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Organocatalysis in aqueous media

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Abstract

While enzymes – nature's catalysts - are the cornerstones of all living systems, the introduction of artificial catalytic systems in biology has been challenging. In this review, we critically evaluate if organocatalysis could be a future tool to selectively access new chemical transformations and provide new possibilities for chemical biology and possibly biomedicine. Organocatalysts are well-suited for modification and design, since compared to enzymes and metal-based catalysts they are simple, often less toxic and widely accessible. Divided by activation mechanisms, we structure and extensively discuss organocatalytic literature examples in aqueous media and compare organocatalysis to enzymatic catalysis. The specific organocatalysed reactions are evaluated for their *biological compatibility* and *in vivo* applicability. We establish the boundary conditions for organocatalysis to work in such environments and subsequently highlight promising organocatalytic reaction candidates for biological settings. Overall, catalyst characteristics (functionalities, pK_a) and reaction engineering (catalytic intermediate microenvironment) are key in the development of efficient organocatalysis in aqueous media, which shows much resemblance with enzymatic catalysis. So far, this rapidly evolving field has only a limited set of organocatalytic reactions with biological potential. Still, based on recent developments we expect a bright future for organocatalysis in biological settings, enriching the field of chemical biology and possibly biomedicine.

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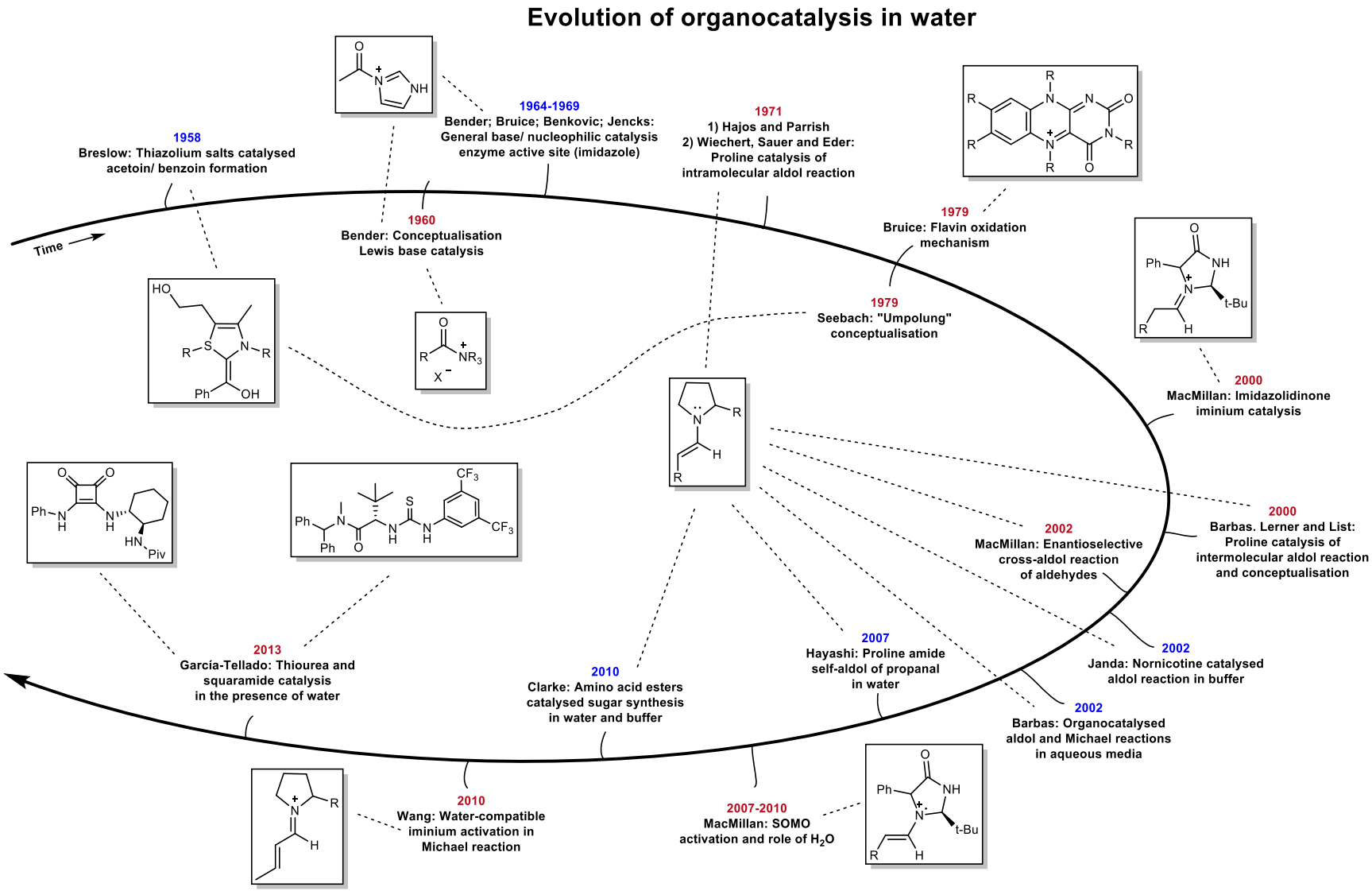
Enzymes – nature's catalysts - are crucial parts of living systems. By definition a catalyst accelerates the progression of a chemical reaction towards equilibrium without being noticeably consumed and hence does not alter the thermodynamics of the overall process.[1] Biocatalysis and transition metal catalysis have seen widespread use under biologically relevant conditions.[2-9] In contrast, the application of organocatalysis in biological systems is still in its infancy. Organocatalysis is a powerful tool to selectively access new chemical transformations, opening up new opportunities in chemical biology. There, for example, by constructing protein-bioconjugates we can gain better understanding of the biochemistry of the cell and eventually come closer to the creation of *de novo* life. Organocatalysts are well-suited for this task, since compared to enzymes and metal-based catalysts they are simple, often less toxic, widely accessible and easily designed and modified. Organocatalysts can even be considered minimalistic biocatalysts, since both structural functionalities - e.g. amino acids or co-factors – as well as catalytic mechanisms can be similar. Nonetheless, where biocatalysed reactions commonly proceed in aqueous environments, performing organocatalytic reactions in water remains a considerable challenge.[10-13] Major drawbacks for using water - a solvent with a high surface tension, polarity and hydrogen bonding ability - are the insolubility of most organic compounds, the hydrolytic stability of chemical compounds and catalytic intermediates and destabilisation of transition states by disruption of hydrogen bonds.[11,14,15] Despite these challenges, organocatalysis in aqueous media has made considerable progress over the years, with landmark developments provided as a timeline in BOX 1. The incentive for this review is to evaluate the potential of organocatalysis in biological settings and to structure the literature on organocatalysis in water. We anticipate to complement existing reviews[11-13,16-20] by establishing a comprehensive overview of aqueous phase organocatalysis, discussing potentially interesting organocatalytic bond-making and breaking reactions for biological settings. Each organocatalytic activation mode will be discussed regarding mechanism, history, noteworthy examples, and where relevant a comparison with enzymatic catalysis. Next, these reactions are evaluated for their *biocompatibility* and *in vivo* applicability*,* according to pre-defined boundary conditions for implementing promising candidates in biological environments. Finally, we discuss were the application of organocatalysis in biology may be headed. In this review, we limit the discussion to reactions where all participating reactants, co-solvents, reagents and formed products are dissolved homogeneously in water or buffer as the major solvent. In addition, whereas for biocatalysts and transition metal catalysts loadings are typically far below stoichiometric, for organocatalyst loadings differ largely on a case to case basis. Hence, catalyst loadings will be critically evaluated throughout this review. Altogether, organocatalysis in aqueous and even biological environments can lead to many important applications, ranging from smart materials (e.g. soft robotics or self-healing materials) to more biomedically relevant applications (e.g. controlled drug-delivery systems or on demand drug synthesis in a tumour cell).

Organocatalytic activation

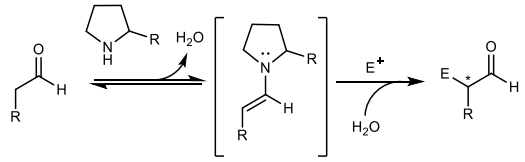
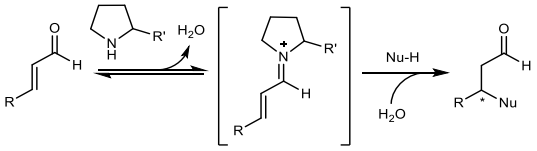
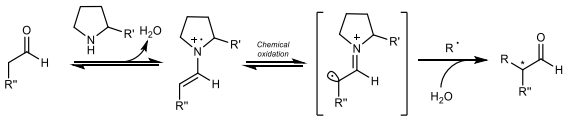
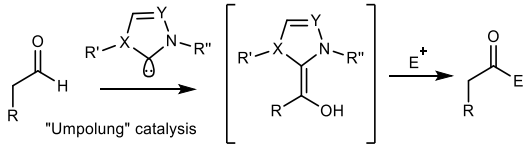
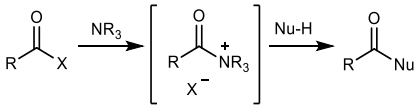
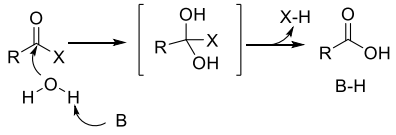
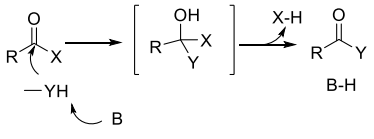
Popularized by seminal studies from Barbas, List and MacMillan, organocatalysis has evolved as a third strategy in asymmetric catalysis, next to transition metal and enzymatic catalysis.[21-23] In the current review, we go beyond asymmetric catalysis and include any bond breaking and forming transformations. According to Melchiorre, building on work by MacMillan, the majority of enantioselective organocatalytic reactions operate through eight covalent and non-covalent activation modes (BOX 2).[24,25] In covalent activation the catalyst forms a covalent intermediate with the substrate. In the aqueous phase, enamine and iminium activation have received major attention, mostly focussing on the aldol reaction.[26,27] Other activation modes, not often discussed for asymmetric

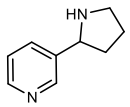
catalysis but important in a water context, are nucleophilic, general and specific acid or base catalysis. In non-covalent activation, the catalyst accelerates the reaction through non-covalent binding to a substrate. Although there are many non-covalent activation modes, these, as well as SOMO activation, will not be discussed in this review, since examples in aqueous media are scarce, likely resulting from the competitive nature of the solvent. Supramolecular catalysis in water was recently reviewed[28] and will thus not be discussed here either.



Box 1: Timeline of organocatalysis in water: highlighting key contributions to the research area. Red years refer to conceptual contributions or organocatalysed reactions in (the presence of) water/ organic solvent mixtures. Blue years signify organocatalytic reactions in water or buffer medium.

Box 2: Covalent-based organocatalysis in aqueous media structured by categories, mechanisms, reactions and catalysts.

Category	Catalyst	Covalent-based organocatalytic reaction mechanisms	Example reaction variants	Table 1 ref.
Enamine Catalysis	1	 **[Enamine – HOMO activation]**	Aldol reaction Michael reaction	1-7 8,9
Iminium Catalysis	1,2	 **[Iminium – LUMO activation]**	Michael reaction	10
SOMO Catalysis	2	 **[Radical cation – SOMO activation]**	No examples in water	
N-hetero-cyclic carbene Catalysis	3	 **[Breslow intermediate]**	Enal coupling Benzoin condensation	21 22
Nucleo-philic Catalysis	4,5,6	 **[Lewis base complex]**	Ester hydrolysis Morita-Baylis-Hillman reaction Acetylation Substitution Hydrazone/ oxime formation Native chemical ligation	11 12,15 13 14 16,17 18
General Base Catalysis	4,5,6	 **[Tetrahedral Intermediate]**	Knoevenagel reaction Multi-component reaction for azapyrrolizidine synthesis	19 20
Specific Base Catalysis		 **[Tetrahedral Intermediate]**		
Catalysts				



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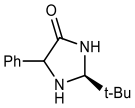

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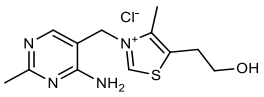

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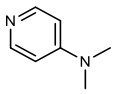

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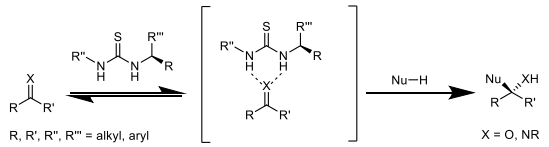
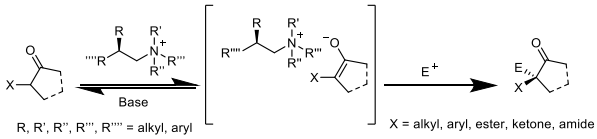
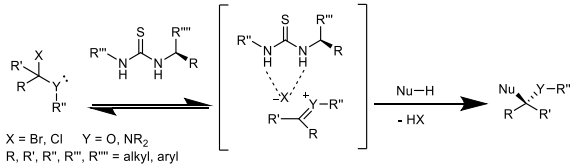
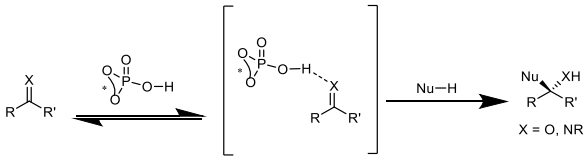
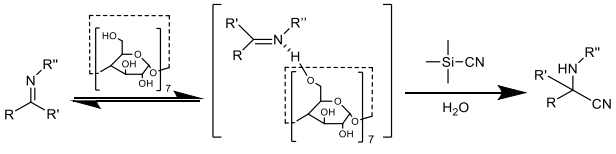
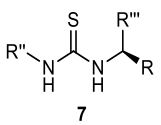
Nornicotine	Imidazolidinone	Thiamine	DABCO	Pyridine	DMAP

Box 2: Non-covalent-based organocatalysis in aqueous media structured by categories, mechanisms reactions and catalysts.

Category	Catalyst	Non - Covalent-based organocatalytic reaction mechanisms	Examples of reaction variants	Table 1 ref.
Hydrogen – Bonding Catalysis	7	 R, R', R'', R''' = alkyl, aryl **[LUMO activation]** X = O, NR	Knoevenagel reaction	25
Phase transfer Catalysis	8	 R, R', R'', R''', R'''' = alkyl, aryl **[Chiral – ion Pair]** X = alkyl, aryl, ester, ketone, amide	No examples in water	
Anion Binding Catalysis	7	 X = Br, Cl Y = O, NR_2 R, R', R'', R''', R'''' = alkyl, aryl **[LUMO activation]**	No examples in water	
Brønsted Acid Catalysis	9	 **[LUMO activation]** X = O, NR	Mannich reaction Michael type Friedel-Crafts addition	23 24
Supra-Molecular Catalysis	10, 11	 **[Host- guest complex]**	See ref.[28]	

Catalysts



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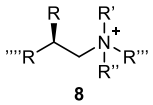

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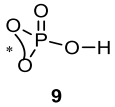

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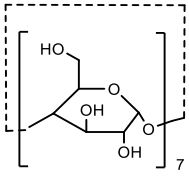

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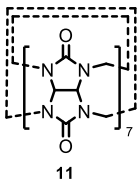
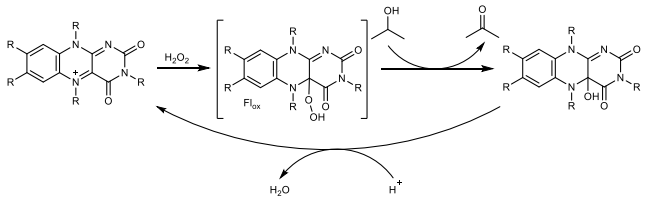
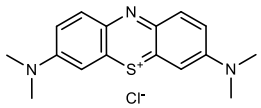
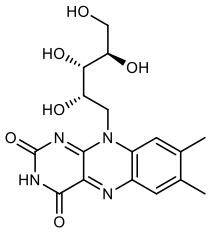


11

Thiourea	Ammonium ion	Brønsted acid	Beta-cyclodextrin (β-CD)	Cucurbit[*7*]uril (CB[*7*])

Box 2: Miscellaneous organocatalysis in aqueous media structured into categories, mechanisms, example reactions and typical catalysts.

Category	Catalyst	Miscellaneous organocatalytic reaction mechanisms	Examples of reaction variants	Table 1 Ref.
Oxidation or Reduction Catalysis	12, 13		Alkene reduction Sulfoxidation	26 27
		[Flavin hydroperoxide intermediate]		
Catalysts				
		 **12**	 **13**	
		Methylene blue	Riboflavin	

Covalent activation

Enamine catalysis

Enamine catalysis refers to electrophilic substitution reactions via the activation of carbonyl compounds by primary or secondary amines, generating enamine intermediates (via deprotonation of carbinolamine and/or an iminium ion).[29,30] Subsequently, the intermediates can react with various electrophiles or undergo pericyclic reactions.[29] The concept has its fundamental roots, amongst others[31] in the biological approach to carbon-carbon bond formation. In nature the direct asymmetric aldol reaction is catalysed via lysine residues[32-34] in the active centre of the enzyme class I aldolases[34] (Lys229, Fig. 1a) and catalytic antibodies (Ab 38C2 or 33F12)[34,35] (the aldolase reaction). In the quaternary protein structure, Lys229 (Fig. 1a) is surrounded by acidic and basic amino acids, which provide the right chemical environment for activation of carbonyl substrates, overall giving rise to high catalytic turnover. Several active site amino acids working together in a very dynamic way enable the selective activation and conversion of specific substrates to specific products. This complex environment, however, is difficult to reconstruct chemically. Hence, mimicking enzyme catalysis with small molecule organocatalysts is challenging and reaction conditions and catalyst characteristics must be carefully screened for. On the other hand, organocatalysts have the advantage to be much less substrate specific, which opens up the opportunity to use them for a plethora of reactions and substrates.

Enamine intermediate **TS1** formation in neutral aqueous conditions is initiated by converting the carbonyl donor into an iminium ion, thereby decreasing its pK_a. Hereafter, the nucleophilic enamine species **TS2** is formed by deprotonation by a proximate tyrosine residue (Tyr363)[36], acting as weak Brønsted base cocatalyst **14**.[31,37] The latter species reacts with the carbonyl acceptor (iminium ion species **TS3**), which upon hydrolysis regenerates the catalytic amine (lysine **15**) and releases the product (Fig. 1c).[30] Concurrently to the proton transfer from the enol to the aldehyde, a C-C bond formed yielding the aldol product.

The essential amino acid proline **16** is an efficient enantioselective catalyst for a variety of organic reactions, such as aldol, Mannich and Michael reactions, commonly in organic solvents.[21] Consequently, **16** and its analogues **17,18**,**20**,**21** were investigated as catalysts for aqueous aldol reactions (Table 1 – reactions 1a,b,c, 2 and 3). Interestingly, **16** as well as other amino acids containing secondary amine functionalities[38] do not catalyse the aldol reaction in pure water.[39] Due to the high pK_a (11.4) of the pyrrolidine ring, **16** is mostly zwitterionic at near-neutral pH, effectively lowering the available catalyst concentration.[40] Additionally, a computational study on the enamine mechanism of zwitterionic **16** in water revealed that enamine formation and hence further aldol reaction products are inhibited at an early stage in the reaction. The thermodynamically and kinetically favourable formation of the ketal from acetone with water is dominant rather than the formation of enamine intermediates regardless of the moderate activation barrier towards aldol products.[41] Nevertheless, Hayashi used proline amide as a catalyst for the self-aldol reaction of propanal in water, achieving moderate yields and enantioselectivities.[42] As it seems, the amide functionality activates the aldol substrate in water the same way as does the carboxylic acid of proline in organic solvents.

Poelarends reported on the unique mechanism of 4-oxalocrotonate tautomerase (Fig. 1b) for catalysis of aldol and Michael reactions.[43] This enzyme does not belong to the classical aldolases which use Schiff's base formation of a lysine primary amine in the mechanism. In contrast the tautomerase

harbours a unique catalytic N-terminal proline residue (Pro1), capable of forming an enamine intermediate, thereby resembling organocatalytic enamine-mediated proline catalysis.

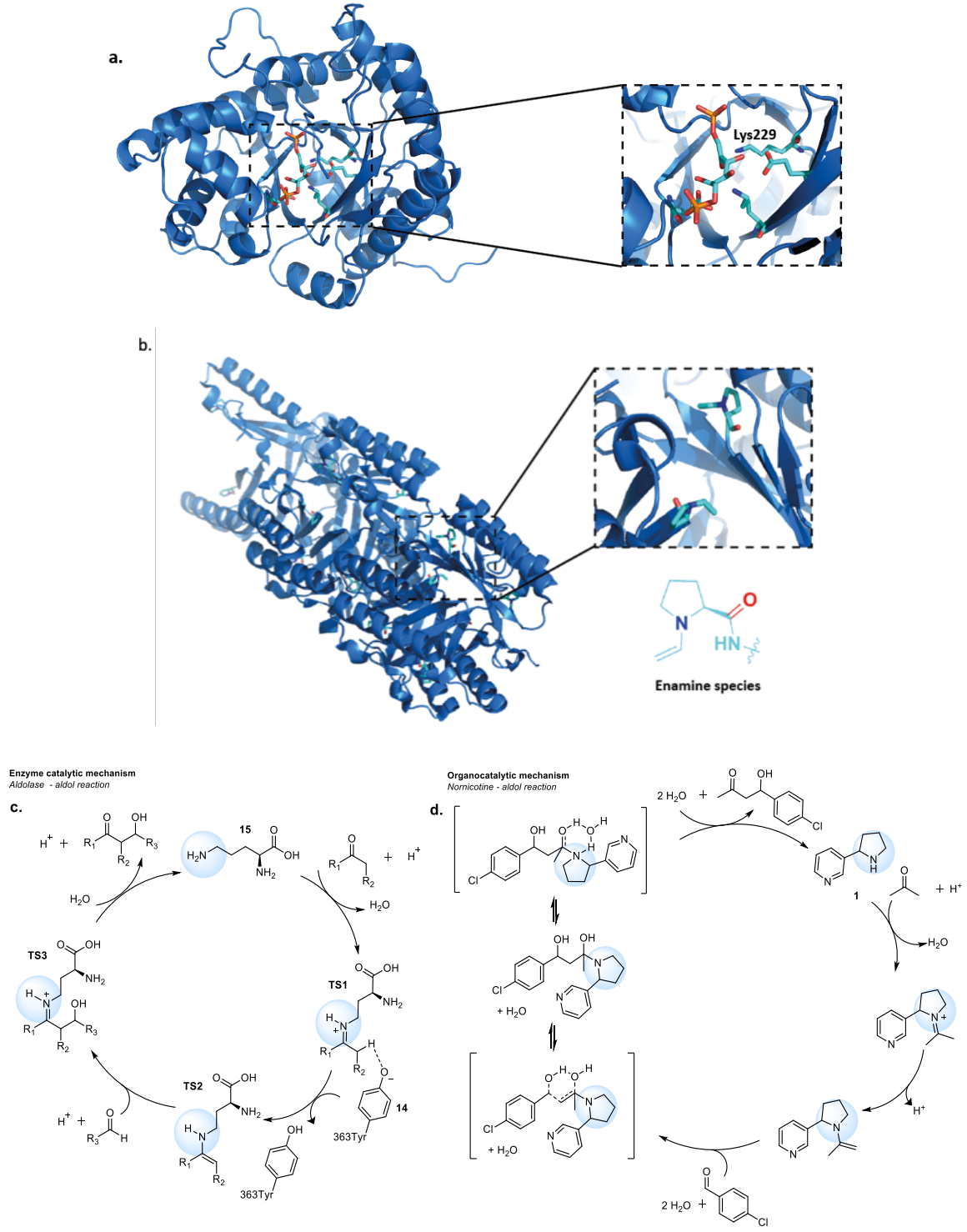


Figure 1: a. Structural details of human muscle aldolase complexed with fructose 1,6-bisphosphate highlighting active site amino acid residues as sticks (Lys229, Lys146, Asp33, Glu187). Lys229 is involved in the Schiff's base formation. The X-ray structure 4ALD[33] was obtained from the PDB[44] and the images were created using the PyMOL Molecular Graphics System[45]. b. Hexameric structure of 4-oxalocrotonate tautomerase (PDB: 4X1C[43]) with N-terminal proline residues bound with acetaldehyde forming the enamine species displayed as sticks. c. Class I aldolases (active site of enzyme: amino acid lysine – Lys 229) direct asymmetric aldolization of unmodified carbonyl compounds[31,46]. d. Mechanistic details for the nornicotine

1 catalysed aqueous aldol reaction of acetone and 4-chlorobenzaldehyde. In brackets: calculated transition states based on density functional theory (DFT) calculations and kinetic isotope studies[47]. N.B. The blue circle highlights the catalytic active centre.

In 2002, Barbas showed that **16** (Table 1 – reaction 1a) catalyses direct intermolecular aldol reactions of ketones in a mixture of buffered aqueous media and organic solvent.[48] Although the reaction was performed in an excess of organic solvent, we deem the work as a fundamental starting point for enzyme inspired (biomimicry) organocatalysis. Indeed, the authors attempted to mimic the hydrophobic environment of the active site[49] of aldolase antibodies[34] using proline derivatives containing hydrophobic groups. Whilst giving good yields and diastereoselectivities in the reaction between cyclohexanone and *p*-nitrobenzaldehyde, enantioselectivities remained poor (Table 1 – reaction 1b).[50] It is questionable if the observed reaction is truly driven by the proposed enamine mechanism. Taking into consideration that the proline derivatives bear secondary amine functionalities (pK_a ~ 11) and are solubilized in non-buffered water, the pH will rise and the aldol reaction will potentially be catalysed by a general base mechanism.[47] Moreover, these results suggest that increased hydrophobicity and a diminished contact between bulk water and the transition state do not necessarily lead to high enantioselectivities. In contrast, Mase showed that the same hydrophobically modified proline at similar reaction conditions can lead to improved aldol product yield and enantioselectivity at low catalyst loading.[51]

Wang[52], Perica[53] and Singh[54] developed novel proline-based catalysts for the direct asymmetric aldol reaction in water, with Singh demonstrating a reduction of the catalyst loading down to 0.5 mol% **20** without compromising selectivity (Table 1 – reaction 2). De Nisco showed in 2009 that proline-based dipeptides **18**, derived from L-proline and apolar β[3]-L-amino acids (Table 1 – reaction 1c) can catalyse the aldol reaction in brine. The highest efficiencies were obtained by catalysts bearing aromatic side chains.[55] The salting out effect caused by brine[56] combined with the efficiency of hydrophobic catalyst residues again points to the importance of creating a hydrophobic environment for efficient enamine catalysis.[50,54] Marx demonstrated the tethering of catalyst and substrate to DNA to increase reaction rates and improve stereoselectivity by bringing the reactants in close proximity.[57] They promoted catalyst/substrate interactions by covalently linking proline to DNA (**21**) and benzaldehyde to its complementary DNA strand (Table 1 – reaction 3).

In a seminal study, Janda showed in 2001 that the nicotine metabolite nornicotine **1** is capable of catalysing aldol reactions in water at physiological pH and temperature (Table 1 – reaction 4).[39] Experimental and computational studies into the nornicotine catalysed aldol reaction validated the enamine catalytic mechanism (Fig. 1d).[58] By replacing the pyridine moiety on nornicotine with electron poor aryl groups, the catalytic efficiency improved. These moieties lower the pK_a of the pyrrolidine nitrogen and hence effectively increase the concentration of available catalyst.[40,59]

Besides catalysts using a pyrrolidine catalytic centre, other types of organocatalysts have been deployed for enamine catalysis in water. For example, L-histidine **23** was demonstrated by Mahrwald as an effective catalyst in asymmetric cross-aldol additions (Table 1 – reaction 5).[60] Since high amounts of reactants were used with equimolar amounts of water, it is questionable if the reaction is truly performed "in water". When larger amounts of water or buffer were used the product yield decreased significantly.[61] Clarke used hydrophobic esters of amino acids such as proline, alanine and leucine **24** to catalyse the hydroxyacetaldehyde self-aldol reaction (Table 1 – reaction 6).[62] This is a remarkable example of the direct aldol dimerization under homogeneous conditions in buffer, a major landmark

towards full stereocontrol building on previous work by Pizzarello and Weber[63]. Again demonstrating the benefit of hydrophobic catalysts, the cross-aldol reaction published by Nakano showed the use of a range of amino amide organocatalysts **19**, for which the highest catalytic activity was obtained with **19** having a bulky and hydrophobic 1-pyrenyl group (Table 1- reaction 1d).[64]

More recently, Juaristi developed novel proline analogues as potential organocatalysts for the asymmetric aldol reaction in water with 10% benzoic acid additive.[65] Their work is an outstanding example in terms of "lessons learned from the past", focused on a novel multifunctional chiral catalyst modified with various reaction performance-enhancing functionalities, reported in past literature. The authors evaluated a second hydrogen bond donor on the chiral organocatalysts to be critical in both rate acceleration and improved stereo-induction. Hydrophobicity, acidic hydrogens adjacent to the nitrogen active centre and hydrogen bonding abilities were essential to enhance the catalytic performance and enantioselectivity.[65] Also working with proline and other N-heterocyclic catalysts, Fascione described the use of organocatalysis for protein modification, in the site selective aldol ligation of protein linked aldehydes to aldehyde donors at 37 °C in phosphate buffer. Although a striking example of organocatalysis under physiological pH and temperature, the catalyst concentration of **16** used was high (100 mol%, Table 1 – reaction 7).[66] Another remarkable example of enzyme inspired aldol reactions is shown by Luo and Cheng, who employed a organocatalyst/cyclodextrin hybrid **22** (Table 1 – reaction 4b). Their catalyst promoted asymmetric direct aldol reactions with excellent enantioselectivities in aqueous acetate buffer (pH 4.8).[67]

Michael and Mannich reactions are other common methods for carbon-carbon bond formation through enamine organocatalysis. To our best knowledge, however, no enantioselective organocatalytic Mannich reactions in water are reported to date. Regarding the organocatalytic aqueous Michael reaction, the reader is referred to the recently published comprehensive review by Pellissier[68], discussing various proline analogues, 1,2-diamine catalysts, cinchona alkaloid catalysts and peptide-based catalysts, amongst others. Consequently, this review is limited to a few outstanding examples, such as the work done by Kumar[69], Miura[70], and Mainkar[71]. Kumar designed several proline-based organocatalysts by modifying L-proline with hydroxyimides for the asymmetric Michael additions of ketones to nitroolefins at room temperature (Table 1 – reaction 8a). Especially, the *N*-hydroxyphthalimide modified L-proline analogue **25** afforded the Michael adduct in high yields and good selectivities. The authors argue that reaction components together with water molecules are favourably coordinated via hydrogen bonding interactions, leading to robust transition state intermediates and hence to high selectivities for Michael adducts.[69] Miura employed perfluorooctanesulfonamide modified catalyst **27** using moderate catalyst loadings (10 mol%) for the Michael addition of benzyl malonate to α,β-unsaturated ketones (Table 1 – reaction 9).[70] Noteworthy, the novel catalyst performs in both solvents cyclohexanone and water at room temperature without additives, whereas slightly better performance was observed in cyclohexanone. Indeed, the perfluoroalkyl group in H_2O is suggested to benefit the Michael addition adduct, due to its hydrophobic nature, sequestering reactants, and strong electron-withdrawing potential, lowering the activation barrier towards the α,β-unsaturated ketones.[70] A significantly lower catalyst loading (0.05 mol%) was reported by Mainkar for the model reaction between cyclohexanone and nitrostyrene (Table 1 – reaction 8b).[71] Using organocatalyst **26** with a peptide bond surrogate triazole connecting a pyrrolidine group to an amino amide, they achieved a moderate yield (70%) whereas enantioselectivities were excellent.[71]

Iminium catalysis

Iminium ion catalysis is an important branch of asymmetric organocatalysis, enabling various reactions such as Knoevenagel condensations, cyclo- and nucleophilic additions, Michael reactions, and cascades thereof. Substrate activation occurs throughout the condensation of the amine catalyst with a substrate carbonyl, forming a reactive iminium ion with an increased polarization similar to that induced by Lewis or Brønsted acid activation of carbonyls.[72] In particular, these transient iminium ions lower the LUMO energy ("LUMO-lowering catalysis") of their substrate upon condensation, which in turn enables them to react with nucleophiles.[73] Iminium catalysis is very general as a large variety of possible nucleophile-electrophile combinations and interactions exists, including nucleophilic additions and cycloadditions, amongst others.[74] Later on in this review, iminium intermediates will come back in transamination reactions catalysed by primary amines such as aniline (nucleophilic catalysis). For iminium catalysis, MacMillan first reported in 2000 the enantioselective organocatalytic Diels-Alder reaction in water/methanol mixtures using chiral imidazolidinone catalysts (Fig. 2).[23] Since then research on iminium catalysis has expanded, mostly aimed at developing catalysts capable of generating high yields and efficient asymmetric induction. From the example of the Diels-Alder reaction, the general mechanism of iminium activation is given in three major steps (Fig 2). In the initial step, the activated iminium ion is formed by condensation of a primary or secondary amine with the α,β-unsaturated carbonyl substrate. This is followed by the catalysed reaction (step 2), ultimately generating the product iminium ion adduct. The latter species regenerates the catalyst and releases the product upon hydrolysis with water in the final step.[75]

Despite its potential, aqueous phase iminium organocatalysis remains rare. As one of the first, Wang reported on the enantioselective Michael addition of malonates to a broad range of enone substrates.[76] The reaction used primary-secondary diamine catalysts bearing various lengths of alkyl chains (20 mol%) and TFA as an acidic additive (20 mol%), at 50 °C. The length of the alkyl chains on the catalyst deemed to be critical to achieve high yields, again pointing to the role of creating a hydrophobic environment.

More recently, Resch showed that lysine **15**, arginine and histidine **23** are suitable catalysts for the Michael addition of water to α,β-unsaturated ketones (Table 1 – reaction 10). Remarkably, the iminium driven Michael addition proceeds in phosphate buffer (pH 7.0) without additives at 40°C using **15** with moderate catalyst loadings (25 mol%). The authors highlight the importance of the carboxylic acid moiety within the amino catalyst, potentially serving as proton acceptor for water.[77]

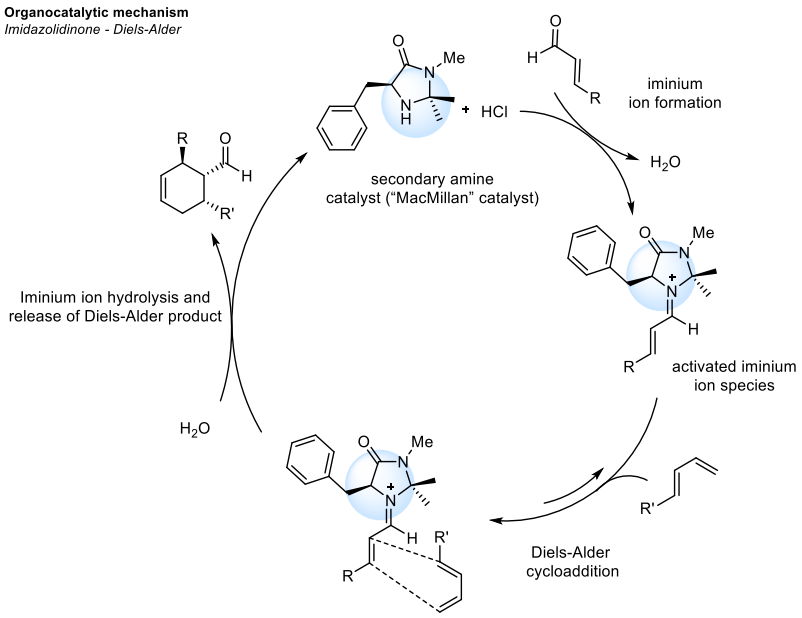


Figure 2. Mechanistic details for the imidazolidinone-catalysed aqueous Diels-Alder reaction of enals and dienes.[23]

Nucleophilic and general/specific base catalysis

An organic base can generally act as a proton acceptor or function as a nucleophile. In catalysis, the former leads to either general or specific basic catalysis, the latter to nucleophilic (Lewis base) catalysis.[78] General and specific acid/base catalysis have been known for a long time, whilst nucleophilic catalysis was conceptualised by Bender in 1960.[78] In the same period, Jencks defined the criteria for nucleophilic catalysis: 1) the catalyst must have a higher reactivity towards the substrate than the acceptor molecule; 2) the reactive intermediate should be more susceptible towards attack by the acceptor than the substrate; 3) the equilibrium constant for the reactive intermediate formation must be smaller than for the product formation.[79] This guarantees a catalytic reaction that is faster than the uncatalysed background reaction and no incorporation of the catalyst in the reaction products.[79] Subsequently, it was the fascination for enzyme catalysis and the elucidation of their mechanisms that fostered studies on the catalytic role of small organic molecules. Bruice, Benkovic and Jencks studied the role of the imidazolyl group of a histidine residue in the active centre of α-chymotrypsin (FIG. 3a) and delineated the nucleophilic catalytic role of imidazole **28** in hydrolytic and acyl-transfer reactions (FIG. 3c and Table 1 – reaction 11a).[80-89] The active site of the serine protease constitutes a catalytic triad of serine, histidine and an acidic amino acid acting collectively in the catalysis of amide or ester hydrolysis (FIG. 3b).[90-92] Here, histidine was recognized as the most important catalytic species with a general acid or base catalysis mechanism via the imidazole ring through serine or water (de)protonation (FIG. 3b).[93] Imidazole is a highly polar, amphoteric aromatic N-heterocycle, capable of both nucleophilic (as Lewis base) and general acid/base catalysis (as imidazolium cation). The catalytic cycle for nucleophilic catalysis proceeds through an acetyl-imidazole reactive intermediate and the formation of this intermediate is rate-limiting (FIG. 3c).[84,94] Catalysis of **28** depends on the leaving group ability of imidazole versus the –OR group.[94] Esters with poorer leaving groups are subject to general base catalysis, whereas activated esters with better leaving groups (such as substituted phenolates) exhibit a nucleophilic mechanism (FIG. 3c).[80,82] Additionally, the nucleophilic catalysis of **28** (pK_a 6.9) is pH-dependent and favours basic conditions, as more imidazole free base species are present.[95]

Besides the well-established hydrolysis of activated esters it was shown that imidazole and/or histidine can catalyse other reaction types: the aldol reaction (enamine-mediated, described above), hydrolysis of *N*-acetylserinamide, RNA cleavage, thioester hydrolysis, amongst others.[95] Additionally, several studies showed effective catalysts designs with imidazole/ histidine residues as part of a nanoparticle[96], polymer[97,98] or protein[93,99-101]. Remarkably, in 2000, Chen described Ser-His as a minimalistic enzyme for amide hydrolysis[102], yet follow-up research proved Ser-His is only able to catalyse activated ester hydrolysis (similar to imidazole activity).[103] Not surprisingly, designing *de novo* catalysts for amide hydrolysis and/or condensation in water is a vibrant research area.[104] In that realm, Ulijn and co-workers used a direct selection approach to screen for catalytically active phages through self-assembly of the product.[105] Counterintuitively, some of the active phages for ester and amide hydrolysis contained no Ser-His sequences or even no His at all, which thus point towards a different catalytic mechanism. Further studies gave no evidence for Michaelis-Menten kinetics and a catalytic mechanism that resembles small molecule organocatalysis was suggested. The selection approach using active phages is an interesting step forward towards identifying efficient new organocatalysts, although their activity does not yet compare to proteases and esterases developed through natural evolution.[104] Up to date the hydrolysis of amide bonds at mild reaction conditions remains the exclusive terrain of hydrolytic enzymes and a simple organic molecule cannot match the rate enhancements achieved with the natural biocatalysts. In that respect, also the conceptual and mechanistic relevance of the comparison between the hydrolysis of amides and activated esters has been subject of debate.[103,106]

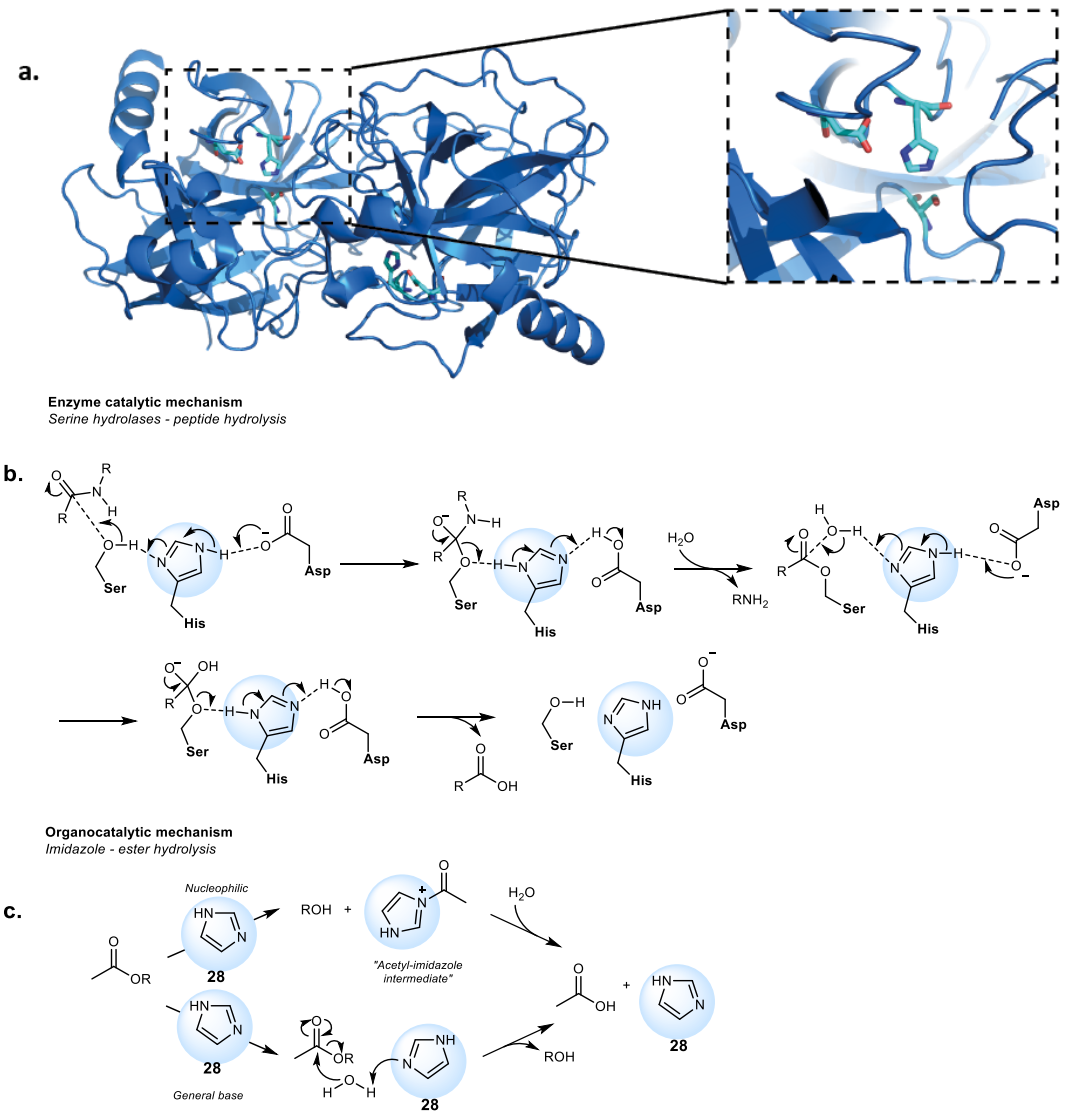


Figure 3: a. Structural details of α-chymotrypsin (from *Bos taurus*) showing the active site with catalytic triad residues (His57, Asp102, Ser195). X-ray dimer structure is 4CHA[107]. b. Serine hydrolase catalytic mechanism with catalytic triad acting collectively in the hydrolysis of the amide bond. c. Mechanistic details for ester hydrolysis catalysed by imidazole 26, either nucleophilic (top) or general base (bottom).

Next to bond breaking reactions, **28** can also catalyse bond formation reactions, such as the Morita-Baylis–Hillman (MBH), where **28** is used as a nucleophilic catalyst. Under basic conditions addition of **28** resulted in rate accelerations of MBH reactions with cyclic enones and aldehydes, leading to high yields, faster reaction times and a larger substrate scope including unreactive and hindered aldehydes.[108] Moreover, other recent work showed that an imidazole derivative – bicyclic imidazolyl alcohol **30** – is a superior catalyst compared to **28** in the MBH reaction of cyclic enones and isatin (Table 1 – reaction 12).[109-111] Experimental and computational evidence proved that an intramolecular proton transfer is responsible for the increased catalytic efficiency of **30**.[111] Here, it should be noted that the reaction of cyclic enones and isatin in water requires a surfactant to create a hydrophobic environment.

Pyridine **5** is another well-known aromatic nitrogen heterocycle and together with its derivatives the catalyst(s) of choice for acetylations. Here, **5** acts as nucleophilic catalyst, creating a reactive acetyl-

pyridinium intermediate. With a pK_a of 5.2, the free base species of **5** is the major constituent at neutral pH, which gives it an advance over other basic amine catalysts.[112] Consequently, **5** is an effective catalyst for the hydrolysis of acetic anhydride (Table 1 – reaction 13). The reaction exhibits a nucleophilic mechanism, as the catalytic activity is completely absent for 2-methyl substituted pyridines caused by steric hindrance to nucleophilic attack.[113] However, the reaction in water competes with a dominant background hydrolysis of the anhydride, making addition of **5** superfluous. **5** is also exploited as catalyst for aryl acetate hydrolysis, yet rate accelerations are not comparable to **5** catalysed acylations with anhydride or **28** catalysed activated ester hydrolysis.[112,114] Additionally, **5** is reported as catalyst for maleimide polymerization in water.[115] The reaction is described as a pyridine-catalysed, non-radical polymerization. However, the mechanism is unclear and **5** might as well act as nucleophilic initiator, as was more recently discovered for other amines in polar solvents.[115,116]

A more reactive and basic analogue of pyridine is DMAP (4-dimethylaminopyridine) **6** (pK_a of 9.2). In aqueous environment, however, the higher basicity poses an immediate problem of protonation and loss of catalytic activity. In a recent study, this was effectively overcome by DMAP incorporation in surface-cross-linked micelles (SCMs) **29**, creating a hydrophobic microenvironment that facilitates efficient catalysis for (phosphate)ester hydrolysis even under acidic conditions (Table 1 – reaction 11b).[117] Other recent literature examples of **6** catalysis include the application of DMAP for affinity protein-labeling[118], the activation of thioesters via a DMAP-SH analogue for acyl transfer reactions[119], a DMAP artificial catalyst system for histone-selective acylation using nucleosome-binding catalysts and acyl donors (in analogy to histone acetyltransferases)[120] and the synthesis of substituted thiophenes via the Gewald reaction in water catalysed by DMAP-functionalized polyacrylonitrile fibers.[121]

Another versatile tertiary amine organocatalyst is DABCO (1,4-diazabicyclo[2.2.2]octane) **4**, a stronger base (pK_as 3.0; 8.8) than **28** and **5**, yet not as strong as **6**. **4**-catalysed MBH reactions of benzaldehyde and acrylonitrile or cyclic enones proceed in aqueous environment and are even accelerated in water due to hydrogen bond stabilization of the enolate reactive intermediate and/or aldehyde activation (Table 1 – reaction 15).[122,123] An analogue of DABCO, 3-quinuclidinol **31** was found as an optimal catalyst for the MBH reaction, demonstrating the largest effect on reaction rate acceleration compared to **4** and **6**.[122] The origin of the rate enhancements in aqueous media were associated with hydrogen bonding, and only marginally with solvent polarity and hydrophobic effects, as both salting-in and salting-out experiments caused an increase in rate.[122]
Along the same line, **4** was deployed in Knoevenagel condensations in water for the formation of α,β-unsaturated carbonyl building blocks.[124,125] Here, **4** is used as a basic catalyst and most likely acts as a general or specific base (Table 1 – reaction 19), deprotonating the active methylene compound (as general base) or water (specific base), as no information on the pH of the reaction is provided and in general hydroxide is known to catalyse these reaction types.[126] However, a concern for this reaction is the solubility of the starting materials and products. The product is usually insoluble in water, which leads to phase separation and drives the reaction to completion. Next to that, Knoevenagel condensations with highly reactive methylene compounds in water often show a fast uncatalysed reaction due to the hydrophobic effect.[127] The polarity of the reactants drives them closer together, causing them to associate into small droplets surrounded by the bulk water phase.[128] This way, the rates of reactions with negative activation volumes are greatly improved. Additionally, the hydrogen-bond donating character of water can also provide a rate enhancement[129], as was observed in the

MBH reaction example catalysed by **31.**[122] Another example of a Knoevenagel reaction in water is in a multicomponent reaction catalysed by piperidine **35** affording azapyrrolizidine compounds (Table 1 – reaction 20).[130,131] The reaction occurs with high regio-, chemo-, and diastereoselectivity. It is not clear whether **35** catalyses the Knoevenagel condensation via iminium formation or base catalysis. Again, the product in this reaction is insoluble, making this a heterogeneous reaction system. A more exotic literature example where **4** is used as nucleophilic catalyst is the allylic substitution of vinyl phosphonates with N- and S-nucleophiles (Table 1 – reaction 14).[132]

Besides tertiary amines, primary amines can also function as nucleophilic organocatalysts in water. A commonly applied primary amine organocatalyst is aniline **32** (see ref.[133] for review). **32** catalyses the popular bioconjugation strategies of hydrazone and oxime formation through a transamination mechanism (Fig.4, Table 1 – reaction 16). The bioconjugation enables amongst others functionalization of polymers[134] and biomolecules for *in vitro* and *in vivo* studies.[133,135] The nucleophilic catalytic mechanism of **32** was elucidated by Cordes and Jencks back in 1962 (FIG. 4a).[79]

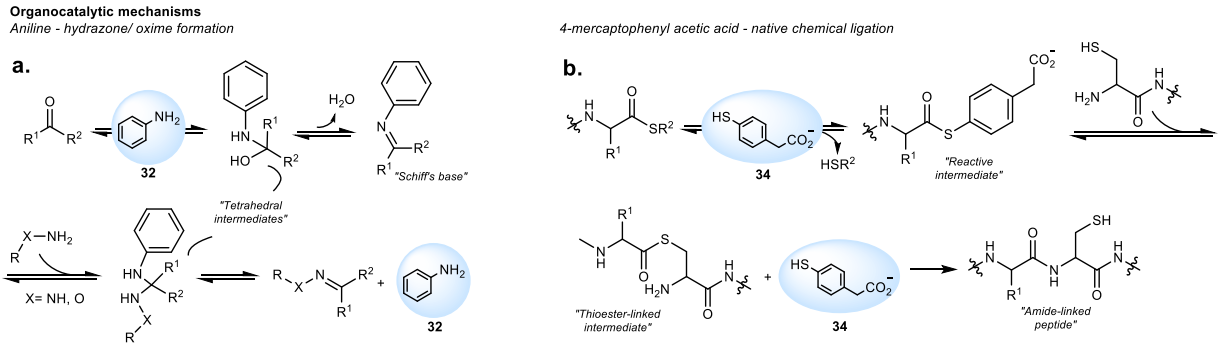


Figure 4: Proposed catalytic mechanisms: a. Aniline (32) -catalysed hydrazone (X=NH)/ oxime (X=O) formation. b. Aryl thiol-catalysed native chemical ligation (NCL).

The aldehyde or ketone and **32** react to form the first reactive tetrahedral intermediate, followed by the formation of the Schiff's base (the imine) by elimination of water. The imine is subsequently attacked by the hydrazide (semicarbazide in ref.[79]) or alkoxyamine nucleophile producing a second tetrahedral intermediate, whereupon aniline is expelled and the hydrazone (semicarbazone in ref.[79]) or oxime product formed.[135] Despite being a carbonyl condensation reaction, the equilibrium favours the hydrazone formation in aqueous environment and hence fulfils the criteria of Jencks for nucleophilic catalysis.[79] Yet, the reaction is severely hampered by slow reaction kinetics under neutral conditions, while accelerated under acidic conditions by acid catalysis[136], making application in most biological systems challenging. Besides, high concentrations of **32** (super-stoichiometric) are required to realise significant rate accelerations.[137] In that respect, lately the development of improved aniline catalysts has become popular and so-called second and third generation catalysts designed for bioconjugation (in biological settings) have been developed.[135] Especially Kool and co-workers have made tremendous efforts to increase organocatalytic efficiency and shed light on the importance of the pK_a and substituent effects of the catalyst.[135,138-140] In general, higher basicity promotes protonation of the Schiff's base and accelerates the reaction.[135,141] Next to catalyst engineering, the choice of the reactants appears to be crucial and for example *ortho*-substituents on aryl aldehydes can greatly enhance the catalytic efficiency (e.g. by intramolecular general acid catalysis with *o*-phosphate groups[142])[135,143]. Successful examples of organocatalysed hydrazone/ oxime formation under (more) biological relevant conditions have been reported by Dawson, who studied hydrazone

and oxime ligations of peptides at pH 7.0 and showed significant rate enhancements (up to 40-fold) with super-stoichiometric aniline concentrations.[141,144] However, the compatibility of **32** in high concentrations with biomolecules and living systems has been called into question and in that context *p*-aminophenylalanine (pFA) was used as alternative biocompatible catalyst by Bane.[145] In another biologically interesting example, Cha used aniline-terminated DNA as a catalyst for DNA-hydrazone formation under physiological conditions and achieved significant rate improvements by further catalyst engineering.[146] Recently, Xia used organocatalyst **33** to promote rapid hydrazone crosslink exchange (Table 1 – reaction 17) in hyaluronan hydrogels in the presence of HUVEC cells at physiological pH and temperature.[67] Besides, in a very recent study by Fascione, the aldehyde product of the protein aldol ligation was further functionalised in a tandem organocatalyst-mediated β-hydroxy oxime ligation catalysed by *p*-methoxy aniline at neutral pH (see enamine chapter for the aldol ligation part).[66] Strikingly, for this organocatalysed oxime formation an unexpected pH effect rate was observed and the yield of conjugation product was higher at pH 7.5 than at pH 4.5. A possible explanation may be the establishment of H-bonding between the β-hydroxy moiety and the protonated aldehyde or Schiff's base intermediate.[66] Finally, very recently Roelfes described the design of an artificial enzyme with pFA incorporated as unnatural catalytic amino acid.[147] The protein environment with hydrophobic binding pocket resulted in extreme rate accelerations, outperforming aniline by a factor >550 for a model hydrazone formation.[147]

Organocatalysed click chemistry reactions are not only limited to hydrazone and oxime formation, as demonstrated by the use of thiol catalysts in native chemical ligation (NCL). Native chemical ligation is an effective method for the chemoselective formation of a covalently linked ligation product from two unprotected peptides under aqueous conditions.[148,149] Specifically, a peptide-thioester reacts with peptide with an N-terminal cysteine, yielding a polypeptide with a native amide bond at the ligation site (Table 1 – reaction 18).[148] Classically, mixed catalyst systems (benzyl mercaptan/ thiophenol) or 2-mercaptoethanesulfonate sodium salt (MESNA) were deployed to accelerate the reaction, yet reaction times were still unsatisfactory for peptides with unhindered ligation sites, making them susceptible to side-reactions.[148,150,151] Therefore, Kent explored different alkyl and aryl thiols as catalysts with varying pK_as.[148] Aryl thiols with higher pK_as were more effective, while the opposite trend was observed for alkyl thiols, such as MESNA and benzyl mercaptan. Overall, 4-mercaptophenyl acetic acid **34** (MPAA) was reported as optimal catalyst showing improved water solubility and no offensive odor.[148] **34** has been used extensively to speed up NCL reactions ever since, although the quest for better performing catalysts has not ended. Recently, mercaptobenzyl sulfonates were investigated and while not providing a catalytic advantage in terms of rate, the increased polarity resulted in improved water solubility and also aided the purification process.[152] Akin to aniline catalysis, the thiol-catalysed NCL seems an example of nucleophilic catalysis, as a reactive thioester intermediate is formed from the reactant peptide-thioester and the thiol catalyst (FIG. 4b).[148,150,153] The intermediate formation is followed by the thiol-thioester exchange with the thiol moiety of N-terminal cysteine sidechain.[148,150] Finally, the amide-linked peptide ligation product is formed via a spontaneous and irreversible intramolecular rearrangement of the thioester linked intermediate. The rate-determining step for aryl thiol catalysts is the initial catalyst-thiol-thioester exchange similar to the aniline Schiff's base formation for hydrazone and oxime formation, while the opposite holds true for the alkyl thiols, where the transthioesterification with the thiol of the cysteine-peptide is rate-determining.[148,150] Yet, the full mechanistic details have not been elucidated.[148] New studies also focused on catalyst systems suitable for *N*,*S*-acyl shift based ligations. In that context, very recently

Melnyk used water-soluble alkyldiselenol catalysts for thioester-thiol exchange and ligation. Their catalyst systems was superior to MPAA based systems and specifically efficient at pH 4.0, enabling complex peptide syntheses.[154]

N-heterocyclic carbene catalysis

In contrast to the common electrophilic carbonyl activation, N-heterocyclic carbene (NHC) catalysts are special in the sense that they exhibit an opposite aldehyde activation mechanism with an inverted nucleophilic reactivity. Upon creation of the 'Breslow intermediate' by nucleophilic attack of the carbene to the carbonyl, the aldehyde shows a charge-reverse "Umpolung" reactivity (BOX.2), a concept that was introduced by Seebach in 1979.[155-157] The catalytic mechanism for NHC-catalysis was elucidated in 1958 by Breslow, based on the earlier work of Lapworth with cyanide catalysis (FIG. 5a).[156,158] In the NHC catalysed benzoin condensation, the thiazolium salt is deprotonated at the acidic C-2 position[159], typically by a strong (in)organic base, generating the highly (catalytically) active carbene. This carbene in turn attacks the carbonyl of the aldehyde, generating a thiazolium salt adduct. Deprotonation of the formerly aldehydic proton, which is rendered acidic due to the negative inductive effect of the cationic NHC group, forms the enamine-like 'Breslow intermediate'. This intermediate can attack a second electrophile, like the carbonyl of another aldehyde, which is followed by the elimination of the benzoin product and regeneration of the carbene.[155,160] Chirality can be induced by deploying chiral NHC-catalysts, this way there is a preference for the formation of one geometric 'Breslow intermediate' isomer and the electrophile is directed to the least hindered enantiotopic site.[160]

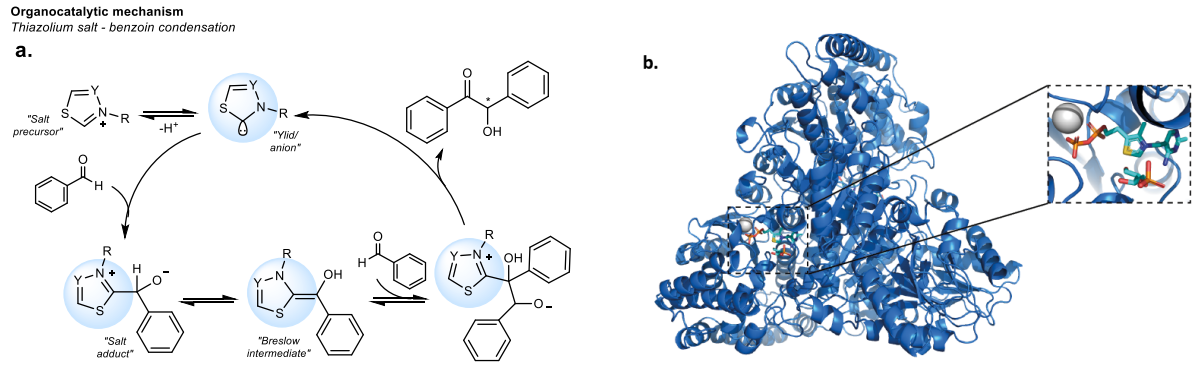


Figure 5: a. Catalytic mechanism of the benzoin condensation catalysed by thiazolium salt as active carbene (postulated by Breslow[155,156]). b. X-ray dimer structure of transketolase from *Saccharomyces cerevisiae* 1NGS[161,162] with the thiamine diphosphate cofactor (top) and erythrose-4-phosphate substrate (bottom) highlighted as sticks and a calcium ion as grey sphere.

The organocatalytic NHC catalysis finds its roots in the thiamine diphosphate (ThDP)-dependent enzymes, such as transketolases (FIG. 5b), amongst others.[163-165] These enzymes are prevalent in biocatalytic pathways and use a thiamine coenzyme (water-soluble vitamin B_1) as NHC catalyst precursor together with a metal ion. ThDP-dependent enzymes catalyse a plethora of lyase and ligase reactions and follow the mechanism as postulated by Breslow (FIG. 5a).[163,164] The enzyme provides a hydrophobic environment and the coenzyme binding site is located in a deep cleft (FIG. 5b), that leads to stabilisation of the ionic or radical transition states and overall regulates the substrate scope, catalytic activity and enantioselectivity.[161,163] As such, these biocatalysts work very efficiently in aqueous media. On the contrary, performing NHC-organocatalysed reactions in water has proven to

be a major challenge due to the extremely reactive, unstable and moisture sensitive "naked" carbene and the risk of undesired side-product formation.[166] Most NHC catalysts have a pK_a higher than water and get easily protonated in water, which results in a very low concentration of actual carbene. Nonetheless, these disfavourable thermodynamics can be overruled by fast deprotonation kinetics and equilibrium-shifting by reaction with the electrophile.[166,167] In this light, considerable efforts in the past decades have led to successful literature examples of NHC-organocatalysed reactions under aqueous conditions (see review[166] and references therein). For instance, Chi and co-workers were able to use water as the only solvent in NHC-catalysed reactions with enals and enones, aldehydes or isatins (Table 1 – reaction 21), generating lactones or cyclopentenes by decarboxylation. Both are examples of conjugate Umpolung reactivity with α,β-unsaturated aldehydes.[168] In the same vein, Ganesan used NHCs as catalysts for heterocycle and carbocycle formation in water.[169] In this example, brine was used as reaction medium to increase the reaction rate via hydrophobic hydration. Considering the classical benzoin condensation, Iwamoto was able to use benzimidazolium salts **37** in water as catalysts in the presence of an (in)organic base (Table 1 –reaction 22a).[170] The N-substituent of **37** proved to be crucial and catalysts with long aliphatic chains were found to be superior in terms of product yield. It was proposed that these long alkyl chains facilitated the creation of micelles in the water phase. This way, various benzaldehydes were coupled, affording α-hydroxy ketones in moderate to excellent yields without further purification. However, in this example no efforts on the preparation of chiral substances were made and until recently the asymmetric benzoin condensation could not be successfully accomplished in water. Yet, a very recent example proved otherwise and reported on a chiral NHC-catalysed asymmetric benzoin reaction (Table 1 –reaction 22b), forming α-hydroxy ketones in good yield and high enantioselectivities.[171] Here, inorganic bases were essential and as with the previous example from Ganesan, brine could accelerate the homodimerization of benzaldehyde. Furthermore, water was postulated to act as proton shuttle in the catalytic mechanism.

Non-covalent activation

Organocatalysis through non-covalent activation is a highly active field, mainly focused on enantioselective catalysis in organic solvents (sometimes in the presence of water[14]).[172] In contrast, in buffered water only very few examples have been reported, likely because Brønsted acid catalysis is hindered by the buffer and hydrogen bonding catalysis suffers from strong competition with the water solvent. Still, Cai[173] and Chimni[174] demonstrated Brønsted acid catalysis using sulfonated amino acids **39** and camphor sulfonic acid catalyst **40**, respectively. They employed water as the only solvent, achieving excellent yields and high diastereocontrol at room temperature in the three-component Mannich-type reaction and in the Michael type Friedel-Crafts reaction, respectively (Table 1 – reaction 23 and 24).[173,174] Gao demonstrated hydrogen bonding catalysis in water using phenylalanine–urea **41** for an organocatalytic Knoevenagel condensation (Table 1 - reaction 25).[175]

Miscellaneous

Oxidation/ reduction organocatalysis

With the aim of developing environmentally benign, safe, sustainable and economically attractive redox catalysts, organocatalysis has been acknowledged as a valuable tool in oxidation and reduction chemistry. Taking inspiration from nature's flavin-dependent enzymes, especially much research has been devoted to the development of biomimetic flavin catalysts that use oxygen and hydrogen peroxide as terminal oxidants.[176-178] These catalysts constitute a flavin moiety able of catalysing redox reactions, akin to the flavin-dependent enzymes which harbour a (non)-covalently bound flavin

prosthetic group that mediates the electron-transfer catalysis (oxygen transfer or dehydrogenation).[178,179] Examples of these enzymes include monooxygenases (e.g. Baeyer-Villiger monooxygenase in FIG. 6a), halogenases (e.g. Tryptophan 7-halogenase) and oxidases (e.g. glucose oxidase).[92] For one oxygen atom insertion reactions (monooxygenase activity), the flavin cofactor (FMN or FAD), previously reduced by the nicotinamide cofactor (NAD(P)H), reacts with oxygen via a Michael-type addition yielding a hydroperoxide. Notably, in the organocatalytic route hydrogen peroxide functions as direct oxidant. Finally, oxygen is transferred to the substrate and regeneration of flavin leads to the elimination of water (FIG. 6b).[92,176] The active flavin hydroperoxide species of the enzyme are hidden in a hydrophobic pocket and were found to be very unstable outside the enzyme.[176,177] Yet, pioneering work by Bruice and co-workers demonstrated that 5-alkyl analogues of flavin hydroperoxides are significantly more stable and could subsequently be used for heteroatom oxidations.[180,181] In general, the flavin organocatalytic reactions can be carried out under mild conditions and most examples are performed in mixtures of water and alcohol or acetonitrile. Reactions that use buffered water as the sole solvent are somewhat less common. For instance, bridged flavinium organocatalysts **42** were used in the diimide mediated reduction of electron rich alkenes in aqueous media (Table 1 –reaction 26).[182] Although catalyst loadings were low (5 mol%), the reaction was conducted at reflux and 10 equivalents of toxic hydrazine hydrate were required. In other organocatalytic redox examples, enantioselective sulfoxidations of aromatic and aliphatic sulfides with H_2O_2 have successfully been carried out in phosphate buffer with N5-ethyl flavin catalysts **43** constituting a chiral auxiliary made of cyclodextrin (Table 1 –reaction 27).[183-185] Rate enhancement, high conversions and very high enantioselectivities were accomplished due to pre-coordination of the substrates inside the cyclodextrin cavity with minimal catalyst loadings (0.3-5 mol%) and no overoxidation was observed.

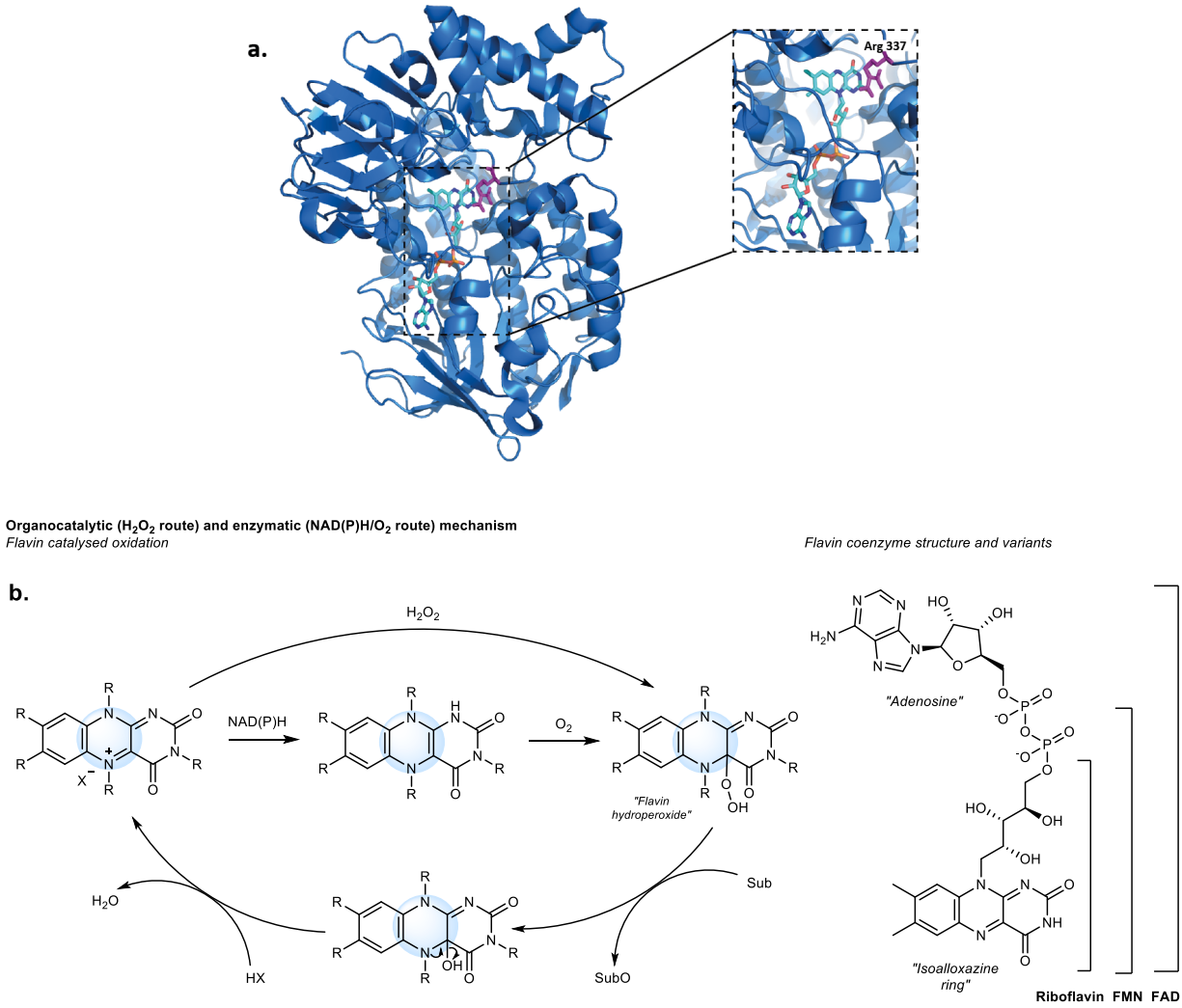


Figure 6: a. X-ray monomeric structure of phenylacetone monooxygenase, a Baeyer–Villiger monooxygenase from the thermophilic bacterium *Thermobifida fusca*, 1W4X[186] with FAD (flavin adenine dinucleotide) cofactor highlighted as sticks and the arginine residue of the active site (Arg337) on the *re* side of the flavin ring in magenta. In this position Arg337 can stabilise the flavin peroxide species. b. Catalytic cycle for flavin-catalysed substrate oxidation (Sub to SubO) by H_2O_2 (organocatalytic) or NAD(P)H/ O_2 (enzyme-mediated). In the final step water gets eliminated on regeneration of the flavin cofactor.

Whereas the flavin inspired organocatalytic redox catalysts are by far the most studied, other catalytic systems are also reported. For example Raines showed disulfide reduction catalysed by small molecule thiols and selenols as electron-relay catalysts and used this approach to reduce disulfides *in vitro*.[187]

A final more unusual example of an organocatalysed reaction in aqueous media is the catalysis of simple 2-oxoacid salts **44** with cyanamide in intramolecular phosphate ester formation (Table 1 – reaction 28).[188] Glyoxylate and pyruvate are shown to catalyse this reaction by *in-situ* formation of a cyclic reactive intermediate with cyanamide. Moreover, the reaction is accelerated by divalent metal cations that promote the breakdown of the urea product and regeneration of **44**.

Organocatalytic reactions for non-living and living biological settings

Although we already have seen some successful examples of *biocompatible* organocatalysis, such as the organocatalyst-mediated protein aldol ligation[66], the use of DMAP for affinity protein-labeling[118] and the artificial enzyme for hydrazone or oxime formation[147], only a fraction of currently known organocatalytic reactions can operate in biological settings. For potential *biocompatible* applications, we identified three criteria: (1) temperature and pH set to physiological conditions (temperature between 25 (RT) and 40 °C, pH 7.0 – 7.4); (2) potential reactions should be performed in water and preferentially in buffered media with a maximum of 10% miscible co-solvent; (3) in addition, no additives, such as surfactants, should take part in the reaction. Two additional criteria for *in vivo* applicability are the toxicity of (4) the catalyst and (5) the reactants and products. Due to lack of available toxicological data, the evaluation is based on LD_{50} and IC_{50} values. In cases those values were not available, the catalyst building blocks or similar structures were evaluated accordingly.

Overall, we came down to 19 reactions (Table 1) that, based on the applied criteria, are potentially *biocompatible*, where catalyst and reagent toxicity is of less concern. Specifically, for enamine catalysis we selected aldol reactions 3, 4a and 6 and Michael addition 9, while for iminium catalysis only reaction 10 (Michael addition of water) is considered applicable. For nucleophilic catalysis, reaction 11 (ester hydrolysis), 14 and 15 (DABCO **4** or 3-quinuclidinol **31** catalyzed additions or substitutions), reactions 16 and 17 (hydrazone formation) and reaction 18 (native chemical ligation) were selected. Reactions 19 and 20 remained for general/specific base catalysis and for NHC catalyzed reactions 21, 22a and 22b apply. For non-covalent catalysis, reactions 23 and 24 (Brønsted acid) and reaction 25 (hydrogen-bonding) seem to be applicable. For redox catalysis only reaction 27 with the flavin-cyclodextrin conjugate **43** fits the criteria.

Toxicity issues greatly reduce the number of reactions with *in vivo* potential, leaving reactions 6 (self-aldol – enamine catalysis), 14 (vinylphosphonate allylic substitution – nucleophilic catalysis), 17 (hydrazone formation – nucleophilic catalysis), 21 (enal coupling – NHC catalysis), 22b (benzoin condensation – NHC catalysis), 24 (Friedel-Crafts – Brønsted acid catalysis) and 25 (Knoevenagel – hydrogen-bonding catalysis) as possible candidates.

Conclusion and outlook

When considering organocatalysis in aqueous media, the discussed examples demonstrate that catalyst pK_a and catalyst reactivity compared to the reactants is essential, in line with Jencks's criteria.[79] Additionally, organocatalysis in aqueous media seems to benefit substantially from improved catalyst designs, by either attaching or varying substituents on the catalyst and changing the reactivity (e.g. for aniline derivatives[135,138-140]) or by engineering the catalysts to work under unusual conditions by creation of a favourable microenvironment (e.g. DMAP in SCMs[117] or the designer protein with an unnatural organocatalytic residue[147]). The latter examples show much resemblance with natural enzymes, where for example a hydrophobic pocket can provide the optimal chemical environment, as we have seen in the various biocatalytic examples. Moreover, specifically for the aldol reaction, catalysts designed with increased hydrophobicity (e.g. primary amine catalyst with cyclodextrin[189]), acidic hydrogens adjacent to the nitrogen active site and hydrogen bonding abilities, enhance catalytic performance.[65] Hence, whilst the catalytic engineering of organocatalysts is still in its initial stages, there is tremendous potential for organocatalysis to be exploited in biological environments. In that context, we underlined the boundary conditions for organocatalysis in biological settings and evaluated the existing literature examples for potential *biocompatible* and *in vivo* applications. Altogether, we expect a bright future for cleverly designed organocatalytic processes and encourage applications in biological systems, enriching the field of chemical biology and possibly biomedicine.

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Author contributions

M.P.v.d.H and B.K. researched data for the review and wrote the manuscript. R.E. revised the manuscript. All authors commented on the work and the manuscript. M.P.v.d.H and B.K. contributed equally.

Competing interests statement

The authors declare no competing interests.

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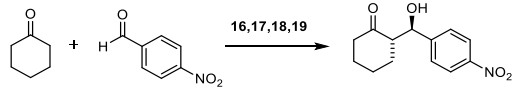
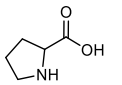
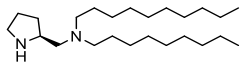
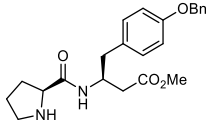
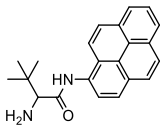
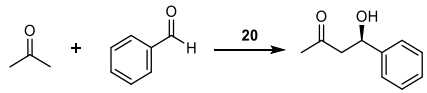
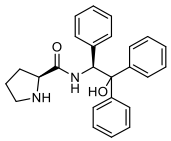
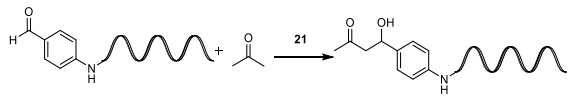
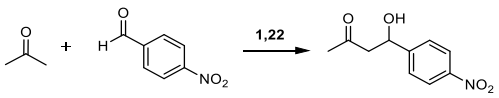
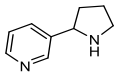
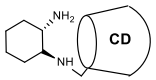
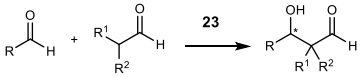
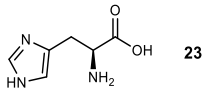
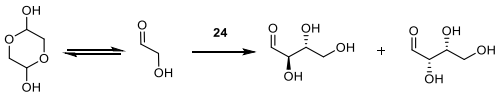
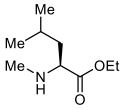
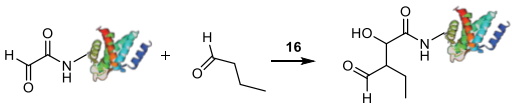
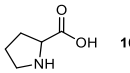
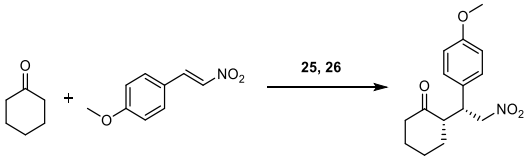
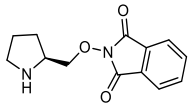
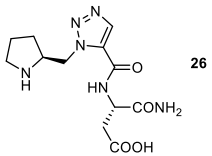
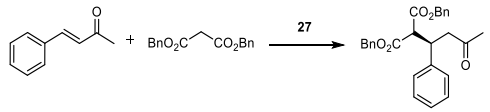
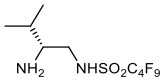
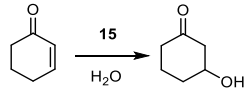
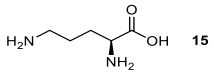
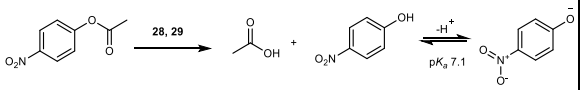
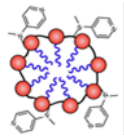
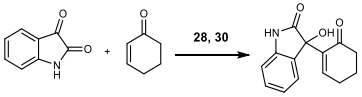
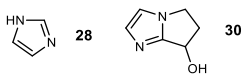
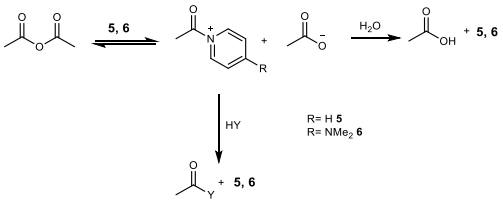
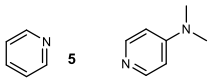
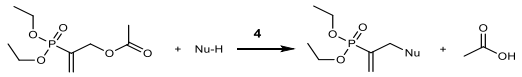
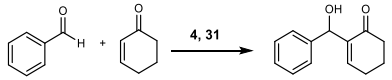
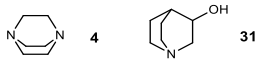
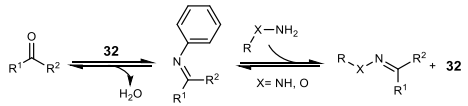
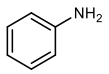
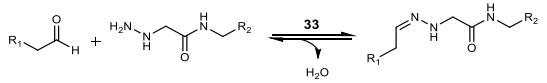
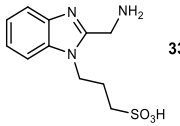
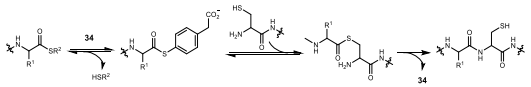
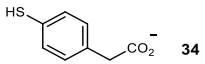
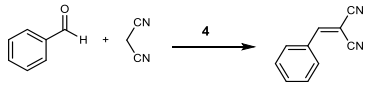
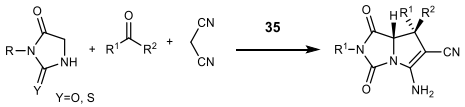
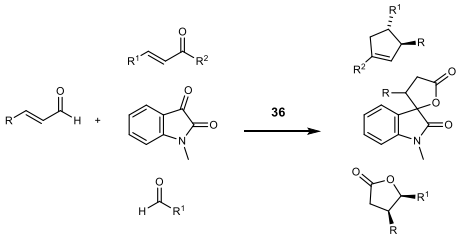
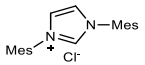
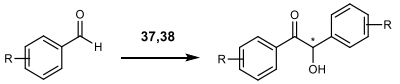
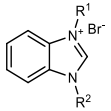
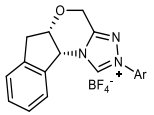
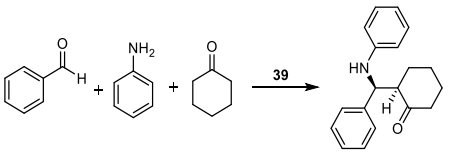
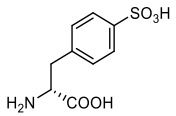
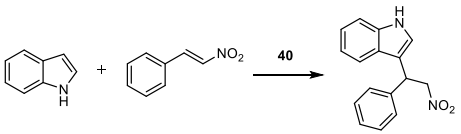
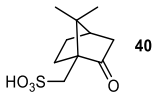
Table 1: Overview of organocatalysed reactions and criteria: reaction conditions (T, pH), catalyst (loading), reaction time, activation mode, compound toxicity (in different contexts).

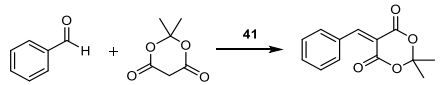
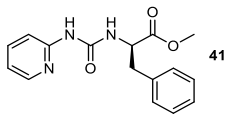
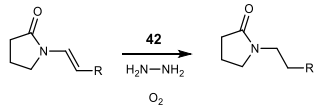
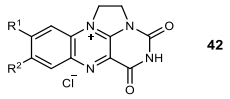
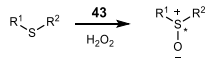
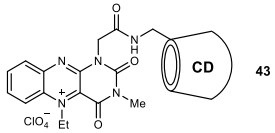
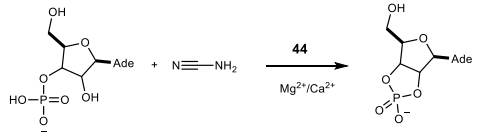
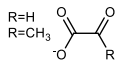
#	Reaction variant	Activation-mode	Organocatalyst* (loading)	Reaction conditions				Catalyst toxicity LD_{50}/IC_{50} value**	Remarks	Ref.
				pH	T (°C)	Time	Additives			
1a	 Direct asymmetric aldol reaction	Covalent – enamine	 **16** L-Proline (20 mol%)	n.d.	RT	1-72 h	0.1 eq. SDS	L-Proline see reaction 2 below.	Use of PBS buffer	[48]
1b			 **17** Proline derivative with aliphatic side group (10 mol%)	n.d.	RT	5 h	None	L-Proline see reaction 2 below.		[50]
1c			 **18** L-Proline dipeptide catalyst (3 mol%)	7.0	RT	48 h	None	Dipeptides modified with hydrophobic side group	Use of brine	[55]
1d			 **19** Amino amide catalyst (3 mol%)	n.d.	RT	48 h	None	Amino acid	Use of seawater	[64]
2	 Direct asymmetric aldol reaction	Covalent – enamine	 **20** Proline derivative with aromatic groups (0.5 mol%)	n.d.	10	7 h	None	L-Proline: No adverse effects > ~2.8 g/kg (oral, rats oral)[190] 1,1,2-Triphenyl-ethanol: highly toxic (IC50: 0.4 uM, NIH/3T3)[191]	Use of brine	[54]
3	 Direct asymmetric aldol reaction	Covalent – enamine	 **21** DNA modified proline ($1.5 * 10^{-5}$ mol%)	7.2	25	4 h	None	L-Proline see reaction 2 above.	Use of phosphate buffer (100 mM)	[57]

4a	 Direct asymmetric aldol reaction	Covalent – enamine	 **1** Nornicotine (30 mol%)	7.4	37	12 h	None	Nornicotine LD50: 3.409 mg/kg (intravenous, mouse)[192]	Use of Phosphate buffer; reaction proceeded 6% yield without catalyst	39
4b			 **22** Chiral diamine catalyst (5 mol%)	4.8 (optimal ph)	RT	12 – 48 h	None	1,2-diaminocyclohexane LD50: >50 mg/kg Cyclodextrin LD50: 356 mg/kg	Use of acetate buffer	189
5	 Cross-aldol reaction of enolizable aldehydes	Covalent – enamine	 **23** L-Histidine (10 mol%)	7.0	RT	16 h-7 days		Histidine, essential amino acid, safe in food products and not carcinogenic[193]	Equimolar amounts of water	60,61
6	 Organocatalysed self-aldol reaction	Covalent – enamine	 **24** N-methyl leucine ethyl ester (10 mol%)	7.0	RT	5 h	None	No growth inhibition at > 1.7 mM leucine ethyl ester in algae[194]	Use of phosphate buffer	62
7	 Organocatalyst-mediated protein aldol ligation (OPAL)	Covalent - enamine	 **16** L-Proline (100 mol%)	7.5	37	6 h	None	L-Proline see reaction 2 above.		66
8a	 Michael addition of ketones to nitroolefins	Covalent - enamine	 **25** Pyrrolidine-oxyimide catalyst (10 mol%)	n.d.	RT	15 h	None	N-hydroxy phthalimide LD50: 178 mg/kg (intravenous, mouse)		69
8b			 **26** Peptidomimetic triazole-based catalysts (5 mol%)	n.d.	RT	30 h	None	Possible growth inhibitor, IC50: 40 uM for ((S)-methyl 2-(methyl((1-undecyl-1H-1,2,3-triazol-4-yl)methyl)amino) Propanoate)[195]		71

9	 Asymmetric Michael additions of malonates to enones	Covalent - enamine	 **27** Perfluoroalkane-sulfonamide catalyst (10 mol%)	n.d	RT	24 h	None	Derivative (Perfluorooctane sulfonic acid) LD50: 251 mg/kg (oral, rat)		[70]
10	 Michael addition of water to α,β-unsaturated ketones	Covalent – iminium ion	 **15** L-Lysine (25 mol%)	7.0	40	3 h	None	L-Lysine LD50: 10.1 g/kg (oral, mouse)[196]	Use of sodium phosphate buffer (250 mM)	[77]
11a	 Ester hydrolysis (and phosphate ester)	Covalent - Nucleophilic base catalysis	 **28** Imidazole (super-stoichiometric)	> 7 (basic favourable)	RT	Fast (minutes)	Organic co-solvent for ester	Imidazole LD50: 880 mg/kg (oral, mouse) / 220 mg/kg (oral, rat) and suspected of reproductive toxicity	Use of imidazole buffers	[80-82]
11b			 **29** DMAP-SCM (50 mol%)	4-8	35	Fast (minutes)	Organic co-solvent for ester	DMAP LD50: 350 mg/ kg (oral, mouse)	Use of HEPES buffer	[117]
12	 Morita–Baylis–Hillman (MBH) reaction	Covalent - Nucleophilic base catalysis	 **28** **30** Imidazole or bicyclic imidazolyl alcohol (10 mol%)	n.d.	RT	4 h	Sodium Dodecyl Sulfate (SDS)	Imidazole see reaction 11a above. For bicyclic imidazolyl alcohol no toxicity information available.		[109-111]
13	 Acetylation/ hydrolysis with acetic anhydride	Covalent - Nucleophilic base catalysis	 **5** **6** Pyridine or DMAP (super-stoichiometric)	5.5	25	Fast (minutes)	Organic co-solvent for acetic anhydride	Pyridine LD50: 891 mg/kg (oral, rat) DMAP see reaction 11b above.	Use of pyridine buffers	[112,113]

14	 Synthesis of functionalised vinylphosphonates	Covalent - Nucleophilic base catalysis	 DABCO (20 mol%)	n.d.	RT	Overnight		DABCO LD50: 1700 mg/kg (oral, rat)[197]	Sulfur and nitrogen-based aliphatic and aromatic nucleophiles	132
15	 Morita–Baylis–Hillman (MBH) reaction (also acrylate instead of enone)	Covalent - Nucleophilic base catalysis	 DABCO or 3-hydroxyquinuclidine (stoichiometric)	n.d.	RT	4 h		DABCO see reaction 14 above. For 3-hydroxy quinuclidine no toxicity info reported.	Purification by flash chromatography.	122
16	 Hydrazone/ oxime formation with aniline	Covalent - Nucleophilic base catalysis	 Aniline derivative (super-stoichiometric)	2.5-7.4 (acidic favourable)	RT	Minutes to hours (depending on catalyst and reactants)	Some cases co-solvent (DMF) for reactants	Aniline LD50: 464 mg/kg (oral, mouse)		79,134,135,138-140,143
17	 Hydrazone formation with benzimidazole derivative	Covalent - Nucleophilic base catalysis	 2-(aminomethyl)benzimidazole derivative (super-stoichiometric)	7.4	37	Minutes to hours (depends on concentrations)		HUVEC cell viability after 24 h: 87%	Use of PBS buffer	67
18	 Native chemical ligation (NCL)	Covalent - Nucleophilic base catalysis	 4-mercaptophenyl acetic acid (MPAA) (super-stoichiometric)	7.0	25	Few hours (depending on catalyst and reactants)	TCEP (to maintain thiols in reduced form)	Benzyl mercaptan LD50: 493 mg/kg (oral, rat) 4-mercaptophenylacetic acid irritative chemical, no further toxicity information available	HPLC purification	148,152
19	 Knoevenagel condensation	Covalent – General/ specific base catalysis	 DABCO (10 mol%)	n.d.	RT	2 min	/	DABCO see reaction 14 above	Recycling of catalyst (six times) by recovery from the filtrate water.	124

20	 Multi-component reaction	Covalent – iminium and/ or base	 **35** Piperidine (10 mol%)	n.d.	RT-80	0.5-8 h		Piperidine LD50: 337 mg/kg (oral, rats)		[130]
21	 Coupling of enals with enone, isatins or aldehydes	Covalent - NHC (conjugate Umpolung)	 **36** Azolium salt (NHC precursor) (5-15 mol%)	n.d.	RT-40	24 h	NaOH as inorganic base (2 eq.)	No info available. Yet, the thiamine cofactor of enzymes is a vitamin (Vitamin B_1).	Reactions performed in air	[168]
22a	 (Asymmetric) benzoin condensation	Covalent - NHC (Umpolung)	 **37** Benzimidazolium salts (NHC precursor) (20 mol%)	n.d.	RT	1-30 h	TEA/ DBU/ NaOH as base	Benzimidazolium salts are toxic[198]	Large hydrophobic N-subtituents on catalyst formed micelles	[170]
22b			 **38** Triazolium salts (NHC precursor) (10 mol%)	n.d.	RT	7-24 h	Inorganic bases required	Triazolium salts found with low-toxicity[199]	Brine accelerated the reaction	[171]
23	 Mannich reaction	Non-covalent - Bronsted Acid catalysis	 **39** sulfonated amino acid (0.010 mol%)	n.d.	RT	5 h	None	Benzenesulfonic acid LD50: 890 mg/kg (oral, rat)		[173]
24	 Michael type Friedel-Crafts addition of indoles to nitro-olefins and enones	Non-covalent - Bronsted Acid catalysis	 **40** D-CSA (Camphorsulfonic acid) (20 mol%)	n.d.	RT	10 h	None	D-CSA LD50: 2502 mg/kg (subcutaneous, mouse)		[174]

25	 Knoevenagel condensation	Non-covalent - Hydrogen-bonding catalysis	 phenylalanine–urea catalyst (10 mol%)	n.d.	RT	12 h	None	Phenylalanine LD50: ~5.3 g/kg (acute, rat) Urea: No acute death < 1g/kg (oral, rat)		[175]
26	 Diimide mediated reduction of alkenes	Redox	 Bridged flavinium salts (5 mol%)	n.d.	100	18 h	10 eq. of hydrazine hydrate required. LD50 129 mg/kg (oral, rat)	No specific data available. Flavins are used as food additives.	Reaction under reflux conditions	[182]
27	 Enantioselective sulfoxidation	Redox	 Flavin-cyclodextrin conjugate (0.3-5 mol%)	7.5	RT	<2 h	2.3 eq. of H_2O_2 as oxidant	No specific data available. Flavins and cyclodextrins are used in food products.	Use of phosphate buffers	[183-185]
28	 Phosphate activation with cyanamide (intramolecular phosphate ester formation)	Redox	 2-oxocacid salts (glyoxylate and pyruvate) (stoichiometric)	5.0-5.2 (optimum)	40	24 h	Divalent metal cations (to regenerate catalyst)	Prebiotic building blocks: pyruvate and glyoxylate intermediates in metabolic pathways.	In-situ generation of a cyclic reactive intermediate from cyanamide and the 2-oxoacid salt	[188]

Abbreviations: T=temperature, RT=room temperature, n.d. = not determined, Nu-H = nucleophile, DME = dimethoxyethane, CAN = ceric ammonium nitrate, SCM=surface-crosslinked-micelle, DMF=N,N-dimethylformamide, TCEP=Tris(2-carboxyethyl)-phosphine, Ade=adenine, Mes=mesityl, TEA=trimethylamine, DBU= 1,8-Diazabicyclo[5.4.0]undec-7-ene, Ar=aryl, Me=methyl, Et=ethyl, CD=cyclodextrin.

Notes: *Catalyst are numbered regardless of their ionization state or different R-groups. **If the source of catalyst toxicity is not listed, the Material Safety Data Sheet (MSDS) has been consulted. If no toxicity information for the exact chemical structure was available, toxicity values of similar structures are listed when available and relevant.

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