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A novel thermoresponsive polypeptide: synthesis and characterization

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S1. Materials

Reagents for monomer synthesis, polymerization and modification, namely, triphosgene (98%), glutamic acid γ-methyl ester (Glu(γOMe), 99%), α-pinene (99%), *n*-hexylamine (99%) and isopropylamine (≥99.5%) were purchased from Sigma-Aldrich (Germany).

Solvents, namely, 1,4-dioxane, petroleum ether, diethyl ether and dimethylformamide (DMF), as well as inorganic acids and alkalis used to prepare solutions were purchased from Vekton (Russia). 1 M HEPES buffer solution was prepared by dissolving commercial kit (PanEco, Russia) in deionized water and additionally filtered through Millipore Merck membrane filters (Germany) with a pore size of 0.22 μm.

S2. Synthesis and Modification

To obtain P[Glu(γOMe)] **2**, an NKA monomer **1** of the corresponding amino acid was synthesized with triphosgene in anhydrous 1,4-dioxane in the presence of α-pinene according to the previously published procedure [R.Wilder, S. Mobashery, *J. Org. Chem.*, 1992, **57**, 2755].

The polymerization reaction was carried out in a 4% solution of the monomer in dry 1,4-dioxane for 96 h at 22 °C. The monomer (**1**)/initiator ($HexNH_2$) ratio for P(Glu(γOMe)) **2** was [M]/[I] = 100. After polymerization, polymer **2** was precipitated with diethyl ether, washed and dried *in vacuo*. The yield of product **2** was 78%.

To remove the protective (OMe)-group, a 250 mg polymer **2** sample was saponified with 1M NaOH in a dioxane-water mixture (6:1) for 1 h at 22 °C under vigorous stirring. After that, the resulting polymer solution was transferred to a dialysis bag and subjected to dialysis using a membrane with MWCO 1000 against deionized water. The resulting polymer was then lyophilized and the removal of protecting groups was monitored by ^{1}H NMR and FTIR spectroscopy. The yield of polymer product **3** (PGlu) was 60%.

The modification of PGlu **3** with isopropylamine was carried out by complete activation of the carboxy groups in the polymer using HOBt and DCC. For this purpose, PGlu **3** was dissolved in DMF, a 2-fold molar excess of HOBt relative to the amount of carboxy groups was added and incubated for 15 min. The mixture was incubated for 30 min and then a solution of DCC in DMF (2 eq. relative to carboxy groups) was added. The concentration of polymer in the reaction mixture was 10 wt%. The reaction was left under stirring for another 45 min at room temperature, and then isopropylamine (4 equiv.) regarding carboxy groups was added to the activated polymer. After 20 h of stirring, the reaction mixture was diluted with water and transferred to a dialysis bag (MWCO 1000). Dialysis was carried out sequentially against DMF/deionized water mixture (50:50, v/v) and then deionized water (48 h in total). The obtained aqueous polymer solution was freeze dried. The yield of modified polymer **4** was 80%.

S3. Polymer Characterization

Size Exclusion Chromatography

The molecular weight characteristics of polymer were analyzed by size-exclusion chromatography (SEC) using a Prominence Shimadzu LC-20 system with a refractometer detector RID 10-A (Kyoto, Japan) and containing a tandem of two columns Agilent PLgel MIXED-D (7.5 × 300 mm, 5 µm) (USA). A 0.1 M solution of LiBr in DMF was used as eluent. The mobile phase flow rate was 1.0 ml min^{-1}, and the analysis temperature was 40 °C. Number average (M_n) and weight average (M_w) molecular weights, as well as dispersity ($Đ$) of the obtained polymer were calculated using calibration curve built on polymethylmethacrylate standards with molecular masses in the range from 17,000 to 250,000 and dispersity below 1.14. The calculations were performed using GPC LC Solutions software (Shimadzu, Kyoto, Japan).

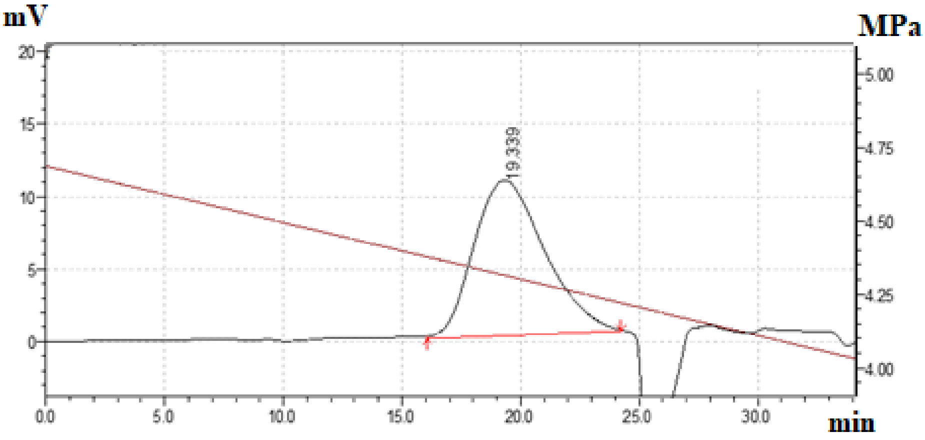
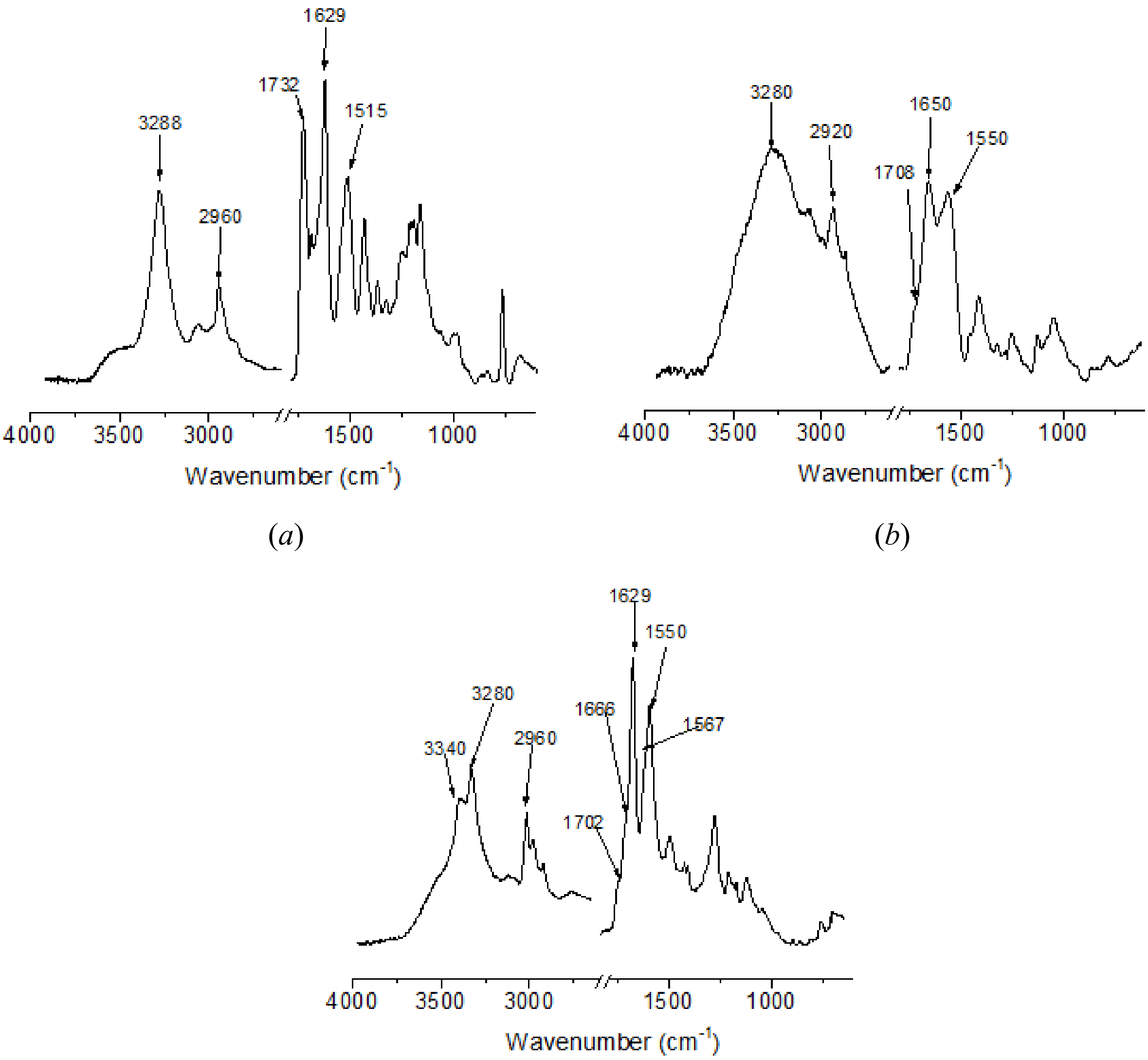


Figure S1. SEC trace of P[Glu(γOMe)] **2** in DMF.

Fourier-Transform Infrared Spectroscopy

The spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer (Germany) supplied with a console for total internal reflection with a working element made of ZnSe. The resolution was 4 cm^{-1}, the number of scans was 60. During the spectra registration, a correction for the penetration depth as a function of wavelength was applied.



(*a*) (*b*)

(*c*)

Figure S2. FTIR spectra of synthesized (*a*) P[Glu(γOMe)] **2**, (*b*) its deprotected **3** and (*c*) modified with Pr^iNH_2 **4** derivatives.

In FTIR spectrum of P[Glu(γOMe)] **2**, the characteristic bands corresponding to valent vibrations of the NH bond of the amide group (3288 cm^{-1}), vibrations of the C=O ester group (1732 cm^{-1}), vibrations of the C=O bond of the amide group (1629 cm^{-1}, amide I) and strain vibrations of the NH bond of the amide group (1515 cm^{-1}, Amide II) are detected (Figure S2, *a*). The band at 2960 cm^{-1} corresponds to the CH_3 vibrations of the methyl ester group. The positions of the characteristic bands of Amide I and Amide II correspond to *β*-folded conformation of the polypeptide chain.

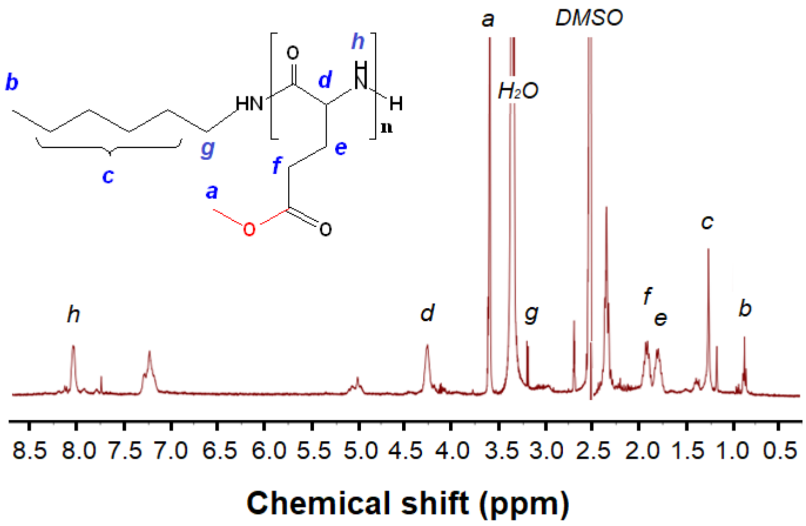
After alkaline hydrolysis (polymer **3**), the bands at 2960 cm^{-1} and 1732 cm^{-1}, characteristic to methyl ester, disappeared (Figure S2, *b*). The appeared broad band with a maximum at 3280 cm^{-1} includes the OH vibrations of glutamic acid and the valence vibrations of the NH bond of the amide group. The shoulder at 1708 cm^{-1} is characteristic of the C=O vibrations of the glutamic acid group. The characteristic bands at 1650 cm^{-1} and 1550 cm^{-1} correspond to the amide bond when the polypeptide chain is in the α-helical conformation.

FTIR spectrum of the polypeptide **4** modified with Pr^{i}NH$_2$ contains the 3280 cm^{-1}, 1629 cm^{-1}, and 1550 cm^{-1} bands characteristic of the polypeptide chain (Figure S2, *c*). The 2960 cm^{-1} band corresponds to the CH$_3$ group of isopropylamide moiety. In addition, some background rise in the region of 3500-3200 cm^{-1} is noticeable, which along with the 1702 cm^{-1} band indicates the presence of glutamic acid fragments in the sample. The bands at 3400 cm^{-1}, 1666 cm^{-1} and 1567 cm^{-1} may be attributed to the side chain amides.

^{1}H NMR spectroscopy

The spectra of polymers were recorded using a Bruker AC-400 NMR spectrometer (400 MHz) (Germany) in DMSO-d$_6$ at 25 °C. The substitution degree was calculated as the ratio of the integral intensity of the signals of amide protons of isopropylamide group (NH, 7.71 ppm) to the signal of methanetriyl group proton of PGlu (CH, 4.25 ppm). Spectra of P[Glu(γOMe)], PGlu and P[Gln(Pr^i)] are presented below.

(*a*)



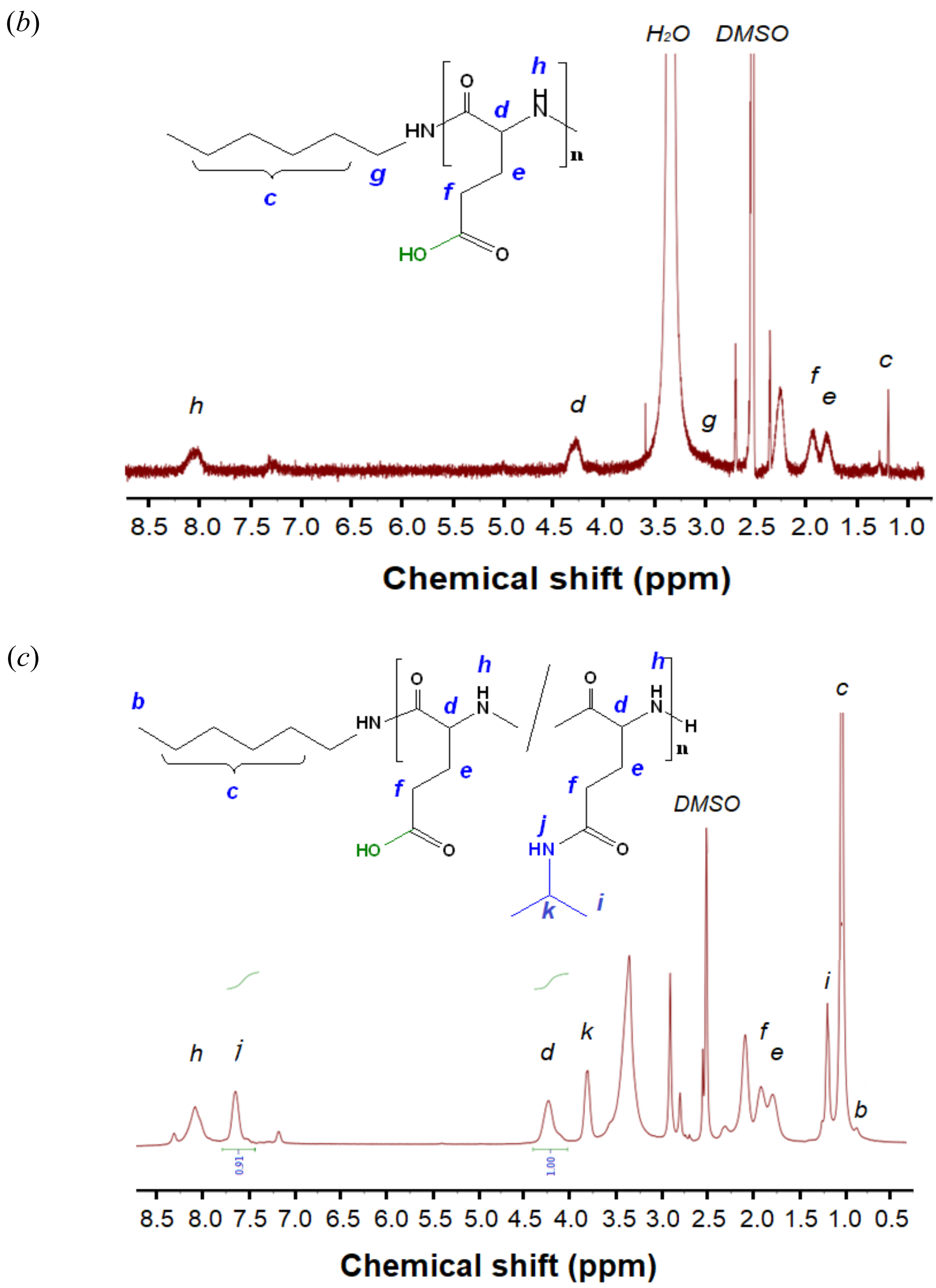


Figure S3. ^{1}H NMR spectra of (*a*) P[Glu(γOMe)] **2**, (*b*) its derivative deprotected by alkaline saponification **3** and (*c*) modification with Pr^{i}NH$_2$ **3**.

S4. Light Scattering and Thermoresponsive Properties

Light scattering was studied on a Photocor Complex instrument (Photocor Instruments Inc., Russia); the light source was a Photocor-DL diode laser (power, 5–30 mW; wavelength, λ=659.1 nm). The calibration of the device, that is, the determination of the instrument coefficient, was carried out using toluene (R_V=1.38×10^{-5}cm^{-1}). The measurements were performed at scattering angles θ in the range 45–135°. The correlation function of the scattered light intensity was recorded using a PhotocorPC2 correlator with 288 channels and processed using DynalS software (ver. 8.2.3, SoftScientific, Tirat Carmel, Israel). The experiments were carried out at a temperature of 20 °C.

The temperature-sensitive behavior of polymer aqueous solutions was investigated by light scattering and turbidimetry methods on a Photocor Complex unit equipped with a Photocor-PD sensor for recording the intensity of transmitted light. Solutions with different concentrations were investigated at temperatures from 15 to 75 °C in deionized water. The temperature was varied discretely in steps of 2 – 4 °C at low temperatures and in steps of 1.0 °C near the phase separation temperature. The temperature and concentration dependences of the scattered light intensity *(I),* optical transmittance *(I*),* and hydrodynamic radii (R_h) of scattering objects in solutions were determined. For all investigated polymer solutions, the time required to achieve time-constant values of their characteristics at low temperatures was 600-1000 s, and sometimes exceeded 2500 s near the phase separation area.