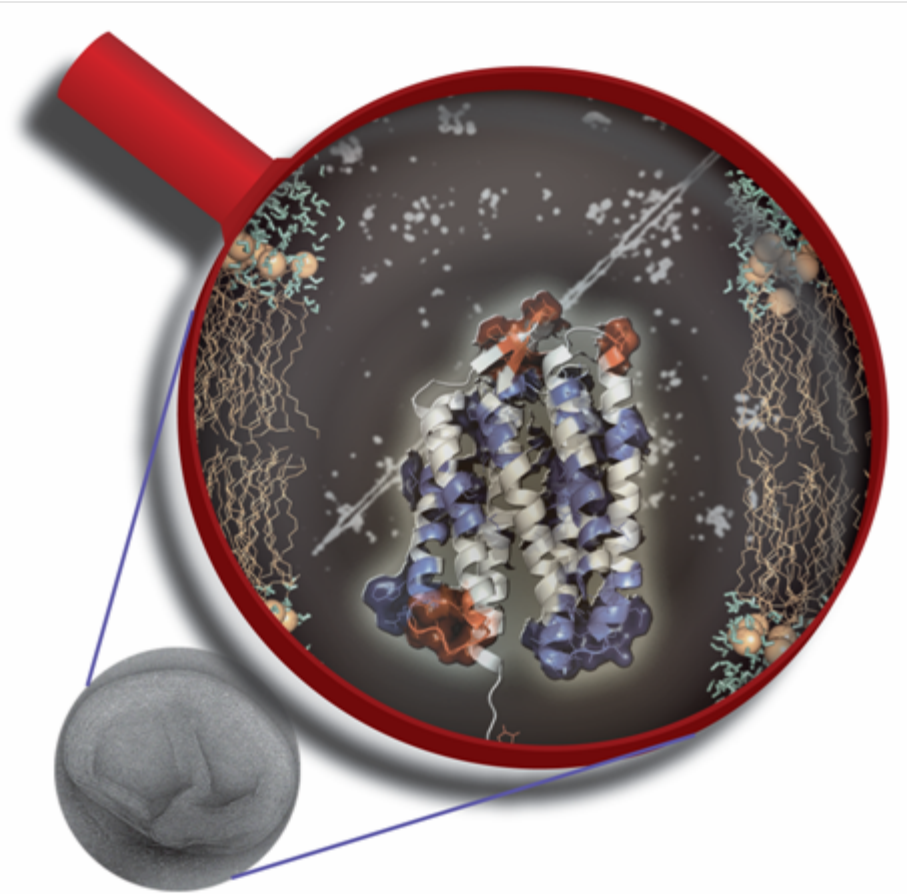
Studying structure and dynamics of protein complexes by solid-state NMR spectroscopy

Christian Ader, Gitta Angerstein, Manuel Etzkorn, Itzam de Gortari, Henrike Heise, Ashutosh Kumar, Henrik Müller, Robert Schneider, Karsten Seidel, Marc Baldus

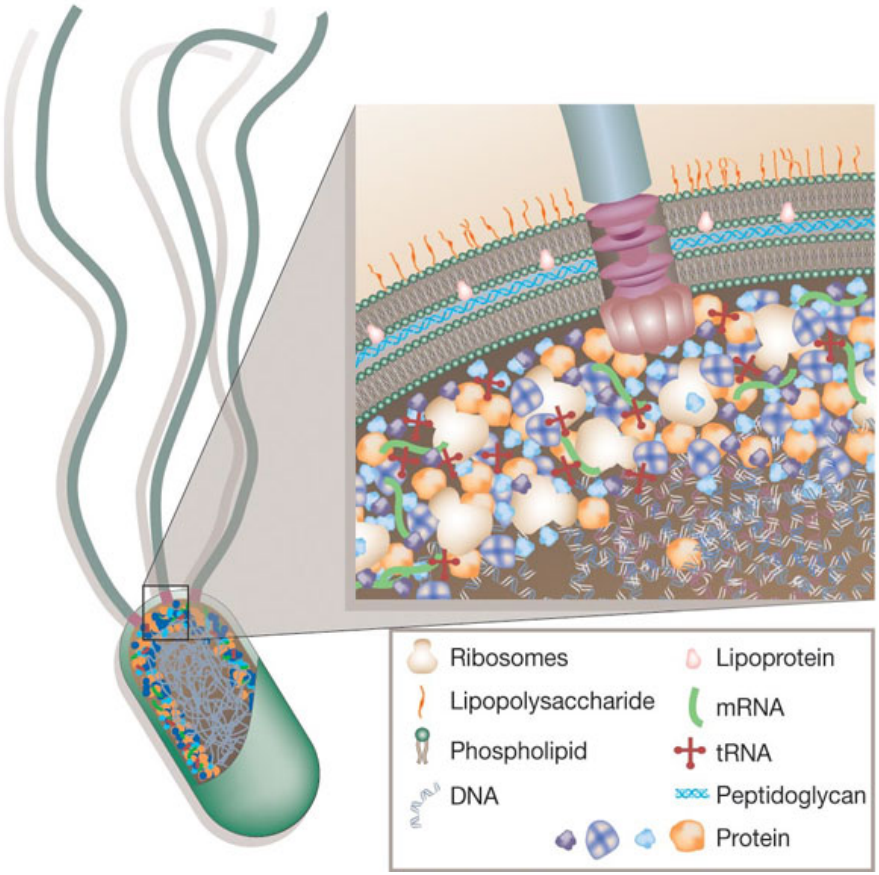


Solid-state NMR group
Max Planck Institute for Biophysical Chemistry
37077 Göttingen, Germany

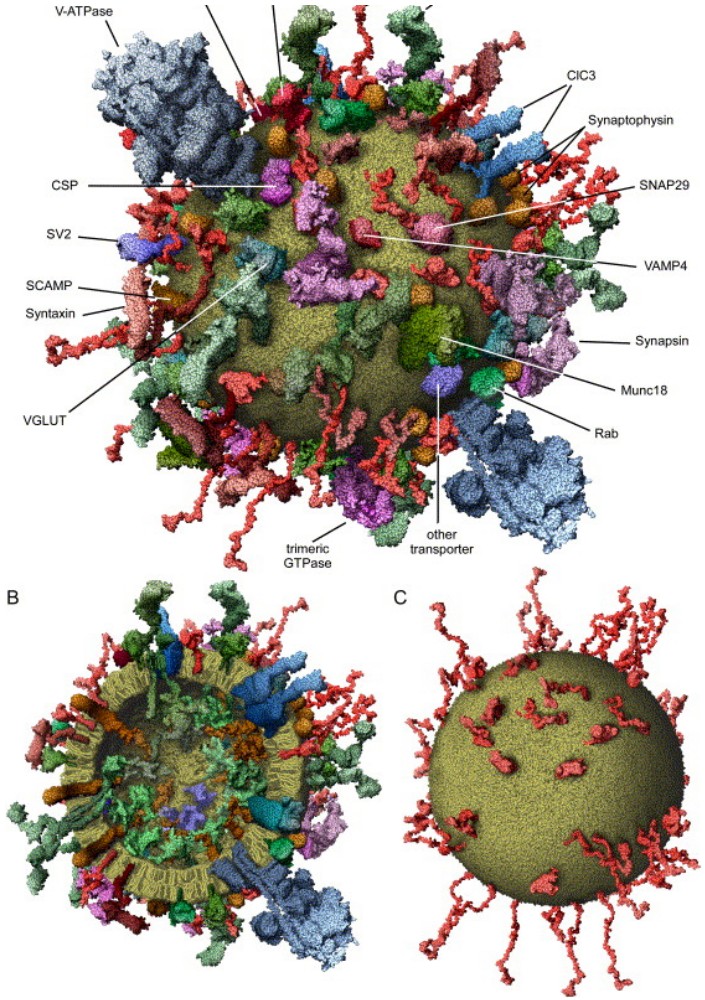
The EBSA prize lecture

July 18th 2007

Life can be crowded…



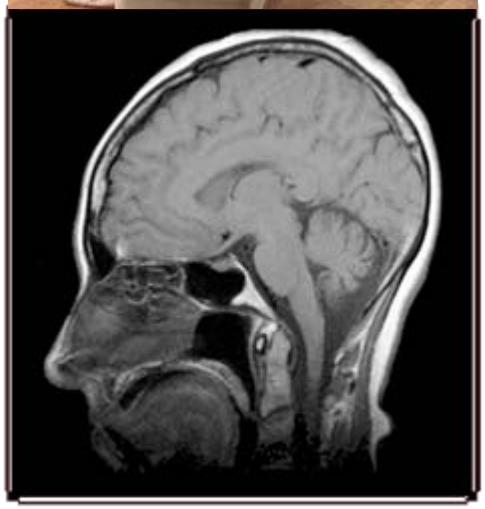
Schematic representation of a crowded cell.
Dobson Nature 2004



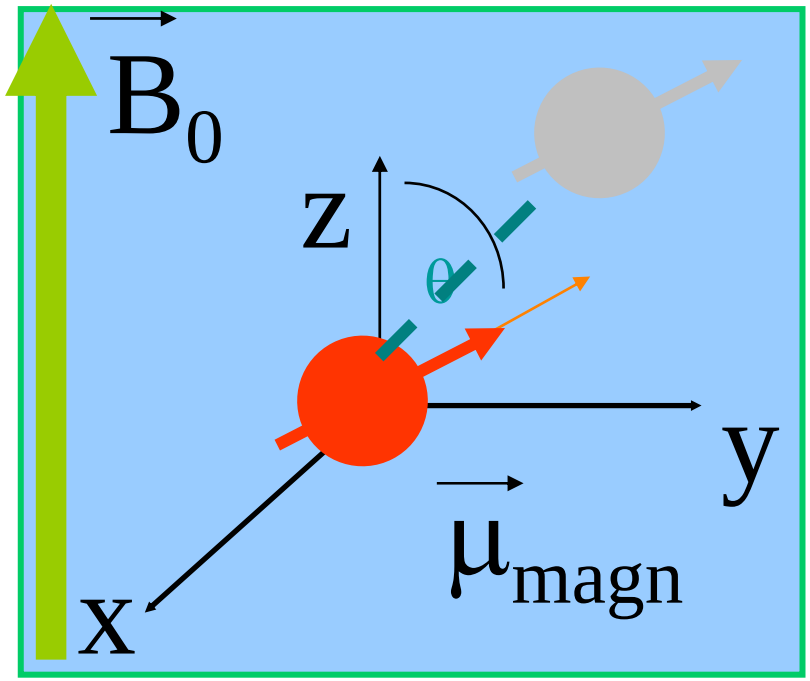
Molecular Model of an Average synaptic vesicle
Jahn et al., Cell 2006

Magnetic resonance

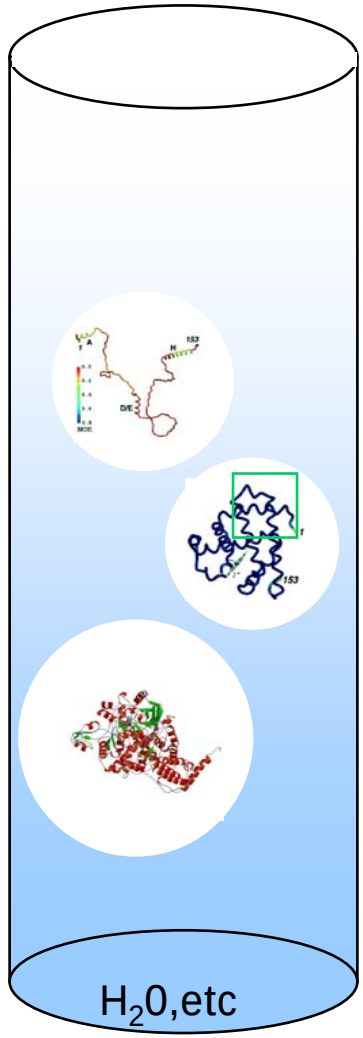
"magnetic resonance imaging"



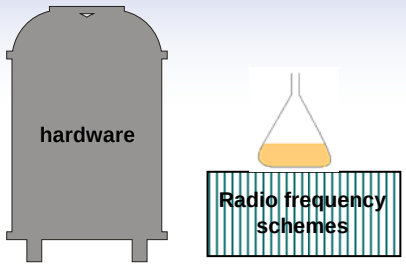
Interactions on the molecular level



"high-resolution NMR"

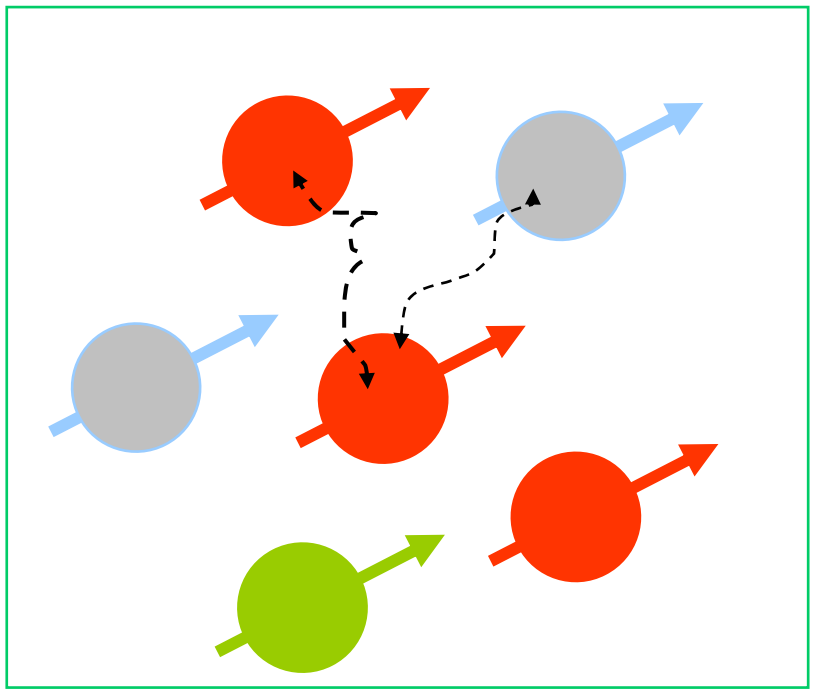


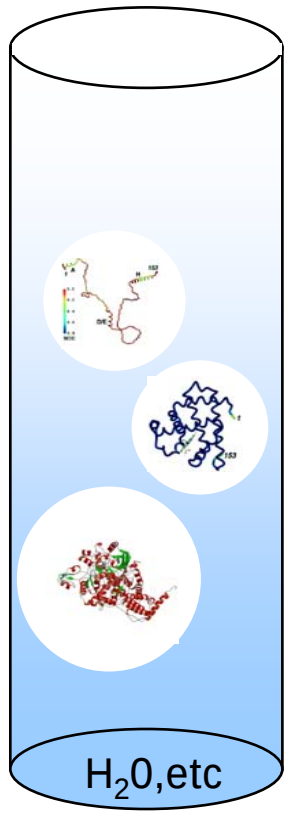
High-resolution NMR



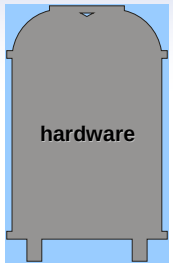
Solution-state NMR

Interactions are relatively weak due to motion

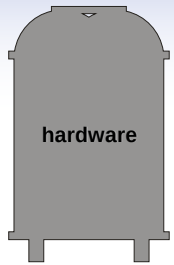


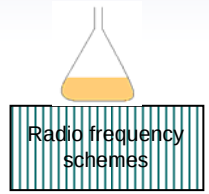


Magnetic resonance: solution vs. solid-state NMR



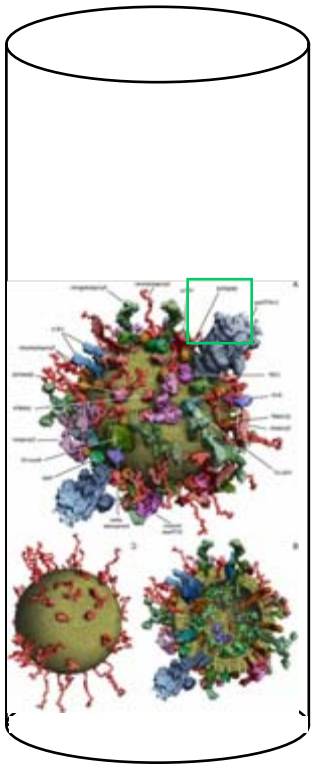
Radio frequency schemes

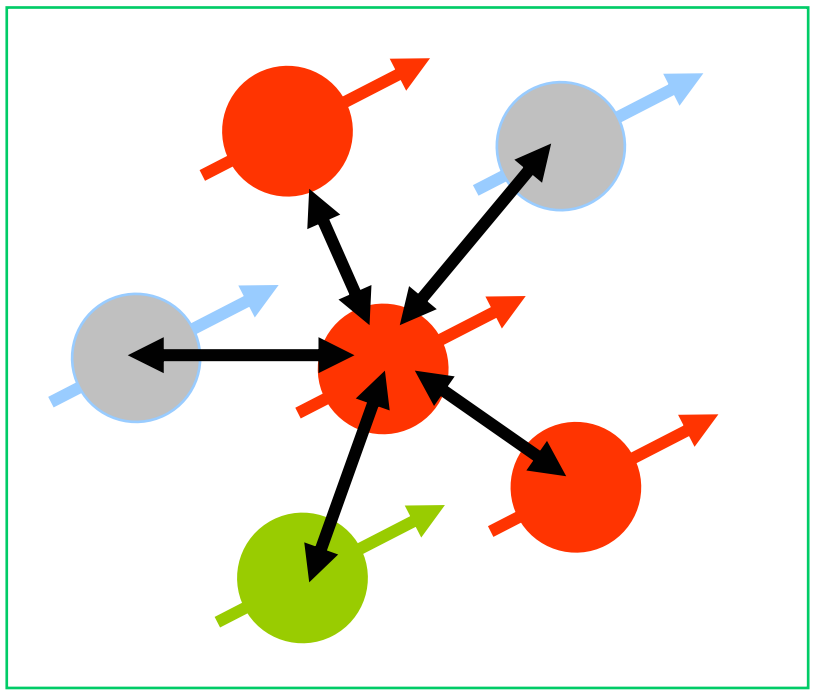




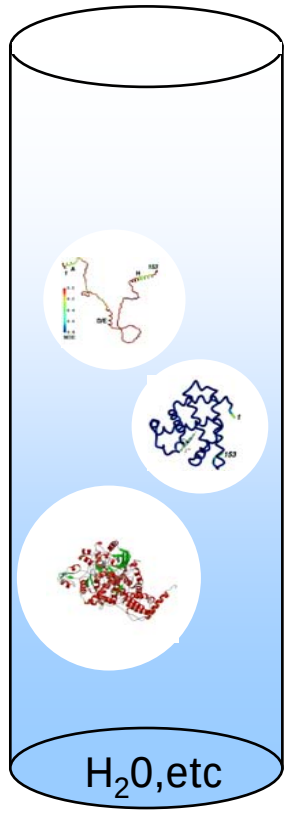
Interaction strength increases

Solid-state NMR

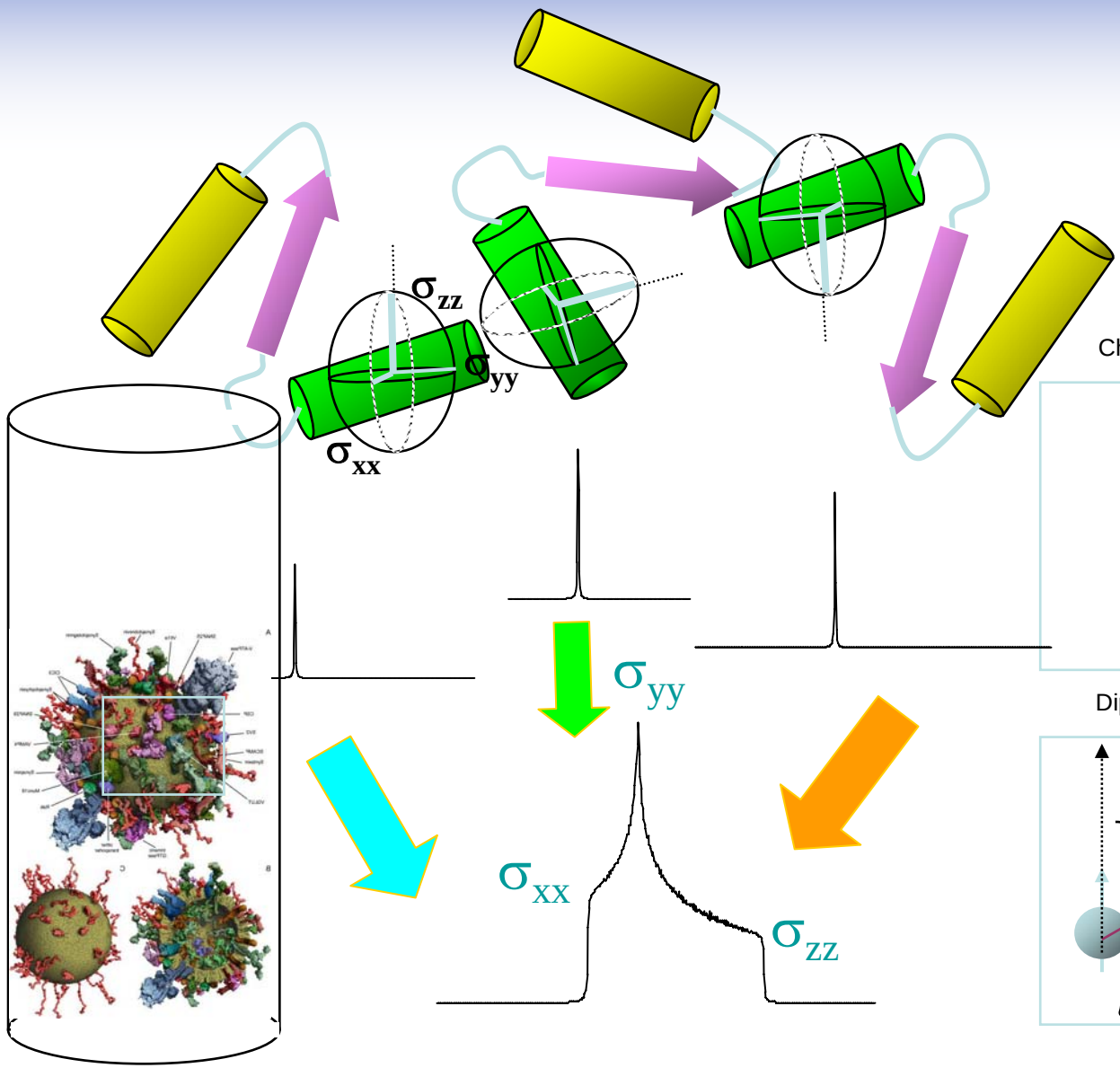


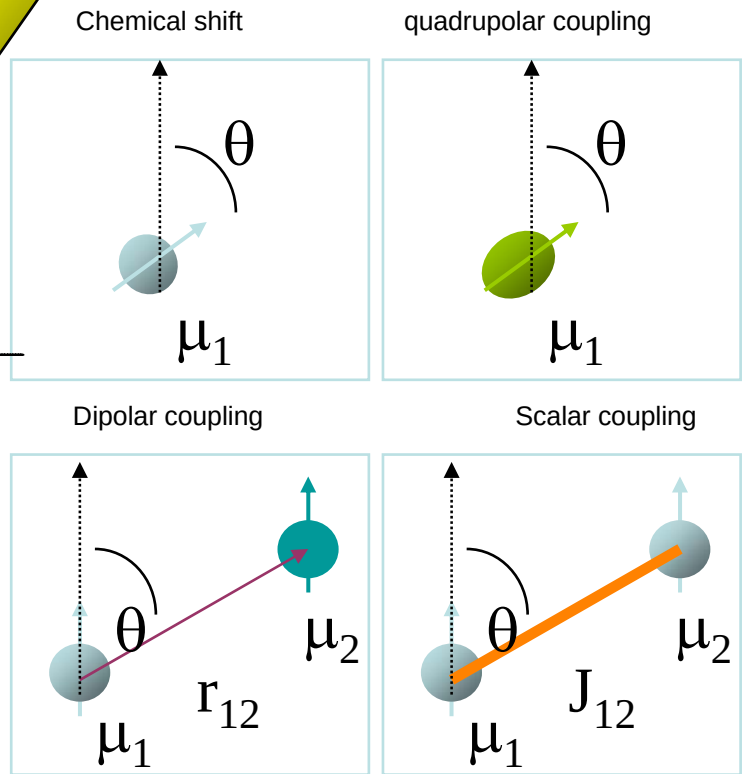


Solution-state NMR



ssNMR: Interactions are stronger and anisotropic



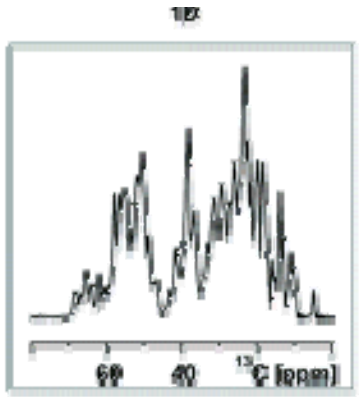


ssNMR Methods: Structural parameters

Radio frequency schemes

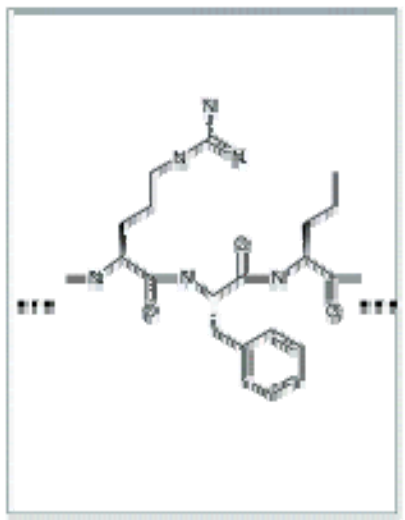
MAS

EXPERIMENTS

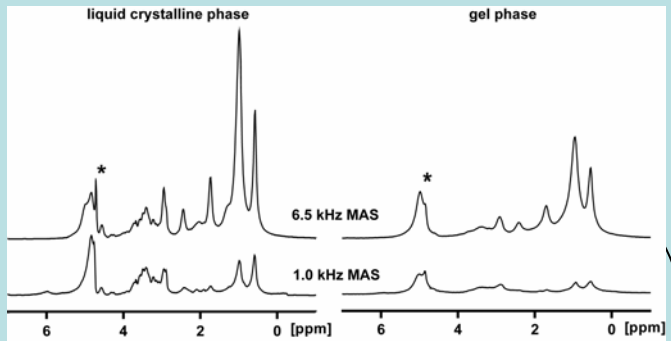


1. Sample preparation

STRUCTURE



^{1}H ssNMR exhibits limited resolution



S. Luca, H. Heise, M. Baldus, ***Acc. Chem. Res.*** 2003, *36*, 858-865.

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hardware

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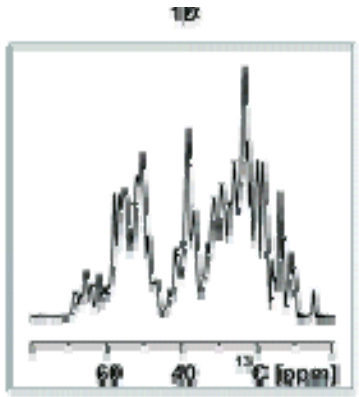
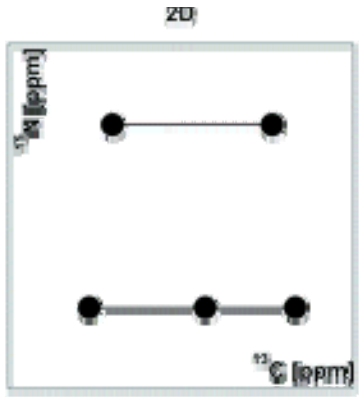
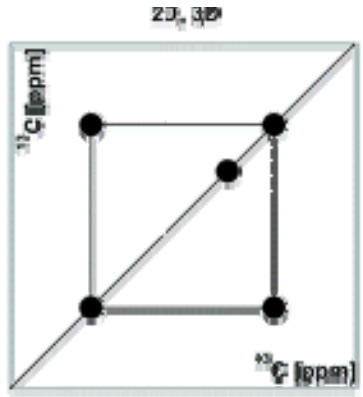


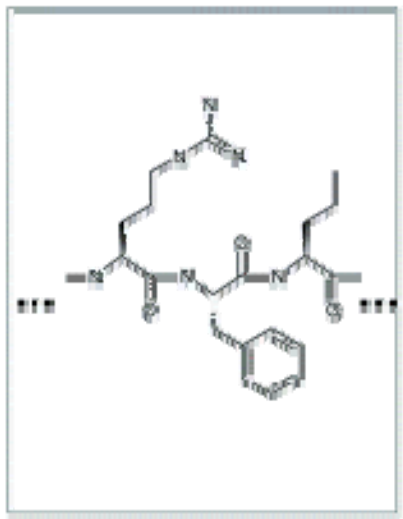


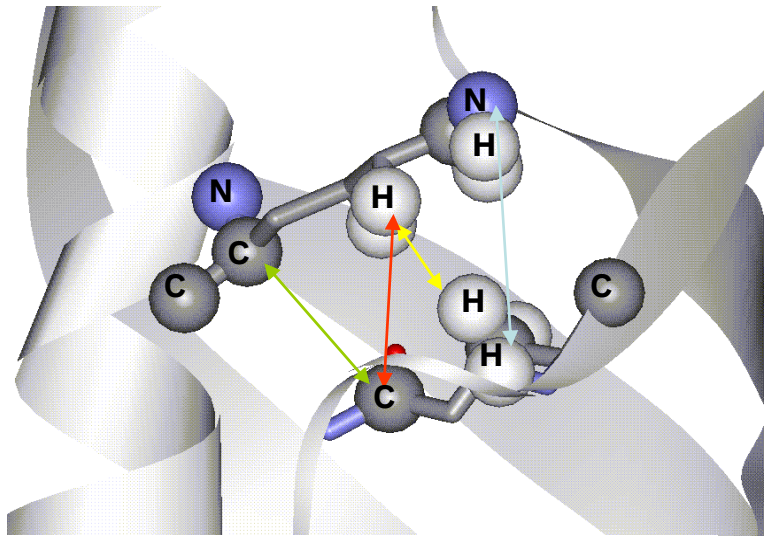


1. Sample preparation

2. Chemical Shift assignments & Structural parameters

STRUCTURE





S. Luca, H. Heise, M. Baldus, ***Acc. Chem. Res.*** 2003, *36*, 858-865.

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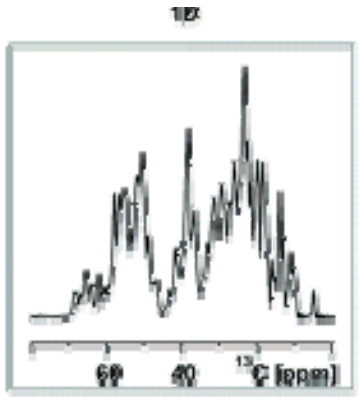

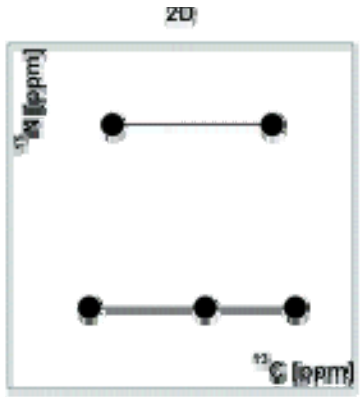

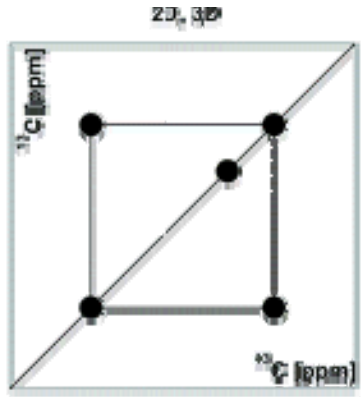

1. Sample preparation

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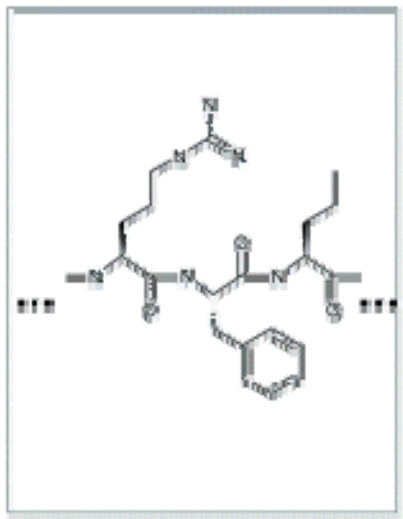
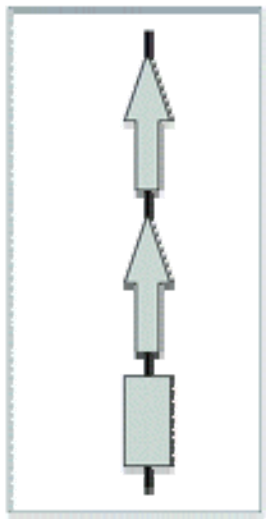
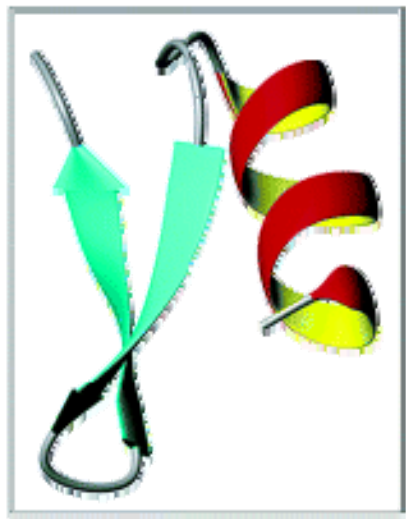




SECONDARY STRUCTURE



TERTIARY STRUCTURE

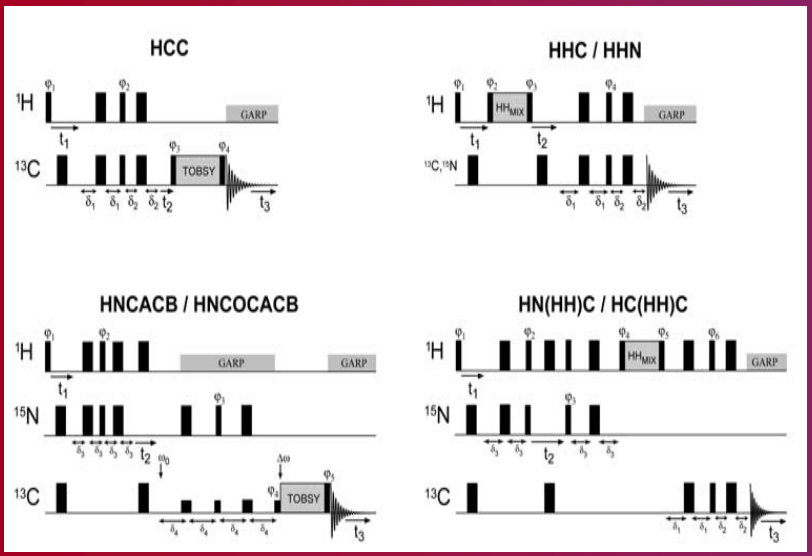
3D structure

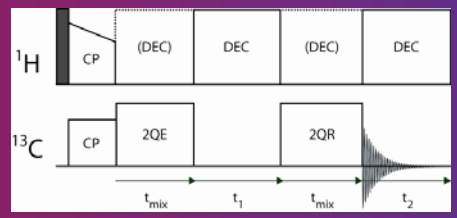
S. Luca, H. Heise, M. Baldus, ***Acc. Chem. Res.*** 2003, *36*, 858-865.

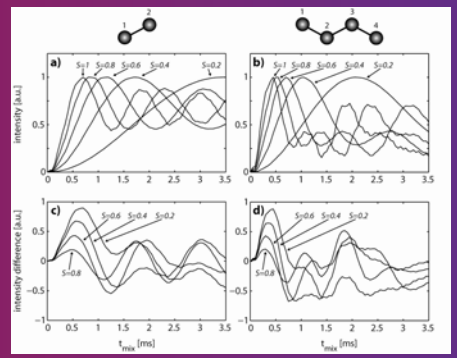
ssNMR: Structure and Dynamics

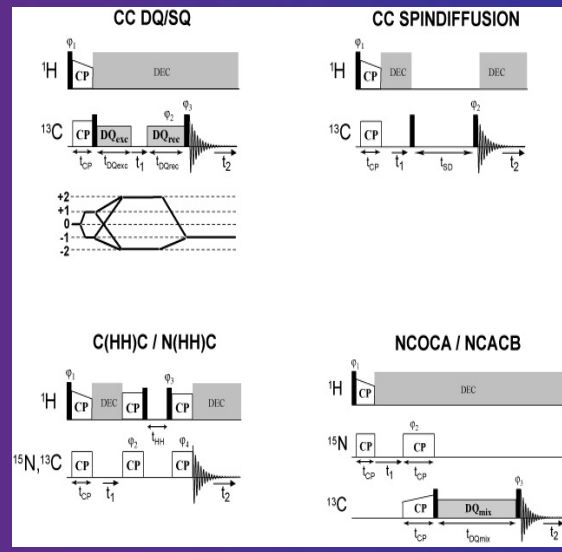
Mobile

Rigid









spin-spin interactions

ns μs ms s

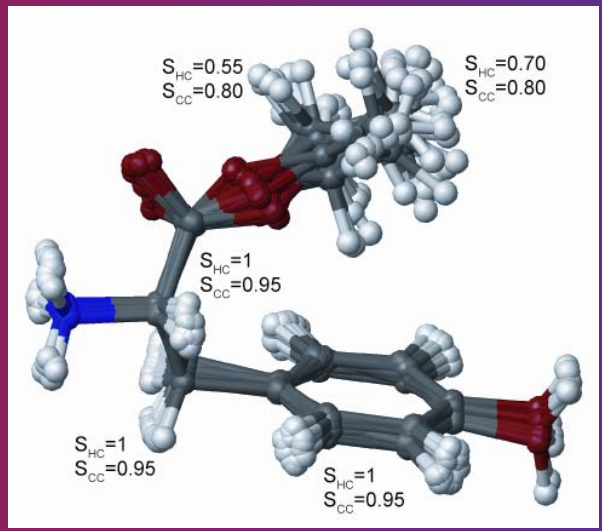
M. Baldus, ***J.Biomol. NMR.*** 2007, in press.

ssNMR: Structure and Dynamics

Mobile

Rigid

...



...

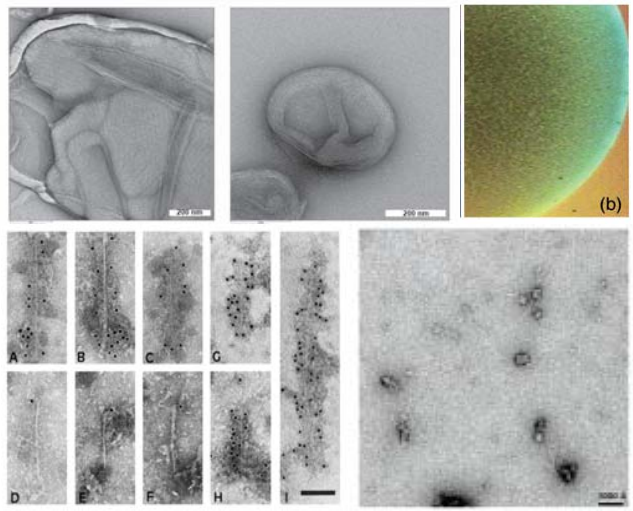
spin-spin interactions

ns μs ms s

Molecular complexes investigated by solid-state NMR spectroscopy

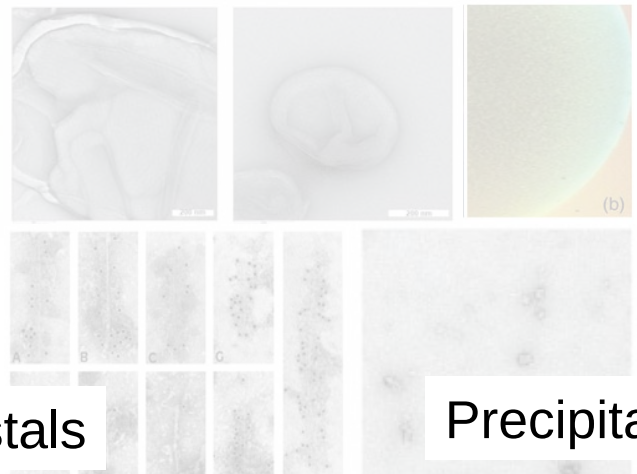
• For a large range of molecular sizes and correlation times

- Proteoliposomes
- Powders
- frozen solutions
- microcrystals
- gels
- precipitates
- aggregates
- etc.

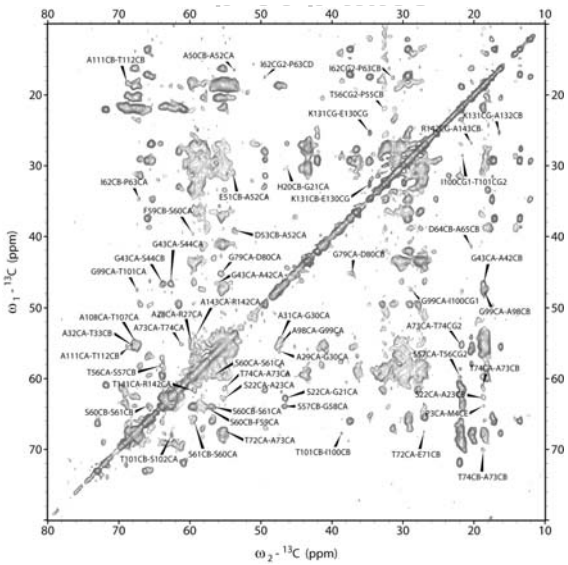


Molecular complexes investigated by solid-state NMR spectroscopy

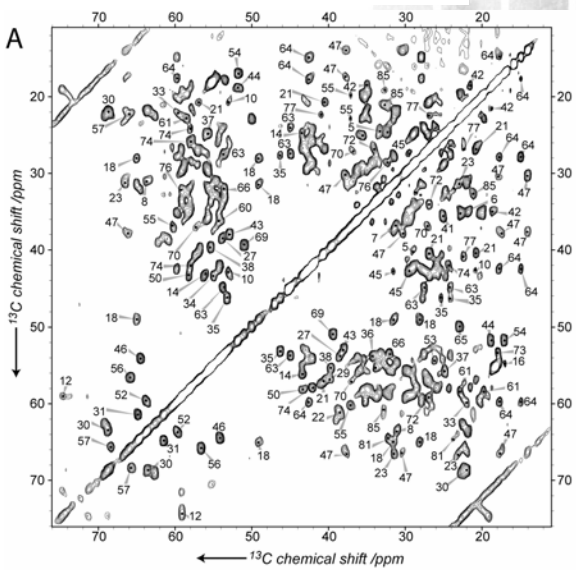
- For a large range of molecular sizes and correlation times
  - Proteoliposomes
  - Powders
  - frozen solutions
  - microcrystals



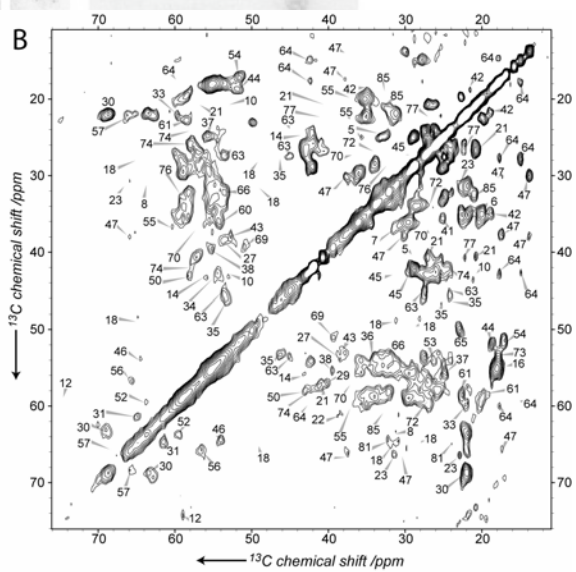
Proteoliposomes



Microcrystals

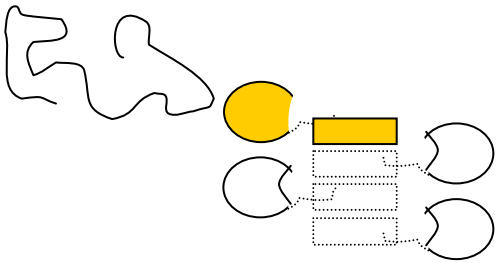


Precipitates

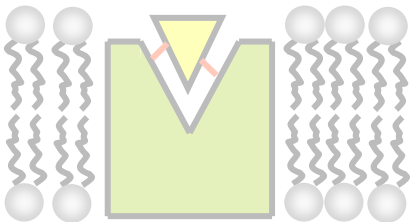


Baldus M, ***Curr. Opin. Struct. Biol.*** 2006, 16, 618-623.

Outline



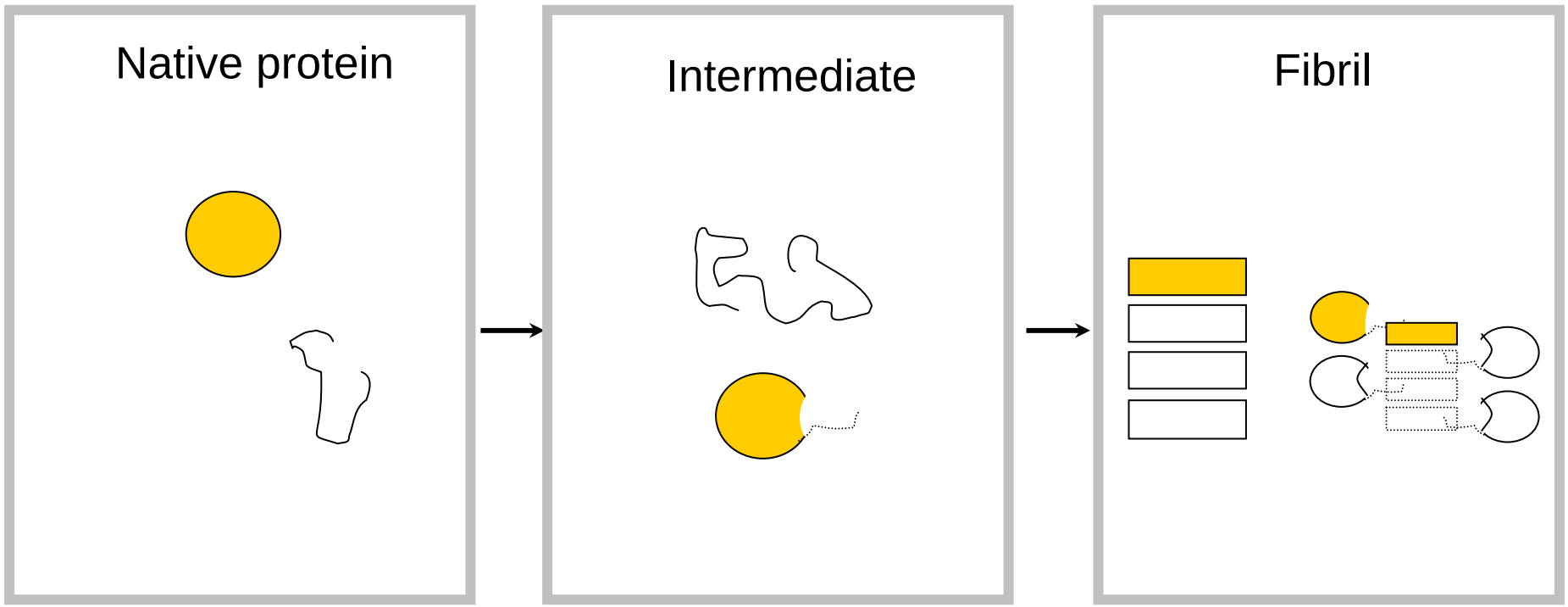
Protein Folding & Aggregation



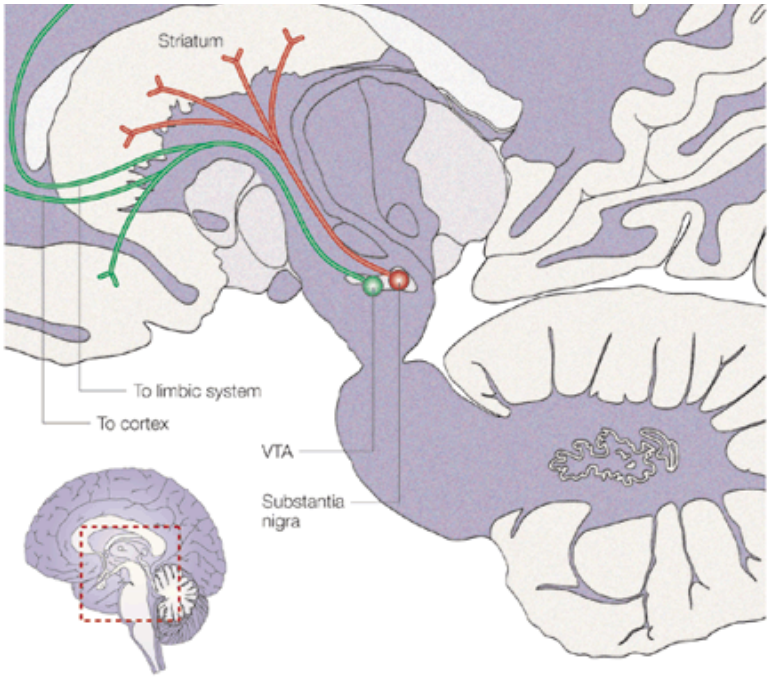
Ligand – Membrane Protein interactions

Membrane Protein complexes

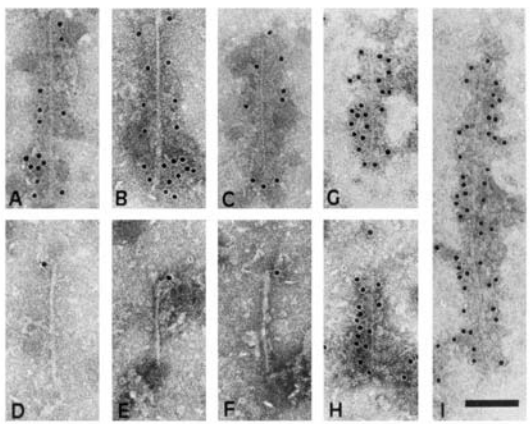
Protein folding & aggregation



α-synuclein (AS)



α-synuclein fibrils are found in brains of patients with Parkinson disease.
(intracellular inclusions in dopaminergic neurons)



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KTKEGVLYVGS
KTKEGVVHGVATVAE
KTK**EQVTNVGG**
AVVTGVTAVAQ
KTVEGAGSIAAATGFV
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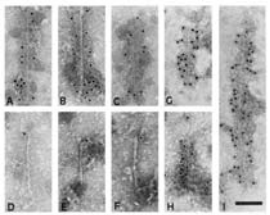
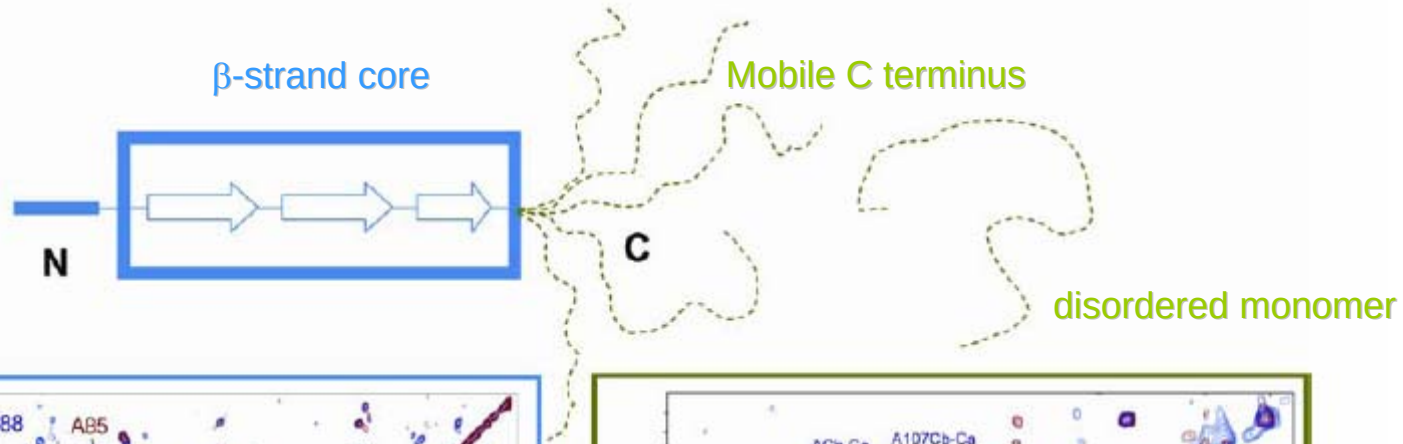
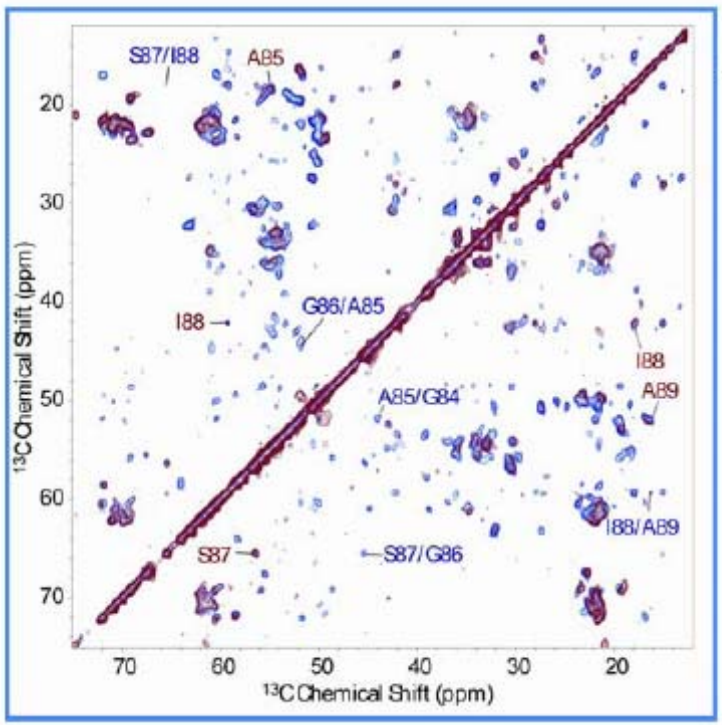
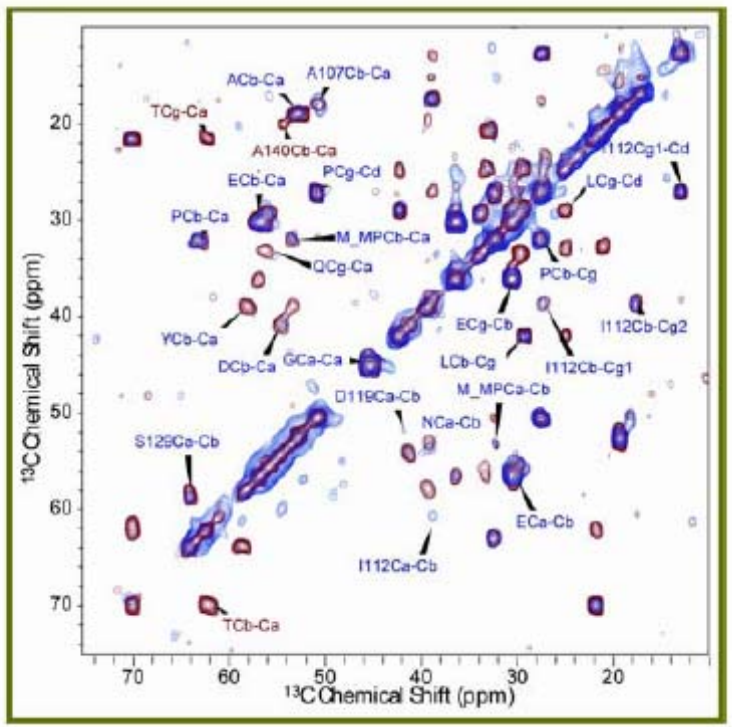
DPDNEAYEMPSEEGYQDYEPEA

ssNMR methods for α synuclein fibrils and beyond

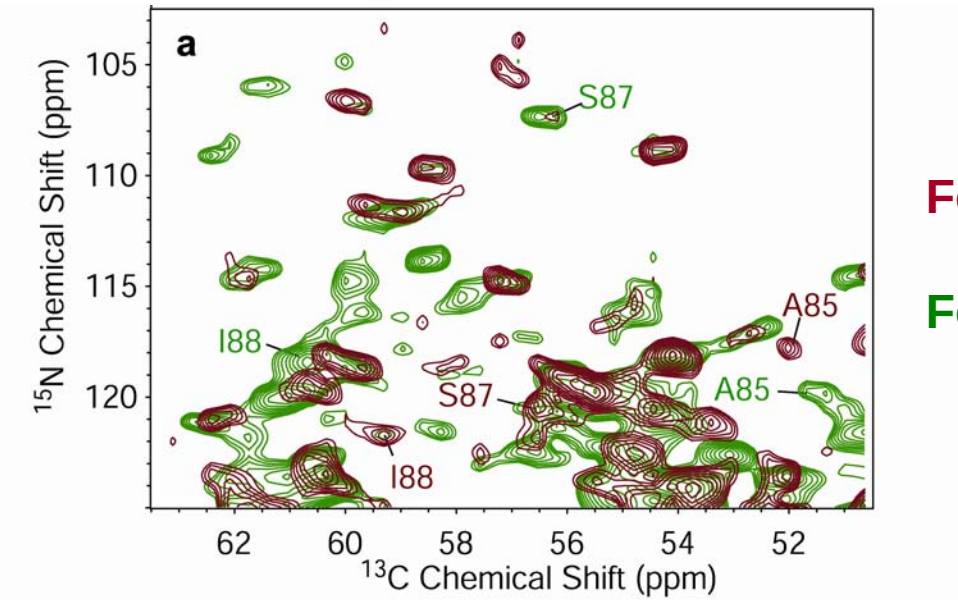








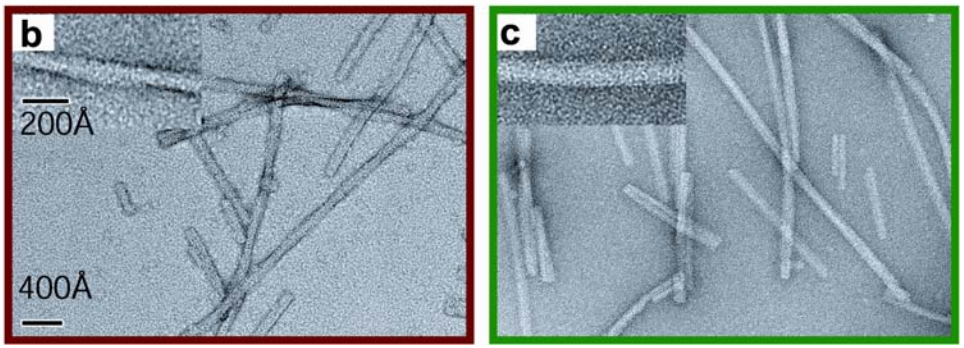
AS: Correlation between molecular structure and fibril morphology



Solid-state NMR

Form A

Form B



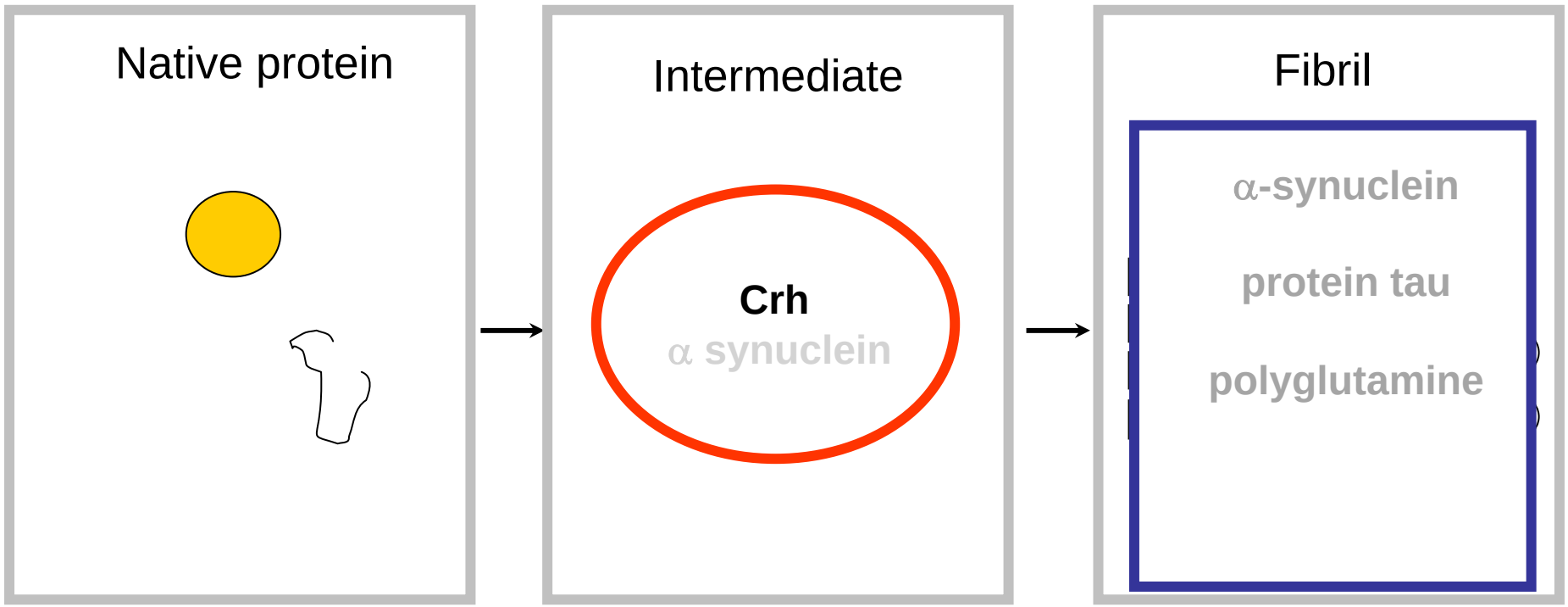
Form A

Form B

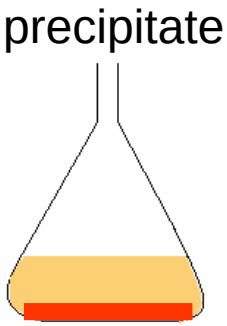
EM

Heise, H.; Hoyer, W.; Becker, S.; Andronesi, O. C.; Riedel, D.; Baldus, M. **PNAS** 2005, *102*, 15871-15876.

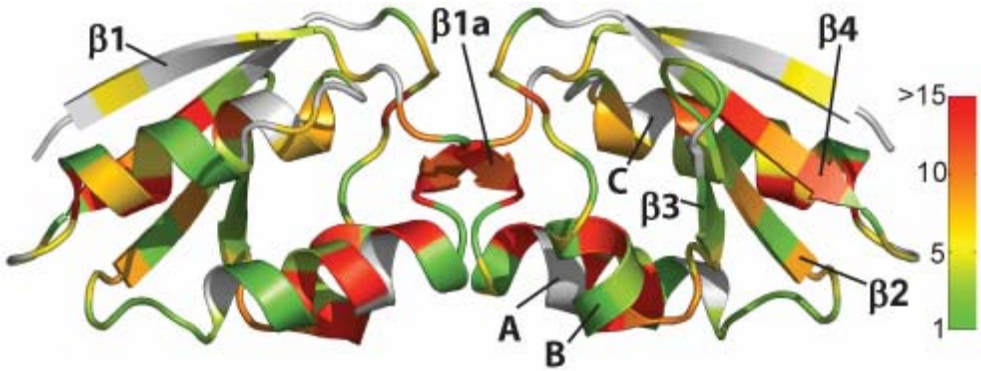
Protein aggregation and fibril formation



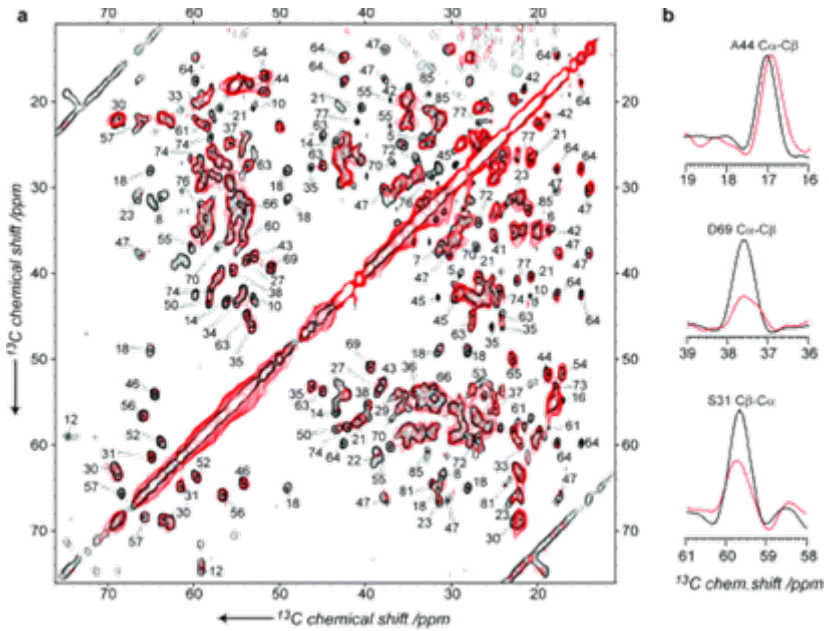
Characterize folding intermediate by 2D ssNMR



Catabolite repression Histidine-containing phosphorcarrier protein (Crh)

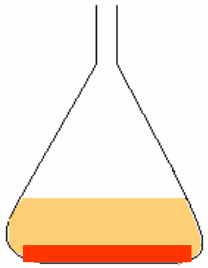


Precipitate vs.
Micro Xtals



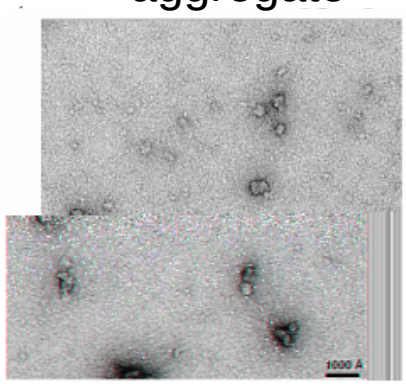
Characterize folding intermediate by 2D ssNMR

precipitate

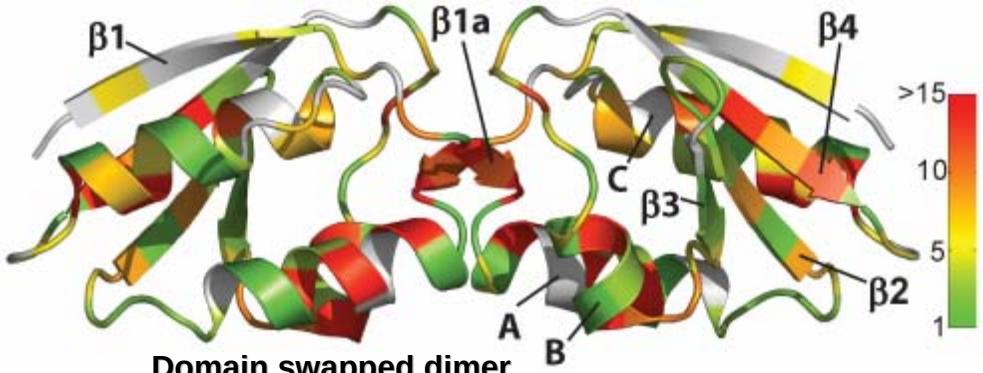


269 K ⇨ 282 K

aggregate

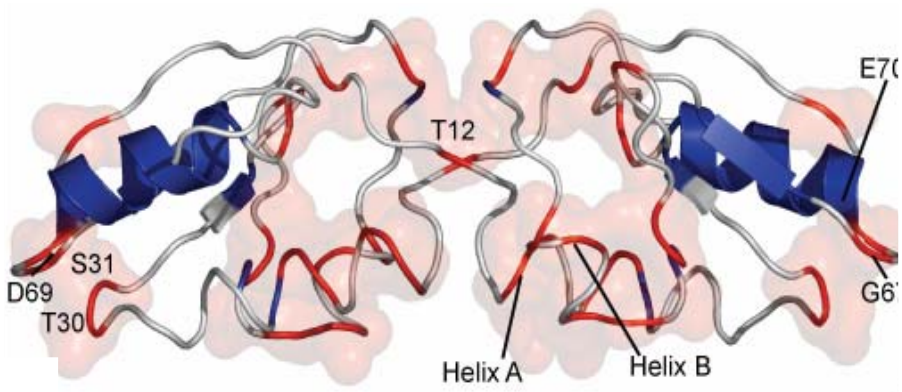


Catabolite repression Histidine-containing phosphorcarrier protein (Crh)

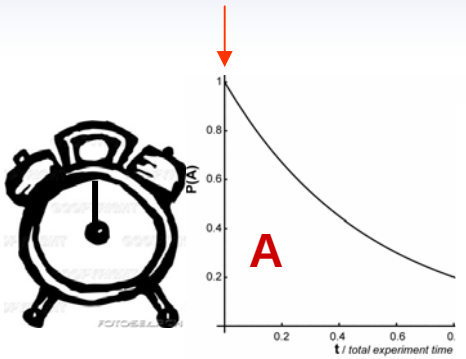


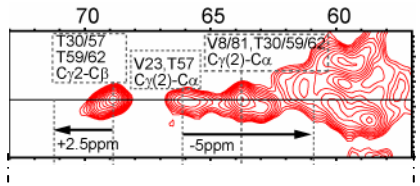
Domain swapped dimer

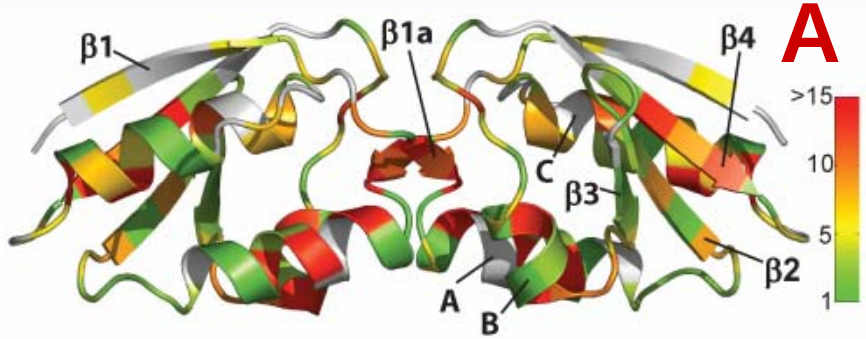
?



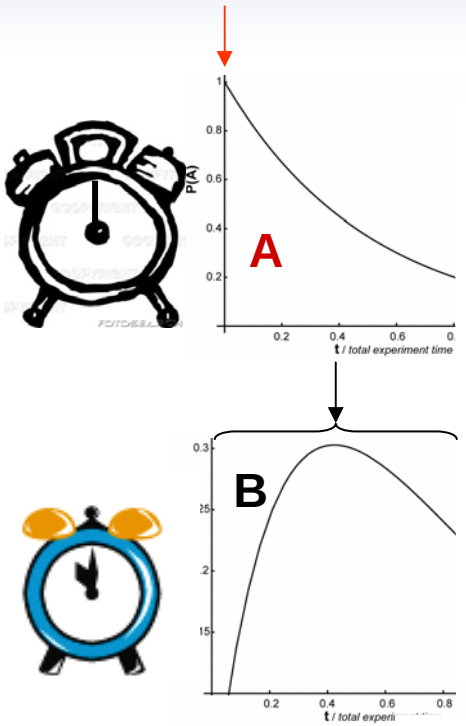
Refolding according to time-resolved 2D ssNMR

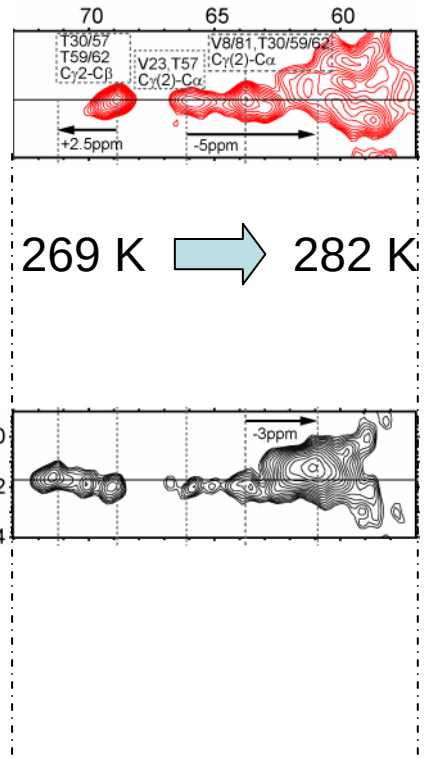


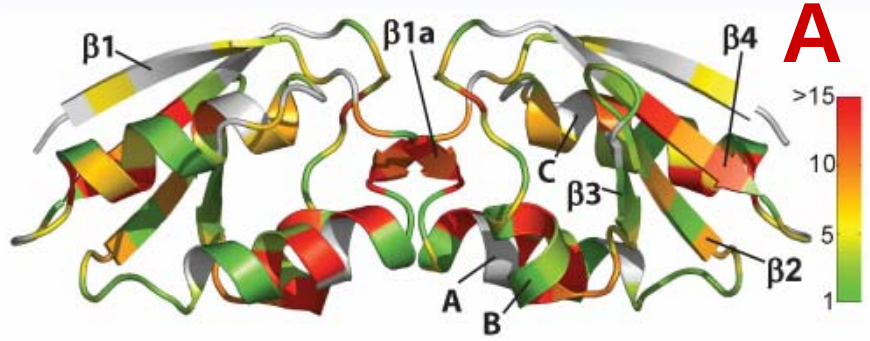




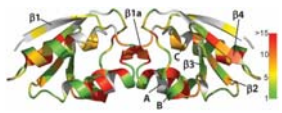
Refolding according to time-resolved 2D ssNMR

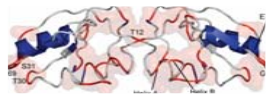




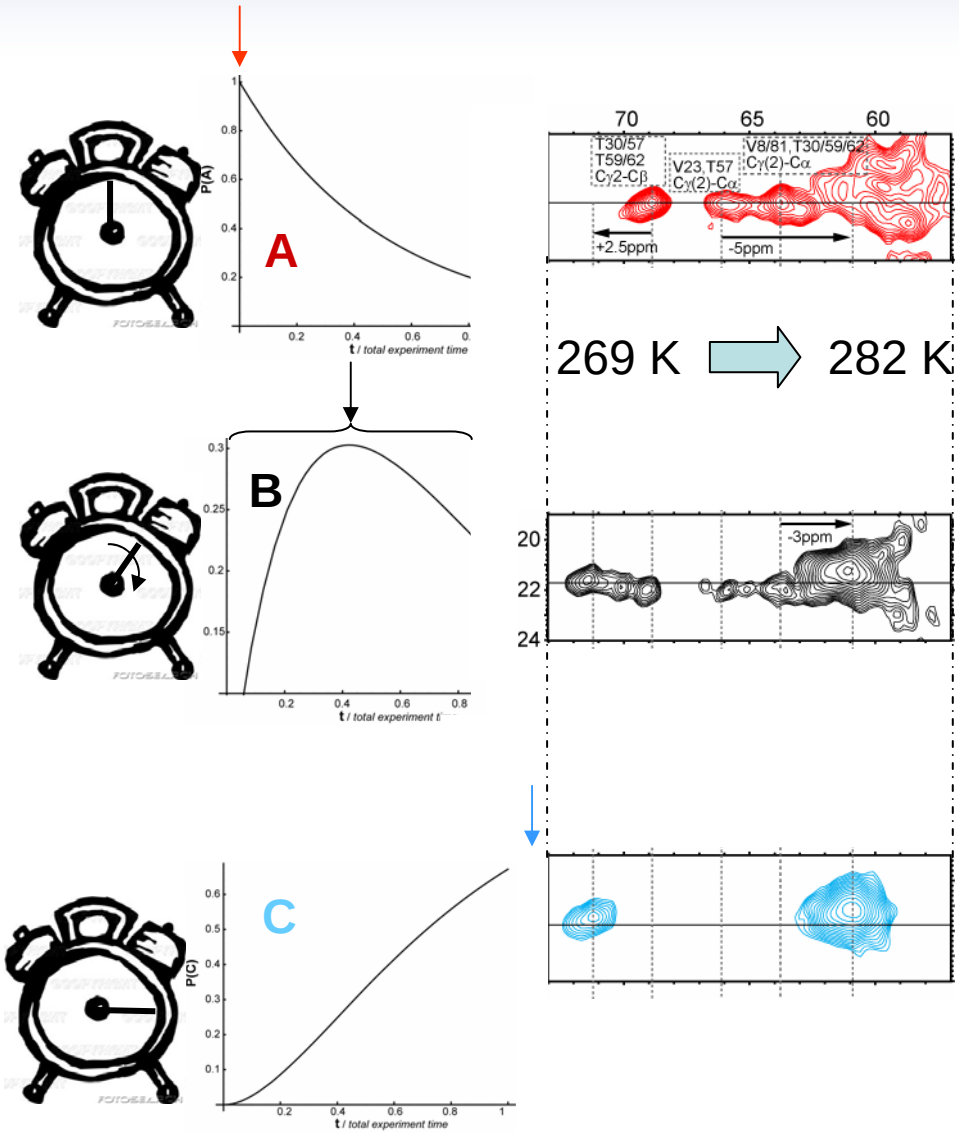


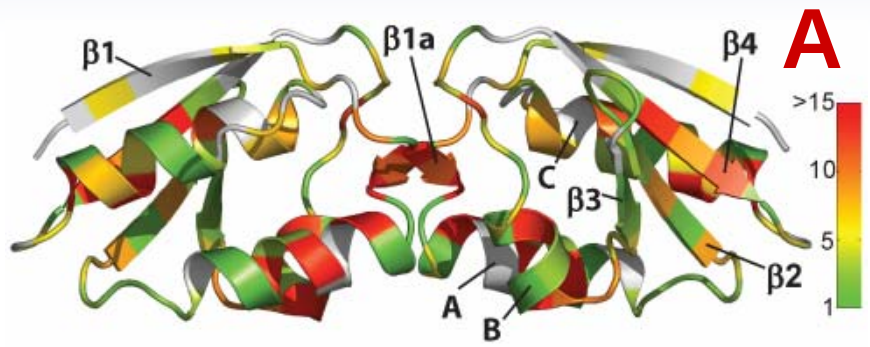
B + “Unfolded“ + intermediate



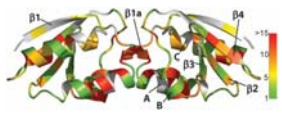


Refolding according to time-resolved 2D ssNMR

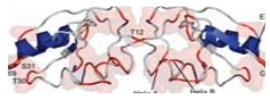




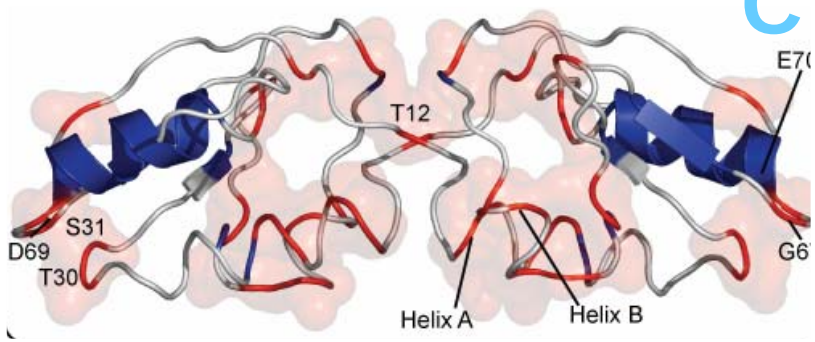
B



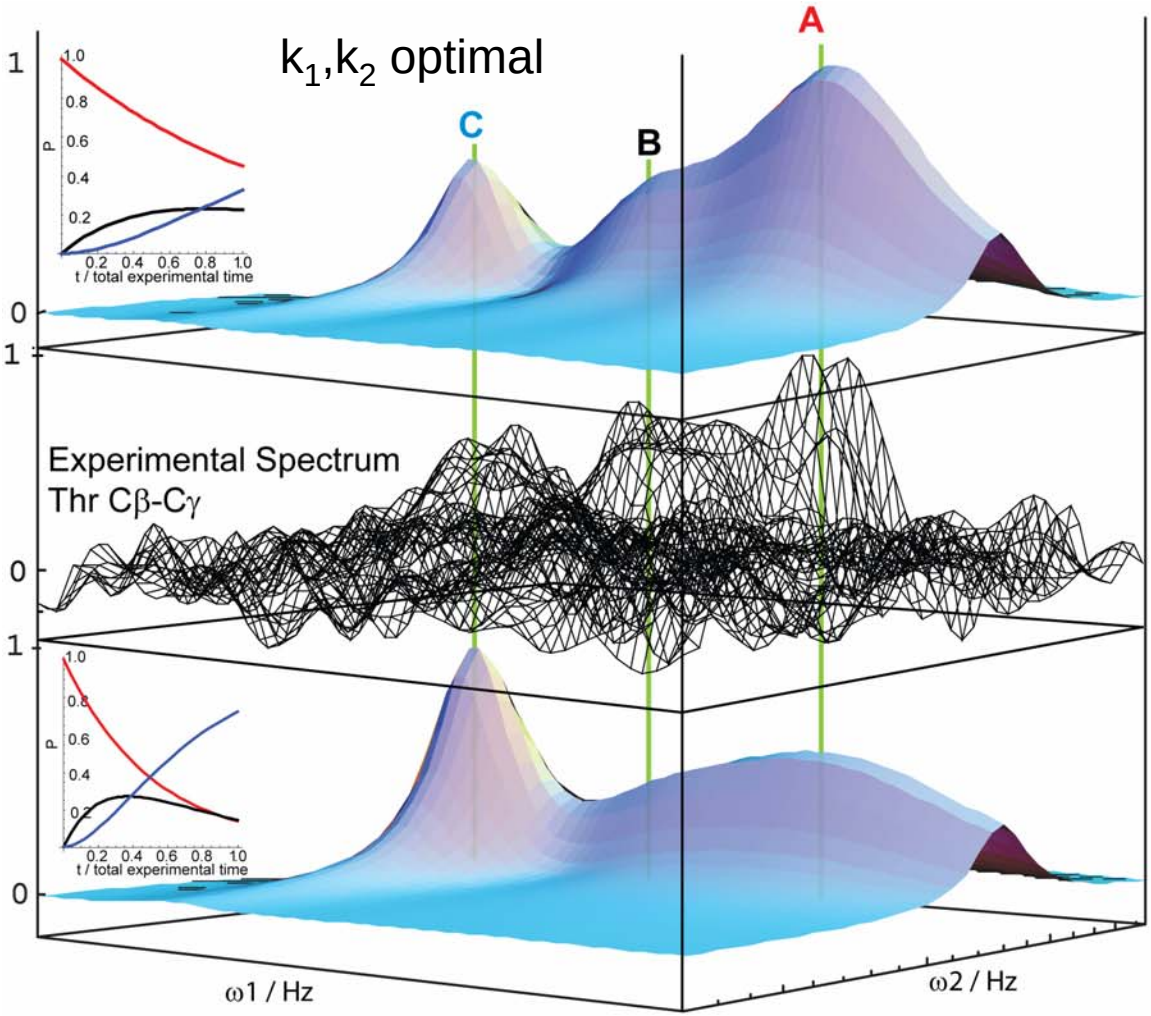
\+ "Unfolded" + intermediate

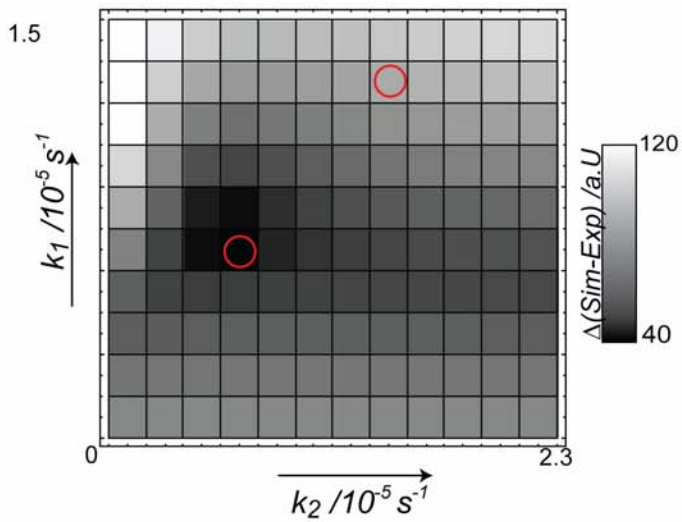


C



2D ssNMR data are sensitive to aggregation kinetics



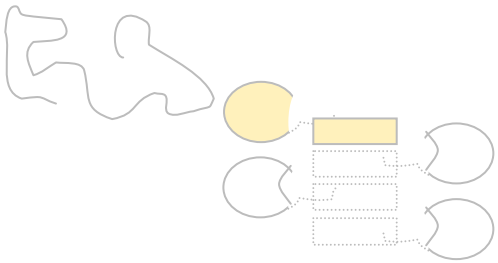


$$L_n(\omega_2) = \frac{\alpha_{n,2}}{\alpha_{n,2}^2 + (\omega_2 - \omega_{n,2})^2} \quad (n=A,B,C)$$

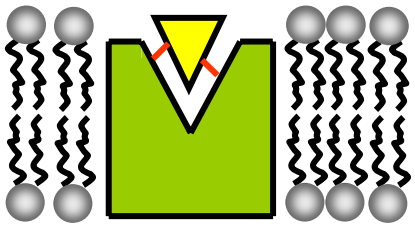
$$\begin{aligned} S(\omega_1,\omega_2) = & \int_0^\infty a_A \cdot e^{-k_1 t_1} \cdot e^{(i\omega_{A,1} - \alpha_{A,1})t_1} \cdot L_A \\ & + a_B \cdot \left(1 - e^{-k_1 t_1}\right) e^{-k_2 t_1} \cdot e^{(i\omega_{B,1} - \alpha_{B,1})t_1} \cdot L_B \\ & + a_C \cdot \left(1 - e^{-k_1 t_1}\right)\left(1 - e^{-k_2 t_1}\right) \cdot e^{(i\omega_{C,1} - \alpha_{C,1})t_1} \cdot L_C \\ & \cdot e^{-i\omega_1 t_1} dt_1 \end{aligned}$$

Etzkorn, M., Böckmann, A., Penin, F., Riedel, D., and Baldus, M. (2007) **J. Amer.Chem. Soc.,** 129, 169-175.

Outline



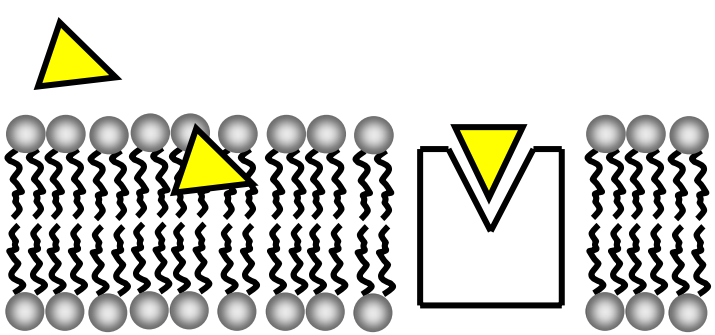
Protein Folding & Aggregation

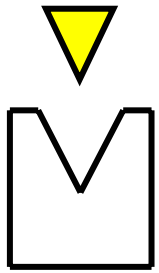


Ligand – Membrane Protein interactions

Membrane Protein complexes

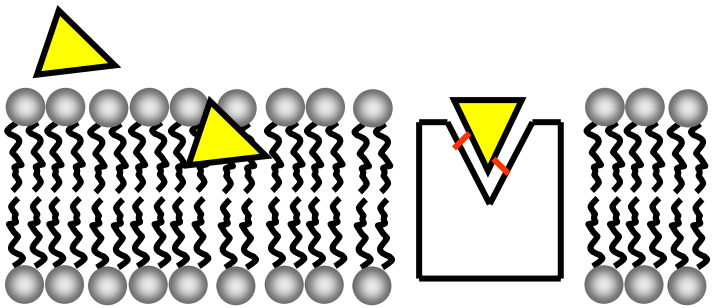
Ligand – membrane protein interactions by ssNMR

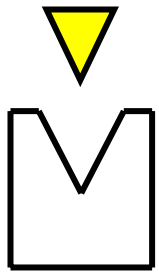




Neurotensin

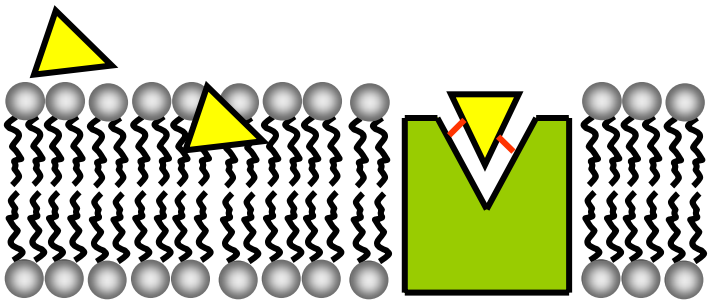
Neurotension receptor (GPCR)

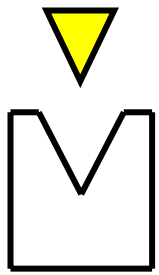




Phospholamban

Ca-ATPase





Kaliotoxin

KcsA-Kv1.3

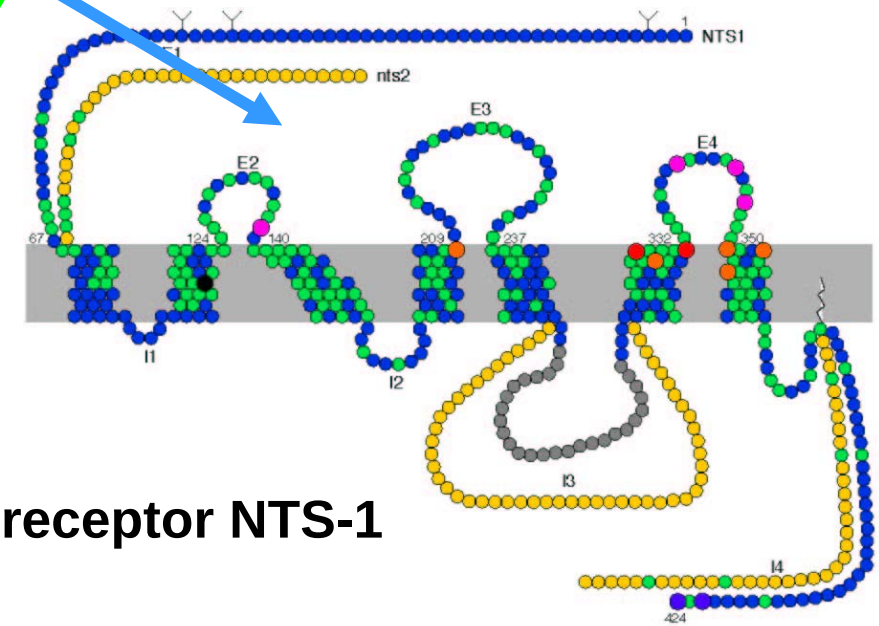
The Neurotensin – NTS-1 System

Neurotensin (NT)

ELYENKP**R**[8]**R**[9]**P**[10]**Y**[11]**I**[12]**L**[13]

Psychosis, schizophrenia
Parkinsons disease

Sub-nanomolar binding affinity of NT and NT(8-13) to NTS-1



Rat Neurotensin receptor NTS-1

Vincent, J. P.; Mazella, J.; Kitabgi, P. *Trends Pharmacol. Sci.* **1999**, *20*, 302.

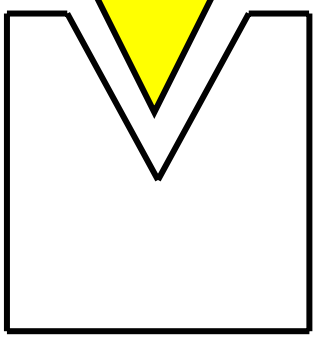
Neurotensin bound to a G-protein coupled receptor

$^{13}C/^{15}N$

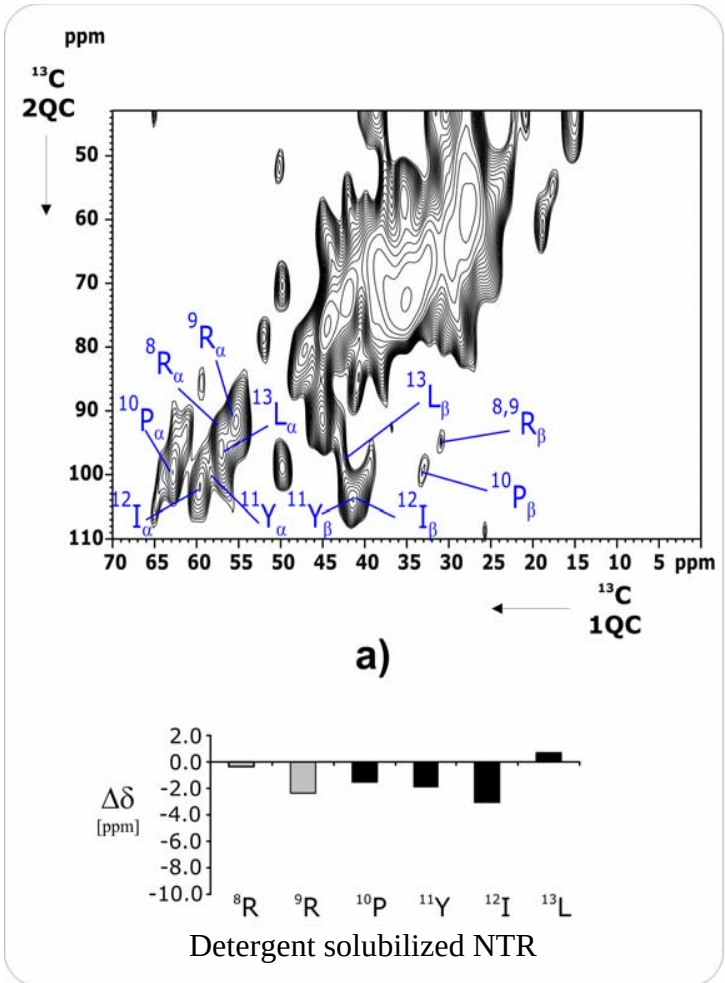
neurotensin

ELYENKP$R^{8}R^{9}P^{10}Y^{11}I^{12}L^{13}$

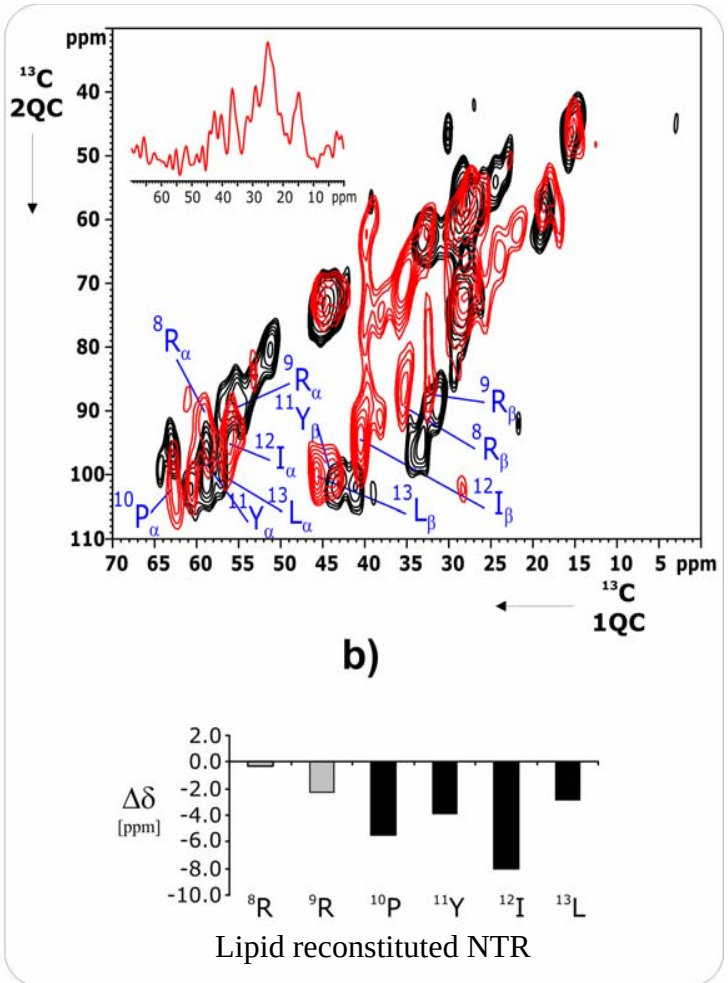
$^{13}C/^{15}N$



U[^{13}C,^{15}N] NT(8-13)
10 μg – 22 μg



Detergent solubilized NTR



Lipid reconstituted NTR

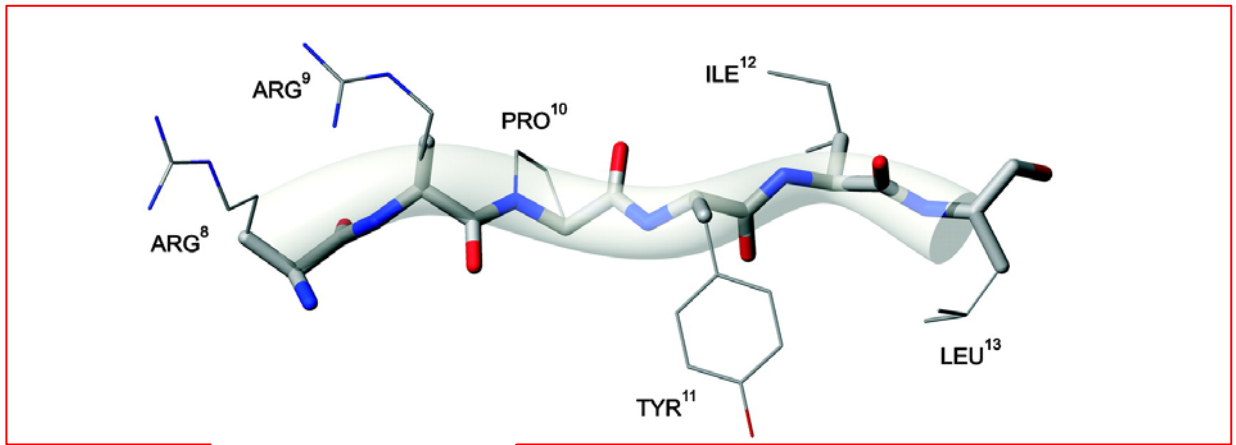
Neurotensin bound to a G-protein coupled receptor

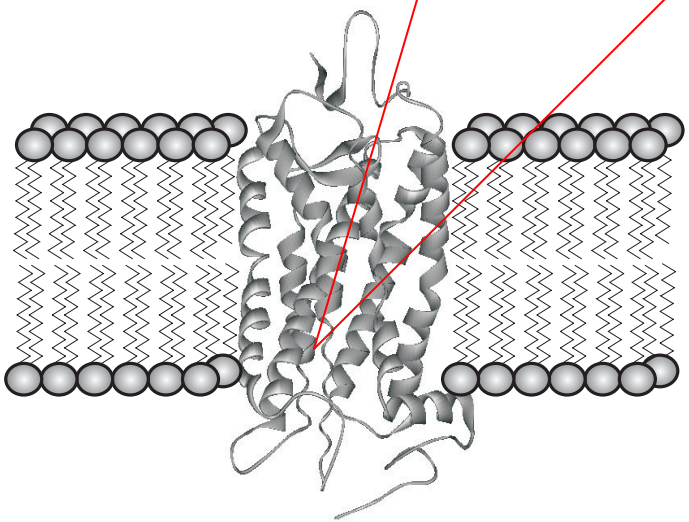
$^{13}C/^{15}N$

neurotensin

ELYENKP**R**^{8}**R**^{9}**P**^{10}**Y**^{11}**I**^{12}**L**^{13}

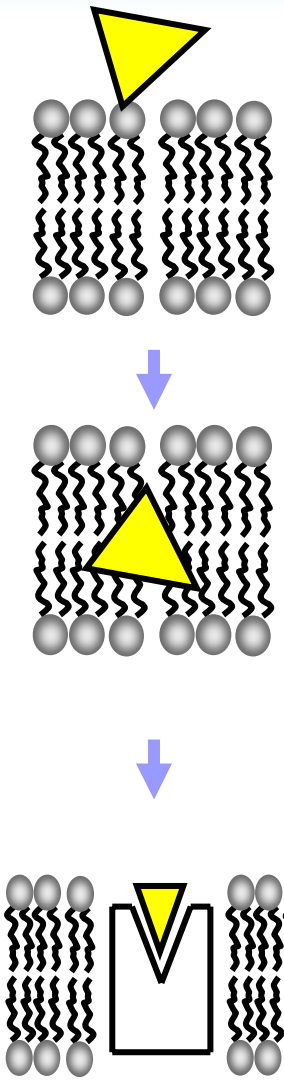
$^{13}C/^{15}N$

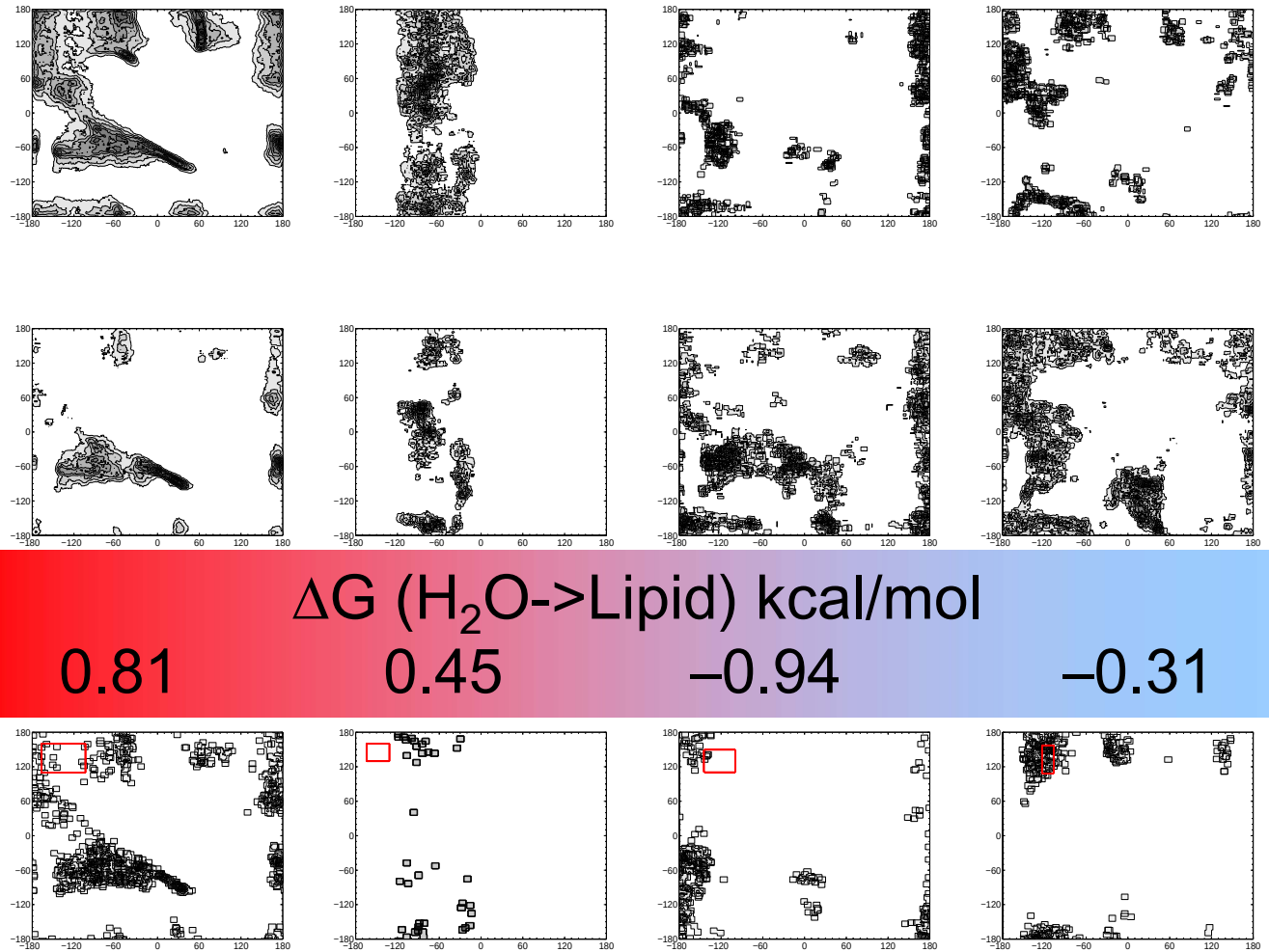




Luca, S., White, J.F., Sohal, A.K., Filipov, D.V., van Boom, J.H., Grisshammer, R., and Baldus, M., 2003, **PNAS**, *100,* 10706

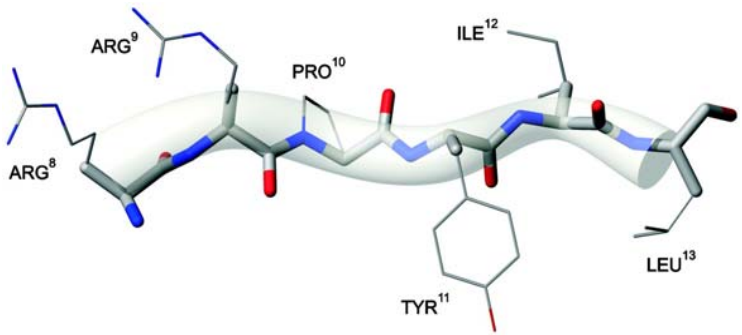
Conformational disorder of Neurotensin



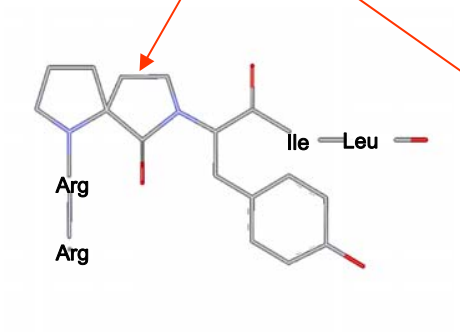


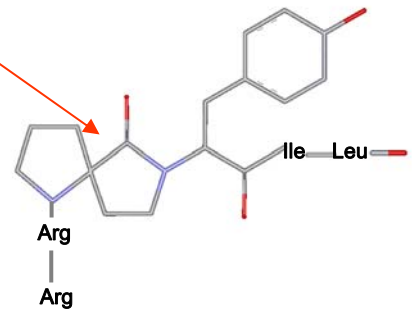
Heise, H., Luca, S., de Groot, B. L., Grubmueller, H. & Baldus, M. (2005) ***Biophys. J.*** 89**,** 2113-2120.

Comparison: Binding affinities of NT and rigidized NT



4,4-spirolactam





ψ (Pro)	146 ± 15°	~ 120 °	~ -120 °
K_D [nM]	0.27	12	16000

→ A ssNMR-structure / affinity relationship !

Bittermann, H.; Einsiedel, J.; Hubner, J.; Gmeiner, P. *J. Med. Chem.* **2004**, *47*, 5587-5590.

Ligand – membrane protein interactions by ssNMR

Neurotensin

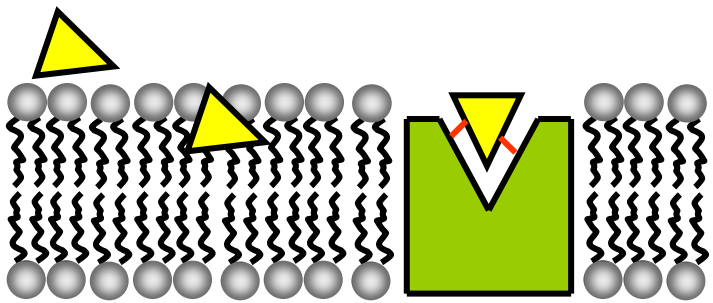
Neurotension receptor (GPCR)

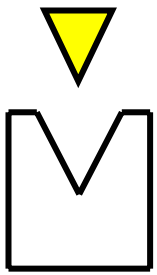
Luca, S. et al., 2003, **PNAS**,100, 10706.

Heise et al, 2005 **Biophys. J.** 89, 2113.

Phospholamban

Ca-ATPase

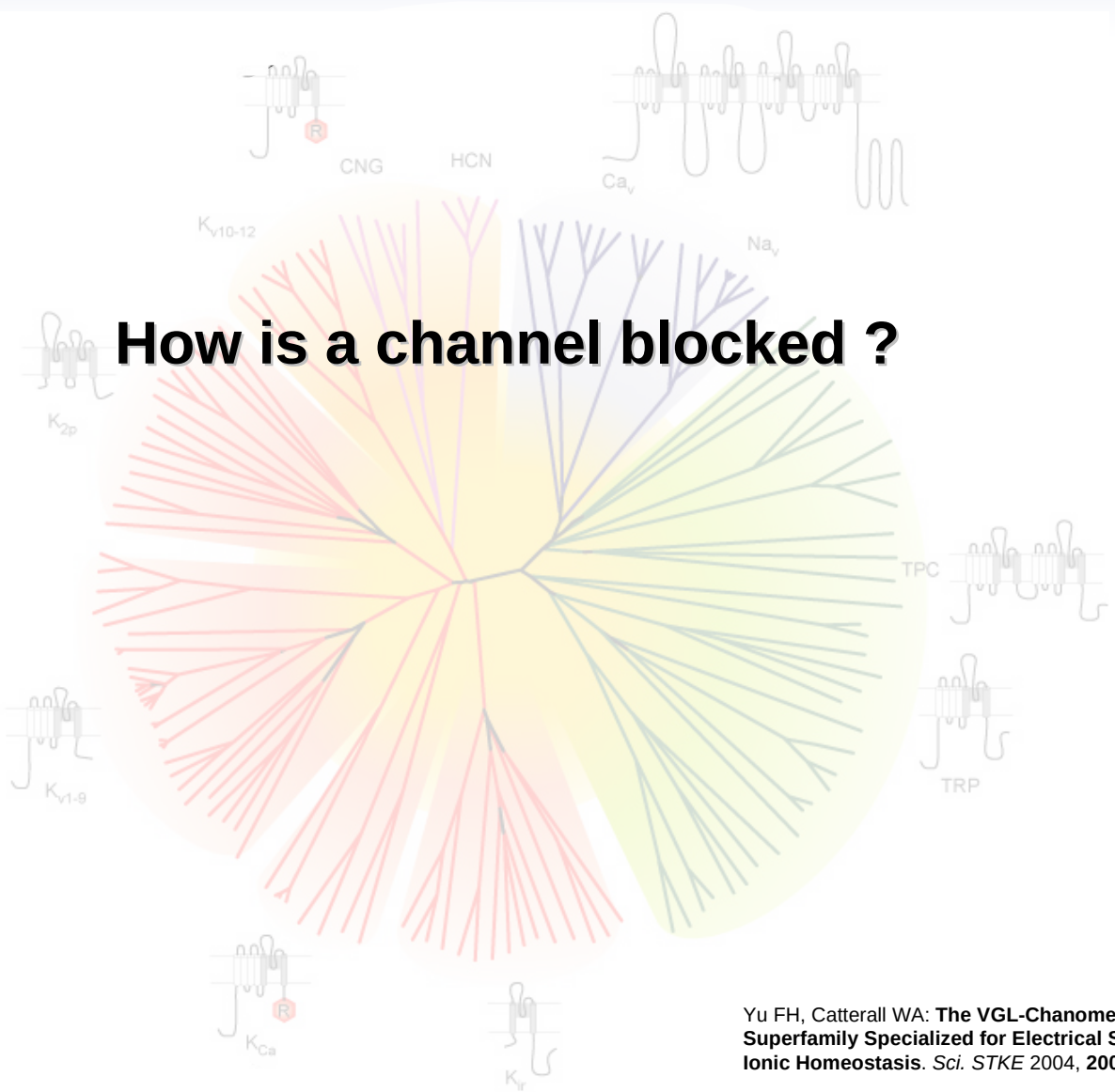




Kaliotoxin

KcsA-Kv1.3

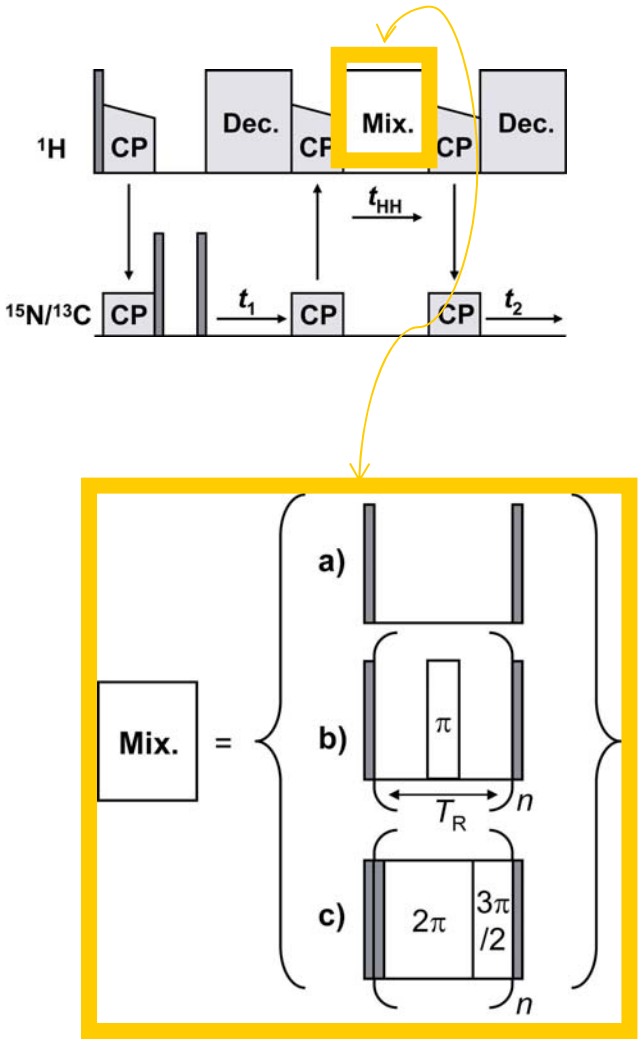
Voltage-gated ion channels

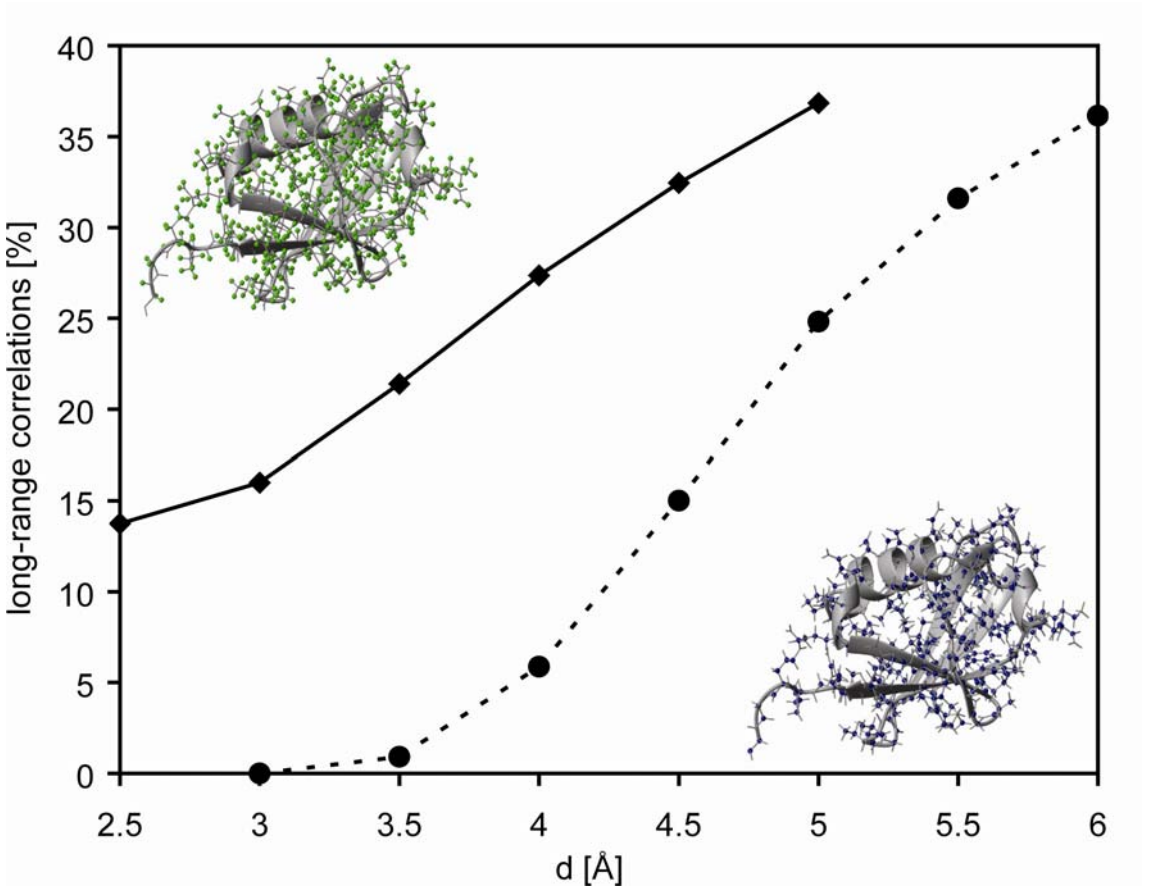


How is a channel blocked ?

Yu FH, Catterall WA: **The VGL-Chanome: A Protein Superfamily Specialized for Electrical Signaling and Ionic Homeostasis**. *Sci. STKE* 2004, **2004**:re15-

Relative fraction of long-range correlations

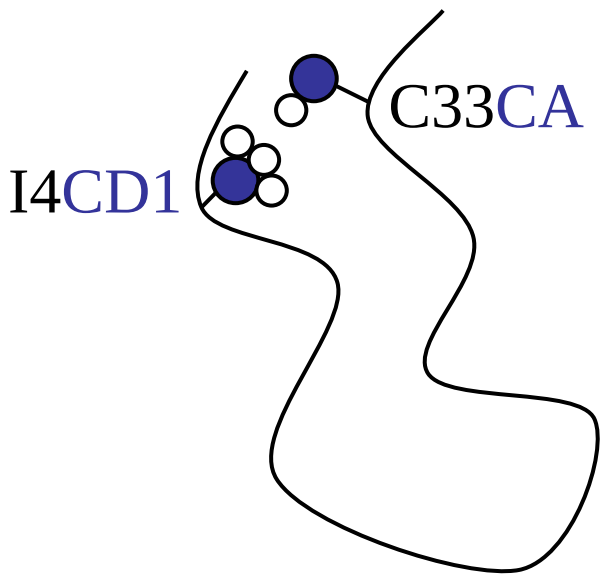


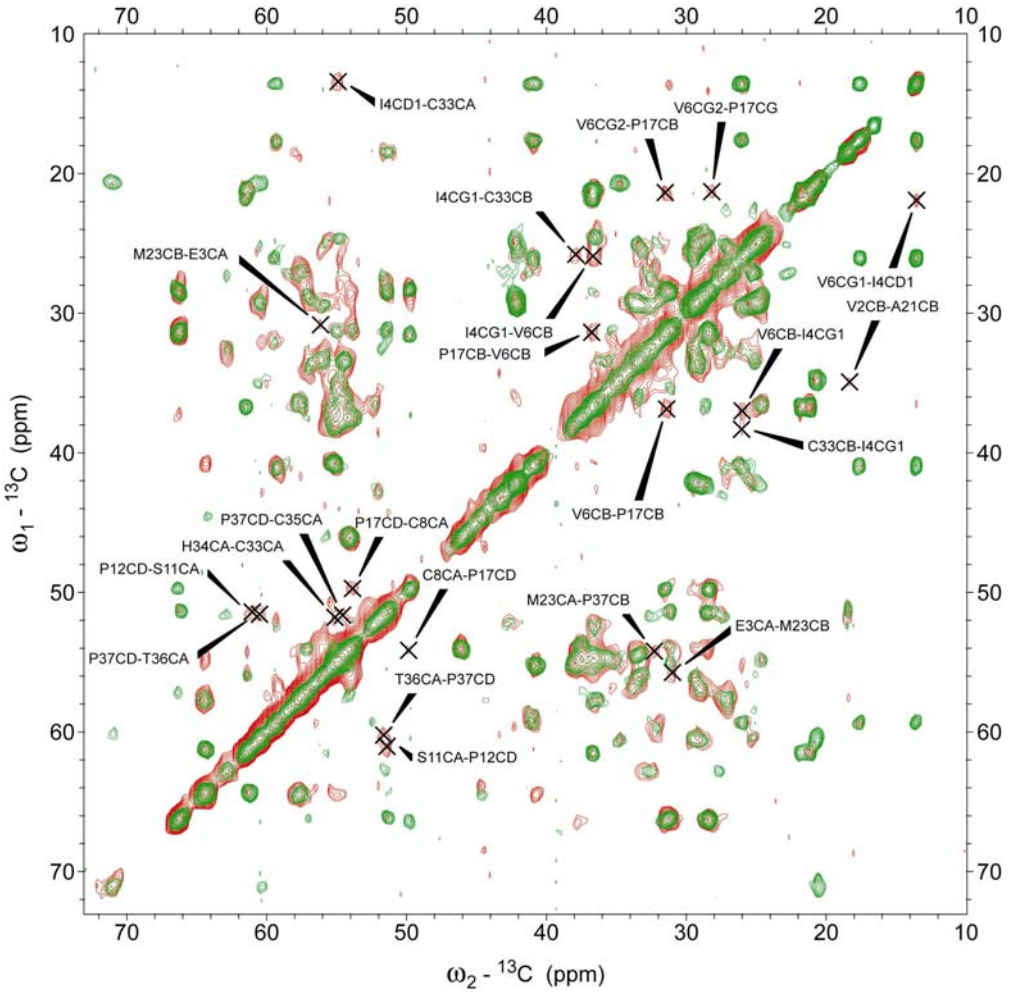


A. Lange, S. Luca, M. Baldus, *J.Am.Chem.Soc.* 2002, *124*, 9704-9705.
Lange, K. Seidel, L. Verdier, S. Luca, M. Baldus, *J.Am.Chem.Soc.* 2003, *125*, 12640-12648.
K. Seidel, M. Etzkorn, C. Griesinger, A. Sebald, M. Baldus, *J.Phys.Chem. A.,* 2005, 109, 2436-2442.

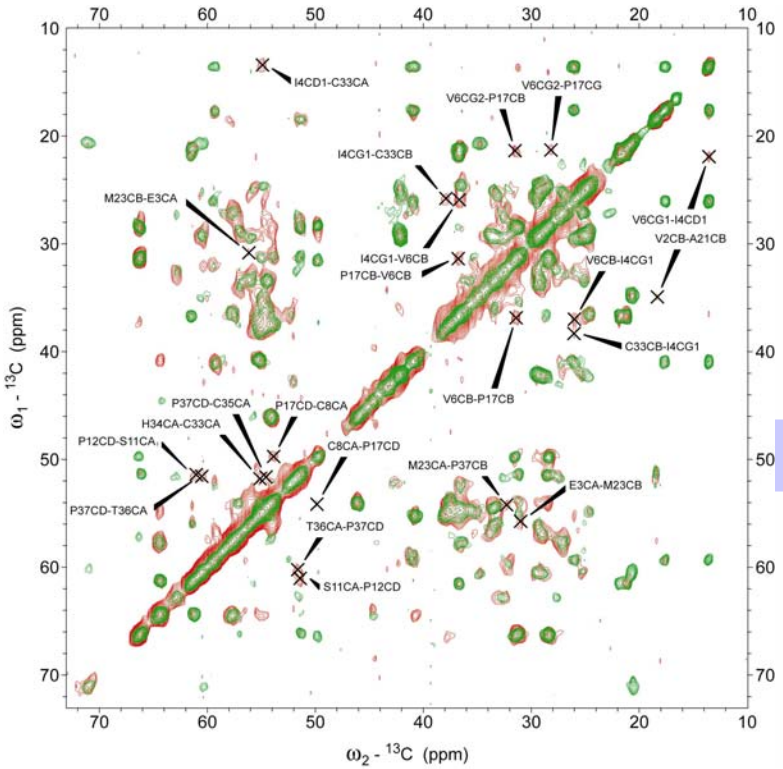
Obtaining the 3D ssNMR structure of KTX

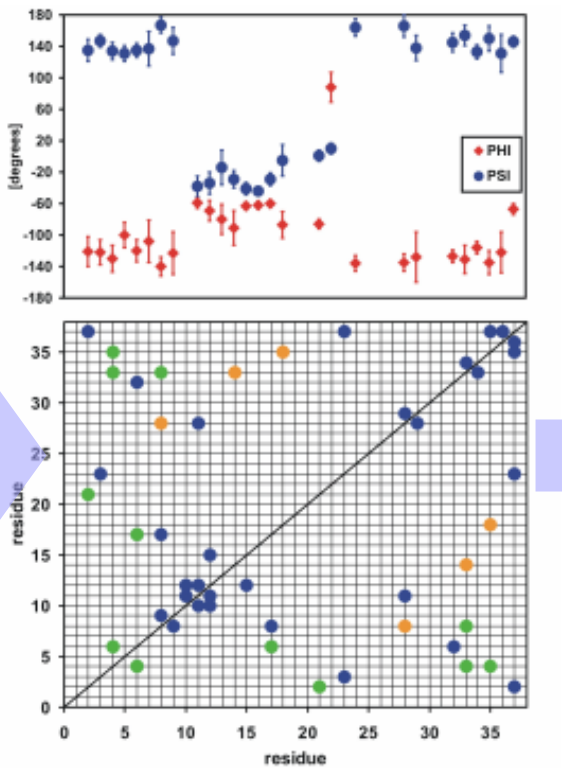
CC vs. CHHC 2D

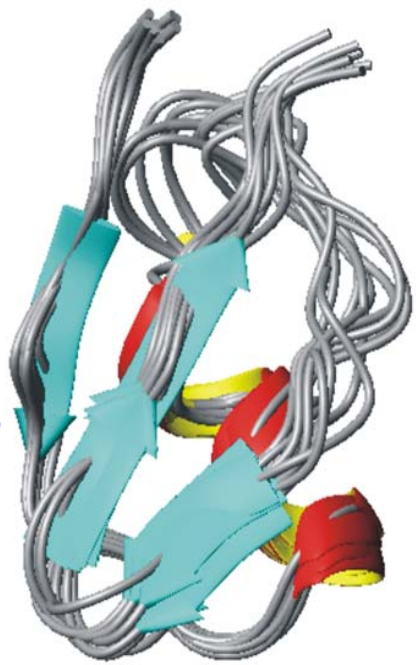




Obtaining the 3D ssNMR structure of KTX







PDB: 1XSW

CC vs CHHC on KTX

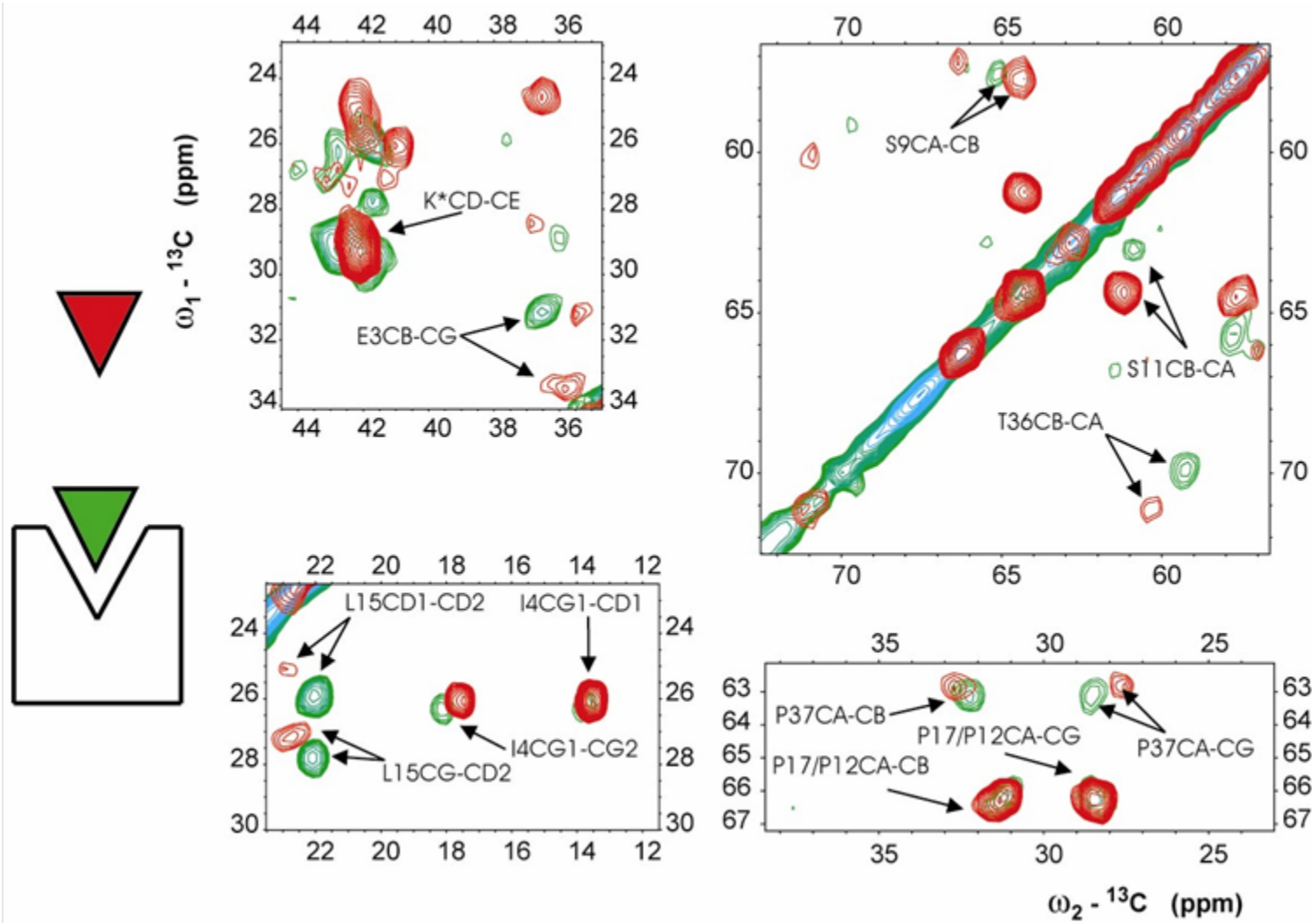
Backbone RMSD: 0.8 Å

Backbone RMSD (residues 4-38) between solid KTX and KTX in solution:1.9 Å

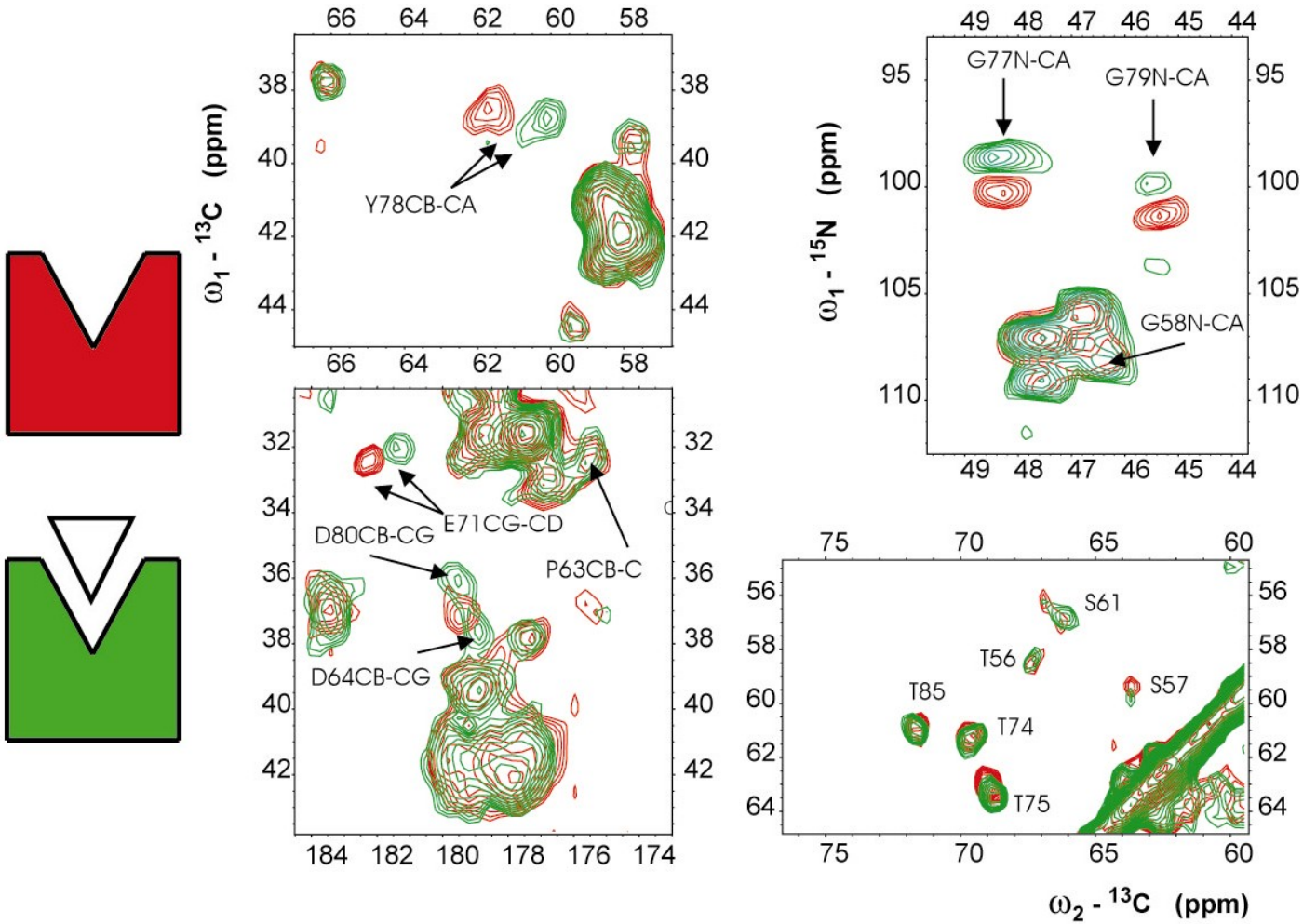
Lange A, Becker S, Seidel K, Pongs O, Baldus M. 2005. **Angew. Chem.-Int. Edit.** 44:2089.

M. Gairi, R. Romi, I. Fernandez, H. Rochat, M. F. Martin-Eauclaire, J. Van Rietschoten, M. Pons, E. Giralt, 1997. **J. Pept. Sci.,** 3: 314.

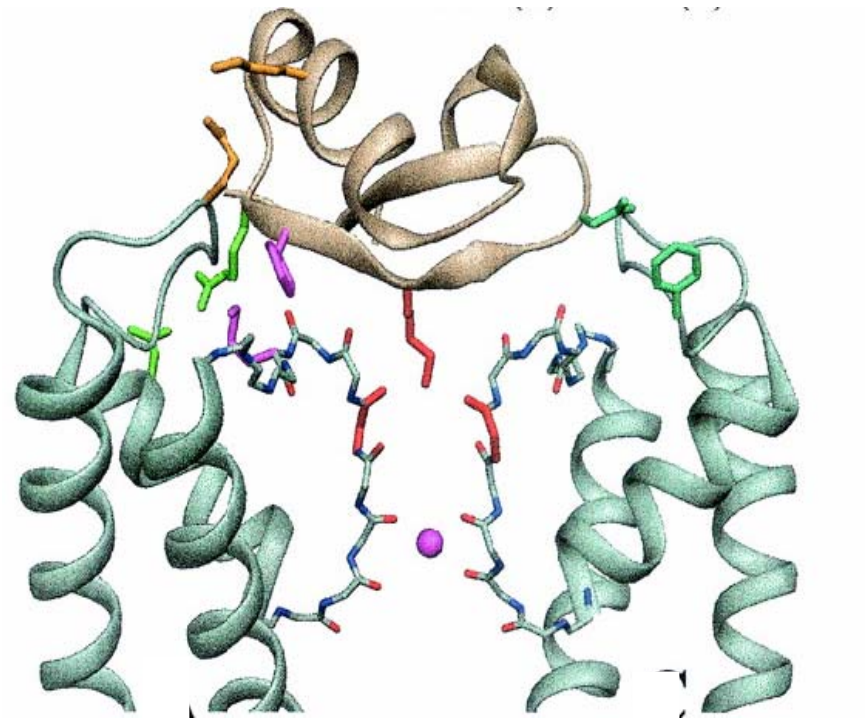
Free vs. Channel-bound U-[^{13}C,^{15}N] KTX



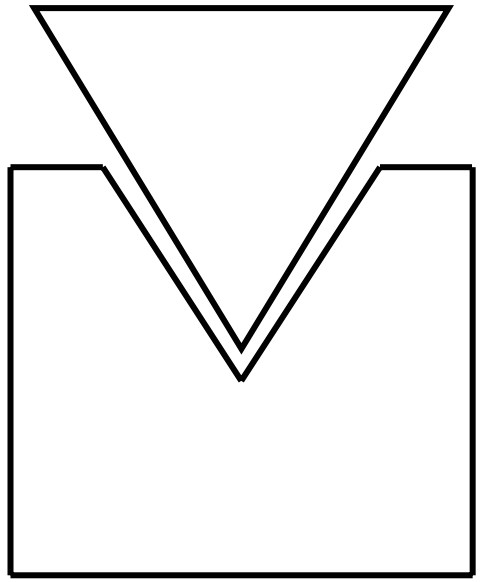
Free vs. KTX-bound U[^{13}C,^{15}N] KcsA-Kv1.3



Toxin – Ion channel complex according to MD

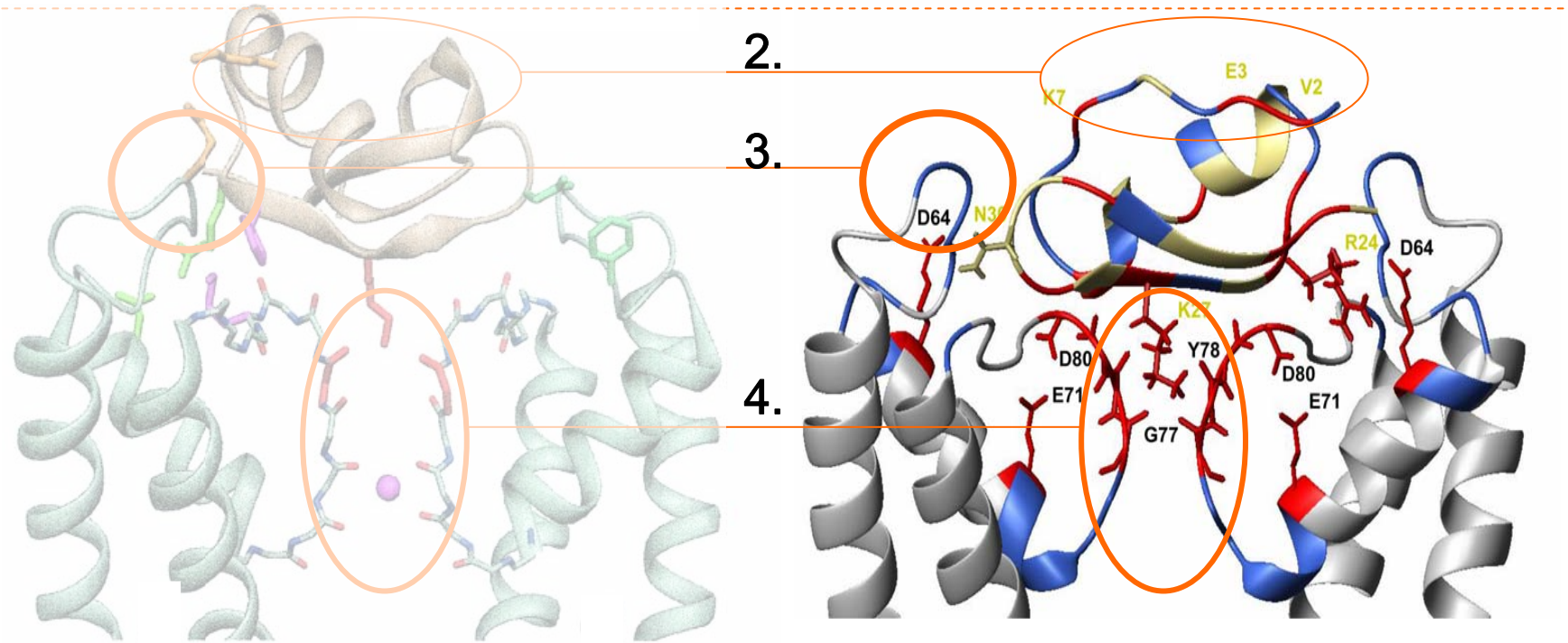


Lock – Key interaction



Eriksson, M. A. L.; Roux, B. *Biophys. J.* **2002**, *83*, 2595-2609.

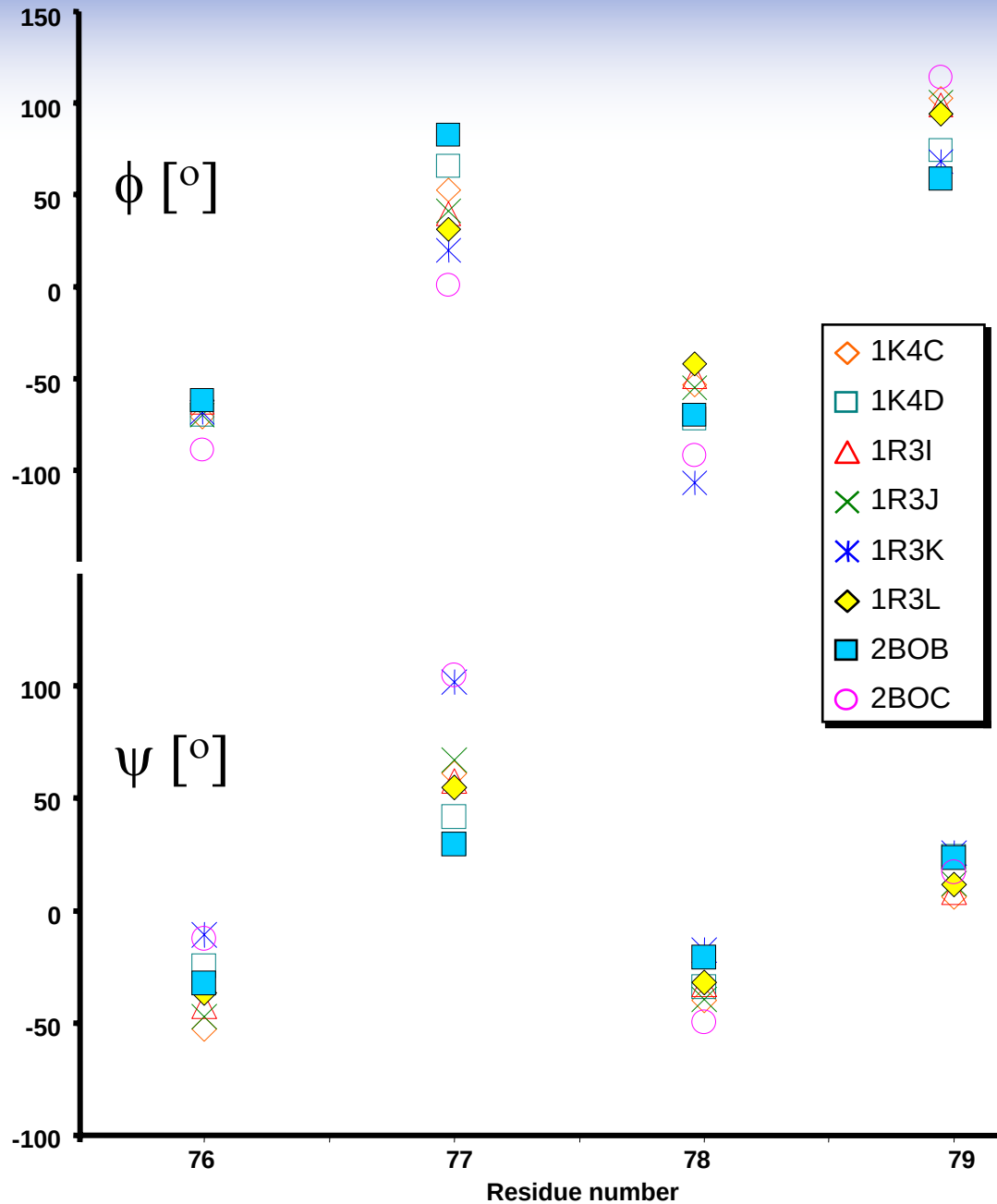
Toxin – Ion channel complex according to ssNMR

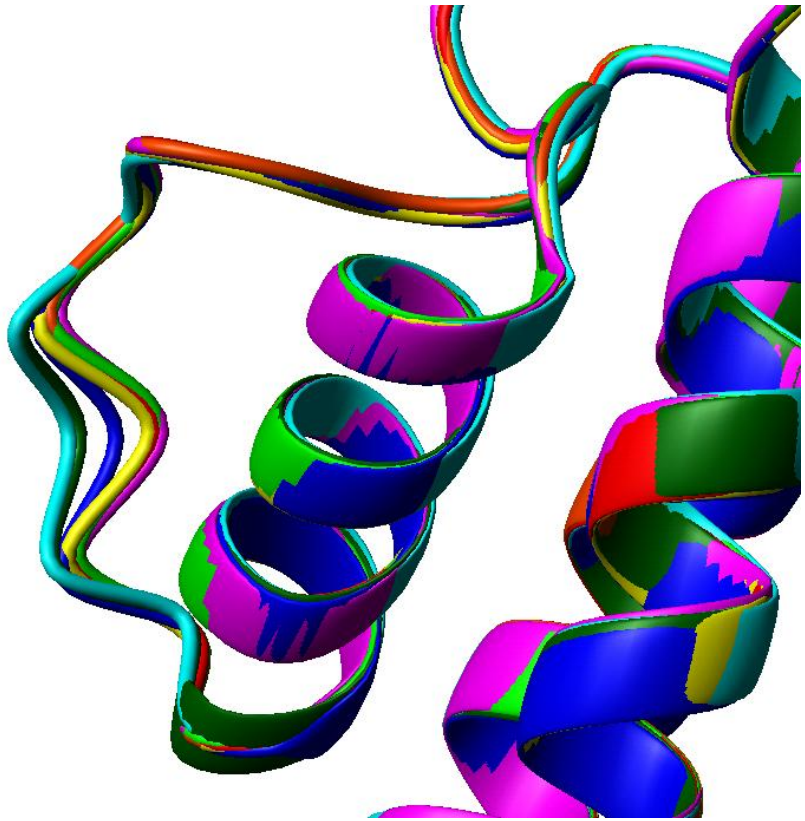


1. Toxin inserts deeper into pore
2. Toxin structure altered.
3. Turret not directly involved in binding interface
4. Selectivity filter changes conformation

Lange, A.; Giller, K.; Hornig, S.; Martin-Eauclaire, M. F.; Pongs, O.; Becker, S.; Baldus, M. **Nature** (2006) 440, 959-962.

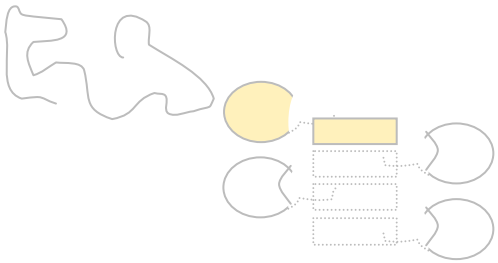
Channel: Intrinsic conformational flexibility



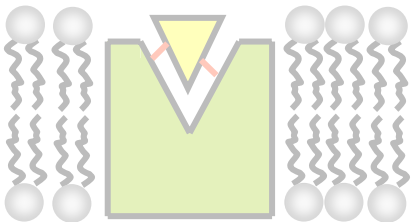


Lange A, Giller K, Pongs O, Becker S, Baldus M (2006), **J. Recept. Signal. Transduct. Res**., 26, 379-393

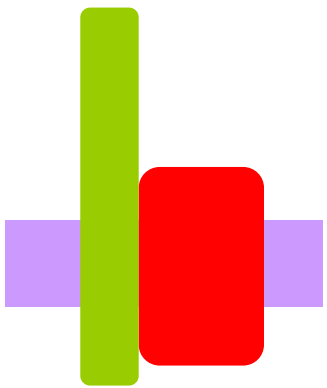
Outline



Protein Aggregation

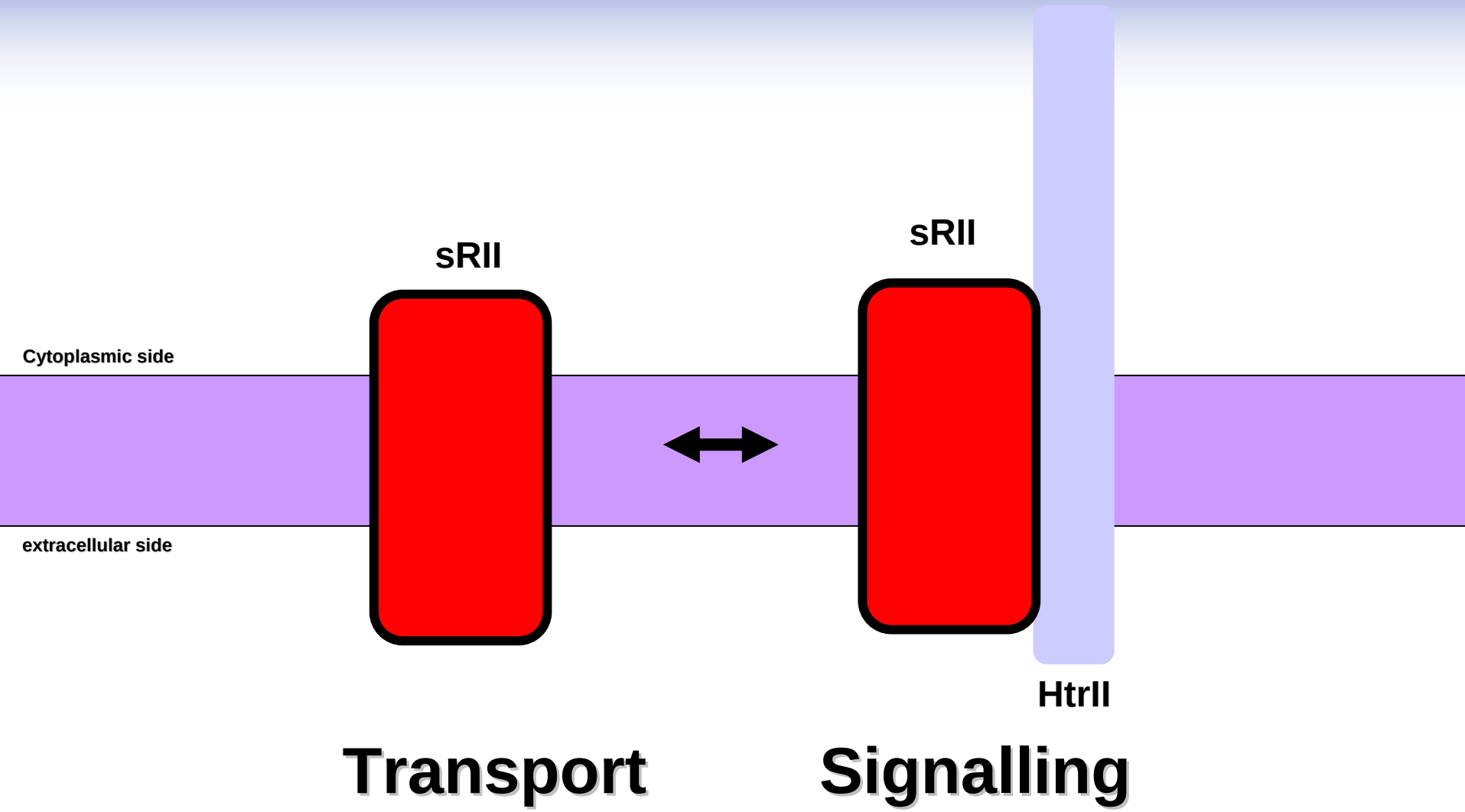


Ligand – Membrane Protein interactions



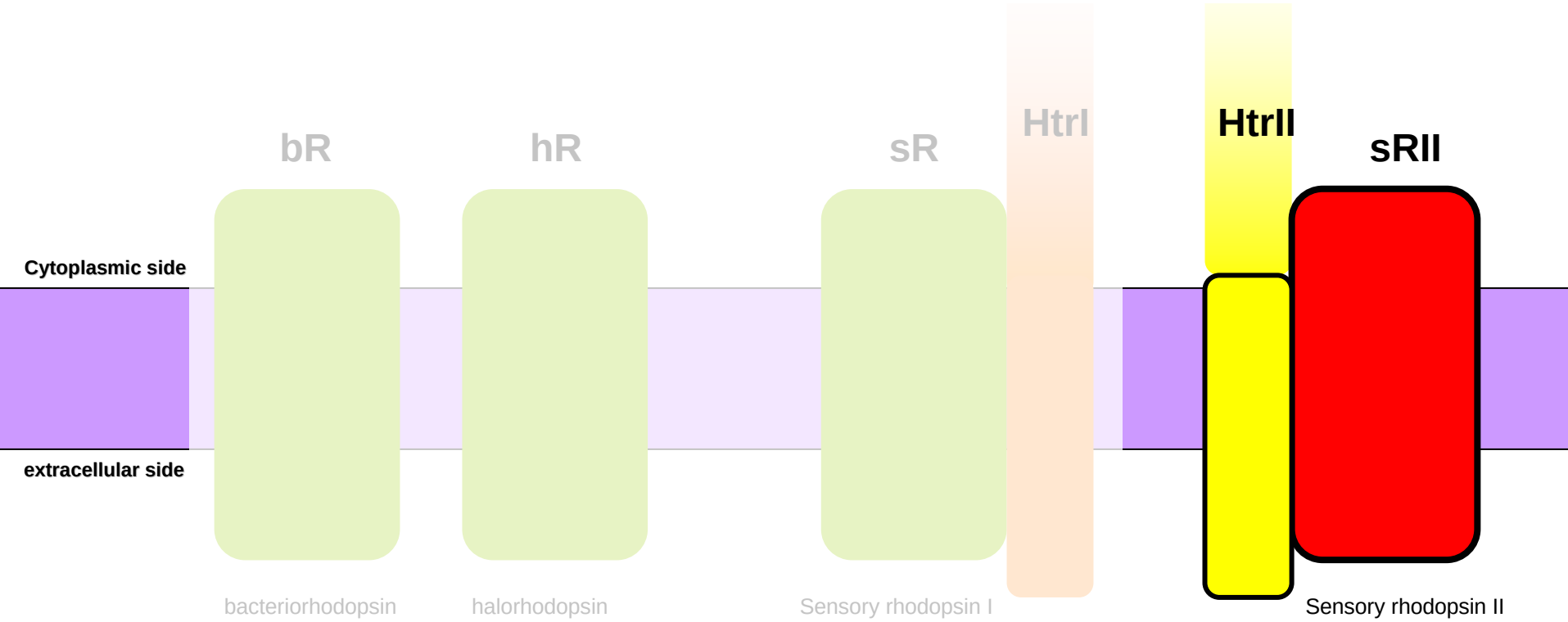
Membrane Protein complexes

How can one receptor exert two different functions ?



Bogomolni, R. A., Stoeckenius, W., Szundi, I., Perozo, E., Olson, K. D., and Spudich, J. L. (1994) *PNAS* 91, 10188-10192
Schmies, G., Engelhard, M., Wood, P. G., Nagel, G., and Bamberg, E. (2001) *PNAS* 98, 1555-1559
Sudo, Y., Iwamoto, M., Shimono, K., Sumi, M., and Kamo, N. (2001) *Biophys. J.* 80, 916-922
Sudo, Y., and Spudich, J. L. (2006) *PNAS* 103, 16129-16134

Sensory rhodopsin II belongs to the family of Retinal proteins



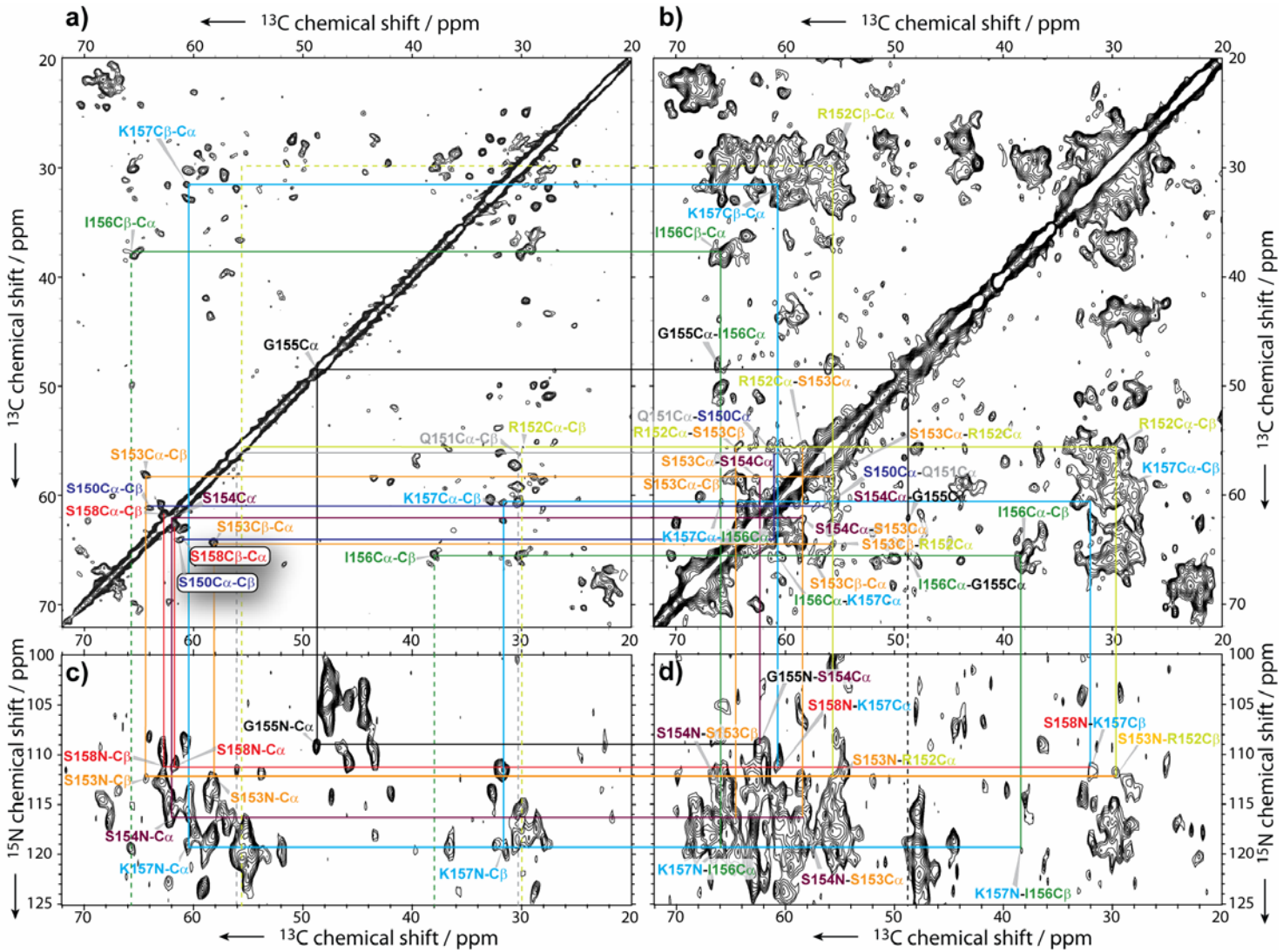
Ion channels

photo-sensors

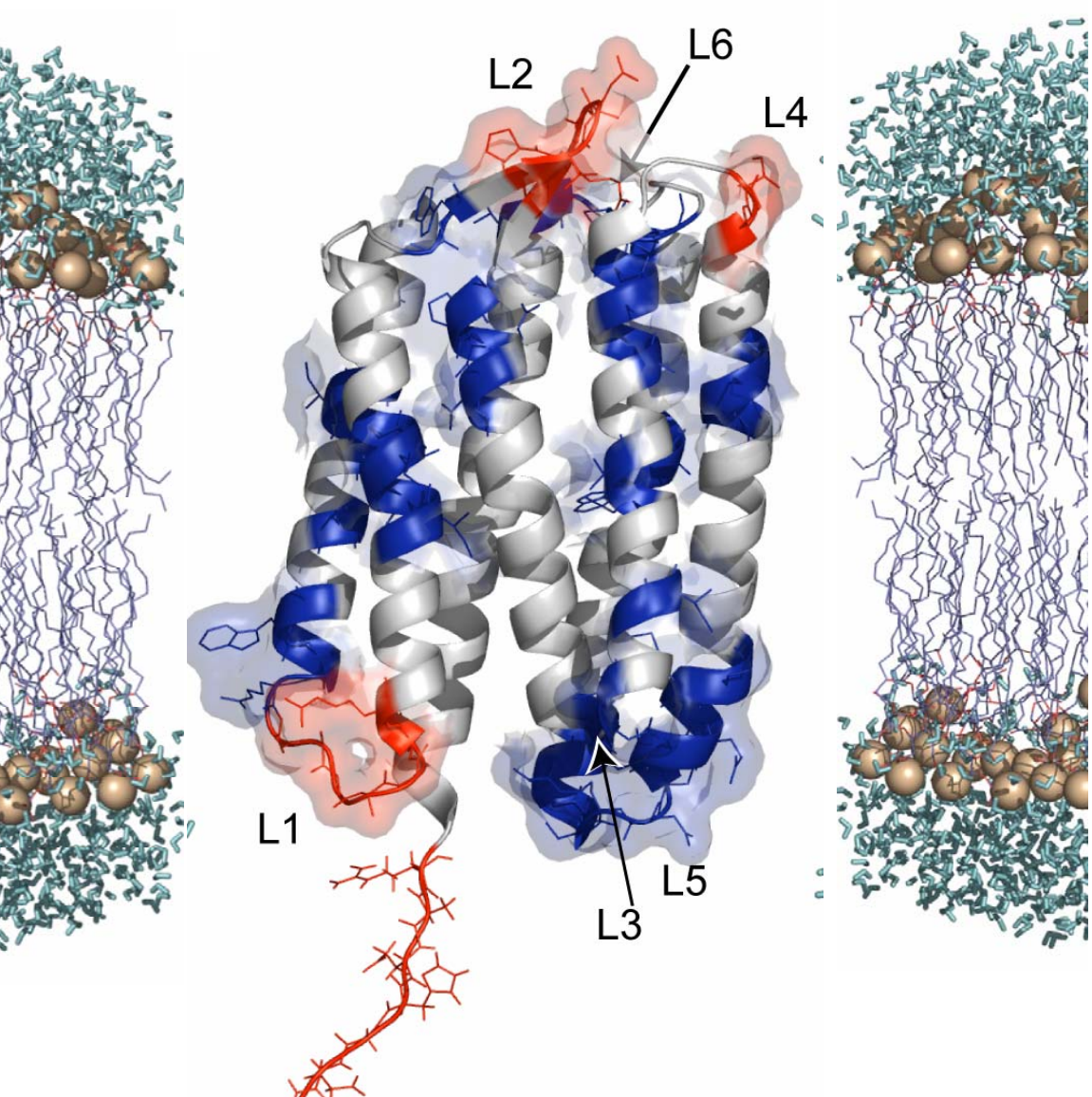
Gordeliy, V. I. et al., **Nature** 2002, *419*, 484-487., **Nature** 2006, 440, 115-119
E. Bordignon, J. P. Klare, M. Doebber, A. A. Wegener, S. Martell, M. Engelhard, H.-J. Steinhoff, **J. Biol. Chem.** 2005, *280*, 38767-38775.

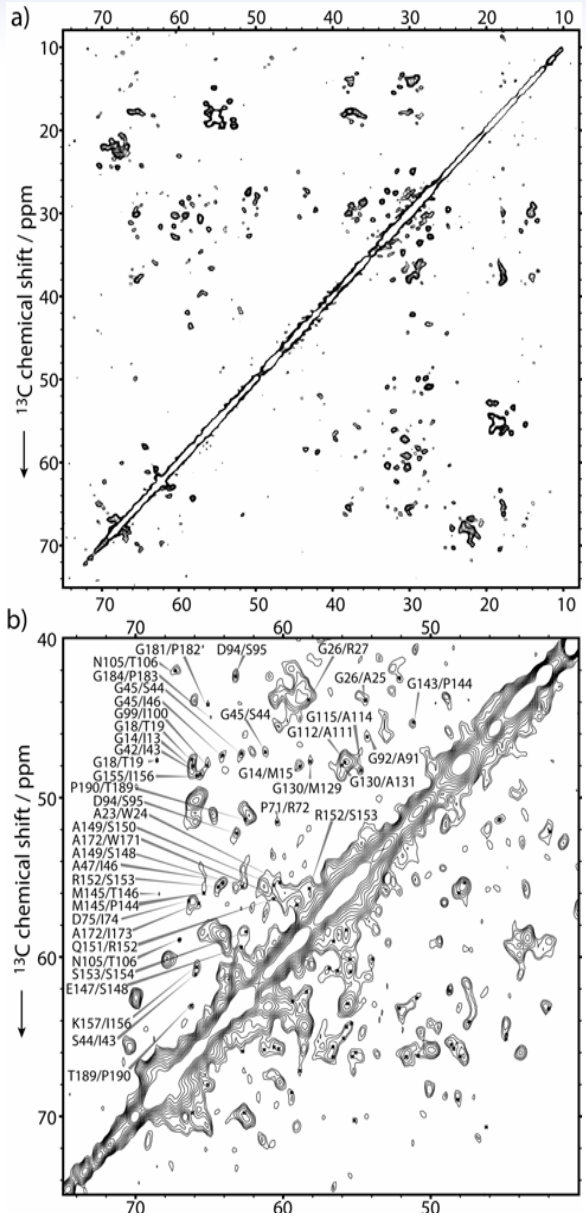
adapted from: Y. Sudo, M. Yamabi, S. Kato, C. Hasegawa, M. Iwamoto, K. Shimono, N. Kamo, **J. Mol. Biol.** 2006, *357*, 1274-1282.

SRII proteoliposomes: ssNMR assignments

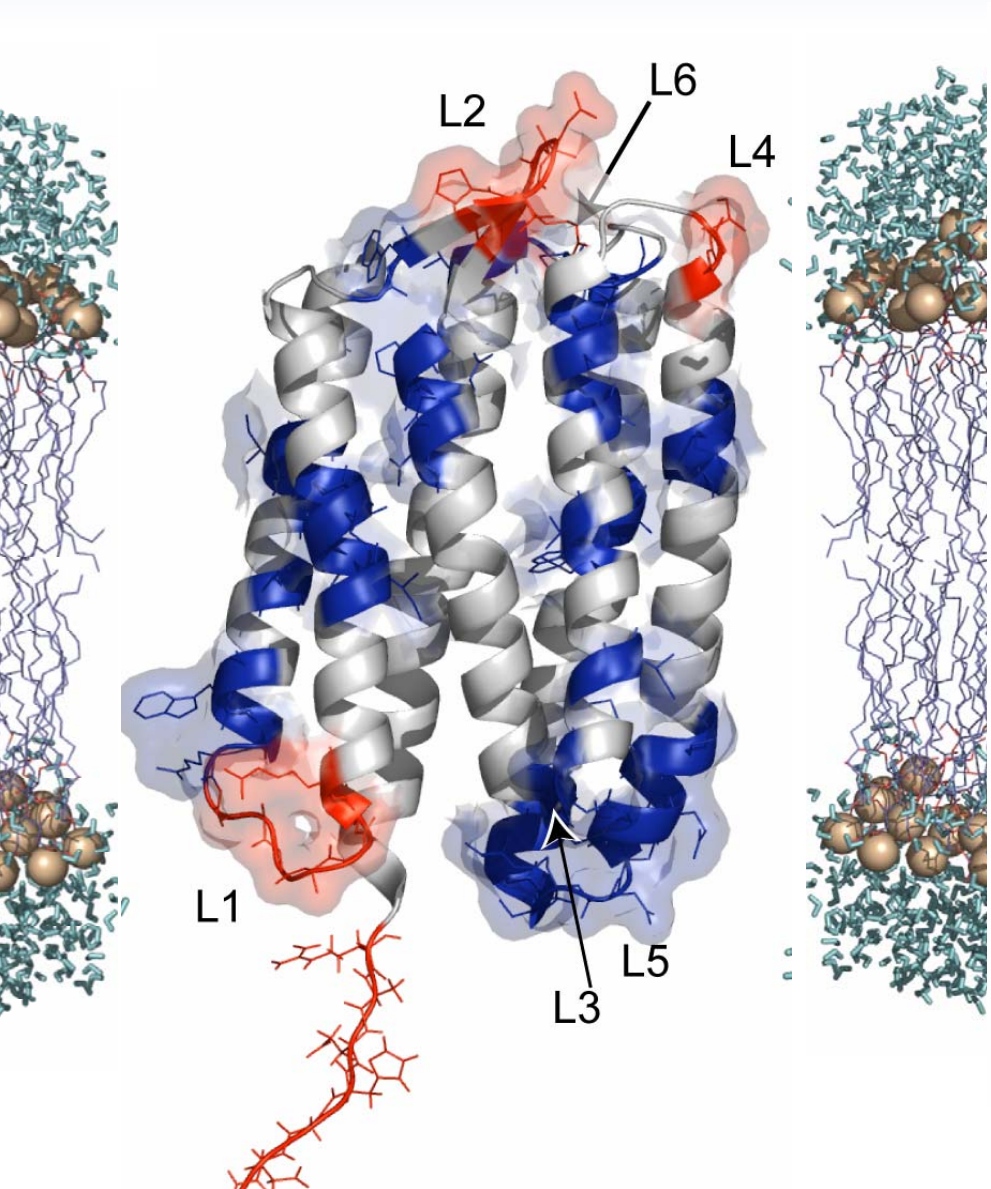


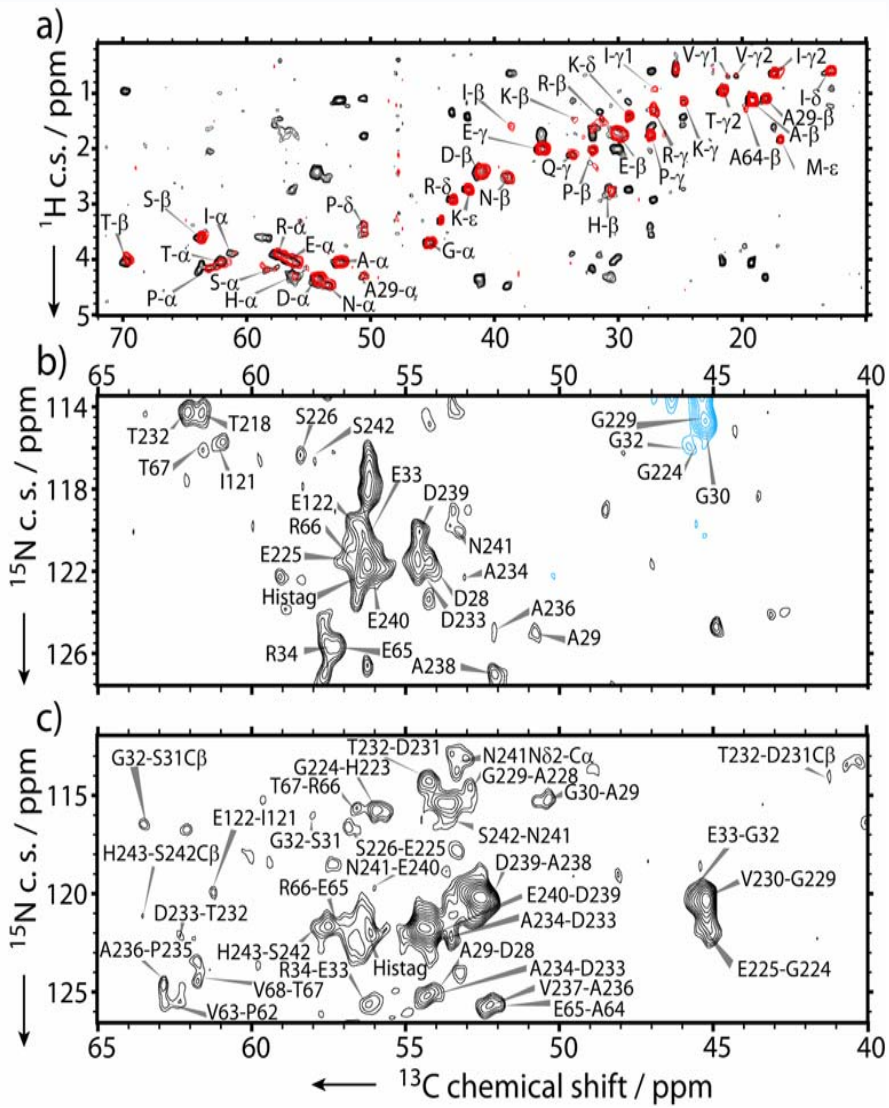
Static protein residues, SRII proteoliposomes



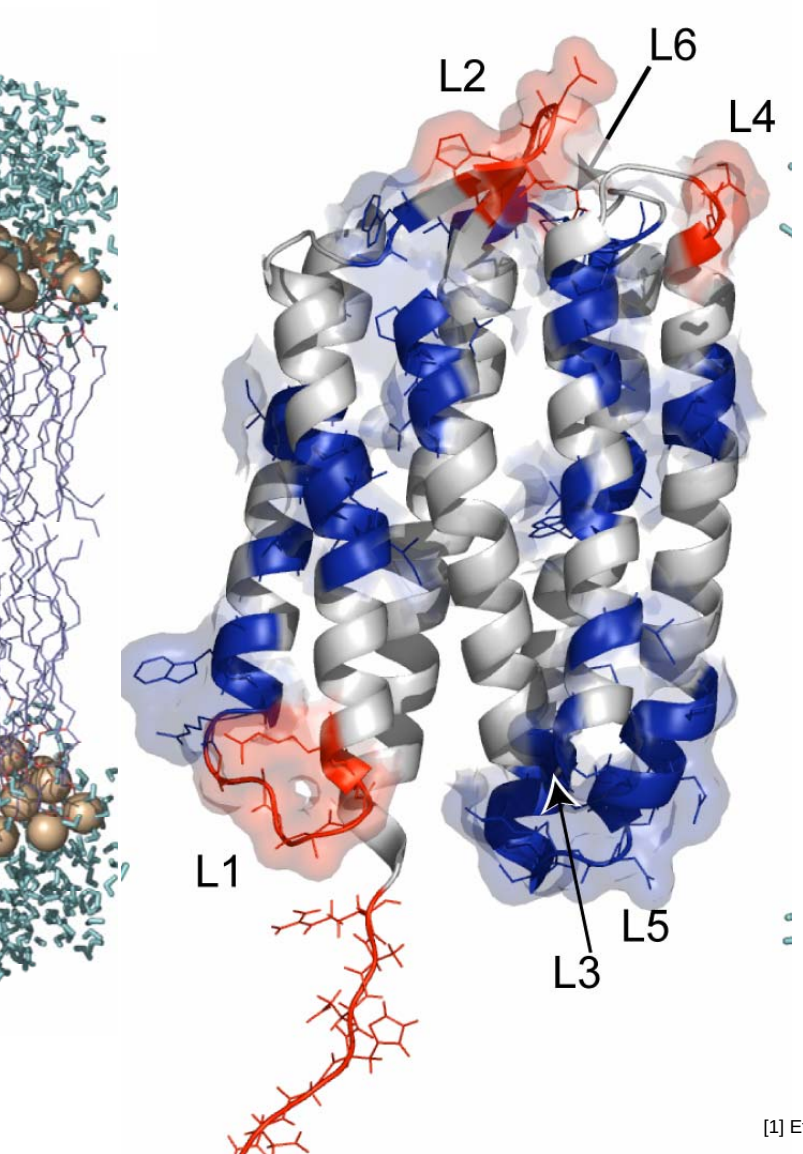


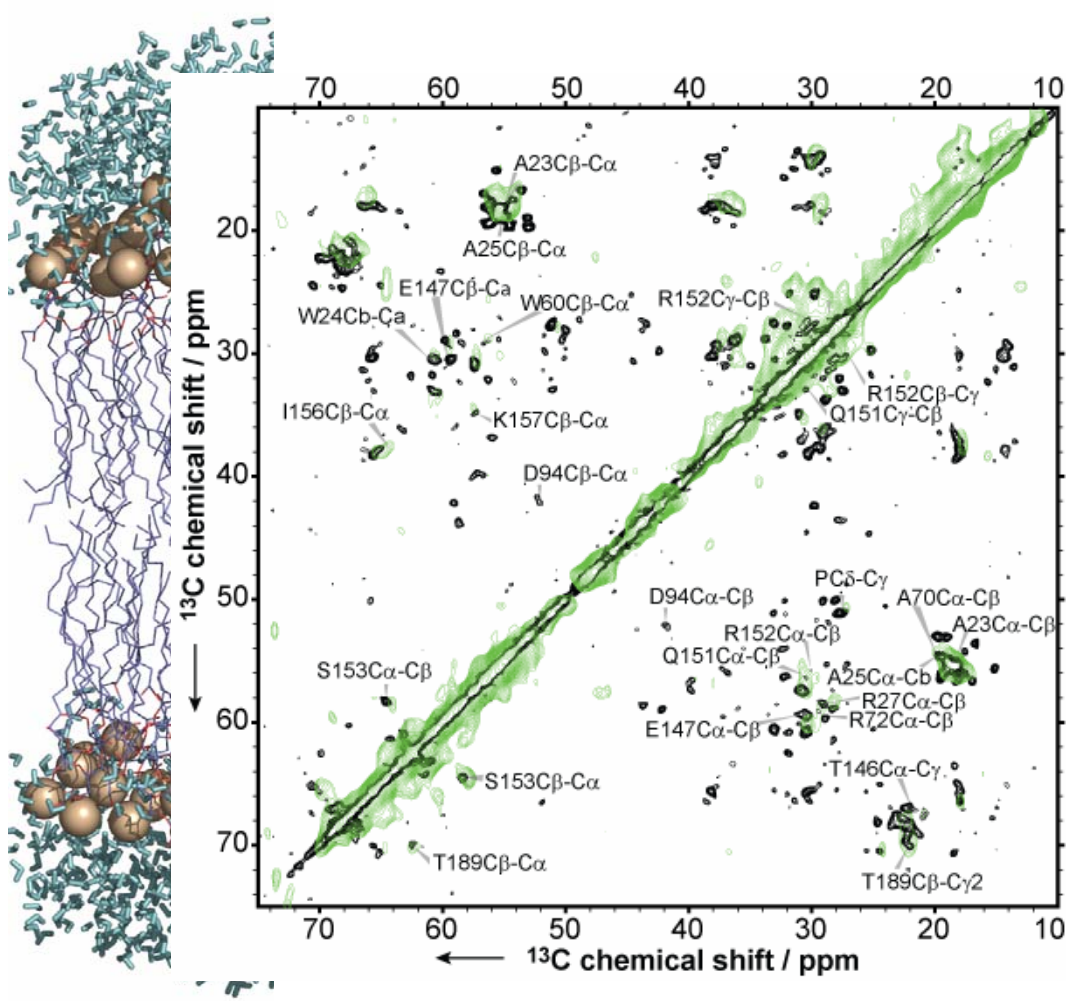
Dynamic protein residues, SRII proteoliposomes





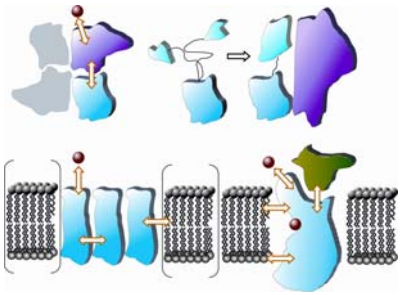
Water exposed protein residues, SRII proteoliposomes

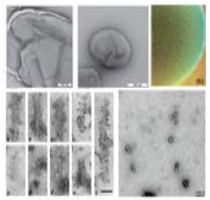




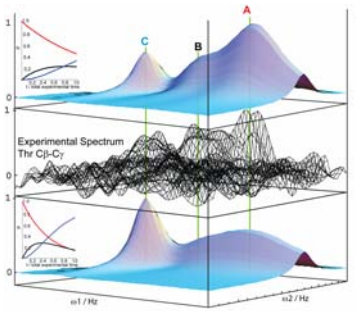
[1] Etzkorn, M., Matell, S., Andronesi, O.C., Seidel, K., Engelhard, M., and Baldus, M. (2007):, **Angew. Chem.-Int. Edit.,** *46,* 459-462.

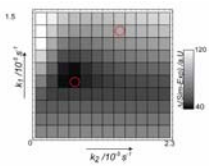
Summary



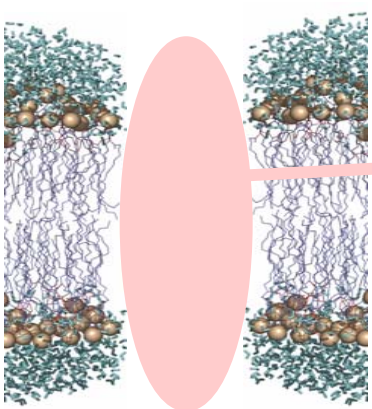


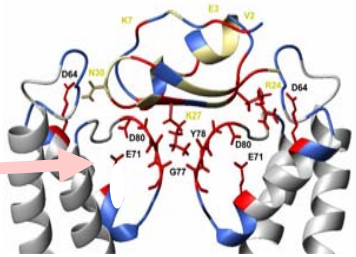
Solid-state NMR can be applied to protein complexes under a variety of experimental conditions





Protein folding and aggregation can be studied at atomic resolution and in real time





Molecular plasticity plays an important role in high-affinity ligand binding, complexation events and protein functionality in membranes

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