Endeavors to make the photophore, diazirine easy to use: An application to biochemical methodology.

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Photoaffinity labeling enables the direct probing of a target protein through a covalent bond between a ligand and its binding protein, and even a complex formed by weak interactions can be isolated. We used 3-aryl-3-trifluoromethyl-diazirine as a photophore because it appears to come closest to satisfy the criteria required for useful photophore.

We have developed three biochemical method using the diazirine: Firstly, we developed a method for an efficient cloning of display phages using photoreactive ligand. The photoreactive ligand baring carbene-generating photophore, diazirine functions as a barb on a hook, onto which ligands are placed as fish bait. Thus, the cross-linked phages stay on the ligand even after intensive washing, whereas almost all the unspecific binding phages are removed. As a result, the photochemical panning can separate target displayed phages from the mixture 1000 times more efficient than the traditional panning does. Secondly, we developed a photochemical electrophoretic mobility shift assay (EMSA) using photoreactive DNA. The photochemical EMSA could inform a number of DNA-binding proteins and their molecular weight. Thirdly, we developed diazirine unit for accelerating the structural elucidation of the ligand-binding region by photoaffinity labeling. The cleavable photophore, S-[4-(3-trifluoromethyl-*3H*-diazirin-3-yl)-benzyl]-methane thiosulfonate leaved a small tag on the protein, and the feature is suitable for determining the crossliked site by mass spectrometry.