Supporting information for article:

Probing the effectiveness of spectroscopic reporter unnatural amino acids: a structural study

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<table>
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<tr>
<th>pBAD vector</th>
<th>WT</th>
<th>133TAG</th>
<th>149TAG</th>
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<td>pDULE vector</td>
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**Figure S1** Coomassie stained tris-glycine SDS-PAGE of purified proteins post Ni-NTA affinity chromatography illustrating efficient, site-specific incorporation of 1 and 2 with high fidelity at either the 133 or 149 sites. The protein constructs were expressed from pBAD-sfGFP (WT in lane 2); pBAD-sfGFP-133TAG and pDULE-pCNF/pCCF in the presence (lane 3) or absence (lane 4) of 1; pBAD-sfGFP-149TAG and pDULE-pCNF/pCCF in the presence (lane 5) or absence (lane 6) of 1; pBAD-sfGFP-133/149TAG and pDULE-pCNF/pCCF in the presence (lane 7) or absence (lane 8) of 1 and presence (lane 9) or absence (lane 10) of 2.
Figure S2  Surface representations in the vicinity of the 133 (A) or 149 (B) sites in the sfGFP-133-pCNF or sfGFP-149-pCNF crystal structures, respectively. The 133pCNF residue (orange) is solvent accessible with a solvent accessibility of 89% and the 149pCNF residue (cyan) is partially buried with a solvent accessibility of 58%. Surface representations of surrounding residues were made in PyMol.
Figure S3  Final refined structure of sfGFP-133/149-pCNF. The overall structure of one of the molecules in the asu is shown in (a.) with 2F$_o$-F$_c$ map at 1$\sigma$ (blue mesh). Focused views including 2F$_o$-F$_c$ map around the 133 (b.) and 149 (c.) sites.
Figure S4  Refinement progression for sfGFP-133/149-pCCF. Electron density 2Fo-Fc map at 1σ (blue mesh) and difference density Fo-Fc map at +3σ (green mesh) are shown for the 133 (a. – c.) and 149 (d. – f.) sites throughout refinement. Initial structure from molecular replacement with alanine modeled at the 133 (a.) and 149 (d.) sites, refinement following incorporation of phenylalanine at the 133 (b.) and (e.) sites, and final maps at the 133 (c.) and 149 (f.) sites.
**Figure S5** Structural flexibility of the loop region containing pCCF at the 133 site. Alignment of each of the four chains in the asymmetric unit of sfGFP-133/149-pCCF with wild type sfGFP (in green, PDB ID 2B3P) in the loop around the 133 site. The four unique chains of sfGFP-133/149-pCNF are shown in magenta, orange, blue, and yellow and WT sfGFP is shown in green. All atoms for residues 131 – 135 are shown in ball and stick.
Figure S6  Decarboxylation of E222 in the sfGFP-149-pCNF structure. The GFP chromophore (CRO) and residues near E222 are shown in stick with atoms colored by atom type (carbon in cyan, nitrogen in blue, oxygen in red). 2F_o-F_c electron density contoured at 1σ is shown in blue mesh. The E222 residue has been decarboxylated and truncated at the $\gamma\square C$ and the dissociated linear CO$_2$ molecule is shown in clear electron density above the $\gamma\square C$. The distance between the $\gamma\square C$ of E222 and the carbon in CO$_2$ is 3.2 Å.