CHAPTER 1: INTRODUCTION

1.1 Introduction

1.1.1 Chemistry and the brain

The brain is the most complex organ of a vertebrate's body. It is the central command center that governs our perceptions, thoughts, feelings, movements and memory. The human brain weighs approximately 1.4 kg and contains roughly one hundred billion neurons.[1] Each neuron makes an estimated ten thousand connections, or synapses, with other neurons, forming a communication network of staggering complexity that has been the focus of intense study. Each neuron has one axon and many dendrites. Signaling between neurons occurs between the axon of one cell and a dendrite of another at a gap called the synapse cleft

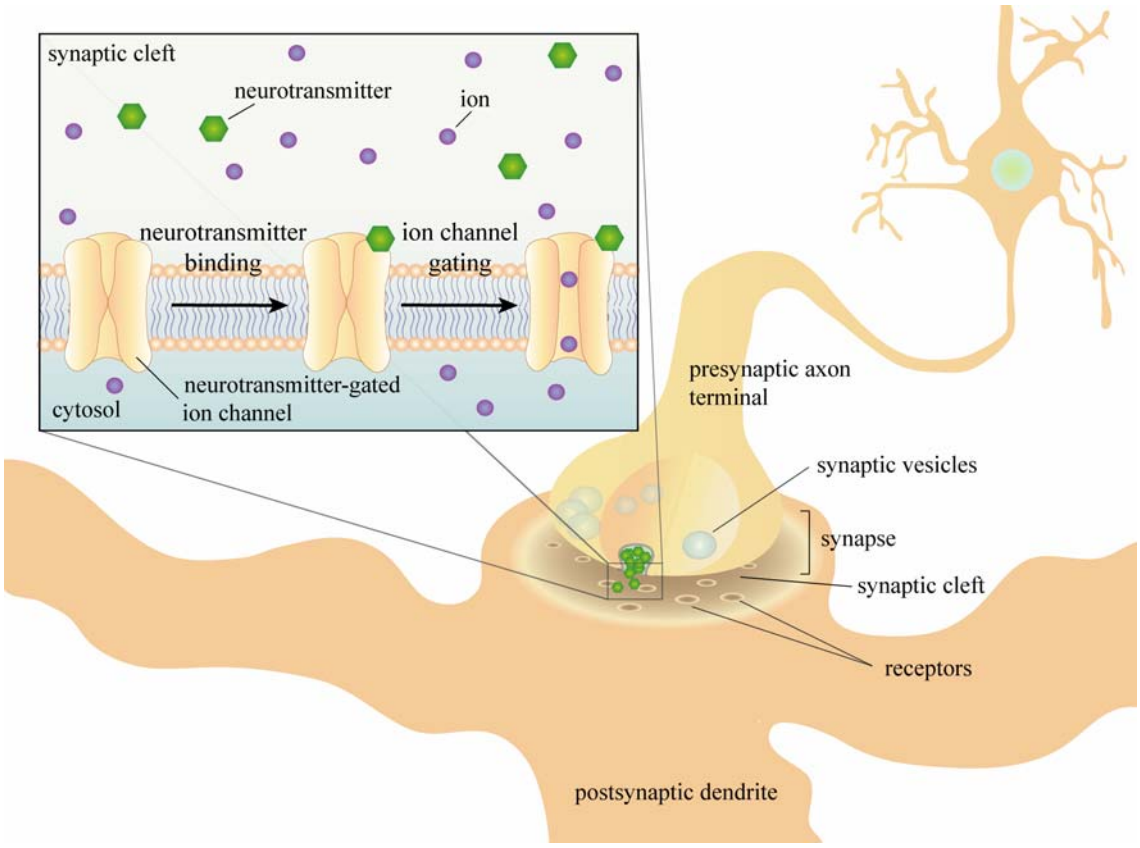


Figure 1.1. Synaptic transmission between neurons. Neurotransmitters are released from the presynaptic axon terminal into the synaptic cleft. The neurotransmitters bind to ion channels embedded in the membrane of the postsynaptic dendrite of a neighboring neuron. The ion channel is activated and gated, allowing for ions to flow, thus generating an electrical current.

(figure 1.1). Synaptic transmission is initiated when the presynaptic axon terminal releases small molecules called "neurotransmitters" from synaptic vesicles. These neurotransmitters are released at the synapse where they diffuse across the synaptic cleft to the postsynaptic dendrite of a neighboring neuron. The neurotransmitters bind to receptors embedded in the membrane of this dendrite, causing activation of the receptor, conveying the synaptic signal across the cell membrane.

Ion channels are a specialized class of receptors that form a pore across the membrane. They undergo an opening and closing process, known as gating, in response to stimuli such as neurotransmitter binding, voltage, or mechanical stress. During synaptic transmission, neurotransmitters bind to their partner ion channels which results in the gating of these ion channels (figure 1.1, inset). Ions pass through the pore of the channel constituting an electrical current, thus converting a chemical signal into an electrical signal. This electrical signal can then effect other changes in the neuron resulting in release of neurotransmitters. In this manner a signal is propagated from neuron to neuron via a sequence of chemical signals and electrical signals.

1.2 Chemical methodologies aimed at understanding protein function

1.2.1 Unnatural amino acid incorporation

Understanding protein function is at the heart of experimental biology. This endeavor frequently requires the generation of proteins that are modified to contain chemical probes or proteins that are homogeneous in naturally occurring posttranslational modifications, such as glycosylation or phosphorylation. The generation of these modified proteins is not available via standard ribosomal synthesis. Two methodologies have emerged as powerful methods to

generate modified proteins—native chemical ligation/expressed protein ligation and nonsense suppression. Developed by the Kent laboratory in 1994, native chemical ligation involves the reaction of two fully unprotected synthetic fragments under aqueous conditions, at physiological pH, resulting in the formation of a native peptide bond at the ligation site.[2] Expressed protein ligation is an extension of native chemical ligation where a synthetic or recombinant peptide fragment is chemoselectively ligated to a recombinantly expressed protein.[3, 4] This methodology allows for the generation of larger quantities of modified proteins than nonsense suppression; however, it does not allow for *in vivo* biochemical studies. Furthermore, the application of native chemical ligation/expressed protein ligation in the synthesis of membrane proteins has been limited due to several major technical challenges. While nonsense suppression may not afford the quantities needed for some biochemical studies, it does allow for the *in vivo* modification of proteins for biological studies.

For over a decade, the use of nonsense (stop codon) suppression methodology to incorporate unnatural amino acids, *in vitro* and *in vivo*, has expanded our ability to manipulate protein structure. Modification of proteins by conventional mutagenesis is limited by the twenty natural amino acids, whereas the incorporation of unnatural amino acids is limited by our synthetic ability. Consequently, precise changes can be made to the chemical properties of a specific amino acid enabling one to study electrostatic and steric effects (e.g., hydrogen bonding, cation-π, polarity, size, nucleophilicity, and redox potential). Furthermore, this methodology has been used to site-specifically introduce spectroscopic probes, posttranslational modifications, photoaffinity labels, and other chemical moieties in a protein of interest.[5-10] It has also been used to introduce "chemical handles" with orthogonal

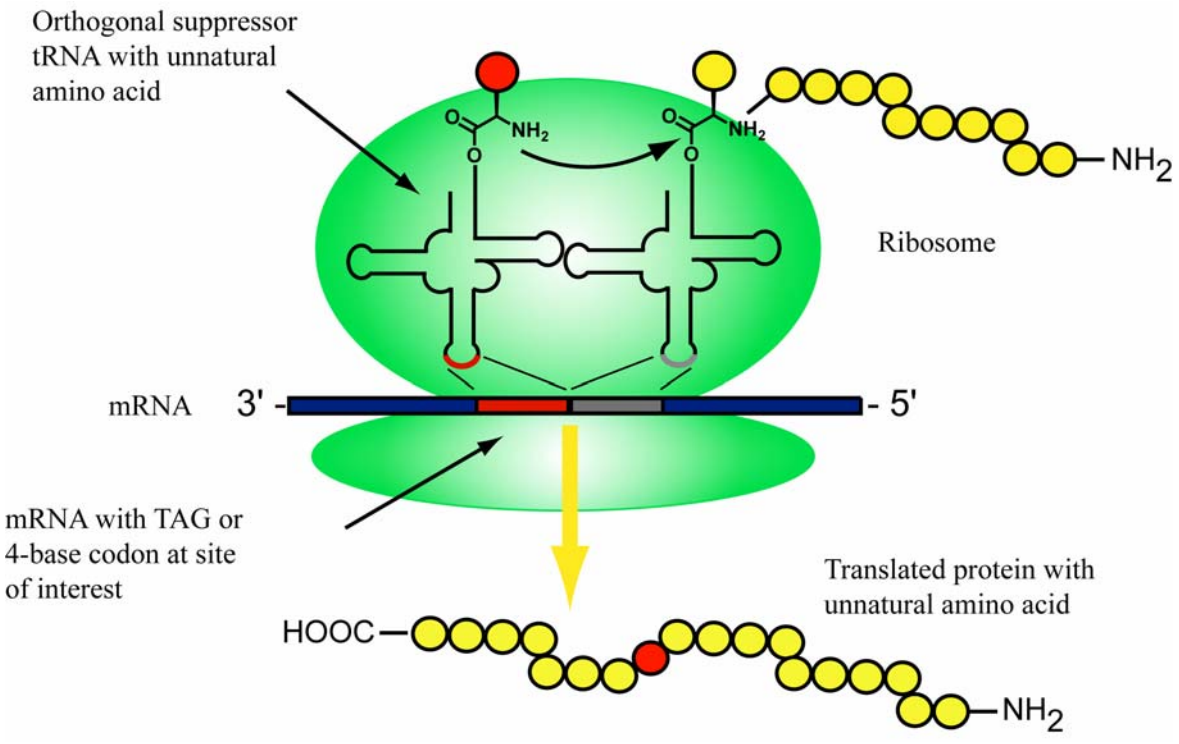


Figure 1.2. Nonsense suppression methodology.

chemical reactivities such that a native protein can be selectively modified under mild conditions with a variety of reagents without protecting groups.[9]

In 1989, Schultz et al. first introduced the biosynthetic incorporation of an unnatural amino acid using nonsense suppression and an *in vitro* translation system.[11-13] The fundamental approach to unnatural amino acid incorporation through nonsense suppression is shown in Figure 1.2. This methodology utilizes a noncoding or "nonsense codon"—typically a stop codon—to replace the codon for the amino acid of interest within a particular gene. This is achieved through conventional site-directed mutagenesis. Subsequently, a tRNA against this "nonsense codon" is chemically acylated with the desired unnatural amino acid and provided to the translational machinery with the modified gene of interest.[14] The translational machinery proceeds with protein translation, site-specifically incorporating the

desired unnatural amino acid within the desired gene; thus producing the full length modified protein. This methodology has been extended for *in vivo* systems and has been demonstrated in a variety of biological systems including *E. coli*,[15] yeast,[16] mammalian cells,[17] and *Xenopus laevis* oocytes.[18] Additionally, the evolution of specific aminoacyl-tRNA synthetases has allowed for the *in vivo* acylation of the suppressor tRNA with intracellular levels of the unnatural amino acid in *E. coli*.[8]

1.2.1.1 Nonsense suppression in Xenopus laevis oocytes for the expression of ion channels

The extension of nonsense suppression methodology to incorporate unnatural amino acids to living cells was first demonstrated in 1995.[19] The basic methodology is shown in Figure 1.3. First, site-directed mutagenesis is used to replace the amino acid codon of interest with the amber stop codon TAG in the desired gene. *In vitro* transcription is then used to generate the corresponding mRNA with the appropriately placed UAG. A suppressor tRNA, containing the anticodon CUA, is then chemically acylated with the desired unnatural amino acid. The mRNA and the amino-acylated tRNA are subsequently co-injected into *Xenopus laevis* oocytes. The oocytes are incubated for a period ranging from 24 to 72 hours during which protein synthesis, transport and surface assembly occurs. The result is the generation of the desired protein with the unnatural amino acid incorporated at the site of interest. The channel can then be characterized by electrophysiology. A limitation of this methodology is that the quantity of the modified protein is determined by the amount of aminoacylated tRNA that is injected since the tRNA is not reacylated once it has been used in protein translation. Therefore, the amount of modified protein is stoichiometric to the amount of tRNA injected. This limitation is minimized by the use of electrophysiology for

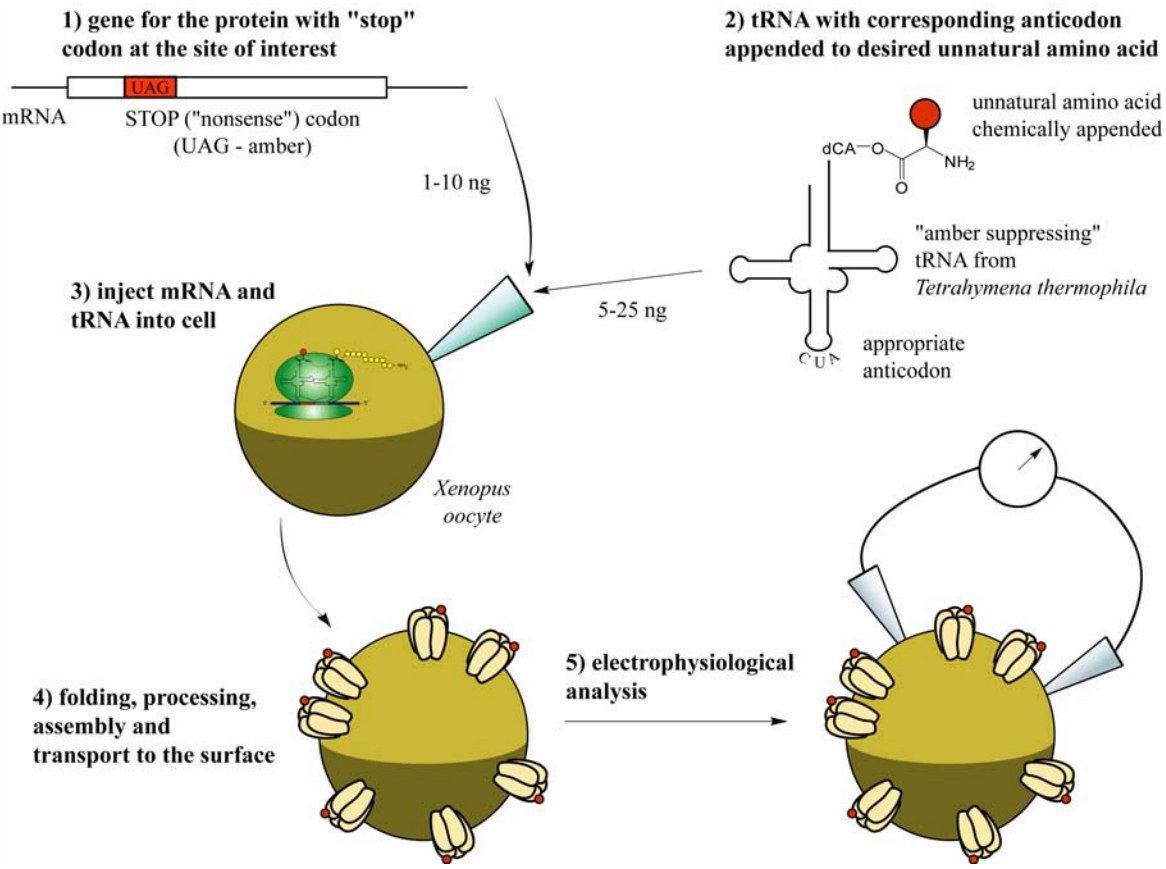


Figure 1.3. Schematic of *in vivo* nonsense suppression methodology using *Xenopus* oocytes for structure-function studies of ion channels.

the analysis of ion channels. Electrophysiology is a highly sensitive technique, allowing for functional characterization with very small amounts of expression ion channels.

The use of nonsense suppression for unnatural amino acid incorporation in *Xenopus laveis* oocytes requires the generation of an activated precursor—namely the aminoacyl suppressor tRNA (figure 1.4). This process begins with the synthesis of the desired unnatural amino acid. The free amino acid is amino protected, typically with a protecting group that can be removed with photolysis, such as a nitroveratryloxycarbonyl (NVOC) group. Conversion of the carboxylic acid to a cyanomethyl ester activates the amino acid for coupling to the dinucleotide dCA. Coupling of the unnatural amino acid is achieved via

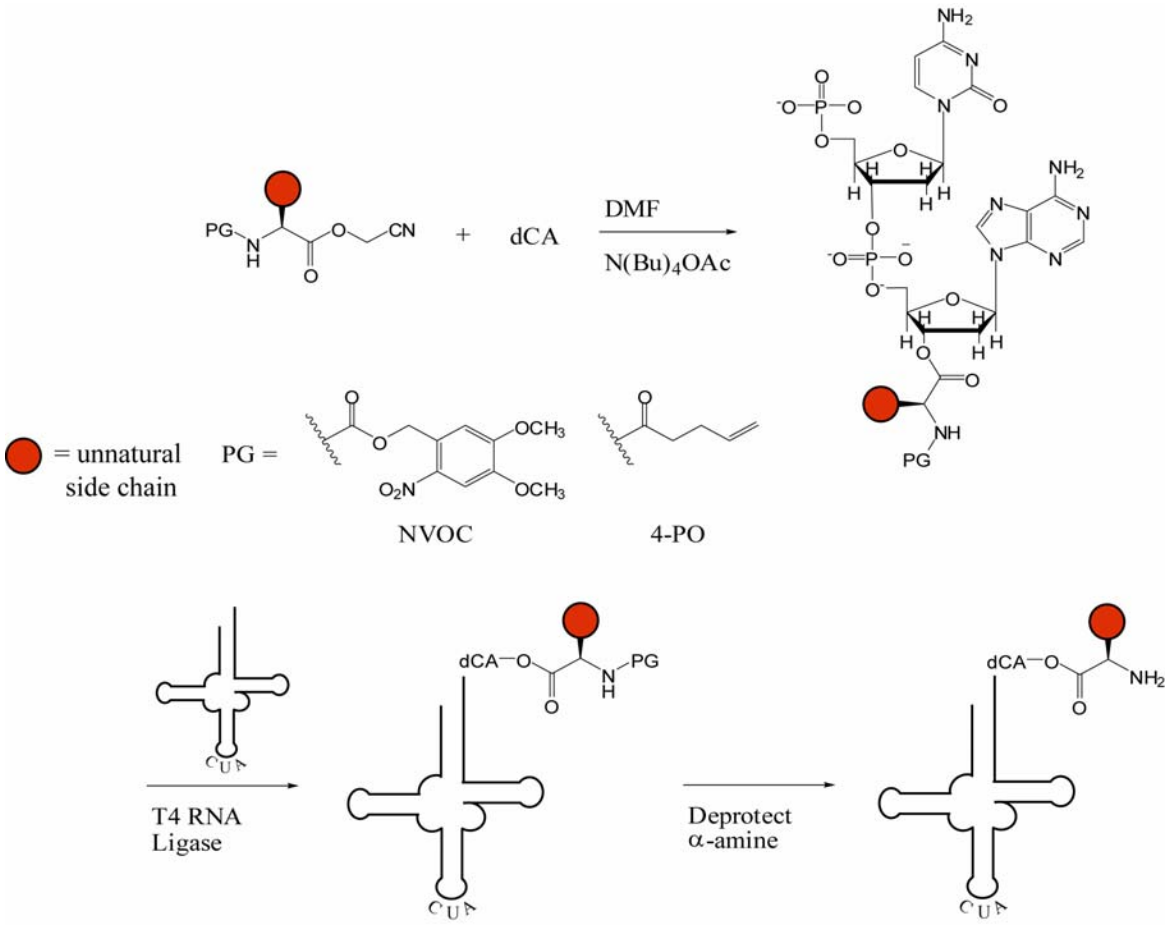


Figure 1.4. Chemical acylation of tRNA to append unnatural amino acids.

treatment with *tert*-butyl ammonium in the presence of dCA in dimethyl formamide (DMF). The dCA amino acid is enzymatically ligated to expressed 74-mer tRNA to produce the desired full length 76-base-pair aminoacyl tRNA. Immediately prior to injection of the charged tRNA, the amino-protecting group of the unnatural amino acid is removed by photolysis.

To date, over one hundred unnatural amino acids have been incorporated into proteins for *in vivo* biological studies in *Xenopus laevis* oocytes (figure 1.5). Nonsense suppression

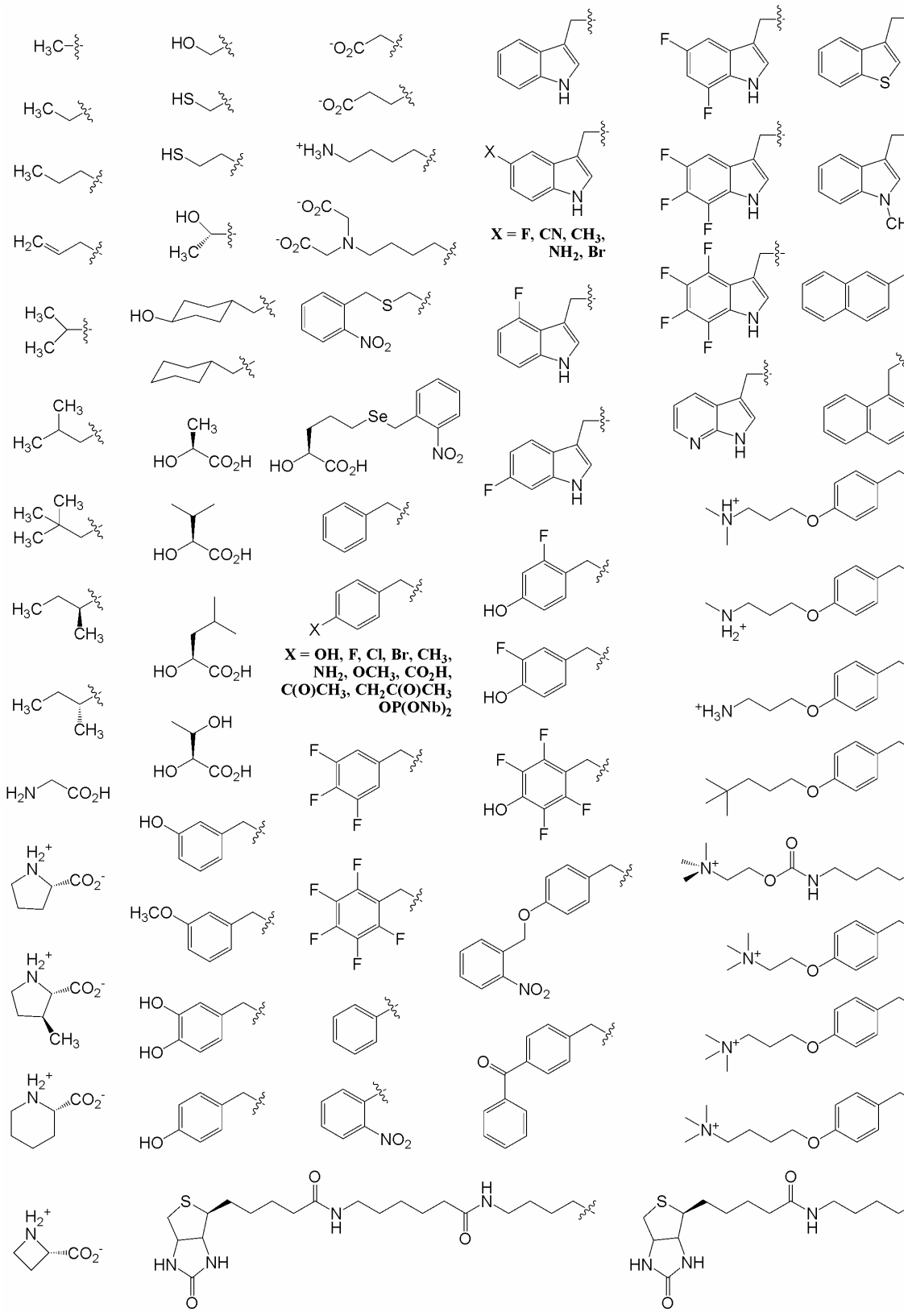


Figure 1.5. Representative unnatural amino acids that have been incorporated into membrane proteins using *in vivo* nonsense suppression in *Xenopus* oocytes.

has been used to study a variety of chemical interactions such as cation-pi interactions and hydrogen bonding in ion channels. It has also been used to incorporate backbone mutations as well as biophysical probes and fluorophores. Recently, multiple unnatural amino acids were site-specifically incorporated into proteins expressed in *Xenopus* oocytes using a combination of nonsense suppression and frameshift suppression.[20] This advancement should allow for greater structure-functions studies and FRET studies in ion channels.

1.2.2 Photoaffinity probes

The specific recognition of a bioactive ligand by its binding partners is a major event in biological processes; therefore the elucidation of the binding partners often provides invaluable information in the understanding of biological processes. The identification of binding partners may also facilitate the development of therapeutics. A variety of biological techniques have been developed aimed at identifying binding partners of a specific interaction; however the identification of binding partners is often hampered by low binding affinities due to the dynamic nature of these interactions. Photoaffinity labeling has emerged as a powerful technique to identify binding partners/proteins as well as to study how these binding partners interact.[21] This method overcomes low binding affinities by photochemically introducing a covalent bond between the ligand and binding partner.

Three photoreactive groups are commonly used in photoaffinity labeling—phenylazide, phenyldiazirine, and benzophenone (figure 1.6). Phenylazides are the mostly widely used photoaffinity label and upon photolysis at 300 nm they generate a highly reactive nitrene intermediate that forms nitrogen-carbon bonds or a more labile nitrogen-heteroatom bond. A drawback to the use of phenylazides is that the singlet phenylnitrenes

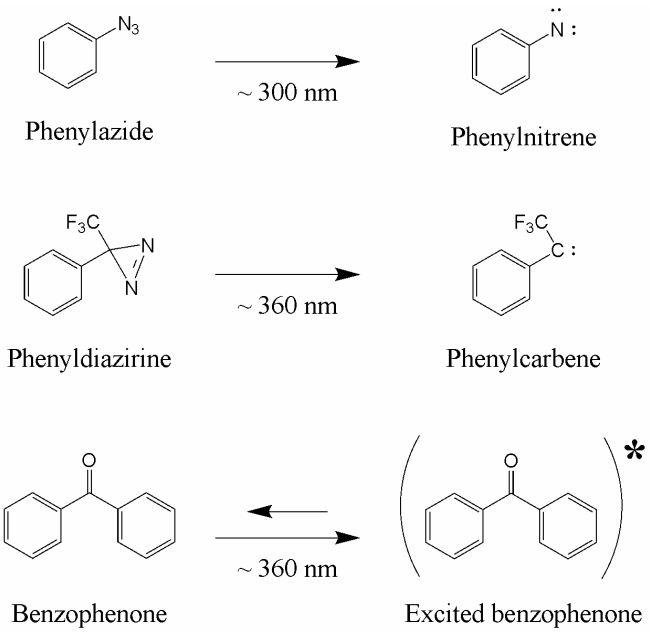


Figure 1.6. Three main classes of photoreactive reagents.

are short lived and rapidly rearrange into long-lived electrophiles, ketenimines, which are unreactive to nonactivated C-H bonds.[22] This can lead to nonspecific labeling. Furthermore, the lower wavelength of activation for azides of 300 nm can cause damage to proteins which is not seen with photoreactive groups with an activation wavelength of 360 nm. Phenyldiazirines form a carbene intermediate when photolyzed at 360 nm. The carbine is considered to be more reactive than a typical nitrene.[23] Similar to phenylazides, photolysis of phenyldiazirines also leads to long-lived electrophiles, diazo-isomers, which lead to nonspecific labeling. The formation of long-lived electrophiles in the case of both phenylazides and phenyldiazirines has been eliminated with the use of electron-withdrawing fluorine. Fluorination of the phenyl ring in phenylazides was shown to prevent the formation of ketenimines during photolysis.[24, 25] For phenyldiazirines, the trifluoromethyl substitution was shown to stabilize the diazo-functionality as shown by Brunner et al. with 3-trifluoromethyl-3-phenyldiazirine.[26] Benzophenone is part of the "carbonyl" photoreactive group and is unique from other photoreactive agents in that photo-dissociation does not occur. Excitation of benzophenone at 360 nm generates a reactive triplet state of the carbonyl that can undergo C-H bond

insertion. The excited state of the carbonyl relaxes to the ground state if C-H bond insertion does not occur; consequently, a higher cross-linking yield often can be achieved with repeated excitation.[27]

1.2.2.2 Applications of photoaffinity reagents in biological studies

The aforementioned photoaffinity probes have all been used in a variety of biological applications. They have been used to generate photoreactive DNA, photoreactive proteins, and photoreactive small molecules that act as probes. Both phenylazides and benzophenone derivatives have been extensively used for biological studies due to the ease of accessibility. Phenylazide derivatives are easily synthesized or are commercially available. Benzophenone derivatives are also commercially available. The use of phenyldiazirines in biological studies has been limited since the synthesis of phenyldiazirines is difficult and few are commercially available.

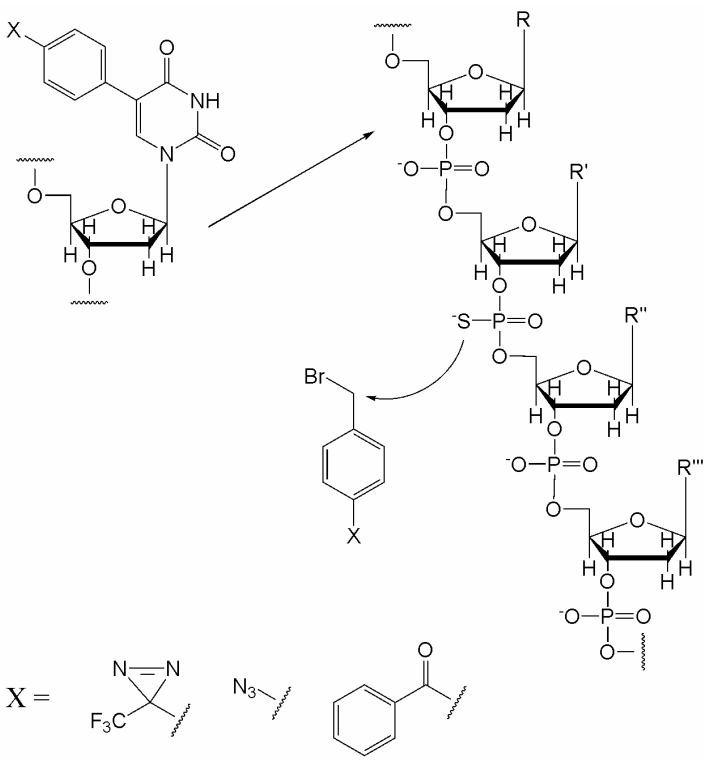
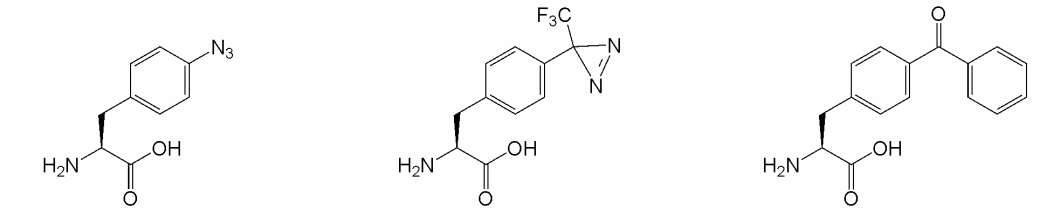


Figure 1.7. Two methods of generating photoreactive DNA.

The incorporation of photoreactive moieties into DNA has been used extensively to study interactions between nucleotides and proteins. Photoreactive nucleobases, in which the photoreactive bases

are substituted for normal bases, have been used to study transcriptional complexes,[28, 29] chromatin remodeling complexes[30, 31] and chromatin structure.[32] Photoreactive moieties have also been incorporated into DNA via the phosphate backbone in which one more of the desired phosphodiesters have been replaced with phosphorothioates. The sulfur undergoes nucleophilic deplacement of a bromide derivative of the photoreactive moiety, thus attaching the photoreactive moiety at the desired site (figure 1.7). This method of generating photoreactive DNA has been used to study binding interactions in transcriptional complexes and of transcriptional regulators.[33-36]

The use of photoaffinity reagents has also contributed extensively to the study of protein-protein interactions.[37-42] They have been incorporated into peptides and proteins to determine contact regions between binding partners. For example, this methodology has been used to determine the inner-strand contact region of β-amyloid peptide.[43] Typically photoreactive amino acids are incorporated into peptides using standard solid-phase synthesis or solution-phase synthesis (figure 1.8).[37-42, 44, 45] For larger proteins, photoreactive moieties have been incorporated using three main methods: 1) chemoselective incorporation via reaction of the bromide reagent with cysteine residues within the protein,[46-48] 2) with the use of a cell-free translation system,[49-54] and 3) *in vivo* using nonsense suppression



p-azido-L-phenylalanine

L-4'-(3-(trifluoromethyl)-3*H*-diazirine-3-yl)phenylalanine

p-benzoyl-L-phenylalanine

Figure 1.8. Photoreactive amino acids.

methodology.[5, 6, 55] The last methodology was used to incorporate *p*-benzoyl-L-phenylalanine site-specifically into human Grb2 protein in mammalian Chinese hamster ovary cells.[56] Irradiation of the cells resulted in the cross-linking of human Grb2 with its known binding partner, epidermal growth factor (EGF).

An extension of photoaffinity labeling is the generation of multifunctional probes for the identification and isolation of binding partners to proteins and ligands. This methodology utilizes the photoaffinity reagent to overcome weak binding affinities that often hamper conventional methods of isolating and identifying ligand/protein-protein binding partners and couples this reactivity to a purification handle, such as biotin, to allow for the facile isolation of the cross-linked complex. The isolated complex can then be analyzed using mass spectrometry to identify binding partners. A variety of biotinylated photoreactive probes has been synthesized and successfully used to study and isolate protein-substrates. Probes ranging from biotinylated photoactivated γ-secretase[57] inhibitors to bis-mannose photolabels to study glucose transporter isoform 4 (GLUT4)[58] have been synthesized. This methodology is particularly promising in the area of carbohydrate research in which lectins often exhibit low binding affinities.

1.3 Dissertation work

The studies contained in this dissertation are aimed at utilizing chemistry to understand neurobiology and neuronal communication. Chapters 2 and 3 both address the gating of ion channels, describing structure-function studies to shed light on the gating mechanisms of two classes of ion channels. Chapter 2 studies the gating mechanism of the mechanosensitive channel of small conductance (MscS). MscS is voltage modulated and

Chapter 2 describes studies to elucidate the role of arginine residues in the voltage sensitivity of MscS. In Chapter 3, we utilized nonsense suppression to incorporate unnatural amino acids to study the gating of the cation-selective Cys-loop family of ion channel receptors. Specifically, it describes work aimed at elucidating the role of *cis-trans* isomerization of a proline residue in the gating mechanism of the serotonin-gated 5-hydroxy-tryptamine receptor 3A ($5\text{-}HT_{3A}$) and the nicotinic acetylcholine receptor. Lastly, Chapter 4 addresses the role of fucose-galactose carbohydrates in learning and memory. It aims to identify lectins to fucose-α(1-2)-galactose as well as identify the corresponding glycoproteins bearing fucose-α(1-2)-galactose.

1.4 References

1. Blinkov, S. M. i.; Glezer, I. i. a. I., *The human brain in figures and tables; a quantitative handbook*. Basic Books: [New York], 1968; p xxxii, 482 p.
2. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B., Synthesis of proteins by native chemical ligation. *Science* **1994,** 266, (5186), 776-9.
3. Muir, T. W.; Sondhi, D.; Cole, P. A., Expressed protein ligation: a general method for protein engineering. *Proc Natl Acad Sci U S A* **1998,** 95, (12), 6705-10.
4. Severinov, K.; Muir, T. W., Expressed protein ligation, a novel method for studying protein-protein interactions in transcription. *J Biol Chem* **1998,** 273, (26), 16205-9.
5. Chin, J. W.; Martin, A. B.; King, D. S.; Wang, L.; Schultz, P. G., Addition of a photocrosslinking amino acid to the genetic code of Escherichiacoli. *Proc Natl Acad Sci U S A* **2002,** 99, (17), 11020-4.
6. Chin, J. W.; Santoro, S. W.; Martin, A. B.; King, D. S.; Wang, L.; Schultz, P. G., Addition of p-azido-L-phenylalanine to the genetic code of Escherichia coli. *J Am Chem Soc* **2002,** 124, (31), 9026-7.
7. Chin, J. W.; Schultz, P. G., In vivo photocrosslinking with unnatural amino Acid mutagenesis. *Chembiochem* **2002,** 3, (11), 1135-7.
8. Wang, L.; Xie, J.; Schultz, P. G., Expanding the genetic code. *Annu Rev Biophys Biomol Struct* **2006,** 35, 225-49.
9. Wang, L.; Zhang, Z.; Brock, A.; Schultz, P. G., Addition of the keto functional group to the genetic code of Escherichia coli. *Proc Natl Acad Sci U S A* **2003,** 100, (1), 56-61.
10. Zhang, Z.; Gildersleeve, J.; Yang, Y. Y.; Xu, R.; Loo, J. A.; Uryu, S.; Wong, C. H.; Schultz, P. G., A new strategy for the synthesis of glycoproteins. *Science* **2004,** 303, (5656), 371-3.
11. Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G., A general method for site-specific incorporation of unnatural amino acids into proteins. *Science* **1989,** 244, (4901), 182-8.
12. Cornish, V. W.; Mendel, D.; Schultz, P. G., Probing Protein-Structure and Function with an Expanded Genetic-Code. *Angewandte Chemie-International Edition in English* **1995,** 34, (6), 621-633.
13. Cornish, V. W.; Benson, D. R.; Altenbach, C. A.; Hideg, K.; Hubbell, W. L.; Schultz, P. G., Site-specific incorporation of biophysical probes into proteins. *Proc Natl Acad Sci U S A* **1994,** 91, (8), 2910-4.
14. Nowak, M. W.; Gallivan, J. P.; Silverman, S. K.; Labarca, C. G.; Dougherty, D. A.; Lester, H. A., In vivo incorporation of unnatural amino acids into ion channels in Xenopus oocyte expression system. *Ion Channels, Pt B* **1998,** 293, 504-529.
15. Ryu, Y.; Schultz, P. G., Efficient incorporation of unnatural amino acids into proteins in Escherichia coli. *Nat Methods* **2006,** 3, (4), 263-5.
16. Chin, J. W.; Cropp, T. A.; Anderson, J. C.; Mukherji, M.; Zhang, Z.; Schultz, P. G., An expanded eukaryotic genetic code. *Science* **2003,** 301, (5635), 964-7.
17. Monahan, S. L.; Lester, H. A.; Dougherty, D. A., Site-specific incorporation of unnatural amino acids into receptors expressed in Mammalian cells. *Chem Biol* **2003,** 10, (6), 573-80.
18. Beene, D. L.; Dougherty, D. A.; Lester, H. A., Unnatural amino acid mutagenesis in mapping ion channel function. *Curr Opin Neurobiol* **2003,** 13, (3), 264-70.

19. Nowak, M. W.; Kearney, P. C.; Sampson, J. R.; Saks, M. E.; Labarca, C. G.; Silverman, S. K.; Zhong, W.; Thorson, J.; Abelson, J. N.; Davidson, N.; Schultz, P. G.; Dougherty, D. A.; Lester, H. A., Nicotinic Receptor-Binding Site Probed with Unnatural Amino-Acid-Incorporation in Intact-Cells. *Science* **1995,** 268, (5209), 439-442.
20. Rodriguez, E. A.; Lester, H. A.; Dougherty, D. A., In vivo incorporation of multiple unnatural amino acids through nonsense and frameshift suppression. *Proc Natl Acad Sci U S A* **2006,** 103, (23), 8650-5.
21. Brunner, J., New photolabeling and crosslinking methods. *Annu Rev Biochem* **1993,** 62, 483-514.
22. Li, Y. Z.; Kirby, J.; George, M.; Poliakoff, M.; Schuster, G. B., Photochemistry of Aryl Azides - Formation and Reactivity of Dehydroazepines. *Abstracts of Papers of the American Chemical Society* **1988,** 196, 322-ORGN.
23. Smith, R. A.; Knowles, J. R., Letter: Aryldiazirines. Potential reagents for photolabeling of biological receptor sites. *J Am Chem Soc* **1973,** 95, (15), 5072-3.
24. Chehade, K. A.; Spielmann, H. P., Facile and efficient synthesis of 4-azidotetrafluoroaniline: a new photoaffinity reagent. *J Org Chem* **2000,** 65, (16), 4949-53.
25. Karney, W. L.; Borden, W. T., Why does o-fluorine substitution raise the barrier to ring expansion of phenylnitrene? *Journal of the American Chemical Society* **1997,** 119, (14), 3347-3350.
26. Brunner, J.; Senn, H.; Richards, F. M., 3-Trifluoromethyl-3-phenyldiazirine. A new carbene generating group for photolabeling reagents. *J Biol Chem* **1980,** 255, (8), 3313-8.
27. Galardy, R. E.; Craig, L. C.; Printz, M. P., Benzophenone triplet: a new photochemical probe of biological ligand-receptor interactions. *Nat New Biol* **1973,** 242, (117), 127-8.
28. Forget, D.; Langelier, M. F.; Therien, C.; Trinh, V.; Coulombe, B., Photo-cross-linking of a purified preinitiation complex reveals central roles for the RNA polymerase II mobile clamp and TFIIE in initiation mechanisms. *Mol Cell Biol* **2004,** 24, (3), 1122-31.
29. Tate, J. J.; Persinger, J.; Bartholomew, B., Survey of four different photoreactive moieties for DNA photoaffinity labeling of yeast RNA polymerase III transcription complexes. *Nucleic Acids Res* **1998,** 26, (6), 1421-6.
30. Sengupta, S. M.; Persinger, J.; Bartholomew, B.; Peterson, C. L., Use of DNA photoaffinity labeling to study nucleosome remodeling by SWI/SNF. *Methods* **1999,** 19, (3), 434-46.
31. Sengupta, S. M.; VanKanegan, M.; Persinger, J.; Logie, C.; Cairns, B. R.; Peterson, C. L.; Bartholomew, B., The interactions of yeast SWI/SNF and RSC with the nucleosome before and after chromatin remodeling. *J Biol Chem* **2001,** 276, (16), 12636-44.
32. Pruss, D.; Bartholomew, B.; Persinger, J.; Hayes, J.; Arents, G.; Moudrianakis, E. N.; Wolffe, A. P., An asymmetric model for the nucleosome: a binding site for linker histones inside the DNA gyres. *Science* **1996,** 274, (5287), 614-7.
33. Mayer, A. N.; Barany, F., Photoaffinity cross-linking of TaqI restriction endonuclease using an aryl azide linked to the phosphate backbone. *Gene* **1995,** 153, (1), 1-8.
34. Musier-Forsyth, K.; Schimmel, P., Acceptor helix interactions in a class II tRNA synthetase: photoaffinity cross-linking of an RNA miniduplex substrate. *Biochemistry* **1994,** 33, (3), 773-9.

35. Westerheide, S. D.; Boss, J. M., Orientation and positional mapping of the subunits of the multicomponent transcription factors RFX and X2BP to the major histocompatibility complex class II transcriptional enhancer. *Nucleic Acids Res* **1999,** 27, (7), 1635-41.
36. Yang, S. W.; Nash, H. A., Specific photocrosslinking of DNA-protein complexes: identification of contacts between integration host factor and its target DNA. *Proc Natl Acad Sci U S A* **1994,** 91, (25), 12183-7.
37. Escher, E.; Schwyzer, R., p-Nitrophenylalanine, p-azidophenylalanine, m-azidophenylalanine, and o-nitro-p-azido-phenylalanine as photoaffinity labels. *FEBS Lett* **1974,** 46, (1), 347-50.
38. Fischli, W.; Caviezel, M.; Eberle, A.; Escher, E.; Schwyzer, R., [The synthesis of 4'-azido-3',5'-ditritio-l-phenylalanine peptides as "photo-affinity probes" for ligand-receptor interaction (author's transl)]. *Helv Chim Acta* **1976,** 59, (3), 878-9.
39. Kauer, J. C.; Erickson-Viitanen, S.; Wolfe, H. R., Jr.; DeGrado, W. F., p-Benzoyl-L-phenylalanine, a new photoreactive amino acid. Photolabeling of calmodulin with a synthetic calmodulin-binding peptide. *J Biol Chem* **1986,** 261, (23), 10695-700.
40. Nassal, M., 4'-(1-Azi-2,2,2-Trifluoroethyl)Phenylalanine, a Photolabile Carbene-Generating Analog of Phenylalanine. *Journal of the American Chemical Society* **1984,** 106, (24), 7540-7545.
41. Schwyzer, R.; Caviezel, M., p-Azido-L-phenylalanine: a photo-affinity 'probe' related to tyrosine. *Helv Chim Acta* **1971,** 54, (5), 1395-400.
42. Shih, L. B.; Bayley, H., A carbene-yielding amino acid for incorporation into peptide photoaffinity reagents. *Anal Biochem* **1985,** 144, (1), 132-41.
43. Egnaczyk, G. F.; Greis, K. D.; Stimson, E. R.; Maggio, J. E., Photoaffinity cross-linking of Alzheimer's disease amyloid fibrils reveals interstrand contact regions between assembled beta-amyloid peptide subunits. *Biochemistry* **2001,** 40, (39), 11706-14.
44. Escher, E. H.; Nguyen, T. M.; Robert, H.; St-Pierre, S. A.; Regoli, D. C., Photoaffinity labeling of the angiotensin II receptor. 1. Synthesis and biological activities of the labeling peptides. *J Med Chem* **1978,** 21, (9), 860-4.
45. O'Neil, K. T.; Erickson-Viitanen, S.; DeGrado, W. F., Photolabeling of calmodulin with basic, amphiphilic alpha-helical peptides containing p-benzoylphenylalanine. *J Biol Chem* **1989,** 264, (24), 14571-8.
46. Hixson, S. H.; Hixson, S. S., P-Azidophenacyl bromide, a versatile photolabile bifunctional reagent. Reaction with glyceraldehyde-3-phosphate dehydrogenase. *Biochemistry* **1975,** 14, (19), 4251-4.
47. Kaneda, M.; Sadakane, Y.; Hatanaka, Y., A novel approach for affinity-based screening of target specific ligands: application of photoreactive D-glyceraldehyde-3-phosphate dehydrogenase. *Bioconjug Chem* **2003,** 14, (5), 849-52.
48. Kenyon, G. L.; Bruice, T. W., Novel sulfhydryl reagents. *Methods Enzymol* **1977,** 47, 407-30.
49. Driessen, A. J.; Manting, E. H.; van der Does, C., The structural basis of protein targeting and translocation in bacteria. *Nat Struct Biol* **2001,** 8, (6), 492-8.
50. High, S.; Martoglio, B.; Gorlich, D.; Andersen, S. S.; Ashford, A. J.; Giner, A.; Hartmann, E.; Prehn, S.; Rapoport, T. A.; Dobberstein, B.; et al., Site-specific photocross-linking reveals that Sec61p and TRAM contact different regions of a membrane-inserted signal sequence. *J Biol Chem* **1993,** 268, (35), 26745-51.

51. Hohsaka, T.; Ashizuka, Y.; Taira, H.; Murakami, H.; Sisido, M., Incorporation of nonnatural amino acids into proteins by using various four-base codons in an Escherichia coli in vitro translation system. *Biochemistry* **2001,** 40, (37), 11060-4.
52. Hohsaka, T.; Sisido, M., Incorporation of non-natural amino acids into proteins. *Curr Opin Chem Biol* **2002,** 6, (6), 809-15.
53. Samuelson, J. C.; Chen, M.; Jiang, F.; Moller, I.; Wiedmann, M.; Kuhn, A.; Phillips, G. J.; Dalbey, R. E., YidC mediates membrane protein insertion in bacteria. *Nature* **2000,** 406, (6796), 637-41.
54. Scotti, P. A.; Urbanus, M. L.; Brunner, J.; de Gier, J. W.; von Heijne, G.; van der Does, C.; Driessen, A. J.; Oudega, B.; Luirink, J., YidC, the Escherichia coli homologue of mitochondrial Oxa1p, is a component of the Sec translocase. *Embo J* **2000,** 19, (4), 542-9.
55. Wang, L.; Brock, A.; Herberich, B.; Schultz, P. G., Expanding the genetic code of Escherichia coli. *Science* **2001,** 292, (5516), 498-500.
56. Hino, N.; Okazaki, Y.; Kobayashi, T.; Hayashi, A.; Sakamoto, K.; Yokoyama, S., Protein photo-cross-linking in mammalian cells by site-specific incorporation of a photoreactive amino acid. *Nat Methods* **2005,** 2, (3), 201-6.
57. Li, Y.-M., Photoactivatable gamma-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* **2000,** 405, 689-694.
58. Koumanov, F., Cell-surface Biotinylation of GLUT4 Using bis-Mannose Photolabels. *Biochemical Journal* **1998,** 330, 1209-1215.