Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supplementary Information

Sortase A catalyzed reaction pathways: A comparative study with six SrtA variants

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Schematic representation and amino acid sequences of SrtA variants used in the present study

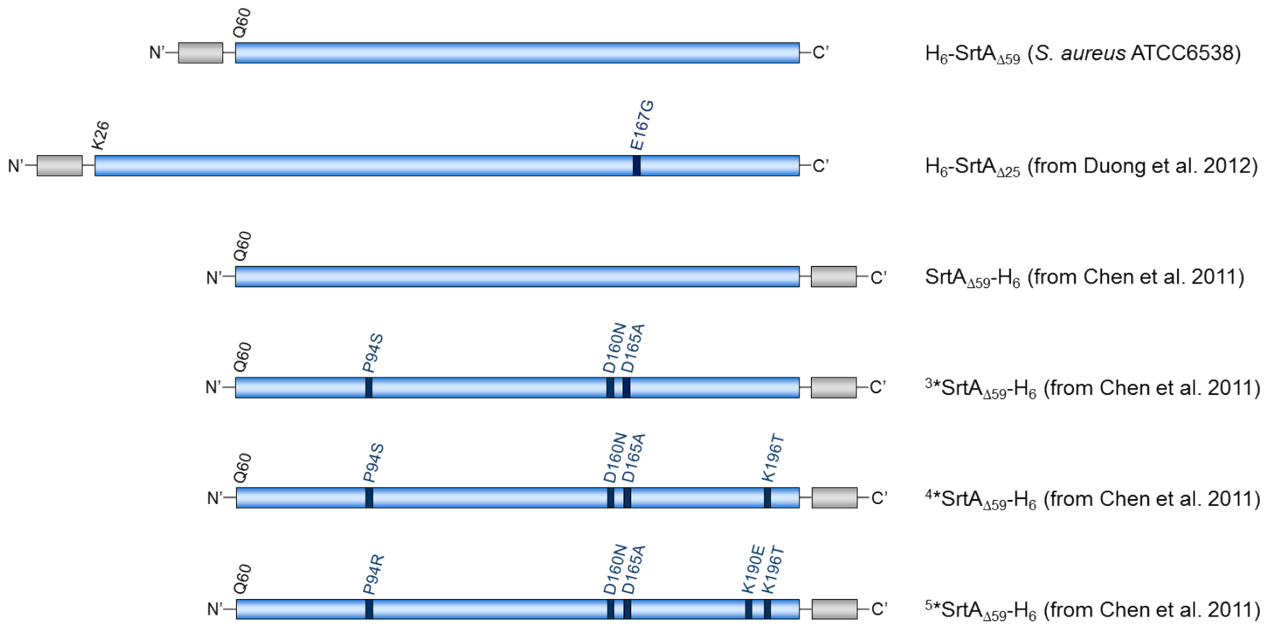

Figure S1: Overview of SrtA variants used in the present study. Amino acid changes relative to the standard SrtA from *S. aureus* ATCC6538 are indicated in dark blue. Hexahistidine tags added for purification of the proteins by IMAC are represented by grey boxes.

Amino acid sequences of SrtA variants

H_6-$SrtA_{\Delta59}$ (159 amino acids; MW: 18.1 kDa; ε_{280}= 14440 $M^{-1}cm^{-1}$):
MRGSHHHHHHGSQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATPEQLNRGVSFAEENES
LDDQNISIAGHTFIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRDVKPTDVEVLD
EQKGKDKQLTLITCDDYNEKTGVWEKRKIFVATEVK

H_6-$SrtA_{\Delta25}$ (202 amino acids; MW: 23.0 kDa; ε_{280}= 17420 $M^{-1}cm^{-1}$):
MGSSHHHHHHSSGLVPRGSHMKPHIDNYLHDKDKDEKIEQYDKNVKEQASKDKKQQAKP
QIPKDKSKVAGYIEIPDADIKEPVYPGPATPEQLNRGVSFAEENESLDDQNISIAGHTFIDRPN
YQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRDVKPTDVGVLDEQKGKDKQLTLITCDDY
NEKTGVWEKRKIFVATEVK

$SrtA_{\Delta59}$-H_6 (156 amino acids; MW: 17.9 kDa; ε_{280}= 14440 $M^{-1}cm^{-1}$):
MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATPEQLNRGVSFAEENESLDDQNISIAGHT

FIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRDVKPTDVEVLDEQKGKDKQLTLI
TCDDYNEKTGVWEKRKIFVATEVKLEHHHHHH

[3*]$SrtA_{\Delta 59}$-H_6 (156 amino acids; MW: 17.8 kDa; ε_{280}= 14440 $M^{-1}cm^{-1}$):
MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATSEQLNRGVSFAEENESLDDQNISIAGHT
FIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRNVKPTAVEVLDEQKGKDKQLTLI
TCDDYNEKTGVWEKRKIFVATEVKLEHHHHHH

[4*]$SrtA_{\Delta 59}$-H_6 (156 amino acids; MW: 17.8 kDa; ε_{280}= 14440 $M^{-1}cm^{-1}$):
MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATSEQLNRGVSFAEENESLDDQNISIAGHT
FIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRNVKPTAVEVLDEQKGKDKQLTLI
TCDDYNEKTGVWETRKIFVATEVKLEHHHHHH

[5*]$SrtA_{\Delta 59}$-H_6 (156 amino acids; MW: 17.9 kDa; ε_{280}= 14440 $M^{-1}cm^{-1}$):
MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATREQLNRGVSFAEENESLDDQNISIAGHT
FIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRNVKPTAVEVLDEQKGKDKQLTLI
TCDDYNEETGVWETRKIFVATEVKLEHHHHHH

Schematic representation and amino acid sequences of target protein variants used in the present study

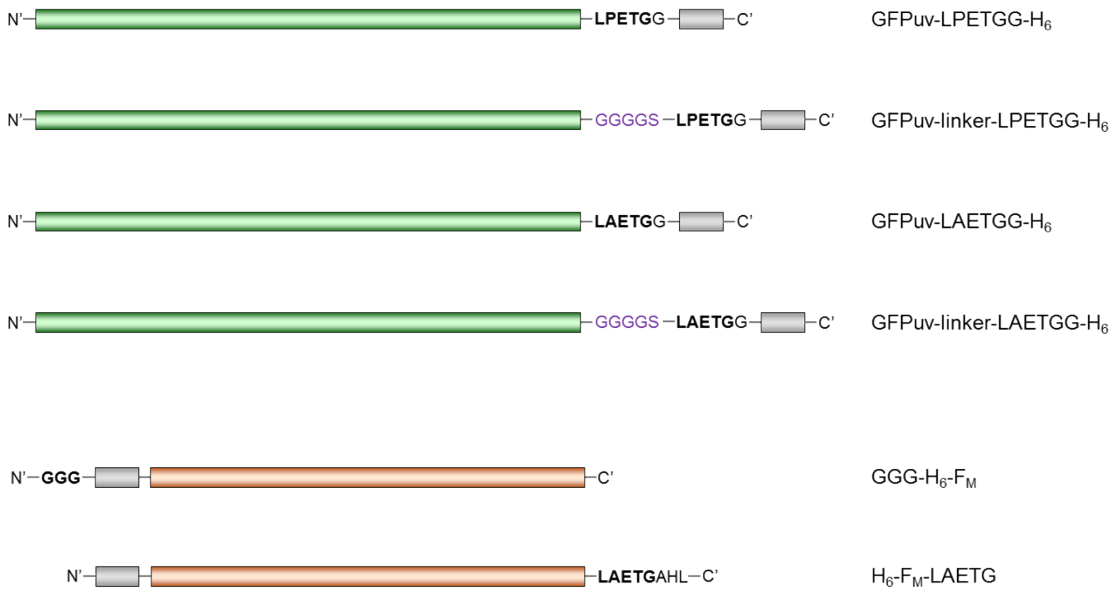

Figure S2: Overview of GFPuv variants and oligoglycine-modified F_M protein used as substrates for SrtA-catalyzed coupling reactions. Hexahistidine tags added for purification of the proteins by IMAC are represented by grey boxes.

Amino acid sequences of target protein variants

GFPuv-LPETGG-H_6 (250 amino acids; MW (non-mature): 28.2 kDa; ε_{280}= 21890 $M^{-1}cm^{-1}$):
MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVT
TFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRI
ELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQ
NTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYKLPETGGH
HHHHH

GFPuv-LAETGG-H_6 (250 amino acids; MW (non-mature): 28.1 kDa; ε_{280}= 21890 $M^{-1}cm^{-1}$):
MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVT
TFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRI
ELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQ
NTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYKLAETGGH
HHHHH

GFPuv-linker-LPETGG-H_6 (255 amino acids; MW (non-mature): 28.5 kDa; ε_{280}= 21890 $M^{-1}cm^{-1}$):
MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVT
TFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRI
ELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQ
NTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYKGGGGSLP
ETGGHHHHHH

GFPuv-linker-LAETGG-H_6 (255 amino acids; MW (non-mature): 28.5 kDa; ε_{280}= 21890 $M^{-1}cm^{-1}$):
MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVT
TFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRI
ELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQ
NTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYKGGGGSLA
ETGGHHHHHH

GGG-H_6-F_M (132 amino acids; MW: 14.7 kDa; ε_{280}= 14440 $M^{-1}cm^{-1}$):
MGGGHHHHHHYPYDVPDYAAMAEFMGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDG
KKMDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPH
ATLVFDVELLKLE

H_6-F_M-LAETG (137 amino acids; MW: 15.3 kDa; ε_{280}= 14440 $M^{-1}cm^{-1}$):
MHHHHHHYPYDVPDYAAMAEFMGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKM
DSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATL
VFDVELLKLELAETGAHL

SDS-PAGE gels of SrtA catalyzed reactions

H_6-$SrtA_{\Delta59}$ (18.1 kDa):

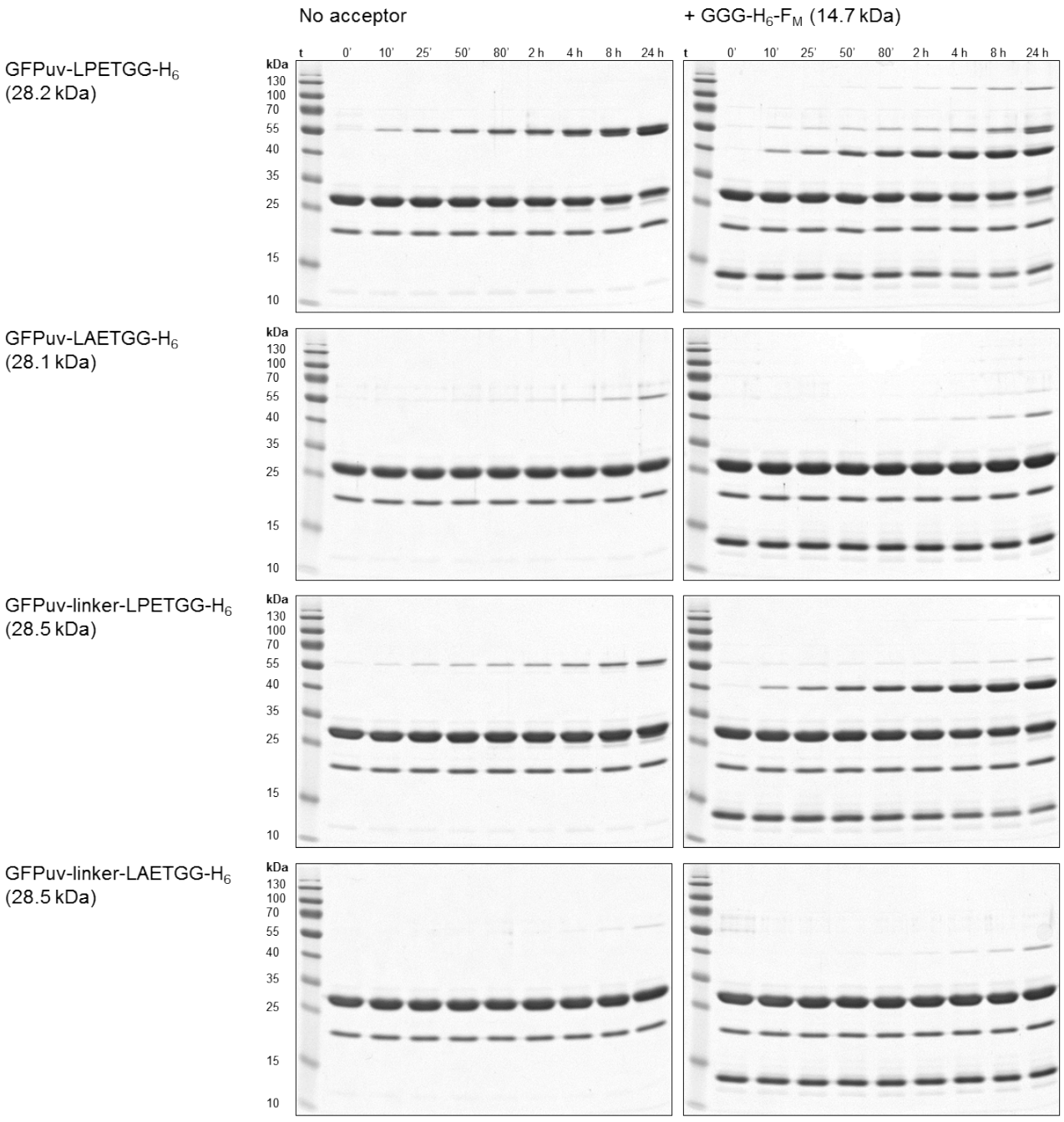

Figure S3a: Left: Reactions of GFPuv target proteins (10 μM) catalyzed by H_6-$SrtA_{\Delta59}$ (2 μM). Right: Reactions of GFPuv target proteins (10 μM) and the acceptor protein GGG-H_6-F_M (10 μM) catalyzed by H_6-$SrtA_{\Delta59}$ (2 μM).

$\underline{H_6\text{-}SrtA_{\Delta25}}$ (23.0 kDa):

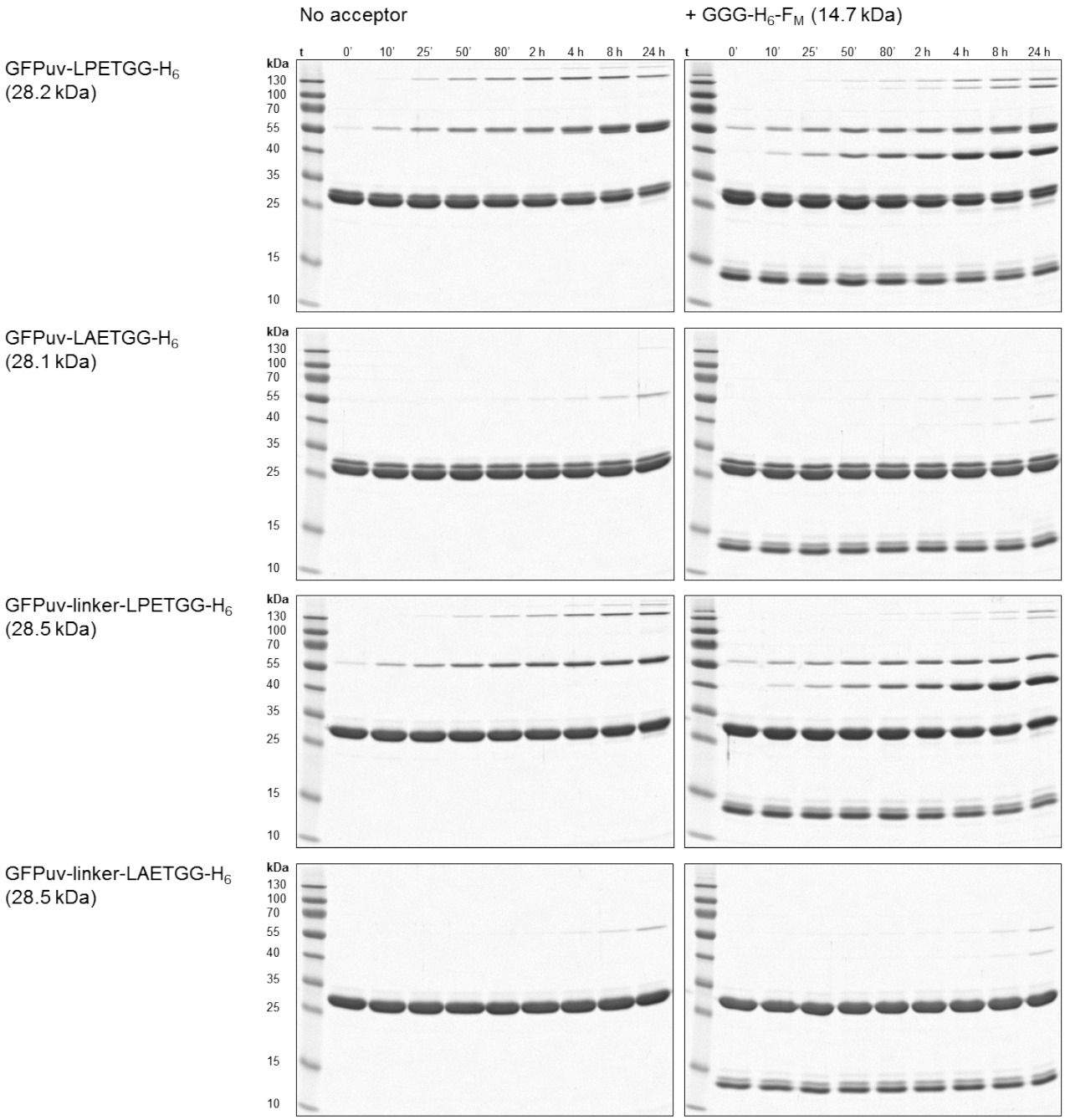

Figure S3b: Left: Reactions of GFPuv target proteins (10 μM) catalyzed by $H_6\text{-}SrtA_{\Delta25}$ (2 μM). Right: Reactions of GFPuv target proteins (10 μM) and the acceptor protein GGG-H_6-F_M (10 μM) catalyzed by $H_6\text{-}SrtA_{\Delta25}$ (2 μM).

$\underline{SrtA_{\Delta59}\text{-}H_6\ (17.9\ kDa)}$:

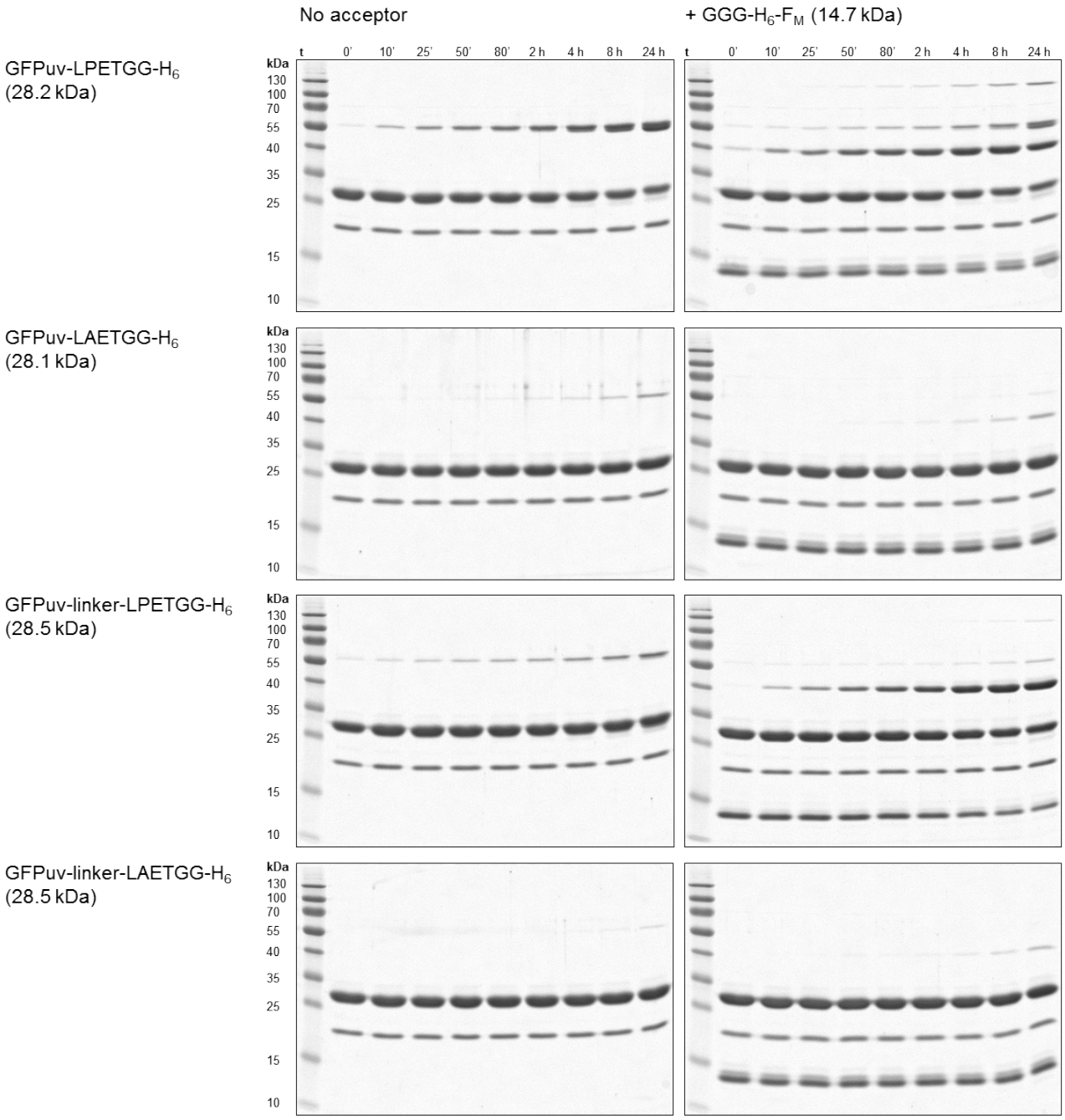

Figure S3c: Left: Reactions of GFPuv target proteins (10 μM) catalyzed by $SrtA_{\Delta59}\text{-}H_6$ (2 μM). Right: Reactions of GFPuv target proteins (10 μM) and the acceptor protein GGG-H_6-F_M (10 μM) catalyzed by $SrtA_{\Delta59}\text{-}H_6$ (2 μM).

$^{3*}SrtA_{\Delta59}$-H_6 (17.8 kDa):

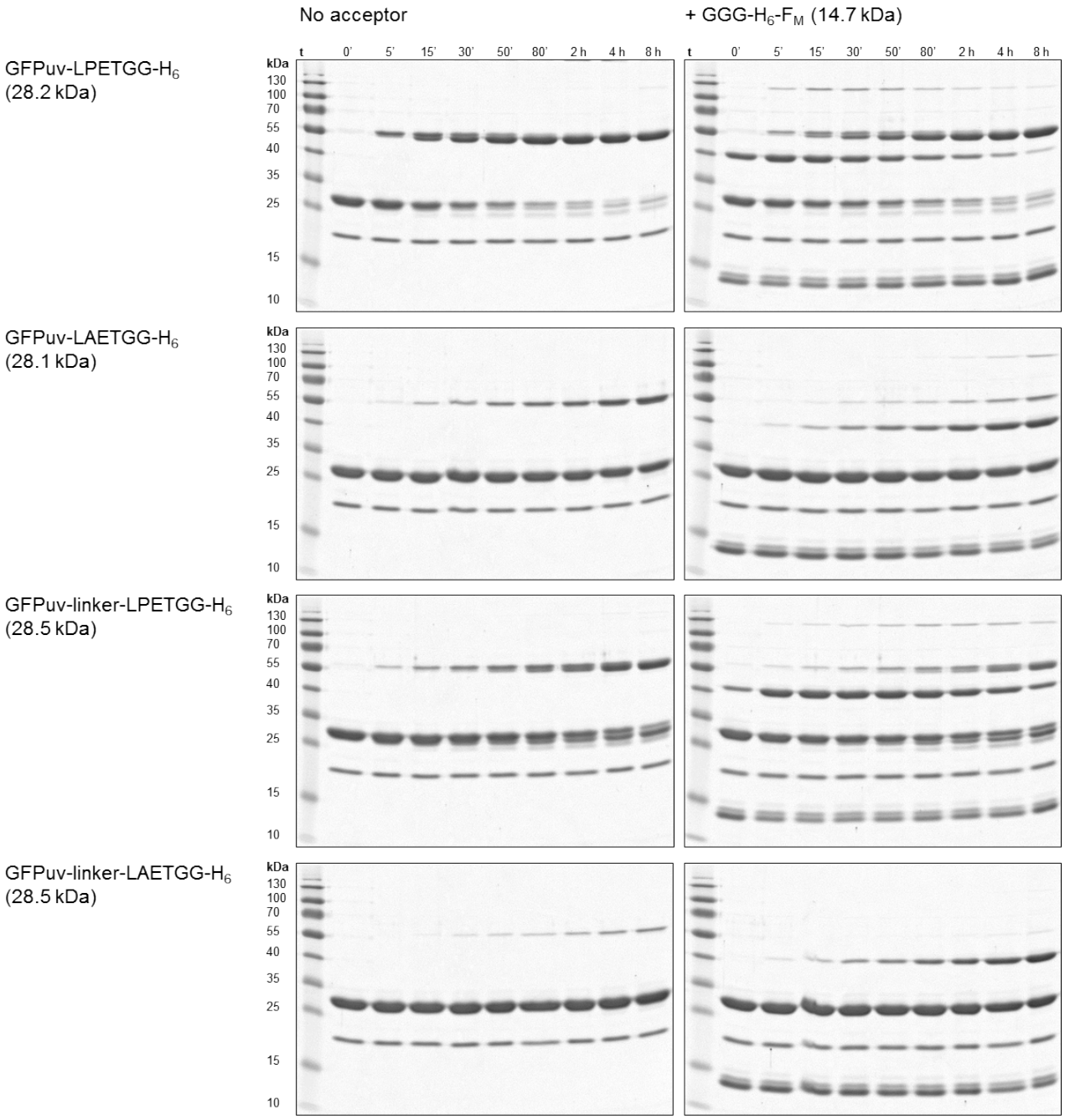

Figure S3d: Left: Reactions of GFPuv target proteins (10 μM) catalyzed by $^{3*}SrtA_{\Delta59}$-H_6 (2 μM). Right: Reactions of GFPuv target proteins (10 μM) and the acceptor protein GGG-H_6-F_M (10 μM) catalyzed by $^{3*}SrtA_{\Delta59}$-H_6 (2 μM).

^{4*}SrtA$_{\Delta59}$-H$_6$ (17.8 kDa):

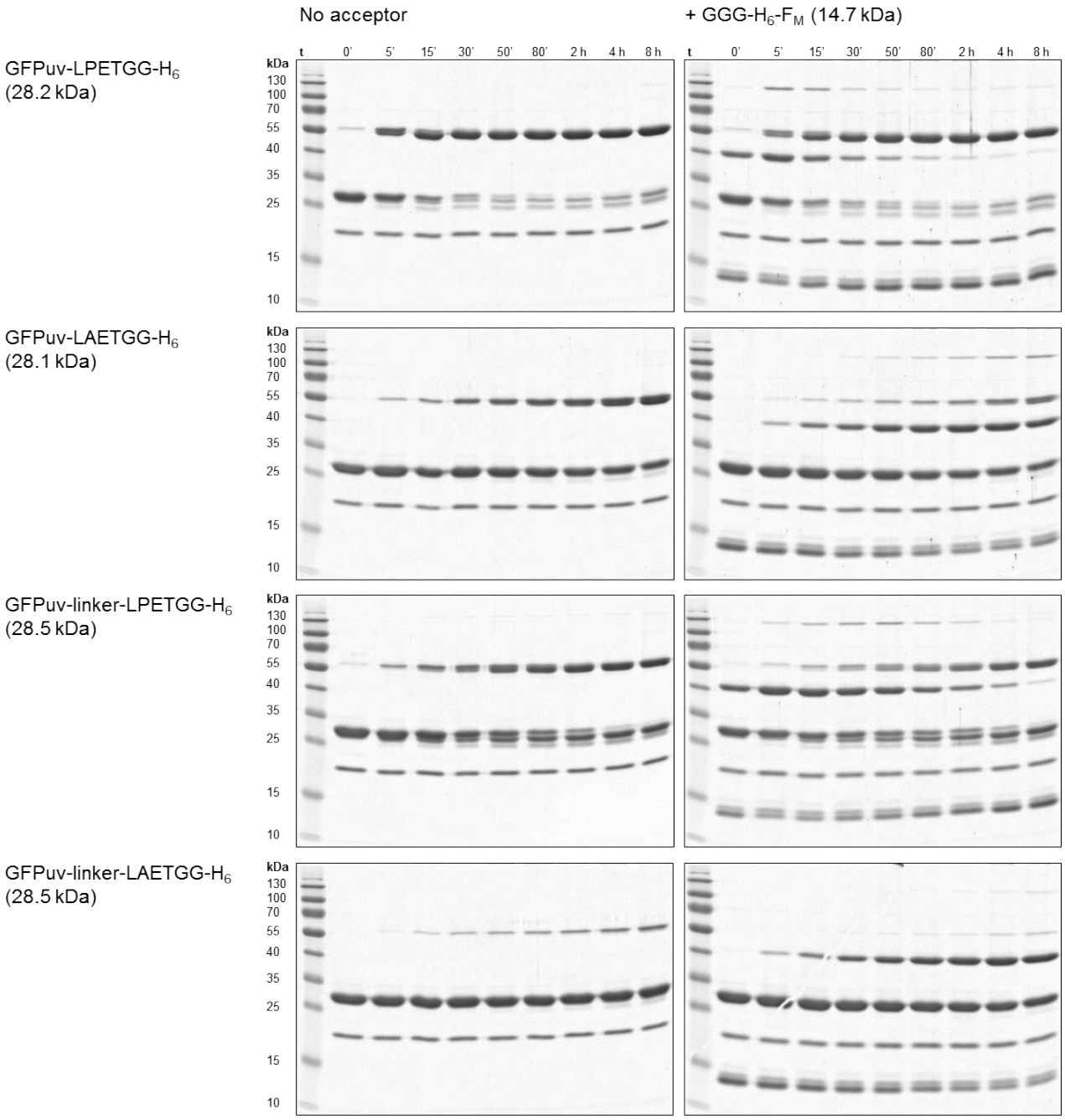

Figure S3e: Left: Reactions of GFPuv target proteins (10 μM) catalyzed by ^{4*}SrtA$_{\Delta59}$-H$_6$ (2 μM). Right: Reactions of GFPuv target proteins (10 μM) and the acceptor protein GGG-H$_6$-F$_M$ (10 μM) catalyzed by ^{4*}SrtA$_{\Delta59}$-H$_6$ (2 μM).

^{5*}SrtA$_{\Delta59}$-H$_6$ (17.9 kDa):

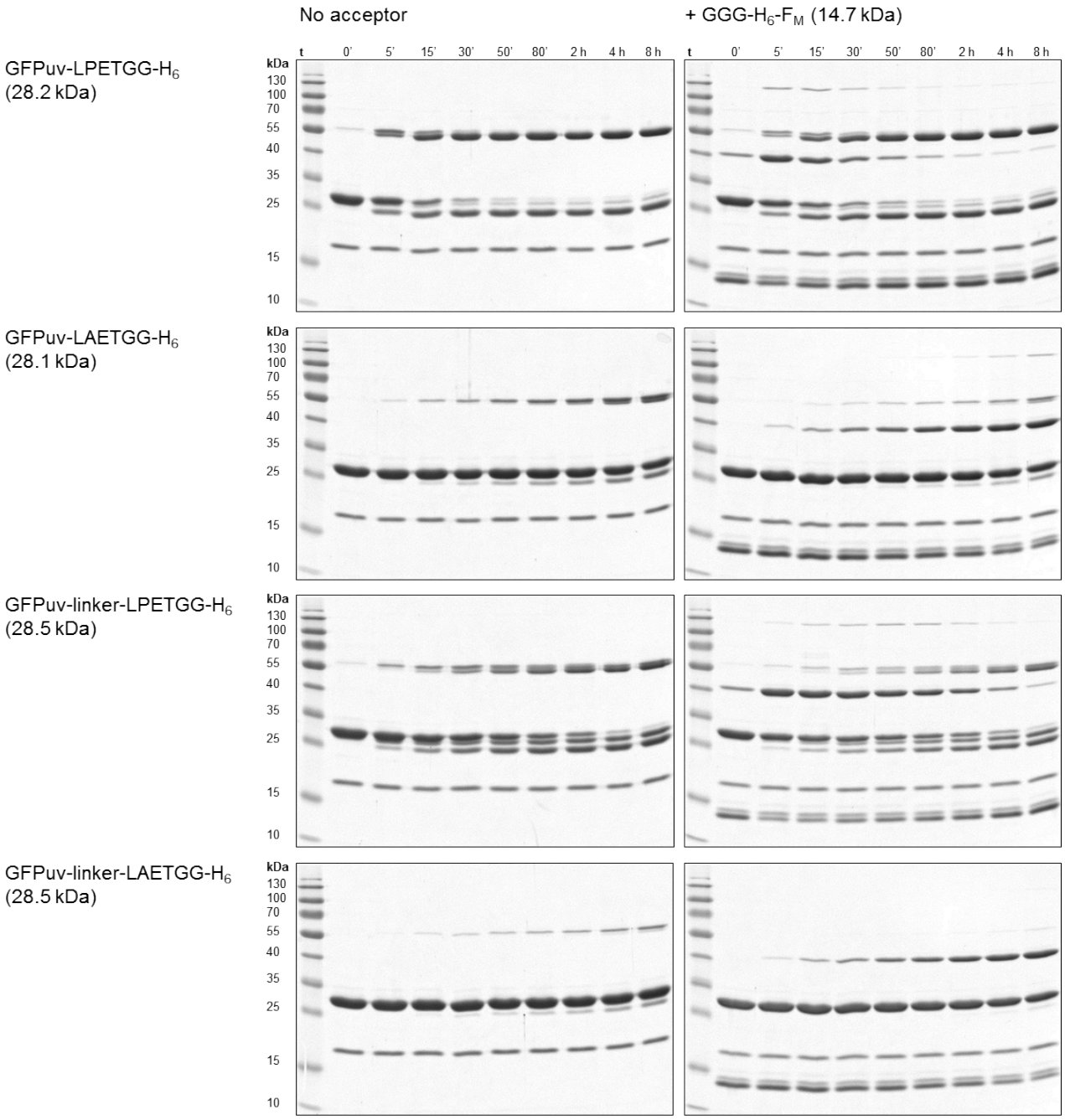

Figure S3f: Left: Reactions of GFPuv target proteins (10 μM) catalyzed by ^{5*}SrtA$_{\Delta59}$-H$_6$ (2 μM). Right: Reactions of GFPuv target proteins (10 μM) and the acceptor protein GGG-H$_6$-F$_M$ (10 μM) catalyzed by ^{5*}SrtA$_{\Delta59}$-H$_6$ (2 μM).

Evaluation of SDS-PAGE gels (see Suppl. Figures S3a-f)

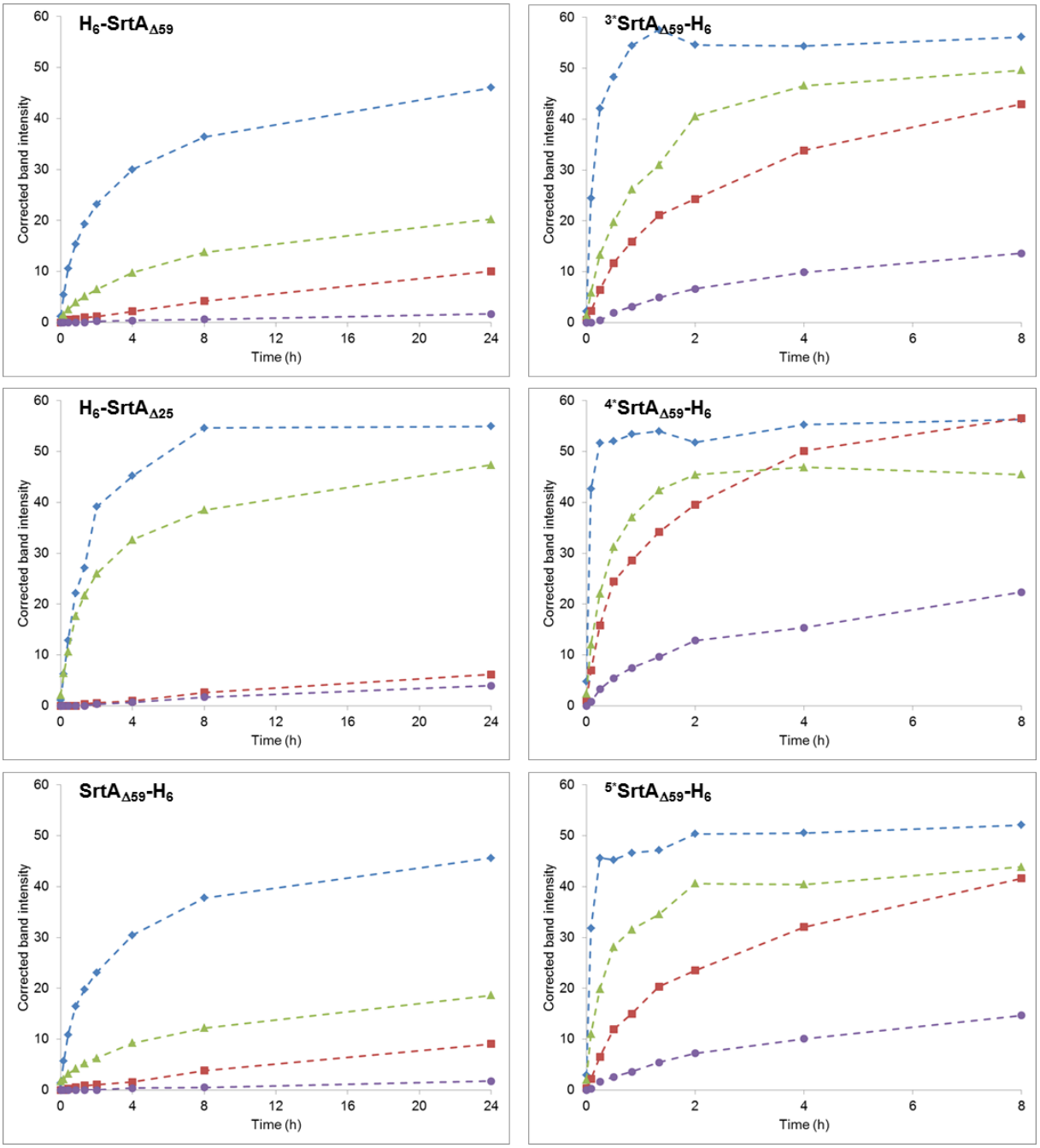

Figure S4a: Intermolecular crosslinking of the GFPuv target proteins GFPuv-LPETGG-H_6 (blue), GFPuv-LAETGG-H_6 (red), GFPuv-linker-LPETGG-H_6 (green) and GFPuv-linker-LAETGG-H_6 (purple) by SrtA variants. Band intensities of crosslinked protein species were obtained by digital image analysis of the respective SDS-PAGE gels (Suppl. Figures S3a-f, left column) and corrected for the band intensity and the molecular weight of the employed SrtA variant as described in the experimental section.

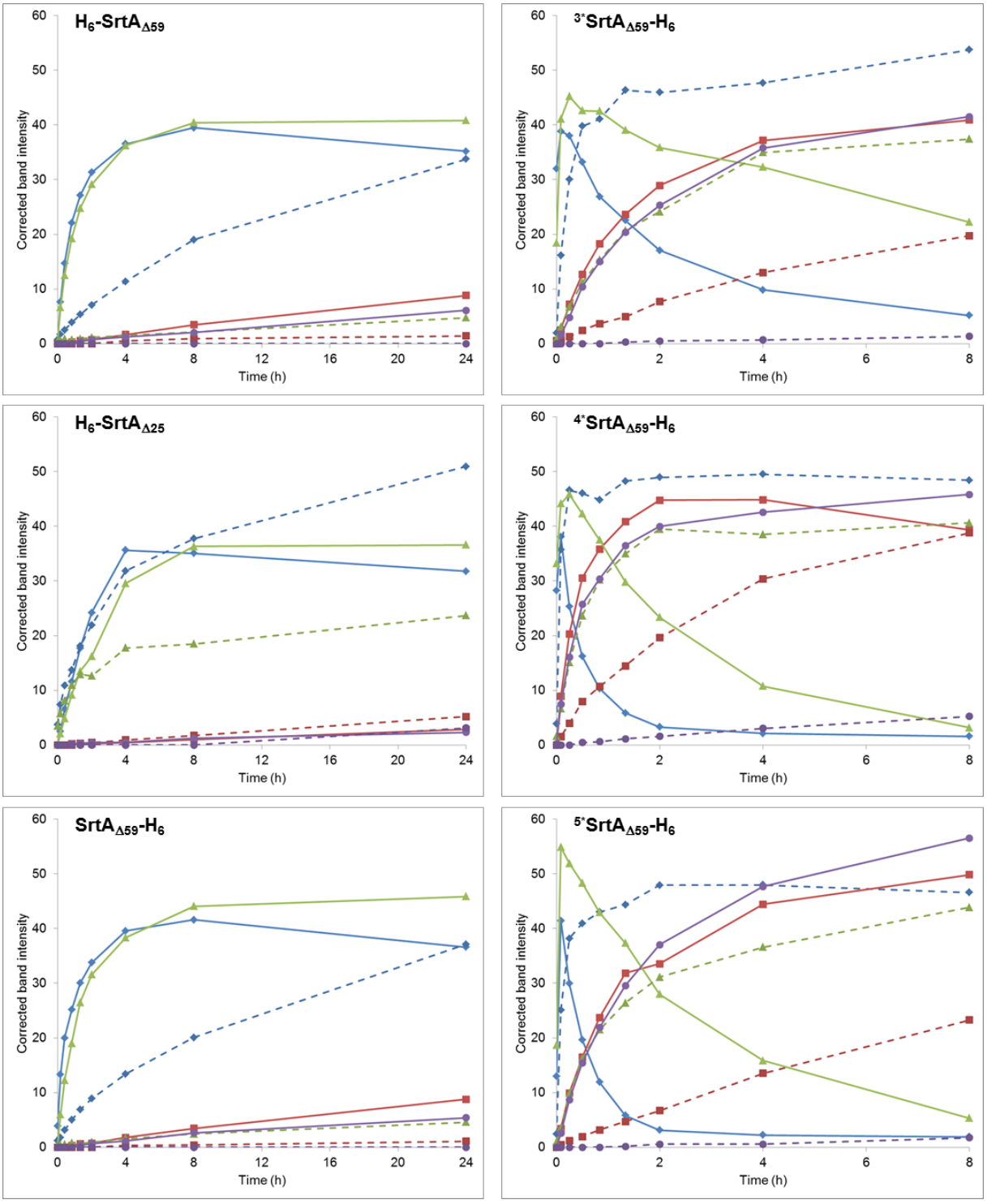

Figure S4b: Transpeptidation reactions between the GFPuv target proteins GFPuv-LPETGG-H_6 (blue), GFPuv-LAETGG-H_6 (red), GFPuv-linker-LPETGG-H_6 (green), GFPuv-linker-LAETGG-H_6 (purple) and the acceptor protein GGG-H_6-F_M (GFPuv-F_M product: solid lines). Byproducts, *i.e.* crosslinked homodimeric and -oligomeric species of GFPuv are depicted as dashed lines. Band intensities of the formed protein species were obtained by digital image analysis of the respective SDS-PAGE gels (Suppl. Figures S3a-f, right columns) and corrected for the band intensity and the molecular weight of the employed SrtA variant as described in the experimental section.