Intestinal Tract collaboration was funded by the European Commission.[14]

The gut microbiome is now a popular target in the biotechnology industry owing to intensified interest in the physiological benefits. Using the CAS Content Collection™, we have undertaken a tailored analysis to investigate advances in knowledge relating to the human gut microbiome, its complexity and functionality, its communication with the central nervous system, and the effect of the gut microbiome-brain axis on mental and digestive health.

1670
Anntonie van Leeuwenhoek discovers the microorganisms, making use of his newly developed microscopes

1729
Pier Antonio Micheli classification of plants and fungi

1875
Ferdinand Cohn lays the foudations of bacteriological taxonomy (Drews, 2000)

1890
Robert Koch published four postulates, establishing the causative relationship between the presence of a microorganism and a specific infectious disease (Segre, 2013)

1907
Ilya Metchnikov presents a theory on the role of gut microbiome in health and disease (Metchnikoff, 1923)

1917
Alfred Nissle isolated E. coli Nissle 1917 strain, probiotic that antagonized pathogens (Sonnenborn, 2016)

1928
Discovery of antibiotics by Alexander Fleming (Tan, 2015)

1944
Pioneering work of Robert E. Hungate on the refinement of anaerobic culture techniques which enabled first isolation of human-associated anaerobes (Hungate, 1969)

1958
Ben Eiseman reported a case of 4 patients with pseudomembranous enterocolitis cured with a fecal enemas, before C. difficile was found as the cause (Eiseman, 1958)

1959
Germ-free mice reared (Reyniers, 1959)

1965
Gut microbiota transfer in germ-free animals (Schaedler, 1965)

1972
Microbiota affects metabolism of host-directed drugs (Peppercorn, 1972)

1981
Microbiota progression in early life reported (Stark, 1982)

1995
First complete genome sequences of the bacteria Haemophilus influenzae and Mycoplasma genitalium (Fleischmann, 1995)

1996
First human fecal sample sequenced (Wilson, 1996)

2003
Studies of host-associated microorganisms other that bacteria (Guarner, 2003)

Figure 1. Timeline of major research and development milestones related to the microbiome

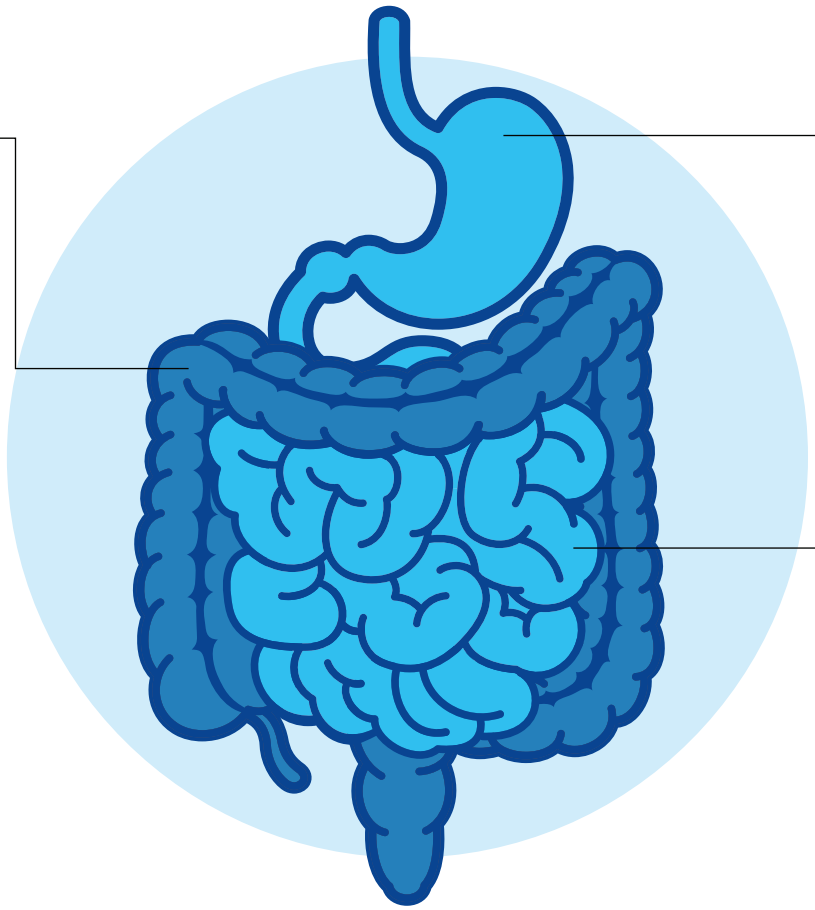
A closer look at our gut bacteria

The gut microbiome differs according to the gastrointestinal (GI) anatomy and other factors relating to its physiology, pH and O_2 tension, flow rates, substrate availability, and host secretions.[15] The microbiome is affected by many factors, including diet, medications (especially antibiotics), ethnicity, age, and general health.[16] The four dominant phyla that reside in the human gut are (**Figure 2**):[16]

- Firmicutes (which contain Lactobacilli)
- Bacteroidetes
- Actinobacteria (which contain Bifidobacteria), and
- Proteobacteria

Other phyla found in lower numbers are Fusobacteria and Verrucobacteria.

Research to date has uncovered relationships between e.g. metabolic, neurological, and autoimmune disorders, allergies, infections, cancers, and the microorganisms that live on and in humans.



Large Intestine

10^{10}-10^{12} CFU/ml

Firmicutes:
Lactobacilli
Streptococci
Eubacteria
Colstridia
Veilonella
Staphylococci

Bacteroidetes:
Streptococci

Actinobacteria:
Bifidobacteria

Proteobacteria:
Proteus
Enterobacteria
Pseudomonads

Fusobacteria:
Akkermansia

Stomach

10^{1}-10^{3} CFU/ml

Firmicutes:
Lactobacilli
Streptococci

Bacteroidetes
Proteobacteria
Fusobacteria
Actinobacteria

Small Intestine

10^{4}-10^{8} CFU/ml

Firmicutes:
Lactobacilli
Streptococci

Bacteroidetes:
Streptococci

Actinobacteria:
Bifidobacteria

Fusobacteria:
Akkermansia

Figure 2. Gut microbiota participant bacteria

Role of the gut microbiome in health and disease

Human microbiota collaborates closely with the digestive tract to exert five predominant functions:[17]

01 **Promote digestion** by assisting the absorption of nutrients by gut cells or the fermentation of some food fractions, which generate important metabolites, including short-chain fatty acids.

02 **Support digestive tract maturation** by participating in the assembly of GI mucus and promoting the enzymatic activity of the mucosa.

03 Provide a **barrier function** against pathogens and toxins; some bacteria release antimicrobial agents that protect from pathogenic bacteria.

04 Play a **protective role** in **promoting immune system development.**

05 Support the **synthesis of essential vitamins**, including vitamin B.

Gut microbes contribute to important physiological activities, including immunomodulation and the regulation of various hormones and metabolites (**Figure 3**). Alterations to these processes can disturb the balance in the microbiome (a process termed dysbiosis)[18] and trigger a range of pathological processes fundamental to mental health, metabolism, and many organ systems (**Table 1**).

There is substantial evidence supporting a relationship between gut microbiota and **digestive system disorders** (disorders of gut-brain interaction, **DGBIs**), particularly irritable bowel syndrome (IBS), inflammatory bowel disease (IBD, including Crohn's disease and ulcerative colitis), and functional constipation.[19–21]

Owing to its wide prevalence, IBS is considered the **prototype of DGBI**. IBS typically presents in early adulthood with symptoms including abdominal pain, bloating, and altered bowel habits. IBS can be classified into four subtypes: constipation-predominant (IBS-C), diarrhea-predominant (IBS-D), mixed type (IBS-M), and undefined IBS (IBS-U). Pathophysiological processes in IBS involving gut microbiota are thought to influence pathophysiological processes involving the immune system, the hypothalamic-pituitary-adrenal (HPA) axis, the gut-brain axis (GBA) and the enteric nervous system (ENS).[22] A large genome-wide analysis suggests shared pathogenic pathways between IBS and mood and anxiety disorders.[23] While it is widely accepted that alterations in gut-brain interactions are fundamental to IBS pathology, a causative role of the microbiome must still be elucidated. The heterogeneity of healthy gut microbiota presents challenges in identifying a clear IBS microbial signature.

Owing to altered transmission within the GBA, changes in the levels of gut microbial metabolites have been reported to be associated with **neurological diseases** such as Parkinson's disease, Alzheimer's disease, autism spectrum disorders,[24] and **mental health disorders** including anorexia nervosa,[25] chronic stress, and depression[26] however, the underlying mechanisms remain unclear.

Chronic stress is an inherent part of modern living. A link between stress and the abundance of Lactobacilli in mice was discovered for the first time more than 40 years ago.[26] A growing number of animal and human studies have verified the bidirectional relationship between stress and modulation of gut microbiota however, issues remain owing to limited translatability from animal models to humans and host heterogeneity.[27] Modulation of the gut microbiome has emerged as a possible way to improve stress resilience and mental health. In 2013, Dinan et al. coined the term **psychobiotic** — live microorganisms which when ingested in adequate amounts, produce a health benefit in patients suffering from psychiatric illness.[28] The term psychobiotic has since been widely adopted by neuroscientists to describe the use of different biotics to tackle depression, stress, anxiety, and other mental health complaints through the GBA.

Obesity and diabetes, both of which are regarded as **metabolic disorders**, are believed to be strongly affected by gut microbiota status.[29] Research also suggests a correlation between gut microbiota composition and levels of cytokines and inflammatory markers in patients with **COVID-19**.[30]

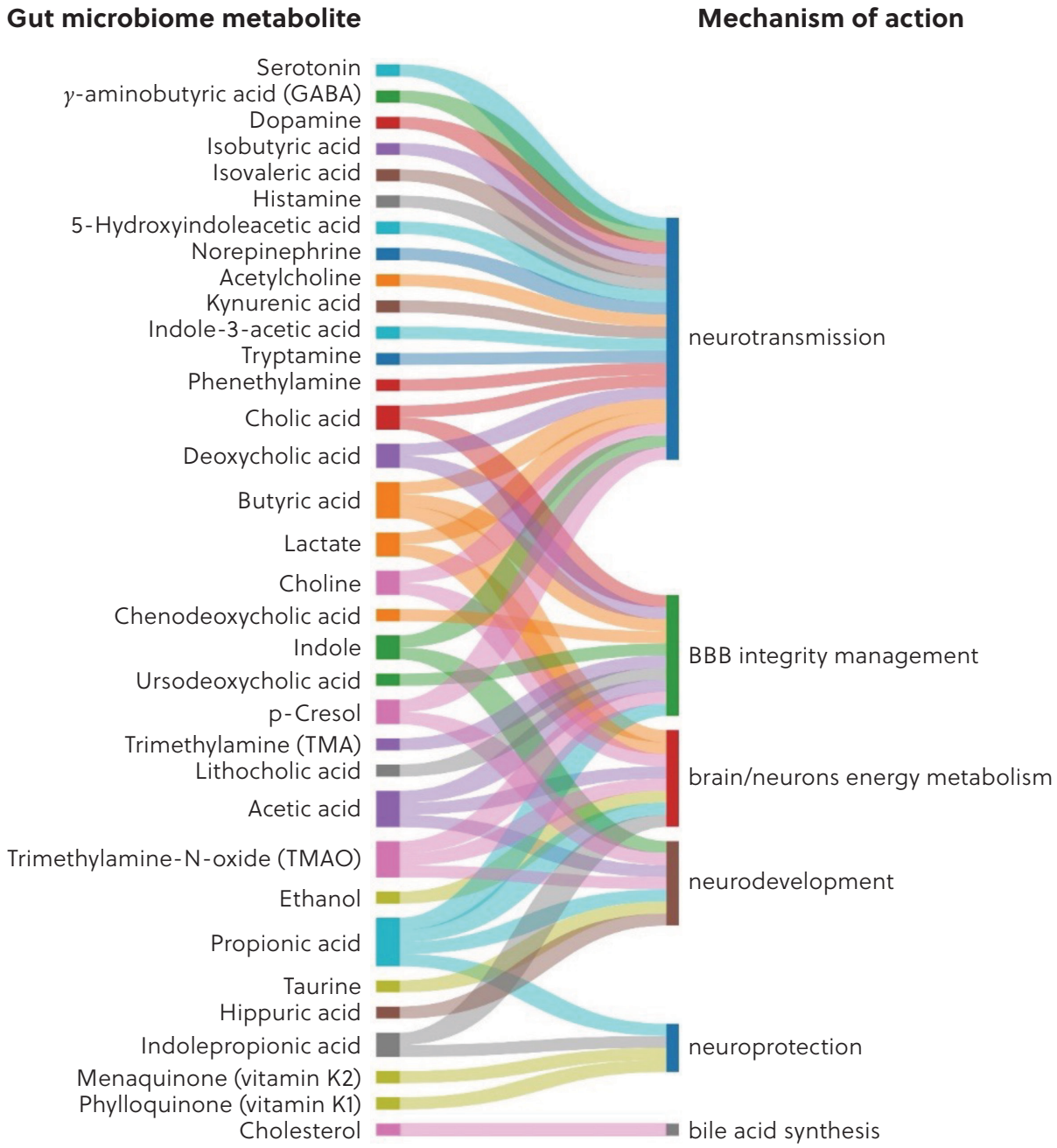


Figure 3. Exemplary gut microbiome metabolites and their mechanism of action in gut-brain communications

Table 1. Gut dysbiosis in digestive system diseases, mental health, and metabolic disorders

Diseases	↓ Decreasing bacteria	↑ Increasing bacteria
Digestive system diseases		
Irritable bowel syndrome	Bifidobacterium Faecalibacterium Bacteroides	Ruminococcus Dorea Enterobacteriaceae Lactobacillaceae Bacteroides Firmicutes:Bacteroidetes ratio
IBD: Crohn's disease	Bacteroides Faecalibacterium prausnitzii Bifidobacterium adolescentis	
IBD: Ulcerative colitis	Bifidobacteria Roseburia hominis Faecalibacterium prausnitzii Lachnospiraceae Ruminococcaceae	
Mental health disorders		
Anxiety disorder	Bacteroidetes Ruminococcus gnavus Fusobacterium	Bacteroidaceae Enterobacteriaceae Burkholderiaceae
Post-traumatic stress disorder	Actinobacteria Lentisphaerae Verrucomicrobia	
Depression	Prevotella Dialister	Eggerthella Holdemania Turicibacter Paraprevotella
Neurodegenerative disorders		
Alzheimer's disease	Actinobacteria Bacteria Firmicutes Proteobacteria	Bacteroidetes Firmicutes Verrucomicrobia
Parkinson's disease	Anaerostipes Blautia Clostridium Eubacterium Faecalibacterium Fusicatenibacter Prevotella Roseburia Ruminococcus	Acidaminococcus Actinomyces Bifidobacterium Clostridium Hungatella Lactobacillus Methanobrevibacter Streptococcus Porphyromonas
Metabolic disorders		
Type 1 diabetes	Alistipes Akkermansia Bifidobacterium Eubacterium rectal Prevotella Firmicutes Verrucomocrobia	Alistipes Clostridium Firmicutes Lactococcus Lachnospiraceae Roseburia Veillonella
Type 2 diabetes	Clostridia Faecalibacterium prausnitzii Firmicutes Eubacterium rectale Roseburia	Akkermansia muciniphilia Bacteroides Betaproteobacteria Clostridia Desulfovibrio Eggerthella E. coli Firmicutes:Bacteroidetes ratio
Obesity	Ruminococcus flavefaciens Bifidobacterium Methanobrevibacter	Firmicutes:Bacteroidetes ratio Actinobacteria Bacteroides Prevotellaceae

The gut microbiome and particularly its links between the digestive and nervous systems present an attractive target for the development of novel therapeutics for an ever-growing list of disorders.

An overview of microbiome therapies

One such way of targeting the GBA to impart health benefits is through the use of biotics, as defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP):[31]

- **Probiotics** are live microorganisms.
- **Prebiotic**s are substrates selectively utilized by host microorganisms.
- **Synbiotics** are a mix of live microorganisms and substrate(s) selectively used by host microorganisms.
- **Postbiotics** comprise the preparation of inanimate microorganisms and/or their components

Fecal microbiota transplantation (FMT), or fecal transplantation, is another specialized research area that has received considerable interest, particularly in the prevention of *Clostridium difficile (C. difficile)* infection.[32] The process involves the transplantation of healthy fecal bacteria from a donor to a recipient via colonoscopy.[32]

Research and development highlights

Journal and patent activity

There are more than 250,000 scientific articles (mainly journal articles and patents) in the CAS Content Collection relating to gut/intestinal microbiome/microbiota, with nearly 15,000 linked to mental and gut health aspects. Microbiome-related literature has sharply increased over the last decade, overcoming other "omics" topics, e.g., proteomics. A steady, exponential growth in the number of journal articles over time was observed from 1997 to 2021, with further accelerated interest in 2021 and 2022 (**Figure 4**).

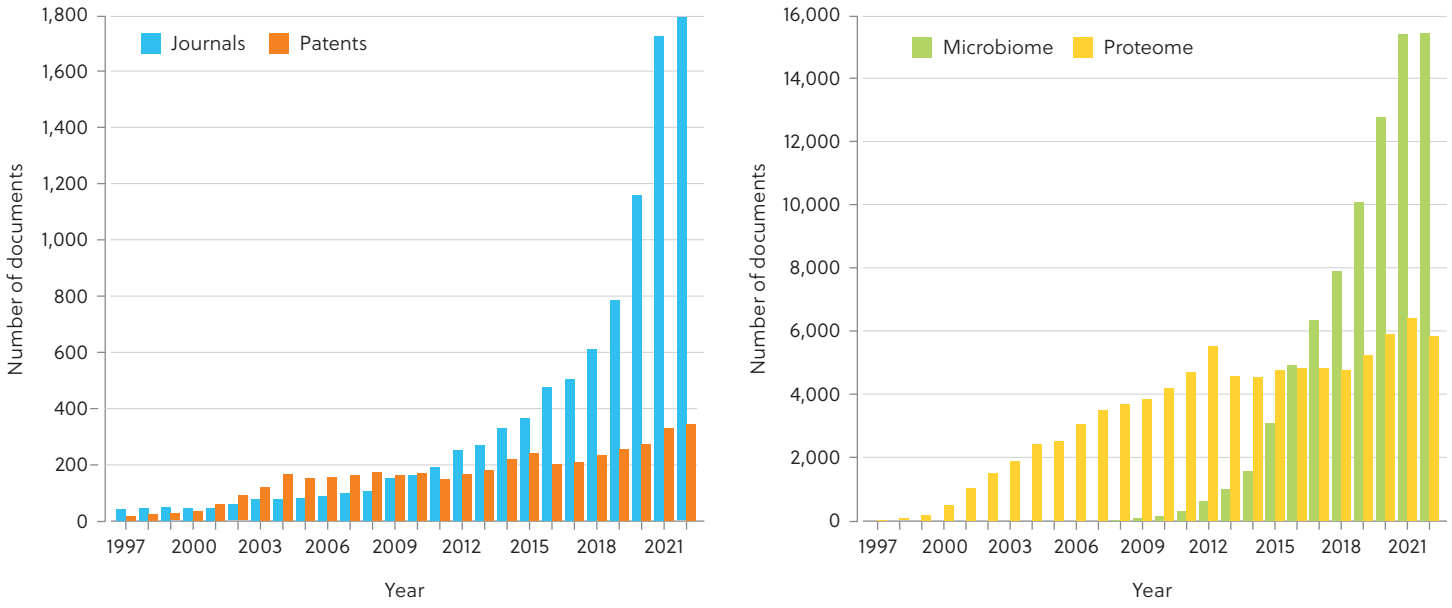


Figure 4. Journal and patent publication trends in gut microbiome research related to mental and gut health; Inset: Microbiome vs. Proteome document yearly trends

The number of patents grew rapidly until 2004, possibly correlating with the initial accumulation of knowledge and transfer into patentable applications; after this time, activity plateaued (**Figure 4**). The United States, China, Japan, and Korea were the leading countries responsible for published journal articles and patents associated with the gut microbiome in mental and gut health (**Figure 5**). The most active countries in terms of patent filing between 1990 and 2022 were the United States (n=14,919), followed by Germany (n=1754), Switzerland (n=1520), and the United Kingdom (n=1501).

Research themes

An examination of key publication concepts (approx. total 4500) relevant to gut microbiome research in mental and gut health revealed **"immunity"** (>4000 documents) and **"gut microbiome"** (>3500 documents) as top concepts in the area (**Figure 6**). The **"gut-brain relationship"** concept exhibited the greatest growth rate between 2021 and 2022.

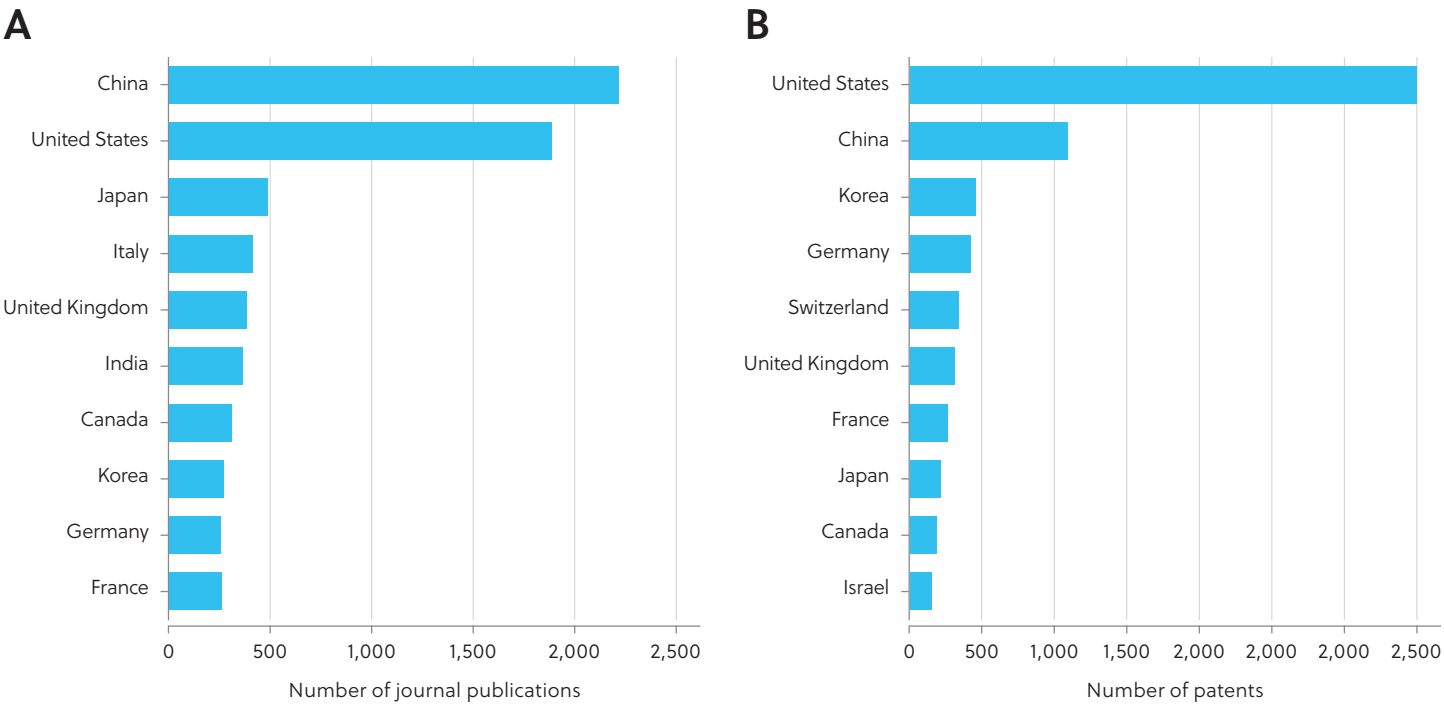


Figure 5. Top countries publishing journal articles (A) and patents (B) related to gut microbiome research in mental and gut health

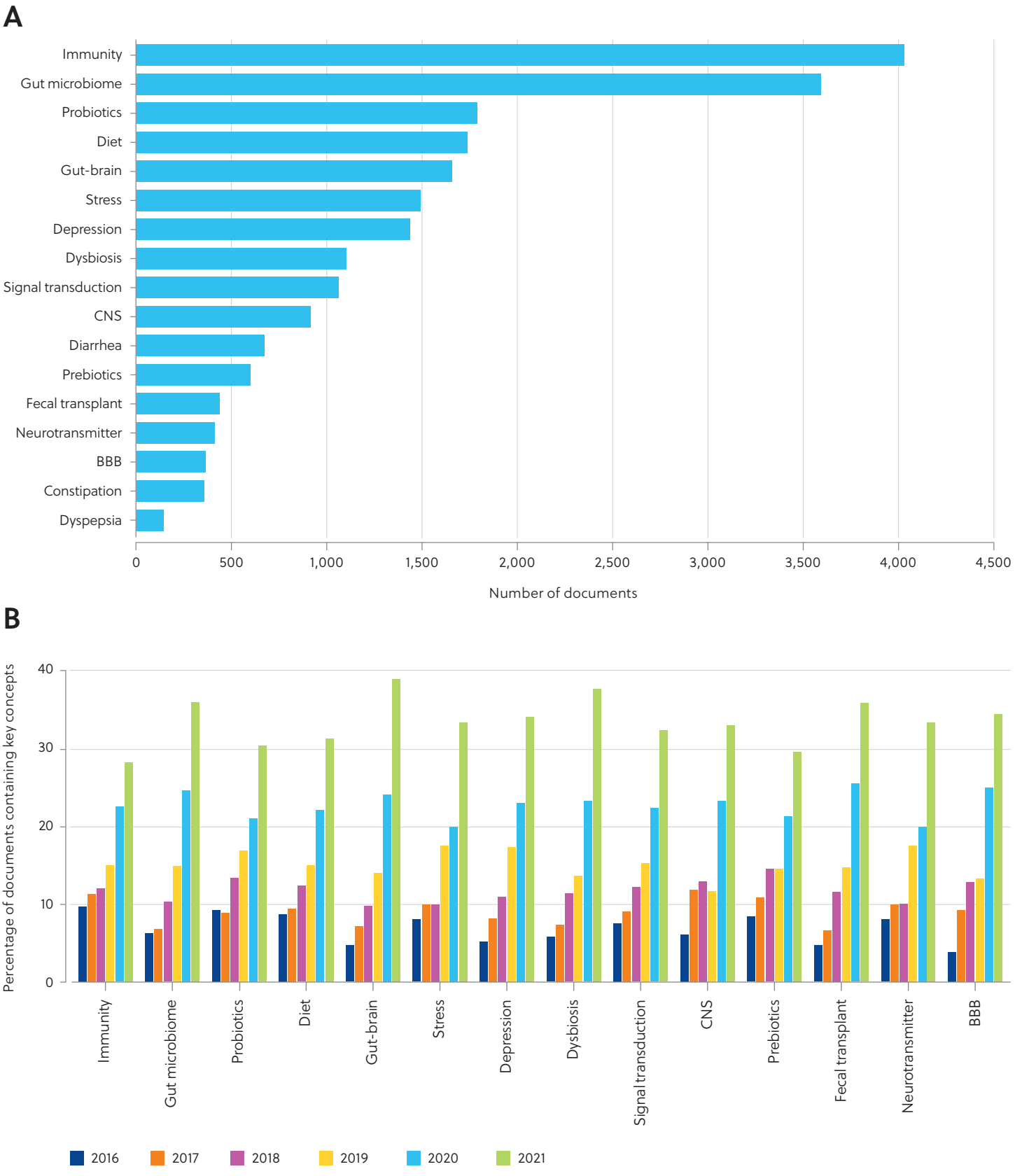


Figure 6. Key concepts in the scientific publications relevant to the gut microbiome research in mental and gut health: (A) Number of publications exploring key concepts related to gut microbiome research in mental and gut health. (B) Trends in key concepts presented in the articles related to gut microbiome research in mental and gut health during 2016–2021. Percentages are calculated with yearly publication numbers for each key concept, normalized by the total number of publications for the same concept in the same period

Our findings identified thousands of studies reporting correlations between gut microbiota and mental, metabolic, and digestive system disorders, cardiovascular and neurodegenerative diseases, various cancers, and immune and autoimmune diseases (**Figure 7**).

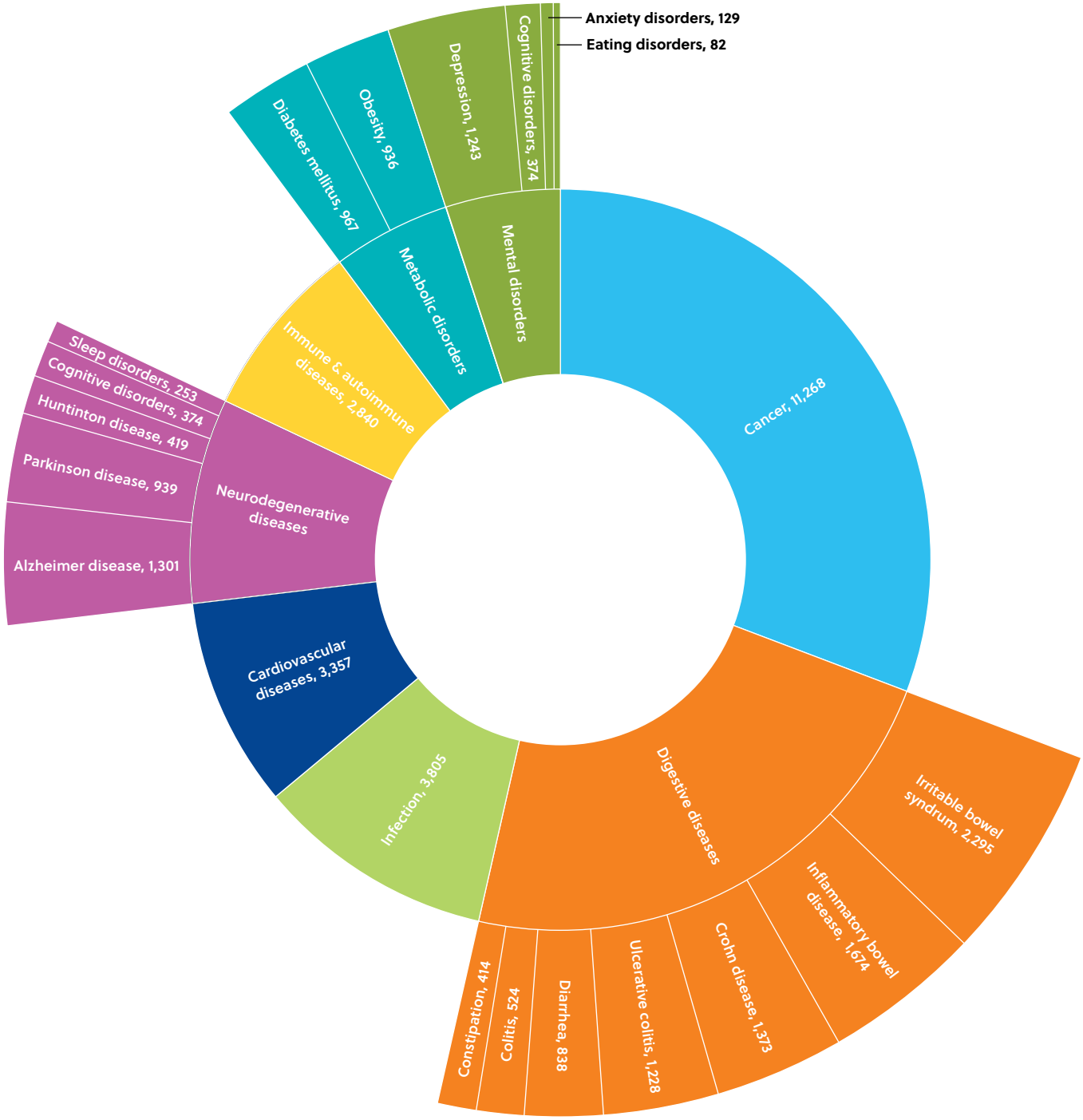


Figure 7. Distribution of the publications in the CAS Content Collection related to gut microbiome-associated diseases

CAS analyzed trends in the number of publications related to various diseases from 2016 to 2021, with all conditions experiencing substantial increases in research output (**Figure 8**). The theme of **dysbiosis** experienced the biggest surge in publications during this time. Other areas of research focus based on publication numbers were **depression, Alzheimer's disease**, and **Parkinson's disease.**

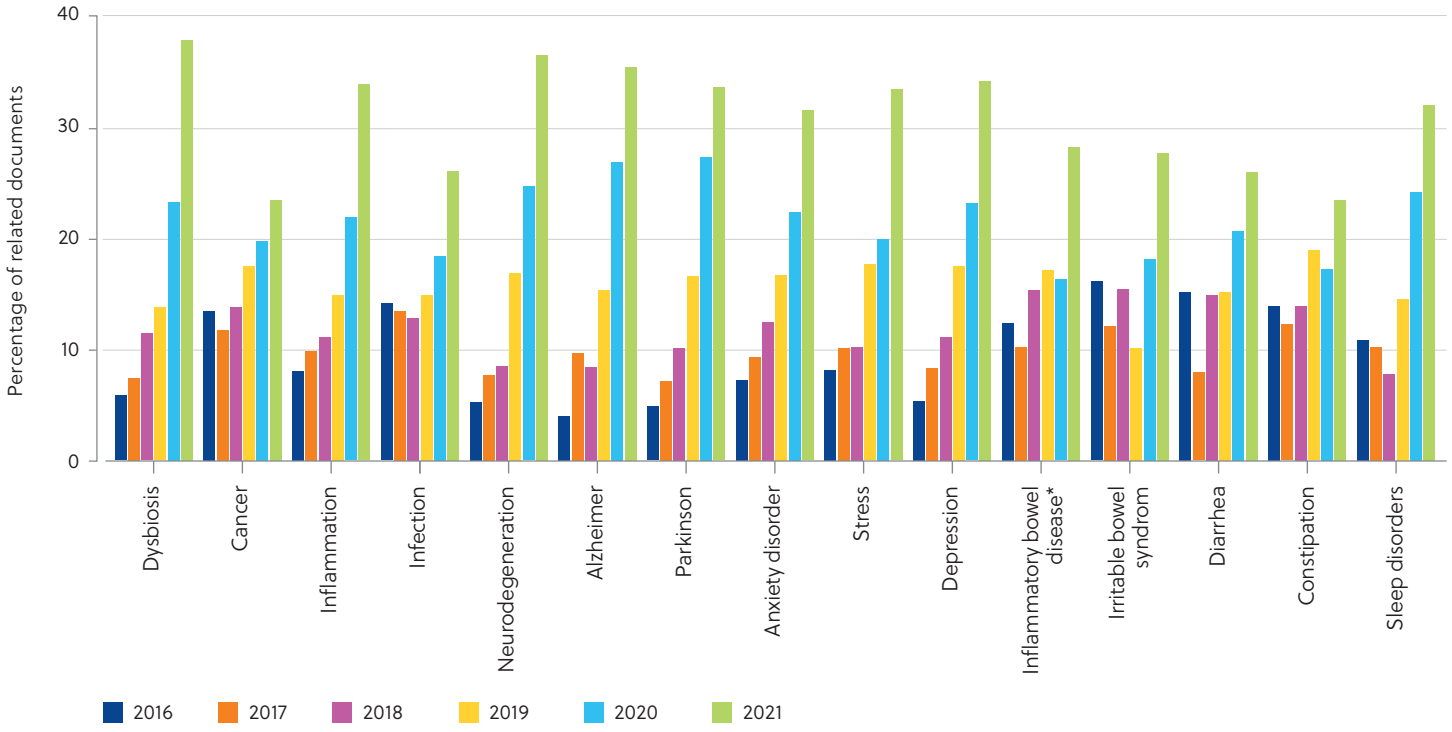


Figure 8. Trends in the number of publications concerning gut microbiome-related conditions during 2016–2021. Percentages are calculated with yearly publication numbers for each condition, normalized by the total number of publications for the same condition in the same period

Our analysis highlighted common gut microbiome metabolites that feature in the literature: **neurotransmitters,** serotonin (n=389 documents), and γ-aminobutyric acid (GABA; n=194 documents); and the **short-chain fatty acids** butyric acid (n=384 documents) and acetic acid (n=372 documents), which affect brain function and digestive function respectively.

Probiotics continue to be the leading focus of research interest in the global community, whereas the number of organizations evaluating **prebiotics** was significantly lower. **Postbiotics** have been investigated the least, owing to their small commercial presence however, they do exert some benefits over probiotics owing to longer shelf life and more specific targeting capabilities.

Key players in the microbiome field

The top academic organizations responsible for journal publications were universities and research institutes, with the University College Cork, the Chinese Academy of Science, the University of California, and McMaster University leading the field (**Figure 9**). Regarding patent activity, lead universities and medical centers included the University of California, Johns Hopkins University, and the University of Texas (**Figure 9**).

A

Universities	Journal Publication
University College Cork	135
Chinese Academy of Sciences	65
University of California	53
McMaster University	50
Huazhong University of Science & Technology	41
Jiangnan University	40
Ningbo University	37
Kyung Hee University	34
Nanjing Agricultural University	33
China Agricultural University	33
University of Calgary	31
Huazhong Agricultural University	31
Chinese Academy of Fishery Sciences	31

B

Universities	Patents
University of California	36
Johns Hopkins University	25
University of Texas	22
Cedars-Sinai Medical Center	18
Yale University	15
Harvard College	14
Southeast University	12
University of Florida	10
Jiangnan University	10
California Institute of Technology	9
Duke University	8
Massachusetts Institute of Technology	7
Vanderbilt University	7

Figure 9. Top universities, research institutes, and hospital publications (until December 2022) related to gut microbiome research in mental and gastrointestinal health. A. Journal publications. B. Patents

Private investment is growing rapidly in the industry,[33] which endorses the clinical potential of prebiotics, probiotics, and the gut microbiome overall. From 2014 through 2018, the total yearly investment increased from $250 million to approximately $1 billion (**Figure 10**). Investment activity continued to increase up to 2021, when more than $2 billion was raised in capital, followed by a decrease in investment in 2022. Notable active investors include the French venture capital group Seventure Partners, US life science innovators Flagship Pioneering, UK biotechnology firm Microbiotica, and Swedish probiotics firm Biogaia.[34–37]

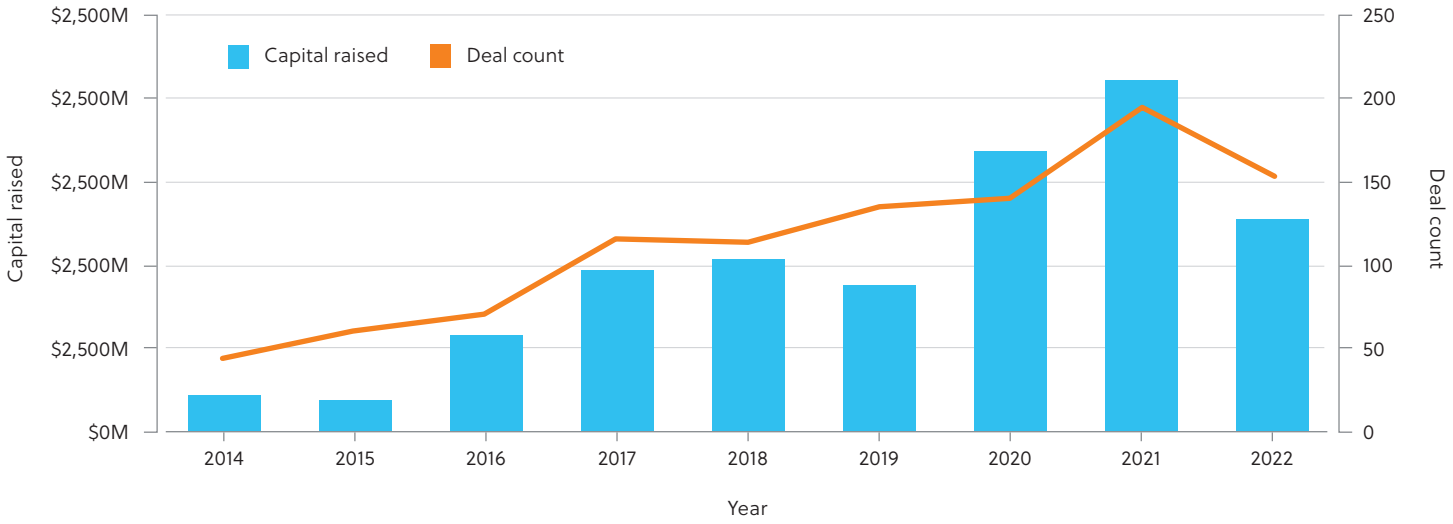


Figure 10. Overall capital raised and deal counts of venture capital investment for the prebiotic, probiotic, and the microbiome field [$]

Clinical development pipeline

A 2022 report estimates that >130 microbiome companies are evaluating >200 pipeline therapies in various stages of development.[38] An analysis of ClinicalTrials.gov between 2004 and 2022 showed that clinical trials utilizing probiotics or prebiotics for treating DGBIs are focused in the areas of functional dyspepsia, functional constipation, functional diarrhea, and IBS.[39] The most studied mental health disorders in relation to clinical trials of probiotic and prebiotic use for the same period are stress, followed by depression, anxiety, cognitive impairment, and sleep impairment.[39]

There has been a recent burst of activity in the approval of FMT therapies for the prevention of recurrent *C. difficile* infection:

- SER-109 (Seres Therapeutics) was granted priority review by the US FDA in October 2022.[40]
- REBYOTA® (Ferring Pharmaceuticals) was approved by the US FDA in November 2022.[41]
- BIOMICTRA (BiomeBank) was approved by the Australian Register of Therapeutic Goods in November 2022.[42]

Some examples of completed or ongoing studies in the microbiome space are summarized in **Table 2**.

Table 1. Gut dysbiosis in digestive system diseases, mental health, and metabolic disorders

Area of interest	Treatment	Study overview
Functional constipation	Retrograde colonic enema with fecal supernatant	Randomized clinical trial (RCT) investigating the efficacy and safety of retrograde colonic enema with FMT in the treatment of pediatric functional constipation (NCT05035784)[43]
IBS	Healthy feces microbiota	FMT intervention led to significantly fewer IBS symptoms and fatigue, and a greater quality of life both at two and three years (NCT03822299)[44]
	Blautia hydrogenotrophica	Data from Phase II RCT supports the use of Blautia hydrogenotrophica (given for 8 weeks) in both IBS with constipation and IBS with diarrhea (NCT03721107)[45]
	Eight strains encompassing Streptococcus, Lactobacillus, Bifidobacteria	PROAGE study: The use of daily probiotics for 45 days in hospitalized elderly patients was associated with a significant reduction in diarrhea and constipation, and a significant increase in serum albumin, prealbumin, and protein in patients ≥80 years old (NCT00794924)[46]
	Multi-strain probiotic capsules containing four Bifidobacterium, five Lactobacillus, and one Streptococcus species	The RCT that showed eight weeks of probiotic treatment was associated with significant improvements in IBS severity and symptoms in patients with diarrhea-prominent IBS (NCT04662957)[47]
	Probiotic drink containing Lactobacillus casei strain Shirota	12 weeks of daily probiotic consumption was associated with a significant reduction in acute diarrhea in children aged 1–5 years old in a developing country vs. a regular nutrient drink (NCT00534170)[48]
Mental health disorders	Probiotic mixture containing Lactobacillus helveticus, Bifidobacterium longum, and Lactiplantibacillus plantarum	Proof-of-concept RCT showed that probiotics consumed for four weeks led to subtly altered brain activity and functional connectivity in healthy subjects performing an emotional task without major effects on the fecal microbiota composition (NCT03615651)[49]
	Lacticaseibacillus paracasei	Sisu study: After five weeks of treatment, Lacticaseibacillus paracasei was shown to improve psychological and physiological markers of stress and anxiety in healthy adults (NCT03494725)[50]
	Bifidobacterium adolescentis or combination of Lactocaseibacillus rhamnosus LGG, and Bifidobacterium BB-12	A pilot RCT investigating the efficacy of two probiotics given for 12 weeks in adults with depressive symptoms (defined as a score of 20–40 on the Beck's Depression Inventory [BDI-II]; NCT05564767)[51]
Insomnia	FMT capsules	A RCT to investigate whether FMT capsules, administered for four weeks, can improve sleep in patients with insomnia, and their effect on gut microbiota and its metabolites, inflammatory factors, neurotransmitters, and sex hormones in peripheral blood (NCT05427331)[52]

Notable patents

A diverse and growing number of patents related to probiotics and prebiotics were identified within the CAS Content Collection, including:

- Actinia chinesis, the golden kiwifruit, is being investigated for the treatment of **constipation and IBS** (patent number: WO2016085356A1)
- Evaluation of bacteria such as the probiotic Enterococcus faecalis for use in the treatment of social behavioral deficit symptoms such as **depression** by increasing social behavior and decreasing corticosterone levels along with **c-Fos** expression in the brain (patent number: WO2022182908A1)
- A fermented supernatant of Lactobacillus casei or paracasei species for the promotion of human health and prevention of **inflammatory disorders.** The postbiotic was shown to stimulate peripheral blood mononuclear cells and protect from endotoxic shock and Salmonella infection (patent number: WO2019149941A1)
- Methods and probiotic compositions that can be used to modulate **serotonin** levels by adjusting the composition of gut microbiota, along with adjusting the level of serotonin-related metabolites (patent number: US20160058808A1)
- Probiotic composition for the prevention and/or treatment of a mental disorder with **memory impairment** by gut microbiome modulation for an increase in memory scores (EP3932415A1)

Summary

The gut microbiome is a well-recognized player in the state of our health today, owing to its functions in metabolism, pathogenic protection, and immunomodulation. Research findings on the GBA suggest there are a plethora of conditions and processes dependent on the state of the gut microbiome. The microbial therapeutics market has grown to become a multimillion-dollar industry, and biotics are a commonplace approach to improving gut health.

Several fecal transplant therapies have been recently approved or are in late-stage approval for treatment of the *C. difficile* infection, a condition that causes almost 500,000 illnesses in the US annually.[40–42,53] We've seen further advances in IBS, UC, and neurodegenerative diseases with investigational therapies,[54] of which have significant implications for how we treat these conditions in the future. GI conditions have proven to be an important proof-of-concept stepping stone for microbial therapies, although we should expect to see much more activity in dermatologic, respiratory, and oncology pipelines.[54]

Knowledge gaps to be addressed as a priority include understanding the immunological function of specific microbes in the human gut microbiota, their role in neurodegenerative/psychiatric disorders, and how microbial metabolites influence brain function. Recent advances in next-generation sequencing technologies, along with innovations in metagenomics, metabolomics, multi-omics, bioinformatics, and artificial intelligence tools, provide the opportunity to characterize the microbial populations and their functions better and help with better correlation prediction.

To uncover the various interactions occurring within the gut microbial community, the research focus must shift from individual microbes to the gut microbiome ecosystem. Other logistical challenges for the scientific community to overcome include a lack of standardized protocols and analytical methods and, perhaps most importantly, the variable nature of microbiota composition between individuals. This will make it even more challenging to understand the complexities of this forgotten organ, yet may pave the way for personalized healthcare through harnessing the power of microbiomology.

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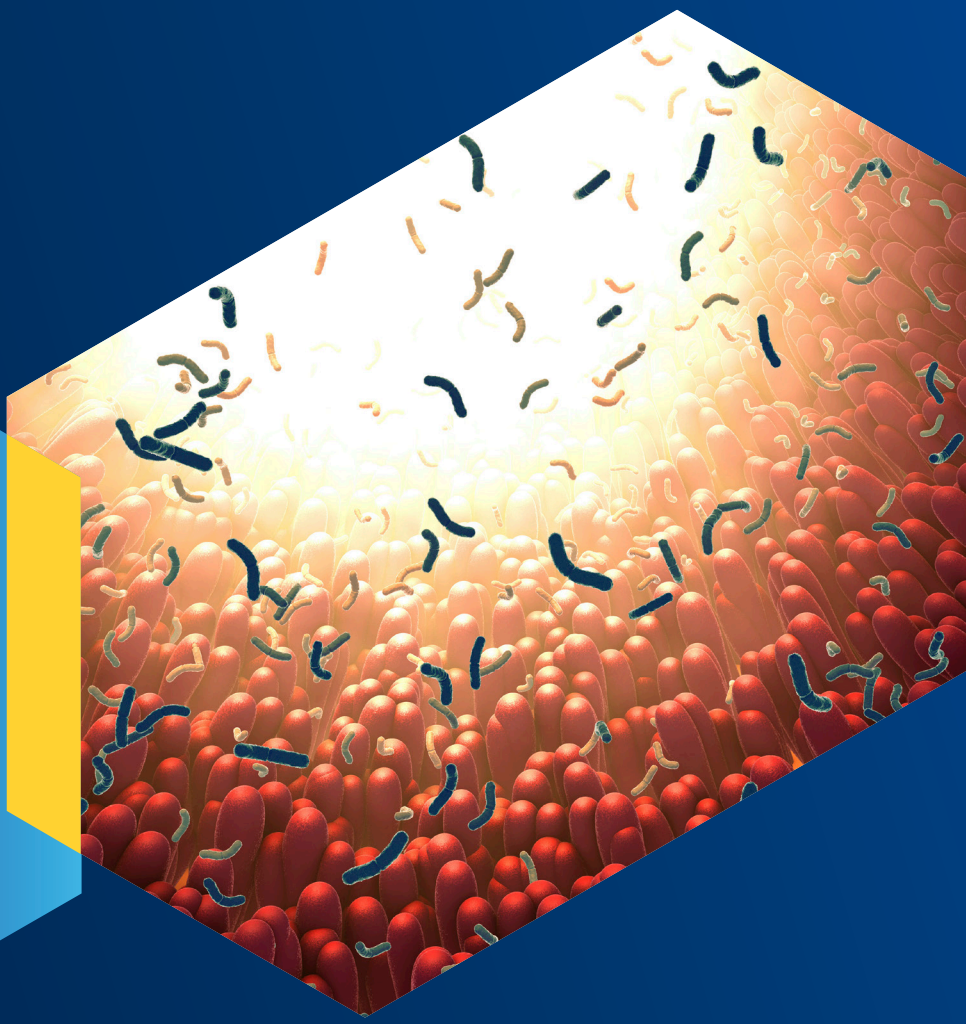

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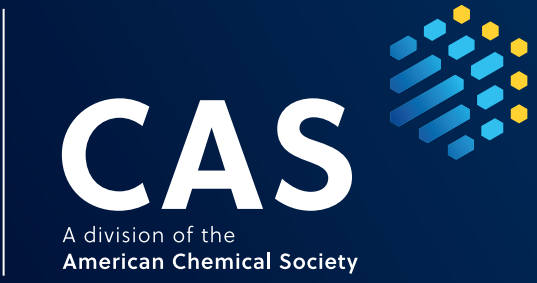

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