ACS Macro Letters

Letter

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Thiol-Functionalized Block Copolymer Vesicles

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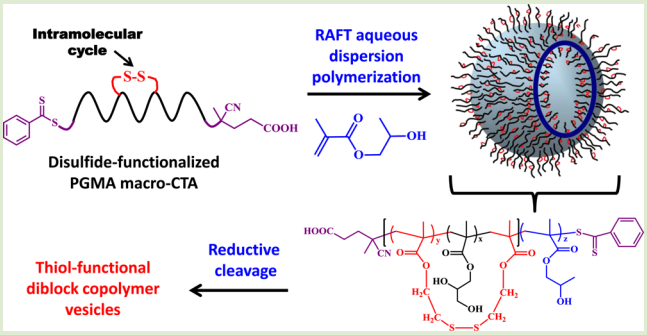
Supporting Information

ABSTRACT: Thiol-functionalized block copolymer vesicles are readily prepared via RAFT-mediated polymerization-induced self-assembly (PISA). More specifically, a disulfide-functionalized poly(glycerol monomethacrylate) macro-CTA is chain-extended using 2-hydroxypropyl methacrylate): the growing water-insoluble poly(2-hydroxypropyl methacrylate) chains drive in situ self-assembly to produce diblock copolymer vesicles in concentrated aqueous solution. The disulfide bonds in the poly(glycerol monomethacrylate) stabilizer chains are reductively cleaved in situ using either tributyl phosphine or tris(2-carboxyethyl)phosphine to generate thiol groups, which react immediately with either a quaternary acrylate to introduce cationic character or with rhodamine B acrylate or rhodamine B isothiocyanate to confer a convenient fluorescent tag. In addition to such facile derivatization, such thiol-functionalized vesicles may offer some potential for drug delivery applications, because enhanced muco-adhesion is anticipated for these nano-objects.



Thiol-disulfide chemistry is of considerable importance, because it confers covalent stabilization of the supramolecular structures of various proteins such as keratin, which is a major constituent of hair, nails and hooves.[1] From the viewpoint of synthetic polymer chemistry, there is increasing interest in exploiting thiol-disulfide chemistry because it offers orthogonality, reversibility, and redox activity.[2−7] Such attributes are potentially useful for various biomedical applications.[8−11] Fundamental "proof-of-structure" studies are also feasible. For example, we have recently shown that a disulfide-based dimethacrylate (DSDMA) comonomer is useful for the synthesis of branched vinyl copolymers via living radical polymerization, because selective cleavage of the disulfide bond under mild conditions provides compelling evidence that the polydisperse branched copolymer comprises randomly coupled near-monodisperse primary chains.[12−14] Recently, we realized that DSDMA can act as an atom-efficient protecting group for thiol functionality, which cannot be used directly in radical-based vinyl polymerizations because it acts as a highly efficient chain transfer agent.[15] Moreover, we found that DSDMA undergoes predominantly intramolecular cyclization (rather than intermolecular branching) when statistically copolymerized with a monovinyl monomer such as methyl methacrylate in relatively dilute solution.[16] Thus, it is feasible to prepare a DSDMA-containing methacrylic copolymer with minimal branching. This is important in the context of polymerization-induced self-assembly (PISA),[17−27] because linear polymer chains are expected to have better packing efficiencies within coronal stabilizer layers. Herein we have extended our recent studies[17−22] of the formation of block copolymer "nano-objects" via PISA to produce thiol-functional vesicles using DSDMA-containing block copolymers. Post-polymerization derivatization of these vesicles can be conveniently achieved in aqueous solution so as to introduce either cationic character or fluorescent labels for confocal microscopy studies. Moreover, thiol-functional vesicles are expected to exhibit enhanced muco-adhesion,[28] which suggests potential drug delivery applications.

It is well-known that relatively asymmetric amphiphilic diblock copolymers can self-assemble to form vesicles (aka polymersomes) in aqueous solution provided that the volume fraction of the hydrophobic block is sufficiently large.[29−31] Unlike surfactant-based liposomes, block copolymer vesicles have thicker, more resilient membranes, which can lead to longer circulation times if appropriate surface functionality is conferred, such as PEGylation.[32,33] This has led to a number of biomedical applications being suggested for these nano-objects, including delivery vehicles,[34−39] nanoreactors,[40,41] or enhanced contrast agents for magnetic resonance imaging.[42,43] In principle, controlling vesicle surface chemistry is also a prerequisite for targeting specific organs or biomolecules.[44−48] Desirable surface functionality includes amine or avidin groups, because these allow facile conjugation of oligopeptides and proteins.[46,48] Hillmyer et al. have recently reported the

Received: June 22, 2012
Accepted: July 25, 2012
Published: July 30, 2012

   dx.doi.org/10.1021/mz300318a | *ACS Macro Lett.* 2012, 1, 1041−1045

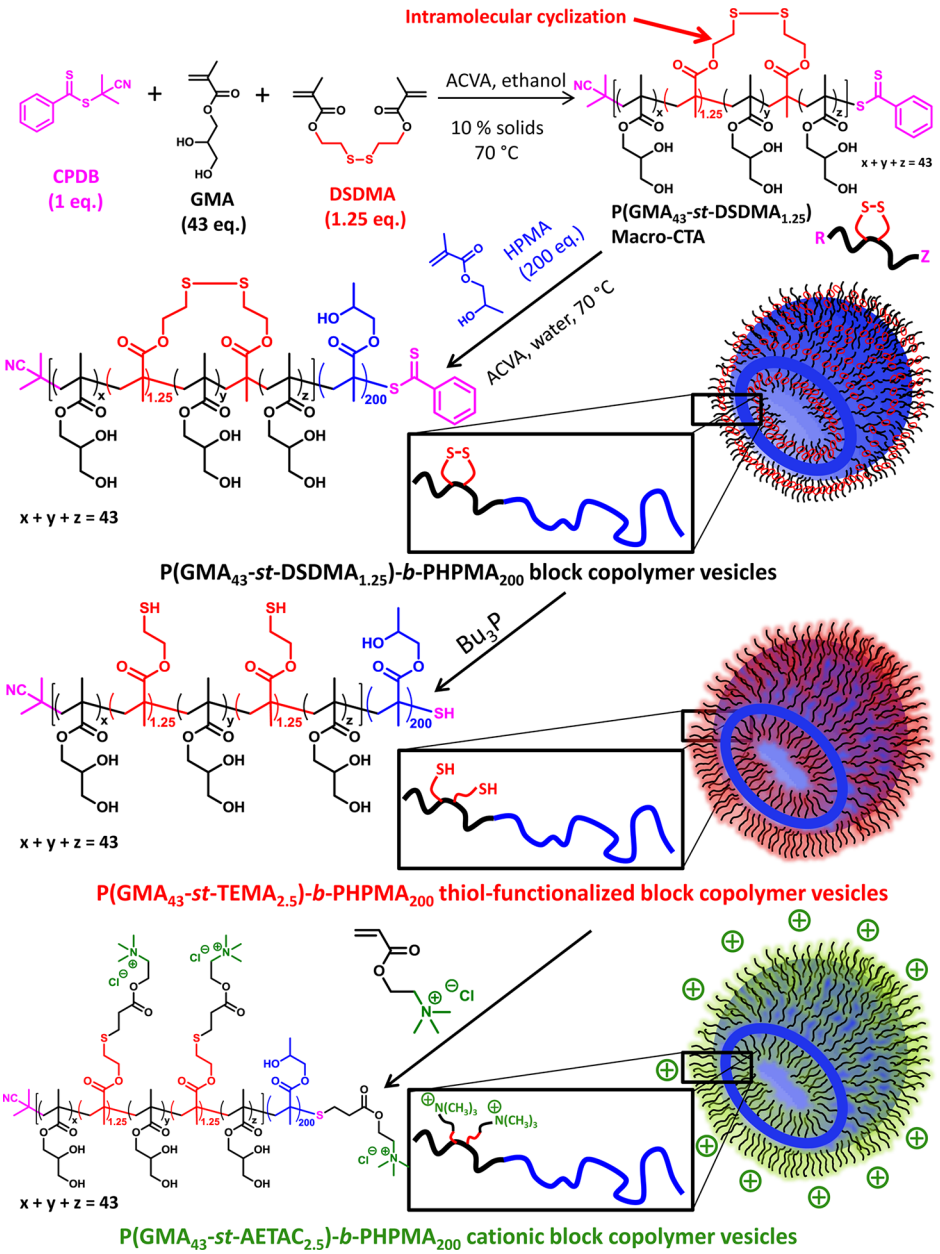


Figure 1. RAFT synthesis of thiol-decorated block copolymer vesicles and their subsequent derivatization. (1) Statistical copolymerization of GMA and DSDMA via RAFT using CPDB chain transfer agent and ACVA initiator in ethanol at 70 °C. Conditions: $[GMA]_0$ = 10 w/w %; [GMA]/[DSDMA]/[CPDB]/[ACVA] relative molar ratios = 40:1.25:1.0:0.25. (2) The resulting linear $PGMA_{43}$-$DSDMA_{1.25}$ macro-CTA was then utilized for the RAFT aqueous dispersion polymerization of HPMA at 70 °C at 10 w/w % solids to produce disulfide-functionalized block copolymer vesicles. (3) Reductive cleavage of the disulfide bonds in the stabilizer chains affords thiol groups that readily react with 2-[(acryloyloxy)ethyl] trimethylammonium chloride (AETAC) to produce cationic vesicles or react with either rhodamine B acrylate or rhodamine B isothiocyanate to produce fluorescent vesicles.

synthesis of peptide-conjugated vesicles using thiol chemistry, but in this case, the thiol moiety was located on the peptide rather than on the vesicles.[49] As far as we are aware, there have been few, if any, reports of *thiol-decorated* vesicles being utilized in this context. Following our recent work on the microstructure of branched copolymers,[16] we realized that the propensity of a divinyl monomer (DSDMA) to undergo intramolecular cyclization when copolymerized with a monovinyl comonomer under certain conditions offered a facile, atom-efficient route for the preparation of thiol-functional nano-objects via disulfide-functional precursors.

More specifically, reversible addition−fragmentation chain transfer (RAFT) polymerization[50−52] was utilized to statistically copolymerize glycerol monomethacrylate (GMA) with a small amount of DSDMA at 10 % w/w solids in ethanol to produce a $P(GMA_{43}$-*stat*-$DSDMA_{1.25})$ macro-CTA, see Figure 1. DMF GPC analysis (vs poly(methyl methacrylate) calibration standards) of this disulfide-containing macro-CTA indicated a relatively low polydispersity (see Figure 2), which confirmed that the DSDMA comonomer had reacted almost exclusively intramolecularly with very little branching, as expected.[16] Cleavage of the disulfide bonds in this macro-CTA using excess tributylphosphine led to a modest narrowing of the

dx.doi.org/10.1021/mz300318a | *ACS Macro Lett.* 2012, 1, 1041−1045

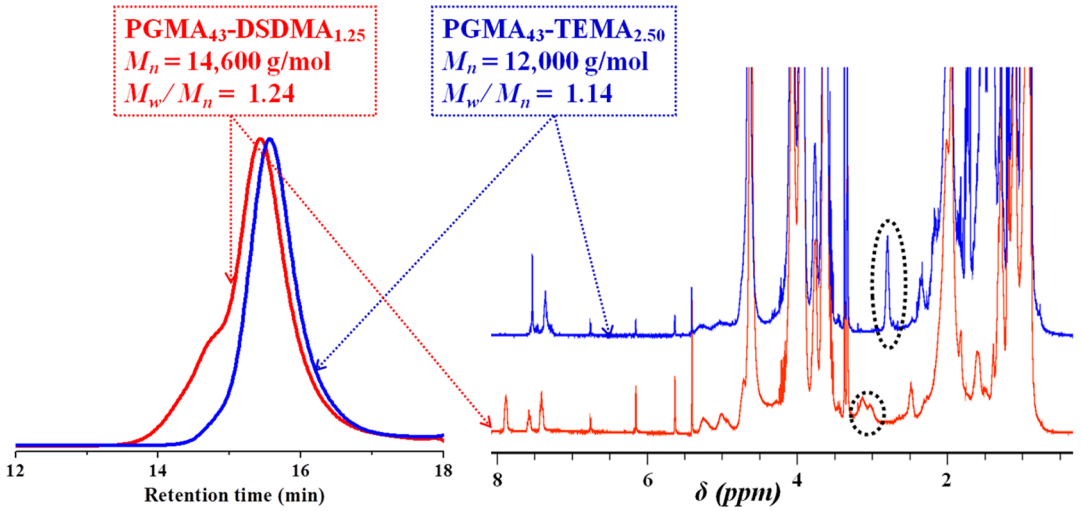


Figure 2. Left: DMF GPC curves obtained for the P(GMA_{43}-$DSDMA_{1.25}$) macro-CTA before and after disulfide cleavage using tributyl phosphine. Note the modest reduction in M_n and M_w/M_n for the thiol-functional derivative due to the elimination of the relatively light branching that is present in this macro-CTA. Right: ^{1}H NMR spectra (CD_3OD) recorded for the same macro-CTA and its corresponding thiol-functional derivative, which confirms the formation of the 2-thioethyl methacrylate (TEMA) residues, as expected.

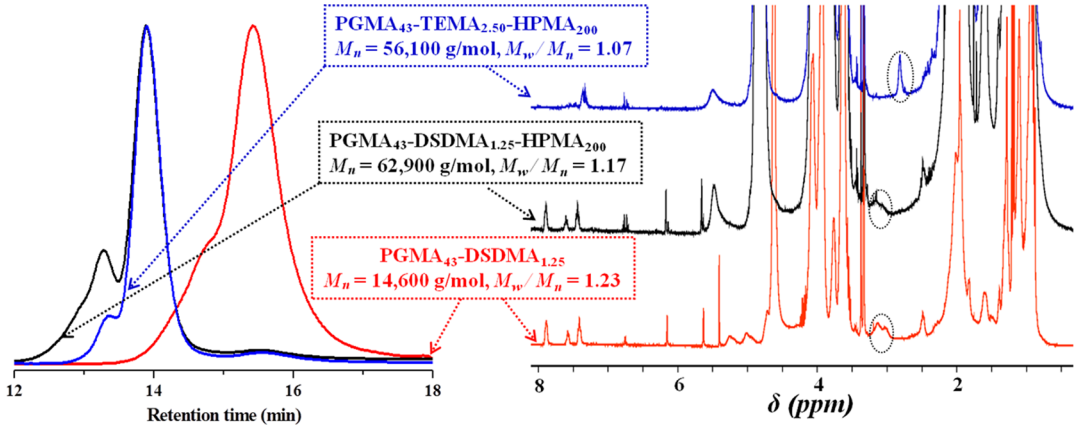


Figure 3. DMF GPC curves (left) and ^{1}H NMR spectra (right; recorded in CD_3OD) obtained for the P(GMA_{43}-$DSDMA_{1.25}$) macro-CTA (red curve), the P(GMA_{43}-$DSDMA_{1.25}$)-*b*-$PHPMA_{200}$ diblock copolymer (black curve) and the corresponding P(GMA_{43}-$TEMA_{2.50}$)-*b*-$PHPMA_{200}$ diblock copolymer obtained after disulfide cleavage (within the aqueous vesicle dispersion) using tributyl phosphine (blue curve).

molecular weight distribution: the M_w/M_n is reduced from 1.24 to 1.14 for the resulting P(GMA_{43}-*stat*-$TEMA_{2.5}$), where TEMA denotes 2-thioethyl methacrylate residues. The success of this reaction was confirmed by ^{1}H NMR studies (see Figure 2), because the broad signal at 2.9−3.2 ppm due to the thiamethylene protons of the polymerized DSDMA residues disappears and is replaced by a sharp signal at around 2.75 ppm due to the HS-C$\underline{H}_2$ methylene protons of the TEMA residues.[16]

This macro-CTA was then chain-extended with 2-hydroxypropyl methacrylate (HPMA) under aqueous dispersion polymerization conditions[17,18] to produce P(GMA_{43}-*stat*-$DSDMA_{1.25}$)-*block*-$PHPMA_{200}$ diblock copolymer vesicles (see Figure 1). Vesicle phase space was achieved by targeting an appropriately high degree of polymerization (DP) for the membrane-forming PHPMA chains. Reproducible syntheses of this type require a detailed knowledge of the phase diagram for a given macro-CTA type and mean DP of the hydrophobic block and also the total solids concentration at which the HPMA polymerization is conducted. Without this prior knowledge, there is always a risk that ill-defined mixed phase regions comprising vesicles and worms may be inadvertently targeted. The comprehensive phase diagram study that informed the present work has been published elsewhere.[53] The GPC curves shown in Figure 3 confirm a high blocking efficiency (>95%) for the P(GMA_{43}-*stat*-$DSDMA_{1.25}$) macro-CTA and low polydispersities for both the disulfide-functionalized P(GMA_{43}-*stat*-$DSDMA_{1.25}$)-*block*-$PHPMA_{200}$ diblock precursor (M_w/M_n = 1.17) and the final thiol-functionalized P(GMA_{43}-*stat*-$TEMA_{2.5}$)-*block*-$PHPMA_{200}$ copolymer (M_w/M_n = 1.07). The corresponding ^{1}H NMR spectra again confirms the efficient cleavage of all the disulfide bonds on addition of excess tributyl phosphine so as to generate thiol (TEMA) moieties on the PGMA stabilizer chains. Because the ^{1}H NMR analysis is conducted in CD_3OD, the copolymer chains are molecularly dissolved under these conditions. However, the disulfide cleavage is actually conducted in aqueous solution, which produces thiol groups on the PGMA-based stabilizer chains expressed at the outer and inner leaflets of the vesicle membrane.

A transmission electron micrograph of linear disulfide-functionalized P(GMA_{43}-*stat*-$DSDMA_{1.25}$)-*block*-$PHPMA_{200}$ di-

dx.doi.org/10.1021/mz300318a | *ACS Macro Lett.* 2012, 1, 1041−1045

block copolymer vesicles prepared via this route is shown in Figure 4a. The characteristic spherical, polydisperse nature of

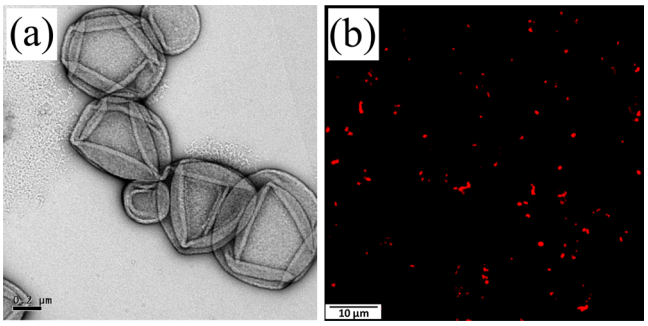


Figure 4. (a) Transmission electron micrograph obtained for P(GMA_{43}-*stat*-$DSDMA_{1.25}$)-*b*-$PHPMA_{200}$ vesicles prepared by RAFT aqueous dispersion polymerization at 70 °C. (b) Fluorescence microscopy image obtained for the corresponding rhodamine-labeled vesicles produced after reductive disulfide cleavage followed by in situ addition of a reactive rhodamine B label.

these nano-objects is clearly evident and pronounced buckling has occurred under the high vacuum conditions required for sample imaging. Moreover, no evidence for any contamination of other diblock copolymer phases, for example, worms or spheres, was found in electron micrographs recorded at lower magnifications (images not shown). DLS studies of a dilute aqueous dispersion of these vesicles indicated an intensity-average diameter of 565 nm and a relatively broad size distribution (polydispersity = 0.15). Crosslinked thiol-functional vesicles were also readily prepared by the addition of ethylene glycol dimethacrylate (EGDMA) after the HPMA polymerization. This bifunctional crosslinker locks in the vesicle superstructure, enabling these nano-objects to survive a challenge with excess methanol. In contrast, the corresponding linear copolymer vesicles are completely destroyed by this alcohol challenge, because methanol is a good solvent for the hydrophobic membrane-forming PHPMA chains. EGDMA cross-linking also causes a subtle change in morphology, with nanophase separation occurring within the vesicular membrane, see Supporting Information, Figure S1. We have recently reported similar observations for cross-linked vesicles prepared using epoxy-amine chemistry.[21] In the present case, EGDMA cross-linking presumably converts the linear diblock copolymer chains into star-like diblocks, which leads to the evolution of granularity on the nanoscale.

A fluorescence microscopy image of rhodamine-labeled vesicles prepared by reacting the thiol-functional vesicles with the rhodamine B isothiocyanate reagent[54] is shown in Figure 4b. The vesicle dimensions are quite close to the detection limit of the instrument. Nevertheless, the polydisperse nature of the vesicles and their (mainly) submicrometer dimensions is apparent. We have also obtained video clips of such vesicles diffusing across the field of view (data not shown).

Finally, the thiol functionality can also be exploited to confer weakly cationic character on the vesicles. Conducting a thia-Michael addition reaction using a commercial cationic acrylic reagent, 2-[(acryloyloxy)ethyl] trimethylammonium chloride (AETAC), leads to a significant change in the electrophoretic footprint of the vesicles, see Figure 5. Both the original disulfide-based P(GMA_{43}-*stat*-$DSDMA_{1.25}$)-*block*-$PHPMA_{200}$ copolymer vesicles and also corresponding the thiol-functional P(GMA_{43}-*stat*-$TEMA_{2.5}$)-*block*-$PHPMA_{200}$ vesicles exhibit sole-

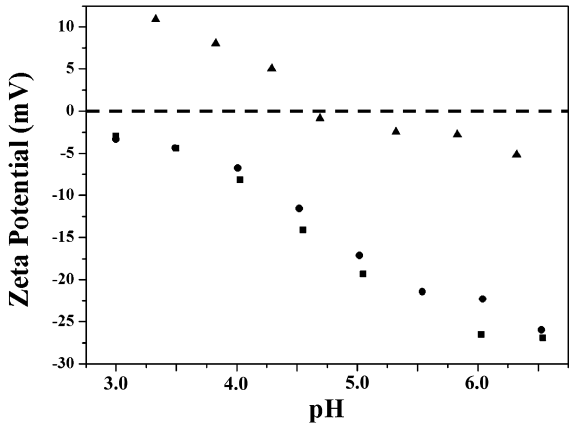


Figure 5. Zeta potential vs pH curves obtained for (■) the disulfide-functionalized diblock copolymer vesicle precursor; (●) the corresponding thiol-functionalized diblock copolymer vesicles after reaction with excess tributylphosphine; (▲) the final weakly cationic vesicles obtained after in situ reaction of the 2-[(acryloyloxy)ethyl] trimethylammonium chloride reagent with the surface thiol groups via thia-Michael addition.

ly negative zeta potentials across the pH range studied, whereas the AETAC-derivatized vesicles possess an isoelectric point at around pH 4.3, with zeta potentials as high as +11 mV being observed at around pH 3.3. TEM studies after AETAC derivatization confirm retention of the vesicular morphology after disulfide cleavage and thia-Michael addition (see Figure S2 in the Supporting Information). This surface functionalization is expected to affect the adsorption behavior of the vesicles, since it should promote stronger electrostatic interactions with oppositely charged solid surfaces (e.g., silica, mica, glass, etc.) and perhaps also with the air/water interface, which is believed to possess both anionic and hydrophobic character.[55] Thus interfacial adsorption studies will be explored in future work.

In summary, intramolecular cyclization of a disulfide-based dimethacrylate during its statistical copolymerization with glycerol monomethacrylate using RAFT chemistry is demonstrated to be an atom-efficient route for introducing latent thiol functionality. This disulfide-functional macro-CTA can be utilized to polymerize 2-hydroxypropyl methacrylate in concentrated aqueous solution and hence prepare diblock copolymer vesicles via RAFT-mediated polymerization-induced self-assembly (PISA). The disulfide bonds within the poly(glycerol monomethacrylate) stabilizer chains may be reductively cleaved to generate thiol groups, which can be reacted in situ with functional acrylates or isothiocyanates to introduce either cationic character or a convenient fluorescent tag, respectively. Such thiol-functionalized vesicles may offer some potential in the context of drug delivery because enhanced muco-adhesion is anticipated for these novel "nano-objects".

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full experimental section and TEM images of both AETAC-derivatized and EGDMA-cross-linked vesicles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

S.P.A. acknowledges EPSRC for postdoctoral support of A.B. and J.R. (Platform Grant EP/J007846/1). Yorkshire Forward is also thanked for partial support of P.C.

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