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# Modelling multi-protein complexes using PELDOR distance measurements for rigid body minimisation experiments using XPLOR-NIH

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ABSTRACT

Crystallographic and NMR approaches have provided a wealth of structural information about protein domains. However, often these domains are found as components of larger multi domain polypeptides or complexes. Orienting domains within such contexts can provide powerful new insight into their function. The combination of site specific spin labelling and Pulsed Electron Double Resonance (PELDOR) provide a means of obtaining structural measurements that can be used to generate models describing how such domains are oriented. Here we describe a pipeline for modelling the location of thio-reactive nitroxyl spin locations to engineered sties on the histone chaperone Vps75. We then use a combination of experimentally determined measurements and symmetry constraints to model the orientation in which homodimers of Vps75 associate to form homotetramers using the XPLOR-NIH platform. This provides a working example of how PELDOR measurements can be used to generate a structural model.



## 1. Introduction

To date there have been nearly 100,000 structures deposited in the protein data bank (PDB). This rich source of structural biological information is utilised by many laboratories to gain functional insights. Many structures deposited in the PDB are of individual protein domains, which are constituents of larger macromolecular complexes or proteins. Orientation of domains of known structure within such larger assemblies can often add significantly to the understanding of how different protein modules function together.

In this paper, a procedure is described which utilises Pulsed Electron Double Resonance (PELDOR) [alternatively referred to as double electron–electron resonance (DEER)] distance measurements as restraints to dock PDB structures together. This procedure can easily be extrapolated to utilise other sources of distance information, such as residue specific crosslinking information obtained from cross-linking MS/MS experiments. The protocol focusses on a molecular modelling workflow and assumes PELDOR distance measurements have been obtained, and requires access to the molecular visualisation package PyMol (http://pymol.org/) [1]. An additional plugin, MTSSLwizard [2], is required to dock the locations of spin labels onto previously determined structures. This is available at http://www.pymolwiki.org/index.php/MtsslWizard and installed by placing the python script in the appropriate directory. XPLOR-NIH [3] is then used to model spin labelled structures using experimentally obtained distance constraints. XPLOR-NIH is available at, (http://nmr.cit.nih.gov/xplor-nih/), in versions designed to run on Linux and Mac operating systems.

The working example referred to in this paper is that of a histone chaperone called Vacuolar Protein Sorting 75 (Vps75). X-ray crystallography has been used to show that Vps75 adopts homodimeric "headphone" fold conformations [4–6]. However, in solution Vps75 was recently discovered to adopt a tetrameric conformation [7]. In order to obtain insight into how two Vps75 homo-dimers were arranged within the tetrameric particle a series of PELDOR distance measurements were made at moderate salt concentrations [7]. These measurements were used to dock together two identical Vps75 dimer crystal structures as rigid bodies using the molecular modelling software XPLOR-NIH. The final model was further validated by mutagenesis experiments.

*Abbreviations:* Cys, cysteine; Glu, glutamic acid; Tyr, tyrosine; Vps75, vacuolar protein sorting protein 75.

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## 2. Methods

### 2.1. In silico MTSL 'R1' labelling of Vps75 with MTSSLwizard and formatting pdb files for XPLOR-NIH

PELDOR is an Electron Paramagnetic Resonance (EPR) experiment in which the distance between two spin labels is measured. Spin label pairs are usually incorporated into proteins by the cross-reaction of cysteine residues with a sulfhydryl reactive nitroxide radical containing compound such as S-(2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate (MTSL). This spin labelled side chain is usually referred to as R1 and is prefixed by the amino acid number. For example, Vps75 E56R1 refers to Vps75 in which Glu 56 which has been mutated to Cys and cross-reacted with MTSL (Fig. 1A).

Load the PDB structure of Vps75 [4] by typing 2ZD7 into the PDB loader service plugin if available or by downloading the 2ZDZ.pdb file from http://www.rcsb.org/. To remove solvent and select the relevant chains of Vps75 for modelling purposes execute the following PyMol commands:

```
extract Vps75a, chain a
extract Vps75b, chain b
remove HETATM
delete 2ZD7
```

XPLOR-NIH uses segment IDs to select different polypeptide (or other) chains within a macromolecular assembly to allow various operations to be performed on these segments in isolation. To assign new segment IDs to each chain of Vps75 perform the following commands in PyMol:

```
alter chain A, segi='A'
alter chain B, segi='B'
```

Next, spin labels are introduced at the site E56R1 in Vps75 using MTSSLwizard [2]. Under the wizard menu in PyMol open the pre-installed "MTSSLwizard". It is recommended to use the default settings of MTSSL Wizard for the initial R1 labelling of sites in PyMol. If no spin label ensemble is obtained try increasing the thoroughness or reducing the VdW restraints for the conformer search. For introducing spin labelling sites the following default settings were used:

**Table 1**
List of PDB files required for creating the starting PDB and PSF files for molecular modelling with XPLOR-NIH. Segment ID and residue numbers are noted for each file which can be checked for consistency as deviations from values quoted above may affect subsequent steps in the protocol.

| File | Segment ID | Residue sequence | |
|---|---|---|---|
| | | Start | End |
| Vps75aN.pdb | A | 4 | 225 |
| Vps75bN.pdb | B | 10 | 227 |
| Vps75cN.pdb | C | 4 | 225 |
| Vps75dN.pdb | D | 10 | 227 |
| E56R1aN.pdb | S | 1 | 1 |
| E56R1bN.pdb | S | 2 | 2 |
| Y35Rx2a.pdb | S | 3 | 3 |
| E56R1cN.pdb | T | 1 | 1 |
| E56R1dN.pdb | T | 2 | 2 |
| Y35Rxb.pdb | T | 3 | 3 |

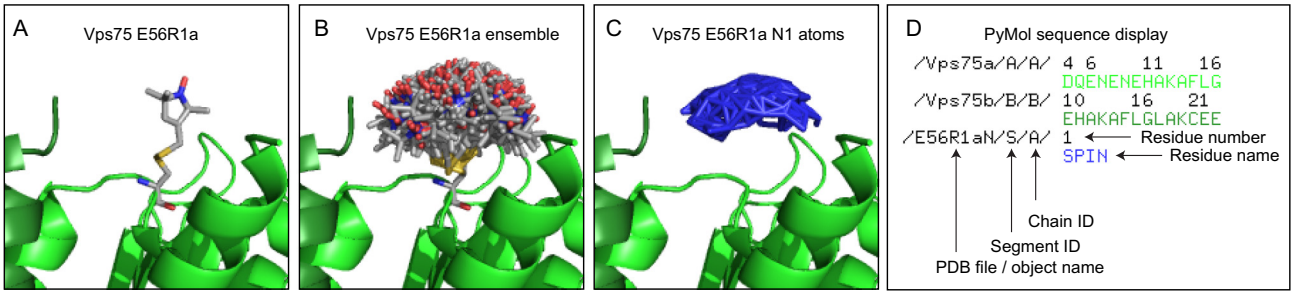


**Fig. 1.** Extraction and formatting of the nitroxide nitrogen coordinates of the E56R1a spin label ensemble. (A) 1/200 conformers produced as a result of labelling E56 of Vps75 chain A with MTSL using MTSSLwizard. (B) The full spin label ensemble, "set all_states, on", at position E56R1a. (C) The ensemble of 190 nitroxide N1 atoms extracted into the E561aN.pdb file. (D) An annotated screen from PyMol, with sequence display on, highlighting key identifiers within each PDB file which are utilised in subsequent XPLOR-NIH modelling steps.

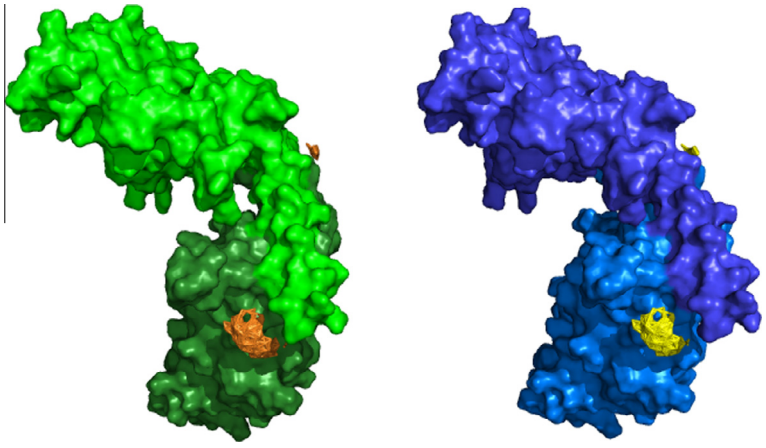

**Fig. 2.** Starting coordinates of the two Vps75 dimers (green and blue) with associated nitroxide nitrogen atoms of spin label ensembles (orange and yellow) as per Vps75tet.pdb.