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# Magnetic resonance imaging of tumor with a self-traceable polymer conjugated with an antibody fragment

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ABSTRACT

A $^{13}$C-enriched phosphorylcholine polymer ($^{13}$C-PMPC) as a self-traceable MR (magnetic resonance) tag was conjugated with a fragment (scFv) of Herceptin, a clinical antibody against antigen Her2. When injected in model mice bearing Her2(+) (gastric) and Her2(−) (pancreatic) tumors, the antibody-tag conjugate $^{13}$C-PMPC-scFv selectively accumulated in the Her2(+) tumor with a rapid build-up/decay (accumulation/clearance) profile and, with the use of the $^{1}$H–$^{13}$C double-resonance (heteronuclear correlation) technique, the Her2(+) gastric tumor was clearly MR imaged.



Current MRI (magnetic resonance imaging) is based on the $^{1}$H NMR relaxation of water and affords morphological images of the body.[1] New directions of MRI focus on specific detection of tumors, which may be MR-imaged using either tumor targeters appropriately labeled with MR-responsible moieties such as $^{19}$F nuclei[2] or paramagnetic contrast agents,[3] or specific signal amplification techniques such as hyperpolarization[4] and chemical exchange saturation transfer[5] for tumor-responsible small molecules such as pyruvate, $H_2O_2$, or glucose. Recently, we reported a different approach.[6] A phosphorylcholine polymer (PMPC) enriched with $^{13}$C at the methyl groups with a mean molecular weight of $M_n$ = 63,000 was found to accumulate highly selectively and efficiently in the tumor (colon 26) of tumor-bearing mice primarily by the so-called EPR (enhanced permeability and retention) effect, and the tumor could be clearly MR-visualized with this $^{13}$C-concentrated polymeric nano-probe by the $^{1}$H–$^{13}$C double-resonance[7] (heteronuclear correlation) technique.[6c] This probe thus provides a novel example of a self-traceable EPR polymer that is free from foreign labeling. However, there are still some issues that need to be addressed if this probe is to have wider applicability as a tumor imager. One issue is the slowness of accumulation and clearance. Another involves the general utility of the EPR effect[8], that is, the size-allowed invasion and retention of nano-particles in tumor tissues which usually have a defective vascular wall with a wide opening and undeveloped lymphatic drainage. Although the EPR effect has been widely used for the passive targeting of tumors,[9] the EPR-susceptibility of tumor tissues depends on the type of tumor.[10] While colon 26, a mouse rectal cancer cell line, is highly susceptible to EPR, this is not necessarily true for other types of tumors.[11] In the present work, we prepared a conjugate of $^{13}$C-PMPC as an MR signal generator with an antibody fragment as an active tumor targeter, and investigated the performance of this conjugate with the above issues in mind. We report here that the antibody-functionalized probe can selectively image an antibody-responsive but otherwise less EPR-susceptible tumor with a rapid build-up/decay profile.

Methacryloyloxyethylphosphorylcholine (MPC) enriched with $^{13}$C at the choline methyl groups was polymerized under the

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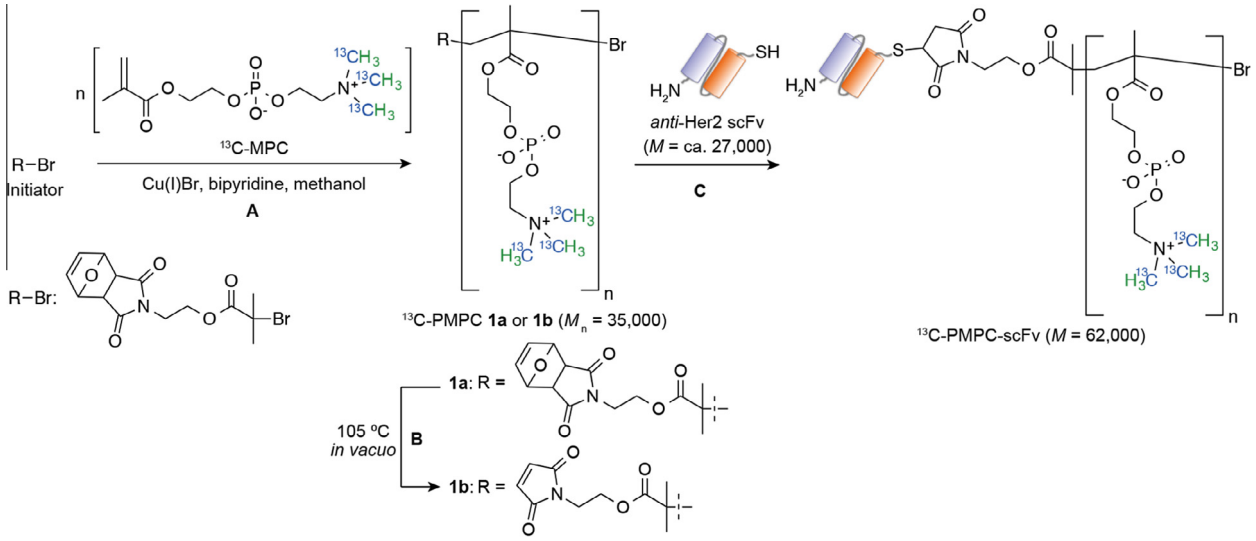


**Scheme 1.** Preparation of $^{13}$C-PMPC-scFv. (A) Atom-transfer radical polymerization of monomer $^{13}$C-MPC to afford polymer $^{13}$C-PMPC **1a** ($M_n$ = 35,000), (B) retro-Diels Alder reaction of protected polymer **1a** to give deprotected one $^{13}$C-PMPC **1b** with an active maleimide terminus, (C) coupling of the maleimide of **1b** with a fragment (scFv, $M$ = ca. 27,000) of Herceptin to afford $^{13}$C-PMPC-scFv ($M_n$ = 62,000).

atom-transfer radical polymerization (ATRP) conditions[12] using furan-protected maleimide bromide (R-Br) as an initiator and CuBr/2,2′-bipyridine as a catalyst, according to the scheme n(MPC) + R-Br → R-(MPC)$_n$-Br (Scheme 1).[6c] The polymer thus obtained ($^{13}$C-PMPC) had a mean molecular weight of $M_n$ = 35,000 ($n$ = 118) and a polydispersity index of $M_w/M_n$ = 1.4. This was subjected to coupling with a fragment (scFv) of Herceptin, a well-known clinical antibody against antigen Her2 that is expressed in breast cancer as well as gastric cancer.[13] The actual fragment used (scFv) was a 257-amino acid sequence (from M1 to C257) that contained fragments of the heavy and light chains of Herceptin, conjugated with a nonapeptide ($H_6G_2C$) composed of a His-tag ($H_6$) linked with a tripeptide glycine-glycine-cysteine ($G_2C$) at the C-terminus (Supplementary information for details).[14] The genetically engineered single-chain fragment variable (scFv, $M$ = ca. 27 kDa) was expressed in *Escherichia coli* and purified by His-tag-targeted affinity chromatography. For polymer-antibody coupling, $^{13}$C-PMPC was carefully heated in vacuo at 105 °C in the absence of any solvent to allow deprotection (retro-Diels Alder removal of the furan ring) of the terminal initiator moiety into an active maleimide form. Addition (Michael addition) of the terminal cysteine residue in scFv to the maleimide at a molar ratio of $^{13}$C-PMPC/scFv = 24.5 afforded conjugate $^{13}$C-PMPC-scFv with a mean molecular weight of 35,000 + 27,000 = 62,000, which was freed from $^{13}$C-PMPC in excess on treatment with a His-tag column, purified by size-exclusion chromatography (GPC) (Fig. 1a), and characterized by electrophoresis (SDS–PAGE) (Fig. 1b) and $^1$H-{$^{13}$C} double-resonance NMR analysis (Fig. 1c).

Antigen Her2 is known to be highly expressed in gastric cancer as well as in breast cancer, but not in pancreatic cancer.[15] Thus, N87 (a human gastric cancer cell line) and SUIT2 (a human pancreatic cancer cell line) were used here as Her2(+) and Her2(−) tumor cells, respectively.[16] Model mice were obtained by transplanting the Her2(+) and Her2(−) tumor cells at the right shoulder and the contralateral left shoulder, respectively, of healthy mice (~20 g). Probe $^{13}$C-PMPC-scFv (113.5 mg/kg = 1.83 μmol/kg body weight or 2.27 mg/20-g mouse) was intravenously injected into the tail vein of tumor-bearing mice. In vivo MR images of the mice were taken under probe-optimized double-resonance ($^1$H–{$^{13}$C}) conditions in essentially the same manner as described elsewhere[6c] (Supplementary information for details). Figure 2a shows a typical image (double-resonance image overlaid on a $T_2$-weighted single-resonance ($^1$H) morphological image)[17] taken at 22 h after administration of the probe. We can clearly see that (1) the Her2(+) gastric tumor is clearly visualized while the Her2(−) pancreatic tumor is hardly visible, and (2) there is no appreciable accumulation of the probe in the liver, although some unidentified, noise-looking spots are still noticed. The observed

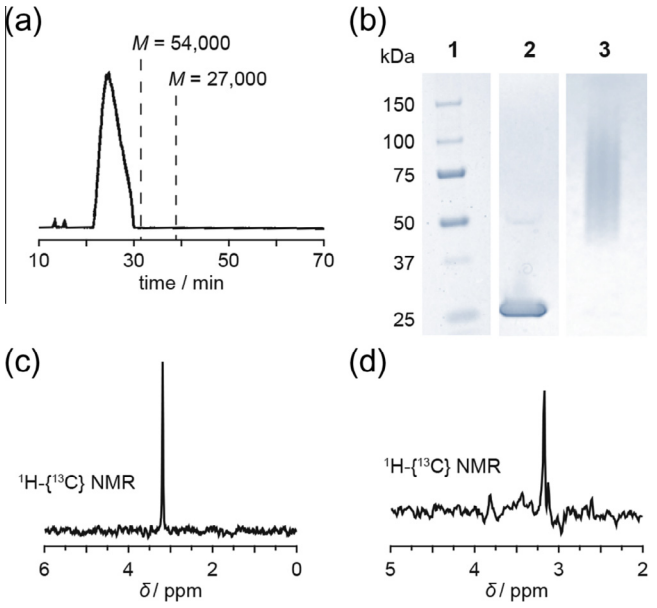


**Figure 1.** (a) GPC trace for $^{13}$C-PMPC-scFv. Retention times for scFv ($M$ = 27,000) and its S–S (disulfide) dimer ($M$ = 54,000) (see Supplementary information) are also indicated. (b) SDS–PAGE analysis for $^{13}$C-PMPC-scFv. Lane 1, molecular-weight marker. Lane 2, monomeric scFv ($M$ = ca. 27 kDa). Lane 3, $^{13}$C-PMPC-scFv ($M$ = 62 kDa). The broad band observed for $^{13}$C-PMPC-scFv is due to molecular-weight distribution ($M_w/M_n$ = 1.4) for the PMPC moiety. (c) One-dimensional $^1$H–{$^{13}$C} double-resonance NMR spectrum for $^{13}$C-PMPC-scFv in $D_2O$, showing a single signal for the $^{13}$C-enriched methyl protons. The spectrum was obtained at 25 °C with a Bruker Avance 700 spectrometer equipped with a 5 mm TCI CryoProbe in a similar manner as reported previously.[6c] (d) Ex vivo double-resonance NMR spectrum of the Her2(+) tissue removed from a probe-administered mouse at a time point of 48 h from injection of the probe.