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DyNAs: Constitutional Dynamic Nucleic Acid Analogues

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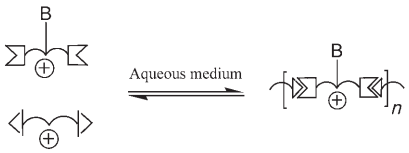
Abstract: Dynamic cationic polymers were generated in aqueous media from functionally complementary monomers bearing nucleobase groups. ^{1}H NMR spectroscopy was used to follow the polycondensation reaction of the nucleobase-appended dihydrazides **1** and **2** with the dialdehydes **B** and **C**. The reversibility of these polymers was established by proton NMR spectroscopy through exchange of the dihydrazide **2** with polymer **1B**. The polymers **1B**, **2B**, **1C**, and **2C** represent dynamic biopolymers of nucleic acid type, DyNAs. Electrostatic interaction of these polymers with polyanionic entities, such as polyphosphates, polynucleotides, and polyaspartic acid, was shown to take place. It induces a change in size of the dynamic polymer, as it responds by an increase in degree of polymerization to an increase of the overall anionic charge introduced, that is, to the total electrostatic interaction.

Keywords: combinatorial chemistry · dynamers · imines · polymers · supramolecular chemistry

Introduction

Constitutional dynamic chemistry[1] implements the reversibility of noncovalent and covalent connections to generate constitutional variation on both the supramolecular and the molecular levels through component exchange. It allows the development of adaptive chemical systems that are responsive to external factors and chemical effectors. It is emerging as a tool[2] to develop novel materials[1c] as well as to find biologically active molecules.[3,4]

Dynamic covalent polymers are reversible polymers (dynamers),[1c,5] as are supramolecular polymers,[1b,c] but formed by polycondensation between ditopic monomers bearing complementary functional groups that undergo a reversible covalent reaction (Scheme 1). They are capable of undergoing component reorganization and exchange under controlled conditions, so that redistribution of constituents can be induced by external effectors, such as metal-ion binding[7a,b] or protonation.[7c] They thus present tunability after polymerization. The exchange reaction through the reversible covalent bonds in the polymer backbone can be controlled by external factors such as pH and temperature as well as by the binding of target molecules, so that the process may yield novel materials of variable constitution depending on the conditions.[7d] The extension of this approach to biologically interesting component molecules, such as amino acids, peptides,[8] carbohydrates,[9] and nucleic acids units[10] (present work) should allow the generation of dynamic biopolymers, *biodynamers*, in aqueous solution. Polycationic dynamic polymers bearing nucleobase residues, generated by polycondensation of suitable cationic monomers through reversible covalent reaction would represent dynamic nucleic acid analogues (DyNAs). They could be of interest for complementary binding to nucleic acid strands, while being potentially also able to function as vectors for nonviral gene delivery applications. The nucleobase recognition processes could be of interest for areas such as drug delivery and gene thera-



Scheme 1. Schematic representation of the formation of polycationic dynamic covalent polymers from functionally complementary monomers; B represents a nucleobase.

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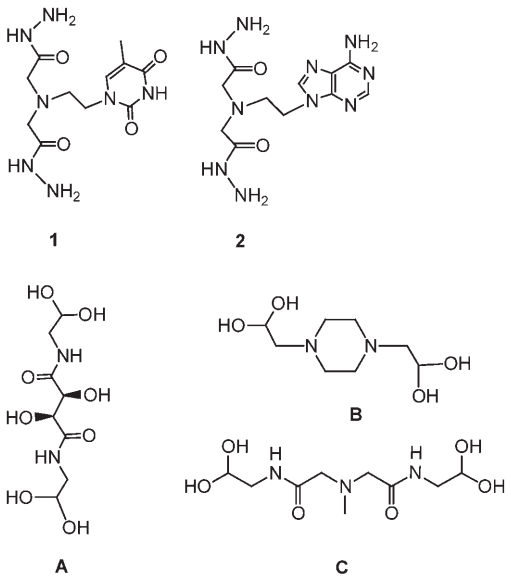
py systems. In recent years, significant progress was made in understanding the complex formation between polycations and the nucleic acid polyanions for delivery applications.[11] However, cationic polymers suffer from long-term cytotoxicity and biocompatibility remains an important issue for their use in therapeutic applications in vivo, such as gene transfer.[12,13] The incorporation of nucleobases and their derivatives into polymers may become increasingly important, in particular for the preparation of biocompatible materials. Nucleic acid analogues,[14] in particular peptide nucleic acids,[15] have been developed as artificial agents for selective binding and recognition of nucleic acid strands.

Results and Discussion

Following the considerations above, we considered generating nucleobase-containing polycationic dynamic polymers. Nucleobases are involved in many biological recognition processes so that it may be interesting to develop dynamic polymers bearing nucleobases appended on the polymer backbone. To this end, we synthesized the nucleobase containing dihydrazides **1** and **2**, as described in Scheme 2.[16] These structures were chosen as they are achiral and symmetrical molecules, containing tertiary amine groups expected to be protonated at pH 6–7, thus favouring water solubility as well as electrostatic interaction with the nucleic acid polyanions. The polycondensation of **1** and **2** with dicarbonyl compounds such as **A**–**C** is expected to yield polyacylhydrazones soluble in aqueous medium. The acylhydrazone functionality has been implemented in a number of dynamic combinatorial chemistry studies.[2–5,17] It introduces several interesting features into the polymer backbone: 1) a high percent of acylhydrazone formation with carbonyl groups in aqueous media near physiological pH, 2) hydrogen-bonding features mimicking the peptide bond, and 3) control of the reversibility of the connections by pH.

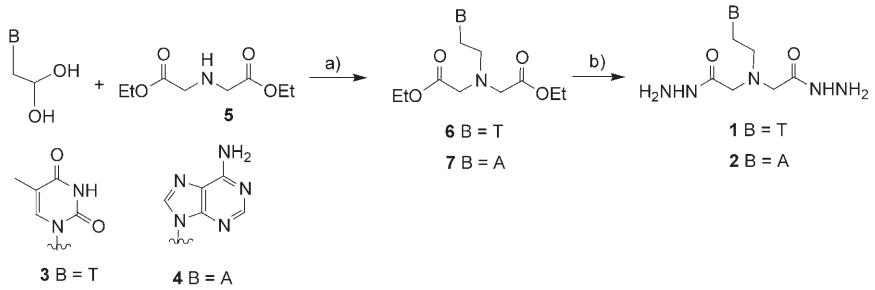


Synthesis of the monomers 1 and 2: The diesters **6** and **7** were obtained by reductive amination of aldehydes **3** and **4** with the imino diaceticacid diethylester **5**, using sodium cyanoborohydride in methanol at room temperature. Treatment of the nucleobase appended diesters **6** and **7** with hydrazine monohydrate in methanol gave the corresponding hydrazide derivatives **1** and **2**, respectively (Scheme 2). Compounds **A**, **B**, and **C** were prepared as described in the literature.[8]

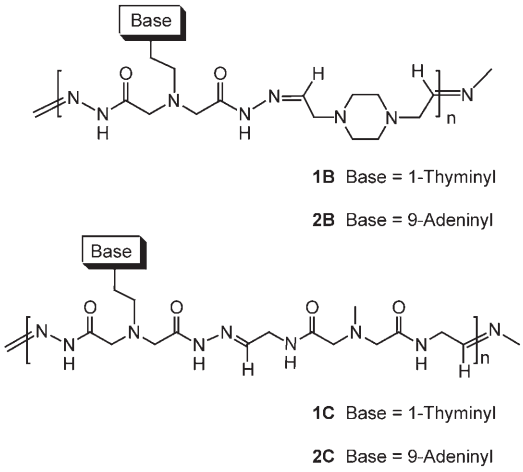
Generation of dynamic nucleic acid analogues: Adding together equimolar amounts of the tartaric acid derived dialdehyde **A** and of the thymine bearing dihydrazide **1**, each in 20 mM 0.5 M sodium acetate buffer solution at pD 6, resulted in precipitation of the polymer formed. To ensure solubility, we turned our attention to the polymerization reaction with the positively charged dialdehyde derivatives **B** and **C**. Thus, equimolar amounts of 20 mM piperazine dialdehyde **B** and thymine dihydrazide **1** were mixed and their polycondensation to polymer **1B** was investigated by ^{1}H NMR spectroscopy (Figure 1).

After reaching equilibrium ($\geq$ 90 min), the average degree of polymerization DP_n was calculated by integrating the terminal hydrated aldehyde $-CH(OH)_2$ proton signal of the polymer with respect to the polyacylhydrazone imine (HC=N) proton signal, giving a lower limit of polyacylhydrazone molecular weight M_n.[5a] The polymer **1B** obtained at 20 mM concentration of the reacting monomers has DP_n of about 29 and therefore an average molecular weight M_n of about 13369 g mol^{-1}. The polymers **1C**, **2B**, and **2C** were

Abstract in Telugu: కేంద్రక క్షార భరిత పరస్పర క్రియాశీలక మోనోమర్ల చేత చైతన్యవంతమైన ధనావేశిత పాలిమర్లు జలద్రావణం లో ఉత్పత్తి చేయబడినవి. కేంద్రక క్షారం కల్గిన డైహైడ్రజిడ్ 1, 2, మరియు డై ఆల్డిహైడ్ B మరియు C యొక్క బహుళ సంఘనన చర్యను ప్రోటాన్-యన్. యం. ఆర్. వర్ణపట మాపకము ద్వార అనుసరించడం జరిగినది. ప్రోటాన్- యన్. యం. ఆర్. ఉపయోగించి డైహైడ్రజిడ్ 2 మరియు పాలిమర్ 1B ల పరస్పర మార్పిడి వల్ల జరిగే ద్విగత ప్రక్రియను స్థిరీకరించడమైనది. ఈ 1B, 2B, 1C, మరియు 2C పాలిమర్లు న్యూక్లియిక్ ఆమ్ల రకాలకు చెందిన డైనమిక్ బయోపాలిమర్లు (DyNAs) గా సంకేతించవచ్చును. ఈ పాలిమర్లు బహుళ బుడాపేశ పూరిత అణువులైన పాలిఫాస్ఫేట్లు, పాలిన్యూక్లియోటైడ్లు, మరియు పాలి ఆస్పార్టిక్ ఆమ్లంతో స్థిరవిద్యుత్ సంకర్షణలు గలవని నిరూపితమైనది. ఈ సంకర్షణల ప్రేరేపణల వల్ల పాలిమరీకరణ శాతం లో పెరుగుదల జరుగుతుంది, అది ప్రవేశ పెట్టబడిన బుడాపేశం మొత్తం మీద అనగా వాటి స్థిరవిద్యుత్ సంకర్షణల పైన ఆధార పడి ఉంటుంది.



Scheme 2. Synthetic procedure : a) $NaCNBH_3$, MeOH, acetic acid, RT; b) Hydrazine hydrate, EtOH, RT for **1**, reflux for **2**.



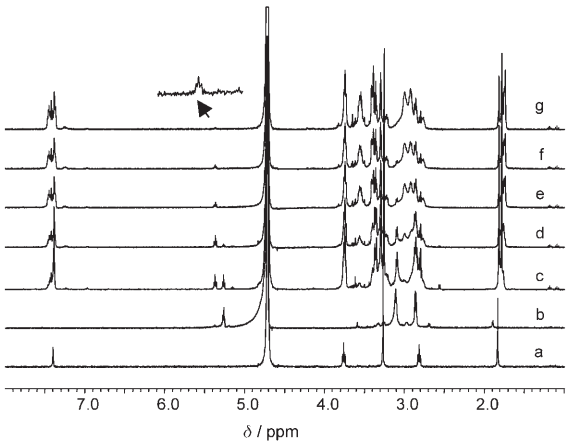


Figure 1. ^{1}H NMR spectrum of polymer **1B** obtained by mixing solutions of the dihydrazide **1** and the dialdehyde **B** (each 20 mM in 0.5 M sodium acetate buffer at pD 6 and 22 °C); a) dihydrazide **1**; b) dialdehyde **B**; c) spectra of (**1**+**B**) taken at time intervals after mixing: 5 min, d) 10 min, e) 30 min, f) 60 min, g) 90 min. The inset of spectrum g (arrow) shows the amplified signal of the terminal - C*H*$(OH)_2$ proton.

also prepared under similar reaction conditions and the results are summarized in Table 1.

The effect of concentration on the degree of polymerization was studied by ^{1}H NMR spectroscopy. Equimolar amounts of thymine dihydrazide **1** and piperazine-derived dialdehyde **B** were mixed in buffer solution at pD 6 and 22 °C, at gradually increasing concentrations from 20 mM up to 100 mM (Figure 2). As expected, DP_n was found to increase markedly with concentration from about 29 at 20 mM to 158 at 100 mM, giving M_n = 82002 g mol^{-1} for the latter concentration.

The multiangle laser light scattering (MALLS) technique may be used to determine the average molecular weight of polymers in solution from the intensity of the scattered light.[18] The weight-averaged molecular weights (M_w) of the present polymers obtained by MALLS measurements are summarized in Table 1.

Table 1. Degree of polymerization (DP_n), molecular weight (M_n) and weight-averaged molecular weight (M_w) of the dynamic polymers **1B**, **1C**, **2B**, and **2C**.[a]

Entry	Polymer	DP_n	M_n [g mol^{-1}]	M_W	M_W/M_n
1	**1B**	29	13369	15400	1.2
2	**1C**	46	23920	1.88×10^5	7.8
3	**2B**	4	1880	8000	4
4	**2C**	25	13225	1.2×10^6	–

[a] 20 mM of each monomeric component **1**, **2**, **B**, and **C** in sodium acetate buffer (0.5 M) at pD 6 and 22 °C.

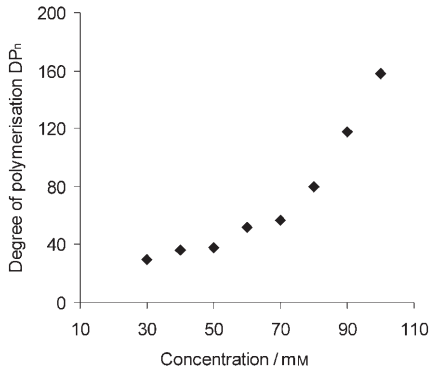


Figure 2. Effect of concentration on the molecular weight of the dynamic polymer **1B** generated from equimolar amount of the dihydrazide **1** and the dialdehyde **B** at concentrations ranging from 20 mM to 100 mM in sodium acetate buffer at pD 6 and 22 °C.

Dynamic features—component exchange: To test the dynamic character of these polymers, the adenine dihydrazide **2** (10 mM) was added to the polymer **1B**, prepared at 100 mM concentration at pD 6 at 22 °C. The exchange reaction was completed in less than about 4 h. The incorporation of dihydrazide **2** into polymer **1B** was easily observed in the ^{1}H NMR spectra on following the changes in the H-1 and H-8 proton signals of the adenine group. The change in aspect and the broadening of these signals indicated incor-

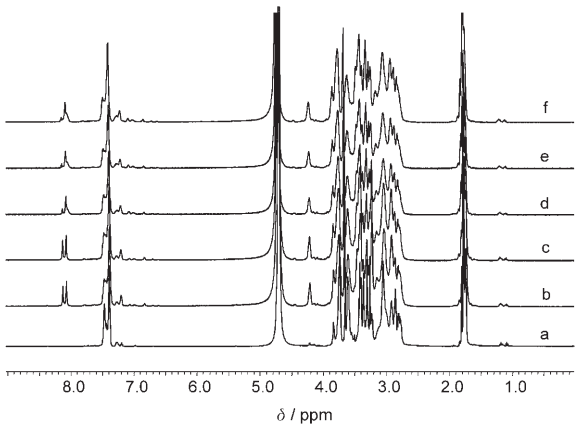


Figure 3. Exchange reaction of polymer **1B** generated from 100 mM of each monomer with adenine dihydrazide **2** (10 mM) monitored by ^{1}H NMR spectroscopy, in sodium acetate buffer (0.5 M) at pD6 and 22 °C: a) spectrum of polymer **1B**; spectra at different times after addition of dihydrazide **2** (10 mM); b) 5 min, c) 30 min, d) 60 min, e) 180 min, f) 24 h.

poration of **2**, thus testifying for the dynamic nature of the polymer (Figure 3).

Interaction with polynucleotides: It was expected that the present polycationic polymers would bind to polyanionic polymers like DNA or RNA by electrostatic interactions. Surface plasmon resonance (SPR) can be used to study such interactions. The biotin-containing oligonucleotide dA_{40} was immobilized onto a sensor chip that contained a streptavidin-loaded carboxymethyl dextran matrix. As a control, a second flow cell surface that contained only carboxymethyl dextran was left underivatized. The dynamic polymers generated from equimolar amounts of monomers were injected over both surfaces for a range of concentrations (1–20 mM) and pH (4.5–7.0). The response curves are shown in Figure 4.

Both the polymers **1B** and **2B** showed high affinity for the polydeoxyribonucleotide dA_{40} at pH 4.5, presumably through nonspecific electrostatic interactions. Upon increasing the pH from 4.5 to 6.0, the binding of these polymers significantly decreased. On further increase of the pH up to 7, binding became undetectable even at 10 mM. The strong decrease in affinity observed as the pH increased indicated that the dynamic polymers **1B** and **2B** were binding through Coulombic interactions with dA_{40}.

Experiments were next conducted with poly(A) and poly(U) with about 700 kD, 2100–2300 nucleotides. The addition of 1 wt% solution of complementary poly(A) to the polymer **1B** formed at 20 mM gave a stable hydrogel, indicating strong binding of poly(A) with the cationic polymer **1B** by electrostatic interaction. Although base pairing and stacking interactions of complementary nucleobases might also be present, there is no evidence for their contribution. On the other hand, the addition of 1% wt solution of non-complementary poly(U) to the polymer **1B** (20 mM) gave a viscous solution in 30 min, and after 2 h the formation of a fine precipitate was observed. No hydrated aldehyde proton

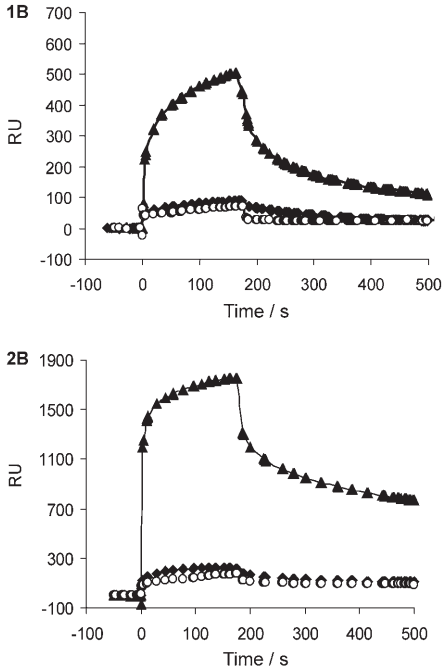


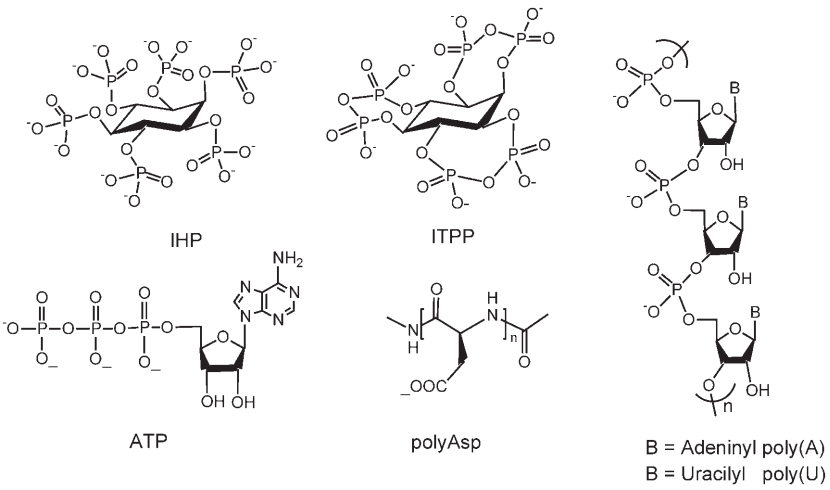
Figure 4. Surface plasmon resonance SPR sensograms for binding of polymers **1B** (left) and **2B** (right) to the polydeoxyribonucleotide dA_{40} in the pH range 4.5 (▲) 5.0 (◆), and 6.0 (○) at 1 mM in sodium acetate buffer (120 mM) and 25 °C. RU: response units. The lines are drawn through the data points to show the evolution of the response.

NMR signal could be detected, indicating a high degree of polymerization.

Taken together, the results above indicate that the binding of the polycationic dynamers **1B** and **2B** with polynucleotides was mainly of electrostatic nature, with little or no effect of potential Watson–Crick hydrogen bonding between complementary nucleobases. This is not unexpected in view of the noncommensurate character of the two types of polymers. The use of neutral strands, for which electrostatic interactions would be much less dominant, could reveal such effects.

Adaptive features of the DyNAs: Our next goal was to demonstrate the adaptability of these polymers in the presence of various biologically interesting polyanionic target molecules, such as inositol hexaphosphate (IHP), inositol tripyrophosphate (ITPP),[19] polyaspartic acid (polyAsp), and ATP. It was expected that strong interaction with these polyanionic species would drive the present dynamic polycations towards an increase in positive charge through a size increase. The target molecules were chosen as there is no interference of proton NMR signals with those of the polymer **1B**, thus facilitating the calculation of the DP_n by integration of the (CH=N) imine proton signal. The results are shown in Figure 5.

When the polymerization reaction of **1**+**B** (20 mM each, sodium acetate buffer, pD 6, 22 °C), was conducted in the presence of 1 wt% polyAsp, the DP_n, determined by NMR



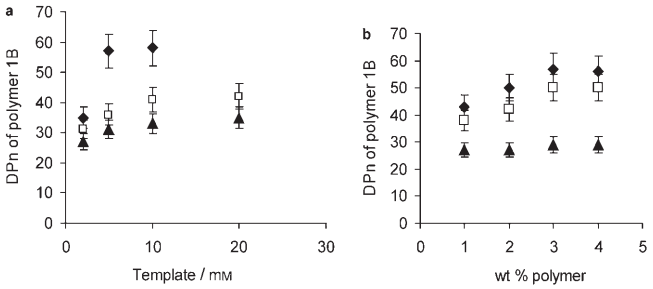


Figure 5. Increase in degree of polymerization DP_n of polymer **1B** (from 20 mM **1** + **B** each, sodium acetate buffer, pD6, 22 °C) on addition of various concentrations of polyanionic target species (concentration, DP_n) : a) ▲ ATP (2 mM, 31), (5 mM, 36), (10 mM, 41), (20 mM, 42); □ ITPP (2 mM, 27), (5 mM, 31), (10 mM, 33), (20 mM, 35); ◆ IHP (2 mM, 35), (5 mM, 57), (10 mM, 58), (20 mM, not detectable by NMR spectroscopy); b) □ polyAsp M_w 5000–15000 (1%, 38), (2%, 42), (3%, 49), (4%, 49); ◆ polyAsp M_w 15000–50000, (1%, 43), (2%, 50), (3%, 57), (4%, 56); ▲ polyethylene glycol (1%, 27), (2% 27), (3%, 29), (4% 29).

spectroscopy was significantly increased to 38, while in the absence of template it was 29. Increasing the polyAsp concentration up to 4 wt%, gave DP_n of 49. Next, the polymerization reaction was run in presence of high molecular weight (M_w 15000–50000) polyAsp as a function of concentration at pD 6. The DP_n increased up to 57 at 3 wt% of template, while on further increase to 4%, the DP_n was unchanged. The changes may be assigned to an increase in electrostatic binding of the polycationic polymer **1B** to the polyanionic aspartic acid polymer as its length increases.

As a control experiment, the polymerization reaction **1**+ **B** was conducted in presence of polyethylene glycol (PEG M_w 15000) as a function of concentration (Figure 5). The DP_n, determined by NMR spectroscopy, remained unchanged, indicating that neutral molecules had no effect on the polymerization reaction.

Similarly, we have also tested the change in DP_n of the dynamic polymer **1B** in presence of IHP, ATP, and ITPP as a function of template concentration. As expected, in the presence of the target of high, negative charge density IHP, a high DP_n value of 58 was found at 10 mM of template. When the concentration of IHP was increased up to 20 mM, the DP_n could not be determined as no hydrated aldehyde peak could be observed.

These results show that the DP_n of **1B**:

1) Increases with the maximum negative charge of the target species (Figure 5a) : ATP(4−) < ITPP(6−) < IHP-(12−).
2) Increases with the molecular weight of the anionic polymer (Figure 5b).
3) Levels off at high target concentration.

This behavior may be explained on the basis of an increase in electrostatic interaction between cationic **1B** and anionic target on increase of 1) the density of charge in the target, and 2) the number of charges, accompanying size increase of the anionic polymer. Furthermore, the observed levelling off may be ascribed to the fact that the increment of binding provided by an additional positively charged residue of **1B** decreases as the size of **1B** increases. One may note that, at an excess of template (more negative charge than positive charge) partial dissociation could in principle take place. Thus, the curves in Figure 5 might present a maximum. This is not observed at the highest concentrations at which the DP_n could be determined. At higher concentrations (i.e., 20 and 100 mM IHP) the DP_n was too high for determination by NMR spectroscopy (see above).

The observed increase in size of the dynamic polymer demonstrates its adaptability, that is, its ability to respond to the presence of a target entity; the response being a function of the features of the target entity in a sort of electrostatic (charge) recognition process.

Conclusion

Nucleobase appended dynamic polycationic polymers have been generated in aqueous medium and shown to undergo constitutional exchange with monomers at physiological pH. They are able to respond to the presence of anionic target species by changing their size in an adaptation process driven by electrostatic interaction and presenting charge-recognition character. The present results thus extend to dynamers[1c,5] incorporating biological residues, the behavior displayed by the constituents of dynamic combinatorial libraries.[2] Such polymers bear features of dynamic versions of peptide nucleic acids[15] and may have potential applications in the fields of nucleic acid recognition and analysis, gene transfer, and material sciences. Further work is being pursued along these lines, directed towards designing

DyNAs commensurate to nucleic acids that might bind to them with structural and/or sequence specificity.

Experimental Section

Materials: ATP, IHP, dA_{40} (Proligos) poly(A), poly(U) (about 700 kD, 2100–2300 nucleotides), and the aspartic acid (M_w: 5–15000 and 15000–50000 and polyethylene glycol (15000) polymers were obtained commercially (Sigma–Aldrich) unless stated otherwise and used without further purification. ITPP has been synthesized in the course of other work.[19]

Spectroscopic measurements: NMR spectra were recorded on a Bruker 400 MHz spectrometer. The chemical shifts are reported in ppm downfield from tetramethylsilane; coupling constants are in Hz. Electrospray ionization mass spectrometry (ESI-MS) was carried out on a Bruker Micro-TOF mass spectrometer coupled with liquid chromatography.

Deuterated buffer solutions for NMR measurements were prepared in D_2O using a desired concentration of deuterated [D_4]acetic acid (for pD 4–6) and then the p^2H (pD) was adjusted by using a solution of NaOD in D_2O. The pD of the solution was monitored with a pH meter and the pD of buffer solution is equal to the pH meter reading + 0.4.

Preparation of components 1 and 2:

{Ethoxycarbonylmethyl-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)ethyl]amino}acetic acid ethyl ester (6): Sodium cyanoborohydride (98 mg, 1.59 mmol) was added to a solution of compounds **5** (200 mg, 1.07 mmol) and **3** (100 mg, 0.53 mmol) in methanol (15 mL) and the resulting solution was stirred for 24 h. Acetic acid (4 equiv) was then added and the solution stirred for a further 48 h. The reaction mixture was concentrated under reduced pressure; diluted with chloroform (100 mL); washed with saturated $NaHCO_3$ solution, water, and brine; and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude material purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 95:5) to give the diester **6** (100 mg, 60%). White solid; m.p. 86–87°C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.58$ (br s, 1H), 7.38 (s, 1H), 4.21 (q, $J = 8.0$ Hz, 4H), 3.82 (t, $J = 4.0$ Hz, 2H), 3.53 (s, 4H), 3.05 (t, $J = 4.0$ Hz, 2H), 1.94 (d, $J = 1.0$ Hz, 3H), 1.30 ppm (t, $J = 8.0$ Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 171.1$, 164.2, 150.8, 141.9, 109.6, 60.7, 56.0, 53.8, 50.1, 47.1, 14.2, 12.2 ppm; ESI-MS: m/z: 341.8 $[M]^+$; elemental analysis calcd (%) for $C_{15}H_{23}N_3O_6$: C 52.78, H 6.79, N 12.31; found : C 52.76, H 6.78, N 11.92.

{[2-(6-Amino-purin-9-yl)ethyl]ethoxycarbonylmethylamino}acetic acid ethyl ester (7): Sodium cyanoborohydrate (85 mg, 1.38 mmol) was added to a solution of compounds **5** (230 mg, 1.16 mmol) and **4** (100 mg, 0.46) in methanol (25 mL), and the resulting solution was stirred for 24 h. Acetic acid (4 equiv) was then added and the solution stirred for a further 48 h. The reaction mixture was concentrated; diluted with chloroform (100 mL); washed with saturated $NaHCO_3$ solution, water, and saturated brine; and then dried over Na_2SO_4. Evaporation under reduced pressure gave a crude product which was purified by flash chromatography (98:2 CH_2Cl_2/MeOH) to give the diester **7** (70 mg, 43%). White solid; m.p. 140–141°C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.35$ (s, 1H), 8.16 (s, 1H), 6.08 (br s, 2H), 4.30 (t, $J = 8.0$ Hz, 2H), 4.14 (q, $J = 8.0$ Hz, 4H), 3.50 (s, 4H), 3.21 (t, $J = 8.0$ Hz, 2H), 1.26 ppm (t, $J = 4$ Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 171.0$, 155.5, 155.4, 152.7, 150.0, 141.8, 141.7, 119.4, 60.7, 55.8, 54.5, 42.5, 14.2 ppm; ESI-MS: m/z: 351.1 $[M]^+$; elemental analysis calcd (%) for $C_{15}H_{22}N_6O_4$: C 51.42, H 6.33, N 23.99; found: C 51.84, H 6.55, N 23.51.

{Hydrazinocarbonylmethyl-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)ethyl]amino}acetic acid hydrazide (1): Hydrazine hydrate (90 μL) was added to a solution of ethyl ester **6** (90 mg, 0.26 mmol) in EtOH (10 mL), and the resulting solution was stirred at RT for four days. The resulting precipitate was filtered and dried under reduced pressure to give the dihydrazide **1** (41 mg, 50%). White solid; m.p. 200–201°C; ^{1}H NMR (400 MHz, D_2O): $\delta = 7.39$ (s, 1H), 3.75 (t, $J = 8.0$ Hz, 2H), 3.26 (s, 2H; CH_2), 2.81 (t, $J = 5.6$ Hz, 2H; CH_2), 1.82 ppm (s, 3H; CH_3); ^{13}C NMR (101 MHz, D_2O + tBuOH): $\delta = 171.7$, 166.9, 152.2, 143.2, 110.5, 56.6, 52.9, 46.2, 11.2 ppm; ESI-MS (pos.): m/z: 336.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{11}H_{19}N_7O_4$: C 42.17, H 6.11, N 31.29; found: C 42.41, H 6.13, N 31.20.

{[2-(6-Aminopurin-9-yl)ethyl]hydrazinocarbonylmethylamino}acetic acid hydrazide (2): Hydrazine hydrate (51 mg, 70 μL, 1.00 mmol) was added to a solution of diethyl ester **7** (60 mg, 0.17 mmol) in ethanol (10 mL), and the resulting solution refluxed for 5 h. Evaporation under reduced pressure gave the dihydrazide **2** (50 mg, 92%). White solid; m.p.204–206°C; ^{1}H NMR (400 MHz, D_2O): $\delta = 8.06$ (s, 1H), 7.99 (s, 1H), 4.17 (t, $J = 4.0$ Hz, 2H), 3.18 (s, 4H), 2.99 ppm (t, $J = 4.0$ Hz, 2H); ^{13}C NMR (101 MHz, D_2O + tBuOH): $\delta = 171.6$, 155.0, 152.1, 148.7, 142.5, 118.1, 56.6, 53.9, 42.2 ppm; ESI-MS (pos.): m/z: 324.1 $[M+2H]^+$; elemental analysis calcd (%) for $C_{11}H_{18}N_{10}O_2$: C 40.99, H 5.63, N 43.46; found: C 40.66, H 5.72, N 44.70.

Biacore experiments: The surface plasmon resonance (SPR) measurements were performed by using BIAcore X system thermostated at 25°C with streptavidin-coated sensor chips (SA) for all experiments. The sensor chip consisted of a gold surface and streptavidin covalently immobilized on a carboxymethylated dextran layer at the surface. Oligonucleotides were purchased from Proligo. For interaction measurements, injections were performed at 30 μL min^{-1} for 180 s followed by running buffer at 30 μL min^{-1} for a further 300 s. After each injection the surface was regenerated by successive injections of either 0.1 M aq HCl or 10–25 mM aq NaOH. The running buffer was NaOAc 120 mM (pH 4.5, 5.0 and 6.0) and it was thoroughly degassed prior to use. For immobilization the flow cell 1 was injected with biotin dA_{40} (123 μM in water) at 5 μL min^{-1} to give a final response of 1250 RU. As control, flow cell 2 was left underivatized.

Multiangle laser light scattering (MALLS) measurements—static light scattering: Laser light scattering is a common technique[20] for determining the shape of polymers through the mean square radius of giration $\langle RG \rangle_Z$ and the particle scattering factor $P_Z(q)$.[21] Wide- and small-angle static light scattering experiments were carried out. The light scattering intensity was measured by using an in-house apparatus[22] equipped with 1) a red He-Ne laser of wavelength $\lambda_0 = 632.8$ nm in vacuum, 2) a discrete-angle goniometer acting within the range from 20° to 155°, 3) a Hamamatzu type photomultiplier as detector, 4) a photo-counting device, and 5) a toluene matching bath. The vertical polarization of the incident beam with respect to the scattering plane has been used. The analyzer, arranged between the measuring cell and the photomultiplier, could assume both the vertical and the horizontal orientations. The first position allows measurement of the isotropic I_{VV} scattering, while the second was applied in the study of the depolarized I_{VH} scattering intensity.[23] The excess of light scattering intensity $I_{VV}(q) = I_{VVsolution} - I_{VVsolvent}$ was measured as a function of scattering vector $q = \frac{4\pi n}{\lambda_0} \sin\frac{\theta}{2}$ with an accuracy of 1% (θ is the scattering angle). The values of Raleigh excess scattering intensity $R(q)$ were obtained from light scattering intensity I_{VV} through calibration with a benzene standard. Taken as an example, the water refractive index and the average refractive index increment of our samples relative to water are equal to $n = 1.33$ and $dn/dc = 0.181$ mL g for the **1B** polymer. For spherically symmetric objects, we applied the following formula for $R(q)$ [Eq. (1)] in which M is the molecular weight, K is an optical constant, $K = K_{Calibration}(dn/dc)^2$, dn/dc is the refractive index increment, C_{polym} is the polymer concentration, $P(q)$ is the form factor of the chain, and $S(q)$ is the long-range interferences from distant scatterers.

$$R(q) = K\, c_{polym}\, M\, P(q)\, S(q) \qquad (1)$$

General procedure for the preparation of polyacylhydrazones and proton NMR determination of the average degree of polymerization (DP_n): Stock solutions (300 mM) of the dialdehydes (**B**, **C**) and dihydrazides (**1**, **2**) were prepared by dissolving a given compound in ^2H_2O or deuterated buffer solution (0.5 M sodium acetate, pD 6.0). The dialdehydes **B** and **C** were obtained from the corresponding dimethyl acetals by in situ deprotection using 0.6 N DCl. Then the pD of the dialdehyde **B** solution was carefully adjusted to 2–3 using diluted NaOD solution, prior to use for the preparation of polymers. This stock solution (300 mM) was used for preparing polymers at desired concentration by serial dilutions. Equimolar amounts of monomers dialdehyde **B** (33.3 μL) and dihydrazide **1**

(33.3 μL) were mixed in a NMR tube to make up a final 20 mM concentration each for a final volume of 500 μL in 0.5 M sodium acetate buffer at pD 6 (22 °C). The polycondensation reaction was completed in 3–4 h at room temperature and after reaching the equilibrium, the proton NMR spectrum was recorded. The average degree of polymerization (DP_n) was determined by integrating the CH proton signal of the hydrate $-CH(OH)_2$ of terminal aldehyde groups with respect to the imine protons (CH=N−), giving $M_w = 13369\ g\,mol^{-1}$, degree of polymerization $DP_n = 29$ for polymer **1B**. This NMR method allows the determination of the average DP_n and the polymer molecular weight M_n. M_n is the lower limit of the polyacylhydrazone molecular weight, based on the average DP_n.[5a]

General procedure for the exchange of polyacylhydrazones with monomers: Typically, a 100 mM solution of polymer **1B** was prepared by mixing equimolar amounts of aldehyde **B** and dihydrazide **1** solutions in a NMR tube making up to the volume 500 μL in 0.5 M sodium acetate buffer at pD 6 and 22 °C. After reaching the equilibrium, the polymer **1B** was charged into a NMR tube with dihydrazide **2** as a solid to give a dihydrazide concentration of 10 mM. The resulting mixture was kept at room temperature and followed for component exchange by proton NMR spectroscopy, looking at the adenine H-1 and H-8 proton signals. These signals broadened over time, indicating that the dihydrazide **2** was incorporated into the polymer **1B** through exchange of dihydrazide components at room temperature.

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[1] a) J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4763–4768; b) J.-M. Lehn, *Science* **2002**, *295*, 2400–2403; c) J.-M. Lehn, *Prog. Polym. Sci.* **2005**, *30*, 814–831.

[2] Reviews on dynamic combinatorial chemistry see: a) J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455–2463; b) G. R. L. Cousins, S.-A. Poulsen, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **2000**, *4*, 270–279; c) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **2002**, *6*, 321–327; d) J.-M. Lehn, A. V. Eliseev, *Science* **2001**, *291*, 2331–2332; e) O. Ramström, J.-M. Lehn, *Nat. Rev. Drug Discovery* **2002**, *1*, 26–36; f) O. Ramström, T. Bunyapaiboonsri, S. Lohmann, J.-M. Lehn, *Biochim. Biophys. Acta* **2002**, *1572*, 178–186.

[3] a) I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106–2110; b) O. Ramström, J.-M. Lehn, *ChemBioChem* **2000**, *1*, 41–48; c) T. Bunyapaiboonsri, O. Ramström, S. Lohmann, J.-M. Lehn, L. Peng, M. Goeldner, *ChemBioChem* **2001**, *2*, 438–444; d) T. Bunyapaiboonsri, H. Ramström, O. Ramström, J. Haiech, J.-M. Lehn, *J. Med. Chem.* **2003**, *46*, 5803–5811; e) S. Zameo, B. Vauzeilles, J.-M. Beau, *Angew. Chem.* **2005**, *117*, 987–991; *Angew. Chem. Int. Ed.* **2005**, *44*, 965–969; f) M. S. Congreve, D. J. Davis, L. Devine, C. Granata, M. O. Reilly, P. G. Wyatt, H. Jhoti, *Angew. Chem.* **2003**, *115*, 4617–4620; *Angew. Chem. Int. Ed.* **2003**, *42*, 4479–4482.

[4] a) M. Hochgürtel, H. Kroth, D. Piecha, M. W. Hofmann, C. Nicolau, S. Krause, O. Schaaf, G. Sonnenmoser, A. V. Eliseev, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3382–3387; b) O. Ramström, S. Lohmann, T. Bunyapaiboonsri, J.-M. Lehn, *Chem. Eur. J.* **2004**, *10*, 1711–1715; c) R. Larsson, Z. Pei, O. Ramström, *Angew. Chem.* **2004**, *116*, 3802–3804; *Angew. Chem. Int. Ed.* **2004**, *43*, 3716–3718; d) H. Li, P. Williams, J. Micklefield. J. M. Gardiner, G. Stephens, *Tetrahedron* **2004**, *60*, 753–758.

[5] a) W. G. Skene, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 8270–8275; b) T. One, T. Nobori, J.-M. Lehn, *Chem. Commun.* **2005**, 1522–1524; c) E. Kolomiets, J.-M. Lehn, *Chem. Commun.* **2005**, 1519–1521; d) J.-L. Schmitt, J.-M. Lehn, *Helv. Chim. Acta* **2003**, *86*, 3417–3426.

[6] a) *Supramolecular Polymers*, 2nd ed. (Ed.: A. Ciferri), Taylor & Francis, Boca Raton **2005**; b) L. Brunsveld, B. J. B. Folmer, E. W. Meijer, R. P. Sijbesma, *Chem. Rev.* **2001**, *101*, 4071–4098; c) J.-M. Lehn, *Polym. Int.* **2002**, *51*, 825–839; d) J.-M. Lehn in *Supramolecular Polymers*, 2nd ed. (Ed.: A. Ciferri), Taylor & Francis, Boca Raton **2005** Chapter 1.

[7] a) N. Giuseppone, J.-M. Lehn, *J. Am. Chem. Soc.* **2004**, *126*, 11448–11449; b) N. Giuseppone, J.-M. Lehn, *Chem. Eur. J.* **2006**, *12*, 1715–1722; c) N. Giuseppone, G. Fuks, J.-M. Lehn, *Chem. Eur. J.* **2006**, *12*, 1723–1735; d) for the effect of gelation on constitutional exchange and selection, see: N. Sreenivasachary, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5938–5943.

[8] S. Lohmann, **2003**, Ph.D. thesis, Université louis Pasteur, Strasbourg (France).

[9] a) Y. Ruff, J.-M. Lehn, unpublished results; b) I. Nakazawa, S. Suda, M. Masuda, M. Asai, T. Shimizu, *Chem. Commun.* **2000**, 881–882.

[10] For nucleic acid systems involving a reversible bond, see: a) J. T. Goodwin, D. G. Lynn, *J. Am. Chem. Soc.* **1992**, *114*, 9197–9198; b) Y. Krishnan-Ghosh, A. M. Whitney, S. Balasubramanian, *Chem. Commun.* **2005**, 3068–3070; c) A. Bugaut, J.-J. Toulmé, B. Rayner, *Angew. Chem.* **2004**, *116*, 3206–3209; *Angew. Chem. Int. Ed.* **2004**, *43*, 3144–3147; for dynamic selection by binding to DNA, see: d) B. Klekota, M. H. Hammond, B. L. Miller, *Tetrahedron Lett.* **1997**, *38*, 8639–8642; e) B. Klekota, B. L. Miller, *Tetrahedron* **1999**, *55*, 11687–11697; f) C. Karan, B. L. Miller, *J. Am. Chem. Soc.* **2001**, *123*, 7455–7456.

[11] a) I. Chemin, D. Moradpour, S. Wieland, W. B. Offensperger, E. Walter, J.-P. Behr, H. E. Blum, *J. Viral Hepatitis* **1998**, *5*, 369–375; b) P. Erbacher, J. S. Remy, J.-P. Behr, *Gene Ther.* **1999**, *6*, 138–145; c) W. Zauner, M. Ogris, E. Wagner, *Adv. Drug Delivery Rev.* **1998**, *30*, 97–113; d) B. Ochietti, N. Guérin, S. V. Vinogradov, Y. S. Pierre, P. Lemieux, A. V. Kabanov, V. Y. Alakhov, *J. Drug Targeting* **2002**, *10*, 113–121.

[12] a) A. V. Kabanov, V. A. Kabanov, *Bioconjugate Chem.* **1995**, *6*, 7–20; b) O. Boussif, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix, J.-P. Behr, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7297–7301.

[13] a) D. Putnam, R. Langer, *Macromolecules* **1999**, *32*, 3658–3662; b) Y. Lim, Y. H. Choi, J.-S. Park, *J. Am. Chem. Soc.* **1999**, *121*, 5633–5639.

[14] a) J. Summerton, D. Weller, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 187–195; b) O. Chakhmakhcheva, M. Andrianov, A. Buryakova, M. Choob, V. Efimov, *Nucleosides Nucleotides* **1999**, *18*, 1427–1428; c) T. Vilaivan, G. Lowe, *J. Am. Chem. Soc.* **2002**, *124*, 9326–9327; d) A. D. Mesmaeker, K.-H. Altmann, A. Waldmer, S. Wendeborn, *Curr. Biol. Curr. Opin. Stru. Bio.* **1995**, *5*, 343–355; e) Ö. Almarsson, T. C. Bruice, J. Kerr, R. N. Zuckermann, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7518–7522; f) J. Micklefield, *Curr. Med. Chem.* **2001**, *8*, 1157–1179; g) S. M. Freier, K.-H. Altmann, *Nucleic Acids Res.* **1997**, *25*, 4429–4443.

[15] a) M. Egholm, O. Buchardt, P. E. Nielsen, R. H. Berg, *J. Am. Chem. Soc.* **1992**, *114*, 1895–1897; b) M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, P. E. Nielsen, *Nature* **1993**, *365*, 566–568 c) O. Buchardt, M. Egholm, R. H. Berg, P. E. Nielsen, *Trends Biotechnol.* **1993**, *11*, 384–386.

[16] Related compounds were also prepared: D. T. Hickman, N. Sreenivasachary, J.-M. Lehn, unpubllished results.

[17] a) G. R. L. Cousins, S.-A. Poulsen, J. K. M. Sanders, *Chem. Commun.* **1999**, 1575–1576; b) R. L. E. Furlan, Y.-F. Ng, S. Otto, J. K. M. Sanders, *J. Am. Chem. Soc.* **2001**, *123*, 8876–8877; c) L. F. Bornaghi, B. L. Wilkinson, M. J. Kiefel, S.-A Poulsen. *Tetrahedron Lett.* **2004**, *45*, 9281–9284; d) R. L. Furlan, Y.-F. Ng, S. Otto, J. K. M. Sanders, *J. Am. Chem. Soc.* **2001**, *123*, 8876–8877; e) R. Nguyen, I. Huc, *Chem. Commun.* **2003**, 942–943.

[18] a) P. Wyatt, *Anal. Chim. Acta* **1993**, *272*, 1–40; b) R. Mendichi, G. Giammona, G. Cavallaro, A. G. Schieroni, *Polymer* **1999**, *40*, 7109–7116.

[19] K. C. Fylaktakidou, J.-M. Lehn, R. Greferath, C. Nicolau, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1605–1608; L. F. Johnson, M. E. Tate, *Can. J. Chem.* **1969**, *47*, 63–73.
[20] a) P. Debye, *J. Phys. Coll. Chem.* **1947**, *51*, 18–32; b) B. H. Zimm, *J. Chem. Phys.* **1948**, *16*, 1099–1116.
[21] H. C. Benoit, J. S. Higgins, *Polymers and Neutron scattering*, Oxford Science, Clarendon, Oxford **1994**; B. Chu, *Laser light scattering*, 2nd ed., Academic Press, New York, **1991**.
[22] A. Libeyre, D. Sarazin, J. François, *Polym. Bull.* **1981**, *4*, 53–60.
[23] G. G. Fuller, *Optical Rheometry of Complex Fluids: Theory and Practice of Optical Rheometry*, Oxford University Press, Oxford, **1995**.

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