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In situ real-time monitoring the mechanism of self-assembly of short peptide supramolecular polymers

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EXPERIMENTAL SECTION

Reagents and materials. N-Fluorenylmethoxycarbonyl-diphenylalanine (Fmoc-FF), N-Fluorenylmethoxycarbonyl-dialanine (Fmoc-AA) and N-Fluorenylmethoxycarbonyl-diglycine (Fmoc-GG) was purchased from Bachem Co., Switzerland and was used without further purification. Calcium carbonate and cesium carbonate ≥99.9%, were purchased from Alfa Aesar, Germany. Sodium hydroxide (for analysis) was purchased from Merck, Germany. **AQui** fluorophore (9-(azetidin-1-yl)-5-butyl-4H-benzo[de][2,6]naphthyridine-4,6(5H)-dione[S1] was provided by Dr. Rosario Herranz and Dr. Francisco Fueyo-Gonzalez at Instituto de Química Médica-CSIC (IQM-CSIC).

Hydrogel preparation. Fmoc-FF peptide was weighed into vial and deionised water was added to obtain a final concentration of 20 mM. This suspension was then sonicated (in a HSt Powersonic 603-ultrasonic bath) for 1 hour. NaOH solution 0.5 M was then added dropwise. After each addition, the solution was sonicated until get a clear solution at a pH of approximately 10.6. The pH was measured using a HACH sensiont PH 3 pH meter. The pH meter was calibrated using pH 4, pH 7 and pH 10 buffer solutions. This solution was again diluted with deionised water to obtain a final Fmoc-FF concentration of 10 mM and 5 mM. Gelation was induced by adding different salts at concentrations of 50 mM (for the 20 mM peptide solution), 25 mM (for the 10 mM peptide solution) and 12.5 mM (for the 5 mM peptide solution). The mixture was vortexed for 5 seconds using an LBX V05 series vortex stirrer.

For FLIM, FLCS and fluorescence studies, the peptide-hydrogel was prepared adding the **AQui** fluorophore to a clear peptide solution for a final fluorophore concentration of 25 μM. The mixture was vortexed for 5 seconds. Gelation was induced by adding the corresponding salt of Cs^+ or Ca^{2+}. The mixture was again vortexed for 5 seconds.

Transmission electron microscopy (TEM). The hydrogels used in this work were studied with LIBRA 120 PLUS Carl Zeiss. Hydrogels were vortexed and diluted 1/10 with water. A drop of an aqueous suspension of the hydrogel was placed on a 300-mesh copper grid. The TEM simple was stained with 1% aq. uranyl acetate solution. The sample was dried at room temperature for 30 min.

High-resolution transmission electron microscopy (HR-TEM). HR-TEM images were made using a HAADF FEI TITAN G2 microscope, with a maximum resolution TEM 0.8 Å and resolution STEM 2 Å. A drop of an aqueous suspension of the hydrogel was deposited on a copper grid previously covered with a thin layer of carbon.

Atomic Force Microscope (AFM). The measurements were obtained with a Park NX20 atomic force microscope (Park Systems) operating at 25ºC and atmospheric pressure. The data resulting from each study was processed using the XEI program (Park Systems).

Circular dichroism (CD). The CD spectra were recorded using a Jasco J-815 spectropolarimeter with a xenon lamp of 150 W. The hydrogels were gelified into a 0.1 mm quartz cell (Hellma 0.1 mm quartz Suprasils) using the protocol described above. Spectra were obtained from 200 to 320 nm with a 1 nm step and 0.5 s integration time per step at 20 ºC.

X-ray diffraction (XRD). Samples of the hydrogels were deposited on a flat glass sample holder and dried overnight prior to data collection. XRD patterns were generated with Cu Ka radiation (l = 1.5418 Å) on a PANalytical X'Pert PRO diffractometer equipped with a PIXcel detector operating at 45 kV and 40 mA. The 2y range was from 51 to 801 with a step size of (2y) 0.0391. By varying the scattering angle, the explored momentum transfer vector (q) was in the range of 1 o q (nm^{-1}) o 28.0, with q = (4p/k)sin h, where h is the scattering angle.

Fourier-transform infrared (FTIR) spectroscopy. **FTIR** spectra were recorded using a Bruker IFS-66 spectrometer (Bruker, Ettlingen, Germany) equipped with a liquid nitrogen-cooled MCT detector and an Attenuated Total Reflection (BioATR-II) accessory thermostated at 25ºC. The hydrogels were deposited on the ATR cell and spectra were scanned every 5 min over the range between 4000 and 900 cm^{-1} with a 2 cm^{-1} resolution and 128 accumulations. Spectra were recorded with salt solutions without the hydrogel under identical conditions and subtracted from each spectrum of the hydrogels. Spectral contributions from residual water vapor were reduced using the atmospheric compensation filter built in the Bruker OPUS software.

Fluorescence. Fluorescence emission spectra and gelation kinetics were obtained on a Jasco FP-8300 spectrofluorimeter (Jasco, Tokyo, Japan), at the excitation wavelength λ_{ex} of 470 nm. We studied the temporal evolution of the fluorescence emission spectra of peptide-salts hydrogels in the presence of the **AQui** fluorophore. For the study of gelation kinetics, peptide-salts hydrogels were formed *in situ* and gelling was studied for 24 h.

Rheology

- **Rheological characterization of the hydrogels under shear**

. Mechanical properties were characterized under oscillatory shear stress at a temperature of 25.0 ± 0.1ºC, using a Haake MARS III controlled-stress rheometer (Thermo Fisher Scientific, Waltham, MA, USA), provided with a plate-plate sensor of 35 mm of diameter. First we obtained the values of the storage (G') and loss (G'') moduli as a function of the amplitude of the oscillatory shear strain, γ_0, at a constant frequency of 1 Hz (amplitude sweeps). Then we subjected the samples to frequency sweep tests of fixed shear strain amplitude (γ_0 = 0.0002) within the Linear Viscoelastic Region (LVR), and increasing frequency in the range 0.1–15 Hz. From these tests we obtained the values of the viscoelastic moduli (both G' and G'') corresponding to the LVR as a function of frequency. We carried out at least three repetitions for different aliquots.

- **Self-healing studies**

For the investigation of the self-healing behavior of the hydrogels at 20 mM peptide concentration we used the Haake MARS III rheometer provided with the plate-plate sensor of 35 mm of diameter, and proceeded as it follows. For these studies hydrogels were prepared and left at rest during 24 hours. Then, the hydrogels were placed in the measuring geometry and subjected to a shear rate of 20 s^{-1} during 120 s to provoke their fracture. Immediately afterwards, the shear rate was stopped and the samples were subjected to oscillatory shear strain of γ_0 = 0.001 and 1 Hz of frequency, for a total time of 6000 s, and the evolution of the viscoelastic moduli was monitored as a function of time. We carried out at least three repetitions for different aliquots.

Differential Scanning Calorimetry (DSC). DSC experiments were performed in a DASM-4 microcalorimeter[S2] equipped with capillary platinum cells. The reference cell was filled with

volume of a salt solution in water, and the sample cell was filled with hydrogel-precursor mixture prepared immediately prior to its introduction into the instrument. The samples were scanned up to 100 °C, cooled and rescanned again to check the reversibility of the thermograms. The scan rate was varied between 0.5 and 2 °C min^{-1} to investigate specifically the dependence with time of the observed thermal effects. Instrumental baselines were recorded with both cells filled with salt solution in water and subtracted from the experimental thermograms. The corrected thermograms were normalized to heat capacity relative the water using the Fmoc-FF molar concentrations.

Multidimensional FLIM microscopy and FLCS spectroscopy instrumentation. These experiments were carried out on a MicroTime 200 instrument (PicoQuant GmbH, Berlin, Germany), previously described in detail.[S1] The excitation source was a pulsed, 470-nm diode laser (LDH-P-C-470, PicoQuant), operated at a repetition rate of 20 MHz. The excitation beam was focused on the sample through a ×100, 1.4NA oil immersion objective. Fluorescence emission was collected back, filtered through the main dichroic mirror and a cleanup cutoff filter (500LP) and focused onto the 75-μm confocal aperture. The emitted light was split in an orange channel and a red channel using a 600DCXR dichroic mirror and appropriate bandpass filters to define I_{550} (Thorlabs 550/40 bandpass filter) and I_{630} (Chroma 630/60 bandpass filter). Two avalanche single photon detectors (Perkin Elmer) were used to detect individual photons and a TimeHarp 200 photon-counting module (PicoQuant) was used for individual photon time tagging. FLIM images were obtained with a spatial resolution of at least 19.5 nm/pixel and a temporal resolution of 29 ps/channel in the microtime scale. Images were analyzed using SymPhoTime 64 (PicoQuant) and home-coded scripts in FIJI (distribution of ImageJ).[S3] FLIM images were obtained by fitting all pixels of the image to a monoexponential decay function, using a reconstructed instrument response function, after applying a 5-pixel spatial binning and an 8-channel temporal binning. Segmentation was performed by subtracting the background defined by a 50-pixel median filter[S4] and applying the *robust automatic threshold selection* tool in FIJI.

For FLCS experiments, the excitation laser was focused 10 μm inside the aqueous solution of Fmoc-FF dipeptide (at either 2.5, 5 or 10 mM) and the studied counterion,

Ca^{2+} or Cs^{+} (at the concentrations of 6.25, 12.5 or 25 mM). Fluorescence fluctuation traces were collected during 16 min, in 2 min steps. At least three repetitions with different preparations of each sample were performed. FLCS curves were obtained by applying a time-weighted filter to account for fluorescence photons during the autocorrelation of the fluctuation signal. Then, the reconstructed FLCS autocorrelation curves, $g(t)$, were fitted to the diffusion equation with two components, with the short diffusion time, $\tau_{D,fast}$, fixed at 55.2 μs.

$$g(t) = g(0) \sum_{i\,=\,slow,fast} p_i \left[1 + \frac{t}{\tau_{D,i}}\right]^{-1} \left[1 + \frac{1}{s^2}\left(\frac{t}{\tau_{D,i}}\right)\right]^{-1/2}$$

where $\tau_{D,i}$ represents the diffusion time of either one of the components, with a specific contribution of p_i; g(0) is the limiting amplitude; and s is the geometrical parameter accounting for the relative size of the vertical and lateral axes of the excitation volume, as previously defined.[S4] The reconstruction of the time-weighted FLCS autocorrelation curves and the subsequent fittings were performed in SymphoTime 32 (PicoQuant).

We performed a global analysis of the FLCS curves to obtain a consensus $\tau_{D,fast}$ value for the Fmoc-FF monomer, obtaining 55±3 μs. We then used this value as a fixed parameter to analyze individually all the curves, avoiding overparametrization of the FLCS curve fitting process.

FIGURES

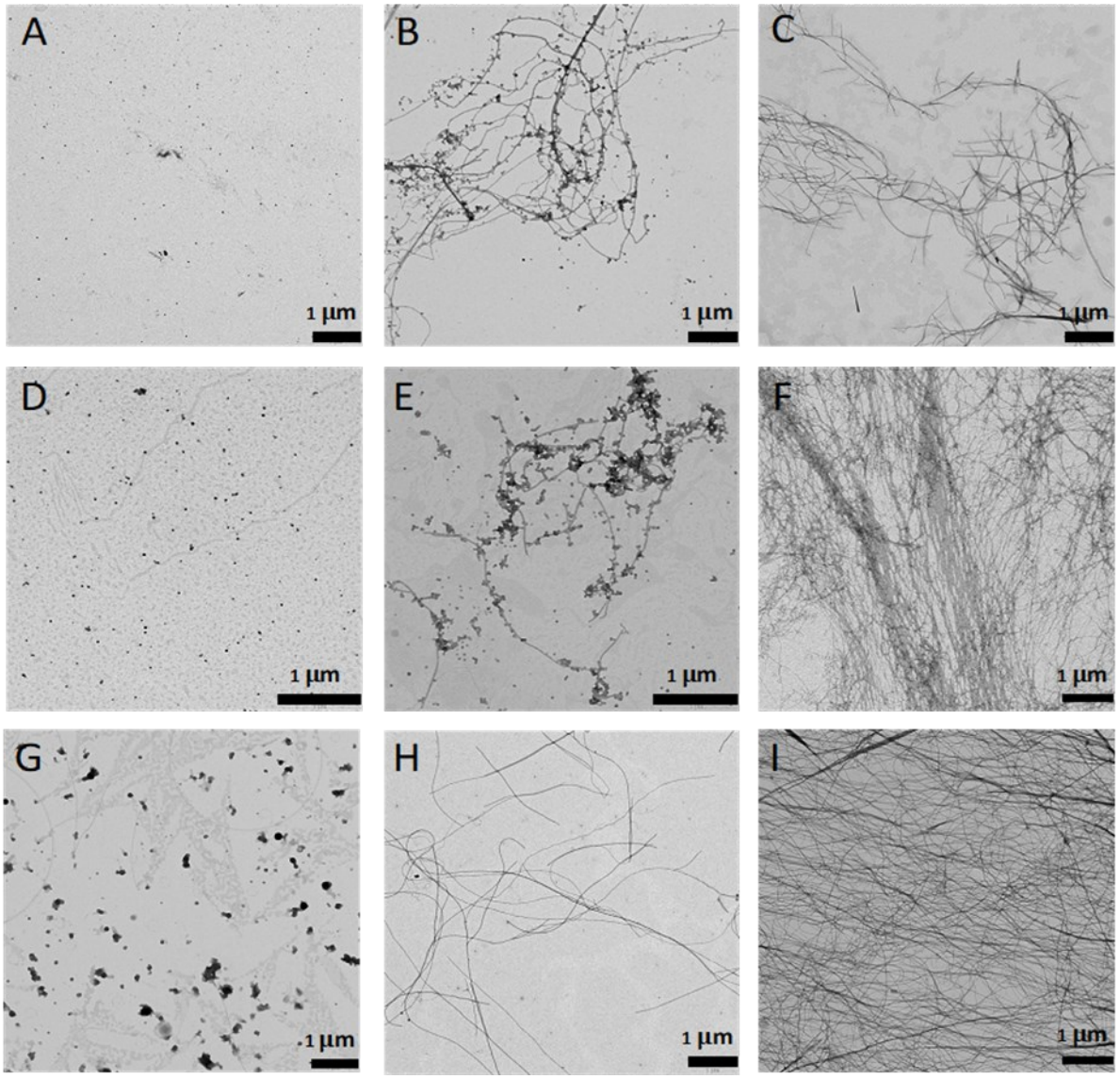


Figure S1. TEM Images of Fmoc-FF Na^+ salt at 2.5 mM (A), 5 mM (B) and 10 mM (C); TEM images of Fmoc-FF Cs^+ salt at 2.5 mM (D), 5 mM (E) and 10 mM (F); TEM images of Fmoc-FF Ca^{2+} salt at 2.5 mM (G), 5 mM (H) and 10 mM (I) peptide conc.

Experiments using Na^+ and Cs^+ salts showed the presence of similar nanospheres of homogeneous sizes with diameters of 120 to 160 nm at 2.5 mM, being most abundant in the case of Cs^+, (Figure S1A and S1D). At 5 mM amorphous fibers of higher aspect-ratio coexisted with nanospheres observed at 2.5 mM. Such fibers have been formed by the coalescence of nanospheres, having both the same diameter. At 10 mM, mature fibers of higher crystallinity were found (Figures S1C and S1F). In the case of the peptide Na^+ salt, the presence of short and

narrow fibers and ribbons with well-defined edges could be observed. Notably, the presence of Cs^+ significantly altered the morphology of the aggregates (Figure S1F). Images showed a greater number of thinner fibers that in some cases collapsed to form sheets. The structures observed in Figures S1B and S1E (amorphous nanofibers) differed significantly to the final fibers found in Figure S1C and S1F. On the contrary, TEM images of the peptide solution at 2.5 mM in the presence of Ca^{2+} showed aggregates of bigger sizes formed by the coalescence of droplets (Figure S1G), suggesting the emergence of a liquid-liquid phase separation process at very early stage (Figure S2).[S5] At higher concentration (5 mM), the presence of narrow fibrils of 14 to 20 nm of diameter and well-defined edges was clearly observed. This cation presented a clearly different behavior from the previous one, affecting the peptide self-assembly process at an early stage. The appearance of a lasting liquid-liquid separation phase process when Ca^{2+} was present, promoted the formation of well-defined fibers at lower concentrations, improving the kinetics of the process. We also analyzed the morphology of these aggregates in the gel state. TEM images of Fmoc-FF Ca^{2+} salt at 10 mM (Figure S1I), showed the presence of a higher number of fibers and ribbons of similar aspect to those observed in Figure 3H. Fibers of higher diameters were formed by the intertwining of two or more individual fibers. In this case, the morphology of the fibers found at 5 mM (Figure S1H) was similar to those found at higher oncentrations (Figure S1I), suggesting that Ca^{2+} was able to promote peptide self-assembly more efficiently than Na^+ or Cs^+.

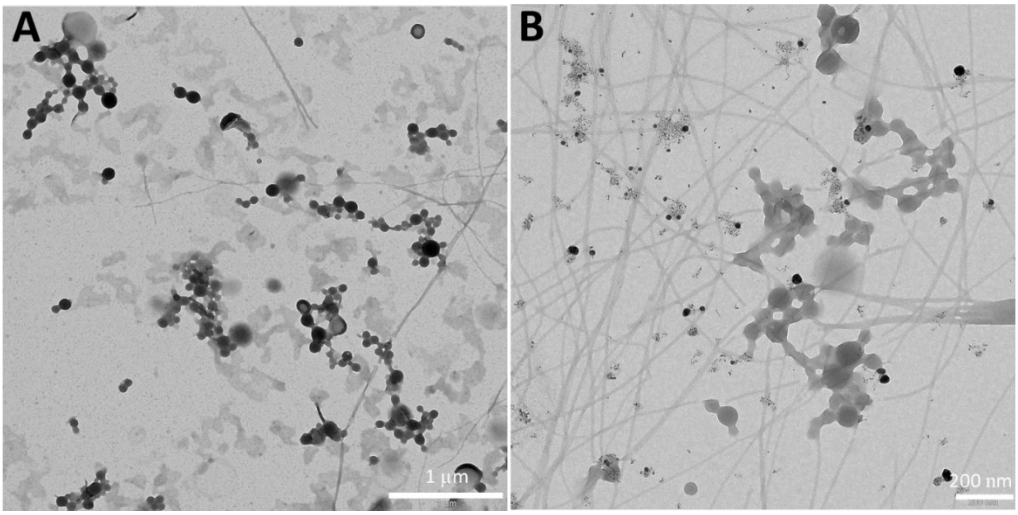


Figure S2. TEM images of liquid-liquid phase formed by Fmoc-FF hydrogels in the presence of $CaCO_3$ (A) and Fmoc-AA in the presence of Ag_2CO_3 (B). It has been reported that Fmoc-AA in the presence of Ag_2CO_3 forms liquid-liquid phases.[S5] We show in here as a control to demonstrate that similar structures are formed using $CaCO_3$ in our conditions and visualized with the same TEM apparatus.

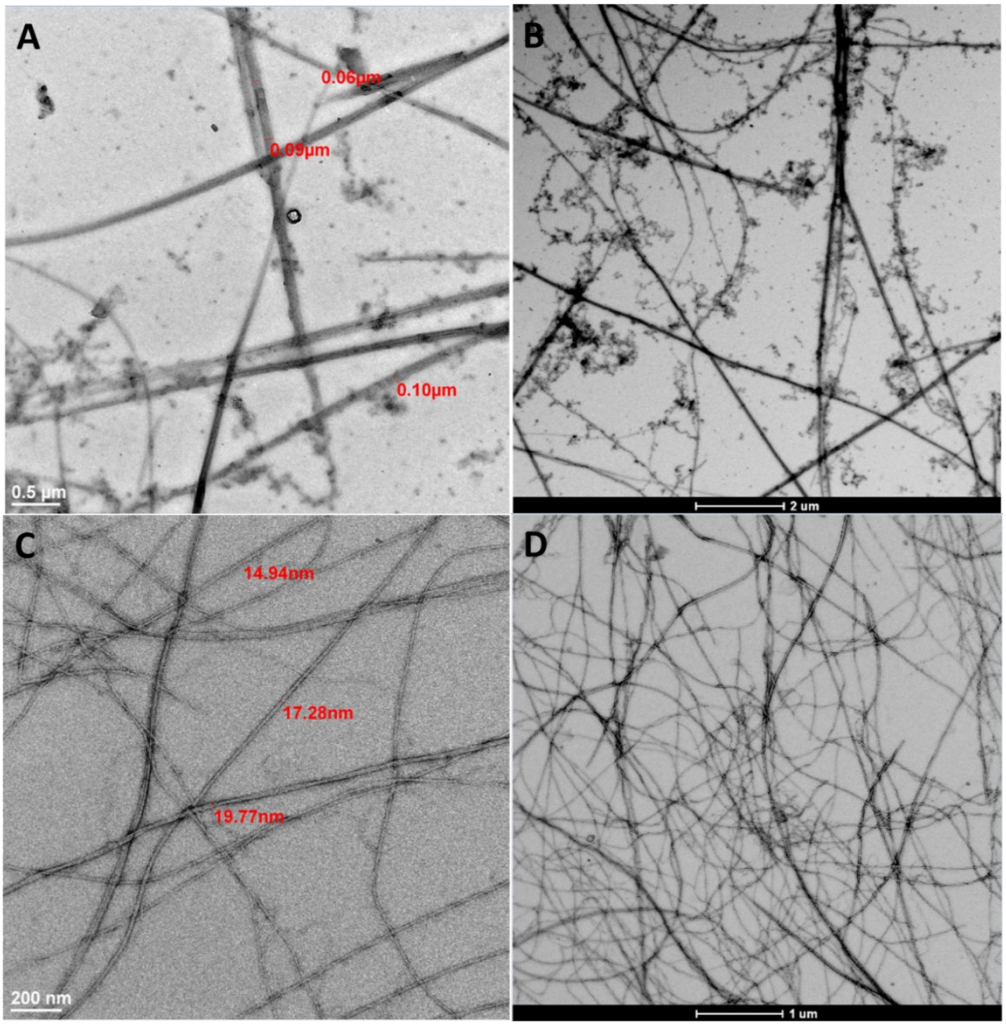


Figure S3. HR-TEM images; (A-B) 10 mM of Fmoc-FF and 25mM of Na_2CO_3 hydrogels; (C- D);10 mM of Fmoc-FF and 25mM of $CaCO_3$ hydrogels.

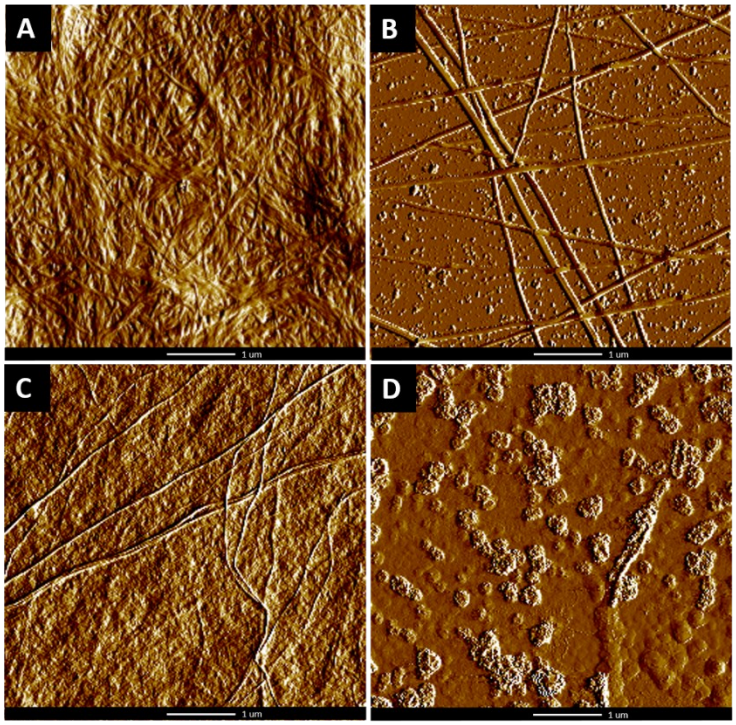


Figure S4. AFM images of 10mM Fmoc-FF hydrogels; A) 25 mM $CaCO_3$; B) 25 mM $CaCO_3$ prepared at 1/100 dilution in deionized water; C) 25 mM Cs_2CO_3; D) 25 mM Cs_2CO_3 prepared at 1/100 dilution in deionized water.

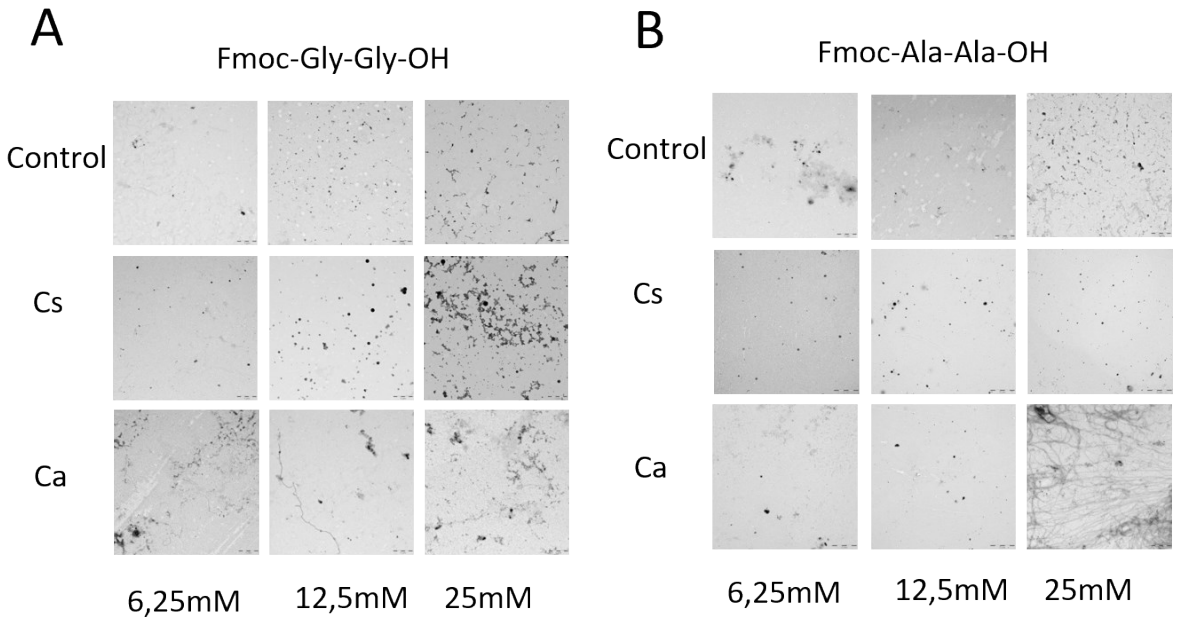


Figure S5. TEM images peptide hydrogels at different concentrations of peptide-salt: 2.5/6.25 mM, 5/12.5 mM and 10/25 mM; A) Fmoc-GG peptide with Cs_2CO_3 and $CaCO_3$ salts; B) Fmoc-AA with Cs_2CO_3 and $CaCO_3$ salt. In the case of Fmoc-GG the formation of fibers were not

observed. With Fmoc-AA only at the highest concentration of peptide and Ca^{2+} the formation of fibers were observed. In this case, similar to Fmoc-FF, Ca^{2+} was promoting the formation of fibers more efficiently than Cs^{+}.

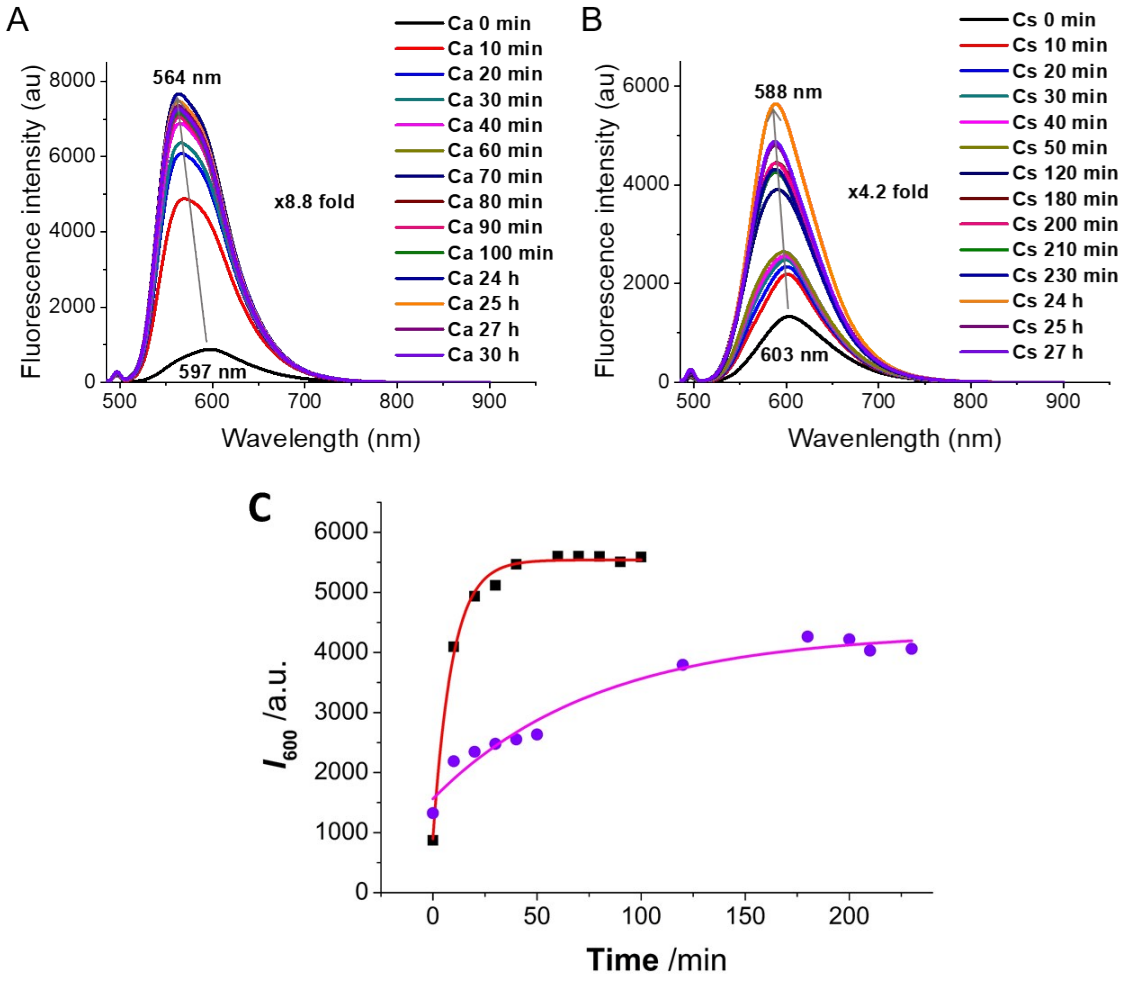


Figure S6. A and B) Steady-state fluorescence spectra (λ_{ex} = 470 nm) of **AQui** in incubating 10 mM Fmoc-FF in the presence of 25 mM Ca^{2+} (A) or Cs^{+} (B) as a function of the incubation time. C) Fluorescence emission intensity at 600 nm from spectra in panels A) and B) of **AQui** in incubating 10 mM Fmoc-FF in the presence of 25 mM Ca^{2+} (black squares) or Cs^{+} (violet circles). The lines represent fittings to a single exponential function ($I_{600} = I_{600,max} + (I_{600,0} - I_{600,max})\cdot\exp[-k_{obs}\cdot t]$, where t is the time and k_{obs} is the apparent rate constant).

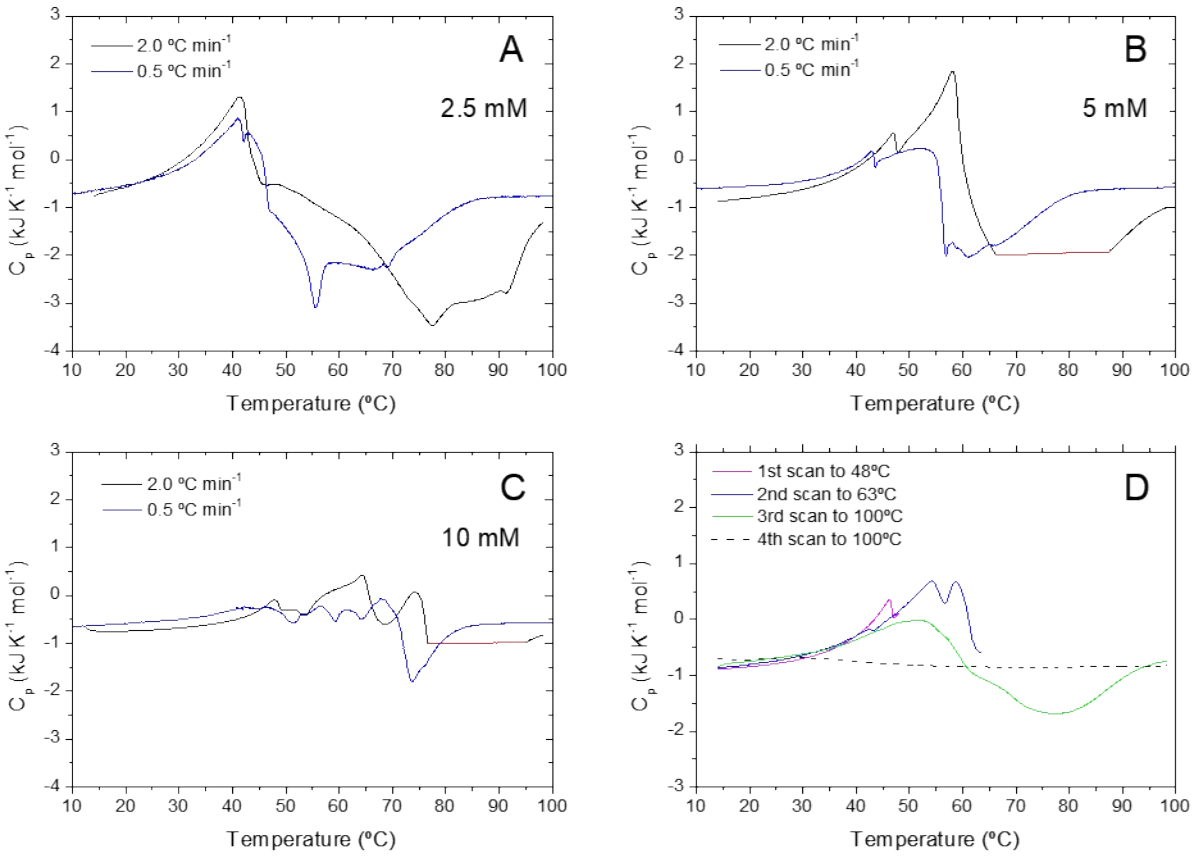


Figure S7. **(A-C):** DSC thermograms measured at two different scan rates with: A) 10 mM Fmoc-FF and 25 mM Cs_2CO_3; B) 5 mM Fmoc-FF and 12.5 mM Cs_2CO_3; C) 2.5 mM Fmoc-FF and 6.25 mM Cs_2CO_3. The flat red segments correspond to signal over range of the calorimeter. D) Consecutive DSC scans at 2°C·min^{-1} with 5 mM Fmoc-FF and 12.5 mM Cs_2CO_3 obtained by heating up to the indicated temperatures, cooling and rescanning again.

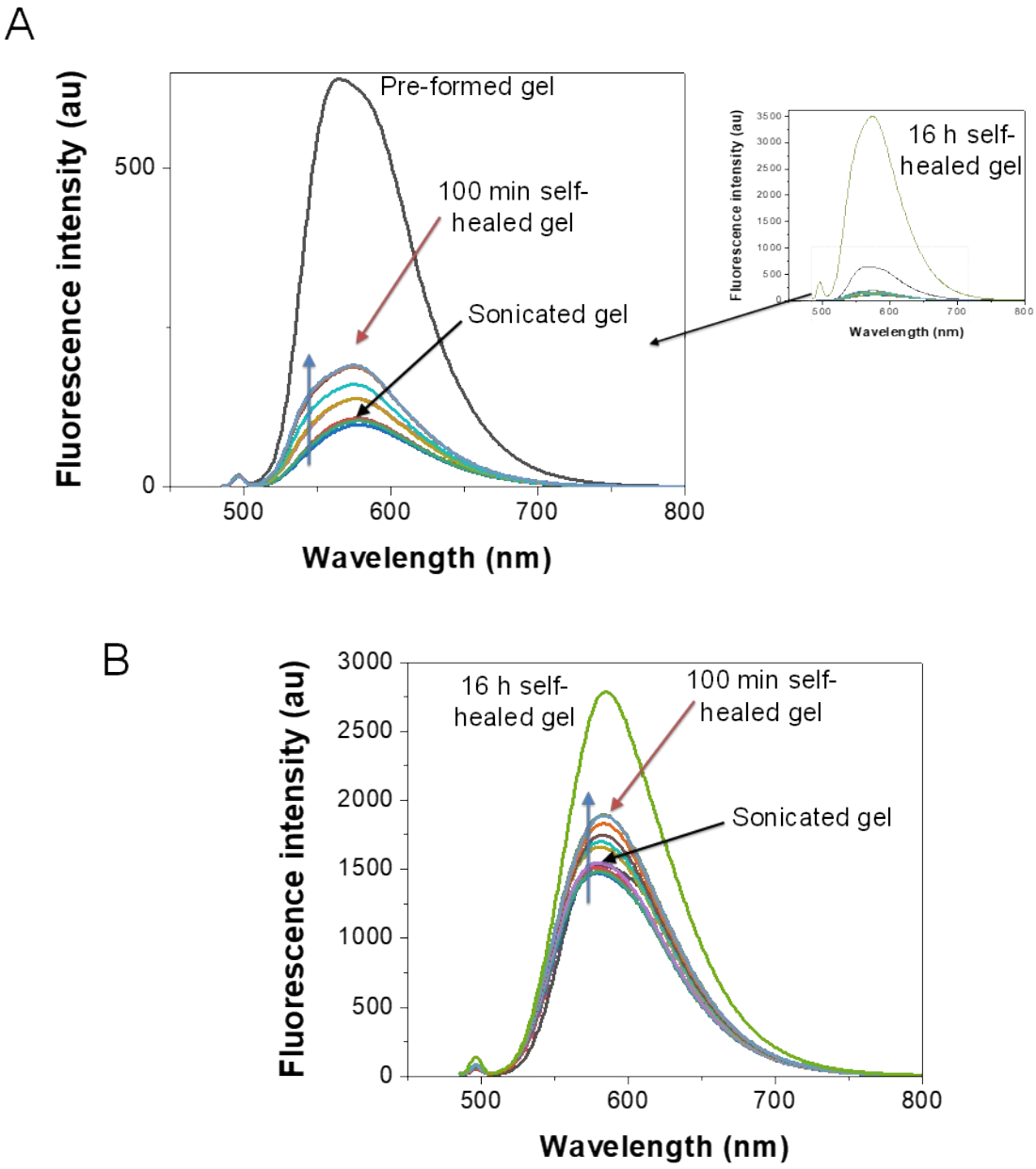


Figure S8. Time recovery of the emission spectra (λ_{ex} = 470 nm) of **AQui** in Fmoc-FF Ca^{2+} (A) and Fmoc-FF Cs^{+} (B) gels, after ultrasound treatment.

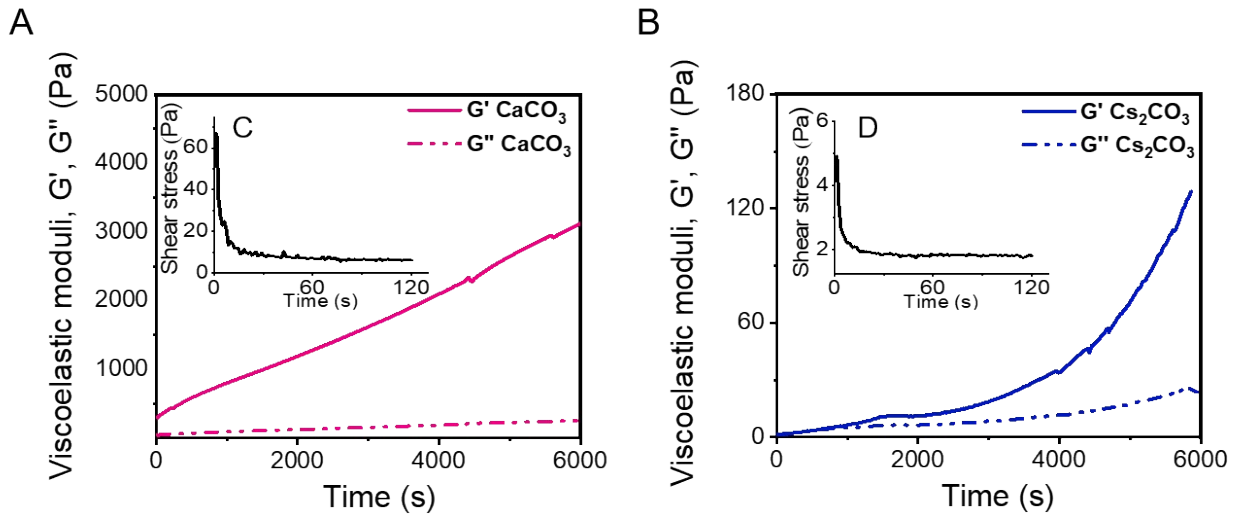


Figure S9. Self-healing of Fmoc-FF Ca^{2+} (10 mM) (A) and Fmoc-FF Cs^{+} (10 mM) (B). (A) and (B) show the evolution of the viscoelastic moduli after fracture of the hydrogels by 120 s of shearing at a constant shear rate of 20 s^{-1}. (C) and (D) show the evolution of the shear stress, τ, during the shearing at constant shear rate -observe that the shear stress decreased strongly upon fracture of the hydrogels.

DFT CALCULATIONS

Theoretical calculations were performed by DFT methods using the Gaussian 09 software[S6] at the ωB97XD/def2SVP level of theory.[S7] The LanL2DZ basis set and pseudopotential was used for Na^+, Cs^+ and Ca^{2+}.[S8] The calculations were carried out with an ultra-fine integration grid and in water as solvent applying the polarizable continuum model with the integral equation formalism (IEFPCM) available in Gaussian 09.[S9] Thermochemistry data were obtained through frequency calculations, which also confirmed that the optimized structures corresponded to an energy minimum. Basis set superposition error (BSSE) was corrected for the Fmoc-FF complexes with Na^+, Cs^+ and Ca^{2+} using the counterpoise method.[S10]

The coordinates of the minimized structures along with the thermochemistry data are shown in Tables S1-S12.

Fmoc-FF

For Fmoc-FF as carboxylate (terminal carboxylic acid deprotonated) two different geometries have been considered, an extended one derived from a fragment of a β-sheet structure (Fig S10 left) and a more folded one derived from the X-ray structure of Fmoc-FF (Fig S10 right).[S11] The latter was found to be around 2.86 kcal/mol more stable than the β-sheet-like one.

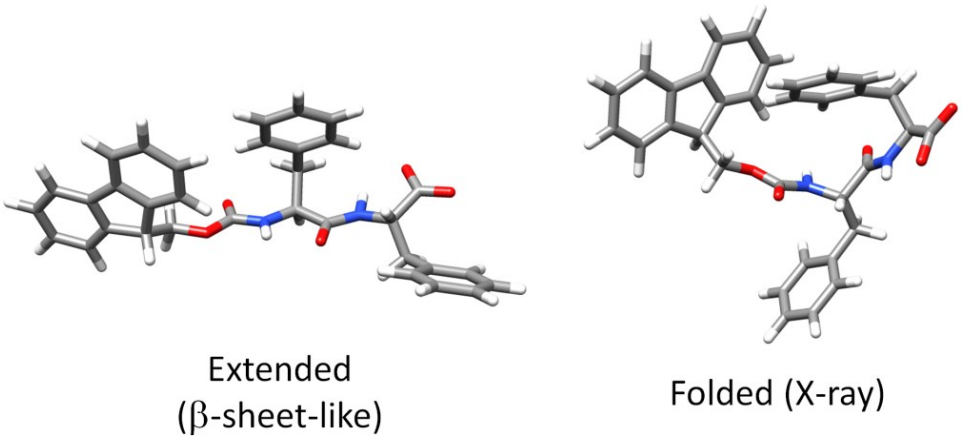


Figure S10. Calculated structures for deprotonated Fmoc-FF. Color coding: C, gray; N, blue; O, red; H, white.

Interaction of Fmoc-FF with metal cations

Three different geometries have been considered for the complexes resultant from the coordination of Na^+, Cs^+ or Ca^{2+} as metal ions to deprotonated Fmoc-FF (Figure S11). One is derived from the β-sheet-like structure of Fmoc-FF with the metal ion coordinated to the terminal carboxylate as bidentate ligand (Figure S11 top left). The other two arise from the coordination of the metal ion to the folded conformation and differ on the carboxylate acting as a monodentate (Figure S11 top right) or bidentate ligand (Figure S11 bottom). For Fmoc-FF Na^+ our calculations predict that, among the structures tested, the most stable complex (*ca.* 5.5 kcal/mol respect to the next in energy) is that derived from the β-sheet-like structure (Fig S11 top left).

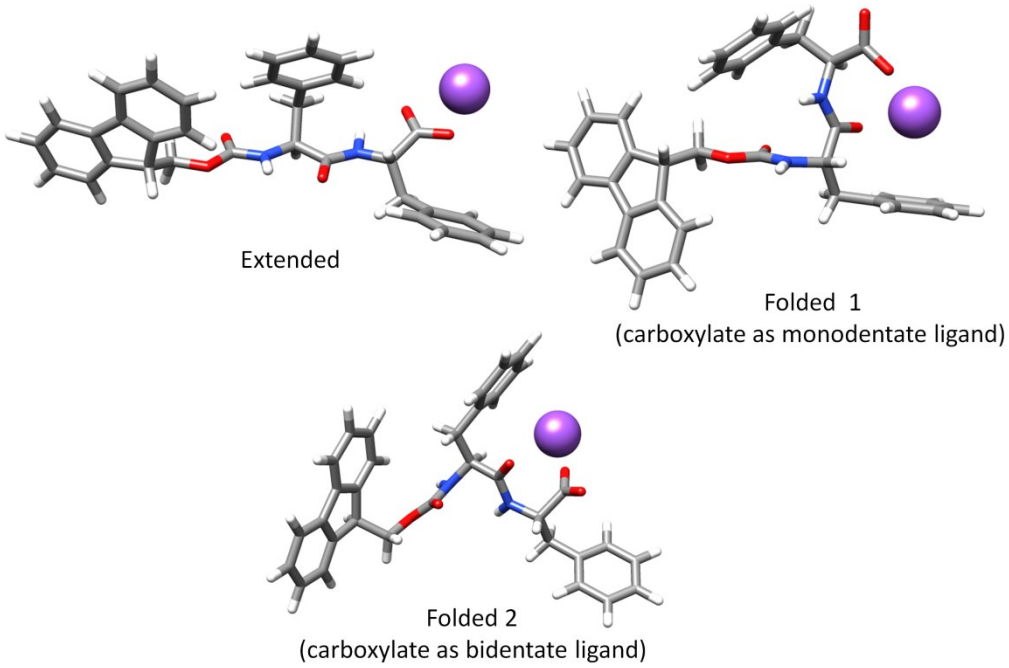


Figure S11. Structures minimized for Fmoc-FF Na^+. Color coding: C, gray; N, blue; O, red; H, white, Na, purple.

Finally we studied the exchange of Na^+ by Cs^+ or Ca^{2+}. The corresponding ΔG for the exchange process was calculated as: $\Delta G = G(\text{Fmoc-FF } M^{n+}) + G(Na^+) - G(\text{Fmoc-FF } Na^+) - G(M^{n+})$. The BSEE-corrected values of $G(\text{Fmoc-FF } M^{n+})$ and $G(\text{Fmoc-FF } Na^+)$ were used and the most stable of the structures tested was considered for Fmoc-FF Na^+.

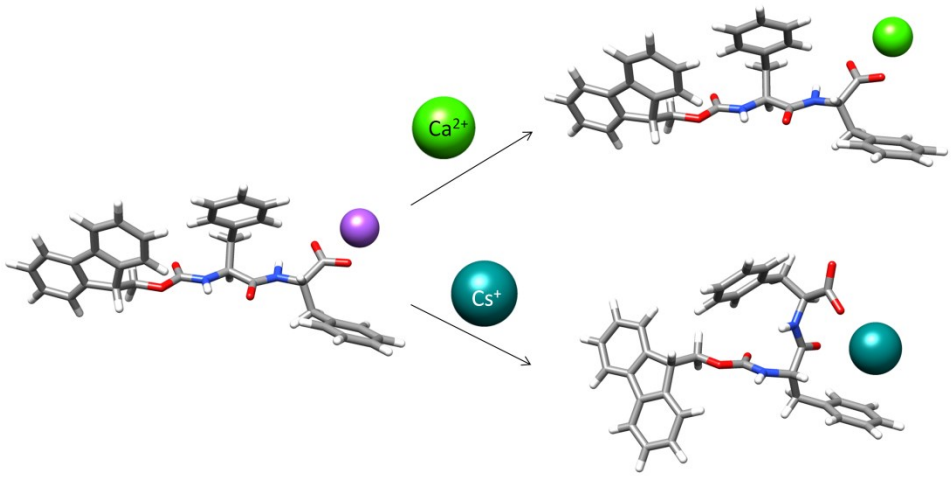
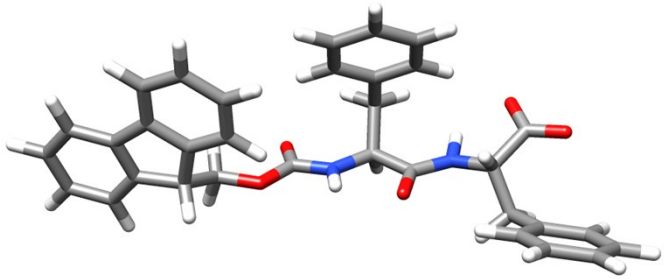


Figure S12. Cation exchange process from Fmoc-FF Na^+ studied by DFT calculations. Color coding: C, gray; N, blue; O, red; H, white, Na, purple, Ca, green, Cs, green-blue.

Table S1. Atomic coordinates for the calculated structure of Fmoc-FF (extended form)

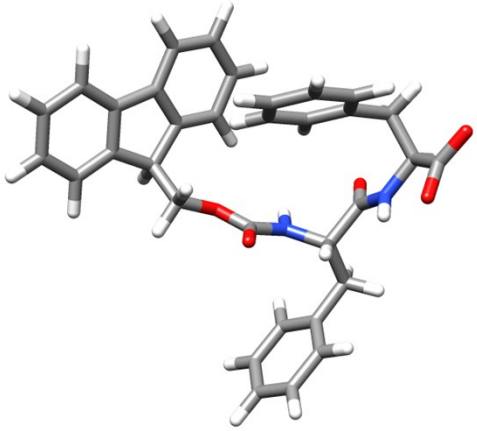


Atom	X	Y	Z
N	-0.242923	-1.033472	-0.982668
C	0.894514	-0.713468	-1.803482
C	2.123066	-0.791212	-0.881598
O	1.996736	-1.038562	0.314936
C	0.780180	0.670954	-2.485779
C	0.771691	1.823461	-1.514385
C	1.962915	2.481153	-1.175573
C	-0.420397	2.237512	-0.906197
C	1.960559	3.520284	-0.244723
C	-0.424453	3.280158	0.020203
C	0.767569	3.922143	0.357476
H	-0.046965	-1.149049	0.008239
H	1.011705	-1.468939	-2.599818
H	1.609541	0.783569	-3.199754
H	-0.144668	0.656228	-3.078873
H	2.900384	2.174402	-1.650585
H	-1.361016	1.746551	-1.170519
H	2.897368	4.022985	0.007248
H	-1.365961	3.594863	0.475694
H	0.765160	4.739121	1.082648
N	3.298801	-0.548548	-1.470025
C	4.513867	-0.290179	-0.730468
C	5.423907	0.611679	-1.620277
O	4.901447	1.033269	-2.682711
C	5.206366	-1.588701	-0.271093
C	6.061078	-1.423228	0.959843
C	5.447996	-1.196693	2.199890
C	7.456842	-1.492271	0.910990
C	6.207693	-1.039602	3.358543
C	8.221748	-1.341449	2.068039
C	7.600381	-1.112783	3.296600
H	3.355622	-0.147925	-2.408517
H	4.253413	0.295433	0.169287
H	4.411948	-2.315260	-0.041100
H	5.798801	-2.007880	-1.098690
H	4.356112	-1.144571	2.248300
H	7.948639	-1.655011	-0.050552
H	5.710203	-0.864169	4.315547
H	9.311442	-1.399805	2.009505
H	8.199001	-0.993261	4.202645
O	6.566830	0.834340	-1.193514
C	-1.488538	-1.213257	-1.459400
O	-2.336974	-1.511626	-0.457874
O	-1.826251	-1.114638	-2.623314
C	-3.720776	-1.566619	-0.766094
H	-3.972997	-0.792561	-1.506258
H	-3.963133	-2.548379	-1.202802
C	-4.497198	-1.347725	0.527858
C	-5.993616	-1.406836	0.299306
H	-4.185865	-2.122718	1.249746
C	-4.314214	0.033833	1.125642
C	-6.759765	-2.440042	-0.229440
C	-6.598833	-0.202064	0.698055
C	-3.150042	0.660129	1.561277
C	-5.552993	0.694440	1.216410
C	-8.140615	-2.259835	-0.356543
H	-6.297437	-3.380915	-0.537897
C	-7.976137	-0.022793	0.573233
C	-3.237035	1.950702	2.092398
H	-2.186021	0.154481	1.489105
C	-5.637895	1.984118	1.740218
C	-8.742128	-1.061961	0.041421
H	-8.755197	-3.063047	-0.768803
H	-8.450138	0.911020	0.884057
C	-4.468363	2.607454	2.179009

H	-2.332298	2.448402	2.448109	H	-9.822230	-0.938663	-0.064056
H	-6.599390	2.497728	1.811552	H	-4.515721	3.615413	2.597057

Charge = −1; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.560957 (Hartree/Particle)
Thermal correction to Energy = 0.594497
Thermal correction to Enthalpy = 0.595442
Thermal correction to Gibbs Free Energy = 0.489216
Sum of electronic and zero-point Energies = –1758.481112
Sum of electronic and thermal Energies = –1758.447571
Sum of electronic and thermal Enthalpies = –1758.446627
Sum of electronic and thermal Free Energies = –1758.552853

Table S2. Atomic coordinates for the calculated structure of Fmoc-FF (folded form)



Atom	X	Y	Z	C	3.612362	4.937979	-1.481959
O	4.385472	-2.522215	-1.893262	H	4.003671	5.186176	-2.471260
O	4.522678	-4.531424	-0.902481	C	3.894275	3.694942	-0.916713
O	2.709461	-0.660527	2.203009	H	4.503744	2.973948	-1.469133
O	0.401053	1.453178	-1.271019	C	3.663412	1.986741	0.927875
O	-0.881072	1.689294	0.572479	H	3.632565	2.014097	2.028191
N	1.286864	1.318840	0.830938	H	4.662813	1.628151	0.637208
H	1.050163	1.239909	1.813685	C	2.628633	0.958138	0.439849
N	3.425528	-1.310224	0.144106	H	2.638942	0.924782	-0.657572
H	3.602380	-1.074454	-0.835028	C	2.933399	-0.428605	1.016731
C	3.407582	3.355565	0.351928	C	3.678715	-2.710602	0.386109
C	2.627530	4.291534	1.040969	H	4.446351	-2.829913	1.170630
H	2.238242	4.040146	2.030920	C	4.259168	-3.316425	-0.934957
C	2.341993	5.536175	0.478810	C	2.421956	-3.479046	0.840308
H	1.732590	6.254581	1.032010	H	2.708337	-4.539354	0.882390
C	2.833500	5.863108	-0.785152	H	2.153910	-3.150999	1.854091
H	2.611982	6.837627	-1.226036	C	1.245990	-3.283332	-0.083662

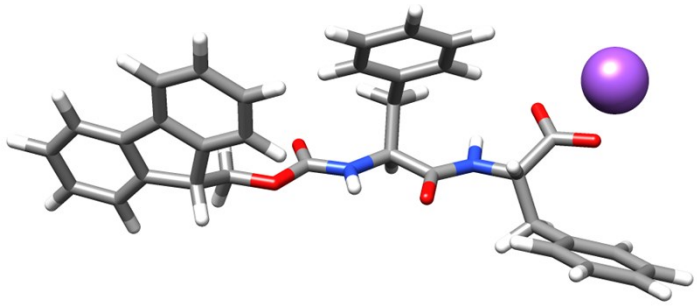
C	1.125546	-4.028411	-1.265386	H	-4.704856	3.295421	-1.030821
H	1.889100	-4.775888	-1.496315	C	-6.251183	1.944841	-1.719318
C	0.056815	-3.819970	-2.136750	H	-6.736724	2.700388	-2.340785
H	-0.021321	-4.413476	-3.051071	C	-6.777047	0.650570	-1.664754
C	-0.913570	-2.860221	-1.841971	H	-7.668848	0.404883	-2.245519
H	-1.754601	-2.700050	-2.520616	C	-6.174922	-0.330292	-0.874074
C	-0.804715	-2.111545	-0.670553	H	-6.590101	-1.339824	-0.833060
H	-1.565453	-1.369489	-0.417606	C	-5.037958	0.006077	-0.140328
C	0.267644	-2.321027	0.196930	C	-4.209042	-0.794754	0.775035
H	0.344468	-1.730123	1.113787	C	-4.317331	-2.126789	1.173160
C	0.285604	1.480879	-0.063103	H	-5.112282	-2.765794	0.782084
C	-2.052021	1.729454	-0.227177	C	-3.390561	-2.630000	2.087719
H	-1.968655	0.997644	-1.044644	H	-3.458561	-3.671471	2.409515
H	-2.160030	2.729125	-0.676853	C	-2.379189	-1.812069	2.599566
C	-3.256929	1.404222	0.654016	H	-1.664650	-2.220886	3.317290
H	-3.353011	2.184447	1.427755	C	-2.267176	-0.478563	2.196936
C	-4.508285	1.307565	-0.195487	H	-1.471596	0.154735	2.591573
C	-5.109770	2.281570	-0.984700	C	-3.180434	0.023777	1.275117

Charge = −1; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.561138 (Hartree/Particle)
Thermal correction to Energy = 0.594802
Thermal correction to Enthalpy = 0.595746
Thermal correction to Gibbs Free Energy = 0.489071
Sum of electronic and zero-point Energies = –1758.485344
Sum of electronic and thermal Energies = –1758.451680
Sum of electronic and thermal Enthalpies = –1758.450736
Sum of electronic and thermal Free Energies = –1758.557411

Table S3. Thermochemistry parameters of calculated Na^+, Cs^+ and Ca^{2+} ions

	Na^+	Cs^+	Ca^{2+}
Charge (multiplicity)	1 (0)	1 (0)	2 (0)
Zero-point correction	0.000000	0.000000	0.000000
Thermal correction to Energy	0.001416	0.001416	0.001416
Thermal correction to Enthalpy	0.002360	0.002360	0.002360
Thermal correction to Gibbs Free Energy	-0.014429	-0.016914	-0.015212
Sum of electronic and zero-point Energies	-0.159212	-19.847052	-36.456445
Sum of electronic and thermal Energies	-0.157796	-19.845636	-36.455029
Sum of electronic and thermal Enthalpies	-0.156852	-19.844691	-36.454085
Sum of electronic and thermal Free Energies	-0.173641	-19.863966	-36.471657

Table S4. Atomic coordinates for the calculated structure of Fmoc-FF Na^+ in its extended form

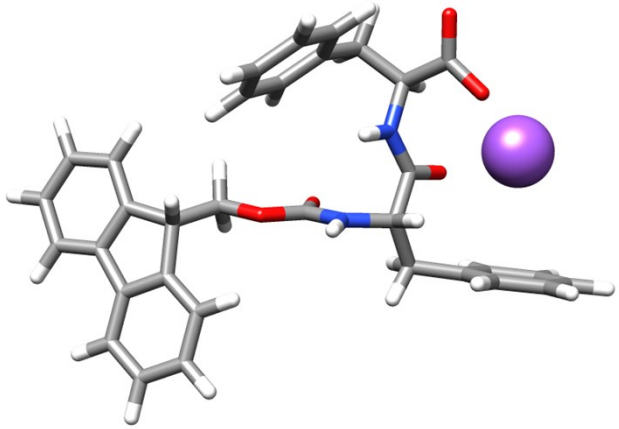


Atom	X	Y	Z
N	-0.522175	-0.672736	-1.126485
C	0.648251	-0.129482	-1.761395
C	1.844550	-0.559221	-0.899624
O	1.680892	-1.172875	0.149862
C	0.590652	1.409197	-1.922471
C	0.604643	2.151690	-0.610916
C	1.810586	2.611868	-0.063314
C	-0.580631	2.363208	0.105186
C	1.830785	3.259185	1.172145
C	-0.562630	3.015226	1.337989
C	0.644083	3.461802	1.877630
H	-0.370197	-1.112045	-0.222592
H	0.768953	-0.566580	-2.767606
H	1.434349	1.732226	-2.550481
H	-0.324529	1.633044	-2.488196
H	2.744018	2.459182	-0.613669
H	-1.532418	2.023062	-0.311809
H	2.779739	3.611497	1.583305
H	-1.498776	3.180420	1.875752
H	0.658254	3.972908	2.842887
N	3.046987	-0.189201	-1.359058
C	4.247179	-0.275726	-0.558771
C	5.221522	0.820924	-1.038587
O	4.819459	1.582196	-1.956973
C	4.865052	-1.689416	-0.599426
C	5.759742	-2.011495	0.570648
C	5.204793	-2.152814	1.849833
C	7.138279	-2.189562	0.419495
C	6.006522	-2.455041	2.949675
C	7.944552	-2.497348	1.515837
C	7.382005	-2.628822	2.785955
H	3.140948	0.453203	-2.143801
H	3.983283	-0.045529	0.487857
H	4.023981	-2.399410	-0.597709
H	5.403460	-1.829048	-1.549298
H	4.125678	-2.026164	1.978693
H	7.586491	-2.077536	-0.570830
H	5.554736	-2.561177	3.938863
H	9.019866	-2.633440	1.377365
H	8.012534	-2.868627	3.645187
O	6.337765	0.881623	-0.486705
C	-1.745484	-0.666790	-1.689394
O	-2.639670	-1.260204	-0.878372
O	-2.025195	-0.189027	-2.771695
C	-4.006918	-1.200709	-1.253541
H	-4.228564	-0.227970	-1.717322
H	-4.220884	-1.988696	-1.992813
C	-4.846072	-1.400887	0.003731
C	-6.329149	-1.369503	-0.303599
H	-4.564886	-2.368971	0.453540
C	-4.701192	-0.284660	1.019903
C	-7.061962	-2.172753	-1.170627
C	-6.960467	-0.348879	0.428758
C	-3.563804	0.157667	1.689312
C	-5.946792	0.325032	1.256110
C	-8.435990	-1.947946	-1.300290
H	-6.579019	-2.970647	-1.739998
C	-8.331062	-0.125637	0.301059
C	-3.684712	1.212515	2.599192
H	-2.594648	-0.309394	1.507996
C	-6.065216	1.381181	2.159152
C	-9.063617	-0.933564	-0.570779
H	-9.024755	-2.571847	-1.976253
H	-8.825400	0.665069	0.869997
C	-4.922656	1.820121	2.830407
H	-2.802285	1.560121	3.140784
H	-7.032218	1.854738	2.343536

H	-10.137984	-0.772521	-0.683680	Na	6.984375	2.649577	-1.953721
H	-4.996600	2.642470	3.545413				

Charge = 0; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.562301 (Hartree/Particle)
Thermal correction to Energy = 0.597899
Thermal correction to Enthalpy = 0.598844
Thermal correction to Gibbs Free Energy = 0.487072
Sum of electronic and zero-point Energies = –1758.664925
Sum of electronic and thermal Energies = –1758.629327
Sum of electronic and thermal Enthalpies = –1758.628383
Sum of electronic and thermal Free Energies = –1758.740155
BSSE correction: 0.007223333439
BSSE-corrected Free Energy: –1758.732932

Table S5. Atomic coordinates for the calculated structure of Fmoc-FF Na^+ in its folded form 1 (carboxylate as monodentate ligand)

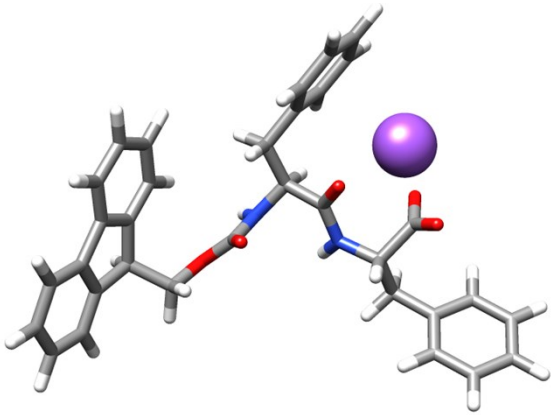


Atom	X	Y	Z	C	6.324498	-3.846854	-0.100048
O	4.918664	1.855161	0.401811	H	7.371091	-4.159274	-0.087941
O	4.451543	4.014965	0.046763	C	5.619185	-3.793611	-1.303782
O	3.488664	-0.383874	-1.404776	H	6.113297	-4.064413	-2.239835
O	-0.098187	-0.588554	-1.420015	C	4.285943	-3.387241	-1.317854
O	-1.466610	-0.294078	0.352772	H	3.747325	-3.326688	-2.266234
N	0.669080	-0.762359	0.721480	C	2.195781	-2.547096	-0.150058
H	0.378178	-0.782435	1.690731	H	1.585357	-3.196294	0.497182
N	2.333594	1.197164	-0.272525	H	1.778990	-2.609409	-1.164506
H	1.682075	1.354694	0.489222	C	2.030252	-1.114695	0.380145
C	3.629715	-3.027621	-0.135421	H	2.596336	-1.030697	1.321530
C	4.341984	-3.104145	1.067927	C	2.671163	-0.064642	-0.542177
H	3.849053	-2.845686	2.009770	C	2.959312	2.358212	-0.865304
C	5.678927	-3.504137	1.087928	H	3.361887	2.033510	-1.837862
H	6.216326	-3.551863	2.037877	C	4.219511	2.800554	-0.044783

C	1.922802	3.465219	-1.109649	C	-5.237519	1.647412	-1.144714
H	2.484051	4.391337	-1.288252	H	-4.480417	2.435519	-1.128361
H	1.351939	3.226862	-2.019284	C	-6.438654	1.839847	-1.834161
C	0.962668	3.640442	0.042963	H	-6.615691	2.780561	-2.359942
C	1.352529	4.296428	1.218972	C	-7.415895	0.839929	-1.854888
H	2.358804	4.719068	1.271446	H	-8.348465	1.007551	-2.398163
C	0.480333	4.389640	2.303259	C	-7.212416	-0.369419	-1.187367
H	0.798427	4.906758	3.211694	H	-7.979065	-1.147287	-1.205148
C	-0.794015	3.822939	2.234329	C	-6.013844	-0.561772	-0.501111
H	-1.475092	3.895434	3.085113	C	-5.523306	-1.707631	0.281772
C	-1.191068	3.162438	1.072060	C	-6.114647	-2.939079	0.561441
H	-2.185801	2.715107	1.009015	H	-7.108444	-3.183100	0.179050
C	-0.317404	3.073287	-0.011912	C	-5.410697	-3.858378	1.341478
H	-0.625822	2.547819	-0.920671	H	-5.857784	-4.828279	1.570402
C	-0.285105	-0.559927	-0.222030	C	-4.138768	-3.548972	1.833042
C	-2.554711	0.010848	-0.508772	H	-3.602534	-4.280049	2.442172
H	-2.608464	-0.722107	-1.327527	C	-3.546414	-2.313119	1.555198
H	-2.398294	1.006587	-0.953542	H	-2.552967	-2.074306	1.938822
C	-3.829785	-0.008972	0.323940	C	-4.244354	-1.395019	0.776905
H	-3.679364	0.652236	1.195366	Na	5.657012	-0.052121	-0.573687
C	-5.031566	0.443395	-0.480107				

Charge = 0; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.563015 (Hartree/Particle)
Thermal correction to Energy = 0.598240
Thermal correction to Enthalpy = 0.599184
Thermal correction to Gibbs Free Energy = 0.491292
Sum of electronic and zero-point Energies = –1758.661040
Sum of electronic and thermal Energies = –1758.625815
Sum of electronic and thermal Enthalpies = –1758.624871
Sum of electronic and thermal Free Energies = –1758.732763
BSSE correction: 0.008651093543
BSSE-corrected Free Energy: –1758.724112

Table S6. Atomic coordinates for the calculated structure of Fmoc-FF Na^+ in its folded form 2 (carboxylate as bidentate ligand)

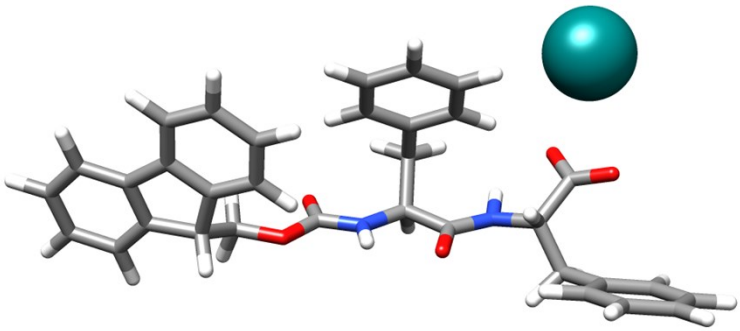


Atom	X	Y	Z
O	-4.446997	0.793093	0.534609
O	-4.678055	-0.039086	-1.523447
O	-1.774240	1.127544	-0.814918
O	1.162673	-0.552699	-0.245219
O	2.133779	-1.210612	1.695274
N	0.426398	0.204900	1.770847
H	0.649715	0.235080	2.758062
N	-2.028962	-0.579882	0.640916
H	-1.717261	-0.969254	1.523463
C	-0.649103	3.572873	0.275299
C	-1.546845	4.112513	1.204976
H	-1.596307	3.705079	2.218496
C	-2.390474	5.168950	0.858572
H	-3.088537	5.569027	1.597541
C	-2.339382	5.715966	-0.423754
H	-2.998500	6.543022	-0.696718
C	-1.433317	5.200894	-1.352738
H	-1.379933	5.624356	-2.358419
C	-0.599783	4.139108	-1.003869
H	0.092961	3.728662	-1.742780
C	0.253023	2.416505	0.640829
H	0.970843	2.748119	1.408676
H	0.838604	2.101708	-0.233919
C	-0.474962	1.196725	1.221637
H	-1.101110	1.527425	2.064827
C	-1.466033	0.565551	0.232341
C	-3.184897	-1.161116	-0.019157
H	-2.880809	-1.638764	-0.964461
C	-4.212308	-0.046940	-0.367085
C	-3.801272	-2.210052	0.914383
H	-3.026559	-2.948700	1.177443
H	-4.101531	-1.698209	1.842222
C	-4.987237	-2.914357	0.305518
C	-4.808141	-4.028391	-0.523492
H	-3.797770	-4.405653	-0.705434
C	-5.900458	-4.662467	-1.114980
H	-5.741670	-5.532209	-1.756745
C	-7.193155	-4.189897	-0.884254
H	-8.050207	-4.687008	-1.344169
C	-7.384030	-3.080558	-0.059812
H	-8.392537	-2.704585	0.127555
C	-6.288429	-2.448662	0.528184
H	-6.440748	-1.575811	1.168563
C	1.242481	-0.522210	0.964080
C	3.145169	-1.936110	1.008918
H	2.870606	-2.037336	-0.049593
H	3.184528	-2.933117	1.467964
C	4.505095	-1.240873	1.143177
H	4.772006	-1.199140	2.212615
C	5.551138	-1.987487	0.340429
C	5.984030	-3.302301	0.472971
H	5.586196	-3.948128	1.259529
C	6.948108	-3.788076	-0.415970
H	7.301124	-4.817263	-0.321468
C	7.465773	-2.968104	-1.423186
H	8.218203	-3.364092	-2.108711
C	7.032244	-1.647866	-1.560070

H	7.440699	-1.009830	-2.347038	C	4.035759	2.456142	0.128800
C	6.071931	-1.163260	-0.672896	H	3.490137	3.363703	0.396769
C	5.444666	0.163356	-0.561136	C	3.850676	1.291104	0.878745
C	5.632108	1.325822	-1.308081	H	3.173353	1.292037	1.735142
H	6.329211	1.344728	-2.148890	C	4.546528	0.140641	0.521062
C	4.914694	2.471411	-0.958220	Na	-3.763049	2.376521	-1.149766
H	5.047923	3.390390	-1.533139				

Charge = 0; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.562959 (Hartree/Particle)
Thermal correction to Energy = 0.598192
Thermal correction to Enthalpy = 0.599137
Thermal correction to Gibbs Free Energy = 0.490764
Sum of electronic and zero-point Energies = –1758.655277
Sum of electronic and thermal Energies = –1758.620043
Sum of electronic and thermal Enthalpies = –1758.619099
Sum of electronic and thermal Free Energies = –1758.727472
BSSE correction: 0.010421491585
BSSE-corrected Free Energy: –1758.717051

Table S7. Atomic coordinates for the calculated structure of Fmoc-FF Cs^+ in its extended form

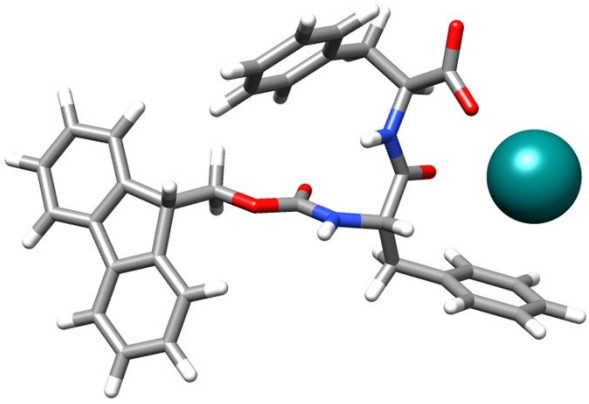


Atom	X	Y	Z	H	0.042417	1.211668	-2.890411
N	1.356138	1.220448	-1.260116	H	-0.947360	-0.962875	-2.522660
C	0.117056	0.809891	-1.864869	H	0.811073	-1.095597	-2.553115
C	-1.001548	1.439461	-1.017502	H	-2.212107	-1.335929	-0.456372
O	-0.740659	2.087258	-0.008854	H	2.074657	-1.653187	-0.480036
C	-0.034257	-0.728837	-1.955270	H	-2.273818	-2.370934	1.819897
C	-0.068070	-1.410774	-0.611631	H	2.019049	-2.707436	1.759681
C	-1.286543	-1.630307	0.045893	H	-0.156892	-3.064911	2.924230
C	1.115955	-1.803565	0.023892	N	-2.247896	1.196815	-1.443793
C	-1.319124	-2.214753	1.311556	C	-3.414494	1.390825	-0.611438
C	1.085073	-2.399610	1.284770	C	-4.420941	0.255240	-0.942061
C	-0.132205	-2.601932	1.935300	O	-4.063820	-0.554314	-1.838182
H	1.266347	1.734191	-0.387284	C	-4.013041	2.802510	-0.769516

C	-4.907940	3.226299	0.367783	C	7.169595	0.939013	-0.289259
C	-4.354167	3.483846	1.629109	H	5.615223	2.319216	0.316028
C	-6.288019	3.380499	0.203533	C	5.337424	0.306249	1.060325
C	-5.157295	3.876775	2.698911	C	8.060730	1.506931	-1.193355
C	-7.095974	3.778416	1.269058	C	7.577495	-0.114524	0.547704
C	-6.533987	4.026003	2.522040	C	4.124092	0.151165	1.724204
H	-2.426256	0.494272	-2.162719	C	6.437144	-0.506338	1.391584
H	-3.111950	1.261901	0.441544	C	9.367483	1.012876	-1.254365
H	-3.164710	3.502395	-0.826409	H	7.752797	2.328570	-1.844560
H	-4.549064	2.869521	-1.729051	C	8.881110	-0.606084	0.488548
H	-3.274536	3.375377	1.769939	C	4.021891	-0.820341	2.724594
H	-6.736320	3.177257	-0.772171	H	3.266575	0.775112	1.468480
H	-4.705636	4.072925	3.674328	C	6.332264	-1.480264	2.384080
H	-8.172317	3.893951	1.120189	C	9.772863	-0.033912	-0.420730
H	-7.165760	4.336848	3.357270	H	10.078642	1.449997	-1.958624
O	-5.467968	0.218166	-0.276537	H	9.201850	-1.422885	1.139122
C	2.570451	0.964067	-1.781501	C	5.114226	-1.630272	3.049721
O	3.537793	1.464648	-0.991102	H	3.077559	-0.945443	3.258731
O	2.787861	0.359011	-2.813573	H	7.185564	-2.111881	2.641598
C	4.879731	1.128840	-1.307635	H	10.797672	-0.406831	-0.481159
H	4.930291	0.096464	-1.684602	H	5.014422	-2.384203	3.833711
H	5.251087	1.799734	-2.098357	Cs	-5.046905	-3.098458	-0.289885
C	5.714340	1.279091	-0.040169				

Charge = 0; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.561865 (Hartree/Particle)
Thermal correction to Energy = 0.597783
Thermal correction to Enthalpy = 0.598727
Thermal correction to Gibbs Free Energy = 0.484571
Sum of electronic and zero-point Energies = –1778.349234
Sum of electronic and thermal Energies = –1778.313316
Sum of electronic and thermal Enthalpies = –1778.312371
Sum of electronic and thermal Free Energies = –1778.426527
BSSE correction: 0.005450954395
BSSE-corrected Free Energy: –1778.421076

Table S8. Atomic coordinates for the calculated structure of Fmoc-FF Cs^+ in its folded form 1 (carboxylate as monodentate ligand)

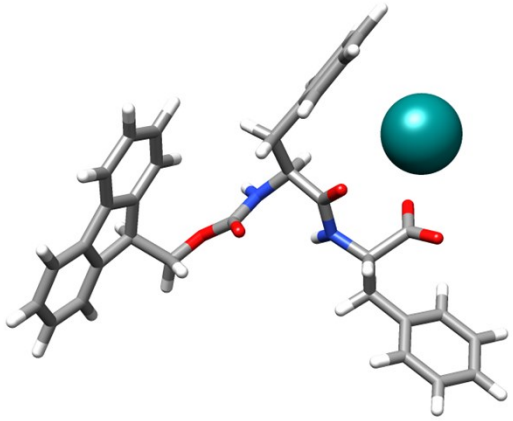


Atom	X	Y	Z
O	3.748672	2.193280	0.999648
O	3.515284	4.248111	0.132881
O	2.630228	-0.216201	-1.522187
O	-0.899148	-0.499679	-1.477381
O	-2.263091	-0.318785	0.312175
N	-0.111438	-0.745674	0.649334
H	-0.394459	-0.829232	1.617591
N	1.529242	1.290206	-0.236468
H	0.929832	1.386855	0.575233
C	2.846703	-2.974513	-0.262182
C	3.443527	-3.234178	0.979080
H	2.876016	-3.066817	1.899065
C	4.755204	-3.703818	1.063195
H	5.201907	-3.892914	2.041681
C	5.493130	-3.929378	-0.099727
H	6.521216	-4.292133	-0.037794
C	4.907005	-3.682130	-1.342899
H	5.477199	-3.850457	-2.259136
C	3.597671	-3.207688	-1.420293
H	3.153336	-2.996376	-2.394801
C	1.428406	-2.456709	-0.332735
H	0.772642	-3.135195	0.234431
H	1.073305	-2.445815	-1.371620
C	1.252155	-1.057519	0.276909
H	1.826057	-1.013532	1.216514
C	1.858865	0.048216	-0.606176
C	2.143662	2.491431	-0.748161
H	2.697080	2.194327	-1.652655
C	3.231467	3.040463	0.239736
C	1.080163	3.526656	-1.143348
H	1.612045	4.464262	-1.349147
H	0.582700	3.198553	-2.068522
C	0.044613	3.728605	-0.063760
C	0.307562	4.540699	1.048229
H	1.267020	5.060915	1.099855
C	-0.627742	4.667228	2.075108
H	-0.407863	5.307306	2.932962
C	-1.840108	3.977758	2.012493
H	-2.571841	4.076234	2.817420
C	-2.110344	3.160986	0.914687
H	-3.055547	2.616300	0.859759
C	-1.174243	3.039103	-0.112346
H	-1.381757	2.391266	-0.969333
C	-1.077218	-0.527327	-0.277575
C	-3.369033	-0.051696	-0.539402
H	-3.424098	-0.806703	-1.338145
H	-3.236407	0.933578	-1.014094
C	-4.631100	-0.071113	0.313102
H	-4.483609	0.621682	1.160224
C	-5.853850	0.327691	-0.488005
C	-6.096098	1.503473	-1.190003
H	-5.357150	2.308402	-1.211868
C	-7.310653	1.645841	-1.868085
H	-7.515860	2.563964	-2.422745
C	-8.265490	0.624749	-1.840749
H	-9.209005	0.753207	-2.375713
C	-8.025593	-0.556381	-1.135867

H	-8.774799	-1.351030	-1.116210	C	-4.842149	-3.562113	1.947878
C	-6.813685	-0.698830	-0.460938	H	-4.281995	-4.259594	2.574630
C	-6.287307	-1.806406	0.353183	C	-4.280729	-2.324505	1.618700
C	-6.847793	-3.039649	0.683981	H	-3.287825	-2.050885	1.979547
H	-7.841191	-3.318165	0.324866	C	-5.008817	-1.449516	0.818721
C	-6.113474	-3.915730	1.485445	Cs	5.438196	-0.130718	-0.060852
H	-6.536185	-4.886259	1.754529				

Charge = 0; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.562263 (Hartree/Particle)
Thermal correction to Energy = 0.598053
Thermal correction to Enthalpy = 0.598997
Thermal correction to Gibbs Free Energy = 0.488032
Sum of electronic and zero-point Energies = –1778.354811
Sum of electronic and thermal Energies = –1778.319021
Sum of electronic and thermal Enthalpies = –1778.318077
Sum of electronic and thermal Free Energies = –1778.429042
BSSE correction: 0.006468482187
BSSE-corrected Free Energy: –1778.422574

Table S9. Atomic coordinates for the calculated structure of Fmoc-FF Cs^+ in its folded form 2 (carboxylate as bidentate ligand)

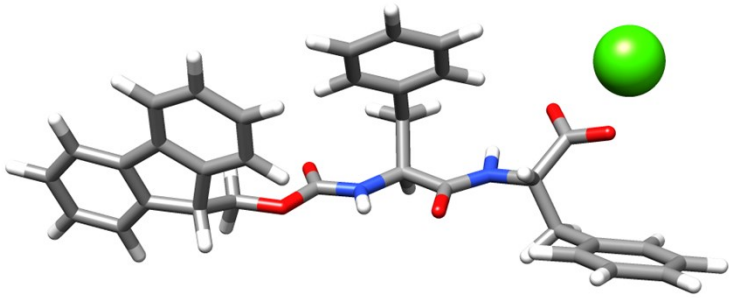


Atom	X	Y	Z	H	0.918542	1.476873	1.528245
O	3.929353	0.535316	1.208614	C	0.517336	-3.240292	0.301371
O	4.474203	1.206917	-0.859847	C	1.220431	-3.800666	1.376326
O	1.449788	-0.573696	-0.786209	H	1.062369	-3.417944	2.388467
O	-1.807830	0.654022	-0.270818	C	2.119163	-4.850162	1.179299
O	-2.847951	1.158613	1.679505	H	2.660055	-5.267635	2.031298
N	-0.965464	-0.015622	1.735745	C	2.325703	-5.364625	-0.101671
H	-1.183395	-0.092272	2.721584	H	3.030699	-6.183449	-0.259352
N	1.295459	1.172320	0.638460	C	1.629847	-4.816113	-1.180937

H	1.789762	-5.204628	-2.189108	C	-3.945402	1.756027	1.002452
C	0.737897	-3.762860	-0.978523	H	-3.688724	1.901956	-0.055350
H	0.208170	-3.329899	-1.829876	H	-4.110054	2.735336	1.471731
C	-0.488231	-2.136531	0.527576	C	-5.206252	0.892871	1.129872
H	-1.266032	-2.514865	1.209504	H	-5.464078	0.805848	2.198801
H	-0.985618	-1.873771	-0.415592	C	-6.339479	1.509976	0.335141
C	0.069455	-0.859163	1.172754	C	-6.936166	2.757433	0.482241
H	0.718360	-1.140043	2.017589	H	-6.623592	3.439708	1.276472
C	0.986550	-0.067026	0.228504	C	-7.954645	3.126880	-0.402045
C	2.380908	1.926284	0.041441	H	-8.435451	4.101747	-0.295956
H	2.165454	2.095348	-1.024959	C	-8.364658	2.259361	-1.418907
C	3.726920	1.146453	0.135745	H	-9.161940	2.564256	-2.100300
C	2.483410	3.273526	0.768903	C	-7.766806	1.006662	-1.570408
H	1.504028	3.776480	0.718555	H	-8.091778	0.330830	-2.364662
H	2.693642	3.066028	1.830913	C	-6.751907	0.638137	-0.688172
C	3.549977	4.178637	0.208141	C	-5.961151	-0.599244	-0.591406
C	3.276707	5.016016	-0.880023	C	-5.999577	-1.766748	-1.352921
H	2.270331	5.028141	-1.308426	H	-6.688222	-1.863532	-2.195348
C	4.268814	5.833799	-1.420890	C	-5.143025	-2.816353	-1.015587
H	4.035983	6.482275	-2.268804	H	-5.158568	-3.737768	-1.601721
C	5.554106	5.827308	-0.877333	C	-4.273440	-2.703091	0.073183
H	6.331766	6.469528	-1.296993	H	-3.617950	-3.537970	0.331170
C	5.838152	4.997552	0.208121	C	-4.237193	-1.533062	0.837192
H	6.841325	4.986519	0.640861	H	-3.565815	-1.457740	1.695029
C	4.843251	4.180201	0.743444	C	-5.073031	-0.475689	0.492147
H	5.071349	3.524690	1.588174	Cs	4.137702	-2.144292	-0.491845
C	-1.875111	0.602133	0.938319				

Charge = 0; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.562181 (Hartree/Particle)
Thermal correction to Energy = 0.598023
Thermal correction to Enthalpy = 0.598968
Thermal correction to Gibbs Free Energy = 0.487182
Sum of electronic and zero-point Energies = –1778.349609
Sum of electronic and thermal Energies = –1778.313767
Sum of electronic and thermal Enthalpies = –1778.312822
Sum of electronic and thermal Free Energies = –1778.424608
BSSE correction: 0.007801174657
BSSE-corrected Free Energy: –1778.416807

Table S10. Atomic coordinates for the calculated structure of Fmoc-FF Ca^{2+} in its extended form

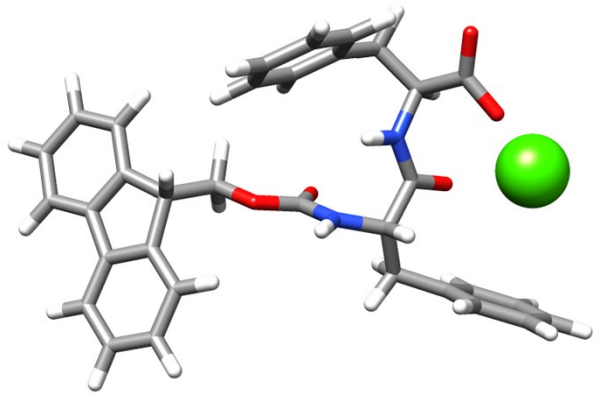


Atom	X	Y	Z
N	-0.724899	-0.698304	-1.271628
C	0.466605	-0.088881	-1.799329
C	1.633191	-0.638756	-0.964913
O	1.438429	-1.392890	-0.018753
C	0.423607	1.458782	-1.768867
C	0.336192	2.033017	-0.378230
C	1.495409	2.385564	0.326883
C	-0.905207	2.195880	0.249817
C	1.415456	2.876949	1.629847
C	-0.987891	2.693267	1.550062
C	0.173135	3.030543	2.246448
H	-0.597391	-1.260776	-0.434671
H	0.618957	-0.397027	-2.848498
H	1.312006	1.849351	-2.287066
H	-0.446401	1.761402	-2.367233
H	2.470964	2.277804	-0.155932
H	-1.821195	1.943734	-0.291396
H	2.329462	3.146982	2.163984
H	-1.966180	2.820131	2.018558
H	0.108999	3.419854	3.265018
N	2.852132	-0.211813	-1.327276
C	4.021652	-0.425662	-0.506946
C	4.966838	0.765158	-0.698202
O	4.627489	1.662197	-1.516070
C	4.696686	-1.781273	-0.808921
C	5.603062	-2.283071	0.286599
C	5.053142	-2.695905	1.507596
C	6.989168	-2.358909	0.119668
C	5.867120	-3.164688	2.537587
C	7.807909	-2.832115	1.145606
C	7.249976	-3.234394	2.359431
H	2.965242	0.519130	-2.024636
H	3.706432	-0.426487	0.549708
H	3.881411	-2.506904	-0.950594
H	5.239004	-1.716241	-1.764574
H	3.968899	-2.650489	1.647495
H	7.433313	-2.036742	-0.825523
H	5.419555	-3.482543	3.482225
H	8.888962	-2.885439	0.996258
H	7.890148	-3.604272	3.163560
O	6.024906	0.800836	-0.033982
C	-1.949034	-0.547967	-1.812615
O	-2.867394	-1.218780	-1.094101
O	-2.212103	0.105818	-2.803317
C	-4.232090	-1.017380	-1.428201
H	-4.397592	0.031700	-1.715529
H	-4.499583	-1.652912	-2.287079
C	-5.074792	-1.378734	-0.209569
C	-6.552670	-1.185883	-0.482373
H	-4.862972	-2.428672	0.057251
C	-4.844406	-0.472797	0.984574
C	-7.346473	-1.768558	-1.464410
C	-7.102900	-0.263502	0.424946
C	-3.676500	-0.242830	1.705610
C	-6.039601	0.177719	1.341899
C	-8.699447	-1.421778	-1.532709
H	-6.927359	-2.489277	-2.170755
C	-8.452442	0.081291	0.358127
C	-3.715439	0.640589	2.788713
H	-2.746141	-0.741741	1.430801
C	-6.075988	1.064337	2.417666
C	-9.246275	-0.505140	-0.629494
H	-9.335355	-1.872515	-2.297743
H	-8.883721	0.796056	1.062677
C	-4.902616	1.290367	3.139690

H	-2.807358	0.823078	3.367375	H	-4.912733	1.977600	3.988590
H	-7.003403	1.570134	2.695846	Ca	6.696288	2.993164	-1.033254
H	-10.305235	-0.246285	-0.696847				

Charge = 1; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.562568 (Hartree/Particle)
Thermal correction to Energy = 0.598135
Thermal correction to Enthalpy = 0.599079
Thermal correction to Gibbs Free Energy = 0.487403
Sum of electronic and zero-point Energies = –1794.969683
Sum of electronic and thermal Energies = –1794.934116
Sum of electronic and thermal Enthalpies = –1794.933172
Sum of electronic and thermal Free Energies = –1795.044847
BSSE correction: 0.008224504206
BSSE-corrected Free Energy: –1795.036622

Table S11. Atomic coordinates for the calculated structure of Fmoc-FF Ca^{2+} in its folded form 1 (carboxylate as monodentate ligand)

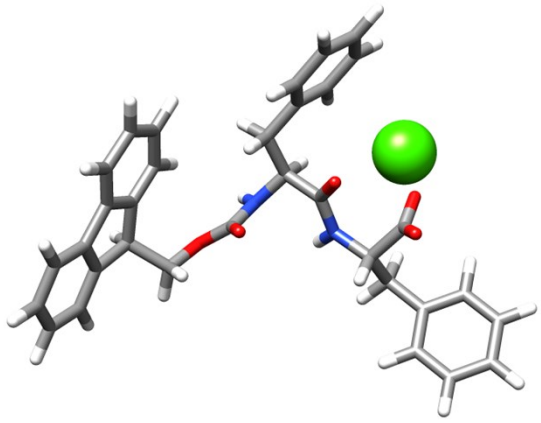


Atom	X	Y	Z	H	6.001678	-3.659843	2.037244
O	4.831975	1.875731	0.361028	C	6.041220	-3.998811	-0.096518
O	4.216736	4.023038	0.294307	H	7.071302	-4.361970	-0.103470
O	3.453976	-0.357102	-1.158644	C	5.309078	-3.933195	-1.283303
O	-0.219236	-0.544589	-1.373222	H	5.765074	-4.245006	-2.225859
O	-1.647391	-0.275992	0.354837	C	3.996996	-3.462345	-1.271721
N	0.484828	-0.703696	0.791346	H	3.437505	-3.394648	-2.207870
H	0.161777	-0.743421	1.749940	C	1.975530	-2.513268	-0.077874
N	2.177034	1.220997	-0.169886	H	1.341545	-3.156237	0.552896
H	1.445765	1.382699	0.516416	H	1.555834	-2.538199	-1.092637
C	3.388885	-3.050542	-0.080472	C	1.845401	-1.089185	0.486976
C	4.126665	-3.139309	1.106564	H	2.382887	-1.049318	1.447936
H	3.671910	-2.841890	2.055941	C	2.548780	-0.036427	-0.379192
C	5.442949	-3.603159	1.100198	C	2.801284	2.382183	-0.772188
				H	3.208598	2.054288	-1.742230

C	4.053623	2.825733	0.052504	C	-5.194239	0.419536	-0.585995
C	1.761020	3.483063	-1.027334	C	-5.388163	1.584141	-1.320735
H	2.317262	4.417307	-1.178255	H	-4.631778	2.372981	-1.334683
H	1.223559	3.252073	-1.958504	C	-6.576458	1.736030	-2.041855
C	0.757878	3.639673	0.091840	H	-6.744132	2.645605	-2.622598
C	1.099142	4.281134	1.290759	C	-7.552836	0.735243	-2.024506
H	2.102678	4.700925	1.390765	H	-8.475344	0.870982	-2.593244
C	0.181628	4.364568	2.337458	C	-7.361089	-0.434825	-1.287229
H	0.461867	4.870690	3.264263	H	-8.126982	-1.213557	-1.275751
C	-1.090330	3.803367	2.207228	C	-6.175386	-0.586800	-0.569269
H	-1.807482	3.869329	3.028310	C	-5.699733	-1.686175	0.286043
C	-1.439109	3.157571	1.021799	C	-6.297296	-2.899510	0.625388
H	-2.432313	2.716906	0.910349	H	-7.283896	-3.165657	0.239219
C	-0.520079	3.076833	-0.024751	C	-5.609435	-3.771405	1.471369
H	-0.792218	2.563754	-0.952003	H	-6.061787	-4.726279	1.748110
C	-0.445911	-0.520253	-0.182257	C	-4.347257	-3.433253	1.969019
C	-2.717086	-0.006093	-0.541895	H	-3.823876	-4.126992	2.630718
H	-2.756701	-0.775972	-1.327112	C	-3.748698	-2.215503	1.631245
H	-2.548094	0.967403	-1.028923	H	-2.763377	-1.953839	2.020688
C	-4.008062	0.014720	0.265492	C	-4.430396	-1.344855	0.786845
H	-3.877062	0.722369	1.102883	Ca	5.794159	0.024121	-0.710550

Charge = 1; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.563818 (Hartree/Particle)
Thermal correction to Energy = 0.598674
Thermal correction to Enthalpy = 0.599618
Thermal correction to Gibbs Free Energy = 0.493119
Sum of electronic and zero-point Energies = –1794.965842
Sum of electronic and thermal Energies = –1794.930986
Sum of electronic and thermal Enthalpies = –1794.930042
Sum of electronic and thermal Free Energies = –1795.036541
BSSE correction: 0.009366287473
BSSE-corrected Free Energy: –1795.027175

Table S12. Atomic coordinates for the calculated structure of Fmoc-FF Ca^{2+} in its folded form 2 (carboxylate as bidentate ligand)



Atom	X	Y	Z
O	-4.365208	0.659509	0.262886
O	-4.090556	-0.261155	-1.734100
O	-1.664200	1.102073	-0.545283
O	1.198868	-0.627991	-0.062825
O	2.271575	-1.299176	1.817536
N	0.527587	0.067427	1.996013
H	0.792897	0.090304	2.973346
N	-1.940148	-0.651255	0.860742
H	-1.592408	-1.086290	1.708931
C	-0.483422	3.486894	0.552160
C	-1.506036	3.963347	1.381755
H	-1.698776	3.487452	2.346779
C	-2.296740	5.045637	0.994387
H	-3.093862	5.396287	1.654013
C	-2.069413	5.681045	-0.226853
H	-2.688129	6.528350	-0.530512
C	-1.040556	5.227437	-1.053489
H	-0.848654	5.719567	-2.009799
C	-0.258538	4.140321	-0.665113
H	0.534956	3.780838	-1.325652
C	0.379324	2.311139	0.947296
H	1.076465	2.626673	1.741089
H	0.992372	1.996571	0.091400
C	-0.369194	1.088456	1.492250
H	-0.984412	1.395458	2.351911
C	-1.367781	0.497795	0.486480
C	-3.020899	-1.312018	0.143600
H	-2.611816	-1.932998	-0.669062
C	-3.926737	-0.239847	-0.494073
C	-3.805682	-2.189710	1.125193
H	-3.107352	-2.886692	1.615132
H	-4.222434	-1.531335	1.902592
C	-4.906342	-2.966572	0.446425
C	-4.655891	-4.231460	-0.098957
H	-3.659212	-4.671119	-0.001779
C	-5.662232	-4.936921	-0.758349
H	-5.450070	-5.924517	-1.174282
C	-6.937533	-4.384464	-0.882841
H	-7.727730	-4.936839	-1.396212
C	-7.198239	-3.123621	-0.345045
H	-8.194357	-2.684581	-0.436604
C	-6.189145	-2.420955	0.313079
H	-6.393590	-1.431034	0.729355
C	1.332329	-0.621758	1.142206
C	3.258910	-1.997685	1.068618
H	2.922592	-2.104493	0.028808
H	3.350010	-2.993410	1.522560
C	4.608481	-1.271047	1.119358
H	4.946992	-1.233036	2.168384
C	5.611254	-1.988662	0.238091
C	6.079960	-3.294745	0.327654
H	5.753854	-3.954376	1.135430
C	6.986889	-3.753394	-0.632972
H	7.367435	-4.775327	-0.572977
C	7.412598	-2.915614	-1.668180
H	8.121328	-3.290608	-2.409781
C	6.942753	-1.604094	-1.761978

H	7.279412	-0.952050	-2.571120	C	4.000878	2.425992	0.179548
C	6.039736	-1.146426	-0.803247	H	3.460782	3.321253	0.495965
C	5.395736	0.166043	-0.634430	C	3.891595	1.250922	0.928938
C	5.506920	1.338008	-1.381638	H	3.278754	1.231597	1.832518
H	6.140635	1.377361	-2.270522	C	4.578964	0.116562	0.509615
C	4.796615	2.467011	-0.968982	Ca	-3.615198	2.234929	-1.570667
H	4.870996	3.393419	-1.542592				

Charge = 1; multiplicity = 0; (0 imaginary frequencies)
Zero-point correction = 0.564633 (Hartree/Particle)
Thermal correction to Energy = 0.599040
Thermal correction to Enthalpy = 0.599985
Thermal correction to Gibbs Free Energy = 0.495713
Sum of electronic and zero-point Energies = –1794.958959
Sum of electronic and thermal Energies = –1794.924551
Sum of electronic and thermal Enthalpies = –1794.923607
Sum of electronic and thermal Free Energies = –1795.027879
BSSE correction: 0.011345277318
BSSE-corrected Free Energy: –1795.016534

REFERENCES

S1 Fueyo-González, F.; González-Vera, J. A.; Alkorta, I.; Infantes, L.; Jimeno, M. L.; Aranda, P.; Acuña-Castroviejo, D.; Ruiz-Arias, A.; Orte, A.; Herranz, R. *ACS Sensors* 2020, **5** (9), 2792–2799.

S2 Privalov, P. L.; Plotnikov, V. V. *Thermochim. Acta* 1989, **139**, 257–277

S3 Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J. Y.; White, D. J.; Hartenstein, V.; Eliceiri, K.; Tomancak, P.; Cardona, A. *Nat. Methods.* 2012, **9**, 676-682.

S4 Rüttinger, S.; Kapusta, P.; Patting, M.; Wahl, M.; Macdonald, R. *J. Fluoresc*. 2010, **20** (1), 105–114.

S5 Yuan, C.; Levin, A.; Chen, W.; Xing, R.; Zou, Q.; Herling, T.W.; Challa, P. K.; Knowles, T. P. J.; Yan, X. *Angew. Chemie - Int. Ed.* 2019, **58**, 18116–18123.

S6 Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision B.01, Gaussian, Inc., Wallingford CT* **2010**.

S7 Chai, J. D.; Head-Gordon, M. *Phys. Chem. Chem. Phys.*, 2008, **10**, 6615-6620.

S8 Wadt W. R.; Hay, P. J. *J. Chem. Phys.*, 1985, **82***,* 284-298.

S9 Tomasi, J.; Mennucci, B.; Cammi, R. *Chem. Rev.*, 2005, **105**, 2999-3093.

S10 Boys, S. F.; Bernardi, F. *Mol. Phys.*, 1970, **19***,* 553.

S11 Raeburn, J.; Mendoza-Cuenca, C.; Cattoz, B. N.; Little, M. A.; Terry, A. E.; Zamith Cardoso, A.; Griffiths, P. C.; Adams, D. J. *Soft Matter*, 2015, **11**, 927-935.