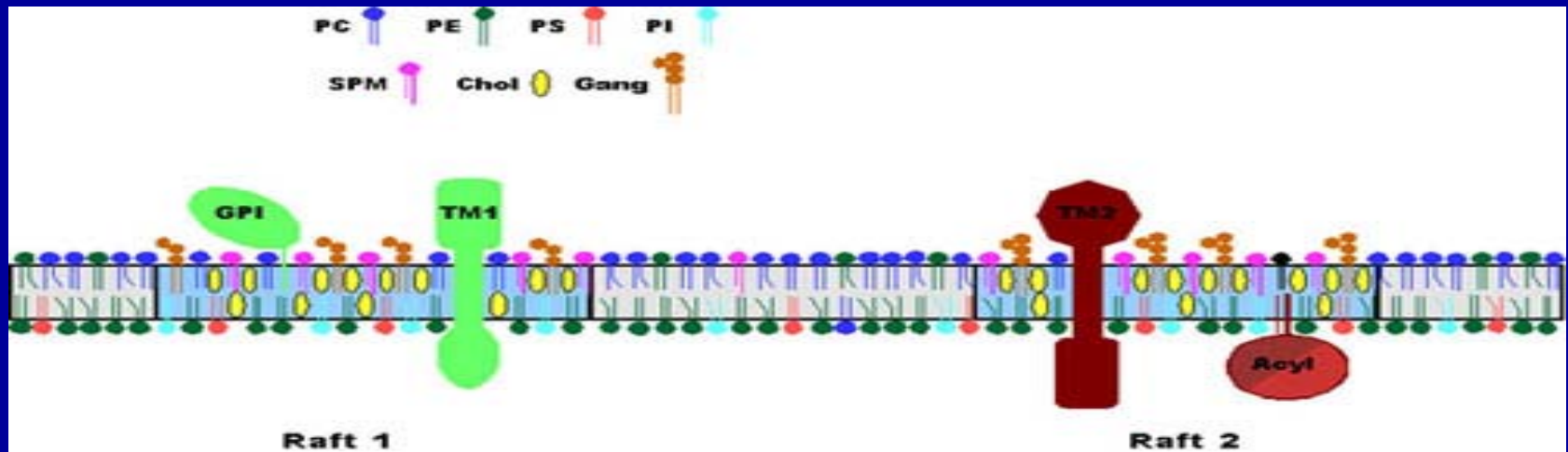


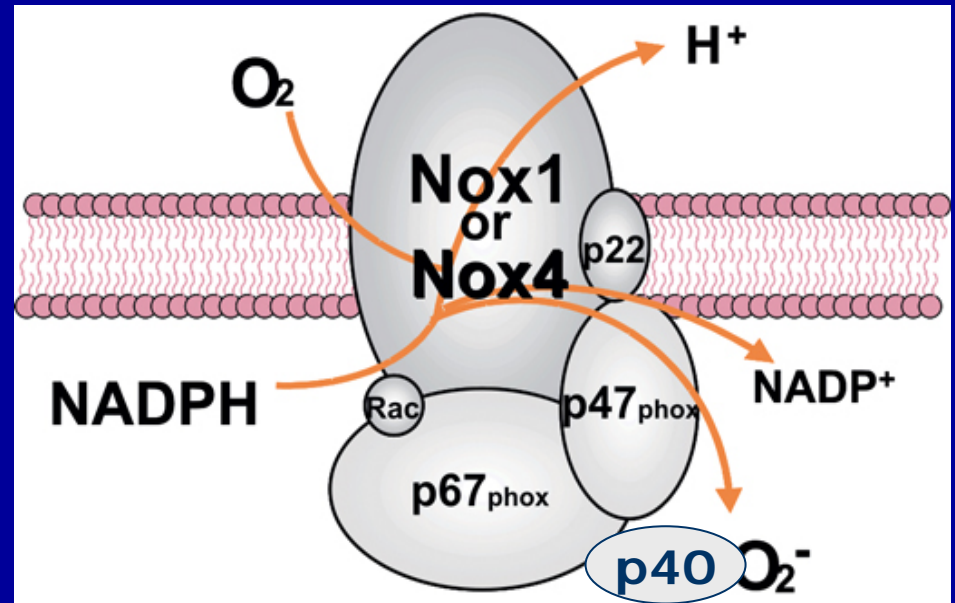
Lipid Rafts

- A **lipid raft** is a cholesterol-enriched microdomain in cell membranes.
- They contain: cholesterol, glycolipids, sphingolipids, and proteins
- Lipid rafts are involved in molecular trafficking, immune system functions, as well as signaling transduction
- They can be stimulated to cluster by: tumor necrosis factor α , Fas ligand, endostatin, *et al.*



NADPH Oxidase-Derived Superoxide in the Kidney

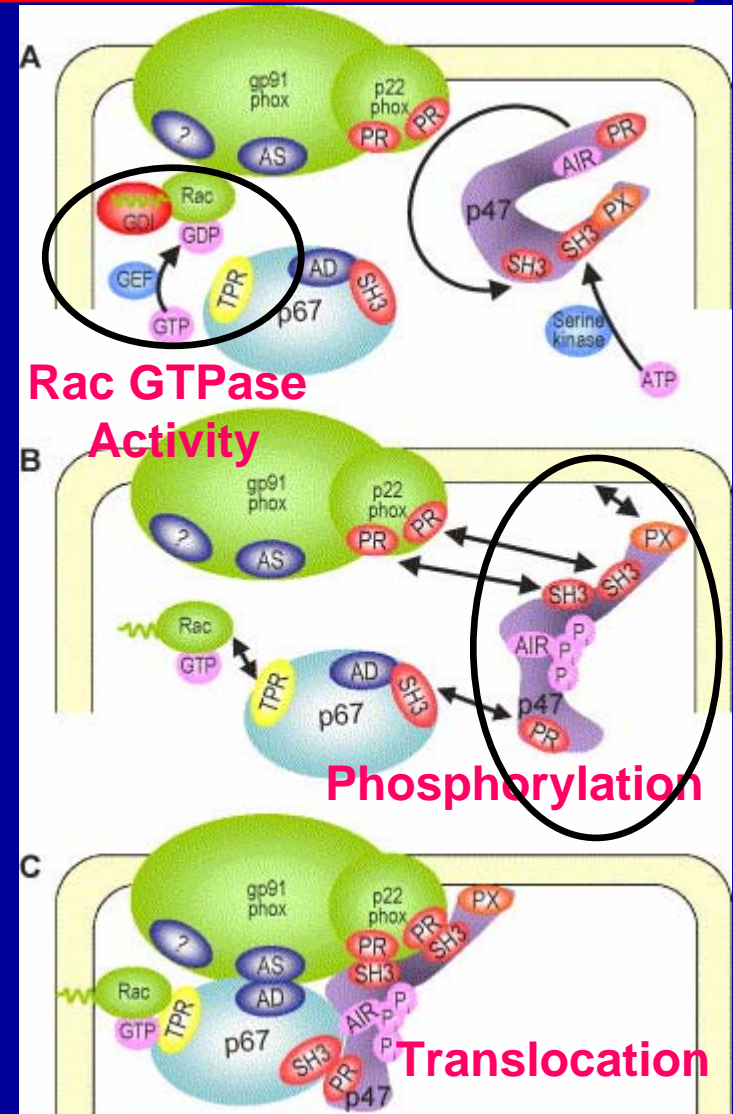
- NAD(P)H oxidase is a membrane associated enzyme that generate superoxide
- 5 subunits: p47, p67, p40, p22, Nox isoform, and Rac
- one of major enzymes responsible for O_2^- production in the kidney under physiological conditions



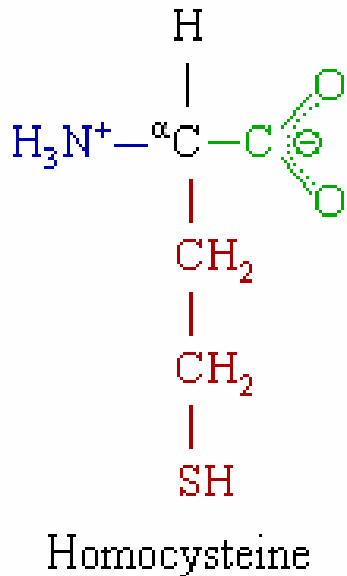
[memorias.ioc.fiocruz.br/100\(Suppl\)/5253NO01.html](http://memorias.ioc.fiocruz.br/100(Suppl)/5253NO01.html)

Activation Mechanisms of NADPH Oxidase

- P47 Translocation
- Rac GTPase activity



Hyperhomocysteinemia



Hyperhomocysteinemia

a critical pathogenic factor in the progression of ESRD and in the development of cardiovascular complications related to ESRD

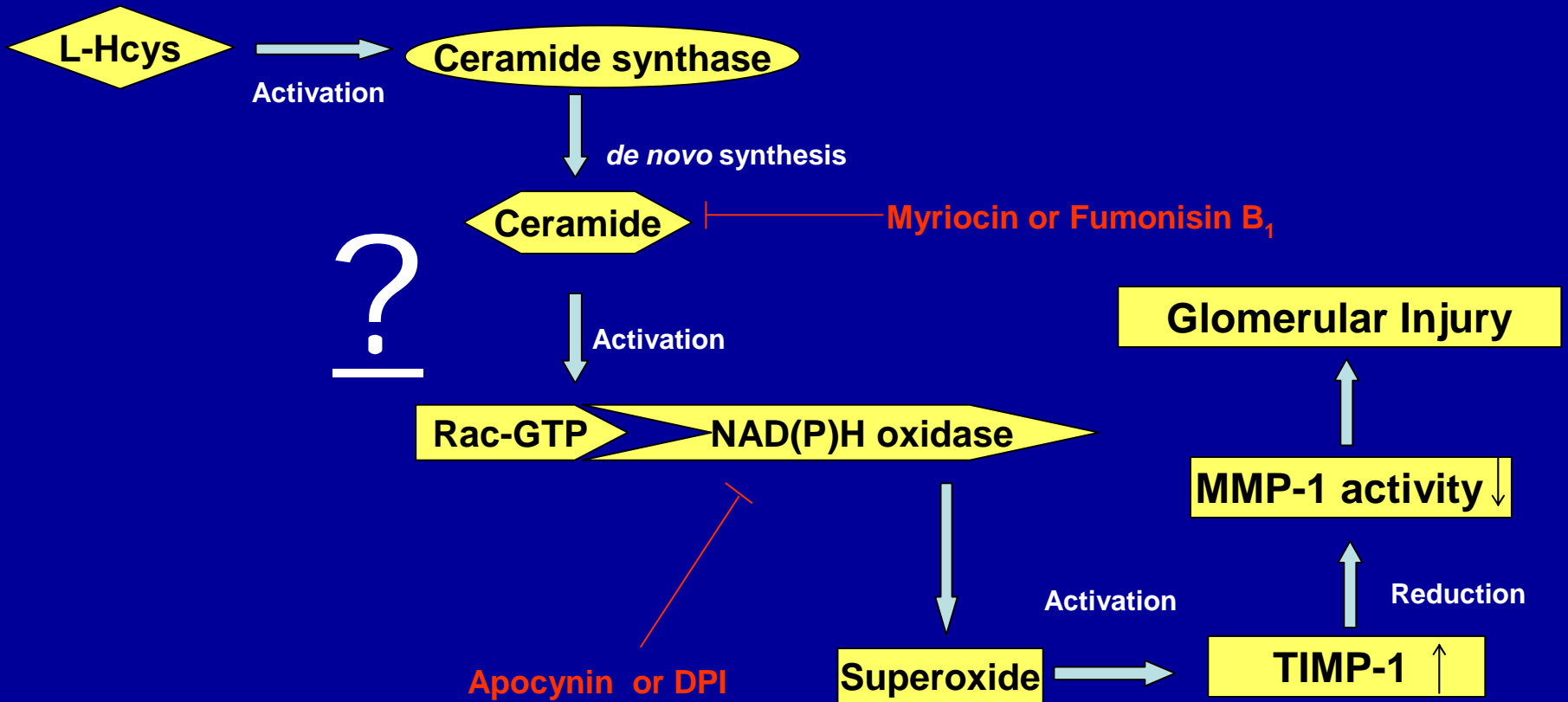
Hyperhomocysteinemia

occurs in 85%-100% of patients in ESRD

Hyperhomocysteinemia

< 10	µmol/L	Normal
15-30	µmol/L	Mild
30-60	µmol/L	Moderate
> 80	µmol/L	Severe

Background



Background

Fas Ligand



Lipid raft formation



Redox platform formation on the lipid raft

p47 translocation to lipid raft

Gp91 aggregation on the lipid raft

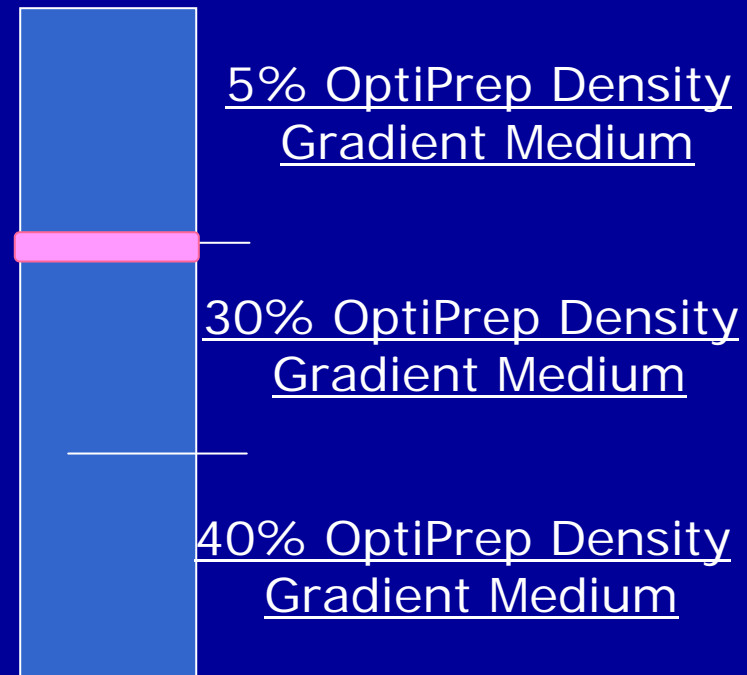
Rac GTPase translocation to lipid raft

Question:

Whether redox signaling platforms formed on the lipid raft are involved in the regulation of Hcys-induced NADPH oxidase activity?

Methods: Isolation of lipid rafts

Gradient
centrifugation

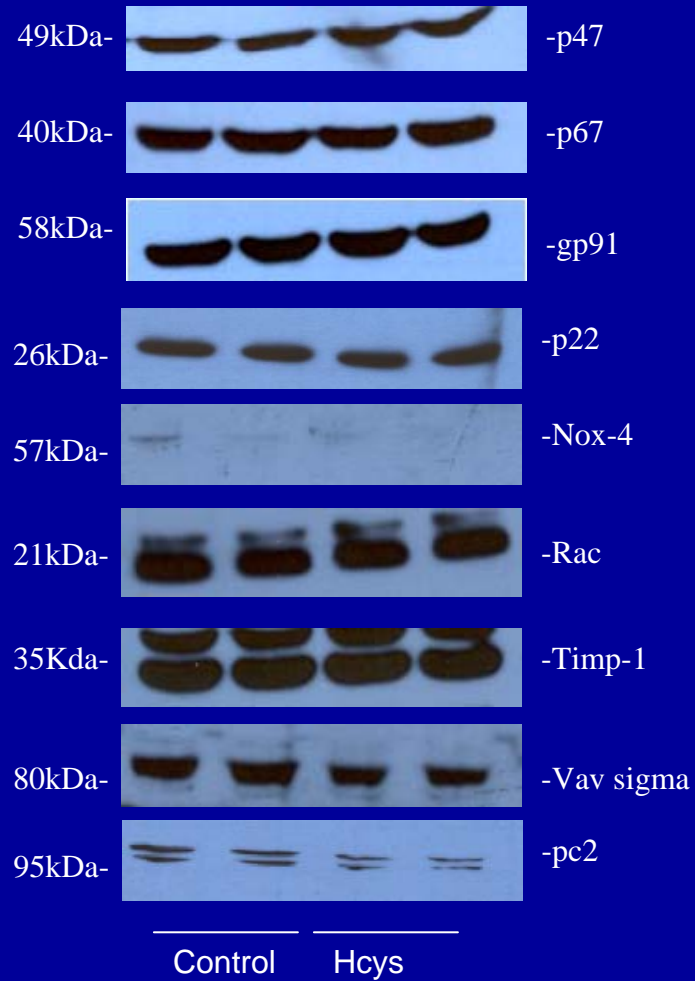


Methods: Western Blot

- Seperation of proteins via eletrophoresis
- Proteins in gel are transferred to nitrocellulose membrane
- Membrane is probed with antibodies

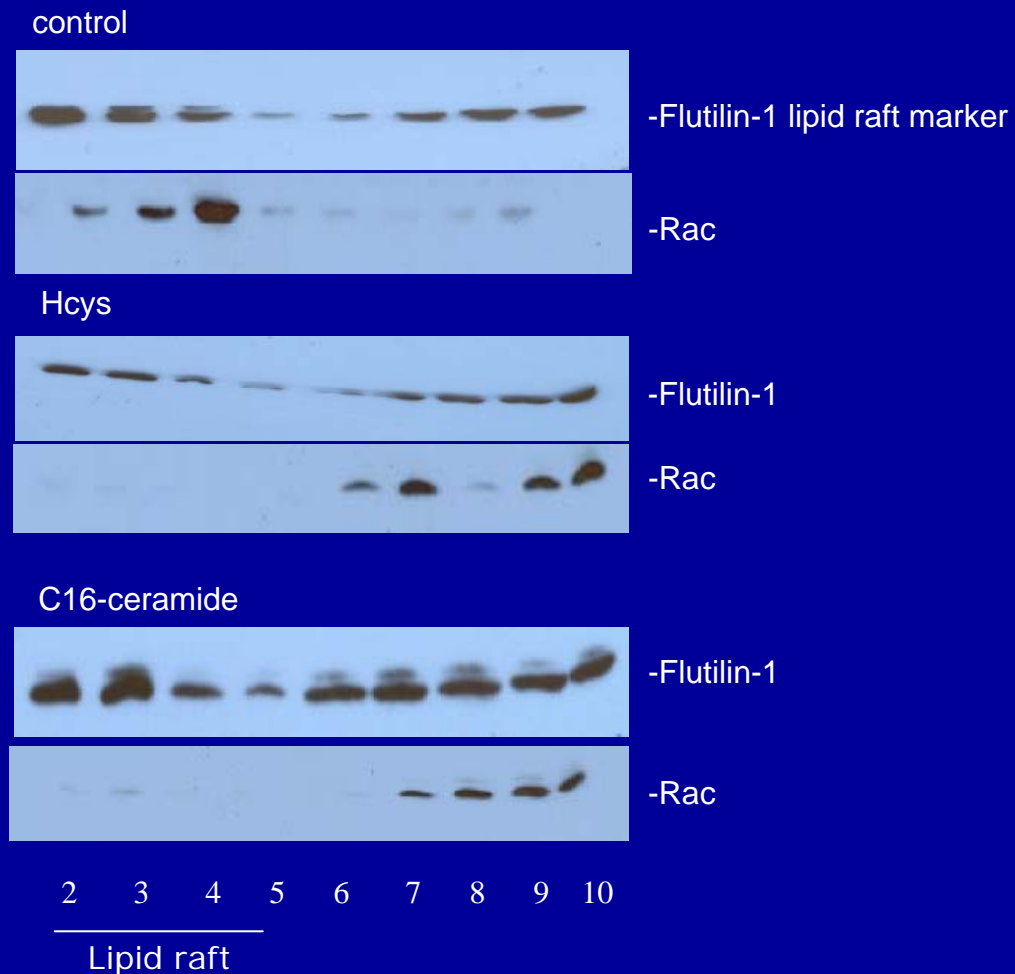
Results

Characterization of target enzymes in glomerular endothelial cells

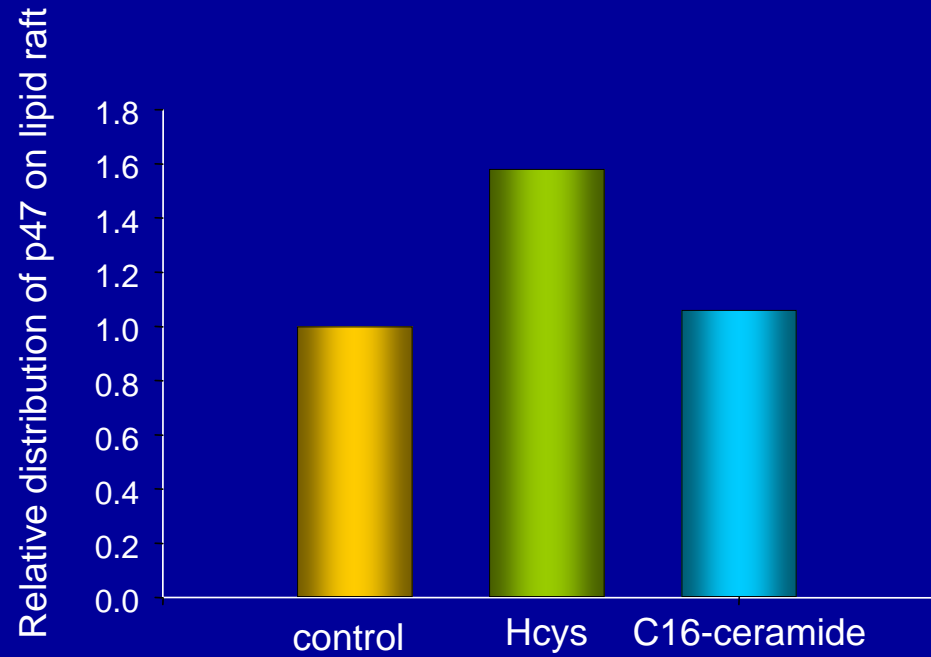
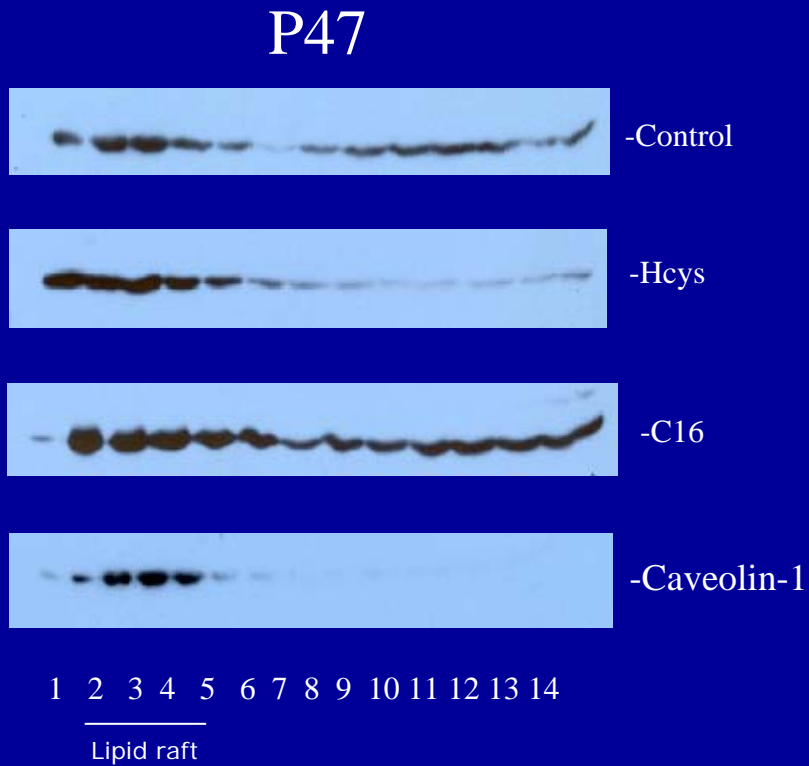


Results

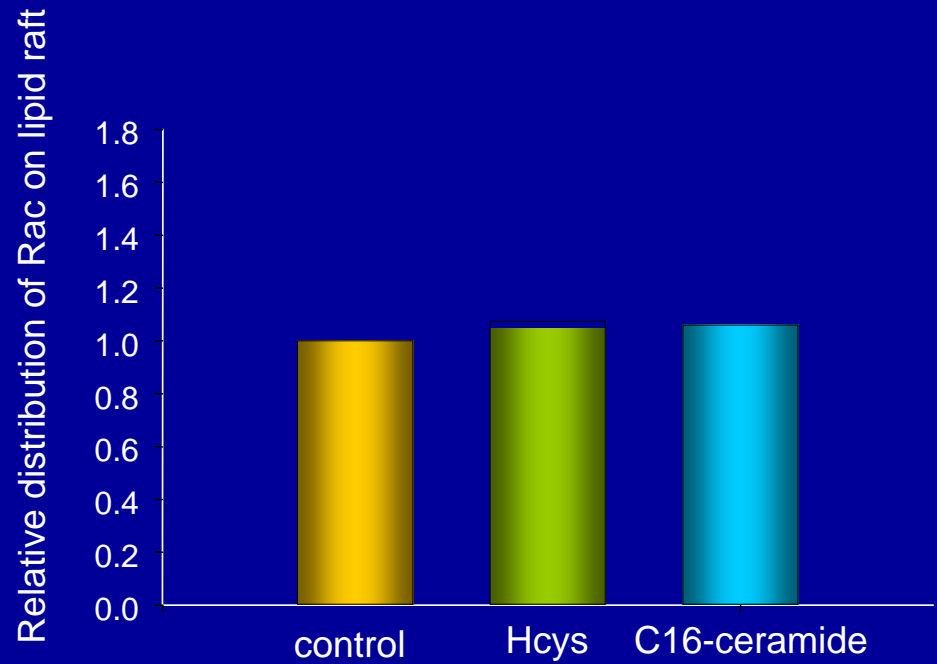
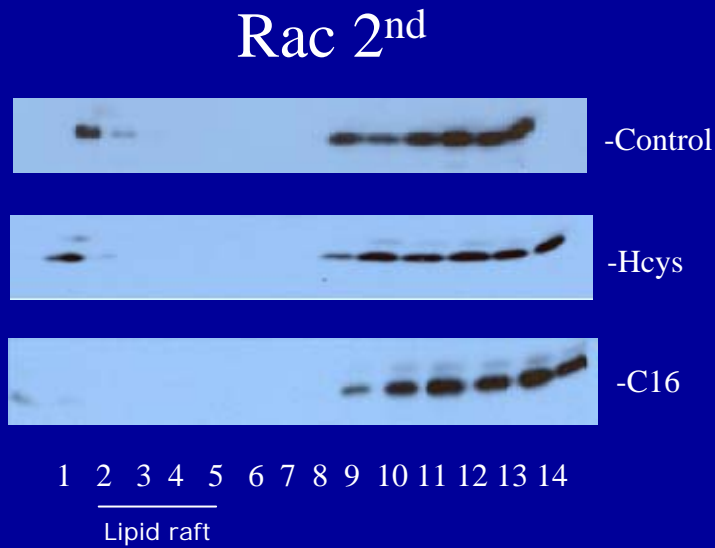
Isolation of lipid raft from glomerular endothelial cells



Results



Results



Conclusion

- Possible that lipid raft-redox platform is involved in Hcys induced NADPH oxidase by p47 translocation
- Need more data to confirm these findings

Acknowledgment

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