Structure and dynamics of adapter proteins at the interface of cell membrane and actin cytoskeleton

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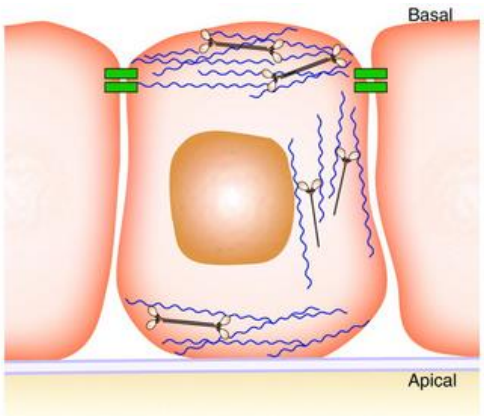
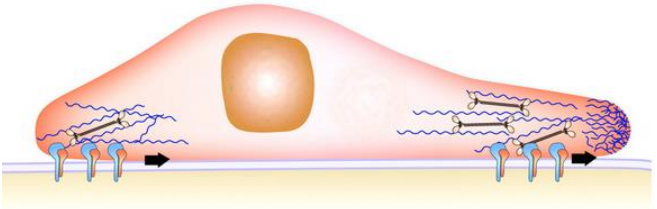
SSRL SAXS Workshop
March, 2016

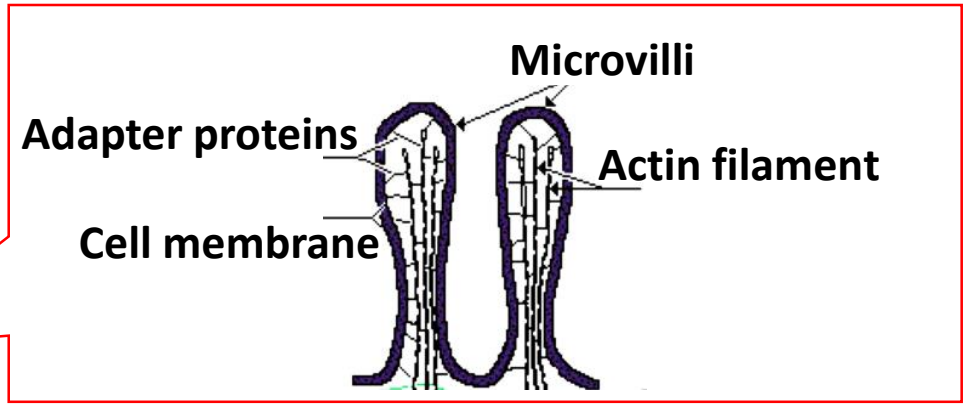
The interface of cell membrane and cytoskeleton

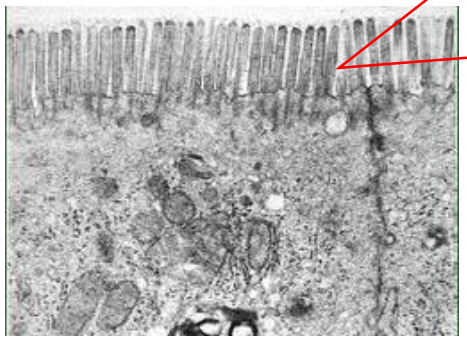
Cell adhesion



Cell migration





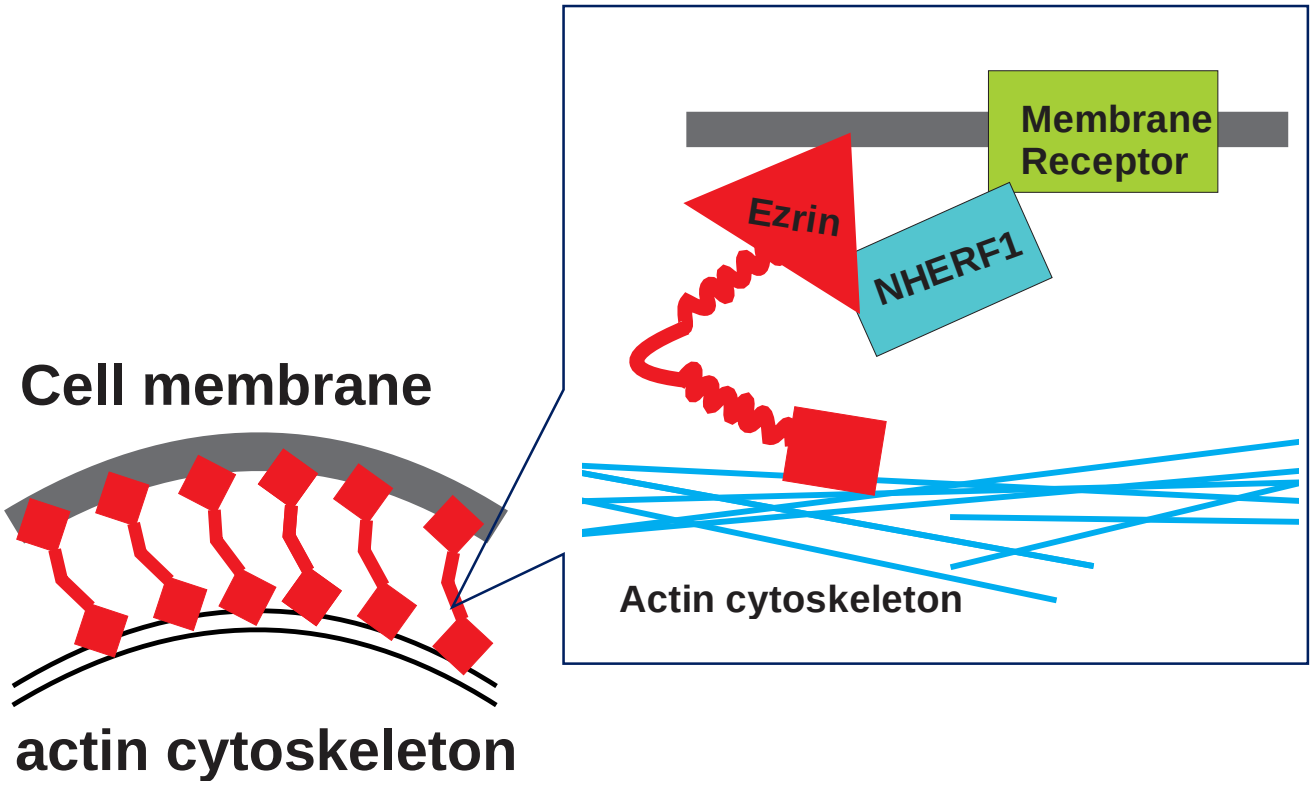


Polarized epithelial cells

Adsoption
Secretion
Mechanotransduction

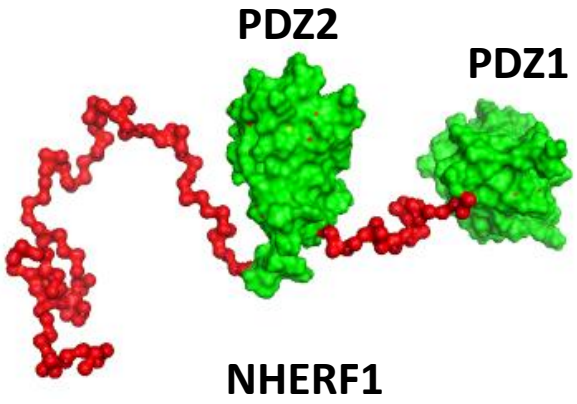
Receptor trafficking

Adapter proteins NHERF1 and Ezrin regulate membrane receptor and ion channel assembly, trafficking and signaling



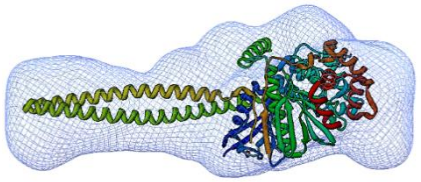
Linking the cell membrane to the F-actin cytoskeleton

Multi-domain adapter proteins: Intramolecular interactions regulate protein-protein assembly



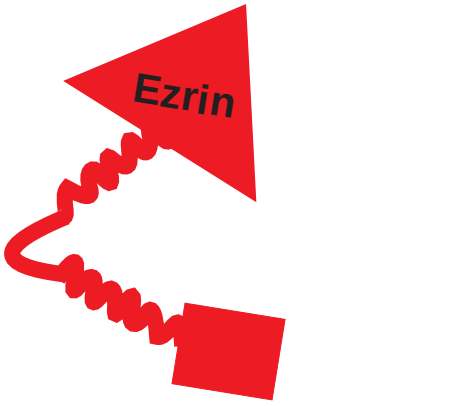
"Supra-tertiary Structure"

Ezrin

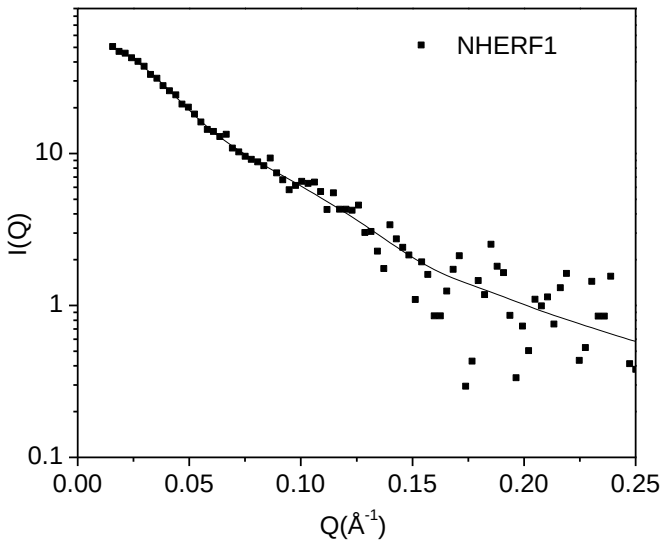


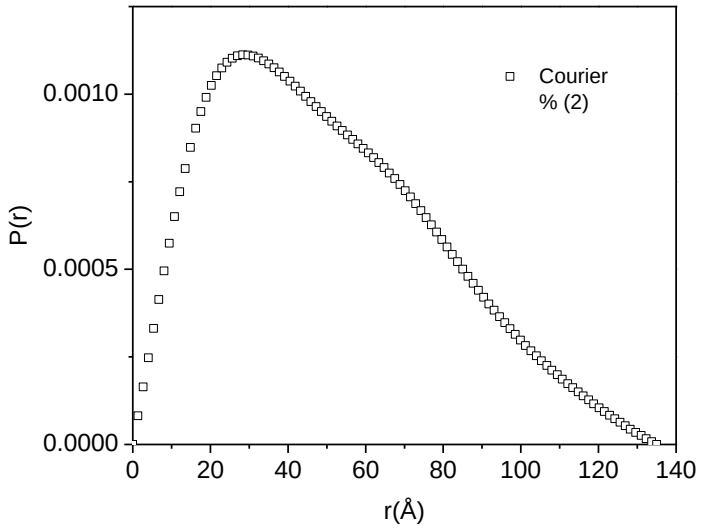
Binding to signaling
Lipid PIP2

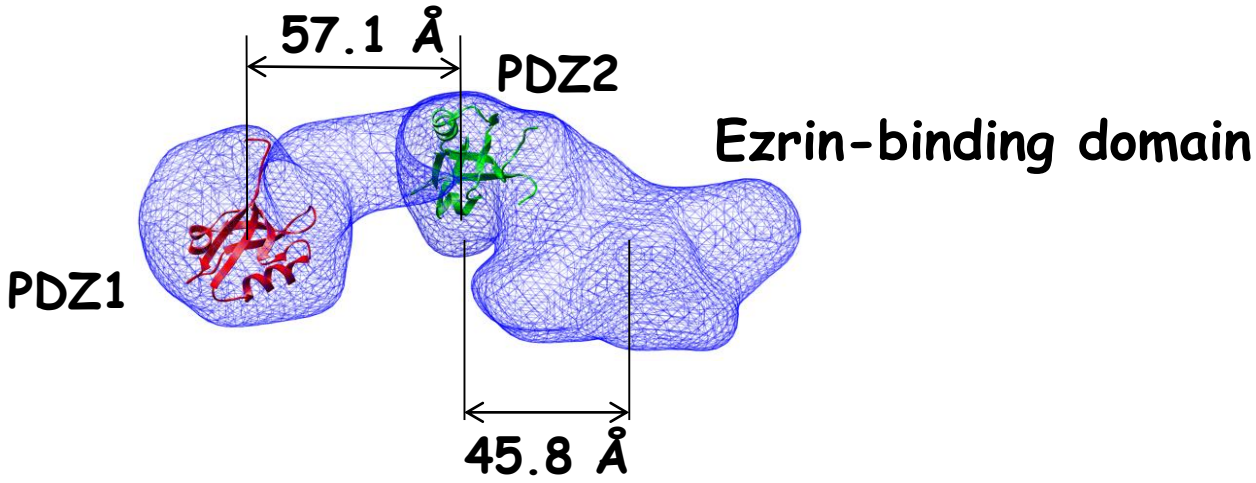
phsphorylation



NHERF1 alone in solution by SAXS

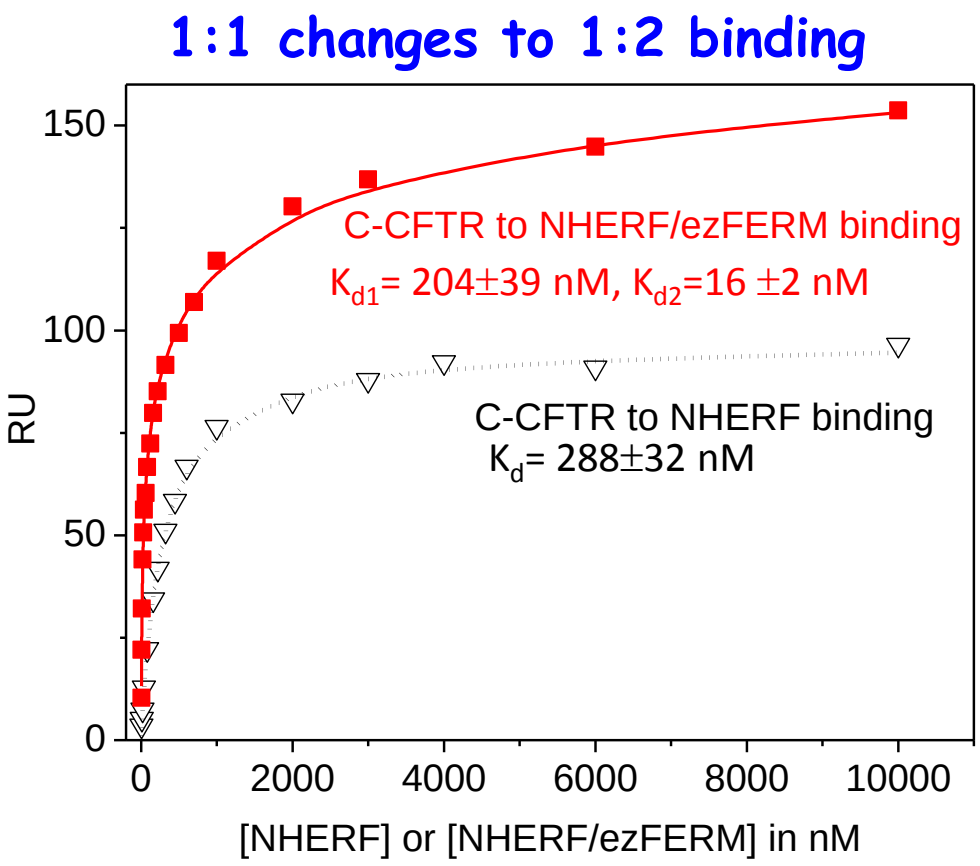


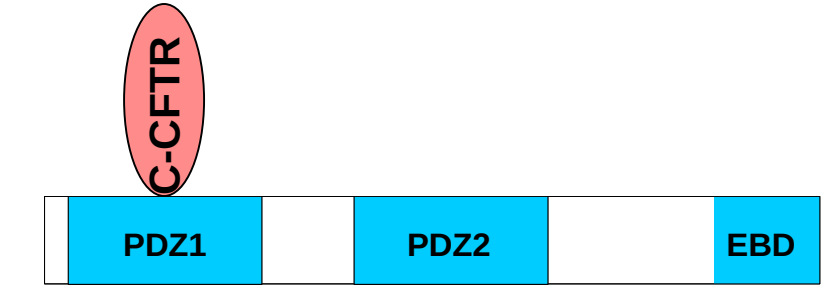


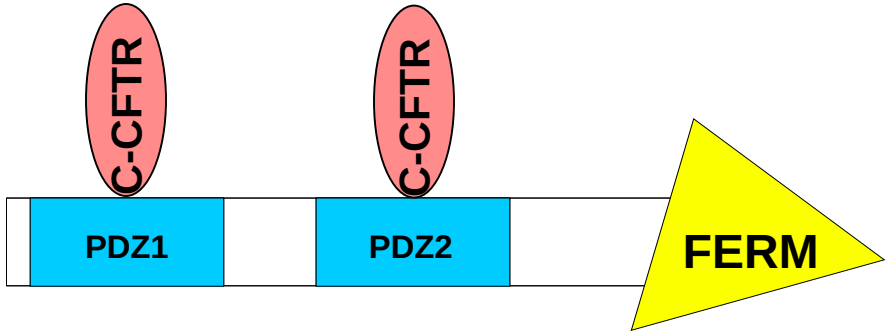


Ezrin positively modulates NHERF1 to assemble protein complexes

Changes stoichiometry of NHERF1 binding to C-CFTR

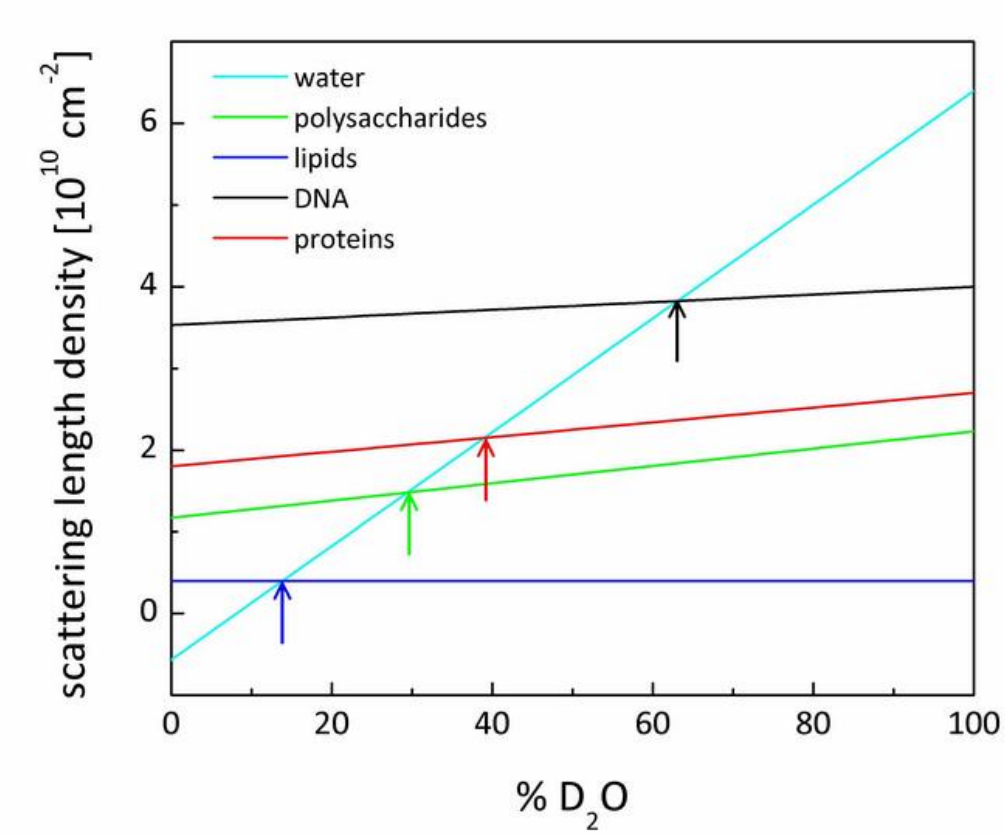






Li et al, JBC, 2005, 2007

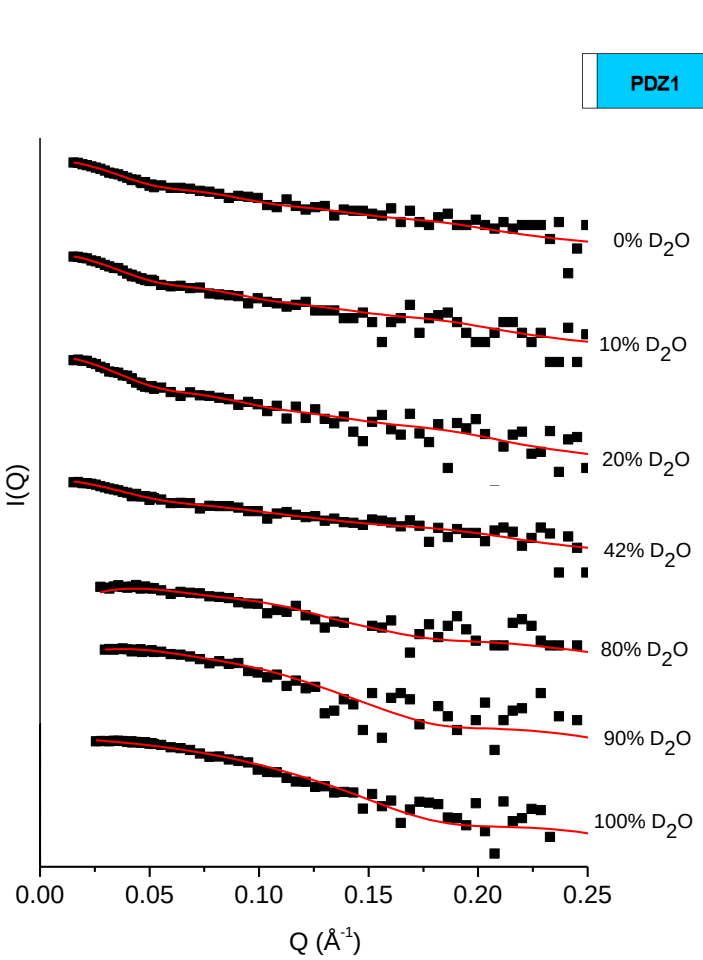
Selective deuteration and contrast variation SANS to study protein complexes

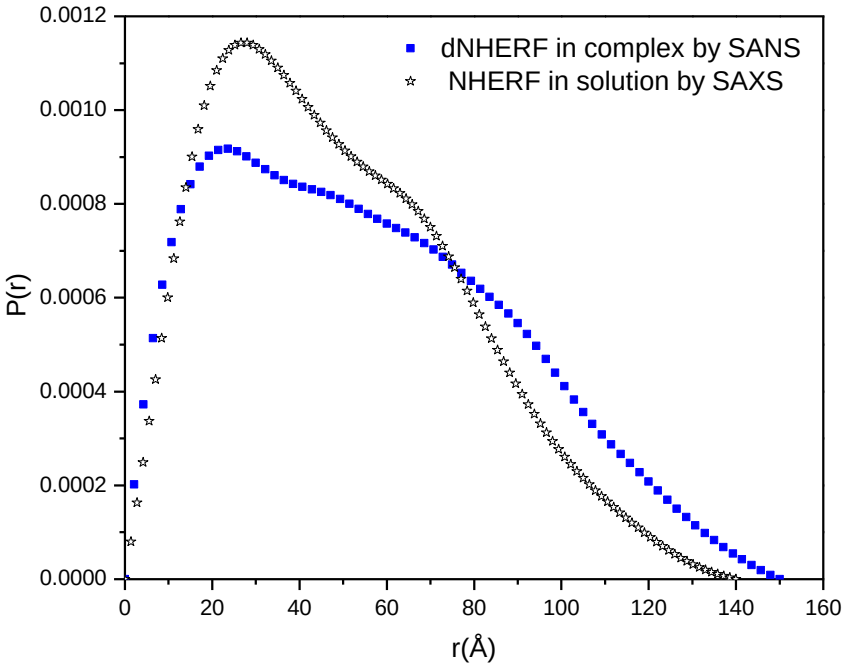


H	D	C	N	O	P	S
-0.3742	0.6671	0.6651	0.940	0.5804	0.517	0.2847

Contrast variation SANS: Conformational changes in NHERF1 upon binding to ezrin FERM domain

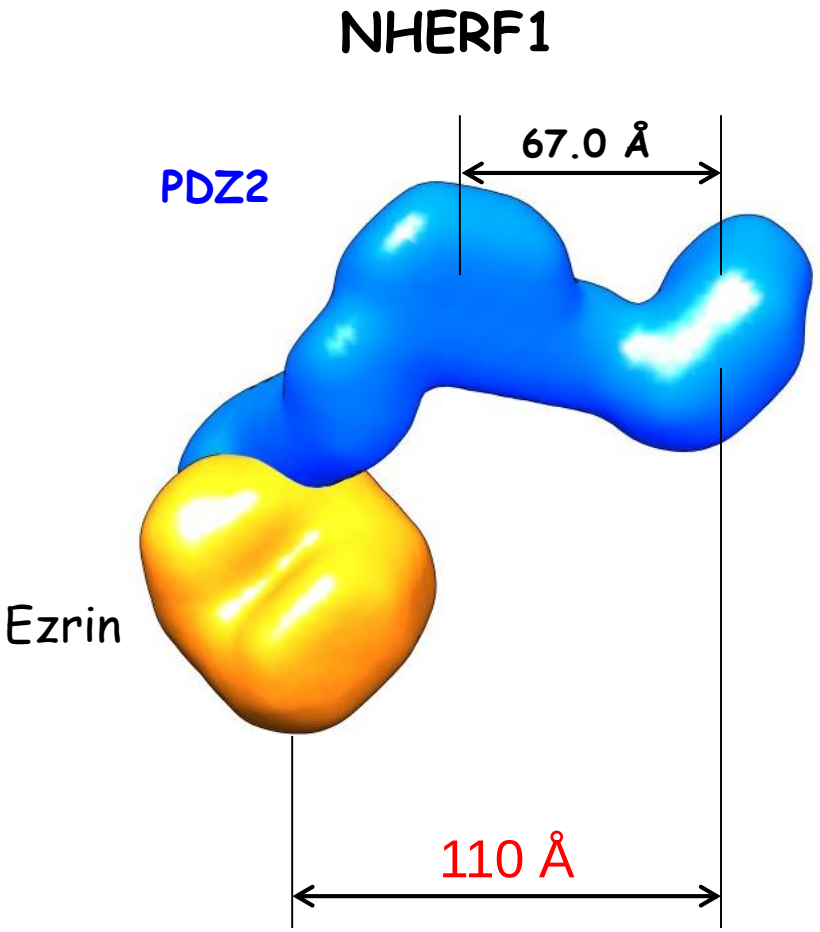
In 40% D2O, at the contrast match point of unlabeled FERM

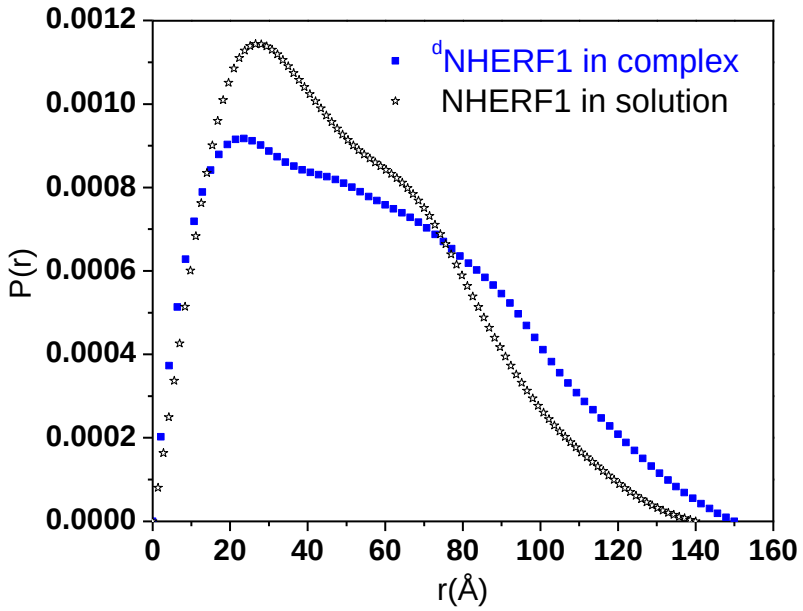




Li et al, JMB, 2010

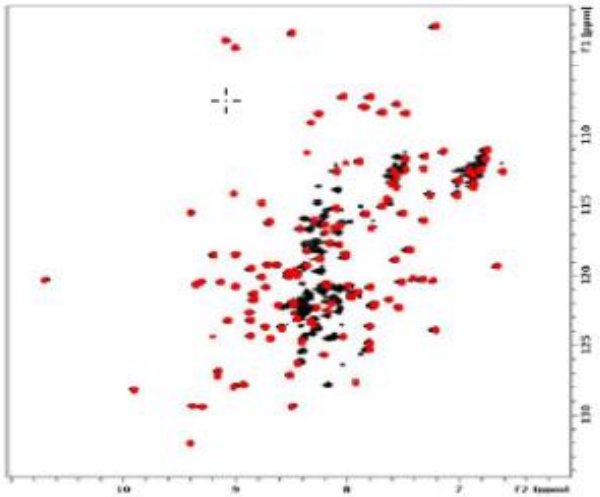
Structural changes in NHERF1 upon binding to ezrin

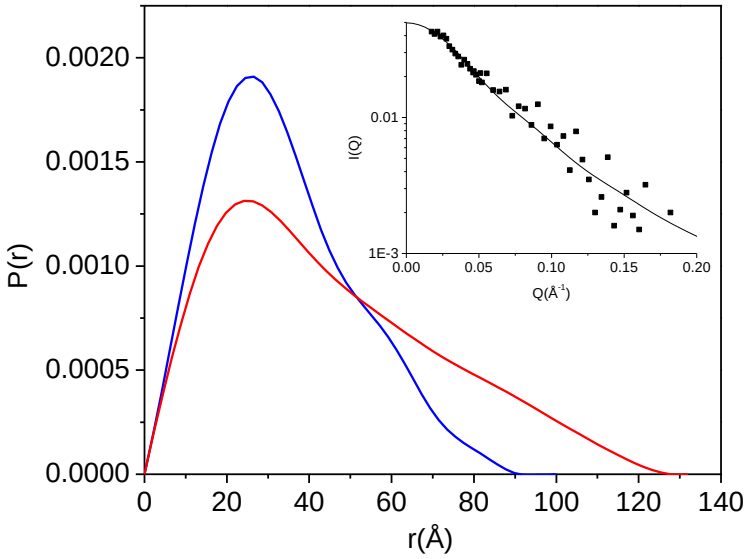




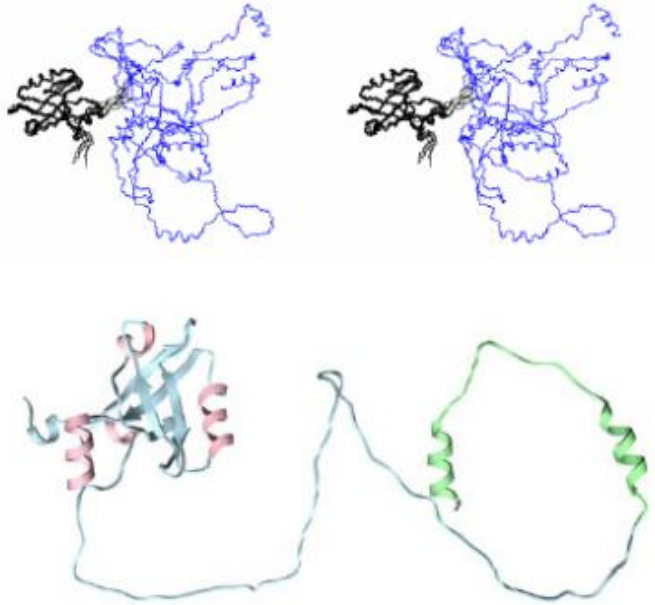
Ezrin-induces long-range interdomain allostery in NHERF1 (Li et al, JMB, 2009)

A conformational switch in the scaffolding protein NHERF1 controls autoinhibition and complex formation.



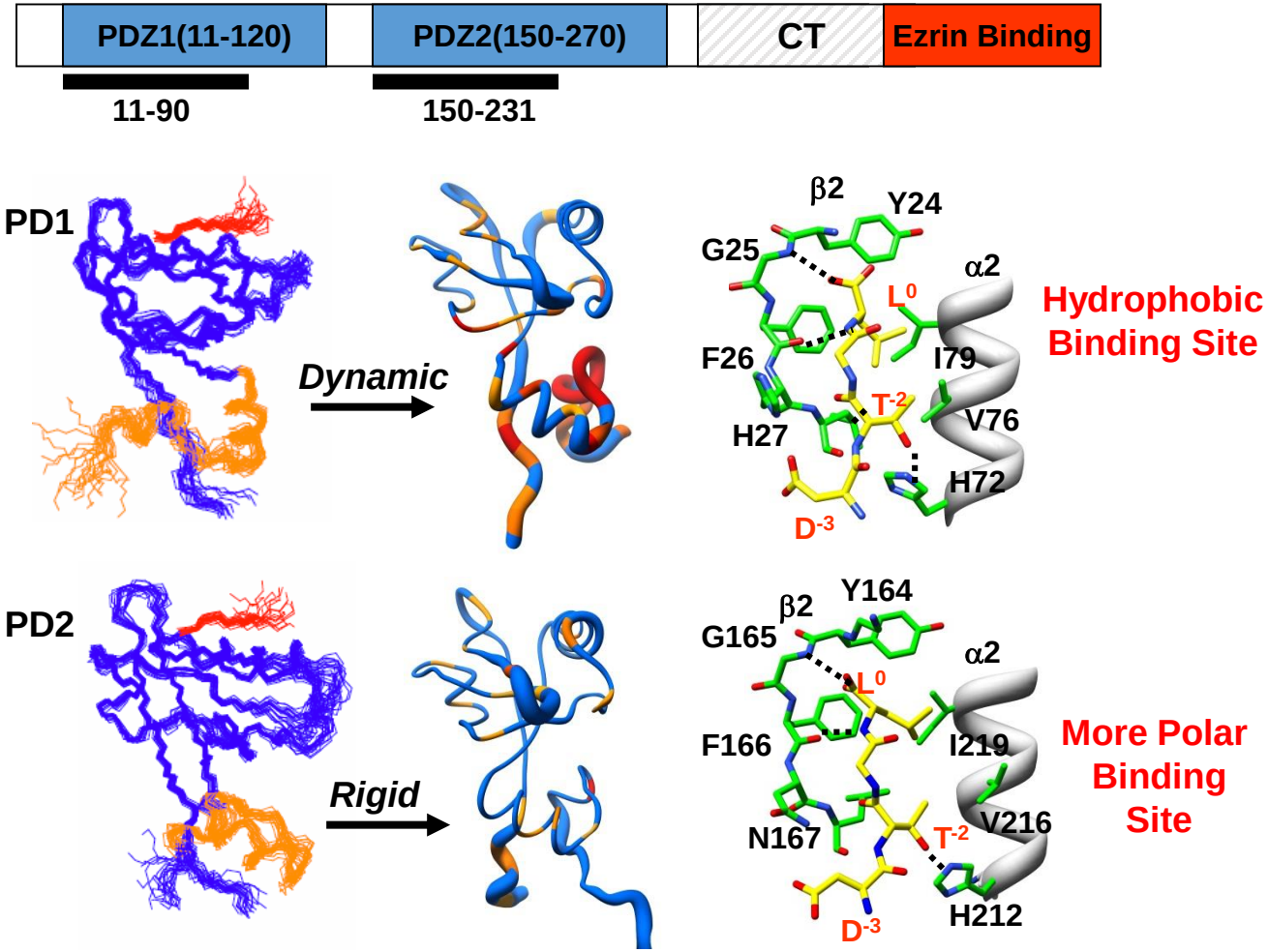


Structural transition in PDZ2CT upon binding to FERM—joint NMR and SANS refinement



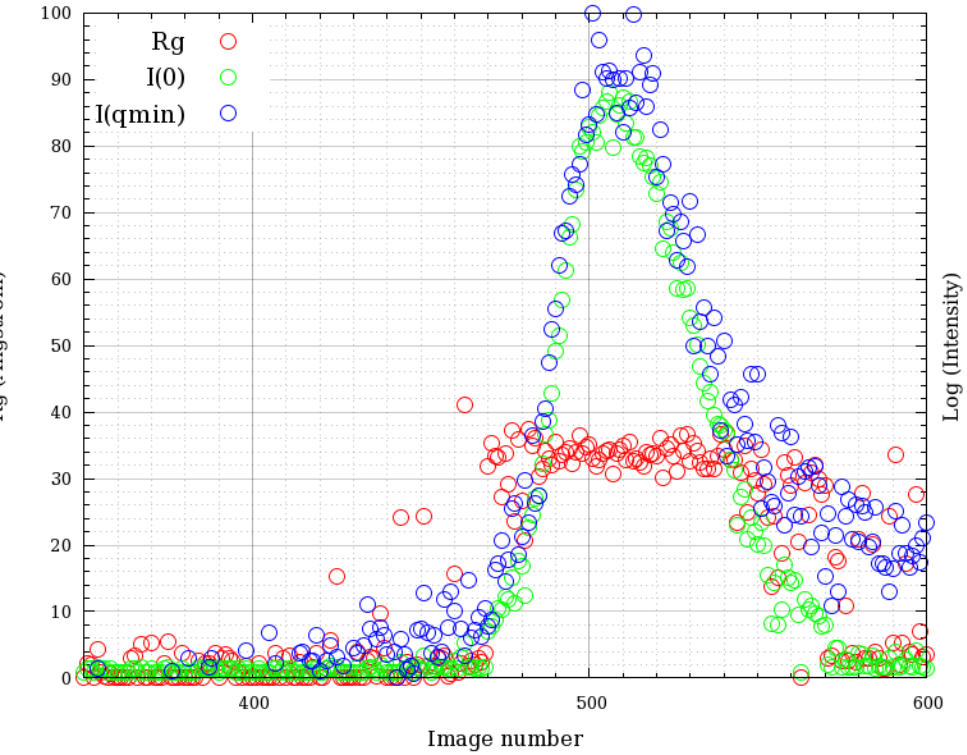
Bhattacharya et al, JBC, 2010

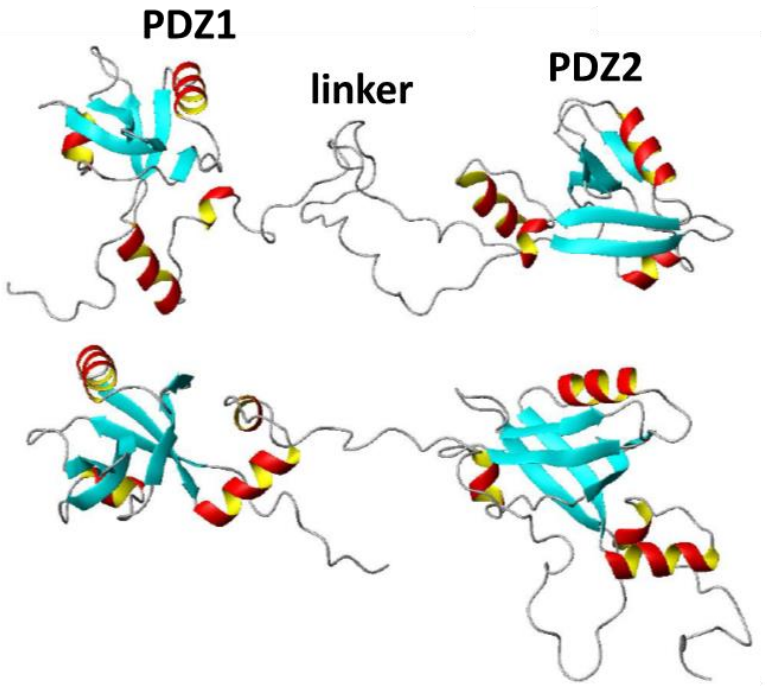
NMR Study of PDZ dynamics upon ligand binding



S. Bhattacharya et, al, JMB, 2013

SEC-SAXS of PDZ1-PDZ2

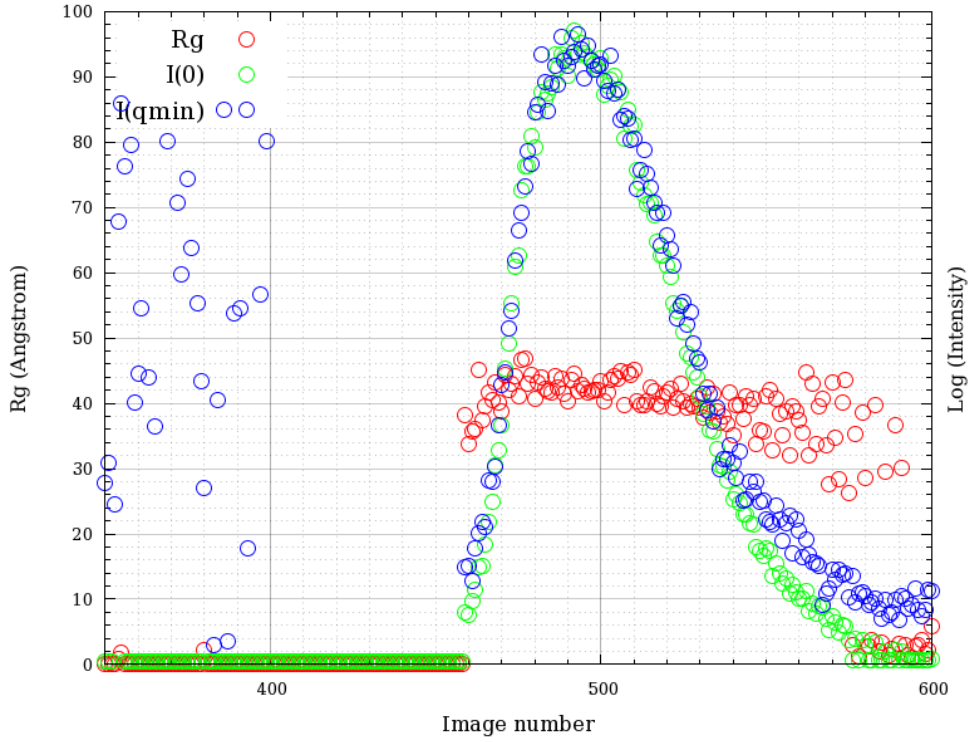


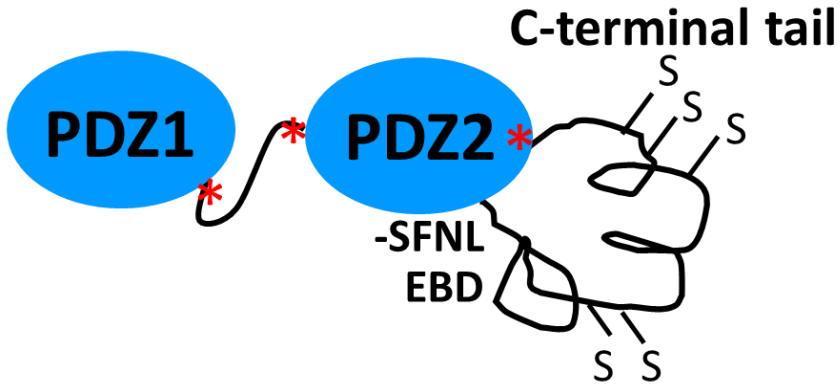


Preliminary NMR Structure

March, 2016, SSRL

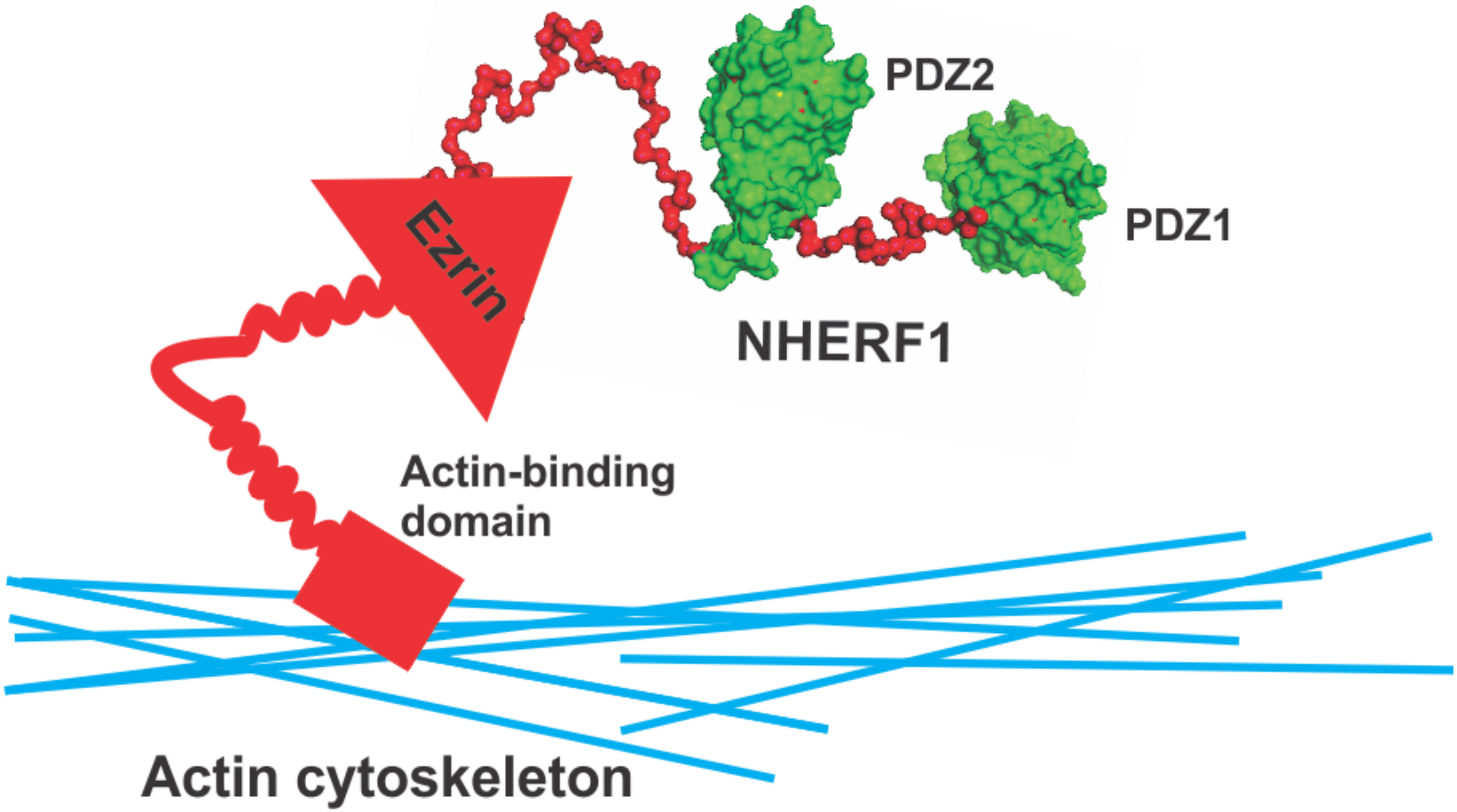
Full-length NHERF1



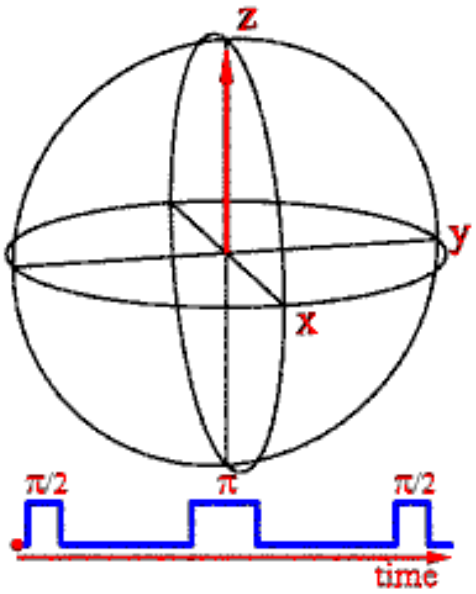


SEC-SAXS January, 2016, SSRL

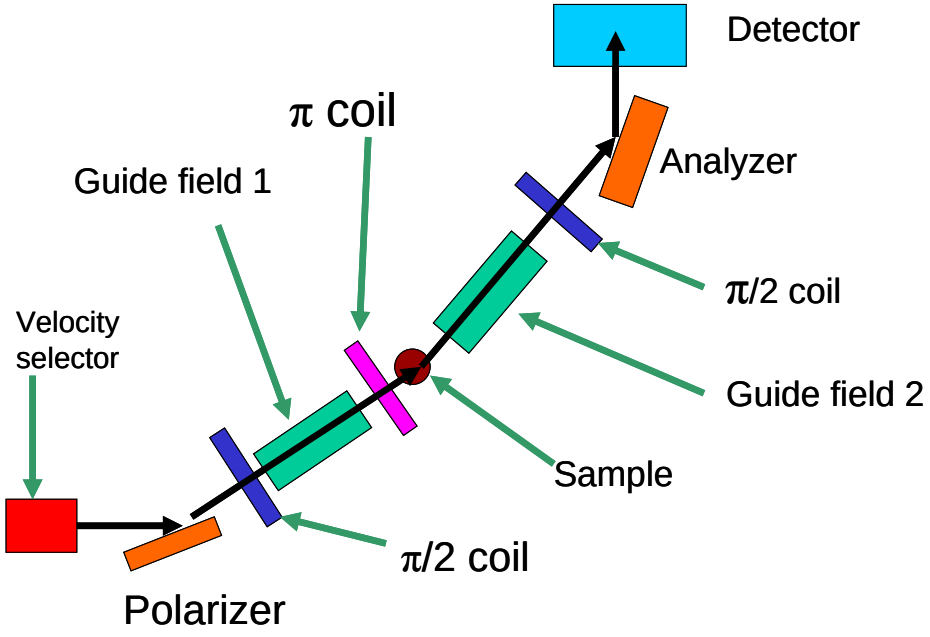
Propagation of long-range allostery



Applying neutron spin echo spectroscopy to study long-range protein domain motion



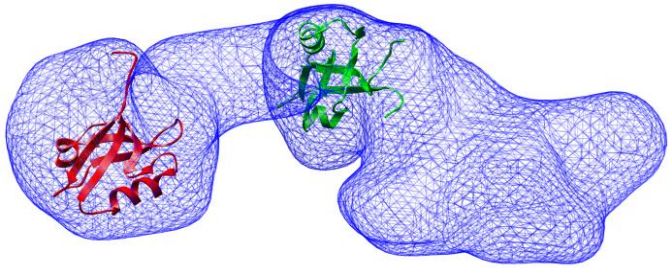
Ferenc Mezei

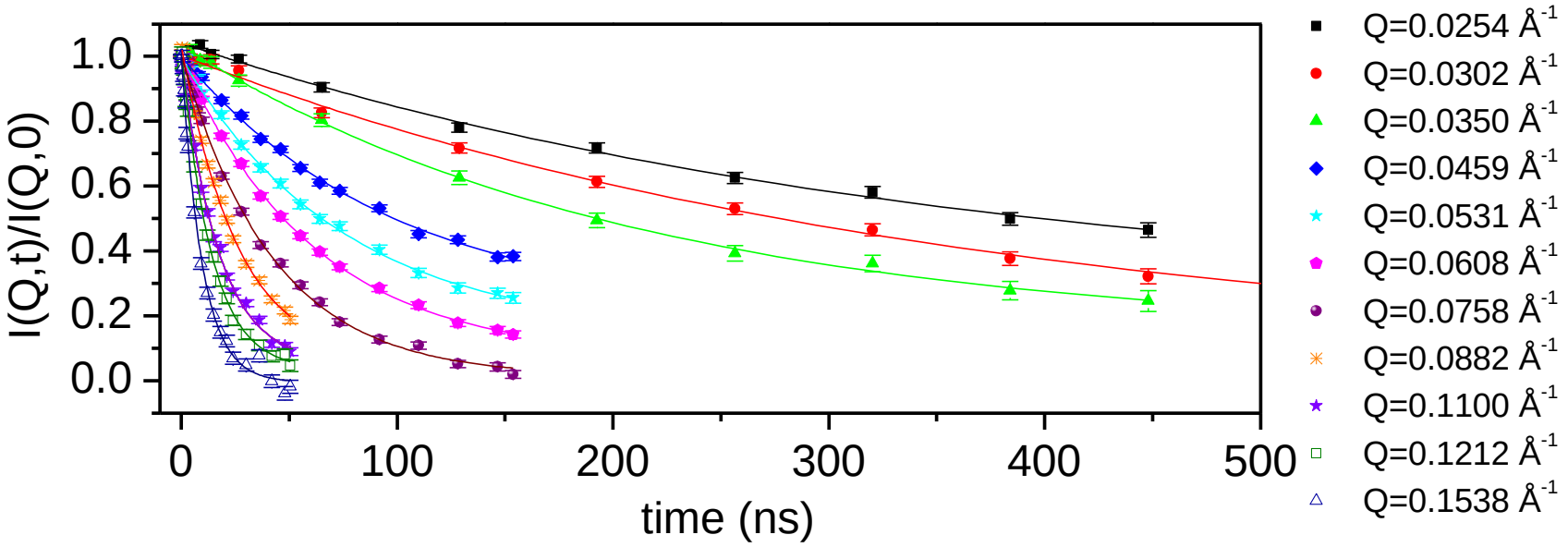


Nanosecond to microsecond time scales

10-1000 Å: nano length scales—**nanoscale motion**

NSE spectra on NHERF1 alone



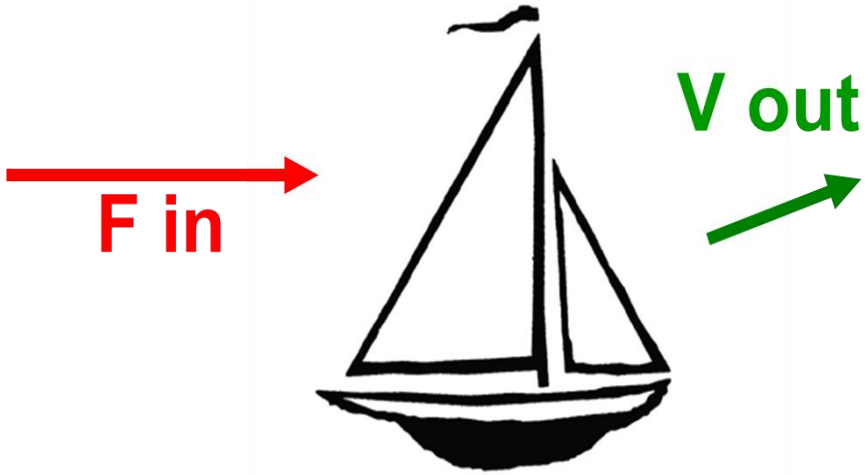


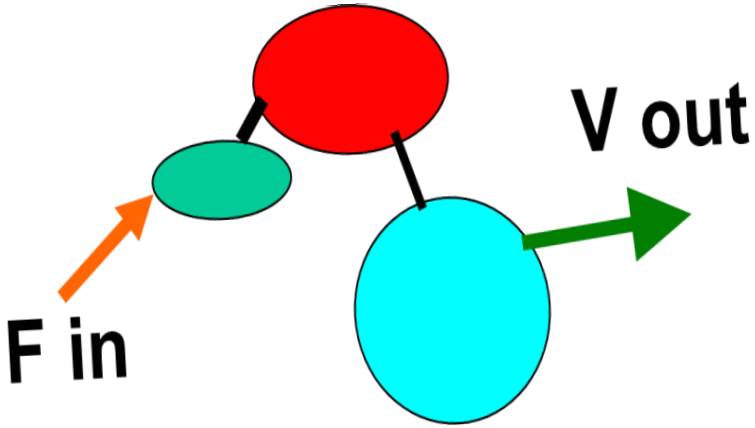
Protein motion—low Reynolds number

Overdamped creeping motions-

Effective diffusion constant $D_{eff}(Q)$

Mobility tensor $\vec{v} = \overleftrightarrow{H}\,\vec{F}$



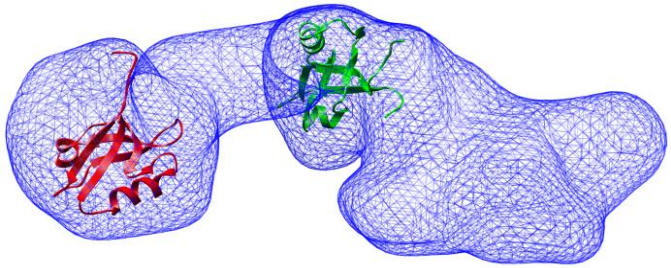


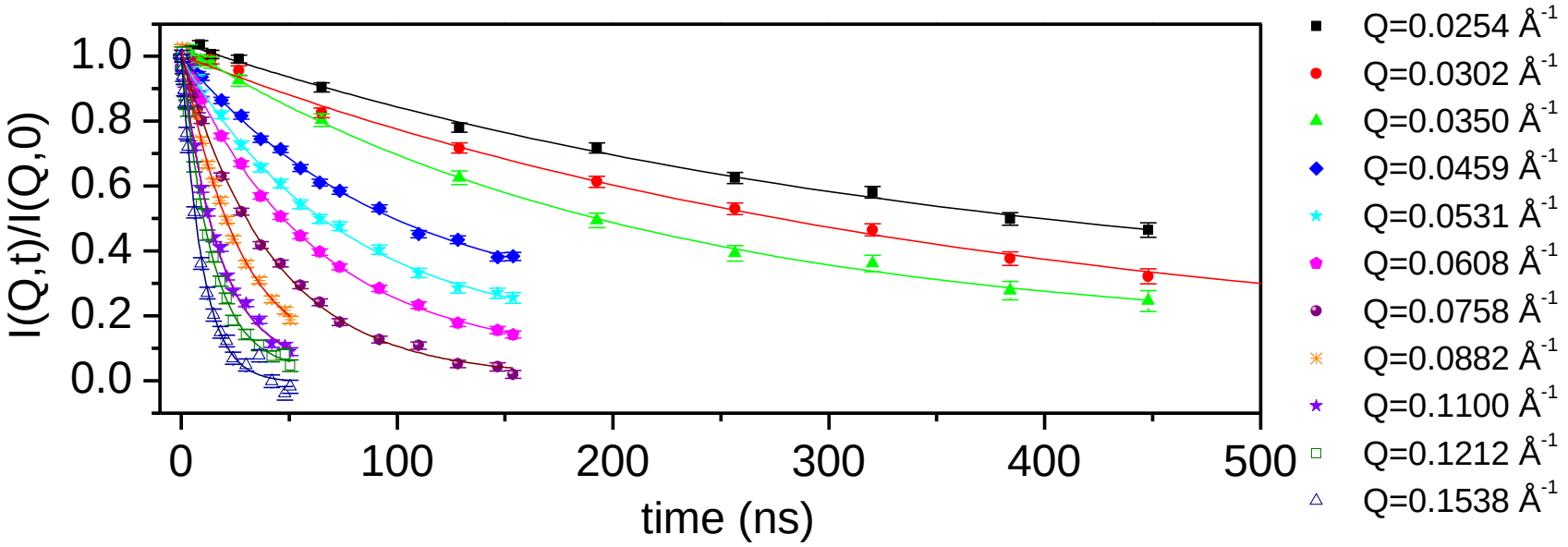
H is the mobility tensor, and yields the velocity of a domain given the force applied on it or another subunit. NSE yields H, given structure.

Bu et al, PNAS, 2005

Farago, Biophys. J., 2010

NSE spectra on NHERF1 alone





The Q dependence of the decay rates of the NSE measured correlation functions is defined by the mobility tensor

$$D_{eff}(Q) = \frac{k_B T}{Q^2} \frac{\sum_{jl} \left\langle b_j b_l \left(Q \cdot H_{jl}^{\ T} \cdot Q + L_j \cdot H_{jl}^{\ R} \cdot L_l \right) e^{iQ\cdot(r_j - r_l)} \right\rangle}{\sum_{jl} \left\langle b_j b_l e^{iQ\cdot(r_j - r_l)} \right\rangle}$$

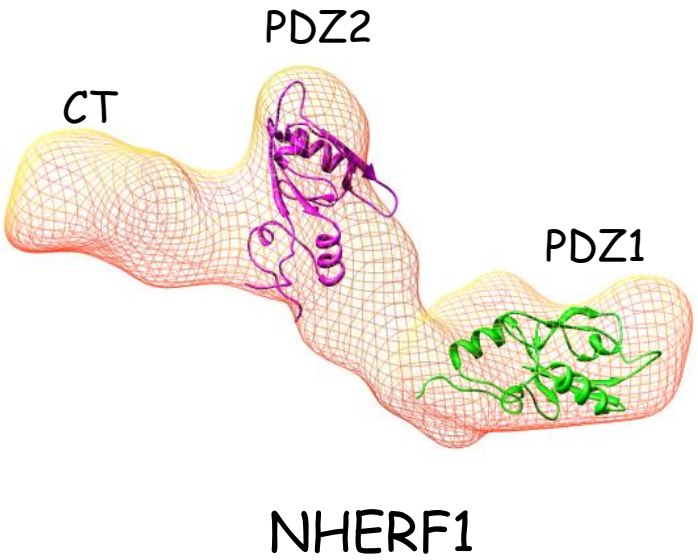
Mobility tensor H defines dynamics

$\mathbf{L}_j = \mathbf{r}_j \times \mathbf{Q}$ is the angular momentum vector.

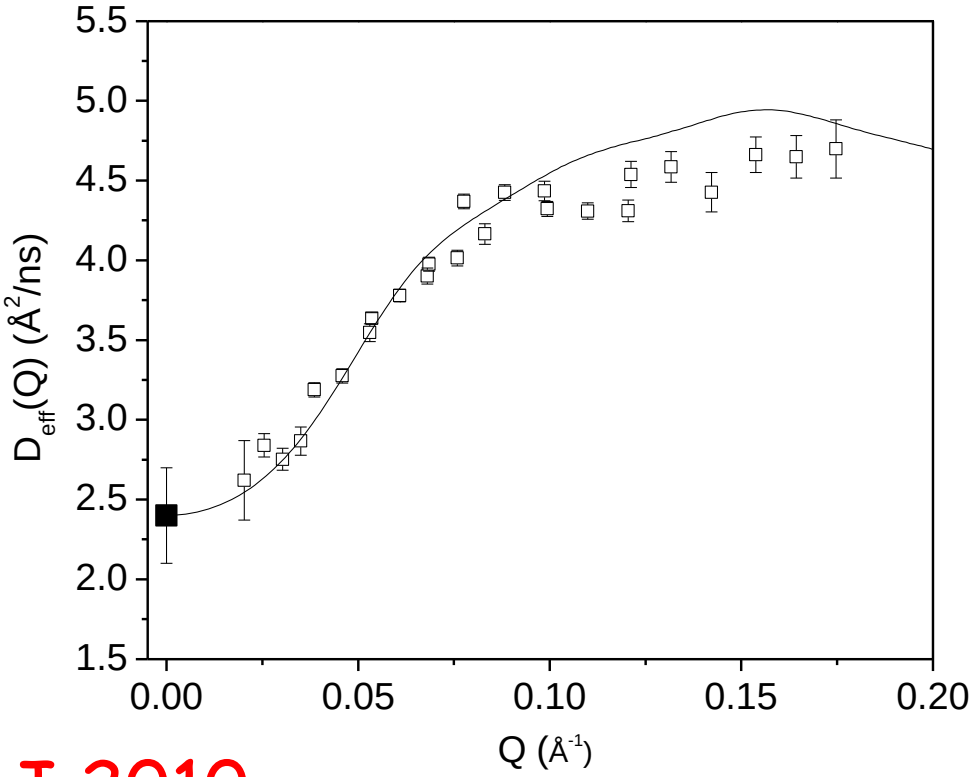
The angular velocity vector is given by $\boldsymbol{\omega} = H^R \boldsymbol{\tau}$, with the torque $\boldsymbol{\tau} = \Sigma_n \mathbf{r}_n \times \mathbf{F}_n$.

$\boldsymbol{\omega} = H^R{}_{\Sigma mn}\, \mathbf{r}_m \times (N^2 H^T)^{-1}{}_{mn}\, (\boldsymbol{\omega} \times \mathbf{r}_n)$

The dynamics of (unbound) NHERF1 alone is well described by a rigid-body model



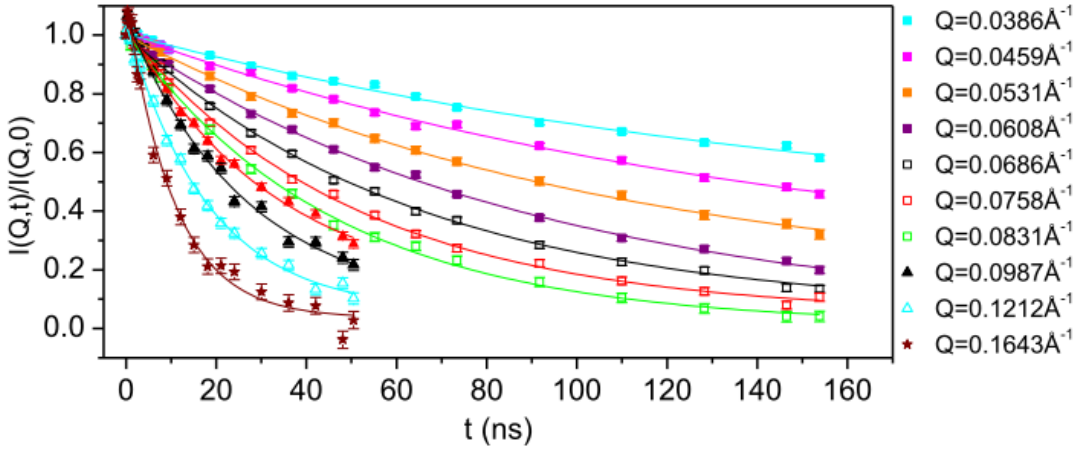
NHERF1



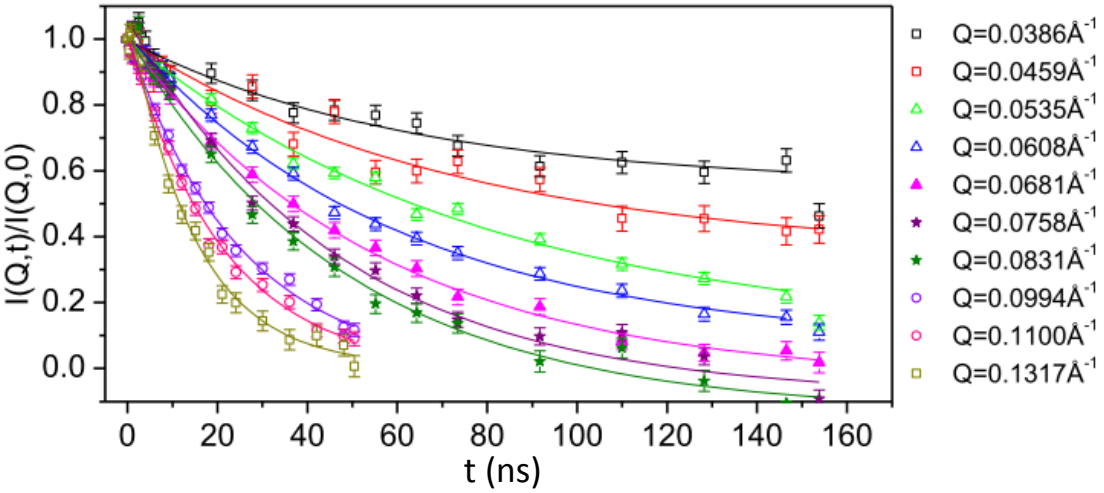
Farago et al Biophys J 2010

Only inputs to calculations are diffusion constant from PFG NMR and SANS coordinates—no need to fit NSE data or use MD!

NSE spectra on NHERF1-FERM complex



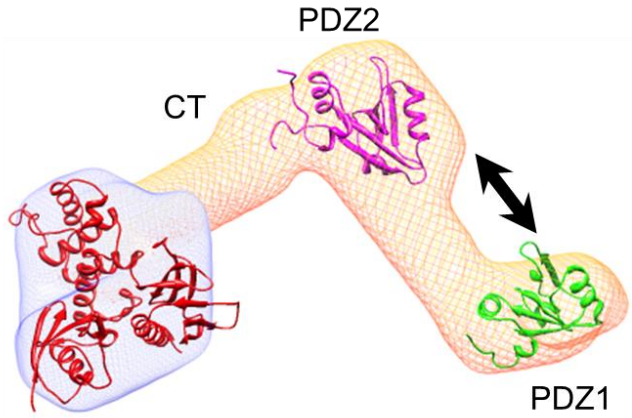
^{h}NHERF1-^{h}FERM complex



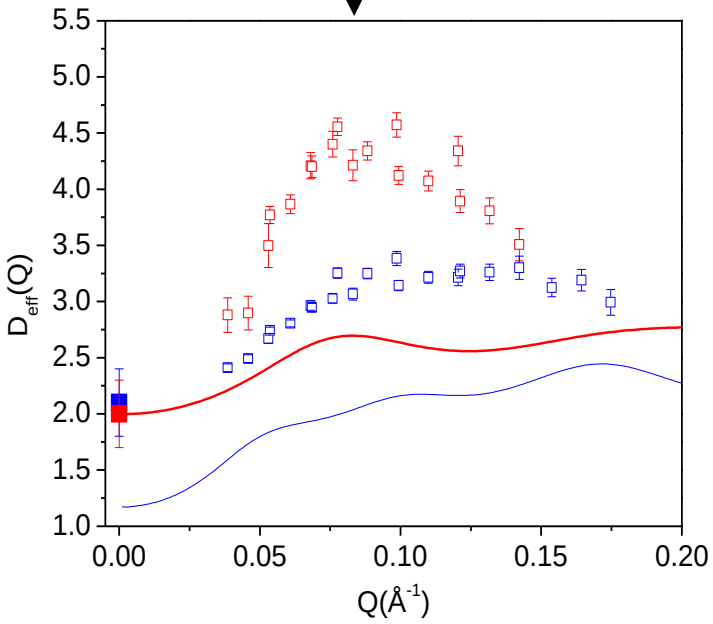
^{h}NHERF1-^{d}FERM complex

in D2O solution

Binding to ezrin activates inter-domain motions in NHERF1 more than 100 Å away!!

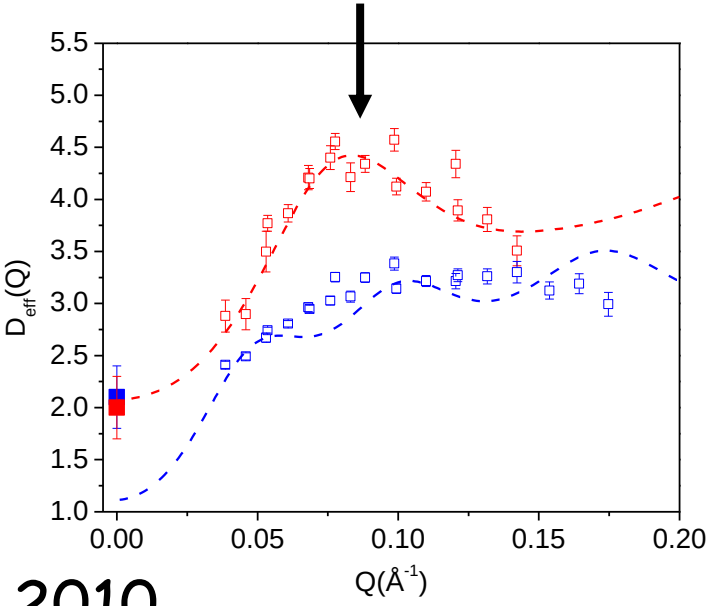


Rigid body



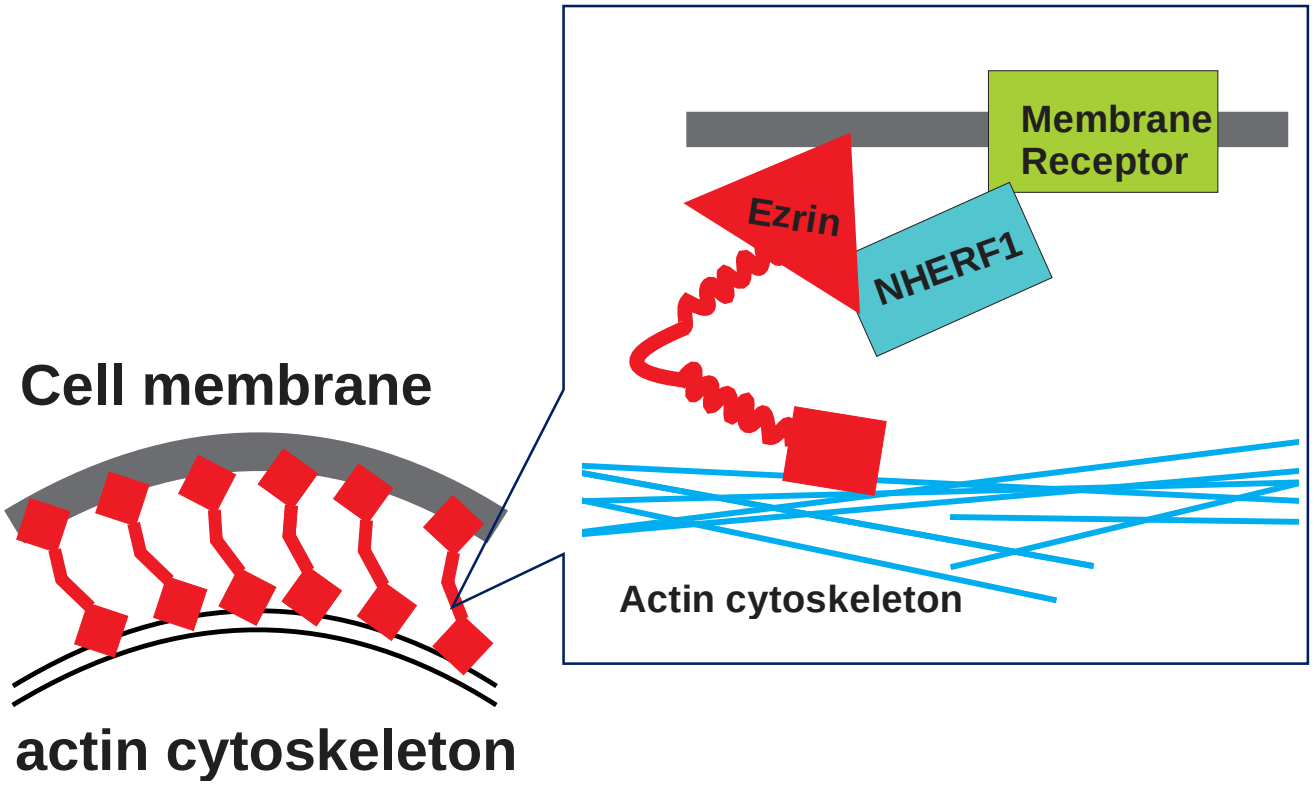
FERM

Inter-domain motion



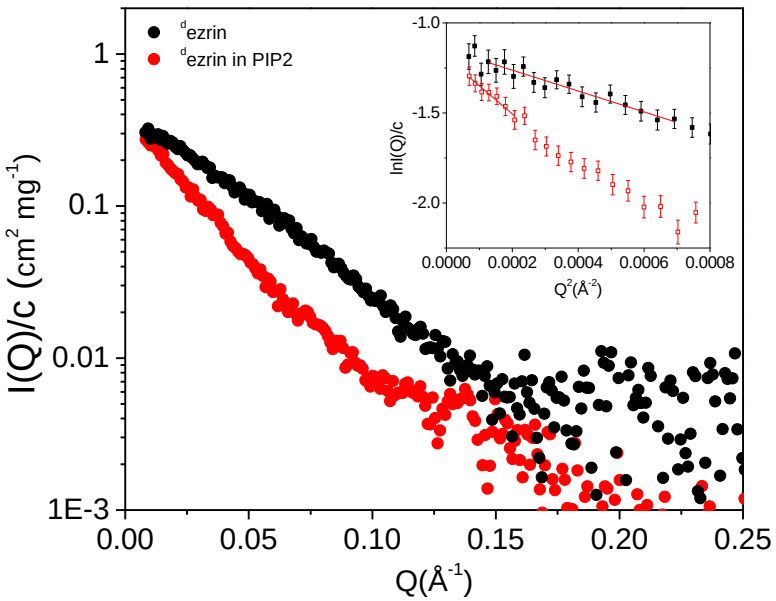
Farago et al Biophys J 2010

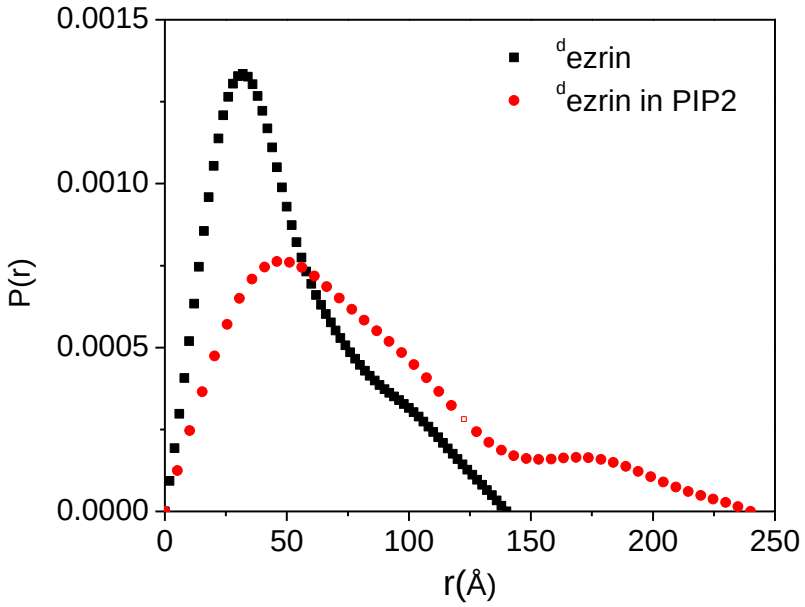
Adapter proteins NHERF1 and Ezrin regulate membrane receptor and ion channel assembly, trafficking and signaling

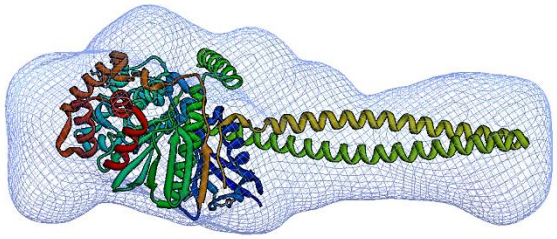


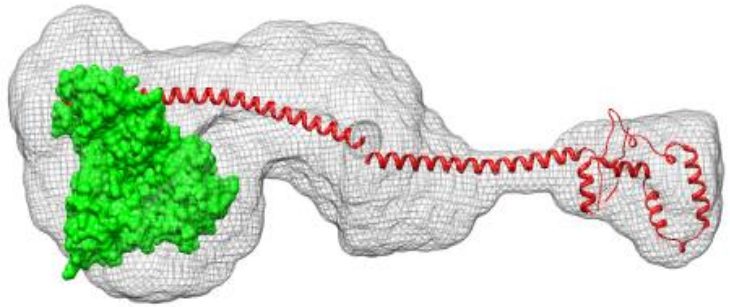
Linking the cell membrane to the F-actin cytoskeleton

SANS reveals the open conformation of Ezrin in PIP2 in 20% D2O

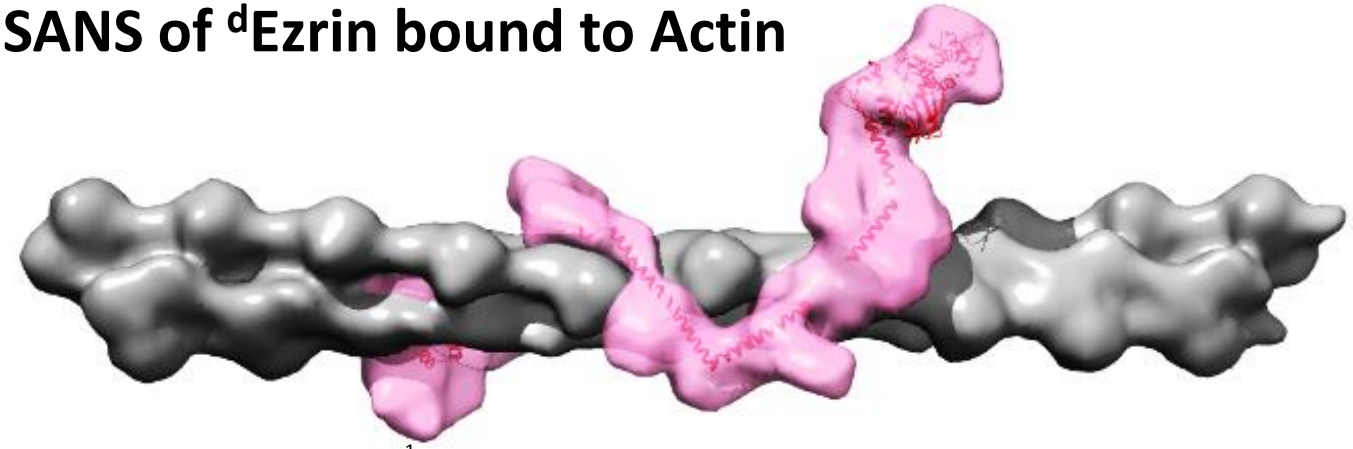


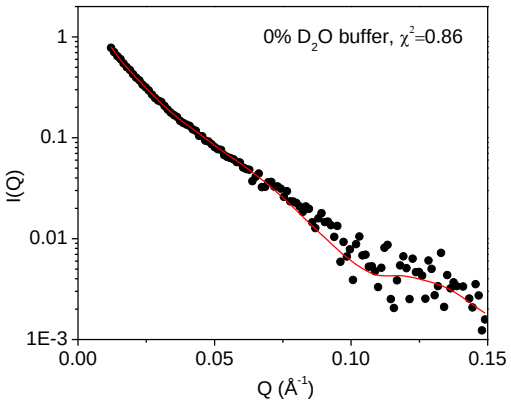


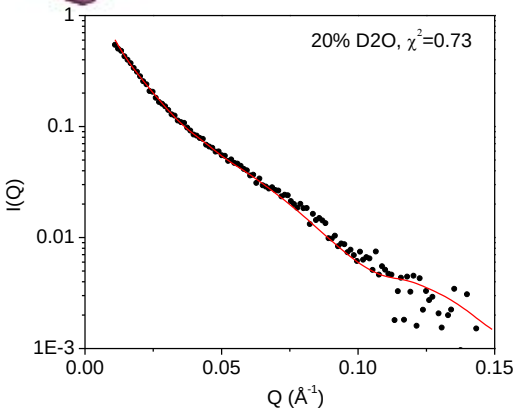


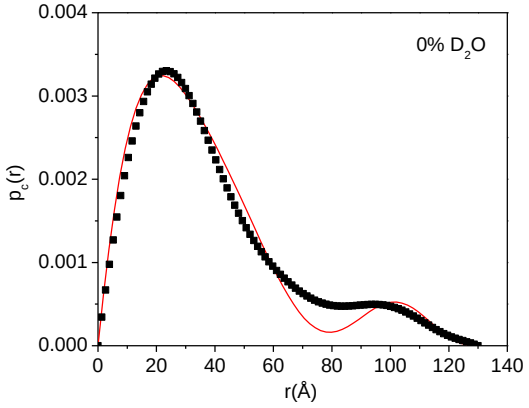


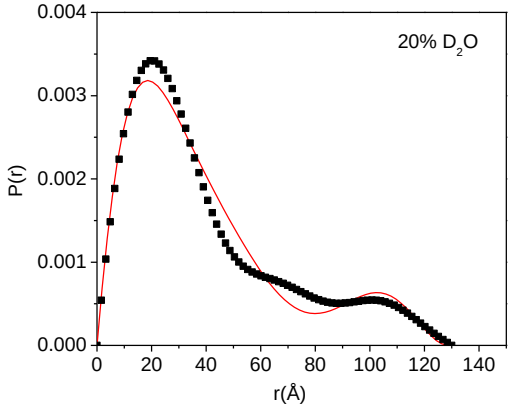
Contrast variation SANS of ^{d}Ezrin bound to Actin





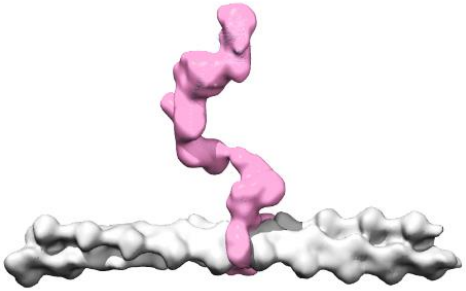


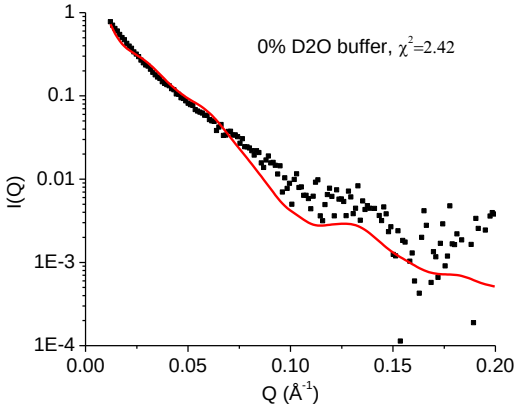


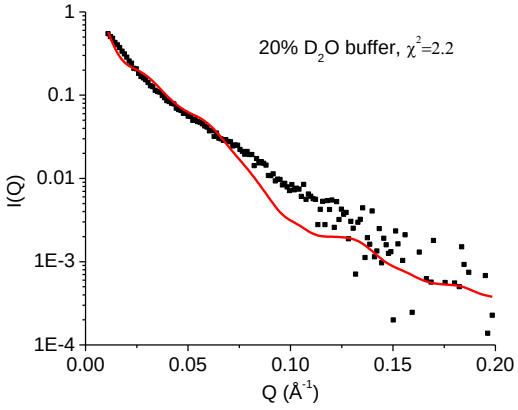


Jayasundar et al, JBC, 2012

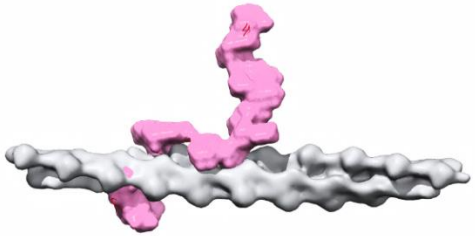
(A)

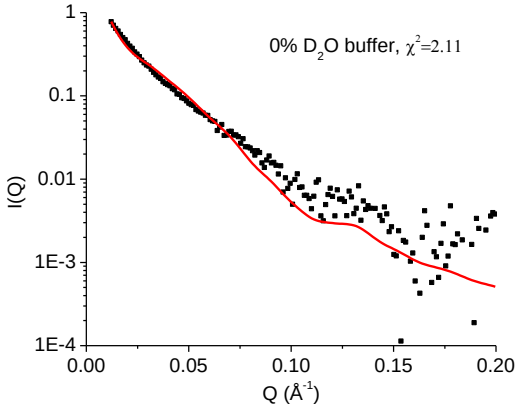


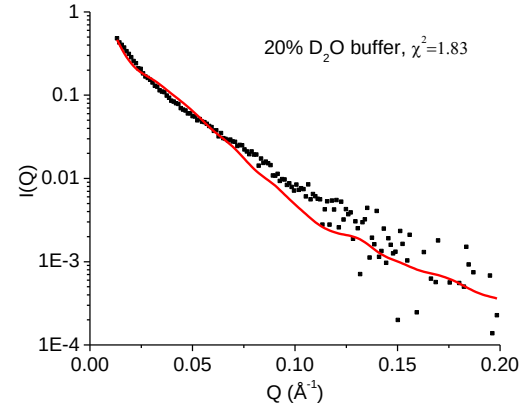

0% D2O buffer, χ^2=2.42
I(Q)
Q (Å^{-1})


20% D_2O buffer, χ^2=2.2
I(Q)
Q (Å^{-1})

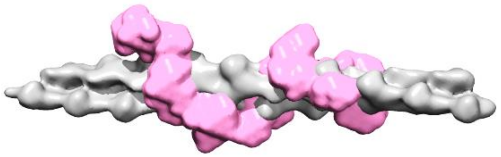
(B)

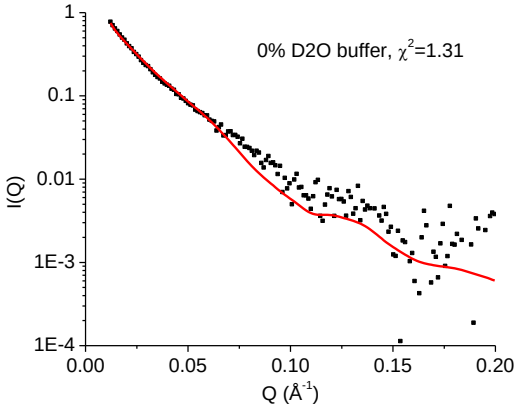


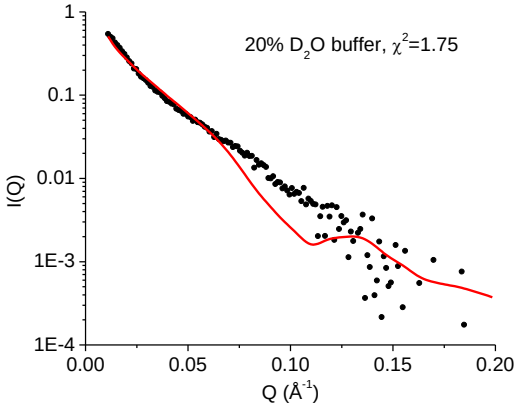

0% D_2O buffer, χ^2=2.11
I(Q)
Q (Å^{-1})


20% D_2O buffer, χ^2=1.83
I(Q)
Q (Å^{-1})

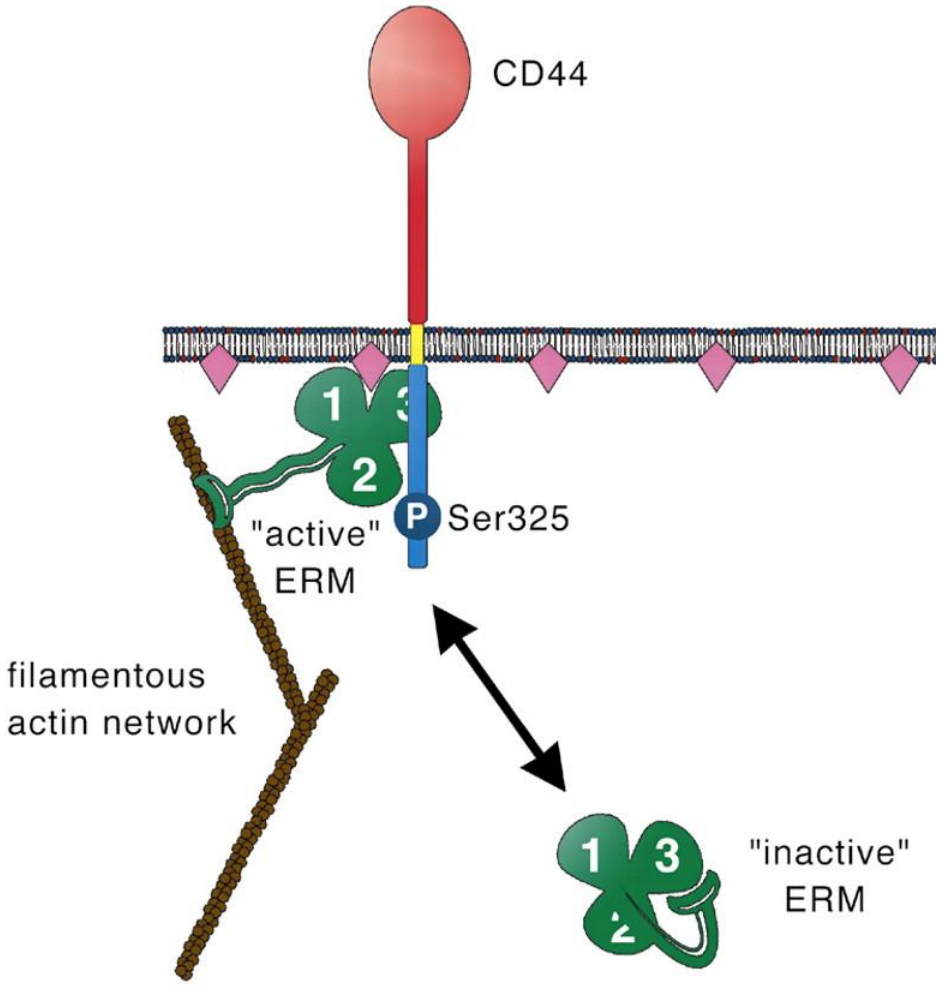
(C)



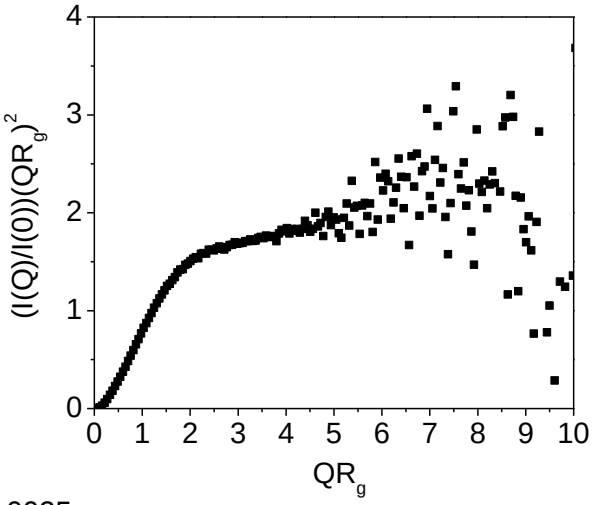

0% D2O buffer, χ^2=1.31
I(Q)
Q (Å^{-1})

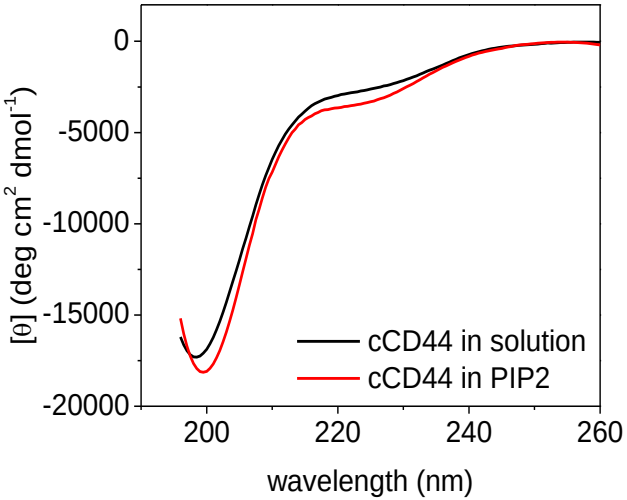

20% D_2O buffer, χ^2=1.75
I(Q)
Q (Å^{-1})

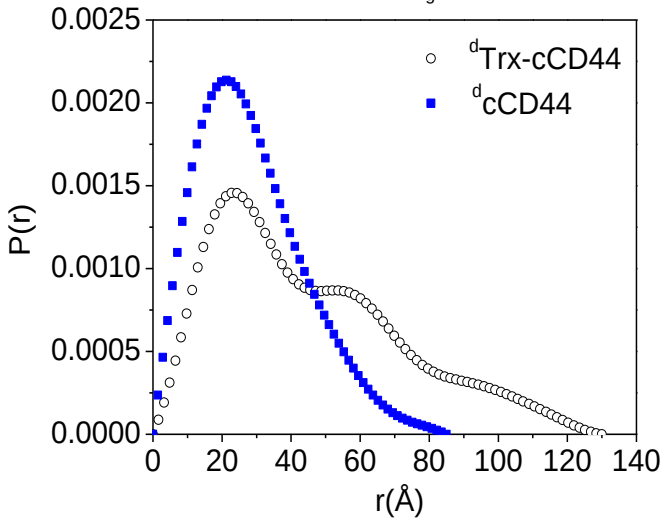
CD44 a single transmembrane protein involved in cell migration cell-cell adhesion and cell-matrix adhesion

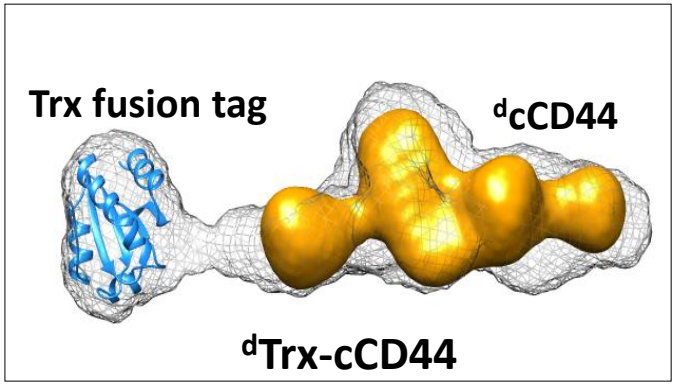


The cytoplasmic tail of CD44 adopts a collapsed and molten globule like conformation



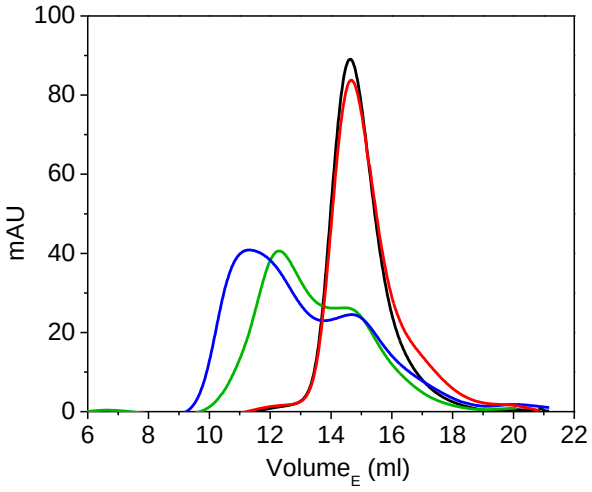


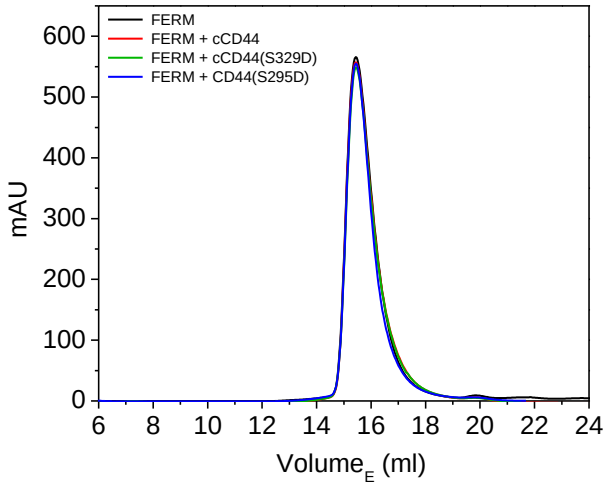


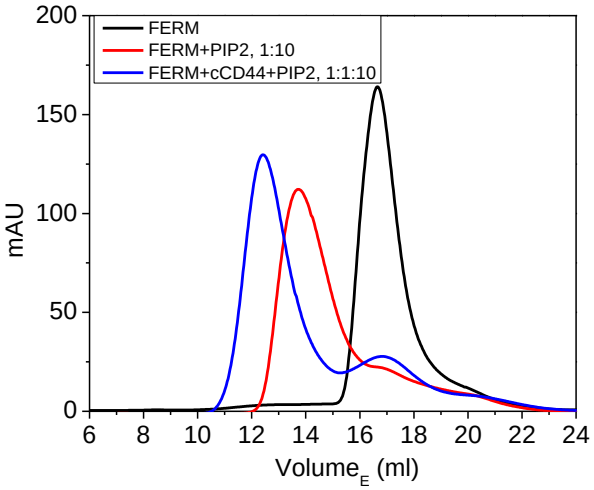


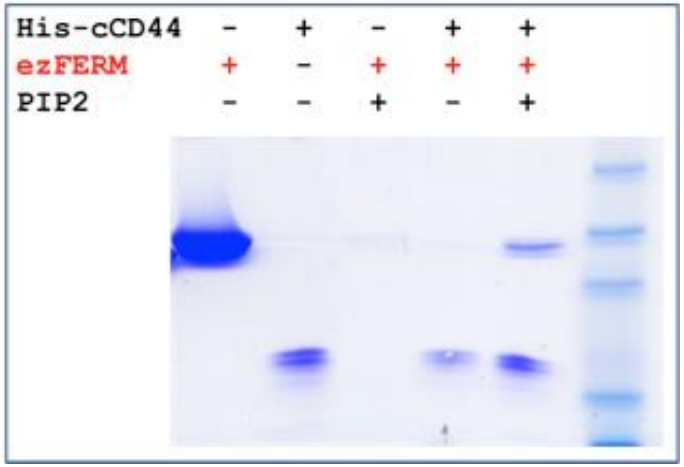
BNL, 2013

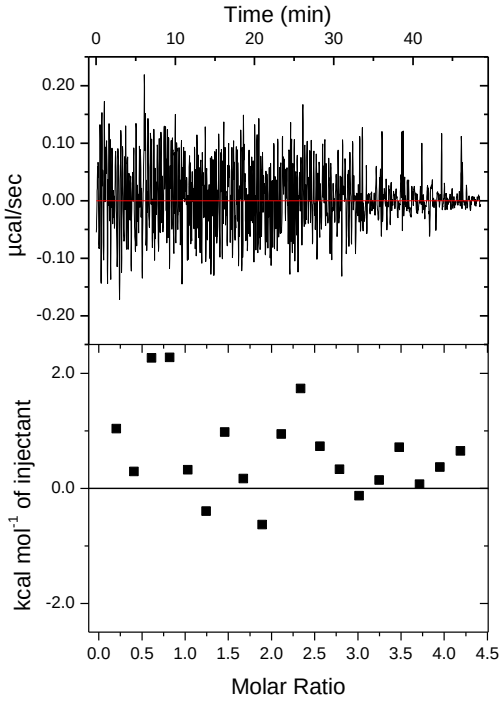
Only in PIP2, CD44 binds to Ezrin, or the FERM domain of Ezrin



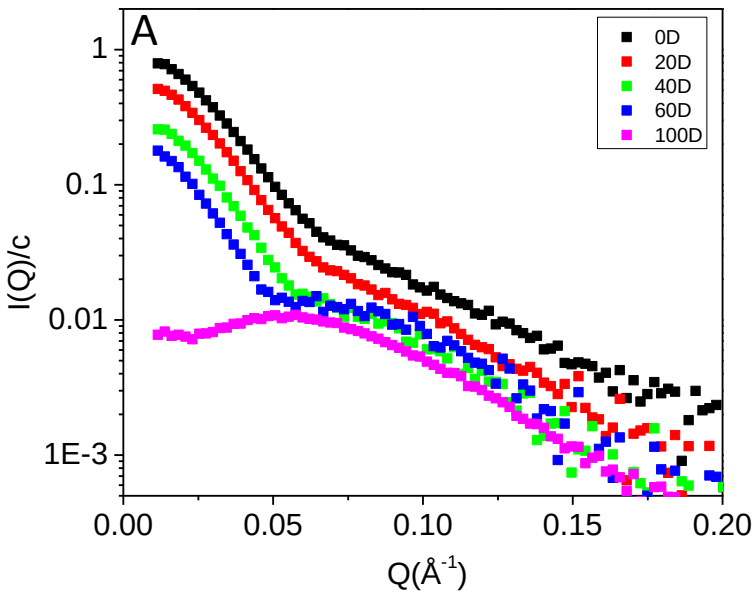


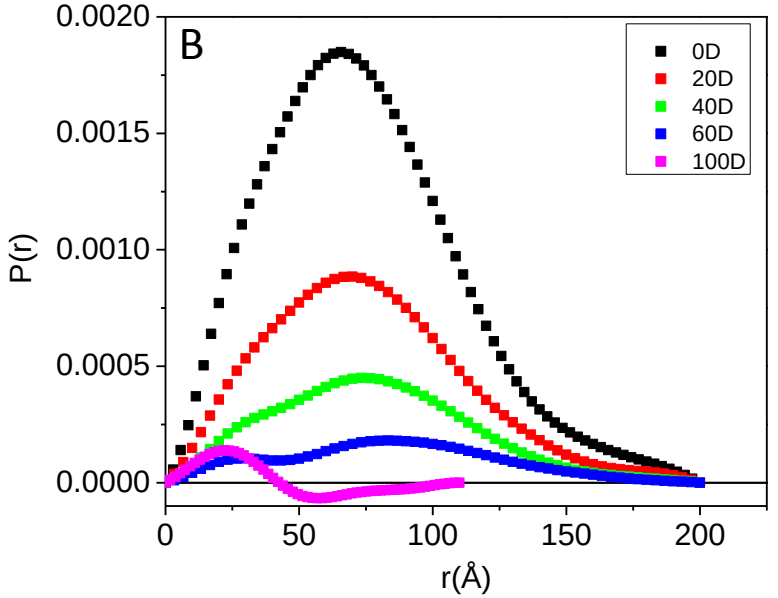


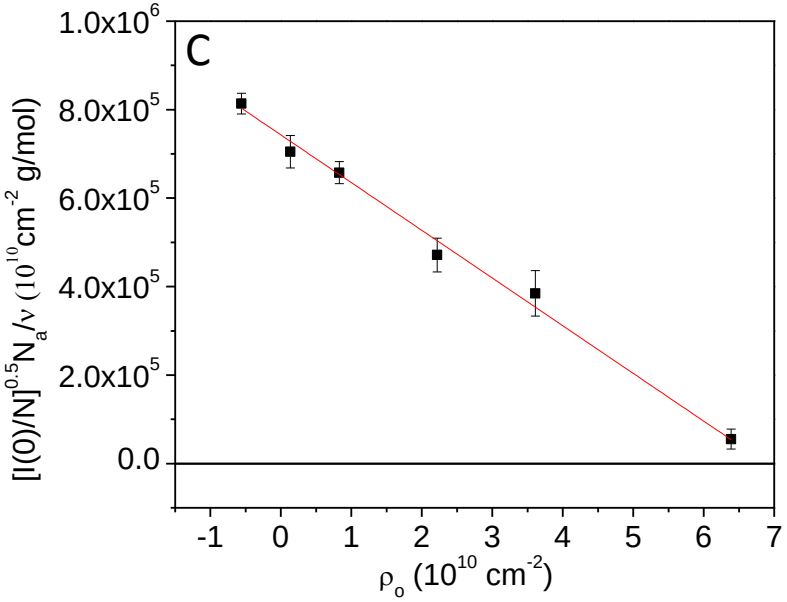


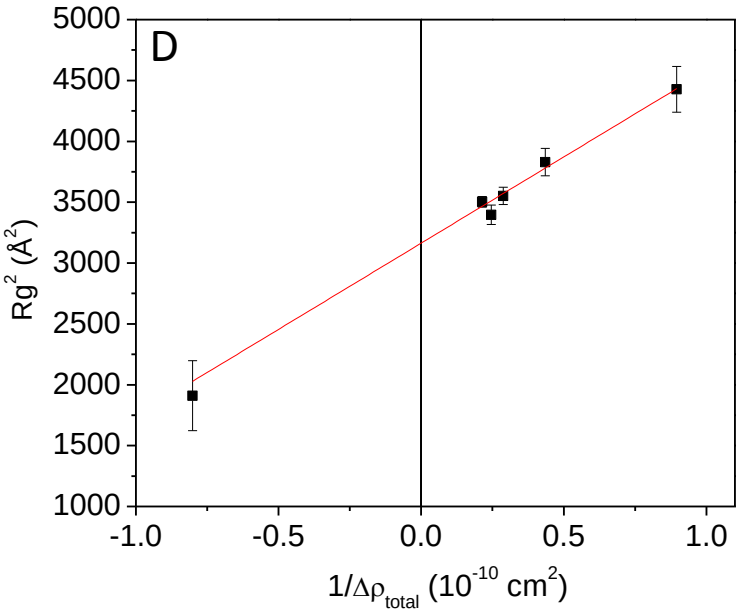


Contrast variation SANS on PIP2/^{d}cCD44/^{d}FERM

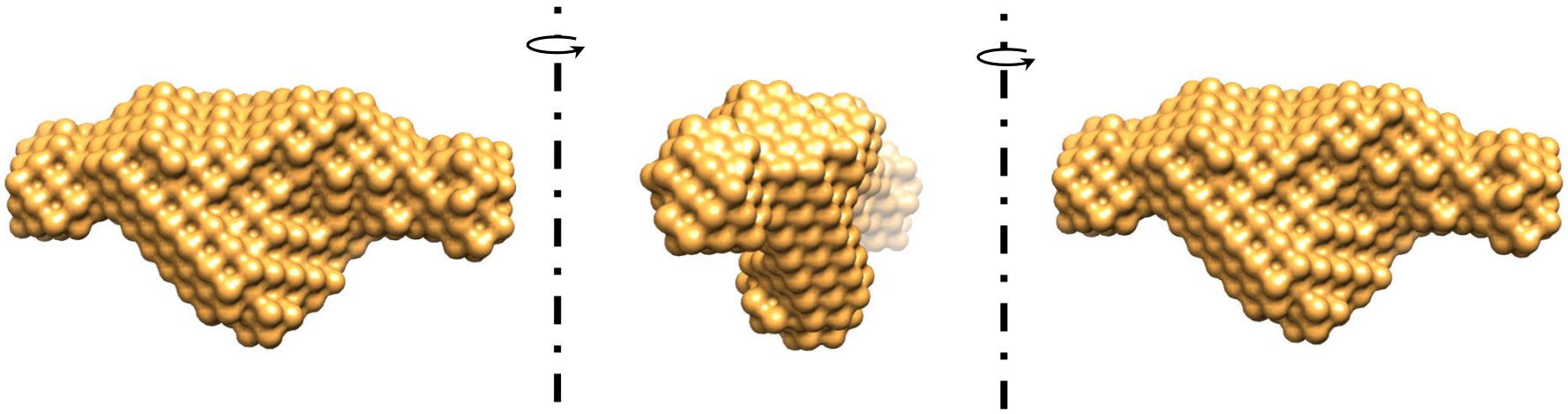






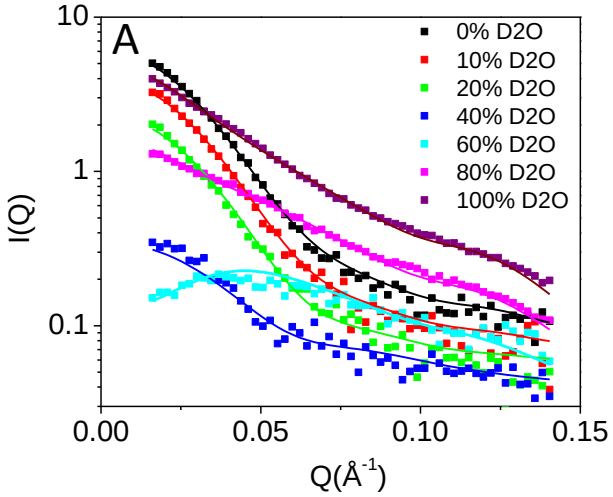


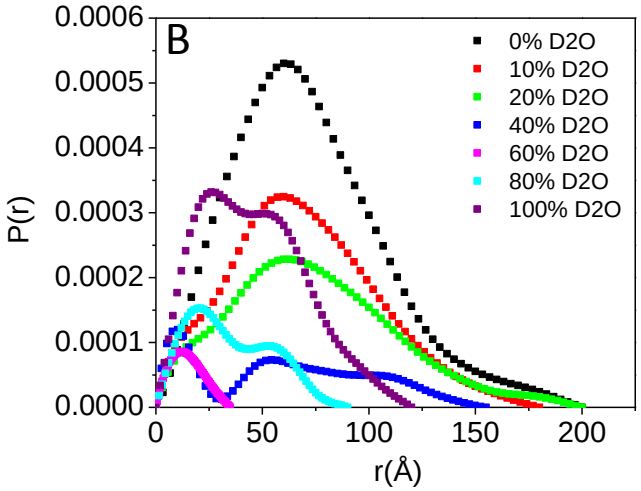
3-D image of PIP2/^{d}cCD44/^{d}FERM

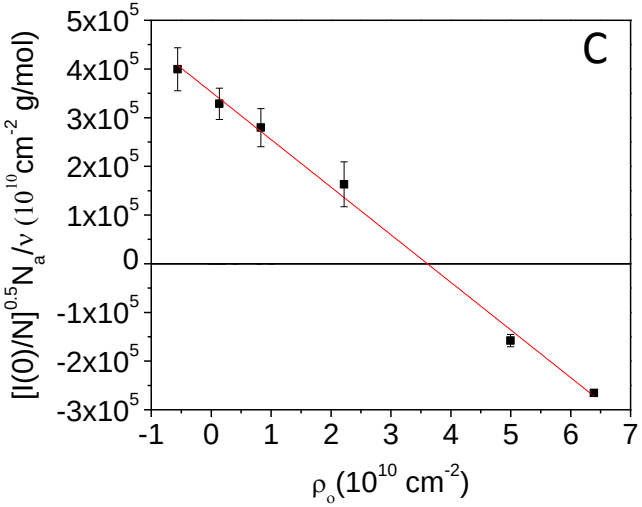


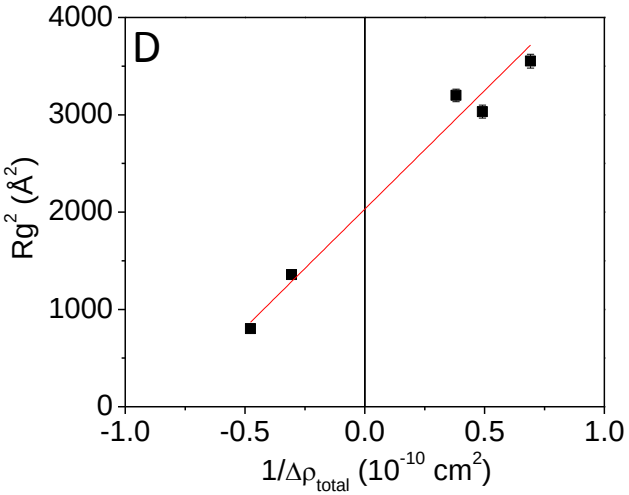
Dammin of SANS data in 20% D2O at the contrast-matching point of PIP2

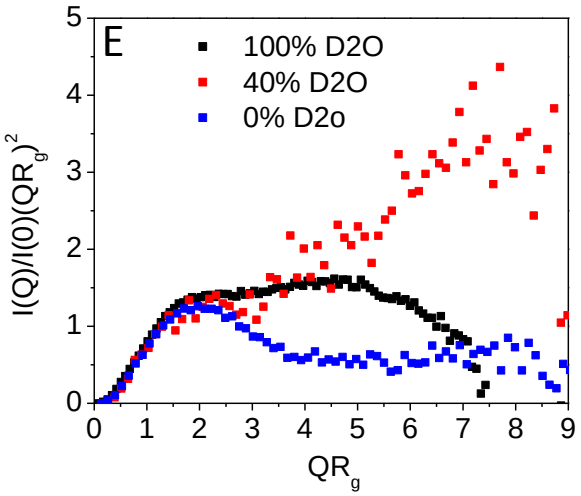
Contrast variation SANS on PIP2/^{d}cCD44/^{h}FERM



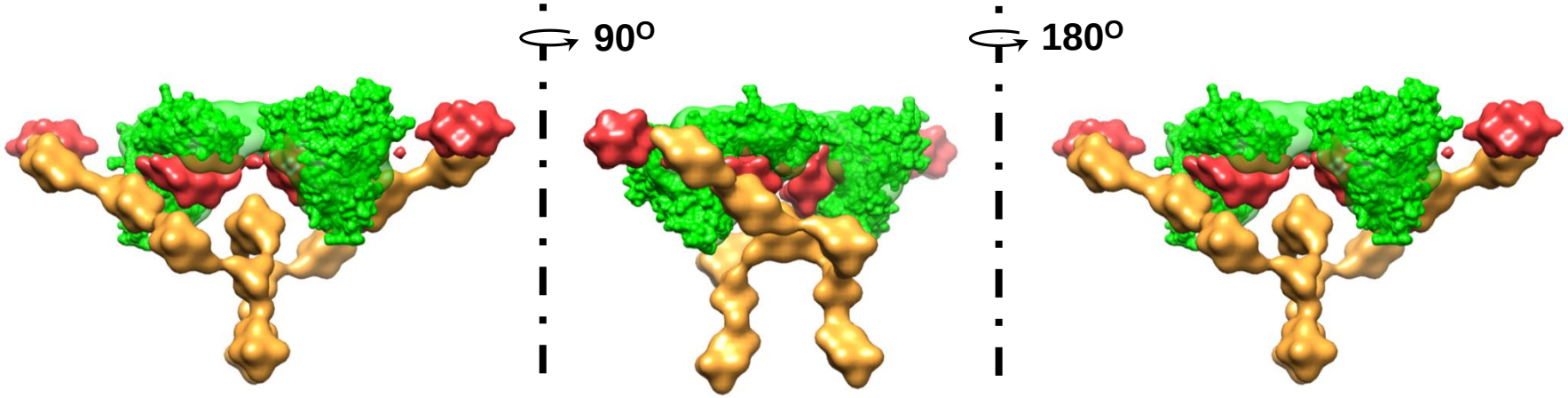




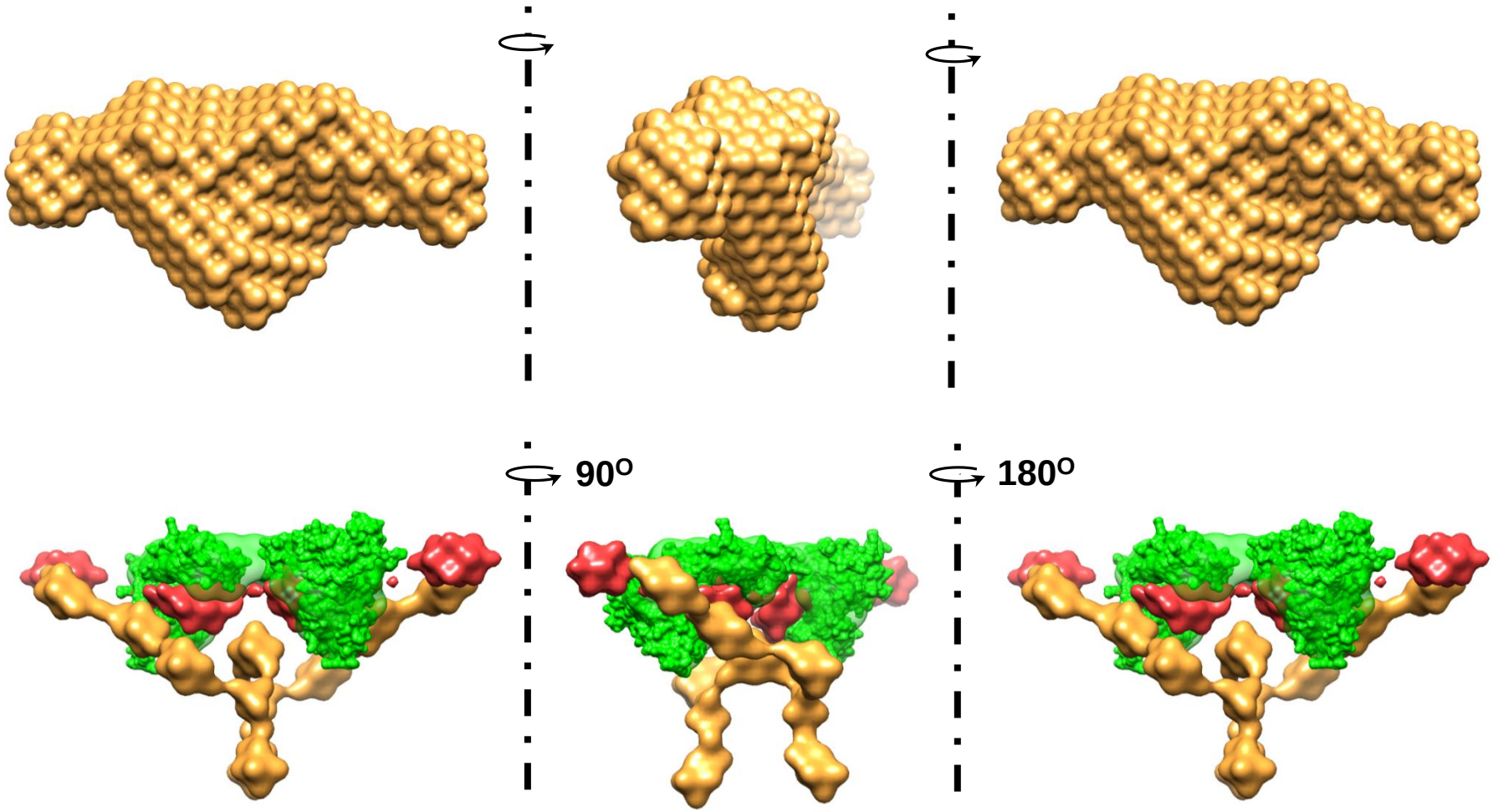


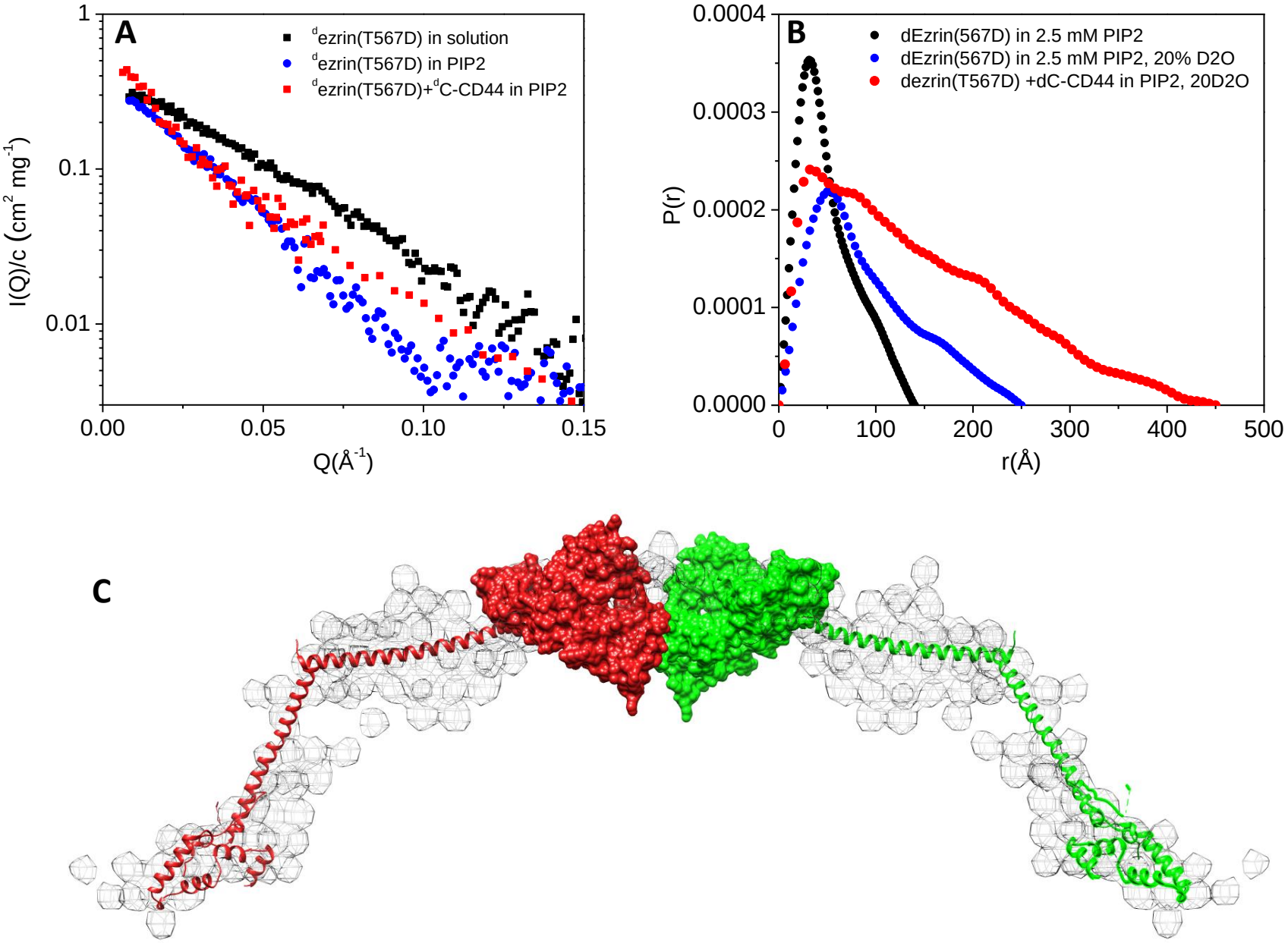


3-D image of PIP2/^{d}CD44/^{h}FERM



MONSA using SANS data at all contrasts


90°
180°


A
dezrin(T567D) in solution
dezrin(T567D) in PIP2
dezrin(T567D)+dC-CD44 in PIP2
I(Q)/c (cm2 mg-1)
Q(Å-1)
B
dEzrin(567D) in 2.5 mM PIP2
dEzrin(567D) in 2.5 mM PIP2, 20% D2O
dezrin(T567D) +dC-CD44 in PIP2, 20D2O
P(r)
r(Å)
C

PIP2 activates the cytoplasmic tail of CD44 and facilitates the assembly of a CD44·Ezrin hetero-tetramer complex

Acknowledgements

Lab members
Jianquan Li
James Jayasundar
Jeong Ho Ju
Jahan Khajeh
Sabine Baxter
Xiaodong Chen

NYU School of Medicine
David Callaway

ILL
Bela Farago

BNL
Marc Allaire

ORNL
William Heller
Chris Stanley
Changwoo Do
Lilin He
Flora Meillieur

SSRL
Thomas Weiss
Tsutomu Matsui
Ivan Rajkovic

New York Structural Biology Center
David Cowburn
Shibani Bhattacharya