

**Biochemical and biophysical
characterisation of the genetically
engineered Type I
restriction-modification system,
EcoR124I_{NT}**

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Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusion embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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Abstract

The EcoR124I_{NT} restriction-modification (R-M) system contains the genes HsdS3, HsdM and HsdR. S3 encodes the N-terminal domain of the wild-type S subunit and has been shown to dimerise in solution (Smith et al., 1998). Following purification of the subunits of the EcoR124I_{NT} R-M system, complexes of the methyltransferase S3/M and restriction endonuclease S3/M/R were formed and shown to have activity *in vitro*, methylating and hydrolysing a symmetrical DNA recognition sequence, respectively. The DNA mimic OCR (overcome classical restriction) protein inhibited the methyltransferase activity *in vitro*, with maximum inhibition at a 1:2 molar ratio of (S3/M)₂ to an ocr dimer.

Dynamic light scattering (DLS), sedimentation equilibrium (SE) and sedimentation velocity (SV) experiments showed S3 to exist as a dimer and S11 (the central conserved domain of S) to exist as a tetramer in solution. M was found to be dimeric in solution, whilst the R protein was monomeric. A complex of S3/M was found to have a stoichiometry (S3/M)₂ and a complex of S3/M/R had a stoichiometry of S3/M/R₁, even when a 2:1 molar ratio of R to S3/M, was added.

Small angle neutron scattering (SANS) experiments provided values for the radius of gyration (R_g), which for S3 was comparable to that calculated for the recently published crystal structure of the S subunit from *Methanococcus jannaschii* (Kim *et al.*, 2005). These experiments also showed a decrease in the D_{max} in the presence of the 30 bp DNA recognition sequence from 200Å to 140Å, suggesting a similar conformational change in the positioning of the subunits as has been detected for the wild-type M.EcoR124I and a related type I ½ system AhdI. This change following DNA binding was also observed by SV experiments. Furthermore *ab initio* modelling from the SANS data has provided a low-resolution structure for the EcoR124I_{NT} MTase and its complex with DNA.

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Abbreviations

1D	one-dimensional
3D	three-dimensional
Å	Angstrom/s
ABC	active biological control
<i>A. hydrophila</i>	<i>Aeromonas hydrophila</i>
Amp	ampicillin
Amp ^r	ampicillin resistant
APS	ammonium persulphate
Ard	anti-restriction protein
AFM	atomic force microscopy
ATP	adenosine triphosphate
AU	absorbance units
AUC	analytical ultracentrifugation
bp	base pairs
BSA	bovine serum albumin
CD	circular dichroism
C protein	controller protein
CcrM	cell cycle-regulated methyltransferase
CV	column volumes
D	diffusion coefficient
Da	Daltons
Dam	DNA adenine methyltransferase
Dar	defence against restriction
DEAE	diethylaminoethyl
dH ₂ O	distilled water
DLS	dynamic light scattering
dNTP	deoxynucleoside triphosphate
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA

DTT	dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EOP	efficiency of plating
EMSA	electrophoretic mobility shift assay
ENase	restriction endonuclease
EtBr	ethidium bromide
EtOH	ethanol
Exo III	exonuclease III
F	frictional ratio
FB	freezing buffer
FPLC	fast protein liquid chromatography
g	gram/s
h	hour/s
HgCl	mercury (I) chloride
HIC	hydrophobic interaction chromatography
His	histidine
HEPES	(N-[2-hydroxyethyl] piperazine-N ⁺ - [2-ethanesulphonic acid])
Hsd	host specificity of DNA
HTH	helix-turn-helix
ID	insertion device
IEX	ion exchange
IPTG	isopropyl- β -D-thiogalactopyranoside
IVC	<i>in vitro</i> compartmentalization
k	Boltzmann constant
K _a	equilibrium association constant
KAc	potassium acetate
Kan	kanamycin
Kan ^r	kanamycin resistant
kbp	kilobase pairs
K _d	equilibrium dissociation constant
kDa	kiloDaltons

L	litre/s
Lla	<i>Lactococcus lactis</i>
μg	microgram/s
μL	microlitre/s
μM	micromolar
M	molar
MCS	multiple cloning site
MeOH	methanol
mg	milligram/s
mL	millilitre/s
mM	millimolar
min	minute/s
M subunit	modification subunit
M _B	benchmark protein ladder (Amersham)
M _r	molecular mass
mRNA	messenger RNA
MS	mass spectrometry
MTase	methyltransferase
MWCO	molecular weight cut-off
m/z	mass to charge ratio
N	any nucleotide
N ₂	nitrogen gas
NaAc	sodium acetate
Na ₂ EDTA	ethylenediamine tetra-acetic acid (disodium salt)
NaBr	sodium bromide
ng	nanogram/s
nm	nanometre/s
nM	nanomolar
nt	nucleotide/s
OD	optical density
OD _x	optical density at x nm
ORF	open reading frame
pac site	packaging site

PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pET	plasmid for expression by T7 RNA polymerase
PNK	polynucleotide kinase
Q	quaternary amine
R	a purine
R subunit	restriction subunit
RA	restriction alleviation
R _h	hydrodynamic radius
R-M	restriction-modification
RNA	ribonucleic acid
rpm	revolutions per minute
s	seconds
S	Svedberg
SAM	S-adenosylmethionine
SANS	small angle neutron scattering
SAXS	small angle X-ray scattering
S subunit	specificity subunit
SC	supercoiled
SDS	sodium dodecyl sulphate
SE	sedimentation equilibrium
SPR	surface plasmon resonance
ssDNA	single-stranded DNA
SV	sedimentation velocity
TAE	tris, acetate, Na ₂ EDTA
TE	tris, Na ₂ EDTA
TEMED	N, N, N', N'-tetramethylethylenediamine
TNE	tris, sodium chloride, Na ₂ EDTA
TRD	target recognition domain
Tris	tris (hydroxymethyl)-aminomethane
tRNA	transfer RNA
U	units
UV	ultraviolet
V	volts

\bar{V}	partial specific volume
v/v	volume/volume
w/v	weight/volume
w/w	weight/weight
Y	a pyrimidine