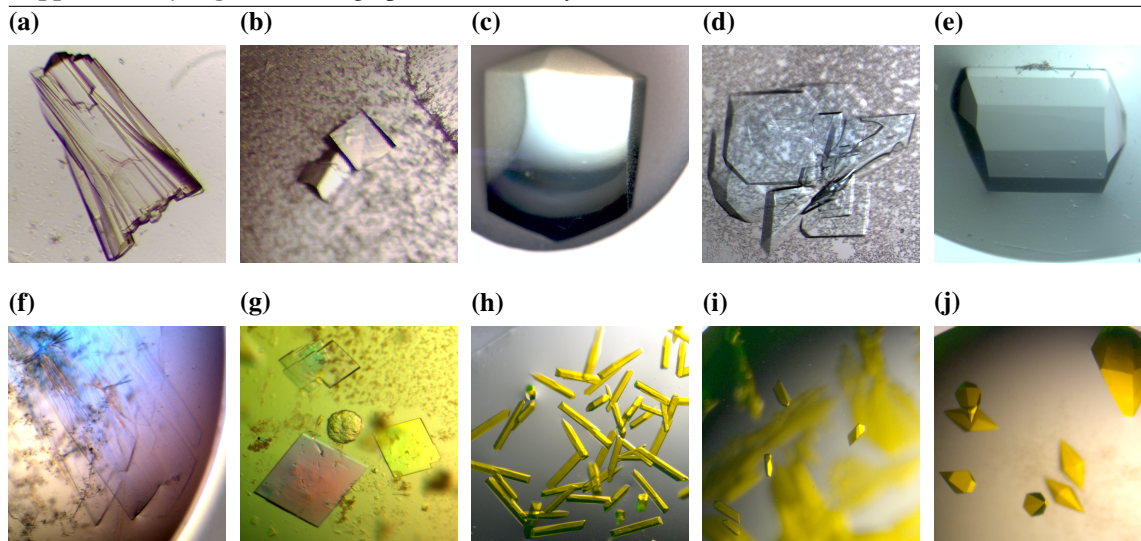
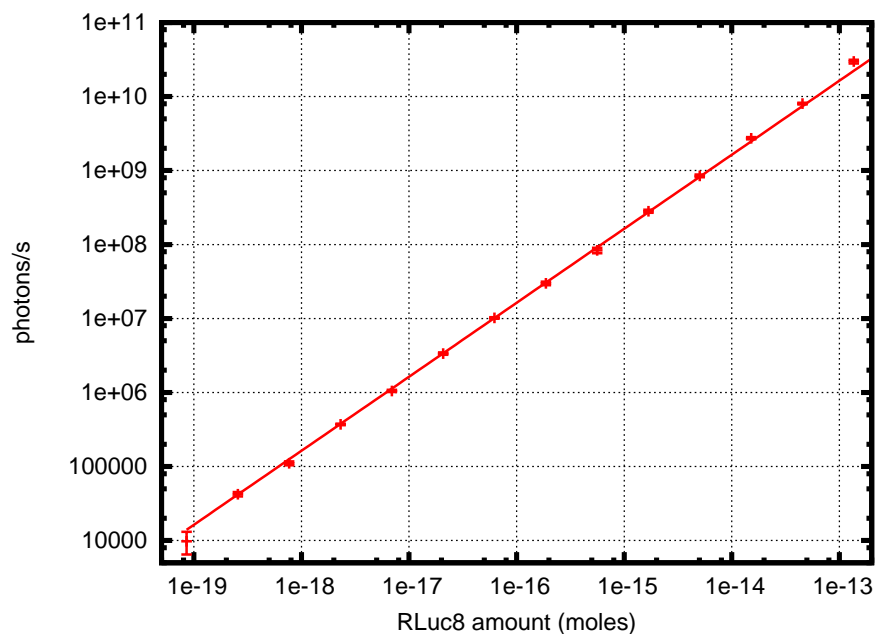

Supplementary Figure 1 Photographs of various crystals.



Conditions referred to by a label are given in Table 1 of the main text. **(a)** RLuc8:diammonium. **(b)** RLuc8:KSCN. **(c)** Protein: RLuc8 at 450 mg/ml, Mother Liquor: 0.3 M NaCl, 1.25 M diammonium phosphate, 0.1 M imidazole pH 8.0, Time: 8 months. Crystal was $\sim 100 \mu\text{m}$ in size, and diffracted to 1.6 \AA . **(d)** S3RLuc8:thiomaltoside. **(e)** Protein: S3RLuc8 at 30 mg/ml, Mother Liquor: 0.5 M NaCl, 1.55 M diammonium phosphate, 0.1 M imidazole pH 8.0, Time: 4 months. **(f)** RLuc8/K25A/E277A:PEG/isopropanol. **(g)** RLuc8:PEG/isopropanol. **(h)** RrGFP:PEG/MPD. **(i)** Protein: RrGFP at 48 mg/ml, Mother Liquor: 0.1 M MES pH 6.5, 12% w/v PEG 20,000, Time: 30 min. **(j)** Protein: RrGFP at 48 mg/ml, Mother Liquor: 2.0 M ammonium sulfate, 5% v/v isopropanol, Time: 2 days. Cryoprotectant: mother liquor containing 35% MPD. These crystals are not optimal for diffraction, as the particularly long C axis of the crystal cell ($\sim 250 \text{ \AA}$) would require a significant loss of diffraction resolution in order to keep the diffraction spots properly separated for the purposes of peak integration.

Supplementary Figure 2 Linearity of the luciferase enzymatic reaction.



Serial 1:2 dilutions of RLuc8 were made in nickel affinity elution buffer (300 mM NaCl, 250 mM imidazole, 20 mM HEPES, pH 8) containing 1% human serum albumin as a carrier protein. The samples were then assayed in triplicate by adding 1 μ l of each sample to 100 μ l room temperature 100 mM sodium phosphate buffer (pH 7), adding 1 μ l of 0.5 μ g/ μ l coelenterazine, manually mixing, and measuring the average photons/s emitted over 10 s. Luminometer calibration was as previously described (Loening *et al.*, *Protein Eng. Des. Sel.* 19:391-400, 2006), and background luminescence was subtracted from the measured values. Error bars are SEM.

Supplementary Table 1 Yield and specific activity of cytoplasmically expressed RLuc8 and the surface mutation constructs

Protein	Yield ($\mu\text{g/ml}$)	Specific Activity (relative to RLuc)
1 RLuc8	528	4.8
2 RLuc8/K12A/E106A	392	5.0
3 RLuc8/K25A/E277A	312	6.1
4 RLuc8/E183A/K227A	418	5.6
5 RLuc8/K12A/K25A/E106A/E277A	140	4.0
6 RLuc8/K25A/E183A/K227A/E277A	40	3.0
7 RLuc8/812A/E106A/E183A/K227A	232	3.4
8 RLuc8/K12A/K25A/E106A/E183A/K227A/E277A	20	4.0

Yield represents μg of recovered protein per ml of culture. Specific activities were measured using coelenterazine, and are reported as relative to that of periplasmically expressed, C-terminal 6xHis tagged RLuc that has an activity of 3.2×10^{22} photons/s/mole enzyme (Loening *et al.*, *Protein Eng. Des. Sel.* 19:391-400, 2006).