**Project Title:**

# Biomembranes - 10N-Nonyl Acridine Orange Inhibits Cardiolipin Polymorphism

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## 1. Background and purpose

Cardiolipin (CL) is a unique, four acyl-chain anionic phospholipid. The interesting features of this lipid include its structural uniformity and molecular symmetry. In eukaryotic cells, CL is restricted to mitochondria, the powerhouse of the cell. The formation of local non-bilayer structures has been proposed to be crucial for the mitochondrial membrane dynamics and is thought to control the function of mitochondria membrane proteins.

10*N*-nonyl acridine orange (NAO) is a fluorescent dye, which preferentially binds to CL. Consequently, NAO is commonly used as a mitochondria specific dye in fluorescence microscopy. On the other hand, it is known, that micromolar concentrations of NAO inhibit cristae formation in mitochondria, leading to cell death.

Recently, we measured the effect of NAO on the morphology of CL membranes by means of electron microscopy, small angle X-ray scattering, scanning transmission x-ray microscope and $^{31}$P-NMR. Our results indicate that NAO inhibits $Ca^{2+}$ induced CL polymorphism. Interestingly, we could demonstrate the ability of NAO to rescue CL from non-bilayer structures, for the first time.

## 2. Usage status and calculation methods

Primarily quantum mechanics (QM) simulations utilizing the Gaussian 03 software package have been performed. Additionally the NAMD software package has been employed for molecular dynamics (MD) simulations.

## 3. Results

Suitable force field parameters (CHARMM) for NAO were developed, based on the results of the QM simulations. Additionally, tetraoleylcardiolipin (TOCL) as well as tetralinoleylcardiolipin models were established. This enabled the creation of hydrated membrane patches consisting of 72 TOCL as well as TLCL molecules. Furthermore, membrane patches featuring additional 144 molecules of NAO were created and the initial equilibration as well as production runs are currently underway.

## 4. Conclusion

Based on our previously established experimental data in combination with the preliminary MD simulations we are currently developing a new model describing the interaction of NAO and CL interaction in lamellar phase from a molecular point of view.

## 5. Schedule and prospect for the future including aims for the next usage term

During the next usage period, the equilibration and productions runs for the parameterized membrane patches will be completed (NAMD software package). Additionally, QM simulations (Gaussian software package) of two additional molecules, namely doxorubicine (DOX) as well as pirarubicine (PIR) will be conducted to develop suitable CHRAMM force field parameters.

In combination with our experimental data the interaction of DOX as well as PIR with TOCL membranes will be studied utilizing MD simulations. This will significantly contribute to a better understanding of the interaction of NAO, DOX and PIR with TOCL, a prerequisite to clarify some of the underlying molecular mechanism of the side effects of anthracycline chemotherapeutics.

## 6. Concerning research achievements

Preliminary results have been presented at national and international meetings. Currently, initial preparations of a manuscript for a peer reviewed journal are underway to facilitate a timely submission once the MD simulations and final data evaluation have been completed.

**Fiscal Year 2011 List of Publications Resulting from the Use of RICC**

**[Publication]**

**[Proceedings, etc.]**

**[Oral presentation at an international symposium]**

**[Others]**

Poster/Scientific exhibit presentations

1. "Unraveling the Influence of 10-*N*-Nonyl Acridine Orange on Cardiolipin Polymorphism"
   P. Greimel, M. Murate, H. Takahashi, T. Kobayashi
   International ERATO Symposium on Lipid Structures, Osaka, Japan, 11.-14. November **2011**.
2. "Scanning Transmission X-Ray Microscopy: Unraveling the Influence of 10-*N*-Nonyl Acridine Orange on Cardiolipin Polymorphism"
   P. Greimel, T. Kobayashi
   12th Extreme Photonics Symposium, Japan, 30. June **2011**.