

## SUPPORTING INFORMATION

### **Mass spectral charting of neuropeptidomic expression in the stomatogastric ganglion at multiple developmental stages of the lobster *Homarus americanus***

**Xiaoyue Jiang<sup>a</sup>, Ruibing Chen<sup>b</sup>, Junhua Wang<sup>a</sup>, Anita Metzler<sup>c</sup>, Michael Tlusty<sup>c</sup>, and  
Lingjun Li<sup>a,b,\*</sup>**

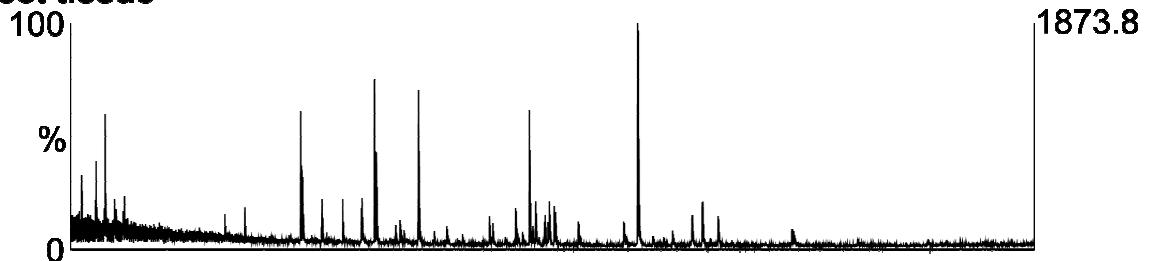
<sup>a</sup>School of Pharmacy, University of Wisconsin, 777 Highland Avenue, Madison, WI 53705-2222, USA

<sup>b</sup>Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, WI 53706-1396, USA

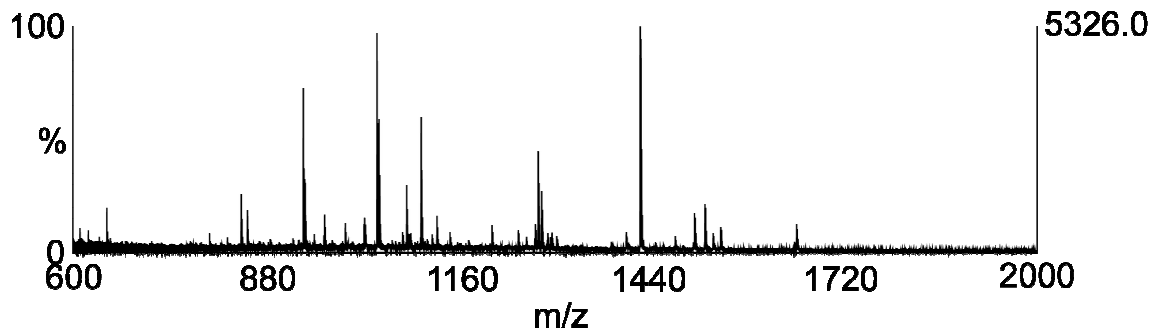
<sup>c</sup>Lobster Research and Rearing Facility, Edgerton Research Laboratory, New England Aquarium, Central Wharf, Boston, MA 02110-3399, USA

**Supporting Figure S1**

**(a) Direct tissue**



**(b) Tissue extraction**



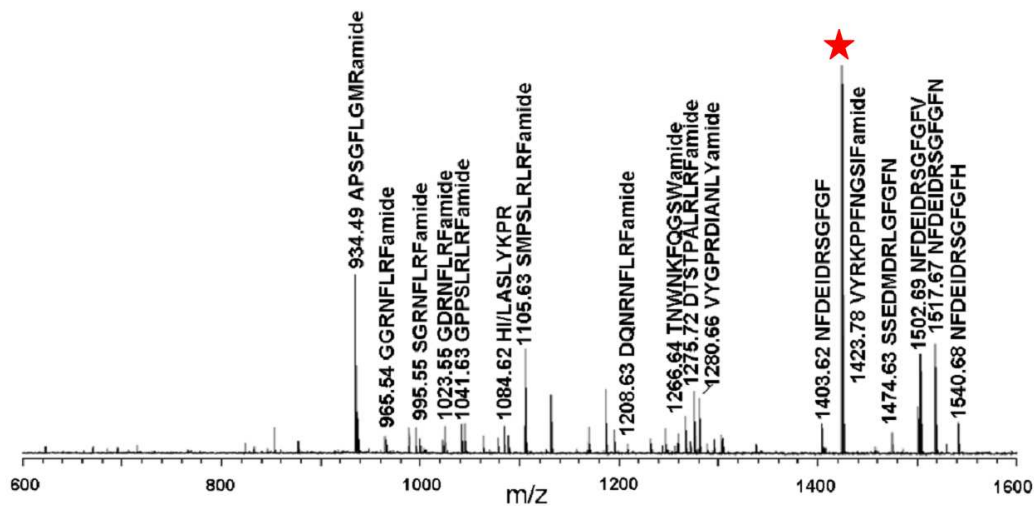
**Supporting Figure S1 :** Entire mass range analysis of (a) a single adult STG by direct tissue

analysis and (b) tissue extract of 15 pooled adult STGs using MALDI TOF/TOF MS. Most

neuropeptides have masses between  $m/z$  900-1600. The right axis shows the absolute intensity of

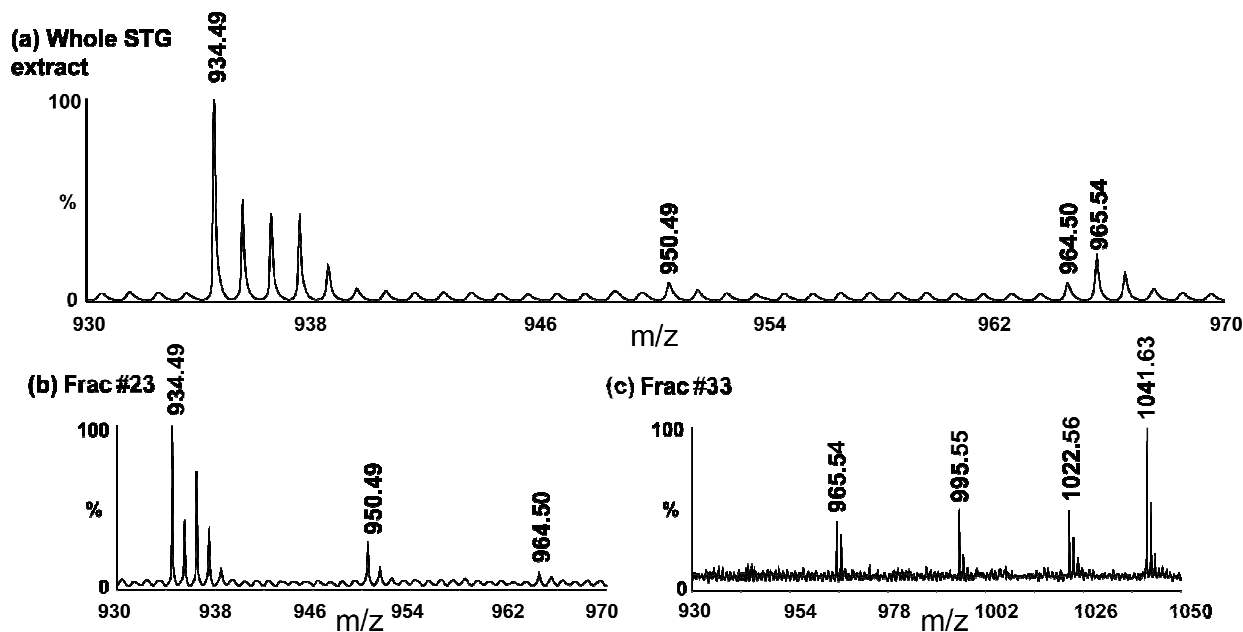
the spectrum.

## Supporting Figure S2



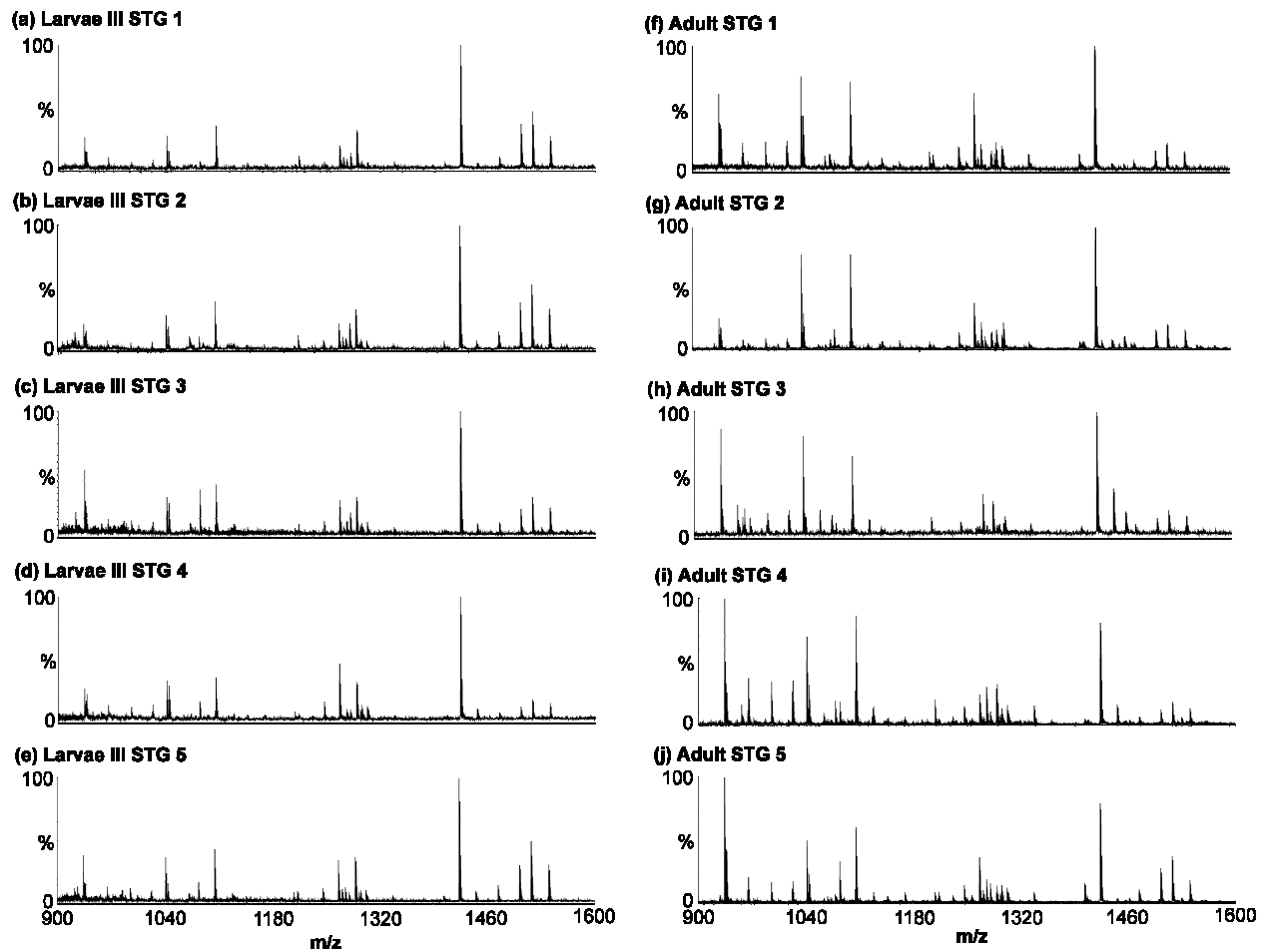
**Supporting Figure S2** : FT direct tissue analysis of the STG of American lobster. As shown, peak  $m/z$  1423.78 is the most intense peak in the spectrum. The highest signal response of VYRKPPFNGSIFamide in MALDI MS measurement might be due to the presence of multiple basic residues in its sequence.

### Supporting Figure S3 :



**Supporting Figure S3 :** Mass spectral analysis of CE separation and identification of three tachykinin peptides APSGFLGMRamide ( $m/z$  934.49), APSGFLGM(O)Ramide ( $m/z$  950.49) and TPSGFLGMRamide ( $m/z$  964.5) in the adult STG extract. (a) The peak intensities for  $m/z$  950.49 and  $m/z$  964.50 are too low to be identified unambiguously as putative neuropeptide peaks. It is also noted that  $m/z$  964.50 peak is detected together with  $m/z$  965.54 peak (GGRNFLRFamide). After separation, in (b) fraction #23, peak abundances for  $m/z$  950.49 and  $m/z$  964.50 are increased, with the elimination of interfering  $m/z$  965.54 peak. (c) In fraction #33,  $m/z$  965.54 is eluted, together with other neuropeptide isoforms belonging to the FaRP family.

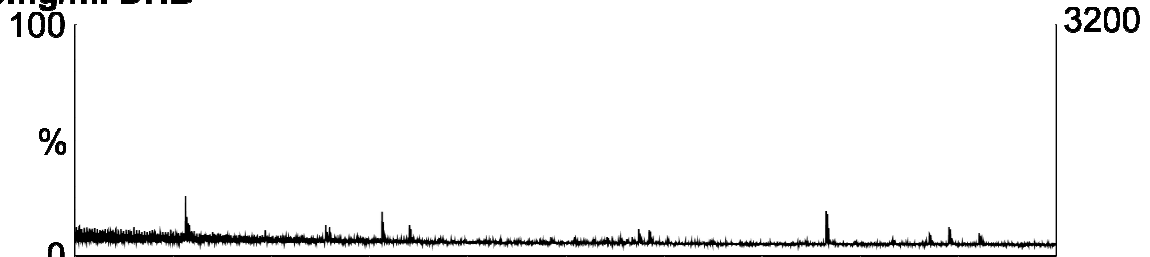
## Supporting Figure S4 :



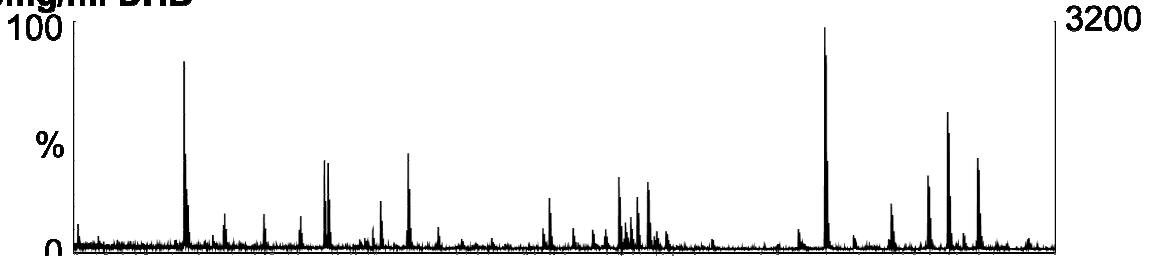
**Supporting Figure S4 :** Demonstration of spectral variability which is caused by sample preparation and animal individual variation. (a-e) Direct tissue analysis of STGs from five larvae III lobsters. (f-j) Direct tissue analysis of STGs from five adult lobsters.

### Supporting Figure 5

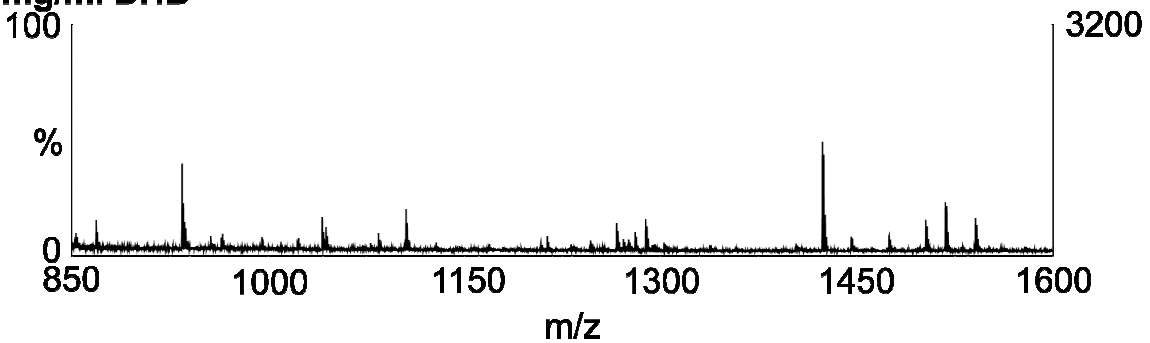
(a) 100mg/ml DHB



(b) 20mg/ml DHB



(c) 10mg/ml DHB



**Supporting Figure 5 :** Comparisons of different concentrations of DHB for analyzing the STG of early stage lobster (stage V). The STGs were detected using (a) 100mg/ml DHB; (b) 20mg/ml DHB; and (c) 10mg/ml DHB. The y axis has been normalized to the same absolute intensity for each spectrum. 20mg/ml DHB could give the highest peak intensities for the sample. However, the matrix concentration does not affect the relative peak intensities of the peptides.