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SLDMOL: A tool for the structural characterization of thermally disordered membrane proteins☆

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ABSTRACT

SLDMOL is a program for modeling the 1-D scattering length density (SLD) profile of proteins at the lipid membrane–solution interface or adsorbed to other surfaces. The program reads experimental SLD data from neutron or *X*-ray reflectivity measurements and compares the results to a trajectory of protein structures, finding the conformation and orientation that best fits the experimental data. SLDMOL is a freely distributed open source program written in python that can be run independently using command lines or a GUI. SLDMOL has also been integrated into the larger SASSIE package extending molecular modeling capabilities. Sample environment conditions can be replicated including H_2O/D_2O solvent contrasts, specific amino acid deuteration and complex molecular assemblies. Ensembles of protein conformations can be generated independently (e.g. molecular dynamics simulations) or with SASSIE. For each individual structure a best-fit SLD profile is outputted along with a goodness of fit parameter, protein depth penetration and surface coverage. In addition to individual comparisons SLD profiles can be calculated over ensemble averages of protein structures. As a result, SLDMOL provides a detailed molecular interpretation of reflectivity data or conversely can be used to predict experimental outcomes for different protein conformation and specific deuteration schemes prior to measurements.

Program summary

Program title: SLDMOL

Catalogue identifier: AETX_v1_0

Program summary URL: http://cpc.cs.qub.ac.uk/summaries/AETX_v1_0.html

Program obtainable from: CPC Program Library, Queen's University, Belfast, N. Ireland

Licensing provisions: GNU General Public License, version 3

No. of lines in distributed program, including test data, etc.: 330 2107

No. of bytes in distributed program, including test data, etc.: 125 715 760

Distribution format: tar.gz

Programming language: Python.

Computer: PC/Mac.

Operating system: 32- and 64-bit Linux (Ubuntu 10.04, Centos 5.6) and Mac OS X (10.7–10.9).

RAM: 1 GB

Classification: 3.

External routines: Python 2.6.5, numpy 1.4.0, scipy 0.8.0, Tcl 8.5, Tk 8.5, Mac installation requires Xcode 3 development tools.

☆ This paper and its associated computer program are available via the Computer Physics Communication homepage on ScienceDirect (http://www.sciencedirect.com/science/journal/00104655).

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Subprograms used:

CatId	Title	Reference
AEKL_v1_0	SASSIE	CPC 183(2012)382

Nature of problem:
Reflectivity is a powerful technique to study the conformation of biological molecules at surface and interfaces. Open source software that facilitates computational modeling and interpretation of experimental results in terms of detailed molecular structure is currently lacking.

Solution method:
SLDMOL takes one or an ensemble of atomistic configurations of proteins and compares them to 1-D scattering length density (SLD) profiles determined from reflectivity experiments. Protein structures can be generated independently (e.g. molecular dynamics simulations) or through the SASSIE software package. SLDMOL performs an optimization to determine which structure best fits the SLD profile. Structures can also be averaged either directly or through a Monte-Carlo weighting algorithm. Finally SLDMOL allows the modeling of reflectivity experiments through the in silico selective deuteration of amino acids and the calculation of SLD profiles in a number of different aqueous solvent contrasts.

Additional comments:
!!!!Due to the large file size, SLDMOL is not delivered directly when download or Email is requested. Instead an html file giving details of how the program can be obtained is sent.!!!!

Running time:
Varies depending on application. Typically 10 min to 24 h depending on the number of structures to be evaluated.

1. Introduction

High resolution structural characterization of membrane bound proteins, is itself a significant challenge, but only provides part of our understanding with regards to function. The spatial organization of these proteins with respect to the lipid membrane is also important for their activity in biological processes. However, information such as the binding orientation of peripheral membrane proteins, conformational changes and subunit organization of protein complexes are often lacking in high-resolution structures. Because biological membranes are thermally disordered environments, non-crystallographic scattering approaches are required. *X*-ray and neutron reflectivity have been applied in the past to characterize thin fluid films at the sub-nanometer level [1]. Lately, reflectivity has emerged as powerful tools to study the complex molecular architecture of biological membrane systems [2–7].

Typical analysis methods of measured specular reflection provide a 1-D scattering length density (SLD) profile normal to the membrane plane. The SLD profile represents an envelope structure sensitive to the distribution of protein atoms projected along the normal vector (*z*-axis). A particularly important advancement has been the integration of high-resolution data and molecular modeling with reflectivity to yield a 3-D view of proteins on the membrane [3–5]. The SLDMOL program is intended as a tool to facilitate refinement of reflectivity results with atomistic protein models. In addition the incorporation of SLDMOL as a module in the SASSIE software package [8] provides extended molecular modeling capabilities. Installation of SLDMOL is handled by an installation python script, further details are provided in the CPC program library.

A chief characteristic of neutron scattering is the contrast in intensity between hydrogen and deuterium isotopes. As a result, for biological samples, isomorphic replacement provides a non-invasive probe allowing specific structures within a molecular complex to be highlighted. Furthermore "contrast variation" of aqueous buffers consisting of different H_2O/D_2O mixtures can further serve to enhance scattering from the regions of interest. However, selective deuteration of protein amino acids involves complex cloning and expression procedures. SLDMOL can model multiple amino acid deuteration schemes showing which ones provide the largest changes in SLD profiles aiding in the design of protein constructs.

In addition to membrane proteins, the interaction of proteins with different surfaces has significant importance in pharmaceutical and biotechnological research. SLDMOL can easily be used to refine reflectivity measurements of protein structure at any surface interface. Here we introduce the main features of SLDMOL and provide examples of its use on the membrane bound HIV-1 Gag protein that is important for viral assembly [9].

2. Features and usage

2.1. Overview

SLDMOL can be used either as a fitting utility to find protein conformations that best match experimental scattering length density profiles or as a modeling utility to generate hypothetical density profiles with differing conditions. Both functions result in scattering length density profiles of protein structures on an experimentally measured membrane film.

Protein conformations in standard pdb or dcd formats are read in one by one, profiles are generated by calculating the scattering length and the cross-sectional area of the protein structure projected along the membrane surface normal (*z*-axis). Each protein amino acid is approximated as a sphere with a tabulated volume previously reported for proteins in solution [10]. The total scattering length of each amino acid is distributed evenly throughout the sphere and is calculated based on the amino acid's chemical composition. For neutron scattering profiles additional terms: specific deuteration of residues, number of exchangeable hydrogens and the H_2O/D_2O ratio of the bulk aqueous solvent are also taken into account.

In fitting mode, the SLD profile calculated for each protein structure can be averaged together or evaluated on an individual basis. Two parameters are optimized when fitting the SLD profiles to experimental data, the *z*-position of the protein and the total

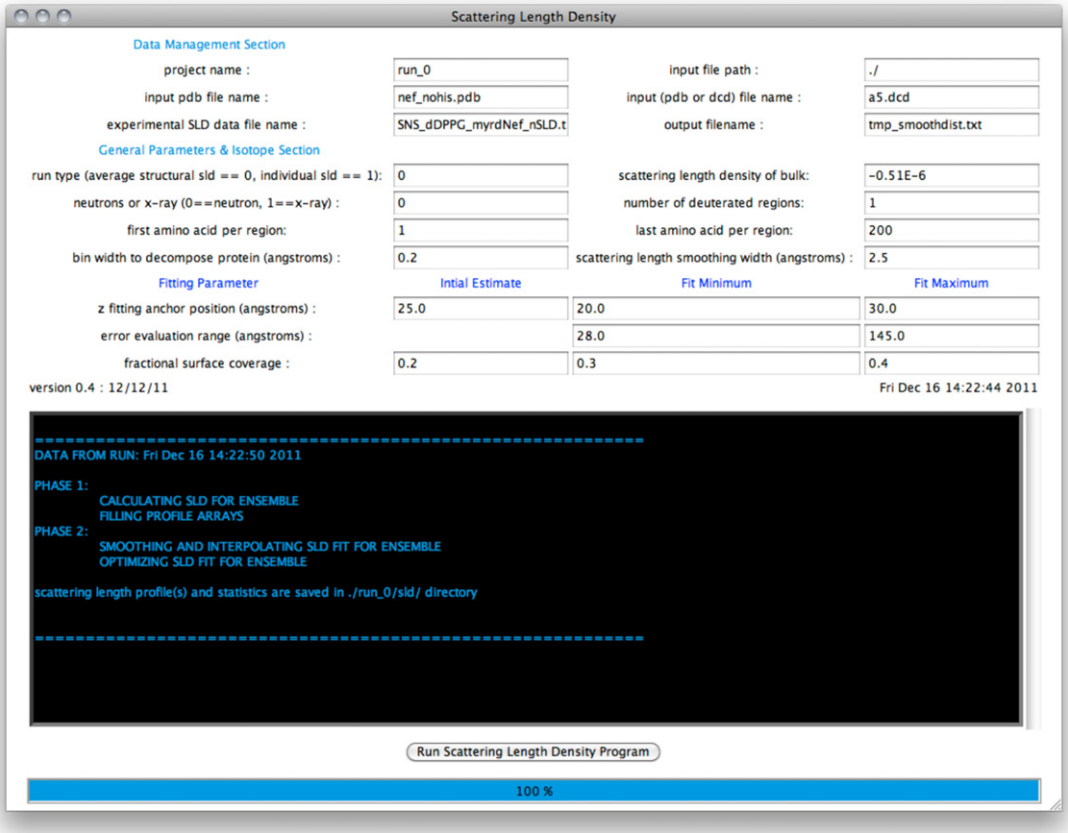

Fig. 1. Snapshot of SLDMOL GUI integrated in the SASSIE software package.

surface coverage. When modeling, these values can be specified and fixed for all the structures. A snapshot of the SLDMOL GUI shown in Fig. 1 indicates entry places for filenames and parameter values required for SLD calculation and fitting. These entries will be explained in greater detail in later sections.

SLDMOL outputs SLD profiles for each structure as well as the best-fit parameter values and a goodness of fit metric. A DCD file containing the protein structures aligned along their best-fit z-position is also generated. As an initial analysis a plot of the goodness of fit versus frame number as well as of the best, worst and average SLD profile are both plotted and saved as an ASCII file.

2.2. Fitting utility

The first section, "Data Management Section", shown in the GUI of Fig. 1 defines important input and output files for setting up a run. Each session is given a *project name* that will be used to create a file folder in the current working directory. All output files generated by SLDMOL will be saved in this folder. Input files can reside in different folders, the user must define a directory path. The first *input pdb file* field requires the file name of a pdb structure file of the protein and will provide the program with a correlation between atom # with its corresponding atom name and amino acid residue. The next field, *input (pdb or dcd) file,* requires the name of the file that contains the ensemble of protein conformations to be fit. This file can be either in standard pdb file format or in the CHARMM dcd trajectory file format. Several programs exist for interconverting different file formats from molecular dynamics trajectories [11] to those supported by SLDMOL. The 1-D SLD profile resulting from reflectivity measurements that the protein conformations will be compared to is given in the *experimental SLD data file name* field. The output data files will be described in a later section.

The "General Parameters & Isotope Section" is used to define the universal behavior of the SLDMOL program. The list of parameters and a brief description is given below:

- *run-type*: Sets whether a trajectory of structures will be evaluated individually or on average. (0 == calculate average SLD profile, 1 == calculates individual SLD profiles).
- *model or fit mode*: determines whether SLDMOL will attempt to fit each protein conformation to experimental SLD profiles or not (0== model mode, 1== fit mode).
- *neutron or X-ray*: calculate neutron density profiles or electron density profiles (0==neutron, 1 == X-ray)
- *scattering length density of bulk*: SLD value for the aqueous solvent used in the experiment, units of Å^{-2} for neutron measurements and $e/\text{Å}^3$ for X-ray. This value can be obtained from the experimental SLD profile data and will determine the H_2O/D_2O ratio of the bulk solvent for neutron measurements. This will impact the scattering contribution of exchangeable hydrogens in the protein.
- *number of deuterated regions*: how many amino acid regions of the protein are specifically deuterated (must be a number >= 0)
- *first amino acid per region*: a list of amino acid numbers that define the beginning residue for each set of specifically deuterated regions. The length of the list should be equal to the integer value given for *number of deuterated regions*. Residue numbering is based on that given in the input pdb file.
- *last amino acid per region*: a list of amino acid numbers that define the last residue for each set of specifically deuterated regions.

- *bin width to decompose protein*: spatial resolution along z with which the SLD profile will be calculated
- *scattering length smoothing width:* Gaussian function sigma value for smoothing the calculated SLD profile, used to approximate instrument resolution limit.

The final section of the SLDMOL GUI defines the fitting parameters used by the optimization algorithm (*z fitting anchor position & fractional surface coverage*). For the aqueous region above the membrane surface the SLD profile is a linear combination of the contribution of both the bulk solvent SLD and the contribution from the bound protein. The protein contribution depends on the molecular volume along z, which is defined by the protein structure and on the total surface coverage. This last term then is defined as a free fit parameter. The z anchor position adjusts the placement of the protein SLD profile to best match that of the experiment. The user provides an initial estimate and minimum and maximum value constraints for the fit variables.

The *error evaluation range*, parameter is used to define the region of the experimental SLD profile to compare with the protein SLD. Usually a z range starting at the boundary between the lipid headgroup and aqueous phase and ending where the SLD reaches a value of the bulk aqueous phase is used.

SLDMOL provides several output results and preliminary data analysis after completing the fitting procedure. For each protein conformation, parameter fit values, a goodness of fit value and a SLD profile is written to a file using a '_sld[frame number]' suffix to the filename prefix defined in *output filename*. SLDMOL also writes a new dcd trajectory file with each protein conformation aligned with a z-value that corresponds to the fit-results. An ASCII text file containing the goodness of fit versus frame number is written with the suffix '_chisq_vs_frm'. Finally a second ASCII file containing the SLD profile of the best-fit protein structure, worst fit protein structure and average over all structures is written to disk and plotted on the screen.

2.3. Fitting utility example: HIV-1 Gag

As an example of the fitting module we show its application to neutron reflectivity (NR) data collected for the retroviral assembly protein HIV-1 Gag bound to a solid supported lipid membrane [12,13]. HIV-1 Gag is the single critical factor that drives the formation of new viral particles. Initially expressed in the cellular cytoplasm Gag proteins target the plasma membrane where they laterally self-organize and nucleate new viral assembly sites. Gag also incorporates the viral genomic RNA into the newly forming virion. Gag consists of three functional domains, listed from N to C terminus; they are the matrix (MA), capsid (CA) and nucleocapsid (NC) and are joined by flexible unstructured linkers. Cryo-electron microscopy images of immature virions showed the three Gag domains linearly extended about 200 Å from the membrane surface [14].

Neutron reflectivity measurements found that the Gag protein binds to the membrane surface in two distinct conformations [2], one configuration found Gag in a compact structure with dimensions of roughly 90 Å from the membrane surface. Fig. 2 shows the SLD profile of compact Gag (blue circles) and of the solid supported tethered membrane (gray line). Upon the inclusion of short single-stranded DNA segments the Gag protein underwent a dramatic conformational shift to an extended structure approximately 180 Å from the membrane surface. A proposed model for Gag assembly suggests that on the membrane Gag is originally folded over with both terminal domains MA and NC bound to the membrane surface. However in the presence of nucleic acid, the NC domain detached from the membrane surface to form energetically more favorable interactions with the single-stranded DNA. Thus forming

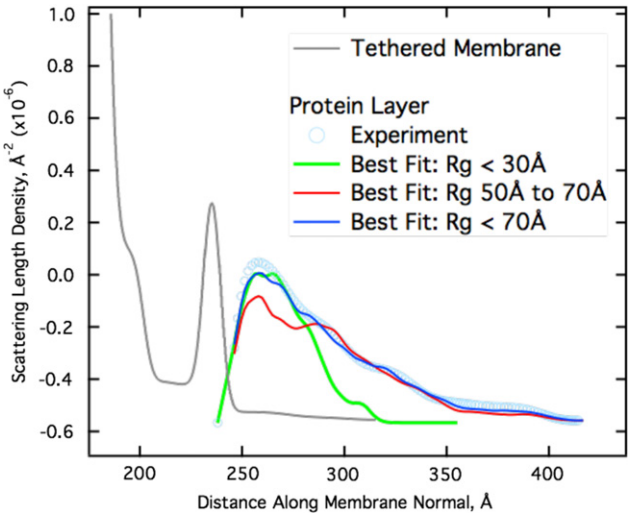

Fig. 2. Molecular modeling of Gag protein structure on the membrane using SLDMOL to fit experimentally derived SLD profiles. The experimentally derived SLD profiles for Gag, light blue circles, are shown in the compact conformational state, panel A, and the extended state, panel B. Using the SASSIE software package 100,000 independent conformations of the Gag protein were generated and subsequently filtered based on Rg values. An average SLD profile using a Monte-Carlo optimization to weight the structures provided a best fit for each Rg range and can be seen as solid lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the extended conformation observed in the earlier Cryo-EM studies. Because a high-resolution structure of the full length Gag protein has not been resolved the dimensional information obtained from NR was not rigorously interpreted in terms of molecular detail.

Using SLDMOL in combination with the SASSIE software package we further analyze our previous NR results to determine the organization of Gag domains that are capable of creating the compact SLD profile of Gag bound to the lipid membrane. SASSIE uses a Monte-Carlo search approach and backbone dihedral move sets to efficiently sample allowable conformational space of flexible protein regions. SASSIE also provides analysis tools for conformational ensembles, such as calculating and grouping independent structures according to their radius of gyration. Here we used SASSIE to sample the flexible protein linkers in Gag and generated 100,000 independent structures of the protein saved in dcd format. The fitting module of SLDMOL was then used to screen these structures against the experimentally derived SLD profile. However due to the intrinsically disordered regions in the Gag protein, it is unlikely that the experimental SLD profile represents a single protein conformation. In fact the Gag protein likely exists as an ensemble of intra-converting conformers on the membrane surface.

Gag structures were evaluated as an average SLD profile and fit to the experimental data. In contrast to a simple average, a Monte-Carlo optimization algorithm was used to determine the optimal set of individual weights to each Gag structure that resulted in the best fit to the experimental SLD. The best-fit average SLD profile was calculated by scaling the individual SLD profiles using the weight factors and then summing them together. The MC algorithm is implemented as a separate software utility supplied with the SASSIE package. For this analysis the Gag structures were divided into three categories based on protein radius of gyration, Rg. The categories are (i) Rg $<$ 30 Å, (ii) 50 Å $<$ Rg $<$ 70 Å, (iii) Rg $<$ 70 Å. The MC weighting algorithm was then applied to each category of structures separately and the resulting SLD profiles shown in Fig. 2. The results clearly show that only compact structures of the Gag protein, Rg $<$ 30 Å, can well approximate

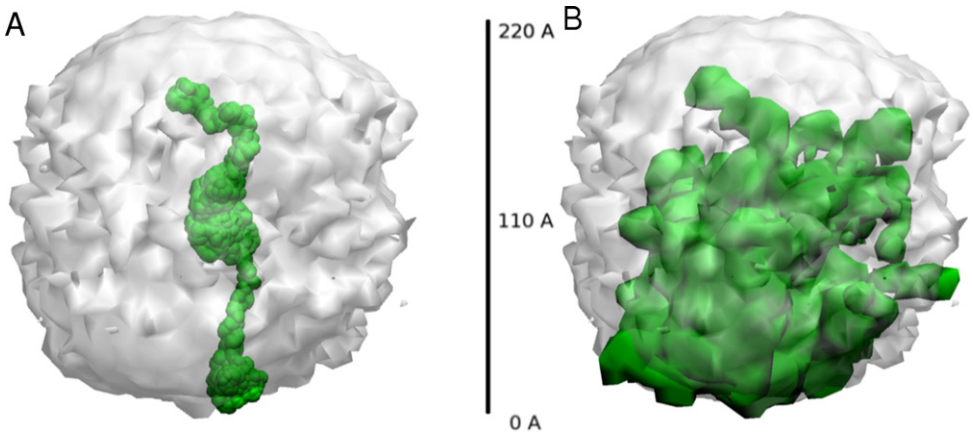

Fig. 3. The ensemble of Gag protein conformations represented as a 3-D density plot. The gray density depicts the volume sampled by all 100,000 conformations generated by the SASSIE software package with all structures aligned to the N-terminal MA domain. The solid-dark green Gag structure in panel A shows an extended conformation of the protein. Panel B portrays the ensemble of configurations used to fit the compact structure of Gag using the MC weighting algorithm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the peak near the membrane surface but completely miss the extra protein density distal to the bilayer (Fig. 2, green curve). Considering only extended structures, 50 Å $<$ Rg $<$ 70 Å, can match the tail protein density but poorly approximate the peak density (Fig. 2, red curve). The final category, Rg $<$ 70 Å, includes all Gag structures both compact and extended and closely matches the experimental data (Fig. 2, blue curve). Despite requiring a range of structures for an accurate fit of the experimental SLD profile, the MC algorithm gave stronger weight to compact and intermediate structures as shown in Fig. 3. A 3-D density distribution of the volume occupied by Gag over all conformations generated by SASSIE is shown in Fig. 3, panel A in light gray color. Superimposed on the density distribution is a single structure of Gag in the extended state, where the MA domain at the bottom anchors to the membrane surface and the NC domain reaches ∼200 Å away. Fig. 3, panel B shows in place of the Gag structure the 3-D density distribution of structures weighted by the MC algorithm. This subset of conformations is more restricted, with only a few extended structures contributing to the average. By using atomistic models to interpret NR results we are able to gain a better insight into the range of protein configurations present on the membrane surface and the relative population that best re-creates the measured data.

2.4. Modeling utility

The modeling utility uses most of the same parameters as the fit utility except that the *model* field in the GUI is set to 0 to indicate that SLDMOL is operating in model mode. In this mode, the *z* anchor position and the fractional area coverage parameters are not fit, but instead the user provides these values in the initial value column. Minimum and maximum parameter values are ignored. SLDMOL will then calculate SLD profiles based on these fixed values. Analogous to the fitting options, SLD profiles can be calculated for each individual protein structure in a trajectory or as an average over all structures. The program will output the SLD profiles along with the parameter values used for generating the profile.

The application of neutron scattering to biological systems is particularly appealing due to the powerful non-invasive labeling afforded by the replacement of hydrogen with deuterium isotopes within a molecule. Yet, to achieve selective labeling within a protein or even a supra-molecular complex presents a number of technical challenges. In addition not all deuteration schemes are effective and therefore must be chosen carefully before investing significant resources and time in developing samples. The modeling utility provides an important means by which trial deuteration schemes of the protein can be modeled to observe real changes in the SLD profile.

2.5. Modeling utility example: HIV-1 Gag deuterated MA and CA-NTD domains

The interpretation of the experimentally determined SLD profiles of membrane bound Gag suggest that Gag can adopt either compact or extended conformational states indicated by the dimensions of the membrane normal SLD profiles. Presumably the membrane bound MA domain of Gag remains as a fixed anchor point while domains further downstream in sequence reorganize in the different Gag configurations. This hypothesis and the nature of domain rearrangement can potentially be tested through selective deuteration highlighting the position of individual Gag domains. This experiment can be tested *in silico* to at least provide the magnitude of changes that can be expected in the SLD profiles and whether they are within the regime of instrument sensitivity.

The standard SLDMOL interface allows a direct averaging of protein conformation with selectively deuterated regions. First using the SASSIE module we divided the ensemble of Gag configurations into two categories: (i) 'compact ensemble' defined as structures with an Rg $<$ 45 Å and (ii) 'extended ensemble' defined as having an Rg $>$ 45 Å. For the two ensembles SLDMOL was used to calculate the average SLD profile of the fully protonated Gag protein, the MA deuterated Gag protein and the CA-NTD deuterated Gag protein. Fig. 4A and B show the compact and extended averaged profiles respectively for Gag in a H_2O contrast aqueous buffer. The CA-NTD domain of Gag is separated from the MA domain by a 30 amino acid highly flexible linker region. To adopt extended configurations this linker likely needs to be extended and as a result the CA-NTD is distal from the N-terminal MA domain (Fig. 4B) while for compact structures the CA-NTD region actually overlaps partially with the MA domain (Fig. 4A). The MA and CA-NTD distributions can be seen more clearly by taking the difference profile between the selectively deuterated protein and the fully protonated protein for both the extended and compact ensembles (Fig. 4C). As expected, Fig. 4C shows the MA distribution to be nearly identical for the extended and compact conformational states whereas the CA-NTD is much sharper and overlaps the MA distribution in the compact state and is much broader and further from MA in the extended states.

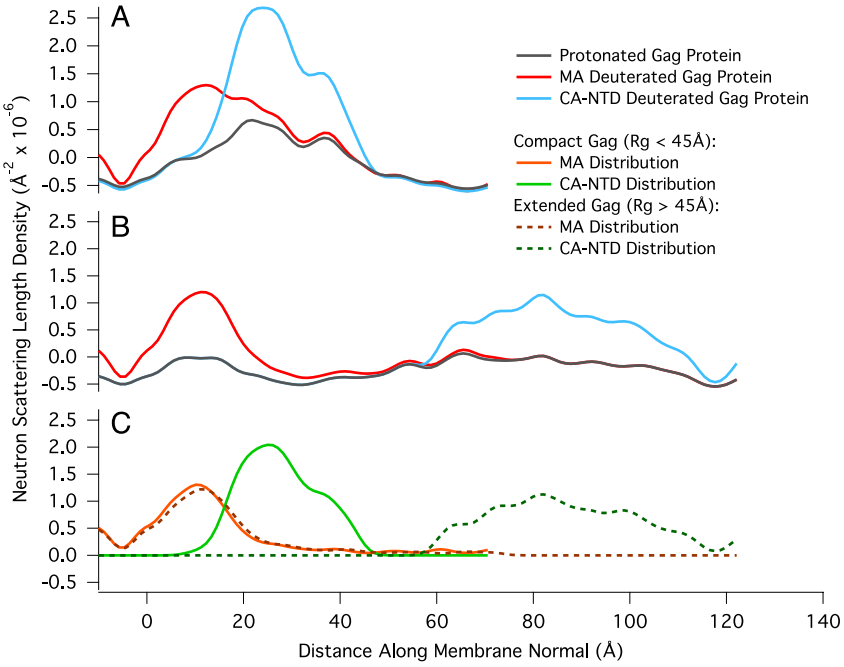

Fig. 4. Modeling the SLD profile of Gag protein with selectively deuterated protein domains and two different conformational ensembles of the protein. Profiles were calculated for a fully protonated Gag molecule (gray line) and for the same conformation ensemble except with the MA domain (red line) or the CA-NTD domain (light blue) selectively deuterated. Panel A shows profiles for a compact ensemble of Gag with an Rg $<$ 45 Å whereas panel B shows profiles for extended Gag conformations with an Rg $>$ 45 Å. Panel C shows the distribution of the MA (orange line) and the CA-NTD (green lines) domains calculated by taking the difference between the selectively deuterated and the protonated SLD profiles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The modeling also suggests that experiments on deuterated MA and CA-NTD have a strong likelihood of revealing the position of C-terminal domains with respect to the membrane. In addition by adjusting the solvent contrast of the aqueous buffer, the scattering contribution from only the deuterated domain can be highlighted while the rest of the protein is masked. This requires finding the right combination of H_2O and D_2O buffer. SLDMOL can compute the expected SLD profile over a range of mixtures to determine the optimal experimental conditions for resolving the deuterated domains of a protein system.

3. Summary

The heterogeneous environment required for the proper folding and function of membrane-associated proteins has made their characterization a significant problem for structural biology. Neutron reflectivity has emerged as a powerful method to not only resolve the conformational state of membrane bound proteins but also to reveal their spatial orientation and position with respect to the membrane layer. Yet for most measurement schemes structural information is limited to a 1-D profile normal to the membrane plane. SLDMOL has been developed to provide greater molecular detail in NR data interpretation and allow access to 3-D information. SLDMOL can be used to find both protein structures and orientations that best fit NR data. In addition for flexible protein systems SLDMOL can determine a weighted ensemble of structures capable of representing the average density profile of a membrane bound protein. As deuteration schemes for biomolecular systems are becoming more sophisticated a need to vet out plausible protein constructs prior to expensive wet-lab commitment is desired. The modeling facilities of SLDMOL are capable of performing virtual selective deuteration as well as contrast exchange allowing for prior experimental optimization. In addition to membrane systems the software is easily adaptable to interpret reflectivity data from any surface associated biomolecular system.

Acknowledgments

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