Supporting Information

Natural Tripeptide-Based Inhibitor of Multifaceted Amyloid β Toxicity

K. Rajasekhar, Chilakapati Madhu and T. Govindaraju*

Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O., Bengaluru 560064, Karnataka, India

Email: tgraju@jncasr.ac.in

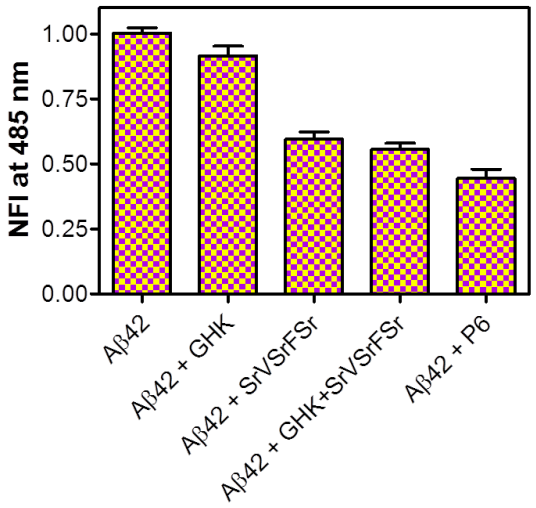


Figure S1. Inhibition of Aβ42 aggregates studied by thioflavin (ThT) assay. Aβ42 (10 μM) was incubated alone and independently with peptides GHK, SrVSrFSr, GHK and SrVSrFSr, and **P6** in 1:2 stoichiometry and their influence on aggregation is quantified by measuring ThT fluorescence intensity, which is represented as normalized fluorescence intensity at 485 nm for a given time point (48 h).

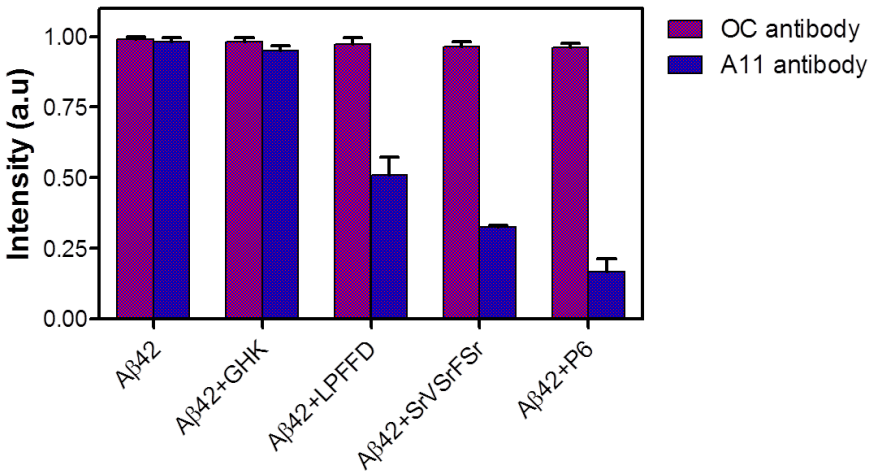


Figure S2. Plot of normalized chemiluminescence intensity's obtained from dot blot analysis of Aβ42 (10 μM) aggregates incubated with 50 μM peptides (GHK, LPFFD, SrVSrFSr and **P6**) at 37 °C. OC or A11 (1:3000) is used as primary antibody followed by HRP conjugated anti-mouse antibody (1:10000) as secondary antibody. Each experiment was repeated three times (n = 3). Error bars represent the standard deviation (SD) of the measurement.

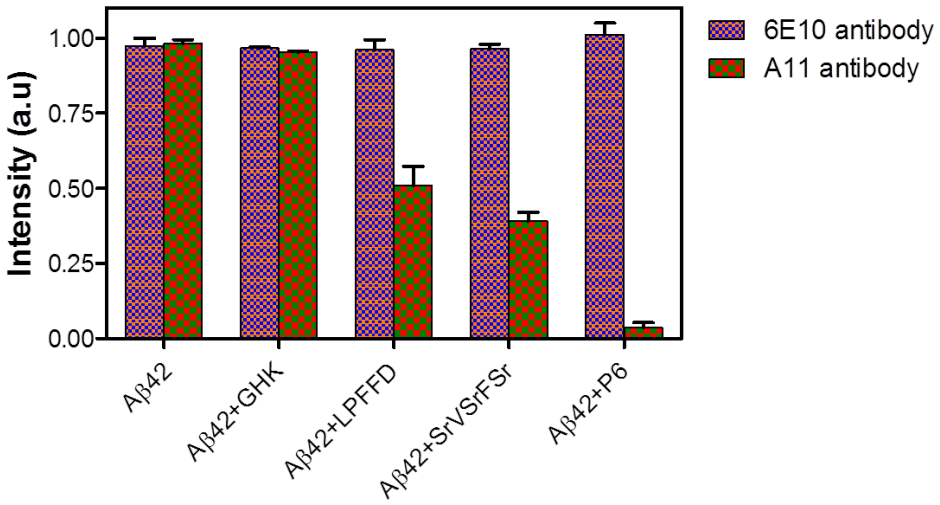


Figure S3. Plot of normalized chemiluminescence intensities obtained from dot blot analysis of Aβ42 (10 μM) incubated with 50 μM peptide (GHK or LPFFD or SrVSrFSr or **P6**) at 37 °C. 6E10 or A11 (1:3000) is used as primary antibody followed by HRP conjugated anti-mouse antibody (1:10000) as secondary antibody. Each experiment was repeated three times (n = 3) and error bars represent the standard deviation (SD) of the measurements.

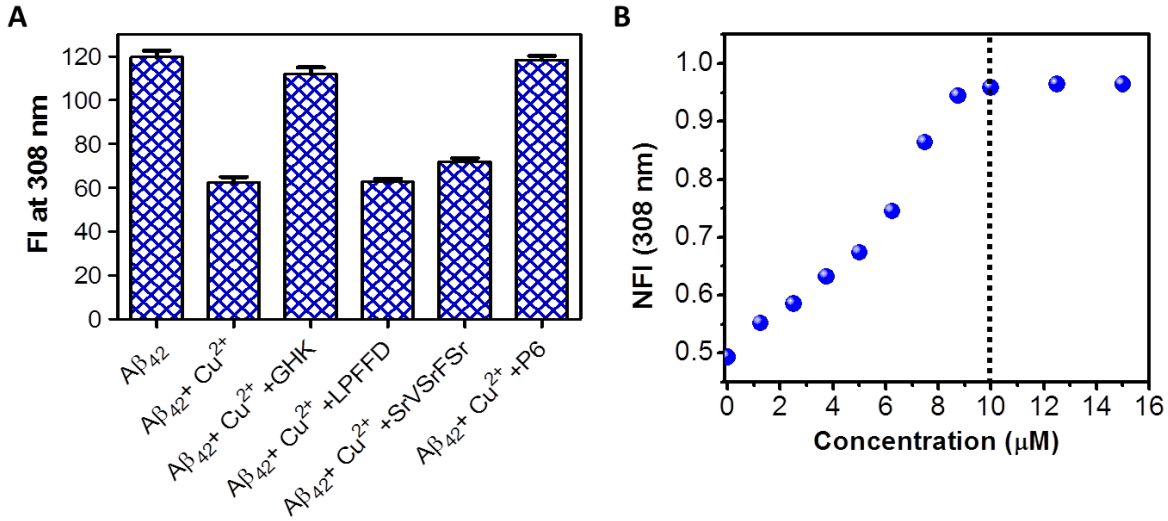


Figure S4. Extraction of metal ion from Aβ42-Cu^{2+}complex. (A) Tyr fluorescence measured for Aβ42-Cu^{2+} (10 μM) in presence of 20 μM peptide (GHK or LPFFD or **P6**). The fluorescence intensity was measured at 308 nm with excitation at 285 nm. (B) Fluorescence enhancement at 308 nm upon addition of **P6** to Aβ42-Cu^{2+} complex in a concentration-dependent manner. **P6** and Cu^{2+} exhibit 1:1 binding stoichiometry.

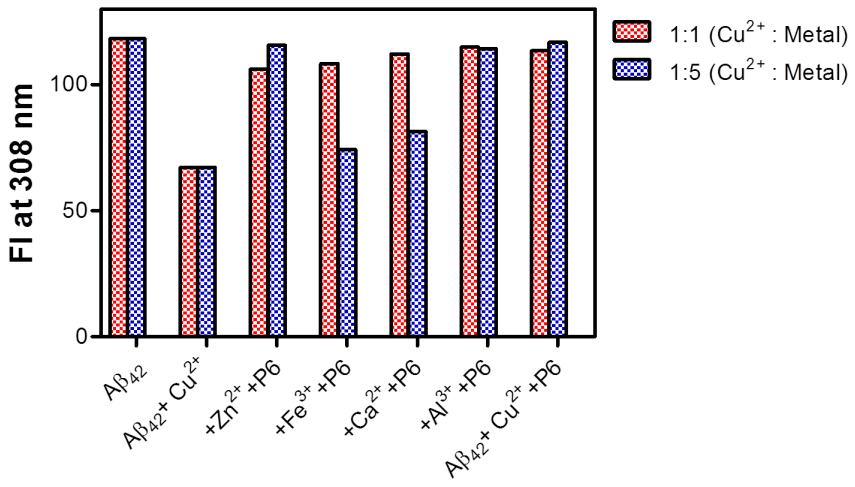


Figure S5. Plot of fluorescence enhancement at 308 nm upon treating Aβ42-Cu^{2+} complex with **P6** in presence of different metal ions (Zn^{2+}, Fe^{3+}, Ca^{2+} and Al^{3+}).

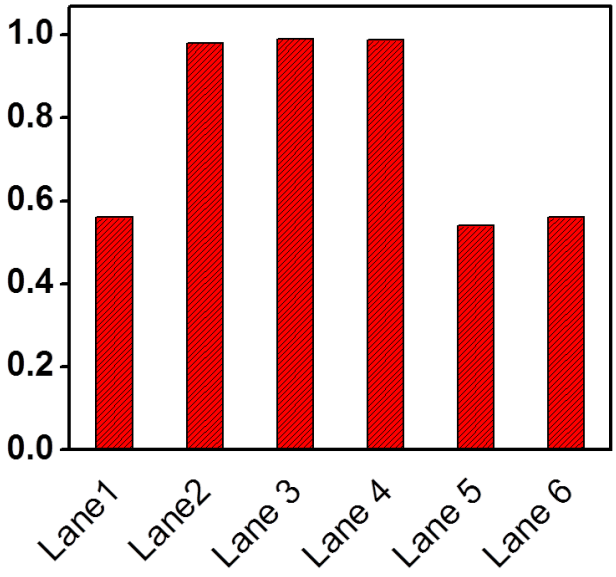


Figure S6. Plot for band intensities of c-DNA (pBR322) band in agarose gel (Figure 6). Each lane (1, 2, 3, 4, 5 and 6) has plasmid DNA (pBR322), Aβ42 (1.1 μM), Cu^{2+} (1 μM), ascorbate (150 μM) treated with peptides GHK (lane 5, 2 μM), LPFFD (lane 3, 2 μM), SrVSrFSr (lane 4, 2 μM) and **P6** (lane 6, 2 μM) incubated at 37 ºC and ran on agarose gel.

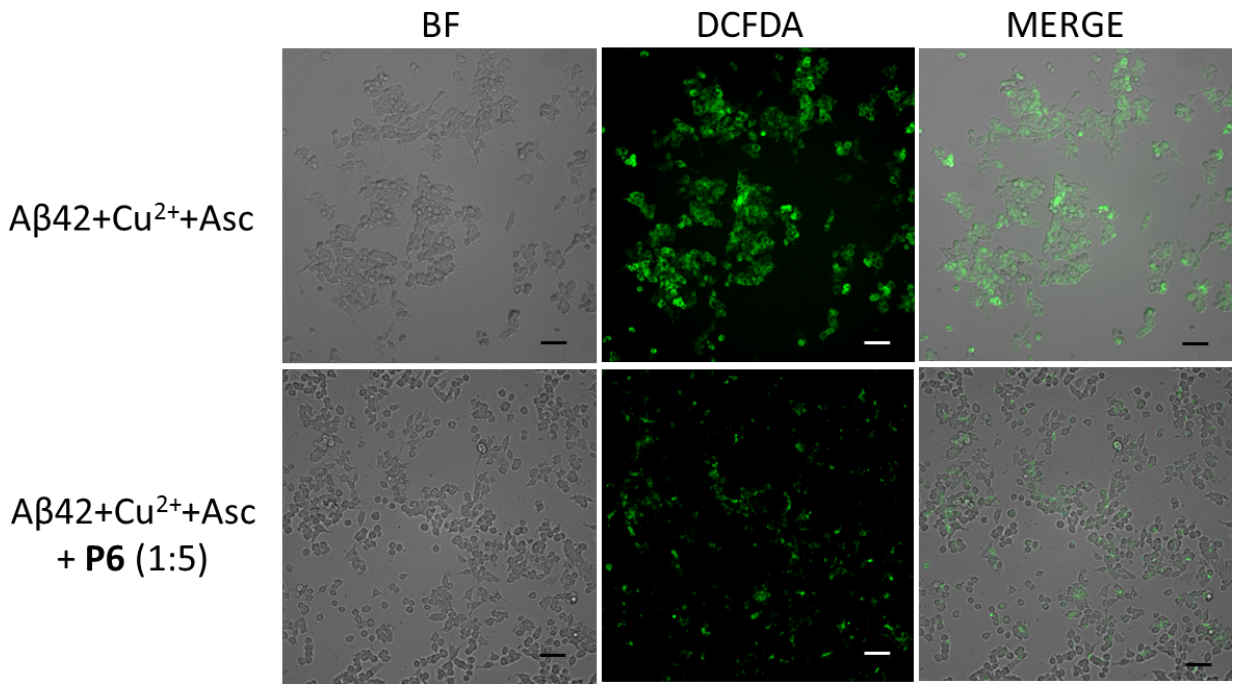


Figure S7. Fluorescent microscope images of PC12 cells used in DCFDA assay (scale bar 50μM)

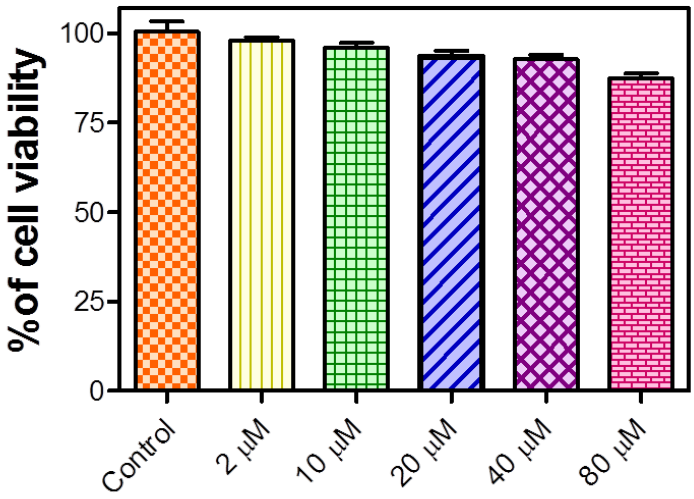


Figure S8. MTT assay (cell viability assay) for **P6** with varying concentrations in PC12 cells.

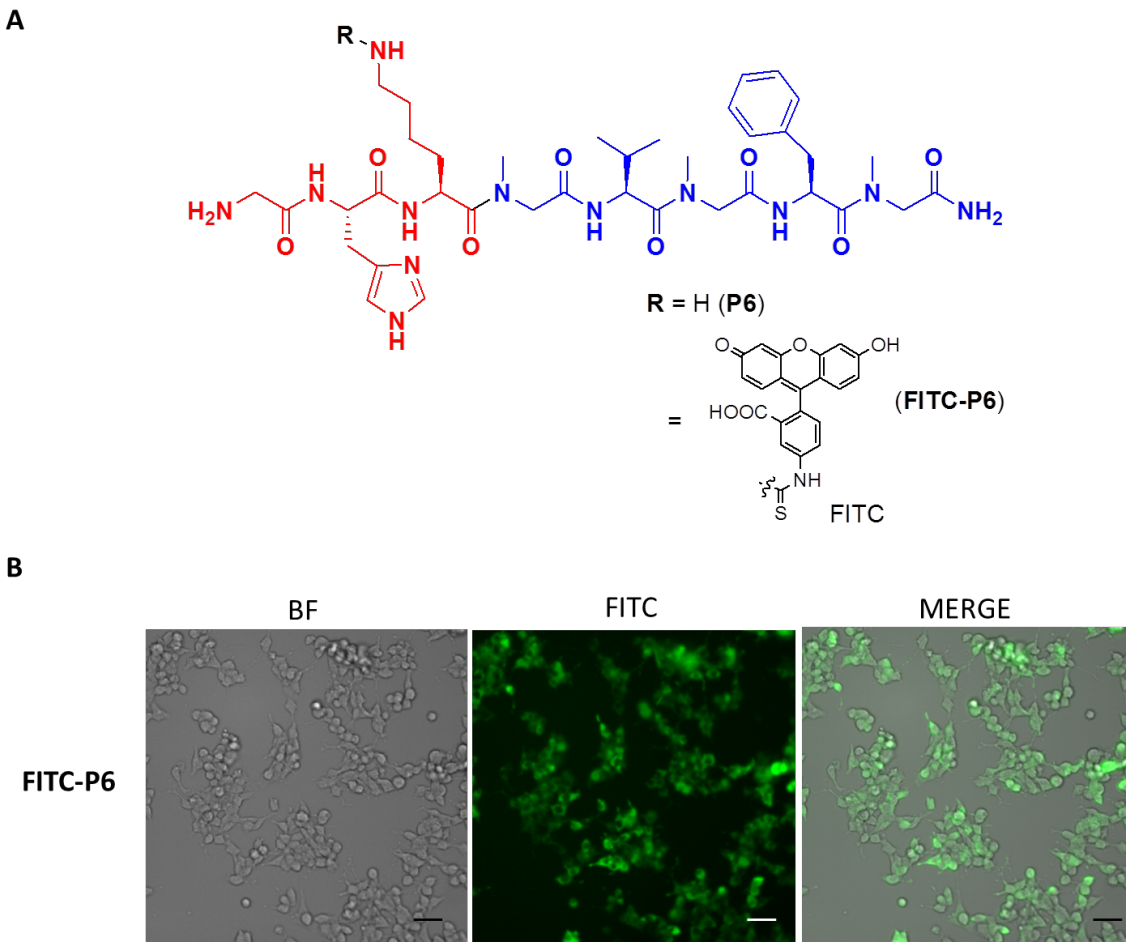


Figure S9. Cell permeability of **P6**. (A) Structure of **FITC-P6** (B) Fluorescent microscope images of PC12 cell incubated with FITC-P6 (20 µM) for 2h at 37 °C. Scale bar 50 µM

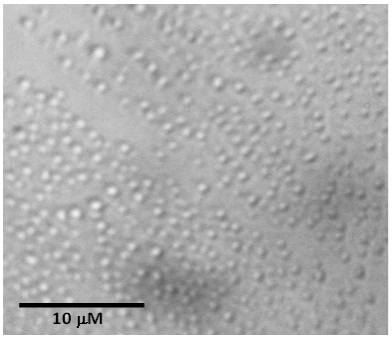


Figure S10. Bright field microscopic image of synthetic vesicles in 10 mM PBS buffer, used for liposome leakage assay.

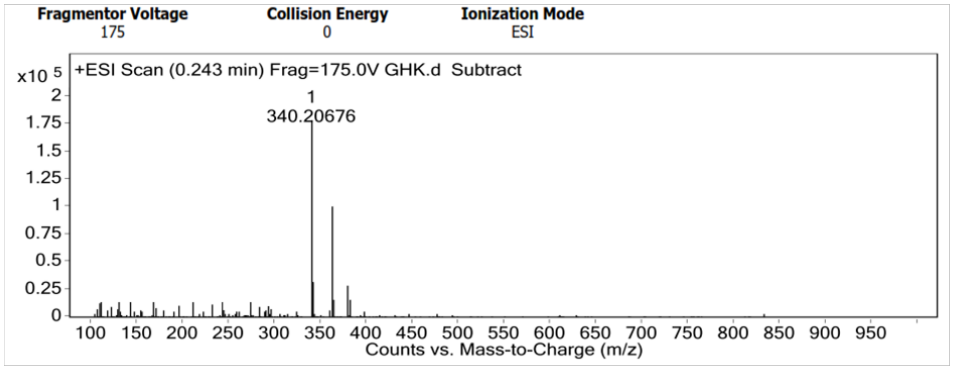
HRMS and HPLC traces

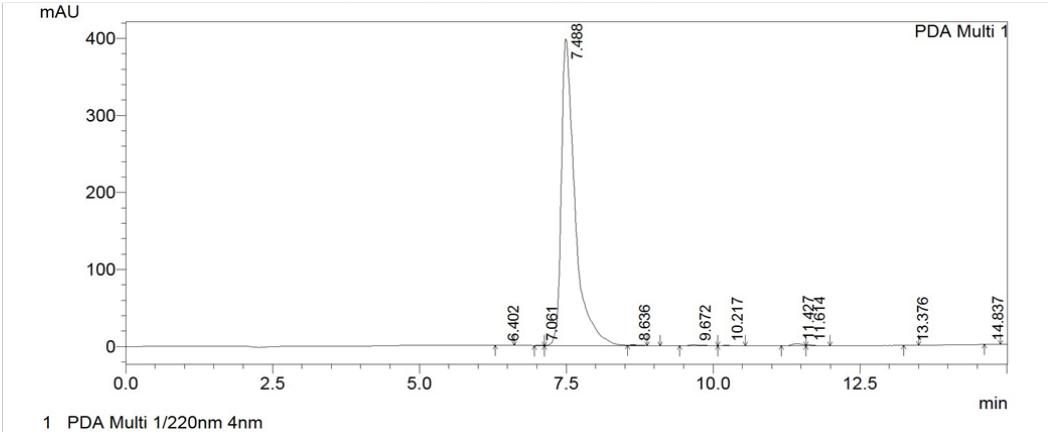
Name	Sequence	Exact (calculated) mass	Observed mass
GHK	Gly-His-Lys	340.1848	340.1859 **[M+H]$^+$**
LPFFD	Lys-Pro-Phe-Phe-Asp	637.3344	637.3348 **[M+H]$^+$**
SrVSrFSr	**Sr**-Val-**Sr**-Phe-**Sr**	499.2645	499.2648 **[M+Na]$^+$**
P6	Gly-His-Lys-**Sr**-Val-**Sr**-Phe-**Sr**	821.4398	821.4391 **[M+Na]$^+$**
FITC-P6	**FITC-** Gly-His-Lys-**Sr**-Val-**Sr**-Phe-**Sr**	1188.4726	1188.4896 **[M+H]$^+$**

Sr = Sarcosine (N-methylglycine).

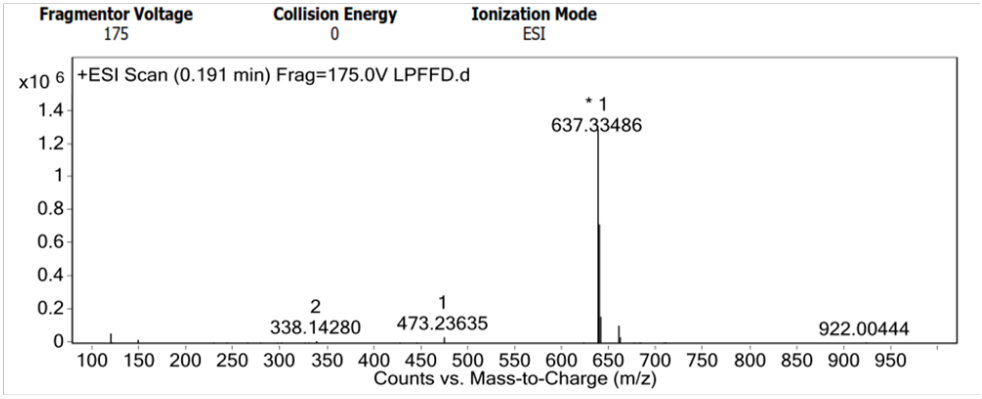
Peptide	Gradient	Flow rate (ml/min)	Retention time (R_t)
GHK	0-40% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 15 min	8	7.48 min
LPFFD	0-100% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 15 min	8	9.72 min
SrVSrFSr	20-100% MeCN (0.1% TFA)in H_2O (0.1% TFA) over 15 min	8	9.61 min
P6	0-100% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 15 min	8	8.93 min
FITC-P6	20-100% MeCN (0.1% TFA)in H_2O (0.1% TFA) over 20 min	8	12.5 min

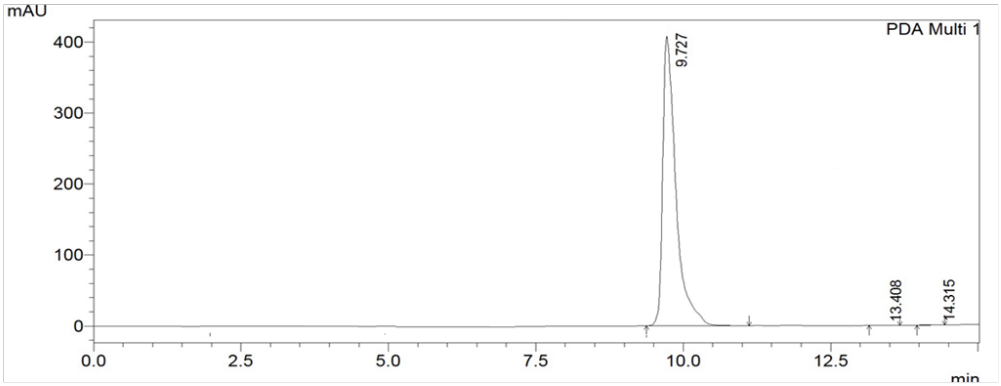
GHK


Fragmentor Voltage
175
Collision Energy
0
Ionization Mode
ESI
+ESI Scan (0.243 min) Frag=175.0V GHK.d Subtract
x10 5
1
340.20676
Counts vs. Mass-to-Charge (m/z)

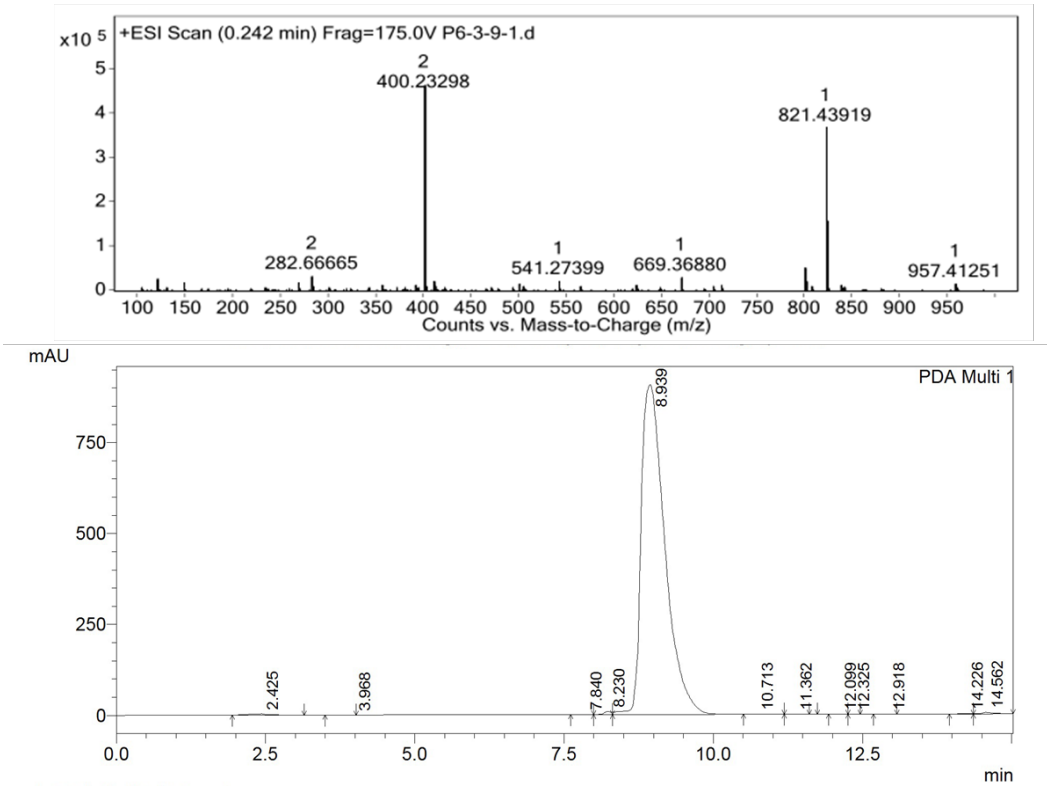

mAU
PDA Multi 1
7.488
6.402
7.061
8.636
9.672
10.217
11.427
11.614
13.376
14.837
min
1 PDA Multi 1/220nm 4nm

LPFFD

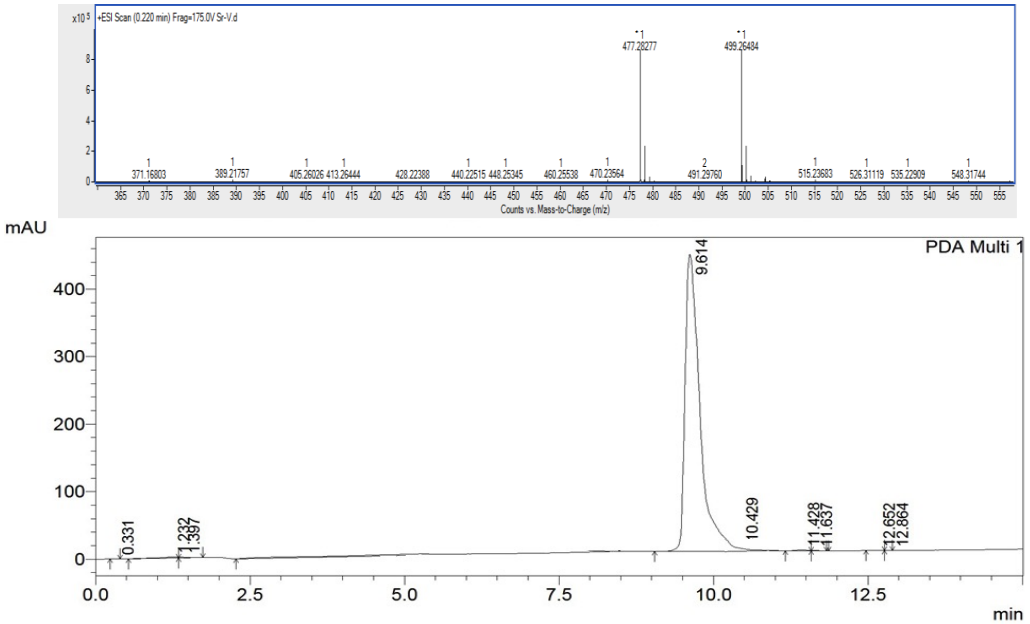

Fragmentor Voltage
175
Collision Energy
0
Ionization Mode
ESI
+ESI Scan (0.191 min) Frag=175.0V LPFFD.d
x10 6
* 1
637.33486
2
338.14280
1
473.23635
922.00444
Counts vs. Mass-to-Charge (m/z)


mAU
PDA Multi 1
9.727
13.408
14.315
min

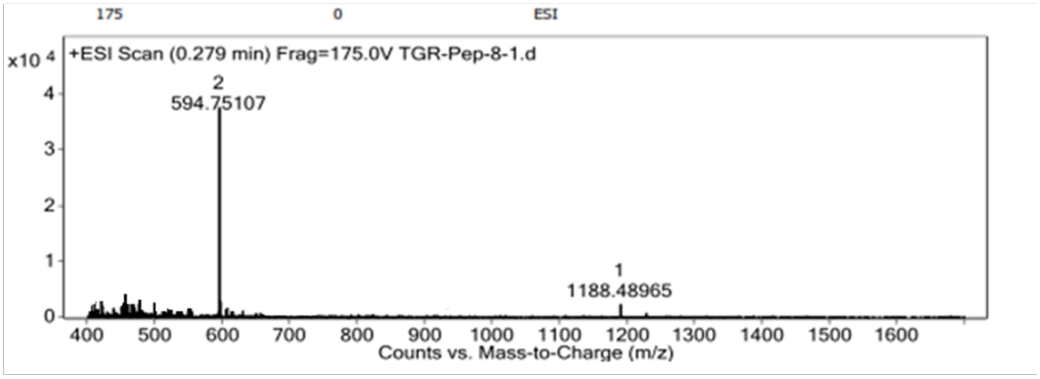
P6

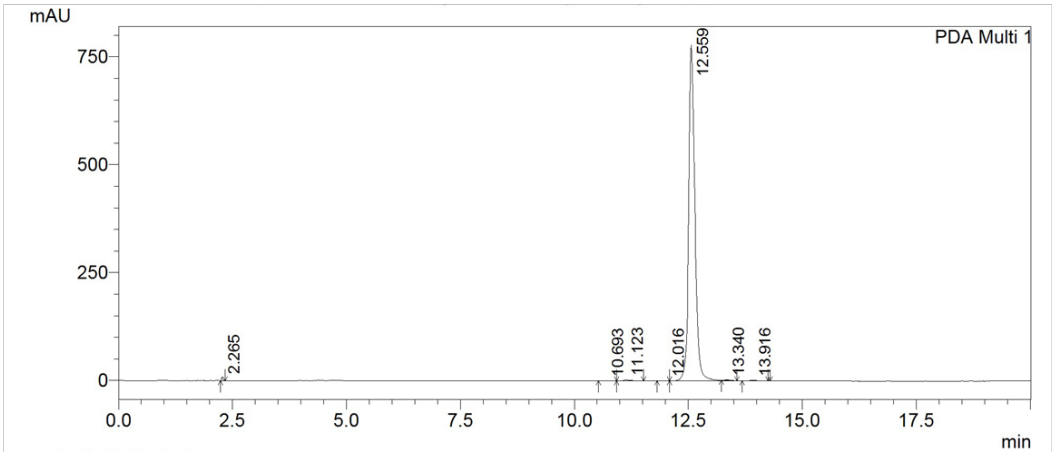

+ESI Scan (0.242 min) Frag=175.0V P6-3-9-1.d
x10 5
2
400.23298
1
821.43919
2
282.66665
1
541.27399
1
669.36880
1
957.41251
Counts vs. Mass-to-Charge (m/z)
mAU
PDA Multi 1
8.939
2.425
3.968
7.840
8.230
10.713
11.362
12.099
12.325
12.918
14.226
14.562
min

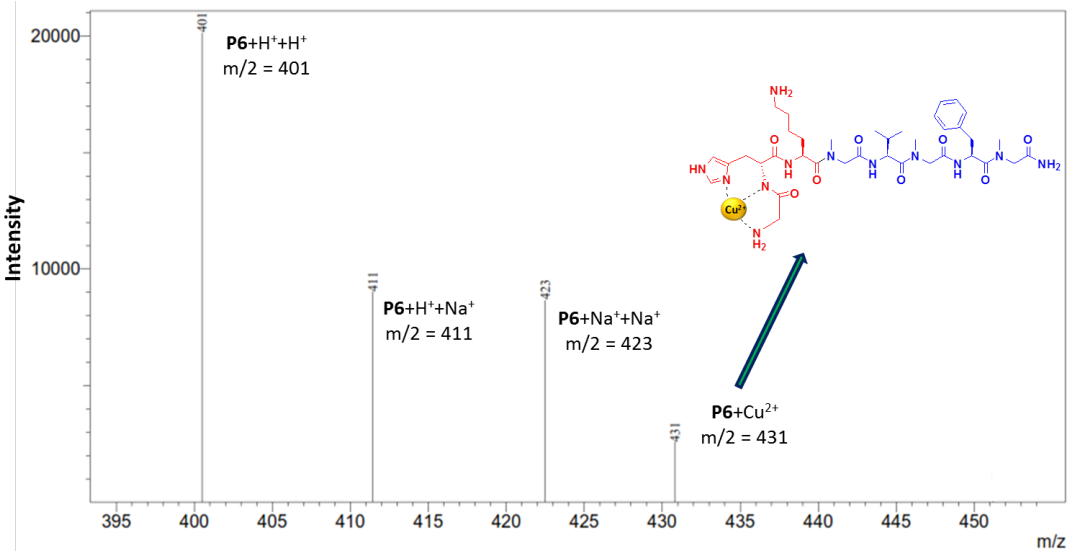
SrVSrFSr


+ESI Scan (0.220 min) Frag=175.0V Sr-V.d
477.28277
499.26484
Counts vs. Mass-to-Charge (m/z)
mAU
PDA Multi 1
9.614
0.331
1.232
1.397
10.429
11.428
11.637
12.652
12.864
min

FITC-P6







LCMS mass spectrum of complex **P6**+Cu^{2+} in PBS buffer (1 mM)