Trends in Chemistry

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Review

The hidden hand of molecular chirality in marine biogeochemistry

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Marine organic matter cycling, driven by microbial activities, involves largely unexplored chiral compounds. Despite their prevalence, the role of these compounds in marine processes remains understudied. This review dives into the intriguing world of marine enantiomers, mirror-imaged compounds with identical physical properties but often distinct biological fates. The review explores the diversity, biological functions, microbial transformation, and enzymatic enantioselectivity of these enantiomers, with a particular focus on amino acids and sulfonates, highlighting their significance in marine biogeochemistry. Additionally, the review discusses recent advances in chemical and biological analytical methods, addressing the challenges and strategies for chiral analysis of marine dissolved organic matter (DOM). Future research directions to bridge the gap between chemistry and ocean science are also proposed.

Highlights

While chirality has been extensively studied in various fields, its role in marine biogeochemistry remains relatively unexplored.

Chiral compounds can act as both carbon sources and carbon sinks in the ocean, influencing carbon cycling processes.

Marine microorganisms have evolved distinctive enzymatic strategies to metabolize enantiomers.

The development of advanced experimental and analytical techniques has significant implications for the discovery and identification of novel chiral metabolites in the ocean.

Chiral molecules in the biosphere

Chirality is prevalent in nature, with the building blocks of biomacromolecules like protein and nucleic acids being chiral [1] and chiral structures commonly observed in biomineralized materials across the nanoscale to the macroscale [2]. Enantiomers, mirror-imaged chiral molecules, share identical physicochemical properties but can have distinct biosynthetic origins, fates, and functions [3]. Hundreds of enantiomer pairs have been discovered in various ecosystems, including terrestrial, aquatic, and atmospheric environments [3,4]. For example, carvone enantiomers exhibit distinct scents, with (+)-carvone smelling of caraway and its antipode producing spearmint odor, and are produced by higher plants [3]. Atmospheric α-pinene, the most abundant biogenic volatile organic compound, emitted from the Amazon rainforest exhibits chiral bias, with (+)-α-pinene dominating at the canopy level and (–)-α-pinene at the tower top [5]. Only specific enantiomers of pyrisulfoxin compounds from *Streptomyces albolongus* exhibited significant cytotoxicity against various cancer cell lines [6]. Approximately 794 natural products with potential pharmaceutical applications existing as racemic mixtures were collected, with only a small fraction (approximately 5%, 42 enantiomeric pairs) originating from oceanic organisms (Table S1 in the supplemental information online) [4]. Despite the vast diversity of compounds (likely composed of millions of molecules) in the ocean, the chiral properties of these compounds have been largely overlooked.

The ocean holds a vast reservoir of **DOM** (see Glossary), estimated at a staggering 642 Pg carbon (1 Pg = 10^{15} g) – roughly equivalent to the amount of CO_2 in the atmosphere [7]. This ancient pool, aged between 4000 and 6000 years, acts as a crucial long-term **carbon sink**, storing carbon in the deep ocean for millennia [8,9]. By **carbon sequestration**, DOM plays a vital role in mitigating climate change [7]. Oceanic biogeochemical processes, such as **autochthonous production**, **heterotrophic consumption**, **photomineralization**, and **aggregation**, significantly influence DOM compositions [10–12]. Essential nutrients like nitrogen, phosphorus, sulfur, and iron are cycled through these processes, supporting marine life [13,14]. Heterotrophic processes driven primarily by oceanic microorganisms involve the breakdown of DOM into usable forms for organisms: incorporation into

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biomass, respiration that releases inorganic carbon back into the environment, and the creation of more persistent organic matter [7,10]. Untargeted mass spectrometry and NMR measurements have identified 165 microbial-origin DOM compounds that serve as carbon and energy sources or signal molecules, shaping microbial community dynamics within the oceanic food web [15,16]. Fluorescence-induced thermal ionization mass spectrometry has revealed over 20 000 molecular formulas in seawater DOM compositions, each potentially representing more than 30 isomers [10]. Current techniques are sufficient to isolate and identify these isomeric compounds but are challenging due to the low abundance of many chiral molecules, hindering our understanding of their transformations and cycling in the ocean. Thus, chirality remains enigmatic in the marine metabolite pool.

While traditional understanding of chiral molecules in the ocean has primarily focused on amino acids, recent discoveries have expanded this knowledge. D-amino acids, primarily derived from bacterial cell walls, are significantly less abundant than L-amino acids. D-amino acids can be utilized by deep-sea bacteria, while also being considered potential carbon storage molecules [17,18]. Recent studies have shown that phytoplankton, the primary ocean producers, synthesize two enantiomeric forms of organic sulfur dihydroxypropanesulfonate (DHPS) [19]. These chiral molecules are widely distributed in marine environments and play a crucial role in carbon and sulfur cycling [13,19]. This review synthesizes recent research on marine enantiomers, highlighting their biological function, nutrient cycling, and enzyme-driven transformation mechanisms. Key enantiomers, D/L-amino acids and *R*/*S*-DHPS being classical examples, are introduced, along with emerging technologies for future studies. By acknowledging the importance of enantiomers in marine ecosystems, this review aims to stimulate interdisciplinary research, particularly between chemistry and ocean science, to uncover hidden aspects of biogeochemical cycling.

Diversity and functions of enantiomers in marine environments

Enantiomers, such as amino acids, sulfonates, and natural secondary metabolites from marine organisms, as well as anthropogenic molecules from abiotic sources, have been discovered in the ocean (Figure 1 and Table S1). These chiral molecules are essential for biomolecular compositions, cellular processes, and microbial interactions, but can also act as pollutants, negatively impacting marine ecosystem health (Figure 2A–D).

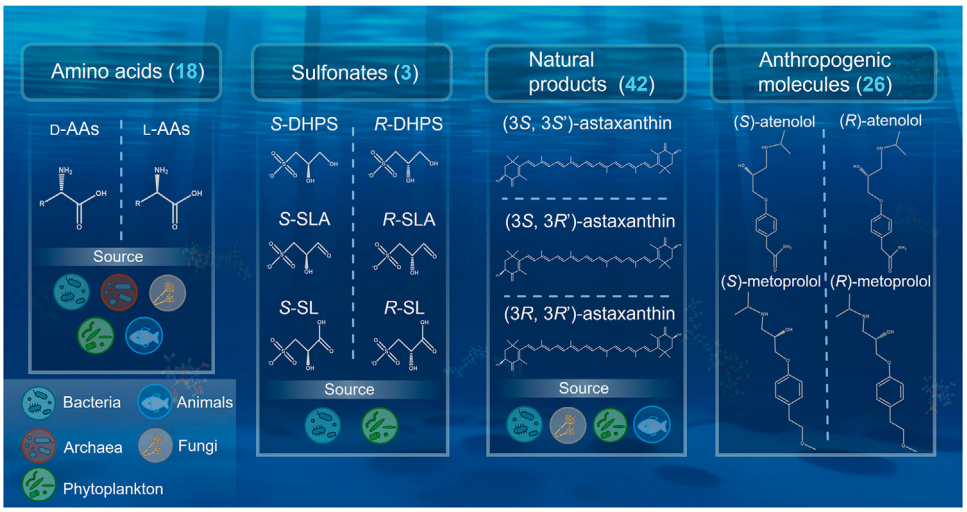


Figure 1. Classification and number of enantiomers in the ocean according to published literature. The number following each classification indicates the current counts of known enantiomers in the ocean, with representative enantiomer compounds shown. Abbreviations: AA, amino acid; DHPS, dihydroxypropanesulfonate; SL, sulfolactate; SLA, sulfolactaldehyde.

Glossary

Aggregation: particulate organic matter, derived from living organisms and their decomposition products, can come together to form larger particles. Aggregation affects particle settling velocities and interactions with marine organisms, ultimately influencing the vertical flux of carbon in the ocean.
Autochthonous production: the generation of organic matter within an ecosystem by its own organisms, such as algae and cyanobacteria performing photosynthesis in a body of water. It involves the conversion of inorganic substances (CO and H O) and energy (e.g., sunlight, chemical energy) into organic compounds, serving as a primary energy source in the ecosystem.
Carbon sink/carbon sequestration: a process or component that absorbs and stores more carbon than it releases, reducing atmospheric CO_2 concentrations. In the ocean, this occurs through four main mechanisms: the microbial carbon pump, the biological carbon pump, the carbonate counter pump, and the solubility carbon pump. Together, these processes make the ocean a vital carbon sink.
Carbon sources: refers to a process or component that releases CO or other carbon compounds into the atmosphere or ocean, increasing overall carbon levels. Carbon sources in the ocean include respiration by marine organisms and the decomposition of organic matter through biotic or abiotic processes.
Dissolved organic matter (DOM): organic substances dissolved in seawater that pass through a filter typically with a pore size of 0.22–0.7 μm.
Enzymatic promiscuity: the ability of an enzyme to catalyze an unexpected side reaction in addition to its main reaction.
Heterotrophic consumption: a biological process where organisms obtain energy and nutrients by consuming organic matter produced by other organisms.
Labile DOM: a fraction of dissolved organic matter that is readily available and easily degradable by microorganisms. It typically includes simple organic compounds like sugars and L-amino acids.
Microbial carbon pump: a process by which marine microbes convert labile organic carbon into refractory dissolved organic carbon through a series of physiological and ecological processes.

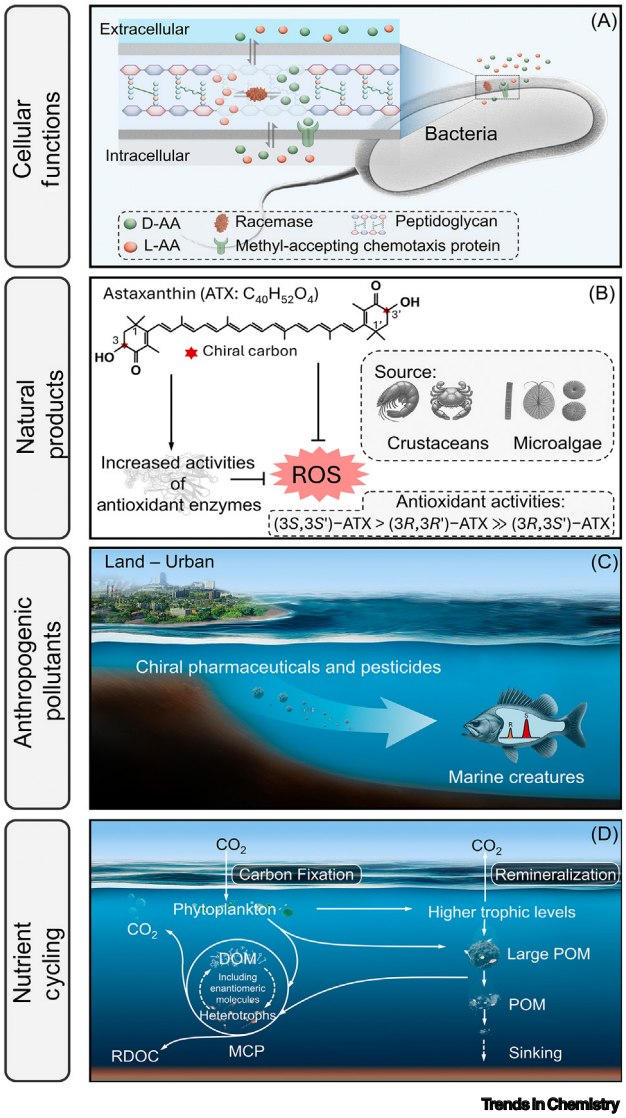


Figure 2. Functions of chiral molecules in the ocean. (A) Schematic diagram illustrating the secretion of D-amino acids in *Vibrio cholerae* as chemotactic repellent signals that can control the motility of *V. cholerae*, enabling them to shift away from low-nutrient or adverse conditions [23]. (B) The source and function of astaxanthin. (C) Anthropogenic enantiomers from pharmaceuticals, pesticides, and other sources enter marine ecosystems. (D) Diagram of dissolved and particulate organic matter cycling in the ocean. Abbreviations: AA, amino acid; DOM, dissolved organic matter; MCP, microbial carbon pump; POM, particulate organic matter; RDOC, refractory dissolved organic carbon; ROS, reactive oxygen species.

This transformation helps to sequester carbon in the ocean for extended periods, contributing to climate regulation.

Microbial loop: a complex food web involving bacteria, phytoplankton, and zooplankton, efficiently recycling nutrients and energy in marine ecosystems.

Photomineralization: a process by which organic compounds are converted into inorganic minerals through the action of light, typically UV light.

Refractory dissolved organic carbon: a type of organic carbon compound that is highly resistant to microbial degradation and can persist in the ocean for millennia.

While proteins predominantly comprise L-amino acids, marine organisms can produce a range of D-amino acids. These D-amino acids can be found in free form or incorporated into various biomolecules, including peptidoglycan and peptides [20]. A total of 17 D-amino acid isomers of proteinogenic amino acids, as well as the metabolite intermediate D-ornithine, have been detected in marine seawater and organisms (Figure 1). D-tryptophan and D-glutamine have not yet been detected. Free D-amino acids exhibit a wide range of biological functions, such as facilitating biofilm formation and degradation, promoting spore germination and growth, maintaining peptidoglycan homeostasis, and providing carbon and nitrogen for cellular metabolism [21]. Marine organisms, such as invertebrates, utilize D-aspartic acid and D-alanine for neurotransmission and osmoregulation [22]. D-amino acids also shape microbial communities. For example, D-arginine and D-lysine, recognized by *Vibrio* species chemoreceptors, repel *Vibrio* from unfavorable conditions (Figure 2A) [23]. Additionally, *Vibrio*-secreted D-arginine can inhibit the growth of other bacteria by interfering with their phosphate transport and being mistakenly incorporated into their protein,

presumably resulting in proteotoxicity [24]. Recently, in the coral system, newly discovered symbiotic microbes have been found to possess an extremely reduced genome, lacking normal carbohydrate metabolism [25]. These microbes may rely solely on L-arginine provided by their coral host for energy [25]. D-alanine and D-glutamic acid are major components of peptidoglycan in the bacterial cell wall, with smaller amounts of D-serine and D-aspartic acid also present [17]. D-aspartic acid may also be a component of archaeal cell walls and is frequently detected in marine algae [26]. D-alanine, D-valine, D-leucine, and D-serine are among the most common D-amino acids found in bioactive peptides produced by marine algae and fungi via non-ribosomal peptide synthesis and ribosomally synthesized and post-translationally modified peptide synthesis [20].

Marine natural products, including enantiomeric compounds, exhibit diverse bioactivities such as antimicrobial, cytotoxic, enzyme inhibitory, anti-inflammatory, and antioxidant properties [27]. For example, astaxanthin, a potent antioxidant primarily derived from marine crustaceans, fish, and algae, exists in three isomers [(3*S*, 3′*S*), (3*R*, 3′*R*), and (3*R*, 3′*S*)] (Figure 1) [28,29]. The (3*S*, 3*S*′)-isomer, with its optimal spatial arrangement, demonstrates the highest bioactivity [28]. Located at the cell membrane, astaxanthin directly interacts with reactive oxygen species, neutralizing free radicals and protecting cells from oxidative damage (Figure 2B) [28]. Reactive oxygen species are omnipresent in the ocean and can directly affect the growth of marine organisms, thereby shaping microbial communities [30]. Algal blooms are prominent hotspots for substantial reactive oxygen species production [30]. Marine bacteria and algae can produce astaxanthin [29], which may help to alleviate oxidative stress.

Anthropogenic chiral pollutants, primarily pharmaceuticals and pesticides, also contaminate marine environments through various pathways: river import, atmospheric deposition, and oceanic transport (Figure 2C) [31,32]. These pollutants, such as chiral organochlorine pesticides, have been detected in surface waters, deep-sea waters, and sediments [33,34]. They can be transported to the deep sea via biological, physical, and particle-mediated processes [33]. Once in the marine food web, chiral pollutants can be selectively bioaccumulated by organisms, leading to various levels of ecotoxicity. For example, chiral pharmaceuticals like atenolol and metoprolol exhibit enantioselective accumulation and toxicity in marine organisms [35].

Phytoplankton including algae and cyanobacteria, responsible for half of Earth's photosynthesis, produces organic matter that fuels the marine **microbial loop** (Figure 2D) [16]. Their metabolites, such as sugars, amino acids, and sulfur compounds, including DHPS, serve as metabolic currencies exchanged between phytoplankton and bacteria. Diatoms may provide DHPS to bacteria as a carbon and sulfur source, receiving vitamin B_{12} in return [36]. DHPS also functions as an antioxidant and osmoprotectant, accumulating to millimolar concentrations in diatoms [37]. Both *R*- and *S*-enantiomers of DHPS are widely distributed in marine environments and can be utilized by aerobic bacteria in surface seawater [19]. Even in anoxic marine environments, such as sediments and the intestinal tracts of fish, sulfur derived from DHPS can be metabolized by sulfate and sulfite-reducing bacteria to hydrogen sulfide, posing a threat to organismal health [38]. Additionally, microbes have been observed to degrade anthropogenic chiral pollutants and unusually occurring L-glucose as **carbon sources** [39,40]. These findings suggest that microbes possess a remarkable ability to adapt and utilize a wide range of chiral molecules, highlighting their evolutionary flexibility and the significant role of chirality in marine biogeochemical cycles.

D-amino acids: a microbial carbon paradox

Amino acids constitute a major component of the marine DOM pool, with estimated inventories of approximately 89 ± 29 Pg carbon and 35 ± 11 Pg nitrogen, respectively [41]. The majority of marine L- and D-amino acids exist in combined forms, both high- (>2500 Da) and low-molecular-weight

molecules [42]. Marine microbes possess a diverse array of extracellular and intracellular peptidases and proteases that cleave peptide bonds between L-amino acid residues, facilitating the catabolism of polymers of amino acids [43]. Free L-amino acids and their oligopolymers are readily utilized by marine microbes, serving as carbon and nitrogen sources in both seawater and sediment environments [44,45]. By contrast, the dual role of D-amino acids in marine carbon cycling requires further clarification, as they may function as either a carbon source or a carbon sink.

Bacteria and archaea utilize D-aminopeptidases to selectively break down bacterial peptidoglycan di- and tripeptides, releasing free D-amino acids [45,46]. These D-amino acids can be either released extracellularly or metabolized intracellularly during cell wall remodeling [47]. Hydrolyzable D-amino acids derived from samples hydrolyzed by hydrochloric acid are generally less abundant than hydrolyzable L-amino acids in sunlit surface waters, often with differences in concentrations of four- or fivefold [42]. In the deep sea, concentrations of hydrolyzable D-amino acids are even lower, with their free forms often falling below detectable limits [48]. Four peptidoglycan-derived D-amino acids (D-alanine, D-glutamate, D-aspartic acid, and D-serine) are relatively abundant in surface seawater, with concentrations in the tens of nanomoles and D:L ratios ranging from 0.2 to 0.7, D-alanine being the predominate one [42]. These amino acids also constitute a significant portion of hydrolyzable amino acids in marine sediments [49]. The D:L ratio of amino acids in marine sediments tends to increase with depth [50,51]. This accumulation of D-amino acids in sediments is primarily attributed to the presence of peptidoglycan in bacterial cell walls and the preservation of bacterial macromolecular remnants [50]. This suggests that D-amino acids are more likely to be preserved in sediments than L-amino acids. In older, deeper coastal Chilean sediments, they can constitute over half of the total hydrolyzable amino acids, with the majority of D-amino acids existing in combined forms [49]. This suggests that incomplete peptidoglycan degradation contributes to the formation of refractory organic carbon. Additionally, enzymes capable of hydrolyzing bonds between D-amino acids or between D- and L-amino acids are relatively rare in microbes. Thus, while high-molecular-weight compounds containing D-amino acids can potentially be degraded into smaller fragments, these low-molecular-weight compounds often resist further breakdown by microbial enzymes (Figure 3A) [52]. This property has significant applications for drug design, particularly in the development of peptide-based therapeutics [53]. For instance, replacing L-amino acids with D-amino acids in antibacterial peptides can significantly increase their resistance to microbial degradation [54,55].

L-amino acids in living organisms serve as substrates for D-amino acid synthesis, reactions catalyzed by enzymes termed racemase, which alters the stereochemistry of the chiral α-carbon [56]. Free D-amino acids can be used for the synthesis of cell wall peptidoglycan and are primarily catabolized by marine bacteria through two main pathways: reversible reactions catalyzed by racemases, converting D-enantiomers to L-enantiomers, or deamination by D-amino acid dehydrogenases, transaminases, ammonia-lyases, and oxidases, yielding α-keto acids and ammonia (Figure 3B) [57,58]. α-Keto acids further enter central metabolism. Additionally, some bacteria and archaea, such as Rhodobacterales and halobacteria, use an acetylation pathway involving *N*-acetyltransferase and *N*-acetyl-D-glutamate racemase to convert D-glutamate to the L-form (Figure 3B) [18]. Racemases and oxidases for D-alanine, D-aspartic acid, and D-glutamate are common in marine microbes (Table S2 in the supplemental information online). These findings suggest that relatively abundant free D-amino acids can be labile substrates for microbes, serving as potential carbon sources in these environments.

Most marine microbes preferentially utilize free L-amino acids, but some can use free D-amino acids. Cultures of bacteria and archaea have shown the consumption of all free D-isomers of

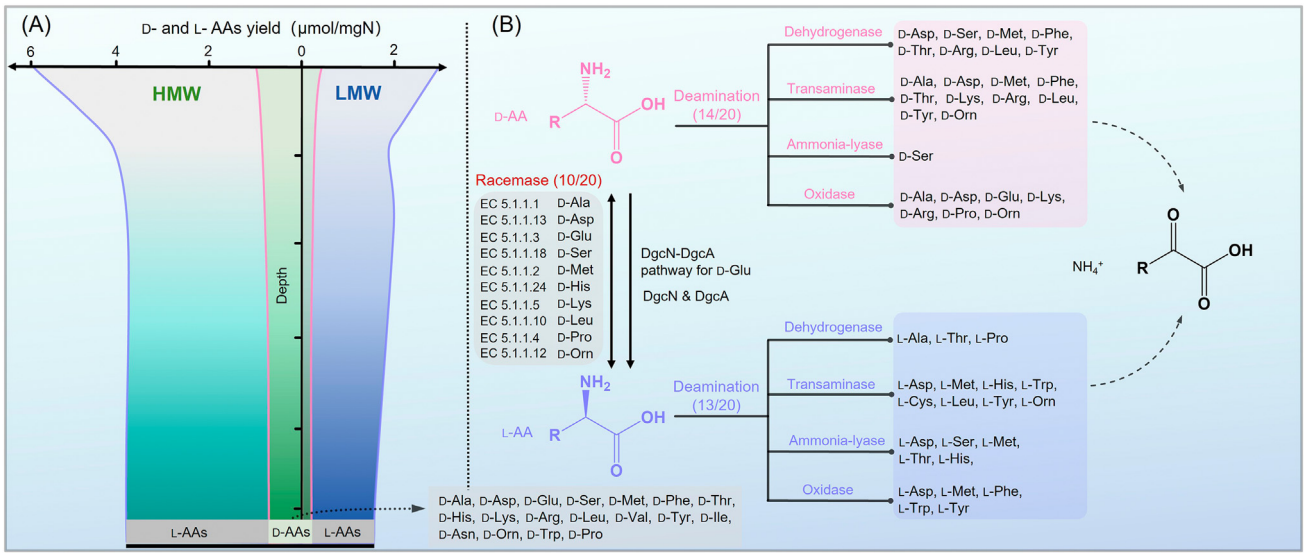


Figure 3. Occurrence and transformation of amino acids in marine environments. (A) Diagram of the trendline of D-amino acid and L-amino acid concentrations in the water column. The trendline refers to the research by Ianiri *et al.* [42]. The horizontal axis represents the yields of D- and L-amino acids varying with depth, with the light-blue curve representing L-amino acids and the pink curve representing D-amino acids. HMW and LMW represent high- and low-molecular-weight organic matter, respectively. The molecular weight cutoff for LMW/HMW is 2500 Da. (B) Currently known racemization, deamination of amino acids in marine microbes. Amino acid metabolism enzymes are shown in Table S2 in the supplemental information online. The primary degradation manner of amino acids (racemization and deamination) is displayed. Enzyme Commission (EC) numbers of racemases for amino acid metabolism are shown. All of the enzymes are retrieved from the Integrated Microbial Genomes and BioCyc databases [97,98]. The numbers in brackets represent the amounts of known catalyzable amino acids out of all 20 amino acids. Abbreviations: AA, amino acid; DgcA, *N*-acetyl-D-glutamate racemase; DgcN, *N*-acetyltransferase. The DgcN–DgcA pathway converts D-glutamate to the L-form [18].

proteinogenic amino acids, although individual species typically utilize only a subset (Table S2). Additionally, some species, like the deep-sea bacterium *Nautella* sp. strain A04V, even grow better with D-valine than L-valine as a sole carbon source [59], and archaea marine Crenarchaeota Group I preferentially takes up D-aspartate over L-aspartate in deep-sea environments [60]. Bacteria and archaea actively take up exogenous free L-amino acids through various amino acid transporters in the cellular membrane. One of the smallest bacteria in the ocean, the ubiquitous SAR11, exhibits ultrahigh affinity of its solute-binding proteins in the transporter system for 16 L-amino acids, ranging from several hundred picomolar to nanomolar [61]. This affinity is consistent with the average concentration of free L-amino acids in the ocean. When these bacteria become abundant, they will be responsible for up to 50% of L-amino acid assimilation in the surface seawater [62]. While amino acid transporters often possess broad substrate specificity, capable of transporting both L- and D-amino acids (e.g., D-alanine and L-alanine) [63], the number of D-amino acids with dedicated transporters is limited. To date, only transporters for D-alanine, D-aspartic acid, D-serine, D-arginine, D-leucine, D-methionine, and D-histidine have been identified in bacteria [64]. Some transporters for D-alanine show a higher affinity for D-alanine than for L-alanine [61], suggesting a specialized transport system for D-amino acids. By contrast, substrate-binding proteins of D-methionine transporters typically exhibit low affinity in the millimolar range [65]. Given the extremely low concentrations of most non-peptidoglycan-derived free D-amino acids in seawater (below 1 nM) [48], which often fall below microbial uptake thresholds, these compounds may contribute to the pool of refractory organic carbon, especially in the deep sea. This suggests that, while microbes can utilize D-amino acids under laboratory conditions with high D-amino acid concentrations, their uptake of ambient free D-amino acids in natural environments might be limited.

Overall, the degradability of D-amino acids can vary depending on specific biogeochemical conditions. While these compounds can contribute to the formation of **refractory dissolved organic carbon** within the marine **microbial carbon pump** [7], their bioavailability is often limited. The low concentration of most free D-amino acids in deep-sea seawater limits their direct microbial uptake, and their incorporation into complex compounds reduces their degradability. However, abundant D-amino acids in bacterial peptidoglycan, like D-alanine [42], can serve as carbon sources for marine microbial communities.

Chiral compound metabolism: implications for enzymatic promiscuity

Microorganisms have evolved remarkable abilities to adapt to diverse chiral environments. A key factor in this adaptation is the presence of enzymes that can metabolize chiral compounds. This evolutionary process, shaped by the interaction between enzymes and their chiral substrates, has led to the development of complex metabolic networks [10]. A significant characteristic of enzymes that catalyze chiral metabolites is their promiscuity, which allows them to act on a variety of substrates.

Similar to amino acid transport, both enantiomers of DHPS can be taken up by a common transporter in bacteria [37]. However, the metabolic pathway of DHPS in aerobic bacteria, such as Roseobacteraceae, involves individual enzymes that are specific for the DHPS enantiomers (Figure 4) [19]. Two dehydrogenases, HpsO and HpsP, stereoselectively catalyze *R*-DHPS and *S*-DHPS, respectively [19]. *R*-DHPS can also be the substrate of dehydrogenase HpsN, but not the *S*-enantiomer (Figure 5) [19,66]. By contrast, the dehydrogenase GabD has catalytic activities for *R*- and *S*-sulfolactaldehyde, the products of DHPS oxidization by HpsO and HpsP (Figure 4) [19]. The dehydrogenase DhpA from an anoxic bacterium, *Desulfovibrio* sp. DF1, can potentially metabolize both *R*- and *S*-enantiomers of DHPS [67]. These enzymes (HpsO, HpsP, and HpsN) in Roseobacteraceae also exhibit promiscuous activities toward D-gluconate, D-sorbitol, and L-histidinol, respectively [19]. For instance, HpsO and D-gluconate dehydrogenase both belong to the short-chain dehydrogenase/reductase superfamily and share similar overall protein structures and common active-site architecture, including catalytic triad serine, tyrosine, and

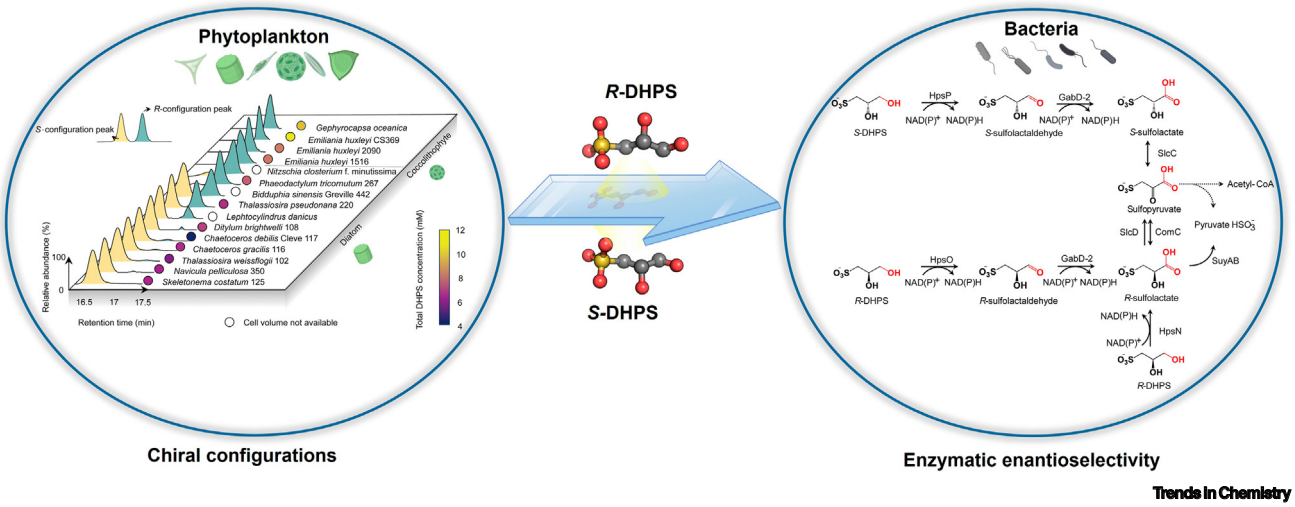


Figure 4. Production of dihydroxypropanesulfonate (DHPS) in marine phytoplankton and chirally selective DHPS catabolic pathway in marine Roseobacteraceae. The chromatographic peaks represent *R*- and *S*-DHPS derived from diatoms and coccolithophores. In this catabolic pathway, the enzymes and intermediates are shown and the reactive groups are colored in red. Abbreviations: GabD-2, *R*- and *S*-sulfolactaldehyde dehydrogenase; HpsN, *R*-DHPS dehydrogenase; HpsO, *R*-DHPS dehydrogenase; HpsP, *S*-DHPS dehydrogenase; SlcC, *S*-sulfolactate dehydrogenase; SlcD and ComC, *R*-sulfolactate dehydrogenase; SuyAB, *R*-sulfolactate sulfolyase. Broken lines indicate that the multiple-step reactions involve the conversion of sulfopyruvate to pyruvate, acetyl-CoA, and bisulfite.

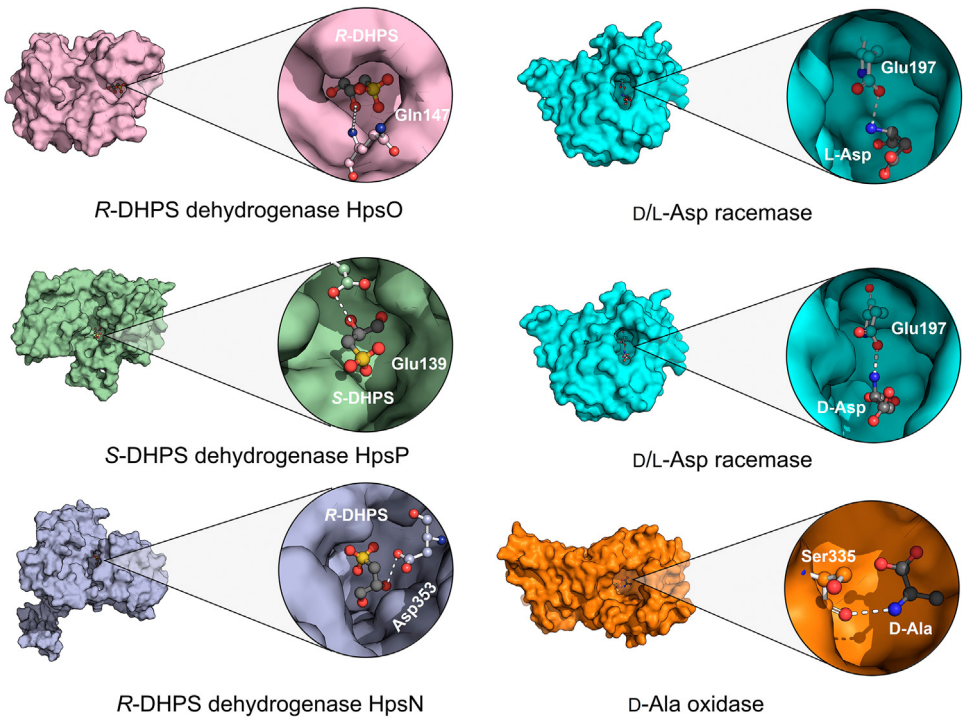


Figure 5. Structural analysis of chirally selective enzymes. To illustrate clearly, only hydrogen bonds formed between functional groups at chiral carbon of ligands and essential residues are shown as white broken lines. The ligand-docked protein structures of *R*-dihydroxypropanesulfonate (DHPS) dehydrogenase HpsO (Protein Data Bank ID: 8WWF), HpsN (8WWE), and *S*-DHPS dehydrogenase HpsP (8WWD) are from marine Roseobacteraceae [19]. D/L-Asp racemase (5WXY and 5WXZ) is from *Microcystis aeruginosa* [69]. D-Ala oxidase (1C0P) is from *Rhodotorula toruloides* [99].

lysine [19]. All HpsO, HpsP, and HpsN contain ancient and conserved Rossmann-fold domains [19], suggesting that they may have evolved from a common generalist ancestor within this superfamily that diverged to give a range of different families with different functions. Additionally, there are other known enzymes involved in chiral sulfonate metabolism, such as CuyA and CuyB catalyzing L- and D-cysteate, respectively [68].

Amino acid racemases, in addition to acting on both amino acid enantiomers by accommodating both substrates within their structure and lowering the energy barrier between them – such as McyF binding L- and D-aspartate in the same active pocket through stable hydrogen bonding and hydrophobic interactions with conserved residues (Figure 5) [69] – also exhibit broad substrate specificity. For instance, BsrV from *Vibrio cholerae* can reversibly racemize ten of the proteogenic amino acids [70]. Intriguingly, it exhibits enhanced catalytic efficiency toward lysine, arginine, and methionine, likely due to its larger substrate entry channel, which can accommodate a diverse range of L-amino acids [70]. However, hydrogen bond interactions with the hydroxy or amino groups on the chiral center with conserved residues provide the rationale for substrate D-specificity and prevent the binding of L-amino acid, such as D-alanine oxidase (Figure 5).

Enzymatic promiscuity, the ability of enzymes to catalyze reactions beyond their primary function, is a key driver of enzyme evolution [71]. This versatile property allows enzymes to adapt to new substrates and environments, leading to the emergence of specialized enzymes through processes such as gene duplication and divergence. Directed evolution and high-throughput

screening are powerful techniques that can rapidly accelerate the evolution of enzymes with desired properties, such as high enantioselectivity [72]. By introducing random mutations and selecting for specific phenotypes, we can engineer enzymes with remarkable chiral selectivity. For example, lipases with no initial chiral preference can be evolved to favor specific enantiomers of esters [73]. Similarly, mutations in alcohol dehydrogenases and ketoreductases can confer the ability to produce single-enantiomer compounds [74,75]. Even short-term evolutionary processes can enable bacteria to adapt to non-native chiral carbon sources like D-xylulose [76]. This is possible because promiscuous enzymes can be recruited to metabolize these non-native enantiomers [76]. Site-directed mutagenesis can enhance enzymes' selectivity by altering protein structure at key residues in the active sites or substrate-binding pocket. For example, wild-type cyclohexanone monooxygenase from *Acinetobacter* sp. NCIMB 9871 has poor enantioselectivity for 4-hydroxycyclohexanone (9% *R*), but a mutant in which phenylalanine was converted to serine reverses stereoselectivity to 79% (*S*) [77].

Considering the vast diversity of organic compounds in the ocean, estimated to exceed 4 million [10], it is plausible that enzymatic promiscuity plays a crucial role in microbial metabolism. By enabling the utilization of diverse chiral compounds, these enzymes contribute to the efficient cycling of organic matter in marine environments.

Challenges and strategies in chiral analysis of marine DOM

The analysis of molecular chirality typically involves sample preparation and detection. Sample preparation is a necessary and crucial step to separate chiral molecules from matrices that interfere with detection and to concentrate the chiral molecules. The concentration levels of chiral molecules in biological and environmental samples are usually very low, and the backgrounds are highly complex [78]. The concentration of seawater DOM is generally only 80–200 μM, with individual compounds present at picomolar to nanomolar levels [10]. Additionally, the salinity of seawater is extremely high, whereas other salt solutions, such as those used for metabolite analysis, typically have much lower salt concentrations. For example, the 1–10 g/l salt solutions found in blood and urine are approximately 30 times lower than that of seawater [16]. Nonvolatile salts can interfere with ion formation, leading to reduced spectral quality in mass spectrometry, and salts decrease the sensitivity of the NMR probe. The metabolites in seawater, especially small and highly polar organic molecules (<200 Da), constituting the majority of the **labile DOM** in the ocean, are difficult in enrichment and purification, which hinders the detection of marine chiral molecules. While strategies like tangential flow filtration [79], reverse osmosis/electrodialysis [80], and solid-phase extraction can remove salts [81], they capture only a small fraction (10–40%) of marine DOM, primarily targeting moderate- (>300 Da) to high-molecular-weight compounds [16].

The techniques for chirality analysis have been comprehensively summarized in the literature [4,78]. Currently, due to their versatility in applications, as well as their sensitivity and reproducibility in providing qualitative and quantitative data, chiral liquid chromatography and 2D liquid chromatography–mass spectrometry are the most widely used methods for chiral separation [78]. Derivatization is one powerful strategy in chiral liquid chromatography for the analysis of chiral metabolites. By reacting with nonpolar chiral derivation agents before chromatography, small molecules of interest become more hydrophobic, improving their recovery from seawater. Furthermore, derivatization disrupts the symmetry of enantiomers, converting them into diastereomers that can be separated chromatographically. For example, free D- and L-amino acids have been extensively analyzed using chiral thiol *N*-isobutyryl-L-cysteine followed by chromatography [42]. Similarly, DHPS enantiomers from seawater can be effectively separated after derivation with the chiral phosphine reagent diisopropyl phosphoryl L-alanine [19]. The incorporation of phosphorus enhances mass spectrometry sensitivity, leading to improved detection [82]. Chiral

phosphine reagents can also undergo dehydration condensation reactions with hydroxyl, amino, and thiol functional groups, making them promising tools for the discovery of additional chiral enantiomers in seawater. However, not all target chiral molecules possess suitable functional groups for derivatization, requiring the development of distinct derivatization reagents for different types of analytes. Moreover, impurities in chiral derivatizing reagents and unwanted side reactions may interfere with the detection [78]. Another strategy is to use chromatography columns with chiral stationary phases. This method does not require chiral derivatization and involves fewer sample preparation steps. However, chiral columns are not suitable for all chiral compounds and are more expensive than non-chiral columns. The combination of chiral mobile phase additives with achiral stationary phases can also effectively separate chiral enantiomers. Liquid chromatography using chiral mobile phases can separate and subsequently detect 18 chiral amino acids present in plasma at picomolar concentrations [78]. The caveat is that the chiral mobile phase must be enantiomerically pure and not interfere with the detection. 2D and 3D high-performance liquid chromatography have been developed and can separate trace amounts of chiral metabolites. This approach involves connecting two or three chromatographic columns in series, with the first dimension using an achiral column, the second dimension using a chiral column, and an additional column, such as an anion-exchange column [83], potentially used in the third dimension to further purify the enantiomers. Moreover, a novel method for the direct separation and analysis of enantiomers using dual alternating-current excitation to control the ion rotation direction, leveraging differences in collision cross-sections of enantiomers in an electric field, provides a potential strategy for chiral separation in mass spectrometry without column-based analysis [84]. However, the selectivity of this method is limited, making it unable to be applied to chiral analytes with very low enantiomeric ratios.

High-throughput metagenomics, metatranscriptomics, and metaproteomics can help in deciphering novel metabolites involved in biogeochemical cycles. The Earth Microbiome Project aims to profile the metabolic content of all known species on Earth [85]. While public databases like Global Natural Products Social Molecular Networking contain a large number of metabolites (approximately 52 496) [85,86], the lack of available standards significantly hampers the qualitative and quantitative analysis of novel compounds. Screening potential chiral metabolites from these databases, in combination with chemical analysis, can guide further research into their role in marine ecosystems (Figure 6). Labile DOM is rapidly consumed by heterotrophic bacteria within minutes to hours, forming a highly dynamic metabolite pool. Isotope-labeled metabolic rate measurements, such as ^{3}H, ^{13}C, and ^{15}N labeling of substrates (Figure 6) [87,88], are promising strategies for tracking rapid metabolic transformations and fluxes of chiral compounds in natural environments, providing insights into the role of chirality in biogeochemical cycles.

Concluding remarks and future perspectives

In conclusion, the nature of enantiomers significantly impacts their biological roles. From influencing cellular processes to shaping marine carbon cycling, enantiomers exhibit diverse biological activities. While some are readily metabolized by microorganisms, others resist degradation, potentially acting as long-term carbon reservoirs. The catalytic promiscuity of enzymes involved in chiral compound metabolism highlights the evolutionary adaptability of microorganisms, enabling them to exploit novel substrates and contribute to the dynamic interplay between biology and the environment.

Although the analysis of chiral compounds in marine environments is hindered by factors such as low concentrations and complex matrices, recent technological advances in chiral analysis provide promising avenues for future research (Figure 6). These advances enable the development of techniques to identify and enrich specific enantiomers in the ocean. While many studies on chiral compound metabolism are conducted in laboratory settings, *in situ* biological incubator instruments

Outstanding questions

What specific biological functions are associated with each enantiomer? How do marine microorganisms metabolize enantiomers?

How can we develop more advanced techniques to discover novel marine enantiomers and determine their absolute configurations?

How can we design *in situ* biological incubation experiments to study the real-time transformation of chiral compounds in extreme environments such as the deep sea and hydrothermal vents?

How can we utilize advanced computational tools and experimental techniques to discover and characterize novel enantioselective enzymes?

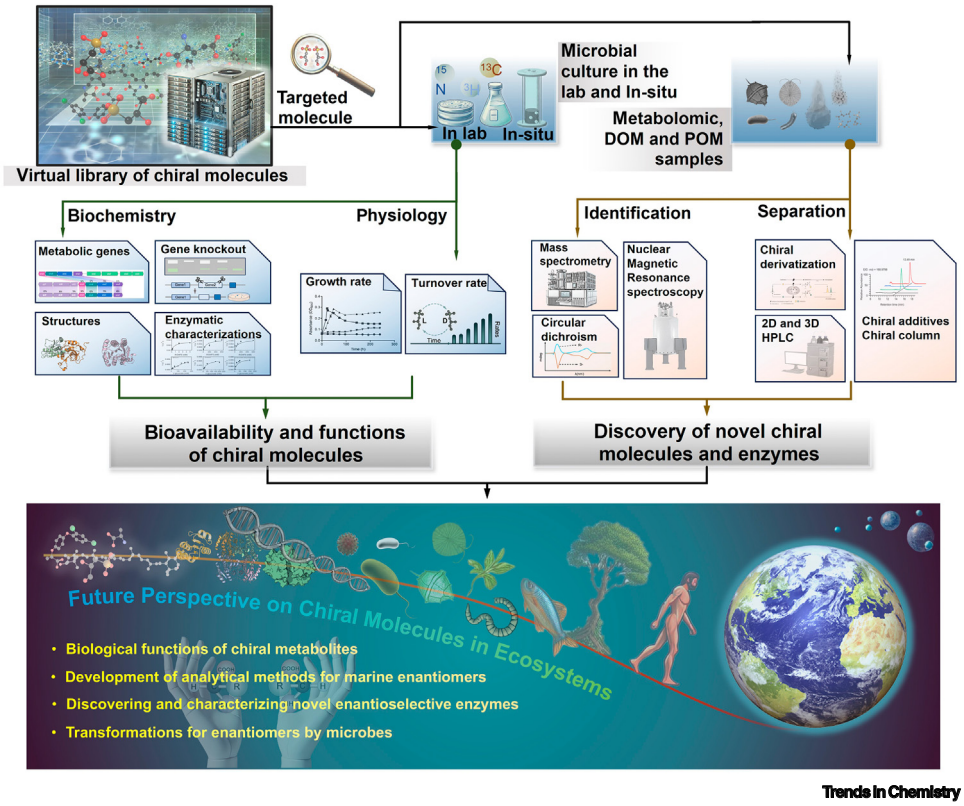


Figure 6. Framework for advanced analytical methods for chiral molecules in the ocean and perspectives on future research directions. Abbreviations: DOM, dissolved organic matter; HPLC, high-performance liquid chromatography; POM, particulate organic matter.

offer a promising approach to study the transformation of chiral enantiomers under various environmental conditions, such as amino acids in the high-pressure, oxidative-stress environments of the deep sea (Figure 6) [88]. More than half of the ocean occurs in regions below 3000 m [89], representing a largely undiscovered pool of chiral compounds. In addition to the deep sea, hydrothermal vents are another key area of interest for future research into chiral compounds, given their potential role in the evolution of life. Bacteria inhabiting these extreme environments have been found to produce DHPS [90]. Understanding how marine organisms synthesize and degrade enantiomers can provide insights into their ecological and biological roles, such as homeostatic mechanisms between D- and L-amino acids, as well as potential applications in biotechnology. The advent of the omics era has yielded large datasets of marine metabolic enzymes, such as *Tara* Oceans and The Global Ocean Microbiome [91,92]. These datasets provide a foundation for the mining of enzyme information, construction of metabolic pathways for marine DOM, and identification of enantiomer-specific transporters, such as those for D-amino acids. Improving the enantioselectivity of biocatalysts remains a challenge. The discovery of new chiral enzymes, coupled with advances in protein engineering, is expanding the substrate scope and catalytic performance of biocatalysts. Recent breakthroughs in protein structure prediction, such as AlphaFold3 and associated tools like Foldseek and domain analysis [93–95], provide a deeper structural understanding of enzyme catalysis and the mechanism of chiral enzymes recognizing chiral substrate. Additionally, recent studies have used machine learning to predict the enantioselectivity of an amidase enzyme toward novel substrates. These data-driven approaches highlight the potential of machine learning to expedite biocatalyst development and optimize biocatalytic processes.

Chirality, a fundamental concept with a 176-year history, continues to shape our understanding of the natural world. The creation of mirror bacteria has recently been highlighted as carrying potential risks, such as immune evasion and ecosystem invasion [96]. By contrast, chemically synthesized chiral molecules entering the environment are unlikely to significantly alter marine biogeochemistry, as microbes may develop mechanisms to degrade them over time. This natural process, combined with sustainable synthesis practices, helps to minimize their ecological impact. As chemists delve deeper into the chiral world of the ocean in the future (see Outstanding questions), it will foster interdisciplinary collaboration between chemistry and marine science, unveiling invisible dimensions of biogeochemical cycling and assisting in the development of novel biocatalysts with potential applications in chemistry.

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Declaration of interests

The authors declare no interests.

Supplemental information

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