Site-specific modification of nanobodies targeting ovarian cancer and innovative bioconjugation methods for the efficient detection of cancer at early stage

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Abstract

Ovarian cancer (OC) is one of the most common cancer types in woman. The incidence of OC increases with age. OC can be successfully treated if detected early but the diagnosis of OC at early stages is difficult since there are no obvious symptoms and no screening test has proven to be effective. Several biomarkers have been identified for the diagnosis and therapy of ovarian carcinomas such as Cancer Antigen 125 (CA125) or Human Epididymis protein 4 (HE4). Furthermore, Secretory leukocyte protease inhibitor (SLPI) and Progranulin (PGRN) are both overexpressed markers related to survival in ovarian cancer. PGRN has been described as a prognostic biomarker for the advanced stages while SLPI has been considered as an early detection marker of OC. However, those biomarkers´ sensitivity is still poor in the early stages of the disease, with an average of 50% for stage I and 90% for the stage II or higher. Since the selectivity and sensitivity for the biomarkers dedicated to OC are still insufficient for the detection at early stages and for monitoring the treatment and the recurrence of the disease, we attempt to develop a more efficient biosensor strategy based on the simultaneous detection of the biomarkers HE4, SLPI and PGRN. Several approaches, by which a bio-orthogonal functional group will be introduced site-specifically in the nanobody structure, will be explored. This might pave the way to develop a biosensor platform on which all nanobodies have a uniform orientation at the biosensor surface. This should lead to an improved sensitivity since all nanobodies will have their active regions accessible for binding the biomarker. The best strains of each kind of nanobody (HE4, SLPI and PGRN) were characterized by measuring the binding affinity of these Nbs with their antigens via enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR) and epitope mapping.

Methods

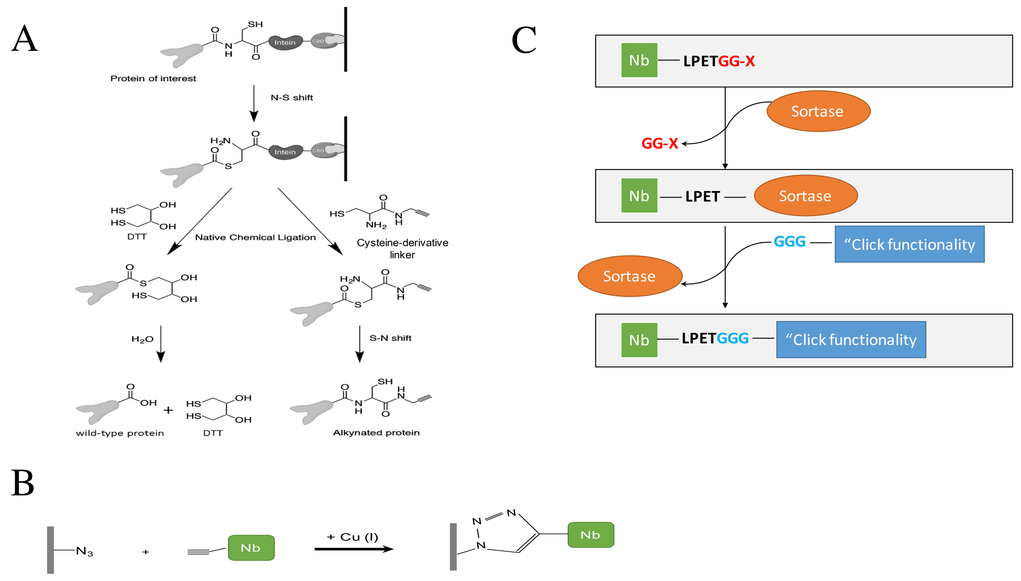

Figure 1. Site-specific functionalization and conjugation methods of Nanobodies.
(A) Expressed protein ligation (EPL). **(B)** Copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC). **(C)** Sortase-A mediated protein ligation (SPL). **Nb:** Nanobody

Results

Clone	Estimated K_D (nM)
2183	4.62
2203	509.68
2204	353.86
2205	6.78
2209	198.08
2210	4.56
2211	48.15
2212	28.72
2213	-
2214	23.31
2309	16.04
2310	-
2364	746.75

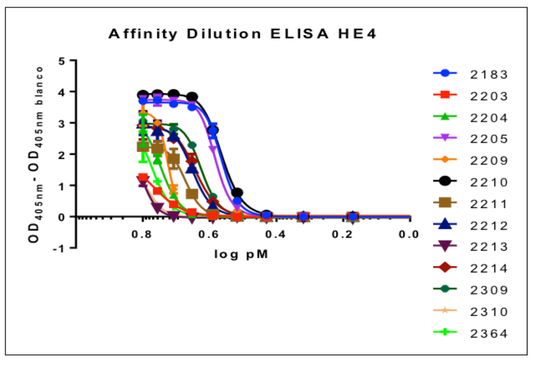

Figure 2. Affinity of HE4 Nanobodies by ELISA
HE4-2R22.1(2183); HE4-2R49.3(2203); HE4-2R50.2(2204); HE4-2R42.1 (2205); HE4-2R5.1(2209); HE4-2R8.1(2210); HE4-2R71.3(2211); HE4-2R78.3 (2212); HE4-2R36.3(2213); HE4-1R50.1(2214); 1R1.1(2309); HE4-1R47.1(2310); HE4-2R57.4(2364)

Clone	Estimated K_D (nM)
2252	4.06
2253	4.33
2258	32.4
2262	78.9
2275	3.44
2276	1.96
2277	91.5
2311	4.04

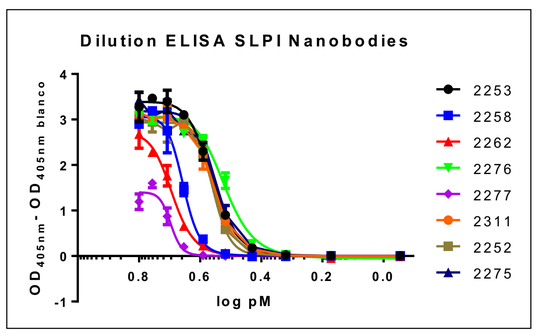

Figure 3. Affinity of SLPI Nanobodies by ELISA
Nb_1SLPI-2R-2-2 (2252); Nb_1SLPI-2R-4-1 (2253); Nb_1SLPI-2R-33-1 (2258); Nb_1SLPI-2R-94-2 (2262); Nb_1SLPI-3R-23-1 (2275); Nb_1SLPI-2R-37-2 (2276); Nb_1SLPI-2R-7-1 (2277); Nb_1SLPI-2R-60-1 (2311).

Clone	Estimated K_D (nM)
3223	768
3232	198
3233	175
3234	15
3237	2.7
3238	-
3239	1.13
3246	2.06
3250	3.48
3274	122
3276	1.77
3277	-

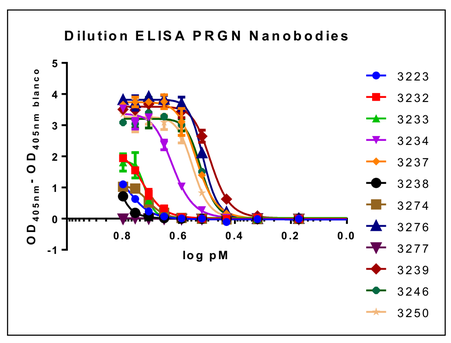

Figure 4. Affinity of PGRN Nanobodies by ELISA
Nb_1PRGN-1R-22-1 (3223); Nb_1PRGN-2R-43-1 (3232); Nb_1PRGN-2R-48-1 (3233); Nb_1PRGN-2R-90-1 (3234); Nb_1PRGN-2R-4-1 (3237); Nb_1PRGN-2R-8-2 (3238); Nb_1PRGN-3R-14-1 (3239); Nb_1PRGN-3R-33-2 (3246); Nb_1PRGN-3R-13-2 (3250); Nb_1PRGN-3R-25-2 (3274); Nb_1PRGN-3R-27-3 (3276); Nb_1PRGN-3R-43-2 (3277).

		HE4		SLPI		PGRN		
Clone		2183	2210	2252	2276	3237	3239	3276
Expression		4.4 mg/L	7.72 mg/L	20.87 mg/L	0.82 mg/L	10.45 mg/L	0.53 mg/L	11.31 mg/L
ELISA		+++	+++	+++	+++	+++	+++	+++
Biacore	k_{on}	5.2E+6	3.2E+6	3.2E+6	2.1E+6	1.0E+5	1.1E+6	5.2E+5
	k_{off}	1.9E-3	8.9E-3	5.6E-2	3.5E-3	3.3E-3	7.9E-4	2.2E-3
K_D		3.6E-10	2.8E-9	1.8E-8	1.7E-9	3.3E-8	7.2E-10	4.2E-9
Epitope mapping		- independent of **2211** & **2212** - interferes with **2205** & **2309**	Not measured but same behavior expected as **2183**	- independent of **2276** - interferes with **2253**; **2258** & **2311**	- independent of **2252; 2258** - interferes with **2253** & **2311**	- independent of **3239; 3246** & **3250** - competitive with **3276**	- independent of **3237; 3246** & **3276** - competitive with **3250**	- independent of **3239; 3246** & **3250** - competitive with **3237**

Figure 5. Characterization of best clones of HE4; SLPI and PGRN Nanobodies

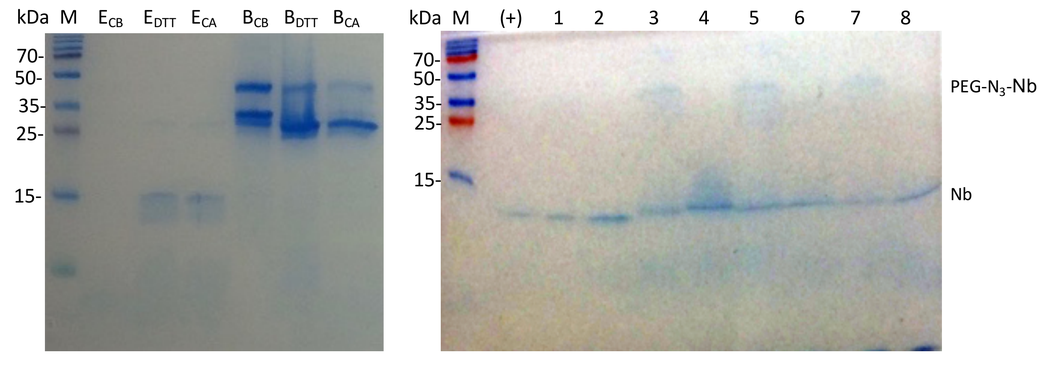

Figure 6. Purification of Nb-mediated by alkynation and CuAAC of Nb with polymer PEG-N3 10000 and analysis by SDS-PAGE . E: Elution fraction; B: Bead fraction. (+) positive control; 1,3,5,7: Alkynated Nbs; 2,4,6,8: non-alkynated Nbs.

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