In Vivo Activation of Azi-propofol Prolongs Anesthesia and Reveals Synaptic Targets*

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*Running title: Light-induced prolongation of anesthesia

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Background: Azi-propofol is a photoactive analogue of the general anesthetic propofol.

Results: In vivo photolabeling of tadpoles results in covalent ligand binding to neuronal proteins and prolongation of anesthesia.

Conclusion: Reconciling time-resolved gel proteomics with behavioral state allows identification of potential anesthetic targets.

Significance: In vivo activation of efficacious photolabels provides a novel approach to investigate mechanisms of general anesthetics.

SUMMARY

General anesthetic photolabels have been instrumental in discovering and confirming protein binding partners and binding sites of these promiscuous ligands. We report the in vivo photoactivation of meta-azi-propofol, a potent analogue of propofol, in Xenopus laevis tadpoles. Covalent addition of meta-azi-propofol in vivo prolongs the primary pharmacologic effect of general anesthetics in a behavioral phenotype we termed ‘optoanesthesia’. Coupling this behavior with a tritiated probe, we performed unbiased, time-resolved gel proteomics to identify neuronal targets of meta-azi-propofol in vivo. We have identified synaptic binding partners, such as synaptosomal-associated protein 25, as well as voltage-dependent anion channels as potential facilitators of the general anesthetic state. Pairing behavioral phenotypes elicited by the activation of efficacious photolabels in vivo with time-resolved proteomics provides a novel approach to investigate molecular mechanisms of general anesthetics.

General anesthetics bind many proteins with low affinities (µM Kᵩ values), hindering analyses of ligand-target interactions. Photoactive analogues of clinically used general anesthetics have been developed to aid molecular studies (1-5). These probes share physicochemical properties with their parent molecules, retain anesthetic activity, and undergo photolysis under long-wave ultraviolet light (UVA) (315-400 nm), a feature that limits damage to cellular macromolecules upon irradiation following equilibration with the ligands. With these compounds, anesthetic binding sites have been mapped on integrin LFA (3, 6), Torpedo nicotinic receptors (7, 8), β-tubulin (9), PKC (10), and GABA_A receptors (11, 12), among others. Direct identification of anesthetic substrates from complex homogenates has proceeded with a neurosteroid analogue (2) and halothane (13), the latter an unaltered general anesthetic containing a carbon-bromine bond broken by shorter UV wavelengths to create reactive carbon-centered radicals (14).

Despite anesthetic efficacy in vivo, the use of these probes has been limited to in vitro preparations. Electrophysiological evidence supports the concept that covalent incorporation of photoactive anesthetics to binding sites can result in prolonged modulation of functional proteins (15). While alkylphenol anesthetics are thought to
act in part through GABA$_A$ receptors, genetic studies prove other 'on-pathway' targets exist (16, 17). Thus, we tested the feasibility of activating the photoaffinity probe meta-azi-propofol (AziPm) in vivo as a tool to identify novel molecular substrates that contribute to alkylphenol general anesthesia. AziPm is an analogue of propofol (2,6-diiisopropylphenol) that contains an alkyl diazirinyl group in the meta position of the phenol ring (Figure 1) (4). In vivo photolabeling of *Xenopus laevis* tadpoles equilibrated with AziPm results in a previously unreported behavioral phenotype that we call 'optoanesthesia'. We describe this in conjunction with unbiased, time-resolved gel proteomics employing a tritiated version of the photolabel.

**EXPERIMENTAL PROCEDURES**

**Materials.** 2,6-diisopropylphenol was acquired from Sigma-Aldrich, and AziPm was synthesized by W. P. Dailey (University of Pennsylvania) through published methods (4). AziPm was radiolabeled by AmBios Labs (Boston, MA) by iodinating the ring and reducing with tritium under catalytic conditions. The final product was purified with HPLC. Ecolite(+) liquid scintillation cocktail (MP Biomedicals) was used with a PerkinElmer Tri-Carb 2800TR instrument; a Varian Cary 300 Bio UV-VIS spectrophotometer was used for spectroscopy. First and second dimension gels, electrophoresis apparatuses, and molecular weight markers were from Bio-Rad. UVA was generated by filtering a 100 W arc mercury lamp through colored glass UV-visible broadband (~340-615 nm) and UV bandpass (~250-375 nm) filters (lamp and filters from Newport, Stratford, CT). Light intensities (measured with an optical power meter [Thorlabs, Newton, NJ]) were 28.1 µW/mm$^2$ and 27.7 µW/mm$^2$ at 350 nm and 375 nm, respectively. Albino *X. laevis* tadpoles (stage 45-47) were purchased from Nasco (Fort Atkinson, WI) and housed in supplied pond water for at least 24 hours prior to experiments. Animal protocols were approved by IACUC of the University of Pennsylvania.

**Tadpole immobility studies.** Tadpoles were placed in Petri dishes with propofol or AziPm dissolved in pond water. The same physicochemical parameters determine anesthetic passage across the gills and skin as does passage across the blood brain barrier (18, 19). In some experiments, after 30 minutes equilibration, tadpoles were transferred to fresh water; in others, after equilibration, tadpoles remained on the bench for a sham control or were exposed to UVA (photolabeled in vivo) before transfer to fresh water. Immobility was defined (and scored) as the percentage of tadpoles that did not swim, twitch, or right themselves throughout a thirty-second time window preceding every ten-minute interval. Tadpole assays are established measures of anesthetic potency (18, 19), and reversible immobility is the most commonly used general anesthetic endpoint. Alternative causes of immobility in our study (e.g., muscular toxicity) were not ruled out, but should have had additional and toxic features that would have been observed (e.g., cardiac muscle dysfunction, etc). The water temperature was 21-22°C for experiments and changed < 0.5°C throughout any experiment.

**In vivo photolabeling and isolation of neuronal membranes.** Tadpoles were incubated for 30 minutes with 4 µM [3H]AziPm and treated ± UVA for 10 minutes. After transfer to fresh water, tricaine methanesulfonate (500 mg/L) was added immediately for the 0 time point or at 165 minutes for the emergence time point, and the tadpoles were placed on ice. After decapitation, brains and spinal cords were removed with forceps under a dissecting microscope, and placed in ice-cold 0.32 M sucrose, 5 mM tris, pH 7.4 supplemented with protease inhibitors. Tissue isolation required less than 15 minutes following each time point; central nervous system (CNS) tissue was homogenized every 3-5 minutes using a Teflon/glass homogenizer.

CNS homogenates were centrifuged at 100,000 x g for 10 minutes, washed with isolation buffer, and re-centrifuged. The pellet was homogenized in 5 mM tris, pH 7.4 and centrifuged at 100,000 x g for 10 minutes, washed, and centrifuged again before resuspension in 2 mM tris, pH 7.4. An aliquot was removed for a protein assay prior to freezing at -80°C.

**In vitro photolabeling.** Unexposed tadpoles were anesthetized with tricaine methanesulfonate and neuronal tissue, dissected as above, was homogenized in sucrose buffer, centrifuged at 100,000 x g for ten minutes, washed, and re-centrifuged. The pellet was
suspended in isolation buffer, protein concentration determined, then diluted to 1 mg/ml in a microcentrifuge tube. 4 μM [3H]AziPm ± 400 μM propofol was added and, after a brief vortex, was incubated at 21°C in the dark for 10 minutes. After transfer to a quartz cuvette (pathlength, 1 mm), the tissue was photolabeled for 10 minutes using the same light source as above. The homogenates were then centrifuged at 100,000 x g, homogenized in 5 mM tris, re-centrifuged at 100,000 x g, washed, and stored at -80°C in 2 mM tris.

**Scintillation counting of neuronal tissue.** Dissected CNS tissue from tadpoles treated with 4 μM [3H]AziPm ± UVA was placed in 1 ml ice-cold 2% SDS, 1% triton x-100, 5 mM tris, pH 7.4 supplemented with protease inhibitors. Following homogenization, the protein concentration was determined. 5 and 10 μl of the homogenates were added to separate vials in scintillation fluid. The disintegrations per minute (dpm) from each vial were normalized to the corresponding protein amount, and the mean of the two values was used for a single experimental measurement.

**IEF/SDS-PAGE.** After thawing, 100 μg of neuronal membrane protein was centrifuged for 15 minutes at 15,000 x g. Following removal of the supernatant, the pellet was dissolved in 125 μl 7 M urea, 2 M thiourea, 20 mM dithiothreitol, and 0.2% carrier ampholytes. Isoelectric focusing and SDS-PAGE proceeded according to the manufacturer’s instructions, with 3-10 non-linear pH strips (7 cm) and 4-15% SDS-PAGE. Tissue from ~25 tadpoles sufficed for a single gel.

**Spot intensity quantitation and liquid scintillation counting.** Gels were washed with water and fixed overnight in 15% trichloroacetic acid before staining with Coomassie G-250. After destaining, the gels were scanned on a Bio-Rad GS-800 Calibrated Densitometer with quantitation performed using the accompanied Quantity One software. Background was subtracted with a box drawn between the 50 and 75 kDa molecular weight markers, and mean optical density (O.D.) multiplied by spot area was recorded from contoured spots.

Spots were excised with a 1.5 mm cylindrical hole punch and placed into scintillation vials. 400 μl of 30% hydrogen peroxide was added and the sealed vials were incubated overnight at 65°C to dissolve the polyacrylamide. These were cooled to room temperature before adding scintillation fluid.

**Mass spectrometry analysis.** Trypsin digested samples were separated on a nanoLC column before online electrospray into a Thermo LTQ linear ion trap. Raw data was acquired with Xcalibur, and a database downloaded from NCBI (November, 2011) with the search term ‘Xenopus’ was searched with Sequest. Parameters were 1 amu parent ion tolerance, 1 amu fragment ion tolerance, and 1 missed cleavage. The search result files were combined with Scaffold 3 and filtered with the criteria: Xcorr scores (+1 ion) 1.7, (+2) 2.3, (+3) 2.8; protein identification confidence 99.9%; peptide identification confidence 95%; 2 peptide minimum. Spectra were manually inspected to ensure quality and confidence.

**Statistics.** GraphPad Prism 5 was used for figure preparation and data analysis. Student’s t-test, one-way ANOVA, two-way ANOVA, and Bonferroni’s correction were calculated within the GraphPad software. Significance is expressed as *p < 0.05 and **p < 0.01.

**RESULTS**

**Optoanesthesia in Xenopus tadpoles.** Albino tadpoles anesthetized with 3 μM propofol or 4 μM AziPm (approximate EC99 doses) recovered on similar time scales following transfer to fresh pond water (Figure 1A, left). The tadpoles are equilibrated with the alkyl phenol, and recovery under these conditions is largely a function of drug diffusion back into the water.

In this study, we hypothesized that covalent occupation of ligand binding sites in vivo would result in prolonged anesthetic effects following washout of unadducted compound. The diazirine of AziPm has a peak absorbance of ~370 nm, undergoing photolysis to form a reactive carbene, while propofol absorbs wavelengths less than 300 nm. Albino tadpoles immobilized with AziPm and exposed to UVA before transfer to fresh water exhibited prolonged immobility not observed with propofol control groups (Figure 1A, right). Further, a relationship between lamp exposure time and recovery time was evident, suggesting progressive occupancy of functionally relevant sites. No toxicity (premature death) or differences in body mass were observed between
tadpoles treated with either alkylphenol anesthetic ± UVA following emergence (measured up to ten days).

Covalent adduction would concentrate ligand into protein sites with infinitely low off-rates, increasing the apparent potency of the molecule. In vivo photolabeling for ten minutes after equilibration with a sub-EC₉₀ AziPm dose markedly increased the population of immobilized tadpoles (Figure 1B). Further, we hypothesized that retained attachment of AziPm in functionally relevant targets after washout and emergence would manifest as a decrease in the effective concentration of propofol for immobility. Thus, 20 hours after emergence, tadpoles treated as above were exposed to 2 µM or 0.8 µM propofol. Animals photolabeled in vivo displayed increased sensitivity (more rapid induction, slower emergence, and induction with a lower dose) relative to controls (Figure 1C).

Lastly, 4 µM AziPm in pond water was photolysed for a period corresponding to twice the diazirine half-life (i.e., to a final concentration of ~1 µM [measured by absorption spectroscopy] plus whatever the product[s] of photolysis are). Tadpoles were then placed in this solution, and after 30 minutes, immobility was not observed, ruling out the possibility that a more potent, 'caged' anesthetic with slower washout kinetics was released with light (data not shown). Together, these data suggest prolonged anesthetic influence due to photoadduction of ligand in vivo, a phenomenon we termed optoanesthesia.

[^3H]AziPm in tadpole neuronal tissue. Because general anesthetics are assumed to exert their effects through CNS targets, retention of photoactivated AziPm in neural tissue was measured following optoanesthesia. Brains and spinal cords from control tadpoles and those photolabeled in vivo with[^3H]AziPm were isolated to quantify radioactivity after recovery in fresh water (Figure 2A). Following[^3H]AziPm induction, without washout, no difference is seen between groups treated ± UVA. However, eight fold more radioactivity was noted in the neuronal tissue of photolabeled animals at 165 minutes, the point of emergence for all tadpoles exposed to 4 µM AziPm and 10 minutes of UVA.

Identification of photolabeled proteins. Optoanesthesia indicates that neuronal substrates photolabeled in vivo are relevant targets of AziPm and possibly propofol anesthesia. For protein identifications, neuronal membrane protein from tadpoles equilibrated with[^3H]AziPm and photolabeled for 10 minutes was subjected to IEF/SDS-PAGE. Duplicate gels were stained, and one hundred random spots were excised for scintillation counting (Figure 2B). Mean background radiation (from three spots excised from each gel containing no detectable Coomassie staining in a region through which the SDS-PAGE separated proteins migrated) was 18.0 dpm, and the mean for all 100 spots was 18.8 dpm. Seven spots contained dpm greater than two standard deviations from the background mean (Figure 2C). No protein spots from control tadpoles incubated with[^3H]AziPm but not exposed to the lamp contained counts exceeding this background threshold.

Response to in vivo photolabeling. We hypothesized that for tadpoles to regain movement ('emerge'), the cellular components contributing to mobility must adapt by removing photolabeled proteins whose activity is altered, and/or by replacing these photolabeled macromolecules with newly synthesized proteins. To test this, neuronal membranes were isolated 165 minutes after tadpoles were photolabeled (when all had emerged) as above for IEF/SDS-PAGE, with the previously identified spots from duplicate gels assayed for dpm. The mean from three spots contained counts within 10% of the initial value (12, 22, and 33) while decreases of 46%, 35%, 42%, and 28% were noted in spots 4, 6, 41, and 85 respectively. Coomassie intensity was quantified to assess changes in protein expression, and with the exception of spot 4, little variation was observed (Figure 2D).

Spot dpm was normalized to corresponding Coomassie intensities for the in vivo experiments. We proposed that proteins with decreased radioactivity content coincident with emergence gain additional credibility as functionally important targets. Thus, we calculated the ratio of normalized photolabel incorporation at 165 minutes to that at the 0 time point for each spot (Figure 2E). A ratio of 1 would indicate that the fraction of adducted protein did not change over the 165 minutes. We found that the ratio from spots 6, 33, 41, and 85 are significantly less than 1, suggesting potential relevance in emergence from
optoanesthesia.

Conserved specificity and target identification. In vitro photolabeling of neuronal homogenates with 4 μM [3H]AziPm ± 400 μM propofol was performed to investigate the conserved, saturable specificity of protein sites. Protein spots identified as photolabeled in vivo were analyzed, and all contained dpm above background. A significant effect of propofol on normalized dpm was revealed with decreased photolabel incorporation ranging from 2-75% in each spot (p < 0.05, two-way ANOVA; n = 3 and n = 2 for each spot [-] and [+] propofol, respectively) (Table 1). A separate gel was run for protein identification. Six spots were unambiguously identified as containing a single protein, while two high confidence identifications were possible in the seventh (Table 1).

DISCUSSION

We describe optoanesthesia, a light-induced anesthetic potentiation, and present a method through which novel general anesthetic targets can be discovered. Reconciling proteomic data with a behavioral phenotype provides a powerful means to assign relevance to identified binding partners. The mechanisms of recovery from optoanesthesia are likely to reside in adducted, relevant proteins being targeted for accelerated degradation and/or being replaced by newly synthesized protein. An alternative hypothesis, not tested here, is that the activities of proteins that are not targets of AziPm are altered to compensate for the covalent modification of the alkylphenol binding partners. Although emergence pathways may be distinct from induction pathways (20), emergence must still require an offloading of the anesthetic from induction targets; thus we view our initial hypothesis, that label intensity should decrease in functionally relevant targets, as reasonable.

Performing photolabeling in live organisms assured that molecular targets were in a functional state, and because the primary effect of the anesthetic (immobility) was prolonged, confirmed that relevant targets were adducted. Further evidence for ligand incorporation to relevant general anesthetic sites was seen by the increased sensitivity to propofol after in vivo photolabeling. Despite label attachment to the identical proteins in vitro, substantial displacement of photoactive ligand by the parent propofol was most evident with VDACs. These mitochondrial porins with multiple phosphorylation states were also the most prominently labeled proteins in vivo. Protection of photolabeling by propofol suggests conserved and specific alkylphenol site(s). An alternative explanation for propofol competition is allosterism, which would require separate specific cavities for AziPm and for propofol, a possibility we view as unlikely.

Although VDACs are highly abundant proteins, this binding is not interpreted as 'nonspecific'. Specificity can be viewed as specific to a particular physiological outcome or, on the molecular level (and favored here), high occupancy, saturable binding. Other general anesthetics have been shown to bind VDACs in vitro, but functional consequences have yet to be reported. VDACs bind GABAA receptors (21, 22), known propofol targets, but knockout of VDAC-1 and VDAC-3 do not appear to alter anesthetic sensitivity (22). Knockout of VDAC-2 in mice is embryonic lethal, and interestingly, our time-resolved approach implicates VDAC-2 over VDAC-1 and VDAC-3 (Figure 2E). However, this may reflect a general 'protective' effect of VDAC-2 from cellular apoptosis (23).

Of the other identified proteins, SNAP-25 and Gβ4 exhibited decreases in the ratio of photolabeled to unmodified protein at the time of emergence. Published evidence has suggested anesthetic interactions with SNAP-25 and/or Gβ4 might contribute to depressed neuronal signaling. For example, SNAP-25, a component of the ternary SNARE complex, binds volatile anesthetics at physiologically relevant concentrations (24), and isoflurane and propofol inhibit neurotransmitter release through interactions with SNAREs or associated proteins (25, 26). Mutagenesis in SNARE complex proteins (including SNAP-25) alters organism sensitivity to general anesthetics (27). Mammalian studies suggest SNAP-25 may be predominantly expressed in excitatory neurons (28, 29), and this protein negatively regulates voltage-gated calcium channels independent of its role in exocytosis (30, 31). Gβ (as part of Gβγ) can also directly inhibit presynaptic voltage-gated calcium channels (32, 33) and binds to SNAP-25 and syntaxin to inhibit neuronal exocytosis (34, 35).

The lack of [3H]AziPm displacement by...
propofol on some proteins can be interpreted in several ways. For instance, anesthetic site(s) (e.g., on SNAREs) may not be conserved amongst ligands; isoflurane and halothane bind to the SNARE complex in a non-competitive and non-saturable manner (24). The hydrophobic interior of the coiled-coil complex likely harbors multiple sites of varying affinities, each capable of binding ligands with low occupancy. Alternatively, protein substrates (including Gβ) may be specific to AziPm but not propofol (propofol specificity was tested as the photolabel 'parent'). Regardless, these interpretations do not preclude functional involvement in hypnosis or optoanesthesia, as we did not test competition with non-tritiated AziPm, but also little evidence suggests saturable binding underlies these states.

With our approach in a model vertebrate organism, the tadpole, we provide in vivo evidence for the functional involvement of synaptic targets previously suspected only from in vitro or lower organism studies. Proving this involvement will require extensive genetic manipulations. Additionally, we have identified only a subset of targets bound by AziPm in vivo, not including ion channels (such as the GABA_A receptor) that are the object of some general anesthetic hypotheses. Resolving low abundance proteins with multiple transmembrane domains is not feasible with IEF/SDS-PAGE (36, 37), and thus a priori we did not anticipate their identification. The development of proteomic approaches complimentary to IEF/SDS-PAGE, and those that are capable of expanding the dynamic range of neuronal protein detection, will further the investigative power of optoanesthesia.

In conclusion, we anticipate the translation of in vivo photolabeling and behavior-paired proteomics to a wider variety of model organisms and photoactive molecules to investigate molecular mechanisms of general anesthetic pharmacology.

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FOOTNOTES

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2The abbreviations used are: long wave ultraviolet (UVA), *meta*-azi-propofol (AziPm), central nervous system (CNS), disintegrations per minute (dpm)
Light-induced prolongation of anesthesia

FIGURE LEGENDS

FIGURE 1. (A) Time course of recovery for tadpoles following anesthetic equilibration and (left) sham treatment or (right) UVA exposure. 3 µM propofol (open symbols; left structure in right panel) or 4 µM AziPm (closed symbols; right structure in right panel) was used. Treatment times were 3 minutes (diamonds), 10 minutes (circles), or 20 minutes (triangles), and the water was changed at time 0. Data shown is the mean ± SE from 3-4 experiments per group. (B) In vivo photolabeling for 10 minutes after equilibration with a sub-EC99 dose of 3 µM AziPm increased the immobile fraction of tadpoles. The water was changed at time 0, with photolabeling from -10 to 0 minutes. A one-way ANOVA found a significant difference between the three means (p < 0.01), and Bonferroni’s post-hoc found a significant decrease in the percent of mobile tadpoles after lamp exposure and water change (blue bar, p < 0.01). Data represents the mean ± SE from three experiments per treatment (± UVA). After equilibration, the tadpoles were randomly assigned to sham or UVA treatment, with the data at -10 minutes representing both groups, and sham-treated animals represented by the black bar. (C) Induction and recovery of tadpoles treated with (left) 2 µM propofol or (right) 0.8 µM propofol 20 hours after the indicated treatments. Error bars represent SE.

FIGURE 2. (A) Quantitation of dpm normalized to protein amount in CNS tissue of tadpoles treated with AziPm ± UVA for ten minutes. Data is from 3 experiments per treatment (10 tadpoles per experiment). Mean ± SE is shown, and data was analyzed by one-way ANOVA with Bonferroni’s post-hoc comparing dpm within each time point (p < 0.01). (B) Representative Coomassie stained gel of tadpole neuronal membrane protein. (C) Mean dpm of spots excised from gels of tissue isolated immediately after in vivo [3H]AziPm ± UVA treatment. Dpm values were arranged in ascending order, with measurements from select spots indicated. The dashed line indicates background mean from + UVA gels with the dotted line indicating two standard deviations. (D) Coomassie stain intensity quantified from in vivo gel spots. Spot 4 was found to decrease with a two-tailed student t-test (p < 0.05). (E) The ratio (dpm/intensity)_{165 min} divided by (dpm/intensity)_{0 min} shows the change in the fraction of photolabeled protein over the emergence period. Standard deviation is shown, and a ratio of 1 would indicate no change.

TABLE 1: Protein spot analysis and LC-MS/MS identification

| Spot | % Disp | Protein ID | NCBI Accession # | MW (Da) | MW (Da) | pI | pI | % Seq Coverage | Spectra Count |
|------|--------|------------|------------------|---------|---------|----|----|----------------|--------------|
| 4    | 40.1   | VDAC-2     | gi|62826006 | 30183   | 27937  | 8.36 | 8.99 | 29 | 18 |
| 6    | 52.0   | VDAC-2     | gi|62826006 | 30183   | 27448  | 8.36 | 8.27 | 23 | 14 |
| 12   | 46.5   | VDAC-1     | gi|28302268 | 30627   | 28671  | 6.85 | 6.71 | 20 | 11 |
| 22   | 74.5   | VDAC-1     | gi|28302268 | 30627   | 29160  | 6.85 | 6.21 | 26 | 16 |
| 33   | 3.4    | SNAP-25    | gi|33416802 | 23172   | 26468  | 4.74 | 4.89 | 30 | 15 |
| 41   | 1.7    | Gβ4        | gi|49257618 | 37504   | 33084  | 5.70 | 5.78 | 20 | 11 |
| 85   | 26.6   | PDIA3      | gi|28302197 | 56086   | 54992  | 5.72 | 5.91 | 30 | 25 |
| 26.6 | VHA-55 |              | gi|28436920 | 56411   | 54992  | 5.56 | 5.91 | 20 | 21 |

1[^3]H[AziPm displacement by propofol in vitro photolabeling experiments. 2Theoretical values were computed with ExPASy Compute pI/Mw tool (http://web.expasy.org/compute_pi/). Monoisotopic molecular weights are shown. 3Observed values were estimated from molecular weight markers and IEF resolving estimations published by the manufacturer of the gels.
Figure 1

A

B

C

prior treatment (20 hr)

- 3 μM propofol + UVA
- 4 μM AziPm
- UVA
- 4 μM AziPm + UVA
Figure 2

A

B

C

D

E

Light-induced prolongation of anesthesia

[Image of graphs and figures related to light-induced prolongation of anesthesia]
In Vivo Activation of Azi-propofol Prolongs Anesthesia and Reveals Synaptic Targets
Brian P. Weiser, Max B. Kelz and Roderic G. Eckenhoff

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