The NMR solution structure of the VMA-7 subunit of the vacuolar H^+-ATPase from *Saccharomyces cerevisiae*.

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Introduction

Vacuolar H^+-ATPase (VATPase) is a membrane spanning proton pump which is found in virtually all eukaryotic cells. The action of the pump results in translocation of protons across vacuolar membranes, allowing the pH of intracellular compartments to be regulated. Errors in the function of the pump have been implicated in the pathology of a number of diseases including osteoporosis, diabetes and several common cancers including cervical cancer. A structural understanding of this complex at the atomic level will open up paths to new therapeutics for these diseases by control of its activity. Though the structure of the V-ATPase enzyme complex has been shown to be related to that of the F_1F_0 ATPase, there are major differences in the subunits and the composition of the two enzymes. Therefore, a significant effort has been started at Leeds under the MASIF scheme to determine the structure of the enzyme subunits and the complete complex using modern structure determination methods: X-ray crystallography, NMR spectroscopy and electron microsocopy.

The VATPase complex contains at least ten distinct subunit types and four of these are of a size amenable to modern NMR techniques. The current target, VMA-7, is a 14kDa subunit of the complex, which has unknown function and fold, and can be over expressed in *E. coli* and purified as a GST fusion protein. Preliminary structural studies using FT-IR, CD and NMR showed that it is folded in aqueous solution and contains ~30% alpha helix. We have now optimised the over-expression of the protein in M9 minimal media to give 4mg of fully purified protein per litre of culture and have produced sufficient quantities of pure protein for NMR structural studies.

Good quality ^1H-^{15}N HSQC, ^1H-^{15}N HSQC NOESY (Fig. 1), ^1H-^{15}N HSQC TOWNY and ^1H-^{15}N HMQC-NOESY-HSQC spectra of the protein collected at 25°C in a non-micellar detergent have allowed us to complete approximately 30% of the backbone assignment of VMA-7 in a matter of a few weeks. Complete assignment of all structured regions of the protein is expected to be completed within the next few months. The 3D structure of the complex will be calculated using a hybrid approach using both residual dipolar coupling and NOESY restraints; and is expected to be complete late this year. To allow us to collect the dipolar coupling and NOESY restraints required for the calculation of the 3D structure, we have transferred the VMA-7 cDNA to a new GST Precision Protease Fusion vector which will allow us to produce protein free from low levels of contaminating proteases found in thrombin; giving a stable ^{15}N-^{13}C doubly-labelled protein.

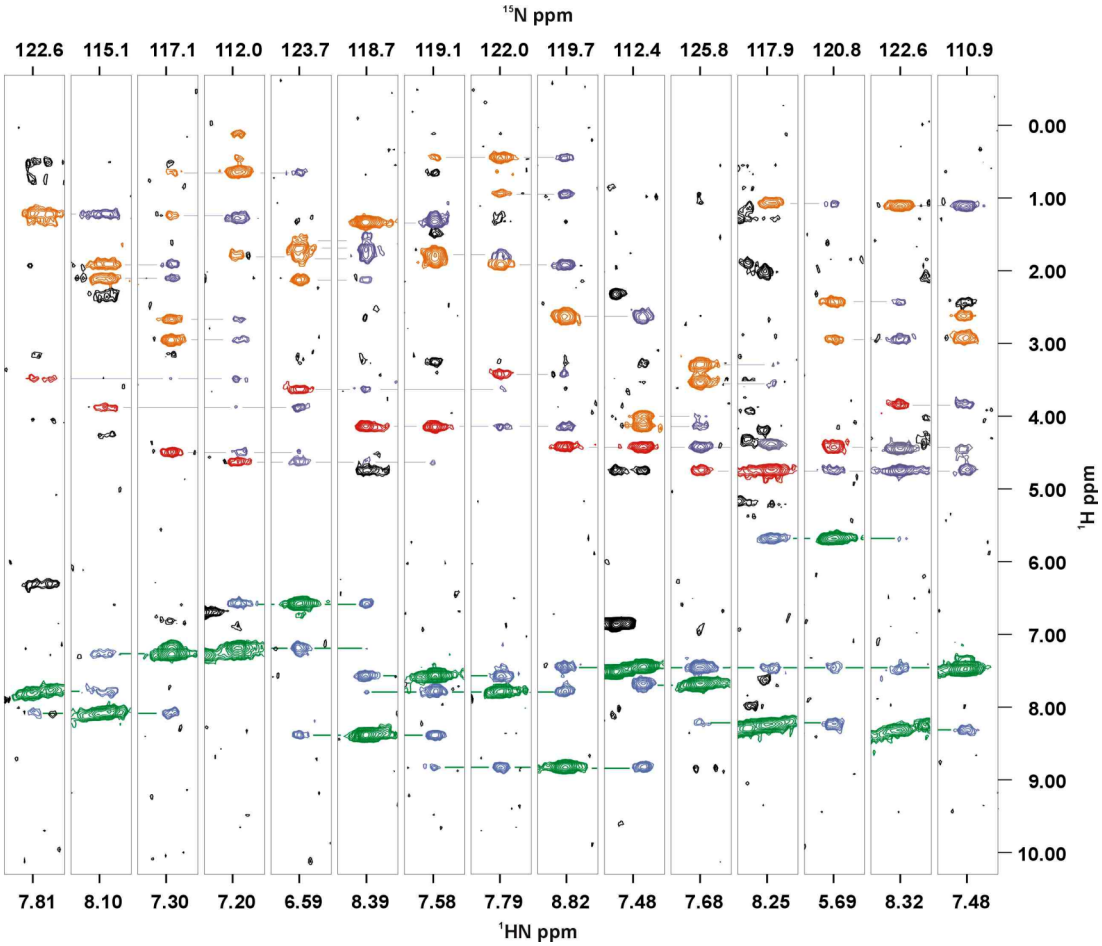

Fig 1 Backbone assignments of a helical region from the ^{1}H-^{15}N HSQC-NOESY spectrum of VMA-7 derived from *Saccharomyces cerevisiae.*

Key: diagonal peaks are in green, HNi-Hαi peaks are in red, sidechain HNi-HXi peaks are in orange and HNi-HXi-2..i+4 peaks are in electric blue. Inter-residue connections used in the assignment are shown as blue lines .

To provide new targets for solution NMR studies, research on high yield over-expression of other subunits from VATPase in a number of different hosts is being undertaken.

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References

Jones, R.P.O., Hunt, I.E., Jaeger, J., Ward, A., O'Reilly, J., Barratt, E.A., Findlay, J.B.C. & Harrison, M.A. (2001), Expression, purification and secondary structure analysis of *Saccharomyces cerevisiae* vacuolar H^{+}-ATPase subunit F (VMA7p), *Molecular Membrane Biology*, **18**, 283-290.