One-pot three-component double-click method for synthesis of [^{67}Cu]-labeled biomolecular radiotherapeutics†

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A one-pot three-component double-click process to prepare tumor-targeting agents for cancer radiotherapy is described here. By utilizing DOTA (or NOTA) containing tetrazines (DOTA: 1, 4, 7, 10-tetraazadodecane-1, 4, 7, 10-tetraacetic acid, NOTA: 1, 4, 7-triazacyclononane-1, 4, 7-triacetic acid) and the TCO-substituted aldehyde (TCO: trans-cyclooctene), the two click reactions, the tetrazine ligation (an inverse electron-demand Diels-Alder cycloaddition)[1] and the RIKEN click (a rapid 6π-azaelectrocyclization),[2–8] could simultaneously proceed under mild conditions to afford the covalent attachment of the DOTA or NOTA, which forms a bioavailable stable complex with copper (II), to biomolecules such as albumin and anti-IGSF4 antibody without altering their activities (Fig. 1).

Subsequently, the radiolabeling of DOTA- or NOTA-attached albumin and anti-IGSF4 antibody (a tumor-targeting antibody) with ^{67}Cu as a promising β^-/γ-e-mitting theranostic radionuclide having a half-life of 62 h, which is compatible with radioimmunotherapy, could be achieved by mixing DOTA- or NOTA-attached albumin and anti-IGSF4 antibody with RIs and subsequent purification by Amicon filtration; a separate experiment with ^{65}Zn was conducted for comparison (Fig. 2 and Table 1). ^{67}Cu and ^{65}Zn could be produced in the ^{70}Zn$(d,\alpha n)^{67}$Cu and ^{nat}Cu$(d,x)^{65}$Zn reactions at the AVF cyclotron. Our work provides a new and operationally simple method for introducing ^{67}Cu to biomolecules, which is an important process for preparing clinically relevant tumor-targeting agents.

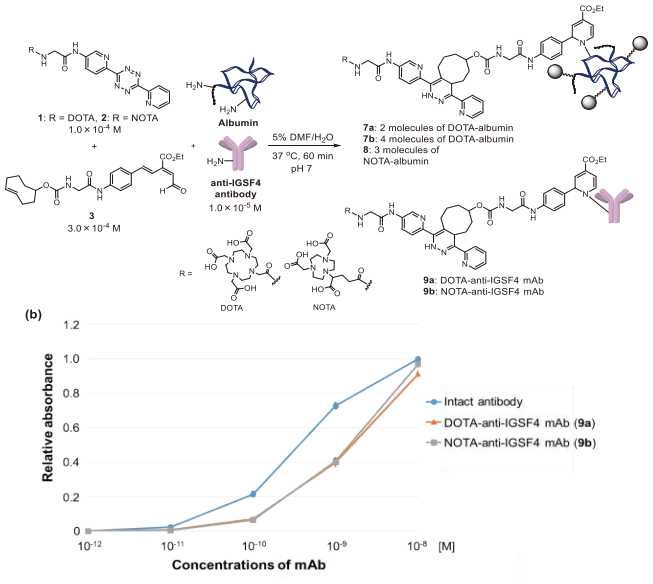

Fig. 1. (a) One-pot three-component click labeling of albumin and anti-IGSF4 antibody as a cancer-targeting agent. (b) Affinities of intact and labeled anti-IGSF4 antibodies to IGSF4 analyzed by ELISA. DMF = N,N-dimethyl formamide, ELISA = enzyme-linked immunosorbent assay.

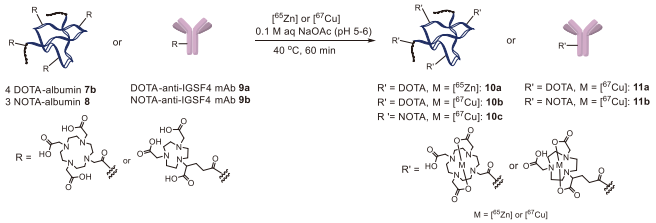

Fig. 2. Radiolabelings of DOTA or NOTA-attached albumins and anti-IGSF4 antibody.

Table 1. Radiochemical yields (RCY) of ^{67}Cu and ^{65}Zn.

Entry	Chelator-attached biomolecules	Added [^{65}Zn] or [^{67}Cu][a] (Radioactivities)	RCY[b] (%)
1	DOTA-albumin **7b**	[^{65}Zn] (300 kBq)	80
2	DOTA-albumin **7b**	[^{67}Cu] (11 MBq)	72
3	DOTA-anti-IGSF4 mAb **9a**	[^{67}Cu] (11 MBq)	51
4	NOTA-albumin **8**	[^{67}Cu] (11 MBq)	19
5	NOTA-anti-IGSF4 mAb **9b**	[^{67}Cu] (11 MBq)	7

[a] Specific activities of ^{67}Cu and ^{65}Zn were 110 MBq/μg and 125 MBq/μg, respectively.
[b] RCY (Radiochemical yield) was obtained from the radioactivity of the purified radiolabeled product against the added [^{65}Zn] or [^{67}Cu].

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