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Group 14 Metallafluorenes for Lipid Structure Detection and Cellular Imaging †

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Abstract: Fluorescent compounds have been shown to be useful in probing lipid dynamics, and there is ongoing interest in nontoxic, photostable, and sensitive dyes. Recently we evaluated a number of 2,7-disubstituted-alkynyl(aryl)-3,6-dimethoxy-9,9-diphenyl-sila- and germafluorenes for their potential as cellular fluorescent probes. These compounds exhibit remarkable quantum yields in hydrophobic environments and dramatic increases in emission intensity in the presence of surfactants. Here we show that they exhibit significant emission enhancements in the presence of small unilamellar vesicles and are nontoxic to *E. coli*, *S. aureus*, and *S. cerevisiae.* Further, they luminesce in *S. cerevisiae* cells with strong photostability and colocalize with the lipid droplet stain Nile Red, demonstrating their promise as lipid probes.

Keywords: fluorescence; lipid; metallafluorene

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1. Introduction

The introductory understanding of the role of biological membranes is that they are barriers and are used to regulate transport and for energy processes. Only fairly recently has there been a developing understanding that membranes are dynamic in their lipid composition and properties, and that these local differences participate in cellular processes in a profound way and have been linked to disease states [1, 2], including cellular stress [3].

Fluorescence spectroscopy is an accessible and powerful tool for characterizing membrane composition and behavior [4]. This technique relies on the development of suitable probes. Commonly used probes such as Nile Red, dansyl and NBD [5] vary with respect to relevant properties such as excitation and extinction wavelength, extinction coefficient, working concentration (sensitivity), photostability, and quantum yield, all of which can impact their utility. When this is coupled with the rapidly expanding research area, it is no surprise that a call for more probes to meet expanding needs is prominently articulated [4].

Recently we examined the potential of a small library of sila- and germafluorenes (metallafluorenes, or MFs) containing alkynyl(aryl) substituents at the 2,7- position ([6,7]; Fig. 1) for their potential as fluorescent probes of surfactants. These compounds are soluble and luminescent in aqueous solution and exhibit high quantum yields and dramatic emission enhancements in the presence of various surfactants (5-25 fold) [8]. These results suggested that MFs could have potential as probes of lipids both in vitro and in vivo. Here we examine the sensitivity, toxicity, and photostability of MFs toward lipids in both settings and demonstrate the potential of these compounds as lipid probes. Indeed, they are sensitive to DOPC small unilamellar vesicles (SUVs) with significant fluorescence enhancements. These dyes show no toxicity to Gram-positive bacteria, Gram-negative bacteria, and yeast cells and demonstrate high photostability. When compared to the commercially available lipid droplet dye Nile Red, these MFs show strong colocalization with more punctate staining, demonstrating their potential as lipid probes.

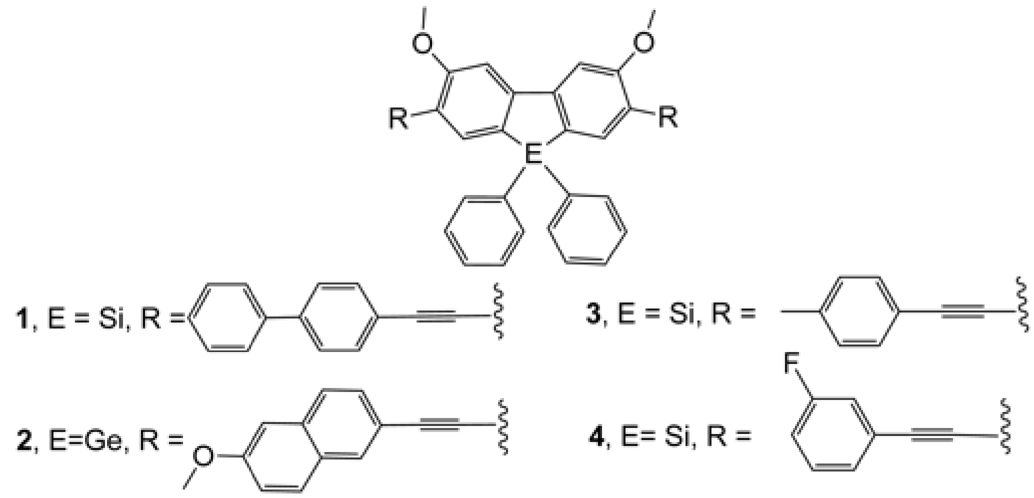

Figure 1. Structures of 2,7-disubstituted sila- and germafluorenes used in this study.

2. Materials and Methods

2.1. Materials

Phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL). 200 proof ethanol was purchased from Decon Labs (King of Prussia, PA). All chemicals used were of reagent grade and were used as received without further purification. DMSO and Nile Red were obtained from Millipore Sigma. Xylene was obtained from ThermoFisher. **1**-**4** were synthesized as previously described using an appropriate alkynyl(aryl) precursor in a palladium-catalyzed Sonagashira cross-coupling reaction [6,7] and dispensed from stocks in DMSO as previously described [8].

2.2. Preparation of Small Unilamellar Vesicles (SUVs)

At 25 ˚C, a stock concentration of 4.2 mM DOPC was prepared by drying under inert gas and then resuspended in 10 mM Tris buffer. After 30 minutes, DOPC was sonicated for 27 minutes at 25 ˚C until cloudy. The DOPC-SUV solution was then passed through an Avanti Mini Extruder eleven times to make uniformly-sized 0.1 µm DOPC-SUVs at 25 ˚C. DOPC-SUVs were then diluted to 0.1 mM in a quartz cuvette for fluorescence measurements [9,10].

2.3. Spectroscopy

Absorbance spectra were recorded on a Shimadzu 1800 with slits (bandpass) set to 1 nm. Emission spectra were collected in an acid-washed quartz cuvette on a Fluorolog-3 (SPEX) spectrofluorimeter. The temperature was maintained at 25 °C with a thermostatted cell holder equipped with a magnetic stirrer. Emission spectra were collected with the indicated excitation wavelength and slits (bandpass). MF photostability in xylene was observed at the indicated emission maximum.

2.4. Microbial Toxicity

Culture tubes containing LB media or YPD media were inoculated with *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), or *Saccharomyces cerevisiae,* respectively. 1-4 were added such that the final DMSO concentration was 2-10% and the MF at its solubility limit in the media. The tubes were incubated at either 37ºC (bacteria) or 30ºC (yeast) overnight and visually inspected for growth.

2.5. Confocal Laser Scanning Microscopy

Samples were prepared by smearing a small amount of cells onto a glass microscope slide and heat-fixed by passing the slide through flame no more than 5 times. Then **1-4** or NR was applied to heat-fixed cells at 15 μM and incubated at room temperature for 15 min for MFs and 10 min for Nile Red. Slides were then rinsed with 2-3 mL of deionized water, topped with coverslips and sealed with clear nail polish. Cells were imaged with a Zeiss LSM 900 confocal microscope with an excitation wavelength of 405 nm. For photostability, the sample was illuminated with 1% laser power and images collected periodically.

3. Results and Discussion

3.1. Spectroscopic Studies

To assess their sensitivity to a biologically relevant membrane, the emission spectra of **1-4** were compared in the absence and presence of DOPC-SUVs. As shown in **Fig. 2**, fold-enhancements range from 2-7 fold, with **1** and **2** showing the most dramatic changes.

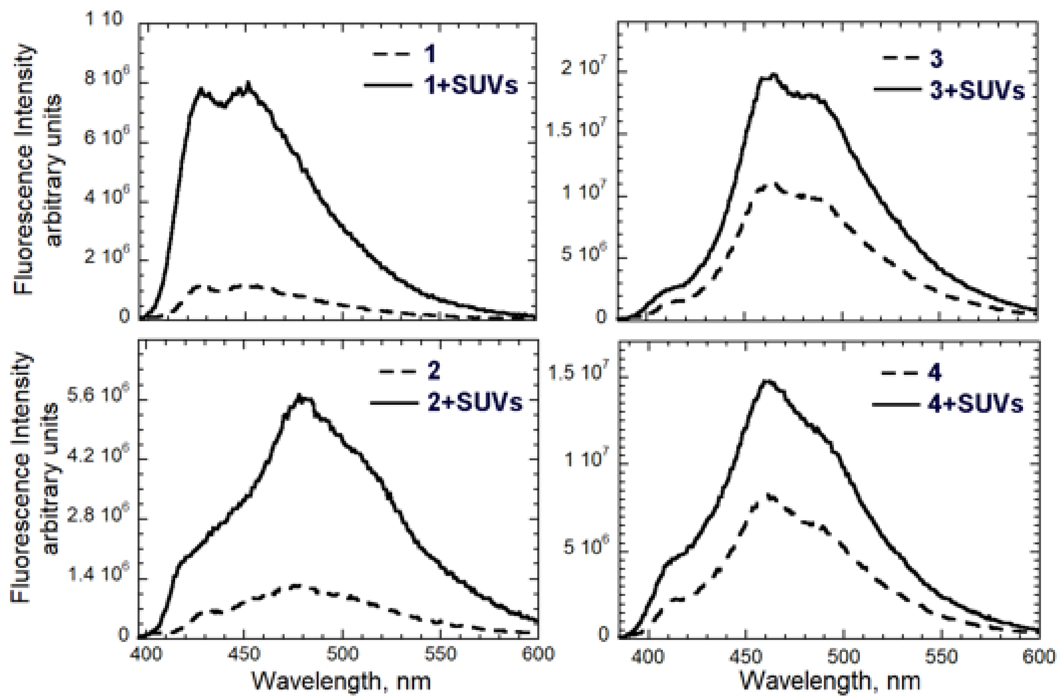

Figure 2. Emission spectra of 1-4 in the absence (dashed) and presence (solid) of 0.1 mM DOPC-SUVs. Conditions: 1 μM MF, 0.1 mM DOPC, 10 mM Tris pH 8, 25 °C. The excitation wavelength was 387 nm and the slits (bandpass) set to 1.0 nm. 3 min incubation.

The photostability of these MFs was initially probed by observing the emission signal as a function of time in xylene, which is used to mimic the interior of membranes [11]. As summarized in Figure. 3, these signals are remarkably stable over two hours of continuous excitation. Together, the responsiveness to SUVs and photostability in xylene indicate promise for MFs as probes of lipids *in vivo*.

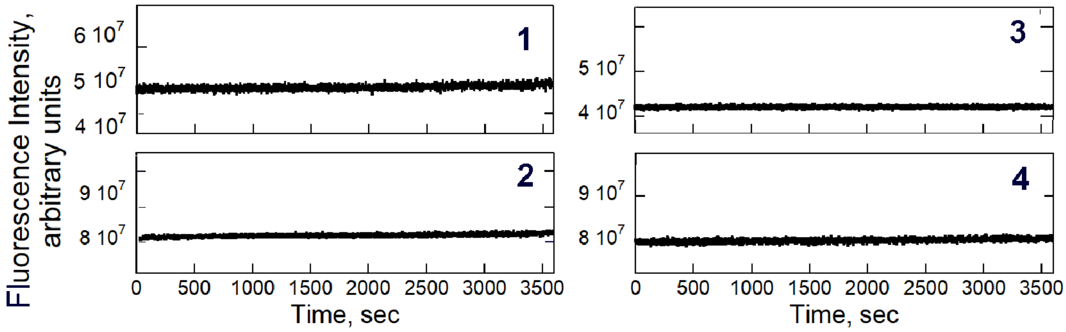

Figure 3. Photostability in vitro. Compounds 1-4 were diluted into p-xylene and excited continuously. Conditions: 1 μM MF, 0.1-0.4% DMSO. 1: excitation at 387 nm, slits 1 nm; 2: excitation at 390 nm, slits 0.8 nm; 3: excitation at 376 nm, slits 0.9 nm; 4: excitation at 376 nm, slits 0.9 nm.

3.2. Microbial Toxicity Studies

To assess their potential for use in cellular imaging, MFs were screened for toxicity against microorganisms. At the solubility limit for these compounds in media (at least 50 μM), there was no inhibition of yeast growth. For E. coli and S. aureus no inhibition of growth was observed at the MF solubility limit in media.

3.3. Imaging of S. cerevisiae with Metallafluorenes

To determine if these MFs can be used to stain cells, **1-4** were introduced to yeast cells and subsequently imaged using confocal microscopy. **Fig. 4** illustrates that in all cases, MF emission intensity is visible inside fixed yeast cells.

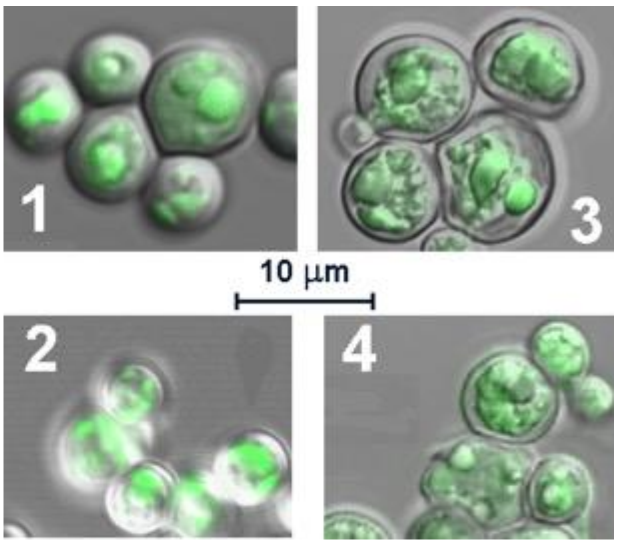

Figure 4. Confocal Imaging of MF in Yeast Cells**.** Conditions: 15 μM MF as indicated, 63x. The excitation wavelength was 405 nm and the scan range 400-600 nm.

To assess MF photostability in yeast cells, excitation was applied and fluorescence observed as a function of time. As summarized in Figure. 5 for 1 and 2, fluorescence persisted for over 2 min, with 2 showing greater photostability. See Supplemental Fig. S1 for photostability studies of 3 and 4.

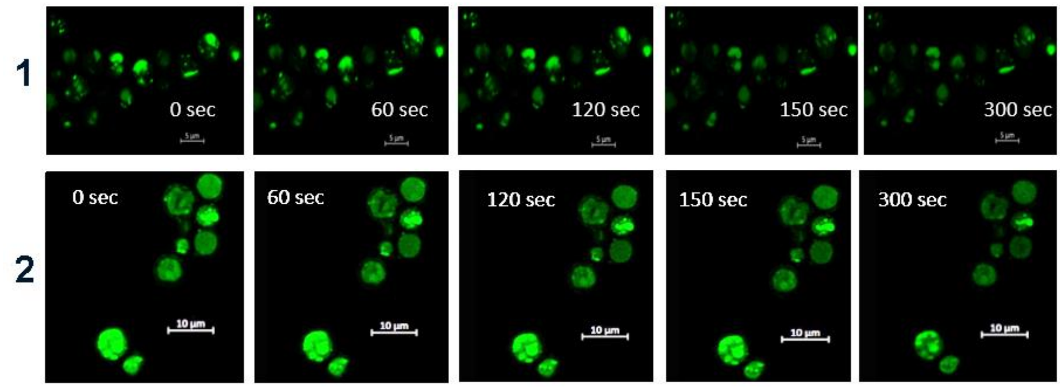

Figure. 5. Photostability of 1 and 2 in Yeast Cells. See Methods for details. S. cerevisiae were stained for 15 minutes with 15 μM **1** or **2** and then imaged periodically during continuous excitation. Magnification is 63x.

Finally, to determine where these MF localize in yeast, we costained with Nile Red, a well known lipid droplet stain [12]. As shown in Figure. 6, 1 colocalizes with this probe and demonstrates clear specificity for *S. cereviseiae* organelles, including the vacuole and possibly lipid granules. See Supplemental Fig. S2 for a colocalization study of 2 and 4.

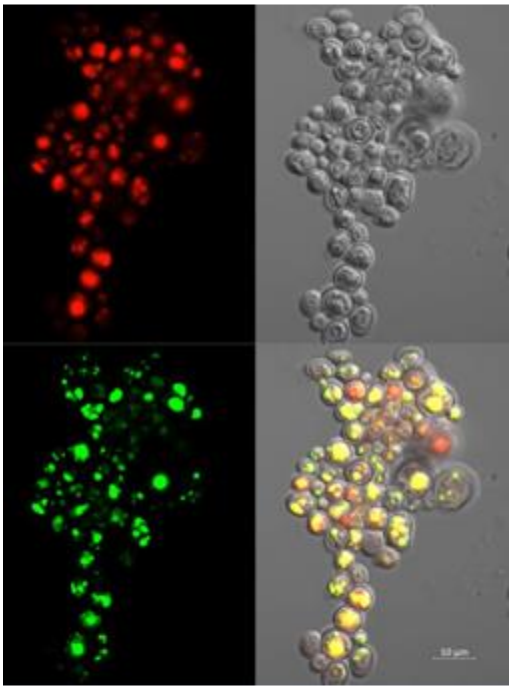

Figure. 6. Compound 1 Colocalizes in Yeast with Nile Red. Red, Nile Red; green, **1**; top right, transmitted light; bottom right, yellow indicates colocalization. 15 μM probe, 63x magnification.

4. Conclusions

We show here that these metallafluorenes have good photostability and are sensitive to lipid structures in vitro, demonstrating impressive fold enhancements in the presence of SUVs. Further, they are non toxic to cells and can enter cells and colocalize with Nile Red but yield more punctate images. In addition, the higher extinction coefficients of MFs and competitive quantum yields [8] make them more sensitive. All of these observations bode well for the application of MFs as lipid probes both in vitro and in vivo. The synthetic scaffolding of these MFs provides convenient tuning of desired properties by changing the 2,7 substituent. This feature facilitates designs that incorporate optimal solubility, emission spectra, dipole moment, and solvatochromism for specific applications.

5. Patents

WO/2020/210416; PCT International Patent Application No.: PCT/US2020/027355

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Photostability of **3** and **4** in Yeast Cells. Figure S2: **2** and **4** Colocalize in Yeast with Nile Red.

Author Contributions: Conceptualization, C.D., H.S. and S.J.-N; Investigation, H.S. and S.J.-N.; Resources, C.D., J.B.-W, S.G. and W.O.; Data Curation, S.J.-N.; Writing – Original Draft Preparation, C.D. and H.S.; Writing – Review & Editing, C.D. and S.J.-N.; Supervision, C.D.; Project Administration, C.D.; Funding Acquisition, J.B.-W. and C.D. H.S. and S.J.-N. contributed equally to the preparation of this manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

References

1. Levental, I.; Levental, K.R.; Heberle, F.A. Lipid Rafts: Controversies Resolved, Mysteries Remain. *Trends Cell Biol* **2020**, *30*, 341-353, doi:10.1016/j.tcb.2020.01.009.
2. Carravilla, P.; Nieva, J.L.; Eggeling, C. Fluorescence Microscopy of the HIV-1 Envelope. *Viruses* **2020**, *12*, doi:10.3390/v12030348.
3. Ashoka, A.H.; Ashokkumar, P.; Kovtun, Y.P.; Klymchenko, A.S. Solvatochromic Near-Infrared Probe for Polarity Mapping of Biomembranes and Lipid Droplets in Cells under Stress. *J Phys Chem Lett* **2019**, *10*, 2414-2421, doi:10.1021/acs.jpclett.9b00668.
4. Klymchenko, A.S.; Kreder, R. Fluorescent probes for lipid rafts: from model membranes to living cells. *Chem Biol* **2014**, *21*, 97-113, doi:10.1016/j.chembiol.2013.11.009.
5. Klymchenko, A.S. Solvatochromic and Fluorogenic Dyes as Environment-Sensitive Probes: Design and Biological Applications. *Acc Chem Res* **2017**, *50*, 366-375, doi:10.1021/acs.accounts.6b00517.
6. Hammerstroem, D.W.; Braddock-Wilking, J.; Rath, N.P. Synthesis and characterization of luminescent 2,7-disubstituted silafluorenes. *J. Organomet. Chem.* **2016**, *813*, 110–118. 10.1016/j.jorganchem.2016.04.004.
7. Hammerstroem, D.W.; Braddock-Wilking, J.; Rath, N.P. Luminescent 2,7-disubstituted germafluorenes. *J. Organomet. Chem.* **2017**, *830*, 196–202. 10.1016/j.jorganchem.2016.12.021
8. Spikes, H.J.; Jarrett-Noland, S.J.; Germann, S.M.; Braddock-Wilking, J.; Dupureur, C.M. Group 14 Metallafluorenes as Sensitive Luminescent Probes of Surfactants in Aqueous Solution. *J Fluoresc* **2021**, doi:10.1007/s10895-021-02730-3.
9. Amaro, M.; Filipe, H.A.; Ramalho, J.P.; Hof, M.; Loura, L.M.S. Fluorescence of nitrobenzoxadiazole (NBD)-labeled lipids in model membranes is connected not to lipid mobility but to probe location. *Phys Chem Chem Phys* **2016**, *18*, 7042-7054.
10. Mazeres, S.; Joly, E.; Lopez, A.; Tardin, C. Characterization of M-laurdan, a versatile probe to explore order in lipid membranes. *F1000Res* **2014**, *3*, 172, doi:10.12688/f1000research.4805.2.
11. Shaya, J.; Collot, M.; Benailly, F.; Mahmoud, N.; Mely, Y.; Michel, B.Y.; Klymchenko, A.S.; Burger, A. Turn-on Fluorene Push-Pull Probes with High Brightness and Photostability for Visualizing Lipid Order in Biomembranes. *ACS Chem Biol* **2017**, *12*, 3022-3030, doi:10.1021/acschembio.7b00658.
12. Greenspan, P.; Mayer, E.P.; Fowler, S.D. Nile red: a selective fluorescent stain for intracellular lipid droplets. *J Cell Biol* **1985**, *100*, 965-973, doi:10.1083/jcb.100.3.965.