Staudinger Ligation Method for Rapid and Reliable Chemical Synthesis of Proteins

**INVENTORS** • Ronald Raines, Laura Kiessling, Bradley Nilsson

**THE WARF ADVANTAGE**

Since its founding in 1925 as the patenting and licensing organization for the University of Wisconsin-Madison, WARF has been working with business and industry to transform university research into products that benefit society. WARF intellectual property managers and licensing staff members are leaders in the field of university-based technology transfer. They are familiar with the intricacies of patenting, have worked with researchers in relevant disciplines, understand industries and markets, and have negotiated innovative licensing strategies to meet the individual needs of business clients.

**OVERVIEW**

Researchers and drug development companies have traditionally obtained proteins by using recombinant DNA technology to express them in genetically engineered organisms. However, this approach can take years to produce a given protein. In addition, the proteins may not work correctly due to improper folding or biological contamination, or may not be produced in sufficient quantities.

An alternative is the total chemical synthesis of proteins. This approach is much faster, taking only days or weeks. It produces homogeneous, correctly folded and biologically active proteins with nearly 100 percent reliability.

However, current methods of chemically synthesizing proteins have major limitations. In particular, native chemical ligation and expressed protein ligation require the presence of a cysteine residue at the ligation junction. But few peptides naturally have a cysteine at this junction, and adding a cysteine residue is often undesirable because cysteine reacts readily with disulfide bonds, O2 and other electrophiles.

**THE INVENTION**

UW-Madison researchers have developed an improved method of synthesizing proteins that does not require a cysteine residue at the ligation junction. This method expands the utility of total protein synthesis by removing the limitation inherent in native chemical ligation and expressed protein ligation. It is inspired by the Staudinger reaction, in which a phosphine molecule is used to reduce an azide to an amine.

In this method, a phosphinothioester and an azide are united to form an amide bond. A phosphinothiol reagent is used to efficiently generate a phosphinothioester from an amino acid, peptide or protein fragment. An azido group then can be formed at the N-terminus or a basic side group of another amino acid, peptide or protein. Then the phosphinothioester is reacted with the azido group to ligate the amino acids, peptides or proteins.
proteins.

This reaction allows the formation of an amide bond among a wide variety of chemical species. It can be used to repeatedly ligate natural and/or nonnatural amino acids to synthesize proteins or peptides. Large peptides or proteins can be formed by ligating two or more small peptides or proteins.

**APPLICATIONS**

- Total chemical synthesis of proteins, which provides a rapid, reliable route to homogeneous, correctly folded proteins with full biological activity
- Ligation of biological molecules, including amino acids, peptides and protein fragments
- Synthesis of peptides and proteins, including the stepwise synthesis of peptides on solid supports

**KEY BENEFITS**

- Eliminates need for cysteine residue at the ligation junction
- Traceless - leaves no residual atoms in the ligated peptide product
- Allows the formation of an amide bond between a wide variety of chemical species

**ADDITIONAL INFORMATION**

**Related Technologies**
The inventors have expanded their original technology by

- Improving their methods of synthesizing phosphinothiol reagents for use in Staudinger ligation (see WARF reference number P02304US).
- Developing a method of performing traceless Staudinger ligation in water (see WARF reference number P08350US02).
- Developing a molecule with two functional groups in which one of the functional groups is an azide that reacts well with the phosphinothioesters used in Staudinger ligation (see WARF reference number P04016US).

**Tech Fields**
Research Tools - Synthesis & purification
Materials & Chemicals - Synthesis

**CONTACT INFORMATION**

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854.