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Membrane targeted nanoactuators for cellular photostimulation

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Optical technologies for non-genetic cell photostimulation are becoming increasingly influential in the translational medicine field (1). In this regard, Ziapin2, an amphiphilic alkyl-substituted 4,4'-diaminoazobene was recently developed. Ziapin2 inserts properly in the cell membrane persisting in a *trans* configuration. The insertion in the membrane and the consequent formation of Ziapin2 dimers lead to a thinning of the membrane and an increase of the membrane capacitance. Light stimulation induces a trans-cis isomerization with a significant perturbation of the cell membrane potentials, able to trigger action potential firing in excitable cells without directly affecting ion channels or local temperature (2–4). Moreover, capacitance increase after Ziapin2 portioning in the membrane induces stable changes in cellular physiology even under dark conditions.

In the present project, we propose to modify Ziapin2 to obtain a push-pull molecule able to overcome such limitations. The new molecule is more soluble in water to improve its biocompatibility and clearance. The substitution of the amino group with a nitro group increases the red-shift absorption avoiding the need to use UV light, not suitable for applications in living cells.

Here, we report a study of push-pull molecule biological functions, going through biocompatibility assays and electrophysiological characterization using an immortalized (HEK) cell line model. In accordance with the theoretical design of the molecule, the absorbance/emission spectra display a significant red-shift with a peak absorbance at 517nm and an emission peak at 600nm. The preliminary biological investigation reveals that the push-pull molecules are able to enter properly in cells after a few minutes of incubation with no evident cytotoxicity at two different concentrations (5 and 10μM) persisting for up to 5 days after exposure. In addition, after molecule internalization cells exposed to an acute light stimulation lasting 30s do not display evidence of significant phototoxicity. We also demonstrated that in cells loaded with the compound, millisecond pulses of visible light induce a transient and defined depolarization of the membrane potential followed by a delayed and slight hyperpolarization. The amplitude of the membrane depolarization is dependent on both the light power density and the molecule concentration.

While further studies of the push-pull photophysical features and biological behavior are in progress, data so far available indicate that the push-pull molecule is a promising tool for photo-excitation of cells for both *in vitro* and *in vivo* applications.

References

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