CHEMBIOCHEM

Supporting Information

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for

N-Methyl Scanning Mutagenesis Generates Protease-Resistant G Protein Ligands with Improved Affinity and Selectivity

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Table of Contents:

Peptide Synthesis

Analysis by MALDI-TOF MS:

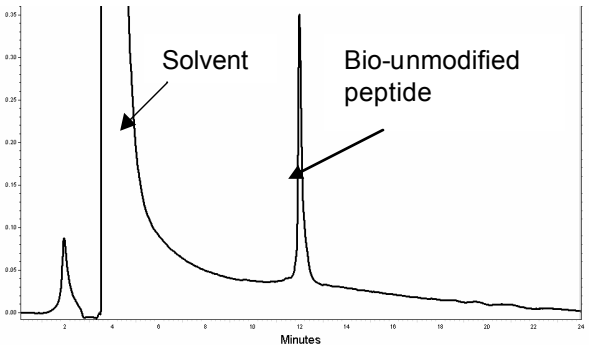
Unmodified peptide Expected $[M+H]^+$ = 1871.0, obs. $[M+H]^+$ = 1870.4 The expected mass of peptides with 1 *N*-methyl residue $[M+H]^+$ = 1885.4, N-Me-D obs. $[M+H]^+$ = 1885.3 N-Me-K obs. $[M+H]^+$ = 1884.5 N-Me-L obs. $[M+H]^+$ = 1884.7 N-Me-Y obs. $[M+H]^+$ = 1885.9 N-Me-W10 obs. $[M+H]^+$ = 1884.6 N-Me-W11 obs. $[M+H]^+$ = 1884.7 N-Me-E obs. $[M+H]^+$ = 1885.3 N-Me-F obs. $[M+H]^+$ = 1885.7 N-Me-L obs. $[M+H]^+$ = 1885.6

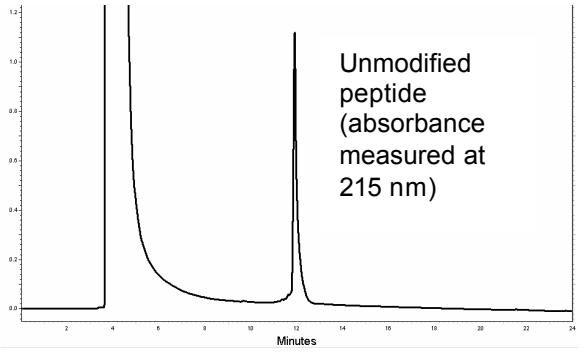
Bio-Unmodified peptide Expected $[M+H]^+$ = 2095.2 , obs. $[M+H]^+$ = 2096.0 The expected mass of all N-methyl peptides $[M+H]^+$ = 2110.1, N-Me-D obs. $[M+H]^+$ = 2110.9 N-Me-K obs. $[M+H]^+$ = 2110.6 N-Me-L obs. $[M+H]^+$ = 2110.2 N-Me-Y obs. $[M+H]^+$ = 2110.6 N-Me-W10 obs. $[M+H]^+$ = 2109.5 N-Me-W11 obs. $[M+H]^+$ = 2110.6 N-Me-E obs. $[M+H]^+$ = 2110.0 N-Me-F obs. $[M+H]^+$ = 2110.0 N-Me-L obs. $[M+H]^+$ = 2109.8

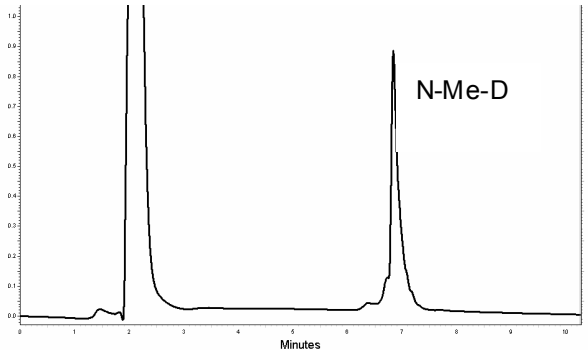
Digest product LYWWEFL Expected $[M+H]$ = 1056.2, obs. $[M+H]$ = 1056.6

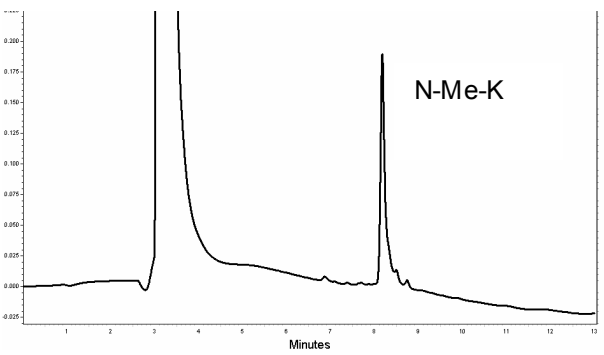
R6A Expected $[M+H]$ = 2276.6, obs. $[M+H]$ = 2276.9 Bio-R6A Expected $[M+H]$ = 2501.7, obs. $[M+H]$ = 2501.2

HPLC traces.






N-Me-D
Minutes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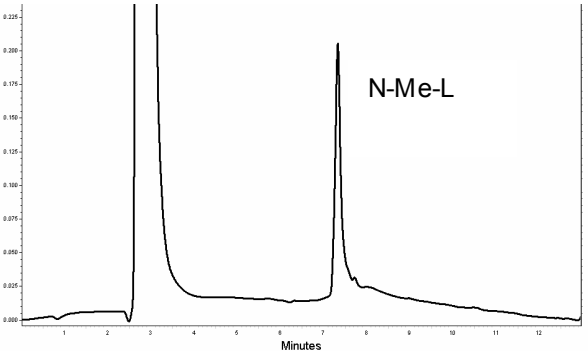
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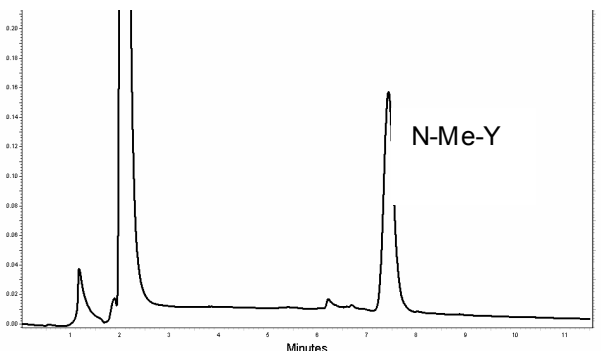
N-Me-Y
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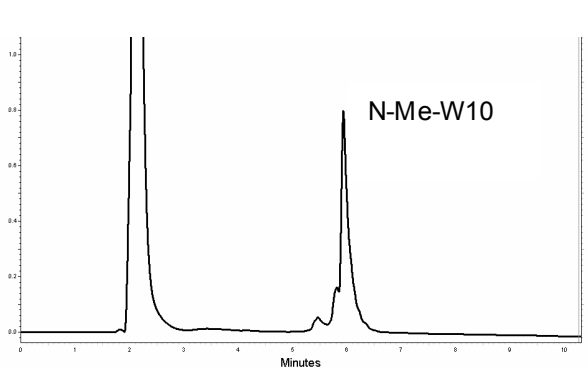
N-Me-W10
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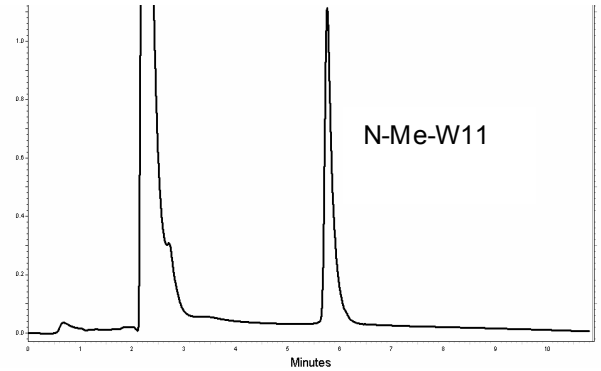
N-Me-W11
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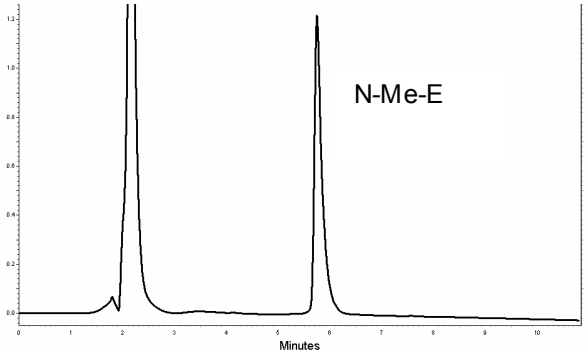
N-Me-E
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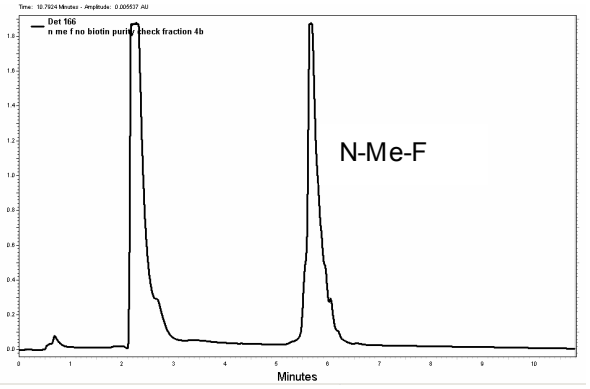
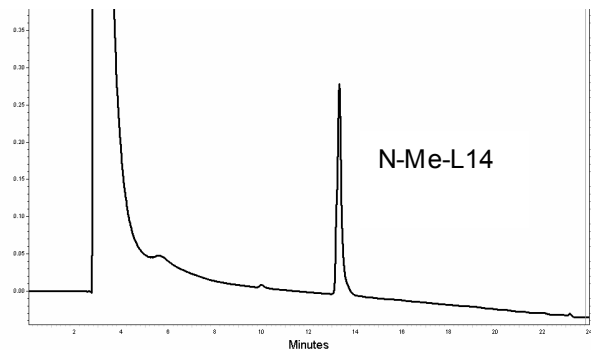

Det 166
n me f no biotin purity check fraction 4b
N-Me-F
Minutes


N-Me-L14
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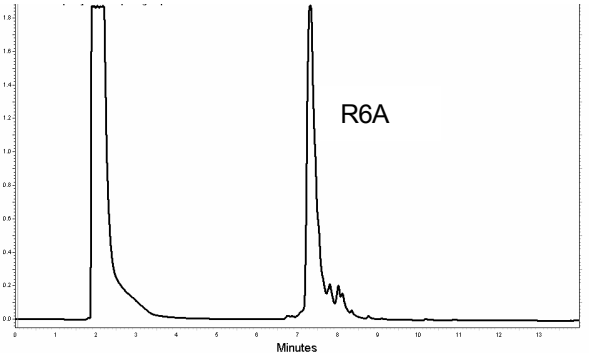
R6A
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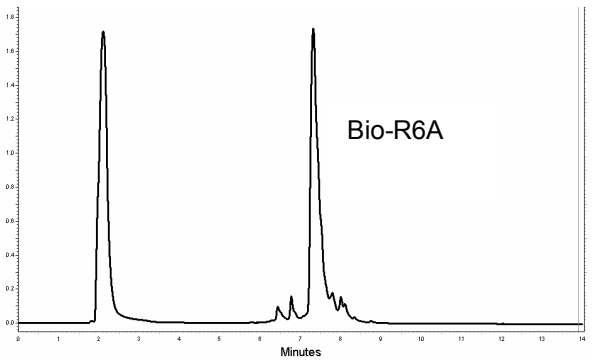
Bio-R6A
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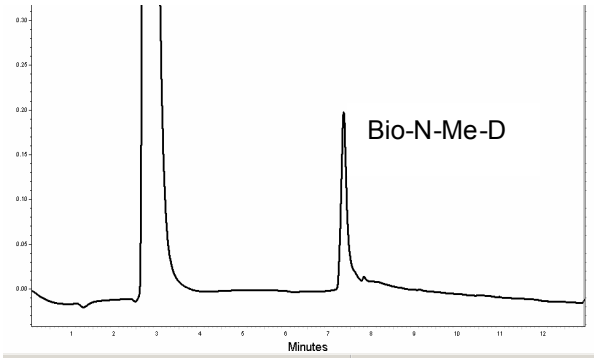
Bio-N-Me-D
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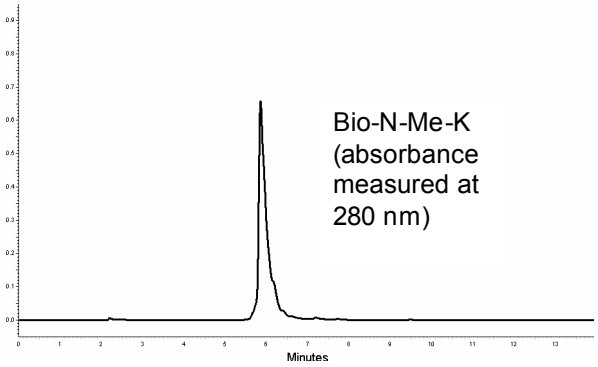
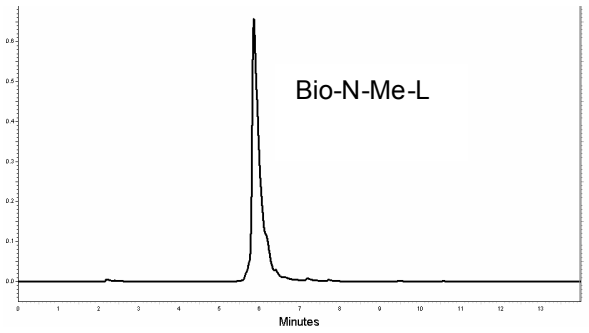

Bio-N-Me-K
(absorbance
measured at
280 nm)
Minutes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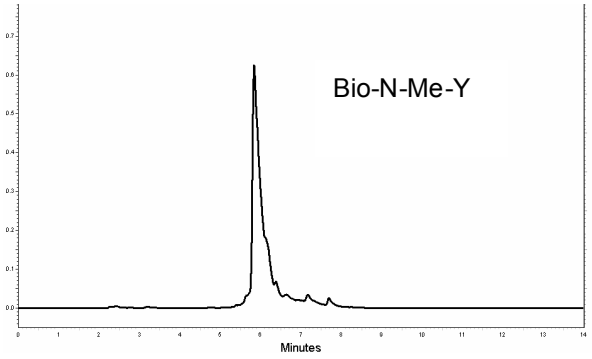
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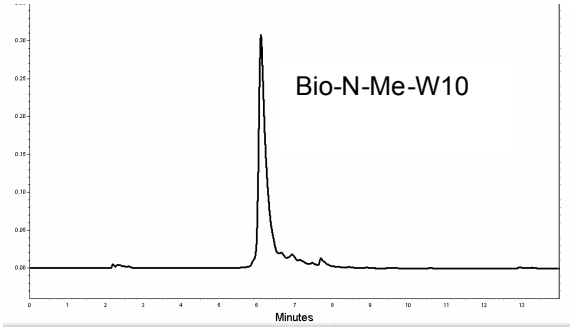
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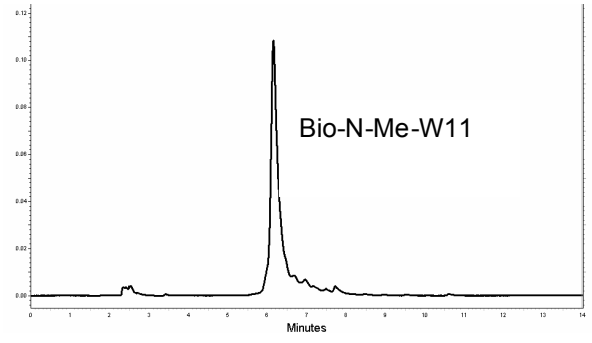
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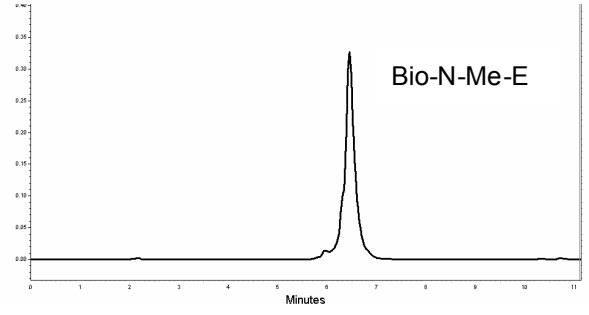
Bio-N-Me-E
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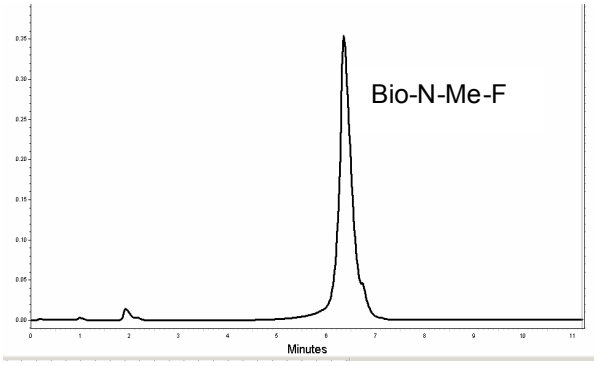
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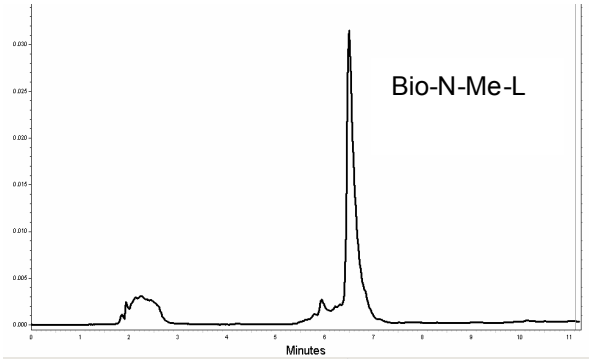
Bio-N-Me-L
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N-Methyl Selectivity

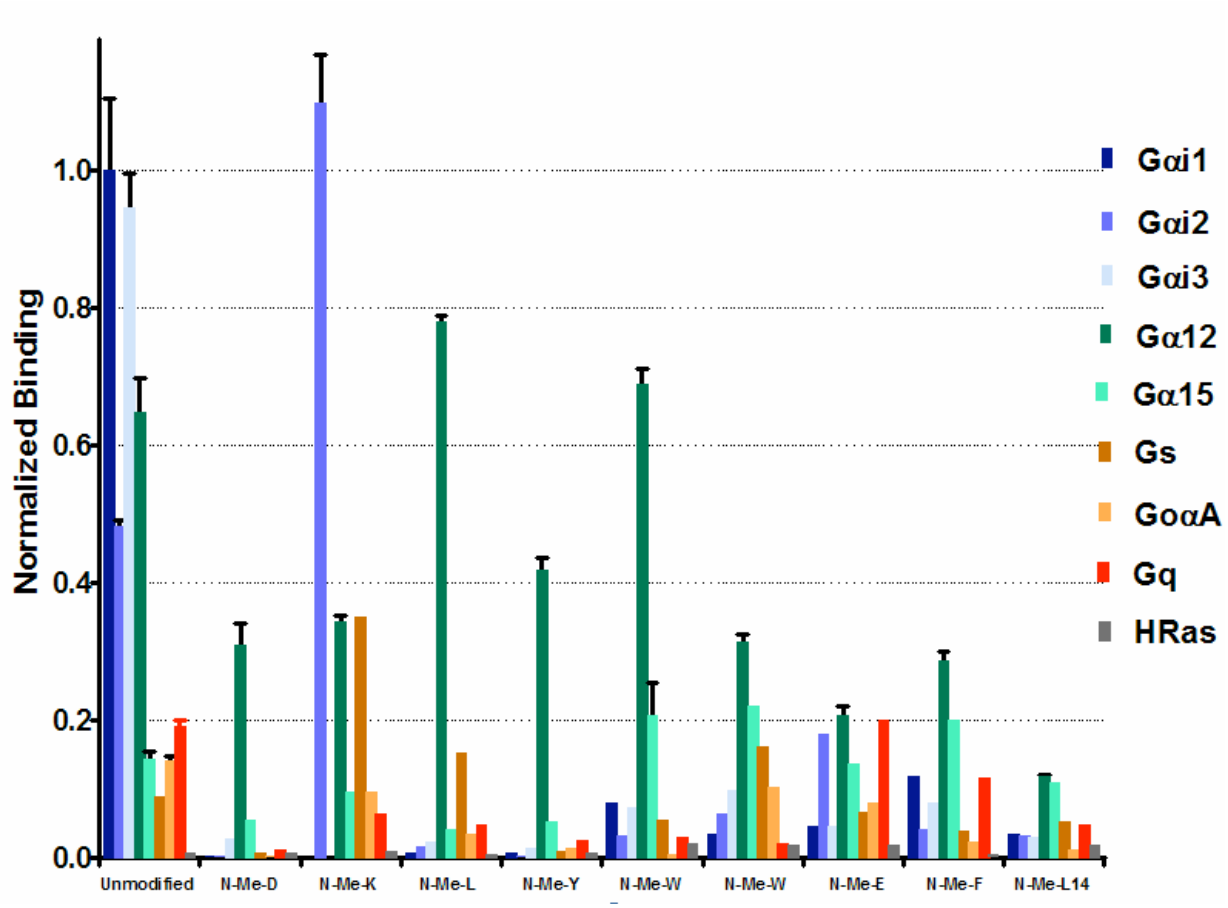

Figure S1: Selectivity for the N-methyl peptide library. G protein subunits are color coded. Binding is normalized by dividing the samples cpm by the average cpms obtained for the unmodified peptide binding to its target Gαi1. Two N-Me-K data points have been removed in order to scale the graph in a way to resolve weaker binding interactions.