title and keywords give an instant sense of the niche for this work

writer started with diseases related to genetics and narrowed to photochemistry quickly and effectively

IM and BI as outcomes of research

detailed but organized research plan; here in one big paragraph

not every single step is clear yet; that's OK!

Title: Viral photosensitivity: cellular photoregulation through synthetic biology approaches
Key words: Photochemistry, photoregulation, synthetic biology, unnatural amino acids, photolabile, cage, superfolding proteins

Many parts and processes of organisms, including those which may be defective or deleterious, are determined by gene sequences, and genetic therapies are the utilization of emergent technologies to prevent or amplify the expression of these genes. With such capabilities, scientists can exhibit greater control over diseases both between and within organisms. These genetic engineering approaches have been increasingly supplemented with advances in photochemistry and synthetic biology, among other disciplines. From the first "caging" of adenosine-triphosphate in 1978[1] to more recent efforts to use light for controlling natural mammalian genetic regulatory systems[2, 3, 4], the use of photolabile inactivating groups has developed over three decades into many sophisticated mechanisms of spatiotemporal genetic control. As an example, I am currently conducting research for a thesis project regarding the site-specific modification of oligonucleotides for use with photocage groups. The project will increase the efficiency with which cage molecules can potentially direct antisense techniques such as ribonucleic acid interference (RNAi). Photochemical treatments of many varieties imply immense potential not only for novel, potent molecular therapies, but also for elucidating intricate cellular pathways, as they offer near-immediate control over for various genetic mechanisms. Within the realm of synthetic biology, researchers have developed libraries of unnatural amino acids with novel functionalities – new reactivities, fluorescence, or photosensitivity, for example. These have been created along with a host of highly specific, orthogonal unnatural codons and transfer ribonucleic acid (tRNA) complexes with which to incorporate modified amino acids. Indeed, Schultz shows cysteine, serine, and tyrosine residues modified with photolabile nitrobenzyl "cage" compounds bound to their side chains; he successfully uses these along with necessary other molecules to translate the new codons into latent residues within common proteins[5]. These "unatural amino acid" techniques are recent developments, and have yet to be applied in mammalian cells or for therapeutic purposes.

intro

My hypothesis is that synthetic genomic additions can be utilized both generate cells which become autonomously photosensitive by photocaging residues of critical proteins and to generate novel, exceptionally functional proteins. The first step in my research plan would be to enumerate proteins whose sudden activation *en masse* would trigger clear or dire responses. For example, members of the cysteine-aspartic acid protease family, such as Caspase 3, may be successful as a photoactivated agent of cell necrosis. Those proteins determined to have sufficient apoptotic or therapeutic potential when activated would then be run through a series of further selections. Each peptide's primary structure would be examined for possible locations for insertion of an unnatural codon – at present, most likely cysteines, serines, or tyrosines, as perviously mentioned[5]. The tertiary and quarternary structures would then be analyzed *in silico* and determined to understand precisely the steric, chemical, and other effects of one or multiple cage groups along the protein. This step would involve selecting a suitable host cell line, a line susceptible to each protein's functionality specifically. This susceptibility would be confirmed experimentally by up-regulating each protein independently in a series of assays with the cell line in culture. Following affirmation of the toxicity or efficacy of large influxes of the selected peptides, the effectiveness of the photocaging mechanism with each peptide would be established. A codon, tRNA, and synthetase complex would be chosen or engineered, most likely one corresponding to the frequently-manipulated amber "stop" codon[5]. Each protein would be up-regulated in culture through the modified amino acid pathway with at least three species of modified amino acids in separate assays. Following irradiation with 365 nm light for nitrobenzyl cage groups, each protein-amino acid combination would be assessed for efficiency in inducing the desired effect. This screening would be conducted via myriad controls, comparing activity of the caged protein to positive, non-caged controls and fully negative controls, and the cellular response induced by photoactivation of each complex combination to the rate of cell response prior to photoactivation and, again to positive and negative controls equally irradiated. Final options would be selected for minimum footprint of the caged species and maximum efficacy upon photorelease of the cage compound. These final candidates would be optimized, possibly with directed evolution methods, and a final, optimal chemical species selected. A potential pitfall which might arise is activity of a fraction of the inactive proteins, either in the caged state or as a result of ambient light. Such issues could be addressed by caging additional amino acid residues or determining alternate chemistries such that cage groups are more stable.

methods

discussing control experiments and work-arounds further increases credibility as a clear-thinking scientist

explicit labels for IM and BI

The **intellectual merit** of the project is multivariate. If successfully virally transfected along with caged amino acid species, the genes coding for a photocage-modified protein could be a stable, reproducible source of photosensitivity for malignant tissue. Currently, much genetic engineering work is conducted on a post-translational level,[2] but this method of synthetic photobiology modulates cells on a pre-transcription level; it would be a more direct, fundamental approach to genetic and cellular work. The technology could also be used to produce large modified-protein-expressing cultures of mammalian cells to generate the photosensitive peptides for traditional delivery to be followed by spatiotemporal photoactivation. Such proteins would be a significant new weapon with which oncologists can fight superficial cancers and other malignancies. Beyond therapeutics, the success of this project could accelerate laboratory studies across the field of molecular engineering by providing proteins capable of near-instantaneous activation and modified proteins with "superfolding" or other useful functionalities.

IM

The project will have **broader impacts**, as well, and will be widely inclusive of disciplines and peoples. Successful completion will hinge on contributions from analytical chemists, stereochemists and biochemists; cell biologists, synthetic biologists and microbiologists; and genetic, chemical, cellular and molecular engineers. It will also be most successful in a large, multilevel academic laboratory setting, offering educational opportunities for both undergraduates (transfection and purification assays) and graduate students (experimental, genetic and molecular design), as well as high school students (cell culture and purification methods). Involving undergraduate and high school students in the project will allow the encouragement of underrepresented students to pursue higher science and engineering by giving them the thrill and confidence of discovery. The project will benefit from inter-university collaboration, compiling parallel ongoing work to minimize unnecessary overlap and maximize the potential of resulting products and processes. I certify that this essay is my own work.

BI

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