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## Chiral selection under swirling: From a concept to its realization in soft-matter self-assembly

Josep Claret, Jordi Ignés-Mullol, Francesc Sagués\*

Departament de Ciència de Materials i Química Física, Facultat de Química, Universitat de Barcelona, Barcelona, Catalonia

**Summary.** Chirality, i.e., the absence of mirror symmetry, is evident in nature and has profound implications in the field of Biology but also in Materials Science. The aim of this contribution is: (i) to concisely present the basic concepts and definitions related to chirality and (ii) to briefly summarize our own research into the induction of chiral selection under vortical motion during the self-assembly of soft-matter aggregates. [*Contrib Sci* 11(2): 199-205 (2015)]

**\*Correspondence:**

Francesc Sagués  
Departament de Ciència de Materials  
i Química Física  
Facultat de Química  
Universitat de Barcelona  
Martí i Franquès, 1  
08028 Barcelona, Catalonia  
E-mail: f.sagues@ub.edu

### A brief introduction to chirality: thoughts, concepts and definitions

Chirality is a pervasive concept in nature and is a feature of structures and phenomena at disparate scales, with relevance for disciplines ranging from Cosmology to the Biological Sciences. The world, as we know it, is intrinsically asymmetric. Indeed, we directly quote the opening sentences of G.H. Wagnière in his book *On Chirality and the Universal Asymmetry* [26]: “That the world is asymmetric is a trivial observation. How can the world be anything but asymmetric?”

If asymmetry is a fundamental property of the universe, then a basic question has yet to be satisfactorily answered. According to most laws of physics, any asymmetric object or any asymmetrically moving system could, in principle, exist with equal probability as the corresponding mirror object or mirror system. The laws of gravitation and electromagnetism together with the strong interactions governing the structure of atomic nuclei predict that image and mirror image should have exactly the same energy and, consequently, the same probability of occurring. So why are we living in the world as it is, and not in the mirror world? Or why do the actual world

**Keywords:** chirality · enantiomorphic · vortical stirring · helicity · soft matter

and the mirror world not coexist and be simultaneously perceptible?”

Probably the deeper and more challenging questions on chirality refer to the homochirality of life. In the following, we thus briefly focus first on this context before turning to scenarios of chirality that are closer to our experience in Physical Chemistry. One of the most trivial ways to experience chirality in life is by observing our hands. They look similar, certainly, but they are not identical. In fact, they are in-plane mirror images. Objects that are distinct from, that is, not superimposable onto, their mirror images are referred to as chiral, while any macroscopic chiral object and its mirror image can be enantiomorphic (enantiomeric). Now let us consider the elementary components that living entities are built from. The essential molecules on which life, as we know it today, is based, such as DNA, RNA, proteins, and sugars, are all chiral. Moreover, one of the most striking observations in Biology is that this biological or biomolecular homochirality is the same everywhere, whether in a virus or in a human cell. Quoting again Wagnière: “Suppose that in the universe there exists an as yet unknown planet..., and on which atmosphere and climatic conditions resemble those on Earth. In the course of history of this planet, life chemically resembling that on Earth has evolved. Will these living organisms be molecularly homochiral to those on Earth? In other words, is the biological homochirality the direct consequence of universal and thus fundamental laws or is it due to chance—whatever that means—and thus mainly depends on local conditions?”

When referring to molecules, one is obligated to first adopt a chemical perspective. In fact, and from a purely thermodynamics standpoint, one could easily conclude that a chiral molecule and its enantiomer should basically have the same energy. Under conditions of thermodynamic equilibrium, both forms should thus display equal existing probabilities. This would be exactly true if we ignored subtle effects rooted in elementary particles theories. To be precise, the so-called parity-violating weak forces would subtly break the aforementioned balance, favoring one chiral form over the other. However, whether that biological homochirality arises from such elementary particle interaction effects is unclear, and nowadays mostly disregarded. We are thus led, and probably will be for many years, to look for new responses to the intriguing question about the origin of homochirality in life. But let us skip this challenging question to concentrate on chirality as it appears in non-living matter. A series of concepts and a brief historical account of how this concept has become rooted in general sciences are probably worth reviewing in the first part of this contribution.

Although chirality is a term more often associated with Chemistry than with Physics, physicists are also acquainted with it, since the implications extend over a disparity of problems covering many different and very actively investigated disciplines. We have already mentioned the fundamental question of parity violation at the level of weak interactions among elementary particles. The history dates back to 1927, when E.P. Wigner [27] formulated the principle of conservation of parity according to which “all interactions in nature are invariant with respect to space inversion.” With the discovery of new interactions, this statement was questioned theoretically by T.D. Lee and C.N. Yang in 1956 [12]. Only one year later, parity violation was confirmed in a brilliant experiment by C. S. Wu et al. [28].

Closer to our times and interests, chirality is often mentioned when referring to Materials Science, for example, in relation to supramolecular assemblies [15], soft-matter-based systems [9,23], or to Biophysics and Developmental Biology [3,8,24], among others.

## Definitions of chirality

The term chiral was adopted by Lord Kelvin in 1904, when he stated “I call any geometrical figure, or group of points, chiral, and say it has chirality, if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself “[22]. Mathematically formulated, one could use a definition based on group theory, by specifying the criterion for an object to display chirality as the absence of any rotation-reflection (or improper rotation) axes  $S_n$  [10]. This supposes, in particular, the lack of planes of reflection and inversion points, the more natural symmetries of achiral objects. Notice also that all totally asymmetric bodies, as well as those objects displaying only rotation symmetry axes  $C_n$ , are chiral [10]. With a more modern perspective, the concept of chirality has been extended to encompass not only static objects, but also time-dependent physical vector fields eventually related to translational or rotational dynamic processes. In this respect and already more than 25 years ago, L.D. Barron [2] proposed to unambiguously distinguish between “true” and “false” chirality, by establishing that true chirality is exhibited by systems that exist in two enantiomeric states that are inter-converted by space inversion, but not by time reversal combined with any proper spatial rotation. In fact, this last requirement is what enforces true chirality in a broad sense. For example, false chirality is exhibited by co-linear electric and magnetic fields, while true chirality appears when a

magnetic field is applied parallel to the propagation direction of a light beam of arbitrary polarization.

## Louis Pasteur's dissymmetric forces

A particularly important discovery that advanced the modern history of chirality in the natural sciences was that of the French chemist and microbiologist Louis Pasteur (1822–1895). In the mid-19th century he was working with solutions of sodium ammonium tartrate that were non-optically active; that is, they did not rotate the plane of a polarized light. After recrystallization, he found, however, that the crystals could be distinctively distinguished in terms of their chirality. In solutions of one type of crystals, the plane of polarization was rotated to one side; the reverse was true with the enantiomorphic crystals. Thus, one could say that Pasteur was able to resolve a racemic mixture, containing equal amounts of both chiral forms, into its pure enantioselective components. Referring back to Wagnière [26], it is clear that Pasteur was fortunate to have dealt with a striking example of racemic resolution, since very often racemic solutions also form racemic crystals, in which case resolution must be attempted using other, more sophisticated procedures. It is also worth remembering at this point that Pasteur was the first to propose a theory to explain the origin of biomolecular homochirality. He did so by conceiving of the existence of chiral, or what he referred to as dissymmetric, forces [14].

In spite of its speculative origin, the idea of chiral forces as conjectured by Pasteur has by now gained wide recognition. In this regard, one should bear in mind that in the absence of chiral influences, achiral compounds can be transformed only into other achiral compounds, a racemic mixture, or an enantiopure component with equal probability of the two enantiomers. This conserved symmetry can, in principle, only be broken by using chiral components (catalysts, promoters, or chemical modifiers), by applying special sorts of confinements that may favor chiral arrangements from atomic to meso-scales (oriented reacting surfaces, aligning boundaries, or similar controlling boundaries), or, finally, by the application of the aforementioned chiral forces.

According to Guijarro et al. [10], the list of such chiral forces is rather limited in nature but also in laboratory experiments. Based on what we know today, in this category we can above all include the action of a circularly polarized light, as it is probably the most representative influence. A well-known example of its practical utilization as a chiral selector within a chemical context is the asymmetric

photodegradation of racemic leucine [7]. The two remaining effects are much less widely recognized: (1) the magneto-chiral influence [20], mentioned above as the combination of a static magnetic field with a co-linearly applied light beam of arbitrary polarization, and (2) the vortical stirring effect.

The demonstration of the chiral nature of swirling has an interesting history of its own. In fact, as quoted in Guijarro et al. [10], Pasteur himself was determined to find the “cosmic force of dissymmetry” inspired by the motion of the Earth. He therefore tried, albeit in vain, to induce selection in the dextro- or levo-optical rotation of chemically synthesized molecules by conducting reactions in a centrifuge. These attempts were long considered as complete nonsense and Pasteur's efforts were totally disregarded. This skepticism was not totally unfounded, since from a superficial point of view one could sustain that stirring a fluid does nothing but impart a simple rotary motion to it. However, the situation turns out to be somewhat more subtle. Vortices in fluids are the more familiar signatures of turbulence and are thus associated with complex, or more precisely, chaotic hydrodynamic motions. Using dynamic concepts, we can say that vortical flows result from the superposition of circular and translational motions. The first component or, to be more precise, its associated angular momentum, is represented by a time-odd, axial (unchanged under space inversion  $P$ ) vector, whereas the translational momentum is clearly time-odd but polar (reversed under the operation  $P$ ). Because this combination is endowed with helicity, vortices are true chiral dynamic structures. In the following, we restrict our discussion to the role of vortical stirring in chiral selection, since this has been the main goal of our recent research on chiral effects.

## Vortical stirring as a selection mechanism for the chirality of soft aggregates

The possibility to break the chiral symmetry of solid materials when crystallized under stirring has been widely recognized since it was first observed nearly 25 years ago for supersaturated solutions of sodium chlorate [11]. More recently, similar observations were published for the same system when initial solutions containing both enantiomers were ground with glass beads [25].

In the case of soft materials, the possibility is even more intriguing. In 2001, members of our group coauthored with J. M. Ribó a study demonstrating not only signatures of

induced chirality under stirring, but what turned out to be the first experimental evidence of chiral selection conditions [19]. Here, the term chiral selection has to be understood in the sense that in addition to the realization of chiral symmetry-breaking, more importantly, the effective sign of the desired enantiomeric excess can be chosen at will, according to the desired handedness, right or left. Note that in this respect chiral selection, as compared to pure chiral symmetry-breaking in which both signs are a priori equally realized, is a different, deeper, and broader scenario for the spontaneous emergence of chirality. The investigated system consisted of the self-assembly of homoassociates of achiral porphyrin derivatives during rotatory evaporation. For that particular system it was demonstrated that the helicity sign of the circular dichroism spectra of the dissolved aggregates correlated, within statistical confidence levels, with the handedness of the applied stirring, either clockwise or counterclockwise. Evidence for the formation of helical bundles following this association process was obtained a few years later from atomic force microscopy images [6]. A more recent discussion pointed out the need to better discern the intrinsic chirality of the aggregates [21], while other authors revisited the question by considering the reversible or dynamic nature of chirality [5,29].

The need to extend the scenario first reported by J. M. Ribó and coworkers and to exclude any artifact arising from the employed observation technique led us to devise a different experimental approach. We intended to prove chiral selection of soft-matter condensates, by employing more direct in situ characterization techniques that would make any further interpretation of the measurements unnecessary. Our research is summarized in four consecutive publications [4,16–18]. All of them refer essentially to the same experimental setup but address three complementary aspects. These are briefly summarized below.

### Chiral-symmetry selection in interfacial systems induced by vortical flows

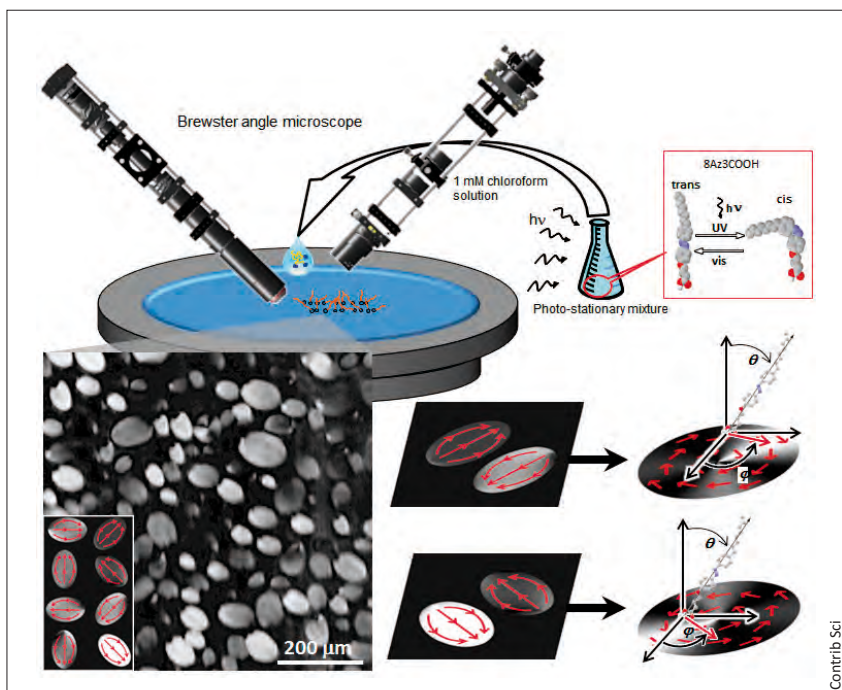
The chosen system refers to surfactant monolayers spread at the air/water interface under moderate stirring of the underlying aqueous subphase. This scenario intrinsically differs in many respects from the one considered by Ribó et al. First, in this case chiral selection is sought at the level of a soft interface lying on the boundary separating two different phases, while flow shearing is applied only to one of them. We thus searched for the possibility that a genuine

three-dimensional centimeter-scale stirring mechanism, when applied to the water subphase, could be effectively imprinted into a two-dimensional monomolecular-thick monolayer on top of it. In short, this would be a signature of an effective scaling-down of chirality encompassing different dimensions and disparate length-scales. On the other hand, observation of the reflectivity patterns displayed at the interface, here realized via simple polarized-light (Brewster angle) microscopy (BAM), would provide a direct measure of the orientations of the amphiphilic molecules residing at the monolayer, in which case the chiral symmetry-breaking measure would be free of any ambiguity.

The chosen system was a Langmuir monolayer of a photosensitive azobenzene derivative (Fig. 1). This system is particularly interesting because, under suitable illumination conditions, a very slowly evolving interfacial emulsion forms at the aqueous-air interface; at the same time, micron-size circular domains (like two-dimensional droplets) enriched with the *trans* isomer progressively coarsen, while embedded in a continuous and unstructured mostly *cis* phase. The self-assembled circular domains display a long-range orientational order of the stretched *trans* azo-molecules, a feature that BAM distinctively resolves in so-called bend-like textures: Tilted molecules organize tangentially to the droplet's contour around a central singularity or defect of positive topological charge. This system intrinsically supposes a symmetry-breaking scenario. However, as expected, domains of either sign, i.e., clockwise and counterclockwise bends, are equally realized, since the azobenzene molecule is itself achiral. Observations change drastically when the monolayer is led to evolve under gentle stirring of the aqueous subphase [16,17] or in the presence of chiral dopants [4,18]. Following this intervention, the previous delicate balance of equally realized chiralities is strikingly broken.

To elucidate the mechanism of chiral selection, we first need to understand how circular bend domains, with resolved but random orientational (clockwise or counterclockwise) chirality, are formed. The answer can be found by following the spontaneous evolution of an initially pure *cis* component into a mixed *cis-trans* monolayer that, eventually, will feature the desired bend domains. Unlike *cis* isomers, the geometry of the *trans* azobenzene amphiphile favors supramolecular assembly. Moreover, the two isomeric forms perform very differently as surfactants. As mentioned above, this leads to their spontaneous phase separation at the air/water interface and to the initial formation of sub-micron agglomerates with elliptical, rather than circular, shape. The reason why these domains are not circular at the





**Fig. 1.** A droplet of a chloroform solution of the azobenzene derivative is spread at the air/water interface. The solution is irradiated with UV light prior to spreading in order to maximize the presence of the *cis* isomer. Brewster angle microscopy (BAM) observation reveals the formation of segregated domains rich in the *trans* isomer. Inside the domains, surfactant molecules organize with long-range orientational order, as interpreted from BAM image analysis. The coalescence of antiparallel elliptical domains results in the formation of circular domains whose bend texture has an orientational chirality, correlated with the arrangement of the doublet of the parent elliptical domains. (Details are provided in [16]).

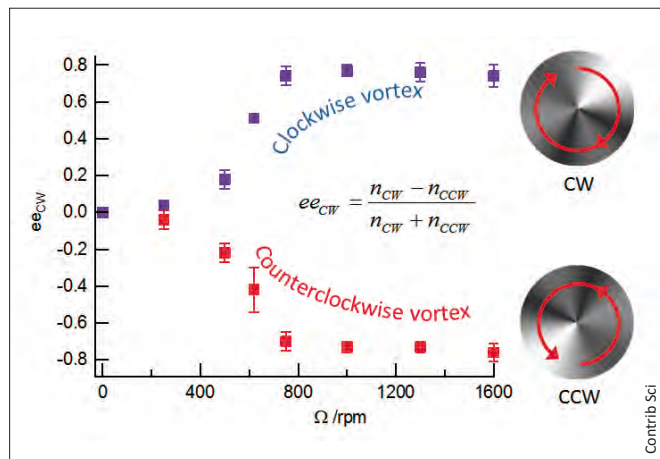
very beginning is because the assembled *trans* amphiphiles organize with their hydrophobic tails mostly aligned along the same direction. This in turn promotes the stretching of the two-dimensional droplets into ellipses. Moreover, since polar heads and hydrophobic tails in the aligned *trans* amphiphiles are clearly different, the way they assemble at either tip of an elliptical domain differs as well. This is an important result that helps in understanding the mechanism leading to the coupling of subphase stirring and the orientational chirality of monolayer domains. BAM imaging unambiguously shows towards which tip of the ellipse the hydrophobic tails are tilted.

Just as oil droplets suspended in water tend to coalesce spontaneously, elliptical domains surrounded by the *cis* phase in a monolayer of our azobenzene amphiphile tend to merge, prompted by the tendency to decrease the length of the contact region between the two dissimilar monolayer phases. Remarkably, the outcome of this process depends on the relative orientation of the ellipses in a merging pair. If the two elliptical domains are initially parallel, that is, if the hydrophobic tails inside either ellipse are tilted towards the same direction, the resulting domain after coalescence will be a larger ellipse with the same inner configuration as the two parent domains. Conversely, if the two elliptical domains are antiparallel, the coalescence results in a circular domain with a bend inner texture, that is, with a resolved orientational

chirality. The resulting handedness depends on the spatial arrangement of the parent domains, which constitute a chiral doublet. If the northern-most domain is aligned eastwards, then the outcome is a domain with clockwise handedness. If the southern-most domain is aligned eastwards, the resulting domain will be counterclockwise. Since the elliptical domains are randomly oriented, on average the same number of bend domains of either handedness is formed.

The above scenario is drastically altered when the monolayer is spread and is allowed to develop on a stirred, instead of a quiescent, air/water interface. After a period of incubation that can be as short as a few minutes, stirring is stopped and the configuration of the monolayer is monitored under BAM observation. The result is that the population of chiral bend domains is biased towards a handedness that is correlated with the sign of stirring. We quantify this result by defining an enantiomeric excess parameter,  $ee_{CW}$ , as the statistically meaningful difference between the fraction of domains with clockwise handedness and the fraction with the opposite one. Although the difference is hardly measurable at low stirring rates (racemic mixture), >90% of the domains have the same handedness as the stirring vortex for rates of about 1000 rpm [16,17] (Fig. 2).

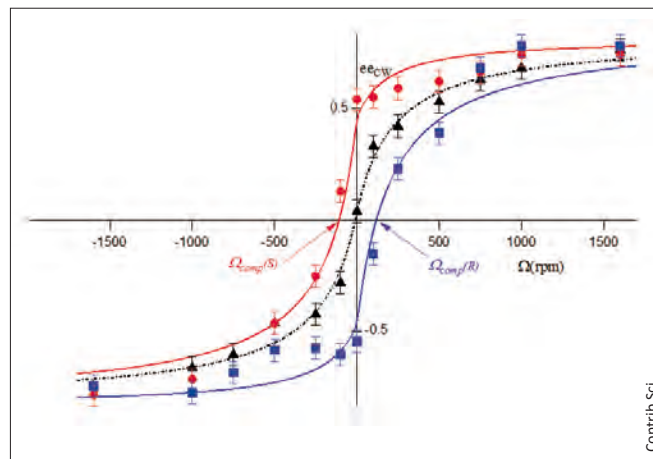
From this intriguing observation we can ask: What is the nature of the mechanism that leads to this chiral selection? We propose a description that is based on the asymmetric



**Fig. 2.** Chiral selection as a function of the stirring rate is quantified by means of an enantiomeric excess parameter,  $ee_{CW}$ , obtained by exploring a statistically meaningful number of bend domains. The orientational chirality individual bend domains can be directly assessed from BAM imaging by comparing them with the motifs shown in the figure. (Details are provided in [16,17]).

nature of the elliptical domains detailed above. When two antiparallel domains merge to generate a chiral bend domain, the configuration of the ordered surfactant molecules undergoes a dramatic rearrangement, which can be described using the framework of creation and annihilation of topological defects that is often invoked when describing the dynamics of soft materials, most notably liquid crystals [13]. Suffice it to say in the present case that this rearrangement is faster under stirring when the sign of the vortex coincides with the handedness of the doublet of antiparallel ellipses, thus with the handedness of the resulting bend domain. Although bend domains of either chirality may form, those with the same handedness as the underlying vortex are kinetically favored and therefore comprise the majority [16].

Less dramatic than the physical chiral selection described above is the possibility to achieve a similar effect by doping the surfactant monolayer with a chiral dopant whose molecular structure is similar to that of the original amphiphile. In this case, we chose custom-synthesized azobenzene surfactants with a chiral center of either sign. When the monolayer is prepared, as explained above, over a quiescent subphase and doped with a racemic mixture of the dopants, the result is a racemic mixture of chiral bend domains. That is,  $ee_{CW} = 0$ . On the other hand, an imbalance of either chiral dopant results in the formation of a monolayer with the majority of bend domains of the selected handedness. Just as above, we can provide a mechanism for the chiral selection, which it is now of chemical origin. Once again, the clue is found using




**Fig. 3.** Coupling between chiral dopants and vortical stirring in the chiral selection process. Stirring rates in the range  $-1600$  rpm (counterclockwise) to  $+1600$  rpm (clockwise) are explored for monolayers including 6% S dopant (red circles), 6% R dopant (blue squares), and a 3%/3% racemic mixture of S and R dopants (black triangles). Solid lines are fit to a kinetic model. (Details are provided in [18]).

BAM image analysis. We observe that, when the chiral dopant has a significant enantiomeric excess, the evolved elliptical domains are distorted, adopting a shape like a kidney bean. In fact, opposite signs of the enantiomeric excess of the chiral dopant result in bean-like domains with enantiomeric (mirror-image) configurations. Because of their shape, merging encounters of two antiparallel bean-like domains are dissimilar when they lead to a clockwise, rather than a counterclockwise, bend domain. This enhances the kinetics of coalescence into one particular type of bend domain, resulting in the observed enantiomeric excess. A definitive and very valuable advantage of working with this chemically doped system is that it also permits confrontations with chemical and physical chirality-inducing effects when the chemically modified monolayer is allowed to evolve under stirring conditions. The two chiral influences are forced to compete and situations of improved or reversed chiral sign selection are observed [18] (Fig. 3). The result is remarkable: The two chiral influences couple such that at low stirring rates the chemical effect dominates, while at high stirring rates the physical force determines the selection. The single main result that evidences this coupling is the existence of a compensation point indicating the existence of a subphase stirring rate that exactly compensates the chiral influence of the chiral dopant of opposite sign.

A similar chiral selection of bend domains can be achieved when a non-amphiphilic chiral solute is present in the subphase during monolayer incubation. We have performed experiments

in which different concentrations of amino acids (available in either chirality) are dissolved in the water subphase prior to monolayer spreading. The underlying physical mechanism is less clear in this case. Nevertheless, the chiral environment present in the water subphase may couple with the surfactant configuration during the domain rearrangement following ellipse coalescence, resulting in the kinetic dissymmetry that leads to chiral selection [4]. No effects of stirring have been investigated yet for this particular situation.

In summary, in the second part of this contribution we presented a soft-matter system in which orientational chirality, as it appears in domains of self-assembled surfactants, can be selected using either a chemical influence (chiral dopants) or a physical effect (vortical stirring). From this modest perspective and by advancing into the selection control of the chirality exhibited during the self-organization of soft materials, our aim is to provide new perspectives on the emergence of chirality in Nature. These same perspectives could be eventually invoked to solve the intriguing mystery of the biological homochirality observed in the living universe. 

**Competing interests.** None declared

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