Circular Dichroism Study of Orexin B under Oxidative Stress Conditions

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Supplementary Materials

Table S1. *Analysis of the orexin B secondary structure content in the investigated solvent environments.*

	α-helix	β-strand	Turns	Unordered	$\theta_{MRW,205}$	$\theta_{MRW,220}$	Ratio $\theta_{MRW,205}/\theta_{MRW,220}$
Buffer	9.3%	24.3%	24.2%	42.2%	-1062.95	-289.80	n.d.
TFE 20%	36.8%	17.9%	21%	24.3%	-1893.02	-1162.31	0.61
TFE 80%	64.8%	2.6%	13.9%	18.7%	-2885.51	-2027.57	0.70
SDS	43.4%	11.5%	17.7%	27.4%	-1746.05	-1147.82	0.66
DMPG/DMPC	31%	14.8%	22.4%	31.8%	-1484.19	-1387.94	0.94

n.d. : non determined

Table S2. *Analysis of the orexin B secondary structure content when irradiated and with DEA NONO-Ate.*

	Orexin B in	α-helix	β-strand	Turns	Unordered
phosphate buffer, irradiated	0 min	9.3%	24.3%	24.2%	42.2%
	5 min	8.5%	27.5%	23.5%	40.5%
	10 min	8.5%	28.1%	23.2%	40.2%
	15 min	8.7%	27.2%	23.7%	40.4%
DMPG/DMPC, irradiated	0 min	31%	14.8%	22.4%	31.8%
	15 min	36.5%	6.7%	20.6%	36.2%
	30 min	27.1%	19.2%	22.8%	30.9%
	45 min	26.6%	18.2%	24.2%	31%
DEA NONO-Ate in phospate buffer	0 min	9.3%	24.3%	24.2%	42.2%
	30 min	8.2%	27.3%	23.5%	40.9%
	60 min	7.3%	28.7%	22.8%	41.1%
	90 min	7.9%	27.5%	23%	41.7%
	120 min	8.5%	26.8%	23.5%	41.2%
DEA NONO-Ate in TFE	0 min	64.8%	2.6%	13.9%	18.7%
	30 min	66.8%	2.7%	8.9%	21.6%
	60 min	67.3%	4.7%	6.8%	21.2%
	90 min	64.9%	4.2%	7.8%	23.1%
	120 min	64.8%	4.2%	7.8%	23.2%

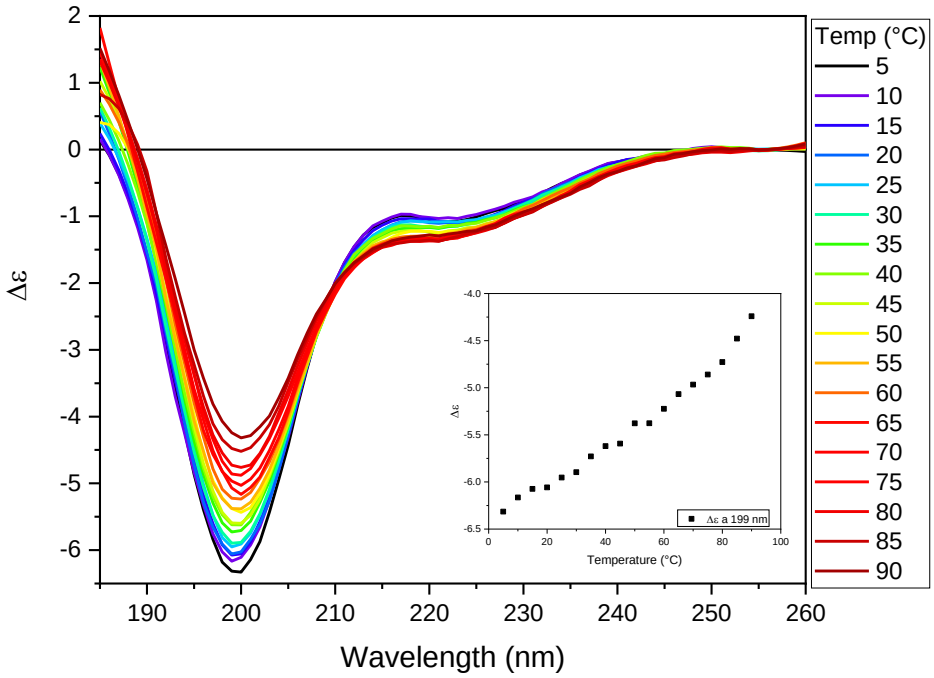


Figure S1. *CD melting plot of orexin B (34 µM in phosphate buffer). Spectra were recorded on a Jasco J-1500 dichrograph in the 185-260 nm range in the 5-90°C temperature range every 5°C, with a temperature gradient of 2°C/min.* ***Insert****: plot of Δε at 199 nm versus temperature.*

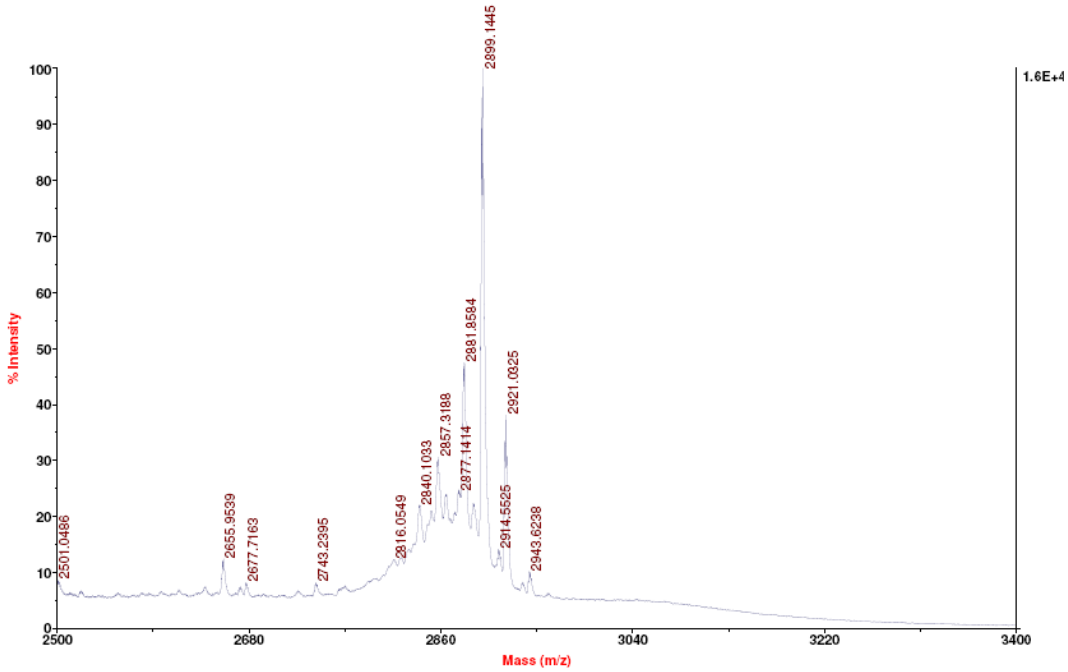


Figure S2. *MALDI-TOF MS analysis of orexin B in phosphate buffer, irradiated. Matrix: α-Cyano-4-hydroxycinnamic acid in 9: 1 = MeCN: H_2O + 0.1% TFA. Orexin B [M+H$^+$] =2899 m/z; Orexin B Met(O) [M+H$^+$] =2915 m/z.*

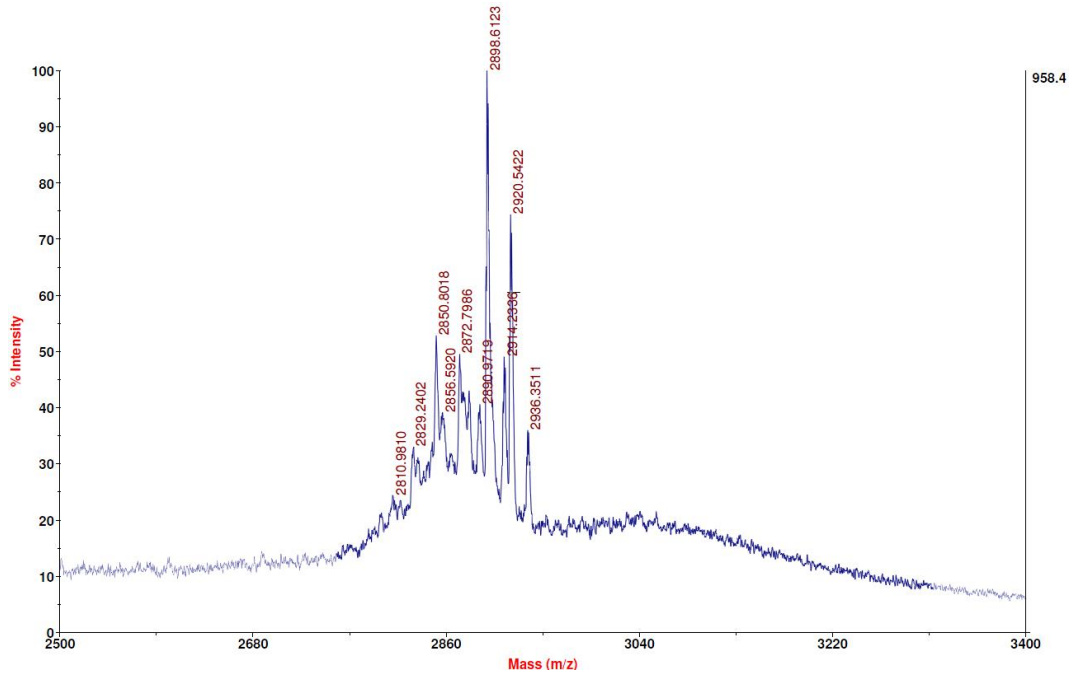


Figure S3. *Orexin B in phospholipids, irradiated. Matrix: α-Cyano-4-hydroxycinnamic acid in 9: 1 = MeCN: H_2O + 0.1% TFA. Orexin B [M+H^+] =2899 m/z; Orexin B Met(O) [M+H^+] =2915 m/z.*

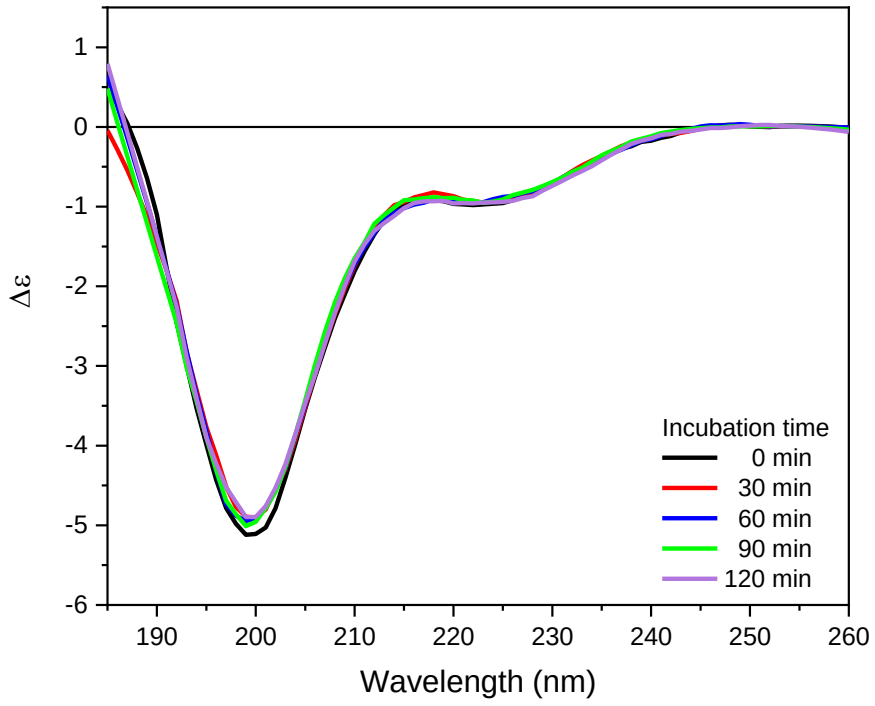


Figure S4. *Far-UV CD of orexin B (34 μM) in 20 mM phosphate buffer, added of 20 eq. of DEA NONOate. Spectra were recorded on a Jasco J-1500 dichrograph at increasing incubation times (indicated).*

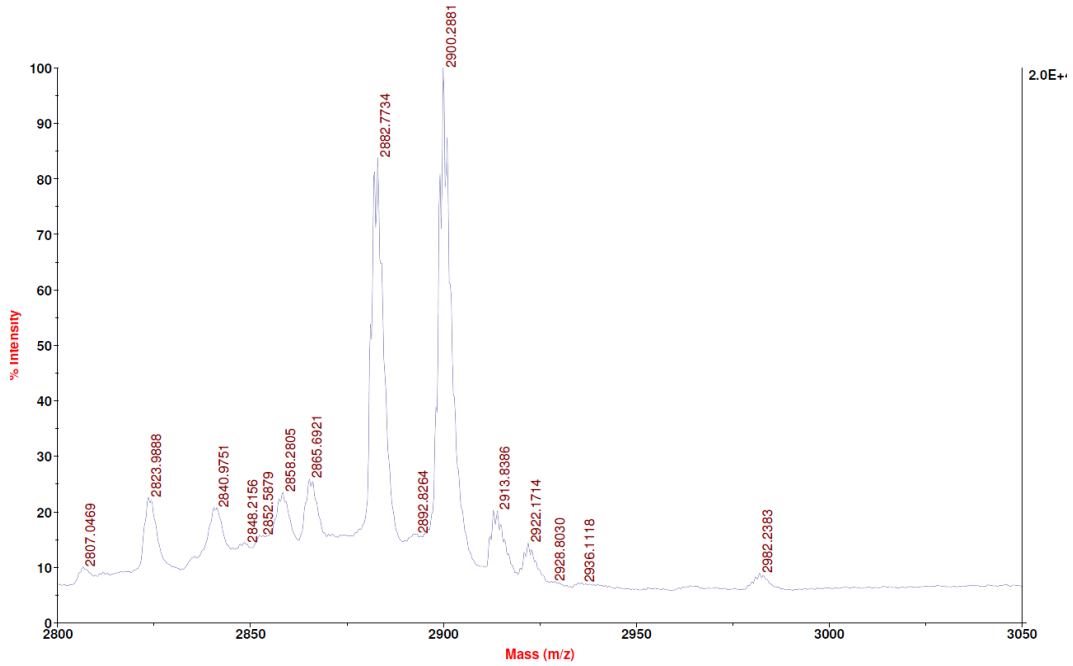


Figure S5. *MALDI-TOF MS analysis of orexin B in presence of DEA NONO-Ate in phosphate buffer. Matrix: α-Cyano-4-hydroxycinnamic acid in 9: 1 = MeCN: H_2O + 0.1% TFA. Orexin B [M+H^+] =2899 m/z.*

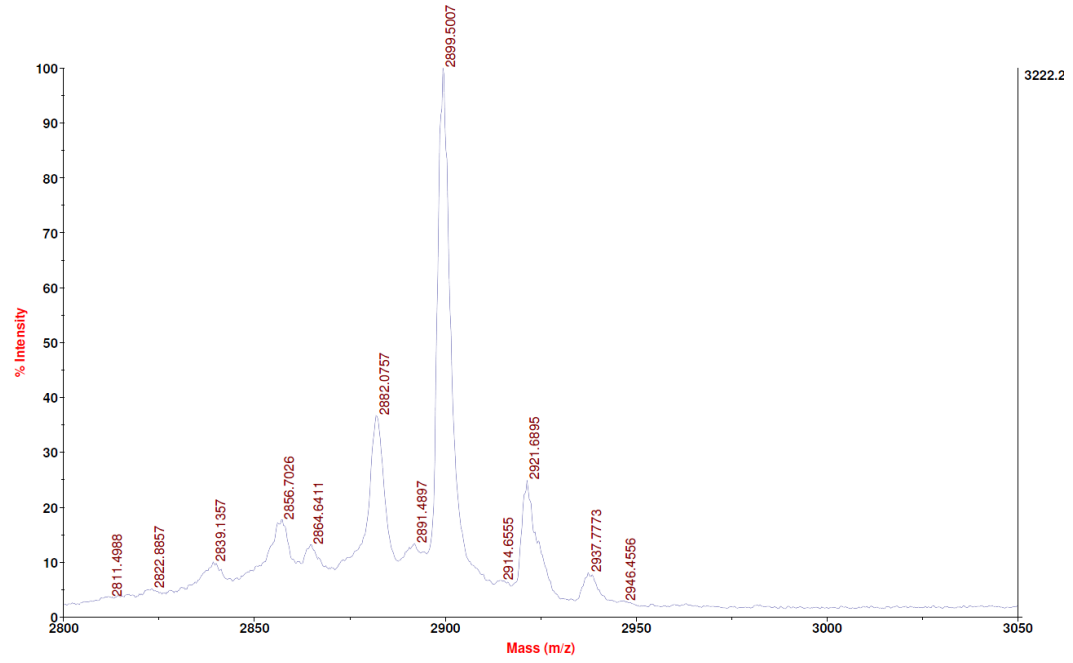


Figure S6. *MALDI-TOF MS analysis of orexin B in presence of DEA NONO-Ate in 80% TFE. Matrix: α-Cyano-4-hydroxycinnamic acid in 9: 1 = MeCN: H_2O + 0.1% TFA. Orexin B [M+H^+] =2899 m/z.*

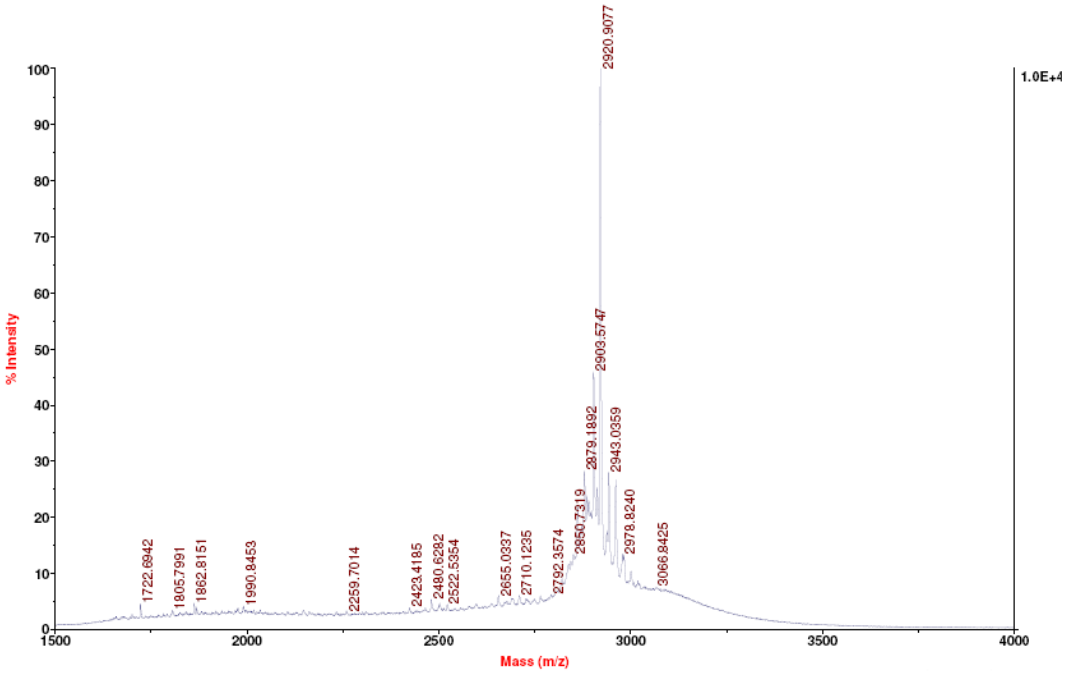


Figure S7. *MALDI-TOF MS analysis of orexin B in presence of glyoxal in phosphate buffer. Matrix: α-Cyano-4-hydroxycinnamic acid in 9: 1 = MeCN: H_2O + 0.1% TFA. Orexin B [M+H^+] =2899 m/z.*

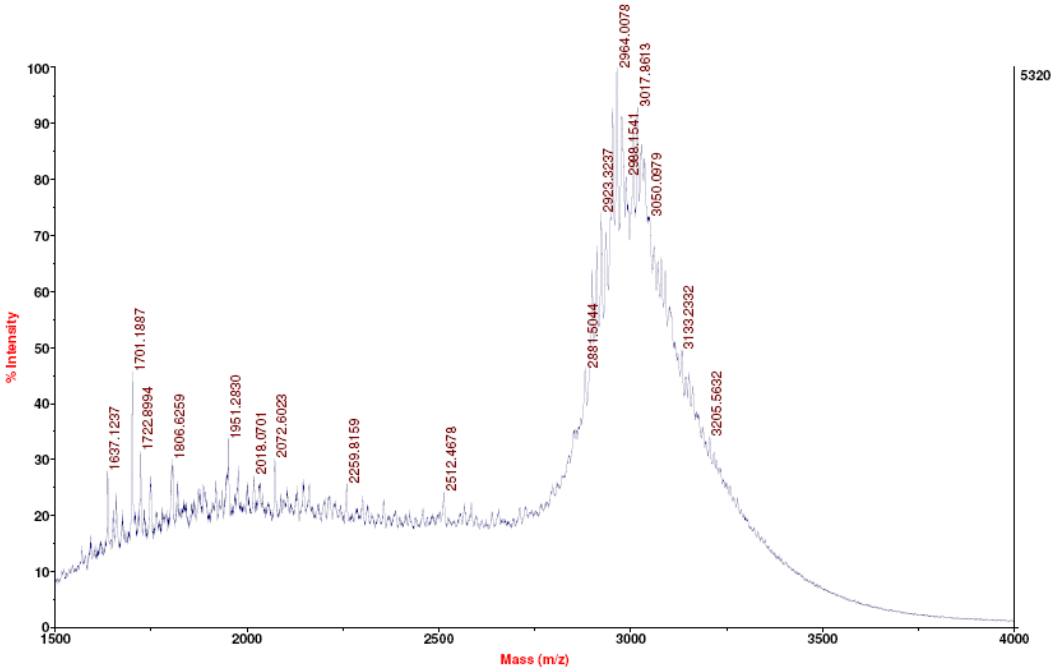
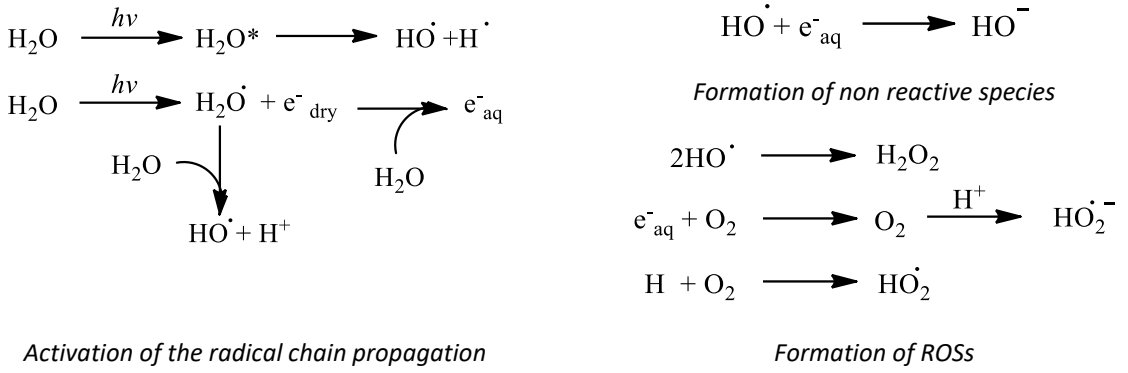
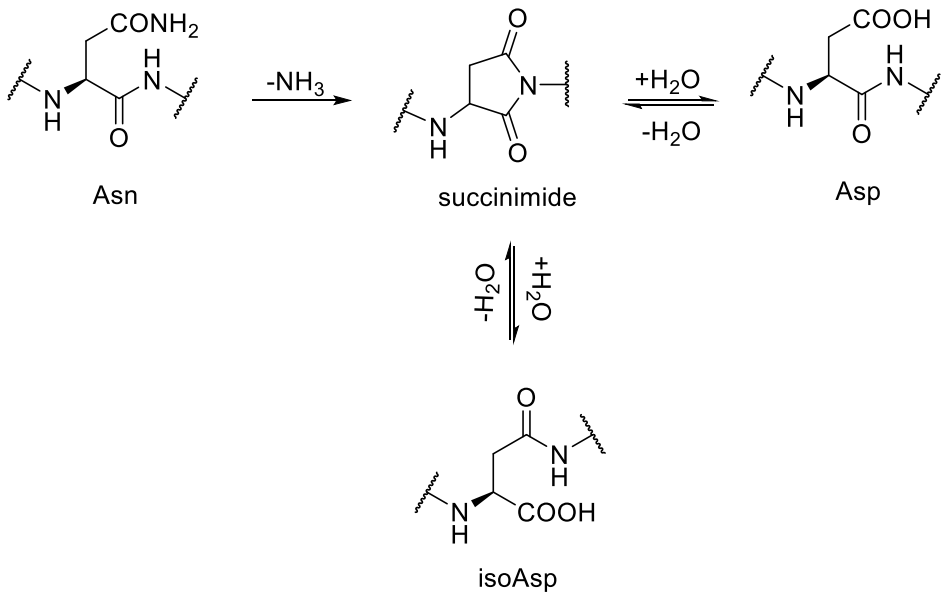


Figure S8. *MALDI-TOF MS analysis of orexin B in presence of pyruvic aldehyde in phosphate buffer. Matrix: 2,5-dihydroxybenzoic acid in 9: 1 = MeCN: H_2O + 0.1% TFA. Orexin B [M+H^+] =2899 m/z.*

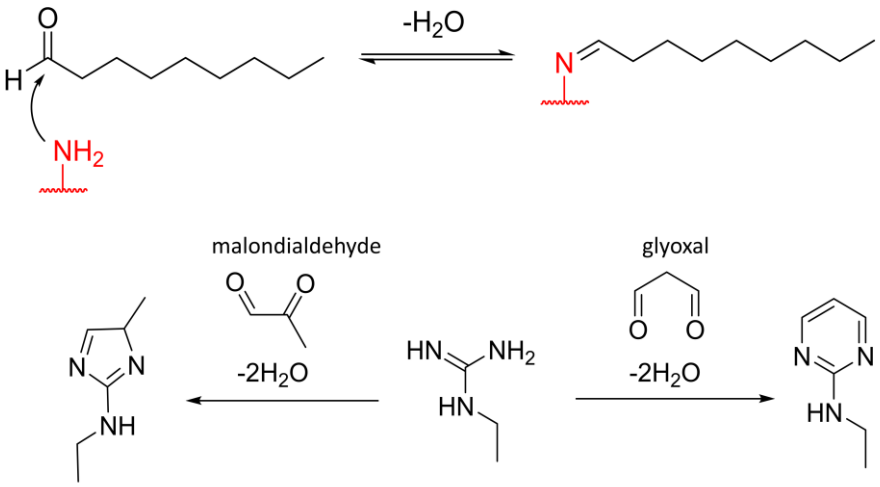


Scheme S1. *Mechanism of ROS generation in aqueous medium due to UV irradiation.*

Scheme S2. *Mechanism of NO· radical formation due to DEA NONO-Ate.*



Scheme S3. *Mechanism of Asn residue deamination with the formation of a succinimide intermediate.*



Scheme S4. *Mechanism of stable adducts formation among glyoxal or pyruvic aldehyde with amino acid residues.*