Electronic Supplementary Material (ESI) for Materials Advances.

Supplemental Information: Understanding the relationships between solubility, stability, and activity of silicatein

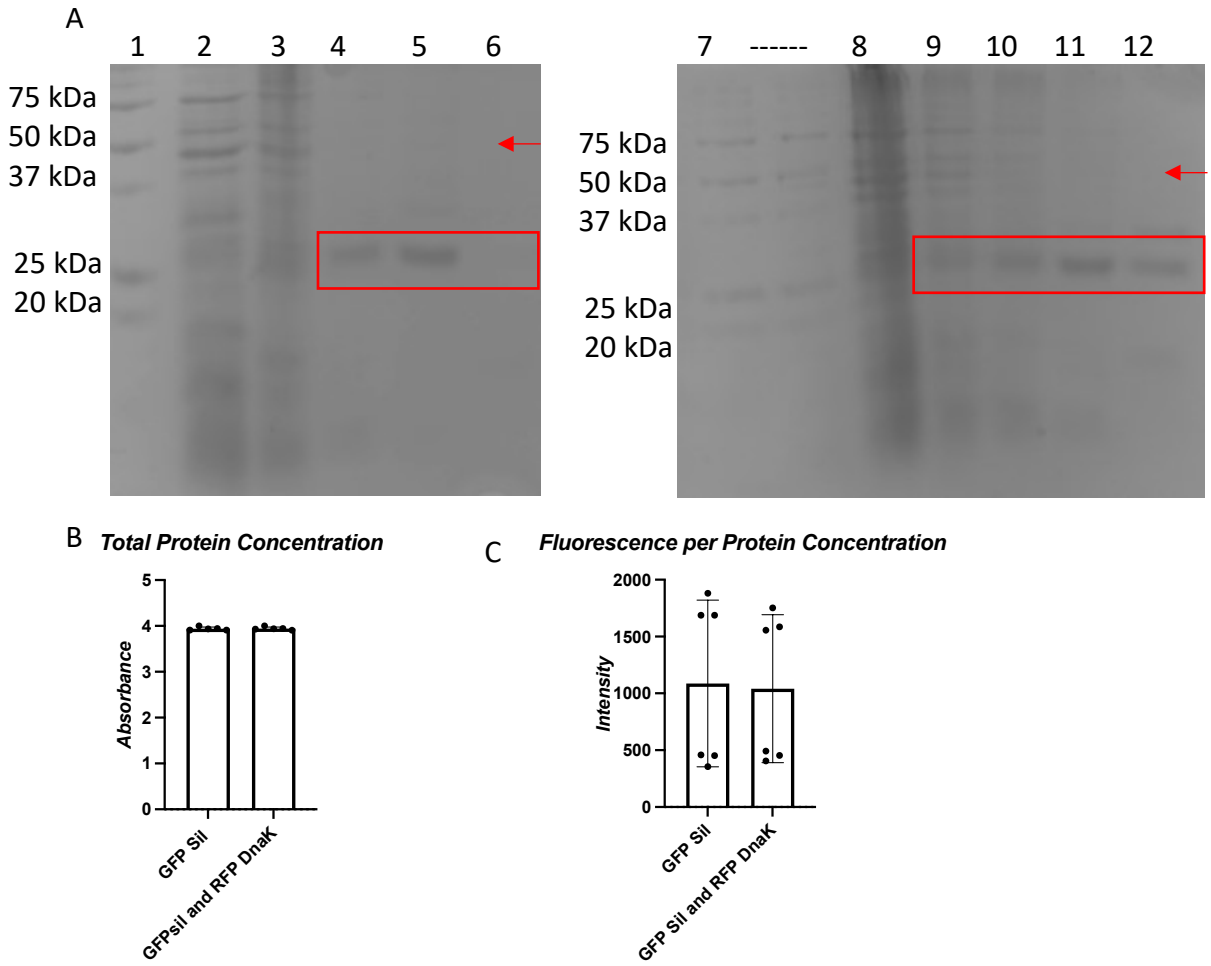
Toriana N. Vigil,[a] Mary-jean C. Rowson, [b] Abigail J. Frost,[a] and Bryan W. Berger[a]*

Table of Contents

Table 1. Mass Spectrometry Analysis 2
Figure 1. SDS PAGE eGFPsil, eGFPsil/DnaK 3-4
Table 2. Silicatein fusion yields 5
Figure 2. Native PAGE for biomineralization activity, graphical explanation 6
Figure 3. Native PAGE for biomineralization activity, all fusion proteins 7
Table 3. Nanoceria mass recovery 8
Supporting SDS-PAGE gel for Fig. 1 9
Supporting Native PAGE gel for Fig. 2 10

Table 1. Mass spectrometry analysis of 26 kDa band showed 6 potential *E. coli* chaperone matches

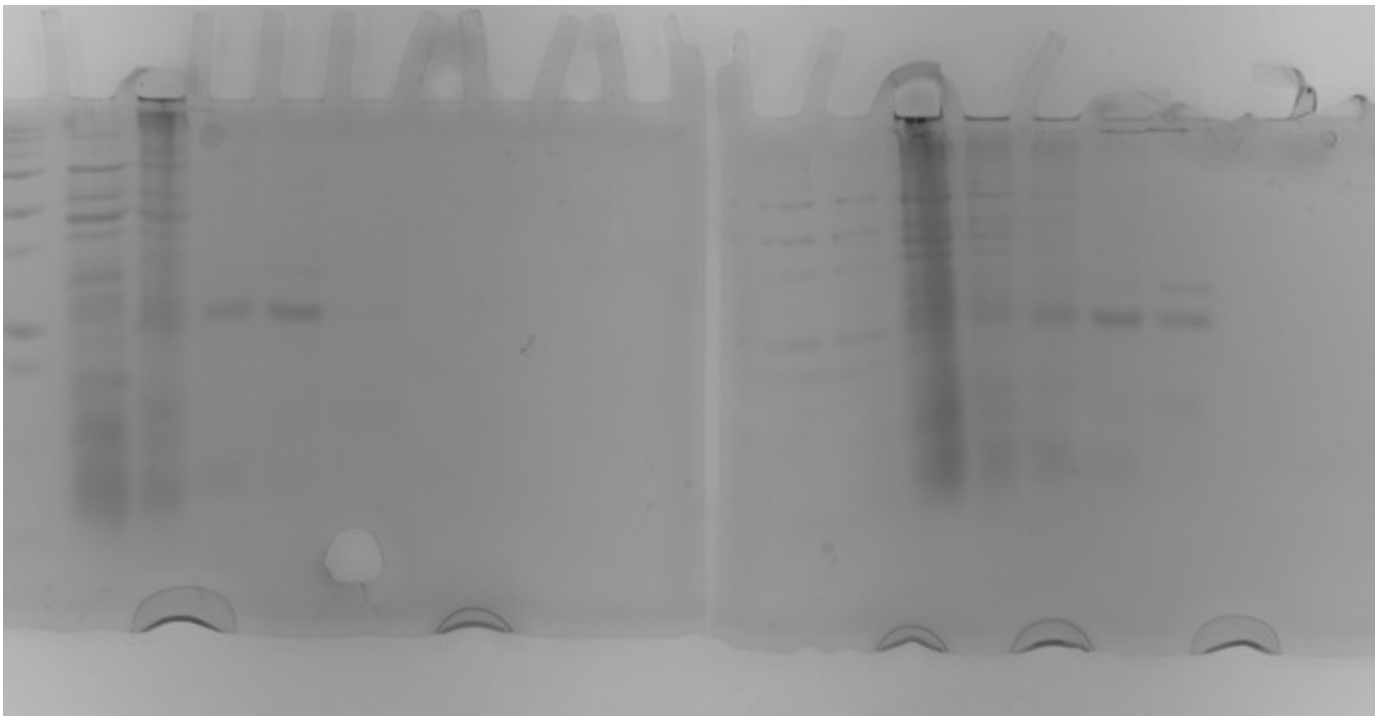
Protein	Peptide	Probability	MW	Percent coverage of AA
10 kDa chaperonin	GEVLAVGNGR	88	10 kDa	10%
	SAGGIVLTGSAAAK	87		14%
Chaperone protein DnaK	ASSGLNEDEIQK	98	69 kDa	2%
	DVSIMPFK	81		1%
	FQDEEVQR	99		1%
	KFEELVQTR	42		1%
	MQELAQVSQK	78		2%
	VIENAEGAR	97		1%
	VIENAEGDR	89		1%
FKBP-type 22kDa peptidyl prolyl cis trans isomerase	EGVNSTESGLQFR	100	22 kDa	6%
	FQAMAAEGVK	100		5%
	HPAVPVDVVHR	100		5%
	LIDGTVFDSSVAR	94		6%
	VINQGEGAIPAR	100		6%
	YLEENAK	18		3%
Protein GrpE	AAMVTVAK	98	22 kDa	4%
	GYTLNGR	85		4%
	RTELDIEK	80		4%
	SMLDVVR	83		4%
	TELDIEK	80		4%
FKBP type peptidyl prolyl cis trans isomerase SlyD	DLVVSLAYQVR	41%	21 kDa	6%
	DVFMGVDELQVGMR	100%		7%
	FNVEVVAIR	100%		2%
Cluster of Trigger factor	EVIEFYSK	90%	48 kDa	2%
	FGVEDGSVEGLR	63%		3%
	GKVPMNIVAQR	100%		3%
	INPAGAPTYVPGEYK	100%		3%
	MIPGFEDGIK	75%		2%
	NFIDAIIK	66%		2%
	QALELPR	90%		2%
	QDVLGDLMSR	99%		2%
	SELVNVAK	98%		2%
	SQAIEGLVK	22%		2%
	VPMNIVAQR	99%		2%



SI Fig. 1. A. SDS PAGE gels for eGFP-sil expression and eGFP-silicatein/DnaK expression. 1. ladder, 2. eGFPsil FT, 3. eGFPsil 10 mM, 4. eGFPsil 25 mM, 5. eGFPsil 50 mM, 6. eGFPsil 500 mM, 7. ladder, 8. eGFPsil RFPDnaK FT, 9. eGFPsil RFPDnaK 10 mM, 10. eGFPsil RFPDnaK 25 mM, 11. GeFPsil RFPDnaK 50 mM, 12. eGFPsil RFPDnaK 500 mM

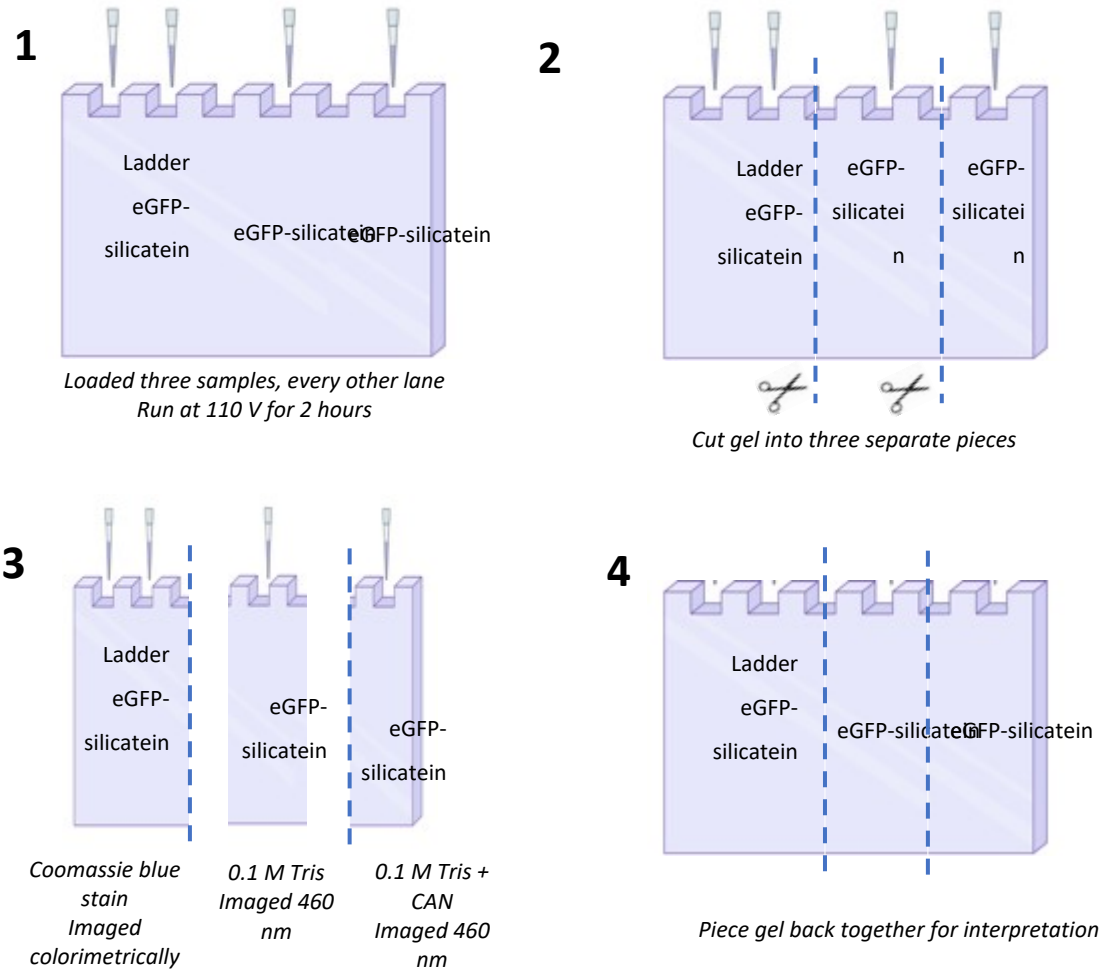
6) The anticipated DnaK chaperone bands (26kDa substrate binding domain) are evident with eGFP-silicatein expression, as highlighted by red outline. eGFP-silicatein has a calculated molecular weight of 52 kDa, and is not evident on SDS PAGE. 12) DnaK chaperone bands are qualitatively more intense, however, eGFP-silicatein is still not observable at 52 kDa (red arrows).
B. Comparison of total protein concentration by A280 shows no significant difference between total protein in purified GFP-silicatein and GFP-silicatein RFP DnaK solutions. **C.** Comparison of fluorescence (ex. 485 nm, em. 512 nm) per total protein concentration shows no significant differences between purified GFP-silicatein and GFP-silicatein RFP DnaK solutions.

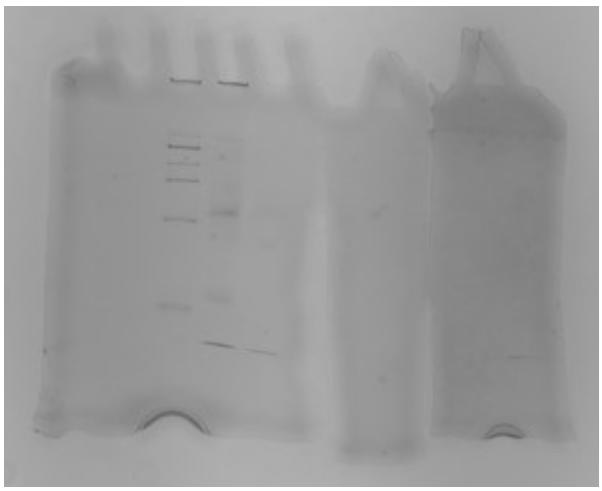
Completely raw and unedited SDS PAGE gels for SI Fig 1.

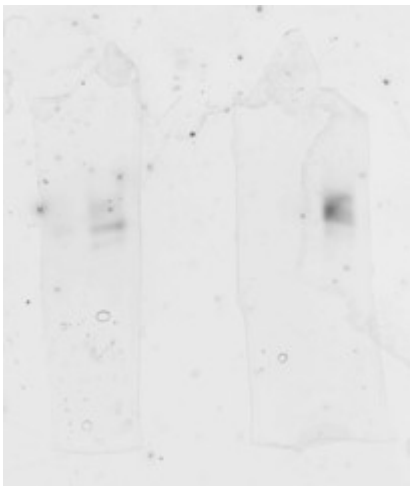


SI Table 2. Comparison of various silicatein fusion yields.

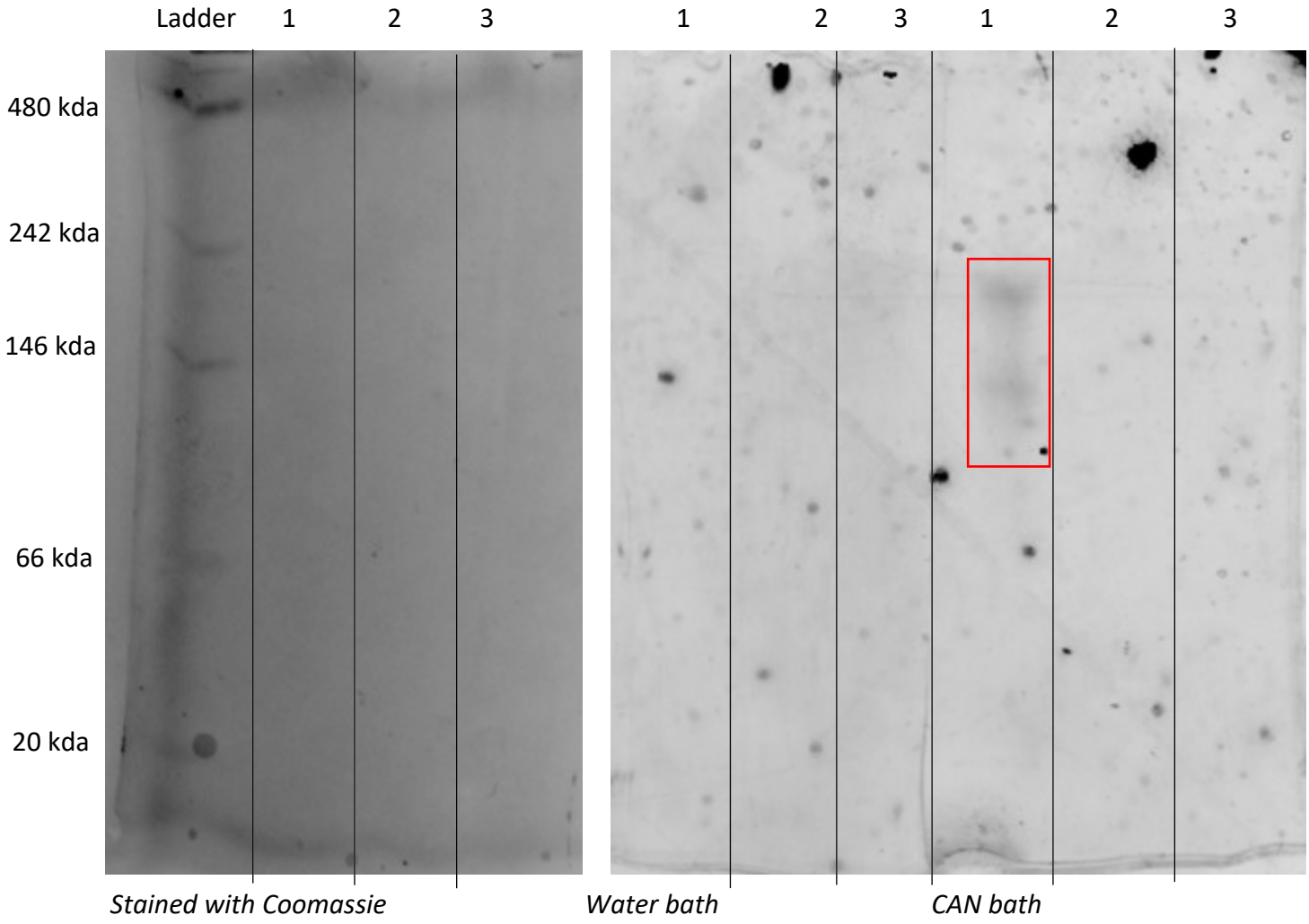
Replicate	**TF-silicatein (nmol / L)**	**eGFP-silicatein (nmol / L)**	**msGFP2-silicatein (nmol / L)**
1	826	92.7	68.7
2	1,950	68.3	277
3	2,120	224	303
4	658	150	310
5	337	185	3.5
6	873	469	367
7	1,630	433	210
Average	1,200 +/- 690	232 +/- 160	722 +/- 1,200



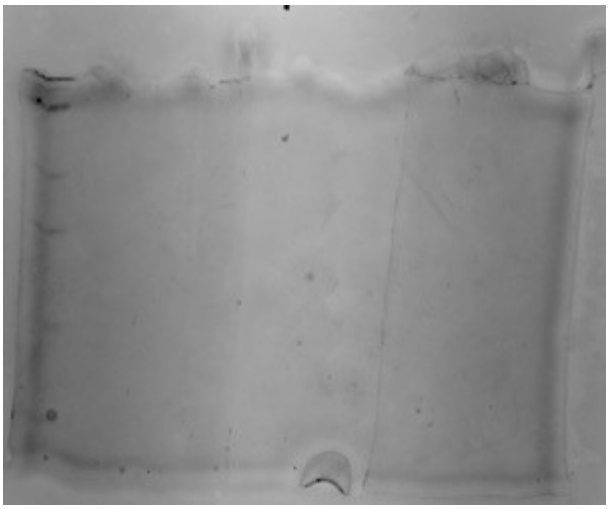


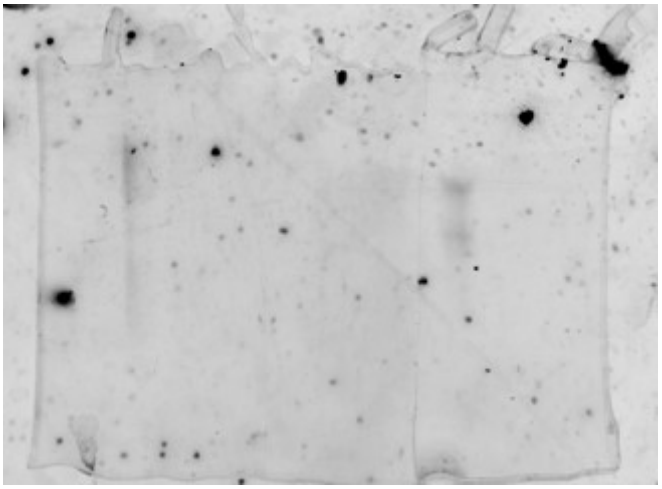


SI Fig. 2. Native PAGE for biomineralization activity procedure. 1) Samples are loaded onto gel in triplicate, run at 110 V for 2 hours. 2) Gel is sliced into 3 pieces (with one replicate per piece) 3) Each piece is incubated in either Coomassie stain, 0.1 M Tris bath, or 0.1 M Tris + CAN bath, then imaged colorimetrically or at 460 nm respectively. 4) The gel is pieced back together for interpretation. (Created with Biorender) Complete raw and un-edited images of gel. 5) imaged colorimetrically, 6) imaged with fluorescence (slices 2 and 3 of gel pictured)



SI Fig. 3. Native PAGE with 1) eGFP-silicatein, 2) msGFP2-silicatein, 3) TF-silicatein. Gel sections were stained with Coomassie blue and imaged colorimetrically, or incubated in water bath and imaged at 460 nm, or incubated in CAN bath and imaged at 460 nm highlighting a dense patches of ceria coinciding with protein, indicating biomineralization activity (as highlighted by red boxes). Additional information regarding this native PAGE procedure can be found in Materials and Methods and SI Fig. 2. Completely raw and unedited images below.

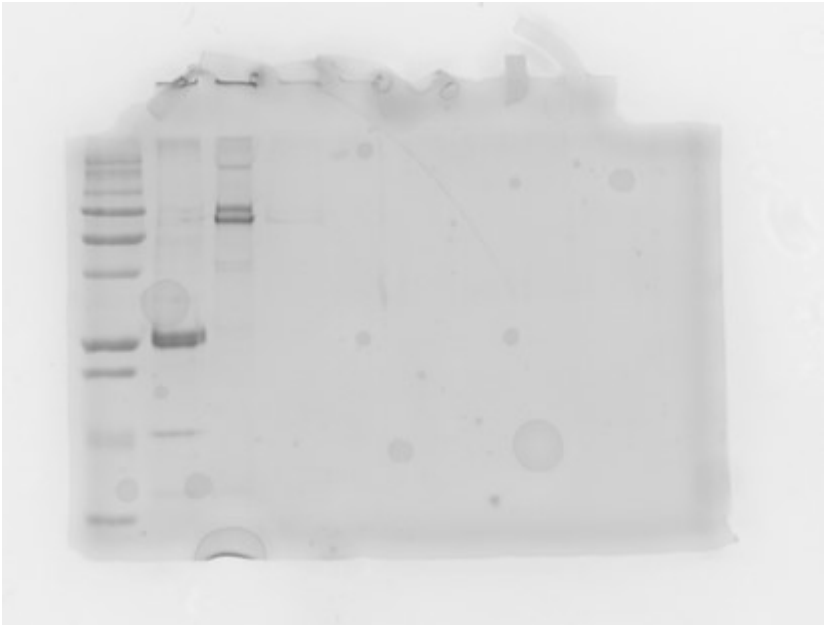




SI Table 3. Nanoceria recovery as measured by mass (mg) with Mettler-Toledo XPR-56 micro-analytical balance after ultra-centrifugation, ethanol wash, and drying. Error is significant, however silicatein fusion products were confirmed as mineralized nanoceria via XRD.

	eGFP-silicatein	msGFP2-silicatein	TF-silicatein	No protein control
Average (mg)	0.66	0.87	0.83	0.56
Standard deviation (mg)	0.39	0.26	0.31	0.36
N =	24	6	14	7

Supporting gel images, Fig. 1
Completely raw and unedited



Supporting gel images, Fig. 2
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