# Development of a Generalized Platform for Hydrophobic Peptide Synthesis by Utilizing WuXi TIDES-Solubilizing Tag

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## Introduction

In 2025, WuXi TIDES Peptide Discovery team had synthesized over 50,000 peptides, with ~20% classified as highly hydrophobic. Such peptides with **poor aqueous solubility** impede SPPS due to resin-bound aggregation, resulting in poor solvation and inefficient coupling. This necessitates excessive reagents, prolonged reaction time, and risk of truncated products, thus results in the challenge of synthesis, purification and scale-up.[1]

To address this issue, we have developed a novel solubilizing platform via screening of 20 solubilizing tags.[2-5] Our optimized **WuXi TIDES- Solubilizing Tag** enables high-yield synthesis of challenging highly hydrophobic peptides to support fast SAR studies and potential future scale-up.

## Strategy

Our innovation involves pre-installing solubilizing tags on resin before peptide elongation, enhancing solubility and coupling efficiency while enabling mild tag removal. As shown in Figure 1, the solubility tag is immobilized via SPPS, followed by affinity tag/linker addition and peptide chain elongation. Post-cleavage yields a hydrophilic peptide with tag (HPLC-purified).

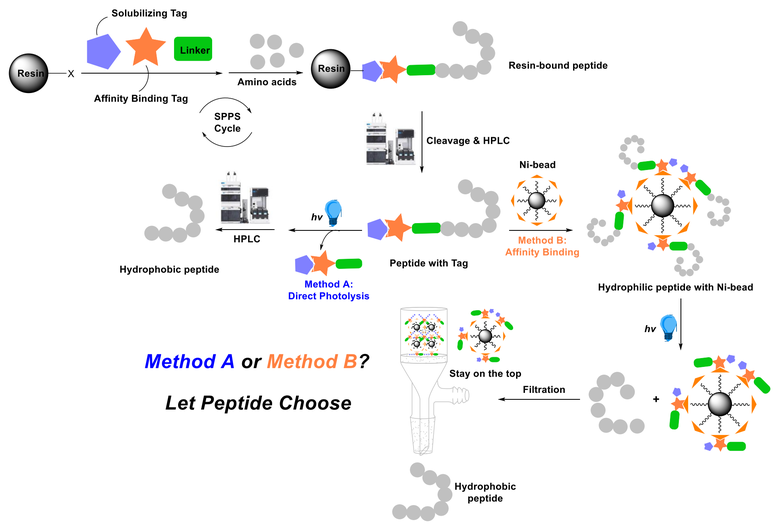



**Figure 1.** Strategy design for the synthesis of hydrophobic peptides with Solubilizing Tags.

For final peptide isolation, two methodologies were employed: **Method A:** The peptide with tag undergoes UV photolysis, followed by additional HPLC purification to generate the pure target peptide. **Method B:** The peptide with tag is subjected to Ni-NTA affinity bead, followed by photolytic release, to directly yield the target hydrophobic peptide without requiring additional HPLC purification.

## Result

### 1. Screening of Solubilizing Tags: Performance Evaluation

To screen solubilizing tags, we tested eight variants on a model peptide. Tags 1-8 were linked to resin via SPPS, followed by linker addition, peptide elongation, cleavage, and purification. Solubility was assessed by precipitation/Tyndall effect observation (Figure 2 and Table 1). After photolysis/HPLC purification, model peptide yields were calculated.

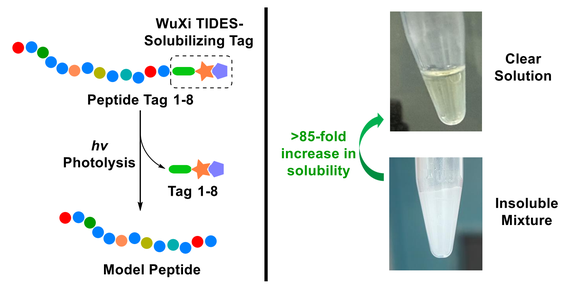



**Figure 2.** Solubilizing tag screening.

**Table 1**. The result comparison of the model peptide with/without solubilizing tag.

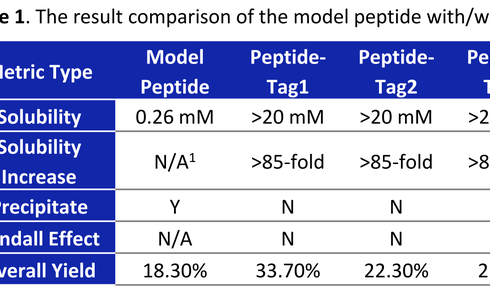
| Metric Type | Model Peptide | Peptide-Tag1 | Peptide-Tag2 | Peptide-Tag3 | Peptide-Tag4 | Peptide-Tag5 | Peptide-Tag6 | Peptide-Tag7 | Peptide-Tag8 |
|---|---|---|---|---|---|---|---|---|---|
| Solubility | 0.26 mM | >20 mM | >20 mM | >20 mM | 17 mM | 19 mM | 0.05 mM | 14 mM | 0[2] |
| Solubility Increase | N/A[1] | >85-fold | >85-fold | >85-fold | 68-fold | 75-fold | N/A | 27-fold | N/A |
| Precipitate | Y | N | N | N | N | N | Y | N | N |
| Tyndall Effect | N/A | N | N | N | Y | Y | N/A | Y | Y |
| Overall Yield | 18.30% | 33.70% | 22.30% | 2.90% | 8.90% | 17.70% | 13.20% | 14.40% | 12.50% |

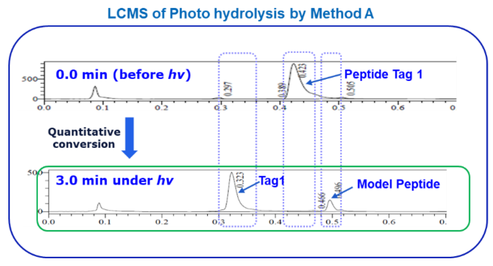
**Key results**: Tags 1-3 were advanced to secondary screening, identifying 10 effective tags (not shown here).

### 2. Efficient Photolytic Cleavage Evaluation

Using optimized WuXi TIDES-Solubilizing Tag, we assessed two cleavage methods (Figure 3):

**Method A:** Photolysis quantitatively converted tagged peptide to target in 3 min (LCMS-confirmed), yielding 33.7% pure product with the purity of 97.2% after HPLC purification.
**Method B:** Immobilization on Ni-beads enabled photolytic release in 15 min; filtration directly isolated target peptide (42.2% yield, 94.3% purity) with simultaneous tag-resin retention (reusable). Method A offers speed but requires HPLC, while Method B integrates tag absorption/isolation. Both provide complementary production strategies.



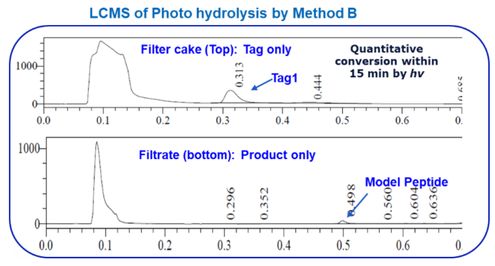




**Figure 3.** Efficiency evaluation for model peptide with Method A and B.

## Case Studies

This strategy was applied to troubleshoot difficulties encountered in real cases. Comparative experiments were employed to synthesize cyclic peptides 1-5, both with and without the **WuXi TIDES-Solubilizing Tag**. Comparative analysis revealed that tagged peptides consistently achieved (see table below): 1) >10-fold higher yields; 2) Elevated purity; 3) Reduced purification rounds. This comparison clearly indicates that the **WuXi TIDES-Solubilizing Tag** strategy offers significant advantages.

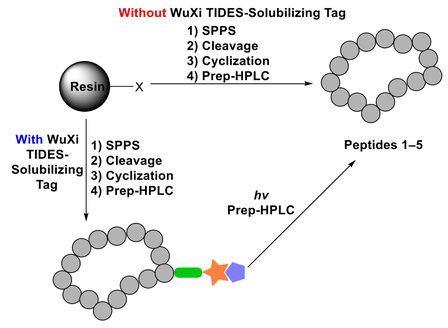



**Figure 4.** Comparative synthesis of peptides with/without **WuXi TIDES-Solubilizing Tag.**

**Table 2**. The results of Peptides 1-5 with/without **WuXi TIDES-Solubilizing Tag**

| Compound ID | Strategy | Overall Yield | Purity | Number of HPLC Purifications |
|---|---|---|---|---|
| Peptide 1 | Without Tag | 1.5% | 95% | 5 |
| | With Tag | 8.8% | 99% | 2 |
| Peptide 2 | Without Tag | 1.8% | 97% | 3 |
| | With Tag | 21.2% | 95% | 2 |
| Peptide 3 | Without Tag | 0.9% | 80% | 2 |
| | With Tag | 3.0% | 98% | 2 |
| Peptide 4 | Without Tag | 0.7% | 70% | 2 |
| | With Tag | 4.2% | 97% | 2 |
| Peptide 5 | Without Tag | 0.7% | 69% | 2 |
| | With Tag | 2.5% | 92% | 2 |

## Conclusion

WuXi TIDES has developed a generalized solubilizing tag strategy to address the synthesis and purification of poorly aqueous soluble peptides. We have identified 10 highly effective solubilizing candidates. Application of this strategy to challenging peptides significantly improved yields and purity while reduced the number of purification cycles. Moving forward, we will further optimize the solubilizing tags along with the affinity purification process and cleavable linkers to broaden applicability, ultimately enabling scale-up and manufacturing implementation.

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