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Experimental & Computational Approaches in Unraveling Mechanisms Of Amyloid Formation

**Intrinsically Disordered Proteins share a
common molecular mechanism in membranes
damages: the lipid-chaperone hypothesis.**

Carmelo La Rosa

University of Catania, Italy

Email: carmelo.larosa@unict.it

Recently, a phenomenological model¹⁻⁶ based on experimental findings was proposed to simulate the transfer kinetics of a lipid-protein complex from water to the lipid-bilayer phase. We find that the water-soluble lipid-protein complex inserts into the membrane faster than the free protein due to the hydrophobic differences between the lipid-protein complex and the bare protein. This model is supported by several experiments carried out on human IAPP⁷.

Importantly, our results carried out on human IAPP offer a novel mechanistic explanation as to why bilayer thickness is inversely correlated to membrane damage induced by amyloid proteins. The increased bilayer damage seen for thin bilayers is caused by an increase of lipid-bound proteins causing faster insertion and membrane disruption as there are more free lipids in solution for the shorter acyl-chains (high CMC). Thus, it is not the thickness of the bilayer that is important in causing membrane damage, but rather the relative free lipid concentration in solution, coupled to the stability of the lipid-protein complex and its hydrophobicity.

Question: Are the results obtained an exclusive behavior of the human IAPP, or can they be extended to other IDPs?

An answer to this question can be given by investigating other IDPs. In this light, different amyloidogenic proteins, including A β peptide and α -synuclein was investigated. Further, we also investigated two non-amyloidogenic proteins, rat-IAPP and β -synuclein, as controls.

Remarks on human IAPP

Self-assembled phospholipids into LUVs are in chemical equilibrium with free lipids in solution.

Free lipids in solution interacting with hIAPP form a stable lipid-protein complex

hAIPP interacting with LUVs containing **high cmc** phospholipids (**μM**) form pores and not fibrils.

hIAPP interacting with LUVs containing **low cmc** phospholipids (**nM**) form fibrils in aqueous phase and not pores

Free phospholipids, by forming a **phospholipid-hIAPP** complex act as chaperon and transport hIAPP into the bilayer

Question: is this molecular mechanism exclusively of hIAPP or it is a general mechanism common to Intrinsically Disordered Proteins?

In order to answer this question, we have investigated A β 40, A β 42, rIAPP, α -synuclein and β -synuclein interacting with LUVs containing phospholipids with different **cmc**.

Dye leakage and ThT assay of A β (1-40) interacting with model membranes containing phospholipid having different CMC (phospholipid used; from PC 14 (1:0) to PC 20 (1:0) CMC PC 14 (1:0), PC 18 (1:0) and PC 20 (1:0): 1×10^{-7} M, 2×10^{-8} M and 6.3×10^{-9} M respectively.

A β s form pore with a dimension of 1.8 nm, so to determine leakage we use the couple Ca⁺⁺/fura-2, instead carboxy-fluorescein.

Results: A β (1-40) show the same behavior of hIAPP

In model membrane containing phospholipid with **high CMC** pore formation is enhanced and fibril formation is repressed

in model membrane containing phospholipid with **low CMC** pore formation is repressed and fibril formation is enhanced.

α -synuclein show the same behavior of hIAPP and A β (1-40).

Is known that both rat IAPP and β -synuclein do not form fibrils and are not toxic for cells. Here we report ThT assay and carboxy-fluorescein dye leakage of rat IAPP and β -synuclein. Results: at protein concentrations of 10 μ M β -synuclein and rat IAPP do not form fibrils. Rat IAPP forms pores according to data from Raleigh lab⁹. Also rat IAPP in some conditions is toxic. β -synuclein does not form pores.

Experiments show how pore formation of hIAPP, rIAPP and α -synuclein does not depend on bilayer protein-mismatch and bilayer thickness.

Addition of a relatively high concentration of free PC14 (1:0) (CMC 1×10^{-7}) to PC 22 (1:0) model membranes allows for the formation of the lipid-protein complex, promoting insertion into the bilayer, pore formation and increasing leakage.

NMR and CD measurements suggest the formation of lipid-protein complex.

On the increased hydrophobicity of lipid-protein complex respect bare protein

Link between our biophysical investigations and in vivo data: literature data¹⁰.

Analyzing literature data emerges that possible source of free phospholipids should be lipid oxidation.

CONCLUSIONS

Free lipids form a stable complex with IDPs

IDS interacting with membranes share common molecular mechanisms in pore and fibril formation

Lipids transport IDPs into bilayer (Lipid-Chaperone hypothesis)

**Lipid-Chaperone hypothesis is a more general molecular model
that include both amyloid and oligomer hypothesis**

Oxidized lipids are candidate to trigger Diabetes, Alzheimer and Parkinson

References

- (1) La Rosa, C.; Condorelli, M.; Compagnini, G.; Lolicato, F.; Milardi, D.; Do, T. N.; Karttunen, M.; Pannuzzo, M.; Ramamoorthy, A.; Fraternali, F.; Collu, F.; Rezaei, H.; Strodel, B.; Raudino, A. Symmetry-Breaking Transitions in the Early Steps of Protein Self-Assembly. *Eur Biophys J* **2020**. <https://doi.org/10.1007/s00249-020-01424-1>.
- (2) Sciacca, M. F. M.; Kotler, S. A.; Brender, J. R.; Chen, J.; Lee, D.; Ramamoorthy, A. Two-Step Mechanism of Membrane Disruption by A β through Membrane Fragmentation and Pore Formation. *Biophys. J.* **2012**, *103* (4), 702–710. <https://doi.org/10.1016/j.bpj.2012.06.045>.
- (3) Brender, J. R.; Salamekh, S.; Ramamoorthy, A. Membrane Disruption and Early Events in the Aggregation of the Diabetes Related Peptide IAPP from a Molecular Perspective. *Acc. Chem. Res.* **2012**, *45* (3), 454–462. <https://doi.org/10.1021/ar200189b>.
- (4) Sciacca, M. F. M.; Tempira, C.; Scollo, F.; Milardi, D.; La Rosa, C. Amyloid Growth and Membrane Damage: Current Themes and Emerging Perspectives from Theory and Experiments on A β and HIAPP. *Biochim Biophys Acta Biomembr* **2018**. <https://doi.org/10.1016/j.bbamem.2018.02.022>.
- (5) Scalisi, S.; Sciacca, M. F. M.; Zhavnerko, G.; Grasso, D. M.; Marletta, G.; La Rosa, C. Self-Assembling Pathway of HiApp Fibrils within Lipid Bilayers. *ChemBioChem* **2010**, *11* (13), 1856–1859. <https://doi.org/10.1002/cbic.201000090>.
- (6) La Rosa, C.; Scalisi, S.; Lolicato, F.; Pannuzzo, M.; Raudino, A. Lipid-Assisted Protein Transport: A Diffusion-Reaction Model Supported by Kinetic Experiments and Molecular Dynamics Simulations. *J Chem Phys* **2016**, *144* (18), 184901. <https://doi.org/10.1063/1.4948323>.
- (7) Scollo, F.; Tempira, C.; Lolicato, F.; Sciacca, M. F. M.; Raudino, A.; Milardi, D.; La Rosa, C. Phospholipids Critical Micellar Concentrations Trigger Different Mechanisms of Intrinsically Disordered Proteins Interaction with Model Membranes. *J. Phys. Chem. Lett.* **2018**, *9* (17), 5125–5129. <https://doi.org/10.1021/acs.jpclett.8b02241>.
- (8) Korshavn, K. J.; Satriano, C.; Lin, Y.; Zhang, R.; Dulchavsky, M.; Bhunia, A.; Ivanova, M. I.; Lee, Y.-H.; Rosa, C. L.; Lim, M. H.; Ramamoorthy, A. Reduced Lipid Bilayer Thickness Regulates the Aggregation and Cytotoxicity of Amyloid- β . *J. Biol. Chem.* **2017**, *292* (11), 4638–4650. <https://doi.org/10.1074/jbc.M116.764092>.
- (9) Cao, P.; Abedini, A.; Wang, H.; Tu, L.-H.; Zhang, X.; Schmidt, A. M.; Raleigh, D. P. Islet Amyloid Polypeptide Toxicity and Membrane Interactions. *Proceedings of the National Academy of Sciences* **2013**, *110* (48), 19279–19284. <https://doi.org/10.1073/pnas.1305517110>.
- (10) Söderberg, M.; Edlund, C.; Kristensson, K.; Dallner, G. Fatty Acid Composition of Brain Phospholipids in Aging and in Alzheimer’s Disease. *Lipids* **1991**, *26* (6), 421–425. <https://doi.org/10.1007/BF02536067>.
- (11) Westermark, P.; Andersson, A.; Westermark, G. T. Islet Amyloid Polypeptide, Islet Amyloid, and Diabetes Mellitus. *Physiol. Rev.* **2011**, *91* (3), 795–826. <https://doi.org/10.1152/physrev.00042.2009>.
- (12) Lorenzo, A.; Yankner, B. A. Beta-Amyloid Neurotoxicity Requires Fibril Formation and Is Inhibited by Congo Red. *PNAS* **1994**, *91* (25), 12243–12247. <https://doi.org/10.1073/pnas.91.25.12243>.
- (13) Pike, C. J.; Walencewicz, A. J.; Glabe, C. G.; Cotman, C. W. In Vitro Aging of SS-Amyloid Protein Causes Peptide Aggregation and Neurotoxicity. *Brain Research* **1991**, *563* (1–2), 311–314. [https://doi.org/10.1016/0006-8993\(91\)91553-D](https://doi.org/10.1016/0006-8993(91)91553-D).
- (14) Okada, T.; Wakabayashi, M.; Ikeda, K.; Matsuzaki, K. Formation of Toxic Fibrils of Alzheimer’s Amyloid β -Protein-(1–40) by Monosialoganglioside GM1, a Neuronal Membrane Component. *Journal of Molecular Biology* **2007**, *371* (2), 481–489. <https://doi.org/10.1016/j.jmb.2007.05.069>.
- (15) Novitskaya, V.; Bocharova, O. V.; Bronstein, I.; Baskakov, I. V. Amyloid Fibrils of Mammalian Prion Protein Are Highly Toxic to Cultured Cells and Primary Neurons. *J. Biol. Chem.* **2006**, *281* (19), 13828–13836. <https://doi.org/10.1074/jbc.M511174200>.
- (16) Nalbantoglu, J.; Tirado-Santiago, G.; Lahsaïni, A.; Poirier, J.; Goncalves, O.; Verge, G.; Momoli, F.; Welner, S. A.; Massicotte, G.; Julien, J.-P.; Shapiro, M. L. Impaired Learning and LTP in Mice Expressing the Carboxy Terminus of the Alzheimer Amyloid Precursor Protein. *Nature* **1997**, *387* (6632), 500–505. <https://doi.org/10.1038/387500a0>.
- (17) Morris, R. G. M.; Garrud, P.; Rawlins, J. N. P.; O’Keefe, J. Place Navigation Impaired in Rats with Hippocampal Lesions. *Nature* **1982**, *297* (5868), 681–683. <https://doi.org/10.1038/297681a0>.
- (18) Bennett, E. J.; Shaler, T. A.; Woodman, B.; Ryu, K.-Y.; Zaitseva, T. S.; Becker, C. H.; Bates, G. P.; Schulman, H.; Kopito, R. R. Global Changes to the Ubiquitin System in Huntington’s Disease. *Nature* **2007**, *448* (7154), 704–708. <https://doi.org/10.1038/nature06022>.
- (19) Katzman, R.; Terry, R.; DeTeresa, R.; Brown, T.; Davies, P.; Fuld, P.; Renbing, X.; Peck, A. Clinical, Pathological, and Neurochemical Changes in Dementia: A Subgroup with Preserved Mental Status and Numerous Neocortical Plaques. *Ann Neurol.* **1988**, *23* (2), 138–144. <https://doi.org/10.1002/ana.410230206>.
- (20) Chiti, F.; Dobson, C. M. Protein Misfolding, Functional Amyloid, and Human Disease. *Annu. Rev. Biochem.* **2006**, *75* (1), 333–366. <https://doi.org/10.1146/annurev.biochem.75.101304.123901>.
- (21) Sciacca, M. F. M.; Lolicato, F.; Di Mauro, G.; Milardi, D.; D’Urso, L.; Satriano, C.; Ramamoorthy, A.; La Rosa, C. The Role of Cholesterol in Driving IAPP-Membrane Interactions. *Biophys. J.* **2016**, *111* (1), 140–151. <https://doi.org/10.1016/j.bpj.2016.05.050>.
- (22) Milardi, D.; Sciacca, M. F.; Randazzo, L.; Raudino, A.; La Rosa, C. The Role of Calcium, Lipid Membranes and Islet Amyloid Polypeptide in the Onset of Type 2 Diabetes: Innocent Bystanders or Partners in a Crime? *Frontiers in endocrinology* **2014**, *5*.
- (23) Sciacca, M. F.; Pappalardo, M.; Milardi, D.; Grasso, D. M.; La Rosa, C. Calcium-Activated Membrane Interaction of the Islet Amyloid Polypeptide: Implications in the Pathogenesis of Type II Diabetes Mellitus. *Archives of biochemistry and biophysics* **2008**, *477* (2), 291–298.
- (24) Sciacca, M. F.; Milardi, D.; Messina, G. M.; Marletta, G.; Brender, J. R.; Ramamoorthy, A.; La Rosa, C. Cations as Switches of Amyloid-Mediated Membrane Disruption Mechanisms: Calcium and IAPP. *Biophysical journal* **2013**, *104* (1), 173–184.
- (25) Sciacca, M. F. M.; Monaco, I.; Rosa, C. L.; Milardi, D. The Active Role of Ca²⁺ Ions in A β -Mediated Membrane Damage. *Chem. Commun.* **2018**, *54* (29), 3629–3631. <https://doi.org/10.1039/C8CC01132J>.
- (26) Brender, J. R.; Krishnamoorthy, J.; Messina, G. M. L.; Deb, A.; Vivekanandan, S.; Rosa, C. L.; Penner-Hahn, J. E.; Ramamoorthy, A. Zinc Stabilization of Prefibrillar Oligomers of Human Islet Amyloid Polypeptide. *Chem. Commun.* **2013**, *49* (32), 3339–3341. <https://doi.org/10.1039/C3CC40383A>.