

Supporting Information

FRET-Paired Hydrogel Forming Silk-Elastin-Like Recombinamers by Recombinant Conjugation of Fluorescent Proteins

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Table S1. Abbreviated amino acid sequence and molecular weight (M_w) of each SELR-FP. The sequence corresponding to elastin-like blocks is represented in blue, while the one for silk-like domains is denoted in purple. The sequence of each FP is written in green (AcEGFP) or red (eqFP650).

ELR	abbreviated amino acid sequence	M_w (Da)
SELR-AcEGFP	MESLLP- {[(VPGVG)₂-VPGEG-(VPGVG)₂]₁₀-(VGIPG)₆₀-[V(GAGAGSG)₅]₂G} - VMASKGEELFTGVVPILVELDGDVN GHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTL TYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFFEDDG NYKSRAEVKFEGLTLVNRIELTGTDFKEDGNILGNKMEYNY NAHNVIYIMTDKAKNGIKVNFKIRHNIEDGSVQLADHYQQ NTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMILLEFVT AAGITHGMDELYKV	128,737
SELR-eqFP650	MESLLP- {[(VPGVG)₂-VPGEG-(VPGVG)₂]₁₀-(VGIPG)₆₀-[V(GAGAGSG)₅]₂G} - VMGEDSELIENMHMKLYMEGTVN GHHFKCTSEGEKPYEGTQTAKIKVVEGGPLPFAFDILATSF MYGSKTFINHTQGIPDFFKQSFPEGFTWERITTYEDGGVLT TQDTSLQNGCLIYNV KINGVNFPSNGPVMQKKT LGWEAST EMLYPADSGLRGHSQMALKLVGGGYLHCSLKT TYRSK KPAK NLKMPGFYFVDRKLERIKEADKETYVEQHEMAVARYCDLPS KLGHSV	128,048

Table S2. Theoretical and calculated absolute amino acid composition of SELR-AcEGFP. Data regarding Cys (C) and Trp (W) are missing due to experimental issues.

amino acid	theoretical	calculated	difference (%)
D+N	18+14	21.53	-32.7
E+Q	38+7	49.00	8.9
S	31	24.74	-20.2
H	9	6.30	-30.0
G	524	562.18	7.3
T	18	11.66	-35.2
R	6	7.49	24.8
A	50	50.55	1.1
Y	11	8.68	-21.1
C	2	-	-
V	321	329.23	2.6
M	8	7.73	-3.4
W	1	-	-
F	12	8.44	-29.7
I	133	136.07	2.3
L	21	18.91	-10.0

K	18	13.68	-24.0
P	231	231.93	0.4
TOTAL	1473	1488.12	1.0

Table S3. Theoretical and calculated absolute amino acid composition of SELR-eqFP650.

amino acid	theoretical	calculated	difference (%)
D+N	9+9	18.69	3.8
E+Q	39+8	52.19	11.0
S	36	33.13	-8.0
H	8	5.50	-31.3
G	528	547.70	3.7
T	18	15.31	-14.9
R	6	10.63	77.2
A	51	52.51	3.0
Y	11	8.63	-21.5
C	4	4.56	14.0
V	317	315.42	-0.5
M	11	10.63	-3.4
W	2	1.97	-1.5
F	12	8.38	-30.2
I	129	127.02	-1.5
L	20	18.38	-8.1
K	20	15.25	-23.8
P	232	232.17	0.1
TOTAL	1470	1478.07	0.5

Table S4. Fold increase of the storage modulus (G') of SELR-based hydrogels in comparison to each SELR-FP at two different concentrations.

concentration (mM)	G' fold increase compared to SELR only		
	SELR-AcEGFP	SELR-eqFP650	1:1 mixture
1.13	2.63	2.35	2.36
1.73	1.30	1.36	1.40

Table S5. FRET efficiencies of SELR-FPs 1:1 molar mixtures at different concentrations and temperatures. FRET efficiencies are represented as mean \pm SD (n = 2).

concentration (mg/mL)	temperature (°C)	FRET efficiency (mean \pm SD)
10	15	0
	37	0
50	15	0.247 \pm 0.008
	37	0.330 \pm 0.156
100	15	0.201 \pm 0.026
	37	0.246 \pm 0.043
200	15	0.182 \pm 0.014
	23	0.185 \pm 0.012
	37	0.212 \pm 0.007

Table S6. Comparison between the predicted value of each type of hydrogen in SELR-AcEGFP and the experimental values found by integration of each peak in the corresponding H-NMR spectrum (see Figure S4).

type of hydrogen	predicted value	measured value
-CH3	1026	Reference
-CH- and -CH2-	1591	1383
-NH2	454	371

Table S7. Comparison between the predicted value of each type of hydrogen in SELR-eqFP650 and the experimental values found by integration of each peak in the corresponding H-NMR spectrum (see Figure S4).

type of hydrogen	predicted value	measured value
-CH ₃	1012	Reference
-CH- and -CH ₂ -	1594	1330
-NH ₂	451	413

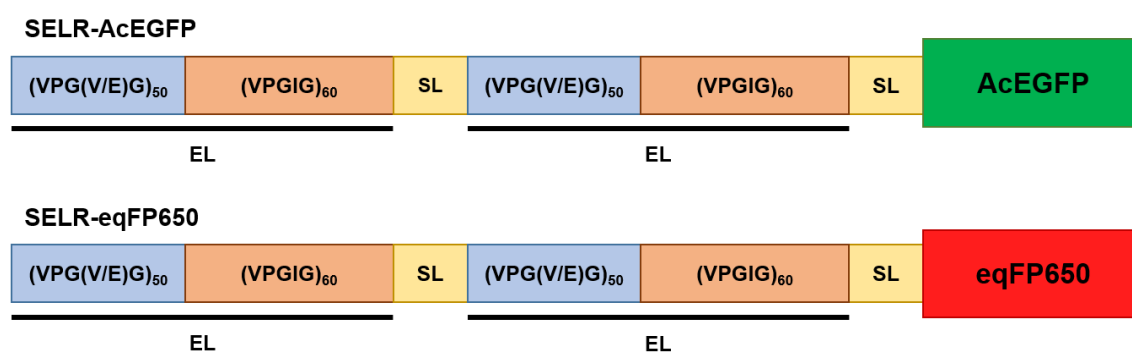


Figure S1. Schematic representation of both SELR-FPs. SL stands for silk-like, while EL means elastin-like.

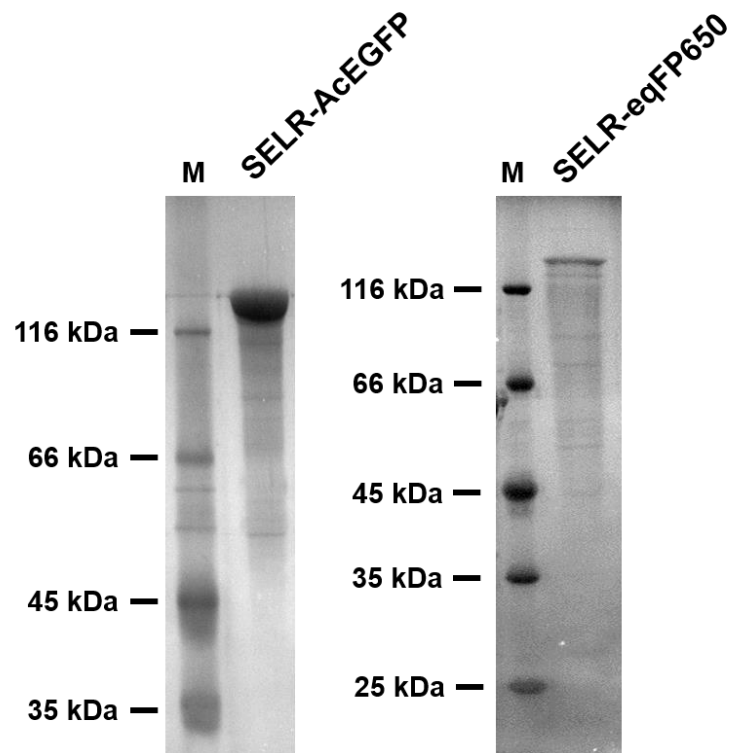


Figure S2. SDS-PAGE of both SELR-FPs showing a good correlation between the observed M_w and the theoretical one: 128.7 and 128.1 kDa for SELR-AcEGFP and SELR-eqFP650, respectively.

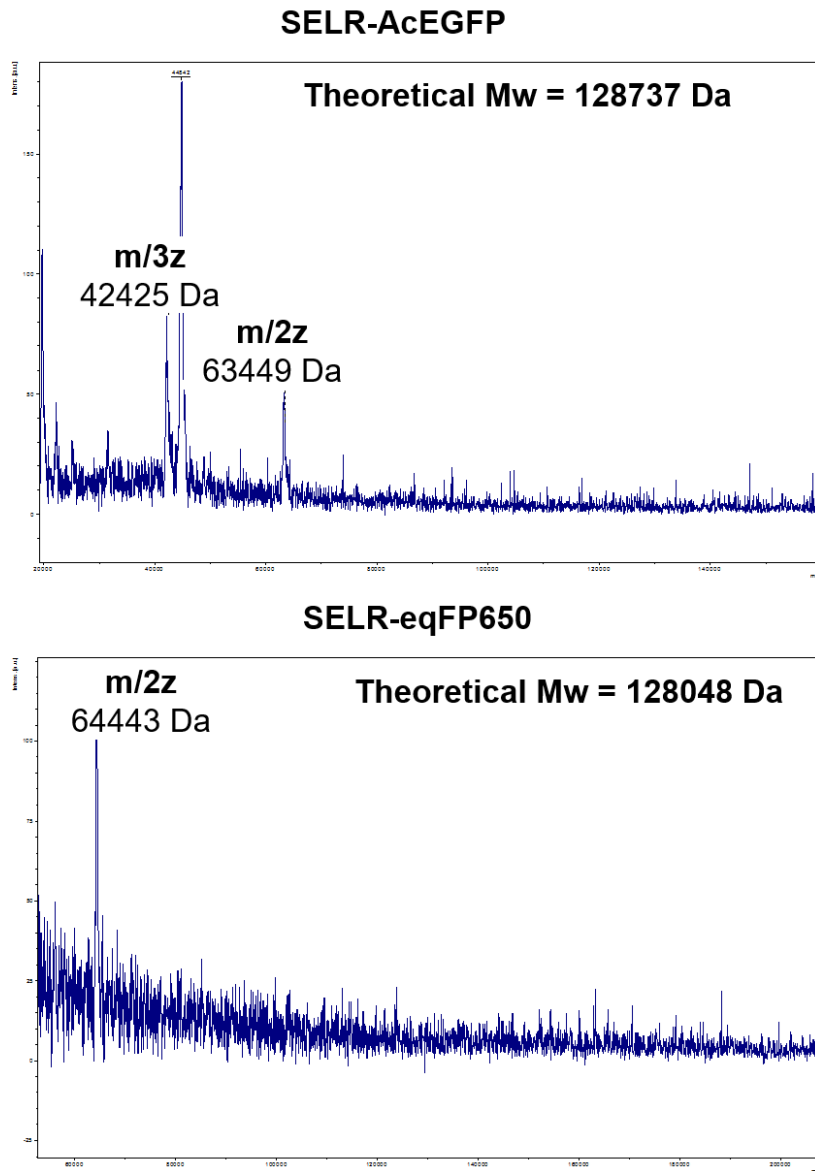


Figure S3. MALDI-TOF spectra of both SELR-FPs. It can be observed that only the doubly charged recombinamers were detected, but it confirms the agreement between the experimental and the expected M_w .

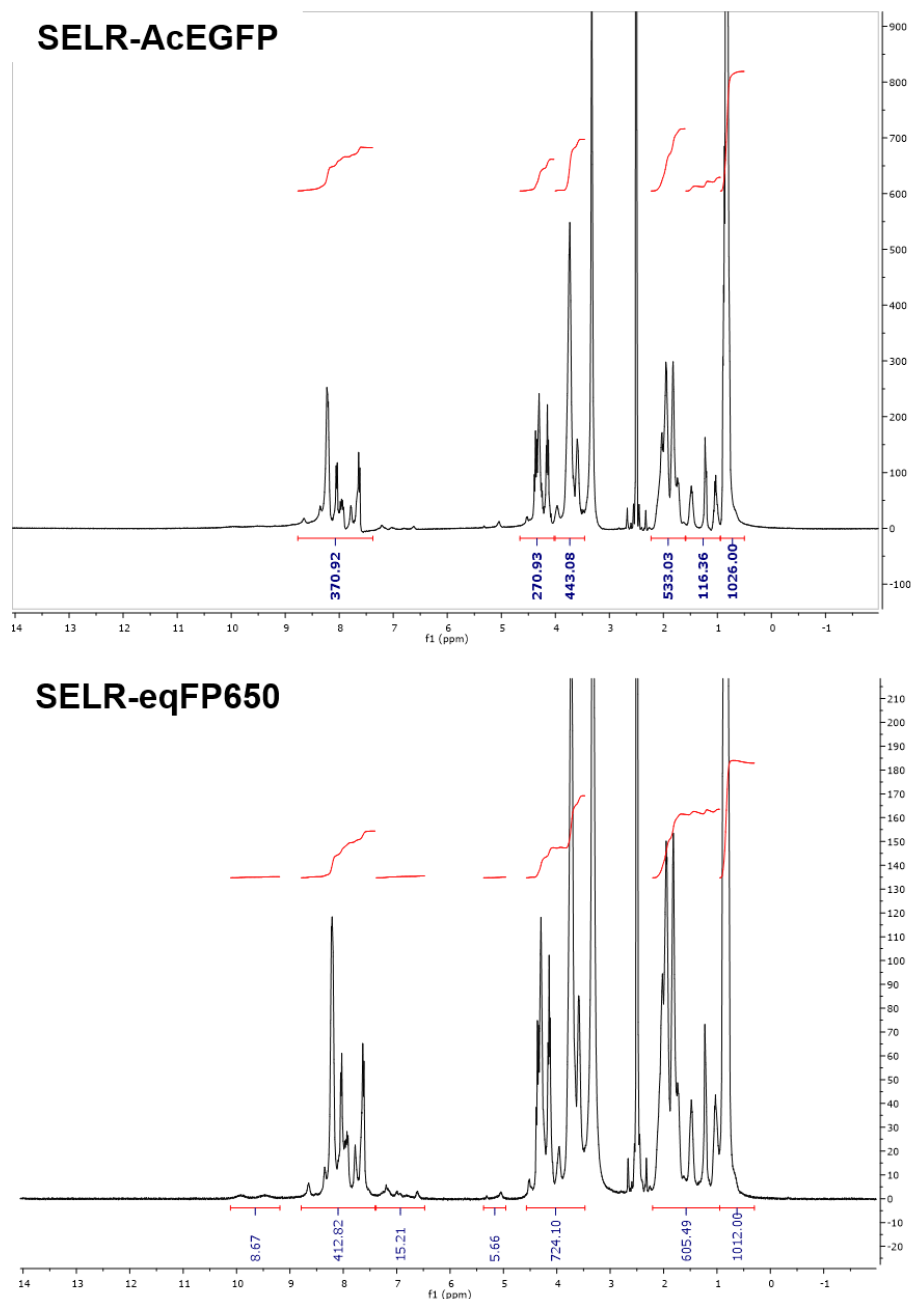


Figure S4. $^1\text{H-NMR}$ spectra of both SELR-FPs. The peak corresponding to protons in -CH_3 groups (0.5-0.95 ppm) is used as integration reference by assigning the theoretical proton number. No contaminants derived from the bioproduction and purification processes could be observed in any case.

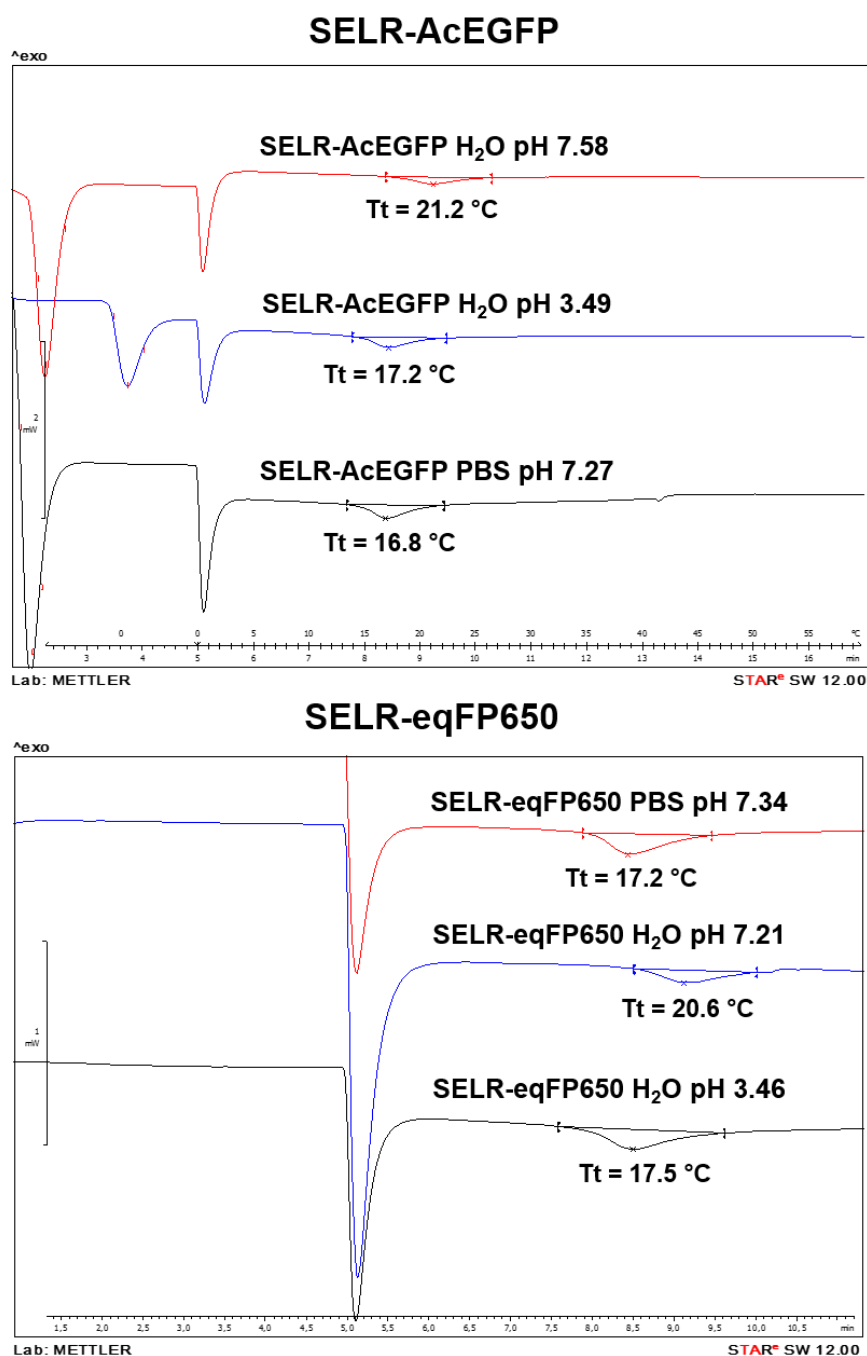


Figure S5. DSC spectra of both SELR-FPs indicating the T_t at 50 mg/mL and different solvent conditions. As expected, the T_t is lower at acid pH in ultra-pure water due to protonation of the carboxyl group present in glutamic acid residues included in the elastin-like blocks. This result was also observed for the SELRs dissolved in the presence of salts (PBS, 137 mM NaCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, and 2.7 mM KCl) due to the salting out effect.

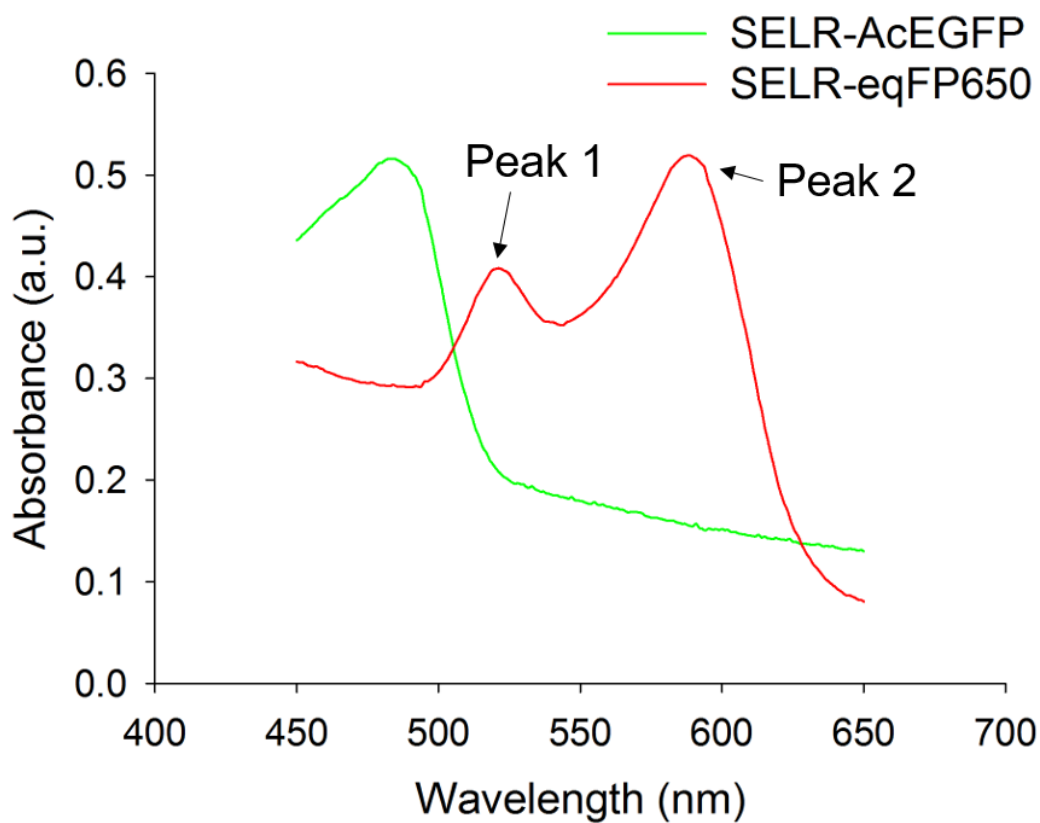


Figure S6. Absorbance spectra of both SELR-FPs dissolved in ultra-pure water at 10 mg/mL ($7.77 \cdot 10^{-5}$ and $7.81 \cdot 10^{-5}$ M for SELR-AcEGFP and SELR-eqFP650, respectively). Both measurements were performed in 1-cm light path cuvettes at 37°C. SELR without fusion to FPs was used as reference.

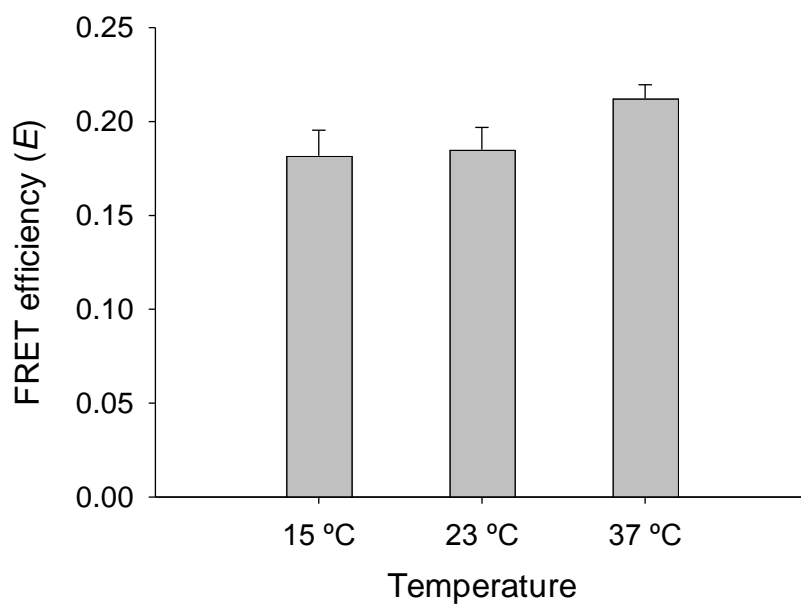


Figure S7. Comparison of FRET efficiencies at 200 mg/mL at different temperatures, below (15°C) and above the T_t (23 and 37°C). Not significant differences were found in every case.