increasing the error bars on the line positions to give a χ^2 value of 1 and then calculating the covariance matrix. The temperature in the slow-exchange regimes was calibrated with a methanol thermometer (*13*) and a 10-mm NMR dual-tube assembly in which the outer tube contained undiluted methanol and the inner tube contained the sealed methylcyclohexane solution. The chemical shifts were found to be linear in temperature, and the correlation of the chemical shift difference between the equatorial C-Me and C-2,6 spectral lines was used to measure the temperature in other low-temperature experiments. The dual-tube assembly was used to directly measure the temperature in the intermediate- and fast-exchange regions. The spectrometer response was found to be linear under similar operating conditions by using a solution of methylcyclohexane (major conformation) in cyclohexane under concentrations that were approximately equal to the major and minor conformers. The filter response of the spectrometer was measured, and intensity corrections of up to 3% were measured and applied to the outermost resonances. Spin-lattice relaxation times T_1 for the proton magnetization varied from 0.3 to 0.55 s at 180 K to 0.14 to 0.25 s at 143 K. Relaxation delays between scans were varied between 2 and 8 s.

17. This measurement of the free energy is consistent with an earlier measurement of $\Delta A_0 = 6317 \pm 837$ J mol^{-1} and $d\Delta A(T)/dT = 6.7 \pm 5.4$ J mol^{-1} K^{-1} (*8*, *14*) but is more precise.
18. Linearity of the temperature dependence of the chemical shifts with respect to a cyclohexane reference was previously reported for the conformers of methylcyclohexane and for the related compounds *cis*- and *trans*-1,4-dimethylcyclohexane, where the chemical shifts are linear over the entire measured region of 70 K for the cis conformation and 120 K for the trans conformation (*14*).
19. P. R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences* (McGraw-Hill, New York, ed. 1, 1969). The extent to which the reduced χ^2 is >1 allows a quantitation of the probability that the observed deviations of the data from the best fit are consistent with the random experimental errors, the determination of which is described in the caption of Fig. 2. For the global fits presented here, 225 data points are included: 57 for the slow-exchange equilibrium constants, 16 for the fast-exchange averages, and 152 for the slow-exchange chemical shifts of the major and minor conformers.
20. A conceivable systematic error would be caused by the neglect of a hypothetical third conformer, which might be present at undetectable levels in slow exchange but would contribute significantly in fast exchange. Cryogenic trapping experiments on methylcyclohexane from an initial temperature of 873 K (*15*) set an upper bound at 300 K of 10^{-4} for the ratio of a hypothetical third conformer to that of the equatorial form, whereas a population of several percent would be needed to account for the present observations with reasonable chemical shift parameters.
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23. These contributions to the unitary evolution may be viewed as resulting from ALBATROSS. The description of the spectral densities in terms of system and bath susceptibilities (*21*) is not elaborated here.

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Chain Length Recognition: Core-Shell Supramolecular Assembly from Oppositely Charged Block Copolymers

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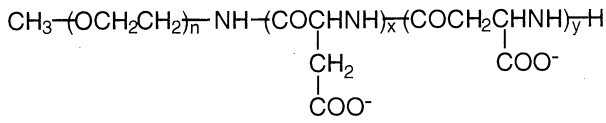
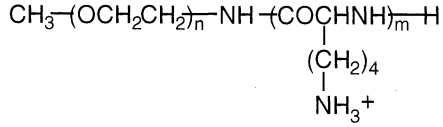
Molecular recognition based on length was found to occur between oppositely charged pairs of flexible and randomly coiled block copolymers in an aqueous milieu. Matched pairs with the same block lengths of polyanions and polycations exclusively formed even in mixtures with different block lengths. These assemblies of the charged segments with matched chain lengths then formed larger core-shell–type supramolecular assemblies with an extremely narrow size distribution due to the strict phase separation between core- and shell-forming segments.

Recently, molecular recognition and the resulting supramolecular assembly processes have received considerable attention in chemistry, biology, and applied fields. Carefully designed chemical architectures promote precise molecular recognition, often leading to an assembly process (*1*–*6*). A common strategy for the formation of supramolecular assemblies is the construction of spatially ordered networks of noncovalently bonded constituent molecules. Most of studies in this area to date have been focused on the design of spatially ordered structures in which small differences in the steric factors of the constituent molecules crucially affect the thermodynamic stability and assembly of the system (*7*). Here we introduce a molecular recognition system that uses assembly of coiled block copolymers. Exclusive pairwise recognition of oppositely charged polymer strands occurs selectively on the basis of length, creating multimolecular micellization of pairs of oppositely charged block copolymers in aqueous solution. The block copolymers used here were composed of oppositely charged pairs of poly(ethylene glycol)-b-poly(α,β-aspartic acid) (Scheme 1)



Scheme 1

and poly(ethylene glycol)-b-poly(L-lysine) (Scheme 2)



Scheme 2

We reported previously that mixing this pair of block copolymers with the same degree of polymerization (DP) in aqueous media led to the spontaneous formation of polyion complex (PIC) micelles having diameters of several tens of nanometers with an extremely narrow size distribution (*8*). A core-shell architecture with the PIC core surrounded by a poly(ethylene glycol) (PEG) corona was proposed for these PIC micelles.

Block copolymers were synthesized by the ring-opening polymerization of *N*-carboxyanhydride of the amino acids β-benzyl-L-aspartate or ε-benzyloxycarbonyl-L-lysine, initiating from the ω-NH_2 group of α-methoxy-ω-aminopoly(ethylene glycol) (*8*). The molecular weight (M_w) of the PEG segment was fixed at 5000 g/mol for all block copolymers examined in this study. After polymerization, protecting groups on the poly(amino acid) segments were removed either by alkali treatment (β-benzyl-L-aspartate) or by acid treatment (ε-benzyloxycarbonyl-L-lysine). Block copolymers thus obtained were confirmed to have a fairly narrow M_w distribution [ratio of the weight-averaged to number-averaged molecular weight (M_w/M_n) < 1.10] by gel filtration chromatography (GFC). For both anionic and cationic partners, two sets of copolymers with different DP of poly(amino acid) segments (18 and 78) were prepared and were abbreviated as A-18 and A-78 (Scheme 1) and as C-18 and C-78 (Scheme 2), respectively.

Recognition and selectivities for specific chain lengths upon micellization were directly monitored by GFC (Fig. 1). Gel filtration of the 1:1 mixture of the anionic polymers A-18 and A-78 [based on the aspartic acid (Asp) content]

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provided the chromatogram with two clearly separated peaks corresponding to A-18 (elution volume, 17.4 ml) and A-78 (elution volume, 15.3 ml), respectively (Fig. 1A). Then a given amount of the cationic polymer C-18 was added to this mixture so as to neutralize 0.5 equivalents of Asp units in the solution. Complete disappearance of the A-18 peak from the chromatogram was observed, with the A-78 peak remaining totally intact without any change in GFC peak intensity (Fig. 1B). A peak corresponding to the PIC micelle was clearly observed in the exclusion volume of the chromatogram. The chemical composition of the fractionated PIC micelle was determined by ^1H nuclear magnetic resonance (NMR) in D_2O containing 1.0 M NaCl. The molar ratio for Asp and Lys residues ([Asp]/[Lys]) in the PIC micelles was determined to be 1.0, and the observed ratio of PEG to Asp and Lys residues was consistent with the calculated ratio, assuming that the micelle exclusively includes paired A-18 and C-18. However, addition of C-78 to the mixture of A-18 and A-78 resulted in complete selection of A-78, as shown in Fig. 1C. NMR analysis of the micelle fraction also revealed that PIC micelle formation involved only A-78 and C-78. Thus, a pair of oppositely charged block copolymers exclusively selects matching partners with the same length of charged segments upon micellization.

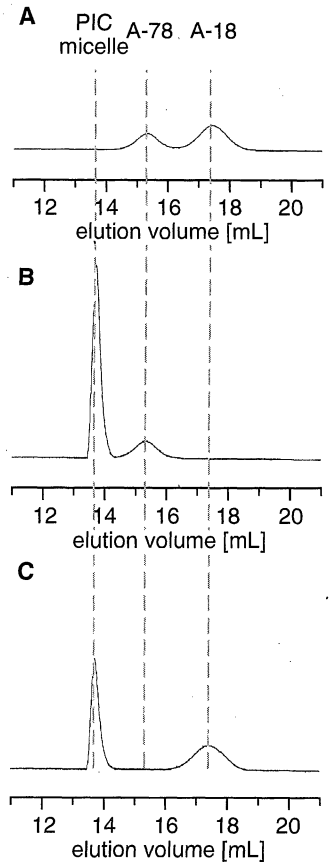

Fig. 1. Chain length–dependent recognition of block copolymers monitored by GFC. (**A**) A-18 (3.02 mg/ml, 6.06 mmol of Asp per milliliter) and A-78 (1.22 mg/ml, 6.06 mmol of Asp per milliliter). (**B**) A-18 (3.02 mg/ml, 6.06 mmol of Asp per milliliter), A-78 (1.22 mg/ml, 6.06 mmol of Asp per milliliter), and C-18 (3.34 mg/ml, 6.06 mmol of Lys per milliliter). (**C**) A-18 (3.02 mg/ml, 6.06 mmol of Asp per milliliter), A-78 (1.22 mg/ml, 6.06 mmol of Asp per milliliter), and C-78 (1.65 mg/ml, 6.06 mmol of Lys per milliliter). A Superdex 75 HR column (Pharmacia) and TSK gel G3000PW$_{XL}$ (Tosoh, Japan) were used for GFC measurements. The eluent used was 10 mM phosphate buffer with 50 mM NaCl (pH 7.4); the flow rate was 0.3 ml/min. Detection was done by refractive index at room temperature.

Chain length–dependent recognition through micellization was further supported by data from dynamic light scattering (DLS). As summarized in Table 1, PIC micelles prepared under charge-neutralized conditions ([Asp]/[Lys] = 1) have a significant difference in their average DLS size between A-18/C-18 and A-78/C-78 pairs: The former was approximately 10 nm less in diameter than the latter, reflecting differences in the micelle association number.

Addition of either C-18 or C-78 to the mixture of A-18 and A-78 resulted in the formation of narrowly distributed PIC micelles with diameters corresponding to those of micelles prepared from the matched pair, as did the analogous experiment for polyanion addition (Table 1). These results are consistent with GFC results shown in Fig. 1 and strongly suggest a selective micelle formation mechanism that operates through chain length recognition.

Because PIC micelles were confirmed to have a narrow size distribution that was nearly monodisperse in nature, static light-scattering (SLS) measurements were then carried out to determine the micelle weight-averaged M_w as well as the micelle association number. All of the SLS results (the last column in Table 1) were quite consistent with DLS results, supporting selective formation of PIC micelles between matched polymer pairs. PIC micelles from A-18/C-18 had a M_w of about 5×10^5 g/mol, which corresponds to an association number of approximately 40 chains of both A-18 and C-18. The M_w increased to 3×10^6 g/mol for the A-78/C-78 pair, corresponding to a total of 180 chains

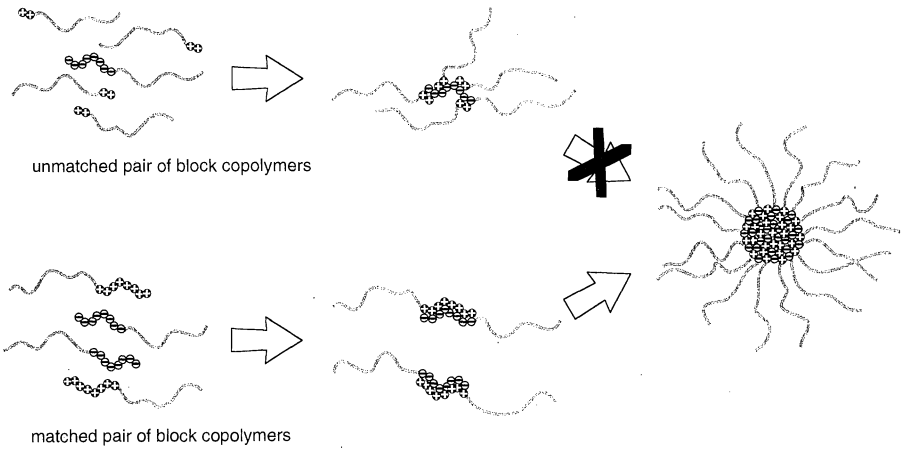

Fig. 2. Schematic model for chain length–dependent recognition through the formation of PIC micelles.

Table 1. Size and M_w for mixtures of block copolymers. ND, not detected.

Polyanion	Polycation	Average diameter* (nm)	Polydispersity index*	M_w† (g/mol)
		Matched pairs		
A-18	C-18	31.5	0.0532	4.97×10^5
A-78	C-78	40.5	0.0265	3.12×10^6
		Unmatched pairs		
A-18	C-78	ND	ND	3.95×10^4
A-78	C-18	ND	ND	4.33×10^4
		Addition of polycation to mixed polyanions		
A-18 and A-78	C-18	30.8	0.0654	5.03×10^5
A-18 and A-78	C-78	40.3	0.0534	3.13×10^6
		Addition of polyanion to mixed polycations		
A-18	C-18 and C-78	30.9	0.0731	5.00×10^5
A-78	C-18 and C-78	40.9	0.0698	3.13×10^6

*Average diameter and polydispersity indices were obtained by cumulant analysis of DLS using a DLS-700 (Otsuka Electronics, Japan) at 25.0° ± 0.1°C. †Molecular weights were determined by SLS using DLS-700 at 25.0° ± 0.1°C.

(90 chains each for A-78 and C-78).

The system with unmatched polymer length pairs (A-18/C-78 and A-78/C-18) had a M_w somewhat greater than the simple average of constituent block copolymers (3.9×10^4 g/mol and 4.3×10^4 g/mol for the A-18/C-78 and A-78/C-18 combinations, respectively), indicating an association of ionomeric block copolymers, yet much smaller than that of PIC micelles. Block copolymers in these combinations may assume a minimum number of anion-cation associations to compensate for their charge. If aggregates formed consisting of one longer chain (DP = 78) with four shorter chains (DP = 18), then the M_w of such an association would be $\sim 4 \times 10^4$ g/mol, which is similar to the observed value for the A-18/C-78 and A-78/C-18 systems. As shown in Fig. 2, unmatched pairs form only the minimal charge-neutralized polyion complex, which is unable to grow further into larger PIC micelles, whereas matched length pairs of block copolymers spontaneously assembled into PIC micelles with a considerable association number. Polymer complexes formed between unmatched pairs should be less stable than those formed between matched pairs, and consequently, in the coexisting competitive condition of matched and unmatched pairs, only the matched pairs form bimolecular complexes that grow into PIC micelles. The remaining block copolymers of unmatched length are left in isolated form.

Circular dichroism spectra of resulting PIC micelles indicated that the poly(L-lysine) block, which is optically active (*9*), assumed no particular secondary structure in the micelle. Thus, both poly(α,β-aspartic acid) and poly(L-lysine) segments in the block copolymer behave as polymer strands without any ordered structures during the process of micellization, which indicates that the charge-neutralizing selective recognition observed here is indeed due to the difference in the chain length itself.

The key determinant in this recognition process is the strict phase separation between the PEG corona and the PIC core domain, requiring regular alignment of the molecular junctions between PEG and poly(amino acid) segments at the interface of the two domains. The requirement for charge stoichiometry (neutralization) in the core is another essential factor in this recognition process. This eventually determines the number ratio of participating polyanion and polycation strands in the core and restricts the spatial arrangement of segments in the core. Ion pairs should have an uniform distribution in the PIC core, and unmatched length pairs cannot achieve this without phase mixing of PEG corona and PIC core domains.

The length selection system demonstrated with these flexible ionomer polymer strands provides a new approach for controlling supramolecular assembly. Recently, higher ordered assembly of block copolymers in selective solvents to form spherical, rodlike, and univesicular or lamellar structures was reported (*10*, *11*). Assembly of charged block copolymers in aqueous medium may lead to the formation of similar higher ordered structures through precise recognition based on the chain lengths of charged segments, which may be useful for constructing self-assembled layers based on electrostatic interaction (*12*). Chain length recognition based on PIC formation at two-dimensional interfaces may yield self-assembled layers of block copolymers with definite layer thicknesses modulated by chain lengths in the charged segments of the block copolymer.

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Requirement for Diverse, Low-Abundance Peptides in Positive Selection of T Cells

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Whether a single major histocompatibility complex (MHC)–bound peptide can drive the positive selection of large numbers of T cells has been a controversial issue. A diverse population of self peptides was shown to be essential for the in vivo development of CD4 T cells. Mice in which all but 5 percent of MHC class II molecules were bound by a single peptide had wild-type numbers of CD4 T cells. However, when the diversity within this 5 percent was lost, CD4 T cell development was impaired. Blocking the major peptide–MHC complex in thymus organ culture had no effect on T cell development, indicating that positive selection occurred on the diverse peptides present at low levels. This requirement for peptide diversity indicates that the interaction between self peptides and T cell receptors during positive selection is highly specific.

The immune systems of higher vertebrates generate a diverse population of potential T cell receptors (TCRs) through random rearrangement of gene segments within the TCR loci. The dilemma is in choosing which T cells will contribute to protective immunity without knowing the antigens that they may eventually encounter (*1*). This problem is addressed by evaluating the TCRs of developing thymocytes based on their recognition of the thousands of different self peptides bound to MHC molecules in the thymus. Potentially autoreactive T cells with TCRs that bind with too high an affinity to self peptide–MHC complexes are eliminated by a process called negative selection. The process of positive selection, by which T cells are chosen to mature, results in a population of T cells that interacts with MHC molecules with sufficient, albeit weak, affinity to permit a strong interaction with a particular nonself peptide presented by self MHC. Whether specific interaction with the self peptide is needed to select such a T cell repertoire has been hotly debated. Of particular interest is whether a given self peptide selects a limited number of different TCRs and thus truly shapes the specificity of T cells during positive selection.

A role for particular self peptide–MHC ligands during positive selection of T cells was demonstrated when specific peptides or increasingly complex peptide mixtures were added to

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