# JoVE: Science Education

## Solid Phase Synthesis

--Manuscript Draft--

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**Science Education Title**: Solid Phase Synthesis

## Overview:

Merrifield's solid-phase synthesis is a Nobel Prize winning invention where a reactant molecule is bound on a solid support and undergoes successive chemical reactions to form a desired compound. When the molecules are bound to a solid support, excess reagents and byproducts can be removed by washing away the impurities, while the target compound remains bound to the resin. Specifically, we will showcase an example of solid-phase peptide synthesis (SPPS) to demonstrate this concept.

## Principles:

Solid-phase synthesis is a method used to streamline the synthesis of molecules. It is often used in combinatorial chemistry, a technique used to prepare a large number of molecules in a short period of time, to generate libraries of compounds due to the ease of purification and overall chemical synthesis. Solid-phase synthesis typically involves the use of a resin; a non-soluble, polymer-based material which is prefunctionalized so the starting building block can easily bind. The building blocks are generally protected once they are added onto the resin and they can be easily deprotected and treated with the next desired building block in solution (**Figure 1**). Once the desired molecule has been synthesized, it can easily be cleaved from the resin.

Because it is robust, solid-phase synthesis has been used to synthesize nucleic acids, oligosaccharides, and most commonly, peptides. Discovered and reported by Robert Bruce Merrifield in 1963, solid-phase peptide synthesis (SPPS) has become the most widely used method to generate libraries of peptides. Merrifield won the 1984 Nobel Prize for the invention of SPPS. SPPS can easily take advantage of Fmoc (base sensitive) or Boc (acid sensitive) *N*-protecting groups on the amino acids to build up libraries of peptides in a short amount of time. HBTU (coupling agent) and *i*-$Pr_2EtN$ (base) activate the *C*-terminus of the amino acid for coupling with another amino acid. Fmoc protecting groups can be removed by 4-methylpiperidine, while Boc protecting groups can be removed by strong acids such as trifluoroacetic acid. In this experiment, we will demonstrate SPPS through the synthesis of a dipeptide. We will use the Kaiser test, a qualitative method to test for the presence of primary amines, to monitor the progress of the reaction.

## Procedure:

1. Loading the Resin

    1.1. To a 100mL peptide synthesis vessel ~~manual SPPS synthesizer~~, add 2-chlorotrityl chloride (CTC) resin (1.1 mmol/g, 0.360 g, 0.400 mmol). Add 20 mL DMF and allow them to swell for 30 min under $N_2$. ~~(20 mL) was added and the beads swelled for 30 min under $N_2$.~~

**Commented [ASW1]:** There's a lot of information in this procedure that has to be inferred by the reader/ viewer. There is no explanation of the various reagents, what their role is in the synthesis, and some discrepancies between Figure 1 and the reagents used. Provide more information about the steps of SPPS.

**Commented [K2]:** I have changed Figure 1 to reflect the reaction conditions.

**Commented [ASW3]:** What is this?

**Commented [ASW4]:** How do these work?

**Commented [ASW5]:** This should be written in the present-tense imperative mood. So, "Add 20 mL DMF to the beads and allow them to swell for 30 min under N2."

**Commented [ASW6]:** What is this?

**Commented [K7]:** This is vessel where the rxn is done manually as opposed to an automated machine. Here is the link to the glassware https://www.chemglass.com/product_view.asp?pnr=CG-1866#CG-1866-04

1.2. ~~The beads were drained~~Drain the beads under vacuum and add 10 mL DMF. ~~(10 mL) was added.~~

1.3. Add 500 mg Fmoc-Ala-OH (~~500 mg,~~ 1.60 mmol) and 2.5 mL *i*-$Pr_2EtN$ ~~(2.5 mL) were added~~ and ~~allowed to~~ mix under $N_2$ for 15 min.

1.4. ~~The solvent was drained~~Drain the solvent under vacuum and ~~the loading~~repeat the loading with Fmoc-Ala-OH ~~was repeated again~~ for 15 min.

1.5. ~~The solvent was drained~~Drain the solvent under vacuum and wash the beads ~~the beads were washed~~ with 10 mL DMF ~~(10 mL)~~ under $N_2$ and ~~drained~~ drain under vacuum 3x.

2. Deprotection of the Fmoc Group

2.1. Add 10 mL 20% 4-Methylpiperidine in DMF ~~(10 mL) was added~~ and ~~the beads stirred~~stir the beads under $N_2$ for 15 min.

2.2. ~~The solvent was drained~~Drain the solvent under vacuum and ~~-~~ repeat the deprotection~~the deprotection was repeated again~~.

2.3. Wash t~~T~~he beads with 10 mL ~~s were washed with~~ DMF ~~(10 mL)~~ under $N_2$ and drain~~ed~~ under vacuum 3x.

3. Performing the Kaiser Test

3.1. Perform the Kaiser test by adding 1-2 drops of solution A (0.5 mL 0.01 M KCN, 24.5 mL pyridine), B (1 g ninhydrin, 20 mL *n*-butanol), and C (20 g phenol, 10 mL *n*-butanol) each into two test tubes. One test tube will be the control while the other will monitor the reaction.

3.2. Add a few beads of the resin from the reaction vessel to ~~the~~ reaction test tube and heat up the two test tubes to 110 °C.

3.3. If the deprotection is complete, the contents of the test tube will turn a dark blue/purple color. If the deprotection is incomplete or failed, the solution will remain yellow. Compare the reaction test tube with the control test tube.

4. Coupling the Next Building Blocks

4.1. Drain the solvent under vacuum.

4.2. ~~The beads were washed once~~Wash the beads with 10 mL *N*-methyl-2-pyrrolidone ~~(NMP) (10 mL)~~ under $N_2$ and ~~the solvent was drained~~drain the solvent under vacuum.

4.3. To begin the next coupling, add 10 mL NMP ~~(10 mL)~~, 620 mg Fmoc-Phe-OH (~~620 mg,~~ 1.6 mmol~~)~~), 610 mg ~~,~~HBTU ~~(~~(610 mg, 1.6 mmol), and 2.5 mL *i*-$Pr_2EtN$ ~~(2.5 mL)~~ and allow ~~were added and~~ the resin to bubble~~n bubbled~~ under $N_2$ for 30 min.

4.4. Drain the solvent under vacuum.

4.5. Wash the~~The~~ beads ~~were washed~~ with 10 mL DMF ~~(10 mL)~~ under $N_2$ and drain~~ed~~ under vacuum 3x.

4.6. Perform the Kaiser test (see step 3.1-3.3) to look for the completion of the coupling. The beads and solution in the test tube should be yellow.

**Commented [ASW8]:** What is this? What does it do in the reaction? In Figure 1, it seems you're using Et3N.

**Commented [ASW9]:** Why do you use this instead of just piperidine?

**Commented [K10]:** Piperidine is a regulated chemical so it makes it more difficult to obtain. 4-methylpiperdine is a comparable substitute. I have changed the figure to reflect this.

5. Cleaving the Peptide Off the Resin

5.1. Cleave the remaining Fmoc group using steps 2.1-2.3.
5.2. After the solvent is drained under vacuum, ~~to the resin was added~~add 40 mL cleavage solution (95% TFA, 2.5% $H_2O$, 2.5% TIPS) to the resin and bubble ~~, and the resin bubbled~~ under $N_2$ for 3 h.
5.3. A new receiving flask was replaced on the peptide synthesizer and the TFA solution containing the desired peptide was drained under vacuum into the new flask.

6. Precipitation and Isolation of the Peptide

6.1. ~~The TFA solution was separated~~Separate the TFA solution into 4 conical vials and add 25 mL cold ether (–20 °C) to each vial ~~was added~~ to precipitate the peptide.
6.2. ~~The vials were centrifuged~~Centrifuge the vials (3,000 rpm, 0–4 °C) for 20 min. ~~The~~ Decant the remaining TFA and ether solution ~~was decanted~~ from the conical vials and ~~the peptide precipitate~~concentrate the peptide precipitate ~~was concentrated~~ to afford the desired dipeptide as a white solid.

## **Representative Results**:

Representative results for solid phase peptide synthesis for Procedure 3.

| Procedure Step | Color of solution |
|---|---|
| 3.1 | Control - Clear, light yellow<br><br>Reaction – Clear, light yellow |
| 3.2 | Control - Clear, light yellow<br><br>Reaction – Dark blue |
| 3.3 | Dark blue solution, beads blue – complete deprotection or coupling failed<br><br>Colorless, beads yellow – deprotection failed or completing complete<br><br>Colorless solution, beads red – incomplete coupling or |

| | |
|---|---|
| | incomplete deprotection |

**Table 1. Representative results for procedure 3.**

## Summary:

In this experiment, we have demonstrated an example of solid-phase synthesis via SPPS through the synthesis of a dipeptide.

## Applications

Solid-phase synthesis is widely used in combinatorial chemistry to build up libraries of compounds for rapid screening. It has been commonly used to synthesize peptides, oligosaccharides, and nucleic acids. Moreover, this concept has been and implemented in chemical synthesis. Because it is heterogeneous, -these solid-supported reagents can often be recycled and reused in subsequent reactions.

**Commented [K11]:** Generally all very similar in their methods (activation, coupling, deprotection, cleavage), however, oligonucleotides are general more sensitive.

**Commented [ASW12]:** 1) What are the differences between this peptide synthesis, and, say oligonucleotides? 2) Are there non-biochemistry uses for solid phase synthesis?

## Legend:

**Figure 1. Concept behind the solid phase peptide synthesis (SPPS)**

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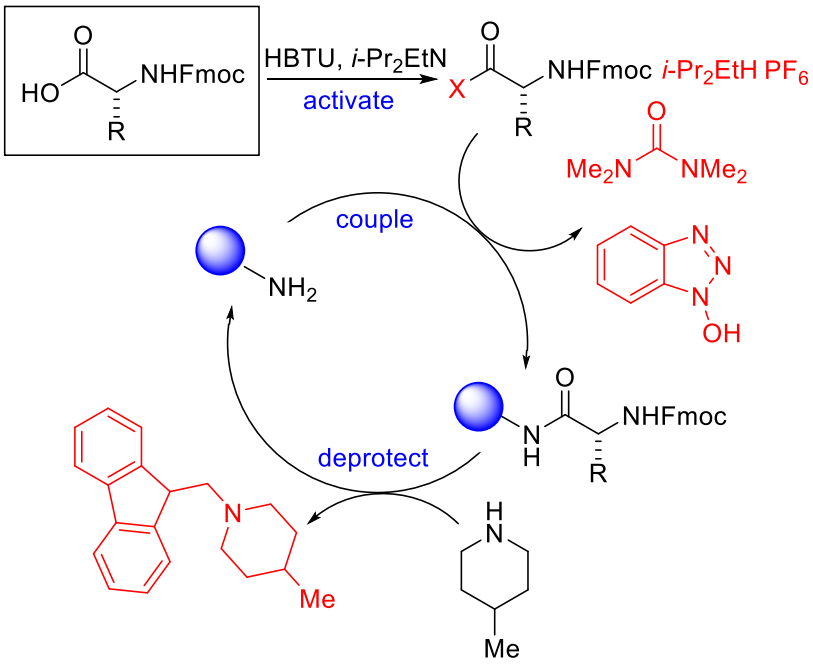


Figure 1