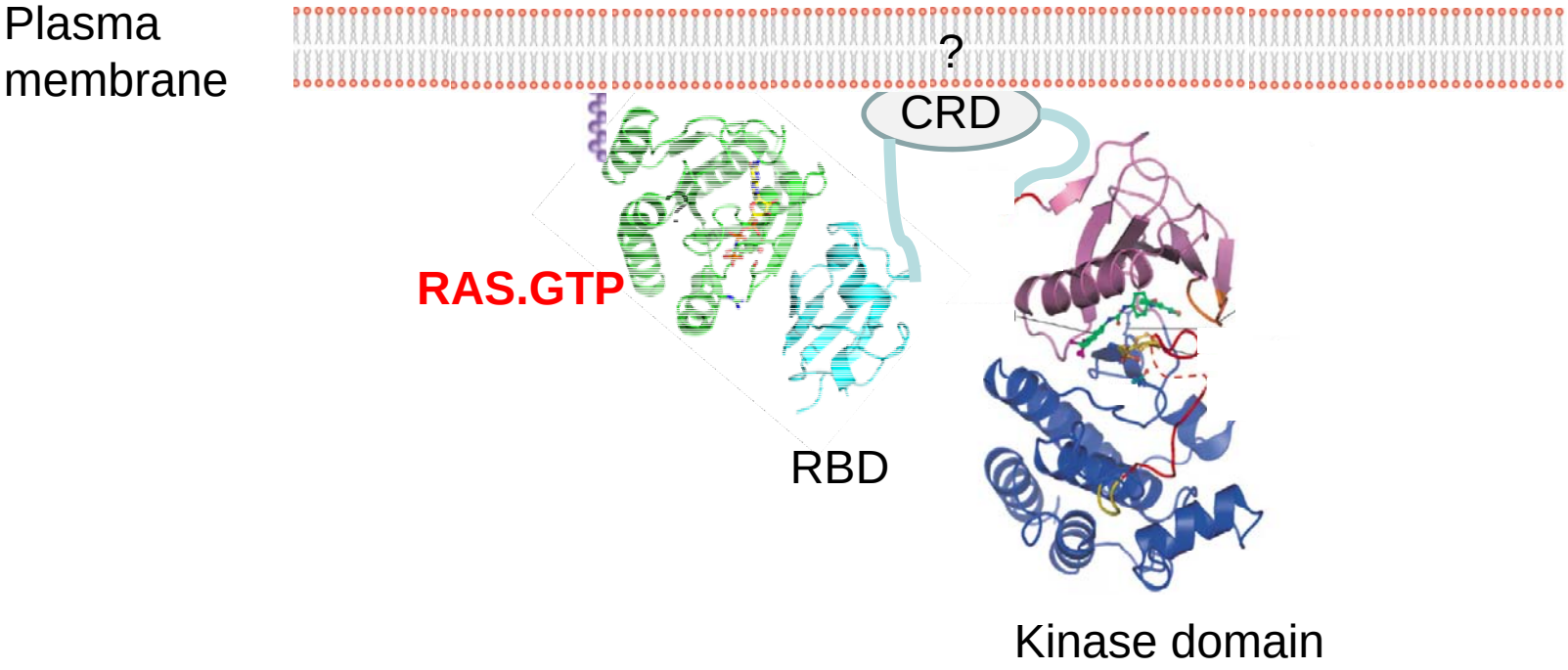
Frederick National Laboratory
for Cancer Research

Pilot 2 Introduction: Frank McCormick

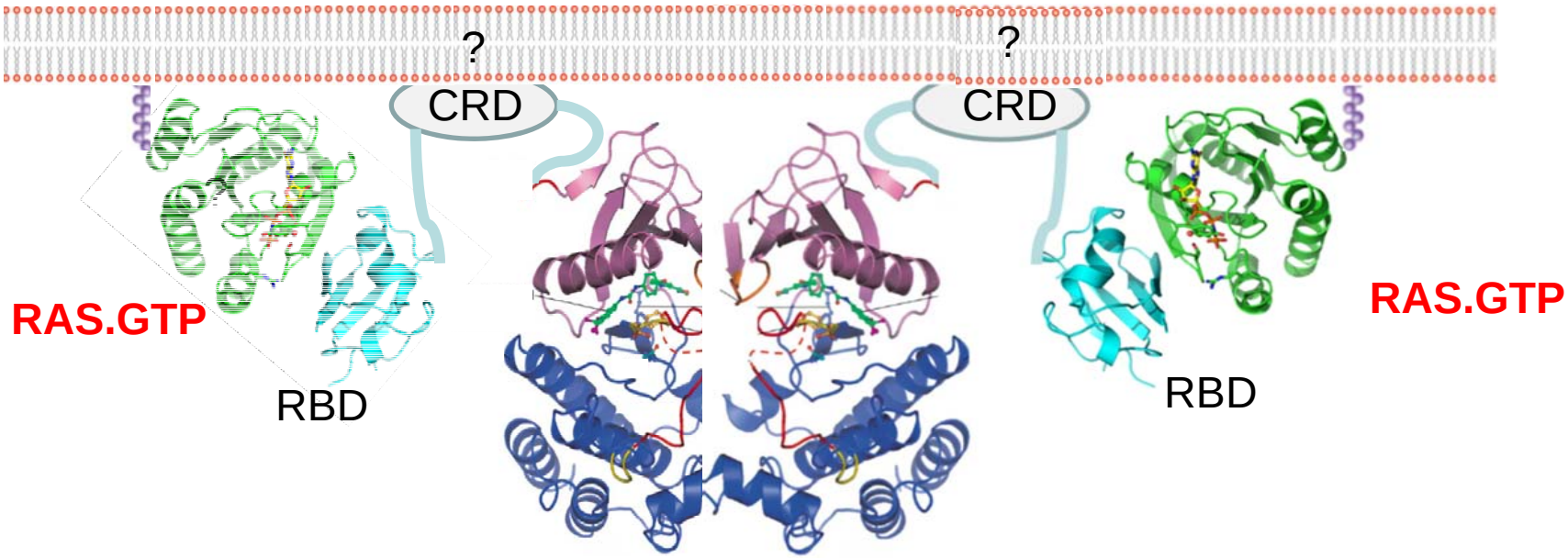
DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute
The Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute

RAS.GTP recruits RAF to the plasma membrane

Frederick National Laboratory for Cancer Research



Raf dimerization and activation



Dimerized Active Kinase domain

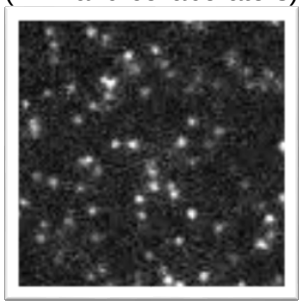
↓

Mek

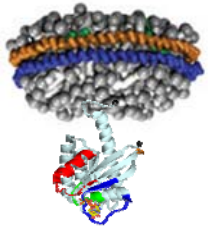
A multi-pronged approach to KRAS-RAF membrane interactions

Frederick National Laboratory for Cancer Research

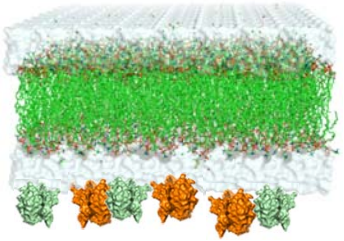
In vivo
(FNL and collaborators)



In vitro
(FNL and collaborators)



In silico
(DOE)

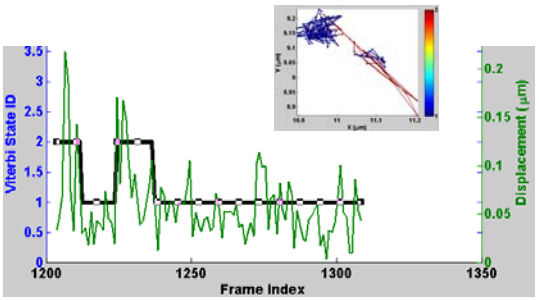


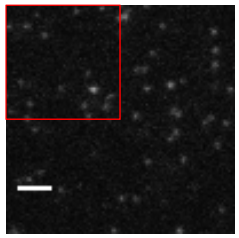
Frederick National Laboratory for Cancer Research

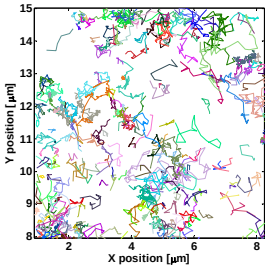
Tommy Turbyville

Andy Stephen

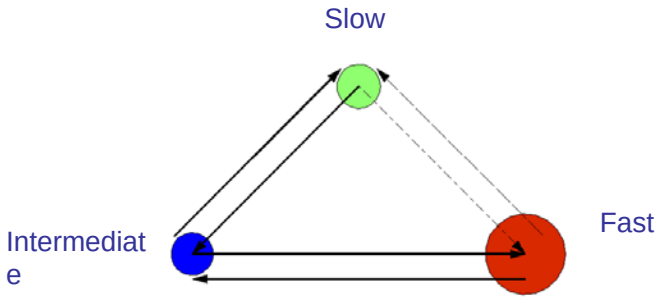
Frantz Jean-Francois







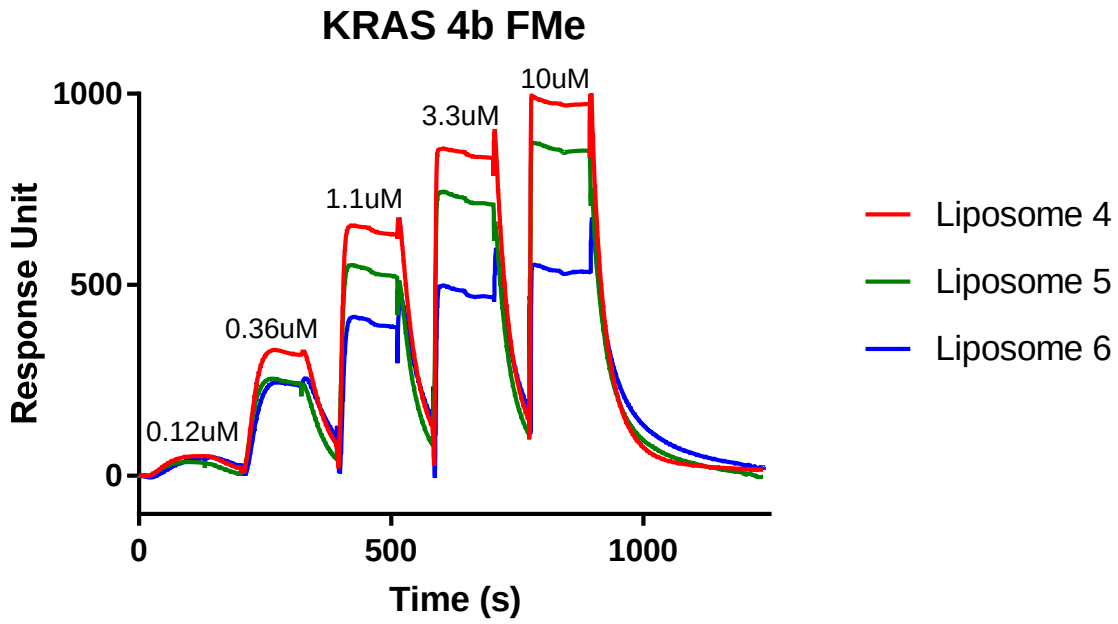
Component	Diffusion (um^2/s)	Fraction
Fast	0.97	48%
Intermediate	0.22	26%
Slow	0.039	26%

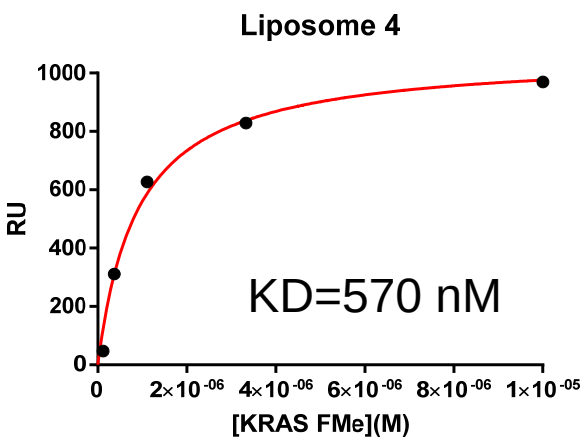


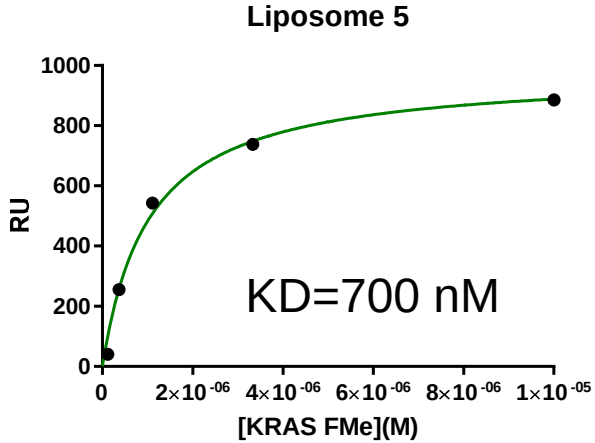
Dataset: RASless-MEF, HaloTag-KRAS4b; # Tracks: 29, 165; # Cells: 12; Avg. Track Length = 13.1 frames (frame = 10 ms)

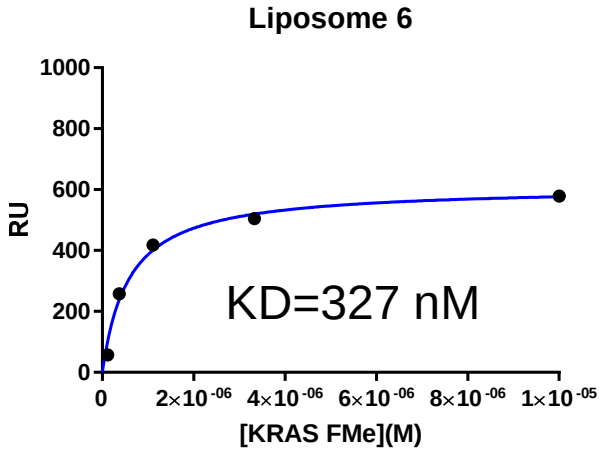
Accurate determination of binding constants

Frederick National Laboratory for Cancer Research





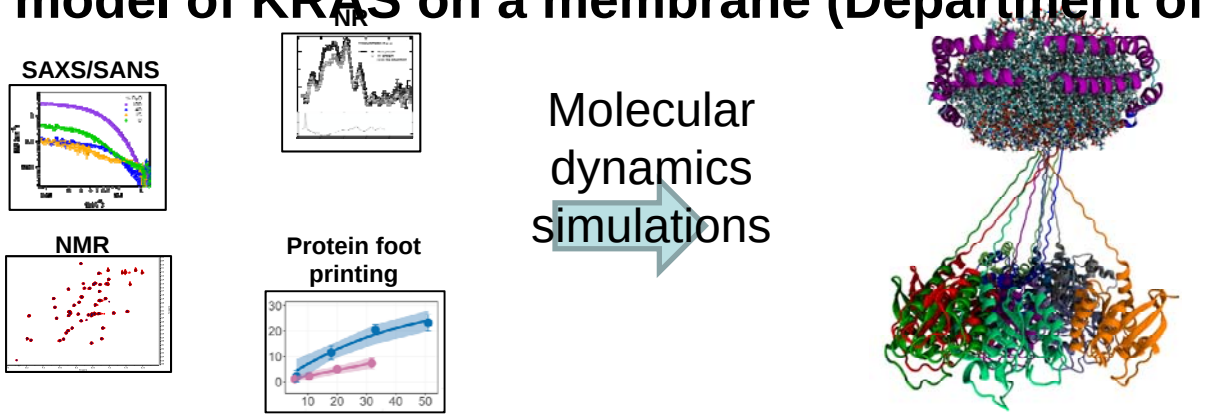




KRAS:membrane – structure and orientation

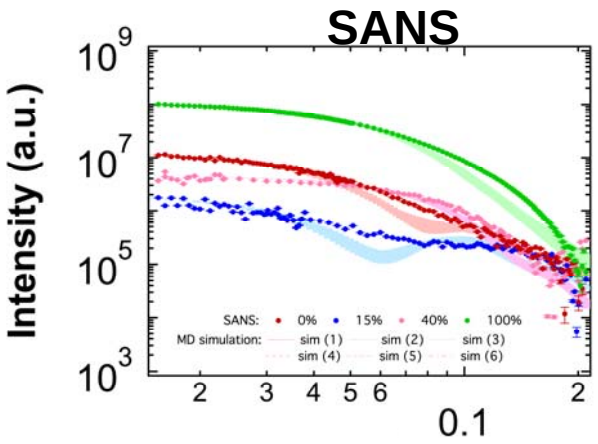
Frederick National Laboratory for Cancer Research

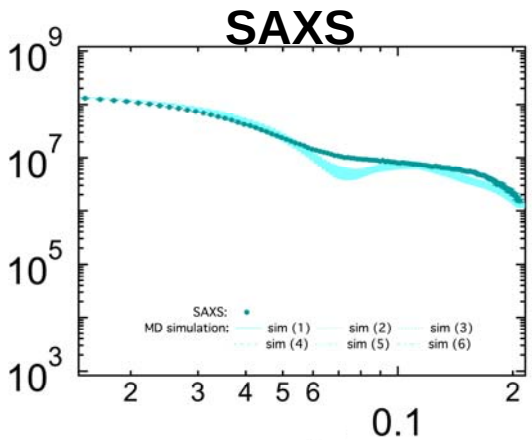
- **KRAS4b protein shape and orientation on the membrane**
  - Neutron reflectivity (National Institute of Standards and Technology)
  - Small Angle Neutron/X-ray Scattering (Oak Ridge National Laboratory)
- **Identify KRAS4b:membrane interacting residues**
  - Protein foot printing (Washington University in St. Louis)
  - NMR (National Magnetic Resonance Facility at Madison)
- **Develop a model of KRAS on a membrane (Department of Energy)**



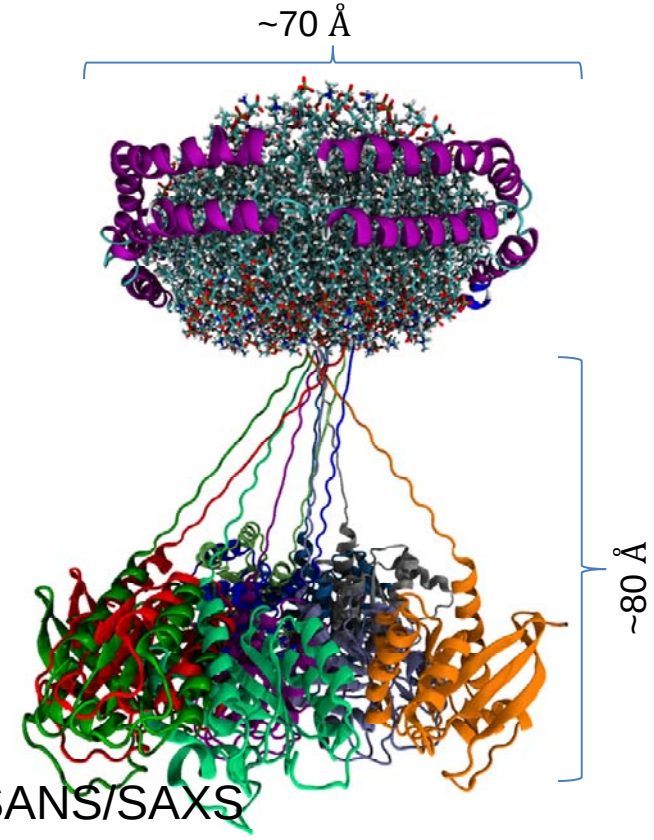
SAXS and SANS indicate KRAS exists in an extended conformation

Frederick National Laboratory for Cancer Research





Methods	R_g (Å)	D_{max} (Å)
SANS	41.6±1.4	~120
SAXS	48.3±0.1	~145
MD simulation	48.9±0.7	~130.9±3.9

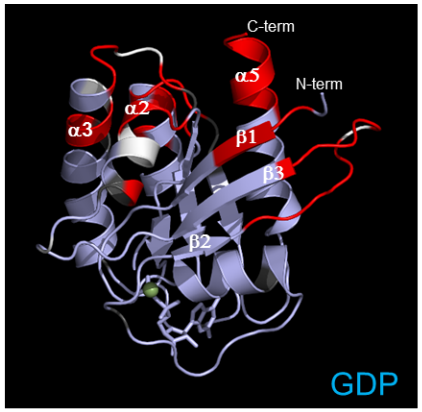


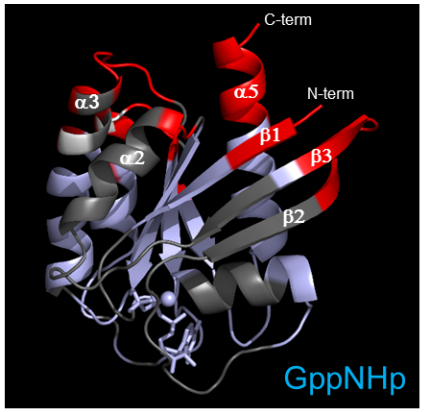
- Ensemble MD simulations agree qualitatively with SANS/SAXS

Chris Stanley, Sindhu Bhowmik, Blake Wilson, Arvind Ramanathan (ORNL)

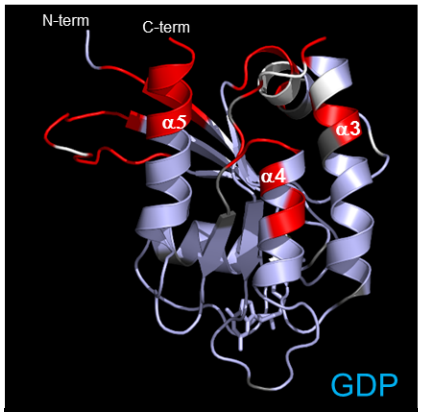
Beta strand 1-3 and helix 2-5 are close to the membrane

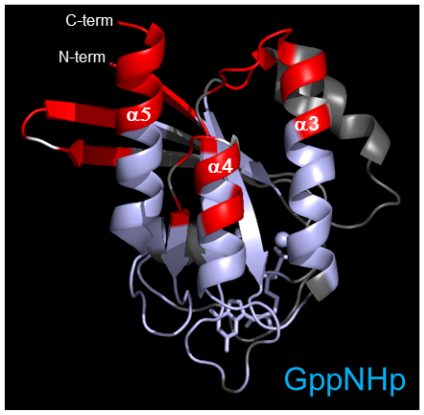
Frederick National Laboratory for Cancer Research





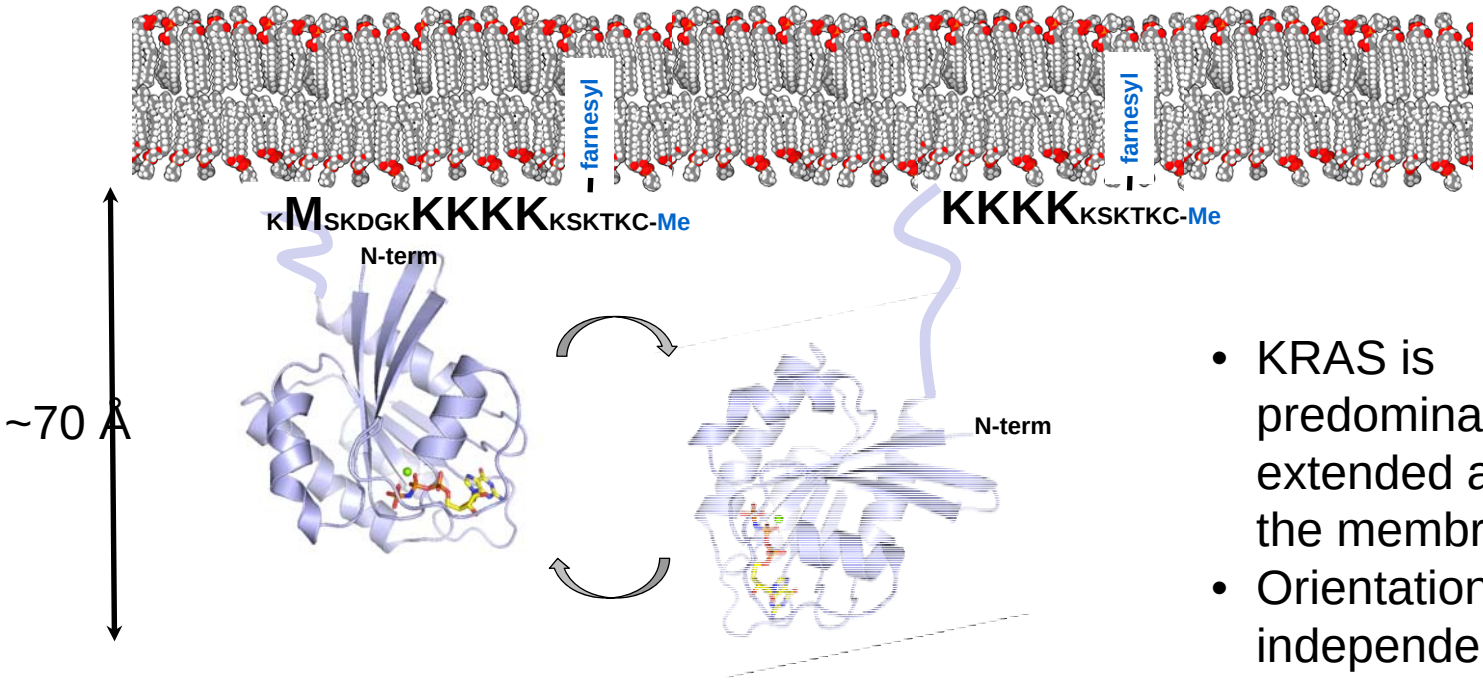
Red ≤60% signal remaining (ratio of +Gd/-Gd)
White excluded
Gray unassigned





Biophysical data indicates KRAS exists in an extended conformation at the membrane

Frederick National Laboratory for Cancer Research



- KRAS is predominantly extended away from the membrane.
- Orientation independent of nucleotide state
- N-terminal beta strands 1-3 and regions of helix 2-5 are proximal to the membrane