A Nanometric Platform for Live Cell Multiplex Screening of Non-natural Peptides

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Abstract

The unique available *in vivo* high throughput screening system was proposed by Ruoshlati and Pasqualini [1]. The system is based on the use of a library of filamentous phages expressing at their surface a high number of degenerated peptide sequences. Each individual phage expresses only one peptide sequence. The selection and identification of peptide sequences on phages associated with a tumor or other tissue after IV administration of the library, permits identification of novel tumor/tissue targeted peptide sequences. However, this approach is intrinsically limited to peptides. The aim of our work is to develop a new chemistry-based technology to overcome the limitation of phage libraries, thus extending the scope of *live cell*/in *vivo* biological panning to non-natural peptides using a synthetic multiplex "phage-like" system. In this presentation we have developed a nanometric monodispersed system substituted with a panel of fluorescent probes and candidate D-peptides both tethered using click chemistry. As a first prove of concept we have synthesized a library of all-D peptides that were conjugated and screened using flowcytometry, in a variety of cancer cell lines. A positive peptide has been further validated directly using FACS and microscopy analysis.

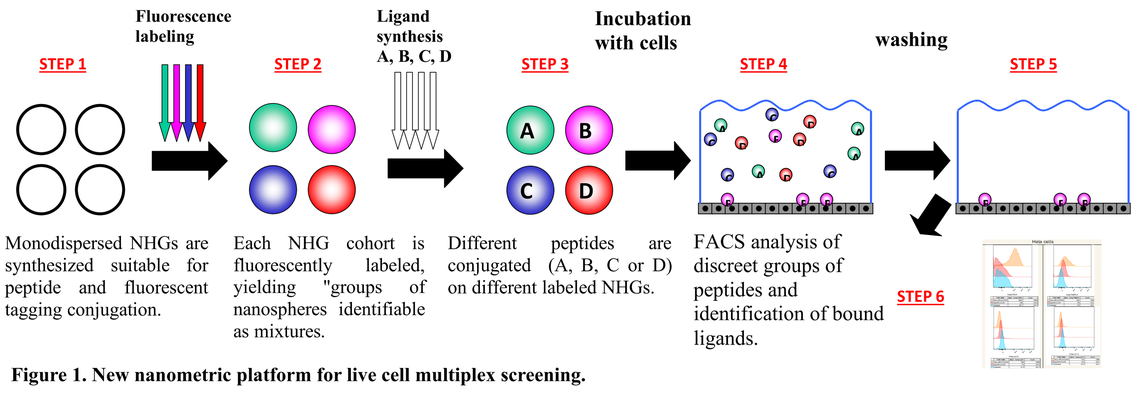


Figure 1. New nanometric platform for live cell multiplex screening.

Results and Discussion

For our system, we used monodispersed NHGs of 400 nm [2]. The NHGs possess free amino groups that are modified with propiolic acid amide. Candidate D-peptides are synthesized on a peptide synthesizer and an azido tail is placed at their N-terminus. Azido peptides and Azido probes are mixed and clicked onto the NHGs (figure 2). As a proof of concept, we have synthesized a library of 16 D-peptides that were conjugated with 4 different fluorescent probes to result in 4 multiplexed groups of 4 peptides. The groups were screened in a panel of cancer cell lines using flow-cytometry. Positive hits are defined as cells displaced into the fluorescent quadrant of the histogram at a given emission wavelength.

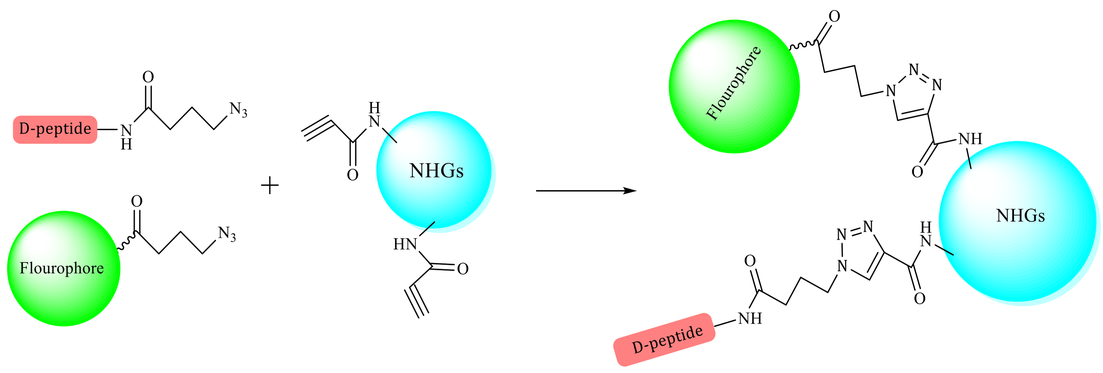
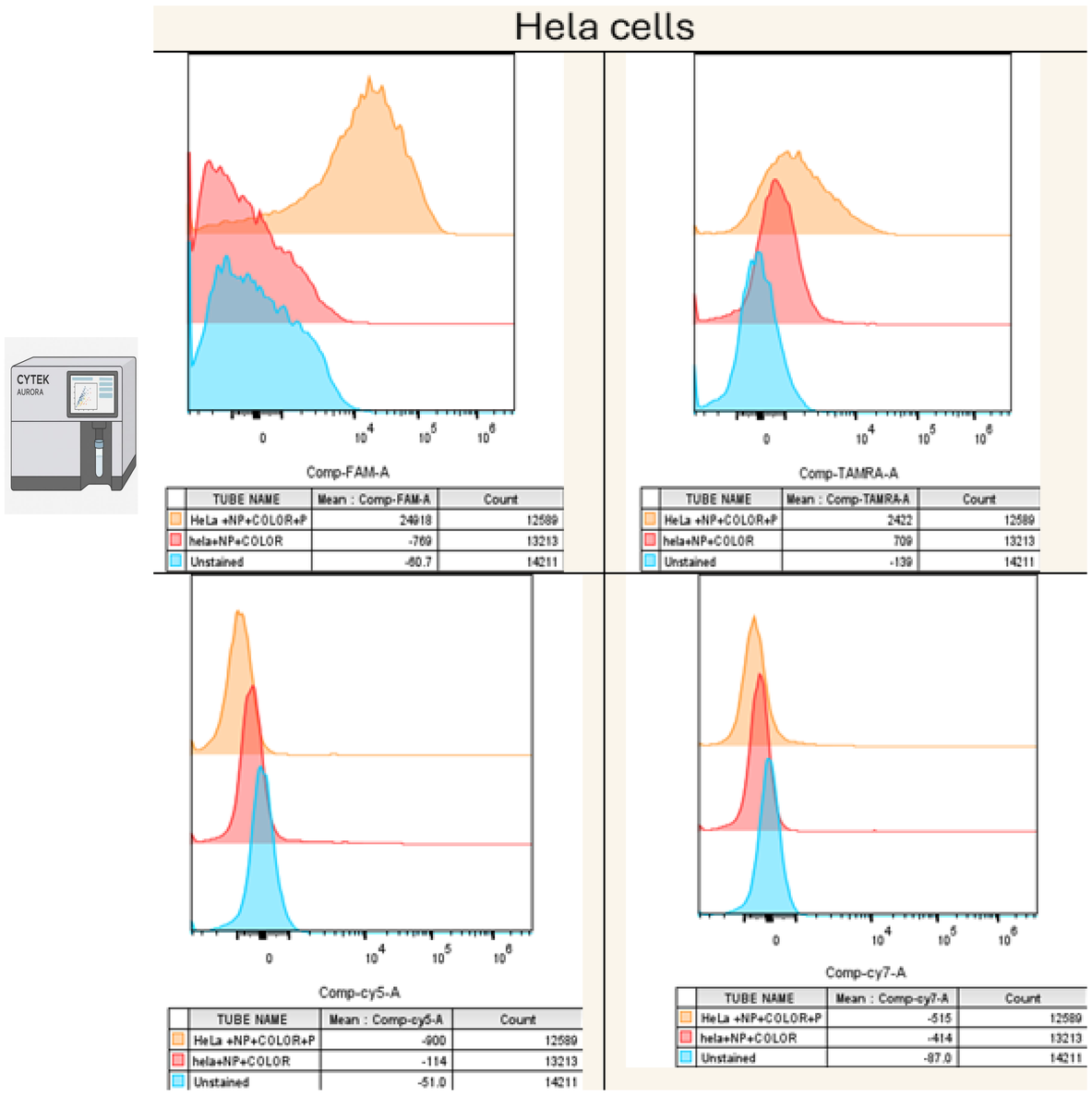


Figure 2. Strategy for conjugation to nanohydrogels using click chemistry

Peptide #	Probe on NHG	Sequence on NHG
9	FAM	n-t-a-s-y-d-w-NH_2
10	FAM	v-l-p-k-w-s-a-NH_2
11	FAM	m-p-k-e-s-q-f-NH_2
12	FAM	w-g-k-d-t-f-v-NH_2
5	TAMRA	l-t-v-a-p-w-y-NH_2
8	TAMRA	l-t-v-s-p-w-a-NH_2
13	TAMRA	f-t-k-m-r-g-d-y-a-l-h-g-NH_2
14	TAMRA	g-h-l-a-y-d-g-r-m-k-t-f-NH_2
1	*Sulfo Cy-5*	l-t-v-s-p-w-y-NH_2
4	*Sulfo Cy-5*	l-t-a-s-p-w-y-NH_2
6	*Sulfo Cy-5*	l-t-v-s-a-w-y-NH_2
15	*Sulfo Cy-5*	v-y-t-e-h-p-r-f-a-t-k-g-NH_2
2	*Sulfo Cy-7*	a-t-v-s-p-w-y-NH_2
3	*Sulfo Cy-7*	l-a-v-s-p-w-y-NH_2
7	*Sulfo Cy-7*	l-t-v-s-p-a-y-NH_2
16	*Sulfo Cy-7*	g-k-t-a-f-r-p-h-e-t-y-v-NH_2

Figure 3. Peptide/probes conjugated to nanohydrogels used in this study.



TUBE NAME	Mean : Comp-FAM-A	Count
HeLa +NP+COLOR+P	24918	12589
hela+NP+COLOR	-769	13213
Unstained	-80.7	14211

TUBE NAME	Mean : Comp-TAMRA-A	Count
HeLa +NP+COLOR+P	2422	12589
hela+NP+COLOR	709	13213
Unstained	-139	14211

TUBE NAME	Mean : Comp-cy5-A	Count
HeLa +NP+COLOR+P	-900	12589
hela+NP+COLOR	-114	13213
Unstained	-51.0	14211

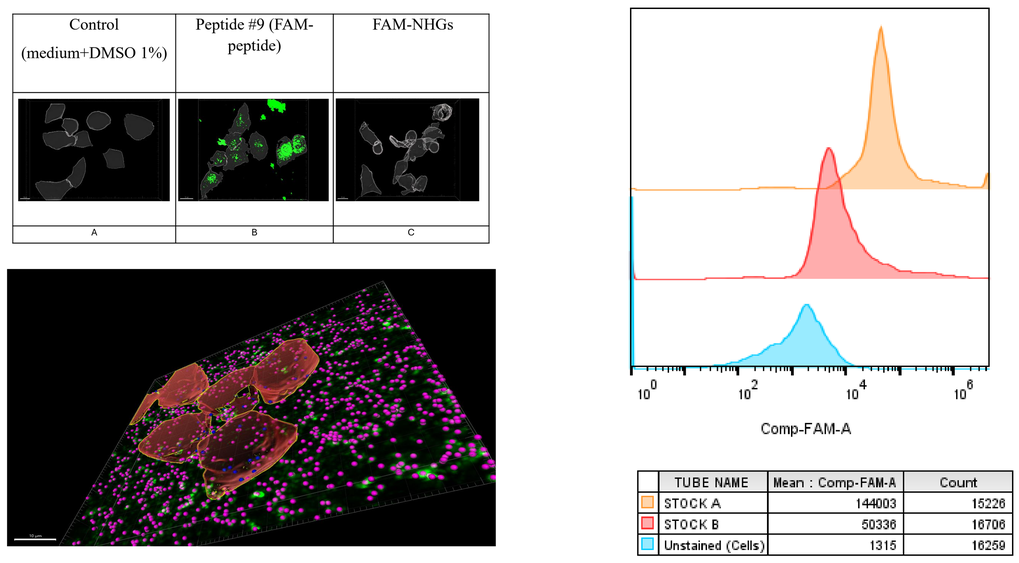
TUBE NAME	Mean : Comp-cy7-A	Count
HeLa +NP+COLOR+P	-515	12589
hela+NP+COLOR	-414	13213
Unstained	-87.0	14211

Figure 4. Example of multiplex screening using Cytek flow cytometer: peptide #9 co-labeled with 5-FAM, peptide #14 co-labeled with 5-TAMRA, peptide #4 co-labeled with Sulfo-Cy5 and peptide #2 co-labeled with Sulfo-Cy7. Blue= non labelled NHGs, Red= NHGs labelled only with probe, Orange= NHG co-labeled with peptide and probe.

Peptide #	Sequence	HeLa cells	PC-3 cells	MCF-7 cells
1 (S1-3)	l-t-v-s-p-w-y-NH_2	-	-	-
2 (S7-9)	a-t-v-s-p-w-y-NH_2	-	-	-
3 (S1-3)	l-a-v-s-p-w-y-NH_2	-	-	-
4 (S7-9)	l-t-a-s-p-w-y-NH_2	-	-	-
5 (S1-3)	l-t-v-a-p-w-y-NH_2	-	-	-
6 (S10-12)	**l-t-v-s-a-w-y-NH_2**	**-**	**+**	**+**
7 (S10-12)	l-t-v-s-p-a-y-NH_2	-	-	-
8 (S10-12)	l-t-v-s-p-w-a-NH_2	-	-	-
9 (S7-9)	**n-t-a-s-y-d-w-NH_2**	**+**	**-**	**-**
10 (S1-3)	v-l-p-k-w-s-a-NH_2	-	-	-
11 (S4-6)	m-p-k-e-s-q-f-NH_2	-	-	-
12 (S10-12)	w-g-k-d-t-f-v-NH_2	-	-	-
13 (S4-6)	f-t-k-m-r-g-d-y-a-l-h-g-NH_2	-	-	-
14 (S7-9)	**g-h-l-a-y-d-g-r-m-k-t-f-NH_2**	**+**	**-**	**-**
15 (S4-6)	v-y-t-e-h-p-r-f-a-t-k-g-NH_2	-	-	-
16 (S4-6)	g-k-t-a-f-r-p-h-e-t-y-v-NH_2	-	-	-

Figure 5. Multiplex screening of 16 peptides conjugated to NHGs on three cancer cell lines. Screening results were confirmed by two separate experiments.

Direct validation of peptide 9#



TUBE NAME	Mean : Comp-FAM-A	Count
STOCK A	144003	15226
STOCK B	50336	16706
Unstained (Cells)	1315	16259

Figure 6. Validation of FAM-peptide #9 incubated with HeLa cells during 1 h. Left Upper panel: Internal stack of confocal microscopy (A) Control cells, (B) FAM#9 100 µM, (C) FAM-NHGs (no peptide). Left lower panel: 3D illustration of fluorescence using Imaris: pink dots=extracellular peptide, blue dots= intracellular peptide. All pictures were taken in the FAM channel using the same sensitivity parameters. Right panel: FACS analysis of FAM-labeled peptide 9 at 10 (red) and 100 (orange) µM.

Conclusion

We have developed a new technique for simultaneous screening of a number of small ligands. The technique is based on the establishment of a phage –like synthetic system composed of an inert biocompatible nanohydrogel (400nm), a panel of fluorescent tags and the ligands to be tested. The technique is suitable for the detection using flow-cytometry. By this method we could identify several peptides with spefic affinity for certain cancer cell lines. Here we present the validation of peptide 9# that was directly labelled and studied under confocal microscopy and flowcytometry. We have clearly shown that a cell-based combinatorial (multiplex) screening can be performed using a mixture of different small molecules and/or peptides linked to appropriately tagged nanohydrogels. Since the hydrogels are biocompatible, the next step would be to use them directly in vivo.

Acknowledgements

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