Immobilisation of active enzymes on novel GFP protein particles

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ABSTACT

Inclusion bodies were previously thought to be aggregations of inactive, mis-folded proteins. However, there is a growing body of evidence that suggests otherwise. In 2011 Jahns et al demonstrated a self-assembling GFP protein particle (GFP particle) that not only exhibited fluorescence, but was also able to display functional antibody and ligand binding sites. These functional GFP particles exhibited reasonable activity, and in many cases outperformed commercially available particles. The GFP particles consisted of an aggregation of fusion proteins. These fusion proteins in turn consisted of an N-terminally extended enhanced GFP protein which was fused at its C-terminus to an inactive polyester synthase (PhaC(C319A)) from *Ralstonia eutropha*, and a functionality, e.g. antibody/ ligand binding site. In this study, GFP particles were investigated to ascertain whether they could serve as a support for the immobilization and display of active enzymes; and provide a technology that is potentially more efficient and cost-effective than other enzyme immobilization methods. Furthermore, their inherent fluorescence would provide an additional advantage. The enzymes used for functionality tests were: a thermostable α-amylase from *Bacillus licheniformis* that lacked its signal sequence (Bla(-ss)); *N*-acetyl-D-neuraminic acid aldolase (NanA) from *Escherichia coli*; and organophosphohydrolase (OpdA) from *Agrobacterium radiobacter*. These enzymes were chosen for their differing quaternary structure- monomer, tetramer, and dimer, respectively- and were fused to the C-termini of GFP fusion proteins. The results of this investigation showed that it is possible to generate fluorescent GFP particles inside recombinant *E. coli* BL21(DE3) cells which are also able to display active enzyme. These enzyme-bearing GFP particles exhibited considerable stability across a range of temperature, pH, and storage conditions, and could also be reused. The activity of the particles was also compared to a similar technology- functionalized PHA beads; however, the PHA beads consistently exhibited stronger enzyme activity under all conditions tested. GFP protein particles represent a novel method for the immobilization and display of enzymes. Their ability to immobilise and display active enzymes of different quaternary structure under a range of conditions makes GFP particles particularly attractive to industrial biocatalysis processes. Potential applications include diagnostic assays, food production, pharmaceutical production, and bioremediation.

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Where do I start? As I'm sure every post-grad can appreciate, undertaking a Master's degree is not to be considered lightly. All those who have gone before can tell you how much work is required: the long days; the lack of sleep; the repetition of assays that fail for unforeseen reasons (or foreseen reasons). My project was no exception, and there are many people I'd like to thank for their words of encouragement and advice for which I'm deeply grateful. First, I'd like to thank my supervisor Professor Bernd Rehm for giving me the opportunity of studying under him. Considering that he did not know me beforehand, he took a chance on an eccentric guy from Wellington, and gave me advice and direction that not only helped me with this study, but will also be of great use in my future career. Next, I'd like to thank David Hooks for his endless support and technical knowhow. I was very much a novice in molecular biology when I started, but thanks to David, and his patience, I finish my Master's with a wealth of knowledge- and some fancy squash techniques. A thank you must also go out to Jason Lee for making the weekend work and breaks a lot more enjoyable, and Iain Hay for having the answer I need no matter how obscure the question. A special thanks to the whole Rehm lab and Polybatics for being a great bunch of people to work beside; Trevor Loo for his HPLC expertise; and Rosie Bradshaw's lab for letting me steal their equipment. A big thanks to my flatties Bex Smith for being a whirlwind of energy and laughs; Bob Stewart for helping me get in touch with my Upper Hutt gangsta roots; and Kim Green for the fly-by hugs. As always I'm indebted to my family for their support and encouragement, and financial assistance. I don't want to think how much money they've given me over the years, but I promise it's been worth it.

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ABBREVIATIONS

AGE	Agarose Gel Electrophoresis
Ap^r	Ampicillin resistance gene
AVTS	Amino acids Alanine-Valine-Threonine-Serine that constitute a short N-terminal extension of GFP in GFP fusion proteins
BLA	α-amylase from *Bacillus licheniformis*
Bla(-ss)	α-amylase from *Bacillus licheniformis* that lacks the signal sequence required for extra-cellular export
Bla(-ss)-PhaC	α-amylase/ PHA synthase fusion protein
BSA	Bovine Serum Albumin
bp	Base pair
˚C	Degrees Celsius
CFP	Enhanced cyan-fluorescent protein
CLEA	Cross-linked enzyme aggregate
CLEC	Cross-linked enzyme crystal
Cm^r	Chloramphenicol resistance gene
dATP	Deoxyadenosine triphosphate
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
dTTP	Deoxythymidine triphosphate
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
ELISA	Enzyme-linked immunoabsorbant assay
extGFP	N-terminally extended green fluorescent protein
FHKP	Amino acids Phenylalanine-Histidine-Lysine-Proline that constitute a short N-terminal extension of GFP in GFP fusion proteins
GB1	IgG binding domain of Protein G from *Streptococcus* Group G
[GB1]3	Triple-repeat of GB1
GFP	Green fluorescent protein
GFP particle	GFP fusion protein particle

GiCL	Fusion protein consisting of ext(AVTS)GFP-inactive PHA synthase-pentaglycine linker.
GiCLB	Fusion protein consisting of ext(AVTS)GFP-inactive PHA synthase-pentaglycine linker-α-amylase. Also denotes resultant GFP particle.
GiCLN	Fusion protein consisting of ext(AVTS)GFP-inactive PHA synthase-pentaglycine linker-[*N*-acetyl-D-neuraminic acid aldolase]. Also denotes resultant GFP particle.
GiCLO	Fusion protein consisting of ext(AVTS)GFP-inactive PHA synthase-pentaglycine linker-organophosphohydrolase. Also denotes resultant GFP particle.
GiCLZ	Fusion protein consisting of ext(AVTS)GFP-inactive PHA synthase-pentaglycine linker-ZZ domain. Also denotes resultant GFP particle.
GNL	Fusion protein consisting of ext(AVTS)GFP-[*N*-acetyl-D-neuraminic acid aldolase]-pentaglycine linker.
GNLN	Fusion protein consisting of ext(AVTS)GFP-[*N*-acetyl-D-neuraminic acid aldolase]-pentaglycine linker-[*N*-acetyl-D-neuraminic acid aldolase]. Also denotes resultant GFP particle.
GNLZ	Fusion protein consisting of ext(AVTS)GFP-[*N*-acetyl-D-neuraminic acid aldolase]-pentaglycine linker-ZZ domain. Also denotes resultant GFP particle.
h	Hour
HcR	Far red protein HcRed
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	High Performance Liquid Chromatography
IB	Inclusion body
IgG	Immunoglobulin G
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	Kilodalton
LAVG	Amino acids Leucine-Alanine-Valine-Glycine that constitute a short N-terminal extension of GFP in GFP fusion proteins
LB	Luria-Bertani
M	mol per litre

µg	Microgram
µl	Microliter
mA	Milliampere
ml	Millilitre
mM	Millimol per litre
MALDI-TOF/MS	Matrix-assisted Laser Desorption/ Ionisation Time-of-Flight Mass Spectrometry
MalE	Maltose binding protein
ManNAc	*N*-acetyl-D-mannosamine
min	Minute
MOPS	3-Morpholinopropanesulfonic acid
MW	Molecular weight
NanA	*N*-acetyl-D-neuraminic acid aldolase from *E. coli*
NanA-PhaC	*N*-acetyl-D-neuraminic acid aldolase/ PHA synthase fusion protein
Neu5Ac	*N*-acetyl-D-neuraminic acid
ng	Nanogram
OpdA	Organophosphohydrolase from *Agrobacterium radiobacter*
PCR	Polymerase Chain Reaction
pET14b-GiCL	pET14b plasmid that encodes the fusion protein GiCL.
pET14b-GiCLB	pET14b plasmid that encodes the fusion protein GiCLB.
pET14b-GiCLN	pET14b plasmid that encodes the fusion protein GiCLN.
pET14b-GiCLO	pET14b plasmid that encodes the fusion protein GiCLO.
pET14b-GiCLZ	pET14b plasmid that encodes the fusion protein GiCLZ.
pET14b-GNL	pET14b plasmid that encodes the fusion protein GNL.
pET14b-GNLN	pET14b plasmid that encodes the fusion protein GNLN.
pET14b-GNLZ	pET14b plasmid that encodes the fusion protein GNLZ.
PHA	Polyhydroxyalkanoate
PhaC	PHA synthase
PhaC (C319A)	Inactive PHA synthase
PhaC-OpdA	PHA synthase/ Organophosphohydrolase fusion protein
PHB	Polyhydroxybutyrate
rpm	Revolutions per minute
R.T.	Room temperature (22 ˚C- 25 ˚C)

SDS	Sodium dodecylsulfate
SDS-PAGE	Sodium dodecylsulfate Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
U	Enzyme units
V	Volts
x g	Unit denoting centrifugal force as a multiple of standard gravity on Earth
YFP	Enhanced yellow fluorescent protein
ZZ	IgG binding domain of Protein A from *Staphylococcus aureus*

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