Supporting Information

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

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Contents:

Acknowledgements:

We thank Ms. Yukiko Isono (National Institutes of Natural Sciences) for her help with the preparation of recombinant proteins.

This research was supported by the KAKENHI, Grant-in-Aid for Scientific Research on the Specially Promoted Research (No. 24000009) from the Japan Society for the Promotion of Science (JSPS), for which M.F. is the principal investigator, and also in part by KAKENHI, Grant-in-Aid for Scientific Research (No. 23107509, 25107708) on the Innovative Areas: "Fusion Materials" (Area no. 2206) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Grant-in-Aid for Scientific Research (No. 25102007) on the Innovative Areas: "Dynamical Ordering & Integrated Functions" (Area no. 2501) from MEXT, and Grant-in-Aid for Young Scientists (A) (No. 24685010) from MEXT. This research, including the NMR experiments on a 920 MHz system, was performed with the aid of Nanotechnology Network Project (A102, B102, A101, B100, and A100) & Nanotechnology Platform Program (B106, Molecule and Material Synthesis) of MEXT, at Institute for Molecular Science.

1. General

NMR spectra were obtained using the following spectrometers.

300 MHz: JEOL RESONANCE JNM-ECA300 spectrometer equipped with a TH5 probe with a z-field gradient coil.

500 MHz: Bruker AV500 spectrometer equipped with a TCI CryoProbe with a z-field gradient coil.

600 MHz: JEOL RESONANCE JMN-ECA600 spectrometer equipped with a HCN Cold Probe with a z-field gradient coil (53040HCNVC).

600 MHz (HR-MAS): Bruker AVIII600 spectrometer equipped with a HRMAS probe.[S1]

920 MHz: JEOL RESONANCE JNM-ECA920 spectrometer equipped with a HCN probe with a z-field gradient coil.[S2]

Data were processed with JEOL DELTA software (version 5.0.2) when measurements were performed on JEOL spectrometers, and TOPSPIN (version 1.3) when on Bruker spectrometers. Solvents and reagents were purchased from TCI Co., Ltd., WAKO Pure Chemical Industries Ltd., and Sigma-Aldrich Co. All the chemicals were of reagent grade and used without further purification unless noted otherwise. For separation of compounds, column chromatography using Wakosil C-300 (Wako reagents) was performed. For separation of compounds using Gel Permeation Chromatography (GPC), Japan Analytical Industry Co. Ltd. LC-908 Recycling Preparative HPLC was used with chloroform as the eluent. High-resolution mass spectrometry (HR-MS) was measured on Bruker maXis. Elemental analyses for carbon, hydrogen, and nitrogen were performed on a Yanaco MT-6. Melting points were determined on a Yanaco MP-500V apparatus. IR spectra were measured as KBr pellets or by ATR using a DIGILAB FTS-7000 instrument. The single crystal X-ray diffraction data were recorded on a Bruker APEX-II/CCD diffractometer equipped with a focusing mirror (MoKα radiation $\lambda = 0.71073$ Å) using a cryostat system equipped with a N_2 generator (Japan Thermal Eng. Co., Ltd.). Preparation of protein Gly76Cys ubiquitin was based on a procedure described elsewhere.[S3] The dimer, formed via the oxidation between the Cys76 residues of two ubiquitin monomers, was purified by Size Exclusion Chromatography (SEC) using GE Healthcare Hiload 16/600 Superdex 75 prep grade. Purity was confirmed using SDS-PAGE. Preparation of the PUB protein was described elsewhere.[S4]

S1. Bruker Biospin K.K. Demonstration Laboratory.

S2. Institute for Molecular Science, Nanotechnology Network Project & Nanotechnology Platform Project.

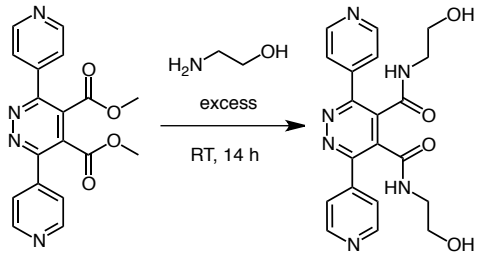
S3. D. Fujita, K. Suzuki, S. Sato, M. Yagi-Utsumi, E. Kurimoto, K. Kato and M. Fujita, *Chem. Lett.*, 2012, **41**, 313.

S4. Y. Kamiya, Y. Uekusa, A. Sumiyoshi, H. Sasakawa, T. Hirao, T. Suzuki and K. Kato, *FEBS Lett.*, 2012, **586**, 1141.

2. Synthesis of bidentate ligands and complexes

All NMR spectral data in this section were collected at 300 K and the chemical shift values reported here are with respect to an internal standard: a TMS standard for $CDCl_3$, the residual solvent signal for DMSO-d_6, and a $CDCl_3$ solution of TMS sealed inside a glass capillary tube for D_2O.

• Synthesis of *N,N'*-Bis(2-hydroxyethyl)-3,6-di(4-pyridinyl)pyridazine-4,5-dicarboxamide (bidentate ligand 2b)



2a **2b**

Compound **2a** (308 mg, 879 μmol) was stirred in 2-aminoethanol (3.0 mL) for 14 h at room temperature. The resulting precipitate was filtered and washed with cooled methanol to give ligand **2b** (299 mg, 731 μmol) as a white powder in 83% yield. ^{1}H NMR (300 MHz, DMSO-d_6, 300 K): δ (ppm) = 8.76 (d, J = 6.0 Hz, 4H, Py*H*α), 8.69 (t, J = 5.5 Hz, 2H, N*H*), 7.74 (d, J = 6.0 Hz, 4H, Py*H*β), 4.61 (t, J = 5.3 Hz, 2H, O*H*), 3.30 (td, J = 5.6, 5.3 Hz, 4H, $NHCH_2CH_2$), 3.17 (td, J = 5.6, 5.5 Hz, 4H, $NHCH_2CH_2$); ^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 163.3 (*C*O), 154.6 (C_q), 150.0 (Py*C*α), 143.2 (Py*C*γ), 132.6 (C_q), 123.1 (Py*C*β), 59.0 ($NHCH_2CH_2$), 41.6 ($NHCH_2CH_2$); HRMS: m/z ([M+H]$^+$): Calcd. for $C_{20}H_{21}N_6O_4$: 409.1619, Found: 409.1615; E.A.: Calcd. for $C_{20}H_{20}N_6O_4$•0.4(H_2O): C, 57.80; H, 5.04; N, 20.22, Found: C, 58.06; H, 5.04; N, 20.07; m.p.: 250 °C (decomposed); IR (KBr, cm^{-1}): 3277, 2909, 2845, 1647, 1589, 1562, 1425, 1400, 1337, 1271, 1081, 825.

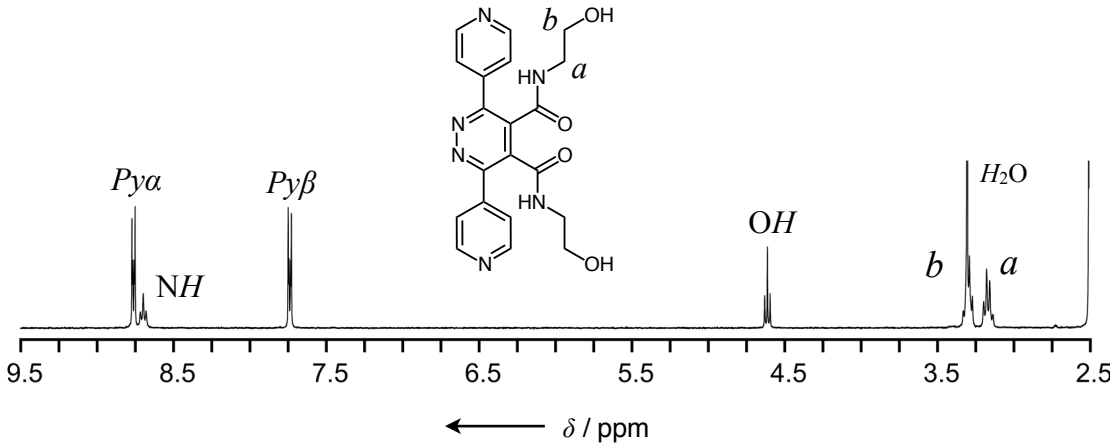
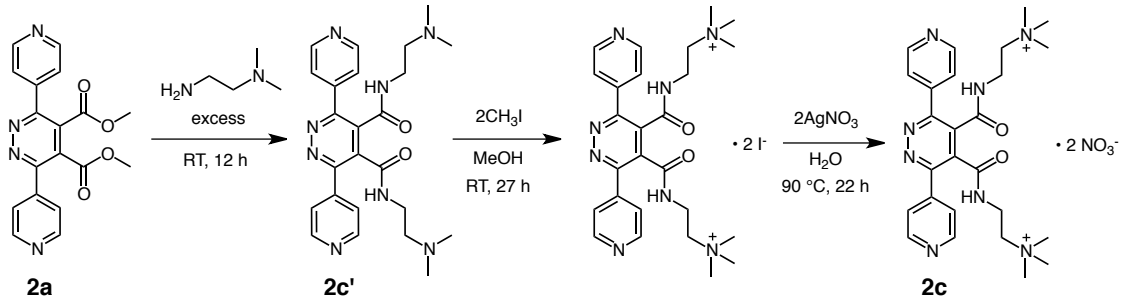


Figure S1. ^{1}H NMR spectrum of bidentate ligand **2b** (300 MHz, DMSO-d_6, 300 K).

• Synthesis of 2,2'-((3,6-Di(4-pyridinyl)pyridazine-4,5-dicarbonyl)bis(azanediyl))bis(*N*,*N*,*N*-tri methylethanaminium) nitrate (bidentate ligand 2c)



Compound **2a** (805 mg, 2.58 mmol) was stirred in *N*,*N*-dimethylethylenediamine (2.0 mL) for 12 h at room temperature. After evaporation of excess *N*,*N*-dimethylethylenediamine, the crude mixture was washed with cooled acetonitrile to give compound **2c'** (812 mg, 1.76 mmol) as a pale brown powder. Iodomethane (275 mg, 1.94 mmol) was added to a methanol solution (5.0 mL) of the whole amount of compound **2c'** and stirred for 27 h at room temperature. The precipitate was filtered off and the filtrate was evaporated to yield 1.08 g of yellow powder as a crude mixture. The whole amount of the crude mixture and silver nitrate (492 mg, 2.91 mmol) were dissolved in water (3 mL). The reaction mixture was stirred in the dark at 90 °C for 22 h. The pale yellow precipitate was filtered off through celite, washed by distilled water, and the filtrate was evaporated. The crude mixture was recrystallized from methanol at 5 °C to yield the title compound **2c** as a white powder (358.69 mg, 581 mmol) in 22% yield. ^{1}H NMR (300 MHz, DMSO-d_6, 300 K): δ (ppm) = 9.15 (t, J = 5.8 Hz, 2H, N*H*), 8.83 (d, J = 6.1 Hz, 4H, Py*H*α), 7.72 (d, J = 6.1 Hz, 4H, Py*H*β), 3.57 (td, J = 6.6, 5.8 Hz, 4H, NHC*H*$_2$CH$_2$), 3.23 (t, J = 6.6 Hz, 4H, NHCH$_2$C*H*$_2$), 3.03 (s, 18H, C*H*$_3$); ^{13}C NMR (125 MHz, DMSO-d_6, 300 K): δ (ppm) = 164.4 (*C*O), 154.5 (C_q), 150.3 (Py*C*α), 142.7 (Py*C*γ), 132.1 (C_q), 123.1 (Py*C*β), 62.6 (NHCH$_2$*C*H$_2$), 52.5 (*C*H$_3$), 33.4 (NH*C*H$_2$CH$_2$); HRMS: m/z ([M–NO$_3$]$^-$): Calcd. for $C_{26}H_{36}N_9O_5$: 554.2834, Found: 554.2836; E.A.: Calcd for $C_{26}H_{36}N_{10}O_8$•1.5(H_2O): C, 48.52; H, 6.11; N, 21.76, Found: C, 48.37; H, 6.09; N, 21.54; m.p.: 250 °C (decomposed); IR (KBr, cm^{-1}): 3418, 3217, 3031, 1665, 1543, 1492, 1371, 1271, 1218, 1142, 1074, 937, 829.

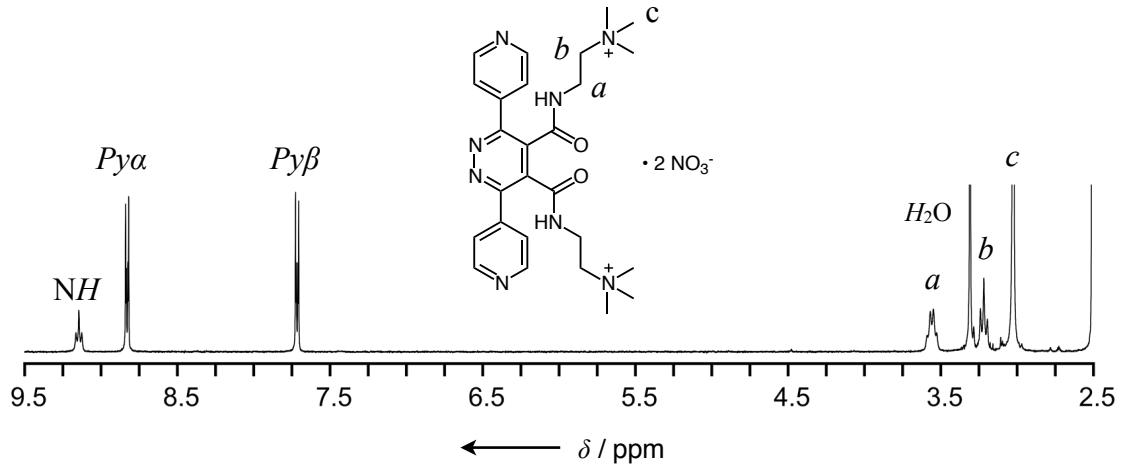
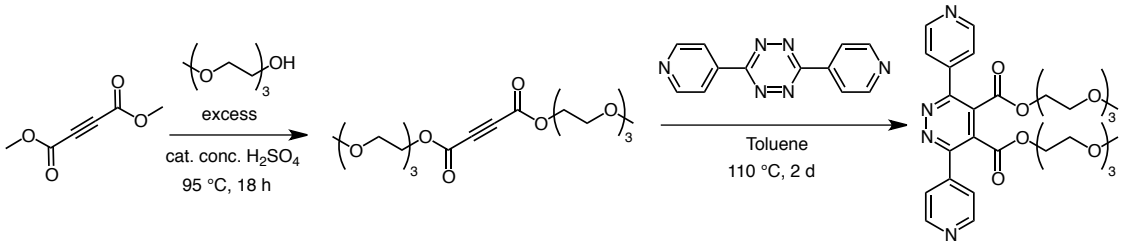


Figure S2. ^{1}H NMR spectrum of bidentate ligand **2c** (300 MHz, DMSO-d_6, 300 K).

• Synthesis of bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl) 3,6-di(pyridin-4-yl)pyridazine-4,5-dicarboxylate (bidentate ligand 2d)



2d

Under argon, dimethyl acetylenedicarboxylate (5.90 mL, 48.0 mmol) and concentrated sulfuric acid (2.10 mL, 59.6 mmol) was added to distilled triethylene glycol monomethyl ether (62.0 mL, 396 mmol), and stirred at 95 °C for 3 d. Upon cooling to room temperature, the reaction mixture was neutralized using 0.5 M $NaHCO_3$ aq. After extraction by diethylether and washing by brine, drying over anhydrous Na_2SO_4 and evaporation of the solvent *in vacuo* gave a brown liquid as a crude mixture. Purification by silica gel column chromatography by gradient elution using ethyl acetate and hexane (10:1), gave the acetylene compound (1.18 g, 5.00 mmol) as a pale yellow liquid. The whole amount of the obtained compound and 3,6-di(4-pyridyl)-1,2,4,5-tetrazine (1.18 g, 5.00 mmol) were added to toluene (20 mL), and stirred under reflux at 110 °C for 2 d. The black oil obtained after evaporation of the solvent *in vacuo*, was purified by silica gel column chromatography eluted by ethyl acetate, followed by GPC, to give the title compound **2d** (1.87g, 3.04 mmol) as a brown oil in 6% yield. ^{1}H NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.81 (d, J = 6.1 Hz, 4H, Py$H\alpha$), 7.66 (d, J = 6.1 Hz, 4H, Py$H\beta$), 4.38 (t, J = 9.4 Hz, 4H), 3.56 (m, 20H), 3.35 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, 300 K): δ (ppm) = 164.4 (CO), 155.5 (C_q), 150.5 (Py$C\alpha$), 143.1 (C_q), 129.3 (C_q), 123.3 (Py$C\beta$), 72.0 (CH), 70.6(CH), 68.3(CH), 66.4(CH), 59.2(CH); HRMS: m/z ([M+H]$^+$): Calcd. for $C_{30}H_{39}N_4O_{10}$: 615.2661, Found: 615.2653; IR (ATR, cm^{-1}): 2882, 1739, 1601, 1397, 1250, 1101, 943, 841.

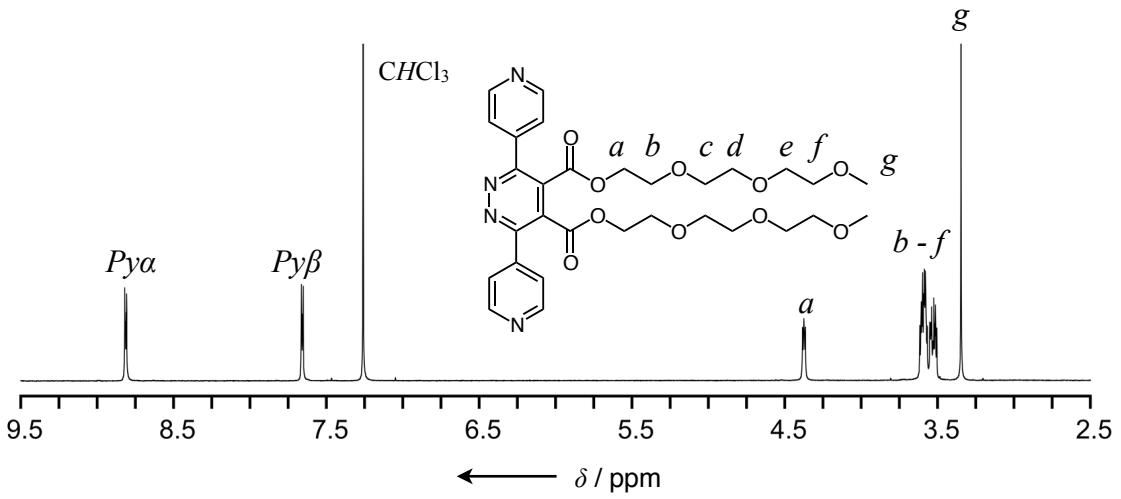
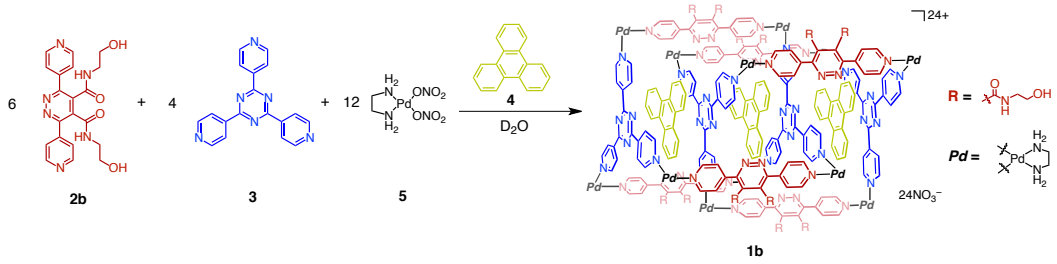


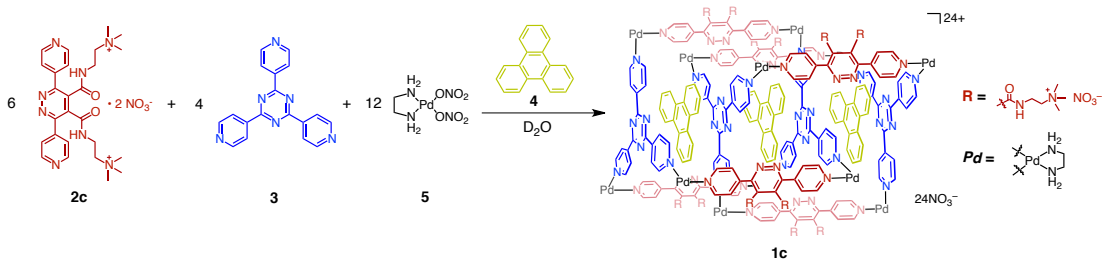
Figure S3. ^{1}H NMR spectrum of bidentate ligand **2d** (500 MHz, $CDCl_3$, 300 K).

• Synthesis of complex 1b



A mixture of bidentate ligand **2b** (51.7 mg, 126 mmol), tridentate ligand **3** (26.3 mg, 84.2 mmol), triphenylene **4** (19.3 mg, 84.6 mmol), and (en)Pd(NO_3)$_2$ **5** (73.5mg, 253 mmol) were stirred in D_2O (1.4 mL) at 40 °C for 2 h to give a yellow suspension. After removal of excess triphenylene by centrifugation, ^{1}H NMR analysis of the supernatant solution revealed the selective formation of complex **1b**. After filtration to remove the excess triphenylene, the resulting yellow solution was freeze-dried. **1b** was isolated as a yellow powder (153 mg, 19.4 μmol) in 92% yield. ^{1}H NMR (300 MHz, D_2O, 300 K): δ (ppm) = 9.30 (d, J = 6.8 Hz, 12H), 9.15 (d, J = 6.8 Hz, 12H) 8.79 (d, J = 6.4 Hz, 12H), 8.62 (d, J = 6.2 Hz, 12H), 8.37 (d, J = 6.8 Hz, 12H), 8.27 (d, J = 6.6 Hz, 12H), 7.79 (d, J = 4.7 Hz, 12H), 6.70 (m, 24H), 6.01 (d, J = 9.4 Hz, 6H), 5.60 (d, J = 8.5 Hz, 12H), 4.87 (d, J = 9.0 Hz, 6H), 3.74-3.68 (m, 24H), 3.60-3.54 (m, 24H), 3.10 (m, 12H), 3.07 (m, 12H), 2.95-2.93 (m, 12H), 2.88-2.86 (m, 12H); ^{13}C NMR (125 MHz, D_2O, 300 K): δ (ppm) = 167.6 (C_q), 165.4 (C_q), 164.7 (*C*O), 164.6 (*C*O), 154.9 (C_q), 154.5 (C_q), 152.5 (*C*H), 152.3 (*C*H), 151.7 (*C*H), 151.4 (*C*H), 147.1 (C_q), 147.1 (C_q), 144.1 (C_q), 142.6 (C_q), 133.0 (C_q), 132.9 (C_q), 127.3 (C_q), 126.9 (*C*H), 126.8 (C_q), 126.7 (*C*H), 126.6 (*C*H), 125.5 (*C*H), 125.0 (*C*H), 124.2 (*C*H), 122.2 (*C*H), 121.3 (*C*H), 59.8 (*C*H$_2$), 59.8 (*C*H$_2$), 47.5 (*C*H$_2$), 47.2 (*C*H$_2$), 47.1 (*C*H$_2$), 42.6 (*C*H$_2$); E.A.: Calcd for $C_{270}H_{300}N_{108}O_{96}Pd_{12}$•24($H_2O$): C, 39.05; H, 4.23; N, 18.22, Found: C, 39.21; H, 3.95; N, 18.02; m.p.: 220 °C (decomposed); IR (KBr,cm^{-1}): 3416, 3238, 3078, 2959, 1659, 1517, 1376, 1063, 822.

• Synthesis of complex 1c



A mixture of bidentate ligand **2c** (55.5 mg, 90.0 mmol), tridentate ligand **3** (18.8 mg, 60.1 mmol), triphenylene **4** (13.7 mg, 59.8 mmol), and (en)Pd(NO_3)$_2$ (52.3mg, 180 mmol), and were stirred in D_2O (1.5 mL) at 40 °C for 27 h to give a yellow solution. ^{1}H NMR analysis of the solution revealed the selective formation of septuple aromatic stack **1c**. After filtration to remove the excess triphenylene, the resulting clear yellow solution was freeze-dried. **1c** was isolated as a yellow powder (113 mg, 12.4 μmol) in 83 % yield. ^{1}H NMR (300 MHz, D_2O, 300 K): δ (ppm) = 9.31 (d, J = 6.7 Hz, 12H), 9.11 (d, J = 6.5 Hz, 12H), 8.73 (d, J = 6.3 Hz, 12H), 8.61 (d, J = 6.1 Hz, 12H), 8.30 (d, J = 6.7 Hz, 12H), 8.09 (d, J = 6.7 Hz, 12H), 7.81 (d, J = 5.8 Hz, 12H), 6.90 (d, J = 6.3 Hz, 12H), 6.76 (d, J = 9.0 Hz, 12H), 6.17 (d, J = 9.7 Hz, 6H), 5.70 (d, J = 8.2 Hz, 12H), 5.11 (d, J = 9.0 Hz, 6H), 3.91-3.81 (m, 24H), 3.62-3.54 (m,

24H), 3.29 (s, 54H), 3.28 (s, 54H), 3.10-3.04 (m, 24H), 2.96-2.94 (m, 12H), 2.88-2.86 (m, 12H); ^{13}C NMR (125 MHz, D_2O, 300 K): δ (ppm) = 167.6 (C_q), 165.3 (C_q), 165.3 (*C*O), 165.1 (*C*O), 154.9 (C_q), 154.5 (C_q), 152.6 (*C*H), 152.3 (*C*H), 151.7 (*C*H), 151.5 (*C*H), 147.3 (C_q), 147.2 (C_q), 144.0 (C_q), 142.7 (C_q), 132.6 (C_q), 132.4 (C_q), 127.4 (C_q), 126.9 (C_q), 126.8 (*C*H), 126.7 (*C*H, *C*H), 125.8 (*C*H), 125.1 (*C*H), 124.5 (*C*H), 122.3 (*C*H), 121.6 (*C*H), 63.3 (CH_2), 63.3 (CH_2), 53.7 (CH_3), 47.6 (CH_2), 47.2 (CH_2), 47.1 (CH_2), 34.5(CH_2), 34.4(CH_2); E.A.: Calcd for $C_{270}H_{300}N_{108}O_{96}Pd_{12}$•36($H_2O$): C, 37.62; H, 4.83; N, 18.93, Found: C, 37.78; H, 4.50; N, 19.01; m.p.: 220 °C (decomposed); IR (KBr,cm^{-1}): 3425, 3208, 3075, 1669, 1515, 1373, 1144, 1055, 939, 820.

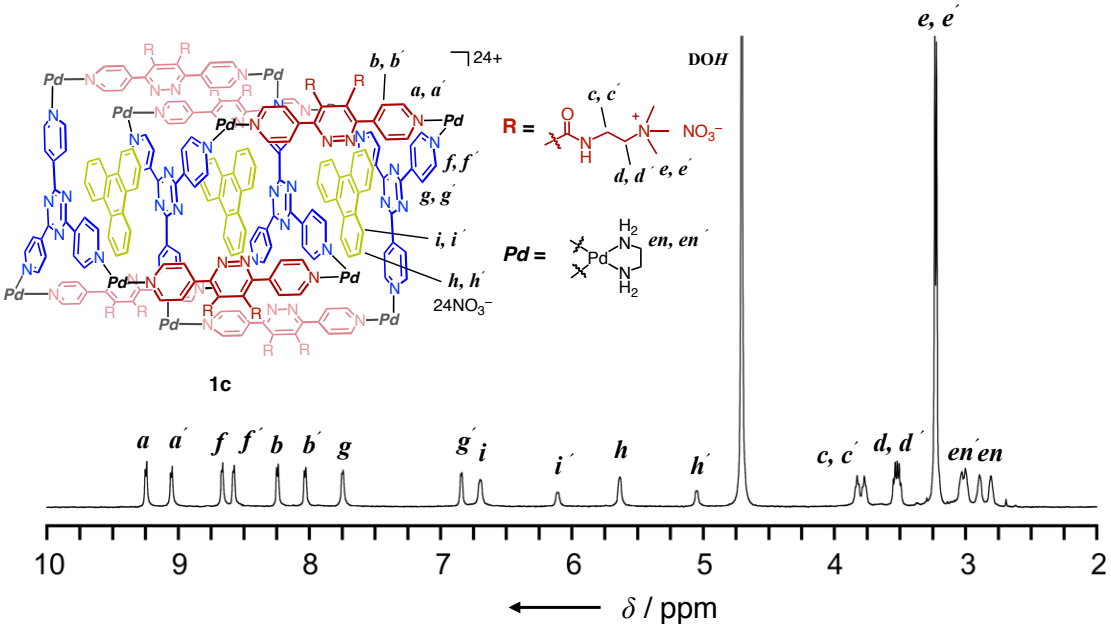
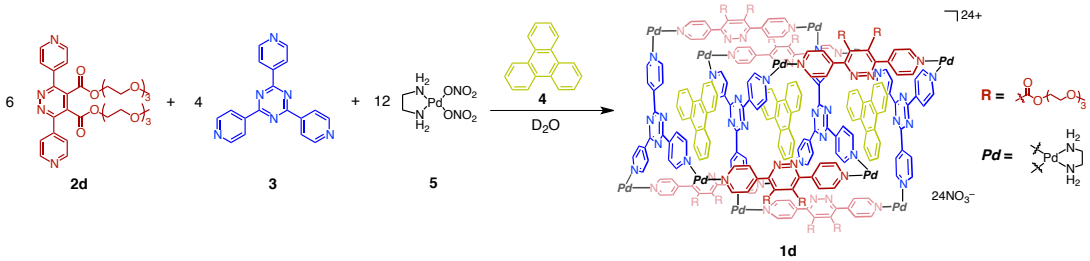


Figure S4. ^{1}H NMR spectrum of complex **1c** (300 MHz, D_2O, 300 K).

• Synthesis of complex 1d



A mixture of bidentate ligand **2d** (191 mg, 311 mmol), tridentate ligand **3** (64.9 mg, 208 mmol), triphenylene **4** (47.4 mg, 208 mmol), and (en)Pd(NO_3)$_2$ **5** (181 mg, 623 mmol), and were stirred in D_2O (5.2 mL) at 40 °C for 23 h to give a yellow suspension. After removal of excess triphenylene by centrifugation, ^{1}H NMR analysis of the supernatant solution revealed the selective formation of complex **1d**. After filtration to remove the excess triphenylene, the resulting yellow solution was freeze-dried. **1d** was isolated as a yellow powder (432 mg, 47.4 μmol) in 91% yield. ^{1}H NMR (500 MHz, D_2O, 2.5 mM, 300 K): δ (ppm) = 9.34 (d, J = 7.0 Hz, 12H), 9.17 (d, J = 6.3 Hz, 12H), 8.81 (d, J = 5.5 Hz, 12H), 8.66 (d, J = 5.3 Hz, 12H), 8.32 (d, J = 7.0 Hz, 12H), 8.22 (d, J = 6.3 Hz, 12H), 7.81 (br, 12H), 6.78 (d, J = 7.0 Hz, 12H), 6.70 (br, 12H), 6.10 (br, 6H), 5.62 (br, 12H), 4.81 (br, 6H), 4.59 (br, 12H), 4.57 (br, 12H),

3.83 (br, 12H), 3.82 (br, 12H), 3.78 (m, 48H), 3.76 (m, 12H) 3.72 (t, J = 8.8 Hz,12H), 3.70 (t, J = 8.8 Hz,12H), 3.61 (t, J = 8.8 Hz, 12H), 3.44 (s, 18H), 3.33(s, 18H) , 3.07 (br, 12H), 3.03 (br, 12H), 2.96 (br, 12H), 2.87 (br, 12H); ^{13}C NMR (125 MHz, D_2O, 300 K, 2.5 mM): δ (ppm) = 167.5 (C_q), 165.2 (C_q), 164.4 (*C*O), 164.3(*C*O), 156.2 (C_q), 155.9 (C_q), 152.3 (*C*H), 151.9 (*C*H), 151.6 (*C*H), 151.3 (*C*H), 147.9 (C_q), 147.7 (C_q), 144.0 (C_q), 142.4 (C_q), 129.7 (C_q), 129.6 (C_q), 127.2 (C_q), 127.2 (*C*H), 127.1 (*C*H), 126.8 (C_q), 126.6 (*C*H), 125.4 (*C*H), 124.9 (*C*H), 124.2 (*C*H) , 122.2 (*C*H), 121.1 (*C*H), 71.3 (CH_2), 71.2 (CH_2), 69.9 (CH_2), 69.8 (CH_2), 69.7 (CH_2), 69.7 (CH_2), 69.6 (CH_2), 68.0(CH_2), 68.0 (CH_2), 58.4 (CH_3), 58.3 (CH_3), 58.2 (CH_2), 58.2 (CH_2), 47.4 (CH_2), 47.2 (CH_2), 47.0 (CH_2), 47.0 (CH_2) E.A.: Calcd for $C_{330}H_{408}N_{96}O_{132}Pd_{12}$•36($H_2O$): C, 40.62; H, 4.96; N, 13.78, Found: C, 40.60; H, 4.75; N, 13.84; m.p.: 190 °C (decomposed); IR (KBr,cm^{-1}): 2882, 1739, 1601, 1397, 1250, 1101, 943, 841.

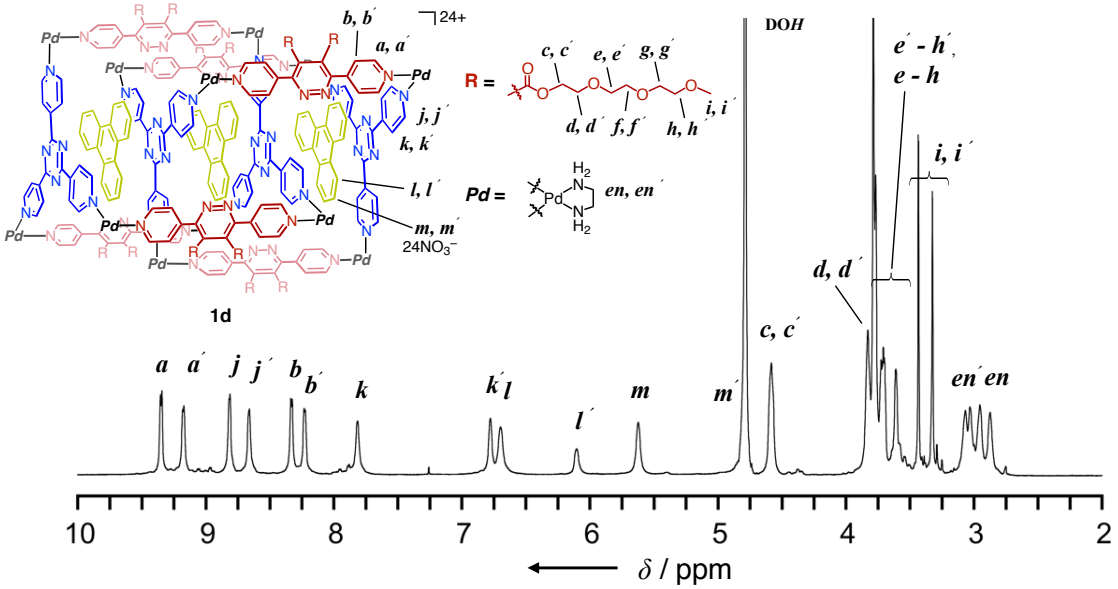


Figure S5. ^{1}H NMR spectrum of complex **1d** (500 MHz, D_2O, 300 K).

3. X-ray crystallographic structure of complex 1b'

To obtain a single crystal of the complex, (*N*,*N*,*N*',*N*'-tetramethylethylenediamine)Pd(ONO_2)$_2$ was used as a metal source for the complexation, affording complex **1b'** which has a structure analogous to the hydroxyl side chain bearing complex **1b**. Gradual evaporation of water from its aqueous solution produced yellow needle-like crystals. Single crystal X-ray diffraction of the obtained crystal was measured at 90 K. The collected diffraction data were processed with the Bruker APEX-II software. The structure was solved by the charge flipping method using SUPERFLIP[S5] and refined by full-matrix least-squares methods using the SHELXL-97[S7] module running on the Yadokari-XG 2009[S6] software. All hydrogen positions were calculated using a riding atom model. DFIX and SIMU as restraints were used during the refinement for the side chains, capping ligands of palladium, and nitrate ions. Additionally, solvent water correction (SWAT) was used.

The complex takes up only half of the cell volume and the water molecules and nitrate ions occupying the remaining space were severely disordered, leading to data with a low resolution. Therefore, all the atoms were refined isotropically. The structure of the complex composed of seven aromatic molecules stacked in a parallel manner was clearly determined. Furthermore, the side chains located orthogonal to the stacking of the aromatic molecules were also observed, although the terminal atoms were not modeled for some of the side chains due to severe disordering. CCDC 927291 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

S5. L. Palatinus and G. Chapuis, *J. Appl. Cryst.*, 2007, **40**, 786.
S6. G. M. Sheldrick, *SHELXL-97 – A program for the Refinement of Crystal Structures*, University of Göttingen, Germany **1997**, release 97-2.
S7. C. Kabuto, S. Akine, T. Nemoto and E. Kwon, *J. Cryst. Soc. Jpn.*, 2009, **51**, 218.

Table S1. Crystal data and structure refinement for complex **1b'**.

CCDC No.	927291	
Empirical formula	$C_{316.50}\,H_{366}\,N_{93}\,O_{111}\,Pd_{12}$	
Formula weight	8525.82	
Temperature	90(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	$P\bar{1}$	
Unit cell dimensions	a = 23.379(3) Å	α = 107.7170(10)°
	b = 27.150(3) Å	β = 92.383(2)°
	c = 43.649(5) Å	γ = 103.950(2)°
Volume	25415(5) Å^3	
Z	2	
Density (calculated)	1.114 Mg/m^3	
Absorption coefficient	0.485 mm^{-1}	
F(000)	8712	
Crystal size	0.19 × 0.04 × 0.02 mm^3	
Theta range for data collection	1.06 to 20.78°	
Index ranges	–23<=h<=23, –27<=k<=27, –43<=l<=43	
Reflections collected	153910	
Independent reflections	52489 [R(int) = 0.1284]	
Completeness to theta = 20.78°	99.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9904 and 0.9135	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	52489 / 155 / 2195	
Goodness-of-fit on F^2	1.502	
Final R indices [I>2σ(I)]	R_1 = 0.1695, wR_2 = 0.4435	
R indices (all data)	R_1 = 0.2795, wR_2 = 0.4931	
Largest diff. peak and hole	2.047 and –1.042 e•Å^{-3}	

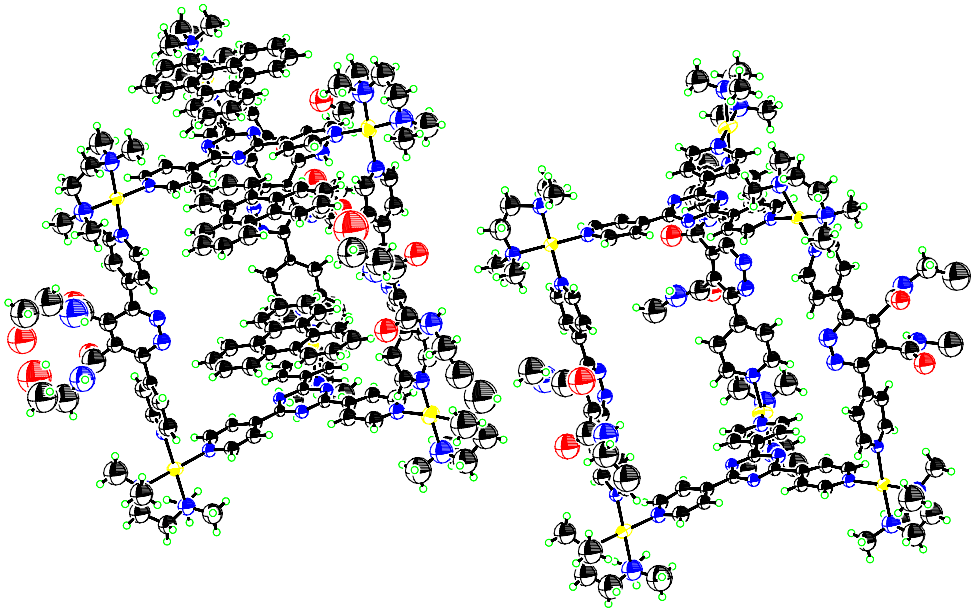


Figure S6. ORTEP drawing (50% probability ellipsoids) of complex **1b'**. The counter ions (NO_3^-) and solvent molecules are omitted for clarity.

4. Concentration-dependent aggregation behavior of complexes

• Concentration-dependent ^{1}H NMR spectra of complexes

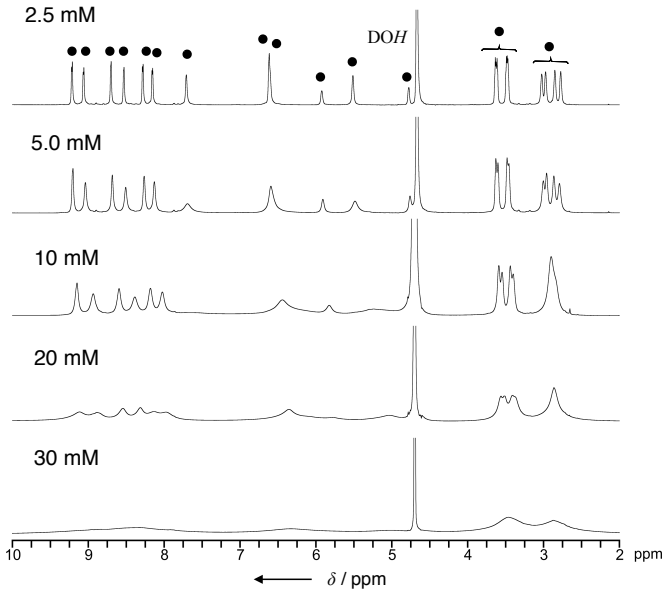


Figure S7. ^{1}H NMR spectrum of complex **1b** (300 MHz, D_2O, 300 K) at various concentrations.

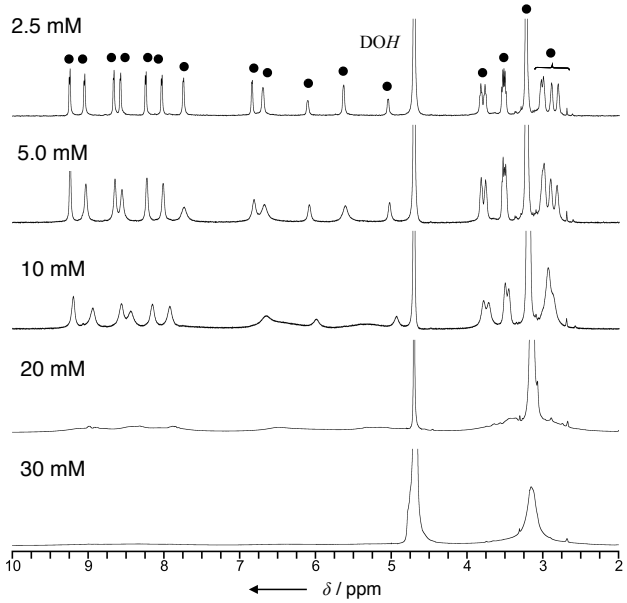


Figure S8. ^{1}H NMR spectrum of complex **1c** (300 MHz, D_2O, 300 K) at various concentrations.

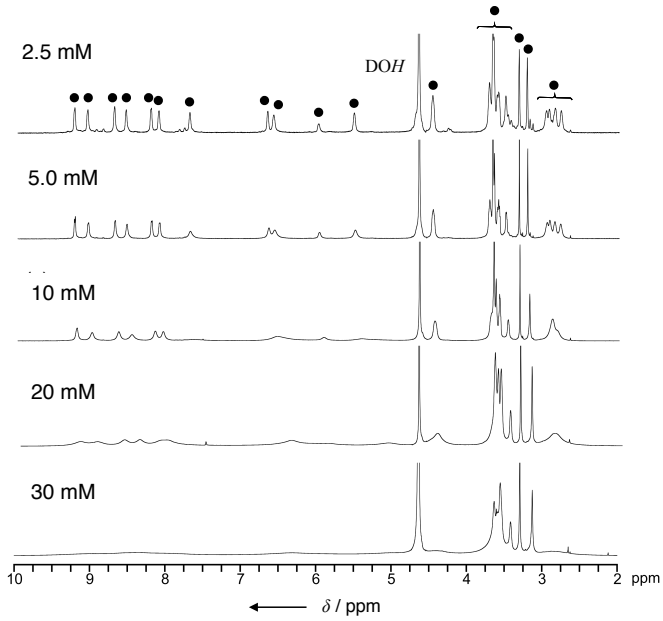


Figure S9. ^{1}H NMR spectrum of complex **1d** (300 MHz, D_2O, 300 K) at various concentrations.

• Concentration-dependent diffusion coefficients and degree of aggregation of complexes

Diffusion coefficient *D* was measured using DOSY NMR at concentrations of 2.5, 5.0, 10, 20, 30 mM. The degree of aggregation *n* was estimated using the approximation for rod-like molecules according to the reported procedure[S8] using d = 3 nm for the diameter of the complex (from the core aromatic structure to the amide or ester of the side chains) and L = 2.4 nm as the length of a single complex in the stacking direction. Due to the limits of the approximation for rod-like molecules,[S9] for concentrations of 2.5 and 5.0 mM the degree of aggregation was estimated using the Stokes-Einstein equation for a rigid sphere.

Table S2. Concentration-dependence of diffusion coefficient *D* and the degree of aggregation *n* for complexes **1b**, **1c**, and **1d**.

Complex	2.5 mM		5.0 mM		10 mM		20 mM		30 mM	
	D / 10^{-11} m^2•s^{-1}	n	D / 10^{-11} m^2•s^{-1}	n	D / 10^{-11} m^2•s^{-1}	n	D / 10^{-11} m^2•s^{-1}	n	D / 10^{-11} m^2•s^{-1}	n
1b	14	1	12	1	8.1	3	2.9	18	-[b]	
1c	13	1	1.1	1	6.4	5	1.3[a]	57[a]	-[b]	
1d	13	1	11	1	7.8	3	2.5	23	1.5[a]	45[a]

a: Due to severe broadening of signals of the aromatic molecules and ethylenediamine of the palladium complexes, only the diffusion coefficients of the side chains were determined. b: Due to severe broadening of signals, diffusion coefficients were not determined.

S8. Y. Yamauchi, Y. Hanaoka, M. Yoshizawa, M. Akita, T. Ichikawa, M. Yoshio, T. Kato and M. Fujita, *J. Am. Chem. Soc.*, 2010, **132**, 9555.

S9. W. Eimer and R. Pecora, *J. Chem. Phys.*, 1991, **94**, 2324.

5. HR-MAS of 30 mM complex 1b solution

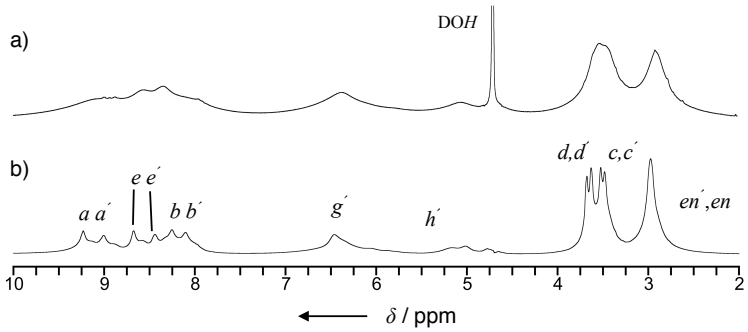


Figure S10. ^{1}H NMR spectra of 30 mM complex **1b** solution: a) solution-state ^{1}H NMR (500 MHz, D_2O, 300 K) and b) HR-MAS NMR with a MAS rotation of 10 kHz (600 MHz, D_2O, 300 K).

The 30 mM solution of complex **1b** was measured using High-Resolution Magic-Angle-Spinning (HR-MAS) NMR with a MAS rotation of 10 kHz with presaturation of the solvent residual signal. The MAS reduces the effect of $3\cos^2\theta-1$ of the dipolar tensor that is responsible for the fast relaxation, and thus the signals are sharpened. Signals that can be assigned to the complex on MAS conditions clearly show the maintenance of the structure of the complex. Additionally, the results demonstrate that the rise in viscosity upon the increase of concentration is not the dominant factor responsible for the broadening of signals, because between Figure S10a and S10b there is no difference in viscosity, but even so the signals are sharper on MAS conditions.

6. Measurement of apparent coupling values J_{app}

•Measurement and processing

For the measurements of apparent coupling values J_{app} of complex **1b**, two-dimensional ^{13}C-coupled ^{1}H-^{13}C HSQC spectra were recorded with acquisition times indicated in Table S3. Time domain data on both dimensions were apodized with a squared sine bell function and zero-filled prior to 2D Fourier transformation to yield a digital resolution indicated in Table S3.

Table S3. Measurement conditions for apparent coupling values J_{app} on each NMR system.

NMR system / MHz	*t*2 (^{1}H) acquisition time / ms	*t*1 (^{13}C) acquisition time / ms	digital resolution after zero-filling / Hz	
			*F*2 (^{1}H)	*F*1 (^{13}C)
300	92	19	0.1	25.8
500	137	23	0.1	21.6
600	91	19	0.1	25.9
920	59	13	0.2	38.0

• Magnetic-field dependent J_{app}

For the 2.5 mM solution of complex **1b**, J_{app} was measured using 300, 500, 600, and 920 MHz spectrometers in order to obtain the inherent $^{1}J_{CH}$ coupling constant, by estimating the intercept through

linear approximation of the squared magnetic field B_0^2 vs. J_{app} plot, according to reported procedures.[S10] Experimental errors are shown as the root-mean-square uncertainty ΔJ for each of the signals using,

$$\Delta J = LW / SN \quad \ldots(S1)$$

where LW is the line width at half height (in the ^{1}H dimension) and SN is the signal-to-noise ratio.[S11] The intercept, and thus the inherent $^1J_{CH}$ for signal $C^{h'}$-$H^{h'}$ of complex **1b** was determined to be $^1J_{CH}$ = 156.4 Hz.

Table S4. Measured apparent coupling values J_{app} on each NMR system.

NMR system / MHz	field strength B_0 / T	B_0^2 / T^2	J_{app} / Hz	ΔJ / Hz
300	6.99 T	48.8601	156.2	0.8
500	11.7 T	137.8276	155.6	0.1
600	14.1 T	198.5281	155.4	0.1
920	21.6 T	467.4244	153.9	0.2

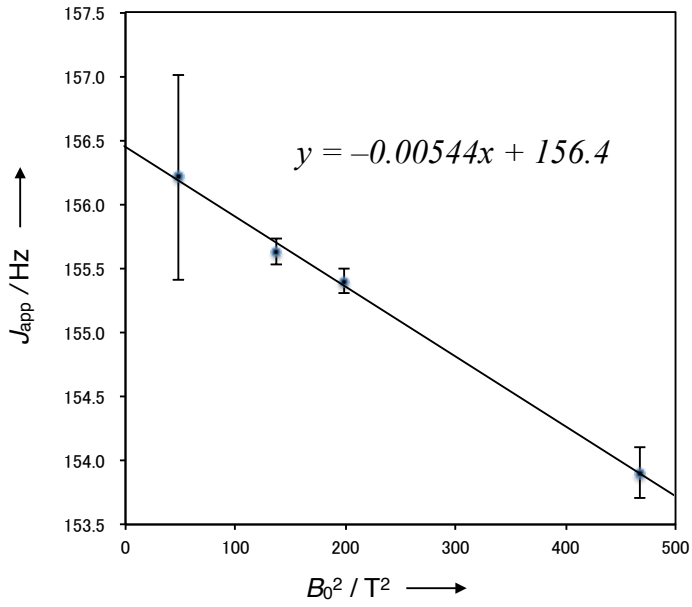


Figure S11. Apparent coupling values J_{app} plotted against the squared field strength B_0^2 for the $C^{h'}$-$H^{h'}$ pair in a 2.5 mM solution of complex **1b**. The equation represents the linear approximation of the plot.

S10. S. Sato, O. Morohara, D. Fujita, Y. Yamaguchi, K. Kato and M. Fujita, *J. Am. Chem. Soc*., 2010, **132**, 3670.

S11. A. Bax, G. Kontaxis and N. Tjandra, Dipolar couplings in macromolecular structure determination. In *Nuclear Magnetic Resonance Of Biological Macromolecules, Part B***;** T. L. James and V. Dötsch, U. Schmitz, Eds.; Academic Press: London, UK, 2001; pp 127-174.

Table S5. Measured apparent coupling values J_{app} at each concentration of **1b** solution.

concentration / mM	J_{app} / Hz	ΔJ / Hz	D [Hz] (= J_{app} - $^1J_{CH}$)
2.5	155.4	0.11	-1.0
5.0	155.0	0.11	-1.4
10	153.7	0.37	-2.7

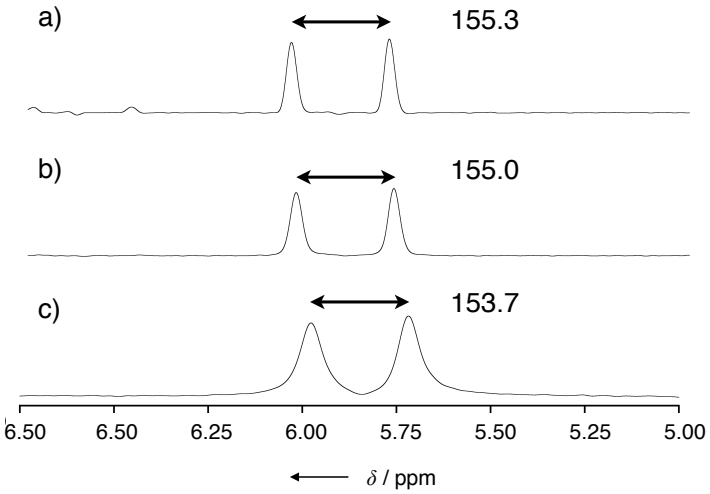


Figure S12. Slices of the ^{1}H dimension containing signal $C^{h'}$-$H^{h'}$ at concentrations of a) 2.5, b) 5.0, and c) 10 mM of complex **1b,** measured by ^{13}C-coupled ^{1}H-^{13}C HSQC NMR**.** (600 MHz, D_2O, 300 K) J_{app} are indicated in Hz.

Spectra for complex **1b** at a concentration of 2.5, 5.0 and 10 mM were obtained by ^{13}C-coupled HSQC measurements at 300 K on a 600 MHz spectrometer. The apparent J coupling values (J_{app}) were read following the reported procedure.[10]

7. Induction of RDC upon a coexisting protein: Cys76Gly ubiquitin dimer.

•Measurement and processing of data

For the measurement of the ^{1}H-^{15}N coupling values of G76C ubiquitin dimer in a complex **1b** H_2O:D_2O = 9:1 solution, two-dimensional ^{1}H-^{15}N HSQC was measured with acquisition times of 137 ms and 60 ms for the $t1$ and $t2$ dimensions, respectively, and ^{1}H-^{15}N TROSY spectra were recorded with acquisition times of 137 ms and 30 ms for the $t1$ and $t2$ dimensions, respectively. For the measurement of ^{1}H-^{15}N coupling values for G76C ubiquitin dimer H_2O:D_2O = 9:1 solution, two-dimensional ^{1}H-^{15}N HSQC and ^{1}H-^{15}N TROSY spectra were measured with acquisition times of 137 ms and 60 ms for the $t1$ and $t2$ dimensions, respectively. All time domain data were apodized with a squared sine bell and were zero-filled prior to 2D Fourier transformation to yield a digital resolution of 3.7 Hz ($F2$) and 0.5 Hz ($F1$). The apparent coupling value J_{app} in half magnitude was measured by comparing the chemical shifts of the HSQC and TROSY spectrum.

Table S6. Observed coupling values and calculated RDC values for ^{15}N-^{1}H bonds (500 MHz, 95% H_2O/5% D_2O, 300 K).

Residue	$1/2(^1J_{NH} + D_{NH})$ [Hz][a]	$1/2\,^1J_{NH}$ [Hz][b]	$1/2D_{NH}$ [Hz]	D_{NH} [Hz]
F4	47.6	48.0	–0.4	–0.8
T7	45.3	47.3	–2.0	–4.0
T9	43.6	47.9	–4.3	–8.6
K11	47.4	48.0	–0.6	–1.2
V17	44.5	47.2	–2.7	–5.4
S20	43.5	46.7	–3.2	–6.4
K27	49.5	48.0	1.5	3.0
D32	50.4	48.1	2.3	4.6
K33	44.5	48.1	–3.6	–7.2
Q41	47.2	46.0	1.2	2.4
L43	50.4	47.8	2.6	5.2
I44	49.2	47.5	1.7	3.4
F45	48.4	47.1	1.3	2.6
G47	41.2	46.7	–5.5	–11
L50	49.4	46.7	2.7	5.4
D52	47.1	47.6	–0.5	–1.0
R54	44.3	47.6	–3.3	–6.6
L56	44.0	48.2	–4.2	–8.4
Y59	49.1	47.1	2.0	4.0
N60	48.0	46.7	1.3	2.6
I61	47.5	47.6	–0.1	–0.2
E62	51.2	47.4	3.8	7.6
S65	46.0	46.8	–0.8	–1.6
T66	48.9	46.6	2.3	4.6
H68	50.8	47.9	2.9	5.8

a: Measured for ubiquitin dimer in a 35 mM complex **1b** solution. b: Measured for ubiquitin dimer in water.