SUPPORTING INFORMATION

Water Soluble Nanoparticles from PEG based Cationic Hyperbranched Polymer and RNA That Protects RNA from Enzymatic Degradation

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Experimental Section

RNA isolation: Total cellular RNA was isolated from yeast cells using a slightly modified hot phenol method. Briefly, cells were harvested by centrifugation at 3000 r.p.m for 5 mins, rinsed in water and lysed in the presence of glass beads and lysis buffer (50 mM Tris.Cl, pH 7.0, 1 mM EDTA and 1% SDS) by alternating ten rounds of vigorous vortexing with incubation on ice for 30 secs each. Water saturated phenol at 65 °C was used for phenol extraction followed by extraction with chloroform and precipitation in 0.5 M LiCl and 100% chilled ethanol. RNA precipitate was pelleted by centrifugation at 12,000 rpm for 15 mins, washed with 70% alcohol dried and resuspended in water.

Preperation of RNA/HP Complexes: Molar amine content of HP was taken as positive units of HP and Molar phosphate content of RNAwas taken as negative units of RNA Complexes of HP and RNA were obtained by mixing aqueous solutions of HP and RNA at different positive units of HP vs negative unit of RNA or charge ratios ($Z_{+/-}$).

Gel Electrophoresis: The electrophoretic mobility of the polymer/RNA complexes at different polycation/RNA ratios was determined by gel electrophoresis using 1.2% agarose gel containing 5% formaldehayde and 0.5 μg/ml ethidium bromide at 120V. Electrophoresis buffer consisted 50 mM Sodium acetate, 10 mM EDTA and 0.2 mM MOPS, pH 7. The gels were visualized under UV illumination and gel pictures were taken by a Gel Doc system (Biorad).

Atomic Force Microscopy: Complexes at different $Z_{+/-}$ were prepared as described above. 2-3 μl of each complex was deposited on a freshly split untreated mica strip

(Molecular Imaging, Tempe, AZ), and allowed to adsorb for 5 min at room temperature. The mica surface was then imaged using a PicoSPM system (Molecular Imaging) operating in MAC mode. A 225 μm long magnetically coated cantilever (MAC lever) with a spring constant of 2.8 N/m and a resonance frequency of 65 kHz was used. The cantilever is made to oscillate due to the magnetic force resulting from the solenoid placed under the sample plate. The image is generated by the change in amplitude of the free oscillation of the cantilever as it interacts with the sample.

Nuclease Resistance of RNA within the Complexes: Total cellular RNA (35 μM) and different amount of polymer were mixed as described above to prepare RNA/HP complexes of different $Z_{+/-}$. After addition of 10 μg of RNase to 1 mL of complex solution at 25 °C, the absorbance change at 260 nm was monitored in order to follow RNA degradation by RNase, using an UV/visible spectrophotometer(Cary 400, Varian)

Synthesis and characterization of the hyperbranched polymer

Experimental part

Materials. All reagents were purchased from Aldrich and used as received except the following, Glycidol (96 %) was purified by vacuum distillation and stored in a refrigerator (2-4 °C). 1,1,1- Tris(hydroxymethyl) propane (Fluka) and potassium methylate solution (25 wt % in methanol, Fluka) were used as such. Epoxide terminated poly(ethylene glycol) monomethyl ether was synthesized by reaction of MPEG(Mn - 350), sodium hydroxide and epichlorohydrin.[1] Molecular weights, intrinsic viscosity [η], hydrodynamic radii (R_h) and radii of gyration (R_g) were determined by GPC with a triple detector and DAWN-EOS multi angle detector in aqueous 0.1N $NaNO_3$ solution; the

details have been described earlier.[2] The dn/dc value for polyglycidol was determined to be 0.12 cm^3/g and was used for the calculation of molecular weight of other polymers.

Synthesis of copolymer of hyperbranched polyglycidol and PEG

Polymerizations were carried out in a three-necked glass reactor equipped with a mechanical stirrer and a syringe pump under argon atmosphere. Tris(hydroxymethyl)propane (TMP, 120 mg) was stirred with 0.2 mL potassium methylate solution and excess methanol was removed under vacuo at 50 °C. Glycidol (5 mL) was added dropwise at 95 °C over 10 hrs. The ratio of glycidol to TMP corresponds to a degree of polymerization of 100. After the addition of glycidol, the mixture was stirred for additional 2 hrs, after which 20 mL MPEG-epoxide was added dropwise over 12 hrs. The mixture was stirred for an additional 3 h. The viscous polymer was dissolved in methanol and passed though cation exchanges resin to remove potassium ions. Polymer was precipitated twice from diethyl ether to remove unreacted PEG-epoxide and subsequently dried at 70 °C in vacuo. Yield was 85 %.

GPC analysis (non-dilaysed product): Mn- 53000 , Mw/Mn- 6.01

^{1}HNMR analysis in d_6-DMSO (Figure S1)

PG-PEG: δ 3.2 (OCH_3 groups from PEG) , 3.25-3.8 (broad, peaks from glycidol and PEG main chain protons), 4.2-5.0 (broad , peaks from hydroxyl groups)

Amine terminated PG-PEG (PG-PEG-amine)

PG-PEG (16 gm) dissolved in 100 mL THF was reacted with methane sulfonyl chloride (1.17 mL, ~ 20 % of OH groups) in presence of triethylamine (3 mL) for 12 hrs. The salts were filtered off and polymer was isolated by precipitation in ether. The dried polymer was dissolved in 100 mL dioxane to which 40 mL Tris(2-aminoethyl)amine was added and refluxed for 24 hours. Dioxane was removed by rotary evaporation. Solid then was dissolved in minimum amount of methanol and the polymer were twice precipitated in diethyl ether. The obtained polymer (15 gm) was dissolved in 100 ml water and added to stirred solution formic acid (90 % w/w) and formaldehyde(37 % w/w)(15 each) at 0 °C. The reaction mixture was refluxed at 95 °C overnight. The volatiles were removed in vacuo. Polymer was extracted with dichloromethane after adjusting the pH of the aqueous solution to 10 with sodium hydroxide. Finally, the polymer was purified by dialysis using regenerated cellulose acetate membrane (MWCO 1000). Yield 10g. The product is characterized by ^{1}HNMR, GPC analysis and conductometric titrations. We were not able to calculate the absolute amine content of the polymer from ^{1}HNMR analysis as intensity of peaks varied largely upon changing the solvent as shown in the Figure S1. So we adopted a conductometric titration method using HCl and NaOH for this purpose. Representation of reactions occurs during conductometric titrations are given in the Scheme S1 and conductometric titration curves are given in the Figure S2. Values obtained from conductometric titrations agree very well with the charge neutralization point as measured by DNA binding (see main text).

^{1}HNMR analysis: d_6-DMSO

PG-PEG mesylate: (Figure S2) δ 3.12 (methyl from mesylate), 3.2 (-OCH3 from PEG), 3.25-3.8 (broad- peaks from glycidol and PEG main chain protons), 4.2-5.0 (broad, peaks from hydroxyl groups) : Approximately 20 % of the hydroxyl groups have been converted to mesylate group as calculated from the proton intensity.)

PG-PEG-amine: (Figure S3) δ 2.08 (-N-CH_3), 2.16 & 2.22 (N-CH_2-), 3.20 (-OCH_3, from PEG), 3.25-3.8 (broad, peaks from glycidol and PEG main chain protons), 4.2-5.0 (broad , peaks from hydroxyl groups)

GPC Analysis (dialyzed final product): Mn- 116700, Mw/Mn- 1.72 Rg- 24.5 nm

Synthesis of Quaternized amines:

The tertiary amine groups in PG-PEG amine were quaternized using ethyl bromide. For a typical reaction PG-PEG amine (1.2 g) was dissolved in acetonitrile (16 ml) and methanol (8 ml) and ethyl bromide (150 mg) was added. The solution was refluxed over night and the solvent was removed in a rotary evaporator. The final product is dissolved in water and freeze dried and is characterized by HNMR and conductometric titrations. Percentage of quaternization was calculated by ^{1}HNMR (**Figure S4**) and from conductometric titrations of the quaternized amine product and it was observed from NMR and conductometric titration (shown bellow) that all the tertiary amine was

converted to quaternized group. We took the advantage of the protonation behavior of tertiary amine compared to quaternary ammonium salt in the case of conductometric titrations (**Figure S5**).

Figure S1: ^{1}HNMR spectra of PG-PEG polymers in d_6-DMSO

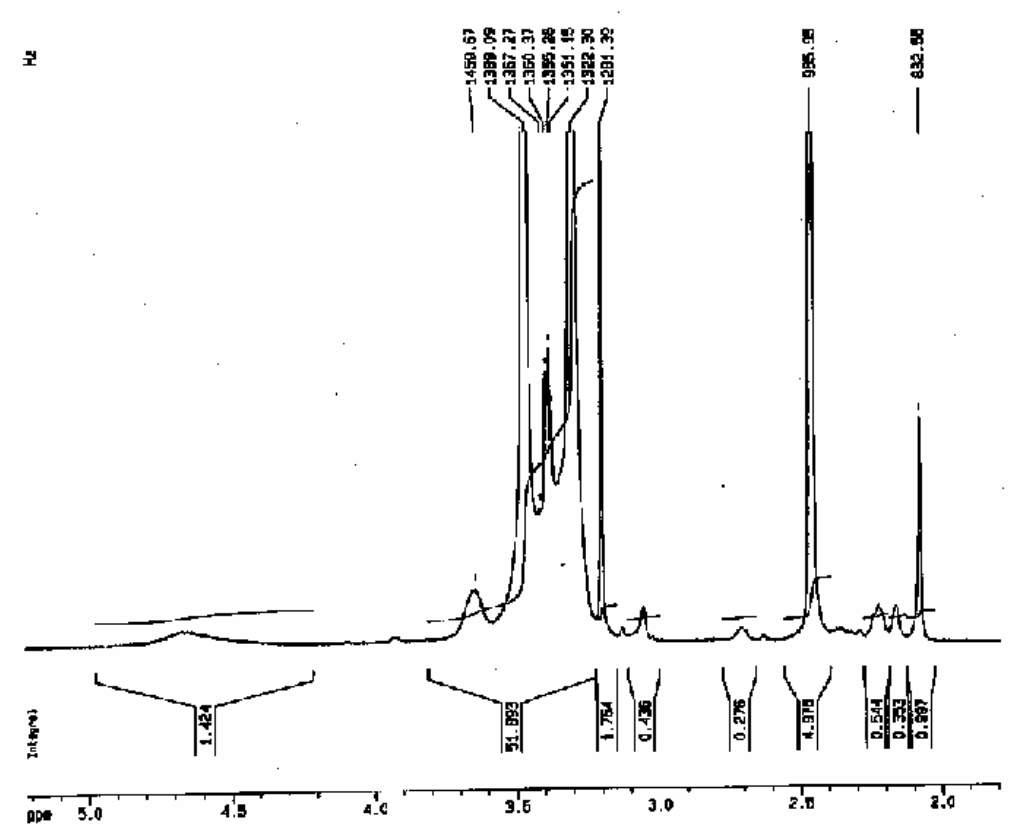


Figure S2: **^{1}HNMR spectra of PG-PEG mesylate polymers in d_6-DMSO**

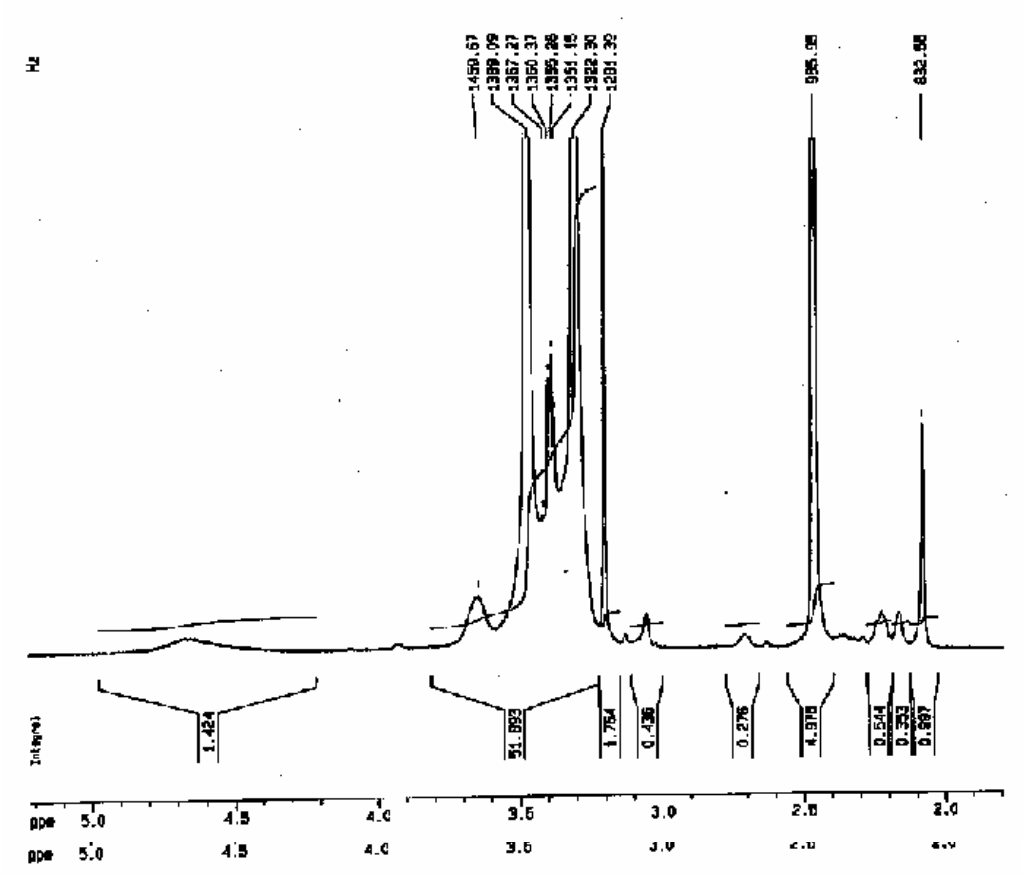


Figure S3: ^{1}HNMR spectra of Hyperbranched PG-PEG amine in d_6-DMSO.

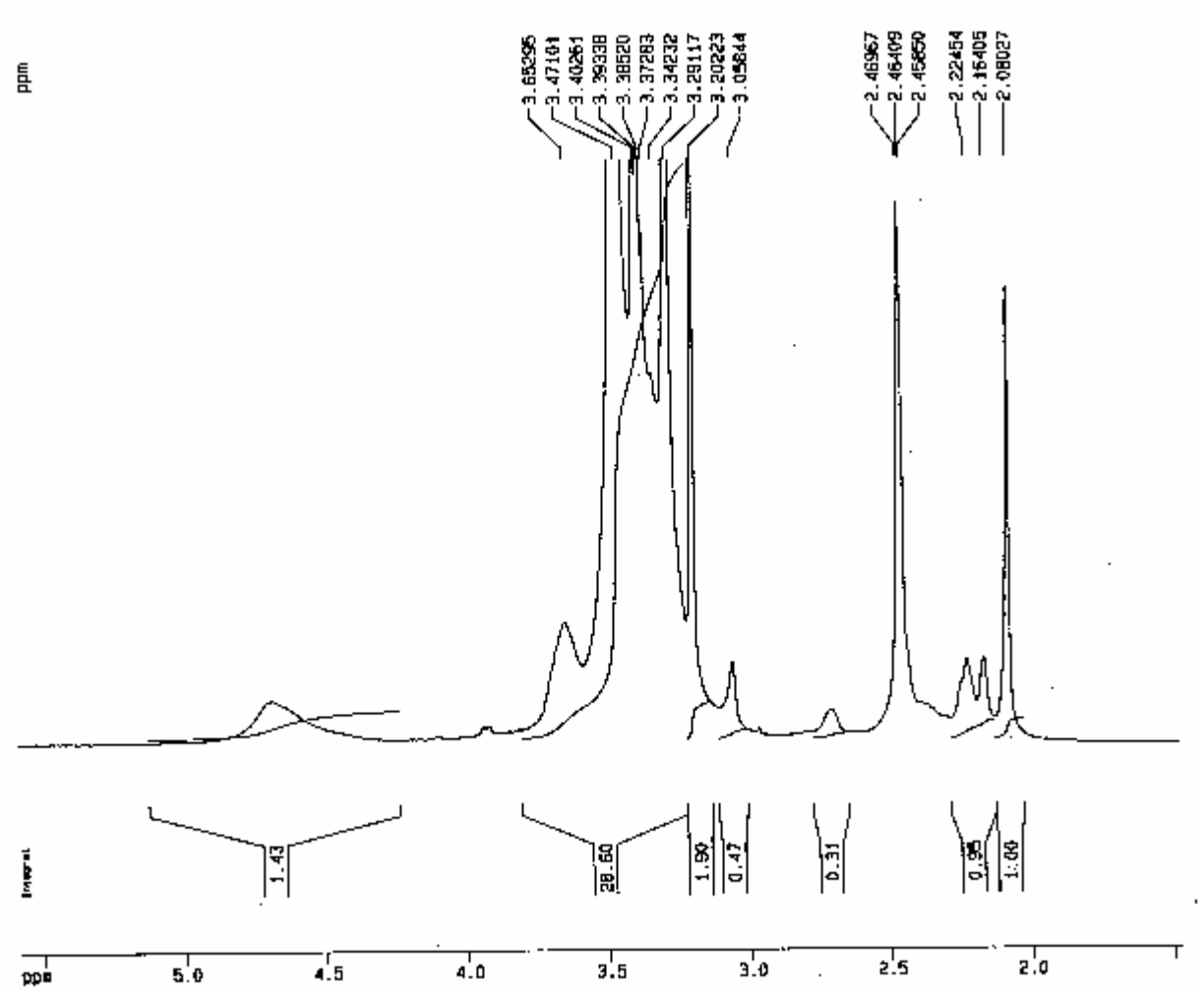


Figure S4: ^{1}HNMR spectra of PG-PEG amine and quaternized polymers in d_6-DMSO.

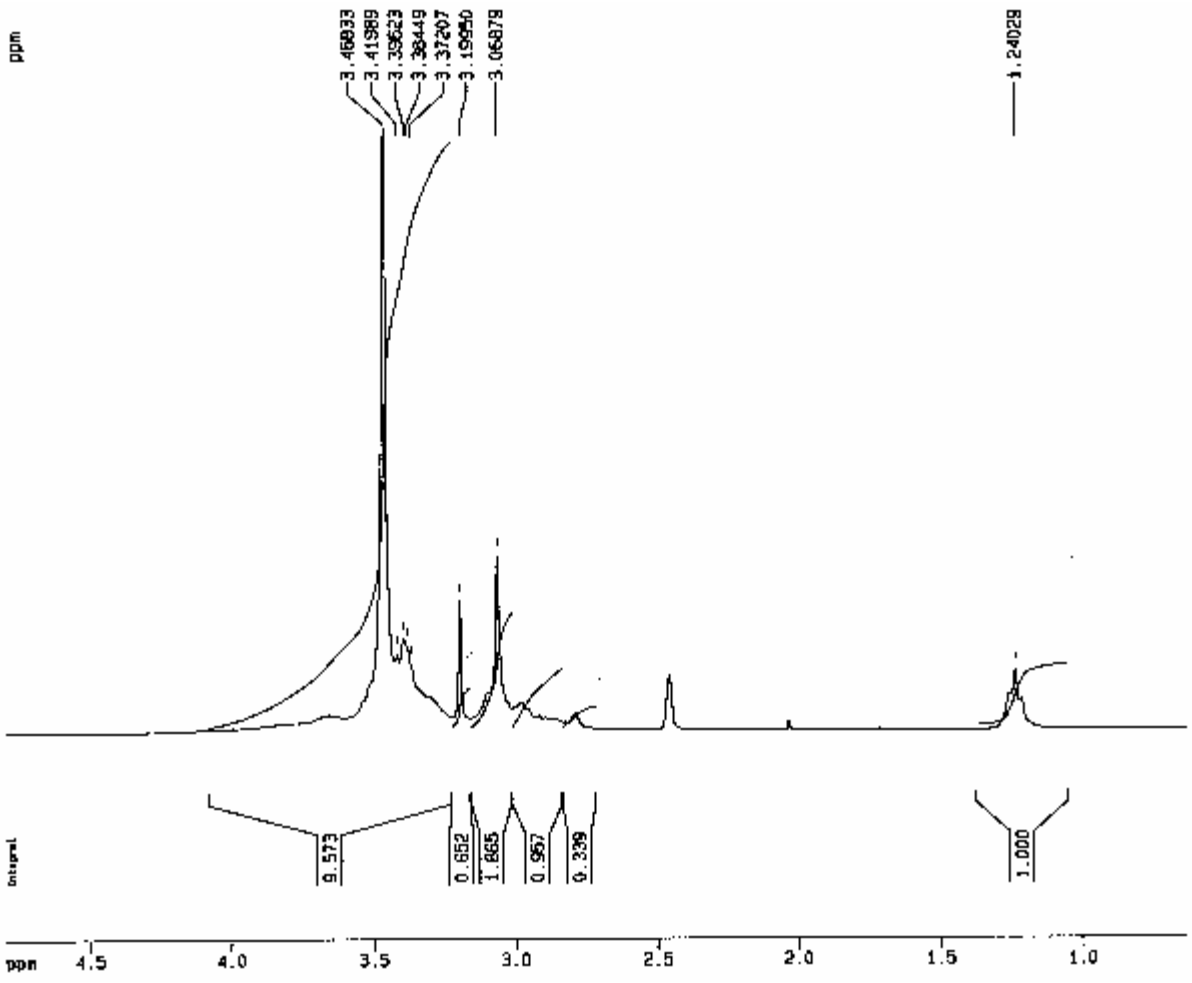
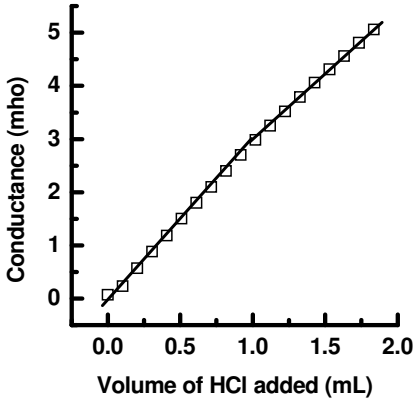
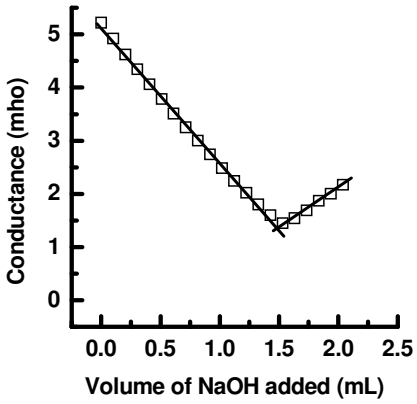


Figure S5: Representative Conductometric Titration Curves for **PG-PEG Amine:**

A) direct titration



B) back titration



References:

1) US patent 6,221,977

2) K. R. Kumar, J. N. Kizhakkedathu, D. E. Brooks, *Macromol. Chem. Phys.* 2004, *205*, 567.