Let’s try to figure out the concentrations of ACMA we need by doing some literature reading.

<https://www.jbc.org/content/257/16/9365.full.pdf>

-The assay medium at 30 "C contained in a final volume of 1.5 ml: 10 m~ MES, the reconstituted vesicles (2 mg of asolectin, 2.78 pg of protein), 50 mM CH.,COOK adjusted to pH 6.0 with KOH, and 5 u1 of ACMA (0.5 mM stock solution in ethanol). All assay components, except MgATP, were added and incubated for 3 min in the spectrophotometer at 30 "C. The fluorescence intensity was taken as 100% for a full scale reading on the recorder after correction for the light-scattering contribution fmm the vesicles and the signal due to the buffer. An aliquot of 60 pl of 150 mM MgATP (10 mM MES adjusted to pH 6.0 with KOH) was added to start the reaction. ACMA fluorescence measurements were taken with a Beckman ACTA V spectrophotometer equipped with a homemade thermostated cell. Excitation wavelength was 400 nm and the emission light was screened by a PIL Lienenfdter (460 nm

ACMA conc calc: (0.5 mM)(5 uL) = C2(1500 uL)

C2 = 0.0016 mM = 1.6 uM

**They do stock soln in ethanol**

<https://reader.elsevier.com/reader/sd/pii/S0005273605000829?token=9778114C344C0154A9D15F8A59A8F655427F709B0776F52D17A117805C8DE30E8DE2D28C835CCAA4176F5FB8E77AA092>

1 uM ACMA (what I tried)

I thought ACMA wouldn’t work in bulk??

<https://ediss.uni-goettingen.de/bitstream/handle/11858/00-1735-0000-0023-3E83-7/Dissertation_Miriam-Schwamborn.pdf?sequence=1>

but otherwise:

Excitation and emission spectrum of ACMA in ATPase buffer (0.5 mM MOPS, 100 mM KCl, 2 mM MgCl2, pH 7.3). At this pH, the mono-cationic species is predominat.

0.9 µM ACMA (3.5 µL of 0.2 mM in DMSO

Need to be protonated, which makes logical sense - ACMA is a weak base (pKa = 8.6) and membrane permeable in its deprotonated form, but upon protonation, the fluorescence is quenched and the membrane permeability is reduced drastically. [72,126,129] Th

excited with 410 nm and the fluorescence emission at 490 nm was detecte

**can also try to just read the vesicles in biotek afterwards? What would happen in bulk?**

**EXPERIMENTS**

First try extract, buffer, 1 uM ACMA and put into biotek for like 2 hrs

Then try 2 uM ACMA and 50 uM ACMA in vesicles and image

Then think of bulk experiments u can do for atp stuff if that helps ☹