Supporting Information

Development of specific L-methionine sensors by FRET-based protein engineering

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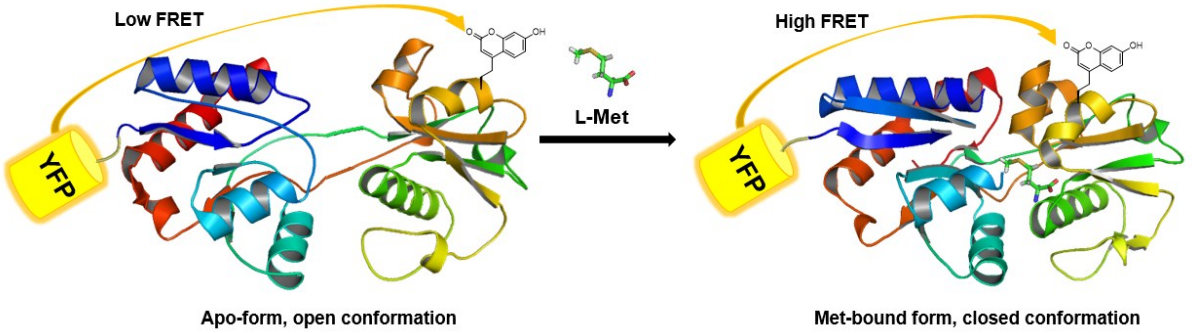


Figure S1. Schematic representation of the designed sensor protein. YFP is fused to the N-terminus of MetQ, and CouA is incorporated into the position where the greatest change in FRET ratio is produced upon L-Met binding.

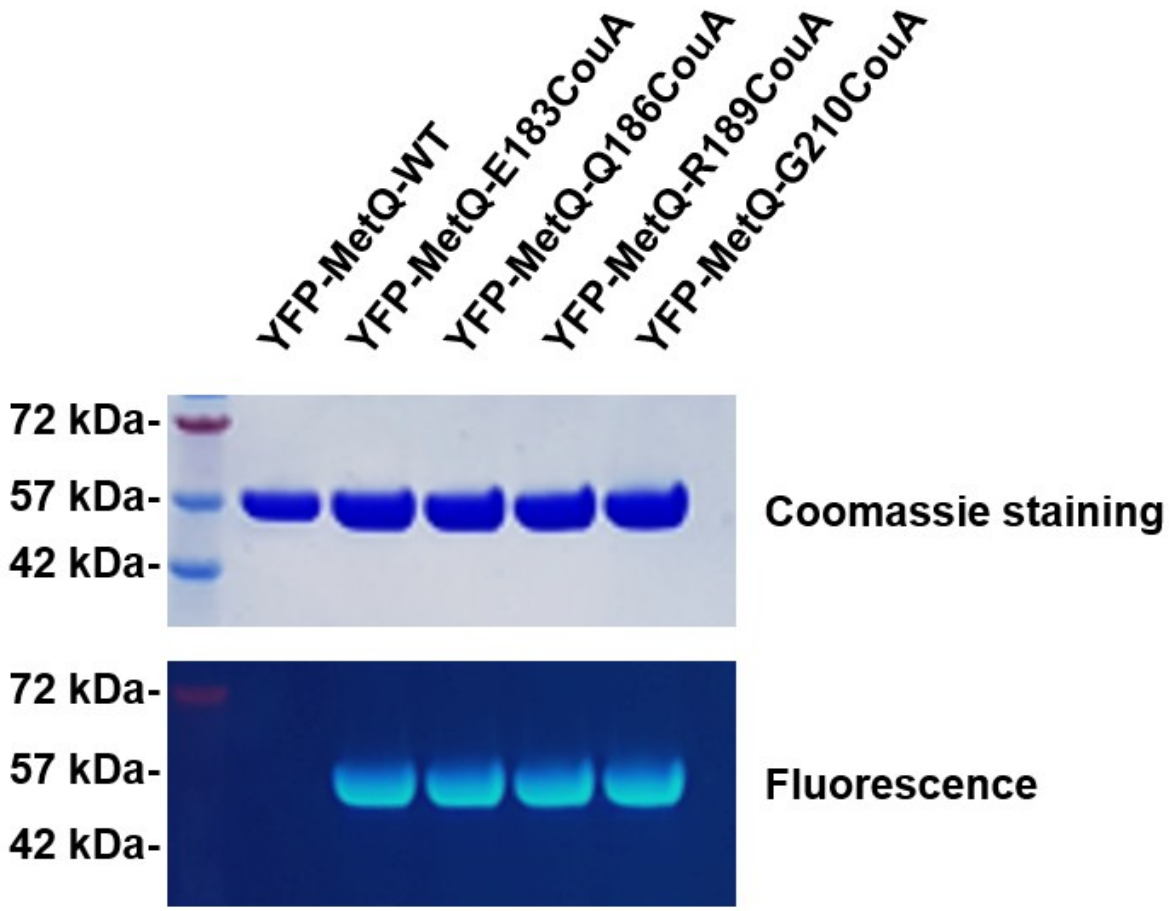
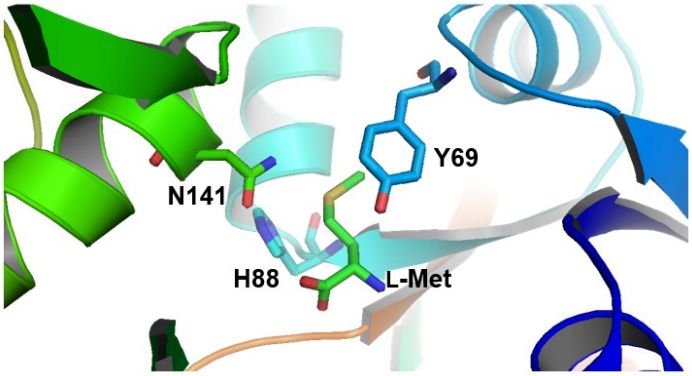


Figure S2. SDS-PAGE analyses of the purified YFP-MetQ mutant proteins containing CouA at the indicated position. The proteins were expressed in the presence of CouA (1 mM) and the corresponding tRNA/CouRS pair and purified using Ni-NTA affinity chromatography.



Mutations introduced into the selected residues in the Met-binding site of MetQ

WT	Y69	H88	N141
Mutations	Ala		
	Val	Ala	Ala
	Ile	Leu	Leu
	Leu	Phe	Asp
	Met		

Figure S3. Crystal structure of the ligand binding site of MetQ complexed with L-Met (PDB 4YAH). The residues, Y69, H88 and N141, were mutated as shown in the table to improve the binding specificity of MetQ.

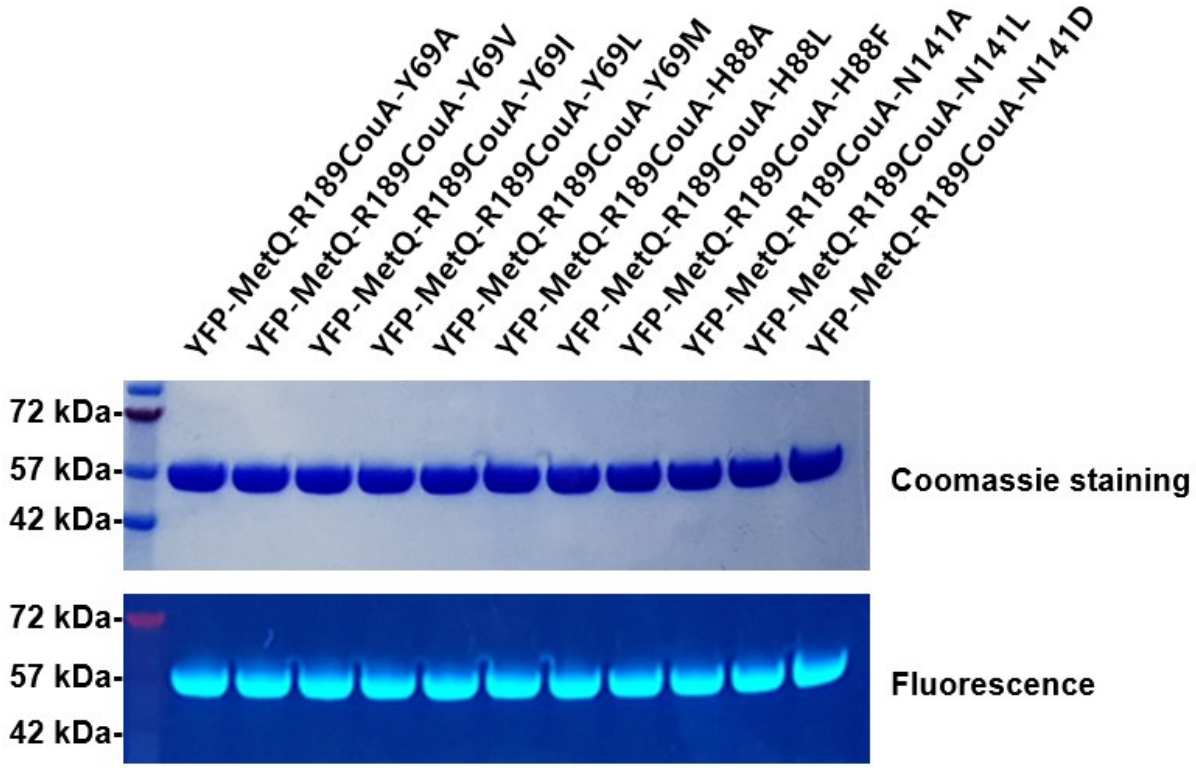


Figure S4. SDS-PAGE analyses of the purified YFP-MetQ-R189CouA mutants with the indicated mutations. The proteins were expressed in the presence of CouA (1 mM) and the corresponding tRNA/CouRS pair and purified using Ni-NTA affinity chromatography.