

End-Point Immobilization of Recombinant Thrombomodulin *via* Sortase-Mediated Ligation

Rui Jiang, Jacob Weingart, Hailong Zhang and Xue-Long Sun*

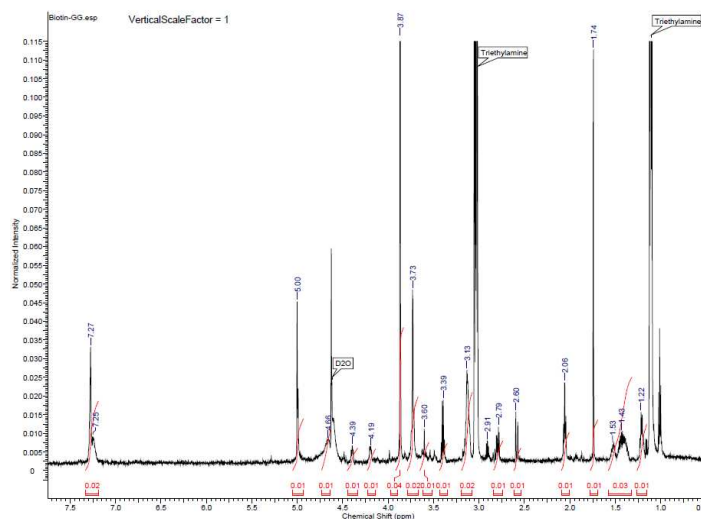
SUPPORTING INFORMATION

1. ^1H NMR and FTIR diGly-Biotin and diGly-Dansyl Spectrum Results

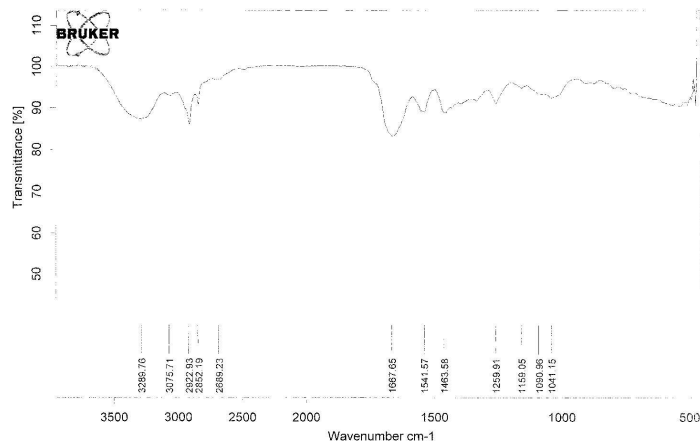
diGly-Biotin

^1H NMR (CD_3OD) d: 4.39 (m, 1 H, CH-1-Biotin), 4.19 (m, 1 H, CH-4-Biotin), 3.87 (s, 2H, $\text{CH}_2\text{-Gly}$), 3.73 (s, 2H, $\text{CH}_2\text{-Gly}$), 3.39 (t, 2H, CH_2CH_2), 3.13 (t, 2H, CH_2CH_2), 3.60 (dd, 1 H, CH-Biotin), 2.91 (m, 1H, CH-Biotin), 2.79 (dd, 1 H, CH-Biotin), 2.60 (m 1 H, CH-Biotin), 2.06 (t, 1 H, $\text{CH}_2\text{CO-Biotin}$), 1.53, 1.43, 1.22 (m, 6 H, $(\text{CH}_2)_3\text{-Biotin}$). FTIR (cm^{-1}): 3290, 3075, 2922, 2852, 2689, 1667, 1541, 1463, 1259, 1090.

^1H NMR



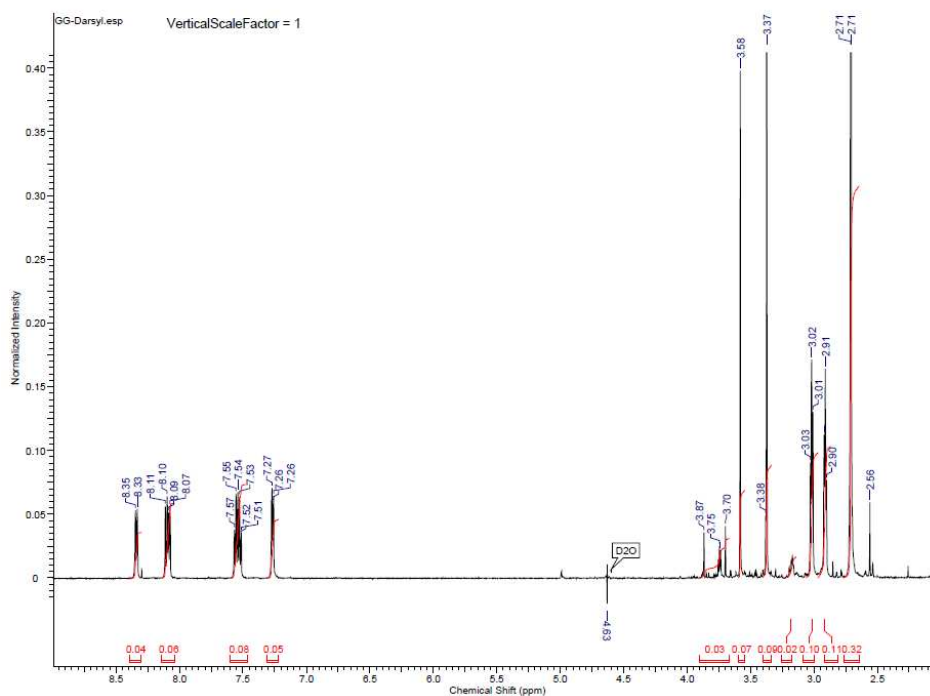
FTIR



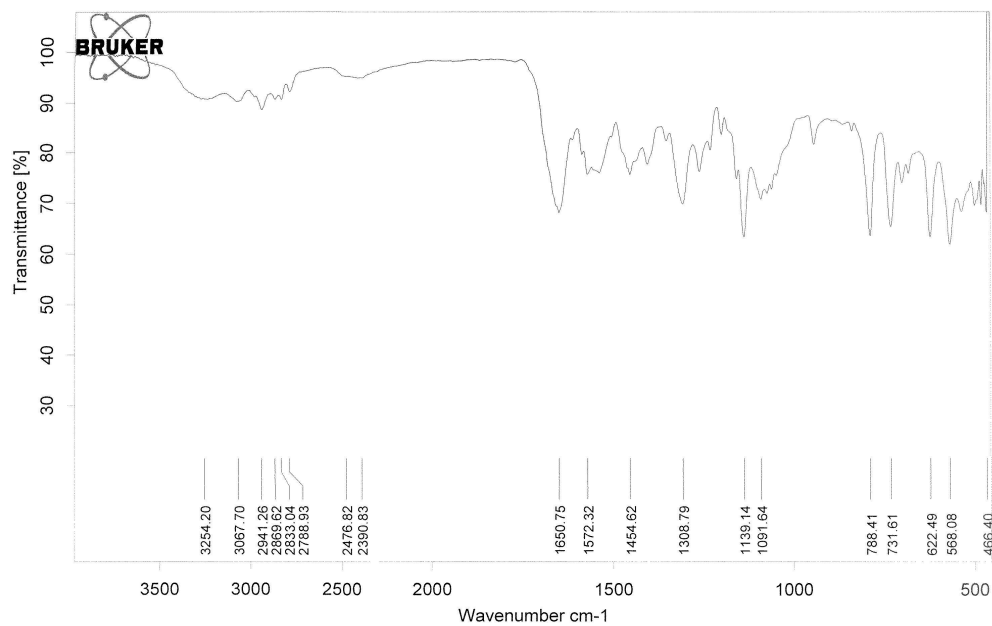
diGly-Dansyl

$^1\text{H NMR}$ (D_2O) δ : 8.34 (d, 1H, aromatic), 8.09 (m, 2H, aromatic), 7.55 (m, 2H, aromatic), 7.26 (m, 1H, aromatic), 3.58 (s, 2H, $\text{CH}_2\text{-Gly}$), 3.37 (s, 2H, $\text{CH}_2\text{-Gly}$), 3.02 (t, 2H, CH_2CH_2), 2.91 (t, 2H, CH_2CH_2), 2.71 (s, 6H, $\text{CH}_3\times 2$). FTIR (cm^{-1}): 3254, 3067, 2941, 2869, 2833, 2788, 2390, 1650, 1572, 1454, 1308, 1139, 1091.

$^1\text{H NMR}$



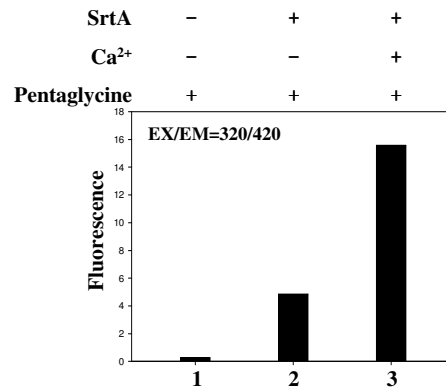
FTIR



2. Full amino acid sequence of TM₄₅₆ fusion protein (dITM₄₅₆)

EGF4
GIEGRSVEPVDP**CFRANCEYQCQPLNQ**TSYLCVCAEGFAP**IPHEPHRCQLFCNQ**TACPADC
EGF5 **EGF6**
DPNTQASCECPEGYILDDGF**ICTDIDE**CENGGF**CSGVCHNLP**GT**FEFECICG**PDSALAGQ**IGT**
DCDSGKVDGGDSGLPETGGSHHHHHH

3. Enzymatic activity assay of recombinant SrtA



For activity determination, SrtA substrate Abz-LPETG-Lys (Dnp)-NH₂ (1 mM) from Anaspec was incubated with pentaglycine (2 mM) in assay buffer (20 mM Tris, 150 mM NaCl and 5 mM CaCl₂, pH 8.0). Reactions were initiated by the addition of recombinant SrtA (1 μM) and were monitored by measuring the increase in fluorescence for 15 min (EX = 320 nm, EM = 420 nm, 37 °C).