Phosphorodiamidate Morpholino Scaffold for Improved Nanosensor Stability

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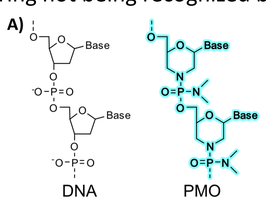
Research Question

This project aims to use the nucleic acid analog phosphorodiamidate morpholino oligo (PMO) as a scaffold to increase nanosensor stability *in vivo*.

Introduction

- Acetylcholine (ACh) is a neurotransmitter proven to be significant in neurodegenerative diseases like Alzheimer's [1].
- Previous ACh nanosensors used DNA as a scaffold due to ease of chemical synthesis and predictable base-pairing mechanism; however, DNA strands are susceptible to exonuclease digestion *in vivo* [2].
- PMO is a nucleic acid analog with increased stability against nuclease digestion due to uncharged backbones and the six-membered morpholine ring not being recognized by cellular enzymes (Fig. 1A) [3].



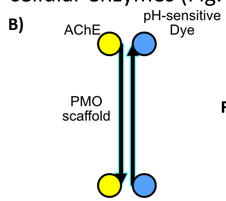


Figure 1: Chemical structures of DNA and PMO (A) and PMO nanosensor design (B).

- To explore PMO scaffold's structural potential, a simple PMO:DNA duplex scaffold will be loaded with components needed for ACh-sensing; Acetylcholinesterase and pH-sensitive dye (Fig. 1B). This will serve as proof of concept for similarly structured scaffolds, such as the dendrimeric ACh nanosensor used in the Clark Lab (Fig. 5).

Acetylcholinesterase (AChE) purification

HEK293T-expressed rAChE and *E.coli*-expressed dAChE4 were purified using affinity chromatography (Fig. 2), and characterized using spectrophotometer, HPLC, and SDS PAGE (Fig. 4).

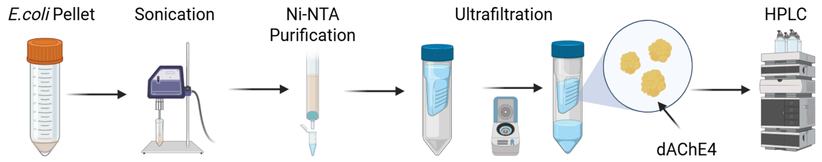


Figure 2: Workflow of AChE purification using *E.coli* pellets. Steps include sonication, Ni-NTA resin purification, ultrafiltration, and HPLC.

PMO-based ACh nanosensor preparation

- **Coupling:** dAChE was coupled to thiol-modified PMO via Maleimide-PEG_{12}-succinimidyl ester linker. Amine-reactive pH-sensitive dye (pHrodo Deep Red TFP ester) was conjugated to amine modified PMO with complimentary sequence.
- **pHrodo Deep Red-PMO:DNA confirmation:** TBE-PAGE and HPLC were used to characterize nanosensor conjugates.
- **Photoacoustic imaging:** TriTom system (PhotoSound) was used to obtain photoacoustic and fluorescence signals.

Results

- **Characterization and calibration of free pHrodo Deep Red**

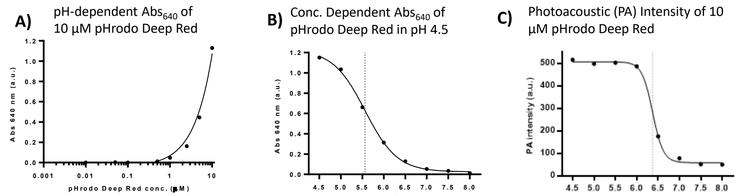


Figure 3: Characterization of pH-sensitive dye, pHrodo Deep Red. (A) pH-dependent Abs_{640} change of 10 µM pHrodo Deep Red dye was measured on spectrophotometer. (B) Concentration-dependent Abs_{640} change of pHrodo Deep Red dye at pH 4.5. (C) Photoacoustic intensity of 10 µM pHrodo Deep Red in a range of pH (4.5 - 8.0) was measured with the TriTom system. The dye solution was loaded in a phantom with PTFE tubing.

- **ACh sensor preparation and confirmation**

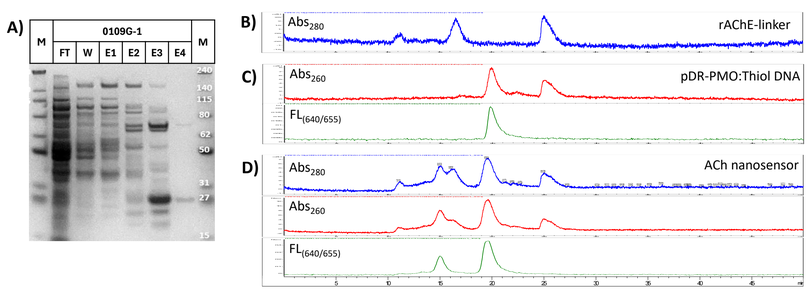


Figure 4: PMO-based ACh nanosensor preparation. (A) SDS PAGE confirmation of IMAC-purified dAChE4. Ni-NTA resin purification yielded dAChE4 (78 kDa) that were further purified via ultrafiltration and HPLC (protein ladder (M), flowthrough (FT), wash (W), and elution fractions (E)). (B-D) Analytical SEC-HPLC of rAChE-linker, pHrodo Deep Red-PMO:thiol DNA, and ACh sensor. Nucleic acid (Abs_{260}), Protein (Abs_{280}), and pHrodo Deep Red($FL_{(640/655)}$) absorbances are shown to characterize PMO, dAChE, and pHrodo deep red concentrations respectively.

Discussion

- **AChE purification:** Multiple purification rounds and further purification using ultrafiltration and HPLC was needed to remove non-targeted proteins (Fig. 4A).
- **Testing pHrodo Deep Red as photoacoustic dye:** Free pHrodo Deep Red showed clear pH-dependent Abs640 changes and photoacoustic signal up to 10 µM concentration. (Fig. 3) The nanosensor concentration needs to be within the tested range for sufficient photoacoustic signal measurement.
- **PMO-based ACh sensor synthesis:** HPLC analysis of synthesized nanosensors suggests concluded that large amounts of unreacted pHrodoDR-PMO:DNA remained within samples, resulting in limited concentrations and florescence (Fig. 4B).

Next Steps

1. Further refinement for AChE purification is needed to obtain sufficient amount of nanosensor.
2. Optimize nanosensor synthesis efficiency for sufficient photoacoustic signal from pHrodo Deep Red.
   - Better coupling and purification yield
3. Explore PMO use in the other more complex nanosensor scaffolds (Fig. 5).

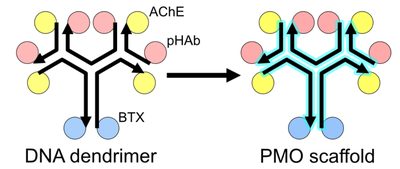


Figure 5: Schematics of ACh nanosensors with PMO scaffolds. Prevously reported DNA dendrimeric structure could be replaced with PMO strands for higher stability

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References

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