

## Supporting Information

### **Structure–property relationships of photoresponsive inhibitors of the kinesin motor**

Ammathnadu S. Amrutha, K. R. Sunil Kumar, Kazuya Matsuo, Nobuyuki Tamaoki\*

Research Institute for Electronic science, Hokkaido University, N20, W10, Kita-Ku, Sapporo-001-0020, Hokkaido, Japan

\*Corresponding author:

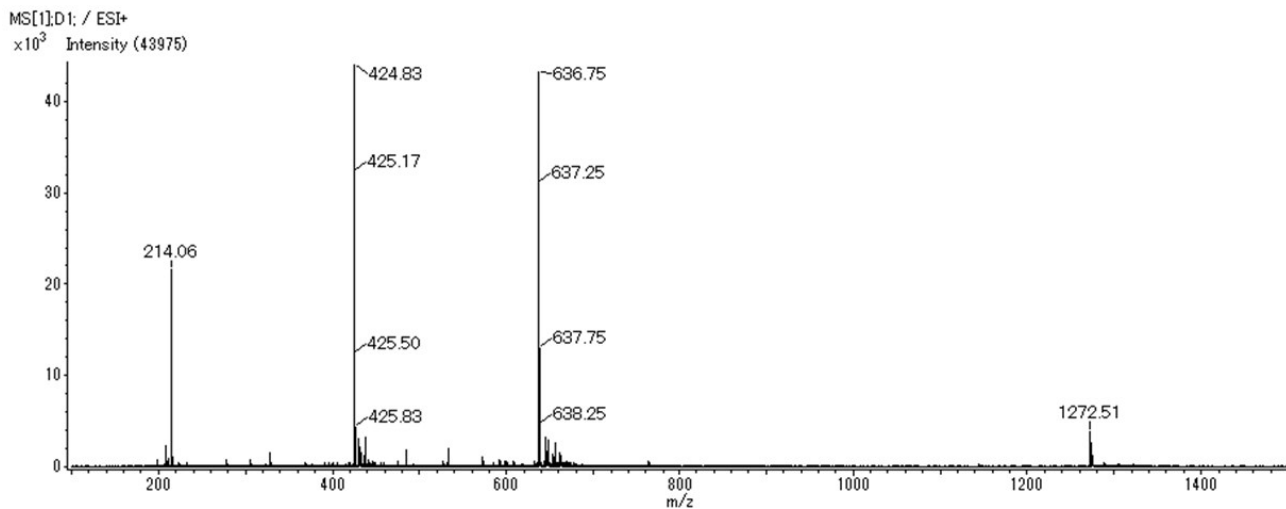
E-mail: [tamaoki@es.hokudai.ac.jp](mailto:tamaoki@es.hokudai.ac.jp)

Phone: +81-11-706-9356

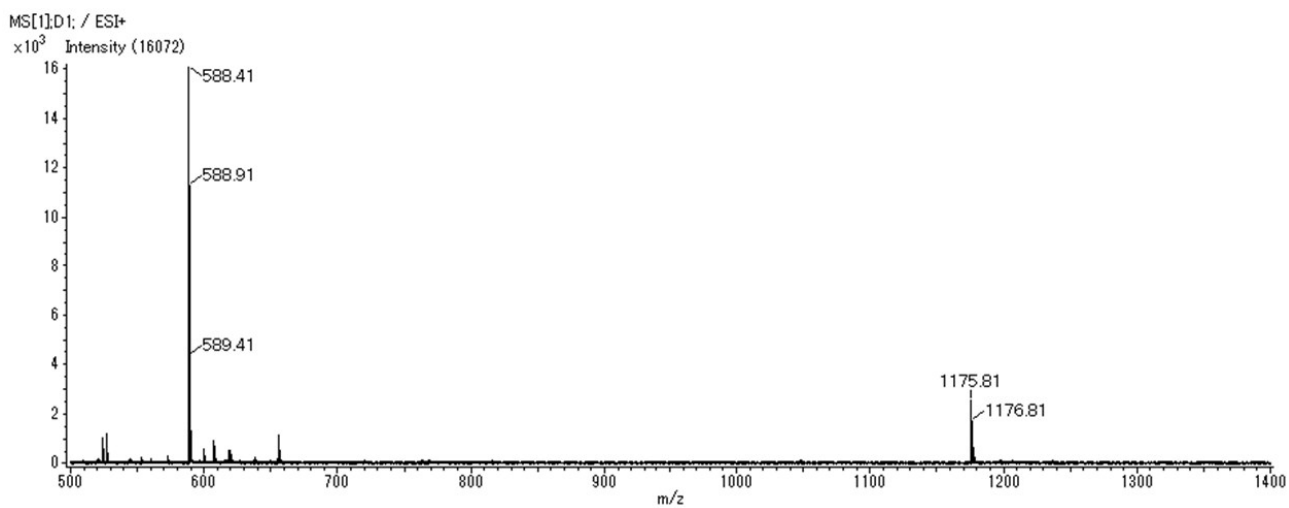
## Table of contents

1. Mass spectra of azo-peptides	-1-
2. Purity of the azo-peptides by HPLC analysis	-9-
3. Photoisomerization of azo-peptides	-14-
4. Thermal stability of the <i>cis</i> isomers	-15-
5. HPLC analysis on the conversion ratio from <i>trans</i> to <i>cis</i> and <i>cis</i> to <i>trans</i> forms	-16-
6. Structural comparison between the <i>Drosophila melanogaster</i> kinesin-1 and human kinesin-1 motor domains	-19-

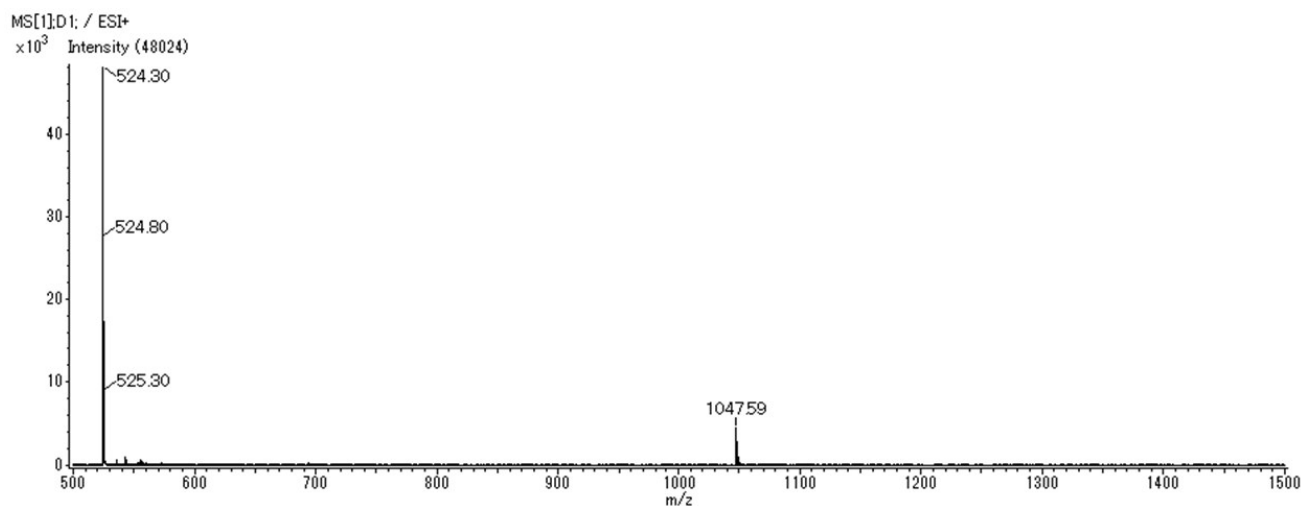
## 1. Mass spectra of azo-peptides



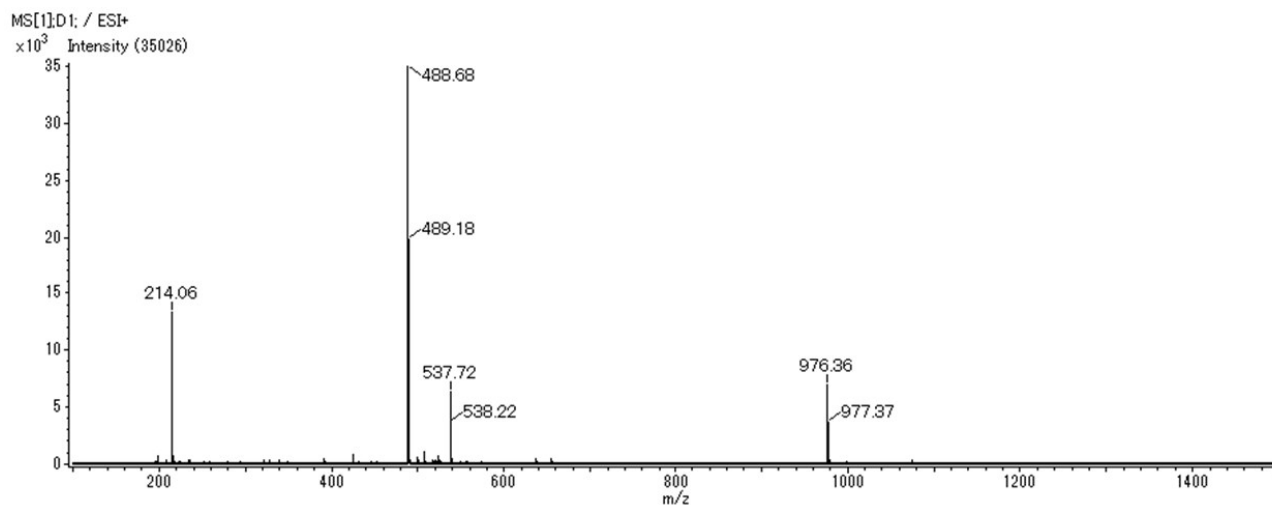
**Figure S1.** ESI<sup>+</sup> mass spectrum of azo-peptide **2**:  $m/z = 1272.51$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 1272.65)



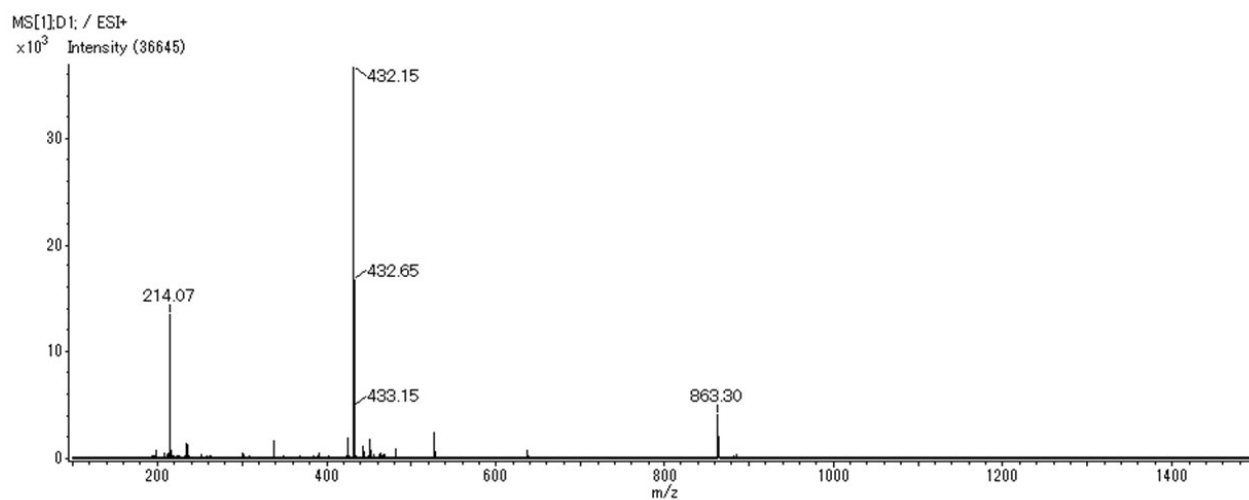
**Figure S2.** ESI<sup>+</sup> mass spectrum of azo-peptide **3**:  $m/z = 1175.81$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 1175.6)



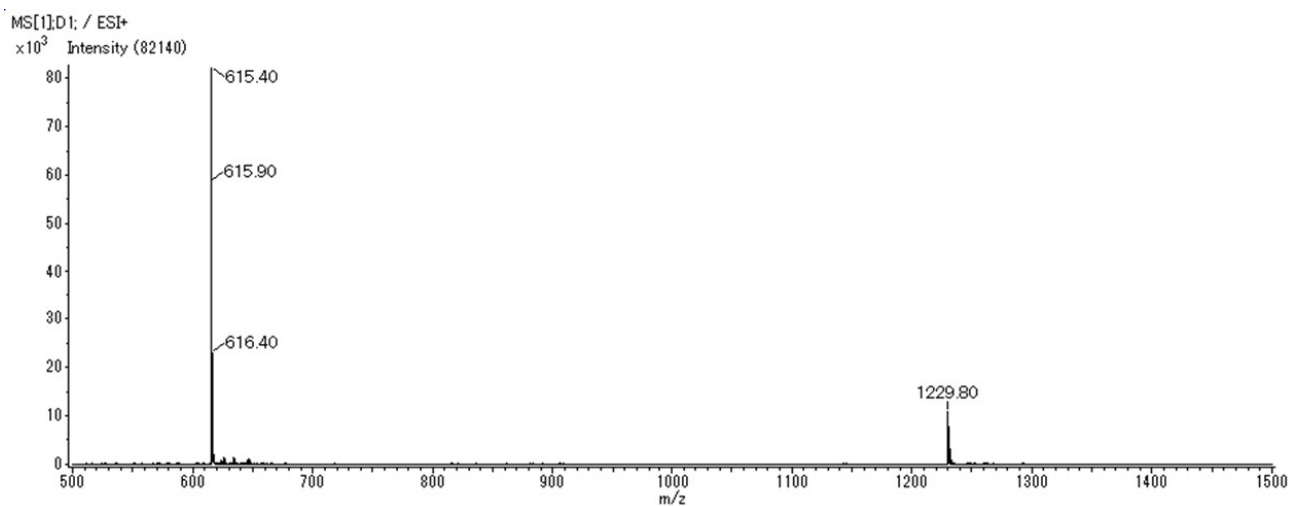
**Figure S3.** ESI<sup>+</sup> mass spectrum of azo-peptide **4**: m/z = 1047.59 [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 1047.50)



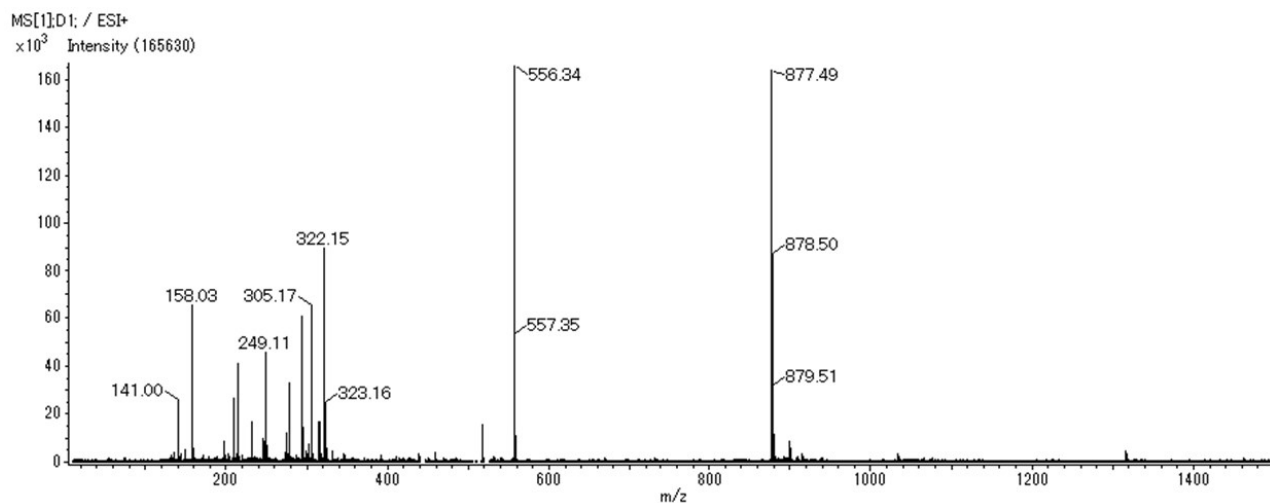
**Figure S4.** ESI<sup>+</sup> mass spectrum of azo-peptide **5**: m/z = 976.36 [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 976.47)



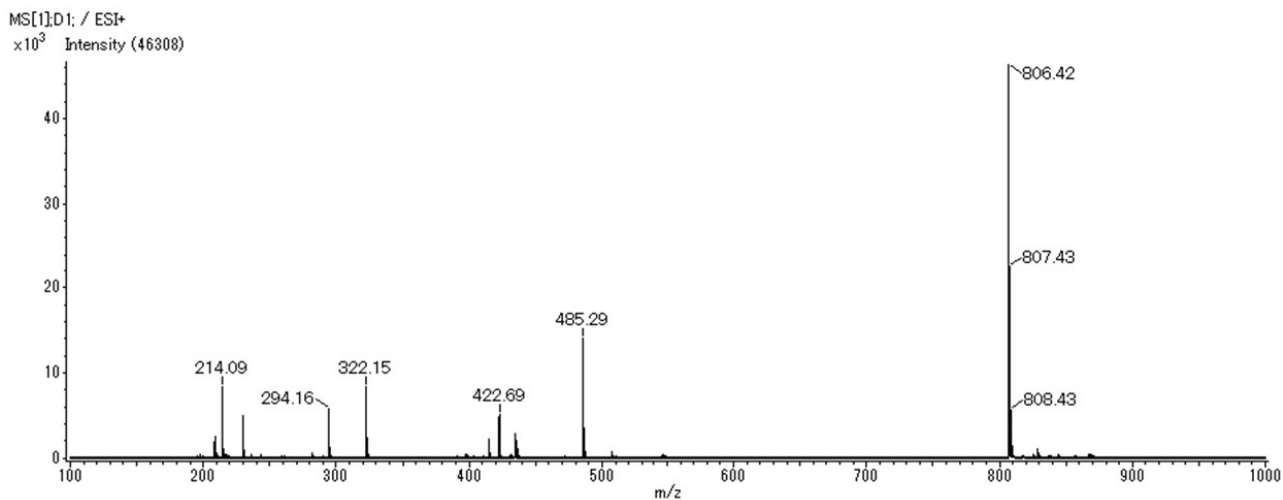
**Figure S5.** ESI<sup>+</sup> mass spectrum of azo-peptide **6**: m/z = 863.30 [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 863.38)



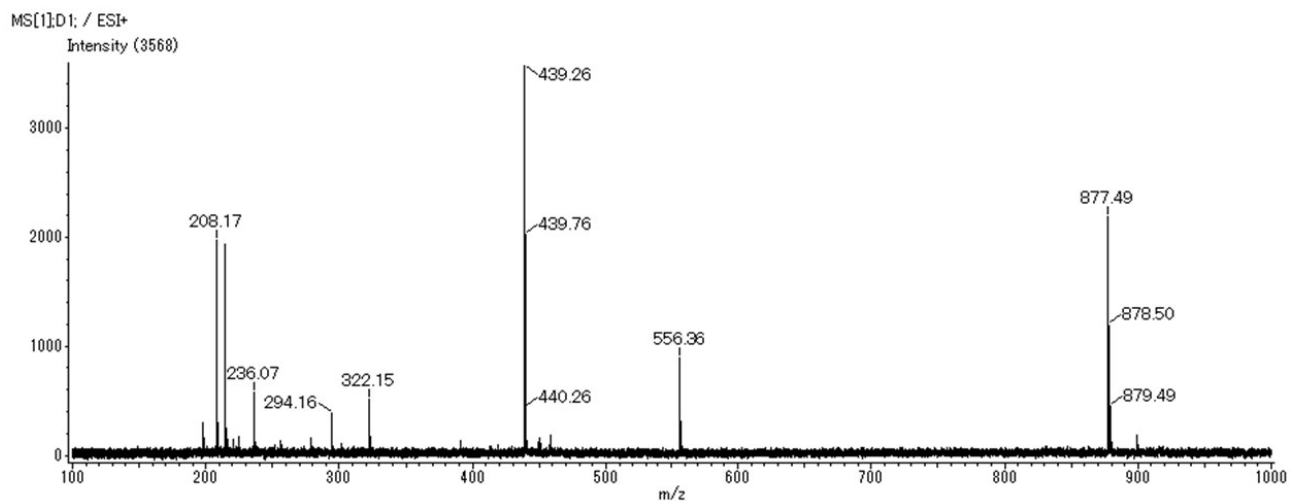
**Figure S6.** ESI<sup>+</sup> mass spectrum of azo-peptide **7**: m/z = 1229.80 [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 1229.64)



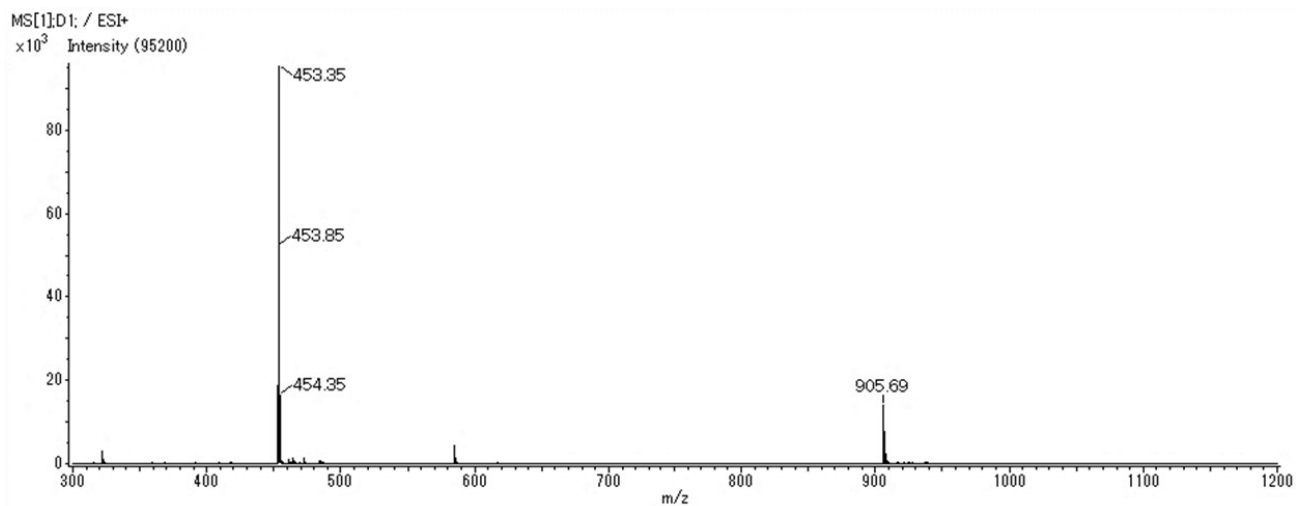
**Figure S7.** ESI<sup>+</sup> mass spectrum of azo-peptide **8**:  $m/z = 877.49$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 877.49),  $m/z = 556.34$  [Pro-Lys-Ala-Ile-Gln+H]<sup>+</sup> and  $322.15$  [Azo-Ile]<sup>+</sup> (calculated mass for the fragments of the parent azo-peptide **8** cleaved at the amide linkage between the Ile and Pro : 556.34 and 322.15 respectively)



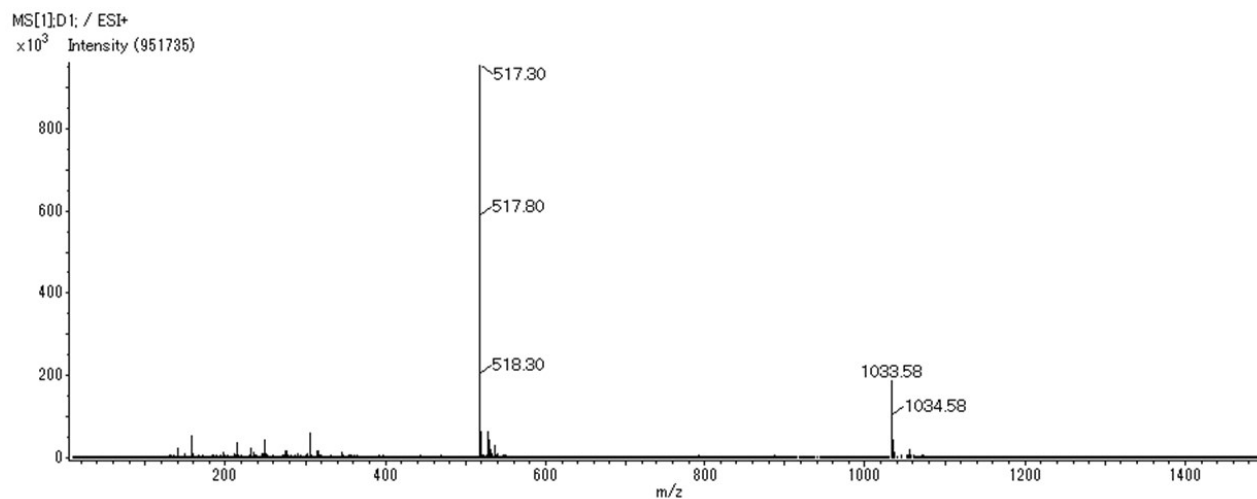
**Figure S8.** ESI<sup>+</sup> mass spectrum of azo-peptide **9**:  $m/z = 806.42$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 806.45),  $m/z = 485.29$  [Pro-Lys-Ala-Ile-Gly+H]<sup>+</sup> and  $322.15$  [Azo-Ile]<sup>+</sup> (calculated mass for the fragments of the parent azo-peptide **9** cleaved at the amide linkage between the Ile and Pro : 485.30 and 322.15 respectively),  $m/z = 422.69$  [M+H+K]<sup>2+</sup> (calculated mass for the most abundant isotope: 422.77).



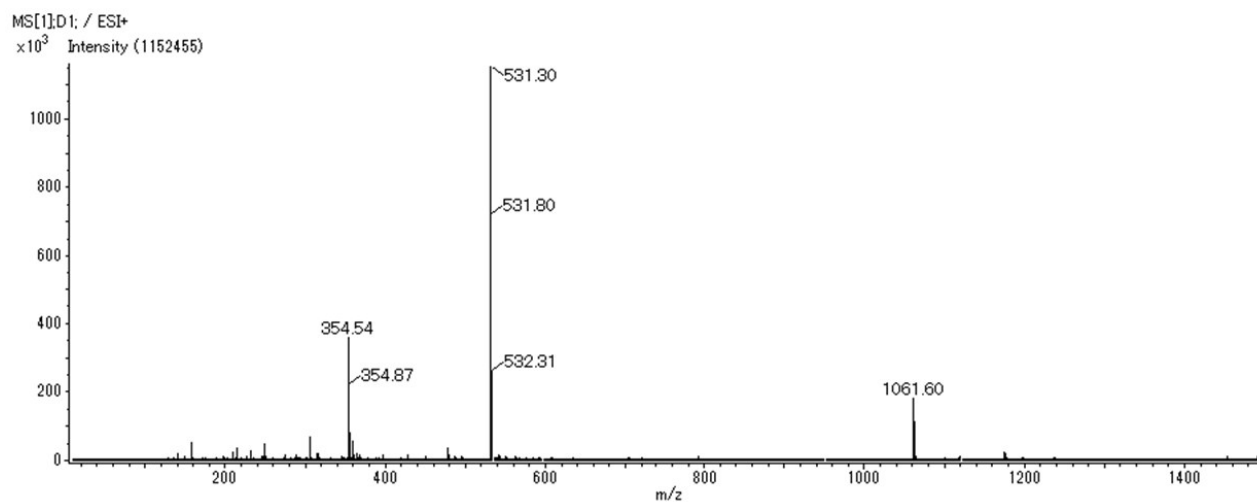
**Figure S9.** ESI<sup>+</sup> mass spectrum of azo-peptide **10**:  $m/z = 877.49$   $[M+H]^+$  (calculated mass for the most abundant isotope: 877.52)



**Figure S10.** ESI<sup>+</sup> mass spectrum of azo-peptide **11**:  $m/z = 905.69$   $[M+H]^+$  (calculated mass for the most abundant isotope: 905.53)

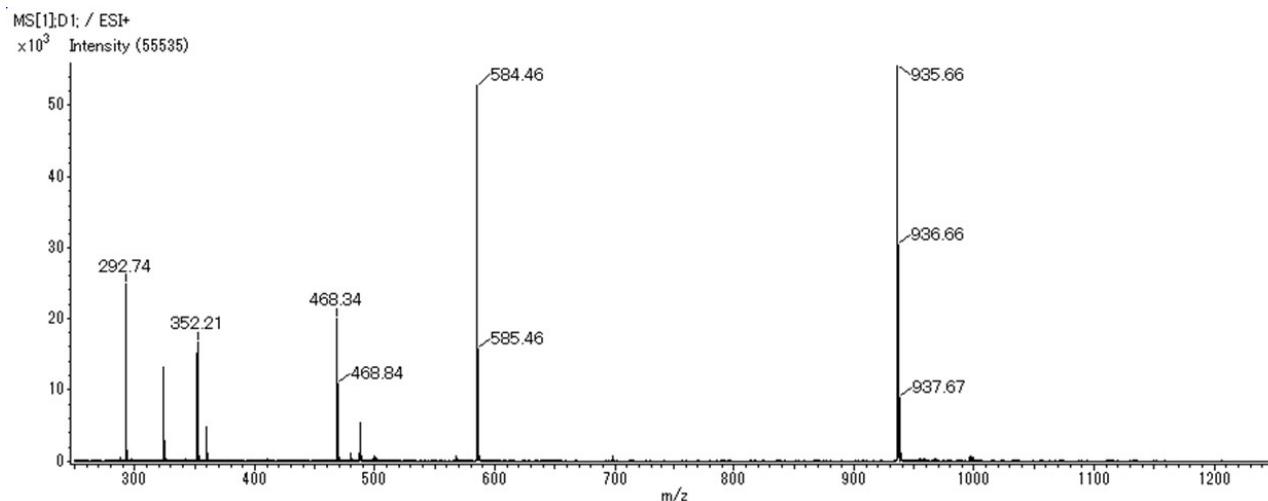


**Figure S11.** ESI<sup>+</sup> mass spectrum of azo-peptide **12**:  $m/z = 1033.58$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 1033.59)

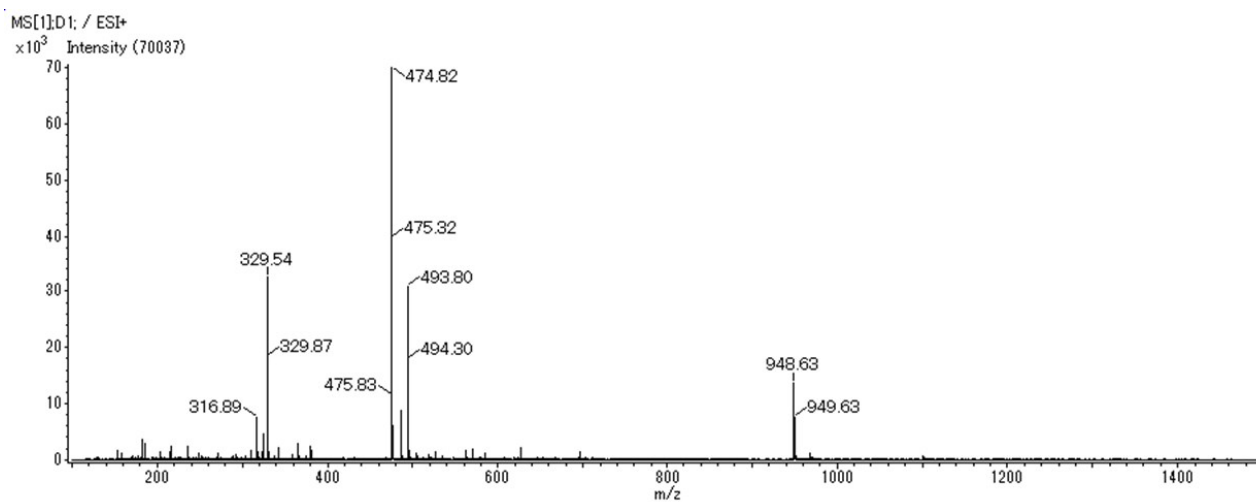


**Figure S12.** ESI<sup>+</sup> mass spectrum of azo-peptide **13**:  $m/z = 1061.60$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 1061.63)

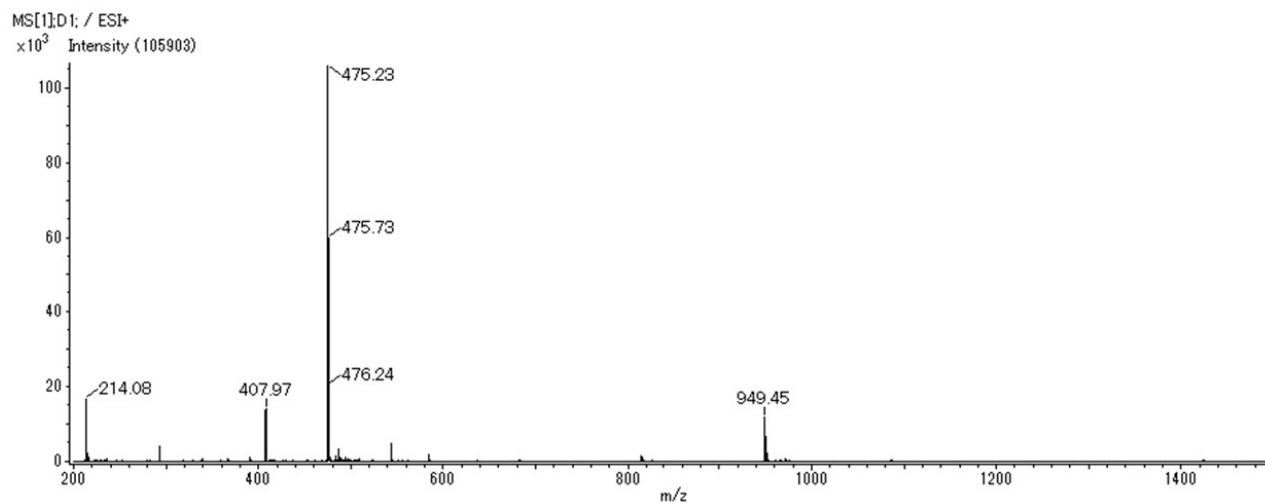




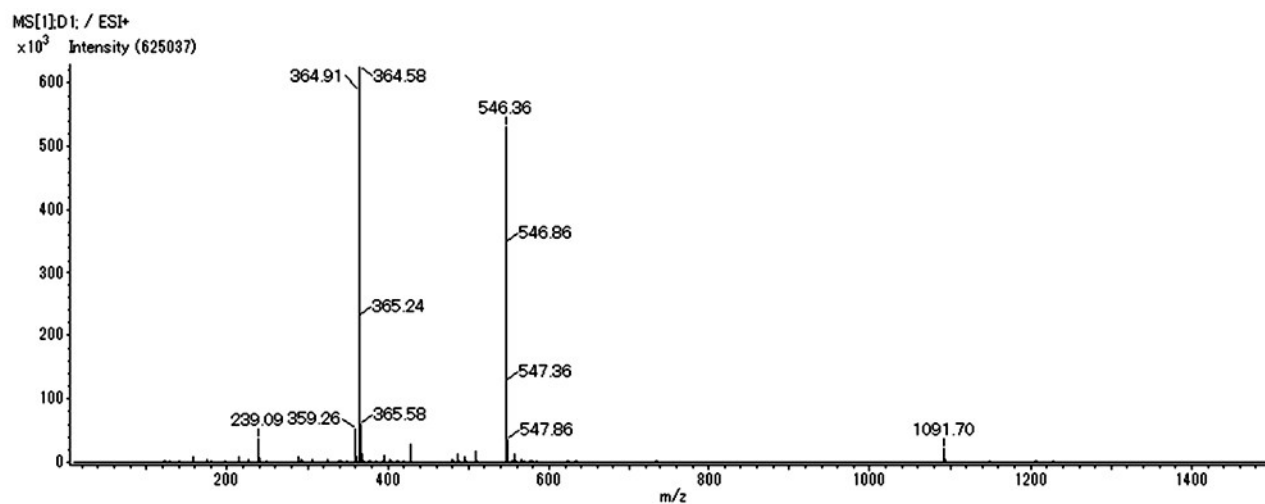
**Figure S13.** ESI<sup>+</sup> mass spectrum of azo-peptide **14**:  $m/z = 935.66$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 935.54),  $m/z = 584.46$  [Pro-Lys-Ala-Ile-Arg+H]<sup>+</sup> and 352.21 [MeO-Azo-Ile]<sup>+</sup> (calculated mass for the fragments of the parent azo-peptide **14** cleaved at the amide linkage between the Ile and Pro : 584.38 and 352.16 respectively),  $m/z = 468.34$  [M+2H]<sup>2+</sup> (calculated mass for the most abundant isotope: 468.27 ).



**Figure S14.** ESI<sup>+</sup> mass spectrum of azo-peptide **15**:  $m/z = 948.63$  [M+H]<sup>+</sup> (calculated mass for the most abundant isotope: 948.57)



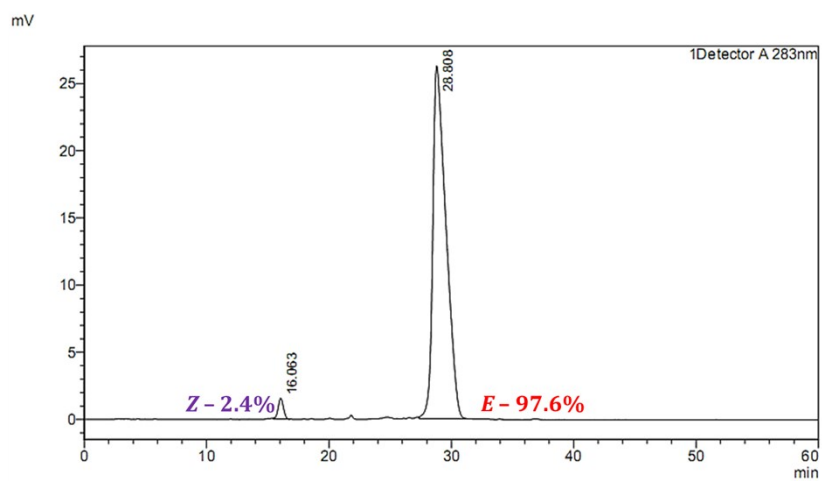
**Figure S15.** ESI<sup>+</sup> mass spectrum of azo-peptide **16**:  $m/z = 949.45$   $[M+H]^+$  (calculated mass for the most abundant isotope: 949.52)



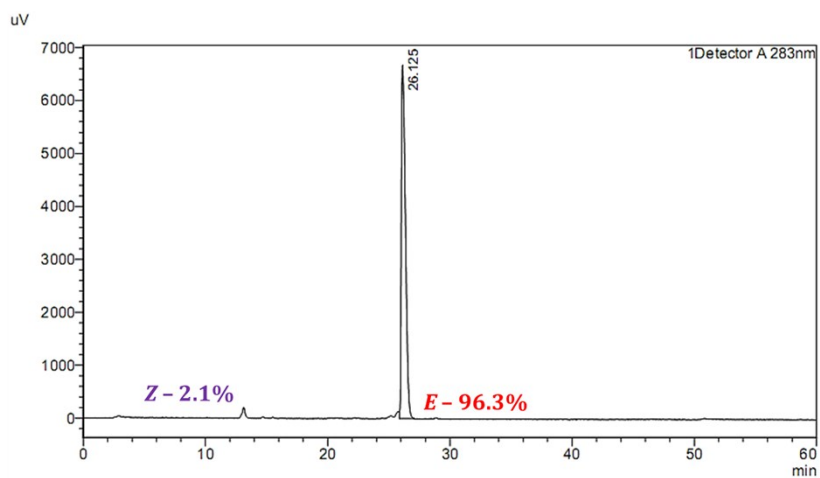
**Figure S16.** ESI<sup>+</sup> mass spectrum of azo-peptide **17**:  $m/z = 1091.70$   $[M+H]^+$  (calculated mass for the most abundant isotope: 1091.64)

## 2. Purity of the azo-peptides by HPLC analysis

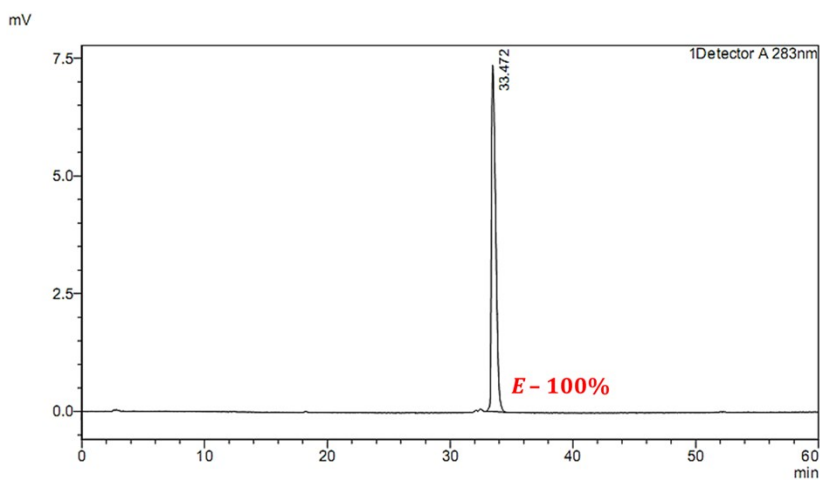
(a) azo-peptide 2



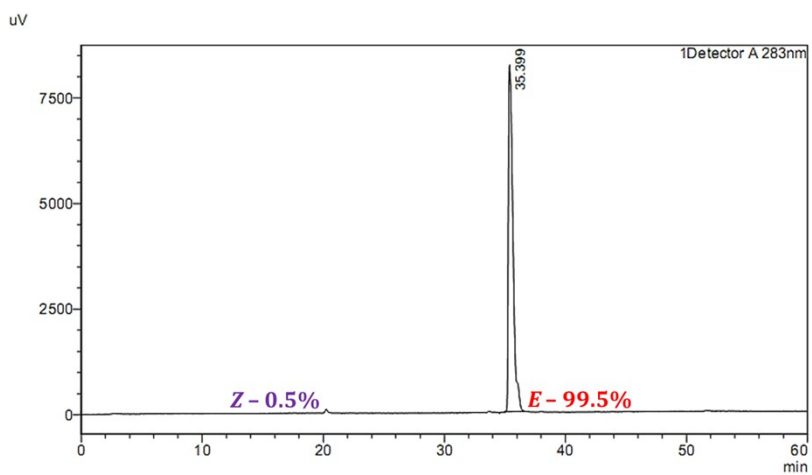
(b) azo-peptide 3



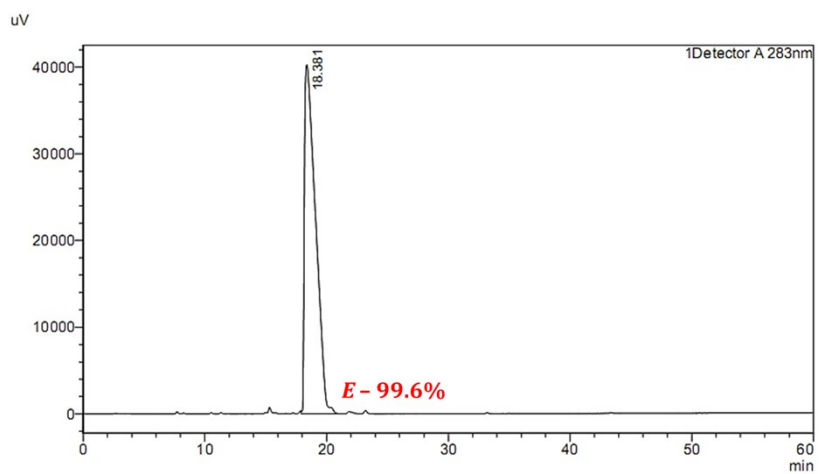
(c) azo-peptide 4



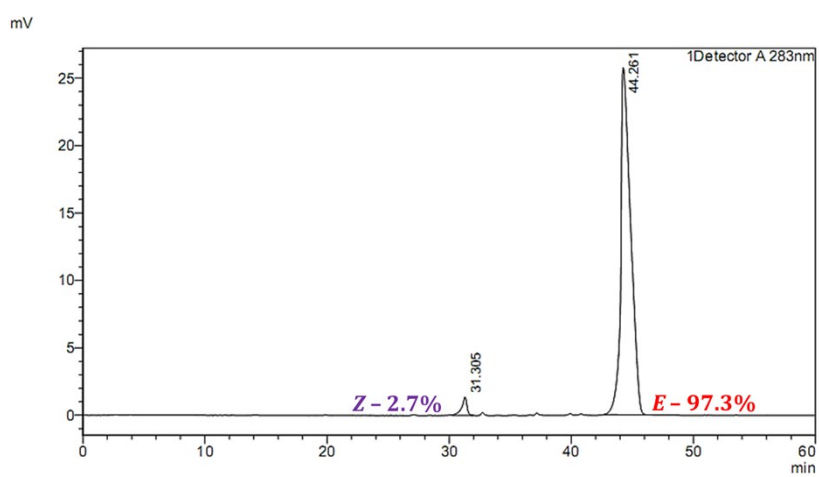
(d) azo-peptide 5



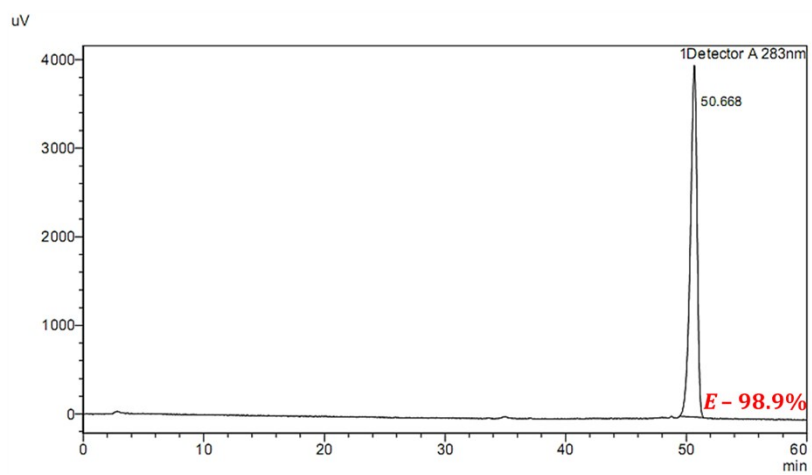
(e) azo-peptide 6



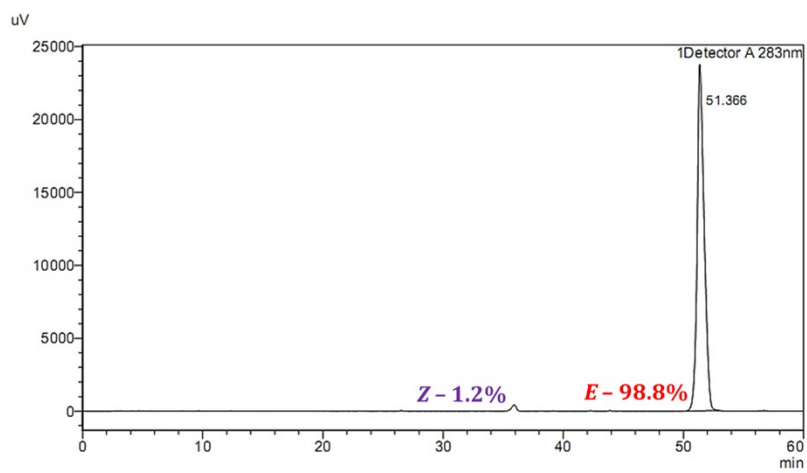
(f) azo-peptide 7



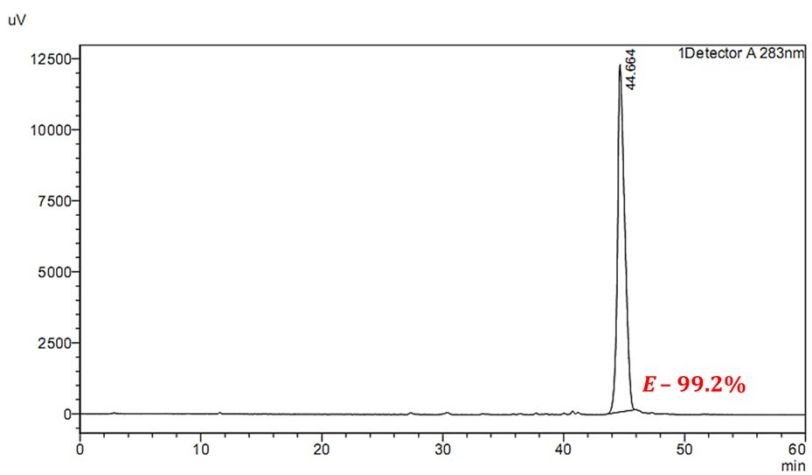
(g) azo-peptide 8



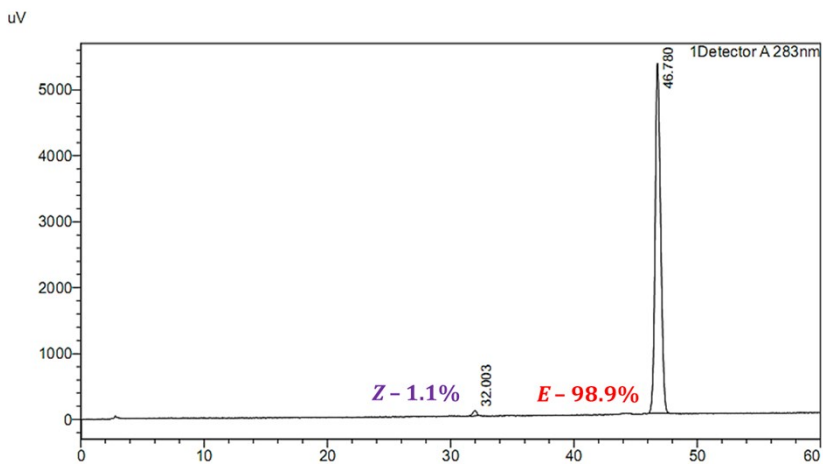
(h) azo-peptide 9



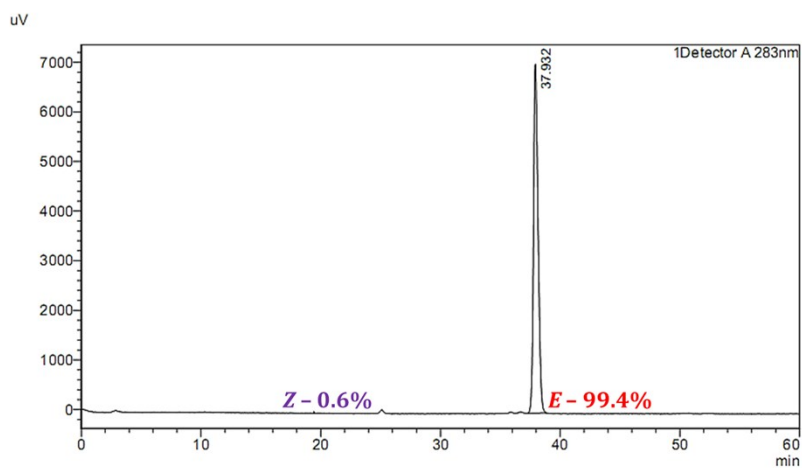
(i) azo-peptide 10



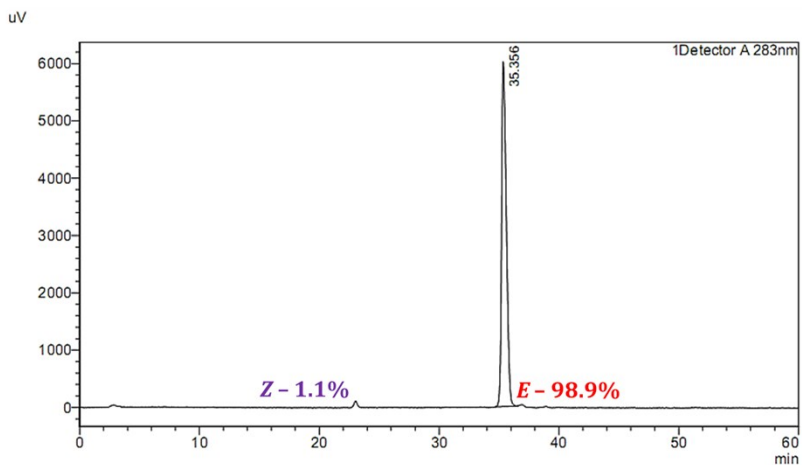
(j) azo-peptide 11



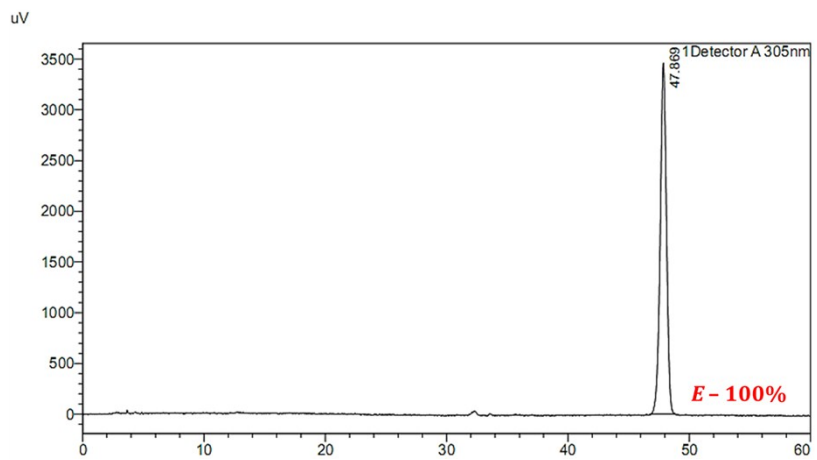
(k) azo-peptide 12



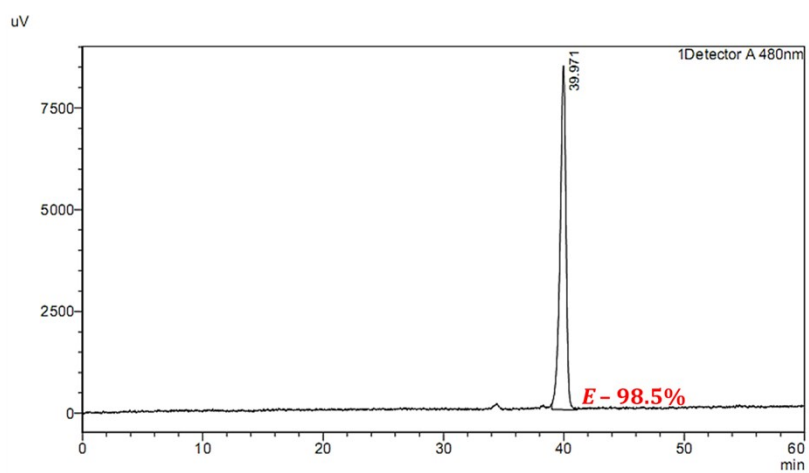
(l) azo-peptide 13



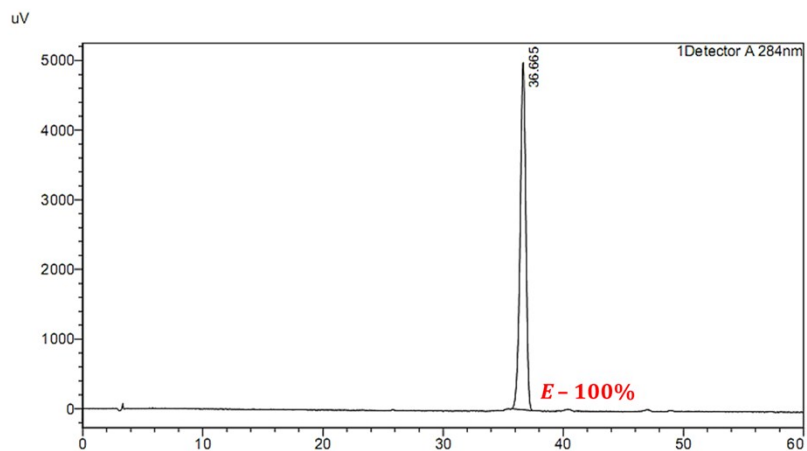
(m) azo-peptide 14



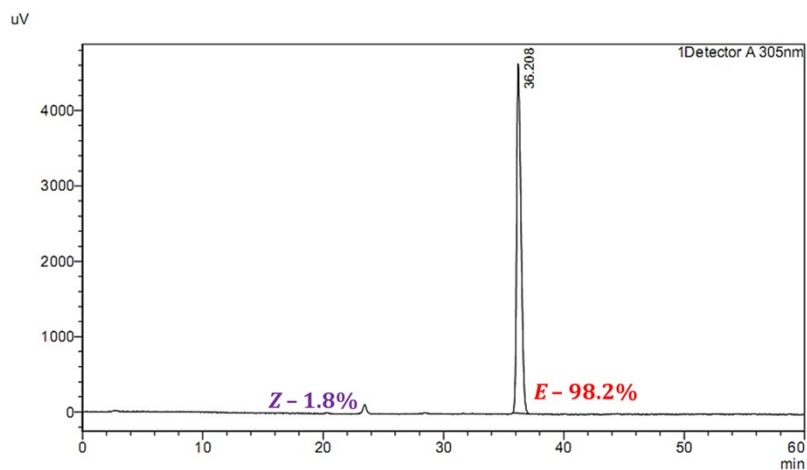
(n) azo-peptide 15



(o) azo-peptide 16



(p) azo-peptide 17

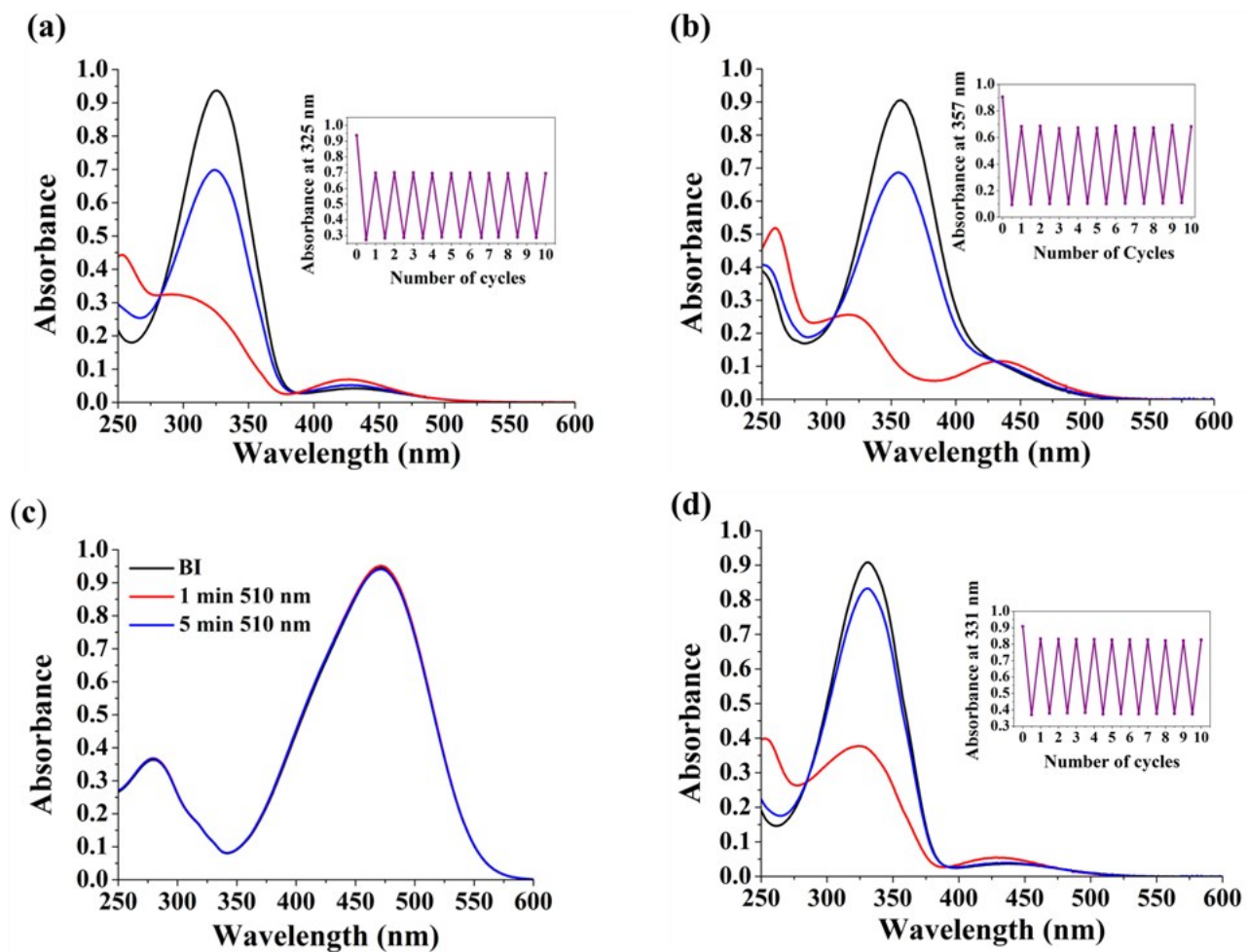


**Figure S17.** HPLC chromatograms are showing > 95% purity. Conditions of the RP-HPLC analysis; Column - 5C<sub>18</sub>-MS-II, 4.6×250 mm (Nacalai Tesque, Inc.); Eluent - CH<sub>3</sub>CN/H<sub>2</sub>O containing 0.1% TFA; Solvent gradient - 20 to 45% of acetonitrile in water for 1 h; Flow rate - 1 mL/min. Injection volume - 20 μL was used to analyze the purity of each azo-peptide.



### 3. Photoisomerization of the azo-peptides:

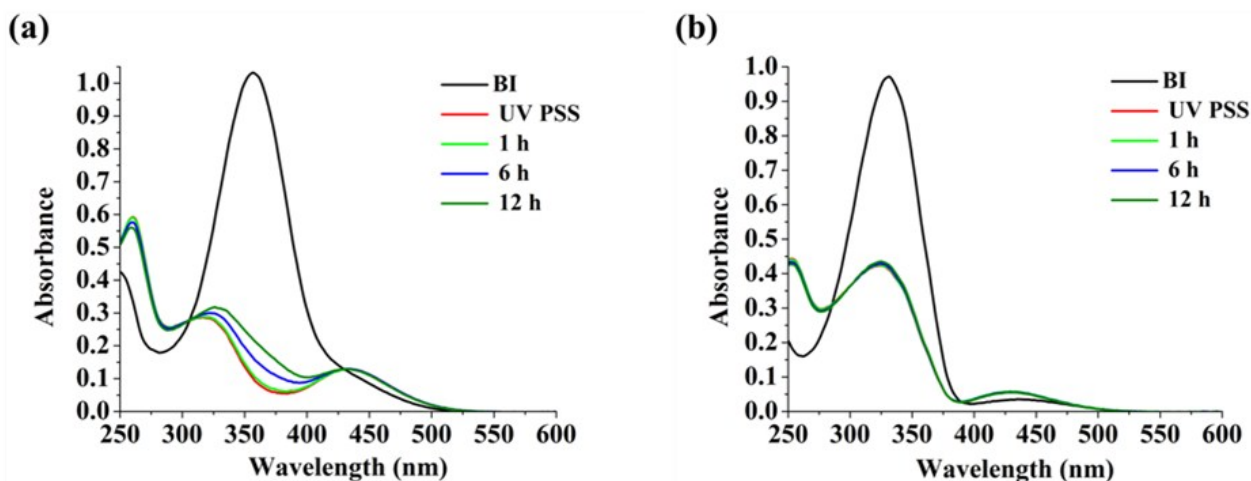
Photoisomerization experiments of compounds **11**, **14**, **15**, **16** were performed in BRB-80 buffer at 25 °C.



**Figure S18.** (a), (b), (c) and (d) are the UV-Vis absorption spectra of azo-peptides **11**, **14**, **15** and **16** respectively in BRB-80 buffer solution at 25 °C; before irradiation (black line), UV PSS (red line), Vis PSS (blue line). Inset of (a), (b), and (d): Absorbance changes at 325 nm, 357 nm and 331 nm respectively after the alternate irradiations of 365 and 436 nm for azo-peptides **11** and **16**, 365 and 510 nm for azo-peptide **14** for 10 cycles. Azo-peptide **15** was irradiated with 510 nm light.

#### 4. Thermal stability of the *cis* isomers:

Thermal stability studies of the *cis* isomers of azo-peptides **14** and **16** were performed in BRB-80 buffer at 25 °C.

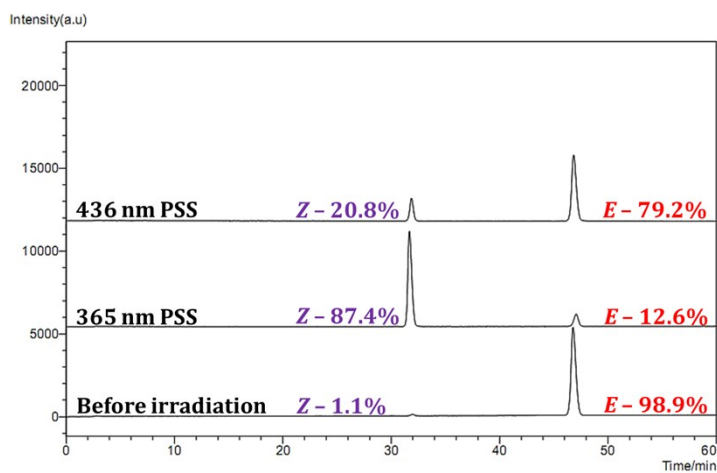


**Figure S19.** UV-Vis absorption spectra of azo-peptides **14** and **16** showing their thermal stability in BRB-80 buffer solution are represented by (a) and (b) respectively after irradiating with 365 nm light up to their PSSs and then incubation in the dark at 25 °C.

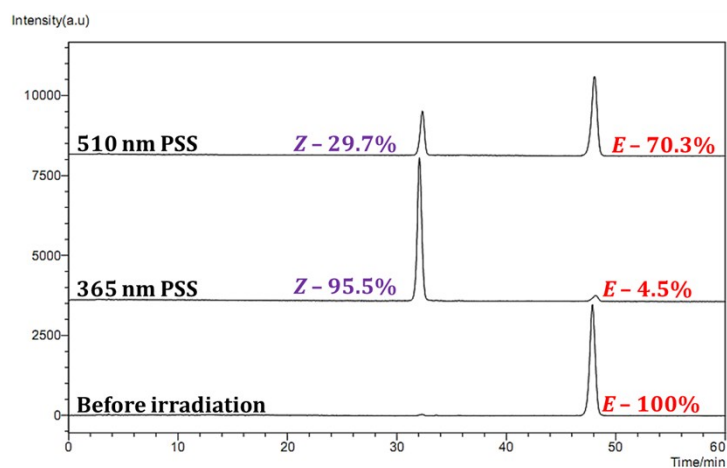
#### 5. HPLC analysis on the conversion ratio from *trans* to *cis* and *cis* to *trans* forms:

The photo conversion ratio from *trans* (*E*) to *cis* (*Z*) and *cis* (*Z*) to *trans* (*E*) of the azo unit in azo-peptides **11**, **14** and **16** upon irradiation with 365 nm light and 436 nm or 510 nm light was measured with HPLC analyses. Conditions of the RP-HPLC analysis; Column - 5C<sub>18</sub>-MS-II, 4.6×250 mm (Nacalai Tesque, Inc.); Eluent - CH<sub>3</sub>CN/H<sub>2</sub>O containing 0.1% TFA; Solvent gradient - 20 to 45% of acetonitrile in water for 1 h; Flow rate – 1 mL/min. Injection volume -20 μL was used to analyze the isomer ratio and the isosbestic point in this eluent condition (283 nm for **11**, 305 nm for **14**, 284 nm for **16**) was used as the monitoring wavelength.

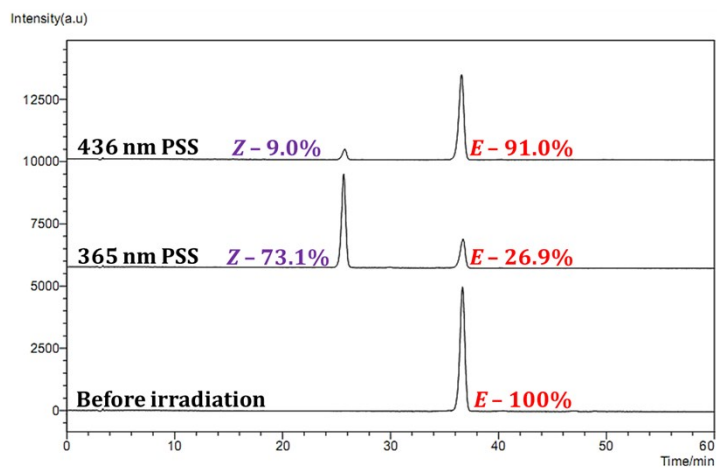
(a) azo-peptide 11



(b) azo-peptide 14



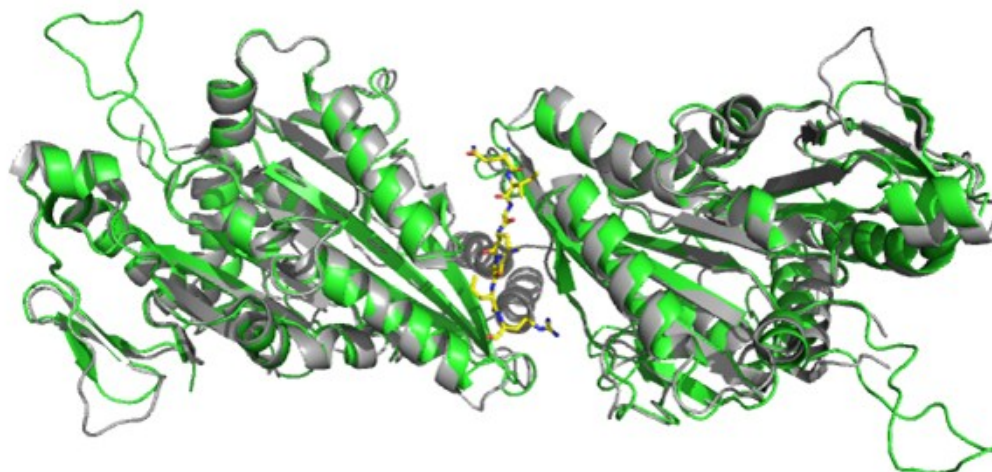
(c) azo-peptide 16



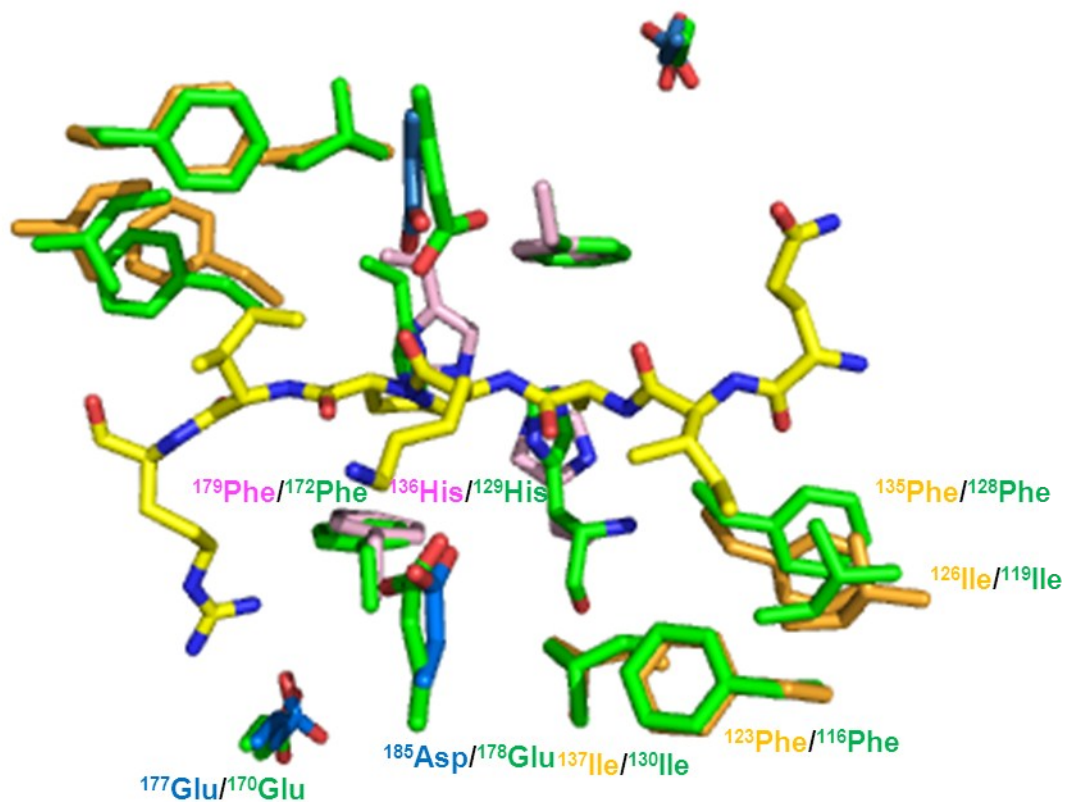
**Figure S20.** HPLC chromatograms showing the *cis* (*Z*) and *trans* (*E*) isomer ratio at before irradiation, after 365 nm light irradiation at PSS and after 436 nm or 510 nm light irradiation at PSS.

## 6. Structural comparison between the *Drosophila melanogaster* kinesin-1 and human kinesin-1 motor domains

(a)



(b)



**Figure S21.** (a) Overlay of *Drosophila melanogaster* kinesin-1 (gray, PDB: 2Y65) and human kinesin motor domains (green, PDB: 1BG2). (b) Detailed similarity in critical amino acids for tail binding.