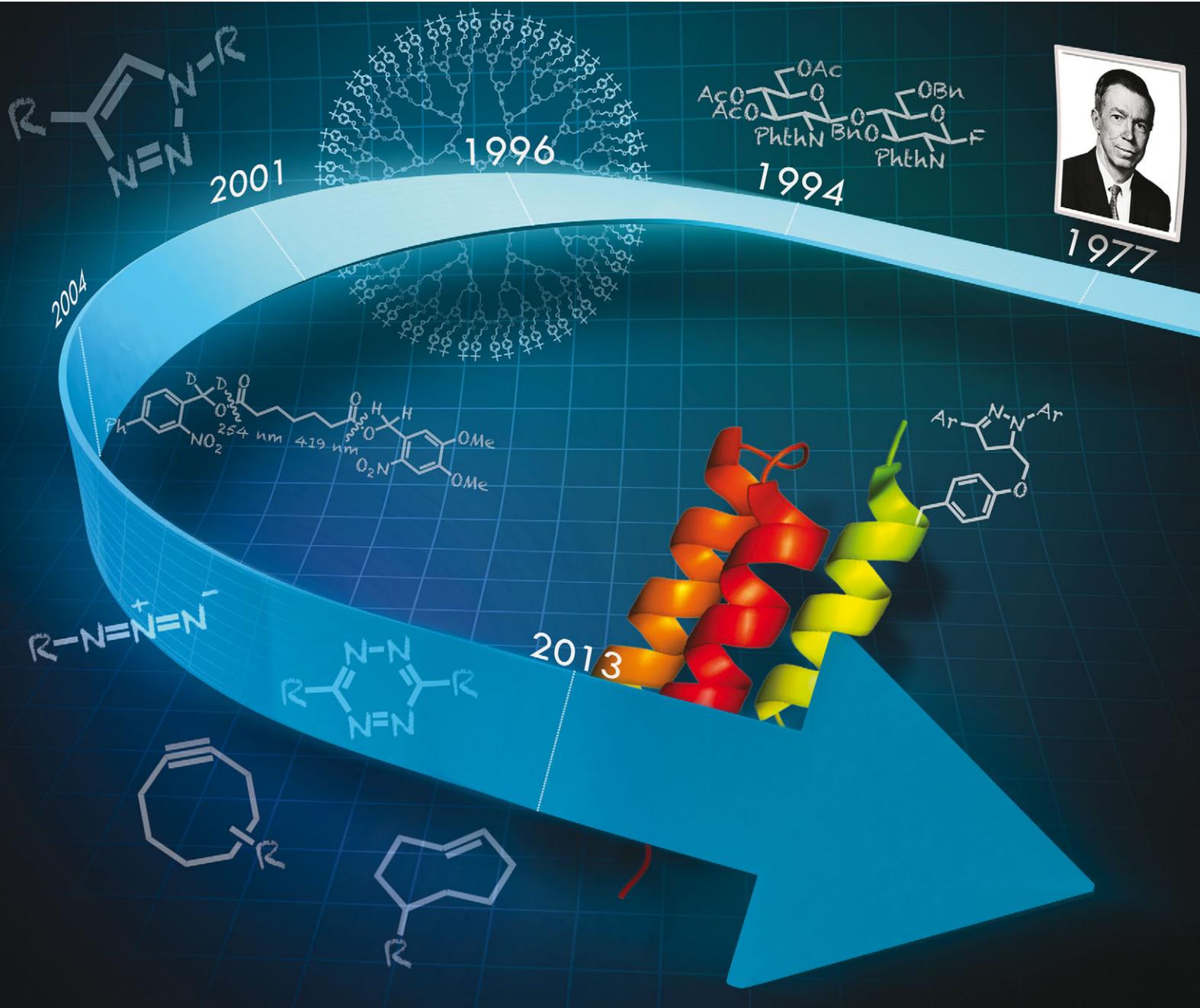


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FEATURE ARTICLE

Chun-Ho Wong and Steven C. Zimmerman
Orthogonality in organic, polymer, and supramolecular chemistry: from Merrifield to click chemistry



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Orthogonality in organic, polymer, and supramolecular chemistry: from Merrifield to click chemistry

Chun-Ho Wong and Steven C. Zimmerman*

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The concept of orthogonality has been applied to many areas of chemistry, ranging from wave functions to chromatography. But it was Barany and Merrifield's orthogonal protecting group strategy that paved the way for solid phase peptide syntheses, other important classes of biomaterials such as oligosaccharides and oligonucleotides, and ultimately to a term in widespread usage that is focused on chemical reactivity and binding selectivity. The orthogonal protection strategy has been extended to the development of orthogonal activation, and recently the click reaction, for streamlining organic synthesis. The click reaction and its variants are considered orthogonal as the components react together in high yield and in the presence of many other functional groups. Likewise, supramolecular building blocks can also be orthogonal, thereby enabling programmed self-assembly, a superb strategy to create complex architectures. Overall, orthogonal reactions and supramolecular interactions have dramatically improved the syntheses, the preparation of functional materials, and the self-assembly of nanoscale structures.

1. Introduction

As with many terms in chemistry, the term orthogonality, as applied to protecting groups, began with a very precise definition in a limited setting. Thus, in the context of his revolutionary

advance in peptide synthesis, Merrifield first reported the term in 1977 as a protecting group removal strategy.¹ It was a simple but powerful concept that one of multiple protecting groups could be removed in the presence of all others by using a cleavage reaction with a different mechanism.

Since then the usage of the term orthogonality has broadened dramatically. Indeed, the term is now used in supramolecular chemistry for systems where two non-covalent interactions occur with no crosstalk as well as in accelerated

*Department of Chemistry, University of Illinois at Urbana-Champaign,
600 S Mathews Avenue, Urbana, Illinois 61801, USA.
E-mail: sczimmer@illinois.edu; Tel: +1 (217)-333-6655*



Chun-Ho Wong

Chun-Ho Wong was born in Hong Kong in 1982. He received his first-class BSc (Hons) in Chemistry in 2004 and MPhil in 2006 from the Chinese University of Hong Kong, working with Prof. Hak-Fun Chow on dendrimer and supramolecular chemistry. He then spent a year as an editorial assistant at the SYNLETT (HK) regional editorial office. In 2007, he received the Croucher Foundation Scholarship

and commenced his PhD research at the University of Illinois at Urbana-Champaign under the guidance of Prof. Steven C. Zimmerman working on drug discovery for myotonic dystrophy. His research interests include drug design and chemical biology.



Steven C. Zimmerman

Steven C. Zimmerman was born in Evanston, Illinois, in 1957. He received his BS from the University of Wisconsin, his PhD from Columbia University, and was an NSF-NATO Post-doctoral Fellow at Cambridge University. He joined the University of Illinois faculty in 1985, served as interim or permanent Head of the Department for eight years, and is currently the Roger Adams Professor of Chemistry. He has broad interests in

organic, polymer, and supramolecular chemistry and their interface with biology and materials chemistry. He dedicates this article to his father-in-law, Prof. Isaiah Shavitt (1925–2012) and his father, Prof. Howard E. Zimmerman (1926–2012).

polymer and dendrimer syntheses. Recently, the widely-used, copper-catalyzed alkyne–azide coupling (CuAAC) has been described as a highly orthogonal reaction and in biological contexts as a bioorthogonal conjugation method. Although the click reaction by itself does not fit the classical Barany and Merrifield's definition of orthogonality (*vide infra*), its use in this context is pervasive and the clearest indication that as a chemical term orthogonality has achieved polysemic status.²

This feature article traces the history of orthogonal chemistry and summarizes its current application in several areas of chemistry with a focus on levels of orthogonal complexity.

2. Chemoselectivity

...“The ability to discriminate among the reactive sites is referred to as chemoselectivity.”...

Trost (1983)³

Although orthogonality predates chemoselectivity, it is helpful to discuss the latter term first. Chemoselectivity was coined by Trost in 1983 to describe the ability of a chemical reagent to discriminate among reactive sites.³ For example, in compound **1** the reaction with the sodium salt of dimethyl malonate in DMF occurs exclusively with displacement of the bromide to give **2** (Fig. 1).^{3,4} But in the presence of tetrakis(triphenylphosphine)-palladium(0), the allylic acetate is activated and undergoes displacement to **3** with no formation of **2**. The high chemoselectivity of these two reactions originates from their quite different mechanisms.

Chemoselectivity may also be achieved with a *single* mechanism and two structurally similar sites, provided that there is precise control of the reaction conditions. An example would be two acetal groups that cleave in acid, but have graduated reactivity; so increasing acidity entirely removes one and then the other. This strategy commonly employed in protecting group chemistry is referred to as the *modulated lability approach* (Fig. 2a).⁵ This approach takes advantage of the reactivity difference of functional groups A and B toward a mild and more forcing reagent in the same type of reaction. Indeed, the early peptide synthesis developed by Merrifield used the 1000 : 1 reactivity difference of the *t*-butyloxycarbonyl (Boc) and benzyl groups toward TFA to allow differential deprotection of the growing peptide at the amino terminus and without deprotecting the peptide side chains.⁶ The use of a stronger

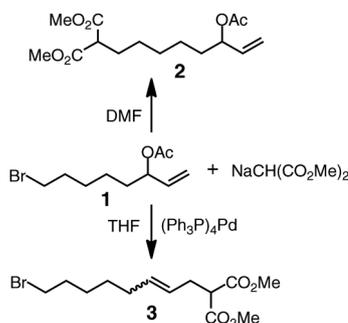


Fig. 1 Synthetic scheme showing chemoselective reaction of compound **1** at either the bromide or allylic acetate sites.^{3,4}

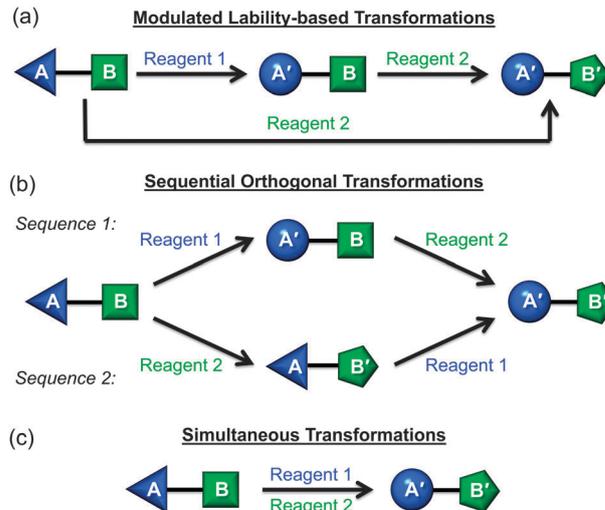


Fig. 2 Three examples of chemoselective transformation of two functional groups: (a) modulated lability approach, (b) sequential orthogonal transformations and (c) simultaneous transformations. Functional groups A and B can be on two separate molecules.

acid (*e.g.*, HF) removes both groups and cleaves the peptide from the resin. In this and the previous example, it is not possible to reverse the order of the deprotection steps. Thus, the sequence of the reaction is controlled by the different reactivity of the two functional groups.

3. Orthogonal protecting group chemistry

...“An orthogonal system is defined as a set of completely independent classes of protecting groups. In a system of this kind, each class of groups can be removed in any order and in the presence of all other classes.”...

Barany and Merrifield (1977)^{1b}

3.1 Orthogonality vs. chemoselectivity

The orthogonal reactions developed by Barany and Merrifield involve two protecting group removal steps that occur with high chemoselectivity (Fig. 2b). In practice, each of the deprotection reactions occurs by a *different mechanism* designed so that each is able to effect removal of one protecting group exclusively. Thus, each reaction is chemoselective so each may occur in any order. As shown in Fig. 2b, the use of either sequence affords the same product. Orthogonality can be considered as a subset of chemoselectivity. However, the terms “orthogonal” and “chemoselective” are often used interchangeably in the literature. In addition, terms such as partial or quasi-orthogonal are occasionally seen, sometimes referring to partial chemoselectivity.

3.2 Increasing complexity: multiple dimensions

The discovery of the 9-fluorenylmethoxycarbonyl (Fmoc) group by Carpino meant that for the first time an amino protecting group could be removed under mildly basic conditions.⁷ Because the conditions are orthogonal to the acidic *t*-Bu removal and high yielding, the Fmoc–*t*-Bu strategy (orthogonality-based; Fig. 3) has largely replaced Merrifield's classic peptide

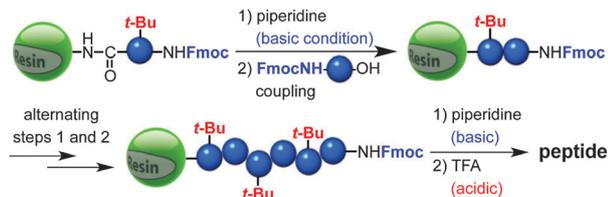


Fig. 3 A general representation of an Fmoc–*t*-Bu strategy (orthogonality-based) for peptide synthesis.

synthesis using the Boc–benzyl strategy (modulated lability-based). The Fmoc and *t*-Bu groups represent an excellent pair of orthogonal protecting groups.

The number of “dimensions” of an orthogonal system can be increased. For example, in his Nobel Lecture, Merrifield described a pentapeptide containing three orthogonal protecting groups (Fig. 4).⁶ The peptide is linked to the resin by an *o*-nitrobenzyl (ONb) group and protected by two additional functional groups (*t*-Bu and Dts) that can each be cleaved without affecting the other.

Another way in which the complexity of a set of orthogonal reactions can be increased is by conducting their operation simultaneously in a single flask (Fig. 2c). This scenario represents a more stringent example of chemical orthogonality because beyond the reagents being selective for their respective functionality they must not affect one another.⁸ This type of transformation, illustrated in Fig. 2c, can offer higher efficiency, with reduced time and waste; allowing us to envision a multistep synthesis where all the reagents are added at once. The simultaneous, multi-functionalization of macromolecules and the dual labeling of cellular targets for imaging are already possible and will be discussed below (*vide infra*).

Higher dimensional orthogonal systems are possible. Indeed, some synthetic targets benefit from multiple orthogonal protecting group strategies. For example, the challenges in oligosaccharide synthesis are significant because the many hydroxyl groups have similar reactivity. A wider range of orthogonal

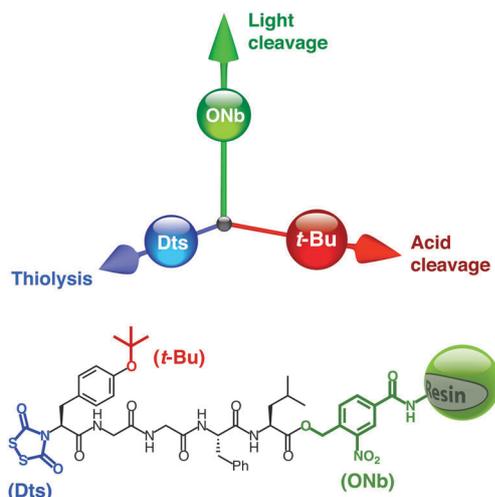


Fig. 4 Three orthogonal protecting groups removed independently by acid (*t*-Bu), reductive cleavage (Dts), and light (ONb).⁶

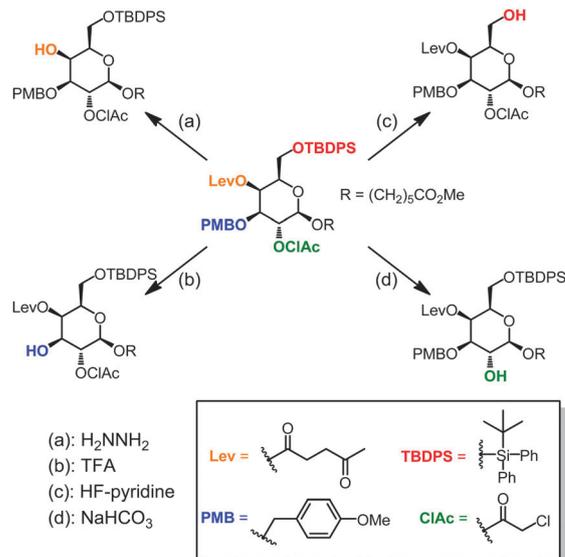


Fig. 5 An orthogonal set of four protecting groups used for the synthesis of a library of oligosaccharides.^{9a}

protecting groups is now available and this enabled Wong and co-workers to report the synthesis of a library of oligosaccharides containing 38 416 members.^{9a} The four dimensional system consisted of chloroacetyl, *p*-methoxybenzyl, levulinyl, and *t*-butyldiphenylsilyl protecting groups that were selectively deprotected using four different mechanisms: basic hydrolysis, acidic hydrolysis, hydrazinolysis, and fluoride-based deprotection, respectively (Fig. 5).^{9a} Later, this orthogonal strategy was combined with a one-pot sequential strategy for the preparation of branched oligosaccharides.^{9b}

Boons and co-workers reported another set of orthogonal protecting groups for oligosaccharides that are chemoselectively removed using DDQ oxidation, Pd-catalysis, hydrazinolysis, and fluoride-based desilylation.¹⁰ It was also found that the selected protecting group pattern on the mannose rings is important for obtaining high yields and selective β -mannosylation. Reviews on protecting group strategies and the influence on stereoselectivity in oligosaccharide synthesis are available.¹¹

3.3 Chromatic orthogonality

...“Orthogonality is defined as the possibility of making one functional group react selectively in the presence of others under specific conditions...a differentiation based on the color of a light beam could be named chromatic orthogonality.”...

Bochet (2004)¹²

A powerful approach to spatiotemporal orthogonal deprotection avoids reagent-based reactions, using photolytic cleavage reactions instead to achieve the desired chemoselectivity. Deprotection can be performed using a wide variety of photolabile groups (*e.g.*, *o*-nitrobenzyl, *p*-hydroxyphenacyl, benzophenone)¹³ in the presence of other reagent-based protecting groups. In general, photolabile groups form an orthogonal system with a wide range of chemical-based protecting groups

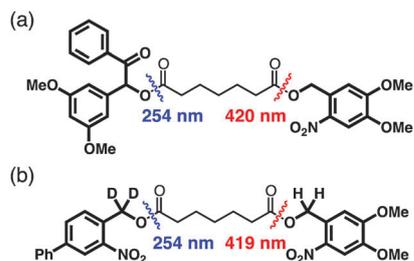


Fig. 6 Examples of photolabile groups for wavelength-selective deprotections.^{14b,15}

(*e.g.*, silyl and allyl groups) because light can be absorbed with considerable selectivity. Indeed, many functional groups do not absorb light from common laboratory light sources and many that do are not photoreactive. It is further possible to create a set of photolabile groups that each react with light of a different wavelength – a type of orthogonality referred to as chromatic orthogonality.^{12,14} For example, the 3',5'-dimethoxybenzoin and 2-nitroveratryl esters shown in Fig. 6a are photo-deprotected at 254 and 420 nm, respectively. The approach was later refined with a pair of *o*-nitrobenzyl-based photolabile groups that can be photo-deprotected orthogonally by fine-tuning using both substituent- and isotopic effects on quantum yields (Fig. 6b).¹⁵

The use of orthogonal protecting group strategies is now commonplace in the synthesis of a range of complex molecules. These include natural products and biomolecules such as oligosaccharides, glycoproteins, and nucleic acids. The high level of dimensionality is most evident in Kociński's classification of a large number of protecting groups into 13 orthogonal sets; members of each set are removable by a unique mechanism.¹⁶

4. Orthogonal coupling reactions

Avoiding protection–deprotection steps can dramatically streamline the construction of complex molecules.^{17,18} The potential of this approach was made particularly apparent in Baran's seminal report of a protecting-group-free synthesis of hapalindole Q in 2004.¹⁹ The use of orthogonal coupling reactions is one powerful strategy for avoiding protecting groups. As with orthogonal protecting groups, this approach is not limited to a pair of reactions, rather multiple reactions can be orthogonal and performed in any order.

4.1 Orthogonal glycosylation

...“We investigated the possibility of using two sets of chemically distinct (orthogonal) glycosyl donors and activation conditions... The chosen set of reactions is therefore shown to be orthogonal.”...

Ogawa *et al.* (1994)²⁰

To our knowledge, the earliest example of an orthogonal coupling strategy is the orthogonal glycosylation reported by Ogawa and co-workers in 1994 (Fig. 7).²⁰ In this approach, two distinct anomeric groups (phenylthio and fluoride) can be orthogonally activated without affecting one another. This

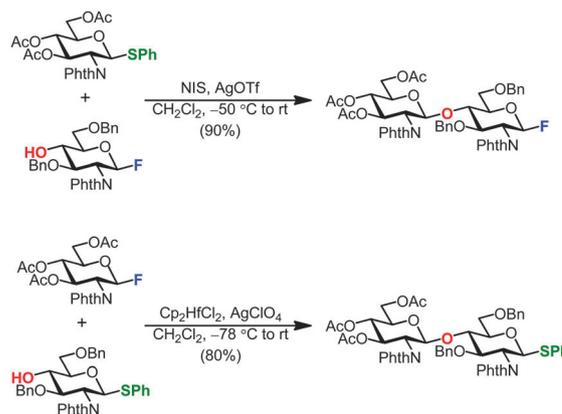


Fig. 7 Orthogonal glycosylation developed by Ogawa and co-workers.²⁰

approach has also been adopted in solid phase oligosaccharide syntheses^{21,22} and in the production of a combinatorial library of oligosaccharides.²³

4.2 Orthogonal dendrimer syntheses

...“It was anticipated that both pairs of functional groups and their resulting coupling products would be inert to the conditions of the other coupling reaction, orthogonality that would allow 4 and 5 to be employed consecutively in either order.”...

Zeng and Zimmerman (1996)²⁴

The second reported example of an orthogonal coupling approach to prepare a complex molecule came in the context of polymer synthesis. Our group disclosed the first orthogonal dendrimer synthesis featuring two AB₂-type monomers (*i.e.*, AB₂ and CD₂) that couple in a convergent approach utilizing alternating Mitsunobu esterification and Sonogashira coupling steps.²⁴ Because these two reactions are orthogonal, deprotection or activation steps could be avoided for the first time (Fig. 8). Thus, each coupling reaction added one layer to the dendrimer. The approach was extended with the use of AB₄ and CD₄ monomers (Fréchet's branched-monomer approach),²⁵ so that each orthogonal coupling reaction adds two layers to the dendrimer (Fig. 9). This remains the fastest dendrimer synthesis to date in terms of number of steps from the monomer units. Indeed, the MW of 4 and 5 are just 592 and 416, respectively, but they combine in three synthetic steps to produce a sixth generation dendrimers with a MW = 20 896. This is a rate of growth (MW per step) not even observed in nature.

By using the Mitsunobu esterification and Sonogashira coupling reactions, the layers of the dendrimer alternate. Similarly, Majoral and co-workers reported an orthogonal dendrimer synthesis in a divergent approach using alternating hydrazone formation and Staudinger reaction.²⁶ To produce a homogeneous dendrimer, where the layers are chemically identical, Yu and co-workers combined the Horner–Wadsworth–Emmons and Heck reactions as two orthogonal coupling reactions so that the linkages prepared are all vinylene-type.²⁷

Although the orthogonality aspect of click reactions will be discussed in Section 4.5, it should be noted here that click

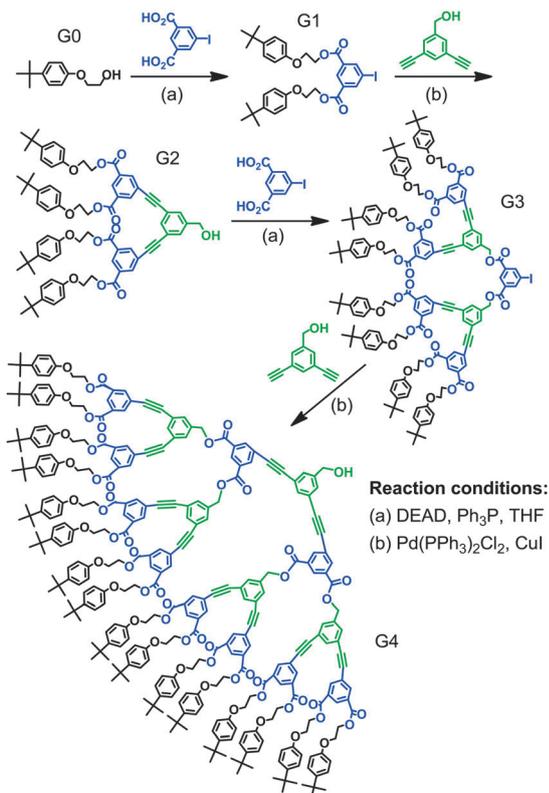


Fig. 8 Orthogonal synthesis of a G4 dendron using alternating Mitsunobu and Sonogashira couplings.²⁴

chemistry is emerging as an efficient method to construct dendritic structures.²⁸ Hawker *et al.* reported several approaches involving the use of CuAAC²⁹ and thiol–ene reactions^{30,31} coupled to “non-click” reactions to construct dendritic structures. They also reported the use of two orthogonal click reactions (CuAAC and thiol–ene reactions) to accelerate the dendrimer synthesis such that a G6 dendrimer can be prepared in a single day (Fig. 10).³² Recently, Monteiro demonstrated the use of two orthogonal click reactions – CuAAC and nitroxide radical coupling (NRC) – to prepare dendrimers in

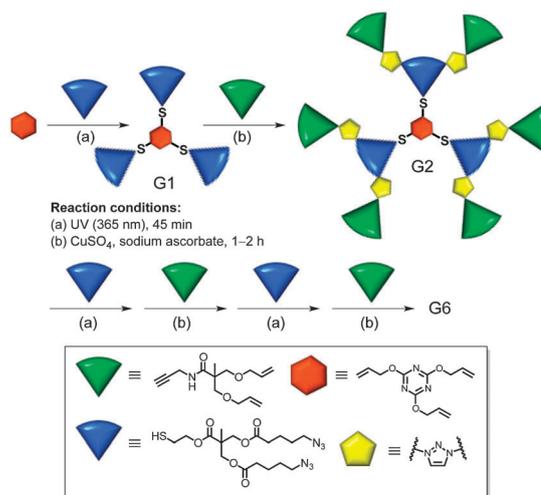


Fig. 10 Accelerated divergent synthesis of a G6 dendrimer using two orthogonal click reactions.³²

either convergent or divergent approaches.³³ Under appropriate conditions, it is possible to synthesize G3 dendrimers with a variety of building blocks in a one-pot setting. Other orthogonal click syntheses of dendrimers are also reported.^{34,35}

4.3 Chemical ligation of peptides

Although peptides containing up to 100 amino acids are now synthesized routinely, larger peptides and proteins (*i.e.*, >150 amino acids) cannot be readily prepared in high yield and purity because the peptide coupling efficiency decreases with sequence length. Chemical ligation provides a convenient method to link two peptide segments covalently.^{36,37} In particular, the native chemical ligation has been widely adopted because it produces a natural peptide bond (Fig. 11).³⁸ The method uses a Cys–thioester ligation with an entropically favored intramolecular transacylation. At pH 7–8, the thioester is reactive toward thiols, not hydroxyl nucleophiles. Internal cysteine residues can reversibly react with the thioester but this reaction is unproductive. As a result, the C-terminal thioester

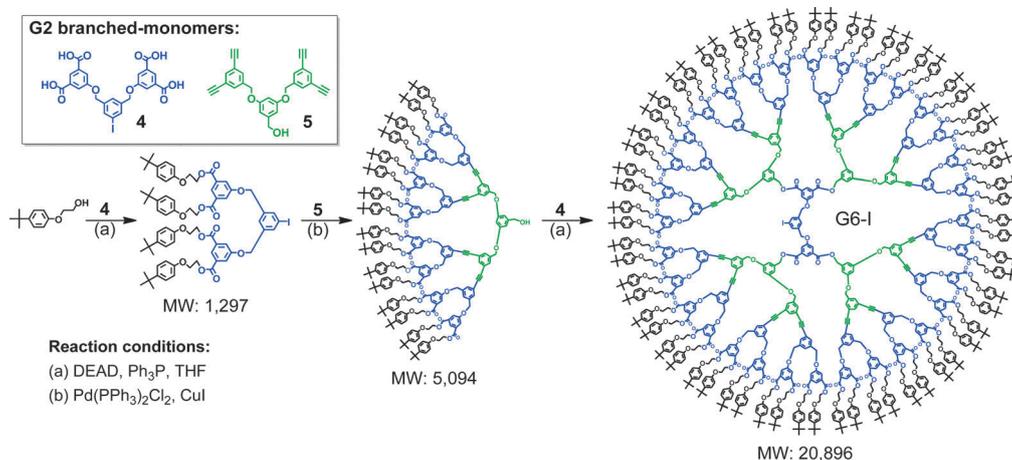


Fig. 9 Rapid synthesis of a G6 dendron using a combination of orthogonal coupling strategy and branched-monomer approach.²⁴

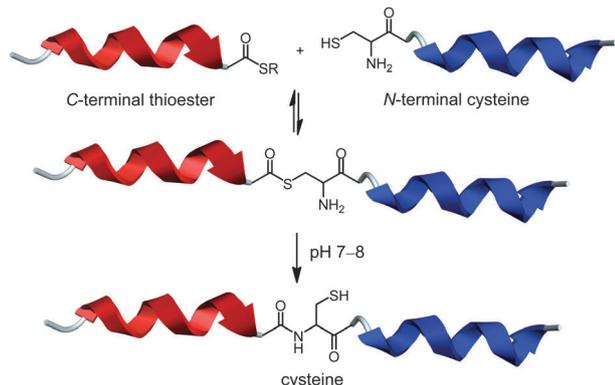


Fig. 11 Cys-thioester ligation as an example of orthogonal ligation.³⁸

will permanently connect only to the N-terminal cysteine, forming a native peptide linkage. The reaction therefore is highly chemoselective. As with other highly chemoselective reactions (see click reactions; Section 4.5), the chemical ligation of peptides has been termed orthogonal ligation.³⁶

4.4 Orthogonal catalysis

The concept of orthogonal reactivity can also be applied to catalysis and catalytic cycles. For example, Buchwald and co-workers developed Pd- and Cu-based catalysts that effect chemoselective C- or N-arylation reactions, respectively (Fig. 12).³⁹ Likewise, switching metal catalysts from Pd- to Cu-based allowed the selective N- and O-arylation, respectively, of various aminophenols.⁴⁰ The site of arylation of aminoalcohols can also be controlled by the choice of ligands with high selectivities from 15 : 1 to >50 : 1.⁴¹ In each of these systems the two chemoselective transformations are expected to be orthogonal even if orthogonality was not demonstrated experimentally.

In orthogonal catalysis there are also different levels of complexity from the sequential catalytic transformations just described to systems where two or more catalytic cycles operate simultaneously. The latter arguably represents the most demanding of all orthogonal systems because each entire catalytic cycle must be fully orthogonal. Thus, the substrate and product and each reactive intermediate and catalytic species must be compatible unless the kinetics of the cycles are vastly different in which case a few of the species may not be present at the same time. Chung and co-workers reported a three-step, one-pot synthesis of fenestranes using Co nanoparticles and Pd(II) catalysts in which the two catalysts operate simultaneously (Fig. 13).⁴² The first step is carried out prior to the addition of the palladium catalyst. Nonetheless, this is an example of one-pot, orthogonal catalysis made possible by the mutual independence of the Pd(II) catalyst for the allylic

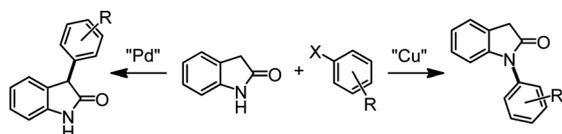


Fig. 12 Orthogonal Pd- and Cu-based couplings of oxindoles via C- and N-arylation, respectively.³⁹

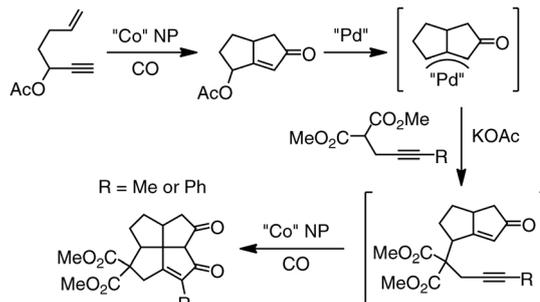


Fig. 13 One-pot three-step orthogonal tandem catalysis in the synthesis of fenestranes by Co nanoparticles and allyl palladium chloride.⁴²

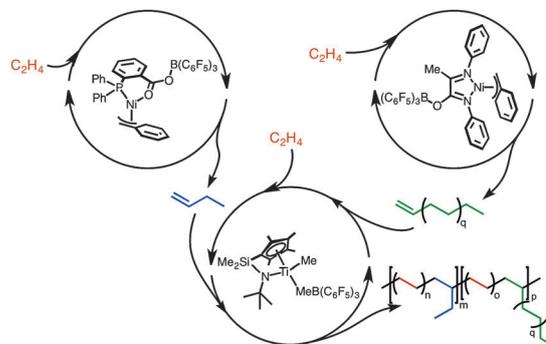


Fig. 14 Orthogonal-tandem catalysis for the preparation of branched polyethylenes in the presence of three catalysts.⁴⁵

alkylation in the second step and the Co catalyst for the last Pauson–Khand reaction. Fogg and dos Santos have termed such processes orthogonal tandem catalysis.⁴³ Similarly, Hidai, Uemura and co-workers reported the use of ruthenium and platinum as orthogonal tandem catalysts to synthesize a series of substituted furans.⁴⁴ Another impressive example is the synthesis of branched polyethylenes using three catalysts that operate simultaneously in a one-pot procedure (Fig. 14).⁴⁵ Polyethylenes of different properties can be prepared simply by varying the mole ratios of the catalysts.

4.5 Click reactions, orthogonal click reactions, and bioorthogonality

The objective of this section is not to provide a comprehensive review of click chemistry but to focus on its relationship to orthogonality. In discussing click reactions generally, Sharpless noted that “although click reaction components are necessarily highly reactive, their chemoselectivity profiles are quite narrowly defined, that is, the reactions are “orthogonal” to an unusually broad range of reagents, solvents, and other functional groups”.⁴⁶ Thus, he correctly noted the implicit orthogonality of click reactions as originating in the high reactivity of the coupling components with one another but broad tolerance of other reagents and functional groups. The prototypical click reaction, the copper-catalyzed alkyne–azide cycloaddition (CuAAC), illustrates these ideas well. It is a highly chemoselective reaction that proceeds in high yield under mild conditions⁴⁶ yet the azide and alkyne groups are relatively unreactive.

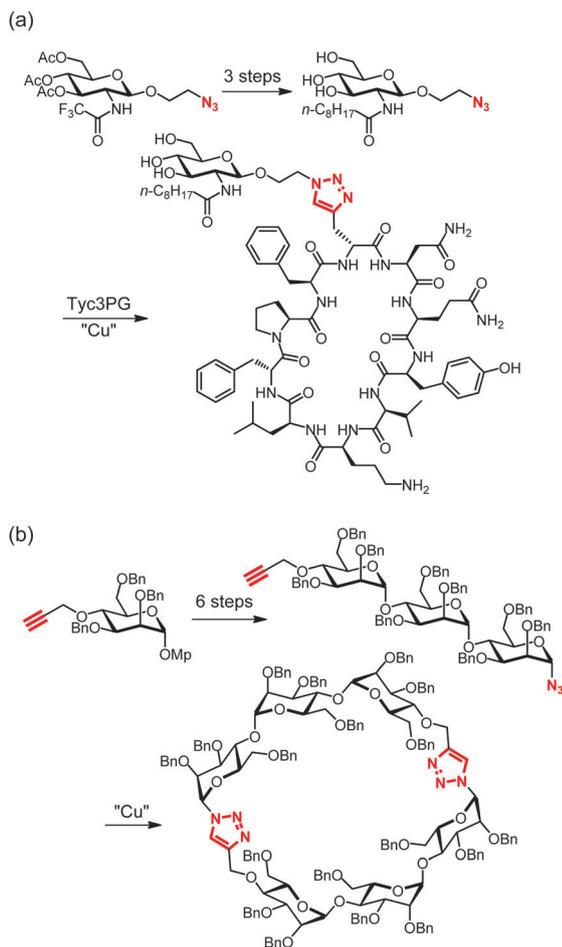


Fig. 15 Two examples illustrating the orthogonality profiles of alkynes and azides under a wide range of conditions.^{49,50}

The low reactivity profile of organic azides^{47,48} and terminal alkyne groups is illustrated in Fig. 15a and b, respectively. Thus, Lin and Walsh prepared a series of glycopeptide antibiotics by the chemoselective click reaction of various azido sugars with an alkyne-containing cyclic peptide (Tyc3PG; Fig. 15a).⁴⁹ The azide group is carried through several steps prior to reaction with the alkyne group. Analogously, Gin reported the synthesis of a cyclodextrin analog by double click reaction of a trisaccharide carrying both click partners.⁵⁰ In this example, the terminal alkyne is carried through six steps prior to the cyclization reaction (Fig. 15b).

4.5.1 Orthogonal functionalization of polymers using click chemistry. The orthogonality of the click reaction is well illustrated in the post-functionalization of polymers. Indeed, click methodology has the ability to create new polymers by introducing functionality at polymer chain ends or along the backbone.⁵¹ Preparation of alkyne- or azide-containing polymers and their subsequent click modifications will not be covered here; readers are directed to several recent reviews.⁵² This section focuses on orthogonal, simultaneous, one-pot post-polymerization modifications of polymers using click reactions. This approach minimizes the number of synthetic steps and reduces the number of work-up and purification operations.^{53,54}

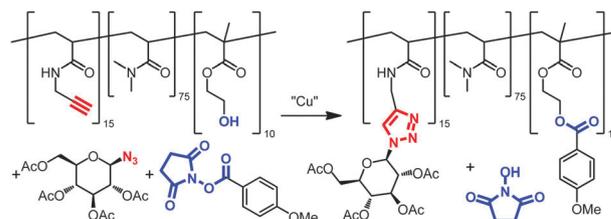


Fig. 16 Simultaneous functionalization of a water-soluble terpolymer through CuAAC and activated ester-based functionalizations.⁵⁵

Hawker reported the one-pot, simultaneous and cascade functionalization of polymers.⁵⁵ Both strategies combined CuAAC and an esterification reaction as a highly orthogonal pair. For example, a simultaneous functionalization was performed on a water-soluble polymer containing acetylene- and hydroxyl-moieties for CuAAC and esterification, respectively (Fig. 16).⁵⁵ NMR and IR analysis of the product polymer indicated >95% conversion for both reactions. The cascade-type functionalization occurs when a side-chain modification adds new functionality that can undergo a further transformation. The authors described the reaction of a polymer carrying active ester groups with propargylamine and an organic azide under CuAAC conditions. The amide formation reaction and alkyne click can occur in any order to furnish the cascade-functionalized polymer.

Lyon described a related approach to the formation of multi-responsive gels.⁵⁶ Thus, EDC-based amidation and the CuAAC reaction were the orthogonal reactions used to link fluorescein and rhodamine dyes simultaneously to methacrylate-based microgels. Both FT-IR and epifluorescence microscopy demonstrated the synthesis of the desired materials.

Yang and Weck reported the one-pot, simultaneous polymer modification using two orthogonal click reactions – CuAAC and hydrazone formation.⁵⁷ The polymer modification is highly modular with a wide range of substrates including those with biological interest (Fig. 17). Quantitative functionalization was observed by NMR and IR analysis.

Tunca and co-workers reported an analogous process using the CuAAC and Diels–Alder reaction.⁵⁸ In this report, two end-functionalized polymers (maleimide–PMMA and acetylene–PEG) were attached to polystyrene polymers containing anthracene and azide moieties with >90% grafting efficiency based on NMR analysis. In an approach that has been reviewed recently, the combination of Diels–Alder and CuAAC has been used to prepare

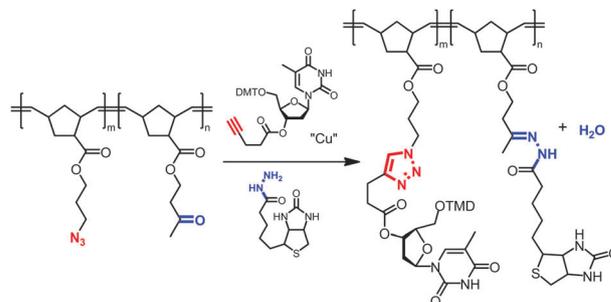


Fig. 17 One-pot dual post-polymerization functionalization using two click reactions (CuAAC and hydrazone formation).⁵⁷

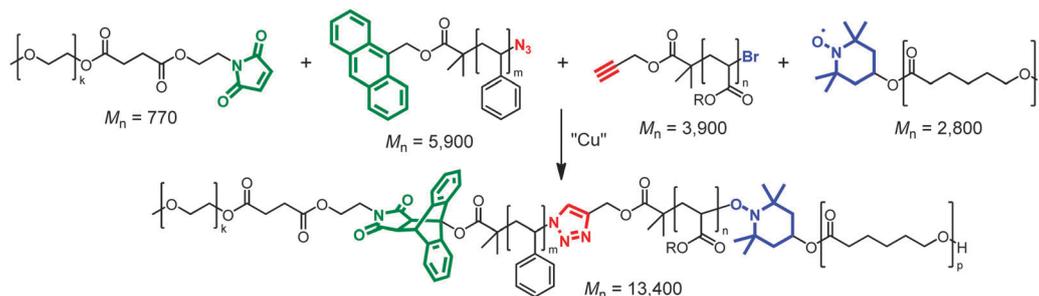


Fig. 18 One-pot tetrablock copolymer synthesis using CuAAC, Diels–Alder, and nitroxide radical coupling reactions.⁶¹

polymers of different architectures, including star polymers, cyclic polymers, and dendrimers.⁵⁹ The one-pot, cascade functionalization of polystyrene using a combination of CuAAC and Diels–Alder reactions was also reported by Yagci and co-workers.⁶⁰

A final example of polymer-based click chemistry comes from Tunca who reported a combination of three orthogonal reactions (CuAAC, Diels–Alder, and NRC) in a one-pot preparation of linear tetrablock copolymers (Fig. 18).⁶¹ The isolated yields of the polymers were 50–55%, with GPC and NMR analysis showing the M_n values of the isolated polymers to be in good agreement with the sum of the M_n of the reactant polymers.

4.5.2 Click chemistry beyond CuAAC. As seen above, CuAAC is a powerful reaction with a wide range of applications. The high chemoselectivity of the CuAAC allows it to form a highly orthogonal reaction pair with many other reactions. Indeed, there are only a few reactions reported not to be fully compatible with CuAAC and these often involve the instability of organic azides toward heat, light, or phosphine.⁶² It has been reported that the copper catalyst may be partially poisoned by nucleophilic thiol species⁶³ and that it is not fully compatible with oxime formation.⁶⁴ Residual copper can also be problematic in certain applications.⁶⁵

The radical-mediated addition of thiols to terminal alkene groups, called the “thio-click” by Schlaad in 2007,⁶⁶ has emerged as a useful complement or alternative to the CuAAC reaction. Radical “thio-click” reactions can be initiated thermally and photochemically where the latter has a higher efficiency.⁶⁷ The utility of the thiol–ene, thiol–yne, and thiol–Michael addition reactions is evident in their widespread applications, including in polymer and materials synthesis and as a tool in chemical biology studies.^{59,68,69} Nitroxide radical coupling is also considered a click-like reaction.^{59,70} As noted previously, CuAAC and NRC form an orthogonal reaction pair allowing the preparation a variety of polymeric G3 dendrimers with $M_n > 20\,000$ in a one-pot fashion.³³

4.5.3 Orthogonal dynamic combinatorial libraries using click-type chemistry.

...“The resulting double-level dynamic libraries are therefore named orthogonal. . .the two processes, in principle, do not interfere, and analogously to the orthogonal protecting groups in organic chemistry”...

Lehn *et al.* (2001)⁷¹

Together with the CuAAC reaction, certain imine-forming condensations⁴⁶ have been classified by Sharpless as fulfilling the

criteria of a click reaction. Similar to the CuAAC process, these condensation reactions can be orthogonal to a range of other reactions, but a key difference is that imine formation is reversible under some conditions. With this orthogonal and reversible nature, Eliseev and Lehn reported the first double-level, dynamic combinatorial library (DCL) based on the orthogonality of two reversible processes: imine formation and metal–ligand exchange.⁷¹ Other pairs of orthogonal exchange reactions have been reported, for example hydrazone formation–disulfide exchange⁷² and imine formation–nitroxide formation.⁷³ The complexity of DCLs has also been raised to a triple-level with the use of three orthogonal exchange processes. Thus, Otto and Nitschke reported a DCL with imine, disulfide, and metal–ligand exchanges.⁷⁴ Furlan and co-workers reported a DCL with hydrazone, disulfide, and thioester exchanges.⁷⁵ For more details, readers are directed to several recent reviews.⁷⁶

4.5.4 Bioorthogonal ligation using click chemistry.

...“Selective chemical reactions that are orthogonal to the diverse functionality of biological systems are now recognized as important tools in chemical biology. . .these bioorthogonal reactions have inspired new strategies”...

Bertozzi *et al.* (2004)⁷⁷

The past decade has seen an increasing focus on developing highly chemoselective reactions in biological settings. These click reactions were termed *bioorthogonal* and have proven useful new tools for studying biological processes by functionalizing biomolecules selectively.⁷⁸ Beyond working in aqueous medium, bioorthogonal reactions may need to occur on or within living cells and ultimately in organisms.⁷⁹ One example is the modified Staudinger reaction reported by Saxon and Bertozzi in 2000,⁸⁰ which involves the selective functionalization of cell surface glycoproteins (Fig. 19). Thus, Jurkat cells were incubated with *N*-azido-acetylmannosamine so that they displayed azido groups on their surface. The Staudinger–Bertozzi ligation, as it is now known,⁸¹ shows no cross-reactivity between the phosphine and cellular disulfide bonds. Although the Staudinger–Bertozzi ligation is relatively slow ($k_{\text{obs}} \approx 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$),⁸² the reaction has shown to be applicable in bacterial cultures⁸³ and in mice.⁸⁴

The CuAAC reaction was also applied to cellular bio-labeling.⁸⁵ The issue of copper cytotoxicity can be circumvented by using polytriazole ligands⁸⁶ to accelerate the reaction and protect the cells from reactive oxygen species (ROS) generated by the Cu-catalyzed reduction of oxygen. Nonetheless, considerable

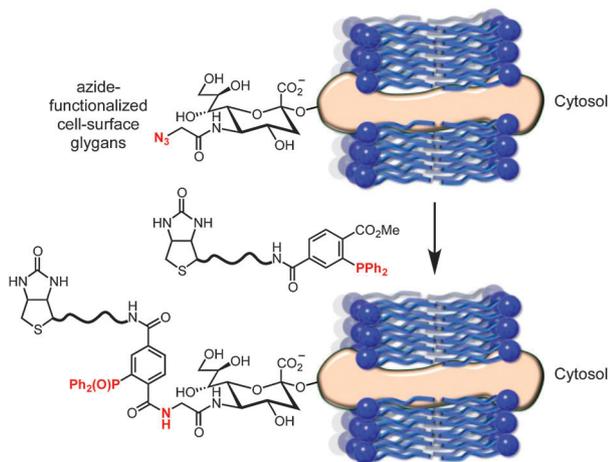


Fig. 19 Bioorthogonal Staudinger–Bertozzi ligation.⁸⁰

cell death would be expected when the copper concentration is above the maximum tolerated level of *ca.* 500 μM .^{78c}

To overcome the toxicity of copper ions, Bertozzi and co-workers developed a number of cyclooctynes for the copper-free, strain-promoted alkyne–azide cycloaddition (SPAAC).⁷⁸ The triple bond in the cyclooctyne (OCT) ring is more reactive (strain-promoted) than common terminal alkyne groups and allows the cycloaddition to occur under physiological conditions without the presence of copper catalyst. Boon's dibenzocyclooctynes (DBCO) were also found to undergo SPAAC and were applied successfully to live cell labeling.⁸⁷ The rates of the first generation cyclooctyne derivatives were found to be comparable to that of Staudinger ligation, and about 70–80-fold slower than the standard CuAAC reaction.^{78,79}

Computational studies⁸⁸ provided insights into the origin of the rate enhancement (*e.g.*, electronic *vs.* strain effects) for a

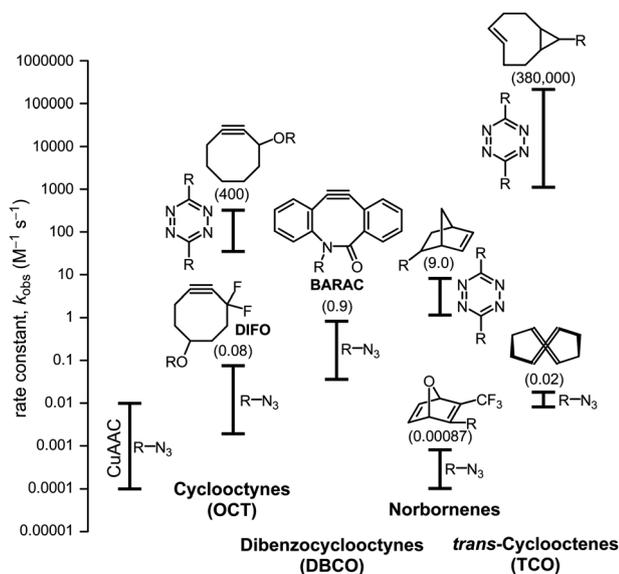


Fig. 20 Comparison of range of rate constants of bioorthogonal reactions. Numbers in parentheses are the largest rate constants reported in the series. Rate constants depend on the actual conditions and substituents on the substrates; the ranges are served as ballpark comparison only. Structures represented the best in the series. Adapted with permission from ref. 89.

range of click partners (Fig. 20).⁸⁹ With a difluoro substituted cyclooctyne moiety (DIFO), the rate of SPAAC is found to be comparable to CuAAC under pseudo-first-order conditions.⁹⁰ Similarly, the DBCO systems reported by Boons and Popik were found to have similar reaction rates.⁹¹ Apparent drawbacks of these systems are their lengthy syntheses and hydrophobicity. The latter can limit aqueous solubility and lead to membrane or serum proteins localization. Sletten and Bertozzi reported the dimethoxyazacyclooctyne (DIMAC) system as having an improved $\log S$ value (-2.7 *vs.* -3.1 for OCT) and hence showing higher water-solubility.⁹² DIMAC also showed much less non-specific background labeling compared to its parent compounds. The system does require a 9-step sequence from a known pyranoside (overall yield $\sim 6\%$) and the rate is comparable to that of cyclooctyne.⁹²

The tetrazine-based inverse-electron-demand cycloaddition is receiving considerable attention as a new member in the bio-orthogonal reaction toolbox because of its faster reaction rate (see Fig. 20).⁹³ Schultz and Lemke showed the incorporation of *trans*-cyclooctene (TCO) containing lysine into proteins and the subsequent Diels–Alder reaction with fluorogenic tetrazine-functionalized dyes in living cells.⁹⁴ The strain-promoted inverse-electron-demand Diels–Alder cycloaddition (SPIEDAC) occurs with a rate constant as high as $35\,000\ \text{M}^{-1}\ \text{s}^{-1}$. An even higher rate constant ($380\,000\ \text{M}^{-1}\ \text{s}^{-1}$) was recently reported in a study on *in vitro* DNA labeling using (*E*)-bicyclo[6.1.0]non-4-ene⁹⁵ derivatives.⁹⁶ In general, tetrazine cycloadditions with cyclooctynes⁹⁴ and norbornenes⁹⁷ also proceed more rapidly compared to the same reaction with organic azides.⁹⁸

The broad range of rate constants in Fig. 20 (note logarithmic scale) suggests that some of the bioorthogonal reactions can be used simultaneously without cross talk. Indeed, Jäschke recently reported a one-pot, *in vitro* dual labeling of DNA using CuAAC and tetrazine-based cycloaddition as an orthogonal reaction pair.⁹⁶ Also very recently, Hilderbrand reported the use of two copper-free cycloadditions (DBCO–azide and TCO–tetrazine) as an orthogonal reaction pair for selective multi-target imaging of cancer cells (Fig. 21).⁹⁹

The development of “photo-click” chemistry offers spatio-temporal control over the reaction initiation.^{78b} Thus, Lin and co-workers developed the photo-initiated high efficiency bioconjugation of proteins using 2,5-diaryltetrazoles.¹⁰⁰

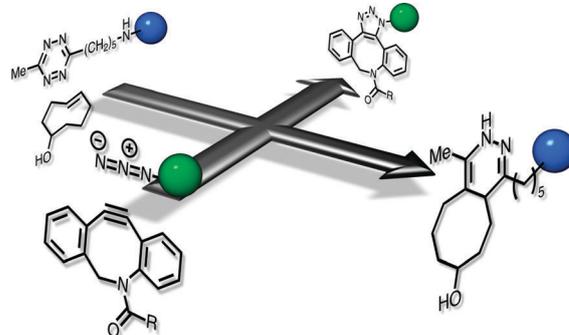


Fig. 21 Two orthogonal click cyclization reactions can be used simultaneously in a biological setting.⁹⁹

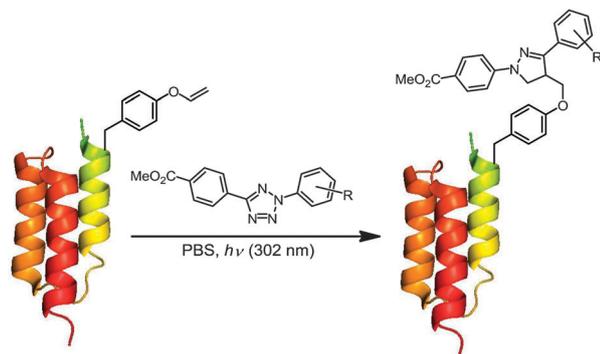


Fig. 22 Photo-triggered functionalization of O-allyl-tyrosine-containing Z-domain protein by photo-click chemistry.^{100a}

The diaryltetrazole undergoes nitrogen extrusion to form a nitrile–imine dipole that undergoes a [3 + 2]-cycloaddition with alkene dipolarophiles (Fig. 22).^{100a} Boons and Popik also developed a photo-inducible system in which the dibenzocyclooctyne ring is masked by a cyclopropenone motif.⁹¹ The photo-triggered decarbonylation offers the ability to label living organisms in a spatio-temporally controlled manner. This system has also been applied successfully in the modification of surfaces (*vide infra*).¹⁰¹

5. Orthogonality (fidelity) in supramolecular chemistry

5.1 Metal–ligand coordination combined with hydrogen bonding

...“From a synthetic point of view it is important that the two types of interactions [*metal–ligand coordination and hydrogen bonding*] are “orthogonal”, that is, mutually compatible.”...

Reinhoudt *et al.* (1997)^{102a}

The orthogonality discussed thus far is based on chemical reactions, *i.e.*, two highly chemoselective functional group inter-conversions. The concept of orthogonality can be extended to non-covalent interactions. Indeed, the remarkable complexity of biological systems is built upon a series of intricate non-covalent interactions that are an exceptional example of a high-dimension orthogonal system. Because supramolecular systems are at equilibrium, the orthogonality is continually being tested, unlike the case of an irreversible chemoselective reaction. Although this possible complication can be a challenge, it also represents an opportunity because unwanted supramolecular interactions may self-correct. So there is a proofreading mechanism possible in self-assembling systems in general.

Reflecting on some of these ideas, Reinhoudt reported in 1997 the first discrete nanostructure assembled by two different supramolecular interactions (Fig. 23).^{102a} Metallo-dendrimers as large as 28 kDa were characterized making these among the largest discrete self-assembled polymers (dendrimers) known.^{102b} The importance of the orthogonality between the metal–ligand coordination and hydrogen bonding was explicitly recognized by Reinhoudt (see the above quote). The pincer coordination¹⁰³

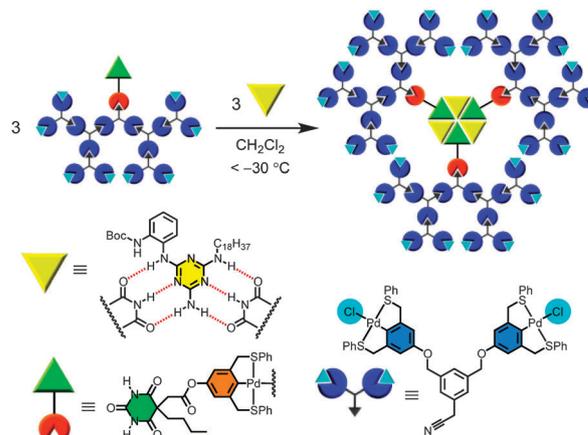


Fig. 23 Reinhoudt's self-assembled dendrimer featuring orthogonal Pd–pincer coordination and cyanuric acid (CA)–melamine hydrogen bonded rosette motifs.^{102a}

seen in Fig. 23 is now extensively used in combination with other hydrogen bonding recognition modules by the Weck group.¹⁰⁴

Other commonly used coordination complexes include 2,2'-bipyridine (bpy) and 2,2':6',2''-terpyridine (tpy) metal complexes that are orthogonal to many of the same hydrogen bonding modules used with the Pd–pincer complex. These units have been particularly useful in the design of main-chain and side-chain modified supramolecular polymers.¹⁰⁵ For example, Schubert *et al.* reported the formation of hydrogen-bonded, metallo-supramolecular polymers containing the UPy hydrogen bonding module and terpyridine as end-groups in the presence of the Zn(II) ion.¹⁰⁶ The same concept was applied to telechelic polymers to yield high molecular weight supramolecular polymers.¹⁰⁷ Noting that the UPy dimer is stable only in non-polar solvents, Schmuck prepared a monomer containing a terpyridine and a guanidiniocarbonyl pyrrole carboxylate zwitterion.¹⁰⁸ The zwitterion is known to form a highly stable dimer in DMSO ($K_a > 10^8 \text{ M}^{-1}$)¹⁰⁹ and the resulting polymer is observed in both DMSO and aqueous solutions displaying typical ring-chain equilibrium.¹¹⁰ The self-assembly of the hydrogen-bonding and metal-coordination is found to be fully reversible. This orthogonal approach to supramolecular polymers has been reviewed recently.¹¹¹

5.2 Orthogonal hydrogen bonding interactions

...“These non-standard nucleotides and the pairs that they form have had particular value as ‘orthogonal binders’, recognition elements that bind with DNA-like specificity, but without interference by natural DNA.”...

Benner *et al.* (2007)¹¹²

The very high fidelity of DNA replication is often associated with highly selective hydrogen bonding.¹¹³ In fact, DNA replication is not perfect and the high fidelity originates not only in base-pair selectivity but also in shape complementarity^{113b,c} and subsequent proofreading processes.¹¹⁴ The issue of selectivity in the pairing of DNA bases and their analogs has been examined in multiple contexts. Benner and co-workers have developed base analogs as a way to expand the “genetic alphabet,”

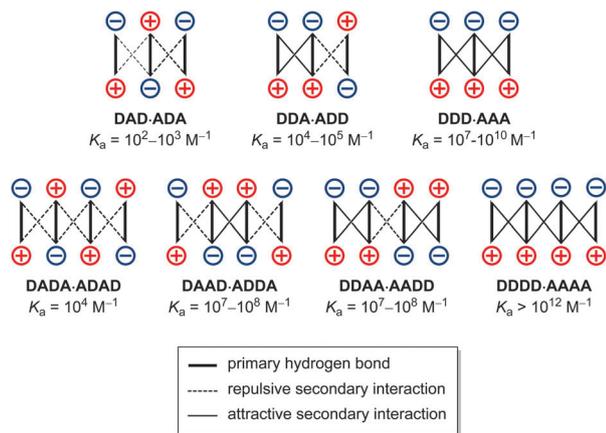


Fig. 24 Possible arrangement of hydrogen bond donor and acceptor groups in triply and quadruply hydrogen bonded complexes and ranges for measured association constants.^{116,117d}

to create a new base that is orthogonal to A-T and G-C.¹¹⁵ Jorgensen used computational methods to explore the origin of base-pair stability and proposed that, in addition to the number of hydrogen bonds, the stability could be explained by the arrangement of the donor and acceptor groups and particularly the resultant differences in “secondary electrostatic interactions” (Fig. 24).¹¹⁶

We reported in 1992^{117a} the first experimental data that supported the Jorgensen proposal and have since collected considerable additional data and this knowledge has been used in the design of more highly orthogonal and more stable DNA base-pair analogs.^{117b,c} For example, the UG-DAN heterocomplex^{118–121} and DeAP dimer^{122,123} represent two hydrogen-bonded pairs that are highly orthogonal and significantly more stable than DNA base-pairs (Fig. 25, see also Section 5.3).

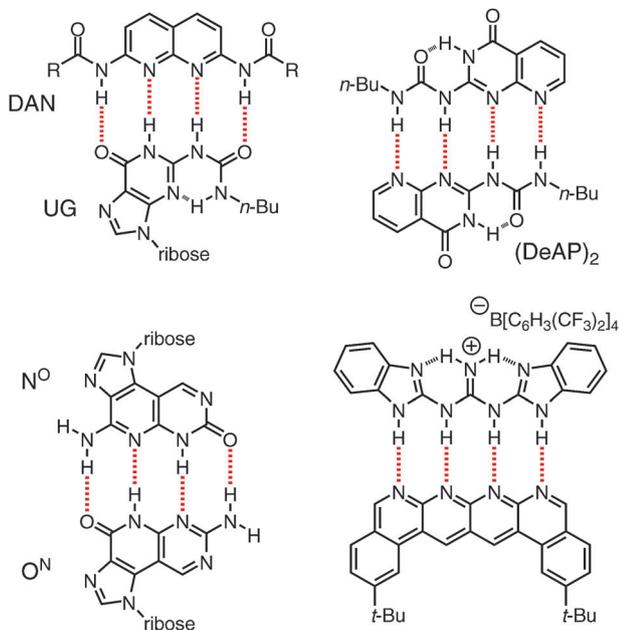


Fig. 25 Four stable quadruply hydrogen-bonded complexes each with different motifs: UG-DAN heterocomplex,^{118–121} DeAP dimer,^{122,123} Minakawa and Matsuda's novel deoxyribonucleotides,¹²⁴ and Leigh's AAAA-DDDD complex.¹²⁵

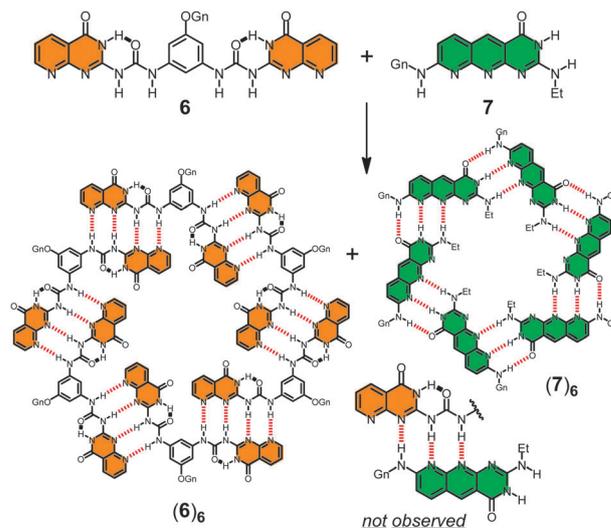


Fig. 26 Orthogonal self-assembly of hexameric dendrimers. Gn = Fréchet-type dendron.¹²³

Minakawa, Matsuda and co-workers found that these quadruply hydrogen bonded base pairs, depending on the sequence arrangement, are capable of increasing the thermal stability of oligonucleotides.¹²⁴ Leigh and co-workers reported in 2011 a highly stable AAAA-DDDD quadruple hydrogen bonded complex with $K_a > 10^{12} \text{ M}^{-1}$ in CH_2Cl_2 .¹²⁵

One area that has not been fully explored is the degree of orthogonality between the various hydrogen bonding modules represented by the arrays in Fig. 24. Nonetheless, these modules have proven quite useful in a wide range of orthogonal assembly studies. For example, we reported the orthogonal self-assembly of two dendritic systems consisting of bis-ureidodeazapterin (**6**) and pyrimido-naphthyridinone (**7**) units (Fig. 26).¹²³ Strikingly no mixing of the two heterocycles was observed even though the AAD and DDA arrays in **7** could interact with the AADD array in **6** with the formation of three hydrogen bonds.

Related areas where these high-affinity and high-fidelity hydrogen-bonded complexes have been applied are, *e.g.*, in the formation of main-chain supramolecular polymers, in the noncovalent side-chain modification of polymers, and the formation of supramolecular polymer blends. An example of the latter involves two immiscible polymers (PS and PBMA), prepared with a few mol% of UG and DAN units on the polymer backbone of PBMA and PS, respectively.^{120,126} The properties (such as T_g and viscosity) of the supramolecular blend were found to be tunable and thermoreversible. The same recognition pair was reported to form supramolecular alternating block copolymers with PEG and PBMA blocks.¹²¹ Again, the properties of the copolymers and the polymeric structures are found to be reversible because of the supramolecular nature of the recognition process.

Weck and co-workers demonstrated the orthogonal nature of UG-DAN and Hamilton receptor-cyanuric acid (Hamilton receptor-CA) and applied the units for the preparation of supramolecular ABC triblock copolymers.¹²⁷ The triblock copolymer can be prepared by stepwise additions of each block or by a

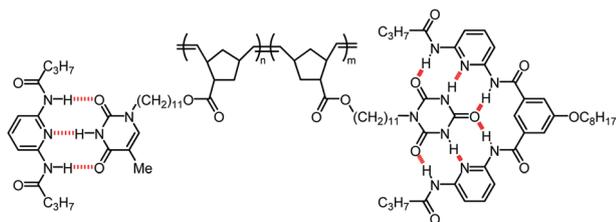


Fig. 27 Weck's supramolecular polymer side-chain functionalization displaying orthogonal self-assembly of Hamilton receptor-CA and T-DAP motifs.¹²⁷

one-pot self-assembly. Recently, Lünig reported the use of Hamilton receptor-CA and complementary ADDA-DAAD units for orthogonal assembly of a G2 supramolecular dendrimer.¹²⁸ Weck reported the supramolecular side-chain functionalization of copolymers using a combination of Hamilton receptor-CA and T-DAP motifs (Fig. 27) but noted non-specific interactions between the Hamilton receptor and T.¹²⁷ Similarly, hydrogen-bonding and metal coordination might not be fully orthogonal, depending on the particular system.^{129,130} Reviews on side-chain supramolecular polymers can be referred to for more details.^{131,132}

5.3 Quantifying orthogonality in supramolecular systems

In the case of orthogonal reactions, the extent of orthogonality can be measured by the degree of chemoselectivity, which, in turn, is measured by the chemical yield of desired and undesired products. In supramolecular systems, the extent of orthogonality can be directly observed. For example, in a remarkable example of “self-sorting” Isaacs has reported the ¹H NMR spectrum of a multicomponent supramolecular mixture.¹³³ The self-assembling systems, including Rebek's calixarene capsule, Meijer's ureido-pyrimidinone dimer, Reinhoudt's rosette, and Isaacs's cucurbituril dimer, all faithfully formed their designed complex without interference from the other components. The same group has defined different types of self-sorting and examined quantitatively the process under thermodynamic control using a combination of simulation and experiment.^{134–136}

As a framework for discussing and quantifying supramolecular orthogonality, we defined the fidelity (F) of a supramolecular system as the mole fraction of desired complexes (Fig. 28).^{119,137,138} If each of the possible equilibrium constants can be measured or estimated, F can be calculated analytically at all concentrations and stoichiometries of interest. Importantly, a three-dimensional fidelity plot allows these systems to be understood in considerable detail (Fig. 28). To better measure the inherent orthogonality of a system, F at a 1 : 1 ratio of the compounds or complexes of interest was measured and this was termed *intrinsic fidelity* (intrinsic orthogonality).¹³⁷

Two fidelity plots that examine the orthogonality of a pair of complexes are illustrated in Fig. 28. First, using a mixture of triply hydrogen-bonded complexes G-C and T-DAP, high fidelity ($F > 0.96$) is observed over a broad concentration range ($1.0\text{--}10^{-6}$ M), indicating that the hydrogen-bonding arrays in G-C and T-DAP are capable of leading to highly selective binding. This is consistent with the fact that the homodimerizations and other undesired complexations are relatively weak ($<10\text{ M}^{-1}$). Similarly, a quadruply hydrogen-bonded system

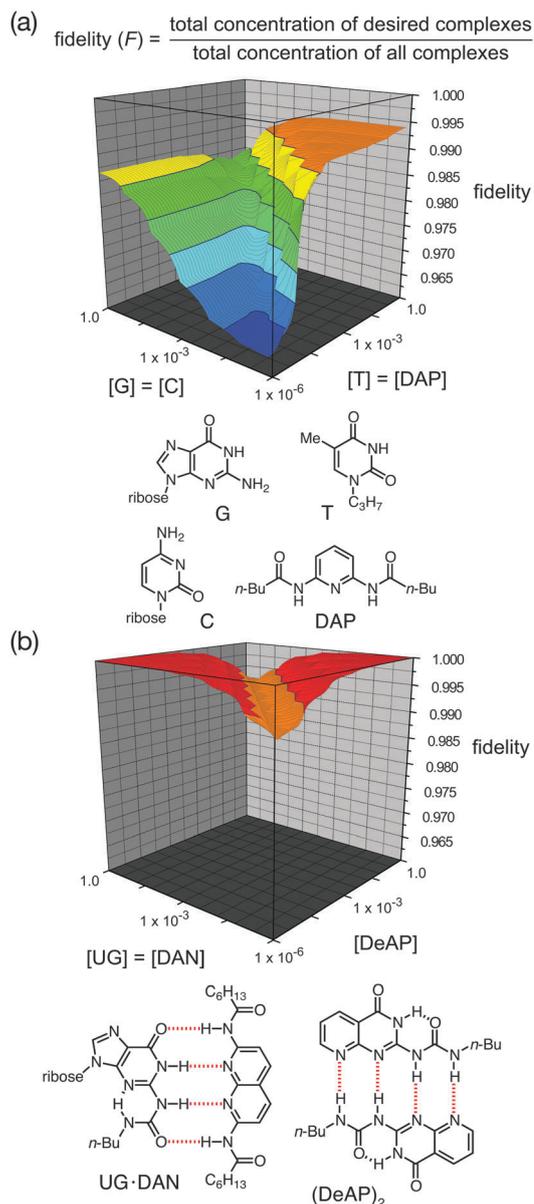


Fig. 28 Fidelity plots for mixtures of (a) G-C and T-DAP and (b) UG-DAN and (DeAP)₂ complexes. Concentrations are expressed in molar (M). Adapted with permission from ref. 119. Copyright (2005) American Chemical Society.

consisting of UG-DAN and (DeAP)₂ forms a very high fidelity system ($F \geq 0.99$) over a wide range of concentrations and stoichiometries.¹¹⁹ Again, although both complexes are quadruply hydrogen-bonded, the difference in the arrays (DAAD-ADDA and DDAA-AADD) contributes significantly to the orthogonality in the recognition process.

6. Orthogonal surface modifications

The modification of surfaces is important in many areas, including the fabrication of microelectronics, optoelectronics, and sensors.¹³⁹ Modified surfaces also play a key role in various aspects of biological engineering, for example as scaffolds for tissue engineering, stem-cell differentiation, and cell culture.¹⁴⁰

Various lithographic techniques allow the creation of patterned surfaces with micron to nanometer scale features. The ability to orthogonally alter the surface chemistry allows precise control over the properties and patterns that are displayed.

6.1 Surface modifications by self-assembly

In 1989 Whitesides and co-workers reported the formation of patterned self-assembled monolayers, employing an orthogonal set of hard–soft acid–base interactions.¹⁴¹ In particular, the selective adsorption of alkanethiols on gold and organic acids on alumina surfaces was observed on gold surfaces with a micro-patterned silver overlay (Fig. 29). Two additional orthogonal self-assembly systems were reported, one using alkanethiols on gold and carboxylic acids or alkane phosphonic acids on indium tin oxide (ITO)¹⁴² and the second system alkane disulfides on gold and alkane isocyanides on platinum.¹⁴³ The latter system did not achieve full orthogonality as it requires a non-equimolar solution of the disulfide and isocyanide (40 : 1) to achieve selective surface functionalization because of the undesired competitive adsorption of isocyanide on a gold surface.¹⁴³

Recently, Hoepfner reported the self-assembly of Si and Au nanoparticles (NPs) and hydroxyl-containing micelles on a multi-functionalized silicon substrate with $-\text{NH}_2$, $-\text{SH}$, or $-\text{CO}_2\text{H}$ groups, with potential selectivity based on their chemical reactivity.¹⁴⁴ Full orthogonality was not possible because undesired interactions such as adsorption of Au NP onto the ammonium surface occur. To avoid cross reactivity, the functionalization was done in a sequential manner: (1) self-assembly of negative charged Si NP on the ammonium functionalized surface, (2) Au NP on the thiol surface, and (3) hydroxyl-containing micelles on the carboxylic acid surface.

6.2 Surface modifications *via* click chemistry

Not surprisingly CuAAC has emerged as a powerful method to covalently attach various entities to surfaces. By combining with other orthogonal reactions, CuAAC has further allowed selective surface functionalization to give patterned surfaces. For example, Gleason used capillary force lithography (CFL) to create a nanopattern of amine and alkyne groups from a bilayer consisting of 50 nm thick non-crosslinked poly(propargyl methacrylate) (PPMA) film laid by initiated chemical vapor deposition (iCVD) on top of a 100 nm thick poly(allylamine) (PAAm) film using plasma enhanced chemical vapor deposition

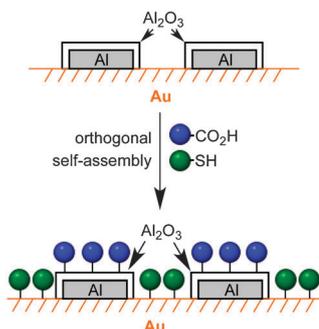


Fig. 29 Orthogonal surface self-assembly based on hard–soft acid–base principle.¹⁴¹



Fig. 30 Simultaneous dual surface functionalization using a combination of CuAAC and carbodiimide chemistry. Reproduced with permission from ref. 145. Copyright (2008) American Chemical Society.

to achieve cross-linking.¹⁴⁵ The functionalization was accomplished in one step with a solution of a Cu(I) salt, tetramethylrhodamine-5-carbonyl azide and 5/6-carboxyfluorescein, succinimidyl ester (Fig. 30).¹⁴⁵ The orthogonal coupling of alkyne to azide and amino group to NHS-ester was evident in the microscopic images showing the red and green fluorescence in the same pattern created by the CFL.

Maynard reported the use of two orthogonal click reactions to pattern proteins on surfaces (Fig. 31).¹⁴⁶ The initial patterned surface was prepared by electron-beam lithography.¹⁴⁷ Subsequently, oxo-myoglobin was attached to the hydroxyl-amine-coated areas through oxime formation followed by the attachment of ubiquitin azide using CuAAC (Fig. 31). The simultaneous immobilization of three different proteins (streptavidin, bovine serum albumin, and myoglobin) on surface patterned with biotin, maleimide, and aminoxy functionalities was also reported.¹⁴⁸ Similarly, Chi used CuAAC and thiol–ene reactions as an orthogonal set of coupling reactions to pattern proteins into nanostripes on Si wafers.¹⁴⁹

Hawker reported a novel set of orthogonal copper-free, thermal reactions for selective surface modification through thermal microcontact printing (μCP).¹⁵⁰ Three different dyes were anchored to the surface using orthogonal thermal azide–alkyne cycloaddition (TAAC) and acyl ketene formation followed by nucleophile trapping. It was found that acyl ketene was formed at 150 °C from its precursor and reacted with amines but not azides; the azide was shown to be stable even at 150 °C.¹⁵⁰

Another example of surface modification using a copper-free, dibenzocyclooctyne-based SPAAC was reported by Popik

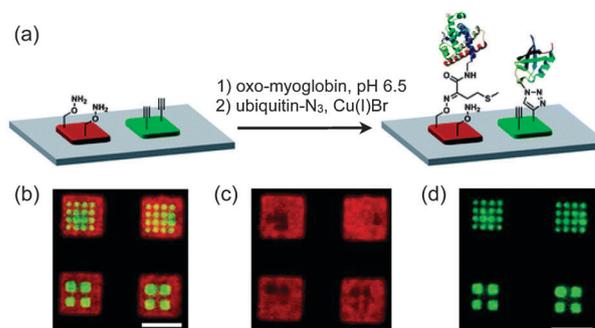


Fig. 31 (a) Dual protein patterning using oxime formation followed by CuAAC. (b) Overlay of myoglobin (green) and ubiquitin (red) fluorescence images. (c) Ubiquitin reacts with alkyne patterns. (d) Myoglobin reacts with aminoxy patterns. Scale bar = 5 μm . Adapted with permission from ref. 146.

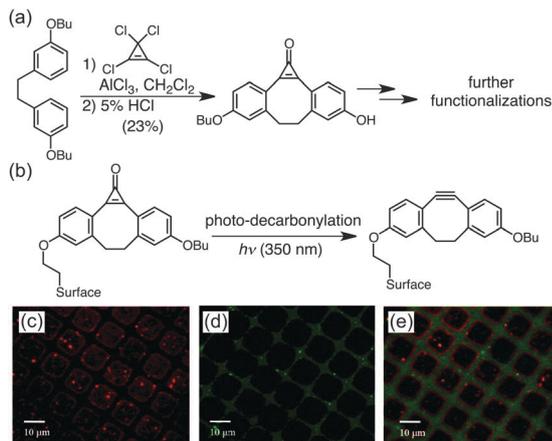


Fig. 32 (a) Synthesis of cyclopropanone-masked DIBO derivatives, (b) photo-decarbonylation upon irradiation at 350 nm, (c)–(e) fluorescence microscope images of a photo-patterned surface prepared by sequential photo-activations and labeling with rhodamine (red) and fluorescein (green). Scale bar = 10 μm . Adapted with permission from ref. 151. Copyright (2010) American Chemical Society.

and co-workers.¹⁵¹ This DIBO-based immobilization has also been applied successfully to functionalize microbead surfaces.¹⁵¹ One of the appealing features of the DIBO system is that the reactive triple bond can be masked as a cyclopropanone motif (Fig. 32a).¹⁰¹ Photo-decarbonylation can be easily performed upon irradiation at 350 nm, thereby revealing the reactive triple bond for copper-free cycloaddition (Fig. 32b). Spatially controlled immobilization can be achieved simply using a shadow mask. Areas protected by the mask will not undergo cycloaddition, thus allowing spatial functionalization on a surface substrate (Fig. 32c–e). Bowman and co-workers also reported the spatiotemporally controlled alkyne–azide cycloaddition using the photochemical reduction of Cu(II) to Cu(I) with photolithographic techniques.¹⁵²

6.3 Surface modifications via supramolecular interactions

An extremely active area of research applies the principles of Section 5 – orthogonal supramolecular connections – to the patterning of surfaces and the formation of functional mono- and multi-layers. This area has been reviewed very recently by Yilmaz and Huskens and, thus, will not be covered here.¹⁵³

7. Conclusions

The concept of orthogonality has come a long way from the protecting group strategy first described by Barany and Merrifield in 1977. Indeed that very same notion of two chemoselective reactions operating with a complete lack of interference from one another has become pervasive in organic chemistry. Indeed, the complexity has multidimensional protecting group strategies available and one can envision a similar development in orthogonal coupling reactions and orthogonal catalytic cycles. Ultimately, this would facilitate more efficient syntheses and perhaps even lead to a day when multi-step synthesis can be carried out in a single pot with simultaneous addition of the reagents.

The orthogonal approach has moved well beyond the realm of synthetic organic chemistry into materials and polymer chemistry and also become a powerful tool for chemical labeling of biomolecules within living cells. The remarkable rate at which click chemistry and bioorthogonal reactions have been adopted is testimony to their powerful ability to link two entities in high yield and without interference even in complex systems. The expansion of the click chemistry toolbox by having additional orthogonal click reactions will undoubtedly further accelerate progress in many of the areas described herein. Where will the next big advance come? Predictions along these lines are always challenging, but more sophisticated strategies for controlling the spatial and temporal aspects of orthogonal processes, especially in synthetic organic and biological contexts, would represent a major advance.

Acknowledgements

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