Supplemental materials

Does Argnine Aggregate Formation in Aqueous Solutions Follow Two-Step Mechanism?

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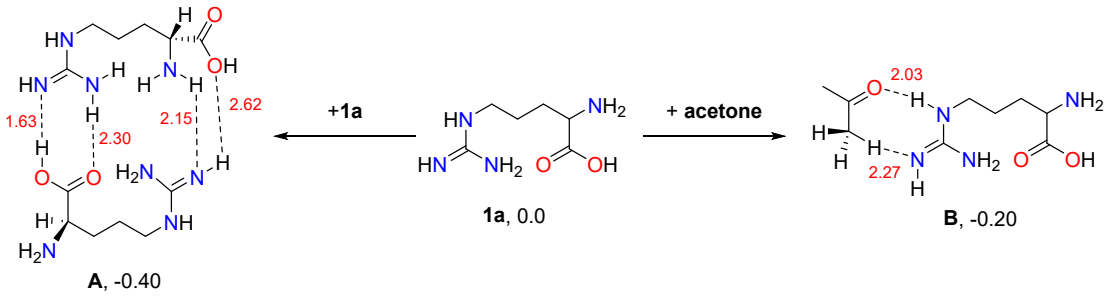
Materials and methods

Density functional theory calculations

DFT calculations with the B3LYP/6-31G(d) method were done by running Gaussian 09 to study the possible interactions between two arginine molecules and the arginine and the acetone molecules. It was found that the dimerization of two arginine molecules (1a) via hydrogen bonding to complex A is

exergonic by 0.4 kcal mol^{-1}, while the formation of complex B from 1a and acetone is exergonic only by 0.2 kcal mol^{-1}. This indicates stronger interaction is expected between two arginine molecules, probably because more H-bonds could be formed in this case as demonstrated by the dashed lines in the following scheme (distances are in Ångstrom). Only the most stable complex was shown among different possibilities.



A, -0.40 **1a**, 0.0 **B**, -0.20

Table S1 Parameters utilized in light scattering assessment at 25oC.

Mass (%)	□ ($\times 10^3$ Pa s^{-1})	Refractive index
1% NaCl with 0.50 mol L^{-1} *d*-Arg	1.4732	1.5607
2% NaCl with 0.50 mol L^{-1} *d*-Arg	1.4902	1.5607
4% NaCl with 0.50 mol L^{-1} *d*-Arg	1.5239	1.5607
1% Acetone with 0.50 mol L^{-1} *d*-Arg	1.4822	1.5607
5% Acetone with 0.50 mol L^{-1} *d*-Arg	1.6153	1.5607
10% Acetone with 0.50 mol L^{-1} *d*-Arg	1.7753	1.5607
0.25 mol L^{-1} *d*-Arg	1.0807	1.5607
0.50 mol L^{-1} *d*-Arg	1.3041	1.5607
1.00 mol L^{-1} *d*-Arg	1.8623	1.5607
1.00 mol L^{-1} *l*-Ala	1.0403	1.4334
1.50 mol L^{-1} *l*-Ala	1.1222	1.4334
1.70 mol L^{-1} *l*-Ala	1.1557	1.4334
0.26 mol L^{-1} *l*-His	0.9474	1.5805
0.43 mol L^{-1} *l*-Val	0.9879	1.4434
0.51 mol L^{-1} *l*-Val	1.0098	1.4434
0.10 mol L^{-1} *l*-His	1.0033	1.5805
0.15 mol L^{-1} *l*-His	1.0617	1.5805
0.20 mol L^{-1} *l*-His	1.1230	1.5805
0.25 mol L^{-1} *l*-His	1.1900	1.5805

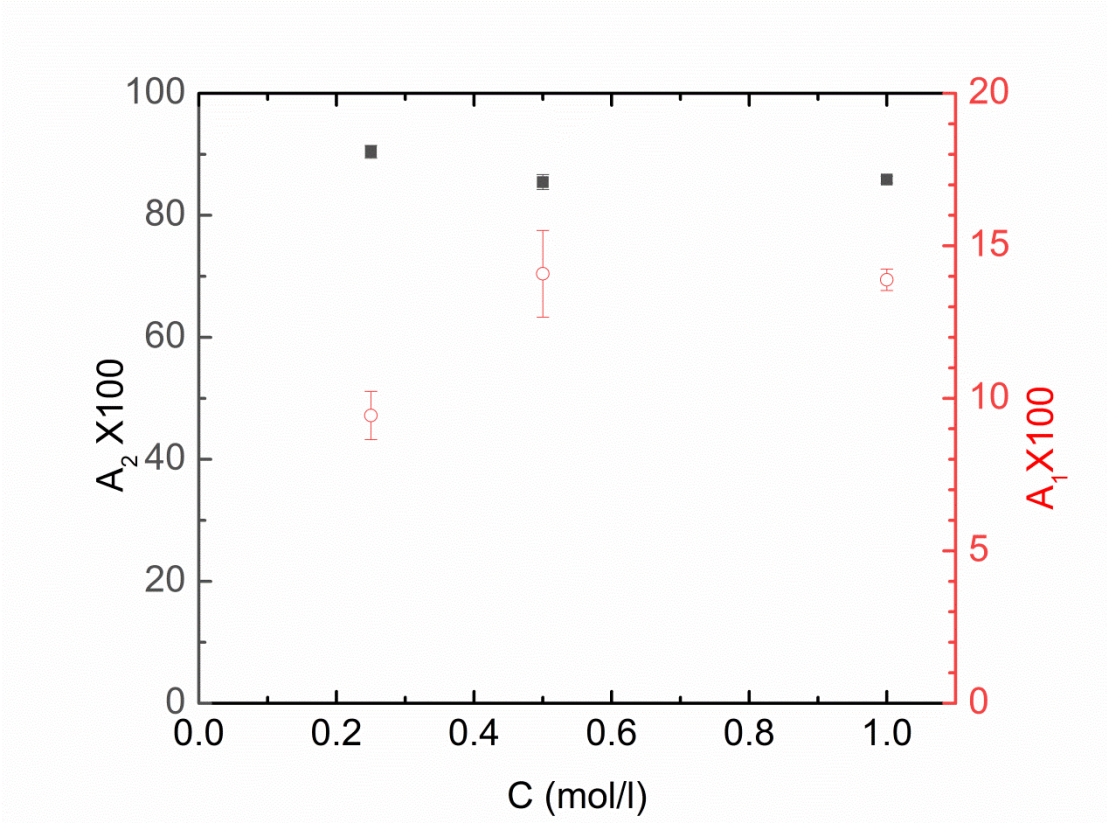


Figure S1 The light intensity population distributions of 0.5 mol L^{-1} aqueous *d*-Arg solutions at pH 7 characterized by the amplitudes in Eq. (s1). The solid black square stands for clusters with the large characterized decay times while the open red circle does for that with the small value of characterized decay times.

Table S2 The ratio of concentration of large aggregates to small clusters *vs*. Arg concentration. The subscribe 2 stands for the concentration of clusters with the large size while 1 does for that of the small size

mol L^{-1}	C_2/C_1
0.25	$6.65\times10^{-14}\pm3.98\times10^{-14}$
0.50	$6.00\times10^{-14}\pm2.47\times10^{-14}$
1.00	$1.05\times10^{-14}\pm0.57\times10^{-14}$

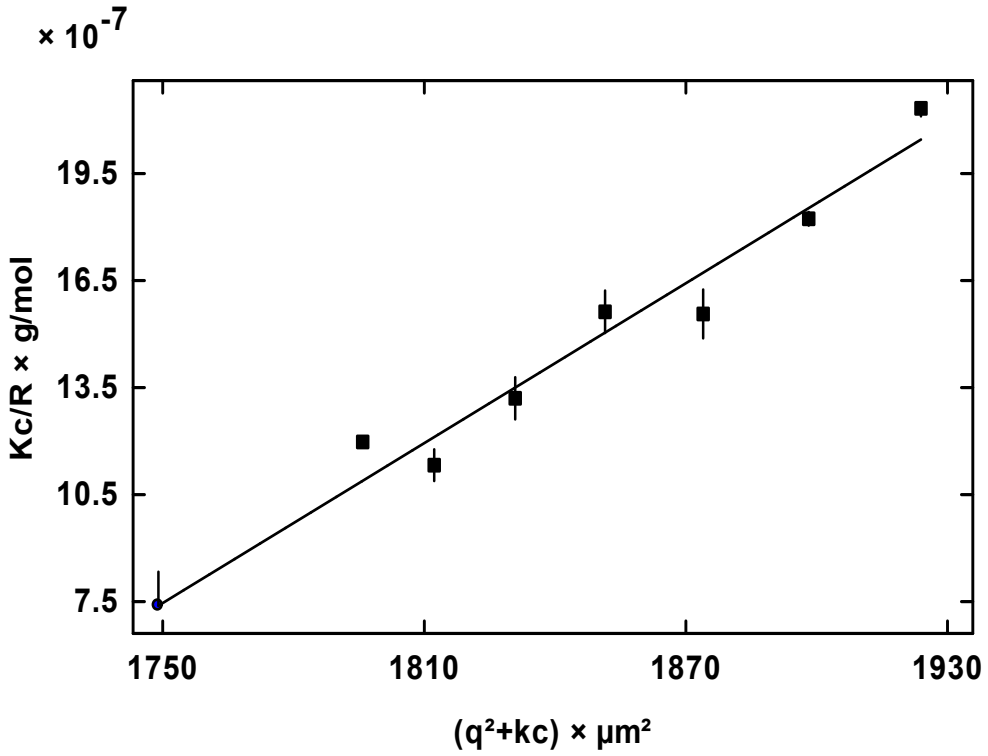


Figure S2 Zimm plot of 0.25 mol L^{-1} *d*-Arg aqueous solution at pH 7.

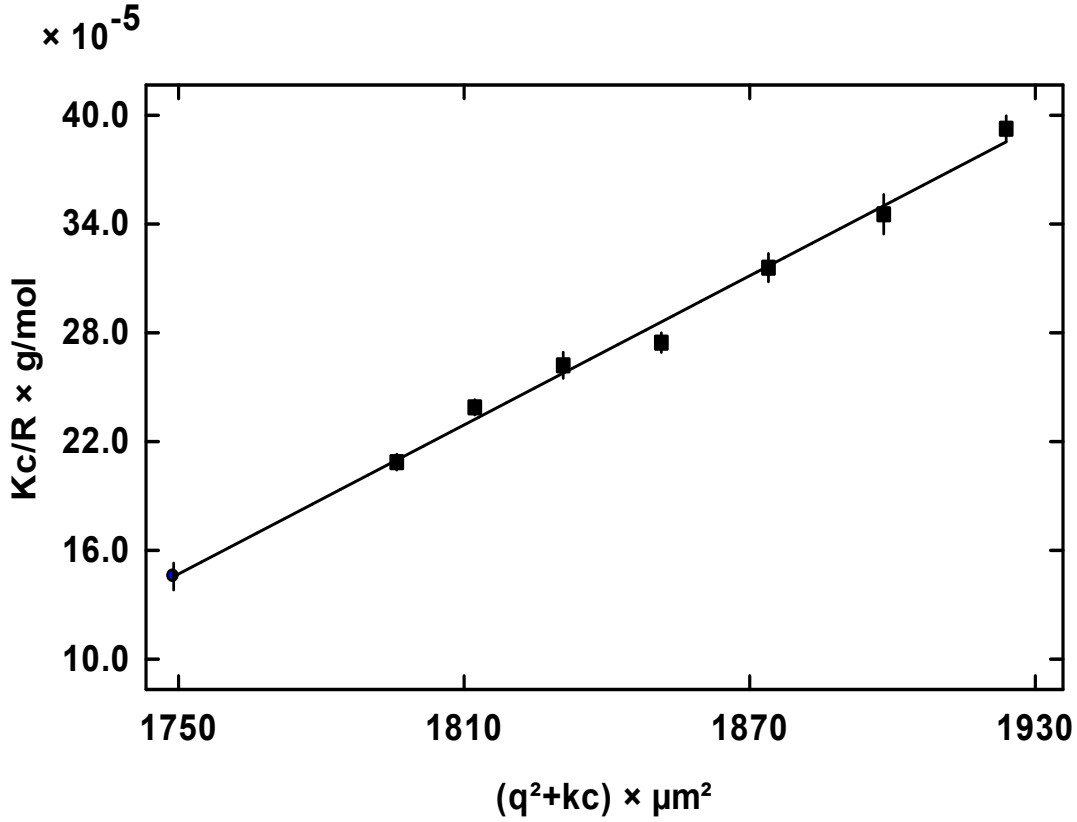


Figure S3 Zimm plot of 0.5 mol L^{-1} *d*-Arg aqueous solution at pH 7.

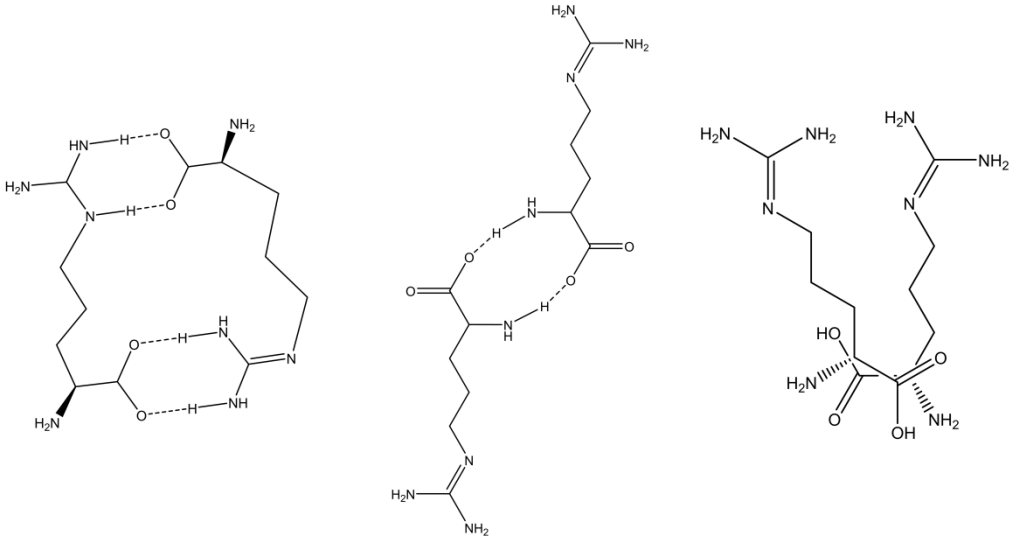


Figure S4 The possible dimers formed in aqueous solutions based on molecular dynamic simulation[1].

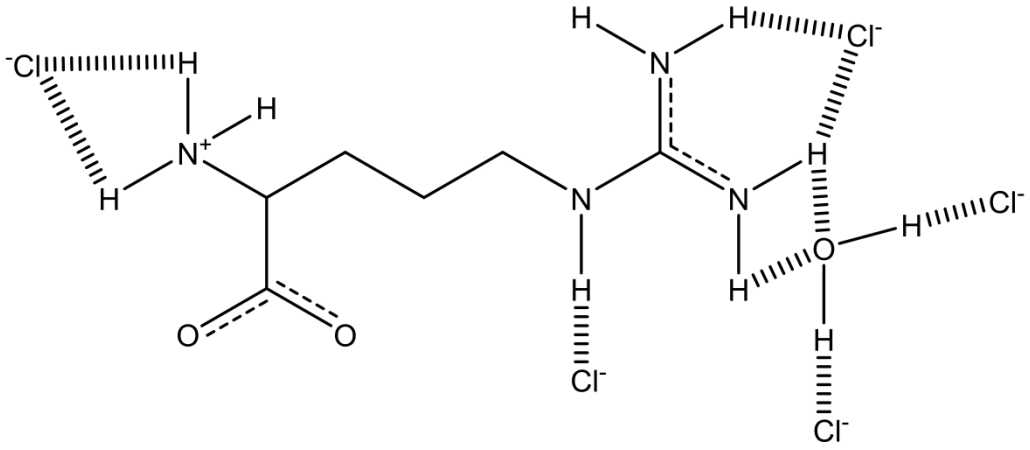


Figure S5 The possible Arg-Cl- pairs in aqueous solutions based on MD simulations[1].

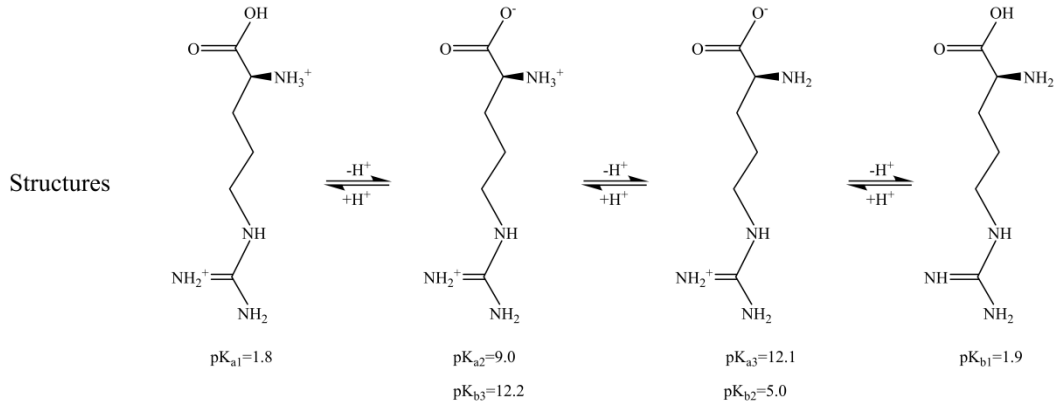


Figure S6 Structure and pk_a value of Arg in aqueous solutions.

Table S3. 11 *l*- amino acids in aqueous solutions

Amino acid	C (mol L^{-1})	pI	Charge (pH=7.0)	Hydrophobicity	R_H (nm)	Tightly bound water[2]	Hydration water[2]	Loosely bound water[2]	$N_{dangling}$[2]
l-arginine	1.00	10.76	+	-4.5	111±9	6.33±0.35	NA	NA	NA
l-histidine	0.10	7.59	+	-3.2	104±3	0.43±0.02	26.7±1.3	26.3	5.9± 0.3
l-glutamic acid	0.05	3.22	-	-3.5	39±3	0.30±0.02	NA	NA	NA
l-alanine	1.00	6.00	x	1.8	108±9	0.23±0.01	10.3±0.5_	10.1	0.8± 0.2
l-valine	$4.3x10^{-4}$	5.96	x	4.2	93±7	~0.09	21.0±1.0	20.9	4.8±0.2
l-threonine	0.10	6.16	x	-0.7	115±6	0.44±0.02	34.3±1.7	33.9	1.9±0.1
l-lysine	0.03	9.74	+	-3.9	—	4.02±0.20	NA	NA	NA
l-aspartic acid	0.10	2.77	-	-3.5	—	0.13±0.01	NA	NA	NA
l-tyrosine	0.10	5.66	x	-1.3	—	~0.05	NA	NA	NA
l-glutamine	0.10	5.65	x	-3.5	—	0	NA	NA	NA
l-asparagine	0.10	5.41	x	-3.5	—	1.31±0.07	NA	NA	NA
l-cysteine	0.10	5.05	x	2.5	—	0.18±0.01	8.6±0.4	8.4	1.2±0.06

l-serine	0.10	5.68	x	-0.8	—	1.13±0.06	44.0±2.2	42.9	1.7±0.2

x means that the amino acid does not carry a charge.

The definitions of tightly bound water, hydration water, loosely bound water, and dangling water could be consulted to the reference 2.

References

1. Shukla, D.; Trout, B. L., Interaction of Arginine with Proteins and the Mechanism by Which It Inhibits Aggregation. *Journal of Physical Chemistry B* **2010,** *114*, 13426–13438.
2. Shen, Y.; Liu, L.; Zheng, Q.; Zhao, X.; Han, Y.; Guo, Q.; Wang, Y., Quantitative insights into tightly and loosely bound water in hydration shells of amino acids. *Soft Matter* **2021,** *17*, 10080-10089.