



Figure.S1 Multiple sequence alignment of 27 seed sequences of Pfam family of GFP originated from different organisms with both fluorescent and non-fluorescent proteins. The 27 seed sequences of Pfam family of GFP were retrieved in fasta format and the multiple sequence alignment were performed in BioEdit sequence alignment editor. The alignment was prepared with respect to GFP sequence from *Aequorea Victoria* (P42212).

n-GFP	MSKGEELFTGVVPIVLVELDGDVNGHKFSVSGEGDATYGKLTLLKFICTTGKLPVPWPTL	60
n-GFP (+15-17)	MSKGEELFKGVVPIVLVELDGDVNGHKFSVSGEGDAKEGKLTLLKFICEEGLKLPVPWPTL	60
n-GFP (+5-6)	MSKGEELFTGVVPIVLVELDGDVNGHFSVSGEGNGDATYGKLTLLKFICTTGKLPVPWPTL	60
n-GFP	VTTFGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV	120
n-GFP (+15-17)	VTTFGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV	120
n-GFP (+5-6)	VTTFGYGVQCFARYPSGMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGNTLV	120
n-GFP	NRIELKGFKEEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD	180
n-GFP (+15-17)	NRIELKGFKEEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD	180
n-GFP (+5-6)	NRIELTGINFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIENGSVQLAD	180
n-GFP	HYQQNTPIGDGPVLLPDNHYLSTQSALSNDPNEGRDHMVLEFVTAAGITHGMDELYK	238
n-GFP (+15-17)	HYQKNTPIGDGPVLLPDHHLSTRSALSNDPNEGRDHMVLEFVTAAGITHGMDELYK	238
n-GFP (+5-6)	HYQQNTPIGSGPVLLPDNHYLSTQSALSNDPNEGRDHMVLEFVTAAGITHGMDELYK	238

Figure.S2 Primary structures of n-GFP and its variants with mutations for surface charge variation. Red marked residues in n-GFP are the amino acids on protein surface whose side chains are >50% exposed to solvent. The signs ‘*’ and ‘•’ in n-GFP denote the 23 charged amino acids and 14 polar uncharged residues on the surface respectively. The mutations increase or decrease the surface charge numbers are indicated green and pink in n-GFP(+15-17) and n-GFP(+5-6) respectively.

s-GFP(+10-13)	MSKGEELFTGVVPIVLVELDGDVNGHKFSVRGEGEGDATNGKLTLLKFICTTGKLPVPWPTL	60
s-GFP (+15-17)	MSKGEELFKGVVPIVLVELDGDVNGHKFSVRGEGEGDAKEGKLTLLKFICEEGLKLPVPWPTL	60
s-GFP (+5-6)	MSKGEELFTGVVPIVLVELDGDVNGHFSVSRGEGNGDATNGKLTLLKFICTTGKLPVPWPTL	60
s-GFP(+10-13)	VTTLGYGVQCFARYPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYYKTRAEVKFEGDTLV	120
s-GFP (+15-17)	VTTLGYGVQCFARYPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYYKTRAEVKFEGDTLV	120
s-GFP (+5-6)	VTTLGYGVQCFARYPSGMKRHDFFKSAMPEGYVQERTISFKDDGTYYKTRAEVKFEGNTLV	120
s-GFP (+10-13)	NRIELKGFKEEDGNILGHKLEYNFNHSHKVYITADKQKNGIKANFKIRHNVEDGSVQLAD	180
s-GFP (+15-17)	NRIELKGFKEEDGNILGHKLEYNFNHSHKVYITADKQKNGIKANFKIRHNVEDGSVQLAD	180
s-GFP (+5-6)	NRIELTGINFKEDGNILGHKLEYNFNHSHKVYITADKQKNGIKANFKIRHNVENGSVQLAD	180
s-GFP(+10-13)	HYQQNTPIGDGPVLLPDNHYLSTQSVLLKDPNEGRDHMVLEFVTAAGITHGMDELYK	238
s-GFP (+15-17)	HYQKNTPIGDGPVLLPDHHLSTRSVLLKDPNEGRDHMVLEFVTAAGITHGMDELYK	238
s-GFP (+5-6)	HYQQNTPIGSGPVLLPDNHYLSTQSVLLSDPNEGRDHMVLEFVTAAGITHGMDELYK	238

Figure.S3 Co-introduction of stabilizing mutations along with mutations for surface charge variations into the primary structures of n-GFP rescued the problem of protein misfolding. In s-GFP(+10-13), the amino acids highlighted in blue are the mutations which enhance folding efficiency and stability. The mutations increase or decrease the surface charge numbers are indicated green and pink in s-GFP(+15-17) and s-GFP(+5-6) respectively.