Covalent Capture: Merging Covalent and Noncovalent Synthesis
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This Review is concerned with the search of molecules to find a suitable partner with whom to form a bond. Whether an initial encounter results in a fixed bond depends on many critical issues, such as reciprocal compatibility in terms of size and shape, complementary reactivity, proper alignment of reactive groups, and medium effects. The chemist acts as the master of ceremony and finds a high reward in the case of a successful marriage. Giotto’s painting “The Wedding of Mary and Joseph” illustrates the principles of supramolecular chemistry involved.

1. Introduction

Organic chemistry is founded on covalent synthesis, that is, the formation of covalent bonds between molecules by the reaction of functional groups.[1] Supramolecular chemistry[2] now allows chemists to construct molecular structures through noncovalent interactions, such as hydrogen[3] and coordination bonds[4] as well as π–π stacking[5] and hydrophobic interactions.[6] This approach is termed noncovalent synthesis.[7,8] Covalent and noncovalent synthesis have many orthogonal characteristics, which means that, generally, the target structure determines which approach is most suitable for its preparation; therefore, both approaches are generally considered as alternatives. In this Review we describe a trend in current day organic chemistry, referred to as covalent capture, that relies on the combined use of covalent and noncovalent synthesis by taking advantage of their complementarity (Figure 1).

![Figure 1](image)

Figure 1. Covalent capture by the irreversible or reversible formation of a covalent bond.

The covalent capture strategy is inspired by nature. Biological processes frequently involve a molecular recognition event followed by the formation of a covalent bond between the two components. This sequence of events gives nature the possibility to perform error corrections, alter substrate reactivity, or increase structural stability.

Peptide synthesis requires very high precision since a single amino acid mismatch may result in the complete deactivation of the protein. Ribosomal peptide synthesis relies on the very selective recognition of the mRNA codon by the anticodon of an aminoacyl-tRNA unit in the ribosomal A site, after which the terminal NH₂ group forms a peptide bond with the activated C terminus of the peptide chain bound in the ribosomal P site.[9] The high fidelity of peptide synthesis is guaranteed by two mechanisms, both relying on the molecular recognition event:[10] 1) The formation of the peptide bond requires the release of the elongation factor Tu from aminoacyl-tRNA, which is induced by the hydrolysis of GTP to GDP. This takes several milliseconds, which is a time scale on which mismatched aminoacyl-tRNAs dissociate. 2) The hydrolysis of GTP induces a conformational change which alters the codon–anticodon interaction. Only the correct aminoacyl-tRNA interacts strongly with mRNA in both conformations, an incorrect one does not.

Enzyme catalysis frequently involves the formation of a transient covalent bond between the substrate and the enzyme, which is referred to as covalent catalysis.[11] For example, the enzymatic decarboxylation of acetoacetate to acetone and carbon dioxide involves the formation of a Schiff base between a lysine residue and the carbonyl group. In this way, the highly unfavorable expulsion of a highly basic enolate ion at neutral pH values, which occurs in the uncatalyzed pathway, is circumvented.[12] Nucleophilic catalysis frequently involves the temporary acylation of a serine or histidine residue. For example, the use of a serine hydroxy group rather than a water molecule is preferred in hydrolysis reactions not only because of the higher nucleophilicity of the alcohol, but also because the nucleophilic attack occurs in a “pseudo”-intramolecular manner and is, therefore, favored entropically. In this context, it is of interest to note that Zhang and Houk recently stated boldly that the high proficiency of enzymes in all cases is a consequence of the formation of a temporary covalent bond between the substrate and enzyme.[13] Although this concept has been questioned,[14–16] it is undoubtedly true that enzymatic reactions very frequently follow a pathway quite different from that of the...
uncatalyzed reaction and involves the formation of a covalently bound intermediate.\[17\]

Protein folding is a delicate interplay between noncovalent interactions and the formation of covalent disulfide bonds.\[18\] The structures of native proteins are destabilized by the loss of conformational entropy associated with protein folding.\[19\] Stabilizing covalent disulfide bonds are used to compensate for that loss. In addition, disulfide bonds reinforce the stability of the noncovalent interactions in the vicinity by augmenting the local concentration of the functional groups involved in recognition. In this case the disulfide bonds add the novelty of reversibility. In their classical contributions on protein folding, Anfinsen and Haber showed that denatured ribonuclease containing random disulfide bonds spontaneously converted into the thermodynamically more stable native ribonuclease upon addition of trace amounts of the reducing agent β-mercaptoethanol.\[20\]

Nature benefits greatly from the covalent capture principle, and this has inspired chemists to apply the same strategy in artificial systems.\[21\] The purpose of this Review is to show that the covalent capture strategy is important for many areas of research and that it allows chemists to tackle problems which are difficult to address in other ways. In fact, the examples are so numerous that we can by no means give an exhaustive review. Therefore, we have applied the following two stringent criteria: 1) the elements involved in molecular recognition must be present before and after the formation of the covalent bond. This excludes the formation of covalent bonds catalyzed by metals\[22–26\] or small organic molecules,\[27\] as well as substrate-directed organic synthesis.\[28,29\] Here, the noncovalent interactions that drive stereo- or regioselective bond formation are induced by transient species (catalysts, directing groups) which are not part of the final product. 2) Almost exclusively, examples will be discussed in which only two molecules are involved in the molecular recognition and which become united in one new molecule. This excludes the covalent capture of oligomeric or multimeric structures\[30–32\] and also template-directed reactions.\[33\] Intriguing examples of the latter can be found in the areas of dynamic combinatorial chemistry\[34,35\] and self-replication.\[36\] Preference is given to examples that illustrate the advantages and challenges of this approach.

2. Concept

This Review is divided in two major parts based on whether the covalent bond formed is irreversible or reversible, since this causes a major conceptual difference. In the two concepts, the product formation or distribution in the case of a competition experiment is determined by different parameters (Figure 2).

The principle behind irreversible covalent capture is that the formation of the complex accelerates the formation of the covalent bond relative to the reference (ref) bimolecular reaction ($\Delta G_{\text{capture}} < \Delta G_{\text{ref}}$), where the effect is proportional to the rate acceleration observed for the intra- versus the intermolecular reactions in entirely covalent systems. The origin of this rate acceleration has been the topic of intense debate over the past decades. Here we will not enter into this
Covalent Capture


Theoretical discussion, which itself has been the topic of several reviews. The most frequently used experimental parameter to evaluate the efficiency of an intramolecular reaction is effective molarity (EM). The effective molarity represents the concentration of the reagents (or one of the reagents) at which the intermolecular reaction proceeds at the same rate as the intramolecular reaction. Mandolini showed that, in the absence of ring strain, the effective molarity in ring-closure reactions of difunctional chain molecules can be correlated to the number of rotatable bonds in the linker. A maximum EM is expected for short rigid linkers that position the reactive groups in exactly the right position to form the transition state.

Compared to entirely covalent systems, enthalpy contributions in pseudo-intramolecular reactions in complexes play a much more dominant role in determining the efficiency of the formation of the covalent bond. In fact, it was shown for many supramolecular systems that the experimentally observed EM values were significantly lower than those calculated based on entropy contributions only. A critical feature in irreversible covalent capture is the compatibility of the complex geometry with the formation of the transition state. Incompatibility will lead to (partial) rupture of the complex, which inevitably means an increase in the \( \Delta G^\text{capture} \) value, with an enthalpic contribution equal to that required for (partial) breaking of the noncovalent bonds. Such a negative effect is more likely to occur for short rigid linkers in combination with highly directional noncovalent interactions, such as hydrogen bonding, which is exactly opposite to the entropy effect. The above poses a challenge for using irreversible covalent capture and, more importantly, for applications in which irreversible covalent capture is used as a covalent “read out” of the binding strength of the noncovalent interaction. It must be mentioned that this discussion is valid in the case where the formation of the covalent bond is the rate-determining step, which is true for the majority of experimental cases discussed later. In exceptional cases, however, the formation of the complex is the rate-determining step (\( \Delta G^\text{complex} > \Delta G^\text{capture} \)), which creates new opportunities for applying the covalent capture strategy (Section 3.4.1).

The past decade has witnessed the emergence of dynamic covalent chemistry which relies on the formation of a reversible covalent bond. Dynamic covalent chemistry unites the advantages of noncovalent recognition (self-organization, error correction) with the intrinsic stability of a covalent bond. The application of dynamic covalent chemistry opens up new perspectives in the application of covalent capture. The presence of reversible covalent bonds in reversible covalent capture implies that these systems are under thermodynamic control. Under these conditions, the stability of transition states (and thus rate acceleration) is no longer of importance. Rather, the product distribution is now determined by the relative thermodynamic ground-state stabilities of the free components, that is, the complex (\( \Delta G^\text{complex} \)) and the reversibly captured complex (\( \Delta G^\text{complex} + \Delta G^\text{capture} \)). From a design point of view, this is rather attractive since it eliminates knowledge about the exact nature of the transition-state structures. On the other hand, reversible covalent capture does not directly correlate the “captured” product to the stability of the initial complex for the simple reason that the geometrical constraints imposed by the covalent bond may not be compatible with the noncovalent interaction. Therefore, similar to irreversible capture, applications in which reversible capture is used to quantify the binding strength in the initial complex require careful system design and analysis of the results.

Reversible covalent capture is exploited in two different experimental regimes. In the first case, it is used as a tool to shift the complex equilibrium towards the product. Under conditions (for example, high dilution, highly polar solvents) where \( \Delta G^\text{complex} \) is too small to induce formation of significant amounts of complex, the formation of a reversible covalent bond (\( \Delta G^\text{complex} + \Delta G^\text{capture} \)) can push the equilibrium towards the formation of the complex. In the second case, reversible covalent capture is used to evaluate the differences in the thermodynamic stabilities between covalent adducts. In these cases, and assuming isoenergetic covalent bonds, it is the strength of the noncovalent interaction in the adduct that determines the product distribution in mixtures.

The different nature of the two covalent capture strategies sets different demands for the chemical reaction used. Apart from an essential compatibility with the recognition units and the medium, intrinsically no limitations exist for the reactions used for irreversible capture. However, the practical choice is determined by the criterion that the formation of the covalent bond must be very slow in the absence of the molecular recognition event. The high concentration of building blocks required for weak complexes typically require a reaction between weak nucleophiles (-OH, -NH₂, or -SH) and electrophilic acceptors such as epoxides, anhydrides, maleimides, and \( \alpha,\beta \)-unsaturated aldehydes. However, in the case where complexes persist even at very low concentrations, highly reactive functional groups can also be used, since the second-order background reaction is very slow under highly dilute conditions (see Section 3.4.1).

The formation of a covalent bond in dynamic covalent capture has the prerequisite of being reversible under the experimental conditions. Many reversible covalent bonds are known, for example transesterifications, aldol exchange, Michael/retro-Michael reactions, Diels–Alder/retro-Diels–Alder reactions, and alkene metathesis. The two predominantly used reversible reactions are disulfide exchange and transamination reactions. Both exchange reactions are fast, occur under mild conditions, and are highly compatible with other functional groups and the use of polar media (including water). An additional important aspect is that the reversibility can be “turned off” by acidification or reduction, which facilitates characterization of the captured product.

3. Irreversible Covalent Capture

3.1. Artificial Enzymes

The development of catalysts able to compete with enzymes both in terms of activity and selectivity continues to be one of the main challenges for chemists. It is
evident that the concept of covalent capture cannot lead to a catalyst, since, by definition, the formation of a covalent bond between the catalyst and the substrate kills the catalyst, unless a further fast reaction allows turnover (for a different approach see Section 3.4.2). Nonetheless, it is a particularly valuable method for mimicking enzymes that use covalent catalysis, in particular in the reaction steps leading to the formation of the covalently modified enzyme intermediate. Although numerous beautiful examples, both in terms of design and activity, have been reported, we will focus our discussion on the classical cyclodextrin-based catalysts. The simplicity of these catalysts has allowed structural studies which shed light on fundamental issues related to the covalent capture strategy.

Cyclodextrins (CDs) are water-soluble cyclic oligomers of \( \alpha\)-d-glucose with a hydrophobic cavity that is lined on both rims by hydroxy groups (Figure 3a).\(^{[51]} \) The most readily available cyclodextrins \( \alpha\)-CD, \( \beta\)-CD, and \( \gamma\)-CD differ in the number of \( \alpha\)-d-glucose units (6, 7, or 8, respectively). The cavity is slightly wider on the side of the secondary hydroxy groups, which, together with some steric hindrance from the primary \( \alpha\)-CH\(_2\)OH groups, means that small organic molecules enter the cavity on that side. It was already observed in the 1960s by Bender and co-workers that the inclusion of \( m\)-nitrophenylacetate in \( \beta\)-CD results in the transfer of the ester group to the hydroxy groups of the cyclodextrin about 100 times faster than the hydrolysis rate under the same conditions.\(^{[52]} \) Mechanistically, this transfer resembles the first step of a serine protease, which involves acyl transfer of the substrate to a serine hydroxy group of the enzyme. Importantly, Bender and co-workers observed that the Hammet plot of the cyclodextrin-catalyzed transacylation reactions of several substituted benzoates was not linear, thus suggesting that the rate accelerations were not determined exclusively by the electronic properties of the substrates.\(^{[53]} \) Subsequent molecular modeling studies by Breslow and co-workers indicated that formation of the tetrahedral intermediate required the substrates to be partially pulled out of the cavity.\(^{[54]} \) In other words, the geometrical positioning of the ester and \( \beta\)-CD hydroxy groups are far from optimal, and a large part of the favorable binding energy between the host and guest is lost upon formation of the transition state. This is further illustrated by the difference in the rate of acyl transfer between adamantane- and ferrocene-based \( p\)-nitrophenylesters 1 and 2 (Figure 3b).\(^{[55,56]} \) Although the complex formed between adamantane derivative 1 and \( \beta\)-CD is significantly stronger than that formed between ferrocene derivative 2 and \( \beta\)-CD \( (K_{\text{cat}} = 3300 \text{ and } 175 \text{ m}^{-1}, \text{respectively}), \) the rate of deacylation of 1 is several orders of magnitude lower \( (k_{\text{cat}}/k_{\text{uncat}} = 2150:1 \text{ versus } 5.9 \times 10^6:1). \) The huge rate enhancement observed for 2 indicates that the transition state is bound as strongly as the substrate, which was supported by molecular models. The importance of geometrical issues was further emphasized by observations by both Breslow et al.\(^{[56]} \) and Menger et al.\(^{[57]} \) which revealed a strong decrease in the rate of deacylation for less-activated substrates. For poor leaving groups, the rate-determining step becomes the departure of the leaving group \( (k_3), \) instead of the attack on the hydroxy group for activated substrates \( (k_1, \text{Figure 3c}). \) Consequently, for poorly activated substrates, the transition state comes after the formation of the tetrahedral intermediate, instead of before. Since this requires rotation of the ester bond, this comes with a high energy penalty because of the rigidity of the system. In fact, the insertion of a rotor into the substrate solved this problem.\(^{[58]} \)

The examples discussed here clearly show that the efficiency of product formation does not simply reflect the association constant between the two components, but rather the extent to which transition structures are stabilized. An important parameter is the formation of the productive complex, in which the reactive groups are optimally oriented for the formation of a covalent bond. Only in this case is maximum benefit obtained from preorganizing the reactive

Figures

Figure 3. a) Structure of \( \beta\)-cyclodextrin. b) Substrates 1 and 2. c) Acyl transfer from an ester bound in the CD cavity to a hydroxy moiety of the CD.
groups in a complex. So, although important, exploiting entropy may not be sufficient to guarantee the covalent capture of the “best” substrate in terms of binding strength. These studies show that EM values necessarily also contain (to a different extent) an enthalpic contribution.

3.2. Structural Stabilization of Supramolecular Aggregates and Foldamers

Self-assembly is the spontaneous association of molecules into a well-defined structure that is held together by weak interactions, such as hydrogen bonds, coordinative bonds, electrostatic interactions, or hydrophobic interactions.[59–62] Over the past 15 years self-assembly has emerged as the best strategy for obtaining molecular architectures with dimensions on the nanoscale. The key features of the self-assembly strategy are the small synthetic effort needed to make the building blocks, the possibility of correcting errors during the assembly process, and the adaptability of the resulting assembly to external stimuli.[34,35] On the other hand, an inherent weakness of noncovalent interactions is that the kinetic stability of the aggregates is often low. As an extension of templated synthesis,[63] the covalent capture of dynamic aggregates, referred to as covalent postmodification, has been developed as an elegant tool to convert the aggregates into kinetically inert structures.[64]

The covalent capture of an assembly composed of two cyclic peptides by ring-closing metathesis reported by Ghadiri and co-workers is taken as an illustrative example. (Figure 4)[65,66] The eight-residue cyclic peptide 3 alternates with amino acids of opposite chirality which predispose the amide bonds for the formation of intermolecular hydrogen bonds.[67] Selective N-methylation of the amide backbone ensures that dimer 3₂ is the exclusive aggregation product—otherwise oligomeric cylindrical nanotubes are obtained. Peptide 3 is equipped with two l-homoallylglycine residues for use in olefin metathesis reactions. As a result of the C₂ symmetry of 3, dimer 3₂ exists in two interconverting diastereomeric forms 3₃₂ and 3₃₂, of which only in 3₃₂ do the allyl moieties have the correct alignment for covalent capture. Despite its low association constant in CDCl₃ (99 ± 1M⁻¹), it was observed that a ring-closing metathesis reaction followed by reduction of the double bonds in the formation of dimer 4 in an impressive yield of 65 % after 48 h. Attempts to perform a covalent capture in polar solvents yielded no metathesis products, thus indicating that hydrogen-bond-induced dimerization is essential for increasing the local alkene concentration. Evidence that the hydrogen bonds persisted in 4 was obtained from 2D ROESY experiments and FTIR spectroscopy.

The covalent capture method has also been applied extensively to increase the structural stability of foldamers. Foldamers can be defined as oligomers with a characteristic tendency in solution to spontaneously adopt a secondary structure which is stabilized by noncovalent interactions between non-adjacent subunits.[66–70] The most frequently studied foldamers are α-helical peptide structures, as well as other helices and β sheets. Covalent stabilization of a foldamer allows the secondary structure to persist, even in very short sequences and outside of a protein context. Predominant strategies are the cross-linking of peptidic side chains[71–73] and the use of covalent hydrogen-bond surrogates.[74–76] The latter strategy has the advantage that the solvent-exposed surface of the α helix remains intact and that no side-chain functionalities have to be “sacrificed”. In addition to structural stabilization, covalent capture has the additional advantage of improving the proteolytic stability with respect to the unconstrained analogue.[77]

3.3. Covalent Modification of Biomolecules

3.3.1. Site-Selective DNA Alkylation

Molecules that are able to interfere with gene expression are of great therapeutic value.[78] Based on the natural compounds distamycin and netropsin, Dervan et al. developed a unique class of pyrrole-imidazole-based polyamides that were able to bind in a predictable manner in the minor groove of DNA with nanomolar affinity.[79] The binding selectivity originates from a combination of hydrogen-bond formation with the Hoogsteen edges of the DNA base pairs and steric complementarity. The ability of polyamides to interfere with DNA expression in vitro and also in vivo has been illustrated by numerous examples.[80,81] Amongst other compounds, polyamides have been used to inhibit gene expression by binding to promoter and enhancer regions,
thus preventing the formation of the transcription machinery. Any noncovalently bound molecule is simply expelled by the RNA polymerase during the transcription of DNA. It has been shown by various research groups that this problem can be solved by conjugating the polyamide to an DNA-alkylating agent, such as chlorambucil. In this way the polyamide is anchored in a precise location on the DNA through an irreversible covalent bond. Expulsion is hence no longer possible, and the molecule may inhibit gene expression.

Interestingly, in the course of these studies Dervan and co-workers accidentally discovered that a relatively small structural change in the polyamide–chlorambucil conjugate dramatically changed its effectiveness in arresting cancer proliferation in mice. The two polyamide–chlorambucil conjugates 5-Chl and 6-Chl are constitutional isomers that differ in the hairpin turn moiety used (α- or γ-diaminobutyric acid, respectively; Figure 5a). The polyamide fragment targets the 5'-WWGGGWG-3' sequence (W = A or T) located in the coding region of the histone H4c gene of the human colon carcinoma cell line SW620, which is subsequently alkylated by the chlorambucil moiety (Figure 5b). Polyamide 5 binds the target site with an association constant of \( K_{\text{ass}} = 6.7 \times 10^{10} \text{M}^{-1} \), with a 94- and 26-fold selectivity over two target sequences with a single base-pair mismatch. The γ turn present in parent polyamide 6 allows for a better folding of the polyamide in the DNA minor groove, which is reflected by the 2.4-fold higher affinity (\( K_{\text{ass}} = 1.6 \times 10^{10} \text{M}^{-1} \)) for the target site, but with significantly lower selectivity (15- and 6-fold) over the mismatch sites.

The alkylation behavior of conjugates 5-Chl and 6-Chl is completely different in terms of both specificity and activity. Up to a concentration of 30 nM, conjugate 5-Chl exclusively alkylates the target site, whereas 6-Chl is not specific and alkylates both the target and mismatch sites at concentrations as low as 3 nM. A time-dependent study of the alkylation revealed that under identical conditions 5-Chl reacts 120 times slower than 6-Chl, which is remarkable considering the structural similarity between the two conjugates. Finally, in vivo studies revealed that 5-Chl arrested cancer growth in a SW620 xenograft nude mouse tumor model, whereas 6-Chl proved lethal to the mice. Although the exact origin of this difference has not yet been disclosed, successful covalent capture in this case occurs through a combination of high binding selectivity and, counterintuitively, less-efficient covalent bond formation.

### 3.3.2. Irreversible Enzyme Inhibitors

Molecules able to irreversibly bind to the active site of enzymes are not only useful as pharmaceuticals, insecticides, and pesticides, but also as activity profilers or mechanistic probes. This is by far the largest and commercially most important research area in which the covalent capture strategy is applied, with the first examples dating back to the 1960s.

The reaction of an active-site-directed irreversible inhibitor, also referred to as affinity label, involves the initial formation of an enzyme–inhibitor complex followed by formation of a covalent bond. This sequence is identical to the first step of any enzymatic transformation that comprises formation of an intermediate, with the important difference that the reaction stops at this stage and, hence, the catalyst (the enzyme) does not turn over. A plot of the initial reaction rate against substrate concentration corresponds to a typical Michaelis–Menten mechanism and, consequently, the reaction shows saturation kinetics.

An affinity label also has other characteristic features: the rate of enzyme inactivation by the affinity label is lowered by competing reversible inhibitors and substrates, the reaction rate shows a similar pH dependence as the rate of the corresponding catalytic transformation, and the inhibitor forms a 1:1 covalent complex with the enzyme. The principal reactive groups in proteins are nucleophiles (the -OH group of serine, tyrosine, and threonine; the -NH2 group of lysine; the imidazole ring of histidine; the -SH group of cysteine) if necessary activated by general base catalysis. Consequently, affinity labels are often electrophilic species such as epoxides, anhydrides, malonimides, aldehydes, or haloalkanes. The reactivity is in general not very high as this would lead to nonselective formation of a covalent bond. The low reactivity is compensated for by the fact that the formation of a covalent bond in the active site occurs pseudo-intramolecularly, which is a characteristic feature of covalent capture.
Nonetheless, a much higher selectivity is obtained when the catalytic properties of the target enzyme itself are used to generate the reactive species.[91,92] These types of inhibitors are referred to as suicide inhibitors. An effective suicide inhibitor has the following characteristics: it is chemically inert in the absence of enzyme, activated specifically by the target enzyme, and, after activation, the formation of the covalent bond occurs more rapidly than dissociation. A textbook example is the suicide inhibition of the enzyme β-hydroxysterdecanoyldehydrase by 3-decynoyl-N-acetylcysteamine (7; Figure 6).[93] The enzyme activates the substrate by proton abstraction to form a highly reactive Michael acceptor, which is then alkylated on the histidine residue of the active site to give the covalent adduct E-7.

Apart from pharmaceutical applications, covalent capture is also finding application in the field of proteomics, which deals with the study of the structure and function of proteins both in assays and in vivo.[94] Activity-based protein profiling (ABPP) is defined as a chemical strategy that utilizes active-site-directed covalent probes to profile the functional state of enzymes in a complex proteome, that is, the entire arsenal of proteins expressed by prokaryotic or eukaryotic genomes (Figure 7a).[95–97] Typically, classes of enzymes are targeted rather than a specific enzyme. Activity-based probes have the same characteristics as irreversible inhibitors, but contain an additional tag, which allows detection of the covalent capture event (Figure 7b). For this purpose, fluorophores, biotin, or inert chemical handles (azides and alkynes for click reactions or Staudinger reactions) are frequently used. Cravatt and co-workers prepared a series of fluorophosphonate (FP) probes equipped with fluorescein or biotin for the visualization of catalytically active serine hydrolases in proteomes (Figure 7c).[98] These probes label covalently active serine hydrolases through attack of the nucleophilic -OH group of serine on the phosphorus center. Cysteine, aspartyl, and metallohydrolases do not react with the fluorophosphonate moiety. Therefore, exposure of a proteome to a probe results in the selective labeling of any active serine hydrolases present. The ability of ABPP to resolve complex patterns was illustrated by comparing the profiles of phosphorylated proteins from extracts of several rat organs, which resulted in the unexpected discovery of a serine hydrolase in the prostate.

Schultz, Harris, and co-workers showed that enzyme activity can be quantified by measuring the amount of enzyme trapped by a mechanism-based inhibitor on a microarray.[99] Multifunctional activity probes were composed of a small peptide for inhibition of cysteine protease, an acrylate moiety for covalent capture, a peptide nucleic acid (PNA) for encoding, and a fluorophore for optical readout (Figure 7d). Exposure of a crude cell lysate to the probes resulted in the covalent capture of active cysteine proteases; unreacted probes were then removed by using a size-exclusion filter. The captured enzymes were directed to specific locations on a oligonucleotide microarray by PNA-DNA hybridization, and then a fluorescent readout of the encoded regions was used to quantify the amount of cysteine protease present.

Although theoretical (Section 2) and experimental model studies (Section 3.1) indicate that a direct correlation between

![Figure 6. Mechanism for suicide inhibition of the enzyme β-hydroxysterdecanoyldehydrase (E) by 3-decynoyl-N-acetylcysteamine (7).](image)

![Figure 7. a) Strategy of activity-based protein profiling. A proteome is exposed to an activity probe that selectivity reacts with the target protein. The tag serves for isolation (for example, biotin) or visualization (for example, a fluorophore). b) An activity probe generally consists of a targeting device for selective binding, a "warhead" for forming the covalent adduct (frequently a Michael acceptor), and an analytical handle. c,d) Examples of activity probes by Cravatt and co-workers[98] and Schultz and co-workers.[99]](image)
the strength of binding and the rate of covalent bond formation does not exist, this is hardly an issue for ABPP. The differences in the active site and catalytic mechanisms between different enzyme classes are so large that the risk of trapping an enzyme that does not belong to the target group is very low. In fact, the affinity label of the probe identified by Schultz, Harris, and co-workers showed a clear inhibition of lymphocyte-mediated cell death.

3.3.3. Probing Interactions between Biomacromolecules

The elucidation of interactions between biomacromolecules (protein–protein or protein–DNA/RNA) is of key importance for understanding biological processes and the development of new pharmaceuticals. The identification of protein–protein interactions is a daunting task for which the covalent capture strategy appears very promising. In its most rudimentary form, exogenous nonspecific cross-linkers are added to a protein mixture to yield very complex mixtures of coupled proteins. The complexity can be largely reduced by conjugating the linker to the (engineered) protein of interest, which then covalently captures its partner upon interaction. Additional complications result from the fact that many protein–protein interactions are regulated by post-translational modifications (PTMs) of the proteins which are both transient and substoichiometric in nature. For example, the Smad2 signaling protein forms stable homotrimer in response to the receptor-mediated phosphorylation of Ser465 and Ser467, whereas monophosphorylation at Ser465 affords a homotrimer with lower stability. The homotrimer is disrupted by the addition of the SARA binding domain of the SARA protein. Muir and co-workers used this system to illustrate that a PTM-mediated protein–protein interaction can be covalently captured by engineering both a photoactive amino acid cross-linker and a phosphorylated Ser465 residue in the MH2 domain of the Smad2 protein. The successful covalent capture of the homotrimer was evident from the observation that irradiation of the complex led to the formation of species with molecular weights corresponding to the dimeric and trimeric versions of Smad2. These species were barely detectable without irradiation or in the control protein. Additionally, the amount of dimers and trimers formed diminished greatly when the proteins were pretreated with an excess of SARA-SBD, thus indicating that covalent capture requires the formation of trimers.

An elegant application of covalent capture for the detection of a protein/RNA/ligand complex was recently provided by Arndt and co-workers (Figure 8). Thiostrepton is an antibiotic with a sub-nanomolar affinity for the 50S subunit of the bacterial ribosome. Upon binding, thiostrepton locks the conformation of the flexible ribosomal protein L11 on the ribosomal 23S rRNA and blocks the action of ribosomal GTPases. Nosipeptide has a similar activity and molecular structure as thiostrepton, but the exact location and molecular structure of the complex have yet to be determined. In this context, covalent capture becomes attractive as a mechanistic tool. Both thiostrepton and nosipeptide contain dehydroalanines that are able to act as Michael acceptors for a nucleophilic thiol. Therefore, a series of mutants of the L11 protein were generated with single Cys residues inserted by site-directed mutagenesis and their reactivity towards thiostrepton and nosipeptide in the presence of 23S rRNA was tested. Whereas four reactive positions on L11 could be identified for thiostrepton, nosipeptide only gave a covalent adduct with two mutants. Presumably this is related to the presence of only one Michael acceptor in nosipeptide as opposed to three in thiostrepton. The observed reactivity pattern of covalent capture gives a readout of the orientation of the ligand in the binding site, which is a valuable mechanistic tool in combination with data from other sources (for example, NMR spectroscopy, X-ray crystallography, or molecular modeling).

3.4. Irreversible Combinatorial Chemistry
3.4.1. DNA-Templated Organic Synthesis

DNA-templated organic synthesis (DTS) recently experienced further interest. The elegance of DTS relies on its conceptual straightforwardness: DNA hybridization is used to increase the effective molarity of reactive groups attached to the DNA strands. This allows common synthetic organic reactions to be studied at nanomolar concentrations, at which the rate of the nontemplated reaction is infinitely small. Of all the known self-assembly motifs, DNA hybridization can be controlled and programmed to the highest extent. The high fidelity and robustness of DNA hybridization gives complete control over the relative position of both DNA strands, thereby giving a unique opportunity to systematically vary the position of the reactive groups.

Studies by Liu and co-workers of the rate of a wide variety of organic reactions as a function of the number of nucleotides separating the functional groups gave very surprising results (Figure 9a). In terms of effective molarity, a decrease in the rate of bond formation would be expected upon increasing the distance. However, for many reactions (for example, formation of an amide bond, nitro-aldol addition, Wittig olefination, Heck coupling) it was observed that the rate of bond formation was independent of the distance separating the reactive groups (from 0 up to 10 bases, or even 30 bases in the case of thiol addition to maleimide or α-iodoacacetamide). This finding was rationalized with the hypothesis that the rate-determining step is not the formation of the covalent bond, but the annealing of the two DNA strands. This hypothesis was confirmed by the observations that the formation of the covalent bond followed second-order kinetics (first order in each of the DNA strands) and that lowering the concentration of both strands resulted in a decreased rate of bond formation. This observation led to the proposal of a model in which DNA annealing is the rate-
determining step when the separation is small, which results in the covalent bond forming at a constant rate (Figure 9a). Increasing the distance results in a continuous decrease in the effective molarity of the reactive groups until the formation of the covalent bond becomes the rate-determining step (thus inducing distance dependence).

Distance dependence is a negative feature of DTS as it limits the positions on a DNA template that can encode the reagents. This problem was resolved by using a so-called \( \omega \) (omega) architecture for the template strand, which contains a small number of nucleotides (3–5) at the reactive 5' end that complement an appending region on the reactive 3' end of the reagent. This number of base pairs is too small to induce annealing, which occurs only in the case when the reagent contains an additional coding region that is complementary to a distant coding region on the template. This results in the formation of a bulge (\( \omega \)) in the template, which brings the two reactive groups in proximity. With this setup, the distance-dependent 1,3-dipolar cycloaddition and reductive amination reactions proceeded efficiently even when the coding region was 15–25 bases away from the reactive ends.

The enormous potential of DTS in the elucidation of organic reactions was illustrated by studying the formation of covalent bonds in a 144-membered combinatorial library obtained by mixing two reagent pools A and B containing 12 substrates each (Figure 9b).[113] The substrates in pool A were connected to the 5' terminus of a DNA strand containing a region that encoded for the specific substrate. In addition, the DNA strands contained an annealing region for reagents of pool B. The substrates in pool B were attached through a biotin-containing linker to the 3' terminus of a DNA strand containing a coding region for the attached substrate. Mixing pools A and B resulted in the formation of 144 heteropairs, which were analyzed for productive combinations, that is, combinations of substrates leading to the formation of a covalent bond. Covalent capture results in a transfer of the biotin unit to the A substrates, which are successively captured with magnetic streptavidin beads and amplified by the polymerase chain reaction (PCR) with a DNA primer containing the cyanine fluorophore Cy3. A DNA microarray containing all the possible encoding combinations was then used to identify the active substrate combinations. As an example, this procedure led to the discovery of a new palladium(II)-mediated alkyne-alkene macrocyclization reaction.

3.4.2. Identification of Peptide Catalysts

The combinatorial synthesis and screening of catalysts eliminates, at least partly, the demanding task of rational design.[114] This is a daunting challenge since the screening protocol has to detect catalytic activity rather than a static molecular property such as binding affinity. Successful methods for on-bead catalyst screening rely either on IR thermography,[115] the formation of insoluble colored reaction products,[116] fluorescent pH indicators,[117] or the use of gels that prevent product diffusion.[118,119]

The covalent capture strategy has been implemented by setting up the system in such a way that the formation of the covalent bond only occurs when the peptide sequence displays catalytic activity. For example, Tanaka, Fuller, and Barbas prepared a 6.4 /C148 107-membered peptide library by phage display in which six amino acids were randomly appended to the C terminus of an 18-residue \( \alpha \)-helical peptide known to catalyze the decarboxylation of oxaloacetic acid.[120,121] The selection of peptides from this library was performed by covalently trapping those peptides able to form a stable enamino upon reaction with 1,3-diketones labeled with bovine serum albumin (BSA). The selected peptides...
catalyzed aldol and retro-aldol reactions with rate accelerations of up to 1400 ($k_{cat}/k_{uncat}$).

A very elegant screening approach was developed by Wennemers and co-workers for the identification of small peptides as catalysts for asymmetric aldol reactions. The screening methodology was based on the co-immobilization of the catalyst and one of the reagents on a resin (Figure 10a). A 3375-membered library of potential tripeptide catalysts was prepared by encoded split-mix synthesis on a resin, after which reagent A (a ketone derived from levulinic acid) was attached through a spacer.[123] The addition of reagent B (a dye-marked benzaldehyde) resulted in covalent attachment to the resin only when a catalytically active peptide sequence was present (Figure 10b). Isolation and analysis of the colored beads revealed two main consensus sequences, of which one (H-L-Pro-L-Pro-L-Asp-NH₂) outperformed L-Pro as a catalyst for the asymmetric aldol reaction. Conceptually, this approach represents a large step forward since it does not require knowledge about the reaction mechanism or the intermediates involved.

4. Reversible Covalent Capture

4.1. Hydrogen-Bond-Driven Self-Assembly in Aqueous Solution

The hydrogen-bond-driven self-assembly of molecules into well-defined aggregates in water remains one of the great challenges in the field of noncovalent synthesis. Considering the fact that biological self-assembly processes often involve the formation of multiple hydrogen bonds, it is evident that designed self-assembly processes in water have not yet reached the sophistication of those found in nature. The main successes in inducing the formation of hydrogen bonds in water have been obtained by creating hydrophobic micro-environments, for example, micelle-like or rodlike polymeric stacks.[126,127] Very recently, it was shown by Gong and co-workers that the combined use of hydrogen bonds and dynamic covalent bonds results in the spontaneous formation of discrete structures in water, where the hydrogen bonds induce sequence-selectivity.[128,129] Oligoamides 8 and 9 associate in nonpolar media to give a highly stable duplex 8-9 held together by six hydrogen bonds (Figure 11). The complementary arrangement of hydrogen-bond donors and acceptors in 8 (DADADA) and 9 (ADAADA) ensures that the heteroduplex 8-9 is the only product. Similarly, oligoamides 10 (DDAAD) and 11 (AADDAA) give exclusively the heteroduplex 10-11. In aqueous media, the formation of the hydrogen bond alone is not enough to confer sufficient thermodynamic stability to the dimers. However, in combination with the reversible formation of a disulfide bond, the exclusive formation of duplex 8-9 was observed when 8 and 9 were mixed in an equimolar ratio in a 9:1 H₂O/THF mixture and treated with iodine. The question remains as to what extent duplex formation is driven by the formation of hydrogen bonds. Control experiments indicate that in the absence of the complementary strands both 8 and 9 form a homocyclic structure through formation of an intramolecular disulfide bond. The presence of oligoamide 10 with a noncomplementary array of hydrogen-bond donors and acceptors did not interfere in the formation of the product: duplex 8-9 was the only product observed. On the other hand, combining 10 with its complementary partner 11 resulted in the exclusive formation of duplex 10-11.

The importance of the recognition event was even more evident from a subsequent experiment in which six oligoamides (present as three couples of complementary strands with the ability to form either 2, 4, or 6 hydrogen bonds) were mixed in an equimolar ratio.[129] Although a large number of duplexes could be formed statistically, at thermodynamic equilibrium each strand had exclusively dimerized with its complementary partner. This phenomenon is called self-sorting, which refers to the situation in which a system under thermodynamic control displays a nonstatistical composition.[130,131] Rather uniquely, the self-sorting in this system is based on a combination of noncovalent and dynamic covalent bonds. Self-sorting occurs because this gives rise to a situation with a maximum number of hydrogen bonds and covalent bonds.

The importance of the spatial positioning of the reactive groups and the recognition modules in determining the thermodynamic stability of the system was also nicely demonstrated by Bennet, Philp, and co-workers. In a simple model system they employed a reversible Diels–Alder
reaction between diene \( \text{12} \) and the dienophiles \( \text{13–15} \), which contain spacers of different length (Figure 12a).\(^{[132,133]} \) The reversible covalent bond is accompanied by the formation of two hydrogen bonds between the amidopyridine and the carboxylic acid moieties. The hydrogen bonds have a stabilizing effect on all the Diels–Alder products \( \text{16–18} \), but is maximal for product \( \text{17} \), in which the spacer length allowed the optimal geometry for the formation of the hydrogen bonds (Figure 12b). So, similar to the observation by Gong and co-workers, the strength of the noncovalent bonds is the determining factor in regard to the thermodynamic stability of the product.

However, the study by Bennes and Philp is also interesting for another reason, as it sheds light on a fundamental difference between irreversible and reversible covalent capture. Apart from studying the thermodynamic stability, the effect of the recognition event on the rate of product formation was also studied. Here, a different order was

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Figure 11. Sequence-specific association of oligoamides in aqueous media.

Figure 12. a) Reversible Diels–Alder reaction between diene \( \text{12} \) and dienophiles \( \text{13–15} \). b) X-ray crystal structure of Diels–Alder adduct \( \text{17} \).
observed and a maximum rate acceleration was determined for dienophile 13, which has the shortest spacer, not for 14. Clearly, the single carbon atom spacer allows easiest access to the Diels–Alder transition state, but does not provide sufficient flexibility to permit the retention of two hydrogen bonds in the final product. This accounts for the fact that 13 reacts faster than 14, while 17 is the most stable product. Consequently, the kinetically favored product in this model system is 16, whereas compound 17 is the thermodynamically most stable. It is important to notice that capturing the kinetic product is the equivalent of irreversible covalent capture (Section 3). In other words, an irreversible covalent capture in the model system of Bennes and Philp would have given 16 as the major product, whereas under reversible conditions the thermodynamic product 17 is dominant despite the fact that the noncovalent recognition event is the same in both of them.

4.2. Measuring Stability and Order in Biological Structures

4.2.1. Peptides

Knowledge of the forces that drive the folding of peptide strands into proteins is of crucial importance for understanding the mechanisms behind diseases related to protein misfolding and for the engineering of artificial peptide constructs. Protein-folding processes are generally measured by monitoring changes in a spectroscopic indicator of the higher order structure, such as a CD signal, upon imposing conditions that induce denaturing of the protein (concentration, temperature, ionic strength). A possible problem is that the intrinsic stabilities of the limiting conformational states, native and denatured, are affected by the experimental conditions.

Driven by this concern, Woll and Gellman developed a new strategy for measuring higher order stability in polypeptides. The so-called backbone thioester exchange (BTE) approach relies on the replacement of a backbone amide bond in the polypeptide with a thioester, which can undergo thioester/thiol exchange in aqueous solution at a neutral pH value (Figure 13). The validity of this model was initially tested on bovine pancreatic polypeptide (bPP), a 36-residue protein that adopts a well-defined tertiary structure in which the C-terminal segment is independent of the tertiary structure. In other words, systems in which secondary and tertiary structures are formed in a synergetic manner are much more complicated to analyze. This is not the case for bPP, which for a large part also preserves the α helix in the absence of the tertiary structure.

The elegance of this approach is that it allows for a rapid study of the effect of single mutations in the peptide chains on the stability of the folded structure. Such an analysis was performed for bPP by examining the K_{BTE} values upon Ala substitution of the Tyr_{93} and Tyr_{125} residues, which are known from NMR studies to play a critical role in the formation of the tertiary structure. In fact, the positive AG_{fold,BTE} values suggest that removal of these Tyr residues causes a loss of favorable contacts.

In follow-up studies, the same research group exploited the BTE approach to study the effect of substituting Phe with F-Phe on the conformational stability of the chicken villin headpiece subdomain (cVHPP) and for identifying preferential side-chain interactions at antiparallel coiled-coil interfaces. In regard to the latter case, it is interesting to note that in their classic paper on the chiroselective replication of peptides Ghadiri and co-workers had already used the same dynamic approach to demonstrate the stereospecificity of coiled-coil formation. The greater stability of a homochiral coiled coil over its heterochiral analogue was studied by the slow oxidation of a racemic mixture of coiled peptides 21^{DD}-SH and 21^{DD}-SH in air as well as by promoting disulfide exchange of heterochiral dimer 21^{DD}-S-S-21^{DD} in a glutathione redox buffer (Figure 14a). On statistical grounds, a 1:1 ratio of homochiral (21^{DD}-S-S-21^{DD}, 21^{DD}-S-S-21^{DD}) and heterochiral (21^{DD}-S-S-21^{DD}) coiled coils would be expected, but HPLC analysis clearly showed that in both experiments the heterochiral 21^{DD}-S-S-21^{DD} dimer was completely absent at thermodynamic equilibrium (Figure 14b).

Recently, the Gellman research group expanded the BTE approach to secondary peptide structures, in particular, the formation of β sheets. In a conceptually related prior study by Krishnan-Ghosh and Balasubramanian, the thermodynamic equilibrium was examined between two peptides 22 and 23 containing 1 and 4 Leu-Lys repeats, respectively, which are known to predispose peptides for β-sheet formation (Figure 15). With conventional disulfide exchange they observed a strong amplification of the dimeric peptide 23-S-
Covalent Capture

4.2. Bilayer Membranes

The hypothesis that clusters (or domains) of lipids are formed within fluid biological membranes, in which the lipid distribution is not random, has been the subject of much controversy. A number of studies indicate that these clusters, also referred to as “lipid rafts”, may play key roles in the control of a variety of cellular processes, including signal transduction and membrane trafficking. Furthermore, they have been associated with the production and cellular entry of viral particles. Cholesterol is a major component of mammalian cell membranes, and despite numerous investigations involving membranes comprising cholesterol and phospholipids, the structural role of this sterol in producing condensed, fluid membranes has remained a mystery.

Davidson and Regen have established a nearest neighbor recognition (NNR) procedure to quantify the tendency of lipids to cluster on the basis of the ability of a single lipid to react with its nearest neighbor. The technique relies on the reversible covalent capture of two adjacent lipids (or lipid and cholesterol) through the reversible formation of a disulfide bond. The principle of the strategy is very straightforward: if lipids A and B are connected to each other through a reversible bond, equilibration will result in the formation of a mixture of homo- (AA and BB) and heterodimers (AB). In the absence of any thermodynamic preference the equilibrium distribution is such that AA:BB:AB is 1:1:2, which corresponds to an equilibrium constant of $K = 4$ (Figure 16).

Any bias of the equilibrium in favor of the hetero- or homodimers is indicative of a nonstatistical distribution in the fluid bilayer. By using NMR spectroscopic studies, the authors were able to prove the preference of cholesterol for lipids with long hydrocarbon chains (C16 and C18) when the sterol concentration reaches biologically relevant levels. This has provided experimental support to the theory of the formation of “complexes” between cholesterol and lipids within biological membranes. Furthermore, evidence was obtained for the condensing effect of cholesterol on lipid bilayers. This implies that the flexible acyl chains of phospholipids are able to complement the shape of neighboring cholesterol molecules, thereby resulting in a large number of hydrophobic contacts and tight packing. Thus, cholesterol appears to act as a rigid hydrophobic template for the membrane lipids to maximize their interactions.

4.3. Reversible Combinatorial Chemistry

4.3.1. Drug Discovery

In all the previous examples of reversible covalent capture a small number of species and equilibria were studied. However, as will be shown next, dynamic covalent capture is also an attractive strategy for combinatorial drug discovery and catalyst selection.

Erlanson, Braisted, Wells et al. at Sunesis Pharmaceuticals have laid the conceptual foundation for a combinatorial screening through the report of a tethering strategy for ligand
discovery (Figure 17a).\textsuperscript{151–153} The approach consists of using disulfide exchange between a Cys residue located near the target site and a small library of potential disulfide-containing ligands. Most of the library members will show no intrinsic affinity for the protein and the disulfide bond between the ligand and protein will be easily reduced. However, binding interactions between a ligand and the target site of a protein will stabilize the disulfide bond and shift the thermodynamic equilibrium towards the modified protein. Determination of the composition at thermodynamic equilibrium then reveals the library member with the highest affinity for the target.

The validity of the tethering concept was first tested on thymidylate synthase (TS), which has a Cys residue located in the active site.\textsuperscript{151} This enzyme is involved in the synthesis of thymidine monophosphate (dTMP), and is thus a prominent anticancer drug target. Potential ligands were screened in sets of 10 with sufficient mass differences to allow identification of the captured components by mass spectrometry. Tethering experiments were performed in the presence of an excess of 2-mercaptoethanol to impose reducing conditions and thus reversibility. The screening of a total of 1200 disulfide-containing fragments resulted in a strong selection of N-arylsulfonamideproline derivatives, such as N-tosyl-D-proline. Importantly, as before, it is the noncovalent interaction between the ligand and the protein that determines the thermodynamic stability. This is evidenced by the fact that the observed distribution of kinetic products (in the absence of the reducing agent) is entirely different from the composition at thermodynamic equilibrium, thus showing only a moderate selection of N-tosyl-D-proline. The high sensitivity of the tethering strategy in detecting weak interactions is illustrated by the high value obtained for the dissociation constant (1.1 mM), which is at a level that would be hard to detect with conventional high-throughput screening.

The scope of the tethering strategy was further increased by two subsequent developments: fragment assembly\textsuperscript{154} and extended tethering.\textsuperscript{155} Fragment assembly implies that the final drug is obtained by a merger of fragments selected individually from different screening experiments.\textsuperscript{156} The binding of interleukin-2 (IL-2) to its receptor induces T-cell proliferation and is an important target for immune disorders. A series of interleukin-2 (IL-2) mutants where prepared in which Cys residues were introduced around the region of the protein involved in binding to its receptor. A screening of all the mutants against a library of 7000 disulfide-containing fragments resulted in the selection of a series of structurally related fragments with affinity for the area around the receptor binding site. A focused set of 20 compounds was next prepared in which the selected fragments were merged with a known inhibitor for IL-20 obtained from a traditional drug-discovery approach. Inhibition experiments revealed a 5–50-fold increase in the affinity for 8 out of 20 components compared to the original inhibitor, thereby bringing the IC\textsubscript{50} value down to the nanomolar region.

The extended tethering strategy implies the introduction of a Cys residue near the active site which is then covalently modified with an “extender”, a small molecule with an affinity for the protein but also containing a protected thiol group (Figure 17b). Deprotection liberates a thiol which can be used to perform a dynamic screening as described before. The selected fragments are then merged covalently with the extender to yield the final drug. The use of the extended tethering strategy led to the discovery of a novel inhibitor for caspase-3, a protein involved in apoptosis.

The results reported by Erlanson et al. and others,\textsuperscript{157,158} illustrate the added value of using reversible covalent capture in a drug-discovery program. The main advantage of the tethering strategy is the ability to detect weak interactions between the target protein and library members. By itself, this can be useful for discovering new lead compounds, but the improvement in the binding affinity of known inhibitors by adding self-selected fragments or the unification of multiple weakly binding fragments into a single high-affinity binder is also important.
4.3.2. Catalyst Discovery

The search for catalysts by dynamic chemistry offers the advantage of rapid access to different ligand systems without the hassle of synthesis. Although out of the scope of this Review, a lot of attention has recently been directed towards self-assembled catalysts in which the addition of a metal ion to a dynamic ligand system induces a redistribution of the composition in favor of the catalyst. For example, addition of Rh ions to a 2-pyridone/2-hydroxypyridine tautomer system equipped with diphenylphosphine ligands shifted the equilibrium fully towards the nonsymmetrical dimer, which is the noncovalent equivalent of a traditional covalent bidentate ligand.\[^{159}\] This strategy has been successfully exploited for the rapid generation and screening of libraries of supramolecular catalysts.\[^{160,161}\]

Recently, our research group has shown that reversible covalent capture has potential to identify new catalytic units (Figure 18a).\[^{162}\] In this case a ten-component hydrazide library was screened for components able to interact with a phosphonate target, which is a transition-state analogue of the basic hydrolysis of an ester moiety (Figure 18b). Functional groups that undergo stabilizing interactions with the phosphonate group should also stabilize the transition state and, hence, accelerate ester hydrolysis. Screening is straightforward, since the presence of stabilizing interactions enhances the thermodynamic stability of the corresponding hydrazone and leads to an increased concentration compared to a control scaffold that lacks the target. Changes in the composition of such a dynamic system can be conveniently monitored by \(^1\)H-\(^{13}\)C HSQC spectroscopy by following the characteristic imine signals in the spectrum.\[^{163}\] These studies revealed a correlation between the thermodynamic amplification and the efficiency of the selected chemical function to assist in intramolecular catalysis. For example, the screening experiment revealed a maximum amplification of hydrazone 24 containing an ammonium group, which accelerated the hydrolysis of the ester moiety in 25 by up to 60-fold with respect to a reference compound. Control experiments were in strong support of the assumption that rate acceleration resulted from stabilization of the transition state. Naturally, this study did not yield a true catalyst, since the intramolecular ester bond was required. Nonetheless, it is expected that this approach will be very useful when combined with an independent search for substrate affinity or when used to improve the properties of existing catalysts.

However, an in-depth study of the same system also shed light on the experimental boundaries in the application of reversible covalent capture.\[^{164}\] It was observed that thermodynamic amplification completely disappeared at high sample concentration or when a large excess of hydrazides was used. This was rationalized by the argumentation that under these conditions intramolecular interaction is disfavored over intermolecular interaction.

5. Summary and Outlook

The covalent capture strategy is a unifying theme for many areas of research. In its irreversible form, it is mainly used to gain information about the strength of the molecular recognition between various partners. It can be used to study simple host–guest to substrate–enzyme, and protein–protein interactions. More recent studies have shown that irreversible

Figure 18. a) Application of dynamic covalent capture for catalyst discovery. b) Target reaction. c) Selection of the functional group by the phosphonate transition-state analogue. d) Positioning of the selected group near the target bond.
covalent capture can be used to covalently “freeze” the dynamic products of self-assembly.

Traditionally, the covalent capture strategy was a kinetic approach that relied on the rate acceleration in the formation of the covalent bond through complex formation. The development of dynamic covalent chemistry has led to the application of the covalent capture strategy under thermodynamically controlled conditions. In both ways, a successful covalent capture strongly depends on the compatibility of the formation of a covalent bond with the formation of a complex. The examples discussed in this Review clearly reveal that the covalently captured product, obtained under either kinetic or thermodynamic control, does not necessarily correlate to the strongest noncovalent complex. This is an important issue to keep in mind when applying the covalent capture strategy.

The particularly successful application of the covalent capture strategy to protein systems results from the intrinsically high substrate selectivity. This largely offsets any suboptimal covalent capture arising from geometry issues, either kinetic or thermodynamic control, does not necessarily correlate to the strongest noncovalent complex. This is an important issue to keep in mind when applying the covalent capture strategy.

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[17] The comparison of enzyme-catalyzed reactions (and those of systems operating similarly to enzymes) with the corresponding reference uncatalyzed ones is always complicated because the two systems behave in quite different ways. This has led to many controversies: The heated debate between D. J. Cram and R. Breslow 25 years ago is a prominent example (see the letters exchange in Chem. Eng. News 1983, April 11).


