Edited by
Maurice Goeldner, Richard Givens

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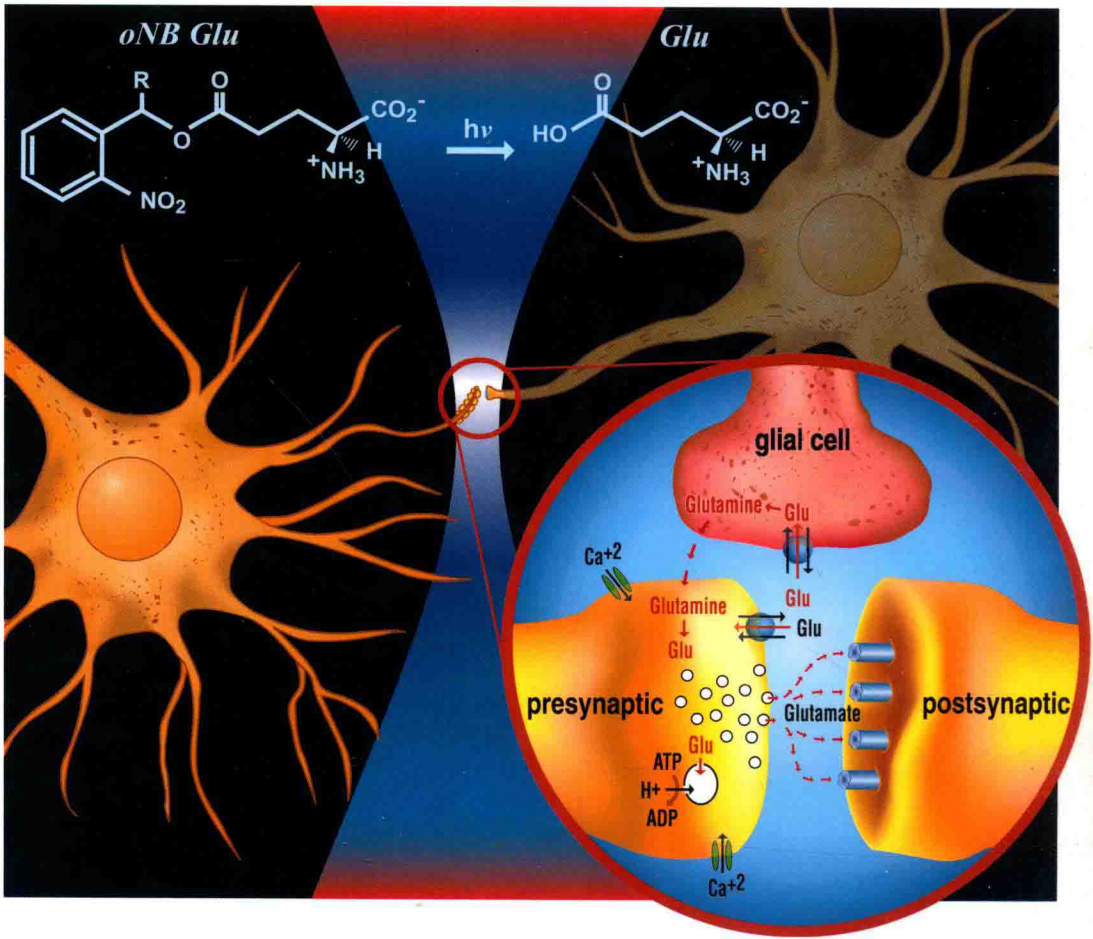
Dynamic Studies in Biology

Phototriggers, Photoswitches and Caged Biomolecules



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Foreword

I came to the United States at the end of 1975 and joined the laboratory of Professor Joseph Hoffman at Yale. This was a particularly fortunate time for someone who had trained as an organic chemist and biophysicist and was interested in applying a chemical-based approach to interesting physiological problems. I began a collaboration with Biff Forbush, and for the next couple of years we embarked on trying to make a caged ATP that would efficiently release ATP rapidly on illumination under physiological conditions. The driving force for this approach came from the idea that in order to study an ion pump, or P-type ATPase, in a sided preparation, it would be ideal to be able to generate ATP within a closed compartment. The particular experiments that had been originally planned for this new tool (if it worked) have in fact still not been carried out, as their significance was made redundant by our increasing knowledge about the sodium pump.

As with most synthetic chemical approaches, the literature provided encouragement. Work on the photorelease of carboxylic acids from nitrobenzyl esters by Baltrop had shown that lengthy illumination in the ultraviolet range of these esters in acetone produced quantities of the free carboxylic acids. Not surprisingly, this was also an area (among so many) that had received attention from R. B. Woodward, who had demonstrated the utility of photodeprotection of nitrobenzyl phosphate esters. Indeed, photodeprotection had been appreciated as a potentially useful strategy in peptide and nucleic acid synthesis.

Biff Forbush and I then spent much of the next two years in the dark! Our initial synthetic efforts provided a clue that caged ATP was an achievable goal as our multi-step synthesis provided very small quantities of a long wavelength-absorbing material that yielded ATP on photolysis. Eventually we improved the synthetic route and could produce reasonable quantities of our new reagent. One of the first benefactors of this approach was David Trentham, who had recently come to the United States. He used some of our early samples in initial studies in skeletal muscle fibers. These led to studies over the next several years, from the laboratories of David Trentham and Yale Goldman among others, that have made significant steps forward in our understanding of the details of the contractile machinery, and also introduced David and his colleagues, including John Corrie, to the photorelease area in which they have made major mechanistic advances.

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I then initiated studies of sodium pump partial reactions using the photorelease of caged ATP inside resealed red cell ghosts. These proved to be quite difficult and technically demanding. This led to me giving a series of seminars over the next couple of years on the "potential" of the caged ATP strategy, without being able to demonstrate any new biological information derived from its use. Eventually we were successful and were able to put to good use our new tool. During this time it became apparent to me that there would now be many variations on the caged ATP theme, as the chemistry was apparently relatively straightforward and photorelease could be applied to many different biological substrates and many different biological situations. Sure enough, the photorelease strategy was subsequently applied to other nucleotides, sugars, cyclic nucleoside monophosphates, neurotransmitters, etc. etc., using the same photochemical approach. Several of these approaches and applications are discussed in this monograph, and the reader is encouraged to discover the new developments in substrate release that are presented here.

Around this time it was becoming abundantly clear that the regulation and rapid alteration of cellular Ca levels was an essential and widely used factor in a large number of intracellular signaling systems. Furthermore, rapid changes in intracellular Ca are used as a trigger to activate a variety of physiological reactions. Many scientists were engaged in designing and evaluating reagents to reliably monitor intracellular Ca concentrations in a number of physiological systems. I realized that a reagent that could rapidly release Ca and generate rapid signaling transients or elevations in Ca might be a very useful tool in cellular and molecular biophysical studies. Obviously this is conceptually somewhat different than cleaving off a covalent protecting group from a substrate, but as usual the key elements were already in the literature. It was known that EDTA was a very effective chelator of polyvalent cations such as Ca, Mg etc., with dissociation constants in the nanomolar range. I also knew that iminodiacetic acid, half of the EDTA coordinating center, bound the same cations, but much more weakly (with a dissociation constant in the millimolar range). Thus if we could use light to bifurcate the Ca-EDTA complex to two iminodiacetic acid molecules, the bound Ca would be released. The synthetic chemistry proved to be very challenging, and, some ten years later, with the input of a post-doctoral Fellow, Graham Ellis-Davies, DM-nitrophen was successfully synthesized and had just the predicted properties for a caged Ca reagent base on EDTA. The kinetics of release turned out to be fairly rapid, and increases in Ca were achieved in around 60 microseconds. The selectivity of these chelators mirrored that of the parent compound EDTA, so they could as easily be used as photochemical sources of Ba, Mg etc. Subsequently, a similar approach led us to a molecule with higher selectivity for Ca, based on EGTA. Since then Graham Ellis-Davies has made some refinements to these initial caged Ca reagents. While Ca and other ion release using caged chelators are not a subject covered in this monograph, the reader is directed to a leading reference on this topic [G. C. R. Ellis-Davies "Development and application of calcium cages" in "Biophontonics" (Academic Press) eds. G. Marriott and I. Parker, Meth. En. 2003 360A, 226–238].

The particular advantages of photorelease from caged compounds as an experimental strategy are many. The rapidity with which substrates are released and hence reactions initiated now can be accomplished in the millisecond and sub-millisecond time range. The activating signal, high intensity light, is instantly available (using flash lamps or lasers) and can be readily switched off. Since prior to activation the caged substrates are relatively inert, they can be used to activate processes that are within closed compartments (vesicles or ghosts) or in ordered structures (muscle fibers or crystals). They can also be employed to synchronize arrays of proteins or enzymes. The technique provides high spatial and temporal resolution, and by virtue of the skills and imagination of a number of synthetic chemists, has a very broad versatility. There have been several recent and important developments that have extended the scope of this technology; these have included the introduction of multiphoton approaches, the application to "caged" fluorescence, the use of caged peptides or proteins, and the continuing development of newer photochromic moieties with more desirable (faster or longer wavelength) chemical or photochemical properties.

This volume brings together a group of expert practitioners in this field who have contributed greatly to its broader application and potential. This is the first time that a single volume has been dedicated to this important experimental strategy. I believe it will prove to be of considerable value to many biological scientists who already employ this approach as well as to those who are considering its application to their particular systems. I hope that it will also trigger the imagination of experimentalists and enable them to carry out studies that are made more accessible by access to caged compounds and photoactivation.

July 1, 2004

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Preface

Cage compounds have been known in biochemistry and physiology for more than two decades [1]. The past few years have witnessed a considerable increase in interest in caged derivatives because of their high degree of temporal and spatial controllability. Light-initiated cage release is now extensively employed to investigate molecular processes in biochemistry and biophysics as well as to initiate other physiological phenomena. More recently, these same approaches have been extended to the release of substances in building or isolating libraries in combinatorial chemistry, in photolithography, and in the photorelease of caged reagents in chemical transformations. In parallel with recent developments for biological and medicinal technological innovations, the miniaturization of devices for delivery of reagents in analysis and chemical processing and in real-time spectral and physical measurements is a rapidly developing technology. These developments have placed increased demands on the need for even greater spatial and temporal control on processes including chemical transformations. Consequently, the use of light-activated processes becomes much more appealing to those working in the life sciences [2].

As will be discussed in this volume, a very extensive range of substrates and reagents has been delivered by the photolysis of photoactivated protecting groups. This monograph both reviews the recent accomplishments in the field of caged compounds and also looks forward to future inroads into other fields.

Much of what is presented here, in fact, refers to processes in many other fields where the control of reagent or substrate delivery is a key element in a biological study. Initially in this field, only small, low-molecular-weight compounds were "releasable". Now, whole proteins and oligonucleotides are the object of "caging" applications that can range from molecular beacons to enzyme switches.

In spite of the increased interest, the number and range of useful and practical cages remains very limited, with less than a handful that have found sustained interest within the life sciences. In fact, over 80% of the published discoveries employing photoremovable protecting groups are based on a single photochemical process, the photoredox chemistry of the 2-nitrobenzyl chromophore [3]. While this photochemical reagent has proven to be versatile, robust, and generally efficient, it has well-documented limitations, such as the slow rate

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of substrate release and generation of a reactive nitroso functionality, that significantly restrict its application in biological studies. These limitations have stimulated the design and development of cages, leading to an expansion in the availability of usable caging groups. Caging chromophores must have several key properties or attributes. Among these are a strong absorption in the near UV-vis region, an efficient photorelease process, a hypsochromic shift of the absorption spectrum due to the photoproduct, ease of attachment of the chromophore without introducing new added stereocenters, and a cage and photoproduct that are biologically inert. There are currently four principal chromophores, used in caged reactions, that are included in this monograph. These four are evaluated and their applications presented. The newer applications of multiphoton decaging have also been included. These have shown particular promise because of the improvement in spatial resolution for controlled release.

This monograph provides a timely assessment and overview of the state of the field and recent investigations of cage photorelease for a wide variety of applications. It remains to be discovered what new cages will replace or augment those chromophores now in use and what expanded repertoire of applications will emerge. Future research will undoubtedly extend both areas and require a reinvestigation of the accomplishments achieved with the aid of photoremovable protecting groups.

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Strasbourg, France, July 2004 — Maurice Goeldner
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