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Synthesis and semi-synthesis of selenopeptides and selenoproteins

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Abstract

The versatile chemistry of the genetically encoded amino acid selenocysteine (Sec) is employed in Nature to expand the reactivity of enzymes. In addition to its role in biology, Sec is used in protein engineering to modify folding, stability, and reactivity of proteins, to introduce conjugations and to facilitate reactions. However, due to limitations related to Sec's insertion mechanism in Nature, much of the production of Sec containing peptides and proteins relies on synthesis and semisynthesis. Here, we review recent advances that have enabled the assembly of complicated selenoproteins, including novel uses of protecting groups for solid phase peptide synthesis, rapid selenoester driven chemical ligations and versatile expressed protein ligations.

Graphical abstract

Introduction

Selenium is used in Nature in nucleic acids, sugars, small compounds and proteins, but its most significant utilization is in the form of the genetically encoded amino acid selenocysteine (Sec). Sec and Cys share many physiochemical properties, but Sec is a better nucleophile and electrophile because of selenium's electronic structure.[1,2] Another marked difference to their sulfur containing counterparts is the much lower redox potential of selenocompounds.[1,3] Thus, Sec and Cys can not only play similar roles in proteins, but because Sec is more reactive it broadens the range of chemical reactions that are facilitated by enzymes.[1,2] Accordingly, the rapidly growing interest in proteins and peptides

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containing selenium originates from two overlapping areas: Firstly, the study of selenoproteins in their own right, as proteins with unique and specialized cellular roles[4], and secondly from protein engineering for which Sec's particular properties offer unique chemical approaches towards the production, manipulation, and augmentation of macromolecules.

For such protein engineering applications, it is particularly beneficial that Sec, due to its specific chemistry, can be manipulated in ways that leave any of the 20 canonical amino acids unaffected. In that sense Sec is often considered a "hetero amino acid" in the protein and its high reactivity can be exploited to diversify and control peptide and protein chemistry (Fig. 1).[5] For example, Sec can be employed directly for site-specific protein labeling and conjugation[6] or it can be selectively converted into dehydroalanine[7] - an electrophilic handle for natural post translational modification or other bioconjugations.[8,9] In addition, selenoproteins and selenopeptides have been used to probe the kinetics and dynamics of protein's oxidative folding,[10] as well as to alter chemical properties of proteins.[11] Finally, ^{77}Se -Sec is utilized to study proteins by NMR spectroscopy.[12] The interested reader can find more detailed discussions of selenoproteins and selenopeptides and their fascinating role in biology, biochemistry and protein engineering in references[4,13,14] and[3,15].

Despite the great promise that Sec offers for protein engineering, its full potential has yet to be unlocked. One of the major challenges is the peculiar bioincorporation of Sec in Nature. Unlike the 20 canonical amino acids, which are genetically encoded by unique codons, Sec relies on dedicated ancillary proteins that enable the alternative use of the stop codon UGA for translational incorporation into the polypeptide chain.[16,17] Unfortunately, termination of transcription is thus a common side reaction when overexpressing an organism's native Sec-incorporation machinery. To overcome this limitation, novel approaches have been recently developed in which Sec-specific tRNA was engineered and the native selenium incorporation path was altered.[18–25] Despite the promise of such approaches, obtaining high yield of protein can be challenging due to low efficiency and fidelity of Sec loading. Thus, this technique is not yet routinely employed for preparation of selenopeptides or selenoproteins.[26]

Nevertheless, in recent years the reliable production of complex selenopeptides and selenoproteins has been achieved by several methods, that combine automated solid phase peptide synthesis (SPPS), recombinant expression of fragments, and native chemical ligation (NCL). The latter, is a widely used synthetic method that relies on an amide-forming reaction to generate proteins from their respective fragments. Sec-mediated NCL reactions take place between a N-terminal peptide fragment with a C-terminal reactive thioester (or, as discussed below, now selenoester), and a C-terminal peptide fragment containing Sec at the N-terminus. The nucleophilic Sec attacks the N-terminal fragment and forms a transient thioester between the two fragments, which then rapidly forms the peptide bond through an intramolecular acyl Se→N shift.[27–30] Relative to Cys-mediated NCL, Sec-mediated ligations exhibit faster kinetics and are compatible with a wider pH range. Sec's high nucleophilicity facilitates fast reaction rates and its low pK_a allows for ligations at low pH to minimize undesired side reactions such as thioester hydrolysis.[29] Furthermore, following

the reaction Sec can be selectively converted into the abundant amino acids Ala or Ser under mild conditions. Thus, Sec offers more choices for the ligation site.[31,32]

Numerous articles and book chapters have surveyed the different approaches for preparing selenoproteins.[5,15,26,33,34] Here, we review recent advances in the field of synthetic and semi-synthetic preparation of selenopeptides and selenoproteins. We highlight new methods that either ease the preparation or increase the complexity of proteins of interest.

Enzymatic incorporation of Sec at the N-terminus of protein fragments

Foregoing the complicated native bioincorporation of Sec, Patterson and coworkers introduced Sec enzymatically by *E. coli* aminoacyl transferase (AaT) at the N-termini of protein fragments.[35] Normally, AaT recognizes an N-terminal Arg or Lys on the protein fragment and inserts a Leu or Phe beforehand. To hijack this mechanism for Sec incorporation, the Patterson group synthesized a Sec-adenosine analog accepted by AaT as substrate. AaT incorporated Sec successfully into both peptides and expressed protein fragments, as long as the N-terminal residue was Arg or Lys. This enzymatic conversion approach has increased the options available for generating the desired protein fragments for Sec-mediated NCL to build complex proteins.

Sequential ligations of multi-fragments using selenozolidine

Complex proteins can be produced using consecutive NCLs of several peptides. The need for several separate reactions leads to a decline in yield. In such cases, the more efficient Sec-mediated reactions are advantageous. The major challenge for multiple Sec-NCL is to avoid self-ligation. This can be done using appropriate protection and deprotection of either the Sec (C to N ligation) or the thioester surrogate (N to C ligation). In the past, p-methoxybenzyl (PMB) was frequently chosen as the Sec protection group [36]. However, its use required harsh reaction conditions or excess thiols which need to be removed before the next ligation step can occur. The Hondal group introduced treatment with ascorbate for milder deprotection of Sec residues.[37] Recently, Metanis and coworkers have introduced selenozolidine (Sez) as a new protecting group that greatly benefits multiple ligation schemes because it can be unmasked to reveal Sec using MeONH₂ with high efficiency under mild conditions (Fig. 2).[38] Additionally, Sez has proven to be a good choice as a general protection group during Sec peptide synthesis.[39]

The authors demonstrated their method by producing the human enzyme phosphohistidine phosphatase 1 (PHPT1) via two sequential ligations followed by a deselenization step. The Sez approach was also used in the total chemical synthesis of human selenoprotein W (SELENOW) and selenoprotein M (SELENOM). The SELENOM synthesis (Figure 2) used three C to N Sec-mediated NCLs.[40]

Improving the kinetics and efficiency of amide forming reactions: Sec-selenoester ligations

Sec-mediated reactions are highly efficient, however to form the reactive selenolate, the diselenide bond (Se-Se) between Sec-fragments needs to be broken first. While this requires the presence of a strong reducing reagent (the redox potential of selenocystine is –381

mV[41]), its choice remains challenging. Strong reducing agents, such as phosphines, can lead to selenium deselenization and the formation of undesired side products causing a decline in overall yield. However, weaker reducing reagents, such as dithiothreitol, are inefficient and thus decrease the generation of the selenolate. This slows down the reaction, permits the hydrolysis of the thioester and once again affects efficiency and yield.

The Payne group resolved this bottleneck by forgoing the use of reducing agents altogether. This is made possible by ligating the Sec-containing fragment to a selenoester (instead of thioester) fragment, because the diselenide bond directly takes part in the reaction (Figure 3).[42] In these diselenide-selenoester ligations peptides containing an N-terminal selenocystine are ligated to peptides with C-terminal aryl selenoesters. The resulting reaction rates are significantly faster (within minutes) than those for conventional Sec-thioester ligations and overall yield is greatly increased. This paved the way for the construction of complex proteins via 'one-pot synthesis' of multi-step ligations of peptides. In such reactions, the different steps can be carried out in one vessel without the need to purify reagents between successive ligations. This reduces both the cost and time of preparing complex proteins via multi-step assembly and has enabled NCL to generate complex, multi-domain, and large proteins. Notable examples of one-pot reactions include early secretory antigenic protein-6 (ESAT-6) made from three fragments [42] and superoxide dismutase-1 (SOD-1) made from four fragments.[43]

Capitalizing on the rapid reaction between selenoester and diselenides peptides, Payne and colleagues developed an additional way of placing Sec in peptides and proteins. They introduced the amino-acid derivatives β -selenoaspartate and γ -selenoglutamate at the N-terminus, which are utilized for rapid diselenide-selenoester ligations. The β or γ positioned Se can be deselenized quickly and selectively, even in the presence of an unprotected Sec. [44] The ability to use the rapid kinetics of selenium-driven ligations, while leaving Sec at selected positions unperturbed, is useful for a wide range of applications, including the preparation of native selenoproteins or augmentation of proteins with post-translational or other modifications.[45] To demonstrate the generality of the method the group prepared the membrane enzyme selenoprotein K, which contains a native diselenide bond,[46] and the thrombin inhibiting hyalomins.[44]

As diselenide-selenoester ligations gain popularity, ways by which selenoesters are incorporated into peptides are also evolving and several such methods have recently been reported.[47,48] The aryl selenoesters were found to react with N-terminal primary amines without catalysts, thus controlling the order in which multi-step ligations proceed.[43] In addition, bis(2-selenylethyl)amido (SeEA) was shown to facilitate Sec-mediated ligations but offers higher stability and increased ease of preparation.[49]

Expressed Sec-mediated protein ligation

So far, the exploitation of Sec's high nucleophilicity in NCL has been restricted to selenopeptides synthesized by SPPS. However, not all peptides can be prepared effectively through solid phase peptide synthesis as some may start aggregating on the resin, while others suffer from limited solubility which interferes with later steps. Even if synthesis of a selenopeptide is viable, commercially available, protected Sec remains expensive and overall

production costs can become prohibitive as peptide length increases. Custom synthesis of protected Sec derivatives on the other hand requires careful handling to avoid toxic byproducts. This renders Sec-ligations, although powerful, still not routine for the non-expert.

To bypass these obstacles associated with SPPS of selenium containing peptides, our group recently introduced an expressed protein ligation (EPL) method in which Sec is incorporated in recombinant protein fragments (Fig. 4).[7,26] This biochemistry friendly and inexpensive method relies solely on expression of the protein fragments in bacteria that take up selenite or selenocystine from the growth medium. To overcome possible solubility and aggregation problems of the fragments, the sequence of interest is expressed as fusion protein with a soluble protein partner such as the maltose binding protein (MBP). This lifts the constraints on the size and chemical properties of the Sec-containing segment and makes it possible to utilize a diverse set of peptides with variable physiochemical properties. Just prior to ligation, the selenol-fragment is released by a protease from its fusion partner and then immediately reacts with the thioester fragment in a one-pot reaction. These ligations reactions can be carried out under both native and denaturing conditions. The former allowing the proteins to refold immediately after the ligation and to gain native function. In addition, Sec-mediated EPL reduces the use of harsh chemicals and by forgoing synthesis it requires no familiarity with selenium chemistry thus alleviates potential toxicity. Thus, Sec-mediated EPL is not only cheaper and faster but also greener.

Nevertheless, while our method expands the type of fragments now amenable for NCL and EPL, in its current form it cannot accommodate Cys in the Sec-containing fragment because all Cys are replaced by Sec. For cases where the final product requires multiple Sec and Cys, we envision a multi-step EPL strategy. The challenge lies in generating a thioester from the appropriate fragment for the second ligation step. Solutions for this challenge may emerge from enzymatic conversion or through variations of hydrazide-derived thioester formation using intein technology.[50,51]

To demonstrate the abilities of our method, we prepared thioredoxin, SELENOW, as well as SELENOM.[7,26] The latter was prepared in a one-step ligation instead of the three steps needed when SPPS-derived peptides are used.[40] In addition, we demonstrated the ability to selectively deselenize Sec in the presence of other Cys residues to form dehydroalanine (DHA) using bis-alkylation-elimination by α,α' -dibromo-adipyl(bis)-amide (DBAA). This allows the site-specific introduction of dehydroalanine which, as mentioned earlier, is used for radical initiated carbon-carbon bond formation and thus an entry point for radical initiated incorporation of post translational and other modifications.[8,9]

Conclusions and perspective

Sec-mediated ligation is now the leading option for both sequential and one-pot multiple step ligations. Along the way, milder protection and deprotection schemes in SPPS simplify preparation of fragments[15] and the transition from thioesters to selenoesters resolved much of the previous problem of maintaining seleno-fragments reduced and primed for attack. The benefits of diselenide-selenoester ligations will also be advantageous for

ligations with other selenol-derivatives of natural amino acids such as selenoproline and selenophenylalanine.[52,53] Finally, Sec-mediated EPL expands the repertoire and solubility of fragments that can be produced for ligations. It would be highly beneficial to merge the Sec-mediated EPL and the diselenide-selenoester ligation by finding a biochemical, i.e. enzymatic or recombinant approach, to prepare selenoester. In addition, as advances in synthetic biology and reprogramed cell lines assist in harnessing biology for chemistry, and vice versa, the near future may already see complex selenol-fragments for EPL that are prepared in high yields by engineered Sec insertion pathways.

While the focus has so far been primarily on Sec for the preparation of seleno-peptides and proteins, there is also ample room for exploration of other seleno- amino acids to diversify chemistry in proteins. Particularly exciting is the potential of selenomethionine for conjugations. It introduces yet another “hetero amino acid” with minimal perturbation to structure and function but with the capability for site-specific protein modifications.[54] Overall, as Sec-mediated ligations and conjugations become simpler, greener and more popular, native and non-native selenoproteins are transitioning from niche to chemistry mainstream.

Acknowledgments

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References and recommended reading

- of special interest
 - of outstanding interest
1. Reich HJ, Hondal RJ. Why Nature chose selenium. *ACS Chem Biol.* 2016; 11:821–841. [PubMed: 26949981]
 2. Arner ES. Selenoproteins-what unique properties can arise with selenocysteine in place of cysteine? *Exp Cell Res.* 2010; 316:1296–1303. [PubMed: 20206159]
 3. Hondal RJ, Marino SM, Gladyshev VN. Selenocysteine in thiol/disulfide-like exchange reactions. *Antioxid Redox Signal.* 2013; 18:1675–1689. [PubMed: 23121622]
 4. Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: molecular pathways and physiological roles. *Physiol Rev.* 2014; 94:739–777. [PubMed: 24987004]
 5. Metanis N, Hilvert D. Natural and synthetic selenoproteins. *Curr Opin Chem Biol.* 2014; 22:27–34. [PubMed: 25261915]
 6. Hofer T, Thomas JD, Burke TR Jr, Rader C. An engineered selenocysteine defines a unique class of antibody derivatives. *Proc Natl Acad Sci U S A.* 2008; 105:12451–12456. [PubMed: 18719095]
 7. Liu J, Chen Q, Rozovsky S. Utilizing selenocysteine for expressed protein ligation and bioconjugations. *J Am Chem Soc.* 2017; 139:3430–3437. [PubMed: 28186733]
 8. Wright TH, Bower BJ, Chalker JM, Bernardes GJ, Wiewiora R, Ng WL, Raj R, Faulkner S, Vallee MR, Phanumartwiwath A, et al. Posttranslational mutagenesis: A chemical strategy for exploring protein side-chain diversity. *Science.* 2016; 354
 9. Yang A, Ha S, Ahn J, Kim R, Kim S, Lee Y, Kim J, Soll D, Lee HY, Park HS. A chemical biology route to site-specific authentic protein modifications. *Science.* 2016; 354:623–626. [PubMed: 27708052]
 10. Metanis N, Hilvert D. Harnessing selenocysteine reactivity for oxidative protein folding. *Chem Sci.* 2015; 6:322–325. [PubMed: 28757941]

11. Arai K, Takei T, Okumura M, Watanabe S, Amagai Y, Asahina Y, Moroder L, Hojo H, Inaba K, Iwaoka M. Preparation of selenoinsulin as a long-lasting insulin analogue. *Angew Chem Int Ed Engl.* 2017; 56:5522–5526. [PubMed: 28394477]
12. Liu J, Rozovsky S. ⁷⁷Se NMR spectroscopy of selenoproteins. In: Hatfield LD, Schweizer U, Tsuji AP, Gladyshev NV, editors *Selenium: Its Molecular Biology and Role in Human Health*. Springer International Publishing; 2016. 187–198.
13. Hatfield DL, Tsuji PA, Carlson BA, Gladyshev VN. Selenium and selenocysteine: roles in cancer, health, and development. *Trends Biochem Sci.* 2014; 39:112–120. [PubMed: 24485058]
14. Liu J, Rozovsky S. Membrane-bound selenoproteins. *Antioxid Redox Signal.* 2015; 23:795–813. [PubMed: 26168272]
15. Mousa R, Notis Dardashti R, Metanis N. Selenium and selenocysteine in protein chemistry. *Angew Chem Int Ed Engl.* 2017; 56:15818–15827. [PubMed: 28857389]
16. Rother M. Prokaryotic Selenoprotein Biosynthesis and Function. In: Hatfield DL, Schweizer U, Tsuji PA, Gladyshev VN, editors *Selenium: Its Molecular Biology and Role in Human Health*. Springer International Publishing; 2016. 47–58.
17. Gladyshev VN, Carlson BA, Hatfield DL. Pathways in de novo biosynthesis of selenocysteine and cysteine in eukaryotes. In: Hatfield DL, Schweizer U, Tsuji PA, Gladyshev VN, editors *Selenium: Its Molecular Biology and Role in Human Health*. Springer International Publishing; 2016. 39–45.
18. Cheng Q, Arner ES. Selenocysteine insertion at a predefined UAG codon in a release factor 1 (RF1)-depleted *Escherichia coli* host strain bypasses species barriers in recombinant selenoprotein translation. *J Biol Chem.* 2017; 292:5476–5487. [PubMed: 28193838]
19. Miller C, Brocker MJ, Prat L, Ip K, Chirathivat N, Feiock A, Veszpremi M, Soll D. A synthetic tRNA for EF-Tu mediated selenocysteine incorporation in vivo and in vitro. *FEBS Lett.* 2015; 589:2194–2199. [PubMed: 26160755]
20. Arner ES, Sarioglu H, Lottspeich F, Holmgren A, Bock A. High-level expression in *Escherichia coli* of selenocysteine-containing rat thioredoxin reductase utilizing gene fusions with engineered bacterial-type SECIS elements and co-expression with the selA, selB and selC genes. *J Mol Biol.* 1999; 292:1003–1016. [PubMed: 10512699]
21. Thyer R, Filipovska A, Rackham O. Engineered rRNA enhances the efficiency of selenocysteine incorporation during translation. *J Am Chem Soc.* 2013; 135:2–5. [PubMed: 23256865]
22. Fan Z, Song J, Guan T, Lv X, Wei J. Efficient expression of glutathione peroxidase with chimeric tRNA in amber-less *Escherichia coli*. *ACS Synth Biol.* 2018; 7:249–257. [PubMed: 28866886]
23. Aldag C, Brocker MJ, Hohn MJ, Prat L, Hammond G, Plummer A, Soll D. Rewiring translation for elongation factor Tu-dependent selenocysteine incorporation. *Angew Chem Int Ed Engl.* 2013; 52:1441–1445. [PubMed: 23193031]
24. Thyer R, Robotham SA, Brodbelt JS, Ellington AD. Evolving tRNA(Sec) for efficient canonical incorporation of selenocysteine. *J Am Chem Soc.* 2015; 137:46–49. [PubMed: 25521771]
25. Rakauskaitė R, Urbanaviciute G, Ruksenaite A, Liutkeviciute Z, Juskenas R, Masevicius V, Klimasauskas S. Biosynthetic selenoproteins with genetically-encoded photocaged selenocysteines. *Chem Commun (Camb).* 2015; 51:8245–8248. [PubMed: 25679146]
26. Liu J, Chen Q, Rozovsky S. Selenocysteine-mediated expressed protein ligation of SELENOM. *Methods Mol Biol.* 2018; 1661:265–283. [PubMed: 28917051]
27. Dawson P, Muir T, Clark-Lewis I, Kent S. Synthesis of proteins by native chemical ligation. *Science.* 1994; 266:776–779. [PubMed: 7973629]
28. Quaderer R, Sewing A, Hilvert D. Selenocysteine-mediated native chemical ligation. *Helvetica Chimica Acta.* 2001; 84:1197–1206.
29. Hondal RJ, Nilsson BL, Raines RT. Selenocysteine in native chemical ligation and expressed protein ligation. *J Am Chem Soc.* 2001; 123:5140–5141. [PubMed: 11457362]
30. Gieselman MD, Xie L, van Der Donk WA. Synthesis of a selenocysteine-containing peptide by native chemical ligation. *Org Lett.* 2001; 3:1331–1334. [PubMed: 11348227]
31. Dery S, Reddy PS, Dery L, Mousa R, Dardashti RN, Metanis N. Insights into the deselenization of selenocysteine into alanine and serine. *Chem Sci.* 2015; 6:6207–6212. A mechanistic study of deselenization of Sec to alanine and serine under anaerobic and aerobic conditions. This provides

the ability to transform the site of ligation in Sec-mediated NCL into those two common amino acids and thus render it traceless. [PubMed: 30090236]

- 32•. Malins LR, Mitchell NJ, McGowan S, Payne RJ. Oxidative deselenization of selenocysteine: applications for programmed ligation at serine. *Angew Chem Int Ed Engl.* 2015; 54:12716–12721. Oxidative deselenization of Sec to serine by phosphines. Published at the same time as reference 31, the work provides a route for deselenization of Sec after NCL, turning it into the common amino acid serine. [PubMed: 26384718]
33. Malins LR, Mitchell NJ, Payne RJ. Peptide ligation chemistry at selenol amino acids. *J Pept Sci.* 2014; 20:64–77. [PubMed: 24285588]
34. Mousa R, Reddy PS, Metanis N. Chemical protein synthesis through selenocysteine chemistry. *Synlett.* 2017; 28:1389–1393.
- 35•. Wang YJ, Szantai-Kis DM, Petersson EJ. Chemoselective modifications for the traceless ligation of thioamide-containing peptides and proteins. *Org Biomol Chem.* 2016; 14:6262–6269. A proof of concept that Sec can be added to peptides via an enzymatic insertion before Lys and Arg. This suggests that evolution of different enzymes to recognize selenocompounds for peptide modification presents a general path to straight forward selenofragment preparation. [PubMed: 27264841]
36. Harris KM, Flemer S Jr, Hondal RJ. Studies on deprotection of cysteine and selenocysteine side-chain protecting groups. *J Pept Sci.* 2007; 13:81–93. [PubMed: 17031870]
37. Ste Marie EJ, Ruggles EL, Hondal RJ. Removal of the 5-nitro-2-pyridine-sulfonyl protecting group from selenocysteine and cysteine by ascorbolysis. *J Pept Sci.* 2016; 22:571–576. [PubMed: 27480992]
38. Reddy PS, Dery S, Metanis N. Chemical synthesis of proteins with non-strategically placed cysteines using selenazolidine and selective deselenization. *Angew Chem Int Ed Engl.* 2016; 55:992–995. [PubMed: 26636774]
39. Whedon SD, Markandeya N, Rana ASJB, Senger NA, Weller CE, Turek F, Strieter ER, Chatterjee C. Selenocysteine as a latent bioorthogonal electrophilic probe for deubiquitylating enzymes. *J Am Chem Soc.* 2016; 138:13774–13777.
- 40•. Dery L, Reddy PS, Dery S, Mousa R, Ktorza O, Talhami A, Metanis N. Accessing human selenoproteins through chemical protein synthesis. *Chem Sci.* 2017; 8:1922–1926. In this work selenazolidine was used as Sec's protecting group for both peptide synthesis and directed NCL multiple ligations. The method was utilized to prepare two native selenoproteins. [PubMed: 28451306]
41. Besse D, Siedler F, Diercks T, Kessler H, Moroder L. The redox potential of selenocystine in unconstrained cyclic peptides. *Angew Chem Int Ed Engl.* 1997; 36:883–885.
- 42•. Mitchell NJ, Malins LR, Liu X, Thompson RE, Chan B, Radom L, Payne RJ. Rapid additive-free selenocystine-selenoester peptide ligation. *J Am Chem Soc.* 2015; 137:14011–14014. A seminal study that established additive free diselenide-selenoester ligation. This method achieves rapid ligations. The reactions require no additives, thus minimizing side reactions. Furthermore, it enables ligations at sterically hindered junctions. This method is rapidly becoming the preferred path for Sec-mediated ligations. [PubMed: 26487084]
- 43•. Takei T, Andoh T, Takao T, Hojo H. One-pot four-segment ligation using seleno- and thioesters: synthesis of superoxide dismutase. *Angew Chem Int Ed Engl.* 2017; 56:15708–15711. This work exploits aryl selenoester's directly reaction with primary terminal amines to prepare superoxide dismutase from four fragments in a one pot reaction. This demonstrates that complex and multidomain proteins can be produced by designing sequential reactions that liberate the assembly from purifications between the individual steps. [PubMed: 29048715]
- 44•. Mitchell NJ, Sayers J, Kulkarni SS, Clayton D, Goldys AM, Ripoll-Rozada J, Barbosa Pereira PJ, Chan B, Radom L, Payne RJ. Accelerated protein synthesis via one-pot ligation-deselenization chemistry. *Chem.* 2017; 2:703–715. Introduces diselenide-selenoester ligations using β -selenoaspartate or γ -selenoglutamate and demonstrates their rapid selective deselenization in the presence of unprotected Sec. Thus, this route exploits selenium's power in rapid ligations while leaving Secs within the protein untouched.

45. Mitchell NJ, Kulkarni SS, Malins LR, Wang S, Payne RJ. One-pot ligation-oxidative deselenization at selenocysteine and selenocystine. *Chemistry*. 2017; 23:946–952. [PubMed: 27859731]
46. Liu J, Zhang Z, Rozovsky S. Selenoprotein K form an intermolecular diselenide bond with unusually high redox potential. *FEBS Lett*. 2014; 588:3311–3321. [PubMed: 25117454]
47. Hanna CC, Kulkarni SS, Watson EE, Premdjee B, Payne RJ. Solid-phase synthesis of peptide selenoesters via a side-chain anchoring strategy. *Chem Commun*. 2017; 53:5424–5427.
48. Temperini A, Piazzolla F, Minuti L, Curini M, Siciliano C. General, mild, and metal-free synthesis of phenyl selenoesters from anhydrides and their use in peptide synthesis. *J Org Chem*. 2017; 82:4588–4603. [PubMed: 28414443]
- 49••. Raibaut L, Cargoet M, Ollivier N, Chang YM, Drobecq H, Boll E, Desmet R, Monbaliu JM, Melnyk O. Accelerating chemoselective peptide bond formation using bis(2-selenylethyl)amido peptide selenoester surrogates. *Chem Sci*. 2016; 7:2657–2665. The work shows that bis (2-selenylethyl)amido (SeEA) is an alternative to selenoester for rapid ligation reactions that is easier to synthesis and more stable. The method was used for a one-pot assembly of three peptides that exploited the large reactivity difference between SeEA and SEA to control reaction kinetics. [PubMed: 28660038]
50. Fang GM, Wang JX, Liu L. Convergent chemical synthesis of proteins by ligation of peptide hydrazides. *Angew Chem Int Ed Engl*. 2012; 51:10347–10350. [PubMed: 22968928]
51. Thom J, Anderson D, McGregor J, Cotton G. Recombinant protein hydrazides: application to site-specific protein PEGylation. *Bioconjug Chem*. 2011; 22:1017–1020. [PubMed: 21618993]
52. Townsend SD, Tan Z, Dong S, Shang S, Brailsford JA, Danishefsky SJ. Advances in proline ligation. *J Am Chem Soc*. 2012; 134:3912–3916. [PubMed: 22332757]
53. Malins LR, Payne RJ. Synthesis and utility of beta-selenol-phenylalanine for native chemical ligation-deselenization chemistry. *Org Lett*. 2012; 14:3142–3145. [PubMed: 22642500]
54. Dardashti RN, Metanis N. Revisiting ligation at selenomethionine: Insights into native chemical ligation at selenocysteine and homoselenocysteine. *Bioorg Med Chem*. 2017; 25:4983–4989. [PubMed: 28526476]

Highlights

- When selenoester is used for ligation reactions with diselenides, kinetics are accelerated and side reactions are minimized.
- Selenozolidine is emerging as the preferred selenocysteine protecting group for solid phase synthesis of peptides and their subsequent ligations.
- Using recombinant seleno-fragments in expressed protein ligation increases its scope and versatility.
- Selenocysteine-driven native chemical ligation can now be used effectively to create complex proteins by multi-fragment ligation.

Figure 1.

Selenopeptides and Selenoproteins: Their synthesis, semi-synthesis and applications. (A) Fragments with N-terminal Sec are most commonly prepared either by solid phase peptide synthesis (right) or by growing bacteria with selenocystine in the growth medium (left). In standard native chemical ligation reactions, an amide bond is formed between fragments containing a selenolate and a thioester, respectively. (B) Selenopeptides and selenoproteins can be utilized for a wide and diverse range of applications.

Figure 2.

The three steps synthesis of SELENOM exemplifies the use of the Sez protecting group for Sec-mediated NCL. (Adapted from reference[40]).

Figure 3.

Principle of Sec-selenoester ligations. (Adapted from references [42,44]).

A. Conventional Sec ligation takes place between a fragment containing thioester and fragment with an N-terminal Sec. The latter dimerizes forming a diselenide bond and strong reductant is necessary to break it and to generate the selenolate to initiate the reaction. Adduct may lead to deselenization, lowering the yield and creating side products.

B. In contrast, aryl-selenoesters directly attack the diselenide bond to generate the selenolate for the ligation reaction, thus removing the need for additives. These reactions are significantly faster, cleaner and result in a higher yield.

C. Also other, non-natural, selenium-containing amino acids can be employed for ligations. This allows a free Sec to remain in the protein after any trace of the ligation is removed by deselenization of the ligation site.

Figure 4.

Principle of Sec-mediated expressed protein ligation. (A) The seleno-fragment of interest is expressed in *E. coli* as a fusion with maltose binding protein in selenocysteine enriched medium. The complementary thioester protein fragment is prepared by established intein technology. Immediately prior to the ligation with the thioester-fragment, the seleno-fragment is cleaved from maltose binding protein by tobacco etch virus protease.[7] The selenolate attacks the thioester and the native protein is spontaneously generated. (B) Scheme illustrating the construction of SELENOM by a one-step Sec-mediated expressed protein ligation.