Investigation of the Propensity for Self-Association of the N-Terminal Domain of Annexin A2 in the Presence of Anionic Lipids

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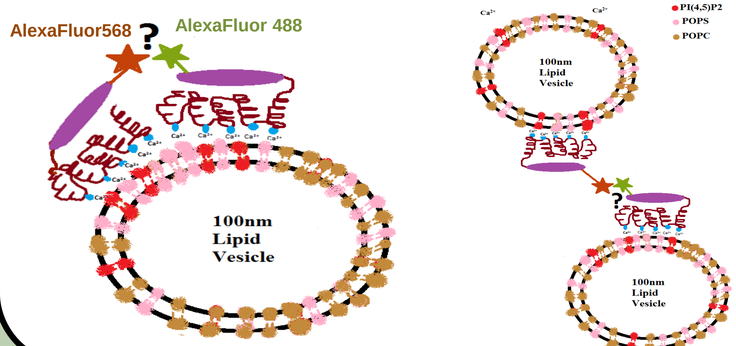
*All emission spectra were dilution corrected to better display changes in fluorescence intensity upon lipid/CaCl2 addition.

Abstract

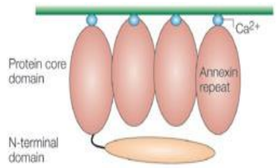
Annexin A2 (ANXA2), a calcium-dependent membrane binding protein shown to promote membrane domain formation, is implicated in many cellular processes such as endocytosis and exocytosis. In order to determine if ANXA2 promotes domain formation by self-associating with the N-terminal domain of adjacent ANXA2 proteins upon binding anionic phospholipids, Forster Resonance Energy Transfer (FRET) assays were performed using ANXA2 proteins fluorescently labeled at the singular, exposed N-terminal cysteine residue. No FRET transfer was observed, suggesting that under these experimental conditions the N-terminal domains do not interact.

Hypothesis



Background

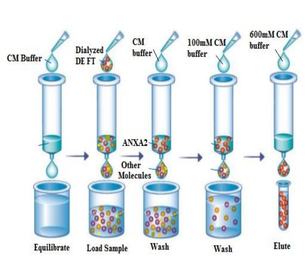
- **ANXA2 is the annexin protein most relevant in human health and disease[2]**
- **ANXA2 binding to the plasma membrane is believed to cause cytosolic anionic phospholipids to cluster[2]**

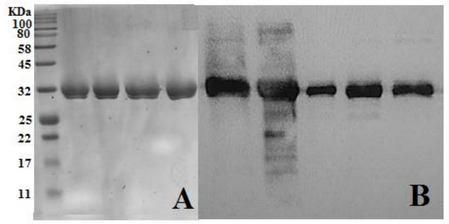


N-terminal domain[1]:

- **regulates function by altering calcium affinities at protein interaction sites**
- **has sites for protein-protein interactions and post-translational modification**

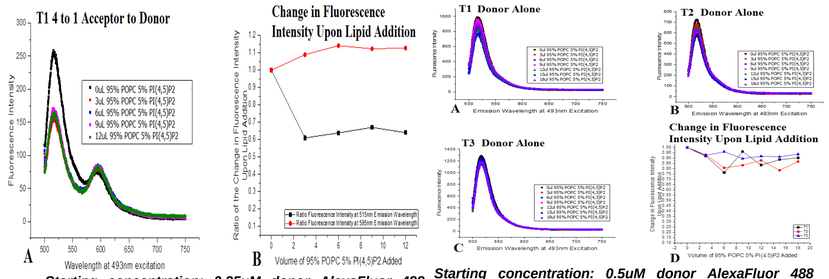
ANXA2 Purification





(A) Denatured SDS-PAGE Gel of ANXA2 Purification. Left to Right: 6 HEPES Buffer, CM Fraction 6 MES Buffer, CM Fraction 7 HEPES Buffer, CM Fraction 7 MES Buffer. (B) Western Blot after ANXA Purification. Left to Right: (1) French Press Flow through, (2) French Press pellet, (3) DE Flow Through, (4) CM Flow Through Fraction 6, (5) CM Wash Fraction 6.

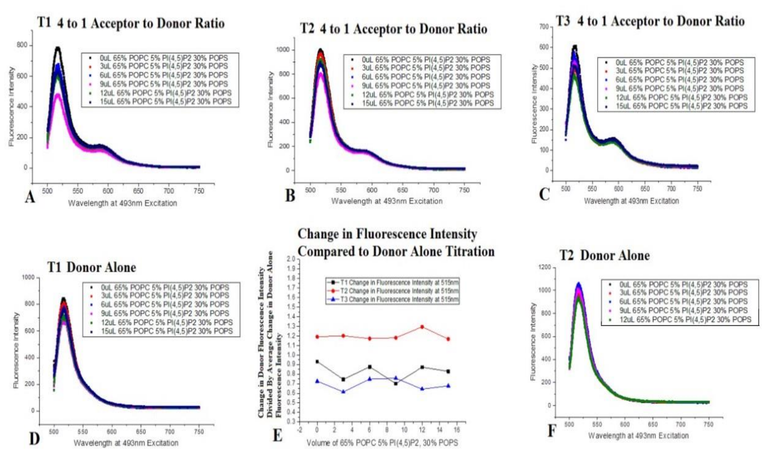
95% POPC 5% PI(4,5)P_2



Starting concentration: 0.25uM donor AlexaFluor 488 ANXA2, 1.0uM AlexaFluor 568 ANXA2 acceptor, and 0.5mM CaCl2. (A) T1 Change in Fluorescence intensity of the Emission Spectra Upon Lipid Addition (B)Change in Ratio of Fluorescence Intensity Upon Lipid Addition, all values for each trial were divided by the fluorescence intensity when there were no lipids present.

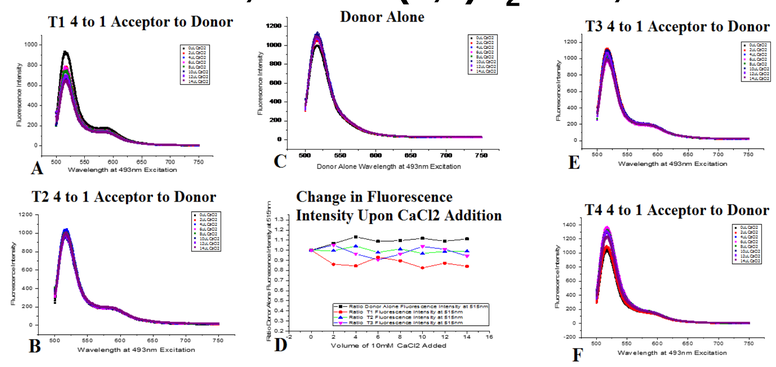
Starting concentration: 0.5uM donor AlexaFluor 488 ANXA2 and 0.5mM CaCl2. (A-C) T1-T3 Change in Fluorescence intensity of the Emission Spectra Upon Lipid Addition (D)Change in Ratio of Fluorescence Intensity Upon Lipid Addition, all values for each trial were divided by the fluorescence intensity when there were no lipids present.

65% POPC 5% PI(4,5)P_2, 30% POPS



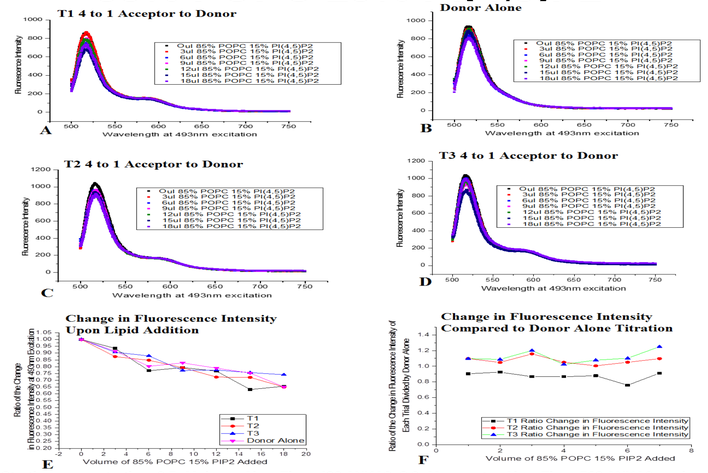
Starting concentration: 0.5uM donor AlexaFluor 488 ANXA2, 2.0uM acceptor AlexaFluor 568 ANXA2, and 0.5mM CaCl2. (A-D, F) Change in fluorescence intensity of the emission spectra upon lipid addition (E) Change in Ratio of Fluorescence intensity upon lipid addition divided by donor alone fluorescence intensity.

55% POPC, 15% PI(4,5)P_2 30%, POPS



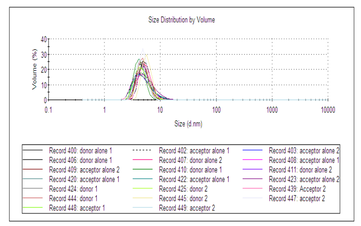
Starting concentration: 0.5uM donor AlexaFluor 488 ANXA2, 2.0uM acceptor AlexaFluor 568 ANXA2, 0.5mM EGTA. (A-C, E-F) Change in fluorescence intensity of the emission spectra upon $CaCl_2$ addition (D) Change in the fluorescence intensity of the emission spectra upon $CaCl_2$ addition divided by fluorescence intensity without lipids.

85% POPC 15% PI(4,5)P2

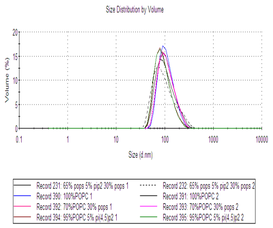


Starting concentration: 0.5uM donor AlexaFluor 488 ANXA2, 2.0uM acceptor AlexaFluor 568 ANXA2, and 0.5mM $CaCl_2$. (A-D,) Change in fluorescence intensity of the emission spectra upon lipid addition (E) Change in fluorescence intensity upon lipid addition intensity divided by fluorescence intensity before lipid addition (F) Change in fluorescence intensity upon lipid addition divided by donor alone fluorescence intensity.

Dynamic Light Scattering (DLS) Data



Acceptor and Donor Size Before Experimentation



Lipid Vesicle Size After Extrusion

Conclusion

- **No FRET transfer observed between the two protein fragments.**
- **Aggregation and light scattering by lipids altered the FRET signal**
- **At this point, the hypothesis cannot be confirmed, however, a different donor/acceptor pair with a larger Forster distance R^0 and better spectral overlap might yield different results.**

Future studies:

- **Perform FCS-FLIM experiments in cells**

Principal References

1. Gerke, V., Creutz, C. E., & Moss, S. E. (2005). Annexins: linking Ca2+ signalling to membrane dynamics. *Nature reviews Molecular cell biology, 6*(6), 449-461.
2. Gerke, V., & Moss, S. E. (2002). Annexins: from structure to function. *Physiological reviews, 82*(2), 331-371.
3. Illien, F., Piao, H.-R., Coué, M., Di Marco, C., & Ayala-Sanmartin, J. (2012). Lipid organization regulates annexin A2 Ca 2+-sensitivity for membrane bridging and its modulator effects on membrane fluidity. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1818*(11), 2892-2900.
4. Patel, D. R., Isas, J. M., Ladokhin, A. S., Jao, C. C., Kim, Y. E., Kirsch, T., . . . Haigler, H. T. (2005). The conserved core domains of annexins A1, A2, A5, and B12 can be divided into two groups with different Ca2+-dependent membrane-binding properties. *Biochemistry, 44*(8), 2833-2844.