

Engineering synthetic Lsm rings for applications in nanotechnology

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Declaration

Where appropriate, work done in collaboration with other groups or individuals has been acknowledged. Outside these contributions, the material in this thesis is entirely my own work and to the best of my knowledge original. No part of this thesis has been submitted for a higher degree to any other university or institution. I consent to this thesis being made available for photocopy and loan.

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Abstract

The structural diversity of proteins makes them highly attractive as self-assembling bio-bricks or ‘tectons’ for the fabrication of biocompatible materials. In the current study, ring-forming Lsm proteins were exploited to construct novel nanostructures via supramolecular engineering. The Lsm family of proteins comprise the core of the ribonucleoprotein (RNP) complexes, crucial to RNA metabolism. They assemble into oligomeric rings of six to eight protein chains *in vitro* to generate an RNA-binding scaffold.

Synthetic ring tectons composed of individual or fused Lsm sequences from *S.cerevisiae* were engineered so as to self assemble into simplified ring structures. My work focused on two polyprotein forms, fusions of Lsm[4+1] and Lsm[2+3]. In solution these recombinant Lsm polyproteins self-organise into robust ring structures (7.5 nm x 5.0 nm), that maintain their intrinsic RNA-binding ability. Elevated levels of overlaid ring pairs are favoured at low ionic strength, indicating electrostatic mediated packing of Lsm rings. SAXS output reveals these ring pairs to be assembled in a staggered conformation rather than coaxially stacked.

Using chemical modification, Lsm tectons were further fabricated into new coherent architectures. Cys residues engineered into two opposing tecton faces promoted covalent interactions between rings, generating disulfide-bonded Lsm[4+1]₄ clusters (11 – 12 nm) that were readily disengaged by reduction agents. This cluster assembly displayed enhanced thermal stability across a range of solvent conditions.

Conjugation between Lsm tectons was also mediated through metal-His coordination, resulting in organisations that are sensitive to EDTA, salt concentration and pH. A Ni²⁺-chelated Lsm[4+1]₄ cluster displayed structural similarities to its Cys-

linked counterpart; this four-ringed assembly possessing an increased stability compared to Lsm[4+1]₄, although RNA-binding was compromised. In the presence of Cu²⁺ or Co²⁺ the tecton Lsm₃₈ organised as ring pairs that were coaxially organised, so displaying potential as a precursor to an Lsm-based tubule.

This thesis also describes crystallisation progress towards an atomic-resolution structure of the Lsm[4+1]₄ tecton. With highly pure samples, screening and optimisation across a range of conditions and crystallisation avenues allowed collection of six native datasets (at ~ 3 Å). This data could serve to solve the first crystal structure of an Lsm polyprotein ring at high resolution.

The use of Lsm rings to construct new and functional nanostructures is still in its infancy. However, this project illustrates their potential as robust and highly stable tectons, suitable for engineering endeavours. The construction of Lsm tectons into controllable higher-order superstructures, discussed in this thesis, may have future applications in RNA housing and delivery capsules or as next-generation bio-inspired nanosensors.

List of abbreviations

Å	angstrom
A ₂₈₀	absorbance at 280 nm
A ₂₆₀	absorbance at 260 nm
AFM	atomic force microscopy
AI	autoinduction
ASU	asymmetric unit
bp	base pair
BMC	bacterial microcompartment
CCP4	collaborative computational project number 4
CD	circular dichroism
cv	column volume
D _{max}	maximal particle diameter
DMSO	dimethyl sulfoxide
dn/dc	refractive index increment
Dps	DNA-binding protein
DSF	differential scanning fluorimetry
DTT	dithiothreitol
EYFP	enhanced yellow fluorescent protein
FAD	flavin adenine dinucleotide
<i>g</i>	standard acceleration due to gravity
GFP	green fluorescent protein
GroEL	heat shock protein 1
Hcp1	haem carrier protein 1
Hfq	bacterial Lsm protein
His ₆	hexa-Histidine
HSP 60	heat shock protein 60
IMAC	immobilised metal affinity chromatography
ITC	isothermal titration calorimetry
JCSG	joint centre for structural genomics
K _{av}	size exclusion distribution coefficient

kDa	kilo Dalton
LB	lysogeny broth
LIC	ligation-independent cloning
Lsm	like-Sm
Lsm[4+1]	polyprotein composed of fused Lsm4 and Lsm1
Lsm[2+3]	polyprotein composed of fused Lsm2 and Lsm3
MALLS	multi-angle laser light scattering
mRNA	messenger RNA
Mt Lsm α	<i>Methanothermobacter thermoautotrophicum</i> Lsm α
MW	molecular weight
NCS	non-crystallographic symmetry
NDS	normalised spatial discrepancies
OD ₆₀₀	optical density at 600 nm
PA-Sm1	<i>Pyrococcus abyssi</i> Sm1
P-body	processing body
PDB	protein data bank
PEG	polyethyleneglycol
PHENIX	python-based hierarchical environment for integrated crystallography
pKa	acid dissociation constant
p(r)	electron distance distribution function
R ²	coefficient of determination
R _g	radius of gyration
RNP	ribonucleoprotein
rRNA	ribosomal RNA
SAXS	small angle x-ray scattering
SEC	size exclusion chromatography
SeMet	L-selenomethionine
SmAP	Sm archaeal protein
snoRNA	small nucleolar RNA
snRNP	small nuclear ribonucleoprotein
SOC	super optimal broth with glucose added
SP1	stable protein 1

SPR	surface plasmon resonance
Tacsimate	mixture of titrated organic acid salts
TBP	titanium-binding peptide
TEM	transmission electron microscopy
TRAP	<i>trp</i> RNA-binding attenuation protein
T _m	melting temperature
v/v	unit volume per unit volume
V ₀	void volume of size exclusion chromatography
w/v	unit weight per unit volume
w/w	unit weight per unit weight
XDS	x-ray detector software
