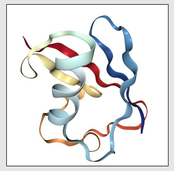
A NOVEL RESIN FOR CHEMICAL PROTEIN SYNTHESIS PART I: THE THIOESTER

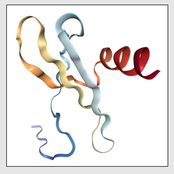
Tyler Siegford[1], Joachim Weidmann[2], Elena Dimitrijevic[2], Philip E. Dawson[2], and Darren A. Thompson[1,3]
[1]UNIVERSITY OF IDAHO, [2]THE SCRIPPS RESEARCH INSTITUTE, [3]PEPTIDAHO RESEARCH CONSORTIUM

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].

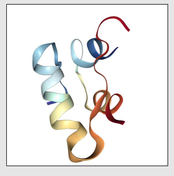
EXAMPLES OF CHEMICALLY SYNTHESIZED PROTEINS



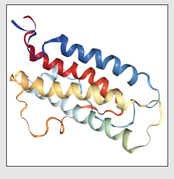
Ubiquitin:
A common post translational modifier. Has large impact on the roles of other human proteins [5].



SDF1-α:
A cytokine that directs white blood cell migration. Shown to inhibit HIV and metastatic tumors, and to have cardioprotective properties [3].



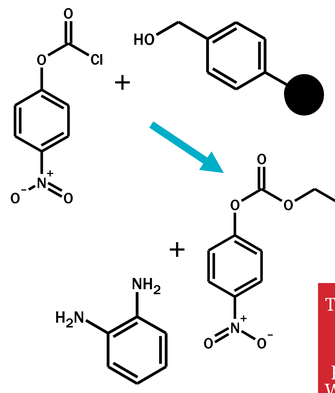
Insulin:
A hormone that regulates the amount of glucose in the blood. Used as a life saving diabetes treatment [4].



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

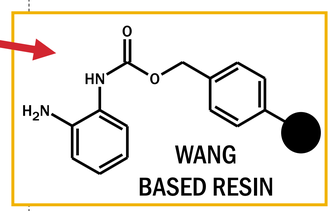
IDAHO INBRE
IDeA Network of Biomedical Research Excellence

SYNTHESIS OF WANG BASED RESIN



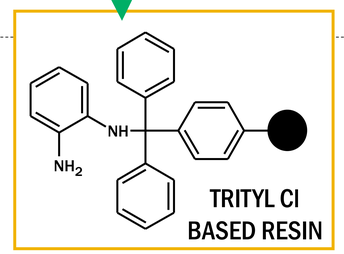
Commonly used polystyrene "Wang" resin was reacted with 4-nitrophenyl chloroformate in the presence of lutidine to produce "NPC-Wang". Alternatively, "NPC-Wang" from Rapp Polymer was also employed in this synthesis.

The "NPC-Wang" made during the last step was reacted with phenylene diamine in the presence of DIEA to produce our Wang based *o*-aminoanilide resin.

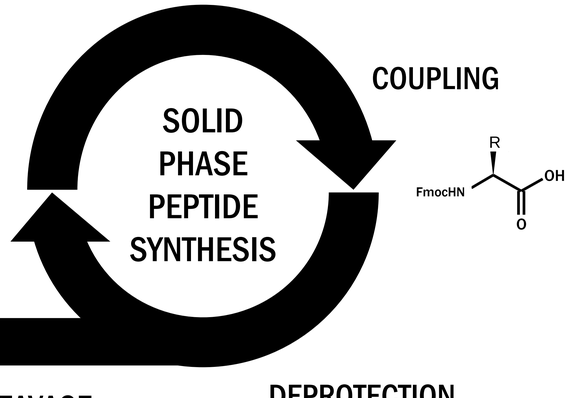


SYNTHESIS OF TRITYL CL BASED RESIN

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

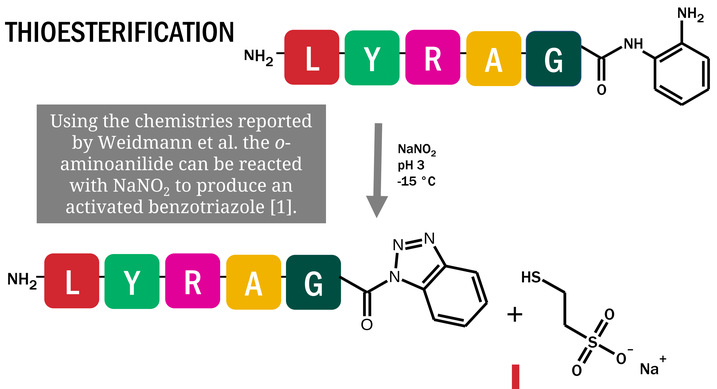


WANG VS TRITYL CL
C-terminal *o*-aminoanilide peptides can be produced from Wang resin with purity (90%). However, using the reactions above, the resin synthesized peptide yield was unacceptable (10%). Trityl Chloride based resin can produce peptides with yields as high as 49% (may be falsely low see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



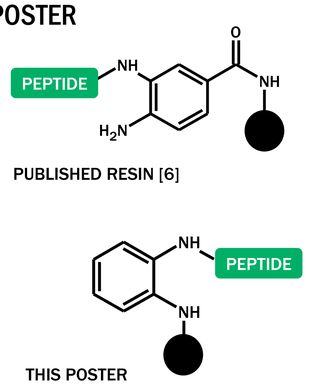
Solid Phase Peptide Synthesis (SPPS) is a robust method routinely used in chemical protein synthesis. The method relies on the cyclic coupling and deprotection of amino acids to create a precisely ordered and natively structured poly-peptide. Our resins function with normal coupling, deprotection, and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

THIOESTERIFICATION

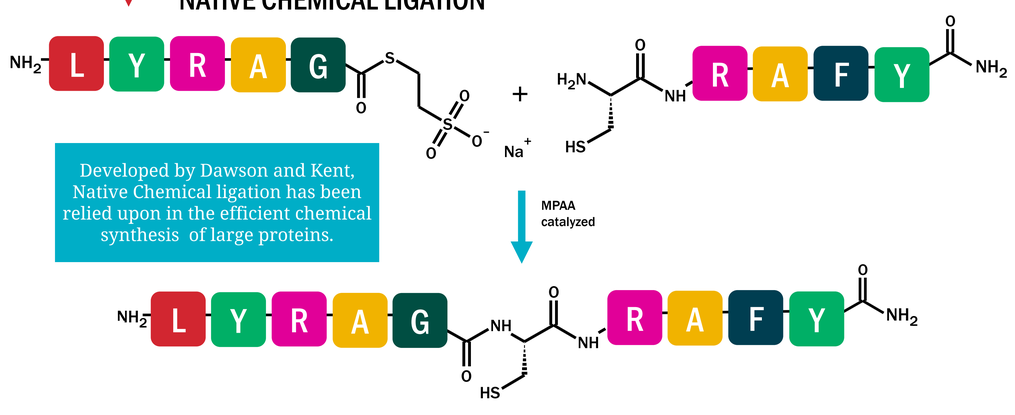


Using the chemistries reported by Weidmann et al. the *o*-aminoanilide can be reacted with $NaNO_2$ to produce an activated benzotriazole [1].

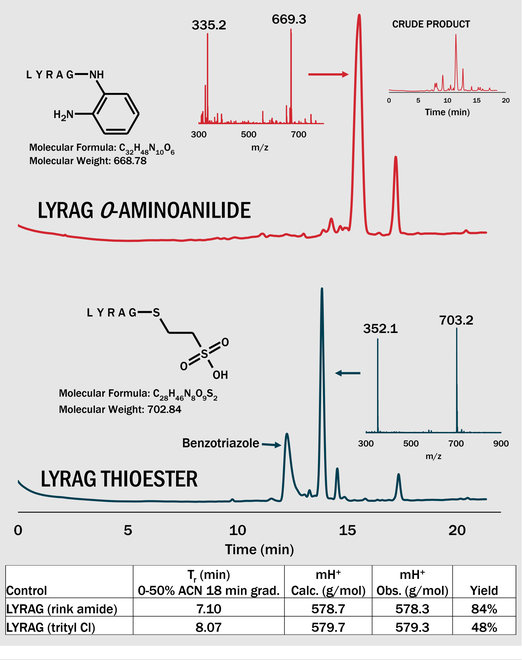
PUBLISHED RESIN VS THIS POSTER



NATIVE CHEMICAL LIGATION



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



Control	T$_r$ (min) 0-50% ACN 18 min grad.	mH$^+$ Calc. (g/mol)	mH$^+$ Obs. (g/mol)	Yield
LYRAG (rink amide)	7.10	578.7	578.3	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
- Protected peptide resin unreactive to acetic anhydride

Future Experiments:
- Part II- Native Chemical Ligation
- Synthesize all twenty genetically encoded amino acid resins (have Ala and Gly)
- Other nucleophiles (NaSH, benzylamine, etc.)

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)

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University of Idaho
Coeur d'Alene