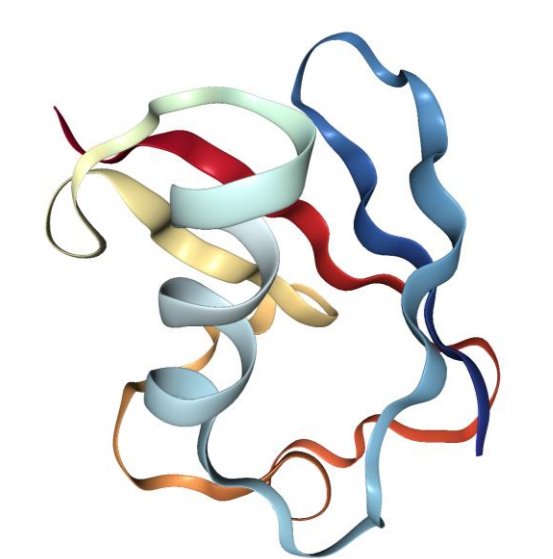


Tyler Siegford<sup>1</sup>, Joachim Weidmann<sup>2</sup>, Philip E. Dawson<sup>2</sup>, Kristopher Waynant<sup>1</sup> and Darren A. Thompson<sup>1,3</sup>  
<sup>1</sup>UNIVERSITY OF IDAHO, <sup>2</sup>THE SCRIPPS RESEARCH INSTITUTE, <sup>3</sup>PEPTIDAHO RESEARCH CONSORTIUM

## ABSTRACT

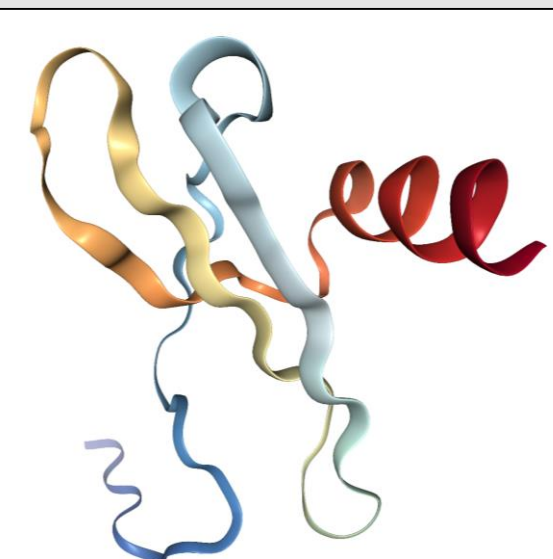
Therapeutic proteins have successfully treated a range of diseases and offer potential to treat many more. Using chemical synthesis, proteins can be fabricated uniformly with precise control over chemical structure. High purity synthesis of proteins larger than 50 amino acids relies on the use of ligation strategies to link smaller peptides. The most notable of these ligation strategies, Native Chemical Ligation, requires peptides with C-terminal thioesters. We have produced a novel resin for solid phase peptide synthesis (SPPS) by coupling 1,2-phenylenediamine directly to several different types of resin, which when cleaved, leaves a C-terminal *o*-aminoanilide. Using the chemistries reported by Weidmann et al. this *o*-aminoanilide can be activated and substituted with a variety of thiols giving a peptidyl C-terminal thioester primed for ligation [1].

## EXAMPLES OF CHEMICALLY SYNTHESIZED PROTEINS



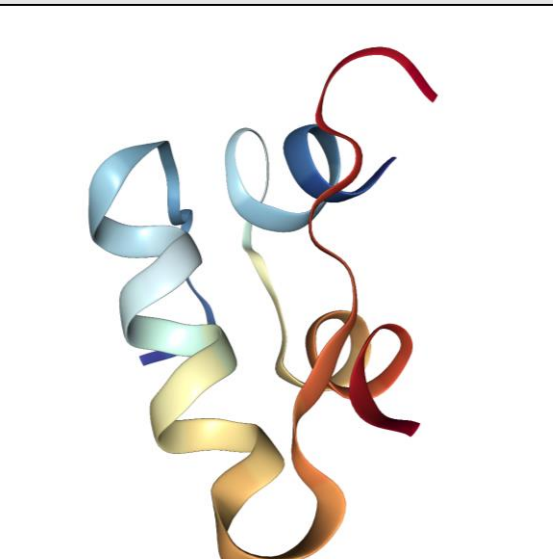
### Ubiquitin:

A common post translational modifier. Has large impact on the roles of other human proteins [5].



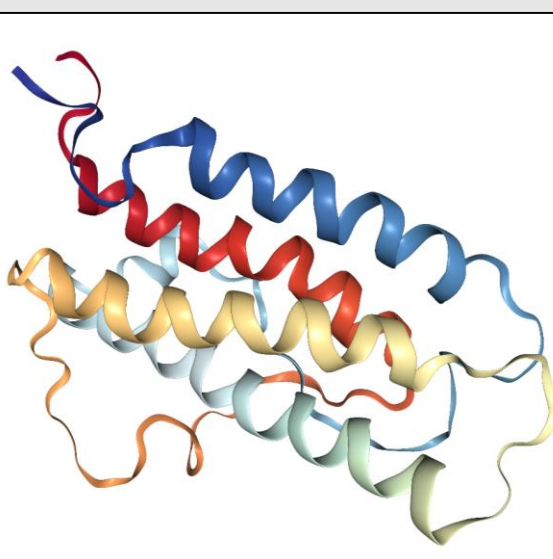
### SDF1-α:

A cytokine that directs white blood cell migration. Shown to inhibit HIV and metastatic tumors, and to have cardioprotective properties [3].



### Insulin:

A hormone that regulates the amount of glucose in the blood. Used as a life saving diabetes treatment [4].



### Erythropoietin:

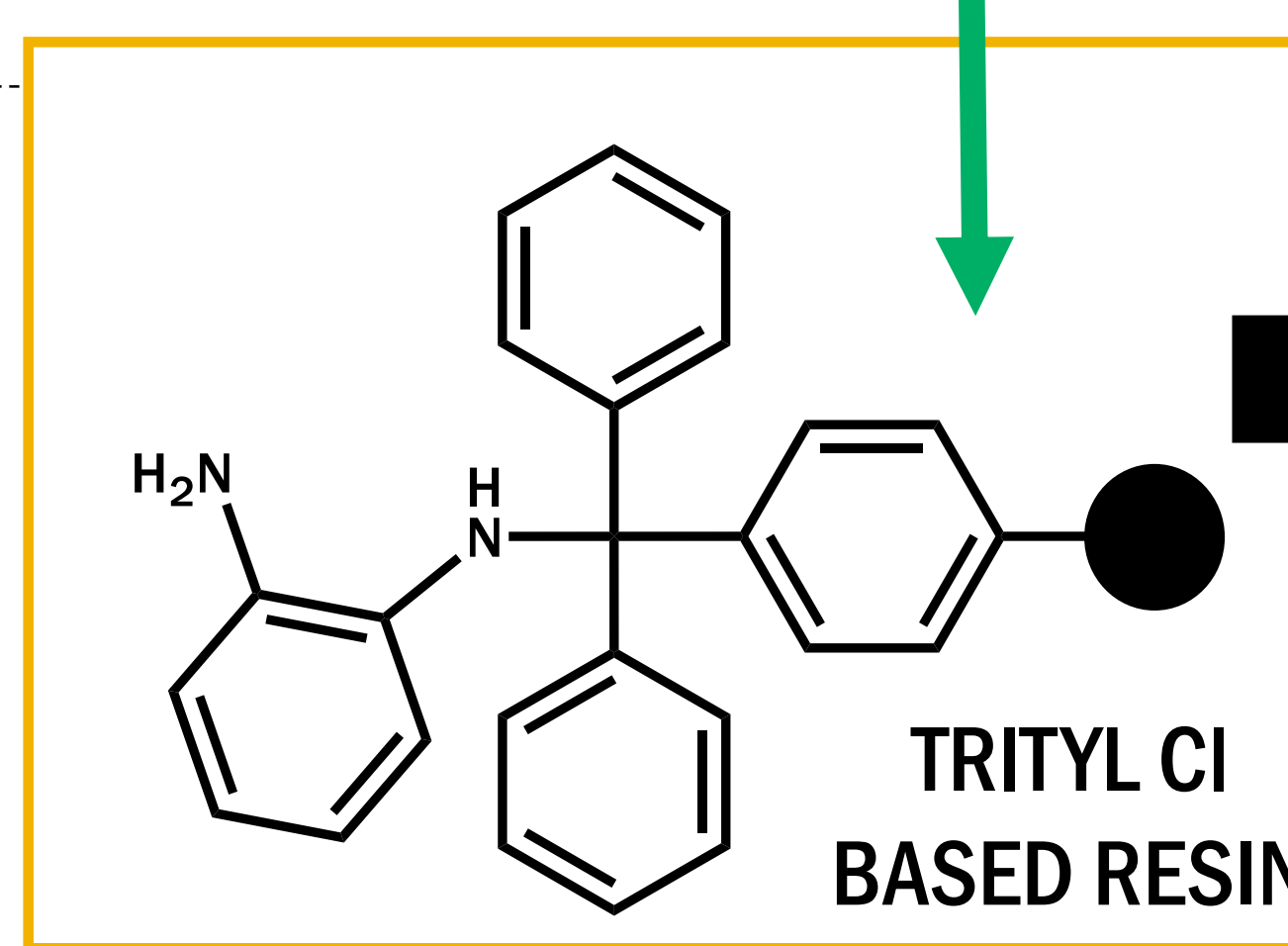
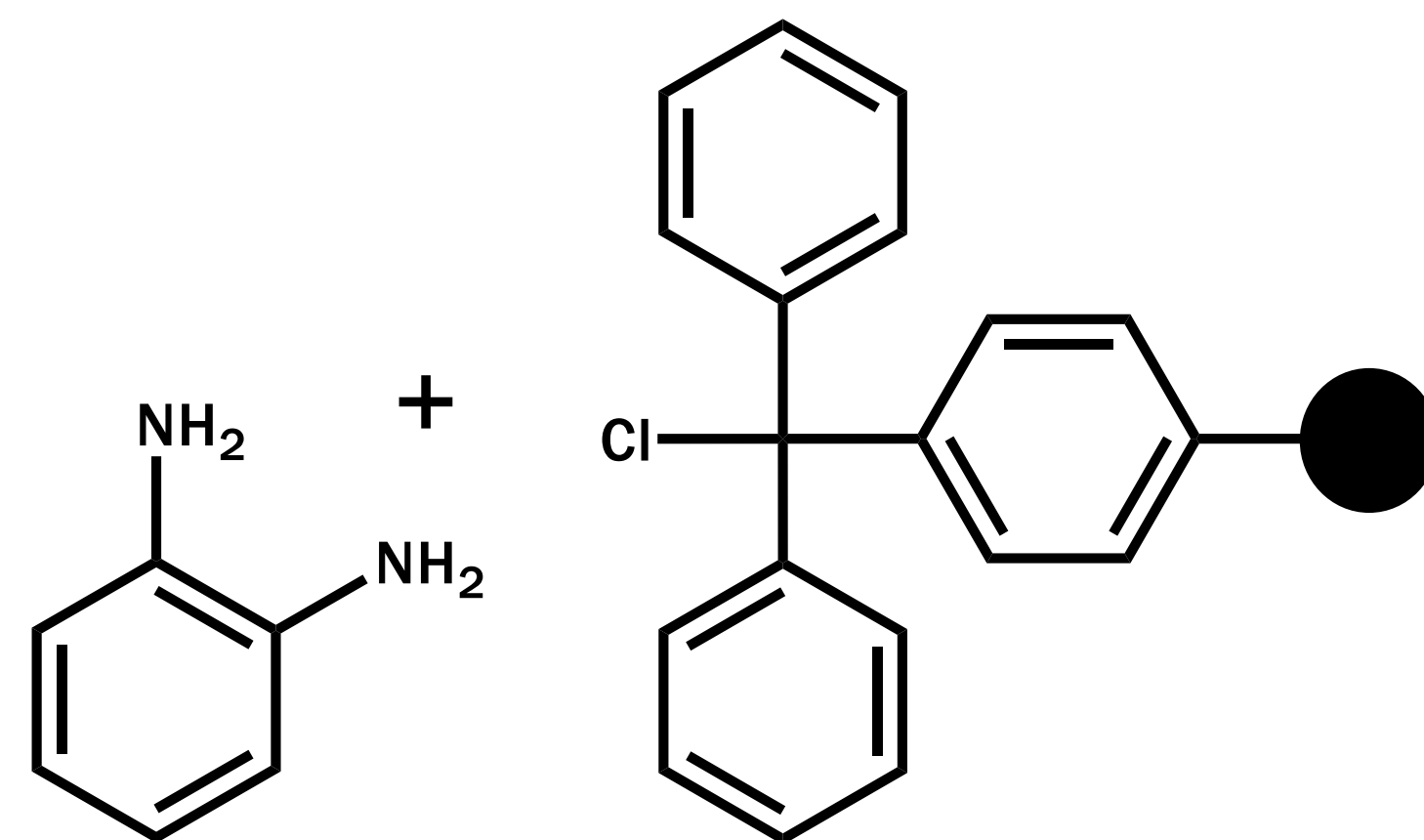
A hormone responsible for the production of red blood cells. Used globally as a treatment for anemia [2].

## Acknowledgements

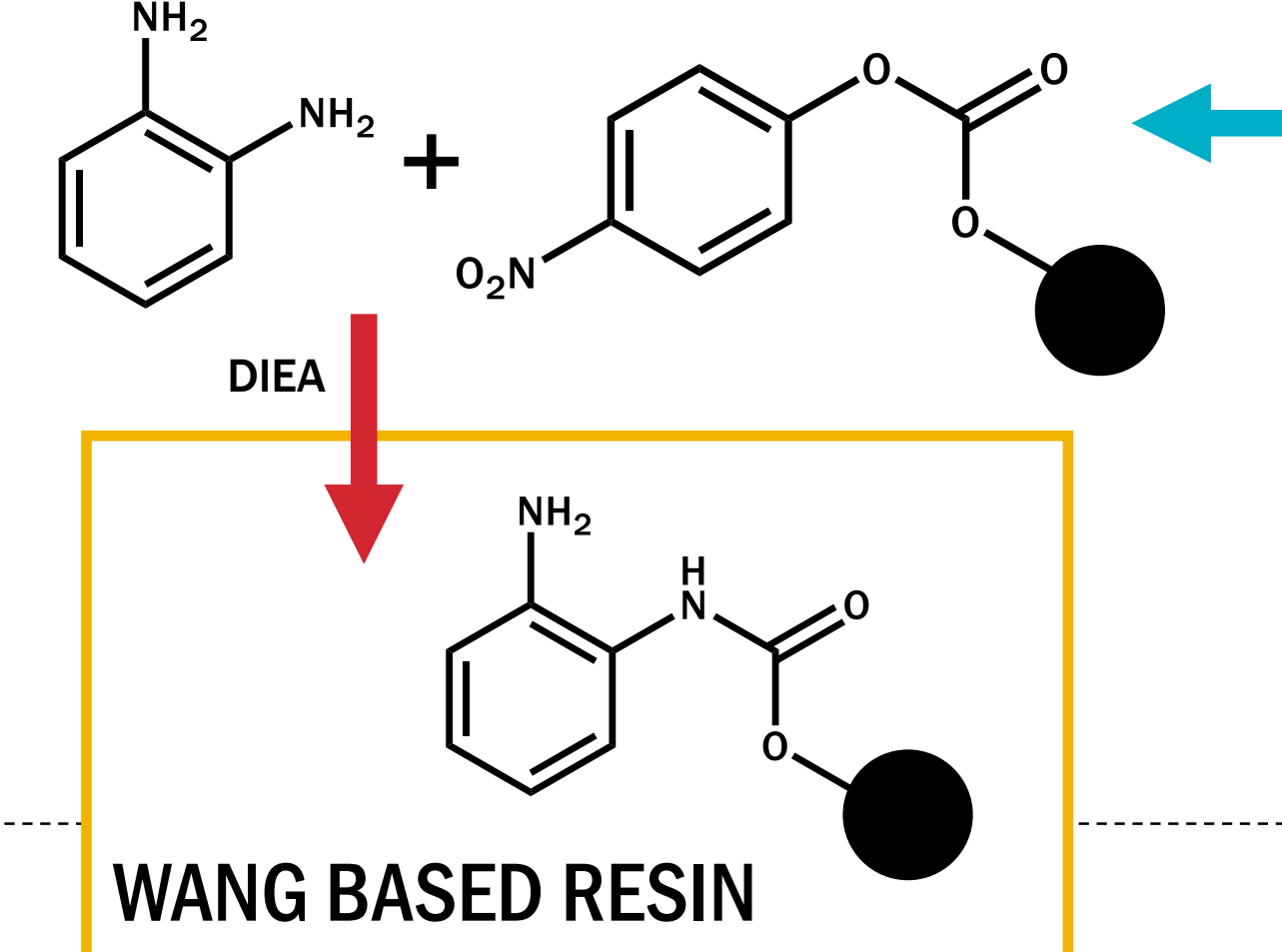
The project described was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant #P20GM103408  
Other support provided by the Peptidaho Research Consortium and John Wieser  
Special thank you to Gonzaga University for the use of specialized analytical equipment (NSF grant CRIF:MU grant #0741868)

## SYNTHESIS OF TRITYL CL BASED RESIN

Trityl Cl resin, another commonly used polystyrene resin, was reacted in the presence of DIEA to produce our trityl chloride based *o*-aminoanilide resin.



## SYNTHESIS OF WANG BASED ALTERNATIVE RESIN

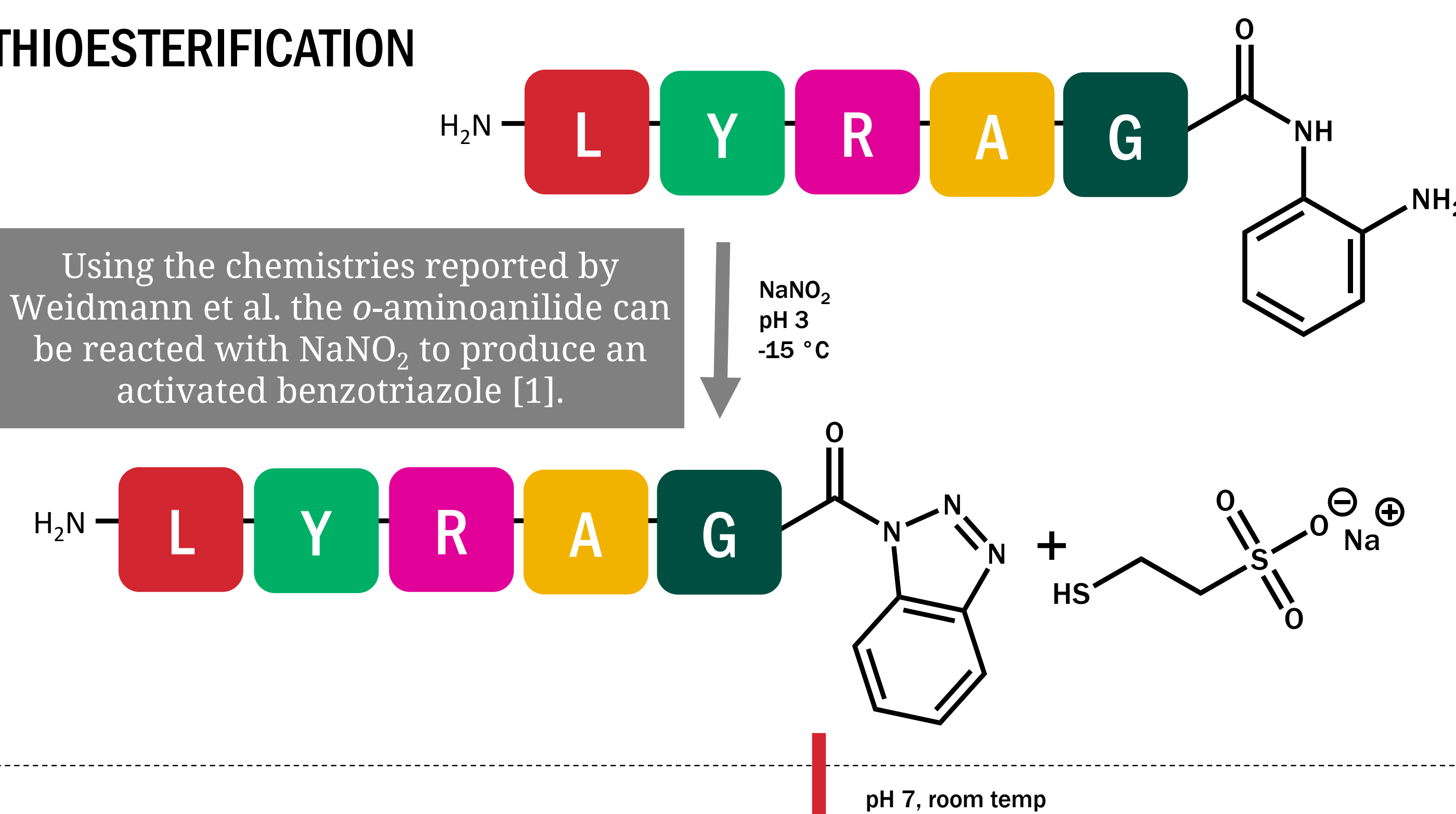


Polystyrene "Wang" resin was reacted with 4-nitrophenyl chloroformate in the presence of lutidine to produce "NPC-Wang". Alternatively, "NPC-Wang" from Rapp Polymer was also used in this synthesis.

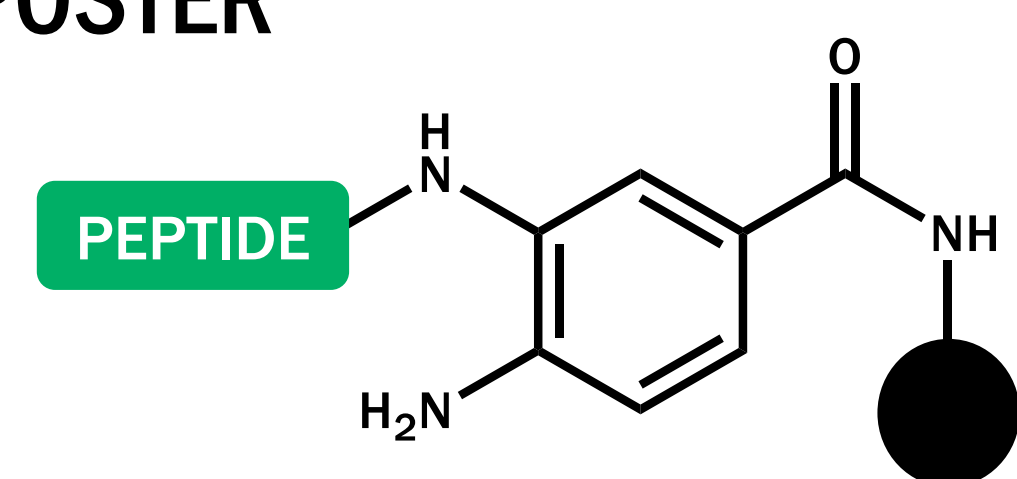
## SOLID PHASE PEPTIDE SYNTHESIS

CLEAVAGE

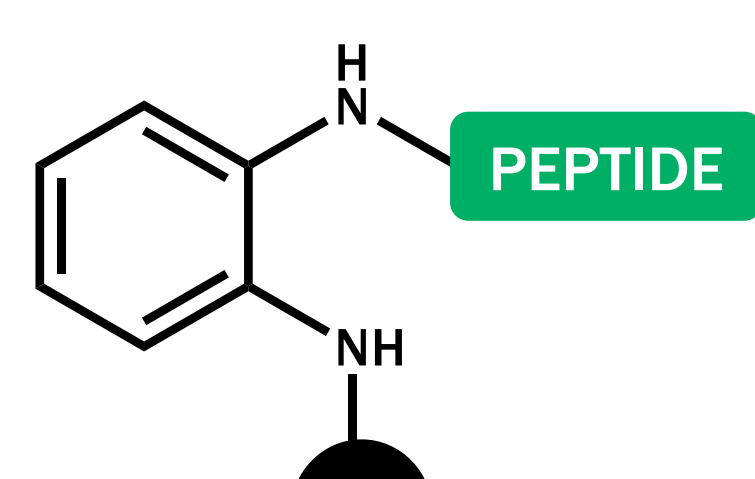
## THIOESTERIFICATION



## PUBLISHED RESIN VS THIS POSTER

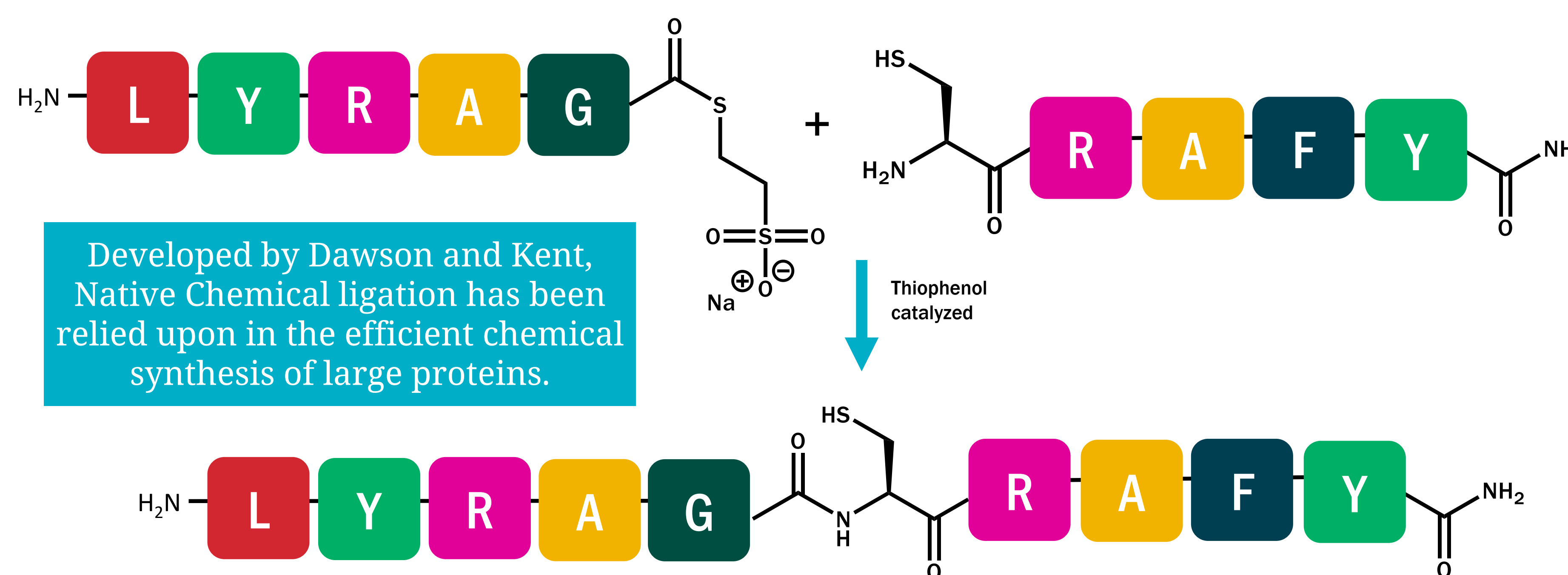


PUBLISHED RESIN [6]



THIS POSTER

## NATIVE CHEMICAL LIGATION



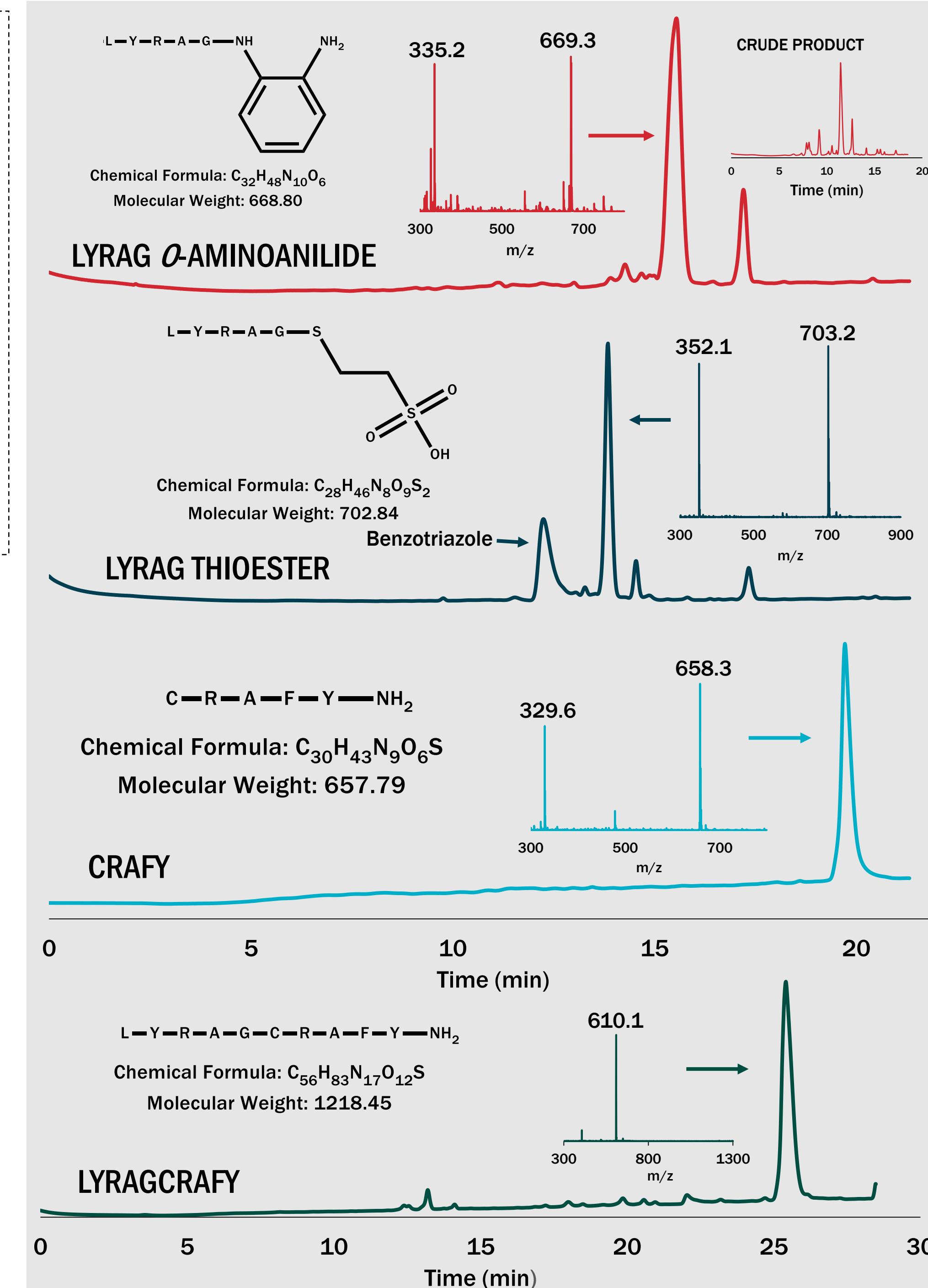
Developed by Dawson and Kent, Native Chemical ligation has been relied upon in the efficient chemical synthesis of large proteins.

## WANG VS TRITYL CL

C-terminal *o*-aminoanilide peptides can be produced from Wang resin with purity (90%). However, the resin synthesized peptide yield was unacceptable (10%). Trityl Chloride based resin can produce peptides with yields as high as 49% (may be falsely low see control) and reasonable purity (73%).

## FMOC SOLID PHASE PEPTIDE SYNTHESIS

(SPPS) is a robust method routinely used in chemical protein synthesis. The method relies on the cyclic coupling and deprotection of amino acids to create a precisely ordered and natively structured poly-peptide. Our resins function with normal coupling, deprotection, and cleavage conditions to produce custom peptides with C-terminal *o*-aminoanilides.



Control	T <sub>r</sub> (min)	mH <sup>+</sup> Calc. (g/mol)	mH <sup>+</sup> Obs. (g/mol)	Yield
LYRAG (rink amide)	0-50% ACN 18 min grad.	7.10	578.7	84%
LYRAG (trityl Cl)	8.07	579.7	579.3	48%

## CONCLUSION

Benefits of new resin:

- Straightforward, easily synthesized
- Inexpensive/commercially available materials
- Compatible with Fmoc SPPS
- Resin suitable for acetic anhydride capping

Future Experiments:

- Synthesize metabolically relevant tandem repeat from Mucin7 protien
- Other nucleophiles (NaSH, benzylamine, etc.)

## Works Cited

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- [6] Blanco-Canosa, J. B. & Dawson, P. E. An Efficient Fmoc-SPPS Approach for the Generation of Thioester Peptide Precursors for Use in Native Chemical Ligation. *Angewandte Chemie International Edition* **47**, 6851–6855