Improved fluorescent proteins for Synthetic Biology

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BioBricks

Standard parts for building with biology

BioBricks [1] are standardised pieces of DNA flanked by prefix and suffix sequences. Advantages for synthetic biology include:

Standardisation: the standard assembly process can put together any two BioBricks

Modularity: combination of two BioBricks creates a new standard BioBrick due to the formation of a scar (mixed site) (Fig.1)

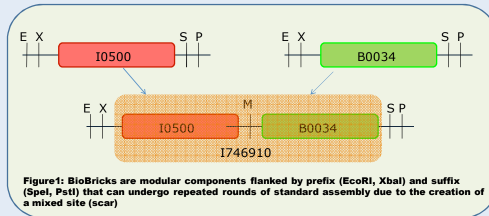

Figure1: BioBricks are modular components flanked by prefix (EcoRI, XbaI) and suffix (SpeI, PstI) that can undergo repeated rounds of standard assembly due to the creation of a mixed site (scar)

Parallel or automated assembly to combine large numbers of BioBricks

Characterisation: An open source online database contains sequences, assemblies, design considerations, part and device characterisations

Physical part repository: The Registry of Standard Biological Parts [1] physically hosts thousands of BioBricks available to researchers

Modelling and Design of systems based on the characterisation of basic parts

Abstraction: Creation of higher-order devices isolated from underlying detail (Fig. 2)

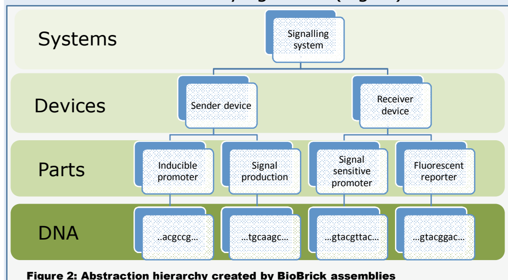

Figure 2: Abstraction hierarchy created by BioBrick assemblies

Introduction

Green Fluorescent Protein (GFP) [2] offers efficient and convenient means of visualising the dynamic process of gene expression and of obtaining a readout of the current state of complex gene regulatory networks – features of major interest for synthetic biology.

Despite the potential applications of improved variants of GFP in synthetic biology, such as for protein secretion and in vitro gene expression, the only GFP variant currently characterised in the Registry of Standard Biological Parts is the somewhat outdated GFP mut3b [3] .

In this project two recently developed GFP variants, P7 GFP [4] and superfolder GFP [5], were chosen for standardisation into BioBrick format, characterisation and contribution to the registry.

Results

Superfolder GFP fluorescence increases 3.5 to 4 fold faster than P7 or mut3 GFP in vivo

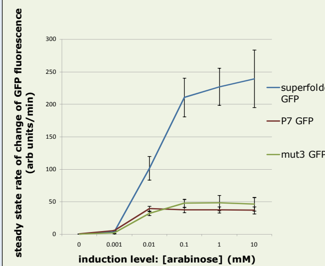

Figure 3: Rates of increase of in vivo GFP fluorescence 2.5 to 4hrs after induction of the pBAD promoter with different levels of arabinose.

Superfolder GFP yields up to four fold more total fluorescence than mut3 or P7 GFP when expressed in vivo

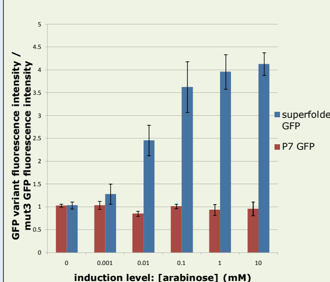

Figure 4: in vivo fluorescence intensities of GFP variants were compared at steady state (between 3hrs – 5hrs after induction). The fluorescence intensity of superfolder and P7 GFP is expressed as a fraction of the intensity of mut3 GFP.

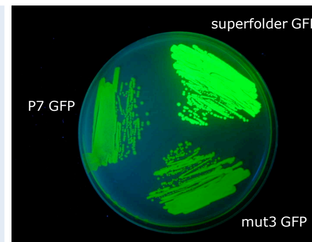

Figure 5: E.coli BW27783 growing on LB agar plate containing 10mM arabinose. The three streaks are expressing the GFP variant indicated in the picture driven by the pBAD promoter (see Methods for construct). Plate was illuminated with handheld UV lamp and photographed.

Superfolder GFP displays improved performance in vivo including absolute fluorescence levels in liquid culture and on colony plates as well as the rate of increase in GFP fluorescence while both P7 and superfolder GFP show improved in vitro folding and stability.

P7 GFP has the fastest refolding kinetics of the three variants investigated.

After denaturing in vitro, P7 GFP renatures faster than superfolder or mut3 GFP

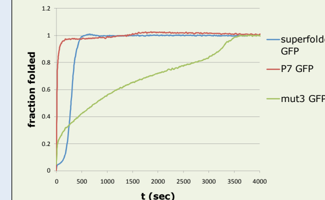

Figure 6: Short term in vitro refolding of 6-his purified GFP samples after complete denaturing. Data shown is the fraction folded at each time point compared to the maximally refolded sample.

Excitation and emission spectra vary slightly between the GFP variants

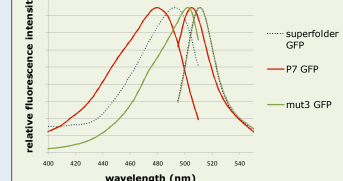

Figure 7: Excitation and Emission spectra determined for 6-his purified GFP variants. Excitation was at 480nm for emission spectra; emission was at 520nm for excitation spectra.

P7 and superfolder GFPs are more resistant to denaturing in vitro than is mut3 GFP

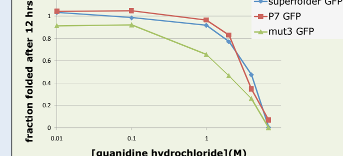

Figure 8: Resistance of 6-his purified GFP variants to denaturing after 100fold dilution into TNG buffer containing different concentrations guanidine hydrochloride Data shown is the fraction folded after 12 hours compared to a fully folded sample.

In vitro renaturing ability is stronger in superfolder and P7 than in mut3 GFP

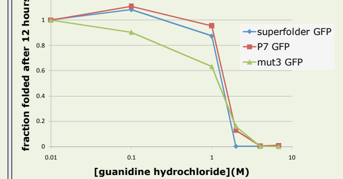

Figure 9: Ability of 6-his purified GFP variants to refold in different concentrations guanidine hydrochloride after complete denaturation. Data shown is the fraction folded after 12 hours compared to a fully folded sample.

Methods

mut3 GFP was obtained from the 2007 distribution of the Registry of Standard Biological Parts [1]. P7 GFP [4] was kindly provided by Dr Adam C. Fisher from Cornell University, New York. Superfolder GFP [5] was created by de novo DNA synthesis (Geneart). All three GFP variants were cloned – as non tagged and 6-his tagged versions - into the same basic construct residing in pSB1A2:

I0500 pBAD promoter | B0034 RBS | GFP variant coding region | B0015 terminator

Amino acid changes with respect to mut3 GFP were confirmed by sequencing:

	30	39	64	65	68	99	105	124	145	153	163	171	206
mut3	S	Y	F	G	V	F	N	E	Y	M	V	I	A
P7	S	Y	L	A	L	F	Y	V	F	M	V	I	A
super-folder	R	N	L	T	V	S	T	E	F	T	A	V	V

Table 1: relevant amino acid positions of GFP variants showing all mutations of superfolder and P7 GFP as compared to mut3 GFP

Conclusion

Two improved GFP variants were standardised into BioBrick format and their in vivo and in vitro properties were compared to mut3 GFP:

Superfolder GFP represents a major improvement for in vivo GFP expression and will hence be extremely useful for synthetic biology applications.

P7 GFP may be a promising candidate for in vitro experiments.

Both variants were contributed to the Registry of Standard Biological Parts and are as such available for wide use in the synthetic biology community.

References

[1] MIT Registry of Standard Biological Parts: http://partsregistry.org

[2] Chalfie, *et al.* (1994). "Green Fluorescent Protein as a Marker for Gene Expression," *Science* 263, 802-805.

[3] Cormack et al (1996) "FACS-optimized mutants of the green fluorescent protein (GFP)", *Gene*, 173, 33-38

[4] Fisher et al (2008) "Laboratory Evolution of Fast-Folding Green Fluorescent Protein Using Secretory Pathway Quality Control", PLoS ONE 3(6)

[5] Pédelacq et al (2006) "Engineering and characterization of a superfolder green fluorescent protein", Nature Biotech 24 (1), 79-88