The Career of Tom W. Muir

Scott Simonovich

MacMillan Group Meeting

February 24th 2010

Education and Career

Education

B.S. Chemistry - University of Edinburgh (1989)

Ph.D. Chemistry - University of Edinburgh (1993)

Post-doctoral research - Stephen Kent, The Scripps Research Institute

Senior Research Associate - The Scripps Research Institute

Independent Career - Rockefeller University

Assistant Professor (1996 - 2000)

Associate Professor (2000 - 2002)

Professor (2002 - 2005)

Richard E. Salomon Family Professor (2005 - present)

Director of Pels Family Center for Biochemistry and Structural Biology

Education and Career

Awards

Blavatnik Award for Young Scientists

Vincent du Vigneaud Award

Irving Sigal Young Investigator Award

Leonidas-Zervas Award

Burroughs Wellcome Fund New Investigator Award

Research Focuses

Protein function in complex systems of biological interest

Protein semi-synthesis and total synthesis

Ligation and protein splicing

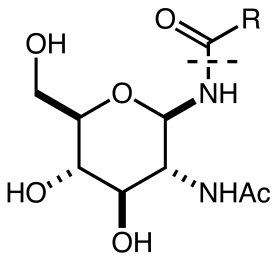
Post-translational modifications

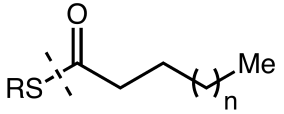
Isotope and fluorescence labeling

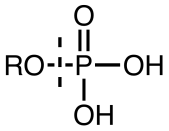
Structure - function relationships in K channels

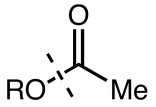
General Overview of Research

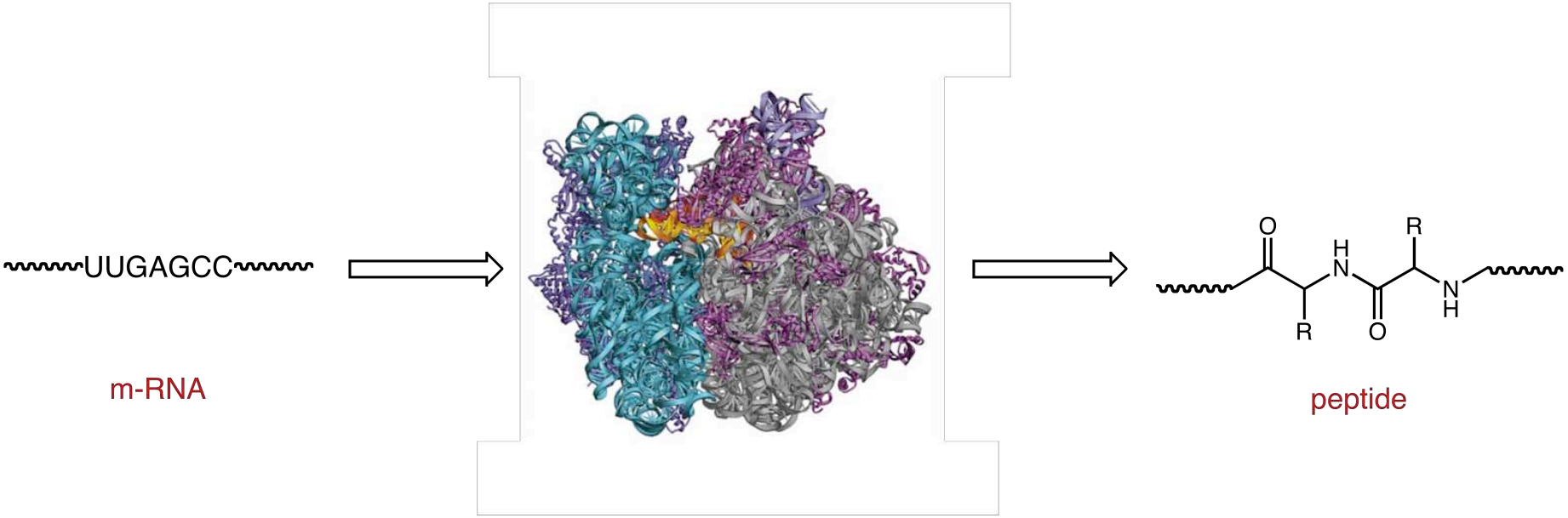
- Protein function central in experimental biology
- Description of post-translational modifications
  - Function
  - 3D structure
  - Stabilty
  - Interactions
- Accelerate acquisition of protein function information
- Access useful peptides unattainable by ribosomal synthesis



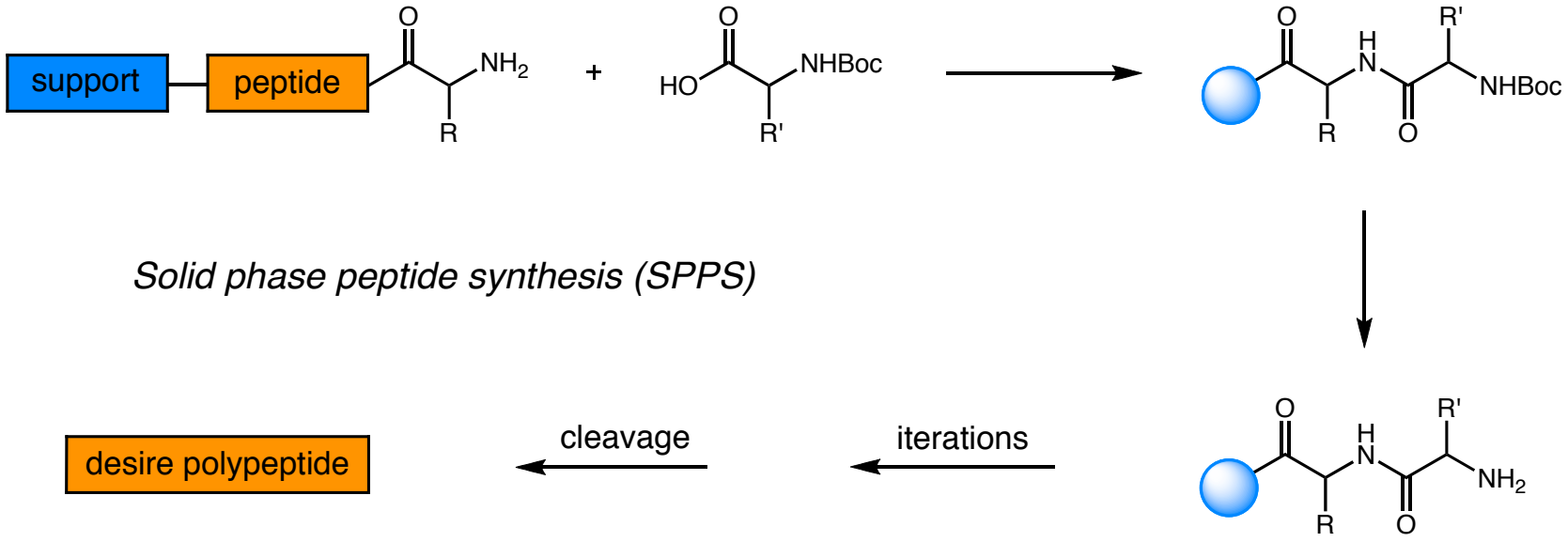








Previous Methods for Peptide Synthesis

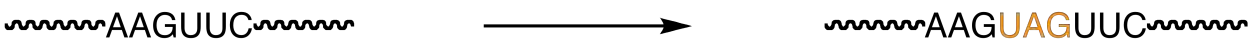


Solid phase peptide synthesis (SPPS)

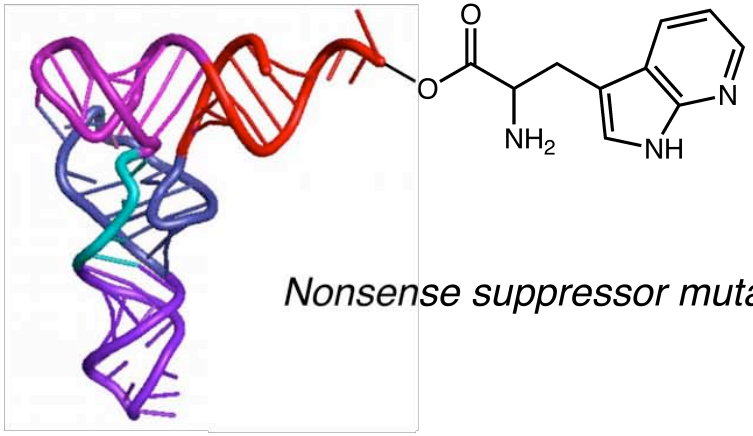
- Useful method with significant drawbacks
  - Incorporate unnatural or D-amino acids
  - Each step must be very efficient

    35 residue polypeptide:
    99% yield per step = 49% IY
    95% yield per step = 3% IY
  - Maximum length of ~50 amino acids
  - Can be very time consuming
  - Many peptides not suitable for SPPS

Previous Methods for Peptide Synthesis

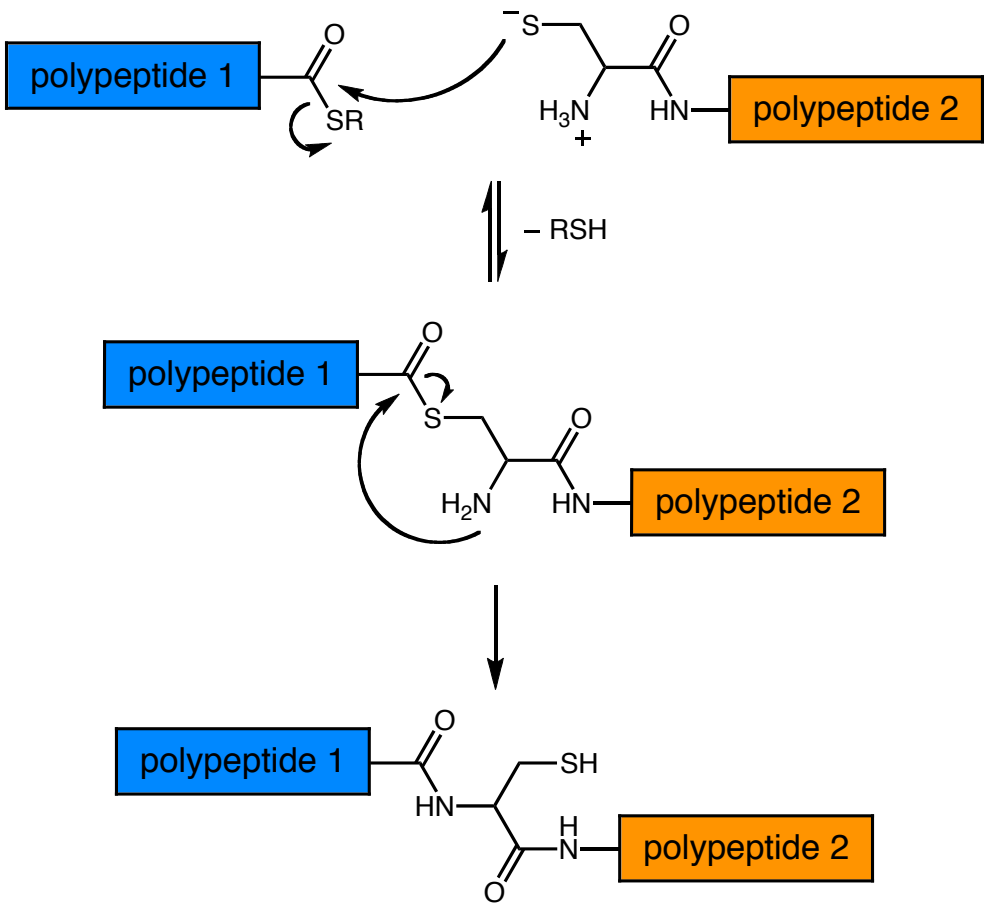


- Install nonsense mutation into mRNA sequence
- Construct tRNA with corresponding anti-codon to incerpt "stop" codon
- Site selective incorporation of unnatural amino acid at "stop" codon
- Difficult to prepare and deliver appropriate tRNA with acylated residue



Nonsense suppressor mutagenesis

Chemical Ligation



Native Ligation (NL)

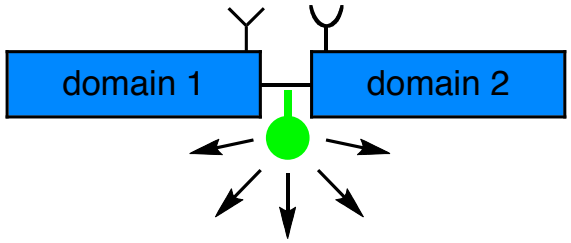
- Coupling of unprotected AA's
- Compatible with side chains
- Nearly quanitative yield
- General tool for semi-synthesis
- Works at physiological pH
- Numerous improvements

Figure adapted from Muir, T. W. *Annu. Rev. Biochem.* **2003**, *72*, 249.

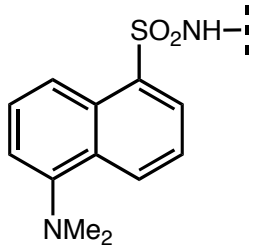
Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776.

Chemical Ligation

- Applications in protein engineering to study biological systems
  - Insertion of synthetic peptide into recombinant protein
  - Biosensor with properties dependent upon state of system



fluorescent probe

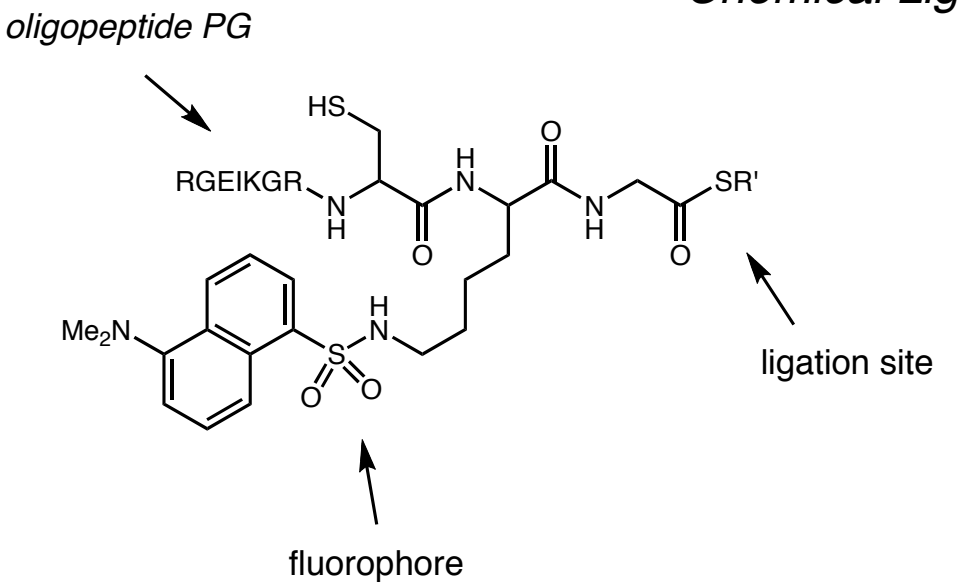


(dansylamide)

- Abelson nonreceptor protein tyrosine kinase (Abl)
- src Homolgy 2 domain (SH3) and src Homolgy domain 2 (SH2)

Cotton, G. J.; Ayers, B.; Xu, R.; Muir, T. W. *J. Am. Chem. Soc.* **1999**, *121*, 1100.

Chemical Ligation



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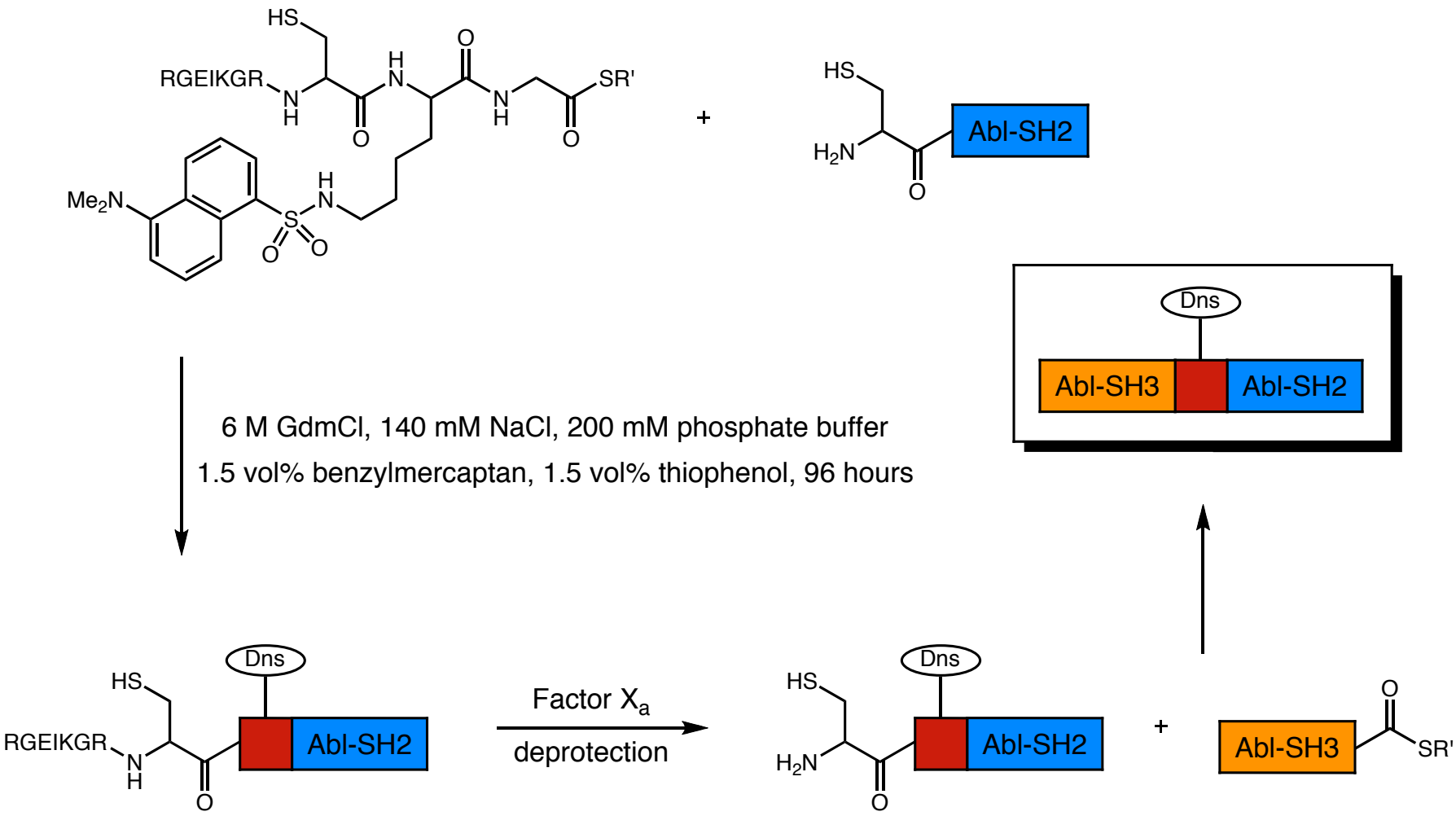

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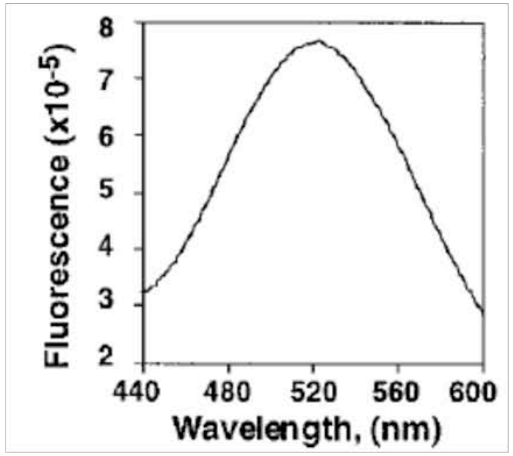

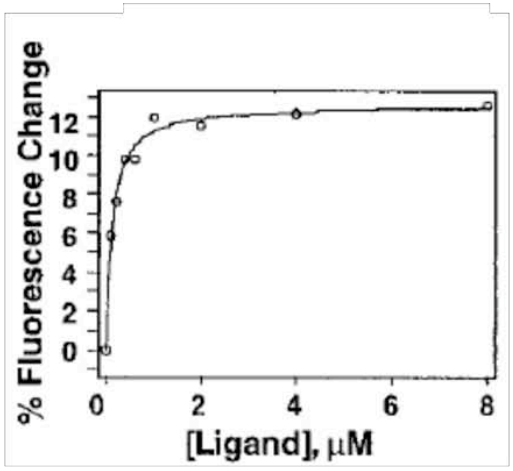

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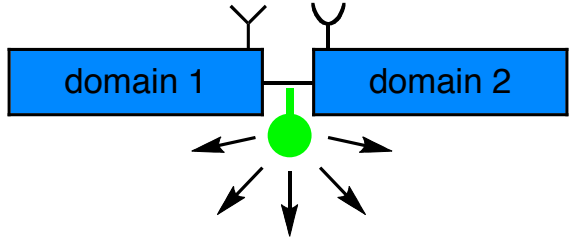
Chemical Ligation

- $K_d = 0.123 \pm 0.017\ \mu M$
- Monodentate ligands led to insignificant fluorescence increases
- Useful biosensor for future investigations
  - High affinity, bidentate ligands
  - in vitro screening of combinatorial peptide libraries
  - Characterize protein-protein interactions that regulate Abl function

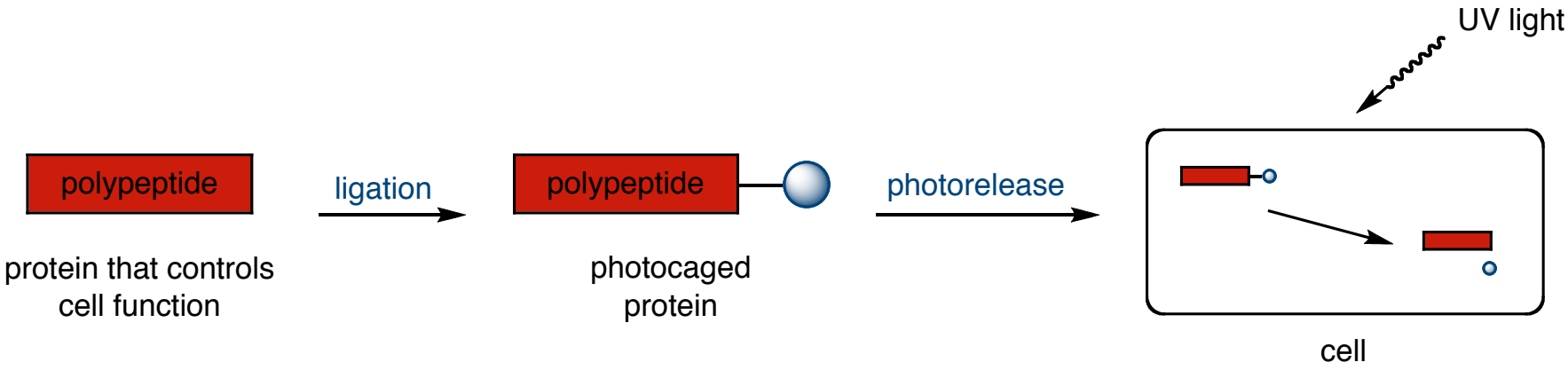


Can native ligation regulate molecular processes to study protein function?

Cotton, G. J.; Ayers, B.; Xu, R.; Muir, T. W. *J. Am. Chem. Soc.* **1999**, *121*, 1100.

Chemical Ligation

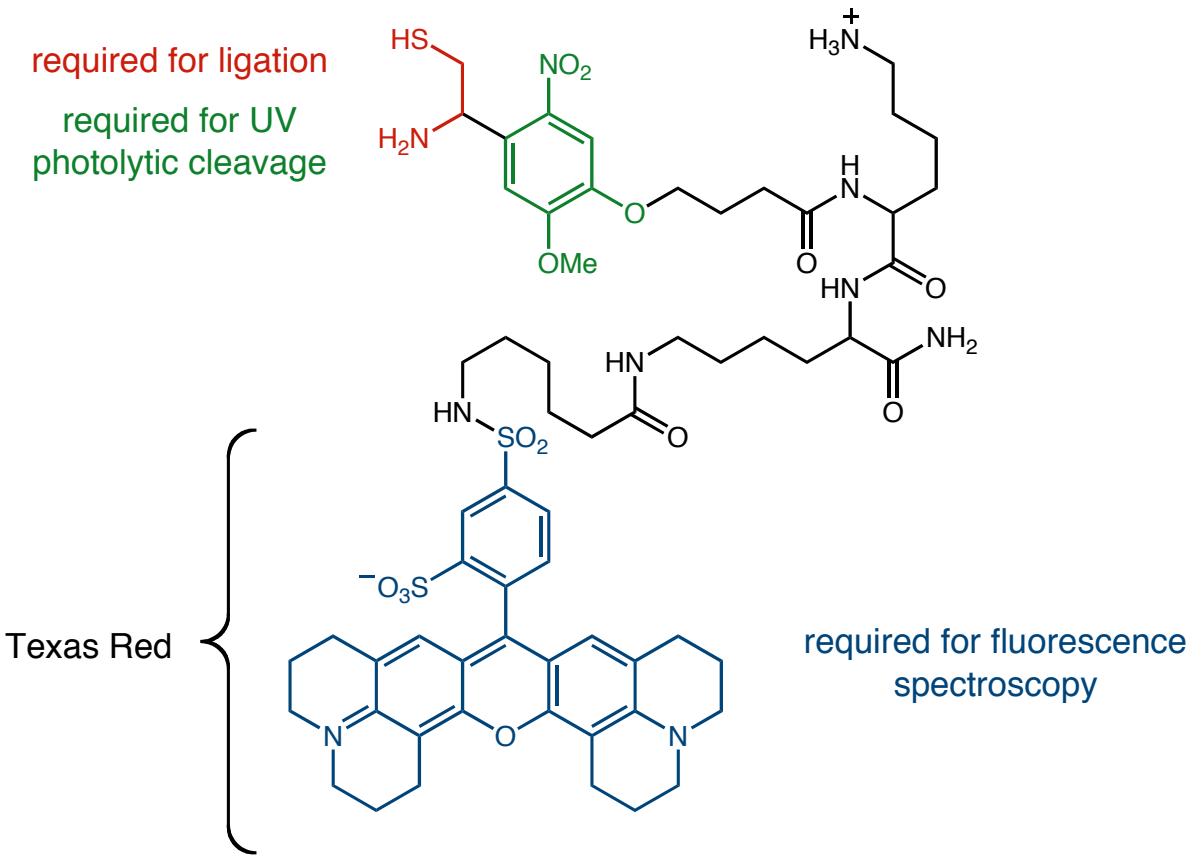
- Chemical modification of proteins + external impulse
- Semi-synthesis through native ligation with photolabile PG
- Traditionally difficult to prepare photocaged systems



- Control of protein function through subcellular localization with light

Pellois, J-.; Muir, T. W. *Angew. Chem. Int. Ed.* **2005**, *44*, 5713.

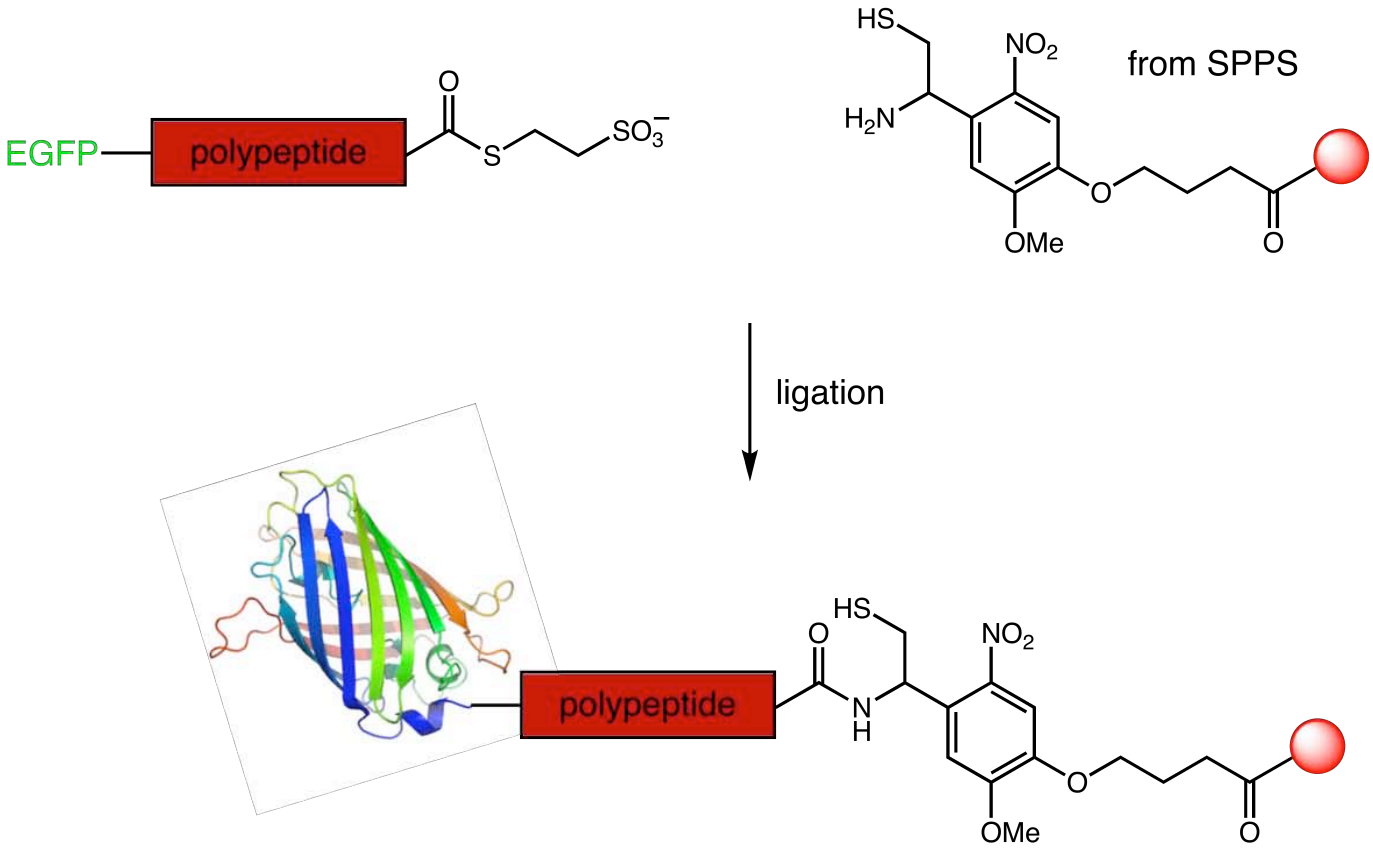
Chemical Ligation



- Synthesize fluorophore-bearing fragment and ligate to recombinant protein

Pellois, J-.; Muir, T. W. *Angew. Chem. Int. Ed.* **2005**, *44*, 5713.

Chemical Ligation



- Ligated product shows 33% FRET between TR and EGFP at 488 nm

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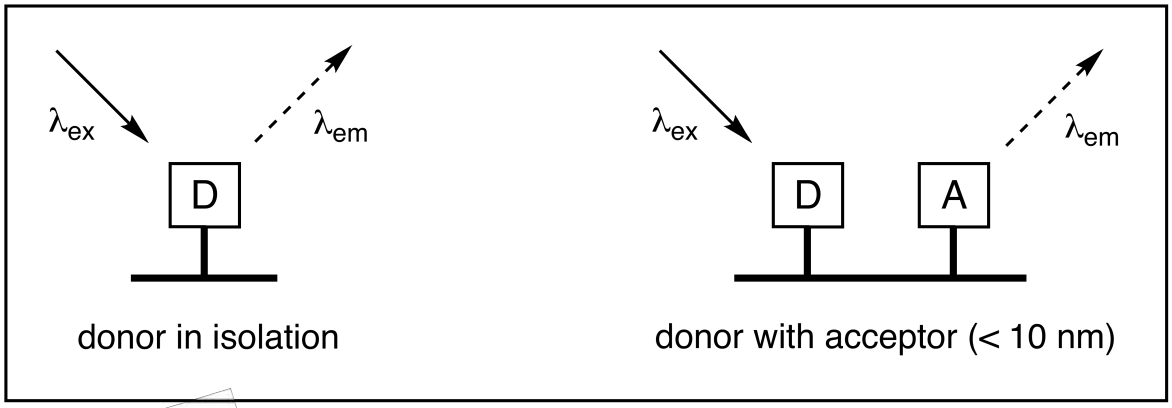
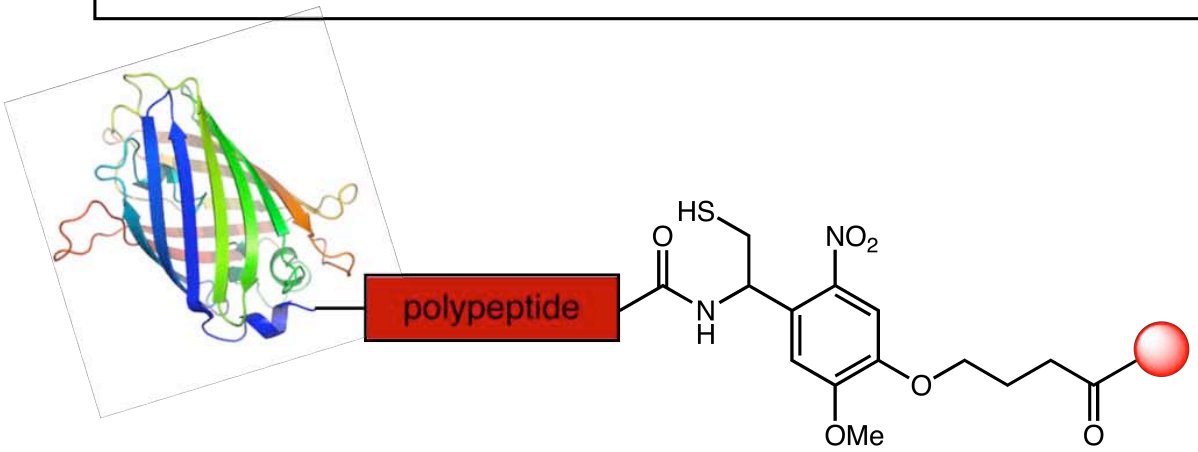
Chemical Ligation

Fluorescence Resonance Energy Transfer (FRET)





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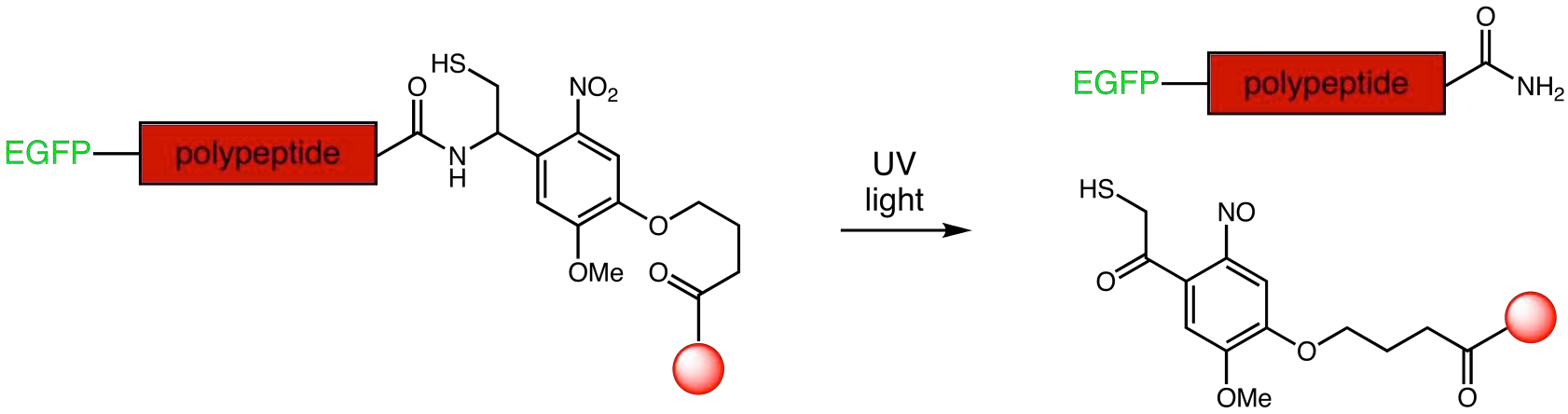
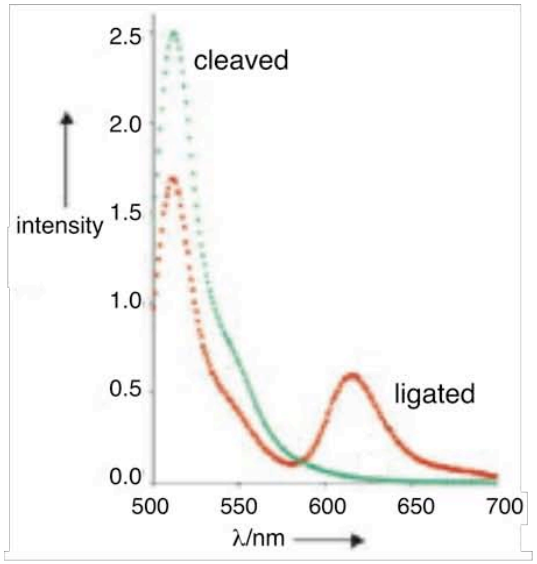
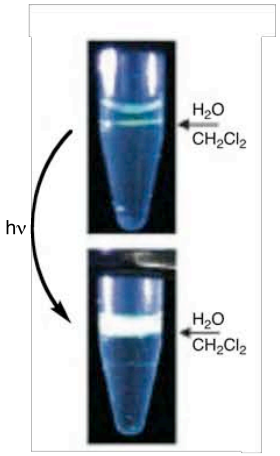
Chemical Ligation

- *in vitro* photocleavage







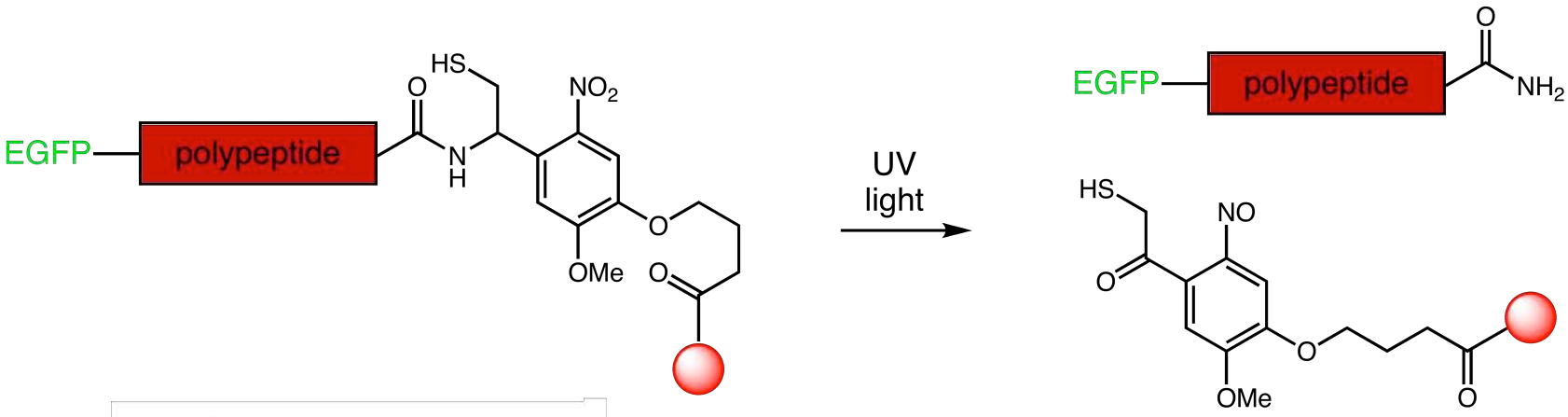
protein at interface

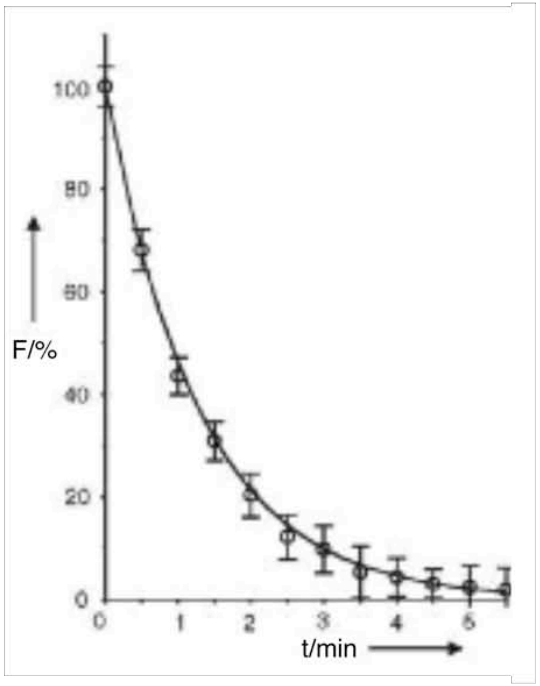
protein in aqueous layer

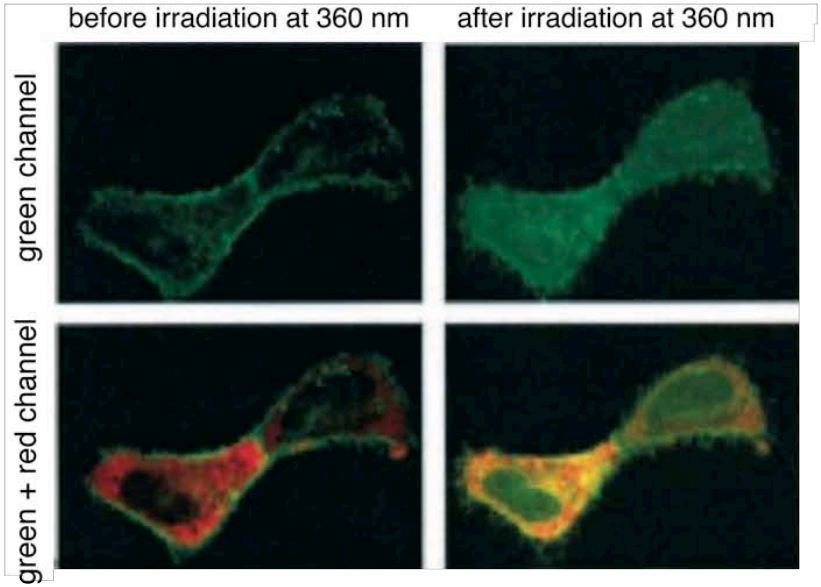
Pellois, J-.; Muir, T. W. *Angew. Chem. Int. Ed.* **2005**, *44*, 5713.

Chemical Ligation

- *in vivo* photocleavage (microinjection into Hela cells)

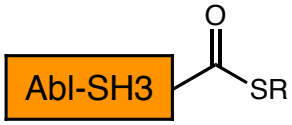






Chemical Ligation

- Photolysis of protein in a cell
- Dosable manner with low-intensity UV light
- Valuable if different protein concentrations trigger different responses
- Photolysis of small molecule can change function of protein



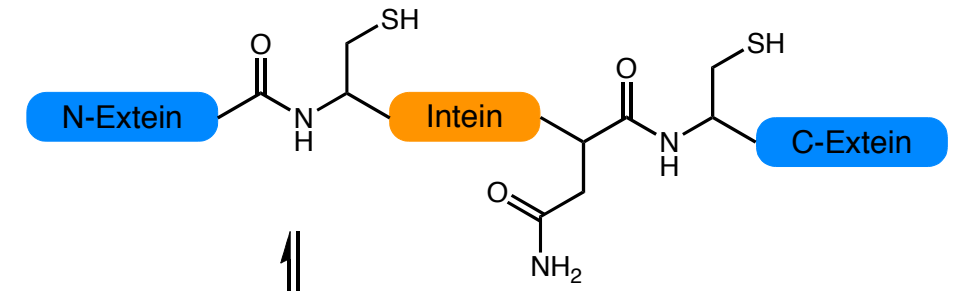
How are protein-bearing thioesters constructed?

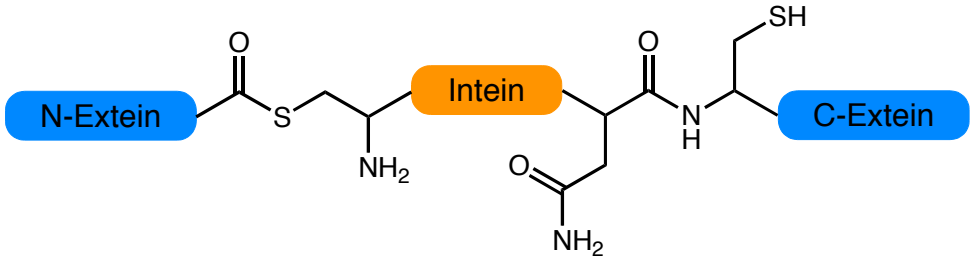
- Total synthesis
- Expression

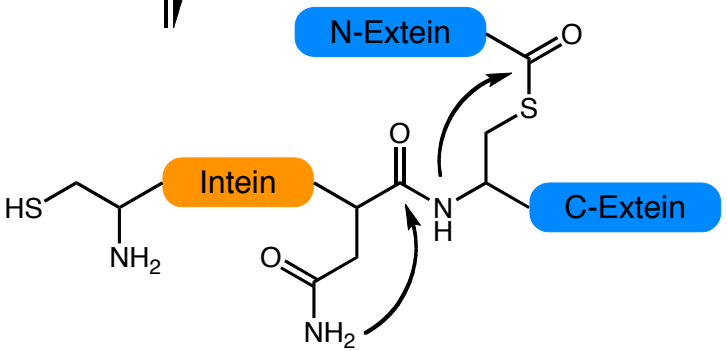
Expressed Protein Ligation

Expressed Protein Ligation (EPL)

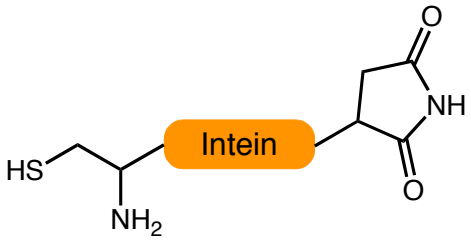
Protein Splicing

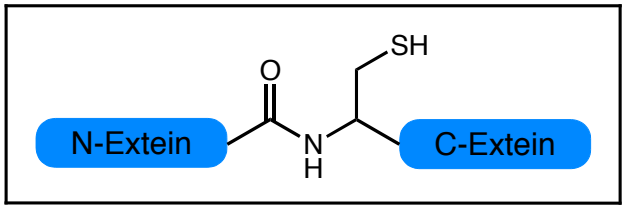






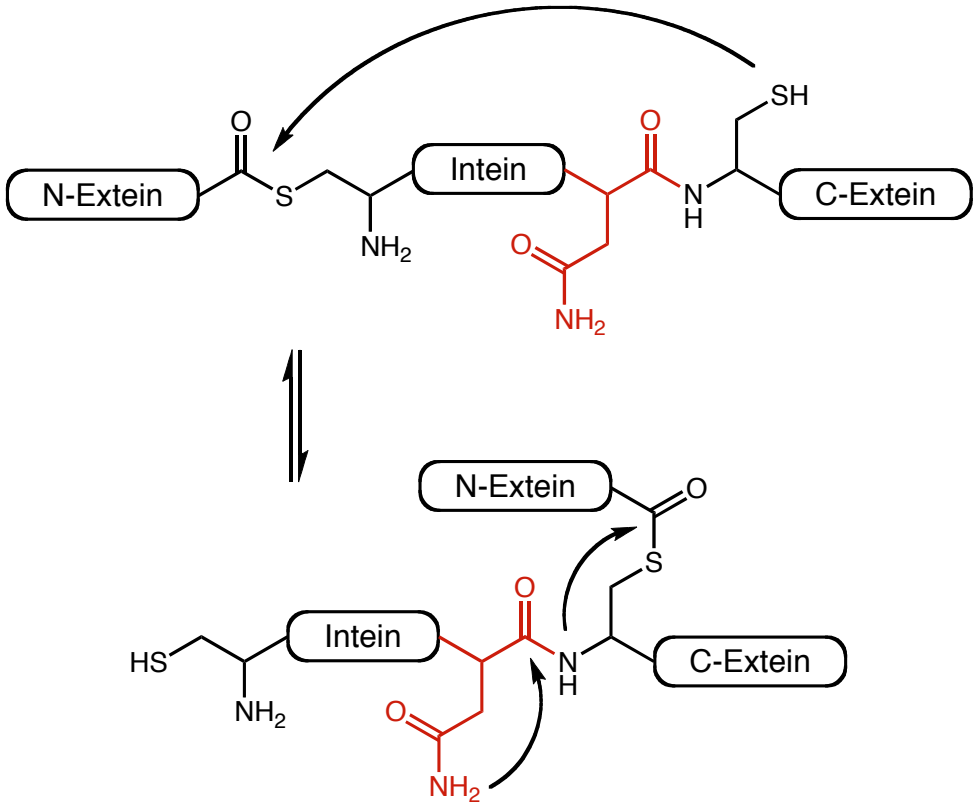
■ No sequence requirements on exteins





Muir, T. W. *Annu. Rev. Biochem.* **2003**, *72*, 249.

Expressed Protein Ligation (EPL)



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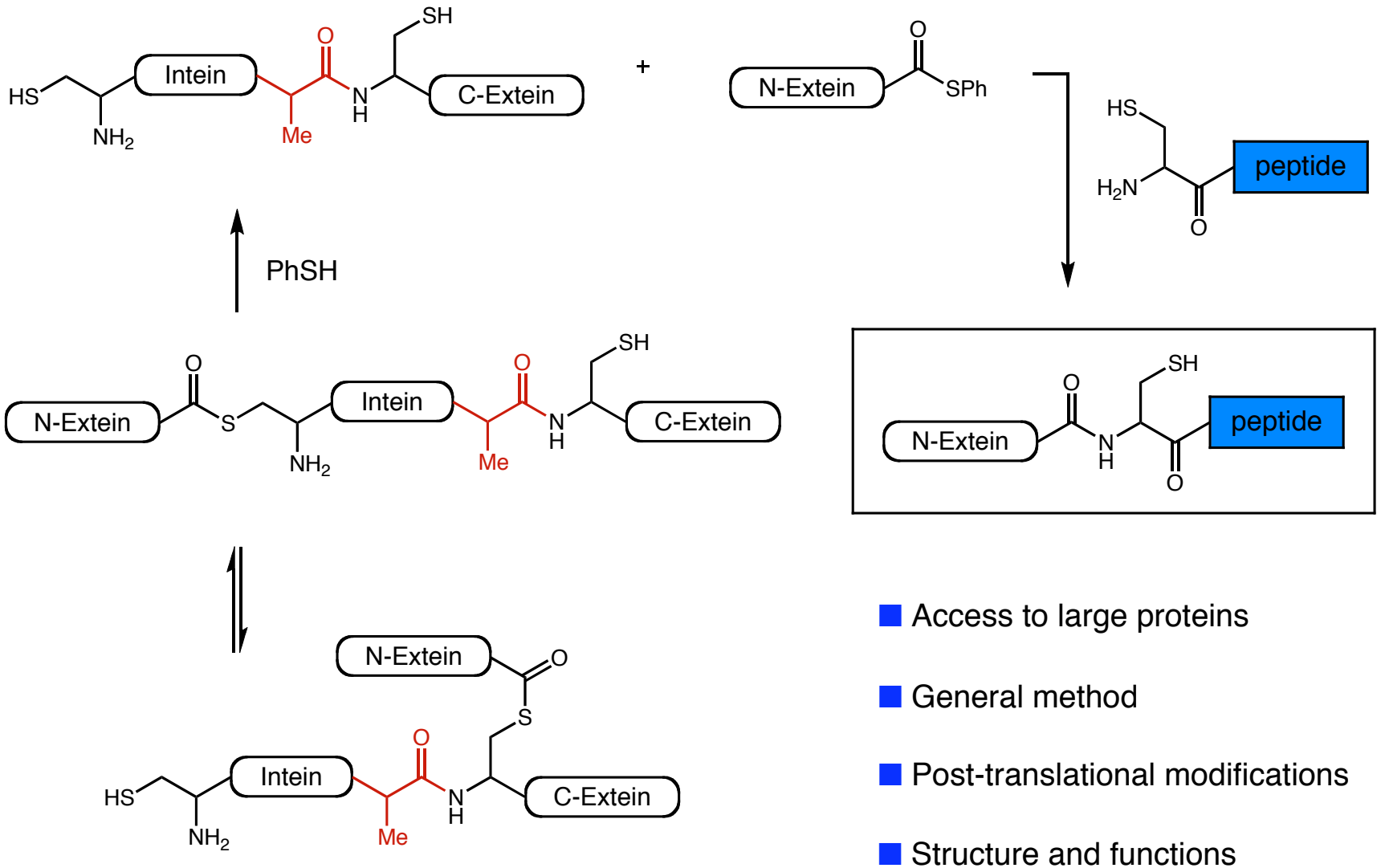
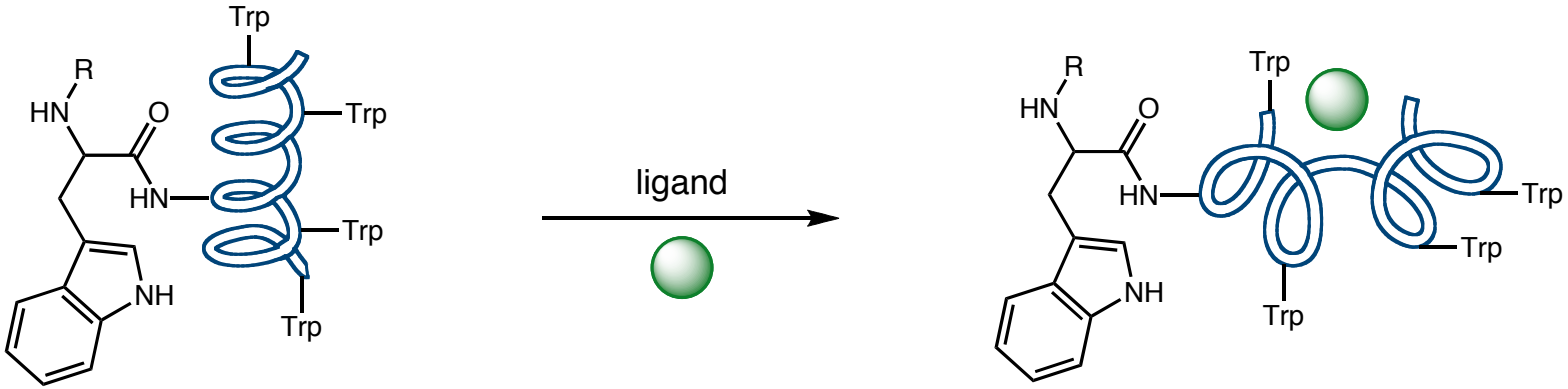


- Access to large proteins
- General method
- Post-translational modifications
- Structure and functions

Expressed Protein Ligation (EPL)

Protein Conformation

- Trp intrinsic fluorophore - sensitive to local environment



- Risks of destabilizing protein or altering function
- Trp analogs well suited for studying structure (lack of techniques)

Expressed Protein Ligation (EPL)

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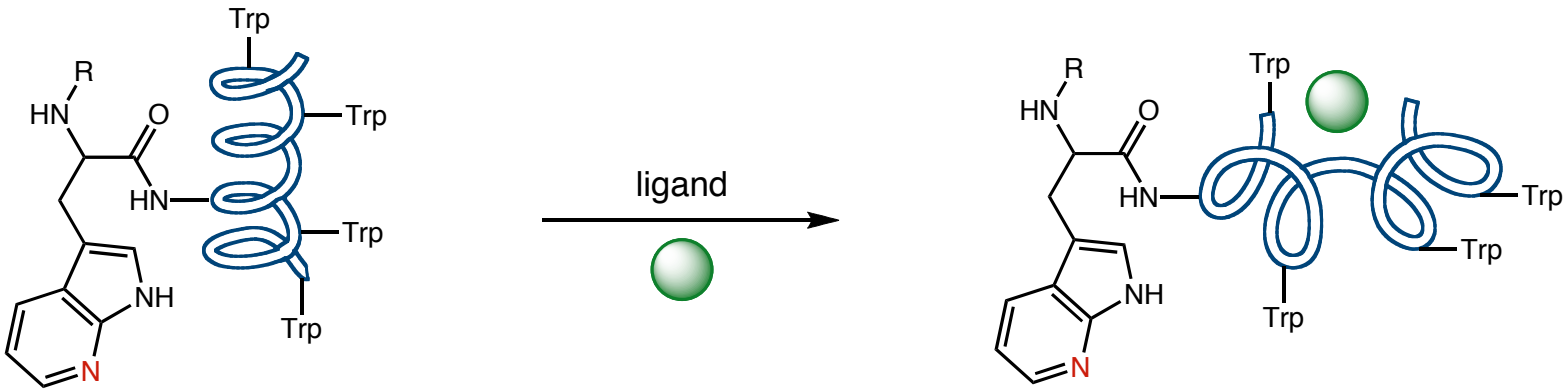

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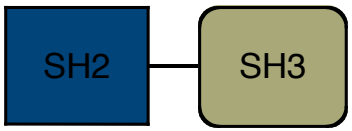
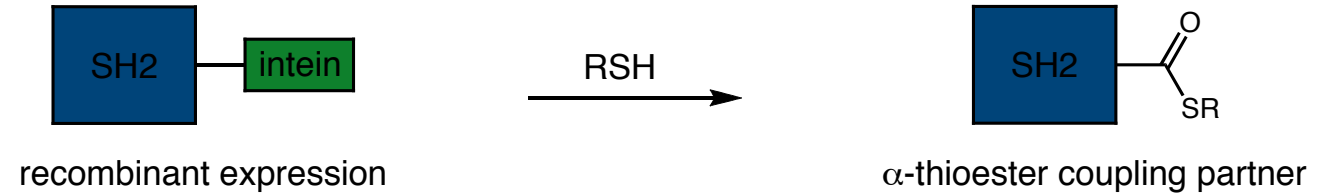


c-Crk-I

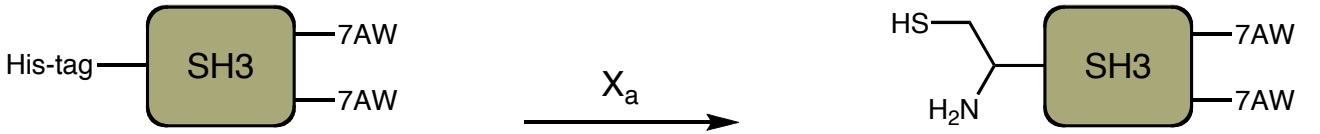
- Dynamic properties of SH3 domain
- Previously studied in isolation
- Domain-specific biophysical information

Expressed Protein Ligation (EPL)



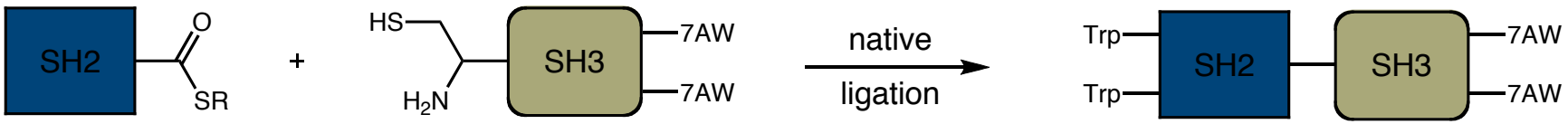
recombinant expression

α-thioester coupling partner



recombinant expression
(*E. coli* Trp auxotroph)

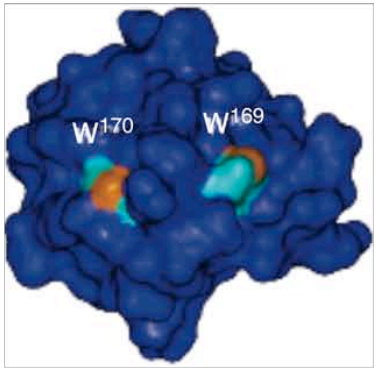
Terminal cys coupling partner



c-Crk-I

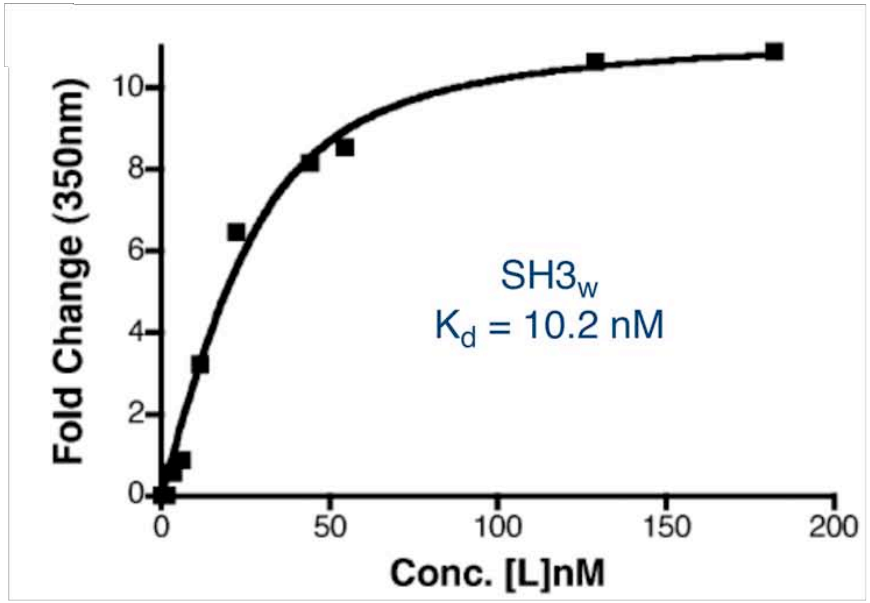
Muir, T. W.; *et al. J. Am. Chem. Soc.* **2004**, *126*, 14404.

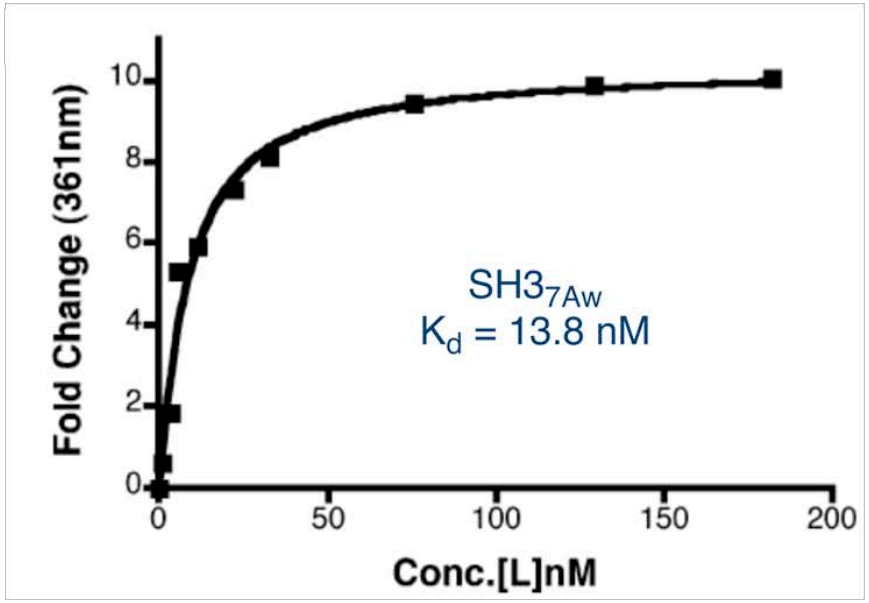
Expressed Protein Ligation (EPL)

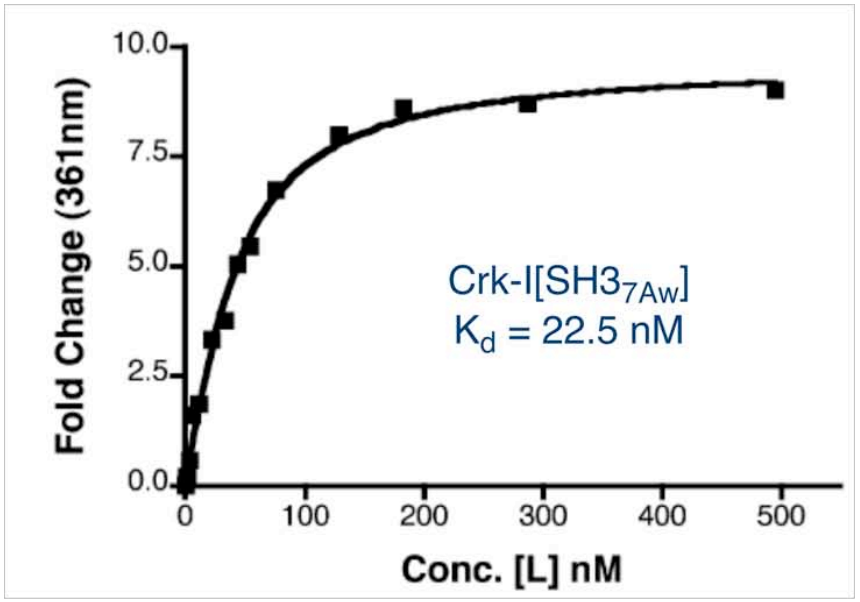


SH3 domain of c-Crk-I adapter protein

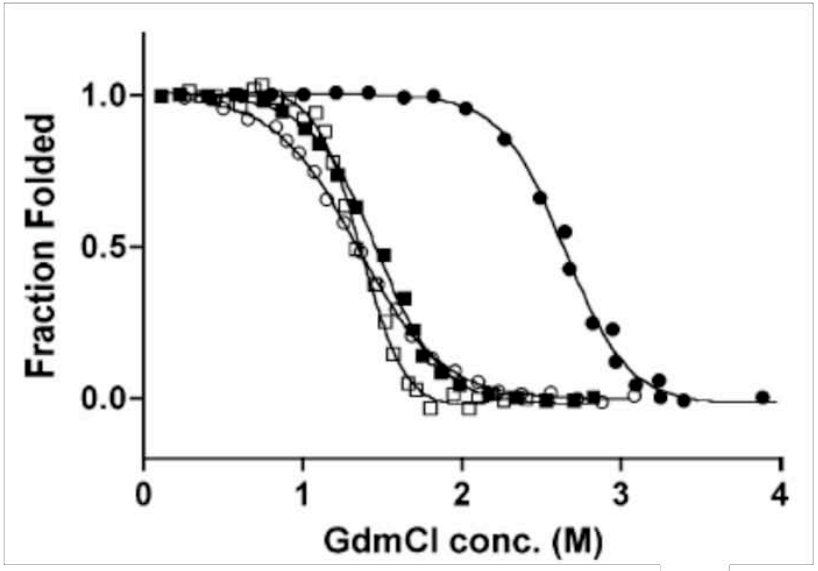
- W^{169} involved in ligand binding (poly-Pro peptide)
- W^{170} component of hydrophobic core







Expressed Protein Ligation (EPL)

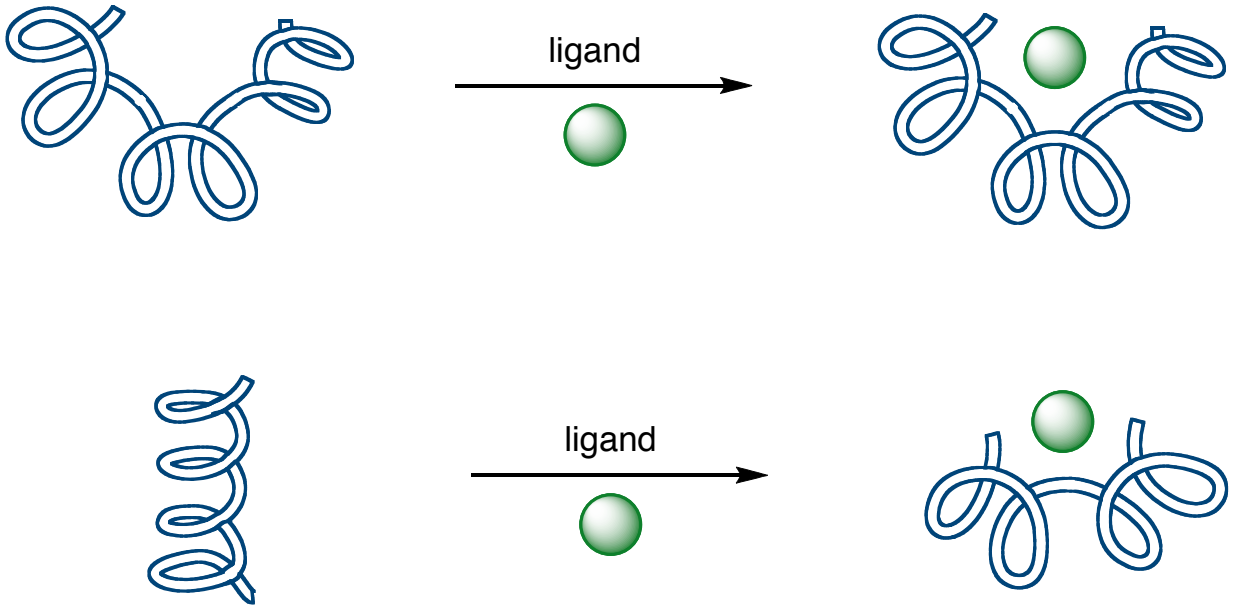


- Stability stidies validate Trp substitution
- Isolated SH3 and c-Crk-I protein have similar thermodynamic properties
- First direct comparison of SH3 to multidomain protein
- Application of EPL with two recombinant peptide fragments

Small Molecule Protein Splicing

- Temporal control over protein function with small molecules
- Turning protein on/off is difficult with standard genetic techniques

Chemical Genetics



Mootz, H. D.; Muir, T. W. *J. Am. Chem. Soc.* **2002**, *124*, 9044.

Small Molecule Protein Splicing

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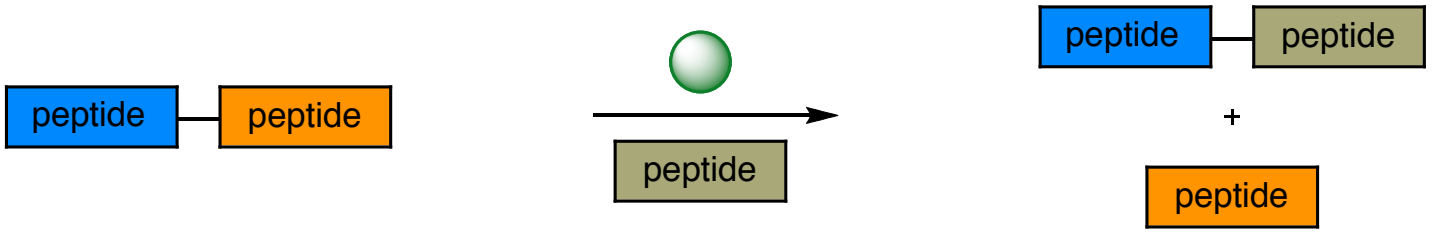
- Turning protein on/off is difficult with standard genetic techniques

Chemical Genetics

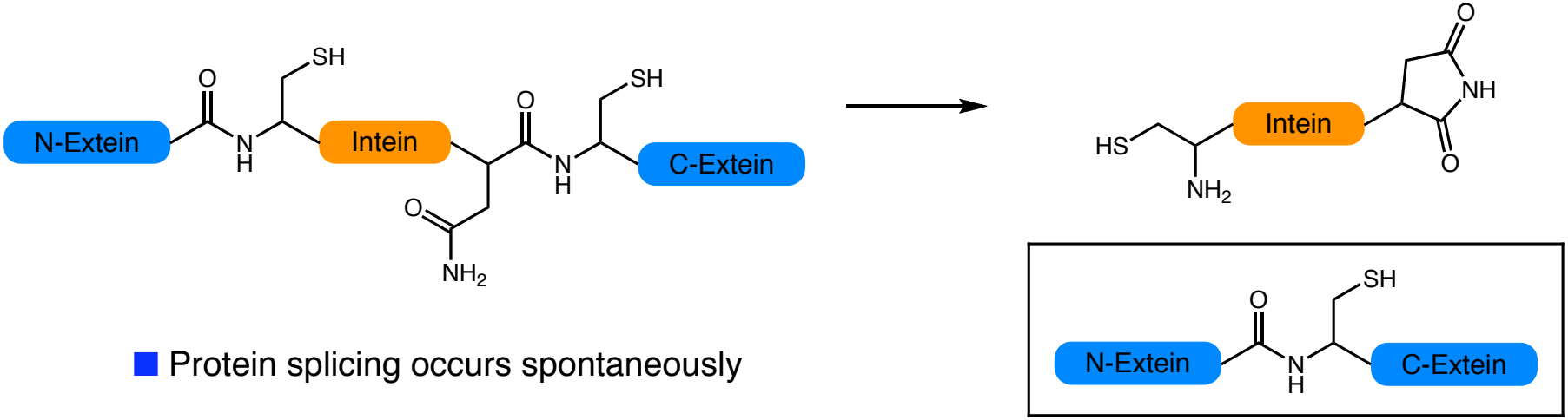


- Dramatic changes in primary structure lead to changes in function

Conditional Protein Splicing

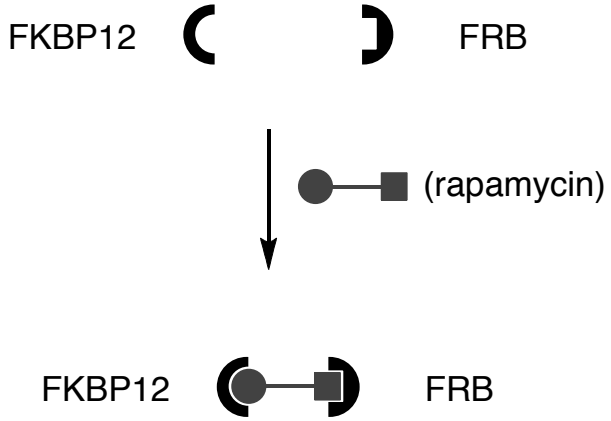
Mootz, H. D.; Muir, T. W. *J. Am. Chem. Soc.* **2002**, *124*, 9044.

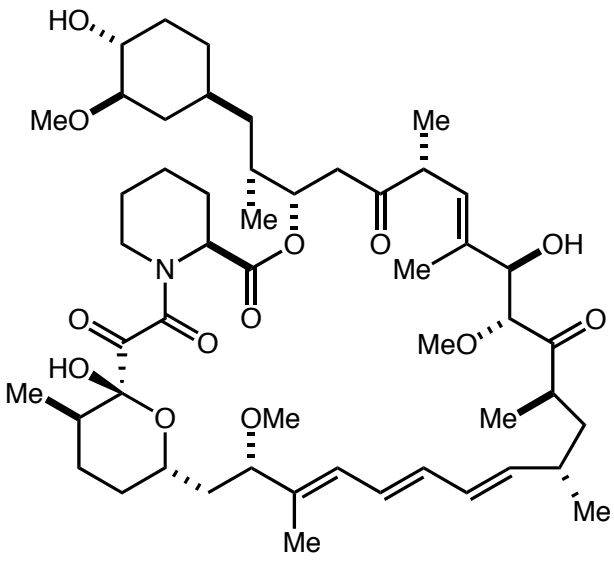
Small Molecule Protein Splicing



■ Protein splicing occurs spontaneously

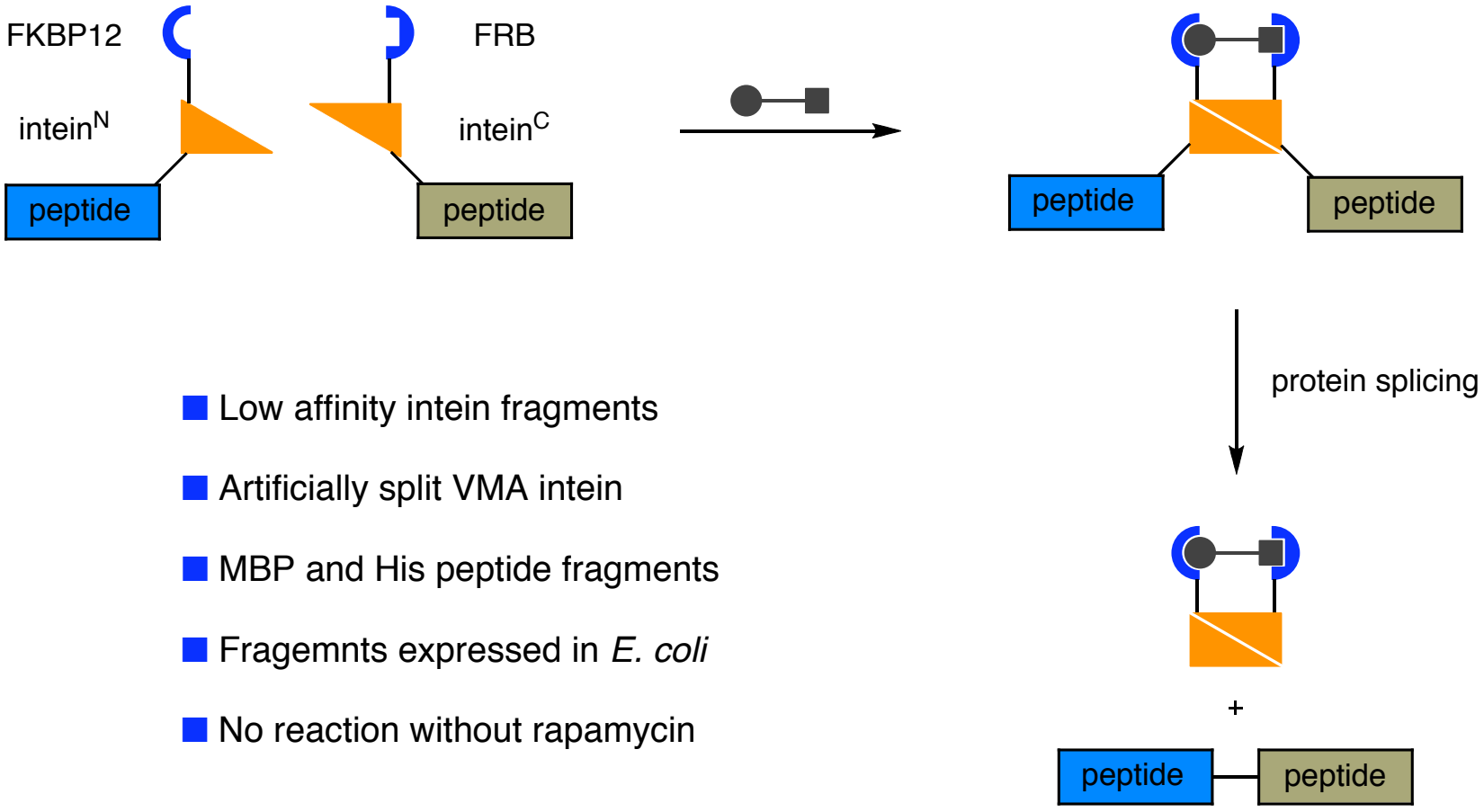
Conditional Protein Splicing = Protein Splicing + Molecule-Induced Heterodimerization





Small Molecule Protein Splicing

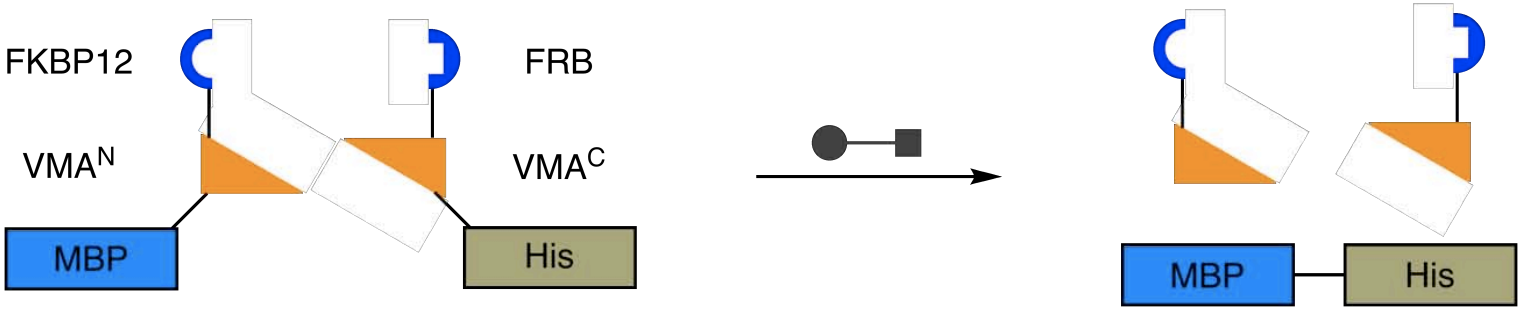
Conditional Protein Splicing = Protein Splicing + Molecule-Induced Heterodimerization

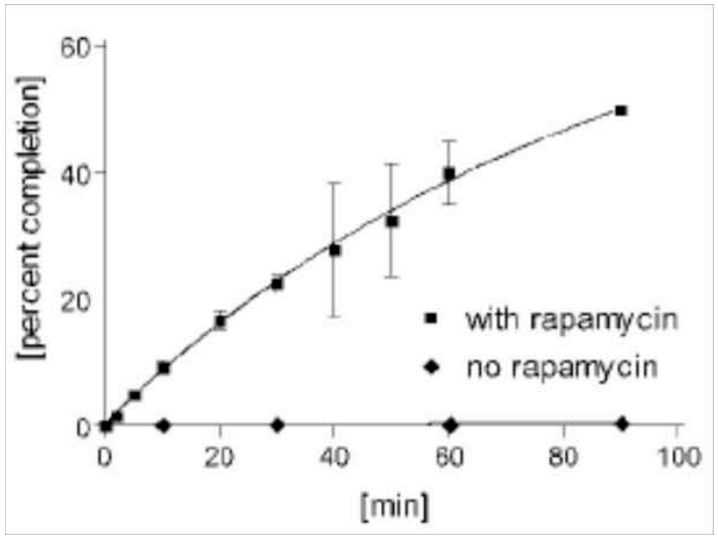


- Low affinity intein fragments
- Artificially split VMA intein
- MBP and His peptide fragments
- Fragemnts expressed in *E. coli*
- No reaction without rapamycin

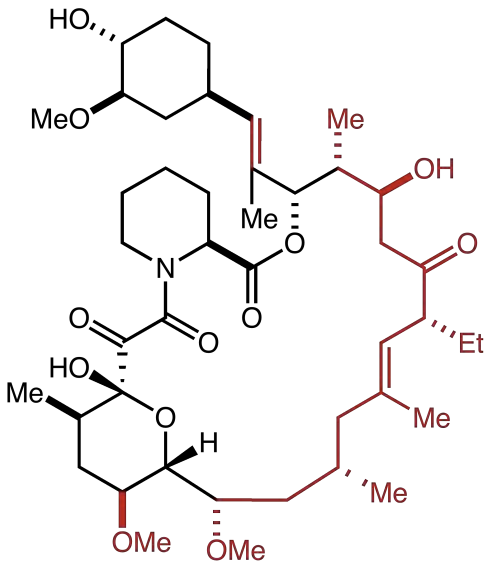
Figures adapted from Mootz, H. D.; Muir, T. W. *J. Am. Chem. Soc.* **2002**, *124*, 9044.

Small Molecule Protein Splicing





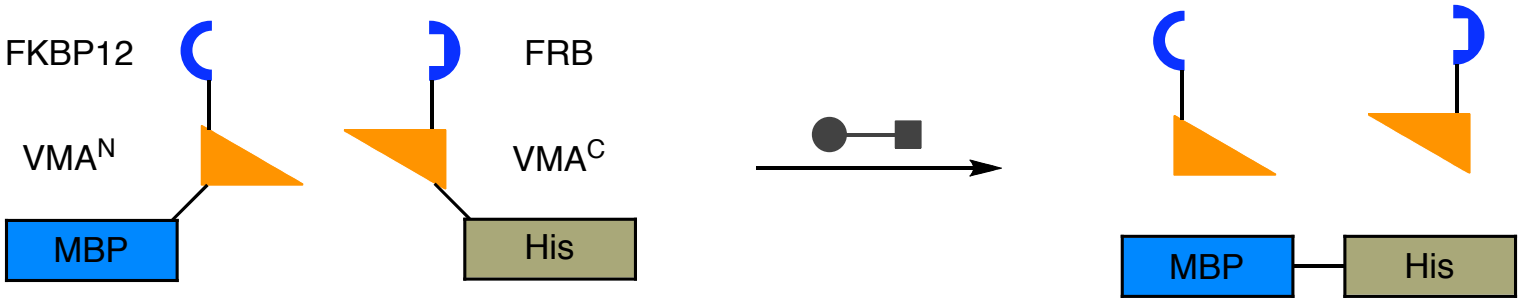
4-fold decrease with ascomycin



ascomycin

(only binds FKBP)

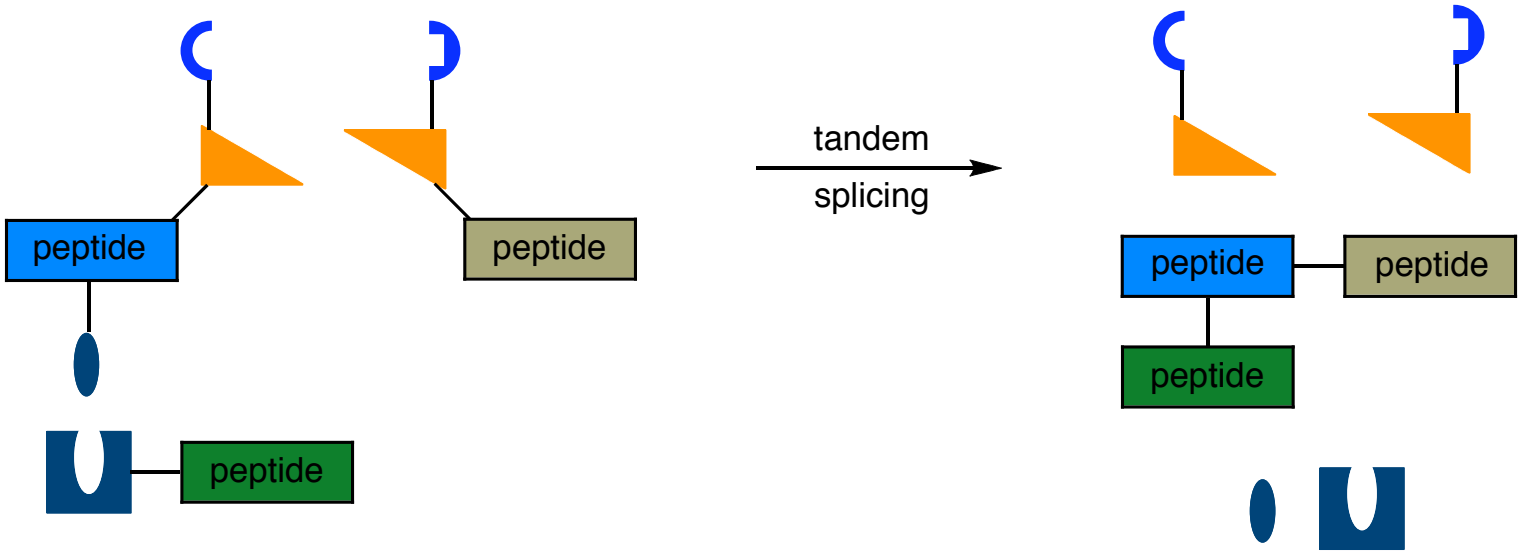
Small Molecule Protein Splicing



- First example of protein splicing by small molecule
- MBP and His are model protein
- No structural or sequence restrictions to exteins

Is this technique limited to the coupling of two peptide fragments?

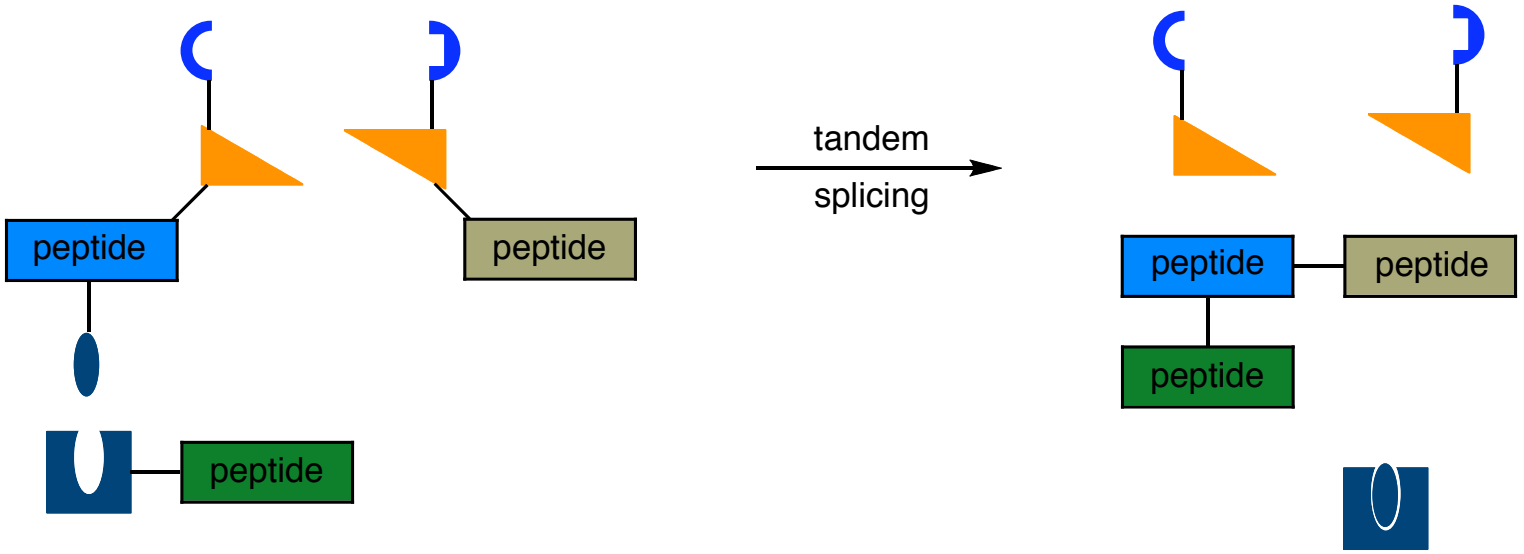
Small Molecule Protein Splicing



Potential difficulties:

- Intein specificity
- Timing of splicing events
- Occurs under physiological conditions
- Starting materials only required in low concentrations

Small Molecule Protein Splicing

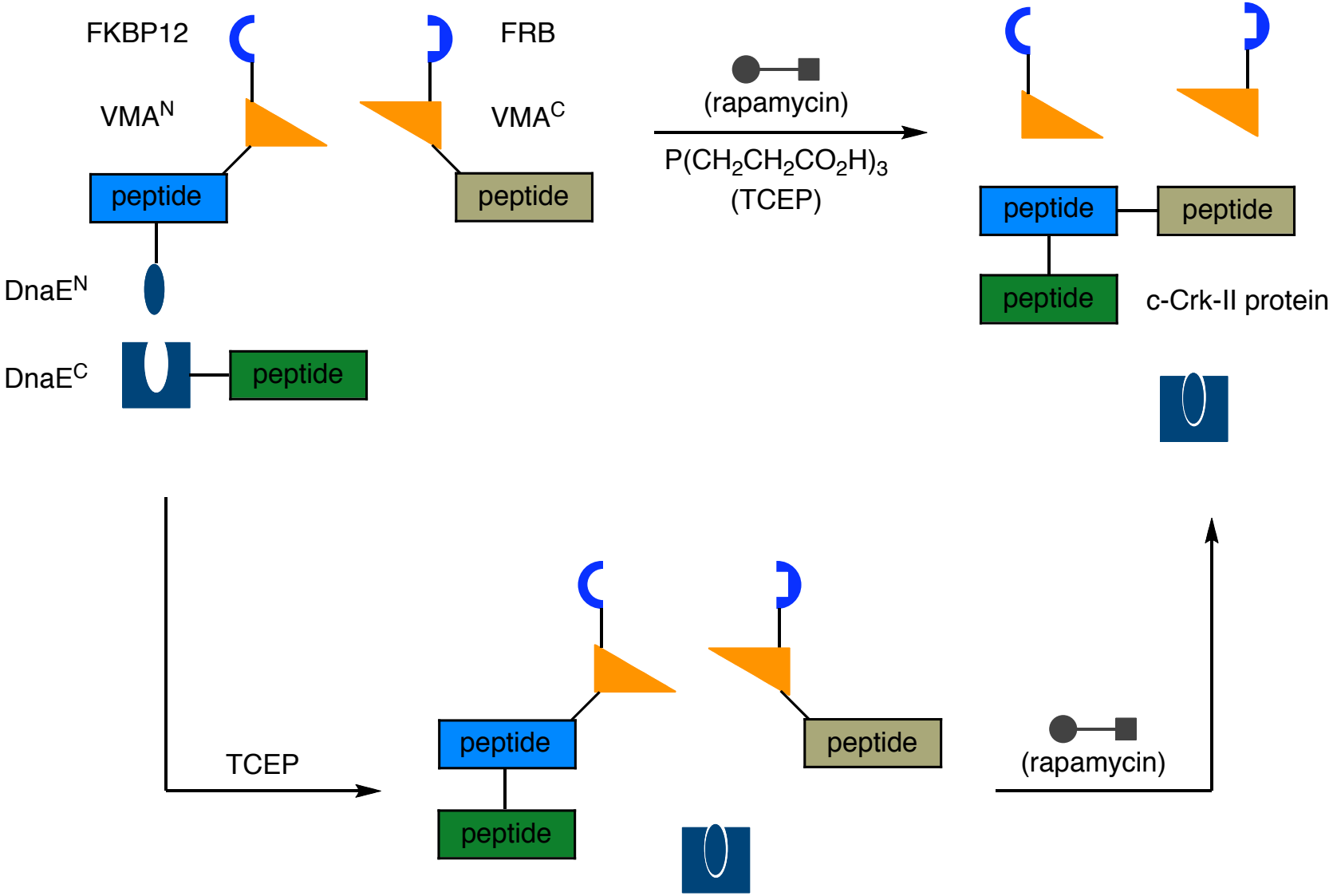


DnaE^{N} DnaE^{C}

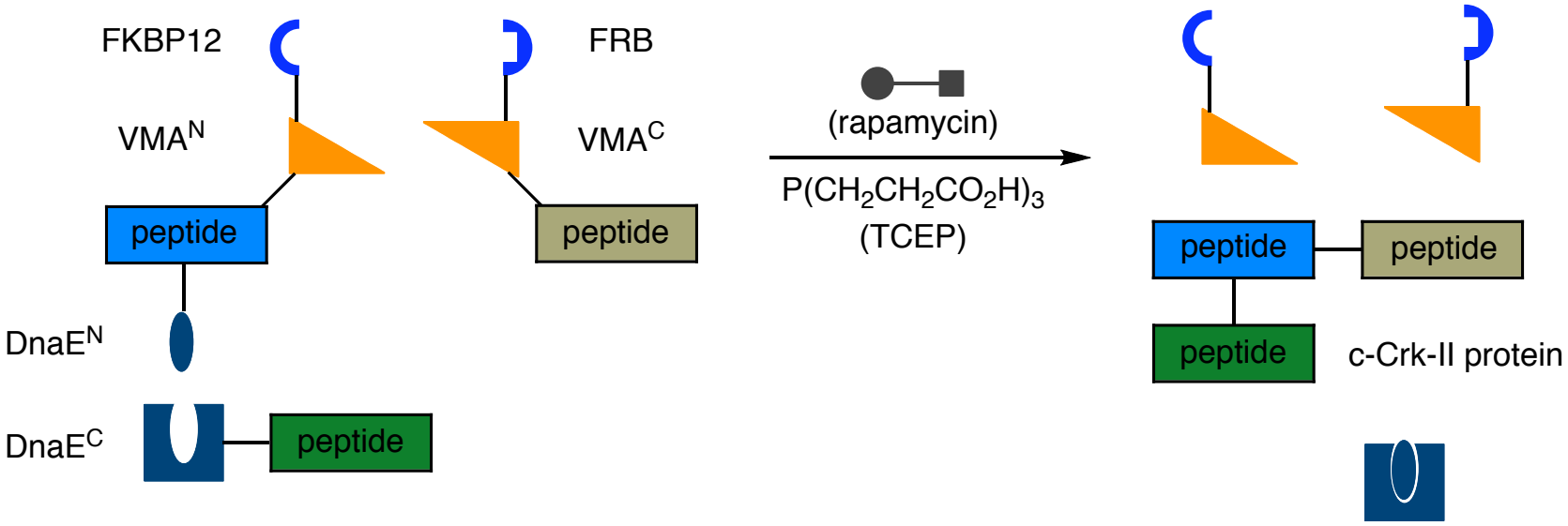
naturally split intein

- Essentially nothing is known about molecular recognition
- Why do naturally split intein spontaneously splice?
- Suggests intrinsic affinity difference between two intein types

Small Molecule Protein Splicing

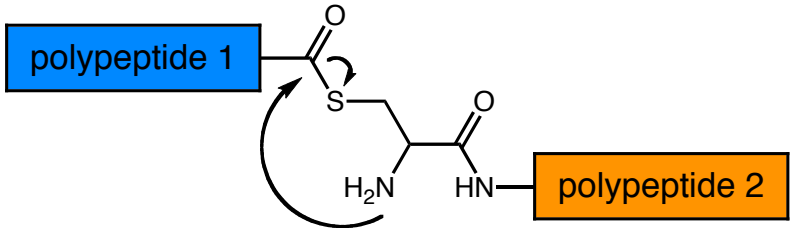


Small Molecule Protein Splicing

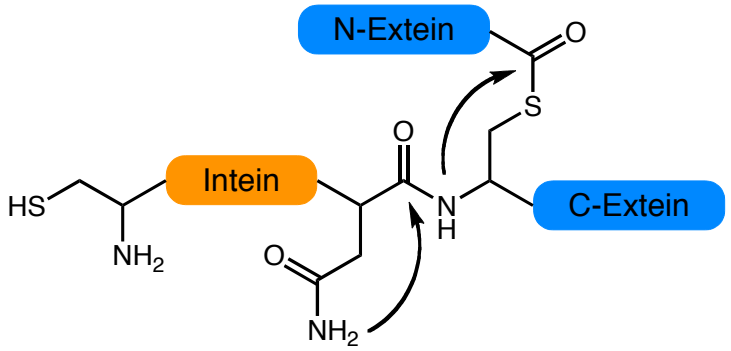


- Splicing can occur simultaneously or in a stepwise fashion
- Additional tags and protecting groups for easy isolation purification
- Proficient with purified fragments or crude cell lysates
- Streamlined process this great generality

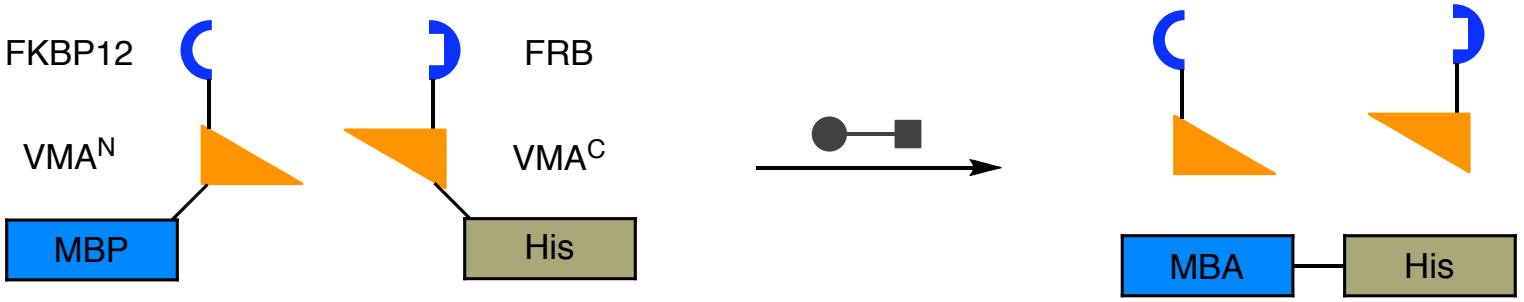
Summary



Native Chemical Ligation



Expressed Protein Ligation



Conditional and Tandem Protein Splicing