Electornic Supplementary Information (ESI) for:

Inhibition of *Escherichia coli* RecA by rationally redesigned N-terminal helix

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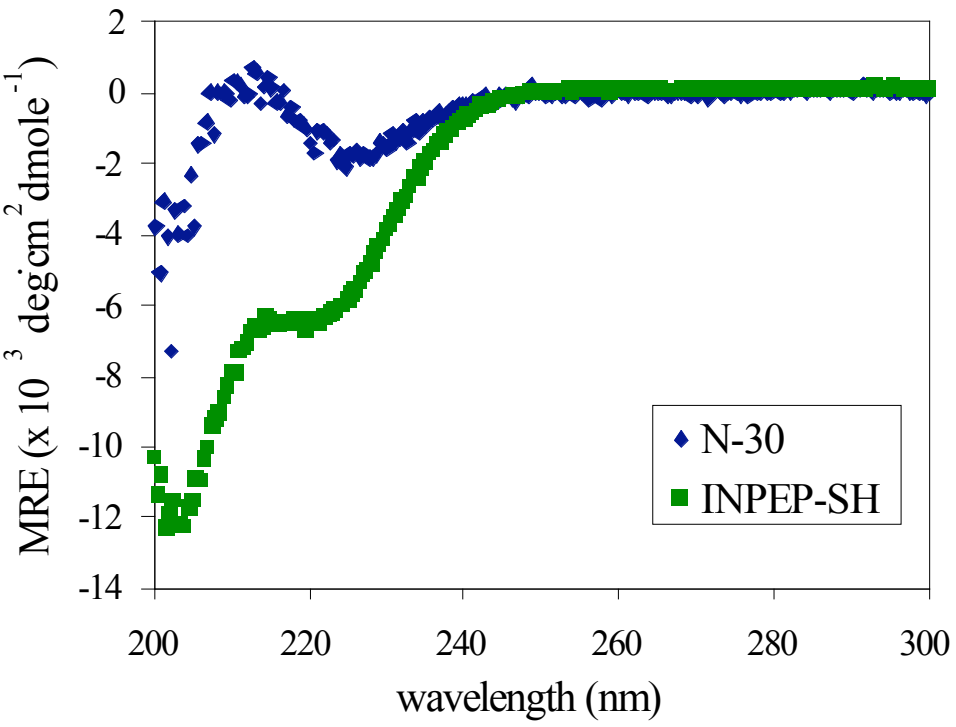
Contents:

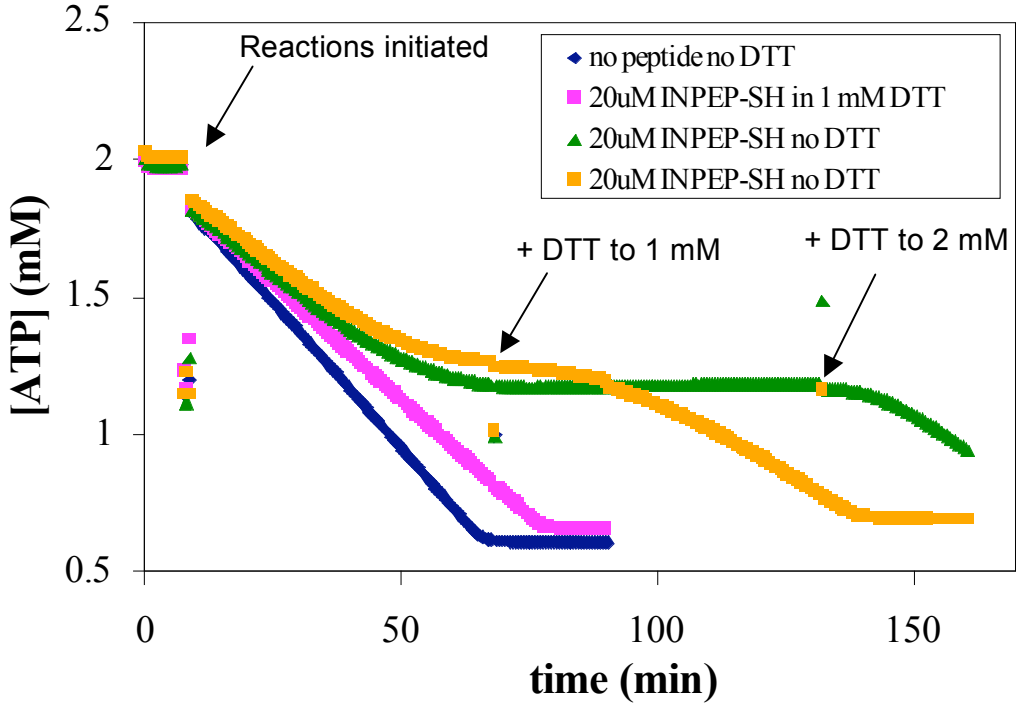


Supplementary Figure S1 Circular Dichroism Data. The circular dichroism spectra of RecA N-30 peptide and INPEP-SH were recorded using an Aviv model 202-01 Spectropolarimeter. Assays were performed using 100 μM peptide (50 mM potassium phosphate, pH 7.5, 25 °C) in 1-mm pathlength quartz cells. Mean residue ellipticity [θ] was calculated using the equation [θ] = (θ_{obs}/10*lc*)/*r*, where θ_{obs} is the measured ellipticity in millidegrees, *l* is the length of the cell (cm), *c* is the concentration (M), and *r* is the number of residues. Percent helicity values are based on fraction helix (f_h) at 25 °C. Fraction helix was calculated according to Rohl and Baldwin[1] where f_h = ($[\theta]_{222}$ + 485)/([-39375(1 - 3/Nr)] + 485), where Nr is the number of residues in a helical conformation (16 for N-30, 17 for INPEP-SH).

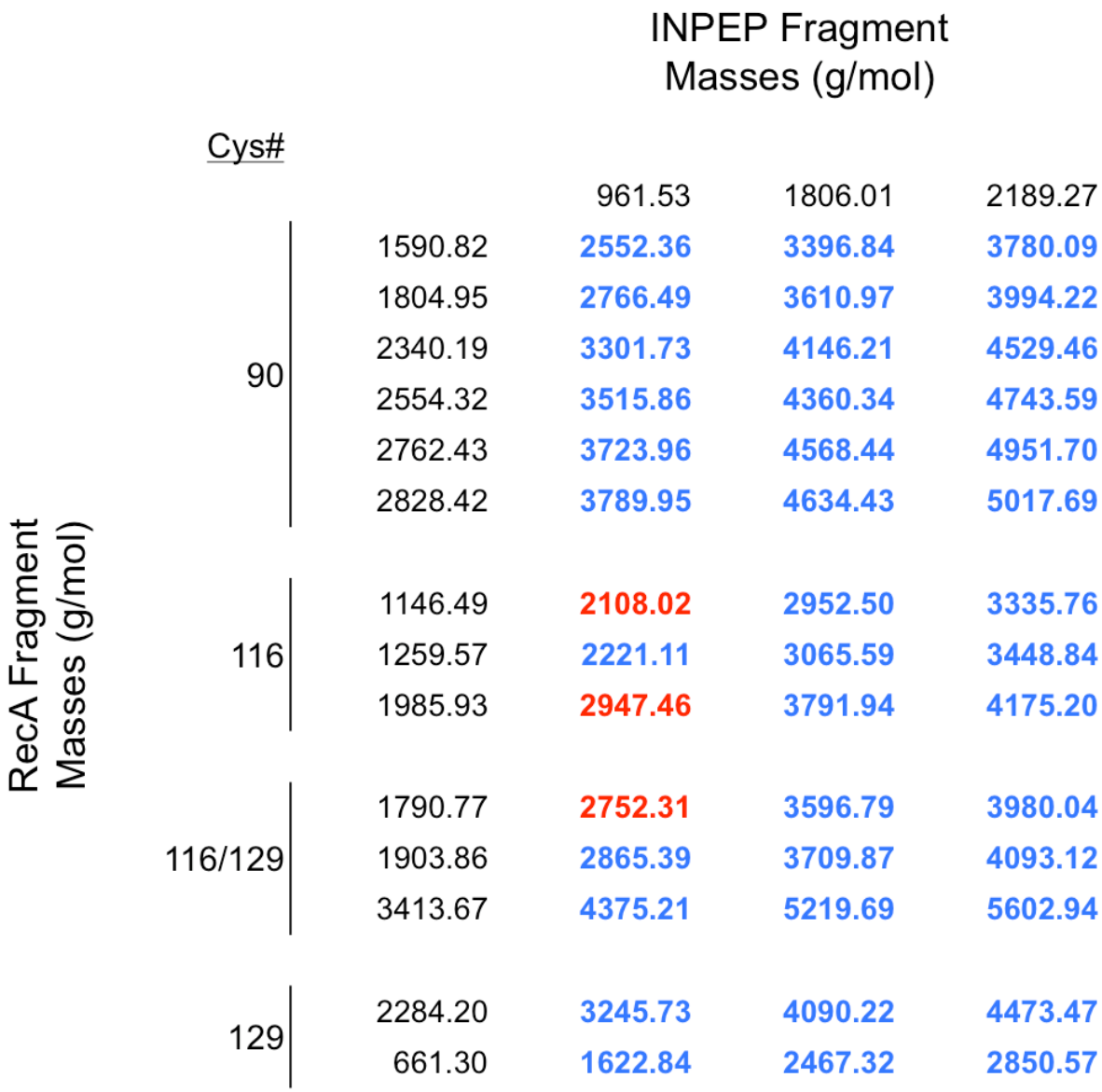
For RecA N-30: f_h = 1.9%

For INPEP-SH: f_h = 18%

[1] Rohl, C. A.; Baldwin, R. L. Deciphering rules of helix stability in peptides. *Energetics of Biological Macromolecules, Pt B, volume 295*; 1998: 1.

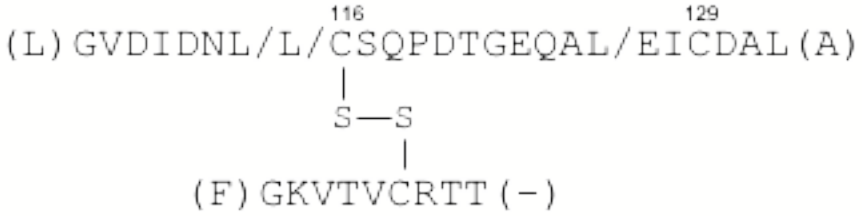


Supplementary Figure S2 ATPase assay—effect of reductant. The ATPase activity of RecA was assayed using a PerkinElmer Lambda 60 spectrophotometer equipped with a six-position cell changer and peltier heating device set at 37 °C. Assay conditions were 1 μM RecA protein in 25 mM Tris-HOAc, pH 7.1, 5 mM $Mg(OAc)_2$, 5% v/v glycerol with 2.3 mM Phosphoenolpyruvate, 2 mM NADH, 5 U/mL Lactate Dehydrogenase, 5 U/mL Pyruvate Kinase, 15 μM-nts poly(dT). INPEP was added just prior to assay start, and the ATPase reaction initiated after 5 min thermal equilibration. Rates of ATP hydrolysis of inhibited RecA were similar at the onset, but reactions without DTT slowly decreased in rate as the peptide made disulfide bonds with the protein. (Note linearity of the slope of RecA inhibited with 20 μM INPEP-SH in the presence of DTT.) The activities were, however, partial reconciled with the *addition* of DTT at the indicated time points: **magenta** = 80% activity remaining; **gold** & **green** ≈ 60% activity recovered.

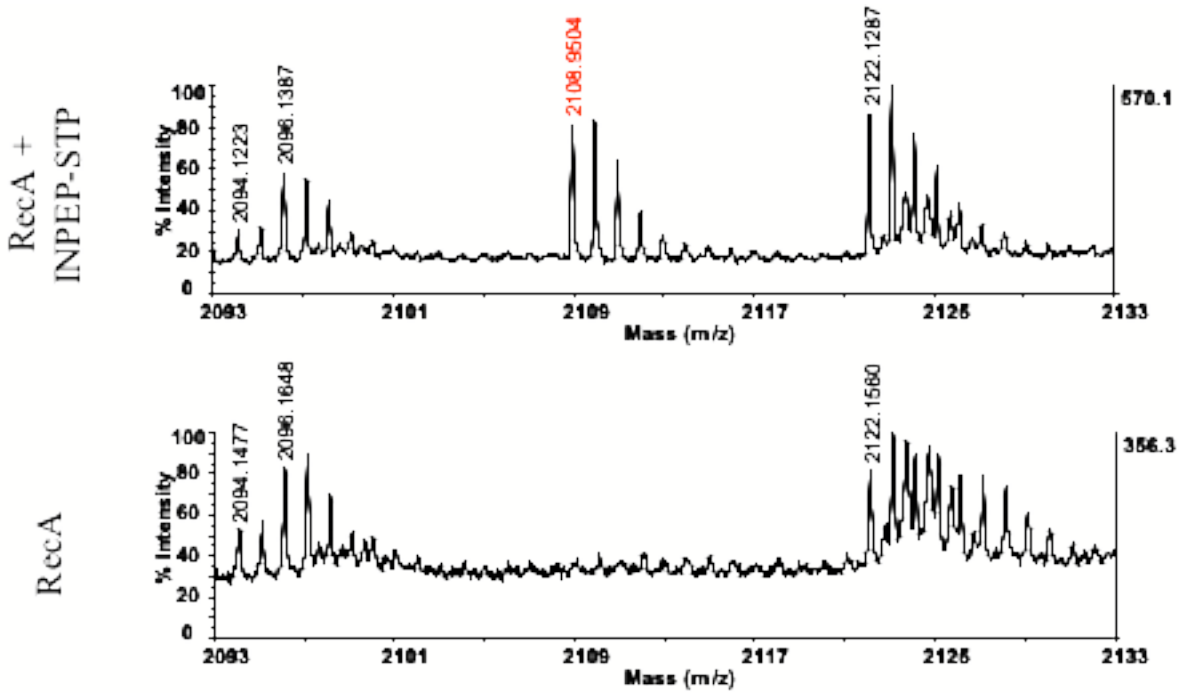


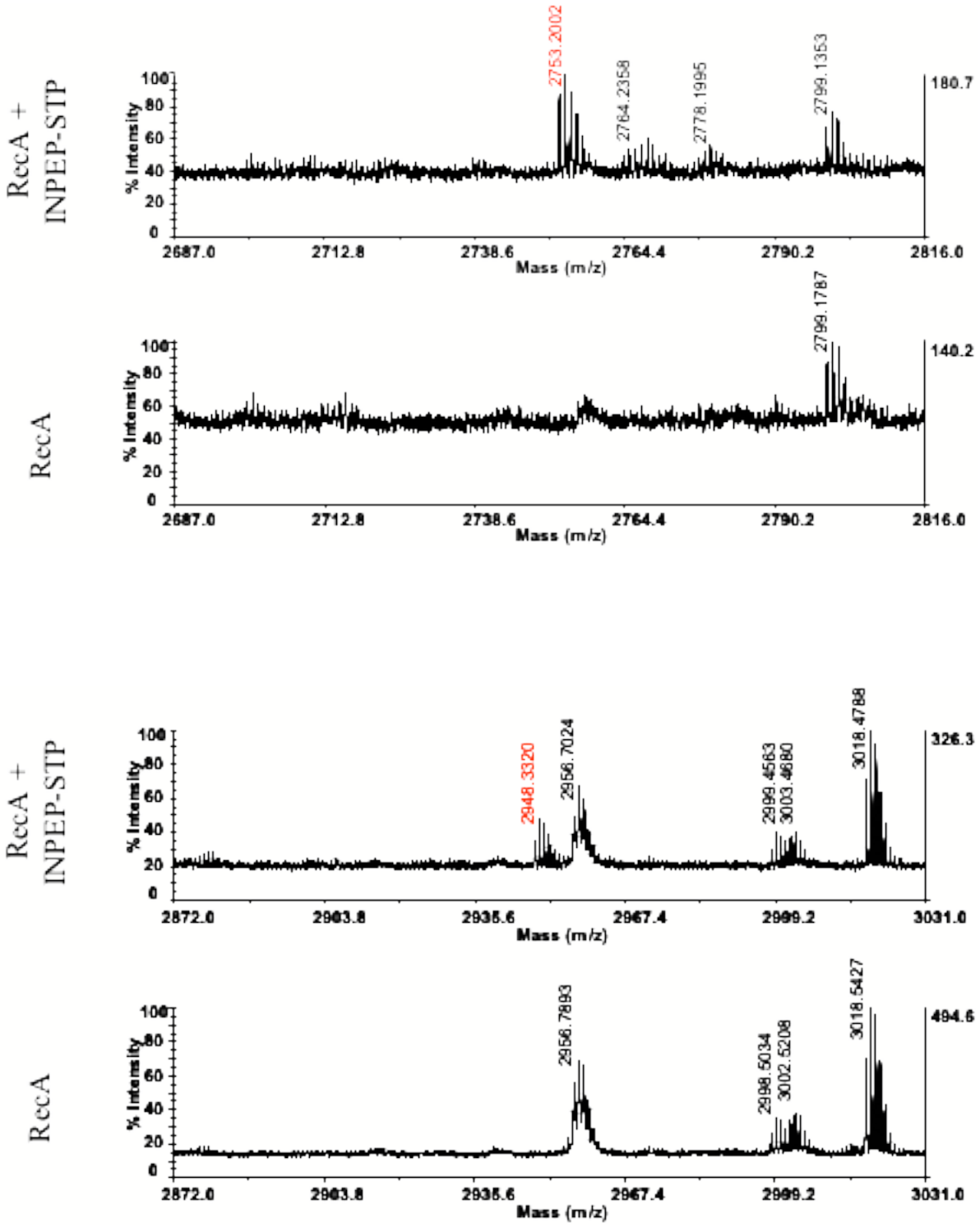
Supplementary Figure S3 Mass Spectral analysis. RecA protein that was inhibited by INPEP-STP was digested in-gel with chymotrypsin and the resultant fragments were analysed using MALDI-TOF mass spectrometry at UNC's Hooker Proteomics Core Facility. Based on the expected chymotrypsin cleavage sites, fragments containing the possible cysteines (90, 116, or 129) were crossed with expected fragments from INPEP to generate the table of possible mass fragments (blue text). Comparison of the inhibited RecA mass spectra to the uninhibited RecA mass spectra (see spectograms on pages S4 and S5), three possible mass fragments were found (red text).

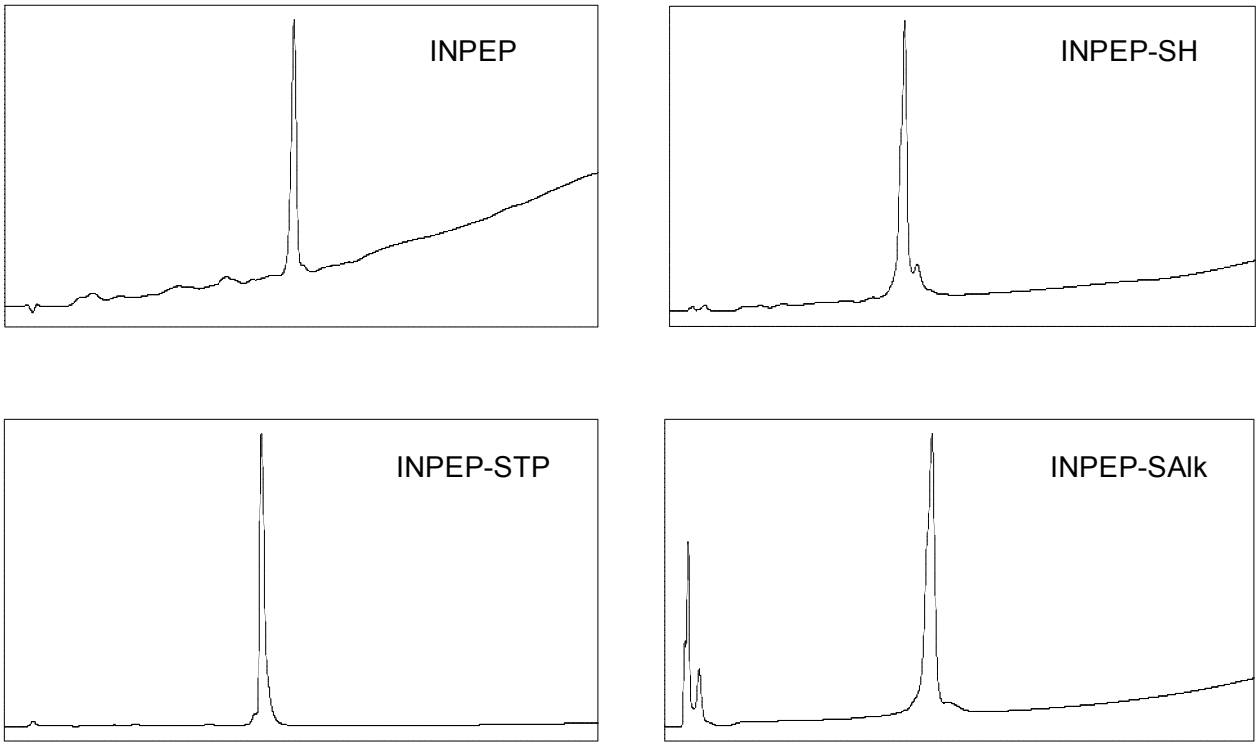
RecA chymotrypsin fragment(s)



INPEP chymotrypsin fragment




RecA + INPEP-STP
2753.2002
2764.2358
2778.1995
2799.1353
180.7
% Intensity
Mass (m/z)
RecA
2799.1787
140.2
RecA + INPEP-STP
2948.3320
2956.7024
2999.4563
3003.4680
3018.4788
326.3
RecA
2956.7893
2998.5034
3002.5208
3018.5427
494.6



Supplementary Figure S4 INPEP purity assessed by HPLC. The HPLC traces at 220 nm of purified INPEP peptides are shown above. Traces were acquired on a Waters 600E system with a 2487 dual wavelength detector set at 220 and 280 nm. Lyophilized peptides were dissolved in 0.1% TFA or 10 mM NH_4OAc buffer (INPEP-SAlk). Peptides were eluted from Ascentis C-18 reverse phase column with a linear gradient from 0 to 100% HPLC solvent B over 100 minutes. All peptide peaks were determined to be >98% pure by their HPLC profiles. INPEP-SH HPLC trace also shows disulfide dimer as small post peak.