# Crystallization and preliminary X-ray analysis of bacteriophage T4 UvsY recombination mediator protein

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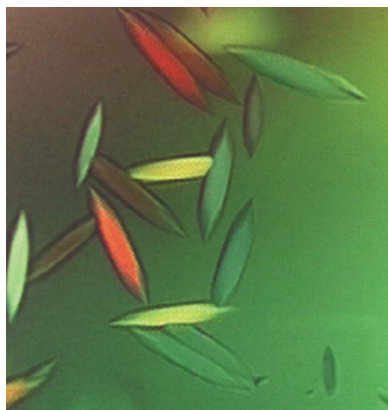College of Medicine, Burlington, VT 05405,
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Bacteriophage T4 UvsY protein is considered to be the prototype of recombination mediator proteins, a class of proteins which assist in the loading of recombinases onto DNA. Wild-type and Se-substituted UvsY protein have been expressed and purified and crystallized by hanging-drop vapor diffusion. The crystals diffract to 2.4 Å using in-house facilities and to 2.2 Å at NSLS, Brookhaven National Laboratory. The crystals belong to space group $P422$, $P4_222$, $P42_12$ or $P4_22_12$, the ambiguity arising from pseudo-centering, with unit-cell parameters $a = b = 76.93$, $c = 269.8$ Å. Previous biophysical characterization of UvsY indicates that it exists primarily as a hexamer in solution. Along with the absence of a crystallographic threefold, this suggests that the asymmetric unit of these crystals is likely to contain either three monomers, giving a solvent content of 71%, or six monomers, giving a solvent content of 41%.

## 1. Introduction

Homologous genetic recombination involves the physical exchange of DNA strands between two different homologous DNA molecules or regions. This process is important as a mechanism for achieving genetic diversity during eukaryotic meiosis and bacterial conjugation (Camerini-Otero & Hsieh, 1995; Bianco *et al.*, 1998). It is also required for the error-free repair of DNA double-strand breaks (Jackson, 2002; Valerie & Povirk, 2003), post-replicative daughter-strand gaps and interstrand cross-links. Typically, homologous recombination is initiated by the invasion of a segment of single-stranded DNA into a homologous region of duplex DNA, forming a D-loop intermediate. The strand-invasion reaction is catalyzed by specific recombinase enzymes, which form filamentous structures known as presynaptic filaments on the invading single strand. Presynaptic filaments are the essential functional units in homologous recombination and their assembly is modulated by other components of the recombination machinery, including ssDNA-binding proteins (SSBs) and recombination mediator proteins (RMPs). SSBs stimulate recombination by removing inhibitory secondary structure from ssDNA, thereby maintaining the polynucleotide in an optimum conformation for presynaptic filament assembly. Conversely, SSBs can also inhibit recombination by competing with recombinase for binding sites on ssDNA. Overcoming this inhibition is the function of RMPs, which promote presynaptic filament assembly on ssDNA molecules previously complexed with SSBs.

RMP function has been studied most thoroughly in the bacteriophage T4 system (Beernink & Morrical, 1999; Bleuit *et al.*, 2001). The major proteins of the T4 recombination system required for presynaptic filament assembly include UvsX, the phage recombinase and ortholog of *Escherichia coli* RecA protein; Gp32, the phage SSB; and UvsY, the phage RMP. UvsY is considered to be the prototype RMP and was first discovered as a factor promoting UvsX-catalyzed DNA strand-exchange reactions by facilitating presynaptic filament assembly (Harris & Griffith, 1989; Kodadek *et al.*, 1989; Morrical & Alberts, 1990; Yonesaki & Minagawa, 1989). UvsY alleviates inhibition of recombinase activities caused by the ssDNA-binding protein, a relationship recapitulated by RMPs in other systems including *E. coli* RecO/R complex, eukaryotic Rad52 proteins and certain eukaryotic Rad51 paralogs (Beernink & Morrical, 1999).

# crystallization communications

Biochemical and biophysical studies of UvsY protein have provided important clues pertaining to the mechanism of recombination mediation. This protein can bind to both single-stranded and double-stranded DNA, but has a much higher affinity for the former (Yonesaki & Minagawa, 1989; Sweezy & Morrical, 1997). UvsY interacts specifically with Gp32 (Jiang *et al.*, 1993), UvsX (Formosa & Alberts, 1984) and other T4 recombination proteins (Bleuit *et al.*, 2001). UvsY has a monomeric molecular weight of 15.8 kDa; however, it exists in solution as a stable hexamer in both ssDNA-bound and unbound forms at salt concentrations $\geq 0.2\,M$ NaCl (Beernink & Morrical, 1998). At lower salt concentrations UvsY hexamers reversibly associate into larger species. UvsY destabilizes Gp32–ssDNA interactions and stabilizes UvsX–ssDNA interactions (Liu *et al.*, 2006; Sweezy & Morrical, 1999). Both effects require UvsY–ssDNA interactions. A model of UvsY mediator activity has been proposed in which wrapping of ssDNA around UvsY hexamers destabilizes Gp32–ssDNA interactions while creating an ssDNA conformation that nucleates UvsX–ssDNA filaments (Beernink & Morrical, 1998; Sweezy & Morrical, 1999; Liu *et al.*, 2006).

In order to reveal the molecular basis of UvsY function, the structure of UvsY is valuable. In addition, as the prototype of recombination mediator proteins (Beernink & Morrical, 1999), the structure of UvsY will lead to a greater understanding of how RMPs facilitate the assembly of presynaptic filaments and related structures in DNA-recombination and DNA-repair transactions. This work is focused on how crystallization of UvsY protein is achieved and on preliminary X-ray diffraction studies of UvsY crystals.

## 2. Materials and methods

### 2.1. Materials

The *UvsY* gene of bacteriophage T4 cloned in the pTL251W plasmid was a gift from Dr T. C. Lin of Yale University. The UvsY protein was purified as described previously (Kodadek *et al.*, 1989; Sweezy & Morrical, 1997) and SeMet-substituted UvsY was expressed following the protocol of 'methionine-pathway inhibition' described by Doublié (1997). The UvsY overexpression strain was streaked at 303 K. 1 ml LB medium was inoculated with a single colony and grown overnight. The cells were spun down and resuspended in 1 ml minimal medium (M9 formula with 5 g l$^{-1}$ dextrose and 2 mg l$^{-1}$ thiamine) to remove LB components. 1 l minimal
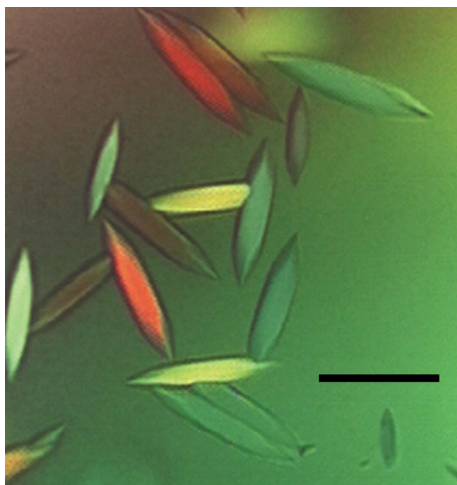
**Table 1**
SeMet MAD data-collection statistics.

Values in parentheses are for the outermost resolution shell.

| Beam source | X25, NSLS, BNL | | |
|---|---|---|---|
| Space group | $P422$, $P4_2 22$, $P42_1 2$ or $P4_2 2_1 2$ | | |
| Unit-cell parameters (Å) | $a = b = 76.93$, $c = 269.8$ | | |
| Resolution (Å) | 20–2.2 | | |
| Wavelength (Å) | 0.9790 | 0.9794 | 0.9611 |
| Total observations | 669253 | 679492 | 675479 |
| Unique reflections | 41441 | 41872 | 41813 |
| Redundancy | 16.1 | 16.2 | 16.2 |
| Average $I/\sigma(I)$ | 29.7 (3.4) | 33.0 (3.7) | 29.3 (3.1) |
| Data completeness (%) | 98.2 (87.8) | 98.5 (90.6) | 98.4 (88.3) |
| $R_{\mathrm{merge}}$ | 0.102 | 0.087 | 0.095 |

medium was subsequently inoculated with the resuspended cells for large-scale culture. At mid-log phase, 100 mg l$^{-1}$ lysine, phenylalanine and threonine, 50 mg l$^{-1}$ isoleucine, leucine and valine and 60 mg l$^{-1}$ selenomethionine were added and induction was carried out after 15 min. The induction and harvesting were the same as for wild-type UvsY, while all the buffers used in the purification steps were degassed. Purified wild-type and Se-substituted UvsY protein were stored at 253 K in 20 m$M$ Tris pH 7.4, 50% glycerol, 2 m$M$ BME and 100 m$M$ NaCl. Prior to crystallization, UvsY protein was dialyzed into 1 $M$ ammonium acetate solution and concentrated using a Centricon filter (Amicon; molecular-weight cutoff 10 kDa). The resulting 10–20 OD$_{280}$ protein was either used directly or dialyzed against appropriate buffers for crystallization.

### 2.2. Crystallization

Initial crystallization screening was carried out with commercial kits (Hampton Research). UvsY was crystallized by hanging-drop methods. After dialysis against and concentration in 1 $M$ ammonium acetate, UvsY protein was dialyzed into 100 m$M$ HEPES pH 7.5, 500 m$M$ NaCl and adjusted to 4 OD$_{280}$. 2 µl of the UvsY solution was combined with 2 µl reservoir solution [100 m$M$ HEPES pH 7.5, 6%(*w/v*) hexanediol and 1.5 $M$ lithium sulfate] and suspended over a well containing 1 ml reservoir solution. Rod-like crystals grew to their full length in ~10 d at 293 K.

### 2.3. Data collection

UvsY crystals were cryoprotected with a solution of 2 $M$ lithium sulfate and 5%(*w/v*) glycerol, mounted in nylon cryoloops and flash-frozen in liquid nitrogen. The high concentration of lithium sulfate serves as the primary cryoprotectant. X-ray diffraction data from wild-type UvsY crystals were collected in-house on a MAR 345 detector and Rigaku RUH3R X-ray generator with Xenocs multilayer optics and Cryoindustries crystal-cooling system. A complete MAD data set from a single Se-substituted UvsY crystal was collected at beamline X25 at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. Data were reduced and scaled with *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

## 3. Results

UvsY crystallizes with a rod-like morphology (Fig. 1), with typical dimensions of $500 \times 50 \times 50$ µm. Wild-type UvsY crystals diffract to 2.4–2.7 Å. In addition, SeMet-substituted UvsY has been successfully purified and crystallized in the same manner as the wild type. The six methionine residues per UvsY protomer were all substituted by SeMet as confirmed by MALDI–TOF mass spectrometry (data not
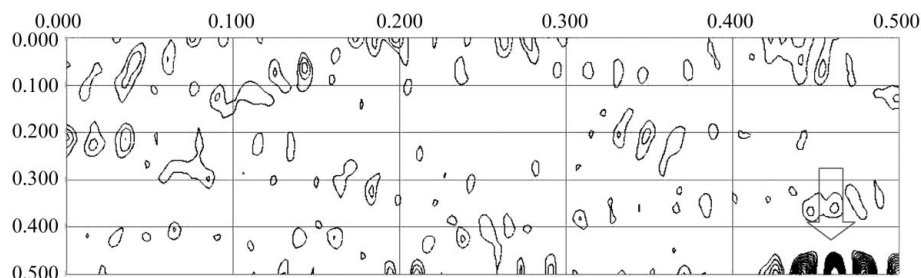
**Figure 2**
$v = 0.5$ section ($u = 0$–$0.5$, $w = 0$–$0.5$) of the self-Patterson map using reflections in the resolution range 20–2.2 Å and contoured in $1\sigma$ intervals starting at $1\sigma$. The strong peak (1/3 of the origin peak at $u = 0.5$, $v = 0.5$, $w = 0.463$) indicates the presence of noncrystallographic translational symmetry.

shown). A complete MAD data set was collected at NSLS beamline X25. Statistics regarding the MAD data set are presented in Table 1.

Since previous ultracentrifugation studies (Beernink & Morrical, 1998) strongly suggest the hexamer to be the fundamental oligomeric state and since the space group of these crystals lacks a crystallographic threefold, we anticipate that there are either three monomers per asymmetric unit, giving a solvent content of 71%, or six monomers, giving a solvent content of 41%.

Translational noncrystallographic symmetry is apparent from the (self-) Patterson map (Fig. 2). The strong peak at ($u = 0.5$, $v = 0.5$, $w = 0.463$) indicates pseudo-body-centering. Since this symmetry modulates the diffraction intensities in such a way that low- and medium-resolution reflections with ($h + k + l$) odd are attenuated, thus giving rise to patterns along the principal reciprocal-space axes characteristic of screw axes, the space group is ambiguously identified as either $P422$, $P4_222$, $P42_12$ or $P4_22_12$. Self-rotation functions failed to reveal the presence of other noncrystallographic symmetry.

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