Innovative bioconjugation methods for an improved detection of ovarian cancer at early stage using multiple biomarkers

Lan-Huong Tran[1], Natalia Smiejkowska[2], Cecile Vincke[2], Nick Devoogdt[2], Serge Muyldermans[2], Peter Adriaensens[1,3], and Wanda Guedens[1]

[1] Biomolecule Design Group, Institute for Materials Research (IMO), Hasselt University, Agoralaan - Building D, BE-3590 Diepenbeek, Belgium

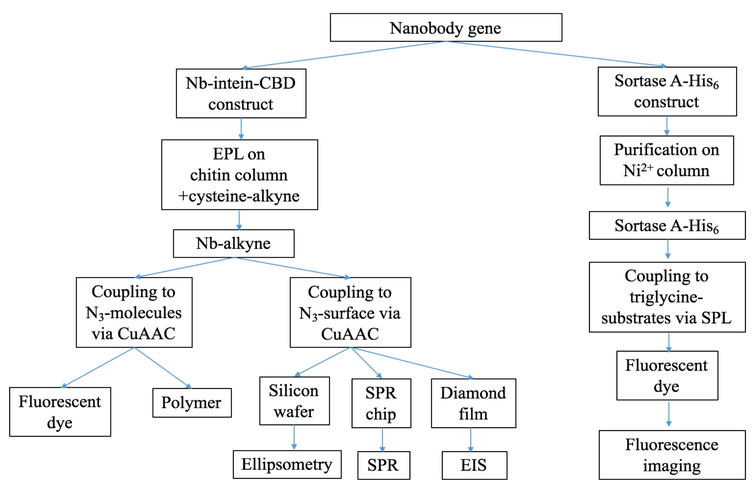
[2] Cellular and Molecular Immunology, Vrije Universiteit Brussel, Boulevard de la Plaine 2, 1050 Ixelles

[3] Applied and Analytical Chemistry, Institute for Materials Research (IMO), Hasselt University, Agoralaan - Building D, BE-3590 Diepenbeek, Belgium

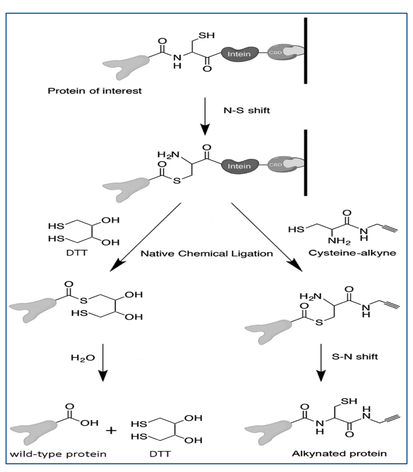
Abstract

Ovarian cancer (OC), whose incidence increases with age, is one of the most common cancer types in women. 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 and 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 of 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 more efficient biosensor detection strategies based on the bioconjugation of nanobodies with the biomarkers HE4, SLPI and PGRN. Several approaches, by which the introduction of a site-specific and bio-orthogonal functional group can pave the way to a uniform orientation of the nanobodies on the biosensor surface, will be explored. This should lead to an improved sensitivity since all nanobodies will have their active regions accessible for binding the biomarker.

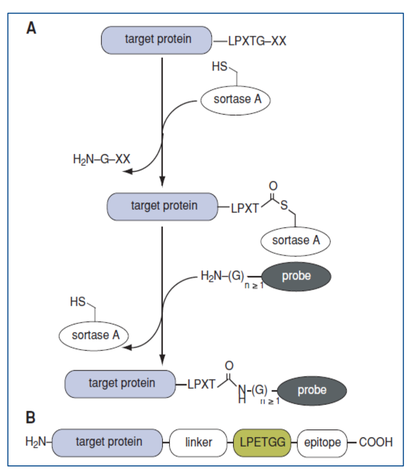
Methods



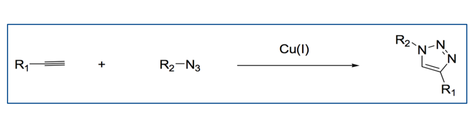
CuAAC: Copper (I)-catalyzed azide-alkyne cycloaddition; **CBD**: Chitin binding domain; **EIS**: Electrochemical impedance spectroscopy; **EPL**: Expressed protein ligation; **SPL**: Sortase A-mediated protein ligation;



Expressed protein ligation - EPL



Sortase A – mediated protein ligation - SPL



Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) Click Chemistry

Results

Seq Family		Estimated K_D (nM)
F3	2183	4.62
F4	2203	509.68
F2	2204	353.86
F5	2205	6.78
F2	2209	198.08
F3	2210	4.56
F6	2211	48.15
F1	2212	28.72
F2	2213	-
F1	2214	23.31
F7	2309	16.04
F8	2310	-
F2	2364	746.75

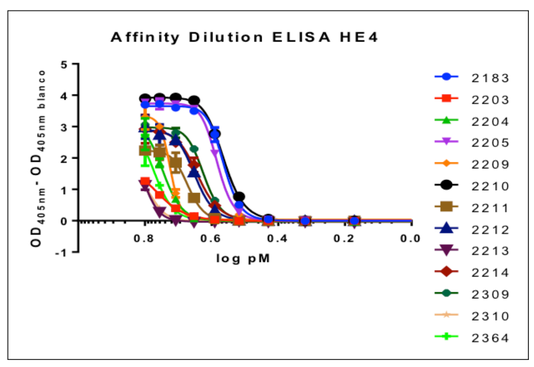


Figure 2. Affinity of HE4 Nanobodies measured by ELISA

Seq Family	Clone	Estimated K_D (nM)
F2	2253	4,33
F4	2258	32,4
F8	2262	78,9
F5	2276	1,96
F10	2277	91,5
F9	2311	4,04
F3	2252	4,06
F1	2275	3,44

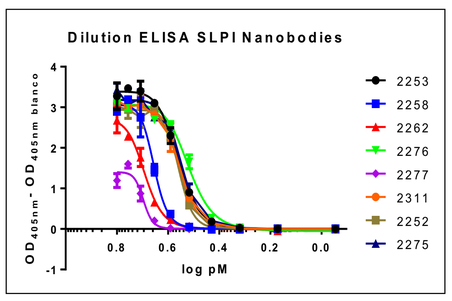


Figure 3. Affinity of SLPI Nanobodies measured by ELISA

Seq Family	Clone	Estimated K_D
F1	3223	768
F2	3232	198
F3	3233	175
F4	3234	15
F5	3237	2,7
F6	3238	-
F10	3274	122
F11	3276	1,77
F12	3277	-
F7	3239	1,13
F8	3246	2,06
F9	3250	3,48

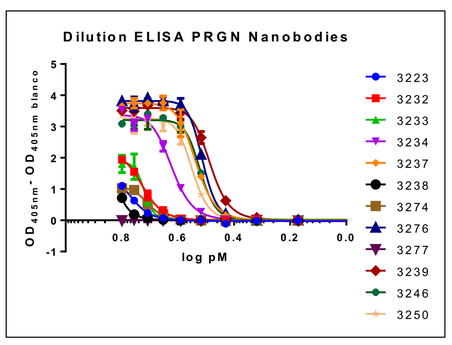


Figure 4. Affinity of PGRN Nanobodies measured by ELISA

		HE4			SLPI		PGRN		
Clone		**2183**	**2205**	**2210**	**2252**	**2276**	**3237**	**3239**	**3276**
Expression		++	+	+++	+++	+/-	+++	+	+++
ELISA		+++	+++	+++	+++	+++	+++	+++	+++
Biacore	k_{on}	5.2E+6	5.1E+6	3.2E+6	3.2E+6	2.1E+6	1.0E+5	1.1E+6	5.2E+5
	k_{off}	1.9E-3	3.1E-3	8.9E-3	5.6E-2	3.5E-3	3.3E-3	7.9E-4	2.2E-3
K_D		3.6E-10	6.1E-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**	- competitive with **2211**; **2212**& **2309** - interferes with **2183**	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

References

(1) American Cancer Society. (2016) *Atlanta Am. Cancer Soc.* Report.
(2) Ta, D. T. et al. (2015) *Protein Eng. Des. Sel. 28*, 351–363.
[3] Steen Redeker, E. et al. (2013) *Bioconjug. Chem. 24*, 1761–1777.
[4] Guimaraes, C. P. et al. (2013) Nat. Protoc. 8(9), 1787–1799.

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Huong.tran@uhasselt.be
Tel: +3211268348
Institute for Materials Research (IMO), Hasselt University, Campus Diepenbeek
Agoralaan - Building D, BE-3590 Diepenbeek, Belgium