Supramolecular Packing and Macroscopic Alignment Controls Actuation Speed in Macroscopic Strings of Molecular Motor Amphiphiles

Franco King-Chi Leung,* Tobias van den Enk,† Takashi Kajitani,§ Jiawen Chen,‡ Marc C. A. Stuart,† Jeroen Kuipers,‖ Takanori Fukushima,* and Ben L. Feringa*‡,†

†Center for System Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
‡Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8503, Japan
¶RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan
§Department of Cell Biology, Molecular Imaging and Electron Microscopy, University Medical Center Groningen, University of Groningen, 9712 CP Groningen, The Netherlands

Supporting Information

ABSTRACT: Three-dimensional organized unidirectionally aligned and responsive supramolecular structures have much potential in adaptive materials ranging from biomedical components to soft actuator systems. However, to control the supramolecular structure of these stimuli responsive, for example photoactive, materials and control their actuation remains a major challenge. Toward the design of “artificial muscles”, herein, we demonstrate an approach that allows hierarchical control of the supramolecular structure, and as a consequence its photoactuation function, by electrostatic interaction between motor amphiphiles (MA) and counterions. Detailed insight into the effect of various ions on structural parameters for self-assembly from nano- to micrometer scale in water including nanofiber formation and nanofiber aggregation as well as the packing structure, degree of alignment, and actuation speed of the macroscopic MA strings prepared from various metal chlorides solution, as determined by electronic microscopy, X-ray diffraction, and actuation speed measurements, is presented. Macroscopic MA strings prepared from calcium and magnesium ions provide a high degree of alignment and fast response photoactuation. By the selection of metal ions and chain length of MAs, the macroscopic MA string structure and function can be controlled, demonstrating the potential of generating multiple photoresponsive supramolecular systems from an identical molecular structure.

INTRODUCTION

Supramolecular polymers are found in many living systems, for example, cytoskeleton filaments (F-actin1 and microtubules2), flagellar filaments of bacteria,3 and polymers of viral proteins and muscles,4 to serve vital roles in key biological functions. While biological systems provide precise control in supramolecular polymerization,5–7,14 synthetic supramolecular polymers5,6,14 in aqueous media allow tunable features due to the design based on synthetic compounds and bioinspired functionality.6,7,16,17,8–15 This delicate molecular design strategy allows the construction of hierarchical supramolecular assemblies along multiple-length-scales. At the microscopic length-scale level,10,11 numerous unimolecular amphiphilic molecules have been shown to assemble into highly ordered one-dimensional (1D) supramolecular systems, through noncovalent interaction, for example, hydrogen bonding18–25 arene interaction,26–33 and electrostatic effects.32,34–37 At macroscopic length-scales, the obtained 1D supramolecular polymers of unimolecular amphiphiles can further assemble, instead of forming a three-dimensional (3D) randomly entangled network, into 3D unidirectionally aligned hierarchical supramolecular structures, providing exciting opportunities toward applications for instance in regenerative (biomedical) materials,38–40 actuators, electronics, and optoelectronic materials.41–43 To further demonstrate the importance of 3D unidirectionally aligned hierarchical supramolecular structures, we recently reported that a photoresponsive hierarchical supramolecular assembled structure derived from an amphiphilic molecular motor with precise control of molecular organization and cooperativity allows energy conversion, accumulation of strain, and amplification of the molecular

Received: October 6, 2018
Published: November 21, 2018

DOI: 10.1021/jacs.8b10778
rotation of motor amphiphile (MA) to macroscopic muscle-like contractive motions. This supramolecular approach provides a complementary method to the existing macroscopic actuators obtained by stimuli-responsive crystals, polymeric gels, and polymeric liquid crystals.

The dynamic nature of the supramolecular polymers provides inherent sensitivity of the assembled structure to the external environment, for example, chemicals, solvents, external shear force, electric and magnetic fields. In addition, the design of molecular amphiphiles allows precise control over the hierarchical structure from microscopic to macroscopic length-scale and their intrinsic functions. Notably, electrostatic screening of amphiphilic self-assembled structures, pioneered by Stupp et al., by careful choice of counterions, provides a mean to control stiffness of 3D randomly entangled supramolecular structures and has enabled to govern important functions, for example, cell proliferation, differentiation, adhesion, and migration. However, the control of 3D unidirectionally aligned hierarchical supramolecular structure by a single non-invasive external stimulus, without covalent chemical modification of the molecular amphiphile structure, at different length-scale and as a consequence its function remains highly challenging.

In our recently reported artificial muscle, the electrostatic interaction between carboxylate groups of MA and Ca$^{2+}$ allows the MA nanofiber stabilization and the formation of a MA macroscopic string using a shear flow method to provide unidirectionally aligned MA strings for photoactuation. We envisioned that by manipulating the electrostatic interaction of the carboxylate groups of MA and its counter-cations (M$^{n+}$) allows further control of the hierarchical assembled structure of the motor amphiphile and elucidates key parameters for supramolecular aggregation (Figure 1). The nature of the cationic counterion effect on the organization of MA might enable the control of induction of nanofibers formation, aggregation of nanofibers, structural order parameters of the unidirectionally aligned structures, and speed of photoactuation of the string of unidirectionally aligned nanofibers. Additionally, the side chains of MA are modified to provide insight in the effect of the chain length effect on the structure and functioning of MA macroscopic strings. By elucidating the key design of supramolecular muscles, ultimately, this could open up new prospects toward the development of controllable stimuli-responsive materials and future soft robotic systems.

### RESULTS AND DISCUSSION

#### Molecular Design and Synthesis

The motor amphiphile was designed with a second-generation molecular motor core, and a dodecyl chain was attached to the upper half, and two carboxyl groups connected with alkyl-linkers to the lower half (Figure 1). In addition to the countercation effect, we envision that various chain lengths of the alkyl-linker, which connected the two carboxyl groups to the lower half of the motor unit, allow for systematic modification of the packing in the resultant MA string and its actuation function. Motor amphiphiles with shorter chain lengths, MAC$_6$ and MAC$_8$, as well longer chain lengths, MAC$_{11}$, were designed (Figure 1). The general synthesis is summarized in Scheme 1. Compounds 1 and 4 were prepared by our reported procedures (Scheme 1). The key step in the synthesis of MAC$_6$, MAC$_8$, and MAC$_{11}$ is the formation of the central overcrowded olefinic bond by

![Figure 1. Molecular structures of molecular motor amphiphiles and the hierarchical organization and photoactuation process of their assembled structures in the macroscopic string.](https://doi.org/10.1021/jacs.8b10778)
diazo-thioketone coupling (Scheme 1). The precursors 2 were obtained by Williamson ether formation of thioxanthone 1 with the corresponding alkyl bromides in the presence of K₂CO₃ in DMF, followed by conversion into the corresponding thioketones 3 with Lawesson’s reagents in toluene. Hydrazine 4 was in situ oxidized with diacetoiodobenzene in DMF into the corresponding diazo compound, and subsequent addition of the freshly prepared thioketones 3 provided the corresponding episulfides 5. Desulfurization with triphenylphosphine in toluene gave the corresponding overcrowded alkenes 6. The motor amphiphiles MAs were obtained by hydrolysis of the ester groups into carboxylic acid groups in the presence of LiOH in water and THF. The structures of all new motor amphiphiles were unambiguously determined by ¹H, ¹³C NMR, and high-resolution ESI-TOF mass spectrometry (Figures S15–S37).

**Ionic Effect of MAC₁₀ Assembled Structure.** Freshly prepared aqueous solutions of MAC₁₀ with 2 equiv of sodium hydroxide were heated at 80 °C for 30 min and cooled down to room temperature to afford a colorless transparent solution, indicating that the deprotonated form is soluble up to 50.0 mM concentration. A Nile Red fluorescence assay (NRFA), which probes the internal hydrophobicity of assemblies, revealed a decrease in blue shift when diluting beyond 0.01 mM and showed a critical aggregation concentration (CAC) of 2.67 μM (Figure 2a). The MAC₁₀ assemblies formed by the water-soluble carboxylates were imaged using cryogenic transmission electron microscopy (cryo-TEM) to capture their solution-state morphologies. MAC₁₀ assembled into fibers hundreds of nanometers to micrometers in length at 5.0 mM concentration (Figure S1a), while no nanostructure was observed below CAC (Figure S1b). To investigate the counterion effect of LiCl, NaCl, KCl, BeCl₂, MgCl₂, CaCl₂, SrCl₂, BaCl₂, and ScCl₃ on nanostructure formation of the MAC₁₀, NRFA was employed to probe the change of internal hydrophobicity of the MAC₁₀ assembly. The blue shift of Nile Red was monitored in the MAC₁₀ solutions (1.01 μM; below CAC) with various concentrations of CaCl₂ (0.01–15.0 mM) (Figure 2b). A gradual increase of the blue shift of the MAC₁₀ assembly was observed with increasing concentration of CaCl₂ reflecting an increase of the internal hydrophobicity. At 1.0 mM of CaCl₂, nanostructure formation was observed below CAC (Figure S1c). The results suggested that the excess amount of counterion (Ca²⁺) promotes the formation of nanofibers below CAC. An increase of internal hydrophobicity of MAC₁₀ assembly for other metal chloride solutions was only observed above 1.0 mM, indicating that Ca²⁺ ions induce nanostructure formation of MAC₁₀ more effectively than the other ions (Figure 2b). Surprisingly, also Mg²⁺ ions (above 1.0 mM) induced nanostructure formation effectively. However, the high charge density Be²⁺ and Sc³⁺ as well as large ionic radii Sr²⁺ and Ba²⁺ ions showed no significant effect on nanofibers formation. Similarly, high concentration of LiCl, NaCl, and KCl showed no significant nanoaggregate formation.

The complementary ionic interaction between the carboxylate moieties of MAC₁₀ and its counterions induces the aggregation of nanofibers. Dynamic light scattering (DLS) was then employed to investigate the aggregation of MAC₁₀ nanofibers with its counterions (concentration: 0.01 mM to 15.0 mM). The molar scattering intensity of MAC₁₀ solution (1.01 mM; above CAC) gradually increased with the concentration of CaCl₂, indicating that aggregation of nanofibers occurred (Figure 3). At 15.0 mM of CaCl₂, 145.3 ± 13 M Counts s⁻¹ M⁻¹ molar scattering intensity was observed. A lower molar scattering intensity 45.9 ± 3 M Counts s⁻¹ M⁻¹ was observed at 1.01 mM concentration of CaCl₂ (Figure S1d). The critical aggregation concentration of MAC₁₀ (concentration: 5.0 × 10⁻³ to 2.0 mM) and for determination of counterion effect to MAC₁₀ (1.01 μM; below CAC) nanofiber formation concentration with various metal chlorides (concentration: 0.01–15.0 mM).

![Figure 2](image2.png)  
**Figure 2.** Nile Red fluorescence assay (a) for determination of the critical aggregation concentration of MAC₁₀ (concentration: 5.0 × 10⁻³ to 2.0 mM) and (b) for determination of counterion effect to MAC₁₀ (1.01 μM; below CAC) nanofiber formation concentration with various metal chlorides (concentration: 0.01–15.0 mM).

![Figure 3](image3.png)  
**Figure 3.** Molar scattering intensity of MAC₁₀ nanofibers (1.01 mM; above CAC) in the presence of metal chlorides (concentration: 0.01 mM to 15.0 mM).
Counts \( s^{-1} \text{ M}^{-1} \) was obtained in MgCl\(_2\) solutions (15.0 mM). Comparable molar scattering intensities of 26.7 ± 1 M Counts \( s^{-1} \text{ M}^{-1} \), 27.1 ± 1 M Counts \( s^{-1} \text{ M}^{-1} \), and 26.3 ± 1 M Counts \( s^{-1} \text{ M}^{-1} \) were obtained in BeCl\(_2\), SrCl\(_2\), and BaCl\(_2\) solutions (15.0 mM), respectively. Considering the binding affinity between carboxylate and \( \text{M}^{2+} \), \( \text{Ca}^{2+} \) is expected to provide more significant aggregation.\(^6\)\(^7\) However, the triple-charged \( \text{Sc}^{3+} \) (15.0 mM) afforded aggregates with a comparable molar scattering intensity 29.4 ± 2 M Counts \( s^{-1} \text{ M}^{-1} \) to that of aggregates based on \( \text{Be}^{2+} \), \( \text{Sr}^{2+} \), and \( \text{Ba}^{2+} \) ions, possibly due to charge mismatch to \( \text{MAC}_{10} \) in the nanofiber structure. No significant aggregation was observed with LiCl, NaCl, and KCl adding up to 15.0 mM (Figure 3).

To further investigate the counterion effect (metal chlorides) on the interbrillar interaction and the structure features of \( \text{MAC}_{10} \) nanofibers, a macroscopic string of \( \text{MAC}_{10} \) was prepared according to our previous reported procedure.\(^4\)\(^4\) Typically, a 50.0 mM solution of \( \text{MAC}_{10} \) was manually drawn into an aqueous solution of MgCl\(_2\) (150 mM) from a pipet, and a noodle-like string with an arbitrary length was formed.

\[ q = 0.1 - 0.45 \text{ nm}^{-1} \]

\( \text{(g)} \) \( \text{(h)} \) \( \text{(i)} \)
Scanning electronic microscopy (SEM) of the string, prepared from the solution of MgCl$_2$, shows arrays of unidirectionally aligned nano fiber bundles (Figure 4a), which was essentially identical to that of the string prepared from CaCl$_2$ solution (Figure S2a,b). Notably, the MAC$_{10}$ strings prepared from the solutions of BeCl$_2$, SrCl$_2$, and ScCl$_3$ also showed similar morphology, that is, arrays of nano fiber bundles with unidirectional alignment, in SEM images (Figures S3a, S4a, and 4b), while no alignments were observed in the MAC$_{10}$ strings prepared from solutions of BaCl$_2$, LiCl, NaCl, and KCl (Figures S5a, S6a, S7a, and 4c). The freshly prepared MAC$_{10}$ string from a MgCl$_2$ solution showed uniform birefringence in the direction of the strings long axis in polarized optical microscopy (POM) images (Figures 4d and S8a), which is essentially identical to POM images of the MAC$_{10}$ string obtained from CaCl$_2$ solution (Figure S2c). A lower birefringence was observed in the POM images of the MAC$_{10}$ strings prepared from solutions of BeCl$_2$, SrCl$_2$, and ScCl$_3$ (Figures S3b, S4b, 4c, and S8b). In addition, no birefringence was observed in the POM images of the MAC$_{10}$ strings prepared from solutions of BaCl$_2$, LiCl, NaCl, and KCl (Figures S5b, S6b, S7b, 4f, and S8c). The results indicated that MAC$_{10}$ nanofibers are aligned unidirectionally in the presence of Mg$^{2+}$ and Ca$^{2+}$ ions, while a lower degree of alignment in MAC$_{10}$ nanofibers is found in the presence of Be$^{2+}$, Sr$^{2+}$, and Sc$^{3+}$ ions. However, no significant alignment of MAC$_{10}$ nanofibers was observed in the presence of Ba$^{2+}$, Li$^+$, Na$^+$, and K$^+$ ions.

To provide the structural parameters and orientational order, that is, degree of alignment, of the MAC$_{10}$ nanofibers in the macroscopic strings, we carried out through-view small-angle X-ray scattering (SAXS) measurements. In the 2D SAXS image of the MAC$_{10}$ string prepared from MgCl$_2$ solution on a sapphire substrate at 25 °C (Figure 4g), a pair of spot-like scatterings is observed in a smaller-angle region ($q = 0.1–0.45$ nm$^{-1}$) (Figure 4g, inset), which is due to scatterings from the unidirectionally aligned nano fiber bundles. The diffraction arcs with $d$-spacings of 5.23, 2.58, and 1.75 nm (Figure 4j), arising from the diffractions from the (001), (002), and (003) planes, respectively, of a lamellar structure, which is constructed by the unidirectionally aligned nanofibers of MAC$_{10}$ with ionic interaction between Mg$^{2+}$ and carboxylates of MAC$_{10}$ as interfibrillar interaction. The layer spacing of the lamellar structure ($c = 5.23$ nm) of the MAC$_{10}$ string prepared from MgCl$_2$ solution is shorter than that of observed MAC$_{11}$ string prepared from CaCl$_2$ solution ($c = 5.48$ nm) (Figure S2d,e), indicating that Mg$^{2+}$ ions induce a closer packing in the nanofibers of MAC$_{10}$. The angular dependency of the peak intensity of the diffraction from the (001) plane converted from the through-view 2D SAXS image of the MAC$_{10}$ string prepared from MgCl$_2$ solution (Figure 4g) showed the intensity maxima at 0° and 180° (Figure S9a). The peak intensity of the diffraction from the (001) plane was quantified by full-width half-maximum (fwhm) to obtain an ∼95° azimuthal angle, with a smaller azimuthal angle representing a larger degree of unidirectional alignment (Table 1). Indeed, a smaller fwhm (∼65°) was observed in the MAC$_{10}$ string prepared from CaCl$_2$ solution (Figure S9b and Table 1), indicating that a higher degree of alignment of MAC$_{10}$ nanofibers is obtained in CaCl$_2$ solution.

A pair of spot-like scatterings was observed in a smaller-angle region ($q = 0.1–0.45$ nm$^{-1}$) in the 2D SAXS image of the MAC$_{10}$ string prepared from solutions of ScCl$_3$, BeCl$_2$, and SrCl$_2$ (Figures 4h, S3c, and S4c, inset). The $d$-spacings of the diffraction arcs from the (001), (002), and (003) planes are summarized in Table 1. In accordance with the ionic radii of the cations, smaller layer spacings of a lamellar structure ($c = 5.07$ nm and $c = 4.99$ nm) were observed in the MAC$_{10}$ strings prepared from solutions of ScCl$_3$ and BeCl$_2$, respectively (Figures 4k and S3d) compared to those observed with Ca$^{2+}$ and Mg$^{2+}$ (Table 1). Meanwhile, a larger layer spacing of a lamellar structure ($c = 5.69$ nm) was observed in the MAC$_{10}$ strings prepared from solutions of SrCl$_2$ (Figure S4d, Table 1). In good agreement with the POM results, a lower degree of alignment was observed in the MAC$_{10}$ strings prepared from solutions of ScCl$_3$, BeCl$_2$, and SrCl$_2$ (fwhm >110°, Table 1, Figure S9).

Consistent with the results obtained in SEM and POM analysis, no spot-like scatterings in a smaller-angle region ($q = 0.1–0.45$ nm$^{-1}$) and an isotropic ring of (001) diffraction plane were observed in the 2D SAXS images of the MAC$_{10}$ strings prepared from solutions of B$_2$O$_3$, LiCl, NaCl, and KCl (Figures S5c, S6c, S7c, and 4i). The results indicated the lack of alignment of MAC$_{10}$ nanofibers in the presence of Ba$^{2+}$, Li$^+$, Na$^+$, and K$^+$ ions. On the basis of monocharged ions, larger layer spacings of lamellar structures ($c = 6.72$, 6.84, 6.88 nm) were observed in the MAC$_{10}$ strings prepared from solutions of LiCl, NaCl, and KCl, respectively (Figures S6d, S7d, and 4i, Table 1). In accordance with the ionic radius of double-charged Ba$^{2+}$ ion, a larger layer spacing of the lamellar structure...
(c = 5.77 nm) was observed in the MA<sub>10</sub> strings prepared from solutions of BaCl₂ (Figure S5d, Table 1). In summary, the order for degree of unidirectional alignment in the MA<sub>10</sub> strings prepared form metal chloride is Ca<sup>2+</sup> > Mg<sup>2+</sup> > Be<sup>2+</sup> ≈ Sr<sup>2+</sup> ≈ Sc<sup>3+</sup> ≈ Ba<sup>2+</sup> ≈ Li<sup>+</sup> ≈ Na<sup>+</sup> ≈ K<sup>+</sup>. A similar order389 of binding constants for low molecular weight organic carboxylates and alkali/alkaline earth metals has been described in the literature. For instance, the binding constant of succinate-alkali/alkaline earth metal complexes follows the order Ca<sup>2+</sup> > Mg<sup>2+</sup> > Be<sup>2+</sup> > Sr<sup>2+</sup> > Sc<sup>3+</sup> > Ba<sup>2+</sup> ≈ Li<sup>+</sup> ≈ Na<sup>+</sup> ≈ K<sup>+</sup>. The results indicated that a higher binding constant of MA<sub>10</sub> and its countermolecules allows a structurally more ordered macroscopic string formation.

**Ionic Effect of MA<sub>10</sub> Actuation Speed.** According to the anticipated photoactuation mechanism of MA<sub>10</sub>, the photochemical isomerization of MA<sub>10</sub> from the stable isomer to the unstable isomer induces the actuation of the MA<sub>10</sub> string toward the light source (Figure 5a). With a comprehensive structural investigation of MA<sub>10</sub> strings prepared from various metal chlorides solutions, the resultant hierarchical supramolecular structure, which seems to be to a large extent governed by the electrostatic interaction of M<sup>n+</sup> and carboxylate groups of MA<sub>10</sub> would be expected to control the actuation speed of a MA<sub>10</sub> string. Next, a freshly prepared MA<sub>10</sub> string was studied in a cuvette containing an aqueous solution of CaCl₂ (150 mM). Upon photoirradiation (λ = 365 nm, power output 15.5 mW), the MA<sub>10</sub> string bent toward the light source from an initial angle of 0° within 15 s, indicating that the actuation speed is 7.94 ± 0.4°/s (Figure 5a,b). It should be noted that a higher power output was employed (0.7 A applied current) than in our previous study (0.2 A applied current, actuation speed = 1.5°/s), providing a wider measuring window of actuation speed investigation. The MA<sub>10</sub> string, prepared from MgCl₂ solution (150 mM), bent with a saturated flexion angle of 90° within 25 s (4.29 ± 0.2°/s). Based on the degree of alignment of MA<sub>10</sub> strings prepared from solutions of CaCl₂ and MgCl₂ (Table 1), consistently, a higher degree of alignment of MA<sub>10</sub> strings provided a faster actuation toward the light source. Meanwhile, a comparable degree of alignment in the MA<sub>10</sub> strings prepared from solutions of ScCl₃, BeCl₂, and SrCl₂ showed a similar photoactuation speed (1.0–1.8°/s, Figure 5b and Table 1). In addition, the MA<sub>10</sub> strings prepared from solutions of BaCl₂, LiCl, NaCl, and KCl revealed no alignment, and no actuation was observed upon photoirradiation (Figure 5b and Table 1). In general, by choosing a particular metal chloride to prepare the MA<sub>10</sub> string, control over the degree of alignment of the MA<sub>10</sub> string and its structural packing was achieved to provide a means to control the actuation speed.

**Chain Length Effect of MA Structure and its Actuation.** To further elucidate key structural parameters, subsequently self-assembled structures based on motor amphiphiles with different chain length, that is, MA<sub>6</sub>, MA<sub>8</sub>, MA<sub>11</sub>, and MA<sub>14</sub>, and their actuation speed were studied. The photochemical isomerization steps of MA<sub>6</sub>, MA<sub>8</sub>, and MA<sub>11</sub> were examined by <sup>1</sup>H NMR and UV–vis spectroscopy (Figures S10 and S11). Essentially identical <sup>1</sup>H NMR signal shifts were observed in CD₂Cl₂ solutions of MA<sub>6</sub>, MA<sub>8</sub>, and MA<sub>11</sub> (Figure S10), and upon extended irradiation time, photostationary states with an unstable/stable isomer ratios of 2:9:1 were formed in CD₂Cl₂ solutions of MA<sub>6</sub>, MA<sub>8</sub>, and MA<sub>11</sub>. In UV–vis absorption studies of CH₃Cl solutions of MA<sub>6</sub>, MA<sub>8</sub>, and MA<sub>11</sub>, an isosbestic point at 327 nm over the course of irradiation indicated that a comparable and selective photoisomerization process occurs (Figure S11). In accordance with the sample preparation method as for MA<sub>10</sub> (vide supra), freshly prepared aqueous solutions of MA<sub>6</sub>, MA<sub>8</sub>, and MA<sub>11</sub> with 2.0 equiv of NaOH were heated at 80 °C for 30 min and cooled down to room temperature to afford colorless transparent solutions, showing that the deprotonated form is soluble up to 50.0 mM concentration. Nanofibers of MA<sub>6</sub>, MA<sub>8</sub>, and MA<sub>11</sub> (1.0 mM) were observed by cryo-TEM with uniform diameter (~5–6 nm) and several micrometers in length (Figure S12). To provide robust and stable macroscopic MA strings, 50.0 mM solutions of MA<sub>8</sub> or MA<sub>11</sub> were manually drawn into an aqueous solution of CaCl₂ (150 mM) from a pipet, and a noodle-like string with an arbitrary length formed, but no aligned string was formed from the solution of MA<sub>6</sub>. Possibly due to an unstable macroscopic structure formed upon addition of CaCl₂ (Figure S13). SEM images of the MA<sub>6</sub> and MA<sub>11</sub> strings prepared from the solution of CaCl₂ showed arrays of unidirectionally aligned nanofiber bundles, (Figure 6a,b), which are essentially identical to that of the MA<sub>10</sub> string (Figure S2a). The freshly prepared MA<sub>6</sub> and MA<sub>11</sub> strings showed a lower birefringence in the direction of their long axis in POM images (Figures 6c,d and S14) compared to that of observed in the MA<sub>10</sub> string (Figure S2c). The structural parameters and degree of alignment of the MA<sub>6</sub> and MA<sub>11</sub> nanofibers in the macroscopic string were again analyzed by SAXS measurement. The 2D image of the MA<sub>6</sub> string prepared from CaCl₂ solution on a sapphire
substrate at 25 °C (Figure 6e,f) revealed a weak pair of spot-like scatterings in a smaller-angle region (q = 0.1−0.45 nm$^{-1}$) (Figure 6e, inset), and the diffraction arc of the (001) plane with d-spacing of 5.38 nm of a lamellar structure was observed (Figure 6g). Higher order diffraction planes (002) and (003) were found in the MA$_{c11}$ string prepared from a CaCl$_2$ solution (Figure 6f,h). The layer spacing of the lamellar structure (c = 5.38 nm) of the MA$_{c8}$ is shorter than that of the MA$_{c10}$ string (c = 5.48 nm) (Figure S2e) and MA$_{c11}$ string (c = 5.71 nm), indicating that the shorter alkyl-linker in MA induces a closer packing of the MA nanofibers in the corresponding macroscopic string. In good agreement with POM results, lower degrees of alignments were observed in the MA$_{c8}$ (fwhm = 110°) and MA$_{c11}$ (fwhm = 108°) strings.
respectively, which were slower than that seen for the 
from an initial angle of 0° to a saturated flexion angle of 90°
with an actuation speed of 1.84 ± 0.2°/s and 2.21 ± 0.1°/s,
respectively, which were slower than that seen for the MAC10
string prepared in the aqueous CaCl2 solution (Table 1).
These results clearly demonstrate that the structure of motor
amphiphile is crucial to the macroscopic responsive behavior
and there is a distinct effect of chain length going from n = 6
(no actuation) to n = 11 (slower actuation).

■ CONCLUSION
Motor amphiphiles with various chain lengths at the lower half
of the motor moiety were synthesized and probed for their self-
assembly properties. Nanofibers of MAC8, MAC9, MAC10, and
MAC11 in water were observed by cryo-TEM. As shown by
NRFA, calcium ions enhanced the formation nanofibers of
MAC10 dramatically, while other ions were shown less effective.
DLS measurements were consistent with NRFS showing that
the calcium-ion-induced nanofiber aggregation of MAC10
is more efficient than with the other ions used in present study.
By applying a shear flow method, macroscopic strings of
MAC10 prepared in the presence of calcium and magnesium
ions provided a higher degree of alignment which facilitated a
faster response to light during photoactuation. The current
approach demonstrates the potential of generating muscle-like
functions with distinct mobility, allowing access to multiple
photoresponsive supramolecular actuation systems from
identical molecular structure. We envisage that a permanent
macroscopic motion powered by light might be feasible by
employing a molecular motor with a lower barrier for the
thermal helix inversion step, and studies toward such systems
are currently in progress.

■ ASSOCIATED CONTENT
2 Supporting Information
The Supporting Information is available free of charge on the
ACS Publications website at DOI: 10.1021/jacs.8b10778.
Synthesis, 1H and 13C NMR spectra, UV–vis spectra,
POM images, XRD profiles, XRD images, SEM images,
cryo-TEM images (PDF)

■ AUTHOR INFORMATION
Corresponding Author
* b.l.feringa@rug.nl

ORCID
Franco King-Chi Leung: 0000-0003-0895-9307
Jiwen Chen: 0000-0002-0251-8976
Marc C. A. Stuart: 0000-0003-0667-6338
Takanori Fukushima: 0000-0001-5586-9238
Ben L. Feringa: 0000-0003-0588-8435

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS
This work was supported financially by the Croucher
Foundation (Croucher Postdoctoral Fellowship to F.K.C.L.),
The Netherlands Organization for Scientific Research
(NWO–CW), the European Research Council (ERC;
advanced grant no. 694345 to B.L.F.), the Ministry of
Education, Culture and Science (Gravitation program no.
024.001.035), and a Grant-in-Aid for Scientific Research on
Innovative Areas “z-figuration” (nos. 26102008 and
15K21721) of The Ministry of Education, Culture, Sports,
Science and Technology (MEXT), Japan. The synchrotron
XRD experiments were performed at the BL45XU in the
Spring-8 with the approval of the RIKEN Spring-8 Center
(proposal no. 20160027). Part of work has been performed at
the Giepmans lab, which is sponsored by ZonMW grant
91111.006 (ATLAS); NMW 175-010-2009-023 (Zeiss con-
 focal) and STW Microscopy Valley 12718 (CLEM).

■ REFERENCES
(1) Fletcher, D. A.; Mullins, R. D. Cell Mechanics and the
(2) Huber, F.; Schnaub, J.; Röncke, S.; Rauch, P.; Müller, K.;
Füster, C.; Kü, J. Emergent Complexity of the Cytoskeleton: From
(3) Lino, T. Assembly of Salmonella Flagellin in Vitro and in Vivo. J.
(4) Simmons, N. S.; Blout, E. R. The Structure of Tobacco Mosaic
Virus and Its Components: Ultraviolet Optical Rotatory Dispersion.
(5) Luhn, J. M. Supramolecular Polymer Chemistry - Scope and
(6) Wojtecki, R. J.; Meador, M. A.; Rowan, S. J. Using the Dynamic
Bond to Access Macroscopically Responsive Structurally Dynamic
6065.
Functional Supramolecular Polymers for Biomedical Applications.
(10) Krieg, E.; Rybtchinski, B. Noncovalent Water-Based Materials:
(11) Li, S. L.; Xiao, T.; Lin, C.; Wang, L. Advanced Supramolecular
2012, 41, 5950–5968.
(12) Aida, T.; Meijer, E. W.; Stupp, S. I. Functional Supramolecular
(13) Palmer, L. C.; Stupp, S. I. Molecular Self-Assembly into One-
(14) Oshovsky, G. V.; Reinholdt, D. N.; Verboom, W. Supra-
 molecular Chemistry in Water. Angew. Chem., Int. Ed. 2007, 46,
2366–2393.
(15) Mulder, A.; Huskens, J.; Reinholdt, D. N. Multivalency in
(16) Hamley, I. W. Self-Assembly of Amphiphilic Peptides. Soft
(17) Smith, K. H.; Tejeda-Montes, E.; Poch, M.; Mata, A.
Integrating Top-down and Self-Assembly in the Fabrication of
2011, 40, 4563–4577.
(18) (a) Yang, S. K.; Zimmerman, S. C. Hydrogen Bonding Modules
(b) Cantekin, S.; de Greef, T. F. A.; Palmons, A. R. A. Benzene-1,3,5-
Tricarbamidoxime: A Versatile Ordering Motey for Supramolecular
(19) Dankers, P. Y. W.; Herrmans, T. M.; Baughman, T. W.;
Camkawa, Y.; Kieltyka, R. E.; Bastings, M. M. C.; Jansen, H. M.;
Popa, E. R.; Fytas, G.; Meijer, E. W. Hierarchical Formation of
Supramolecular Transient Networks in Water: A Modular Injectable


