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# Crystallization and initial crystallographic analysis of the *Streptococcus parasanguinis* FW213 Fap1-NR$_\alpha$ adhesive domain at pH 5.0

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The adhesin fimbriae-associated protein 1 (Fap1) is a surface protein of *Streptococcus parasanguinis* FW213 and plays a major role in the formation of dental plaque in humans. Increased adherence is highly correlated to a reduction in pH and acid activation has been mapped to a subdomain: Fap1-NR$_\alpha$. Here, Fap1-NR$_\alpha$ has been crystallized at pH 5.0 and diffraction data have been collected to 3.0 Å resolution. The crystals belonged to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 122.0$, $c = 117.8$ Å. It was not possible to conclusively determine the number of molecules in the asymmetric unit and heavy-atom derivatives are now being prepared.

## 1. Introduction

Commensal streptococci belong to a group of bacteria which are the primary colonizers of the oral cavity in humans and act as substrates for subsequent adhesion by other bacteria, leading to the formation of biofilms such as dental plaque (Kolenbrander, 2000). Fimbriae-associated protein 1 (Fap1) from the Gram-positive *Streptococcus parasanguinis* is an ∼200 kDa surface fibre that is essential for fimbrial biogenesis, adhesion and biofilm formation (Wu *et al.*, 1998; Wu & Fives-Taylor, 1999; Froeliger & Fives-Taylor, 2001; Stephenson *et al.*, 2002; Zhou *et al.*, 2008). Fap1 comprises an N-terminal signal sequence followed by a unique adhesive region, which is projected away from the bacterial surface by an extensive region of glycosylated serine-rich repeats (Wu & Fives-Taylor, 1999; Ramboarina *et al.*, 2010). At the C-terminus an LPXTG motif anchors the fimbriae to the cell wall. Whilst the specific host receptor has yet to be identified, Fap1 binds to an *in vitro* tooth model composed of saliva-coated hydroxylapatite (SHA; Stephenson *et al.*, 2002) and shows a significant increase in adherence upon shifting towards an acidic environment (Ramboarina *et al.*, 2010).

The adhesive region of Fap1 (Fap1-NR) is composed of two domains: an N-terminal 10 kDa three-helix bundle (Fap1-NR$_\alpha$) and a C-terminal 25 kDa $\beta$-sandwich domain (Fap1-NR$_\beta$) separated by a 27-residue linker. The NMR structure of Fap1-NR$_\alpha$ (PDB entry 2kub; residues 116–231) and the crystal structure of Fap1-NR$_\beta$ (PDB code 2x12; residues 231–437) have both been solved at pH 8.0; furthermore, the respective orientations of these structures were modelled at pH 8.0 and pH 5.0 using SAXS electron-density maps of Fap1-NR (residues 106–437; Ramboarina *et al.*, 2010). At low resolution the adhesive region resembles a curved boomerang-like architecture which becomes much more 'open' under acidic conditions.

NMR $^{15}$N–$^{1}$H HSQC spectra recorded over a range of pH values identified major chemical shift perturbations within the linker region and the adjacent pole of Fap1-NR$_\alpha$ (Ramboarina *et al.*, 2010). NMR relaxation experiments show that the two Fap1-NR domains have some independent motion and that there is likely to be equilibrium between an 'open' and 'closed' form which moves towards the former adhesive arrangement under acidic conditions. This is apparently a survival mechanism of *S. parasanguinis* to outcompete acid-tolerant

**Table 1**
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Beamline | SLS X06DA (PXIII) |
| Space group | $P4_12_12$ or $P4_32_12$ |
| Unit-cell parameters (Å) | $a = b = 122.0$, $c = 117.8$ |
| Resolution range (Å) | 37.39–3.00 (3.16–3.00) |
| Wavelength (Å) | 0.939 |
| Total reflections | 160421 (4668) |
| Unique reflections | 18054 (600) |
| Completeness (%) | 98.3 (92.7) |
| Multiplicity | 8.9 (3.8) |
| $R_{\rm merge}$† (%) | 10.0 (38.9) |
| $\langle I/\sigma(I)\rangle$ | 4.6 (1.9) |

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean intensity of the observations $I_i(hkl)$ of reflection $hkl$.

species under acidic stress in response to the fermentation of ingested sugars by resident bacteria in the oral cavity. Here, we report the preliminary crystallographic analysis of Fap1-NR$_\alpha$ at pH 5.0. Hopefully, this structure will help to address how pH changes lead to a rearrangement of this domain and are propagated out to form an adhesive Fap1 moiety.

## 2. Materials and methods

### 2.1. Protein purification and crystallization

Fap1-NR$_\alpha$ was purified from *Escherichia coli* as described previously (Ramboarina *et al.*, 2010). The sample was then dialysed against 20 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl and concentrated to 5 mg ml$^{-1}$. Conditions for crystallization were initially screened by the sitting-drop method of vapour diffusion at 293 K using sparse-matrix crystallization kits (Hampton Research, USA; Emerald BioSciences, USA; Molecular Dimensions Ltd, USA) in 96-well MRC plates with 100 nl protein solution and 100 nl reservoir solution using a Mosquito nanolitre high-throughput robot (TTP Labtech). Initially, very small crystals were obtained from 1.0 *M* sodium/potassium phosphate pH 5.0 in two weeks. After optimizing the protein concentration (2 mg ml$^{-1}$) and reservoir conditions (0.9 *M* sodium/potassium phosphate pH 5.0) and streak-seeding into 10 µl drops (5 µl protein solution and 5 µl reservoir solution) which had been pre-equilibrated for 1 h over 500 µl reservoir solution in a 24-well Linbro plate, large crystals (∼0.4 × 0.2 × 0.2 mm) were obtained within three months.

### 2.2. X-ray data collection, processing and analysis

Crystals were cryoprotected by transferring them into 5 µl 1.0 *M* sodium/potassium phosphate pH 5.0, 6% D-glucose, allowing 5 min to equilibrate and then repeating with additional 6% increments of D-glucose up to a final cryoprotectant concentration of 1.0 *M* sodium/potassium phosphate pH 5.0, 24% D-glucose. Crystals were immediately mounted in a cryoloop and flash-cooled in liquid nitrogen. Diffraction data from a single native crystal were collected on beamline X06DA (PXIII) of the Swiss Light Source (SLS), Switzerland (Fig. 1). Data were processed with *MOSFLM* (Leslie, 2006) and were scaled with *SCALA* (Evans, 2006). Data-collection statistics are shown in Table 1. The content of the unit cell was analyzed using the Matthews coefficient (Matthews, 1968) and the self-rotation function was calculated using *POLARRFN* (Collaborative Computational Project, Number 4, 1994).

## 3. Results and discussion

Crystals of Fap1-NR$_\alpha$ at pH 5.0 were obtained after three months and grew to ∼0.4 × 0.2× 0.2 mm. Data were collected to 3.0 Å resolution (Fig. 1) and were indexed in point group *P*422; examination of the systematic absences indicated that the true space group was $P4_12_12$ or $P4_32_12$. Analysis of the crystal content suggested that there are four to eight molecules in the asymmetric unit; however, owing to the

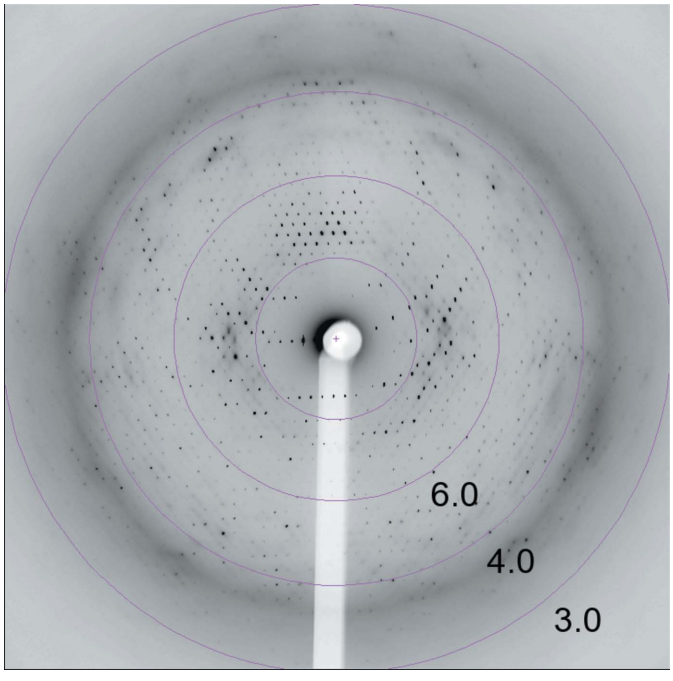


**Figure 1**
Diffraction pattern of a Fap1-NR$_\alpha$ crystal. The resolution rings are at 12, 6, 4 and 3 Å.

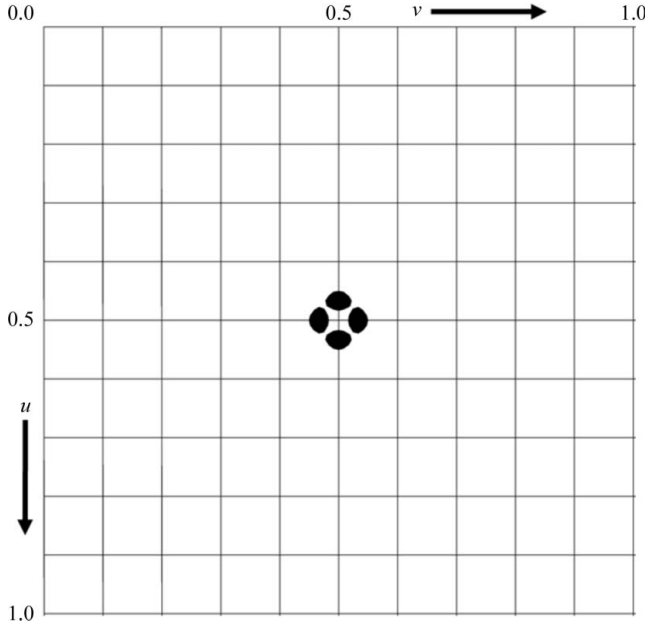


**Figure 2**
Plot of the $w = 0.5$ section of the native Patterson map of a Fap1-NR$_\alpha$ crystal indexed in $P4_12_12$ indicating translational pseudo-symmetry with a peak at $u = 0.469$, $v = 0.5$ and symmetry-related peaks at $u = 0.5$, $v = 0.469$, $u = 0.531$, $v = 0.5$ and $u = 0.5$, $v = 0.531$ with a total height of 20.8% of the origin peak (the origin peak has not been plotted).

large crystal size and low resolution of diffraction it is most likely that four to six molecules are present with Matthews coefficients of 3.80–2.53 Å$^3$ Da$^{-1}$ (Matthews, 1968) and a corresponding solvent content of 67.6–51.5%. Self-rotation functions were calculated, although no significant peaks relating to noncrystallographic symmetry were observed. Examination of a native Patterson map showed an off-origin peak with 20.8% of the height of the origin at (0.469, 0.5, 0.5) and indicates the presence of translational pseudosymmetry (Fig. 2). Data-collection and processing statistics are listed in Table 1.

Molecular replacement with the Fap1-NR$_\alpha$ NMR structure at pH 8.0 (PDB entry 2kub; Ramboarina *et al.*, 2010) as a search model was attempted in *Phaser* (McCoy *et al.*, 2005), *AMoRe* (Navaza, 1994) and *MOLREP* (Vagin & Teplyakov, 2010) using the pseudo-translation option. In addition, to ensure that the systematic absences attributed to crystallographic symmetry were not in fact results of the pseudo-centring, molecular replacement was attempted with all possible space groups of *P*422. No solutions were found and although this may be a consequence of the presence of a large number of molecules in the asymmetric unit, it is more likely to indicate that a change in pH affects the tertiary structure of Fap1-NR$_\alpha$ (Ramboarina *et al.*, 2010). Selenomethionine-labelled Fap1-NR$_\alpha$ has been produced, but the crystals were of insufficient quality for diffraction studies. We are currently preparing heavy-atom derivatives with a view to solving the phase problem by anomalous dispersion techniques. The structure of Fap1-NR$_\alpha$ at pH 5.0 may help to explain how changes in pH result in a conformational rearrangement within Fap1-NR and activate the adhesive properties of Fap1.

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