Workshop on "Recent Advances in Biomacromolecular NMR Spectroscopy"

Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

2011
Feb.17-18
# Program Overview

**Day 1 : February 17th, 2011 (Thursday)**

**Place : B1B Lecture Room**

**Lectures Sessions**

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<td>Lecture II - <strong>Recent Development of Paramagnetic Relaxation Enhancement (PRE) in Studying Protein Solution Dynamics.</strong></td>
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16:20-17:00  Session II : Young Researcher Presentations (II)

Characterization of a Novel Cysteine Protease Inhibitor, Crammer, from Drosophila melanogaster, and its Potential Role in Cathepsin Regulation
Tien-Sheng Tseng (Prof. Ping-Chiang Lyu Lab, NTHU)

Study on complex structure between human synaptotagmin I (syt I) C2B domain and inositol hexakisphosphate (IP6) by NMR
Meng-Che Chuang (Prof. Chin Yu Lab, NTHU)

17:00-18:00  Session III : Invited Young Scientist Talks

New Insights of 12-Tungstophosphoric Acid from Solid-state 31P NMR of Absorbed Trimethylphosphine Oxide and Introduction of the New High-Field NMR Facilities at Instrumentation Center of National Taiwan University
Shing-Jong Huang, PH.D. (Department of Chemistry, NTU)

NMR and X-ray analysis of DNA recognition by Myb3 DNA binding domain from Trichomonas vaginalis
Yuan-Chao Lou, PH. D. (Institute of Biomedical Science, Academia Sinica)

Day 2 : February 18th, 2011 (Friday)
Place : B1A Meeting Room

Practical Sessions

08:30-09:00  Registration

09:00-10:30  Practical Session I- Drug discovery by NMR
by Dr. Wolfgang Jahnke, Novartis Institutes for BioMedical Research, Switzerland

10:30-10:50  Coffee Break

10:50-12:20  Practical Session II- Paramagnetic Relaxation Enhancement (PRE)
by Dr. Chun Tang, Chinese Academy of Sciences Wuhan, China

12:20-14:00  Lunch Break

14:00-15:30  Practical Session III- SAXS Reveals Protein Structures
by Dr. U-Ser Jeng & Dr. Yu-Shan Huang, National Synchrotron Radiation Research Center, Taiwan

15:30-15:50  Coffee Break

15:50-17:20  Practical Session IV: Discussion
Lectures Sessions
NMR in an integrated drug discovery approach

Wolfgang Jahnke
Novartis Institute for Biomedical Research, Basel, Switzerland
wolfgang.jahnke@novartis.com

NMR is a useful and highly versatile biophysical technique that can support the drug discovery process in a variety of ways\(^1\). On one hand, biomolecular NMR is a very robust and reliable method to detect and characterize protein-ligand interactions. On the other hand, NMR can give structural information on proteins and protein-ligand complexes.

In my presentation, I will review the most commonly used NMR experiments to detect ligand binding, and I will present two recent applications of valuable NMR contributions to the drug discovery process: Allosteric non-bisphosphonate inhibitors of FPPS\(^2\), and allosteric inhibitors of Abl kinase\(^3\).

\(^1\) W. Jahnke “Perspectives of biomolecular NMR in drug discovery: The blessing and curse of versatility” J. Biomol. NMR 39, 87-90 (2007)
Recent Development of Paramagnetic Relaxation Enhancement (PRE) in Studying Protein Solution Dynamics

Chun Tang
State Key Laboratory of Magnetic Resonance and Atomic Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan, Hubei 430017, China

Proteins in solution constantly undergo conformational fluctuations, thus to fulfill their specific functions. Most established NMR methods including relaxation dispersion afford only timescales of protein dynamics. Recently, we applied paramagnetic relaxation enhancement (PRE) technique to directly visualize structures of low-populated species in a dynamic system. The technique utilizes large dipole moment of an unpaired electron and \(<r^6>\) distance dependency of PRE effect. I will talk about applications of PRE to visualize protein-protein encounter complexes, protein domain movement, and protein oligomerization. In addition, ongoing work studying the dimerization of ubiquitin will also be presented.

To study protein dynamics using PRE, a paramagnetic probe is introduced at specific site, often through a surface cysteine residue. The flexible linker between the paramagnetic center and protein backbone results in uncertainty of the observation point. We have designed a rigid probe that permits detection of subtle movement. We have improved the PRE technique, called differential scaled PRE (DiSPRE); when using multiple probes of different paramagnetic strengths, we can extract both temporal and spatial characterization for an exchanging process. Taken together, paramagnetic NMR offers new opportunities to visualize protein structure and dynamics in solution.
A small/wide-angle X-ray scattering instrument for structural studies of soft matter at the NSRRC

U-Ser Jeng, Chiu-Hun, Su, Chun-Jen Su, Wei-Chung Chuang, Yu-Shan Huang
National Synchrotron Radiation Research Center, Taiwan

Research on soft matter with time-resolved small/wide-angle X-ray scattering (SAXS/WAXS) at NSRRC is blooming because of the much improved X-ray flux provided at the new SAXS beamline BL23A. The BL23A SWAXS endstation, open since May 2009, provides efficient collection of high-quality data that follow closely the standards of the world’s leading SAXS instruments at ESRF, SOLEIL, APS or SPring-8 in static measurements of equilibrium morphology or slow kinetics in solutions and films. Presented in this talk are the features of the new BL23A SWAXS endstation, including simultaneous, time-resolved SAXS/WAXS with DSC, shear, a stretching device, and a stopped-flow device for structural kinetics of polymers, nanoparticles, liquid crystals and biomacromolecules in solution. SAXS/WAXS applications on biomacromolecular solutions or oriented films will be emphasized, including protein folding-unfolding and peptide-membrane binding. The future X-ray photon correlation spectroscopy (XPCS) beamline with the Taiwan Photon source (TPS), aiming for structural kinetics and dynamics of thermal equilibrium structures on a time scale from hundreds of seconds to milliseconds, will be briefed.

**Figure 1** Schematic of the BL23A SAXS/WAXS instrument.
HFNMRC Users Presentations &
TMRS Activities
Applying diffusion MRI techniques to discover the white matter connectivity in human brain

Yu-Chun Lo 羅仔君 and Wen-Yih Isaac Tseng
Institute of Biomedical Engineering, National Taiwan University

Neuroscience is an interdisciplinary science that collaborates with other fields. Neuroimaging is a part of neuroscience and includes the use of various techniques to either directly or indirectly image the structure, function, or pharmacology of the brain. Neuroimaging studies focus on discovering the primary and cognitive functions in the brain. Recently, magnetic resonance imaging (MRI) has become an important technique to discover the human brain non-invasively. Advances in MRI methodology, such as the functional MRI and the diffusion MRI, and related analytic strategies allow researchers to test the brain connectivity models related to healthy patterns and disease pathology in psychiatric disorders.

There are four main topics targeted to diffusion spectrum imaging (DSI) techniques for clinical applications in this dissertation. First, we introduced the diffusion MRI techniques and related analytic approaches, such as voxel based morphometry (VBM) and tractography-based morphometry. Second, we analyzed white matter tracts in the fronto-striato-thalamic circuitry of the healthy human brain using DSI data and found the gender and handedness factors should be considered when one evaluates the impairment of the fronto-striato-thalamic circuitry. Third, we aimed to further explore white matter abnormalities in patients with obsessive-compulsive disorder (OCD) in DSI and tractography of targeted white matter tracts in the fronto-striato-thalamic circuitry which most probably play an important role in OCD neuropathology. This study supports the white matter abnormalities of patients with OCD, which corroborates neurobiological models that posit a defect in fronto-striato-thalamic circuitry in OCD. Finally, we analyzed the microstructural integrity of the long-range connectivity related to social cognition and language processing with diffusion tractography among adolescents with autism compared to neurotypicals. The loss of leftward asymmetry and reduction of interhemispheric connection in adolescents with autism suggest alterations of the long-range connectivity involved in social cognition and language processing.

In summary, we successfully applied the DSI techniques to healthy participants and patients with mental disorders. The translation of DSI technique from laboratory to clinical setting is potentially feasible. Moreover, the clinical experience and technical strengths provide a strong basis for us to extend to imaging genetics, aiming to determine effective endophenotypes of psychiatric disorders. In the future, it is plausible that brain imaging may serve as effective endophenotypes that link clinical manifestation (phenotypes) and the biological variables (genotypes). The methodology of validating endophenotype will be readily extended to other psychiatric disorders.
Supra-molecular Magnetic Nanoparticles for Contrast Enhancement in MR Molecular Imaging

Chaohsiung Hsu 許朝雄 and Yung-Ya Lin

Taiwan International Graduate Program (TIGP), Academia Sinica (and National Tsing Hua University)
Comprehensive Solid-State NMR Study of Octacalcium Phosphate Incorporated with Succinate

Tim W. T. Tsai and Jerry C. C. Chan
Department of Chemistry, National Taiwan University

Octacalcium phosphate (OCP) is an important model compound in the study of biomineralization. The octacalcium phosphate incorporated with succinate (OCPS) is hypothesized as a component of mineral granules in mitochondria. It is therefore in vitro prepared and characterized mainly by solid-state NMR spectroscopy. Accordingly, the molecular formula of OCPS is determined to be \( \text{Ca}_{7.81}(\text{HPO}_4)_{1.82}(\text{PO}_4)_{3.61}(\text{succinate})_{0.56} \cdot z \text{H}_2 \text{O} \), where \( z \leq 0.5 \). Furthermore, the succinate molecule is found to be in the hydration layer of OCPS. The stability of OCPS is significantly higher than OCP with respect to the hydrolysis reaction at high pH and/or high temperature conditions. We conclude that the hydration layer of OCP is playing the key role in the structural transformation of OCP.

References:
Hydrogen bonding network in Nafion proton exchange membrane

Zhen Wu 呉振¹, Peter Chu² and Shangwu Ding¹
¹Department of Chemistry, National Sun Yat-Sen University
²Department of Chemistry, National Central University

The structural and dynamical properties of Nafion membranes swollen with water molecules in varied hydration level under proton and sodium forms have been investigated by NMR variable temperature experiments and pulse field gradient techniques. By analyzing the spin-lattice relaxation time of water in membranes as a function of temperature, the activation energy is obtained in light of BPP theory. In proton form of membranes, the activation energy is up to 43500 J · mol⁻¹ for dehydrated state (λ=6) and down to 16500 J · mol⁻¹ for fully hydrated state (λ=22), respectively. In sodium form of membranes, the activation energy is up to 35500 J · mol⁻¹ for dehydrated state (λ=8) and down to 15500 J · mol⁻¹ for fully hydrated state (λ=17), respectively. The ab initio calculations from triflic acid (CF₃SO₃H), CF₃SO₃Na and H₂O, as model system, are carried out in order to characterize the dependency of rotational correlation time and activation energy on hydrogen bonding network within Nafion membranes. With less water content, the results indicate that most of water molecules are involved in hydrogen bonding network close to the sulfonate acid groups which is contrary to the case of higher water content. These results are useful in understanding the behavior of dynamics and proton transfer for Nafion proton exchange membrane.
Characterization of a Novel Cysteine Protease Inhibitor, Crammer, from *Drosophila melanogaster*, and its Potential Role in Cathepsin Regulation

Chao-Sheng Cheng 曾天生, Tien-Sheng Tseng, Yu-Nan Liu, Dian-Jiun Chen, Ping-Chiang Lyu

*Institute of Bioinformatics and Structural Biology, National Tsing Hua University*

*Drosophila melanogaster* crammer belongs to a novel class of cysteine protease inhibitors and has been found to be involved in the formation of *Drosophila* long-term memory (LTM). The biophysical properties of crammer remain elusive. Here we found that the oligomeric state of crammer is pH-dependent. At neutral pH, *in vitro*, crammer exists as a disulfide bonded dimer, while at acidic pH, the monomer is predominant. However, *in vivo*, crammer likely exists as a monomer inside the lysosome. Our inhibitory assay shows that only monomeric crammer, but not a dimer, can act as a strong competitive inhibitor to suppress *Drosophila* cathepsin, which is distinct from the conclusions of previous studies. In particularly, Cys72 in crammer is not only responsible for the formation of intermolecular disulfide bond but also crucial for cathepsin inhibition. Finally, we also report the first structure of a propeptide-like inhibitor, crammer, and propose that monomeric crammer, like a molten globular protein, can undergo conformational change from the flexible state to the well-packed state upon binding with cathepsin. We presume the major driving forces for controlling structural change are the packing of aromatic residues and network salt bridges.
Study on complex structure between human synaptotagmin I (syt I) C2B domain and inositol hexakisphosphate (IP6) by NMR

Meng-Je Juang 莊孟哲 and Chin Yu
Department of Chemistry, National Tsing Hua University

Synaptotagmin I (Syt I) is a synaptic vesicle protein that contains two copies of highly conserved protein kinase C homology regions known as the C2A and C2B domains. The C2A domain binds Ca2+ and the C2B domain binds inositol polyphosphates (IP4, IP5, and IP6). It has been reported that Ca2+ regulated exocytosis of secretory vesicles is proposed to be activated by Ca2+ binding to the C2A domain and inhibited by inositol polyphosphate binding to the C2B domain. Inositol hexakisphosphate (IP6) is the principal storage form of phosphorus in many plant tissues. It has been shown that IP6 is protective against Parkinson's disease in vitro. In the present study, we investigated the interaction of IP6 with C2B in the presence and absence of Ca2+ by various biophysical methods including isothermal titration calorimetry (ITC) and multidimensional NMR spectroscopy. These data show that IP6 binds specifically to the lysine-rich region of C2B with similar binding strength both in the presence and absence of Ca2+. The experimental data indicated IP6 binding to C2B is calcium independent. To understand the IP6 induced inhibition in exocytosis of secretory vesicles, we solved the solution structure of C2B-IP6 complex. This information will give clues to design better drugs for neurological disorders.
New Insights of 12-Tungstophosphoric Acid from Solid-state $^{31}$P NMR of Absorbed Trimethylphosphine Oxide and Introduction of the New High-Field NMR Facilities at Instrumentation Center of National Taiwan University

Shing-Jong Huang 黃信炅  
Department of Chemistry, National Taiwan University

The first part of this talk is intended to present our recent works (in collaboration with Prof. Shang-Bin Liu’s group) on Keggin-type heteropolyacids such as 12-Tungstophosphoric acid (H$_3$PW$_{12}$O$_{40}$; HPW) using solid-state $^{31}$P NMR of absorbed trimethylphosphine oxide (TMPO) probe molecule in conjunction with density functional theory (DFT) calculations. It will be shown that various protonated TMPOH$^+$ and (TMPO)$_2$H$^+$ adducts may be unambiguously identified using $^{31}$P-$^1$H Lee-Goldburg cross-polarization (LG-CP) HETCOR and $^{31}$P-$^{31}$P DQ correlation techniques. Moreover, it was found that transport of TMPO absorbate in HPW adsorbent invokes a desorption/absorption process associated with the (TMPO)$_2$H$^+$ adducts. Consequently, three types of protonic acid sites with distinct superacidic strengths, corresponding to the observed $^{31}$P chemical shifts of 92.1, 89.4, and 87.7 ppm, were identified for TMPO/HPW system. Together with the results from DFT calculations, thus, the TMPOH$^+$ complexes are most likely associated with protons located at three different terminal oxygen (O$_d$) sites of the PW$_{12}$O$_{40}^{3-}$ polyanions. Similar techniques have also been applied to other heteropolyacid systems, such as alkali ion-exchanged Cs$_x$H$_{3-x}$PW$_{12}$O$_{40}$ and H$_3$PMo$_{12}$O$_{40}$ (HPMo).

In the second part of this talk, I will briefly introduce the new high-field NMR facilities at Instrumentation Center of National Taiwan University (NTU). This new 800 MHz NMR spectrometer (Bruker AVANCE III), which is located at the Chemistry Building of NTU, was installed just before the end of 2009. This spectrometer (under the supervision of Prof. Ying-Chih Lin) is also equipped with several probeheads for solution-state applications, including a 5 mm TCI cryoprobe, a 5 mm TXI, and a 5 mm BBO probes and also equipped with the solid-state accessories. We are also anticipating a new solid-state Bruker AVANCE III 600 MHz NMR spectrometer (supervised by Prof. Jerry Chun-Chung Chan) to arrive and to be installed in the same facility by mid-2011. With these state-of-the-art instruments, we hope to offer the best services to the NMR communities and to contribute to the NMR-related research in Taiwan.
NMR and X-ray analysis of DNA recognition by Myb3 DNA binding domain from *Trichomonas vaginalis*

Yuan-Chao Lou 羅元超, Shu-Yi Wei, Meng-Ru Ho, Jung-Hsiang Tai and Chinpan Chen
Institute of Biomedical Sciences, Academia Sinica

*Trichomonas vaginalis*, a protozoan parasite, is the causative agent of trichomoniasis, which is a common but often neglected sexually transmitted disease. The *ap65-1* gene of *T. vaginalis*, encoding a 65-kDa surface adhesion protein which is associated with the cytoadherence of *T. vaginalis* to the host cells, is regulated by Myb proteins through the Myb recognition elements (MREs). Among the identified Myb proteins (Myb 1 to 3), Myb3 interacts with the MRE-1 element. We identified that Myb3<sub>53-180</sub>, containing two Myb-like DNA-binding motifs (designated as R2 and R3 motifs) and an extension of 31 residues at C-terminus (Ile<sup>150</sup>-Lys<sup>180</sup>), is the essential fragment for DNA recognition. And interestingly, the removal of C-terminal 31 residues reduces the DNA binding ability significantly. The structural basis of Myb3<sub>53-180</sub>/DNA interaction was investigated by X-ray crystallography and showed that only the third helixes of both R2 and R3 motifs are responsible for DNA recognition. In the complex structure, the C-terminal 31 fragment forms a significant b-hairpin, but no direct interactions between DNA and the 31 residues can be observed. Using SPR, ITC and site-directed mutagenesis, we showed the importance of C-terminal fragment in DNA binding. Also, paramagnetic NMR was applied to investigate the interactions between Myb3<sub>53-180</sub> and DNA, and will be presented in detail in this talk.
Practical Sessions
Drug discovery by NMR

Dr. Wolfgang Jahnke
Drug discovery by NMR - Practical session

Wolfgang Jahnke
Workshop “Recent advances in biomacromolecular NMR spectroscopy”
18 February 2011

Agenda

- How to build a fragment library
  - Presentation and questions/answers
- How to analyze NMR fragment screening data
  - Practical session

Aspects of a fragment library

- Typically between 500 and 5000 compounds
  - Generally high hit rate
- Satisfy “rule of three” (Congreve et al. DrugDiscovToday 8, 876, 2003)
  - MW < 300Da (typically < 250Da)
  - clogP < 3
  - # H-bond donors < 3, # H-bond acceptors < 3
- Satisfy diversity criteria
- Exclude reactive groups
- Fragments contain chemical handles for synthetic follow-up

Process of creating the library

- Solubilize candidate fragments in dmso-δ6
- Collect LC-MS data
  - Continue if purity >85% and mass OK
- Collect NMR spectra (in aqueous buffer)
  - Continue if structure is OK and solubility > 500uM: Fragment accepted
- Peak picking
- Design mixtures based on pick picked spectra
  - Minimize overlap between fragments
- Physically add the accepted fragments into the mixtures
**Flowchart for NMR-based fragment screening**

- Collect reference spectra of free mixtures without protein
  - [fragment] = 200uM
  - T1rho, waterLOGSY, STD
- Collect spectra of mixtures in the presence of target protein
  - [fragment] = 200uM, [target] = 5-10uM
- Compare spectra with and without protein, to obtain preliminary hit list
- Test all preliminary hits as single compounds with and without protein, to obtain final hit list
- If known high-affinity ligand is available, record competition spectra

**Practical session**

*Analysis of three fragment screening mixtures against target X*

- Which fragments bind to target X, and are they competitive with a known high-affinity inhibitor?

**Practical session – how to proceed (1)**

*Analysis of three fragment screening mixtures against target X*

- Using Topspin and spectra directory Rahmel, please process spectra
  - 110,111,112 – 1H, TTr, wL spectra of mixture 1 + targetX
  - 120,121,122 – 1H, TTr, wL spectra of mixture 2 + targetX
  - 130,131,132 – 1H, TTr, wL spectra of mixture 3 + targetX
  - 10,11,12 – 1H, TTr, wL spectra of free mixture 1
  - 20,21,22 – 1H, TTr, wL spectra of free mixture 2
  - 30,31,32 – 1H, TTr, wL spectra of free mixture 3
  - 210,211,212 – 1H, TTr, wL spectra of mixture 1 + targetX + competitor
  - 220,221,222 – 1H, TTr, wL spectra of mixture 2 + targetX + competitor
  - 230,231,232 – 1H, TTr, wL spectra of mixture 3 + targetX + competitor
- Processing commands:
  - "qsin;p" for 1H, waterLOGSY spectra (e.g. 110, 112)
  - Then superimpose procnos 1113 and 1111 to see TTr effect

**Practical session – how to proceed (2)**

- Process spectra of mixtures in presence of targetX, compare to spectra of free mixtures. Decide which peaks show TTr/waterLOGSY effects
- Deconvolute the mixtures into their components
  - Mix 1: 1501, 1509, 1521, 1599, 1775, 1788, 1792, 1828
  - Mix 2: 1503, 1506, 1525, 1531, 1640, 1643, 1798, 1862
  - Mix 3: 1504, 1507, 1524, 1533, 1560, 1804, 1846, 1874
- Superimpose spectra of individual compounds onto mixtures, to decide which fragment is a hit.
- Then analyze the competition experiments to see whether hits are competitive, non-competitive or synergistic
Paramagnetic Relaxation Enhancement (PRE)

Dr. Chun Tang
Outline

1. How to introduce a paramagnetic probe to otherwise diamagnetic protein?
2. How to measure $^1$H $\Gamma_2$ PRE rates?
3. How to apply PRE restraints in Xplor-NIH?
4. How to account for the mobility of paramagnetic probe?
5. Xplor-NIH calculations
6. What happens when the exchange is not so fast?

Part 1

Conjugation method

- protein engineering (ATCUN, LBT)
- chemical cross-linking

Selection of conjugation site (Cys)

- periphery of the interface
- solvent exposed (charged)
- frequently mutated or modified
- does not perturb structure (RDC, functional assay)
- iso-electrical
- too mobile?
- cross-validation

Probes of choice

- Gd$^{3+}$ (DTPA, CLaNP, LBT)
- Mn$^{2+}$ (EDTA)
- spin radical (PROXYL, TEMPO)
- Cu$^{2+}$ (EDTA, ATCUN)

- isotropic g-tensor
- stereo-isomer
Conjugation of a probe

- protein purification
- preparation of probe, metal loading
- desalting, conjugation
- desalting, further purification

**Part 2**

**PRE measurement**

\[ \Gamma_2 = R_{2,para} - R_{2,\text{dia}} \]

\[ \sigma(\Gamma_2) = \frac{1}{T_b - T_a} \times \left( \frac{\sigma_{\text{dia}}}{I_{\text{dia}}(T_a)} + \frac{\sigma_{\text{dia}}}{I_{\text{dia}}(T_b)} + \frac{\sigma_{\text{para}}}{I_{\text{para}}(T_a)} + \frac{\sigma_{\text{para}}}{I_{\text{para}}(T_b)} \right) \]
Single-time-point measurement of PRE

\[ I_{\text{para}} (0) = I_{\text{dia}} (0) \frac{R_{2,\text{dia}}}{R_{2,\text{dia}} + \Gamma_2} \exp(-\Gamma_2 \tau) \]

- \[ \tau_C = (\tau_1^{-1} + \tau_2^{-1})^{-1} = 4 \text{ ns} \]
- \[ \tau_{C,\text{dia}} = 0.8 \text{ s} \]
- \[ \tau_{T,\text{dia}} = 1.2 \text{ s} \]
- \[ \tau_{T,\text{dia}} = 1.6 \text{ s} \]

Complete longitudinal recovery is difficult

- > 5/R\_1 or > 5/(R\_1 + R\_2)
- > deuterated protein

Setting of \( \Delta T = T_b - T_a \)
Part 3

Implementation of PRE restraints

\[ E_{\text{Restraint}} = k \sum w_i (P_{\text{obs}} - P_{\text{calc}})^2 \]

\[ \Gamma_2 = \frac{1}{15} \left( \frac{\mu_0}{4\pi} \right)^2 \gamma_j^2 g^2 \mu_e^2 s(s+1)[4J(0) + 3J(\omega)] \]

for pre in (preSite1, preSite2, preSite3):
    pre.setTcType("fix")
    pre.setTauC(12.8)
    pre.setTiType("fix")
    pre.setTtType("fix")
    pre.setEquType("sb")
    pre.setSbmfType("taut")
    pre.setTauI(0.000)
    pre.setTtI(0.000)
    pre.setTcI(0.000)
    pre.setTnfType("const")
    pre.setRlxType("r2dd")
    pre.setFreq(1000.14)
    pre.setSqn(0.5)
    pre.setGfac(0.4472)
    potList.append(pre)
    rampedParams.append( MultRamp(.01,10,"pre.setForce(VALUE)" ) )
    pass

PRE restraint format

• Ensemble representation
• sb mode and sbmf mode

Part 4

Ensemble representation of paramagnetic probe

XPlor-NIH: SB and SBMF mode

\[ \Gamma_S = S_{PRE}^2 < r^{-6} > f_{SB} (\tau_r) + (1 - S_{PRE}^2) < r^{-6} > f_{SB} (\tau_r) \]

\[ S_{PRE}^2 \approx S_{PRE, angular}^2 S_{PRE, real}^2 \]

\[ < r^{-6} > = \frac{1}{N} \sum_i r_i^{-6} \]

\[ \frac{1}{\tau_r} = \frac{1}{\tau_c} + \frac{1}{\tau_i} \]
Part 5

Xplor-NIH

- Xplor 3.851
- CNS
- CYANA
- python interface

Invoke Xplor

% xplor -py
% xplor
% pyXplor

XPLOR>python

- command line input
- script based

xplor.command('struct @1gb1.psf')

Flow scheme

- PDB (mutant), PSF
- Topology, parameter file
- build hydrogen
- patch thiol linkage
- duplicate
- randomize the paramagnetic probe
- refine against PRE

$xplor_installation/eginput/pre/
2 INI

REMARKS FILENAME="..//Psf/totalPlus3Alt.psf"
REMARKS DATE:21-Aug-03 15:13:32 created by user: tangc

3103 INATOM
1  1  MET  N   NH3 -0.100000 14.0070  0
2  1  MET  HT1 H   C   0.260000  1.0080  0
3  1  MET  HT2 H   C   0.260000  1.0080  0
4  1  MET  HT3 H   C   0.260000  1.0080  0
5  1  MET  CA  CT -0.220000 12.0110  0
6  1  MET  HA  HA -0.100000  1.0080  0
7  1  MET  CB  CT -0.200000 12.0110  0

1246 INBOND: bonds
1  5       5         6         5         7         7         8
7  9       7         10        10        11        10        12
10 13      12        14        14        15        14        16
14 17      5         18        18        19        19        2
3  1       4         1         20        21        20        22
22 23      22        24        24        25        24        26
26 27      27        28        27        29        27        30
30 31      30        32        32        33        32        34
22 35      35        36        18        20        37        38

PDB file of MTSL conjugate

ATOM  1 CA  MTS  79  -0.557  -0.793  99.449  1.00 20.00 CO Ca
ATOM  2 N01 MTS  79  0.353  0.222 101.367  1.00 20.00 CO
ATOM  3 O01 MTS  79  1.044  0.878 102.147  1.00 20.00 CO
ATOM  4 SG  MTS  79  0.128  -2.683  97.596  1.00 20.00 CO
ATOM  5 C02 MTS  79  -1.306  -1.146 100.479  1.00 20.00 CO
ATOM  6 C03 MTS  79  -0.852  -2.566 101.782  1.00 20.00 CO
ATOM  7 C04 MTS  79  -1.092  -1.416 100.131  1.00 20.00 CO
ATOM  8 C05 MTS  79  -0.554  -1.146 100.127  1.00 20.00 CO
ATOM  9 C06 MTS  79  0.460  1.229 100.300  1.00 20.00 CO
ATOM 10 C07 MTS  79  -0.384  -1.655 100.754  1.00 20.00 CO
ATOM 11 C08 MTS  79  -0.002  -0.346 101.349  1.00 20.00 CO
ATOM 12 C09 MTS  79  -0.272  -0.546 100.984  1.00 20.00 CO
ATOM 13 C10 MTS  79  -0.332  -0.332 102.669  1.00 20.00 CO
ATOM 14 C11 MTS  79  0.987  1.737 100.321  1.00 20.00 CO
ATOM 15 C12 MTS  79  -1.558  0.257 100.147  1.00 20.00 CO
ATOM 16 C13 MTS  79  0.243  -0.559 98.620  1.00 20.00 CO
ATOM 17 C14 MTS  79  2.264  2.506 99.765  1.00 20.00 CO
ATOM 18 C15 MTS  79  -0.513  1.981 99.479  1.00 20.00 CO
ATOM 19 C16 MTS  79  0.654  1.555 98.219  1.00 20.00 CO
ATOM 20 C17 MTS  79  -0.388  -2.304 100.315  1.00 20.00 CO
ATOM 21 C18 MTS  79  -1.481  0.840 103.305  1.00 20.00 CO
ATOM 22 C19 MTS  79  -1.323  0.313 103.048  1.00 20.00 CO
ATOM 23 C20 MTS  79  -0.500  -1.229 105.668  1.00 20.00 CO
ATOM 24 C21 MTS  79  -1.877  -1.666 105.384  1.00 20.00 CO
ATOM 25 C22 MTS  79  -1.841  0.171 101.704  1.00 20.00 CO
ATOM 26 C23 MTS  79  -3.131  -3.346 97.848  1.00 20.00 CO
ATOM 27 C24 MTS  79  -3.621  -1.450 98.548  1.00 20.00 CO

Generate topology/parameter for paramagnetic probe

http://xray.bmc.uu.se/hicup/xdict.html

Topology file example

Parameter file example
Generate psf file from pdb file

```plaintext
! delete select (resi 3) endwrite psf output=1xyz.psf endstop
```

Generate psf file for non-peptide

```plaintext
write psf output=MTSL.psf endstop
```

Example of the psf file

```plaintext
write psf output=para_E.psf endwrite coor output=para_E.pdb endstop
```

Build hydrogen atoms

```plaintext
hbuild select=(name H*) phistep=360 endflags exclude * include bonds angle impr endconstraint fix (not name H*) endmini powell nstep 1000 end
```

Patch thiol linkage

```plaintext
patch ES reference=1=(resi 112) reference=2=(resi 502) endwrite psf output=para_E.psf endwrite coor output=para_E.pdb endstop
```
Various energy functions in Xplor-NIH

- noePot - NOE distance restraints
- rdcPot - dipolar coupling
- csaPot - Chemical Shift Anisotropy
- cstMagPot - regne against chemical shift tensor magnitudes and orientations
- jCoupPot - 3J-coupling
- psrPot - Paramagnetic relaxation enhancement
- gyrPot - pseudopotential enforcing correct protein density
- dfPot - refine against rotational diffusion tensor
- residueDistPot - contact potential for hydrogen attraction/hrepulsion
- planeDistPot - distance between atoms and plane
- xplorPot - use XPLOR potential terms
- slowScanPot - potential tor solution X-ray and neutron scattering
- posSymmPot - restrain atomic positions relative to those in a similar structure
- sardcPot - RDCs in steric alignment media

Duplicate paramagnetic probe

```plaintext
parameter @parallhdg_new.pro end
structure @xyz_3atcun.psf end
coor @Random/startup4_13.pdb
vector do (segid "ALTU")
   (name cu or (resi 48 or resi 63) and (name cb or name hb or name sg))
for $an in (1 2 3 4 5 6 7 8 9) loop alts
   evaluate ($al = "ALT" + encode($an) )
   duplicate
      segid = $al
      select = ( segid "ALTU" )
   end
end loop alts
write psf output=xyz_3atcun10.psf end
write coor output=xyz_3atcun10.pdb end
stop
```

Randomize paramagnetic probe

```plaintext
import protocol
duplicate protocol.loadPDB("model.pdb")  #initialize coordinates
coolParams=[[] # a list which specifies potential smoothing
   set up potential terms from NMR experiments, covariant geometry,
   and knowledge-based terms
   # initialize coolParams for annealing protocol for each energy term
from ivs import FIV
   configure which degrees of freedom to optimize
   dyn = FIV()
from simulationTools import AnnealFIV
   coolLoop=AnnealFIV(dyn,...)  #create simulated annealing object, specify temperature schedule
   def calcOneStructure( structData ):
      """ a function to calculate a single structure """
      # [ randomize velocities ]
      # [ perform high temp dynamics ]
      dyn.run()
      # [ cooling loop ]
      coolLoop.run()
      # [ final minimization ]
      dyn.run()
      structData.writeStructure(pdbFile)  #write val pdb record to file
      # with energies, rmsid in headers
      # a separate .vio file also written
from simulationTools import StructureLoop
   StructureLoop(numStructures=100,  #calculate all structures
      pdbTemplate='SCRIPT_STRUCTURE.xm',  # in parallel, if desired
      structLoopAction=calcOneStructure).run()  #also report stats at end
```

Skeleton of a typical script
Multiple conformer/ensemble representation

Two tiers and two timescale

\[ \Gamma^{\text{calc}}(i) = (1 - \lambda)\Gamma^{\text{specific}}(i) + \lambda \sum_{j} \Gamma^{\text{specific}}(i, j) / N \]

stereospecific complex
(population 1-λ)

Encounter complex
(population λ)

N HPr conformers by fixing EIN coordinates

Implementing PRE restraints

Sample script: randomize_init.inp

Duplicate and randomize an entire protein or a portion of a protein

Sample script: randomize_init.inp

Ensemble structure refinement against PRE

Sample script: optimize.py
Evaluate the agreement between observed and calculated $Q$:

$$Q_e = \frac{\sum_{i=1}^{n} Q_i^{2,obs}}{\sum_{i=1}^{n} Q_i^{2,cal}}$$

Cross-validation of PRE restraints

- Perturbation due to mutation / conjugation
- A random subset of PRE restraints
- Perturbation due to mutation / conjugation

Intermediate exchange time-scale (upper is to lower ms):

- Shorter distance
  - Minor population
  - 8Å ($k_B T = 5600 s^{-1}$, $p_B = 1\%$)
- Longer distance
  - Main population
  - 34Å ($k_B T = 2 s^{-1}$, $p_A = 99\%$)

$$Q_{e, obs} = Q_{e, A} \times 0.99 + Q_{e, B} \times 0.01 = 58 s^{-1}$$

Qualitative application of PRE (single structure):

- Ubbink et al. Biochemistry 2008

Part 5
Simulating McConnell equation

\[ I_2 = I_{2,X} = 2 \text{ s}^{-1} \]
\[ I_{2,\text{min}} = 2 \text{ s}^{-1} \]
\[ I_{2,\text{max}} = 61 \text{ s}^{-1} \]

\[ k_{\text{ex}} = 40 \text{ s}^{-1} \]
\[ k_{\text{ex}} = 4,000 \text{ s}^{-1} \]
\[ k_{\text{ex}} = 40,000 \text{ s}^{-1} \]
Structure of protein by SAXS

Dr. Yu-Shan Huang
SAXS Reveals Protein Structures

Huang, Yu-Shan
NSRRC, Taiwan

Methods for 3D Structures of Biomolecules

- Nuclear Magnetic Resonance Spectroscopy
  - Atomic resolution
  - Difficulties in large protein studies
  - Excellence for dynamic studies
- Small-Angle X-ray Scattering
  - Resolution up to 1 nm
  - Excellence for functional solution structures, dynamic studies, and large proteins
- Macromolecule Crystallography
  - Atomic resolution
  - Bottleneck for having a crystal
  - Difficulties in dynamic studies
- Cryo-Electron Microscopy
  - Resolution up to 1 nm
  - Excellence for large proteins

Small-Angle X-ray Scattering

SAXS is capable of revealing the aperiodic system, e.g. protein solutions. The protein particles in solutions are:
- embedded with arbitrary orientation and with irregular distances in a matrix,
- limited in size, non-oriented, and aperiodic,
- assumed to be identical.

Solution structures can be probed by SAXS.
- For diluted monodisperse systems, SAXS can determine size (1 ~ 100 nm), shape, and the internal structure.
- For diluted polydisperse systems, SAXS can evaluate a size distribution under the assumption of a certain shape of the particles.
- For semi-dilute systems, the scattering curve is the product of the particle scattering function and of the interparticle interference function.

Books on SAS

Reviews on SAS


SAXS and NMR

EM wave interacts with materials

Elastic (Thomson-Rayleigh) scattering: one electron

Classical dipole approximation

$$E_{\text{mol}}(R, t) = -E_{0} e^{2} e^{i\lambda R} \cos 2\theta \quad r_{c} = \frac{e^{2}}{4\pi\varepsilon_{0} m c^{2}} = 2.82 \times 10^{-5} \ \text{Å} \quad \frac{I_{0}}{I_{0}} = \frac{\left|E_{\text{mol}}\right|^{2} r_{c}^{2} \Omega}{\mid \Omega \mid}$$

Differential cross-section

$$\frac{d\sigma}{d\Omega} = \left(\frac{I_{0}}{I_{0}}\right)^{2} \Omega = r_{c}^{2} \cos^{2} 2\theta = r_{c}^{2} p \quad \sigma_{\text{mol}} = \frac{\pi}{3} r_{c}^{2} = 0.665 \text{barn}$$

where $p$ is 1, $\cos 2\theta$, or $(1 + \cos 2\theta)/2$ for vertical, horizontal scattering planes of a horizontally polarized source, or unpolarized source, respectively.
Elastic scattering: atom and molecule

Total scattering length of one atom

\[-r_f f(Q) = -r_f \int \rho(r)e^{iQ \cdot r} dV\]

Atomic form factor

\[f(Q, \lambda) = f^0(Q) + f'(\lambda) + i f''(\lambda)\]

Total scattering length of one molecule

\[-r_F f_{mol}(Q, \lambda) = -r_F \sum r_i f_j(Q, \lambda)e^{iQr_i}\]

Based on relativistic Hartree-Fock calculations in IUCr Int’l table vol. C 6.1.

SAXS: elastic scattering

Incident beam \(I_0\)

\[E(\vec{r}, \tau) = E_0(\vec{r})e^{i(k \cdot r - \omega \tau)}\]

Transmitted beam \(I_t\)

\[\rho(\vec{r})\]

\(Q = 4\pi \sin \theta / \lambda\)

Small-Angle X-ray Scattering

\[I(Q) = I_o \tilde{P}(Q) S(Q)\]

\[I_o = n_p (\Delta \rho)^2 V^2\]

\(n_p\): Number density of particles

\(V\): Volume of the particle

\(A_p\): Scattering contrast of particles to the matrix

\(\tilde{P}(Q)\): Particle Form Factor

\(S(Q)\): Structure Factor

It defines the relationship between the positions of the particles
Sample requirements: Ideality and Monodispersity

**Ideality:**
- No intermolecular interaction at infinite dilution
- SAXS pattern is independent of concentration
- AUC, DLS

**Monodispersity:**
- Identical particles
- Purification protocol
- Mass Spec., AUC, DLS

Small-Angle X-ray Scattering

For SAXS measurement, inelastic scattering is a smaller effect than elastic scattering. It only contributes to the background and does not yield any structural information.

\[
-r_v f(Q) = - r_v \int \rho(r) e^{-iQr} dV
\]

If the scatterers take all orientations,

\[
f(Q) = 4 \pi \int_0^\infty \rho(r) \frac{\sin Qr}{Qr} r^2 dr \quad \left( e^{iQr} \right)_\Omega = \frac{\sin Qr}{Qr}
\]

\[
f(0) = 4 \pi \int_0^\infty \rho(r) r^2 dr = Z
\]

Debye formula (1915)

\[
I(Q) = \sum_{i=1}^{N} \sum_{j=1}^{N} f_i(Q) f_j(Q) \frac{\sin Qr_{ij}}{Qr_{ij}}
\]

SAXS Distinguishes Shapes and Sizes

- **Sphere**
  \[\tilde{P}(Q) = (3j_1(QR)/QR)^2\]

- **Ellipsoid**
  \[\tilde{P}(Q) = \int_0^a 3j_1(v)^2 \frac{dv}{v} d \mu\]

- **Rod or disk**
  \[\tilde{P}(Q) = \int_0^1 2f(w) \sin(w)^2 \frac{dw}{w} d \mu\]

Small-Angle X-ray Scattering

\[A(Q) \cdot A'(Q) = \int \rho(\mathbf{r}) \rho(\mathbf{r}) e^{-iQr} dV_i dV_j \quad A(Q) = \int \rho(\mathbf{r}) e^{-iQr} dV\]

\[= \int \rho(\mathbf{r}) \rho(\mathbf{r} - \mathbf{r}) e^{-iQr} dV_i d(V_i - V)\]

\[= V \gamma(\mathbf{r}) e^{-iQr} dV_i = V \gamma(\mathbf{r}) e^{-iQr} dV\]

\[V \gamma(\mathbf{r}) = 4 \pi \int_0^\infty r^2 \rho(\mathbf{r}) \rho(\mathbf{r} - \mathbf{r}) dr_i\]

\[I(0) = \int \rho(\mathbf{r}) \rho(\mathbf{r}) dV_i dV_j = \tilde{P}^2 V^2\]

\[I(0) = 4 \pi \int_0^\infty r^2 \gamma(\mathbf{r}) \frac{\sin Qr}{Qr} dr = 4 \pi \int_0^\infty r^2 \gamma(\mathbf{r}) dr\]
Analytical scattering intensity of globular particles

\[ I(Q) = \left( \frac{3 \sin Q R - Q R \cos Q R}{Q R} \right)^2 \]

\[ p(r) = 12 x^2 (2 - 3x + x^3) \quad x = r / 2R \leq 1 \]

Correlation function

Correlation (characteristic) function (Porod, 1951)

\[ V_\gamma(r) = 4\pi \int_0^r \rho(\tau) \rho(r - \tau) d\tau \]

\[ I(Q) = 4\pi \int_0^\infty r^2 V_\gamma(r) \frac{\sin Q r}{Q r} dr \]

\[ V_\gamma(r) = \frac{1}{2\pi^2} \int_0^\infty I(Q) Q^2 \frac{\sin Q r}{Q r} dQ \]

Porod Invariant \[ \Delta = 2\pi V_\gamma(0) = \int_0^\infty I(Q) Q^2 dQ \]

\[ V_\gamma(0) = 4\pi \int_0^\infty \rho^2(\tau) d\tau = V_\gamma \]

Correlation length (i.e. mean width of \( \gamma(r) \))

\[ I_l = \frac{\pi}{\Delta} \int_0^\infty I(Q) Q dQ \]

Volume of particles \[ V = 2\pi^2 \frac{I(0)}{\Delta} \]

Pair-distance distribution function

Pair-distance distribution function (Guinier, 1955)

\[ p(r) = r^2 V_\gamma(r) \]

\[ I(Q) = 4\pi \int_0^\infty p(r) \frac{\sin Q r}{Q r} dr \]

\[ p(r) = \frac{1}{2\pi^2} \int_0^\infty I(Q) Q^2 \sin Q r dQ \]

\[ I(0) = 4\pi \int_0^\infty p(r) dr = 4\pi \int_0^\infty r^2 V_\gamma(r) dr \]

\[ p(50) = 4\pi(50)^2 \int_0^{50} \rho(\tau) \rho(\tau - 50) d\tau \]

Guinier Law and Radius of gyration

Guinier approximation is applied for the innermost part of the scattering curve.

\[ I(Q) = I(0) \exp(-Q^2 R_g^2 / 3) \quad 0 < Q R_g < 1.3 \]

Guinier plot of \( \log I(Q) \) vs \( Q^2 \) shows a linear descent with a slope \( \tan \alpha \), where

\[ R_g^2 = K^2 \tan \alpha \quad \tan \alpha = \frac{\Delta \log I(Q)}{Q^2} \quad K = \sqrt{\frac{\Delta}{\log e}} = 2.628 \]
Radius of gyration of simple homogenous bodies

Sphere \[ R_g^2 = \left( \frac{3}{5} \right) R^2 \]
Hollow sphere \[ R_g^2 = \left( \frac{3}{5} \right) \frac{R_2^5 - R_1^5}{R_2^5 - R_1^5} \]
Ellipsoid \[ R_g^2 = \frac{1}{5} \left( a^2 + b^2 + c^2 \right) \]
Parallelepiped \[ R_g^2 = \frac{1}{12} \left( a^2 + b^2 + c^2 \right) \]
Elliptic cylinder \[ R_g^2 = \frac{a^2 + b^2}{4} + \frac{h^2}{12} = R_2^2 + \frac{h^2}{12} \]
Hollow cylinder \[ R_g^2 = \frac{R_1^2 + R_2^2}{2} + \frac{h^2}{12} \]

Radius of gyration of the cross section \( R_c \)

For rod-like particles
\[
R_c^2 = \frac{\int r^2 p_r(r) dr}{\frac{1}{2} \int p_r(r) dr} \quad I_c(Q) = I(Q) Q^2 \]
\[
p_r(r) = \frac{1}{2\pi} \int I_c(Q) Q \sin(QrdQ) \]
\[
I_c(Q) = I_c(0) \exp\left(-Q^2 R_c^2 / 2\right) \]
\[
R_c^2 = K_c^2 \tan \alpha \quad \tan \alpha = \frac{\Delta \ln I_c(Q)}{Q^2} \]
\[
K_c = \frac{1}{\sqrt{\log 2}} = 2.146 \]

Cross section
\[
A = \frac{2\pi \int_0^\infty I_c(Q) dQ}{\Delta} \]

Radius of gyration of the thickness \( R_t \)

For disc-like particles
\[
R_t^2 = \frac{\int r^2 p_r(r) dr}{\frac{1}{2} \int p_r(r) dr} \quad I_t(Q) = I(Q) Q^3 \]
\[
p_r(r) = \frac{1}{2\pi} \int I_t(Q) Q \sin(QrdQ) \]
\[
I_t(Q) = I_t(0) \exp\left(-Q^2 R_t^2 / 2\right) \]
\[
R_t^2 = K_t^2 \tan \alpha \quad \tan \alpha = \frac{\Delta \ln I_t(Q)}{Q^2} \]
\[
K_t = \frac{1}{\sqrt{\log 2}} = 1.517 \]

Thickness
\[
T = \pi \frac{\int_0^\infty I_t(Q) dQ}{\Delta} \]

Absolute intensity and molecular mass

\[
I(0)/c \propto M.M. \]

Irreversible aggregation

Gainet plot

1.6 mg/ml
3.4 mg/ml
7 mg/ml
Interaction and 2nd virial coefficient

In the case of intermolecular interactions, \( I(0)c \) is concentration dependant.

\[
I(0, c) = \frac{I(0)_\text{ideal}}{1 + 2A_2Mc + ...} = \frac{Kc}{1 + 2A_2Mc + ...}
\]

Porod Law: asymptotic regime

Hypothesis: The particle has a uniform electron density and a sharp interface with the solvent.

\[
l_\text{lim}_{Q \to \infty} [I(Q)] = 2\pi S \Delta \rho Q^{-4} + B
\]

\( S \) is the area of the solute/solvent interface

Kratky plot: \( Q^2I(Q) \) vs \( Q \)

The degree of compactness

Globular particle: bell-shaped curve (asymptotic behaviour in \( q^4 \))

Gaussian chain: plateau at large \( Q \) (asymptotic behaviour in \( q^2 \))

\[
\lim_{Q \to \infty} [Q^2I(Q)] = \frac{20 - (QR)^{-2}}{R_g^2}
\]

SAXS

Scattering contrast:

\[
I_{\text{contrast}}(Q) = I_{\text{solution}}(Q) - I_{\text{solvent}}(Q)
\]

Scattering length density:

Protein \( \sim 1.21 \times 10^{11} \text{ cm}^{-2} \); Solvent \( \sim 9.4 \times 10^{10} \text{ cm}^{-2} \); Bound solvent \( \sim 1.14 \times 10^{11} \text{ cm}^{-2} \)
Spherical Harmonics Approach

\[ I(Q) = 2\pi^2 \sum_{l=0}^{\infty} \sum_{m=-l}^{l} |A_{lm}(\bar{Q}) - \rho_0 \tilde{E}_{lm}(\bar{Q}) + \delta \rho \tilde{B}_{lm}(\bar{Q})|^2 \]

\[ A_{lm}(\bar{Q}) = 4\pi \theta^2 \sum_{j} f_j(Q) \sum_{l=0}^{\infty} \sum_{m=-l}^{l} j_l(Qr) Y_{lm}^*(\theta, \varphi) \]

Form factor of the atomic group:

\[ f_j(Q) = \sqrt{\sum_{i=1}^{N} \sum_{k=1}^{N} f_i(Q) f_k(Q) \frac{\sin d_{ik}Q}{d_{ik}Q}} \]

Form factor of the dummy solvent atom:

\[ g_j(Q) = G(Q) V_j \exp(-\pi Q^2 V_j^2) \]

Angular envelope function:

\[ F(\theta, \varphi) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} f_{lm} Y_{lm}^*(\theta, \varphi) \]

Crysol: predict solution scattering curve with a pdb

Crysol

Number of points: 250
Fit exp. Curve: n
CRYSOL—predict solution scattering curve with a pdb

Input: pdb file
Output: *log → record parameter used in CRYSOL calc. and also final calc. Rg value
*alm → net partial amplitude
*int → scattering intensities

\[ I_{\text{difference}} = \sqrt{I_{\text{atomic}}^2 - I_{\text{shape}}^2 - I_{\text{border}}^2} \]

Oligomers

SAXS data of lysozyme (6LYZ) by CRYSOL

Primus: data reduction and verification

Click "Tool" to open the "data processing" tab
Click select to input your data, click plot to plot your data.
From SAXS analysis by using ATSAS package (Svergun et al), it is possible to:

- Model the structure evolution of macromolecules during unfolding process (Gasbor, Dammin),
- Validate theoretically predicted models (Crysol),
- Analyze similarities between macromolecules in solution and in the crystal (Crysol),
- Model the quaternary structure of multi-subunit complex by rigid-body refinement against SAXS data (Sasref, Bunch, Monsa).
Dummy Residue Simulation: Gasbor

Re-calculate SAXS profile

Fit p(r) using Gasbor based on ab initio model analysis

CRYSOL + modified PDB

Comparison of crystal structure and solution structure

Unfolding Process of Lysozyme by Temperature

Dummy residues models (beads) fitted to SAXS data at various temperatures and crystal structures of lysozyme (ribbons) from PDB.

Comparison of crystal structure and solution structure

Rigid-body Refinement Reveals Quaternary Structure

SASREF data fitting

Di-domain structure model

Rigid Body Refinement for an integrated PDB of the two sub-units