

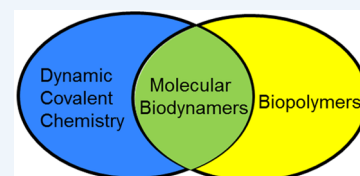
Molecular Biodynamers: Dynamic Covalent Analogues of Biopolymers

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CONSPECTUS: Constitutional dynamic chemistry (CDC) features the use of reversible linkages at both molecular and supramolecular levels, including reversible covalent bonds (dynamic covalent chemistry, DCC) and noncovalent interactions (dynamic noncovalent chemistry, DNCC). Due to its inherent reversibility and stimuli-responsiveness, CDC has been widely utilized as a powerful tool for the screening of bioactive compounds, the exploitation of receptors or substrates driven by molecular recognition, and the fabrication of constitutionally dynamic materials. Implementation of CDC in biopolymer science leads to the generation of constitutionally dynamic analogues of biopolymers, biodynamers, at the molecular level (molecular biodynamers) through DCC or at the supramolecular level (supramolecular biodynamers) via DNCC. Therefore, biodynamers are prepared by reversible covalent polymerization or noncovalent polyassociation of biorelevant monomers.



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In particular, molecular biodynamers, biodynamers of the covalent type whose monomeric units are connected by reversible covalent bonds, are generated by reversible polymerization of bio-based monomers and can be seen as a combination of biopolymers with DCC. Owing to the reversible covalent bonds used in DCC, molecular biodynamers can undergo continuous and spontaneous constitutional modifications via incorporation/decorporation and exchange of biorelevant monomers in response to internal or external stimuli. As a result, they behave as adaptive materials with novel properties, such as self-healing, stimuli-responsiveness, and tunable mechanical and optical character. More specifically, molecular biodynamers combine the biorelevant characters (e.g., biocompatibility, biodegradability, biofunctionality) of bioactive monomers with the dynamic features of reversible covalent bonds (e.g., changeable, tunable, controllable, self-healing, and stimuli-responsive capacities), to realize synergistic properties in one system. In addition, molecular biodynamers are commonly produced in aqueous media under mild or even physiological conditions to suit their biorelated applications.

In contrast to static biopolymers emphasizing structural stability and unity by using irreversible covalent bonds, molecular biodynamers are seeking relative structural adaptability and diversity through the formation of reversible covalent bonds. Based on these considerations, molecular biodynamers are capable of reorganizing their monomers, generating, identifying, and amplifying the fittest structures in response to environmental factors. Hence, molecular biodynamers have received considerable research attention over the past decades. Accordingly, the construction of molecular biodynamers through equilibrium polymerization of nucleobase-, carbohydrate- or amino-acid-based monomers can lead to the fabrication of dynamic analogues of nucleic acids (DyNAs), polysaccharides (glycodynamers), or proteins (dynamic proteoids), respectively. In this Account, we summarize recent advances in developing different types of molecular biodynamers as structural or functional biomimetics of biopolymers, including DyNAs, glycodynamers, and dynamic proteoids. We introduce how chemists utilize various reversible reactions to generate molecular biodynamers with specific sequences and well-ordered structures in aqueous medium. We also discuss and list their potential applications in various research fields, such as drug delivery, drug discovery, gene sensing, cancer diagnosis, and treatment.

1. FROM CONSTITUTIONAL DYNAMIC CHEMISTRY TO DYNAMERS

Importing the concept of constitutional dynamics from supramolecular chemistry into molecular chemistry through the use of reversible covalent bonds instead of supramolecular noncovalent interactions opens up novel perspectives to chemistry and leads to the emergence of constitutional dynamic chemistry (CDC), which leads toward adaptive chemistry.^{1–4} CDC encompasses both dynamic covalent chemistry (DCC)^{5–8} and dynamic noncovalent chemistry (DNCC). It takes advantage of the lability of reversible covalent bonds formed by reversible chemical reactions or of noncovalent

interactions between molecular recognition groups to generate constitutional molecular or supramolecular diversity within constitutional dynamic libraries (CDLs) of chemical species of either molecular or supramolecular type. The resulting CDLs consist of entities that can undergo continuous constitutional changes or adaptations through incorporation/decorporation or reshuffling of components in response to physical or chemical, internal or external stimuli. As a consequence, the resulting systems are chemically diverse, dynamic, and

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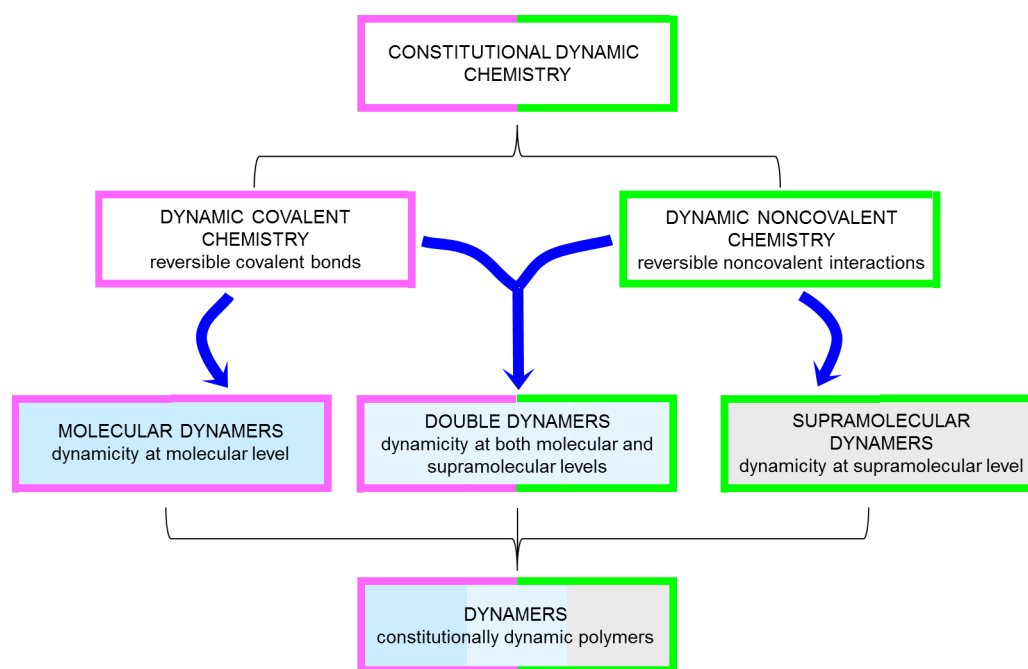


Figure 1. Generation of molecular and supramolecular dynamers through constitutional dynamic chemistry.

adaptable at both molecular and supramolecular levels, providing new possibilities and tools for the screening of bioactive compounds, exploitation of receptors or substrates driven by molecular recognition, and fabrication of constitutionally dynamic materials.^{1,9,10}

The implementation of CDC specifically in polymer science leads to the generation of constitutionally dynamic polymers or “dynamers” (Figure 1) at both molecular and supramolecular levels through DCC and DNCC, respectively.^{11–14} Dynamers are defined as polymers in which the monomers are connected through reversible covalent bonds or noncovalent interactions. By virtue of the properties of reversible linkages and core groups, dynamers possess both dynamic and adaptive features and may undergo spontaneous and continuous constitutional modifications via assembly/disassembly and exchange of their components in response to internal or external stimuli. Compared with constitutionally static polymers, dynamers behave as smart polymers with novel features such as self-healing, stimuli-responsiveness, and tunable mechanical and optical properties.^{12,13}

According to the type of reversible connection, dynamers can be subdivided into three categories (Figure 1): (1) *molecular dynamers*, covalent equilibrium polymers generated by polymerization through the construction of reversible covalent bonds including Diels–Alder linkages, imines, acylhydrazones, oximes, boronate esters, and disulfides;^{15–21} (2) *supramolecular dynamers*, noncovalent reversible polymers produced by polyassociation of ditopic static monomers via formation of noncovalent bonds such as hydrogen bonding, π – π -stacking, electrostatic interactions, metal ion coordination, host–guest recognition, and van der Waals forces;^{15,22,23} (3) *double dynamers*, polymers with constitutionally dynamic properties at both molecular and supramolecular levels, fabricated through a combination of reversible covalent bonds with noncovalent interactions.^{15,24,25} In particular, molecular dynamers are currently receiving extensive research attention.

2. MOLECULAR BIODYNAMERS: MOLECULAR/COVALENT DYNAMERS WITH BIOLOGICALLY RELEVANT MONOMERS

Biopolymers or biomacromolecules are polymeric molecules created by living organisms. Owing to their mode of generation, their molecular constitution, and well-defined 3D structure, they exhibit various functions and biocompatibility. Based on the type of basic building block, they are grouped into three categories: nucleic acids, polysaccharides, and proteins. Extending the principles of dynamers into the field of biopolymers leads to the definition of biodynamers, that is, dynamers implementing biorelevant residues.¹² Biodynamers are prepared by reversible covalent polymerization or noncovalent polyassociation. As a result, biodynamers are constitutionally dynamic analogues of biopolymers at both molecular and supramolecular levels and hold the ability to combine biofunctionality (recognition, catalysis) of biopolymers with the adaptive feature of dynamers leading to synergistic properties. By analogy to the classification of dynamers, biodynamers can be divided into molecular, supramolecular, and double biodynamers.

In contrast to naturally occurring biopolymers or static analogues of biopolymers, molecular biodynamers are based on biorelevant monomers connected by reversible linkages. As a consequence of the inherent dynamic properties of DCC, molecular biodynamers are capable of reorganizing their components, modifying their sequence, or adapting their length in response to various physical or chemical factors even after polymerization. Therefore, unlike either static biopolymers featuring structural stability and unity owing to their irreversible covalent bonds or supramolecular biodynamers displaying chemical lability and diversity resulting from their comparatively fragile noncovalent interactions, molecular biodynamers display an attractive balance by taking advantage of reversible covalent bonds. As a result, molecular biodynamers exhibit an optimal combination of relative structural stability and lability with comparable chemical unity

and diversity. More specifically, the inherent nature of bio-relevant constituents and reversible covalent bonds may confer to molecular biodynamers biocompatible, biodegradable, biofunctional, changeable, tunable, controllable, self-healing, and stimuli-responsive properties.

As biofunctionalities of nucleic acids, polysaccharides, and proteins rely on their highly ordered assembled 3D structures,²⁶ mimicking or modifying biopolymers also provides novel tools to unravel the correlation between biofunctionality and structure of biopolymers. In view of all these considerations, the development of novel molecular biodynamers as adaptive and functional biomaterials is presently receiving considerable attention. Accordingly, the construction of molecular biodynamers, through the incorporation of nucleobase-, carbohydrate-, or amino-acid-derived moieties, gives rise to the formation of covalent dynamic analogues of nucleic acids (DyNAs), polysaccharides (glycodynamers), or proteins (dynamic proteoids), respectively.¹² These molecular biodynamers are created by reversible polymerization in aqueous media under mild conditions, which resemble the physiological environment, for future application as smart biomaterials.

In this Account, we will give a brief review of recent work on molecular biodynamers mainly reported by our groups, namely, the fabrication of DyNAs, glycodynamers, and dynamic proteoids.

3. MOLECULAR BIODYNAMERS: DyNAs, GLYCODYNAMERS, AND DYNAMIC PROTEOIDS

3.1. DyNAs: Dynamic Analogues of Nucleic Acids

DyNAs, with ribose- or non-ribose-backbones, can be classified into main-chain- and side-chain-dynamic categories. The former are made by reversible polymerization of nucleobase-derived monomers, while the latter are prepared by reversibly grafting nucleobase residues through DCC (Figure 2). Hence,

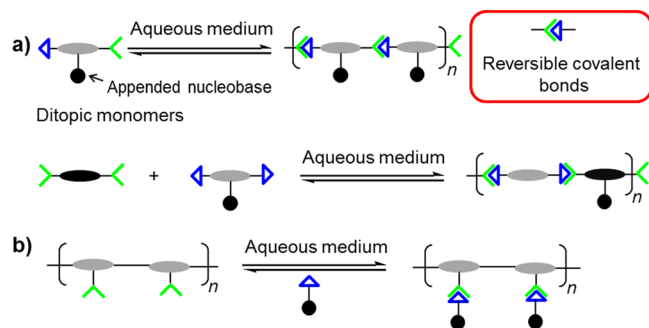


Figure 2. Generation of (a) main-chain DyNAs and (b) side-chain DyNAs.

their constitution and properties are adaptable under given conditions in response to driving forces such as self-folding into stable secondary or tertiary architectures, substrate binding, or addition of target entities, including complementary DNAs (DNA-templated reversible polymerization^{27–30}) or non-DNA targets.³¹

DNA-templated reversible polymerization of nucleobase-modified ditopic monomers allows for the synthesis of main-chain DyNAs (Figure 3a). As DNA template, the complementary DNA acts as catalyst during equilibrium polymerization to facilitate the reaction through specific Watson–Crick base-pairing interactions (DNA hybridization). Therefore,

reversible polymerization cannot take place in the absence of DNA template, and the change of DNA template leads to the fabrication of compounds with a different sequence. In other words, DNA-templated reversible synthesis of DyNAs displays sequence specificity and selectivity such that only sequence-matched DyNAs are generated and amplified.²⁸ Lynn and coworkers pioneered DNA-templated synthesis of main-chain DyNAs with ribose backbones. They accomplished DNA-templated reversible polycondensation of synthetic mono-, di-, and tetranucleotides to produce octamer DyNAs with ribose main chains through formation of reversible imine bonds in aqueous media, affording stable products in high yield (~80%) after reductive amination.³² With this methodology, even sequence-defined DyNAs of main-chain-dynamic type with 33 nucleotides were synthesized.³³ Furthermore, similar polymerization was achieved by using solid-supported DNA templates in high yield (~90%).³⁴ The solid-supported templates can be conveniently prepared by automated solid-phase DNA synthesis and repeatedly utilized for catalysis and purification of products, which saves time and effort for the synthesis of DyNAs.

In comparison to DNA or RNA, peptide nucleic acids (PNAs) have peptide-like (non-ribose) backbones instead of ribose main chains. PNAs still hold the capacity to form stable double-helical structures with DNA, RNA, or themselves in accord with Watson–Crick base-pairing rules.^{35,36} Extending principles of DNA-templated reversible polymerization and the methodology of reductive amination to non-ribose peptide-like backbones leads to the production of dynamic analogues of PNAs.^{29,30} DyNAs of both main-chain- and side-chain-dynamic types were efficiently fabricated (Figure 3a,b) through imine formation, and static products were obtained in high yields after reductive amination.^{29,37,38} In addition to imine condensation, dynamic analogues of PNAs can also be generated by using other types of reversible covalent bonds, such as thioester formation.^{39,40} Consistent with the conclusions of dynamic analogues of DNAs, DNA-templated synthesis of dynamic analogues of PNAs proceeds in a sequence-specific manner, resulting in sequence specificity and chain-length controllability.³⁷ Thus, the use of complementary DNA as template not only provides a driving force for reversible polymerization through DNA base pairing but also results in the sequence-directed synthesis of DyNAs.

In contrast to DNA-templated synthesis, it has been shown that the presence of polyanionic entities can also induce adaptation in chain-length of DyNAs.³¹ Constitutional modifications are, however, mainly driven by electrostatic forces between substrates and polyanionic targets instead of Watson–Crick base-pairing interactions (Figure 3c). Main-chain-dynamic types of DyNAs without ribose backbones were designed and synthesized through reversible polycondensation of dialdehydes with nucleobase-derived dihydrazides in aqueous media under mildly acidic conditions (Figure 4a,b). The formation of polyacylhydrazones was selected due to its synthetic accessibility. Furthermore, the resulting acylhydrazones are doubly functional through reversible imine-bond formation and noncovalent hydrogen-bonding interactions via the amide groups. As a consequence, the resulting dynamic cationic polymers are able to optimize their constitution in response to pH, temperature, or chemical additives to achieve tunability and stimuli-responsiveness even after polycondensation. More importantly, it was shown that anionic target species, such as inositol hexaphosphate (IHP), inositol

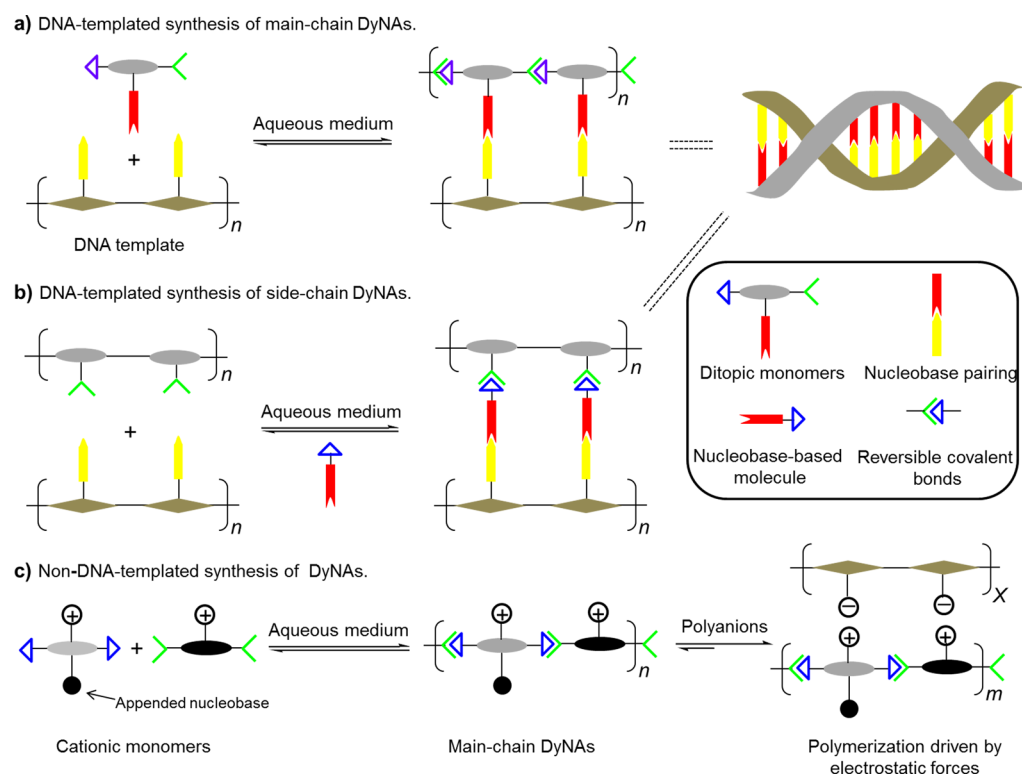


Figure 3. Schematic representation of the synthesis DyNAs with and without DNA templates.

triphosphate (ITPP), poly(aspartic acid), and adenosine triphosphate (ATP) (Figure 4c), trigger modification of their chain length through electrostatic forces. Surface plasmon resonance (SPR) measurements indicated that high binding affinities were induced by electrostatic forces between DyNAs and anionic polynucleotides (Figure 4d).³¹

To conclude, these findings, from both DNA- and non-DNA-templated synthesis of DyNAs, reveal that the utilization of anionic entities (DNA or non-DNA) can initiate constitutional adaption of DyNAs via specific or nonspecific noncovalent interactions between building blocks and target molecules and result in generation and amplification of the best adapted DyNAs. Nucleic acids, including both DNA and RNA, are essential biomacromolecules with biological functionalities, such as storage of genetic information (DNA) and translation of genetic code into proteins (RNA). Thus, DyNAs can provide in principle a novel methodology for designing and producing structural and functional biomimetics of nucleic acids, which can be used as biofunctional materials, for instance, in the areas of nucleic acid sensing and gene delivery.

3.2. Glycodynamers: Dynamic Analogues of Polysaccharides

Saccharide recognition plays a key role in many biological processes, including cell–cell interactions and cell communication,⁴¹ which makes carbohydrates attractive entities to create mimics of carbohydrate-based recognition processes. Given that carbohydrates are associated with numerous diseases, many attempts have been made to design and construct carbohydrate-based species for therapy and diagnosis of saccharide-associated diseases, such as tumors and chronic inflammation.⁴² Application of DCC in glycoscience offers novel opportunities for this field.

CDLs of saccharides are generated by DCC at the molecular level and feature recombination of their components through

reversible covalent bonds and amplification of specific compounds due to receptor-binding processes in response to the addition of target entities. Due to the inherent adaptive nature of dynamic saccharide libraries, such CDLs allow for target-driven and self-screening processes. Dynamic saccharide libraries were designed and generated through the formation of acylhydrazone⁴³ and disulfide bonds⁴⁴ in aqueous media at physiological pH. The CDLs obtained were applied for both rapid generation and efficient identification of ligands targeting lectin with enhanced inhibitory efficiency.

On the other hand, DCC allows one to mimic, modify, or (bio)functionalize polysaccharides through the generation of glycodynamers. As a consequence of the intrinsic dynamic features of DCC and the bioactivity of the carbohydrate-based components used, glycodynamers hold the potential to feature synergistic properties by combining adaptability with biofunctionality (molecular recognition), biodegradability, and biocompatibility of carbohydrates and may thus find application in the field of biofunctional materials science. Through different synthetic approaches, one may envisage to create three types of glycodynamers (Figure 5): (1) glycosidic main-chain, resulting from either (a) polymerization of saccharide residues through reversible covalent reactions or (b) reversible conjugation of small molecules to a static glycosidic backbone; (2) glycosidic side-chain, in which saccharide residues are either (a) irreversibly attached to a dynamic backbone or (b) reversibly appended on a static backbone; (3) glycodynamer containing both a dynamic backbone and reversible side chain(s).⁴⁵

Glycodynamers with a dynamic glycosidic main-chain (type 1a) can be prepared by reversible covalent polymerization of ditopic saccharide residues (Figure 5a). As dynamic mimics of naturally occurring glycans, the resulting materials exhibit both adaptability and biorelevant properties. Oxime-bond formation, through reversible condensation of aldehyde and hydroxyl-

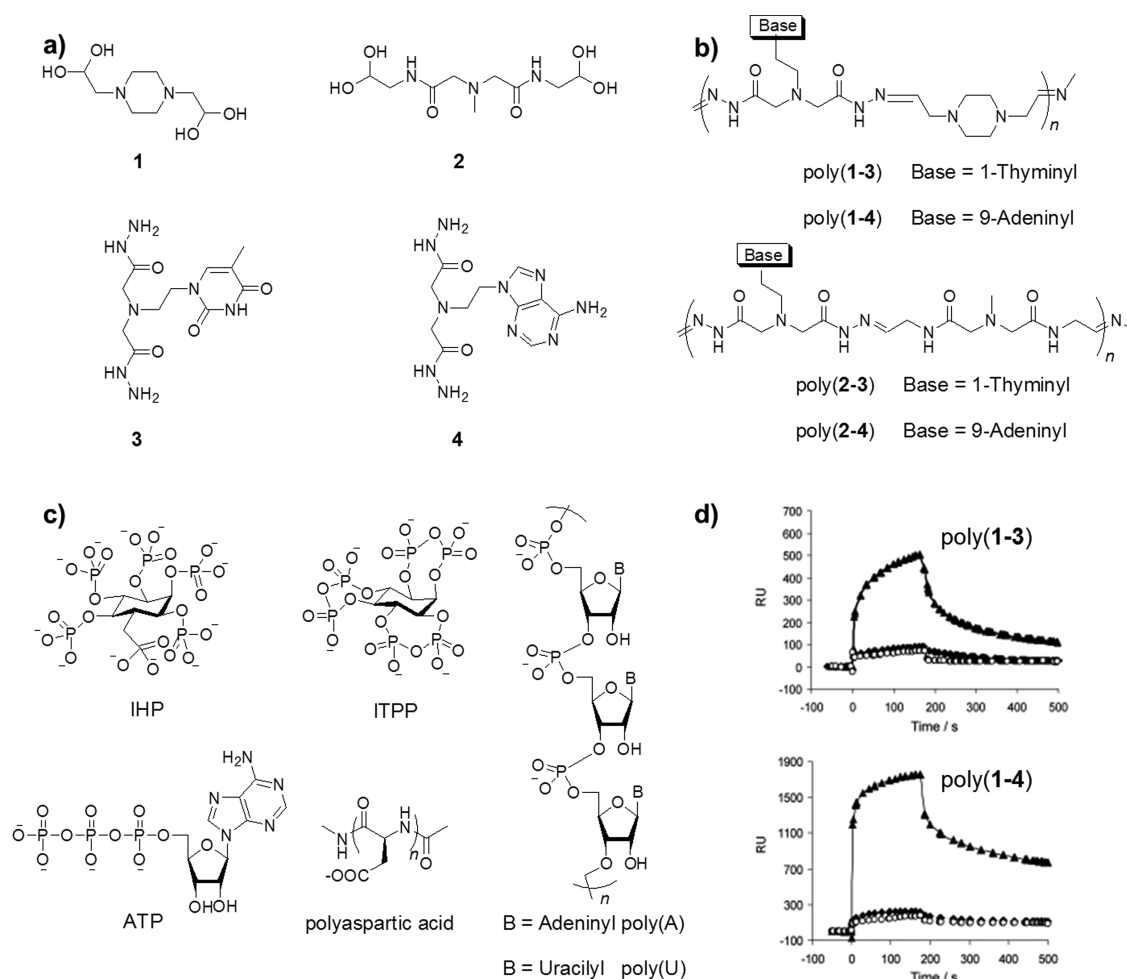


Figure 4. (a) Ditopic cationic monomers used for polyacylhydrazone formation. (b) Structures of generated polyacylhydrazones. (c) Structures of polyanionic targets. (d) Surface plasmon resonance (SPR) for binding of poly(1-3) and poly(1-4) to polyadenine at different pH values (\blacktriangle , pH = 4.5; \blacklozenge , pH = 5; \circ pH = 6). Adapted with permission from ref 31. Copyright 2006 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

amine monomers, is widely employed due to its inherent advantages: (1) efficient formation at mildly acidic pH; (2) higher stability against hydrolysis compared to the corresponding imine; (3) stability in aqueous solution at physiological pH; (4) pH responsiveness.⁴⁶ Oxime polysaccharides fabricated through enzyme-triggered polycondensation both in moderately acidic (pH \approx 5.5) and nearly physiological aqueous media (Figure 6) has been reported.⁴⁷ Many enzymes, however, may lose their catalytic activity under the conditions required for the construction of reversible covalent bonds. Hence, reversible oxime polycondensation was performed without using enzymes.⁴⁵ Monomer 5 contains a protected aldehyde group and an amino-oxy group, which can be polymerized by in situ deprotection–polycondensation, leading to glycodynamer poly9. In contrast, the alternative copolymer poly(7-10) can be obtained by the addition of bisalkoxyamine 7 to a neutralized solution of deprotected dialdehyde 6 (Figure 6). The formation of glycodynamers was confirmed by using diffusion-ordered NMR spectroscopy (DOSY) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. Furthermore, incorporation of *tert*-butylhydroxylamine as a termination agent to the formed polymer solutions was followed by $^1\text{H-NMR}$ and DOSY-NMR spectroscopy. Based on integrations of $^1\text{H-NMR}$ spectra and diffusion coefficients from DOSY NMR spectra, half-lives for exchange at different pD

values were obtained, and the observation that polymer poly(7-10) shortens upon addition of *tert*-butylhydroxylamine (Figures 6d,e,f) demonstrated its dynamicity.

Glycodynamers with a static glycosidic backbone and dynamic side chains (type 1b) can be formed by reversible immobilization of functional species onto a static polysaccharide (Figure 5b), such as vanillin,⁴⁸ peptides,⁴⁹ or flavoring, antimicrobial, antifungal, or antitumoral small molecules.^{50,51} The resulting glycodynamers are endowed with stimuli-responsiveness through the operation of reversible covalent bonds and present valuable properties of both components. In a given environment, specific chemical or physical stimuli can induce controlled release of the appended functional molecules. Thus, this type of glycodynamer provides a tool for the functional modification of saccharides and can be synthesized as biodynamic films^{50,51} or drug-delivery systems⁴⁹ with biofunctionality, biocompatibility, adaptability, and stimuli-responsiveness.

The synthesis of glycodynamers of type 2a is conducted by equilibrium polymerization of monomers featuring irreversibly grafted saccharides (Figure 5c). The resulting glycodynamers consist of a dynamic backbone and glycosidic static side chains. In view of their structural diversity and synthetic accessibility, different carbohydrate-modified dihydrazides and dialdehydes were designed and synthesized as monomers for the formation

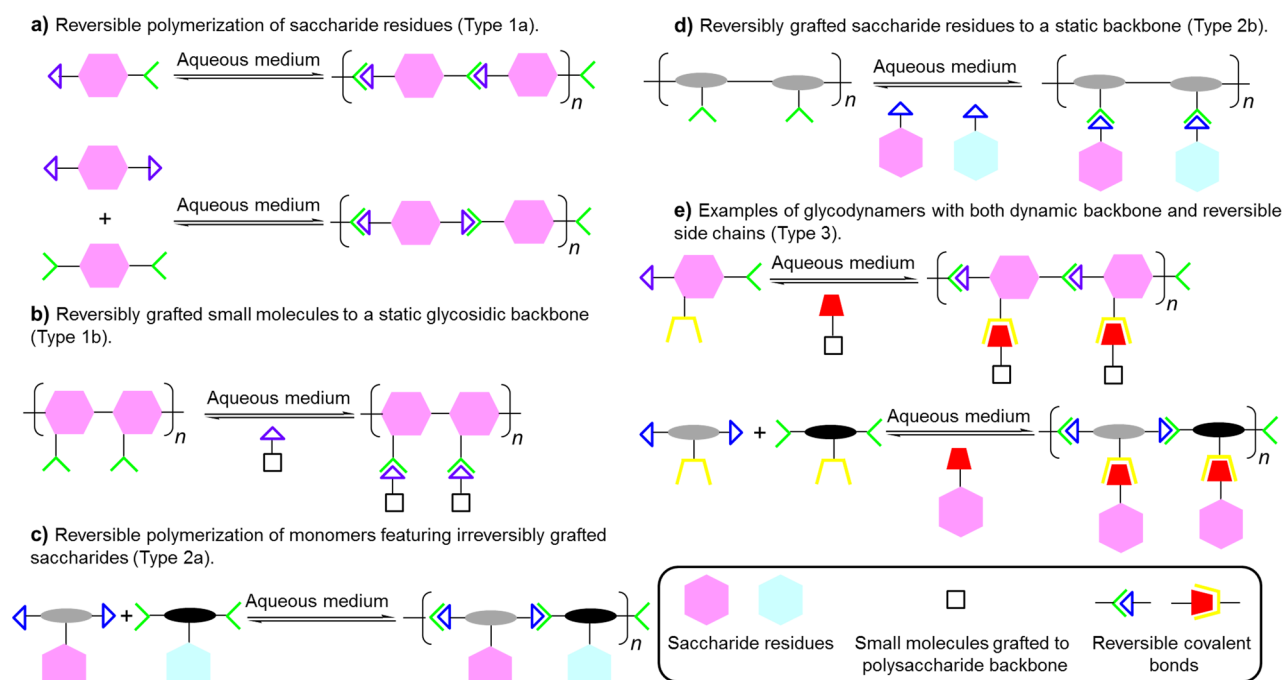


Figure 5. Schematic representation of the generation of different types of glycodynamers.

of polyacylhydrazones and the investigation of this type of glycodynamers (Figure 7a).^{52,53} A reversible polycondensation reaction in aqueous media under mildly acidic conditions afforded a series of glycodynamers with high molecular weights, which feature relevant biofunctional properties owing to their carbohydrate side chains (Figure 7b). Cryo-transmission-electron microscopy (cryo-TEM) and small-angle neutron scattering (SANS) revealed the construction of cylindrical micelle-like and wormlike structures. Moreover, these dynamic glycopolymers displayed intense fluorescence (Figure 7c), which can be attributed to their tightly packed structures mediated by hydrophobic interactions of aromatic chromophores.⁵² Their dynamic properties were demonstrated by adding one equivalent of **14** to glycodynamer poly(**10-13**) and poly(**12-13**) and following monomer replacement through both ¹H-NMR and fluorescence spectroscopy, because the incorporation of **14** to glycodynamer poly(**10-13**) and poly(**12-13**) induced changes in ¹H-NMR spectra and fluorescence properties (Figure 7d).⁵³ In addition, the target-binding ability of these glycodynamers for peanut agglutinin was studied by SPR. Glycodynamers poly(**11-14**) and poly(**12-14**) showed enhanced affinity compared to their corresponding monomers and can be used as efficient ligands for peanut agglutinin (Figure 7e).⁵³ Taking into account that exchange and replacement of monomers also induce the constitutional modification of the polyacylhydrazones, it provides a novel strategy for the preparation of adaptive carbohydrate-based biomaterials with controllable and tunable properties, such as fluorescence and affinity for a biological target.

Finally, glycodynamers of type 2b (Figure 5d), with a static main chain and dynamic glycosidic side chains, are prepared by reversibly grafting saccharide residues to a linear or cyclic backbone. It offers novel tools for reversible postpolymerization modification of static polymers to achieve improved biocompatibility and combined biofunctionality and stimuli-responsiveness. In particular, the reversible modification of linear or cyclic functional polypeptide backbones give rise to

the generation of dynamic analogues of glycopeptides, including both linear and cyclic types (for a review, see ref 54). For instance, cyclopeptide scaffolds for multivalent presentation of saccharides through the formation of oxime bonds were recently fabricated.⁵⁵ On another note, multiple presentation of glycosidic groups has been achieved through the self-assembly of grid-type metallosupramolecular architectures leading to octavalent entities that displayed selective binding of the mannose-functionalized derivative toward concanavalin A.⁵⁶

3.3. Dynamic Proteoids: Dynamic Analogues of Proteins

Various reversible reactions can be employed for the preparation of dynamic proteoids. Given that enzymes are capable of selectively catalyzing peptide synthesis under mild conditions, various dynamic systems based on positively or negatively charged peptides were developed through the reversible enzymatic formation of amide bonds in aqueous media at physiological pH.⁵⁷ In the presence of oppositely charged polysaccharides, substantial increments in product yield were observed due to electrostatic interactions between peptides and templates. Reversible native chemical ligation reactions that selectively occur at *N*-(methyl)-cysteine residues in aqueous solution at physiological pH afforded reversible proteoids in the absence of enzymes.⁵⁸ In this dynamic system, peptide fragments of the resulting product can undergo exchanges in the presence of dithiothreitol (DTT). Furthermore, disulfide bond formation is also widely used for the preparation of dynamic proteoids. For instance, dynamic combinatorial systems consisting of two types of competitive peptide-functionalized compounds have been set up.⁵⁹ Under given conditions, two sets of self-replicating peptide-based macrocycles were created by selective incorporation of their favored building blocks into respective kinetically controlled replicators.

We generated dynamic proteoids using reversible C=N bond formation.^{60,61} Polycondensation of a water-soluble

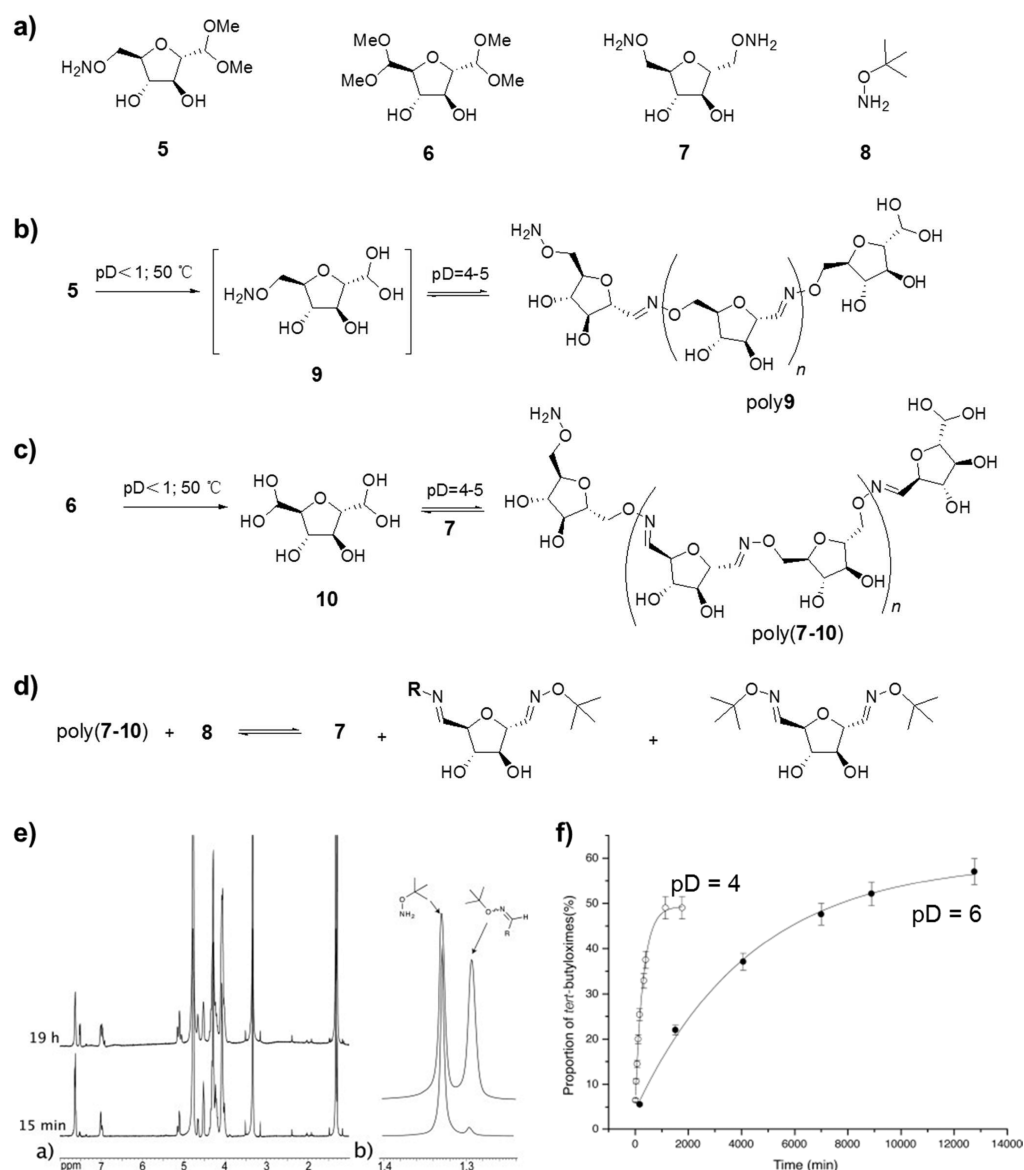


Figure 6. (a) Structures of ditopic monomers 5–7 and the termination agent 8. (b) Generation of the glycodamer poly9. (c) Generation of the glycodamer poly(7–10). (d) Dynamic chain termination: equilibrium upon addition of 8 to poly(7–10). (e) ¹H NMR spectra of the exchange mixture obtained after addition of 8 to poly(7–10) after 15 min and 19 h. (f) pD dependence of the rate of exchange between poly(7–10) and 8. Adapted with permission from ref 45. Copyright 2008 Wiley Periodicals, Inc.

amphiphilic dialdehyde **15** with various bifunctional amino acid hydrazides **16**–**25** in aqueous media (pD ≈ 5, Figure 8) using both imine and acylhydrazone formation affords biodynamers with doubly covalent dynamicity. The dialdehyde features a tricyclic aromatic core to stabilize the resulting biodynamers through π – π -stacking interactions and a hexaglyme chain endowing the structures generated with water solubility. Under mildly acidic conditions, acylhydrazone formation proceeds readily and goes to completion, whereas imines are barely formed. It was found, however, that reversible polycondensation takes place in a nucleation–elongation (N-E) manner⁶² and is driven by self-organization and folding of the dynamic proteoids formed through hydrophobic interactions between the dialdehyde core and the side chains of the amino acid hydrazides used.⁶⁰ The architectures of the polymers were characterized by cryo-TEM, dynamic light scattering (DLS), and SANS, which revealed the generation of three types of nanostructures: globular nano-objects, nanorods, and oligomers

(Figure 8b,c).⁶¹ Furthermore, by studying their polycondensation and monomer exchange via ¹H-NMR spectroscopy, it became apparent that side chains of the amino acid hydrazides affect the rates of polymerization (Figure 8d), structure, and dynamic properties of the resulting biodynamers. Given these findings, we concluded that⁶¹ (1) aromatic rings (**16**, **17**, and **18**) speed up polymerization and stabilize biodynamers through π – π -stacking interactions to build globular nano-objects; (2) positively charged side chains (**19** and **20**) accelerate polymerization and give rod-shaped architectures, whereas negatively charged side chains block polymerization and produce oligomers; (3) the presence of hydroxyl groups (**24** and **25**) stabilizes the polymers and leads to globular nano-objects through hydrogen bonds; (4) electrostatic forces dominate the reversible polycondensation when two oppositely charged species are utilized, leading to equal incorporation of the monomers and to neutral dynamic proteoids; (5) when two amino acid hydrazides exist in a

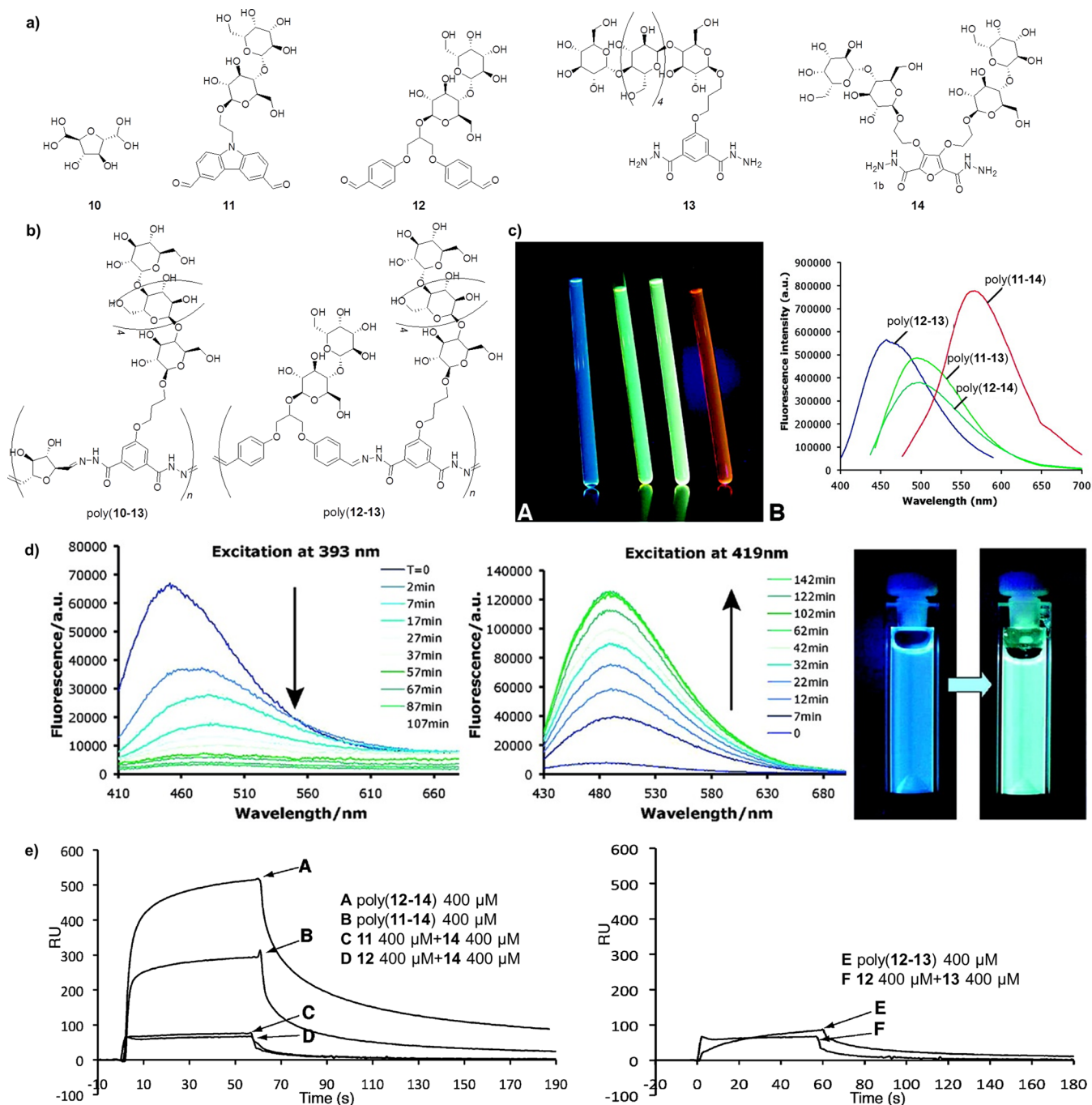


Figure 7. (a) Structures of the dialdehydes and the dihydrazides. (b) Structures of glycodynamers **poly(10-13)** and **poly(12-13)**. (c) Photography of **poly(11-13)**, **poly(11-14)**, **poly(12-13)**, and **poly(12-14)** under UV irradiation (365 nm, left) and their emission spectra (right). (d) Evolution of the fluorescence of **poly(12-13)** after addition of **14** (left) and photography of **poly(12-13)** before and after monomer exchange with **14** (right). (f) Surface plasmon resonance (SPR) results of binding to peanut agglutinin. Adapted with permission from ref 53. Copyright 2010 American Chemical Society.

system, monomers with a faster rate of polymerization are preferably incorporated into the dynamic proteoids formed; and (6) addition of an amino acid hydrazide with a faster rate of polymerization to an existing dynamic proteoid leads to monomer replacement. Our findings set the stage for the rational design and production of various types of well-defined architectures and smart proteoid materials.

Dynamic proteoids combine the properties of all monomers, particularly the biocompatibility of the amino-acid-derived monomers, with the adaptability from the reversible covalent

bonds. Hence, such proteoid materials may be used in both biomedical and bioengineering fields. In addition, proteins play significant roles in numerous biological processes, which are attributed to their unique, specific, and stable 3D architectures obtained through folding. To unravel the relationship between the specific 3D structure of a protein and its related biofunction is essential but remains a challenge, which may be addressed by the construction of dynamic proteoids. Furthermore, protein–protein complexes are an attractive class of drug targets. Unlike traditional ones, containing well-defined pockets for inhibitors

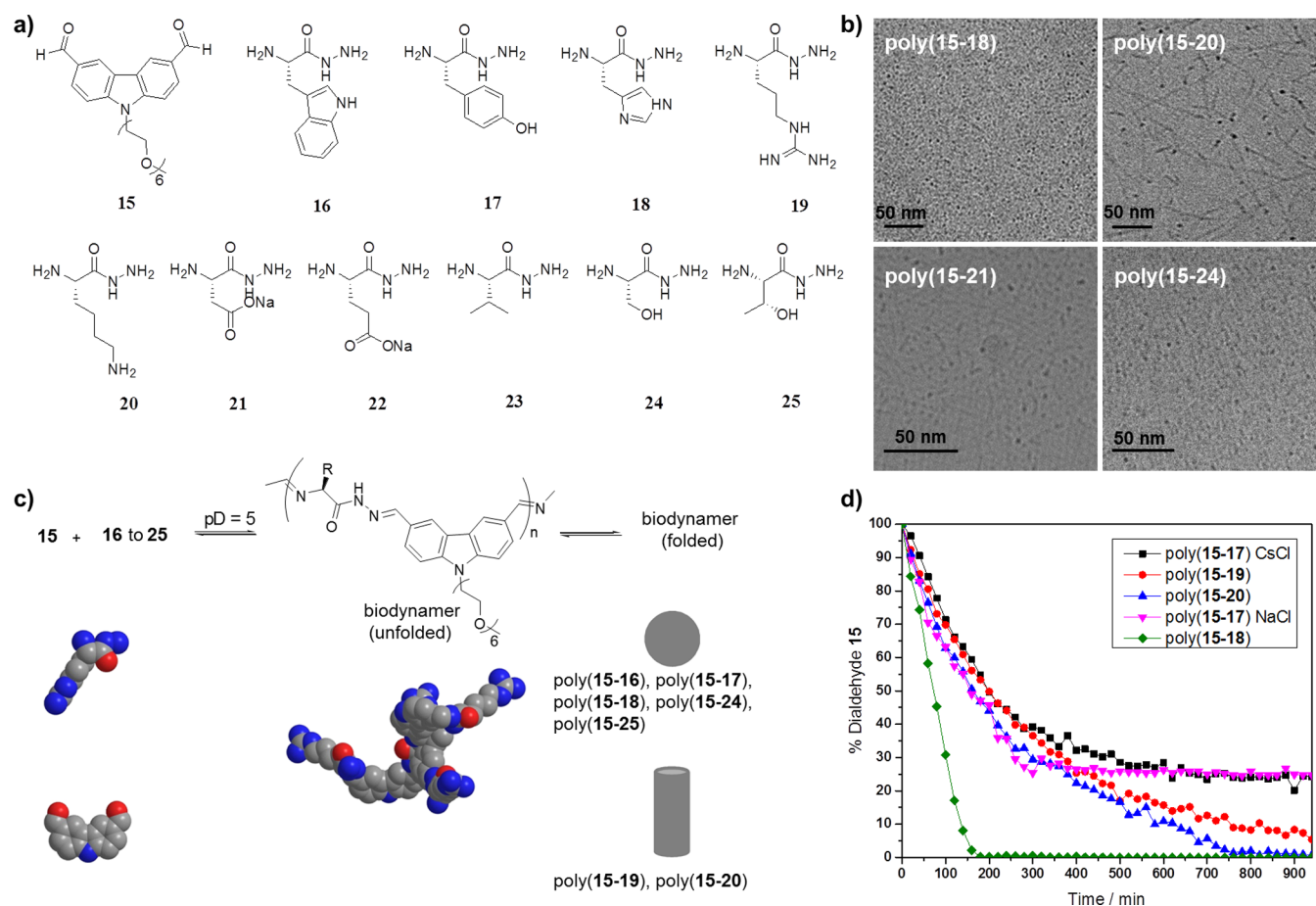


Figure 8. (a) Structures of the dialdehyde **15** and amino acid hydrazides **16–25**. (b) Cryo-TEM images of poly(**15-18**), poly(**15-20**), poly(**15-21**), and poly(**15-24**). (c) Schematic representation of dynamic proteoid generation. (d) Rate of polymerization: percentage of unreacted dialdehyde versus time. Adapted with permission from ref 61. Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

to bind, the contact surfaces of protein–protein interactions are usually large, flat, hydrophobic, and solvent-exposed.⁶³ These features make the design of specific binders for protein–protein interactions particularly challenging. We believe that dynamic proteoids could provide tools for designing, identifying, and fabricating dynamic inhibitors of protein–protein interactions.

4. CONCLUSIONS AND OUTLOOK

Molecular biodynamers offer a combination of chemical, biological, and combinatorial methodologies to design and synthesize dynamic analogues of biopolymers, such as nucleic acids, polysaccharides, or proteins. In contrast to static biopolymers, synthetic molecular biodynamers feature dynamics resulting from the implementation of DCC, leading to synergistic properties, which combine biorelevant features (e.g., biocompatibility, biodegradability, biofunctionality) of the constituent components with dynamism. In response to internal or external stimuli, biodynamers are capable of undergoing self-adaptation of their molecular constitution, 3D architecture, physical features, chemical properties, and function, in order to generate, identify, and amplify the fittest entities. Therefore, molecular dynamers can be employed as adaptive functional biomaterials. The construction of molecular biodynamers through CDC, including DyNAs, glycodynamers, and dynamic proteoids, provides powerful tools to mimic both structure and biofunctionality of nucleic acids, polysaccharides, or proteins and to unravel the correlation between structure

and functionality. However, it is still challenging to characterize the structures obtained and to design the generation of the desirable structural and functional features. Moreover, molecular biodynamers may find applications based on the respective building blocks, namely, nucleobases, carbohydrates, and amino acids. For instance, DyNAs might be of use for gene sensing, glycodynamers for cancer diagnosis and treatment, and dynamic proteoids to understand protein folding and protein–protein interactions (for instance in diseases involving protein aggregation). At present, the surface of the field has just been scratched. One may envisage an increasing emergence of biodynamers fabricated by CDC for the development of adaptive biomaterials and their implementation in the field of biomedicine, bioengineering, biotechnology, and drug delivery.

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Notes

The authors declare no competing financial interest.

Biographies

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Jean-Marie Lehn was born in 1939 in Rosheim (France). In 1963, he received his Ph.D. in chemistry from the University of Strasbourg and became Professor of Chemistry at the Université Louis Pasteur in Strasbourg in 1970. From 1979 to 2010, he was Professor at the Collège de France in Paris. He is currently Honorary Professor at the Collège de France in Paris and Professor at the University of Strasbourg Institute of Advanced Study (USIAS). In 1987, he shared the Nobel Prize in Chemistry for his studies on the chemical basis of “molecular recognition”. He defined a new field of chemistry, which he called “supramolecular chemistry”. Subsequently, he developed the area in the direction of self-organization by design or with selection, and constitutional dynamic chemistry, which then led to adaptive chemistry. He has received numerous international honors and awards and is a member of many academies and institutions.

Anna K. H. Hirsch, born in 1982 in Trier, Germany, studied Natural Sciences at the University of Cambridge (2000–2004) and obtained her Ph.D. from the ETH Zurich in 2008 under the supervision of Prof. François Diederich. After postdoctoral studies with Prof. Jean-Marie Lehn at the Institut de Science et d'Ingénierie Supramoléculaires in Strasbourg, she joined the Stratingh Institute for Chemistry at the University of Groningen as an assistant professor in 2010. In 2015, she became associate professor at the University of Groningen. Her research interests are in the areas of structure-based drug design, solubilization of hydrophobic drugs, and the application of dynamic combinatorial chemistry and target-guided synthesis in drug discovery.

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