Conversion of Supramolecular Clusters to Macromolecular Objects

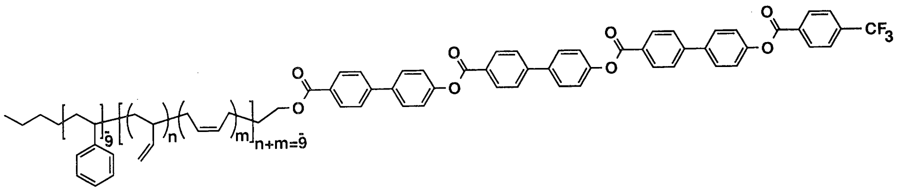
Eugene R. Zubarev, Martin U. Pralle, Leiming Li, Samuel I. Stupp*†

In a reaction proceeding within a nanoscopic volume, supramolecular clusters were transformed to polymer objects while retaining their shape and size. Spatial isolation of the cross-linkable blocks of oligobutadiene that were involved in this stitching reaction was achieved by self-assembly of the molecules that made up the clusters. Thermal activation of cross-linking yielded macromolecules (molecular weight of 70,000) with a narrow size distribution that was similar to that of the supramolecular clusters. The macromolecules obtained have an anisotropic shape (2 nanometers by 8 nanometers), as determined by electron microscopy and small-angle x-ray scattering, and form materials that exhibit a liquid crystalline state.

A key difference between synthetic polymers and proteins is the well-defined folded shape of proteins in solution; polymers, with their simpler composition, tend to exhibit highly symmetrical nanostructures (*1, 2*). Polymers with more sophisticated functions might be realized if synthetic pathways were found to create macromolecules with distinct and permanent shapes (regardless of state) or to create low-symmetry objects that were different from highly symmetrical dendrimers (*3*). Molecular object polymers (*1, 4*) have a permanent chemical map on their surfaces and pack in specific ways to form condensed phases. Self-assembly is a rapid pathway from small molecules to supramolecular nanometer-sized objects that cannot be synthesized by conventional chemical reactions (*5, 6*). We recently synthesized triblock rodcoil molecules that self-assemble into mushroom-shaped nanostructures (*5*), which exist in the solid state but could disassemble in solution or in the melt. Here, we report on the synthesis of soluble nanostructures with a molecular weight (MW) of ~70,000 through cross-linking of supramolecular clusters. The covalent polymers obtained are highly anisotropic, with an aspect ratio of 4, and have a characteristic shape that resembles a mushroom. Furthermore, the precursor molecular aggregates and the resulting macromolecules appear identical when viewed with an electron microscope, which suggests that this cross-linking reaction is essentially a size- and shape-invariant chemical transformation.

Our first approach to creating shape-invariant molecular objects was to synthesize molecules that organized into two-dimensional (2D) structures with the proper orientation to react (*4*). Cross-linkable groups in these supramolecular structures offer the possibility of covalently locking the aggregate's original shape, but in many cases, such reactions resulted in either insoluble materials or in polymer products with very broad MW distributions (*1*). This is due to the difficulty of controlling the planar dimensions (*xy*) of cross-linkable regions in these 2D structures. Thus, it is important to control the size of cross-linkable regions by preassembled nanostructures in a way that limits the volume of reaction and the extent of interconnection. This goal requires spatial isolation of cross-linkable segments within one supramolecular unit.

We synthesized triblock rodcoil molecules that had the structure shown in Scheme 1. The synthesis and complete characterization of this molecule will be reported elsewhere (*7*). Rodcoil molecules **1** are composed of three different blocks: a block of atactic oligostyrene; a block of oligobutadiene with a random sequence of 1,4- and 1,2-addition units, based on nuclear magnetic resonance (NMR) data; and a rodlike block that is identical in all of the molecules. The butadiene block serves as a segment in which carbon-carbon double bonds can generate cross-links at high temperatures (*8–12*). The aromatic identical segments in all molecules are strongly driven to aggregate and crystallize (partly because of π-π overlap between biphenyl units). However, the structural diversity of the triblock molecules prevents the formation of three-dimensional crystals. As previously suggested (*5*), the oligostyrene segment provides steric elements that limit the size of the aggregates. An important feature of molecules **1** is their CF_3-terminus [as opposed to the OH-terminus of the molecule reported in (*5*)], which has a very large dipole moment and is also an extremely hydrophobic functional group (*13*). The CF_3 group also appears to provide an additional driving force for self-assembly.



Scheme 1. Triblock rodcoil molecules **1**.

Annealing precursor **1** in a liquid crystalline (LC) phase at 250°C (*14*) under a nitrogen atmosphere for several hours resulted in the formation of a product with a high molar mass, a narrow polydispersity, and a MW of ~70,000. Gel permeation chromatography (GPC) traces (Fig. 1) of samples that were annealed for different periods of time and then dissolved in tetrahydrofuran (THF) (1 weight %) show that annealing time strongly affects the yield, which can be as high as 80 weight % after annealing for 20 hours. A slight increase in MW and polydispersity [the ratio of weight average ($\bar{M}_w$) to number average ($\bar{M}_n$) MWs] occurred with further annealing. However, the calculated degree of polymerization with polystyrene standards remained constant at 22 ± 2 (MW ≈70,000). Moreover, the material was completely soluble (even after 20 hours of annealing), and the formation of an insoluble gel-fraction was not observed. The GPC traces also show that products with a MW less than that of the precursor do not form, suggesting that decomposition reactions do not occur. The use of conventional flash chromatography resulted in the complete isolation of the new product because it has a considerably lower retention factor relative to that of precursor **1** in most organic solvents. The GPC traces of starting material and isolated product reveal narrow polydispersities, which are on the order of 1.08 and 1.11, respectively (see Fig. 2).

Small-angle x-ray scattering (SAXS) revealed that both the monomeric precursor **1** and the isolated macromolecular product have layered structures in the solid state with a primary *d* spacing of 81 Å. Nonlinear optical experiments showed that supramolecular clusters have a noncentrosymmetric structure (*15*), and therefore the molecules must pack in a parallel (mushroomlike) manner and not in an antipar

---

Department of Materials Science and Engineering, Department of Chemistry, Beckman Institute for Advanced Science and Technology, Materials Research Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

*To whom correspondence should be addressed. E-mail: s-stupp@nwu.edu

†Present address: Department of Materials Science and Engineering and Department of Chemistry, Northwestern University, Evanston, IL 60208, USA.

allel (dumbbell-like) manner. Most important, the layer periodicity and narrow polydispersity suggest a strong correlation between the covalent structure of the product and that of its supramolecular precursor. Moreover, the polymerization is apparently confined to the volume of the supramolecular cluster and is therefore nanoscopic, connecting a nearly discrete number of molecules. Because precursor **1** is in an LC phase at the polymerization temperature, the spatial isolation of cross-linkable blocks that is required to form well-defined objects must prevail in the mesophase.

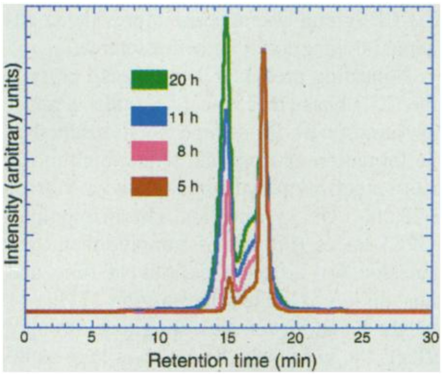
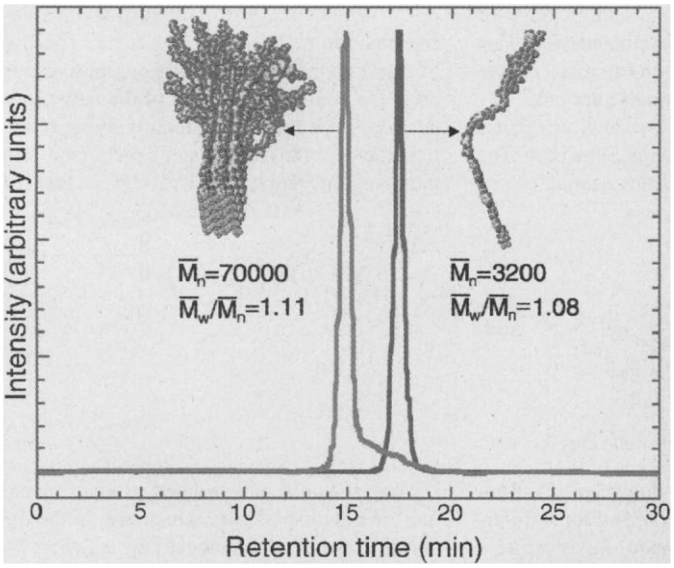


Fig. 1. GPC traces of precursor **1** samples that were annealed for different periods of time. Each trace shows the peak corresponding to **1** ($\bar{M}_n$ = 3200 and $\bar{M}_w/\bar{M}_n$ = 1.08). Molecular weight and polydispersity of the macromolecular product (relative to polystyrene standards) were found to be $\bar{M}_n$ = 63,000 and $\bar{M}_w/\bar{M}_n$ = 1.07, after 5 hours; $\bar{M}_n$ = 67,800 and $\bar{M}_w/\bar{M}_n$ = 1.11, after 8 hours; $\bar{M}_n$ = 72,600 and $\bar{M}_w/\bar{M}_n$ = 1.21, after 11 hours; and $\bar{M}_n$ = 75,200 and $\bar{M}_w/\bar{M}_n$ = 1.35, after 20 hours.

Transmission electron microscopy (TEM) studies of triblock molecules **1** revealed a solid state structure consisting of aggregates ~2 nm in diameter (Fig. 3A). Other similar molecules that were synthesized in our laboratory also formed aggregates but had larger dimensions (*5, 16*). Similar structures of 2 nm were observed for the isolated polymer object material (Fig. 3B), which indicate that aggregation and rod packing were not altered by polymerization. The thickness of layers (8 nm) revealed by SAXS is four times the diameter of the aggregates (2 nm). Thus, the supramolecular units are highly anisometric in shape (8 nm by 2 nm). More important, the electron diffraction patterns show the crystal's primary spacing to be 4.5 Å, which is presumably the distance between biphenyl groups. On the basis of this number and the cross section of nanostructures observed by TEM (20 Å), we estimated that there are (on average) 23 ± 2 molecules in each supramolecular aggregate. On the basis of the electron diffraction pattern, we found that the rod axes in the cluster are normal to the layers and are perpendicular to the plane of the TEM micrographs (before and after polymerization). The combination of SAXS, nonlinear optical experiments, and TEM suggests that triblock molecules **1** pack into the mushroom-shaped nanostructures that lack $D_{\infty h}$ symmetry (*5*).

The observed clusters are smaller by about a factor of 4 in comparison with those described in (*5*). However, their precursor differs chemically in a subtle way (a CF_3-terminus versus an OH-terminus). In order to understand this difference, we synthesized and studied more than 20 analogous molecules with varying chemical structure (*7*), many of which had the same structure as that of precursor **1,** except for the terminal group. This slight structural change resulted in a remarkable difference in properties, and none of the analogous rodcoils could be converted to well-defined molecular objects. Annealing of these molecules leads to products that are either insoluble or have very broad polydispersity ($\bar{M}_w/\bar{M}_n > 8$). However, rodcoil molecules with a CF_3-terminal group but with a different length of either the coil or rod segments have shown similar reactive behavior. Their GPC traces revealed the formation of high-molar-mass products with a narrow polydispersity within the range from 1.12 to 1.25 and MWs of up to 100,000. These data suggest that the CF_3 group has an important role in the observed phenomenon. We think that the small size of the aggregates, possibly as a result of molecular geometry or dipole moment, may result in the observed nanoscopic reaction. Substitution of the CF_3 group by other termini (such as methyl, carboxyl, hydroxyl, vinyl, phenyl, and chromone) causes a substantial change in the wide-angle electron diffraction pattern. This pattern shows a much higher degree of order in the nanosized aggregates of molecules with



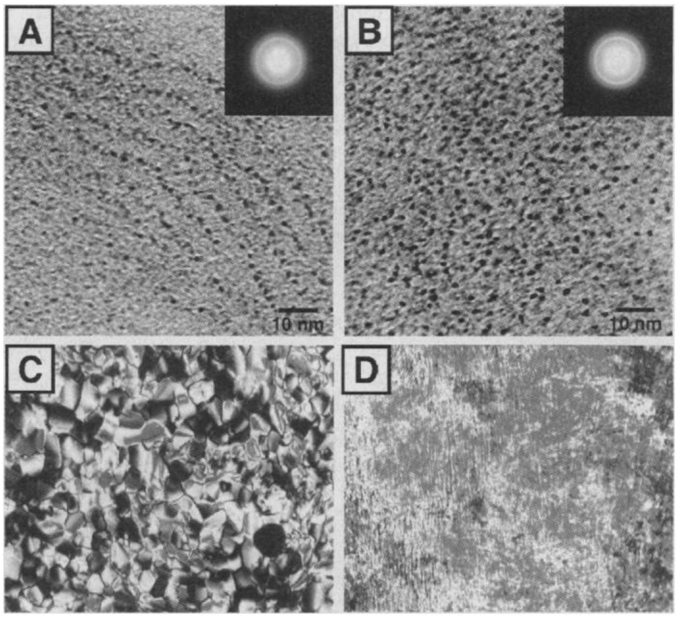


Fig. 2 (left). GPC traces of precursor **1** (blue curve) and the high molar mass product (red curve) isolated by flash chromatography after 8 hours. The molecular representation of the macromolecule contains 20 molecules of precursor **1** stitched by covalent bonds through butadiene blocks. **Fig. 3 (right).** (**A**) A TEM micrograph at high magnification of a film that was formed by regularly sized and shaped supramolecular clusters composed of triblock molecules **1.** (**B**) A TEM micrograph at high magnification of a film that was formed by the isolated molecular objects. The wide-angle electron diffraction patterns are shown in the upper right corner of (A) and (B). The contrast in the image is produced by osmium tetroxide that stains double bonds in polybutadiene segments and also by the crystalline order among rod segments based on the observed diffraction pattern. The diffraction contrast should be substantial, considering the intensity of the Debye rings in the pattern. (**C**) An optical micrograph between crossed polarizers showing the smectic to isotropic phase transition of precursor **1** at 330°C. (**D**) An optical micrograph between crossed polarizers showing the smectic texture of the macromolecular product at 420°C.

a CF_3-terminus. As a result, the spatial isolation of the butadiene block is only achieved in rodcoils with a CF_3-terminus, thus preventing the interconnection of nanostructures.

Nuclear magnetic resonance (NMR) measurements (*17*) revealed that conversion of the clusters into molecular objects by cross-linking is accompanied by a 70% loss of unsaturated bonds. According to the mechanism proposed for this reaction (*8–12*), each butadiene structural unit can produce only one cross-link. On the basis of this information, we ran a multilayered random walk simulation of the nanoscopic reaction using a program that was developed previously in our laboratory (*1*). The top view of the 2D projections of linked molecules for different numbers of layers is shown in Fig. 4. Matching the experimental observation, the cluster used in the simulation contained 25 molecules (5 molecule by 5 molecule lattice) and became completely stitched by covalent bonds with only six layers of reactive groups (corresponding to six butadiene units per molecule). Because the average number of layers in the experimental system is nine, the conversion of supramolecular clusters into polymer objects is feasible, on the basis of the simulation.

Additional information confirming the structure of the polymer objects was obtained by ^{1}H NMR experiments (*17*). On the basis of integration of the NMR spectrum of the cross-linked product, we estimated that annealing causes the formation of six cross-links per molecule of precursor **1**. This result implies that the cross-linking density within each macromolecule is very high and that this feature should contribute to the shape invariance of the formed objects. Furthermore, the relaxation time T_1 of the biphenyl protons increased from 1.5 to 2.9 s after polymerization, and this T_1 value is considerably greater than that of the aliphatic protons (1.1 s) and styrenic aromatic protons (1.4 s), whose T_1 values after polymerization remained constant within experimental error. The observation suggests that, even in solution, rod segments have the strongest interaction within the object. This interaction should also contribute to the shape persistence of the covalent objects as the solid material is dissolved.

Dynamic light scattering experiments for solutions in THF (0.1 weight %) only revealed evidence of nanostructures after cross-linking. Conversion of molecules **1** into covalent polymers results in a strong stabilization of the LC phase of the system. The initial precursor undergoes a phase transition from the smectic to the isotropic state at 330°C (Fig. 3C), whereas the polymerized objects form a highly birefringent melt at 160°C, and isotropization is never observed before chemical decomposition at 430°C (Fig. 3D). Differential scanning calorimetry indicated that, contrary to precursor **1**, the molecular object polymers do not exhibit any first-order phase transitions. The observation suggests that these mushroom-shaped objects cannot be stable in the isotropic state, possibly because their particular shape and surface chemical map would create an isotropic liquid of high free volume and fluctuations between favorable and very unfavorable contacts.

In nature, there is a mushroom-shaped object that is formed by a heptameric aggregate of protein molecules (*18*). This supramolecular structure, known as α-hemolysin, can implant itself into cell membranes, and it is synthesized by the human pathogen *Staphylococcus aureus*. This molecular aggregate also contains a pore to transport molecules and ions across cell membranes (*19*). In contrast to α-hemolysin, our cross-linked aggregate could function as an "undenaturable" molecular object polymer, and with proper modification, it could serve as a macromolecule that modifies cell behavior or creates interesting materials.

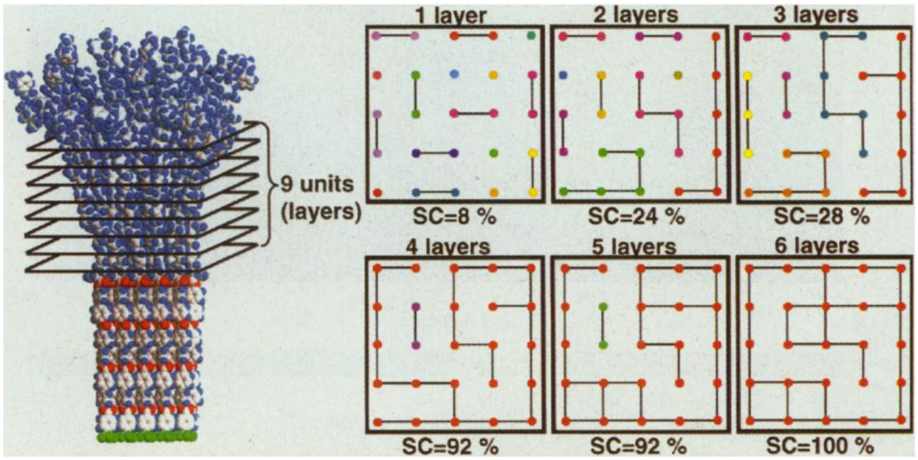


Fig. 4. Two-dimensional projections of molecules linked in the supramolecular cluster (right) based on a random walk computer simulation. The stitching conversion (SC), which is the percentage of covalently linked molecules in the cluster, increases with the number of reactive layers. The number of layers is equal to the number of butadiene structural units in precursor molecules (left).

References and Notes

1. S. I. Stupp *et al.*, *J. Am. Chem. Soc.* **117**, 5212 (1995).
2. M. Muthukumar, C. K. Ober, E. L. Thomas, *Science* **277**, 1225 (1997).
3. D. A. Tomalia and H. D. Durst, *Top. Curr. Chem.* **165**, 193 (1993); J. M. J. Fréchet, *Science* **263**, 1710 (1994); G. R. Newkome, C. N. Moorefield, G. R. Baker, A. L. Johnson, R. J. Behera, *Angew. Chem. Int. Ed. Engl.* **30**, 1176 (1991); J. S. Moore, J. Zhang, Z. Wu, D. Venkataraman, S. Lee, *Makromol. Chem. Macromol. Symp.* **77**, 295 (1994).
4. S. I. Stupp, S. Son, H. C. Lin, L. S. Li, *Science* **259**, 59 (1993).
5. S. I. Stupp *et al.*, *ibid.* **276**, 384 (1997).
6. J.-M. Lehn, *Supramolecular Chemistry* (VCH, New York, 1995); *Pure Appl. Chem.* **66**, 1961 (1994); S. C. Zimmerman, F. Zeng, D. E. C. Reichert, S. V. Kolotuchin, *Science* **271**, 1095 (1996); J. C. M. van Hest, D. A. P. Delnoye, M. W. P. L. Baars, M. H. P. van Genderen, E. W. Meijer, *ibid.* **268**, 1592 (1995); T. W. Bell, *ibid.* **271**, 1077 (1996); V. Percec, G. Johansson, G. Ungar, J. Zhou, *J. Am. Chem. Soc.* **118**, 9855 (1996); V. Percec, G. Johansson, J. Heck, G. Ungar, S. V. Batty, *J. Chem. Soc. Perkin Trans. I* **1**, 1411 (1993); G. R. Newkome, X. Lin, C. Yaxiong, G. H. Escamilla, *J. Org. Chem.* **58**, 3123 (1993).
7. E. R. Zubarev, L. Li, S. I. Stupp, in preparation.
8. D. Doskocilova, J. Straka, B. Schneider, *Polymer* **34**, 437 (1993).
9. I. C. McNeill, L. Ackerman, S. N. Gupta, *J. Polym. Sci. Polym. Chem. Ed.* **16**, 2169 (1978).
10. N. Grassie and A. Heaney, *Polym. Lett.* **12**, 89 (1974).
11. M. A. Golub and M. Sung, *ibid.* **11**, 129 (1973).
12. Thermal cross-linking of polybutadiene above 230°C in an inert atmosphere has been previously studied. Doskocilova *et al.* used high-resolution solid state ^{13}C NMR and suggested that cross-linking involves the reaction of a pendant vinyl group (1,2 units) with the methylene group in 1,4-*cis*-butadiene units (*8*). However, little is known about the actual mechanism of this reaction. Grassie and Heaney found that introducing typical free radical inhibitors does not affect the rate at which vinyl groups disappear (*10*). Therefore, it is thought that cross-linking proceeds through a molecular mechanism and does not involve radical formation. A probable mechanism for cross-linking, which involves an activated intermediate complex between the pendant vinyl group (1,2 unit) and the methylene group of a 1,4-*cis* unit, has been suggested. A hydrogen transfer reaction then results in the simultaneous formation of the covalent bond between two carbon atoms.
13. B. E. Smart, in *Organofluorine Chemistry: Principles and Commercial Applications*, R. E. Banks, B. E. Smart, J. C. Tatlow, Eds. (Plenum, New York, 1994), pp. 57–69; D. F. Eaton and B. E. Smart, *J. Am. Chem. Soc.* **112**, 2821 (1990).
14. An investigation of the annealed samples by NMR and GPC studies indicated that the cross-linking reaction starts at temperatures higher than 230°C. Annealing of precursor **1** at 220°C for 24 hours revealed no cross-linking of butadiene units, and the formation of molecular objects was not observed.
15. Samples were mounted onto a rotation/translation stage and the p- or s-polarized 1064-nm fundamental laser beam was tightly focused on the center of the sample, yielding a peak intensity of 5 MW/cm^2. The 1064-nm fundamental was produced by a Q-switched yttrium-aluminum-garnet-Nd laser with a 20-ns pulse width operating at a 20-Hz repetition rate. The second harmonic at 532 nm was separated from the fundamental by a series of green pass filters and a monochromator. The light was collected in a photomultiplier tube, and the signal was sent to a digital data acquisition system and an oscilloscope. All data were corrected for the transmission of the fundamental and the absorption of the second harmonic. Maker fringes were observed from cast films of the uniform thickness when the incident angle of the p- and s-polarized laser beam was being changed continuously. This result suggests that the films possess ∞mm symmetry and polar order. The sinusoidal fringes that were obtained by plotting the square root of the second harmonic intensity (obtained by scanning an s-polarized laser beam with an incident

angle of 40° across samples) against the thickness also indicated the polar nature of the material.

16. G. N. Tew, L. M. Li, S. I. Stupp, *J. Am. Chem. Soc.* **120**, 5601 (1998).
17. ^{1}H NMR spectra revealed the disappearance of 70% of the vinyl protons after annealing precursor **1** and after isolating the macromolecular product of narrow MW distribution. The disappearance of vinyl protons [δ4.8 to δ5.9 parts per million (ppm)] is accompanied by an increase of 20% in the number of aliphatic protons. These changes reflect the conversion of double to single bonds and are consistent with the expected values. The position of all the aromatic, aliphatic, and residual vinyl peaks were found to be the same after the transformation from precursor **1** to macromolecular objects. However, some broadening and suppression of aromatic resonances were observed (δ7.3 to δ8.5 ppm).
18. L. Song *et al.*, *Science* **274**, 1859 (1996); Y. Fang, S. Cheley, H. Bayley, J. Yang, *Biochemistry* **36**, 9518 (1997); S. Cheley *et al.*, *Protein Eng.* **10**, 1433 (1997).
19. B. Walker and H. Bayley, *J. Biol. Chem.* **270**, 23065 (1995); R. G. Panchal and H. Bayley, *ibid.*, p. 23072; H. Bayley, *Bioorg. Chem.* **23**, 340 (1995).

20. Supported by grants from the Office of Naval Research (N00014-96-1-0515), NSF (DMR 93-12601), the U.S. Department of Energy (DEFG02-91 ER45439, which was obtained through the Materials Research Laboratory of the University of Illinois), and Master Builders Technologies. We acknowledge the use of facilities at the University of Illinois Center for Electron Microscopy and the Visualization Laboratory of the Beckman Institute for Advanced Science and Technology.

26 August 1998; accepted 9 December 1998

Compensation of Horizontal Temperature and Salinity Gradients in the Ocean Mixed Layer

Daniel L. Rudnick* and Raffaele Ferrari

Establishment of the temperature-salinity relationship in the ocean has concerned oceanographers for decades because of its importance for understanding ocean circulation. High-resolution measurements in the ocean mixed layer are used to show that temperature and salinity gradients on horizontal scales of 20 meters to 10 kilometers tend to compensate in their effect on density. These observations support the notion of a horizontal mixing in the mixed layer that depends on density gradient.

Temperature and salinity are the two most important physical properties of seawater. First, temperature and salinity determine the dynamic variable density at fixed pressure. Second, our understanding of the general circulation of the ocean is based to a large extent on the use of temperature and salinity as tracers to infer the flow. The temperature-salinity relationship has therefore been an object of study since the early days of oceanography. Iselin (*1*) noticed the correspondence between horizontal profiles in the surface mixed layer (*2*) and vertical profiles in the thermocline (*3*) and concluded that subsurface water is derived from late winter surface water. Properties set in the winter mixed layer remain essentially unchanged as water is transported along surfaces of constant density (*4, 5*). Direct observations of small vertical diffusivities in the thermocline (*6, 7*) support the idea that water properties are set at the ocean's surface and bottom boundaries. The question of how the temperature-salinity relationship is established in the mixed layer is thus of central importance to oceanography. Further, this relationship is of climatic importance because coupling of the atmosphere and ocean takes place through the mixed layer, and it is of biological importance because biomass in the ocean is concentrated in the upper ocean where sufficient sunlight is available for photosynthesis.

A measure of the relative effect of temperature and salinity on density is the density ratio

$$R \equiv \frac{\alpha\Delta\theta}{\beta\Delta S} \qquad (1)$$

where α and β are the expansion coefficients for temperature and salinity, and the temperature and salinity differences $\Delta\theta$ and ΔS are taken over a spatial interval. A horizontal front that is cold and fresh on one side and warm and salty on the other, so that temperature and salinity have opposing effects on density, is often said to be compensated, having a density ratio of 1. The phenomenon of compensation is well known at certain fronts (*8, 9*). A front where the effect of temperature on density is twice (but opposite) that of salinity, and hence density varies across the front, has a density ratio of 2.

The last published works of Stommel (*10, 11*) suggest a regulating mechanism for the temperature-salinity relationship in the mixed layer. Stommel observes that the mixed-layer

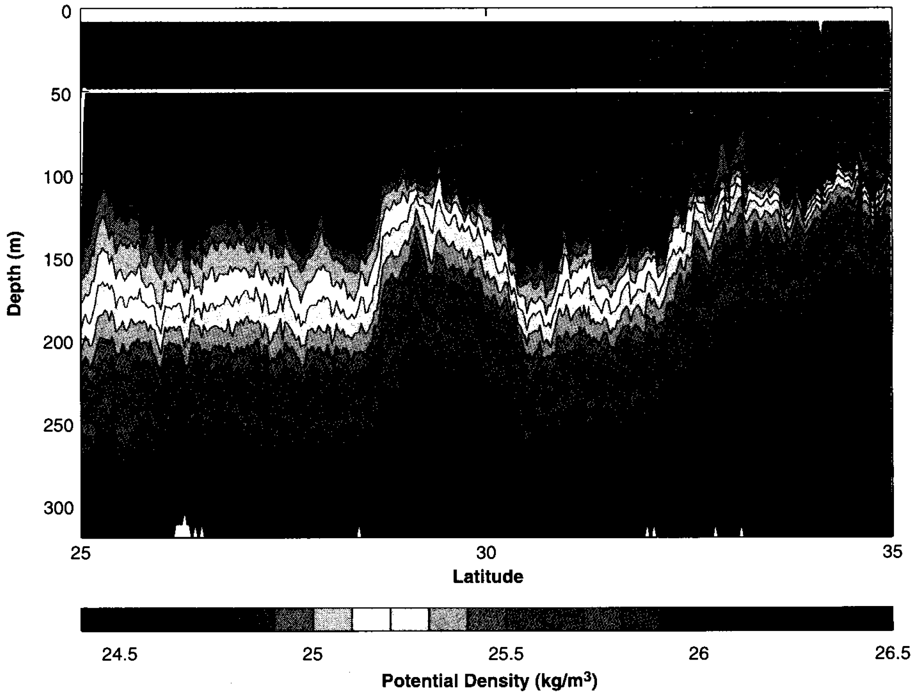


Fig. 1. Potential density section along 140°W as measured by a sawtooth SeaSoar tow. Data are averaged in bins 3 km in the horizontal by 8 m in the vertical before contouring. The white line is a level tow along the 50-dbar surface. The mixed layer as defined by a 0.1 kg m^{-3} difference from the shallowest measurement is indicated by the black line. Mixed-layer depth varies between 100 and 150 dbar, so 50 dbar is always within the mixed layer.

Scripps Institution of Oceanography, La Jolla, CA 92093–0230, USA.

*To whom correspondence should be addressed. E-mail: drudnick@ucsd.edu