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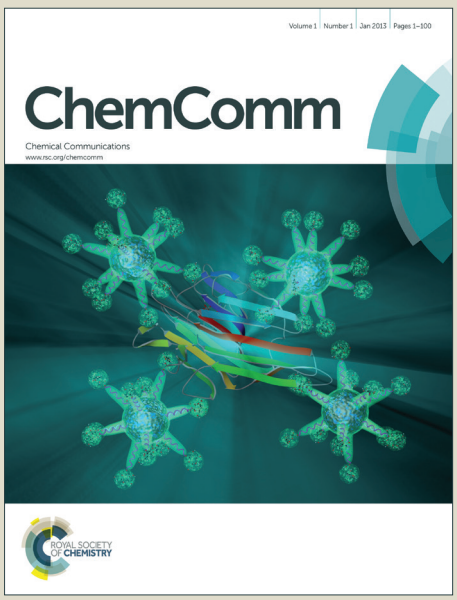

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Self-Assembled, π-Stacked Complex as a Finely-Tunable Magnetic Aligner for Biomolecular NMR Applications

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The number of molecules constituting 1D intermolecular aggregates of the π-stacked self-assembled complex was controlled by altering the concentration and temperature. Enhanced magnetic orientation was observed for the aggregates with larger aggregation number. It was demonstrated that the designable complex aggregates serve as magnetic aligners to induce magnetic alignment upon an analyte protein coexisting in the solution, resulting in the observation of residual dipolar coupling (RDC) of the analyte.

Residual dipolar coupling (RDC)[1] is a magnetically induced spin-spin coupling between nuclei, which is observable by NMR spectroscopy when a molecule is partially oriented against the applied magnetic field. RDC measurement has become a modern NMR method for the structure analysis of biomolecules like protein because it provides angular information about the chemical bond vectors of target proteins.[2] RDC values (Hz) are normally very small but are in practice enhanced by using alignment media such as liquid crystals,[3] bicelles,[1b,4] filamentous phages,[5] and gels.[6] One of the most important features for the aligners is to adjust the degree of induced alignment,[4a,5a,6c,6d,7] and the loss of the control falls in severely broadened NMR signals due to excessive degree of alignment. Recently, self-assembled complex **1**[8] was shown to be magnetically orienting as a discrete molecule, where with a large number of parallel aromatic rings show the accumulated diamagnetic alignment.[9] Because of its unique nature to form one-dimensional aggregates at high concentrations,[10] we expected that the intermolecular-π-stacked structures of complex **1** shows enhanced magnetic alignment and could act as a magnetic aligner with an adjustable aligning ability achieved by designing the functional groups and by adjusting the degree of aggregation. Here, we report that the degree of aggregation of well-defined and highly designable complex **1** is significantly varied by changing the concentration and temperature and, hence, the aggregate is used as a tunable magnetic aligner for a protein to observe enhanced RDC at a suitable range (Fig. 1).

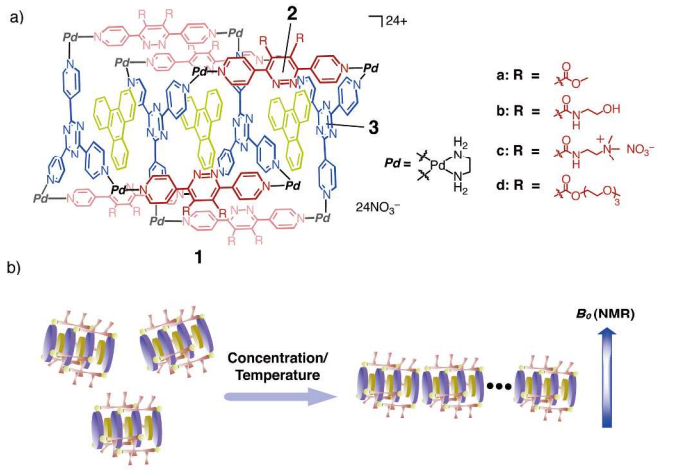

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Fig. 1 (a) Chemical structures of π-stacked complexes **1** with hydrophilic side chains. (b) One-dimensional aggregation of **1** with the nature of magnetic orientation.

Previously reported complex **1a** forms one-dimensional aggregates in saturated aqueous solution (~20 mM at 300 K). To use complex **1** as a magnetic aligner, higher water solubility is required. To enhance the solubility, we designed **1b-d** with hydrophilic side chains, which were easily introduced by the chemical modification of bidentate ligand component **2**. The septuple aromatic stack **1b** quantitatively self-assembled from a mixture of bidentate ligand **2b** (126 μmol), tridentate ligand **3** (80.0 μmol), and triphenylene **4** (84.6 μmol) in a D_2O solution (1.4 mL) of $(en)Pd(NO_3)_2$ (**5**, 253 μmol) stirred at 40 °C for 2 h. The ^{1}H NMR spectrum showed two sets of signals for bidentate ligand **2b** ($H^{a,b,c,d}$ and $H^{a',b',c',d'}$), tridentate ligand **3** ($H^{e,f}$ and $H^{e',f'}$), and triphenylene **4** ($H^{g,h}$ and $H^{g',h'}$) in 1:1, 1:1, and 2:1 integral ratios, respectively (Fig. 2a), which is consistent with the reported structure of complex **1a**. The X-ray crystallographic structure of complex **1b'**, analogous to **1b** except for the tmeda capping ligands on the palladium, showed that the twelve hydrophilic side chains are placed at the periphery of the complex perpendicular to the stacked aromatic rings and will not disturb the intermolecular 1D stacking (Fig. 2b). As expected, complex **1b** showed high water solubility (~40 mM at 300 K). In a similar manner, complexes **1c** and **1d** were synthesized, both of which also showed higher water solubility (~25 mM and ~40 mM at 300 K, respectively) than **1a**.

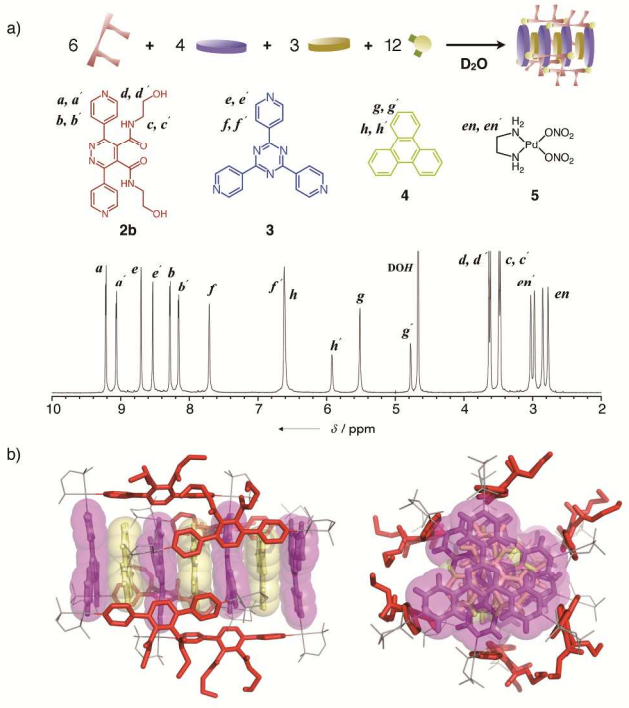

Fig. 2 (a) Synthetic scheme and ^{1}H NMR spectrum (920 MHz, D_2O, 2.5 mM, 300 K) of complex **1b**. The complex **1b** was self-assembled from six bidentate ligands **2b**, four tridentate ligands **3**, three aromatic molecules **4**, and twelve metal ions **5**. (b) Crystal structure of complex **1b'**: side view (left) and top view (right). The counter ions (NO_3^-) and solvent molecules are omitted for clarity. Crystal data: Space group $P\bar{1}$, a = 23.379(3) Å, b = 27.150(3) Å, c = 43.649(5) Å, α = 107.7170(10)°, β = 92.383(2)°, γ = 103.950(2)°, V = 25414(5) Å^3, Z = 2, T = 90 K, R_1 = 0.1695, wR_2 = 0.4435, GOF = 1.502 (CCDC 927291).

The aggregation of complex **1b** was studied at various concentrations by diffusion-ordered NMR spectroscopy (DOSY). At 2.5 mM in D_2O, sharp ^{1}H signals were observed with a diffusion coefficient of $D = 1.4 \times 10^{-10}$ m^{2}s^{-1}, consistent with the monomeric structure. At higher concentrations (10 - 20 mM), the ^{1}H signals of **1b** became broader, and the diffusion coefficients were reduced to $D = 8.1 \times 10^{-11}$ and 2.9×10^{-11} m^{2}s^{-1}, respectively (Fig. 3). From the Stokes-Einstein equation using the approximation for rod-like molecules, the degree of aggregation n was estimated to be 3 and 18, respectively. At 40 mM, the D value could not be determined as the signals were severely broadened. Even at a high concentration of **1b** of 30 mM, however, reasonably sharp signals were observed by high-resolution magic angle spinning (HR-MAS) NMR spectroscopy at 10 kHz MAS rate,[11] indicating that the structure of **1b** remained unchanged (see Fig. S10). The solutions of **1c** and **1d** also showed the same behavior, demonstrating the efficient formation of 1D aggregates and the orthogonal designability of the functional groups to the inherent 1D aggregating nature.

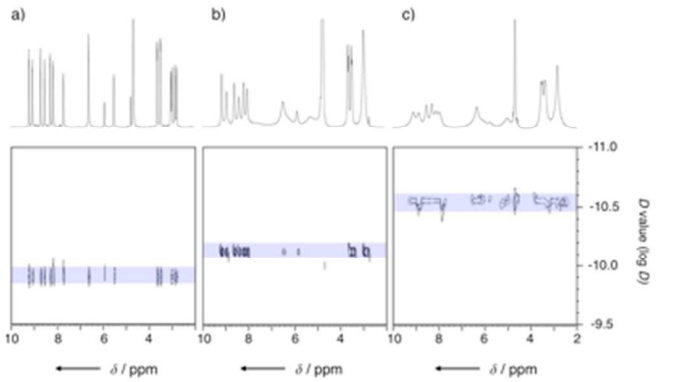

Fig. 3 ^{1}H NMR (above) and ^{1}H DOSY NMR (below) spectra (500 MHz, D_2O, 300 K) of the solution of complex **1b** at (a) 2.5 mM, (b) 10 mM, and (c) 20 mM.

For complex **1b**, the apparent J coupling values (J_{app}), which consist of the inherent $^1J_{CH}$ and RDC values, were measured by ^{13}C-coupled ^{1}H–^{13}C HSQC spectrum at various concentrations. The inherent $^1J_{CH}$ value was determined by magnetic field-dependent J_{app} measurement on 300 to 920 MHz NMR spectrometers (See ESI†).[9] From these values, the RDC values for the $C^{h'}$-$H^{h'}$ pair measured on a 600 MHz NMR spectrometer at 2.5, 5.0, and 10 mM were determined as –1.0, –1.4, and –2.7 Hz, respectively. The increase of the RDC absolute values is ascribed to the enhanced magnetic orientation of the aggregates.

Above 10 mM, RDC was not accurately measured due to severely broadened ^{1}H signals. In this region, the magnetic orientation was indirectly estimated by quadrupole splitting values ($\Delta\nu$) of the solvent D_2O, reflecting the magnetic orientation of D_2O molecules induced by the complex aggregates.[4,5,7,12] At 308 K, we succeeded to prepare up to a 50 mM solution of complex **1b**, and the 40, 45, and 50 mM solutions of complex **1b** showed $\Delta\nu$ = 0.74, 1.48, and 2.27 Hz, respectively (Fig. 4). The 45 mM solution of complex **1b** was measured at lower temperatures of 303 and 300 K, and we observed $\Delta\nu$ = 2.21 and 3.16 Hz, respectively. These results show the enhanced formation of longer one-dimensional aggregation of complex **1** upon the increase of the concentration or decrease of temperature. The observed $\Delta\nu$ values are comparable to that of reported medium,[5a,7a] so we expected the induction of magnetic alignment upon a coexisting analyte is possible.

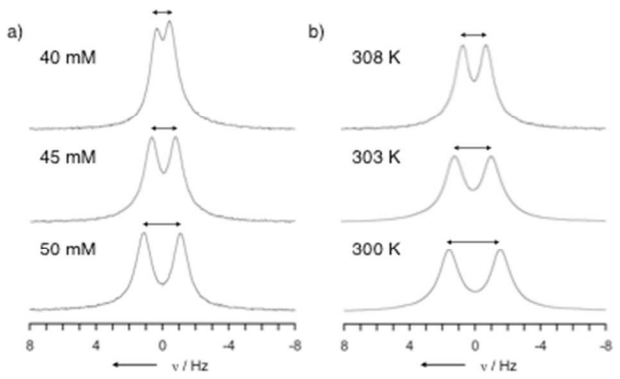

Fig 4 ^{2}H NMR spectra (92.1 MHz, D_2O) of D_2O in complex **1b** solution under (a) different concentrations at 308 K and (b) different temperatures at 45 mM.

To demonstrate the function as an aligner, proteins were chosen as analytes and added to the solution of complex **1b**, and the induction of magnetic alignment by the orienting aggregate of complex **1b** on the proteins was studied. We examined the ^{15}N-labeled dimer of mutated Gly76Cys ubiquitin[13] prepared via the oxidative formation of the disulfide bond between the *C*-terminal thiol groups at the mutated cysteine residues. The ubiquitin dimer was dissolved in a 35 mM solution of complex **1b**, and ^{1}H–^{15}N HSQC and ^{1}H–^{15}N TROSY spectra were measured to determine the apparent *J* coupling values J_{app}, which consist of the inherent $^{1}J_{NH}$ and RDC values of the amide NH bond.[14] The inherent $^{1}J_{NH}$ values were determined in water. The induction of magnetic orientation upon the ubiquitin dimer was effective, and we observed RDC: for example, the amide groups of T9, S20, and G47 showed RDC = –8.6, –6.4, and –11.0 Hz, respectively (Fig. 5). The observed RDC values were distributed in the moderate range of –11.0 to 7.6 Hz depending on the direction of the N–H pairs with respect to the magnetic field (Table S6).[15] In contrast to ubiquitin which has a positively charged surface, PUB,[16] a protein with a negatively charged surface, formed insoluble precipitates upon mixing with complex **1b**, presumably due to the formation of an ion pair with the complex bearing a +24 valence. The present goal to show the induction of RDC on an analyte using the 1D aggregates of our original, synthesized self-assembled complexes was well demonstrated, and the detailed studies including the comparison with widely utilized alignment media for proteins are not within the coverage of the present work, which will be discussed in future.

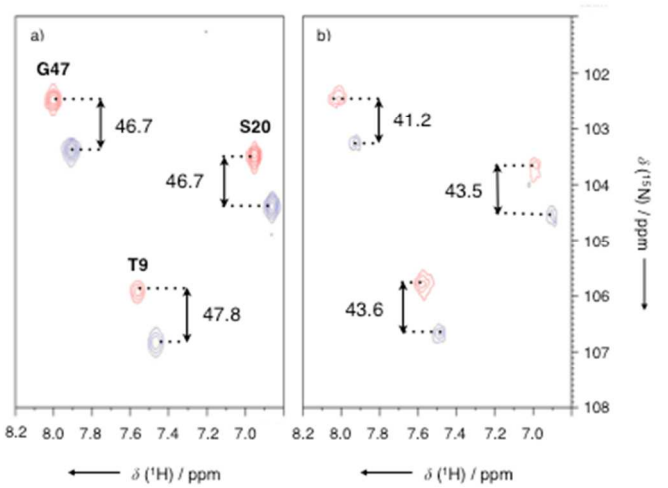

Fig. 5 ^{1}H–^{15}N HSQC spectra (red) and ^{1}H–^{15}N TROSY spectra (blue) (500 MHz, 95% H_2O/5% D_2O, 300 K) of ubiquitin dimer (a) in water and (b) in the 35 mM solution of complex **1b**. The difference of the chemical shifts between HSQC and TROSY signals corresponds to $1/2J_{app}$ for the amide ^{15}N-^{1}H pair.

In summary, we have developed a new magnetic aligner using the 1D aggregates of magnetically orienting molecules, where the diamgnetic alignment is effectively enhanced through intermolecular π-stacking. The structural control on the 1D aggregation degree realized adjustable magnetic alignment of the clusterized complex, and, furhtermore, adjustable induction of magnetic alignment to a coexisting analyte with RDC observation. In the present study, we utilized the surrounding functional groups to improve the water solubility of the complexes and found that their positions do not disturb the inherent 1D aggregating behaviour. This high degree of freedom to select the functional groups would promise to realize a predictable degree of alignment of analytes through designed interaction between the magnetic aligner and the analytes.

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Notes and references

1 (a) J. H. Prestegard, *Proc. Natl. Acad. Sci. U.S.A.*, 1995, **92**, 9279; (b) N. Tjandra and A. Bax, *Science*, 1997, **278**, 1111; (c) N. Tjandra, J. G. Omichinski, A. M. Gronenborn, G. M. Clore and A. Bax, *Nat. Struct. Biol.*, 1997, **4**, 732; (d) J. R. Tolman, J. M. Flanagan, M. A. Kennedy and J. H. Prestegard, *Nat. Struct. Biol.*, 1997, **4**, 292; (e) J. H. Prestegard, H. M. Al-Hashimi and J. R. Tolman, *Q. Rev. Biophys.*, 2000, **33**, 371; (f) E. de Alba and N. Tjandra, *Prog. NMR Spectrosc.*, 2002, **40**, 175.

2 (a) A. Bax, *Protein Sci.*, 2003, **12**, 1; (b) J. H. Prestegard, C. M. Bougault and A. I. Kishore, *Chem. Rev.*, 2004, **104**, 3519.

3 (a) A. Saupe and G. Englert, *Phys. Rev. Lett.*, 1963, **11**, 462; (b) S. R. Prosser, J. A. Losonczi and I. V. Shiyanovskaya, *J. Am. Chem. Soc.*, 1998, **120**, 11010; (c) M. Rückert and G. Otting, *J. Am. Chem. Soc.*, 2000**,** **122**, 7793.

4 (a) A. Bax and N. Tjandra, *J. Biomol. NMR*, 1997, **10**, 289; (b) C. R. Sanders and J. H. Prestegard, *Biophys. J.*, 1990, **58**, 447; (c) C. R. Sanders and J. P. Schwonek, *Biochemistry*, 1992, **31**, 8898.

5 (a) M. R. Hansen, L. Mueller and A. Pardi, *Nat. Struct. Biol.*, 1998, **5**, 1065; (b) G. M. Clore, M. R. Starich and A. M. Gronenborn, *J. Am. Chem. Soc.*, 1998, **120**, 10571.

6 (a) R. Tycko , F. J. Blanco and Y. Ishii, *J. Am. Chem. Soc.*, 2000, **122**, 9340; (b) K. Kobzar, H. Kessler, and B. Luy, *Angew. Chem. Int. Ed.*, 2005, **44**, 3145; (c) C. Naumann, W. A. Bubb, B. E. Chapman and P. W. Kuchel, *J. Am. Chem. Soc.*, 2007, **129**, 5340; (d) J. Ma, G. I. Goldberg and N. Tjandra, *J. Am. Chem. Soc.*, 2008, **130**, 16148.

7 (a) J. Sass, F. Cordier, A. Hoffmann, M. Rogowski, A. Cousin, J. G. Ominchinski, H. Löwen and S. Grzesiek, *J. Am. Chem. Soc.*, 1999**,** **121**, 2047; (b) L. G. Barrientos, C. Dolan and A. M. Gronenborn, *J. Biomol. NMR*, 2000, **16**, 329.

8 (a) M. Yoshizawa, J. Nakagawa, K. Kumazawa, M. Nagao, M. Kawano, T. Ozeki and M. Fujita, *Angew. Chem. Int. Ed.*, 2005, **44**, 1810; (b) Y. Yamauchi, M. Yoshizawa and M. Fujita, *J. Am. Chem. Soc.*, 2008, **130**, 5832; (c) J. K. Klosterman, Y. Yamauchi and M. Fujita, *Chem. Soc. Rev.*, 2009, **38**, 1714.

9 S. Sato, O. Morohara, D. Fujita, Y. Yamaguchi, K. Kato and M. Fujita, *J. Am. Chem. Soc.*, 2010, **132**, 3670.

10 Y. Yamauchi, Y. Hanaoka, M. Yoshizawa, M. Akita, T. Ichikawa, M. Yoshio, T. Kato and M. Fujita, *J. Am. Chem. Soc.*, 2010, **132**, 9555.

11 (a) H. D. H. Stover and J. M. J. Frechet, *Macromolecules*, 1991, **24**, 883; (b) E. E. Romanova, F. Grinberg, A. Pampel, J. Kärger and D. Freude, *J. Magn. Reson.*, 2009, **196**, 110; (c) S. Iqbal, F. Rodríguez-LLansola, B. Escuder, J. F. Miravet, I. Verbruggen and R. Willem, *Soft Matter*, 2010, **6**, 1875.

12 (a) N. J. Salsbury, A. Darke and D. Chapman, *Chem. Phys. Lipids*, 1972, **8**, 142; (b) B. Deloche and E. T. Samulski, *Macromolecules*, 1981, **14**, 575.

13 (a) D. Fujita, K. Suzuki, S. Sato, M. Yagi-Utsumi, E. Kurimoto, K. Kato and M. Fujita, *Chem. Lett.*, 2012, **41**, 313; (b) D. Fujita, K. Suzuki, S. Sato, M. Yagi-Utsumi, Y. Yamaguchi, N. Mizuno, T. Kumasaka, M. Takata, M. Noda, S. Uchiyama, K. Kato and M. Fujita, *Nature Commun.*, 2012, **3**, 1093.

14 (a) G. Kontaxis, G. M. Clore and A. Bax, *J. Magn. Reson.*, 2000, **143**, 184; (b) S. Tate, *Anal, Sci.*, 2008, **24**, 39; (c) S. M. Douglas, J. J. Chou and W. M. Shih, *Proc. Natl. Acad. Sci. U.S.A.*, 2007, **104**, 6644.

15 For ubiquitin monomer, the solution was successfully prepared without precipitation, but RDC was not observed.

16 Y. Kamiya, Y. Uekusa, A. Sumiyoshi, H. Sasakawa, T. Hirao, T. Suzuki and K. Kato, *FEBS Lett.*, 2012, **586**, 1141.

