Supp Fig S1: Protein engineering and in vitro characterization.

x) Sequence alignment. Numbering according to 2VHA.pdb (glutamate binding protein, purple) and 2B3P.pdb (superfolder GFP, orange). Differences between iGluSnFR3.v857 and iAspSnFR are:

GltI: E25K in iAspSnFR, E25D in iGluSnFR3.v857

GltII: S27A in iAspSnFR, S27S in iGlu3SnFR3.v857

GltI: S72P in iAspSnFR, S72S in iGluSnFR3.v857

GltI: A184V in iAspSnFR, A184S in iGluSnFR3.v857

cpSFVenus: S147S in iAspSnFR, S147N in iGluSnFR3.v857

cpSFVenus: N147I in iAspSnFR, N147T in iGluSnFR3.v857

cpSFVenus: T228 in iAspSnFR, G228 in iGluSnFR3.v857

cpSFVenus: L46 in iAspSnFR, F46 in iGluSnFR3.v857

cpSFVenus: GYG chromophore in iAspSnFR, SYG chromophore in iGluSnFR3.v857

1 2 3 4 5 6

0 0 0 0 0 0

MRSAAGSTLDKIAKNGVIVVGHRKSAVPFSYYDSQHKVVGYSQEYSNAIVEAVKKKLNK

1 1 1

7 8 9 0 1 2

0 0 0 0 0 0

PDLQVKLIPITPQNRIPLLQNGTYDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGG

1 1 1 1 1 1

3 4 5 6 7 8

0 0 0 0 0 0

DIKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFM

1 2 2 2 2 2

9 0 1 2 3 4

0 0 0 0 0 0

MDDVLLAGERAKAKKPDDWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAE

2 1 1 1 1 1 1

5 4 5 6 7 8 9

0 7 3 3 3 3 3

KWFDKWFKNPILVSHIVYITADKQKNGIKANFKIHHNVEDGSVQLADHYQQNTPIGDGPV

2 2 2 2 2

0 1 2 3 3

3 3 3 3 8 1 9

LLPDNHYLSYQSVLSKDPNEKRDHMVLLEFVTATTNSLGMDELYKGGTGGSMSKGEELFT

1 2 3 4 5 666

9 9 9 9 9 567

GVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKLICTTGKLPVPWPTLVTTLGYGVQ

1 1 1

7 8 9 0 1 2

9 9 9 9 9 9

CFARYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKLEGDTLVNRIELKGID

1 1 2 2 2

3 4 6 7 7

9 6 0 0 9

FKEDGNILGHKLEYNFNNPLNMNFELSDEMKALFKEPNDKALKLQHHHHHH