Supporting Information

Structural and Energetic Determinants of Tyrosylprotein Sulfotransferase Sulfation Specificity

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Table S1.
The sequences of wild type and mutants of gastrin used to study the correlation between sulfation rate and sulfation energy [1]. The mutated residues are highlighted in bold.

Peptide	Sequence	Sulfation Rate (%)	Sulfation Energy (kcal/mol)
Wild type gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	71.8 ± 7.2	3.36
YE-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-**Glu**-Trp-Met-Asp-Phe-NH_2	59.9 ± 1.5	-0.26
YA-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-**Ala**-Trp-Met-Asp-Phe-NH_2	90.2 ± 1.7	5.28
EDY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-**Asp**-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	100.0 ± 0.0	-2.83
ERY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-**Arg**-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	0.0 ± 0.0	6.48
KAY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-**Lys**-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	86.6 ± 3.9	3.96
AAY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-**Ala**-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	91.1 ± 3.1	1.49
AEAY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-**Ala**-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	61.1 ± 1.7	8.39
AEEAY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-**Ala**-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	70.8 ± 1.4	3.89
AEEEAY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-**Ala**-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	70.7 ± 6.2	4.83
HDHDY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-**His**-**Asp**-**His**-**Asp**-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	100.0 ± 0	0.74
HDHAY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-Glu-**His**-**Asp**-**His**-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	89.6 ± 1.1	2.52
KEEAAY-gastrin	pGlu-Gly-Pro-Trp-Leu-Glu-**Lys**-Glu-Glu-**Ala**-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	87.9 ± 2.4	5.29

Reference:

[1] J. R. Bundgaard, J. Vuust, and J. F. Rehfeld, "New Consensus Features for Tyrosine O-Sulfation Determined by Mutational Analysis," *J. Biol. Chem.*, vol. 272, no. 35, pp. 21700–21705, Aug. 1997.

A

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TPST-1   5    LKQNLLLACLVISSVTVFYLGQHAMECHHRIEERSQPVKLESTRTTVRTGLD----LKAN  60
              +++ LL A   +  V    LGQ  +EC      R+    L S R  +R   +    + N
TPST-2   5    VRRVLLAAGCALVLVLAVQLGQQVLEC------RAVLAGLRSPRGAMRPEQEELVMVGTN  58

TPST-1   61   KT-FAYHKDMPLIFIGGVPRSGTTLMRAMLDAHPDIRCGEETRVIPRILALKQMWSRSSK  119
                 + Y K MPLIF+GGVPRSGTTLMRAMLDAHP++RCGEETR+IPR+LA++Q WS+S +
TPST-2   59   HVEYRYGKAMPLIFVGGVPRSGTTLMRAMLDAHPEVRCGEETRIIPRVLAMRQAWSKSGR  118

TPST-1   120  EKIRLDEAGVTDEVLDSAMQAFLLEIIVKHGEPAPYLCNKDPFALKSLTYLSRLFPNAKF  179
              EK+RLDEAGVTDEVLD+AMQAF+LE+I KHGEPA  LCNKDPF LKS  YLSRLFPN+KF
TPST-2   119  EKLRLDEAGVTDEVLDAAMQAFILEVIAKHGEPARVLCNKDPFTLKSSVYLSRLFPNSKF  178

TPST-1   180  LLMVRDGRASVHSMISRKVTIAGFDLNSYRDCLTKWNRAIETMYNQCMEVGYKKCMLVHY  239
              LLMVRDGRASVHSMI+RKVTIAGFDL+SYRDCLTKWN+AIE MY QCMEVG +KC+ V+Y
TPST-2   179  LLMVRDGRASVHSMITRKVTIAGFDLSSYRDCLTKWNKAIEVMYAQCMEVGKEKCLPVYY  238

TPST-1   240  EQLVLHPERWMRTLLKFLQIPWNHSVLHHEEMIGKAGGVSLSKVERSTDQVIKPVNVGAL  299
              EQLVLHP R ++ +L FL I W+ +VLHHE++IGK GGVSLSK+ERSTDQVIKPVN+ AL
TPST-2   239  EQLVLHPRRSLKLILDFLGIAWSDAVLHHEDLIGKPGGVSLSKIERSTDQVIKPVNLEAL  298

TPST-1   300  SKWVGKIPPDVLQDMAVIAPMLAKLGYDPYANPPNYGKPDPKIIENTRRVYKGEFQLPDF  359
              SKW G IP DV++DMA IAPMLA+LGYDPYANPPNYG PDP +I NT+RV KG+++ P
TPST-2   299  SKWTGHIPGDVVRDMAQIAPMLAQLGYDPYANPPNYGNPDPFVINNTQRVLKGDYKTPAN  358

TPST-1   360  LKEKPQTEQ  368
              LK   Q  Q
TPST-2   359  LKGYFQVNQ  367
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B

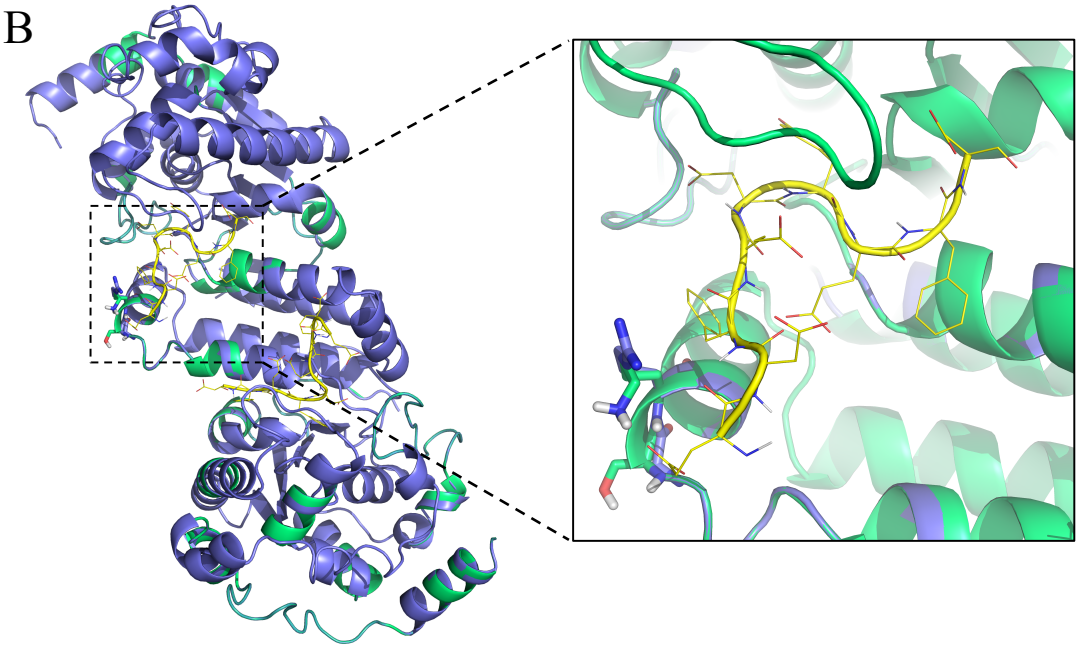

Figure S1. Comparison between two isoforms of TPST. (A) The sequence alignment of two TPST isoforms, TPST-1 and TPST-2. The highlighted region indicates the two mutations near the binding pockets. One of the mutations maintains the charge. The difference between the TPST-1 and TPST-2, with respect to the peptide-binding is thus not significant. (B) The crystal structure of TPST-2 (purple) and a homology model of TPST-1 (green) are very similar. Thus the method described in this work should be transferable to TPST-1.

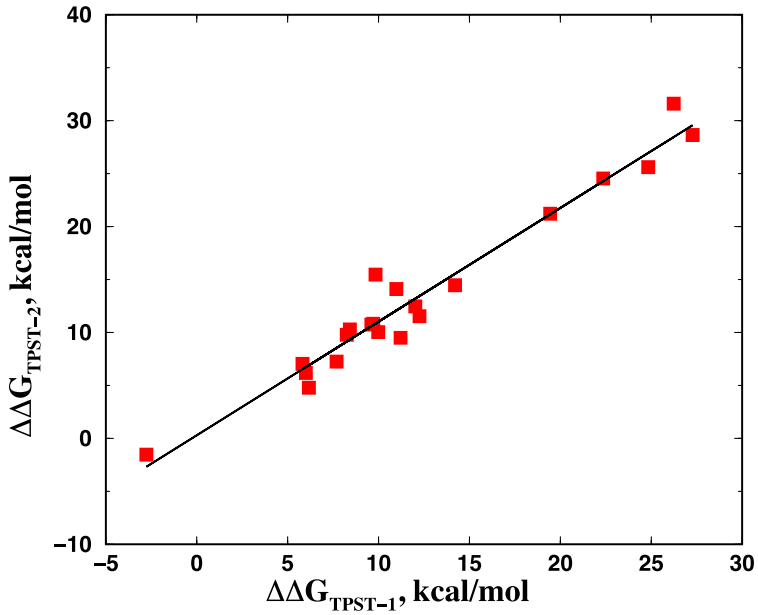

Figure S2. Sulfation energies estimated using two isoforms of TPST. The scatter plot of $\Delta\Delta G_{bind}$ values for TPST-1 and TPST-2 features a high correlation with the Pearson correlation coefficient r = 0.98. A linear regression results in the slope of 1.07 and an offset of 0.28 kcal/mol.