Bioconjugation and crosslinking technical handbook

Reagents for bioconjugation, crosslinking, biotinylation, and modification of proteins and peptides

thermo scientific

Introduction

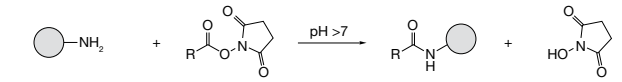
Bioconjugation is the process of chemically joining two or more molecules by a covalent bond where at least one molecule is a biomolecule. This technique utilizes a variety of reagents that contain reactive ends to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules. The availability of several chemical groups in proteins and peptides make them targets for a wide range of applications including biotinylation, fluorescence dye conjugation, immobilization to solid supports, metabolic labeling, and protein structural studies. Bioconjugation reagents, crosslinkers, and modification reagents can be described by their chemical reactivity, molecular properties, or by their applications (Table 1).

Table 1. Key considerations for selecting the right bioconjugation reagent.

Chemical reactivity

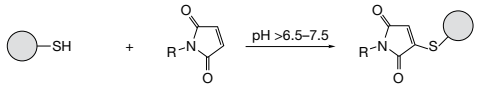
First, select a reagent with the functional group(s) to bind your biomolecules of interest.

NHS ester reaction



Amine-containing molecule | NHS ester compound | Amine bond | NHS

Maleimide reaction

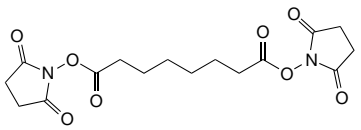


Sulfhydryl-containing molecule | Maleimide compound | Thioether bond

Molecular properties

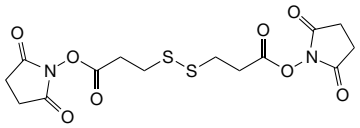
Second, choose which features or characteristics are important for your application.

Functionality



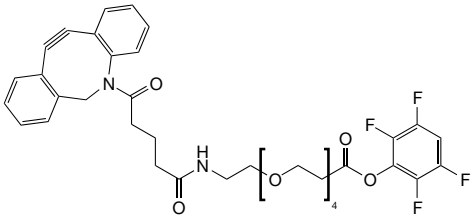
DSS—same reactivity on both ends

Cleavability



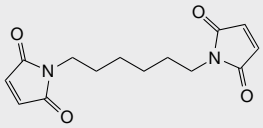
DSP with disulfide linker for cleavage

Structural modifications



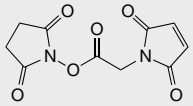
DBCO—copper-free click moiety

Spacer arm composition



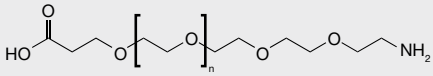
BMH with hydrocarbon spacer

Spacer arm length



AMAS 4.4 Å—short spacer between reactive groups

Spacer arm structure

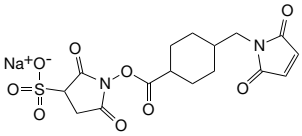


CA(PEG)$_n$—adds solubility in aqueous solutions

Applications

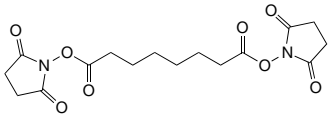
Select the specific reagent depending on the application (e.g., protein detection, immobilization, or interaction studies).

Conjugation



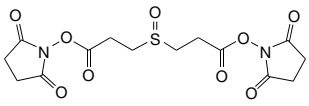
Sulfo-SMCC

Immobilization



DSS—couples proteins to surfaces

Protein interaction studies



DSSO—MS-cleavable crosslinker

Packaging options

Select a package size or grade based on the scale of your reaction or your requirements. These reagents are available from milligram to kilogram quantities.

Milligram

Single tubes

Milligram to gram

Catalog product

Milligram to gram

Premium-grade

Gram to kilogram

Large-volume or custom packages

Contents

Chemical reactivity of crosslinking reagents

Introduction

The most important property of a bioconjugation reagent or crosslinker is its reactive chemical group. The reactive group establishes the mechanism for labeling or crosslinking. Labeling reagents have a reactive moiety at one terminus, such as an NHS ester for amine labeling, and a chemical moiety at the other terminus, such as a fluorescent dye or biotin. Crosslinkers contain at least two reactive groups that target functional groups found in biomolecules. The functional groups that are commonly targeted for bioconjugation include primary amines, sulfhydryls, carbonyls, biorthogonal azides, and alkynes (Figure 1 and Table 2).

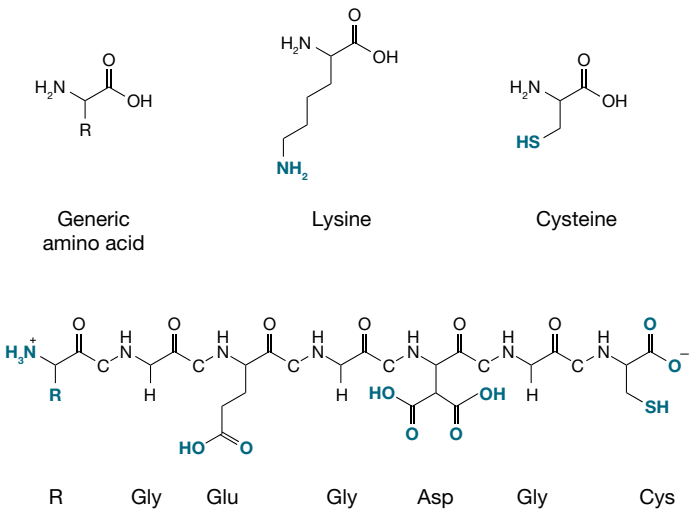


Figure 1. Common amino acid functional groups targeted for bioconjugation.

Table 2. Popular crosslinker reactive groups for bioconjugation.

Reactivity class	Target functional group	Reactive chemical group
Amine-reactive	$-NH_2$	• NHS ester • Imidoester • Pentafluorophenyl ester • Hydroxymethyl phosphine
Carboxyl-to-amine reactive	–COOH	Carbodiimide (e.g., EDC)
Sulfhydryl-reactive	–SH	• Maleimide • Haloacetyl (bromo-, chloro-, or iodo-) • Pyridyl disulfide • Thiosulfonate • Vinyl sulfone
Aldehyde-reactive (e.g., oxidized sugars, carbonyls)	–CHO	• Hydrazide • Alkoxyamine • NHS ester
Photoreactive (i.e., nonselective, random insertion)	Random	• Diazirine • Aryl azide
Hydroxyl (nonaqueous)-reactive	–OH	Isocyanate
Azide-reactive	$-N_3$	• Alkyne • Phosphine

Amine-reactive chemical groups

Primary amines ($-NH_2$) exist at the N terminus of each polypeptide chain (called the α-amine) and in the side chain of lysine (Lys, K) residues (called the ε-amine). Because of their positive charge at physiological conditions, primary amines are usually outward facing (i.e., on the outer surface of proteins), making them more accessible for conjugation without denaturing protein structure. A number of reactive chemical groups target primary amines (Figure 2), but the most commonly used groups are *N*-hydroxysuccinimide esters (NHS esters) and imidoesters.

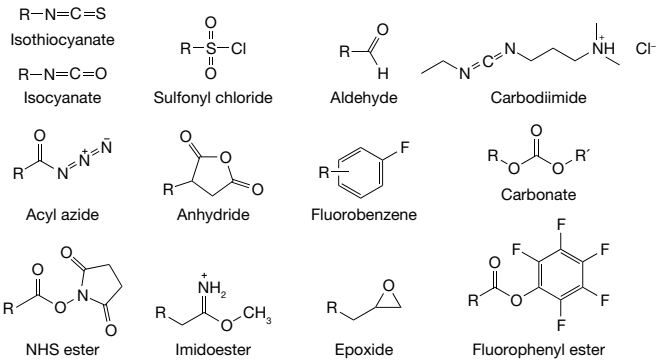
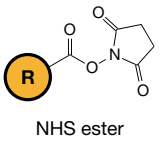


Figure 2. Reactive chemical groups that target primary amines.

N-hydroxysuccinimide esters (NHS esters)



NHS esters are reactive groups formed by EDC activation of carboxylate molecules. NHS ester–activated crosslinkers and labeling compounds react with primary amines in slightly alkaline conditions to yield stable amide bonds (Figure 3). The reaction releases *N*-hydroxysuccinimide, which can be removed easily by dialysis or desalting.

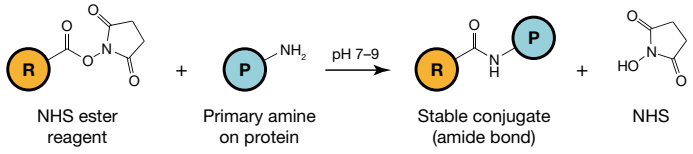


Figure 3. NHS ester reaction scheme for chemical conjugation to a primary amine. R represents a labeling reagent or one end of a crosslinker having the NHS ester reactive group. P represents a protein or other molecule that contains the target functional group (i.e., primary amine).

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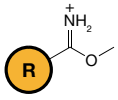
NHS-reactive chemistry

NHS ester crosslinking reactions are most commonly performed in phosphate, carbonate–bicarbonate, HEPES, or borate buffers at pH 7.2–8.5 for 30 minutes to 4 hours at room temperature or 4°C. Primary amine buffers such as Tris (TBS) are not compatible because they compete for the reaction. However, in some procedures, it is useful to add Tris or glycine buffer at the end of a conjugation procedure to stop the reaction.

Hydrolysis of the NHS ester competes with the primary amine reaction. The rate of hydrolysis increases with buffer pH and contributes to less-efficient crosslinking in less-concentrated protein solutions. The half-life of hydrolysis for NHS ester compounds is 4–5 hours at pH 7.0 and 0°C. This half-life decreases to 10 minutes at pH 8.6 and 4°C. The extent of NHS ester hydrolysis in aqueous solutions free of primary amines can be measured at 260–280 nm because the NHS by-product absorbs in that range.

Sulfo-NHS esters are identical to NHS esters except that they contain a sulfonate (–SO_3) group on the *N*-hydroxysuccinimide ring. This charged group has no effect on the reaction chemistry, but it does tend to increase the water solubility of crosslinkers containing them. In addition, the charged group prevents sulfo-NHS crosslinkers from permeating cell membranes, enabling them to be used for cell surface crosslinking methods.

Imidoesters



Imidoester

Imidoester crosslinkers react with primary amines to form amidine bonds (Figure 4). To ensure specificity for primary amines, imidoester reactions are best done in amine-free, alkaline conditions (pH 10), such as with borate buffer.

Because the resulting amidine bond is protonated, the crosslink has a positive charge at physiological pH, much like the primary amine that it replaced. For this reason, imidoester crosslinkers have been used to study protein structure and molecular associations in membranes and to immobilize proteins onto solid-phase supports while preserving the isoelectric point (pI) of the native protein. Although imidoesters are still used in certain procedures, the amidine bonds formed are reversible at high pH. Therefore, the more stable and efficient NHS ester crosslinkers have steadily replaced them in most applications.

Imidoester reaction chemistry

Imidoester crosslinkers react rapidly with amines at alkaline pH to form amidine bonds but have short half-lives. As the pH becomes more alkaline, the half-life and reactivity with amines increases, making crosslinking more efficient when performed at pH 10 than at pH 8. Reaction conditions below pH 10 may result in side reactions, although amidine formation is favored between pH 8 and 10 (Figure 4). Studies using monofunctional alkyl imidates reveal that at pH <10, conjugation can form with just one imidoester functional group. An intermediate *N*-alkyl imidate forms at the lower pH range and will either crosslink to another amine in the immediate vicinity, resulting in *N,N´*-amidine derivatives, or it will convert to an amidine bond. At higher pH, the amidine is formed directly without formation of an intermediate or side product. Extraneous crosslinking that occurs below pH 10 sometimes interferes with interpretation of results when thiol-cleavable diimidoesters are used.

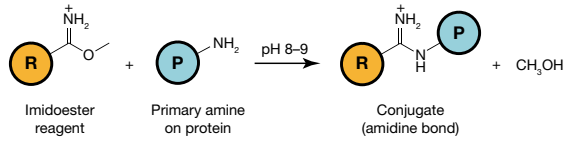
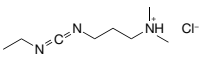


Figure 4. Imidoester reaction scheme for chemical conjugation to a primary amine. R represents a labeling reagent or one end of a crosslinker having the imidoester reactive group. P represents a protein or other molecule that contains the target functional group (i.e., primary amine, –NH_2).

Carboxylic acid–reactive chemical groups

Carboxylic acids (–COOH) exist at the C terminus of each polypeptide chain and in the side chains of aspartic acid (Asp, D) and glutamic acid (Glu, E). Like primary amines, carboxyls are usually on the surface of protein structure. Carboxylic acids are reactive towards carbodiimides.

Carbodiimides (EDC and DCC)



Carbodiimide (EDC)

EDC and other carbodiimides are zero-length crosslinkers. They cause direct conjugation of carboxylates (–COOH) to primary amines (–NH_2) without becoming part of the final amide-bond crosslink between target molecules.

Because peptides and proteins contain multiple carboxyls and amines, direct EDC-mediated crosslinking usually causes random polymerization of polypeptides. Nevertheless, this reaction chemistry is used widely in immobilization procedures (e.g., attaching proteins to a carboxylated surface) and in immunogen preparation (e.g., attaching a small peptide to a large carrier protein).

EDC reaction chemistry

EDC reacts with carboxylic acid groups to form an active *O*-acylisourea intermediate that is easily displaced by nucleophilic attack from primary amino groups in the reaction mixture (Figure 5). The primary amine forms an amide bond with the original carboxyl group, and an EDC by-product is released as a soluble urea derivative. The *O*-acylisourea intermediate is unstable in aqueous solutions. Failure to react with an amine results in hydrolysis of the intermediate, regeneration of the carboxyls, and the release of an *N*-unsubstituted urea.

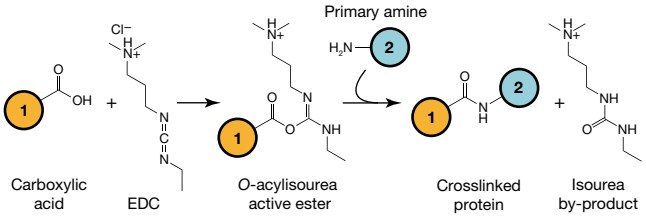


Figure 5. Carboxyl-to-amine crosslinking using the popular carbodiimide EDC. Molecules 1 and 2 can be peptides, proteins, or any chemicals that have respective carboxylate and primary amine groups. When they are peptides or proteins, these molecules are tens to thousands of times larger than the crosslinker and conjugation arms diagrammed in the reaction.

EDC crosslinking is most efficient in acidic (pH 4.5) conditions and must be performed in buffers devoid of extraneous carboxyls and amines. MES buffer (4-morpholinoethanesulfonic acid) is a suitable carbodiimide reaction buffer. Phosphate buffers and neutral pH (up to 7.2) conditions are compatible with the reaction chemistry, but with lower efficiency. Increasing the amount of EDC in a reaction solution can compensate for the reduced efficiency.

N-hydroxysuccinimide (NHS) or its water-soluble analog (sulfo-NHS) is often included in EDC coupling protocols to improve efficiency or create dry-stable (amine-reactive) intermediates (Figure 6). EDC couples NHS to carboxyls, forming an NHS ester that is considerably more stable than the *O*-acylisourea intermediate while allowing efficient conjugation to primary amines at physiological pH.

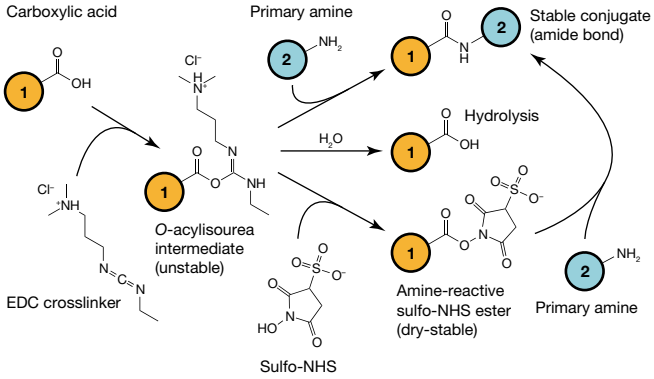


Figure 6. Carboxyl-to-amine crosslinking using the carbodiimide EDC and sulfo-NHS. Addition of NHS or sulfo-NHS to EDC reactions (bottom-most pathway) increases efficiency and enables molecule 1 to be activated for storage and later use.

EDC is also capable of activating phosphate groups in the presence of imidazole for conjugation to primary amines. The method is sometimes used to modify, label, crosslink, or immobilize oligonucleotides through their 5′ phosphate groups.

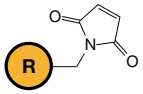
DCC reaction chemistry and applications

DCC (dicyclohexyl carbodiimide) crosslinks carboxylic acids to primary amines in the same manner as EDC. However, because DCC is not water-soluble, it is primarily used in manufacturing and organic synthesis applications rather than in the typical protein research biology laboratory. For example, most commercially available, ready-to-use NHS ester crosslinkers and labeling reagents are manufactured using DCC. Because water is excluded, the resulting NHS ester can be prepared and stabilized as a dried powder without appreciable hydrolysis. DCC is also commonly used in commercial peptide synthesis operations.

Sulfhydryl-reactive chemical groups

Sulfhydryls (–SH) exist in the side chain of cysteine (Cys, C). Often as part of a protein's secondary or tertiary structure, cysteines are joined between their side chains via disulfide bonds (–S–S–). These must be reduced to sulfhydryls to make them available for crosslinking by most types of reactive groups. Sulfhydryls are reactive towards maleimides, haloacetyls, and pyridyl disulfides.

Maleimides



Maleimide

Maleimide-activated crosslinkers and labeling reagents react specifically with sulfhydryl groups (–SH) at near-neutral conditions (pH 6.5–7.5) to form stable thioether linkages. Disulfide bonds in protein structures must be reduced to free thiols (sulfhydryls) to react with maleimide reagents. Extraneous thiols (e.g., from most reducing agents) must be excluded from maleimide reaction buffers because they will compete for coupling sites.

Short homobifunctional maleimide crosslinkers enable disulfide bridges in protein structures to be converted to permanent, irreducible linkages between cysteines. More commonly, the maleimide chemistry is used in combination with amine-reactive NHS ester chemistry in the form of heterobifunctional crosslinkers that enable controlled two-step conjugation of purified peptides and/or proteins.

Maleimide reaction chemistry

The maleimide group reacts specifically with sulfhydryl groups when the pH of the reaction mixture is between pH 6.5 and 7.5, resulting in the formation of a stable thioether linkage that is not reversible (Figure 7). In more alkaline conditions (pH >8.5), the reaction favors primary amines and also increases the rate of hydrolysis of the maleimide group to a nonreactive maleamic acid. Maleimides do not react with tyrosines, histidines, or methionines.

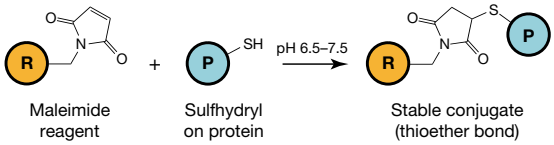
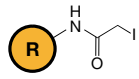


Figure 7. Maleimide reaction scheme for chemical conjugation to a sulfhydryl. R represents a labeling reagent or one end of a crosslinker having the maleimide reactive group. P represents a protein or other molecule that contains the target functional group (i.e., sulfhydryl, –SH).

Thiol-containing compounds, such as dithiothreitol (DTT) and β-mercaptoethanol (BME (also known as 2-mercaptoethanol)), must be excluded from reaction buffers used with maleimides because they will compete for coupling sites. For example, if DTT were used to reduce disulfides in a protein to make sulfhydryl groups available for conjugation, the DTT would have to be thoroughly removed using a desalting column before initiating the maleimide reaction. Interestingly, the disulfide-reducing agent TCEP does not contain thiols and does have to be removed before reactions using maleimide reagents.

Excess maleimides can be quenched at the end of a reaction by adding free thiols. EDTA can be included in the coupling buffer to chelate stray divalent metals that otherwise promote oxidation of sulfhydryls (nonreactive).

Haloacetyls



Iodoacetyl group

Most haloacetyl crosslinkers contain an iodoacetyl or a bromoacetyl group. Haloacetyls react with sulfhydryl groups at physiological to alkaline conditions (pH 7.2–9), resulting in stable thioether linkages. To limit free iodine generation, which has the potential to react with tyrosine, histidine, and tryptophan residues, it is best to perform iodoacetyl reactions in the dark.

Haloacetyl reaction chemistry

Haloacetyls react with sulfhydryl groups at physiological pH. The reaction of the iodoacetyl group proceeds by nucleophilic substitution of iodine with a sulfur atom from a sulfhydryl group, resulting in a stable thioether linkage (Figure 8). Using a slight excess of the iodoacetyl group over the number of sulfhydryl groups at pH 8.3 ensures sulfhydryl selectivity. In the absence of free sulfhydryls, or if a large excess of iodoacetyl group is used, the iodoacetyl group can react with other amino acids. Imidazoles can react with iodoacetyl groups at pH 6.9–7.0, but the incubation must proceed for longer than one week.

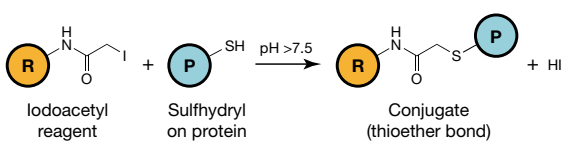
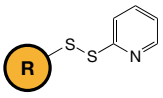


Figure 8. Iodoacetyl reaction scheme for chemical conjugation to a sulfhydryl. R represents a labeling reagent or one end of a crosslinker having the iodoacetyl or bromoacetyl reactive group. P represents a protein or other molecule that contains the target functional group (i.e., sulfhydryl, –SH).

Histidyl side chains and amino groups react in the unprotonated form with iodoacetyl groups above pH 5 and pH 7, respectively. To limit free iodine generation, which has the potential to react with tyrosine, histidine, and tryptophan residues, iodoacetyl reactions and preparations should be performed in the dark. Iodoacetyl compounds should not be exposed to reducing agents.

Pyridyl disulfides



Pyridyldithiol

Pyridyl disulfides react with sulfhydryl groups over a broad pH range to form disulfide bonds. As such, conjugates prepared using these crosslinkers are cleavable with typical disulfide-reducing agents such as dithiothreitol (DTT).

Pyridyl disulfide reaction chemistry

Pyridyl disulfides react with sulfhydryl groups over a broad pH range (the optimum is pH 4–5) to form disulfide bonds. During the reaction, a disulfide exchange occurs between the molecule's –SH group and the reagent's 2-pyridyldithiol group. As a result, pyridine-2-thione is released and can be measured spectrophotometrically (A_{max} = 343 nm) to monitor the progress of the reaction. These reagents can be used as crosslinkers and to introduce sulfhydryl groups into proteins. The disulfide exchange can be performed at physiological pH, although the reaction rate is slower than in acidic conditions (Figure 9).

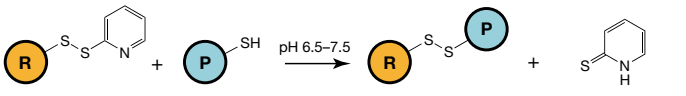


Figure 9. Pyridyldithiol reaction scheme for cleavable (reversible) chemical conjugation to a sulfhydryl. R represents a labeling reagent or one end of a crosslinker having the pyridyl disulfide reactive group. P represents a protein or other molecule that contains the target functional group (i.e., sulfhydryl, –SH).

Carbonyl-reactive chemical groups

Carbonyl (–CHO) groups can be created in glycoproteins by oxidizing the polysaccharide posttranslational modifications with sodium *meta*-periodate. Hydrazide and alkoxyamine reactive groups target aldehydes. These aldehydes also react with primary amines to form Schiff bases that can be further reduced to form a covalent bond (reductive amination).

Carbohydrate modification is particularly useful for creating target sites for conjugation on polyclonal antibodies because the polysaccharides are located in the Fc region. This results in labeling or crosslinking sites located away from antigen binding sites, ensuring that antibody function will not be adversely affected by the conjugation procedure.

Carbonyls (aldehydes) as crosslinking targets

Aldehydes (RCHO) and ketones (RCOR′) are reactive varieties of the more general functional group called carbonyls, which have a carbon–oxygen double bond (C=O). The polarity of this bond (especially in the context of aldehydes) makes the carbon atom electrophilic and reactive to nucleophiles such as primary amines.

Although aldehydes do not naturally occur in proteins or other macromolecules of interest in typical biological samples, they can be created wherever oxidizable sugar groups (also called reducing sugars) exist. Such sugars are common monomer constituents of the polysaccharides or carbohydrates in posttranslational glycosylation of many proteins. In addition, the ribose of RNA is a reducing sugar.

Periodic acid (HIO_4) from dissolved sodium periodate ($NaIO_4$) is a well-known mild agent for effectively oxidizing vicinal diols in carbohydrate sugars to yield reactive aldehyde groups. The carbon–carbon bond is cleaved between adjacent hydroxyl groups. By altering the amount of periodate used, aldehydes can be produced on a smaller or larger selection of sugar types. For example, treatment of glycoproteins with 1 mM periodate usually affects only sialic acid residues, which frequently occur at the ends of polysaccharide chains. At concentrations of 6–10 mM periodate, other sugar groups in proteins will be affected (Figure 10).

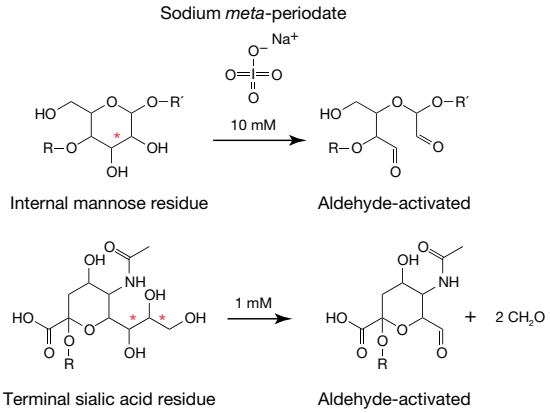
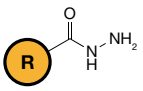


Figure 10. The reaction of sodium periodate with sugar residues yields aldehydes for conjugation reactions. R and R′ represent connecting sugar monomers of the polysaccharide. Red asterisks indicate sites of diol cleavage. Sialic acid is also called *N*-acetyl-D-neuraminic acid.

Hydrazides



Hydrazide

Carbonyls (aldehydes and ketones) can be produced in glycoproteins and other polysaccharide-containing molecules by mild oxidation of certain sugar glycols using sodium *meta*-periodate. Hydrazide-activated crosslinkers and labeling compounds will then conjugate with these carbonyls at pH 5–7, resulting in formation of hydrazone bonds.

Hydrazide chemistry is useful for labeling, immobilizing, or conjugating glycoproteins through glycosylation sites, which are often (as with most polyclonal antibodies) located at domains away from the key binding sites whose function one wishes to preserve.

Hydrazide reaction chemistry

Aldehydes created by periodate oxidation of sugars in biological samples react with hydrazides at pH 5–7 to form hydrazone bonds (Figure 11). Although this bond to a hydrazide group is a type of Schiff base, it is considerably more stable than a Schiff base formed with a simple amine. The hydrazone bond is sufficiently stable for most protein-labeling applications. If desired, however, the double bond can be reduced to a more stable secondary amine bond using sodium cyanoborohydride (see section on reductive amination).

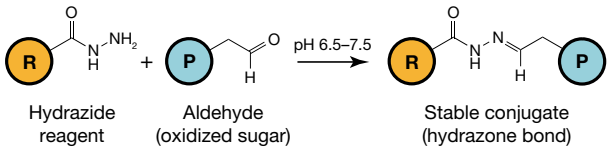
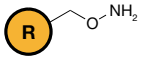


Figure 11. Hydrazide reaction scheme for chemical conjugation to an aldehyde. R represents a labeling reagent or one end of a crosslinker having the hydrazide reactive group. P represents a glycoprotein or other glycosylated molecule that contains the target functional group (i.e., an aldehyde formed by periodate oxidation of carbohydrate sugar groups, such as sialic acid).

Alkoxyamines



Alkoxyamine

Although not currently as popular or common as hydrazide reagents, alkoxyamine compounds conjugate to carbonyls (aldehydes and ketones) in much the same manner as hydrazides.

Alkoxyamine reaction chemistry

Alkoxyamine compounds conjugate to carbonyls to create an oxime linkage (Figure 12). The reaction is similar to that of hydrazides and can also use aniline as a catalyst.

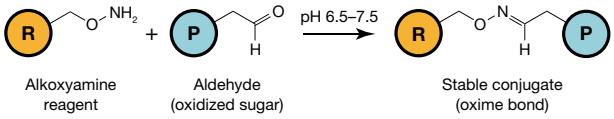


Figure 12. Alkoxyamine reaction scheme for chemical conjugation to an aldehyde. R represents a labeling reagent or one end of a crosslinker having the alkoxyamine reactive group. P represents a glycoprotein or other glycosylated molecule that contains the target functional group (i.e., an aldehyde formed by periodate oxidation of carbohydrate sugar groups, such as sialic acid).

Reductive amination

In reductive amination, the electrophilic carbon atom of an aldehyde attacks the nucleophilic nitrogen of a primary amine to yield a weak bond called a Schiff base bond. Unlike the bond formed with hydrazide or alkoxyamines, the Schiff base formed with ordinary amines rapidly hydrolyzes (reverses) in aqueous solution and must be reduced to an alkylamine (secondary amine) linkage to stabilize it. Sodium cyanoborohydride ($NaCNBH_3$) is a mild reducing agent that performs this function effectively, without reducing other chemical groups in biological samples (Figure 13). Like carbodiimide crosslinking chemistry (carboxyl to amine), reductive amination (aldehyde to amine) is a zero-length crosslinking method.

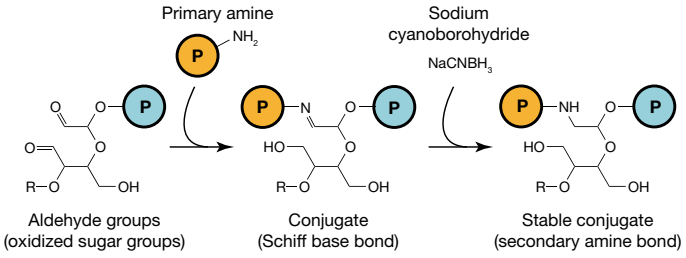


Figure 13. Reductive amination, the conjugation of aldehydes and primary amines. The initial reaction results in a weak, reversible Schiff base linkage. Reduction with sodium cyanoborohydride creates a stable, irreversible secondary amine bond.

Photoreactive crosslinkers

Photoreactive crosslinkers are widely used for nonspecific bioconjugation. While numerous options exist (Figure 14), the two most common photoreactive chemical groups are diazirines and aryl azides. Photoreactive groups are activated by ultraviolet (UV) light and can be used *in vitro* and *in vivo*.

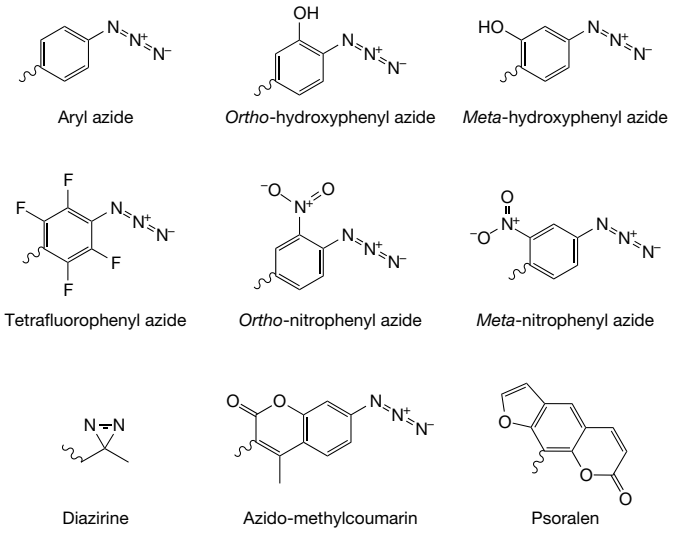
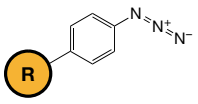


Figure 14. Common photoreactive chemical groups used for bioconjugation.

Aryl azides



Photoreactive reagents are chemically inert compounds that become reactive when exposed to UV or visible light. Historically, aryl azides (also called phenyl azides) have been the most popular photoreactive chemical group used in crosslinking and labeling reagents.

Photoreactive reagents are most often used as heterobifunctional crosslinkers to capture binding partner interactions. A purified bait protein is labeled with the crosslinker using the amine- or sulfhydryl-reactive end. Then this labeled protein is added to a lysate sample and allowed to bind its interactor. Finally, photoactivation with UV light initiates conjugation via the aryl azide group.

Aryl azide reaction chemistry

When an aryl azide is exposed to UV light (250–350 nm), it forms a nitrene group that can initiate addition reactions with double bonds, insertion into C–H and N–H sites, or subsequent ring expansion to react with a nucleophile (e.g., primary amines).

The latter reaction path dominates when primary amines are present in the sample (Figure 15).

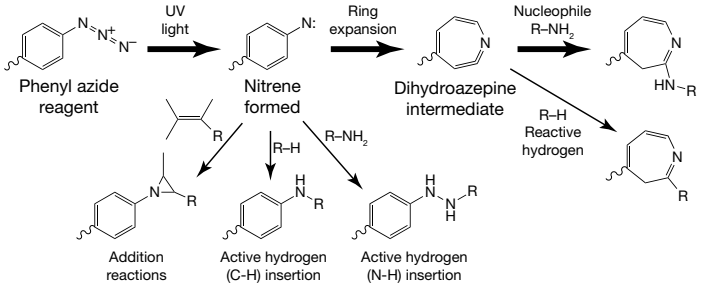


Figure 15. Aryl azide reaction scheme for light-activated photochemical conjugation. Squiggle bonds represent a labeling reagent or one end of a crosslinker having the phenyl azide reactive group. R represents a protein or other molecule that contains nucleophilic or active hydrogen groups. Bold arrows indicate the dominant pathway. Halogenated aryl azides react directly (without ring expansion) from the activated nitrene state.

Thiol-containing reducing agents (e.g., DTT or β-mercaptoethanol) must be avoided in the sample solution during all steps before and during photoactivation because they reduce the azide functional group to an amine, preventing photoactivation. Reactions can be performed in a variety of amine-free buffer conditions. If working with heterobifunctional photoreactive crosslinkers, buffers should be used that are compatible with both reactive chemistries involved. Experiments must be performed in subdued light and/or with reaction vessels covered in foil until photoreaction is intended. Typically, photoactivation is accomplished with a handheld UV lamp positioned close to the reaction solution and shining directly on it (i.e., not through glass or polypropylene) for several minutes.

Three basic forms of aryl azides exist: simple phenyl azides, hydroxyphenyl azides, and nitrophenyl azides. Generally, short-wavelength UV light (e.g., 254 nm) is needed to efficiently activate simple phenyl azides, while long-wavelength UV light (e.g., 365 nm) is sufficient for nitrophenyl azides. Because short-wavelength UV light can be damaging to other molecules, nitrophenyl azides are usually preferable for crosslinking experiments.

Diazirines

Diazirines are a newer class of photoactivatable chemical groups that are being used in crosslinking and labeling reagents. The diazirine (azipentanoate) moiety has better photostability than aryl azide groups, and it is more easily and efficiently activated with long wavelength UV light (330–370 nm).

Diazirine reaction chemistry

Photoactivation of diazirine creates reactive carbene intermediates (Figure 16). Such intermediates can form covalent bonds through addition reactions with any amino acid side chain or peptide backbone at distances corresponding to the spacer arm lengths of the particular reagent. Diazirine analogs of amino acids can be incorporated into protein structures by translation, enabling specific recombinant proteins to be activated as the crosslinker.

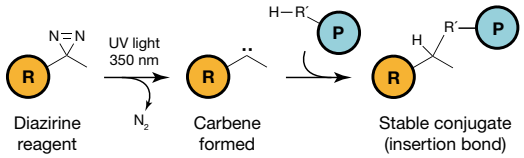
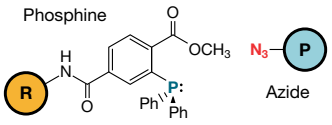


Figure 16. Diazirine reaction scheme for light-activated photochemical conjugation. R represents a labeling reagent or one end of a crosslinker having the diazirine reactive group. P represents a protein or other molecule that contains nucleophilic or active hydrogen groups (R′).

Chemoselective ligation



Chemoselective ligation refers to the use of mutually specific pairs of conjugation reagents. Unlike typical crosslinking methods used in biological research, this reaction chemistry depends upon a pair of unique reactive groups that are specific to one another and also foreign to biological systems. Because these reactions (azide–alkyne or azide–phosphine) do not occur in cells, these functional groups react only with each other in biological samples, thus resulting in minimal background and few artifacts, hence the term “chemoselective”. This specialized form of crosslinking can be applied for both *in vivo* metabolic labeling and bioconjugation using bioorthogonal coupling partners.

Chemoselectivity of azide–alkyne reactions

The reaction between an azide and an alkyne either using a copper catalyst or a copper-free strained alkyne results in the formation of a stable triazole linkage between the coupling partners. This reaction has received much attention because of the bioorthogonal nature of the two coupling partners. In the classic click reaction, an azide is coupled to an alkyne using Cu(I) to bring two coupling partners together and form a stable triazole linkage. One drawback of this approach is that copper ions—both Cu(II) and Cu(I), which are produced in the presence of ascorbate or TCEP—can harm cells, reduce the fluorescence of fluorophores, and impair protein function. To overcome this challenge, strained cyclic alkynes (DIBO/DBCO) have been developed to efficiently react with an azide to form the triazole linkage in the absence of copper under biological conditions. The strain in this eight-membered ring allows the reaction with azide-modified molecules to occur in the absence of catalysts or extreme temperatures, enabling the study of the surface of live cells, and preventing copper-induced damage of fluorescent proteins such as GFP in fixed and permeabilized cells (Figure 17).

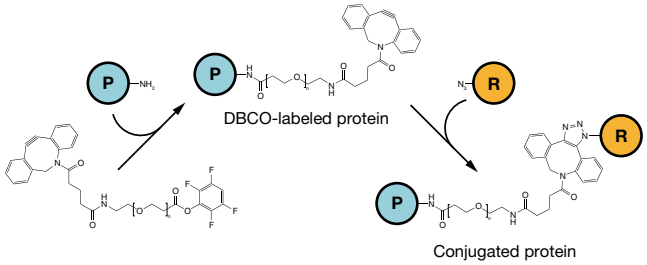


Figure 17. Two-step reaction scheme for conjugating a protein R and azide containing coupling partner P with TFP-PEG$_{(n)}$-DBCO. In this example, the TFP-PEG$_{(n)}$-DBCO is first reacted with the protein to produce a DBCO-labeled protein. After excess nonreacted crosslinker and by-products are removed, the DBCO-labeled protein is reacted with the appropriate molar ratio of azide-coupling partner containing an azide group, forming a stable triazole linkage.

Chemoselectivity of azide–phosphine reactions

The Staudinger reaction occurs between a methyl ester phosphine and an azide (N_3^-) to produce an aza-ylide intermediate that is trapped to form a stable covalent bond (Figure 18).

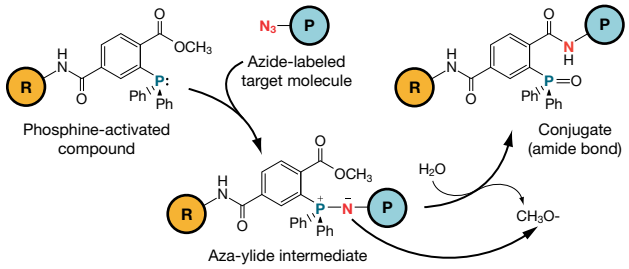


Figure 18. Staudinger ligation reaction scheme (azide–phosphine conjugation). Phosphine-activated proteins or labeling reagents react with azide-labeled target molecules to form aza-ylide intermediates that quickly rearrange in aqueous conditions to form stable amide bonds between reactant molecules.

The Staudinger ligation involves the reaction of azido bioorthogonal probes with phosphine compounds. Similar to click chemistry, the ligation reaction is highly specific and can be performed in aqueous environments at physiological pH. Staudinger ligations do not require copper to be reactive, which increases their biocompatibility; however, these reactions also tend to be slower than click reactions because of the absence of a catalyst.

Molecular properties of crosslinking reagents

Introduction

Bioconjugation and crosslinking reagents are selected based on their chemical reactivities and other chemical properties that affect their behavior in different applications. Key considerations include the chemical specificity of the reactive ends, reaction conditions, and if further modification to the protein or peptide of interest is required to enable bioconjugation. Other important factors that influence the functionality, specificity, and solubility of the bioconjugation reaction include the spacer arm length, cleavability, composition, and structure (Table 3).

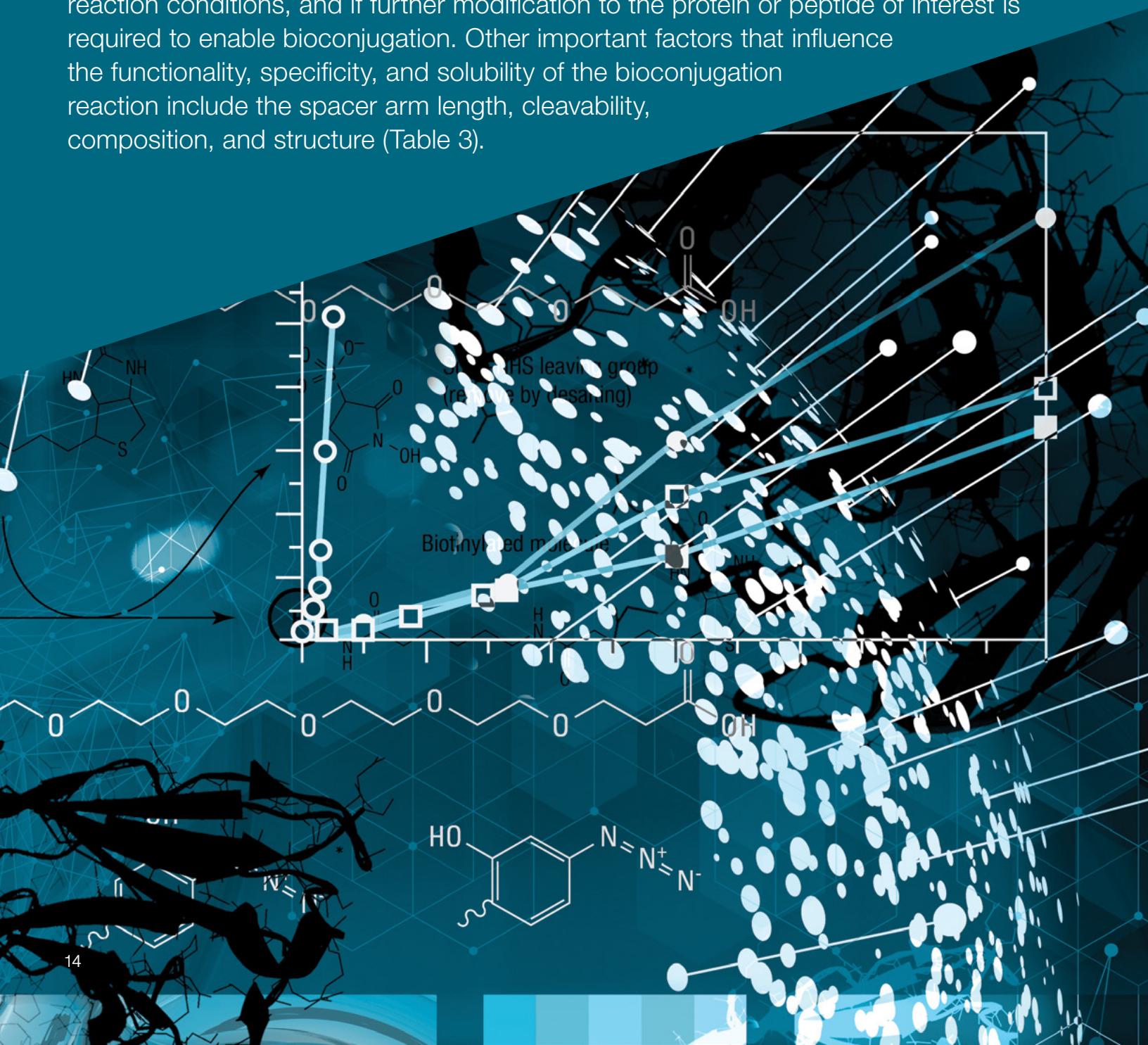


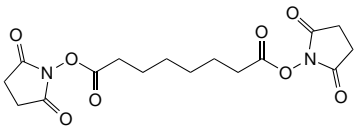
Table 3. Molecular properties of bioconjugation reagents.

Property	Description
Chemical specificity	The reactive target(s) of the crosslinker's reactive ends. A general consideration is whether the reagent has the same or different reactive groups at either end (termed homobifunctional and heterobifunctional, respectively).
General reaction conditions	The buffer system required to perform bioconjugation. Variables include pH, buffer concentration, and protein concentration.
Modifications	These specialized reagents add molecular mass, create a new functional group that can be targeted in a subsequent reaction step, or increase the solubility of the molecule.
Spacer arm length	The molecular span of a crosslinker (i.e., the distance between conjugated molecules). A related consideration is whether the linkage is cleavable or reversible.
Spacer arm composition	The chemical groups found within the spacer arm.
Spacer arm cleavability	The availability of a cleavage site within the spacer arm between the chemical reactive groups.
Chain structure and solubility	The presence of a straight or branched chain that can effect whether a crosslinker or modifier can permeate into cells and/or crosslink hydrophobic proteins within membranes. These properties are determined by the composition of the spacer arm and/or reactive group.

Homobifunctional and heterobifunctional crosslinkers

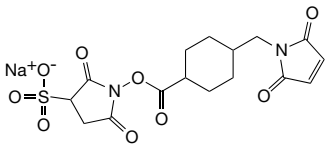
Crosslinkers can be classified as homobifunctional or heterobifunctional. Homobifunctional crosslinkers have identical reactive groups at either end of a spacer arm (Figure 19). Generally, they must be used in one-step reaction procedures to randomly "fix" or polymerize molecules containing like functional groups. For example, adding an amine-to-amine crosslinker to a cell lysate will result in random conjugation of protein subunits, interacting proteins, and any other polypeptides whose lysine side chains happen to be near each other in the solution. This is ideal for capturing a "snapshot" of all protein interactions but cannot provide the precision needed for other types of crosslinking applications. For example, when preparing an antibody–enzyme conjugate, the goal is to link one to several enzyme molecules to each molecule of antibody without causing any antibody-to-antibody linkages to form. This is not possible with homobifunctional crosslinkers.

Heterobifunctional crosslinkers possess different reactive groups at either end (Figure 20). These reagents not only allow single-step conjugation of molecules that have the respective target functional groups, but they also allow sequential (two-step) conjugations that minimize undesirable polymerization or self-conjugation. In sequential procedures, heterobifunctional reagents are reacted with one protein using the most labile group of the crosslinker first. After removing excess unreacted crosslinker, the modified first protein is added to a solution containing the second protein where reaction through the second reactive group of the crosslinker occurs. The most widely used heterobifunctional crosslinkers are those having an amine-reactive group (succinimidyl ester, NHS ester) at one end and a sulfhydryl-reactive group (e.g., maleimide) on the other end. Because the NHS ester group is less stable in aqueous solution, it is usually reacted to one protein first. If the second protein does not have available native sulfhydryl groups, they can be added in a separate prior step using sulfhydryl-addition reagents.



DSS
Disuccinimidyl suberate
MW 368.34
Spacer arm 11.4 Å

Figure 19. Homobifunctional crosslinker example. DSS is a popular, simple crosslinker that has identical amine-reactive NHS ester groups at either end of a short spacer arm. The spacer arm length (11.4 Å) is the final maximum molecular distance between conjugated molecules (i.e., nitrogens of the target amines).



Sulfo-SMCC
Sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate
MW 436.37
Spacer arm 8.3 Å

Figure 20. Heterobifunctional crosslinker example. Sulfo-SMCC is a popular crosslinker that has an amine-reactive sulfo-NHS ester group (left) at one end and a sulfhydryl reactive maleimide group (right) at the opposite end of a cyclohexane spacer arm. This allows sequential, two-step conjugation procedures.

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Certain reagents have three termini, and are referred to as trifunctional crosslinkers or label transfer reagents. These compounds typically possess two chemically reactive functional groups and one label such as a biotin group. A commonly used example is sulfo-SBED (Figure 21).

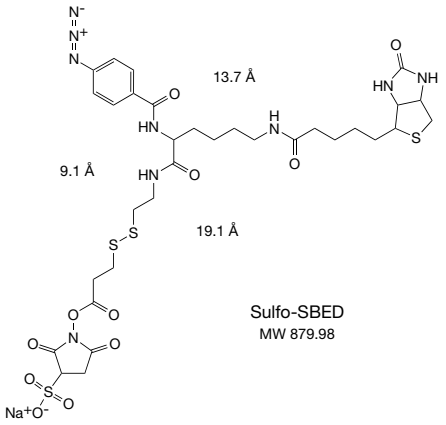


Figure 21. Trifunctional crosslinker example: sulfo-SBED.

General reaction conditions

In many applications, it is necessary to maintain the native structure of the protein complex, so crosslinking is most often performed using near-physiological conditions. Reaction buffers for commonly used reactive groups are summarized in Table 4. Optimal crosslinker-to-protein molar ratios for reactions must be determined empirically, although product instructions for individual reagents generally contain guidelines and recommendations for common applications.

Depending on the application, the degree of conjugation is an important factor. For example, when preparing immunogen conjugates, a high degree of conjugation is desired to increase the immunogenicity of the antigen. However, when conjugating to an antibody or an enzyme, a low to moderate degree of conjugation may be optimal so that biological activity of the protein is retained.

The number of functional groups on the protein's surface is also important to consider. If there are numerous target groups, a lower crosslinker-to-protein ratio can be used. For a limited number of potential targets, a higher crosslinker-to-protein ratio may be required. Furthermore, the number of components should be kept low or to a minimum because conjugates consisting of more than two components are difficult to analyze and provide less information on spatial arrangements of protein subunits.

Modifications

Protein analysis and detection techniques often require more than direct conjugation with a bifunctional crosslinker or activated labeling reagent. For example, in many situations, specialized protein modifications are needed to add molecular mass, increase solubility for storage, or create a new functional group that can be targeted in a subsequent reaction step. Protein modification reagents are chemicals that block, add, change, or extend the molecular reach of functional groups.

Protein sulfhydryls (side chain of cysteine) are important regulators of protein structure and function. Reducing agents are used to prevent intra- and intermolecular disulfide bonds

Table 4. Summary of characteristics of reactive groups.

Reactive group	Target molecules	Reaction buffer	Optimal reaction pH range
Amine	Aldehydes (oxidized carbohydrates) Carboxylic acid (EDC-modified)	PBS (non-amine) MES	pH 7.2 pH 4.5–7.2
Aryl azide	Unsubstituted aryl azides react primarily with amines	PBS (non-amine)	NA
Carbodiimide	Carboxylic acids, hydroxyls	MES or PBS	pH 4.5–7.2
Hydrazide	Aldehydes, ketones Carboxylic acid (EDC-modified)	0.1 M Na-acetate/phosphate MES/non-amine buffer	pH 5.5–7.5 pH 4.5–7.2 (up to pH 7.5)
Imidoester	Amines	PBS, borate, carbonate/bicarbonate, HEPES	pH 8–9
Iodoacetyl	Sulfhydryls	PBS, borate, carbonate/bicarbonate, HEPES	pH 7.5–8.5
Isocyanate (PMPI)	Hydroxyls Amines	Nonaqueous	NA
Maleimide	Sulfhydryls	Thiol-free	pH 7 optimal pH 6.5–7.5
NHS ester	Amines	PBS, borate, carbonate/bicarbonate, HEPES	pH 7.5 optimal pH 7.2–8.5
Pyridyl disulfide	Sulfhydryls	Thiol-free	pH 7–8
Vinyl sulfone (HBVS)	Sulfhydryls	Thiol-free	pH 8

from forming between cysteine residues of proteins in order to enable crosslinking or modification. This reduction is sometimes carried out under denaturing conditions to enhance reactivity of inaccessible disulfide bonds. Dithiothreitol (DTT), β-mercaptoethanol (BME), and Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) are frequently used to reduce the disulfide bonds of proteins. TCEP-HCl is a potent, versatile, odorless, thiol-free reducing agent with broad application to protein and other research involving reduction of disulfide bonds (Figure 22). This unique compound is easily soluble and very stable in many aqueous solutions. TCEP reduces disulfide bonds as effectively as dithiothreitol (DTT), but unlike DTT and other thiol-containing reducing agents, TCEP does not have to be removed before certain sulfhydryl-reactive crosslinking reactions.

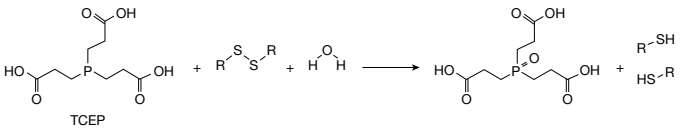
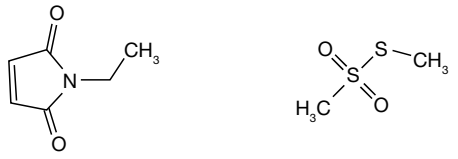


Figure 22. TCEP reduces disulfide bonds within bioconjugation reagents and proteins.

Chaotropic and denaturing chemical agents, including urea and guanidine hydrochloride, disrupt water interactions and promote hydrophobic protein and peptide solubilization, elution, refolding, and structural analysis.

Certain reagents are capable of reacting permanently or reversibly with sulfhydryl groups (e.g., NEM or MMTS, respectively). These reagents add a very small "cap" on the native sulfhydryl, enabling the activity of certain enzymes to be controlled for specific assay purposes (Figure 23).



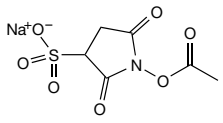
NEM
N-Ethylmaleimide
MW 125.13

MMTS
Methyl methanethiosulfonate
MW 126.20

Figure 23. Sulfhydryls can be blocked using NEM and MMTS.

Sulfo-NHS acetate is a protein modification reagent that reacts with primary amines at pH 7.0–9.0, allowing modifications to occur in many standard buffers without amines (Figure 24). Once reacted, the amine is irreversibly capped with an acyl group.

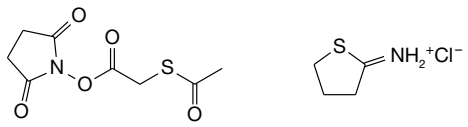
For reversible amine blocking, Thermo Scientific™ Pierce™ Citraconic Anhydride (Cat. No. 15479100) is used. Sulfo-NHS acetate is typically used to prevent polymerization when performing protein crosslinking reactions and when conjugating peptides to carrier proteins for immunogen production. Blocking amines on the peptide allows directed conjugation of carboxylic acids on the peptide to primary amines on the protein using Thermo Scientific™ EDC (Cat. No. 22980, 22981).



Sulfo-NHS acetate
MW 259.17

Figure 24. Sulfo-NHS acetate is a protein modification reagent for blocking primary amines.

SATA and related reagents contain an amine-reactive group and a protected sulfhydryl group. By reacting the compound to a purified protein, the side chain of lysine residues can be modified to contain a sulfhydryl group for targeting with sulfhydryl-specific crosslinkers or immobilization chemistries. The method does not actually convert the amine into a sulfhydryl; rather, it attaches a sulfhydryl-containing group to the primary amine. The effect is also to extend the length of the side chain by several angstroms (Figure 25).



SATA
MW 231.23
Spacer arm 2.8 Å

Traut's reagent
MW 137.63
Spacer arm 8.1 Å

Figure 25. Sulfhydryls can be converted to amines using SATA or Traut's reagent.

Chemically attaching single- or branched-chain polyethylene glycol (PEG) groups to proteins (often referred to as PEGylation) is a form of labeling or modification that is primarily used to confer water solubility and inert molecular mass to proteins. Forms of PEG that have been synthesized to contain reactive chemical groups comprise ready-to-use, activated reagents for PEGylation (Figure 26).

Learn more at **thermofisher.com/proteinmodification**

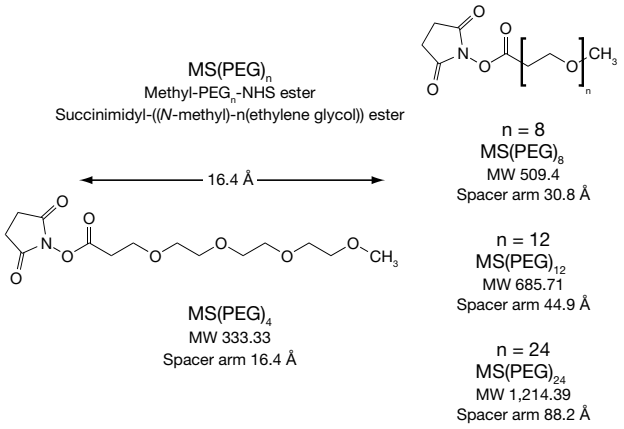


Figure 26. Examples of single-chain, amine-reactive PEGylation reagents.

Spacer arm length

The spacer arm is the chemical chain between two reactive groups or between a reactive group and a label. The length of a spacer arm (measured in Å) determines how flexible a conjugate will be. Longer spacer arms have greater flexibility and reduced steric hindrance. Longer spacer arms have the caveat of possessing more sites for potential nonspecific binding. Spacer arms can range from zero length to >100 Å (Figure 27).

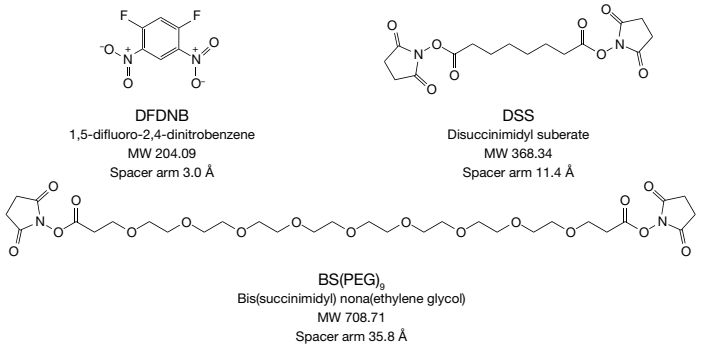


Figure 27. Different lengths of spacer arms.

Spacer arm composition

The molecular composition of a bioconjugation spacer arm can affect solubility and nonspecific binding. Traditional crosslinkers and labeling reagents have spacer arms that contain hydrocarbon chains or polyethylene glycol (PEG) chains. Hydrocarbon chains are not water-soluble and typically require an organic solvent such as DMSO or DMF for suspension. These reagents are better suited for penetrating the cell membrane and performing intercellular crosslinking because they are hydrophobic and uncharged. For example, if a charged sulfonate group is added to the termini of a hydrophobic crosslinker, a water-soluble analog is formed. A good example of this is the comparison of DSS with BS^3. DSS is soluble in organic solvents whereas BS^3 is soluble is aqueous buffers. $BS(PEG)_5$ is also water-soluble because of its PEG spacer (Figure 28).

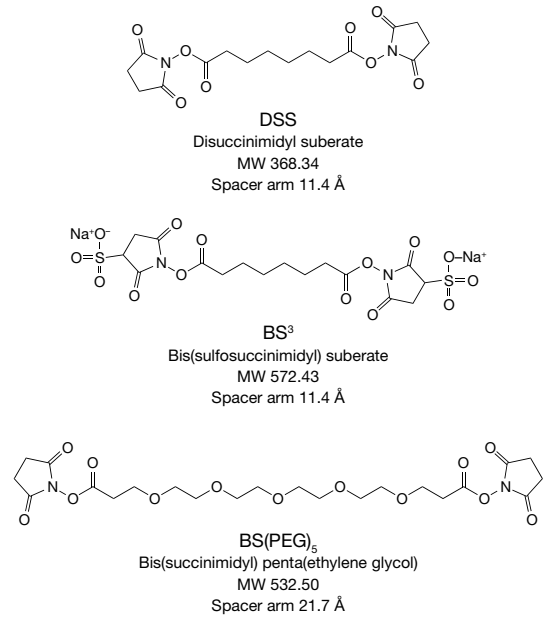


Figure 28. Crosslinkers with various spacer arm compositions.

Spacer arm cleavability

Crosslinkers and protein modification reagents form stable, covalent bonds with the proteins they react with. In certain applications, it is desirable to have the ability to break that bond and recover the individual components. Bioconjugation reagents are available with a cleavage site built into the spacer arm. The most commonly used cleavage site is a disulfide bridge, which can be readily reduced with the introduction of a common reducing agent such as β-mercaptoethanol, dithiothreitol, or TCEP. An example of this is the crosslinker DTBP (Figure 29).

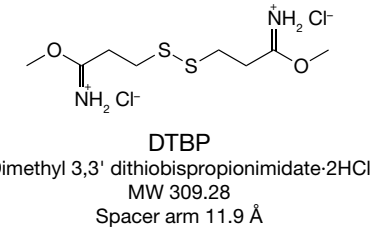


Figure 29. The disulfide bridge built into the spacer arm of DTBP allows easy cleavage of a protein conjugate using standard reducing agents.

Spacer arm structure and solubility

Crosslinkers typically possess a straight chain spacer arm, but protein modification reagents allow more options. A good example is Thermo Scientific™ PEGylation reagents that can be either straight or branched. For example, CA(PEG)$_n$ is a straight-chain PEGylation reagent and TMM(PEG)$_{12}$ is a branched reagent (Figure 30).

Many crosslinkers, by virtue of their hydrophobic spacer arms, have limited solubility in aqueous solutions. These crosslinkers are generally dissolved in DMF or DMSO, then added to the biological system or solution of biomolecules to be crosslinked. Hydrophobic crosslinkers are able to cross cellular and organellar membranes and affect crosslinking both at the outer surface of a membrane and within the membrane-bound space. It is often inconvenient or undesirable to introduce organic solvents into a crosslinking procedure for a biological system. It is also desirable in many instances to affect crosslinking only on the outer surface of a cellular or organellar membrane without altering the interior of the cell or organelles. For such cases, several water-soluble, membrane-impermeant crosslinkers are available.

Some crosslinkers contain a spacer arm formed from PEG subunits, resulting in a polyethylene oxide (PEO) chain with abundant oxygen atoms to provide water solubility. These crosslinkers are designated by a (PEG)$_n$ in their name and are both water-soluble and unable to penetrate biological membranes. They provide the added benefit of transferring their hydrophilic spacer to the crosslinked complex, decreasing the potential for aggregation and precipitation of the complex.

Other crosslinkers obtain their water solubility and their ability to permeate the membrane by virtue of a charged reactive group at either end of the spacer. These charged reactive groups, such as sulfo-NHS esters or imidoesters, impart water solubility to the crosslinking reagent, but not to the crosslinked complex because the reactive group is not a part of the final complex.

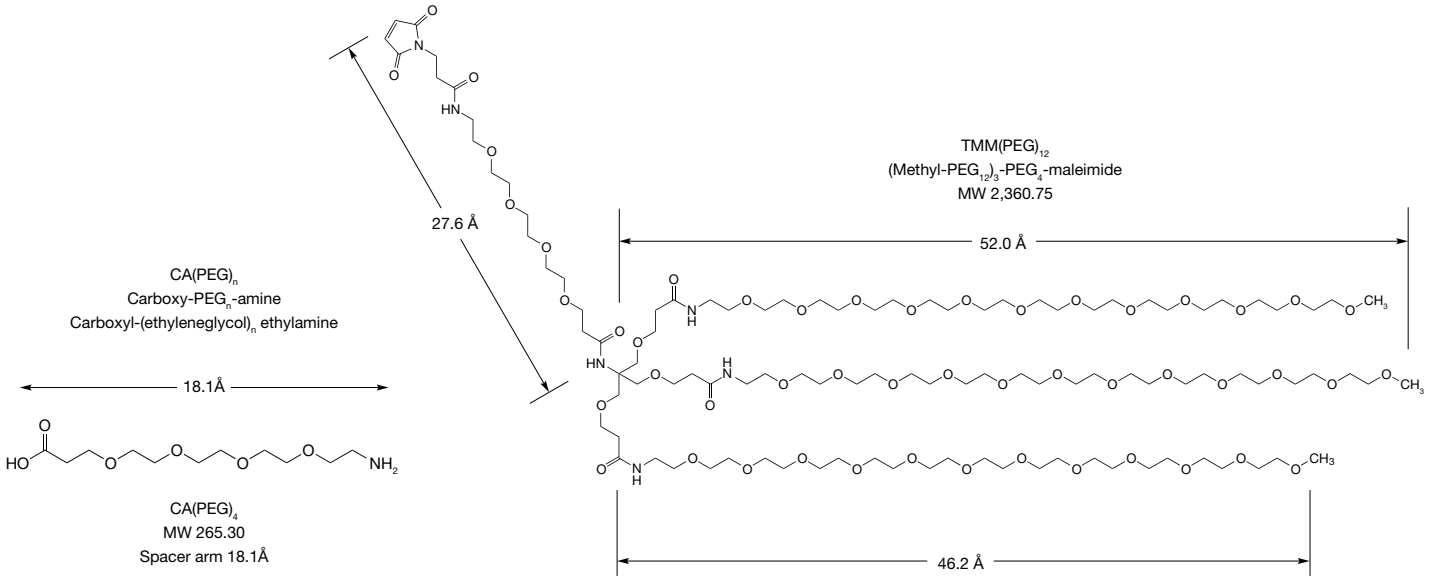


Figure 30. Straight and branched protein modification reagents.

Applications using bioconjugation and crosslinking reagents

Introduction

Bioconjugation and crosslinking reagents have a variety of applications in life science research and assay development. These include protein and peptide biotinylation, antibody labeling with fluorophores or drugs, protein immobilization onto solid supports, protein–protein conjugation, label transfer, protein interaction and crosslinking using mass spectrometry, *in vivo* crosslinking, metabolic labeling, and cell membrane structural studies. Commonly, antibodies are the target of bioconjugation with applications in purification and detection in a complex biological sample. In this process, NHS ester chemistry is the most widely used method for labeling available lysine residues.

Protein and peptide biotinylation

The highly specific interaction of avidin with biotin (vitamin H) can be a useful tool in designing nonradioactive purification and detection systems. The extraordinary affinity of avidin for biotin (K_a = 10^{15} M^{-1}) is the strongest known noncovalent interaction of a protein and ligand, and allows biotin-containing molecules in a complex mixture to be discretely bound with avidin conjugates. Desthiobiotin is a modified form of biotin that binds less tightly to avidin and streptavidin than biotin while still providing excellent specificity in affinity purification methods. Unlike biomolecules that are labeled with biotin, proteins and other targets that are labeled with desthiobiotin can be eluted without harsh denaturing conditions (Figure 31).

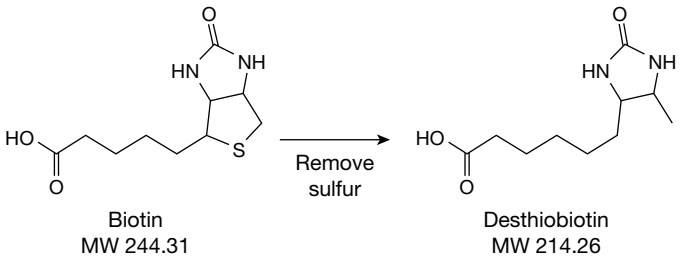


Figure 31. Comparison of the chemical structures of biotin and desthiobiotin.

The extensive line of Thermo Scientific™ biotinylation labeling reagents exploits this unique interaction. Biotin, a 244 Da vitamin found in tiny amounts in all living cells, binds with high affinity to avidin-based molecules. Since biotin is a relatively small molecule, it can be conjugated to many proteins without significantly altering their biological activity. The valeric acid side chain of the biotin molecule can be derivatized to incorporate various reactive groups that are used to attach biotin to other molecules. Using these reactive groups, biotin can be easily attached to most proteins and peptides.

Biotinylation reagents are available for targeting a variety of functional groups, including primary amines, sulfhydryls, carbohydrates, and carboxyls. Photoreactive biotin compounds that react nonspecifically upon photoactivation are also available. This variety of functional group specificities is extremely useful, allowing the choice of a biotinylation reagent that does not inactivate the target macromolecule.

Several cleavable or reversible biotinylation reagents are also available and allow specific elution of the biotinylated molecule from biotin-binding proteins and peptides. The most frequently used biotinylation reagents, *N*-hydroxysuccinimide (NHS) esters and *N*-hydroxysulfosuccinimide (sulfo-NHS) esters, react with primary amines (Figure 32). While NHS esters of biotin are the most frequently used biotinylation reagents, they are not necessarily the best for a particular application. If only a portion of the primary amines on a protein are reacted, reaction with NHS esters of biotin will result in a random distribution of biotin on the surface of the protein. If a particular primary amine is critical to the biological activity of the protein, modification of this critical amine may result in the loss of its biological activity. Depending on the extent of biotinylation, complete loss of activity may occur.

Antibodies are biotinylated more often than any other class of proteins, and it is advantageous to biotinylate in a manner that will maintain immunological reactivity. Thermo Scientific™ Sulfo-NHS-LC-Biotin is an excellent choice for labeling both monoclonal and polyclonal antibodies because it is the simplest and often the most effective method.

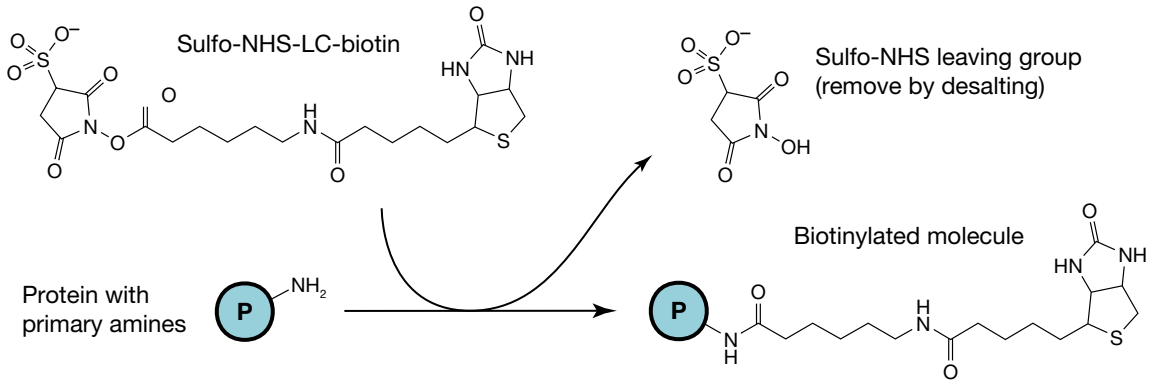


Figure 32. Biotinylation using NHS ester chemistry.

Antibody labeling and crosslinking

Antibodies, like other proteins, can be covalently modified in many ways to suit the purpose of an assay. Many immunological methods involve the use of labeled antibodies and a variety of reagents have been created to allow labeling of antibodies. Enzymes, biotin, fluorophores, and various small molecules are all commonly used to provide a detection signal in biological assays.

The most common target for antibody labeling or conjugation is primary amines, which are found primarily on lysine residues. They are abundant, widely distributed, and easily modified because of their reactivity and their location on the surface of the antibody. Primary amines can be targeted using several kinds of conjugation chemistries. The most commonly used reagent for primary amine labeling is the *N*-hydroxysuccinimidyl ester (NHS ester) reactive group. Many reactive crosslinkers, fluorescent labeling products, or biotinylation reagents are commercially available pre-activated with an NHS-ester group.

Understanding the functional groups available on an antibody is the key to determining a strategy for modification.

- Primary amine groups ($–NH_2$) are found on lysine side chains and at the amino terminus of each polypeptide chain.
- Sulfhydryl groups (–SH) can be generated by reducing disulfide bonds in the hinge region.
- Carbohydrate residues containing cis-diols can be oxidized to create active aldehydes (–CHO).

In any particular antibody clone, lysines (primary amines) might occur prominently within the antigen binding site. Thus, the lone drawback to this labeling strategy is that it occasionally causes a significant decrease in the antigen-binding activity of the antibody. The decrease may be particularly pronounced when working with monoclonal antibodies or when attempting to add a high density of labels per antibody molecule.

A simple alternative to these traditional methods is the Invitrogen™ SiteClick™ antibody labeling system (Figure 33), which allows simple and gentle site-selective attachment of compounds to the carbohydrate domains present only on the heavy chains of essentially all IgG antibodies regardless of isotype and host species. This method also provides excellent reproducibility from labeling to labeling and from antibody to antibody because the N-linked glycans are highly conserved. The Invitrogen™ SiteClick™ Antibody Azido Modification Kit uses enzymes to specifically attach azido moieties to the antibody carbohydrate domains. Once the azide is attached, a variety of sDIBO alkyne labels are available to conjugate with a simple incubation step. A number of different conjugates can be site-selectively attached to the heavy chain glycans—including Invitrogen™ Alexa Fluor™ dyes, pHrodo™ dyes, R-PE, biotin, Invitrogen Qdot™ probes, or independently supplied DIBO/DBCO conjugates.

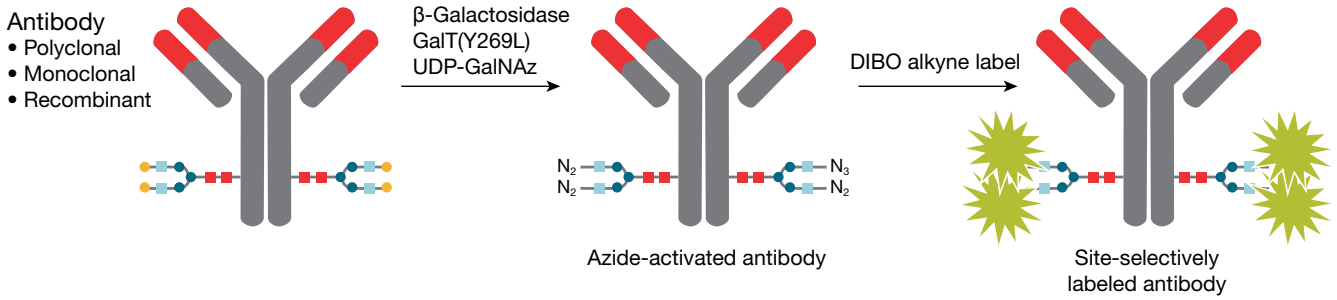


Figure 33. The SiteClick antibody labeling system. The first step in the SiteClick antibody labeling process involves removal of terminal galactose residues from the heavy chain N-linked glycans using β-galactosidase, exposing essentially all possible modifiable GlcNAc residues. Second, the free terminal GlcNAc residues are activated with azide tags by enzymatic attachment of GalNAz to the terminal GlcNAc residues using the GalT(Y289L) enzyme. In the third step, the azide residues are reacted with the dibenzocyclooctyne (DIBO)-functionalized probe of choice (e.g., Alexa Fluor™ 488 DIBO alkyne). The average degree of labeling is 3–3.5 labels per antibody.

Learn more at **thermofisher.com/siteclick**

Synthesis of antibody–drug conjugates

Over the past decade, biologics have been increasingly pursued as therapeutic agents. In the case of antibody–drug conjugates (ADCs), internalization of the antibody is a powerful mechanism of action. Internalization moves the ADC from its binding site at the plasma membrane of the target cell to the lytic environment of a lysosome, resulting in activation of the attached toxin (Figure 34).

The efficiency of this internalization process is directly linked to the therapeutic index of the ADC. We have combined powerful pHrodo dyes and SiteClick antibody labeling technologies to provide easy-to-use antibody labeling tools for creating antibody conjugates and studying internalization. Biomolecules labeled with pHrodo dyes are essentially nonfluorescent outside of cells at neutral pH and become brightly fluorescent in the acidic environment of lysosomes after they are internalized. This feature enables a no-wash, no-quench assay for detection of endocytosis, trafficking of ADCs in live cells, and cell killing by the antibody–drug conjugate (Figure 35). Fluorescent signals are only detectable for antibodies that have been specifically internalized. In addition, the location of the internalized antibody in the endocytic pathway can be studied.

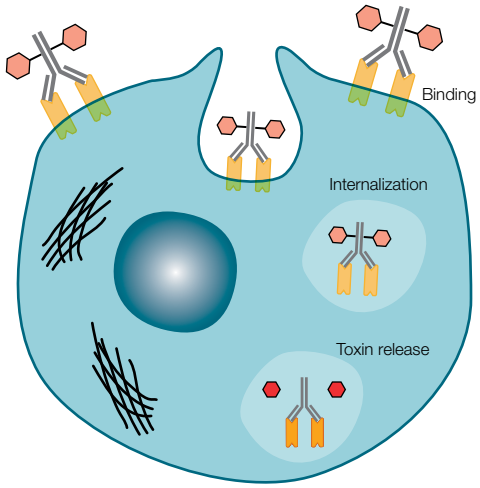


Figure 34. Internalization of an antibody–drug conjugate. An antibody–drug conjugate (ADC) comprises a monoclonal antibody directed, for example, against a tumor cell antigen, coupled to a small cytotoxic molecule. An ADC is designed to specifically bind to target cells, where it is rapidly internalized. Typically the drug is liberated following trafficking to the lysosome, resulting in a highly targeted chemotherapeutic agent.

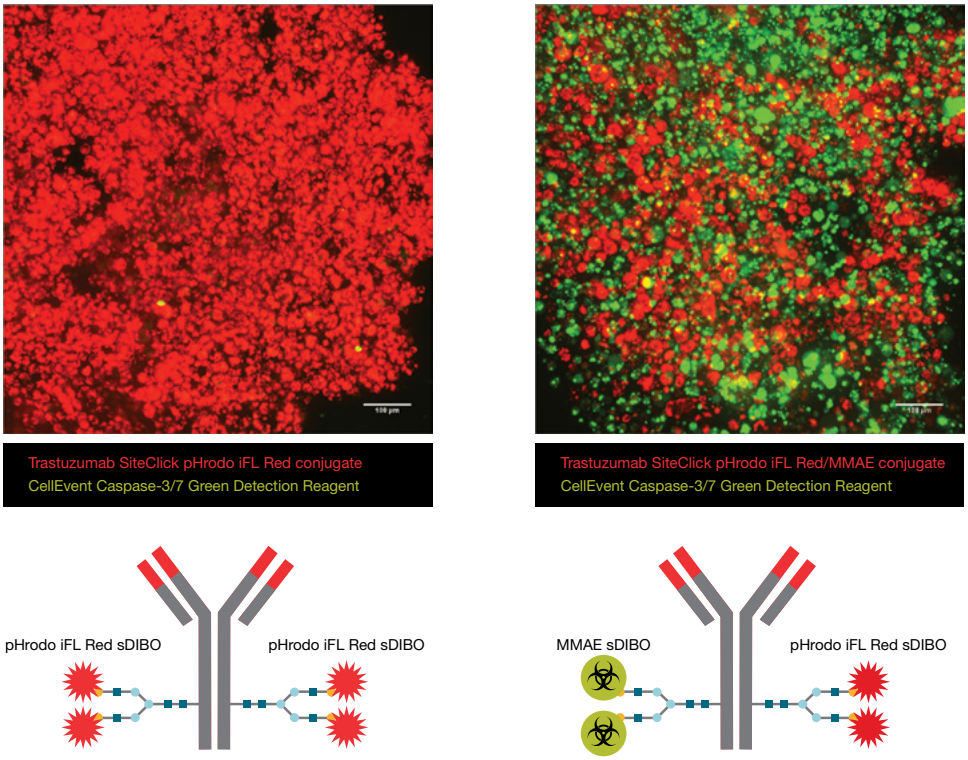


Figure 35. Cell killing with an antibody–drug conjugate. (A) Trastuzumab, a therapeutic antibody targeting HER2, was labeled with Invitrogen™ pHrodo™ iFL Red dye using SiteClick conjugation. A spheroid of SKBR3 (HER2+) breast cancer cells was treated for 48 hours with 30 nM of the labeled trastuzumab and Invitrogen™ CellEvent™ Caspase-3/7 Green sensor. Live-cell imaging was performed on the Thermo Scientific™ CellInsight™ CX7 HCA Platform. Red fluorescence indicates antibody delivery specifically into HER2+ cells. **(B)** Trastuzumab was labeled with both pHrodo iFL Red dye and the tubulin-destabilizing drug MMAE, using SiteClick conjugation. An SKBR3 (HER2+) spheroid was treated for 48 hours with 30 nM of the labeled antibody–drug conjugate and CellEvent Caspase-3/7 Green sensor. Live-cell imaging was performed on the CellInsight CX7 platform. Red fluorescence indicates antibody delivery specifically into HER2+ cells. Green indicates cell killing by the antibody–drug conjugate.

Protein immobilization onto solid supports

Proteins, peptides, and other molecules can be immobilized onto solid supports for affinity purification of proteins or for sample analysis. The supports may be nitrocellulose or other membrane materials, polystyrene plates or beads, agarose, beaded polymers, or glass slides. Some supports can be activated for direct coupling to a ligand. Other supports are made with nucleophiles or other functional groups that can be linked to proteins using crosslinkers. Carbodiimides such as Thermo Scientific™ EDC (Cat. No. 22980, 22981) are very useful for coupling proteins to carboxy- and amine-activated glass, plastic, and agarose supports. Carbodiimide procedures are usually one-step methods; however, two-step methods are possible if reactions are performed in organic solvents, or if Thermo Scientific™ NHS (Cat. No. 24500) or Sulfo-NHS (Cat. No. 24510) is used to enhance the reaction. EDC is useful for coupling ligands to solid supports and to attach leashes onto affinity supports for subsequent coupling of ligands. Useful spacers are diaminodipropylamine (DADPA), ethylenediamine, hexanediamine, 6-aminocaproic acid, and any of several amino acids or peptides. Spacer arms help to overcome steric effects when the ligand is immobilized too close to the matrix to allow access by the receptor. Steric effects are usually most pronounced when the ligand is a small molecule. The aldehyde-activated Thermo Scientific™ AminoLink™ Plus agarose resin (Cat. No. 20501) uses reductive amination, Thermo Scientific™ Pierce™ NHS-activated agarose (Cat. No. 26200) uses NHS ester chemistry, and Thermo Scientific™ SulfoLink™ resin (Cat. No. 20401) uses haloacetyl chemistry to immobilize molecules, whereas Thermo Scientific™ CarboxyLink™ resin (Cat. No. 20266) uses carbodiimide chemistry (Figure 36).

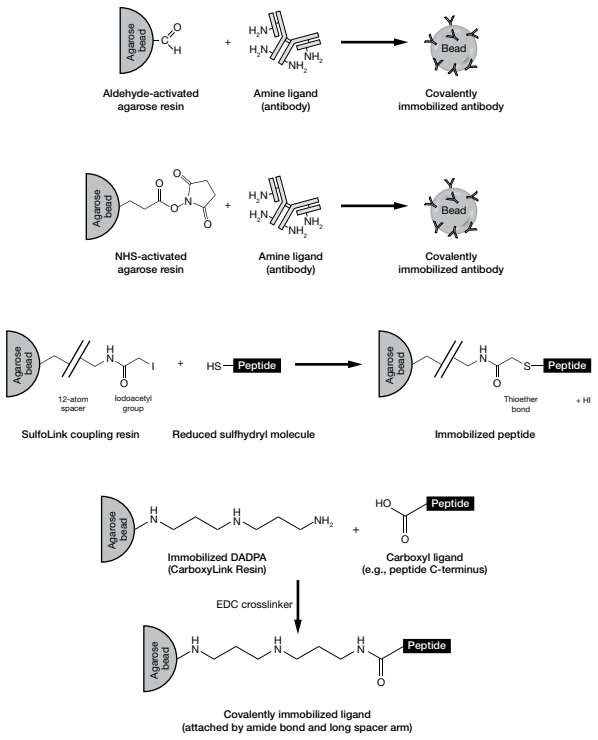


Figure 36. Immobilization of biomolecules onto solid supports using different bioconjugation chemistries.

Thermo Scientific™ crosslinkers DMP (Cat. No. 21666) and DSS (Cat. No. 21555) are used to immobilize antibodies on protein A or protein G supports for antigen purification. After the antibody binds to the Fc-binding proteins, the antibody is oriented so that the Fab region is available for antigen binding. DSS or DMP is applied to the bound antibody column to link the two proteins through primary amines. Thermo Scientific™ Pierce™ Crosslink IP Kit (Cat. No. 26147) is based on this chemistry and utilizes DSS to covalently immobilize the captured antibody to protein A/G agarose resin (Figure 37). The antibody resin is then incubated with the sample that contains the protein antigen of interest, allowing the antibody–antigen complex to form. After washing, the antigen is recovered by dissociation from the antibody with elution buffer supplied in the kit. The entire procedure is performed in a microcentrifuge spin cup, allowing solutions to be fully separated from the agarose resin upon brief centrifugation. Only the antigen is eluted by the procedure, enabling it to be identified and further analyzed without interference from antibody fragments. Furthermore, the antibody resin often can be reused for additional rounds of immunoprecipitation.

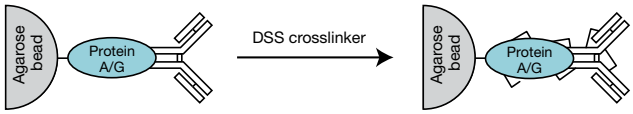


Figure 37. Covalent attachment of captured antibody to protein A/G agarose resin using the crosslinker DSS.

Surface modification using PEG-based reagents

Polyethylene glycol (PEG) compounds of discrete chain length can provide linkers of known molecular dimension for creating biocompatible planar surfaces or particles. In particular, PEG reagents containing a carboxylate group on one end and a thiol or lipoamide group on the other are effective as hydrophilic bridges between an adsorptive surface and an affinity ligand.

Combining CA(PEG)$_{12}$ molecules with MA(PEG)$_8$ derivatives (or CT(PEG)$_{12}$ with MT(PEG)$_8$ as thiol reagents) in surface modification can form a hydrophilic lawn of methyl ether–terminated PEGs with periodic exposed carboxylic acid–containing PEGs (Figure 38). The exposed carboxylic acid groups can be coupled to affinity ligands using the carbodiimide coupling reaction with EDC and sulfo-NHS. In addition, functionalization of solid surfaces with polyethylene glycol spacers significantly reduces nonspecific protein binding.

Thiols readily bind to gold surfaces, forming dative bonds, and have been used extensively for the modification of various surfaces such as quantum dots, self-assembled monolayers, and magnetic particles. Monodentate thiols, however, can be easily removed by compounds such as DTT. Bidentate thiols, such as lipoamides, provide an added level of stability to the nanostructure and are much more resistant to removal from the metal surface by DTT or similar reagents. In addition to their increased stability, bidentate thiols provide added flexibility owing to the fact that they can be used with a variety of metal surfaces (e.g., gold or silver nanoparticles).

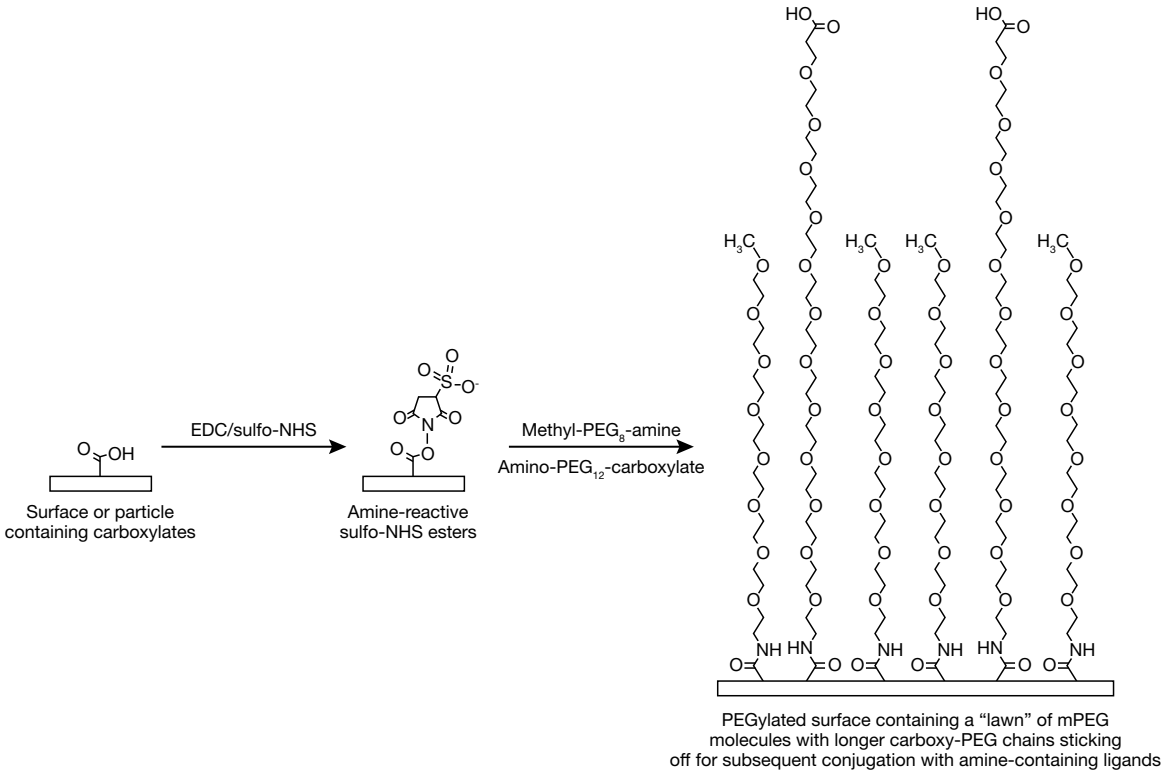


Figure 38. Shorter methyl-PEG$_n$-amine reagents can be combined with longer PEG compounds containing terminal carboxylate groups to create the classic "flowers in the grass" surface modification. The carboxylate groups then can be used to immobilize affinity groups using a carbodiimide coupling procedure with EDC and sulfo-NHS.

Learn more at **thermofisher.com/pegylation**

Hapten–carrier conjugation for antibody production

Several approaches are available for conjugating haptens to carrier proteins. The choice of which conjugation chemistry to use depends on the functional groups available on the hapten, the required hapten orientation and distance from the carrier, and the possible effect of conjugation on biological and antigenic properties. For example, proteins and peptides have primary amines (the N terminus and the side chain of lysine residues), carboxylic groups (the C terminus and the side chain of aspartic acid and glutamic acid), and sulfhydryls (the side chain of cysteine residues) that can be targeted for conjugation. Generally, it is the many primary amines in a carrier protein that are used to couple haptens via a crosslinking reagent.

Many crosslinkers are used for making conjugates for use as immunogens. The best crosslinker to use depends on the functional groups present on the hapten and the ability of the hapten–carrier conjugate to function successfully as an immunogen after its injection. Carbodiimides are good choices for producing peptide–carrier protein conjugates because both proteins and peptides usually contain several carboxyls and primary amines. Carbodiimides such as EDC react with carboxyls first to yield highly reactive unstable intermediates that can then couple to primary amines (Figure 39).

This efficient reaction produces a conjugated immunogen in less than 2 hours. Often peptides are synthesized with terminal cysteines to enable attachment to supports or to carrier proteins using sulfhydryl- and amine-reactive, heterobifunctional crosslinkers. By attaching the crosslinker first to the carrier protein (with its numerous amines) and then to a peptide containing a reduced terminal cysteine, all peptide molecules can be conjugated with the same predictable orientation (Figure 40). This method can be very efficient and yield an immunogen that is capable of eliciting a good response upon injection.

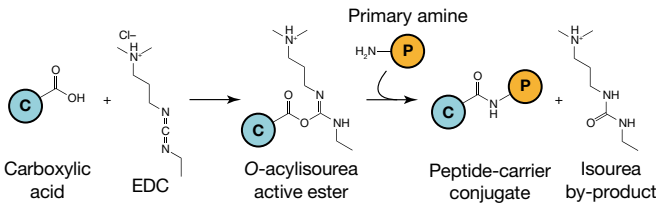


Figure 39. EDC-mediated conjugation of peptides and carrier proteins. Carrier proteins (C) and peptides (P) have both carboxyls and amines, so conjugation occurs in both orientations. Carrier proteins are very large in comparison to typical peptide haptens; therefore, numerous conjugation sites exist on each carrier protein molecule.

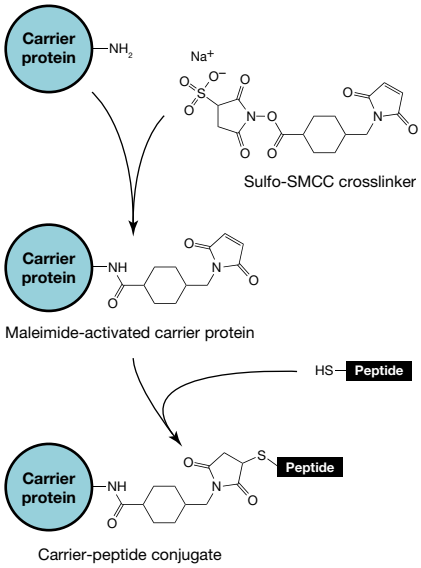


Figure 40. Peptide conjugation to carrier proteins for antibody production.

Learn more at **thermofisher.com/carrierproteins**

Protein–protein conjugation

One of the most common applications for crosslinkers is the production of protein–protein conjugates. Conjugates are often prepared by attachment of an enzyme, fluorophore, or other molecule to a protein that has affinity for one of the components in the biological system being studied. Antibody–enzyme conjugates (primary or secondary antibodies) are among the most common protein–protein conjugates used. Although secondary antibody conjugates are available and relatively inexpensive, enzyme-labeled primary antibodies are usually expensive and can be difficult to obtain. Many reagents are used for the production of antibody–enzyme conjugates. Glutaraldehyde conjugates are easy to make, but they often yield conjugates that produce high background in immunoassays. Carbohydrate moieties can be oxidized and then coupled to primary amines on enzymes in a procedure called reductive alkylation or amination. These conjugates often result in less background in enzyme immunoassays and are relatively easy to prepare; however, some self-conjugation of the antibody may occur (Figure 41).

Homobifunctional NHS ester or imidoester crosslinkers may be used in a one-step protocol, but polymerization and self-conjugation are also likely. Homobifunctional sulfhydryl-reactive crosslinkers such as Thermo Scientific™ Pierce™ BMH (Cat. No. 22330) may be useful if both proteins to be conjugated contain sulfhydryls. Heterobifunctional crosslinkers are perhaps the best choices for antibody–enzyme or other protein–protein crosslinking. Unwanted self-conjugation inherent when using homobifunctional NHS ester reagents or glutaraldehyde can be avoided by using a Thermo Scientific™ Pierce™ reagent such as SMCC (Cat. No. 22360) or Sulfo-SMCC (Cat. No. 22322). Sulfo-SMCC is first conjugated to one protein, and the second is thiolated with SATA (Cat. No. 26102) or Traut's reagent (Cat. No. 26101), followed by conjugation (Figure 42). Alternatively, disulfides in the protein may be reduced, and the two activated proteins are incubated together to form conjugates free of dimers of either protein. Any of the other NHS ester, maleimide, or pyridyl disulfide crosslinkers can be substituted for sulfo-SMCC in this reaction scheme. Heterobifunctional photoactivatable phenyl azide crosslinkers are seldom used for making protein–protein conjugates because of low conjugation efficiencies.

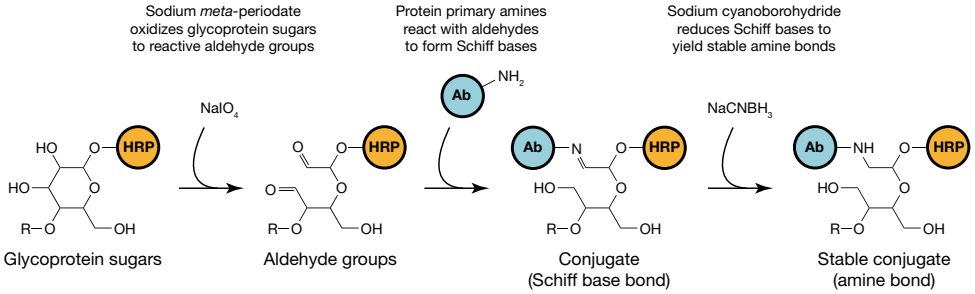


Figure 41. Reaction scheme for labeling antibodies with enzymes such as HRP using reductive amination.

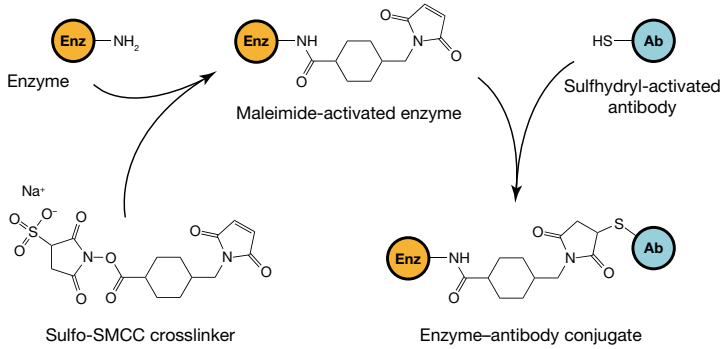


Figure 42. Reaction scheme for labeling reduced antibody fragments with maleimide-activated enzymes.

Synthesis of immunotoxins

Specific antibodies can be covalently linked to toxic molecules and then used to target antigens on cells. Often these antibodies are specific for tumor-associated antigens. Immunotoxins are brought into the cell by surface antigens and, once internalized, they proceed to kill the cell by ribosome inactivation or other means. The type of crosslinker used to make an immunotoxin can affect its ability to locate and kill the appropriate cells. For immunotoxins to be effective, the conjugate must be stable *in vivo*. In addition, once the immunotoxin reaches its target, the antibody must be separable from the toxin to allow the toxin to kill the cell. Thiol-cleavable, disulfide-containing conjugates have been shown to be more cytotoxic to tumor cells than noncleavable conjugates of ricin A immunotoxins. Cells are able to break the disulfide bond in the crosslinker, releasing the toxin within the targeted cell.

Thermo Scientific™ Pierce™ SPDP (Cat. No. 21857) is a reversible NHS ester, pyridyl disulfide crosslinker used to conjugate amine-containing molecules to sulfhydryls. For several years, this has been the "workhorse" crosslinker for production of immunotoxins. The amine-reactive NHS ester is usually reacted with the antibody first. In general, toxins do not contain surface sulfhydryls; therefore, sulfhydryls must be introduced into them by reduction of disulfides, which is common for procedures involving ricin A chain and abrin A chain, or through chemical modification reagents. A second SPDP molecule can be used for this purpose and is reacted with amines on the immunotoxin, then reduced to yield sulfhydryls.

Another chemical modification reagent that is commonly used for production of immunotoxins is Thermo Scientific™ Pierce™ 2-Iminothiolane, also known as Traut's reagent (Cat. No. 26101). Traut's reagent reacts with amines and yields a sulfhydryl when its ring structure opens during the reaction.

Label transfer

Label transfer involves crosslinking interacting molecules (i.e., bait and prey proteins) with a labeled crosslinking agent and then cleaving the linkage between bait and prey such that the label remains attached to the prey. This method allows a label to be transferred from a known protein to an unknown interacting protein. The label can then be used to purify and/or detect the interacting protein. Label transfer is particularly valuable because of its ability to identify proteins that interact weakly or transiently with the protein of interest. New non-isotopic reagents and methods continue to make this technique more accessible and simple to perform by any researcher.

Label transfer reagents can also have biotin built into their structure. This type of design allows the transfer of a biotin tag to an interacting protein after cleavage of a cross-bridge. Thermo Scientific™ Pierce™ Sulfo-SBED reagent (Cat. No. 33033) is an example of such a trifunctional reagent (see Figure 21, page 16). It contains an amine-reactive sulfo-NHS ester on one arm (built off the α-carboxylate of the lysine core), a photoreactive phenyl azide group on the other side (synthesized from the α-amine), and a biotin handle (connected to the ε-amino group of lysine). The arm containing the sulfo-NHS ester has a cleavable disulfide bond, which permits transfer of the biotin component to any captured proteins.

In use, a bait protein first is derivatized with sulfo-SBED through its amine groups, and the modified protein is allowed to interact with a sample. Exposure to UV light (300–366 nm) couples the photoreactive end to the nearest available C–H or N–H bond in the bait–prey complex, resulting in covalent crosslinks between bait and prey. Upon reduction and cleavage of the disulfide spacer arm, the biotin handle remains attached to the protein(s) that interacted with the bait protein, facilitating isolation or identification of the unknown species using streptavidin, Thermo Scientific™ NeutrAvidin™ protein, or monomeric avidin.

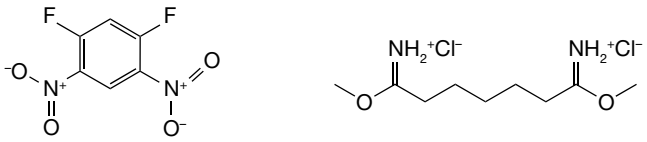
The architecture of this trifunctional label transfer reagent differs substantially from the bifunctional counterparts discussed above. The advantages become almost immediately apparent just by examining the structure.

The reactive moieties are well-segregated within sulfo-SBED. Most importantly, with a biotin label designed into sulfo-SBED, radiolabeling with ^{125}I is no longer necessary. The biotin label can be used to significant advantage in a label transfer application. For example, biotin can operate as a handle for purification of the prey protein or prey protein fragments or as a detection target using streptavidin-HRP and colorimetric or chemiluminescent substrates.

Subunit crosslinking and protein structural studies

Crosslinkers can be used to study the structure and composition of proteins in samples. Some proteins are difficult to study because they exist in different conformations with varying pH or salt conditions. One way to avoid conformational changes is to crosslink subunits. Amine-, carboxyl-, or sulfhydryl-reactive reagents are used for identification of particular amino acids or for determination of the number, location, and size of subunits. Short- to medium-length spacer arm crosslinkers are selected when intramolecular crosslinking is desired. If

the spacer arm is too long, intermolecular crosslinking can occur. Carbodiimides that result in no spacer arm, along with short-length conjugating reagents, such as amine-reactive Thermo Scientific™ Pierce™ DFDNB (Cat. No. 21525), can crosslink subunits without crosslinking to extraneous molecules if used in optimal concentrations and conditions (Figure 43). Slightly longer crosslinkers, such as Thermo Scientific™ DMP (Cat. No. 21666, 21667), can also crosslink subunits, but they may result in intermolecular coupling. Adjusting the reagent amount and protein concentration can control intermolecular crosslinking. Dilute protein solutions and high concentrations of crosslinker favor intramolecular crosslinking when homobifunctional crosslinkers are used.



DFDNB
1,5-Difluoro-2,4-dinitrobenzene
MW 204.09
Spacer arm 3.0 Å

DMP
Dimethyl pimelimidate·2HCl
MW 259.17
Spacer arm 9.2 Å

Figure 43. DFDNB and DMP are used for crosslinking between protein subunits.

For determination or confirmation of the three-dimensional structure, cleavable crosslinkers with increasing spacer arm lengths may be used to determine the distance between subunits. Experiments using crosslinkers with different reactive groups may indicate the locations of specific amino acids. Once conjugated, the proteins are subjected to two-dimensional electrophoresis. In the first dimension, the proteins are separated using nonreducing conditions and the molecular weights are recorded. Some subunits may not be crosslinked and will separate according to their individual molecular weights, while conjugated subunits will separate according to the combined size. The second dimension of the gel is then performed using conditions to cleave the crosslinked subunits. The individual molecular weights of the crosslinked subunits can be determined. Crosslinked subunits that were not reduced will produce a diagonal pattern, but the cleaved subunits will be off the diagonal. The molecular weights of the individual subunits should be compared with predetermined molecular weights of the protein subunits using reducing SDS-polyacrylamide gel electrophoresis.

Protein interaction and crosslinking using mass spectrometry

Chemical crosslinking in combination with mass spectrometry is a powerful method to determine protein–protein interactions. This method has been applied to recombinant and native protein complexes, and more recently to whole cell lysates and intact unicellular organisms, in efforts to identify protein–protein interactions on a global scale.

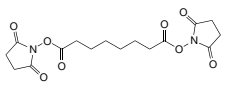
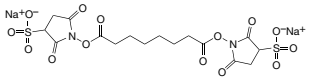
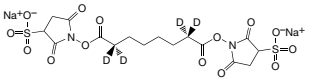
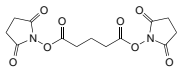
Thermo Scientific™ MS-grade crosslinkers are available with different linker lengths and as isotopically labeled sets to help elucidate protein–protein interactions (Table 5). Simplification of the analysis of crosslinked proteins is essential for successful protein characterization. In addition to traditional crosslinkers, next-generation crosslinkers have been developed to address simplifying MS analysis through crosslinker enrichment and cleavable functionality. These high-quality reagents have been validated in protein–protein interaction studies using Thermo Scientific™ mass spectrometers that use different types of fragmentation (CID, HCD, ETD, and EtHCD) and levels of tandem mass spectrometry (MS^2 and MS^3), in order to improve identification of protein–protein interaction sites.

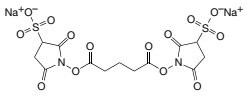
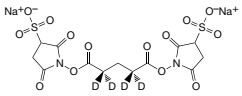
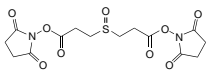
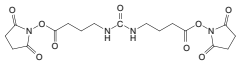
Our MS-grade crosslinkers are high-quality reagents that are available in multiple packaging options and sizes. We offer extensive technical expertise and support for various applications, as well as validation of these products in workflows using Thermo Scientific™ mass spectrometers.

Highlights

- **High quality**—products manufactured in ISO 9001–certified facilities
- **Convenience**—products available in Thermo Scientific™ No-Weigh™ packaging or in multiple pack sizes
- **More choices**—available with different linker lengths, MS cleavability, and deuterium isotope labels
- **Technical support**—extensive web resources and support to help ensure successful results

Table 5. Overview of Thermo Scientific crosslinkers used for studying protein–protein interactions.

Crosslinker	DSS	BS^3	BS^3-d_4	DSG
Structure				
Full name	Disuccinimidyl suberate	Bis(sulfo-succinimidyl) suberate	Bis(sulfo-succinimidyl) 2,2,7,7-suberate-d_4	Disuccinimidyl glutarate
Spacer arm (Å)	11.4	11.4	11.4	7.7
Water-soluble	No	Yes	Yes	No
Isotopically labeled	No	No	Yes	No
MS-cleavable	No	No	No	No

Crosslinker	BS^2G-d_0	BS^2G-d_4	DSSO	DSBU
Structure				
Full name	Bis(sulfo-succinimidyl) glutarate	Bis(sulfo-succinimidyl) 2,2,4,4-suberate-d_4	Disuccinimidyl sulfoxide	Disuccinimidyl dibutyric urea
Spacer arm (Å)	7.7	7.7	10.1	12.5
Water-soluble	Yes	Yes	No	No
Isotopically labeled	No	Yes	No	No
MS-cleavable	No	No	Yes	Yes

MS-cleavable crosslinkers (DSSO and DSBU)

Thermo Scientific™ DSSO (disuccinimidyl sulfoxide) and DSBU (disuccinimidyl dibutyric urea, also known as BuUrBu) are high-quality, MS-cleavable crosslinkers that contain an amine-reactive *N*-hydroxysuccinimide (NHS) ester at each end of a 7-atom and 11-atom spacer arm, respectively (Table 5, page 30). These products are offered in convenient single-use packaging (10 x 1 mg).

Features of DSSO and DSBU include:

- Amine-reactive NHS ester (at both ends) reacts rapidly with any molecule containing a primary amine
- MS-cleavable by collision-induced dissociation (CID)
- High-purity crystalline reagents for protein structure and interaction characterization
- Membrane-permeant, allowing intracellular crosslinking
- Water-insoluble (dissolve first in DMF or DMSO)

The crosslinker facilitates analysis of protein structure and complex interactions using mass spectrometry. DSSO and DSBU have reactivity similar to that of DSS, but contain linkers that can be cleaved in the gas phase during tandem MS (MS^2) using CID. The ability to cleave crosslinked peptides during MS^2 enables MS^3 acquisition methods, which facilitate peptide sequencing using traditional database search engines. The MS cleavage of DSSO and DSBU also generates diagnostic ion doublets during MS^2, which enables identification of crosslinked peptides from dead-end modifications and searching using novel database search engines such as MeroX or XlinkX* (Figure 44).

* Licensed from the Heck group, Utrecht University, The Netherlands.

A. Spectra of DSSO-crosslinked peptide

B. Spectra of DSBU-crosslinked peptide

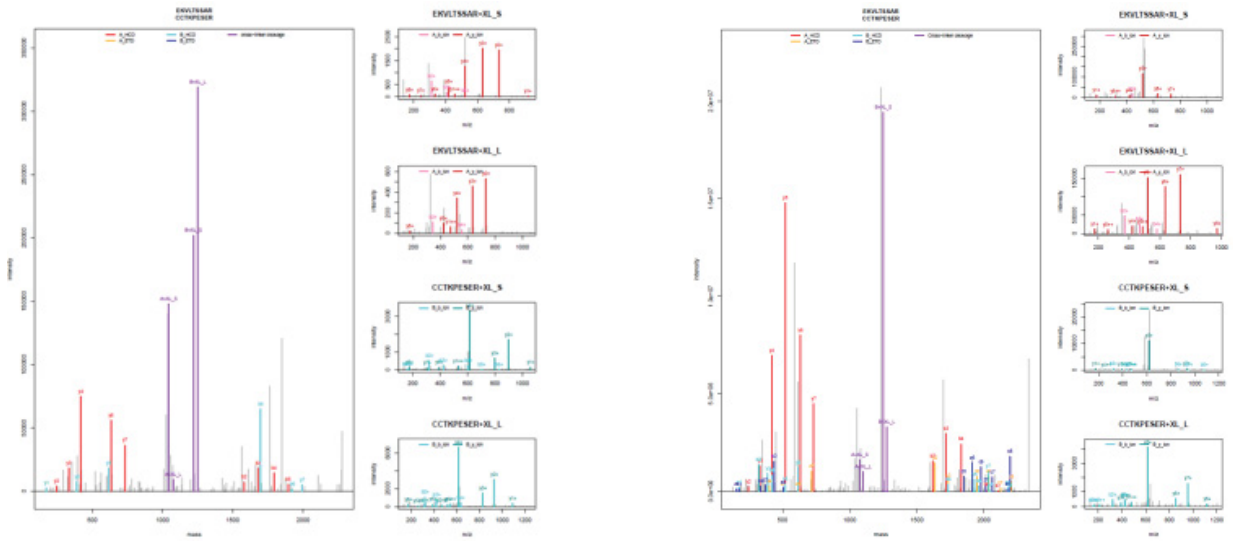


Figure 44. Spectra of BSA-crosslinked peptides identified by MS^2/MS^3 method and XlinkX software. The peptides were crosslinked using **(A)** DSSO and **(B)** DSBU. XlinkX software uses unique fragment patterns of MS-cleavable crosslinkers to detect and filter crosslinked peptides for a database search.

Ordering information

Product	Quantity	Cat. No.
DSSO (Disuccinimidyl Sulfoxide)	10 x 1 mg	A33545
DSBU (Disuccinimidyl Dibutyric Urea)	10 x 1 mg	A35459

Enrichable MS crosslinkers PhoX (DSPP) and TBDSPP

Thermo Scientific™ PhoX (DSPP, disuccinimidyl phenyl phosphonic acid) and TBDSPP (*tert*-butyl disuccinimidyl phenyl phosphonate, tBu-PhoX) are amine-reactive, enrichable crosslinkers designed for mass spectrometry analysis. Both crosslinkers have amine-reactive *N*-hydroxysuccinimide (NHS) esters at the ends of a 7-atom spacer arm containing either a phosphonic acid group, PhoX (DSPP), or phosphonate ester (TBDSPP) for enrichment (Figure 45). These phospho groups are used for enrichment of crosslinked peptides using immobilized metal affinity chromatography (IMAC) or metal oxide affinity chromatography (MOAC). Additionally, TBDSPP is cell-permeant for intracellular crosslinking applications.

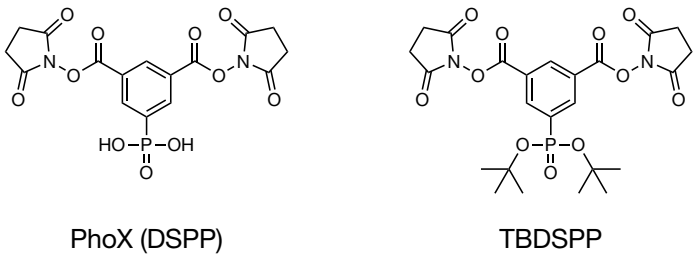


Figure 45. Chemical structure of enrichable crosslinkers PhoX (DSPP) and TBDSPP.

Features of PhoX (DSPP) and TBDSPP include:

- Trifunctional crosslinker—reactive groups: NHS ester (both ends), phosphonic acid (PhoX (DSPP)) or phosphonate ester (TBDSPP) in the spacer for enrichment
- High-purity crystalline reagents for protein structure and interaction characterization
- Membrane-permeant version (TBDSPP) for intracellular crosslinking
- Enrichable using Fe-NTA IMAC or TiO_2 MOAC

Of the two enrichable MS crosslinkers, PhoX (DSPP) is more water soluble than TBDSPP and is better for *in vitro* crosslinking of simple purified proteins and protein complexes. TBDSPP is better for intracelluar crosslinking, being more membrane-permeant. Athough both crosslinkers can be enriched using traditonal phosphopeptide methods, TBDSPP-crosslinked peptides must first be incubated with trifluoroacetic acid (TFA) to remove the *tert*-butyl protection groups (Figure 46).

Chemical crosslinking in combination with MS is a powerful method to determine protein–protein interactions. This method has been applied to recombinant and native protein complexes and to whole cell lysates or intact unicellular organisms in efforts to identify protein–protein interactions on a global scale. Both traditional, noncleavable- and MS-cleavable crosslinkers can be used for identification of protein–protein interaction sites, but phospho-enrichable crosslinkers are advantageous because they can be used to enrich low-abundance crosslinked peptides and thereby improve MS identification rates (Figures 47 and 48).

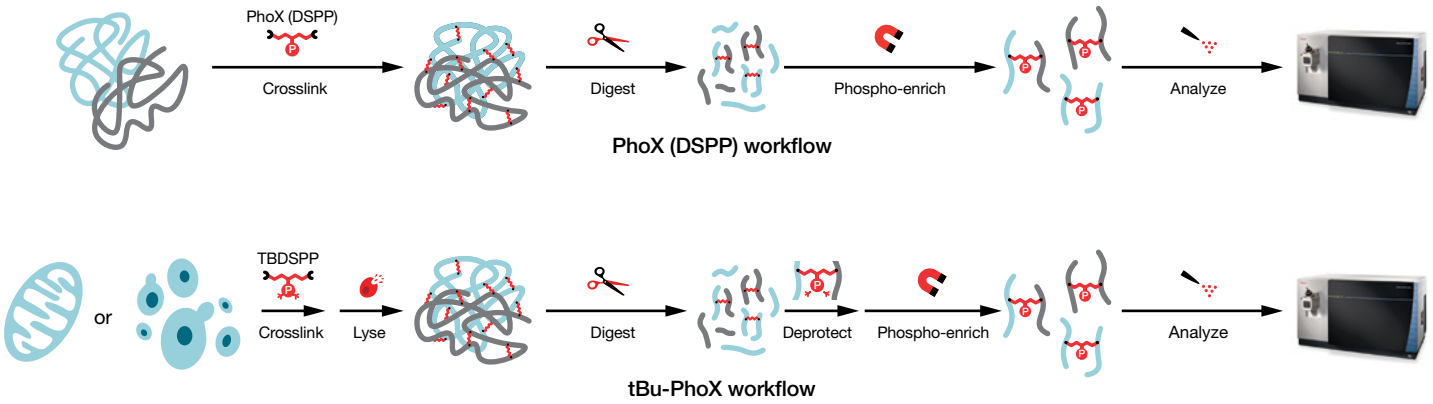


Figure 46. Schematic of PhoX (DSPP) and TBDSPP crosslinking LC-MS workflows. Simple proteins and complexes are crosslinked using PhoX (DSPP) before digestion, phospho-enrichment, and LC-MS analysis. Proteins, organelles, or cells can be crosslinked using TBDSPP but require deprotection using TFA before phospho-enrichment and LC-MS analysis.

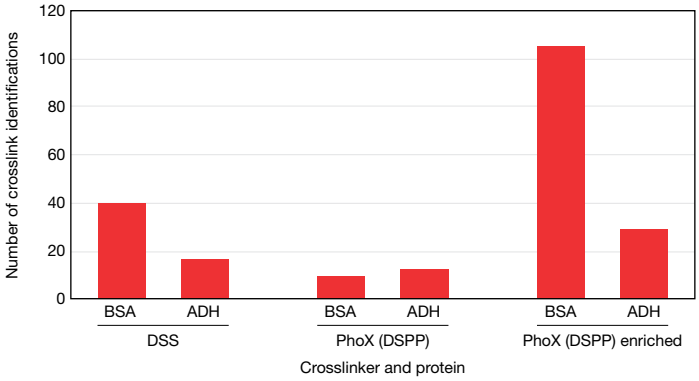


Figure 47. BSA and ADH crosslinking using DSS and PhoX (DSPP). Purified proteins were crosslinked using a 20-fold molar excess and acetone-precipitated before digestion and LC-MS analysis. PhoX (DSPP) samples enriched using the High-Select Fe-NTA Magnetic Agarose Kit had >2-fold more crosslinks identified than DSS samples.

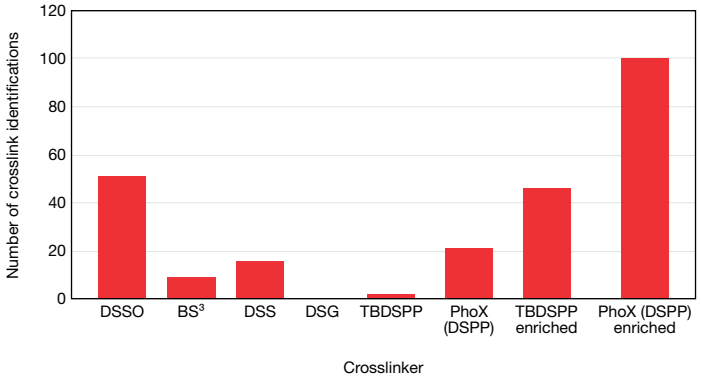


Figure 48. Crosslinking of *E. coli* ribosomes. Ribosomes were crosslinked with a 40-fold molar excess of different crosslinkers for 1 hour at room temperature. Samples were reduced and alkylated before acetone precipitation to remove excess crosslinker. TBDSPP samples were deprotected for 30 minutes at 37°C with 2.5% TFA. All samples were digested overnight before C18 cleanup and LC-MS analysis.

Ordering information

Product	Quantity	Cat. No.
DSPP (Disuccinimidyl Phenyl Phosphonic Acid, PhoX)	50 mg	A52286
TBDSPP (*tert*-Butyl Disuccinimidyl Phenyl Phosphonate, tBu-PhoX)	50 mg	A52287

Learn more at **thermofisher.com/ms-crosslinking**

In vivo crosslinking

Crosslinkers are used for identification of near-neighbor protein relationships and ligand–receptor interactions. Crosslinking stabilizes transient endogenous protein–protein complexes that may not survive traditional biochemical techniques such as immunoprecipitation. The most basic cellular crosslinker is formaldehyde (Thermo Scientific™ Pierce™ 16% Formaldehyde (w/v), Methanol-free, Cat. No. 28906), which is commonly used to stabilize chromatin interactions for chromatin immunoprecipitation (ChIP) assays (Thermo Scientific™ Pierce™ Agarose ChIP Kit, Cat. No. 26156). The crosslinkers chosen for these applications are usually longer than those used for subunit crosslinking. Homobifunctional amine-reactive NHS esters or imidates and heterobifunctional amine-reactive, photoactivatable phenyl azides are the most commonly used crosslinkers for these applications. Occasionally, a sulfhydryl- and amine-reactive crosslinker such as Thermo Scientific™ Sulfo-SMCC (Cat. No. 22322) may be used if one of the two proteins or molecules is known to contain sulfhydryls. Both cleavable or noncleavable crosslinkers can be used. Because the distances between two molecules are not always known, the optimal length of the spacer arm of the crosslinker may be determined using a panel of similar crosslinkers with different lengths. Thermo Scientific™ DSS (Cat. No. 21555) and its cleavable analog DSP (Cat. No. 22585) are among the shorter crosslinkers used for protein–protein interactions.

In contrast to crosslinkers that are introduced to cells exogenously, methods exist to incorporate crosslinkers into the proteome of a cell. This can be accomplished with photoreactive crosslinkers such as Thermo Scientific™ L-Photo-Leucine (Cat. No. 22610) and L-Photo-Methionine (Cat. No. 22615). These amino acid analogs are fed to cells during cell growth and are activated with UV light (Figure 49). In the experiment below, photoreactive amino acids were compared to formaldehyde treatment for identifying endogenous protein complexes (Figure 50).

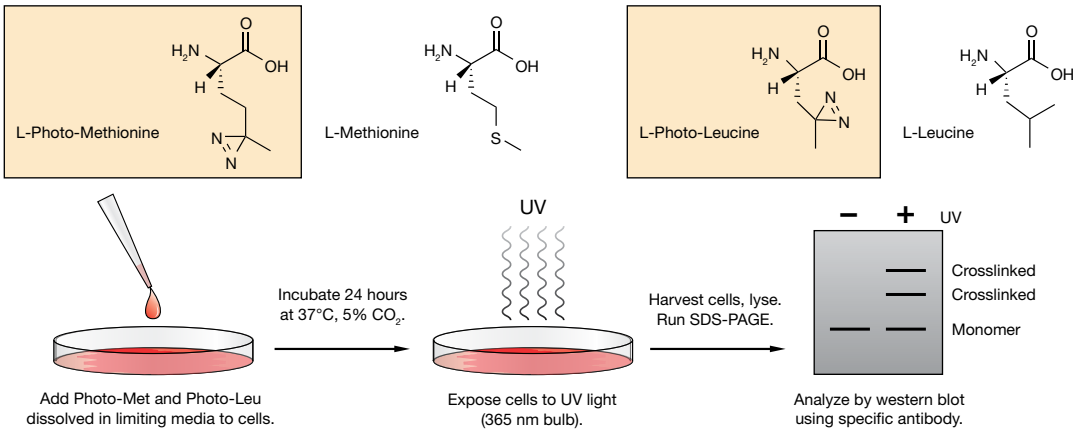


Figure 49. *In vivo* crosslinking with photoreactive amino acids.

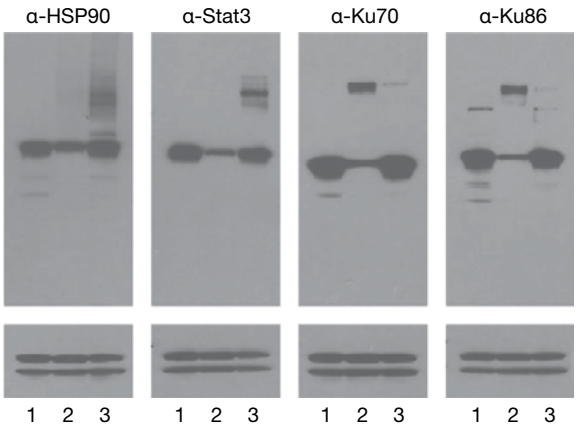


Figure 50. Photoreactive amino acid crosslinking and formaldehyde crosslinking are complementary techniques for protein interaction analysis. HeLa cells were mock treated (lane 1), treated with 1% formaldehyde for 10 minutes (lane 2), or treated with Photo-Methionine and Photo-Leucine followed by UV treatment (lane 3). Cells were lysed, and 10 µg of each was analyzed by SDS-PAGE and western blotting with antibodies against HSP90, Stat3, Ku70, and Ku86. Lower panels are β-actin (upper band) and GAPDH (lower band), which were blotted as loading controls.

Learn more at **thermofisher.com/in-vivo-crosslinking**

Metabolic labeling

Chemoselective ligations use unique chemical functional groups for specific conjugation. Examples of this chemistry include hydrazide–aldehyde condensation, click chemistry (azide–alkyne), and Staudinger ligation (azide–phosphine). Click chemistry is the detection method of choice for samples that would be compromised by direct labeling or antibody-based secondary detection techniques. The click label is small enough to penetrate complex samples easily, and the selectivity and stability of the click reaction provides high sensitivity and low background signal. This gentle sample treatment together with the biocompatible Invitrogen™ Click-iT™ Plus reaction means that detection can be multiplexed with expressed proteins such as GFP, protein labels such as R-PE, and a wide range of organic fluorophores. The Staudinger ligation has the best utility for live-cell labeling and mass spectrometry (MS) applications. The Staudinger reaction occurs between a phosphine and an azide to produce an aza-ylide that is trapped to form a stable covalent bond. Because phosphines and azides are absent in biological systems, there is minimal background labeling of cells or lysates. Unlike click chemistry, Staudinger ligation requires no accessory reagents such as copper.

Metabolic labeling involves incorporation of a chemoselective crosslinker into the proteome of living cells. This facilitates protein isolation or fluorescent labeling. One such example is the metabolic labeling of glycoproteins using azido sugars. Azido groups react with phosphines to create a stable amide bond following the Staudinger reaction. Once incorporated, azido sugars can be labeled with biotin (Figure 51) or DyLight fluorophores (Figure 52). In this experiment, U2OS cells or HK-2 cells were fed azido sugars, fixed, and stained with Thermo Scientific™ DyLight™ 550-Phosphine (Cat. No. 88910) or DyLight™ 650-Phosphine (Cat. No. 88911).

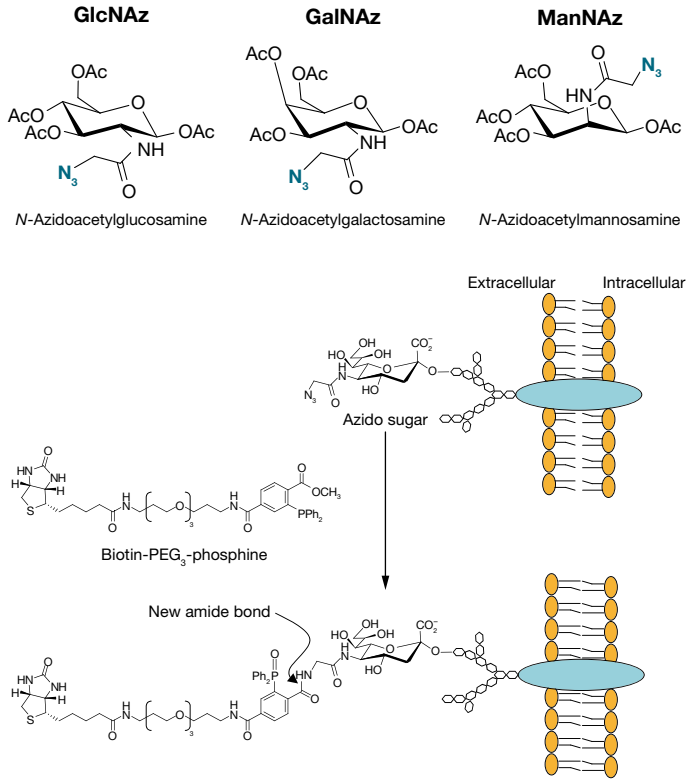
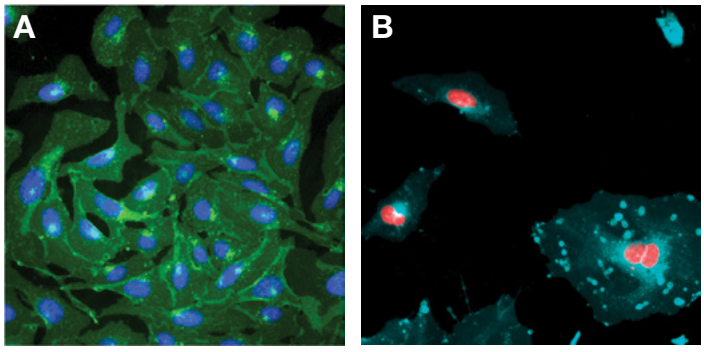


Figure 51. Biotin labeling of azido sugars.



U2OS: azido-galactosamine HK-2: azido-mannosamine

Figure 52. *In vivo* detection of metabolically incorporated azido sugars using DyLight 550- and 650-Phosphine labeling reagents. **(A)** U2OS cells were incubated with 40 µM azido-acetylgalactosamine in cell culture medium for 72 hours, and the live cells were incubated with 100 µM of DyLight 550-Phosphine. The cells were then washed, fixed with 4% paraformaldehyde, and counterstained with Hoechst™ 33342 stain (green: DyLight 550–labeled azido-galactosamine, blue: Hoechst 33342–labeled nuclei). **(B)** HK-2 cells were incubated with 40 µM azido-acetylmannosamine in cell culture medium for 72 hours, and the live cells were incubated with 100 µM of DyLight 650-Phosphine. The cells were then washed, fixed with 4% paraformaldehyde, and counterstained with Hoechst 33342 stain (cyan: DyLight 650–labeled azido-mannosamine, red: Hoechst 33342–labeled nuclei).

Learn more at **thermofisher.com/metabolic-labeling**

Cell surface crosslinking and biotinylation

Crosslinkers are often used to identify surface receptors or their ligands. Membrane-impermeant crosslinkers ensure cell surface–specific crosslinking. Water-insoluble crosslinkers, when used in controlled amounts of reagent and reaction times, can reduce membrane penetration and reaction with inner-membrane proteins.

The sulfonyl groups attached to the succinimidyl rings of NHS esters result in a crosslinker that is water-soluble, membrane-impermeant, and nonreactive with inner-membrane proteins. Therefore, reaction time and quantity of crosslinker are less critical when using sulfo-NHS esters. Homobifunctional sulfo-NHS esters, heterobifunctional sulfo-NHS esters, and photoreactive phenyl azides are good choices for crosslinking proteins on the cell surface (Figure 53).

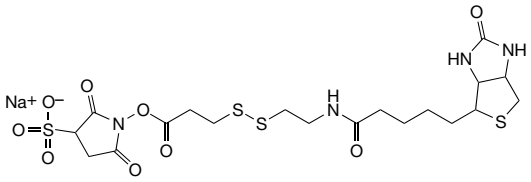


Figure 53. Sulfo-NHS-SS-biotin is used for the biotinylation and isolation of cell surface proteins.

Determination of whether a particular protein is located on the surface or the integral part of the membrane can be achieved by performing a conjugation reaction of a cell membrane preparation to a known protein or radioactive label using a water-soluble or water-insoluble crosslinker. Upon conjugation the cells may be washed, solubilized, and characterized by SDS-PAGE to determine whether the protein of interest was conjugated. Integral membrane proteins will form a conjugate in the presence of a water-insoluble crosslinker, but not in the presence of water-soluble crosslinkers. Surface membrane proteins can conjugate in the presence of water-soluble and water-insoluble crosslinkers. The Thermo Scientific™ Pierce™ Cell Surface Protein Isolation Kit (Cat. No. 89881) is a complete set of reagents that utilizes a cell-impermeant, cleavable biotinylation reagent (Sulfo-NHS-SS-Biotin) for the selective biotinylation and subsequent purification of mammalian cell surface proteins to the exclusion of intracellular proteins. The labeled surface proteins are affinity-purified using Thermo Scientific™ NeutrAvidin™ agarose resin.

Cell membrane structural studies

Cell membrane structural studies require reagents of varying hydrophobicity to determine the location and the environment within a cell's lipid bilayer. Fluorescent tags are used to locate proteins, lipids, or other molecules inside and outside the membrane. Various crosslinkers, with differing spacer arm lengths, can be used to crosslink proteins to associated molecules within the membrane to determine the distance between molecules. Successful crosslinking with shorter crosslinkers is a strong indication that two molecules are interacting in some manner. Failure to obtain crosslinking with a panel of shorter crosslinkers, while obtaining conjugation with the use of longer reagents, generally indicates that the molecules are located in the same part of the membrane, but are not interacting. Homobifunctional NHS esters, imidates, or heterobifunctional NHS ester/photoactivatable phenyl azides are commonly used for these procedures. Although imidoester crosslinkers (imidates) are water-soluble, they are still able to penetrate membranes. Sulfhydryl-reactive crosslinkers may be useful for targeting molecules with cysteines to other molecules within the membrane.

Thermo Scientific™ EDC (Cat. No. 22980, 22981) and other water-soluble and -insoluble coupling reagent pairs are used to study membranes and cellular structure, protein subunit structure and arrangement, enzyme–substrate interactions, and cell-surface and membrane receptors (Figure 54). The hydrophilic character of EDC can result in much different crosslinking patterns in membrane and subunit studies than hydrophobic carbodiimides. Often it is best to attempt crosslinking with a water-soluble and water-insoluble carbodiimide to obtain a complete picture of the spatial arrangements or protein–protein interactions involved.

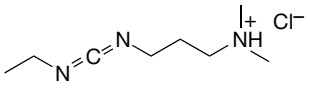
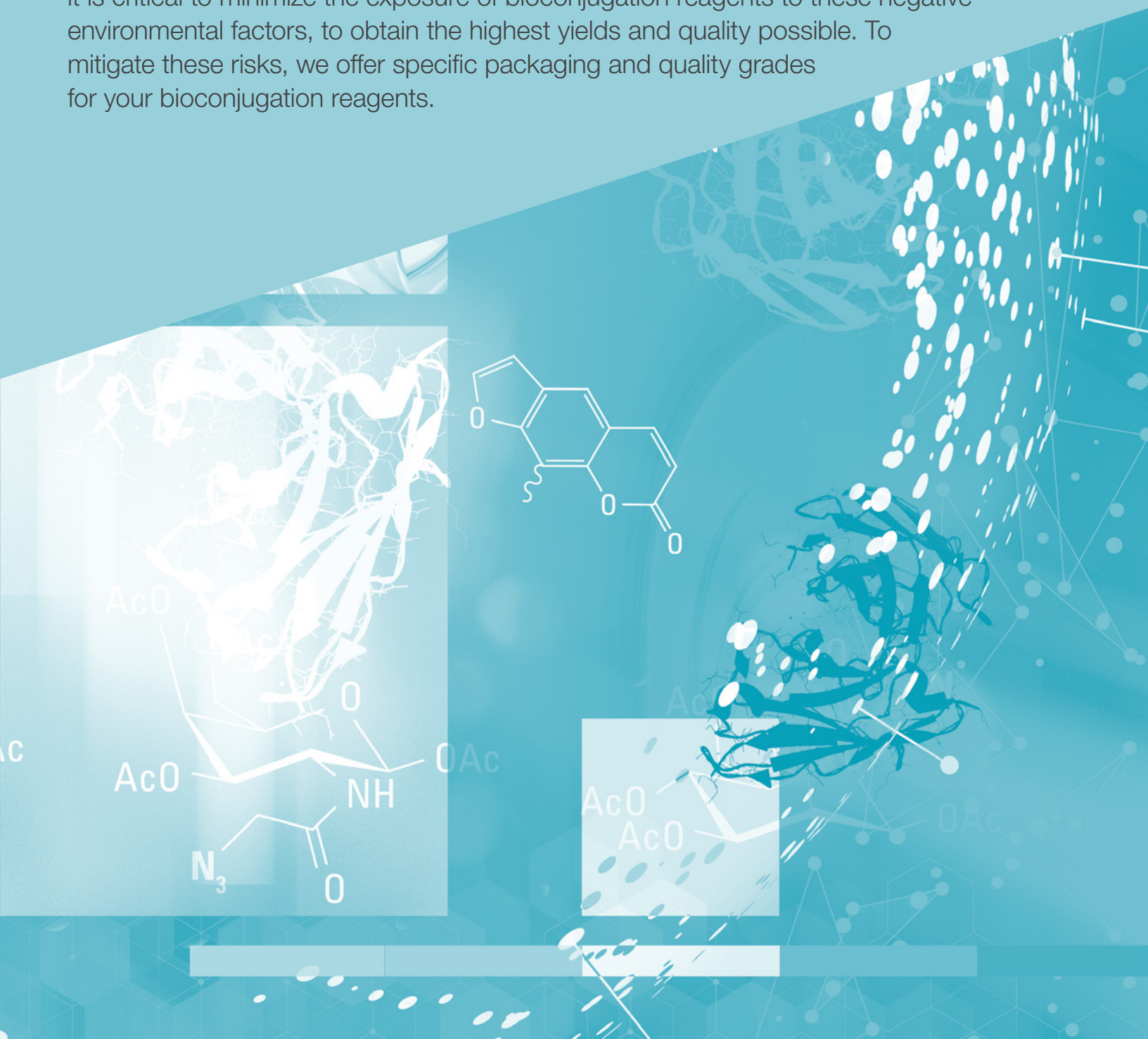


Figure 54. EDC can be used in coupling studies to study cell membrane structure.

Special packaging to meet specific bioconjugation needs

Introduction

Bioconjugation reagents are highly reactive molecules and therefore may be sensitive to water, light, oxygen, or other surrounding conditions. They may form unstable intermediates that lead to undesired side products of the intended reactions. Therefore, it is critical to minimize the exposure of bioconjugation reagents to these negative environmental factors, to obtain the highest yields and quality possible. To mitigate these risks, we offer specific packaging and quality grades for your bioconjugation reagents.



No-Weigh packaging format for bioconjugation reagents

Convenient, ready-to-use vials for single-use applications

With the convenient Thermo Scientific™ No-Weigh™ packaging format, a ready-to-use solution can be made quickly and conveniently. This unique packaging format helps eliminate the need to weigh out small volumes of dry chemicals.

Once reconstituted, the reagent is ready to use at the desired concentration. Avoid weighing hassles and wasting precious reagents with our single-use No-Weigh packaging format.

Highlights

- **Helps save time**—avoid weighing chemicals; just add water, buffer, or solvent to create a working solution in seconds
- **Helps reduce waste**—small working aliquots limit the amount of unused material discarded
- **Always fresh**—working solution is ready to use at desired concentration; no need to store stock solutions

The No-Weigh packaging format is available in convenient, easy-to-handle screw cap vials for our most widely used protein modification reagents, including reducing agents, crosslinkers, and PEG- and biotin-labeling products (Table 6).

Table 6. No-Weigh reagents.

Reagent type	No-Weigh reagents
Amine-to-azide	TFP ester-PEG_4-DBCO
Carboxyl-to-amine crosslinkers	EDC DSG Sulfo-NHS
Amine-to-amine crosslinkers	DSP BS^3 DSS DSSO DSBU $BS(PEG)_5$
Amine-to-sulfhydryl crosslinkers	SMCC Sulfo-SMCC $SM(PEG)_2$ $SM(PEG)_{12}$
Photoreactive	Sulfo-SANPAH
Modification reagents	Iodoacetamide
Reducing agents	TCEP-HCl DTT
Sulfhydryl-to-azide	Maleimide-PEG_4-DBCO

Premium-grade protein bioconjugation reagents

Higher quality and assurance of performance from a trusted supplier

Thermo Scientific™ Pierce™ Premium-Grade Reagents are the reagents of choice for applications where product integrity and risk minimization are critical (Table 7). Compared to standard grade reagents, Pierce Premium-Grade Reagents provide clearly defined quality by including batch-specific information such as quality assurance review, lot sample retention, and change control notification (CCN), as well as an enhanced level of analytical testing and product characterization.

Pierce Premium-Grade Reagents are manufactured to the highest possible specifications to enable data integrity and offer robust consistency. The consistency of each lot of reagent is assessed using thorough testing procedures (Table 8).

Highlights

- **Quality reagents**—high-purity reagents that can be used to create high-quality activated derivatives, labeled proteins, and bioconjugates
- **Product integrity**—enhanced level of testing and characterization
- **Lot retention**—ample supply of past lots retained to ensure future process testing
- **Change management**—change control notification (CCN) service
- **Consistent manufacturing**—batch-specific manufacturing documentation review

Table 7. Pierce Premium-Grade Reagents.

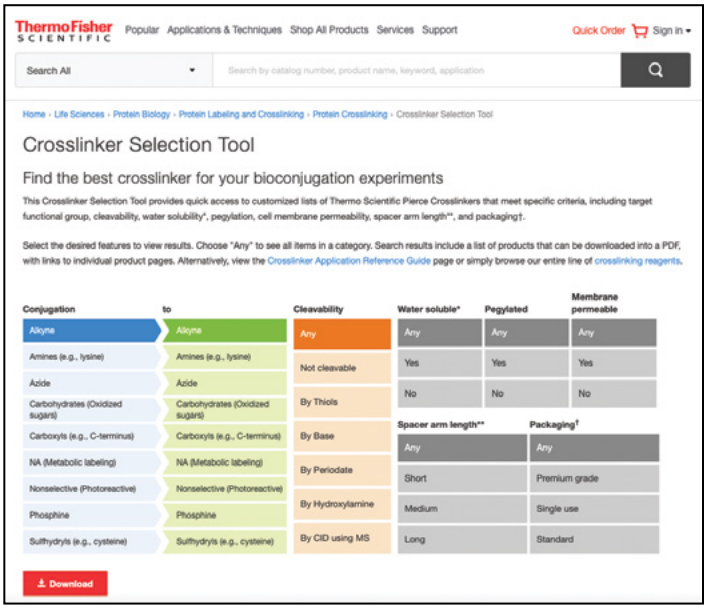
Reagent type	Pierce Premium-Grade Reagent
Carboxyl-to-amine crosslinkers	EDC Sulfo-NHS
Amine-to-amine crosslinkers	BS^3 DSP
Amine-to-sulfhydryl crosslinkers	Sulfo-SMCC SPDP
Biotinylation reagents	Sulfo-NHS-LC-biotin Sulfo-NHS-SS-biotin
Reducing agents	TCEP-HCl

Table 8. Testing for Pierce Premium-Grade Reagents.

Specification	Procedure
Purity	Quantitative NMR using an internal standard
Visual	Color assessment
Solubility	Example: sample dissolves at a specified concentration in a given solvent to yield a clear, colorless solution
Identity	Infrared (IR) spectroscopy
Mass identity	Mass spectrometry
Water content	Karl Fischer titration
Trace metals	Inductively coupled plasma mass spectrometry (ICP-MS)
Elemental analysis	Reported values for C, H, N, O, and S based on combustion analysis
Residual solvent analysis*	Headspace gas chromatography

* Performed upon request at an additional cost.

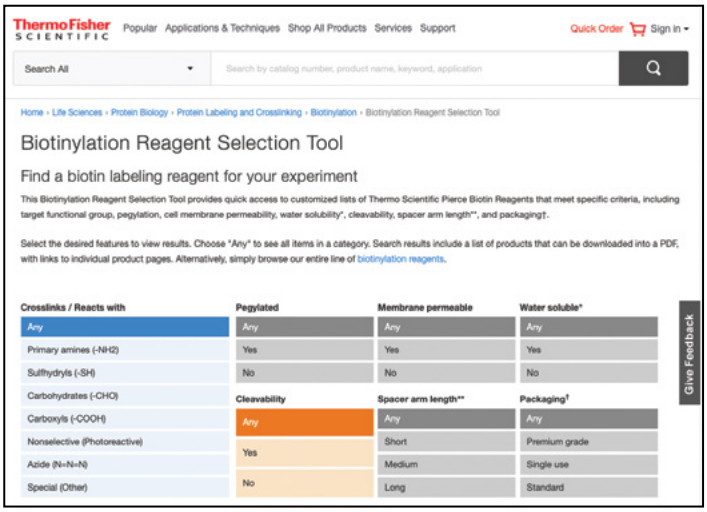
Bioconjugation resources



Crosslinker Selection Tool

The Crosslinker Selection Tool provides quick access to customized lists of Thermo Scientific™ Pierce™ crosslinkers that meet specific criteria, including target functional group, solubility, and cell membrane and the ability to permeate the cell membrane. Use the simple drop-down boxes to easily select the optimal crosslinking reagent for your application.

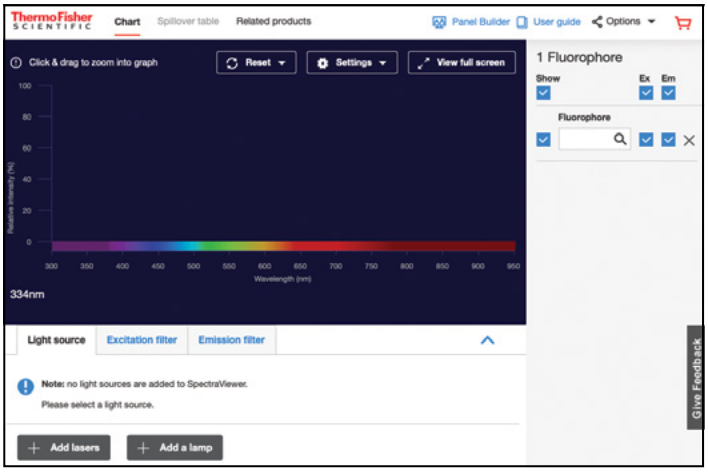
Learn more at **thermofisher.com/crosslinking-tool**



Biotinylation Reagent Selection Tool

The Biotinylation Reagent Selection Tool provides quick access to customized lists of Thermo Scientific™ biotinylation and desthiobiotin reagents that meet specific criteria, including target functional group, spacer arm length, and solubility. Use the simple drop-down boxes to easily select the optimal biotinylation reagent for your application.

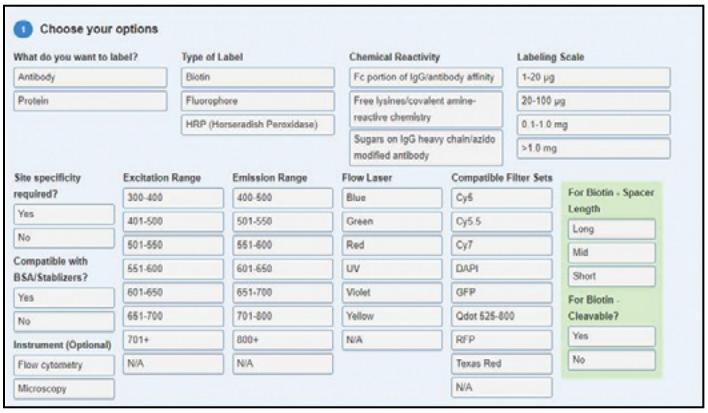
Learn more at **thermofisher.com/biotinylation-tool**



Fluorescence SpectraViewer Tool

The SpectraViewer is a tool to help you plan your experiments and analyses, by assisting you in choosing the best fluorophore based on your application, light sources, and filters. Easily find the right fluorophore to label your protein, antibody, or nucleic acid over a broad range of applications and techniques.

Learn more at **thermofisher.com/spectraviewer**



Labeling kit selection guide

The labeling kit selection guide provides quick access to customized lists of antibody and protein labeling kits for fluorophore, biotin, or HRP labeling. Easily find the right labeling kit based on your application, light sources, sample prep needs, and scale.

Learn more at **thermofisher.com/AbLabelingGuide**

Glossary of crosslinking terms

Acylation: Reaction that introduces an acyl group (–COR) into a compound.

Aryl azide: Compound containing a photoreactive functional group (e.g., phenyl azide) that reacts nonspecifically with target molecules.

Carbodiimide: Reagent that catalyzes the formation of an amide linkage between a carboxyl (–COOH) group and a primary amine ($–NH_2$) or a hydrazide ($–NHNH_2$). These reagents do not result in the formation of a cross-bridge and have been termed zero-length crosslinkers.

Crosslinker: Reagent that will react with functional groups on two or more molecules to form a covalent linkage between the molecules.

Conjugation reagent: Crosslinker or other reagent for covalently linking two molecules.

Diazirine crosslinker: Succinimidyl-ester diazirine (SDA) crosslinkers combine amine-reactive chemistry with an efficient diazirine-based photochemistry for photocrosslinking to nearly any other functional group. The photoactivation of diazirine with long-wave UV light (330–370 nm) creates carbene intermediates. These intermediates can form covalent bonds via addition reactions with any amino acid side chain or peptide backbone at distances corresponding to the spacer arm lengths.

Disulfide bonds: Oxidized form of sulfhydryls (–S–S–); formed in proteins through –SH groups from two cysteine molecules. These bonds often link polypeptide chains together within the protein and contribute to a protein's tertiary structure.

α-Haloacyl: Functional group (e.g., iodoacetyl) that targets nucleophiles, especially thiols. α-Haloacyl compounds have a halogen atom such as iodine, chlorine, or bromine attached to an acyl group on the molecule. These alkylating reagents degrade when exposed to direct light or reducing agents, resulting in loss of the halogen and the appearance of a characteristic color.

Hapten: Molecule recognized by antibodies but unable to elicit an immune response unless attached to a carrier protein. Haptens are usually, but not always, small (<5 kDa) molecules.

Homobifunctional crosslinker: Reagent with two identical reactive groups used to link two molecules or moieties.

Heterobifunctional crosslinker: Reagent with two different reactive groups used to link two molecules or moieties.

Hydrophilic: Substances that readily dissolve in water.

Hydrophobic: Substances with limited solubility in water.

***N*-hydroxysuccinimidyl (NHS) ester:** Acylating reagent commonly used for crosslinking or modifying proteins. These esters are specific for primary ($–NH_2$) amines between pH 7 and 9, but are generally the most effective at neutral pH. They are subject to hydrolysis, with half-lives approximating 1–2 hours at room temperature at neutral pH.

Imidate crosslinker: Primary amine-reactive functional group that forms an amidine bond. The ε-amine in lysine and N-terminal amines are the targets in proteins. Imidates react with amines in alkaline conditions (pH 7.5–10) and hydrolyze quickly, with half-lives typically around 10–15 minutes at room temperature and pH 7–9. At pH >11, the amidine bond is unstable, and crosslinking can be reversed. The amidine bond is protonated at physiological pH; therefore, it carries a positive charge.

Imidoester: Amine-reactive functional group of an imidate crosslinker.

Immunogen: Substance capable of eliciting an immune response.

Integral membrane protein: Protein that extends through the cell membrane and is stabilized by hydrophobic interactions within the lipid bilayer of the membrane.

Ligand: Molecule that binds specifically to another molecule. For example, a protein that binds to a receptor.

Moiety: Indefinite part of a sample or molecule.

Monomer: Single unit of a molecule.

NHS: Abbreviation for *N*-hydroxysuccinimide.

Nitrene: Triple-bonded nitrogen–nitrogen reactive group formed after exposure of an azido group to UV light. Its reactivity is nonspecific and short lived.

Nonselective crosslinking: Crosslinking using reactive groups such as nitrenes or aryl azides, which react so quickly and broadly that specific groups are not easily and efficiently targeted. Yields are generally low, with many different crosslinked products formed.

Nonspecific crosslinking: Another term for nonselective crosslinking.

Oligomer: Molecule composed of several monomers.

Photoreactive: Reactive upon excitation with light at a particular range of wavelengths.

Polymer: Molecule composed of many repeating monomers.

Pyridyl disulfide: Aromatic moiety with a disulfide attached to one of the carbons adjacent to the nitrogen in a pyridine ring. Pyridine 2-thione is released when this moiety reacts with a sulfhydryl (–SH)-containing compound.

Spacer arm: Part of a crosslinker that is incorporated between two crosslinked molecules and serves as a bridge between the molecules.

Substrate: Substance upon which an enzyme acts.

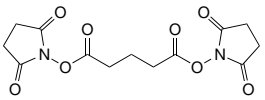
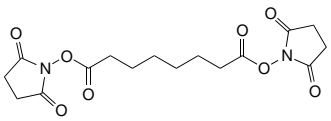
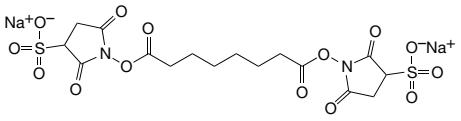
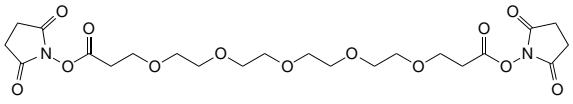
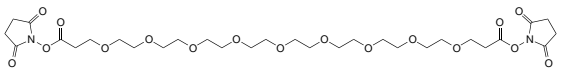
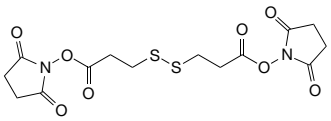
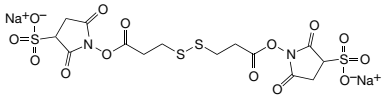
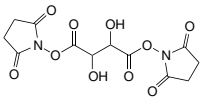
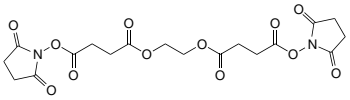
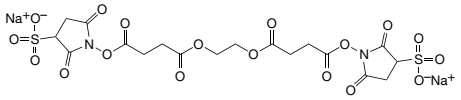
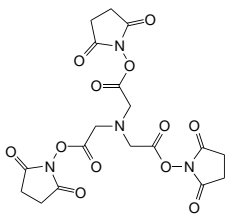
Sulfhydryl: –SH group, present on cysteine residues in proteins.

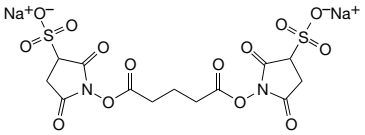
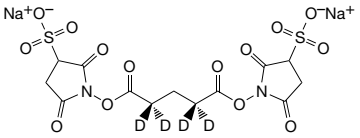
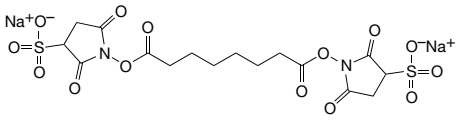
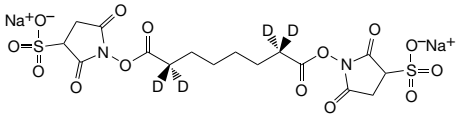
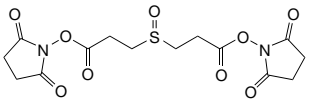
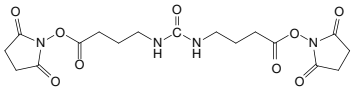
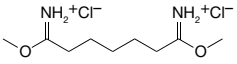
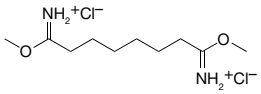
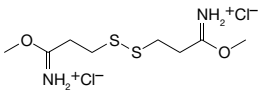
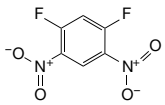
Thiols: Also known as mercaptans, thiolanes, sulfhydryls, or –SH groups, these are good nucleophiles that may be targeted for crosslinking.

Ultraviolet (UV): Electromagnetic radiation of wavelengths between 10 and 390 nm.

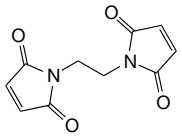
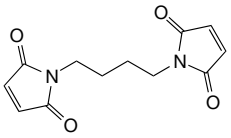
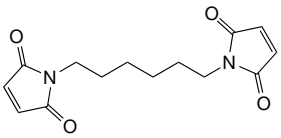
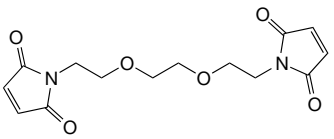
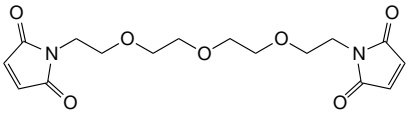
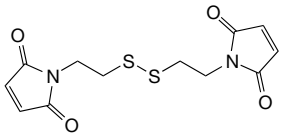
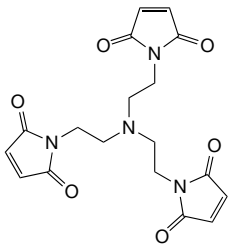
Ordering information

Homobifunctional crosslinkers

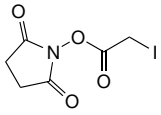
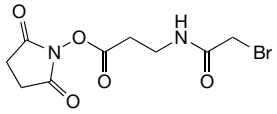
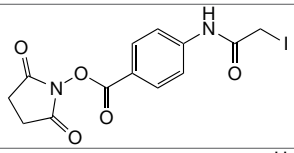
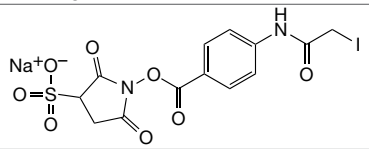
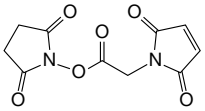
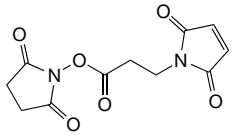
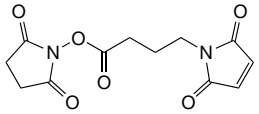
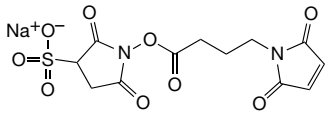
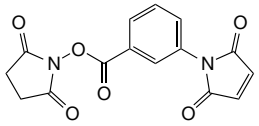
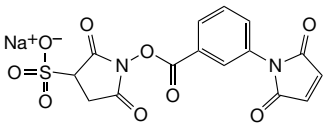
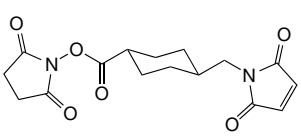
Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Amine-to-amine reactive (NHS ester)					
DSG (disuccinimidyl glutarate)	326.26	7.7		50 mg	20593
Pierce DSG, No-Weigh Format				10 x 1 mg	A35392
DSS (disuccinimidyl suberate)	368.35	11.4		50 mg	21655
				1 g	21555
Pierce DSS, No-Weigh Format				10 x 2 mg	A39267
BS^3 (bis(sulfosuccinimidyl)suberate)	572.43	11.4		50 mg	21580
				1 g	21586
Pierce Premium-Grade BS^3				100 mg	PG82083
				1 g	PG82084
Pierce BS^3, No-Weigh Format				10 x 2 mg	A39266
BS(PEG)$_5$ (PEGylated bis(sulfosuccinimidyl)suberate)	532.50	21.7		100 mg	21581
Pierce BS(PEG)$_5$, No-Weigh Format				10 x 1 mg	A35396
BS(PEG)$_9$ (PEGylated bis(sulfosuccinimidyl)suberate)	708.71	35.8		100 mg	21582
DSP (dithiobis(succinimidyl propionate)), Lomant's Reagent	404.42	12.0		1 g	22585
				50 mg	22586
Pierce Premium-Grade DSP				1 g	PG82081
				10 g	PG82082
Pierce DSP, No-Weigh Format				10 x 1 mg	A35393
DTSSP (3,3′-dithiobis(sulfosuccinimidyl propionate))	608.51	12.0		50 mg	21578
DST (disuccinimidyl tartrate)	344.24	6.4		50 mg	20589
EGS (ethylene glycol bis(succinimidyl succinate))	456.36	16.1		1 g	21565
Sulfo-EGS (ethylene glycol bis(sulfosuccinimidyl succinate))	660.45	16.1		50 mg	21566
TSAT (tris-(succinimidyl) aminotriacetate)	482.36	4.2		50 mg	33063

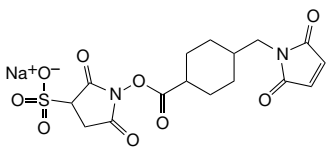
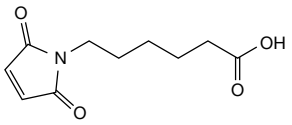
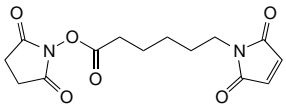
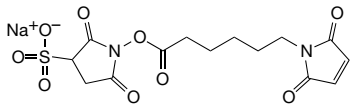
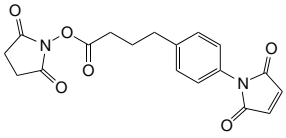
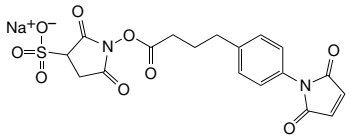
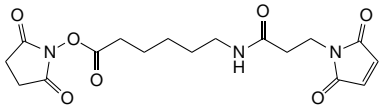
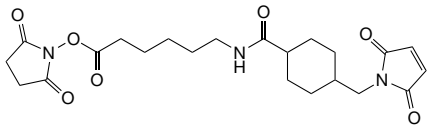
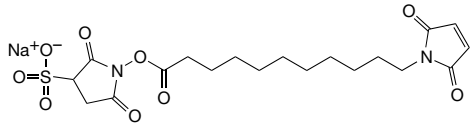
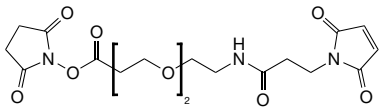
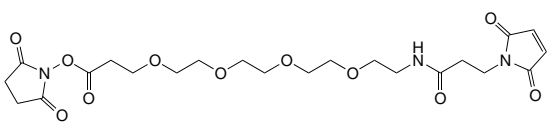
Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Amine-to-amine reactive (NHS ester), deuterated or MS-cleavable					
BS^{2}G-d$_0$ (bis(sulfosuccinimidyl) glutarate-d$_0$)	530.35	7.7		10 mg	21610
BS^{2}G-d$_4$ (bis(sulfosuccinimidyl) 2,2,4,4-glutarate-d$_4$)	534.38	7.7		10 mg	21615
BS^3-d$_0$ (bis(sulfosuccinimidyl) suberate-d$_0$)	572.43	11.4		10 mg	21590
BS^3-d$_4$ (bis(sulfosuccinimidyl) 2,2,7,7-suberate-d$_4$)	576.45	11.4		10 mg	21595
DSSO (disuccinimidyl sulfoxide)	388.35	10.1		10 x 1 mg	A33545
DSBU (disuccinimidyl dibutyric urea)	426.38	12.5		10 x 1 mg	A35459
Amine-to-amine reactive (imidoester or difluoro)					
DMP (dimethyl pimelimidate)	259.17	9.2		50 mg	21666
				1 g	21667
DMS (dimethyl suberimidate)	273.20	11.0		1 g	20700
DTBP (Wang and Richard's Reagent)	309.28	11.9		1 g	20665
DFDNB (1,5-difluoro-2,4-dinitrobenzene)	204.09	3.0		50 mg	21525

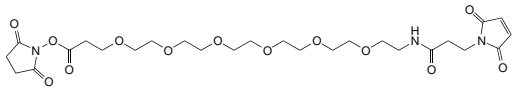
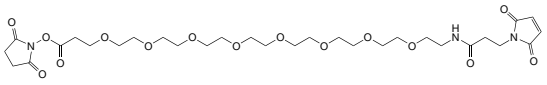
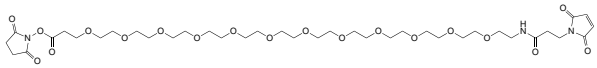
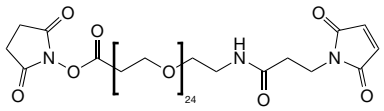
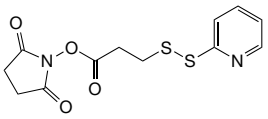
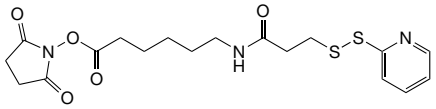
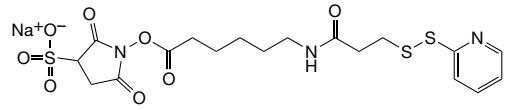
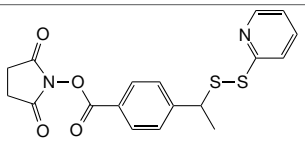
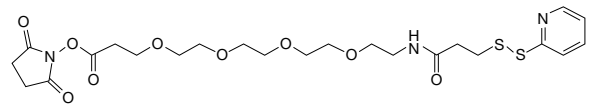
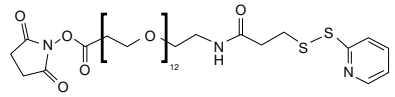
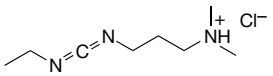
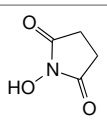
Learn more at **thermofisher.com/proteincrosslinking**

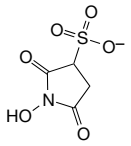
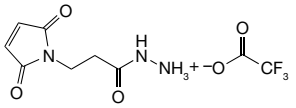
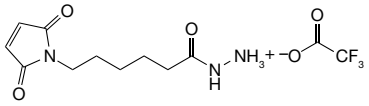
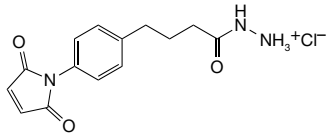
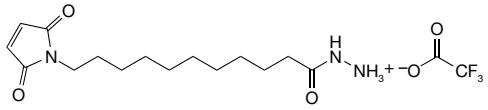
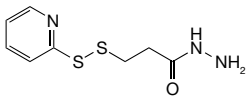
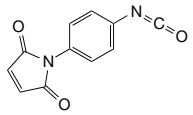
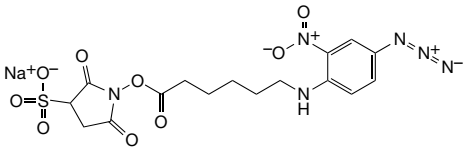
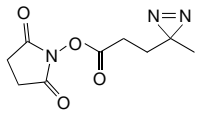
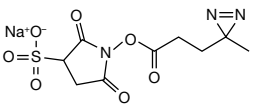
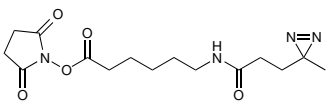
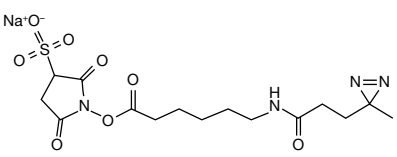
Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Sulfhydryl-to-sulfhydryl reactive (maleimide)					
BMOE (bismaleimidoethane)	220.18	8.0		50 mg	22323
BMB (1,4-bismaleimidobutane)	248.23	10.9		50 mg	22331
BMH (bismaleimidohexane)	276.29	16.1		50 mg	22330
$BM(PEG)_2$ (1,8-bismaleimido-diethyleneglycol)	308.29	14.7		50 mg	22336
$BM(PEG)_3$ (1,11-bismaleimido-triethyleneglycol)	352.34	17.8		50 mg	22337
DTME (dithiobismaleimidoethane)	312.37	13.3		50 mg	22335
TMEA (tris(2-maleimidoethyl)amine)	386.36	10.3		50 mg	33043

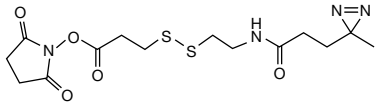
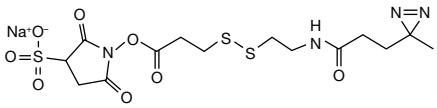
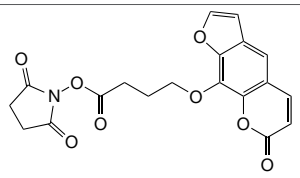
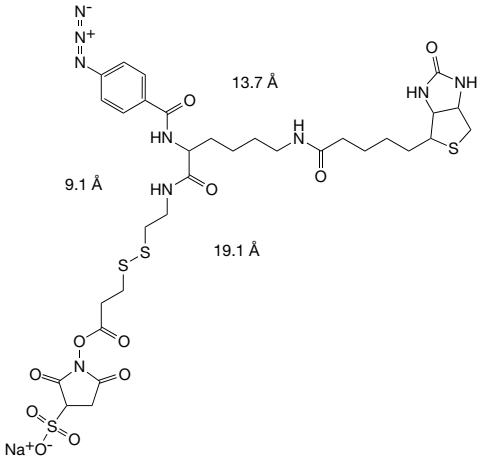
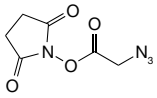
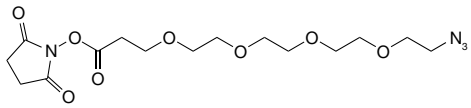
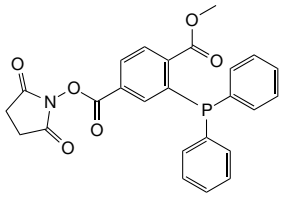
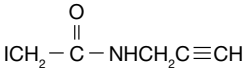
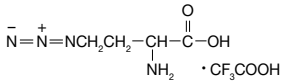
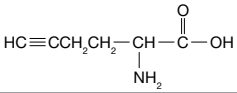
Heterobifunctional crosslinkers

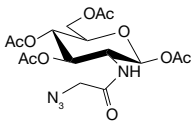
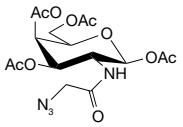
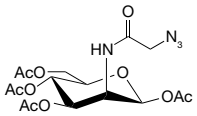
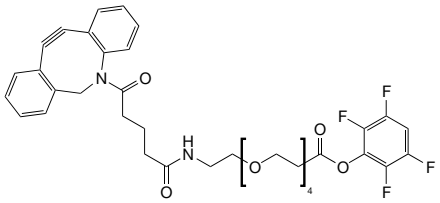
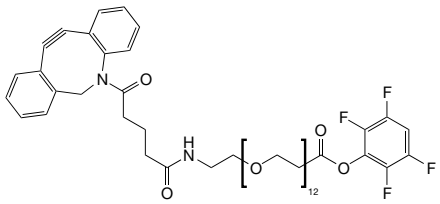
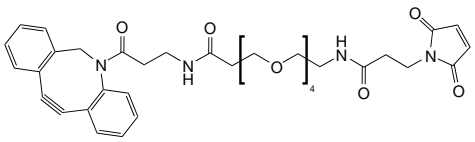
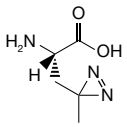
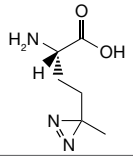
Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Amine-to-sulfhydryl reactive (NHS-haloacetyl)					
SIA (succinimidyl iodoacetate)	283.02	1.5		50 mg	22349
SBAP (succinimidyl 3-(bromo-acetamido)propionate)	307.10	6.2		50 mg	22339
SIAB (succinimidyl (4-iodoacetyl) aminobenzoate)	402.14	10.6		50 mg	22329
Sulfo-SIAB (sulfosuccinimidyl (4-iodoacetyl) aminobenzoate)	504.19	10.6		50 mg	22327
Amine-to-sulfhydryl reactive (NHS-maleimide)					
AMAS (*N*-α-maleimidoacet-oxysuccinimide ester)	252.18	4.4		50 mg	22295
BMPS (*N*-β-maleimidopropyl-oxysuccinimide ester)	266.21	5.9		50 mg	22298
GMBS (*N*-γ-maleimidobutyryl-oxysuccinimide ester)	280.23	7.3		50 mg	22309
Sulfo-GMBS (*N*-γ-maleimidobutyryl-oxysulfosuccinimide ester)	382.28	7.3		50 mg	22324
MBS (*m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester)	314.25	7.3		50 mg	22311
Sulfo-MBS (*m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester)	416.30	7.3		50 mg	22312
SMCC (succinimidyl 4-(*N*-maleimido-methyl)cyclohexane-1-carboxylate)	334.32	8.3		50 mg	22360
Pierce SMCC, No-Weigh Format				10 x 1 mg	A35394

Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Sulfo-SMCC(sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate)	436.37	8.3		50 mg	22322
				1 g	22122
Pierce Premium-Grade Sulfo-SMCC				100 mg	PG82085
				1 g	PG82086
Pierce Sulfo-SMCC, No-Weigh Format				10 x 2 mg	A39268
Pierce EMCA (*N*-ε-maleimidocaproic acid)	211.21	9.4		1 g	22306
EMCS(*N*-ε-maleimidocaproyl-oxysuccinimide ester)	308.29	9.4		50 mg	22308
Sulfo-EMCS (*N*-ε-maleimidocaproyl-oxysulfosuccinimide ester)	410.33	9.4		50 mg	22307
SMPB (succinimidyl 4-(*p*-maleimidophenyl)butyrate)	356.33	11.6		50 mg	22416
Sulfo-SMPB (sulfosuccinimidyl 4-(*N*-maleimidophenyl)butyrate)	458.38	11.6		50 mg	22317
SMPH (succinimidyl 6-((β-maleimidopropionamido)hexanoate))	379.36	14.2		50 mg	22363
LC-SMCC (succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate))	447.48	16.2		50 mg	22362
Sulfo-KMUS (*N*-κ-maleimido-undecanoyl-oxysulfosuccinimide ester)	480.46	16.3		50 mg	21111
$SM(PEG)_2$ (PEGylated SMCC crosslinker)	425.39	17.6		100 mg	22102
				1 g	22103
Pierce $SM(PEG)_2$, No-Weigh Format				10 x 1 mg	A35397
$SM(PEG)_4$ (PEGylated SMCC crosslinker)	513.5	24.6		100 mg	22104
				1 g	22107

Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
SM(PEG)$_6$(PEGylated, long-chain SMCC crosslinker)	601.60	32.5		100 mg	22105
SM(PEG)$_8$ (PEGylated, long-chain SMCC crosslinker)	689.71	39.2		100 mg	22108
SM(PEG)$_{12}$ (PEGylated, long-chain SMCC crosslinker)	865.92	53.4		100 mg	22112
				1 g	22113
Pierce SM(PEG)$_{12}$, No-Weigh Format				10 x 1 mg	A35398
SM(PEG)$_{24}$ (PEGylated, long-chain SMCC crosslinker)	1,394.55	95.2		100 mg	22114
Amine-to-sulfhydryl reactive (NHS-pyridyldithiol)					
SPDP (succinimidyl 3-(2-pyridyldithio) propionate)	312.37	6.8		50 mg	21857
Pierce Premium-Grade SPDP				100 mg	PG82087
				1 g	PG82088
LC-SPDP (succinimidyl 6-(3(2-pyridyldithio)propionamido) hexanoate)	425.52	15.7		50 mg	21651
Sulfo-LC-SPDP (sulfosuccinimidyl 6-(3′-(2-pyridyldithio)propionamido) hexanoate)	527.57	15.7		50 mg	21650
SMPT (4-succinimidyloxycarbonyl-α-methyl-α(2-pyridyldithio)toluene)	388.46	20.0		50 mg	21558
PEG$_4$-SPDP (PEGylated, long-chain SPDP crosslinker)	559.17	25.7		100 mg	26128
PEG$_{12}$-SPDP (PEGylated, long-chain SPDP crosslinker)	912.07	54.1		100 mg	26129
Carboxyl-to-amine reactive (carbodiimide plus NHS ester)					
EDC (1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride)	191.70	NA		10 mg	77149
				5 g	22980
				25 g	22981
Pierce Premium-Grade EDC				1 g	PG82079
				25 g	PG82073
				500 g	PG82074
Pierce EDC, No-Weigh Format				10 x 1 mg	A35391
NHS (*N*-hydroxysuccinimide)	115.09	NA		25 mg	24500

Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Sulfo-NHS (*N*-hydroxysulfosuccinimide)	217.13	NA		500 mg	24510
				5 g	24525
Pierce Premium-Grade Sulfo-NHS				500 mg	PG82071
				10 g	PG82072
Pierce Sulfo-NHS, No-Weigh Format				10 x 2 mg	A39269
Sulfhydryl-to-carbohydrate or -carboxyl (maleimide, pyridyldithiol/hydrazide, or isocyanate)					
BMPH (*N*-β-maleimidopropionic acid hydrazide)	297.19	8.1		50 mg	22297
EMCH (*N*-ε-maleimidocaproic acid hydrazide)	225.24	11.8		50 mg	22106
MPBH (4-(4-*N*-maleimidophenyl) butyric acid hydrazide)	309.75	17.9		50 mg	22305
KMUH (*N*-κ-maleimidoundecanoic acid hydrazide)	295.38	19.0		50 mg	22111
PDPH (3-(2-pyridyldithio)propionyl hydrazide)	229.32	9.2		50 mg	22301
PMPI (*p*-maleimidophenyl isocyanate)	214.18	8.7		50 mg	28100
Photoreactive (NHS ester and aryl azide, phenyl azide, diazirine, or psoralen)					
Sulfo-SANPAH (sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino) hexanoate)	492.40	18.2		50 mg	22589
Pierce Sulfo-SANPAH, No-Weigh Format				10 x 1 mg	A35395
SDA (NHS-Diazirine) (succinimidyl 4,4′-azipentanoate)	225.20	3.9		50 mg	26167
Sulfo-SDA (Sulfo-NHS-Diazirine) (sulfosuccinimidyl 4,4′-azipentanoate)	327.25	3.9		50 mg	26173
LC-SDA (NHS-LC-Diazirine) (succinimidyl 6-(4,4′-azipentanamido) hexanoate)	338.36	12.5		50 mg	26168
Sulfo-LC-SDA (Sulfo-NHS-LC-Diazirine) (sulfosuccinimidyl 6-(4,4′-azipentanamido)hexanoate)	440.40	12.5		50 mg	26174

Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
SDAD (NHS-SS-Diazirine) (succinimidyl 2-((4,4′-azipentanamido) ethyl)-1,3′-dithiopropionate)	388.46	13.5		50 mg	26169
Sulfo-SDAD (Sulfo-NHS-SS-Diazirine) (sulfosuccinimidyl 2-((4,4′-azipentanamido)ethyl)-1,3′-dithiopropionate)	490.51	13.5		50 mg	26175
SPB (succinimidyl-(4-(psoralen-8-yloxy))-butyrate)	385.32	8.6		50 mg	23013
Sulfo-SBED Biotin Label Transfer Reagent	879.97	19.1 13.7 9.1		10 mg	33033
Pierce Sulfo-SBED Biotin Label Transfer Reagent, No-Weigh Format				10 x 1 mg	A39260
Chemoselective ligation (NHS ester and azide-phosphine or -alkyne)					
NHS-Azide	198.14	2.5		10 mg	88902
NHS-PEG_4-Azide	388.37	18.9		100 mg	26130
NHS-Phosphine	461.40	5.4		10 mg	88900
Iodoacetamide Alkyne	223.01	7.8		1 mg	I10189
Click-iT AHA (L-azidohomoalanine)	258.16	NA		5 mg	C10102
Click-iT HPG (L-homopropargyl-glycine)	127.14	NA		5 mg	C10186

Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Chemoselective ligation					
GlcNAz (*N*-azidoacetylglucosamine, tetraacylated)	430.37	NA		5 mg	88903
GalNAz (*N*-azidoacetylgalactosamine, tetraacylated)	430.37	NA		5 mg	88905
ManNAz (*N*-azidoacetylmannosamine, tetraacylated)	430.37	NA		5 mg	88904
TFP Ester-PEG$_4$-DBCO	714.7	17.9		25 mg	C20039
				10 x 1 mg	C20043
TFP Ester-PEG$_{12}$-DBCO	1,067.12	46.3		25 mg	C20040
Maleimide-PEG$_4$-DBCO	647.74	29.75		25 mg	C20041
				10 x 1 mg	C20044
Photoreactive amino acids					
L-Photo-Leucine	143.15	0		100 mg	22610
L-Photo-Methionine	157.17	0		100 mg	22615

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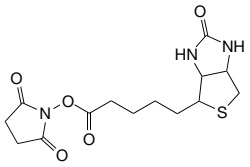
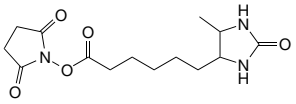
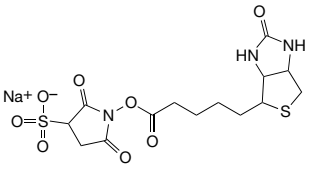
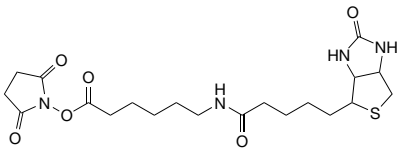
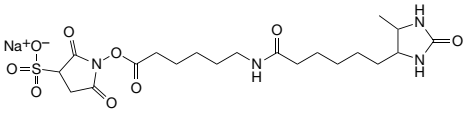
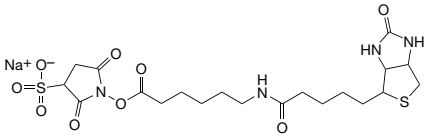
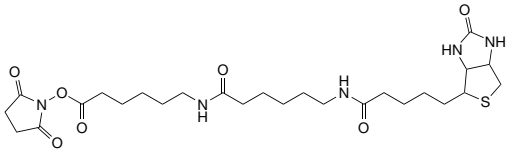
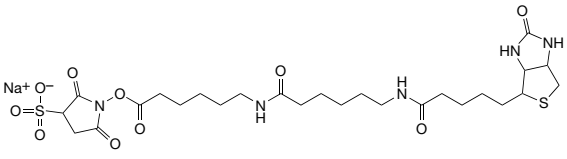
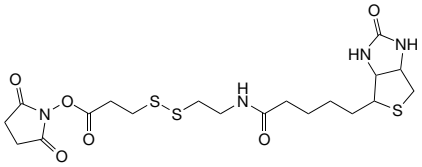
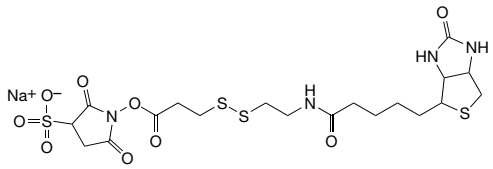
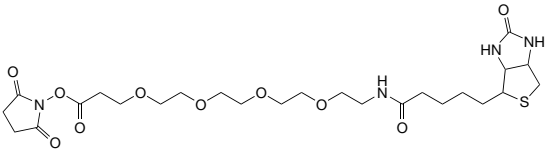
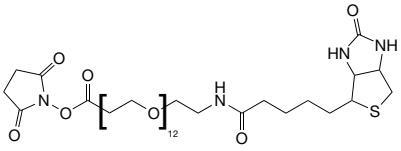
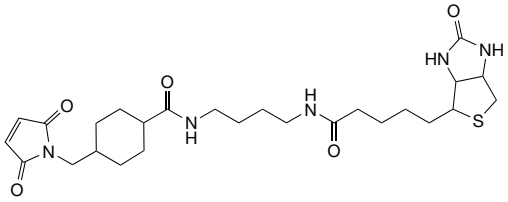
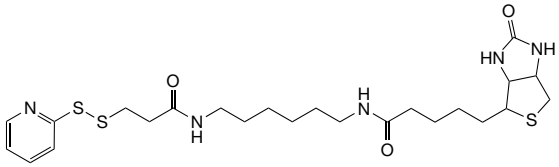
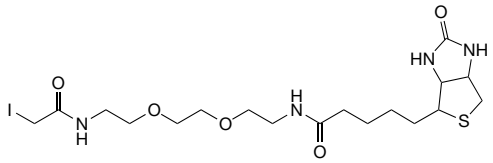
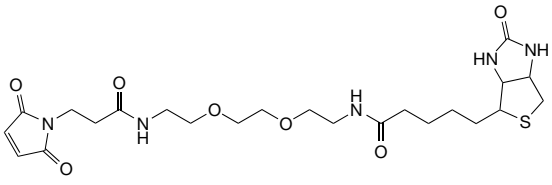
Biotin and desthiobiotin labeling reagents

Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
Amine-reactive					
EZ-Link NHS-Biotin	341.38	13.5		100 mg	20217
EZ-Link NHS-Desthiobiotin	311.33	9.7		50 mg	16129
EZ-Link Sulfo-NHS-Biotin	443.43	13.5		50 mg	21217
EZ-Link Sulfo-NHS-Biotin, No-Weigh Format				10 x 1 mg	A39256
EZ-Link NHS-LC-Biotin	454.54	22.4		50 mg	21336
EZ-Link Sulfo-NHS-LC-Desthiobiotin, No-Weigh Format	526.54	17.3		5 x 1 mg	A39265
EZ-Link Sulfo-NHS-LC-Biotin	556.59	22.4		100 mg	21335
Pierce Premium-Grade Sulfo-NHS-LC-Biotin				100 mg	PG82075
				1 g	PG82076
EZ-Link Sulfo-NHS-LC-Biotin, No-Weigh Format				10 x 1 mg	A39257
EZ-Link NHS-LC-LC-Biotin	567.70	30.5		50 mg	21343
EZ-Link Sulfo-NHS-LC-LC-Biotin	669.75	30.5		50 mg	21338
EZ-Link Sulfo NHS-LC-LC-Biotin, No-Weigh Format				10 x 1 mg	A35358
EZ-Link NHS-SS-Biotin	504.65	24.3		50 mg	21441

Product	MW	Spacer arm (Å)	Structure	Quantity	Cat. No.
EZ-Link Sulfo-NHS-SS-Biotin	606.69	24.3		100 mg	21331
Pierce Premium-Grade Sulfo-NHS-SS-Biotin				100 mg	PG82077
				1 g	PG82078
EZ-Link Sulfo-NHS-SS-Biotin, No-Weigh Format				10 x 1 mg	A39258
EZ-Link NHS-PEG$_4$-Biotin	588.67	29.0		25 mg	21330
				50 mg	21362
				1 g	21363
EZ-Link NHS-PEG$_4$-Biotin, No-Weigh Format				10 x 2 mg	A39259
EZ-Link NHS-PEG$_{12}$-Biotin	941.09	56.0		25 mg	21312
				500 mg	21313
EZ-Link NHS-PEG$_{12}$-Biotin, No-Weigh Format				10 x 1 mg	A35389
Sulfhydryl-reactive					
EZ-Link BMCC-Biotin	533.68	32.6		50 mg	21900
EZ-Link HPDP-Biotin	539.78	29.2		50 mg	21341
EZ-Link HPDP-Biotin, No-Weigh Format				10 x 1 mg	A35390
EZ-Link Iodoacetyl-PEG$_2$-Biotin	542.43	24.7		50 mg	21334
EZ-Link Maleimide-PEG$_2$-Biotin	525.62	29.1		50 mg	21901BID
EZ-Link Maleimide-PEG$_2$-Biotin, No-Weigh Format				10 x 2 mg	A39261

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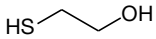
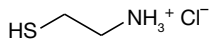
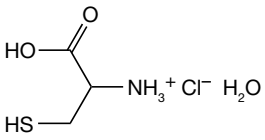
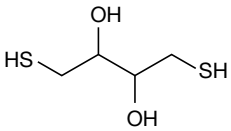
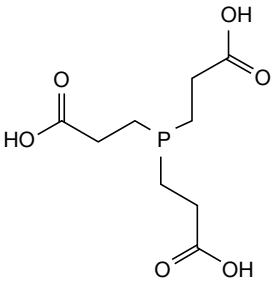
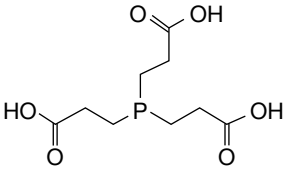
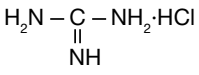
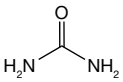
Modification reagents for reduction and denaturation of proteins

Product	MW	Spacer arm (Å)	Reactive group	Structure	Quantity	Cat. No.
Disulfide bond reduction						
Pierce 2-Mercaptoethanol/ (β-mercaptoethanol)	78.13	NA	Thiol		10 x 1 mL	35602
Pierce Mercaptoethylamine-HCl (2-mercaptoethylamine-HCl (2-MEA))	78.13	NA	Thiol		6 x 6 mg	20408
Pierce Cysteine-HCl	175.63	NA	Thiol		5 g	44889
Pierce DTT, Cleland's Reagent/Dithiothreitol	154.25	NA	Thiol		5 g	20290
Pierce DTT, No-Weigh Format					48 x 7.7 mg	A39255
Bond-Breaker TCEP Solution, Neutral pH	286.25	NA	Phosphine		5 mL	77720
Pierce TCEP-HCl	286.65	NA	Phosphine		1 g	20491
					10 g	20490
Pierce Premium-Grade TCEP-HCl					1 g	PG82080
					10 g	PG82089
					100 g	PG82090
Schiff base reduction to alkylamine linkage						
AminoLink Reductant (sodium cyanoborohydride)	62.84	NA	Cyanoborohydride		2 x 1 g	44892
Protein denaturants and chaotropes						
Guanidine-HCl	95.53	NA	NA		500 g	24110
Guanidine-HCl (8 M solution)					200 mL	24115
Urea	60.06	NA	NA		1 kg	29700

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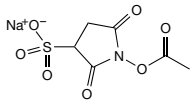
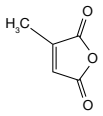
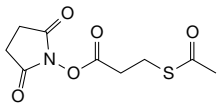
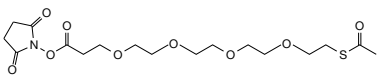
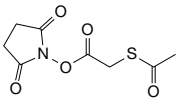
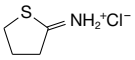
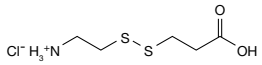
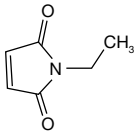
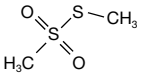
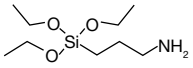
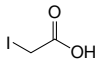
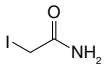
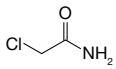
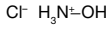
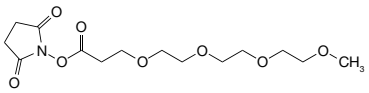
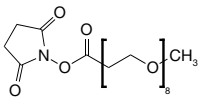
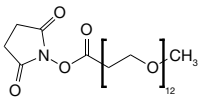
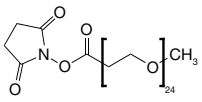
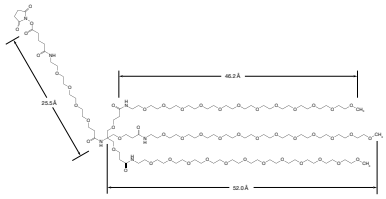
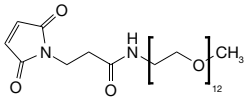
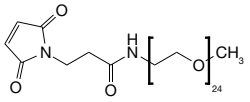
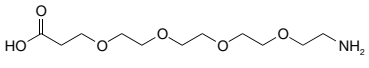
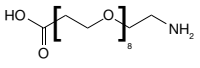
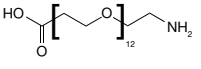
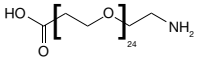
Modification reagents for proteins and peptides

Product	MW	Spacer arm (Å)	Reactive group	Structure	Quantity	Cat. No.
Irreversibly blocks primary amines						
Pierce Sulfo-NHS-Acetate (sulfo-*N*-hydroxysulfosuccinimide acetate)	259.17	NA	NHS ester		100 mg	26777
Reversibly blocks primary amines						
Pierce Citraconic Anhydride (2-methylmaleic anhydride)	112.08	NA	NA		100 g	20907
Modifies primary amines to contain a protected sulfhydryl group						
Pierce SATP (*N*-succinimidyl S-acetylthio-propionate)	245.25	4.1	NHS ester		50 mg	26100
Pierce SAT(PEG)$_4$ (*N*-succinimidyl S-acetyl(thio-tetraethylene glycol)	421.46	18.3	NHS ester/ acetylated sulfhydryl (protected)		100 mg	26099
Pierce SATA (*N*-succinimidyl S-acetylthioacetate)	231.23	2.8	NHS ester		50 mg	26102
Modifies primary amines to contain a free sulfhydryl group						
Traut's Reagent (2-iminothiolane)	137.63	8.1	Iminothiolane		500 mg	26101
Adds amine or carboxylic acid functional group to protein or surface						
Pierce AEDP (3-((2-aminoethyl)dithio) propionic acid-HCl)	217.74	NA	Amine/ carboxylic acid		50 mg	22101
Irreversibly blocks sulfhydryl groups						
Pierce NEM (*N*-ethylmaleimide)	125.13	NA	Maleimide		25 g	23030
Reversibly blocks sulfhydryl groups						
Pierce MMTS (methyl methanethiosulfonate)	126.20	NA	NA		200 mg	23011

Product	MW	Spacer arm (Å)	Reactive group	Structure	Quantity	Cat. No.
Adds primary amine to glass and silica surfaces through silylation						
Pierce APTS (3-aminopropyltriethoxysilane)	221.37	NA	NA		100 g	80370
Oxidizes carbohydrates for reductive amination						
Pierce Sodium *Meta*-Periodate	213.89	NA	Periodate		25 g	20504
Alkylates reduced cysteines						
Pierce Iodoacetic Acid	185.95	NA	Iodoacetyl		500 mg	35603
Pierce Iodoacetamide, No-Weigh Format	184.96	NA	Iodoacetyl		30 x 9.3 mg	A39271
Pierce Chloroacetamide, No-Weigh Format	93.51	NA	Chloroacetyl		10 x 2 mg	A39270
Deprotects SATA-modified molecules						
Pierce Hydroxylamine-HCl	69.49	NA	NA		25 g	26103

PEGylation (PEG labeling) reagents for proteins

Product	MW	Spacer arm (Å)	Reactive group	Structure	Quantity	Cat. No.
Amine-reactive linear PEGylation of protein or surface, terminating with a methyl group						
$MS(PEG)_4$ (methyl-PEG_4-NHS ester)	333.33	16.4	NHS ester		100 mg 1 g	22341 22342
$MS(PEG)_8$ (methyl-PEG_8-NHS ester)	509.40	30.8	NHS ester		100 mg	22509
$MS(PEG)_{12}$ (methyl-PEG_{12}-NHS ester)	685.71	44.9	NHS ester		100 mg 1 g	22685 22686
$MS(PEG)_{24}$ (methyl-PEG_{24}-NHS ester)	1,214.39	88.2	NHS ester		100 mg	22687
Amine-reactive branched PEGylation of a protein or surface, terminating with a methyl group						
$TMS(PEG)_{12}$ (($methyl\text{-}PEG_{12}$)$_3$-PEG_4-NHS ester)	2,420.80	52.0	NHS ester		1 g	22424
Sulfhydryl-reactive branched PEGylation of a protein or surface, terminating with a methyl group						
$MM(PEG)_{12}$ (methyl-PEG_{12}-maleimide)	710.81	51.9	Maleimide		100 mg	22711
$MM(PEG)_{24}$ (methyl-PEG_{24}-maleimide)	1,239.44	95.3	Maleimide		100 mg	22713
PEGylation of a protein or surface, terminating with a carboxylic acid or primary amine						
$CA(PEG)_4$ (carboxyl-(4-ethyleneglycol) ethylamine)	265.30	18.1	Amine/ carboxylic acid		100 mg 1 g	26120 26121
$CA(PEG)_8$ (carboxyl-(8-ethyleneglycol) ethylamine)	441.51	33.6	Amine/ carboxylic acid		100 mg 1 g	26122 26123
$CA(PEG)_{12}$ (carboxyl-(12-ethyleneglycol) ethylamine)	617.72	46.8	Amine/ carboxylic acid		100 mg 1 g	26124 26125
$CA(PEG)_{24}$ (carboxyl-(24-ethyleneglycol) ethylamine)	1,146.35	89.8	Amine/ carboxylic acid		100 mg 1 g	26126 26127

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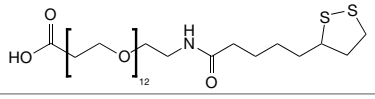
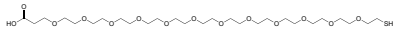
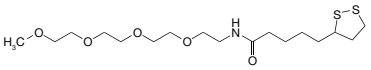
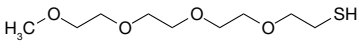
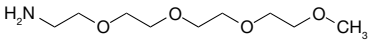
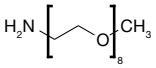
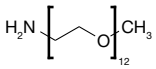
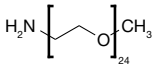
Product	MW	Spacer arm (Å)	Reactive group	Structure	Quantity	Cat. No.
PEGylation of a gold, silver, or metal surface, terminating with a carboxylic acid or methyl group						
$CL(PEG)_{12}$ Carboxy-PEG-Lipoamide Compound	806.03	55.5	Carboxylic acid/ bidentate thiol		100 mg	26135
$CT(PEG)_{12}$ Carboxy-PEG-Thiol Compound	634.77	47.8	Carboxylic acid/ thiol		100 mg	26133
$ML(PEG)_4$ Methyl-PEG-Thiol Compound	395.58	23.6	Bidentate thiol		100 mg	26134
PEGylation of a protein or inert material surface, terminating with a methyl group						
$MT(PEG)_4$ Methyl-PEG-Thiol Compound	224.32	15.8	Thiol		100 mg	26132
PEGylation of a protein, oxidized carbohydrate, or surface, terminating with a methyl group						
$MA(PEG)_4$ (methyl-(4-ethyleneglycol) ethylamine)	207.27	15.5	Amine		100 mg	26110
					1 g	26111
$MA(PEG)_8$ (methyl-(8-ethyleneglycol) ethylamine)	383.48	29.7	Amine		100 mg	26112
					1 g	26113
$MA(PEG)_{12}$ (methyl-(12-ethyleneglycol) ethylamine)	559.69	43.9	Amine		100 mg	26114
					1 g	26115
$MA(PEG)_{24}$ (methyl-(24-ethyleneglycol) ethylamine)	1,088.32	86.1	Amine		100 mg	26116
					1 g	26117

Fluorescent dye labeling reagents and kits

Product	Emission/excitation (nm)	Emission color	Quantity	Cat. No.
Amine-reactive				
Alexa Fluor 350 NHS Ester (Succinimidyl Ester)	346/442	Blue	5 mg	A10168
Alexa Fluor 350 Antibody Labeling Kit, 5 x 100 µg of antibody	346/442	Blue	1 kit	A20180
Alexa Fluor 405 NHS Ester (Succinimidyl Ester)	402/421	Blue	1 mg	A30000
			5 mg	A30100
Pacific Blue Succinimidyl Ester	385/445	Blue	5 mg	P10163
BODIPY 493/503 NHS Ester (Succinimidyl Ester)	500/506	Green	5 mg	D2191
BODIPY FL NHS Ester (Succinimidyl Ester)	505/513	Green	5 mg	D2184
Fluorescein-5-Isothiocyanate (FITC 'Isomer I')	494/518	Green	1 g	F143
Alexa Fluor 488 TFP Ester	495/519	Green	3 x 100 µg	A37570
			25 mg	A37563
Alexa Fluor 488 NHS Ester (Succinimidyl Ester)	495/519	Green	1 mg	A20000
			5 mg	A20100
Alexa Fluor 488 Protein Labeling Kit	495/519	Green	1 kit	A10235
Alexa Fluor 488 Microscale Protein Labeling Kit	495/519	Green	1 kit	A30006
Alexa Fluor 488 Antibody Labeling Kit	495/519	Green	5-rxn kit	A20181
APEX Alexa Fluor 488 Antibody Labeling Kit	495/519	Green	1 kit	A10468
Zip Alexa Fluor 488 Rapid Antibody Labeling Kit	495/519	Green	1 kit	Z11233
pHrodo iFL Green STP Ester, amine-reactive dye	505/520	Green	3 x 100 µg	P36012
			1 mg	P36013
pHrodo iFL Green Microscale Protein Labeling Kit	505/520	Green	1 kit	P36015
Oregon Green 488 Carboxylic Acid, Succinimidyl Ester, 6-isomer	495/524	Green	5 mg	O6149
Oregon Green 488 Carboxylic Acid, Succinimidyl Ester, 5-isomer	495/524	Green	5 mg	O6147
Oregon Green 514 Carboxylic Acid, Succinimidyl Ester	511/530	Yellow	5 mg	O6139
Alexa Fluor 514 NHS Ester (Succinimidyl Ester)	517/542	Yellow	1 mg	A30002
Alexa Fluor 430 NHS Ester (Succinimidyl Ester)	434/539	Yellow	5 mg	A10169
Pacific Orange Succinimidyl Ester, Triethylammonium Salt	400/551	Orange	1 mg	P30253
Alexa Fluor 532 NHS Ester (Succinimidyl Ester)	531/554	Orange	1 mg	A20001
			5 mg	A20001MP
BODIPY 530/550 NHS Ester (Succinimidyl Ester)	534/554	Orange	5 mg	D2187
Alexa Fluor 555 NHS Ester (Succinimidyl Ester)	555/565	Orange	3 x 100 µg	A37571
			1 mg	A20009
			5 mg	A20109
			25 mg	A37564
Alexa Fluor 555 Protein Labeling Kit	555/565	Orange	1 kit	A20174
Alexa Fluor 555 Microscale Protein Labeling Kit	555/565	Orange	1 kit	A30007
Alexa Fluor 555 Antibody Labeling Kit	555/565	Orange	5-rxn kit	A20187
APEX Alexa Fluor 555 Antibody Labeling Kit	555/565	Orange	1 kit	A10470
Zip Alexa Fluor 555 Rapid Antibody Labeling Kit	555/565	Orange	1 kit	Z11234
BODIPY 558/568 NHS Ester (Succinimidyl Ester)	558/569	Orange	5 mg	D2219
BODIPY TMR-X NHS Ester (Succinimidyl Ester)	542/574	Orange/red	5 mg	D6117
Alexa Fluor 546 NHS Ester (Succinimidyl Ester)	556/575	Orange/red	1 mg	A20002
			5 mg	A20102
Alexa Fluor 546 Protein Labeling Kit	556/575	Orange/red	1 kit	A10237
Alexa Fluor 546 Antibody Labeling Kit	556/575	Orange/red	1 kit	A20183
BODIPY 576/589 NHS Ester (Succinimidyl Ester)	576/590	Red	5 mg	D2225
pHrodo iFL Red STP Ester, amine-reactive dye	566/590	Red	3 x 100 µg	P36011
			1 mg	P36010
Alexa Fluor 568 NHS Ester (Succinimidyl Ester)	578/603	Red	1 mg	A20003
			5 mg	A20103
Alexa Fluor 568 Antibody Labeling Kit	578/603	Red	5-rxn kit	A20184
APEX Alexa Fluor 568 Antibody Labeling Kit	578/603	Red	1 kit	A10494
Alexa Fluor 568 Protein Labeling Kit	578/603	Red	1 kit	A10238

Product	Emission/excitation (nm)	Emission color	Quantity	Cat. No.
Amine-reactive				
BODIPY TR-X NHS Ester (Succinimidyl Ester)	589/617	Red	5 mg	D6116
Alexa Fluor 594 NHS Ester (Succinimidyl Ester)	590/617	Red	3 x 100 µg	A37572
			1 mg	A20004
Alexa Fluor 594 NHS Ester (Succinimidyl Ester)	590/617	Red	5 mg	A20104
			25 mg	A37565
Alexa Fluor 594 Protein Labeling Kit	590/617	Red	1 kit	A10239
Alexa Fluor 594 Microscale Protein Labeling Ki	590/617	Red	1 kit	A30008
Alexa Fluor 594 Antibody Labeling Kit	590/617	Red	1 kit	A20185
APEX Alexa Fluor 594 Antibody Labeling Kit	590/617	Red	1 kit	A10474
BODIPY 630/650-X NHS Ester (Succinimidyl Ester)	625/640	Far red	5 mg	D10000
Alexa Fluor 633 NHS Ester (Succinimidyl Ester)	632/647	Far red	1 mg	A20005
			5 mg	A20105
Alexa Fluor 633 Protein Labeling Kit	632/647	Far red	5-rxn kit	A20170
BODIPY 650/665-X NHS Ester (Succinimidyl Ester)	646/660	Far red	5 mg	D10001
Alexa Fluor 647 NHS Ester (Succinimidyl Ester)	650/668	Far red	3 x 100 µg	A37573
			1 mg	A20006
			5 mg	A20106
			25 mg	A37566
Alexa Fluor 647 Protein Labeling Kit	650/668	Far red	1 kit	A20173
Alexa Fluor 647 Microscale Protein Labeling Kit	650/668	Far red	1 kit	A30009
Alexa Fluor 647 Antibody Labeling Kit	650/668	Far red	5-rxn kit	A20186
APEX Alexa Fluor 647 Antibody Labeling Kit	650/668	Far red	1 kit	A10475
Zip Alexa Fluor 647 Rapid Antibody Labeling Kit	650/668	Far red	1 kit	Z11235
Alexa Fluor 680 NHS Ester (Succinimidyl Ester)	679/702	Far red	3 x 100 µg	A37574
			1 mg	A20008
			5 mg	A20108
			25 mg	A37567
Alexa Fluor 680 Protein Labeling Kit	679/702	Far red	1 kit	A20172
Alexa Fluor 680 Antibody Labeling Kit	679/702	Far red	5-rxn kit	A20188
Alexa Fluor 700 NHS Ester (Succinimidyl Ester)	702/723	NIR	1 mg	A20010
			5 mg	A20110
Alexa Fluor 790 NHS Ester (Succinimidyl Ester)	702/723	NIR	100 µg	A30051
Alexa Fluor 790 Antibody Labeling Kit	702/723	NIR	5-rxn kit	A20189
Sulfhydryl-reactive				
Alexa Fluor 350 C_5 Maleimide	346/442	Blue	1 mg	A30505
BODIPY FL Maleimide (BODIPY FL *N*-(2-Aminoethyl))Maleimide)	410/455	Blue	1 mg	B10250
Fluorescein-5-Maleimide	505/513	Green	25 mg	F150
Alexa Fluor 488 C_5 Maleimide	494/518	Green	1 mg	A10254
Oregon Green 488 Maleimide	495/519	Green	1 mg	O6034
Alexa Fluor 532 C_5 Maleimide	495/524	Green	1 mg	A10255
Alexa Fluor 555 C_2 Maleimide	531/554	Green	1 mg	A20346
BODIPY TMR C_5 Maleimide	555/565	Orange	1 mg	B30466
Alexa Fluor 546 C_5 Maleimide	542/574	Orange/red	1 mg	A10258
Tetramethylrhodamine-5-Maleimide, single isomer	556/575	Orange/red	1 mg	T6027
Rhodamine Red C_2 Maleimide	555/580	Orange/red	1 mg	R6029
Alexa Fluor 568 C_5 Maleimide	570/590	Red	1 mg	A20341
Texas Red C_2 Maleimide	580/605	Red	1 mg	T6008
Alexa Fluor 594 C_5 Maleimide	595/615	Red	1 mg	A10256
Alexa Fluor 633 C_5 Maleimide	590/617	Red	1 mg	A20342
Alexa Fluor 647 C_2 Maleimide	632/647	Far red	1 mg	A20347
Alexa Fluor 680 C_2 Maleimide	650/668	Far red	1 mg	A20344
Alexa Fluor 750 C_5 Maleimide	679/702	NIR	1 mg	A30459

Product	Emission/excitation (nm)	Emission color	Quantity	Cat. No.
Carboxyl-reactive				
Alexa Fluor 350 Hydrazide	346/442	Blue	5 mg	A10439
Fluorescein-5-Thiosemicarbazide	494/518	Green	100 mg	F121
Fluorescein Cadaverine	494/518	Green	25 mg	A10466
Alexa Fluor 488 Hydrazide	495/519	Green	1 mg	A10436
Alexa Fluor 488 Hydroxylamine	495/519	Green	1 mg	A30629
Alexa Fluor 488 Cadaverine	495/519	Green	1 mg	A30676
Qdot 525 ITK Carboxyl Quantum Dots	405/525	Green	250 µL	Q21341MP
Qdot 545 ITK Carboxyl Quantum Dots	405/545	Yellow	250 µL	Q21391MP
Qdot 565 ITK Carboxyl Quantum Dots	405/565	Orange	250 µL	Q21331MP
Alexa Fluor 555 Hydrazide	555/565	Orange	1 mg	A20501MP
Alexa Fluor 555 Cadaverine	555/565	Orange/red	1 mg	A30677
5-TAMRA (5-Carboxytetramethylrhodamine), single isomer	555/580	Orange/red	10 mg	C6121
Qdot 585 ITK Carboxyl Quantum Dots	405/585	Red	250 µL	Q21311MP
Qdot 605 ITK Carboxyl Quantum Dots	405/605	Red	250 µL	Q21301MP
5-ROX (5-Carboxy-X-Rhodamine, Triethylammonium Salt), single isomer	580/605	Red	10 mg	C6124
Texas Red Hydrazide, >90% single isomer	595/615	Red	5 mg	T6256
Qdot 625 ITK Carboxyl Quantum Dots	405/625	Red	250 µL	A10200
Qdot 655 ITK Carboxyl Quantum Dots	405/655	Far red	250 µL	Q21321MP
Qdot 705 ITK Carboxyl Quantum Dots	405/705	NIR	250 µL	Q21361MP
Qdot 800 ITK Carboxyl Quantum Dots	405/800	NIR	250 µL	Q21371MP
Chemoselective				
SiteClick Antibody Azido Modification Kit	NA	NA	1 kit	S20026
Click-iT Alexa Fluor 488 sDIBO Alkyne for Antibody Labeling	494/518	Green	1 each	C20027
Alexa Fluor 488 Azide	494/518	Green	0.5 mg	A10266
Alexa Fluor 488 Alkyne	494/518	Green	0.5 mg	A10267
Click-iT Alexa Fluor 488 sDIBO Alkyne	494/518	Green	0.5 mg	C20020
Click-iT Plus Alexa Fluor 488 Picolyl Azide Toolkit	494/518	Green	1 kit	C10641
SiteClick Qdot 525 Antibody Labeling Kit	405/525	Green	1 kit	S10449
SiteClick Qdot 565 Antibody Labeling Kit	405/565	Orange	1 kit	S10450
Alexa Fluor 555 Azide	555/565	Orange/red	0.5 mg	A20012
Alexa Fluor 555 Alkyne	555/565	Orange/red	0.5 mg	A20013
Click-iT Alexa Fluor 555 sDIBO Alkyne	555/565	Orange/red	0.5 mg	C20021
Click-iT Alexa Fluor 555 sDIBO Alkyne for Antibody Labeling	555/565	Orange/red	1 each	C20028
Click-iT Plus Alexa Fluor 555 Picolyl Azide Toolkit	555/565	Orange/red	1 kit	C10642
SiteClick Qdot 585 Antibody Labeling Kit	405/585	Orange/red	1 kit	S10450
SiteClick R-PE Antibody Labeling Kit	565/578	Orange/red	1 kit	S10467
Tetramethylrhodamine (TAMRA) Azide	555/580	Red	0.5 mg	T10182
Tetramethylrhodamine (TAMRA) Alkyne	555/580	Red	0.5 mg	T10183
Click-iT pHrodo iFL Red sDIBO Alkyne for Antibody Labeling	566/590	Red	1 kit	C20034
SiteClick Qdot 605 Antibody Labeling Kit	405/605	Red	1 kit	S10469
Alexa Fluor 594 Azide	590/617	Red	0.5 mg	A10270
Alexa Fluor 594 Alkyne	405/625	Red	0.5 mg	A10275
SiteClick Qdot 655 Antibody Labeling Kit	405/655	Far red	1 kit	S10453
Alexa Fluor 647 Azide	650/668	Far red	0.5 mg	A10277
Alexa Fluor 647 Alkyne	650/668	Far red	0.5 mg	A10278
Click-iT Alexa Fluor 647 sDIBO Alkyne	650/668	Far red	0.5 mg	C20022
Click-iT Alexa Fluor 647 sDIBO Alkyne for Antibody Labeling	650/668	Far red	1 kit	C20029
Click-iT Plus Alexa Fluor 647 Picolyl Azide Toolkit	650/668	Far red	1 kit	C10643
SiteClick Qdot 705 Antibody Labeling Kit	405/705	NIR	1 kit	S10454
SiteClick Qdot 800 Antibody Labeling Kit	405/800	NIR	1 kit	S10455

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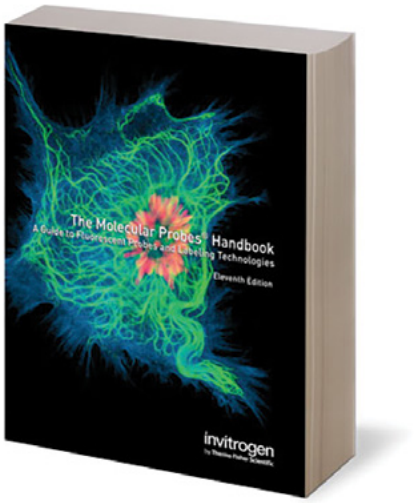

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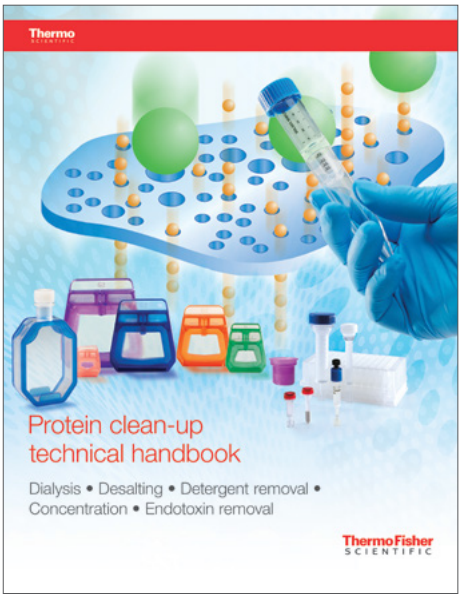

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