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A water-soluble metal–organic complex array as a multinuclear heterometallic peptide amphiphile that shows unconventional anion dependency in its self-assembly†

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Water-soluble metal–organic complex array 1, bearing Ru(II), Pt(II) and Rh(III) complexes at the side residues of the short peptide, exhibits anion and pH-responsive self-assembly behaviours in aqueous media. NaCl-induced aggregation of 1 at neutral pH was suppressed in phosphate buffered saline containing a mixture of Cl$^-$, HPO_4^{2-} and $H_2PO_4^-$, which is unconventional for a peptide amphiphile.

Self-assembly of natural[1] as well as artificially designed[2] amphiphilic peptides is one of the most extensively studied subjects in peptide science, as it covers wide research areas from biomedical[3] to nano-electronics[4] fields. While there are increasing numbers of related studies with metallated peptide amphiphiles,[5] self-assembly of multinuclear heterometallic peptidic systems has been much less explored in spite of its potential utility for the controlled assembly of two or more different metal complexes into a particular arrangement.[6] Here we report a water-soluble metal–organic complex array (MOCA), a multimetallic peptidic array containing a predetermined sequence of metal centres, as a multinuclear heterometallic peptide amphiphile. Due to the presence of positively charged multiple metal complexes in its molecular structure, the water-soluble MOCA exhibited unconventional anion-dependency in its self-assembly, where, in contrast to most of pure organic peptide derivatives, its NaCl-induced aggregation is suppressed by the co-existence of more kosmotropic phosphate anions in phosphate buffered saline (PBS).

In 2011 we reported that sequential coupling of metallated amino acids on a polymeric resin successfully afforded MOCAs containing up to six metal centres of three different metals arranged in a predetermined sequence.[7] Later, this approach was modularized to make larger MOCAs whose molecular weights are close to or even exceed those of small proteins.[8] As a MOCA possesses multiple metal complexes arranged in a specific unique sequence along the peptide backbone, it would be interesting to investigate its interactions with bio-related molecules such as peptides/proteins, nucleic acids and sugars having also a regulated sequence in their structure. Such an idea motivated us to synthesize the first water-soluble MOCA molecule. Here, we designed a heterometallic triad containing Ru(II), Pt(II) and Rh(III) ions as the first generation of water-soluble MOCA molecule (**1** in Fig. 1). Metal complexes were chosen among the already successfully utilized ones in our previous work by considering the possible interactions with bio-related molecules such as DNA.[9] In addition to introducing cationically charged Ru and Pt complexes that are intrinsically water-soluble, we placed one glutamate (Glu) moiety to the neighbouring position of each metallated amino acid moiety and a triethylene glycol (TEG) chain at the N-terminus of the array to give the molecule enough water-solubility (Fig. 1). Coupling of Ru monomer, Glu, Pt monomer, Glu, Rh monomer, Glu and TEG acid in this order and cleavage of the resultant species from the resin afforded a crude mixture containing **1**. Precipitation from DMSO by adding EtOAc and washing the

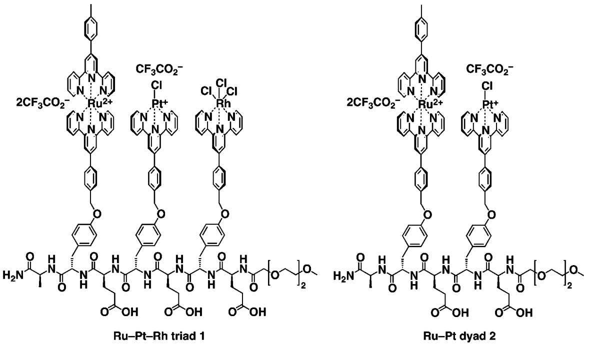

Fig. 1 Molecular structures of water-soluble metal–organic complex array **1** and its reference **2**.

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resultant precipitate with MeOH gave analytically pure **1**, as confirmed by reverse-phase HPLC, in 8% overall yield (ESI†).

As-obtained Ru–Pt–Rh triad **1** was well soluble in DMSO and DMF while it was moderately soluble in MeOH and CH_3CN. Dilution of its CH_3CN solution with Milli-Q grade water (Table 1, condition 1) gave a transparent aqueous solution (10% CH_3CN), in which no aggregation of the triad took place in its concentration range below 50 μM, as confirmed by the dynamic light scattering (DLS) technique. While carboxylic acid moieties of **1** under this condition (pH = 4.52) exist as a mixture of CO_2H and CO_2^-, conversion of the latter into the former by lowering the pH value of the media down to 1.98 with HCl (Table 1, condition 2) made the solution gradually turbid. DLS analysis of the solution after standing for 2 days revealed that **1** aggregated into 50–200 nm sized objects (Fig. 2A, red) over the concentration of 10 μM. Transmission electron microscopy (TEM) of a drop-cast sample revealed that these had a spherical morphology (Fig. 2B). Spheres larger than 70 nm tended to display images suggesting their hollow nature, which were hardly observed in the case of smaller spheres. Cryogenic TEM of the same sample solution gave similar images (Fig. 2C), where the wall was estimated to have a thickness of 20–30 nm which was much bigger than the molecular size of **1**, as calculated from its CPK model to be no larger than 5 nm. These assemblies exhibited a positive zeta potential (ζ) of 27.6 mV, possibly due to the presence of cationically charged Ru and Pt complexes in **1**. The absence of any clear CD patterns in the UV region between 200 and 250 nm (Fig. S10, ESI†) suggests that **1** in those assemblies does not adopt a particular secondary structure such as β-sheet.

When an aqueous solution of **1** (10% CH_3CN) was salted with NaCl (10 mM; Table 1, condition 3), the resultant solution again turned into non-transparent in 2 days at 37 °C to afford a suspension. In contrast to the case of acidification (Table 1, condition 2), however, NaCl-induced assembly of **1** gave only amorphous aggregates larger than 500 nm, as observed by DLS and TEM (Fig. 2A, blue, and Fig. S6A, ESI†). An increase of NaCl concentration by one order of magnitude (100 mM; Table 1, condition 4) only slightly increased the size of aggregates (Fig. S6B and S8, red, ESI†). As **1** intrinsically possesses trifluoroacetate ions as exchange-labile counter anions of its metal complex moieties (Fig. 1), we also tested sodium trifluoroacetate ($NaOCOCF_3$, 100 mM; Table 1, condition 5) as a reference of NaCl, where no aggregate was detected by DLS. $NaOCOCF_3$ even repelled the effect of NaCl, as NaCl-induced aggregates of **1** apparently disappear in a few minutes after the addition of $NaOCOCF_3$ (Table 1, condition 6) where a homogeneous solution without any DLS signals resulted after 3 h.

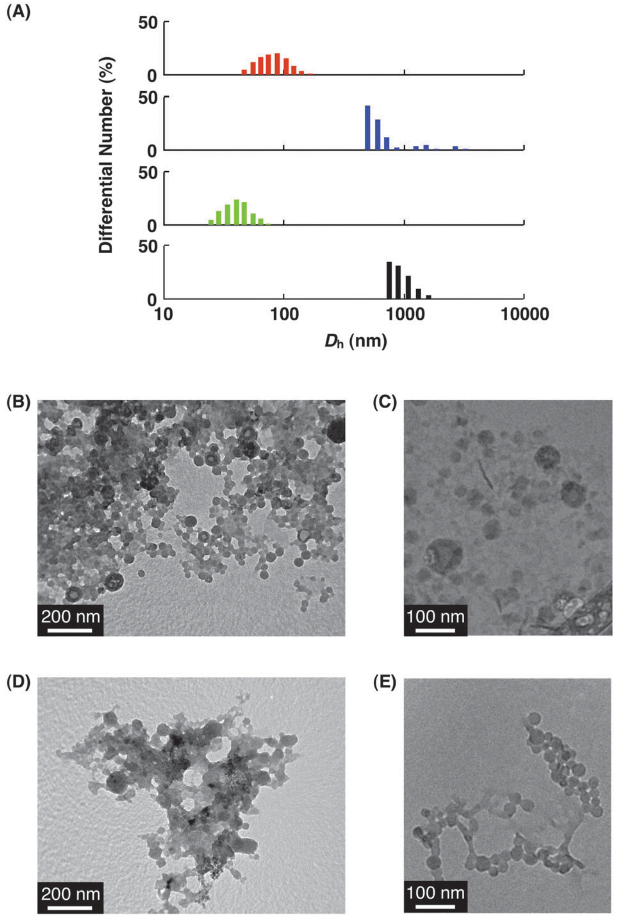

Fig. 2 (A) DLS profiles of **1** under conditions 2 (red), 3 (blue), 7 (green) and 8 (black). (B and D) TEM and (C and E) cryogenic TEM images of non-stained **1** assembled in conditions 2 (B and C) and 7 (D and E).

Being inspired by such anion sensitive self-assembly of **1**, we decided to investigate its behaviour in physiological conditions that generally contain complexed mixtures of anions. As a representative example, we chose PBS (Table 1, condition 7), one of the most commonly used buffer solutions in biological and biomedical research fields. Although PBS contains more than 100 mM concentration of NaCl, the DLS profile of a PBS solution (10% CH_3CN) of **1** displayed no light scattering in the μm-scale region but only at below 100 nm (Fig. 2A, green). Suppression of the NaCl-induced aggregation of **1** in PBS is not

Table 1 Self-assembly behaviours of **1** (50 μM) in aqueous media (10% CH_3CN) in response to additives

No.	Additives	pH	Morphology of the assembly
1	No	4.52	Not detected
2	HCl (10 mM)	1.98	Spheres
3	NaCl (10 mM)	5.42	Amorphous aggregates
4	NaCl (100 mM)	5.34	Amorphous aggregates
5	$NaOCOCF_3$ (100 mM)	5.41	Not detected
6	NaCl/$NaOCOCF_3$ (100/100 mM)	5.44	Not detected
7	NaCl/KCl/Na_2HPO_4/KH_2PO_4 (120/2.4/9/1.6 mM)	7.54	Spheres
8	NaCl/NaOH (100/0.0024 mM)	7.61	Amorphous aggregates

due to the increase of the pH value of the media up to 7.54, as the salting-out effect of NaCl on **1** was preserved at a pH of 7.61 (Table 1, condition 8; Fig. 2A, black, and Fig. S6C, ESI†). TEM as well as cryogenic TEM observations of the PBS solution of **1** (Fig. 2D and E) revealed spheres whose sizes are less than 100 nm. As is the case of spheres obtained under condition 2, those in PBS were positively charged (ζ = 22.2 mV). The observed small salting-out effect of PBS on **1**, less significant than that of NaCl under the same pH condition (condition 8), is unique for the self-assembly of peptide amphiphiles as generally kosmotropic anions such as HPO_4^{2-} and $H_2PO_4^{-}$ in the Hofmeister series[10] promote assemblies of peptides and proteins more efficiently than Cl^-.[11] This is possibly due to the fact that the molecular structure of **1** contains multiple cationic moieties such as doubly charged Ru and singly charged Pt metal complexes, whose interactions with anions could play a decisive role in controlling the self-assembly behaviours of **1**.[12] On the other hand, the presence of the water-insoluble, charge neutral Rh-complex moiety in the molecular structure of **1** is also responsible for its spherical assembly formation in PBS, as reference Ru–Pt dyad **2** (Fig. 1) did not form any assemblies detectable by DLS under the same condition, although **2** as well as **1** aggregated heavily under condition 8 (Fig. S9, ESI,† and Fig. 2A black, respectively). In relation to this, the presence of hydrophobic nile red dye (1 μM) of only 2 mol% with respect to **1** promoted the aggregation of **1** (Fig. S8 blue, ESI†), suggesting that the self-assembly of **1** in PBS is sensitive to the subtle change of the balance between hydrophobic and ionic interactions of **1**. It would be also noteworthy that the Ru–Pt–Rh sequence in **1** can produce a gradient of cationic charge upon dissociation of non-coordinating trifluoroacetate anions (Fig. 1).

In summary, we have reported **1** as the first example of water-soluble MOCA and investigated its self-assembly behaviours in aqueous media. Unconventional anion-dependency of its self-assembly as a peptide amphiphile recommends us to be more conscious of a less considered effect of metallated peptide structures, which is particularly meaningful under physiological conditions where competitive interactions with co-existing anions play a fundamental role. Spherical assemblies of **1** were also observed in an aqueous cell culture medium (Dulbecco's modified Eagle's medium) (10% DMSO) containing fetal bovine serum (Fig. S7 and S8, green, ESI†), suggesting future potential utility of water-soluble MOCAs in the biomedical area. Considering also the observed phosphate-responsive self-assembly behaviours of **1**, its hybridization with nucleic acids for delivery is one of the future research subjects worthy to try.

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