PROJECT FINAL REPORT

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4.1 Final publishable summary report

Executive Summary

The European Commission supported research project RESOLVE focussed on the development of new methods for the purification of molecules with a particular property; that their mirror image cannot be superimposed upon them. This property, called chirality, is of critical importance across a wide range of disciplines and is of significance for society in general.

The purification methods which were the object of the research required understanding of how molecules of different handedness interact at the molecular level. The process of purification is called "resolution" (hence the name of the project) and the six research teams in the consortium managed to discover two important new routes to achieve it. These achievements were the result of fundamental knowledge concerning the synthesis of molecules, understanding of how molecules organise in liquids and at interfaces by both theory and experiment, and the use of state-of-the-art techniques for manipulating surface characteristics.

The project uncovered unique effects in the formation of layers of chiral molecules, and used these layers to separate the mirror image forms of solution-borne mixtures by both adsorption to self-assembled monolayers of molecules as well as by the growth of crystals on specifically patterned surfaces which promote the formation of the solids. These patterns, made by microcontact printing, are quite unique and show fascinating effects on the crystallisation of the organic materials with which have been employed.

The use of the novel resolution technique whereby crystals are ground as they grow giving rise to one enantiomer – so-called attrition-based deracemisation – has been used for the enantioselective synthesis of the drug clopidrogel. Importantly, the technique makes use of all of the material that enters the crystallisation reactor, there is no waste, no separation and recycling, all the material is converted in situ.

An important part of the project involved the identification of enantiomers, and a range of analytical tools were developed to characterise very small quantities of crystalline material so that the handedness of the molecules could be evaluated in a rapid way.

From a purely fundamental point of view, explanations concerning the nucleation – where a crystal starts to grow from a spontaneously formed seed – and growth of chiral structures both in solution and upon surfaces were derived.

It is expected that these unique techniques will give rise in the mid-term to value added products which will be of use in chiral technology. Especially, the recent results on a miniaturised block for screening resolutions is of great potential for the reduction of waste in the screening protocol in resolutions of chiral compounds.

Summary description of project context and objectives

An object which does not have exactly the same arrangement of parts as its mirror image is said to be chiral. Chiral molecules exist in mirror image forms - called enantiomers - which are very often produced as mixtures of “left” and “right” called racemates, as a mixture of left and right gloves or shoes. But, just like shoes and gloves, chiral molecules interact with other objects in different ways, provided they are also chiral, like our hands and feet for the gloves and shoes. Most of the molecules in our bodies that are responsible for all the different functions are chiral. Therefore, the enantiomers of a new molecule will interact in different ways with our body’s molecules.

We can see that pure enantiomers – in the form of medicines and so on - have different activities when they interact with natural systems. So, separating enantiomers is a really important task because they can have different properties when we are exposed to them.

In addition, there are a growing number of chiral man-made materials which have a number of very interesting properties. The mixture of enantiomers has different properties to the pure versions. Therefore being able to isolate the component molecules as a single handedness is very important for materials too.

The separation of enantiomers is called resolution, and is essential in order to ensure the safety and efficiency of chiral compounds, and to discover new properties in materials..

Therefore, the control and exploitation of chirality in solid phase is a critical part of the development of many products in the chemical and pharmaceutical industry. However, the resolution of enantiomers is a time consuming and complex process. It is beneficial for society to improve the speed and sustainability of this process.

With this background, the overall final objective of the RESOLVE project was:

To use a bottom-up philosophy for the separation of enantiomers, and to invent new protocols for use in the identification of resolving agents and resolution of enantiomers.

The reasoning is that by understanding the interactions between molecules at the smallest of levels, by using theory to understand experimental results, and by choosing conditions whereby chirally discriminating interactions are favoured, one can optimise the separation of enantiomers, their resolution.

Crystallisation is usually the most effective way to separate and purify chiral compounds. The growth of crystals on a surface is a way of confining the material and to make separation easier provided they grow preferentially from the topmost layer of the solid. The modification of surfaxces with suitably functionalised chiral layers should be a way to do favour stereoselective growth.

The goals attained on the road to the final objective were:

I. Establish a molecular basis for nucleation and growth and/or inhibition of chiral structures with nanometre scale precision: Little is known concerning the mechanisms of growth inhibition or acceleration, but nanométrica tools can give huge insight into the processes at the molecular level.

II. Transfer the chirality from templates to resolving systems: Use a layer whose chirality is passed from the surface up to the material which is deposited on top of it.
III. Identify diastereomeric products: Use analytical tools to ndetermine what is the handedness of the molecules which can be formed, bearing in mind that small quantities of material are intrinsic to the approach.
IV. Create a rapid screening process for diastereomers: A miniaturised technique with associated tool for analysis of products in a fast way would make a platform for separation of enantiomers which would speed up and make more sustainable the identification and purification of new chiral compounds.

Description of the main S&T results/foregrounds

Setting the scene: The left hand always knows what the right hands doing – Chemical Dexterity

Your left and right hand are different from one another. Your right hand will not be comfortable in a left glove, and vice-versa. Yet the reflection of your right h and in a mirror gives an image of your left hand. Objects which have this property are said to be chiral, and the two objects which have a mirror image relationship are said to be enantiomers

Molecules can have similarities to macroscopic objects, at least in shape and the way they can fit together. Just as there are chiral macroscopic objects, there are all sorts of chiral molecules. Often, as we said in the introduction, one enantiomer works better than the other, or than a combination of the two, and so separating it is really important. The work the RESOLVE consortium has done addresses this problem. So where did we start?

From the Bottom-up

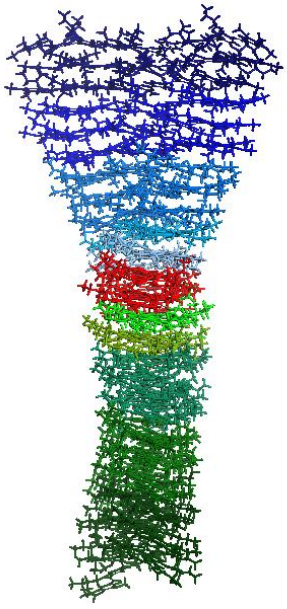
The separation of molecules is often done by crystallization and the way in which a crystal is formed starts with a molecule, which aggregates with others, and eventually – for stability reasons – starts to form a regular packing (rather like a brick wall) which we call a crystal.

If we can control the formation of the crystals from the smallest scale, we can influence how they will grow, rather like training the growth of a plant. So, we say we influence the growth from the bottom-up, from the first stage of growth which influences the next steps.

In a chemical sense, we use concepts like supramolecular chemistry – the chemistry of how molecules come together because of specific forces between them – and self-assembly – the way molecules arrange themselves – to be able to grow crystals where and when we want.

Understanding how molecules get together

Some elongated, aromatic molecules tend to pile up into stacks that can have very peculiar optical and electrical properties: those stacks can act as ‘molecular wires’ and can be used as the active materials in plastic-based photovoltaic devices.
When the molecules possess a bulky group in their periphery, they cannot pack perfectly on top of each other, but have to slightly rotate with respect to each other. If this side group has a specific shape that makes it non superimposable with its mirror image (it is then called chiral), the rotation between adjacent molecules in the stack is well defined, either clockwise or anti-clockwise. As a result, the stack itself is becoming helical, either with a left-



handed or a right-handed helicity, which can be detected optically.
One aspect of the RESOLVE project was to understand, through a joint approach combining experiments and molecular modeling, how the helicity in those stacks can be generated and controlled. This can be done by modifying the solvent in which the stacks form, or by changing the temperature, or with the assistance of chiral additives that interact on the side of the stack and force it to turn into a given helicity. This can be used to detect the presence of the additives in solution via the optical signature of the helical stacks.
The figure illustrates what happens when two stacks with opposite helicities meet in our computer simulations (the helicities are represented with either blue or green molecules, which twist left or right). The red zone represents the fact that the helicity in the 'green' stack forces the first molecules in the 'blue' stack rotate contrary to their initial orientation, to alleviate the stress at the junction. The results we get from the computer back up experimental observations.

Seeing is believing!

In order to gain information on the structure of the materials, it is important to visualize them. Yes, one can use optical microscopes. However, they are of no use when you want to look at the shape of individual molecules. Optical microscopy techniques don't provide the required spatial resolution. At best, they visualize molecules as featureless blobs. Fortunately, there are instruments that do much better than that. Imagine that you are blind and want to find your way. Using your hands or a stick is probably the best approach to get to know the world around you. Or you can read using your finger tips. Scanning probe microscopes are a family of non-optical microscopes that reveal the structure of surfaces by touching that surface with a very sharp tip. The sharper the tip, the smaller the details one can reveal. Tips can be that sharp that it is possible to distinguish not only individual molecules, but also their shape. This family of techniques is particularly useful to image surfaces.
You probably don't realize it but surfaces aren't as dull as you might think. Some surfaces are beautifully structured. In particular, certain sets of molecules will spontaneously organize on very flat (atomically flat!) surfaces. Yes, these molecules feel each other and they feel the substrate and spontaneously, these molecules self-organize on the surface into a very thin film (one molecule thick!). And that's why we need these special microscopes for (Figure 1).

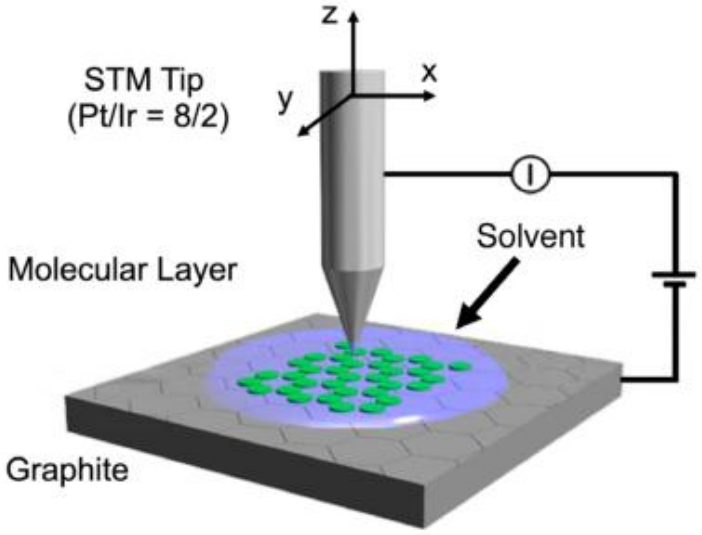


Figure 1. Scheme of the set-up of a scanning tunnelling microscope at the interface between a solvent and an atomically flat substrate (graphite).

Figure 2 is an image of a such a self-assembled monolayer on an atomically flat area of a piece of graphite. The image was obtained with a "scanning tunneling microscope", by raster scanning a very

sharp metallic tip across the surface of graphite, that was exposed to a solution containing the self-organizing molecules. Each bright triangle is the central part of a molecule.

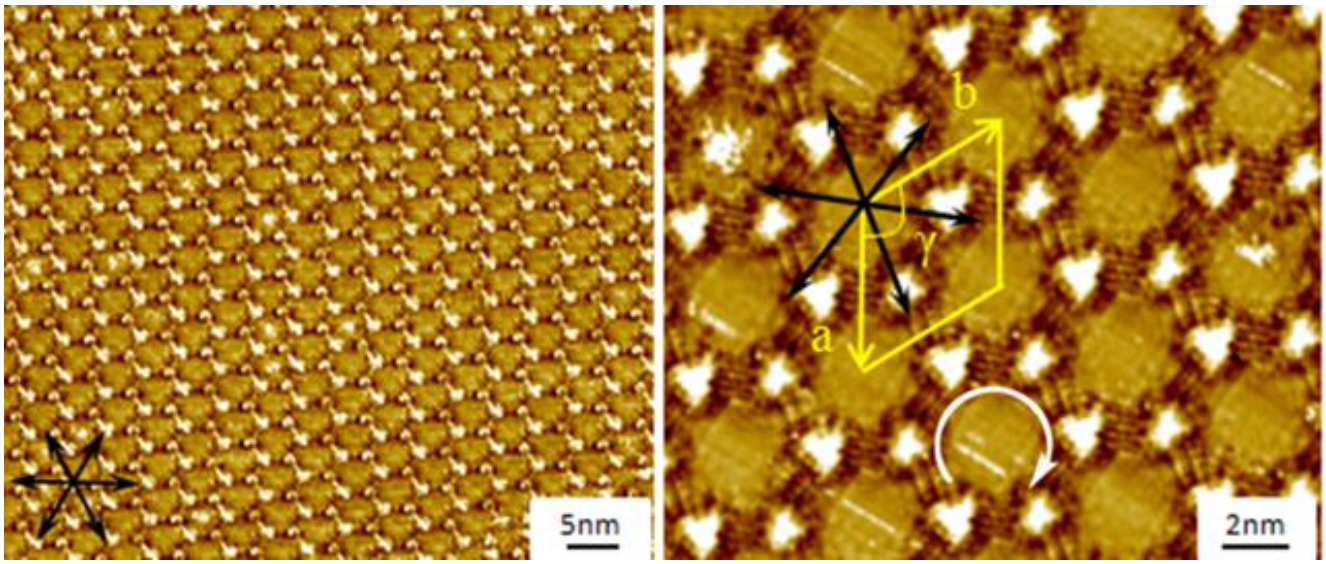


Figure 2. Images of a self-organized monolayer of molecules on top of a piece of graphite recorded by a scanning tunnelling microscopy

Thanks to these great microscopy techniques, we know exactly how such as surface covered by molecules looks like. And this is very important information to design new functional monolayers, in order to "RESOLVE".

It's actually quite simple. What we have done is the following. Imagine that you have a solution of two types of molecules. All molecules are identical to each other - they have the same composition and structure - except for the fact that they are each other's mirror image. Iit is very important to be able to separate these mirror-image molecules from each other, because despite their structural similarity, as drugs they might behave quite differently. One mirror-image form can by an effective drug, while the other mirror-image molecule might be inactive, or worse, poisonous. We have developed a new protocol to separate such mirror-image molecules by selectively adsorbing one of the mirror-image forms on a surface, covered with a regular monolayer of what is called the 'resolving' agent. The resolving agent is a molecule that self-assembles on a surface into a well-ordered monolayer. It is designed to show a strong preference to attract and bind one mirror-image form, but not the other. The concept is shown below (Figure 3). Thanks to the use of these high-resolution microscopes, we could experimentally verify that only one mirror-image form was adsorbed on the surface, while the other was left in solution.

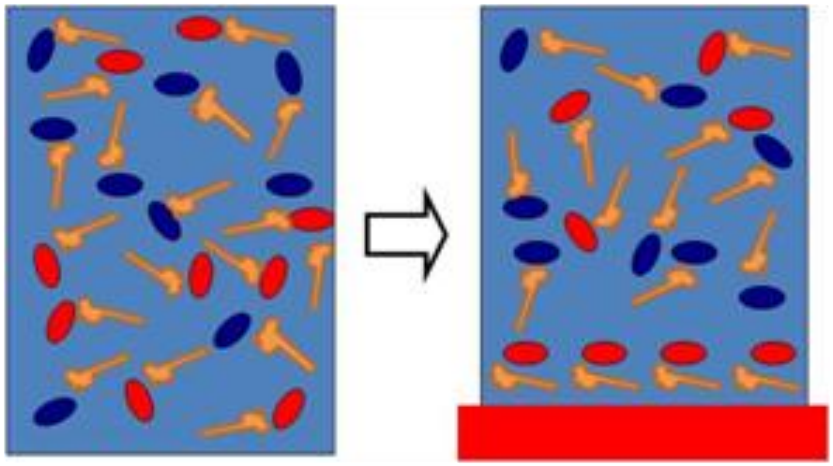


Figure 3. A cartoon depicting crystallisation at the liquid-solid interface. The orange hammer-like features symbolize the enantiopure resolving agents, adsorbedon the surface. The red and blue ovals represent the enantiomers (mirror-image molecules) to be resolved. Here, the red one is segregated from the blue which remains in the solution.

This is illustrated in the following way (Figure 4): A 'resolving' agent forms star-like features on the substrate of graphite, that are part of a larger monolayer. Using scanning tunnelling microscopy, the details of the monolayer structure are revealed. As mentioned before, the self-assembly process goes spontaneously. When exposing this patterned layer to a solution composed of mirror-image molecules, a clear structural change occurs: the star-like features disappear and new rod-like features appear, until the complete surface is covered. These rod-like features are composed of the resolving agent, pre-adsorbed on the surface, and one type of the mirror-image molecule adsorbed from solution. Its mirror image is left in solution

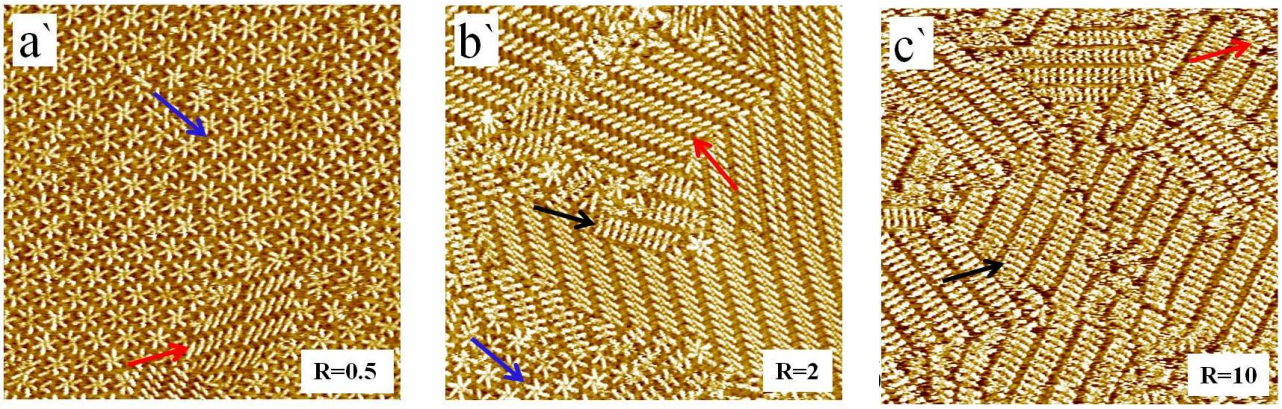
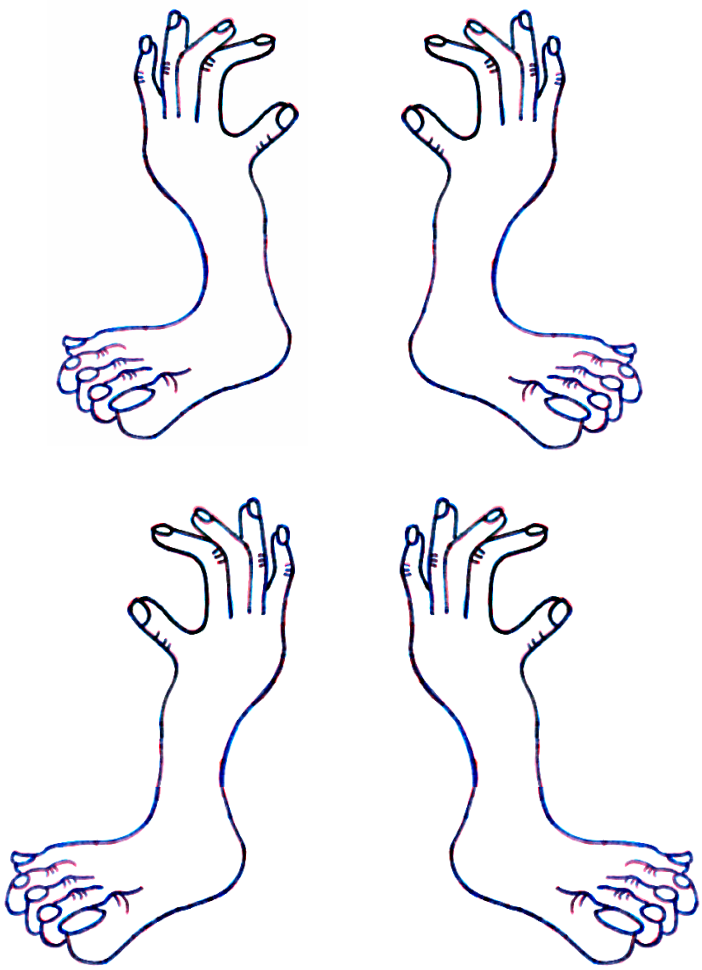


Figure 4. Sequence of scanning tunnelling microscopy images obtained upon exposing the surface of resolving agent to increasing amounts (increasing R) of mirror-image molecules. Only one type of mirror-image molecules is adsorbed on the surface, which is reflected by a change in the monolayer pattern.

Hand and footprints

Molecules can have a handedness intrinsic to them. Let's say a molecule is left handed. The way the molecule attaches to a surface could be influenced by the hand or not! In other words, its foot could organize to be a left foot or a right foot.

The way in which this attachment happens is very important for the chemistry of the layer, because the way in which molecules pack on a surface determines whether enantiomers separate – in which case we can have a resolution process – or if the molecules mix up without any particular order. In the latter case, the chirality is essentially dormant in the molecules and cannot be taken advantage of for separation or any property one might think of, like catalysis for instance.



The "frankenfoot" concept to describe how chiral elements can be transferred from hand to foot print in molecules which adsorb on surfaces. The top beasts are enantiomers, as are the lower ones between them, but the upper and lower creations cannot be interconverted.

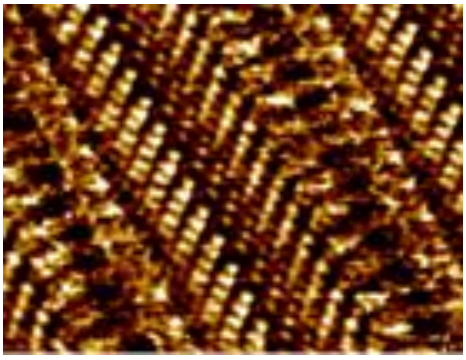
The research carried out in the RESOLVE project has shown that there are molecules whose hand determines the footedness in a very strict way, while certain molecular linkers in between hand and foot can generate a random situation. Knowing the rules for connectivity will help design molecules which can aid in the separation of enantiomers.

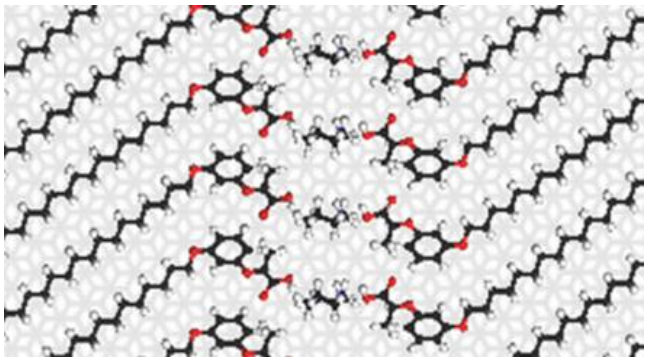
Walking in the steps of Pasteur with nanoshoes – Sorting molecules on graphite

Pasteur is famous for many things, but Chemists remember him especially because he made a discovery which made us understand that molecules can be chiral, remember that they can exist in two forms as left and right hands. He discovered a way of separating a 50:50 mixture of enantiomers by making one of them complex another compound, like showing a glove to the hand. This complex former is called a "resolving agent" these days, that is it allows a resolution to be performed. The

separation is possible because the complexing agent is chiral and preferentially crystallizes with one of the enantiomers, leaving the other one in solution

In the project, we have shown that it is possible to do this kind of separation on graphite with a resolving agent especially designed to do the job by sticking both to the surface and the molecule we want to separate. The microscope that lets us see molecules – the scanning tunneling microscope – was used to see what was happening, in much the same way as Pasteur saw his first chiral crystals with an optical microscope. He took great care, and we needed great care too, it's not easy to see chiral molecules! The trick is in understanding the way the stacks of molecules form. In the image below, one the left is from the microscope (the scanning tunneling), and the image on the right is from a calculation in which the way the molecules assembly has been modelled using an advanced programme for assessing assembly of molecules on surfaces. The molecule with the red parts (oxygen atoms) can be seen to clamp one enantiomer of the molecule from solution onto the surface, through hydrogen bonds. This segregation can be seen as a way of resolving compounds. O nly one enantiomer of the small molecule will form all the right interaction s to complex with our chiral resolver on the surface, where we have a kind of two dimensional crystal



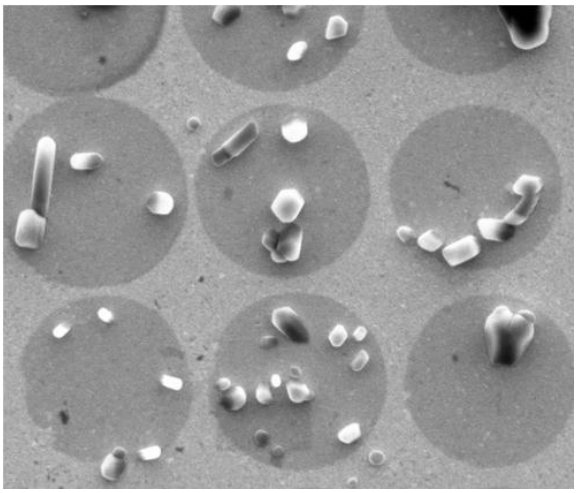


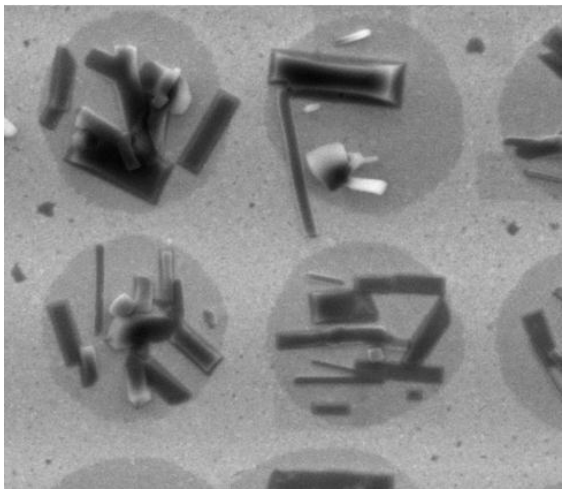
Crystallising molecules from the flat

Did you ever think how crystals form, and why they grow? The molecules of the final crystal come together and at a certain point organize into a periodic structure which continues to grow, a bit like a self-assembling click brick. As we said before, if we can help arrange the bricks, the wall will have to go a certain way.

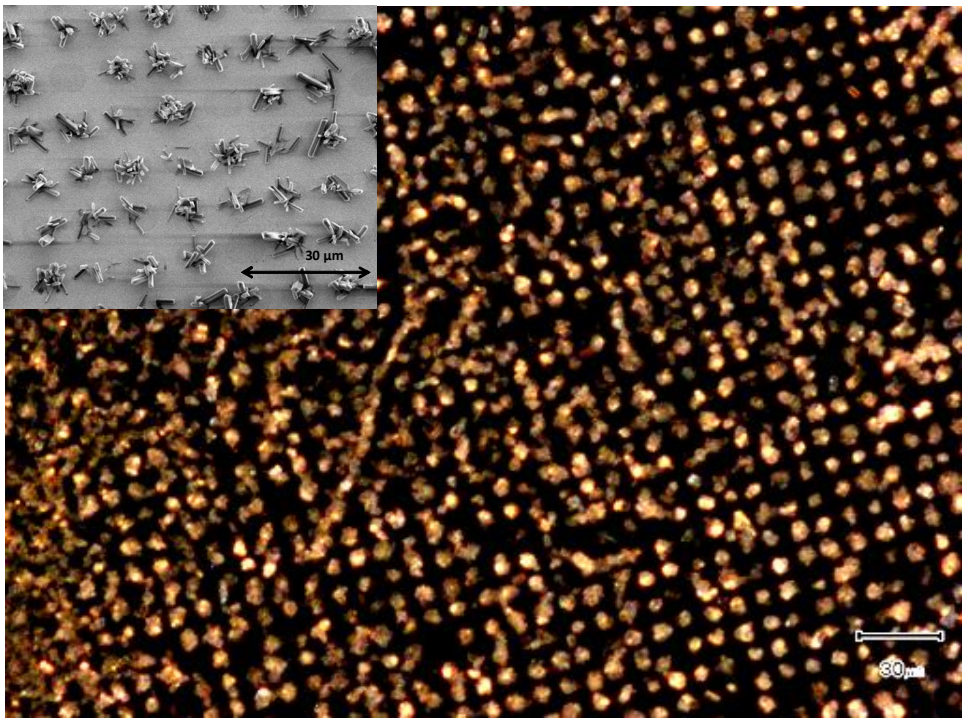
By patterning chiral molecules on a surface we can influence the way chiral crystals grow just by changing the solvent. The electron microscope images shown here are of exactly the same compound, grown on the sane kind of surface. But the crystals in the sample of the left image seem to be growing away from the surface, an the ones on the right look as if they lay flat on the surface.

The difference in the way they can be made to grow standing up or lying down is in the type of solvent we use to grow them. The RESOLVE project showed that it is possible to grow these chiral crystals on a surface, and that the chirality of the molecules and of the molecules attached to the surface where they grow from makes a big difference in the process. This doesn't just happen for pure organic compounds, but also in complexes like the ones that are formed in resolution experiments. This makes us hope that the approach will lead to a surface that can crystallize certain enantiomers and separate them. Now we're working with a rangle of chiral compounds to try just that.





You can see just how well the chiral patterns help make the crystals grow in their place in the microscope image here, where the picture has been taken with a polarizing filter which lets us see only the crystals.



Stirred, not shaken

Crystals of organic compounds are not like rock! They can break, and the molecules at the surface of the crystals can come and go. That effect can be used to turn a mixture of enantiomers into an enantiopure sample. And this effect has allowed the preparation of a drug with one handedness.

The way it works is that in solution a chemical is added that lets left and right hand interconvert, a bit like turning a glove inside out. If a crystal is growing with one handedness there is an effect (called "Ostwald ripening") where the larger crystal will get bigger which means that this handedness will

prevail. That is because the two enantiomers are switching between each other, so one is never in excess, the equilibrium is always re-established.

This technique – called attrition based deracemisation – has huge potential, because all of the the mixture of enantiomers is converted into just one enantiomer. Less waste, and good news for industry!

Potential impact

The way chiral compounds interact at the nanoscale is a transversal phenomenon affecting: a) Catalytic processes – which produce many chiral chemicals of high added value; for b) the pharmaceutical industry (worldwide sales of enantiomer drugs up to 410 billion euros) c) The separation of enantiomers (where chiral polymers are often used, market 30 billion euros); and d) The liquid crystal area interested in ordered layer systems for controlling the chirality of mesophases (the market for cholesterics is around 20 billion euros). The market value of chiral chemicals is increasing at the rate of between 5 and 20 % per annum. There are thus important end-users for the knowledge gained from the research performed in RESOLVE. But there are big challenges.

The academic groups have made very important fundamental discoveries and their results have been published in scientific journals and communicated at conferences, where they have been well received. There are plans to protect certain knowledge generated in the project – especially with regard to two techniques which allow chiral separation.

Syncom is the only company that participated in RESOLVE. One of the major activities of the company is the preparation of the pure enantiomers (mirror images) of chiral compounds. These compounds are prepared, for the most part, for various large pharmaceutical companies in conjunction with their drug development strategies. The technique of Dutch Resolution, which played a key role in the RESOLVE objectives, was developed at Syncom. Separation of racemates (50:50 mixtures of enantiomers) into the pure enantiomers by means of so-called diastereomeric resolution remains probably still the most widely used approach. Dutch Resolution is a variation of diastereomeric resolution. However, despite impressive improvements in recent years the techniques used remain trial and error based. No use is made of modern computer based technologies that in recent years have been applied with breathtaking success to the study of surfaces. It is now possible to see at an atomic level what occurs on a surface. That type of detailed information simply cannot be obtained in solution. It makes therefore eminent sense to marry solution chemistry to solid phase chemistry. This is easily said but not easily done. Chirality on surfaces involves principles that differ from those in solution although the principles can be related.

A goal of RESOLVE was to effect that marriage. This was a long step for Syncom that brought the company far outside of its area of expertise. "Comfort zone" to put it in other terms. However, the clear commercial advantages of an extremely rapid, computer driven, method for screening in order to identify rapidly successful chemical combinations for resolution of racemates into the enantiomers was attractive to the company. Equally important for a small company heavily involved in work that is often pure research is the scientific challenge. This must be emphasized. Knowledge of the behavior of chiral molecules on surfaces is fundamental and is of importance to the company. Survival is based to a large extent on knowledge broader than that of one's competitors.

With RESOLVE Syncom – in close cooperation with our talented and indispensable partners! - has learned how to stack enantiomers onto gold surfaces. In parallel work we have also learned how to work with much cheaper graphite and how to organize certain molecules on that surface. We have learned some of the principles of chirality on surfaces that are a key to success. The ultimate road to automated resolution technologies remains long but the route to be travelled is now clear. Fundamental advances have been made. These advances have also been recognized by the scientific community. The road to commercial application will likely have some unanticipated branches and,

with luck, some profitable shortcuts. We expect to see applications on the longer term of five to ten years.

Syncom has also been involved in the development of an entirely new strategy for the preparation of pure enantiomers. This technique is called attrition-induced grinding and involves, as the name suggests, simply the physical grinding of certain types of racemates in order to provide a single pure enantiomer. Syncom has used this technique in the synthesis of single enantiomers of the anti-hypertensive drug, Clopidogrel (Plavix). These developments are obviously interesting from a commercial standpoint. For the case of Clopidogrel Syncom decided not to apply for a patent but rather published the results. This decision was based on a business case analysis that indicated that more business would be generated by publicity that emphasizes the strength of the company in the area of chirality. Despite the ease of the synthesis it was not expected that a new process that had not been scaled up would be able to compete with existing processes. Huge investments would be necessary for production and Syncom had neither the money nor the facilities. Other companies were not interested. Sober analysis also leads to the conclusion that the pharmaceutical market, the area in which Syncom is active, is doing poorly. Owing to the rising costs of health care, the competition from generics producers in China, India and Israel and the extreme costs involved in development of new drugs the industry has become extremely conservative. The result is general lack of commercial interest in new discoveries. We expect that the best commercial approach will remain the use of hard science to develop new technologies and applications thereof and to use this to strengthen the knowledge base of the firm. Although we are certainly not opposed to commercialization of a new product or technology, we believe that the path will remain difficult in the coming years in this area.

Social impact will come from simple innovations which tackle the big problem of controlling crystallisation which is important in a wider aspect, where small companies high on knowledge portfolio and know-how could make an impact. Support for this kind of initiative could yield novel products for industry. On the other hand, the functionalised surfaces prepared in the framework are unique and offer special surface properties. Identification of users of these effects is important in order to be able to take advantage of this knowledge. The researchers of RESOLVE are in a position to grab this opportunity.

In the long term, the technology resulting from the knowledge and science produced in the project could reduce waste solvent by an order of magnitude, and waste diastereomer mixtures used in chiral separations, and in identifying resolving agents. Therefore, an improvement in the sustainability of the process of identifying diastereomer salts and purifying enantiomeric mixtures will result which is important for the preparation of chiral compounds. But these improvements will need investment in chiral chemistry, and the emergence of promising chiral materials for other applications, such as displays and optical signal processing.

Address of the project public website and contact details

Website: http://www.icmab.es/resolve/

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