

# Pf1 Cosolvent FAQ's

(Frequently Asked Questions) V 2 – 05/2005

## Pf1 for RDC-NMR measurements

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## Pf1 for NMR in general

### What is Pf1?

Pf1 is a 7,349-nucleotide DNA-phage where the circular DNA is packaged with coat protein at a 1:1 nucleotide: coat protein-ratio. It is a filamentous bacteriophage, propagated on *Pseudomonas aeruginosa* Kr. The average dimension is 2000 x 6 nm. Pf1 is negatively charged at pH 7.4, with an approximate linear charge density of 10 e/nm, and has a molecular weight of  $3.75 \times 10^7$  g/mol.

Phages are rebuffed by washing with the desired buffer and centrifuging in a table ultracentrifuge. Supernatant is discarded and phage resuspended. Phage concentrations can be adjusted by weighing appropriate amounts of a 50 mg/ml stock solution and verified by UV absorbance at 270 nm using an extinction coefficient,  $\epsilon = 2.25 \text{ cm}^{-1} \text{ mg}^{-1} \text{ ml}$ . Pf1 is available in highly purified quality (Protease free; RNase free).

### For what is Pf1 good for?

In NMR (nuclear magnetic resonance) measurements Pf1 forms liquid crystals (metamorphe) phases to reduce the motion of the molecules. Pf1 aligns spontaneously in a magnetic field. The observed deuterium quadrupolar splitting in deuterated water scales up with the phage concentration and seems to also be temperature dependent. Application in a wide temperature range and ease of phage - sample - separation or buffer exchange make Pf1 filamentous phage a superior co-solute for NMR experiments.

### Advantages of filamentous phages in dipolar coupling experiments:

- degree of alignment can be easily tuned by changing the phage concentration
- stable under physiological conditions in a wide temperature range
- no effect on the rotational correlation time of nucleic acids
- easy to separate from the macromolecule of interest by ultracentrifugation

## Questions regarding to the NMR application

For which range of pH can you guarantee the stability of Pf1?

pH 6 to 8

Does Pf1 interfere with the protein structure? Or: Is there an impact of Pf1 on the protein structure?

No, there is no interference and no binding; the interaction is due to electric charges

Can I use Pf1 for measuring the structure of Nucleic acids?

Yes

In which range of concentration can I use Pf1?

1 to 50 mg/ml

Can I change the size of the RDC?

Yes, changing the concentration of the phage will change the alignment of the macromolecules.

We use different biological systems in our lab. Could there be a contamination of other probes by recombination processes, etc.?

No, Pf1 is highly specific for *Pseudomonas aeruginosa*.

Is the NMR-sample re-usable?

Separation of Pf1 and the macromolecule is possible by ultracentrifugation, but difficult (UZ: pellet = phage, supernatant = macromolecule).

How high does the magnetic field have to be?

Pf1 is completely aligned in 300MHz

Which amount per sample do you recommend?

5 to 10 mg/ml

Do you need special knowledge for RDC-measurements?

To get the spectra is simple, for analysis you may need more knowledge (see „Varian & Bruker“)

Do you need special knowledge for the analysis or is it compatible with other NMR-methods?

Adjustment is important.

Can Pf1 bind non-specifically to macromolecules?

Pf1 phage particles are highly negatively charged and can bind non-specifically to macromolecules that have a significant positively charged surface patch. This not only results in excessive alignment, but can also shorten the transverse relaxation time and thereby the inherent resolution and sensitivity of multi-dimensional NMR experiments. Lowering the phage concentration to 1–2 mg/ml reduces the degree of induced order, but does not necessarily solve the line broadening problem.

## Precautions

- $\text{NaN}_3$  is highly toxic (R28 – 32; S 28 - 45). Wear goggles and gloves when handling the phage solution.
- Pf1 was isolated from *Pseudomonas aeruginosa* Kr, a potential pathogen. No health risk should emanate from highly purified Pf1 phage. However, use precaution handling the phage material.
- Briefly spin down the phage solution prior to withdrawal, as Pf1 may be distributed across the entire vial.
- Since Pf1 solution is highly viscous at the concentration at hand, use caution in pipeting. You have to pipet the phage solution carefully. We recommend pipeting slowly and using not too small tips to avoid shear stress. **Shear rates can destroy the phages.**
- Avoid proteases.

## Pf1 Storage

Which range of temperature is allowed for measurements with Pf1?

5 to 30 °C, normally 20-27 °C

How long can I store the Co-Solvent?

½ year, but a new charge can be provided within one week.

What are the storing conditions?

From 0 °C to 4 °C (**do not freeze!**)

## RDC

What is RDC?

RDC stands for “residual dipolar coupling”. Residual dipole coupling (RDC) measurements give information about the topology of molecules. The method is used to complement or even substitute classic NOEs (Nuclear Overhauser Effect), which are applied for NMR-structure definition of macromolecules.

Residual dipolar coupling arise from the partial alignment of molecules in orienting media or via an intrinsic anisotropic magnetic susceptibility of the molecule itself (predominantly the magnetic susceptibility of aromatic groups in nucleic acids and protein side chains, paramagnetic ligands, or to a weaker extent of the peptide group (helices!)) when samples are placed in a magnetic field. Practically useful alignment leaves residual (to a large extent averaged) dipolar couplings of up to ca. 30 Hz from the several-kHz-couplings observed in solids where no averaging occurs. Molecules of the solution in an anisotropic medium are oriented by steric clashing, electrostatic interactions and/or weak transient binding. Dipolar couplings are mostly determined for C-H and N-H-groups in non-decoupled, spin-state separated HSQC-like experiments (IPAP, DSSE) and for H-H-couplings in COSY-type experiments. The value is determined by comparison of the splitting in an aligned state with a reference spectrum in isotropic phase where only the J-splitting is detected. Dipolar couplings serve as angular restraints in the structure determination process.

A great advantage of RDC-Measurements is to get geometric information about the arrangement of protein domains or ligand-receptors without doing time consuming NMR-

measurements in a first step. Measuring is carried out in nematic, fluid-crystalline phases, so that the action of the molecules is only partly but not complete inhibited. The characteristic features of the molecules are maintained.

Basis of residual dipole coupling is the magnetic interaction of cores over their space:

The interactions of the cores of different molecules are induced by the magnetic dipole moment of every single atom. A fast rotation movement of the molecules in fluids with low viscosity leads to average forces, means they annul. The magnetic forces reappear by suppressing the motion of the cores. This results in the coupling between the cores and can be visualized as a splitting of the NMR-resonance spectral lines. The structure of the splitting gives information about the geometry of the molecule.

There are liquid crystals (metamorphe) phases, like bicelles or filamentous phages to reduce the motion of the molecules. But the bicelle liquid crystalline phase is unstable in the presence of certain proteins and offers only a limited temperature range. The alignment media for RDC are:

- bicelles consisting of various charged or uncharged lipids
- filamentous phage Pf1
- purple membrane of *Halobacterium salinarum* with bacteriorhodopsin in two-dimensional crystalline arrangement
- mechanically stressed polyacrylamide gels

The following table shows different features between bicelles and Pf1:

Feature	Bicelles	Filamentous phage Pf1
Range of temperature	30-32 °C	5-30 °C (20-27 °C)
pH-range	Predominantly acidic proteins	pH 6-8 with $[\text{NaCl}_2] \leq 100\text{mM}$
Handling	Establishment could be time-consuming	No preparation of the co-solvent; just add it to your sample
Impact on the structure of the molecule	Lipids are partly in solution (not formed as bicelles); this may lead to interaction and deformation of the molecules	No structural changes of the proteins are published so far
Storage conditions of the sample at 4 °C	Bicelles are stable only for defined temperature and salt concentration ranges.	More than a few months

A large advantage from Pf1 is, that you can measure the samples repeated; - also after the samples were stored at 4 °C!

### RDC applications

- Analysis of inter-domain motion
- Analysis of slow dynamics
- Determination of relative domain orientations
- Identification of multimerization state
- Improved assignment
- Structure refinement (proteins, nucleic acids, oligosaccharides)
- Structure determination of protein complexes
- Rapid structure determination
- Validation of structures

## Literature about RDC

1. Markus MA, Gerstner RB, Draper DE, Torchia DA. Refining the overall structure and subdomain orientation of ribosomal protein S4 delta41 with dipolar couplings measured by NMR in uniaxial liquid crystalline phases. *J Mol Biol.* 1999 Sep 17;292(2):375-87.
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4. Clore GM, Starich MR, and Gronenborn Measurement of residual dipolar couplings of macromolecules aligned in the nematic phase of a colloidal suspension of rod-shaped viruses. *AM., 120; J. Am. Chem. Soc., 10571-10572 (1998).*
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6. Saupe, A. and Englert, G., High-resolution nuclear magnetic resonance spectra of orientated molecules. *Phys. Rev. Lett.* 11, 462.465 (1963).
7. Bothner-By, A.A., Domaille, P.J. and Gayathri, C., Ultra-High-Field NMR Spectroscopy: Observation of Proton-Proton Dipolar Coupling in Para-magnetic Bis[tolytris(pyrazolyl)borato]cobalt(II) *J. Am. Chem. Soc.* 103, 5602.5603 (1981).
8. Tolman JR, Flanagan JM, Kennedy MA, Prestegard JH., Nuclear magnetic dipole interaction in field-oriented proteins: information for structure determination in solution. *Proc Natl Acad Sci U S A;* 92(20):9279-83 (1995).
9. Tjandra N, Grzesiek S, and Bax A, Magnetic field dependence of nitrogen-proton J splittings in <sup>15</sup>N-enriched ubiquitin resulting from relaxation interference and residual dipolar couplings. *J. Am. Chem. Soc.* 118; 6264-6272 (1996).
10. Moltke, S. and S. Grzesiek, *J Biomol.* Structural constraints from residual tensorial couplings in high resolution NMR without an explicit term for the alignment tensor. *NMR,* 1999. 15(1): p. 77-82.

## Pf1 Ordering

What can I order??

Pf1 Cosolvent Phage for NMR		
Article	Amount	Article No.
Pf1 Protease free	50 mg	311077
Pf1 Protease and RNase free	50 mg	311079
Pf1 Protease free	100 mg	311059
Pf1 Protease and RNase free	100 mg	311060
Pf1 Protease free	250 mg	311076
Pf1 Protease and RNase free	250 mg	311078
Pf1 Protease free	300 mg	311878
Pf1 Protease and RNase free	300 mg	311879

How can I place an order or contact Profos for further questions?

By phone: + 49 (0) 941 942 62 0

By fax: + 49 (0) 941 942 62 20

By email: [inquiry@profos.de](mailto:inquiry@profos.de)

By post: profos AG  
Josef-Engert-Str. 9  
93053 Regensburg, Germany

## Profos in general

### What is Profos doing and what is "Profos" standing for?

Profos utilizes bacteria's natural enemies (bacteriophages) to design new and innovative tools for the biotech, food and feed industries, e.g. concerning the highly sensitive detection of bacteria or the powerful endotoxin removal. Profos has a wide expertise in protein science, an extended know how in bacteriophage technology, and relies on the broadest platform in phage ligand technology, covered by 11 granted and pending patent families. These skills we want to use for products, which are faster and more effective than any previous – all that, moreover, at a better value.

Profos was established in year 2000 as a spin-off from University of Regensburg and is currently employing 22 people. Profos stands for phage protein folding and stabilization.

Profos AG offers the filamentous phage Pf1 as co-solvent for NMR, other phages like fd and Lf will be developed in near future. We are likely to send you a product description and price list on your request! You are welcome to visit our homepage: [www.profos.de](http://www.profos.de) !