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Hybrid Catalysis in Concurrent Mode: How to Make Catalysts and Biocatalysts Compatible?

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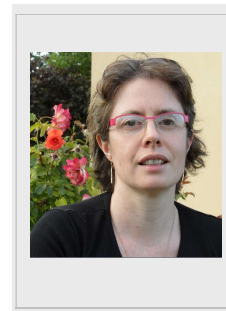
Abstract: For a long time, catalysis and biocatalysis appeared to be in competition, until the organic chemist decided to synergize them for the better. But their inherent differences made the challenge so difficult that most of the hybrid catalysis processes developed turned out to be sequential. However, some processes have shown that the seemingly impossible was achievable, by combining catalysts and biocatalysts in concurrent mode, i.e. by having them work under the same experimental conditions, in the presence of all the reagents, from the start of the reaction cascade. The aim of this review is to show how it has been possible, by drawing on multi-disciplinary fields such as organic-, inorganic chemistry, chemo- and biocatalysis, to make catalysts that are opposed by many parameters coexist, so that, based on a classification of the difficulties encountered, the organic chemist can find rapid ways of overcoming them, as a toolbox, to develop new innovative processes.

1. Introduction

For several years now, chemical synthesis has been looking for new solutions to offer high-performance and eco-compatible routes in the field of cascade reactions. The area of catalysis has a key role to play in this transition, and hybrid catalysis in particular. Indeed, for instance, the combination of chemical and biological catalysts is particularly relevant nowadays as it aims to retain only the best of both worlds, i.e. the use of robust chemical catalysts with a broad spectrum of substrates, together with biocatalysts capable of high stereoselectivity, regioselectivity and chemoselectivity. Nevertheless, it is not a straightforward combination because while the chemo-catalysts are able to work within a broad range of solvents, temperatures and pHs, enzymes are often restricted to aqueous media, ambient temperature and neutral pH. While the first example of hybrid catalysis dates back to 1980, with Van Bekkum's work devoted to the synthesis of mannitol using a combination of an isomerase and a Pt catalyst,^[1] it is only in the last 15 years that numerous examples of hybrid catalysis implementation have emerged, with various reviews highlighting significant advances.^[2–7] To avoid any interference between chemical catalysts and enzymes, one option is to avoid a one-pot process, by compartmentalize them in columns by implementing systems related to flow chemistry. Recent reviews fully illustrate the relevance of this approach.^[8–10] The development of successive reactions in the same pot can also be found in the literature, however, the holy grail, consisting in carrying out reactions in a concurrent mode, i.e. where all the ingredients (catalysts, solvent, reagents etc...) are added from

the start and where the experimental condition are kept unchanged during all the process, drastically reduces the number of articles. This is the reason why most of them are sequential processes where a first series of ingredients is added before a second one at the end of the first step, to ensure the next step. To demonstrate recent fundamental advances in hybrid catalysis in a one pot approach, this review will only deal with systems in true concurrent mode, because it is obvious this mode is by far the most interesting one, but also the most challenging as numerous harmful interactions can prevent the two types of catalysts to work together: interactions i) due to the solvent, ii) to the reagents, iii) to the catalysts themselves or iv) to experimental conditions (pH or temperature incompatibilities). The aim of this paper is to highlight the ingenuity the chemist had to deploy to make the various parameters mentioned above compatible in concurrent mode, during a cascade of one-pot chemical reactions, listing the articles whose authors succeeded in creating a symbiosis between catalysts and biocatalysts. It is noteworthy that this literature was very difficult to find since in many publications the discussion session was unclear concerning the mode used, mainly due to different meanings, forcing to browse in the supplementary data to unambiguously decide whether a given process proceeded via a concurrent mode or not. Moreover, articles without any mentioned incompatibilities will not be included in this review. Finally, articles related to hybrid catalysis involving dynamic kinetic resolution (DKR) or re-generation of co-substrates have not been addressed even though some elegant examples could be found in the literature,^[11–13] focusing instead on multi-stage synthesis processes, precisely on chemo-enzymatic linear cascades, leading to molecular complexity towards high added-value products. In this context, solvent, pH, temperature incompatibilities have been dealt as well as catalysts inactivation phenomena.

Dr. Christine Guérard-Hélaïne received her PhD degree in 1999 from the Clermont-Ferrand university (France) under the supervision of Prof. Bolte. In 1999, she obtained a postdoctoral position at university Mc Gill (Canada) in Pr. Kazlauskas's group. In 2000, she was recruited as assistant professor at Paris VI university. Then she moved in 2008 to the Clermont-Ferrand university after 5 years of childcare obligations. Her research focuses



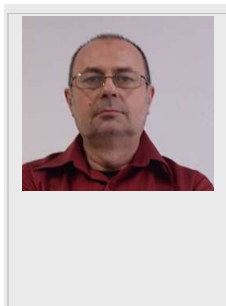
CONCEPT

on the development of enzymatic methods for the preparation of high added value chiral compounds, sometimes involving chemical catalysts (hybrid catalysis). She is also interested in the research of new enzymatic activities via enzyme discovery.

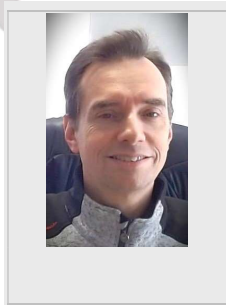
Claude Forano is a full professor at the University Clermont Auvergne and is a full researcher at the Institute of Chemistry of Clermont-Ferrand. He completed his thesis on H⁺ conductors characterized by combined H⁺ conductivity and solid-state NMR at the University Blaise Pascal in 1987. He did a postdoctorate at the University of Exeter on SS-NMR and was invited as a full professor at the University of Ottawa (Canada) in 2010-2011. His current research activities concern the synthesis, characterizations, properties and reactivities of Layered Double Hydroxide materials and their hybrid and biohybrid derivatives for applications in the field of environmental cleaning processes, bio-, photo-, electro-, catalysis and sustainable chemical processes.



Arnaud Gautier obtained his PhD in 1995 and pursued postdoctoral training at Stanford University. In 1997, he joined Professor Istvan Marko's team at the Université Catholique de Louvain, and later became a CNRS member (IRCOF, Rouen, France, 1999) in Professor Serge Piettre's group. In 2005, he relocated to the Université Clermont-Auvergne. His current research interests include the synthesis of metal-carbenes and their application in click chemistry, as well as green processes.

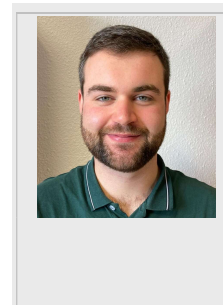


Dr.-Ing. **Virgil Hélaine** is currently lecturer at the Institute for Organic Chemistry at UCA (France). After receiving his PhD in 1999, he joined prof. W.-D. Fessner's group in TU Darmstadt for a postdoctoral internship to work on the development of enzymatic screening assays. Nowadays, his interest in biocatalysts is diverse but mainly focused on enzymatic or hybrid cascade synthesis methodologies involving new enzymes from biodiversity, to offer novel biocatalytic tools for the chemist's portfolio.



Dr. **Cédric Gastaldi** studied chemistry at the University of Clermont-Ferrand where he graduated in 2023, recently completing his Ph.D studies under the supervision of Dr. C. Guérard-Hélaine and Prof. C. Forano working in the

design of concurrent cascades combining enzymes and organometallic catalysts. His main research includes the combination of biocatalysis with metal-catalyzed transformations to develop new synthetic routes and new ways to make these different kinds of catalysts compatible.



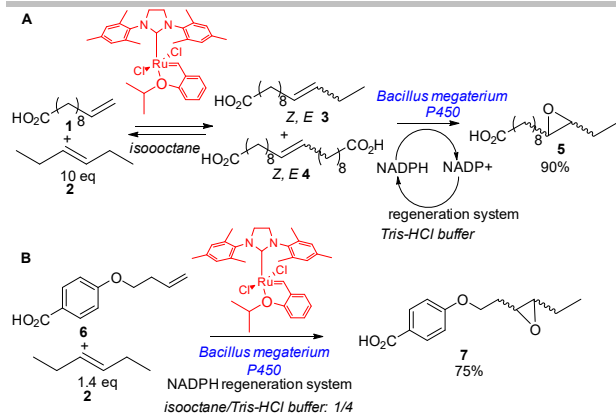
2. Incompatibilities due to the solvent

Using the same solvent to work in conjunction with a chemical catalyst and a biocatalyst is a recurring problem. Indeed, except in a few cases, biocatalysts are often comfortable in water whereas catalysts prefer to work in organic solvents. This issue has already been addressed in a review published in 2020,^[14] but no clear distinction was made between hybrid catalysis in concurrent or sequential mode. Thus, some of these references, relating to the concurrent mode, will be mentioned here, along with more recent ones. They all reveal that solvent incompatibilities have been resolved using three different strategies: the setting-up of i) biphasic systems, ii) deep eutectic solvent (DES) or iii) supercritical CO₂ (sCO₂) solvent.

2.1. Biphasic systems

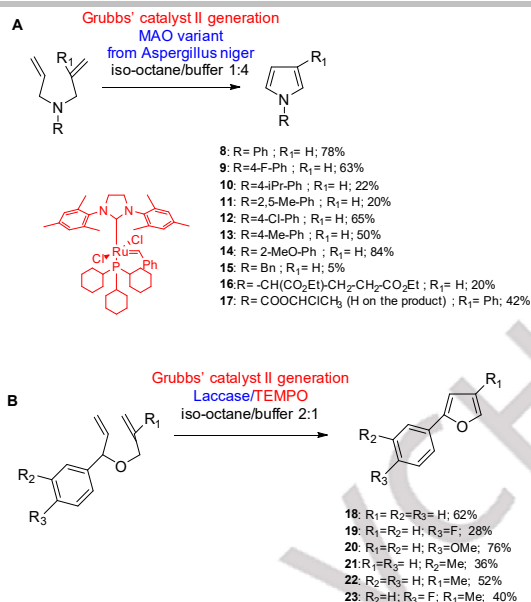
Most of the time, reagents or intermediates exhibit a poor solubility in an aqueous medium often required for the enzyme, and, in addition, can display toxic effects on the biocatalyst. One answer is to build a biphasic water/solvent system with non-miscible liquid phases, ensuring a minimal contact between the biocatalyst and the organic chemical. Regarding the catalyst, this system can be suitable provided that it is not sensitive to air or water. This strategy has been applied for the achievement of a cross-metathesis reaction followed by an epoxidation reaction by combining an organometallic catalyst and a metalloenzyme, respectively.^[15] In a mixture of isooctane and buffered water, the first step was facilitated in isooctane and led to a dynamic equilibration of several alkenes (Scheme 1A): the products of the reactions, from cross- (**3**) and self-metathesis (**4**) as well as the remaining starting alkenes (**1** and **2**). To evolve to a single product, a highly selective P450 from *Bacillus megaterium* was chosen for being five-fold more active towards epoxidation on C13 substrate (**3**) than on C11 one (**1**), without any hydroxylation by-products, whereas a large excess of **2** has been used to avoid self-reaction of **1**. The Hoveyda-Grubbs Ru catalyst, coupled to the P450 and a phosphite dehydrogenase NADPH regenerating system, is a smart example to illustrate the strength of the concurrent mode, since it has enabled to obtain a 90% yield, unfeasible in a sequencing mode due to the equilibrated first step. This work was also successfully extended to another substrate **6** (Scheme 1B) giving the expected product **7** in 75% yield.

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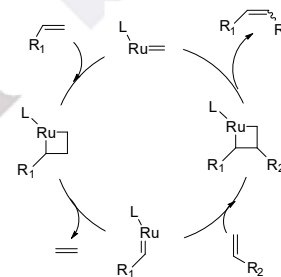


Scheme 1. Cross-metathesis and epoxidation reactions using a mixture of iso-octane and buffered-water as solvents.

The same biphasic system, involving iso-octane/potassium phosphate buffer: 4/1 was also considered,^[16] but, this time, to solve a problem of inhibition of an oxidase by a Grubbs catalyst for pyrroles synthesis. When using several co-solvents in water (DMF, acetone, DMSO, DCM), only the first step of ring closing metathesis was successful, but not the second one involving a monoamino oxidase (MAO) for aromatization to pyrroles derivatives (scheme 2A). An explanation could be found in the mechanism of the metathesis reaction that involve several [2+2] cycloadditions leading to transition metal alkylidenes not particularly crowded for some of them (Scheme 3). The monoamine oxidase from *Aspergillus niger* (uniport.org P46882), containing 15 histidines and 10 cysteines, none of them forming a disulfide bridge, probably interacted with the intermediates mentioned above or directly with the catalyst itself. The biphasic system enabled to compartmentalize the homogeneous catalyst in the organic phase and the enzyme in the buffer (suspension of whole cells), leading to the preparation of ten pyrroles derivatives **8-17** in 5-84 % yields.



Scheme 2. Metathesis—aromatization cascade using a mixture of iso-octane and buffer as solvents



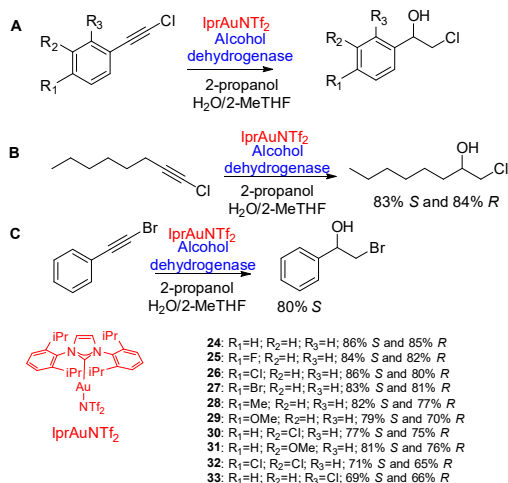
Scheme 3. reminder of the mechanism of the metathesis reaction.

The authors extended this concept to the chemo-enzymatic synthesis of a variety of furan derivatives via a laccase/TEMPO-catalyzed aromatization step (Scheme 2B).^[17] The best solvent found for the laccase-catalyzed reaction (sodium phosphate buffer/MTBE: 1/2) proved to be unsuitable for the metathesis reaction. Again, using iso-octane instead of MTBE, the cascade reaction could be implemented leading to furans **18-23** in good to moderate 28-76% yields.

Recently, hybrid catalysis in concurrent mode was also proposed by coupling an alkyne hydration, catalyzed by a gold *N*-heterocyclic carbene (NHC), to a dehydrogenase in charge of reducing the ketone previously formed^[18] in a biphasic medium composed of water and methyl-THF, this latter allowing to solubilize the aryl-substituted haloalkyne reagent (Scheme 4). A series of aryl- or alkyl-, bromo- or chloro-hydrins **24-33** were prepared in 65-86% yields, as both separate enantiomers, with remarkable enantioselectivities, thanks to stereocomplementary dehydrogenases from the market or home-made overexpressed in *E. coli*. It is noteworthy that this last biocatalysed step was possible using only carbonyl compounds with an electron withdrawing group at the alpha position such as a halogen atom. Indeed, in that case, this activated carbonyl substrate did not

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require adding a large excess of 2-propanol, used in the process as reducing agent for regenerating NADH enzyme cofactor, to shift the equilibrium. Actually, when higher amounts of alcohol were used, larger quantities of by-products were obtained (vinyl ether derivatives for instance). In the same way, the reaction required a catalyst of high reactivity at room temperature, i.e. not a chlorinated complex as seen previously, but a complex with a weaker coordinating anion (triflimide, BF_4^- , PF_6^-).^[19]



Scheme 4. Chemo-catalysed hydration reaction followed by enzymatically assisted reduction of the ketone in a water/ether biphasic system.

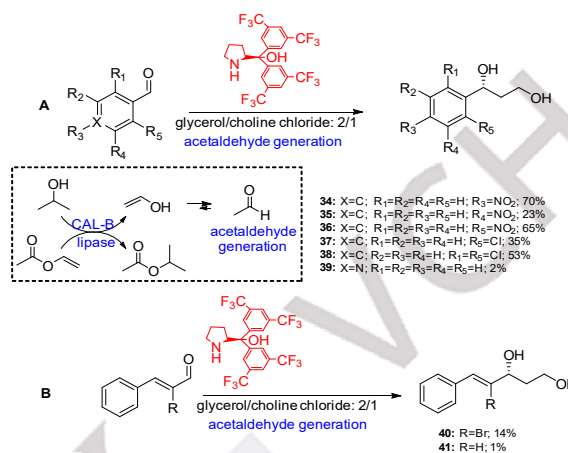
Unfortunately, a biphasic system cannot be the universal answer for hybrid catalysis in concurrent mode, because a fragile enzyme can for instance be deactivated by the organic solvent at the interface with the aqueous medium. Furthermore, a heterogeneous medium remains kinetically unfavorable due to the time required for species to move from one phase to another.

2.2. Deep eutectic solvents

As mentioned above, the solvent of choice for enzymes remains water, but most of the substrates used are rather hydrophobic resulting in their low solubility in an aqueous environment. Therefore, biocatalysis in non-aqueous media has emerged, and, over the past two decades, ionic liquids in particular has gained attention.^[20] However, due to their poor degradability along with high costs, deep eutectic solvents (DES) appeared more recently as a must-have alternative.^[21] They are easily prepared by mixing a weak hydrogen bond donor with a weak hydrogen bond acceptor, resulting in a liquid at ambient temperature, thanks to the intermolecular hydrogen bonds network. They are also qualified as nontoxic, less flammable, nonvolatile and even sometimes biodegradable. Thus, enzymes as diverse as lipases, glucosidases, catalases, haloalkane dehalogenases, epoxide hydrolases, benzaldehyde lyases, peroxidases, proteases etc... successfully converted several substrates in such non-conventional solvents.^[22]

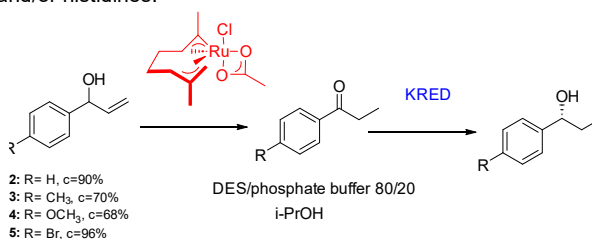
The first example of hybrid catalysis in concurrent mode in DES was published by Dominguez de Maria's group in 2014.^[23] In this paper, acetaldehyde, known to be difficult to handle due to its volatility and toxicity, was *in situ* generated from vinylacetate by the lipase B from *Candida antarctica* (CAL-B) and directly

engaged in a highly selective aldolisation reaction, on various aromatic (scheme 5A) or olefinic (scheme 5B) aldehydes as electrophiles, catalyzed by a proline-based organocatalyst. By using a DES composed of choline chloride and glycerol (1:2 mol ratio), the corresponding aldols **34-41** were prepared with yields ranging from 1 to 70%. To note, the process was not viable without a hydrogen bond donor organocatalyst promoting its solubilization in the DES.



Scheme 5. glycerol/choline chloride DES for achieving a transesterification and a subsequent aldolisation reaction.

Later on, the team of González-Sabín proposed a cascade consisting in a double bond isomerization of various allylic alcohols into their corresponding ketones, catalyzed by a Ru complex catalyst, (Scheme 6) followed by an enantioselective reduction of the carbonyl moiety into the alcohol thanks to a ketoreductase (KRED).^[24] Special attention was paid to the solvent, as only a DES/phosphate buffer mixture gave complete satisfaction (DES = choline chloride and glycerol, 1:2), and interestingly, increasing the proportion of DES increased the enantioselectivity of KRED, enabling to obtain an enantiomeric excess in alcohol up to 99%. Four substrates **2-5** have been investigated in this process with conversion rates from 68 to 96%, but the yields were not given, the products not being isolated. To note, the Ru catalyst is not poisoned by the biocatalyst cysteines and/or histidines.



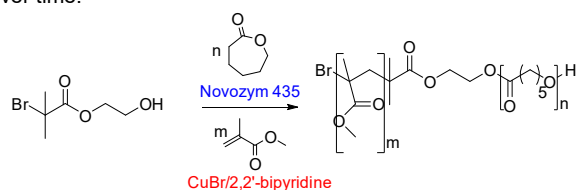
Scheme 6. Ternary solvent (choline chloride/glycerol/phosphate buffer) as the answer in the optimization of an isomerization-reduction cascade.

2.3. Supercritical carbon dioxide (sCO₂)

The last possibility for finding a common solvent for a catalyst and a biocatalyst is the use of supercritical CO₂ (sCO₂). It is an

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environmentally friendly inexpensive, non-toxic, inert and non-flammable fluid. In addition, its critical parameters are easily accessible, i.e. a temperature of 31°C along with a pressure of 7.38 MPa.^[25] A convincing demonstration of a simultaneous one-pot combination of an enzyme and a catalyst has emerged for the formation of block copolymers, in sCO₂.^[26] Thus, a bifunctional initiator was used to build, on its first functionalized site, the first block of the polymer by lipase-assisted (Novozym-435) ring-opening polymerization of ϵ -caprolactone, and, on its second functionalized site, the second block by involving methylmethacrylate (MMA) through a metal-catalyzed atom transfer radical polymerization (Scheme 7). The process was feasible provided that caprolactone was used as both substrate and co-solvent. Indeed, if the caprolactone content was less than 30% volume in sCO₂, formed PMMA precipitated rapidly from the sCO₂ solution causing a complete loss of molecular weight control over time.

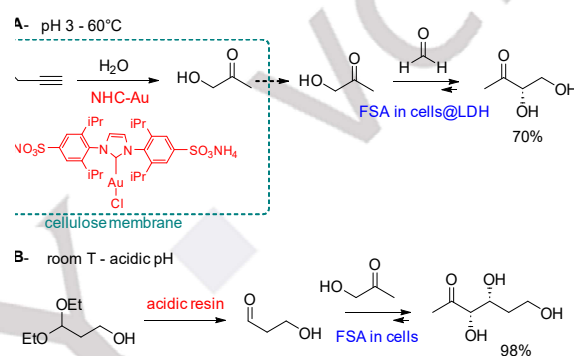


Scheme 7. sCO₂ as a cosolvent with one of the substrates to prepare a copolymer block.

3. Incompatibilities due to the pH

Ongoing research in catalysis has led to the development of several chemical catalysts able to operate in mild conditions i.e. at a neutral pH and at room temperature. These conditions being ideal for enzymes, hybrid catalysis could then be easily implemented. Unfortunately, most of the chemical catalysts require more drastic conditions such as a very acidic pH (pH<4.0), unfavorable to maintain enzyme activity and stability. To circumvent this drawback, sequential processes were applied where conditions were changed between steps, or the second catalyst was added at the end of the previous reaction. To the best of our knowledge, only one example of hybrid catalysis in concurrent mode was successful, and concerned a catalyst, requiring a pH below 3 to work properly (due to the required protodeauration step in the catalytic cycle), that was brought together with an enzyme with an optimum pH of 8. More precisely, a *N*-heterocyclic (NHC) gold complex (Scheme 8A), in charge of the hydration of an alkyne into a ketone, was mixed with fructose-6-phosphate aldolase (FSA), responsible for the subsequent conversion of the ketone into a chiral aldol.^[27] To protect FSA from this low pH, it was kept encapsulated in *E. coli* cells, since it is known that several microorganisms can survive in harsh conditions, maintaining their internal pH close to neutrality.^[28] Unfortunately, even though the aldolization can occur at pH 3 thanks to the cells, two problems arose that needed to be solved i) the NHC gold complex was inactivated in contact of the cell membrane (probably due to the presence of thiols), and ii) the high temperature (60°C) required to achieve the catalyst-assisted hydration reaction caused cells permeabilization, destroying FSA protection. The former was solved introducing another membrane,

in cellulose, for separating cells from the catalyst, whereas the latter was overcome by trapping the FSA-harboring cells inside a clay-like material, namely a LiAl₂-CO₃ layered double hydroxide (LDH) phase. In these conditions, the final monosaccharide was obtained in 70% yield. The authors illustrated that this methodology could be generalized to other systems where acidic pH was an issue, for instance the *in-situ* generation of an aldehyde, via an acid catalyzed deprotection of an acetal, substrate for FSA in a subsequent aldolisation reaction (Scheme 8B). Indeed, aldehydes are generally unstable, and need to be converted rapidly. This is the reason why they are often available as acetals in the market. Thus, a high-added value aldol (with two asymmetrical centers of controlled stereochemistry) was obtained in 98% yield to validate the concept leading to a biocatalysis step at acidic pH.

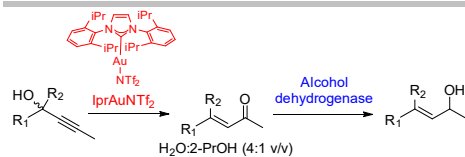


Scheme 8. Process based on several compartmentalizations to make compatible a catalyst and a biocatalyst to both temperature and pH, that could be generalized.

4. Incompatibilities due to the temperature

In some cases, experimental conditions such as temperature may be difficult to find to make catalyst and biocatalyst compatibles, in the same pot and at the same time. One option, as seen previously, could be to choose the best temperature for one catalyst and to protect the other in some way. Another possibility is to design each catalyst so that they can work at a common temperature. This was done in a process consisting of a Meyer-Schuster rearrangement, catalyzed by an *N*-Heterocyclic Carbene Gold(I) complex coupled with a bio-reduction step catalyzed by a dehydrogenase (Scheme 9).^[29] The authors tested several commercial ligands and screened 23 dehydrogenases to get access to the best conversions and complementary stereoselectivity of the chiral secondary alcohol formed from various racemic tertiary alcohols, at a temperature below 40°C. Thus, IPrAuNTf₂ complex and two stereocomplementary dehydrogenases were selected leading to the formation of 30 optically active allylic alcohols **46-60** in 65-86% isolated yields, and 96 to 99% ee for both configurations.

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- 46: R₁=Ph; R₂=Me; 70% S and 78% R
 47: R₁=4-fluorophenyl; R₂=Me; 70% S and 74% R
 48: R₁=4-chlorophenyl; R₂=Me; 65% S and 73% R
 49: R₁=4-bromophenyl; R₂=Me; 70% S and 71% R
 50: R₁=3-fluorophenyl; R₂=Me; 78% S and 80% R
 51: R₁=2-fluorophenyl; R₂=Me; 38% S and 77% R
 52: R₁=4-nitrophenyl; R₂=Me; 80% S and 70% R
 53: R₁=3-nitrophenyl; R₂=Me; 72% S and 86% R
 54: R₁=4-trifluoromethylphenyl; R₂=Me; 72% S and 76% R
 55: R₁=3-trifluoromethylphenyl; R₂=Me; 51% S and 78% R
 56: R₁=2-thienyl; R₂=Me; 69% S and 74% R
 57: R₁=3-thienyl; R₂=Me; 37% S and 73% R
 58: R₁=tBu; R₂=Me; 43% S and 48% R
 59: R₁=Ph; R₂=H; 62% S and 69% R
 60: R₁=Ph; R₂=Ph; 62% R

Scheme 9. Upstream design of catalysts to make them compatible with a given reaction temperature

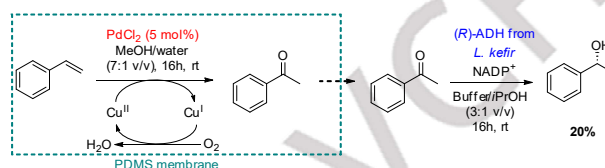
5. Incompatibilities due to unfavourable interactions between catalysts

Hybrid catalysis is composed, in one side, of an enzyme possessing a unique pattern of amino acid residues and, often in another side, of a transition metal ion such as Cu²⁺, Ni²⁺, Zn²⁺ that could bind to the histidine or cysteine side chains. Gratifyingly, the enzyme's catalytic center is usually deeply inserted into the enzyme 3D structure and thus could be hardly accessible for the catalyst, especially when the metal is bonded to a crowded ligand. Nevertheless, some interactions can be highlighted as in the well-studied example concerning *cis*-platin and glutathione where the interactions are responsible for resistance phenomena to anti-cancer therapies.^[30] These potential interactions between the transition metal and the active site of the enzyme should be taken into consideration. Indeed, the resulting S-metal bond formed is strong and should poison the catalyst. Moreover, regarding copper, metal-catalyzed oxidation of histidine and methionine could occur through the generation of reactive oxygenated species, in the presence of oxygen. Other functional groups from the side chain (acid, amine, phenol...) could also interact with the catalyst center. Thus, to answer these potential limitations, physical compartmentalization of chemical and biological catalysts is an effective strategy for hybrid catalysis in concurrent mode.^[5,9] But, the challenge is also to allow the diffusion of reagents (substrates) and products. The ideal construction would be a spatial separation of the catalysts inspired by efficient natural multienzymatic systems encountered in living cells. However, the development of biomimetic artificial cells is still in its early stages and more straightforward approaches are currently explored. Chemical catalysts (organometallic complexes, nanoparticles) and enzymes can be isolated in several ways either by a physical partitioning of two liquid phases (already illustrated before) or by confining them in an appropriate host matrix. Typically, catalysts can be embedded in inorganic host structures while enzymes may be entrapped in natural biological systems (microorganisms for instance), host proteins could be a common host. Artificial membranes can also be used as effective barriers.

5.1. Membranes

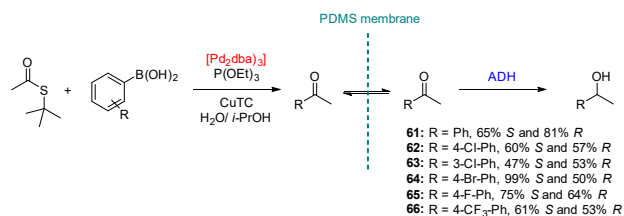
Polydimethylsiloxane (PDMS) membranes are loosely packed barriers with hydrophobic properties and a permeability that allows the diffusion of small organic molecules,^[31,32] charged

reagents being unable to diffuse. A PDMS membrane was used in the one-pot enantioselective chemoenzymatic conversion of styrene into 1-phenylethanol.^[33] The cascade reaction was based on a Wacker-Tsuji oxidation catalysed by PdCl₂/CuCl and a subsequent enzymatic reduction biocatalysed by an alcohol dehydrogenase ((*R*)-ADH). The PDMS membrane avoided enzyme inhibition by Cu²⁺ salts while acetophenone could flux through the hydrophobic membrane driven by the concentration gradient. However, the leaching of methanol, the optimized solvent for the Wacker oxidation, through the membrane, led to a low oxidation of 20% (Scheme 10). For this reason, the process was only efficiently exemplified in a sequential mode, not in a concurrent one.



Scheme 10. Wacker oxidation followed by enzymatic reduction using a membrane separating the catalysts.

A partitioning was also set up using a two chambers glass reactor separated by a PDMS membrane for the synthesis of chiral alcohols using a chemoenzymatic cascade process (Scheme 11).^[34] Again, inhibition of the biocatalyst by transition metals such as copper salts was the most important issue that had to be solved. Indeed, a Liebeskind–Srogl (L–S) coupling reaction (copper mediated, palladium catalysed C–C cross coupling of thioesters with boronic acid) using a *tert*-butylthioester and a phenylboronic acid, catalysed by (tris(dibenzylideneacetone)-dipalladium(0) and triethylphosphite along with copper-(*l*)-thiophene-2-carboxylate (CuTC) (Pd₂(dba)₃/P(OEt)₃/CuTC) produced a ketone that led to an optically pure alcohol through either a (*R*)-selective alcohol dehydrogenase (ADH) from *Lactobacillus kefir* or a (*S*)-selective ADH from *Rhodococcus ruber*. By employing a biomimetic compartmentalization approach, various desired chiral secondary alcohols **61–66** were accessible in 47–99% yield and very high enantioselectivity (99% ee).



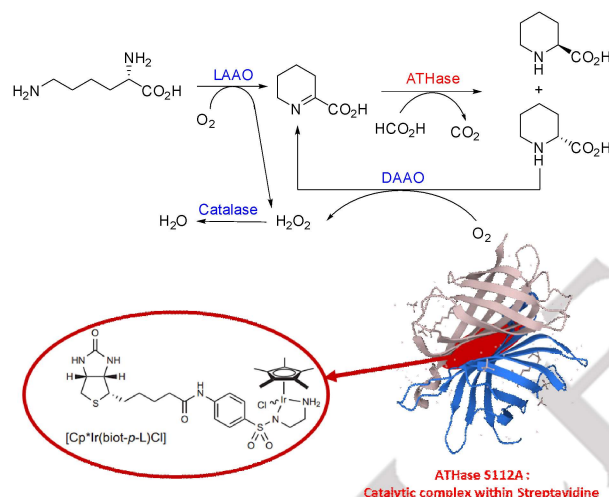
Scheme 11. chambers reactors for implementing Liebeskind–Srogl coupling reaction with a biocatalytic enantioselective reduction.

5.2. Biological systems

Proteins can be used as a common host scaffold for the compartmentalization of organometallic catalysts and metalloenzymes. V. Köhler et al.^[35] implemented an efficient

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concurrent redox orthogonal cascade by combining on one side a biotinylated organometallic transfer-hydrogenation catalyst [Cp*Ir(biotp-L)Cl] within a streptavidin, the resulting construction acting as an artificial transfer hydrogenase (ATHase) by using sodium formate as hydride source, and on the other side various amino-acid oxidases, thus, preventing their mutual inactivation. Addition of catalase to the hybrid system prevented any Ir catalyst poisoning by H₂O₂ due to the oxidases. The concept was first applied to deracemization, that is not the scope of this review, but was enlarged to the synthesis of pipercolic acid from L-lysine, thanks to L-amino acid oxidase (LAO). The enantiomeric excess of the product could be increased by adding a D-selective amino acid oxidase (DAAO), (Scheme 12) in charge to re-oxidize the D-enantiomer. Unfortunately, no yields were given since only an NMR study proved the process. In the same way, this compartmentalization was used to promote an oxyfunctionalization reaction involving a monooxygenase and the ATHase system for the regeneration of NADH.



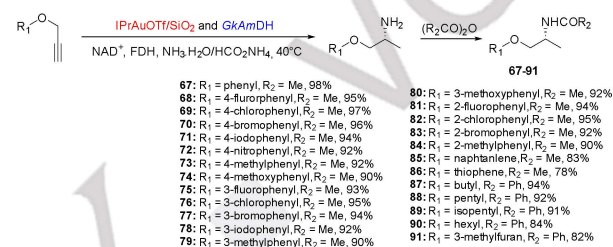
Scheme 12. Concurrent cascade reaction involving an ATHase artificial enzyme, composed of an Ir-based hydrogenation catalyst embedded in a streptavidin scaffold, with an enzymatic cocktail including L-amino acid oxidase (LAO) and catalase.

5.3. Inorganic porous structures

The confinement of chemical catalysts (organometallic complexes, metal or metal oxide nanoparticles)^[36] and biocatalysts (proteins, metalloenzymes)^[37] into porous inorganic structures has been of interest for many years, not only for the purpose of catalyst recycling, but also for their protection against physico-chemical constraints (especially pH and temperature). Nowadays, such porous structures are emerging as efficient barriers for the compartmentalization in hybrid catalysis.

Thanks to their size-sieving properties, 3D-mesoporous structures (3D-MSs), such as mesoporous silica, are good candidates for the partitioning of organometallic catalysts and enzymes. With pore sizes lower than the dimensions of enzymes, 3D-MSs could act as effective physical barriers preventing cross-interactions between the catalysts and the enzymes or even as

host structure to confined organometallic complexes or nanoparticle catalysts (metal, metal oxides, metal sulphides). Mesoporous silica based hollow shell (MS-HS) were used to compartmentalize a chemoenzymatic system.^[38] It was designed for a one-pot concurrent hydration/amination enantioselective cascade reactions to synthesize a series of 25 chiral amines from the corresponding propargyl ethers with high yields and ee ranging from 82 to 97% and 96-99% respectively (Scheme 13). To note, these data were related to the products **67-91** coming from the concurrent mode hybrid catalysis followed by a final chemical acetylation step. More precisely, in this process, an Au/carbene complex IPrAuOTf was encapsulated into MS-HS (255 nm cavity size) built on mesoporous silica walls (35 nm thick) avoiding its cross-interaction with free Gk-amino dehydrogenase (GkAmDH: an engineered thermostable phenylalanine dehydrogenase from *Geobacillus kaustophilus*) left outside.



Scheme 13. A combination of the encapsulated IPrAuOTf and GkAmDH facilitated a hydration/amination cascade to access chiral amines.

The encapsulation of a metal complex within a macromolecular structure^[39] is an appropriate strategy for chemoenzymatic catalysis. Several advantages are targeted: increased reaction speed, adjustment of chemoselectivity and regioselectivity, compatibilization of the active complex in an unfavorable medium such as water, and compartmentalization that prevents direct deleterious interactions with enzymes. To our knowledge, only one example of a one-pot hybrid catalysis in concurrent mode has involved a supramolecular coordination scaffold for encapsulation of an organometallic complex, to prevent its diffusion into the medium and consequently to react with some amino-acid residues, avoiding any enzyme inhibition.^[40] In this molecular architecture, a gold complex [Me₃PAu⁺] was embedded in a tetrahedral Ga₄L₆ (L = *N,N'*-bis(2,3-dihydroxybenzoyl)-1,5-diaminonaphthalene) cluster and combined with an hydrolase for the tandem reaction of enzymatic hydrolysis of an allenic acetate into its corresponding alcohol followed by the gold complex catalyzed cyclization to give various tetrahydrofurans **92-95** from moderate (32%) to very good yields (95%) (Scheme 14A). This process could also be applied to the synthesis of an isoxazolidine (Scheme 14B) with a 56% yield. Finally, the authors also demonstrated it could serve in an isomerization-reduction tandem reaction in which a Ru catalyst (Me₃P)CpRu(NCMe)₂⁺, encapsulated in Ga₄L₆, in charge of isomerizing 1-propenol, could be coupled to an alcohol dehydrogenase along with a FDH regenerating NADPH system, to afford propanol in 61% yield (Scheme 14C). To note all given yields were evaluated by ¹H NMR spectroscopy.

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Conflict of interest

The authors declare no conflicts of interest.

Keywords: hybrid-catalysis • enzyme • catalyst • metallocomplex • concurrent mode.

Scheme 14. Ability of a Ga₄L₆ tetrahedral supramolecular host cluster encapsulating metal guest complexes to catalyse organic reactions collaboratively with enzymes.

6. Conclusion

This paper illustrates the difficulty of getting chemical and biological catalysts, which are intrinsically very different, to work together, in the same environment. As a result, many problems have to be solved due to the numerous reaction parameters (temperature, solvent, pH, catalyst inhibitions), for which hybrid catalysis in sequential mode has proved to be the easiest answer. However, avoiding the accumulation of intermediates in a reaction cascade has many advantages, including reducing their toxicity, or their inhibition with respect to the catalysts, but also shifting equilibria to give better yields, while reducing the possible costs of potential intermediates purifications or reaction media transfers to other containers. This possibility exists via hybrid catalysis in concurrent mode, i.e. in the same pot, with all the reagents and catalysts added from the start, and without any further intervention until the end of the cascade. This is obviously more difficult to implement, but so much more efficient and therefore attractive that it is difficult to find such processes in the literature, with some publications remaining unclear about the mode used. However, effective solutions have been found, involving original solvents or two-phase systems, and ingenious ways of protecting catalysts against the various agents in the reaction mixture or against their counterparts. This is the role of this mini-review: to give the community of chemists interested in hybrid catalysis a rapid access to effective solutions depending on the incompatibilities encountered in their reaction cascade process in concurrent mode.

Acknowledgements

- [1] M. Makkee, A. P. G. Kieboom, H. V. Bekkum, J. A. Roels, *J. Chem. Soc., Chem. Commun.* **1980**, 930–931.
- [2] L. Z. Hessefort, L. J. Harstad, K. R. Merker, L. P. T. Ramos, K. F. Biegasiewicz, *ChemBioChem* **2023**, *24*, e202300334.
- [3] E. Heuson, R. Froidevaux, I. Itabaiana, R. Wojcieszak, M. Capron, F. Dumeignil, *Green Chem.* **2021**, *23*, 1942–1954.
- [4] N. Losada-Garcia, Z. Cabrera, P. Urrutia, C. Garcia-Sanz, A. Andreu, J. M. Palomo, *Catalysts* **2020**, *10*, 1258.
- [5] S. Schmidt, K. Castiglione, R. Kourist, *Chemistry – A European Journal* **2018**, *24*, 1755–1768.
- [6] H. Gröger, W. Hummel, *Current Opinion in Chemical Biology* **2014**, *19*, 171–179.
- [7] C. A. Denard, J. F. Hartwig, H. Zhao, *ACS Catal.* **2013**, *3*, 2856–2864.
- [8] A. D. Clayton, R. Labes, A. J. Blacker, *Current Opinion in Green and Sustainable Chemistry* **2020**, *26*, 100378.
- [9] D. Kracher, R. Kourist, *Current Opinion in Green and Sustainable Chemistry* **2021**, *32*, 100538.
- [10] Y. Liu, P. Liu, S. Gao, Z. Wang, P. Luan, J. González-Sabin, Y. Jiang, *Chemical Engineering Journal* **2021**, *420*, 127659.
- [11] M. J. Takle, B. J. Deadman, K. Hellgardt, J. Dickhaut, A. Wieja, K. K. M. Hii, *ACS Catal.* **2023**, *13*, 10541–10546.
- [12] R. M. Haak, F. Berthiol, T. Jerphagnon, A. J. A. Gayet, C. Tarabiono, C. P. Postema, V. Ritleng, M. Pfeffer, D. B. Janssen, A. J. Minnaard, B. L. Feringa, J. G. De Vries, *J. Am. Chem. Soc.* **2008**, *130*, 13508–13509.
- [13] S. Horino, T. Nishio, S. Kawanishi, S. Oki, K. Nishihara, T. Ikawa, K. Kanomata, K. Wagner, H. Gröger, S. Akai, *Chemistry A European J* **2022**, *28*, e202202437.
- [14] R. Kourist, J. González-Sabin, *ChemCatChem* **2020**, *12*, 1903–1912.
- [15] C. A. Denard, H. Huang, M. J. Bartlett, L. Lu, Y. Tan, H. Zhao, J. F. Hartwig, *Angewandte Chemie International Edition* **2014**, *53*, 465–469.
- [16] N. Scalacci, G. W. Black, G. Mattedi, N. L. Brown, N. J. Turner, D. Castagnolo, *ACS Catal.* **2017**, *7*, 1295–1300.
- [17] C. Risi, F. Zhao, D. Castagnolo, *ACS Catal.* **2019**, *9*, 7264–7269.
- [18] S. González-Granda, L. Escot, I. Lavandera, V. Gotor-Fernández, *ACS Catal.* **2022**, *12*, 2552–2560.
- [19] R. Dorel, A. M. Echavarren, *Chem. Rev.* **2015**, *115*, 9028–9072.
- [20] F. Van Rantwijk, R. A. Sheldon, *Chem. Rev.* **2007**, *107*, 2757–2785.
- [21] J. T. Gorke, F. Srieenc, R. J. Kazlauskas, *Chem. Commun.* **2008**, 1235–1237.
- [22] M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann, *Trends in Biotechnology* **2019**, *37*, 943–959.
- [23] C. R. Müller, I. Meiners, P. D. de Maria, *RSC Adv.* **2014**, *4*, 46097–46101.
- [24] L. Cicco, N. Ríos-Lombardía, M. J. Rodríguez-Álvarez, F. Moris, F. M. Perna, V. Capriati, J. García-Álvarez, J. González-Sabin, *Green Chem.* **2018**, *20*, 3468–3475.
- [25] A. J. Mesiano, E. J. Beckman, A. J. Russell, *Chem. Rev.* **1999**, *99*, 623–634.
- [26] C. J. Duxbury, W. Wang, M. de Geus, A. Heise, S. M. Howdle, *J. Am. Chem. Soc.* **2005**, *127*, 2384–2385.
- [27] C. Gastaldi, V. Hélaine, M. Joly, A. Gautier, C. Forano, C. Guérard-Hélaine, *Catal. Sci. Technol.* **2023**, *13*, 1623–1627.
- [28] H. Richard, J. W. Foster, *J. Bacteriol.* **2004**, *186*, 6032–6041.
- [29] S. González-Granda, I. Lavandera, V. Gotor-Fernández, *Angew. Chem. Int. Ed.* **2021**, *60*, 13945–13951.
- [30] L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, I. Martins, O. Kepp, M. Castedo, G. Kroemer, *Oncogene* **2012**, *31*, 1869–1883.
- [31] A. Ahmad, S.-H. Li, Z.-P. Zhao, *Journal of Membrane Science* **2021**, *620*, 118863.
- [32] S. Armaković, D. Vujić, B. Brkić, *Journal of Molecular Liquids* **2022**, *351*, 118657.
- [33] H. Sato, W. Hummel, H. Gröger, *Angew Chem Int Ed* **2015**, *54*, 4488–4492.
- [34] P. Schaaf, T. Bayer, M. Koley, M. Schnürch, U. T. Bornscheuer, F. Rudroff, M. D. Mihovilovic, *Chem. Commun.* **2018**, *54*, 12978–12981.
- [35] V. Köhler, Y. M. Wilson, M. Dürrenberger, D. Ghislieri, E. Churakova, T. Quinto, L. Knörr, D. Häussinger, F. Hollmann, N. J. Turner, T. R. Ward, *Nature Chemistry* **2013**, *5*, 93–99.
- [36] Q. Yang, Q. Xu, H.-L. Jiang, *Chem. Soc. Rev.* **2017**, *46*, 4774–4808.
- [37] Z. Zhou, M. Hartmann, *Chem. Soc. Rev.* **2013**, *42*, 3894.
- [38] F. Chang, C. Wang, Q. Chen, Y. Zhang, G. Liu, *Angew Chem Int Ed* **2022**, *61*, DOI 10.1002/anie.202114809.

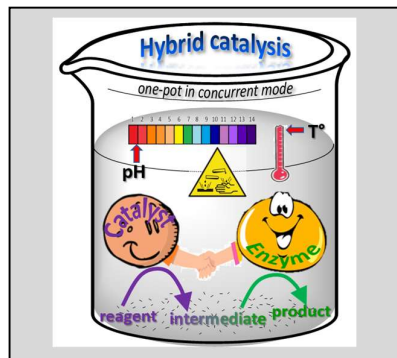
CONCEPT

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- [39] M. J. Wiester, P. A. Ulmann, C. A. Mirkin, *Angew Chem Int Ed* **2011**, *50*, 114–137.
- [40] Z. J. Wang, K. N. Clary, R. G. Bergman, K. N. Raymond, F. D. Toste, *Nature Chem* **2013**, *5*, 100–103.

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Entry for the Table of Contents



This concept covers papers describing how catalysts and biocatalysts can work together under common experimental conditions, without interfering with each other, in the presence of all the reagents from the start of a one-pot hybrid catalysis process, i.e. in concurrent mode.