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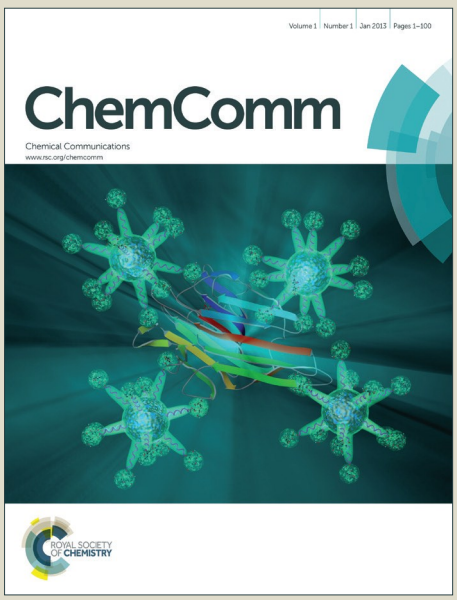

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Enhanced Target Recognition of Nanoparticles by Cocktail PEGylation with Chains of Varying Lengths

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Monodispersed gold nanoparticles (AuNPs) were simultaneously decorated with lactosylated and non-modified shorter poly(ethylene glycol)s (PEGs) to enhance their target recognition. The decoration with sufficiently shorter PEGs dramatically enhanced multivalent binding ability of lactosylated AuNPs to lectin-fixed surface, possibly due to enhanced mobility of the ligands via the spacer effect generated by the shorter PEG chains.

Over the last two decades, poly(ethylene glycol)-coated nanoparticles (PEG-NPs) have been extensively developed for biomedical applications such as biosensing, diagnosis, and drug delivery.[1–6] This is primarily because the hydrophilic and flexible PEG palisades can disperse well in biological media due to the steric repulsive effect, which can also suppress nonspecific protein adsorption and secondary association between NPs. Additionally, PEG-NPs can be modified further for specific binding to target molecules (or receptors) by installing ligand molecules to the distal end of PEG chains.[1–3,5,6] Installation of multiple ligands to PEG-NPs results in remarkably higher binding ability (or avidity) through a multivalent effect compared to that observed with the installation of a single ligand.[3,7–13] Indeed, many previous studies have reported successful target recognition of ligand-installed PEG-NPs, especially in the drug delivery field.[14–22] On the other hand, few studies have focused more on fundamental issues, such as the effect of PEG chain length, flexibility, and conformation on ligand-receptor interactions.[3,10,23,24] One possible drawback of the PEG modification (PEGylation) with single chains is that ligand molecules as well as PEG chains are likely interfered by the neighboring PEG chains particularly on densely PEGylated surface (Figure 1A); the mobility of ligands (or flexibility of PEG chains) is restrained by the neighboring PEG chains to reduce the accessibility of ligands (or avidity of ligand-installed PEG-NPs) to the receptor.[3,10]

To overcome this drawback, the present study was aimed at alleviating the interfering effect of neighboring PEG chains for enhanced avidity of ligand-installed PEG-NPs without compromising their distinctive surface property for negligible nonspecific protein adsorption (or stealthiness). To this end, shorter PEG chains were tethered to NPs along with ligand-installed longer PEG chains. It was assumed that this "cocktail" PEGylation should generate the spacer effect for ligand-installed longer PEG chains, allowing higher mobility of ligands for better accessibility to the receptor (Figure 1B). It is worth noting that the cocktail PEGylation can more effectively suppress nonspecific protein adsorption onto the PEGylated surface, compared to that fabricated with single PEG chains, because of the filler effect of shorter PEG chains.[24] The cocktail PEGylation on PEG-NP surface was herein investigated using a series of model PEG-NPs,[25–29] which were prepared by decorating monodispersed gold NPs (AuNPs) simultaneously with two different PEG molecules, lactosylated PEGs (L-PEGs) with a fixed chain length and non-lactosylated (or methoxy) PEGs (N-PEGs) with shorter chains of varying lengths. As illustrated in Figure 1C, the target-binding ability of cocktail PEG-AuNPs was systematically evaluated using a surface plasmon resonance (SPR) system with a lectin that specifically binds to lactose molecules, Ricinus communis agglutinin 120 (RCA_{120}).[30]

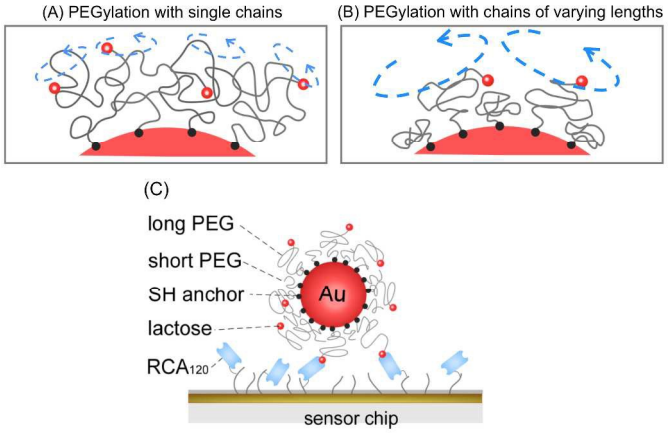

Figure 1. Spacer effects generated by cocktail PEGylation with chains of varying length for enhanced target recognition. (A) Ligand mobility is likely restrained by neighboring PEG chains with the same length. (B) Ligand mobility is rescued by alleviating the restrictive effect using shorter PEG as filler layer. (C) Schematic illustration of the target recognition of cocktail PEG-AuNPs on an RCA_{120}-immobilized SPR sensor chip.

Firstly, a heterobifunctional PEG possessing a lactose group at one end and a thiol (SH) group at the other end (L-PEG-SH) was synthesized for anchoring on gold NPs, as previously described (Scheme S1).[31] The obtained L-PEG-SH had a molecular weight (M_w) of 11 kDa (thus abbreviated as L11) and a lactose inclusion rate of 63% determined by time-of-flight mass spectrometry (ToF-MS) and ^{1}H NMR (data not shown). In addition, three N-PEG-SH derivatives with different chain length (M_w = 2, 5, and 10 kDa, abbreviated as N2, N5, and N10, respectively) were synthesized in a similar manner except for lactose installation. The R_g values of isolated PEG molecules in water were calculated to estimate the size of PEG chains (Table 1).[32,33] Then, a series of PEG-AuNPs were prepared by simply adding AuNPs (20.7 ± 0.1 nm in hydrodynamic diameter) to mixtures containing different molar ratios of L-PEG-SH and N-PEG-SH (L11:NX = 100:0, 75:25, 50:50, and 25:75; X = 2, 5, or 10) in an aqueous solution at ambient temperature.[11,34] After centrifugal purification, the cocktail of PEG-AuNPs was dispersed in phosphate buffered saline (pH 7.4). PEGylation of AuNPs was verified by dynamic light scattering (DLS) as summarized in Table S1, where the hydrodynamic diameter of all the PEG-AuNPs was in the range of 40–60 nm with a narrow distribution, and was decreased with an decrease in M_w of shorter PEGs used in cocktail PEGylation. These values indicate that AuNPs were successfully PEGylated without aggregate formation. Also, there was observed a slight decrease in the diameter with increasing the fraction of shorter PEG in the PEG cocktail. Monodispersed size distribution was further confirmed for all PEG-AuNP samples, as shown in their DLS histograms (Figure S1A–C). Henceforth, the obtained PEG-AuNPs are denoted by the feeding ratio between L-PEG-SH and N-PEG-SH (*i.e.* L11:NX) because the conjugation ratio of each PEG chain cannot be precisely determined after cocktail (or simultaneous) PEGylation.

Table 1. Composition of PEG derivatives used in this study.

Code	Polymer	M_w*	R_g (nm)**
L11	L-PEG-SH	11203	4.4
N10	N-PEG-SH	10225	4.2
N5	N-PEG-SH	5165	2.8
N2	N-PEG-SH	2243	1.7

* Determined by MALDI-TOF MS. ** Gyration radii of isolated PEG in water $R_g = 0.181 \times N^{0.58}$, where N is the degree of PEG polymerization.[32,33]

The target recognition of cocktail PEG-AuNPs was evaluated with RCA_{120} immobilized on a CM3 SPR sensor chip using a Biacore 3000 system at a density of approximately 0.01 molecule/nm^2 at 25°C, as described previously.[9,35] As shown in a typical sensorgram (Figure S2), the cocktail PEG-AuNPs prepared at L11:N5 = 50:50 showed a considerable increase in resonance unit (RU) in a time-dependent manner, whereas such an increase in RU was not observed for the PEG-AuNPs prepared with single PEG chains (L11 or N10). It should be noted that the RCA_{120}–immobilized surface was completely regenerated by adding an excess amount of galactose (100 mg/mL) (Figure S2), suggesting the target-specific interaction between the cocktail PEG-AuNPs and RCA_{120} without non-specific adsorption. This fact enabled the sequential evaluation of different PEG-AuNPs using the same sensor chip surface.

A series of cocktail PEG-AuNPs were systematically evaluated at different particle concentrations and apparently saturated RU values were plotted against the particle concentration (Figures 2A–D). Regardless of the mixing ratio, the cocktail PEG-AuNPs prepared from N10 and PEG-AuNPs prepared at L11:NX = 100:0 showed almost similar RU values, suggesting that N10 did not act as molecular fillers, presumably due to a chain length (or R_g) similar to that of L11. In contrast, the cocktail PEG-AuNPs prepared from even shorter PEGs (N2 and N5) showed larger RU values than PEG-AuNPs prepared at L11:NX = 100:0. Particularly, the RU values of cocktail PEG-AuNPs became larger with the decrease in M_w of N-PEG-SH at all tested L11:NX ratios. The largest values were obtained at L11:N2 = 50:50 and 25:75, which were two orders of magnitude larger than that at L11:NX = 100:0. This significantly enhanced binding ability of cocktail PEG-AuNPs prepared from N2 may be due to the substantially smaller R_g of N2, which enables efficient molecular filling between the longer L11 chains. It should also be noted that a greater binding ability of cocktail PEG-AuNPs was observed at L11:N2 = 25:75 and 50:50 than at L11:N2 = 75:25, suggesting that the filling effect of shorter PEG chains is more critical for the binding ability of PEG-AuNPs than the fed amount of ligand-installed PEG chains. This result can be reasonably explained by considering that the binding ability of ligand-installed PEG-NPs is determined by the number of receptor-accessible ligand molecules.

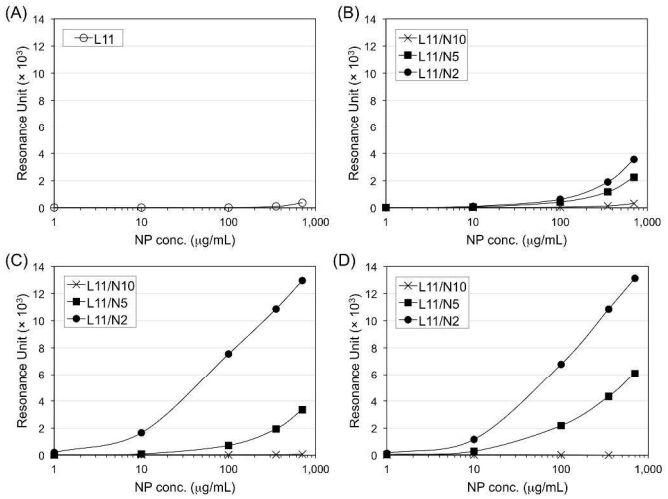

Figure 2. Target-binding abilities of PEG-AuNP cocktails determined by SPR. The feeding ratios of tested cocktail PEG-AuNPs were L11:NX = 100:0 (A), 75:25 (B), 50:50 (C), and 25:75 (D). The resonance unit values at 900 sec after injection (from the respective sensorgram) were plotted against the concentration of injected PEG-AuNPs (Flow rate: 5 μL/min, injection time: 15 min, temperature: 25°C).

In conclusion, cocktail PEGylation using sufficiently shorter PEGs as molecular fillers has been demonstrated to dramatically enhance the target-binding ability of small ligand-installed PEG-NPs. This finding suggests that the mobility of ligands, as well as their density, is crucial for the multivalent binding ability of ligand-installed systems, and thus should be carefully designed. Altogether, cocktail PEGylation is a promising approach to elicit the multivalent potential of ligand-installed PEG-NPs, which may be useful for designing "targeted" drug delivery systems.

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