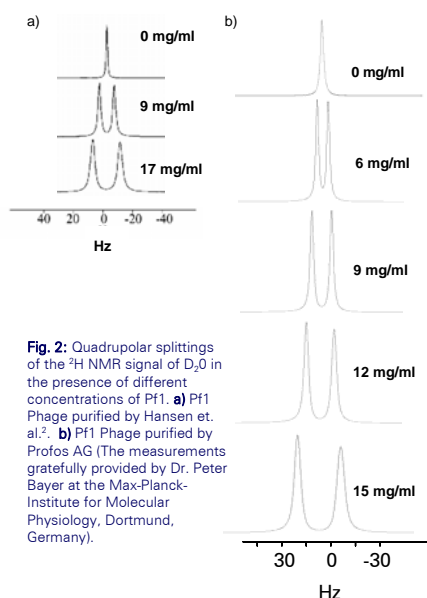


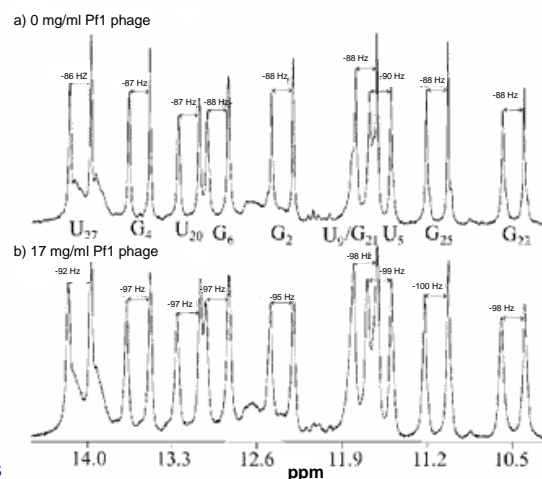
# The filamentous bacteriophage RCD additive Pf1, from Profos AG

Analysis of residual dipolar coupling (RDC) can be optimized by using filamentous bacteriophages like the *Pseudomonas aeruginosa* phage Pf1 from Profos AG.

Publication from several different research groups documented the optimization of RDC measurement by using filamentous bacteriophages. The improvement of the measurements when using Pf1 are documented in Fig. 1. This figure shows a measurement without (a) and with Pf1 (b). When using Pf1 the distance between the single peaks is significant wider. For this measurement, the Pf1 was obtained by the standard purifying protocol (developed by Hansen et al.<sup>1</sup>).



**Fig. 2:** Quadrupolar splittings of the <sup>7</sup>H NMR signal of D<sub>2</sub>O in the presence of different concentrations of Pf1. **a)** Pf1 Phage purified by Hansen et. al.<sup>2</sup>. **b)** Pf1 Phage purified by Profos AG (The measurements gratefully provided by Dr. Peter Bayer at the Max-Planck-Institute for Molecular Physiology, Dortmund, Germany).



**Fig. 1:** 1-D imino proton spectra of a <sup>15</sup>N-labeled IRE-I RNA **a)** with no Pf1 **b)** with 17mg/ml Pf1 (standard purification, modified by Hansen et al.<sup>2</sup>).

The Profos AG is specialized on the innovative use of bacteriophages in biotechnology and owns several patents. Our outstanding expertise in propagation, purification and handling of bacteriophages enables us to produce RNase and protease free Pf1 in best quality.

As one of the leading Pf1 producer in the world we are able to offer as a standard product 1g Pf1. We also developed a new PF1 purification protocol to purify Pf1 in a gentler and better way than the standard protocol. As shown in Fig. 2 the quadrupolar splitting is higher by using Profos Pf1. It is possible to achieve an approximately 30% better splitting when using 9 mg/ml Pf1 from Profos compared to Pf1 purified by the standard protocol.

## Pf1 technical data:

**Storage buffer:** 20 mM K-Phosphate pH 7.6, 2 mM MgCl<sub>2</sub>, 0.02 % azide.

**Storage:** 0 to 4 °C. Do not freeze!

**Concentration:** > 50 mg/ml

**Protease free:** Protease is not detectable.

(Protease contamination was examined by incubation of Pf1 phage with Ready Plate™ 96EnzCheck® Protease Assay Kit according to manufacturers` instructions.)

**Shelf life:** 12 month

## Pf1 compared to bicelles

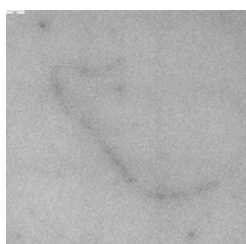
- Can be applied in a wide range of temperature 5-30°C (20-27°C) and pH: pH6-8 with [NaCl] ≤ 100mM
- No preparation of the co-solvent necessary; just add it to your sample
- No structural changes of the proteins are published so far

## Applications

- Alignment of RNA, DNA and protein for NMR
- Structure-activity relationship measurements (SAR by NMR)
- Accurate definition of domain orientation in multi-module macromolecules or complexes

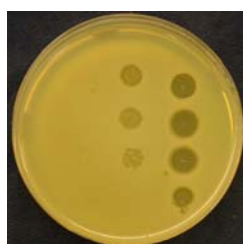
## Advances of PF1

- Degree of alignment can be easily tuned by changing the phage concentration
- Extremely stable under physiological conditions
- No effect on the rotational correlation time of nucleic acids
- Phage macromolecule system is stable for a long time
- Easy to separate from the macromolecule of interest by ultracentrifugation



**Fig. 3:** Electron microscopy of Pf1 phage. Top left: bar length is 100 nm. Image by Profos AG.

**Fig. 4:** Serial dilution of Pf1 phage on strain *Pseudomonas aeruginosa* Kr.



## References on PF1 mediated tunable alignment of proteins and nucleic acids

- <sup>1)</sup> Methods in Enzymology. Vol. 317, 2000 Filamentous Bacteriophage for Aligning RNA, DNA, and Proteins for Measurement of Nuclear Magnetic Resonance Dipolar Coupling Interactions. Hansen M, et al.
- <sup>2)</sup> J Biomol NMR. 2001 Aug;20(4):365-77. Characterization of molecular alignment in aqueous suspensions of Pf1 bacteriophage. Zweckstetter M, et al.
- J Mol Biol. 1999 Sep 17;292(2):375-87. Refining the overall structure and subdomain orientation of ribosomal protein S4 delta41 with dipolar couplings measured by NMR in uniaxial liquid crystalline phases. Markus MA, et al.
- Nat Struct Biol. 1998 Dec;5(12):1065-74. Tunable alignment of macromolecules by filamentous phage yields dipolar coupling interactions. Hansen MR, et al.
- Journal of Biological Chemistry (1986) 261, 1653-1655. The ionic properties of the filamentous bacteriophages Pf1 and fd. Zimmermann et al.